Lab Handbook

BME 251
Undergraduate Laboratory

Spring Semester

DEPARTMENT OF BIOMEDICAL ENGINEERING
THE UNIVERSITY OF TEXAS AT AUSTIN

General Editor:
– Lisa Brannon-Peppas, Ph.D.

Contributors:
– Lisa Brannon-Peppas, Ph.D., Bongsu Jung, Jihoon Kim, Jason Koranek,
Srivalleesha Mallidi, Jim Pollard, Yul Young Park, Jignesh Shah, and Greg Thoreson.

Document and Graphic Design:
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– Lynn Loo, Ph.D., The University of Texas at Austin
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– Ken Diller, Ph.D, Nicholas A. Peppas, Ph.D., and Christine Schmidt, Ph.D.

Contact Information:
Lisa Brannon-Peppas, Ph.D.
1 University Station
CPE 3.168a Mailstop: C0300
Austin, TX 78712

Office: 512.471.4348
Fax: 512.471.4348

Revision A

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BIOMEDICAL ENGINEERING
THE UNIVERSITY OF TEXAS AT AUSTIN

BME UG Lab Policies

This section contains information necessary for getting started in the BME undergraduate laboratory. Laboratory safety is everyone's priority, and you must become familiar with the laboratory safety policies and practices. The section, "Before You Can Work in the Laboratory" on page 1-2, describes what you must do before working in the laboratory.

The last half of this chapter provides the Material Safety Data Sheets (MSDS) for the chemicals that you may be working with in this laboratory. Material Safety Data Sheets are extremely important in the safe handling of hazardous substances. It is your responsibility to become familiar with the specific hazards and safe handling of these chemicals—as well as the rest of the materials described in the MSDS. A glossary of MSDS terms appears at the end of this chapter.

SECTION CONTENTS

BEFORE YOU CAN WORK IN THE LABORATORY

Before you are allowed to work in the laboratory, you must first:

- Read the *Laboratory Handbook* in its entirety.
- Participate in the General Lab Safety brief provided by the Lab Manager during the first lab session.
- Sign and submit the *BME UG Lab Undergraduate Student Contract* with an initialized copy of "BME UG Lab Protocol" on page 1-3.

These prerequisites are mandatory and no exceptions will be made. See "BME 251 Student Contract" on page 1-6 for a copy of the contract you will be required to sign and submit to acknowledge your compliance with the laboratory prerequisites. Refer to http://www.utexas.edu/safety/ehs/train/courses.html for further information regarding online safety training.

LAB SAFETY

Safety is serious business. Any violation of laboratory protocol or general safety practice may result in an F and/or dismissal from the course. The laboratory space is limited, and your actions can potentially endanger others. Always be considerate of others around you.

At all times while you are in the laboratory, you are accountable for the laboratory protocol and safety practices in the following sections:

- "BME UG Lab Protocol" on page 1-3
- "General Laboratory Safety" on page 1-4

BME UG Lab Protocol

Due to the potentially hazardous nature of laboratory work, safe practices are critical to ensure your safe participation in the laboratory course. Any violation of lab protocol or general lab safety practice ("General Laboratory Safety" on page 1-4) may result in an *F* and/or dismissal from the laboratory course.

Read the following table thoroughly, placing your initials next to each box to indicate that you have read and understood the statement. Then turn a copy in with your Student Contract (see "BME 251 Student Contract" on page 1-6 for a checklist of materials that must be turned in before you may work in the lab).

BME UG Laboratory Protocol	Your Initials
When you are in the laboratory, your teaching assistant (TA) is your lab supervisor.	
Before you start any work in the lab, ensure you are familiar with the equipment you will be using—as well as the procedures for operating the equipment. Locate and read the Material Safety Data Sheets (MSDS) for the chemicals you will be using.	
Wear long pants to avoid burns and contact with hazardous chemicals. Shorts, skirts, tank tops, sandals or open-toe shoes are not allowed, and anyone wearing these items will *not* be allowed in the laboratory.	
Food, drinks, candy, or chewing gum are strictly prohibited in the laboratory.	
Never mouth pipette anything.	
Do not wear lab coats, gloves, or other personal protective clothing out of the lab.	
Always wash your hands after working with chemicals and before leaving the lab.	
Misconduct will not be tolerated at any time in the laboratory.	
If you wear contact lenses, you are required to wear eye goggles when working with laboratory chemicals. In general, you should not wear contact lenses in the laboratory, since chemicals or particulates can get caught between the lens and the eye causing severe damage to your eye.	
Clean up after yourself. Label all containers clearly, include your name and the contents of the container.	

General Laboratory Safety

In addition to "BME UG Lab Protocol" on page 1-3, you must observe the following general laboratory safety practices whenever applicable.

Lab Cleanliness

You are accountable for keeping the lab clean.

- Always leave the benchtop clean.
- Put all chemicals and lab materials away when you are finished.
- Throw away paper towels and Kimwipes that you have used.
- Double-check your lab station before you leave to ensure that you are leaving the station in a clean and organized fashion for the next group.

Labels

You must always label any tube, beaker, petri dish, or vial with your initials, the date, and contents *before* use—*even if it's water*. Failure to label containers leads to unknown chemicals, which in turn creates many problems for chemical disposal crews.

Lab Security

Your assistance in keeping the lab secure is essential. Immediately report anything suspicious to Jim Pollard, your TA, or the campus police (#1-4441).

- When the lab is unoccupied, the door must remain closed and locked at all times.
- No lab equipment may leave the lab at any time.

Good Laboratory Practices

1. Avoid loose clothing such as dresses, neckties, and dangling jewelry which can get caught in equipment. Long hair is also a risk around some equipment and should be secured as a precaution.
2. Know where to find and how to operate fire extinguishers, safety showers, eye fountains, and electrical shutoff devices.
3. Materials Safety Data Sheets (MSDS) must be brought into the lab with any chemical.
    - If you pick up a chemical yourself, you may obtain the MSDS when you pick up the chemical from the stockroom. The MSDS should be permanently placed in the yellow binder in the lab.
    - You should always familiarize yourself with the MSDS before using any chemicals.

4. Ventilation hoods should be used when transferring volatile or toxic chemicals.
5. Flammable liquids should be stored in the Flammable Liquid Storage Cabinet when not in use.
    - Minimize the amount of such materials brought into the lab.
    - When working with flammable liquids, keep the liquid in a hood and return the liquid to the *Flammable Liquid Storage Cabinet* at the end of a lab session.
6. Electrical hazards should be eliminated.
    - Frayed or otherwise hazardous electrical cords should be reported and replaced or repaired.
    - Flammable solvents should be kept away from electrical equipment.
    - Electrical equipment should be grounded.
7. Spills must be cleaned up immediately. This includes water, solvents, mercury (broken thermometer), or any other laboratory chemicals. See your lab TA for assistance.
8. Hazardous waste must be properly labeled and presented to the laboratory manager for disposal. This includes organic liquids, flammable materials, toxic chemicals, and solutions of extreme acidity or basicity. *Never* pour these solutions down the drain.

BME 251 STUDENT CONTRACT

Each semester before you may work in the lab, you must submit the following contract and have it initialed by Jim Pollard and signed by Dr. Brannon-Peppas.

Your Information

Name:________________________________ UTEID:_____________________

Current Semester & Year:_________________ Course:_____________________

E-Mail:________________________________ Phone:_____________________

Emergency Contact Information

Contact Name:___________________________ Relationship to You:_____________

Phone Number:__________________________ E-Mail:________________________

Address:____

Required Training for All Students	Date Completed	Initials (Pollard)
Read, initial, and submit copy of "BME UG Lab Protocol" on page 1-3		
Attend *General Lab Safety* (BME UG Lab Manager)	1st Lecture	
Complete *Hazard Communication* (OH 101)[Note 1]		
Complete *Laboratory Safety* (OH 201)[Note 1]		
[Note 1] **These courses are required for students who have NOT completed BME 221. See http://www.utexas.edu/safety/ehs/train/courses.html for course details.**		

Student Signature:____

Lab Supervisor Signature:____

Dr. Lisa Brannon-Peppas

LAB HOURS

Due to fire code restrictions on laboratory occupancy, only one section at a time may use the lab. That is, you may only access the lab when your section is scheduled. *You will not have access the laboratory during any other time*.

The laboratory schedule for the Fall semester is as follows:

- Lecture (all sections): Wednesday 12:00 to 1:00 in GEO 2.216
- Section 1: Monday (AM) 9:00 to 12:00
- Section 2: Monday (PM) 1:00 to 4:00
- Section 3: Wednesday (AM) 9:00 to 12:00
- Section 4: Wednesday (PM) 1:00 to 4:00

LABORATORY SCHEDULE

Monday Morning Lab Section Schedule

Monday morning labs are held in CPE 1.120 from 9 AM to 12 PM on Mondays.

M AM		Assignment	Electromyography	Ultrasound	Glucose Watch
M	**1/24**	Pacemaker Lab			
M	**1/31**	Pacemaker Lab			
M	**2/7**	Pacemaker Exam	**M1**	**M2**	**M3**
M	**2/14**		**M1**	**M2**	**M3**
M	**2/21**	Lab Exam 1	**M1**	**M2**	**M3**
M	**2/28**		**M1**	**M2**	**M3**
M	**3/7**	Lab Report 1 Due	**M2**	**M3**	**M1**
M	**3/14**		**M2**	**M3**	**M1**
M	**3/21**	SPRING BREAK			
M	**3/28**	Lab 2 Exam	**M2**	**M3**	**M1**
M	**4/4**		**M2**	**M3**	**M1**
M	**4/11**	Lab Report 2 Due	**M3**	**M1**	**M2**
M	**4/18**		**M3**	**M1**	**M2**
M	**4/25**	Lab 3 Exam	**M3**	**M1**	**M2**
M	**5/2**		**M3**	**M1**	**M2**
M	**5/11**	Lab Report 3 Due by 1 pm			

Monday Afternoon Lab Section Schedule

Monday afternoon labs are held in CPE 1.120 from 1 PM to 4 PM on Mondays.

M PM		Assignment	Electromyography	Ultrasound	Glucose Watch
M	1/24	Pacemaker Lab			
M	1/31	Pacemaker Lab			
M	2/7	Pacemaker Exam	M4	M5	M6
M	2/14		M4	M5	M6
M	2/21	Lab 1 Exam	M4	M5	M6
M	2/28		M4	M5	M6
M	3/7	Lab Report 1 Due	M5	M6	M4
M	3/14		M5	M6	M4
M	3/21	SPRING BREAK			
M	3/28	Lab 2 Exam	M5	M6	M4
M	4/4		M5	M6	M4
M	4/11	Lab Report 2 Due	M6	M4	M5
M	4/18		M6	M4	M5
M	4/25	Lab 3 Exam	M6	M4	M5
M	5/2		M6	M4	M5
M	5/11	Lab Report 3 Due by 1 pm			

Wednesday Morning Lab Section Schedule

Wednesday morning labs are held in CPE 1.120 from 9 AM to 12 PM on Wednesdays.

W AM		Assignment	Electromyography	Ultrasound	Glucose Watch
W	1/26	Pacemaker Lab			
W	2/2	Pacemaker Lab			
W	2/9	Pacemaker Exam	W1	W2	W3
W	2/16		W1	W2	W3
W	2/23	Lab 1 Exam	W1	W2	W3
W	3/2		W1	W2	W3
W	3/9	Lab Report 1 Due	W2	W3	W1
W	3/16	SPRING BREAK			
W	3/23		W2	W3	W1
W	3/30	Lab 2 Exam	W2	W3	W1
W	4/6		W2	W3	W1
W	4/13	Lab Report 2 Due	W3	W1	W2
W	4/20		W3	W1	W2
W	4/27	Lab 3 Exam	W3	W1	W2
W	5/4		W3	W1	W2
W	5/11	Lab Report 3 Due by 1 pm			

Wednesday Afternoon Lab Section Schedule

Wednesday afternoon labs are held in CPE 1.120 from 1 AM to 4 PM on Wednesdays.

W PM		Assignment	Electromyography	Ultrasound	Glucose Watch
W	1/26	Pacemaker Lab			
W	2/2	Pacemaker Lab			
W	2/9	Pacemaker Exam	W4	W5	W6
W	2/16		W4	W5	W6
W	2/23	Lab 1 Exam	W4	W5	W6
W	3/2		W4	W5	W6
W	3/9	Lab Report 1 Due	W5	W6	W4
W	3/16	SPRING BREAK			
W	3/23		W5	W6	W4
W	3/30	Lab 2 Exam	W5	W6	W4
W	4/6		W5	W6	W4
W	4/13	Lab Report 2 Due	W6	W4	W5
W	4/20		W6	W4	W5
W	4/27	Lab 3 Exam	W6	W4	W5
W	5/4		W6	W4	W5
W	5/11	Lab Report 3 Due by 1 pm			

CONTACT INFORMATION

You may contact laboratory personnel by phone or e-mail, if you require contact outside of normal classroom hours. As a safety precaution, please ensure that your e-mail messages include a descriptive subject heading.

Course Instructor

Lisa Brannon-Peppas, Ph.D.
CPE 3.168a 491-4348
peppas@mail.utexas.edu
office hours: Monday 2:00 to 3:00, Wednesday 10:00 to 11:00,
or by appointment

Special Projects Coordinator/Lab Manager

Jim Pollard
ENS 602 491-0616 & 232-5804
jim.pollard@mail.utexas.edu
office hours: by appointment. Generally available M-F 8:00am to 5:00pm

Technical TAs

Jihoon Kim (EMG Lab)
ENS 20-E 491-9497
jihoonkim@mail.utexas.edu

Yul Young Park (Iontophoresis Lab)
CMA 2.228A 232-1478
pyy@mail.utexas.edu

Srivalleesha Mallidi (Ultrasound)
ENS 613 471-7520
sm3256@engr.utexas.edu

MATERIAL SAFETY DATA SHEETS

The following section provides a list of chemicals that you may be working with in our lab. The material safety data sheets (MSDS) for these chemicals are located in the MSDS notebook located in the laboratory, as well as available on the BME UG Lab web site as PDFs. "MSDS Glossary" on page 1-12 provides definitions for terms used in the MSDS.

You may be required to work with some or all of the following substances:

- Ascorbic Acid
- Galactose
- Gelatin from Porcine Skin, Type A
- Glucose
- Hydroxylapatite
- Maltose

It is your responsibility to read the MSDS information for these chemicals. Do *not* attempt to work with these chemicals until you have read the MSDS information and thoroughly understand the risks.

You may view or download the MSDS in PDF format at:

http://www.bme.utexas.edu/ugrad/UGLab/msds.html

MSDS GLOSSARY

The following glossary was provided by the Environmental Health and Safety Department at the University of Texas at Austin. Please visit their web site for additional MSDS information and resources:

http://www.utexas.edu/safety/ehs/msds/

ACGIH—*A*merican *C*onference of *G*overnmental *I*ndustrial *H*ygienists. An organization of professionals in governmental agencies or educational institutions engaged in occupational safety and health programs. ACGIH develops and publishes recommended occupational exposure limits for chemical substances and physical agents. See *TLV*.

Acid—A substance that produces hydrogen ions (H+) in aqueous solutions. An acid will destroy human tissue on contact. The pH values of acids are between 0 and 6. Strong acids have a lower pH and are more corrosive than weak acids. Examples of strong acids include hydrochloric acid, nitric acid, and phosphoric acid. *See also pH, Bases, Corrosive*.

Acute Effects—Adverse symptoms that occur immediately or shortly after an exposure to a chemical. Common symptoms of acute exposure include headache, dizziness, or nausea.

Acute Toxicity—Acute effects resulting from a single dose of, or exposure to, a substance.

Aerosol—A fine suspension in the air of small particles (*e.g.*, smoke or fog).

Air-Purifying Respirator—A respirator that uses chemicals to remove specific gases and vapors from the air or that uses a mechanical filter to remove particulate matter. An air-purifying respirator must only be used when there is sufficient oxygen to sustain life and the air contaminant level is below the concentration limits of the device. See also *Chemical Cartridge Respirator*.

Alkali—See *Base*.

Allergic Reaction—An abnormal response by the body to chemical or physical stimuli (*e.g.*, hives, sneezing).

Anesthetic—A chemical that causes a total or partial loss of sensation. Overexposure to anesthetics can cause impaired judgment, dizziness, drowsiness, headache, unconsciousness, and even death. Examples include alcohol, paint remover, and degreasers.

ANSI—*A*merican *N*ational *S*tandards *I*nstitute is a privately funded, voluntary membership organization that identifies industrial and public needs for national consensus standards and coordinates development of such standards.

Antidote—A remedy to relieve, prevent, or counteract the effects of a poison.

Appearance—A description of a substance at normal room temperature and normal atmospheric conditions. Appearance includes the color, size, and consistency of a material.

Aquatic Toxicity—The adverse effects to marine life that result from being exposed to a toxic substance.

Asphyxiant—A vapor or gas that can cause unconsciousness or death by suffocation due to lack of oxygen. Most simple asphyxiants are harmful to the body only when they become so concentrated that they reduce oxygen in the air to dangerous levels of 18 percent or lower. The normal level of oxygen in the air is about 21 percent. Asphyxiation is one of the principal potential hazards of working in confined and enclosed spaces.

Asymptomatic—Showing no symptoms.

Atm—*Atmosphere*, a unit of pressure equal to 760 mmHg (mercury) at sea level.

Auto-Ignition Temperature—The minimum temperature at which a substance can ignite without a spark or a flame. Some examples: acetone 538°C (1000°F), ethyl ether 180°C (356°F), phenol 715°C (1319°F).

Base—A substance that produces hydroxide ions (OH-) in aqueous solution. The pH values of bases are between 8 and 14. Strong bases have a higher pH and are more corrosive than weak bases. Examples of strong bases include sodium hydroxide, and ammonium hydroxide. See also *pH, Acid, Corrosive*.

Biodegradable—Capable of being broken down into non harmful products by the action of living things.

Boiling Points (BP)—The temperature at which a liquid changes to a vapor state at a given pressure. The boiling point is usually expressed in degrees Fahrenheit at sea level pressure (760 mmHg, or one atmosphere).

Some examples of boiling points:

Propane	-42°C (-44°F)
Butane	-0.5°C (31°F)
Gasoline	38°C (100°F)
Water	100°C (212°F)
Ethylene Glycol (Antifreeze)	197°C (387°F)

Bonding—The interconnecting of two objects by means of a clamp and bare wire. Its purpose is to equalize the electrical potential between the objects to prevent a static discharge when transferring a flammable liquid from one container to another. The conductive path is provided by clamps that make contact with the charged object and a low resistance flexible cable which allows the charge to equalize.

CAA—*Clean Air Act* was enacted to regulate/reduce air pollution. CAA is administered by the U.S. Environmental Protection Agency (EPA).

Carcinogen—A substance or agent that has been demonstrated to cause or produce cancer in mammals, including humans. Carcinogens are regulated by OSHA and are listed in the National Toxicology Program Annual Report of Carcinogens.

CAS—*Chemical **A**bstracts **S**ervice* is an organization under the American Chemical Society. CAS abstracts and indexes chemical literature from all over the world in *Chemical Abstracts*. *CAS Numbers* are used to identify specific chemicals or mixtures.

Caustic—See *Base*.

cc—*Cubic **c**entimeter* is a volume measurement in the metric system that is equal in capacity to one milliliter (ml). One quart is about 946 cubic centimeters (0.946L).

Ceiling Limit (PEL or TLV)—The maximum allowable human exposure limit for an airborne substance which is not to be exceeded even momentarily. See also *PEL* and *TLV*.

Centigrade—A unit of temperature. To convert from centigrade to Fahrenheit, multiply the temperature given in centigrade degrees by 9, divide that number by 5, then add 32.

Central Nervous System—The brain and spinal cord. These organs supervise and coordinate the activity of the entire nervous system.

CERCLA—*Comprehensive **E**nvironmental **R**esponse, **C**ompensation, and **L**iability **A**ct of 1980*. The Act requires that the Coast Guard National Response Center be notified in the event of a hazardous substance release. The Act also provides for a fund (the Superfund) to be used for the cleanup of abandoned hazardous waste disposal sites.

CFR—*Code of **F**ederal **R**egulations* is a collection of the regulations that have been promulgated under United States Law.

Chemical Cartridge Respirator—A respirator that uses various chemical substances to purify inhaled air of certain gases and vapors. This type respirator is effective for concentrations ten times or more times (depending on the type of respirator) the TLV of the contaminant, if the contaminant has warning properties (odor or irritation) below the TLV. See also *Air-Purifying Respirator*.

Chemical Family—A group of single elements or compounds with a common general name. Example: acetone, methyl ethyl ketone (MEK), and methyl isobutyl ketone (MIBK) are of the "Ketone" family; acrolein, furfural, and acetaldehyde are of the "aldehyde" family.

Chemical Pneumonitis—Inflammation of the lungs caused by accumulation of fluids due to chemical irritation.

CHEMTREC—*Chemical **T**ransportation **E**mergency **C**enter* is a national center established by the Chemical Manufacturers Association (CMA) to relay pertinent emergency information concerning specific chemicals on requests from individuals. CHEMTREC has a 24-hour toll-free telephone number (800-424-9300) to help respond to chemical transportation emergencies.

Chronic Effect—Adverse symptoms of chemical exposure that develop slowly over a long period of time (weeks, months or years) due to repeated long-term exposure to a substance. Examples include cancer or damage to certain internal organs. Also see *Acute Effect*.

Chronic Exposure—Repeated long-term contact with a substance.

Chronic Toxicity—Adverse effects resulting from repeated doses of or exposures to a substance over a long period of time.

Clean Air Act—Clean Air Act (CAA) was enacted to regulate/reduce air pollution. CAA is administered by the U.S. Environmental Protection Agency.

Clean Water Act—Clean Water Act (CWA) Federal law enacted to regulate/reduce water pollution. CWA is administered by the U.S. Environmental Protection Agency.

Combustible—For liquids, a liquid with a flash point above 100°F (37.8°C) but below 200°F (93.3°C). Non-liquid substances such as wood and paper are classified as "ordinary combustibles" by NFPA. Also see *Flammable Liquid*.

Common Name—A name used to identify a chemical other than its chemical name (*e.g.*, code name, code number, trade name, brand name, or generic name). See *Generic*.

Compressed Gas—

a. A gas or mixture of gases having, in a container, an absolute pressure exceeding 40 pounds per square inch (psi) at 70°F (21.1°C); or

b. A gas or mixture of gases having, in a container, an absolute pressure exceeding 104 psi at 130°F (54.4°C) regardless of the pressure at 70°F (21.1°C); or

c. A liquid having a vapor pressure exceeding 40 psi at 100°F (37.8°C) as determined by ASTM D-323-72.

Conc—Concentration.

Concentration—The relative amount of a substance when combined or mixed with other substances. Examples: 2 ppm hydrogen sulfide in air, or a 50 percent caustic solution.

Conditions to Avoid—Conditions encountered during handling or storage that could cause a substance to become unstable.

Corrosive Material—Any solid, liquid, or gas that burns, irritates, or destroys organic tissues such as the skin, lungs, and stomach. Corrosives can also destroy metal and other building materials. The term corrosive includes both acids and bases.

CWA—*Clean Water Act* was enacted to regulate/reduce water pollution. It is administered by the Environmental Protection Agency (EPA).

Decomposition—Breakdown of a material or substance by heat, chemical reaction, electrolysis, decay, or other processes into parts, elements, or simpler compounds.

Density—The mass (weight) per unit volume of a substance. Usually given in pounds per gallon or grams per milliliter. See also *Specific Gravity*.

Depressant—A substance that reduces a bodily functional activity or an instinctive desire, such as appetite.

Dermal—Relating to skin.

Dermal Toxicity—Adverse effects resulting from skin exposure to a substance.

Dike—A barrier constructed to control or confine hazardous substances and prevent them from entering sewers, ditches, streams, or other flowing waters.

DOT—U.S. Department of Transportation regulates transportation of chemicals and other substances.

Dry Chemical—A powdered fire-extinguishing agent usually composed of sodium bicarbonate, potassium bicarbonate, *etc*.

Environmental Toxicity—Information obtained as a result of conducting environmental testing designed to study the effects on aquatic and plant life.

EPA—*U.S.* ***E****nvironmental* ***P****rotection* ***A****gency*.

Evaporation Rate—The rate at which a material will vaporize (evaporate) when compared to the known rate of vaporization of a standard material. The evaporation rate can be useful in evaluating the health and fire hazards of a material. The designated standard material is usually normal butyl acetate (NBUAC or n-BuAc), with a vaporization rate designated as 1.0. Vaporization rates of other solvents or materials are then classified as:

- FAST evaporating if greater than 3.0. Examples:
  - Methyl Ethyl Ketone = 3.8
  - Acetone = 5.6
  - Hexane = 8.3
- MEDIUM evaporating if 0.8 to 3.0. Examples:
  - 190 proof (95%) Ethyl Alcohol = 1.4
  - VM&P Naphtha = 1.4
  - MIBK = 1.6
- SLOW evaporating if less than 0.8. Examples:
  - Xylene = 0.6
  - Isobutyl Alcohol = 0.6
  - Normal Butyl Alcohol = 0.4
  - Water = 0.3
  - Mineral Spirits = 0.1

Explosive—A chemical that causes a sudden, almost instantaneous release of pressure, gas, and heat when subjected to sudden shock, pressure, or high temperature.

Exposure or **Exposed—**Exposure to a chemical occurs when the chemical is taken into the body through inhalation, ingestion, skin absorption, or any other means.

Exposure Limits—The concentration in workplace air of a chemical deemed the maximum acceptable. This means that most workers can be exposed at given levels or lower without harmful effects.

Exposure limits in common use are:

- TLV-TWA: Threshold limit value—time-weighted average.
- STEL: Short-term exposure limit.
- C: Ceiling value.

Extinguishing Media—The fire-fighting substance to be used to control a material in the event of a fire. It is usually identified by its generic name, such as fog, foam, water, *etc*.

Eye Protection—Recommended safety glasses, chemical splash goggles, or face shields to be used when handling a hazardous material.

F Fahrenheit—is a scale for measuring temperature. On the Fahrenheit scale, water boils at 212°F and freezes at 32°F. To convert a temperature from degrees Fahrenheit to degrees Centigrade, subtract 32 from the temperature, multiply that number by five, then divide by 9.

FDA—*U.S.* ***F****ood and* ***D****rug* ***A****dministration*.

Fetus—The developing young in the uterus from the seventh week of gestation until birth.

FIFRA—***F****ederal* ***I****nsecticide,* ***F****ungicide, and* ***R****odenticide* ***A****ct* requires that certain useful poisons, such as chemical pesticides, sold to the public contain labels that carry health hazard warnings to protect users. It is administered by EPA.

First Aid—Emergency measures to be taken when a person is suffering from overexposure to a hazardous material, before regular medical help can be obtained.

Flammable—A chemical that falls into one of the following four categories:

- Liquid—A liquid with a flashpoint below 100°F (37.8°C).
- Solid—A solid, other than a blasting agent or explosive, that is able to cause fire through friction, absorption of moisture, spontaneous chemical change, or retained heat from manufacturing or processing, or which can be ignited readily and when ignited burns so vigorously and persistently as to create a hazard.
- Gas—A gas that, at ambient temperature and pressure, forms a flammable mixture with air at a concentration of 13 percent by volume or less.
- Aerosol—A chemical substance or mixture dispensed from its container as a spray or mist by a propellant under pressure that, when tested by the method described in 16 CFR 1500.45, yields a flame projection exceeding 18 inches at full valve opening, or a flashback at any degree of valve opening.

Flammability Range—The lower and upper concentrations of a chemical vapor in air that will ignite if an ignition source is present. The lower concentration range is called the lower explosive limit (LEL), and the upper concentration range is called the upper explosive limit (UEL).

Some examples of the LEL and UEL for some common chemicals:

acetylene	2.5-80%
acetone	2.6-12.8%
propane	2.4-9.5%
toluene	1.27-7%
diesel fuel	1-5%

Flashback—A flashback occurs when flame from a torch burns back into the tip, the torch, or the hose. It is often accompanied by a hissing or squealing sound with a smoky or sharp-pointed flame.

Flashpoint—The minimum temperature at which a liquid gives off vapor in sufficient concentration to ignite. Used to determine how flammable a liquid is.

Foreseeable Emergency—Any potential occurrence such as, but not limited to, equipment failure, rupture of containers, or failure of control equipment which could result in an uncontrolled release of a hazardous chemical into the workplace.

Formula—The scientific expression of the chemical composition of a material (*e.g.*, water is H2O, sulfuric acid is H2SO4, sulfur dioxide is SO2).

Fume—The particulate, smoke-like emanation from the surface of heated metals.

g—*Gram* is a metric unit of weight. One U.S. ounce is about 28.4 grams.

General Exhaust—A system for exhausting air containing contaminants from a general work area. Also see *Local Exhaust*.

Generic Name—A designation or identification used to identify a chemical by other than its chemical name (*e.g.*, code name, code number, trade name, brand name).

Gestation—The development of the fetus in the uterus from conception to birth; pregnancy.

g/kg—Grams per kilogram is an expression of dose used in oral and dermal toxicology testing to denote grams of a substance dosed per kilogram of animal body weight. Also see *kg*.

Grounding—The procedure used to carry an electrical charge to ground through a conductive path. A typical ground may be connected directly to a conductive water pipe or to a grounding bus and ground rod. See *Bonding*.

Hand Protection—Specific type of gloves or other hand protection required to prevent harmful exposure to hazardous materials.

Hazardous Chemical—Any chemical whose presence or use is a physical hazard or health hazard.

Hazardous Warning—Words, pictures, or symbols, presented on a label to inform of the dangers of a chemical.

HCS—Hazard Communication Standard is an OSHA regulation issued under 29 CFR Part 1910.1200. Also known as HazCom or Right-to-Know.

Health Hazard—A chemical for which there is significant evidence, based on at least one study conducted in accordance with established scientific principles, that acute or chronic health effects may occur in exposed employees. The term *health hazard* includes chemicals that are carcinogens, toxic or highly toxic agents, reproductive toxins, irritants, corrosives, sensitizers, hepatotoxins, nephrotoxins, neurotoxins, agents that act on the hematopoietic system, and agents that damage the lungs, skin, eyes, or mucous membranes.

Highly Toxic—A chemical in any of the following three categories:

- A chemical with a median lethal dose (LD50) of 50 milligrams or less per kilogram of body weight when administered orally to albino rats weighing between 200 and 300 grams each. (ORL-RAT LD50)
- A chemical with a median lethal dose (LD50) of 200 milligrams or less per kilogram of body weight when administered by continuous contact for 24 hours (or less if death occurs within 24 hours) with the bare skin of albino rabbits weighing between 2 and 3 kilograms each. (SKN-RBT LD50)
- A chemical that has a median lethal concentration (LC50) in air of 200 parts per million by volume or less of gas or vapor, or 2 milligrams per liter or less of mist, fume, or dust, when administered by continuous inhalation for 1 hour (or less if death occurs within 1 hour) to albino rats weighing between 200 and 300 grams each. (IHL-RAT LC50)

Hormones—Act as chemical messengers to body organs.

IARC—*International **A**gency for **R**esearch on **C**ancer*.

Ignitable—Capable of being set on fire.

Impervious—A material that does not allow another substance to pass through or penetrate it.

Incompatible—Materials that could cause dangerous reactions by direct contact with one another. Reactions between incompatible chemicals can cause an explosion, a fire, or the release of a toxic gas.

Ingestion—Taking in by the mouth.

Inhalation—Breathing in of a substance in the form of a gas, vapor, fume, mist, or dust.

Inhibitor—A chemical added to another substance to prevent an unwanted chemical change.

Insol—See *Insoluble*.

Insoluble—Not capable of being dissolved in a liquid.

Irritant—A chemical, which is not corrosive, that causes a reversible inflammatory effect on living tissue by chemical action at the site of contact.

kg—Kilogram is a metric unit of weight. One kilogram is about 2.2 U.S. pounds. Also see *g/kg*, *g*, and *mg*.

L—Liter is a metric unit of capacity. A U.S. quart is about 9/10 of a liter.

Lacrimation—Secretion and discharge of tears.

Label—Notice attached to a container, bearing information concerning its contents.

LC—Lethal concentration is the concentration of a substance being tested that will kill.

LCL—Lethal concentration, low, lowest concentration of a gas or vapor capable of killing a specified species over a specified time.

LC50—The concentration of a material in air that will kill 50 percent of a group of test animals with a single exposure (usually 1 to 4 hours). The LC50 is expressed as parts of material per million parts of air, by volume (ppm) for gases and vapors, or as micrograms of material per liter of air (µg/l) or milligrams of material per cubic meter of air (mg/m3) for dusts and mists, as well as for gases and vapors. See also *Highly Toxic* and *Toxic*.

LD—Lethal dose is the quantity of a substance being tested that will kill.

LDL—Lethal dose low, lowest administered dose of a material capable of killing a specified test species.

LD50—A single dose of a material expected to kill 50 percent of a group of test animals. The LD50 dose is usually expressed as milligrams or grams of material per kilogram of animal body weight (mg/kg or g/kg). The material may be administered by mouth or applied to the skin. See also *Highly Toxic* and *Toxic*.

LEL, or **LFL—**Lower explosive limit, or lower flammable limit, of a vapor or gas; the lowest concentration (lowest percentage of the substance in air) that will produce a flash of fire when an ignition source is present. At concentrations lower than the LEL, the mixture is too *lean* to burn. Also see *UEL* and *Flammability Range*.

Lethal Concentration—See LC50.

Lethal Dose—See *LD50*.

Local Exhaust—A system for capturing and exhausting contaminants from the air at the point where the contaminants are produced (welding, grinding, sanding, or other processes or operations). Also see *General Exhaust*.

m—Meter is a unit of length in the metric system. One meter is about 39 inches.

m3—Cubic meter is a metric measure of volume, approximately 35.3 cubic feet or 1.3 cubic yards.

Malaise—A feeling of general discomfort, distress, or uneasiness, an out-of-sorts feeling.

Mechanical Exhaust—A powered device, such as a motor-driven fan or air steam venturi tube, for exhausting contaminants from a workplace, vessel, or enclosure.

Mechanical Filter Respirator—A respirator used to protect against airborne particulate matter like dusts, mists, metal fume, and smoke. Mechanical filter respirators do not provide protection against gases, vapors, or oxygen deficient atmospheres.

Melting Point—The temperature at which a solid substance changes to a liquid state.

Metabolism—Physical and chemical processes taking place among the ions, atoms, and molecules of the body.

Meter—A unit of length, equivalent to 39.37 inches.

mg—Milligram is a metric unit of weight that is one-thousandth of a gram, i.e. 1000 milligrams equals one gram.

mg/kg—Milligrams of substance per kilogram of body weight is an expression of toxicological dose.

mg/m3—Milligrams per cubic meter is a unit for expressing concentrations of dusts, gases, or mists in air.

Micron (Micrometer)—A unit of length equal to one-millionth of a meter; approximately 0.000039 of an inch.

Mist—Suspended liquid droplets generated by condensation from the gaseous to the liquid state, or by breaking up a liquid into a dispersed state, such as splashing, foaming, or atomizing. Mist is formed when a finely divided liquid is suspended in air.

Mixture—Any combination of two or more chemicals.

ml—Milliliter is a metric unit of capacity, equal in volume to 1 cubic centimeter (cc), or approximately one-sixteenth of a cubic inch. One-thousandth of a liter.

mmHg—Millimeters (mm) of mercury (Hg) is a unit of measurement for low pressures or partial vacuums.

Molecular Weight—Weight (mass) of a molecule based on the sum of the atomic weights of the atoms that make up the molecule.

MSDS—*Material Safety Data Sheet*, a document containing information and instructions on the chemical and physical characteristics of a substance, its hazards and risks, safe handling requirements and the actions to be taken in the event of fire, spill or exposure.

Mutagen—A substance or agent capable of altering the genetic material in a living cell. See also *Reproductive Toxin*.

MW—See *Molecular Weight*.

Narcosis—A state of stupor, unconsciousness, or arrested activity produced by the influence of narcotics or other chemicals.

Nausea—Tendency to vomit, feeling of sickness at the stomach.

NFPA—*National **F**ire **P**rotection **A**ssociation* is an international membership organization which promotes and improves fire protection and prevention and establishes safeguards against loss of life and property by fire. Best known on the industrial scene for the National Fire Codes—16 volumes of codes, standards, recommended practices and manuals developed and periodically updated by NFPA technical committees. Among these is NFPA 704M, the code for showing hazards of materials as they might be encountered under fire or related emergency conditions, using the familiar diamond-shaped label or placard with appropriate numbers or symbols.

Neurotoxin—A material that affects the nerve cells and may produce emotional or behavioral abnormalities.

Neutralize—In acid-base chemistry, the process of bringing the pH to between 5 and 8, either by adding an acid to a base or by adding a base to an acid.

NIOSH—*National **I**nstitute for **O**ccupational **S**afety and **H**ealth*, U.S. Public Health Service, U.S. Department of Health and Human Services (DHHS), among other activities, tests and certifies respiratory protective devices and air sampling detector tubes, recommends occupational exposure limits for various substances, and assists OSHA and MSHA in occupational safety and health investigations and research.

Nonflammable—Not easily ignited, or if ignited, not burning rapidly.

Non-Sparking Tools—Tools made from beryllium-copper or aluminum-bronze that greatly reduce the possibility of igniting dusts, gases, or flammable vapors. Although these tools may emit some sparks when striking metal, the sparks have a low heat content and are not likely to ignite most flammable liquids.

NTP—*National **T**oxicology **P**rogram* publishes an Annual Report on Carcinogens.

Odor—A description of the smell of the substance.

Odor Threshold—The lowest concentration of a substance's vapor, in air, that can be smelled.

Olfactory—Relating to the sense of smell.

Oral—Used in or taken into the body through the mouth.

Oral Toxicity—Adverse effects resulting from taking a substance into the body by mouth.

Organic Peroxide—An organic compound that is an oxidizer and contains the bivalent -O-O- structure and may be considered a structural derivative of hydrogen peroxide where one or both of the hydrogen atoms has been replaced by an organic radical.

OSHA—*O*ccupational *S*afety and *H*ealth *A*dministration, U.S. Department of Labor.

Overexposure—Exposure to a hazardous material beyond the allowable exposure limits.

Oxidizer—A substance that readily yields oxygen to or accepts electrons from another substance. By yielding oxygen or accepting electrons, an oxidizer can easily cause or enhance the combustion of other materials. Examples of oxidizers include bleach, which contains sodium hypochlorite, concentrated hydrogen peroxide (greater than 30%), and ammonium nitrate.

Pathologic—Pertaining to or caused by disease.

Pathology—Scientific study of alterations produced by disease.

PEL—*P*ermissible *E*xposure *L*imit is an occupational exposure limit established by OSHA's regulatory authority. It may be a time-weighted average (TWA) limit or a maximum concentration exposure limit.

Percent Volatile—Percent volatile by volume is the percentage of a liquid or solid (by volume) that will evaporate at a temperature of 70°F. Examples: butane, gasoline, and paint thinner (mineral spirits) are 100 percent volatile; their individual evaporation rates vary, but in time, each will evaporate completely.

pH—The symbol relating the hydrogen ion (H+) concentration to that of a given standard solution. A pH of 7 is neutral. Numbers increasing from 7 to 14 indicate greater alkalinity. Numbers decreasing from 7 to 0 indicate greater acidity.

Physical Hazard—Used to describe a chemical that is explosive, flammable, an organic peroxide, an oxidizer, pyrophoric, unstable, or water reactive.

Placenta—A structure attached to the wall of the uterus to nourish the fetus during pregnancy.

Polymerization—A chemical reaction in which one or more small molecules combine to form larger molecules. A hazardous polymerization is such a reaction that takes place at a rate that releases large amounts of energy. If hazardous polymerization can occur with a given material, the MSDS usually will list conditions that could start the reaction and, if the material contains a polymerization inhibitor, the length of time during which the inhibitor will be effective.

ppb—*P*arts *p*er *b*illion. The concentration of a particular chemical in solution. For every one part of the chemical. there are a billion parts of solution. Used to express very low concentrations.

ppm—*P*arts *p*er *m*illion. The concentration of a particular chemical in solution. For every one part of the chemical, there are a million parts of solution. Used to express very low concentrations. One inch in sixteen miles is a common example of the magnitude of parts per million.

Prenatal—Preceding birth.

psi—Pounds per square inch is the pressure a material exerts on the walls of a confining vessel or enclosure. For technical accuracy, pressure must be expressed as psig (pounds per square inch gauge) or psia (pounds per square inch absolute; that is, gauge pressure plus sea level atmospheric pressure, or psig plus approximately 14.7 pounds per square inch). See also *mmHg*.

Pulmonary—Relating to, or associated with, the lungs.

Pulmonary Edema—Fluid in the lungs.

Pyrophoric—A chemical substance that will ignite spontaneously in air at or below a temperature of 130°F (54.4°C).

Reaction—A chemical transformation or change. The interaction of two or more substances to form new substances.

Reactivity—Chemical reaction with the release of energy. Undesirable effects—such as pressure buildup, temperature increase, formation of noxious, toxic or corrosive byproducts—may occur because of the reactivity of a substance to heating, burning, direct contact with other materials, or other conditions in use or in storage.

RCRA—***R****esource* ***C****onservation and* ***R****ecovery* ***A****ct* is environmental legislation aimed at controlling the generation, treatment, storage, transportation and disposal of hazardous wastes. It is administered by EPA.

Reducing Agent—In a reduction reaction, which always occurs simultaneously with an oxidation reaction, the reducing agent is the chemical or substance which combines with oxygen, or loses electrons to the reaction. See *Oxidation*.

REL—***R****ecommended* ***E****xposure* ***L****imit*. The NIOSH REL is the highest allowable airborne concentration which is not expected to injure workers. It may be expressed as a ceiling limit or as a time-weighted average (TWA).

Reproductive Toxin—Substances that affect either male or female reproductive systems and may impair the ability to have children. See also *Mutagen*.

Respiratory Protection—Devices that will protect the wearer's respiratory system from overexposure by inhalation to airborne contaminants. Respiratory protection is used when a worker must work in an area where he/she might be exposed to concentration in excess of the allowable exposure limit.

Respiratory System—The breathing system that includes the lungs and the air passages (trachea or "windpipe," larynx, mouth, and nose) to the air outside the body, plus the associated nervous and circulatory supply.

Routes of Entry—The means by which material may gain access to the body, for example, inhalation, ingestion, and skin contact.

Self-Contained Breathing Apparatus—A respiratory protection device that consists of a supply or a means of respirable air, oxygen, or oxygen-generating material which is carried by the wearer.

Sensitizer—A chemical that causes a substantial proportion of exposed people or animals to develop an allergic reaction in normal tissue after repeated exposure to the chemical.

Silicosis—A disease of the lungs (fibrosis) caused by the inhalation of silica dust.

Skn—Skin.

Skin—A notation sometimes used with PEL or TLV exposure data that indicates that the stated substance may be absorbed by the skin, mucous membranes, and eyes, either by airborne or by direct contact.

Skin Absorption—Ability of some hazardous chemicals to pass directly through the skin and enter the bloodstream.

Skin Toxicity—See *Dermal Toxicity*.

Solubility in Water—A term expressing the percentage of a material (by weight) that will dissolve in water at ambient temperature.

Solvent—A substance, usually a liquid, in which other substances are dissolved. The most common solvent is water.

Specific Chemical Identity—The chemical name, Chemical Abstracts Service (CAS) Registry Number, or any precise chemical designation of a substance.

Specific Gravity—The weight of a material compared to the weight of an equal volume of water is an expression of the density (or heaviness) of a material. Insoluble materials with specific gravity of less than 1.0 will float on water. Insoluble materials with specific gravity greater than 1.0 will sink in water. Some flammable liquids have specific gravities greater than 1.0 and will sink in water. Most flammable liquids have specific gravities less than 1.0 and, if not soluble, will float on water, which is an important consideration for fire suppression.

Spill or Leak Procedures—The methods, equipment, and precautions that should be used to control or clean up a leak or spill.

Splash-Proof Goggles—Eye protection that fits snugly against the face, and may have indirect ventilation ports.

Spontaneously Combustible—Capable of catching fire without an ignition source. Can occur due to oxidation in air or by internal heat buildup.

Stability—The ability of a material to remain unchanged. For MSDS purposes, a material is stable if it remains in the same form under expected and reasonable conditions of storage or use. Conditions that may cause instability (dangerous change) are stated; for example, temperatures above 150°F; shock from dropping.

STEL—*Short-**T**erm **E**xposure **L**imit* (ACGIH terminology). See *TLV*.

Subcutaneous—Beneath the layers of the skin.

Supplied-Air Respirators—Air line respirators or self-contained breathing apparatus.

Sys—System or systemic.

Systemic Poison—A poison that spreads throughout the body, affecting all body systems and organs. Its adverse effect is not localized in one spot or area.

Systemic Toxicity—Adverse effects caused by a substance that affects the body in a general rather than local manner.

Synonym—Another name or names by which a material is known. Methyl alcohol, for example, is also known as methanol or wood alcohol.

Target Organ Toxin—A toxic substance that attacks a specific organ of the body. For example, overexposure to carbon tetrachloride can cause liver damage.

TCL—Toxic concentration low, the lowest concentration of a gas or vapor capable of producing a defined toxic effect in a specified test species over a specified time.

TDL—Toxic dose low, lowest administered dose of a material capable of producing a defined toxic effect in a specified test species.

Temp—Temperature.

Ter—See *Teratogen*.

Teratogen—A substance or agent, exposure to which by a pregnant female can result in malformations in the fetus.

Tfx—Toxic effects(s).

TLV—*Threshold Limit Value* is a term used by ACGIH to express the airborne concentration of material to which nearly all persons can be exposed day after day without adverse effects. ACGIH expresses TLVs in three ways:

- TLV-TWA: The allowable *Time-Weighted Average* concentration for a normal 8-hour workday or 40-hour workweek.
- TLV-STEL: The *Short-Term Exposure Limit*, or maximum concentration for a continuous 15-minute exposure period (maximum of four such periods per day, with at least 60 minutes between exposure periods, and provided the daily TLV-TWA is not exceeded).
- TLV-C: The *Ceiling* exposure limit. The concentration that should not be exceeded even instantaneously.

Torr—A unit of pressure, equal to 1/760 atmosphere.

Toxic—A chemical falling within any of the following three categories:

- A chemical that has a median lethal dose (LD50) of more than 50 milligrams per kilogram but not more than 500 milligrams per kilogram of body weight when administered orally to albino rats weighing between 200 and 300 grams each.

- A chemical that has a median lethal dose (LD50) of more than 200 milligrams per kilogram but not more than 1,000 milligrams per kilogram of body weight when administered by continuous contact for 24 hours (or less if death occurs within 24 hours) with the bare skin of albino rabbits weighing between two and three kilograms each.
- A chemical that has a median lethal concentration (LC50) in air of more than 200 parts per million but not more than 2,000 parts per million by volume of gas or vapor, or more than two milligrams per liter but not more than 20 milligrams per liter of mist, fume, or dust, when administered by continuous inhalation for one hour (or less if death occurs within one hour) to albino rats weighing between 200 and 300 grams each. See also *Highly Toxic*.

Toxic Substance—Any substance that can cause acute or chronic injury to the human body, or which is suspected of being able to cause diseases or injury under some conditions.

Toxicity—The degree of poisoning that can occur from exposure to a chemical.

Trade name—The trademark name or commercial trade name for a material or product.

Transplacental—A chemical that is capable of causing physical defects in the developing embryo because it can easily cross the placental barrier between mother and fetus.

TSCA—***T****oxic* ***S****ubstances* ***C****ontrol* ***A****ct*. Federal Environmental Legislation administered by EPA that regulates the manufacture, handling, and use of materials classified as "toxic substances."

TWA—***T****ime-****W****eighted* ***A****verage* exposure is the airborne concentration of a material to which a person is exposed, averaged over the total exposure time-generally the total workday (8 hours). Also see *TLV*.

UEL, or **UFL—*****U****pper* ***e****xplosive* ***l****imit* or ***u****pper* ***f****lammable* ***l****imit* of a vapor or gas; the highest concentration (highest percentage of the substance in air) that will produce a flash of fire when an ignition source (heat, arc, or flame) is present. At higher concentrations, the mixture is too *rich* to burn. See also *LEL*.

ug—*Microgram*, one millionth of a gram.

Unstable—Tending toward decomposition or other unwanted chemical change during normal handling or storage.

Unstable Reactive—A chemical that, in the pure state, or as produced or transported, will vigorously polymerize, decompose, condense, or become self-reactive under conditions of shock, pressure, or temperature.

Vapor—The gaseous form of a solid or liquid substance as it evaporates.

Vapor Density—The weight of a vapor or gas compared to the weight of an equal volume of air is an expression of the density of the vapor or gas. Materials lighter than air have vapor densities less than 1.0. Some examples are acetylene, methane,

hydrogen. Materials heavier than air, such as propane, hydrogen sulfide, ethane, butane, chlorine, and sulfur dioxide, have vapor densities greater than 1.0. All vapors and gases will mix with air, but the lighter materials will tend to rise and dissipate unless confined. Heavier vapors and gases are likely to concentrate in low places-along or under floors, in sumps, sewers, and manholes, in trenches and ditches where they may create fire or health hazards.

Vapor Pressure—The pressure exerted by a saturated vapor above its own liquid in a closed container. Vapor pressures reported on MSDS are in milliliters of mercury (mmHg) at 68°F (20°C), unless stated otherwise. Some important facts to remember are that vapor pressure increases with temperature, and the lower the boiling point of a chemical, the higher its vapor pressure.

Examples of vapor pressures at 20°C in mm Hg:

Acetone	184
Mercury	0.002
Methylene Chloride	350
Phenol	0.36

Ventilation—See *General Exhaust*, *Local Exhaust*, and *Mechanical Exhaust*.

Vermiculite—Substance used as sorbent for spill control and cleanup.

Viscosity—The tendency of a fluid to resist internal flow without regard to its density.

Volatility—A measure of how quickly a substance forms a vapor at ordinary temperatures. Chemicals with higher vapor pressures and lower boiling points will have higher volatilities and will evaporate quicker.

Water-Reactive—A chemical that reacts with water to release a gas that is either flammable or toxic.

BIOMEDICAL ENGINEERING
THE UNIVERSITY OF TEXAS AT AUSTIN

Pacemakers

In this lab you will build a simplified pacing system using the NI Elvis prototyping board. Then, using a NI oscilloscope, you will monitor, tune, and test your pacing system, as well as solve problems normally associated with pacing systems.

Pacemakers or pacing systems consist of three fundamental parts: a pacemaker, a pacing lead, and a programmer. The pacemaker and pacing lead are placed inside the body while the programmer is used external to the body to monitor and fine-tune the operation of the pacemaker.

This handbook provides some of the fundamentals of pacing systems. For further information, visit the links listed under "Links to Further Pacemaker Information" on page 2-10.

SECTION CONTENTS

INTRODUCTION TO PACEMAKING SYSTEMS

In 1949, Dr. William Bigelow and Dr. John Callaghan at the Banting and Best Institute laboratory in Toronto were studying the effects of extreme cold on the human heart rate. In particular, they were interested in how this might make open heart surgery possible. Although their work had led to a number of successful open heart surgeries, they were unable to re-start the heart if it stopped during surgery.

Enter Dr. John Hopps, electrical engineer and National Research Council researcher. Working with the two physicians, John Hopps and his team discovered that they could apply a gentle electrical stimulus to the heart muscle to simulate the normal nerve stimulation that triggers the heartbeat. Hopps found that the mild stimulus did not appear to harm the heart tissue—even after prolonged exposure. Eventually, Hopps and his team developed a technique that could reliably start a stopped heart, as well as as well as increase or decrease the heart rate, as required.

The first pacemaker was developed in 1950. It was large (about 30 cm long, and several centimeters high and wide), the pulses were generated by vacuum tubes, and the entire unit was powered by 60 Hz household current. Its size meant that it wasn't of immediate practical use but with the advent of transistors and reliable batteries, the package grew smaller and smaller, until a pacemaker was successfully implanted in the chest of a Swedish man in 1957. They have since become a common medical tool.

For photos of some early pacemakers and their specifications, visit:

http://ieee.ca/millennium/pacemaker/pacemaker_pics.html

How a Pacing System Works

A pacing system restores and maintains one of the most essential rhythms of life—the rhythm of the human heart. The two parts of the pacing system inside the body, the pacemaker and the pacing lead, work together to perform two main functions: *pacing* and *sensing*. A third function, communication, is also essential.

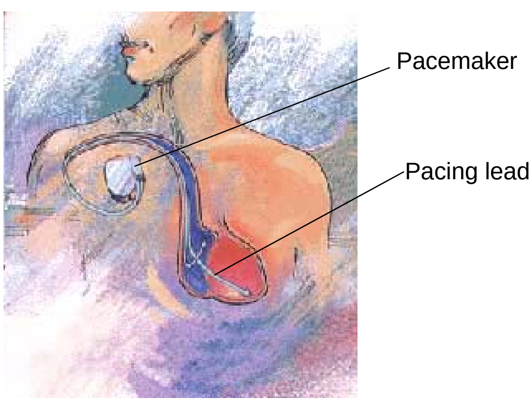


Figure 2-1. *A pacing system*

Pacing

Pacing is the pacemaker function that sends electrical impulses to the heart, causing it to beat. A pacing signal is a tiny electrical potential applied to the heart through a pacing lead. Each tiny electrical signal is called a *pacing pulse* (or *pacing impulse*), and it is this pacing pulse that starts a heartbeat. The pacemaker paces (sends a pacing pulse to) the heart when the heart's own rhythm is interrupted, irregular, or too slow.

Sensing

Sensing is the pacemaker function that monitors the heart's natural electrical activity. It is crucial that when a pacemaker senses a natural heartbeat, it does not send a pacing pulse to the heart; likewise, it is just as important that if the pacemaker does *not* sense a natural heartbeat, the pacemaker does send an electronic impulse to cause a heartbeat.

Communication

The third part of the system, the *programmer*, is a small computer with a soft-panel interface used by a physician to check a pacemaker. One type of programmer uses a magnet (which is placed over the pacemaker) to collect information from the pacemaker.

Figure 2-2. *Medtronic® CareLink™ pacemaker programmer*

Sequence of a Pacing Event

A pacing signal sequence occurs as follows:

1. The pacemaker sends a tiny electrical signal (*pacing impulse*).
2. This impulse travels through the insulated wires of a pacing lead until it reaches the metal electrode at the tip of the lead.
3. The electrode, which is in direct contact with the heart, delivers the electrical impulse to the heart.
4. The electrical impulse triggers the heart tissue to begin a heartbeat.

Rate Responsive Pacing Systems

The pacing pulses are timed so that the heart beats in a manner very similar to a naturally occurring heart rhythm. For some pacemakers, this timing is exactly the same for each heartbeat. In other pacemakers, the timing is based on when the heart beats on its own. For these pacemakers, information about the heart's own electrical activity is sent back to the pacemaker through an electrode on the lead. (This is called sensing.)

If the pacemaker circuitry determines that the heart is beating too slowly, a pacing pulse is sent to start a heartbeat. If the pacemaker circuitry determines that the heart is beating at a proper rate, the pacing pulse is withheld.

In rate responsive pacemakers, a special sensor detects changes in the body such as movement of arms and legs or how often a person breathes. The pacemaker's circuitry interprets these changes and increases the pacing rate (the number of times per minute that a pacing pulse is sent) by either a little or a lot—depending upon the body's need for oxygen.

By increasing the heart rate, the symptoms of bradycardia are often eliminated. This often means patients have more energy and less shortness of breath. However, a pacing system is not a cure, but rather a treatment for a slow heart rhythm. A pacing system will not prevent or stop heart disease (coronary artery disease), nor will it prevent heart attacks.

Currently, pacemakers are the only treatment for bradycardia. This remarkable invention has been benefiting more than 2 million people for over 50 years. Because of a pacemaker, people with bradycardia can expect to lead normal lives.

THE PACEMAKER

When people refer to a pacemaker, they are actually discussing a pacing system: a pacemaker, a pacing lead, and programmer.

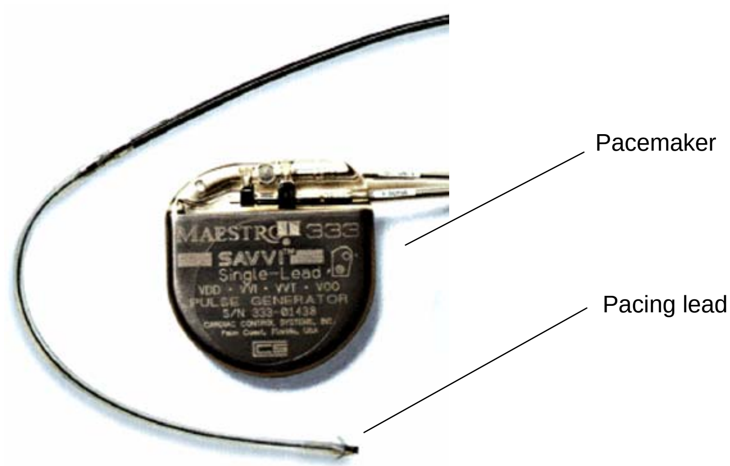


Figure 2-3. *Pacemaker and pacing lead*

- The *pacemaker* is a small metal case containing electronic circuitry and a battery. The pacemaker sends a tiny electrical pulse at a specified time.
- A *pacing lead* is an insulated wire that carries the tiny electrical pulse to the heart—triggering a heartbeat.

A Simple Pacemaking Circuit

Note: The following section is from http://PacemakerProject.com.

Figure 2-4 is the simplest pacemaker circuit. The 510 Ω resistor on the right hand side of the circuit mimics the electrical load the heart places on the pacemaker. The rest of the circuit—battery, resistor, and switch—complete this simple pacemaker circuit. As the switch alternately opens and closes, the capacitor is charged and then discharged into the wires connected to the heart.

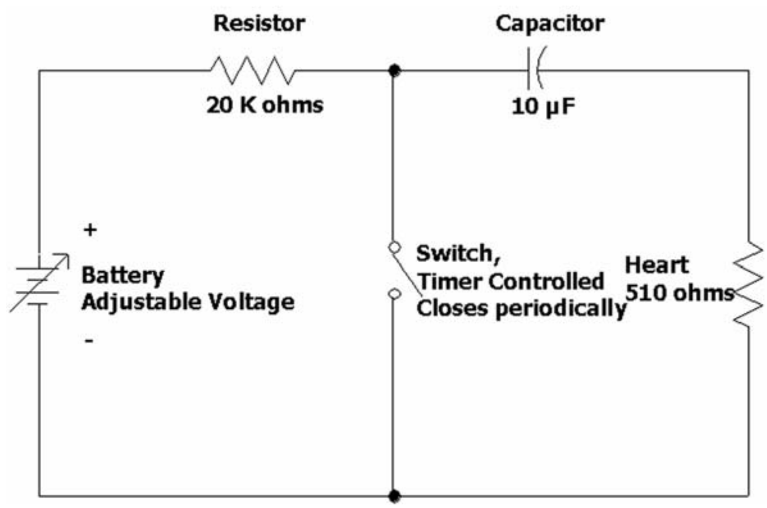


Figure 2-4. *Simple pacing circuit*

Let us assume that the circuit has been assembled. At the time of assembly, the capacitor is presumed to be completely discharged. That is, there is zero voltage on the capacitor. Let us further assume that the switch has not yet been closed.

Current flows from the battery to charge the capacitor (Figure 2-5).

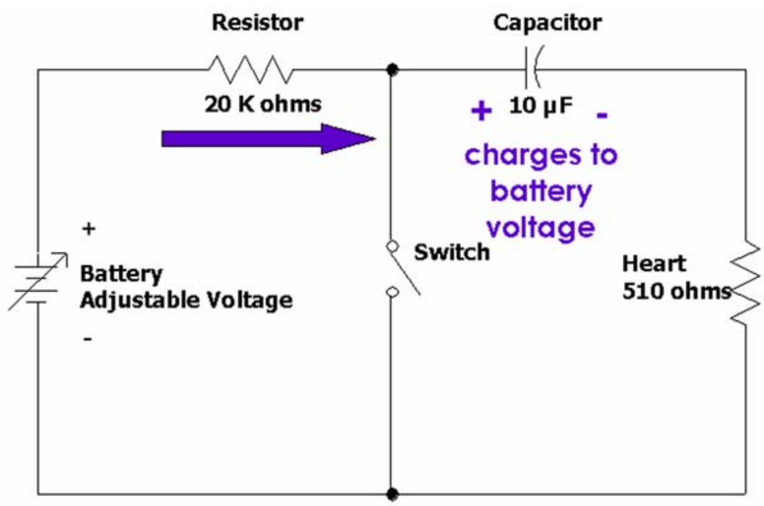


Figure 2-5. *Current flows to charge capacitor*

When the circuit has reached steady state, the capacitor and battery will have equal voltage. Since no current is flowing, there is no voltage generated across the (Heart) resistor (Figure 2-6).

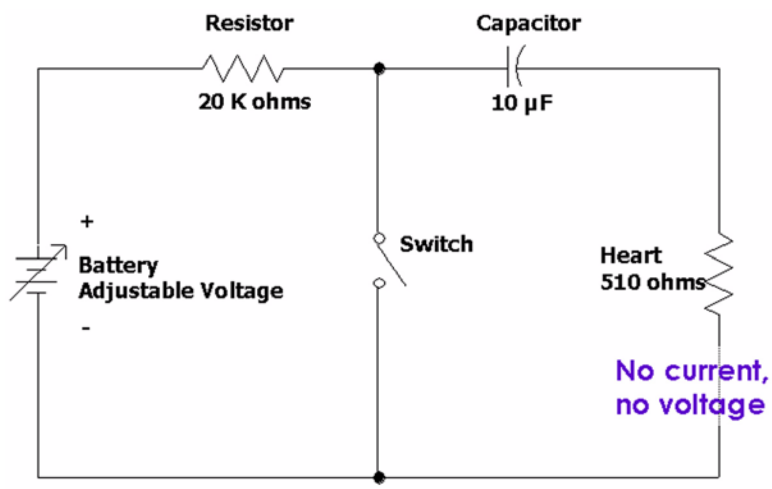


Figure 2-6. *Circuit at steady state*

To generate a pulse from the circuit, the switch closes. When the switch closes, current flows in two loops as indicated by the blue arrows (Figure 2-7).

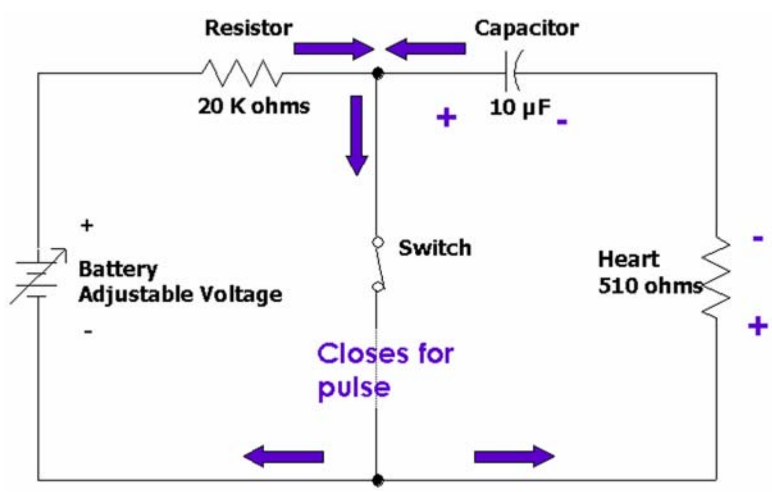


Figure 2-7. *Circuit generating a pacing signal*

In the left hand side of the circuit (Figure 2-7), current flows from the battery through the 20 kΩ resistor, through the switch, and back to the battery. This is wasted current since it has no effect on charging or discharging the capacitor. Nor, does it contribute to the current delivered to stimulate the heart.

In the right hand side of the circuit (Figure 2-7), current flow is caused by the discharge of the 4.7 uF capacitor through wires in the patient's veins to the heart. This is the current that stimulates the heart to contract or beat. Note the polarity of the pacing pulse.

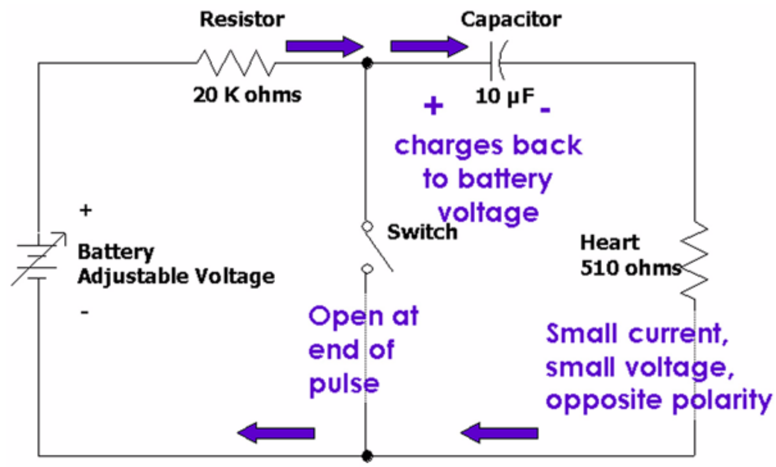


Figure 2-8. *Circuit open at end of pulse*

Given sufficient time, the capacitor will receive and store a charge equal to the battery voltage. Periodically, the switch closes and the charged capacitor delivers an electrical pulse to the heart. When the switch opens, the battery recharges the capacitor (Figure 2-8).

The BME 251 Pacemaking Circuit

We will build our circuit on prototyping boards which mount to the ELVIS stations in the laboratory. The ELVIS stations provide a variable power supply to the prototyping boards, as well as provide test points and a seamless interface with the laboratory computers and PXI test stands. For electrical safety, we will not use the ELVIS onboard variable power supply; rather, we will use batteries to power our circuit.

Stages of the Pacemaking Circuit

We will build our circuit on prototyping boards which mount to the ELVIS stations in the laboratory. The ELVIS stations provide a variable power supply to the prototyping boards, as well as provide test points and a seamless interface with the laboratory computers and PXI test stands. For electrical safety, we will not use the ELVIS onboard variable power supply; rather, we will use batteries to power our circuit.

The circuit that we will build up from the component level is an asynchronous pacemaker and will contain two discrete stages: an oscillator stage and a pulse modulator. An asynchronous pacemaker is one that is free-running. It's electronic stimulus appears at a uniform rate regardless of what is going on in the heart or the rest of the body. It therefore gives a fixed heart rate.

Figure 2-9 provides an overview of a very simple asynchronous pacemaker. The Oscillator and Pulse Output Circuits are the ones that we will be prototyping in this lab.

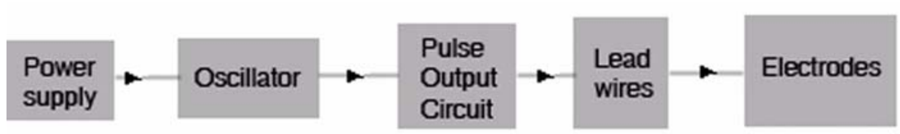


Figure 2-9. *Stages of the pacemaking circuit*

The following sections discuss the Oscillator and Pulse Output Circuit circuit stages in more detail.

Stage 1: Oscillator

The oscillator establishes the pulse rate for the pacemaker; this, in turn, controls the pulse output circuit that provides the stimulating pulse to the heart. The asynchronous pacemaker represents the simplest of pacemakers, because it provides a train of stimulus pulses at a constant rate regardless of the functioning of the heart.

Advanced pacemakers, such as rate-responsive pacemakers, require a timing circuit to control the frequency of stimulus to the heart. But a free-running oscillator is replaced with complex logic circuits, quartz crystal control, and even a microprocessor.

Stage 2: Pulse Output Circuit

Stage two of our pacemaking circuit is the pulse output circuit. The pulse output circuit of the pacemaker generator produces the actual electric stimulus that is applied to the heart. At each trigger from the timing circuit, the output circuit generates an electric stimulus pulse that has been optimized for stimulating the myocardium through an electrode system that is being applied from the generator. Constant-voltage or constant-current amplitude pulses are the two usual types of stimuli produced by the output circuit. Rates for asynchronous pacemakers range from 70 to 90 beats per minute, whereas pacemakers that are not fixed-rate typically achieve rates ranging from 60 to 150 beats per minute.

Output Signal

Before we send our converted and cleaned-up biopotential signal to our measurement device, we pass the signal through a final amplification with a very high gain. The high gain significantly increases the amplitude of our signal, making it easily detectable and manageable. By placing high-gain amplification at the end of our signal chain, we are able to selectively increase our desired signal without amplifying noise because the majority of electrical interference has been filtered out by this point.

Once you have energized your circuit, it is important to monitor your ECG circuit output signal. The output must not go outside of the -9 to +9 range of your batteries. If your signal exceeds these specifications, it will become distorted, or saturated. This phenomena is referred to as railing out and is highly undesirable for electrocardiography applications.

Note: Frequently check the IC chips and your batteries for excessive heat. If you improperly bias your circuit, power will be dissipated inappropriately, and damage may result to your circuit components.

Links to Further Pacemaker Information

http://www.medtronic.com/brady/

Commercial site

http://collections.ic.gc.ca/heirloom_series/volume4/234-237.htm

A page centering on Dr. Wilfred G. Bigelow's contributions to pacemakers

http://ieee.ca/millennium/pacemaker/pacemaker_pics.html

Pacemaker prototypes

http://ieee.ca/millennium/pacemaker/pacemaker_improvements.html

An article from a 1969 edition of Science Dimension

LAB MEETING #1

During this lab we will build and operate a Pacemaking circuit containing two discrete stages: an oscillator stage and a pulse modulator. Both stages are built around separate CMOS 7555 IC chips. If you desire to learn more about these integrated circuit components, specifications sheets can be accessed via the internet:

http://www.engj.ulst.ac.uk/sidk/resources/Data_sheets/ICL7555_6.pdf

Circuit Schematic

The following illustration is a schematic outline of the circuit you will build during this lab.

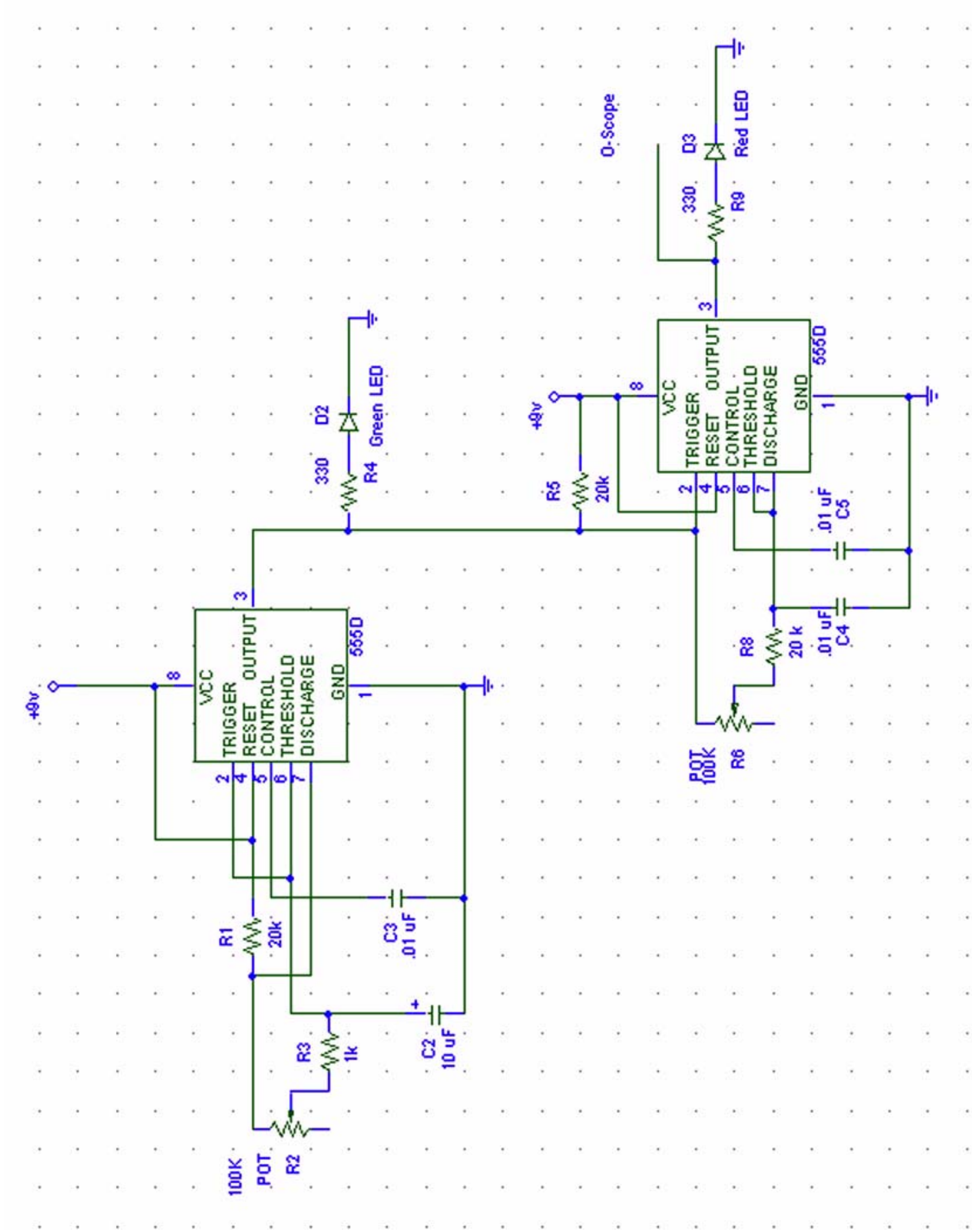


Figure 2-10. *Schematic diagram of the Pacemaker circuit*

Parts List

To build the Pacemaker circuit, you will require the following components (the number in parentheses following the component is the number required):

- 7555 CMOS Timer IC Chip (2)
- 10 uF Polarized Capacitor (1)
- 0.01 uF non-Polar Capacitor (3)
- 1.0 kΩ Resistor (1)
- 100 kΩ Potentiometer (2)
- 20 kΩ Resistor (3)
- 330 Ω resistor (2)
- Green LED (1)
- Red LED (1)
- 9 Volt battery (1)
- 9 Volt battery leads (1)

Lab Meeting #1 Procedure

Start building your pacemaking circuit by placing your two IC chips on the prototyping board. Space the chips out to make room for other components and connections. Figure 2-11 demonstrates an IC chip placed on the prototyping board so that each of the eight terminals of the chip are isolated from one another.

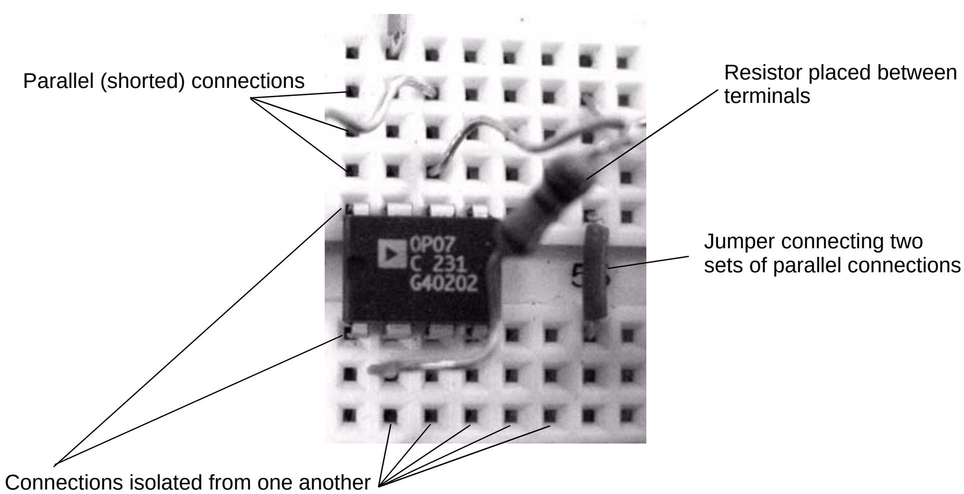


Figure 2-11. *Example of an IC Chip seated correctly on a prototyping board*

Connecting the Power Supply Leads

The pacemaking circuit requires the constant voltage potential of +9 VDC as well as ground. The ELVIS station will provide a ground connection. We will use a 9V alkaline batteries to provide our (+) voltage potentials.

Note: You can damage your circuit if you exceed the +9 VDC required by the circuit. Always ensure that your connection polarities are correct.

Figure 2-12. *Ground connections on the prototyping board*

To Make Power Connection for the Pacemaker Circuit:

Before connecting the power supply leads, you will want to ensure that your batteries are fully charged. Using a multimeter, measure the voltage across the battery terminals. Your battery must read 9 ± 1.5 VDC.

1. Ensure the ELVIS workstation is de-energized.
2. Connect the black lead of the battery connector to the ground terminal on the ELVIS board.

Note: A good habit to develop is to create voltage busses when you will be making several connections to a power supply or ground. For instance, our pacemaking circuit requires several connections to ground on the prototyping board, so you may choose to create a ground bus to facilitate the multiple connection points. This also will ensure that your ground potential is constant and does not float. If you are unsure how to create a ground bus, ask your TA to show you.

3. Use a jumper wire to connect the ground horizontal bus shown above to the first available vertical bus on the ELVIS board.
4. Use a jumper wire to connect the red (positive) lead of the battery connector to the second available vertical bus on the ELVIS board.

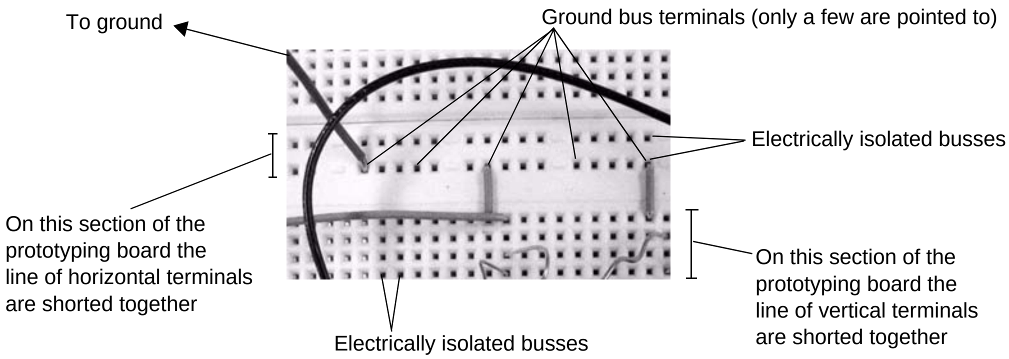


Figure 2-13. *Examples of prototyping board busses*

Connecting the CMOS 7555 Oscillator Stage

Use the pin connections below to identify pins on the CMOS 7555 Timer IC chip.

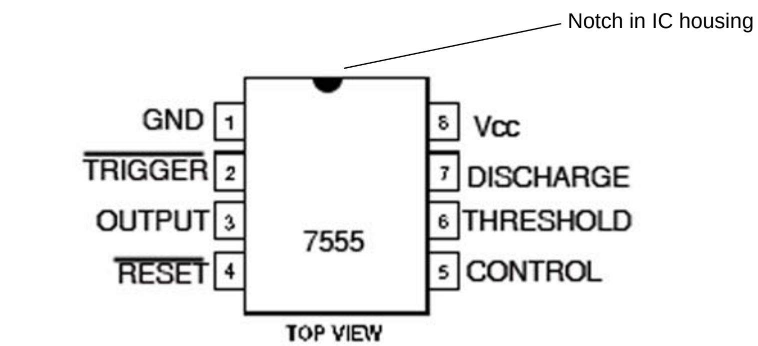


Figure 2-14. *CMOS 7555 Timer pin connections*

To Connect the CMOS 7555 Oscillator Stage:

1. Connect pin 1 of the CMOS 7555 chip to the ground bus which you created earlier
2. Use a jumper wire to connect pin 2 to pin 6 of the CMOS 7555 chip.
3. Connect pin 4 to the +9V voltage bus you created earlier.
4. Use a 0.01 uF capacitor (C3) to connect pin 5 to ground.
5. Use the 10 uF capacitor (C2) to connect pin 6 to ground.

Note: The most important characteristic of electrolytic capacitors is that they have a positive and a negative electrode. If the capacitor is subjected to voltage exceeding its working voltage, or if it is connected with incorrect polarity, it may burst. It is extremely dangerous, because it can quite literally explode.

Electrolytic capacitors generally have markings indicating their polarity. In See Polarity of an electrolytic capacitor., the arrows on the capacitor housing point

toward a "–" sign and the end of the capacitor that must be kept more negative. Ensure that you have correctly identified the polarity of your capacitors before connecting them.

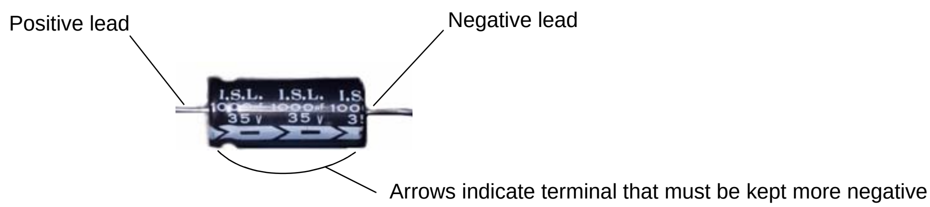


Figure 2-15. *Electrolytic capacitor and identifying markings*

CAUTION:
Explosion Hazard. Electrolytic capacitors may burst if connected incorrectly. Observe the polarity of the capacitors and wear safety glasses at all times when working around electrolytic capacitors.

6. Connect pin 8 to the +9Vvoltage bus you created earlier.
7. Place a 100 KΩ potentiometer (R_2) on the prototyping board so that each lead is connected to a separate terminal.
8. Using a 1KΩ resistor (R_3) connect the positive lead of C_2 to an end lead of R_2.
9. Using a 20 KΩ resistor (R_1) connect the center terminal of R_2 to the +9V voltage bus. (This could also be connected to pin 4 or pin 8 instead of directly to the voltage bus as they are all the same potential.)
10. Connect pin 7 to the center terminal of R_2 using jumper wire.
11. Connect the negative lead of the green light-emitting diode (D_1) to the ground bus.
12. Connect the positive lead of the green light-emitting diode (D_1) in series with a 330 Ω resistor R_4 to pin 3 of CMOS 7555 (IC_1)

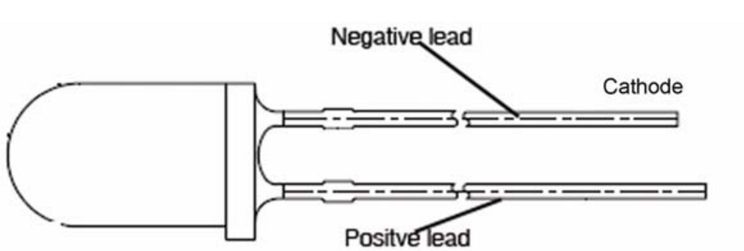


Figure 2-16. *Light-emitting diode (LED)*

Connecting the Pulse-Output Circuit

Beginning with pin 1 and continuing through pin 8:

1. Connect pin 1 of the CMOS 7555 chip to the ground bus which you created earlier.
2. Using a 20 KΩ resistor (R_5) connect pin 2 to the +9V voltage bus.
3. Connect pin 2 of the Pulse-Output Stage to pin 3 of the Oscillator Stage.
4. Connect the negative lead of the Red Light Emitting Diode (D_1) to the ground bus.
5. Connect the positive lead of the Red Light Emitting Diode in series with a 330 Ω resistor (R_4) to pin 3 of CMOS 7555 (IC_2).
6. Use a jumper wire to connect pin 4 to pin 8.
7. Use a 0.01 uF capacitor (C_5) to connect pin 5 to the ground bus.
8. Use a 0.01 uF capacitor (C_5) to connect pin 6 to the ground bus.
9. Use a jumper to connect pin 6 to pin 7
10. Place a 100 KΩ potentiometer (R_6) on the prototyping board so that each lead is connected to a separate terminal.
11. Use a 20 KΩ resistor (R_8) to connect pin 6 to an end lead of potentiometer (R_6).
12. Connect the center lead of potentiometer (R_6) to pin 2
13. Connect pin 8 to the +9V voltage bus.

Checking Your Work

When you have completed creating your pacemaking circuit, you will want to check your wiring. It is relatively easy to cross a connection, and when dealing with sensitive electronic components, such a mistake can damage your circuit permanently.

Using the circuit schematic (Figure 2-10 on page 2-11) and a Fluke 175 multimeter (set to read ohms), check the signal flow of your circuit thoroughly to ensure that pins, leads, and components that are supposed to be shorted together are indeed shorted together, and that pins, leads, and components that should be isolated from one another are isolated.

Connecting the Pacemaker Circuit Output

In our lab, directing the output of our ECG circuit is relatively easy. The prototyping board and ELVIS station are integrated with our lab computers using a DAQ (data acquisition card) installed in the desktop computer and connected to the ELVIS station.

To Route the Pacemaker Circuit Output to a Connected Lab Computer:

1. Connect the output of the Pulse-Output stage (pin 3 on the CMOS 7555 chip) to **ACH0+** on the prototyping board.

2. Connect the output of the Oscillator stage (pin 3 on the CMOS 7555 chip) to **ACH1+** on the prototyping board.
3. Figure 2-17 demonstrates a prototyping board configured to output an analog signal to the DAQ board inputs **ACH0+** and **ACH0-**.

   Note: Contrary to the following figure, you will use the **ACH0+** and **ACH1+**.

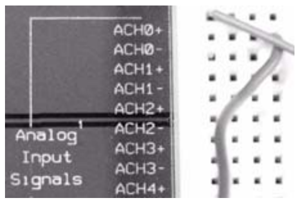


Figure 2-17. *DAQ interface input connections on prototyping board*

4. Ensure you have the ELVIS prototyping station energized and open the National Instruments ELVIS application on your workstation as shown in Figure 2-18.

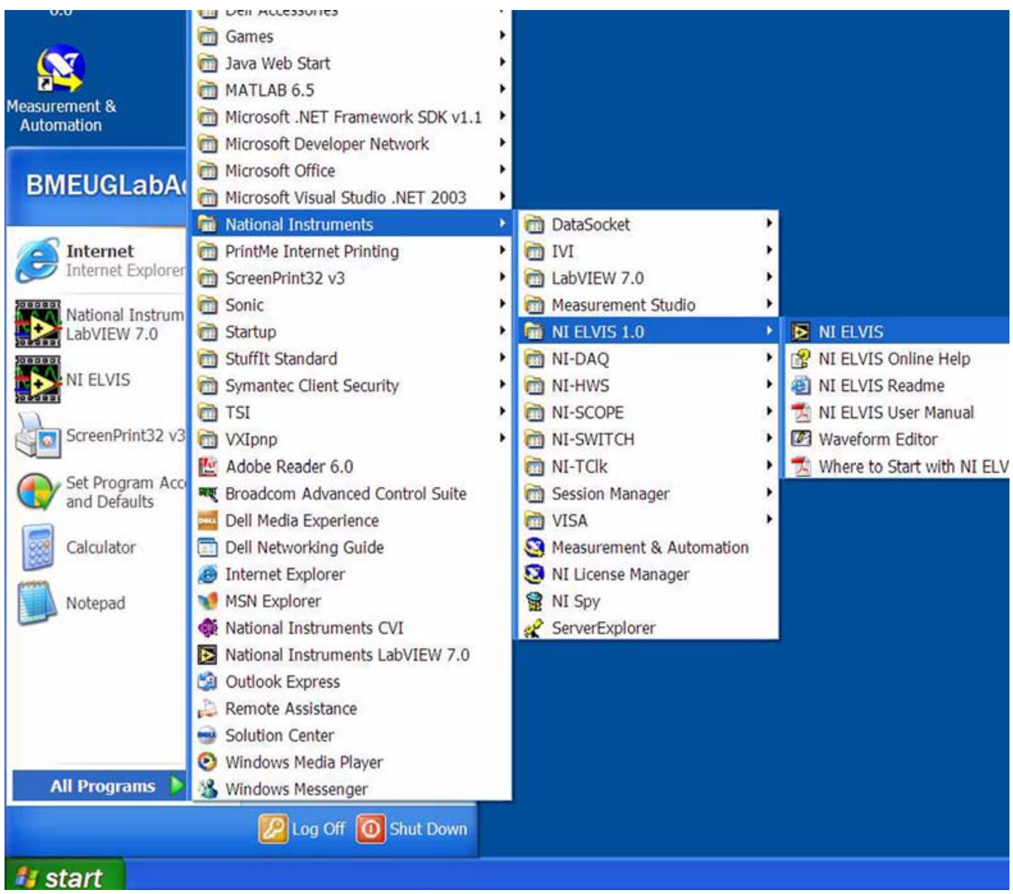


Figure 2-18. *NI Elvis menu location*

5. A dialog box will appear; using your mouse, select **Oscilloscope**.
6. An oscilloscope will appear on your monitor. Configure Channel A as **ACH0** and Channel B as **ACH1**. Ensure both channels are set to **ON**.

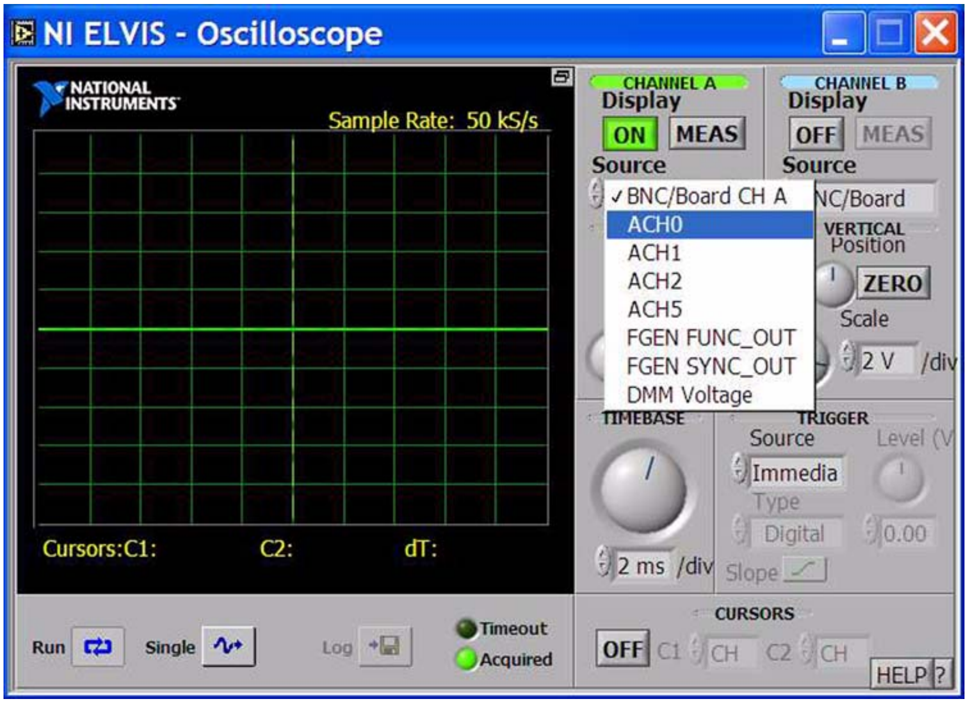


Figure 2-19. *NI oscilloscope soft panel*

7. Connect the 9V battery to your circuit and adjust your oscilloscope to view both waveforms simultaneously.

Note: Please read the next section and complete the prelab assignment before the next lab session.

LAB MEETING #2

Prelab Assignment

During the first part of this lab you have built a very simple pacemaker circuit with no possibilities of response loops. For the second lab session you will work with a simulation (again a very basic one) in Excel to describe the more complicated circuit described earlier in this handbook and during lecture. In preparation for working with this program, you will need to download it from the main laboratory website (http://www.bme.utexas.edu/ugrad/UGLab/) and perform a short homework exercise (also on the main website) before coming to class.

Lab Assignment

During this session you will work with the Excel simulation of a pacemaker to perform analyses including maximizing battery life and other questions. The exercise will be passed out at the beginning of the lab session.

BIOMEDICAL ENGINEERING
THE UNIVERSITY OF TEXAS AT AUSTIN

Medical Ultrasound

The potential of ultrasound as an imaging modality was realized as early as the late 1940s when several people—borrowing sonar technology used in the World War II—started exploring the diagnostic capabilities of ultrasound. However, it did not become widely accepted as a diagnostic tool until the early 1970s when gray scale ultrasound was introduced. Currently, ultrasound scanning has become the second-most utilized imaging modality behind X-rays. It is widely preferred as it does not use radiation, making it safer than alternative means.

SECTION CONTENTS

INTRODUCTION TO MEDICAL ULTRASOUND

The average human ear can detect sound waves with frequencies between 20 Hz and 20 KHz. Sound waves with frequencies higher than 20 KHz are called ultrasound. In, medical ultrasound, frequencies higher than 1.5 MHz and up to 20 MHz are used.

The major clinical applications of ultrasound include:

- Many aspects of obstetrics and gynecology involving the assessment of fetal health
- Acquiring electrocardiographs and the general assessment of heart function
- Intra-abdominal imaging of the liver, kidneys, gall bladder and spleen
- Many musculoskeletal applications
- The detection of compromised blood flow in veins and arteries
- The tracking of needle biopsies

During an ultrasound event, the following events happen:

1. The ultrasound machine transmits high frequency sound pulses into the body using a probe.
2. The sound waves travel within the body and hit a boundary between tissues (for example, between fluid and soft tissue or between soft tissue and bone).
3. Some of the sound waves get reflected back, while some travel on further until they reach some other boundary and get reflected.
4. The reflected waves are picked up by the probe and relayed to the machine. Usually the same transducer is used to transmit and receive.
5. The machine calculated the distance of the probe to the tissue or organ boundaries using the speed of sound in tissue (1540 m/s) and the time of each echo’s return.

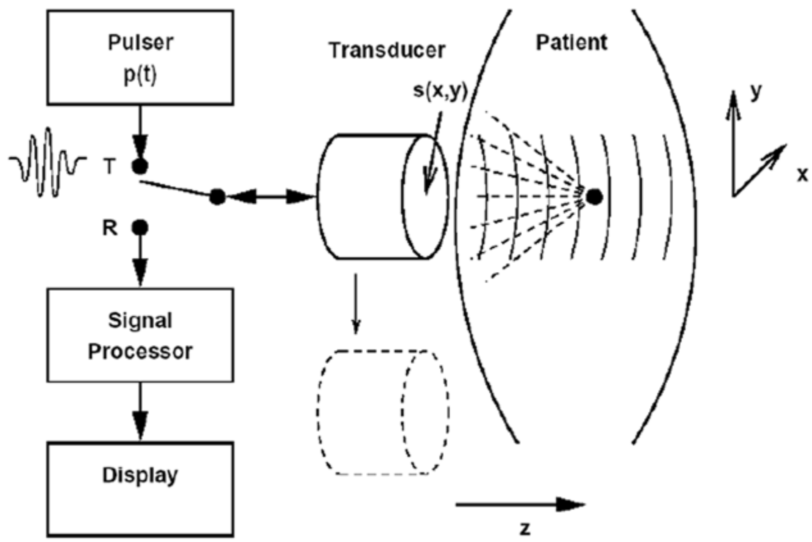


Figure 3-1. *Ultrasound imaging system block diagram*

6. The machine displays the distances and intensities of the echoes on the screen forming a 2 D image as shown in Figure 3-2.

Figure 3-2. *Ultrasound image*

Diagnostic scanning modes:

Current ultrasonic diagnostic imaging systems are mostly operated *pulse-echo mode* (that is, a single probe is used for transmitting the signal and receiving the echoes reflected or scattered back from the tissues). Depending on how the information is displayed, pulse-echo methods can be classified as *A*, *B,* and *M* modes:

A Mode Imaging

The oldest and the most simple imaging mode is the A (amplitude) mode. In this mode, the amplitudes of the pressure waves generated by the transducer are displayed with regard to the position. Today, the A mode is applied in limited areas such as ophthalmology to determine the thickness of the cornea.

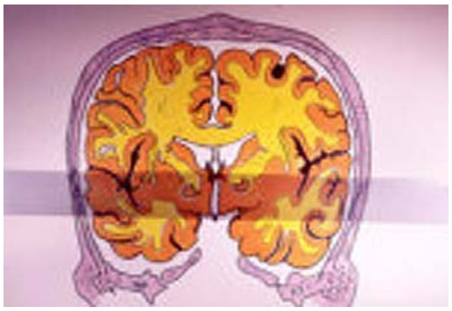


Figure 3-3. *A mode scan*

B mode imaging

In contrast to the A mode, where the amplitudes are represented in the form of peaks (as in a line plot), the amplitudes in B mode are represented as pixels (or images). The brightness of the pixels correspond to the intensity of the electrical signals, that is, the strength of the echoes. This is why B mode is called the *brightness mode*. Modern diagnostic ultrasound systems do normally offer 256 different brightness levels (256 gray scales).

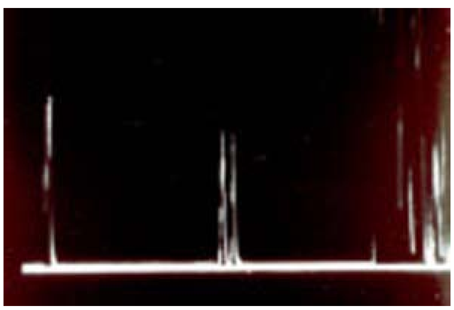


Figure 3-4. *B mode scan*

By shifting the beam after each transmit, one can create a 2 D image as in Figure 3-5.

Figure 3-5. *B mode scan (with beam shited after each transmission)*

M mode imaging

In M (motion) mode, the beam is not shifted as in B mode but held in a fixed position over the tissue to be examined. Now, the generated single lines are arranged to show how the echo data changes over time. Very quick motions such as heart valves can be imaged in this also. Velocity and acceleration measurements are also possible.

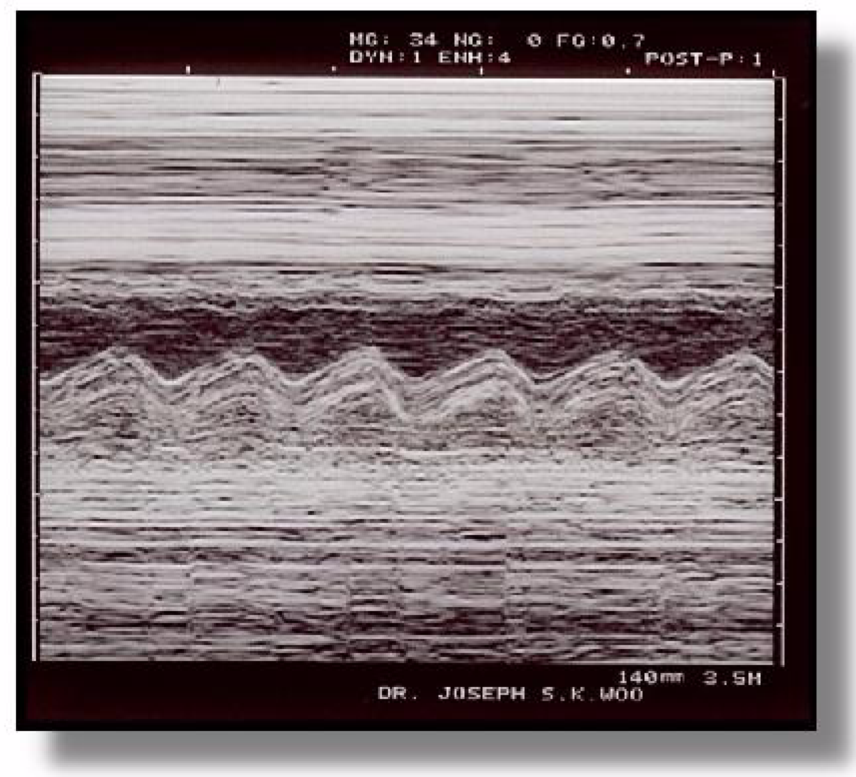


Figure 3-6. *M mode scan*

Transducer probes

The transducer probe is the main part of the ultrasound machine. It makes the sound waves and receives the echoes. It is, so as to speak, the mouth and ears of the machine. The transducer probe generates and receives sound waves using a principle know as piezoelectric effect. The probe has one or more piezoelectric crystals. When an electric current is applied to these crystals, they vibrate rapidly. The vibrations of the crystal produce sound waves which travel outward (transmit). Conversely, when sound or pressure waves hit the crystal, they emit electric currents (receive). Therefore, the same crystals can be used for transmitting and receiving ultrasound waves.

Figure 3-7. *Commercial ultrasound model featuring several transducer probes*

Transducer probes come in many shapes and sizes as shown in the Figure 3-7 above. The shape of the transducer determine the field of view and the frequency of the emitted sound waves determines how deep the sound waves penetrate and the resolution of the image. The transducer may contain a single crystal or many crystals (multi element probes). Multiple element probes may be linear or curved. Multiple element probes have the advantage that the ultrasonic beam can be steered by changing the timing in which each element gets pulsed. In addition the probes maybe moved across the surface of the body, some probes are designed to be inserted though various orifices of the body (vagina, rectum, esophagus), getting closer to the organ for detailed views.

Doppler Ultrasound

Doppler ultrasound is based upon the Doppler Effect. When the object reflecting the ultrasound waves is moving, it changes the frequency of the echoes, creating a higher frequency if it is moving toward the probe and a lower frequency if it is moving away from the probe. How much the frequency is changed depends upon how fast the object is moving. Doppler ultrasound measures the change in frequency of the echoes to calculate how fast an object is moving. Doppler ultrasound has been used mostly to measure the rate of blood flow through the heart and major arteries.

Vocabulary Terms

To ensure you are able to get the most out of your time with the laboratory ultrasound equipment, before the first day of lab you sould be familiar with the general concepts of ultrasound—as well as the following vocabulary terms:

- **TGC (Time gain compensation):** Time gain compensation is essential to amplify returning echoes. The intensity of the ultrasonic beam decreases exponentially as it penetrates the tissue. Also the energy of the beam is lost due to divergence. Therefore, two identical targets with different depths will produce different echoes with the amplitude of the closer target being stronger than the

other. This problem may be avoided by using TGC in which the gain of the amplifier is increased as a function of time or depth to compensate for the loss of energy. The SonoSite system **near** and the **far** gain knobs can be used to adjust this gain.

- **Overall gain:** Overall gain changes the power of the transmitted ultrasound wave. Higher the power, greater the penetration, and higher the returned echoes. However higher power is not always good as lower power. The SonoSite system **gain** knob changes the gain of the complete picture, unlike the TGC where the gain is like a sloped line. Here the gain will increase universally..
- **Depth:** Depth controls the depth up to which the back scattered signals are received and displayed on the screen. Having an excess depth results in the display of multiple reflections, and having less depth results in an incomplete display of the subject under scan.

LAB MEETING #1

The BME UG Lab is equipped with state-of-the-art SonoSite ultrasound systems. We will spend the first lab meeting getting to know the equipment and mastering the techniques required to succesfully operate the system.

Note: The SonoSite ultrasound system equipment is extremely delicate and must be handled with care. The most sensative item is the probe. Take all necessary measures to ensure you do not drop the probe.

⚠ **CAUTION:**
Sensitive Equipment! The ultrasound system probe is delicate. Handle the ultrasound system probe with care at all times.

Objectives

During this lab meeting, you will learn to:

- Operate the SonoSite ultrasound imaging system
- Hold the transducer probe with out damaging it
- Adjust the ultrasound image using the front panel controls of the imaging system
- Change the display patterns on the imaging system screen
- Clearly display a known phantom on the imaging system screen

The SonoSite Ultrasound Imaging System

The SonoSite ultrasound imaging system is a high-quality general imaging system useful in point-of-care ultrasound applications. The system features a digital image LCD screen and direct PC capability. The system weighs 2.6 kg and operates on both battery or AC power.

A few features of the SonoSite ultrasound system that are useful to us are:

- Continuous wave (CW) Doppler and pulsed wave (PW) Doppler
- Extended Resolution Harmonics (ERH) Imaging
- M-mode
- ECG Capability

Ultrasound System Hardare

Figure 3-8. *SonoSite ultrasound system controls*

#	Controls	Description
1	power switch	turns power on and off
2	**near**	affects gain of shallow echoes for 2D
3	**far**	affects gain of deeper echoes for 2D
4	**gain**	affects overall gain in 2D and CPD gain in CPD
5	menu select controls	press **Patient** to access system menus
6	**Menu**, **Depth**, and **Zoom**	allows access to imaging parameters; changes the display depth; increases the image size to 2x
7	trackball	moves objects on the image display
8	**Patient**	displays the system menus
9	**Function** key	used with number keys (1 through 6) to display assigned text; used with the **I** key to display system information
10	battery charge indicators	indicates a fully charged battery when all LEDs are lit
11	LCD brightness control	controls LCD brightness
12	LCD contrast control	controls LCD contrast
13	LCD	adjustable liquid crystal display (LCD)
14	cine arrow keys and **Freeze** key	press to move either way through the cine series of images
15	mode controls	press to access imaging modes (keys **7**, **8**, **9**, **0**)
16	battery release	press to release battery

Lab Meeting #1 Procedure

The SonoSite ultrasound system equipment is extremely delicate and must be handled with care. The most sensative item is the probe. Take all necessary measures to ensure you do not drop the probe.

To Operate the SonoSite Ultrasound System:

1. The transducer is most delicate part of the ultrasound system and extremely expensive. Be gentle and very careful.
   a. Coil the transducer cord around your arm a couple of times. This will help to ensure that even if the transducer slips from your hand, it does not fall on the ground.
   b. Apply the gel provided on the transmission surface of the transducer. The gel forms a critical contact surface between the transducer and the subject.
2. Identify the power switch located on the rear of the system handle. Turn on the system.

   Note: To achieve the best possible image quality, it is important to properly adjust the display, gain, and depth settings.
3. Adjust the view angle of the display.
   a. Adjust the viewing angle during the exam to achieve the best image quality. (The display is attached to a hinged joint that allows you to change the viewing angle.)
   b. Grasp the top of the display and rotate it up and towards you. Do not force the display beyond its maximum viewing angle.
4. Locate your object phantom.
   a. Apply gel on the transducer probe. In this case, you know where the object is inside the phantom.
   b. Position the transducer to see the object on the LCD screen.
5. Adjust the near, far and overall gain to obtain a clear picture of the object inside the phantom.
6. Form an estimate on the size of the object using the scale provided by the depth setting. Spot the edges of the object. (Will you be able to still locate the object if the phantom is totally black and nothing can be seen inside?)
7. Read the characters from the phantom. They should be visible on the screen.
   a. To do this, hold the transducer just above the transparency embedded in the phantom.
   b. Change the angle of the transducer until the characters are visible on the screen.
   c. Once this happens you can freeze the screen and show it to the TA.

      Note: To freeze the image, press the **Freeze** key.

8. Zoom the image.

   Note: Note: Zoom only works during real-time imaging. You will not be able to zoom on a frozen or saved image.

   a. Press the **Zoom** key to magnify the image by a factor of two.

   b. Change the depth while the image is zoomed.

   c. Press the **Zoom** key again to exit zoom.

9. Assume the phantom is totally black and an object is hidden inside it.

   a. Form a written algorithm to detect an unknown object and demonstrate this algorithm to your TA.

      An example algorithm might be:

      1.) Mark one side of the phantom.

      2.) Scan that side to notice any edges of the object, *etc*.

      3.) The adjacent side is next marked, and so on.

Conclusion

By the end of the lab meeting, you should be able to describe the function of the keys on the SonoSite system control panel, as well competently image a given object.

Document what you have learned during this lab meeting in your lab notebook. Ensure that you include your algorithm and any questions you may have concerning ultrasound applications.

Printing Your Images

Ensure the printer is properly set up for operation. Check with your TA if you suspect something is wrong with the system configuration.

To Print an Image:

1. Press the **Freeze** key.
2. Press the **Print/VCR** key or **Print** key to print an image.

To Print all Images:

1. Save the images.
2. Press the **Patient** key.

   A menu appears on which is listed stored images.

3. Select stored images from the on-screen menu.

4. Select print all images from the on-screen menu to print all images.

To Cancel Printing:

Press the **Freeze** key to stop printing.

1.

Saving and Reviewing Images

To Save an Image:

1. Press the **Freeze** key.
2. Press the **Save** key.

   The image memory icon in the lower right section of the screen changes to show that the image was saved.

   The number above the memory icon increases by one to show how many images have been saved.

To Review or Delete Individual Images:

1. Press the **Patient** key.

   A menu appears on which is listed stored images.

2. Select stored images from the on-screen menu.

   A menu appears on which is listed review images.

3. Select review images.

   The last saved image appears with the following menu:

Figure 3-9. *Review images menu*

   Press the **Menu** key (to the left of **Depth**) to turn off or to turn on this menu.

4. Use the cine arrows from the on-screen menu or the cine arrow keys (above **Freeze**), or the Arrow keys (next to the **Enter** Key) to scroll through the images.
5. Select delete from the on-screen menu to remove the selected image from memory.

   A dialog box appears and a menu which present the options **yes** and **no**.

6. Select **yes** to delete the image; select **no** to cancel.

7. When you are finished, press the **Freeze** key to return to the menu.
8. Press **Patient** to return to live imaging.

To Delete All Saved Images:

1. Press the **Patient** key.

   A menu appears on which is listed stored images.

2. Select stored images from the on-screen menu.

   A menu appears on which is listed delete all images.

3. Select delete all images from the on-screen menu.

   A dialog box appears and a menu which present the options **yes** and **no**.

4. Select **yes** to delete all images; select **no** to cancel the operation.
5. Select done from the on-screen menu (twice) to return to live imaging.

Using SiteLink Manager

Starting the SiteLink Image Manager

With SiteLink Image Manager, you can transfer saved images from the SonoSite ultrasound system to the PC. SiteLink Image Manager follows the standard MS Windows XP/2000/NT/98/95 interface.

To start SiteLink Image Manager:

Double-click on the SiteLink Image Manager.exe shortcut.

User Interface Controls

Figure 3-10 lists the SiteLink Image Manager user interface controls.

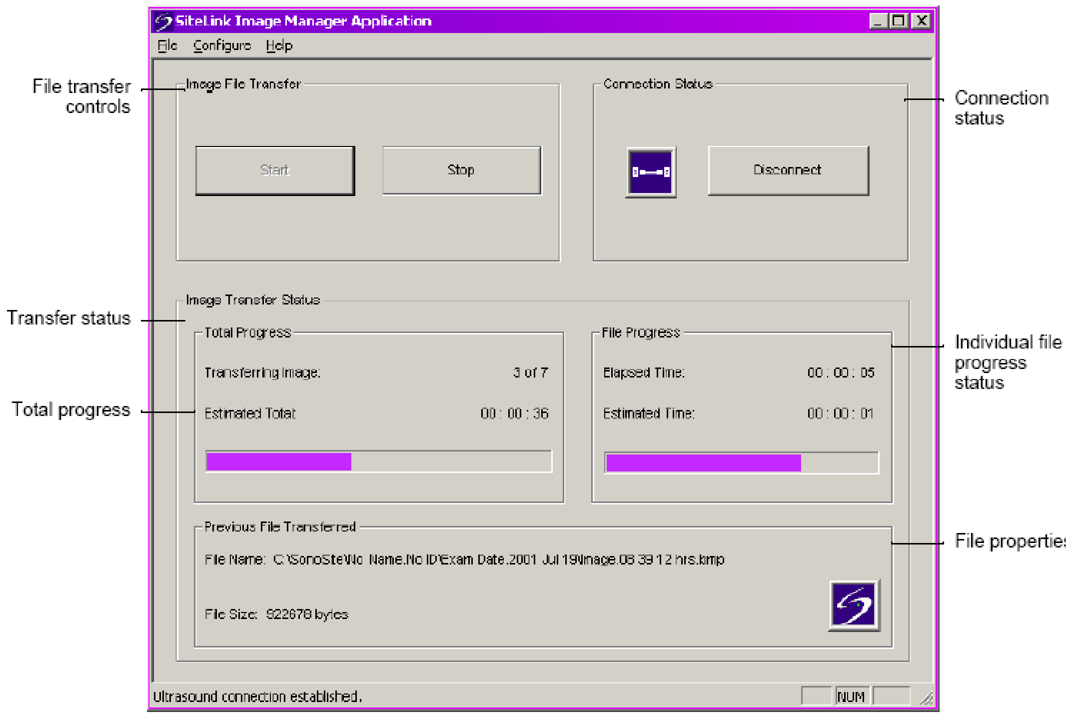
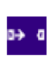
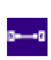


Figure 3-10. *SiteLink user interface controls*

Table 3-1 lists the connection status icons and their meaning.

Table 3-1. *Connection status icons.*

	Suspended (no communication established; animated icon)
	Disconnected
	Connected

Transferring Saved Images From the Ultrasound System

To transfer saved images from the SonoSite ultrasound system to the PC:

1. Press **Start**.
2. When the images begin to transfer, information about the transfer is displayed in the image transfer status area.

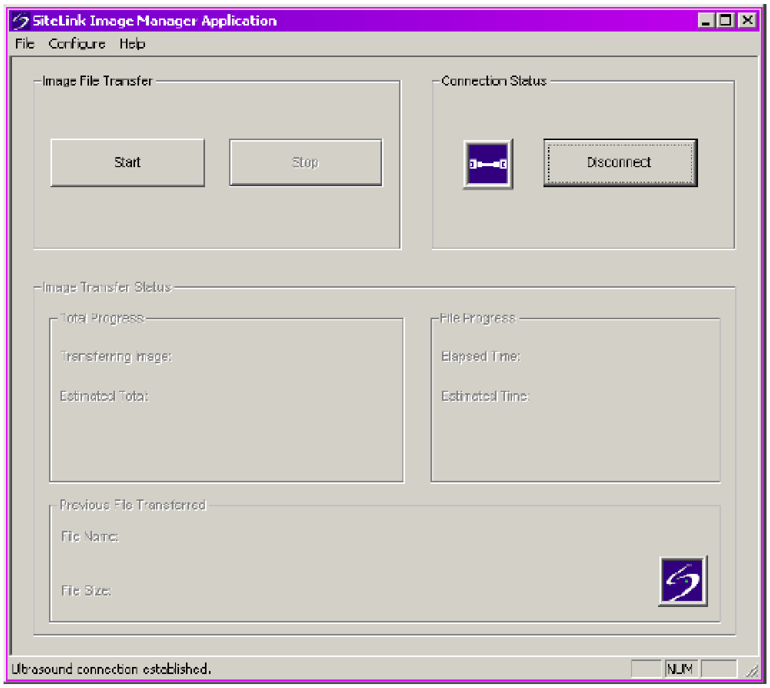


Figure 3-11. *Image file transfer dialogue box*

To locate transferred images:

SiteLink Image Manager stores images into a hierarchy of patient and exam folders using the following information:

- Patient Name and Patient ID
- Exam

Figure 3-12 shows an example of the Microsoft Windows Explore hierarchy created for the transferred images.

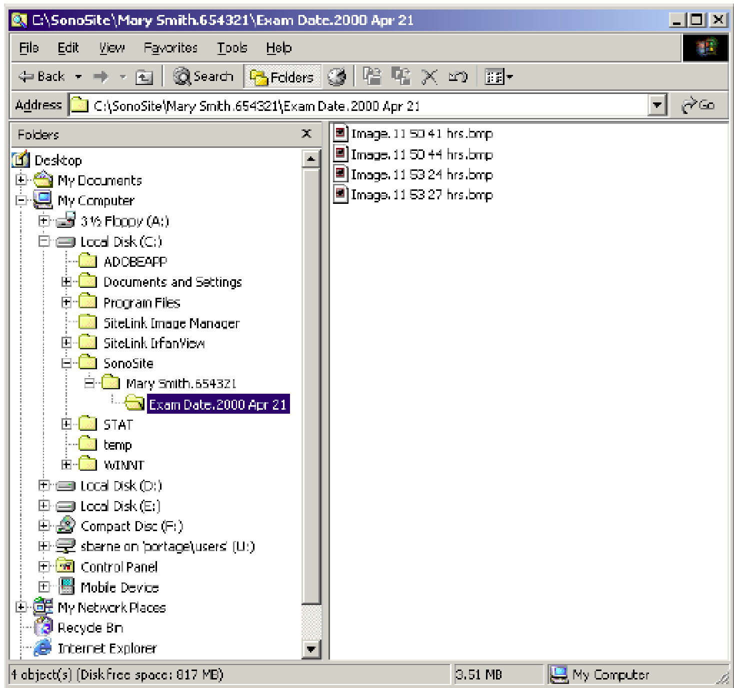


Figure 3-12. *Explorer window*

To Exit SiteLink Image Manager:

1. From SiteLink Image Manager, choose **File**.
2. Then choose **Exit**.

LAB MEETING #2

During this lab, your objectives will be to:

- Continue mastering the use of the ultrasound scanner
- Find objects inside the assigned phantoms
- Estimate the size of the objects using the depth information on the screen
- Recreate the object
- Calculate the time of flight across the object

Lab Meeting #2 Procedure

Locate the phantom you will scan today. This phantom should contain an unknown object. Using only the ultrasound scanner, you will determine the size and shape of the unknown object; then you will create a scale model of the object hidden in the phantom.

1. Using the procedure outlined in "Lab Meeting #1 Procedure" on page 3-11, as well as the algorithm you created in the previous lab, scan across the unknown phantom

   Note: You must scan at least 3 sides to completely scan the phantom and find the object that may or may not be hidden inside it.

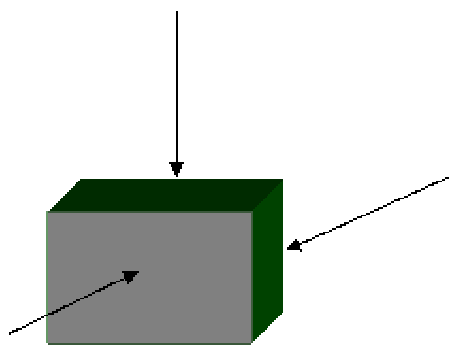


Figure 3-13. *Scanning at least along 3 surfaces to encompass the whole object*

2. The point of the first scan is to detect the location of the object inside the phantom; therefore, the first scan can have big increments.

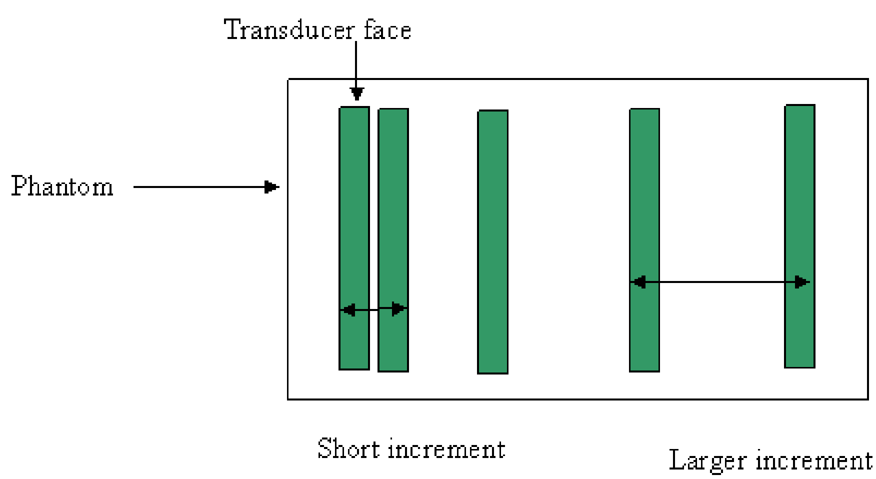


Figure 3-14. *Scanning increments*

Question: Is the scan showed in Figure 3-14 enough? Or do you need to scan in another direction to complete the scan of one surface.

3. After detecting object edges (there can be more than 1 object—just as a patient might have more than 1 tumor), scan the phantom again. This time around, scan with smaller increments to gain a better approximation of the size.
4. Scan the unknown object from 3 *orthogonal* sides, as shown in Figure 3-13. Use the procedures listed under "Measuring Distance" on page 3-19 and "Measuring Area and Circumference" on page 3-19 to aid you in gaining an understanding of the dimensions of the unknown object.
5. Recreate the object, you may use any graphical software or simple hand drawings showing the top, and 2 side views, along with accurate dimensions.

   Question: Since you know the depth of the total image from the screen, can you estimate the size of the object? If yes write a procedure to calculate the dimensions of the unknown object.

Measuring Distance

To perform a distance measurement:

1. On a frozen 2D image, press the **Measure** key.
2. Use the trackball to position the first caliper.
3. Press the **Select** key to activate the second caliper.
4. Use the trackball to position the second caliper.
5. The distance is displayed on the left hand corner of the screen.

Measuring Area and Circumference

To calculate the area and circumference of the object:

1. Perform a distance measurement.
2. Select **Ellipse measurement** from the on-screen menu.
3. The circumference and the area appear in the lower left section of the screen.
4. Use the trackball to adjust the size of the ellipse.
5. Press **Select** to adjust the first caliper.
6. Press **Select** key again to adjust the second caliper.
7. The area and circumference are displayed on the left hand bottom of the screen.

Summary

By accomplishing this lab, you have managed to gain an insight into the use of ultrasound to detect tumors in the body. Additionally, you learned how to hold the transducer, and used your algorithm to detect an unknown object/s in the phantom.

LAB MEETING #3

In this lab, you will measure and analyze a flow using Doppler ultrasound. The Doppler effect describes a phenomenon in which a change in the frequency of sound emitted from a source is perceived by an observer when the source or the observer is moving or both are moving.

Doppler Ultrasound

In medicine, Doppler shift is used to detect and measure blood flow. Red blood cells (RBC's) are the major reflectors in the blood. Ultrasound scanners transmit a series of pulses to detect the blood flow. Echoes from stationary tissue are same from pulse to pulse. Echoes from moving scatterers exhibit slight differences in the time for the signal to be returned to the receiver. These differences can be measured as a direct time difference, or more usually, in terms of a phase shift from which the Doppler frequency is obtained.

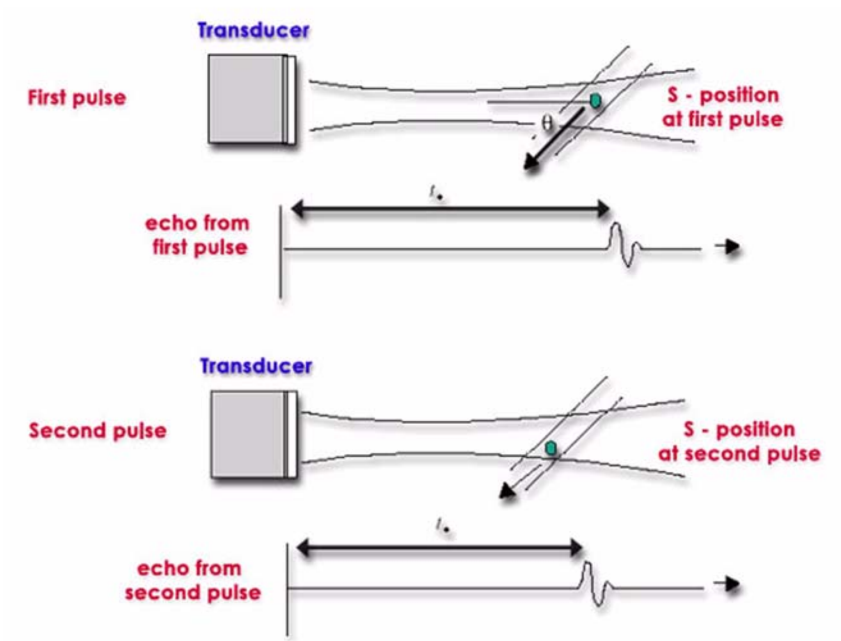


Figure 3-15. *Ultrasound velocity measurement.*

Figure 3-15 shows a scatterer S moving at velocity V with a beam/flow angle q. The velocity can be calculated by the difference in transmit-to-receive time from the first pulse to the second (t2), as the scatterer moves through the beam.

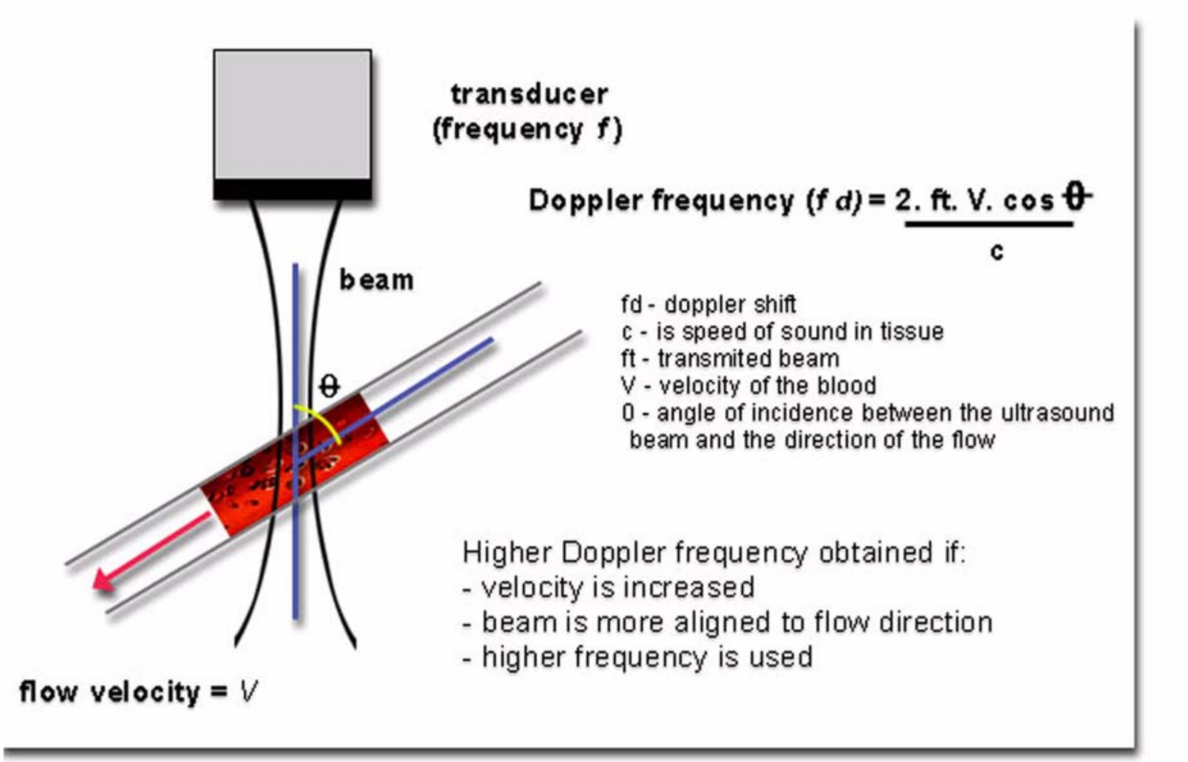


Figure 3-16. *Doppler frequency*

Doppler ultrasound measures the movement of the scatterers through the beam as a phase change in the received signal. The resulting Doppler frequency can be used to measure velocity if the beam/flow angle is known.

Note that since cosine of 90 is 0, if the ultrasound beam is perpendicular to the direction of the blood flow, there will be no Doppler shift. From the formula of Doppler shift, it can be inferred:

- **Blood velocity:** as the blood velocity increases, so does the Doppler frequency.
- **Ultrasound frequency:** higher ultrasound frequency will give increase Doppler frequency, however lower frequencies have better penetration.
- **Angle of insonation:** the Doppler frequency increases as the Doppler ultrasound beam becomes more aligned to the flow direction.

  This can be illustrated by the following figure.

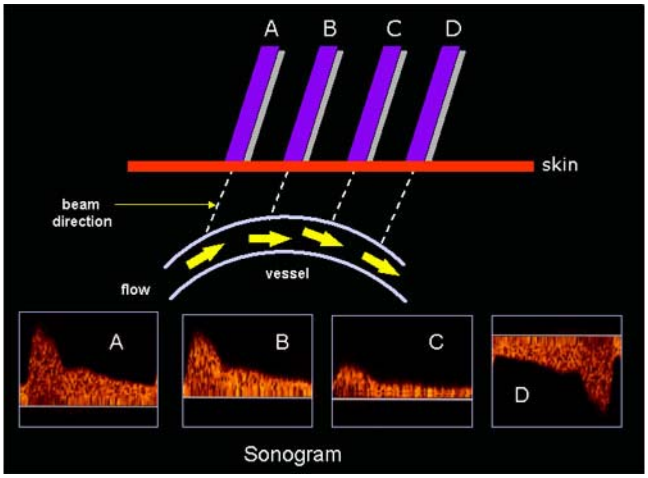


Figure 3-17. *Effect of the Doppler angle in the sonogram.*

- (A) higher-frequency Doppler signal is obtained if the beam is aligned more to the direction of flow.
- In the diagram, beam (A) is more aligned than (B) and produces higher-frequency Doppler signals.
- The beam/flow angle at (C) is almost 90° and there is a very poor Doppler signal.
- The flow at (D) is away from the beam and there is a negative signal.

Types of Doppler Measurements

There are several forms of Doppler measurements for blood flow. We will consider two primary ones: color flow & pulsed doppler.

Color Flow

Color Doppler ultrasound provides an estimate of the mean velocity of a flow within a vessel by color coding the information and displaying it superimposed on the gray scale image. The flow direction is arbitrarily assigned the color red or blue indication flow away or towards the transducer.

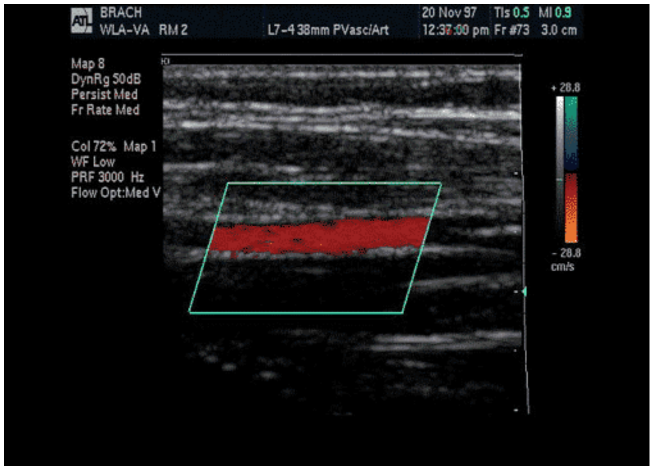


Figure 3-18. *Color Flow Doppler*

Pulsed Doppler

Pulsed Doppler allows a sample volume to be positioned on a vessel visualized as a gray scale image, and then displays a graph of the velocities in the sample volume as a function of time.

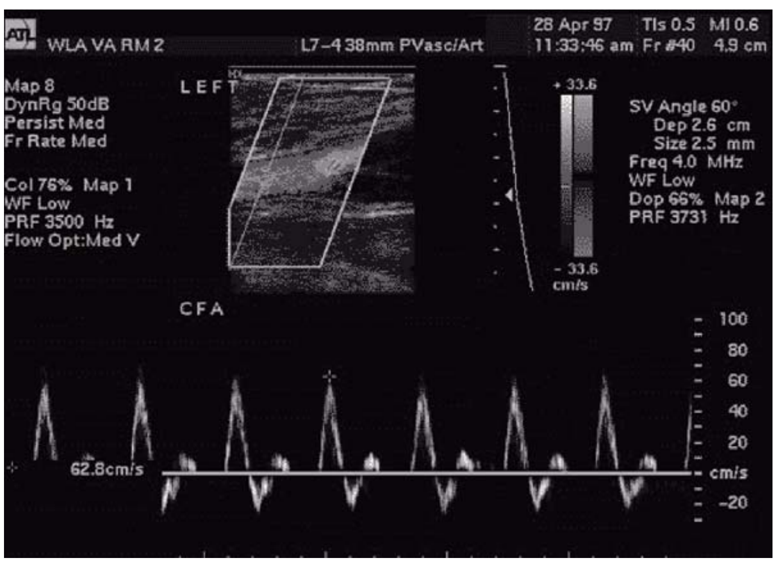


Figure 3-19. *Pulsed Doppler velocities indicated*

Exercise

Consider the following case:

- Ultrasound transducer center frequency: 5 Mhz
- Coronary Blood flow rate: 200 mL/min

- Coronary radii: 0.5 cm
- Angle of the beam w.r.t vein: 30^o
- Speed of the beam: 1540 m/s

Question: Calculate the Doppler shift that will occur.

Note: Does the formula have blood flow or blood velocity? 1 mL = 1 cc

Bonus question: In the formula of Doppler shift, why is there a factor of 2?

Lab Meeting #3 Procedure

During this lab session, you will use the SonoSite ultrasound scanner to perform Doppler measurements. These measurements will include:

- The flow rate and flow velocity of the liquid flowing through the phantom
- An assessment of the importance of the angle of holding the transducer probe on the quality of measurement

To Measure Flow Rate and Velocity:

1. Start the peristaltic pump in position 1 (that is, connect the **In** and **Out** sections of the pump to the two ends of the first pipe in the phantom provided to you by your TA).
2. Set the speed indicator to **6**.
3. Take a beaker of water and place 0.5 to 1 g of amberlite in it.

   Note: Amberlite is used as a scatterer in the ultrasound image.
4. Turn a stirrer on, and place the beaker on it.

   This ensures the amberlite remains in circulation in the water and does not settle down in the water reservoir.
5. Place the transducer over the surface of the phantom.
   - Hold the transducer parallel to the surface.
   - Ensure the transducer is along the plane of the flow and not perpendicular to it.

   Question: Why shouldn't the transducer shouldn't be held perpendicular to the plane of flow?
6. Use a normal 2 D mode to see the image on the screen.

   Note: By default, the screen shows 2D imaging mode. If the screen is not displaying 2D imaging mode, press **0** to enter 2D imaging mode.
7. After making sure you are in the plane of the flow, turn on the color flow Doppler (CPD) by pressing button **8**.

8. Use the trackball to maneuver the color flow to a good spot in the middle of the tube.
9. Press the **Select** key, and then use the trackball to position the “square” seen on the screen.
10. Now turn the Doppler on by pressing button **7**.
11. Use the Doppler measurements to calculate the speed (or *flow*) of the water.
    a. On a frozen Doppler spectral trace, press the **Measure** key.
    b. Use the trackball to position the active caliper to the peak of the waveform.
    c. The velocity measurement is displaced in the upper left section of the screen.
12. Now turn to position **2**, (that is, connect the peristaltic pump to the second pipe in the phantom).

    Note: Make sure that the water flow rate on the peristaltic pump remains the same for both the first and second flow rate measurements.
13. Repeat the procedure and make the measurements required.
    a. Compare the flow rates calculated.
    b. Is there a difference in the velocity measurements in the first and the second pipes?
    c. Why is there a difference? Explain

LAB MEETING #4

During this lab session, you will use MatLAB to load, image process, and process input acoustic data. The objectives for this lab are to:

- Display the given ultrasonic image
- Identify the image
- Hilbert transform the input signal, and display an individual scan lines
- Calculate a mean of scan line

Lab Meeting #4 Procedure

Matlab is a tool for doing numerical computations with matrices and vectors. It can also display information graphically. The best way to learn what Matlab can do is to work through some examples at the computer.

To Load and Process Acoustic Data Using MatLab:

1. Create a directory to store all the files provided.

2. Open the file named: *fillinblanks.m*. This file has details on how to process the data and examples of how to use the commands in Matlab that are required for plotting the image.
3. Change the path in the main command window of Matlab to point to the directory where you have stored the files.
4. Use the **load** command to load the *rf.mat* file supplied to you. Specify the path of the file, or alternatively place the file in the home. For more information on how to use the load command type in "help load" in the command window.
5. Look at the workspace, what is the size of the input image? Which dimension will be the transducer surface? And which is the time dimension?
6. Use **imagesc** command to display the data
7. The image is displayed as a colored image, use colormay to make it a gray scaled image, add a title to your image. Also add x and y labels specifying which is the transducer face, and which is time axis. Use the commands **title**, **xlabel**, and **ylabel**.
8. Plot 128th rf data line of the input data using the **plot** command. Make sure you don't lose the earlier figure. Use the **plot** command.
9. Apply Hilbert transforms on the data, command **b= Hilbert(a)**.
10. Use **hold on** command so you can plot the next graph on the earlier plot.
11. Use **abs** command to find the absolute value of the data obtained from Hilbert transform (the data after Hilbert transform is complex).
12. Plot the absolute value on the same graph as the Hilbert transform data.

    What is the difference in the images?

    What did Hilbert transform accomplish?
13. Now display the whole image which was Hilbert transformed. Again, color map the image to gray scale and Plot the required figures.

EMG

SECTION CONTENTS

MUSCLE CONTRACTION—ELECTROMYOGRAM (EMG)

Muscle is an essential building block of human physiology. Although people typically think of muscle only as something that can be improved through exercise (biceps and quadriceps being among the more well known muscle groups), human muscle has several different structures and functions. There are three types of muscle in the body: skeletal, smooth, and cardiac. Each muscle type has a slightly different composition and is located in distinct parts of the body from the others. For example, cardiac muscle is found in the heart, smooth muscle is found in the hollow organs of the body, and the skeletal muscles are the muscles that most people think of when lifting weights and working out.

Approximately 40% of your body is skeletal muscle, and you can view muscle with various degrees of complexity, from the movement of a whole group of muscles to the interactions between cells within a muscle and the exchange of ions between parts of a muscle. As you might have guessed, a muscle is not a single entity but rather a collection of many smaller parts—fascicles or bundles of individual muscle fibers (elongated cells). Whenever a muscle does work, it goes through a process called contraction. A muscle doing work takes on a number of forms, from swinging a baseball bat to lifting a crate to waving good-bye.

The complete process of muscle contraction is relatively complex and involves a number of physical and chemical interactions. Generally speaking, a contraction starts with an electrical signal (passed along a nerve) which causes the muscle to contract. When an impulse from the nerve reaches the muscle, it sets into motion a chain reaction which results in the contraction of muscle. When a muscle group contracts, literally thousands of individual muscle fibers are contracting. Although the electrical impulse related to any one fiber contracting is actually quite small, the cumulative effect of all of the action potentials of all contracting muscle fibers generates a large enough signal to be measured by electrodes placed on the surface of the skin.

In the human body, skeletal muscles are stimulated to contract by somatic motor nerves that carry signals in the form of electrical impulses from the brain or spinal cord to the skeletal muscle. Axons leave the spinal cord via spinal nerves and the brain via cranial nerves, and are distributed to appropriate skeletal muscles in the form of a peripheral nerve, which is a cable-like collection of individual nerve fibers (axons). Upon reaching the muscle, each nerve fiber branches and innervates several individual muscle fibers. Although a single motor neuron can innervate several muscle fibers, each muscle fiber is innervated by only one motor neuron. The combination of a single motor neuron and all of the muscle fibers it controls is called a motor unit. When a somatic motor neuron generates an action potential, all of the muscle fibers it innervates respond to the secreted neurotransmitter by generating their own action potential and contracting. A whole muscle is made up of thousands of muscle fibers divided up into motor units.

LAB MEETING #1

During this lab session, you will conduct a Biopac tutorial to familiarize yourself with the Biopac system software. Once you have completed the tutorial, you will perform a *Reaction Time* laboratory lesson, developed by Biopac, to further develop the tool skills necessary for the remainder labs in this section.

Lab Meeting #1 Procedure

Biopac Tutorial

The Biopac tutorial is located in Appendix 2 of this manual. All members of the group must complete the tutorial before anyone in the group can start the reaction time exercise. All members of the groups will then serve as subjects for the reaction time exercise.

See "Biopac Tutorial and Reaction Time Lab" on page 7-1 for further information.

Reaction Time Investigation Lab

Some years ago, a world class runner was repeatedly accused by his competitors of "jumping the gun" in races. He could only respond to these accusations by saying that he felt he had done nothing wrong. When film footage of his races was reviewed, it became clear that he was in fact leaving the starting blocks before his competitors. As the evidence against him mounted, he underwent physiological testing which measured how long it took him to respond to external events or stimuli. It was revealed that his reflexes were some five to ten percent faster than those of an average person. This slight advantage enabled him to react faster once he heard the starter's pistol.

The beginning of a race is a classical example of a stimulus-response situation, where people hear a stimulus (the starter's pistol) and react to it in some way (response). There are two key factors in stimulus-response situations: reaction time and learning. Reaction time is the delay between when the stimulus is presented (in this case, we hear it, but there other types of stimuli as well) and when we do something about it. How long it takes to respond to stimuli varies from person to person and from situation to situation. The runner mentioned above had a distinct advantage when it came to reaction time, and most people have delayed reaction times late at night and early in the morning.

Learning is what you are doing while reading this lab exercise; and how well and fast you learn is often related to your reaction times. Imagine someone entering a track event for the first time. When the pistol goes off they might not know what to do, but after everyone around them starts running they would eventually get the idea and start running as well, although they would have to run well to make up for a bad start. On their next race, we would expect them to start at about the same (although not exactly) time as everyone else, assuming some learning took place in the meantime. With practice, and more importantly, learning, their reaction times could be cut from a few

seconds to fractions of a second, once they learn the rules of the game. As people learn what to expect, reaction time typically decreases, as you will hopefully see in this lesson.

In order to control our conscious, voluntary movements when we respond to a stimulus, the information must be processed in the sensory & motor cortex areas of the brain. The neural impulse then moves toward the efferent path down the corticospinal pathway, from the brain into the spinal cord, where the CNS (Central Nervous System) neurons synapse with motor neurons (enters the efferent pathway) which extend out into the periphery of the body. The motor neurons deliver an impulse to the skeletal muscles (effectors) which perform an action. The speed with which sensory and motor impulses move through our nervous systems can be increased through learning how to respond to a stimulus. As we learn, changes in synaptic connections occur as a result of neuronal activity. This process is called potentiation.

However, there are physical limitations to improving reaction time. Once you have learned the proper response to a stimulus, potentiation has occurred and the nervous system has plotted the most efficient pathway for reaction to a stimulus, there will be little or no improvement. The most efficient pathway is the shortest and involves the least number of synapses. However, remember that the speed of a neural impulse is also related to (1) diameter of axons and (2) the degree of axon myelination found in the neurons of the specific neural pathway.

There is evidence that ingestion of small amounts of caffeine (a stimulant) will enhance reaction time. Anecdotal evidence has been presented that ionized air may also enhance reaction time.

Lab Objectives:

- Describe the components of a reflex arc.
- Describe how learning and reaction time act as factors in stimulus-response situations.
- Using Biopac lesson 11, determine if there is a difference in average reaction time when presented with random or non-random stimuli.
- Using Biopac, demonstrate the following hypothesis: “learning may decrease the reaction time”. You will do this by running Lesson 11, then compare the average reaction times generated by each test subject.

Note: During this experiment it is extremely important that the classroom be very quiet since the test subjects will be listening for their stimulus. Therefore, you should talk in very low tones so as not to disturb another group that may be administering the test nearby. The test subject will wear headphones and listen for a click which is a prompt to react by pressing a button.

Overview of Biopac Lesson 11

The lesson looks at how reaction time can vary from situation to situation. There are two parts to this lesson, and both involve pressing a button (response) as soon as possible after hearing a click (stimulus). The only difference between the two parts is that in the first two segments, the stimuli are presented at pseudo-random intervals, and in the third and fourth segments, the stimuli are presented at fixed intervals.

The procedure requires you:

1. Open the Lesson
2. Calibrate the equipment
3. Record your data
4. Review data and print journal

Note: Do not redo/repeat this lesson on the same test subject, or it may skew your results.

Procedure

Follow the setup and recording instructions on the screen of the computer after opening lesson 11.

To Open the Lesson:

1. Your Biopac should already be hooked to the computer and turned on. You may need to open the Biopac application.
2. After opening the Biopac application, a new window opens and requests you "please choose a lesson:".
3. Click on **L11-React-1**, then click **OK**.
4. Your next prompt will be to type in your first name. This will create a folder for you in this lesson.
5. From your box of hardware, obtain an SS10 lead (hand switch) and the headphones.

To Calibrate the Equipment:

The new screen that you see will begin your lesson on Reaction Times. Read and follow the directions at the bottom of the computer screen.

1. Connect the hardware to the MP30 unit:
   a. The hand switch plugs into Channel 1 on the front of the MP30 unit
   b. The headphones plug into the back of the MP30 unit.
2. After you have hooked up the headphones and hand switch, look at the top left of the computer screen for the **Calibrate** button.
3. Click your left mouse button on this when the test subject is ready.

Another prompt will appear before the calibration process begins.

4. A single "click" sound will be given during the 8 second calibration period. When the test subject hears the sound, press the hand switch.

Once calibration is complete, proceed to record the experiment. Follow the instructions at the bottom of the screen.

To Record Reaction Time:

1. During the first two segments of the lesson, the clicks will come at random intervals. The Biopac randomly selects a time and produces a clicking noise that can be heard through the headphones.

   Each time the Biopac produces a click-noise, it will insert a marker (a small downward pointing triangle) on the computer screen just below the measurement windows.

   Each time the subject presses the push-button on the SS10 lead, an upwards pointing *rectangular pulse* will be displayed on the screen indicating your response.

2. After about 60 seconds, 10 clicks will have been produced and the screen will stop moving.
3. Click **Resume** at the top left of your screen to proceed with the second segment of pseudo-random stimuli.
4. When segment 2 is complete, Click **Resume** to start segment 3 of the experiment (non-random stimuli).

   In Segments 3 & 4 the stimuli will be presented at regular intervals. See if your reaction time improves.

5. Continue with segment 4 of the experiment by clicking **Resume**.
6. When done with the fourth segment, the experiment is complete. You should have about 200 seconds of recording.
7. Click on the **Done** button at the top left of the screen.
   a. A window will appear asking you "what would you like to do now?"
   b. Select **copy to floppy or network** and save the data to your folder.

   Note: Always save your data before testing another student.

If there are others in your group who will be tested, you should perform the experiment on them before actually analyzing the data from any of the saved files. You will need to reopen Lesson 11 and re-calibrate. When the last subject in your group has completed all 4 segments of the experiment, you may begin analyzing the data by opening the Lessons folder in Biopac and selecting **Review Saved Data**.

To Review and Save Data:

1. Open the **Review Saved Data** folder.

This is located at the bottom of the window when you open the **Lessons** menu (The **Lessons** folder is at very top of your computer screen).

2. Click on the name of the folder that you want to analyze and print out.
   a. The file is displayed.
   b. Click the file and then click on the **Open** button.
   c. The data displays in a **JOURNAL** at the bottom third of the screen.
3. Print your journal for future reference.
   a. To print your journal, click the **File** menu at the very top of the screen.
   b. Click **Print**.
   c. A new window will ask you to choose what you want to print.
   d. Print only the Journal.
4. After printing the journal data, exit the program**.**

Questions

Answer the following questions after recording the data.

- Calculate the percent change in your mean reaction times:

Table 4-1. *Percent change in mean reaction times*

% dif. between segment 1 & 2	% dif. between segment 2& 3	% dif between segment 3 & 4

- Write some brief instructions describing how you could calculate any of the reaction times byhand on the Biopac screen using the delta T measurement and the I-beam tool.
- What is the approximate length of the neural pathway in the test subject (ie. from the Receptor to the Effector)?
- Calculate your mean impulse speed for segment 1 (m/sec).

  [length of neural path (m) / mean rxn time (sec)]
- Calculate your mean impulse speed for segment 3 (m/sec).
- Discuss your reaction time between the 1st and 10th run.
- Discuss your average reaction time between segment 1 & 2.
- How did your reaction time change when you were presented with non-random stimuli (compare the average times of segments 3 & 4 versus segments 1 & 2)?

- Explain why reactions to non-random stimuli are usually faster than random stimuli that were presented in this experiment.
- Define the term “hypothesis”.
- Review the general components of a “simple nervous reflex” and identify each of the corresponding parts of the nervous system used in today’s reaction time experiment.

  Indicate the specific component of the nervous system that would act as:

  - name of receptor organ
  - name of afferent nerve
  - name of CNS integrating center
  - name of the effector (target)
- Draw and label the components of the neural synapse.Include the neurotransmitter vesicles, pre-synaptic axon terminal, synaptic cleft, post synaptic neuron receptor, and indicate the direction that the neurotransmitter will diffuse through the cleft.
- Explain how and why each of the following factors affect the total reaction time:
  - number of synapses involved in a reflex:
  - degree of myelination of neurons:
  - internal diameter of sensory and motor axons

LAB MEETING #2

Physiologically, the degree of skeletal muscle contraction is controlled by:

- Activating a desired number of motor units within the muscle
- Controlling the frequency of motor neuron impulses in each motor unit

When an increase in the strength of a muscle's contraction is necessary to perform a task, the brain increases the number of simultaneously active motor units within the muscle. This process is known as motor unit recruitment.

Resting skeletal muscles in vivo exhibit a phenomenon known as tonus, a constant state of slight tension that serves to maintain the muscle in a state of readiness. Tonus is due to alternate periodic activation of a small number of motor units within the muscle by motor centers in the brain and spinal cord.

When a motor unit is activated, the component muscle fibers generate and conduct their own electrical impulses that ultimately result in contraction of the fibers.

Although the electrical impulse generated and conducted by each fiber is very weak (less than 100 microvolts), many fibers conducting simultaneously induce voltage differences in the overlying skin that are large enough to be detected by a pair of

surface electrodes. The detection, amplification and recording of changes in skin voltage produced by underlying skeletal muscle contraction is called electromyography. The recording called an electromyogram (EMG).

In today's lab session we will be measuring the amount of electricity generated by your forearm muscles (flexor digitorum muscles) when you clench your fist tighter and tighter, thus demonstrating the action of motor unit recruitment.

Lab Session General Procedure

During Part I, every student will serve as an experimental subject and obtain results for the three experiments described below. Each Biopac Lesson consists of four steps. First, the equipment to be used is set up and attached to the student subject. Then, the actual Biopac computer program is run to carry out the remaining three steps which consist of calibration, recording lesson data, and data analysis. When you do Biopac Lessons, the data you collect are stored in the filename you provide at the start of the lesson. You can come in and review or continue to analyze this data on this computer as needed throughout the semester.

During Part II, you will approach the same phenomenon from the opposite direction. You will voluntarily contract your muscle with increasing tension, and looking at the amount of electrical activity this causes in the muscle (the ElectroMyoGram or EMG). This experiment demonstrates the phenomenon called Multiple Motor Unit Recruitment (MMUR) in a different way than the one you simulated with Part I.

Lab Meeting #2 Procedure (Part 1)

This lesson illustrates the electrical activity that takes place in skeletal muscle tissue when it produces a graded contraction (increasing strength by increasing the number motor units recruited), by clenching your fist tighter and tighter. The experiment will also demonstrate the difference in the number of motor units used in dominant and non-dominant arms when performing the same exercise.

Start the Biopac software and make sure the Biopac Student Lab is powered up and connected to your computer. Upon completion of this exercise, fill in the data tables and answer the exercise questions. Before concluding, be sure that you can manipulate the graphic data that is shown on the computer screen when you are reviewing saved data.

To Set Up for the Biopac EMG Lab:

1. Your computer should already be turned on but the MP30 Acquisition unit may not be turned on.
2. Plug the following hardware into the MP30 unit:

   Electrode lead (SS2 or SS2L) - into Ch 3 (Channel 3)
3. Remove any watches & bracelets from your arms. Attach 3 electrodes to the anterior side of each lower arm.

The left arm should have electrodes placed in exact mirror-image locations. For good adhesion, skin must be dry (not oily) before applying electrodes. Use alcohol wipes to remove any oils if necessary. Allow skin to dry before applying the electrodes.

Note: Do not re-use electrodes if they have been removed from skin.

4. Attach the pinch connectors of the electrode lead set (SS2L) to the electrodes—followingthe color code—to the dominant arm (see Figure 4-1).
5. Start the Biopac Student Lab program on your computer.
6. Choose lesson **L01-EMG-1** and click **OK**.
7. Type in a filename. If you already used this filename for an earlier Biopac lesson, the application will add the new lesson to your pre-existing file. After typing your filename click **OK**.
8. Open the preferences menu and change recording length to 2 minutes.

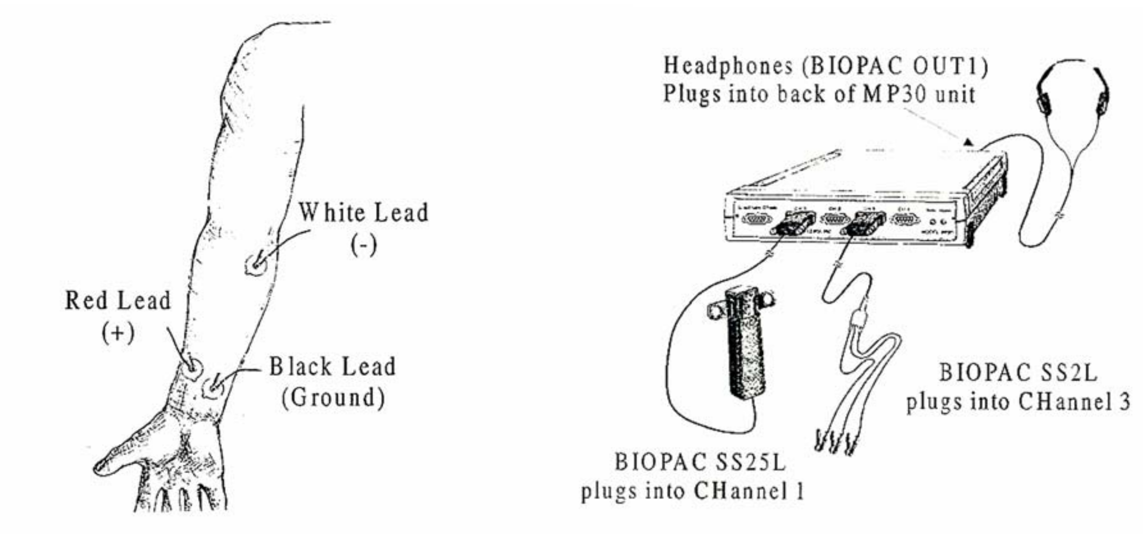


Figure 4-1. *Lead placement and Biopac hardware*

This concludes the set up procedure.

Calibration of the Biopac

To Calibrate the Biopac Lab Equipment:

1. Click **Calibrate**.
2. Read the dialog box and click **OK** when ready.
3. Wait about two seconds, and clench your fist around a tennis ball as hard as possible for about two seconds. The calibration period will stop automatically.

4. If you suspect any problems with the calibration sequence, select **Redo Calibration**.

This concludes the calibration sequence.

Data Recording

Note: The following instructions are slightly different from those printed on the computer screen.

To Record Your Dominant Arm Biopac Data:

1. You are now ready to record data from your dominant arm.
   a. Be sure that you are seated on a stool at the lab bench with your dominant arm connected, extended, and laying on top of several textbooks.
   b. Have your partner use the mouse to click **Record**.
2. The computer will begin recording electrical activity from your arm.

   Begin the exercise by clenching a ball in your fist 4 times as follows:

   - The 1st clench should be very weak
   - The 2nd stronger
   - The 3rd stronger
   - The 4th strongest

   Relax with your hand open for about 2- 3 seconds between each clench.
3. After 4 clenches of increasing strength, the computer operator should click **Suspend**.

   Your data should appear as 4 clusters of waves of increasing amplitude with baseline (relaxing) activity known as tonus, in between.

   Note: If the amplitude of the clusters exceeds the top of the graph, you should redo the calibration step.
4. Now perform a weight lifting exercise with the same arm, by performing wrist flexion, and lifting a 5 lb. Dumbell off of the table.
   a. Start out with arm extended on the books and with dumbell in hand, hanging down in a relaxed position.
   b. The computer operator should now click **Resume** and you should lift the weight by flexing your wrist; stop and hold the weight in the air for about one second then slowly lower the weight back to the resting state by extending your hand at the wrist.
   c. Rest for two seconds and repeat the procedure three more times for a total of four flexion-extensions of the wrist.

   Note: While doing the lifts, use only enough motor units to lift the weight. Don't jerk the dumbell.

5. After 3 repetitions of the exercise, click **Suspend**.

To Record Your Non-Dominant Arm Biopac Data:

1. Remove the leads from your dominant arm and transfer the pinch connectors to electrodes on your non-dominant arm.

Note: Refer to *Set Up* section for proper placement of leads, if necessary.

2. Click **Resume**.
3. Perform the same clenching exercise as you did with your dominant arm and click **Suspend** when complete.
4. Perform the weight lifting exercise with the non-dominant arm.
5. Click **Suspend**.
6. If satisfied with your data, click **Stop** (or **Done**).
   a. A dialogue box ask, **What would you like to do now?**
   b. Select **Copy to floppy or network**, and save the data to a folder on your computer.
   c. You will see a split graph.
7. 11. Print the graph (and *not* the journal) in landscape format.
8. Record data from all members of your group. *You will need to recalibrate and re-run the entire procedure for each test subject*.

Data Analysis

When your group has completed recording, each student should have a printout of their graph (or that of another student). Label each section of your graph as to the activity that was performed and on which arm (R/L). Note that tick marks (V) at the top of your graph indicate breaks (suspend-resume) between the recorded segments. In the 2 weight-lifting sections of the graph, label each flexion and extension activity of the arm (F/E).

Note: You can retrieve any subject's data by entering the **Review Saved Data** mode from the **Lessons** menu.

Use your graph to record amplitude data from the integrated EMG section of the graph.

To determine amplitude of each cluster of waves:

1. Note Channel number (CH) designations near top of graph on computer screen:
   - CH3 = Raw EMG (positive and negative electrical flow from the muscles in millivolts (mV)),
   - CH 40 = Integrated EMG (amount of work done in mV)

2. Set up one of the measurement windows near the top of the screen to read max amplitude on Channel 40 (40 max.).

   Measurement boxes: These boxes are above the marker region in the data window. Each measurement has 3 sections: channel number, measurement type, and the resulting data value. The first 2 sections are pull-down menus that are activated when you click on each box. In other words you can change channels in the first box and measurements in the second box. We will only be measuring maximum amplitude (max) today.

3. In order to perform a measurement of the data on your Integrated EMG graph, you must highlight the area to be measured.

   a. Select the I-beam tool from the boxes at the lower right of your graph (I).

   b. Click on this icon once and move your cursor into the graph; the cursor will change into the I-beam tool.

   c. Move your I-beam tool to the beginning of data region you wish to select and hold down the mouse button.

   d. Drag the mouse until the area you want to select is highlighted (darkened) and release the mouse button.

   e. Read the value from the max measurement window and record it above the corresponding area on your graph (for example, 40 max 0.3172). Three significant numbers is sufficient for recording your data (for example, 0.317).

   Note: If you experience difficulties with this process, ask your TA to demonstrate it for you.

4. Record all of your data for each flexion and extension for both arms on the printout of the graph.

   Note: If you have difficulty locating where one flexion ends and the extension action begins, you may want to use the magnifying tool to enlarge a selected/highlighted region.

   Again, if you need more explanation, ask your TA to demonstrate this process.

5. You should be able to save your data to a shared file on the server. But if not, remember where you saved the data on your computer hard drive.

6. Calculate the mean maximum amplitude of each of the 3 flexions and 3 extensions performed with the dumbell. Record the data the table below.

Data Collection

Table 4-2. *Fist Clenching*

Clench #	Dominant Arm (Max)	Non-Dominant Arm (Max)
1 (25%)		
2 (50%)		
3 (75%)		
4 (100%)		

Table 4-3. *Lifting (5 lb, 10 lb)*

Lifting	Dominant Arm (Max)	Non-Dominant Arm (Max)
1		
2		
3		
4		

Flexion mean for dominant arm:	
Extension mean for dominant arm:	
Flexion mean for non-dominant arm:	
Extension mean for non-dominant arm:	
Flexion difference (%) between 2 arms:	

Part II: Introduction

Recall that skeletal muscle fibers are long and cylindrical, and that each cell has a place on its sarcolemma (cell membrane) where the axon terminal of a motor neuron communicates with it at a synapse called the neuromuscular (myoneural) junction. Every skeletal muscle fiber communicates with only one motor neuron, but each motor neuron has branches, axon collaterals, so that each motor neuron communicates with several muscle fibers. A motor neuron and all of the muscle fibers that it stimulates are called a motor unit.

When a single stimulus is applied to a muscle cell (or muscle) a single contraction called a muscle twitch is elicited. The size and speed of the muscle twitch is always the same for a given muscle. Yet all muscles can contract to various levels of tension (that is, a graded response). Graded response of muscles is accomplished by varying the number of muscle fibers contracting and/or the frequency with which they are stimulated.

The number of muscle fibers in a motor unit depends on the size of the muscle and how precisely its contraction must be regulated. Small muscles requiring precise control of contraction, such as those of the fingers, have very small motor units; while large muscles with little need for precise control of contraction, such as those of the back, have large motor units. At any given time, a few of the motor units in a muscle are being stimulated to contract. This causes a slight tension in a muscle known as tonus. Tonus maintains muscles in a state of readiness for contraction, but also is essential for viability of muscle cells. Persons with spinal cord injury, or neurological disease that eliminates the ability of motor neurons to create tonus suffer muscle atrophy, the degeneration and loss of muscle tissue.

Varying the strength of muscle contraction, graded response, is accomplished by regulating the number of motor units contracting, and/or the number of contractions motor units make in a given time interval (frequency). Increasing the number of motor units contracting increases the strength of motor contraction (tension) by a phenomenon called multiple motor unit recruitment (MMUR); while increasing the frequency of muscle contractions increases the strength of muscle contraction by a phenomenon called temporal summation. When the frequency of muscle contraction allows partial relaxation between contractions it is referred to as wave summation or partial (incomplete) tetanus. When frequency of muscle contraction is sufficiently fast to allow no relaxation between contractions, a form of temporal summation known as fused (or complete) tetanus occurs.

Remember, skeletal muscle cells must always be stimulated to contract by a nerve impulse from a motor neuron. This nerve impulse causes neurotransmitter (acetylcholine) to be released at the neuromuscular junction. Binding of the acetylcholine to receptors of the motor end plate of the muscle fiber causes a wave of depolarization to spread over the sarcolemma and into the muscle fiber along its T-tubules. It is this *excitation* (that is, action potential), that causes the muscle fiber to contract. This process is known as *excitation/contraction coupling*. We cannot measure the time it takes between the arrival of the nerve impulse and the actual contraction of the muscle (that is, latency).

Lab Meeting #2 Procedure (Part II)

Setup

To Prepare for the EMG Lab:

1. Your computer should already be turned on but the MP30 Acquisition unit may not be turned on.
2. Plug the following hardware into the MP30 unit:

   Electrode lead (SS2 or SS2L) - into Ch 3 (Channel 3)
3. Remove any watches & bracelets from your arms. Attach 3 electrodes to the anterior side of each lower arm.

The left arm should have electrodes placed in exact mirror-image locations. For good adhesion, skin must be dry (not oily) before applying electrodes. Use alcohol wipes to remove any oils if necessary. Allow skin to dry before applying the electrodes.

Note: Do not re-use electrodes if they have been removed from skin.

4. Attach the pinch connectors of the electrode lead set (SS2L) to the electrodes—followingthe color code—to the dominant arm (see Figure 4-1.
5. Start the Biopac Student Lab program on the computer.
6. Ensure EMG lesson 2 is running. To start lesson 2, select **L02-EMG-2** from the lesson menu and click **OK**.
7. **Type in** a unique file name. Click **OK**.

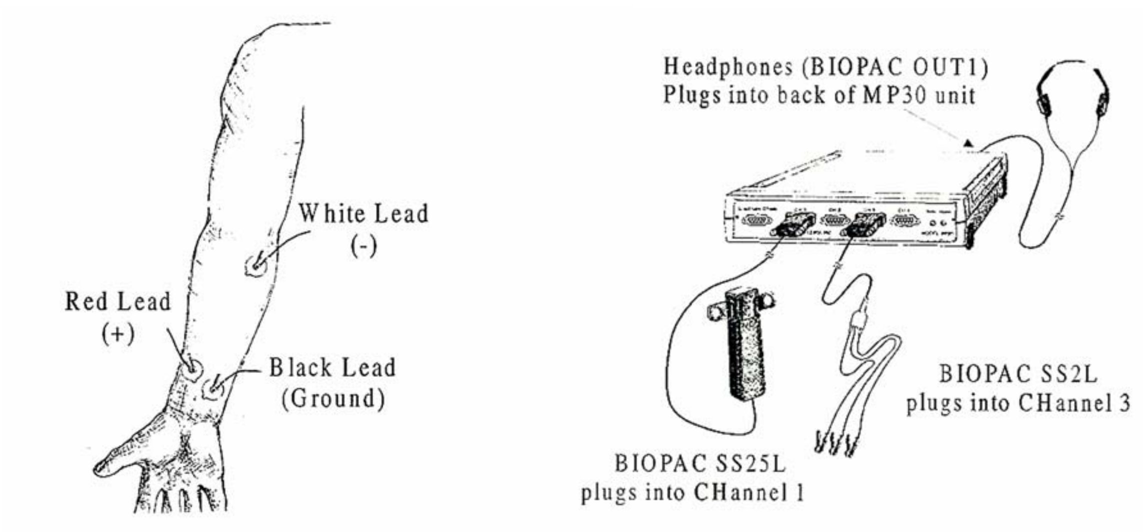


Figure 4-2. *Lead placement and Biopac hardware*

Calibration

To Calibrate Your Equipment:

1. Click **Calibrate** to begin the calibration.
2. Provide the computer a zero force measurement. To measure zero force, set the object that you will clench down on the bench top and let your hand relax. Read the dialogue box and click on **OK** when ready.
3. Provide the computer with a maximum force reading. Wait about two seconds, clench an object as hard as possible, then release. Note your maximum clench force.

4. Wait for the calibration measurement to stop. It takes about 8 seconds for the program to run its calibration procedure. At the end of this 8 seconds your computer screen should resemble Figure 4-3.
5. Make sure the baseline started at zero. If not, repeat the calibration.

When successful calibration is completed, proceed to the next step.

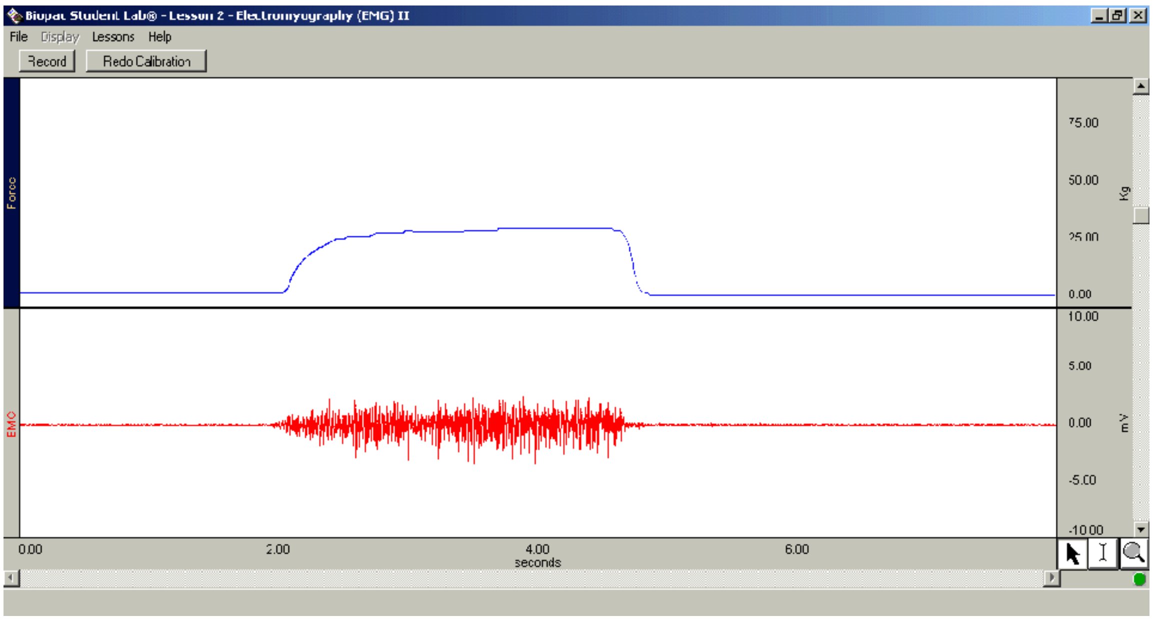


Figure 4-3. *Successful Calibration Screen*

Recording Lesson Data

Each Biopac lab has a set of on-screen instructions that guide you through the experiment.

In this lesson you will record data for your dominant arm, **Forearm 1**, (right arm if you are right handed, left arm if you are left handed) and then your non-dominant arm, **Forearm 2**. The Biopac program will prompt you to switch electrodes from the dominant to the non-dominant arm at the appropriate time. The data for each arm consists of two Procedures. The first Procedure records **Multiple Motor Unit Recruitment (MMUR)**. The second segment records **Muscle Fatigue**. Thus you will record data for four procedures in total.

In the first procedure for the dominant arm you will clench (squeeze) an object four times, increasing the strength of each clench from light to maximum force, in two second intervals. Thus your first clench strength should increase in equal increments of approximately 25%, 50%, 75% and 100% of your maximum clench strength. Observe the clench strengths as you record them in the graph and try to hold each clench steady in order to generate a two second plateau for each clench.

In the second procedure for the dominant arm you will clench an object as hard as you can until the force you can exert decreases by at least 50% as indicated by the computer tracing. This will demonstrate muscle fatigue. (You may also listen to your EMG.)

The third and fourth procedures are the similar recordings for the non-dominant arm.

To Record Forearm 1 (Dominant Arm) Multiple Motor Unit Recruitment:

1. To begin data recording for the first segment click **Record**.
2. Clench (2 seconds) – Release – Wait (2 seconds) and repeat with increasing force four times.
3. Click **Suspend**.
4. Review the data on the screen. Your data recording should resemble Figure 4-4.
5. If your data are good, click **Resume**. If not, click **Redo**, and repeat the procedure.

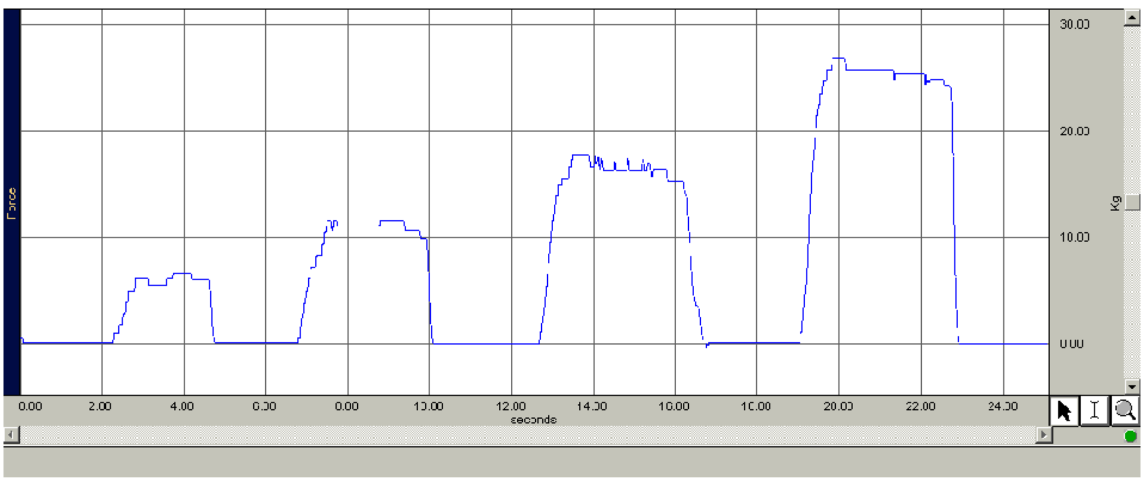


Figure 4-4. *Dominant arm multiple motor unit recruitment graph*

To Record Forearm 1 (Dominant Arm) Muscle Fatique:

1. Click **Resume** while clenching an object as hard as you can. Hold the clench as long as you can.
2. When the force you exert declines by more than 50%, click **Suspend**.
3. Your data recording should should now resemble Figure 4-5.
4. When you click **Suspend** after completing the muscle fatigue procedure for the dominant arm, a dialogue box will appear prompting you to switch the electrode connections to the non-dominant arm. When you have switched the electrodes to the non-dominant arm, click **ok** to close the dialogue box and continue on with the lab procedures.

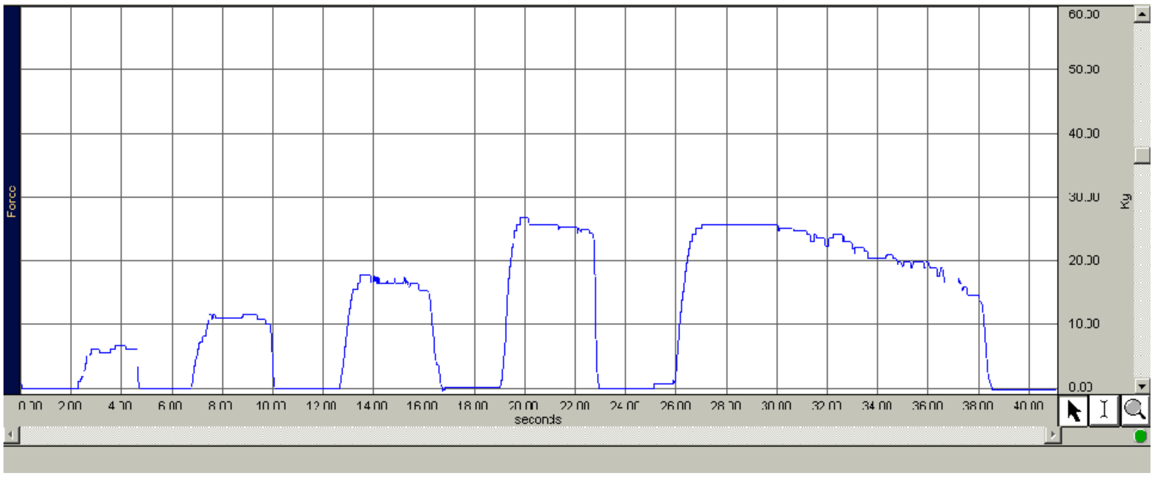


Figure 4-5. *Forearm 1 (dominant arm) muscle fatique graph*

To Record Forearm 2 (non-dominant arm) Multiple Motor Unit Recruitment and Muscle Fatique:

1. Click **Resume** and repeat all the steps you performed with your dominent arm.
2. When finished, you will have completed 4 procedures and your data graph should resemble Figure 4-6.

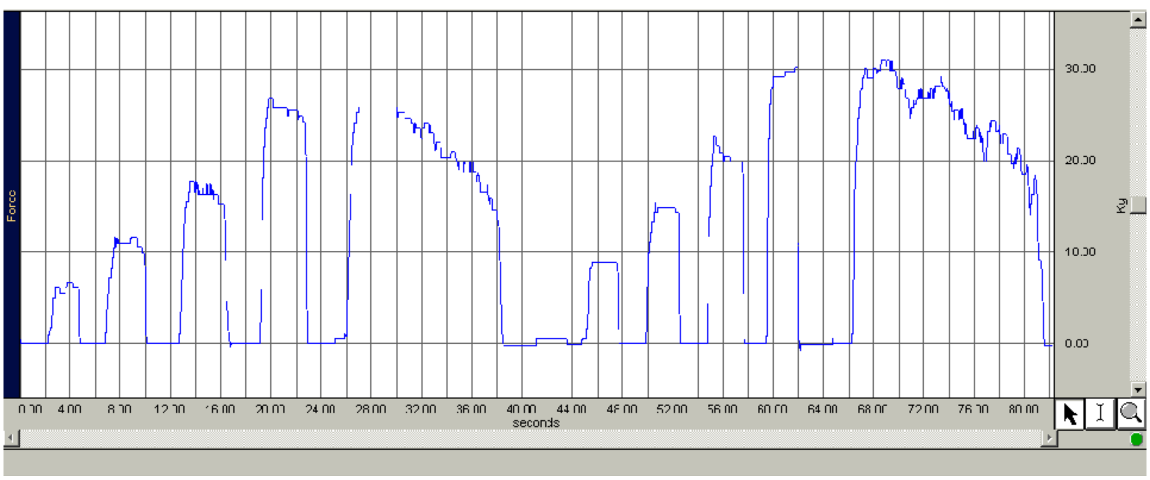


Figure 4-6. *Finished multiple motor unit recruitment and muscle fatique graph*

3. Once you obtain good results type **Stop**.
4. You will be prompted with the question: "Are you finished with both forearm recordings? Pressing **Yes** will automatically save the file and you will not be able to redo."

5. You will be presented with a graph of clench force for both arms, along with two new graphs: EMG and Integrated EMG. Your graph window will look like Figure 4-7.

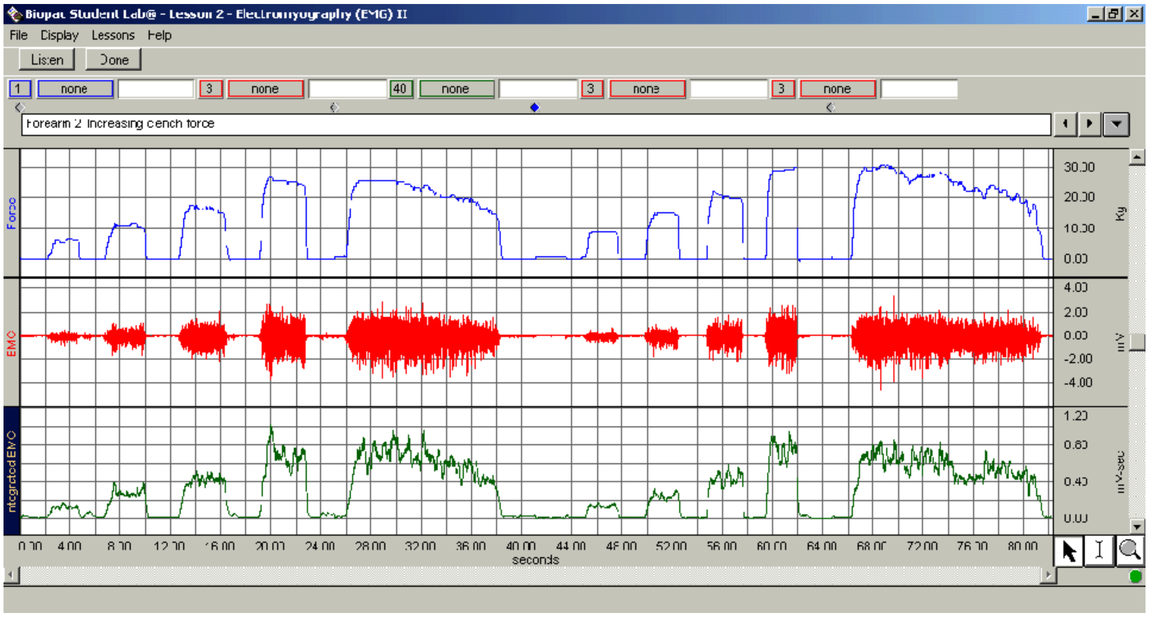


Figure 4-7. *Force, EMG, and Integrated EMG graph*

6. You are also presented with a **Listen** button to hear the EMG data you recorded (optional), and a **Done** button to finish the session.
7. When you click on **Done**, a dialogue box titled **what would you like to do now** will appear. You may choose **Record from another subject** or you may choose **Analyze current data**. Remember, all the data you collected are automatically saved in the file folder you named and you can call it up at any time to complete the analysis.
8. Because of time constraints it is usually best to go ahead and record the data for all subjects in a group and analyze the saved data later.
9. To analyze saved data Choose **Analyze another data file** from the **what would you like to do now** dialogue box, or select the **Lessons** button, and choose **Review Saved Data**.

Data Analysis

Select **Analyze current data** from the **what would you like to do now** menu if you wish to proceed directly to analyze the data you just collected.

If you wish to retrieve saved data to analyze that you collected in an earlier data or laboratory session enter "**Review Saved Data"** from the **Lessons** Menu. Click on the "**Data Lessons**" folder where the data files are stored. The data will be saved in a file with the name you provided at the outset, but with a file extension of "**-L02**". When

you open your data file, notice that a plot of the data you recorded appears in channel 1, along with a plot of the EMG in channel 2 and the plot of the Integrated EMG in channel 3, as in the above screen image.

Before doing any analysis, select **File > Print > Print Graph > Setup > Landscape** to print a hard copy of your data to attach to your lab report.

Near the top of the screen, above the plots, are three measurement boxes. Use the mouse and the drop down menu for each data channel to set the measurement boxes as follows:

CH 1	**mean**	**CH 3**	**p-p**	**CH 40 mean**

Note: You can expand any portion of the graph to better observe it by selecting the magnifying tool at the lower right of the graph screen. Any time you resize the screen with this tool it is a good idea to select **autoscale waveform** from the **Display** menu. This resizes the graphs to optimal dimensions. To return to the original graph screen select **autoscale horizontal** from the **Display** menu.

To Analyze Your Dominant Arm Data:

1. Position the mouse cursor in the **Forearm 1 (dominant)** section of the Journal and click to position the data you will subsequently enter in the Journal.
2. Then, using the mouse, Click the **I-beam** cursor button (at the lower right of the data plot), and select an area on the plateau phase of the first clench.
3. Record this measurement in your Data Journal by selecting **Edit > Journal > Paste Measurements**.
4. Repeat this procedure for each of the three remaining clenches of the dominant arm.
5. Annotate the values you record extensively to construct an organized table so you will be able to understand them when you refer to them later.

   In the example below, the italicized text illustrate the annotations you should make to document your journal:

To Analyze Your Non-Dominant Arm Data:

1. Position the mouse cursor in the **Forearm 2 (non-dominant)** portion of the data Journal.
2. Scroll to the non-dominant arm portion of the data graph and measure its four clenches in the same manner as above.
3. Record and annotate the measurements of the four clenches of the non-dominant arm in segment 3 of the data journal just as you did for the dominant arm.

4. Now record the measurements related to the fatigue data. Set the channel measurements as follows:

   CH 1 value **Channel 40** **delta T**

5. Position the mouse cursor in **Forearm 1 (dominant arm)** portion of your Data Journal.
6. Position the **I beam** cursor (at the lower right of the data plot) on the beginning of the clench recording where maximum clench is occurring for the dominant arm.
7. Paste this measurement into **Forearm 1 (dominant arm)** of your Data Journal by selecting **Edit > Journal > Paste Measurements**. This will record a single value for the maximum clench force. Because you did not highlight a range of the recorded data, there will be no value recorded for delta T.
8. Then select an area starting with the maximum clench force and ending at 50% of the maximum clench force.
9. Record this measurement by selecting **Edit > Journal > Paste Measurements**. This will record the final (50% of maximum) clench force and the time (delta T) it took for this much fatigue to occur.

Your annotated fatigue data will look like this:

Second procedure Forearm 1 (dominant):

Continued maximal clench until fatigue causes 50% reduction in measured force.

	Clench Force	clench time to 50% of max
Start	Value(1)= ____________ Kg	deltaT(40) = N/A
End (fatigue)	Value(1)= = __________ Kg	deltaT(40) = ___________ sec

Position the mouse cursor in **Forearm 2 (non-dominant arm)** of your Data Journal and repeat the above measurement and paste procedures for the other arm. Annotate the data as above.

Select **File > Print > Print Journal** to print a hard copy of your data, your journal log, to attach to your lab report.

Biopac will save your data in the folder with your name for future reference if you tell it to do so upon exiting the program. You may review and/or analyze this saved data at any time. To open your folder at any time you must use the computer in which the data were recorded. Start the Biopac program and open the **Lessons Menu**. Select **Review Saved Data**. Select your folder.

Data Collection

Table 4-4. *Dynamometer Clenching*

	Dominant Arm			Non-Dominant Arm		
Clench #	Mean Force	P-PEMG	Mean EMG Integrated	Mean Force	P-PEMG	Mean EMG Integrated
1 (25%)						
2 (50%)						
3 (75%)						
4 (100%)						

Fatigue (Dominant, Non-Dominant)	
Start: Force	
End: Force	
ΔT	

Discussion

1. Compare the mean measurement for the right and left maximum clench EMG cluster. Are they the same or different?

   Which one suggests the greater clench strength?

2. What factors in addition to sex contribute to observed differences in clench strength?
3. Does there appear to be any difference in tonus between the two forearm clench muscles?

   Would you expect to see a difference? Does subject's sex influence your expectations? Explain your answer.

4. Is there a difference in the absolute values of force generated by males and females in your class?

   What might explain any difference?

5. When holding an object, does the number of motor units remain the same?

   Are the same motor units used for the duration of holding the object?

6. As you fatigue, the force exerted by your muscles decreases. What physiological processes explain the decline in strength?
7. Define *motor unit*.
8. Define *fatigue*.
9. Did your dominant or non-dominant arm perform more work when lifting the same weight?
10. What is the visible difference between the *Raw EMG* and the *Integrated EMG*?
11. Define skeletal muscle tonus and indicate where you could see it in your graph.
12. During the weight lifting portion of the exercise, why did you see differences in the mean amplitude of the muscle voltage between left and right arms?

    What factors might contribute to these differences and those found among different test subjects?
13. What tissue is producing the electrical signals detected by the EMG electrodes?
14. What does the term *motor unit recruitment* mean?
15. Define *electromyography*.

Lab Report Assignment

Attach the data plot you printed out at the beginning of the Data Analysis and the journal log you printed at the end of the Data Analysis.

Mark and label each region on your data plot printout where each of the data measurements recorded in the Journal log occurred. The labels to use are: **MMUR 1-4** (Multiple Motor Unit Recruitment) and **MFat** (Muscle Fatigue) for each arm.

LAB MEETING #3: ELECTROOCULOGRAM (EOG)

During this lab session we will measure eye movement saccades and fixation during reading.

Introduction

What do these three things have in common?

- Two young lovers whose eyes meet across a crowded room
- The final scene of *Casablanca*
- A Michael Chrichton novel

Regardless of what else they have in common, all involve the muscular control of eye movements in your field of view. One of the most important functions your eyes can perform is to “fix” or “lock” on specific objects. When you “fix” on an object, you

position your eyes to that the image of the object is projected onto your retina at the area of greatest acuity, the fovea. Muscular control of your eyes works to keep the image on your fovea, regardless of whether the object is stationary or moving.

There are two primary mechanisms used to fixate on objects in your visual field, defined as the field of view without moving your head:

- *Voluntary fixation mechanism*: Voluntary fixation allows you to direct your visual attention and lock onto the selected object.
- *Involuntary fixation mechanism*: Involuntary fixation allows you to keep a selected object in your visual field once it has been found.

In voluntary types of eye movements, you can fixate on another person from across a crowded room. Voluntary fixation involves a conscious effort to move the eyes. This mechanism is used to initially select objects in your visual field, and once selected, your brain passes the task on to involuntary fixation.

Even when you fixate on a stationary object, your eyes are not still but exhibit tiny, involuntary movements. There are three types of involuntary movements: tremors, slow drifts, and flicking.

- *Tremors*: a series of small tremors of the eyes at about 3080 Hz (cycles/sec).
- *Slow drifts*: involuntary movements that result in drifting movements of the eyes. This drift means that even if an object is stationary, the image drifts across the fovea.
- *Flicking movements*: as the image drifts to the edge of the fovea, the third involuntary mechanism causes a reflex flicking of the eyeball so that the image is once again projected onto the fovea.

The drifting movements and flicking movements will be in opposite directions. If the drifting movement is to the left, the flicking movement will be to the right, although it may not be 180° opposite of the drifting movement.

When you wish to follow a moving object, you use large slow movements or tracking movements. So, as you watch Humphrey Bogart walk away during the final scene of Casablanca, your eyes are following an apparently smooth motion and tracking an object in your visual field. Although you have voluntarily directed your eyes to Humphrey Bogart, tracking movements are involuntary.

Another set of motions is used when you read or when objects are streaming past you, (for example, when you watch the world go by while riding in a train). Rather than a smooth tracking motion, reading usually involves voluntary, larger movements known as saccades, or fixating on a series of points in rapid succession. When this happens, your eye jumps from point to point at a rate of about three jumps per second. During the jumps or saccades, the brain suppresses visual images, so you don't *see* the transitional images between the fixation points.

Typically, the eyes will spend about 10% of the time moving from fixation point to fixation point, with the other 90% of the time fixating on words, although there is much variation.

Eye movement can be recorded as an electrooculogram, a recording of changes in voltage that occur with eye position. Electrically the eye is a spherical “battery,” with the positive terminal in front at the cornea, and the negative terminal behind at the retina of the eyeball. The potential between the front and back of the eyeball is about 0.4 to 1.0mV.

By placing electrodes on either side of the eye you can measure eye movement up to ±70°, where 0° is in front and ±90° is directly lateral or vertical to the eyes. The electrodes measure the changes in potential as the cornea moves nearer or further from the recording electrodes. When the eye is looking straight ahead, it is about the same distance from either electrode, so the signal is essentially zero. When the front of the eyeball, the cornea, is closer to the positive electrode, that electrode records a positive difference in voltage.

Experimental Objectives

- Compare eye movements while fixated on a stationary object and tracking objects.
- Measure duration of saccades and fixation during reading.
- Record spatial position of eye movements during visual examination of materials.

Materials

- BIOPAC electrode lead set (SS2L), Qty2
- BIOPAC disposable vinyl electrodes (EL503), 6 electrodes per subject
- BIOPAC acquisition unit (MP 35)
- Adhesive Tape

Lab Meeting #3 Procedure

To Set Up for the Lab Session:

1. Turn the computer ON.

   The desktop should appear on the monitor. If it does not appear ask the TA for assistance.

2. Make sure the BIOPAC MP35 unit is turned OFF
3. Plug the electrode leads (SS2L) in as follows:
   - Horizontal lead – Ch 1
   - Vertical lead – Ch 2

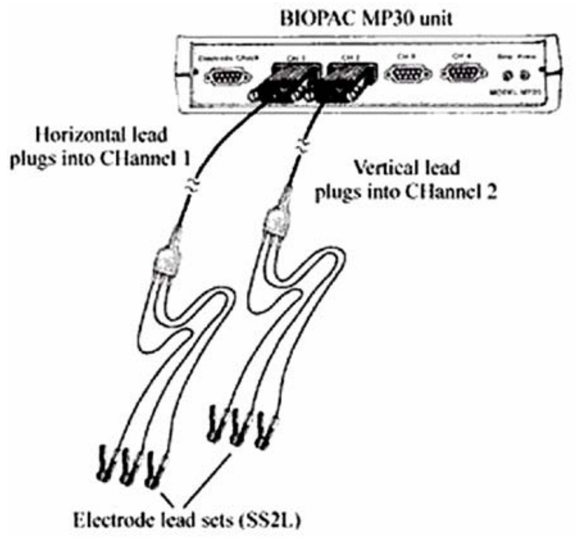


Figure 4-8. *MP30 unit and leads*

4. Turn on the MP35 Data Acquisition Unit
5. Place 6 electrodes on the subject (see Figure 4-9).

   Note: For accurate recordings, attach the electrodes so they are horizontally and vertically aligned.

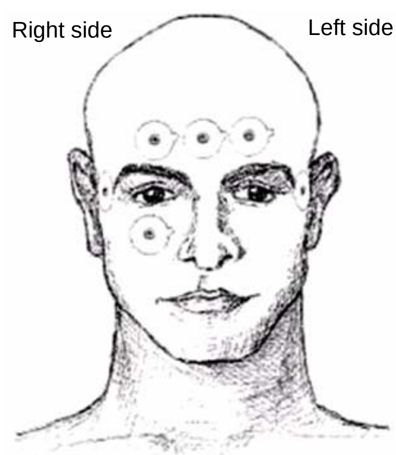


Figure 4-9. *proper electrode placement*

6. Attach the vertical electrode lead set (SS2L) from Channel 2 to the electrodes:
   a. Attach one electrode above the right eye and one below, such that they are aligned vertically.
   b. Attach one electrode to the right of the right eye and one to the left of the left eye, such that they are aligned horizontally.

c. The other two electrodes are for ground, and it is not critical that they be aligned.

d. For optimal electrode adhesion, the electrodes should be place on the skin at least five minutes before the start of the Calibration procedure.

Note: Because these electrodes are attached near the eye, be very careful if using alcohol to clean the skin.

e. Check your work against Figure 4-10 to ensure that you connect each lead to the proper electrode. Run the electrode leads run behind the subject's ears, as shown, to provide a cable strain relief

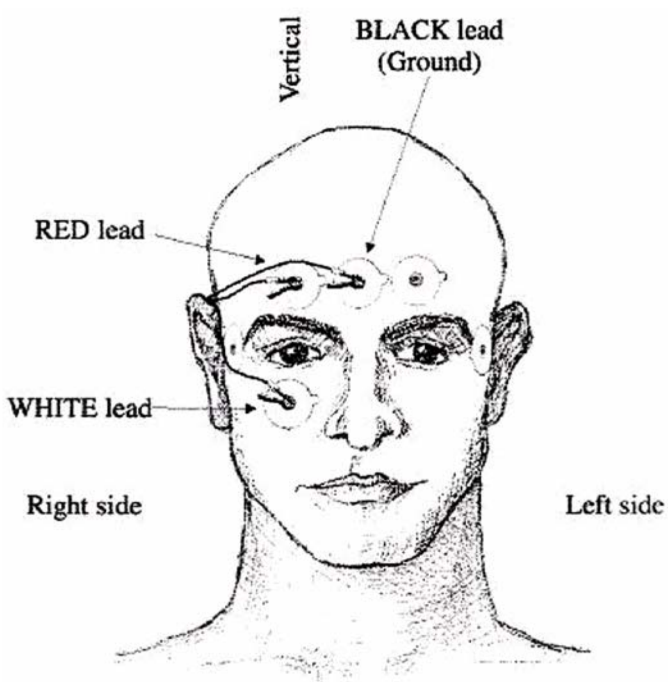


Figure 4-10. *Lead placement for channel 2 (vertical)*

7. Attach the horizontal electrode lead set (SS2L) from Channel 1 to the electrodes as shown in Figure 4-11.

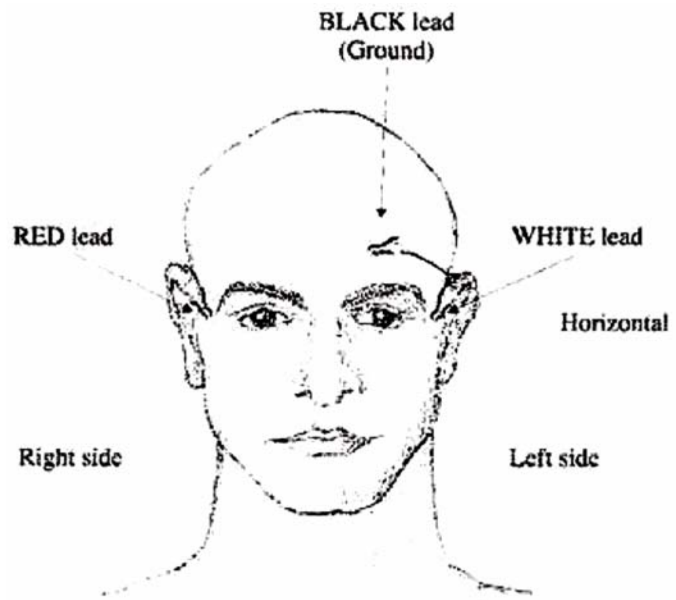


Figure 4-11. *Lead placement for channel 1 (horizontal)*

8. Have the subject adjust the seating position such that his or her eyes are in line with the center of the computer screen.

   The subject should be positioned to see the computer screen easily without moving his/her head. Supporting the head to minimize movement is recommended. The subject should not be in contact with nearby metal objects (faucets, pipes, and so on), and should remove any wrist or ankle bracelets.

   Connect the electrode cable clip (where the cable meets the three individual colored wires) to a convenient location (can be on the Subject's clothes). This will relieve cable strain.

9. Note the distance from the eyes to the computer screen.
10. Start the BIOPAC Student Lab Program
11. Choose lesson **L10EOG1**.
12. Type in your filename
13. Click OK

This concludes the lab set up.

Calibration

The calibration procedure establishes the hardware’s internal parameters (such as gain, offset, and scaling) and is critical for optimum performance. Pay close attention to the Calibration procedure.

To Calibrate Your Set Up:

1. Make sure the subject is seated in the same position as directed in Set Up.

   It is very important that the subject does not move his or her head during the calibration procedure.

2. Click **Calibrate**.
3. Click **OK**.

   After the **OK** button is pressed, a dot will begin a counterclockwise rotation around the screen.

4. The subject should follow the dot on the screen with their eyes while holding their head as still as possible. The procedure will continue for about 10 seconds and then stop automatically.
5. Check the calibration data. There should be fluctuation in the data for each channel, and the data display should resemble Figure 4-12.
   - If similar, proceed to data recording.
   - If different, redo calibration.

   Note: If the subject did not follow the dot on the screen or blinked, giving large data spikes or jitter, or if an electrode was disconnected, giving large baseline drift, then you should redo the calibration by clicking on the **Redo Calibration** button and repeating the entire calibration sequence.

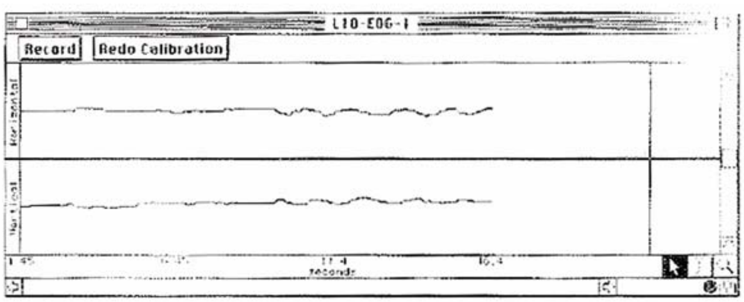


Figure 4-12. *Calibrated data display*

Recording Data

To Prepare for Recording Data:

1. Prepare for the recording.

   In order to work efficiently, read this entire section so you will know what to do before recording.

   Check the last line of the journal and note the total amount of time available for the recording. Stop each recording segment as soon as possible so you don't use an excessive amount of time (time is memory).

Hints for obtaining optimal data:

- Always track the object with you eyes and not your head
- Subject should focus on one point of the object, and maintain that focus while following it around.
- The subject needs to sit so that head movement is minimized during recording.
- There should be enough space near the subject so that an object can be moved around the head at a distance of about 10” (25 cm).
- When moving the object, the director should try to keep it at the same distance from the subject’s head.
- During recording, the subject should not blink. If unavoidable, the recorder should mark the data.
- Make sure the electrodes do not come loose.
- The larger the monitor, the better the data results from the eye tracking portion of this lesson.

2. The subject and director face each other.

   Subject should not be looking at the computer screen

3. The director holds a pen in front of the subject.

   The director should hold a pen in front of the subject’s head at a distance of about 10” (25 cm). The pen should be centered with the subject’s head, so that the subject’s eyes are looking straight ahead.

4. The subject picks a focal point on the pen so that the eyes remain horizontal.

   The subject’s eyes should not move up or down. Ideally, they will only move laterally to follow the object.

To Record Segment I Data:

1. Click **Record**.

   When you click Record, the recording will begin and an append marker labeled “Eyes tracking horizontal” will automatically be inserted.

2. Record for 20 seconds.

   - The subject should fixate and track the object. The subject should try not to blink during recording—though it may be unavoidable.
   - The director should hold object 10” (25 cm) in front of subject for about 5 seconds, then move object laterally to subject’s left, then right, then back to center ($\pm 70^{o}$) in about 3 seconds.
   - The recorder inserts event markers with each change of direction:
     - **L** for left
     - **R** for right

To insert Markers, press the **F9** key. Markers and labels can be edited after the data is recorded.

Note: The director should verbalize the directions so that the recorder knows when to place markers and what direction to label them.

3. Click **Suspend**.

   The recording will halt, and you can review the data. If all went well, your data should look similar to Figure 4-13.

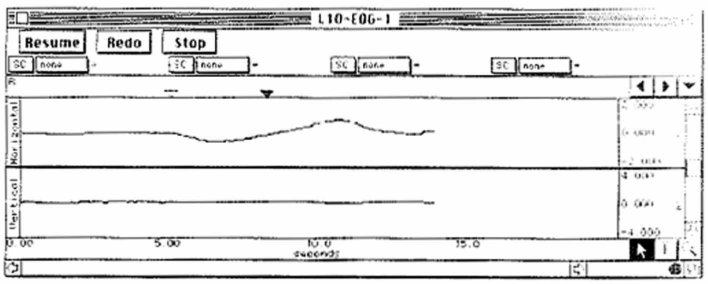


Figure 4-13. *End of segment I (horizontal tracking)*

4. Review the data on the screen.
   - Correct data would:
     - Show that the horizontal EOG (CH1) had large deflections, and the vertical EOG (CH2) had very little deflection.
     - b) Show a positive peak when Subject looked right and a negative peak when the Subject looked left.
   - Incorrect data could result if:
     - Channel connections were incorrect
     - Lead connections were incorrect (for example, the red lead was not connected to the subject's right temple).
     - The suspend button was pressed prematurely
     - An electrode came loose, giving a large baseline drift.
     - The subject looked away or the head moved

   Note: A few blinks may be unavoidable in the data and would not necessitate redoing the recording.

5. If data was incorrect, click **Redo**, otherwise continue on to record segment II.

   Note: Once you press **Redo**, the data you have just recorded is erased.

To Record Segment II Data:

1. The subject and director face one another. the subject should not look at the computer screen.

2. The director holds a pen in front of the subject.

   The director should hold a pen in front of the subject's head at a distance of about 10" (25 cm). The pen should be centered with the subject's head, so that the subject's eyes are looking straight ahead.

3. Subject should pick a focal point on the pen so that the eyes are looking straight ahead (not up or down).

   The subject may need to blink before resuming recording

4. Click **Resume**.

5. When you click **Resume**, the recording will continue and an append marker labeled "Eyes tracking vertically" will be automatically inserted.

6. Record for 20 seconds.

   - The subject should follow the object with his/her eyes without moving her/her head. The subject should try not to blink during the recording—although it may be unavoidable.
   - The director should hold object stationary and center, then move object up, down, and return to center. The director should begin with object about 10" (25 cm) in front of the subject for 5 seconds, and move the object vertically to the edge of the subject's field of vision.
   - Recorder should insert event markers:
     - **U** = Object moved up
     - **D** = Object moved down

     To insert Markers, press the F9 key. Markers and labels can be edited after the data is recorded.

7. Click **Suspend**.

   The recording should halt, giving you time to review the data. If all went well, your data should look similar to Figure 4-14.

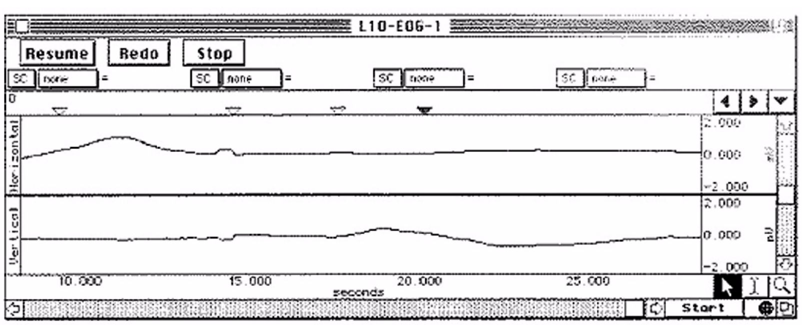


Figure 4-14. *Segment 2 (vertical tracking)*

8. Review the data on the screen. Your data should:

- Show that the vertical EOG (Ch 2) recording had large deflections, and the horizontal EOG (Ch 1) had very little deflection.
- Show a positive peak when subject looked up and a negative peak when the subject looked down.
- Incorrect data could result if:
  - Channel connections were incorrect
  - Lead connections were incorrect (for example, the red lead was not connected to the subject's right temple).
  - The suspend button was pressed prematurely
  - An electrode came loose, giving a large baseline drift.
  - The subject looked away or the head moved

Note: A few blinks may be unavoidable in the data and would not necessitate redoing the recording.

9. If data was incorrect, click **Redo**, otherwise continue on to record segment III.

To Record Segment III Data:

1. The director selects reading and positions it in front of subject. Hold the page in front of the subject at a distance of about 10" (25 cm), centered within his or her line of sight.
2. The subject prepares to read.
3. Click **Resume**.

   When you click **Resume**, the recording will continue and an append marker labeled "Reading" will be automatically be inserted.
4. The subject reads for 20 seconds. The subject should read silently to minimize EMG artifact.
5. *Optional:* Recorder can insert a marker when subject starts reading each line. The subject may signal the recorder (verbally or with hand signals) when starting each new line and the recorder can insert event markers.
6. Click **Suspend**.

   The recording halts, giving you time to review the data. If all went well, your data should look similar to Figure 4-15.

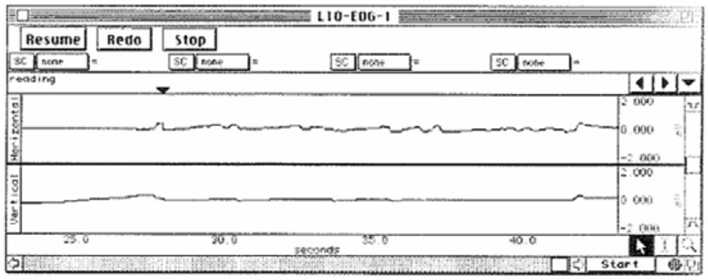


Figure 4-15. *Segment III (reading)*

7. Review the data on the screen

   ▸ Incorrect data could result if:

   - Channel connections were incorrect
   - Lead connections were incorrect (for example, the red lead was not connected to the subject's right temple).
   - The suspend button was pressed prematurely
   - An electrode came loose, giving a large baseline drift.
   - The subject looked away or the head moved

   Note: A few blinks may be unavoidable in the data and would not necessitate redoing the recording.

8. Click **Stop**.

   When you click **Stop**, a dialog box comes up, asking if you are sure you want to stop the recording. Clicking **yes** will end the data recording and automatically save the data. Clicking **no** will bring you back to the *Resume* or *Stop* options. This is simply one last chance to confirm you don't need to redo the last recording segment.

To Record Segment IV Data (Tracking Demonstration):

Note: This part of the recording is for general interest only and data will not be saved. It shows how well the EOG can be used as an eye-tracking device.

1. Reposition the subject as in the set up procedures.
2. Click **DOT PLOT**.

   A new window will appear, similar to the calibration window.

3. The subject should focus on the center of the screen and move his or her head until the dot is also at the center of the screen. Maintain this position throughout the DOT PLOT segment.
4. The subject looks at different points within the data window while holding their head motionless. Note that because of amplifier limitations, the dot can not hold its position for very long and will tend to move toward the center point.

5. Click **Stop**.

   When you click **Stop**, the screen will change to simultaneously display all the dot positions from the last 30 seconds of the dot plot. This segment of data will not be saved.

6. Click **Done**.

   After you press **Done**, a pop-up window with options will appear. Make your choice and continue.

   Note: You may redo the DOT PLOT by clicking **Redo**.

7. Remove the electrodes.

8. If you choose the “Record from another Subject” option:

   a. Attach electrodes per the Set Up procedure and continue the lab

   b. **Each person will need to use a unique file name**.

EOG Data Report

Student’s Name: ____

Lab Section:________________

Date:_________________

Data and Calculations

Subject profile

Name_______________________ Height______________

Age_________________________ Weight______________

Gender: Male / Female

Complete Table 4-5 using Segment 1 data. Be careful to be consistent with units (msec vs secs).

Note: You only need to select one example of a flicking movement

Table 4-5. *Segment 1 Data of Eye Orientation with Object Movement*

Object Position Object Orientation	Stationary Object Fixation	Moving Object Tracking		
Measurement (Ch#)	Flicking	Left	Right	Left
Δ**T** Ch 40				

Table 4-5. *Segment 1 Data of Eye Orientation with Object Movement*

Object Position Object Orientation	Stationary Object Fixation	Moving Object Tracking		
Measurement (Ch#)	Flicking	Left	Right	Left
p-p Ch 40				
Slope Ch 40				

Note: Velocity may be represented with a negative ("-") value because velocity vectors have a magnitude and direction.

Complete Table 4-6 using Segment 1 data.

Table 4-6. *Flicking Movements*

Time	Number of Flicking Movements
0 to 1 seconds	
1 to 2 seconds	
2 to 3 seconds	
3 to 4 seconds	

Complete Table 4-7 using Segment 2 data.

Note: You only need to select one example of a flicking movement.

Table 4-7. *Segment 2 Data of Eye Orientation with Object Movement*

Object Position Object Orientation	Stationary Object Fixation	Moving Object Tracking		
Measurement (Ch#)	Flicking	Left	Right	Left
ΔT Ch 41				
p-p Ch 41				
Slope Ch 41				

Complete Table 4-8 with Segment 3 data.

Note: You may not have seven saccades per line.

Table 4-8. *Segment 3 Reading data*

Time	First Line	Second Line
Measurement		
Number of saccades		

Table 4-8. *Segment 3 Reading data*

Time	First Line	Second Line
Duration of saccade	#1__________ #2__________ #3__________ #4__________ #5__________ #6__________ #7__________	#1__________ #2__________ #3__________ #4__________ #5__________ #6__________ #7__________
Total duration of saccades/ line		
Total reading time/line		
% time of saccades/total reading time		

Questions

- Refer to Table 4-5 data and compare duration (T), relative changes in eye position (Δ), and speed of eye movement (slope) of flicking and tracking movements.
- What is the stimulus for reflex flicking movements?
- Refer to Table 4-7 data and compare duration (T), relative changes in eye position (Δ), and speed of eye movement (slope) of flicking and tracking movements.
- Refer to Table 4-7 data and compare your results with the other two groups in your class. What is the range of variation in% time of saccades per line?
- Describe three types of involuntary movements during fixation on a stationary object.
- Explain how an *electrooculogram* is recorded.
- Define *visual field*.
- Define *saccade*.

LAB MEETING #4: GONIOMETERS AND EMG

Goniometers are devices capable of transforming angular position into a proportional electrical signal. They incorporate gauge elements that measure bending strain along or around a particular axis. The bending strain is proportional to the sum total angular shift along the axis. Because the bending force is extremely small, the output signal is uniquely a proportional function of the angular shift. The twin-axis goniometers used in this study are dual output devices that can simultaneously measure angular rotation about two orthogonal planes.

A goniometer for humans is used to determine the position of a moveable joint and the available range and quality of motion at the joint. Goniometry is useful in assessing abnormalities in joint movement associated with disease, developing treatment strategies and goals, and evaluating progress toward those goals. The combination of goniometry and electromyography (EMG) is prevalent in strength and conditioning studies.

Strength can be defined as the ability of a muscle to produce or resist a physical force. Force generated generated during muscle contraction can be static (force producing no movement) as in isometric contraction, or dynamic (force producing movement) as in isotonic contraction. Dynamic contractions occur when there is visible joint movement and may be classified as either concentric or eccentric muscle contraction.

- *Concentric contraction* occurs if the resistance is less than the force produced by the muscle group. This type of contraction allows the muscle to shorten as it exerts tension (e.g. slowly raising a dumbbell).
- *Eccentric contraction* occurs when the muscle produces a braking force to decelerate rapidly moving body segments or to resist gravity. The lowering of a dumbbell is an example of an eccentric contraction.

Objectives

- To record muscle activity of both the triceps and the biceps muscles.
- To observe limb angular movement of the elbow.

Equipment

- BIOPAC Electrode Lead Sets (2) (**SS2L**)
- BIOPAC Disposable Vinyl Electrodes (**EL503**), 6 per Subject
- BIOPAC Twin Axis Goniometer (180mm) (**SS21L**)
- Adhesive Single-Sided Tape (**TAPE1**)
- Adhesive Double-Sided Tape (**TAPE2**)
- Dumbbell weights (2 different weights)

Lab Meeting #4 Procedure

Hints for Minimizing Measurement Error

Note: Mishandling may result in inaccurate data, reduced equipment life, or equipment failure.

- Apply the electrodes at least 5 minutes before recording. Sweating tends to affect electrode adhesion to the skin.
- Nothing should rub against the electrodes and subject’s clothing should not interfere with electrodes.

- No pressure should be placed on the electrodes or the baseline will be distorted.
- When using the goniometer:
  - Take care to handle/manipulate the sensors as instructed.
  - Observe the minimum value bend radius at all times, particularly when attaching and removing the sensors from the subject.
  - Take care during mounting so that the measurement element always forms a simple bend shape--an "oxbow" shape in the element will reduce accuracy.
  - Remove the endblocks individually and carefully, making sure not to exceed the minimum permissible bend radius, particularly where the measuring element enters the endblocks.
  - Never pull on the measurement element and/or protective spring to remove the goniometer from the subject.

To Set Up the Hardware:

1. The SS21L connects to two channels:
   a. Connect SS21L-X to Channel 1
   b. Connect SS21L-Y to Channel 2
2. This lab requires two SS2L lead sets
   a. Connect one SS2L to Channel 3
   b. Connect the other SS2L to Channel 4
3. Turn on the MP30

Figure 4-16. *MP30 hardware*

To Set Up the Lab Software:

1. Launch the BSL PRO software on the host computer.

   The program will create a new "Untitled1" window .

2. Open the Biomechanics Template by choosing:

File > Open > choose Files of type: Graph Template (*GTL) > File name: "h17.gtl"

The template will establish the required settings.

3. **Save As** the desired file name.

To Calibrate the Equipment:

1. Go to **MP30>Setup Channels** and click on the **wrench** icon for CH1 to change parameters.

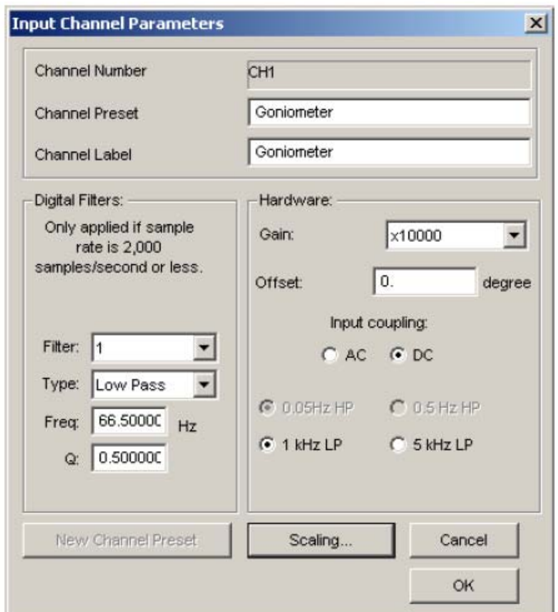


Figure 4-17. *Input channel parameters*

2. Click on the **Scaling...** button to generate the Change Scaling Parameters dialog.

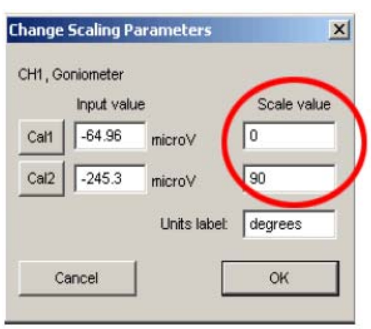


Figure 4-18. *Change scaling parameters*

3. Click on the **Cal1** button.

The scale value should read **0**.

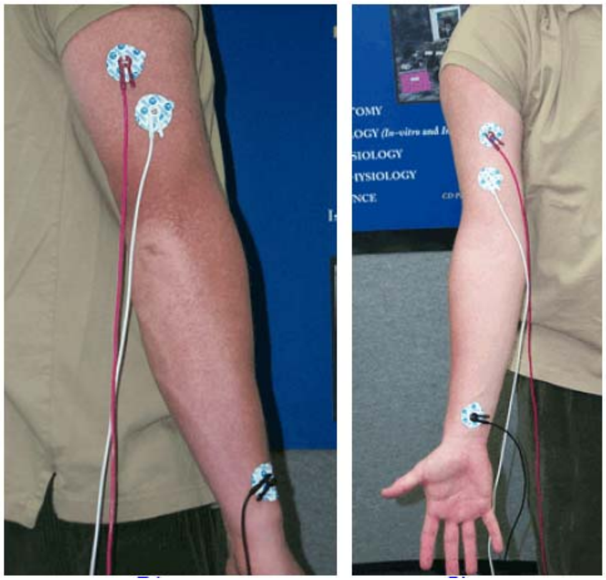
4. Click on the **Cal2** button.

   The scale value should read **90**.

5. Make sure the twin-axis goniometer (**SS21L**) is laying flat and then position it straight at 180^{o} and click **Cal1**.
6. Rotate the top endblock to **90^{o}**, keep it flat, and click **Cal2**.

To Prepare the Subject:

1. Attach six electrodes to the Subject, as shown below.
   a. Abrade each electrode site. It is a good idea to abrade the skin before electrode placement to decrease the amount of impedance between the electrode and the skin's surface.
   b. Apply a small amount of electrode gel (**GEL1**) at each electrode site. A drop of electrode gel will further improve electrode detection.
   c. Apply electrodes.
2. Attach the electrode lead sets (**SS2L**) to the electrodes, paying close attention to the lead colors and positions specified in Table 4-9.
   a. Position the electrode cables so they do not pull on the electrodes.
   b. Clip the electrode cable clip (where the cable meets the three individual colored wires) to a convenient location (such as subject's clothes) to relieve cable strain.



Triceps Biceps

Figure 4-19. *Electrodes properly applied*

Table 4-9. *Electrode lead assignments*

Position	SS2L	Lead Color
Triceps muscle (2)	Ch3	red lead on top white lead on bottom
Wrist—outside	Ch3	black (ground)
Biceps muscle (2)	Ch4	red lead on top white lead on bottom
Wrist—inside	Ch4	black (ground)

3. Attach the twin-axis goniometer (**SS21L**) to the subject's arm as shown Figure 4-20, to measure angular rotation of the elbow.
   a. Attach pieces of double-sided tape to the underside of the goniometer endblocks.
   b. Stick the tape to the subject and allow for the telescoping of the goniometer. The goniometer should be fully extended when the joint is fully flexed.
   c. Press the two endblocks firmly onto the subject and ensure that the goniometer is lying over the top of the joint.
   d. For additional security, pass a single wrap of single-sided medical tape around each endblock and subject's arm. Do not tape too tightly or you will restrict movement.
   e. Secure the cable and connector leaving the goniometer with tape to ensure that they do not pull and detach the goniometer.

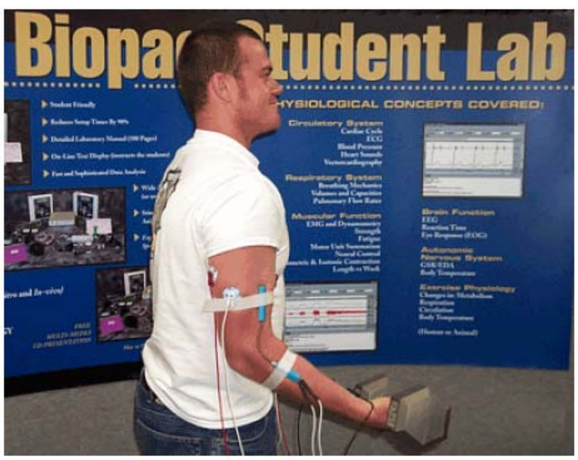


Figure 4-20. *Subject with twin-axis goniometer attached*

Running the Experiement

This recording is set up for the Save Once mode, which tells the MP System to automatically record data into a file, and to store that data into computer memory (RAM) during acquisition.

- To save recorded data, choose **File menu** > **Save As…** > **file type: BSL Pro files (*.ACQ) File name: (Enter Name)** > **Save** button
- To erase all recorded data (make sure you have saved it first), and begin from Time 0, choose: **MP30 menu** > **Setup Acquisition** > Click **Reset**

To Record Segment 1 (No Weight) Control Data:

1. After all the equipment has been properly attached, have the subject stand up.
2. Have the subject slowly lift their arm toward his shoulder, simulating a curl.
3. Have the subject slowly lower his arm toward their leg.

To Record Segment 2 (Lighter Dumbell) Data:

1. The lab partner will hand the lighter dumbbell to the subject.
2. Place a marker (click **F9**) and label it by weight and type.

   The marker text box is above the waveform.
3. Have the subject slowly lift the dumbbell toward their chest.
4. Have the subject slowly lower the dumbbell toward their leg.

To Record Segment 3 (Heavier Dumbell) Data:

1. The lab partner will hand the heavier dumbbell to the subject.
2. Place a marker (click **F9**) and label it by weight and type.

   The marker text box is above the waveform.
3. Have the subject slowly lift the dumbbell toward their chest.
4. Have the subject slowly lower the dumbbell toward their leg.

Analysis

The EMG & Goniometer study will be displayed as follows:

Channel	Displays
CH1	Goniometer
CH2	not displayed
CH3	Triceps (EMG)
CH4	Biceps (EMG)

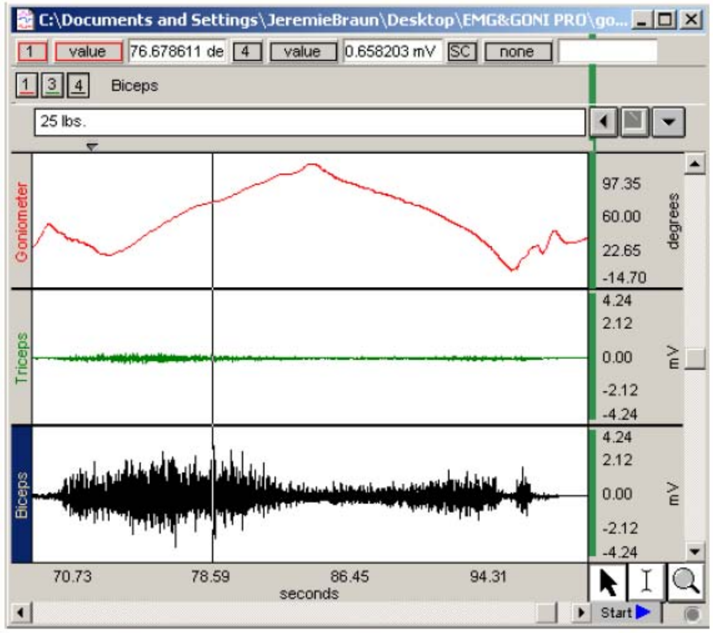


Figure 4-21.

1. Measurement boxes should be established as shown above—CH1 and CH4 as **value**.
2. Use the I-beam tool to locate the angle where the maximum amount of muscle activity was done.

   Notice that this angle is approximately halfway between the ending and starting point of the dumbbell curl.

BIOMEDICAL ENGINEERING
THE UNIVERSITY OF TEXAS AT AUSTIN

Iontophoresis

SECTION CONTENTS

INTRODUCTION

In previous years, Americans have seen the incidence of both type I and type II diabetes rise to epidemic proportions. Consequently, there has been, and continues to be, a considerable investment in the development of glucose monitoring technologies. Self monitoring of glucose concentrations is essential for a safe and effective diabetes therapy.

Traditionally, glucose concentrations have been measured by breaking the skin to draw a sample of blood, but novel technologies that utilize reverse iontophoresis forego this frequent and painful procedure. This method is employed by using a small electric current to drive charged and highly polar compounds through the skin at rates much greater than their passive permeabilities allow. This is made possible by two phenomena: electromigration and electroosmosis. Electromigration is the application of an electric field to drive small ions across the skin. In order to maintain electroneutrality, ionic transport is observed. Uncharged molecules, like glucose, are predominantly transported by Electroosmosis. Because the skin is negatively charged, it is permselective to positive ions and therefore induces an electroosmotic solvent flow that carries neutral molecules in the anode-to-cathode direction (Figure 5-1). It is through this net movement of neutral molecules that glucose can be transported across the skin noninvasively. Our experiments intend to present a mechanistic approach to observing this phenomenon.

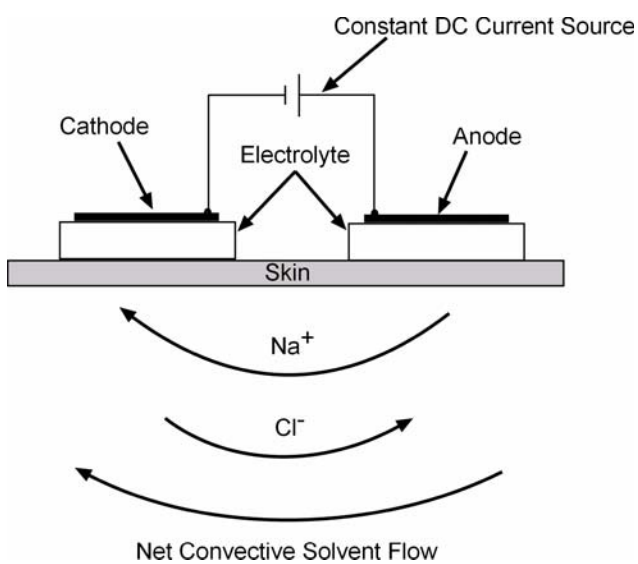


Figure 5-1. *Reverse iontophoresis*

IONTOPHORESIS

By Patrizia Santi, Pharmaceutical Department, University of Parma (I)

Iontophoresis can be defined as a mean of enhancing the flux of ionic compounds across a membrane, by the application of an electric current across it. This technique has been reported useful for the enhancement of transdermal delivery of ionized drugs, including macromolecules. The skin is a multilayered organ delimiting the body. It is constituted of several layers and the outermost layer, stratum corneum, is the main barrier to drug transport. The application of electric current, however, is able to increase the penetration of molecules through this barrier. The two principal mechanisms by which iontophoresis enhances molecular transport across the skin are:

- **iontophoresis**, in which a charged ion is repelled from an electrode of the same charge, and
- **electroosmosis**, the convective movement of solvent that occurs through a charged *pore* in response to the preferential passage of counter-ions when the electric field is applied.

The isoelectric point of the skin is ~4; therefore, under physiological conditions, with the surface of the skin also buffered at or near 7.4, the membrane has a net negative charge and electroosmotic flow is from anode (+) to cathode (-). The phenomenon of electroosmosis has been used as a means to augment the anodic delivery of (in particular) large, positively charged drugs, the transport numbers of which are often extremely small (and whose iontophoretic enhancement therefore depends heavily upon electroosmosis) and to promote the transdermal migration of uncharged, yet polar, molecules, the passive permeation of which is typically very small. In this presentation, some of the applications of iontophoresis are discussed. In particular, iontophoresis will be examined in view of achieving a systemic or a local effect and of extracting analytes from the body though the skin (reverse iontophoresis).

Systemic effect

Iontophoresis can be considered as an interesting alternative to parenteral route, particularly in the case of peptide drugs, macromolecular substances in ionized state at physiological pH values, that are poor absorbed and extensively degraded by proteolytic enzymes in the gastro-intestinal tract, showing extremely low bioavailability when administered orally. The ability of electric current to improve peptide drug transport through the skin is presented, using calcitonin as model drug administered *in vivo* in rabbits. The ypocalcemic response produces was taken as a measure of drug absorption. A particular type of reservoir was used, owing to the poor aqueous stability of calcitonin. From the results obtained it was found that electric current is able to promote drug transport through the skin. Current density and application time resulted the main parameters affecting drug response. Additionally, transdermal administration was compared to i.v. injection in order to propose transdermal iontophoresis as an alternative to injective route. For this reason the hypocalcemic response was related to the dose injected and a sigmoidal relationship was found. A sigmoid relationship was also found between hypocalcemic response and total amount of current applied to the skin. In conclusion, transdermal iontophoresis can reproduce the effect of a calcitonin injection using a reservoir at pH 7.4.

Local effect

Iontophoresis has been extensively investigated for systemic absorption of topically applied drugs, but can be useful also in the therapy of surface tissue diseases. Typical diseases that can benefit of an accumulation into the skin are Herpes Simplex infections and psoriasis. In both cases, the part of the skin involved is the epidermis. This part of the presentation will illustrate the effect of transdermal iontophoresis on the distribution of drugs (acyclovir and khellin) in human skin layers in *in vitro* experiments. The experiments were done *in vitro* using excised human skin. After current application, the skin was thin-sliced (20 µm) and the drug accumulated into each layer was extracted. This allowed the knowledge of the relationship between drug concentration and depth from the surface. The results obtained show that drug concentration into the skin is higher after iontophoresis than after passive diffusion. The application of 30 minutes of iontophoresis gives a drug concentration 3-4 times higher than passive diffusion. In particular, iontophoresis improves the amount of acyclovir present in the skin, particularly at pH 3.0, where an important part of the drug is ionized. Thirty minutes of iontophoresis already give a distribution of ACV into the skin comparable to seven hours of passive diffusion. In the case of the un-ionizable drug khellin, the results obtained show an accumulation of drug in skin layers, demonstrating that not only ionized drug can take advantage of iontophoresis.

Extraction of analytes

Recently, we have demonstrated that electroosmosis can be taken advantage of in *reverse iontophoresis*, whereby the imposition of an electric current across the skin is used to *extract* a substance of interest from within or beneath the skin to the surface. Specifically, it has been shown *in vitro* and *in vivo* (in humans) that the approach can be used to monitor the subdermal concentration variation of glucose, and provide, potentially, therefore, a *noninvasive* technique by which a diabetic might monitor—essentially continuously—the daily fluctuations in blood sugar. Clearly, in order for reverse iontophoresis to realize the maximum number of possible opportunities, it will be necessary to establish the reproducibility of the extraction process and to optimize the experimental conditions so that the largest amount possible of the desired analyte is extracted in the shortest possible time (both of which should enhance the practical utility of the idea). The objective of the research, therefore, was to examine the parameters which determine electroosmotic flow, with a view to maximizing the convective flow of solvent across the skin during iontophoresis. Electroosmotic flow can be manipulated by altering the pH, and by iontophoresing, into the skin, positively charged peptides which can apparently bind avidly to, and neutralize, the negative charge of the membrane (thereby altering permselectivity and the direction and/or extent of electroosmotic flow). The specific aim here, then, is to investigate whether approaches can be identified to reproducibly and significantly enhance electroosmosis. In this initial in vitro study, the non-metabolizable, uncharged, highly polar sugar, D-mannitol, has been used as marker molecule to track the kinetics and extent of electroosmotic flow during reverse iontophoresis.

Further Reading

See “Appendix 3” on page 8-1 for further reading.

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Iontopherous

Patrizia Santi's original article on iontopherous can be viewed at:
http://www.molluscum.com/iontophoresis.htm

Patrizia Santi's cited the following references in her article:

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IONTOPHEROUS LAB OVERVIEW

A number of variables will be evaluated in this laboratory to see what their effect is on the enhanced transport of glucose from gelatin films. In all cases (except time), two values of variable will be tested. The duration (time) that power is applied to the electrodes will be tested three times.

In order to evaluate these variable in an orderly fashion within the time constraints of the lab, a few variables should be evaluated first, and decisions made as to the preferred amounts to be used. Then, during subsequent work (weeks 3 and 4) variables from earlier laboratories will have set values—decreasing the total number of variables in the later labs.

During our four lab sessions, the variables shown in Table 5-1 will be studied.

Table 5-1. *Iontophoresis lab variables*

Glucose Concentration in Film	$\mathbf{C_1}$ = 8 g/160 ml	$\mathbf{C_2}$ = 16 g/160 ml	—
Power Applied	$\mathbf{P_1}$ = 1 mA	$\mathbf{P_2}$ = 3 mA	—
New vs. Old Spots on Gelatin	$\mathbf{S_1}$ (new)	$\mathbf{S_2}$ (old)	—
Water vs. Saline (buffer)	$\mathbf{B_1}$ (saline)	$\mathbf{B_2}$ (water)	—
Distance Between Electrodes	$\mathbf{D_1}$ = 7 cm	$\mathbf{D_2}$ = 3.5cm	—
Time	$\mathbf{T_1}$ = 1 min	$\mathbf{T_2}$ = 3 min	$\mathbf{T_3}$ = 5 min

Week 1

During the week 1 laboratory, you will prepare gelatin films containing two different concentrations of glucose. You will evaluate these films during the week 2 laboratory—analyzing the effects of two different power levels, as well as the difference between using the same spots or new spots for the electrode placement.

Note: The spot placement difference may be important if pulling the glucose from one location depletes the glucose concentration for subsequent readings (and all reading will be done in triplicate).

In summary, during the week 1 lab session, you will need to create six dishes of gelatin (three at each glucose concentration).

Week 2

In week 2 of the laboratory, you will evaluate *new spot* vs. *old spot* placement of the electrodes at a set distance, using saline and a 1-minute power application. Your evaluation will include gelatins of both glucose concentrations and the lower power setting.

The constant distance between electrodes should be the maximum distance achievable in the Petri dishes (7.0 cm). You will need to take into consideration that you must maintain the same distance for all three measurements while ensuring the paths between each pair of electrodes (assume three sets need to be placed on each dish) do not cross or overlap. All studies will be done in triplicate. One reading (sampled three times) from each of three separate spots will be done first and then two additional readings will be taken from one of the previous spots.

This part of the lab will result in a total of 10 measurements. You will then repeat this study at the higher power level. Studies done at the higher power level will result in a total of ten more measurements. You will also need to evaluate the transport of glucose with no power. Three 1-minute, control-puck readings will need to be taken along with readings at 3, 5, 10, 15, 30, 60, and 90 minutes.

Finally, in preparation for the next laboratory session, you will need to prepare three dishes each of gelatin at both high and low concentrations. Additionally, you will need to prepare another sample one with no glucose.

Week 3

In week 3 of the laboratory, the S value has been selected, and now decisions must be made as to optimal distance (D_x) and concentration (C_x). To accomplish this, you will need to run tests on the gelatin films with the electrodes at 1/2 the distance used during the previous session (for both films C_1 and C_2). You will also need to run tests using both the high and low power levels (for both films C_1 and C_2). An analysis should also be completed at the higher power level and greater distance for the gelatin film with no glucose as a negative control. Again, these studies are to be completed with saline.

During this session, you will also make a decision, based on your results from week 2 (and this should be included in your report) which concentration of glucose you will use for the final set of films to be analyzed with 3 and 5 minute power application times. For whichever concentration you choose, prepare six gelatin dishes at that concentration.

Week 4

By week 4 of the laboratory, you will have chosen the values for C and S (during week 2), and based on the results from week 3, you will have chosen the values for D and P. These selected values will be used for all studies during week 4.

The studies with T_1 (1 minute) have been completed in earlier labs. Studies remain for T_2 (3 minutes) and T_3 (5 minutes). At a chosen D and P (given that the power will be applied for a longer time) using saline the experiments will be carried out in triplicate as in previous weeks.

After the saline studies are completed, a final study will be done with the chosen S, D, C and P and a time of 1 minute (T_1) to study the final variable, B. This study will be conducted with water instead of saline—again in triplicate.

Study Schedule and Variable Selection Diagram

Figure 5-2 provides a simplified version of the study schedule and variable selection process.

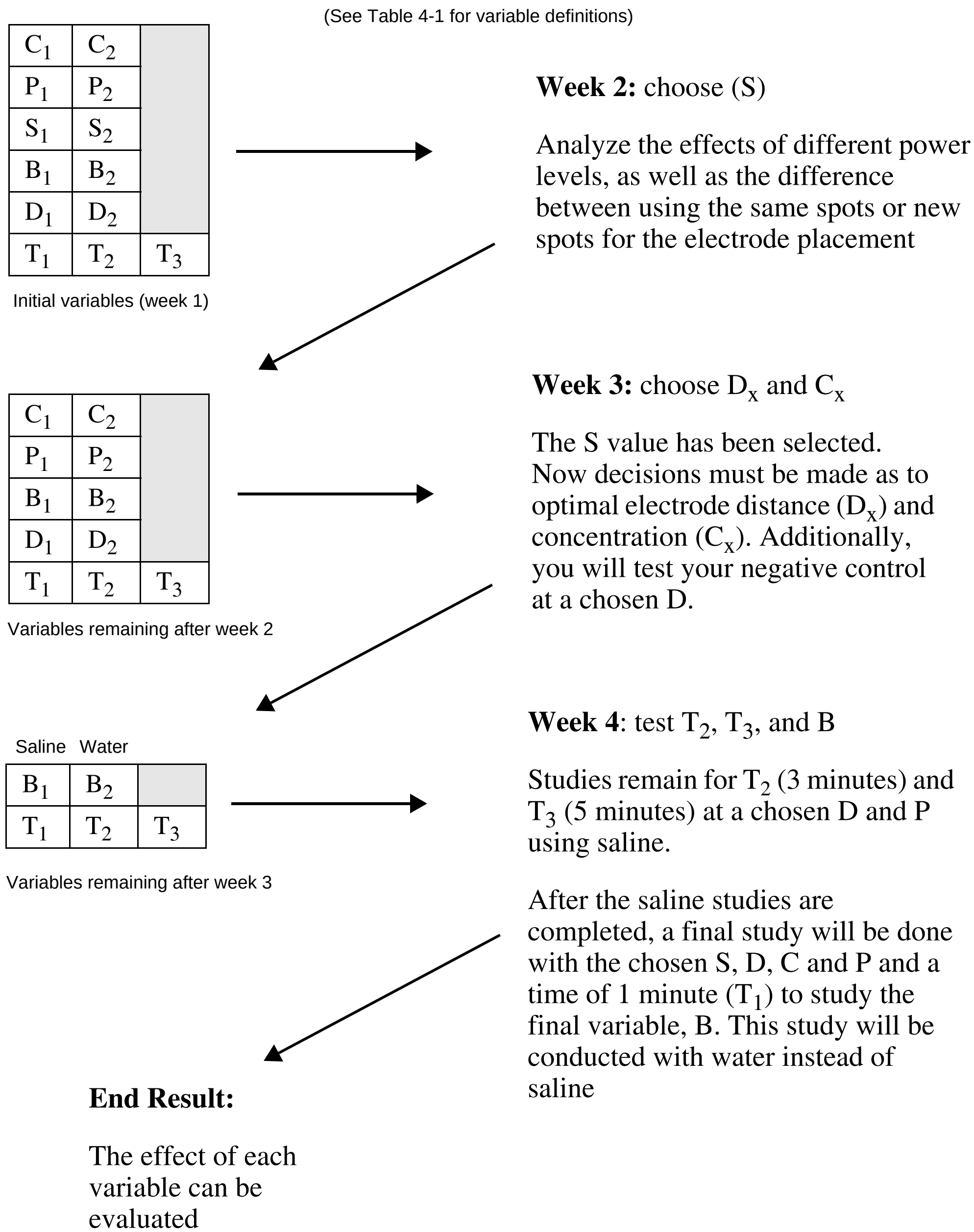

Figure 5-2. *Study schedule and variable selection process*

LAB MEETING #1

During this lab, we will create gelatin formations for use during the next lab. Since the results of your lab depend largely upon the quality of your gelatin formation, we recommend prepare your samples carefully.

Today we are working with gelatin, glucose, and saline solution. *Refer to the respective MSDSs to familiarize yourself with the hazards of these items.*

Overview

During the week 1 laboratory, you will prepare gelatin films containing two different concentrations of glucose. You will evaluate these films during the week 2 laboratory—analyzing the effects of two different power levels, as well as the difference between using the same spots or new spots for the electrode placement.

Note: The spot placement difference may be important if pulling the glucose from one location depletes the glucose concentration for subsequent readings (and all reading will be done in triplicate).

Additionally, during the week 1 laboratory, you will prepare films for diffusion studies that you will conduct during the next laboratory session.

In summary, during the week 1 lab session, you will need to create six dishes of gelatin (three at each glucose concentration).

Materials Required

Chemicals and Solutions

- 320 mL NaCl solution
- Gelatin
- Glucose

Equipment and Supplies

- Heated stir plate
- Beakers (200 mL)
- Large stirbar
- Petri dishes (80 cm)

Lab Meeting #1 Procedure

You will want to prepare and heat your saline solution while simultaneously preparing the gelatin and glucose.

Note: *Never* leave the hotplate-stirrer unattended until the hotplate surface has completely cooled.

Additionally, *never* touch the surface of the hotplate-stirrer to test the temperature of the heating surface.

To Heat Your Saline Solution:

1. Pour 160 ml of saline solution into each of two 200 ml beakers.
2. Place these beakers on the hotplate-stirrers and bring the temperature of the solution to 60ºC.

To Weigh Out the Solution Solvents:

1. Locate your solvents and weigh out the following amounts:
   - 24 g of gelatin (measure two separate amounts of this)
   - 8 g of glucose
   - 16 g of glucose
2. Pour one 24 g sample of gelatin into two separate 200 mL beakers
   a. Place a stirbar in the 200 mL beaker.
   b. Fill each 200 mL beaker with 160 mL of the heated saline solution.
3. Pour the 8 g sample of glucose into the first 200 mL beaker containing gelatin.
4. Pour the 16 g sample of glucose into the second 200 mL beaker containing gelatin.
5. Mix all solutions on a heated stirplate at 350 (cycles/minute) for 10 minutes.
6. Let solutions completely mix then pour into 3 petri dishes that are lying on a flat surface. (Fill to the lower line on the dish.)
7. Let the filled petri dishes cool for 10-15 minutes, then place in the refrigerator.
8. Repeat procedure as necessary to make sufficient dishes.

LAB MEETING # 2

During this lab, you will set up your glucose watch and use it to measure glucose. Your TA has prepared gel solutions and punched out neoprene pucks for you to use during this lab.

Note: This manual assumes you have knowledge of electrical safety and have operated power supplies before. If you do not, ask your TA to describe the equipment and appropriate safety measures before preparing for the lab.

This lab requires astute electrical safety habits. You will be turning on and off the power supply and handling the electrodes frequently. Read "Safety" on page 5-12 and follow strict electrical safety practices.

Overview

In week 2 of the laboratory, you will evaluate *new spot* vs. *old spot* placement of the electrodes at a set distance, using saline and a 1-minute power application. Your evaluation will include gelatins of both glucose concentrations and the lower power setting.

The constant distance between electrodes should be the maximum distance achievable in the Petri dishes. You will need to take into consideration that you must maintain the same distance for all three measurements while ensuring the paths between each pair of electrodes (assume three sets need to be placed on each dish) do not cross or overlap. All studies will be done in triplicate. One reading (sampled three times) from each of three separate spots will be done first and then two additional readings will be taken from one of the previous spots.

This part of the lab will result in a total of 10 measurements. You will then repeat this study at the higher power level. Studies done at the higher power level will result in a total of ten more measurements. You will also need to evaluate the transport of glucose with no power. Three 1-minute, control-puck readings will need to be taken along with readings at 3, 5, 10, 15, 30, 60, and 90 minutes.

Finally, in preparation for the next laboratory session, you will need to prepare three dishes each of gelatin at both high and low concentration glucose concentrations and one without glucose.

Safety

This lab requires you apply current to the gels.

- While preparing the gels, keep the power supply off.
- Never touch the gels while power is being applied to the gels.
- Never turn the power supply on without being certain of the output connections (that is, having checked the output connections for safety and having the output connections in plain site)
- Turn the output to the power supply to **0** before turning the supply on. Then adjust upward.

⚠ **WARNING:**

Electrical Shock. In this lab you will use a power supply capable of life-threatening power levels. If you are unfamiliar with the equipment, ask your TA to explain the equipment to you before preparing the lab.

Do not touch the gels while power is being applied to them.

Materials Required

Chemicals and Solutions

- 0.1 M NaCl solution
- Three petri dishes of Gel 1 (C_1 glucose)
- Three petri dishes Gel 2 (C_2 glucose)
- Deionized water

Equipment and Supplies

- Neoprene ring, (0.678 cm^3) x 6 per 1 gel
- Petri dish, (14 cm)
- Amrel Power Supply Model LPS 301
- Silver-silver chloride (Ag/AgCl) electrode
- Vacuum grease
- Glucose sensor
- Timer

Lab Meeting #2 Procedure

During this session, you will need to:

1. Perform studies to ascertain the optimal placement (old spot vs. new spot) of the electrodes
2. Create two dishes each of gelatin at both high and low concentrations, as well as one other sample with no glucose

To Prepare the Pucks and Gels:

1. Take the neoprene pucks and punch a small hole in the middle of the side (into the thickness) of the puck. This hole is the passage for the electrode to access the center of the puck (see Figure 5-3).
2. Carefully press the electrode into the hole of one puck. Make sure that the electrode rests in the center.
3. Place your second electrode in the second puck.

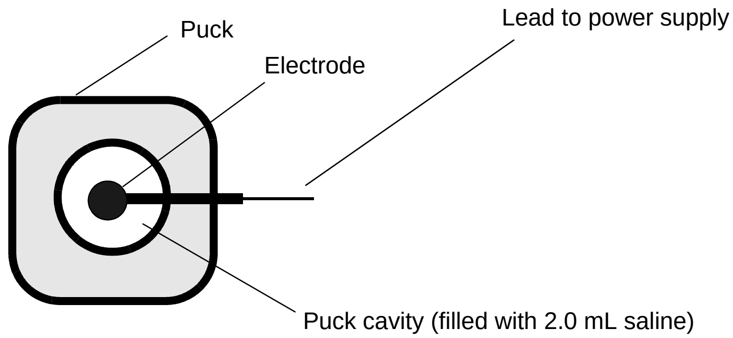

Figure 5-3. *Neophrene puck with electrode inserted (top view)*

4. Apply a generous amount of vacuum grease to the bottom of the puck. This is to create a seal that will prevent the saline solution from leaking out.
5. Put two pucks on the surface of Gel 1 (C_1); place the pucks at a maximum distance apart from one other (7.0 cm) while ensuring the pucks are entirely in contact with the gel.

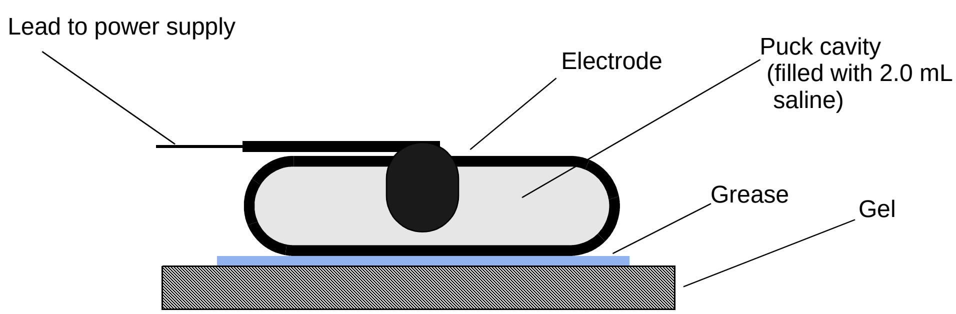

Figure 5-4. *Neophrene puck on gel (cross-sectional view)*

6. Ensure a tight seal is made between the pucks and the gel.
7. After ensuring power is off, connect the electrode lead wires to the lab-supplied power supply.

> ⚠ **CAUTION:**
> **Electrical Shock Hazard! Ensure the power supply is off (that is, de-energized) while making output connections.**

8. Set the electrodes in the center of the pucks.

To Apply Power to the Electrodes:

During this stage you will run a constant current through the cathode (electrode connected to the negative lead of the power supply) and anode (electrode connected to the positive lead of the power supply) with the saline and gel acting as the conductor between the leads.

1. Ensure you have a good seal between your pucks and the gel, that your electrodes are properly connected to the power supply, and that the power supply is de-energized.
2. After checking the connections from the power supply to the electrodes to ensure they are safe, turn on the power supply to 1 mA. The top red light on the power supply should blink.
3. Fill the cavity of one puck with 2 mL of saline solution. Then fill the second puck cavity with 2 mL of saline. Start the timer when the blinking light becomes constant.
4. Run a constant current through the electrodes at 0.1 mA for 1 minute.
5. After 1 minute, turn off the power supply, remove the electrodes from the pucks, and transfer the solution in each puck to a plastic weighing dish using plastic transfer pipets.
6. Reposition the electrodes on the gel—being careful to place the electrodes the same distance as the first study.

   To reposition the electrodes, do the following:

   a. Ensure power is off.
   b. Remove any remaining liquid from the puck cavities with a pipettor.
   c. Lift the pucks from the gel; dry the surface of the gel with a kimwipe.
   d. Clean the pucks with deionized water and dry the pucks with a kimwipe.
   e. Dry the electrodes with kimwipes.
   f. When reapplying the puck, make sure that there is no grease present at the surface in the center of the cavity.
   g. Refer to “To Prepare the Pucks and Gels:” on page 5-13 and place the pucks to the gel in their new positions.

      Repeat the testing process to test a total of three sites.

      To test the same spot, do not lift and replace pucks, but dry as completely as possible between readings.

7. Once you have recorded the results of your study with the electrodes in three different locations and the electrodes in the same location (three separate times), you need to repeat the entire procedure for the second petri dish of Gel 1 (C_1) at the higher power of 3 mA, and then the two petri dishes of Gel 2 (C_2)—one at the lower power level and one at the higher power level.

8. After all studies have been completed with the first C_1 dish, identify three locations on the dish that have not been in a current path and apply pucks. Add 2.0 mL of saline solution to each of these three pucks (no electrodes) and measure the glucose in the solution within the pucks at 1, 3, 5, 10, 15, 30, 60, and 90 minutes. This will provide you with a control for the diffusion of glucose.

   In the end you will have recorded:

   - Three studies with the electrodes in different locations (and three studies with the electrodes in the same location)—petri dish 1 at C_1 and P_1.
   - Three studies with the electrodes in different locations (and three studies with the electrodes in the same location)—petri dish 2 at C_1 and P_2.
   - Three studies with the electrodes in different locations (and three studies with the electrodes in the same location)—petri dish 1 at C_2 and P_1.
   - Three studies with the electrodes in different locations (and three studies with the electrodes in the same location)—petri dish 2 at C_2 and P_2.

   For a total of 20 studies.

9. Record your findings in tables similar to the ones that follow.

Further Instructions

Preparation for Next Laboratory Session

In preparation for the next laboratory session, you will need to prepare three dishes each of gelatin at both high and low concentrations. Additionally, you will need to prepare one other sample with no glucose. (Refer to “Lab Meeting #1 Procedure” on page 5-10 for gel-preparation instructions.)

Clean Up

After completing the lab, carefully remove the electrodes and clean your bench. Return the power supply to the safe condition in which you found it.

Additionally, analyze your results before next lab meeting to ensure that the experiments do not need to be repeated. If repetition is required, gels will need to be remade to be used in the following week.

Observations

C_1 Glucose Concentration (mg/dL)

Solution	After current P_1 mA for T_1 min		Sample
Electrodes in new spots each run	(+)	(-)	Gel 1 (C_1) Sample 1
	(+)	(-)	
	(+)	(-)	
	After current P_1 mA for T_1 min		
Electrodes in same spot	(+)	(-)	Gel 1 (C_1) Sample 1
	(+)	(-)	
	(+)	(-)	

C_2 Glucose Concentration (mg/dL)

Solution	After current P_1 mA for T_1 min		Sample
Electrodes in new spots each run	(+)	(-)	Gel 2 (C_2) Sample 1
	(+)	(-)	
	(+)	(-)	
	After current P_1 mA for T_1 min		
Electrodes in same spot	(+)	(-)	Gel 2 (C_2) Sample 1
	(+)	(-)	
	(+)	(-)	

Questions to Consider

- Where the results close to what you first hypothesized. If not, explain why?
- Is the concentration within the cavity relative to current, time, concentration, or a combination of the three? Explain your answer.
- What do you suppose the affects of the following variations might have on your studies:
  - The thickness of the gels
  - Time of application
  - Intensity of the current

LAB MEETING # 3

During this lab you will choose D_x and C_x.

Note: This lab requires astute electrical safety habits. You will be turning on and off the power supply and handling the electrodes frequently. Read "Safety" on page 5-22 and follow strict electrical safety practices.

Overview

In week 3 of the laboratory, the S value (new spot vs. old spot) has been selected, and now decisions must be made as to optimal distance (D_x) and concentration (C_x). To accomplish this, you will need to run tests on the gelatin films with the electrodes at 1/2 the distance used during the previous session (for both films C_1 and C_2). You will also need to run tests using both the high and low power levels (for both films C_1 and C_2). An analysis should also be completed at the higher power level and greater distance for the gelatin film with no glucose as a negative control. Again, these studies are to be completed with saline.

During this session, you will also make a decision, based on your results from week 2 (and this should be included in your report) which concentration of glucose you will use for the final set of films to be analyzed with 3 and 5 minute power application times. For whichever concentration you choose, prepare three gelatin dishes at that concentration.

Safety

This lab requires you apply current to the gels.

- While preparing the gels, keep the power supply off.
- Never touch the gels while power is being applied to the gels.
- Never turn the power supply on without being certain of the output connections (that is, having checked the output connections for safety and having the output connections in plain site)
- Turn the output to the power supply to **0** before turning the supply on. Then adjust upward.

⚠ **WARNING:**

Electrical Shock. In this lab you will use a power supply capable of life-threatening power levels. If you are unfamiliar with the equipment, ask your TA to explain the equipment to you before preparing the lab.

Do not touch the gels while power is being applied to them.

Materials Required

Chemicals and Solutions

- 0.1 M NaCl solution
- Two petri dishes of Gel 1 (C_1 glucose)
- Two petri dishes Gel 2 (C_2 glucose)
- One petri dish of gelatin (no glucose)
- Deionized water

Equipment and Supplies

- Neoprene rings, (0.678 cm^3) x 6 per 1 gel
- Petri dish, (14 cm)
- Amrel Power Supply Model LPS 301
- Silver-silver chloride (Ag/AgCl) electrodes
- Vacuum grease
- Glucose sensor
- Timer

Lab Meeting #3 Procedure

During this session, you will need to:

1. Perform studies to ascertain the optimal distance for the electrodes on the gel, as well as gel glucose concentration
2. Create gels for the next laboratory session

Distance and Concentration Studies

During this study, you will examine the effects of electrode placement (D) and glucose concentration (S). Additionally, you will run a study of the negative control at D1 (maximum distance studied between electrodes) on a gel containing no glucose.

To Test Distance and Concentration:

This procedure requires astute electrical safety habits. You will be turning on and off the power supply and handling the electrodes frequently. Read “Safety” on page 5-22 and follow strict electrical safety practices

1. You have selected your value for S. Use this value (*same spot* or *new spot)* when determining the placement of the electrodes for this procedure.
2. Prepare your pucks and electrodes as described in the procedure, “To Prepare the Pucks and Gels:” on page 5-13.

3. Place the electrodes on gel 1 (C_1) at D_2.

   Note: D_2 is 1/2 the distance between electrodes as D_1.

4. Apply power to the electrodes as described in the procedure, "To Apply Power to the Electrodes:" on page 5-15

5. Repeat this procedure a total of three times for each of the following:

   - C1 at D2 and P_1
   - C1 at D2 and P_2
   - C2 at D2 and P_1
   - C2 at D1 and P_2

   Record your findings in tables similar to the ones that follow.

To Test the Negative Control Sample:

This procedure requires astute electrical safety habits. You will be turning on and off the power supply and handling the electrodes frequently. Read "Safety" on page 5-22 and follow strict electrical safety practices.

1. Locate your gelatin containing no glucose. This is your negative control.
2. Prepare your pucks and electrodes as described in the procedure, "To Prepare the Pucks and Gels:" on page 5-13.
3. Place the electrodes on the negative control gel at D_1 (the maximum distance between electrodes that you have studied thus far).
4. Apply power to the negative control sample as described in the procedure, "To Apply Power to the Electrodes:" on page 5-15. Use P2 (higher power value of the levels studied thus far) as the study power level.
5. Repeat this procedure a total of three times, and record your findings in tables similar to the ones that follow.

Preparing for Next Lab Session

During this session, you should have decided which concentration of glucose you will use for the final set of films to be analyzed with 10 and 15 minute power application times. For whichever concentration you choose, prepare three gelatin dishes at that concentration. (Refer to "Lab Meeting #1 Procedure" on page 5-10 for gel-preparation instructions.)

Observations

C_1 Glucose Concentration (mg/dL)

Solution	After current P_1 mA for T_1 min		Sample
Electrodes at D_2 and P_1	(+)	(-)	Gel 1 (C_1) Sample 1
	(+)	(-)	
	(+)	(-)	
	After current P_2 mA for T_1 min		
Electrodes at D_2 and P_2	(+)	(-)	Gel 1 (C_1) Sample 1
	(+)	(-)	
	(+)	(-)	

C_2 Glucose Concentration (mg/dL)

Solution	After current P_1 mA for T_1 min		Sample
Electrodes at D_2 and P_1	(+)	(-)	Gel 2 (C_2) Sample 1
	(+)	(-)	
	(+)	(-)	
	After current P_2 mA for T_1 min		
Electrodes at D_2 and P_2	(+)	(-)	Gel 2 (C_2) Sample 1
	(+)	(-)	
	(+)	(-)	

Control Glucose Concentration (mg/dL)

Solution	After current P_2 mA for T_1 min		Sample
Electrodes at D_1 and P_2	(+)	(-)	Negative Control (C_0) Sample 1
	(+)	(-)	
	(+)	(-)	

LAB MEETING #4

During this lab you will test T_2 (3 minutes) and T_3 (5 minutes) at a chosen D and P using saline.

After the saline studies are completed, a final study will be done with the chosen S, D, C and P and a time of 1 minute (T_1) to study the final variable, B. This study will be conducted with water instead of saline

Note: This lab requires astute electrical safety habits. You will be turning on and off the power supply and handling the electrodes frequently. Read "Safety" on page 5-22 and follow strict electrical safety practices.

Overview

By week 4 of the laboratory, you will have chosen the values for C and S (during week 2), and based on the results from week 3, you will have chosen the values for D and P. These selected values will be used for all studies during week 4.

The studies with T_1 (1 minute) have been completed in earlier labs. Studies remain for T_2 (3 minutes) and T_3 (5 minutes) at a chosen D and P using saline.

After the saline studies are completed, a final study will be done with the chosen S, D, C and P and a time of 1 minutes (T_1) to study the final variable, B. This study will be conducted with water instead of saline.

Safety

This lab requires you apply current to the gels.

- While preparing the gels, keep the power supply off.
- Never touch the gels while power is being applied to the gels.
- Never turn the power supply on without being certain of the output connections (that is, having checked the output connections for safety and having the output connections in plain site)
- Turn the output to the power supply to **0** before turning the supply on. Then adjust upward.

WARNING:

Electrical Shock. In this lab you will use a power supply capable of life-threatening power levels. If you are unfamiliar with the equipment, ask your TA to explain the equipment to you before preparing the lab.

Do not touch the gels while power is being applied to them.

Materials Required

Chemicals and Solutions

- 0.1 M NaCl solution
- Three petri dishes of Gel at your chosen glucose concentration
- Deionized water

Equipment and Supplies

- Neoprene rings, (0.678 cm^3) x 6 per 1 gel
- Petri dish, (14 cm)
- Amrel Power Supply Model LPS 301
- Silver-silver chloride (Ag/AgCl) electrodes
- Vacuum grease
- Glucose sensor
- Timer

Lab Meeting #4 Procedure

This procedure requires astute electrical safety habits. You will be turning on and off the power supply and handling the electrodes frequently. Read "Safety" on page 5-22 and follow strict electrical safety practices.

To Test Time (T_2 and T_3):

1. Use your selected values for P, S, D, and C when determining the parameters of this procedure.
2. Prepare your pucks and electrodes as described in the procedure, "To Prepare the Pucks and Gels:" on page 5-13.
3. Place the electrodes on your sample gel (C_x).
4. Apply power to the electrodes as described in the procedure, "To Apply Power to the Electrodes:" on page 5-15.
5. Repeat this procedure a total of three times for each of the following:
   - T_2
   - T_3

   Record your findings in the tables that follow.

To Test Buffer (B):

1. Use your selected values for P, S, D, and C when determining the parameters of this procedure.
2. Substitute water for saline and prepare your pucks and electrodes as described in the procedure, "To Prepare the Pucks and Gels:" on page 5-13.
3. Place the electrodes on your sample gel (C_x).

4. Using T_1 as the time parameter, apply power to the electrodes as described in the procedure, "To Apply Power to the Electrodes:" on page 5-15.
5. Repeat this procedure three times and record your findings in the tables that follow.

Observations

C_{fin} Glucose Concentration (mg/dL): Saline

Variables	After current P_1 mA for T_2 min		Sample
Saline	(+)	(-)	Gel Final (C_{fin}) Sample 1
	(+)	(-)	
	(+)	(-)	
	After current P_2 mA for T_3 min		
Saline	(+)	(-)	Gel Final (C_{fin}) Sample 2
	(+)	(-)	
	(+)	(-)	

C_{fin} Glucose Concentration (mg/dL): Water

Variables	After current P_1 mA for T_1 min		Sample
Water	(+)	(-)	Gel Final (C_{fin}) Sample 3
	(+)	(-)	
	(+)	(-)	

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Appendix

This section contains information about the various forms of scientific writing you will be required to use in BME 251, as well as the rubrics that will be used by your instructor and TAs to evaluate your writing assignments.

SECTION CONTENTS

ACADEMIC INTEGRITY

The University has a strict policy on academic integrity. Any form of plagiarism or academic dishonesty will NOT be tolerated in BME 251. If you have any questions, please consult the web sites below:

- http://www.utexas.edu/depts/dos/sjs/academicintegrity.html
- http://www.utexas.edu/courses/clubmed/plgrism_cit.html

Failure to comply with the University's policy will result in a "zero" in the assignment and an "F" for BME 251.

FORMS OF SCIENTIFIC WRITING

The following sections provide a brief outline of the types of scientific writing you will be required to use in BME 251.

Laboratory Notebooks

Your laboratory notebook is the primary repository for the data you collect this semester. It is therefore important that you adhere to proper note-keeping standards. All entries in your laboratory notebook must be clear and complete; it should contain sufficient information that your teams and your TA can read and understand without any difficulty. See example in Appendix B.

Please pay particular attention to the following points when recording data in your laboratory notebooks:

1. Always use a ballpoint pen and never a pencil when making entries in your lab notebook. You and your team members are responsible for safeguarding your laboratory Black is best, but blue is also acceptable.
2. At the end of a lab session, all the team members should witness and sign the laboratory notebook. Your TA should also witness and sign your laboratory notebook when the entry is complete.
3. Since you will need to have your notebooks with you outside of the lab period (for preparing reports), the notebooks will not be stored in the lab drawers.
4. Under no circumstances should any data be recorded on scraps of paper, backs or palms of hands, shoe soles, etc. Always enter the data in the notebook as the data is being collected.
5. Reserve the first two pages of your laboratory notebook for a table of contents. These pages should be updated periodically. Before starting each experiment, you should have a plan for the day's work (Pre-lab write-up). This plan should already be outlined in your notebook. Your TA will grade your pre-lab write-up before you begin the experiment.

   Note: The Pre-lab write up should be done by the student who will be writing the final lab report.

   The pre-lab write-up should include a general overview of objectives. It should also contain relevant experimental procedures, any unusual safety protocols, and expected/predicted outcomes. In addition to raw data that are collected during the experiment, your laboratory notebook should also contain records of data analysis. While it is not necessary to transcribe information such as equation derivations in your notebook, you should write down the final equation that is used and reference the source. Graphs and figures that result from data analysis should be pasted in your notebook. You should also include a brief discussion that explains the data analysis portion of your records.

Your TA will review the contents of your laboratory notebook each week and provide constructive criticisms. Analyze your data as you conduct your experiment. Do not wait until you have collected [what you think] is all the data before doing so. You may run out of time by the end of the day before you decide that you need to collect some crucial data.

For more information on laboratory note-recording, please refer to:

"Writing the Laboratory Notebook," by Howard M. Kanare (Washington DC: America Chemical Society, 1985). The most relevant sections are pp. 1-8, 14-16, 40-41, 44-45, 53-79, 81-93, and 126-127.

Some additional bits of advice (or reinforcement) are provided below:

1. *The laboratory notebook should be a readable document, not merely a logbook in which numbers are recorded.* Your records must contain everything necessary for someone else to read and fully understand the experiment – it should contain an overview of the experiment, a brief description of the apparatus, all your collected data, an outline of the data analysis (and principal findings), and major conclusions. Your notebooks should be up-to-date at all times: always write down what you are doing or thinking AT THAT TIME, not later. "Pasted-in" items should be limited to data traces acquired by computer or graphs and figures produced by spreadsheets. You should NOT paste in sections of text.
2. *Record your analysis in your notebook.* For example, if you are fitting your data to some theoretical model, your notebook should contain the equations describing the model (do not forget to cite the source from which the equation was taken), the parameters obtained from the fit, and a graph showing the fit superimposed on the data. Although the data fitting itself is done on a spreadsheet, it is essential that you record the method of analysis and the principal findings in your laboratory notebook as they are obtained.
3. *Always try to quantify errors at the time of measurement.* Your error analysis will require you to identify possible sources or error, as well as estimate their magnitudes.
4. *Record your visual observations.* What color is the solution? Is your mixture boiling at a specific temperature? Also record changes in observations.
5. *Properly annotate errors.* You realize that the calculations on page 17 of your laboratory notebook (which was done earlier during the experiment) are incorrect and you are currently up to page 25. You should draw a single line through the erroneous section on p. 17, add a note in the margin that these calculations are incorrect and that the correct version is on p. 25. You should then date and initial the note.
6. *Table of Contents.* The purpose of the table of contents is to help a reader find the page he or she is looking for. Consequently, the entries in the table should be sufficiently descriptive and numerous that a reader can easily identify the proper section and go directly to the point of interest. A subheader such as "Data

Analysis" under "Experiment 1" does not provide sufficient information. Instead, try "Data Analysis: Estimating Stage Efficiencies".

7. *Use your laboratory notebook.* Proper note taking in your laboratory notebook will help you prepare your reports. Use it to your advantage. Do not do the reverse—do not paste your conclusions from your reports into your laboratory notebook. That is *not* what the laboratory notebook is meant for.

Long Report

Each of the three standard experiments will result in a long report assignment. Keep in mind that this is a *group* project. I would like you to write up your results in a manuscript format, suitable for publication as a Research Article following the format of *Biomaterials*. Manuscript writing is an important skill to have, especially if you are going to graduate school, but also if you join industry. Be sure to give sufficient background information so a reader with a science background, but not necessarily an expert in the field, can understand your manuscript. Article from *Biomaterials* have been uploaded to the BME UG Lab web site as examples (http://www.engr.utexas.edu/bme/ugrad/UGLab/resources.html).

The following sections describing the long report have been reproduced from the *Biomaterials* web site [https://www.editorialmanager.com/biomat/]. I'd like for you to abide by these closely.

Manuscript Style and Length

The following sequence is normally required: title, authors, affiliations, abstract, keywords, introduction, materials and methods, results, discussion, conclusions, acknowledgements, appendix (where necessary), figure captions, and tables. The paper should be no more than 10 pages and should be double-spaced.

The Title

The title is obviously the major factor that determines who will find and read the paper and great care should be taken with it. The title should be sufficiently informative so that the reader can immediately assess its likely relevance, but without being excessively long. The title does not have to convey the results or the conclusion, nor indeed does it have to specify the techniques used unless it is a technique—oriented paper. It is best to avoid sentences as titles; the best titles have between six and twelve words with no verbs.

The Authors

Each paper should have a corresponding author, and the affiliations of all authors should *not* be ambiguously stated.

Keywords

Keywords have become very important with respect to literature searches and many search engines operate through the listing of these words. It is in the author's interest to think carefully about the words that will attract interested readers to their paper. There is little point in using very generic terms such as biomaterial, implant, drug, tissue engineering and prosthesis as key words. Equally there is no point in using obscure names, and it is best to avoid the author's own abbreviations.

The Abstract

Next to the title, the abstract will be the second most important point of entry to the paper since most search facilities will print the abstract as part of the service, and far more people will read the abstract than the full papers. The abstract should be concise and informative. It is not the place to expand on techniques or discuss philosophy, and the conclusions that it expresses have to be an accurate reflection on what was found. Abstracts should be not used to exaggerate the significance of the work and they should not contain subjective opinions on this importance or speculate how a material might be used. Very commonly submitted abstracts will include a phrase such as 'material X is very biocompatible and shows promise for use in orthopedic implants'. This is rarely a sensible approach to writing an abstract. The instructions specify a length of 100 to 200 words. Most good abstracts are around 150 words in length.

The Introduction

The Introduction, as the name implies, should introduce the background to the work that has been carried out, effectively providing the scientific rationale. It should contain sufficient citations to the key literature to support this rationale and should lead to a clearly stated hypothesis or set of objectives. Authors should assume that the readership is well-informed and there is no need for any generic educational background. For example, in a paper on wound healing it is not necessary to take the first page to explain the ideal characteristics of wound dressing materials, or in a paper on drug eluting stents it is not necessary to describe all of the competing technologies that address in-stent restenosis. The introduction should rarely be more than two manuscript pages long.

Units

The SI system should be used for all scientific and laboratory data; if, in certain instances, it is necessary to quote other units, these should be added in parentheses. Temperatures should be given in degrees Celsius. The unit 'billion' (10^9 in America, 10^{12} in Europe) is ambiguous and must not be used. If a large number of symbols are used it is helpful if authors submit a list of these symbols and their meanings.

The Materials and Methods

This section should specify exactly what was done experimentally, with sufficient detail for the reader to be able to repeat the experiments if he wishes. All of the experimental work discussed in the paper should be described in this section. Materials used in the work should be described in appropriate detail, including sources

of commercial supply or synthetic routes, and all major equipment should be specified with the manufacturers name, reference number and location. Write in normal prose style; do not list instructions. You should describe what you did in past tense. Describe also measurements and calculations necessary to obtain final results.

The Results

Ideally the Results Section should be separate from the Discussion, but there is some flexibility here. The section should, obviously, be factual and it is best to avoid any philosophy or speculation. Authors should consider very carefully how to present their data. It should not be presented in multiple formats (i.e. the same data should not appear in figures and tables). If the data is displayed very effectively in either a table or figure, it should not be necessary to explain results in great detail in the text, but rather to use the text as a medium for emphasizing the most significant data.

The Discussion

This section should summarize the nature of the observations and attempt to place this data into the context of the existing body of literature and, where appropriate, to express opinions about the significance of the work as far as biomedical engineering is concerned. It should not be repetitive of the Introduction. It is entirely valid to suggest the potential implications of the work but without too much speculation. It is particularly important not to extend the discussion into areas that are not supported by the facts that are in evidence.

The Conclusions

Many authors end the Discussion section with a paragraph on the conclusions. This is not the best way to draw the manuscript to an end, and conclusions should be separated into a distinct section. This should not be too long, nor should it be repetitive of the discussion, and especially should not bring new ideas into the paper. The conclusions have to be based on the facts in evidence and should be limited to reasonable speculation about the significance of the work.

References

Instructions for the preparation of the list of references are given in the guidelines to authors. It is good to have a balance between the older seminal papers that lay the groundwork for that particular area and recent quality papers that have contributed serious input into the subject. Documents that have limited circulation, obscure journals or books, especially those out of date, and electronic sources (for example, web sites) should also be avoided.

All publications cited in the text should be presented in a list of references following the text of the manuscript. In the text refer to references by a number in square brackets on the line (for example*,* Since Peterson[1]), and the full reference should be given in a numerical list at the end of the paper. References should be given in the following form, which is designated Vancouver:

1. Driessens FCM, Boltong MG, Bermudez O, Planell JA. Formulation and setting times of some calcium orthophosphate cements: a pilot study. J Mater Sci: Mater Med 1993;4:503-508.

2. Nancollas H. In vitro studies of calcium phosphate crystallisation. In: Mann S, Webb J, Williams RJP, editors. Biomineralization. Chemical and biochemical perspectives. New York: VCH, 1989. p. 157-182.

3. Brown W, Chow LC. Combinations of sparingly soluble calcium phosphates in slurries and paste as mineralizers and cements. US Patent No. 4612053, 1986.

Figures and Tables

As noted earlier, experimental data should be represented in figures or tables wherever possible. Advice is not given here about the preparation of figures, detail being given in the guide to authors. Authors should note, however, that since figures and tables take up a considerable amount of space, they should be limited in number. Many authors used flow charts to represent experimental strategy or line drawings or photographs of equipment, most of which are unnecessary. Sometimes multiple figures are used with very little data on each, and which could be consolidated.

Figure and table captions should be constructed with care. There should be sufficient information for the reader to understand the subject matter, but it is not necessary to write an extensive text to explain all the detail.

You should consult the web site for more information. The journal itself is also accessible electronically, so feel free to peruse the articles for additional examples. You'll have to access the journal through the university library web site. The online journal web site is http://www.lib.utexas.edu/journals/. Search for "Biomaterials". This will automatically take you to the Biomaterials Journal web site. You can, however, only access the electronic journal from a campus computer. Hard copies are available for your reference in the lab.

Finally, keep in mind that this is a *group* assignment. That means all group members will receive the same grade. You should proof-read the entire report, even sections that were assigned to your group mates, before submitting it. Your name on the report implies that you will take full responsibility of its contents.

Abstract

This is the most important section of your long report. In fact, the reader's first impression of the overall report is frequently formed after reading the abstract. A well-prepared abstract therefore enables the reader to identify the basic content of the document quickly and accurately, to determine its relevance to his/her interest, and to decide whether or not he/she needs to read the document in its entirety.

A useful abstract is a complete, accurate, and concise summary of your long report. It is an actual condensation of the subject matter; the reader should therefore be able to grasp the major findings of the report and their relative importance and relationships from reading the abstract. The abstract should include a quantitative summary of what

you did, the results you obtained, and the conclusions based on these results. The range of numerical values should be given for important parameters, variables, and results. The abstract, however, is not a textual table of contents.

Although the abstract is generally the first section that is read, it should be the last part of the report to be written. The construction of the abstract is the last step of an arduous job. Despite its importance, students are apt to throw the abstract together as fast as possible. For many students, it is a relief to finish the report; hence, they spend very little time on the abstract. The time spent in learning the “general rules” that govern the construction of a good abstract will pay high dividends in the end.

1. Write the abstract last, after you have written the entire report.
2. Make sure you cover these five main points in your abstract:
    a. the principal objectives and the scope of the experiment
    b. the methodology employed
    c. quantitative results
    d. conclusions
    e. recommendations
3. Do not cite references to the literature (this includes references to sections of the main body of the text, figures, tables and bibliographic information) in the abstract. Do not put figures or tables in the abstract. Equations should not be numbered.
4. The abstract should be self-contained. Most abstracts will, at some point, be separated from the parent report. It is therefore important that the significant results, conclusions, and recommendations must be included. The program committees of many engineering conferences will often use abstracts (and only abstracts) as the basis for accepting papers to be presented.
5. The abstract should never give any information or conclusion that is not stated in the main body of the report.

Writing Evaluation Rubrics

The instructor and TAs will evaluate your various writing assignments during the semester using the following rubrics.

Long Report Evaluation

Students: ______________________________	
Project Title:______________________ **Date:**_________ **Evaluated By:**________	
Technical Content	
(a) Introduction/Background (is the problem clearly defined, and placed into proper context within the existing knowledge base? Has the group investigated the problem and the state of current knowledge?):	
(b) Quality and quantity of data (are the data what could be expected from a well-prepared group, and has the group exercised appropriate care in the collection of their data? Is adequate detail given on the experimental design and data collection that someone at another institution could repeat the results?)	
(c) Data analysis (have the data been analyzed in a sensible fashion? Have the major sources of error been identified, and quantified to the extent possible?)	
(d) Technical understanding of the experiment, as reflected throughout (introduction, data analysis, conclusions)	
Comments: __/20	
Abstract	
Is the abstract an effective abstraction of the report, communicating the purpose and principal findings? Does it conform to the length restriction?)	
Comments: ___/5	
Style and Presentation	
(a) Mechanics of organization (does the document conform to the format of a manuscript to be submitted to *Biomaterials*, including length? Does the report effectively convey the information? Is there a logical sequence to the material presented? Is information properly put in the main body, as its importance dictates? Are statements appropriately referenced?)	
(b) Writing style (Does the document read smoothly, and hold the reader's interest? Any particular examples of poor phrasing, word choice, *etc*.)	
(c) Clarity of tables and figures (Are they legible? Is the choice of tables vs. figures to present data appropriate? Are figures constructed so as to make their point at first glance?)	
(d) Appearance (does the report look professional, or at least as if it were prepared with care? Are there significant typographical errors, misspellings, *etc*.?)	
Comments: _____/10	

More on next page

Professional Quality and Format of Report
(a) Does the report use the 3rd person exclusively and present the work as a research effort instead of a laboratory report?
(b) Is the report 10 pages or less, double-spaced, no less then 10 point font. Are all of the margins 1” or less? Are all pages numbered? Are all parts of the paper in the correct order?
(c) Is the tense correct? The bulk of the report should be in the past tense as the work has already been done. The present tense should be used when describing equipment.
(d) Are all references placed where needed and is the correct citation form used?
Comments ____/15

Total	____/50
Performance Grades:	
Student’s Initials/Grade (of 5)	

Further Comments:

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Appendix 2

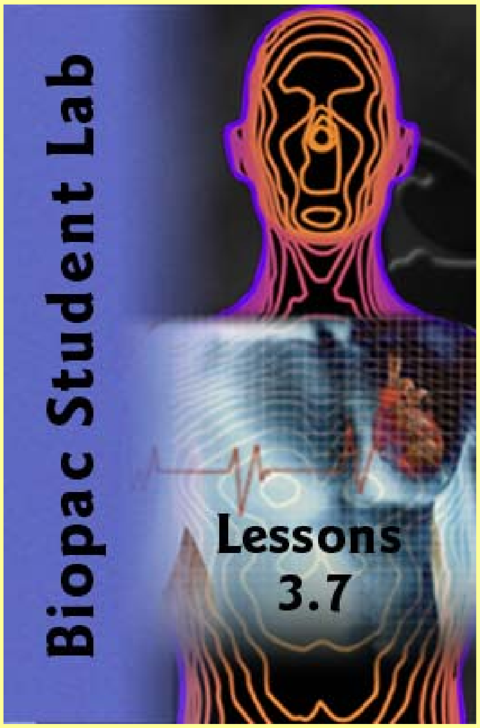
BIOPAC TUTORIAL AND REACTION TIME LAB

The following documents collected here are required materials for week #1 EOG laboratory session.

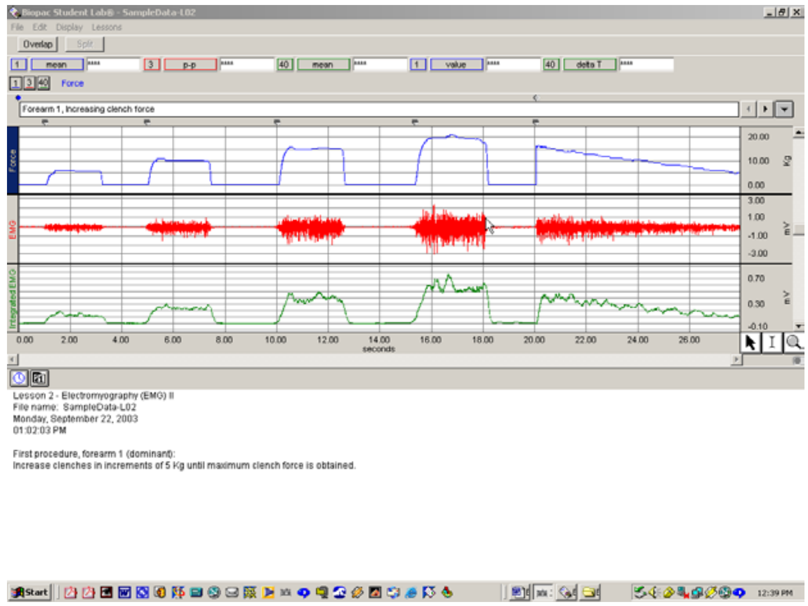
Biopac Tutorial

The Biopac tutorial follows.

BIOPAC Systems, Inc.



BASIC TUTORIAL



PC running Windows® 98SE, Me,
2000 Pro or XP Pro/Home

Jocelyn Mariah Kremer
Documentation
BIOPAC Systems, Inc.

William McMullen
Vice President
BIOPAC Systems, Inc.

BIOPAC Systems, Inc.
42 Aero Camino, Goleta, CA 93117
Phone (805) 685-0066
Fax (805) 685-0067
Email: info@biopac.com
Web Site: http://www.biopac.com

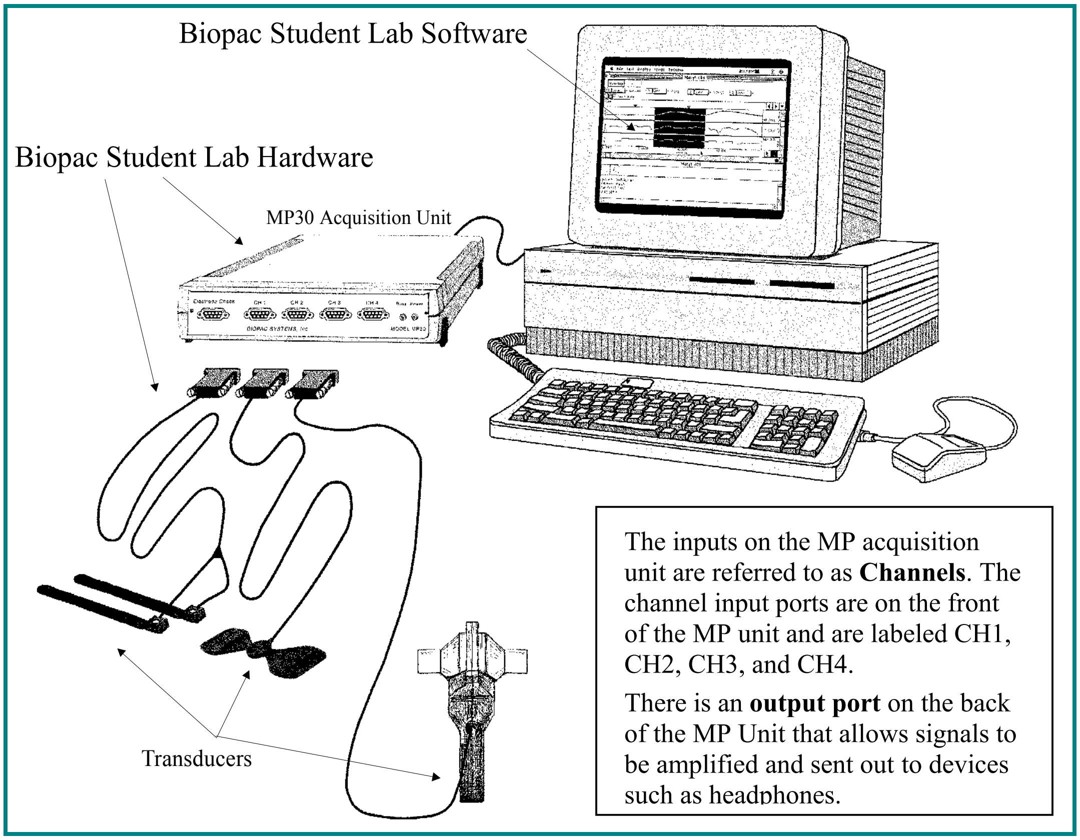
[042304]

Welcome to the Biopac Student Lab!

This short Tutorial covers basic concepts that make the Biopac Student Lab System unique and powerful, and provides detailed instructions on how to use important features of the program for data recording and analysis. You are encouraged to open the Sample Data File and follow along as you complete the Tutorial. Have fun experimenting with the display and analysis functions of the Biopac Student Lab—interacting with the software as the Tutorial explains functionality will ease the learning curve. For more information, see your instructor or review the Software Guide.

Biopac Student Lab System

The **Biopac Student Lab System** is an integrated set of software and hardware for life science data acquisition and analysis.



Software The Biopac Student Lab software includes 17 guided Lessons and BSL *PRO* options for advanced analysis. The software will guide you through each Lesson with buttons and text and will also help you manage data saving and data review.

Hardware Includes the MP30 or MP35 Acquisition Unit, electrodes, electrode lead cables, transducers, headphones, connection cables, wall transformer, and other accessories.

HOW THE BIOPAC STUDENT LAB WORKS

One way to think about how the Biopac Student Lab works is to think about it as being like a video camera connected through a VCR into a television set. In a general sense, a video camera records information about the outside world and then converts the images it collects into an electronic format that can be passed to the VCR and television. The images the VCR captures are stored on videotape to be archived or viewed at a later date.

Like a video camera, the Biopac Student Lab records information about the outside world, although the types of information it collects are different. Whereas cameras record visual information, the Biopac Student Lab records information (“signals”) about your physiological state, whether in the form of your skin temperature, the signal from your beating heart or the flexing of an arm muscle.

This physiological information is transferred via a cable from you (or whomever the information is being recorded from) to the Biopac Student Lab. The type of physiological signals you are measuring will determine the type of device on the end of the cable.

When the signal reaches the Biopac Student Lab, it is converted into a format that allows the data to be read by a computer. Once that is done, the signal can be displayed on the computer screen, much like the video images from the camera are displayed on the television set.

It takes about 1/1,000 of a second from the time a signal is picked-up by a sensor until it appears on the computer screen. The computer's internal memory can save these signals much like the VCR can save the video images. Like a videotaped record, you can use the Biopac Student Lab to recall data that was collected some time ago. And like a video, you can edit and manipulate the information stored in a Biopac Student Lab computer file.

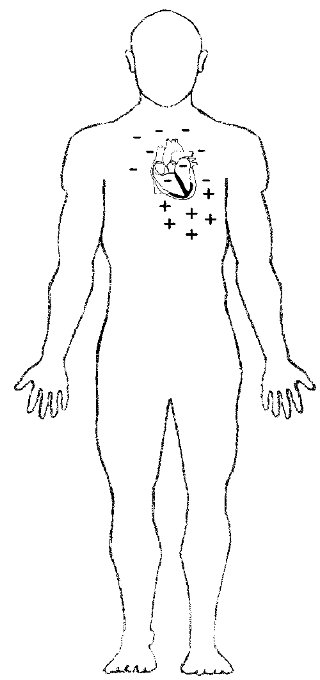
The Biopac Student Lab software takes the signal from the MP unit and plots it as a waveform on the computer screen. The waveform of the signal may be a direct reflection of the electrical signal from the MP channel (amplitude is in Volts) or a different waveform that is based on the signal coming into the MP unit.

- For example, the electrical signal into the MP unit may be an ECG signal, but the software may convert this to display a Beats Per Minute (BPM) waveform.

The Body Electric

When most people think of electricity flowing through bodies, they think of rather unique animals, such as electric eels, or of rare events such as being struck by lightning. What most people don't realize is that electricity is part of everything our bodies do...from thinking to doing aerobics—even sleeping.

In fact, physiology and electricity share a common history, with some of the pioneering work in each field being done in the late 1700's by Count Alessandro Giuseppe Antonio Anastasio Volta and Luigi Galvani. Count Volta, among other things, invented the battery and had a unit of electrical measurement named in his honor (the Volt). These early researchers studied "animal electricity" and were among the first to realize that applying an electrical signal to an isolated animal muscle caused it to twitch. Even today, many classrooms use procedures similar to Count Volta's to demonstrate how muscles can be electrically stimulated.



Electricity is part of everything your body does...from thinking to doing aerobics—even sleeping

Through your lab work, you will likely see how your body generates electricity while doing specific things like flexing a muscle or how a beating heart produces a recognizable electric "signature." Many of the lessons covered in this manual measure electrical signals originating in the body. In order to fully understand what an electrical signal is requires a basic understanding of the physics of electricity, which properly establishes the concept of **voltages**, and is too much material to present here. All you really need to know is that electricity is always flowing in your body, and it flows from parts of your body that are negatively charged to parts of your body that are positively charged.

As this electricity is flowing, sensors can "tap in" to this electrical activity and monitor it. The **volt** is a unit of measure of the electrical activity at any instant of time. When we talk about an electrical signal (or just signal) we are talking about how the voltage changes over time.

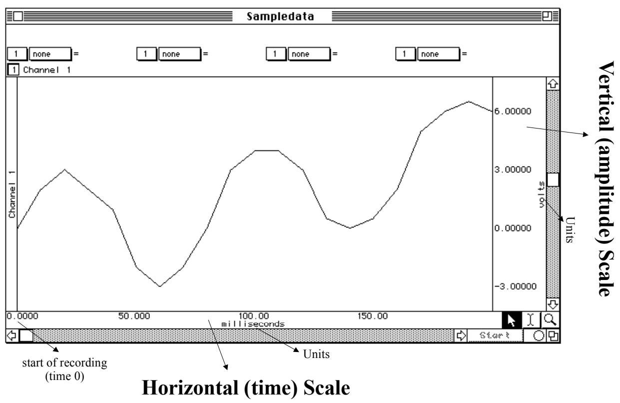
The body's electrical signals are detected with transducers and electrodes and sent to the MP acquisition unit computer via a cable. The electrical signals can be very minute—with amplitudes sometimes in the microVolt (1/1,000,000 of a volt) range—so the MP30 amplifies these signals, filters out unwanted electrical noise or interfering signals, and converts these signals to a set of numbers that the computer can read. These numbers are sent to the computer via a cable and the Biopac Student Lab software then plots these numbers as waveforms on the computer monitor.

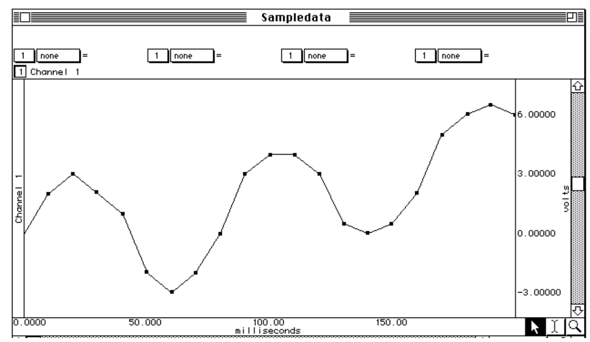
Waveform Concepts

A basic understanding of what the waveforms on the screen represent will be useful as you complete the lessons.

Amplitude is determined by the BSL System based on the type of MP input. The units are shown in the vertical scale region; the unit for this example is Volts.

Time is the time from the start of the recording, which is to say that when the recording begins it does so at what the software considers time 0. The units of time are shown in the horizontal scale region along the bottom of the display; the unit for this example is milliseconds (1/1,000 of a second).





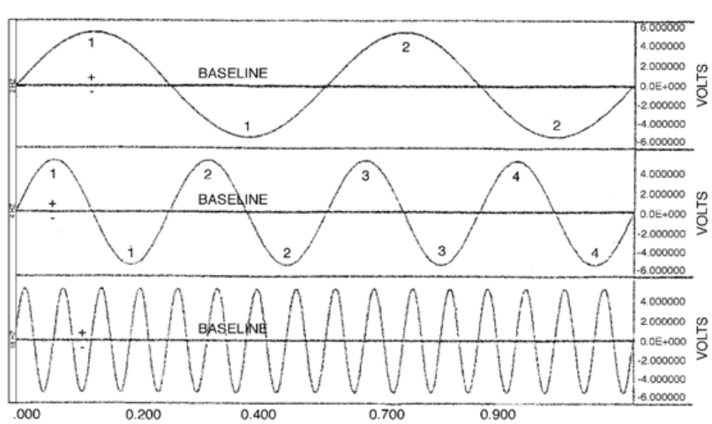
Diving a little deeper into what a waveform represents, you are actually looking at **data points** that have been connected together by straight lines.

These data points are established by the Biopac Student Lab hardware by sampling the signal inputs at consistent time intervals. These data points can also be referred to as **points**, **samples**, or **data**.

The time interval is established by the **sample rate** of the BSL hardware, which is the number of data points the hardware will collect in a unit of time (normally seconds or minutes). The BSL software stores these amplitude values as a string of numbers. Since the sample rate of the data is also stored, the software can reconstruct the waveform.

- Sampling the data is very similar to how a VCR records images from a camera by taking snapshots of the image at specific time intervals. When it plays back the tape, it displays the captured images in quick succession, and our eyes can't see the starting and stopping. Likewise, when you look at the waveform, you see a continuous flow rather than the data points and straight lines.

The Biopac Student Lab Lessons software always uses the same sample rate for all channels on the screen, so the horizontal time scale shown applies to all channels, but each channel has its own vertical scale. A channel's vertical scale units can be in Volts, milliVolts, degrees F, beats per minute, etc. A **baseline** is a reference point for the height or depth ("amplitude") of a waveform.



- Amplitude values above the baseline appear as a "hill" or "peak" and are considered positive (+).
- Amplitude values below the baseline appear as a "trough" or "valley" and are considered negative (–)

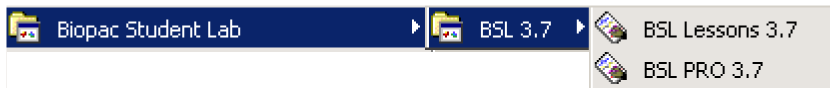
Sample Data File

This Tutorial is designed for you to follow along with a sample data file on a computer without Biopac Student Lab hardware attached. This means that you can complete the Tutorial on a computer outside of the classroom/lab—perhaps at the library, computer lab or home—just as you will always have those options for analyzing data outside the lab. Open the SampleData-L02 file as directed below.

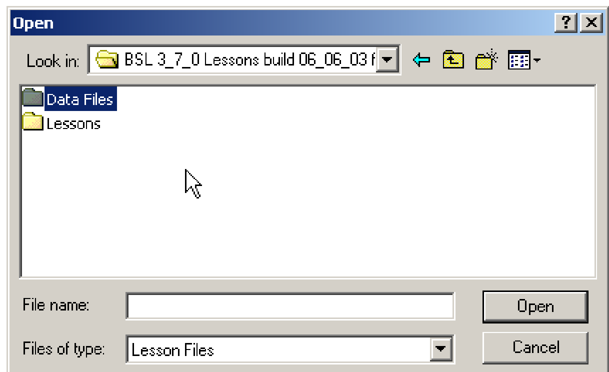
1. Turn the computer ON.
2. Use the desktop icon or the Windows **Start** menu to open **BSL Lessons 3.7**.

To launch the program use desktop icon

or use the Windows® **Start** menu, click Programs and then select:



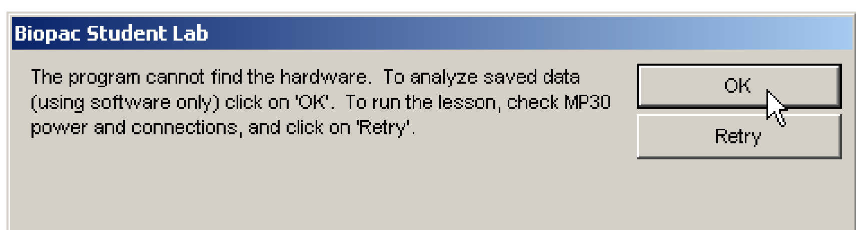
3. In the No Hardware mode, the BSL Program will open to a standard Open Dialog.



Note: A hardware dialog may be generated.

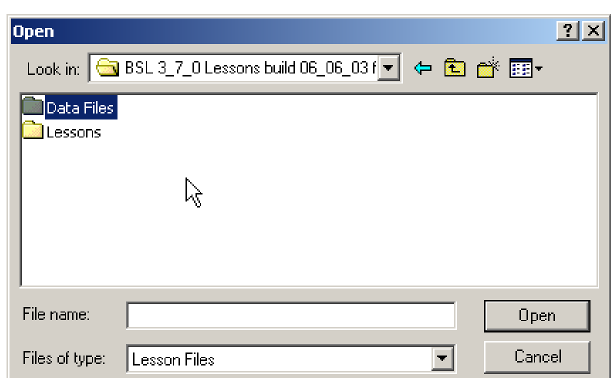
- For this tutorial (and all future analysis), click **OK** to enter the Review Saved Data mode.

If the program was installed with the hardware option but there is no hardware connected, the following dialog may be generated:



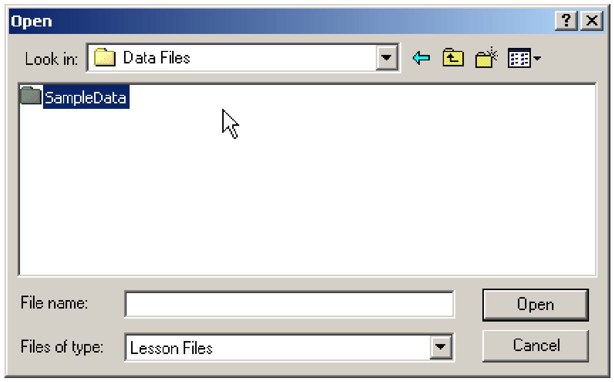
4. Open the **Data Files** folder.
   - The program may open the Data Files for you. If so, skip to the next step.
   - For future analysis, use this dialog to browse to your data files.

Open the **Data Files** folder, which is in the **Biopac Student Lab** program folder.



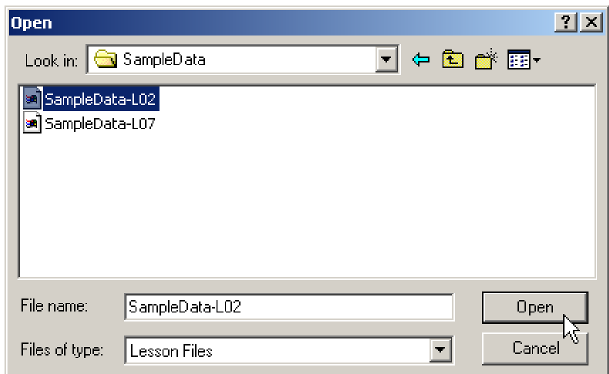
5. Open the **SampleData** folder.

Open the **SampleData** folder, which is in the **Data Files** folder.



6. Open the **SampleData-L02** file.

Select and open the **SampleData-L02** file, which is in the **SampleData** folder.



Don't worry — you can't lose or damage the SampleData-L02 file.

BSL Display

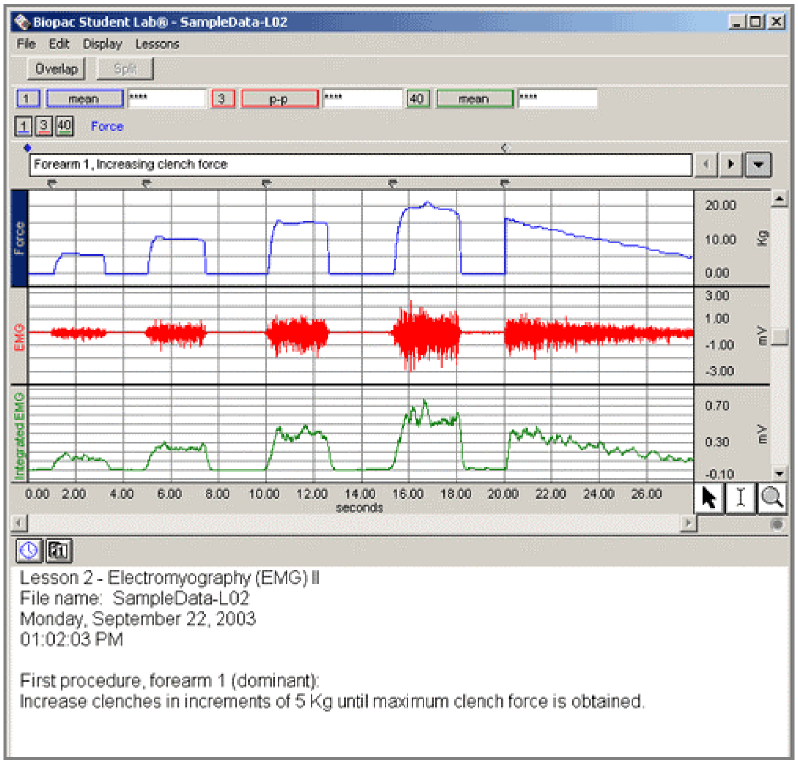
The display includes a Data window and a Journal and both are saved together in one file.

- The **Data window** displays the waveforms and is where you will perform your measurements and analysis.
- The **Journal** is where you will make notes. You can extract information from the Data window and put it in the Journal and you can export the Journal to other programs for further analysis.

The Biopac Student Lab software has a variety of **Display Tools** available that allow you to change the data display by adjusting axis scales, hiding channels, zooming in, adding grids, etc. This can be very useful when you are interested in studying just a portion of a record, or to help you identify and isolate significant data in the record for reporting and/or analysis.

7. Review the display to identify the display elements of the **Data Window** and the **Journal**.
   - The **Data Window** displays waveform(s) during and after recording, and is also called the "Graph Window." Up to eight waveforms can be simultaneously displayed, as controlled by the software and lesson requirements.
   - The **Journal** works like a standard word processor to store recording notes and measurements, which can then be copied to another document, saved or printed.

The SampleData-L02 file should open as shown below:



Biopac Student Lab Display

Top down, the sections of the display are:

- Title Bar (BSL program name and file name)
- Menu Bar (File, Edit, Display, Lessons)
- Tool Bar (lesson specific buttons, such as Overlap and Split)
- Measurement Region (channel, type, result)
- Channel Box(es) and Channel Label(s)
- Marker Region (icons, text and menu)
- Data window — waveform display
- Horizontal scroll for Data Window
- Display Tools (to the right of the Horizontal Scale) — Selection, I-beam, and Zoom icons
- Journal Tool Bar (Time and Date icons)
- Journal

Display Tools

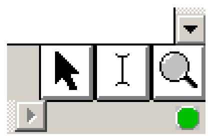
The BSL allows you *complete flexibility* in how the data is viewed. Chart recorders lock you into one view, but with the BSL you can expand or compress the visual scales to aid in data analysis. The Data window display is completely adjustable, which makes data viewing and analysis easier.

- View multiple channels or hide channel(s) from the display view.
- Zoom in on specific segments to take measurements, examine anomalies, etc.
- View the entire record at one time to look for trends, locate anomalies, etc.

Editing and Selection Tools

8. Locate the editing and selection tool icons in the lower right of the Data Window.

A good starting point is to understand the editing and selection tools. In the lower right of the data window there are three icons representing the **Arrow**, **"I-Beam,"** and **Zoom** tools.



To select any of these tools simply click the mouse on the desired icon, and it will appear recessed to indicate it is active (the Selection tool is active/recessed in the picture above).

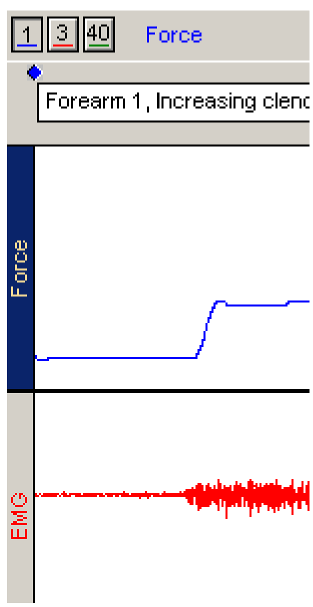
Each tool activates a different cursor in the display window:

Arrow cursor   I-beam cursor   Zoom cursor

Active Channel

9. Click in the CH 1 box to make that the "active channel."
   - The channel box of the active channel will be generated to be recessed, and the label for the active channel will be highlighted on the left edge of the channel display.
   - You can also click on the channel label to make a channel active.

10. Click in the CH 40 box and note how the label changes.



CH 1 active, CH 3 and CH 40 shown (note "Force" is highlighted on the left edge)

CH 1 and CH 3 shown, CH 40 active

Channel boxes are in the upper left of the data window. They enable you to identify the active channel and hide channels from view, so as to concentrate on or print out only specific waveforms at a time.

The display can **Show** one or more data channels, but only one channel can be "active" at any time. The "active" channel box appears recessed.

The Label for the active channel is displayed to the right of the channel boxes and highlighted in the display region.

Show/Hide a channel

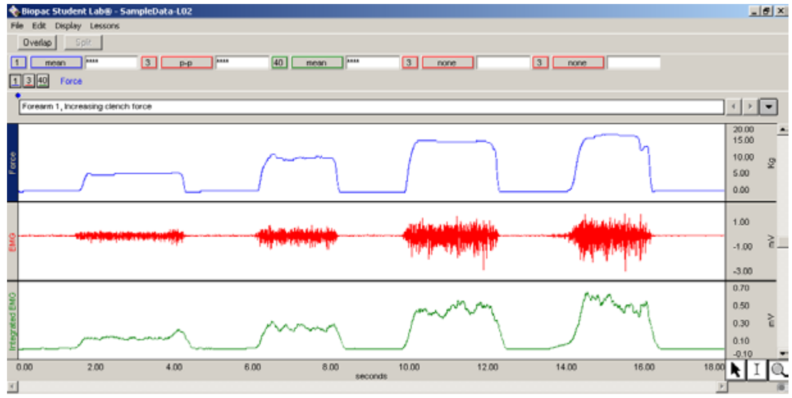
11. To Show or Hide a channel hold the "Ctrl" (Control) key down and click on the Channel box.
    a. Hide CH 40.

When you **Hide** a channel, the data is not lost, but simply hidden, so that you can focus on specific channel(s). Hidden channels can be brought back into view at any time. The channel box displays "slash marks" when it is hidden.

b. Show CH 40.

c. Hide CH 40.

- Showing a channel enables the channel display but does not make it the active channel.
- Hiding an active channel does not prevent it from being the active channel.



Show/Hide Grid Display

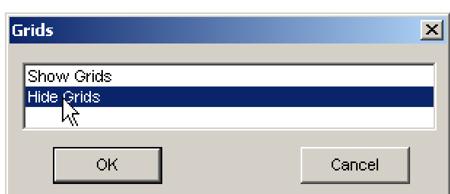
12. Pull down the **File** menu and scroll down to select **Display Preferences**.

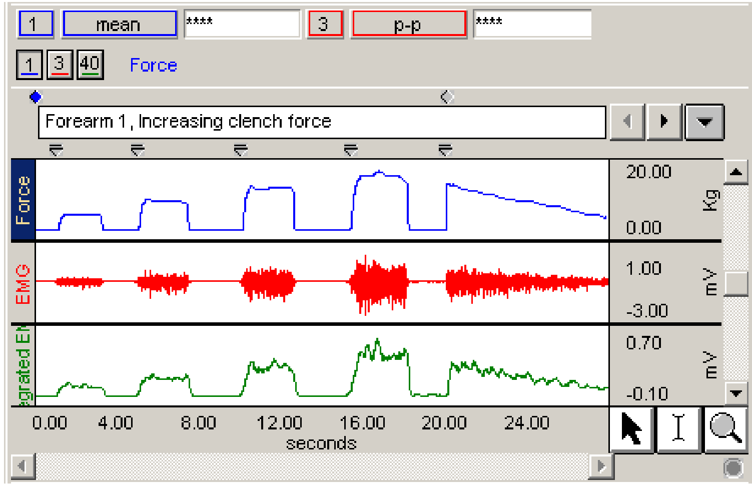
Another powerful feature is the ability to Show or Hide the grid display. A **grid** is a series of horizontal and vertical lines that assist the eye with finding data positions with respect to the horizontal and vertical scales. The horizontal grid is the same for all channels (since they share the Time base), but the vertical grid can be set for each channel.

13. Select **Hide Grids** to turn the grid display OFF, and click **OK**.

14. Review the display without grids.

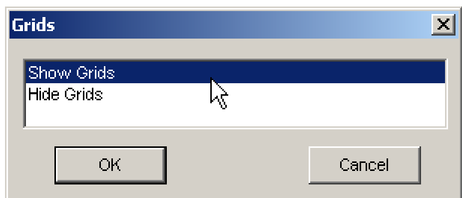
To turn grids on and off in the Review Saved Data mode, simply choose **Display Preferences** from the **File** menu. A Grids dialog will be generated. Make your selection and click OK.





Note that the Grids display affects all channels. If you show a channel that was hidden when Grids were activated, the grid display will show on the channel.

15. **Show Grids**.



To adjust the grids, see Scales & Grids on page 14.

Scroll - Horizontal

16. Locate the **Horizontal Scroll Bar** at the lower edge of the display.

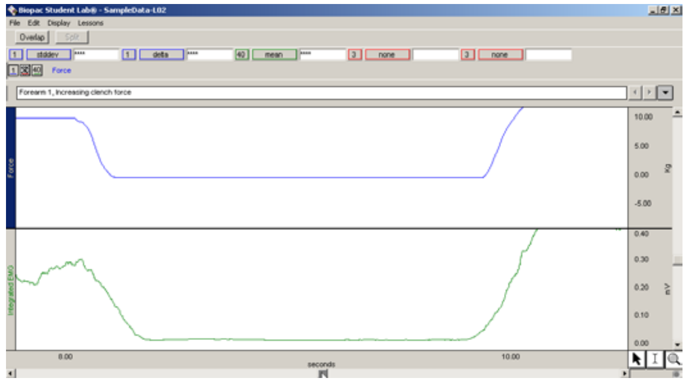
You can move to different locations in the record by using the horizontal scroll bar. Since the horizontal scale applies to all channels in view, it will move every waveform simultaneously.

The scroll bar is active when only a portion of the waveform is in view. To move forward or backward, select and drag the scroll box or click on the left or right arrow. For a continuous scroll, click on the arrow and hold down the left mouse button.

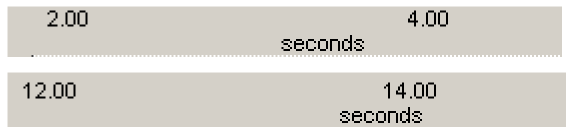
If the entire waveform is displayed, the scroll bar will dim.

17. Use the **Horizontal Scroll** to reposition the data with respect to time.

    - Note that both waveforms moved. This is because the horizontal scale is the same for all channels.



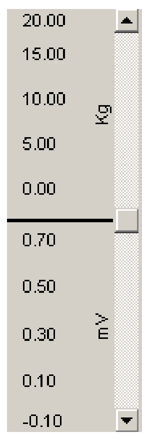
In the sample file, the horizontal scale represents Time in seconds. The software will set the most appropriate Time option for each signal.



Notice the Horizontal Scale range on the bottom changes to indicate your position in the record.

Scroll – Vertical

18. Locate the **Vertical Scroll Bar** along the right edge of the display.

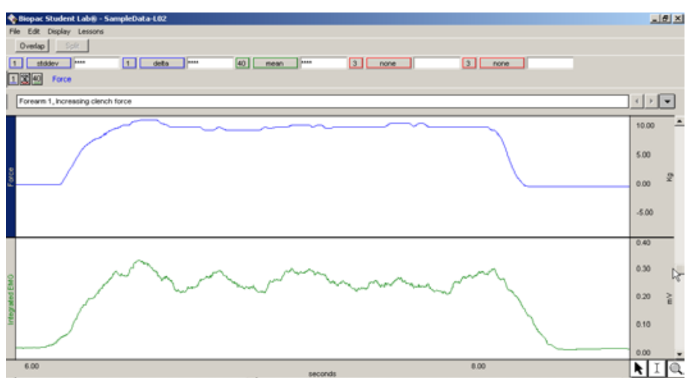


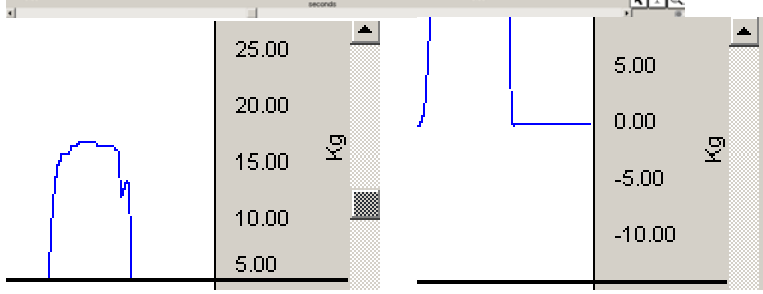
A similar scroll bar can be found next to the vertical scale. This is the **Vertical Scroll Bar**, and it allows you to reposition the waveform in the active channel. The Vertical Scroll Bar runs along the entire right edge of the display window, but only applies to the active channel, which is only a portion of the display if more than one channel is displayed.

The Vertical Scale Range for the active channel changes when you reposition a waveform to reflect the displayed range.

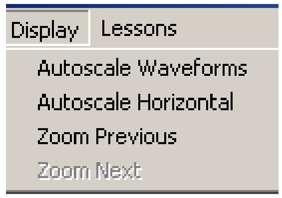
19. Use the Vertical Scroll to reposition the CH 1 Force waveform.

    - Note that the CH 40 Integrated EMG waveform did not move. This is because the vertical scale is independent for each channel.



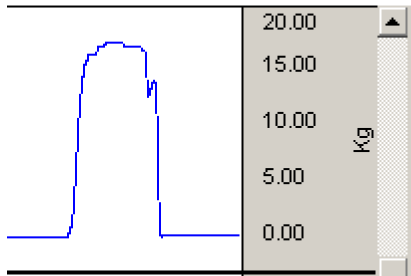


20. Pull down the Display menu and select **Autoscale horizontal** to fit the entire waveform within the data window.

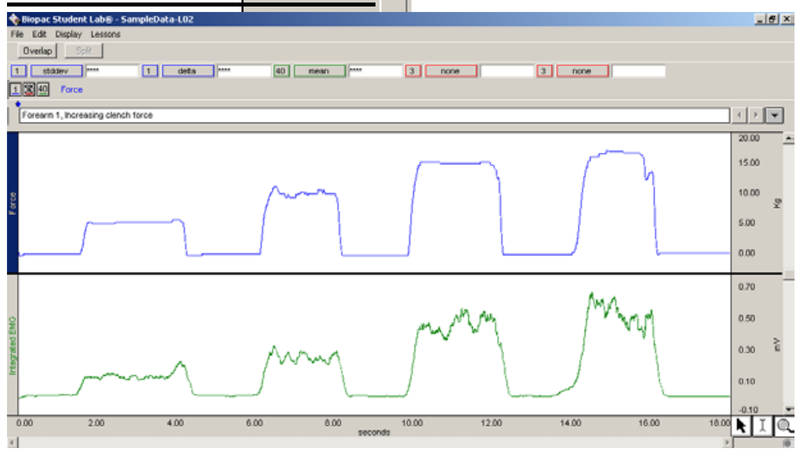


Autoscale horizontal from the **Display** menu is a quick way to fit the entire waveform within the data window. That is, it will adjust the horizontal scale such that the left most portion of the screen is the start of the recording, and the right most portion is the end of the recording. The time per division setting will not necessarily be even numbers, but you can adjust that.

21. Pull down the **Display** menu and select **Autoscale Waveforms** to center waveforms in their display track.



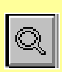
TIP — Selecting **Autoscale Horizontal** and then **Autoscale Waveforms** from the Display menu is the standard way to quickly and easily return to your original data display and view the entire record at once.

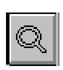


The Autoscale Waveforms option of the Display menu is a very handy tool that performs a "best fit" to each channel's vertical scale. That is, it will adjust the "Scale" and "Midpoint" of each channel's vertical scale, such that the waveform fills approximately two-thirds of the available area.

After autoscaling, the "Scale" will probably not be set to nice even numbers, but you can manually adjust the scale to even numbers.

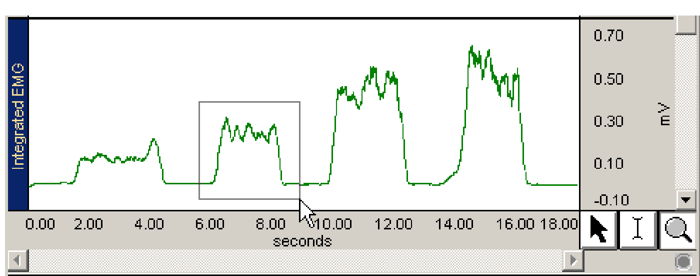
Zoom

22. Click on the **Zoom**  icon to select it.

The **Zoom**  icon is in the lower right of the data window. The **Zoom** function is very useful for expanding a waveform in order to see more detail.

23. Position the cursor in the CH 40 Integrated EMG channel at about 6 seconds, then click and hold the mouse button down and drag the cursor to about 9 seconds. This will draw a box around the area.

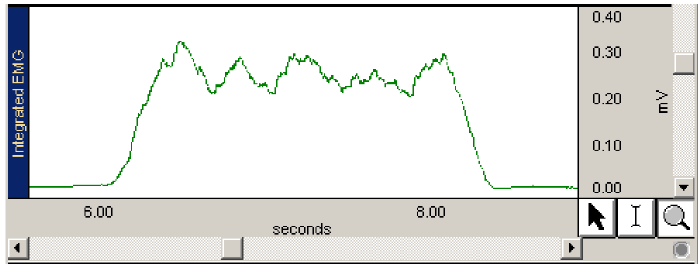
If you know the precise section of the waveform that you'd like to enlarge, you can use the Zoom tool to draw a box around the area.

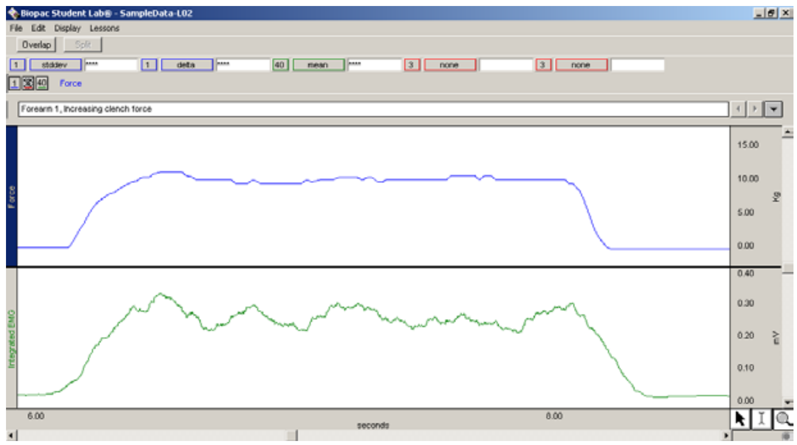


24. Release the mouse button and review the result.

    - Note that the Horizontal and Vertical Scales changed for the selected channel.

When the mouse button is released, the boundaries of the selected area become the new boundaries of the data window.





The Vertical Scale will change for the active channel only, but the Horizontal Scale will change for all channels since it (time) is the same for all channels.

25. Select **Zoom previous** to “undo” the Zoom.

After you have zoomed in on a section of the waveform, you may “undo” the zoom and revert to the scale settings (both horizontal and vertical) established prior to the last zoom by selecting **Zoom previous** from the **Display** menu.

The **Zoom previous** function will only go back one **Zoom** function. You cannot select it 6 times, for instance, to go back 6 zooms.

Scale & Grid Controls

Adjust Scales

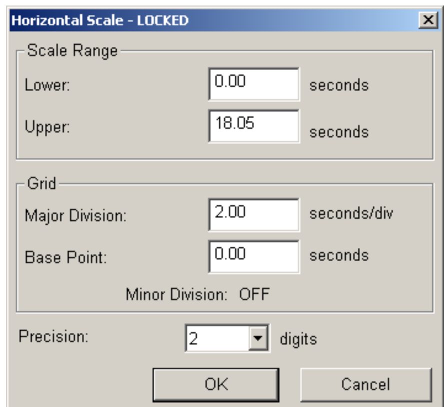
26. Click anywhere in the **Horizontal Scale** region to generate the adjustment dialog.
    - Review the Scale Range and Grid settings.
27. Click anywhere in the **Vertical Scale** region to generate the adjustment dialog.

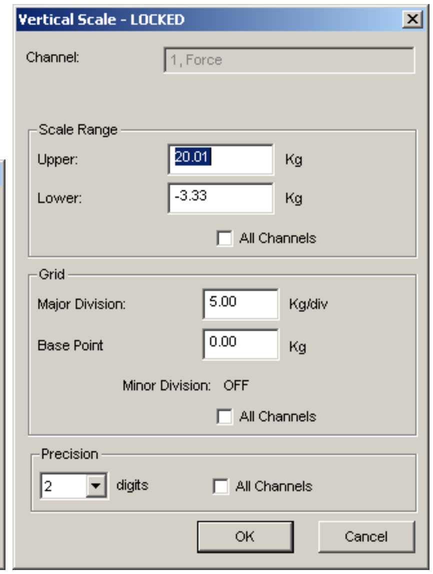
    Note that the Vertical Scale is independent for each channel.

    - Review the Scale Range and Grid settings.

If you click anywhere within the **Scale** region (Horizontal or Vertical) an adjustment dialog will be generated. Any change you make to the Horizontal or Vertical Scale only effects how the display and never alters the saved data file. That is to say, you will never lose any data when you change these settings.

The Vertical Scale is independent for each channel, so you need to select the appropriate channel prior to clicking on the Vertical Scale.





Scale Range: Establishes the boundary values to fit in the display window and is useful for highlighting meaningful segments in the waveform. For instance:

- If the signal has a cycle that repeats every 2 seconds, you could set the Horizontal Scale Range to at 0-8 seconds to show 4 cycles per screen.
- If the signal varies from -2 to +2 Volts, you could set the Vertical Scale Range to match and optimize the display.

Grid: The **Major Division** is the interval for the grid line (i.e., a horizontal line every 2 seconds or a vertical line every 5 Kg). The **Base Point** is the origin for the grid lines, with the Major Divisions drawn above and below (horiz) or left and right (vert) to complete the grid. Minor Divisions are also drawn in relation to the Base Point, if the lesson is set to show them.

All Channels is a quick way to have the scale setting apply to all of the vertical scales in the data window. This is particularly useful when all of the channels are the same type of data (i.e. 2 or 3 channels of ECG data). Repeated clicking in the box will toggle the option on or off.

Precision: Establishes the number of significant digits displayed in the Scale region. Click and hold down the mouse on the precision number to generate a pop-up menu, allowing you to make another selection.

OK: Click on "OK" to initiate the changes made in the dialog.

Cancel: Click on "Cancel" to if no changes are desired.

Measurements

Measurements are fast, accurate, and automatically updated. The measurement tools are used to extract specific information from the waveform(s). Measurements are used in the Data Analysis section of every lesson, so understanding their basic operation is important.

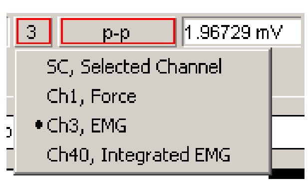
Let's say you wanted to know the force increase between two clenches in the EMG data. You could get a rough estimate by eyeballing the amplitude of the peaks on the vertical scale or by measuring the peak of one and the peak of the next and manually calculating the difference. Or, you could take a much easier and more accurate reading with the "delta" measurement of the Biopac Student Lab. You can use software shortcuts to paste measurement data to the journal or copy waveform data for further analysis in other programs such as Excel.

Measurement tools

28. Review a measurement region to identify the Channel select box, the Measurement type box, and the Result box.

Measurement Region
Channel select, Measurement type, Result

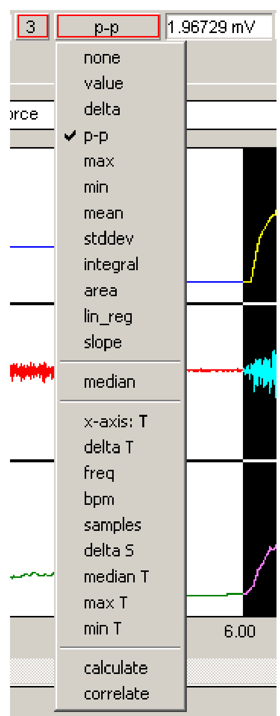
The **Channel select** box includes options for all channels, whether shown or hidden, and an "SC" measurement option. The "SC" performs the measurement on the active channel and is a quick way to step through multiple channels. The "SC" option allows you to make quick measurement comparisons between channels using one region. To take a measurement from another channel, simply click on the desired channel box or click anywhere within the data region for the desired channel using the selection tool.



Channel select options

The **measurement type** box is a pop-up menu next to each channel number box that allows you to choose from 23 Biopac Student Lab measurement functions (or "none").

The **measurement result** is the value that the measurement calculates.



Measurement type options

29. Read about **Measurement Tools** to the right.

To use the measurement tools, you must

a) Set the channel measurement box to the desired channel.

b) Select a measurement type from the pop-up menu.

c) Select an area for measurement.

Note that you can perform these elements in any order, but all three must be completed to achieve a valid measurement.

Two important points regarding measurements:

1. The first is that the measurement only applies to data in the selected area of the waveform that the user specifies.
2. The second is that every lesson contains the same measurement options, but some may not be applicable to that particular lesson. This is because the measurement options are a standard set of tools that are always available, much like a scientific calculator contains a standard set of buttons, many of which may not be necessary for any given problem.

The Selected Area

30. Read about the **Selected Area** to the right.

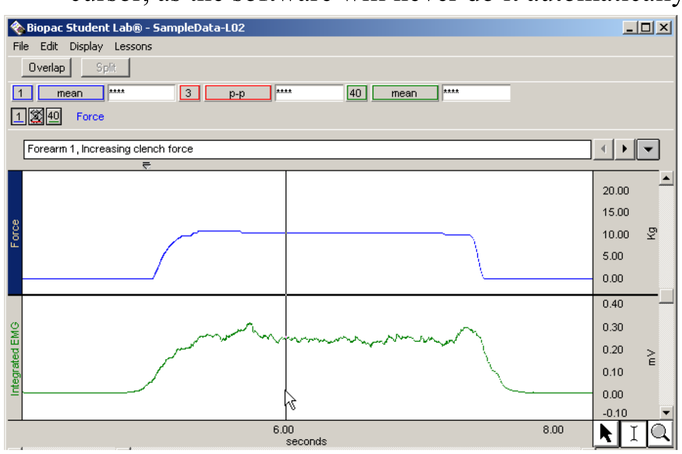
The "**selected area**" for all measurements is the area selected by **the I-Beam tool** (including the endpoints). Note that the "I-beam" cursor position when the mouse button was first pressed defines the starting point and the position at release defines the end position of the selected area.

A critical concept for the measurement tools is that the measurement results only apply to the area established by the "I-Beam" cursor.

- The selected area can be a single point, an area, or the end points of a selected area.
- **If there is no point or area highlighted on the screen, then the measurement results are meaningless**.
- A result of ******** means the selected area is not appropriate or sufficient for the meausurement.
- It is up to you to select a point or an area with the I-Beam cursor, as the software will never do it automatically.

Select a single-point area

31. Click on the **I-beam** [I] icon.



32. Move the cursor over a point on the data.

You will notice that whenever the cursor is over data it is displayed as an "I."

33. Click on the mouse button.

A flashing line should appear at the cursor position.

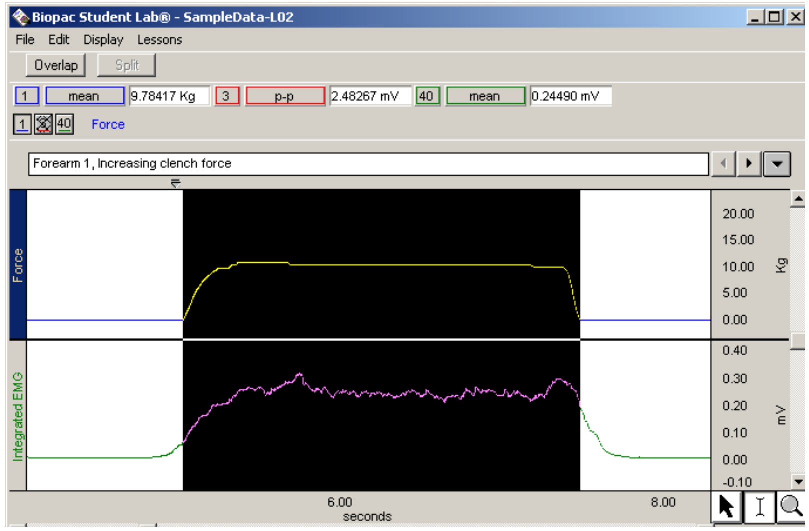
When you have a flashing line, you have one point of data selected. If the line is not flashing, it means that you moved the cursor while the mouse button was pressed, and you actually selected more than one point of data. If this occurred simply click on another portion of data.

34. Click on the selection tool icon to deactivate the point.

When you are finished taking measurements, and wish to deactivate a point, click on the selection tool icon.

Selecting an area (several points)

35. Click on the **I-Beam**  icon.
36. Move the cursor over a point on the data.
37. Hold the let mouse button down and drag the mouse to the right.
38. Release the mouse button.
    - An area should be highlighted.

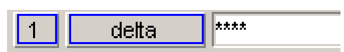


When the mouse button is released, an area should be highlighted (darkened) on the screen, as shown above. This is very similar to how you select words in a word processing program.

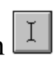
39. Click the mouse on another point of data and then click on the selection tool icon to deactivate the point.

When you are finished taking measurements and wish to deactivate a selected area, click the mouse on another portion of data to select just one point (flashing line appears), and then click on the selection tool icon.

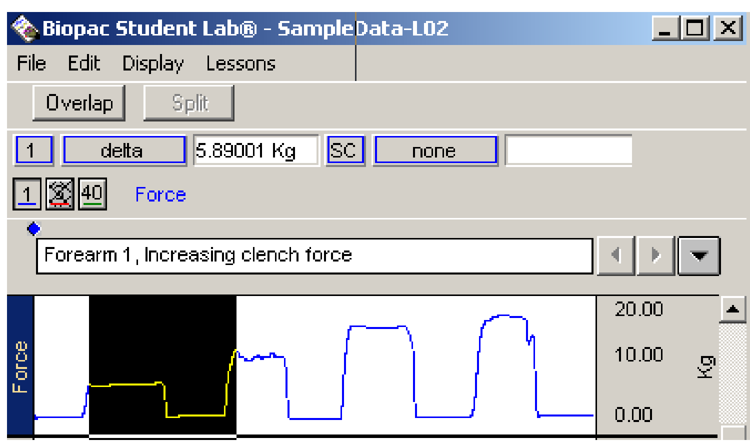
40. Set a region for **CH 1 Force** and **delta**.



41. Click on the I-beam  icon.

Click on the I-beam  icon to activate the I-beam cursor.

42. In the Force channel, use the I-beam to select an area from the peak of one clench to the peak of the next clench.
43. Review the result.
    - This example shows an increase of 5.89 Kg. Your result will vary.



If the correct region is not established by the "**I-Beam**" **cursor** for the measurement type, the result will be meaningless.

Results will update automatically when you change the channel selection or the selected area.

44. You can use the Data Window options in the Display menu to copy measurements to another program.

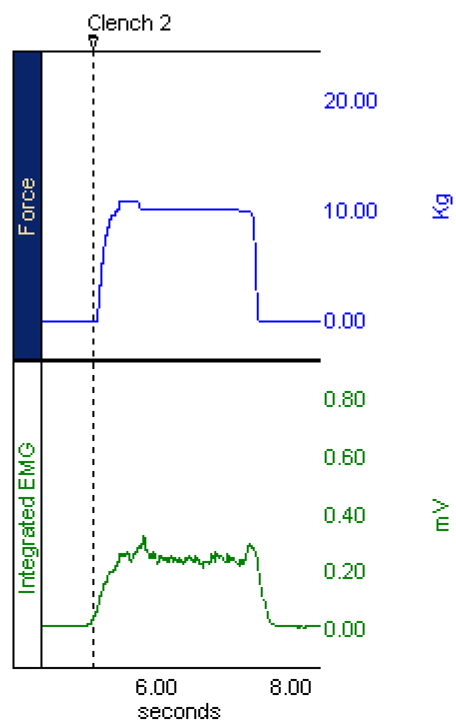
Copy Measurement — Use to copy the measurement values from the data window to another program, such as a Word document or email for your Lab Report.

delta(1) = 0.01392 Kg P-P(3) = 2.48267 mV Mean(40) = 0.15282 mV Value(1) = 0.06469 Kg deltaT(40) = 4.19600 sec

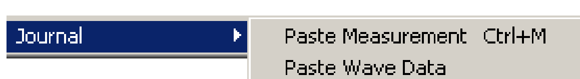
Copy Wave Data — Use when you want to copy waveform data as a set of numbers. The data copied will include all wave data within the area selected by the I-beam cursor for all channels, whether displayed or hidden.

sec	Force	EMG	Integrated EMG
4.526	0.0507694	-0.0258789	0.0121948
4.528	0.0507694	-0.0224609	0.0123364
4.53	0.0507694	-0.0141602	0.0124744
4.532	0.0507694	-0.00268555	0.0124585
4.534	0.0507694	-0.0090332	0.0124524

Copy Graph — Use when you want to copy the waveform data as a picture to be imported into other programs. The Data Window Copy functions will be applied to the selected area, if any. If a single point or no area is selected, the Data Window Copy functions will be applied to the <u>entire data file</u>.



45. You can use the Journal options in the Edit menu to copy measurements to the BSL Journal.



See the Journal section for details.

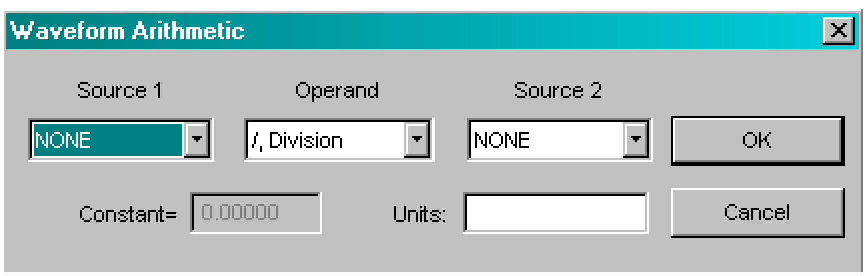
<table>
<tr><th>Measurement Tool</th><th>Definition</th></tr>
<tr><td>area</td><td>Area computes the total area among the waveform and the straight line that is drawn between the endpoints. Area is expressed in terms of (amplitude units multiplied by horizontal units).</td></tr>
<tr><td>bpm</td><td>The Beats Per Minute measurement uses the start and end points of the selected area as a measurement for one beat, calculates the difference in time between the first and last selected points, and divides this value into 60 seconds/minute to extrapolate BPM. This is the result you would obtain if you took $((1/\Delta T)*60)$ for a selected area. If more than one beat is selected, it will not calculate the average (mean) BPM in the selected area.

Note: In order to get an accurate BPM value, you must select an area with the I-Beam cursor that represents one complete beat-to-beat interval. One way to do this is to select an area that goes from the peak of one cycle's R wave to the peak of the next cycle's R wave (R-R interval).</td></tr>
<tr><td>calculate</td><td>Calculate can be used to perform a calculation using the other measurement results. For example, you can divide the mean pressure by the mean flow.

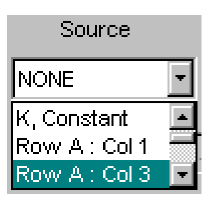
When Calculate is selected, the channel selection box disappears.

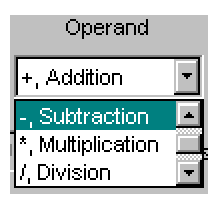
The result box will read "Off" until a calculation is performed, and then it will display the result of the calculation. As you change the selected area, the calculation will update automatically.

To perform a calculation, Ctrl-Click (or on PC, right mouse button click) on the Calculate measurement type box to generate the "Waveform Arithmetic" dialog.



Use the pull-down menus to select Sources and Operand.



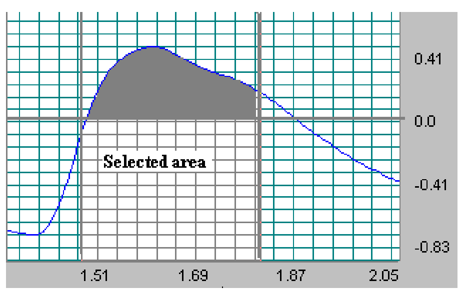


Measurements are listed by their position in the measurement display grid (i.e., the top left measurement is Row A: Col 1). Only active, available channels appear in the Source menu.

You cannot perform a calculation using the result of another calculation, so calculated measurement channels are not available in the Source menu.

The Operand pull-down menu includes: Addition, Subtraction, Multiplication, Division, Exponential.

The Constant entry box is activated when you select "Source: K, constant" and it allows you to define the constant value to be used in the calculation.</td></tr>
</table>

Measurement Tool	Definition
	To add units to the calculation result, select the **Units** entry box and define the unit's abbreviation. Click **OK** to see the calculation result in the calculation measurement box
correlate	**Correlate** provides the *Pearson* product moment correlation coefficient, r**,** over the selected area and reflects the extent of a linear relationship between two data sets: x_i - values of horizontal axis and $f(x_i)$ - values of a curve (vertical axis). You can use Correlate to determine whether two ranges of data move together. *Association* — *Correlation* Large values with large values — Positive correlation Small values with large values — Negative correlation Unrelated — Correlation near zero
delta	The Δ (delta amplitude) measurement computes the difference in amplitude between the last point and the first point of the selected area. It is particularly useful for taking ECG measurements because the baseline does not have to be at zero to obtain accurate, quick measurements.
delta S	The ΔS (delta samples) measurement is the difference in sample points between the end and beginning of the selected area.
delta T	The ΔT (delta time) measurement is the difference in time between the end and beginning of the selected area.
freq	The Frequency measurement converts the time segment between the endpoints of the selected area to frequency in cycles/sec. The Freq measurement computes the frequency in Hz between the endpoints of the selected range by computing the reciprocal of the ΔT in that range. It will not calculate the correct frequency if the selected area contains more than one cycle. You must carefully select the start and end of the cycle. *Note*: This measurement applies to all channels since it is calculated from the horizontal time scale.
integral	**Integral** computes the integral value of the data samples between the endpoints of the selected area. This is essentially a running summation of the data. **Integral** is expressed in terms of (amplitude units multiplied by horizontal units) This plot graphically represents the Integral calculation. The area of the shaded portion is the result. 
lin_reg	**Lin_reg** computes the non-standard regression coefficient, which describes the unit change in <u>*f (x)*</u> (vertical axis values) per unit change in <u>*x*</u> (horizontal axis). **Linear regression** is a better method to calculate the slope when you have noisy, erratic data. *For the selected area*, **Lin_reg** computes the linear regression of the line drawn as a best fit for all selected data points
max	The maximum measurement finds the maximum amplitude value within the selected area (including the endpoints).

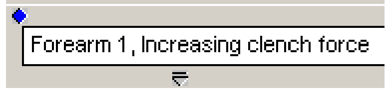
Measurement Tool	Definition
max T	**Max T** shows the time of the data point that represents the maximum value of the data samples between the endpoints of the selected area.
median	**Median** shows the median value from the selected area. *Note* The median calculation is processor-intensive and can take a long time, so you should only select "median" when you are actually ready to calculate. Until then, set the measurement to "none."
median T	**Median T** shows the time of the data point that represents the median value of the selected area. *Note* The median calculation is processor-intensive and can take a long time, so you should only select "median" when you are actually ready to calculate. Until then, set the measurement to "none."
mean	The mean measurement computes the mean amplitude value or average of the data samples between the endpoints of the selected area and displays the average value.
min	The minimum measurement finds the minimum amplitude value within the selected area (including the endpoints).
min T	**Min T** shows the time of the data point that represent the minimum value of the data samples between the endpoints of the selected area.
none	Selecting none turns off the measurement channel and no result is provided. It's useful if you are copying a measurement to the clipboard or journal with a window size such that several measurements are shown and you don't want them all copied.
p-p	The p-p (peak-to-peak) finds the maximum value in the selected area and subtracts the minimum value found in the selected area. P-P shows the difference between the maximum amplitude value in the selected area and the minimum amplitude value in the selected area.
samples	The Samples measurement shows the exact sample number of the selected waveform at the cursor position. Since the Biopac Student Lab handles the sampling rate automatically, this measurement is of little use for basic analysis.
slope	The slope measurement uses the endpoints of the selected area to determine the difference in magnitude divided by the time interval. The slope measurement returns the unstandardized regression coefficient, which describes the unit change in Y (vertical axis values) per unit change in X (horizontal axis). This value is normally expressed in unit change per second (rather than sample points) since high sampling rates can artificially deflate the value of the slope. When the horizontal axis is set to display either frequency or arbitrary units, the slope is expressed as a unit change in vertical axis values per change in Hertz or arbitrary units, respectively. When an area is selected, the slope measurement computes the slope of the line drawn as a best ft for all selected data points.
stddev	Stddev (standard deviation) is a measure of the variability of data points that computes the standard deviation value of the data samples in the selected range. The advantage of the stddev measurement is that extreme values or artifacts do not unduly influence the measurement.
x-axis: T (time)	The Time measurement shows the exact time of the selected waveform at the cursor position. If a range of values is selected then the measurement will indicate the time at the last position of the cursor.
value	The value measurement displays the amplitude value for the channel at the point selected by the I-beam cursor. If a single point is selected, the value is for that point, if an area is selected, the value is the endpoint of the selected area.

Markers

37. Read about **Markers** to the right.

Markers are used to reference important locations in the data. There are two types of markers:

Append markers – Appear as a diamond above the marker text box and are blue when active. Append markers are automatically inserted when you begin each new recording segment and are marked with time data



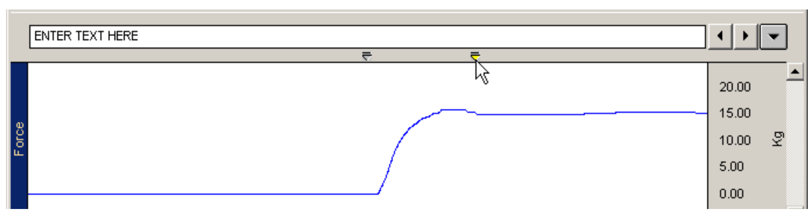
Event markers — Appear as inverted triangles below the marker text region and are yellow when active. Event markers can be manually entered during or after recording by pressing F9 and are pre-programmed for some lessons.

The marker that is darkened/colored is the active marker for which the marker text shown applies.

Add a marker

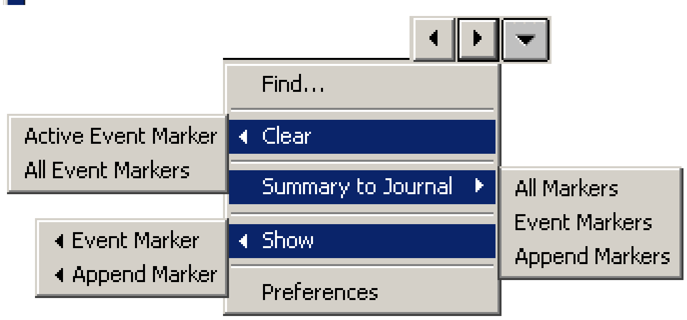
38. Use the selection tool to click in the marker region to the right of the "Clench 3" marker to add a new marker.
39. Label the new marker "test marker" by entering text at the flashing cursor in the marker text region.

You may add markers to your data after it has been recorded simply by clicking within the marker region using the selection tool. This new marker will then become the current active marker, and you may type in the marker text box.



Select a marker

You may change the active marker by using the "marker tools" on the right edge of the marker region.



40. Click on the **right pointing marker tool.**

Click on the **right pointing marker tool** to move to the marker that was placed after the current active marker (if one exists). Notice the marker label and the data position.

41. Click on the **left pointing marker tool.**

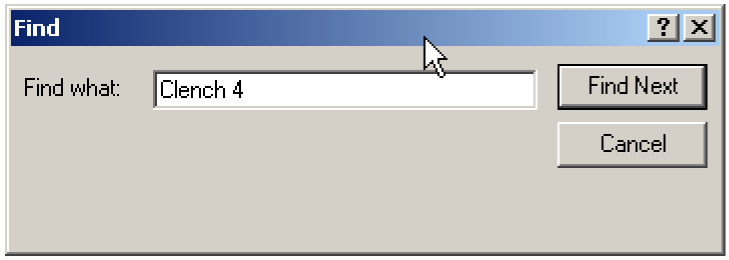
Click on the **left pointing marker tool** to move to the marker that was placed prior to the current active marker (if one exists). Notice the marker label and the data position.

42. Click on the **downward pointing marker tool.**

Click on the **downward pointing marker tool** to generate a pop-up menu as shown above, and drag to select an item or generate a sub-menu.

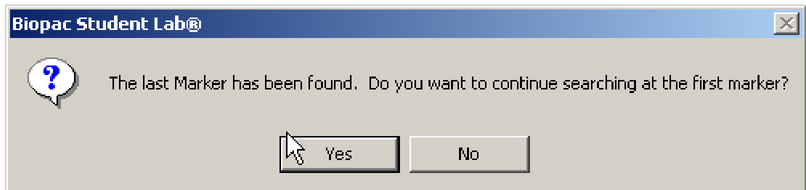
43. Pull down the downward marker arrow and select the **Find…** option.
44. Enter "Clench 4" when prompted and click on **Find Next**.

The marker menu allows you to **Find** certain markers by entering the marker text you want to locate.



Selecting **Find again** will move to the next marker with the same label (if one exists).

45. If prompted, click **OK** to restart marker search from the beginning of the record.



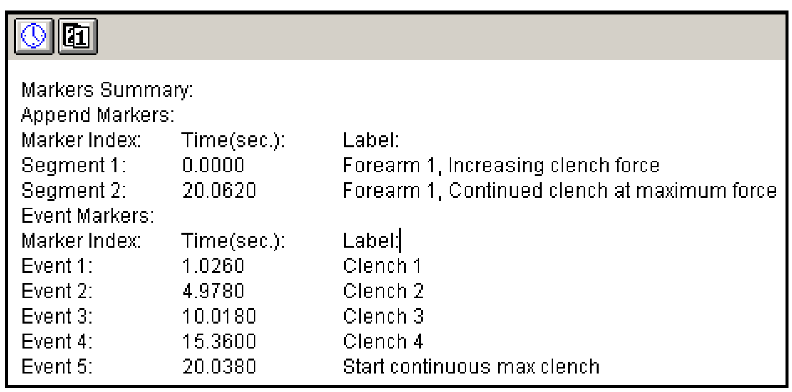
46. Select the **Clear** option to delete the "Test marker."

You cannot clear Append Markers, so these options only apply to Event Markers. **Clear Active Event Marker** will delete the active Event marker if one is selected, and **Clear All Event Markers** will delete all Event markers in the file.

You cannot undo a "Clear" function, so use caution when selecting these functions.

47. Select the **Summary to Journal** option.

These options will paste marker information to the Journal and format the data with an Index, Time, and Label. The "All markers" option will list markers by time, which may mix the Event markers with Append markers.



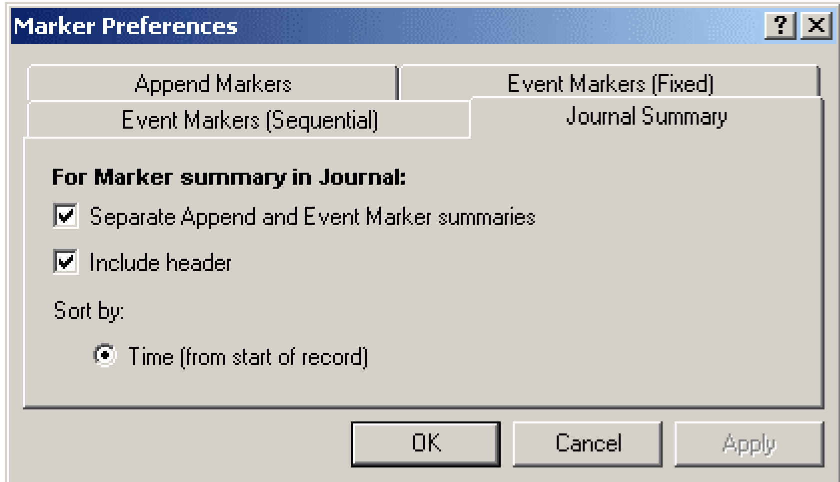
48. Select the **Show** option. Review the list of marker labels at the bottom of the menu and scroll down to select **Clench 4**.

Generate a list of marker. Drag to select a marker and jump to it in the display.

All the marker labels in the record will be listed at the bottom of the menu. The SampleData-L02 file has two markers. You may go to a particular marker by scrolling down to select its label.

- Moving to different markers using this menu may not seem very relevant for the SampleData-L02 file, but when a lot of data has been recorded, it can be a very useful tool.

49. Select the **Preferences** option.

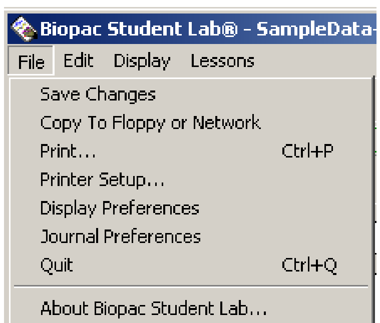


Use the **Journal Summary** tab to set options for ordering and formatting the Summary to Journal option.

Note: The rest of the Marker Preferences are advanced functions for automating marker creation and labeling—your instructor will provide details as needed when you run a Lesson.

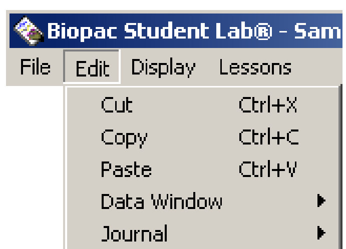
Menu Options

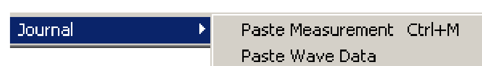
review the options.



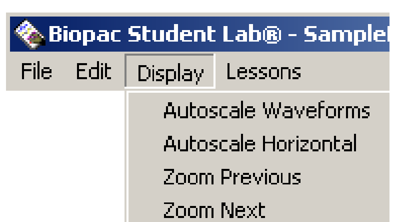
Copy to Floppy or Network allows you to save to your school's network so you can access the file outside of the lab or send Journal reports to your instructor.

51. Click on the **Edit** menu and review the options; drag right to review the sub-options.



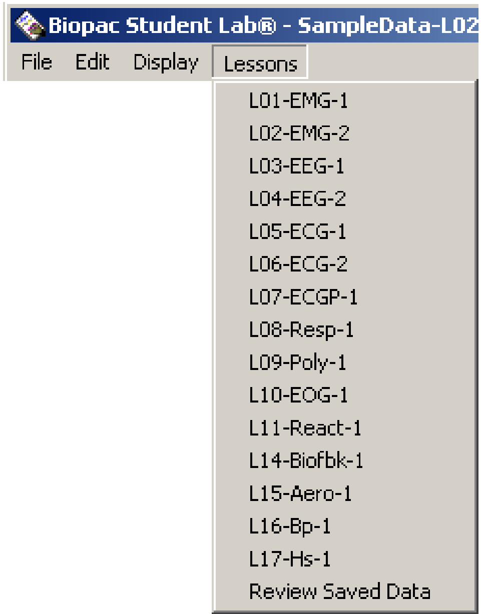


52. Click on the **Display** menu and review the options.



TIP: Use **Autoscale Horizontal** followed by **Autoscale Wave Forms** to view all the data in one screen and restore the data display to its original state.

53. Click on the **Lessons** menu and review the options.



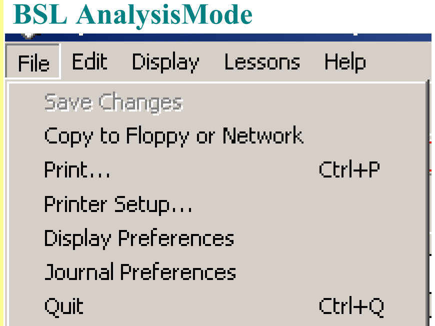
BSL *PRO* Note —

If your instructor set BSL Lesson preferences to activate the BSL *PRO* software controls in the Review Saved Data mode, the menus will include selected *PRO* options for advanced analysis.

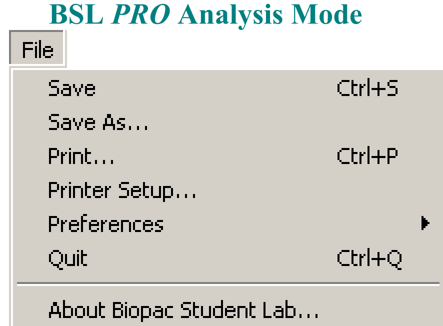
See your instructor or the BSL *PRO* Software Guide for functionality details.

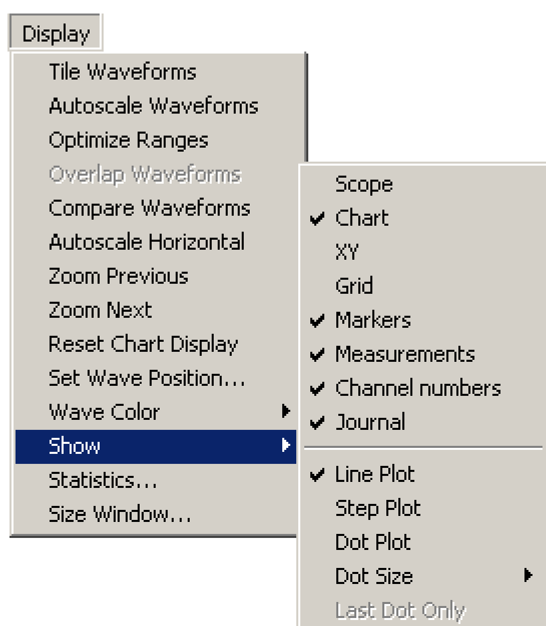
- **Lessons menu stays the same**
- **Help menu stays the same**
- **Transform menu is only available in *PRO* mode**

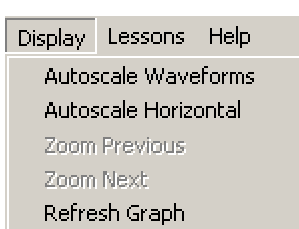
BSL AnalysisMode

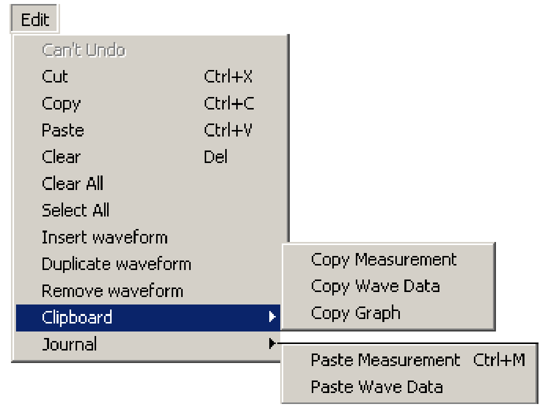


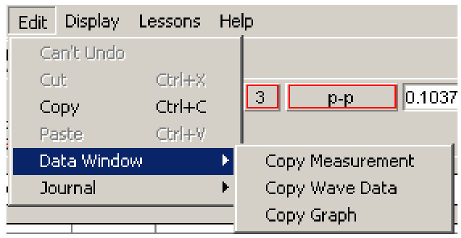
BSL *PRO* Analysis Mode

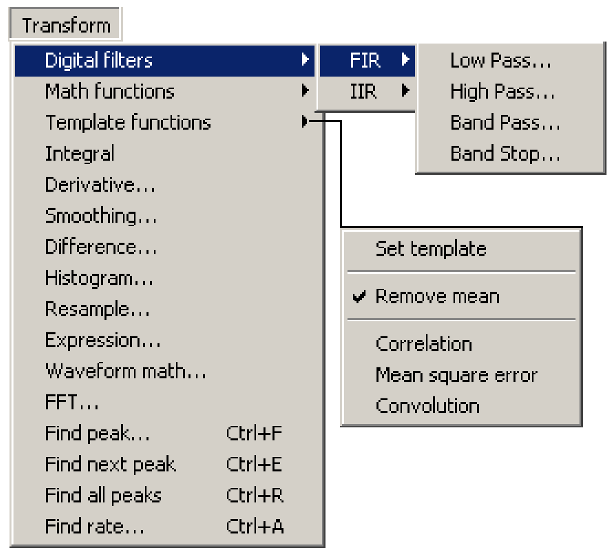







Edit
Can't Undo
Cut Ctrl+X
Copy Ctrl+C
Paste Ctrl+V
Clear Del
Clear All
Select All
Insert waveform
Duplicate waveform
Remove waveform
Clipboard
Journal
Copy Measurement
Copy Wave Data
Copy Graph
Paste Measurement Ctrl+M
Paste Wave Data


Edit Display Lessons Help
Can't Undo
Cut Ctrl+X
Copy Ctrl+C
Paste Ctrl+V
Data Window
Journal
3
p-p
0.1037
Copy Measurement
Copy Wave Data
Copy Graph


Transform
Digital filters
Math functions
Template functions
Integral
Derivative…
Smoothing…
Difference…
Histogram…
Resample…
Expression…
Waveform math…
FFT…
Find peak… Ctrl+F
Find next peak Ctrl+E
Find all peaks Ctrl+R
Find rate… Ctrl+A
FIR
IIR
Low Pass…
High Pass…
Band Pass…
Band Stop…
Set template
Remove mean
Correlation
Mean square error
Convolution

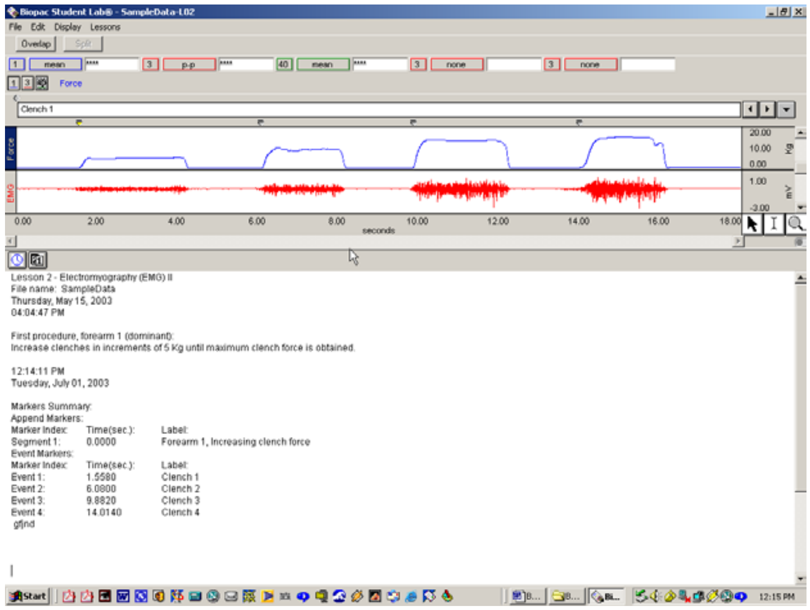
Journal

54. Read about the **Journal** to the right.

The Review Saved Data mode incorporates a **Journal** feature so you can type notes or copy measurements from previously saved data. You can also copy data directly to the Journal. The Journal needs to be the active window for its options to come up.

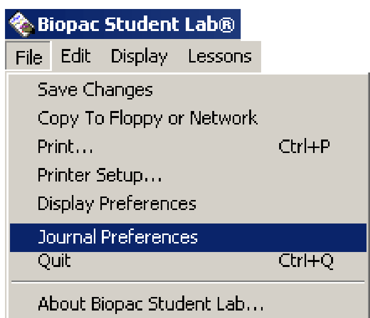
55. Click anywhere in the **Journal** window to activate it.

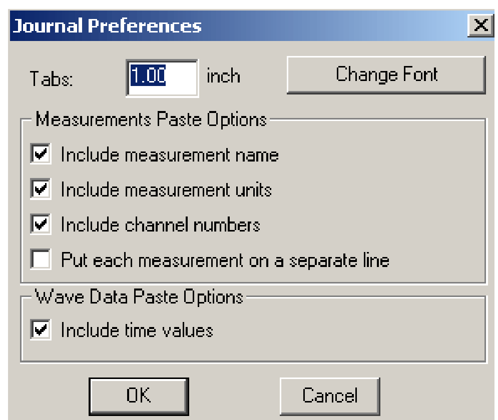
56. Click on the bar separating the Journal window from the Data window and drag up or down to resize the Journal.



Format Journal Entries

57. Pull down the **File** menu, scroll down to **Journal Preferences.**





58. Review the options that can be set to change the way measurements are pasted into the **Journal**.

You can control formatting for text, measurements and wave data. Click on the "Change Font" button to set the text font, style and size.

59. Click in the box next to each measurement paste option box so that all are selected.

Select these options so that when you paste measurements you can easily identify them.

60. Click in the "Include time values" box of the waveform data paste options.

Note When you plan to export the measurements to a spreadsheet program (such as Excel), it is best *not* to select all of the Measurement Paste Options or the Wave Data Paste Option as these will affect the formatting.

61. Click **Ok** to accept the option changes.

Time and Date Stamps

The Journal has stamps for the time and date, which can be useful when creating reports. The icons are on the left edge of the Journal toolbar.

62. Position the cursor at the end of the journal entries and click on the clock icon to activate the **time** stamp.

    Review the result.

The **time** stamp is the "clock" icon at the top left of the Journal window. When you click on the clock icon, the current time (according to your computer's System clock) will be entered in the Journal at the cursor point.

10:36:20 AM

63. Click on the calendar icon to activate the **date** stamp.

    Review the result.

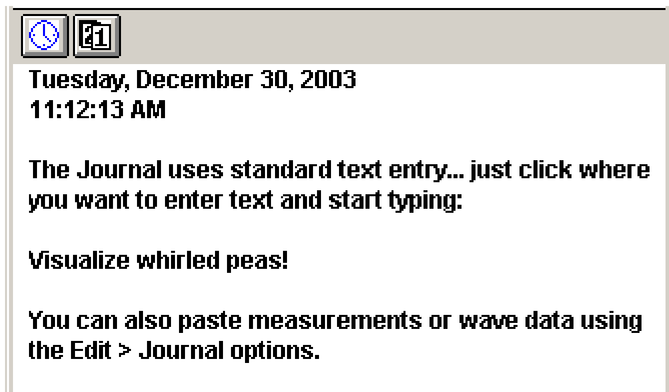
The **date** stamp is the "calendar" button to the right of the time stamp. When you click on the calendar icon, the current date (according to your computer's System calendar) will be entered in the Journal at the cursor point.

Tuesday, December 30, 2003

Text entry

64. Place the cursor at the point you wish to begin typing and enter text using the standard keyboard functions.

It's possible to write anything you want directly in the Journal. Just click on the Journal window and place the cursor at the point you wish to begin typing.



Paste Measurement(s) to Journal

65. Read about the Paste Measurement function.

When you use the **Paste Measurement** function, all the pop-up measurements showing a value will be written to the Journal.

TIP Use the "none" measurement option when you don't want a measurement pasted to the Journal.

To paste a pop-up measurement into the Journal:

1. Select the channel you want to measure by clicking on it with the Selection tool or use the cursor to pick the correct channel number in the boxes just left of each of the pop-up measurements.
2. Choose the appropriate pop-up measurement.
3. Use the I-beam tool to select the portion of the wave you are interested in.

   The pop-up measurement values will update instantly. The pop-up measurements always operate on the selected area you have chosen with the I-beam tool.

   - For instance, if you choose p-p, you will find the peak to peak value of the wave in the selected area. If you choose max, you will get the maximum value of the wave in the selected area.
4. Pull down the Edit menu and select Journal>Paste Measurement. Alternatively, you can use the Ctrl-M keystroke command.

66. Select Channel 1 in the Data Window.

Select the channel you want to measure by clicking on it with the arrow tool or use the cursor to pick the correct channel number in the boxes just left of each of the pop-up measurements.

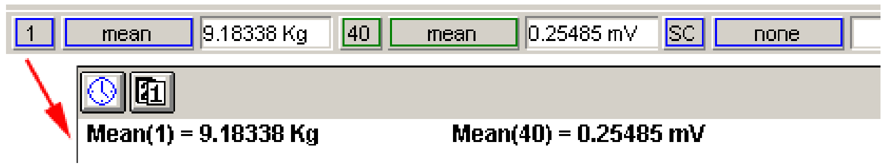
67. Set a pop-up measurement box for CH 1 mean.

68. Set a second pop-up measurement box for CH 40 mean.

69. Use the I-beam tool to select a region from the peak of one R-wave to the peak of the next R-wave.

70. Pull down the **Edit** menu, scroll to **Journal** and slide right to select **Paste Measurement**.

71. Review the Journal entry.



Paste Wave Data to Journal

72. Read about the Paste Wave Data function.

The **Paste Wave Data** function will write all the points that make up the data in the selected wave area to the Journal.

Note It's very easy to put a lot of data into the Journal using this command: One second of a wave that was sampled at 200 Hz will paste 200 numbers into the Journal.

To paste wave data into the Journal:

a. Select the channel you wish to measure by clicking on it using the Selection tool or use the cursor to pick the correct channel in the channel boxes just left of each of the pop-up measurements. This will activate the Data Window.

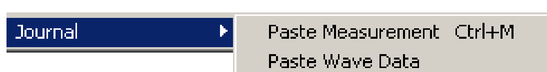
b. Use the I-beam tool to select the portion of the wave you are interested in.

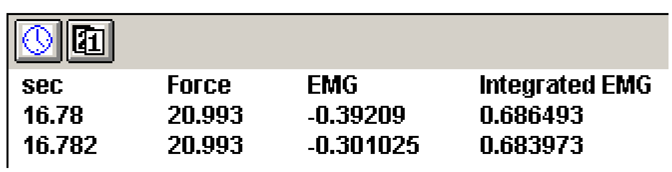
c. Pull down the Edit menu and scroll down to Journal then scroll right to Paste Wave Data.

73. Use the I-beam selection tool to select the portion of the wave you are interested in.

74. Pull down the **Edit** menu, scroll down to **Journal** then scroll right to **Paste Wave Data**.

75. Review the Journal entry.





76. Export Journal Files

The Journal file is saved in standard text format and can be exported to word processing or spreadsheet programs—or any application that accepts Text or ASCII files.

To export Journal text and/or data to another program:

a. Open the BSL file.

b. Select the desired Journal text.

c. Choose **Edit>Copy** to copy the Journal text.

d. Switch to the other program.

e. Choose **Edit>Paste** to paste the copied data.

Note When you plan to place the data into a spreadsheet, it's usually a good idea to remove any extraneous comments, so you just have rows and/or columns of numbers.

Saving the Journal

Use the File menu Save options to save the Journal. The Journal and Graph files are linked and will be opened and saved together.

77. To save with the existing file name and location, click on the **File** menu and select **Save Changes**.
78. To save with a new file name and/or location, click on the **File** menu and select **Copy to Floppy or Network**.

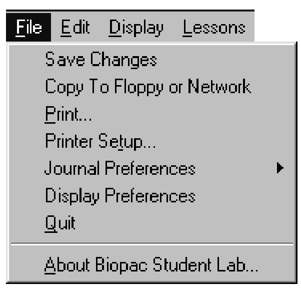
Save Data

79. Read about the **Save** functions to the right.

Each student's recording is automatically saved at the end of each lesson recording. Students do not need to resave files unless they significantly alter the data window settings or make a change to the journal while in the Review Saved Data mode.

Once in the Review Saved Data mode, you may alter the data display window, or enter more information into the journal. When you make changes to the data window, you are only changing how the data appears on the screen — you are not altering the data that has been originally recorded. Saving the changes will never delete any data.

80. Review the save options under the File menu.
    - Save Changes
    - Copy to Floppy or Network



Save Changes

We recommend this option to ensure that lesson files are properly named and saved.
Saves the file with the existing file name and location.

Copy to Floppy or Network

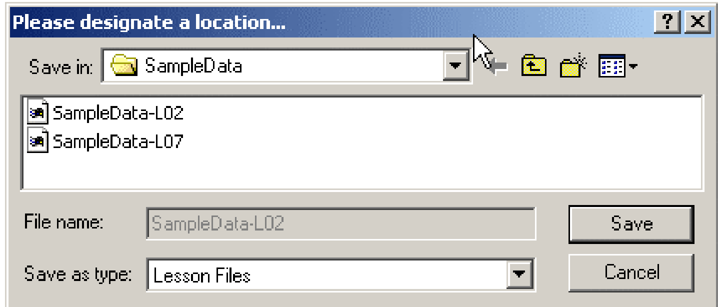
To take data home for analysis, use this option.
Creates a copy of the original file and prompts you for a location to save the copy to — floppy, network, or other media.

81. Pull down the **File** menu and select the **Save changes** option.

The save is automatic, no dialog or confirmation is required.

82. Pull down the **File** menu and select the **Copy to Floppy or Network** option.

The following dialog will be generated so you can designate a location for the file.



File name cannot be altered from the original name. This allows the Review Saved Data mode to work correctly.

Save as type is locked on “Lesson Files,” which is the original way the file was saved and is the waveform display you normally see on screen. This mode copies the journal text as well.

The “**Save**” button instigates the Save function.

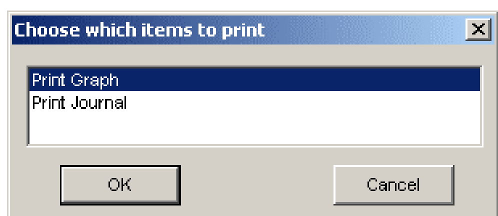
Printing

83. Read about the **Print** functions to the right.

TIP: It’s always a good idea to Save before printing.

You control how the data is presented on the printed page by controlling how it is displayed on the screen prior to selecting Print. All of the options relating to printing the data files apply to the waveforms as they are displayed in the data window. If you’ve zoomed, changed the scale, or hidden a channel, only the portion of data displayed in the data window will be printed. This is actually very useful, because oftentimes you may only want to display a portion of the data.

When you choose **File>Print** you will be prompted to choose which items to print.

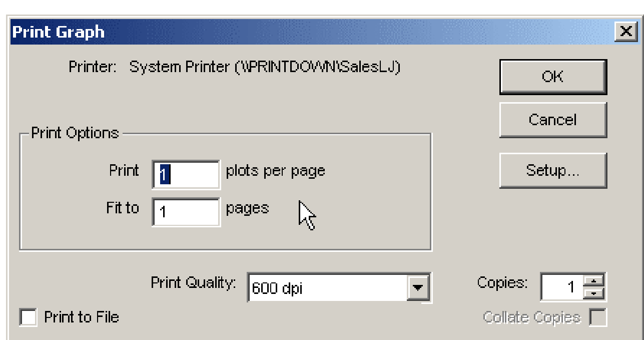


Print Graph

IMPORTANT! The printer only works with the data shown in the data window, which often is not the complete data file.

The following Print dialog is just an example. Your actual Print dialog will depend on the printer and Operating System you are using. The dialog should include **Print Options** that allow you to control how much data is printed on each page.

- Depending on your OS, you may need to access the Print Options via a button in the original Print dialog. Refer to the Users Manual for your computer and/or printer for more detail.



- **Print Options**

Print ___ plots per page

This option determines how each page will be divided up to plot the data displayed in the data window.

- For example, entering “4” will divide each page into four regions.

The printed data may have an expanded time scale if the printer made adjustments to plot what was viewed on the screen evenly across the specified area.

Fit to ___ pages

This option determines how many pages to use to print what is displayed on the screen.

- For example, entering “2” will print the data displayed in the window evenly across two pages. The first (left) part of the data displayed on screen would print on Page 1 and the second (right) part would print on Page 2.

- **Automatic Adjustments**

The software will automatically make the following adjustments prior to printing, which may result in slight differences between what is displayed and what is printed:

- **Vertical Scale** — If a vertical scale in the data window is set to fractional numbers, then the software will slightly adjust the scale to use even numbers for print.
- **Markers** — When markers are displayed on the screen with associated marker text, the data will print with the marker text directly above the appropriate marker and a dashed vertical line will run through the data to indicate the marker’s precise point in time. If the data is compressed, the markers and/or marker text may overlap or be hidden. If this is not acceptable, then you’ll need to expand the time scale so that the markers have enough room to print.

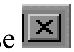
Print Journal

This option generates a standard Print dialog to print the Journal text.

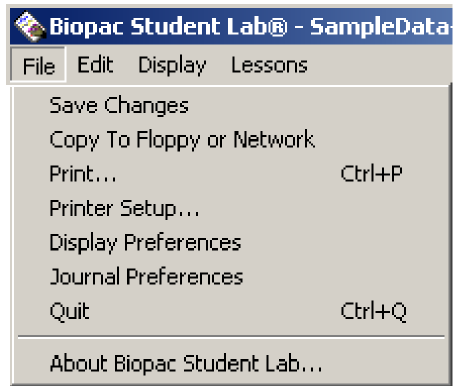
84. Print the entire data file
    a. Click anywhere in the Data Window to make it the active window.
    b. Choose **Display**>**Autoscale Horizontal**.
    c. Choose **Display**>**Autoscale Waveforms**.
    d. Choose **File**>**Print>Print Graph**.
    e. Set Plots per page and Fit to pages as desired.
    f. Click **OK.**
85. Review the printed result.

Quit BSL

86. Pull down the file menu and select **Quit**.

During the recording mode, the application close  icon in the upper right corner of the display window is blocked so you can't inadvertently quit the application when you simply meant to close the data window.

To quit the application, you need to choose **Quit** from the **File** menu.

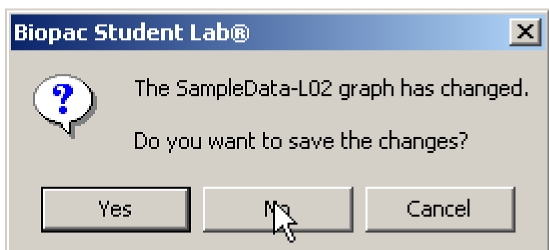


If **Quit** is blocked, click on the STOP button and wait for the recording to end, then try **Quit** again.

87. If prompted, click **Yes** to save all changes.

88. Close the file you opened and close the hard drive window.

When you try to quit the software after altering the data file and/or journal file in any way, the following message will be generated.



Running a Lesson

To run a BSL Lesson, you must have an MP30 or MP35 data acquisition unit connected. If you installed the BSL software with the Hardware option and have an MP unit connected, you can follow along on your computer; otherwise just read this section so you'll know what to expect in the lab.

> **IMPORTANT** — You are free to download the Biopac Student Lab Analysis software for use on your personal computer. The hardware does not have to be attached for you to review and analyze your data files. See www.biopac.com for the BSL Analysis download.

The primary objective of the Recording section is to assist you in obtaining good data, and the primary objective of the Analysis is to help you understand the data and the physiological concept it represents. Lessons are set up so that you can record data in the lab and then analyze the data after class—in the lab or at home.

FAST TRACK	***Detailed Explanation of Steps***
This side of the lesson (left, shaded column) is the "FAST TRACK" through the lesson, which contains a basic explanation of each step.	This side of the lesson contains more detailed information to clarify the steps and/or concepts in the FAST TRACK, and may include reference diagrams, illustrations, screen shots, and/or references to this manual.

Although it is not absolutely necessary, BIOPAC recommends that students work in groups of at least three. Students should choose a **Director**, a **Recorder**, and a **Subject** from their lab group before beginning Set Up (which often involves the Subject).

Director The Director reads the lesson steps and tells the Recorder and Subject what to do. The Director also keeps track of the length of time for each condition.

Recorder During the recording session, the Recorder will insert a marker (press F9 on PC or Esc on Mac) whenever the Subject is requested to do something and will key in the condition to create the marker label. The recording step will indicate what to key in. Note that marker text can be added or edited after the recording is complete.

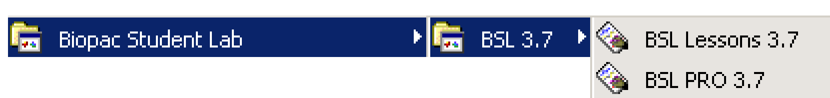
Subject This is the person from whom data will be recorded. The Subject will need to perform the tasks as instructed by the Director. It is suggested that the Subject not look at the computer screen while data is being recorded, as there tends to be a "biofeedback" effect that can bias the heart rate.

1. Turn the computer ON.
2. Use the desktop icon or the Windows **Start** menu to open **Biopac Student Lab 3.7.**.

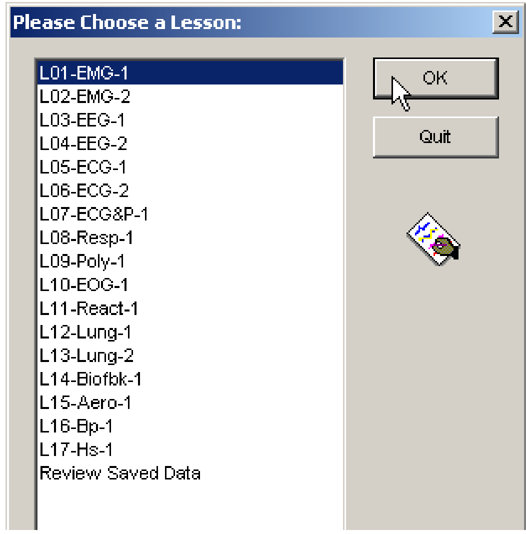
To run a lesson, the computer must have an MP unit connected.

To launch the program, use the desktop icon

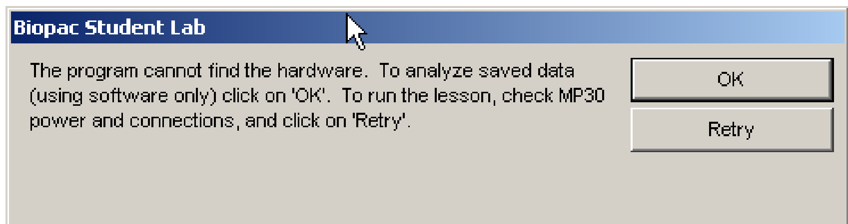
or the Windows® **Start** menu and choose Programs and then select:



3. Click on the **Lessons** menu and select a Lesson.
    - The **Review Saved Data** option will generate a standard Open dialog so you can select a file for analysis.

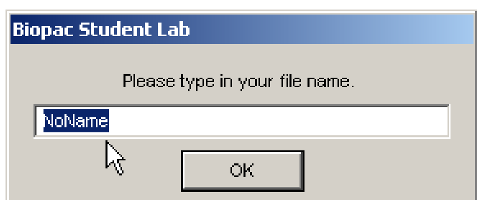


If the hardware is not connected, the following prompt will be generated. To run a lesson, check the power and connections for the MP unit and then click Retry.



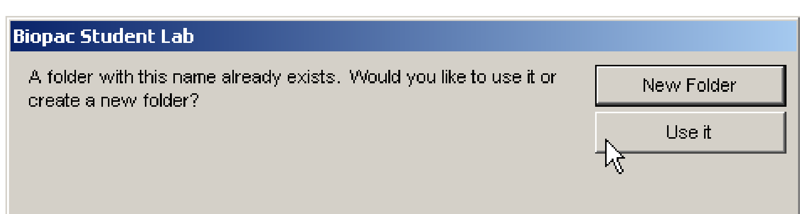
4. Type in your file name.

Type in your name so the BSL System can store all of your data files in one place, and make it easier for you to retrieve data later on.



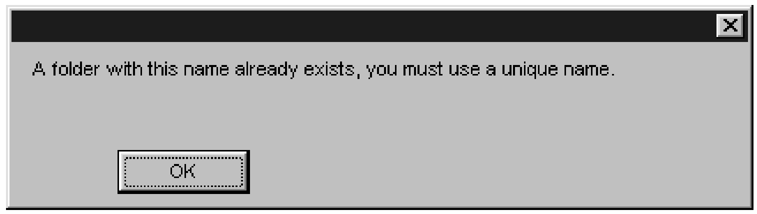
You can enter your real name, a nickname, or the name of your group if you are working with other students. Select and type in a unique identifier, such as the Subject's nickname or student I.D. #, your full name or some combination of your name and other letters and/or numbers (like JohnF or John3). **It is a good idea to use the same log-on name for each lesson**. Be sure to write down the log-on name you choose so that you can keep track of where your data is stored.

The Biopac Student Lab software will let you use the same name ten times. If there are a lot of other students using your computer and you try to log on with a general name ("John" vs. "JohnF") there is a good chance the Biopac Student Lab software will ask you to use a different name.



If you know the existing folder is your own, select **Use it**. This is a convenient way to ensure that all of your lesson files for the class term are stored in one folder. If you Rename the folder, just add a character to the last name you used, i.e. "Lauren" becomes "Lauren2." Once you enter your name and choose **OK**, the Biopac Student Lab software creates a folder inside the "Data Files" folder that is inside the "Biopac Student Lab" folder on your computer (or in an alternate location of your instructor modified setup). This is where all your data will be stored. If you choose the same file name for other lessons, they will also be stored in this folder (with the appropriate lesson number extension).

If you try to use the same file name an eleventh time, the program will insist that you choose a different name.



The files inside your folder can be moved, copied, or duplicated, just like any other file. If you wish, you can copy them to a "floppy disk" as a backup or to be viewed later. Check with your instructor or lab assistant for more information on how to do this.

When you run a lesson, the first step is a prompt for you to enter your name. The Biopac Student Lab software creates a folder with the exact name you enter and places it in the **Data Files** folder of the Biopac Student Lab Program folder. When you press the **Done** button in the lesson to end recording, the BSL program automatically saves your waveform data file to the **Data Files** folder with a file name based on the user name or I.D. given at the start of each lesson.

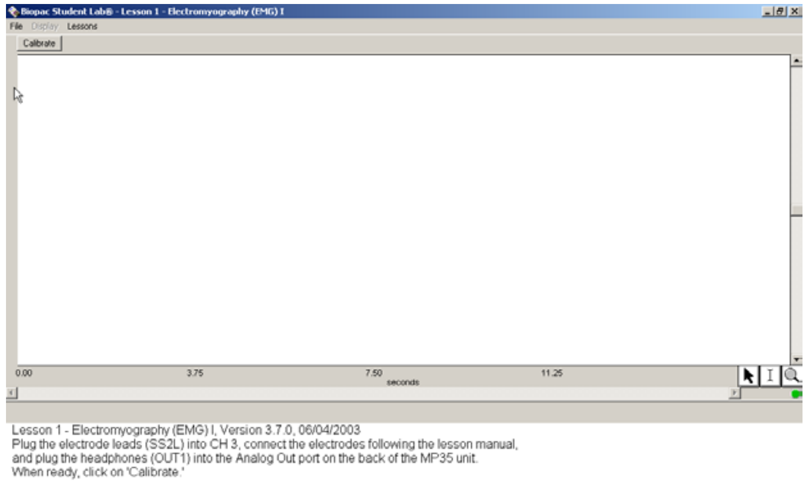
The software will save the data file with the name you entered plus an extension that identifies the lesson number. This extension is very important because the software will key off this extension and open up different tools for the Review Saved Data Mode, depending on the lesson.

Other lesson data files that use the same name will be placed in this same folder, but the software will never allow you to save a data file with the same name and lesson number.

As a general note, because the Biopac Student Lab software automatically is saving the files, you must exercise caution when moving things around into different folders.

It is recommended that you never place other files or folders in the Biopac Student Lab folder, and never take files or folders out of the BSL 3.7 folder with the exception of removing data files from the Data Files folder.

5. Follow the instructions in the lesson manual and in the Journal, or proceed as directed by your instructor.



The recording section is very detailed and includes sample screen shots for comparison—so, even if you don't fully understand what is happening when you're running the lesson or feel pressured by time (as is often the case), you should be able to obtain good, meaningful data. Then, you'll be able to analyze and grasp the important concepts later, during the Analysis section.

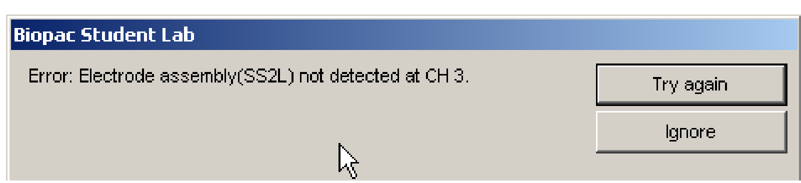
Normally, there are multiple data segments that need to be recorded, with different tasks occurring during and/or between these segments. It is important that you read ahead to the next step in the lesson so it is clear what task(s) you'll need to perform during each recording segment.

6. Step through the lesson as guided in the Lesson manual or by your instructor.

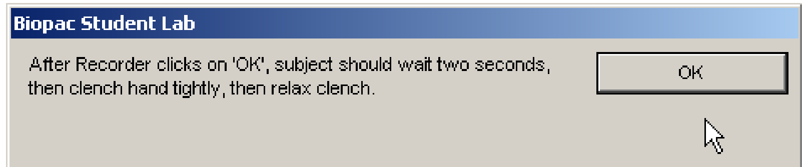
To begin a Lesson, click on the **Calibrate** button. The dynamic calibration procedure will automatically optimize the BSL System for the signal(s) being recorded in the lesson.

- With equipment such as an oscilloscope, you or your instructor needed to "twiddle" with the dials and switches, e.g., gain or amplification settings, position (vertical or horizontal alignment), voltage/division settings, time/division settings, and sweep speed to make sure a "good" signal was recorded. This entire procedure had to be manually repeated for each new subject. The Biopac Student Lab makes the required adjustments for you during the automatic calibration procedure.

If you did not plug in the hardware as directed, you will be prompted to correct it before continuing.



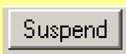
A dialog will help you prepare for the each segment. Read the instructions and click **OK** to continue.



The calibration routine will stop automatically.

If your calibration data does not match the sample screen shot provided, click on **Redo Calibration** to repeat calibration and erase the old data.

If your calibration data matches the sample screen shot, click on **Record** to begin the first recording segment.

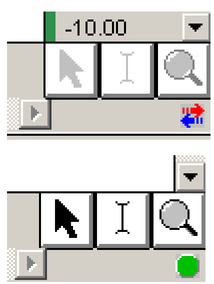


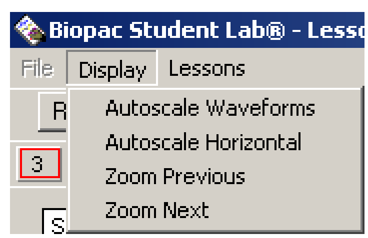
Click on "**Suspend**" when you have completed the assigned segment task(s).

- You should click on "Suspend" as soon as possible once you have completed a segment. This is important because every second wasted is memory used up for the data recording. The more memory used, the slower the program will operate, the more disk space will be used up when the data is saved, and the more possibility of problems.
- When selected, the **Suspend** button toggles to "**Resume**."

Review Recorded Data

When you Suspend recording between lesson segments, you can check that you have good data before continuing. You may want to check your data by zooming in on a segment, taking a measurement, adding grids, etc. Review your data before continuing and read ahead to prepare for the next segment.





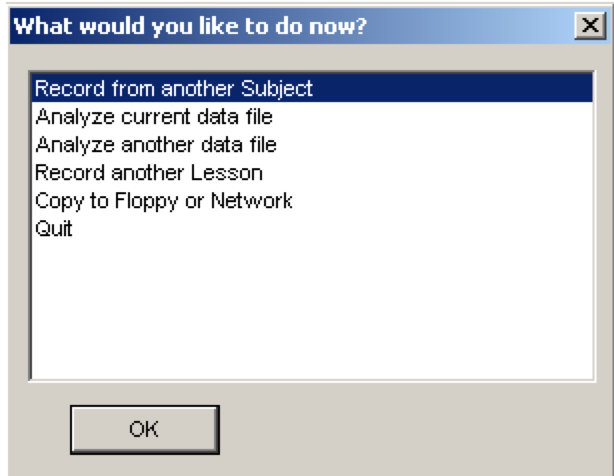
The **Arrow**, "**I-Beam**," and **Zoom** tools are inactive (greyed) during recording but can be used between segments while in the Recording mode. The Display menu items are also available.

To repeat the last recorded section and erase the old data, click on **Redo**.

- The "Redo" feature is very handy and should prevent you from panicking during the recording. You can redo a recording segment if something goes wrong or if you realize you have not performed the proper tasks.

If you are finished with the data recordings, click on **Stop**.

Click the "Done" button in the Lesson display to generate the closing options dialog.



Record from another subject will return you to the first screen of the lesson. Repeat the necessary set up, type in a new Subject name, and repeat the entire recording. Depending on the lesson, you may or may not have to redo the calibration.

Analyze current data file will switch you to the Review Saved Data mode and will automatically open the last recorded data file.

Analyze another data file will switch you to the Review Saved Data mode and will prompt you to find and open a data file for review.

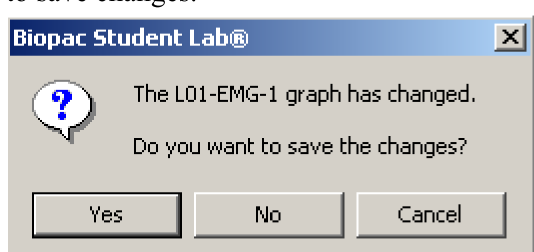
Record Another Lesson will generate the lessons menu.

Copy to Floppy or Network will generate a Save dialog, allowing you to designate the location to save the file to. Note that you cannot change the File name.

Quit will close all lesson windows that are open and then exit the Biopac Student Lab program.

7. Save the recorded lesson data.

Each student's recording is automatically saved at the end of each lesson recording. You do not need to resave files unless you significantly alter the data window settings or make a change to the Journal while in the Review Saved Data mode. You will be prompted to save changes.



Review Saved Data Mode for Data Analysis

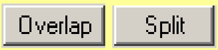
Data Analysis is where you analyze your data to "pull out" measurements that reinforce the concepts presented in the Introduction. Analysis can be done right after the data is recorded, or can be done off-line on another computer (the hardware does not need to be attached). The analysis should be done after all students have recorded their data.

Measurements taken in the Analysis section are to be placed in the Data Report section and may also be placed in the on-screen journal for saving and printing. Whenever a measurement needs to be placed in the Data Report, a reference icon will direct you to the proper section of the Data Report.

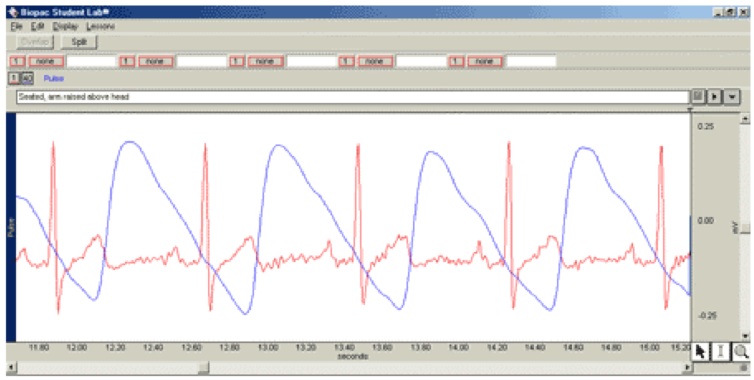
Lesson Specific Buttons

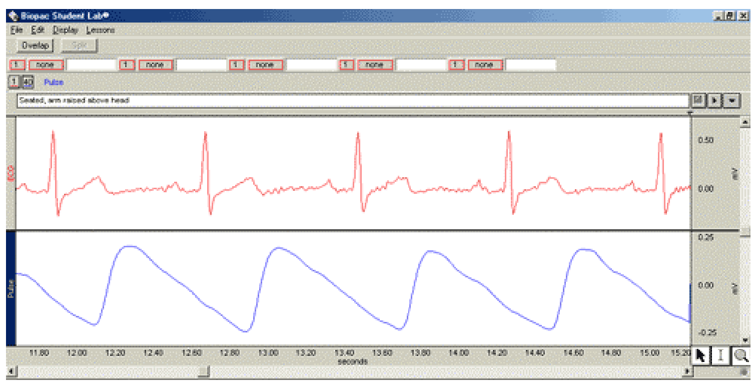
Some lessons have extra buttons (under the menu bar) for features appropriate to the lesson signal and/or protocol. The following buttons are not in the sample file, so just read about them below.

The **Listen** button allows you to listen to the signal as output if you have headphones or other listening hardware connected.

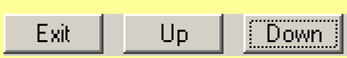


The **Overlap** and **Split** buttons allow you to merge or separate the data channels ("tracks") in the display window.



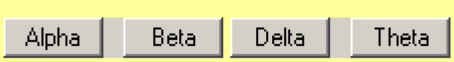


You cannot successfully apply grids after the Overlap function is activated. Grids can change the scale, so begin in the Split mode, then apply grids, then Autoscale, then apply the Overlap.



The **Adjust Baseline** button is only used in *"Lesson 5 ECG I"* and it allows you to position the waveform up or down in small increments so that the baseline can be exactly zero. This is not necessary to get accurate amplitude measurements, but may be desired before making a printout or when using grids.

When you click the **Adjust Baseline** button, **Up** and **Down** buttons will be generated. Simply click on these to move the waveform up or down. The **Exit** button will close the Adjust Baseline options and return to the waveform display.



These buttons are in Lesson 3 and will filter the signal into the bandwidth for alpha, beta, delta and theta analysis.

Legalese

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BIOMEDICAL ENGINEERING
THE UNIVERSITY OF TEXAS AT AUSTIN

Appendix 3

SUPPLEMENTAL READINGS

Opportunities at the Skin Interface for Continuous Patient Monitoring: A Reverse Iontophoresis Model Tested on Lactate and Glucose

Patricia Connolly*, Christopher Cotton, and Fabrice Morin

***Abstract*—Skin has the potential to provide an important noninvasive route for diagnostic monitoring of human subjects for a wide range of applications. Dimensions of surface features in skin suggest that nanodevices and microdevices could be utilized to monitor molecules and ions extracted from the skin. Methods of enhancing extraction from the skin for diagnostics are being developed including reverse iontophoresis, electroporation and sonophoresis. A model system for the simulation of *in vivo* extraction of molecules and ions by reverse iontophoresis is described here that displays similar behavior to skin both in terms of molecular flux levels and electrical impedance characteristics. The device has potential for use in the development of complete reverse iontophoresis/sensor systems, allowing sensor and extraction systems to be studied and optimized before being tested in the complex *in vivo* environment. The system has been tested using glucose and lactate and the results are reported and discussed.**

***Index Terms*—Artificial skin membrane, biosensors, impedance, reverse iontophoresis.**

I. Introduction

In the search for diagnostic devices for continuous monitoring of patients or tissue a great deal of effort has centered on implantable devices. A good example of this is the development of implantable glucose sensors and subcutaneous sensors are currently available with lifetimes of several days [1]. Other implantation systems have been used in the development of artificial retina [2]. Furthermore, substantial experience has been gained in the use of microdevices for *in vitro* measurements such as monitoring of cells on microelectrodes or lab-on-a-chip diagnostics (see [3] and [4] for examples).

However, long-term implantation of biosensors remains an elusive goal. It can be argued that unless the active components of a biosensor can be regenerated *in situ* in the human body, implanted biosensors will always have a limited lifetime.

Until relatively recently, the human skin interface has been neglected as a possible route to continuous patient monitoring. This interface has many advantages, allowing for monitoring without the need for implantation of devices and for extended point of care testing without the need to take frequent blood samples.

There is a large body of work on the transdermal delivery of pharmaceutical substances which gave rise to commercial devices such as nicotine patches (see [5] for examples). In general, the transmission of molecules through the skin and means of enhancing such transdermal transport has been widely studied. In comparison, there is a very small body of literature to date on the direct extraction of molecules from skin for medical diagnostic purposes.

The main barrier to molecular transmission through (or extraction from) skin is the outer layer, the stratum corneum. Transmission of molecules and ions through skin occurs by four routes; transcellular, intercellular, via hair follicles and via eccrine (sweat) glands [6]. The transcellular and intracellular routes depend on specific molecular ability to penetrate cell walls or to move through the lipophilic intercellular regions. Eccrine and hair follicle routes are more accessible and there is evidence that a majority of transmitted molecules and ions cross the skin by this route. The skin structure also displays porosity with pores sizes estimated to be in the region of 10–25 Å. There are $\sim$ 150-cm^{-2} hair follicles and sweat glands on the skin and these are of micro rather than nano dimensions ($\sim$ 40-μm diameter).

Physically enhanced transmission of molecules and ions across the skin has been achieved by several methods including sonophoresis (ultrasound) [7], electroporation (high-voltage impulse) [8] and iontophoresis (low-level direct current) [9]. Iontophoresis is based upon the application of low levels of current to the skin (<1 mA cm^{-2}), delivering ions and charged molecules transdermally as charge carriers in the iontophoretic circuit. In addition, uncharged molecules display enhanced delivery under iontophoresis driven by convective fluid flow which is due to electro-osmosis [9].

The application of iontophoresis to extract analytes (charged and uncharged) from skin is known as reverse iontophoresis. In combination with sensor technology, this has great promise for the noninvasive monitoring of patients. One company has obtained FDA approval for a commercial skin-contact device based on reverse iontophoresis (Glucowatch™, Cygnus Inc.) which allows diabetics to obtain a reading of their glucose levels every 20 min over a 12-h test period using only one finger stick blood test to calibrate the system. The Glucowatch is slightly larger than the average digital watch and is worn on the arm. Gel pads, which must be changed every 12 h, are in contact

Manuscript received June 26, 2002; revised August 13, 2002. *Asterisk indicates corresponding author.*

*P. Connolly is with the Bioengineering Unit, University of Strathclyde, Glasgow G4 0NW, Scotland, U.K. (e-mail: patricia.connolly@strath.ac.uk).

C. Cotton was with the Bioengineering Unit, University of Strathclyde, Glasgow G4 0NW, Scotland, U.K.

F. Morin was a graduate student in the Bioengineering Unit, University of Strathclyde, Glasgow G4 0NW, Scotland, U.K. He is now at the Japan Advanced Institute of Science and Technology, Tatsumokuchi, Tatsumokuchi, Ishikawa 923-1292 Japan.

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with the skin to deliver current from the Glucowatch for reverse iontophoresis and these pads also contain the sensor element (glucose oxidase). The sensor is a platinum-electrode-detecting hydrogen peroxide produced by the glucose oxidase in the presence of glucose and oxygen [10], [11].

Good possibilities exist for microtechnology and nanotechnology to be applied to introduce new devices to this field through the development of noninvasive, wearable sensors and multisensor arrays. The very dimensions of the transmission paths through skin could lend themselves to direct study by miniaturized devices. However, the understanding of the transmission and transport mechanisms (electro-osmosis, diffusion, ion and molecule drift currents) of various analytes will be a critical part of sensor and device development for skin applications. For example, in a reverse iontophoresis/sensor device, uncharged molecules may be extracted from the skin by electro-osmosis due to solvent flow, but once though the skin the molecules arrive at the sensor surface by diffusion mechanisms. This diffusion must take place in a suitable conducting hydrogel which will provide both the skin-hydrating contact and the current path from the iontophoresis electrodes. Sensor behavior in the hydrogel under reverse iontophoresis conditions in such a device would require characterization. This is best carried out in a model system *in vitro* before any *in vivo* studies are made.

Model systems for transdermal transport studies *in vitro* do exist but there are a number of problems associated with their use which make them less than ideal for device and sensor development. The majority of devices in use in pharmaceutical studies of transdermal molecular transmission or extraction are based on the Franz cell [12] which does not readily lend itself to sensor development applications. This cell, when employed for iontophoresis, has the electrodes which drive iontophoretic current placed on either side of a skin sample suspended in bathing solutions. This is very different from the *in vivo* situation where patients have both electrodes mounted close to each other on the surface of their skin. The electric current pathways and applied electric field directions are quite different in both situations and this has practical consequences for measurable parameters such as skin electrical impedance. The realization that the Franz cell did not accurately reproduce the *in vivo* situation for iontophoresis-assisted transdermal drug delivery led Glickfeld *et al.* [13] to develop a new cell where animal tissue could be mounted in a way that more closely replicated the f*in vivo* situation. The design included two iontophoresis chambers mounted on the outer side of the skin sample with a third chamber placed between to aid electrical isolation of the two iontophoresis chambers. It was in this system that the first observations of reverse iontophoresis of molecules were made using animal tissue.

Current *in vitro* systems still largely rely on the use of animal or human tissue for the testing of iontophoretic effects or devices. The continued use of animal or human tissue provides two major problems for groups wishing to develop diagnostic sensor devices for noninvasive patient monitoring. First, the availability of such tissue is now becoming limited in many countries for legal, ethical and safety reasons. Second, trying to study the characteristics of a novel sensor and extraction system during reverse iontophoresis with animal or human tissue allows interference from substances in the skin. Since many of these effects are unexpected it is preferable that the system is characterized in as controlled an environment as possible before skin studies commence.

Another point to consider is that systems using animal or human skin *in vitro* are essentially dealing with dead tissue which does not fully reproduce *in vivo* skin behavior.

Finally, the problem of lateral current leakage from electrode to electrode across the skin rather than through the transdermal route has been given little consideration. Hydration of the stratum corneum from the skin-contacting hydrogel which contains the iontophoresis electrodes will change the resistance of the surface layer of the skin. Excessive sweating will create a complete short circuit between the iontophoresis electrodes ensuring that extraction of substances from the skin is not efficient. Practical devices must cope with this and the Glucowatch™, for example, contains a conductivity sensor mounted against the skin between the two hydrogel pads. If the conductivity rises above a certain threshold (numerical value not given), the device stops issuing glucose readings.

With these problems in mind, a model system for the study of reverse iontophoresis of molecules and ions, inspired by the design of Glikfeld *et al.* [13] and suitable for sensor development and testing has been developed by us which uses a polymer membrane in the place of animal or human skin. The membrane itself has been chosen to mimic some of the key properties of skin which drive the electrical behavior noted *in vivo*, being nanoporous and exhibiting a negative charge at pH 7. Artificial skin membranes have been employed as transdermal models for molecular permeability studies and for iontophoresis drug delivery measurements in the past (see, for examples, [14]–[16]) but no membrane has yet been proposed which might be ideal for reverse iontophoresis studies.

In this new cell, we have chosen not to try and fully isolate the two chambers from each other as this does not accurately reflect the situation *in vivo* where current tracking across the surface of the stratum corneum and along the stratum corneum will occur between the iontophoresis electrodes on the skin. Instead, impedance was measured to estimate the percentage of current that truly participates in transdermal transport.

Importantly, using the polymer membrane as a skin substitute avoids the large sample to sample variation which is encountered in both animal and human skin samples *in vitro* and provides a reliable source of safe test material. The problems of interference from other substances in the skin during reverse iontophoresis for medical diagnostics will, of course, still be encountered and this will necessitate careful *in vivo* studies of any new device. However, the model system is designed to allow the satisfactory development and testing of extraction conditions (voltage, current, sample timing) and sensors before the additional problems of skin *in vivo* are encountered. The essential function of this model system, therefore, is to aid the study of electro-osmosis and reverse iontophoresis of specific analytes under controlled voltage and current conditions and to facilitate the development of iontophoresis-sensor devices for transfer to the clinic for *in vivo* testing.

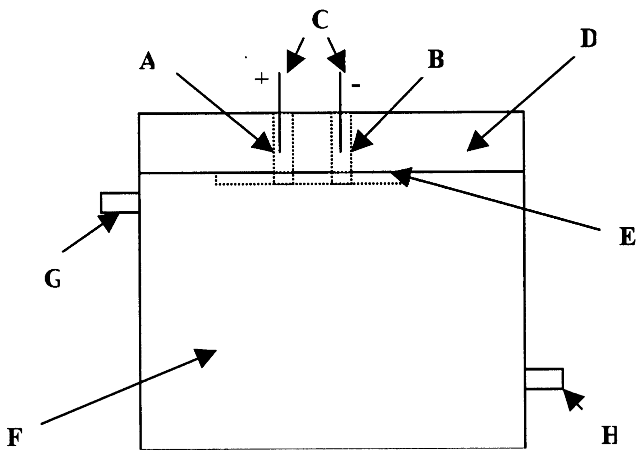

Fig. 1. Model iontophoresis system: A = anode chamber; B = cathode chamber, both chambers 5-mm diameter and 20 mm deep, separated by 4 mm; C = electrodes; D = upper half of cell with drilled out chambers A and B, outer diameter 50 mm; E = separator and gasket (2 mm thick); F = lower chamber with capacity for 22.7 ml of test solution; G and H = inlet and outlet ports.

The results of reverse iontophoresis of glucose and lactate in this new cell and a basic description of the cell construction, are reported here. In addition, an initial study of the impedance of the system is reported and its significance discussed.

II. Materials and Methods

Fig. 1 is a diagram of the iontophoresis cell devised for this study. The model cell is in two halves, comprising an upper and lower section and is machined from polymethylmethacrylate. Liquid in the lower chamber acts as the interstitial fluid under the skin, A and B as the collection chambers in an iontophoresis device in contact with the skin. The Cellulose ester (CE) membrane is placed on a separator which fits into the lower chamber (the separator is drilled through to be open under Chambers A and B). A silicon gasket (also cut so as to be open under chambers A and B) is placed between the membrane and separator to ensure a good lateral liquid seal. When the cell has been assembled with the membrane in place, filling of the lower chamber takes place via the inlet port. CE nanofiltration membrane (Spectrum Laboratories Inc., USA) substitutes for skin in this system. The CE membrane has a negative charge at pH values > 2.2 and a molecular cutoff (for filtration by concentration gradient) of 500 daltons.

Iontophoresis experiments were carried out by filling the lower chamber with HEPES-buffered saline (25 mM HEPES with 133 mM NaCl) with either 5 mM glucose or 10 mM sodium lactate present in the buffer solution. The anode and cathode chambers, A and B, were filled with 300 μL HEPES/NaCl for each iontophoresis test.

A reverse iontophoresis current of 70 uA was supplied under galvanostatic control to chlorided silver wires (1-mm diameter) placed in the buffer solution in chambers A and B using a Solartron 1286 Electrochemical Interface (Solartron, UK). Reverse iontophoresis was carried out at this current setting for periods from 15 to 90 min. In every 15–min period the wires were removed from the chambers, rinsed in deionized water and placed in the opposite chamber before reconnecting to preserve the integrity of the silver-silver chloride layer. i.e., anode and cathode chambers remained the same throughout with A being the anode and B the cathode. In any experiment the 90-min sample was the first test point to ensure good wetting of the membrane. Samples (150 uL) from chambers A and B were taken at appropriate time intervals and stored at 5 °C for up to 24 h before being tested for glucose or lactate as appropriate. After every sample point the chambers A and B were drained of liquid, rinsed with deionized water and refilled with the HEPES/NaCl solution. The lower chamber was drained of lactate or glucose test solution, rinsed and refilled with fresh solution after every sample had been taken. All experiments were carried out at room temperature. A magnetic stirrer bead was placed in the lower chamber and the solution was stirred throughout iontophoresis.

Control experiments were made in the same system with conditions exactly as above but without the application of current or electrodes. This showed the effects of diffusion through the CE membrane without the use of reverse iontophoresis.

Glucose and lactate were assayed using reagents from Glucose Trinder and Lactic Acid standard clinical chemistry kits (Sigma, UK) but sample volume and incubation times were adjusted to take the kits from the normal millimolar clinical range to the micromolar range found in iontophoretic extraction. Glucose and lactate standards for kit calibration were prepared in HEPES-buffered saline.

Low-voltage (200-mV) impedance frequency sweeps were made using a Solartron 1260 Analyzer (Solartron, U.K.) to check the impedance between chambers A and B. The impedance was measured across the Ag/AgCl electrodes suspended in chambers A and B for both filled and empty lower chamber conditions. The two conditions tested were; 1) impedance with the lower chamber completely filled so that a good transmembrane conducting path existed and 2) impedance with the lower chamber emptied of solution so that the only current path was via the leakage current that would occur from chambers A to B along the membrane. For condition 1), chambers A and B were filled with HEPES/NaCl and the lower chamber was empty (newly drained of test solution following a full 90–min iontophoresis cycle). For condition 2), HEPES/NaCl was replaced into the lower chamber and chambers A and B filled with HEPES/ NaCl, frequency sweeps for this test were taken 1 min and 25 min after refilling the device.

Chamber-to-chamber resistance (A–B) was estimated from a complex impedance plot.

III. Results

The results of low current reverse iontophoresis of glucose are displayed in Fig. 2. Glucose was extracted across the CE membrane into the anode and cathode chambers in quantities similar to that found *in vivo* for human skin [10]. In effect, for 5 mM of glucose in the lower chamber up to 10 uM glucose was obtained in the samples taken from the upper chambers. In our system, glucose showed an enhanced extraction effect due to electro-osmosis in both the anode and cathode chambers. Cathodal extraction of glucose by electro-osmosis should be greater than an-

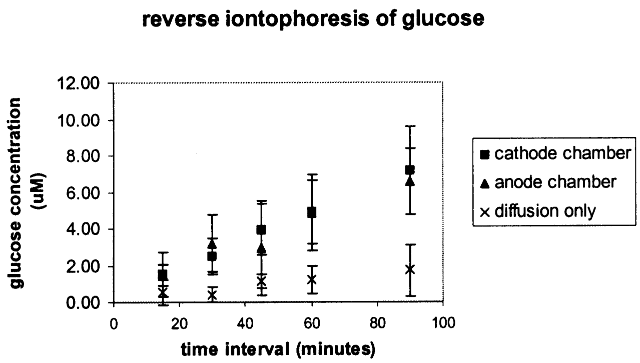

Fig. 2. Average of glucose samples extracted at each chamber over five separate experiments in the model system after iontophoresis for 0–90 min. Error bars show standard deviation in the results.

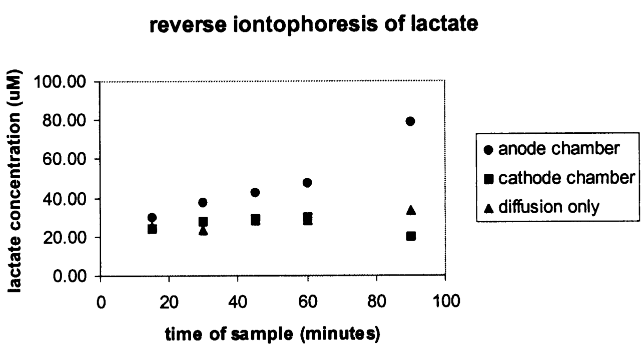

Fig. 3. Average of three separate experiments on lactate sample extraction in the model system following iontophoresis for 0–90 min.

odal extraction [10] in the presence of negatively charged pores. Anodal extraction by electro-osmosis is also significant where neutral or positively charged pores exist in the membrane [17]. It is possible that some masking of negative charge in the CE membrane or some membrane polarization occurred which enhanced the effect of anodal electro-osmosis or reduced the effect of cathodal electro-osmosis. The polarities of voltage on the chambers during the course of the experiment were not reversed. In practice voltage polarity would be changed every few minutes *in vivo* to avoid skin polarization and this has still to be studied in this system. An additional term not considered here is the change in osmotic pressure in the sample chambers due to preferential ion transportation, which in itself could affect solvent flow.

Lactate at 10-mM concentration was also tested for reverse iontophoresis extraction from the lower chamber across the CE membrane. The results shown in Fig. 3 indicate that the rate of extraction of lactate is an order of magnitude greater than glucose under the same conditions. This was expected since lactate has a net negative charge and, therefore, will be transported through the membrane by electric drift current as well as the electro-osmotic and diffusion fluxes that transport glucose.

Impedance analysis of the low voltage frequency sweeps performed allowed an estimate to be made of the chamber to chamber impedance with the lower chamber both filled with liquid and dry. Essentially, if there is a good current path through the anode and cathode chambers and the membrane/lower chamber, then the liquid-filled chamber impedance should demonstrate a much lower resistance between A and B than the dry chamber. Results indicated that the Chamber A to Chamber B resistance (with electrode impedance subtracted) was around 3 kΩ when the lower chamber was filled with buffer solution (both at 1 min and 25 min after filling the chamber) and around 11.5 kΩ just after the chamber had been drained (when the membrane would still be wet). The implication of this is that the major part of the current ($\sim$ 75%) will pass between chambers A and B under dc. iontophoresis but that the remaining 25% of the current is lost as leakage current between the chambers along the membrane never reaching the solution in the lower chamber. This effect is analogous to skin as discussed earlier and this leakage current has to be taken into account in practical devices. The values of resistance for trans-membrane and lateral membrane impedance obtained in this study are within the range of values obtained in skin impedance studies in the literature (see [18] for a discussion of the electrical properties of the skin and impedance measurement).

IV. Conclusion

The model cell and membrane combination when tested with glucose gives similar results to those reported *in vivo* [10]. If this result is indicative of the skin-like properties of the model then the lactate results obtained indicate that *in vivo* reverse iontophoresis of lactate should yield much higher levels of lactate than glucose, neglecting any metabolic effects that might be present during skin transport of lactate *in vivo*.

The current leakage effects in the cell between chambers A and B, demonstrated by the impedance measurements with the lower chamber empty of buffer solution, are also present in real skin and in practice contribute to a loss of transdermal transport power. The results from this study show that even with as much as 25% of current taking a lateral path from chamber to chamber along the membrane, (rather than the assumed path of across the membrane and though the lower chamber solution) good rates of extraction of glucose and lactate can be obtained. This current leakage effect is present when iontophoresis is applied for drug delivery as well as when reverse iontophoresis is used for medical diagnostics. There are no indications in the literature of where the threshold for leakage current (as a percentage of total current delivered by an iontophoresis device) should be set.

The effect of skin impedance on the transport efficacy of iontophoresis in transdermal delivery and extraction should be an essential consideration in the development of new devices but the issues are complex *in vivo*. Hydration of the skin is a critical factor which affects impedance measurement, for example one *in vivo* study showed that use of moisturizers can cause up to 20 times reduction in the surface resistance of the stratum corneum [19]. In addition, it appears that even moderate voltage application can cause electroporation of the skin reducing the transdermal impedance by up to three orders of magnitude [20].

The model cell described in this paper will be used in the future with combinations of glucose and lactate biosensors in hydrogel environments to study sensor behavior and optimize iontophoretic extraction conditions. The upper half of the cell can be modified to accommodate prototype electrode/sensor devices for testing. The aim is to develop and study an optimized, wearable lactate/glucose monitoring system for clinical applications.

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Patricia Connolly received the Ph.D. degree from the University of Strathclyde, Glasgow, U.K., in 1984.

She held a Lectureship/Senior Lectureship at the University of Glasgow, Glasgow, Scotland, from 1984–1992 where she was a founding member of the Bioelectronics Research Group. From 1992–1999, she worked in the medical diagnostics industry in Italy and Switzerland as a Head of Research and Technical Director. She returned to the U.K. to a new Chair in Bioengineering at the University of Strathclyde in 1999 where she founded a research group in Medical Diagnostic Devices and Instrumentation. Her main research interests include noninvasive patient monitoring, rapid diagnostic assays and sensors for cell and tissue engineering.

Christopher Cotton, photograph and biography not available at the time of publication.

Fabrice Morin received the M.Phil. degree in bioengineering from the University of Strathclyde, Glasgow, U.K., in 2000. He is now a Ph.D. degree student at the Japan Advanced Institute of Science and Technology, Tatsunokuchi, Japan.

His research revolves around the design of cell chips for drug screening and neuroprosthetic applications.

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TOPICAL REVIEW

Reverse iontophoresis for non-invasive transdermal monitoring

Benoît Leboulanger[1,2], Richard H Guy[1,2] and M Begoña Delgado-Charro[1,2]

[1] School of Pharmacy, University of Geneva, 30, Quai Ernest-Ansermet CH-1211 Geneva 4, Switzerland
[2] Centre international de recherche et d'enseignement ('Pharmapeptides'), F-74160 Archamps, France

E-mail: richard.guy@pharm.unige.ch

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Abstract

Iontophoresis is the application of a small electric current to enhance the transport of both charged and polar, neutral compounds across the skin. Manipulation of either the total charge delivered and/or certain electrode formulation parameters allows control of electromigration and electroosmosis, the two principal mechanisms of transdermal iontophoresis. While the approach has been mainly used for transdermal drug delivery, 'reverse iontophoresis', by which substances are extracted to the skin surface, has recently been the subject of considerable effort. Glucose monitoring has been extensively studied and other applications, including therapeutic drug monitoring, are contributing to the development of the technique. An internal standard calibration procedure may ultimately render this novel monitoring technique completely non-invasive.

Keywords: iontophoresis, electromigration, electroosmosis, glucose monitoring, therapeutic drug monitoring

1. Introduction

Iontophoresis involves the application of a small and defined electrical current to the skin. This process causes increased molecular transport through the skin and has found application, therefore, in transdermal drug delivery (Burnette 1989). The concept is not new, however; the basic mechanisms were clearly appreciated by Leduc (Leduc 1900, Sage and Riviere 1992) in 1900. Nevertheless, it is only now that approved iontophoretic drug delivery systems are finally reaching the market (Merino *et al* 1997).

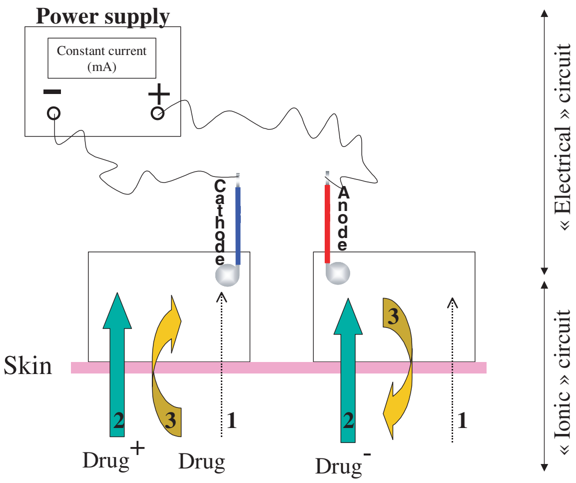

Figure 1. Reverse iontophoresis: a schematic diagram illustrating the experimental set-up. Constant current is delivered to the anode and the cathode from a power supply. Cationic and neutral substances are drawn towards the cathode by electromigration (2), electroosmosis (3) and (to a negligible extent, typically) passive diffusion (1). Anionic compounds are attracted into the anode chamber by electromigration (2), while convective solvent flow (3) opposes this phenomenon (again, passive diffusion (1) is negligible).

(This figure is in colour only in the electronic version)

The symmetry of iontophoresis means that it also enables extraction of solute molecules from within the subdermal compartment to the skin surface. The potential exists, therefore, to use the technique for clinical chemistry without blood sampling. Applications that may be envisaged include general blood chemistry, glucose monitoring, the detection of diagnostic markers and therapeutic drug monitoring (Merino *et al* 1997) . The mechanism of extraction involves either electromigration of charged species to the electrode of opposite polarity, or electroosmosis of polar, neutral or zwitterionic, molecules to the cathode; hence for cations, both mechanisms are operative.

A number of excellent reviews on iontophoresis and its applications have been published (Burnette 1989, Sage and Riviere 1992, Pikal 1992, Ledger 1992, Phipps and Gyory 1992, Scott *et al* 2000, Delgado-Charro and Guy 2001). Here, attention is focused on reverse iontophoresis and its applications in diagnosis and monitoring. After a brief consideration of the basics underlying electrotransport across the skin, a detailed evaluation of the relevant literature and a perspective on the future are presented.

2. Mechanisms of transport during reverse iontophoresis

2.1. Electromigration

Conventionally, in iontophoresis, a constant current is applied, such that the flow of electrons is translated into an ion flux across the skin. A power supply establishes the electric field that causes electrons to migrate in the 'electrical' portion of the circuit and ions to flow in the 'ionic' part (figure 1). It follows that the number of electrons flowing through the 'electrical' portion of the circuit is exactly balanced by the amount of ionic charge flowing through the skin (Sage and Riviere 1992) .

The electromigration contribution to iontophoretic transport is a direct result of current application. Ionic transport proceeds through the skin to maintain electroneutrality (Burnette 1989, Sage and Riviere 1992, Phipps and Gyory 1992). Faraday's law applies to steady-state transport and relates the number of ions crossing the membrane to the electric current, the time of current passage and the charge per ion (Burnette 1989, Sage and Riviere 1992):

$$M_i = \frac{T i_i}{F z_i} \tag{1}$$

where M_i is the number of moles of the ith ion, T is the time (s), z_i is the valence, F is Faraday's constant (96 487 C mol^{-1}) and i_i is the current (A) carried by the ith species.

Given that usually there is more than one ion moving across the barrier, the total number of moles transported (M) by the total current flowing (I) is given by

$$M = \sum_i M_i = \frac{T}{F} \sum_i \frac{i_i}{z_i} \tag{2}$$

where

$$I = \sum_i i_i. \tag{3}$$

This leads to the concept of an ion transport number (efficiency of transport) which is the fraction of the total charge that it transports:

$$t_i = \frac{i_i}{I}. \tag{4}$$

It follows that equation (1) may be rewritten as

$$M_i = \frac{t_i I T}{F z_i}. \tag{5}$$

With respect to the subject of this review, the experimentally measured extraction flux (J_i, mol s^{-1}) is defined by the ratio of the number of moles transported (M_i) to the sampling time (T) (i.e. the duration of reverse iontophoresis):

$$J_i = \frac{M_i}{T} = \frac{t_i}{F z_i} I. \tag{6}$$

Equation (5) shows that iontophoretic extraction is determined by the intensity of current, the time of iontophoresis, the charge and the transport number of the ion of interest. Current intensity (I) is directly and easily controlled by the power supply but is limited, for practical purposes *in vivo*, to not more than 0.5 mA cm^{-2} (Ledger 1992). The time of each extraction period must be sufficiently long to ensure that enough analyte is available for detection but not so long that clinically significant changes in the systemic concentration may have occurred. In any case, it must be recognized that reverse iontophoresis can only provide an estimation of the average level of the analyte in the body during the sampling period.

The charge (z_i) of the extracted ion is dictated by its molecular structure, and determines the polarity of the electrode at which sampling/analysis will be performed. The transport number, however, is difficult to estimate theoretically as it depends on the other ions contributing to the transport of charge across the skin. When an electric field is established across a membrane, ions on either side will migrate in the direction dictated by their charge. The speed of migration of an ion is determined by its physicochemical characteristics and the properties of the media through which the ion is moving (Sage and Riviere 1992, Phipps and Gyory 1992, Scott *et al* 2000, Phipps *et al* 1989). The sum of the individual ionic charges flowing across the skin must equal the number of electrons 'delivered' by the power supply; in other words, there is 'competition' among all the ions present to carry the charge.

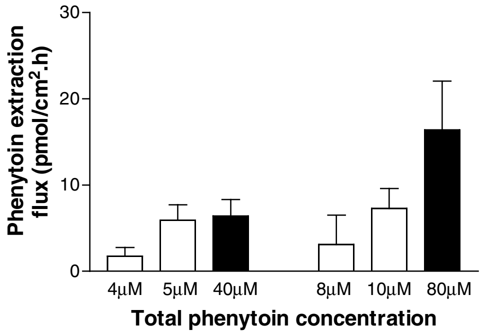

Figure 2. *In vitro* reverse iontophoresis fluxes of phenytoin at the anode illustrate that only free drug is extracted. The open bars (mean ± SD) indicate extraction of drug from a subdermal compartment that did not contain albumin. The filled bars represent data obtained when human serum albumin was present at 44 g l^{-1}. The results are consistent with a free fraction of phenytoin of approximately 0.1. Data redrawn from Leboulanger *et al* (2003c).

The transport number of the ion of interest (the *i*th ion) may also be expressed as follows:

$$t_i = \frac{c_i z_i u_i}{\sum_{j=1}^{n} (c_j z_j u_j)} \tag{7}$$

where c_j is the concentration (mol cm^{-3}), z_j is the valence and u_j is the mobility ($cm^2\ s^{-1}\ V^{-1}$) of each of the '*n*' ions in the system (Phipps and Gyory 1992).

Logically, the transport number depends on concentration (i.e. the available amount of a particular ion to participate in carrying charge across the skin). Likewise, it makes sense that ions, which are more mobile, will play a greater role in the movement of charge through the barrier. Note, however, that the relevant values of concentration (c) and mobility (u) are those inside the skin, rendering their estimation tricky at best and emphasizing the limitations of equation (7) as a predictive tool.

In reverse iontophoresis, the concentration of the analyte is the variable of interest and will depend (in the case of a drug being monitored, for example) on the dosage regimen and the relevant pharmacokinetics. Furthermore, with respect to electromigration, only the ionized fraction of the analyte is extractable and this will depend on the relevant pK_a. Similarly, for analytes that are bound to proteins, it is clear that only the free fraction can significantly contribute to charge transport across the skin. Figure 2 illustrates this point for phenytoin, a drug that is normally ~90% bound to albumin. As far as ionic mobility is concerned, regardless of the medium through which transport is occurring, an inverse dependence upon molecular size can be confirmed (and this is another reason, of course, why only unbound substances are extractable by reverse iontophoresis). In summary, therefore, it can be concluded that an ion can function as a major charge carrier if it is small, fully charged, at high concentration, and not significantly protein-bound. Additionally, and ideally, 'competing' ions are minimized, a situation not practically realizable for reverse iontophoresis where the major charge carriers are Na^+ and Cl^-.

Lastly, it is worth noting that the transport number is a 'formulation-dependent' parameter applicable for a given set of conditions. The transport number t_i can be determined experimentally; for example, by dividing the total amount of the ion transported by the total charge delivered (using Faraday's law, equation (1)), or from the gradient of a graph of ion flux versus current intensity (as in equation (6)) (Luzardo-Alvarez *et al* 2001).

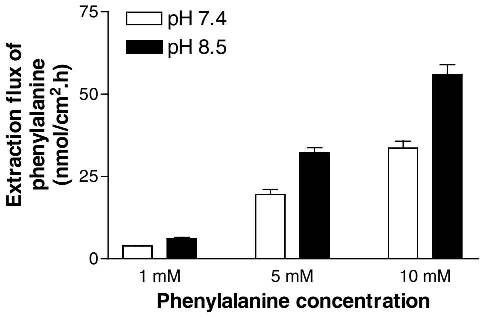

Figure 3. Electroosmotic extraction of phenylalanine as a function of (a) the subdermal concentration of the amino acid, and (b) the pH of the cathodal formulation. Reverse iontophoretic fluxes increased linearly with analyte concentration, and were significantly higher at pH 8.5 than at pH 7.4. Data redrawn from Merino *et al* (1999).

2.2. Electroosmosis

At physiologic pH, the skin is negatively charged and cation permselective. When an electric field is imposed across this type of membrane, there is convective or electroosmotic solvent flow induced in the anode to the cathode direction (i.e. in the direction of counter-ion migration) (Pikal 1990, 1992). This stream of solvent carries along with it dissolved solutes thereby enhancing the transport of neutral and, especially, polar molecules. Electroosmosis thus reinforces the transport of cations while acting against that of anions.

Important characteristics of this mechanism of electrotransport are, first, that the solvent volume flow (J_{vs}, volume $\times$ time^{-1} $\times$ area^{-1}) is proportional to the potential gradient across the skin (Pikal 1990, Burnette and Ongpipattanakul 1987) and, second, that the electroosmotic flux of solute (J_i) is independent of molecular size (at least as long as the solute diameter does not approach that of the transport pathway) (Pikal 1990, 1992). The relationship between the molar flux (J_i) of the solute 'i' and its molar concentration (c_i) is given by Pikal and Shah (1990):

$$J_i = J_{vs} c_i. \tag{8}$$

In an elegant series of experiments, J_{vs} during iontophoresis (Pikal and Shah 1990) was determined to be 6–19 μl h^{-1} mA^{-1}.

In addition to the current density, the pH and the ionic strength are electrode formulation parameters that may modulate electroosmosis (Santi and Guy 1996a). Modifying the pH on either side of the skin can change the charge on the membrane and hence its permselectivity. Practically speaking, only the surface pH can be altered *in vivo*, of course. Figure 3 illustrates that cathodic extraction of phenylalanine is enhanced by as high a pH as can be feasibly maintained in contact with the skin surface (Merino *et al* 1999); in contrast, an acidic pH in the cathode chamber significantly impairs electroosmosis towards the electrode (Santi and Guy 1996a), while favouring extraction in the opposite direction, presumably due to a degree of neutralization of the fixed charge on the skin.

For cathodal extraction, electroosmotic flow is increased by lowering the ionic strength of the electrode formulation (Santi and Guy 1996a, Merino *et al* 1999). This phenomenon is less obvious for anodal extraction (Santi and Guy 1996a). However, it should be remembered that a finite level of electrolyte must be present in the electrode chambers (particularly at the anode) to support the Ag/AgCl electrochemistry.

It has also been found that an anode formulation with $CaCl_2$ or $MgCl_2$, instead of NaCl, increased electroosmotic flow from beneath the skin surface towards the anode (Santi and Guy 1996b). Shielding of the net negative charge on the skin is a possible mechanism for this observation. In contrast, in the cathode chamber, to which electroosmosis predominates, enhanced solvent flow was achieved by formulating the electrode bathing solution with Ca^{++} binding agents (calcein, heparin or EDTA) presumably exposing a greater negative charge on the skin (Merino *et al* 1999, Santi and Guy 1996b).

2.3. The dominant mechanism: electromigration or electroosmosis?

For small mobile ions, electromigration is clearly the principal mechanism; similarly, for neutral, polar substances, electroosmosis dominates as there is no electromigration possible from the electrode. Both mechanisms of electrotransport depend upon the applied current (Phipps *et al* 1989, Padmanabhan *et al* 1990), with the effect being less marked for electroosmosis (Burnette and Ongpipattanakul 1987, Delgado-Charro and Guy 1994). As the size of an ion increases, its mobility is reduced and electromigration is compromised. For cations, this means that the dominant mechanism switches from electromigration to electroosmosis with increasing molecular size (Guy *et al* 2001); for anions, on the other hand, the two contributions will ultimately self-cancel and no transport will be observed. A weak acid, therefore, which is only partially ionized at physiological pH, may be more easily extracted as its neutral form to the cathode (e.g., theophylline (Glikfeld *et al* 1989, Sekkat *et al* 2002)).

3. Advantages and limitations of reverse iontophoresis

Non-invasive sampling methodologies are of obvious benefit to all patients for at least the following reasons: more information is obtainable as sampling can be performed more frequently, decreased pain and discomfort (i.e. better compliance), decreased risk of infection, potential for home-monitoring, etc.

Furthermore, there are special populations for whom non-invasive diagnosis and monitoring would be particularly useful: patients who are repetitively subjected to invasive blood withdrawal procedures, patients who over- or under-respond to standard therapeutic regimens, subjects who are least able to tolerate, recognize or communicate problems with unexpected drug effects and those who, for whatever reason, are either over- or under-dosed. Such patient populations include the ‘critically’ ill under intensive care, cancer and AIDS sufferers, pregnant women, those displaying unusual pharmacokinetics, patients receiving simultaneous, multi-drug dosing regimens (e.g., the elderly) and, of obvious special concern, are pediatric patients, for whom the need for non-invasive diagnosis and monitoring is particularly acute.

Even though reverse iontophoresis is much more efficient and reproducible than passive extraction, the quantities of analyte obtained at the skin surface are necessarily small. Dilution factors are likely to fall in the 10–100-fold range, or higher. Thus, analytical chemistry demands are significant and sampling periods may be so long, as a result, that changes in systemic concentration can occur. While this would be unacceptable in the case of glucose, for therapeutic drug monitoring at ‘steady-state’ the problem is less important (i.e. continuous monitoring is unnecessary, and a prolonged sampling time to obtain an average measure of concentration would be reasonable). Thus, an iontophoretic patch could be worn for a few hours at home, for example, and then sent to the clinical chemistry laboratory. Such an

Table 1. *In vivo* reverse iontophoretic extraction of potassium and sodium ions as a function of different parameters. The study group comprised 98 human volunteers. Data from Benjamin *et al* (1954).

	Potassium (μmol h^{-1})	Sodium (μmol h^{-1})	Ratio
Measurement time (10 measures)	1.8 ± 0.3	4.3 ± 0.5	2.4 ± 0.6
Measurement site			
Volar surface forearm	1.8 ± 0.4	4.3 ± 0.8	2.4 ± 0.4
Back of lower leg	1.6 ± 0.5	4.2 ± 0.8	2.7 ± 0.5
Upper part of abdomen	1.9 ± 0.4	4.5 ± 1.0	2.4 ± 0.5
Ambient temperature			
32–33 °C	1.8 ± 0.3	4.3 ± 0.5	2.4 ± 0.4
21 °C	1.7 ± 0.4	4.4 ± 0.6	2.5 ± 0.4
Gender			
Male	1.7 ± 0.5	4.3 ± 0.8	2.5 ± 0.3
Female	1.7 ± 0.5	4.2 ± 0.6	2.5 ± 0.3
Age			
<24 years	1.8 ± 0.5	4.2 ± 0.7	2.3 ± 0.5
25–49 years	1.7 ± 0.3	4.3 ± 0.5	2.6 ± 0.3
>50 years	1.6 ± 0.3	4.4 ± 0.5	2.7 ± 0.4

‘off-line’ analysis and quantification would be acceptable, and a sampling device simpler and less expensive to design.

An important limitation occurs when the skin accumulates the analyte of interest such that the initial extraction sample contains mostly information about this local ‘reservoir’ (this is the case for glucose (Glikfeld *et al* 1989) and lithium (Leboulanger *et al* 2003a)). A ‘warm-up’ period is necessary, therefore, before readings reflective of systemic levels are obtained.

It is also true that the reverse iontophoretic flux does not reach a constant, ‘steady-state’ rate instantaneously (Delgado-Charro and Guy 2003, Leboulanger *et al* 2003c)—the time to do so depends on the molecule of interest, and the dominant mechanism of electrotransport. However, whether this limitation is significant has not been completely established; for certain analytes, an acceptable correlation between extraction flux and subdermal concentration is also obtained prior to steady-state.

An additional, and significant, limitation is that reverse iontophoresis will simply not work for molecules with particular physicochemical properties. Specifically, proteins, for example, are simply too large to be extracted in amounts that are quantifiable. Extremely lipophilic compounds, with extremely small aqueous solubilities, will also be undetectable; unfortunately, cholesterol falls into this category.

4. Case studies

In 1954, reverse iontophoresis was first applied to the extraction of sodium and potassium ions (Benjamin *et al* 1954). Experiments were performed *in vivo*, in man, using a metal plate as the electrode. A current density of 0.5 mA cm^{-2} was applied over a skin surface of 8.3 cm^2 for 5 min or longer. In a total of nearly 100 subjects, it was shown that age, gender, measurement time, measurement site and ambient temperature did not significantly affect the amounts of the cations extracted (table 1). However, in the longer duration experiments, skin ‘damage’ was observed due to the fact that the pH of the cathodal solution increased from between 6

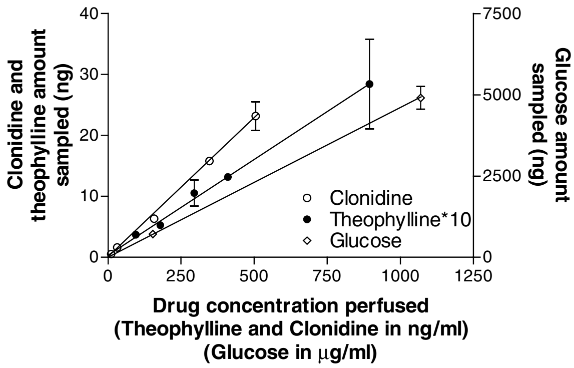

Figure 4. *In vitro* iontophoretic sampling of clonidine, theophylline and glucose. The amounts of the three compounds extracted across hairless mouse skin were linearly correlated with their subdermal drug concentrations. Data redrawn from Glikfeld *et al* (1989).

and 7 to nearly 11. Clearly, therefore, electrolysis of water was taking place at the bare metal electrode and, as a result, it became sensible to henceforth use electrochemically reversible electrodes (e.g., Ag/AgCl) in iontophoresis studies (Cullander *et al* 1993).

The practical potential of reverse iontophoresis was appreciated much later (in 1989) when it was demonstrated that the amount of a substance extracted across the skin in this way was linearly related to the subdermal (and, by extrapolation, the systemic) concentration (Glikfeld *et al* 1989). This relationship was shown for clonidine, theophylline and glucose; that is, for a more or less fully charged cation, for a partially charged anion and for a neutral polar molecule (figure 4).

Subsequently, attention was focused on glucose. Obviously, the availability of a non-invasive tool with which to monitor blood sugar in diabetics would be of immense medical benefit. The conventional, ‘finger-stick’ method, while precise and effective, is rarely used with sufficient frequency to reduce or avoid either hypo or hyper-glycaemic events, despite compelling evidence that such an approach can significantly impact the chronic progression of the disease (The Diabetes Control and Complications Trial Research Group 1993). Reverse iontophoresis experiments *in vitro* (Rao *et al* 1993) and initial *in vivo* studies in non-diabetic subjects (Rao *et al* 1995) established proof-of-concept, and led to the commercial development of an integrated device (the Glucowatch Biographer® (Cygnus, Inc. 2002, Tierney *et al* 2000b)) which is able to extract glucose iontophoretically across the skin and then assay sugar *in situ* with an on-board amperometric biosensor (figure 5). The mechanism of electrotransport of glucose is electroosmosis, meaning that, during each sampling period (20 min, initially, a shorter time in the G2 version of the device), the amount of analyte to be detected is very small. An exquisitely sensitive analytical method is therefore required, and involves a highly optimized adaptation of the Pt-glucose oxidase sensor (Tierney *et al* 1999).

A significant quantity of data has now been published to illustrate the efficiency of this reverse iontophoresis technology to track changes in the blood sugar levels of diabetics over the entire range of glycaemia (Tierney *et al* 2000b, Tamada *et al* 1995, 1999 Garg *et al* 1999, Tierney *et al* 2001, Pitzer *et al* 2001, Potts *et al* 2002). The quality of this information led to the Glucowatch being approved for use in adults by the U.S. Food and Drug Administration in 2001. Additional work in children (7–17 years) allowed this approval to be extended to juvenile diabetics the following year (Eastman *et al* 2002).

The long-term use and usefulness of the Glucowatch remain to be seen; nevertheless, there can be little doubt that this first truly non-invasive approach to the monitoring of blood

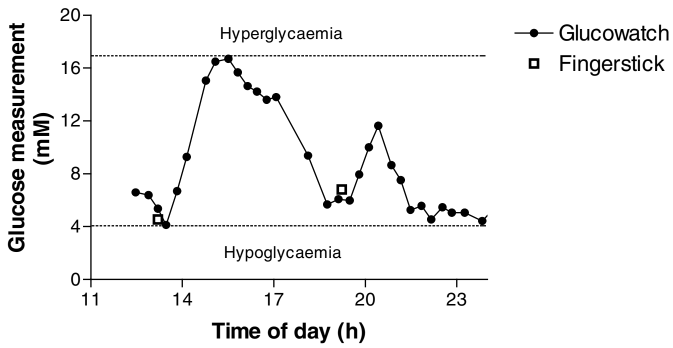

Figure 5. Continuous glucose monitoring *in vivo* with the Glucowatch Biographer® over a 12-h period (closed circles) compared with the typical information available to a diabetic from two 'finger-stick' measurements (open squares) pre-lunch and pre-dinner. Data redrawn from Tierney *et al* (2000b).

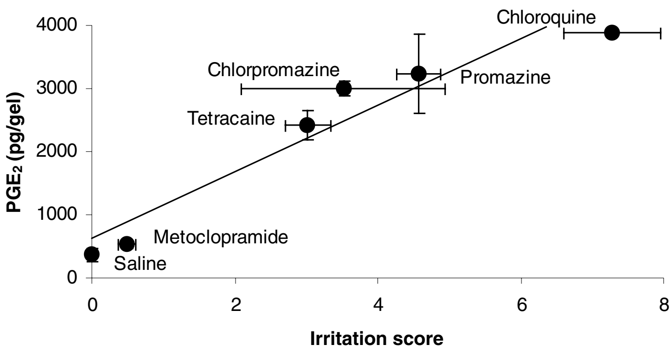

Figure 6. Correlation between PGE_2 efflux and irritation ($r^2 = 0.96$). Iontophoretic systems containing 100 mM drug were applied at 50 μA cm^{-2} for 24 h. Graph redrawn from Mize *et al* (1997).

sugar has made a paradigm shift in the field. It should be said that limitations of the approach are apparent, not least the lengthy, 2–3 h, warm-up time before measurements can be made (due to the need to empty a glucose reservoir in the skin) and the fact that a 'finger-stick' blood measurement is essential to calibrate the device.

Other applications of reverse iontophoresis can be divided into diagnosis/monitoring and therapeutic drug monitoring. An innovative concept was to use the approach as a diagnostic tool for cutaneous inflammation (Mize *et al* 1997). Prostaglandin E2 (PGE_2) was monitored in response to the transdermal delivery of irritant drugs. It was hypothesized and shown that low-level iontophoresis (0.05 mA cm^{-2} over 2 cm^2) of saline did not by itself provoke an increased production of inflammatory markers *in vivo*, in the hairless guinea pig. Subsequently, potentially irritant drugs (chlorpromazine, chloroquine, promazine, tetracaine and metoclopramide) were administered iontophoretically (figure 6). Then, the anodal extraction of PGE_2 from the site of drug administration was monitored and compared to the saline control. Significant increases were observed that correlated well with more classic determinations of irritation (e.g., the Draize test, lesion score).

The reverse iontophoretic extraction of phenylalanine has also been demonstrated (Merino *et al* 1999). In phenylketonuria, a severe metabolic disease, the enzyme which

biotransforms phenylalanine is missing. Early detection of the disease and subsequent control of the diet are therefore essential. Children with the disease are frequently monitored via blood samples and a non-invasive approach would therefore be of interest. Phenylalanine is zwitterionic at physiologic pH and is therefore extracted during reverse iontophoresis by an electroosmotic mechanism. Like glucose, it has been shown that the amounts detected at the cathode are proportional to the subdermal concentrations (figure 3). However, phenylalanine systemic concentrations, even in phenylketonuria, are much less than typical glucose levels in diabetics. It follows that the analytical chemistry challenge for the monitoring of phenylalanine via reverse iontophoresis is considerable. On the other hand, it should be said that continuous and frequent monitoring, as performed by the Glucowatch®, is not necessary for sufferers of phenylketonuria; a device that simply collects the sample (once a day or once a week, for example), which is subsequently sent for analysis at a central analytical laboratory, would be perfectly acceptable.

Very recently, the reverse iontophoretic extraction of urea has been performed in 17 patients (21–35 years) with impaired kidney function (Degim *et al* 2003). Urea was extracted by electroosmosis to the cathode by current application for 5 min. The extracted amounts correlated well with urea levels in the blood ($r^2 = 0.88$). A logical application of this approach is to determine when dialysis should be performed in pediatric patients with kidney disease. A proof-of-concept study was subsequently conducted in six juvenile subjects (aged 9–16 years) for whom it was clearly shown that the amounts of urea extractable pre- and post-dialysis were quite different.

Therapeutic drug monitoring applications of reverse iontophoresis have recently attracted heightened interest. The potential of the approach was first explored using caffeine and theophylline in a model designed to mimic the developing cutaneous barrier in a premature neonate (Sekkat *et al* 2002). While the idea appears feasible for full-term infants, whose stratum corneum performs as well as that of an adult, the technique is less satisfactory when the barrier is impaired (as is the case, of course, for premature babies). The problem is caused by the fact that, superimposed upon the electrotransport of the target analyte being extracted by reverse iontophoresis, there is a significant passive transport which confounds straightforward interpretation of the data. Nevertheless, the non-invasive nature of the technique implies that it may have other useful applications in the case of the sick neonate, at least when skin barrier function is intact.

An important consideration when assessing the feasibility of reverse iontophoresis for therapeutic drug monitoring is the degree to which the compound of interest is protein-bound. Logically, only the free drug is electrotransported across the skin as the protein-bound form is too large to be extracted. This issue has been addressed with two anti-convulsant drugs, valproic acid (Delgado-Charro and Guy 2003) and phenytoin (Leboulanger *et al* 2003c), approximately 90% of which are typically bound to plasma proteins. Reverse iontophoresis extraction of both drugs in a concentration dependent fashion was demonstrated over a wide range encompassing those free levels observed in patients undergoing treatment. Valproate was extracted to the anode, while phenytoin ($pKa = 8.3$) was recovered at both anode (the ionized fraction of the drug being attracted by electromigration) and cathode (the neutral form being carried by electroosmosis). When the level of protein was reduced in the subdermal compartment, the amount of drug extracted was increased consistent with the rise of the free drug level (figure 7). Equally, when monitoring phenytoin at a fixed subdermal protein level, introduction of valproate led logically to an increase in the free amount of the first drug and a higher rate of extraction due to the impact of competitive binding. The sensitivity of the method to respond to changes in free drug concentration in this way supports its potential usefulness for monitoring substances with a narrow therapeutic window. On the other hand, it must be

Phenytoin	80 µM	40 to 80 µM	80 µM
Albumin	44 to 22 g/l	44 g/l	44 g/l
Valproate	-	-	0 to 542 µM
Free Phenytoin	8 to 16 µM	4 to 8 µM	9 to 14 µM

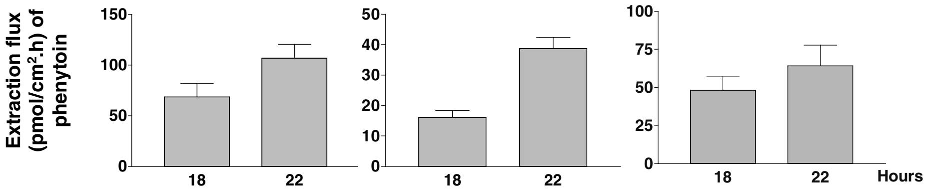

Figure 7. Monitoring of free phenytoin by reverse iontophoresis. The impact of (a) changing albumin concentration (1st panel), (b) changing drug concentration (2nd panel) and (c) addition of a competing drug, valproate (3rd panel) is illustrated. Data from Leboulanger *et al* (2003c).

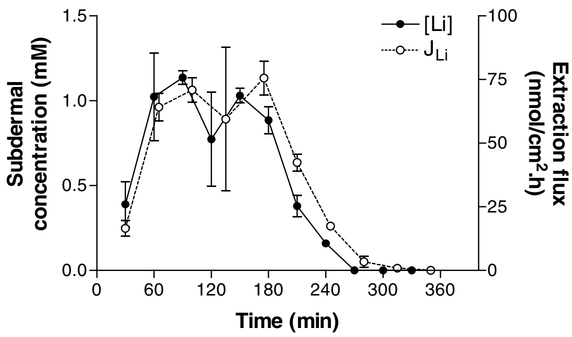

Figure 8. Monitoring of subdermal lithium concentration changes. Continuous line is the subdermal lithium concentration (mM). Dotted line is the cathodal extraction flux (nmol cm^{-2} h^{-1}). Data taken from Leboulanger *et al* (2003b).

recognized that, for lipophilic drugs such as valproic acid and phenytoin, the free systemic concentrations are quite low (50–105 μM and 4–8 μM, respectively) and the amounts extracted by reverse iontophoresis are extremely small (indeed, the *in vitro* experiments described above were performed with radiolabelled drugs). Once more, the analytical challenge *in vivo* will be very demanding.

With lithium, a drug used to treat bipolar disorders, on the other hand, the analytical chemistry problem is much less severe. First of all, as a small, non-protein bound cation, Li^+ is reverse iontophoretically extracted much more efficiently than the aforementioned anti-convulsant drugs (Leboulanger *et al* 2003b). Second, the effective plasma concentrations are much higher for lithium such that the amounts detected at the skin surface can be assayed with existing technology. *In vitro*, the linearity and rapidity of Li^+ extraction from a physiological buffer were simply demonstrated (figure 8); when the concentration of Li^+ in the sub-dermal compartment was varied over time, to simulate a pharmacokinetic profile, the extraction profile closely followed the 'absorption' and 'elimination' phases of the curve. Subsequently, an *in vivo* study (Leboulanger *et al* 2003a) in patients being treated with lithium has shown the potential of reverse iontophoresis to provide a useful clinical tool. The reverse iontophoretic extraction fluxes were extremely well correlated with the corresponding plasma concentrations.

5. Optimization

To expand the range of reverse iontophoresis applications, and improve on the existing technology, three strategies present themselves immediately for consideration.

Most obviously, an improvement in analytical sensitivity is desirable. The lower the extracted amount that can be detected, the larger the number of potential candidates for the technique, and the shorter the time of sampling (and, hence, the lower the total charge passed across the skin). Further discussion of this point, however, is outside the scope of this review.

Second, we may anticipate that optimization of the iontophoretic conditions will lead to maximization of the extracted amount. Thus, choosing the right current, current density, current profile and 'acceptor' phase for the extracted analyte may be expected to improve the extraction efficiency. It is well established that iontophoretic transport is directly proportional to the current and to the time of current application. In practice, however, there are limits to which these parameters can be increased. In terms of current density, it is generally agreed that 0.5 mA cm^{-2} is the maximum tolerable in man (Ledger 1992). It follows that the total current can be increased by increasing the surface area extracted. But, if one maintains the current density fixed, even under these conditions, the degree of sensation experienced by the subject increases with treated area, presumably because a greater number of dermal nociceptors are activated (Rao *et al* 1995). There are at least two other difficulties associated with increasing the total current and the area of skin over which reverse iontophoresis extraction is performed: (i) more current means that the (typically) Ag/AgCl electrodes used must be coated with an augmented layer of AgCl to ensure that the correct electrochemistry operates throughout the sampling period, and (ii) more area implies a greater volume into which the sample is collected, and this may place more demands on the analytical method. While it has been suggested that iontophoresis-induced reddening of skin can be reduced by 'pulsing' the current on/off at different frequencies (Santi and Guy 1996a), the practical result is that the impact of irritation is not proved. In addition, as the extraction is much less efficient during the 'off' period, the total time for sampling has to be increased so that the total desired current can be passed. Alternating current shows no benefits whatsoever; however, switching electrode polarity at the end of each sampling period, as is done in the Glucowatch®, has the distinct advantage of allowing regeneration of the Ag/AgCl electrodes (Tierney *et al* 2000a).

The third strategy is a method to avoid the present necessity to calibrate reverse iontophoretic extraction of an analyte with a blood sample. The amount of the compound of interest recovered at the skin surface is diluted in a certain volume of 'acceptor' fluid. The concentration therein depends on the efficiency of extraction (the analyte's transport number for an ion, the electroosmotic flow for a neutral species) and the volume of the 'acceptor' solution. In the case of the Glucowatch®, for example, in each sampling period, the electroosmotic flow of less than 1 μl is diluted into a volume of 400 μl (Tierney *et al* 2000a); that is, a three order of magnitude dilution of the glucose. Calibration is therefore essential to relate the amount of sugar extracted to the blood concentration. The concept of an 'internal standard' was initially proposed in 1993 (Numajiri *et al* 1993) and has recently been significantly refined and reduced to practice (Delgado-Charro and Guy 2003). The idea is as follows: reverse iontophoresis extracts numerous compounds at the same time; i.e. the process is non-specific and is only rendered specific for a particular compound by using a selective and precise assay. Suppose now that, in addition to the chosen target analyte (A), a second substance is also specifically analysed in each sampling period. Suppose, further, that the blood concentration of this second molecule (IS) is effectively constant. It follows that there should be a proportionality between the measured extraction flux ratio of A and

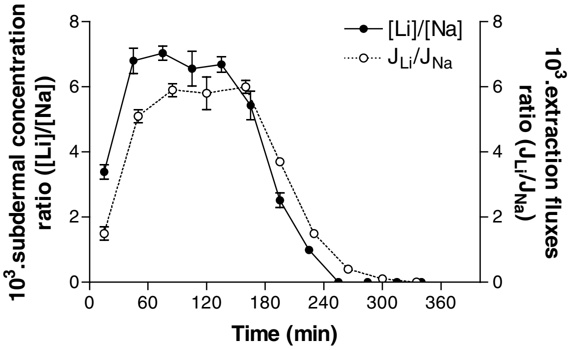

Figure 9. Simultaneous monitoring of lithium and sodium fluxes as a function of time. Data taken from Leboulanger *et al* (2003b).

IS (J_A/J_{IS}) and the ratio of their subdermal, or blood, concentrations (C_A/C_{IS}):

$$\frac{J_A}{J_{IS}} = K\frac{C_A}{C_{IS}}. \tag{9}$$

It follows that, given C_{IS} is fixed and (presumably) known,

$$C_A = \frac{C_{IS}}{K}\frac{J_A}{J_{IS}}. \tag{10}$$

Thus, if the proportionality constant K can be determined and shown to be invariant in a subject population, then an experimental determination of J_A/J_{IS}, together with the known, 'constant' term (C_{IS}/K), allows C_A to be found without the need for blood sampling. The success of this idea rests on the independence of the iontophoretic transport of the analyte and the internal standard. In other words, it is important that a change in the transport number of A (due, for example, to a fall in its systemic concentration) is not compensated by an increase in that of IS. In this case, the validity of equation (9) breaks down. When the concept was proposed initially (Numajiri *et al* 1993), the design of the experiments led to exactly this violation, with the result that the method could not be validated. Recently, however, the principle has been re-visited (Delgado-Charro and Guy 2003), the experimental test designed more carefully, and the technique has been shown to work. The first demonstrated success involved the reverse iontophoretic extraction of valproate using glutamic acid as an anionic internal standard (Delgado-Charro and Guy 2003). Although the concentration of glutamate *in vivo* is not sufficiently constant for this amino acid to be considered as a practical internal standard, it served perfectly to prove the concept in this study. It was shown that (i) the extraction flux of valproate varied linearly with its subdermal concentration, (ii) the extraction flux of glutamate remained constant as the valproate concentration fluctuated and (iii) the ratio of the valproate to glutamate extraction fluxes was proportional both to their subdermal concentration ratio (equation (9)) and, as the glutamate concentration was fixed, to the valproate concentration. The approach allowed experimental variability to be reduced and permitted the subdermal valproate to be found even before the iontophoretic transport achieved 'steady-state'.

Subsequently, similar success was achieved with the Li^+/Na^+(analyte/internal standard) couple (figure 9). The sodium ion is a useful and practical internal standard due to the fact that the concentration of NaCl *in vivo* does not vary outside the range of 125–145 mM (and typically remains within a much narrower window); Na^+ is also the major charge carrier in iontophoresis in the outward direction towards the cathode (much as Cl^- performs the same function towards the anode). *In vivo* measurements, in patients, confirmed the constancy of

the proportionality constant K in equation (9). However, while *in vitro* experiments (Sieg *et al* 2003) indicated that Na^+ may prove a valid internal standard for glucose also, a subsequent *in vivo* study revealed that electroosmotic flow is a much more sensitive phenomenon and can vary by nearly a factor of ten even while the electromigrative flux of Na^+ remains unchanged. It follows that it will be necessary to identify an electroosmotically-extracted internal standard for glucose in order to avoid the need for blood sampling.

6. Conclusion

Recent progress in reverse iontophoresis confirms its considerable potential. The approval and commercialization of the Glucowatch® represents an important milestone for the technology as the first truly non-invasive monitoring device for diabetics. The value of the method is readily appreciated, furthermore, not only as a research tool but also as a practical means by which to improve the quality of care (and life) in patient populations for which repetitive blood sampling represents a significant burden: pediatric, geriatric and chronically ill individuals are obvious examples. Therapeutic drug monitoring is similarly accessible via reverse iontophoresis and it is hoped that the size of the commercial markets here (relative to that for glucose monitoring) does not deter the ultimate realization of practical devices. The future holds promise, in particular, if analytical tools continue to evolve in term of sensitivity, specificity and miniaturization, as they have in the recent past. If this is the case, then the application of reverse iontophoresis in 'smart', feedback drug delivery systems, and in remote sensing, can be foreseen.

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Detection of Hypoglycemia With the GlucoWatch Biographer

KENNETH R. PITZER, DVM[1]
SHASHI DESAI, BS[1]
TIM DUNN, BS[1]
STEVE EDELMAN, MD[2]
YALIA JAYALAKSHMI, PHD[1]
JOHN KENNEDY, BS[1]
JANET A. TAMADA, PHD[1]
RUSSELL O. POTTS, PHD[1]

OBJECTIVE — Hypoglycemia is a common acute complication of diabetes therapy. The GlucoWatch biographer provides frequent and automatic glucose measurements with an adjustable low-glucose alarm. We have analyzed the performance of the biographer low-glucose alarm relative to hypoglycemia as defined by blood glucose ≤3.9 mmol/l.

RESEARCH DESIGN AND METHODS — The analysis was based on 1,091 biographer uses from four clinical trials, which generated 14,487 paired (biographer and blood glucose) readings.

RESULTS — The results show that as the low-glucose alert level of the biographer is increased, the number of true positive alerts (alarm sounds and blood glucose ≤3.9 mmol/l) and false positive alerts (alarm sounds but blood glucose >3.9 mmol/l) increased. When analyzed as a function of varying low-glucose alert levels, the results show receiver operator characteristic curves consistent with a highly useful diagnostic tool. Setting the alert level from 1.1 to 1.7 mmol/l above the level of concern is likely to optimize the trade-off between true positives and false positives for each user. When the same blood glucose data are analyzed for typical monitoring practices (two or four measurements per day), the results show that fewer hypoglycemic events are detected than those detected with the biographer.

CONCLUSIONS — The frequent and automatic nature of the biographer readings allows more effective detection of hypoglycemia than that achieved with current medical practice.

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Hypoglycemia is a common acute complication of diabetes therapy. The frequency of severe hypoglycemia has been shown to increase with more intensive treatment. Increasing the frequency of glucose measurements, regardless of the technique used, makes it possible to detect a greater number of hypoglycemic events. However, as many as seven glucose measurements per day may fail to detect hypoglycemic events (1).

A device providing automatic readings could make frequent monitoring easier and enable an alarm to be sounded in response to glucose readings below user-selected alert levels. Such an alarm could reduce the risk of hypoglycemia, making intensive therapy safer and more acceptable for patients.

The GlucoWatch biographer (Cygnus, Redwood City, CA) provides frequent, automatic, and noninvasive glucose measurements—up to three readings per hour for as long as 12 h after a blood glucose measurement for calibration. Clinical studies in controlled and home environments have demonstrated high accuracy and precision (2,3). The results presented here evaluate the hypoglycemia alert performance in a large and demographically diverse patient population using the biographer both in controlled and normal daily environments. The accuracy and precision results from these studies have been described (4).

The performance of the hypoglycemia alert depends on the selection of a low-glucose alert level that will trigger an audible alarm. The performance of the alert function can be best evaluated by an analysis of the receiver operator characteristic (ROC) curves (5). The ROC curve is a plot of the sensitivity (true positive fraction [TPF]) versus 1.0 − specificity (false positive fraction [FPF]) for a series of possible low-glucose alert levels.

RESEARCH DESIGN AND METHODS

Noninvasive glucose extraction and detection

The biographer extracts glucose through the skin by reverse iontophoresis and measures the extracted sample using an electrochemical biosensor. Iontophoresis is a technique whereby a low-level electric current (0.3 mA/cm^2 in these studies) is passed through the skin between an anode and a cathode (6). The current is carried primarily by the migration of sodium ions toward the cathode. Uncharged molecules (e.g., glucose) are carried along by convective transport (electroosmosis) (7,8). The amount of glucose extracted at the cathode has been demonstrated to correlate with blood glucose in diabetic subjects (9). In the biographer, the extracted glucose is measured by an amperometric biosensor using detection of H_2O_2 generated by the glucose/glucose oxidase reaction. The operating principles of the biosensor are described elsewhere (10–12).

The biographer provides readings every 20 min after calibration using a fingerstick blood glucose measurement taken after a 3-h equilibration period. This sin-

From [1]Cygnus Incorporated, Redwood City; and the [2]UCSD School of Medicine and Veterans Affairs Medical Center, San Diego VA Medical Center, San Diego, California.

Address correspondence and reprint requests to Dr. Pitzer, Cygnus, Inc., 400 Penobscot Dr., Redwood City, CA 94063. E-mail: kpitzer@cygn.com.

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S.E., J.K., and T.D. are paid consultants for and hold stock in Cygnus; and K.R.P., Y.J., J.A.T., R.O.P., and S.D. are employed by and hold stock in Cygnus.

Abbreviations: AUC, area under the curve; FN, false negative; FP, false positive; FPF, FP fraction; PTH, parathyroid hormone; ROC, receiver operator characteristic; TN, true negative; TP, true positive; TPF, TP fraction.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Description of the protocols used*

Study name	Accuracy	Home simulated	Home environment	Lab method comparison
Environment	Controlled clinic	Home simulated	Home	Home simulated
Duration (days)	1	2	5 (Wed. through Sun.)	1
Biographers worn per day	2	1	1	2
Calibrating BG device	HemoCue Photometer	One Touch Profile	One Touch Profile	YSI Analyzer
Comparative BG device	HemoCue Photometer	HemoCue Photometer	One Touch Profile	YSI Analyzer
Frequency of comparative BG measurements (*n*/h)	2	2	1	1
Number of biographer uses	406	212	420	53
Total number of paired points*	7,181	3,829	3,060	417

Data are *n*, unless otherwise indicated. *Includes pairs where the biographer reading and/or the blood glucose value are outside the range of 40–400 mg/dl. BG, blood glucose.

gle-point calibration, which accounts for variability in both biosensor sensitivity and skin permeability, is used to convert subsequent biosensor measurements into glucose readings. A signal-processing algorithm (12) is programmed into the biographer software. The reading lags behind the corresponding blood glucose value by ~17 min.

Data integrity screens in the biographer software detect spurious data points resulting from such sources as electrical noise, high background currents, and open or short circuits. The presence of data points not conforming to objective a priori criteria results in the disregarding (or skipping) of a glucose reading. The biographer also contains skin temperature and skin conductance sensors. The latter is directly related to the amount of sweat on the surface of the skin. Because large temperature changes or the presence of glucose in sweat can confound the glucose measurement, once the output from either of these sensors exceeds a predetermined threshold, the measurement for that cycle will be skipped. These data-screening methods thus prevent potentially inaccurate glucose readings. In the studies presented here, ~20% of all readings were skipped because of data integrity screens.

Study designs

Four clinical studies were performed, each with institutional review board approval. Accuracy was evaluated in a controlled clinic setting, a simulated home environment, and the home environment. An additional laboratory method comparison study evaluated accuracy versus a laboratory standard method (YSI analyzer; Yellow Springs Instruments). Biographer readings were compared to serial capillary blood glucose measurements performed with a variety of instruments. Subjects wore the biographer for 15-h sessions (3-h equilibration and 12-h measurement periods). In the clinic setting, glucose levels were manipulated to produce periods of hypoglycemia and hyperglycemia. All subjects were adults (18 years of age and older) with type 1 or type 2 diabetes requiring insulin treatment. The biographer readings were masked from the patients and investigators during the study period. All dose adjustments for insulin were completed using blood glucose measurements. Table 1 summarizes the design of the clinical studies along with the number of biographer uses and paired points. Patient diaries were used to record the times of meals and insulin injections. The diaries did not require the subjects to record symptoms of possible hypoglycemia.

Data analysis methods

Data download and analyses of accuracy and precision are described elsewhere (2–4). The objective of this analysis was to evaluate the ability of the biographer to correctly detect hypoglycemia as determined by the comparative blood glucose value. This analysis was completed by defining hypoglycemia as blood glucose ≤3.9 mmol/l and varying the biographer low-glucose alert level. At each alert level, each biographer reading was evaluated as either a true positive (TP), false positive (FP), true negative (TN), or a false negative (FN). The definitions of each of the terms are as follows:

- Hypoglycemic event: blood glucose ≤3.9 mmol/l
- Biographer low-glucose alert level: threshold below which the biographer would trigger the audible alarm
- TP: blood glucose ≤3.9 mmol/l and biographer less than or equal to the low alert level
- FP: blood glucose >3.9 mmol/l but biographer less than or equal to the low alert level
- TN: blood glucose >3.9 mmol/l and biographer more than the low alert level
- FN: blood glucose ≤3.9 mmol/l but biographer more than the low alert level

Sensitivity and specificity were defined by the following equations and determined at seven low-glucose alert levels (3.9, 4.4, 5.0, 5.6, 6.1, 6.7, and 7.2 mmol/l).

$$\text{sensitivity} = \text{TP}/(\text{TP} + \text{FN})$$

$$\text{specificity} = \text{TN}/(\text{TN} + \text{FP})$$

These results were then used to prepare the ROC curves.

The TPF is the same as the sensitivity and reflects the frequency with which the biographer correctly identified hypoglycemia of all occasions in which the blood glucose test result was ≤3.9 mmol/l. The FPF is calculated by subtracting the specificity from 1.0. The FPF reflects the frequency with which the biographer incorrectly identified hypoglycemia of all occasions in which the blood glucose test result was >3.9 mmol/l.

Two types of hypoglycemic events were analyzed. First, only paired biographer readings and blood glucose values were analyzed. For this paired-point analysis, the comparative blood glucose result

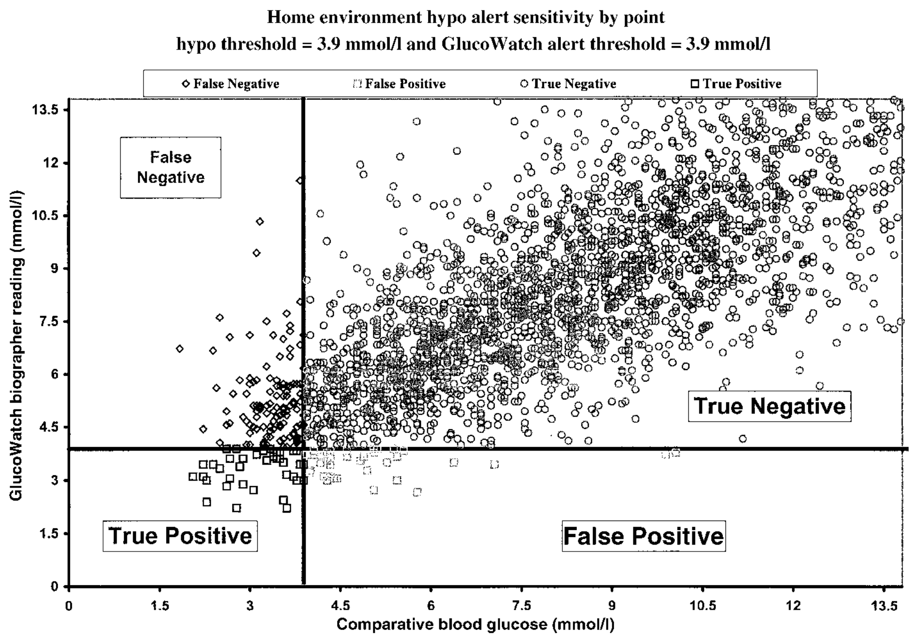


Figure 1—*A correlation plot of the GlucoWatch biographer versus blood glucose values for the home-environment study.*

must have been obtained in the time period between 10 and 20 min before the biographer reading. The results of all studies were analyzed in this manner. In addition, the home-environment data were analyzed for all blood glucose ≤3.9 mmol/l, even if there were no paired biographer readings. This was completed by evaluating biographer readings immediately surrounding the hypoglycemic event.

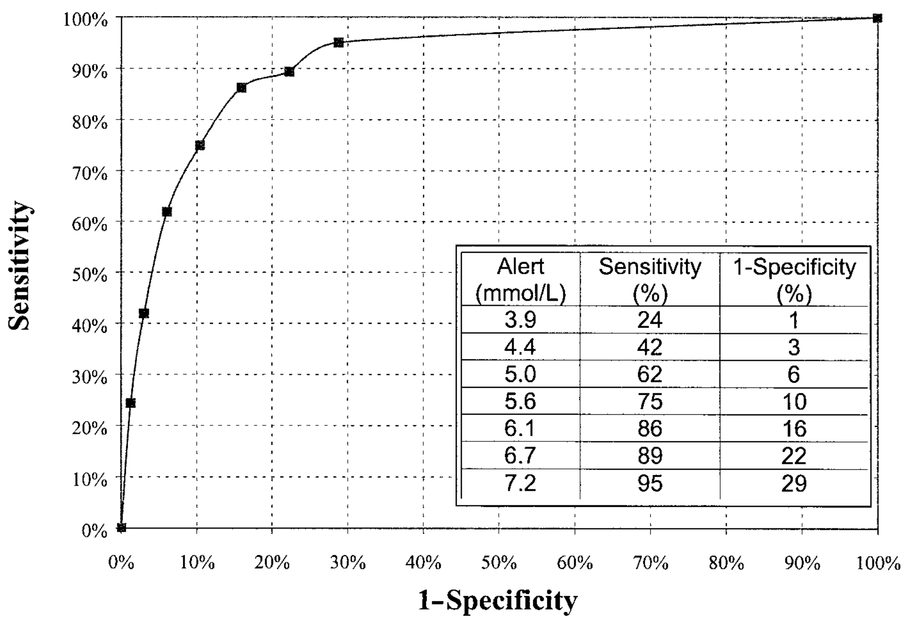
RESULTS — In the home-environment study, there were 247 hypoglycemic events of 5,305 blood glucose measurements. Of those 247 hypoglycemic events, 160 had paired biographer readings of 3,060 paired values.

A plot of the biographer readings versus comparative blood glucose values is shown in Fig. 1. Superimposed on Fig. 1 are lines showing the definition of hypoglycemia (blood glucose ≤3.9 mmol/l) and one choice for the biographer low-glucose alert level (3.9 mmol/l). These lines divide the plot into quadrants of TP, FP, TN, and FN values. Tabulation yields values for TP, FP, TN, and FN of 39, 36, 2,864, and 121, respectively. From these data, values for sensitivity and specificity are 0.24 and 0.99, respectively. Hence, when the low-glucose alert was set at 3.9 mmol/l, the biographer correctly identified 24% (39/[39 + 121]) of the hypoglycemic events. In addition, 99% (2,864/[2,864 + 36]) of the time the biographer correctly identified a value >3.9 mmol/l in the absence of hypoglycemia.

The sensitivity and specificity were obtained at low-glucose alert levels of 4.4, 5.0, 5.6, 6.1, 6.7, and 7.2 mmol/l by a similar graphical tabulation. Figure 2 shows the ROC curve constructed from these data by plotting sensitivity (or TPF) vs. 1.0-specificity (or FPF). A table of the data is shown in Fig. 2. Results show that increasing the alert level increased the sensitivity and 1.0-specificity. The TPF increased from 24 to 95%, and the FPF increased from 1 to 29%, as the alert level increased from 3.9 to 7.2 mmol/l.

A similar analysis was performed for all clinical trials and showed similar results (data not shown). In each case, increasing the low-glucose alert level from 3.9 to 7.2 mmol/l resulted in an increase in TPF to >90% and an increase in FPF to ~30%. Finally, similar results were also obtained when hypoglycemia was defined as blood glucose either <3.3 or 2.8 mmol/l, albeit with fewer events (data not shown).

The analysis of paired data requires that biographer readings exist for each comparative blood glucose measurement. Biographer readings may be unavailable for a number of reasons. For example, the home-environment data shows that of the 247 hypoglycemic events, 160 paired biographer readings were available. Of the remaining 87 hypoglycemic events, 24 biographer readings were unavailable because the event occurred before or during calibration or after the biographer had shut off. The final 63 biographer readings were unavailable because the blood glucose measurement was obtained at a time that differed from the biographer reading



Alert (mmol/L)	Sensitivity (%)	1-Specificity (%)
3.9	24	1
4.4	42	3
5.0	62	6
5.6	75	10
6.1	86	16
6.7	89	22
7.2	95	29

Figure 2—*ROC curve for the data from the home-environment study.*

by $>15 \pm 5$ min or the blood glucose reading coincided with a skipped biographer reading. However, because of the frequent and automatic nature of the data, the nearest biographer readings can be used for analysis. In addition, because perspiration can be associated with hypoglycemia, the biographer is designed to sound an alarm when a reading is skipped because of perspiration. Using the surrounding biographer readings with a low-glucose alert level of 5.6 mmol/l and the perspiration alarm, the sensitivity is 75%, or 47 of 63. This value is identical to that seen in the paired-point analysis.

CONCLUSIONS — The results show that as the biographer low-glucose alert level is increased, TPF increases; however, FPF also increases. In the home-environment study, increasing the alert level from 3.9 to 5.6 mmol/l increases the TPF by ~50%, with a <10% increase in FPF. However, a further increase of the alert level to 7.2 mmol/l yielded a relatively small increase in TPF (<20%), whereas the FPF increased by 16%. Note that the TPF values are an underestimate because the comparative blood glucose meter is assumed error-free and all error is associated with the biographer.

The trade-off between TPF and FPF is best shown in the ROC curves. The optimal alert level is where the slope of the ROC curve is equal to the unity (5). The results of the home-environment study suggest that the optimal alert level (for detection of blood glucose ≤3.9 mmol/l) is ~5.6 mmol/l. Under these conditions, the user obtains the optimal TPF values (75%) relative to the FPF values (10%).

The ROC curves were analyzed for all four studies (data not shown). In each case, a biographer low-glucose alert level near 5.6 mmol/l yields optimal results, with TPF ranging from 75 to 89% and FPF from 10 to 17%. The results are remarkably similar among the studies regardless of differences in the calibrating and comparative meters as well as the study environment; this suggests that the hypoglycemia alert function is robust.

Setting the biographer alert level at 5.6 mmol/l results in an "indifference zone," where the alarm is activated, but the blood glucose value is between 3.9 and 5.6 mmol/l. Although these blood glucose levels are not in the hypoglycemic range, the alert may provide an early warning of a trend downward into that range. Therefore, sounding the alarm is both useful and appropriate. Note that a large number of the FP points are in this indifference zone.

The analysis of TPF and FPF requires both a biographer reading and a blood glucose value within the specified timing window. Because blood glucose was measured only once or twice per hour (depending on the study), it is possible that some hypoglycemic events were excluded from the analysis because no blood glucose test was done at an opportune time. Of greater concern are situations with a known hypoglycemic event (blood glucose ≤3.9 mmol/l) that were excluded from the analysis because of the absence of a biographer reading. For a number of hypoglycemic events, no paired biographer reading was available because of skipped readings or blood glucose data that differed in time from the biographer reading by $>15 \pm 5$ min. Because of the frequent and automatic nature of the biographer data, however, the immediately adjacent readings can be used to detect hypoglycemia. An analysis of those adjacent biographer readings, which also includes the perspiration alarm, yields sensitivity values that are similar to those obtained with paired values. Thus, although the biographer will skip readings that may be aberrant, the alert function remains because of the frequent and automatic nature of the device.

Skipped readings caused by perspiration are of particular interest because perspiration can be a symptom of hypoglycemia. Research subjects report that perspiration skips occur on occasions when there is no doubt that they are sweating. In the home-environment study, there were a total of 13,573 biographer readings from 420 biographer applications. A total of 422 readings (3.1%) were skipped because of perspiration. Of the 247 hypoglycemic events in this study, 11 (4.5%) were associated with biographer skips caused by perspiration. Thus, although perspiration sufficient to cause skipped readings may be more prevalent during hypoglycemia, the overall frequency of readings skipped because of perspiration during hypoglycemia in home use is quite low.

The trade-off between TPF and FPF is a fundamental limitation of any diagnostic test used to screen for a disease or condition of concern. Another limitation specific to the biographer is the lag time between blood glucose levels and biographer readings. With the biographer, the primary component of the lag time is the 20-min measurement cycle that results in a time-averaged glucose reading. An additional source of lag time is the mass transfer between blood and peripheral tissue that has been observed in studies with implanted subcutaneous sensors and microdialysis devices (1). Clearly differentiating between these two sources of lag time will require further studies using controlled environments and standardized variations in blood glucose levels that include insulin-induced hypoglycemia.

In practice, lag time is an additional reason why it is important to select a conservative setting for the low-glucose alert level. In actual use, the patient and/or health professional can change the low-glucose alert level to suit the occasion. For example, if it is crucial that the subject be aware of hypoglycemic events (such as when driving or operating machinery), the alert level could be set higher. Of course, this will also result in increased FP readings. However, by checking the readings immediately preceding the activity and checking for any symptoms, the patient can quickly decide whether further action is needed. Conversely, if it is important to have high certainty of hypoglycemia when the alarm sounds, the alert level can be set lower. In this way, fewer false alerts will occur.

The utility of this new device must be assessed relative to the available alternatives. Currently, patients with diabetes must rely on symptoms of possible hypoglycemia and the limited number of blood glucose tests performed each day. Thus, it is useful to evaluate the detection of hypoglycemia from the same data set using typical blood glucose monitoring practices. Using mealtimes from the patient diaries, the blood glucose data from the home-environment study were analyzed for two commonly recommended testing frequencies.

First the detection of hypoglycemia (blood glucose ≤3.9 mmol/l) was analyzed for standard twice-per-day testing (before breakfast and dinner). In the second, detection was analyzed for four-times-per-day testing (before all meals and at bedtime). These results show that the sensitivity (TPF) is 14 and 39% for twice-per-day and four-times-per-day testing, respectively. There are no FPs in

this analysis because the blood glucose values are assumed to be true.

In contrast, the biographer with an alert level of 4.4 mmol/l has a 42% TPF, with only a 3% FPF (see table inserted in Fig. 2). Thus, even with a less than optimal choice for the low-glucose alert level, the biographer detects more hypoglycemic events than four blood glucose measurements per day, with few FPs. Moreover, the biographer exceeds the detection obtained with two blood glucose measurements per day. Given that the majority of patients with type 1 diabetes measure blood glucose values two or fewer times per day (13), the biographer provides an important improvement over current medical practice.

The area under the ROC curve is often used to judge the value of a diagnostic test. An ideal test would have an area under the curve (AUC) of 1.0. A test with AUC <0.5 has little diagnostic utility. For example, Meloy et al. (14) used a reflectance meter (Accu-chek III; Boehringer Mannheim, Indianapolis, IN) to determine hypoglycemia (defined as blood glucose <2.2 mmol/l) in newborns relative to a laboratory device. Their ROC analysis showed an AUC of ~0.85 for measurements made within 1 h of birth. Boyd (15) discusses the use of parathyroid hormone (PTH), phosphate ion (PO_4^-), and chloride ion (Cl^-) to differentiate patients with primary hyperthyroidism (confirmed by biopsy) from healthy subjects. The ROC analysis showed AUC of 0.98, 0.84, and 0.54 for PTH, PO_4^-, and Cl^-, respectively. Thus, PTH has high diagnostic utility, whereas Cl^- has little value. For the home-environment study presented here, an AUC value of 0.91 was obtained, indicating high diagnostic utility.

It might be argued that the definition of hypoglycemia (blood glucose ≤ 3.9 mmol/l) used in this analysis is higher than that commonly associated with symptoms in many patients. The specific definition of hypoglycemia is somewhat arbitrary. The more important issue is the difference between the definition of hypoglycemia and the selected low-glucose alert level. The implication of this analysis is that the biographer's low-glucose alert level should be set conservatively relative to the actual level of concern for a given patient. Specifically, setting the low-glucose alert from 1.1 to 1.7 mmol/l above the problem level for the patient is likely to optimize the trade-off between TPF and FPF.

In conclusion, the GlucoWatch biographer, after a single blood glucose measurement for calibration, provides 12 h of frequent (3/h) and automatic glucose readings. The frequent and automatic nature of the readings allows for more effective detection of hypoglycemia than that achieved with current medical practice, with a minimal amount of FPs. In addition, it provides low-glucose alert levels that can be adjusted to the patient's need to achieve a higher detection frequency at the expense of additional FP readings. Moreover, by checking previous biographer readings (trend analysis) and evaluating the specific situation, the patient can decide whether action is needed once the alarm sounds.

This device holds great promise in becoming an important tool to help patients aggressively manage diabetes while avoiding or reducing the incidence of severe hypoglycemia. Further studies should be performed to document the ability of the biographer to improve these health outcomes. Additional development should be focused on methods to further improve the balance of TPF versus FPF and thus enable even more effective detection of hypoglycemia.

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