

PREFACE

This book describes measurement methods in medicine and biology. While many books on medical instrumentation cover only hospital instrumentation, this book also encompasses measurements in the growing fields of molecular biology and biotechnology.

As a first course in bioinstrumentation for undergraduate biomedical engineers, this book describes measurement methods used in the growing fields of cell engineering, tissue engineering, and biomaterials. It will also serve as a text for medical and biological personnel who wish to learn measurement techniques.

Chapter 1 covers the basics of instrumentation systems, errors, and the statistics required to determine how many subjects are required in a research study. Because many biomedical engineers are not specialists in electronics, chapter 2 provides the necessary background in circuits, amplifiers, filters, converters, and signal processing.

Chapter 3 describes clinical measurements of molecules, such as oxygen and glucose, to biotechnology measurements such as DNA sequencing. For the fields of biomaterials and tissue engineering, chapter 4 covers measurements on polymers, using surface analysis, protein adsorption and molecular size.

Measurements on blood components are the commonest measurements on cells, as described in chapter 5. Cells are counted and identified using changes in impedance and light scattering. Chapter 6 covers cellular measurements in biomaterials and tissue engineering, such as cellular orientation, rolling velocity, pore size, deformation, shear stress, adhesion, migration, uptake, protein secretion, proliferation, differentiation, signaling and regulation.

Chapter 7 describes measurements on the nervous system—action potentials, EEG, ERG, EOG, EMG and audiometry—and brain imaging using X ray, CT, MRI, nuclear imaging, SPECT, PET, and biomagnetism. Chapter 8 covers heart and circulation, with measurements of cardiac biopotentials, pressure, output, sounds, viability—as well as blood flow and pressure in the periphery.

Measurements of pulmonary volume, flow, diffusion and airway resistance are described in chapter 9, which also includes kidney clearance, bone mineral, and skin water loss. Chapter 10 covers measurements of body temperature, fat, and movement.

Each chapter has references for further study as well as homework problems to test comprehension. A web site contains complete laboratory instructions for 12 laboratories, examination questions, and quiz questions.

Suggested prerequisite courses are calculus, biology, chemistry, and physics. I would welcome suggestions for improvement of subsequent editions.

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Measurement Systems

Kevin Hugo

1.1 Studying biomedical engineering

Edna Jones is a 67-year-old retired teacher who is having difficulty with her vision. She can't remember when it started, but her eyesight has increasingly blurred over the course of the last year. It has now become so poor that last week she drove her car off the road. She hasn't been to a doctor for many years, but now she's decided to see her family physician.

The nurse measured and recorded Edna's vital signs. Although her weight, 90 kg (198 lb.), was measured with an electronic scale, her height, 173 cm (5 ft. 8 in.), was measured manually. She marveled at the device that took her blood pressure (118/76) and pulse (63 beats per minute) simultaneously. Another automated instrument took her body temperature (98.6 °F or 37 °C) from her ear in a few seconds, instead of having to place a thermometer under her tongue for a minute.

Edna related her vision problems and accident to the doctor. She is certain that she did not suffer any injuries. She finds it strange that the doctor asked about how much water she drinks, but she admitted that she has been rather thirsty lately and that she drinks and passes a lot of water. The doctor also noted that that Edna has gained a fair amount of weight.

The doctor began the physical examination by observing Edna's eyes with an ophthalmoscope. There were cotton-like blotches on the normally orange-pink retina. The physician then felt her hands and feet; they were cold, and she has lost some of the sensation of touch in her feet. Finally, samples of urine and blood were taken.

It takes several minutes for the laboratory at the clinic to analyze the urine and blood samples; they use an automated instrument to measure the blood sugar (glucose) in each. The doctor is concerned about the results, and orders an electrocardiogram and further tests on the blood and urine samples before returning to Ms. Jones to discuss her condition.

1-12 Chapter 1 Measurement Systems

so that the majority of the signal will enter the system to be processed and not be lost in the skin impedance.

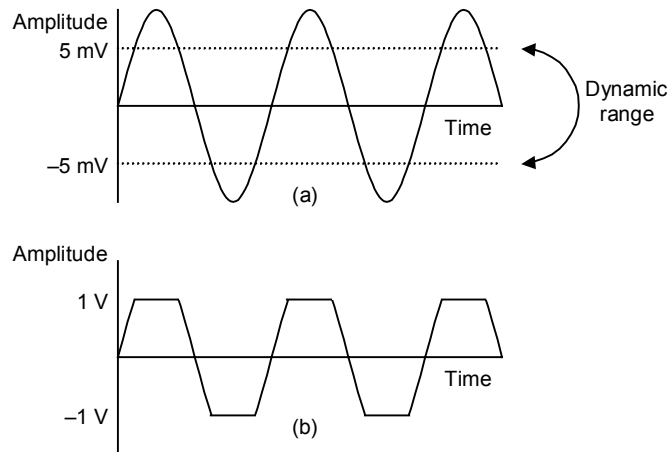


Figure 1.9 (a) An input signal that exceeds the dynamic range. (b) The resulting amplified signal is saturated at ± 1 V.

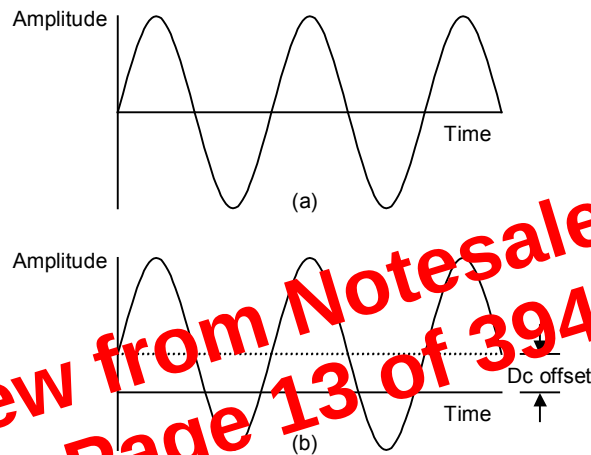


Figure 1.10 (a) An input signal without dc offset. (b) An input signal with dc offset.

Dc lead current is the amount of current that flows through the leads to the patient being observed by the electrocardiograph. It is important that current flowing to the patient not exceed very miniscule amounts (i.e. on the order of microamperes). This is because larger currents can polarize electrodes, resulting in a large offset voltage at the amplifier input.

$$[\text{Lower limit on average weight}] \leq w \leq [\text{Upper limit on average weight}]$$

In the event that we do not know the standard deviation of the population, we can use the sample standard deviation, s , and the Student t distribution t_α .

1.5.3 Hypothesis testing

In hypothesis testing, there are two hypotheses. H_0 , the null hypothesis, is a hypothesis that assumes that the variable in the experiment will have no effect on the result and H_a is the alternative hypothesis that states that the variable will affect the results. For any population, one of the two hypotheses must be true. The goal of hypothesis testing is to find out which hypothesis is true by sampling the population.

In reality, H_0 is either true or false and we draw a conclusion from our tests of either true or false. This leads to four possibilities, as shown in Table 1.8.

Usually, the probability of making a Type I error is designated as α and the probability of making a Type II error is designated as β . Common values for α , the significance level, are 0.01, 0.05, and 0.1. The statistical power of a test is the probability that H_0 will be rejected when it is false. Statistical power is given by $1 - \beta$. Common power levels are above 0.8, indicating that when H_0 is false it will be correctly rejected more than 80% of the time.

Conclusion	Real situation	
	H_0 true	H_a true
Accept H_0	Correct decision	Type II error, $p = \beta$
Reject H_0	Type I error, $p = \alpha$	Correct decision

Table 1.8 The four outcomes of hypothesis testing.

Many scientific studies involving a hypothesis test will report the p value of the test. A very small p value means that the null hypothesis is unlikely to be true. When p is below an arbitrary cut-off value, e.g. 0.05, the result is called statistically significant. A study that reports $p = 0.01$ means that since the result obtained would happen only once in 20 times if H_0 were true, then H_0 should be rejected.

Test result	Has condition?	
	No	Yes
Negative	True negative (TN)	False negative (FN)
Positive	False positive (FP)	True positive (TP)

Table 1.9 Equivalent table of Table 1.8 for results relating to a condition or disease.

Test results about a condition or disease use the terminology in Table 1.9. In this case H_0 states that an individual does not have a condition or a disease, whereas H_a states that an individual does have a condition or disease.

Basic Concepts of Electronics

Hong Cao

Medical instruments are widely used in clinical diagnosis, monitoring, therapy, and medical research. They provide a quick and precise means by which physicians can augment their five senses in diagnosing disease. These instruments contain electric components such as sensors, circuits, and integrated circuit (IC) chips. Modern electronics technology, which includes transistors, ICs, and computers, has revolutionized the design of medical instruments.

Biomedical engineers should have a fundamental understanding of their operations and a basic knowledge of their component electric and electronic systems. Using this knowledge provides a better understanding of the principles of various measurements or even develop new measurements and instruments.

Electrical engineering is too large a topic to cover completely in one chapter. Thus, this chapter presents some very basic concepts in several fields of electrical engineering. It discusses analog components such as resistors, capacitors, and inductors. It then goes on to basic circuit analysis, amplifiers, and filters. From this it moves to the digital domain, which includes converters, sampling theory, and digital signal processing. It then discusses the basic principles of microcomputers, programming languages, algorithms, database systems, display components, and recorders.

2.1 Electronic components and circuit analysis

2.1.1 Current

An atom contains a nucleus surrounded by electrons. Most of the electrons are tightly bound to the atom, while some electrons in the outer orbits are loosely bound and can move from one atom to another. In conductors, there are many free electrons. This process of electron transfer occurs in random directions in materials.

Current can be generated in a circuit by a current source or by a voltage source and resistor in series.

2.1.2 Voltage and potential

In moving a charge (+ or −) from point A to point B in an electric field, the potential energy of the charge changes. That change in energy is the work, W , done by the electric field on the charge. The amount of work is measured in Joules, J. If we let the electric potential, V , be equal to the potential energy, U , per unit charge, q_0 , then we can define a potential difference, or voltage, as

$$\Delta V = V_B - V_A = \frac{W_{AB}}{q_0} \quad (2.3)$$

where V_B and V_A are the potential at points B and A, respectively. The unit of potential is the volt (V), where $1 \text{ J/C} = 1 \text{ V}$. If we choose the potential at infinity to be zero, the absolute potential of a point in an electric field can be defined as the total work per unit charge that has been done to move the charge from infinity to the point.

It is important that potential difference not be confused with difference in potential energy. The potential difference is *proportional* to the change in potential energy, where the two are related by $\Delta U = q_0 \Delta V$.

From Eq. (2.3), we can determine the work needed to move a charge from A to B if we know the potential difference between the two points. We are more interested in potential difference than the absolute potential for a single point. Notice that the potential is a property of the electric field, whether there is a charge in the field or not.

Voltage can be generated in a circuit by a voltage source or by a current source and resistor in parallel. Even though they exist, current sources are much less common than voltage sources in real circuits.

Example 2.1 How much work is needed to move an electron (charge of $1.6 \times 10^{-19} \text{ C}$) from 0 V to 4 V?

Rearranging Eq. (2.3)

$$W_{AB} = q_0 (V_B - V_A) = 1.6 \times 10^{-19} \text{ C} (4 \text{ V} - 0 \text{ V}) = 6.4 \times 10^{-19} \text{ J}$$

2.1.3 Resistors and Ohm's law

When free electrons move in a conductor, they tend to bump into atoms. We call this property resistance and use a resistor as the electric component implementation. The unit of resistance is the Ohm (Ω), in honor of Georg Simon Ohm, who discovered what is now known as Ohm's law. Ohm's law states that for many materials, when a potential difference is maintained across the conductor, the ratio of the current density J (current

$$\sum I = 0 \quad (2.14)$$

or the sum of currents entering or leaving any node is zero, this follows the law of conservation of charge. In other words, the currents entering a node must equal the currents leaving the node (Figure 2.5)

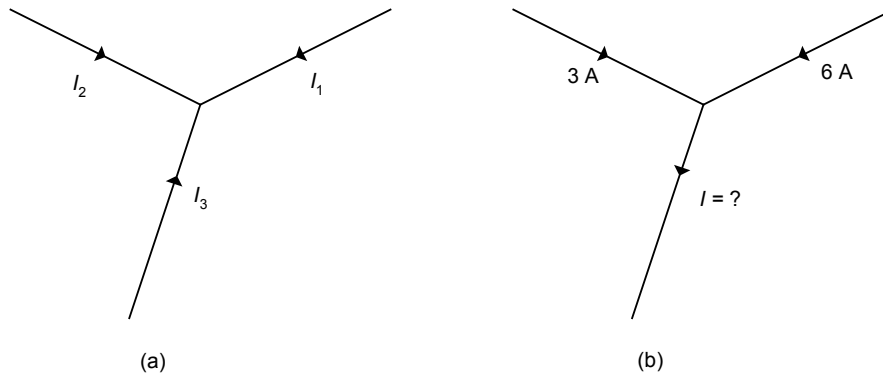


Figure 2.5 (a) Kirchhoff's current law states that the sum of the currents entering a node is 0. (b) Two currents entering and one “negative entering”, or leaving.

When we use Kirchhoff's current law, we can arbitrarily label the current entering the node + and leaving the node -. The sum of the currents entering the node in Figure 2.5(b) is

$$\begin{aligned} +3 + 6 - I &= 0 \\ I &= 9 \text{ A} \end{aligned}$$

Kirchhoff's voltage law and current law are basic laws for circuit analysis. There are two types of circuit analysis, each based on each of Kirchhoff's laws.

We assume unknown current in the loops and set up the equations using Kirchhoff's voltage law and then solve these equations simultaneously.

We can use Figure 2.4(b) to illustrate loop analysis. We assume a current flows through the circuit in the direction already indicated in the figure. If we assumed the current in the other direction, our result would just be negative. Recalling Ohm's law, we have for the sum of the voltages through the clockwise loop

$$\begin{aligned} V_S &= R_1 I + R_2 I \\ 30 &= 10I + 20I \\ I &= 1 \text{ A} \end{aligned}$$

Now that we know the current through the loop, we can find the voltage drop through either resistor using Ohm's law. In this example, the voltage drop for R_1 is 10 V.

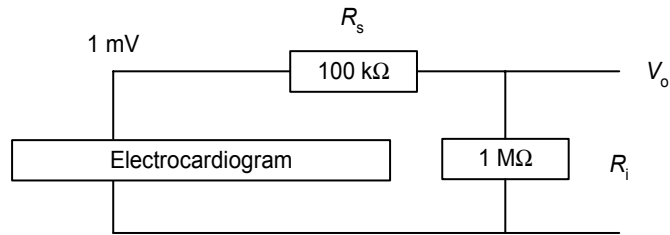


Figure 2.8 The 1 mV signal from the electrocardiogram is attenuated by the resistive divider formed by the 100 kΩ skin resistance and the 1 MΩ input resistance of the oscilloscope.

The current through the resistive divider is

$$i = \frac{V}{R} = \frac{1 \text{ mV}}{100 \text{ k}\Omega + 1 \text{ M}\Omega} = 0.91 \text{ nA}$$

The output voltage V_o is

$$V_o = iR_i = (0.91 \text{ nA})(1 \text{ M}\Omega) = 0.91 \text{ mV}$$

Thus the input voltage (ECG) has been undesirably attenuated by an amount that we can calculate from the voltage divider equation

$$\frac{V_o}{V_i} = \frac{R_i}{R_s + R_i} \quad (2.13)$$

$$\frac{V_o}{V_i} = \frac{R_i}{R_s + R_i} = \frac{1 \text{ M}\Omega}{100 \text{ k}\Omega + 1 \text{ M}\Omega} = 0.91$$

Figure 2.8 is a typical example of voltage divider. With two resistors in series, the output voltage is part of the input voltage. With adjusting the values of R_i and R_s , one can flexibly obtain any percentage of the input voltage.

A potentiometer is a three-terminal resistor with an adjustable sliding contact that functions as an adjustable voltage divider or attenuator. Figure 2.9 shows that if the slider is at the top, $v_o = v_i$. If the slider is at the bottom, $v_o = 0$. If the slider is in the middle, $v_o = 0.5v_o$. Potentiometers are usually circular with a rotatable shaft that can be turned by hand or a screwdriver. The potentiometer is useful to provide a variable gain for an amplifier or for the volume control on a radio. Alternatively, a potentiometer can be used as a two-terminal variable resistor by using the variable resistance between the slider and only one end.

We can also use a resistor and an inductor to make a first-order circuit where $\tau = L/R$.

2.1.10 Frequency

Sinusoidal waves are widely used in electrical circuits. Figure 2.20 shows two sinusoidal waveform, which can be represented as

$$A \sin(2\pi ft + \theta) = A \sin(\omega t + \theta) = A \sin\left(\frac{2\pi}{T}t + \theta\right) \quad (2.37)$$

where A = amplitude of sine wave
 f = frequency of sine wave in hertz (Hz)
 $\omega = 2\pi f$ = angular frequency of sine wave (radians per second)
 θ = phase angle of sine wave (radians)
 $T = 1/f$ = period (seconds)

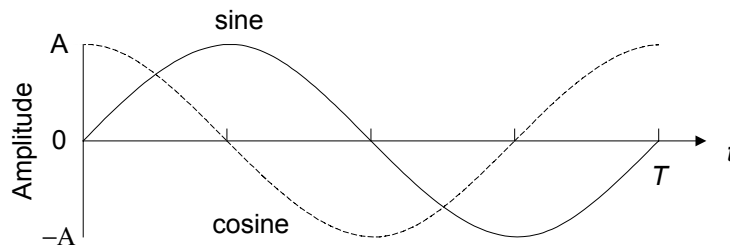


Figure 2.20 One period, T , of the sine and cosine waveforms.

Note the sine function lags 90° in phase behind the cosine function.

Example 2.9 Figures 2.21 and 2.22 show a comparison of a sinusoidal waveform with different frequency and different phase angle.

In Figure 2.21 the solid line shows the function $y = \sin(\omega t)$, the dashed line shows the function with the frequency doubled, $y' = \sin(2\omega t)$. By doubling the frequency the period is reduced by half to $T/2 = 1/2f$.

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The output v_o has the opposite sign of the input v_i and is amplified by an amount R_f/R_i .

When using op amps in circuits it is important to consider the output impedance of any source that they are connected to, as this value will affect the total input resistance and the gain of the op amp.

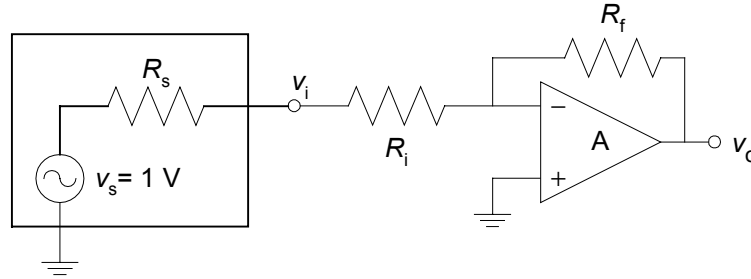


Figure 2.27 Inverter circuit attached to a generator that contains an internal resistance.

Example 2.11 Suppose in Figure 2.27 that $v_i = 1$ V, $R_f = 4$ k Ω and $R_i = 1$ k Ω , we would have, from Eq. (2.52), a gain of -4 and therefore, an output voltage of -4 V. However if the signal generator shown in Figure 2.27 were connected and had output impedance R_s , this impedance would add to R_i of the op amp and the new input impedance would be $R_s + R_i$. R_s would therefore change v_o as well as the gain of the circuit. If $R_s = 150$ Ω , then using Ohm's law we obtain the gain

$$\frac{v_o}{v_s} = -\frac{R_f}{R_i + R_s} = -3.48$$

which is much less than our desired value.

2.2.4 Noninverting amplifier

Figure 2.28(b) shows the circuit for a noninverting amplifier. From rule 1, we know the voltage of the negative input always equals the voltage of the positive input terminal, which in this case is v_i . From Figure 2.28(a) we have

$$\begin{aligned} \frac{v_i - 0}{R_i} &= \frac{v_o - v_i}{R_f} \\ \frac{v_o}{v_i} &= \frac{R_f + R_i}{R_i} \quad \text{or} \quad v_o = \frac{R_f + R_i}{R_i} v_i = v_i \left(1 + \frac{R_f}{R_i} \right) \end{aligned} \quad (2.53)$$

$$\begin{aligned} v_i &= V_m \sin(\omega t + \phi) \\ v_o &= V_m |T(\omega)| \sin(\omega t + \phi + \theta(\omega)) \end{aligned} \quad (2.58)$$

where V_m is the amplitude of the input signal, $|T(\omega)|$ is the amplitude of the transfer function, or filter, and $\theta(\omega)$ is the angle of $T(\omega)$.

Thus the filter multiplies the amplitude of the input sine wave by $|T(\omega)|$ and adds to the phase of the sine wave $\theta(\omega)$. Since $|T(\omega)|$ and $\theta(\omega)$ are functions of frequency, they are named the amplitude response and phase response, respectively, of the filter.

For example, the ideal low-pass filter has a magnitude response of

$$T(f) = \begin{cases} 1 & \text{if } f < f_c \\ 0 & \text{if } f \geq f_c \end{cases} \quad (2.59)$$

where $f = \omega/2\pi$.

The low-pass filter passes all frequency components lower than f_c and rejects all frequency components higher than f_c . Realistically, building a filter with such a sharp transition is not possible since the transition from passband ($T(f) = 1$), to stopband ($T(f) = 0$) takes some finite number of frequencies.

Figure 2.36 shows the magnitude characteristics of four widely used filters: low-pass, high-pass, bandpass and bandstop filter. All these filters have a passband, where the filter passes the frequency components, and a stopband, where the filter rejects or attenuates the frequency components.

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$$\frac{1}{8}V_{\text{ref}} = \frac{1}{2^3}V_{\text{ref}}$$

We call this value the resolution of the DAC. In general, for an n -bit DAC or ADC, the resolution is

$$\text{resolution} = \frac{1}{2^n}V_{\text{ref}} \quad (2.69)$$



Figure 2.40 The ideal static behavior of a 3-bit DAC. For each digital input, there is a unique analog output.

Equation (2.69) shows that if an application calls for high resolution, the DAC needs many bits. DACs are available in 4, 8, 10, 12, 16, 20 or more bits. The more bits a DAC has, the more complex and expensive it is.

A variety of circuit configurations are suitable for a DAC. We present the simplest one: a voltage scaling DAC. Figure 2.41 is a simple 3-bit voltage scaling DAC.

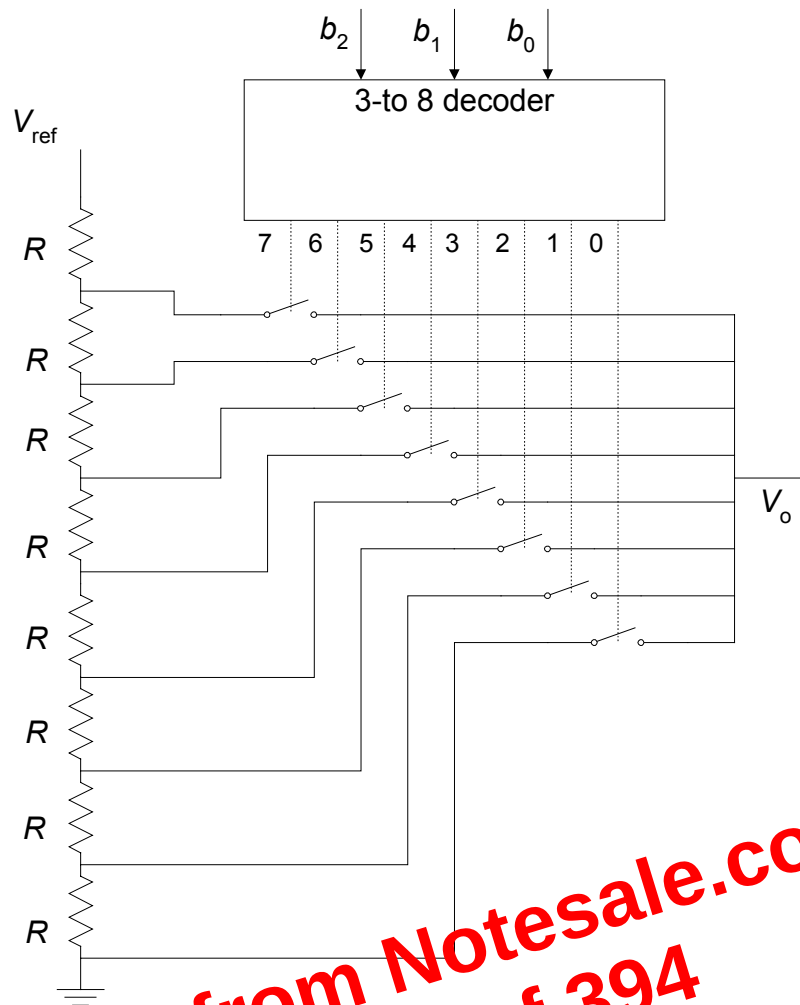


Figure 2.41 A 3-bit voltage scaling DAC converter.

2.4.3 Analog-to-digital converters (ADC)

Many signals used in biomedical engineering such as voltage, temperature, pressure, strain, flow and speed, will originally be in analog form. These signals need to be converted to a digital signal before being transferred to the computer. The objective of an ADC is to convert analog input data to an equivalent output digital word. Since we are going to map a continuous value (infinite number set) to a limited number set, there will be overlap for different input continuous values. Figure 2.42 shows the converting

sequence is processed by algorithms to yield an output sequence. After discrete-time processing, the output sequence is converted back to a continuous-time signal by a DAC.

In some cases, the input samples can be stored in memory and the processing will be implemented after all the samples are obtained. But in other digital signal processing, real-time operation is often desirable, meaning that the system is implemented so that samples of the output are computed at the same rate at which the continuous signal is sampled.

Compared with analog signal processing, digital signal processing has some advantages: (1) A digital filter is highly immune to interference because of the way it is implemented (digital signals are far less susceptible to noise than analog signals). (2) Accuracy is dependent on round-off error, which is directly determined by the number of bits used to represent the digital signal. (3) With the help of modern computers, digital signal processing is much more flexible and powerful than analog signal processing. (4) Performance of a digital signal system is minimally affected by environmental factors such as temperature variation, component aging and power supply fluctuations.

Because of these advantages, digital signal processing is widely used in communication systems, radar and sonar, speech and video coding and enhancement, and biomedical engineering.

There are some limitations of digital signal processing. The greatest drawback is its relatively slow speed. The speed is affected by two factors. One is the sampling speed of the ADC and DAC. The fastest ADCs and DACs work at frequencies in the range of tens of megahertz, while analog signal processing can work at frequencies in the range of a gigahertz. The other is the speed of the signal processing hardware. The speed of analog signal processing is limited by the delay of the circuits. In digital signal processing, it depends on the complexity of the algorithms and the clock speed of the processor.

2.5.1 Digital signals

Analog signals are continuous in time and amplitude. Digital signals are discrete in time and amplitude. They are represented by a sequence of numbers, x , in which the n th number in the sequence is denoted $x[n]$ where n is an integer. Such sequences are usually obtained by the periodic sampling of an analog signal $x_a(t)$. In the sampled sequence, the value of the n th sample in the sequence is equal to the value of $x_a(t)$ (rounded to the precision of the ADC) at time nT , or

$$x[n] = x_a(nT) \quad (2.70)$$

where T is the sampling period and its reciprocal is the sampling frequency. As an example, Figure 2.45(a) shows a segment of a sine wave and Figure 2.45(b) is the sampled sequence of the sine wave.

2.8 Display devices

For improved understanding of the processes of the instrument or of an object, it is important to display the results as either characters or graphically.

The most common device for displaying electric signals is the oscilloscope. The main part of an oscilloscope is a cathode ray tube (CRT). Figure 2.49 is a sketch of a CRT.

The cathode emits electrons. Electrons are accelerated by the axial high voltage, hit the screen, and light is emitted due to fluorescence. The electrons pass through a transverse electric field before hitting the screen and deflect at a certain angle, which is controlled by an electric signal. The deflection control unit controls the location of the spot on the screen. For an oscilloscope, the horizontal deflection control signal is a time swept signal and the vertical deflection control signal is the signal we display.

The heart of a TV monitor is also a CRT, but it is a little different from the CRT for the oscilloscope. In a TV monitor, the signal received from the antenna controls the number of electrons emitted from the cathode and the brightness of the spot on the screen. If many electrons hit the spot, it will emit more light and we observe that the location is white. If no electrons hit the spot, it emits no light and we observe it is black. The deflection control unit controls the location of the spot (scanning) and we can see an image on the screen. For a color TV monitor, there are three colors (red, blue, green) of fluorescent material on the screen. These three colors compose the different colors we observe.

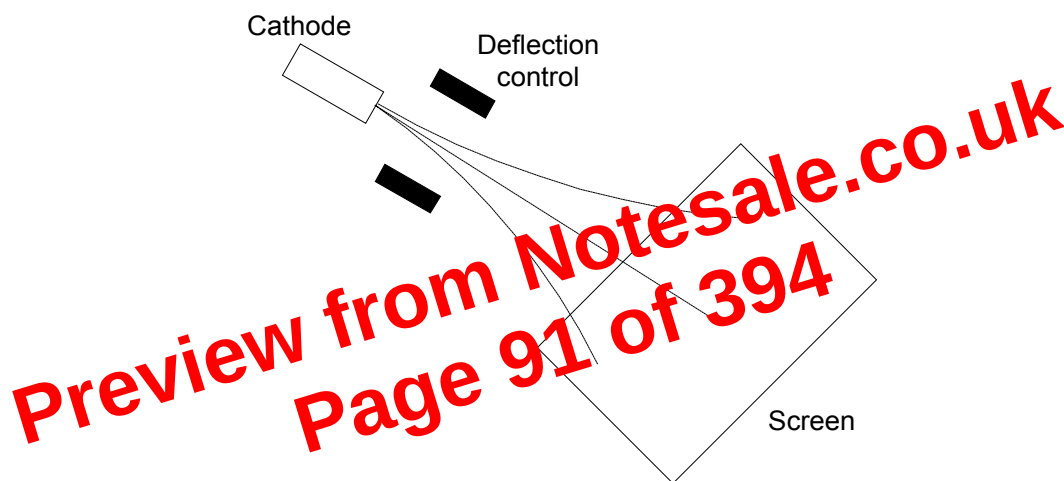


Figure 2.49 Sketch for cathode ray tube (CRT). There are two pairs of electrodes to control the deflection of the electron, but only one pair is shown.

In an alkaline (basic) medium, creatinine and picric acid form a red–orange compound whose structure has been postulated but not confirmed. The hypothesized picrate–creatinine complex forms from a creatinine to picric acid ratio of 1:1. The complex can be measured spectrophotometrically at wavelengths between 505 to 520 nm. The concentration of the hydroxyl ion (alkaline medium) affects the rate of the complex formation. Most methods use a 0.5 M concentration of sodium hydroxide and picric acid in excess of stoichiometric amounts so that picric acid is not the limiting reagent. One of the main problems with the Jaffe reaction is that it is nonspecific when used to measure creatinine in plasma. Molecules that interfere with the specificity of the Jaffe reaction include glucose, protein, ascorbic acid, acetone, and pyruvate. As a result, several modifications exist which increase the specificity of the reaction (Burtis and Ashwood, 1994). However, many interference problems are still unresolved.

3.6 Urea

Urea, $\text{NH}_2\text{--CO--NH}_2$, a nitrogen containing molecule, is a metabolic product of breaking down proteins. Figure 3.2 shows how urea is formed in the liver.

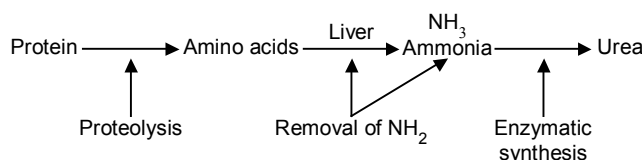
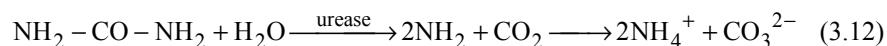


Figure 3.2 Origin of urea in the body (Burtis and Ashwood, 1994). The liver produces urea by first breaking proteins down into their building blocks, amino acids, by a process that breaks the peptide bonds between the amino acids (proteolysis). The amino group (NH_2) of amino acids is removed and ultimately used to form ammonia (NH_3) and urea.

Over 90% of urea is excreted through the kidney in the urine. The body produces urea to rid itself of excess nitrogen.

Ammonia is produced by the liver as a waste product of gluconeogenesis and the liver converts it into urea. Urea is then transported in the blood to the kidneys as blood urea nitrogen (BUN). Although the urea nitrogen measurement is often referred to as BUN, it is never measured from whole blood. Urea nitrogen is most often measured from blood serum (water that is separated from coagulated blood) and sometimes plasma. An above normal amount of urea in the blood is an indicator of decreased kidney function, and therefore possibly kidney disease.

The two primary methods of measuring urea nitrogen are spectrophotometric. The first method measures urea indirectly by quantifying the concentration of the ammonium ion spectrophotometrically. Urea is hydrolyzed by water in the presence of urease (an enzyme) and the resulting ammonium ion is quantified

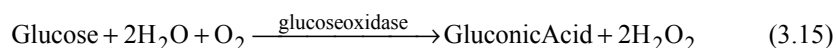


lining of blood vessels (the basement membrane). When glucose denatures collagen, blood vessels are destroyed. This leads to decreased blood flow in the arms and legs (lower perfusion). When glucose denatures proteins associated with neurons, nerve damage occurs and results in a person's inability to feel. Diabetes mellitus is the leading cause of amputation because decreased blood flow causes tissue to die and damaged nerves hinder the sensation thus making widespread damage much more likely.

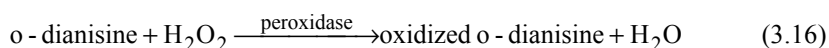
The most common techniques for measuring blood glucose are enzymatic. The glucose oxidase method is a very popular manual procedure used for self-monitoring. The Hexokinase method is widely used in laboratories since the procedures for it are carried out by automated equipment.

3.7.1 Glucose oxidase method

The glucose oxidase method is used in a large number of commercially available strip tests. These simple strip tests allow easy and quick blood glucose measurements. A strip test product, One Touch II (Lifescan, Milpitas, CA), depends on the glucose oxidase- peroxidase chromogenic reaction. After a drop of blood is combined with reagents on the test strip, the reaction shown in Eq. (3.15) occurs.



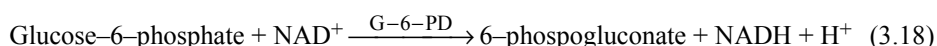
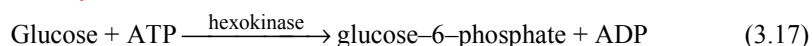
Addition of the enzyme peroxidase and o-dianiside, a chromogenic oxygen results in the formation of a colored compound that can be evaluated visually.



Glucose oxidase chemistry in conjunction with reflectance photometry is used to produce a system for monitoring blood glucose levels (Burtis and Ashwood, 1994). In the Lifescan system (Figure 3.3), a drop of blood is applied to the reagent pad, the strip is inserted into the meter, and the results appear on a digital display screen 45 s later. The device automatically starts and times the test and has a range of 0 to 600 mg/dL.

3.7.2 Hexokinase method

Much of the automated equipment is based on the hexokinase method. The general reactions are



Glucose is phosphorylated to glucose-6-phosphate in the presence of ATP, Mg^{2+} , and the enzyme hexokinase. The glucose-6-phosphate formed in Eq. (3.17) is oxidized to 6-phosphogluconate by the enzyme glucose-6-phosphate dehydrogenase (G-6-PD) in the

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technique for quantifying these elements, because compared to calcium and magnesium, they have low excitation potentials. Figure 3.7 shows a block diagram of a flame photometer. The purpose of the flame is to provide energy to the elements such that their valence electrons will be excited from ground states to higher excited states. Electrons in the excited states are unstable and emit energy as photons of a particular wavelength as they return to their ground states. The wavelength(s) of the light emitted by an element is characteristic of that element. When sodium, potassium, and lithium are present as cations in an aqueous solution and exposed to a flame, sodium produces yellow, potassium violet, and lithium red colors. The intensity of the light given off is directly proportional to the number of photons being emitted, which in turn is directly proportional to the number of atoms, or concentration of cations, in the solution.

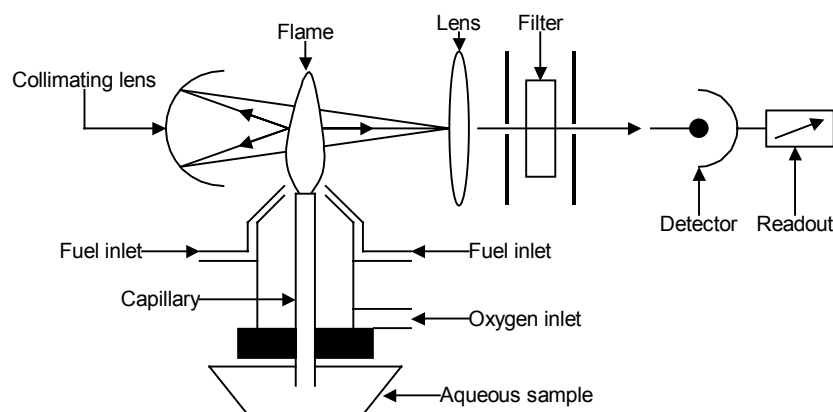


Figure 3.7 The flame photometer aspirates a sample containing metal ions and heats it to incandescence. Detector output is proportional to concentration.

In flame photometry, the monochromator and detector are similar to those found in the spectrophotometer, although the detector is measuring emission as opposed to absorption. The distinguishing features of the system are the flame and the atomizer. The atomizer draws the sample solution containing the cation through an aspirator and into the flame. This is achieved by passing a gas over the upper outlet of a capillary tube at a high velocity, while the lower end of the capillary tube is inserted into the sample. The fuel used to generate the flame can be either propane, natural gas, or acetylene mixed with compressed air or oxygen. Lithium can be used in calibration because it does not naturally occur in the body (provided the patient is not taking lithium medication). A known concentration of lithium is added to the sample. Then a ratiometric technique can be used to compare the ratio of sodium and potassium to lithium.

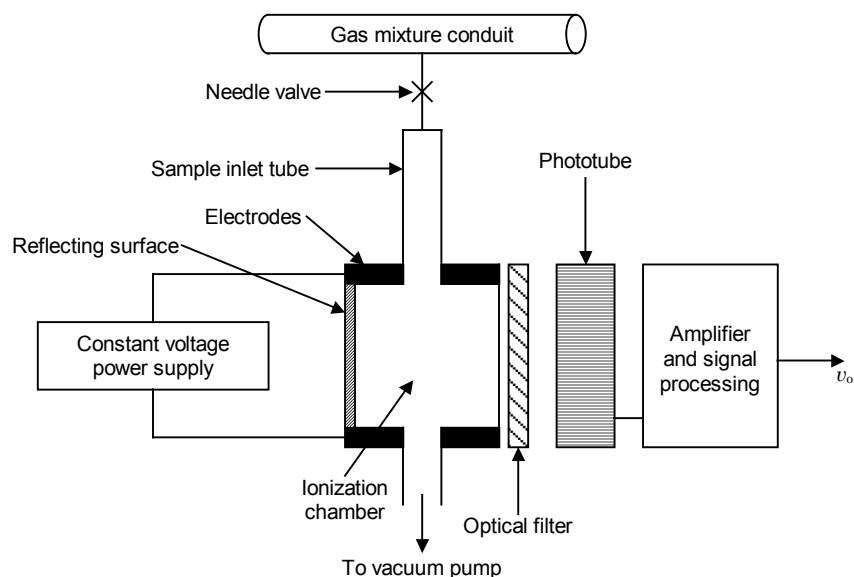


Figure 3.14 Emission spectroscopy measures ultraviolet light emitted by ionized nitrogen (Primiano, 1998).

3.14 Drugs by fluorometry and chromatography

3.14.1 Fluorometry

Fluorescence is the emission of energy in the form of light as a result of the electrons of a molecule returning to their ground state. When certain molecules absorb energy (in the form of electromagnetic radiation) their electrons are raised to higher energy levels. As the electrons of the molecule return to their ground states, they fluoresce. A fluorometer is an instrument that measures the intensity of light produced when the electrons of a molecule return to their ground state. Thus, fluorometry is defined as measuring the relationship between the concentration of a substance and the intensity of the fluorescence produced by that compound when it is excited by radiation. Fluorometry is used to measure therapeutic drugs such as phenobarbital (treatment of epilepsy), enzymes such as proteases (used to break down proteins), hormones such as cortisol, and analytes such as catecholamines (e.g. epinephrine and norepinephrine) and bilirubin. Figure 3.15 shows the components of a fluorometer, which include an excitation source, a primary (excitation) filter, a secondary (emission) filter, a cuvette sample, a detector, and a readout device.

Although the components of a fluorometer are basically the same as those of a spectrophotometer, fluorometry is up to four orders of magnitude more sensitive than spectrophotometry (Wheeler, 1998). In spectrophotometry, the concentration of the sub-

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stance is determined by the difference of the absorbance between a solution that has zero absorbance (the solution used to calibrate the spectrophotometer) and the absorbance of the unknown solution. When the absorbance of the unknown solution is relatively small, small errors in the measurement of the zero absorbance solution can cause relatively large errors in the final determination. Fluorometry avoids this problem by measuring fluorescence directly from the sample without reference to another measurement. Though a disadvantage of fluorometry is that measurements are also susceptible to inner-filter effects. These effects include excessive absorption of the excitation radiation (prefilter effect) and self-absorption of atomic resonance fluorescence (postfilter effect).

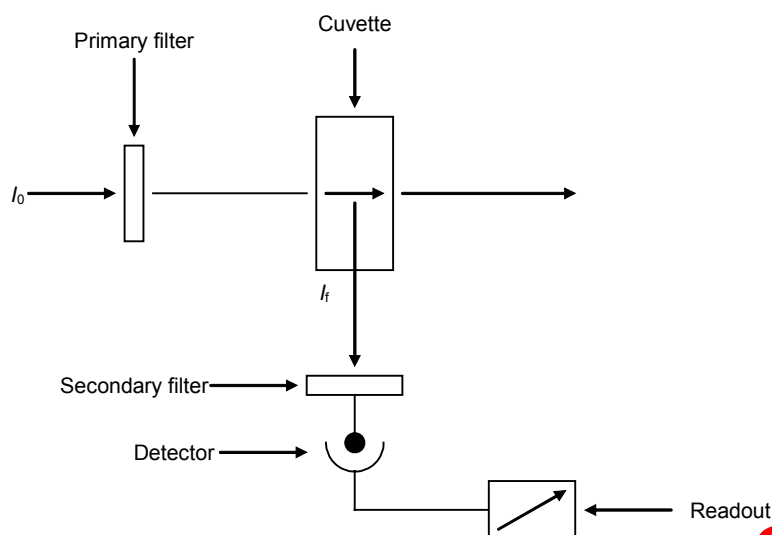


Figure 3.15 Block diagram of a fluorometer. The primary filter passes only wavelengths that excite the fluorescent molecule. The secondary filter blocks all scattered excitation wavelengths and passes only the scattered fluorescent wavelengths. The secondary filter and detector are at a right angle to the primary beam in order to avoid direct transmission of the light source through the sample to the detector.

The theory of fluorometry

The theory behind fluorometry can be derived from the Beer–Lambert law

$$I = I_0 10^{-aLc} \quad (3.31)$$

where

- I = intensity of transmitted light
- I_0 = intensity of the incident light
- a = absorptivity of the sample

We rearrange to determine the amount of light absorbed:

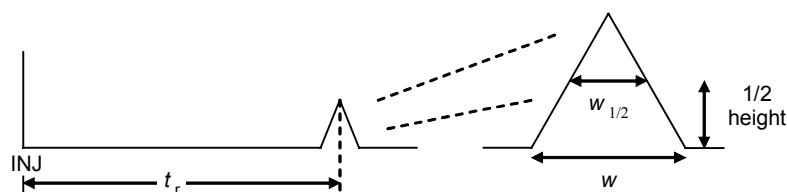


Figure 3.17 In chromatography, the peak appears after retention time t_r .

The resolution of two solute bands is a measure of their degree of separation in terms of relative migration rates and bandwidths and is

$$R_s = \frac{V_r(B) - V_r(A)}{[w(A) + w(B)]/2} \quad (3.33)$$

where $w(A)$ and $w(B)$ are the peak widths measured at the corresponding base. Inadequate separations occur for $R_s < 0.8$ and baseline separation occurs for $R_s > 1.25$ (Bowers et al., 1994).

Example 3.1 For a gas chromatograph with a flow rate of 1.5 L/min, solute A has a retention time of 1 min and solute B has a retention time of 2.5 min. If the peak width for solute A is 0.5 L and the peak width for solute B is 1.5 L, is the resolution sufficient?

Since the flow rate of the solutes and peak widths are known, we only need to find the peak volume before we can use Eq. (3.33) to determine if the resolution is sufficient.

$$w(A) = 0.5 \text{ L}$$

$$w(B) = 1.5 \text{ L}$$

$$V_r(A) = t_{rA} F = 1 \text{ min} \times 1.5 \text{ L/min} = 1.5 \text{ L}$$

$$V_r(B) = t_{rB} F = 2.5 \text{ min} \times 1.5 \text{ L/min} = 3.75 \text{ L}$$

$$R_s = \frac{V_r(B) - V_r(A)}{[w(A) + w(B)]/2} = \frac{3.75 \text{ L} - 1.5 \text{ L}}{[0.5 \text{ L} + 1.5 \text{ L}]/2} = 2.25$$

Since $R_s > 1.25$, the base separation of the bands is sufficient.

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3.18 Problems

- 3.1 Give the equation for Beer's law, define each term, and give units.
- 3.2 Explain the operation of a spectrophotometer and its purpose. List the components of a spectrophotometer.
- 3.3 A sample concentration of 10 mg/dL yields a spectrophotometer transmission of 35%. Assume Beer's law holds and calculate the unknown concentration for a transmission of 70%.
- 3.4 A sample of concentration 20 mg/dL has an absorbance of 0.4 in a spectrophotometer. The sample is then diluted and yields an absorbance of 0.25. Calculate the new concentration.
- 3.5 Define oxygen saturation and state the physiological meaning of SaO_2 .
- 3.6 Search the literature for a plot of SaO_2 versus PO_2 and sketch it.
- 3.7 Describe how NADH is used to measure lactate concentration and why lactate concentration isn't ascertained by measuring lactate directly.
- 3.8 Describe why creatinine is measured and the technique used to measure it.
- 3.9 Describe why and how to measure urea and the body fluids it can be measured in.
- 3.10 Describe why and how to measure glucose from a drop of blood.
- 3.11 Describe how to measure glucose in automated equipment.
- 3.12 Describe amperometry as used in the PO_2 electrode.
- 3.13 Describe the most common enzymatic electrode method for measuring glucose.
- 3.14 Calculate the pH for a hydrogen ion concentration of 10^{-7} mol/L.
- 3.15 Draw a pH electrode and explain its principle of operation. Explain why its amplifier input impedance is important. Explain the relation of the CO_2 electrode to the pH electrode.
- 3.16 Explain the principle of operation and give an example of use for flame photometry.
- 3.17 Explain the principle of operation and give an example of use for mass spectrometry.
- 3.18 Explain why and how CO_2 is measured by infrared transmission spectroscopy.
- 3.19 Explain why and how N_2 is measured by emission spectroscopy.
- 3.20 Explain why and how fluorometry is used. Describe one of the advantages of fluorometry.
- 3.21 Explain why and how chromatography is used. Explain the two principles that are the primary factors affecting interactions in chromatography.
- 3.22 Explain how the glucose sensor minimizes sensitivity to PO_2 variations.

Below the sample plane, the objective lens assembly focuses and magnifies the specimen image. By changing the aperture of the objective lens it is possible to control the specimen contrast and to correct astigmatism. Scattered elements of the electron beam emerging from the sample are also eliminated by the objective lens system (Dykstra, 1992).

To control the magnification of the image being projected, it is necessary to use a projector lens, which will also focus the beam of electrons for appropriate intensity upon the fluorescent screen. This screen can be observed through a viewing window (not shown) or the image can be photographically reproduced by exposing a photographic plate immediately beneath the fluorescent screen (Packer, 1967).

Specimen preparation: A meticulous preparation of the sample is required for suitable TEM observation. This process is lengthy and involves several steps, most of which are chemical processes: fixation, washing, dehydration, infiltration with transitional solvents and with resins, embedding, and curing. Once the specimen is chemically prepared it must be cut into extremely thin slices or sections (from 30 to 60 nm). This procedure is called *ultramicrotomy* and is performed to allow the beams of electrons to pass through the sample material (Bozzola and Russell, 1991). Preparation of suitable thin samples for TEM studies in the area of biomaterials is hard to accomplish due to the difficulty of performing ultramicrotomy on elastomeric materials, such as some polymers (e.g. polyurethanes). There are some alternative methods of specimen preparation that overcome this difficulty. Another problem with polymers is their low contrast. However, this can be overcome by using a defocused conventional TEM. A defocused beam also avoids sample damage or destruction (Goodman et al., 1988).

One major disadvantage of TEM is its limitation for a proper three-dimensional view (Dykstra, 1992). To obtain information on the surface and near surface morphology using TEM, the viewing of the sample must be done at several angles. Using multiple views could permit reconstruction of the three-dimensional structure. However, angle tilt is severely limited by the loss of resolution, especially with thick samples needing large tilt angles (Goodman et al., 1988).

For tissue engineers, TEM represents a powerful tool for studying the intrastructural features of the soft tissue-biomaterials interface, particularly surface related phenomena such as cell adhesion and biomaterial degradation (Sheffield and Matlaga, 1986).

Scanning Electron Microscope (SEM)

SEMs and TEMs have many features in common—they both use electron beams to visualize a sample. However, they differ in the way the beam interacts with the specimen. The principle of SEM operation makes it very useful for topographic analysis, as we will see in the next paragraphs.

Principle: SEM is based upon the interaction of an electron beam with a specimen. The incident beam (primary electrons) displaces orbital electrons from the sample atoms, giving rise to secondary electron emission (Figure 4.5)



Figure 4.6 (a) An STM probe tip made of tungsten magnified 4,000 times. The tip is very small, and can be ruined on a sample, which is seen in Figure 4.6(b). (from

<http://www.orc.soton.ac.uk/~wsb/stm/photos.htm>)

STM instrument: Figure 4.8 shows a block diagram of the STM. Piezotranslators are used to move the sample relative to the tip rather than vice versa. The piezoelectric effect is the mechanical expansion and contraction of a material in response to an electric field. In this way electric voltages are used to generate a three dimensional (3-D) movement of the piezoscanner holding the probe. A widely used piezoscanner made of ceramic material is the piezotube, which has inner and outer electrodes for movements along the three Cartesian axes x , y , and z . The distance between the tip and the sample is kept at 1 nm. The sample is connected to a tunnel voltage source. The tunneling current from the sample to the tip is fed into a current-to-voltage converter. The output of the I/V converter is further processed at the voltage processor block for display. This voltage is also fed back to the system via the scanner voltage block, which provides the necessary voltages for movements along the z axis. A scan controller generates the voltages needed for movements along the x - y plane. Visualization and interpretation of the data provided by

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radiation is captured by an analyzer. The excitation and emission processes take place in an ultrahigh vacuum (UHV) chamber (not shown in Figure 4.12) for minimal sample contamination and for easy passage of the photoelectrons to the analyzer (Paynter, 1988).

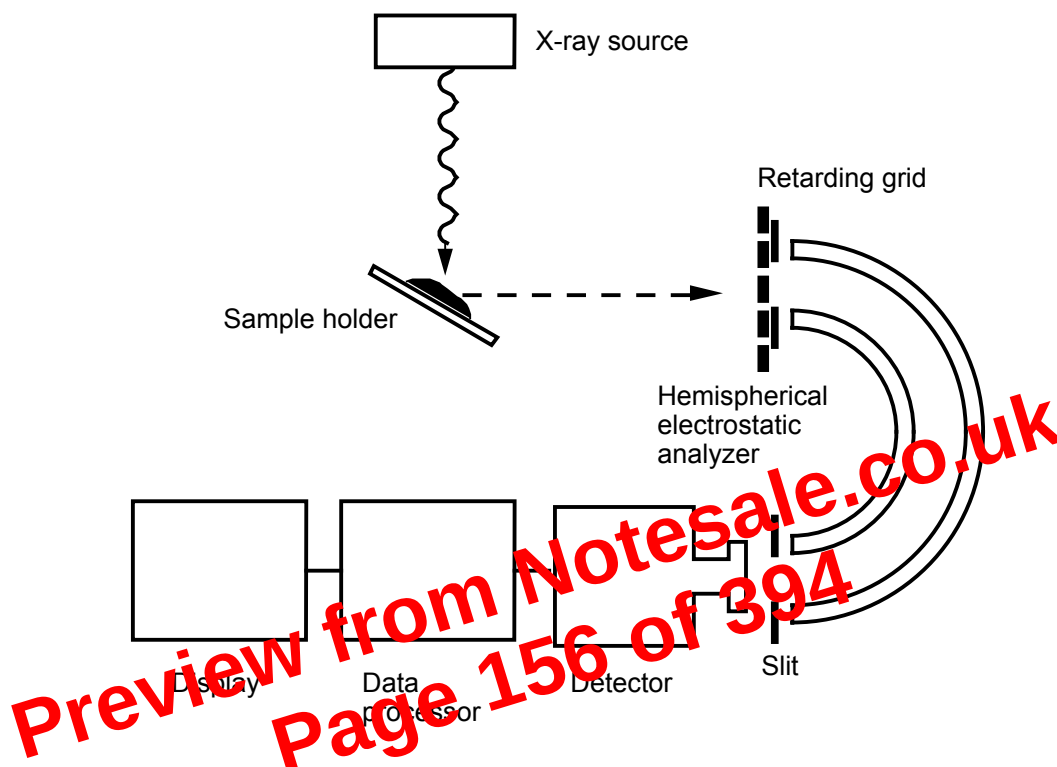


Figure 4.12 Basic schematics of an XPS instrument. An X-ray beam strikes the sample surface, giving photoelectron radiation. These electrons enter the hemispherical analyzer where they are spatially dispersed due to the effects of the retarding grid and of the electrostatic field of the concentric hemispheres. Ramping voltages at the retarding grid allow kinetic energy scanning. At the other end of the analyzer electrons are detected, counted, and a spectrum of photoelectron intensity versus binding energy is displayed.

The analyzer consists of two concentric and electrostatically charged hemispheres. An electrically biased grid at the entrance of the analyzer retards the kinetic energy of the photoelectrons, which are then spatially dispersed as they initiate their semicircular trajectory between the hemispheres. The voltage across the hemispheres is kept constant (fixed analyzer transmission or FAT mode), therefore the energy resolution is also constant. Ramping voltages in the pre-retardation stage allows actual kinetic energy scanning with a constant resolution mode of typically ~ 0.1 to 1.0 eV.

At the other end of the analyzer an electron multiplier detector increases by several orders of magnitude the number of electrons reaching the data processing and display sections, which usually consist of a phosphorescent screen and a detector device

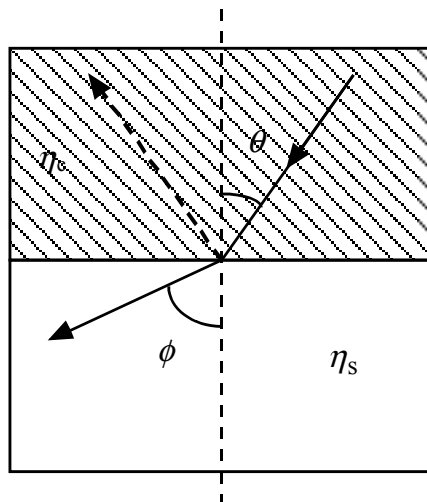


Figure 4.16 When an incident beam traveling at an angle θ in a medium of refractive index η_c encounters another medium of refractive index η_s , it will reflect in a direction given by θ and refract in the direction given by ϕ , verifying Snell's Law of Refraction (Eq. (4.7)).

We have highly simplified the presentation of these phenomena. A more rigorous treatment would have required the use of tools provided by electromagnetic wave theory.

Surface infrared spectroscopy, FTIR-ATR. The combination of the techniques that we have just presented represents a very useful method for studying the surface properties of materials.

The electromagnetic radiation is totally internally reflected (ATR) through an optically transparent material in contact with the sample (see Figure 4.16). The reflectivity exhibited by the surface depends upon the interactions of the electromagnetic field established within the sample. Using infrared spectroscopy (IR) it is possible to obtain a spectrum representing the characteristics of the sample surface within a finite distance of the interface, providing information on its chemical composition (Knutson and Lyman, 1985).

Finally, the addition of the Fourier Transform led the method known as *Fourier Transform Infrared Spectroscopy-Attenuated Total Internal Reflection*, FTIR-ATR, which ultimately combines the versatility of the infrared spectrum and the convenience of computers for fast digital processing, represents a key technique for surface analysis.

4.3.3 Polymer–water interface

6-2 Chapter 6 Cellular Measurements

There are two types of cells: those with a nucleus (eukaryotic cells) and those without (prokaryotic cells). Eukaryotic cells are composed of three main features: a nucleus, surrounded by cytoplasm, which in turn is surrounded by a sack-like plasma membrane. This membrane is made from a phospholipid bilayer and is semipermeable, which means it allows only certain things to pass in or out of the cell. Cells are 90% fluid (cytoplasm), which consists of amino acids, proteins, glucose, and numerous other molecules. On an elemental level, the cell contains 59% hydrogen, 24% oxygen, 11% carbon, 4% nitrogen, and 2% others including phosphorus and sulfur. On a molecular level, the cell contains 50% protein, 15% nucleic acid, 15% carbohydrates, 10% lipids, and 10% other. Table 6.1 shows the contents of the typical cell.

Table 6.1 Typical cell content.

Structure	Description
Cytoplasm	The inside of the cell not including the organelles.
Organelles	Membranous sacs within the cytoplasm.
Cytoskeleton	Structural support made of microtubules, actin and intermediate filaments.
Endoplasmic Reticulum (ER) (two types)	Site of protein and lipid synthesis and a transport network for molecules.
Rough ER	Has ribosomes, and tends to be flat.
Smooth ER	Does not have ribosomes and is tubular.
Golgi Apparatus	Modifies molecules and packages them into small membrane bound sacs called vesicles.
Lysosomes	Main point of digestion.
Microtubules	Made from tubulin and make up centrioles, cilia, cytoskeleton, etc.
Mitochondria	Site of aerobic respiration and the major energy production center.
Nucleus	Location of DNA; RNA transcription.
Peroxisomes	Use oxygen to carry out catabolic reactions.
Ribosomes:	Located on the Endoplasmic Reticulum in the cytoplasm. RNA goes here for translation into proteins.

To provide a rough idea of the scale that cell biologists and other related researchers are working on, Table 6.2 shows the typical size for some of the cell features.

Preview from Notesale.co.uk
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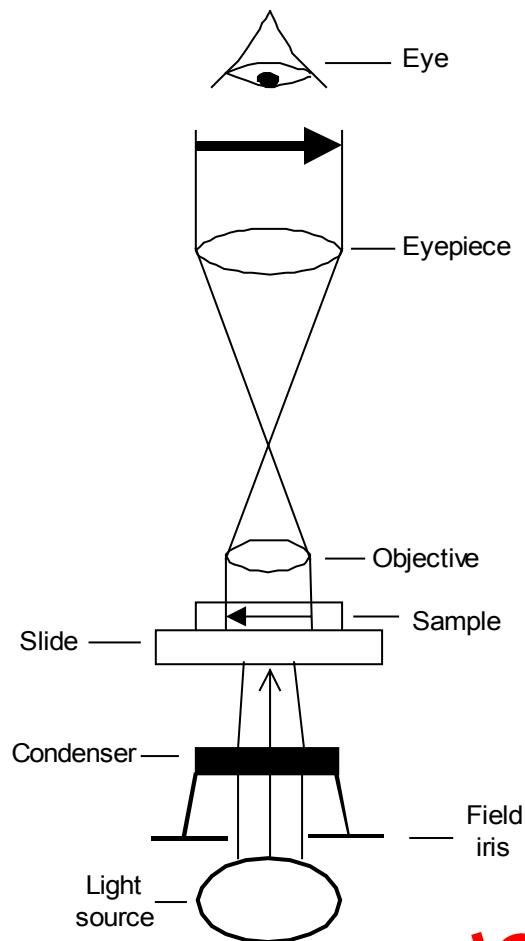


Figure 6.2 Compound microscope. The compound microscope contains a light source for sample illumination, a field iris to control the light field, a condenser to focus the illuminating light, an objective lens, and an eyepiece.

If an electron beam replaces the beam of light, the microscope becomes a transmission electron microscope (Chapter 4). If light is reflected from the object instead of passing through, the light microscope becomes a dissecting microscope. If electrons are reflected from the object in a scanned pattern, the instrument becomes a scanning electron microscope (Chapter 4).

6.2.1 Resolution versus magnification

The microscope's function is to allow viewing of objects that cannot be seen with the human eye. Because we are trying to make an object look larger, the term resolution is

often confused with magnification. As previously discussed in Chapter 4, magnification refers to the size of the image as compared to the original object and is usually expressed as $\times mm$, where mm is the amount of magnification. However, resolution determines whether small objects that are close together can be distinguished as separate objects. As a general rule, the greater the magnification, the greater the resolution. However, there are several practical limitations of lens design that can result in increased magnification without increased resolution. If an image of a cell is magnified by a factor of ten, the image will get larger, but not necessarily any clearer. Without resolution, no matter how much the image is magnified, the amount of observable detail is fixed. Regardless of how much you increase the size of the image, no more detail can be seen. At this point, you have reached the limit of resolution. This is also known as the resolving power of the lens and is only a function of the lens.

The human eye can only distinguish between two objects when they are about 0.1 mm apart at a distance of 100 mm. If two objects are 0.01 mm apart, they cannot be distinguished unless we magnify their image by $\times 10$. In this case, the limit of resolution has changed from 0.1 mm to 0.01 mm, or inversely, our resolving power has increased by a factor of 10.

Unfortunately, a lens can magnify an image without increasing the resolution. Lenses are not perfect and cause objects to become blurry at the edges. Thus, even though they can be made to appear 0.1 mm apart, the edges are so blurry that we lose the ability to see them as two distinct objects. While microscope lenses are typically expressed in terms of magnification, the important value is the resolution.

The resolution of a lens is a function of how it is configured and the wavelength of light that is passed through the lens.

$$\text{resolution} = \frac{0.61\lambda}{n \sin \alpha} \quad (6.2)$$

where λ is the wavelength of the illuminating light, n is the refractive index of the medium between the objective and the specimen, and the cone angle α is 1/2 the angle of the acceptance of light onto the lens (Figure 6.3). The value $n \sin \alpha$ is known as the numerical aperture (n.a.).

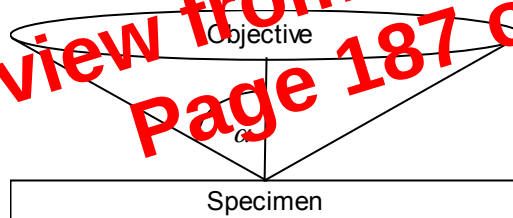


Figure 6.3 Cone angle α .

There are three ways to improve resolution. One is to use shorter wavelengths of light. This is typically done using filters. Another is to increase the cone angle. The cone angle can be increased by placing the objective as close as possible to the specimen. Practically, the largest value of $\sin \alpha$ is 0.95 (GEK, 1997). Finally, the refractive index, n , can

be increased. Immersion oil is sometimes used in place of the air between the objective and the slide to increase n .

With bright field microscopy, work is usually done in the visible light range so the image can be seen directly with the human eye. Since the shortest wavelength of visible light is blue, almost all microscopes have incorporated a blue filter, which is often referred to as a daylight filter. However, the human eye has a maximum sensitivity to green and since specimens contain various colors, the resolution of an image typically varies between 0.2 to 0.35 μm depending on the color of the light source. Resolution can be enhanced by reducing the wavelength to the ultraviolet range. However, ultraviolet (UV) light cannot be seen directly by the human eye. Thus, this form of microscopy requires a detector or photographic film that is sensitive to UV. UV light improves the resolution by a factor of two over visible light.

The best resolution is not attainable, however, unless the lenses are corrected for problems common to lens design. Modern microscopes normally use a set of lenses assembled to maximize the refractive index while minimizing chromatic and spherical distortions. Chromatic and spherical distortions (aberrations) occur in all lenses to some extent due to the fact that a 3-D object is being viewed as a 2-D image. However, aberrations are typically determined by how well the lenses were designed and manufactured. In general, the smaller the optical distortion, the greater the cost.

6.2.2 Light microscope modes

Bright field

The light microscope can use several methods to view objects. Bright field microscopy is used to view stained or naturally pigmented organelles in the cell. It involves the use of color detection. If an object contains colored features, the eye detects those colors. The stains or pigments absorb and alter the light that passes through the sample. Therefore, the light that passes through the sample is seen as a color that is distinctly different from the background or direct light that does not pass through the sample. Most of the features in the cell are relatively colorless and difficult to see under the microscope. The easiest way to enhance the contrast of the relatively transparent cells is to stain them. Different colored dyes are used to stain different parts of the cell. If the cells are dead, this is not a problem except for possible damage to the cellular integrity from the dye.

Further image enhancement can be accomplished by using a specific monochromatic light source. One way to achieve this is by using a filter. The most commonly used filter is one that allows only blue light to pass through. Blue light, about 450 nm, is usually the shortest wavelength used in light microscopy. It determines what cell features you can and cannot see under the microscope. In order to see a specific structure of the cell, it must be large enough to be able to perturb the wave motion of the light rays that strike it. Therefore, mitochondria, about 3 μm , are one of the smallest organelles to be seen through microscopy. Organelles that are smaller than this cannot be resolved be-

There are two general methods for highlighting or marking different structures within a cell or to mark different types of cells. These markers are used for a variety of purposes from locating a certain structure within a cell, identifying which cells contain a particular substance, how much of the substance they contain, where it is located within the cell, how that substance may move over the cell's cycle, if the substance moves between cells, and even how much of the substance is present within a cell.

These markers can be put into the cells in several different ways. Some are naturally taken up by the cells when introduced into their environment. Others are injected into cells using special techniques or devices such as the tiny syringe-like microinjector. Once put into cells, some are analyzed immediately, others are given some time to bond to specific cellular structures, while others are activated at a later time when the researcher wants to observe time specific cellular dynamics.

Fluorescent probes

The use of fluorescent markers or probes is revolutionizing the imaging of live cells and their associated contents, structures, and dynamics. These systems all involve the excitation of a fluorescent material, the subsequent fluorescence emission by the material, and the detection of this fluorescence by a detector (Haugland, 1997).

The probes, also called fluorochromes and fluorophores, are fluorescent materials that are introduced into the cellular environment. There are a number of different ways that these probes are used with the cells:

- the probe binds directly to the cellular structure of interest,
- the probe binds to another molecule that has an affinity or reactivity towards some cellular structure,
- the probe binds to an antibody that in turn binds to some cellular structure,
- the probe does not bind but its fluorescence depends on local concentration of a solute,
- nonfluorescent molecules are converted to fluorescent molecules through an enzymatic reaction.

The use of these probes is especially useful for microscopy for several reasons. First the excitation wavelength is different from the emission wavelength. This allows for easy detection because the excitation wavelength can easily be filtered out. Also, different types of probes emit fluorescence at different wavelengths. This too is especially useful in that several probes can be used to mark different structures within a cell and these can be detected at the same time. In addition, the entire excitation/fluorescence cycle can be repeated.

Two considerations are important regarding probe selection. First, probes must be chosen based on *sensitivity*, or the amount of substance that is labeled within the cell. Also the probe's *specificity* or the ability to only label the substance of interest is important. Specificity is provided by fluorochromes. These are either synthetic chemicals or natural products that can have innate specificity, binding to certain classes of molecules, or they can be attached to molecules that have specificity (for example, antibodies). Some

nating from the focal plane to reach the detector as shown in Figure 6.11. The detector (usually a CCD camera or photomultiplier tube) measures the intensity of light and produces a signal proportional to the light at each point. This signal is then typically sent to an imaging system where the current is converted to a voltage, digitized, processed to reduce noise, and an image is reconstructed for display on a monitor.

CLSM has several advantages over conventional microscopy. CLSM provides high-contrast images of specimens without the image artifacts normally present with conventional contrasting techniques. It also has the ability to view relatively thick specimens and is well suited for fluorescence applications as will be discussed later. The disadvantages of this technique are relatively high cost and possible damage to the specimen from the laser illumination. In an attempt to minimize this damage, each slice may be scanned several times at relatively low laser power and the results integrated.

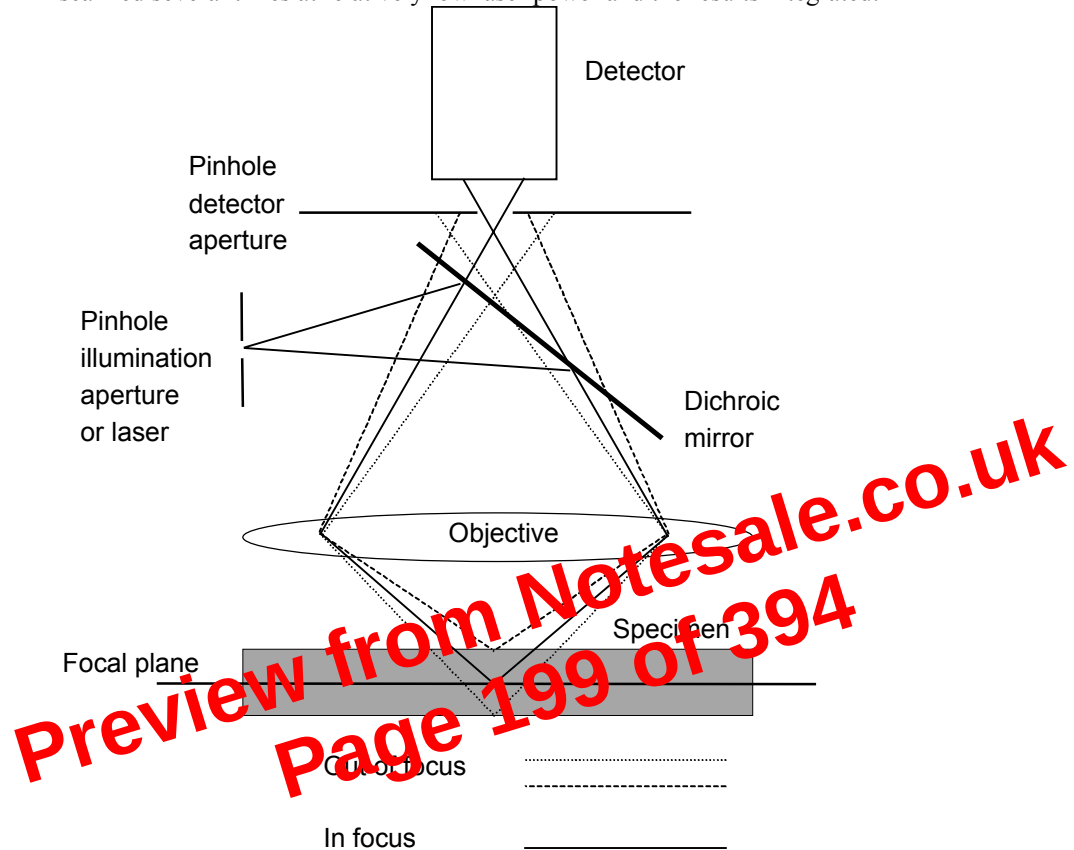


Figure 6.11 Confocal laser scanning microscope. The microscope removes out-of-focus (z-plane) blur by keeping out of focus light from reaching the detector using a pinhole.

6.3 Cell Orientation

Cell orientation refers to both the orientation of cells within tissue and the orientation of cellular components within the cell. There are a number of reasons to measure the orientation of cells within tissue. Understanding how cells are oriented in relation to one another, and thereby the mechanical properties of natural tissue, is an important aspect in the development of artificial tissue (Tranquillo, 1997). Many types of cells have been found to have specific spatial orientations where the top and bottom of a cell can be identified. Another aspect involves the way in which cells orient themselves and migrate in response to different types of stimuli. An understanding of this process will aid in the growth of artificial tissue.

6.3.1 Orientation chamber

One way to measure orientation is by using an orientation chamber. One type of orientation chamber tests the ability of cells to orient themselves in a gradient of chemical attractant. The chamber is similar to a hemocytometer (see Chapter 5). The gradient is set up by diffusion from one well to the other and the orientation of cells toward the well containing chemical attractant is scored on the basis of their morphology or by filming their movement.

6.3.2 Video enhanced contrast microscopy

Video enhanced contrast microscopy (VECM), as shown in Figure 6.13, uses a high-resolution video camera and a light microscope that has differential interference contrast (DIC) optics. By using both the analog and digital contrast enhancement as well as background image subtraction, this method is able to observe objects with dimension an order of magnitude smaller than the resolution limits of the microscope alone (Shotten, 1990). During image subtraction, for example, all identical structures in successive video images can be erased, filtering out any interfering background images. This method involves three steps (Figure 6.14). First the light microscope's DIC optics are adjusted to just under the saturation level of the video camera. The second step involves reducing the background level by adjusting the black-level control. Then the gain control is adjusted to amplify this dark-low intensity signal. The final step is required because the previous one also captures background blms created by lens imperfections and unevenness of illumination. In this step, a specimen free background that has been previously recorded is subtracted from the live image.

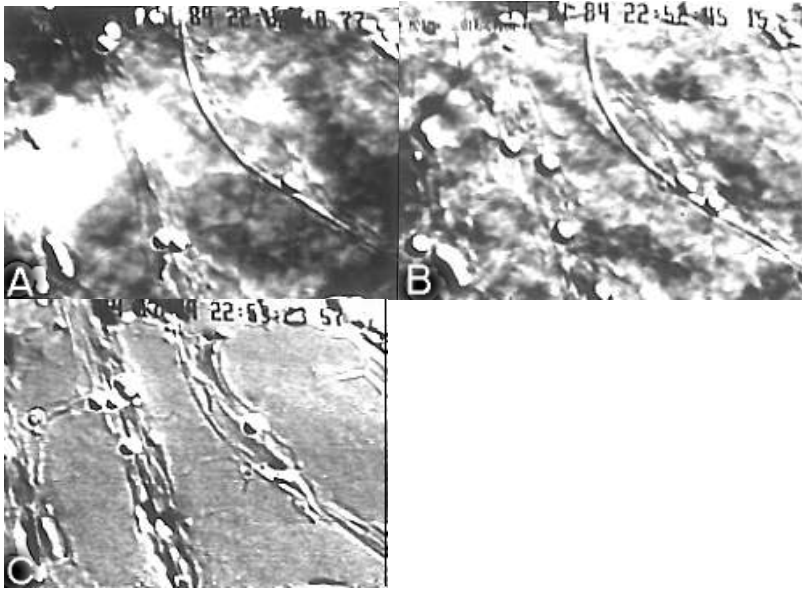


Figure 6.15 (a) An unprocessed photo of cells of the inner epidermis taken through an interference contrast microscope. (b) The same image with digital contrast enhancement, the single structures become apparent. The background fault remains. (c) Subtraction of the background and resulting with further contrast enhancement.

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6.6 Cell Deformation

Cell deformation involves measuring the mechanical properties of cells and the forces involved in cell activation. If a force is applied to a cell and the deformation is measured, the mechanical properties of the cell can be determined. The deformation of cells, in response to some mechanical force outside the cell, is an area of great interest to biomedical researchers. This process is essential in developing mathematical models of the biomechanical aspects of cellular structure, particularly as they apply to the circulatory system. This research involves *in vitro* biological experiments, mathematical modeling of cellular mechanical behavior, and computer simulation of cell mechanics. Looking at cell deformation, researchers are trying to determine the influence of deformation on the growth of cells, how cells sense deformation at their surface, and how they change their function and structure in response to this deformation. Typically video microscopy is used to monitor, record, and measure the amount of deformation in these experiments. The more difficult engineering task with this parameter is the ability to apply a measurable force to deform the cell.

6.6.1 Micropipet technique

Several methods can be used to deform cells (Hochmuth, 1990). The micropipet has been an important tool for cell deformation studies. This technique involves aspirating part or all of a cell into a glass micropipet. A pipet with a diameter of $1\text{ }\mu\text{m}$ is capable of producing a force F of 1 pN . The rigidity and other cell deformation properties can be found as a function of the force on the cell and the displacement of the cell inside the micropipet. By tracking the displacement of the leading edge of the cell as it moves into the pipet, cell deformations as small as $0.1\text{ }\mu\text{m}$ can be measured.



Figure 6.19 In the micropipet technique, force causes a displacement to determine cell deformation properties.

6.6.2 Optical trapping

6.8 Cell Adhesion

Cell adhesion, like cell rolling velocity, is an important parameter in the healing process, in facilitating the growth of tissue, and in the body's ability to assimilate newly implanted biomaterials.

In some cases reduction of adhesion is the aim, in others an increase is desired. Understanding the mechanisms that start the adhesion process, especially in the blood-stream, are of particular interest. In addition, the strength of adhesive bonds is of prime interest to the tissue engineer. Most studies of cell adhesion use force measurements obtained from video and fluorescence microscopy imaging.

6.8.1 Laser trap

The optical tweezer, or laser trap, method is used for generating controlled forces in the piconewton range. It uses a light microscope and the force of radiation pressure from an infrared laser. Although piconewton sized forces are quite small, they are sufficient to stop the motion of highly mobile cells or single enzymes. These optical tweezers allow for measurements of subcellular components including torsional stiffness, flexibility of the cell cytoskeleton, and forces generated by enzymes.

6.8.2 Interferometry

Interference microscopy is used for examining cell adhesion. It provides information on the patterns of adhesion, which in turn provides information on the distribution of forces and how the attached areas of cells move. An interferometer splits a light beam into two beams, which are then recombined so they can interfere (Chapter 5). The difference in optical path length between the two beams causes the interference. Changes in optical path length occur with changes in refractive index (changes in density) of the structure on which the beams are focused. Therefore, the image shows different interference patterns in areas with different densities. Interference microscopes are operated in both the reflectance and transmittance modes.

6.8.3 SEM

The SEM uses an intense electron source known as the field emitter, which provides resolution to diameters less than 1 nm (Morton, 1997). This is used to study the cell adhesion molecule. These molecules are found on the surface of leukocytes and are an important mechanism in the body's response to injury.

- 6.21 Calculate the shear stress for water at 20 °C in a cone and plate viscometer with a diameter of 10 cm, a separation gap of 1 mm at the circumference, and rotation at 1 revolution per second. Give units.
- 6.22 Explain the reason for and the basic principles of measuring cell adhesion.
- 6.23 Explain the reason for and the basic principles of measuring cell migration.
- 6.24 Explain the reason for and the basic principles of measuring cell uptake.
- 6.25 Explain the reason for and the basic principles of measuring cell protein secretion.
- 6.26 Using fluorescence recovery after photobleaching, calculate the fraction of mobile protein where $F(-) = 4$, $F(+) = 1$, $F(\infty) = 2$.
- 6.27 Explain the reason for and the basic principles of measuring cell proliferation.
- 6.28 Explain the reason for and the basic principles of measuring cell differentiation.
- 6.29 Explain the reason for and the basic principles of measuring cell signaling and regulation.

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The X rays travel in all directions, but to prevent patient and operator harm, are shielded by a collimator so only those used in the image proceed. Secondary radiation could fog the film, but is stopped by a grid shaped like Venetian blinds. Phosphor screens emit many light photons for each X-ray photon, thus assisting in darkening the photographic film. To lower X-ray dose, an image intensifier may be used. The X-ray photon strikes a phosphor layer in a vacuum tube. Many light photons stimulate a photocathode to emit many electrons. These are accelerated to strike an output phosphor screen at +25 kV, yielding a good image with low X-ray dose (Siedband, 1998).

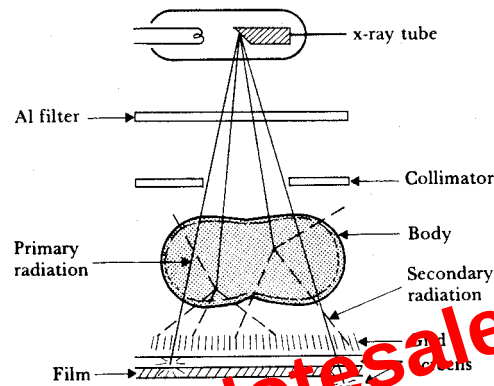


Figure 7.6 The 100 kV X-ray tube generates X rays that form shadow of the body to expose the film. Unlike a camera, there are no lenses.

7.4 Brain Imaging - CT

Figure 7.7 shows a computed tomography (CT) X-ray system. It measures X-ray attenuation of many parallel paths in one plane. Then it repeats this at many angles. The information is processed by computer to yield a 2-dimensional image of a slice through the body. Many slices can be used to create 3-dimensional images. Figure 7.8 shows how the image may be digitally reconstructed by backprojection, in which the attenuation along each path is uniformly assigned along the path. Thus if there is high attenuation at one picture element (pixel), the assignment from many angles increases that pixel more than other pixels. Iterative methods guess at the image, measure the resulting attenuation, correct to that measured by X ray, then back project the revised information. Water is used as the benchmark substance as it has a CT value of zero. Analytic methods use spatial Fourier analysis or convolution techniques. CT can produce cross-sectional images (slices) of anatomic structures without superimposing tissues on each other. Improved CT scanners use multiple simultaneous measurements and X-ray tubes that rotate in 2 s. CT yields images of soft tissue within the brain not possible with conventional X ray, which is largely blocked by the dense skull. The patient is positioned in the center of a donut hole, surrounded by the CT scanner. CT is used in the differential diagnosis of intracranial neoplasms (brain tumors), cerebral infarctions (lack of blood

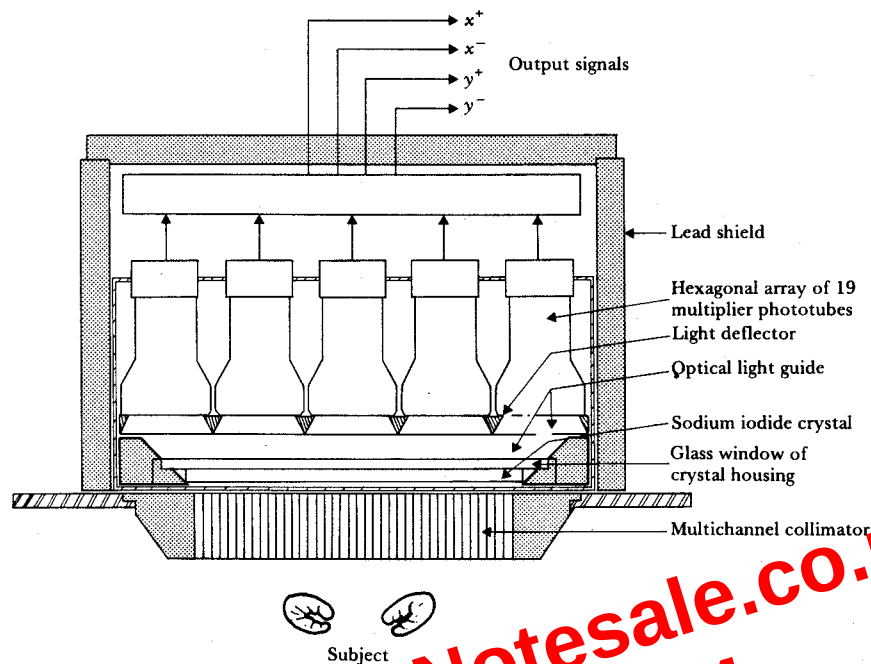


Figure 7.11 In a gamma camera system, radioisotopes emit gamma rays which are collimated, strike a NaI crystal, which emits light measured by photomultiplier tubes.

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7.7 Brain Imaging: Single-Photon Emission Computed Tomography (SPECT)

In single-photon emission computed tomography (SPECT), a scintillation assembly similar to a gamma camera is rotated around the patient. The gamma rays are collected from the patient in a manner similar to CT, but several slices can be obtained at the same time. The resulting multiple slices show depth activity in the volume of interest. It is possible to see anomalies not observable with conventional X rays or gamma camera (Van Heertum and Tikofsky, 1995).

7.8 Brain Imaging: Positron Emission Tomography (PET)

Some isotopes produce positrons that react with electrons to emit two photons at 511 keV in opposite directions. Figure 7.12(a) shows the two detectors on opposite sides of the patient. The detectors determine if the two scintillation effects are coincident and have energy levels close to 511 keV. Additional pairs of detectors permit faster operation. Image reconstruction is similar to that of CT. An advantage of PET is that all of the most common radioisotopes used, ^{15}O , ^{13}N , ^{11}C and ^{18}F , can be compounded as metabolites.

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7.10.3 Visual field

The peripheral vision of the retina reduces rapidly away from the fovea centralis (near the center of the retina). This can be assessed by visual field measurement, in which the examinee detects the targets that stimulate the retina at varying distances from the fovea. Several methods are used to measure visual field, including the confrontation test and methods using perimeters, tangent screens, or an Amsler grid (Newell, 1996).

Confrontation test

For this test the patient fixates their vision straight ahead on the examiner at a distance of 1 m. The patient closes one eye. The examiner puts one hand midway between the patient and the examiner with some fingers extended. The examiner then slowly moves the hand from the periphery toward the center and the patient states the number of fingers displayed. The temporal and nasal fields and the superior and the inferior fields are tested in turn.

Perimeters

A perimeter consists of a half bowl with a radius of curvature of 33 cm so that the eye is at the center of rotation of the hemisphere.

In kinetic perimetry, the patient detects a test object of fixed size and illumination moved from a nonsensing area. In static perimetry, the patient detects a test object of constant size and location whose light intensity is increased until it can be seen.

An isopter, the contour line that connects the points at which an object of certain size and certain light intensity may be recognized, represents a line on the macula lutea that passes through the points of equal visual acuity.

Tangent screen

The evaluation of the central 30° of the visual field is desirable because the photoreceptors of the eye are concentrated in and near the fovea centralis. This can be conducted with a test object of size from 1 to 50 mm on a tangent screen, which contains concentric circles every 5° and radiating lines 15° or 22.5° apart stitched or marked on black cloth 1 m from the subject. This test demonstrates the peripheral isopter, the blind spot, and various scotomas (blind spots in the visual field).

Amsler grid

An Amsler grid is formed by intersecting 21 horizontal and 21 vertical dark lines spaced about 5 mm with one dark dot in the middle. It encompasses the central 20° of the visual field when held at the reading distance. The patient tests an eye by fixating it on the

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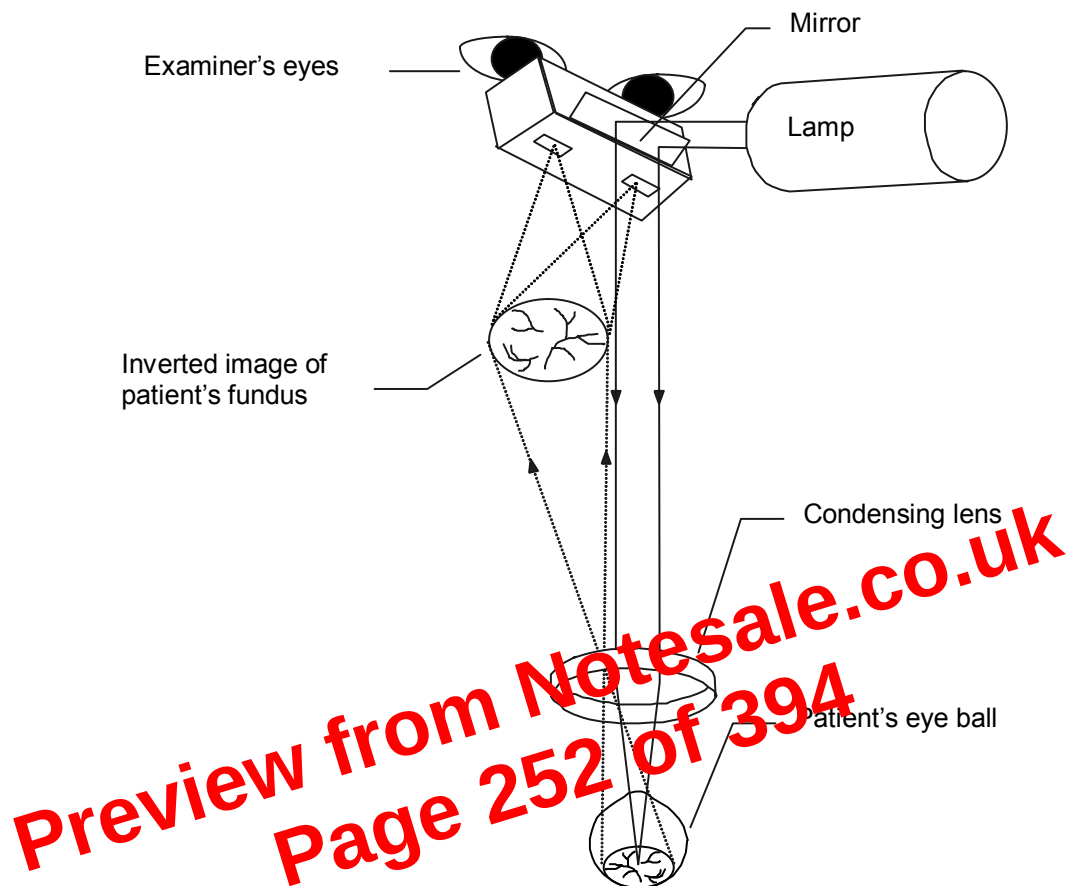


Figure 7.16 In indirect ophthalmoscopy, the examiner stands in front of the patient and holds the condensing lens at an appropriate distance from the patient's eye.

7.11 Ears and Audiometry

7.11.1 The ears

The ear is a sense organ specialized for hearing and balance functions. Anatomically, it is divided into the external ear, middle ear, and inner ear. The external ear is composed of the auricle, the external auditory canal, and the outermost layer of the tympanic membrane. The middle ear, or tympanic cavity, is an oblong cavity bridged by an ossicular chain consisting of three small bones between the tympanic membrane and the inner ear. The inner ear consists of the cochlea and the labyrinth, which are the end organ receptors for hearing and balance (Cody et al., 1981).

The auditory portion of the ear can be physiologically divided into two parts—a sound-conducting apparatus and an electromechanical transducer (Hawke, 1990). The sound-conducting apparatus consists of the external ear, the tympanic membrane, the ossicular chain, and the labyrinthine fluid. The electromechanical transducer transforms the mechanical energy of sound into electric impulses to be transmitted by the auditory nerves to the auditory cortex of the brain.

7.11.2 Audiometry

Audiometry is used to evaluate hearing pathologies in order to provide diagnostic information and rehabilitation.

Pure tone air conduction threshold testing

The audio signal passes through the outer ear, the middle ear, and the inner ear before being further processed by the central auditory system. Pathology of any of these parts of the ear can cause hearing loss. In this test, the subject responds to bursts of single-frequency stimuli presented through calibrated earphones. The signal intensity is adjusted and the threshold with 50% correct responses is recorded (Laszlo and Chasin, 1988).

Pure tone bone conduction threshold testing

A special vibratory transducer is placed on the mastoid process or the forehead and thus stimulates the inner ear directly through the skull. This bypasses the outer and middle ears. If there is a difference between the air conduction and bone conduction responses, this indicates pathology in the outer or middle ear, which may be treatable. Inner ear pathology is difficult to treat (Laszlo and Chasin, 1988).

Speech discrimination testing

In this test, the subject listens to lists of single-syllable speech discrimination words presented through earphones and repeats what he or she hears. The result of this test is scored from 0 to 100% based on the correctness of the subject's answer. In contrast to the pure tone threshold test, which addresses hearing sensitivity, this test assesses the integrity of the entire auditory system's ability in hearing clearly and understanding speech communication. A low score is related to sensorineural loss, and a higher score may be attributed to normal hearing or conductive hearing loss (Laszlo and Chasin, 1988).

Speech reception threshold

The length–force relation of a muscle can be measured isometrically. With this method, a skeletal muscle is maximally stimulated while its length is held constant. The tension generated by the muscle at various constant lengths is measured to generate the length–force curve (Lieber, 1992). Figure 7.19 shows a typical length–force curve of a skeletal muscle (Ozkaya and Nordin, 1991).

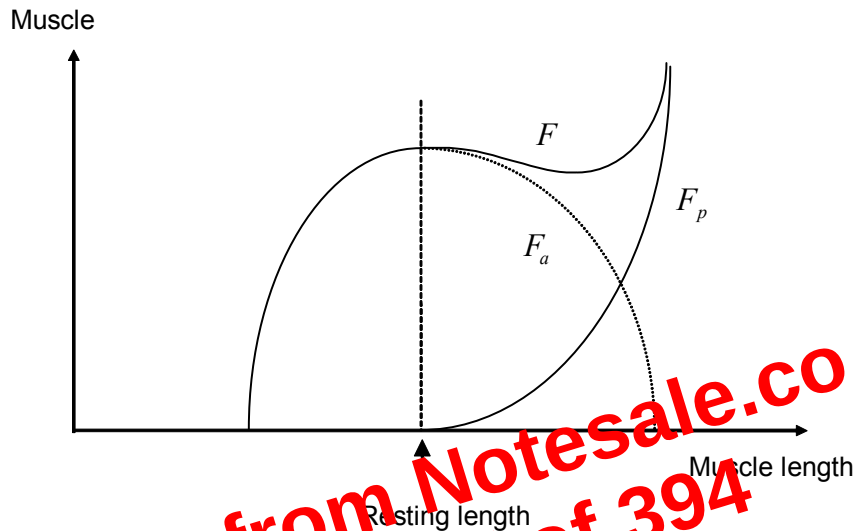


Figure 7.19 The overall force of a muscle, F , is the sum of the active force, F_a , and the passive force, F_p . The active force results from voluntary contraction of the contractile elements of the muscle. The passive force results from elongation of the connective muscle tissue beyond its resting length. No passive force builds up when the muscle is at its resting length or less.

The amount of force generated by a stimulated muscle depends on how its ends are restrained (Woledge et al., 1985). If neither end of a stimulated muscle is fixed, the muscle shortens at its maximum velocity of 33 cm/s, $V_{\max}$, and no force is generated. If we fix one end of the muscle and apply a small force to the other end, the muscle shortens at a steady velocity less than $V_{\max}$. A force of sufficient magnitude, F_0 , will prevent the muscle from shortening and isometric contraction occurs within the muscle. The force–velocity relation is described by Hill's equation (Woledge et al., 1985):

$$(F + a)(V + b) = (F_0 + a)b \quad (7.2)$$

where a and b are constants derived experimentally, F is the muscle force during shortening at velocity V , and F_0 is the maximum isometric force that the muscle can produce. Figure 7.20 shows a hypothetical curve for force–velocity relation according to Hill's equation.

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Shortening velocity

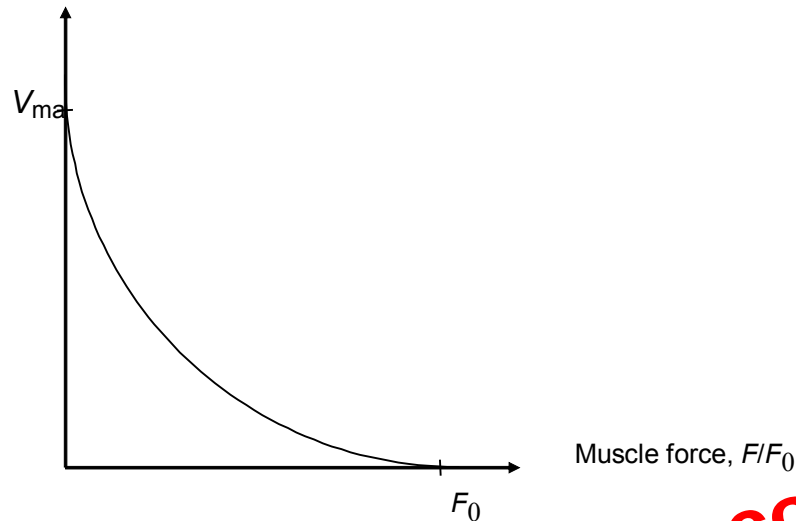


Figure 7.20 The muscle force decreases with increasing shortening velocity.

The force–velocity curve can be obtained experimentally with isotonic measurement (Lieber, 1992). The muscle is allowed to change its length against a constant load during maximal stimulation. The muscle velocity (i.e., shortening velocity, or lengthening velocity) is measured and plotted against the constant load.

7.12.2 Electromyography (EMG)

By placing electrodes into a skeletal muscle, we can monitor the electric activity of the muscle (Keynes and Aidley, 1991). EMG is used to detect primary muscular disorders along with muscular abnormalities caused by other system diseases such as nerve dysfunction (Junge, 1992). Using EMG to study muscle function is also used in various fields such as kinesiology, psychology, and rehabilitation medicine (Basmajian and De Luca, 1985).

The two main types of electrodes for measuring EMG signals from muscles are surface electrodes and inserted electrodes. The inserted type further includes needle electrodes and wire electrodes. Selection of electrode type depends on the particular application and the convenience of use (Loeb and Gans, 1986).

The surface electrode consists of silver disks that adhere to the skin. Saline gel or paste is placed between the electrode and the skin to improve the electric contact. The disadvantages of surface electrodes are that they cannot effectively detect signal from muscles deep beneath the skin and that because of poor selectivity, they cannot eliminate cross-talk from adjacent muscles.

Needle electrodes have a relatively smaller pickup area and thus are more suitable for detecting individual motor unit potentials. Needle electrodes can be

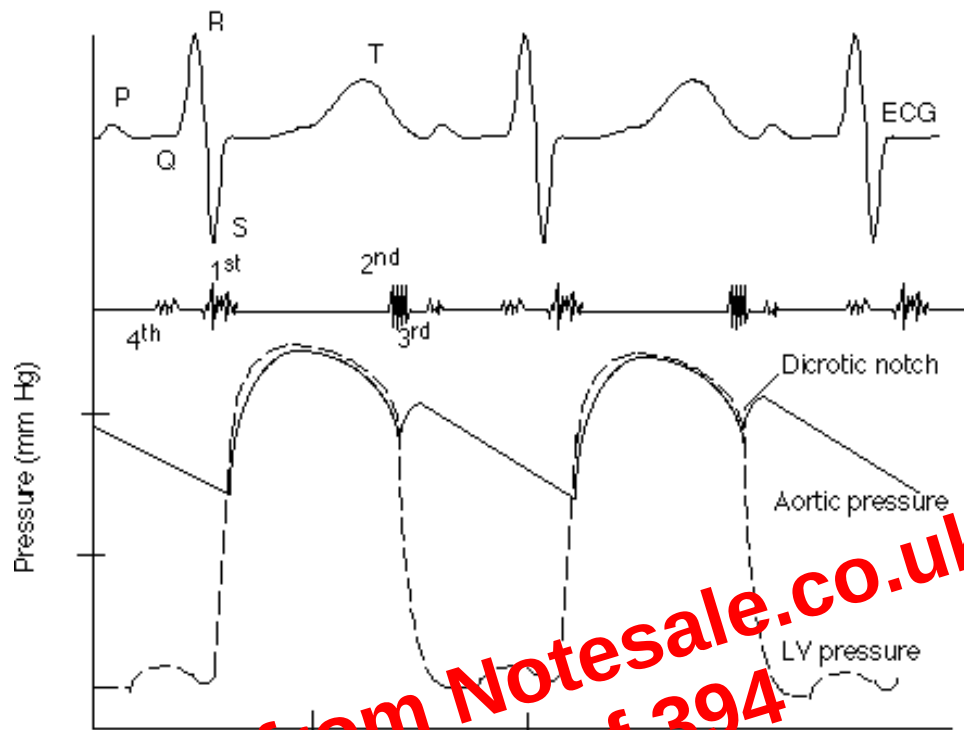


Figure 8.3 In the top figure, the electrocardiogram (ECG) initiates the cardiac cycle. The cardiac impulses (section 8.5) are also shown. The bottom figure shows that ejection occurs when the pressure in the left ventricle exceeds that in the arteries.

During diastole, the pressure in the left ventricle is low and less than that of the arteries. Thus the aortic valve is closed. The blood from the left atrium flows into the left ventricle. The left atrium subsequently contracts due to the excitation from the pacemaker cells in the heart (see section 8.1.3) and further fills the left ventricle. The ventricles contract, which increases the pressure and closes the mitral valve. The pressure increases until it exceeds the aortic pressure, the aortic valve opens, and blood flows into the aorta. The blood continues to flow from the left ventricle into the aorta as long as the ventricular pressure is greater than the arterial pressure, as shown in Figure 8.3. However, if the ventricular pressure is much greater than the aortic pressure, there is a problem with the valve and it may be stenotic (narrow). The dicrotic notch results when the aortic valve slams shut and the AV valve is still closed. Table 8.1 summarizes the events of the cardiac cycle.

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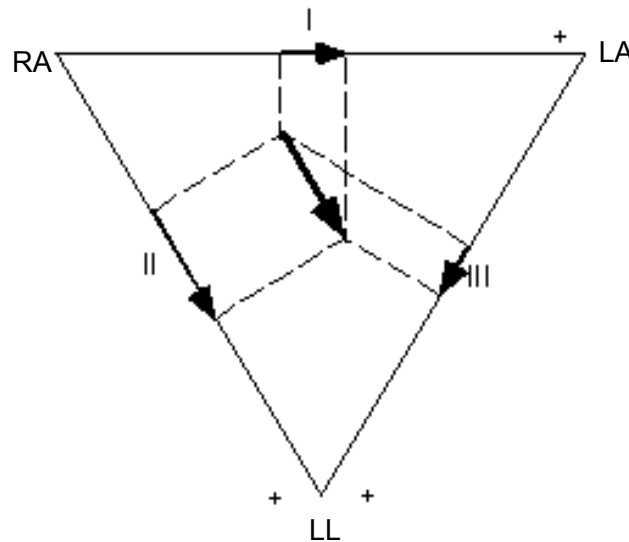


Figure 8.8 Einthoven's triangle. Lead I is from RA to LA, lead II is from RA to LL, and lead III is from LA to LL.

Three additional leads, called the *unipolar leads*, or augmented limb leads, are routinely used in taking diagnostic ECGs. These leads are based on signals obtained from more than one pair of electrode. Unipolar leads consist of the potential appearing on one electrode taken with respect to an equivalent reference electrode, which is the average of the signal from two or more electrodes. The three different unipolar leads are positive electrodes placed on the right arm, augmented right (aVR), the left arm, augmented left (aVL), and the left foot, augmented foot (aVF). The other leads that are frequently measured in clinical ECGs are the *precordial* leads. Physicians place an electrode at various anatomically defined positions on the chest wall (Neuman, 1998b). Required frequency response is 0.05 to 150 Hz.

ECG monitoring

The ECG may be monitored continuously when the patient is in emergency care, in a coronary care unit, an intensive care unit, or during stress tests, by a recorder or telemetry. In these cases, only one lead, usually lead II, is monitored on a display. The system automatically monitors rhythm disturbances and gives an alarm when the heart rate is too slow or too fast. To avoid motion artifacts and muscle noise the frequency response is reduced to 0.5 to 40 Hz.

Ambulatory (Holter) monitor

8.3.2 Catheter

In the cardiac catheterization laboratory, catheters are inserted into the chambers of the heart to measure pressures, flows, and oxygen saturation to determine if valve replacement is required. Catheters can also inject radiopaque dye for X-ray fluoroscopy, which can image vessel narrowing to determine if vessel replacement is required.

A catheter is a flexible tube for insertion into a narrow opening such as the blood vessels, so that fluids may be introduced or removed. Figure 8.9 shows a Swan–Ganz catheter. The first person who passed a catheter into the human heart was Werner Forsmann, a medical student at Eberswalde, Germany. In 1929, at the age of 25, he passed a 65 cm catheter through one of his left antecubital veins, guiding it by fluoroscopy until it entered his right atrium. Forsmann then climbed up the stairs to the Radiology Department where the catheter position was then documented.

Catheters are inserted through sheaths (the layers of connective tissue that envelop structures such as nerves, arteries, tendon, and muscle) into the arteries and the veins of the body then pass up to the heart. The pressures within different areas of the heart are measured. Two approaches may be used to apply a catheter to the heart.

The brachial (in the arm) approach usually utilizes cutdown on the brachial artery (an artery that extends from the axillary artery, down the side and inner surface of the upper arm to the elbow, where it divides into the radial and ulnar arteries) and vasilic vein at the elbow. The direct brachial approach may have advantages in a very obese patient, in whom the percutaneous femoral approach may be technically difficult and bleeding hard to control after catheter removal.

The indirect femoral approach. The percutaneous femoral approach has its own set of advantages. Arteriotomy and arterial repair are not required; it can be performed repeatedly in the same patient at intervals, whereas the brachial approach can rarely be repeated more than two or three times with safety.

Cardiac pressures are usually measured by one of several ways. In the *catheter-type* system, the blood pressure communicates with the pressure sensor via a fluid-filled catheter. The sensor is outside the body. With an appropriate length and diameter, accurate pressure readings can be obtained.

With the *catheter-tip* sensor, the elastic unit is placed in a blood vessel, thus the elastic member is in direct contact with the blood. This arrangement avoids the damping and resonance sometimes encountered with catheter-type systems and is more accurate than the catheter-type sensors (Grossman and Baim, 1991).

8.4 Cardiac Output

Cardiac output is a measure of the well being and performance of the heart. The maintenance of blood flow commensurate with the metabolic needs of the body is a fundamental requirement of human life. In the absence of major disease of the vascular tree, the maintenance of appropriate blood flow to the body depends largely upon the heart's ability to pump blood in the forward direction. The quantity of blood delivered to the systemic cir-

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ulation per unit time is termed the cardiac output, generally expressed in liters/minute (L/min).

Cardiac output of a normal patient is directly correlated with the size of the body. Most investigators use total body surface area as the standardizing variable. The ratio of the cardiac output to area is called the *cardiac index*. Table 8.2 lists several variables related to cardiac output.

Table 8.2 Some physiological variables. The data presented in this table are the average values of a group of subjects.

Variables	Mean ($\pm$ SD)
Weight (kg)	70
Cardiac output (mL/s)	110
Heart rate (min^{-1})	76
Mean velocity, ascending aorta (mm/s)	16
LV end-diastolic volume (mL)	125 ($\pm$ 31)
LV end-systolic volume (mL)	42 ($\pm$ 17)
LV stroke volume (mL)	82 ($\pm$ 20)
LV ejection fraction	0.67 ($\pm$ 0.11)
LV mean wall thickness (mm)	10.9 ($\pm$ 2.0)

$$\text{Cardiac output (CO)} = \text{heart rate (HR)} \times \text{stroke volume (SV)} \quad (8.2)$$

The ejection fraction is defined as

$$\text{Ejection fraction} = \frac{\text{Stroke volume}}{\text{End-diastolic volume}} \quad (8.3)$$

End-diastolic volume is the amount of blood in the ventricle at the end of diastole. End-systolic volume is the volume of blood remaining in the ventricle at the end of systole when ejection is complete.

The stroke volume, the volume of ejected blood from the ventricles, is about 80 mL/beat. The average resting heart rate of human is about 70 beats/min. Thus cardiac output is about $80 \times 70 = 5,600 \text{ mL/min} = 5.6 \text{ L/min}$. Cardiac output is regulated by changes in both HR and SV. Heavy exercise increases both HR and SV, and CO can increase to as high as 25 L/min.

8.4.1 Fick method

In 1870, Fick stated that if the concentration of O_2 in arterial and mixed venous blood is known, and the rate of inhalation of O_2 is also known, the cardiac output, CO, can be computed from the formula (Webster, 1998)

$$\text{CO} = \frac{dm/dt}{C_a - C_v} \quad (8.4)$$

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not only physiological information but also anatomic depiction of intraluminal lesions by ultrasonic imaging. Direct arteriography has also seen new developments with the advent of digital subtraction angiography. While venous digital subtraction angiography has not proven to be as clinically useful as originally hoped, application of digital subtraction techniques to direct arteriography has permitted significant reduction in amounts of contrast agent required. By combining digital subtraction techniques with direct arterial angiography, patients at high risk of renal failure can be studied safely while combined arterial systems such as abdominal and carotid vessels can be simultaneously studied in patients with normal renal function. As for the future, magnetic resonance imaging (MRI) holds promise as an arterial diagnostic tool, particularly with large vessels. However, it appears unlikely that it will replace ultrasound and contrast arteriography.

8.8 Blood Flow

Physical factors that influence blood flow are pressure and resistance. The flow through arterial grafts is measured at the time of surgery to ensure that the graft has been successfully inserted. The flow in peripheral blood vessels is measured as an aid in the diagnosis of peripheral vascular disease.

The flow rate F of a single vessel is the volume of blood moving past a fixed point per unit time. F can be calculated from

$$F = \Delta P / R, \text{ (mL/min).} \quad (8.10)$$

Pressure ΔP is usually measured in mmHg and R is the resistance.

$$R = 8L\eta/\pi r^4, \quad (8.11)$$

where L = length, η = viscosity, r = radius. Thus,

$$F = \Delta P \pi r^4 / (8L\eta) \text{ (Poiseuille's law).} \quad (8.12)$$

ΔP is the difference between the mean arterial blood pressure (MABP) and right atrial pressure. Since the right atrial pressure is about zero, $\Delta P = \text{MABP}$. R is the resistance through all vascular beds in parallel. Fung (1997) provides further information on the biomechanics of the blood vessels.

For detecting or measuring blood flow, ultrasonic Doppler flowmetry has become the main technique of noninvasive investigation, both in the clinical and research laboratory. The continuous wave (CW) and pulsed varieties each have their advocates, the former being easier to use while the latter affords more precise flow interrogation. Although the audible signal suffices for many clinical applications, recordings are required for others and for most research endeavors. Recordings made with the zero-crossing detector are adequate to some extent for real-time frequency spectrum analyzers. These devices not only depict the velocity flow envelope with precision but also allow analysis of the power spectrum at each frequency. Increased velocity and disturbances in

The electromagnetic flowmeter measures the average of the blood velocity across the lumen of the vessel. The outside diameter of the vessel is constrained to be the same as the internal diameter of the probe, so the volume flow through it can be calculated. In practice, this is not an ideal assumption. Most blood vessels display laminar flow—flow is greatest in the center of the vessel and slowest near the walls. It is not uniformly sensitive to the same blood velocity at different positions across the lumen of the vessel. Alterations in the velocity profile across the vessel therefore alter the measured mean velocity, giving rise to error. Fortunately, this induced error is not large enough to cause unacceptable measurements.

8.8.3 Ultrasonic flowmeter

Ultrasonic flowmetry is a commonly used technique for measuring blood velocity in the peripheral arteries. It can measure pulsatile flow. A piezoelectric sensor is used to convert from electric to acoustic signals (see section 8.5.2). Figure 8.17 shows a system for blood flow measurement using an ultrasonic flowmeter. The Doppler effect shifts the frequency of the ultrasonic beam from the oscillator when it intercepts the moving blood cells by an amount Δf , which can be computed from

$$\Delta f = \frac{2uf_0 \cos \theta}{c} \quad (8.14)$$

where f_0 is the fundamental frequency of an ultrasonic wave from the source, traveling at velocity c through the blood. The ultrasonic wave intercepts a stream of moving blood with velocity u crossing the ultrasound beam at an angle θ to produce the frequency shift Δf . The factor of 2 occurs because the Doppler shift arises both on absorption of the sound by the moving blood particles and between the transmitting blood cell and the receiving transducer.

Arterial and venous blood pressure can be measured by inserting a catheter into the blood vessel and maneuvering it until the end is at the site at which the blood pressure is to be measured. In this case the diaphragm of the sensor is mounted at the end of the catheter outside the body (similar to section 8.2). Catheters for use in the artery are usually thin, short, and highly flexible. A catheter can be inserted into the artery inside a needle as well. The alternative method is to use a catheter-tip sensor where the miniature diaphragm is at the tip inside the vessel. Although accurate, these methods are very invasive.

8.9.3 Arterial tonometry

Arterial tonometry is a noninvasive technique for monitoring the arterial blood pressure in a continuous manner. A linear array of pressure sensors is pressed against the radial artery so that at least one sensor is directly over the lumen. Pressure is increased from low to high so that measurements are made when the artery is half collapsed. By selecting the maximal peak-to-peak pressure reading from all sensors, the arterial pressure is determined in the same way as for the applanation tonometer (section 7.10.4). Zorn et al. (1997) compared the Colin Pilot 9200 tonometric blood pressure measurements with intra-arterial blood pressure measurements. Tonometric values were slightly less than the intra-arterial pressure measurements; the mean difference for systolic blood pressure was 2.24 ± 8.7 mmHg and for diastolic pressure was 0.26 ± 8.8 mmHg.

8.10 Vessel Distension

Blood vessels can be distended (stretched) by raising intravascular pressure or decreased in radius by lowering it since they are elastic. The difference between the pressures inside and outside the vessel—the transmural pressure—is one of the factors that controls the radius. The contraction or relaxation of vascular smooth muscle alters vessel diameter by changing the elasticity of the wall and the elasticity of a vascular bed determines how much of the blood volume is accommodated within that region at the existing local pressure.

The distribution of the blood volume among different parts of the system is determined by the relation between local pressure and vascular distensibility. Pressure is high in arteries, but they are not very distensible and the volume of blood in the arterial tree is relatively small. In contrast, a large part of the blood volume resides in the veins, even though their pressure is low, because they are readily distended. The distension of an arterial wall can be calculated from

$$\text{Distension (\%)} = \frac{\Delta d}{d_D} \times 100 \quad (8.15)$$

where Δd is the diameter change and d_D is the diastolic diameter (Nakatani et al., 1995).

8.10.1 Intravascular ultrasound

Intravascular ultrasound provides both *in vitro* and *in vivo* two-dimensional visualization of arteries in real time. Figure 8.22 shows that a rotating ultrasonic transducer illuminates the walls. Reflections from the inner and outer wall surfaces yield accurate determination of artery luminal dimension, cross-sectional area, wall thickness, and wall morphology (Forestieri and Spratt, 1995).

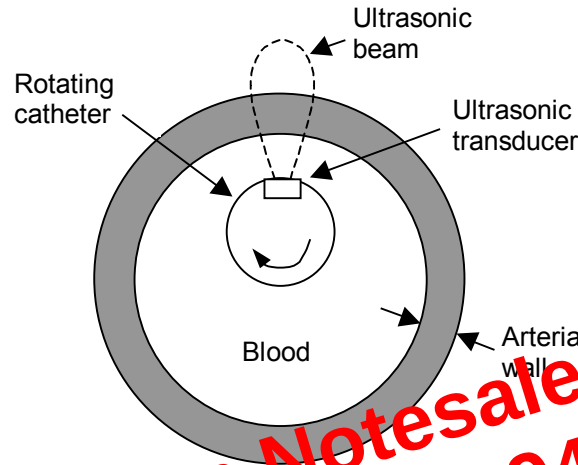


Figure 8.22 A catheter is inserted through the blood vessels. A rotating ultrasonic transducer is attached at its tip and illuminates the walls.

8.10.2 Angiography

Peripheral angiograms are most commonly done to test the arteries that supply blood to the head and neck or the abdomen and legs. Because arteries do not show up well on ordinary X rays, angiograms utilize a *contrast agent* containing iodine, which is injected into the arteries to make them visible on radiographs. A catheter is placed into the artery, via the groin, and is manipulated by the physician into the artery requiring study. Once the catheter is in place, contrast agent, or dye is injected through the catheter into the arteries and a series of X rays taken. The dye allows the doctor to see the inside of the patient's artery and determine how well blood is moving through the vessel.

In digital subtraction angiography (DSA), a first image is taken without contrast agent. Then a second image is taken with contrast agent. The two images are digitized, then subtracted, yielding no image in all regions without contrast agent. Vessels containing contrast agent show very well, so less contrast agent is required.

8.11 Vessel Volume Flow

at the other end. Calculate the blood flow rate in mL/min, assuming that the length of the artery equals 0.5 m.

- 8.15 Sketch an electromagnetic flowmeter and explain its principle of operation.
- 8.16 Sketch an ultrasonic flowmeter and explain its principle of operation.
- 8.17 Draw the block diagram for and describe the automatic indirect auscultatory peripheral blood pressure measurement system.
- 8.18 Draw the block diagram for the automatic indirect oscillometric (not auscultatory) peripheral blood pressure measurement system. Show all pneumatic connections between parts. Show location of all sensors and explain how they work. Describe the measurement cycle. Sketch the resulting waveforms. Explain how the signal processing identifies significant pressures.
- 8.19 Explain why and how we measure direct cardiac pressure using a catheter (catheter-type and catheter tip).
- 8.20 Explain why and how we measure vessel pressure using arterial tonometry.
- 8.21 Explain why and how we measure vessel pressure distensibility.
- 8.22 Explain why and how we measure vessel volume flow.

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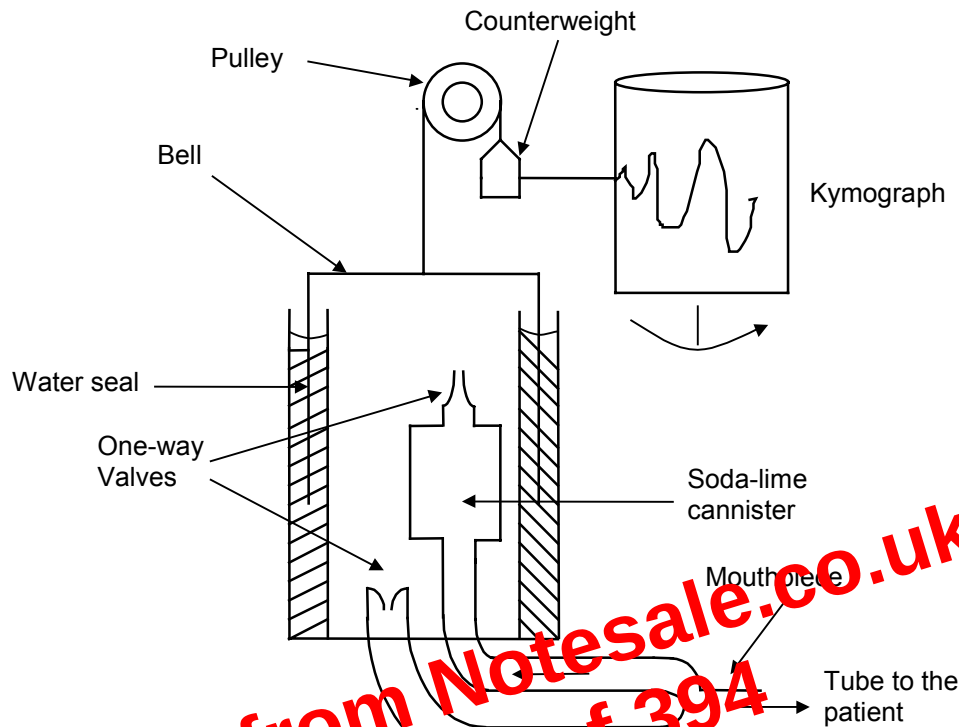


Figure 9.2 In the water seal spirometer, expired CO₂ is removed in the soda-lime cannister.

The mouthpiece of the spirometer is placed in the mouth of the subject whose nose is blocked. As the gas moves into and out of the spirometer, the pressure of the gas in the spirometer changes, causing the bell to move.

The system can be modeled as two gas compartments connected such that the number of moles of gas lost by the lungs through the airway opening is equal and opposite to the number gained by the spirometer. For rebreathing experiments, most spirometer systems have a chemical absorber (soda lime) to prevent build up of carbon dioxide. With the exception of water vapor in a saturated mixture, all gases encountered during routine respiratory experiments obey the ideal-gas law during changes of state:

$$P = \frac{N}{V} RT = \rho RT \quad (9.5)$$

where P is the pressure of the ideal gas, R is the universal gas constant, T is the absolute temperature, ρ is the mole density, which for a well-mixed compartment equals the ratio of moles of gas N in the compartment to the compartment volume V .

The amount of gas transferred from the lung to blood is a function of the pressure gradient and the diffusion capacity (D_L) if the gas is diffusion limited. The equation is

$$-V_g = D_L(P_{Ag} - P_{cg}) \quad (9.10)$$

where V_g is the rate of transfer of gas (g) from alveoli (A) to the capillaries (c), and P is the pressure. D_L is the diffusing capacity in mL/(min·mmHg) and includes the parameters of the surface area of gas transfer, the thickness of the membrane across which the gas is transferred, and the properties of the gas such as the molecular weight and solubility. Since D_L is measured from the gas side, V_g is negative since the gas is moving away from the lungs (Primiano, 1998).

Carbon dioxide diffuses across the alveolar membrane much more easily than oxygen, so a diffusion defect affects oxygen transfer first. Because of this and because of the important role oxygen plays in sustaining life, it is important to evaluate a diffusion capacity for oxygen. But obtaining the P_{aO_2} requires a sample of arterial blood. To avoid this, clinicians use carbon monoxide as the tracer gas as its properties are quite close to those of oxygen, and so its diffusion capacity provides a reasonable estimate of the diffusion capacity of oxygen. In addition, it also has affinity for hemoglobin at low concentrations; hence all carbon monoxide that enters the blood chemically combines with the hemoglobin in the red blood cells. One of the many methods that have been used to find out the diffusion capacity of carbon monoxide is the single breath technique. The subject inspires a mixture of air, 9.5% (vol. less) carbon monoxide and helium (approximately 10%) from RV to TLC. The subject holds his breath at TLC for about 10 s and then forcefully exhales down to RV. Although it requires subject cooperation, it can be performed quickly and can be repeated easily. Also, it does not require samples of arterial blood flow. The computation of D_{LCO} from measurements made during this single breath technique are based on a one compartment model of the lung. If a well-mixed alveolar compartment is filled with a mixture of gases containing some initial alveolar fraction F_{ACO} , then during breath holding with the airway open, the CO diffuses into the blood in the pulmonary capillaries, and the alveolar F_{ACO} decreases exponentially with time (Primiano, 1998).

$$\hat{F}_{ACO}(t_2) = \hat{F}_{ACO}(t_1) \times \exp \left[- \frac{D_L CO (P_{atm} - P_{AH_2O})(t_2 - t_1)}{V_A} \right] \quad (9.11)$$

9.5 Pulmonary Airway Resistance

Resistance to air flow is determined by the same factors governing the flow of fluid of low viscosity in tubes. Resistance to air flow is a calculated quantity instead of a directly measured one. The equation used is as follows

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$$\gamma = \frac{\Delta L}{L} \quad (9.26)$$

Shear stress, τ , can be calculated using the formula

$$\tau = \frac{F}{A} \quad \tau = F/A \quad (9.27)$$

where ΔL is the distance of shear deformation, L is the original length, A is the cross-sectional area and F is the acting force.

The shear modulus, G , can be calculated using τ and γ using the relationship:

$$G = \frac{\tau}{\gamma} \quad (9.28)$$

See more information about measuring shear stress and strain in Chapter 6.

9.12.3 Strain gage

The strain gage is a variable resistance sensor whose electric resistance is

$$R = \frac{\rho l}{A} \quad (9.29)$$

where R = resistance in Ω , ρ = resistivity in $\Omega \cdot \text{m}$, l = length of the wire in m, and A = cross-sectional area in m^2 . An increase in length causes an increase in resistance. The sensitivity is expressed by the gage factor

$$G = \frac{\Delta R / R}{\Delta L / L} = (1 + 2\mu) + \frac{\Delta \rho / \rho}{\Delta L / L} \quad (9.30)$$

where μ is Poisson's ratio, which can be expressed as

$$\mu = -\frac{\Delta D / D}{\Delta L / L} \quad (9.31)$$

where D is the diameter of the cylindrical specimen. Poisson's ratio is the ratio between the lateral strain and axial strain. When a uniaxial tensile load stretches a structure, it increases the length and decreases the diameter.

Figure 9.13 shows four strain gage resistances that are connected to form a Wheatstone bridge. As long as the strain remains well below the elastic limit of the strain gage resistance, there is a wide range within which the increase in resistance is linearly proportional to the increase in length.

$$R_T = R_0 \exp \left[\beta \left(\frac{1}{T} - \frac{1}{T_0} \right) \right] \quad (10.1)$$

where β is the characteristic temperature of the material, R_0 is the resistance at $T_0 = 298$ K. The β parameter is temperature dependent, however, it can be considered as temperature independent for medical applications because the range is only 37 ± 5 °C.

The temperature coefficient, or sensitivity, α , can be defined by differentiating Eq. (10.1), which yields

$$\alpha = R_T \left(\frac{dR_T}{dT} \right) = -\frac{\beta}{T^2} \quad (10.2)$$

where α is temperature dependent and exhibits the nonlinearity of thermistors. At 27 °C (300 K), given $\beta = 4000$ K, α is $-4.4\%/K$.

In contrast to the homogeneous composite of dissimilar materials for the thermistor, a thermocouple is composed of dissimilar materials that are constructed as fused junctions of two materials (e.g., copper and constantan wires). Bare wires have a rapid response time. Wires contacting a metallic sheath have a slower response time. Wires enclosed in an insulator have the slowest response time. When the thermocouple is heated, a current flows from the measuring side (hot junction) to the reference side (cold junction). It develops an electric potential, which can be measured when we place a voltmeter between open ends as shown in Figure 10.3. In 1833, Seebeck first reported this phenomenon called the Seebeck effect. Figure 10.4 shows the measuring principle of a J type thermocouple. An approximate formula for the Seebeck voltage that is related to junction temperatures T_1 and T_2 is

$$V = C_1(T_1 - T_2) + C_2(T_1^2 - T_2^2) \quad (10.3)$$

where C_1 and C_2 are constants that depend on the thermocouple pair with T in kelvins. If the temperature difference $T_1 - T_2$ is small, the second term of Eq. (10.3) can be dropped. The sensitivity or thermoelectric power of the thermocouple is the derivative of Eq. (10.3) with respect to T_1 is

$$\alpha = \frac{dV}{dT_1} \quad (10.4)$$

For example, the sensitivity for the copper–constantan thermocouple is $45 \mu V/^\circ C$ at 20 °C (Cobbold, 1974). To prevent changes in $T_1 - T_2$ due to ambient temperature change, an electronic cold junction is used.

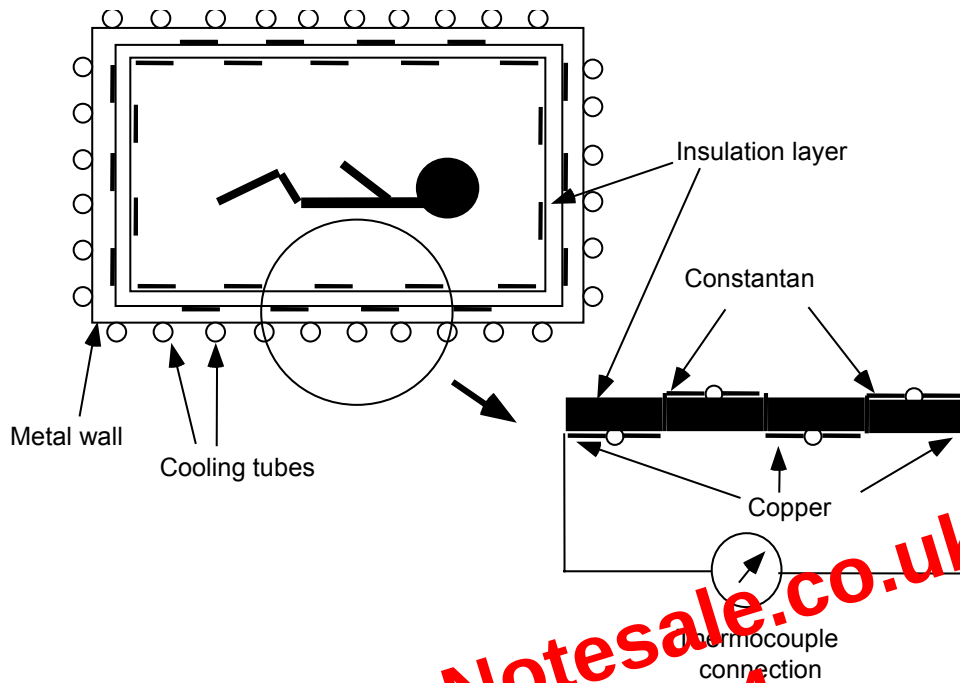


Figure 10.6 In a gradient layer calorimeter, thermocouples measure the difference in temperature across the wall. Ventilating system and measurements not shown.

Air flow calorimeter

An air-flow calorimeter is a convection calorimeter where heat loss through the surface is prevented by insulation. Heat loss from the subject is carried away from the chamber by air-flow. The rate of heat removal by the air-flow is estimated from the mass flow rate and temperature rise of the air. An important objective for the design of an air-flow calorimeter is to prevent any water condensation on the surface of the chamber. Figure 10.7 shows the principle of an air-flow calorimeter. The seated subject is confined in a chamber with polyurethane insulation applied to the outsides. The heat loss from the subject is transferred to the ventilating air, whose temperature is measured at the inlet and the outlet of the chamber. The heat loss ($\dot{Q}_a$) from the subject is the product of air mass flow rate ($\dot{m}$), specific heat (c_a), and temperature change ($T_2 - T_1$) of the ventilating air:

$$\dot{Q}_a = \dot{m} c_a (T_2 - T_1) \quad (10.12)$$

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10.3.2 Indirect calorimetry

Indirect calorimetry estimates heat production by quantitative measurements of the oxidative processes of substrates, especially the measurement of the rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$). Indirect calorimetry constitutes a noninvasive and nonintrusive technique that can be applied in basic research, clinical and field studies of energy requirements for nutritional support in patient care, and sports medicine. We discuss three commonly used methods of indirect calorimetry in this section: open-circuit systems, closed-circuit systems, and the double-labeled water method. The closed-circuit system and the open-circuit system are further grouped into the respiratory gas exchange method. Most ambulatory, laboratory, and bedside uses for the estimation of energy expenditure are based on measurements of respiratory gas exchange. Respiratory exchange methods depend mainly on the measurement of oxygen consumption ($\dot{V}O_2$), either alone or combined with measurement of carbon dioxide ($\dot{V}CO_2$) and methane production ($\dot{V}CH_4$), and sometimes urinary nitrogen excretion ($\dot{N}$). The apparatus that collects gas includes a mouthpiece with a nose clip, a ventilated hood or bed canopy, or a whole body chamber. The whole body chamber system sometimes is considered as a new category. Because the whole body chamber is all of a closed-circuit system but conducted in a confined environment, we consider it as one of the closed-circuit systems. The differences among these methods are in response time, accuracy, duration of measurement, and the degree of confinement. In general, the whole body chamber system is used for physiological research over long periods of time (24 h or longer). The double-labeled water method allows a free living environment but requires expensive isotopes for the measurement. A detailed review of techniques for the measurement of human energy expenditure and commercially available product information are given by Murgatroyd et al. (1992) and Branson et al. (1995).

Open-circuit system

The open-circuit system determines $\dot{V}O_2$ from the minute ventilation rate ($\dot{V}_E$) and the difference (ΔF) between inspired (FI) and expired (FE) gas concentrations. This calculation of $\dot{V}O_2$ includes a correction for change in barometric pressure, temperature, and relative humidity of the inspired air for adjustment of the volume to standard temperature, pressure, and dry air condition (STPD: 0 °C, 760 mmHg, dry). Energy expenditure (EE, kcal/day) is estimated by the standard (Weir, 1949) equation:

$$EE = [(\dot{V}O_2)(3.941) + (\dot{V}CO_2)(1.11)] \times 1440 \quad (10.13)$$

where $\dot{V}O_2$ and $\dot{V}CO_2$ are expressed in L/min, and 1440 = the number of minutes in a day. It assumes that 12.3% of the total calories arise from protein metabolism. When urinary nitrogen excretion ($\dot{N}$) is measured, a third term ($-\dot{N}(2.17)$) in g/min is added to the bracket of Eq. (10.13) to account for protein correction of energy expenditure.

Open-circuit calorimeters use either the mixing chamber or the dilution technique to measure inspired and expired concentrations of oxygen (FIO_2 , FEO_2) and

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volume ($\dot{V}_T$) and whose $\dot{V}O_2$ is 250 mL/min. The respiratory quotient (RQ, given by the ratio $\dot{V}CO_2 / \dot{V}O_2$) of this patient is 0.7. This means that 25 mL per breath of O_2 produces 17.5 mL per breath of CO_2 . In this case, the difference between $\dot{V}_I$ and $\dot{V}_E$ is 75 mL. If $\dot{V}O_2$ is measured at an O_2 concentration of 60% ($FIO_2 = 0.6$) assuming that $\dot{V}_I$ is equal to $\dot{V}_E$, an error of 18% would be obtained for $\dot{V}O_2$ measurement (205 mL/min). When RQ reaches 1.0, this error becomes small. Measurement of $\dot{V}CO_2$ is much simpler. When inspired air is room air, it contains only very small amount of CO_2 ($< 0.03\%$). $\dot{V}CO_2$ may be calculated accurately from a simpler form

$$\dot{V}CO_2 = \dot{V}_E (FECO_2) \quad (10.19)$$

This simpler form is also applied to measurement of $\dot{V}CO_2$ for patients with mechanical ventilation where $FICO_2$ is 0.0.

Most commercially available open-circuit systems use mixing chambers as in Figure 10.10. Expired air from the patient passes through a facemask or mouthpiece and is directed to the mixing chamber. At the end of the mixing chamber, a small sample is drawn by a vacuum pump through O_2 and CO_2 sensors that measure FEO_2 and $FECO_2$. A pressure transducer and thermistor are necessary for pressure- and temperature-compensated gas measurements. A volume transducer is used to measure minute ventilation ($\dot{V}_E$). A microprocessor controls the instrument and provides minute-by-minute data on O_2 consumption and CO_2 production, $\dot{V}O_2$ and energy expenditure of the subject.

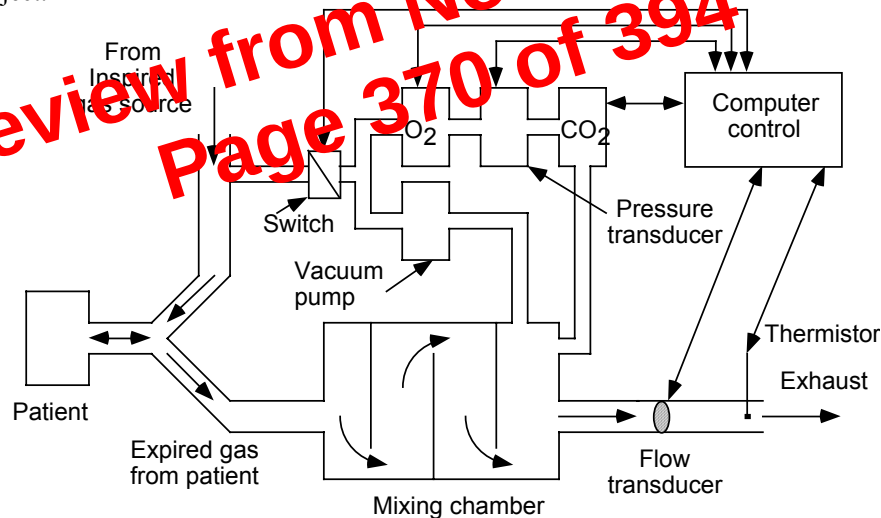


Figure 10.10 A microcomputer-based open-circuit system includes a mixing chamber, O_2 and CO_2 analyzers, and the various variables (pressure, flow, temperature) used to calculate $\dot{V}O_2$.

When measuring respiratory exchange gas, there are two broadly used methods: the air may be directly expired through a mask or mouthpiece as mentioned above or it may be drawn from a hood or canopy enclosing the subject's head. The latter is sometimes called a flow-by system. It needs a pump to draw constant air over a subject's

stable isotopes: deuterium (^2H) and oxygen 18 (^{18}O). Since the early 1980s (Schoeller and van Santen, 1982), the use of doubly labeled water has provided a technique where the total energy expenditure can be measured in a free-living individual over 1 to 2 weeks. It also has considerable appeal for field studies to estimate the energy cost of activities and thermogenesis by subtracting the basal metabolic rate from the total energy expenditure. The principle of the method is based on six assumptions listed as follows:

1. The body is in steady state; i.e. the volume of total body water pool remains constant over time.
2. The turnover rates of water and carbon dioxide are constants over time.
3. The volume of labeled water distribution is only equal to total body water.
4. The isotopes are lost only as water and carbon dioxide.
5. Losses of labeled water and carbon dioxide in urine and exhaled carbon dioxide have the same level of enrichment as in body water.
6. The background levels of the isotopes remain constant.

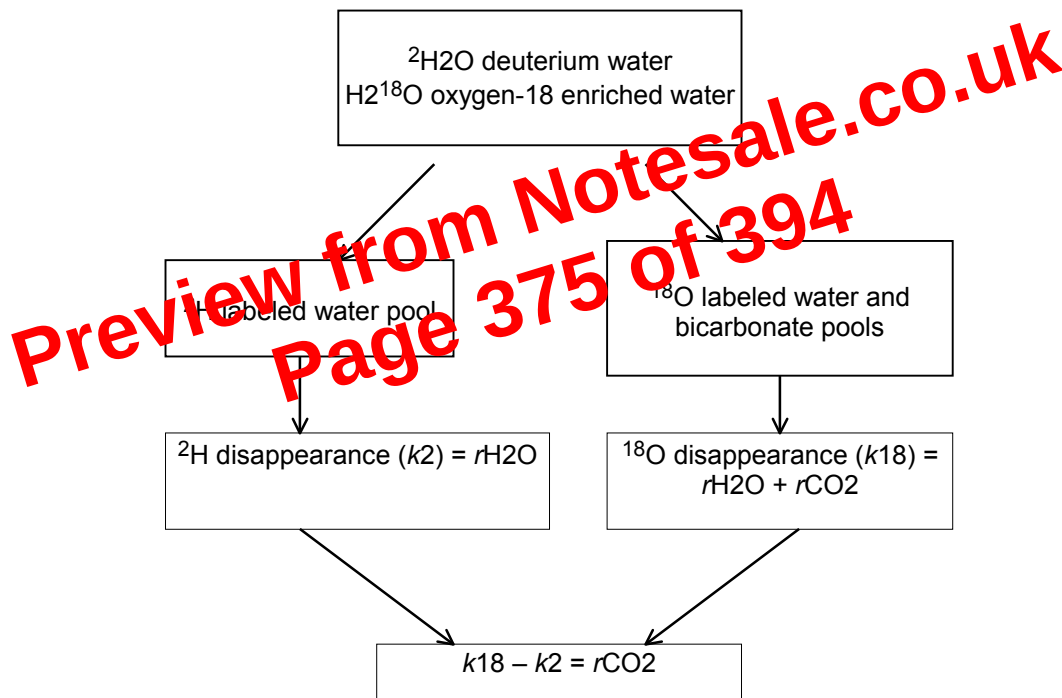


Figure 10.13 Principle of the doubly labeled water method. r is the production rate, k represents rate constants determined from the experiment.

Figure 10.13 shows the principle of the method. After a subject drinks a dose of DLW, the deuterium mixes with the body water pool and the ^{18}O mixes with both water

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and bicarbonate pools. The production rate of CO₂ is calculated as the difference between ¹⁸O and ²H disappearance rates. The formula for this calculation is:

$$r\text{CO}_2 = \dot{V}\text{CO}_2 = \left(\frac{W}{2}\right) \times (k_{18} - k_2) \times 22.4 \quad (10.23)$$

where r (in L/h) is the production rate, W is the size of total body water in mol and k_{18} and k_2 are fractional disappearance rates for ¹⁸O and ²H, respectively. They are computed from measurements of the isotopic concentrations c_{18} and c_2 using $k = (1/c) \times (\Delta c / \Delta t)$. The factor 2 in the denominator of Eq. (10.23) is the molar proportion of oxygen in carbon dioxide in relation to water. In practice, Lifson et al. (1955) modified Eq. (10.23) by including the fractional factor: f_1 (0.93) for ²H₂O gas/²H₂O liquid, f_2 (0.99) for H₂ gas/H₂¹⁸O liquid, and f_3 (1.04) for C¹⁸O₂ gas/ H₂¹⁸O liquid to take a natural fraction of the isotopes between different body fluids into account. Eq. (10.23) becomes

$$r\text{CO}_2 = \dot{V}\text{CO}_2 = \left[\frac{W}{2f_3} (k_{18} - k_2) - \left(\frac{f_2 - f_1}{2f_3} \right) \frac{\dot{m}}{18} \right] \times 22.4$$

or

$$r\text{CO}_2 = \dot{V}\text{CO}_2 = \left[\frac{W}{2.08} (k_{18} - k_2) - \frac{\dot{m}}{624} \right] \times 22.4 \quad (10.24)$$

where $\dot{m}$ is the rate of water loss as vapor in g/h. A minimum of two urine samples (one a few hours after the dose and another at the very end of the experiment) are collected to compute the rate constants (k_{18} and k_2) and the total body water (W). A number of cross-validation studies have been performed by comparison with gas exchange analysis and the whole body chamber under resting condition as well as during sustained heavy exercise (see reviews in Ritz and Coward, 1995; Mclean and Tobin, 1987; Prentice et al., 1991; Ravussin and Rising, 1992). Results showed that the doubly labeled water method is a suitable technique for assessing energy expenditure in free-living circumstances with an accuracy of $\pm 5\%$. However, large underestimates were found in obese subjects (Ravussin and Rising, 1992). Advantages of the doubly labeled water method are that it is noninvasive (oral dosing), nonintrusive (urine sampling), and suitable for noncompliant subjects and field studies. In addition, deuterium distribution space can be used to estimate total body water for an estimate of body composition (see section 10.4.1). Nevertheless, the high cost for the expense of ¹⁸O-enriched water and technical complexity are disadvantages of the doubly labeled water method. The amount of dosage and its resulting cost depend on how precise the mass spectrometer is. In-depth knowledge of the assumptions of the doubly labeled water technique is required to apply it to various physiological and pathological situations.

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skills (such as skating) and providing efficient methods of using the body in daily life skills (e.g., walking and running). Analysis of body movement requires observation and measurement. Many methods are available, ranging from simple visual observation to complicated computerized systems. Typical research or clinical gait analysis laboratory setups include goniometers, accelerometers, electromyography (EMG, see Chapter 7), force plates, and kinematic (motion) analysis systems. A kinematic analysis system varies from simple and subjective visual observation to more expensive and complicated video or optoelectronic systems that can provide objective, quantitative data of three-dimensional motion of selected points on the body. With additional force plate outputs, the kinematic analysis system can be used as a kinetic analysis system to provide force and moment information for human limb movement. In this section, we discuss working principles and applications for goniometers, accelerometers, and video and optoelectronic kinematic analysis systems. For additional information, refer to texts by Allard et al. (1995), Whittle (1996), Winter (1990), and Medved (2001).

10.5.1 Goniometers and accelerometers

Goniometers

A goniometer is a device that measures relative angles of a joint that connects two body segments. The goniometer (see Figure 10.16(a)) consists of two attachment arms, which are fixed to two limb segments of the subject on either side of joint to measure the joint angle without interfering with natural movement. Traditionally, a goniometer uses a resistance potentiometer to convert changes in rotation to an electric output proportional to the positions of two attachment arms. The potentiometer is aligned with the joint axis to establish the zero position and is calibrated to measure the joint angle in degrees. Multiaxial measurements of the joint are possible with two or three potentiometers mounted orthogonally in different planes. The output of the goniometer provides continuous analog data of joint motion at relatively low cost. However, to ensure accurate measurements, fixation to the body with cuffs around the soft tissues must be performed carefully to prevent off-axis problems (Chao, 1980). The measurement error caused by the translation of the joint center during movement can be improved by replacing the rigid arms of the traditional goniometer with a flexible parallelogram linkage designed to accommodate the motion change outside the measurement plane (Thomas and Long, 1964). A triaxial parallelogram electrogoniometer (see Figure 10.16(b)) has been used to record motions in more than one plane for the lower limb (Isacson et al., 1986). Strain gages have also been used as an alternative to the potentiometer. They deform when they are stretched or compressed and the electric output is proportional to the change in joint rotation. The working principle of strain gage and its related circuitry is discussed in section 8.3.1.

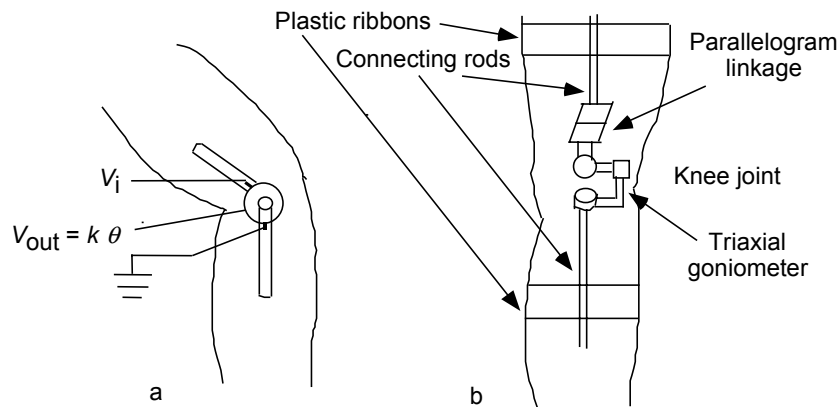


Figure 10.16 (a) A goniometer attached to the shank and thigh to measure knee rotation. V_i is the input voltage. V_{out} is the output voltage that is proportional to the angle of knee rotation. (b) Subject wearing a triaxial goniometer on knee joint.

Accelerometers

Accelerometers are instruments that measure acceleration. Basically, they all contain a small mass and make use of Newton's law ($F = ma$) to measure the force that is required to accelerate the known mass, as shown in Figure 10.17. There are two classes of accelerometers used in the analysis of body movement: strain gage (usually piezoresistive) and piezoelectric accelerometers. A strain gage accelerometer consists of strain gages (wires) bonded to a cantilevered mass and a base to which the cantilever beam is attached. When the base is accelerated, the cantilever beam deforms due to the inertia of the mass, thus changing the strain in the wires. The strain in the wires changes their resistances. We measure the change of resistance with a Wheatstone bridge circuit (see section 9.11.2), which requires a differential amplifier. The resulting electric output is proportional to the acceleration of the mass. A piezoresistive accelerometer works under the same principle as a strain gage accelerometer. Instead of using strain sensitive wire, it uses piezoresistive strain elements as sensors. Piezoelectric accelerometers measure the force directly. The piezoelectric sensor converts the force produced by the acceleration to an electric charge or voltage (see Eq. (8.7) and section 8.5.2 for the working principle and its charge amplifier). For the analysis of body movement, the accelerometer is attached to a body segment at a specific point to measure acceleration in one direction. Two- or three-dimensional measurements are possible with several accelerometers grouped together orthogonally. The use of accelerometers for the analysis of body movement provides an alternative way to measure the velocity and displacement of the limb segment when the initial values of the limb's velocity and displacement are known (Morris, 1973). Some practical limitations prevent widespread use of the accelerometer in body motion analysis: necessity to exclude the effect of the field of gravity from true kinematic acceleration, difficulty in extracting the rotational acceleration, and low frequency noise caused by the baseline drift in the measurement output.

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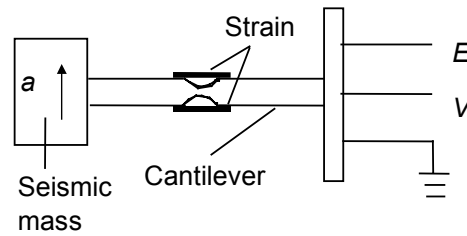


Figure 10.17 Vertical acceleration of the accelerometer frame bends the cantilever beam because the seismic mass remains at rest. Voltage output (V) is proportional to the acceleration (a). E is the supply voltage.

10.5.2 Video and optoelectronic systems

Gait can be measured on a single film using sequential strobe lights and a camera. Gait can be measured by video recorder. Figure 10.18 shows that a video or optoelectronic kinematic analysis system consists of active or passive markers placed at selected surface locations on bony landmarks, sensors that track markers' positions, and a computer system to control the timing for active marker display and signal processing of markers' tracings. The sensors, either optoelectronic circuitry or a video system, collect kinematic data from active (infrared LED) or passive markers and low-pass filter the data before an analog-to-digital converter (A/D), which feeds into a computer for later computation of the position and velocity of the rigid body in three-dimensional (3-D) space. A computerized analysis system often yields graphic displays that can provide information on joint positions, joint angles, segment angles, velocity, and acceleration as a function of time.

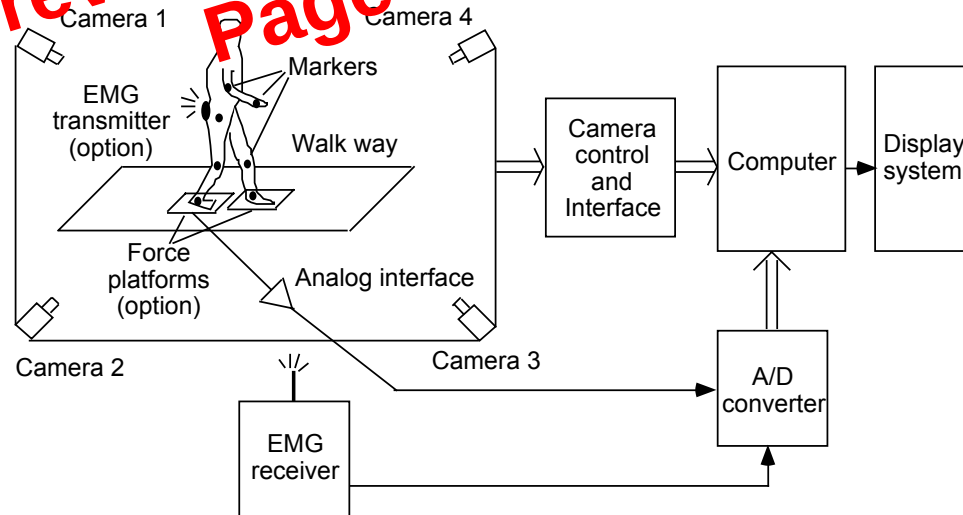


Figure 10.18 An example of a gait analysis setup includes a four-camera kinematic system, two force platforms, and an electromyogram (EMG) telemetry system.