EXPERIMENT 4: NORMAL AND ABNORMAL HUMAN KARYOTYPES

DAY ONE: INTRODUCTION TO THE LIGHT MICROSCOPE

OBJECTIVES:

Today you will practice adjusting your microscope for optimal resolution for bright field and phase viewing. It is important that you learn to adjust your microscope correctly before next time.

- ☐ **Know the parts of the microscope and be able to properly adjust them for optimal resolution.**
- ☐ **Know how to find a specimen on a slide and to get it in focus.**
- ☐ **Know when and how to use phase optics.**
- ☐ **Know how to care for your microscope and slides properly.**

INTRODUCTION:

Cytogenetics is a branch of biology that combines the methods and subject matter of genetics and cytology (the study of cell structures). It has been important since the rediscovery of Mendel's findings in 1900, because the structure and behavior of chromosomes provide the physical bases for Mendel's rules, as well as later discoveries on the organization and expression of genes.

The first reported microscopic observations of organisms were made by Robert Hooke (1665) in England and Anton von Leeuwenhoek in Holland. Both published their observations in the Proceedings of the Royal Society. The instruments that they used were either simple (single lens) or compound (multiple lens) microscopes. Many other observations were published at that time by investigators in England, Holland, and Italy. Although von Leeuwenhoek's lenses were remarkably fine, the quality of the lenses that most investigators had was relatively low. It was not until about 1820 that better quality lenses at lower prices became available. The result was that biologists could observe not only cells but also substructures within cells, which were not visible before.

The ability to discern cellular substructure led to the definition of various organelles (such as the nucleus, chloroplasts, and mitochondria), to the formulation of the Cell Theory, and to the discovery of the origin of cells. One must realize that this was a long, drawn out development of ideas, based on the contributions of many observers. Mitosis was not well described until the 1880s and meiosis even later. The higher plant life cycle was not completely understood until about 1892. In essence, it required about 80 years to understand the basic unit of life, the cell, on the microscopic level. Many hypotheses about cell division, what happened to the contents of the nucleus in mitosis and meiosis, and how fertilization occurred were developed in long papers, often exceeding 100 pages. These were vigorously attacked or supported by other papers of equal length.

This whole period was influenced by several other developments. One was a long period of relative peace in Europe (from about 1815-1914). Another was the Industrial Revolution and the formation of a stable middle class. These conditions led to a rapid expansion of the sciences at the universities, not only in biology, but even earlier, in physics and chemistry. By about 1870, the light microscope had reached a resolving power equal to any instrument on the market now. Synthetic dyes were introduced by chemical synthesis also at this time. This allowed cytologists to fix and stain tissues and cells, thereby arresting the living processes and making them visible in the microscope.

Johann Gregor Mendel started his breeding experiments in about 1855. He announced the results of his work in 1865, and they were published in 1866. Considering the stage of understanding of meiosis and fertilization at that time, you can see why the biologists could not comprehend the significance of Mendel's results. In 1900 when Correns, Tschermak, and DeVries each independently rediscovered Mendel's work, it made sense to the biologists in the context of what was then known about cellular structures and activities.

In 1902-1903, Sutton and Boveri independently published papers that pointed out the correlation of the behavior of chromosomes during meiosis and the behavior of what Mendel called "factors," or what we now call genes. This was the **Chromosome Theory of Inheritance**. It basically pointed out that Mendel's "factors" behaved like chromosomes during meiosis and are therefore likely to be located on chromosomes. This, together with other discoveries (like the X and Y sex chromosomes) led to a widespread study of chromosomes, their abnormalities, and how these were related to genetic phenomena.

Cytogenetics was used in two main ways to study and confirm biological phenomena. One way involved chromosomes that could be distinguished by microscopic examination within a species. With these, contributions of chromosomes and alleles from each parent could be correlated. Bridges and Sturtevant, both students of T.H. Morgan, were especially active in this area; they led the way to a long series of discoveries using *Drosophila* and *Zea mays* (corn) in the early 1900s. In 1933, Painter developed the characterization of giant polytene chromosomes in *Drosophila*; each chromosome had a unique and constant pattern of crossbands when stained. The observation of rearranged patterns provided independent, cytological confirmation of chromosomal inversions, translocations, and deletions, which had been identified by ratios of offspring produced by crosses. These essentially demonstrated that parts of chromosomes could be correlated with genes. Similar work was also done by McClintock in *Zea mays*.

Another important area in which cytogenetics was used was taxonomic studies, based on the similarities and differences among chromosomes of variously related organisms. The underlying assumption was that more closely related organisms should share more of a common genetic background, and therefore have more similarities in chromosome make-up (number, size, shape, bands). This area developed rapidly and many volumes have been published describing the chromosomes of plants and animals. Again, there is a remarkable correlation between species relationships determined by traditional morphological studies, by chromosomal similarities, and by DNA sequence analysis.

Some of the specific subjects studied in cytogenetics include:

1. Morphology and behavior of normal chromosomes during mitosis and meiosis
2. Abnormal behavior of chromosomes in mitosis and meiosis
3. Abnormal chromosome morphology and its correlation with abnormal phenotypes
4. Changes in chromosome number and morphology in relation to evolution
5. Chromosome structure and molecular constitution in relation to gene expression

In the 1940s and 1950s, cytogenetics was somewhat overshadowed by the emergence of microbial and molecular genetics. The development of animal tissue culture techniques changed the approach to cytogenetics in the late 1950s by making genetic material from large organisms more readily available. With the introduction of new staining techniques, detailed areas of mammalian chromosomes could be studied. This, combined with methods of localizing genes by the use of probes for particular informational sequences in the DNA, now allows for the study of chromosomes in greater detail than ever. These techniques have been particularly valuable in studying the taxonomic relationships of organisms and the role of genes in various diseases. The latter area has shown a dramatic increase in activities during the last 20 years, as finer and finer alterations of chromosomes have become detectable. Knowing what chromosomal regions carry what information allows identification of the DNA base sequences carrying the information and the potential for diagnosing and treating the condition. Several journals now specialize in this area and many pharmaceutical companies have developed standard testing kits to identify particular defective genes.

THINGS TO DO:

From the cabinet, find the microscope labeled with the same number as your laboratory station. You will be responsible for the proper use and care of that microscope. Carry it with two hands, one on the arm and one under the base. Plug the cord into the outlet and turn the light source on when you are ready to use it. Turn the light off to save the filament when the scope is not in use. Keep the excess cord tucked out of the way, and always keep the stage clean and dry. With your instructor and this manual, learn the parts (Figs. 2-1 and 2-2) and the operation of the microscope. Your success in viewing specimens will depend on your mastery of proper illumination-- getting the light focused on the specimen and cutting down stray light.

Eyepieces or oculars hold the lenses nearest your eyes. They slip in and out of the tube and are engraved with numbers indicating their magnifying power.

The **tubes** are the inclined cylinders that hold the eyepieces. They allow you to adjust the interpupillary (between the eyes) distance between the eyepieces, so that you get an image from both eyes together.

The **nosepiece** holds the **objective lenses**. When it is rotated a different objective is moved into the light path. You should hear a click when an objective has been properly rotated into place.

Objective lenses are those nearest the specimen. They are engraved with several numbers, including their magnification power. You should find lenses of 3.4x, 10x, 40x, and 100x (oil immersion). A lens with the marking "40/0.65/160/0.17" is a 40 power lens with a numerical aperture of 0.65, a tube length of 160mm, and a preferred coverglass thickness of 0.17 mm.

The **specimen stage** is the platform on which the slide is placed. It has a **slide holder** that allows positioning of the slide by adjustment of **2 stage knobs**. The stage knobs are found under the stage and move **indicators on the stage next to 2 numbered rulers**. Thus, when you find an interesting specimen, you can record the position of the indicators when it is in view and you will easily be able to come back to that spot on that slide.

The **condenser** is just below the stage and should always be positioned almost all the way up (Fig 5). The **knob for moving the condenser** up and down is on the side of the microscope, below the stage. The condenser focuses light from the light source through the specimen. There are **two knobs, toward the back of the condenser for centering** it. There is also a lever for swinging in and out a **condenser lens**, which sits between the condenser and stage. The condenser lens is used with all but the 2.5x objective, since it would narrow the cone of light too much to illuminate the full 2.5x field. In addition, you will find a **condenser iris diaphragm**, for reducing stray light on the edges of the field. The condenser iris diaphragm can only be seen if you remove an ocular and look down the tube when the condenser is set for **bright field ("J")**, rather than phase optics. There are wheels and knobs toward the front of the condenser for centering the condenser iris diaphragm and phase rings and for adjusting the size of the condenser iris diaphragm. As you look at the condenser from one side, you will see a white, vertical indicator line, with a letter or number above it. You can rotate a plate in the condenser so that the symbol above the white line changes. The **symbol above the white line** shows what optical system is in place: "J" indicates bright field optics (the usual optics for stained specimens), while "1," "2," or "3" indicates which phase ring is in place.

The **focusing knobs** are found on the side, where the arm joins the base. The larger knob is the coarse focus, while the smaller knob is the fine focus. Each changes the distance between the stage and the objective. Turning the coarse focus knob moves the objective in greater increments. So, the coarse adjustment is used for the initial focusing, usually with a low powered objective. Once the specimen is in view, the fine focus knob is used. On 40x and 100x, only the fine focus should be used.

The **base** is the bottom of the microscope. It holds the **light source**. Above the light source is a **lamp field iris diaphragm or field stop**; it helps to reduce stray light on the edges of the field. Near one side of the base is the **rheostat knob**. This can be turned to increase or decrease the brightness of the light illuminating the specimen. When your optical system is all adjusted, you should only use the rheostat to control the brightness of illumination.

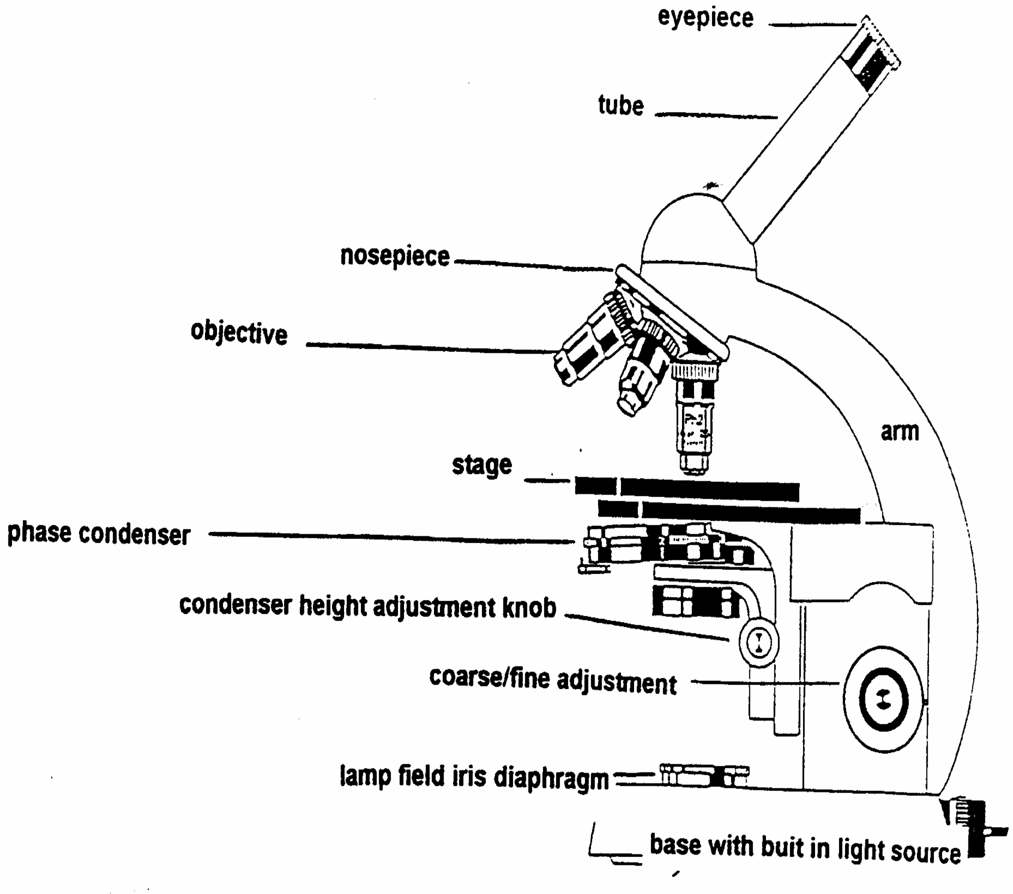


Figure 2-1. Parts of the compound, phase contrast microscope.

Now practice the proper operation of the microscope. The following adjustments are made to maximize resolution, which is the ability to recognize two separate spots as being distinct:

1. Plug in your microscope and turn on the light source. Slowly turn the rheostat up until the light is moderately bright.
2. Obtain or prepare a slide, making sure to wipe the slide and coverglass free of smudges, using a tissue. Place the slide on the specimen stage, in the slide holder. Often you can spot the specimen by holding the slide up to the light and viewing with your naked eye, then make sure you have that area of the slide centered over the light source.
3. Turn the nosepiece so that the 10x objective is in the light path. While you watch from the side, move the objective very close to (but not touching) the slide.
4. Fully crank up the condenser with the condenser lens swung into place. Set the condenser on "J" for bright field viewing.
5. Adjust the interpupillary distance so that you can comfortably view through both eyepieces at the same time. Focus on the slide, using the coarse focus and then the fine focus, taking care to move the objective away from the slide. This initial focusing must be done so that the following optical adjustments are made with the objective near the correct distance from the stage.
6. While viewing the specimen, close the lamp field diaphragm in the base of the microscope stand. The image of the closed diaphragm (a small, bright, multisided spot) should be sharp. If it is not, lower the condenser slightly (1-2 mm) until it is in optimum focus.
7. The image of the field stop should also be in the center of your field of view. If it is not, use the two centering screws on the back of the condenser to move the spot into the center.
8. Slowly open the field stop so that the field of view is fully and evenly illuminated. Do not open it any further. As you open the diaphragm, you should see the dark border recede uniformly all around the field of view. If you do not, you can adjust the centering screws some more.
9. Remove one eyepiece, look down the tube, and view the condenser diaphragm. Make sure it is centered (using the 2 centering wheels at the front of the condenser) and then close the condenser diaphragm (using a third wheel at the front of the condenser) until 3/4 of the field of view is filled with light. The circle of light should be near the outer edge of the dark ring.
10. Put the eyepiece back into the tube, and adjust the intensity of your light with the rheostat, to a level that is comfortable to your eyes. The microscope is now ready to operate under bright field illumination.

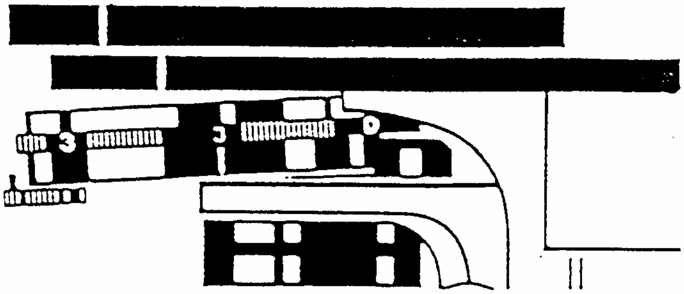


Figure 2-2. Parts of the condenser apparatus.

When you have a specimen of interest on the slide, your first task will be to find the specimen. It is usually easiest to locate it with the 10x objective, which gives you a large depth of focus and field of view. Once you have located your specimen and are sure your optical system is adjusted to give you the best resolution, you can view more detail at a higher magnification. The higher power lenses will magnify just the center of the lower power field of view, so make sure that your specimen is centered in the low power field. Then turn the nosepiece so that the next objective clicks into place. Because the objectives are **parfocal,** when you have focused with one objective, only minor adjustments should be needed to be in focus with other objectives. You may have to turn up the illumination with the rheostat, but you should only have to use the fine focus to get a sharp image. Notice that since the depth of focus is smaller with a higher magnification, a small adjustment can make a big difference.

You will, however, have to adjust the diameter of the lamp field iris diaphragm each time you change objectives. Simply close down the diaphragm, make sure you have a focused hexagon that is centered within the eyepiece field, and then reopen the diaphragm until the light just barely fills the eyepiece filed. Also, when you use the 100x objective, you will have to use a drop of oil between the slide and the 100x objective. After centering and focusing your specimen with the 40x objective, swing it out of the way, add a small drop of oil over the specimen, and then carefully swing the 100x objective into place. The oil has a refractive index closer to that of glass than does air. So, there is less loss of illumination due to light bending when the light passes from the slide to the oil and then to the objective. When you are done with the oil immersion viewing, you need to wipe off the oil from the slide and the lens. **Use a kimwipe tissue on the slide but USE LENS PAPER TO CLEAN THE LENS**. Add a small amount of xylene to the wipe or paper, which will help dissolve the oil. The solvent xylene is harmful for you to breathe and get on your skin. So minimize its use.

Phase Contrast Microscopy

The microscopes that you are using are phase contrast microscopes. They have optics that allow you to see things that cannot be visualized in some bright field situations. When light passes through a specimen, the light waves are retarded to different degrees, depending on the density of the parts of the specimen. If there are only slight differences in retardation, the human eye is unable to detect them. However, when there is a phase ring in the condenser and a matched phase ring in the objective, the differences in retardation are exaggerated and we can discriminate them as contrast differences. This is especially advantageous for viewing living, unstained material or lightly stained specimens. Proper use of good phase optics can make a great difference.

To use phase contrast, do the following: First look at your objectives. Three are labeled with "Ph," which indicates that they contain phase rings. Following "Ph" is a number (1, 2, or 3). For our microscopes, the 10x is Ph-1, the 40x is Ph-2, and the 100x is Ph-3. The number indicates the phase ring in the condenser that should be used with the particular objective. If you examine the condenser, you will see that it is like a large wheel or plate on top. You also will see the numbers 1, 2, and 3 as well as the letter "J" engraved at various intervals on the side of the wheel. The numbers or letter will line up with a small line when you rotate the wheel; each position is a click-stop for a phase ring or no phase ring ("J"). Thus, for bright field illumination with any objective, the condenser should be at the "J" position. For phase contrast optics with the 10x objective, the condenser should be in the "1" position; with the 40x objective, in the "2" position; and with the 100x objective, in the "3" position.

The phase ring in the objective and the corresponding ring in the condenser have to be precisely aligned. Otherwise you do not get a good phase image. The frequency of adjustment required depends on the microscope and how it is handled. Since other laboratory sections will be using the same microscopes, it is a good idea to adjust your scope whenever you use it. There are 3 knobs or wheels on the condenser (Fig. 2-2). One is to adjust the size of the condenser diaphragm for bright field optics. The other 2 are used to align or center one phase ring image over the second one. One of the knobs has a locking nut at the end, which has to be loosened before moving and then tightened back. The other is a small, wheel-like knob that rotates. One moves the condenser along the X-axis, the other along the Y-axis.

To use the alignment knobs, focus on a transparent specimen. Remove one eyepiece and look into the tube. You should see both rings, one bright and one darker. If they are aligned, they are superimposed on each other (Fig. 2-3). If not, you will see two partially overlapping rings. Loosen the locking nut and move both knobs until the rings are superimposed. Then tighten the nut again and replace the eyepiece. You can do this with your bare eye at 10x and, if you are experienced, at 40x. If you are not experienced and if you want to check the 100x objective, you will need a focusing telescope to look down the tube. If a telescope is not available, you can often align the rings by viewing the specimen with the eyepieces in place and adjusting the aligning knobs until you get the most contrast.

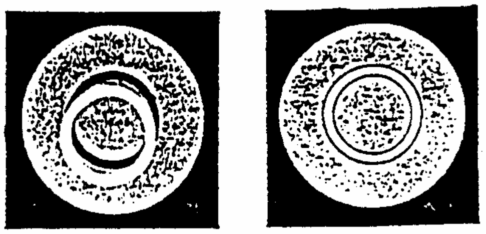


Figure 2-3. View of phase rings through the tube after the eyepiece has been removed. Left shows rings not aligned; right shows aligned (superimposed) rings.

When you work with the microscopes, follow some basic precautions:

- ✓ Always keep the lenses clean.
- ✓ Do not get water, stains, fingerprints, etc. on them; do not get oil on any but the 100x objective.
- ✓ To clean lenses, use lens paper, which does not contain abrasives, like silicon.
- ✓ Use clean slides and coverglasses; they should be wiped with regular tissues.
- ✓ Remember that the working distance (between the front lens of the objective and the specimen when it is in focus) decreases with increasing magnification. Thus, the higher magnification objectives have a very short working distance. If a specimen is too thick, then it may hit the lens if you try to focus on its lower side.

DAY TWO: HUMAN KARYOTYPING

OBJECTIVES:

Today you will view prepared slides of chromosomes from normal human peripheral white blood cells and normal human karyotypes from such cells. After today's lab you should be familiar with

- ☐ **The basic procedure for preparing a human karyotype and the difficulties that may be encountered in preparing one.**

- ☐ **The function of mitogens, microtubule inhibitors, and hypotonic solutions in karyotype preparation.**
- ☐ **The appearance of a normal human karyotype.**
- ☐ **The difference between a normal male and female karyotype.**
- ☐ **Different methods of creating banding patterns in human chromosomes.**

INTRODUCTION:

Good techniques for studying mammalian chromosomes were not developed until the mid-1950s. Then by about 1955, methods to culture mammalian cells were partially standardized. This allowed investigators to use cultured cells for experiments and to get consistent results. Chromosomes must be visible in the light microscope and well spread in order to be studied. This means that dividing cells must be used. Further, because animal chromosomes are often very small, they must be studied with high quality microscopes, using oil immersion objectives.

J.H. Tjio and A. Levar (1956) established that the chromosome number for humans is 2N = 46. Various other investigators confirmed this, after much confusion resulting from the difficulty in counting overlapping chromosomes. In 1959 it was discovered that Down's Syndrome was due to an extra chromosome 21. Since that time human cytogenetics has been developing rapidly and has become very important in medical genetics. Various types of cells can be used to get suitable chromosomes for study. White blood cells are most commonly used. They are separated from red cells by centrifugation, placed in a tissue culture medium, and induced to divide by adding a mitogenic (mitosis inducing) agent, such as **phytohemagglutinin**. The cells are incubated until they divide, which is usually in about 72 hours. At that time **colchicine** is added to the culture. This alkaloid binds to the microtubule-forming proteins, preventing the formation of a spindle apparatus. Thus, cells are arrested at metaphase. The cells are next ruptured and spread out so that the chromosomes do not overlap. This is accomplished by placing them in a **hypotonic solution** to gently burst them open. The cells are then fixed, stained by various techniques, and made into permanent mounts. Then they are ready for microscopic examination. Suitable metaphases are photographed and a karyotype (or arranged set of chromosomes) is prepared from the prints. In most modern cytogenetics laboratories, "a television and a computer are linked to the microscope. As metaphase chromosomes are located using the microscope, an image is recorded by the camera, digitized, and transmitted to the computer, where it is processed into a karyotype and printed" (Cummings, M., Human Heredity, 5th edition, Brooks/Cole, Pacific Grove, 2000). Other types of tissues can be used to obtain cells for karyotyping. Fetal cells from amniotic fluid work well because they also respond to mitogenic agents. Amniocentesis is especially important for women over 40, who have a greatly increased chance of giving birth to babies with abnormal karyotypes.

In a **karyotype**, chromosomes are placed in homologous pairs and arranged in order of descending size. At a meeting in Denver in 1960, it was agreed that the standard human karyotype would contain 7 groups (A-G) based on chromosome size and shape. The shape of a chromosome is defined primarily by the position of its centromere: **acrocentric** chromosomes have the centromere near one end of the chromatids, **metacentrics** have their centromere about halfway down the chromatids, and **submetacentrics** have their centromeres about a third of the way down the chromatids. The centromere position can be defined more exactly by referring to the **centromere index** of the chromosome. The centromere divides the sister chromatids into a short arm (called the p arm) and a long arm (called the q arm), and the centromere index is just the length of the p arm divided by the total length times 100 or $C.I. = [p/(p+q)] \times 100$. Thus the centromere index for a metacentric is about

45-50; for a submetacentric, about 15-44; and for an acrocentric, around 14 and below. In addition, acrocentric chromosomes of humans may have, visible on the ends of their p arms, a narrow stalk (called a secondary constriction) and a blob of chromatin (called a **satellite**).

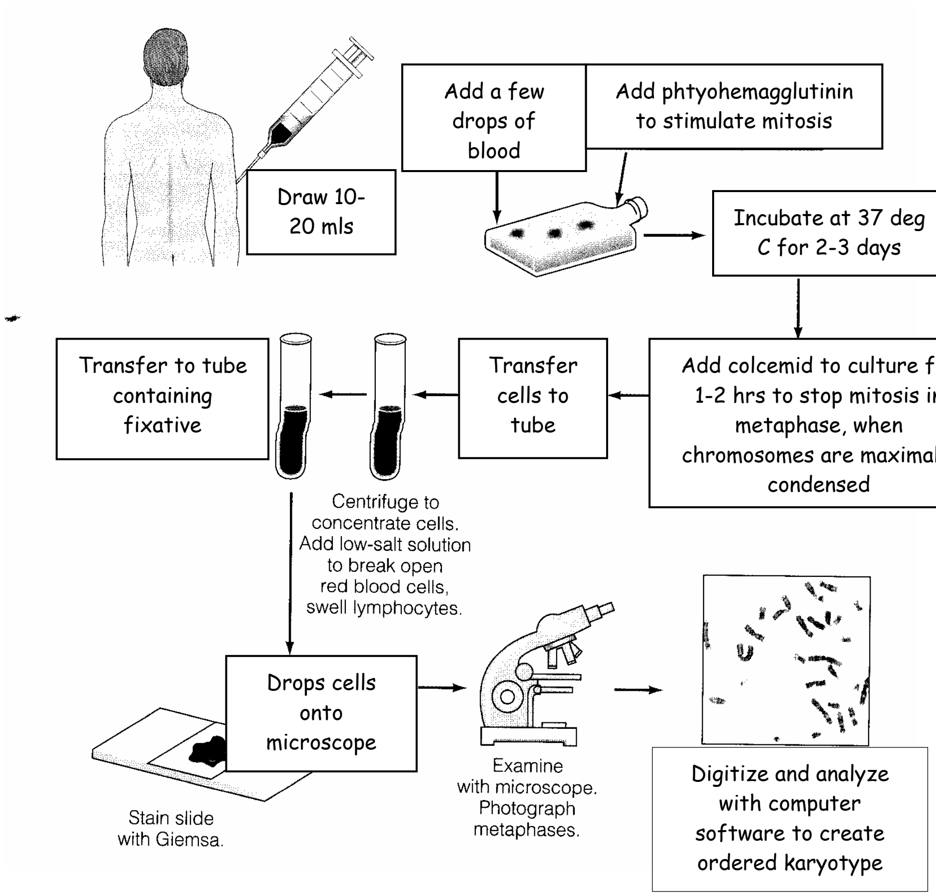


Figure 2-5. Procedure for performing a karyotype of an adult. From Genetics in Medicine, 6th Ed. by Nussbaum, McInnes, and Willard. Thompson & Thompson, Philadelphia, 2001.

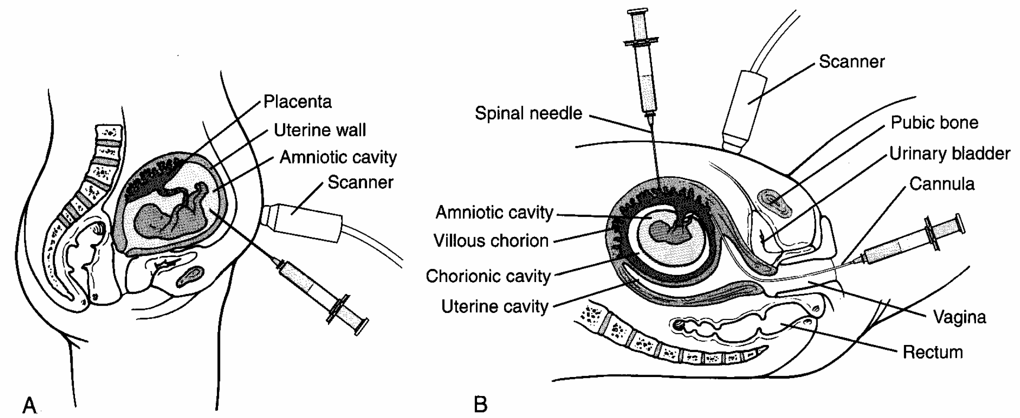


Figure 2-6. Two procedures for performing karyotyping of a fetus. (A) *Amniocentesis.* A needle is inserted transabdominally into the amniotic cavity and a sample of amniotic fluid (~20 mls) is withdrawn. Typically performed at 15-16 weeks of gestation. (B) *Chorionic Villus Sampling.* Can be performed either transcervically or transabdominally. Is associated with a higher rate of miscarriage than amniocentesis but has the advantage that it can be performed earlier (at 10-12 weeks of gestation, when a termination of the pregnancy can still be performed on an outpatient basis). From Genetics in Medicine, 6th Ed. by Nussbaum, McInnes, and Willard. Thompson & Thompson, Philadelphia, 2001.

The compositions of the karyotype groups are as follows:

Group	Chromosome #'s	Size and Shape
A	1 to 3	largest, metacentric and submetacentric
B	4 and 5	large, submetacentric
C	6 to 12	medium, submetacentric
D	13 to 15	medium, acrocentric
E	16 to 18	small, submetacentric
F	19 to 20	small, metacentric
G	21 to 22	smallest, acrocentric

The X sex chromosome resembles the C group, while the Y sex chromosome resembles the G group.

While the standard karyotype makes it easy to detect whether there are abnormalities in chromosome number, only very gross structural mutations are evident. Up to 1970 standard stains were used for karyotypes. Examples are Feulgen, aceto-orcein, and aceto-carmine stains. These interact with nucleic acids in general. To detect structural mutations, an experienced eye and special staining techniques are required. Various staining procedures have been developed that reveal characteristic patterns of cross bands on each chromosome. High resolution banding of human chromosomes yields about 3,000 bands, for a genome of about 30,000-50,000 genes.

G-banding is a technique for producing ligh and dark banding patterns in eukaryotic chromosomes. Bands are produced by staining with Giemsa stain after pretreating chromosomes with trypsin. Each homologous chromosome pair has a unique pattern of g-bands, enabling recognition of particular chromosomes so that karyotypes can be generated. Some of the slides you will be viewing today contain g-banded chromosomes. Compare these with chromosome spreads that have been untreated. Can you see the difference?

Fluorescent In Situ Hybridization (FISH) involves treating the condensed chromosomes to denature the DNA as it sits in the chromosomes, then exposing the denatured DNA to single-stranded DNA probes of specific sequences. Each particular probe sequence is attached to a colored fluorescent dye; different probes are attached to different colored dyes. When a probe pairs with its complementary sequence in the chromosomal DNA, the fluorescent dye can be detected at that location.

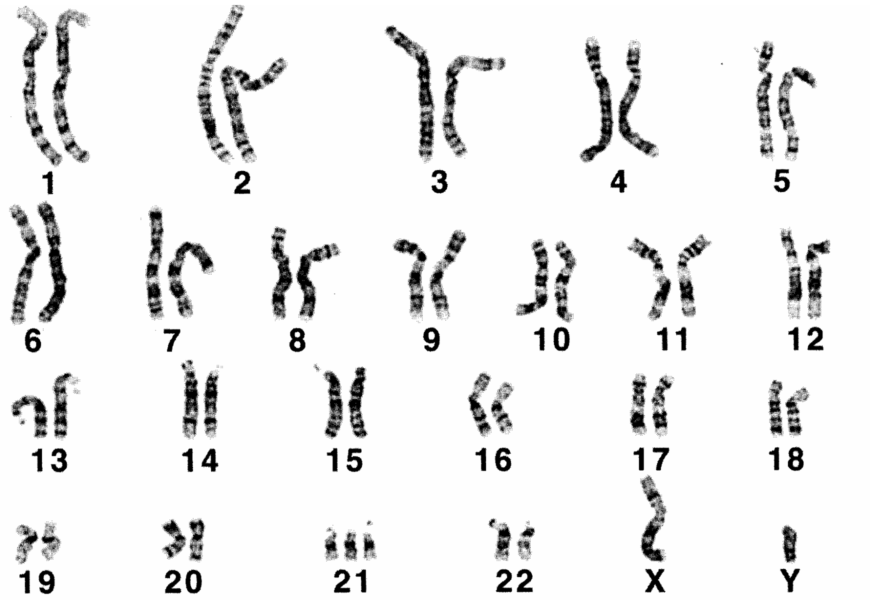


Figure 2-7. Karyotype of a male fetus with Down Syndrome. Note third copy of chromosome 21 and presence of one X and one Y chromosome. From Genetics in Medicine, 6th Ed. by Nussbaum, McInnes, and Willard. Thompson & Thompson, Philadelphia, 2001.

When a particular band varies a lot from one individual to another, we say that the population is polymorphic ("many formed") with respect to that band. Both band and morphological polymorphisms allow chromosomes and the genes they carry to be traced from one generation to the next. With the development of FISH, the tracing of chromosomes and genes has been extended even further.

THINGS TO DO:

1. Get your compound microscope and a prepared slide with human chromosomes. Adjust your microscope and clean the slide thoroughly. Place the slide on the stage and scan it under low power (10x). You should see various scattered cells and nuclei in interphase. Once in a while you will find a spot in which the chromosomes of a metaphase nucleus (now burst open) are scattered. Keep in mind that human chromosomes are very tiny (2-10 µm); so the chromosomes will just look like specks under low power.
2. Pick a chromosome spread where they do not seem to overlap and center it in your 10x field. Go up to 40x and center the chromosomes in that field. Then turn your nosepiece so that you can put a drop of oil on the slide without getting it on any of the objectives. Carefully turn the oil immersion (100x) objective into place. It should make contact with the oil and not trap any air bubbles. Switch your condenser ring to 3, for phase 3, and bring the image into focus using the fine focus knob only.
3. Study the chromosomes carefully, noting their relative sizes and shapes. In some of the spreads the chromosomes appear as single chromatids. This is because they were fixed in late prophase before the duplicated chromatids had time to separate. In other spreads you will be able to discern the 2 chromatids held together by a centromere.
4. Try to count the total number. You may find fewer than 46 if some chromosomes have been spread outside of the field of view. Or you may find more than 46, if two nuclei have burst in the same area. Sometimes cultured cells also spontaneously gain or lose chromosomes. When chromosome counts are done for clinical purposes, many clear spreads are examined from the same individual.
5. Also try to count the number of small acrocentrics and determine the sex of the individual from that. Remember that a female will have 4 small acrocentrics in her G group, while a male has the 4 small acrocentrics of the G group plus a Y chromosome. You can check your guess against the slide's label.
6. Then see if you can detect satellites on the acrocentric chromosomes.
7. Examine slides of males and females, with and without G banding.

Since it is difficult to examine chromosomes directly under the microscope, most clinical examinations are done by taking photographs of banded spreads. Photographs of spreads from many of the individual's cells are made to ensure that a representative sample has been achieved.

DAY THREE: ABNORMAL HUMAN KARYOTYPES AND THEIR ASSOCIATED PHENOTYPES

OBJECTIVES:

Today you will examine abnormal human karyotypes and photographs of children and adults who have these disorders. By the end of today's lab you should

☐ **Be familiar with several different kinds of numerical and structural chromosomal mutations.**
☐ **Be able to recognize such mutations in human karyotypes.**
☐ **Be able to associate clinical phenotypes with their correct karyotypes.**

INTRODUCTION:

The recognition of standard sets of chromosomes for each species made clear that one kind of mutational event involves changes large enough to be seen under the microscope. These are called **chromosomal mutations**, and may involve either a change in number or structure of the chromosomes.

The two large subsets of **numerical abnormalities** are polyploidies and aneuploidies. **Polyploids** (literally "many sets") have one or more of every kind chromosome in the set. These are represented 3n (**triploid**), 4n (**tetraploid**), etc., where n = the number of different kinds of chromosomes in a set. This condition results from a failure of whole sets of chromosomes to separate. It has played an important role in the formation of some new plant species, but is less well tolerated in higher animals. Polyploidies cause sets of phenotypic abnormalities from some of the products of genes being present in higher doses than normal. **Aneuploids** (literally "not true set") have one or two extra or missing chromosomes. For example, a **trisomic** (2n+1) has 3 of one kind of chromosome and 2 of all the rest, while a **monsomic** (2n-1) has just 1 of one kind of chromosome and 2 of all the rest. Aneuploids result from non-disjunction (failure to separate) of chromosomes during meiosis or mitosis. Because the genes on the extra or missing chromosome are present in abnormal numbers relative to other genes, there will again be abnormal doses of gene products for many genes, leading to sets of phenotypic abnormalities. Aneuploidy is also less well tolerated by higher animals than by plants. The reason is probably that higher animals have more complicated developmental programs, in which the right proportions of the right gene products must work together for normal development.

With **structural chromosomal abnormalities**, the chromosome number is normal, but the size, shape and/or arrangement of genes on a chromosome is altered. Structural mutations involve segments of many thousands of nucleotides, which have been gained, lost, or moved around. These include:

- **duplications (dup)**, in which a chromosomal segment is present in multiple copies;
- **deletions (del)**, in which a chromosomal segment is missing;
- **inversions (inv)**, in which a chromosomal segment is flipped around in orientation in the same chromosome; and
- **translocations (t)**, in which a chromosomal segment moves to another chromosome or a different location on the same chromosome. (May be reciprocal: segments switch locations)

All of these structural changes are caused by chromosome breaks that are incorrectly repaired; all are large enough to include many genes in the altered segment and are therefore detectable as changes in the banding pattern. In a karyotype the abnormality is denoted by one of the above abbreviations, following the chromosome #, and followed by the chromosome arm, and bands involved. So, 13 inv q12->21 refers to an inversion of the segment including bands 12 through 21 on the long arm of chromosome 13.

The phenotypic effects of structural mutations vary widely. Duplications and deletions affect several traits, since several genes' products will be present in abnormal doses. Inversions may have effects due to loss of coding sequences if the breakpoints at the ends of the inverted segment are in the middles of genes. Similarly, translocations may be associated with the loss of coding sequences due to interruption by breakpoints. In addition, in individuals with one normal and one rearranged chromosome, the abnormal gene orders caused by inversions and translocations lead to retarded synapsis of homologous regions and to duplications and deficiencies if certain events occur during meiosis. Consult your text for further details, or even better, try to draw an inverted and normal chromosome synapsing, undergoing a single crossover in the inverted segment, and then segregating.

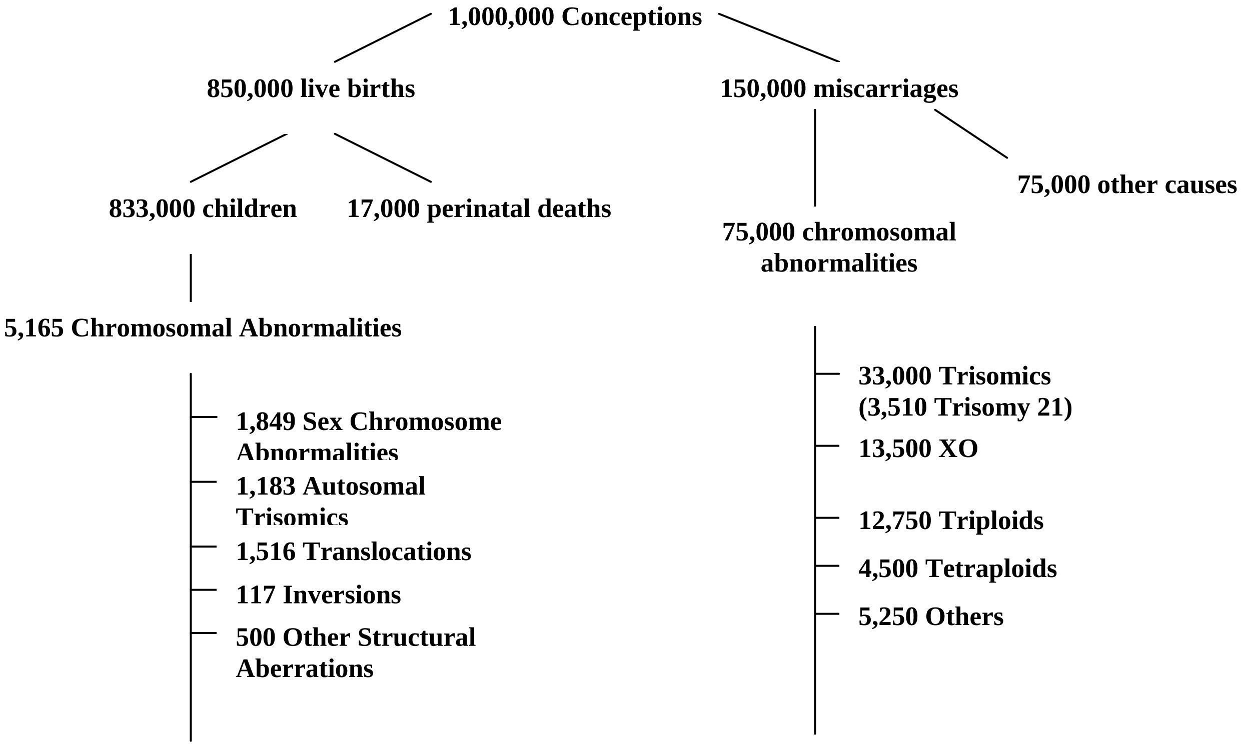
THINGS TO DO:

1. View the following abnormal human karyotypes displayed along the side benches of the laboratory. Most of these are accompanied by a photograph of an individual who has the abnormal karyotype. Notice the various symptoms displayed by the affected individual and be able to list at least three abnormal phenotypic characteristics associated with each karyotype. Below is a list of the abnormal karyotypes you will be responsible for knowing:

Karyotype	**Name of associated clinical disorder**	**Popular name of disorder (if any)**
47, +21	trisomy 21	Down Syndrome
47, +13	trisomy 13	Patau Syndrome
47, +18	trisomy 18	Edwards Syndrome
47, XXY	male X disomy	Klinefelter Syndrome
45, XO	monosomy X	Turner Syndrome
46, 5p-	partial monosomy 5p	Cri du Chat Syndrome
46, 13q-	partial monosomy 13q	None
69, XXX or XXY	triploidy	None; not viable

2. The diagram below shows the frequencies of human chromosome abnormalities among the total number of conceptions.

Frequency of Human Chromosome Abnormalities



Use the diagram above to answer the following questions:

a. What fraction of conceptions are spontaneously aborted? ____________ Do you think this number is likely to be accurate? Too high? Too low? Explain.

b. What fraction of spontaneously aborted fetuses have Down Syndrome? ___________

c. What is the % chance that a pregnant woman of normal childbearing age with no family history of genetic illness will give birth to a child who will die perinatally (soon after birth) *or* have a major chromosomal abnormality of some kind?