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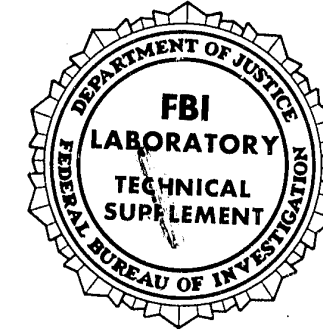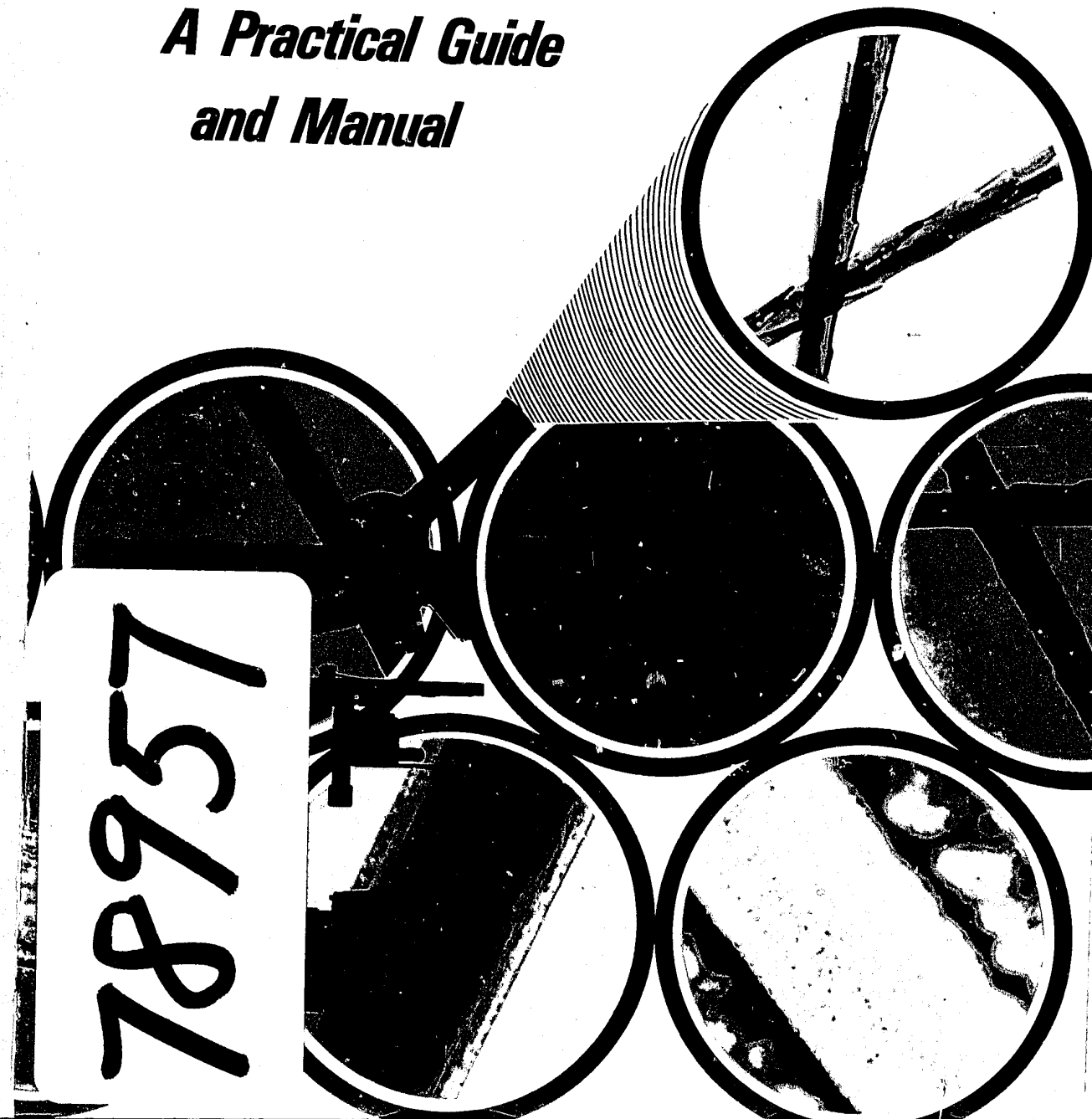FEDERAL BUREAU OF INVESTIGATION

MICROSCOPY of HAIR

A Practical Guide and Manual

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MICROSCOPY OF HAIRS

A Practical Guide
and Manual

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This work is an introductory manual to the microscopic examination
and identification of hairs. Information set forth herein is intended as
a practical guide to provide a foundation on which to build further
knowledge and experience.

UNITED STATES DEPARTMENT OF JUSTICE

FEDERAL BUREAU OF INVESTIGATION

WASHINGTON, D.C. 20535

This technical supplement on "Microscopy of Hairs" was written by the Microscopic Analysis Unit of the Laboratory Branch, Scientific and Technical Services Division, Federal Bureau of Investigation. It is intended for the use of the personnel of law enforcement crime laboratories.

The value of properly collected and later scientifically examined physical evidence by the crime laboratory cannot be over emphasized. It is an essential element of our criminal justice system.

Each photomicrograph contained in this manual depicts actual hair specimens enlarged up to 320 times and illustrates the subtle variations in color between specimens and within individual specimens. These variations will serve as a basis for accurate individualization.

It is hoped the "Microscopy of Hairs" will be valuable as a guide in the crime laboratory to facilitate and speed the examination process and further promote maximum use of physical evidence in our criminal justice system of America.

Clarence M. Kelley
Director, FBI

CONTENTS

BASIC STRUCTURE
OF HAIR

A hair may be defined as a slender threadlike outgrowth from the follicles of the skin of mammals, composed essentially of keratin and having three anatomical regions: The cuticle, the cortex and the medulla. These regions are depicted in the following diagram along with some of the basic structures found within those regions. Note that the cuticular scales always point from the proximal or root end of the hair to the distal or tip end of the hair (Fig. 1).

It should be noted that the sketch below as well as others included in the text are diagrammatic in nature and are used to emphasize structural features being discussed. Certain structures may be omitted and others enhanced for purposes of illustration.

A growing hair is generated from the papilla and, with the exception of that point of generation, consists of dead, cornified cells. Its basic constituents are keratin (a protein) and melanin (a pigment) with trace quantities of metallic elements. These elements may be deposited in the hair during its growth or may be contaminants absorbed and adsorbed by the hair from an external environment. After a period of growth, the hair will remain in the follicle in a "resting" stage and will eventually be sloughed from the body.
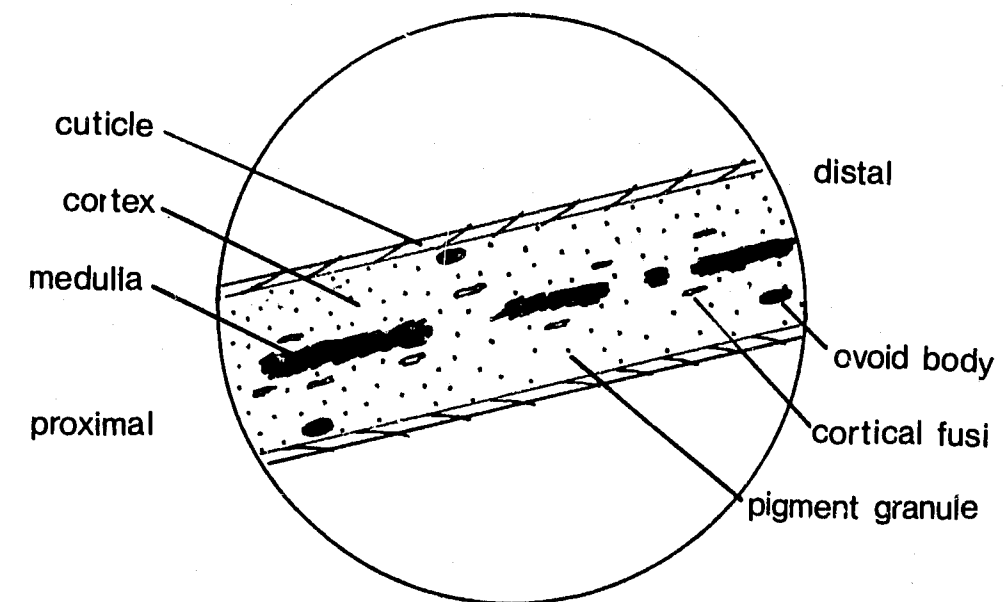

Figure 1

1

hair shaft

epidermis

dermis

sebaceous gland

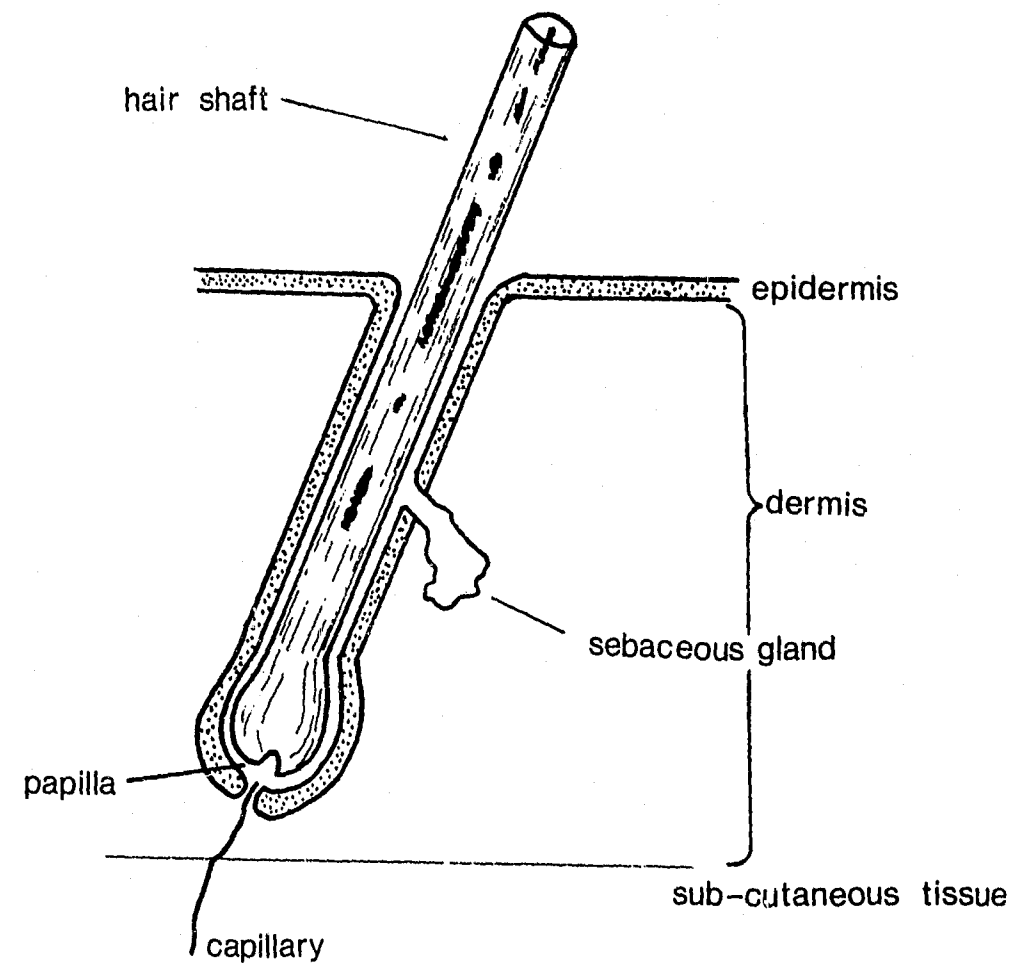papilla

sub-cutaneous tissue

capillary

Figure 2

The hair consists of a shaft which projects above the skin and a root which is imbedded in the skin. The lower end of the root expands to form the root bulb (Fig. 2).

The **cuticle** consists of a layer of scales covering the hair shaft. There are three basic scale structures (although there may be many more combinations and modifications of these structures) and these are:

1. Coronal or crown-like. These are characteristic of hairs of very fine diameter and resemble a stack of paper cups (Fig. 3). Coronal scales are commonly encountered in the hairs of small rodents and bats and only rarely in human hairs.
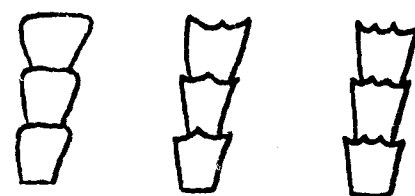

Figure 3

2. Spinous or petal-like. These are more or less triangular-shaped scales which frequently protrude from the hair shaft (Fig. 4). Spinous scales may be found on the proximal end of mink hairs, on the fur hairs of seals, cats and certain other animals. They are never found in human hairs.
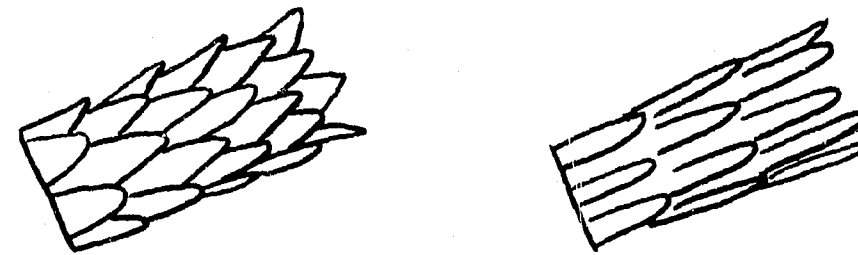
Figure 4

3. Imbricate or flattened. These are overlapping scales with narrow margins and are found on the hairs of humans and other animals (Fig. 5).
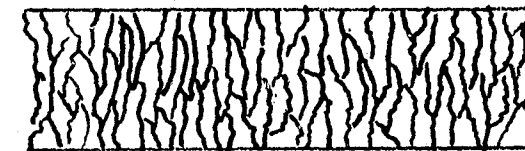
Figure 5

The **medulla** is a central core of cells which may or may not be present in the hair. It may be air-filled and, if so, will appear as a black or opaque structure under transmitted light or a white structure under reflected light. It may also be filled with mounting medium or some other clear substance in which case the structure may appear clear or translucent in transmitted light and nearly invisible in reflected light. If the medulla is present, its structure may be described as (1) fragmentary (or trace), (2) discontinuous (or broken), or (3) continuous throughout the hair shaft.

fragmentary

discontinuous

continuous
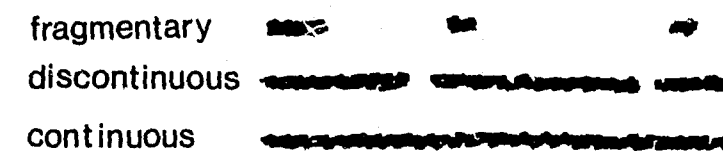
Figure 6

In human hairs the medulla is generally amorphous in appearance whereas in hairs from lower animals its structure is frequently very regular and well-defined. These medulla types may be defined as:

1. uniserial or multiserial ladder (both types found in rabbit hairs)

2

3

uniserial

multiserial
ladder

vacuolated

lattice

amorphous
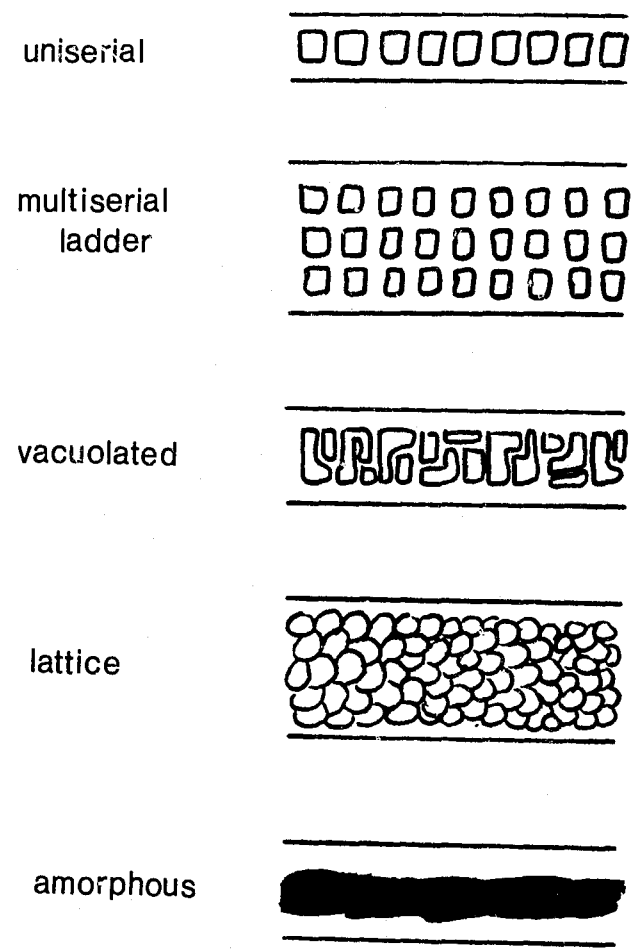
Figure 7

2. cellular or vacuolated (common in hairs of many animals)
3. lattice (deer family hairs).

The **cortex** is the main body of the hair and is composed of elongated and fusiform (spindle-shaped) cells. The cortex may contain air spaces called cortical fusi, pigment granules, and large oval-shaped structures referred to as ovoid bodies.

Cortical fusi (Fig. 9) are irregular-shaped, air spaces of varying shapes and sizes. They are normally found near the root of mature human hairs and their presence may persist throughout the hair shaft.

Pigment granules are small, dark, solid structures which are granular in appearance and considerably smaller than cortical fusi. They may vary in color, size, and distribution within a single hair and among animal species.

Ovoid structures (Fig. 8) are large (much larger than pigment granules), solid bodies which are spherical to oval in shape with very regular margins. They may be encountered in abundance in some cattle hairs and seen occasionally in human hairs from certain individuals.

4

Figure 8
Ovoid bodies—cattle hair (313X)

Figure 9
Cortical fusi—Caucasian Head hair (313X)

5

HUMAN HAIRS — INTRODUCTION

Human hairs may be distinguished from hairs of other mammals in several respects. Hairs of lower animals may be classified into three basic types: (1) the guard or "beard" hairs which provide protection (2) the fur or "wool" hairs which provide insulation and (3) the tactile hairs or whiskers which p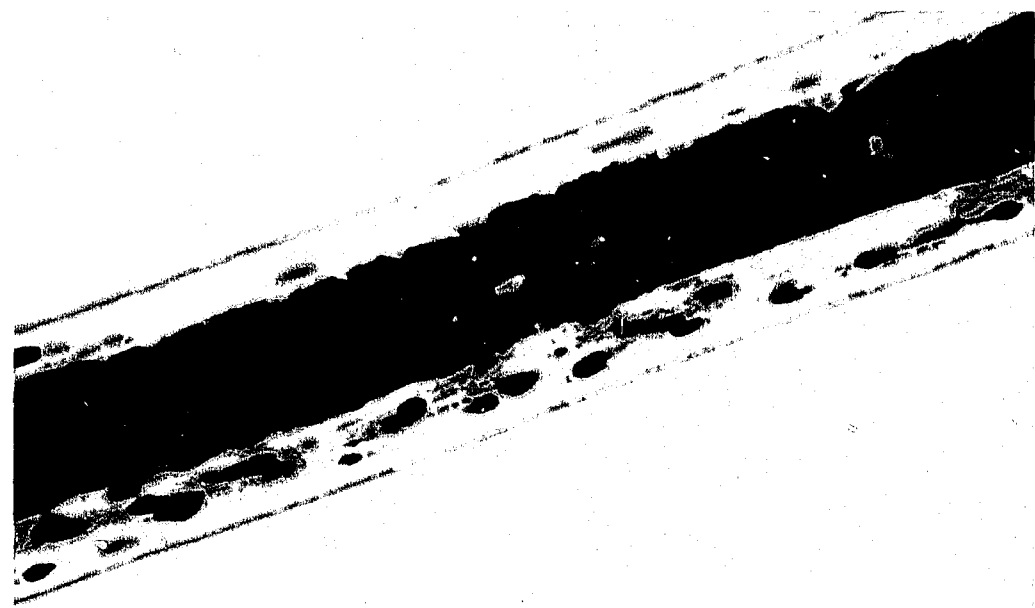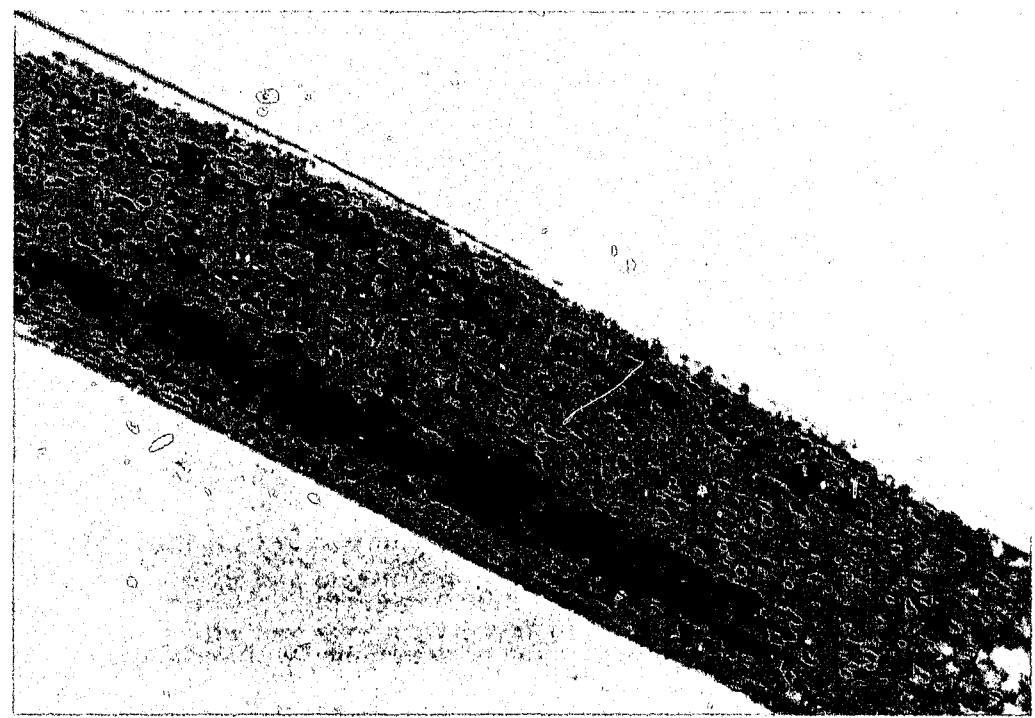rovide for sense enhancement. The hair covering of humans is not so differentiated and might be described as a modified combination of the characteristics of guard hairs and fur hairs.

Human hairs are generally consistent in color and pigmentation throughout the length of the hair shaft whereas hairs of lower animals may exhibit radical color changes within a short distance (banding). Across the hair shaft, the pigmentation in human hairs is evenly distributed or slightly more dense towards the cuticle whereas in hairs of lower animals, the pigmentation is centrally distributed (more dense toward the medulla).

The medulla when present in human hairs is amorphous in its structure and its width is generally less than one third the overall diameter of the hair shaft. The medulla in hairs of lower animals is normally continuous and very regularly structured and generally occupies an area of greater than one third the overall diameter of the hair shaft.

There are other differences such as in the size and shape of the root structures, the scale patterns and the configuration of the hair shaft itself which serve to distinguish human hairs from other animal hairs.

There are a number of possible determinations which may be made from a microscopic examination of hair. A human hair may be classified according to its racial characteristics as being of Caucasian, Negroid, or Mongoloid origin. In some instances, the racial characteristics exhibited by the hair specimen may not be clearly defined indicating the source of the particular hair may be of mixed racial origin.

The region of the body from which a hair came can be determined with considerable accuracy from its gross appearance and microscopic characteristics.

Other determinations include: whether the hair was forcibly removed; indications of damage such as being burned or crushed; and signs of artificial treatment such as dyeing or bleaching.

The age or sex of an individual cannot be determined with certainty from a microscopic examination of hair.

The hair specimen may be compared with hairs from a known source in consideration of a number of variations in structure which may occur. It is es-

sential that a comparison microscope be utilized for this stage of the examination so that the specimens may be compared on a direct, side-by-side basis. The variations or characteristics enable an experienced examiner to distinguish between hairs from different individuals. Based on this comparison, the examiner may conclude (1) that the hairs are consistent or similar and could have come from the same source, (2) that the hairs are dissimilar and did not come from the same source or (3) that the hairs possess characteristics which are not sufficiently defined to arrive at a meaningful conclusion.

These results and conclusions are significant in many applications. Hairs may be the only available means of identification in unidentified deceased cases. Hairs from the windshield on the driver's side may identify the operator of a stolen vehicle which has crashed when occupants of the vehicle accuse one another. Hairs found on the rape victim's clothing which match the suspect's hair serve to corroborate her statements when the suspect denies any contact. In questions of consent in rape cases, the presence of hairs like the victim's which have been forcibly removed suggests force was involved.

Hairs like the victim's found on a hammer or club near the crime scene may serve to identify that instrument as the murder weapon. In some instances, hair may provide investigative lead value such as when hairs of a particular race, color and length are found in a ski mask discarded by a fleeing robber.

These are just a few of the ways in which the results of hair examinations and comparisons, even though not a positive means of personal identification, may be of value to the successful investigation and subsequent prosecution of a crime.

DETERMINATION OF BODY AREA AND RACIAL ORIGIN

The following outline sets forth certain key characteristics which serve as indicators of racial origin (Caucasian, Negroid and Mongoloid). It should be understood that these indicators are generalities and apply primarily to head hairs. The examiner will, of course, encounter specimens which cannot be easily associated with a particular racial group either due to poorly defined characteristics, limited sample or specimen size, or inconsistent indicators. These must be identified as apparent racial mixtures or as not classifiable. Even though the hair specimen may not be classifiable as to race, the specimen may still be of value for comparison purposes. This racial admixture may serve to further individualize the hair and its source, particularly if the same mixed racial characteristics are observed in both the questioned and known samples.

DETERMINATION OF RACIAL ORIGIN

Caucasian (Fig. 13)
1. Shaft diameter moderate with minimal variation (mean diameter for human head hairs: 80 μ).
2. Pigment granules sparse to moderately dense with fairly even distribution.
3. Oval cross-sectional shape.

Negroid (Fig. 14)
1. Shaft diameter moderate to fine with considerable variation.
2. Pigment granules densely distributed (hair shaft may be opaque) and arranged in prominent clumps.
3. Shaft with prominent twist and curl.
4. Flattened cross-sectional shape.

Mongoloid Fig. 15)
1. Shaft diameter coarse and usually with little or no variation.
2. Pigment granules densely distributed and often arranged in large patchy clumps or streaks.
3. Prominent medulla (broad and continuous).
4. Cuticle thick.
5. Round cross-sectional shape.

The following outline lists features of individual hairs which serve to identify the region of the body from which they come. Again, the features listed are generalities and one must consider racial origin of the specimen when analyzing features such as the degree of diameter variation or the medullary structure. Body area determinations may be made with considerable accuracy; however, variations may occur which make this determination difficult or impossible. These particular hair specimens may be nonclassifiable due to immaturity or changes caused by artificial treatment or damage. The hairs may be "transitional" hairs; i.e., from an area of the body between two identifiable regions such as the sideburn, or they may simply be fragmentary and not of sufficient size for an adequate examination.

BODY AREA DETERMINATION

Head hairs
1. Long with moderate shaft diameter and diameter variation.
2. Medulla absent to continuous and relatively narrow when compared to its structure in hairs from other body areas.
3. Often with cut or split tips.
4. May show artificial treatment, solar bleaching, or mechanical damage, such as, caused by backcombing.
5. Soft texture (pliable).

Pubic hairs
1. Shaft diameter coarse with wide variations and "buckling" (Fig. 10).
2. Medulla relatively broad and usually continuous when present (Fig. 12).
3. Root frequently with follicular tags (Fig. 11).
4. Tip usually rounded or abraded.
5. Stiff texture (wirey).

Limb hairs (Arm or Leg)
1. Diameter fine with little variation.
2. Gross appearance of hair is arclike in shape.
3. Medulla is broad, discontinuous and with a granular appearance.
4. Soft texture.

Beard or Mustache Hairs
1. Diameter very coarse with irregular or triangular cross-sectional shape.
2. Medulla very broad and continuous.

Chest Hairs
1. Shaft diameter moderate and variable.
2. Tip long and fine, arclike.
3. Stiff texture.

Axillary or Underarm Hairs
1. Resemble pubic hairs in general appearance.
2. Diameter moderate and variable with less "buckling" than pubic hairs.

Figure 10
Caucasian pubic hair—"buckle" (156X)

Figure 11
Caucasian pubic hair—follicular tag (156X)

3. Tips long, fine.
4. Frequently with bleached appearance.

Other

1. Eyebrow: stubby, some diameter fluctuation. Saber like in appearance.
2. Eyelash: short, stubby with little shaft diameter fluctuation. Saberlike in appearance.
3. Trunk: a combination of features of limb and pubic hairs. A "transitional" hair.

COMPARISON CHARACTERISTICS

We distinguish between two individuals through recognition of certain features about them; i.e., sex, size, age, shape, eye color, hair, etc. None of these features is peculiar to only one individual, but the general appearance and arrangement of all of these features serve as criteria for identification. There are, likewise, a number of features or characteristics which may be present within a given hair specimen which, when considered collectively, provide a basis for individualization.

There is no standard for the importance or weight which should be assigned to a particular characteristic. Such a determination can be made only by the individual examiner and must be based on his experience in conducting hair examinations. These characteristics do not lend themselves to

studies of frequency of occurrence due to the variations which may occur within a single sample and the inherent difficulty in attempting to assign standard values for degrees of variation. If, however, particular characteristics are seen within a hair sample which appear with regular frequency throughout the sample, then they must be considered as significant for individualization purposes.

The process of identification or association involves distinct stages through which one must pass either consciously or unconsciously in the course of an examination:

ANALYSIS

The individual hair specimen must be visually separated into its component parts or characteristics. The color, size, and configuration of these characteristics, as well as their relationship to one another, are observed.

COMPARISONS

The characteristics of the questioned hair determined through analysis are compared with characteristics present in hair samples of known origin for consistencies or inconsistencies.

EVALUATION

Similarities or dissimilarities in the characteristics exhibited by the questioned and known specimens will have a certain value to the examiner based on his experience in conducting similar examinations. His conclusion will be based on his evaluation of those characteristics.

HUMAN HAIR COMPARISON CHARACTERISTICS

The following list is a guide to areas of examination which should be considered in a comparison of hair specimens. The list has been organized into fifteen different features or characteristics. In the literature, one may encounter other lists of identifying features in hair which may enumerate 25 or more different characteristics. These other lists generally do not disagree in substance with the following list but differ only in manner of organization. The characteristics listed are observable in whole mount.

RACE

Those features which serve to determine racial origin have been discussed previously. Again, it is pointed out that even when racial characteristics are

not clearly defined, it is significant when these characteristics are consistent between the hair in question and the hair of known origin.

BODY AREA

Body area characteristics have been discussed previously. As a general rule, most comparisons are conducted using head hair samples and pubic hair samples. Hairs from some other body areas may be of limited value for comparison purposes.

COLOR

There are many variations among individuals in hair color. The particular hue (color shade), value (lightness or darkness) and intensity (saturation) of a specimen are enhanced through microscopy so that even very subtle differences may be distinguished.

LENGTH

Length is considered, keeping in mind that hairs may have been cut between the time of deposit of a questioned specimen and collection of a known
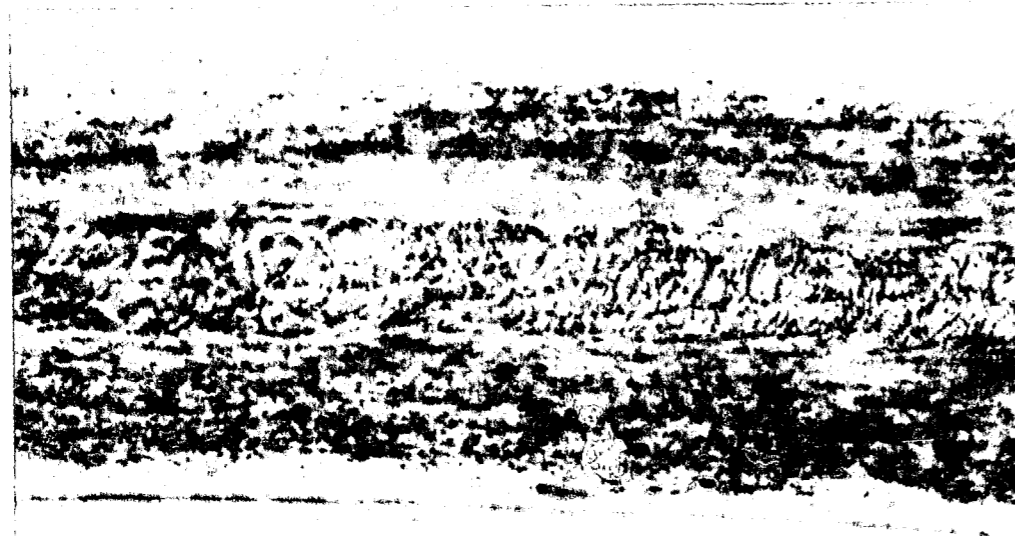

Figure 13
Caucasian head hair (313X). Note opaque and amorphous fragmentary medulla.

Figure 12
Caucasian pubic hair (313X). Note conspicuous vacuolated or cellular medulla
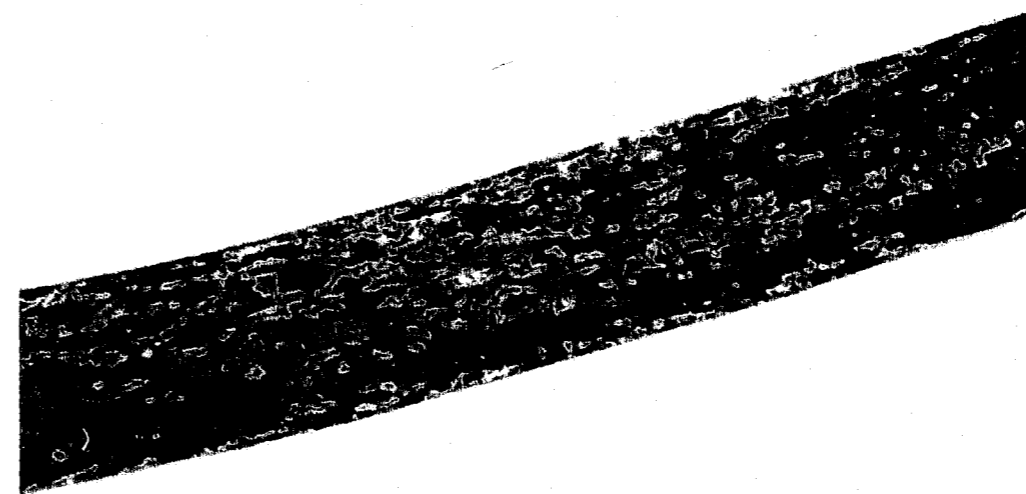

Figure 14
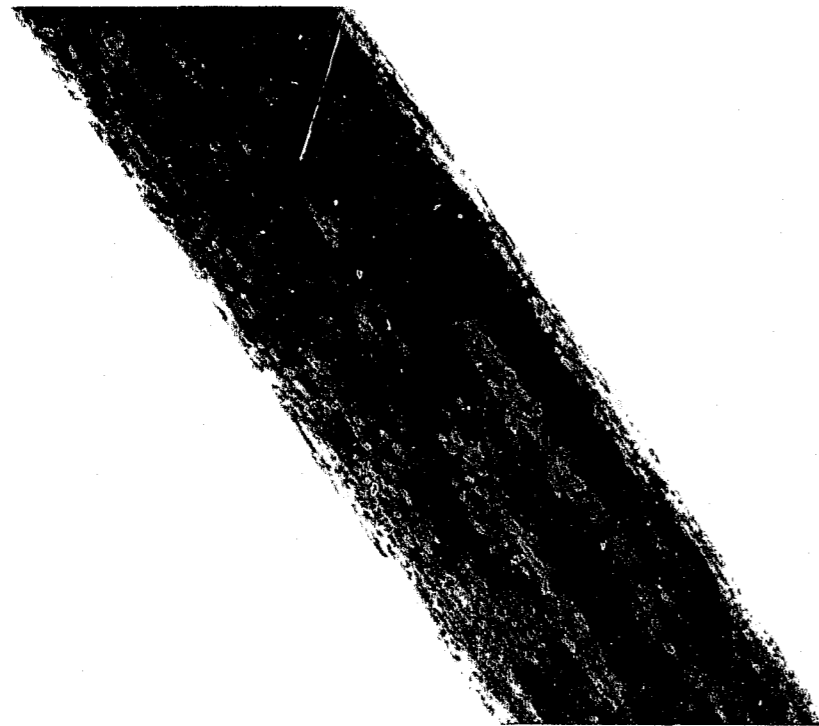Negroid head hair—(313X). Note pigment clumps.

Figure 15
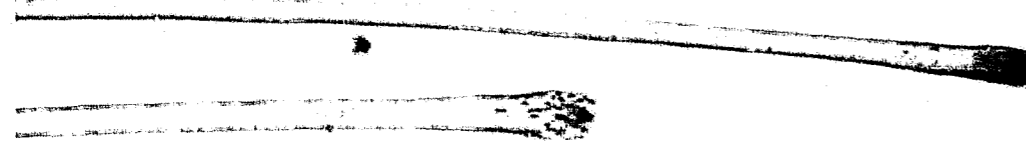Mongoloid head hair—thick cuticle (313X). Note clear definition of inner margin of cuticle.

Figure 16
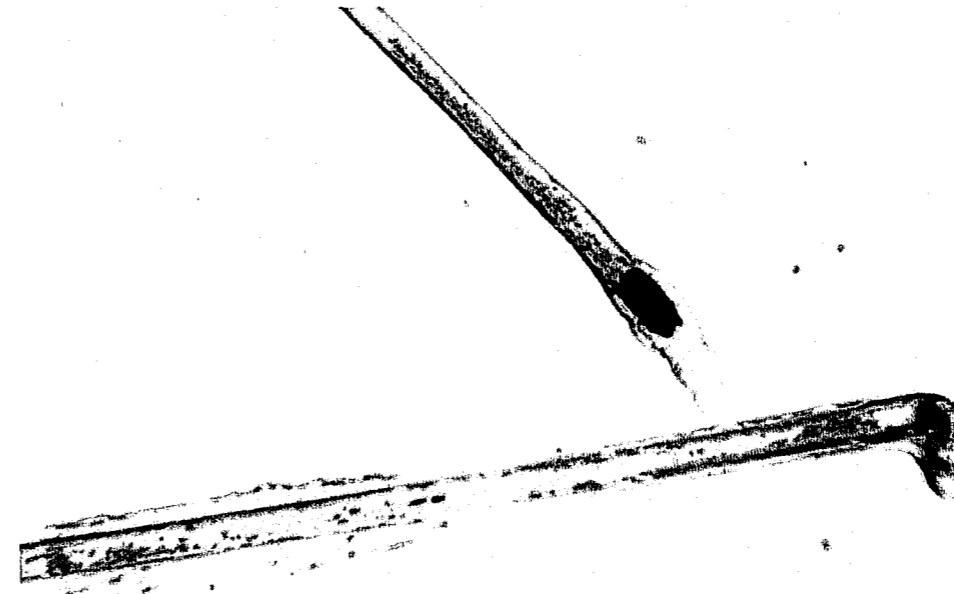Caucasian head hairs—mature roots (44X)

Figure 17
Caucasian head hairs—follicular tag and forcibly removed with root sheath attached (44X)

sample. In addition, there may be a wide range between the length of the shortest and longest hairs on an individual's scalp.

TIP

The tip may be cut, broken, split, abraded (rounded) or finely pointed. These features may be affected by an individual's grooming and hygiene and may even be a product of health and nutrition.

ROOT

Hairs are naturally sloughed from the body periodically. The mature hair root (Fig. 16) will be hardened, have a bulbous shape and will have little or no follicular tissue adhering to it. Pigment is sparse and absent in the mature root and there is frequently an abundance of cortical fusi. A root which has been plucked prior to maturation (Fig. 17) will be soft, have a distorted appearance, and may have tissue adhering to it. Pigment is present and there are rarely cortical fusi.

A root near maturity (at the hardened stage) which has been plucked may exhibit the bulbous shape with a "tag" of soft tissue attached (Fig. 17).

DIAMETER

The overall shaft diameter may range from very fine (40-50μ) to very coarse (110-120μ). Consideration should be given to the range of variation

within a particular sample and the variation in a single hair shaft. The degree of shaft diameter variation as well as the rate of change between variations should also be considered. The phenomenon of abrupt and radical changes is referred to as buckling.

CUTICLE

The inner margin of the cuticle may be very clearly defined (Fig. 15) or may be variable and without sharp delineation. Its thickness may vary from very thick and prominent to so thin as to be indiscernible. The cuticle color may be very clear or somewhat cloudy in appearance. There may be some variation in cuticle thickness within a sample and along a single hair shaft.

SCALES

A scale cast is not necessary to observe features of the scales. The scale margins are visible within the cuticle in whole mount (Fig. 20) and their overall length can be considered. The scales may be undisturbed and closely aligned with the hair shaft or may protrude outwards from the shaft. Scale damage and protrusion are associated with a mechanical action such as backcombing or harsh chemical action such as dyeing or bleaching. The scales may protrude out from the hair shaft and then recurve back to the shaft, giving a looped appearance(Fig. 31).
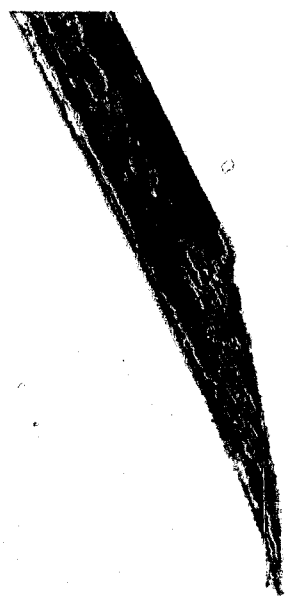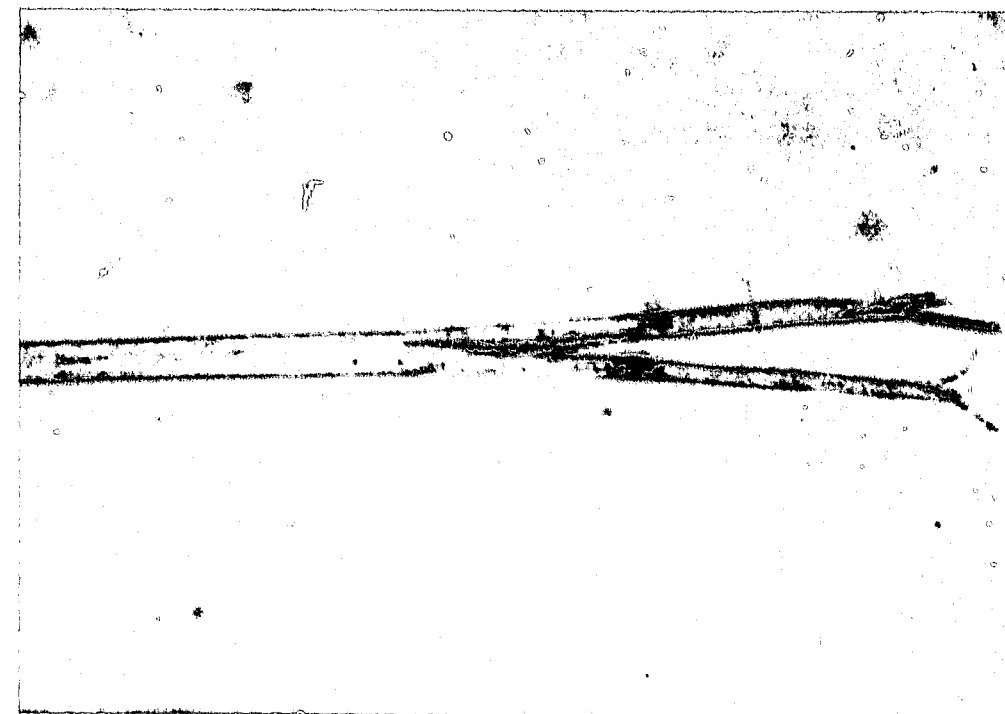
Figure 18
Caucasian head hair—tip razor cut (156X)

Figure 19
Caucasian head hair—tip split (156X)

PIGMENT

The pigment granules may be absent as in "gray" hair or may be so dense as to obscure the inner structural detail of the hair specimen. Granule size may range from very fine so that individual granules are difficult to discern to very coarse. Consideration is given to local distribution of the pigment across the hair shaft as well as to variations in distribution and density along the shaft from proximal end to distal end. The granules may be regularly arranged in streaks or clumps and the size, distribution and density of these groupings (Fig. 14) of pigment should also be considered.

MEDULLA

The medulla may vary from a continuous structure through the center of the hair shaft to fragmentary to absent altogether. It may be opaque or transluscent and may have a vacuolated or a completely amorphous appearance. When the medulla is in its fragmentary form, the cell structures may have a fusiform or spindle-shaped appearance. The width of the medulla in relation to the overall shaft diameter should be considered.

CORTEX

The general appearance of the cortex should be considered. The margins of the elongated cells comprising the cortex may be poorly defined or may be

distinct (Fig. 20). These cells are prominent particularly in hairs which have been bleached and result in a strawlike appearance.

ARTIFICIAL TREATMENT

Bleaching will remove pigment from the hair and give the hair a characteristic yellow cast (Fig. 22). The cortical cell margins may become more prominent and cortical fusi may develop. In addition, harsh or repeated treatments may make the hair shaft brittle and the scales will appear disturbed. Artifical bleaching can be distinguished from solar bleaching in that a clear line of demarcation is visible in artificially treated hairs, i.e., there is an abrupt change between untreated and treated portions. In hairs which have been bleached by the sun and natural elements, there is no point where change can be detected; however, a comparison of the appearance of the proximal and distal ends of the specimen reveals a bleached condition.

To the experienced examiner, dyed hairs possess an unnatural cast or color. In addition, the dye will be present in the cuticle as well as throughout the cortex. Keeping in mind that hair grows at the rate of about 1/2 inch per month, one may measure the distance from root to the line of demarcation of the dyed portion and estimate the time since dye.

Repeated dyeing or bleaching results in seve   lines of demarcation at various time intervals. This would serve to make the particular specimen unusual and unique.

DAMAGE

Cutting with scissors produces a sheared or squarecut appearance, whereas a razorcut (Fig. 18) is angular in appearance and very straight and clean. The length of time since cutting is subject to many variables and, hence, no reliable determination can be made. Crushed hairs (Fig. 23) will exhibit a widening of the hair shaft and the cortical cells may appear ruptured and separated. Broken hairs (Fig. 24) will exhibit a fairly square tip with elongated "tags" or fragments attached. Burned or singed hairs (Fig. 25) are charred and brittle and exhibit round vacuoles at the point of burning.

SPECIAL CHARACTERISTICS

There are other structures which may be encountered and should be considered in a comparison of hair specimens.

Ovoid bodies (Fig. 26) are dark, solid, oval-shaped structures of varying sizes which are very uniform in their general appearance. When they are consistently present in a given hair sample, they should be considered as significant for comparison purposes.

When cortical fusi (Fig. 27) are present, their size, shape, distribution and density should be considered.

18

Figure 20
Caucasian head hair—red color (313X). Note central distribution of pigment, prominent cortical cells and scale margins.

Figure 21
Caucasian head hair (156X). Note line of "trough" or "shoulder". The cross-sectional shape of this hair would be irregular or kidney-shaped.

19

Figure 22
Caucasian head hair (156X)—bleached. Note color change from natural brown to artificial yellowish cast.

Figure 23
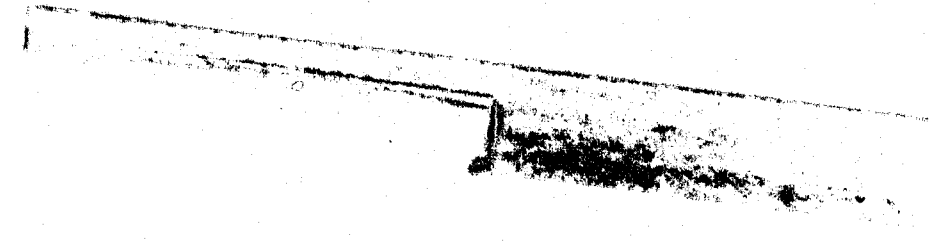Caucasian head hair—crushed (156X)

Figure 24
Caucasian head hair—tip broken (156X)

Figure 25
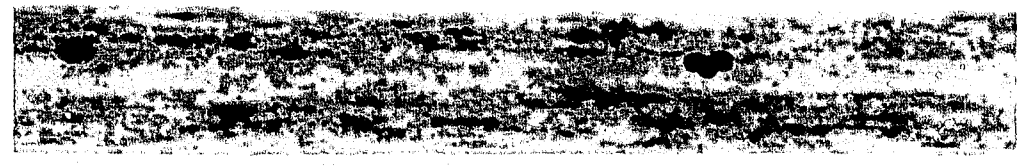Caucasian head hair—tip singed (313X)

Figure 26
Caucasian head hair—large ovoid bodies (156X)

Figure 27
Caucasian head hair—small cortical fusi (313X)

Figure 28
Caucasian head hair—"ringed" or "banded" hair (44X)

Certain diseases or deficiencies may result in changes in the appearance of hair such as "ringed" or "banded" hairs (pili annulati) (Figs. 28 and 29), conspicuous nodes (trichorrhexis nodosa) or regular diameter fluctuations (monilethrix). Egg sacks of parasitic lice (Fig. 30) may be attached to the base of the hair shaft. All these serve to further individualize the hair specimens.

A double medulla is encountered on occasion (usually in beard hairs); however, unless it is a regularly occuring feature within a sample, it is of little value for individualization.
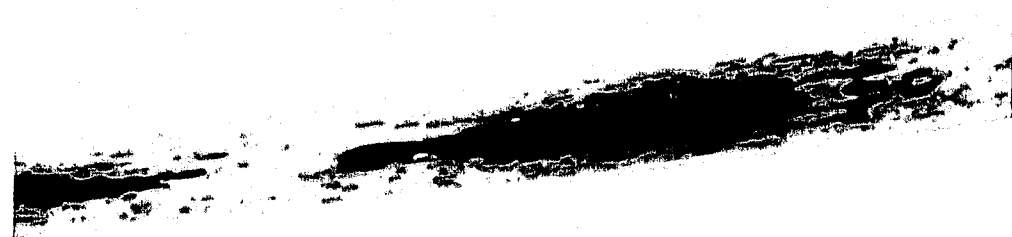

Figure 29
Caucasian head hair—ringed hair (156X)

Figure 30
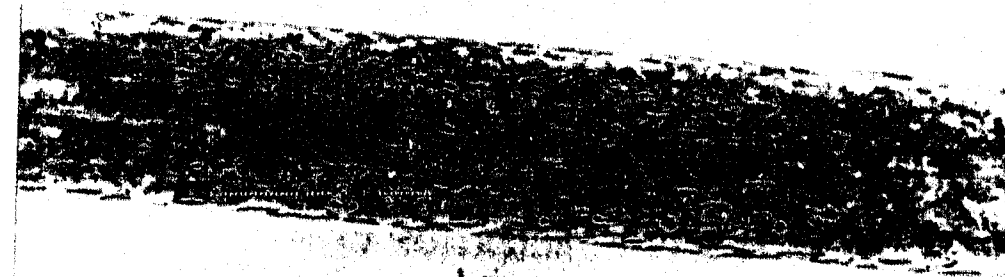Negroid pubic hair—parasite egg casing (44X)

Figure 31
Caucasian head hair—"looped" cuticle (156X)

CONCLUSIONS

There are three basic conclusions which may be derived through a microscopic examination and comparison of hairs. These are:

1. That the hairs from the questioned source are consistent with the hairs in a given known sample with respect to their microscopic characteristics and, therefore, could have come from the source of the known sample.

2. That the hairs from the questioned source are dissimilar to the hairs in the given known sample and, therefore, did not come from the source represented by the known sample, or

3. That the questioned hairs and hairs in a given known sample exhibit both similarities and unaccountable differences in their microscopic characteristics. It may be that, in the opinion of the examiner, the differences are not sufficient to eliminate the source represented by the known sample as being a possible source of the hairs in question. At the same time, the presence of these differences precludes an association being made between the questioned and known specimens. In other words, no conclusion could be reached as to whether or not the questioned hair specimen could have come from the source represented by the known sample.

Note in conclusion (1) above, it is stated that the questioned hair "*could have*" come from the source of the known sample. One must keep in mind that hairs are biological specimens and, accordingly, subject to variation. During his analysis, the examiner must establish the range of variation within the sample and then determine whether the questioned hair fits within that range. It has been found that when two hair samples are randomly selected from the population and compared microscopically, it is very unusual that they cannot be distinguished. However, the possibility cannot be dismissed that there may be two hair samples whose ranges of variation overlap and, therefore, a positive identification cannot be made.

OTHER TECHNIQUES
FOR INDIVIDUALIZATION OF HAIRS

Methods for individualization of hairs have been reported in the literature as possible alternatives to microscopic techniques. Some of these methods are set forth below along with a brief discussion of each.

NEUTRON ACTIVATION ANALYSIS (NAA)

NAA is an instrumental technique for trace elemental analysis. It has been used to demonstrate the presence of some 29 elements in hair samples. It has been reported that if two hair specimens match in relative concentrations of eight to ten of these elements, the probability that these hairs originated from a single person approaches 100%. Since these early reports, it has been demonstrated that hair specimens from one individual may vary periodically in their trace elemental content according to the individual's diet, health, hygiene, and environment. Accordingly, most recent scientific investigators agree that the results obtained from NAA are difficult, if not impossible, to interpret and are unreliable for purposes of individualization. With additional research, NAA may prove to be of value in this area.

ABO BLOOD GROUP DETERMINATION FROM HAIR

It has been demonstrated that antigenic substances (those substances which induce the production of antibodies) are present in tissue other than blood, including keratinized tissue such as hair. The detection of these substances provides the basis for determining the ABO blood group. Researchers have reported some success in this technique; however, the procedure does not permit alternate confirmatory tests on single specimens and results have been erratic, especially with respect to hairs from group "O" individuals. It is felt that the results of this procedure are not sufficiently reliable to be used in criminal cases. Again, further research may show some promise in this area.

SCALE INDEX; MEDULLARY INDEX

The scale index is defined by Hausman (1930) as the ratio of the scale length (distance between scale margins) to the overall diameter of the hair shaft. It appears that this ratio is a function of shaft diameter and has been shown to be of little value for purposes of individualization.

The medullary index is defined as the ratio of the width of the medulla to the width of the overall hair shaft. Again, it appears that this index depends on overall diameter; however, there are significant variations in this figure within a given sample so as to make the medullary index a meaningless figure.

SCALE COUNT

The scale count, as the name implies, is the number of scale margins observed along a given segment of the hair shaft. The technique described by Kirk requires that counts be taken from numerous segments along each hair in a sample and an average of these counts from all hairs in the sample be used for statistical purposes. The arithmetic mean of values from this technique is used to demonstrate differences or similarities between specimens. However, Kirk points out that the range of values overlap considerably between samples so that when analyzing samples of limited size, as in criminal cases, little significance can be attached to the scale count.

REFRACTIVE INDEX

The refractive index of the hair cuticle may be measured and values ranging from 1.545 to 1.556 have been reported (Kirk). Some differences were demonstrated by Kirk in the ranges of the mean values of the refractive indices between Caucasian males and Caucasian females and, hence, it was proposed this technique may be used for sex determination from hair. However, there is a considerable overlap in these ranges and results were erratic when they included members of other races and various age groups.

Accordingly, the determination of refractive index for hair individualization purposes is not sufficiently reliable for use in criminal cases.

SEX DETERMINATION FROM HAIRS

Articles have appeared in the literature concerning sex determination from hairs. Dr. Cecil Jacobsen, George Washington University Medical School, has stated that sex can only be determined from tissue at the root of the hair. Further, the tissue to be examined must be immediately fixed in an ether-alcohol solution upon plucking to insure accurate results. This requirement makes this determination impracticable in criminal cases for obvious reasons.

ION MICROPROBE MASS ANALYSIS

Ion Microprobe Mass Analysis is another technique for determination of trace elemental content. It has the same shortcomings as Neutron Activation Analysis with respect to the interpretation of data derived from the analysis.

CROSS SECTIONS

As a training aid, cross sections of hairs may be of value in demonstrating the cross-sectional shape of the specimen. However, with experience, the examiner is able to approximate the cross-sectional appearance of a hair by observation of the specimen in whole mount (Fig. 21). Determinations such as cuticle thickness, pigment distribution and medullary structure are readily made without resorting to cross-sectioning of the hair.

SCANNING ELECTRON MICROSCOPY

Scanning Electron Microscopy utilizes a beam of electrons rather than light to magnify the image of a specimen. It is used to examine surface detail and is capable of magnifications up to 40,000 times. It is felt by most workers in this field that magnifications beyond 600 times for purposes of individualization yield little or no information of value.

Wildman (1961) makes the following statement which seems appropriate in a discussion of other techniques for the examination of hairs:

". . .contrary to the suggestions made in some publications, no measurement method, such for example the measurement of distance between successive external scale margins or the measurement of fiber diameter, will itself reveal the precise origin of a fiber. . . .chemical tests do not distinguish between animal fibers, since all animal fibers consist of the same substance, namely keratin. The only satisfactory procedure is to use the method of microscopy with a sound knowledge of fiber morphology and careful interpretations of the observations made."

ANIMAL HAIRS—
MICROSCOPIC IDENTIFICATION

The animal hairs discussed in this manual will be limited to those animals most likely to be encountered in actual casework. An adequate reference collection is essential for accurate identification of questioned specimens. In most cases, specific identification can only be accomplished with guard hair specimens. In some instances however, as with some commercial garment furs, specimens can be identified on the basis of the microscopic appearance of the fur or "down" hairs alone.

The animal hairs presented here can be classified into three major groups on the basis of their microscopic appearance. These are:
1. the deer family and antelope
2. the commercial fur animals
3. the domestic animals.

For individual identification within these groups, deer family and antelope hairs are distinguished on the basis of their scale patterns, commercial fur animals are distinguished on the basis of their color, colorbands, scale patterns and medullary structure, and the domestic animals are distinguished primarily through their root structure, medullary structure and pigmentation.

Group characteristics are outlined below:

Deer Family and Antelope
1. Very coarse overall diameter (approximately 300μ).
2. Medulla composed of spherical cells which occupy entire hair (Fig. 33).
3. Diameter constant throughout most of hair.
4. "Wineglass" shaped root (Fig. 32).
5. Regular wave or crimp.

Figure 32
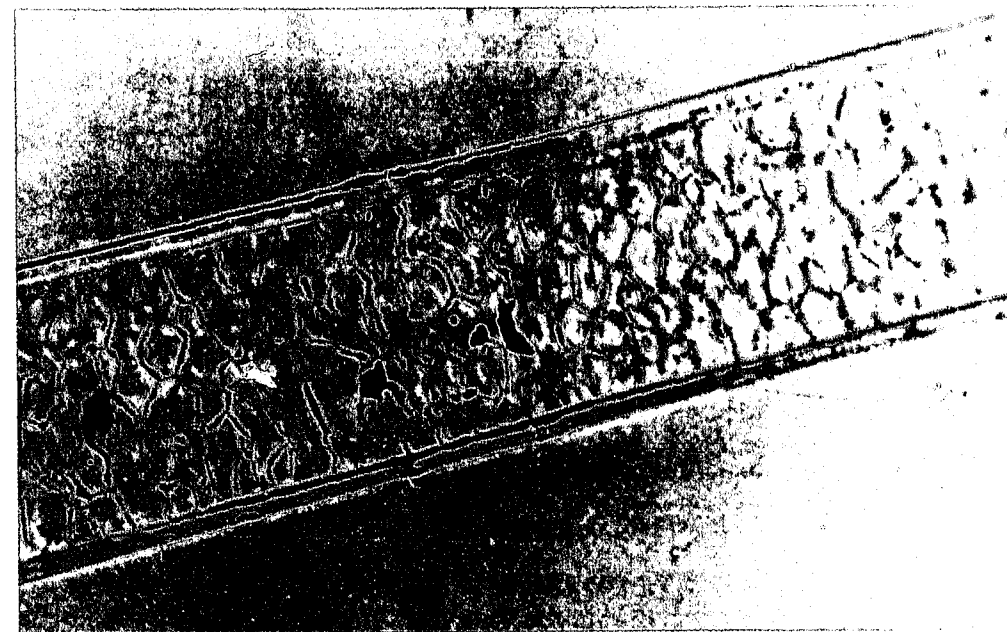"Wine-glass" root—deer hair (44X)

Figure 33
Lattice medulla—deer hair (156X)

Commercial Fur Animals
1. Very fine to medium overall diameter (20μ to 150μ).
2. Characteristic medullary formations (serial or vacuolated).
3. Wide diameter variations within single hair.
4. Hairs generally banded.

Domestic Animals
1. Medium overall diameter (75μ to 150μ).
2. Medulla generally amorphous.
3. Moderate diameter variation within single hair.
4. Hairs generally unbanded.
5. Characteristic root shapes.

DEER FAMILY AND ANTELOPE

The deer family and antelope hairs are not readily distinguishable on the basis of their gross appearance or microscopic appearance in whole mount. However, when scale cast impressions are carefully studied, certain patterns become apparent which can be used to separate the different members of this group. It is emphasized that these patterns are impressions perceived through general observation of the entire hair specimen.

Deer (Figs. 34 and 39):
(White-tailed deer and mule deer): scale margins are round and isodiametric and resemble fish scales.
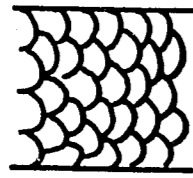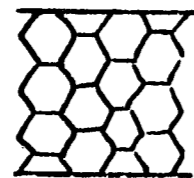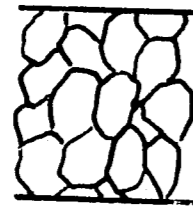
Figure 34

Figure 35

Figure 36

Figure 37

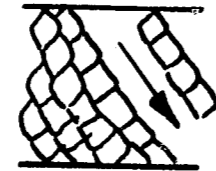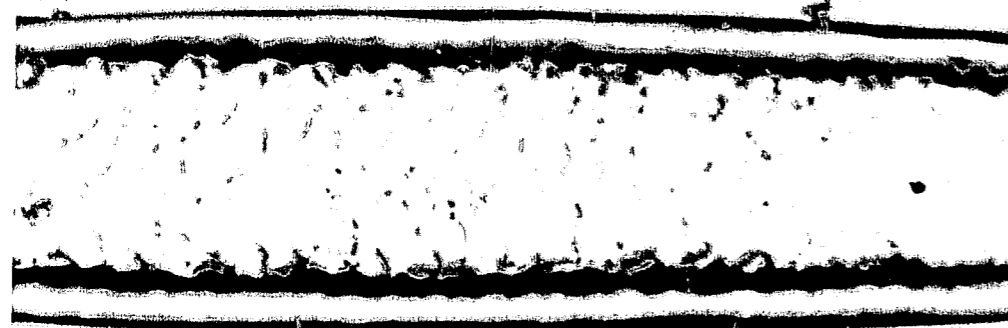Figure 38

Figure 39
Whitetail deer—scale cast (156X)

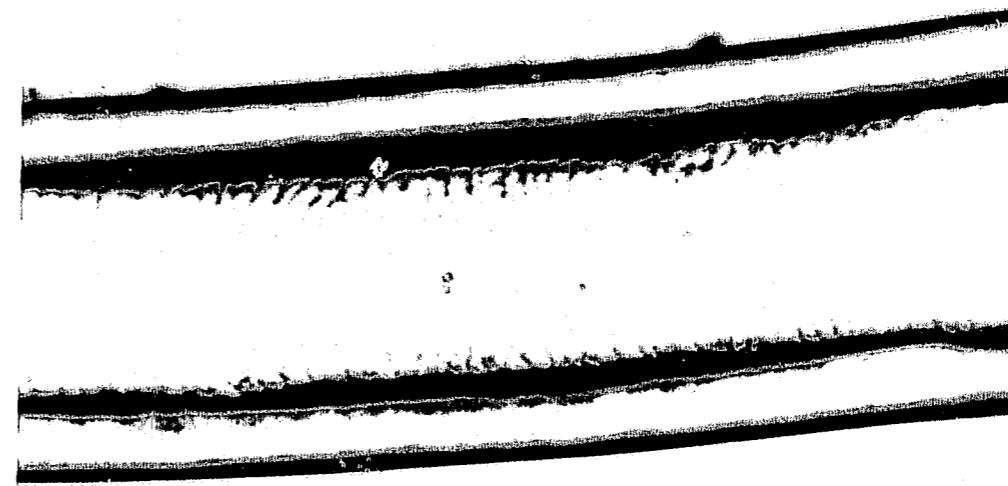Figure 40
Caribou—scale cast (156X)

Figure 41
Elk—scale cast (156X)

Caribou (Figs. 35 and 40):
Scales are hexagonal and usually longer than wide.

Elk (Figs. 36 and 41):
Scales are elongated and five- or six-sided. Scale margins are narrow and ends are pointed.
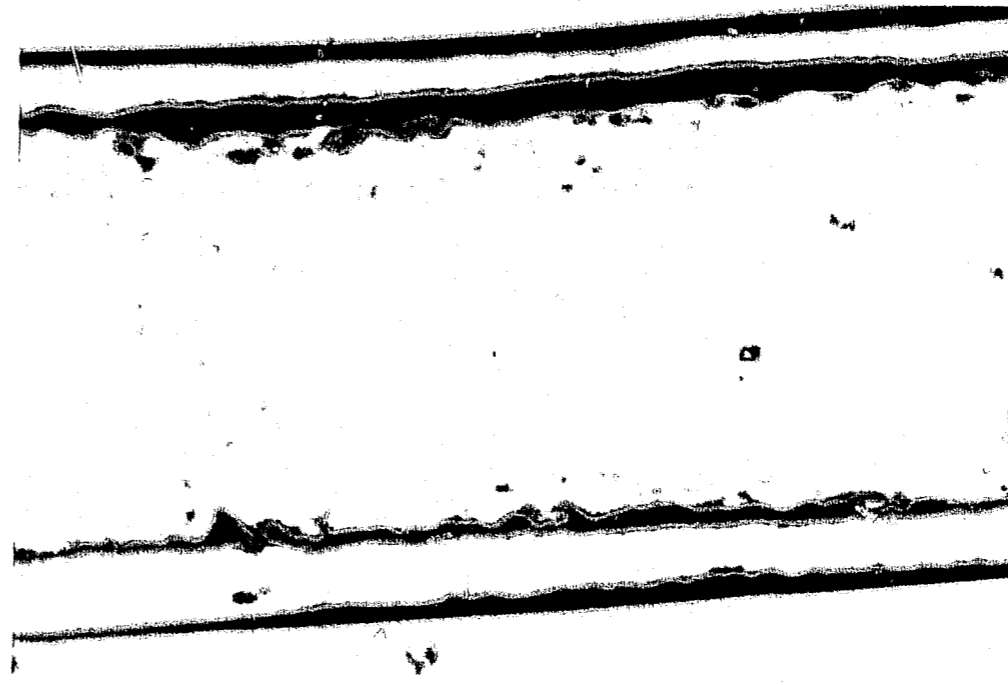

Figure 42
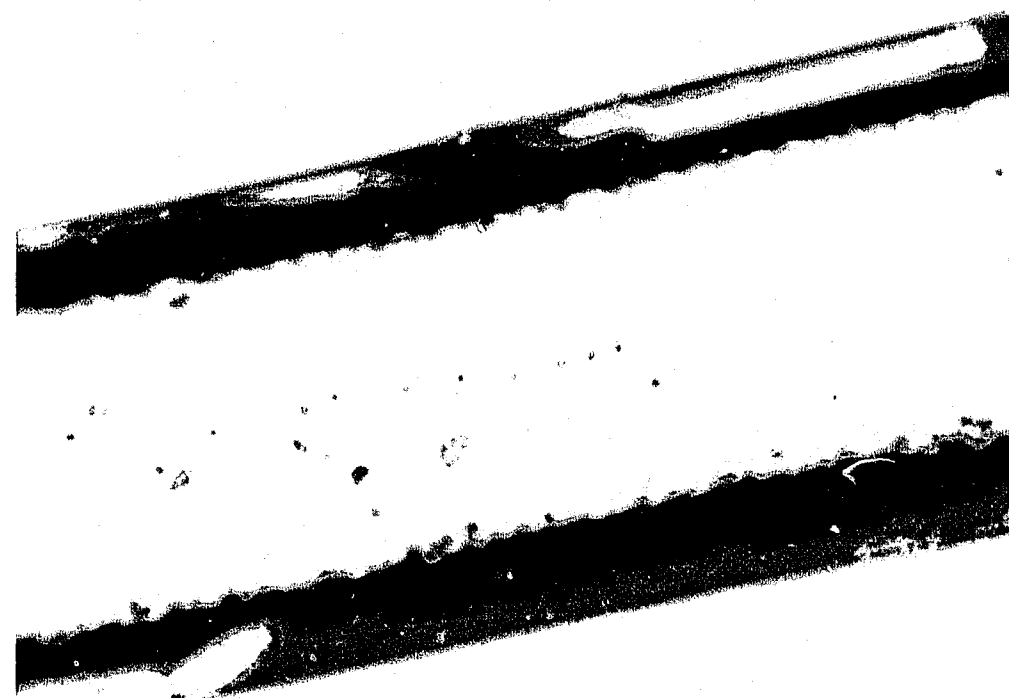Moose—scale cast (156X). Note large overall diameter.

Figure 43
Antelope—scale cast (156X)

Moose (Figs. 37 and 42):

Scales are relatively large and irregular polygons. Overall hair diameter is considerably larger than other members of this group.

Antelope Figs. 38 and 43):

Scales are diamond-shaped and frequently give impression of being arranged in diagonal rows.

Note that goat hairs exhibit similarities to deer family hairs in general form (medullary structure). However, goat hairs are generally finer in overall diameter (approximately 220μ) and will exhibit a narrow, pigmented cortex. No cortex is apparent in deer family hairs (cortex is only occasionally visible in elk hairs). The scale pattern of goat hairs shows flattened scale margins and no regular pattern.

COMMERCIAL FUR ANIMALS

The commercial fur hairs group includes several specimens which are commonly encountered in fur garments and which are fairly distinctive in their microscopic appearance. These are rabbit, seal, mink, muskrat and chinchilla. Of these, seal and chinchilla are identified on the basis of the appearance of their "down" or fur hairs and not by their guard hairs. This is due

to the fact that guard hairs are frequently plucked from seal pelts for a more pleasing appearance. Chinchilla pelts have few if any guard hairs.

The remaining specimens in the commercial fur hairs group are identifiable largely on the basis of their characteristic colors and color banding.

Rabbit: Extensively used in felted fabrics, glove linings, fur trim, coats. Sheds very readily.
1. Medulla: multiserial ladder (guard hairs) (Fig. 44).
2. Ribbon-like appearance. Cross section flattened.
3. May be various colors and lengths.

Seal: Coats
1. Medulla: absent.
2. Diameter: very fine and uniform.
3. Scales: spinous (elongated-petal shape) and easily visible even in whole mount (Fig. 45).
4. Pelts usually dyed and sheared (guard hairs frequently plucked).

Mink: Coats, hats, stoles, trim
1. Characteristic shape: basal 1/3 (root portion) of hair very fine, rapidly widening to four or five times basal diameter and then reducing to a pointed tip (spear-shaped).
2. Scales: spinous (elongated-petal shape) through fine basal portion of hair, changing to imbricate through "blade" or "shield" portion (Figs. 46 and 47).
3. Characteristic dark brown color (other color phases possible).
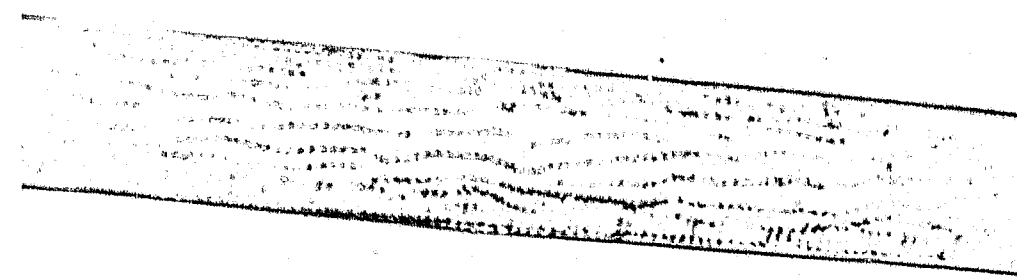4. Maximum length: 30mm. Maximum shield diameter: 143μ.

Figure 44
Rabbit (156X)

Figure 45
Seal (313X)

Figure 47
Mink—scale cast at distal end (313X)

Figure 46
Mink—scale cast at proximal end (313X)

Muskrat: Coats, trim
1. Similar to mink in general shape and appearance.
2. Scales imbricate throughout full length.
3. Maximum length: 43mm, Maximum shield diameter: 110 μ.

Chinchilla: Coats, hats, trim
1. Diameter: very fine and uniform.
2. Medulla: uniserial ladder (fur hairs) (Fig. 48).
3. Pigment usually clumped in segments between medullary cells.
4. Very dense, even coat. Several hairs may emanate from a single follicle (Fig. 49). Few, if any, guard hairs.

Raccoon (Procyon): Coats, trim
1. Medulla: unbroken; amorphous through basal portion changing to vacuolated; broad.
2. Banded: white base to dark brown midshaft to yellow (or white) to black tip.
3. Maximum length: 74mm. Maximum diameter: 154 μ.
4. Scales: diamond-petal shape (basal) to imbricate.
  Note: Raccoon-dog (Nyctereutes), sold commercially as "Russian raccoon" is very similar in structure and appearance to Raccoon. Generally, "Russian raccoon" hairs are longer than raccoon.
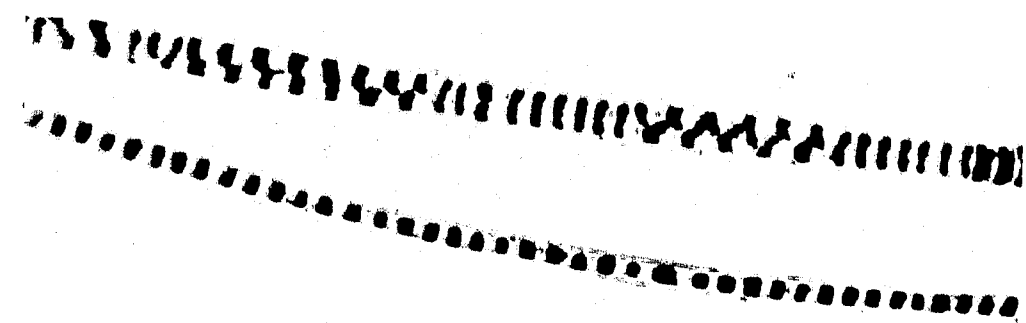
Figure 48
Chinchilla (313X)
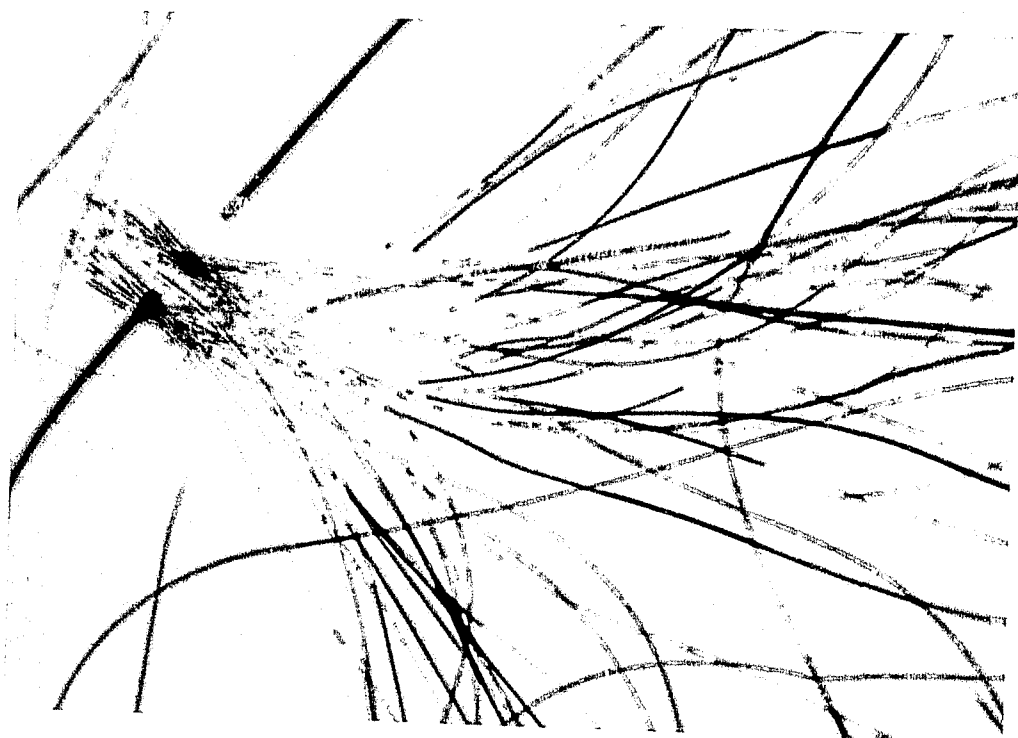
Figure 49
Chinchilla (44X). Note bundles.

Red Fox: Stoles, trim
1. Medulla: unbroken; amorphous or vacuolated; broad (Fig. 51).
2. Banded: brownish-gray base to yellow (or white) to reddish-brown tip.
3. Maximum length: 156mm. Maximum diameter: 110μ.
4. Scales: vary throughout shaft. Mosaic pattern (basal) to diamond-petal shape to elongated-petal to imbricate.

Beaver: Coats, hats
1. Medulla (fur hairs): continuous, cellular (beaded appearance) (Fig. 50). In guard hairs medulla is unbroken, cellular and usually relatively narrow.

2. Unbanded.
3. Diameter: fine, uniform.
4. Pelts always sheared.

Bear (Black and Grizzly): Coats, rugs
1. Medulla: continuous; amorphous; usually less than 1/2 overall shaft diameter.
2. Unbanded; pigment coarse, granular and fairly even distribution.
3. Scales: imbricate.
4. Maximum length: 108mm (Black); 70mm (Grizzly). Maximum diameter: 153μ.

Figure 50
Beaver (500X)

Figure 51
Red fox—proximal end (313X)

DOMESTIC ANIMALS

There are wide variations in color and length of most of the hair specimens in this group. The identifying characteristics given are general and apply in most cases. In order to distinguish between dog and cat and between beef (cattle) and horse, it is usually necessary that the root be present.

Cat
1. Diameter: fine; little variation.
2. Medulla: uniserial ladder (fur hairs) continuous; occasionally vacuolated in coarser hairs.
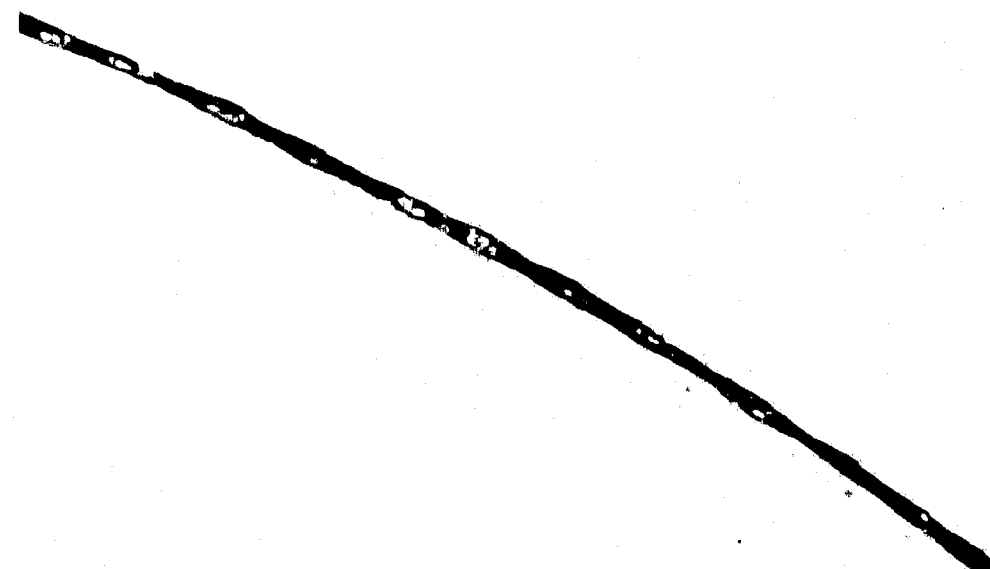3. Scales: spinous, very prominent.
4. May be banded.
5. Root: elongated; no distinct shape; fibrils frayed at base of root (Figs. 52 and 53).

Dog
1. Diameter: fine to coarse (usually coarser than cat hairs); diameter may vary to give short hairs a barrel-like appearance.
2. Medulla: continuous; vacuolated to amorphous; occasionally very broad.
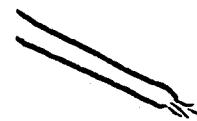
Figure 52
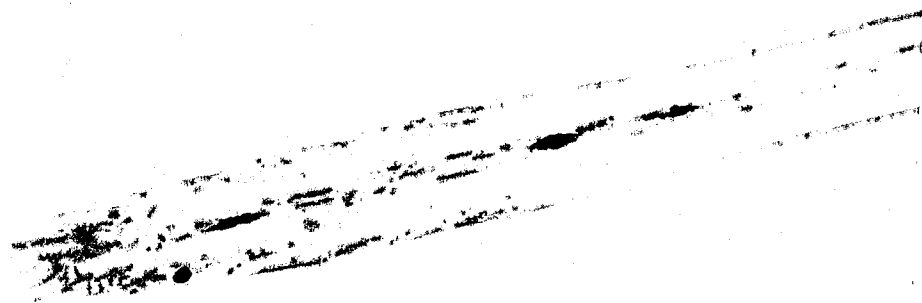
Figure 53
Cat (156X)

Figure 54
Dog (156X)

Figure 55   Figure 56   Figure 57

3. Scales: generally not prominent.
4. Unbanded (pigment occasionally very coarse and extending into root).
5. Root: spade-shaped (Figs. 54 and 55).

Cattle
1. Diameter: coarse.
2. Medulla: absent or continuous; amorphous or vacuolated; width may be narrow to very broad (without mosaic pattern).
3. Scales: imbricate and with no protrusions from hair shaft.
4. Unbanded; ovoid structures abundant; pigment coarse.
5. Root: elongated; medullary structure continues into root area; traces of follicular tissue may be present (Fig. 56).

Horse
1. Diameter: very coarse.
2. Medulla: absent to unbroken; cellular or amorphous (mosaic pattern).
3. Scales: imbricate; without protrusions from hair shaft.
4. Characteristic color; pigment fine, evenly distributed; no ovoid structures.
5. Root: area adjacent to root tapers to bulb-shaped root (Fig. 57).

Hog

1. Diameter: coarse; uniform.
2. Medulla: absent to continuous; amorphous; generally broad to very broad.
3. Scales: imbricate; without protrusions from main shaft.
4. Root: not remarkable.
5. Stiff texture, brittle; tips split.

Goat

1. Diameter: coarse.
2. Medulla: unbroken lattice; occupies nearly entire width of hair shaft.
3. Scales: imbricate (absence of characteristic scale shapes or patterns distinguishes goat from deer family and antelope hairs).

Figure 58
Cattle (156X)

REPORT WRITING AND TESTIMONY

THE REPORT

The report should be clear, concise and easily understandable. It would rarely serve any useful purpose to include technical terminology foreign to the layman such as a detailed de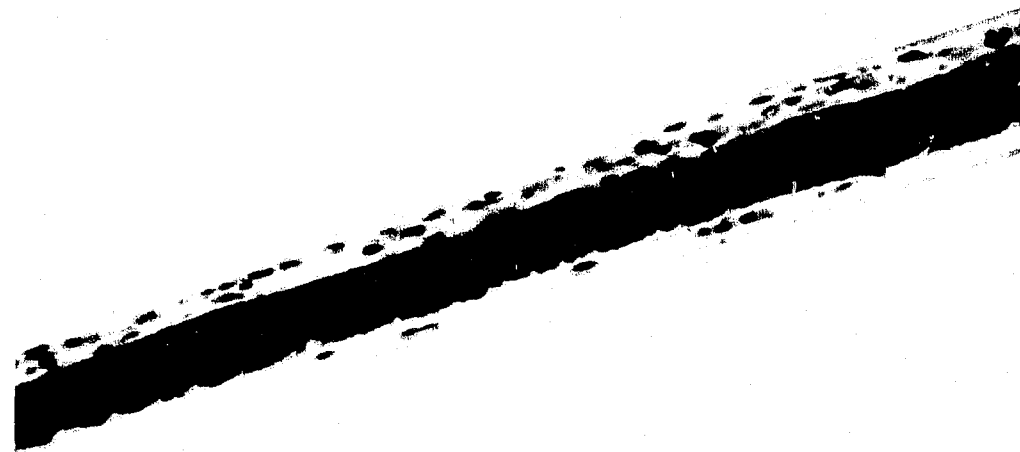scription of individual characteristics. It should contain information pertinent to the requests made by the contributor of the evidence and to the examinations conducted. The evidence examined should be clearly listed and identified as to origin, either through description or contributor's reference numbers. The results of the examination should be set out and followed by a statement giving the examiner's conclusion. It may be desirable to include a clarifying statement in which the limiting factors of an examination are set forth.

The following is given as an example:

Specimens:

Q1   Knit cap
K1   Head hair sample from suspect

Results:

Four brown head hairs were found on specimen Q1. These hairs are of Caucasian origin and have been dyed and were found to exhibit the same microscopic characteristics as the hairs in specimen K1. Therefore, the four brown head hairs found in the Q1 cap could have come from the source represented by specimen K1.

It is pointed out that hairs do not possess a sufficient number of unique individual microscopic characteristics to be positively identified as having originated from a particular person to the exclusion of all others.

Note that the statement of results (the first two sentences) sets forth fairly completely those determinations that can be made; i.e., that the hairs came from the head, that they exhibit Caucasian characteristics, that they have been dyed, and that the Q hairs are consistent with the K hairs in microscopic appearance. The conclusion follows, then, that given these results, the Q hairs could have come from the source of the known sample. The last paragraph is optional and is given so that the lay reader may better understand the nature of the identification. It may be modified in any number of ways to accurately describe the limits or exclusions of a particular conclusion.

TESTIMONY

Testimony of the expert witness should proceed with the same basic constituents as found in the report; namely, the statement of results and the conclusions derived from those results. The witness should be prepared to discuss the process by which his results were obtained and this, of course, should justify the ultimate conclusion. He should endeavor to promote a better understanding on the part of the court and jury into the method of his examination.

SLIDE PREPARATION

Hair specimens are permanently mounted on glass microscope slides in a synthetic resin medium such as Permount (Fisher Scientific Company), Histo-clad (Clay-Adams Company), or Pro-Texx (Scientific Products Company). Temporary mounts may be made using water or xylene if desired.

In most cases, hair specimens may be mounted directly after recovery; however, on occasion it may be necessary to clean debris from the hair specimens in order to better observe structural detail.

Blood may be rinsed or soaked from the hairs with a saline solution. Care should be taken after washing to dry the hairs thoroughly as moisture droplets may result on the specimens when mounted in a medium immiscible with water. Oily or other debris-contaminated specimens may be cleansed in xylene or an ether-alcohol solution.

For permanent mounts, the hair specimens are positioned on the glass slide using a few drops of xylene to hold them in place. Excess xylene used for positioning of hair specimens should be removed with a blotter and a few drops of the mounting medium added. The specimen and medium are then sealed under a cover slip. With particularly stiff-textured or wirey hairs, the mounting medium itself may be used in place of xylene to position the hairs as desired.

It may be necessary to weight the cover slip in order to insure a thin mount. The number of hair specimens mounted on a single slide is optional; however, excessive overlapping should be avoided so as not to obscure observation of underlying specimens. In addition, the limited working distance of high power objectives should be kept in mind. Long hairs should not be cut but may be arranged in a "figure eight" pattern.

Care should be taken to identify the prepared slide according to case and specimen number and initials of the examiner.

SCALE CASTS

It may be necessary or desirable to make a scale cast of the hair specimen, particularly in the identification of some animal hairs. A quick and easy cast may be made using a Polaroid black and white land film print coater.* A thin film is applied to a glass slide with 2 or 3 passes of the Polaroid print coater. The hair specimen is pressed onto the film and allowed to let stand until the

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* Ogle, R.R., B.A. and G.T. Mitosinka, B.S., M. Crim., *Journ of Forensic Sciences*, Vol. 18, No. 1, Jan. 1973.
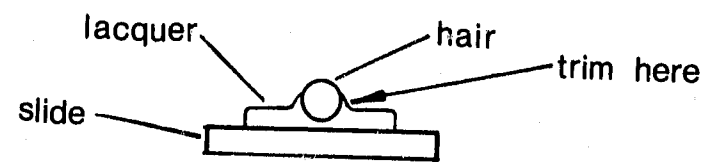
Figure 59

film is dry. The hair is then pulled from the film and the cast may be observed.

Scale casts may also be prepared using a clear lacquer such as fingernail polish. A film may be "painted" on a glass slide or, if the lacquer is thinned with acetone, a drop may be allowed to run down the surface of the slide. The hair is then placed in the film and removed when the lacquer dries. Due to "wetting" of the hair surface it may be necessary to trim the surface of the cast to remove fragments of lacquer which obscure the cast.

SOME PRINCIPLES OF MICROSCOPY

The ability of a microscope to render minute details as separate features (the resolving power) depends on the numerical aperture of its objective. The numerical aperture (N.A.) is defined as:

$$(1) \qquad N.A. = \eta \cdot Sin\, \alpha$$

Where α is the angle formed by the outermost ray of light admitted by the objective and the optical axis and n is the refractive index of the optical medium (air, water, immersion oil) through which this ray must pass between cover glass and front lens.

$$\alpha = \frac{A}{2}$$
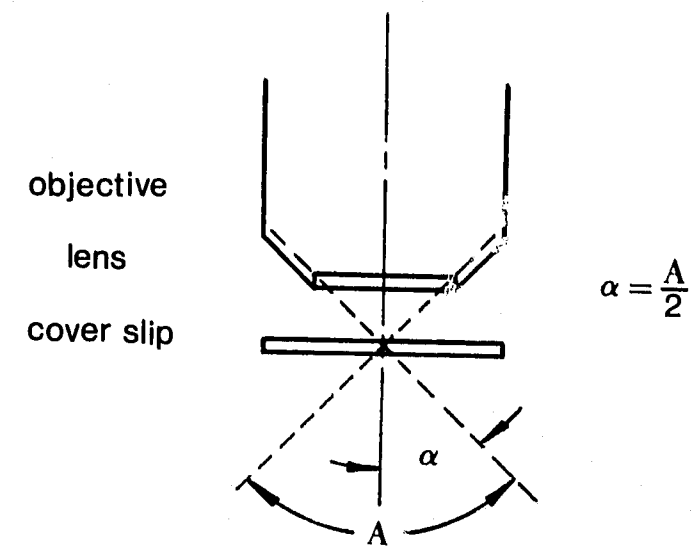
Figure 60

The resolving power (R) is measured in nanometers (1 x 10^{-9}m.) and is defined as:

$$(2) \qquad R = \frac{\lambda}{2\, N.A.}$$

Where λ is the wavelength of the illuminant in nanometers.

The maximum useful magnification of a light microscope is approximately 1000 times the numerical aperture of its objective. Efforts to increase the total magnification beyond this figure will yield no additional detail and is referred to as empty magnification.

It can be seen from formula (2) above that resolution may be improved by increasing the numerical aperture of the objective and/or decreasing the

wavelength of the illuminant. These principles are applied in the electron microscope. Due to physical limitations, the maximum N.A. for dry systems is 0.95. Numerical apertures as high as 1.4 may be utilized through oil immersion techniques.

The following table shows the values for R (resolving power) and maximum useful magnification for given numerical apertures.

N.A.	R. (λ=550nm)	Approximate Maximum Useful Magnification
0.10	2.75 μ	110
0.30	0.92 μ	320
0.65	0.42 μ	690
0.85	0.32 μ	900
1.32	0.21 μ	1400

BASIC PARTS OF
THE COMPOUND MICROSCOPE

ILLUMINATION

It is important to obtain illumination of uniform intensity over the entire field of view with independent control of light intensity, size of illuminated field of view and angular aperture. Two systems of illumination used in ordinary light microscopy are called (1) critical illumination; and (2) Kohler illumination. In critical illumination the lamp filament is imaged in the plane of the specimen and must be diffused through the use of a ground glass plate. In Kohler illumination, the lamp filament is imaged in the plane of the condenser diaphragm and does not require the use of a ground glass plate to evenly illuminate the specimen field. Kohler illumination offers greater versatility in all aspects of light microscopy.

The specimen field is illuminated with a low-voltage tungsten filament lamp. A color-correcting blue filter is used in order to approximate white light.

FIELD DIAPHRAGM

The field diaphragm protects the specimen against unnecessary heating and prevents flare. It should be opened only as far as is necessary to clear the
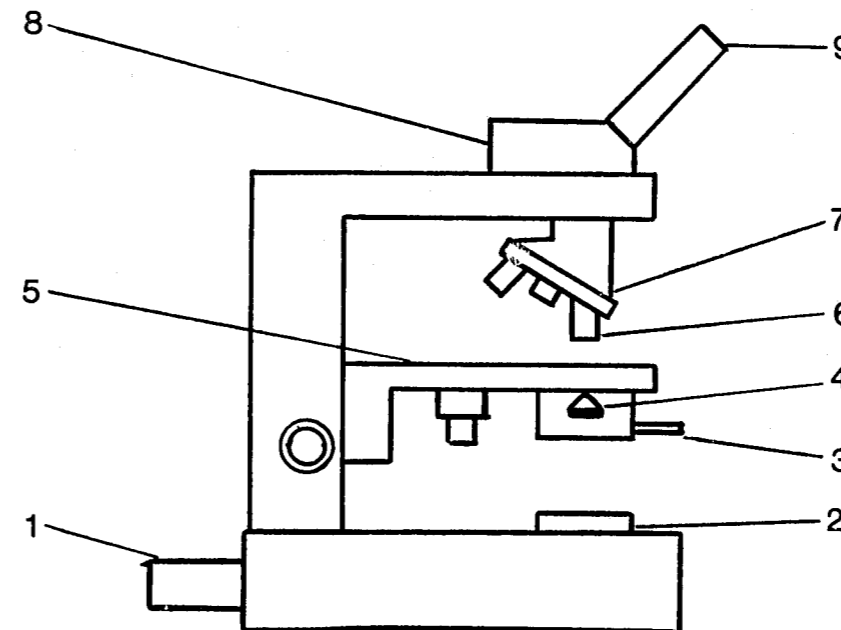
Figure 61
(1) Illumination; (2) field diaphragm; (3) aperture diaphragm; (4) substage condenser; (5) stage; (6) objective; (7) nosepiece; (8) headset; (9) eyepiece.

field of view. If opened too far, the excess light will cause the image to lose its sharpness and contrast.

APERTURE DIAPHRAGM (condenser diaphragm)

The aperture diaphragm determines resolution and contrast of the microscopic image provided it is smaller than the objective diaphragm. To observe specimens of normal contrast, stop the aperture diaphragm so that the objective aperture is reduced by approximately one-third. This adjustment can be made by removal of the eyepiece and observation of the aperture diaphragm on the back lens of the objective.

SUBSTAGE CONDENSER

The condenser lens concentrates the light on the object specimen. It should be in position with objectives having a numerical aperture (N.A.) larger than 0.25. The condenser lens may be swung out of position with objectives having an N.A. of less than 0.25.

MECHANICAL STAGE

The object specimen is placed on the stage for observation.

OBJECTIVE LENS

The objective lens forms an inverted and side-reversed intermediate image of the objective in the diaphragm of the eyepiece. The objectives desirable for scientific use are flat-field objectives and may be classified as achromats, fluorite systems, or apochromats. The achromats are constructed of glass and are limited in quality of correction for color and spherical aberration. Fluorite systems are constructed of fluorspar and offer improved color and spherical correction over achromatic lens systems. The apochromats are complex in structure and have the highest degree of correction.

Markings on the objective indicate the mechanical tube length, the thickness of the cover glass for which the objective was designed (in mm), the magnification of the intermediate image, and the numerical aperture. Markings also identify the system as apochromatic (Apo), fluorite (Fl), or oil immersion (Oel). The absence of such characters indicates an achromatic system.
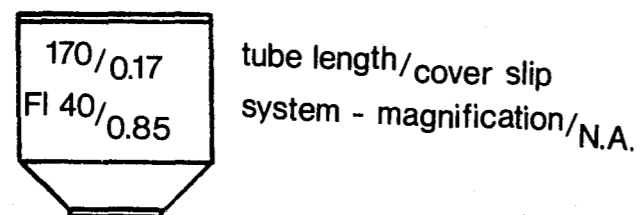
170/0.17   tube length/cover slip
Fl 40/0.85   system - magnification/N.A.

Figure 62

REVOLVING NOSEPIECE

The revolving nosepiece allows the convenient changing of objective lenses. The different objectives are designed to be parfocal and therefore require only fine adjustment of focus on changing.

BINOCULAR TUBE OR HEADSET

The binocular tube allows the use of both eyes in observing the object specimen. It may be adjusted according to the interpupillary distance of the user.

EYEPIECE OR OCULARS

The eyepiece gathers the intermediate image produced by the objective and magnifies the image for the observer. The product of the magnification of the objective and the magnification of the eyepiece yields the resulting or total magnification. Eyepieces may be classified as Huyghens or compensating. Huyghens eyepieces are the simplest type while the compensating eyepieces are more complex and are designed to complement the image quality produced by high power objectives.

GLOSSARY

Achromat: An objective lens system constructed entirely of glass. Limited in quality of correction for color and spherical aberration.

Apochromat: A complex objective lens system generally of wider aperture than fluorite systems. Apochromats produce brilliant images which are corrected to the highest degree for color and spherical aberration.

Chromatic Aberration: The inability of a lens to focus light of different colors (or wavelengths) at a single point.

Coaxial: On the same axis.

Color Band: A band of color on a hair which is sharply separated from adjacent areas of different colors above and below the band.

Comparison Microscope: An instrument which consists of two separate specimen stages joined by an optical bridge. It provides for simultaneous side-by-side viewing of two specimens.

Cortex: The primary tissue in hair which extends from the outer layer of scales (cuticle) to the central core (medulla).

Cortical Fusi: Elongated or spindle-shaped air spaces found in the cortex of hairs.

Cuticle: The outer layer of overlapping scales which cover the hair shaft.

Dispersion: The variation of the refractive index of a medium with changes in color (or wavelength) of light.

Fluorite System: An objective lens system constructed of fluorspar. Offers improved color and spherical correction over achromatic lens systems.

Fur Hairs: Fine diameter hairs which comprise the undercoat of mammals and provide for warmth.

Guard Hairs: Coarse diameter hairs which provide protection and are usually longer than the fur hairs.

Keratin: Any of various sulphur-containing fibrous proteins that form the chemical basis for horny epidermal tissues such as hair, nails, and feathers.

Medulla: The central part of certain structures such as the core of hair.

Melanin: Any of a group of brown or black pigments occurring in plants and animals.

Numerical Aperture (N.A.): A term which indicates the ability of the objective lens to make fine structural detail in the specimen distinct.

$$N.A. = n \cdot \operatorname{Sin} \alpha$$

Oil Immersion: A technique enabling greater resolution at higher magnifications by introduction of a medium (oil) with higher refractive index than air between the objective aperture and specimen cover slip.

Papilla: A small nipple-like eminence; that part of generation of the hair from its follicle.

Parfocal: An objective system of different magnifications designed to have similar focal distances or working distances.

Periplanatic: A compensating type of eyepiece designed for flat and wide field observation.

Plano: An objective designed to eliminate the curvature of the image. Also called "flat field" objectives.

Refractive Index: The ratio of the velocity of light in air to its velocity in another medium.

Resolving Power: The smallest distance between two points which can be seen as separate in the image. It is dependant on the wavelength of the illuminant and the numerical aperture of the objective lens.

$$R = \frac{\lambda}{2 \, N.A.}$$

Spherical Aberration: The inability of a lens to converge light rays from all points on a lens surface to a common focal point.

Tactile Hairs: Coarse, stiff hairs with specialized erectile tissue found on all mammals except man; whiskers.

Vellus: Fine body hair that is present until puberty.

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