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A NEWSLETTER FOR FORENSIC EXPERTS IN MICROANALYSIS

Preparation and Use of Needles and Micropipets for Handling Very Small Particles

To successfully isolate 1-100 µm samples for microscopical examination or analysis by other instruments, one needs a good set of microtools with the most essential being the needle and the micropipet. This paper will describe how to make them and how they are used to solve various sample preparation problems.

TUNGSTEN NEEDLES

The three most commonly used needle types are the Fine, Medium and Curved. Less frequently used are the Flat, Polyethylene and Eyelash needles. Relative sizes of the six needle types are shown in Figure 1.

A procedure for making tungsten needles has been carefully described in The Particle Atlas, Edition Two, Volume I, by McCrone and Delly. The procedure is as follows:

24-gauge, 520 µm, tungsten wire is cut into one-inch lengths using wire nippers to minimize split ends because the wire is very brittle.

The tip of the wire is heated over a Meker burner or alcohol lamp until it is red hot; then it is quickly placed in $NaNO_2$. The ensuing exothermic reaction is allowed to proceed for 1-5 seconds. The end of a freshly cut tungsten wire may require 5 seconds to etch and form a sharp tip. One second may be sufficient to resharpen a damaged tip.

Over the past 20 years, minor changes have been made in the procedure described in The Particle Atlas. Now, a large number of needles are sharpened at one time. They are handled with tweezers and are not placed in a needle holder for sharpening.

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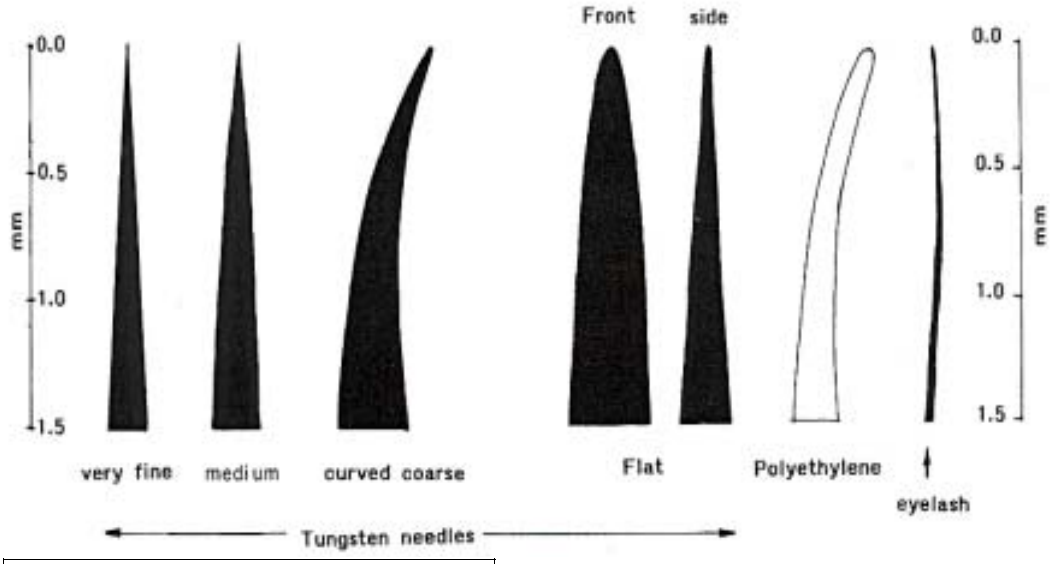

Figure 1:
Relative sizes of the six needle types.

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The use of a sodium nitrite stick (as described in The Particle Atlas) is suitable for sharpening freshly cut tungsten wire. The relatively broad, unsharpened tip will stay hot long enough to put the glowing tip into the nitrate stick. This transfer of the needle from the flame to the $NaNO_2$ stick must be done very quickly. If the tip is allowed to cool even slightly, the exothermic reaction will not be initiated.

For finer tips such as those needing resharpening, the required heat is lost too rapidly and they are bent when their cooled tips are pushed against the sodium nitrite stick. That is why the alternative method using the molten sodium nitrite is preferred for resharpening needles (see Figure 2).

TYPES OF TUNGSTEN NEEDLES

No special techniques are necessary for making Fine, Medium, Coarse or Flat needles. If a large quantity (more than 50) are made at one time, one will obtain approximately equal amounts of each type plus rejects. Rejects are needles with round tips, double tips (resulting from split wire at the tip), and needles with an uneven taper. These can be resharpened.

The freshly made needles are placed in a single pile in a

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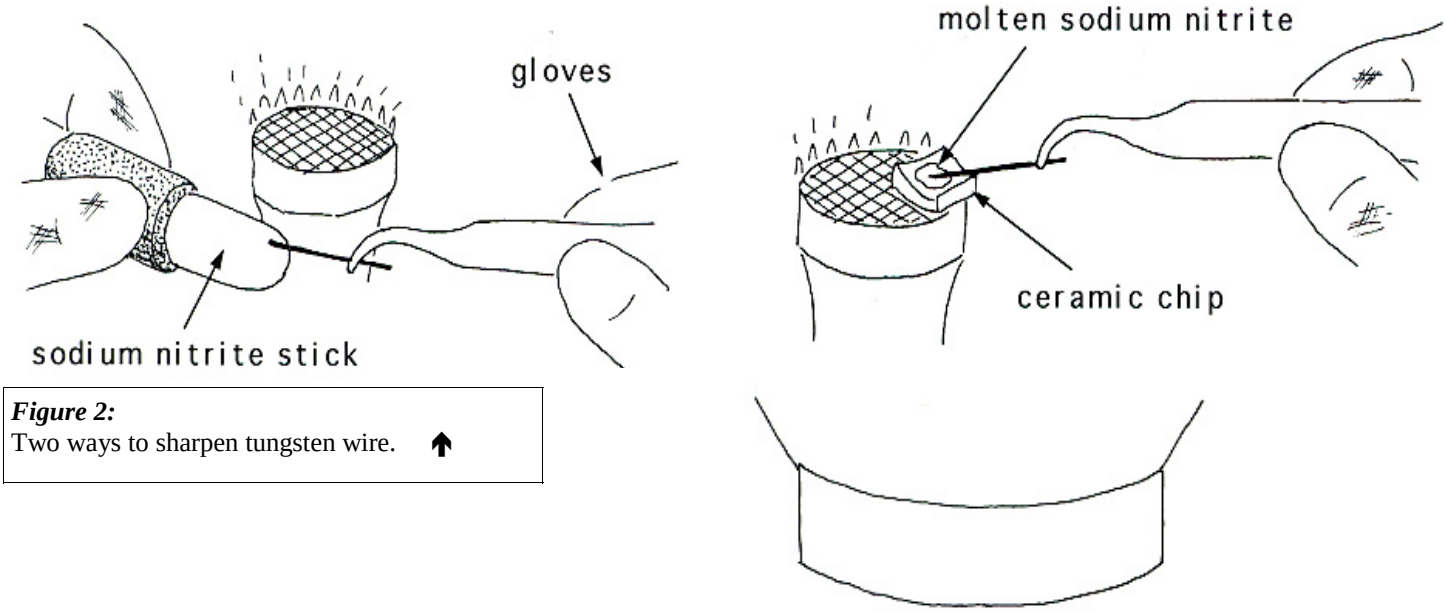

Figure 2:
Two ways to sharpen tungsten wire. ↑

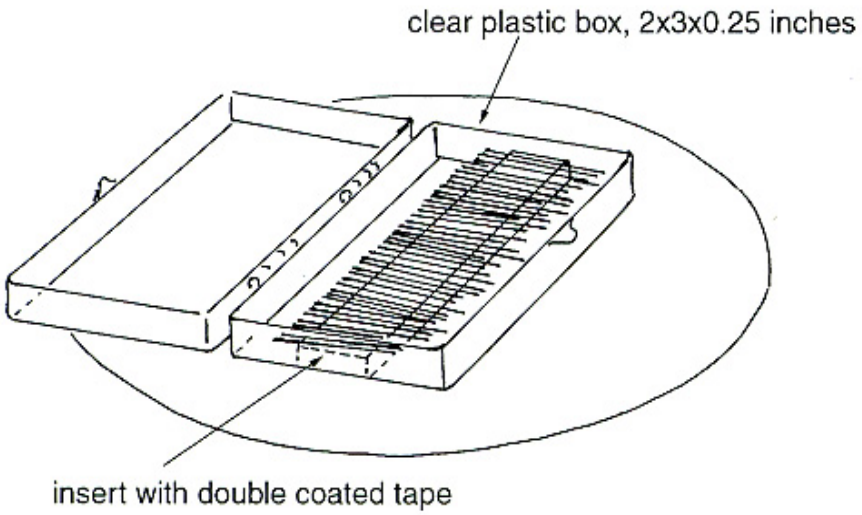

Figure 3:
Proper storage of sharpened tungsten needles.
→

Preparation and Use of Needles and Micropipets for Handling Very Small Particles….*Continued*

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petri dish lined with paper. Excess NaNO2 is removed by flowing a very fine stream of warm water into the petri dish for a few minutes. After the water is decanted, the needles are sorted into the five categories (Fine, Medium, Coarse, Flat and rejects). The good ones are immediately placed into flat clear plastic boxes with an elevated strip of adhesive tape to keep them in place (see Figure 3). The sharpened tips should be in a straight line so that one can compare the tips and make the proper choice of a needle for the task at hand.

A few of the coarse needles can be curved by applying pressure with a tungsten carbide scribe just above the tip as shown in Figure 4.

POLYETHYLENE NEEDLES

Polyethylene needles can be made from high density narrow polyethylene tubing. A 2-3 inch piece of tubing is rotated and heated over an alcohol lamp and pulled out once it has softened. The pulling may have to be done in two or three stages to get a 5 µm tip within 2-3 cm (see Figure 5). These needles are very durable and a few of them can last up to a year of routine use.

EYELASH NEEDLE

A relatively straight Eyelash needle can be made by cutting 3 mm off the tip of an eyelash and attaching it to a medium tungsten needle tip with epoxy. The Eyelash needle can be cleaned by dipping the tip in ethanol or xylene. The needles are also stored, together with the tungsten needles, in clear plastic boxes (see Figure 3).

Table I lists the primary uses for the needles described above.

NEEDLE HOLDERS

The 24-gauge tungsten wire will fit most needle holders. The aluminum needle holders shown in Figure 6 are pre-

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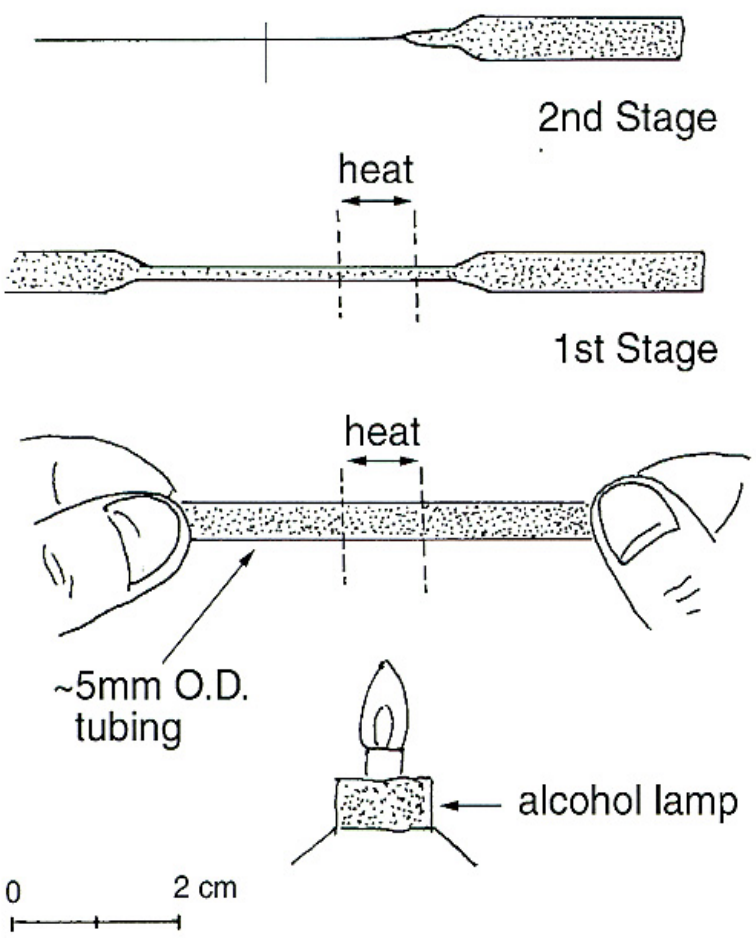

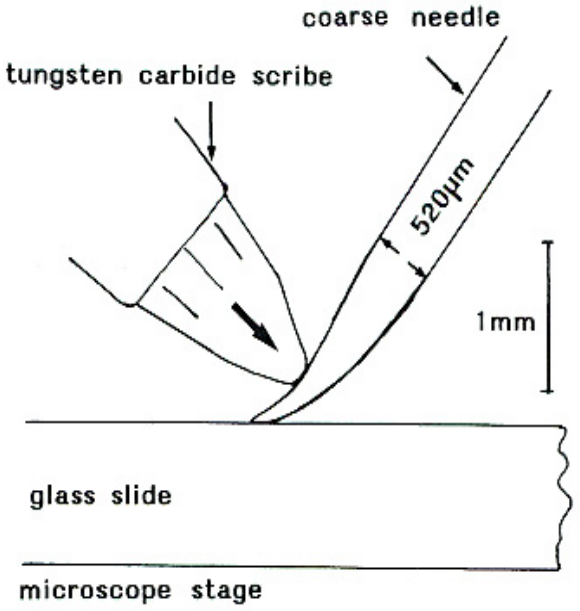

Figure 4:
Making a curved needle for manipulating solvents. ↑

Figure 5:
Making a polyethylene needle. ←

Preparation and Use of Needles and Micropipets for Handling Very Small Particles….*Continued*

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ferred because they are light and have just the right length. A number of them should be on hand, one for each of the various types of needles.

Since polyethylene needles do not fit into these holders, it is helpful to fit them with colorful micro vacuum cups (obtained from an auto supply store) so they can be readily located on the microscope bench (see Figure 7).

Table 1:
Needles for Micro Sample Preparation.

Needle Type	Primary Use
Very Fine Tungsten	Manipulating particles <20 um
Medium tungsten	Manipulating >20 um-100 um
Coarse, curved tungsten	Manipulating drops of solvents
Flat tungsten	Scraping fine residues off
Polyethylene	Performing aqueous extractions
Eyelash	Manipulating samples on very fragile surfaces

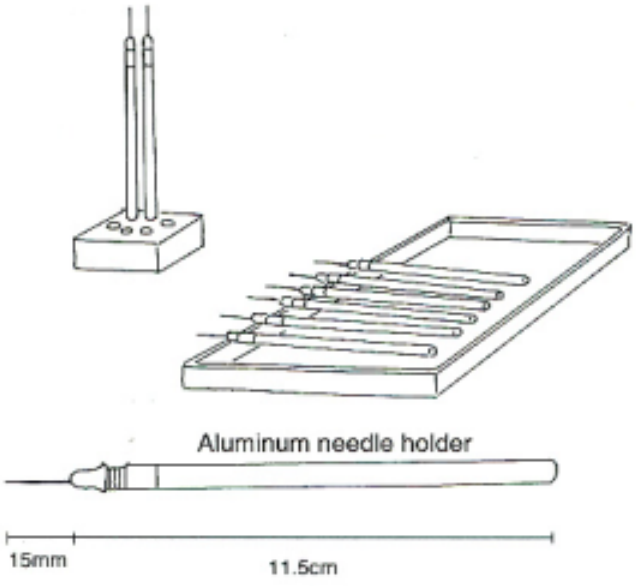

Figure 6:
Needle holder, storage tray, and lucite holder.

TUNGSTEN NEEDLE CLEANING

Before a fresh tungsten needle is used, it should be cleaned thoroughly by passing it a number of times through a needle cleaner made from a sheet of cleanroom paper held by the lid of a small canning jar. (see Figure 8). The paper is wetted and the soft, wet paper cleans the needles very well as they are passed through. Dry paper does not clean as efficiently and may damage a fine tip. By putting a drop of amyl acetate on the paper, one can remove excess collodion from contaminated needles by passing the tips a number of times through the solvent-treated paper fibers.

POLYETHYLENE MICROPIPETS

Polyethylene micropipets are essential for most sample preparation techniques. Because they are very small, they are only used with the stereomicroscope. They deliver small drops of solvent by capillary action and are filled by capillary action as well. They can be made from high density polyethylene tubing by heating the tubing in stages. The procedure is similar to that used in making the polyethylene needles, except the tubing is not fused and the tip is

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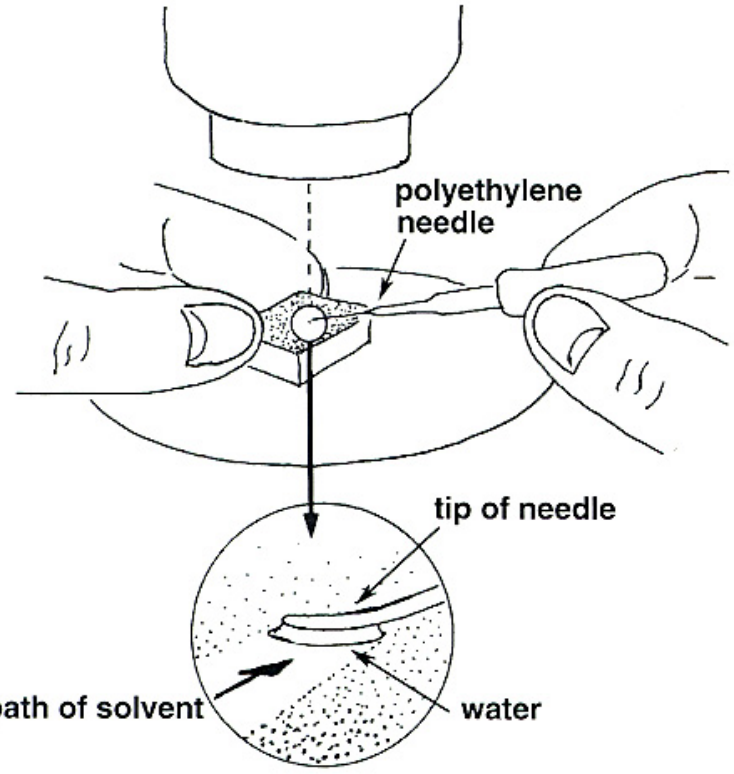

Figure 7:
Extracting a water soluble residue with a polyethylene needle.

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trimmed so that it will deliver a drop of solvent every time it is touched to a surface (see Figures 5 and 9).

These micropipets can also be made from 1-10 µL pipetter tips made of low density polyethylene. They pipets are not as durable as the ones made from high density tubing, but they are much easier to make (see Figure 9) and fit well into our solvent dispensers described below.

The micropipets are designed to be used under a stereomicroscope. They are small and obscure little of the field-of-view. When properly held, the tip of the micropipet will remain in focus. They reliably deliver a small drop of solvent when the tip is touched to a glass slide (see Figure 10). The size of the drop depends on the size of the tip and the amount of liquid in the pipet. The size of the drop can also be controlled by the position of the tungsten needle. As the needle is lowered, it will pull more solvent from the micropipet.

The micropipet can be filled from a 15 mL ground glass bottle by capillary action. It is convenient to have a dozen or more bottles of common solvents on the microscope bench.

A more practical method to keep these micropipets filled with frequently used solvents is to put them in their own solvent dispensers. The solvent dispensers consist of 6 x 50 mm culture tubes placed inside small vials attached to a 1 inch aluminum block. Alternatively, the tubes can be put directly into a heavy plastic block (see Figure 11). It is preferable to have four such dispensers for four micropipets. Keep amyl acetate in one dispenser since it is a good solvent for thin films of flexible collodion used in handling small particles. Nonane is also a good solvent to keep on hand. It does not evaporate quickly, allowing sufficient working time for doing extractions or manipulations. One dispenser should be left empty so that its micropipet can be filled with the desired solvent from a 15 mL bottle. The last dispenser contains n = 1.662 oil. It is convenient to be able to dispense 1 mm drops of the oil when examining micro sam-

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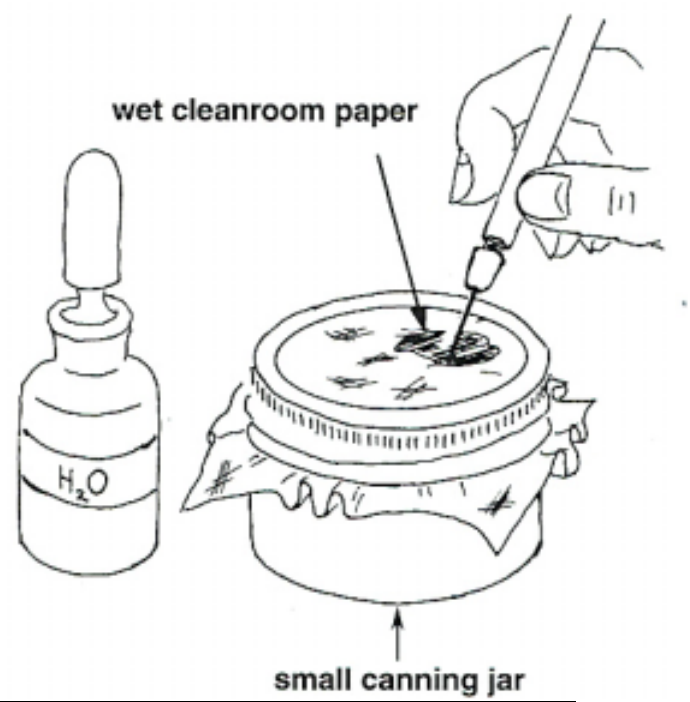

Figure 8:
A Needle cleaner. ➚

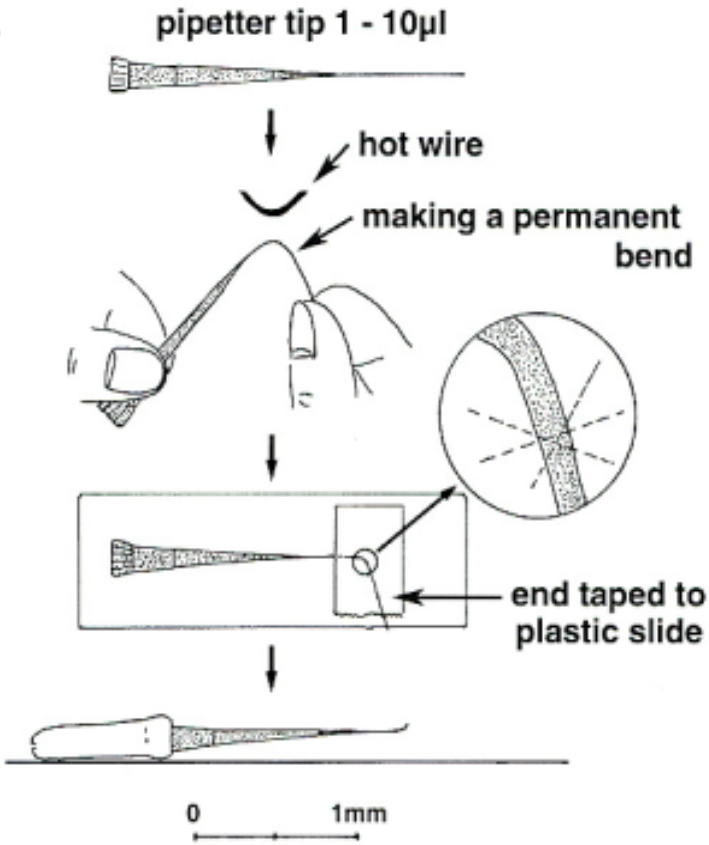

Figure 9:
Micropipets from 1010 uL pipet tips. ➔

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ples under a 1-3 mm coverglass. Again, the micropipets and dispensers can be color-coded using the micro vacuum caps.

The advantage of having these dispensers is that they keep the micropipets filled with solvent all the time and only one hand is required to pick up the pipet. To fill a micropipet from a 15 mL ground glass bottle requires two hands. When manipulating small samples, one may only have one hand free.

Another advantage of these dispensers is that they emit less organic vapors than a 15 mL ground glass bottle that is opened and closed throughout the day. Note that less than one-quarter of the glass vial is filled with solvent. Surpris-

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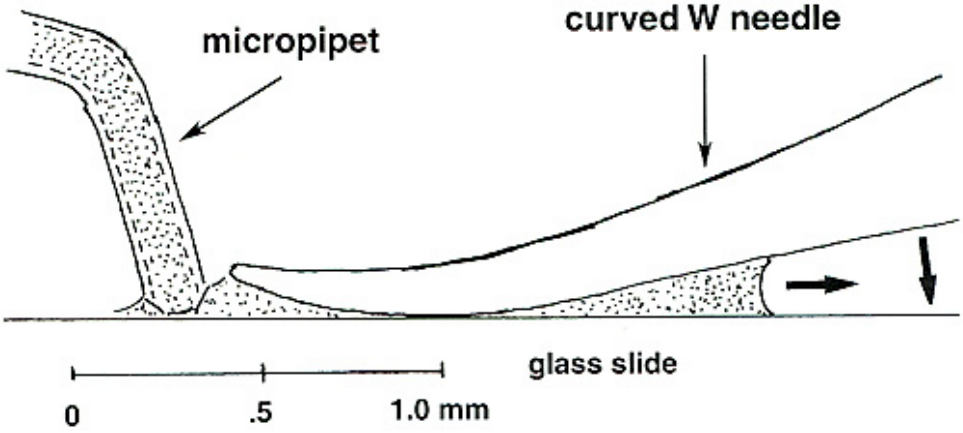

Figure 10:
Increasing the amount of solvent delivered by a micropipet. As the needle is lowered more solvent will flow beneath it..

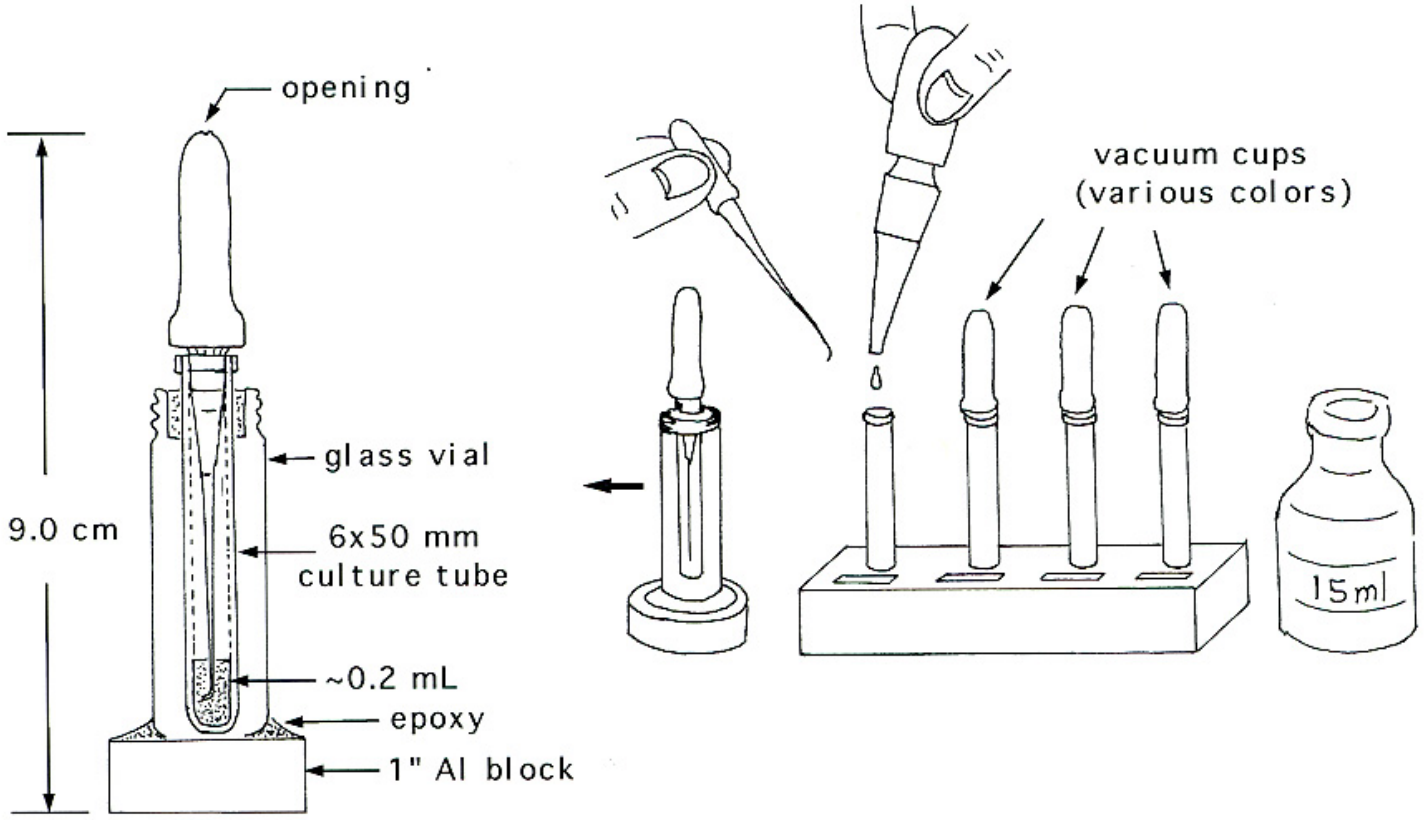

Figure 11:
Solvent dispensers for micropipets.

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ingly, that amount of amyl acetate or nonane will take 2-3 days to evaporate.

A FEW COMMON USES FOR THE VARIOUS TYPES OF NEEDLES

Very Fine tungsten needles are used to pick up a loosely held, 1-10 µm particle directly from a surface and deposit it on a substrate for further analysis. These needles are usually used only once because the tip is frequently damaged in the process.

Medium needles can be used to pick up larger particles, either directly or with soluble gum. Because of their greater strength, these needles are used most frequently. However, they quickly develop slight imperfections which are hard to observe. Since these damaged needles may not release particles properly, they should be changed often, even though they may look undamaged.

The Curved needle is used mainly to manipulate 1 mm drops of solvent on substrates because it can hold a large volume of solvent beneath it due to its large diameter and curved tip. Also, it is used to transfer embedding media for micro replication (see Figure 12).

The Flat needle is ideal for removing fine precipitates from smooth, soft polycarbonate filters. Since the needle has no sharp tip and will not scratch a surface, it can be used like a spatula or a knife. It is very sturdy and can be reused many times (see Figure 13).

The Eyelash needle is used to disperse, without using any solvents, fine powders on a carbon-coated copper grid for analysis by transmission electron microscopy (see figure 14). It requires little skill and gives very nice results. The eyelash needle, unlike the tungsten needle, is not strong enough to break the thin carbon film on the grid. The needle can be rinsed and reused many times .

SOME APPLICATIONS OF THE MICROPIPET AND NEEDLES

The micropipet is not used by itself; it is always used with the needle. This is referred to as the "two-handed technique" (see Figure 15). The two are used together to tack small samples on carbon or beryllium surfaces for further analysis. Frequently, a drop of solvent is all that is necessary to keep a fine powder or a few flakes in place. Larger samples may require a little collodion or soluble gum to hold them down.

Small drops of solvent may help remove a small particle or a fine powder off the tip of a tungsten needle. Groups of small particles can be concentrated or dispersed in small, 1 mm, drops of solvent for further analysis.

Small drops of solvent are used to make approximate solubility checks on nanogram sized samples as shown in Figure 16. The edge of the drop is moved back and forth over the particles, dissolving those that are soluble and leav-

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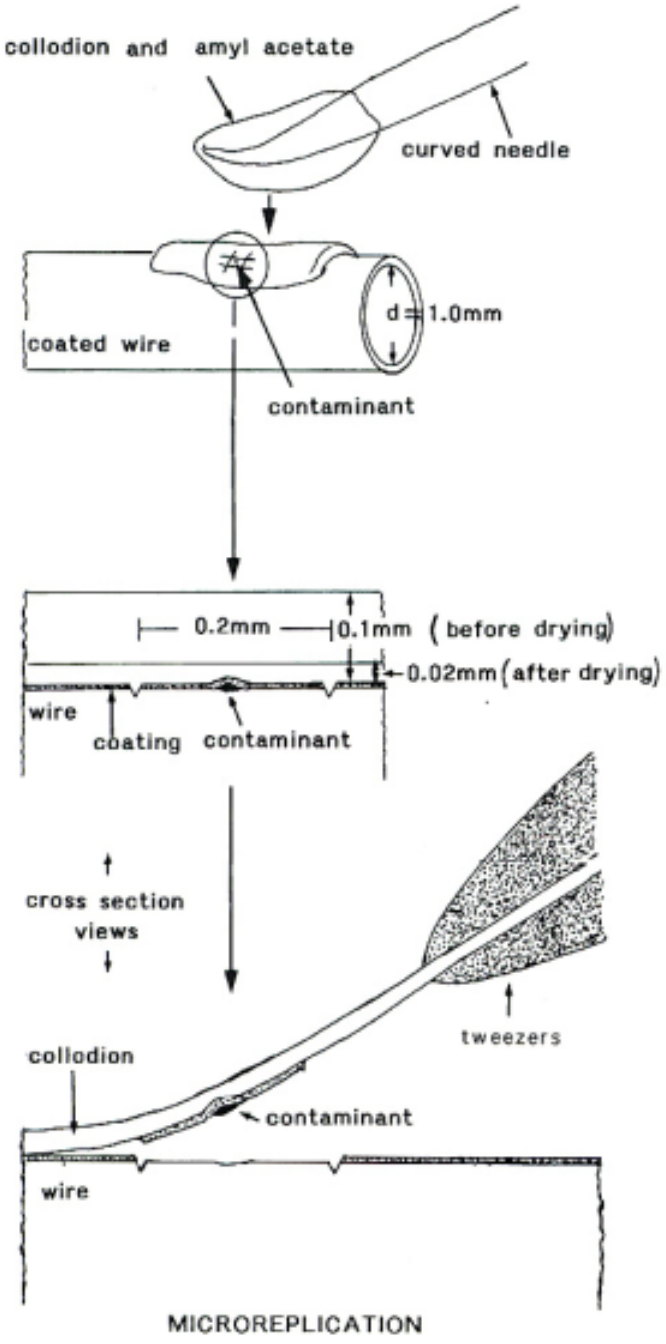

Figure 12:
Removing a small defect with a collodion film.

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ing the others in place. Only the edges of the drop should be used, because the center portion of the drop beneath the tungsten needle has too much turbulence and may dislodge the particles from the glass slide. This makes it difficult to tell if the particles have dissolved or simply moved out of the field-of-view.

The needle and micropipet are ideal for doing micro extraction. Extraction of oil from a small particle serves as an example. A small particle can be placed on a 4 x 5 mm KBr crystal and, with small drops of nonane carefully guided with a needle over the particles, any oil in them can be extracted right on the crystal for IR analysis (see Figure 17). The reason that this extraction works is because the whole drop deposited by the micropipet, as well as the particle, is in the field-of-view at 10 or 20X. One can watch the drop going to dryness and one is able to observe a small amount of oily residue after the nonane evaporates. One can immediately mark the position of the residue with a tungsten needle and run a blank next to the residue by placing a drop of pure solvent to check for a deposit. Large drops, such as those taken directly from the 15 mL ground glass bottles, would not work on the small KBr crystal or any other surface if the sample is 100 times smaller than the drop. Also, it would be difficult to keep the large drop together as it goes to dryness.

Frequently, a tiny piece of polyester filter may be used to remove a micro drop of oil from a hard-to-reach place. To extract the oil from this filter for further analysis, a small drop of nonane is deposited on the surface of the KBr and immediately the filter, held on the tip of a needle, is dipped in the solvent. Most of the oil will remain with the solvent and, as the solvent evaporates, one will see an oily drop appear on the surface of the crystal. The position of the drop is marked with the needle because small drops are hard to relocate once the field-of-view is changed.

These are just some of the ways that tungsten needles and micropipets have been used. There are many more.

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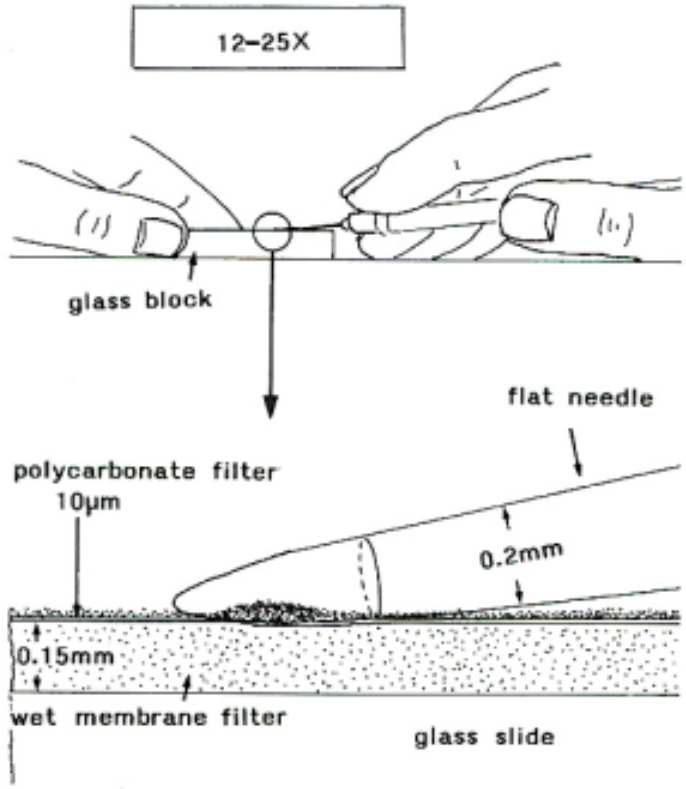

Figure 13:
Collecting nanogram residue from a polycarbonate filter with a flat tungsten needle. ↑

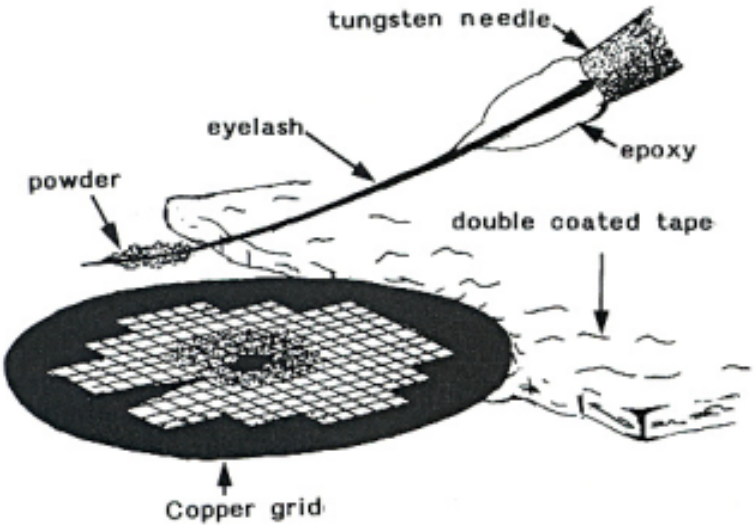

Figure 14:
Dispersing a powder on a copper grid with an eyelash needle. →

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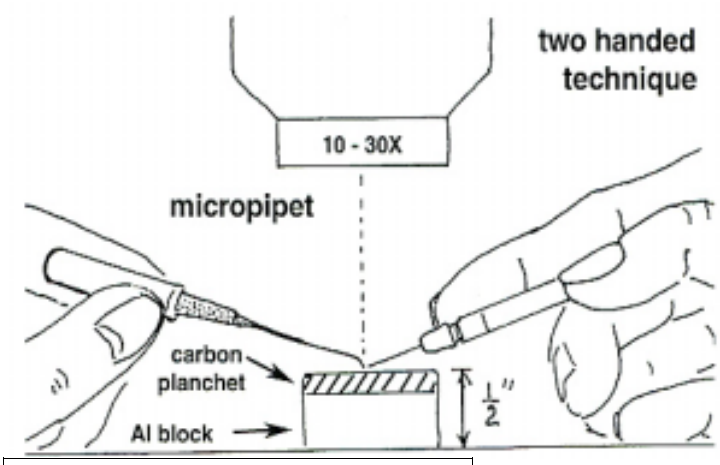

Figure 15:
Preparing a sample for electron microprobe analysis. ↑

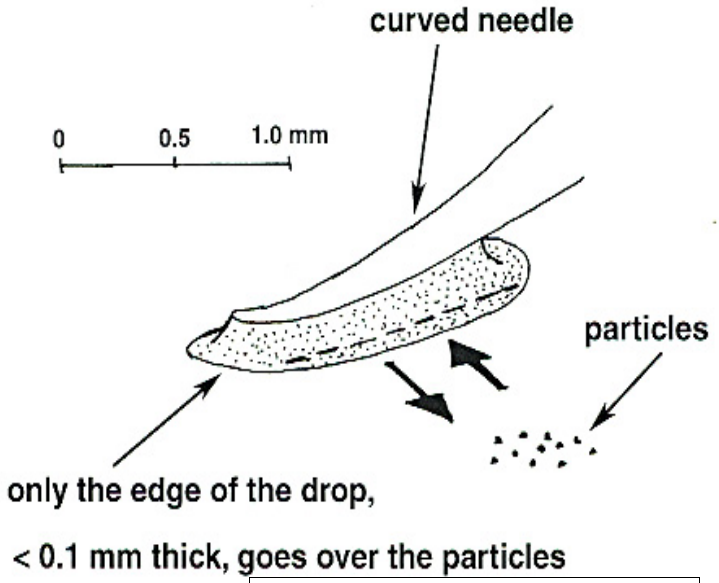

Figure 16:
Checking the solubility of nanogram samples. ↑

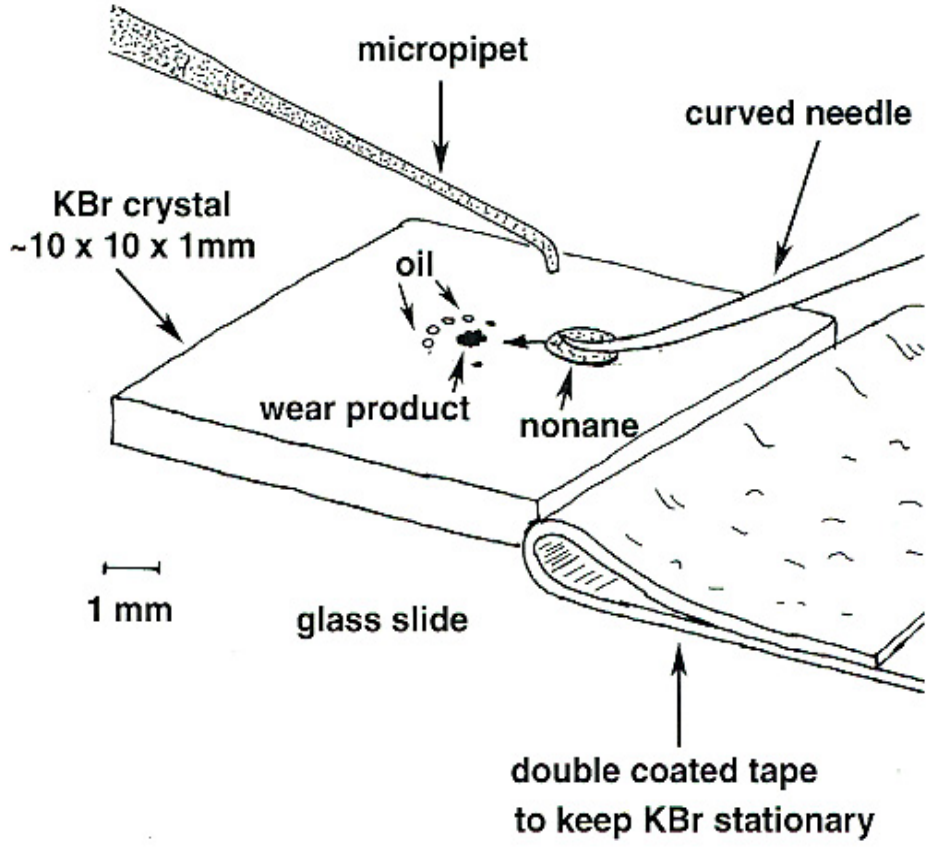

Figure 17:
Extracting a nonane soluble fraction from a wear product.
←

Hair Microscopy in the Age of DNA Testing

While struggling with microscopic hair comparisons over the years, I would be thinking that some day there would be a better way to do this. "Some day" is here. Nuclear DNA testing of hair root tissue is a highly specific and objective method for linking a specific hair to a specific person, albeit only with some hairs; the chances of successful DNA results are increased when less specific mitochondrial DNA testing of the shaft is included. So what happens next? Do we continue to devote resources to microscopic hair comparison? If we don't, what do we lose, and what new sources of error may arise? If we do continue, how should the emphasis change? I addressed some of these issues in two talks I presented to the AAFS at the February 2001 meeting. When this topic arose in discussions with Tim Fallon and Mike Martinez, I sent them the abstracts. Mike wanted to publish them in the newsletter, so here is the abstract of the first talk, along with some words of discussion first.

It is well recognized that many types of information can be provided by microscopy but not by DNA testing. Most microscopists who have worked with human hair would list the following: somatic (body area) origin, the growth stage and any putrefaction of the root; adhering debris, and whether the hair itself is likely to be older debris; chemical treatment and mechanical damage; decomposition and insect damage; and so on. These types of information can provide a time line for the hair deposit and assist in determining its significance. The examinations can be performed even if the microscopist does not have extensive training in morphology-based hair comparison.

Less recognized is a more urgent analytical problem: the selection of samples for DNA testing. Since it is seldom possible with current technology to test every hair via DNA analysis, selection of adequate samples is crucial. Significant error can arise from sampling, so that even if the DNA results are accurate, any interpretation of significance may be skewed if the basis for selecting hairs is faulty. Unless a skillful microscopic examination is performed, the current basis for sample selection is the suitability of the hairs for testing. In other words, the testing determines the sample selection instead of the other way around.

Testing – of any kind - should be performed on samples from materials or stains selected for their potential value as evidence. The deposits, areas, or sets to be sampled should first be selected for their potential significance. It is only then that specific samples should be chosen for their potential specificity or the likelihood of yielding usable results. Error arises when evidential value is conferred on a sample simply because the test is likely to work on that sample. This is equally true of hair deposits or biological stains. When body fluid deposits are sampled for DNA testing, the samples should be selected after some interpretation of the blood or body fluid samples has been performed, even if the interpretation is preliminary. If this is not done, the DNA testing will not provide the desired information about whose blood is associated with which action. Entire stain sets may be missed, especially if they are thin deposits, very tiny, or mixed with other materials, yet these are the stains that may be from an assailant or from another party in the case.

A similar situation obtains with hairs. If hairs from several different individuals are represented on an item of evidence, the sampling process should ensure that at least one hair from each person be sampled, and that the somatic origin be considered. The practices of searching only for hairs with anagen roots, or only for hairs that may be from a specific individual, can lead to potentially misleading results. Each is a potential source of error.

For example, if 15 hairs are found in a stocking cap left at the scene of a crime, two of them may be dark brown hairs having roots with tissue suitable for DNA testing. If these are the only hairs tested, and the DNA is attributed to the dark-haired suspect, no one would know from the DNA report that the remaining 13 lighter hairs are from a different person having chemically treated hair that tends to break off above the root. However, in evaluating which person was last wearing the cap, knowing about the 13 lighter hairs would be essential. A second example is from hairs on the clothing of a homicide victim. A suspect whose hairs were deposited on the clothing may have been with the victim, but at another time, or may simply have been in the same residence. If the hairs are to be used as evidence, it is important to know if there are hairs from anyone else, even if there is no other current suspect.

One way to control for the aforementioned sources of error is to use different criteria for the selection of hairs for DNA testing. Just as the bloodstains in a particular spatter pattern are a logical set from which to select a stain for DNA testing, so microscopically sorted groups of hairs from an item are logical sets from which to select hair samples. A selection based upon microscopic groupings or sets allows for an evaluation of the significance of hairs later linked with specific persons via DNA testing. Grouping the hair requires less time than microscopically comparing all the hairs with control samples, and can form the basis for decisions to perform further testing, whether by microscopic comparison or DNA analysis. Unlike comparisons, microscopic grouping of hairs can be done even without control samples. The questions underlying grouping of hairs are, "do these hairs look as if they could have come from the same person?" and "do they look as if they may have come from different people?" It is what one would do when using secondary controls in a kidnapping or missing-persons case, i.e., hairs from a hairbrush or from the upper clothing. Typically, most of these hairs will be from the person who used the hairbrush and wore the clothing. However, there may be a few stray hairs from someone who borrowed the brush, or from secon-

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Hair Microscopy in the Age of DNA Testing....*Continued*

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dary transfer of another person's hairs adhering to the hair of the person who uses the brush. When deciding which of the hairs to include in a secondary control and which to consider separately, the hair examiner would ask the same questions, i.e., do the hairs look as if they were from the same person or from different people.

The process of grouping described in this article is a sorting process. It is not a comparison process that could lead to a conclusion about common origin. When applied to a representative sample of hairs from the same somatic area of one individual, the grouping process may well yield more than one group. This would also occur with shed hairs collected from a garment or other item. Whether groups of hair originated from one person or several is not important at the sampling stage, as the goal is to have samples that can be described and treated and tested separately. Since each group would be sampled and tested, any overlap would be apparent in the DNA results. If the DNA results are inconclusive, or if there are no hairs in a particular group that are suitable for DNA testing, microscopic comparison could be used instead.

In the example of the hairbrush, the major grouping would be considered a secondary control (i.e., presumed, but not known, to be from a particular individual), and the minor grouping(s) would not. The microscopist would not make a decision about the minor groupings, except to note any correspondence should there be similar hairs from an evidence item. DNA testing of a small number of hairs from each group should be able to resolve whether the major and minor subsets are from one or more individuals.

This same sorting process can be applied to hair from an evidence item. Many hair examiners do this anyway, informally. A grouping process is often reflected in case notes, where the description of one hair refers to another questioned hair. For example: "Hair #2: see description for Hair #1, except darker with denser pigment granules" or "except no medulla". The examiner has grouped Hair #2 with Hair #1 based upon the morphological features described for Hair #1. The grouping process just described could be made more explicit and formal in order to provide a basis for sampling. A good starting point would be to record a detailed description using the terminology for hair characteristics published in the SWGMAT guidelines for human hair comparison. There are usually several groups or sub-sets of hair among the hairs adhering to a piece of clothing, bedding, furniture or carpet. These often represent more than one individual, and one individual may be represented by more than one sub-set or grouping. One hair or several hairs from each group can be subjected to nuclear DNA testing, unless there is a group consisting of hairs without suitable roots. For those hairs, a full microscopic comparison should be performed, whether or not a sample of those hairs would then be subjected to mitochondrial DNA testing. Since m-DNA testing requires the destruction of the hair shaft, any microscopy must be completed beforehand, and should be performed because of the possibility of inconclusive results, heteroplasmy (sub-populations of DNA), or different but maternally related source individuals (which m-DNA would not distinguish).

The grouping approach need not preclude microscopic comparison, which is an efficient method for evaluating large numbers of hairs. However, it would free the microscopist from spending additional hours in struggling with difficult comparisons that can often be resolved by DNA testing. The more modest time investment of grouping hairs would insure that information not be lost or misinterpreted. It would control for biases introduced when sample selection is based primarily upon suitability for DNA testing.

The abstract follows:

Questions of inference: I. Microscopic grouping of hairs and selection of hairs for DNA testing

LEARNING OBJECTIVES: This presentation should stimulate the listener to think about evaluating the significance of hair evidence from the point of view of rudimentary set theory, and to provide a framework for deciding which hairs to test via DNA analysis.

This paper has three objectives: (1) To stimulate thinking about hairs as members of sets; (2) to provide criteria for grouping evidence hairs into sub-sets that may represent different individuals; and 3.) to provide a logical basis for combining microscopy and DNA testing in the analysis and evaluation of hair and its transfer.

The usual subject of forensic comparison of human hair, whether via microscopy or DNA analysis, is the possible origin of the hair from a specific individual. However, when an evidence hair is found to correspond with a hair standard or a DNA standard, it is equally important to evaluate the significance of the correspondence. The questions which arise include 1.) how closely a correspondence of the data correlates with correspondence of origin; 2.) in the case of microscopic comparison, whether the correspondence arises from an overlap of characteristics with hair from a person not represented by the standards; 3.) in the case of DNA testing, whether the hairs tested are representative of the evidence hairs as a group, whether there are sub-groups of hairs not represented in the sample selected for testing, and whether the hairs selected for DNA analysis represent a major or minor contributor to the parent group of evidence hairs; and 4.) whether the evidence hairs were transferred directly from the person of origin, from that person's clothing, or from a more distant intermediary source (the subject of a companion presentation). Each of these questions can be stated as a problem in rudimentary set theory. In considering the various sets of

(Continued on page 12)

Hair Microscopy in the Age of DNA Testing....*Continued*

(Continued from page 11)

which a given hair may be a member, and the sets that the data of examination comprise, the forensic scientist is faced with deciding what constitutes a set.

Although set theory is not yet part of the common parlance in the forensic sciences, forensic scientists make determinations regarding sets in routine decisions about sampling. Forensic hair microscopists typically address this question when sources of secondary hair standards are submitted in cases where primary standards cannot be obtained. When a person is missing and foul play suspected, a hair brush or upper clothing from the missing person is often submitted as a secondary source of that person's hair. In comparing possible evidence hairs with secondary standards, the examiner applies a process of grouping to decide which of the hairs from the secondary source constitute a set that can represent the missing person. It would be useful to articulate criteria for this grouping process so that it could be used more widely.

With the advent of DNA testing of hair tissue and hair shafts, new sources of error are introduced that can affect the interpretation of significance. Significant errors in inference can arise if hairs are selected for DNA testing based primarily upon their suitability for DNA testing rather than upon how well they represent the hairs to be tested. The proportion of hairs on an item having roots and tissue may not be evenly distributed among the source individuals, some of whom may contribute only broken fragments A microscopic grouping process can be used in determining how many subsets of hair are found on specific items of evidence, i.e., approximately how many individuals and which somatic regions (body areas) may be represented as sources of hair. When hairs are selected for DNA testing, it is important to select hairs from each group or sub-set, and if there are no hairs from a given sub-set that are suitable for DNA analysis, to report this separately via rigorous microscopic comparison. Unless each hair on an item is subjected to DNA testing and interpretable results are obtained for most of the hairs, or unless grouping is performed via light microscopy, DNA results alone may be technically correct but misleading.

When a group of hairs shares many morphological characteristics of the type that are often seen throughout a the hairs of a standard and can be considered to typify the standard, one can consider that group to be a sub-set that may be from the same individual, even if there is no standard being used for comparison. The goal of such grouping is not a conclusion about common origin; rather, it is a sorting process for further testing and hypothesis formation. It provides a selection basis for DNA analysis, and if similar groupings of hair are found on other items, provides a basis for evaluating transfer. Whether a sub-set of hair may originate from a certain individual can be addressed by comparison with standards. If only a part of the sub-set from an item exhibits correspondence with a given standard, the examiner would be prompted to request additional standards and to consider the possibility of overlap with hair from another person who is the true source. It is also possible that two sub-sets are from one person.

When a single hair is being compared with the sub-set from a person submitted as a standard, it is sufficient that it lies within the sub-set that the standard comprises. Evidence hairs are rarely compared with all the hair from a given individual, rather, with a sample of hairs from that person. If a hair is just outside the range of a standard, hair examiners. allowing for the limited nature of the standard, may decline to exclude the possibility that the hair could have come from the same person as the standard. A sample is something incomplete, a portion of the whole, and hairs at the extremes of the range of characteristics found in a given individual may not be represented in even a good sample. It is important that these hairs, not only the typical hairs, be tested using DNA. If that is not feasible (the hairs may not be suitable, or DNA results may be inconclusive), additional standards should be requested for microscopic study.

When a number of hairs from an item is being compared with an adequate standard, and there are recognizable groups or sub-sets within the hairs from the item, one would expect few hairs in the sub-set to be outside the range of the standard if the hairs truly originated with the same individual. If the area of overlap of microscopic characteristics is partial only, the examiner does not know whether the sub-set from the item represents more than one individual, or originates with a individual other than the person represented by the standard. DNA testing of hairs both within and outside the overlap of would be useful. If that cannot be done, then a study of transfer may be used to approach this question a different way; this is the subject of a companion presentation.

In summary, potential errors and omissions in interpretation about transfer that may arise from microscopic comparison or DNA analysis of individual hairs can be controlled for by a context-based examination that takes into account subsets of hair and sub-sets of debris from the items being studied. The implication for casework is that the results of comparisons of individual hairs be evaluated in the context of overall transfer, that the primary basis for sample selection for DNA testing or more detailed microscopic examination be the sub-sets of hair on an item, and that results be reported primarily upon the sub-sets rather than upon degree of specificity.

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Requiem for a Hair Examiner

I was once asked by an incredulous attorney, while testifying on the results of a hair comparison, whether my opinion was based solely on having just looked at the hairs. I was completely caught off guard by such a simple question. My reply must have been unremarkable due to the fact that I do not remember my answer though I have never forgotten the question. I was asked that question many years ago. At the time I did not know that it was to portend the current standing forensic hair comparisons has within the criminal justice system. Hair comparisons are treated much like the eccentric uncle that everyone tolerates because he's family but just ignores and wonders how much longer he'll live. I once told a colleague that I would fight to the last for the respect that forensic hair comparisons deserve. And so, I pen my own eulogy.

The microscopical examination of human head hair predates most other modern forensic sciences. Its pedigree is that of the long line of empirical sciences such as taxonomy and astronomy. Throughout history man has acquired useful data by disciplined observation or in the vernacular, by just looking at it. As survival mechanisms, humankind has had to distinguish between harmful and helpful plants, discern seasonal patterns for planting and determine the signs of game in a hunt. Early civilization depended heavily on celestial observation to predict rain and drought cycles. Copernicus and Galileo used their observation of the world to challenge the hierarchy of popular belief. Regardless of the "ology", study began by examining the natural world with our eyes and categorizing the accumulated data with our brains. Not so now. We live in a scientific world of "virtual data". Scientists have become so dependent on data from computers and instruments that we look on with measured disbelief at anything not confirmed by an inanimate object. Before you dismiss my reasoning as the ranting of a Ludite, instruments and computers are extremely useful in measuring and describing our physical world. The question is "who is the master?"

The downfall of forensic hair comparisons came in two assaults. One assault took place when managers, in an effort to save a few bucks, quit hiring trained microscopists and turned hair comparisons over to technicians poorly trained in microscopy. These technicians are criminalists principally working in other areas. Most of these technicians that I have encountered have little interest in doing hair comparisons and rarely were confident enough in their own ability to testify in a cognitive science. The technician hair examiner pooh-poohed the science and sought ways to undermine its value. The second assault appeared in the form of the zealot hair examiner. The true believers that overstated the science with hyperbolic testimony such as "this match is so unique that in all my years as a hair examiner, I've never seen hairs look like these." The zealot, by overstating the microscopical data, set hair comparison on a collision course with disaster. Prosecutors used zealots to convict persons on no more evidence than a single hair. When this type of testimony was finally held up to rigorous scientific scrutiny, the hair-examining technician that pooh-poohed the analysis moved in for the kill.

Forensic hair comparisons, when done properly by a trained examiner, have valuable information to offer an investigation. Hair comparisons are not a positive means of identification. A positive association must be explained clearly so as not to either over emphasize or diminish its value. Unfortunately, the very few respected microscopists doing hair comparisons such as Barry Gaudette, Peter De Forest, Hal Deadman, Dick Bisbing, Chesterene Cwiklik and Ann Reed were drowned out by the chorus of naysayers many years ago.

Where do we go from here? There is nowhere to go but DNA technology. Some forensic scientists, unwilling to do the demanding work of microscopy, have finally gotten their wish. Hair can now be treated like any other biological sample; one kit, one technology, many uses. The investigative value of DNA profiles is far superior to hair comparisons. To continue microscopical examinations on human hair is a waste of resources. I do not even advocate screening hairs, as do Houck and Adams. Hairs should be treated solely as biological evidence relative to the DNA profiles that can be developed. Hair examinations should be phased out completely. Not because hair comparisons have lost their scientific value but because the false perception in the courts and within the general forensic community is that human hair comparisons are unreliable. We may trust our lives to a clinical pathologist who by microscopically examining a biopsy sample determines whether we have a malignant cancer but we cannot trust a well trained forensic microscopist to examine hairs for their value in a criminal investigation. After all, both the pathologist and the forensic microscopist "just look at it."

It is appropriate that in the year that arguably the greatest microscopist ever, Walter McCrone, passed away, I've thrown in the towel. So, stow the slides, cap the mounting medium, turn out the light, there's no one left to fight. Transfer the samples to DNA and let the serologists deal with it. I'm done.

Timothy C. Fallon
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Bexar County Criminal Investigation Laboratory
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San Antonio, Texas
78229-4565

FYI!

A reminder of upcoming events:

SEM Image of a Telogen Root End Human Hair.
IAMA Collection

2003 Meeting - Columbus, OH - October 18-24, 2003
MAFS 2003 FALL MEETING
Hyatt Regency, Greater Columbus Convention Center, Columbus, Ohio
Local Arrangements Co-chairs: Jennifer Duvall and Diane Larson
Program Chair: G. Michele Yezzo
Sail into Columbus ... and see what there is to discover!

The Forensic Labs in the Fort Worth Area
would like to invite you to
"The City Where the West Begins"
SWAFS 2003 Training Conference 2003 in
Ft. Worth, TX
November 3rd-6th
815 Main Street, Fort Worth
800-333-3333
www.radisson.com/ftworthtx
The conference will be held at the Radisson Plaza Hotel, 20 minutes from DFW Airport, walking distance to The Bass Hall, Barnes and Noble Bookstore, two multi-screen AMC theatres and many other unique shops and restaurants. The Fort Worth Trolley provides easy access to the Historic Stockyards and the Art District.

A nightly room rate of $80.00/single-$170.00/quad has been secured.
The final agenda will be provided with the registration packet sent to all SWAFS members in the next few months.
For more information contact**:** Michelle O'Neal,
Tarrant County Medical Examiners Office
817-920-5700 ext 163

It seems no more than right that men should seize time by the forelock, for the rude old fellow, sooner or later, pulls all their hair out.
George Dennison Prentice, Prenticeana, 1860

IAMA Focus and Membership Form On-Line

Anagen Root End Human Hair.
Tim Fallon

IAMA has recently expanded its focus from providing its subscribers with free newsletter publications regarding primer gunshot residue (P-GSR) to addressing all aspects of forensic microscopy in its newsletters to registered IAMA members. In order to create a membership registry, existing subscribers who wish to continue receiving the IAMA newsletter must complete a membership application.

Upon completion, the registered member will be provided with a unique user name and password that will all allow the user to access the secured members only publication web page section, (http://www.iamaweb.com/newsletters/publication.html), containing the current IAMA newsletter, archived newsletters and additional resources. You may download additional applications from the link provided below:

http://www.iamaweb.com/membership/membership.html

Thank you for your support.

Michael V. Martinez
IAMA Founder
Bexar Co. Criminal Investigation Laboratory
webmaster@iamaweb.com

International Association for Microanalysis (IAMA)

APPLICATION FORM

Directions:

1. Complete application form. Only completed forms will be processed.
2. Enclose a $10.00 U.S. nonrefundable application fee made payable to "IAMA" along with the completed application and forward to the Membership Chairperson at the address shown below.

Michael V. Martinez
Bexar County Criminal Investigation Laboratory
7337 Louis Pasteur
San Antonio, Texas 78229-4565

Application as: **Regular Member** ❒ **Associate Member** ❒ **Student Member** ❒
(Checkmark Applicable Level)

Name:_____DOB:_____

Title:_____ Date of Employment:_____

Employer:_____

Mailing Information: *(Fill in Preferred Mailing Address)*

Business ❒ Home ❒
(check one)

Address:_____

City: _____ State: _____ Zip Code:_____

Country: _____

Country Code:_____ Phone: (___)_____ Fax : (___)_____

Email: _____

Supervisor's Name: _____

Title: _____

Applicant's Area of Expertise: *(Check All That Apply)*

Biology ❒	**Breath Alcohol** ❒	**Crime Scene** ❒	**Documents** ❒
Drugs ❒	**Firearms** ❒	**Latent Prints** ❒	**Management** ❒
Toxicology ❒	**Photography** ❒	**Trace** ❒	**Training** ❒

Submit a Curriculum Vitae or Resume Outlining the Following:

Education
Professional Employment History
Professional Training
Professional Organization Memberships

References: *(Must be Regular or Distinguished Members. **REQUIRED FOR APPLICATION PROCESS**)*

Name	Employer	Telephone
1.		
2.		

Signature: _____ **Date:**_____

I certify that to the best of my knowledge all of the statements contained herein and on any attachments are true, correct, complete, and made in good faith.

International Association forMicroAnalysis

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A NEWSLETTER FOR FORENSIC EXPERTS IN MICROANALYSIS

TO: