# WORKING WITH DIATOMS

Techniques and Mounting Methods Explained

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Mounting diatoms can be challenging, especially when using the high refractive media needed to obtain good images. Some of you may be aware of the mounting method suggested by Bill Daily and the use of his brand new Zrax. You can obtain very good and well-cleaned fossil diatoms from Bill upon request (contact him at: HYPERLINK "mailto:dailey@sas.upenn.edu" dailey@sas.upenn.edu). First let me say that Zrax is a wonderful mounting media and you will probably not find better. Some people find the high refractive mounting media hard to work with, the Naphrax and Hyrax of the past were just as difficult so there is no escape to an easier mounting media. However, I do use it a little differently then Bill does with somewhat the same results. The first thing you need to do is perfect the strewn mounts and not go for the arranged slides quite yet. You can certainly get to that a bit later. You first need to get very comfortable with the use of the mounting media. Most of the time when first using the Zrax the diatoms go in all different direction. Ultimately, when arranging individual diatoms you will need to apply the use of a mounting adhesive (fixative), which sets the diatoms in place before applying the mounting media. Later on we will talk about a formula that can be used for this purpose. First let me try and walk you through a strewn slide using the method that I have developed.

To begin with, always mount your diatoms on the cover glass and not the slip. Best optical results are obtained if the diatoms are attached to the cover and not sunk down to the bottom of the media. You will need to suspend your cleaned diatoms in distilled (di) water and collect a drop with a pipette. The drop should not be to thick or crowded with diatoms as they will end up on top of each other. Proper suspension in the di water is needed in this case, that is why you maintain in your "working" vial only about a 1/16 of an inch of cleaned diatoms on the bottom of the vial. With the vial 2/3ed filled with di water, suspend the sample by shaking and collect with pipette. By the way my vials are about 2.5 inched tall and 5/8ths wide (basically). Add your small drop to the cove and then add a drop of (I use) 91% alcohol to the diatom drop. The alcohol will break the water tension and allow the diatoms to spread properly. I often use at least two drops of the alcohol to get the job done as you should end up with a spread that covers all or most of the cover. The cover should be sitting on a slide that can be readily transferred to a hot plate (or held over an alcohol flame). Leave on the hot plate until the diatoms are very dry and fixed firmly to the cover. In the mean time take

a cleaned slide and set it out on a smooth working surface in front of you. Apply to the very center of the slide a small drop of Zrax. By the way I always use round covers!! Important, the consistency of the Zrax should be that of normal syrup (reduced in toluene), not the thick slow moving consistency in which it comes. One additional small point is that a glass rod can be used to apply the Zrax to the slip. Now take the slide that is holding the cover with its fixed diatoms and place it near the slide with the drop of Zrax. With a very fine pair of tweezers (I use the kind that when you squeeze the points open instead of close) and nudge the cove to the edge of the slide. Using the tweezers lift the cove and turn it in preparation for mounting on the Zrax. With one finger to the left of the Zrax, (this to help stabilize the cover in the proper place) lower the cover onto the drop very carefully and slowly. It will come down at an angle as the left side of the cover is already in contact with the surface of the slide and held in place by your finger (left hand finger, this all assuming you are right handed). You will now have a cover that should have mounting media spread to the edges but not extending out under the margins of the cover at any point. Next, pick up the slide and place it on the hot plate. This is the tricky part; I keep the slide only about half way on the plate. Allow the Zrax to very gently begin to boil, I know this sounds a bit strange but try it. The Zrax should boil for long enough to work out all the bubbles (a minute or so). It should stop or slow down at some specific point. Do not let it boil violently as you may then have little splashes on the outer margins of the cover. It is quite helpful to hold the slide with a wooden cloths pen or spring slide holder, as the glass can get a bit warm. I next put the slide on a cold ceramic tile and it quickly cools and hardens. You can now turn the slide upside down if you wish, it can still be smeared or moved if to much pressure is applied but you will now have a very firm and good mount ready for study under the microscope. Note: when you are finished with your mounting session you can rapidly bring the finished mount to maturity by turning the hot-plate to a very low setting and leave the mount on top for an over night curing period. The hot plate should be able tolerate a touch by the finger. The next morning the slide can then be placed on the cold tile and left to cool.

All of this may take a few tries and a bit of practice but believe me this is the best way to go, as I have been doing this for 25 years. Practice this for a while and I will then tell you the (secret) method for mounting arranged diatoms. If you don't have a hot plate then the "careful" use of an alcohol lamp will do the trick. If you don’t leave the slide on the hot plate overnight it will after a few weeks in storage be ready for ringing. If you have a ring-table you will be able to apply with a 000 brush a ring of black shellac around the outer margin of the cover. This is done after the slide is centered and spun on the ring-table. If you cannot fined black

shellac you can add a bit of nicrosin to normal shellac. The compound needs to be made of dewaxed and degummed shellac flakes dissolved into a syrup by Methyl Alcohol. If that cannot be found then just black enamel paint will do, but the mounting media on your slide must be perfectly dry and cured in order to use paint that contains xylem. Shellac is reduced in alcohol and will not penetrate the mounting media or in this case the Zrax.

**Fixative for mounting individual or arranged diatoms.**

In the last session we talking about mounting strews of diatoms and then finishing the preparation for permanent storage. I did not go into the points of labeling, as I'm sure most of you need not be lectured on such basic maters. For a proper fixative I would not recommend gum of tragacanth, as I have never had much success with it. It is intended more for dry mounts such as forams, seeds and insect-eggs. It is also popular for setting rings for mounting cells.

Now that you have thoroughly practiced and perfected the art of making strewn mounts we can now talk a little about mounting single or arranged diatoms. The recommended fixative for adhering the diatoms firmly to the cove-glass consists of a mixture of agents that fortunately still remain available to the amateur. It can be described as a Gelatin-Acetic Acid-Ethyl Alcohol mixture and is prepared as follows.

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| Gelatin | 6 grams |
| Distilled Water | 50 grams |
| Glacial Acetic Acid | 50 grams |
| Ethyl Alcohal | 8 grams |

Place the Gelatin and Distilled Water in a 200ml flask, then place the flask in a hot water bath and agitate until the Gelatin is in solution. When cool add the Acetic Acid and Alcohol. Filter through filter paper, discarding the first few drops that pass through the filter. The fixative is stored in tightly stoppered bottles, filtered occasionally.

The procedure for mounting one or more diatoms on a cover presents only a little more difficulty then mounting strews with the addition of some manipulative techniques. After the covers have been cleaned and dried a very thin layer of fixative is needed. This is rolled over the cover by holding the dried cover between the thumb and index finger and rolling a thin glass rod previously dipped into the fixative. If you intend to fix the diatoms to the 1x3 slide then you

can also add a tiny drop to the center and with a second slide spread it over the surface (spread much like a blood smear). Once the fixative is spread it needs to be left to dry completely. One thing I would suggest is that before applying the fixative you should create a small black guide ring. What I do is use a true ink pen such as the Koh-I-Noor Rapidograph pen (No. 0/.35), you can use regular India ink. A slide is centered on a turn-table and spun, the ink is added to the very center and aloud to thoroughly dry. The ink circle should be about 1/8 inch in diameter when finished. You can make what ever size you like but the idea is to use the circle as a guide for quickly finding the diatom when placing the slide under the microscope. If you intend to make a large arrangement then the circle guide is not needed.

Before you can arrange a selection of diatoms you must prepare a storage slide. All the diatoms you intend to use must first be made ready for quick access. They should be assembled on a normal 1x3 slide that has been thoroughly cleaned. The working material if stored in di water should be available and will be your original source. With the storage slide on a warming table, add several drops of distilled water, then several drops of your working solution (a pasture pipette is needed for the transfer of material). The liquid is gradually evaporated with the aid of the alcohol burner and allowed to completely dry. The dried diatoms that have been distributed over the slide are searched and the forms you need are found then picked up with your bristle and transferred to the storage slide. Sometimes if you have trouble with the forms sticking to the glass you can cement a thin sheet of mica in place, diatoms normally do not stick to this material. It is also a very good idea to have the surface of the storage slide covered with a piece of mica. If you store your original material dry then the above is a bit simpler. You can simply spread the dried forms on you pick slide and move them to your storage slide. I do all of this work under the stereoscope and don't bother with a mechanical finger, as no precision work is needed. You now place both the pick slide and the storage slide together on the surface of a flat stereomicroscope stage. The two slides are held between the thumb and the middle finger with the three-inch sides held together. As a unit they are carefully moved back and forth under the axis of the optical path of the scope. Your hand bristle (wooden stick with one end having a cut flat surface and a bristle attached with beeswax) is only moved up and down as you bring the pick slide into place. While looking through the scope you center a chosen form and lower the bristle until it come in contact with your choice. The diatom should be easily picked up and held only about two millimeters above the slide. You now move both slides together in the opposite direction and deposit your choice on the storage slide. The idea is to simple have usable forms ready and quickly available. You can help your self by trying to

deposit your selections in some sensible order, this being centric forms on one side and pennate forms on the other. A well-seasoned worker will actually place a collection of the same species together in groups. This speeds up the selection process when making the final arrangement. The storage slide is kept in such a way that the samples are protected from dust and passing gusts of air. If you cut out a 1 by 3 piece of 1/16" thick cardboard and glue additional 1 by 2/3 inch pieces on each end you will have a bridge that is always kept over your storage slide. Even when storing in the slide cabinet it is a good idea to keep the storage slide covered with this additional protection. Each cardboard cover and storage slide should be numbered and a note book or card file maintained to describe the source and type of diatoms available.

Once your stored material is ready and available you can begin the process of making the arrangement (or individually mounted form). If the storage slide has a sheet of mica about the thickness of a cover you will have no trouble with focusing the microscope when actually making the arrangement. At this point it is best to use the compound microscope and what every mechanical finger you have available. By the way, this can also be done with a bristle and the careful up and down movement of the hand. But it takes a great deal of practice and probably a bit of natural skill. Success comes much sooner with a proper mechanical finger. You must begin with a perfectly clean No.1 cover-glass, sometimes best cleaned in a Sulphuric Acid-Potassium Bi-chromate mixture. The slips then need to be rinsed in several changes of tap water to eliminate all traces of acid, then in two or more changes of distilled water. The covers, now chemically clean, are stored in distilled water and handled only by their edges. If you add small guild rings the slip is allowed to dry, the rings added and then stored under cover waiting for use. Next the fixative is added to the cove as described above. What need to be done next is to develop a method of temporarily securing the cover to a normal slide. What I have done in the past is now place a very tiny bit of petroleum jelly (Vaseline) on the back of the cover and seat it in the center of a 1 by 3 slide. This is done to make sure the cove stays in place when moving the slide back and forth. This must be the tiniest spot of jelly as you will need to be able to easily lift the cove from the slide when you are done. You now place both the target and the storage slide together on the surface of a flat microscope stage. The two slides are held between the thumb and the middle finger with the three-inch sides together (they are now a pair). As a unit they are carefully moved back and forth under the axis of the optical path of the scope. The mechanical finger is only moved up and down as you bring the storage slide into place. While looking through the scope you center a chosen form and lower the bristle until it come in contact with your choice. The diatom

should be easily picked up and held only about two millimeters above the slide. With your fingers you move both slides together in the opposite direction and center your target slide, which is holding the cover. Depending on the orientation of the diatom held by the bristle, you might turn the target slide to an angle that best suits the lowering of your chosen form. The diatom is lowered onto the cover, set in place and the process then repeated. After a few deposits you need to carefully hold the target slide in a position you can gently exhale on the mount. This gentle puff of moist air from the lungs will help soften the fixative and seat the forms (the diatoms will not properly seat without this action). The above process can go on until your satisfied with your arrangement. Sometimes I have found it helpful to gently heat the work when your completely done with the arrangement. Care needs to be taken, as only the slightest heat is needed. Bear in mind that your work will greatly improve with time and effort.

To finish your preparation you will need to permanently mount your work. The target slide needs to be placed in a position that allows the worker very comfortable access. I like to have the slide on a flat surface with a hand rest just off to the right side, this helps maintain greater control over the glass rod when applying the mounting media. You should have an application rod that has a very small bead or ball at the end. The ball of glass should be a little bigger then a 1/16 of an inch but not as big as a 1/8. You will need to place a small drop of Zrax media very gently on the arrangement. The drop should be released as close to the arrangement as possible. The result should be a drop that covers an area of about $1/8^{th}$ of an inch; if it were a little bigger it would not be a problem. The main idea is to avoid disturbing the arrangement; the slide is then left on the warming table so the solvent (toluene) can be driven off. Very gentle, and I mean gentle boiling should not bother the arrangement. You may in fact spend more time lifting one end of the slide off the table then flat giving the slide relief from the heat source. If you wish to be extra cautious you can reduce the heat on the table below its ability to cause boiling and let the drop stand for a while. Occasionally, some of the diatoms will retain air inside the frustules. This is due to the fact that the media is not able to migrate into the diatom. With the use of adequate heat and more aggressive boiling this is not much of a difficulty but with an arrangement you must be very gentle. What can help in this situation is if you apply a very tiny drop of toluene over the arrangement before applying the Zrax. You should also remember that the fixative must no longer have any moisture, as this will cause problems associated with the mounting media much later on. This can be recognized by the development of extremely tiny bubbles quite some time after the slide has been in storage. If moisture is a problem you can add a few drops of toluene to the mount and then let it evaporate. This should

eliminate any remaining moisture but you will need to then add the "tiny" drop just before mounting. The fresh toluene helps draw in the media into the diatoms. If your not having trouble with internal bubbles then avoid this procedure.

The next step requires that you apply a second drop of mounting media to the very center of a clean slide (the final mount). It helps if you have a template that is the same shape of the slide with a cross-hair indicating the proper center. Around the cross-hair you can draw a circle the same size as the anticipated cover-glass. This drop should be a little bigger then the drop over the arrangement and should be calculated to fill the cove once it is merged with the drop that is already on the cover. The best way to merge the two drops together is to position your new slide upside down and above the slide with the cover (target slide) allowing one end of the new slide to rest at the bottom edge of the target slide and slowly lower the other end until the two drops come in contact. The cover can then be gently lifted off the target slide and is now in contact with the drop on the second slide. The two-drops have a bit of an hourglass configuration, and can be merged by placing the slide on the hot plate. Enough heat is used to soften the media to the point the two drops become one and spread completely under the cover. Any bubbles can be driven off with the application of gentle heat. You will wish to avoid vigorous boiling of course. After the slide cools and is allowed to sit over night you can clean the top surface with a cotton swab and a bit of acetone. In a few days you can ring the slide and add the proper label.

As a final word, it is my hope that the above methods can be used to guild your efforts in a direction that permits great success. However, if you are like most workers you will ultimately make adjustments and modifications that best suet your own needs and level of skill. The natural beauty of diatoms leaves you with much to work with and affords great potential for aesthetic achievement.

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