Diatom Arranging - Different Strokes

By Steve Beats - June 2015

Introduction

I've been into diatoms almost as long as I've been into microscopy; something like [cough] forty [cough] five years. I used to collect antique slides, including diatom arrangements, but prices rose steeply a few years ago and I couldn't afford them any more. The desire to buy new equipment outweighed the desire to buy slides, and the hobby fund could only stretch so far.

In fact, prices got so "good", I decided to sell my collection to raise cash for more accessories and upgrades. I spent too much time looking at the same slides over and over again anyway, so they weren't hard to sell. I thought I'd regret selling the diatom arrangements though, so I elected to hang on to those even though they fetched the best prices.

Then I found Raymond Hummelink's excellent Micscape article on making selected diatom slides. This led me to believe that with some practice I could make arrangements too. So I sold my remaining slides with a promise that I'd learn the art and make replacements of my own. It's all Raymond's fault!

That was a little over two years ago. Sure enough, I can make arrangements now, but it took rather more than "some" practice. Two years and 2000 hours of effort later I'm still learning, still practicing, and still making mistakes. But it's a truly satisfying (and ongoing) experience that I'd strongly encourage others to try.

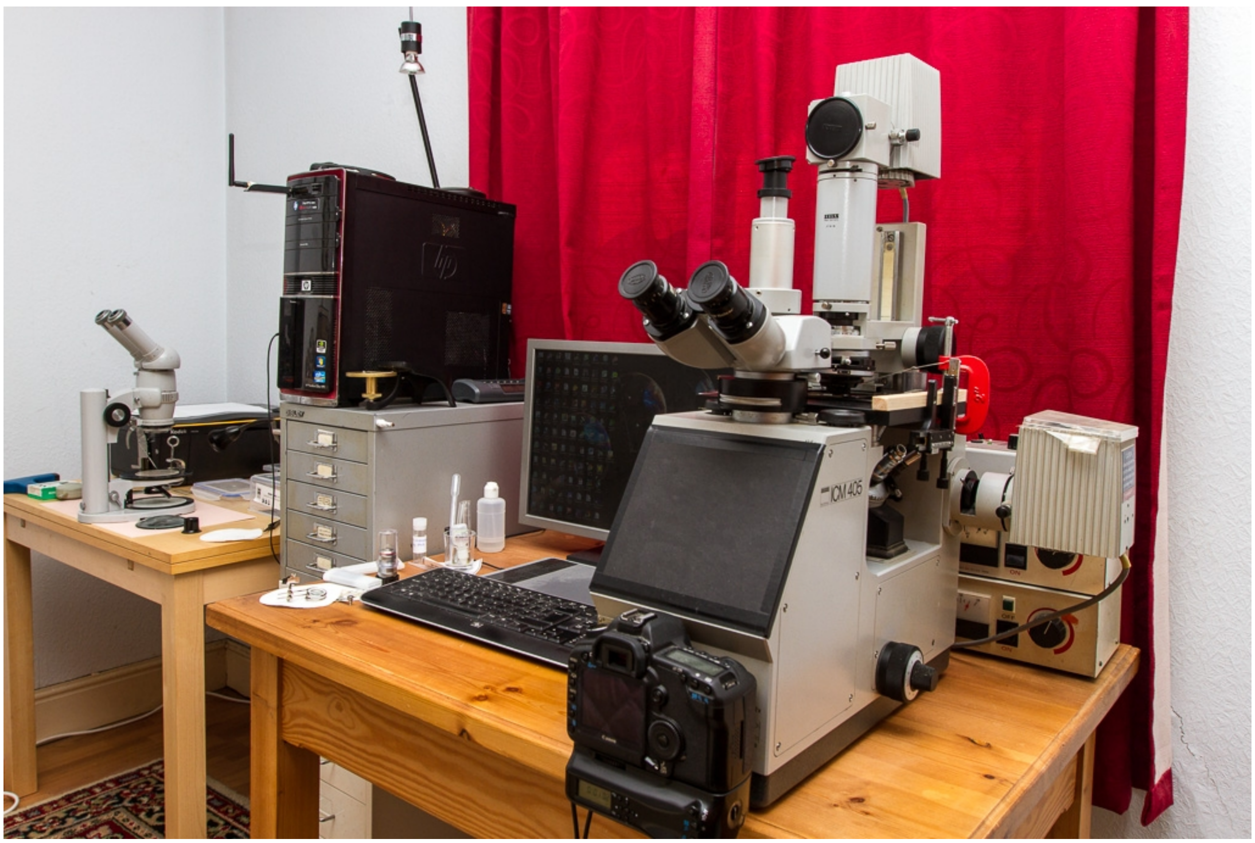
This article is not a "how to" guide (see Raymond's article and other sources listed in the references for that). Rather, it is a grab-bag description of some approaches I developed while learning the craft, interspersed with images of my work. I've tried to describe a few aspects that I haven't seen documented elsewhere in the hope this will be of interest to those already mounting diatoms as well as those who'd like to try.

Main Equipment

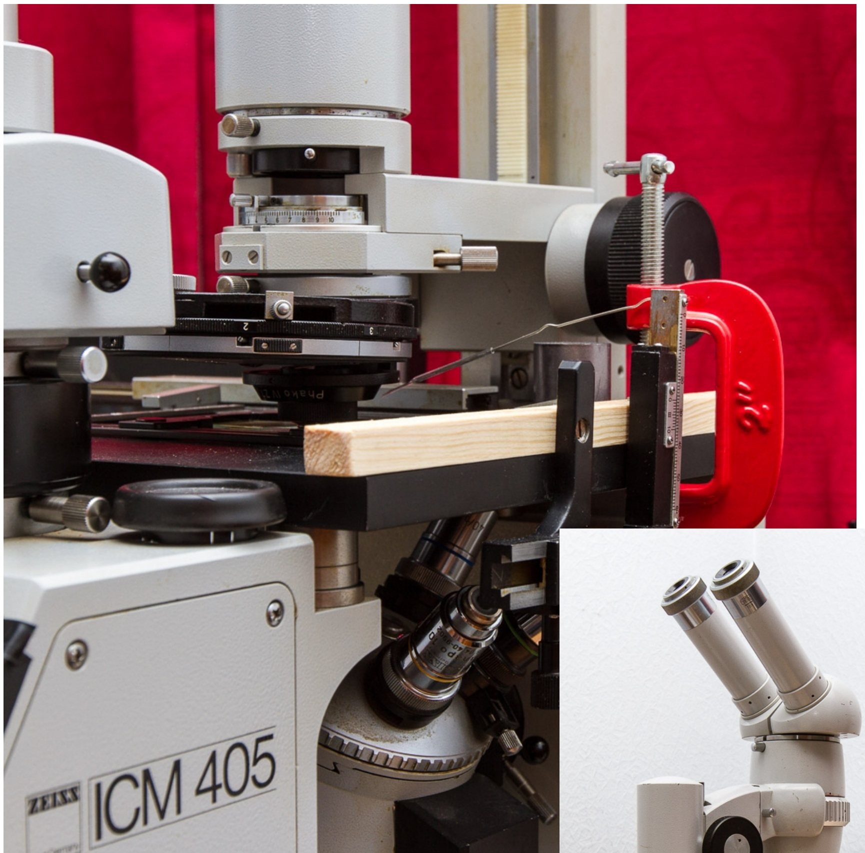
Assuming you already have cleaned material, the only tools you need for making diatom arrangements are a micromanipulator, a microscope and a fixative-coated coverslip to arrange the diatoms on (plus the usual equipment for making mounted slides of course). Almost any microscope will do, but here's what I use.

My micromanipulator is almost exactly as described in Raymond's original article. A glass needle (instead of a bristle) on a straightened paper clip attached to an old x,y stage mechanism. This, in turn, is attached sideways on the stage of the working microscope. It provides precision up-down movement of the needle while a strew or arrangement is moved around the stage by hand. Fine positioning of the tip (and any diatom in front of the tip) is achieved by pressing the needle onto the glass surface so it flexes and extends a tiny distance. Glass needles are hand-pulled from 2mm capillary melting point tubes.

I actually have two of these micromanipulators as I use two microscopes for arranging. One is attached to my [Zeiss ICM 405] inverted and is used for selecting diatoms from strews on coverslips. The other is fixed to a Zeiss stereo zoom microscope and is used mainly for arranging previously-selected frustules (working at 40x to 100x).



The big Zeiss is very good for high resolution views of open strews at moderate to high magnification (100x-630x). I put the strews onto large cover slips so the objectives are working through the right thickness of glass to deliver their best performance. Any small cracks or chips on frustules are well resolved and easy to spot, often not the case with the lower resolution of a stereo microscope. The Zeiss also has a nice x,y stage which makes methodical searching of strews a breeze. No forms are missed as I can guarantee 100% coverage of every strew. The other advantage of the ICM 405 is the illumination methods it offers (brightfield, darkfield, phase, COL, DIC, etc). These are useful in certain situations, mainly related to tiny frustules at the lower size limit for practical manipulation (around 10-15µm).

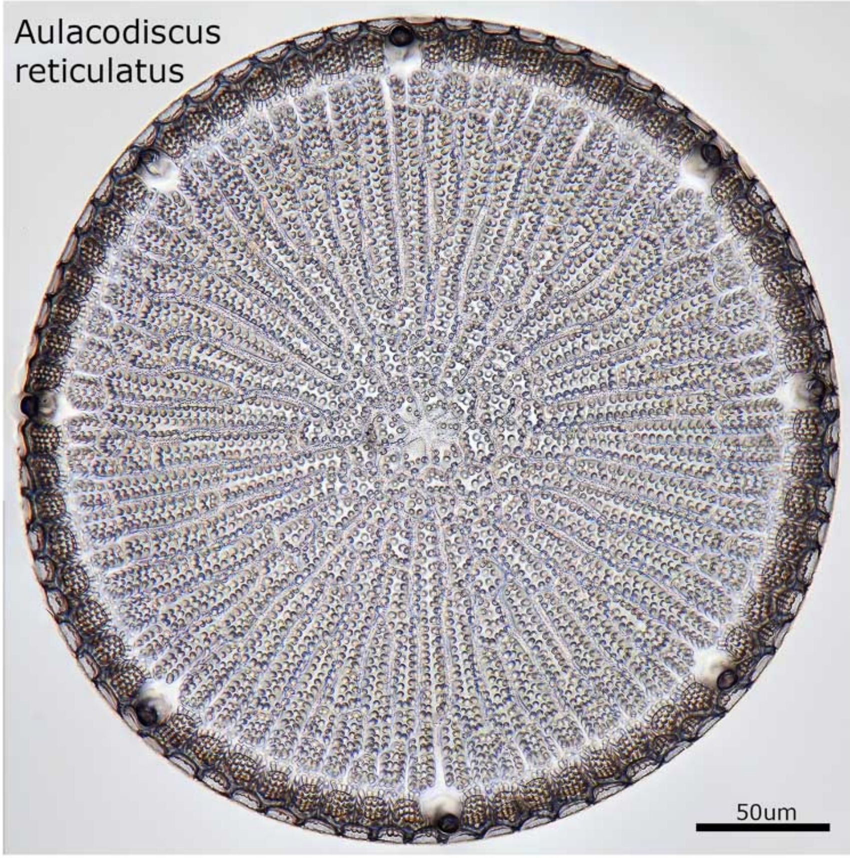


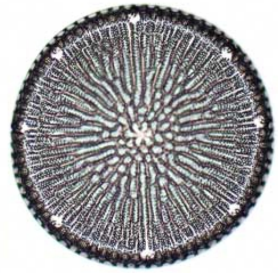
The stereo microscope is better suited for arranging frustules once they have been selected but can be used for selecting from strews too (albeit less methodically as it is too easy to just “fish around” when the strew is moved freehand). Perhaps the greatest advantage is having the stage sat closer to the table-top, making it a far more ergonomic and comfortable setup for extended arranging sessions. It has a greater depth of field for any given magnification too - invaluable for using design templates under the coverslip (more on that later). I use two LED desk lamps for lighting on the stereo one for direct epi-illumination (most of the time) and one for transmitted light via the substage mirror (occasionally).

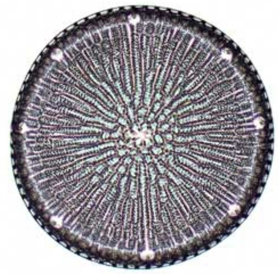
First steps and static stress

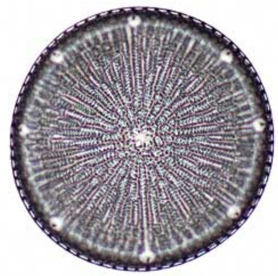
When I first started out, getting selected diatoms even stuck on the slide was cause for celebration in itself. My first few attempts comprised loose “groups” of diatoms attached to a slide then mounted in zrax.

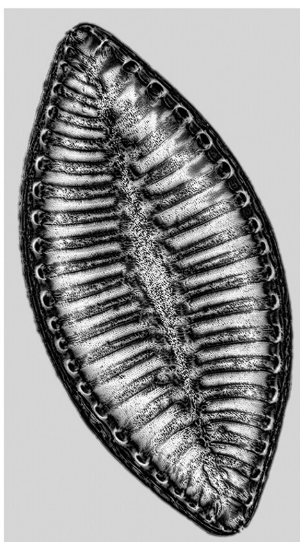
Despite their simplicity, these first slides were every bit as interesting and useful as those I had previously collected. I could study individual frustule details outside of the usual clutter of a strew and get good images of near-perfect diatoms to boot. Being “all my own work” made it doubly satisfying.











Modest though they were, these early efforts were *much* more of a challenge than I expected. The “how to” guides scattered around the internet tend to describe the picking and placing of diatoms as if it’s the easiest thing in the world. Apparently, you touch a diatom with the tip of the needle and it attaches. Then move to where you want the diatom placed and simply “put it down”.

Hah! Maybe I was spectacularly clumsy, but my initial experience was more like...

1. Find a diatom, touch it with the tip of the needle and watch it ping out of sight
2. Go back to step 1 - repeating as many times as necessary
3. Diatom didn’t go “ping”, but now it won’t stick to the needle
4. Try to get the needle under an edge, chase the diatom all over the slide
5. Finally, the diatom sticks to the needle - sometimes at the tip, often higher up
6. Move to place the diatom, discover it has super-glued itself to the needle
7. Rub needle back and forth to dislodge diatom. Ping! Back to step 1
8. At last, the diatom is placed where you want but it but needs slight adjustment
9. Nudge with the tip of the needle - the diatom sticks on again. Go back to step 6

OK, it doesn't happen like that all the time but you get the idea. Picking and placing diatoms is something you have to practice - a lot - before it goes anything like the descriptions in most "how to" articles. It demonstrates the adage that "knowledge is cheap, experience is expensive". You may know what to do in theory, but you have to actually do it countless times to learn the nuances. Some species will stick more than others. Some have "special places" that you just shouldn't touch or they stick like limpets. And so on. It takes time to get proficient but with patience and persistence you will eventually learn how to pick and place adeptly.

These sticking issues are caused by static electricity. Static is your friend of course, there would be no useful force holding diatoms onto the slide or needle without it. But when static gets too strong it causes problems. Diatoms can get stuck fast or may be repelled (pinged) away from the needle. I spent nearly two years doing things the hard way before discovering a "secret weapon" that made things easier (be patient, all will be revealed) but it was good experience as I discovered other ways to alleviate these issues too.

- Don't try arranging with a freshly made glass needle. The tip is too sharp and the resultant electron density will magnify the static effects. Instead, wear the tip down by using it for selecting from strews for a few hours first. Run the tip roughly through the strew several times and it will eventually become blunter and a little rounded. It will work far better for precision placement of individual frustules after wearing in. A "broken-in" needle is a precious thing - look after it well.
- Pick up frustules on the tip *gently* and ensure some of the frustule hangs below the tip so it touches the glass before the needle when it is lowered again. Do not try to "rub" a diatom off the needle - it only makes things worse. Instead, lower the needle very slowly so the frustule (but not the needle) just makes a kissing contact with the glass, then hold it there a few seconds. This seems to bleed off some charge and the diatom will often (but not always) just drop off.
- If everything is sticking, rubbing the needle on some paper fibres stuck to the glass will (counterintuitively) reduce some of the charge. I put a tiny pinprick of super glue on my keeper slides and stick some filter paper on it. When dry, pull off the filter paper to leave behind a tiny tuft of stiff fibres. These are ideal for scraping stuck diatoms from the needle, and for removing some charge by stroking the needle across them a few times. Stroke backwards so the tip can't jam into the fibres and break - they are very hard after super-glueing.
- Earth the microscope (e.g. wire to a water pipe). If the slide surface is charged up then this won't help much in the short term as the glass is an insulator. But it does stop the scope being the source of static and will generally drain charge from a slide sat on the stage, but it can take a few hours to do so.

Despite the tricks above, there are still days when nothing seems to work. Usually cold days when humidity is low but cleaning the needle with a water or alcohol soaked brush can have same effect in any weather. I struggled to place even two diatoms per hour in tightly packed arrangements when static got really bad. The smallest touch to adjust a frustule would attach it to the needle again and it would pull out of place when the needle was retracted, sometimes displacing other diatoms in the process. Patience! Just keep trying, and trying, and trying. Eventually it goes correctly, but it does severely extend the time needed to finish an arrangement.

Thankfully, I recently found a (no longer) “secret weapon” that solves these problems!

I’d wondered for ages if an anti-static gun used for vinyl LPs could help but baulked at the price for such a simple device (£50 for the one with the best reviews). I found articles on how to make them from piezo gas lighters but I didn’t fancy a Heath Robinson solution. After a particularly frustrating session, I ignored the exorbitant cost and bought one. It worked like magic!

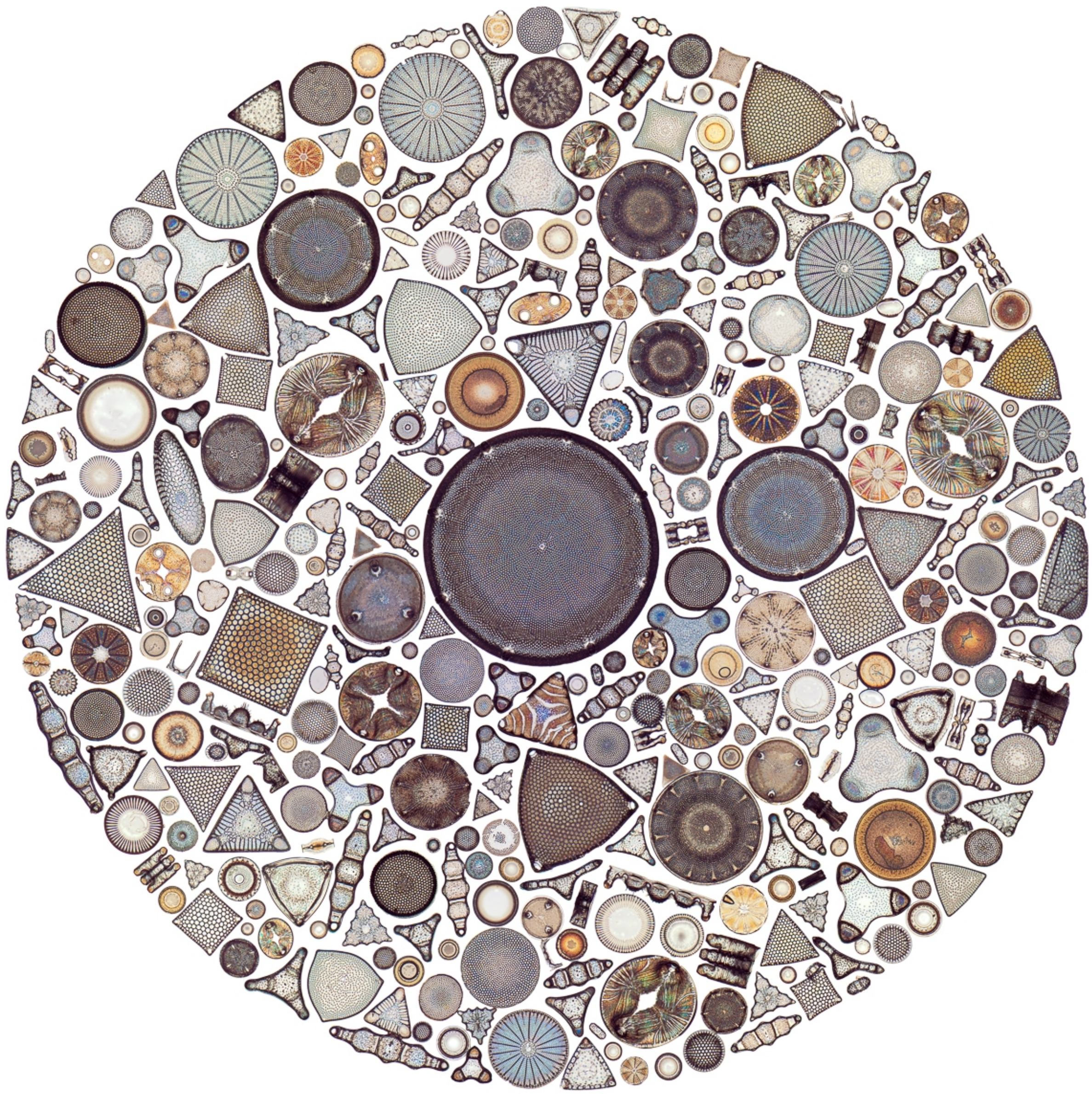
When static problems occur, a couple of gentle squeeze and release cycles on the trigger makes static issues all but vanish. Don’t hold the gun too close or you’ll be treated to the dance of the diatoms! It is mildly entertaining at first, until you discover your best diatoms have leaped up and stuck to the stereo microscope’s front lens! Holding the gun 30-40cm away works well and will keep a slide virtually static-free for days after treatment. In fact, you can now “touch with the tip of the needle and put down” most diatoms as described in the “how to” guides I mentioned earlier. There’s still the odd awkward, sticky diatom to contend with, but the gun makes things so easy it doubled my arranging speed at a stroke. It works even better if you say “do you feel lucky, punks?” in a menacing Clint Eastwood drawl before squeezing the trigger. Well - that’s not really true - but I do it anyway :)

Ramping up and fixative woes

Once I'd partially mastered the basics of cleaning and manipulating diatoms, I felt ready to tackle "proper" arrangements and start replacing some of the slides I had sold. My first serious attempt using material from Oamaru came out like this.

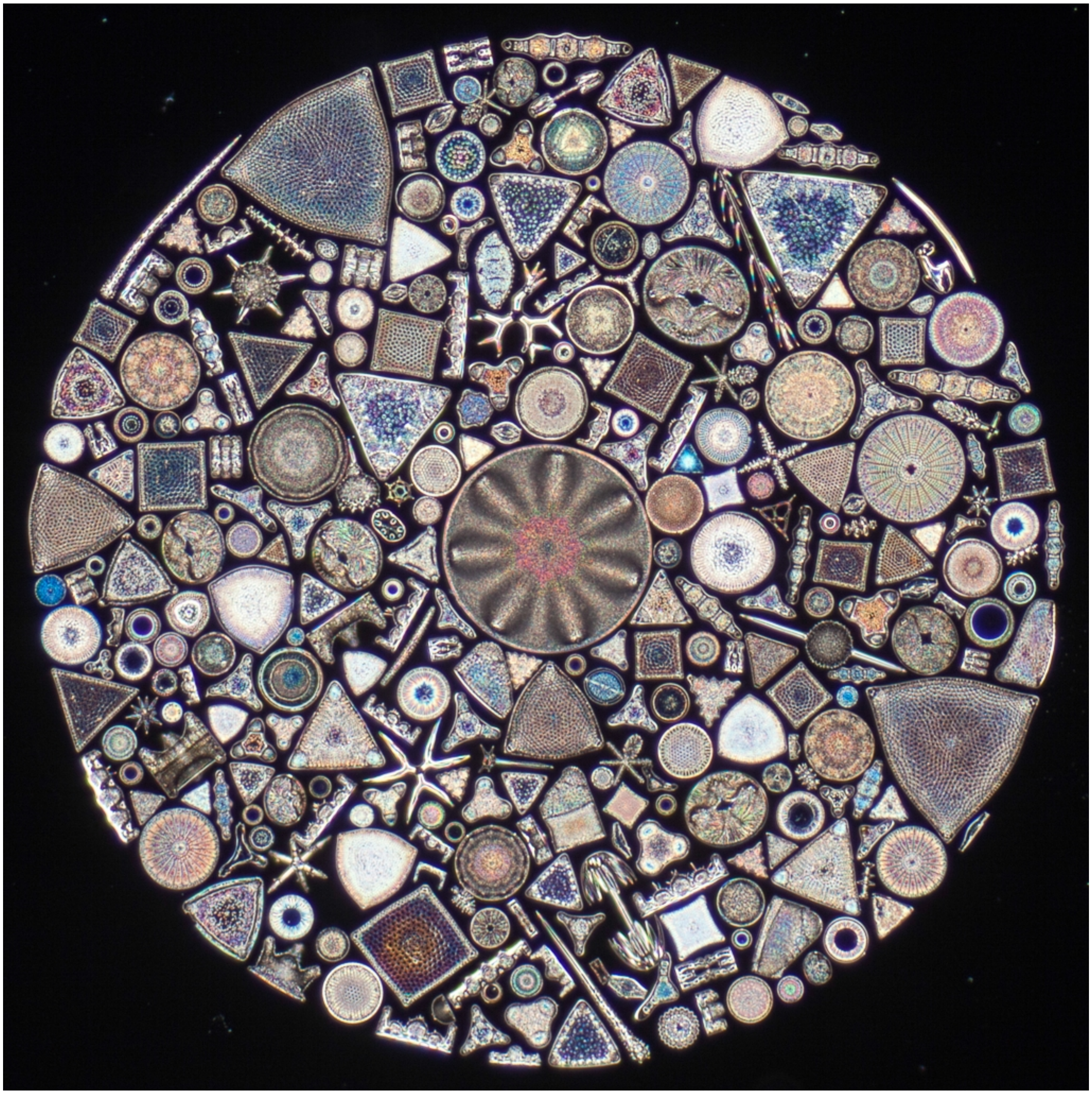
Not too bad, but rather spoiled by floaters dislodged during mounting and nasty bubbles in a couple of frustules. At this stage, I was mounting on the slide rather than the cover slip as I was using a fixative recipe after Solliday which tended to soak into the diatoms somewhat. This was particularly noticeable if the diatoms were stuck face down in the fixative, as they would have to be if mounted on the cover slip. But I kept trying new ways to apply the fixative and was eventually able to apply a thin-enough film such that the soaking-in wasn't too pronounced. No worse than on some vintage slides anyway.

Fixative problems aside, after making more modest circles I got ambitious and decided, at last, to attempt a really big arrangement of Oamaru material. It came out like this.



I was very pleased with the outcome but had not been prepared for the time it would take, nor the number of diatoms needed to fill a 2mm circle this way. The tight tessellation really made it slow work to position the forms, and required far more frustules than I thought it would (I had to select over a thousand to have enough variety of shapes and sizes to fit together as the arrangement progressed). After 130 hours spent meticulously placing 400 diatoms - I was somewhat wiser.

I tackled more large circles after this (now mounting on the coverslip) but still had slight problems with fixative soaking into diatoms or not holding. I lamented the problems on a forum, and up popped Raymond suggesting Debe's fixative would be a better choice. He was right - it's a magical concoction that holds the diatoms firmly with minimal soaking into frustules - although I could still see a bit on certain diatoms at high magnification.



More big circles and types slides (in rows) followed, but I was still irked by the problem of fixative being visible on some diatoms. Eventually, I spent a long time experimenting with variations on Debe's formula looking for one that provided good strength while minimising visible artifacts. The result is my "Modified Debe's Fixative" which uses much less gelatin and a slightly different ratio of alcohols to acid - as follows.

25ml glacial acetic acid
12g isopropyl alcohol
6g isobutanol
0.3g high quality, white, bovine gelatin (pH5.0 - fine powder)

Note: photographic gelatin is recommended but hard to find. The powdered gelatin sold by laboratory suppliers (with the specification above) is equivalent and works just as well. Ordinary *(food) gelatin is not recommended. It appears to work well enough at first but it doesn't bond diatoms very strongly and doesn't store well or remain usable for very long.*

Form the gelatin powder into a small pile and mix with a few drops of distilled water until it is the consistency of thick wall paper paste. Use a minimum amount of water but ensure the "paste" is clear with no white lumps of powder remaining. Drop this moistened gelatin into the acetic acid in a screw top bottle and shake until all the gelatin dissolves (this may need up to an hour of intermittent, vigorous shaking). Do not heat the mixture as this will subtly change the molecular structure of the gelatin and can reduce its strength.

Mix the alcohols in a separate container. When the gelatin is dissolved, add the alcohols to the acetic acid a few drops at a time, mixing well between drops. Use a separate pipette for dropping the alcohol and do not get any of the acid/gelatin mix into the pure alcohol or gelatin may precipitate out.

When all the alcohol is mixed in, filter the finished Debe's through a 0.2um Whatman filter into smaller storage vials. You can use filter paper and funnel, but cover the top with silver foil to reduce the evaporation of the alcohol while filtering. The recipe makes about 50ml of Debe's (a lifetime of arrangements). Store in a cool, dark place, but not the refrigerator.

So far, this mixture has proved to be very stable and it doesn't seem to suffer solidification or appearance of specks of gelatin over time like the stronger formulation can. On top of that, it sticks diatoms very firmly with virtually no detectable soaking-in when the film is applied thinly enough.

The Debe's is applied to a clean coverslip by placing a drop in the centre of the slip using a small glass rod or similar. The fixative will spread to the edge and form a thin flat film on it's own. If the Debe's doesn't spread evenly then the coverslip is not clean enough. I now clean my coverslips using Pirahna mix (dangerous) followed by 5 minutes in an ultrasonic bath of isopropyl alcohol and then heat dry *immediately* before applying the Debe's.

Coating thickness is controlled by the size of the drop and by the size of the cover slip to some extent. If a drop spreads well but doesn't reach the edge, then you get the thinnest possible coating, but it will only just hold onto the diatoms. Only use the thinnest coating for very delicate frustules or very small arrangements. Normally you want the Debe's to spread right to the edge to get a thicker strong coating that holds frustules very firmly.

Venturing beyond the traditional

With circles and simple rows of diatoms pretty much mastered and my arrangement times dropping rapidly, I wanted to do something different and develop a recognisable "style" of my own. But what to do?

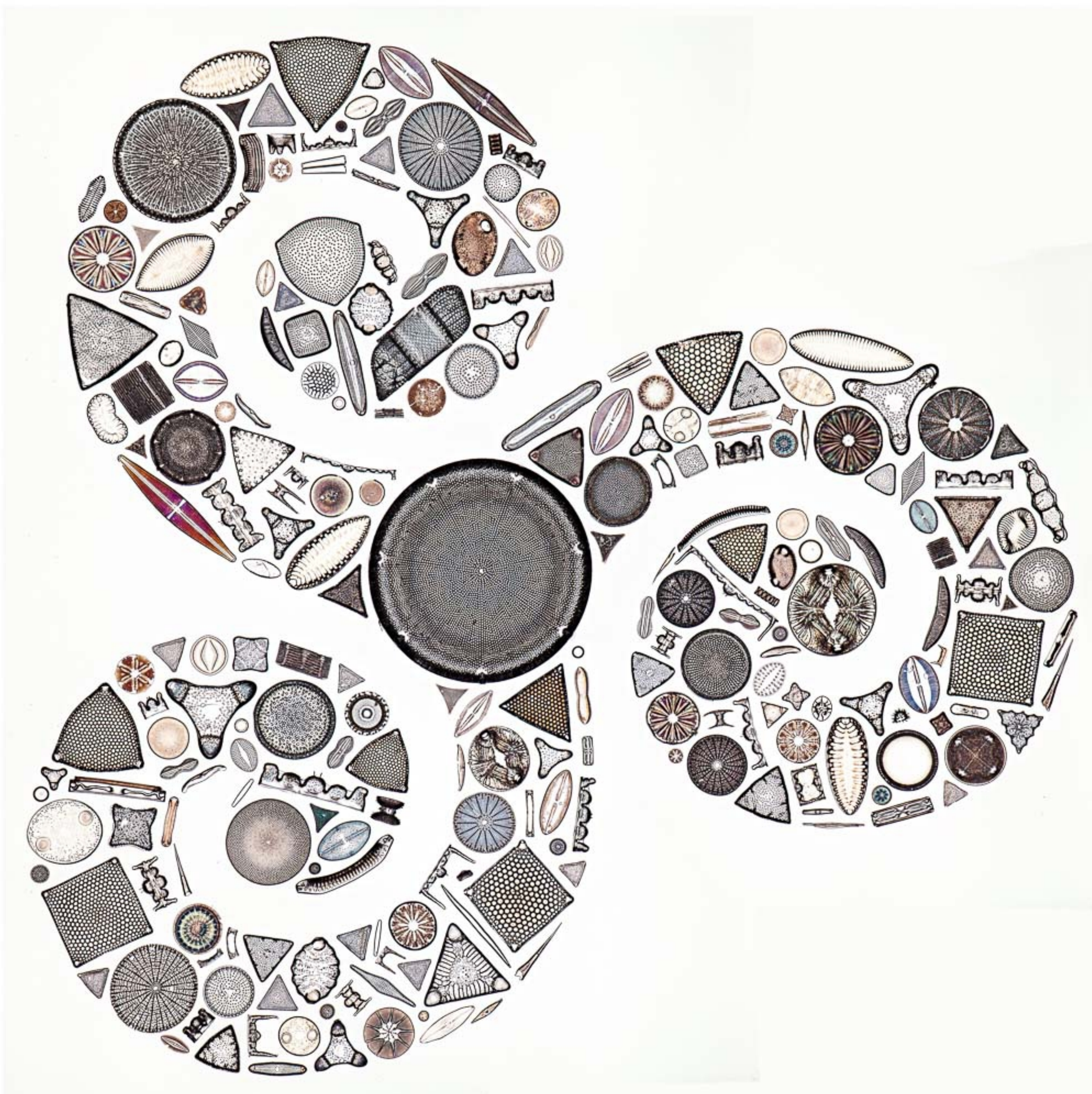
I'm not too keen on rosette or kaleidoscope arrangements. To my eye, they distract from the diatoms and there are already loads of them around anyway. So that wasn't an option. I hit on the idea of keeping the basic tessellation approach I was using for circles, but to apply it to interestingly shaped arrangements instead. Easier said than done...

I couldn't arrange diatoms into shapes by eye and keep everything properly neat, tidy and precise. I already drew circles on the backs of coverslips (with a ringing table) so I had a line to follow when making circular mounts. I'd definitely need something similar for more complex shapes. But how could I draw something more complex at the scale required?

The answer was microfilm! After locating someone to transfer my Photoshopped designs onto microfilm, I found it to be an ideal solution. I could finally take my arranging beyond traditional circles and lines to make something genuinely new and eye-catching. My first successful attempt, using diatoms from Newport Beach, CA , came out like this.

I had a run of failures after this, losing some very big arrangements to small accidents like a coverslip breaking post-arranging, or crushing diatoms by using too little mountant. Silly mistakes which wasted a depressing amount of work. But each mistake turned out to be a valuable learning experience - helping me modify methods or workflow to prevent them happening again.

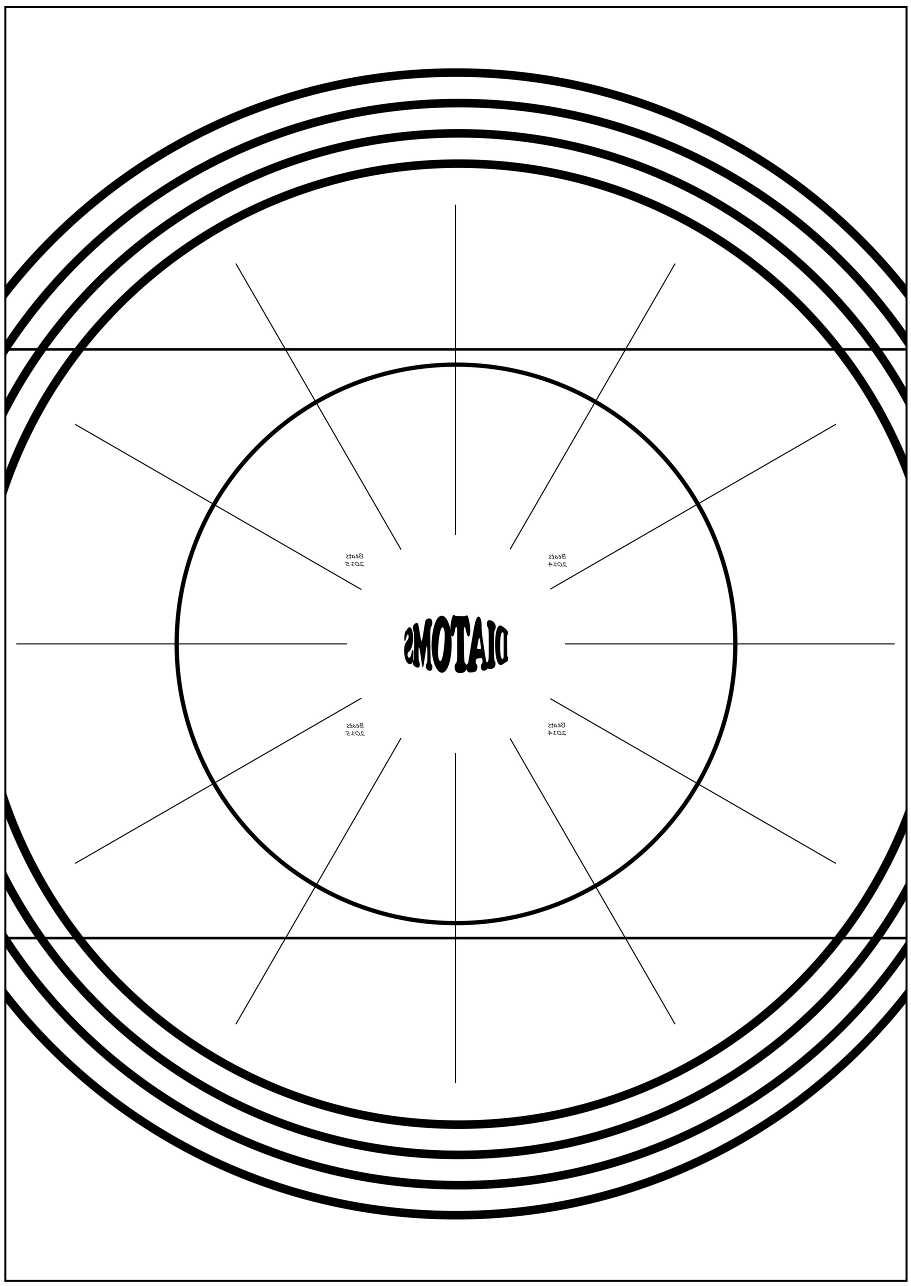
Thankfully, I'm back on track, making less stupid mistakes, and now using the microfilm technique for all my arranging. It works just as well when setting out type-slides in lines or grids as it does for circles and, of course, the more complex shapes. I'm making mostly type slides at the moment, but here's the last unusually shaped arrangement I made. A 'triskele' of fossil diatoms from various locations.



Making and using microfilm templates is pretty easy, once you find a company to produce films for you. I was lucky enough to find someone willing to tack my small jobs onto the ends of the films used for larger contracts at a cost of around 50p a frame (one template per frame). Look for companies offering microfilm or microfiche services.

I use Photoshop to create templates which are then printed onto A3 paper before they are photographed at 20:1 reduction, one sheet placed in the middle of each film frame. I get the negative and positive films sent back to me - but so far I have only used the positive images. Anything that was white (i.e. the background) in the original is clear on the film. Anything that was black comes out dark brown and opaque (i.e. the lines of the design).

Here's a template to give you an example (it's the next big arrangement I plan to make, using diatoms from different countries in each letter). The design is reversed (mirror image) as the diatoms will be viewed through the other side of the coverslip after mounting. The big circles are used for aligning the coverslip over the template.



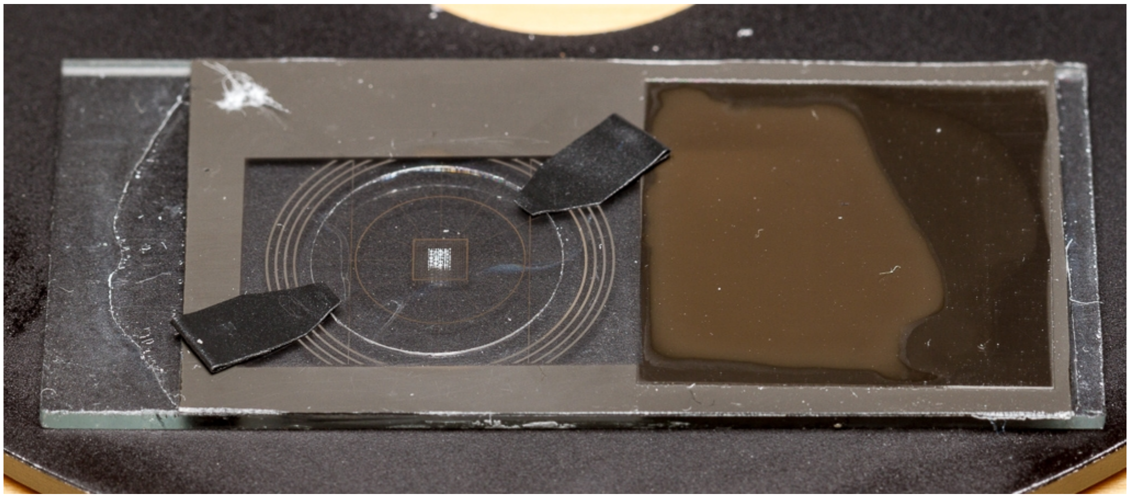
I won't go into great detail here, but in order to use a template, it is stuck to a glass slide, emulsion side up, to give a flat surface to put the working coverslips on. One coverslip is the "keeper" where the selected diatoms are kept and picked from when arranging. The other is Debe's coated and will receive the arrangement.

After much trial and error, I found that a 50/50 mix of Canada balsam and xylene is by far the best adhesive to use. It leaves the surface of the film perfectly flat. But it takes ages to dry (in fact it never dries). 24-48 hours on a hotplate at about 85C will dry it enough for careful use but it needs another 3-4 weeks in a warm place to become "robustly dry".

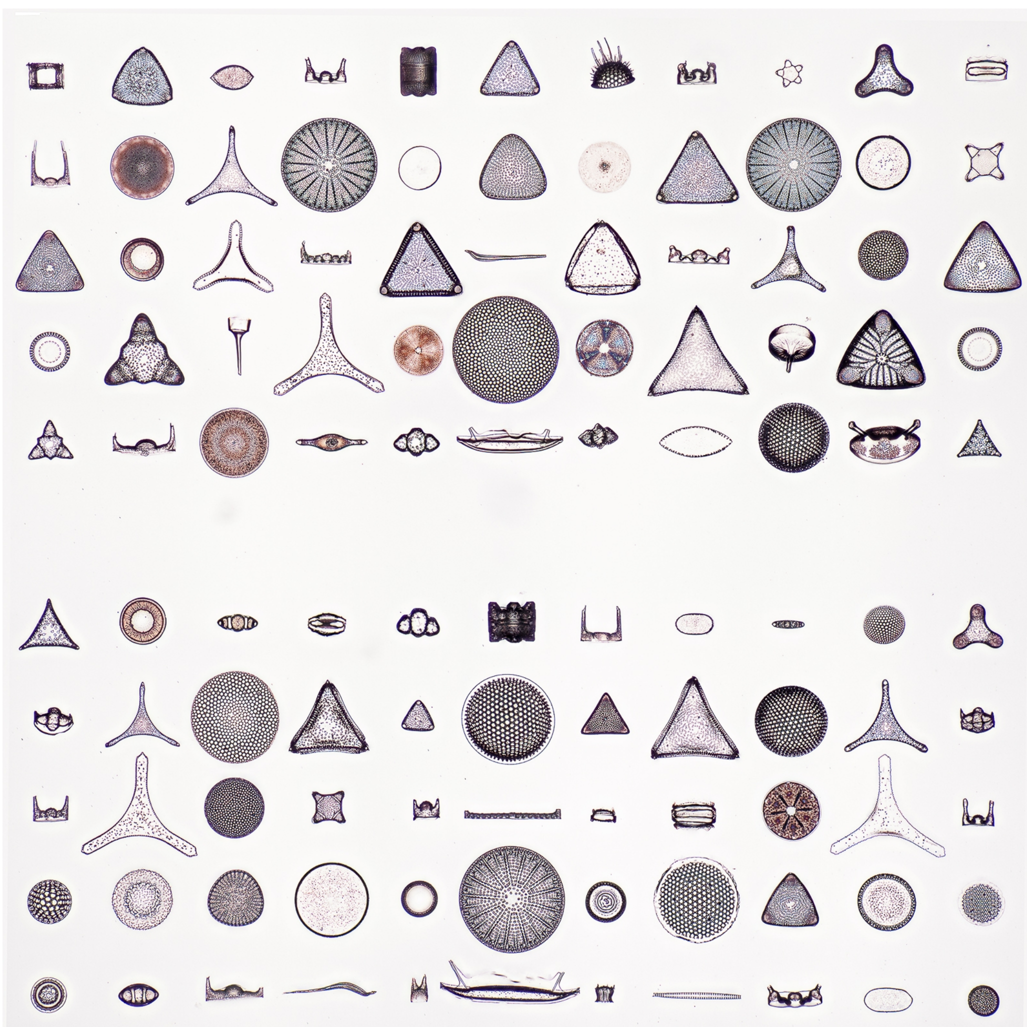
Here are the things needed for making a template slide using the microfilm. Note that the film roll has been cut to put the template at one end of the frame and an equal sized dark area (for the keeper) at the other end. The insulation tape is used to hold one end of the film in place prior to applying a couple of small drops of balsam. The film is pressed down to touch the balsam where surface tension and air pressure take over to pull the film onto the glass and hold it flat.

Don't forget, the film should be stuck with the emulsion side up (shiniest side down), so the imagery will be in direct contact with the underside of the arrangement coverslip. This ensures it is not too out of focus when you are focused on diatoms sitting on the surface of the arrangement coverslip.

Here's a finished type slide still attached to a grid template. Note how the coverslip is held with little tabs of electrical tape. The square "keeper" coverslip on the right is held fast by placing it on a tiny drop of water. A superglued "tuft" of paper fibres is at the top left.



And the resultant finished arrangement (selected types from Kamyshlov, Russia).



Future plans and goals

Not long after making the 400-form Oamaru circle, I got curious about the market value of contemporary diatom arrangements versus antique ones. Were the high prices being paid due to the slides being antique or by famous mounters? Or was it the arrangement and the diatoms that attracted the buyers? I listed my 400-form circle on eBay to find out.

A week later, it sold at a record price for a contemporary slide (and a near-record for any slide)! Given how long it took to make, it was a poor return in monetary terms. But that record price coupled with my improving arranging speed encouraged me to make and sell more. The outcome has been positive in every case.

It's a highly unexpected twist in my plans, but now I intend to make one or two slides a month for sale - in addition to slides for my own collection. It will only ever be a modest cottage industry of course, but will help keep me focused on producing interesting, high quality mounts while improving my technique. And who knows, after several years, I may save enough to afford a second-hand desktop SEM after all :)

In terms of goals - my ultimate one is to make a slide like the Möller Typenplatten. It will be a "modest" thousand form version first, but then I'd like to exceed the achievements of the man himself and produce a 5000-form slide containing perfect specimens from every genus with as many species as I can get my hands on. I already started a keeper slide for this, but it still has less than 500 species on. Obtaining new and interesting material is a slow process and quite a limiting factor at times.

To that end, please permit me a cheeky request. If anyone has any spare diatom material they are willing to sell or barter, please get in touch (my email address is below). I'm sure we can swing a deal for interesting samples from known locations. Raw diatomite is much preferred, but already-cleaned material is welcome too.

So - that's my journey so far. I hope you enjoyed the article and got something useful out of it. Most of all I hope I've encouraged someone to try diatom arranging for themselves. The art will die out if new people don't join in. Have fun.

References

Raymond Hummelink's Micscape article on making selected diatom slides
http://www.microscopy-uk.org.uk/mag/artoct06/rhu-diatoms.html

Fixatives - including Debe's
http://www.diatomsireland.com/diatom-mounting/diatom-mounting-adhesives/

Making glass needles (bottom of page)
http://www.diatomsireland.com/micromanipulator-intro/connectors-needles-and-holder/

Cleaning diatoms
http://www.diatomsireland.com/diatom-cleaning/

Comments to the author Steve Beats are welcomed:
Email: steve.m.beats AT gmail DOT com

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