Microscope Protocol - Qualitative analysis (The "hey, what's in the water" approach)

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Preface: The Instrument techs have made a great protocol for the use of the Nikon E800 that should be used as your primary reference when using the stations microscope This is a companion protocol that will speed you along the path to pretty pictures of Phytoplankton.

Materials Needed:

- Microscope slides
- Cover slips
- Long stem Pasteur pipettes
- Kim wipes
- Immersion oil
- Lens paper
- Big, scary microscope

Making slides

- After doing a net tow, put the cod end in the refrigerator to allow the cells in the water to settle. Thick waters (like brown ice or blooms) will take much less time to settle. A few hours to one day should do the trick. If you aren't seeing anything at all in your slides after two days than you probably need to concentrate it. Take a longer net tow or gravity filter on a 1 micron filter prior to scoping. Note: Sediment analysis is biased toward large, heavy cells.

- Taking the sample: Being careful not to agitate the water in the cod end, draw out a few milliliters from the bottom using a long stemmed Pasteur pipette. The majority of the sediment tends to collect around the edges of the jar.

- Put a few drops on a clean slide (you can strategically select drops that look thick) and place a cover slip over it. Note: Thick water films reduce the image resolution, and stuff will tend to move around on you in the slide. You can use a Kimwipe to wick away excess water from the edge of the cover slip (if desired).

Scoping

- Log onto the computer. Turn on the Power supply for the microscope lamp (Nikon) and the power supply for the camera (Spot) (if needed).
- Microscope Setup (this has given the most success with pictures)
  - Swing the polarizer out of the light path.
  - Set the filter cube to Vis
  - Light source set to DIA

- Set F-stop to open
- Set the condenser slider off to the right. This tends to give better contrast when viewing diatom shells in particular.
- Adjust the lamp as needed. High light tends to work better for pictures.
- Filters for Transmitted illumination- Play around with them. The camera manufacturer suggests using at least a couple neutral density (ND) filters. I have had success with the D and ND8 filters in some applications.
- Objectives- most of the cells we look at are viewed well under the 60x oil immersion lens. Other objectives are good for scanning and selecting an area of the slide to focus on.

Taking Pictures

- If all you want to do is take a quick picture to show the Boss lady, Read on. If you are interested in taking high quality images, refer to the manuals for the camera stored in the protocols folder (spot getting started, spot users guide). On page 8 of the getting started guide, there is a pretty simple stepwise guide to taking nice pictures. If you are really serious about this, check out pages 19-65 of the Spot users guide.

Note: If you decide to play with the camera settings, you may have trouble changing them without administrator access to the computer. At Palmer Station, there are notes on this on the main drive under SOPs.

- Make sure the camera power source is on.
- Click on the Spot icon on the desktop.
- After focusing your image in the microscope, pull out the upper slider out to direct the light path to the camera.
- In the Spot software, click the Get Image button on the upper right end of the toolbar. It will do its thing and cycle through various filters, then spit out your image.
- At this point you can switch back and forth between the scope and camera, playing with settings on the instrument to get an image you like.
- When you are done taking pictures, you can save the images by closing them and saving them as you do. When you are all through, just make sure that you turn off the power sources for the camera, lamp, and scope. Also make sure you clean any immersion oil off the lenses with lens paper. Cover the scope to keep dust off.