

*"Empirical Studies for Improving the
Contrast of Transparent Objects in
Light Microscopy"*

Definitions

- ▣ Hobby:

“an activity done regularly in one’s leisure time for pleasure” (Oxford US English Dictionary)

Quotes:

- ▣ “Hobbies of any kind are boring except to people who have the same hobby.”

David Barry

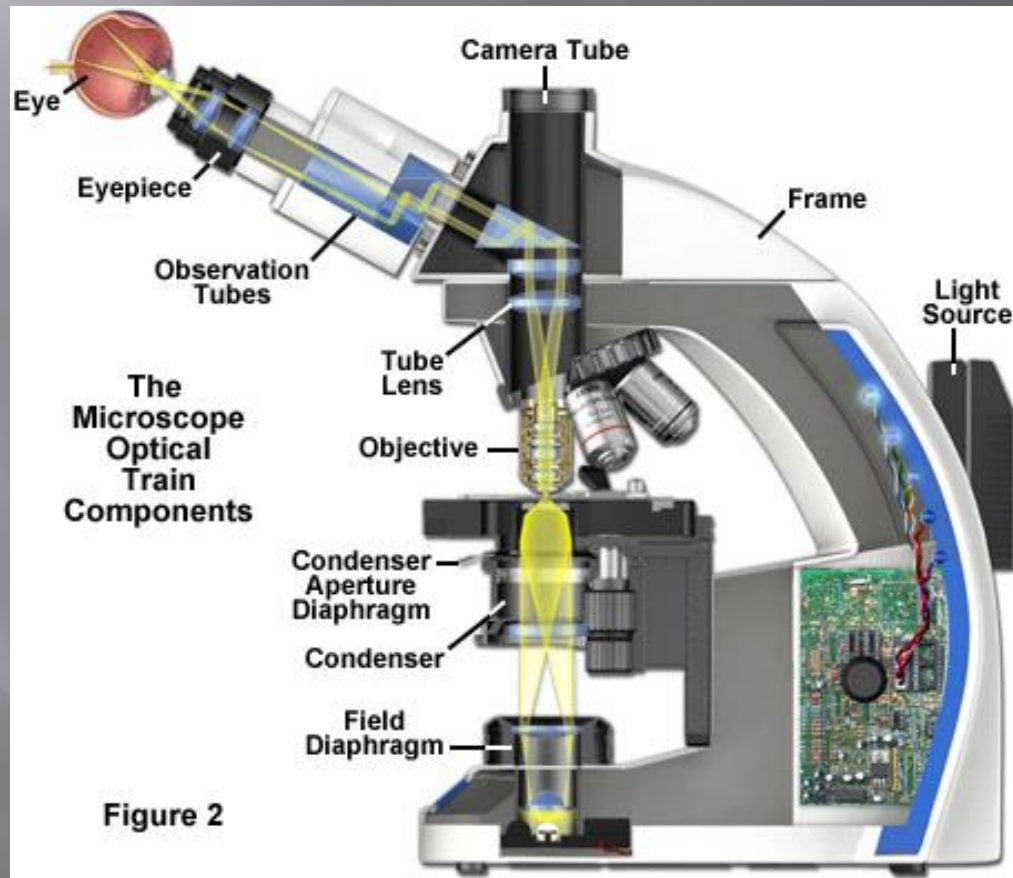
- ▣ “When a habit begins to cost money, it's called a hobby.”

Jewish Proverb quotes

- ▣ “How much did that ***** thing cost? Can't you find a cheaper hobby, like fixing the faucet?”

My very practical wife

The Microscope



Resolution :

- ▣ “The resolution of an optical microscope is defined as the shortest distance between two points on a specimen that can still be distinguished by the observer or camera system as separate entities.”

(Nikon MicroscopyU www.microscopyu.com)

Resolution and Diffraction Limited Systems

- Optical resolution in a diffraction limited system can be described using the following:

$$r = \frac{\lambda}{(2NA)}$$

Where:

λ = illumination wavelength

NA is the numerical aperture of the system
(condenser and objective)

Resolution and Diffraction Limited

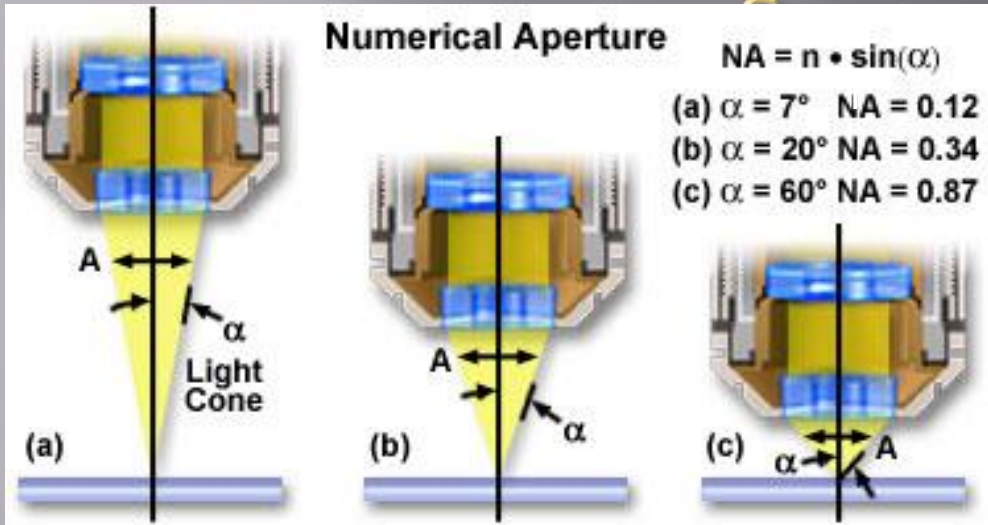


Figure 1

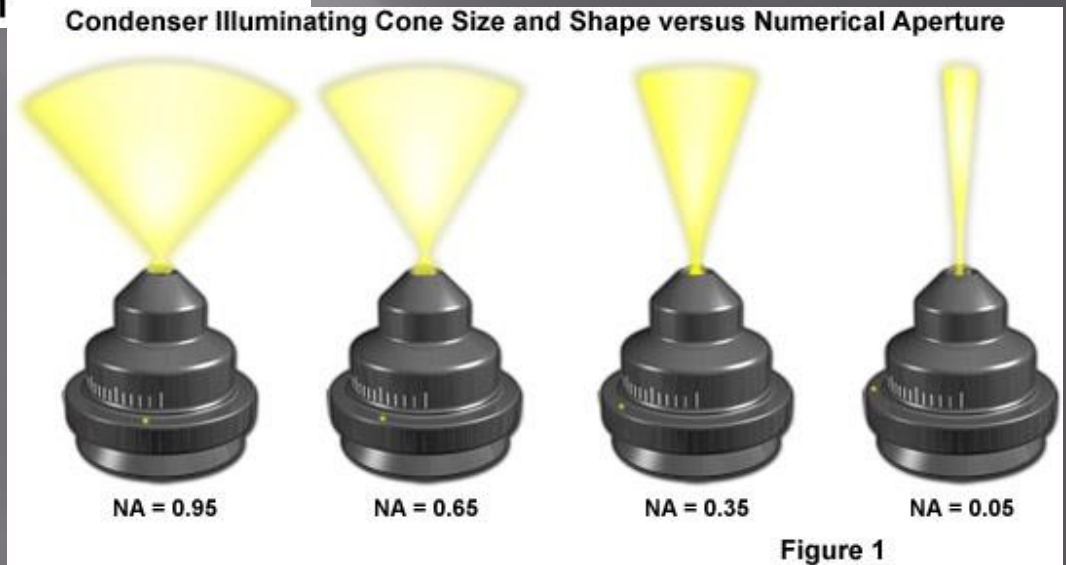


Figure 1

1 <http://www.microscopyu.com/articles/formulas/formulasna.html>

2 <http://zeiss-campus.magnet.fsu.edu/articles/basics/opticaltrain.html>

What does this mean???

- ▣ Theoretical vs. Practical
 - Theoretical limit of ~ 200 nm
 - Practical limit ~ 250 nm

Contrast

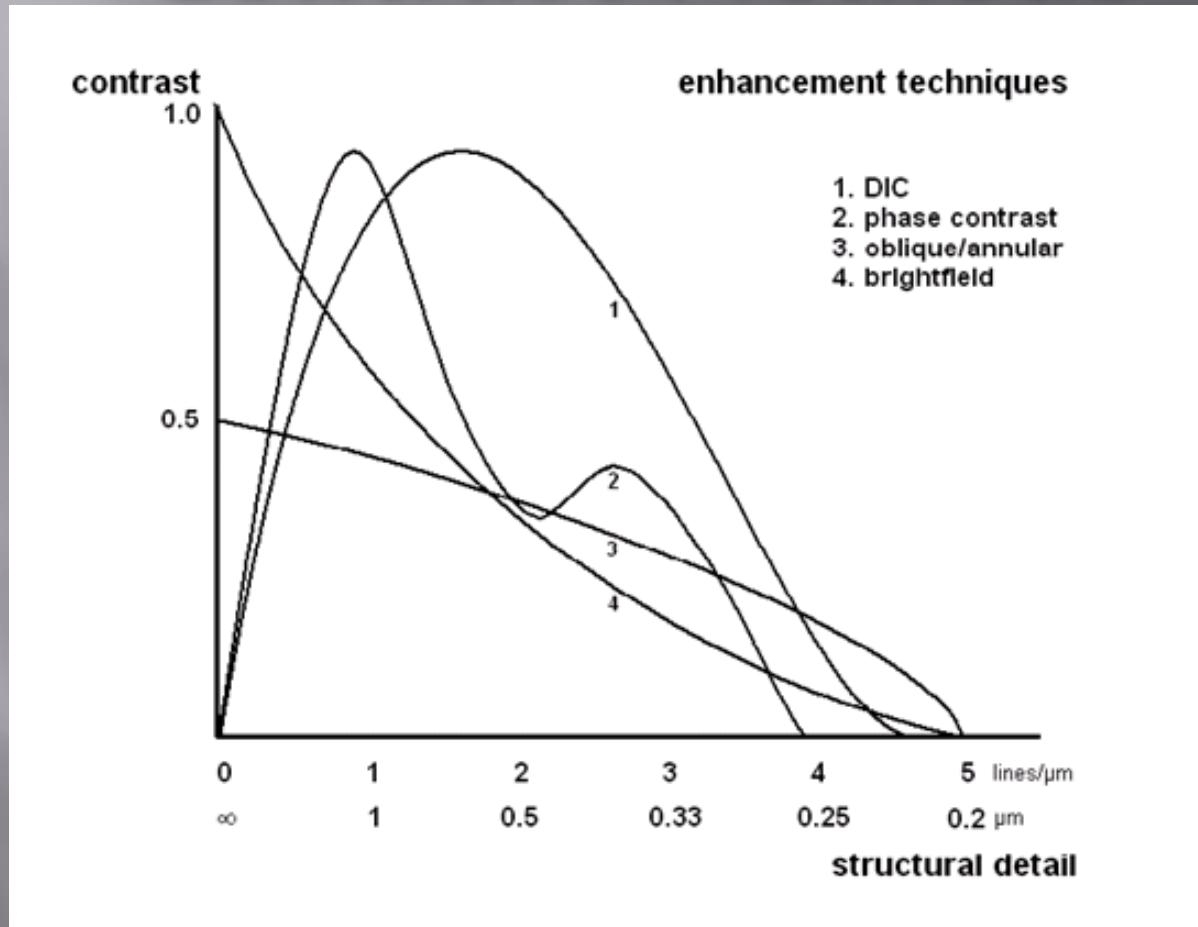
- ▣ “Contrast is defined as the difference in light intensity between the image and the adjacent background relative to the overall background intensity.”

(Nikon MicroscopyU www.microscopyu.com)

Contrast Methods

Specimen Type	Imaging Technique
<p>Transparent Specimens Phase Objects Bacteria, Spermatozoa, Cells in Glass Containers, Protozoa, Mites, Fibers, etc.</p>	<p>Phase Contrast Differential Interference Contrast (DIC) Hoffman Modulation Contrast Oblique Illumination (Razor Edge)</p>
<p>Light Scattering Objects Diatoms, Fibers, Hairs, Fresh Water Microorganisms, Radiolarians, etc.</p>	<p>Rheinberg Illumination Darkfield Illumination Phase Contrast and DIC</p>
<p>Light Refracting Specimens Colloidal Suspensions powders and minerals Liquids</p>	<p>Phase Contrast Dispersion Staining DIC</p>
<p>Amplitude Specimens Stained Tissue Naturally Colored Specimens Hair and Fibers Insects and Marine Algae</p>	<p>Brightfield Illumination</p>
<p>Birefringent Specimens Mineral Thin Sections Liquid Crystals Melted and Recrystallized Chemicals Hairs and Fibers Bones and Feathers</p>	<p>Polarized Illumination</p>

Contrast Methods



<http://www.microscopy-uk.org.uk/mag/indexmag.html>?<http://www.microscopy-uk.org.uk/mag/artapr09/rvw-contrast.html>

The Price of Contrast

Technique	DIY	Cost Range (for used equipment)
Brightfield Illumination	No	Usually the cost of entry \$100 - \$3000.00
Oblique Illumination	Yes	\$0.25 - \$200.00
Annular Illumination (COL)	Yes	\$0.25 - \$2000.00
Rheinberg Illumination	Yes	\$5.00- \$50.00
Darkfield Illumination	Yes/ No	\$0.25 - \$300.00
Polarized Illumination	Yes/ No	\$15.00 - UP
Phase Contrast	No	\$300.00 - \$700.00
Differential Interference Contrast (DIC)	No	\$1200.00 - \$3000.00
Hoffman Modulation Contrast	No	\$1000.00 - \$3000.00

What does this mean???

- ▣ Resolution vs. Contrast
 - Both are needed for best observation
 - Contrast can adversely impact resolution
 - The best method does not always cost the most

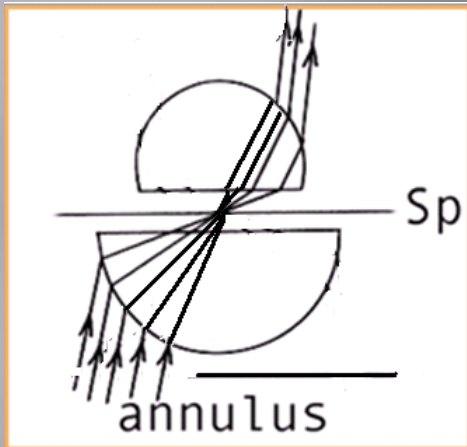
My Foray into Annular or Circular Oblique Illumination (COL) Variants

The low cost DIC

Annular or COL

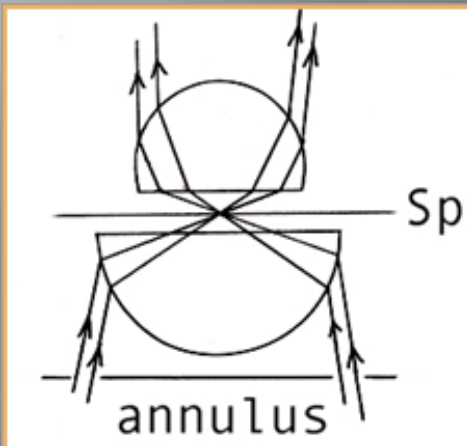
OBLIQUE PRINCIPLE

- Block the light from one side
- Increases higher order diffracted light illuminating specimen
- Produces 3D like shading



COL PRINCIPLE

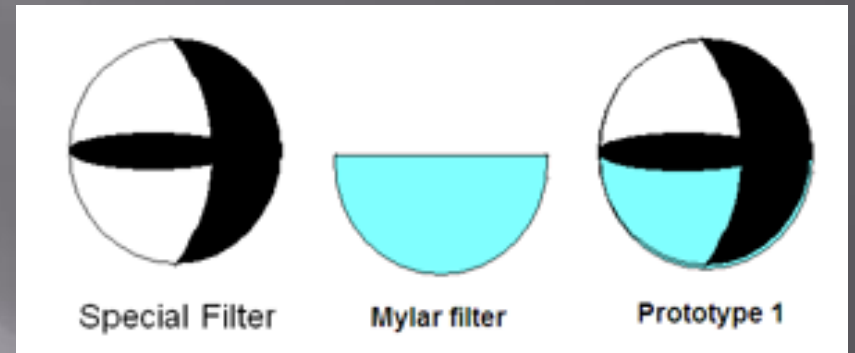
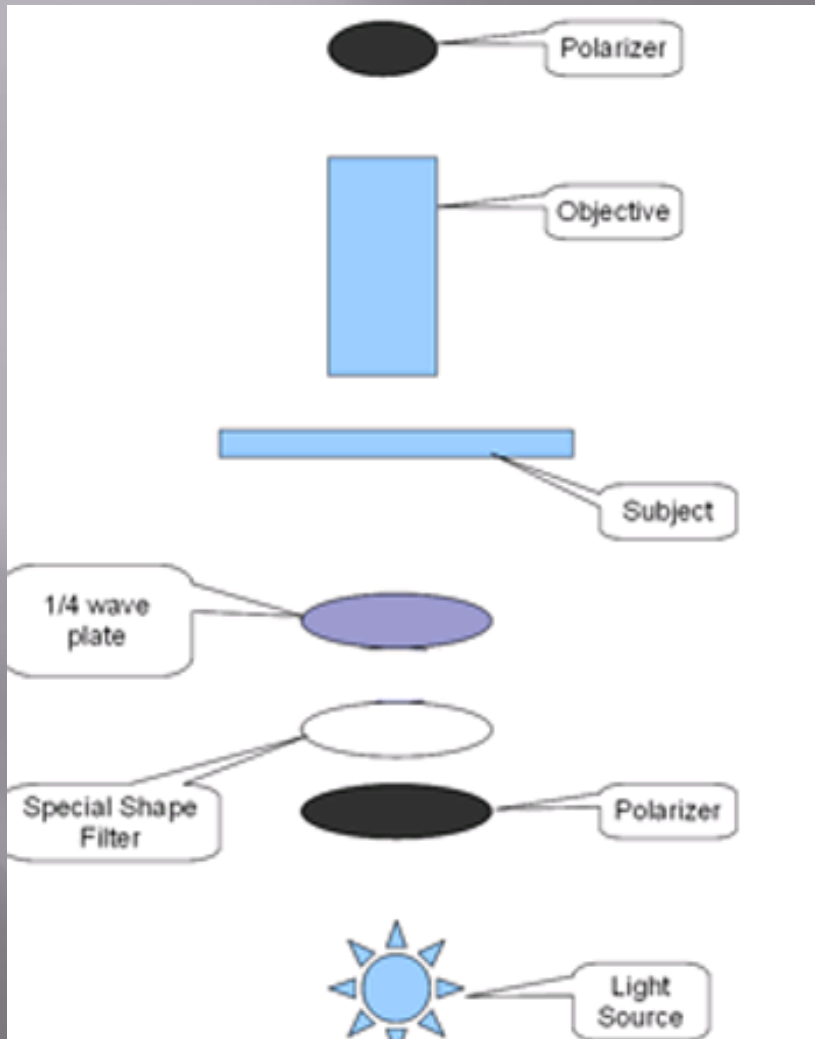
- ▣ Block the intense central light cone
- ▣ Increases higher order diffracted light illuminating specimen



Combining COL and Oblique

- ▣ Modern Microscopy “How To” articles
 - The first article combined several techniques into one method (<http://www.modernmicroscopy.com/main.asp?article=100>)
 - ▣ COL
 - ▣ Oblique
 - ▣ Polarized light
 - The second article (<http://www.modernmicroscopy.com/main.asp?article=106>)
 - ▣ Simplified the components
 - ▣ Added more varied light

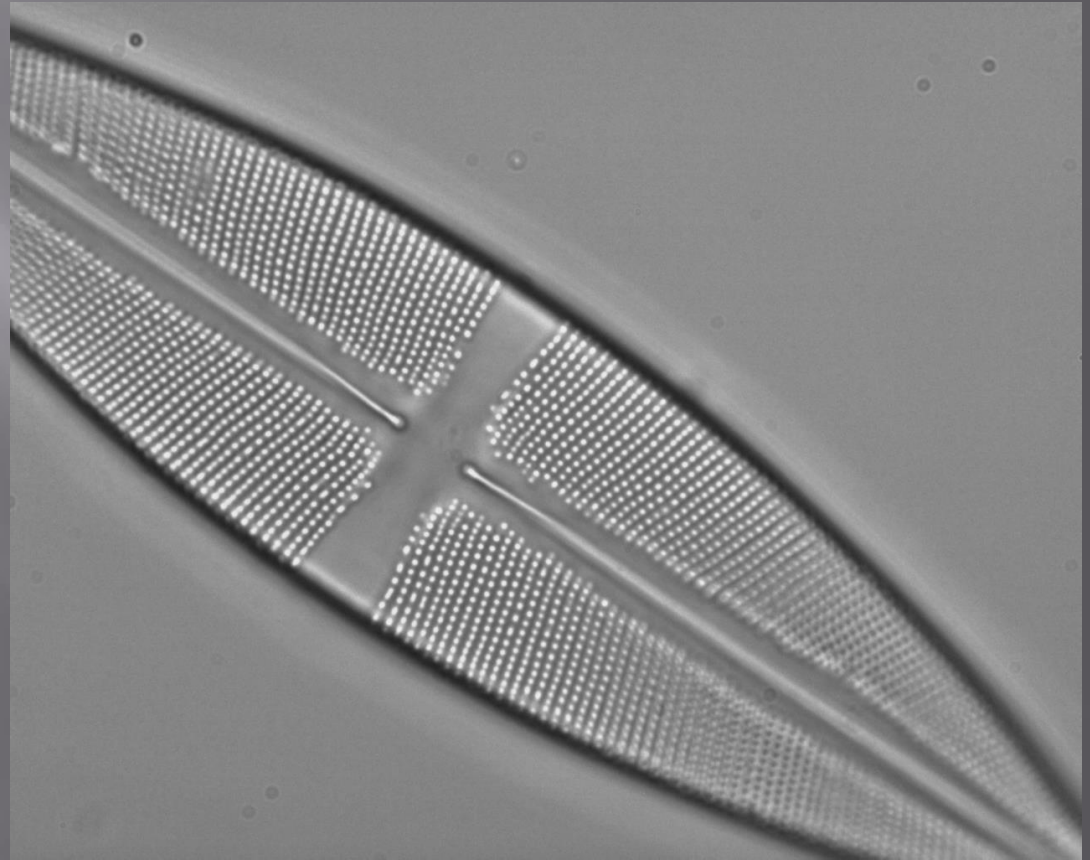
Article I



Article I Actual Setup

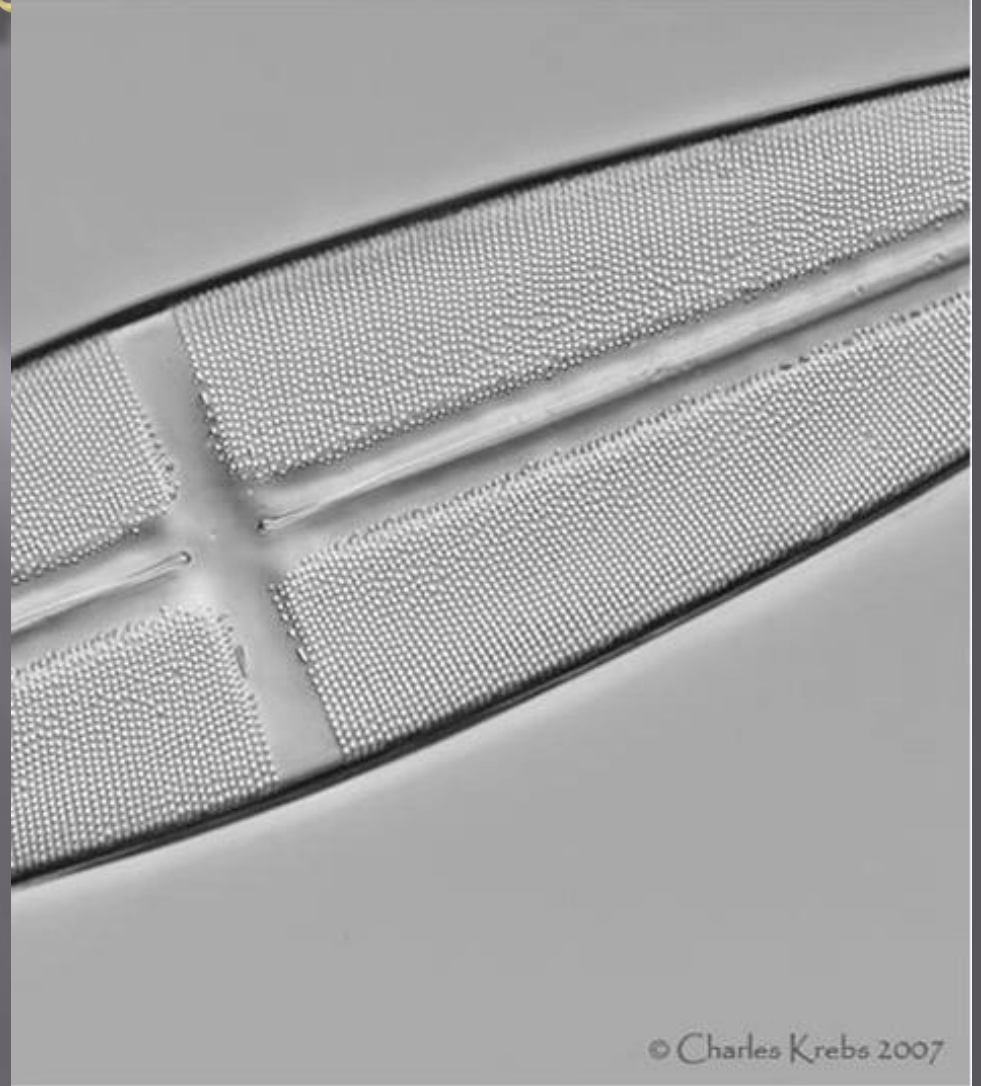


Image Comparison Article I



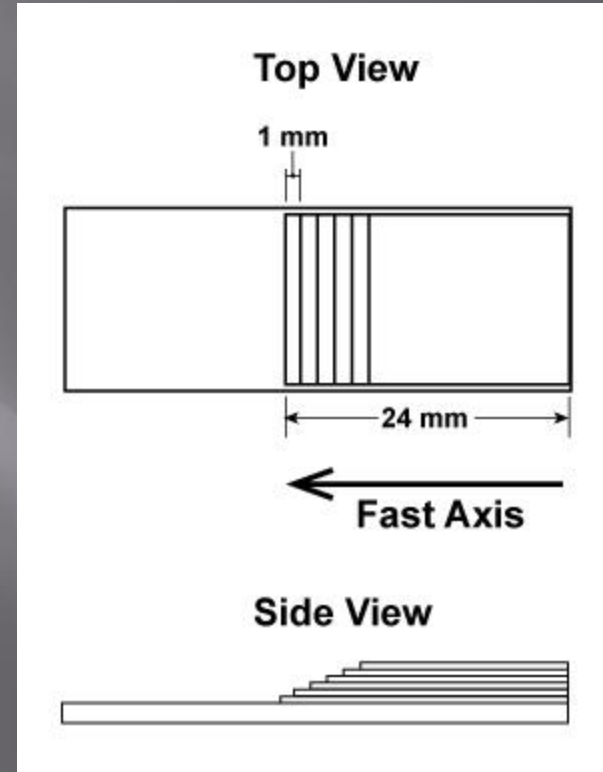
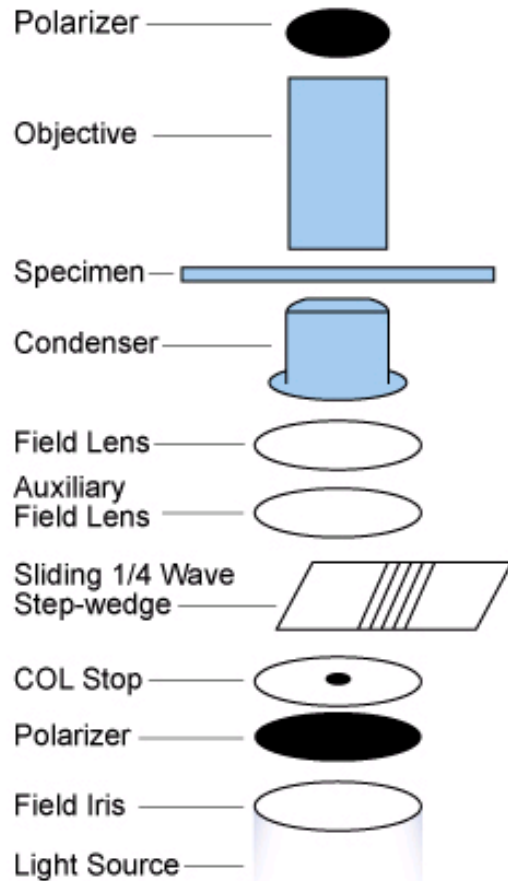
Stauroneis

Image Comparison Article I

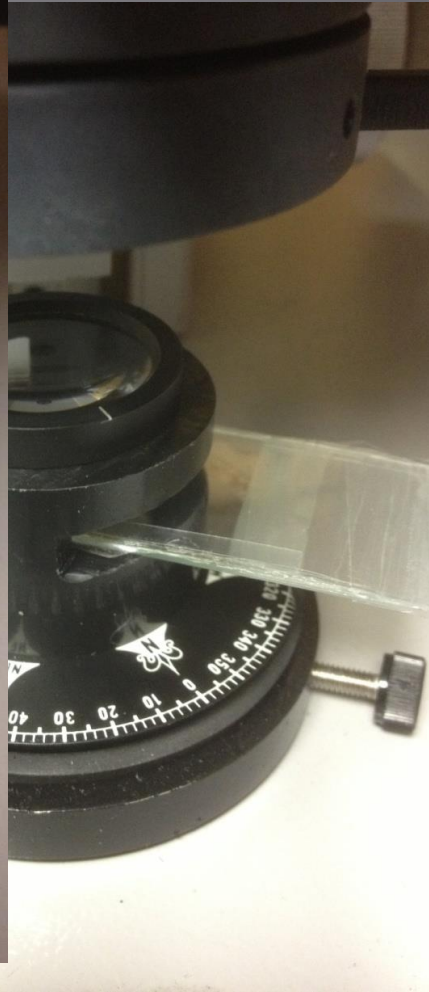


Stauroneis

Article II



Article II Actual Setup

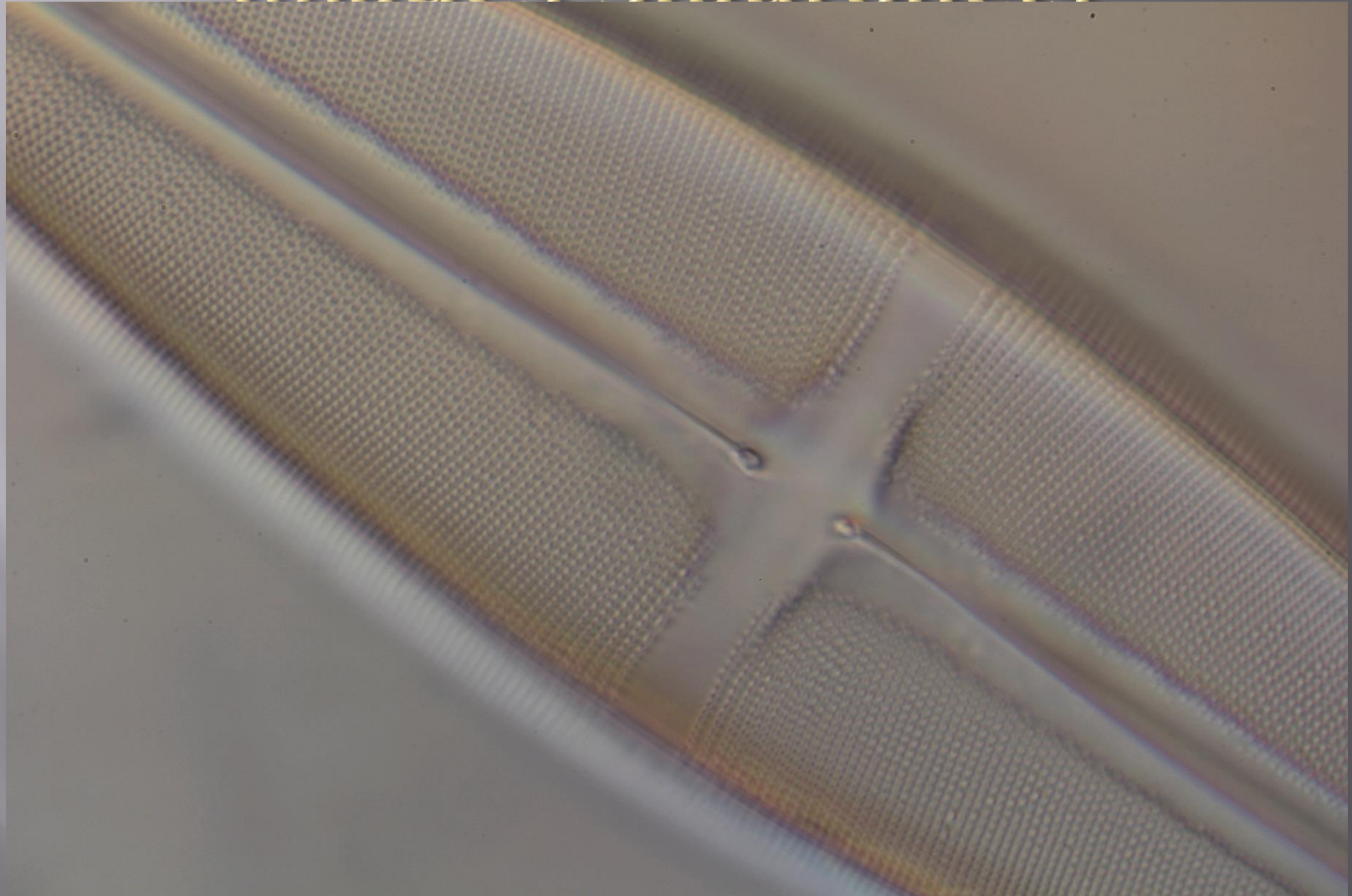


Eight-form and ten-form diatom test slides were selected as the test subject. Striae spacing for test diatoms are given in the table below:

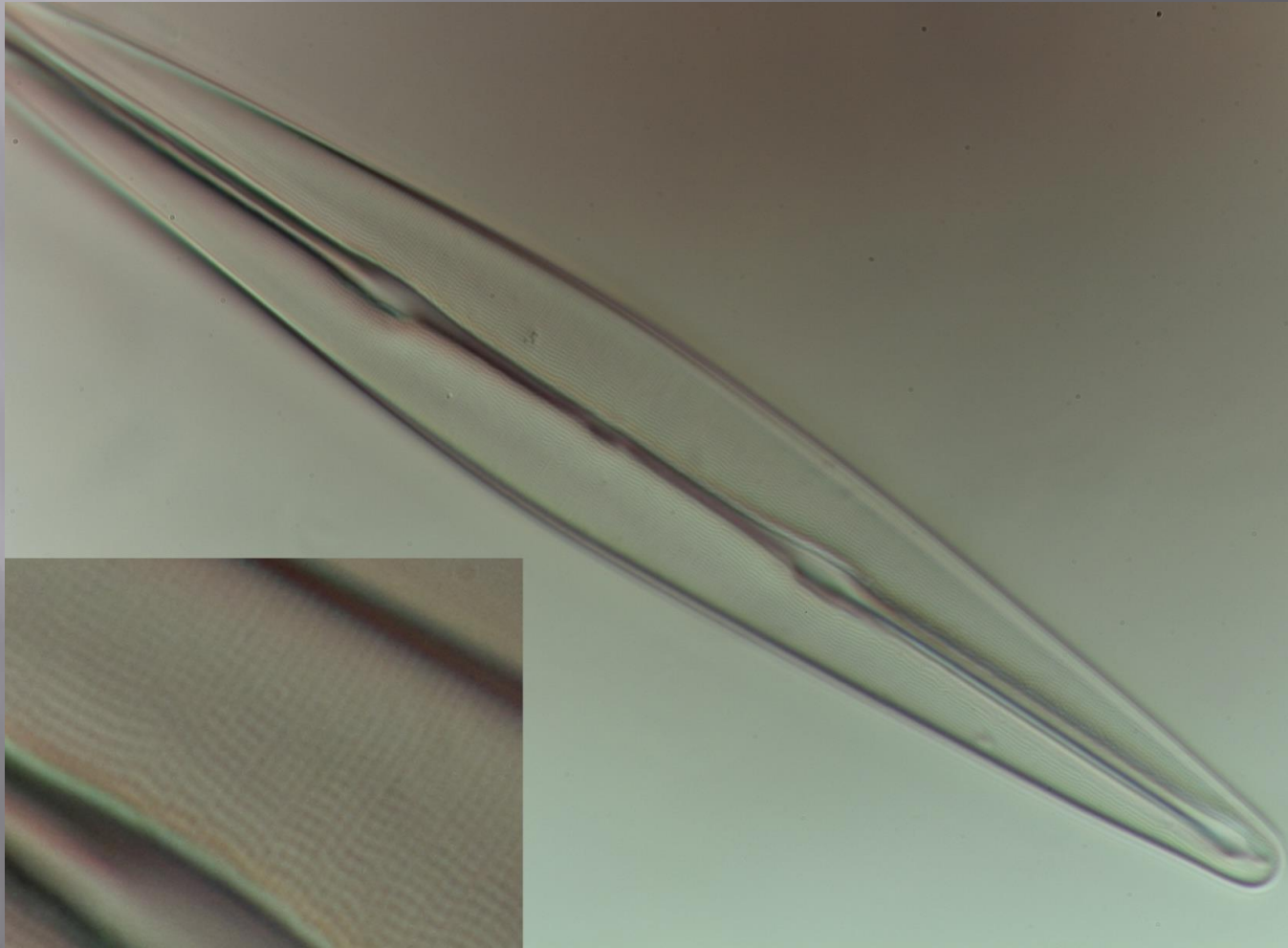
Table 1. Diatoms imaged with critical parameters.

Diatom	Length (mm)	Striae Spacing
<i>Amphiplura pellucida</i>	80	0.27^3 , $0.21 (0.22-0.25)^4$
<i>Amphipleura lindheimeri</i>	100	0.36^5
<i>Surirella gemma</i>	60-100	$0.33 (0.40-0.50)^4$
<i>Nitzschia sigma</i>	150	0.435^3
<i>Stauroneis phoenocenteron</i>	170	0.714^3

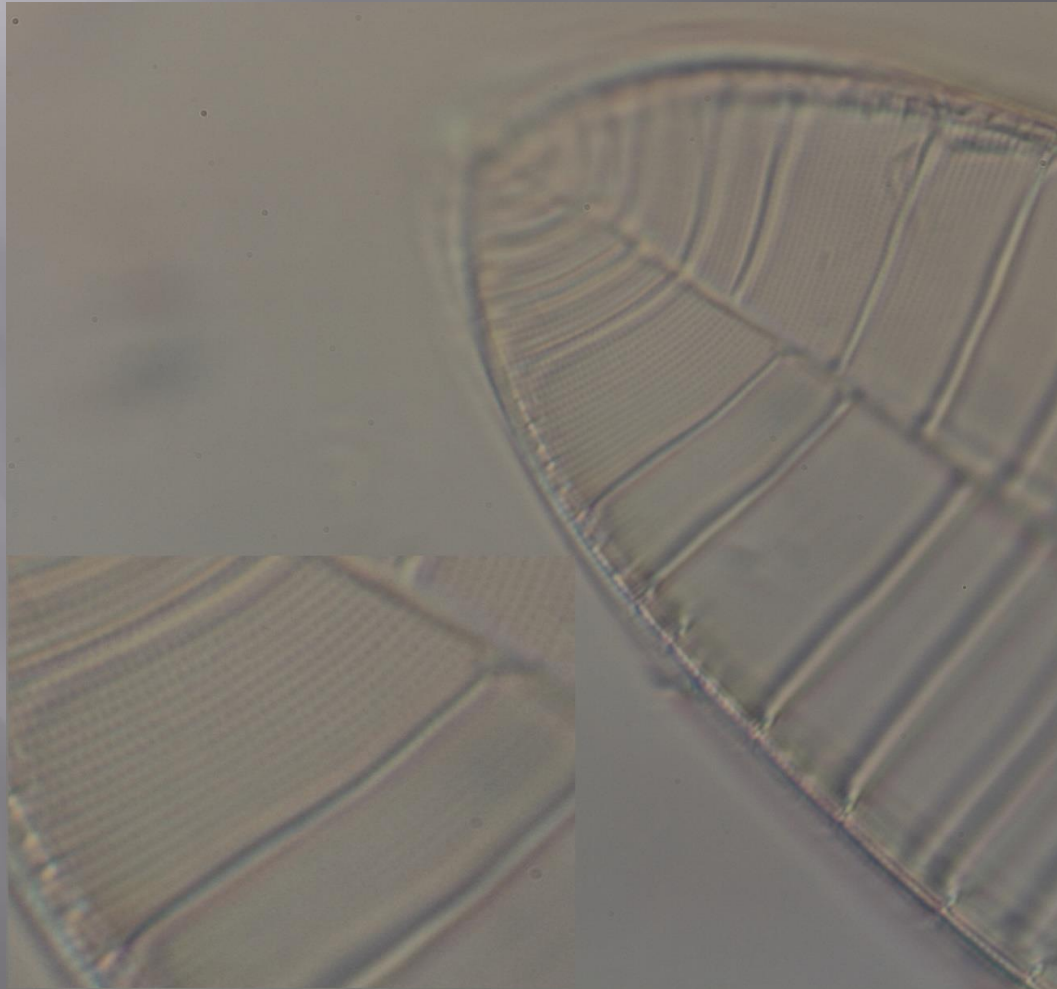
Images Stauroneis



Images Amphiplura



Images Surirella



Images *Amphiplura Pellucida*

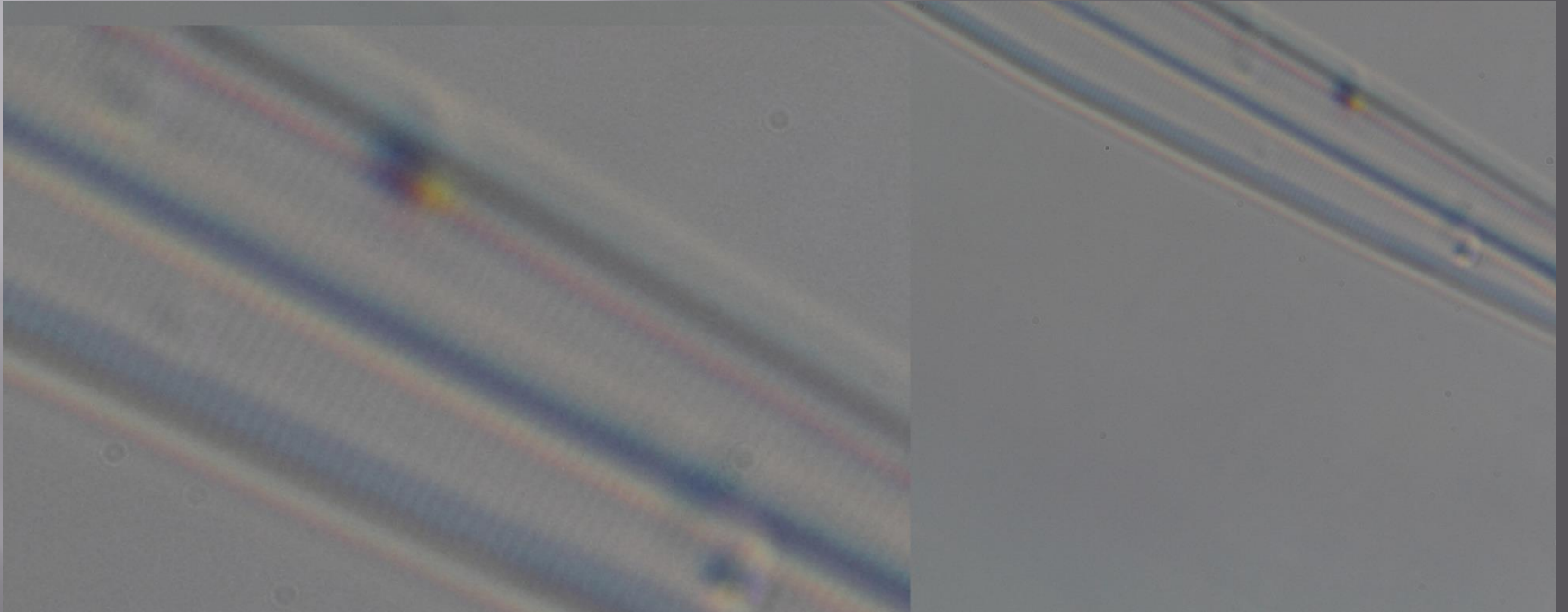
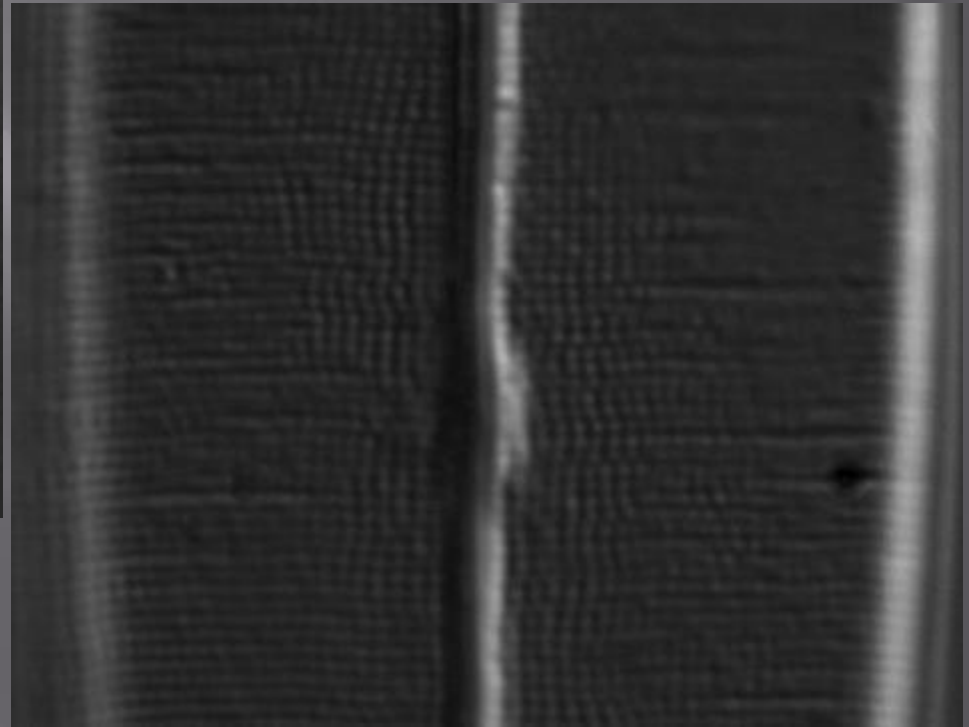
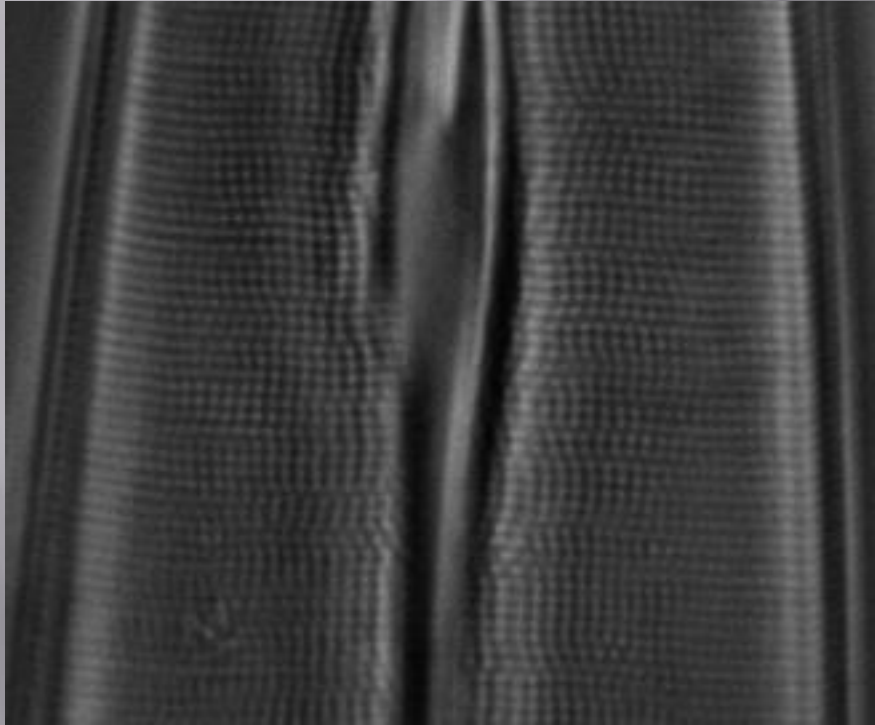


Image Comparison Article II



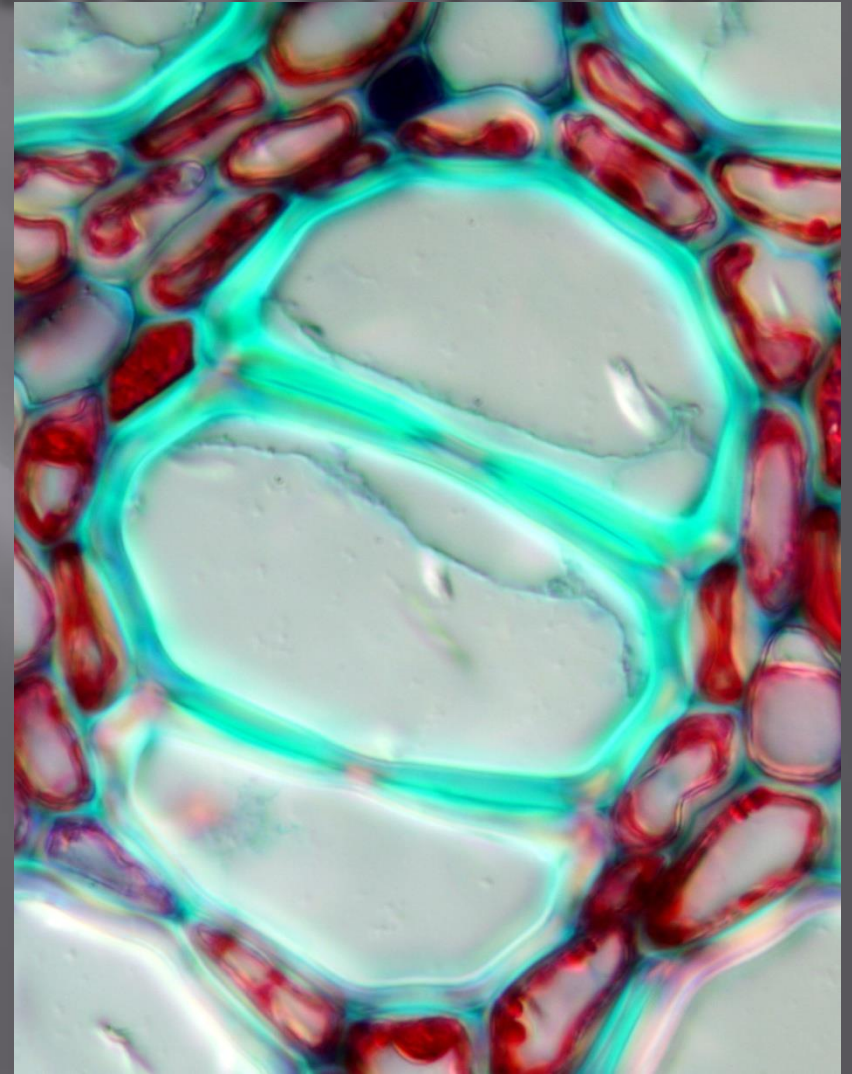
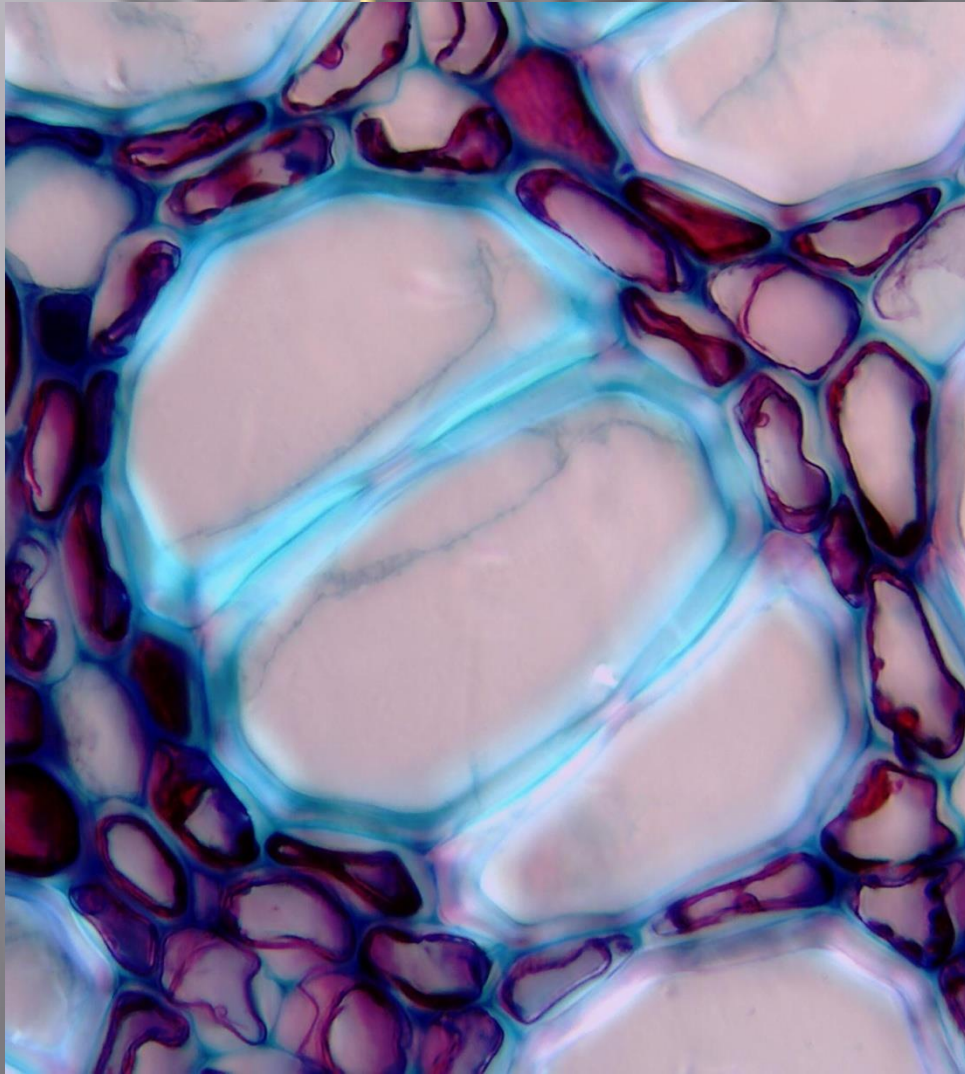
Stauroneis

Image Comparison Article II

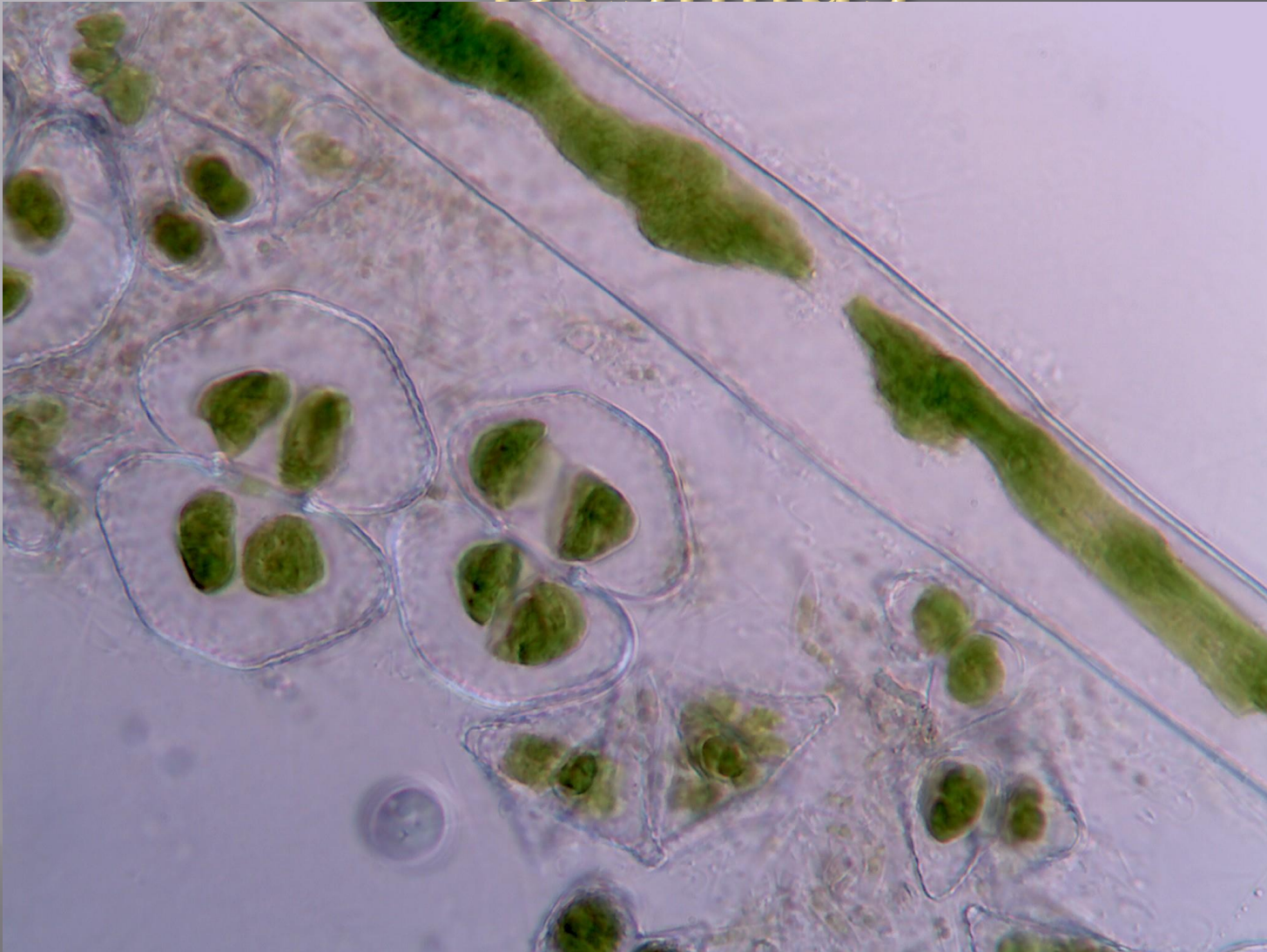


Amphiplura

Comparison to DIC Fern Root



Desmids



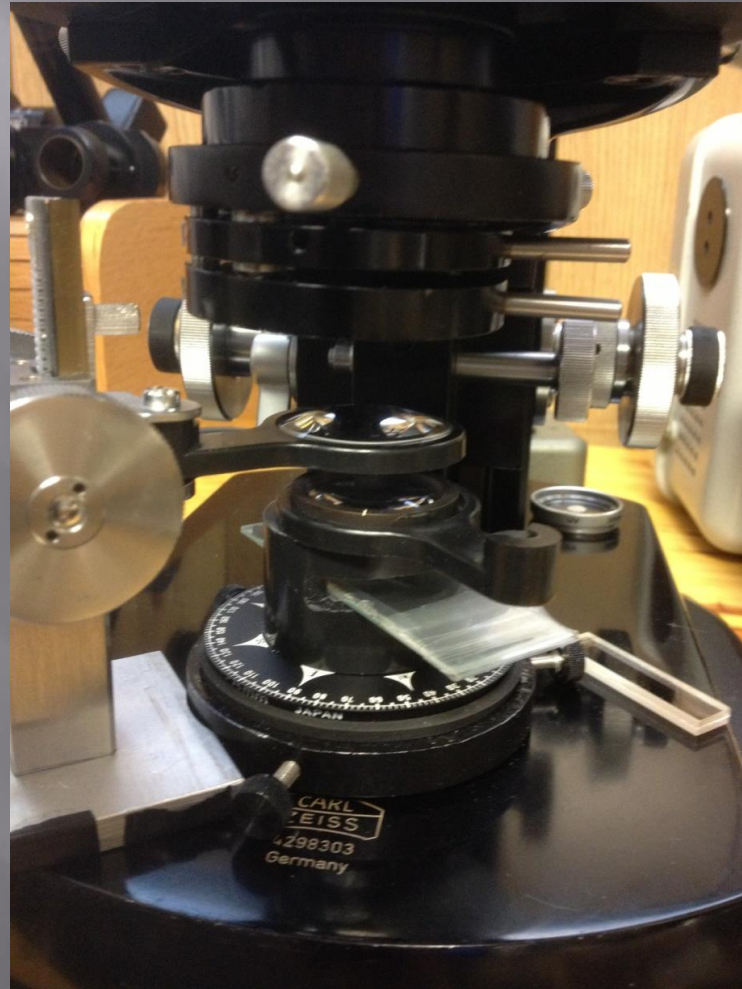
Desmid



Desmid



The Future???



Conclusions

- ▣ All prototypes improved contrast over brightfield
- ▣ Prototypes with the central round stop gave the best resolution enhancement
- ▣ Some prototypes offer image quality and an overall appearance similar to those obtained via DIC at a cost of around \$20.00 (USD)

- ▣ “Legendary innovators like Franklin, Snow, and Darwin all possess some common intellectual qualities — a certain quickness of mind, unbounded curiosity — but they also share one other defining attribute. They have a lot of hobbies.”

Steven Johnson, *Where Good Ideas Come From: The Natural History of Innovation*