Coverslips and Oil: a resolutionary experiment in light microscopy

by Michael Hopping

BEGINNING MYCROSCOPISTS HAVE LOTS OF THINGS TO SORT OUT, including a list of "mechanical" issues. Two of those are discussed here. What coverslips are best? What's the optimal magnification for detailed viewing of really tiny stuff? The answer to the latter will depend on whether you're using a camera-equipped microscope.

I assessed both questions using an AmScope trinocular microscope with "Plan" objective lenses and an 18 megapixel camera supported by AmScope software installed on my laptop. My slides, and yours, should be made of 1 x 3 inch clear glass. Coverslips for mycological microscopy also need to be glass. Use square slips, 22x22 mm. But these can be bought in different thicknesses. Three were compared in the experiment.

- #0 0.08-0.12 mm thick, expensive and uncommon but most forgiving of thick specimens
- #1 0.13-0.16 mm thick, cheap and readily available
- #1.5 0.16-0.19 mm thick, can be cheap but harder to find, not good with thick material but the best fit for the 0.17 mm thickness optimization used with objective lenses

A few magnification basics are in order here. Microscope lenses have much in common with the lenses in cameras, binoculars, loupes, etc., but they operate toward the extreme end of what's resolvable with visible light.

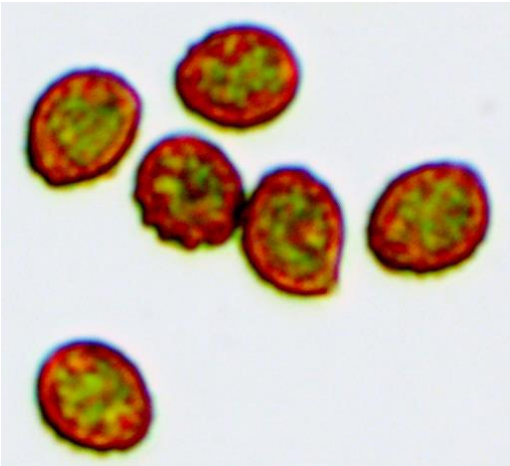
- At 1000x magnification the edges of objects blur due to the appearance of multicolored haloes at those edges. Good quality lenses help but don't eliminate the problem.
- The higher the magnification, the closer the objective lens must be to the material. At 1000x, the objective lens must be within a few microns (thousandths of a millimeter) of the specimen. A thick specimen and thick coverslip combination prevents the 100x objective lens from getting close enough to focus. When rotating the 100x lens into position the cover slip might be knocked aside. Or, if using the focus knobs, the material moves in response to fine focus adjustments—the lens is mashing the coverslip.
- Depth of field: Increased magnification decreases the thickness of material in focus. At 1000x, depth of field might be no more than a micron. At lower magnifications it becomes progressively wider.

Two questions presented themselves.

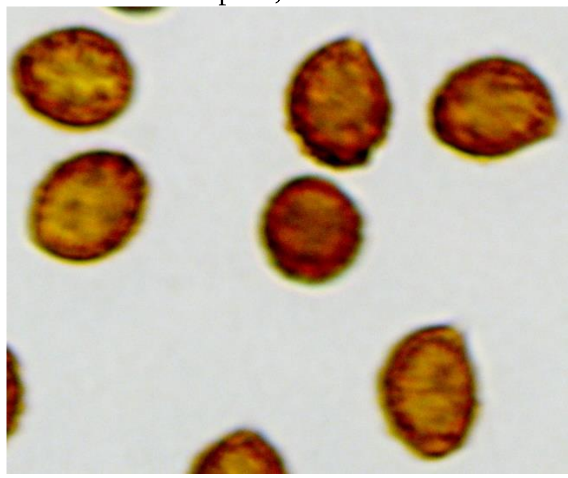
- What's the visible difference in sharpness for coverslips of the three thicknesses?
- The camera and software package let me enlarge images several times over what appears when looking directly down the scope. I can take a snapshot and use it for measurements. How does the greater depth of field with the dry 40x lens compare with the 100x lens + oil for overall image sharpness?

I made three spore slides, one for each thickness of coverslip, from the same cap of *Cortinarius squamulosus*, a species with "warty" spores. Each slide was mounted in KOH, viewed and photographed at both 400x (dry) and 1000x (oil) with the condenser diaphragm set to scope recommendations for each. The resulting photos were examined for the sharpest cross-sectional images of spores, then cropped to equalize spore size. There's not much difference in image resolution at 400x. At 1000x the #1.5 coverslip did outperform thinner ones in the anticipated stepwise fashion. But for everyday purposes the improvement over the #1 coverslip ain't much. For me, the camera and software mean that examining spores at 400x means sharper images and less hassle. Folks lacking the camera option still need to go to the oil lens.

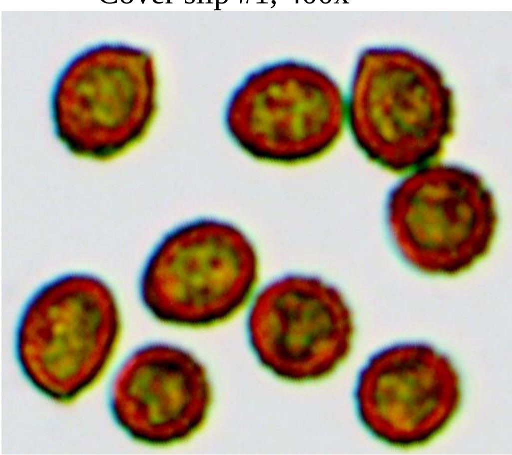
Cover slip #0, 400x



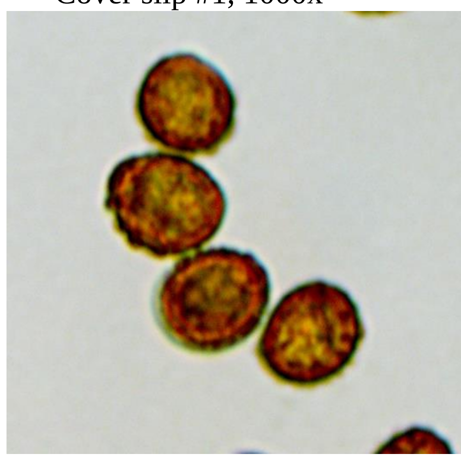
Cover slip #0, 1000x



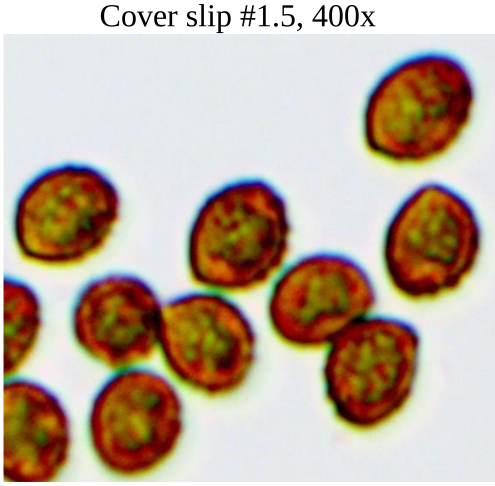
Cover slip #1, 400x



Cover slip #1, 1000x



Cover slip #1.5, 400x



Cover slip #1.5, 1000x