

#1.5 Coverslips for Microscopy

Douglas Cromeey, MS – September 2015

Overview

Optical microscopy is a powerful tool in biological research, but it is important to remember that there are some basic optics/physics principles involved. To best prepare samples for microscopy we need to use these optical principles to our advantage and not to our disadvantage.

There are a number of markings on a microscope objective lens. In the image shown here are examples of 40x oil objectives from all of the major microscope vendors. On every lens is the number 0.17¹. This number is the expected thickness of the coverslip on the microscope slide

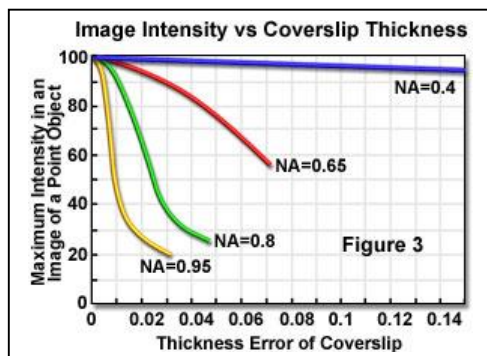


(or at the bottom of a culture dish) given in millimeters. This thin piece of glass is factored into the optical calculations when the lens is designed. Plastic coverslips and/or culture dish bottoms are not acceptable substitutes for a glass coverslip.

Coverslip #	Thickness
0	0.085 to 0.13 mm
1	0.13 to 0.16 mm
1.5	0.16 to 0.19 mm
1.5H	0.17 to 0.18 mm
2	0.19 to 0.23 mm
3	0.25 to 0.35 mm
4	0.43 to 0.64 mm

DIN ISO 8255 standard

Commercially available coverslips come in a variety of thicknesses, but the thickness closest to 0.17mm is a #1.5 coverslip. For the newer superresolution optical microscopes, a #1.5H (high performance) is the required thickness. Using the incorrect coverslip thickness reduces your ability to get the most information out of your sample using an optical microscope.



Coverslip thickness is less important when using lenses that have a numerical aperture (NA) of 0.4 or lower, which on many microscopes would only be the lenses between 1x-10x. As magnification (20x or higher) and NA increases, the loss from having an incorrect thickness coverslip can become significant.² The graph shown used a fluorescent bead for this test and it clearly shows that higher numerical aperture lenses are much more prone to intensity loss with even small deviations from the optimal coverslip thickness. The intensity losses are due to optical aberrations and the effect on transmitted light images is similar.

¹ NOTE: there are some lenses that do not require coverslips, or have an adjustable collar to compensate for variations in coverslip thickness. The majority of microscope lenses expect a 0.17mm thickness glass coverslip.

² <https://www.microscopyu.com/articles/formulas/formulascoverslipcorrection.html>

The bottom line is that the best microscopy images can only be captured when using the correct (#1.5) thickness glass coverslip. Given that coverslips are inexpensive, why use anything else?

Vendors for #1.5H coverslips

The #1.5H coverslips can be a bit more difficult to locate, since this thickness is a relatively new standard. With much tighter thickness tolerances, these coverslips are required for superresolution microscopy. Given that these coverslips can improve images with every microscope, why not standardize on this thickness?

Cost comparison: a 22x22mm high precision (#1.5H) coverslip costs \$65/box of 200 (Bioscience Tools), which comes to \$0.33 each. A regular #1.5 coverslip of the same size from Sigma-Aldrich is \$28.80/pack of 200, which comes to \$0.15 each.

US Distributor information	Available sizes for #1.5H
Azer Scientific (distributor for Marienfeld-Superior.com) Morgantown, PA http://www.azerscientific.com/catalogsearch/result/?q=1.5H&x=0&y=0	Rectangular: 18x18mm, 22x22mm, 24x50mm, 24x60mm Circular: 10mm, 12mm, 18mm
Applied Microarrays, Inc. (distributor for Schott.com) Tempe, AZ http://www.us.schott.com/nexterion/english/products/coverslips.html https://appliedmicroarrays.com/product/high-performance-coverslip-1-5h-cleanroom-cleaned/	Rectangular: 18x18mm, 22x22mm, 24x60mm, 25x65mm, 25x75mm Other dimensions are available to special order
Bioscience Tools San Diego, CA http://www.biosciencetools.com/catalog/Coverslips.htm	Rectangular: 18x18mm, 22x22mm, 24x50mm, 25x60mm Circular: 10mm, 12mm, 13mm, 18mm, 24mm, 25mm
Carl Zeiss Microscopy LLC Thornwood, NY https://www.micro-shop.zeiss.com/index.php?s=207271689a48d43&l=en&p=us&f=s&o=0&h=25&q=cover+glass	Rectangular: 18x18mm, 22x22mm
Ibidi Madison, WI http://ibidi.com/search-results/?q=1.5H	Several types of cell culture dishes/chambers with #1.5H glass coverslips
MatTek Ashland, MA http://glass-bottom-dishes.com/catalog/index.php?main_page=advanced_search_result&search_in_description=1&zenid=b7bb001b3d9699364edde5160fbcde45&keyword=high+tolerance http://glass-bottom-dishes.com/catalog/index.php?main_page=product_info&cPath=56_58&products_id=137	35mm culture dish with 14mm round coverslip opening (#1.5H, "high tolerance") Rectangular: 18x18mm

Data is as of September 2015. Being listed here does not imply a recommendation of the particular vendor.