

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

There are a number of markings on a microscope objective lens. Shown here are examples of 40x oil immersion objective lenses from all of the major microscope vendors. Every lens in this image is clearly marked with the number 0.17. This number is the expected thickness of the coverslip on the microscope slide (or the bottom of a culture dish)



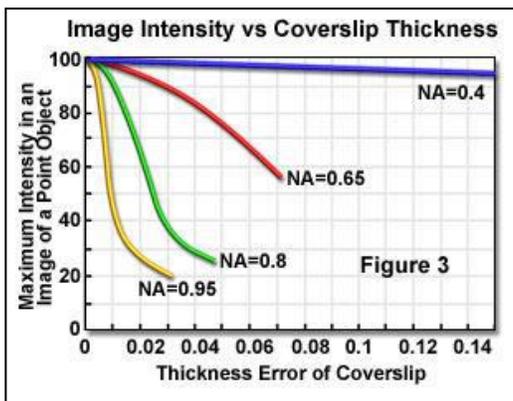
given in millimeters. This thin piece of glass is factored into the optical calculations when the lens is designed. Plastic coverslips and/or culture dish bottoms are not acceptable substitutes for a glass coverslip.

Coverslip #	Thickness
0	0.085 - 0.13 mm
1	0.13 - 0.16 mm
1.5	0.16 - 0.19 mm
1.5H	0.17 - 0.18 mm
2	0.19 - 0.23 mm
3	0.25 - 0.35 mm
4	0.43 - 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 any of the superresolution optical microscopy techniques, a #1.5H (high performance) is the required thickness. **Using the incorrect coverslip thickness can greatly reduce your ability to get the most information out of your sample using an optical microscope.**

Coverslip thickness is less important when using objective lenses that have a numerical aperture (NA) of 0.4 or lower, which on most microscopes would only be lenses between 1x-10x. As magnification (2x or higher) and NA increases, the loss from having an incorrect thickness coverslip can become significant. The graph shown used a fluorescent bead for this test and it clearly shows a significant drop in the maximum intensity of the bead (which could easily be a structure in a cell) 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.



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

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.

Graph is from: <https://www.microscopyu.com/articles/formulas/formulascoverslipcorrection.html>

Standardize on the #1.5H

The #1.5H coverslips can be a bit more difficult to locate, since this is a relatively new ISO standard thickness. Because the manufacturing tolerance is might more stringent, these coverslips are required for superresolution microscopy. Given that these coverslips can improve images with every microscope, why not standardize on this thickness?

US Distributor information <i>(This is information I found online, it is not an endorsement. Reviewed 08/2020)</i>	Available coverslip sizes for #1.5H
Azer Scientific (distributor for Marienfeld-Superior.com), Morgantown, PA http://www.azerscientific.com/ search for <u>high precision</u>	Rectangular: 18x18mm, 22x22mm, 24x50mm, 24x60mm Circular: 10mm, 12mm, 18mm, 25mm <i>Note: their coverslip bottom multi-well plates and culture dishes are #1.5, not #1.5H.</i>
Applied Microarrays, Inc. (distributor for Schott.com), Tempe, AZ https://appliedmicroarrays.com/product/high-performance-coverslip-1-5h-cleanroom-cleaned/	Rectangular: 18x18mm, 22x22mm, 24x60mm, 25x75mm <i>Other dimensions are available to special order</i>
Bioscience Tools , San Diego, CA http://www.biosciencetools.com/catalog/Coverslips.htm (CSHP-No1.5)	Rectangular: 18x18mm, 22x22mm, 24x50mm, 25x60mm Circular: 10mm, 12mm, 13mm, 18mm, 24mm, 25mm
Carl Zeiss Microscopy , White Plains, 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: 22x22mm Circular: 17mm
Cellvis , Mountain View, CA https://www.cellvis.com/product_search.php?cat_num=all&vessel_size=all&well_size=all&coverslip=%231.5H	Coverslip bottom chamber slides , round culture dishes , multi-well plates all with #1.5H
Ibidi , Madison, WI http://ibidi.com/ search for <u>1.5H</u> . You will need to read the product specifications to ensure that you order the #1.5H thickness. Please note, the polymer (non-glass) coverslips offered by ibidi may be incompatible with and/or have not been tested with some microscope immersion oils, see: https://ibidi.com/search?controller=search&s=1.5H	Several types of cell culture dishes/chambers with #1.5H glass coverslip bottoms
MatTek , Ashland, MA https://www.mattek.com/product-category/cultureware/	35mm culture dish with 14mm round #1.5h coverslip opening (<i>P35G-0.170-14-C</i>) Rectangular: 18x18mm (<i>PCS-170-1818</i>)
Neuvitro , Vancouver, WA https://www.neuvitro.com/precision-coverslip-super-resolution	Circular: 12mm, 18mm, 25mm
Ted Pella , Redding, CA https://www.tedpella.com/section_html/pelco-glass-bottom-dishes.htm.aspx#clear_wall	35mm and 50mm round plastic culture dishes w/ #1.5H coverslip bottom
ThorLabs , Newton, New Jersey https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=9704	Rectangular: 22x2mm, 24x50mm Circular: 12mm, 25mm

Mr. Crome is grateful for the support he receives from the SWEHSC (P30-ES006694) and the UACC (P30-CA023074).

From the **UA Microscopy Alliance** - <http://microscopy.arizona.edu/learn/printable-materials>
 ©2020 The University of Arizona, Tucson, AZ *(original 09/2015, updated 08/2020)*
