

The importance of #1.5 thickness coverslips for Microscopy



Douglas Cromeey, MS (ARL Imaging Cores, UACC TACMASR, SWEHSC)
 Modified European Version: Angela Naumann, Life Imaging Center Freiburg (MIAP)



Optical microscopy is a powerful tool in biological research. It is important to remember that there are some basic optical/physics principles involved, which you should definitely take into account to get good imaging results. 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.

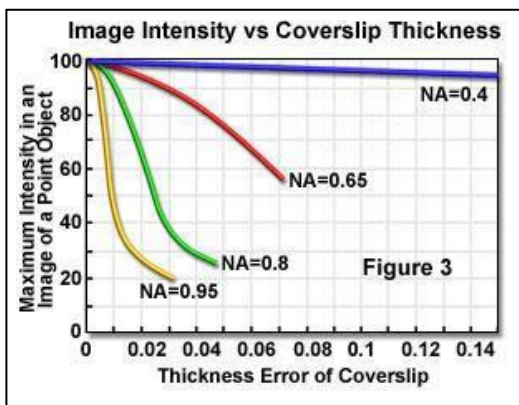
This thin piece of glass is included for the calculation of the optical performance of a microscope objective. Plastic coverslips and/or culture dish bottoms are **not** acceptable substitutes for a glass coverslip.



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

Commercially available coverslips come in a variety of thicknesses, but the thickness closest to 0.17mm is a #1.5 coverslip. For the newer, super resolution optical microscopes, 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. However, as magnification (2x or higher) and NA increases, the loss from having an incorrect coverslip thickness will become significant.



The graph shows a significant drop in the maximum intensity of a fluorescent test 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, highest resolution)** microscopy images can only be captured when using the correct (#1.5) coverglass thickness. 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 are designed for 0.17mm thickness of the glass coverslip.

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

Standardize on the #1.5H

Buying the particular #1.5H coverslips can be a bit tricky, since this is a relatively new ISO standard thickness. However, even in super resolution microscopy, slight deviations from the cover glass thickness show strong negative effects. In addition, also at all other systems using these coverslips will improve your recording. Therefore, why not use these coverslips with the lowest deviation tolerance as standard for all imaging sessions, even if they are a bit more expensive.

European Distributor Information (checked 07/2019)	Available coverslip sizes for #1.5H (link to specific product)
<p>Schmidt Laborbedarf 38518 Braunschweig, Germany WWW: http://www.schmidt-laborbedarf.de/</p> <p>Rectangular Cover Glasses (0.170 +/- 0.005 mm) for high performance microscope (Mariefeld Superior)</p>	<p>18x18 mm, Nr. 1,5H 22x22 mm, Nr. 1,5H 24x50 mm, Nr. 1,5H 24x60 mm, Nr. 1,5H</p>
<p>Schmidt Laborbedarf 38518 Braunschweig,, Germany WWW: http://www.schmidt-laborbedarf.de/</p> <p>Circular Cover Glasses (0.170 +/- 0.005 mm) for high performance microscopes (Mariefeld Superior)</p>	<p>10 mm Ø, Nr. 1,5H 12 mm Ø, Nr. 1,5H 13 mm Ø, Nr. 1,5H 18 mm Ø, Nr. 1,5H 24 mm Ø, Nr. 1,5H 25 mm Ø, Nr. 1,5H</p>
<p>Fisher Scientific GmbH (part of Thermo Fischer Scientific) Im Heiligen Feld 17 - D-58239 Schwerte</p> <p>WWW: https://www.fishersci.de/de/de/home.html</p> <p>Carl Zeiss™ Rectangular Cover Glasses, High Performance - related tolerance D=0.17 mm +/- 0.005 mm</p>	<p>Carl Zeiss™ 18 x 18mm 1,5H</p>
<p>Thorlabs GmbH 85221 Dachau/Munich, Germany</p> <p>WWW: https://www.thorlabs.de</p>	<p>Several types of #1.5H glass coverslips, also small amounts</p>
<p>ibidi GmbH 82166 Gräfelfing, Germany</p> <p>WWW: https://ibidi.com</p>	<p>Several types of cell culture dishes/chambers with #1.5H glass coverslips</p>

Mr. Cromeys 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) - <http://microscopy.arizona.edu/learn/printable-materials>
 ©2017 The University of Arizona, Tucson, AZ (adapted 2020)