Thickness of cover slips and glass slides

Moderators: Chris S., Pau, Beatsy, rjlittlefield, ChrisR

dragonblade

Posts: 275
Joined: Sat Oct 18, 2014 11:16 pm

Thickness of cover slips and glass slides

by **dragonblade** » Sun May 25, 2025 4:01 am

Previously, I had some little boxes of slides and cover slips that were stored inside a foam box. These items were included with my light compound microscope purchase. Oddly, my bedroom contents were rearranged by other family members while I was away on a trip (without my knowledge.) I thought my microscope accessories would be intact within the foam box but I was wrong.

So I'm on the look out for new slides and cover slips.

I note on the objective that it mentions the recommended thickness of the cover slips that are to be used in conjunction with them. In my case, it's 0.17. Though are there different thicknesses of glass slides or are they all about the same thickness? I thought that the thickness of the slides would be a more critical factor.

And would there be any recommendations for online stores that sell cover slips and slides (particularly within Australia)?

Re: Thickness of cover slips and glass slides

by **Beatsy** » Sun May 25, 2025 10:39 am

Assuming you buy reasonable quality slips and slides, you'll find that thickness varies a little more for slides than it does for coverslips (within a brand and within

Beatsy
Site Admin

Posts: 2564
Joined: Fri Jul 05, 2013 3:10 am
Location: Malvern, UK

the same batch/box).

The thickness of slides is far less important than that of coverslips. The "0.17" marked on (most) objectives is designed into the lens and they become far less tolerant of variation as N.A. increases. In practice, the coverslip is the front element of the lens. At N.A. 1.4 you don't want much more than 0.005mm (5µm) deviation from the nominal 0.17mm thickness or spherical aberration will noticeably degrade image quality. Yes, even with oil immersion lenses. At lower power/N.A. (let's say at mag <=20x and NA <= 0.5) it is less important.

I used to use Zeiss 0.17H square coverslips which are 0.17 +/- 0.005 mm thick (H=high precision). I cut my own circular slips from those because the Zeiss round ones are prohibitively expensive, and I couldn't get 13mm diameter ones anyway. Thickness is very consistent with those. However, they're relatively expensive and I switched to Academy brand #1.5 circular coverslips instead. Like all "non-precision" coverslips there is a lot of thickness variation between coverslips in a given box. They (#1.5's) usually range from 0.16mm to 0.19mm thick, with a few outside that range. However, by measuring the thickness of each, I can usually extract between 20-40 "good" coverslips from a box of 100 - "good" being 0.17mm +/- 0.002! There are outliers here too - I had one box that only yielded 6 good slips and another that yielded 80! On average though, they work out cheaper than Zeiss high precision ones - even if you aim for a tighter thickness tolerance and bin the out-of-range ones, as I do. I just buy packs of a thousand at a time (10 boxes of 100 slips) and separate out the gooduns.

With slides, it depends on your preferences really. I prefer slides to be about 1mm thick and use the Clarity brand (they always come out of the box nice and clean - but they'll still need a proper clean and flaming if you plan to make permanent mounts with them). In my experience, over several years of use, thickness variation has never been more than +/- 0.1mm but is normally much tighter tolerance than that. Thicker slides (other brands) tend to be around 1.2mm, which you might prefer as they are less prone to break if dropped.

Hope that helps.

Cheers
Beats

Knowledge is cheap. Experience isn't.

Re: Thickness of cover slips and glass slides

Lou Jost

Posts: 6597

by **Lou Jost** » Sun May 25, 2025 11:18 am

Joined: Fri Sep 04, 2015 7:03 am
Location: Ecuador
Contact:

I've always wondered about slide thickness. The condenser must be designed for a given slide thickness, no? At least for high NA.

Also, you mentioned that coverslip thickness is important even in oil immersion. I had thought that if the oil is well-matched to the glass' refractive index, the thickness of the coverslip shouldn't matter. It that wrong? Maybe it is difficult to match the RI, but if it were precisely matched, maybe it would be true?

Lou Jost
www.ecomingafoundation.wordpress.com
www.loujost.com

Re: Thickness of cover slips and glass slides

by **rjlittlefield** » Sun May 25, 2025 12:37 pm

rjlittlefield
Site Admin

Posts: 24715
Joined: Tue Aug 01, 2006 8:34 am
Location: Richland, Washington State, USA
Contact:

> **Lou Jost wrote:** ↑ Sun May 25, 2025 11:18 am
> Maybe it is difficult to match the RI, but if it were precisely matched, maybe it would be true?

Yes. But of course the issue gets complicated in the fine points. I like this summary by ChatGPT 4o:

> In Practice:
> 1. Mismatch in Refractive Index
>
> - Typical immersion oils are designed to match the refractive index of BK7 glass, which is about 1.515 at 546 nm (green light).
> - In reality, cover glasses vary — most are soda-lime glass, not BK7, and their refractive indices can range from 1.515 to 1.525 or even slightly beyond.
> - Moreover, the immersion oil's refractive index is temperature dependent, with changes on the order of ~0.0004/°C. So even a small temperature difference from calibration conditions (~23°C) can introduce a mismatch.
>
> 2. Dispersion (Chromatic Effects)
> Even if the refractive indices match at one wavelength, they likely diverge at others. Oils and glasses have different Abbe numbers, meaning they disperse light differently. This leads to chromatic aberration, especially if the objective is used over a broad wavelength range.

--Rik

René

Posts: 473
Joined: Sun Oct 08, 2006 4:22 am

Re: Thickness of cover slips and glass slides

by **René** » Sun May 25, 2025 1:29 pm

mmhm, this is all very theoretical. Beats is right, however he mounts his diatoms directly to the underside of the coverslip. Most microscopy work is done with a layer of mountant between slip and specimen. That's why mostly #1 slips are sold, which are slightly thinner than 0.17, thereby compensating somewhat for the layer of mountant.
Used together with immersion oil it all hardly matters anymore for 99% of microscopy. imho.

chat gtp is wrong in saying coverslips are lime glass, they are borosilicate as far as I'm aware. Slides are lime glass. They attract dirt far more than borosilicate, also in unopened cartons. I'll keep my supply over silicagel because of that.

Best, René

Lou Jost

Posts: 6597
Joined: Fri Sep 04, 2015 7:03 am
Location: Ecuador
Contact:

Re: Thickness of cover slips and glass slides

by **Lou Jost** » Sun May 25, 2025 4:58 pm

Thanks Rik, I had forgotten about the effects of dispersion.

Lou Jost
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dy5

Posts: 151
Joined: Sun Feb 07, 2010 7:50 pm
Location: College Park, MD

Re: Thickness of cover slips and glass slides

by **dy5** » Mon May 26, 2025 5:59 am

Interesting, informative discussion. Some higher N.A. objectives have adjustment collars that can compensate at least to some extent for different cover slip thickness. For instance, on my Nikon plan apo 40x/0.95, the collar is labeled 0.11 - 0.23.

The optics for photographing protozoa in water must be much more complicated. I find that adjustment of the collar can make a huge difference in resolution, but determining the correct setting for any given situation is trial and error. It's not at

all simple (at least for me ...) and is complicated by the accompanying change in focus. I've had best settings ranging from about 0.13 to 0.18. Any suggestions for other strategies for finding the correct compensation would be most welcome!

Cheers, David

Re: Thickness of cover slips and glass slides

by **Beatsy** » Mon May 26, 2025 11:50 am

Beatsy
Site Admin

Posts: 2564
Joined: Fri Jul 05, 2013 3:10 am
Location: Malvern, UK

> **dy5 wrote:** ↑ Mon May 26, 2025 5:59 am
> ...Any suggestions for other strategies for finding the correct compensation would be most welcome!

Hi David,

The "textbook" method of finding the best correction setting is to find a speck of something, just a tiny dot, and adjust the collar until the OOF image of the speck looks identical on both sides of the focus plane. Of course, this only works well if you're viewing or photographing stationary subjects at the same level in a (probably permanent or semi-permanent) mount.

If you're chasing critters around in a wet mount, then dynamically judging in-focus image quality by eye is faster, but not really an ideal use for such objectives. Basically the trick is to turn fine focus and the objective's correction collar simultaneously so the subject stays in focus with only the image quality of the in-focus feature(s) changing. After some practice, you should be able to rack back and forth through the best image - from blurred (by SA) to sharp to blurred again and back. With practice, you'll be able to zone in on the best setting quite quickly - or at least very close to it.

You need a very stable scope to do this though (too much wobble makes it really difficult to keep the subject consistently in focus). If that's not the case then it might be easier to restrict the vertical motion of subjects so a single, static correction collar setting is enough. Something like waiting for water to evaporate from the mount so critters get trapped between the slip and slide would work. You'll have to be quick to take photos before things get crushed, and they may look unnatural if compressed too much, but it's one workable alternative - among others.

Cheers
Beats

Knowledge is cheap. Experience isn't.

Re: Thickness of cover slips and glass slides

by **dy5** » Tue May 27, 2025 3:17 am

dy5

Posts: 151

Joined: Sun Feb 07, 2010 7:50 pm
Location: College Park, MD

Thanks, Beats - extremely helpful! It'll be great to try some systematic strategies rather than my 'hit or miss' method.

Cheers, David

BudP

Posts: 107
Joined: Fri Mar 12, 2021 10:33 am
Location: Douglasville, GA

Re: Thickness of cover slips and glass slides

by **BudP** » Wed May 28, 2025 10:14 am

Hi Beats. I am not familiar with “flaming” a slide. Would you mind explaining how you do it? Also what is your preferred method of cleaning a slide. Thanks.

Thanks for your time and consideration,,
BudP

Beatsy
Site Admin

Posts: 2564
Joined: Fri Jul 05, 2013 3:10 am
Location: Malvern, UK

Re: Thickness of cover slips and glass slides

by **Beatsy** » Wed May 28, 2025 11:44 am

> **BudP wrote:** ↑ Wed May 28, 2025 10:14 am
> Hi Beats. I am not familiar with “flaming” a slide. Would you mind explaining how you do it? Also what is your preferred method of cleaning a slide. Thanks.

Hi Bud,

I flame slides with a chefs blowtorch, refillable with butane. Just pass the flame over the surface of the slide a few times. As well as sterilising, it also makes the glass hydrophilic, so drops of water spread out instead of beading up.

Over the years, I tried practically every cleaning method there is but now I'm settled on a very quick and simple method (for both slides and coverslips). This involves cleaning with ROR (Residual Oil remover - recommended to me by Bill Dailey) then flaming. A small squirt of ROR is applied to a lint free cloth which is used to scrub the glass surface - then polish dry and streak-free with another lint-free cloth and flame. This applies to slides straight out of the box. If I re-use slides I pre-clean them in normal washing up liquid and dry them first.

Note: ROR is *ridiculously* expensive for what it is! However, the manufacturer was kind enough to list the ingredients and their ratios in the safety data sheet - so I cloned my own at a tiny fraction of the cost per ml. It works exactly the same.

The recipe for roughly a litre of Bootleg-ROR is...

Deionised water 850ml
20-30% Ammonia 8ml
Salt 8g
90%+ Isopropyl Alcohol (IPA) 45ml
Liquid soap 80ml

I use a pure, vegetable-based soap and would recommend that, but others have reported good results with synthetic soaps too. This is the one I use - https://utilitygreatbritain.co.uk/produ ... iquid-soap

Hope that helps.

Cheers
Beats

Knowledge is cheap. Experience isn't.

BudP

Re: Thickness of cover slips and glass slides

Posts: 107
Joined: Fri Mar 12, 2021 10:33 am
Location: Douglasville, GA

by **BudP** » Wed May 28, 2025 6:17 pm

Thank you. You are very kind to share your knowledge, experience and The Formula! I still have two bottles of ROR from the film days. It will be interesting to see if it is still good after 30 years. Do you flame all of your slides or just the slides for permanent mounts? Again thanks for taking the time to educate a newbie who is enjoying exploring the many facets of microscopy.

Thanks for your time and consideration,,
BudP

Re: Thickness of cover slips and glass slides

Beatsy
Site Admin

Posts: 2564
Joined: Fri Jul 05, 2013 3:10 am
Location: Malvern, UK

by **Beatsy** » Thu May 29, 2025 1:40 am

Only slides for permanent mounts or open strews of diatoms (used for searching and picking out good forms) actually need flaming. It isn't necessary for things like a temporary pond sample. However, I do end up flaming all my slides after cleaning - mainly out of habit and inertia from so many repetitions of the routine.

Cheers
Beats

Knowledge is cheap. Experience isn't.

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