Between Objective & Slide – Getting Better Images from Your Dry Objectives[1]

Steve Neeley (USA)

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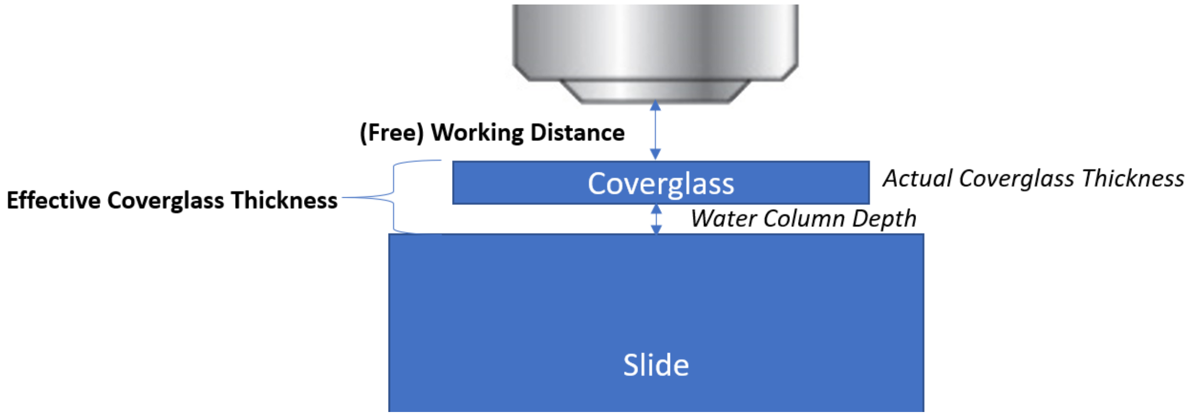
1 The situation for immersion objectives is similar but with important differences. I have little experience with immersion objectives and am not qualified to discuss them. But see page I-34, Better Microscopy Series, Compendium Edition, by D. J. Jackson, for a detailed discussion of these. Get it in bound form at LuLu or download in the form of five pdf files for free from Dave Jackson's site (See the Monday, August 26th, 2019 entry).



Above: A illustration of the important distances and thicknesses between objective and slide

Preface

This article builds on, and extends, Paul James' helpful Micscape article – ['A Case for Thin Coverslips'](). Reading Paul's article is well worth your time.

Note: I use a Leitz Ortholux I and my usual specimen(s) live in pond water. I wrote with that situation in mind, but it is much the same for your scope and whatever you 'mount' your specimens in.

Effective Coverglass Thickness

Leitz was good enough to publish detailed information about its objectives at one point in its history. The most important information for this article being found on pages 22 and 42 of [Image-forming and Illuminating Systems of the Microscope]().

Above: Leitz's extremely helpful catalog and guide to its lenses

Page 22 gives the technical explanation, but page 42 has the bottom line:

[1]) D: to be used with coverglass D = 0.17mm (adhere to coverglass thickness within + − 0.05mm)
O: to be used without, DO: with or without coverglass
DI: adhere to 0.17mm coverglass thickness to within ± 0.01mm, or, where the objective has a correction mount, set this exactly at the actual coverglass thickness.

Above: Screen-capture of the important objective effective coverglass thickness ranges for Leitz objectives.

But Leitz was not really writing about actual coverglass thickness, but rather 'effective' coverglass thickness as page 22 attests.

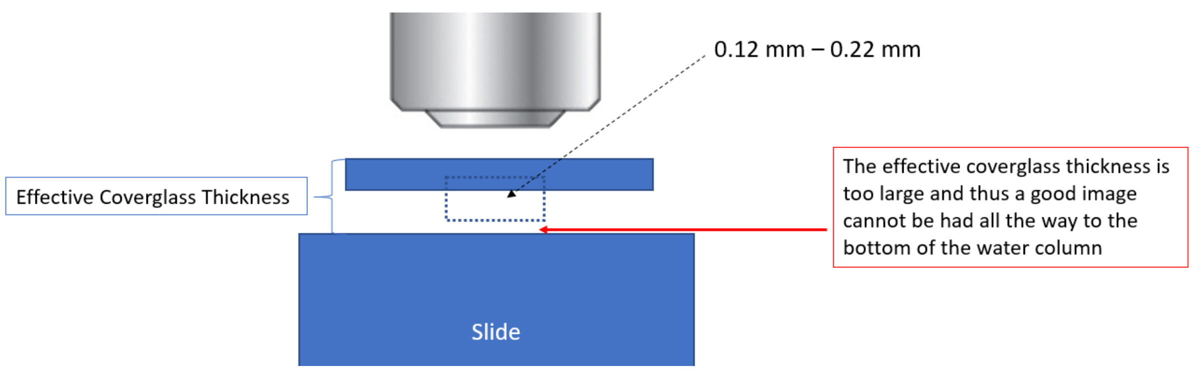
There is a concept called 'effective coverglass thickness'[2] and for my objectives to give an acceptable image (per Leitz's definition of 'acceptable') the effective coverglass thickness must be between 0.12mm and 0.22mm[3]. Period. I suspect it is the same for other brands of objectives, but here I have the actual manufacturer's advice. First a definition:

Effective Coverglass Thickness = Actual Coverglass Thickness + Water Column Depth

Wait, you say! Is that a No. 1 or No. 1-1/2 coverglass . . . or a No. 0 coverglass?

Dave Jackson explains:

"To allow for the presence of at least some mountant in the image path, the "No. 1" coverglasses were standardized at a nominal thickness of 0.13 mm, instead of the expected 0.17mm thickness specified for most objectives. However, for more precise work, where the object may be mounted directly in contact with the underside of the coverglass, a "No. 1-1/2" coverglass thickness was created, having the correct 0.17 mm nominal thickness." [4]



Above: The effective coverglass thickness is too large to allow a good image at the bottom of the water column.

2 Ibid, p. I-33

3 I do not have any 'DI' objectives. If I did, the range would be much more limited, being: 0.16mm – 0.18mm!

4 Ibid, p. I-34

So, the answer to the question is that it could be any of them, or even none of them. You use whatever thickness of coverglass. that when placed over your usual mountant. gets you within an effective coverglass thickness of 0.12mm and 0.22mm. But:

- If the objective was not designed to be used without a coverglass, you do need one.
- There are more stringent constraints for high N.A. objectives discussed later in this article.

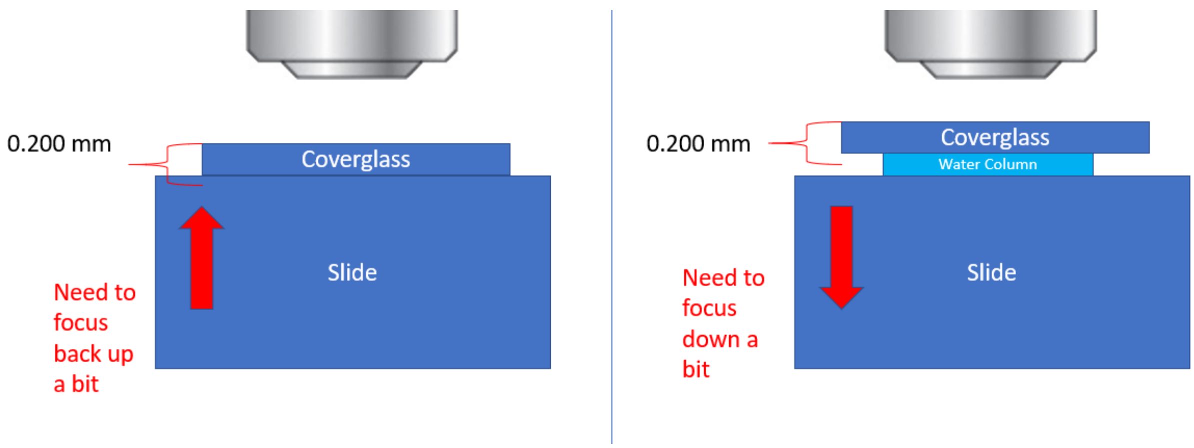
Question: Now that I know my bounds, how do I know if I am within them?

Answer: Measure using the fine focus graduation on your scope.

Measuring Effective Coverglass Thickness

The procedures below let you quickly know where you sit in (or out of) the range. That is the goal.[5]

It involves focusing on the top of the coverglass, at the coverglass edge, where you can simultaneously see good portions of both the coverglass top and the top of the slide. Next, you focus downward exactly 0.200 mm deeper using the fine focus graduations. You do not look in the eyepiece to do this – you just keep your attention on the fine focus graduations. That should put you in within 0.020 to 0.050 mm from the top of the slide in both cases – (1) the bare coverglass case, and, (2), the fully loaded sample case. Since the measurements are in air (refractive index = 1.00) there are no corrections to be made and the subtraction/addition from 0.200 mm can be done in your head[6].



Above: Focusing 0.200 mm down puts you within just a few mm of the top of the slide in both cases – (1) the bare coverglass case, and, (2), the fully loaded sample case.

First, Measure Actual Coverglass Thickness

I struggle with my coverglass supplier. They advertised No. 1 coverglasses, with a range of 0.13mm – 0.17mm but on checking . . . well, the actual variation seems more than that, and the average seems to be nearer the high end. To check a coverglass for thickness before you lay it over the top of a specimen, do this:

1. Lay the coverglass on your bare slide and position the slide on your microscope stage.

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5 *For serious vertical measuring work, using higher power objectives and tight tolerances, see [Gregor Overney's article](#) on Micscape for guidance.*

6 See Note 1 if you want to measure someplace other than an edge.

2. Observe in any of Darkfield, Phase, or Oblique. In Brightfield it is harder for me, at least, to find the tops of the coverglass and slide with any confidence.
3. Using your 10x lens[7], find an edge of the coverglass – any edge will do. With the 10x, at the edge, you will be able to see good portions of both the bare slide top and coverglass top at the same time, although not in focus at the same time.
4. Focus on the top of the coverglass at that edge. There will be dust, flaws, and 'undesirable things' that will stand out there and let you focus.
5. Record the reading on your fine focus graduations.
6. Now, without looking through the eyepiece, but staring intently at the fine focus knob graduations, focus downward two laps – two complete turns of the focus that have you sitting on your initial reading from step 5 above.
    a. This is a distance of 0.200 mm.
    b. Your focus should have gone beyond the top of the bare slide by perhaps .030mm. If not, the coverglass is probably too thick and should be discarded because it leaves -0- room for your specimen.
7. Next, looking in the eyepiece, carefully focus upward (back the way you came!) until you locate the top of the slide using the 'undesirable things' that will surely be there.
8. Finally, do the subtraction from your first reading to this final reading (You should be within 0.020 – 0.050 mm of your 0.200 mm position from step 6 above). This is your actual coverglass thickness.
9. Is the thickness acceptable to you? Does it use an acceptable amount of the 0.12mm and 0.22mm range?
    a. If not, set it aside or throw it away. Get another coverglass from the package and try again!
    b. If so, great. You have 'a keeper', and you can introduce your specimen knowing you have not compromised your image quality from the start before you even began.

Next, Measure <u>Effective</u> Coverglass Thickness

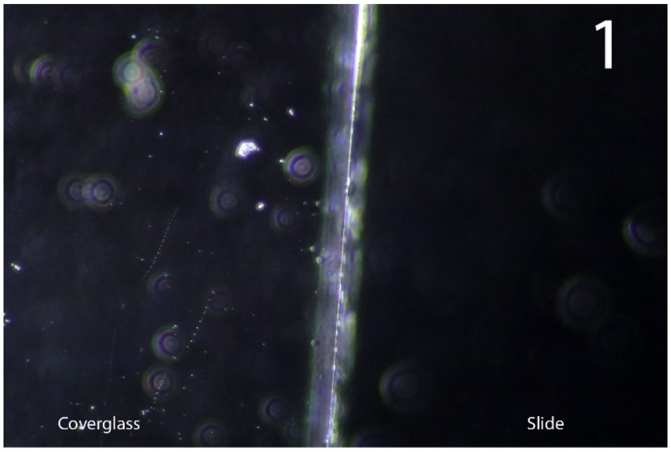
It is the much the same procedure as above, only there is a step 0, and some changes – see bold blue italics below:

1. ***Introduce your specimen (drops of pond water in my case) to the top of the slide***
2. Lay the coverglass on your ***specimen*** and position the slide on your microscope stage
3. Observe in one of Darkfield, Phase, or Oblique. In Brightfield it is harder for me, at least, to find the tops of the coverglass and slide with confidence.
4. Using your 10x lens, find an edge of the coverglass – any edge will do.
5. Focus on the top of the coverglass at that edge. There will be dust, flaws, and 'undesirable things' that will stand out there and let you focus.
6. Record the reading on your fine focus graduations.
7. Now, without looking through the eyepiece, and staring intently at the fine focus knob graduations, focus downward two laps – to complete turns of the focus until you lap once and continue until you just reach your initial reading from step 5 above.
    a. This is a distance of 0.200 mm.
    b. Your focus should ***still be above the top of the slide no more than 0.030mm***.

7 You need a lens with a good amount of working distance – maybe 0.5 mm – just to be safe. A 10x lens usually has at least 10 times that minimum working distance.

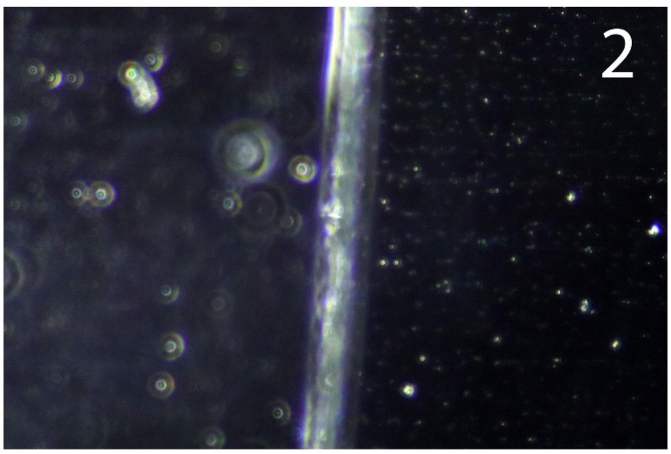
8. Next carefully focus **downward**, looking in the eyepiece, until you locate the top of the slide using the 'undesirable things' that will surely be there.
9. Finally, do the **addition** from your first reading to this final reading (You should be within 0.020 – 0.050 mm of your 0.200 mm position from step 6 above). This is your ***effective*** coverglass thickness.
10. Is the thickness acceptable to you?
    a. If not, **wait for evaporation to thin the water column, or just start over with a new, thinner specimen.**
    b. If so, great. ***Observe away.***

Measuring Example-- with Pictures

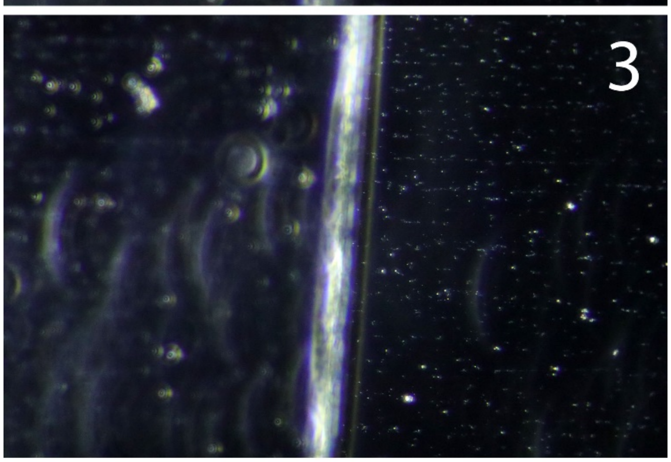


I grabbed the next coverglass on the stack and placed it on the bare slide. Then I focused on the top of the coverglass, right at the edge. Graduation reading was 46. See image 1 on the left.

Note: I was using Darkfield in this sequence.



Now, without looking in the eyepiece, but staring intently at the focus knob, I turned the fine focus knob to focus downward <u>two complete revolutions</u>, coming back to a reading of 46.   At this point, I was focused beyond the top of the slide and into the glass, and so I needed to focus back. See image 2 on the left.

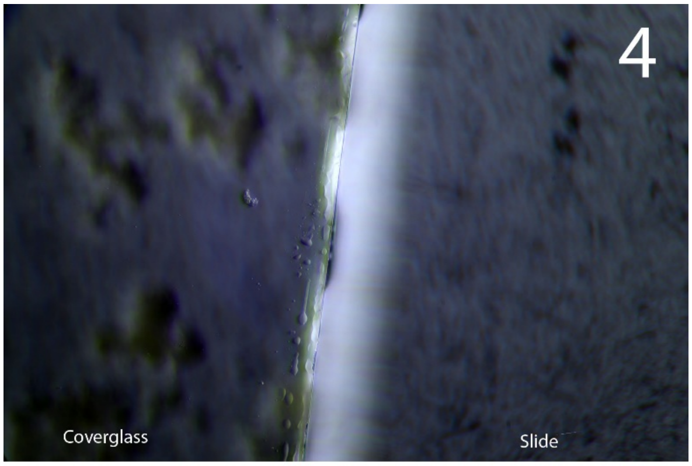


Now, I looked in the eyepiece and focused <u>upward</u> (back the way I came) to find the top of the slide.   There it was. Reading was 21. See Image 3 on the left.

46 – 21 = 25. And so, my coverglass was 200 – 25 = 175. The Ortholux I's graduations are in .001mm increments so that translated to 0.175mm in thickness.   Hmm. Thicker than I had hoped.

Notice that I did not need to memorize any equations or refractive indices. All I needed to do was to keep my initial graduation reading in mind (i.e. 46). The rest was almost mechanical.   In fact, if you don't want to do the subtraction, you can just count off the difference right there on the graduations of the fine focus knob (i.e. 10, 20, 30, 40 . . . and 6 more = 46) and make it mechanical!

Although the coverglass was not the 0.15mm or 0.16mm thick example that I wanted, I used it anyway.

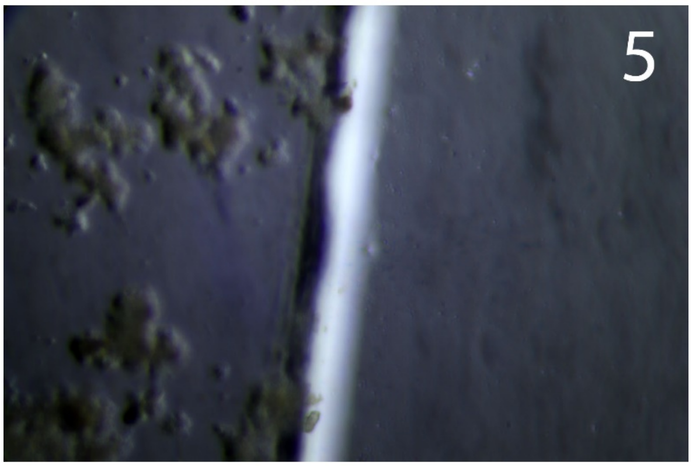


Next, I introduced my sample of pond water onto the slide and placed my coverglass over my sample. I tried NOT to add too much sample – that is often a failure of mine.

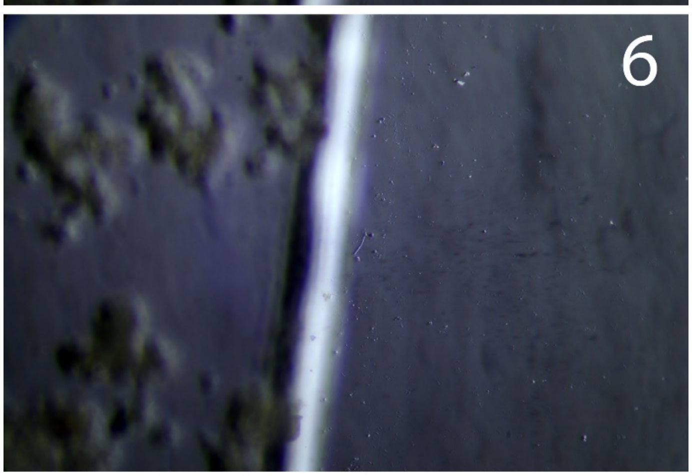
Then I repeated the procedure by again focusing (at an edge) on the top of the coverglass.

Graduation reading was 83. See Image 4 on the left.

Note: I was using Oblique in this sequence just for variety. Phase works very well too but not everyone will have access to Phase. But Darkfield and Oblique[8] *are available to everyone.*



Now, without looking in the eyepiece, but instead looking at the focus knob and its graduations, I turned the knob two complete revolutions and thus focused downward by 0.200 mm. Ok, I was back at 83. See Image 5 on the left.



Now, I looked in the eyepiece and focused <u>downward</u> to find the top of the slide (outside the coverglass and any excess from the sample – I wanted to see the top of the bare slide in the air). See Image 6 on the left.

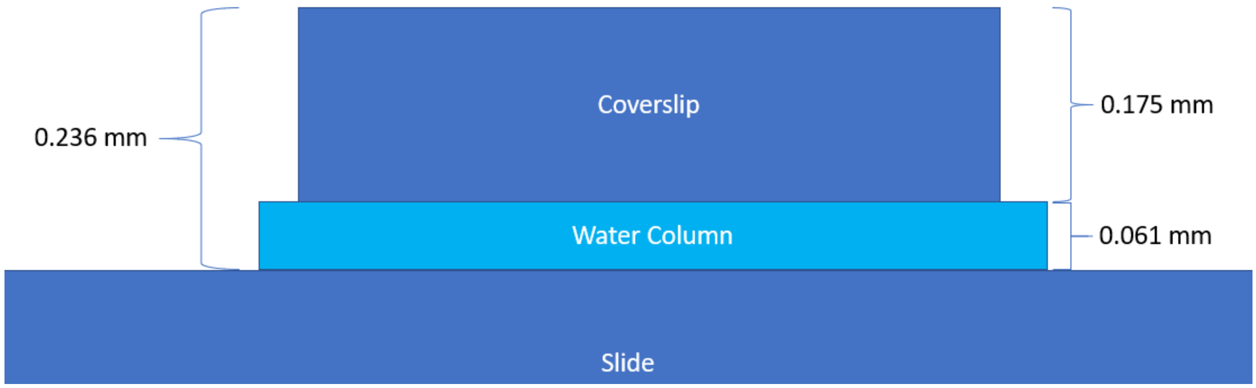
There it was – reading was 19. Thus my 83 reading went to 100 (i.e. 0), so that is 17, and then on to 19. That was a total of 17 + 19 = 36. And so, my effective coverglass thickness is 200 + 36 = 236, which translated to 0.236 mm.

Not so good. I am out of the range!

And finally, I calculated the water column depth (you do not have to do this, I just wanted to do so for the diagram below). It is the (effective coverglass thickness – actual coverglass thickness), or 0.236 mm - 0.175mm = .061 mm.

8 Micscape has many, many good articles on both techniques. Do a search. You will not be disappointed.

Here was the situation in my example displayed in a diagram:



Above: My example with its thicknesses illustrated.

It is amazing how thin the water column is compared to the coverglass. But another way to look at it is to notice how much better it would have been had I had a 0.15 mm coverglass to start with. That would have given an effective coverglass thickness of 0.211 – clearly inside the range.

Since my coverglass supplier claims a range of 0.13 -0.17mm, you would think that 0.15 mm would be the average. I have not found that to be the case.

The Realities of the Water Column . . .

In the climate I live in, especially in the winter, the air is very dry, and evaporation seems fierce. As the water column thins, the coverglass squashes ostracods, copepods, and plant matter too. The thinning water column may trap and immobilize worms, rotifers, nematodes, and even the larger protozoa, under the coverglass. And at some point, compression may slow or even stop, but the drying continues. Sand grains are the worst! They are, of course, not compressible. The effective coverglass thickness may no longer be uniform. Your coverglass may tilt (high-centered on something). If you are using a very thin coverglass, you might even go under the 0.12 minimum for effective coverglass thickness (not likely, though, unless you are using No. 0 coverglasses).

You can always add water near the edge of the coverglass to support your 'micro-aquarium' as needed. And this may raise the water column and coverglass beyond the recommended effective coverglass thickness range again. And then the entire process of evaporation begins anew. S*igh*[9].

But In practice . . .

You do not have to drive yourself crazy with measuring.

Measure <u>actual</u> coverglass thickness <u>often</u> unless experience shows that your coverglass supplier is more consistent than mine. But measure <u>effective</u> coverglass thickness <u>just enough to get a feel</u> for things. After that, measure just to check occasionally, or when you know you are pushing the limits. If you want

9 Yes, there are ways around this. Using paraffin, or Vaseline, to prop-up the coverglass with 'feet', or to enclose it entirely giving some permanence. I do not have much experience here. Or a well-slide, which brings its own problems . . . and I do have experience with these and find them to be useful but not in every circumstance.

the best images from your objectives (e.g. for photography) you may need to be more attentive to the limits for effective coverglass thickness, and measure more often. Then again if the Working Distance (i.e. WD. More on this later) is an issue with a particular objective, you might have to worry ***every time*** you start a new sample, or when you add water at the coverglass edge.

Higher N.A. Lenses Demand Much Thinner Effective Coverglass Thicknesses

Starting with an effective coverglass thickness of 0.236 mm is clearly outside Leitz's design range.   But, really, how much trouble am I in?

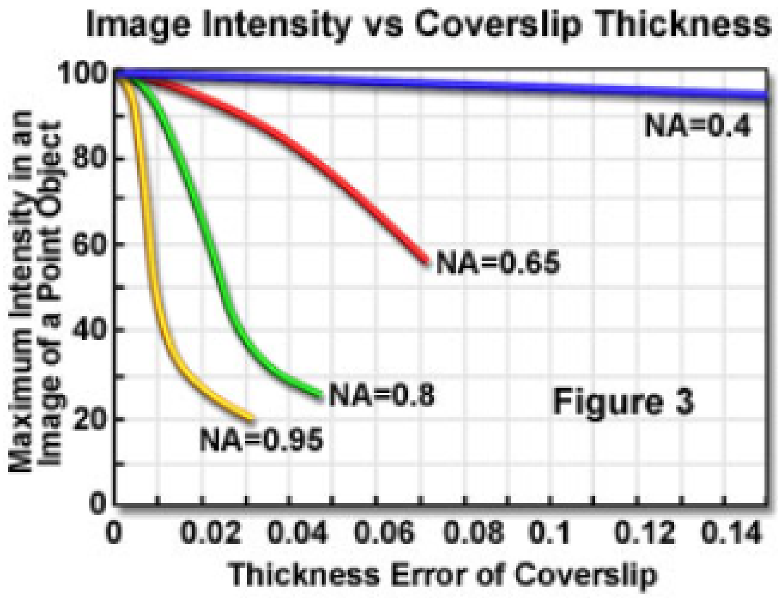
For objectives that have an N.A of < 0.40, not much.   But starting around 0.40 N.A. there is another set of boundaries on effective coverglass thickness that apply -- tighter boundaries than 0.12 mm – 0.22mm. Dave Jackson summarizes it as follows[10]:

Objective NA	Coverglass Thickness*	Range**
0.25 (or less)	0.00 to 0.50mm.	n/a
0.40	0.10 to 0.25mm.	+/- 0.07mm.
0.50	0.12 to 0.22mm.	+/- 0.05mm.
0.60	0.14 to 0.20mm	+/- 0.03mm.
0.70	0.15 to 0.19mm	+/- 0.02mm.
0.80	0.16 to 0.18mm.	+/- 0.01mm.
0.90	0.165 to 0.175mm	+/-0.005mm.
0.95	0.167 to 0.173mm.	+/-0.003mm.

Above: Dave Jackson's table of N.A. vs. allowable range in effective coverglass thickness.

Here, Dave is talking effective coverglass thickness, and as you can see, the acceptable range narrows the greater the N.A. of the objective.   A graphical way to look at it is provided by the Olympus Microscopy Resource Center and you can see that Dave has picked more forgiving values than their graph would suggest.

10 Ibid, p. I-33



Above: Graph from Olympus showing the severe effects of coverglass thickness errors on high N.A. objectives.

Do I Have a Problem?

My example effective coverglass thickness was 0.226 mm. Here are my 'go-to' objectives:

Objective	**N.A.**
10x NPL Phaco Fluotar	0.30
16x NPL Phaco	0.40
25x NPL Phaco Fluotar	0.55
40x NPL Phaco Fluotar	0.75

At 0.236 mm, I am in trouble with the 25x and in a lot of trouble with the 40x. I am not going to be able to get a good image all the way down the water column with either one. I can get a good image maybe half-way down into the water column with the 25x, but with the 40x I cannot get much past the bottom of the coverglass with a good image. This is sad. Amoebas are my favorite 'target' and you usually find them in the detritus at the bottom, and just on top of the slide. It might have been better had I stuck with a more ordinary pedigreed 40x of N.A. 0.65!

What Can I Do About It?

What can I do that does not involve getting rid of the N.A. 0.75 lens?

- Work with thinner samples – if that is possible.
    - It is a hard fight to resist the tendency to add more to the sample (i.e. pond water) than you really should – I well know it. Just a drop or two, if that, for a 22mm x 22mm coverglass. More than that, and you are over the effective coverglass thickness limit and your coverglass is 'swimming'.
    - Waiting a few minutes should bring a thinner sample thanks to evaporation. But results can be spotty, and evaporation is not always uniform.

- Use thinner coverglasses. I could try a No. 0, but all I have right now are No. 1.
    - But I *can* pick a thinner No. 1 coverglass– there is always variation – by measuring before introducing the sample. I should never have accepted a 0.175 mm thick coverglass!
        - If effective coverglass thickness is a continual problem, you may want to do this routinely as it gives you a chance to reject those over your thickness target. I should have done this in my example. I really want coverglasses <= 0.16mm if possible.
- In Brightfield, you can defeat at least some of the problem by closing your condenser diaphragm a bit. By closing the condenser diaphragm, you lower the effective N.A. of your objective and thus increase its depth-of-field[11]. In other words, you trade resolution for depth-of-field. Some consider this a sacrilege. I promise not to tell.

I found J. Delly's explanations[12] particularly insightful. He notes:

"With a layer of mounting medium between the specimen and the bottom of the cover glass, some spherical aberration is bound to be introduced. Spherical aberration in images in the 400x to 600x range is commonly seen in published literature. Photomicrography at higher and lower magnification is comparatively much easier. Photomicrography with high-dry objectives is most difficult".

He also explains that while correction collars are helpful in dry objectives of the N.A. 0.70 to 0.95 range, switching to an immersion objective of similar N.A. may be a better solution.

As an example, here is the complete page 42 of Image-forming and Illuminating Systems of the Microscope:

11 See p. 16 of 'Photography Through the Microscope', by J. Delly, for a full explanation and supporting data, but I reproduced his table in Note 2.

12 Ibid, pp. 16 – 19.

Standard objectives, achromats fluorite systems, and apochromats

170/0.17/45mm **

Type of objective	Engraved: reproduction ratio	/aperture	Free working distance mm	Focal length mm	Coverglass correction[1]	Type of eyepiece[3]	Code No.
Achromatic dry system	4	0.12	24	32	DO	P	519292
	10	0.25	6.8	17	DO	P	519293
	25	0.50	0.44	7.2	D	P	519301
	40	0.65	0.42	4.6	D	P	519419
Apo	40	0.95 korr.			D 2	P	519306
Fl	63	0.85	0.14	3.0	D !	P	519342
Oil immersion objective	Oel 100	1.30	0.10	1.9	D	P	519295

[1] D: to be used with coverglass D = 0.17mm (adhere to coverglass thickness within + − 0.05mm)
O: to be used without, DO: with or without coverglass
D!: adhere to 0.17mm coverglass thickness to within ± 0.01mm, or, where the objective has a correction mount, set this exactly at the actual coverglass thickness.
[2] These objectives have an adjustable correction mount. Image sharpness is almost completely preserved when this correction is operated. Ideal possibility for optimum setting when the coverglass thickness is not known.
[3] H = Huygens eyepieces, P = PERIPLAN or widefield eyepieces must be used.

Unless stated otherwise these figures apply to all the tables.

* Free working distance is the distance between the bottom edge of the objective mount and the top surface of the coverglass (0.17mm) or the top of an uncovered object.

** Tube length/coverglass thickness/adjustment length mm
Objectives of 45 and 37mm adjustment length can be combined on the revolving nosepiece, if the latter are screwed together with an 8mm long adapter, Code No. 519 164.

20385-519 R

42

Above: P. 42 from Image-forming and Illuminating Systems of the Microscope:

I have highlighted a couple of rows in red. Notice that the APO 40x at 0.95 has a correction collar. This means you can correct for variations, within reason, for effective coverglass thickness, but WD will be tight. But that the Fluorite 63x with an N.A. of 0.85, with no correction collar, has *very* stringent effective coverglass restrictions. Also, note how tight its WD is -- only 0.14mm! That is within the glass of a thin No. 1 coverglass. Oh My! To J. Delly's point, Leitz made 50x water immersion objectives of N.A. 1.00 with 0.68 mm WD (using a coverglass) and 0.75mm WD (dipping, without coverglass)[13]. Sadly, these are rare and expensive.

Working Distance Cuts Effective Coverglass Thickness Short

The definition you will find in the literature for WD[14] is:

13 P. 54 and P. 56 of Image-forming and Illuminating Systems of the Microscope:

14 The Leitz literature refers to WD as 'Free' WD, but it is the same thing.

The distance between the bottom edge of the objective mount and the top surface of the cover glass *with the object in focus and in contact with the bottom surface of the coverglass*, or the top of an uncovered object.

An equivalent, but more useful, definition for a 'pond water' hobbyist like me is:

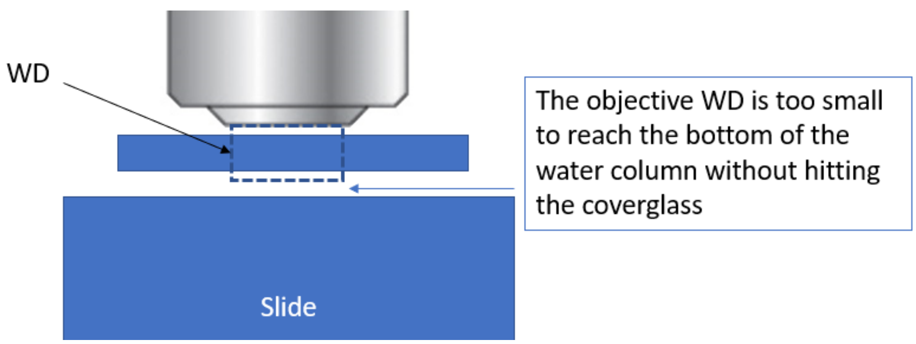
Working distance is the maximum thickness of water column PLUS coverglass that you can bring into focus with a given objective.

In other words, it is the maximum effective coverglass thickness that you can bring into focus, top to bottom. Thicker than that, and you will crash your objective onto the top of the coverglass at some point (or water column if you are going without a coverglass) while focusing deeper and deeper into the water column.

You've probably felt that sickening feeling when you realize – specimen movement is the dead give-away -- that you are, in fact, in contact with the top of the coverglass and are compressing water column and specimen with your focusing while endangering your valuable objective![15]

- If WD is greater than the effective coverglass thickness, good.

- If it is at all close or less than the effective coverglass thickness, you have a problem. This time, the issue is not that you will not get a good image near the bottom of the water column, it is that you physically cannot reach the bottom of the water column!



Above: Too small an objective WD, and you cannot physically reach the bottom of the water column.

How Do You Know Your Objective's WD?

First, do you really need to know it?

Answer: No, not really if it is always obviously greater than any effective coverglass thickness you ever meet.

For me, even though I know the exact WDs for the 10x and 16x objectives – thank you Leitz -- that knowledge is not so critical because I never experience effective coverglass thicknesses of 0.5mm or

15 Happily, many, but not all, high power/high N.A. objectives have spring-loaded barrels and slightly recessed front lenses that prevent damage in just this scenario.

greater.   But that is not always true for the 25x at a WD of 0.38mm, or the 40x at a WD of 0.42 mm. So, I am grateful I know the WDs for these lenses.

It seems that only recently, microscope makers have been including the WD on the side of objectives. This is frustrating because without that, you must rely on catalogs, often old, and sometimes unavailable. Leitz produced so many objective designs through the years that even given you have a 40x objective, say, of a given N.A., there might be more than one such objective – with differing WDs – that seem a match.

I only know of one way to measure WD directly ***and it is not a safe way***. BUT, If you have a lens where WD is unknown but critical, it probably means that you have experienced that "sickening feeling" referenced above where you know you are "in contact with the top of the coverglass"?   Well, if that happens, back away and measure the effective coverglass thickness right then (with your 10x lens!). At least this gives an upper bound – you know your WD is less than this value. Not the way you want to measure WD, but you have taken good advantage of an unpleasant situation to try to avoid it the next time?

Do I Have a WD Problem?

Here is that same table of objectives with the WD added

Objective	**N.A.**	**WD. (in mm)**
10x NPL Phaco Fluotar	0.30	0.53 (This is very tight for a 10x!)
16x NPL Phaco	0.40	0.50
25x NPL Phaco Fluotar	0.55	0.38
40x NPL Phaco Fluotar	0.75	0.42

For my current effective coverglass thickness example (i.e. 0.236 mm), No. But a particularly thick sample combined with a poor luck-of-the-draw coverglass (i.e. that is also particularly thick), and the 25x may be a problem as well as the 40x.   Measuring is my best guard.

If WD is a constant problem for you, you really need to seek other objectives with a more forgiving design. If you have access to special long working objectives – see Paul James' Micscape Article – they can be a good alternative and for more reasons than just the increased WD as Paul explains there. You do give up N.A., though, in exchange for that vastly increased WD – *sigh* – nothing is free – see Note 3.

Summary

- An overarching point in this article is that if you have a graduated fine focus, use it! Your scope is a precision measuring instrument that can measure, very accurately, ridiculously small vertical distances and very quickly too. You will become so expert at this that it will become second nature to you.
- There are limitations that hinge on those same, 'ridiculously small vertical distances', that, once known, empower you. They include:
    - Although there will always be some image degradation if there is a water column between the bottom of the coverglass and your specimen, you do best when you adhere to the manufacturer's recommended effective coverglass thickness.

- Not all objectives belong on your scope (i.e. not all ‘shiny things’ are worth pursuing). Before you jump to an upgraded objective (e.g. bid on eBay), you should consider your observing habits and whether or not that objective really ‘fits’ with the methods you use, and the specimens you observe, as far as possibly more stringent effective coverglass requirements and/or very tight WD.[16]

Notes:

1. The edge, <u>in air</u>, is by far the most convenient and fastest, place to measure. But you can do it anyplace if you consider refractive index. For glass, this is 1.52. For water, it is 1.33. So, to measure effective cover glass thickness in the ‘middle’ of the coverglass, it would be:

   1.33 * (The difference from the top of the slide to the top of the coverglass – i.e. the measured water column thickness).

   PLUS

   1.52 * (The difference from the bottom of the coverglass to the top of the coverglass- i.e. the measured actual coverglass thickness)

   For pond water, you can usually find those three planes fairly easily, i.e. the top of the slide, the bottom of the coverglass, and top of the coverglass. As an interesting experiment, measure in air, and then do the measurement in the middle of the slide using the formula above to see which you prefer. I already know which I prefer.

2. J. Delly’s table on how much you can increase depth-of-field by restricting the condenser diaphragm (and thus lowering an objective’s N.A.) is reproduced below:

Variation in Depth of Field with Change in NA (green light)

NA	0.25	0.30	0.50	0.65	0.85	0.95
Depth (in μm)	8.52	5.83	1.91	0.99	0.40	0.19

3. Here are some long-working distance (LWD) objectives that no doubt came from a Leitz Diavert inverted microscope where having such lenses are necessary. You can see that in the higher powers, N.A. is lower than expected while WD is much, much greater than for ‘normal’ lenses.

16 After drafting this article, I decided to put the N.A. 0.75 40x Fluorite away and swapped back in the prior N.A. 0.65 40x Achromat. I took my own advice! I should have known better. Oh well. The 40x Fluorite can come out of its case when I have a proper specimen to use it on. But I admit, it *<u>was</u>* shiny!

Above: Some of Leitz's long working distances objectives.

Objective	**N.A.**	**WD**	**Typical non-LWD N.A**	**Typical non-LWD WD**
10x Phaco	0.25	6.8 mm	0.25	6.8 mm
L 20x Phaco	0.32	6.7 mm	0.50	1.4 mm
L 32x Phaco	0.40	6.4 mm	0.60	0.6 mm

Of course, just because you are now relieved of WD concerns does not mean the objective's effective coverglass range restrictions are magically widened. See page 57 of Image-forming and Illuminating Systems of the Microscope where you'll see that they are not. But they do have other advantages as Paul James' Micscape article explores.

Comments to the author are welcomed, email psneeley AT xmission DOT com

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