

A Modified Cheshire's Apertometer for Measuring the N.A. of Oil Immersion Objectives

Building a home-made apertometer to measure the numerical aperture (NA) of oil immersion objectives and testing a few antique and modern high-performance objectives

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Having collected a few antique objectives without clear markings and curious about testing the actual numerical aperture of those and other modern and antique oil immersion objectives, I spent a few months looking for an apertometer to perform such testing. Seeing a few antique Abbe's Apertometers pass me by on eBay auctions, selling well above my budget, I decided to have a go at making my own apertometer. Perhaps not as desirable a collector's item as the Abbe Apertometer, but capable of measuring NA with useful accuracy. In this article, I will describe how to make such an instrument and the results testing objectives such as those shown in figure 1.

An easy-to-make apertometer was described by Dushan Grujich in the February 2013 issue of Micscape¹: A version of Frederic J. Cheshire's apertometer described in his 1904 and 1914 articles published in the Journal of the Quekett Microscopical Club^{2,3}. This instrument is a printed card with markings to show the NA of the objective being measured up to about NA = 0.9.

In practice the apertometer is placed on the microscope stage and the centre mark of the apertometer brought into focus using the microscope as in normal operation. Then, the objective is to be raised a fixed distance and the image of the apertometer observed using a phase telescope to view the upper focal plane of the objective. The NA is read off the scale as the outermost marking visible. The theory and operation is well described in the referenced articles. Dushan Grujich's article provides a printable scale for use with a distance (Δ) of 12.5mm, which is practical for use on a typical microscope stand.



Figure 1 - Two ca. 1890's Zeiss high NA objectives

As this apertometer uses air as the medium between the objective and the scale, it is limited to the theoretical limit for a dry objective of NA = 1.0, and practically to about 0.9 given the size of the scale. To measure the NA of oil immersion objectives, the same design principle will work, however the air gap needs to be replaced by a medium with a refractive index similar to or higher than the glass and immersion oil the objectives are designed to work with. Such media are of course glass slides, cover glasses, immersion oil and Canada balsam. Typically with refractive indices (RIs) around 1.52. Therefore to make a modified Cheshire's apertometer for use with these objectives, a 12.5mm thick block of glass or other medium of uniform RI with a modified scale may then be viewed using the same methodology as with the air-gap apertometer to read the higher NA's using oil immersion. Using the formulas in Cheshire's original articles, I created a scale with markings for a distance (Δ) of 12.5mm with a medium of RI = 1.52. Figure 2 shows a full scale, printable copy made to fit on a standard 1" x 3" slide. It is capable of measuring objectives' NAs up to about 1.4.

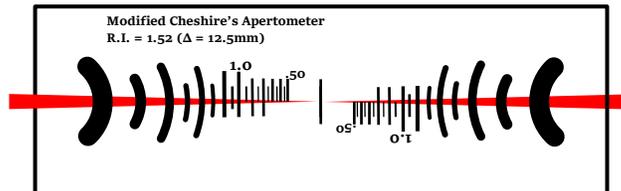


Figure 2 - A modified Cheshire's apertometer scale for oil immersion

As a suitable block of glass with known RI is not an easy or inexpensive item to source, an alternative could be to use a stack of microscope slides and Canada balsam built to the desired thickness. I decided to try this method to build an apertometer, reasoning that if the RI of the glass and balsam is reasonably close it should be good enough for my purposes – a few percent accuracy would be fine. Having access to a good Abbe refractometer, I made the effort to measure the RI of a standard 1mm thick slide and my stock of Canada

balsam diluted with a little toluene for regular slide mounting – both having RIs very close to 1.52. I followed these steps to build the instrument:

- 1) Select a stack of microscope slides from the same box that together achieve close to 12.5mm. In my case I used twelve 1mm thick slides, which stacked to about 12.3mm measured with a micrometer.
- 2) Clean the slides well, and place the first one on a few sheets of lint-free lens paper (probably a sheet or two of regular paper would do).
- 3) Place about 8 drops of balsam evenly over the slide, then place a second slide evenly on top. To avoid making a mess and getting sticky hands I wore disposable latex surgical gloves to do this!
- 4) Carefully build up the whole stack. There will be some balsam squeezing out and running down the side of the stack. The goal is to get a thin layer of balsam between the slides with as little to no air bubbles as possible. While wearing latex gloves and trying to avoid touching too much balsam it is easy to push the stack gently to get it into a nice straight block (figure 3).

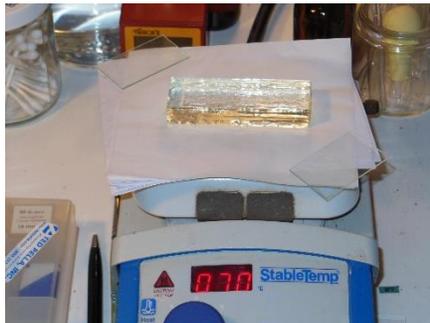


Figure 3 - Stack of slides and balsam

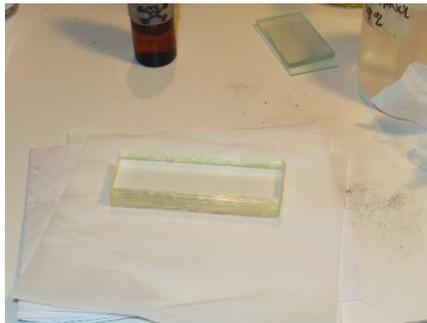


Figure 4 – Cured and cleaned stack of slides



Figure 5 – Scale cemented to block

- 5) Leave the stack on a hotplate at about 60-70°C for about 3 hours to help cure the balsam, carefully manipulating it if the stack is slanted at all to keep it straight, then let it cool.
- 6) Remove the stack from the paper and clean the bottom with a knife and solvent. Place the other side up on fresh paper and leave on hotplate for a further 3 hours or so. Let cool and clean the top and bottom surfaces (figure 4). Measure the thickness of the stack with a micrometer.
- 7) Print out the scale on good printer photo paper if possible with the best quality settings for your printer. Cut to fit a standard slide.
- 8) Cement the scale to a clean slide with balsam then cement the block of stacked slides on top of this. Cure again on a hotplate for a few hours, then let cool.
- 9) In my case the stack was 12.3mm thick after cementing and curing, so to make it up to 12.5mm I cemented a 0.17mm cover glass on top of the stack.
- 10) As a finishing touch, I also drew a scribe line across the centre of the cover glass, to have a feature to focus the objective to the top of the stack (and 12.5mm to the scale). Some marking is desirable to have an easy subject to focus the objective on and could even be a pen mark.

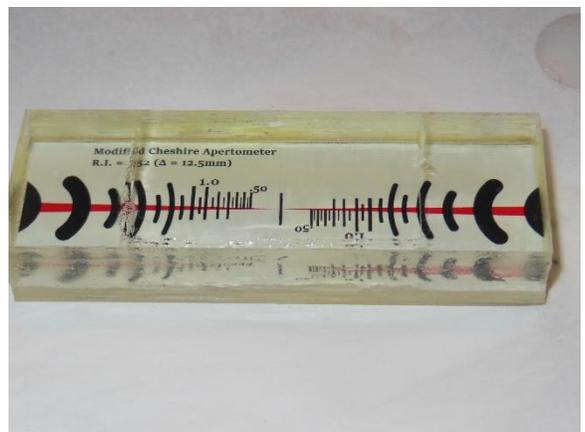


Figure 6 - Finished apertometer

Taking time to cure the balsam and let it harden over at least a day or two is worthwhile. While the balsam is a little soft, the stack can be squeezed to make the sides square if needed (latex gloves can help avoid sticking to it!).

To use the apertometer, simply place on the stage and add a drop of immersion oil, and coverslip if desired, with another drop of oil on top as if setting up with a normal slide. With the objective in position, focus on the top of the block – a marking such as a scribe line will make this easy with substage lighting. Then replace the eyepiece with a phase telescope or a built in telescope (such as the Optovar “PH”

setting on Zeiss Photomicroscopes), and adjust to focus on the scale. I then used an LED light shining on the slide at the side of the stage for top illumination of the scale. The outermost marking visible will provide the measured NA. The apertometer is shown in use on a Zeiss Photomicroscope II below (figure 7).

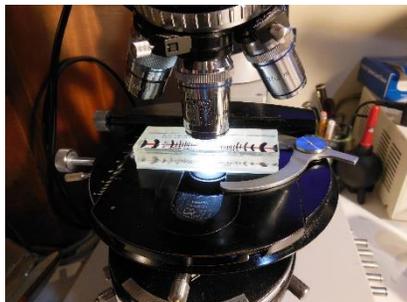


Figure 7 - Apertometer in use

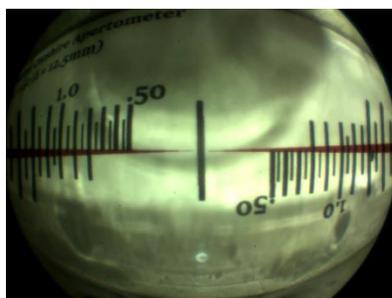


Figure 8 – Zeiss Planapo 1.4NA

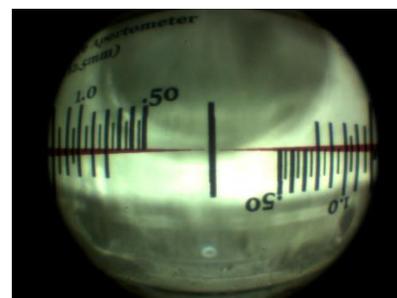


Figure 9 – Zeiss Plan-Neofluar 1.25NA

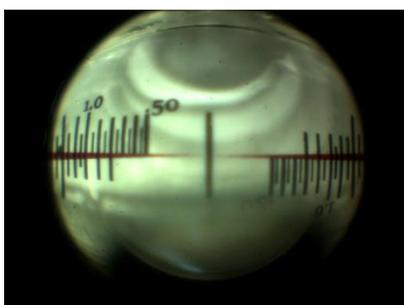


Figure 10 – Leitz Fluorite 80x/1.3NA

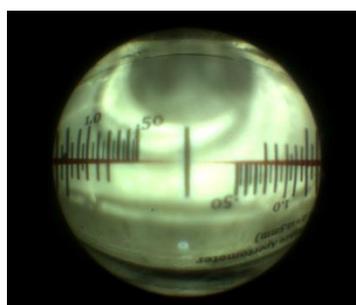


Figure 11 – Antique Zeiss 2mm 1.3NA

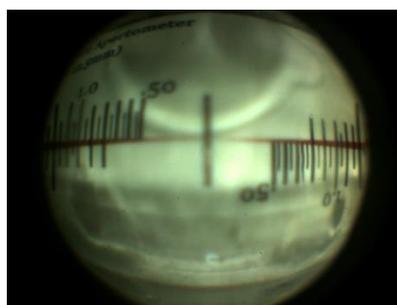


Figure 12 – Antique Zeiss 3mm 1.4NA

To test out the apertometer, I tried a 160mm tube-length Zeiss 63x/1.4NA Planapo, a 63x/1.25NA Plan-Neofluar and a 1950's vintage Leitz 215mm tube-length 80x/1.3NA metallurgical objective. Trusting that the manufacturers' specifications would be accurate enough as a calibration test, they did show the expected results within the legibility of the scale markings (figures 8 to 10).

On testing out the antique Zeiss objectives shown at the beginning of the article, the results were interesting: The 2mm 1.3NA objective showed a measured NA of about 1.35 and the 3mm 1.4NA objective a measured NA of about 1.3. So the lower NA objective read higher than specification and the 1.4NA measured less than specification. I wondered if perhaps the front lenses had been switched on these objectives, but on further inspection that was clearly not the case. Both objectives perform well at tasks such as resolving *Amphipleura pellucida*, although with age and some very minor signs of decentering, this may have affected the resolution and NA as measured. I would have expected a 1890's era Zeiss 1.4NA objective, when new, to have a NA as claimed.

So after a bit of effort cementing glass with balsam a useful and easy to use apertometer can be made by the hobbyist for testing out oil immersion objectives. I keep mine alongside the original type as described by Dushan Grujich to cover the range of objective NAs from low resolution, dry objectives up to high-performance oil immersion.

Comments to the author are welcomed, email: dowcam AT rogers DOT com

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www.micscape.org

References

1. [Measuring NA of the microscope objective - Dushan Grujich, Micscape February 2nd. 2013](#)
2. [Journal of the Quekett Microscopical Club, ix. \(1904\) p. 1 - 8](#)
3. [Journal of the Quekett Microscopical Club, xii. \(1914\) p. 283-6 \(2 figs.\).](#)