## Letters to the Editor

### Comments on Which to Use: No.1 or No.1 1/2 Cover Glasses?

Dear Mr. Editor,

In the May 1999 *Microscopy Today* (No. 99-4 pp 12-13), Gary W. Gill has a short article entitled "Cover Glass Perspectives." Although the article has a lot of interesting information, at one point the author states that one should not use No. 1 1/2 cover glasses even though they have a nominal thickness of 0.16 to 0.19 mm. He says to use No. 1 cover glass to make up for the "substantial and variable thickness of the mounting medium." This is counter to everything I was ever taught. I always use the minimum mounting media possible and press the slide down firmly on to the coverslip to ensure this is kept as thin as possible.

Is it not standard to use No. 1 1/2 coverslips? Is there really a school of thought that No. 1's should be used?

This is a response to the reply Gary Gill posted after I posted the above question to the microscopy listserver. My response, as I interpreted Gary's, should be viewed as a contribution to friendly scientific disagreement and not as a personal criticism of Gary. Furthermore, let me point out that Gary and I may disagree about which size cover glass to use because we have very different preparations. He points out in his most recent posting that he is primarily looking at whole mounts of pap smears which vary in thickness. My laboratory uses mostly 0.5 µm thick semi-thin plastic resin sections and some 8 µm thick paraffin sections.

I pulled out a micrometer and did some measurements on some batches of cover glasses sitting around:

Fisher No. 1 1/2 (22 x 22) cover glasses (No. 12-541B) came in at 180 µm (no variation in the 5 tested).

Fisher No. 1 1/2 (22 x 50) cover glasses (No. 12-544B) ranged from 175 to 180 µm.

Corning No. 1 1/2 (22 x 22) cover glasses came in at 180 µm (no variation in the 5 tested).

Corning No.1 (22 x 22) measured 150 µm (no variation in the 5 tested).

Corning No. 1 (22 x 60) ranged from 140 to 150 µm (average 145 µm).

I should point out that 2 years ago I measured the thickness of the Fisher brand coverslips and they were all about 170 µm and that is why I chose that brand. Obviously there is some inter-batch variation as well as intra-batch variation.

As an aside, I will point out the microscope slides sitting around my lab showed much more variation:

Fisher Superfrost 3" x 1" x 1 mm (12-550-12): 0.95, 1.0, 1.01, 0.98, 0.975.

Fisher Frosted 25 x 75 x 1 mm (12-552): 0.99, 1.00, 0.99, 1.0, 1.0

Clay Adams Gold Seal Rite-on Micro Slides 25 x 75 mm – 0.97 to 1.07 mm thickness (No. 3050): 0.95, 0.96, 0.995, 1.04, 0.95.

More disturbingly, the flatness of the microscope slides also varied along their length.

Back to the question of the cover glass thickness: I then took 5 Fisher Frosted slides that all measured 1.00 mm thick in the center of the slide (exact position marked with a diamond pen). These slides all had 0.5 µm semi-thin plastic resin sections. They were all coverslipped with a set of cover glasses that all measured

180 µm by placing a small drop of Permount (fresh - not overly viscous from sitting around for ages) and pressing the slide down on top of the cover glass with hand pressure for a few seconds and then heating on a slide warmer for a couple of hours. When I remeasured them at the center point, I got something very close to 1180 µm. My micrometer is marked at 10 µm intervals so greater precision than 5 µm is somewhat dicey.

If I used glass slides that had 8 µm paraffin sections (this was the thickness set on the microtome, which I realize isn't precise) and coverslipped them using the same method, I got a number equal to the thickness of the glass + cover glass + 10 µm (presumably the section thickness + mounting medium). Assuming the paraffin section was close to 8 µm, it would imply the mounting medium added about 2 µm. Although it would have been better if the No. 1 1/2 cover glasses had been closer to 170 µm thick, the percent error in using ones that were 180 µm would be less than starting with No. 1 cover glasses that were 150 µm thick and hoping to get an even 20 µm thick layer of mounting medium.

Finally, let me end with some published comments on cover glass thickness by notable authorities:

"It is therefore best to prepare a microslide with the No. 1 1/2 cover glass." John Gustav Daly in *Photography Through The Microscope* (1988) 9th edition, p. 20; Eastman Kodak Co.

"Standard coverslip are assumed to be 0.17 ± 0.01 mm thick (with a refractive index of 1.515). Number 1 1/2 coverslips are nominally selected for this standard thickness." The author goes on to state that coverslips should be measured for the most critical work. Shinya Inoue 1986) *Video Microscopy* 1st edition. p. 134; Plenum Press, NY.

"No. 1 1/2 generally gives the greatest yield of usable cover glasses." G.P. Berlyn, J. Miksche (1976) *Botanical Microtechnique and Cytochemistry".* pg. 8. Iowa State Univ. Press, Ames.

Regards,
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University of Missouri
Columbia, MO

Dear Editor,

I include in my response to his initial query, with an added example of when No. 1 1/2 cover glasses are suitable (last paragraph) and a new last paragraph that addresses his follow-up post:

Correction: No. 1 cover glasses range 0.13 – 0.16 mm thickness; No. 1 1/2, 0.17-0.19 mm (American Society for Testing Materials. Standard Specification for Cover Glasses and Glass Slides for Use In Microscopy. ASTM Designation (E211-70, Effective 12-24-70).

From a different standard: No. 1 = 0.13 – 0.17 mm; No. 1 1/2 = 0.16 – 0.19 mm (Interim Federal Specification Cover Glass, Microscope. NNN-C-001434A, 01/08/71). No significant difference.

Thickness of mounting medium for tissue sections, 3 sets of 4 slides broken across the section, the broken edges trued up and polished and measured with a micrometer microscope (Aumonier FJ, Setteringron R. Some notes on the mounting of histological sections. Proc Roy Micr. Soc. 1967;2:428-9):

Cover glass applied routinely (no pressure) = 10, 51, 63, and 76 µm

Cover glass weighted with 30 g for 2 days = 18,18, 20, and 30 µm

Cover glass with spring loaded clothespin for 72 hours = 5,10, 10, and 20 µm

Therefore, the thickness of mounting medium is substantial relative to the difference between the range of thickness for No. 1 cover glass and the tolerance of high dry achromat objectives to deviations from optimal thickness of 0.17 mm (± 15 µm and more). Ergo, my recommendation to use No. 1 thickness cover glasses. A modest bonus is getting more No. 1 cover glasses per ounce for the same price as for No. 1 1/2.

Fluorite and apochromat objectives have higher NAs power for power than do achromats and so are even more sensitive to cover glass (and mounting medium) thickness. Objectives start to show intolerance to cover glass deviations at approximately 0.6 NA (40X achromat).

Cytologic preparations (*e.g.*, conventional Pap smears, my field) are more problematic than histologic sections. Pap smears can sometimes require up to 12 or more drops of mounting medium to fill in all the valleys of thick preparations.

No. 1 1/2 cover glasses are suitable when there is little or no mounting medium between the specimen and cover glass, such as cells grown in culture on cover glasses, blood films spread on cover glass, cells on Nuclepore filters dissolved on a cover glass, and covered slides that have been "cooked" a la Dr. Ruth Graham in the 1950s (*i.e.*, heated briefly on a hot place to drive off the solvent, which results in a hardened mount that can be handled immediately, beautiful imaging under 40X objectives, and long term stain preservation).

Even if one works with histologic preparations, I still recommend the No. 1 thickness cover glass. As the unique measurements above show, mounting medium thickness is sufficiently great when cover glasses are simply laid on the specimen to bring the combined thickness up to, and into, the 0.17 mm thickness neighborhood. Starting off with a No. 1 1/2 thickness cover glass is likely to result, more often than not, in total thicknesses that exceed the tolerance range of the high numerical aperture objective in use.

And a final repetitive note for emphasis: microscopes that have dirty lenses and are not adjusted to produce Koehler illumination will not reveal the beneficial contribution of correct cover glass/mounting medium. It will be lost in the noise.

Yours truly,
Gary W. Gill,
Diagnostic Cylology
Laboratories, Inc.

## Protecting Inverted Lenses

Steve Sands, Pfizer Central Research

Inverted microscope objectives are expensive, so it pays to protect them. Unfortunately, being located below little chambers filled with buffers means that these objectives are subject to spills. A trick to help protect your objectives is to obtain small, powder free (latex or otherwise) gloves. Cut off one of the fingers (or thumb, depending on the obj.), cut a small hole in the tip, and then carefully full the glove over the objective such that the objective lens is exposed. It's not perfect, but if fitted properly, most liquid will run over the glove, and not into the objective. A small piece of lint free absorbent material, placed on the nosepiece can then absorb small amounts of runoff, and helps prevent liquid from entering the scope body. ■