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TRICKS OF THE TRADE

Make Your Own Central-Stop Dispersion Staining Objective

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Many microscopists have learned the dispersion staining methods in the classroom (or have at least heard of them) only to quickly forget them because they don't have access to a dispersion staining (DS) objective of their own. When applied correctly, it is, however, very useful for the detection and identification of small particles.

Central-stop dispersion staining, where a small opaque disk is placed in the objective back focal plane, is the most common DS technique used in industrial hygiene laboratories throughout the world, mostly for asbestos identification. But it is also very well suited for identifying mixtures or distinguishing between particles with similar refractive indices. Some might say that it is the type of technique that detects the proverbial "needle in the haystack." So, if this is a technique you'd like to try, here's an easy way to get started and save a bundle of money!

Dispersion staining is the type of technique that detects the proverbial "needle in the haystack."

Although the concept for making a DS objective has been mentioned in microscopy classrooms and in the literature of the past, I thought it might be useful to describe a more detailed procedure for making your own central-stop DS objective. I've also included a few tricks that I've learned and a review of the correct way for setting up your microscope for central-stop DS.

An extra 10X objective, or one that is not being used, is the perfect candidate to convert into a DS objective. It need not be of high quality, as DS is not a high-resolution technique; it is more often used to enhance and produce color. Although it may seem reasonable to use a higher magnification objective for DS, it does not necessarily yield better dispersion staining results. In fact, higher magnifications produce DS colors that appear less vibrant and more difficult to interpret. If more magnification is needed, consider a higher magnification eyepiece instead.

There are two simple and cost-effective ways to make your own central-stop DS objective.

1. Place an opaque spot, 4-5 mm in diameter, on the upper lens inside your objective.

2. Place an opaque spot, 4-5 mm in diameter, on a circular (round) coverslip that fits just inside the barrel at the back of your objective.

Of the two methods, the second is the simplest, least invasive and reversible; therefore it is recommended. The method, described below, uses an ordinary 18 mm round glass coverslip and works well for most microscope makes and models. I found that this size coverslip fits safely in infinity-corrected models of Leica, Olympus and Zeiss objectives and microscopes, as well as older, finite-tube length Nikon and Olympus microscopes, and can be made to stay in position after the objective is screwed into the nosepiece. Care must be taken to center and secure the coverslip without breaking it before screwing it into the nosepiece.

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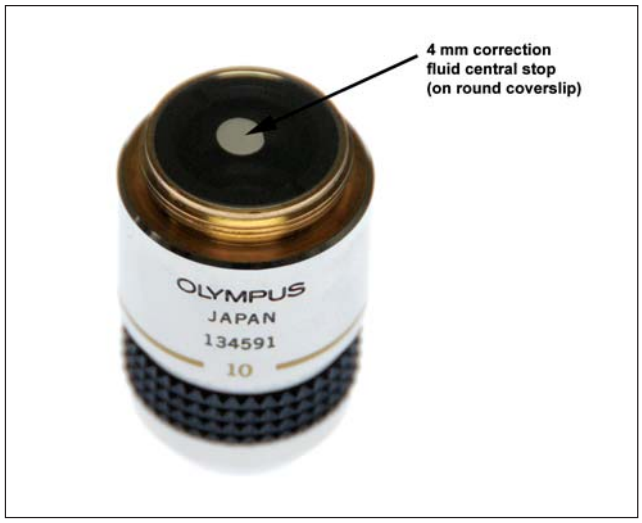

Figure 1. Central-stop dispersion staining (DS) objective. A drop of correction fluid was used to make an opaque spot on a round coverslip and placed on the rim of the objective.

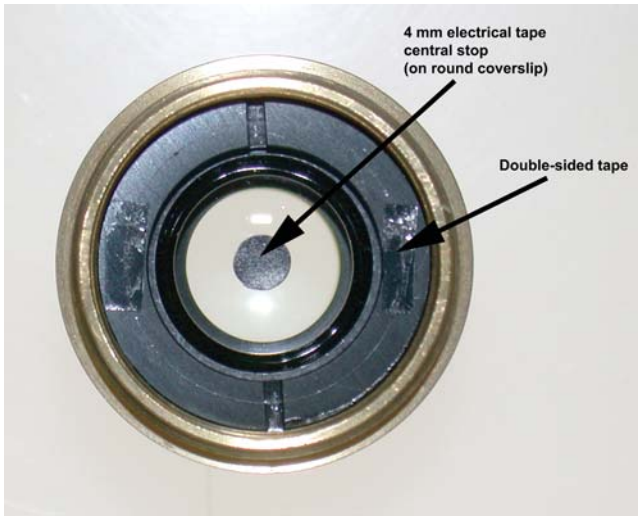

Figure 2. Central-stop DS objective. A 4 mm diameter piece of electrical tape was cut out using a cork borer and placed on a round coverslip. The coverslip is centered then secured with double-sided sticky tape on a flat area inside the objective.

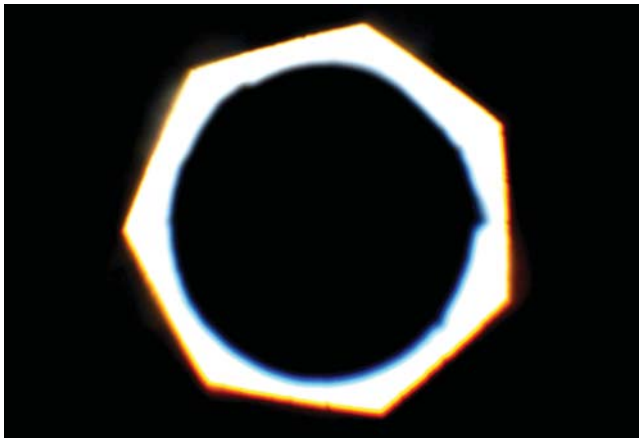

Figure 3. Back focal plane of DS objective. The central stop is centered with respect to the condenser aperture.

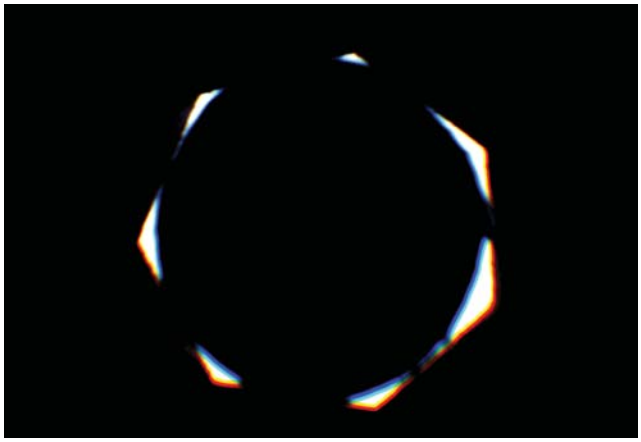

Figure 4. Back focal plane of DS objective. Condenser aperture is closed to an "eclipse" position.

The general idea is to place an opaque central spot inside the objective. This can be achieved in a number of ways. Correction fluid, India ink, enamel paint and electrical tape will all work. In these trials, I found that electrical tape works wonderfully. It's not messy, it's easy to get a round spot and it can be easily repositioned, if necessary.

Here is the procedure for making a DS objective:

1. Construction of the central spot: Place a small piece of electrical tape onto a cutting surface, such as a plastic cutting board or smooth piece of wood. Use a small diameter cork borer to cut out a small circular spot (a 4-5 mm diameter is ideal for most microscopes). An alternative is to apply a small, single drop of correction fluid, India ink or enamel paint directly to the center of the coverslip. Prepare a number of coverslips with spots of varying size, as some trial and error is necessary.

2. Placement of the coverslip into the objective: When the coverslip is the same or has a slightly smaller diameter than the barrel opening on your objective, carefully place the coverslip on the inner rim of the objective (Figure 1). If you have an objective with a wide barrel opening, apply two small pieces of double-

sided sticky tape to the barrel surface then position a round coverslip on top (Figure 2). Once it is secure, use forceps to centrally position the stop.

3. Carefully screw your new DS objective into place.

4. Ensure the microscope is in Köhler illumination. Focus on a sample that has a refractive index matching, or nearly matching, the mounting medium.

5. Use the Bertrand lens (or remove an eyepiece) to observe the back focal plane of the objective and check how well-centered your stop is with respect to the condenser aperture (Figure 3). Close down the condenser aperture to an "eclipse" position (Figure 4). If it is too far off center, you will need to reposition the spot. If the spot is too small, you will not be able to reach the eclipse position.

6. Remove the Bertrand lens and, if possible, flip out the top lens of the condenser. The light intensity will also likely need to be increased.

7. If everything is correct, you should be able to observe colored edges on your particles, against a uniform dark field. (Figures 5 and 6).

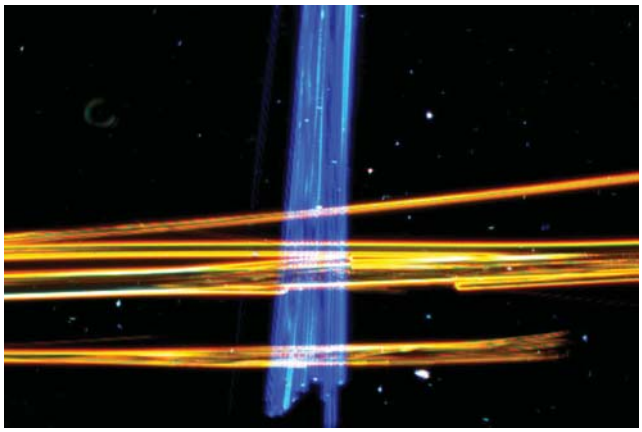

Figure 5. Central-stop DS image of amosite asbestos mounted in n=1.680 high-dispersion liquid.

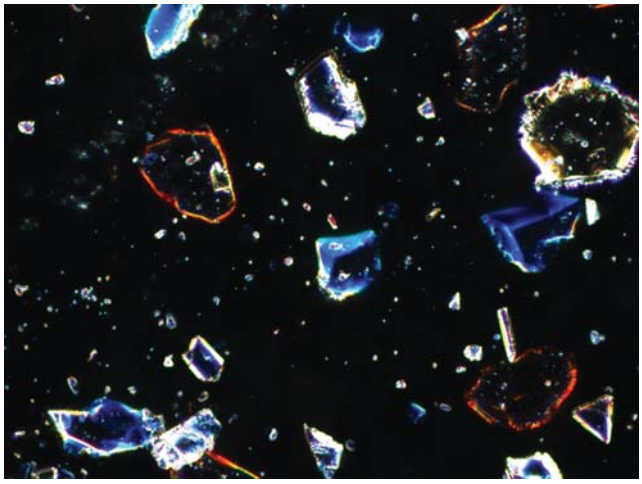

Figure 6. Central-stop DS image of a mixture of glass particles mounted in n=1.520 high-dispersion liquid.

NOTES

• You must be able to close down the condenser aperture to an "eclipse" just behind the central stop. If it won't close sufficiently, make a larger spot.

• Make sure the stop is centered within the condenser aperture. Usually this means centering it within the objective. Occasionally, if the condenser is slightly misaligned or tilted, it causes the condenser aperture to also be misaligned. If this occurs, you will need to reposition your stop to align with the condenser aperture, otherwise you will see uneven bright areas in your field of view.

• Coverslips and slides must be kept very clean when using DS; dust and oil can impede the image.

• Coverslips used on the sample preparation need to be flat and level. A tilted coverslip can offset the image of the aperture diaphragm making centration impossible and colors uneven. Make sure that the *entire* area underneath the coverslip is filled with mounting medium.

• Partially closing down the field diaphragm can sometimes help with small particles by eliminating glare from the surroundings.

• The success of DS depends on the sample and mounting medium having dissimilar dispersion and at least one matching refractive index in the visible spectrum. If the sample is not mounted in a medium with a matching, or close-to-matching refractive index, you will not observe DS colors but only white edges against a dark field.

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