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Dispersion Staining Using a 1.2-1.3 NA Cardioid Darkfield Condenser[1]

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KEYWORDS

Dispersion staining, cardioid darkfield condenser, critical darkfield illumination, central stop darkfield illumination, brightfield illumination, numerical aperture (NA), asbestos, chrysotile, Calidria, amosite, Monolux microscope, LOMO Biolam microscope, microscope objectives

ABSTRACT

A 2007 Inter/Micro presentation (1) and subsequent article in *The Microscope* (2) demonstrated that the LOMO darkfield cardioid condenser provides good darkfield when used with the 60X 1.00 NA LOMO apochromatic objective, with the punctae of diatom Frustulia *rhomboides* resolved. A presentation at Inter/Micro 2009 (3) demonstrated dispersion staining with the same combination of condenser and objective, which is the subject of this article. Comparable imaging was obtained with the 90X 1.25 NA LOMO achromatic objective when the funnel insert (NA-reducing cone) for this objective, included with the condenser, had the end bore diameter increased by 20%. Uniform darkfield required more precise centering of the reduced diameter at the end of the funnel insert. This precise centering was obtained with a homemade insert that has a closer fit to the guiding surfaces in the bore of the 90X objective.

BACKGROUND

My first experience with transmitted light microscopy occurred while I was building a universal student microscope with both transmitted and reflected light capability for hobby use. This included examination of lake-water organisms with transmitted light in both brightfield and darkfield modes. It was during the construction and testing of this microscope that darkfield stops for the transmitted illumination system were made with the minimum NA of the illuminating annulus just high enough to give a dark background, which I call critical darkfield. Transmitted polarized light capability with compensating wave retarders was a key requirement. While testing this polarized light capability, asbestos and olivine slides in matching high index mountant gave dispersion staining colors that matched those obtained with central stop darkfield.

The resulting article in *The Microscope* (4) notes that this critical darkfield dispersion staining worked for all of the objectives, including the 40X 0.65 NA achromat. I subsequently equipped a LOMO Biolam with a similar fiber-optic source external illumination system and polarized light capability used in a study of a red film organism on the lake surface, a study presented at Inter/Micro 2001 (5). At about that time, Andrew A. "Tony" Havics, an environmental microscopist, first evaluated the critical darkfield dispersion capability with a modified Biolam and the 40X objective. He recorded photomicrographs of chrysotile in wall board powder samples containing 0.5% and 0.1% additions by weight. Havics subsequently did a literature search in preparation for his Inter/Micro 2002 presentation on dispersion staining (7), including results using the modified Biolam. His 2002 presenta-

[1]Presented at Inter/Micro 2009, Chicago.

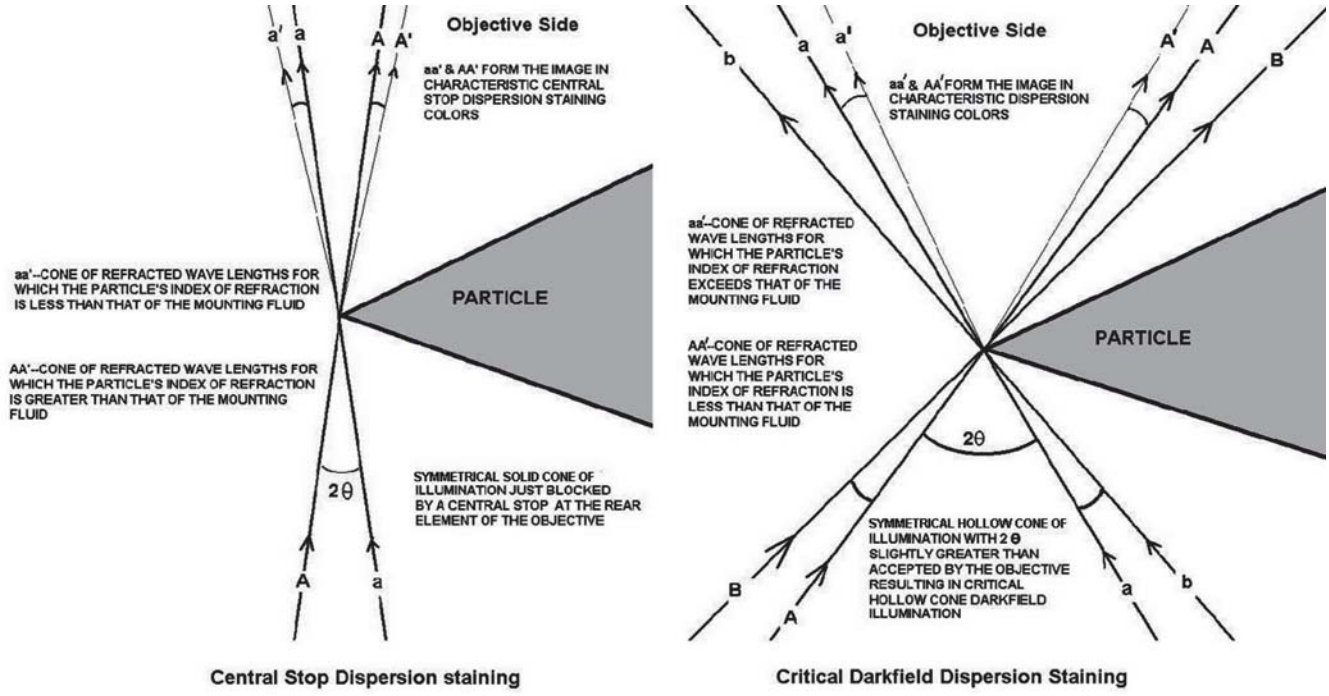

Figure 1. Schematic ray diagrams for both central-stop and critical-darkfield dispersion staining.

tion revisted a 1948 article by Nelson B. Dodge demonstrating critical darkfield dispersion staining with a 40X objective. (See related article on page 155).

A 2003 article in *Microscopy Today* (7) on rediscovery of darkfield dispersion staining references the Nelson Dodge article published in *The American Mineralogist* (8) and Havics's results using my modified Biolam. I subsequently obtained a LOMO cardioid darkfield condenser and 90X 1.30 NA apochromat to pursue my interest in high NA circular oblique illumination. This new capability was used to evaluate the Olympus E-330 DSLR camera for photomicrography with the results presented at Inter/Micro 2007 (1) followed by publication of a paper in *The Microscope* (2). That study also included evaluation of the cardioid condenser with a 60X 0.70-1.00 NA apochromat and found that a good dark ground was obtained with the objective's iris fully open at 1.00 NA. That finding led to this evaluation of darkfield dispersion staining using the cardioid condenser.

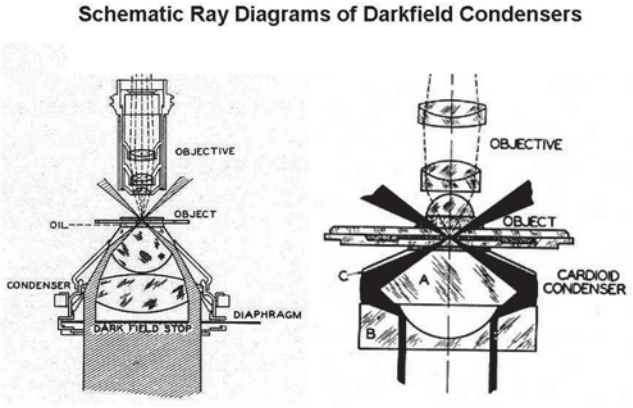

Figure 2. Schematic ray diagrams for darkfield illumination with a refractive condenser (left) and a reflective cardioid condenser (right). Diagrams originally published in Photomicrography: An Introduction to Photography with the Microscope *(Eastman Kodak Company).*

CRITICAL DARKFIELD AND DISPERSION STAINING

My curiosity about how critical darkfield could result in the same dispersion colors as the conventional central stop method led me to construct the schematic ray diagrams shown in Figure 1. These diagrams were used in Havics's Inter/Micro 2002 presentation (6). Figure 2 (9) shows ray diagrams for a refracting condenser with a darkfield stop inserted, as well as

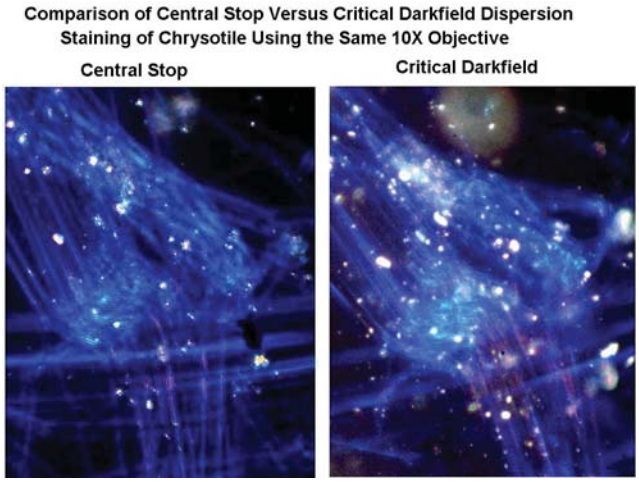

Figure 3. The same field of chrysotile asbestos recorded with plane polarized light and a 10X central-stop objective (left) and critical darkfield (right).

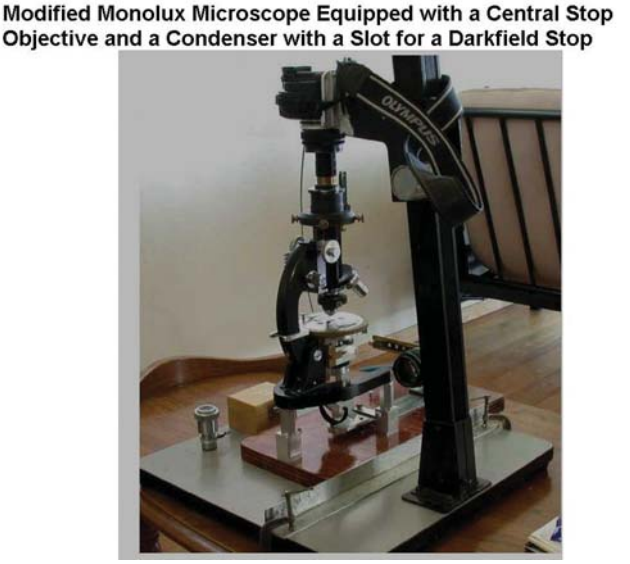

Figure 4. View of the modified Monolux system equipped with a 10X central-stop objective.

the reflective cardioid condenser. The cardioid design is inherently both aplanatic as well as achromatic, a key feature leading me to purchase a cardioid darkfield condenser for my modified Biolam. An article on building a universal student microscope (7) contains dispersion staining images of chrysotile demonstrating high contrast diffraction fringes in the field recorded with a central stop 10X objective. These dark fringes were not present in the same field recorded with critical darkfield (Figure 3).

The resolution of the two darkfield methods with the diatom Surirella *gemma* in a Klaus Kemp 8 Form test slide were compared. The first objective used was the Monolux 10X objective fitted with a 2 mm central stop. It was surprising to see how much more complex the diatom structure was in the image obtained with central stop darkfield than what was evident in the complementary brightfield and critical darkfield images. This discrepancy may have resulted from some deficiency in the inexpensive Monolux objective.

I then used an Olympus 10X dedicated central-stop dispersion staining objective for these experiments with the Monolux system (Figure 4). The resulting images, shown in Figure 5, were the same as with the Monolux objective. The central stop was not removed for the images recorded with brightfield and critical darkfield. A 90X 1.30 NA LOMO apochromat was used with circular oblique illumination from the cardioid condenser to check against the apparently false diatom structure produced with central stop darkfield. The resulting image in Figure 6 agrees with the brightfield and critical darkfield images, except the rows of pores are now resolved in the in-focus portions of this image of a diatom known to have a highly curved, rather than flat, valve face. Therefore, it is not surprising that Havics found blocks 1 and 2 of the HSE/NPL test slide were resolved with critical darkfield using the 10X objective, and no blocks were resolved with central stop darkfield.

SETTING UP THE CARDIOID CONDENSER

The modified Biolam with the cardioid condenser installed is shown in Figure 7. The 1.40 NA condenser was first used to align the illumination system for Köhler illumination before switching condensers. The Biolam has a fully alignable, external fiber-optic source illumination system like the one I built for the Monolux microscope.

The cardioid condenser has centering screws. It was easy to center and use the cardioid condenser for the first time by following the instructions. The ray schematic for a cardioid condenser in the right view of Figure 2 is helpful for understanding the alignment procedure. If the specimen plane is somewhat above or below the condenser focus, the specimen plane will exhibit a dark disk near the center of the field surrounded by an annular, illuminated zone. When the condenser is properly focused, the center of the field will exhibit a bright, uniformly illuminated disk.

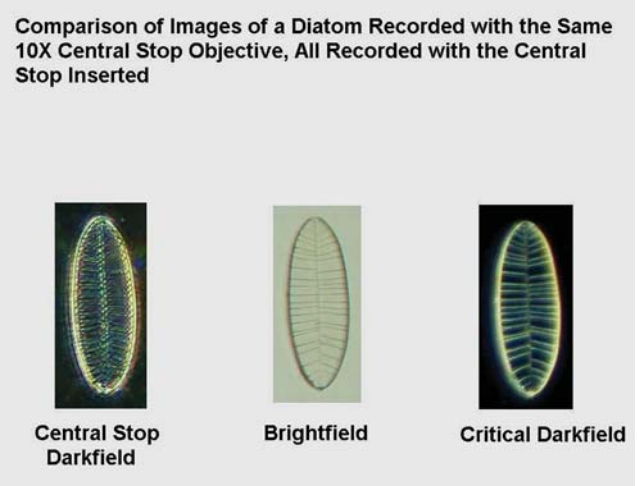

Figure 5. Comparison of photomicrographs of the same diatom Surirella gemma *recorded with the Monolux system shown in Figure 4.*

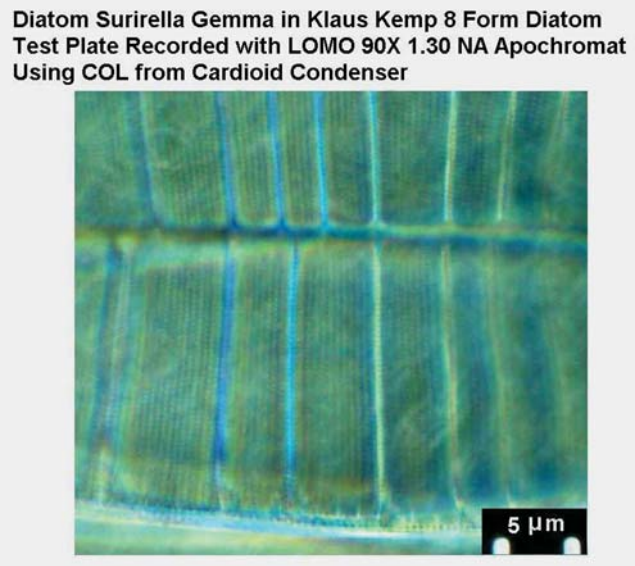

Figure 6. High magnification photomicrograph of the diatom shown in Figure 5.

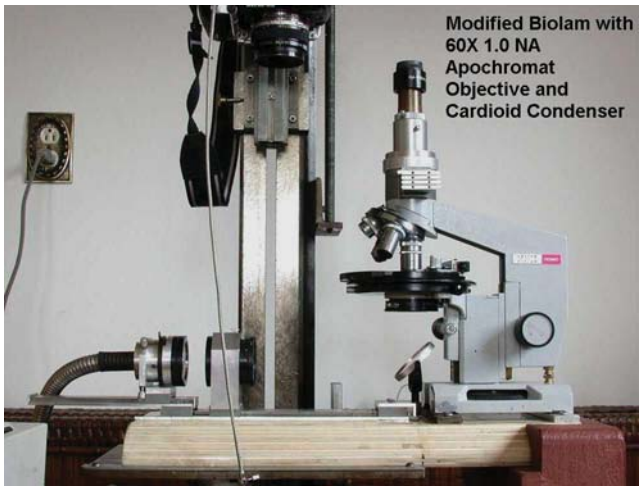

Figure 7. View of the Modified LOMO Biolam with the cardioid condenser and 60X 0.70-1.00 NA objective installed.

A 4X objective and reticule eyepiece were used for this procedure; the wing of a house fly was used as the specimen. Figure 8 shows the off-center illuminated annular zone on the specimen plane, Figure 9 shows that the condenser is focused and centered, and Figure 10 shows a smaller bright disk on the specimen plane after closing the field diaphragm. A field diaphragm was important to eliminate stray white light from nonasbestos particles located just outside the field of view. There is no reference of using the field diaphragm with darkfield.

DISPERSION STAINING OF AMOSITE USING THE CARDIOID CONDENSER

The cardioid condenser does not have a filter holder, so the analyzer was inserted above the 1.00 NA objective. A slide of amosite asbestos in 1.680 RI high dispersion Meltmount was provided by Peter Cooke, an environmental microscopist. Amosite was used for a dispersion staining study with the 60X LOMO objective with its iris fully open to 1.0 NA. Despite the numerous bubbles in the mountant and a large particle scattering white light nearby, a fiber exhibiting the characteristic dispersion staining colors for amosite was clearly evident. Figures 11 and 12 show the splayed end of an amosite fiber bundle with blue-green color in the perpendicular and golden yellow color in the parallel orientations.

DISPERSION STAINING WITH THE 40X, 60X AND 90X OBJECTIVES

Cooke also provided a slide of short fiber Calidria (chrysotile) asbestos in 1.550 HD Meltmount for these dispersion staining studies. The only available image of this short fiber asbestos is the high-resolution, critical darkfield, dispersion staining photomicrograph in Figure 13. It was recorded with the 40X planachromat of Cooke's Olympus BH-2, with a darkfield insert made for its Abbe condenser. Cargille 1.550 HD liquid mountant was used, and a characteristic red-purple

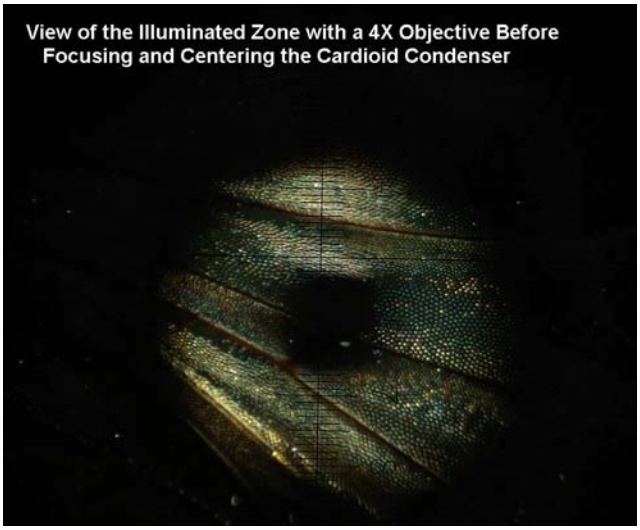

Figure 8. Low magnification photomicrograph showing the off-center, annular illuminated region before the cardioid condenser is focused and centered.

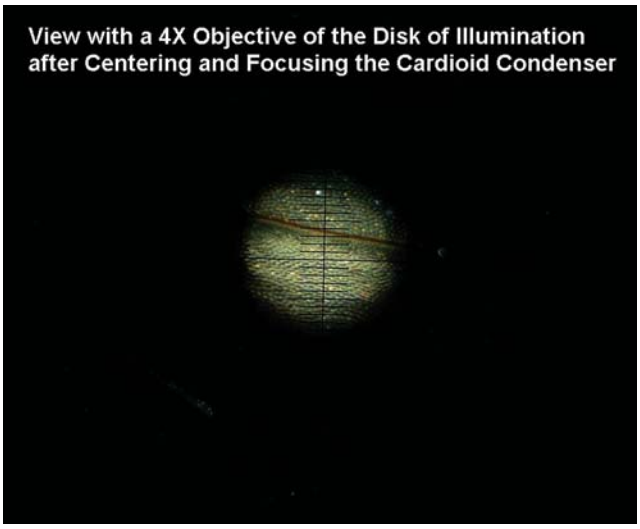

Figure 9. Low magnification photomicrograph showing a centered disk of illumination after focusing and centering the cardioid condenser.

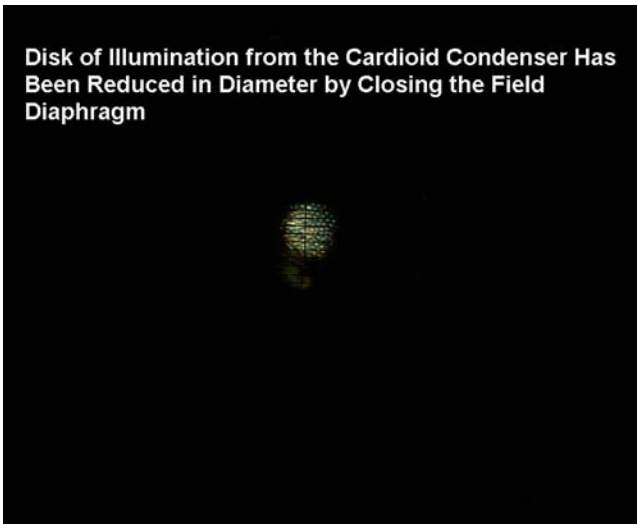

Figure 10. Low magnification photomicrograph showing a smaller illuminated disk than in Figure 9 after closing the field diaphragm.

dispersion staining color is evident in the image even though the slide was being examined at an elevated ambient temperature.

I initially used the 60X 1.0 NA objective with critical darkfield illumination from the cardioid condenser to examine the Calidria in Meltmount. Blue dispersion staining colors were clearly visible in the specimen shown in Figure 14. I then used the 90X 1.25 NA LOMO objective with the funnel insert provided with the cardioid condenser. The instructions with the condensers state that the condenser is not suitable for objectives with an NA higher than 0.85. Because I had an extra funnel insert, I bored out about 20% of the aperture in the end of one of them. This insert did not give a uniform dark ground with the slide of Calidria asbestos, but examination of the image of the rear focal plane of the objective showed incomplete darkfield due to the end of the funnel not being properly centered. After measuring the diameters inside the 90X objective, which allow the funnel to be centered, I made a new insert that was a close, sliding fit inside the objective. The funnel inserts and objective are shown in Figure 15. The blue colors are as apparent with the new insert in the 90X objective as they are with the 60X 1.00 NA objective; compare Figures 14 and 16.

CONCLUSIONS

Critical darkfield dispersion staining first demonstrated by Dodge with a 40X objective in 1948 can be applied with significantly higher resolution using a cardioid darkfield condenser and 1.0 NA objective. Critical darkfield with the 40X 0.65 NA objective can resolve all of the blocks of the HSE/NPL phase-contrast test slide required for counting airborne asbestos fibers at 400X with the phase contrast microscope. This is shown in Figure 17 using critical darkfield and the 40X 0.65 NA objective of the modified Biolam.

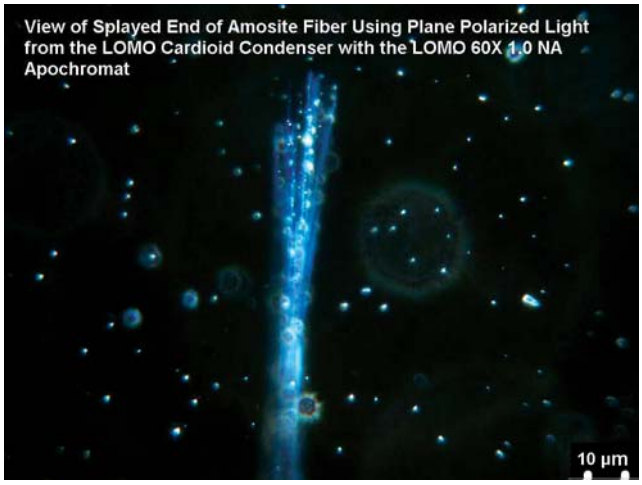

Figure 11. A perpendicular-oriented amosite fiber bundle using the 60X objective at 1.00 NA using plane polarized illumination from the cardioid condenser.

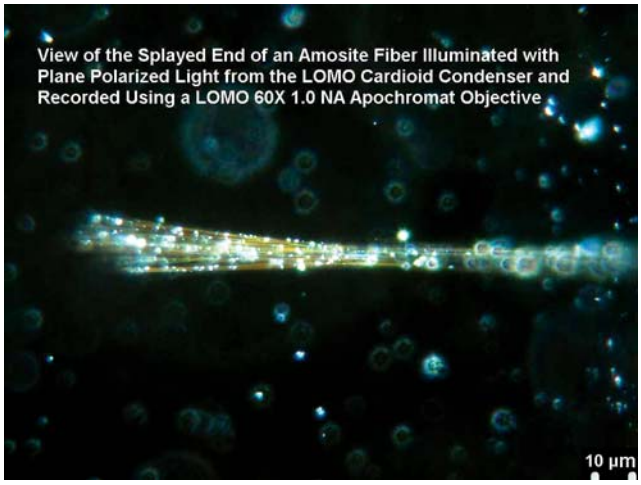

Figure 12. The same amosite fiber in Figure 11 after rotating it to a horizontal orientation parallel to the polarization direction.

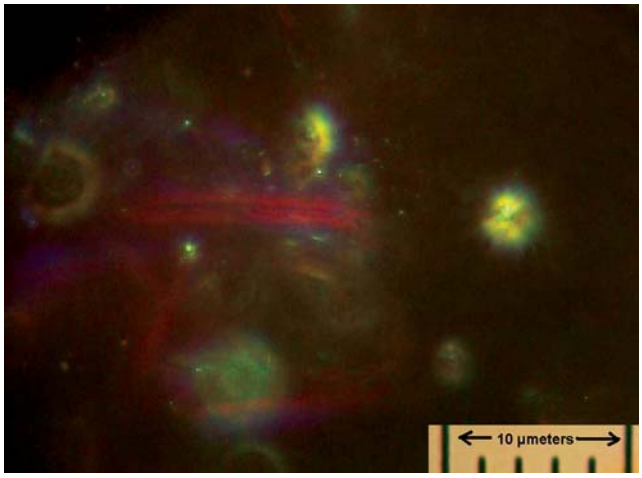

Figure 13. Calidria asbestos in 1.550 HD liquid recorded with the Olympus BH2 microscope with the 40X 0.65 NA objective using plane polarized critical darkfield.

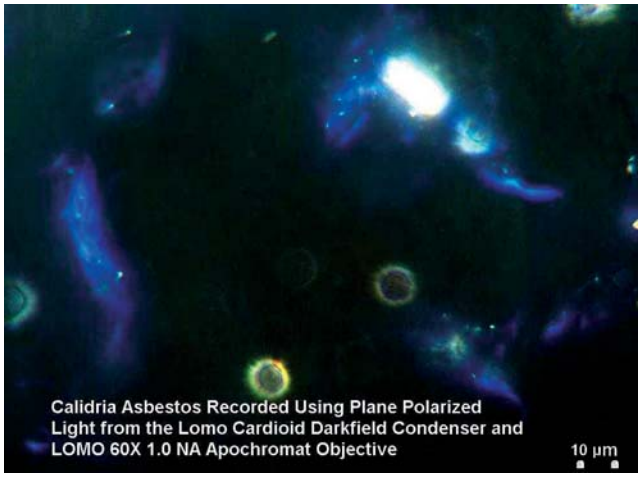

Figure 14. Calidria asbestos in 1.550 HD Meltmount recorded with the 60X objective at 1.00 NA using plane polarized darkfield illumination from the cardioid condenser.

The Japan Association for Working Environment needed dispersion staining with the resolution of 400X phase contrast microscopy to both count and differentiate asbestos from nonasbestos fibers. Nikon met this need with a 0.75 NA objective with an opaque annulus in place of a phase retarder annulus. Critical darkfield dispersion staining with a 1.0 NA objective and cardioid darkfield condenser should provide higher performance than the Nikon objective. The LOMO cardioid condenser in this study can also be used with the LOMO Polam, and a 50 0.70-1.00 NA objective is available for the Polam.

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Figure 15. View of the LOMO 90X 1.25 NA objective with funnel inserts to reduce its operating NA. The precision-made funnel insert is on the right.

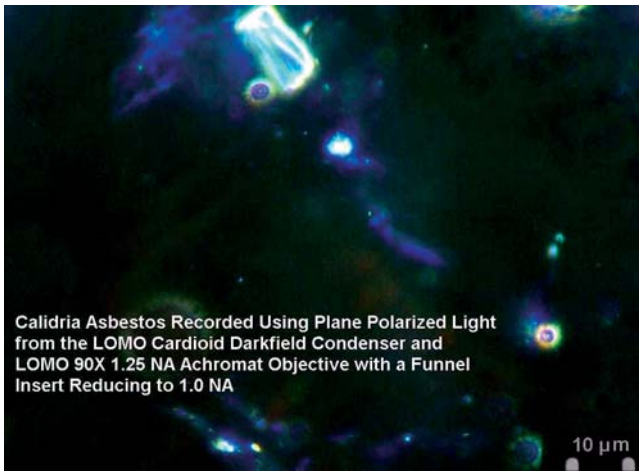

Figure 16. Photomicrograph taken with the 90X objective and the precision-made funnel insert (see Figure 15) of a smaller portion of the field with Calidria asbestos.

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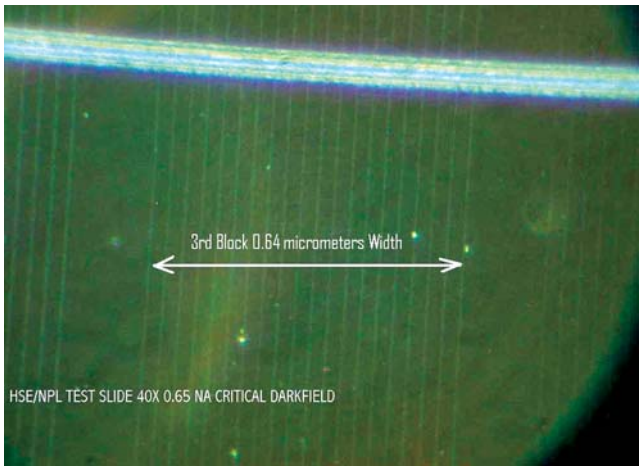

Figure 17. Photomicrograph taken by Tony Havics with the modified Biolam and 40X 0.65 NA achromat and critical darkfield showing that blocks 2, 3 and 4 of the HSE/NPL phase contrast test slide are resolved.