

Microscope Illumination techniques

Possibly the most used microscope lighting, by amateurs, is brightfield. It is the basic default system and is often the only possibility on entry level microscopes. I am fortunate in owning an Olympus BH2/BHS with accessories that allow for other lighting methods as well as brightfield. I show here two common subjects illuminated by brightfield, differential interference contrast (DIC), and polarization. The BHS also allows for phase contrast but I do not have suitable objectives.

For polarization an analyser is required in the light path between the objective and eyepieces. Olympus uses an 'intermediate tube' which also acts as a 1.25x magnifying lens. It has a slot for inserting a wave plate for polarization. This extra tube is not needed for brightfield illumination but I leave it on the microscope.

For DIC, Olympus has a specific substage condenser which has prisms for DIC and an empty aperture for brightfield. DIC also requires a Nomarski prism to be placed in the slot in the intermediate tube.

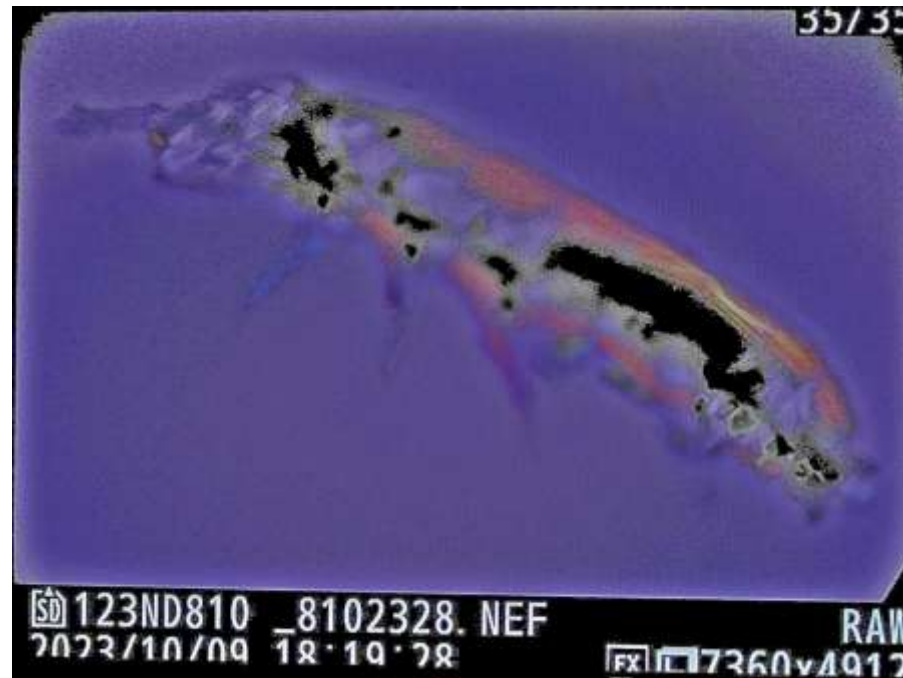
Brightfield setup: substage condenser, open aperture; 4x objective for the Louse; 10x for the Springtail; 1.25x intermediate tube; 2.5x projection eyepiece; Nikon D810 camera.

Polarized – darkfield: as brightfield but with a polarizer filter below condenser; no wave plate in intermediate tube.

Full polarization: as in darkfield but a wave plate inserted into the slot of the intermediate tube.

DIC: green IF550 interference filter below condenser; condenser's polarizer engaged, 10x prisms selected in condenser (there is no 4x prism, thus no DIC for the louse); Nomarski prism in the slot of the intermediate tube.

Several frames were taken of each specimen and combined into one using Zerene Stacker. Each frame displayed on a TV monitor via the D810's Live View feature:



Booklouse - length 1.86 mm

These insects are not often seen but are likely common in most houses; this one was walking across my workshop table. Collected by touching it with a wet artist's paint brush (fine artist's brushes are the best tools for picking up small delicate insects). It was immediately transferred to absolute methyl alcohol and left for three days to 'harden'. Moved to a microscope slide, a drop of Euparal Essence to remove the alcohol and then mounted in Euparal.



brightfield

polarized darkfield

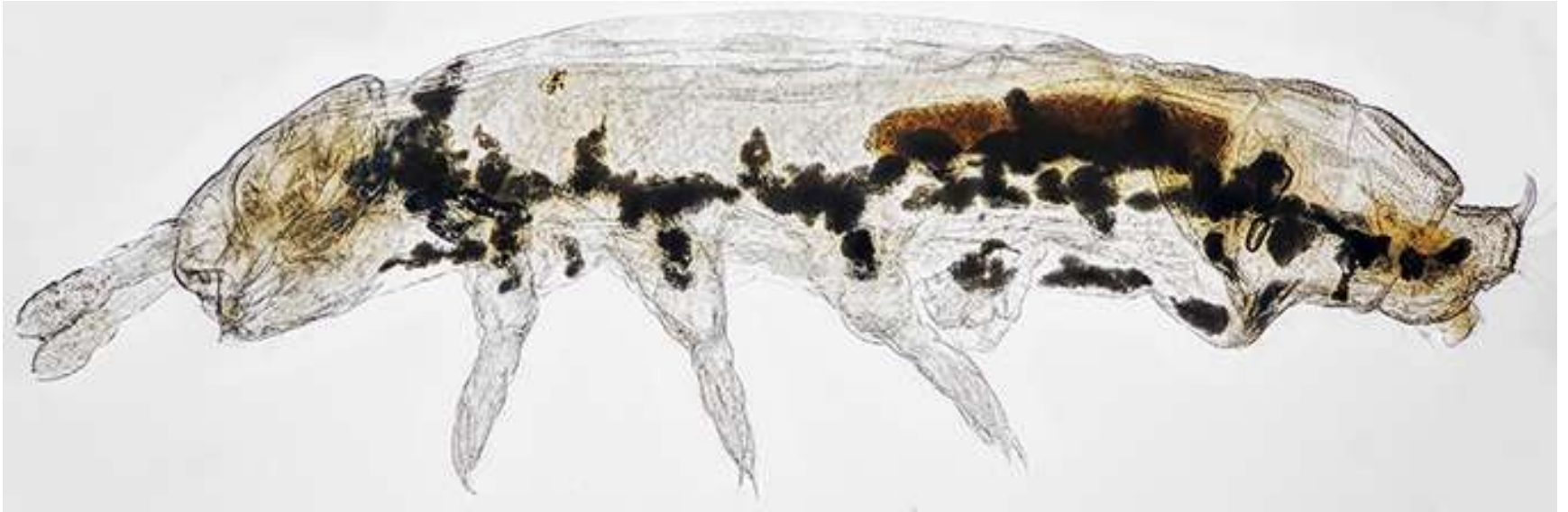
full polarization

The darkfield image shows the internal organs more clearly than the brightfield, and especially the muscles in the head and legs (glowing white 'strands'), but the antennae 'disappear'.

The full polarized image shows the muscles in yellow, green, and red; it also clearly shows the hairs on the posterior abdomen ('lost' in the other images) but fails to show that the abdomen is segmented. Segmented abdomen seen best, but poorly, in the darkfield image.

Springtail – length 1.38 mm

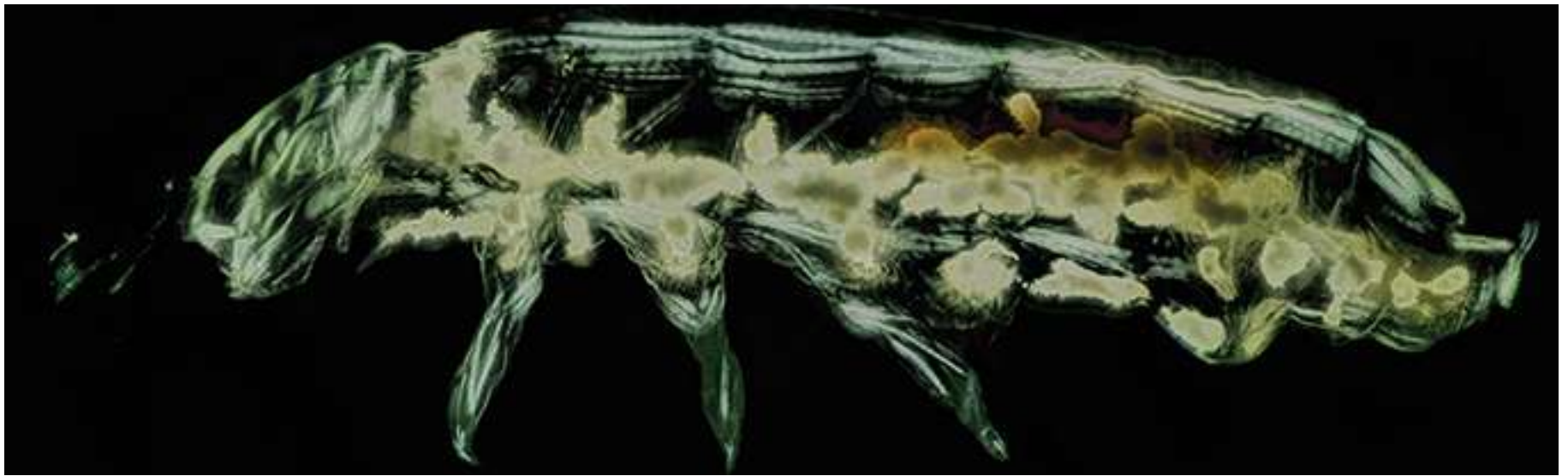
These tiny Hexapods were once considered insects (Class: Insecta) but are now in their own class (Collembola). They differ from insects in several ways including mouthparts within the head (not obvious even under microscopic examination), in insects the mouthparts are conspicuous and external. Springtails are very common in damp litter. They are readily collected using a Berlese funnel. Dehydrated in alcohol, mounted in Euparal.



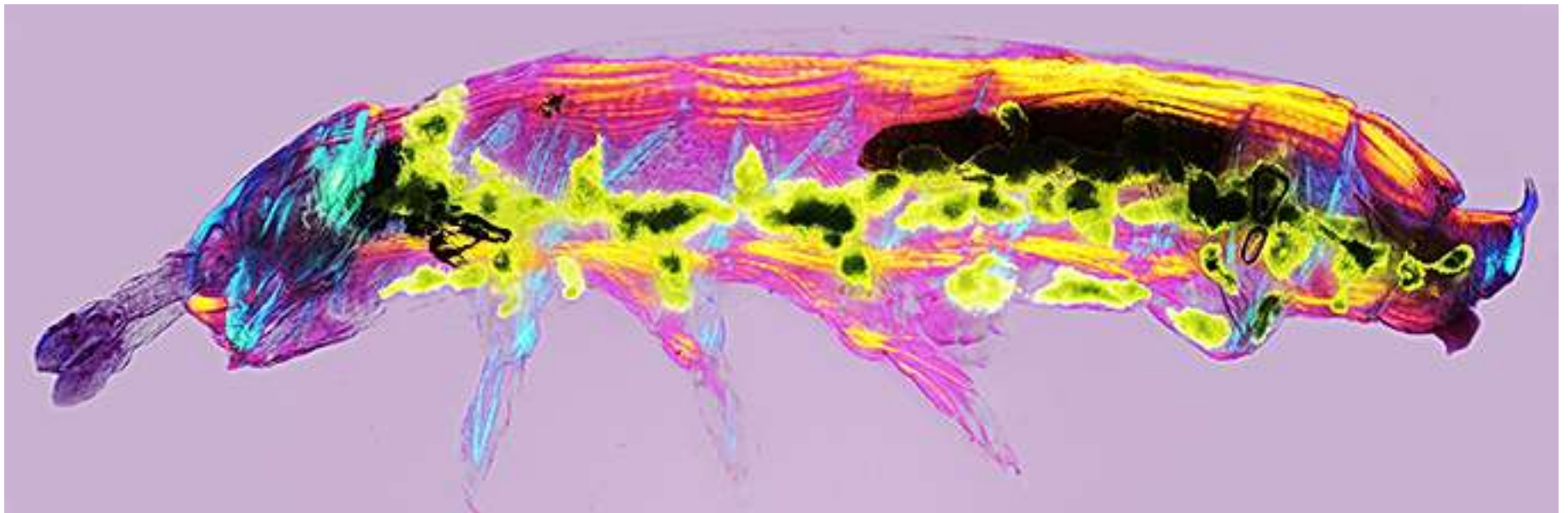
brightfield



DIC



darkfield polarization



full polarization

Springtail images

The black irregular patches in the brightfield and DIC images are likely food storage, possibly fat; show white in reflected light. They brownish cylinder seen dorsally/posteriorly in the brightfield image in food in the digestive system.

Musculature is vaguely visible in brightfield, slightly more obvious in DIC; most obvious in darkfield polarization where it shows as glowing white bands.

In the full polarized image the muscles show as yellow and blue structures.

Each technique has benefits, but if I had to choose just one for detail I believe the darkfield polarization shows the better detail in both specimens.

Comments to the author Anthony Thomas are welcomed, email - at5535587 AT gmail DOT com.

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