Photomicrography workshop for Positive View
Tuesday 16th July 2024

Using reflected light for photomicrography

Most microscopes are designed for use with transmitted light and specimens that are thin, translucent or transparent, and mounted under a coverslip on a glass slide. There is usually a very small distance between the tip of the objective and the specimen, and this makes it difficult or impossible to light the specimen from the top.

However, the major manufacturers make attachments and special objectives for observing opaque or reflective specimens using incident light that is transmitted through the objectives. The illumination can be either bright-field (transmitted through the lenses of the objective) or dark-field (transmitted through a channel outside the lenses of the objective).

Today I have brought my Olympus CH-2 microscope fitted with a BH-RLA vertical illuminator that can be switched between bright-field and dark-field, with a set of 5×, 10×, 20× and 40× Neo objectives that can be used for bright-field and dark-field.

The vertical illuminator is designed for subjects such as reflective metallurgical specimens, and bright-field and dark-field provide different ways of viewing these specimens. However, bright-field gives a poor image for biological specimens such as scales on butterfly wings, pollen on stamens, feathers and lichen, and for non-reflective specimens such as postage stamps and bank notes, although polarisers can give some improvement. Dark-field works much better with these subjects.

For viewing deep specimens, it is easy to use the coarse and fine focus knobs to focus up and down through a specimen.

For photomicrography, the small depth of field (around 0.05 mm for the 5× NA 0.1 objective, even smaller for higher magnifications) was a serious problem with 35 mm and other film formats. Fortunately, digital cameras can be used to produce images with good depth of field. This is achieved by taking a series of photos at different depths through the specimen, and then using image stacking software that selects the sharpest parts of each image and produces a composite image with good depth of field.

A stack of 40 images with the 5× objective can give a composite photograph with 2 mm depth of field. To decide how far to turn the fine focus knob between each image for a stack, I use Rik Littlefield's table at the bottom of this page:
http://zerenesystems.com/cms/stacker/docs/tables/macromicrodof

The working distance with the 5× Neo objective is 22 mm, and 7.5 mm with the 10×, so they allow quite deep specimens to be used, although you do have to be careful that they don't touch the front element. The working distance of the 20× Neo objective is just 1.7 mm (and only 0.62 mm for the 40× Neo), so they can only be used with flat specimens.

Alan Wood