

Photomicrography

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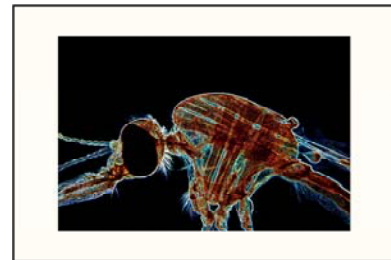
Photography's popularity and its pervasiveness throughout contemporary society flows from its ability to serve as a means of documenting the world (and universe) around us and providing a form of creative expression that allows the photographer to present reality as she or he sees it. Our fascination with "macro" photography is, perhaps, due to its ability to allow us to document, interpret and present a level of detail that exists in reality but otherwise goes unnoticed in our daily lives. It strikes me then, why so few photographers explore photography in the realm of the "micro" and confine their work to just full-scale and macro. All it need take is a modest investment in a decent quality light microscope, comparable in price to a good SLR lens (~\$300 – \$1,500) and a working knowledge of light microscope image formation. Mastering photomicrography technique can open up a new world of exhibit and competition photography and also provide a lucrative niche in an increasingly crowded and competitive stock photo marketplace.



Human Head Louse (40X)



Salmonella bacteria (1,000X)



Mosquito (40X)

Microscope Optics and Image Formation

Microscope image formation involves, quite simply, having a specimen to be observed, an illumination source, and a set of three lens systems, the condenser lens, objective lens, and ocular lens. The condenser lens serves to focus the illumination source (light) onto the specimen so that it is intense enough to pass through the specimen to form a virtual image of the specimen. The image is formed by the interaction of the light waves with the specimen. Where regions of the specimen are dense or opaque, less light will pass through creating a corresponding dark region in the image. Where regions of the specimen are selectively colored or stained, only certain wavelengths of light will be blocked resulting in corresponding colors of the final image. Whereas standard photography (or normal vision) involves forming images from reflected light (light bounced off an object), the microscope forms images from transmitted light (light passed through an object). This limits most light microscope observations to either very small, very transparent, or very thinly cut objects.

The light, once generated from the illumination source, focused on the specimen by the condenser lens, and passed through the specimen to form the virtual image, is then passed

through an objective lens which will magnify the virtual image. Most quality microscopes have a selection of three or four objective lenses allowing for magnifications of 4X, 10X, 40X, and 100X. The objective lens is designed to transmit parallel rays of light, carrying the magnified image, from what will appear to be a distance of infinity.

The final lens, the ocular or eyepiece lens, is designed to focus the virtual image to a point approximately 10mm away for viewing by the human eye. This point of focus of the ocular lens is called the exit pupil, or eyepoint. Most ocular lenses provide an additional 10X magnification resulting in final image magnifications of 40X, 100X, 400X, and 1,000X.

Photography Through the Microscope

In order to photograph the image formed by the objective lens, the photographer has two main options. The first is to physically mount the camera to the microscope, a procedure that requires a trinocular microscope; a microscope with a third viewing tube specifically for photography. Camera mounting equipment will typically include some combination of a T-mount connected to the camera's lens mount, an extension tube, an eyepiece lens, and a mounting connection to the microscope. Alternatively, specially designed cameras for photomicrography can be used and will typically attach through a C-mount.

A more straightforward, less expensive, and simple technique, and one that requires no additional connecting hardware, is to simply replace the human eye with a camera. In normal microscope viewing, the front surface of the eye (cornea) is placed at the ocular lens focal point, or the eyepoint, typically about 10mm from the top of the microscope. Simply placing the front lens element of the camera at this point will, rather than projecting the image on the retina of the eye, project the image on the camera's CCD or CMOS sensor for photographing. This requires some special considerations however, which are listed below:

- DSLR lens must be a "macro" with a very close focus range. I use a Canon 50mm macro, f2.5.
- The DSLR lens should be set to focal length of infinity as the microscope's objective lens forms a virtual image of parallel light rays seeming to come from infinity.
- The DSLR lens aperture should be set to wide open (f2.5 in my case) and the camera mode should be set to aperture priority. The microscope image, being a transmitted image rather than a reflected image, has no depth of field.
- ISO should be set to 100.
- Metering mode should be determined by the image properties (ie. Is the image uniform and filling the field or is the image small and surrounded by a large bright white background).
- Focusing on the camera's ground glass is challenging and focusing is greatly enhanced by using the *live view* function, if available.

- The camera must be firmly mounted and placed so that the front lens element is at the eyepoint and that the camera lens surface lies on the same plane as the viewing surface of the microscope ocular lens.
- If, when mounted as above, space is present between the camera lens and ocular lens, the photograph should be taken in the dark or a lens hood or black velvet fabric should be used to block extraneous light.
- Photograph should be taken with mirror lockup or using the timer mode to reduce camera shake.



Image Processing:

Standard Photoshop techniques of sharpening, shadow-highlight adjustment, dirt and dust removal by cloning, and cropping will be needed. It is very difficult to avoid dirt and dust contamination if not working in a “clean room” setting.

Three-dimensional looking images approximating the look of phase contrast or light microscopy can be created through the use of layers and the *Stylize-Emboss* filter.

Darkfield images can be simulated through the use of either, or a combination of the *Image-Adjustment-Inverse* and the *Stylize-Emboss* filter.

Attractive results can be obtained by using the *Stylize-Glowing Edges* filter.

Resources:

Shots with a Microscope.

http://www.funsci.com/fun3_en/upic/upic.htm

Photography in Your Science Fair Project (Kodak)

http://www.sciencebuddies.com/science-fair-projects/webpage_archive/Kodak_PhotoInSciFair.pdf

Two Basic Methods for Photomicrography.

<http://krebsmicro.com/photomic1/photomic1.html>

Photomicrography with 35mm Cameras (Zeiss)

[http://www.zeiss.com/c1256f8500454979/0/e1523220b44599d0c1256f8f00383cde/\\$file/b-40-046photomicrography35mm.pdf](http://www.zeiss.com/c1256f8500454979/0/e1523220b44599d0c1256f8f00383cde/$file/b-40-046photomicrography35mm.pdf)

Attaching Cameras to Microscopes

http://www.gxoptical.com/GX_Optical_Camera_Adapter_Helpsheet.pdf