

Microscopy and ImageJ

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Preparing a photomicrograph for ImageJ analysis is not as straightforward as a simple photograph taken by a handheld-camera. Usually the field of vision when viewing a disc under the stereo microscope is limited to a very small portion of the disc even at the lowest magnification. This tutorial will help the student in taking photos of the disc under the binocular and in analysing them later with ImageJ. You can use a microscope camera or a smartphone mounted on the stereo microscope.

1. The most important thing to do is to **photograph a reference scale**, which you can later use for the image analysis. First, place the disc under the stereo microscope. To have the same amount of refraction in all images, make sure that the disc is immersed in water. Choose the magnification with which you would like to photograph the disc and focus correctly.
2. Remove the disc from under the stereo microscope. Without changing the magnification, place a calibrating instrument under the binocular microscope and focus. For precise measurements, a stage micrometre is required. Professional stage micrometres are very expensive but there are cheaper alternatives for schools like the Motic 4-dot calibration slide, which costs about 22€, or a calibration slide from AmScope for \$25.
3. Take a photograph of a reference dot (Motic) or scale on the calibration slide.
4. Without changing the magnification, place the disc under the stereo microscope and take pictures of random spots on the disc. Take at least 20 different photographs. Due to the high magnification, systematic randomisation is quite difficult when making photomicrographs. Depending on the magnification, the area in the field of vision is just on the order of μm^2 .
5. When analysing the photograph with ImageJ, use the picture of the calibration slide to set the scale in the software to the calibration scale of the slide. Click on **“Global”**.
6. When the scale is set, load the pictures you want to analyse. Further calibration is not necessary.
7. Make the necessary preparations of the photograph, outlined in the different tutorials.

