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 stereomicroscope is limited to a very small portion of the disc even at the lowest magnification. This tutorial will help the student to make photos of the disc under the binocular and how they can analyse these photos later with ImageJ. You can use a microscope camera or a smartphone mounted on the stereomicroscope.

1. The most important thing to do is to **photograph a reference scale**, which you can later use for the image analysis. Place the disc under the stereomicroscope. Be 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 stereomicroscope . Without changing the magnification, place a calibrating instrument under the binocular and focus. For this, a stage micrometre is necessary. Professional stage micrometres are very expensive but for schools there are cheap alternatives like the Motic 4-dot calibration slide, which costs about 22€ or a calibration slide from AmScope for $25.

3. Make a photograph of a reference dot (Motic) or scale on the calibration slide.

4. Again without changing the magnification, place the disc under the stereo-microscope and make pictures of random points in 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 in the order of µm^2.

5. When analysing the photograph with ImageJ, set the scale using the picture of the calibration slide. Set the scale to the known scale of the calibration slide. Click on **“Global”.**

6. When the scale is set, load the pictures you want to analyse. Further calibration is not necessary.

7. Now load a picture of the disc you want to analyse. Make the necessary preparations of the photograph. Click on Analze – Tools – Grid.

8. Set the grid size to the size of the squares you want depending on the size of organisms on the image. Count the individuals in a square. (See tutorial on Biofilms and Biodiversity and the “ImageJ” Software by Adam Frederick).

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