# Automated cropping and scale bars in imageJ

**Setting the Global Scale**

First set the global scale using a 10X image of the Neubauer chamber. This should be every time before running macros.

1. **Open the Reference Image**:
   * Start Fiji and open the image of the Neubauer chamber (or any other reference image you use to set the scale).
   * Use **File > Open** and navigate to the location of your reference image.
2. **Draw the Line for Scale**:
   * Select the straight line tool from the toolbar.
   * Draw a line across a known distance (e.g., spanning 4 squares of the Neubauer chamber which equals 1mm/1000µm).
3. **Set the Scale**:
   * Go to **Analyze > Set Scale**.
   * In the **Set Scale** window, enter the known distance in microns (1000 µm if the line represents 1mm) in the **Known Distance** field.
   * Enter the unit of length (µm).
   * Make sure to check the **Global** checkbox to apply this scale to all images you open in this session.
   * Click **OK** to set the scale.
4. **Save the Settings** (Optional):
   * You might want to save these settings for future use.
   * Go to **File > Save As** and choose an appropriate format to save your reference image with the scale set.

**Running the Macros**

1. **Open the Macro for Opening Images**:
   * Plugins > macros > edit > open the “open\_pics.ijm” macro. Edit this to the location of the photos. Run the macro, and all images should open.
2. **Open the Macro for Cropping and Adding Scale Bars**:
   * Once all images are open, open the “crop\_scale\_bar.ijm” macro in the same way
   * Do any editing of crop size and/or scale bar options.
   * Choose a folder to save the edited photos in.
   * Before running the macro, ensure all images are open and the global scale is set.
   * Run this macro by clicking **Run**. It will prompt you to select the cropping area on each image (use rectangle tool and draw a rectangle in the center of the spheroid, see picture below), then crop the image, add a scale bar, and save it.
3. **Save Cropped Images**:
   * The cropped images with the scale bar will be automatically saved in the specified directory.
   * Make sure you have the correct path set in the **outputDirectory** variable of your macro.

**Closing Remarks**

* Remember to **close any images** you don't need before starting a new macro to avoid confusion.
* Each time you start a new Fiji session, **repeat the global scale-setting process** with the reference image to ensure accuracy.
* The **cropping size and scale bar size** are set in the macro. Adjust these values if your requirements change.
* For **batch processing**, ensure that the file extensions in the macro match the image files in your directory.

A close-up of a microscope

Description automatically generated