Topic 03 – Basic tools 2

One point why ImageJ is popular is that it can easily be extended by macros, scripts and plugins. In this part you will learn how to use and record macros, how to batch process images, how to install and use new plugins, how to use tool-sets and how to work with 3D images.

Exercise 3.1: Using macros

We will use a macro from the ImageJ-website. The macro ROI_Color_Coder.ijm allows to color code the outlines of particles according to a measurement (area, intensity, perimeter, etc.)

a) Open the image *A4 dapi 1.tif* from the folder *01 – opening images.* Use the *Help* menu of ImageJ to open the macros web-page. Look for the macro and drag it onto the ImageJ-launcher window. The macro needs the selections in the roi-manager and the corresponding measurements in the results-table. Color-code the nuclei by area. Try again and color-code them by roundness. Hint: Use the menu *Macros>Run* from the macro-editor window to run the macro.

Exercise 3.2: Recording macros and batch processing

With the help of the macro-recorder (*Plugins>Macros>Record...*) you can record the commands you run and create a macro from them.

a) Run some commands (smooth, find edges, …) and record them with the macro-recorder. Press the create button to create the macro. Revert the image (*File>Revert* or *ctrl+r*) and run the macro from the macro-editor.

b) Record a macro to measure the features (are, mean intensity, etc) of the nuclei in the image *A4 dapi 1.tif* from the folder *03 - basic tools 02/in*. It should contain the following steps:

- set the measurements you want to have in the result

- set an auto-threshold on the image

- run the particle-analyzer (with the display result option)

- run the command (*Analyze>Label*) to show which objects have been found

Apply the macro to all the images in the folder *03 - basic tools 02/in,* one after the other.

c) Run the macro on the whole folder using *Process>Batch>Macro....* Copy and paste the macro into the window, select the input and output folder and press *Process*. What do you get in the output folder? What do you get in the results-table?
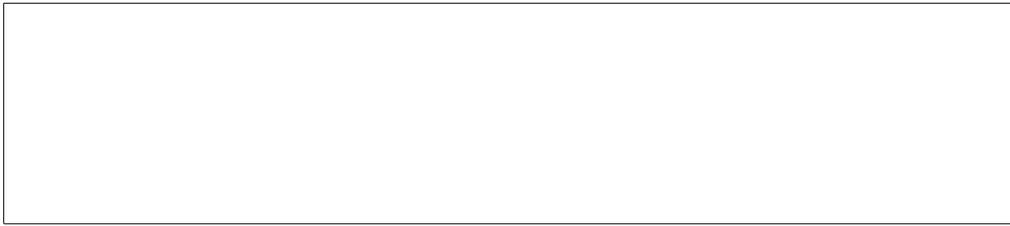
Exercise 3.3: Using Plugins

Plugins are java modules that can be used to add functionality to ImageJ. A large number of plugins concerning microscopy is available.

a) Install the SIOX plugin from the ImageJ website. Open the plugins page from *Help>Plugins.* Search for SIOX and click on the link. Drag the link *siox_.jar* onto the ImageJ launcher. Create a subfolder *Segmentation* in the ImageJ plugins folder and save the plugin into it.

b) Open the image *Pot104Cycle0083.jpg* and try to use the SIOX plugin to segment (seperate from background) the plant.

c) Create a mask using the SIOX plugin. From the mask create a selection (*Edit>Selection>Create Selection*, *Edit>Selection>Make Inverse*). Put the selection onto the original image (*Edit>Selection>Restore Selection*) control the quality of the segmentation.

d) Measure the area of the plant.

Exercise 3.4: Working with stacks

Stacks can either represent volume data or time series. Open the example stack *t1-head.tif* from the folder *03 - basic tools 02*. Note the slider below the image. You can use it to select the visible slice of the stack. The play and pause button next to the slider allow to start and stop the animation of the stack. A right click on the same button opens the options-dialog for the animation.

a) Start the animation. Change the animation speed.

b) Try the commands *z project*, *3d-project* (select mean-value as projection-method) and *make montage* from the *stk* tool-button.

c) Make one 3d-projection around the x-axis and one around the y-axis using the mean-value projection-method. Rename the resulting images, otherwise they will both have the same name, which can lead to problems. Try the commands combine and concatenate from the *stk*

tool-button. Hint: - For concatenate the images must have the same size in the x and y dimensions. You can adjust the canvas size of the image using *Image>Adjust>Canvas Size....*

d) Try the Volume Viewer (*Plugins>3D>Volume Viewer*).

e) Try the ImageJ 3D Viewer. Set the resampling factor to 1 and press ok when you are asked if the image should be converted to 8 bit. As long as the scrolling tool is selected, you can turn the object in the image. You can zoom with the magnifying glass tool. If you loose the object call *View>Reset View* from the 3D viewer window.
   Make a freehand 2d selection across the head. Select the object from the *Select* menu and then call *Edit>Fill Selection*. Turn the object. What happened?