Topic 02 – Basic tools 1

The ImageJ-launcher window consists of three parts. A menu-bar, a toolbar and a status-bar. You will now get to know the basic tools of ImageJ in more detail.

## Excercise 2.1: Pixel information, zooming and scrolling

Open the image *A4 dapi 1.tif* from the folder *01 – opening images*.

- Note that some information about the image is displayed at the top of the image-window. The size of the image in pixel, the type and the size of the image in the memory of the computer.

- Select the magnifying glass tool on the toolbar and click into the image. A left-click does a zoom-in, a right-click a zoom-out. You can use the "+" and "-" keys on the keyboard to do the zooming.

- Note the position-indicator in the upper-left corner, when the image is bigger than the window.

- Zoom-in so that the position-indicator appears. Select the scrolling-tool on the toolbar. Click in the image and without releasing the button move the mouse. Go to the lower-right corner of the image.

- Select the magnifying glass tool again. Zoom-in so that the position-indicator appears. You can drag the image by holding down the space-bar instead of using the scrolling-tool. The advantage is that another tool can be active in the same time. Try to scroll to the lower left corner.

- Zoom in to the maximum zoom and move the mouse over the image. Look at the status-bar of the ImageJ-launcher window. The position of the pixel under the mouse-pointer and its grey-value are diaplayed.

a) What is the maximal possible zoom? What is the minimal zoom?

b) What is the grey-value of the pixel with the coordinates x=549 and y=305? What is the grey-value of the pixel with the coordinates x=58 and y=246?

c) What is the width and height of the image? What is its type? What is its size in the memory?
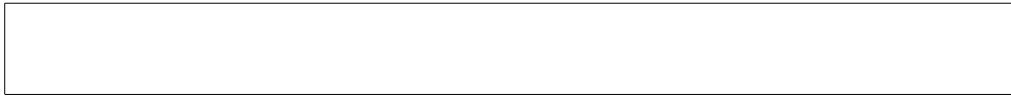
d) Move the mouse over the image. In which direction does the x-coordinates of the pixels become bigger. In which direction does the y-coordinates become bigger? How does the coordinate-system look like?
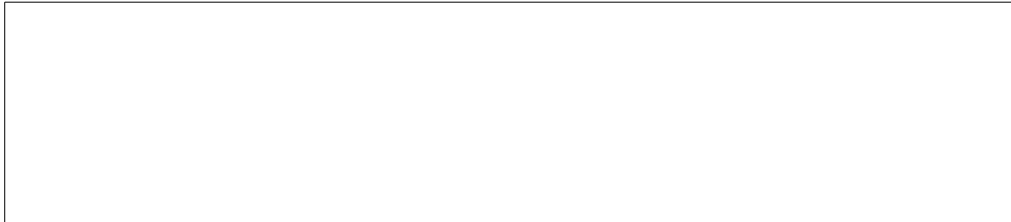
e) You can change the orientation of the y-axis. Open the dialog *Analyse>Set Measurements...* and select *Invert Y coordinates*. Move the mouse over the image again. How did the coordinate-system change?

Exercise 2.2: Area selection tools

Make sure that the rectangular selection tool is active. You can now make a rectangular selection in the image by clicking at a point and dragging the mouse. While doing this the width, the height and the aspect ratio of the selection are displayed. Once you release the mouse button you made a selection on the image. You can still change its position by clicking into it and dragging and its size by using the handles.

a) Make a rectangular selection on the image. Move it around. Change its size. Find out in the ImageJ documentation (ImageJ User Guide) which modifier keys exist for the rectangular selection tool and what they do. Try them.
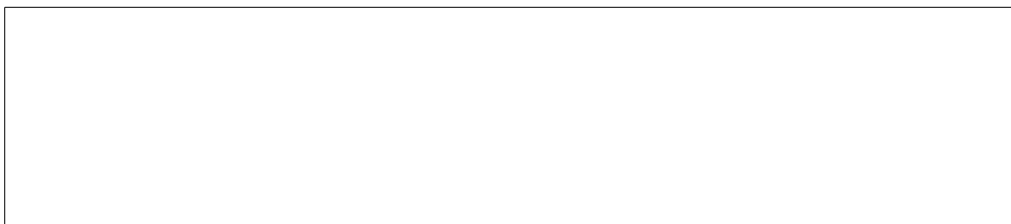
b) Note that you can create complex selections by using the shift and the alt key. Shift adds to a selection. Alt removes from a selection. Try it. Move the complex selection around. Note

that you create **one** selection, even if the parts may be separated.

c) Change the selection color to magenta (*Edit>Options>Colors...*).

d) Right-click on the rectangular-selection tool-button and select the rounded-rectangle-tool. Make a selection. Note that all selection tools use the same color setting except for the rounded-rectangle selection that has its own color setting. You can change it by double-clicking on the tool-button.

e) Double-click on the rounded-rectangle-tool-button. Change the value of the corner-arc size and draw a new selection.

f) Try the other area-selection tools up to and including the freehand-selection tool. Note that when using the freehand-selection tool you should not try to close it yourself. Just go near to the beginning and release the mouse button. The selection will be closed automatically.

g) Make a selection and run the command *Image>Crop*. What happens?
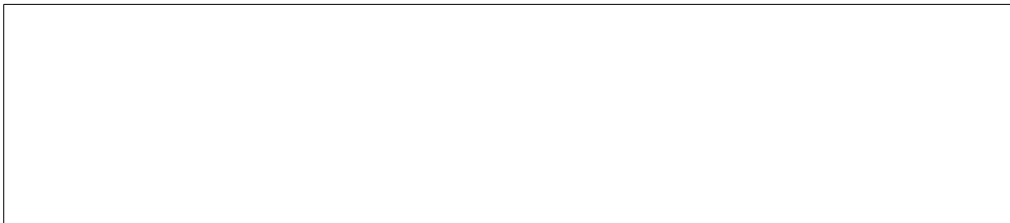
You can reload the original image using *File>Revert* (ctrl+r). Make a selection again and run the command *Image>Duplicate* (shift+d). What happens?
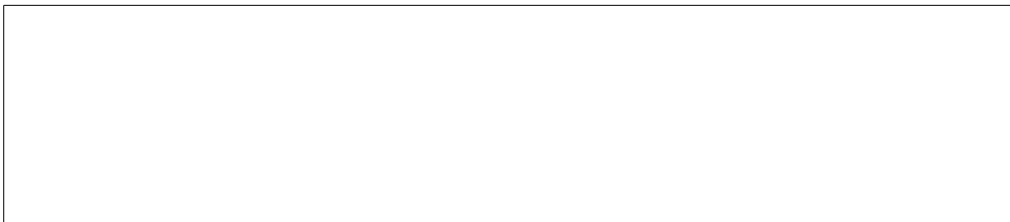
Exercise 2.3: Measuring and the results table

The command *Analyze>Measure* (m) measures either properties of the whole image or of the selection on the image, if there is one. With the dialog under *Analyze>Set Measurements* you can choose which properties will be measured. Measurements are written into the global results table.

a) Use the freehand-selection to select a nucleus, then measure its area and mean gray-value. Do the same thing for multiple nuclei. Zoom into the image to make a precise selection.

b) Call *File>Duplicate* from the results table to make a copy of the results. Measure a new selection. To which table is it added?

c) Use *File>Rename* and rename the original results table (Results) to "old". Make a new

measure. Into which results table is it added?

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d) Clear the results table using *Results>Clear Results* and measure at least 3 nuclei again. What is the mean, the standard-deviation, the min, the max and the mode of the distribution of the area-values? Use *Results>Summarize* and *Results>Distribution...* to get the answers.

Exercise 2.4: Line selection tools

Try the line selection tools straight-line, segmented-line and freehand-line. Note that while creating the line for the straight-line and segmented-line the length of the current segment and the angle with the x-axis are displayed in the status-bar.

a) Use the straight-line tool to estimate the distances between the centers of nuclei.

b) Use the straight-line tool to estimate the diameters of the nuclei.

c) Use the segmented line tool to estimate the perimeters of the nuclei.

d) Use the freehand-line tool to estimate the perimeters of the nuclei.

e) Use the angle tool to estimate the angles between three nuclei.

Exercise 2.5: Profile plots

You can use the line selection tools to create profile plots. A profile plot contains the intensity values along the line. First make a line selection across a nucleus, then press the key *k* (the shortcut for *Analyze->Plot profile*). Note that, when the line width is bigger than one, the profile will be averaged over the width. You can change the line-width by double-clicking on the line-selection tool-button.

a) Use a profile plot to estimate the diameter of a nucleus. Note that the plot is an image itself so that you can use ImageJ tools on the plot. Is there an advantage of using the profile plot rather than measuring the diameter in the original image?

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b) Create a profile plot across a nucleus. Without changing the selection, change the line-width and create a new plot. What has changed?
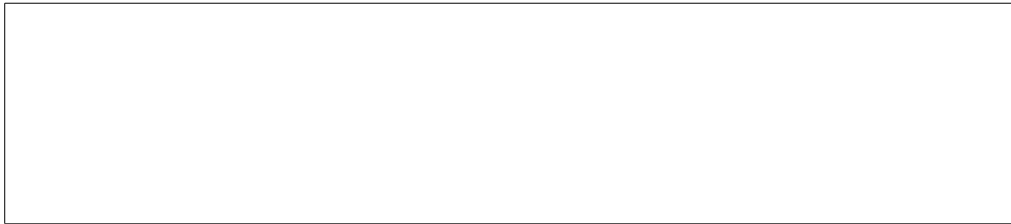
c) Create a profile plot across a nucleus, then make the line longer and create another profile plot (The easiest way to do this without changing the direction is using a horizontal or vertical line with shift pressed.). What is the difference between the plots? Measure the distances between the same two interesting points in the two plots. Do you get the same result in the two plots? How is this possible? - Hint: Look at the pixel width and height in the properties of the two plot images (*Image>Properties...*).
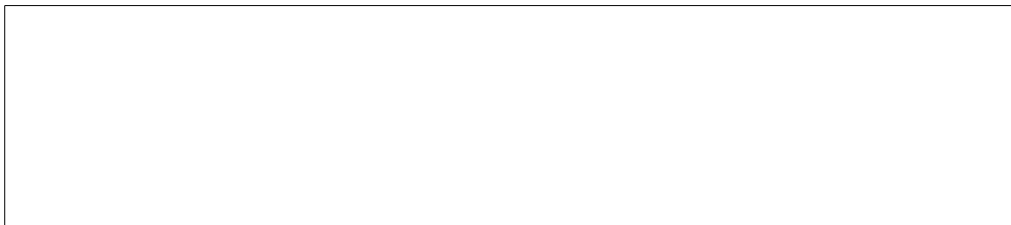
d) Create a profile plot across a nucleus. Click the *List* button on the profile window. Copy the values into the clipboard (ctrl+a to select all and ctrl+c to copy). Open the *Curve Fitter* from *Analyze>Tools>Curve Fitting...* Delete the values in the curve fitter and paste the values from the clipboard into it (ctrl+v). Make a gaussian-fit of the measurement-points.

Exercise 2.6: Point selections

You can use the point selection tools to manually count objects in an image. With the point selection tool use shift to add points or use the Multi-point tool. You can delete points using alt.

a) Make a point selection on three nuclei, then make a point somewhere in the background and make point selections on the remaining nuclei. Delete the point on the background using alt-click. What happens to the numeration of the points?

b) Save an image with a point selection somewhere in the format tif (*File>Save As>Tiff...*). Close the image and reload the saved image (*File>Open...*). What happened to the selection? Can you still modify it?
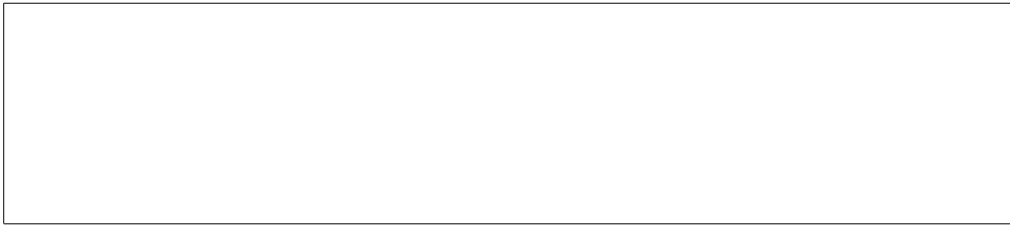
c) Check the option *Auto-Measure* of the point tool. What does it do? Hint: Look at the results table.

d) Can you use the point-selection tool with the *Auto-Measure* and *Auto-Next Slice* options to trace the yellow particle in the time series image *moving-particles.tif* from the folder *02 - basic tools*?

Exercise 2.7: Thresholding and the magic wand tool

The magic wand tool allows to make a selection of connected pixels in an image. There are two different ways to determine which pixels. Either the tool selects all connected pixels with the same intensity value (eventually plus-minus a given tolerance) or it selects all thresholded pixels that are connected, i.e. all pixels with intensities bigger than a given minimum and smaller than a given maximum. The minimum (and maximum) threshold values can be set using the threshold-adjuster. You can open it from *Image>Adjust>Threshold* (shift+t). Switch the display-mode from *Red* to *Over/Under*.

Every click with the wand-tool creates a selection. If you want to remove the selection from an image use *ctrl+shift+a*.

a) Use the wand-tool in combination with the threshold adjuster to select nuclei. Measure the nuclei. How long are the biggest and the smallest perimeters and how long are the biggest and smallest diameters?
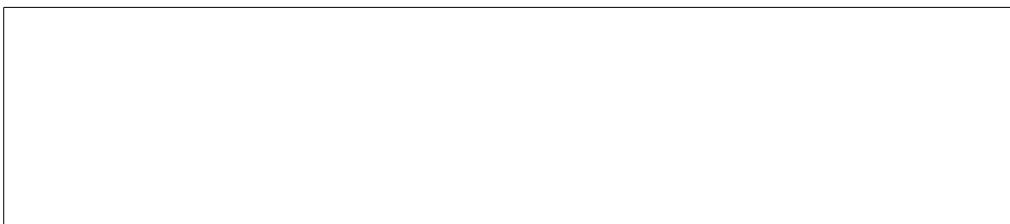
b) Use the wand-tools to select all nuclei as one selection (shift-click). Find out what

percentage of the image area is covered by nuclei. Hint – you can use *ctrl+a* to select the whole image.
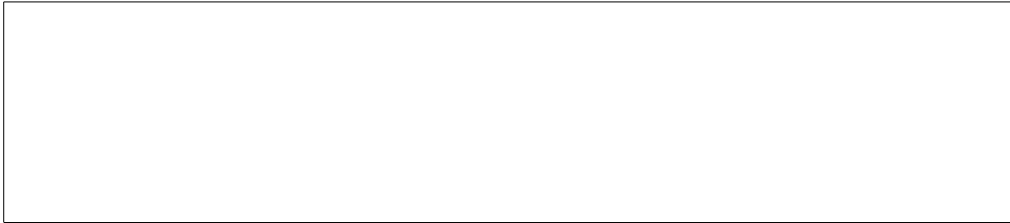
Exercise 2.8: The roi-manager

The roi-manager is a tool that helps to work with selections. You can keep an unlimited number of selections, display, modify and measure them whenever you want. Commands from the roi-manager always work either on the selected rois or on all rois in the manager if none of them is selected.

a) Use the wand-tool to select a nucleus, then press 't' (shortcut for *add to roi-manager,* you find the roi-manager under *Analyze>Tools>ROI Manager....*). Select the *Show All* option on the roi-manager. Select the other nuclei and them to the roi-manager.

b) Click on one of the labels (for example 5) in the image. Note that the corresponding line in the roi-manager is selected. Modify the selection (remove a part using freehand-selection with alt pressed). Press the update button in the roi-manager to make the change permanent.

c) Select one or multiple lines in the roi-manager (you can use ctrl/shift) and press the measure-button. Press the deselect-button, close or clear the results-table and press the measure-button again.

d) What does the flatten button do? Hint – Have a look at the image type of the resulting image.
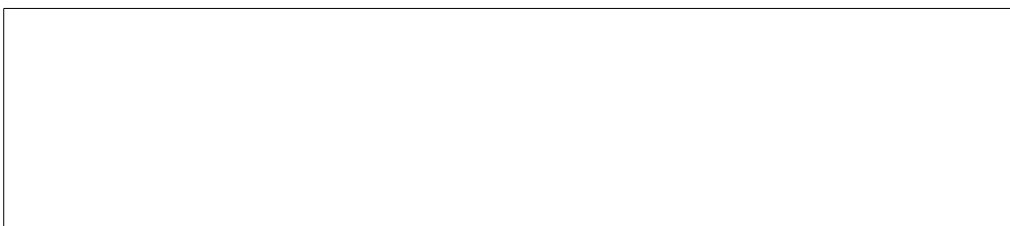
e) Set the lower threshold so that the background becomes blue and there are no holes in the nuclei. You can create a selection from the thresholded values by using *Edit>Selection>Create Selection.* Run the split command from the roi-manager. What happens?
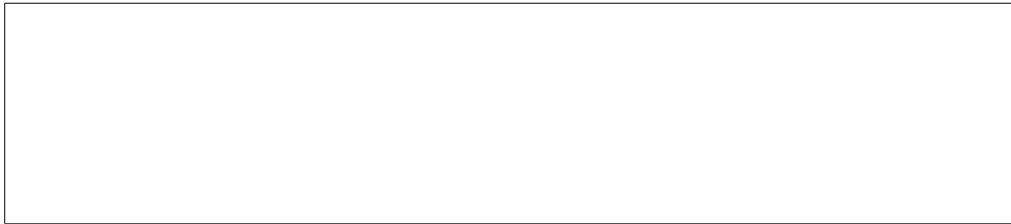

f) Add the selections of all nuclei to the roi-manager. Now open the image *A4 Rhod 1.tif* from the folder *02 - basic tools*. With the newly loaded image active, deselect *Show All* from the roi-manager and select it again. Press the measure button. What happens?
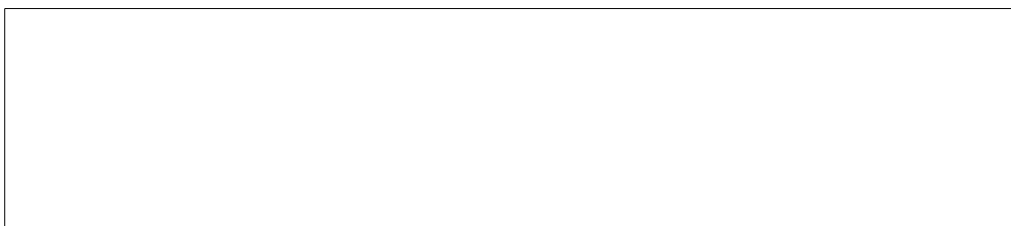
Exercise 2.9: Measuring objects with the Particle Analyzer

Until now all selections have been created individually. The particle-analyzer allows to automatically create a selection for each object (particle), i.e connected region of pixels above the minimum threshold and below the maximum threshold.

a) Use the threshold-adjuster (*Image>Adjust>Threshold* or *shift+t*) and the particle-analyzer to add the nuclei to the roi-manager (deselect everything on the particle-analyzer except *Add to Manager)*.

b) Delete all from the roi-manager and use the particle-analyzer again. This time select the *Exclude on edges* option. Can you explain what this option is good for and when it should be used?
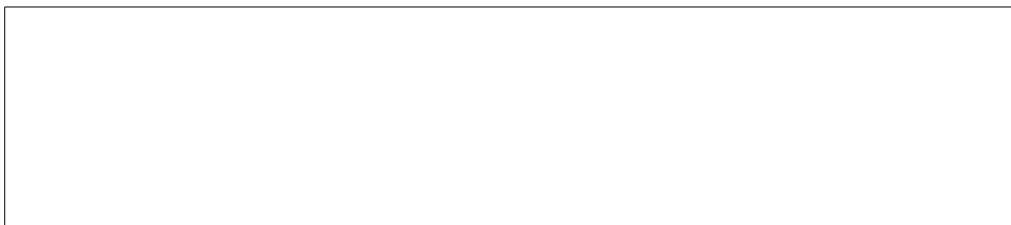
c) Use the particle-analyzer again. This time try to exclude objects that are too small to be nuclei by setting a minimum size in the dialog of the particle-analyzer.

d) Use the particle-analyzer to select only nuclei that have a high circularity value. What is the value that excludes the two nuclei with the lowest ciruclarity?

e) Open the image *inclusion.tif.* Imagine that the inner and the outer white rectangles are particles. Use the particle-analyzer with and without the *Include holes* option. What is the difference?

f) Use the particle-analyzer to find out how many bright blobs there are in the image blobs.gif from the folder *02 - basic tools.* What is the average area of the blobs?

Exercise 2.10: Arrows, annotations and overlays

Images can be annotated in a non-destructive way, using overlays. Arrows and other selection can be added to the overlay by pressing *b* (*Image>Overlay>Add Selection*).

a) Use the arrow tool (right-click on the line-selection tool-button) to create to arrows that point to a nucleus each. Before creating the arrows, use the options of the arrow-tool to set the color of the arrows to magenta.

b) Use the text tool to write an annotation for each arrow (nucleus one and nucleus two). Use *ctrl+alt+b* to add the text to the overlay.

c) Modify the arrows. Hint: Call *Use Image>Overlay>To ROI Manager* first. Don't forget to click the Update-button on the roi-manager after modifying an arrow.

d) Replace the text *nucleus one* by another text (first nucleus). Hint: - You can not modify the text itself. Select the text in the roi-manager, write a new text with the text-tool and press the update-button.

e) Add a third annotation. Hint: Convert the selections back into an overlay, add the new annotation to the overlay.

f) Use *Image>Overlay>Flatten* to create an image containing the annotations that you can copy into your presentation. In this image the annotation is not an overlay or roi, but added by changing pixel values.