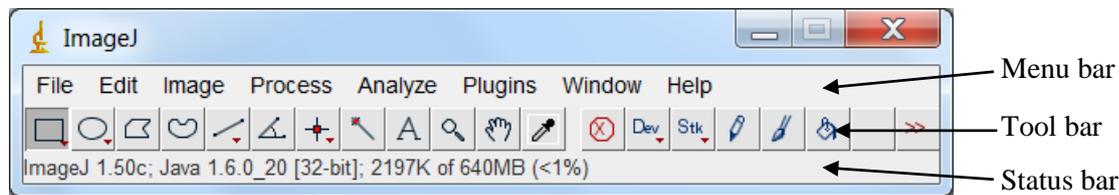


ImageJ

Ver. 1.50c (1.10.2015)



ImageJ (IJ): a public domain software for scientific image processing and analysis written in Java

- Platform independent (Windows, Mac, Linux)
- Developed by Wayne Rasband at NIH
- Web site: <http://imagej.nih.gov/ij/>
- Install version of IJ bundled with the JRE (java runtime environment).
- Run the installer and follow the instructions on the screen.
- For updating, click *Help>Update ImageJ*, select the latest version from the list and rerun IJ.

Help>About ImageJ

Basics

- Select *Plugins>Examples>Scripts>AlwaysOnTop* to keep the IJ main window on top of other running applications.
- Open image **Blobs.tif** by drag&dropping it on IJ window.
- You can always retrieve the saved version of an image by typing **CTRL + r** (or just **r**) (= *File>Revert*).
- You can make a duplicate of an image by typing **SHIFT + d** (= *Image>Duplicate*).
- Menu bar basic tools (12) for working with images
 - Make selection on the image with the rectangular and elliptical selection tools, move/resize the selection; you can control the color of the rectangle/ellipse by selecting *Edit>Options>Colors>Selection*.
 - Try the polygon and freehand selection tools.
 - The line selection tools: straight-, segmented-, freehand line and arrow tool can be used to measure length and angles with the horizontal x-axis and draw arrows.
 - Plot an intensity profile using a line selection tool with the line width = 1 and typing **k** (= *Analyze>Plot Profile*). Try also the Live button.
 - Use the point (or multi-point) selection tool to manually count objects in an image; first click on *Analyze>Tools>ROI Manager* to enable an efficient management of multiple objects.
 - Perform measurements on several objects (nuclei): select the first one using the wand tool¹ ()¹, define which properties to measure using *Analyze>Set Measurements* and type **m** (shortcut for *Analyze>Measure*). Repeat selection and measurement, results will be displayed in the Results table. Note that the selection (outline) of individual nuclei is not perfect.
 - Try the magnifying glass (zooming in/out; keyboard shortcuts: +/-, up/down keys) and scrolling tools.
 - Experiment with the color picker options – specifying foreground- and background color, swapping them, B&W – and their effect on *Edit>Fill/Draw* or *Edit>Clear/Clear*

¹ Set Mode to Legacy and Tolerance to 50 (double-click on the wand tool!)

Outside (note that 8-bit GS images first have to be converted into a color one via *Image>Type>RGB Color*).

- **Macros**
 - Scripts in the ImageJ-macro language that can be used to automate (repetitive) tasks.
 - A collection of macros is available in IJ Plugins subfolders.
 - Much larger number can be accessed from IJ web site: click *Help>Macros*.
 - Drag&Drop **Mandelbrot.txt** macro on IJ window and run it using *Macros>Run Macro* command.
 - A user can record a macro (*Plugins>Macros>Record*), write his own one from scratch (*Plugins>New>Macro*) or modify an existing one (*Plugins>Macros>Edit*).
- **Plugins**
 - Similar to macros but are faster and require knowledge of Java programming language.
 - Can be found in Plugins subfolders or on IJ web site (*Help>Plugins*).
- **Image stacks**
 - Represent volume data (e.g. RGB or n-dimensional images) or time series.
 - Open **t1-head.zip** file by Drag&Dropping.
 - Try the *Image>Stacks* commands *Make Montage*, *Orthogonal Views*, *Z Project* and *3D Project*. For a quicker access to individual commands, "tear them off" after typing **SHIFT + u** (= *Plugins>Utilities>Control Panel*). This option is also useful on other occasions.
 - Also try ImageJ 3D Viewer (from *Plugins>3D*).
- **IJ help**
 - Help for the menu commands: <http://rsb.info.nih.gov/ij/docs/index.html>; the page can also be accessed from within IJ via *Help>Documentation*.
 - The IJ user guide: <http://rsbweb.nih.gov/ij/docs/user-guide.pdf>
 - Further information at the ImageJ Documentation Wiki: <http://imagejdocu.tudor.lu/doku.php>

1 – Digital image

- 2D grid of *width x height* cells – picture elements (pixels). To each pixel, an (integer) value between 0 and a maximum value is associated. These values can be interpreted as intensities: 0 usually means no intensity, yielding a black pixel. Higher values mean lighter pixels and lower values mean darker pixels.
- Open image **Example1.tif**, zoom it in to see individual pixels. Access the image information by typing **i** (= *Image>Show Info*).
- Move the mouse pointer over the image; coordinates and values are displayed in the status bar.
- The coordinate system origin is in the upper left corner of the image with increasing coordinates from left to right and top to bottom.
- Save the image in one of the numerous formats available (*File>Save As*). You can also save it as a text image (*File>Save As>Text Image*) on your hard disk and open it by drag&dropping or with the IJ text editor (*File>Open>Example1.txt*).
- **Spatial sampling**: scale the image with *Image>Scale* selecting X Scale = 0.5 and Y Scale = 0.5. Enlarge – maximize – both images to see the sampling effect.
- **Intensity and binary images**: open intensity (grayscale) image **Oxford.tif**. Type **i** (= *Image>Show Info*) to make sure that the image is indeed a grayscale one and to access its general information. Convert it to a binary one with automatic thresholding: *Process>Binary>Make Binary*. Compare

its histogram – type **h** (= *Analyze>Histogram*) – with that of the grayscale image. Other grayscale→binary image conversion options will be discussed in the later sessions.

- **RGB color images**: open image **Flowers.tif**. It can be considered as consisting of a stack of three planes (channels): red, green and blue. These can be created with *Image>Color>Split Channels*.
- You can convert an RGB color image to a grayscale one using command *Image>Type>8-bit* depending on the following setting:
 - if in *Edit>Options>Conversions* the checkbox "Weighted RGB Conversions" is enabled, the RGB color→grayscale image transformation proceeds according to the following equation: $\text{Intensity} = 0.299 \cdot R + 0.587 \cdot G + 0.114 \cdot B$
 - if the checkbox "Weighted RGB Conversions" is not enabled, the transformation is done according to the following equation: $\text{Intensity} = (R + G + B)/3$
- **Indexed images**: change the display of an intensity image by mapping its values to colors using an appropriate look-up table – LUT.
- Using image **Oxford.tif**, select *Image>Lookup Tables* and then *Spectrum* LUT.
- Indexed image can also be obtained from an RGB image using a color reduction algorithm, such as Median Cut. Open image **Flowers.tif** and select *Image>Type>8-bit Color*. Choose an appropriate number of colors (e.g. 100) to see how the resulting image is of a considerably lower quality in regions with smooth transitions among hues, such as in red flowers.
- To see in a numerical form how each color of the LUT is mixed from the R, G and B components, click on *Image>Color>Show LUT>List*. Experiment with various LUTs.