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# Basic Image Analysis with ImageJ

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# Goals of Imaging

1. Image Processing: overcome limitations in image quality and make the really interesting image content more clearly visible.
2. Image Analysis: compute meaningful measurements



## ImageJ2

The current version of ImageJ focuses on analysis of scientific multidimensional image data. ImageJ2 includes ImageJ1 (see right) with a compatibility layer, so that old-style plugins and macros continue to work.

**For users:** ImageJ2 is a supercharged version of ImageJ1 with new features.

**For developers:** ImageJ2 is an entirely redesigned, more powerful set of software libraries.

## ImageJ1

ImageJ 1.x, sometimes shortened to ImageJ1 or IJ1, is the stable version of ImageJ developed by Wayne Rasband at NIH since 1997.

ImageJ grew organically over time as Wayne Rasband continuously added features according to user requests. Now there are thousands of plugins written by members of a diverse community.



<https://imagej.nih.gov/ij/>

<http://fiji.sc/Fiji>



## Fiji

Fiji is a distribution of ImageJ for the life sciences. It provides a large number of additional plugins to facilitate analysis of life sciences images, particularly microscopy images.

The Fiji distribution is built on the ImageJ2 platform.

Pixel values: measurements of the light being emitted by a sample

## Bit Depth

8 bit –  $2^8$  gray levels: 0 to 255

16 bit –  $2^{16}$  gray levels: 0 to 65535

Color (RGB) – typically 24 bits/pixel (8 bits each for red, green and blue)

## Color

“False” color image: uses a look-up table, LUT (maps intensity to color for display; has nothing to do with the actual imaging data) to assign a display color to each intensity value

## Stages of Image Analysis

1. (Pre)processing, e.g. background subtraction, noise reduction
2. Detection, e.g. thresholding, refinement, ROIs
3. Measurement, e.g. quantify intensities, count pixels to determine areas or volumes

# Image Pre-processing

## *Background correction:*

- Image ▶ Lookup Tables ▶ HiLo LUT
- Relatively even background: Brightness/Contrast > inc Minimum value > Apply
- Uneven background: Process > Subtract background (rolling ball algorithm)  
*The radius should be set to at least the size of the largest object that is not part of the background*

# Image Pre-processing

## *Filters:*

- Gaussian blur: This filter uses convolution with a Gaussian function for smoothing.  
*Sigma (Radius) is the radius of decay to  $\exp(-0.5) \sim 61\%$ , i.e. the standard deviation of the Gaussian*
- FFT
- Median: Reduces noise in the active image by replacing each pixel with the median of the neighboring pixel values.
- Unsharp Mask: Sharpens and enhances edges by subtracting a blurred version of the image (the unsharp mask) from the original.

## Detection: *Segmentation/Thresholding*

- Thresholding: identifies pixels with intensity values in particular ranges
- Result is a binary image  
*binary mask: image with 2 values: 0 and 255*
- Noise is one problem that affects thresholds, especially in live cell imaging



## *Refinement:* Morphological Operations

- Refine or modify shapes of objects in binary images
- fill holes inside objects or make them more compact.
  1. Erosion (minimum filtering)
  2. Dilation (maximum filtering)
  3. Open - erosion followed by a dilation
  4. Close - dilation followed by an erosion
- Watershed transform

# Measuring Images

- By default, measurements are made over the entire area of the currently-selected 2D image slice, and added to a results table

*Important!*

*Size measurements are automatically scaled according to the pixel sizes in Image > Image Properties..., so these must be correct!*

## *Regions of Interest (ROIs)*

- can be used to define specific parts of an image that should be processed independently or measured, and so only pixels within any ROI we draw will be included in the calculations when we run Measure.

## *The ROI Manager*

- The ROI Manager: convenient way to store multiple ROIs in a list, allowing you to easily access, edit and measure them.

Analyze > Tools > ROI Manager....

or draw ROI, press *T*

- The additional Measure command within the manager is then like applying Analyze > Measure to each ROI in turn.
- *Show All* option: show all the ROIs simultaneously

# Measuring objects in binary images

## Generating & Measuring ROIs: 2D image

### *Method 1*

- Analyze > Analyze Particles...detects and measures all the foreground regions as individual objects.
- Make sure to check “Add to Manager”; Measure

### *Method 2*

- Edit > Selection > Create Selection makes a single ROI containing all the foreground pixels
- Disconnected regions can be separated by adding the ROI to the ROI Manager and choosing More >> Split.

## Measuring objects in binary images

### Generating & Measuring ROIs: 3D image

- The Analyze Particles procedure can be applied to 3D but it will label objects in each slice not taking into account the third dimension. Objects that look separated in 2D may be part of the same object in 3D. Hence a true 3D labeling should be performed.
- True 3D labeling: plugin *ObjectCounter3D* will both threshold and label the objects in 3D.
- 3D Segmentation and 3D quantification in 3D Manager

## Best practices for scientific data

- Never change original “raw” data.
- Keep a tag to the original data with any processed image.
- Keep records of steps in any image analysis procedure.
- In preparing images for presentation, resample as little as possible. To create a smaller image, downsample and apply the Unsharp Mask filter.
- To avoid the need for resampling, scan or create the image at high resolution.

