

IMAGEJ

An open platform for scientific image analysis

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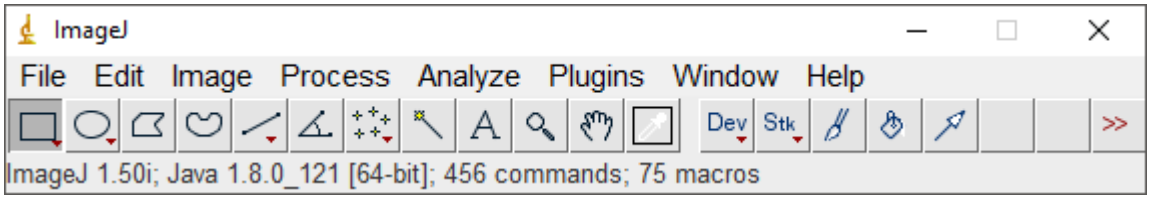
Università di Roma
Tor Vergata

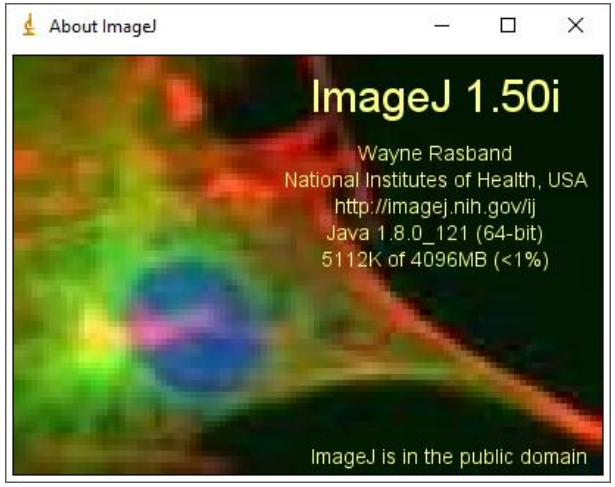
AGENDA

- **Introduction**:
  - What is ImageJ;
  - System of macros & plugins;
  - ImageJ[1] vs. ImageJ[2] *vs.* FIJI.
- **ImageJ**:
  - Toolbar & menubar;
  - Automatizing processing with macros;
  - Image analysis.
- **Integration MATLAB/FIJI**:
  - How to setup;
  - How to launch ImageJ from MATLAB;
  - ~~Sending and~~ receiving data from MATLAB;
  - TrackMate/FIJI: tracking articles;
  - Comments on memory and data types.
- **Questions? Comments?**

INTRODUCTION TO IMAGEJ

- What is **ImageJ**?
  - Platform for image processing;
  - Open source (written in Java language);
  - Huge community involved;
  - Several plugins and macros available on the internet;





INTRODUCTION TO IMAGEJ

- An “ecosystem” of plugins attached…

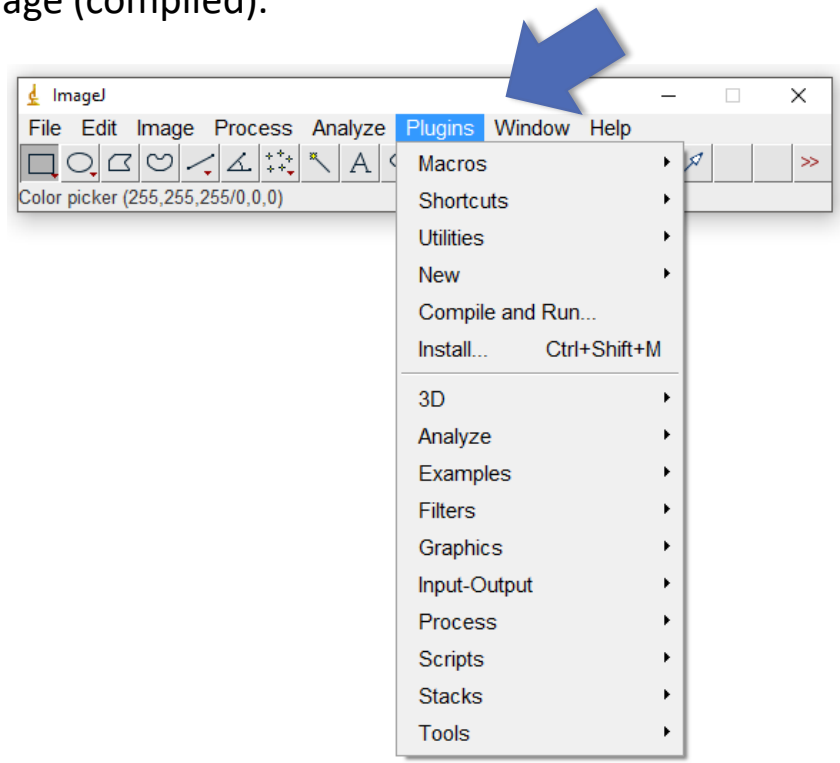




INTRODUCTION TO IMAGEJ

- **Fully customized**:
  - *Macros*:
    - simple scripts created to do same task;
  - *Plugins*:
    - More robust than the macros;
    - Written in Java language (compiled).

- How to create a macro?
- How to create a plugin?



IMAGEJ[1] *VS.* IMAGEJ[2] *VS.* FIJI

- Which are the differences?



ImageJ[1]



ImageJ[2]



FIJI

- Not only about plugins…

- Which one will we use (for now):



  - Easier to use;
  - But still powerful!

IMAGEJ VARIANTS

NATURE METHODS | VOL.9 NO.7 | JULY 2012

NIH Image to ImageJ: 25 years of image analysis

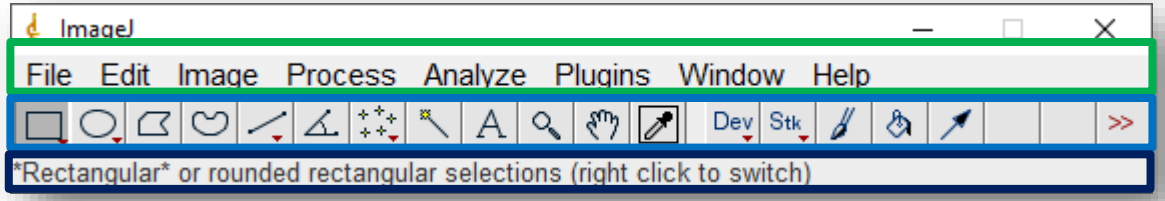
Caroline A Schneider, Wayne S Rasband & Kevin W Eliceiri

Table 1 | List of NIH Image and ImageJ variants

	Date initiated	Description
NIH Image	1987	The predecessor of ImageJ, created by Rasband; made for the Macintosh; no longer under active development
ImageSXM	May 1993	A version of NIH Image for OSX extended by Steve Barrett; intended to handle loading, display and analysis of images from the scanning microscope
ImageJ	1997	The current version of ImageJ developed by Rasband; sometimes called ImageJ1
ImageJ2x	Unknown	An offshoot of ImageJ; modified to use Swing interface; no longer under active development
ImageJA	July 2005	An offshoot of ImageJ developed by Johannes Schindelin; used as the core of Fiji
Fiji	December 2007	A 'batteries included' distribution of ImageJ popular in the life sciences
ImageJX	March 2009	Created by Grant Harris to discuss improvements to ImageJ; formed the basis of an application to NIH that launched ImageJDev
ImageJ2 (ImageJDev)	December 2009	Under development by the ImageJDev project; a complete rewrite of ImageJ; includes ImageJ1 to allow for old-style plug-ins and macros
MBF_ImageJ	2005	Bundle developed by Tony Collins for light microscopists; plug-ins from MBF_ImageJ can be installed on Fiji, combining the programs
SalsaJ	Unknown	An offshoot of ImageJ intended for astronomy applications; designed for use in classrooms; available in over 30 languages
CellProfiler	2006	Free, open-source software started by Anne Carpenter and Thouis Jones; aids biologists without computer-vision training to quantitatively measure cell images automatically
Icy	2011	Created by the Quantitative Image Analysis Unit at Institut Pasteur, Icy provides integrated software to bridge the gap between users and developers through open-source software and a central website
Bio7	Unknown	Application used for ecological modeling; integrated development environment; focuses on individual-based modeling and spatially explicit models
μManager	2005	Open-source microscopy software; controls automated microscopes; comprehensive imaging solution when used with ImageJ; developed by Arthur Edelstein, Ziah Dean, Henry Pinkard and Nico Stuurman

IMAGEJ – GRAPHICAL USER INTERFACE (GUI)

- The simplest GUI (Graphical User Interface):



- Menubar:



- Toolbar:

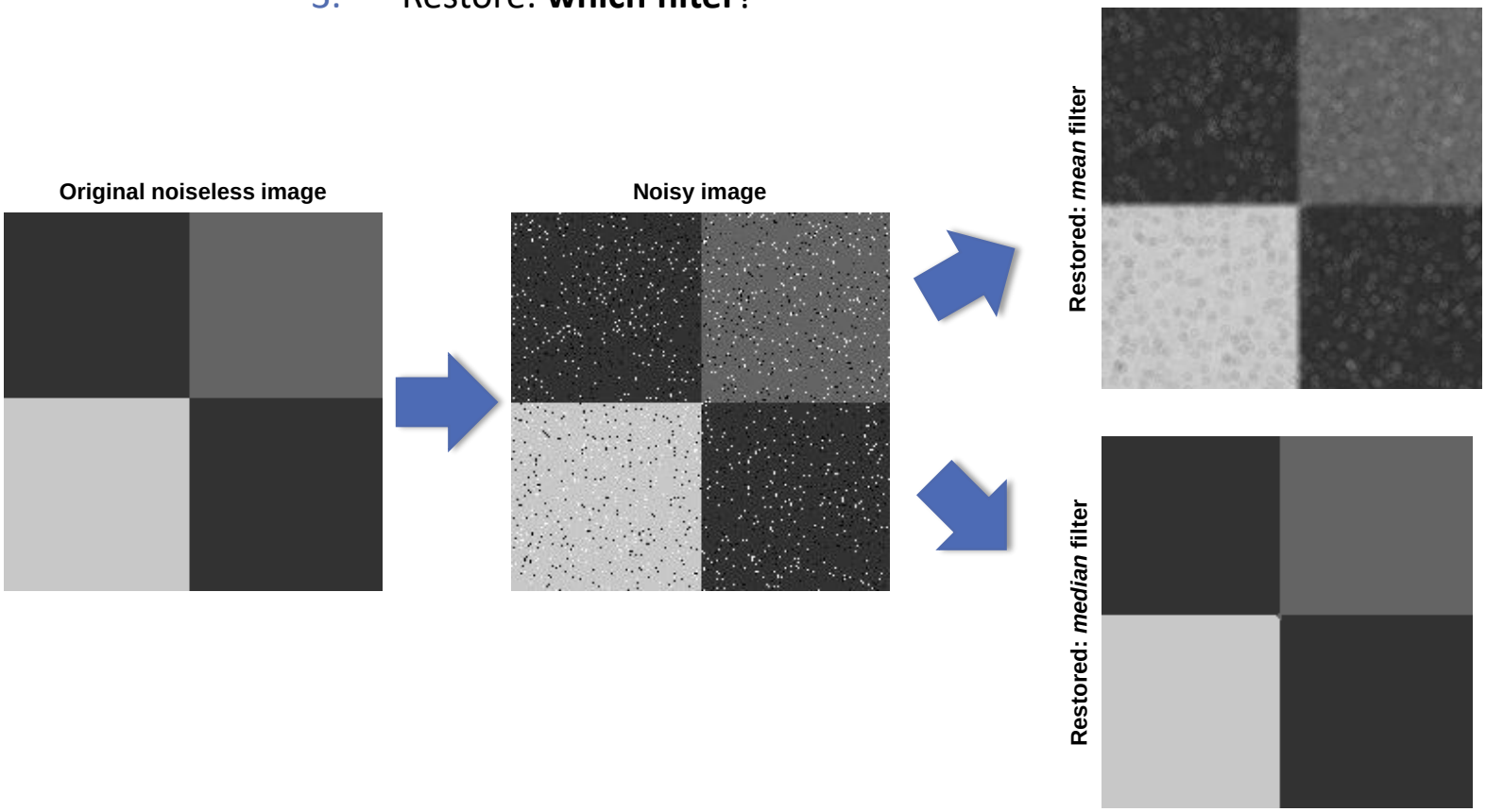


- Statusbar:



IMAGEJ – IMAGE PROCESSING

- **Example #1**:
  - Goal: Load image, apply noise and restore it:
    1. Load the sample image (`chess.bmp`);
    2. Apply "Salt & Pepper" noise (*Process > Noise > Salt and Pepper*);
    3. Restore: **which filter**?



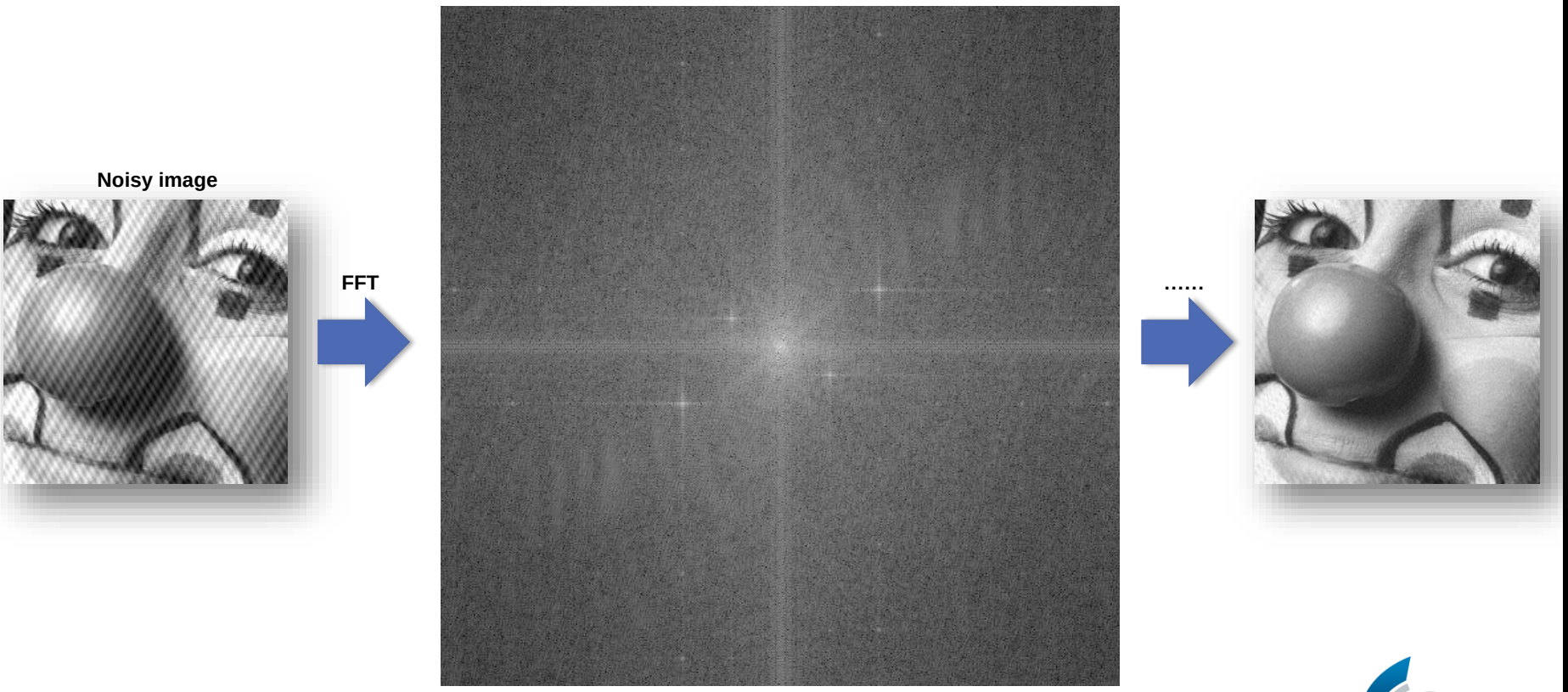
IMAGEJ – IMAGE PROCESSING

- **Example #2**:
  - Goal: restore an image with periodic noise:
    1. Load the sample image (`clown.bmp`):



IMAGEJ – IMAGE PROCESSING

- **Example #2**:
  - Periodic noise → Fourier transform!



IMAGEJ - MACROS

- What are macros?
  - What do they do?
- Creating the first macro:
  - *Plugins > Macro > Record…*

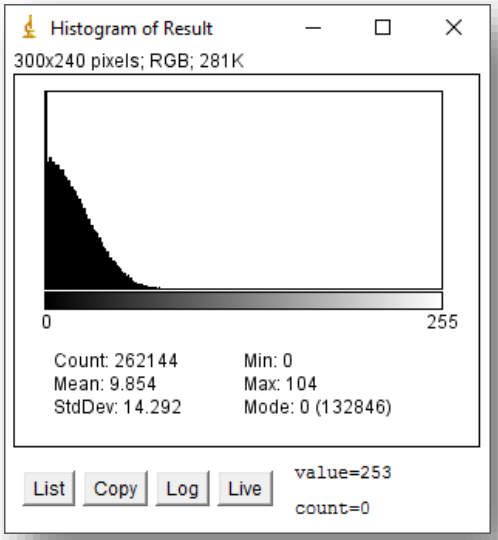


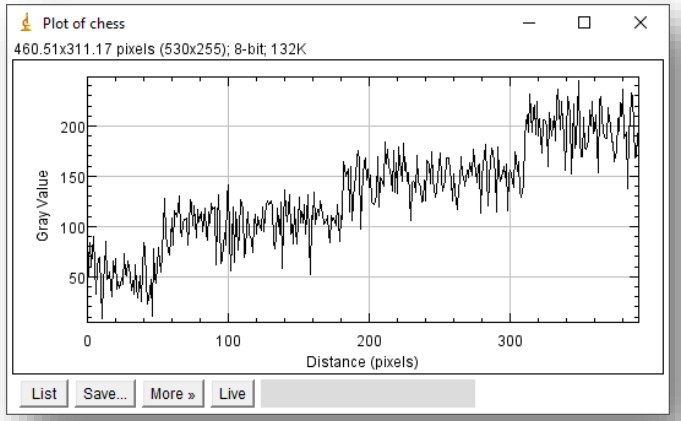
  - Do whatever you want and click on *Create*;

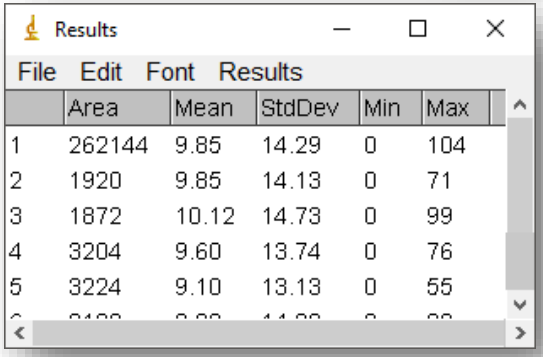
- **Example #3**: Automatizing the process of **Example #2**.

IMAGEJ – IMAGE ANALYSIS

- Histogram;
- Radiometric profile;
- Measurements: mean, max, min, standard deviation, …

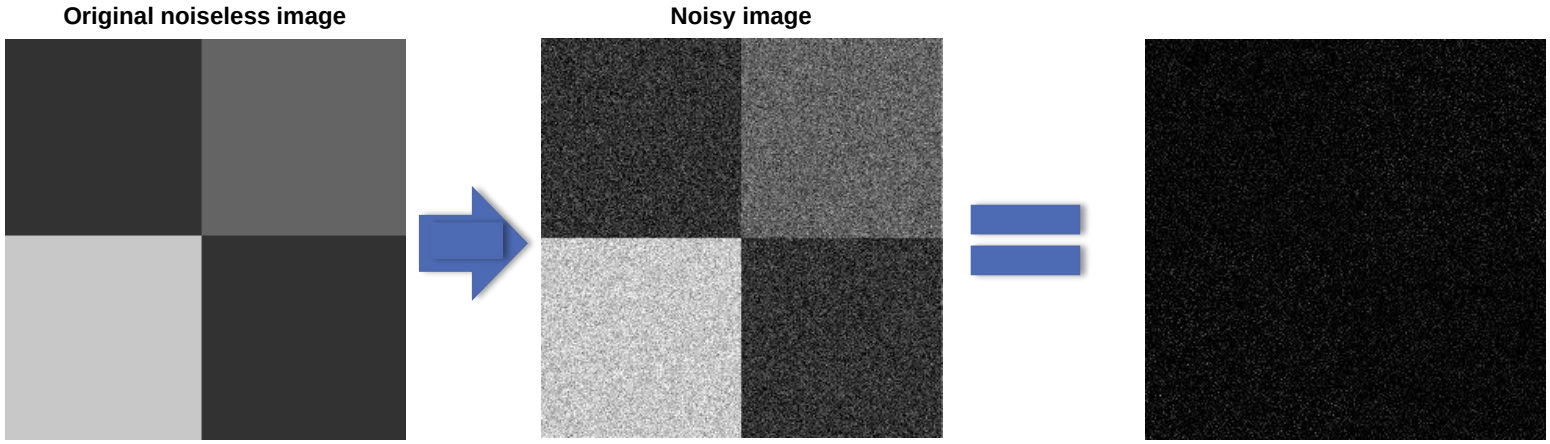






IMAGEJ – IMAGE ANALYSIS

- **Example #4**:
  - Goal: Identify noise;
  - *Process > Noise > Add noise*;
  - Subtract original original image from noisy one;
  - Analyze the result (noise):
    - Variance, histograms…



IMAGEJ & MATLAB - SETUP

- Preliminary observations:
  - You can use either ImageJ or FIJI;
    - We will use Fiji;
  - Pay attention to the required Java version;
  - MATLAB and FIJI **have to** run under the same (version) *Java Virtual Machine* (JVM):

MATLAB
v2017a


v1.51n

JVM
v1.7... → JAVA 7

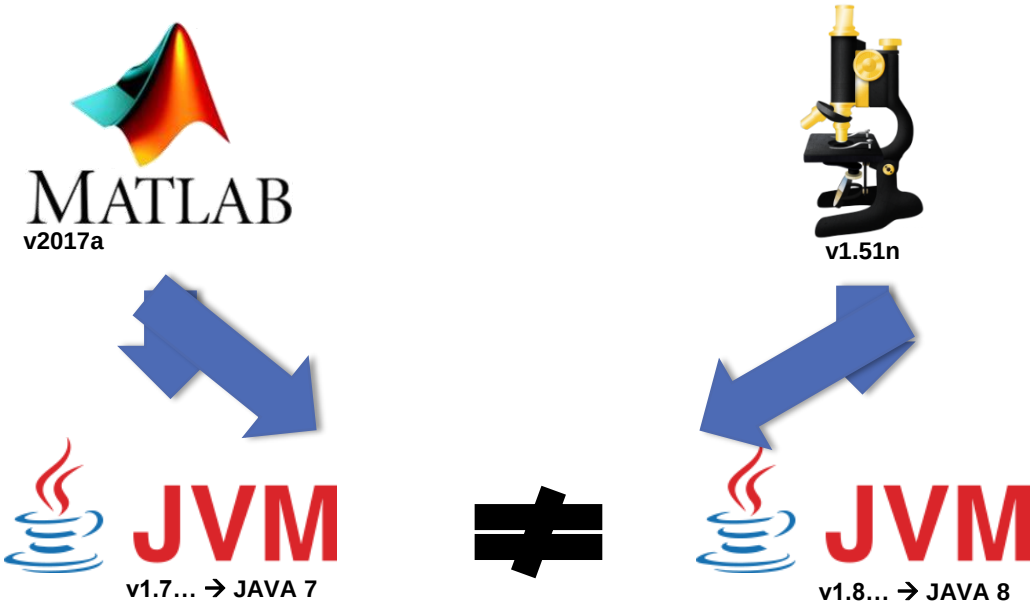
≠

JVM
v1.8... → JAVA 8

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IMAGEJ & MATLAB - SETUP

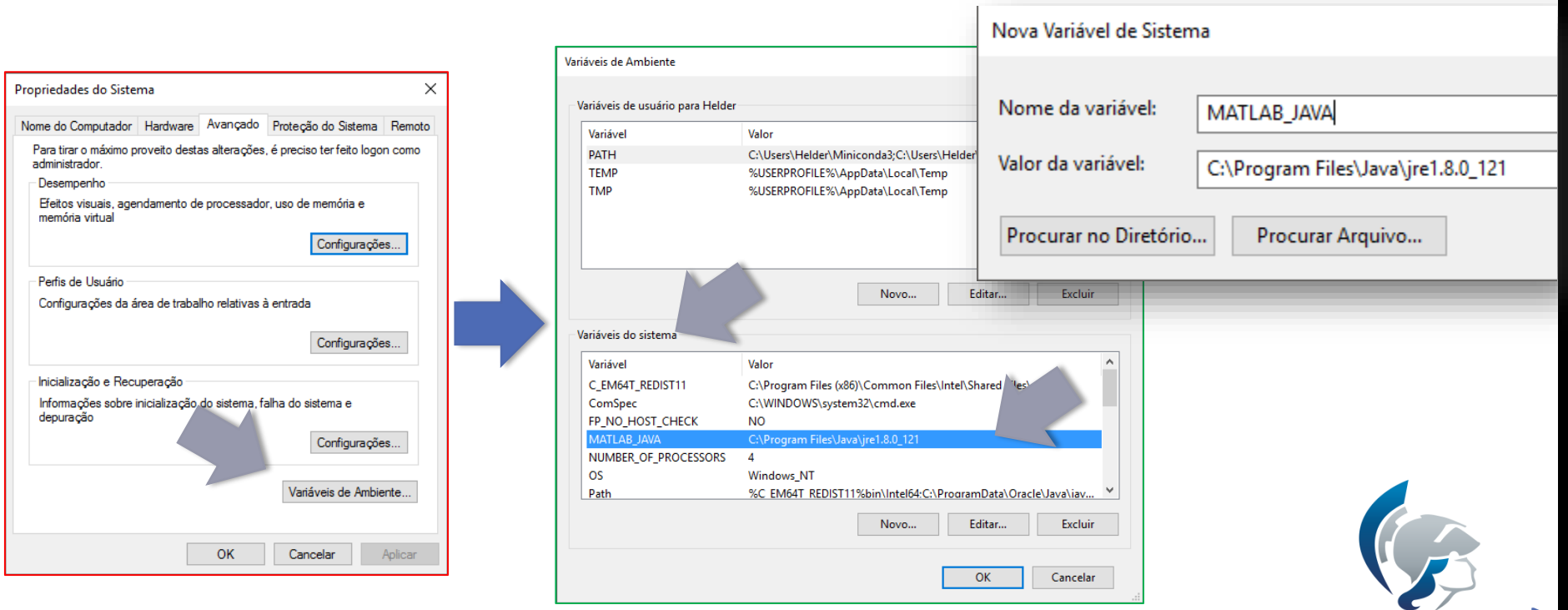
- How to check which version of JVM is being used?
  - MATLAB: `>> version -java`
  - FIJI: *Help > About ImageJ…*
- Changing the JVM used:
  - TIP: download install the latest one from Oracle's webpage;



  - This process doesn't work with MATLAB 2016{a,b}

IMAGEJ & MATLAB - SETUP

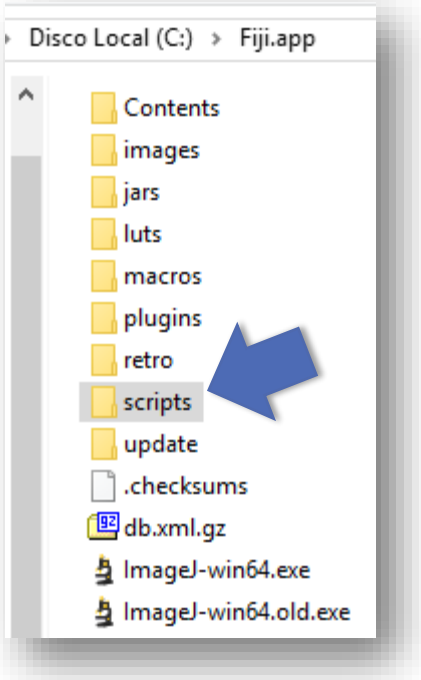
- Changing the JVM used on Windows:
  - Just set a new *Environment Variable* (MATLAB_JAVA) which will points to the desired JVM;
  - Control Panel > System > Advanced System Settings > System Variables:
    - Click on “New..” and set the variable MATLAB_JAVA...

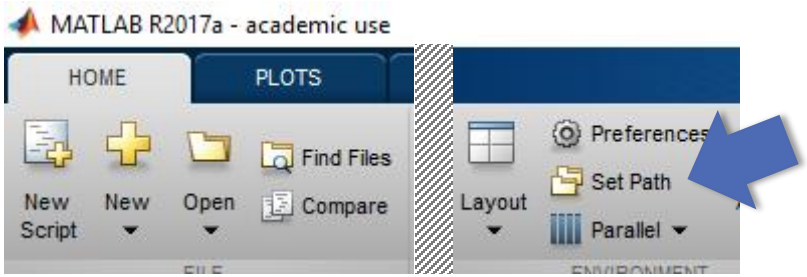


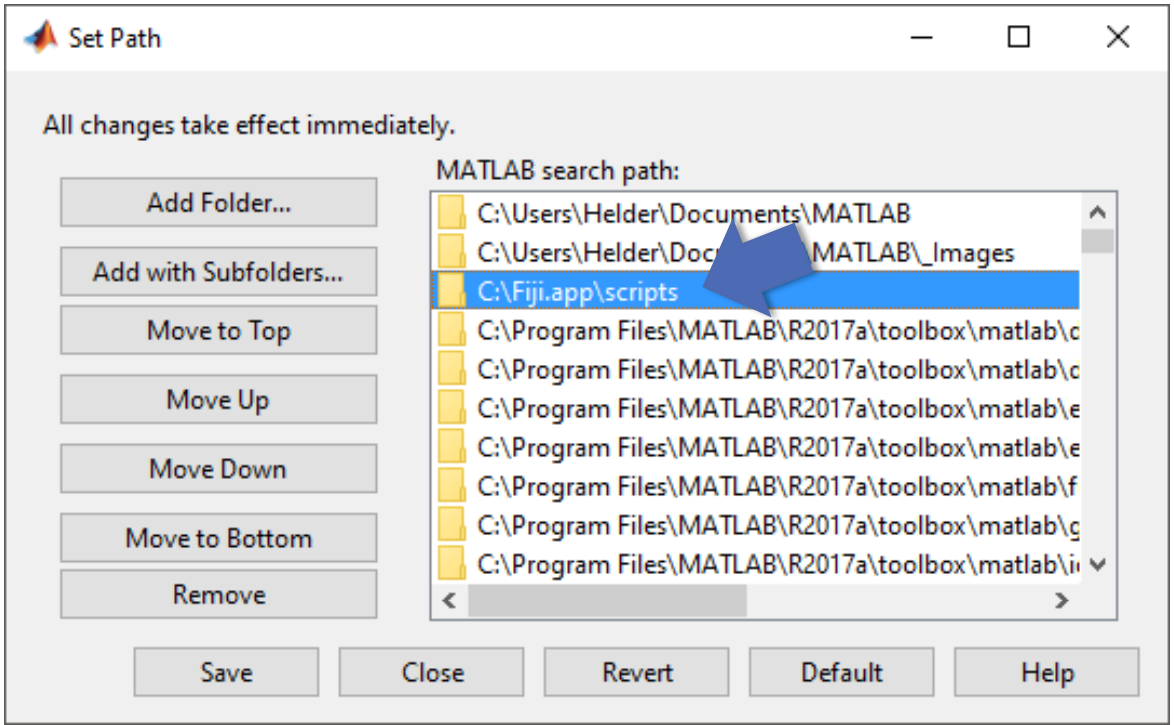
IMAGEJ & MATLAB - SETUP

- Add "*Scripts*" folder to the MATLAB's *path*:

Fiji's installation folder:







IMAGEJ/MATLAB - EXAMPLE #5

- Goal: Perform simple image processing on ImageJ from MATLAB;
- The *methods* and *classes* available are listed on ImageJ documentation[1];
- Preliminaries:

```
% starting ImageJ
ImageJ();
% importing ImageJ Java class
import ij.*


%% Test 01: loading image
im = ij.ImagePlus('../../_Images/Cameraman256.png');
im.show();
```

- Histogram:

```
%% Test 02: Getting and Visualizing the Histogram
im_processor = im.getProcessor();
imhist = im_processor.getHistogram();
bar(imhist);
```

[1] ImageJ documentation: http://javadoc.imagej.net/

IMAGEJ/MATLAB - EXAMPLE #5

- Thresholding:

```
%% Test03: Performing thresholding with IsoData [1] method
% [1] http://imagej.net/Auto_Threshold

im2 = im.clone();
im2_processor = im2.getProcessor();
fprintf('\nThreshold: %.2f\n', im2_processor.getAutoThreshold());

% applying threshold
im2_processor.autoThreshold();
im2.setProcessor(im2_processor);
im2.show();
```

- Filtering:

```
%% Test 04: Gaussian blurring
im3 = im.clone();

im3_processor = im3.getProcessor();
im3_processor.blurGaussian(2);

im3.setProcessor(im2_processor);
im3.show();
```

IMAGEJ/MATLAB - SENDING AND RECEIVING DATA

- ~~Sending and~~ receiving data:

```
% starting ImageJ
ImageJ();

% importing ImageJ Java class
import ij.*

fn = '../../../_Images/Cameraman256.png';

%% Test 01: loading image
im = ij.ImagePlus(fn);
im.show();



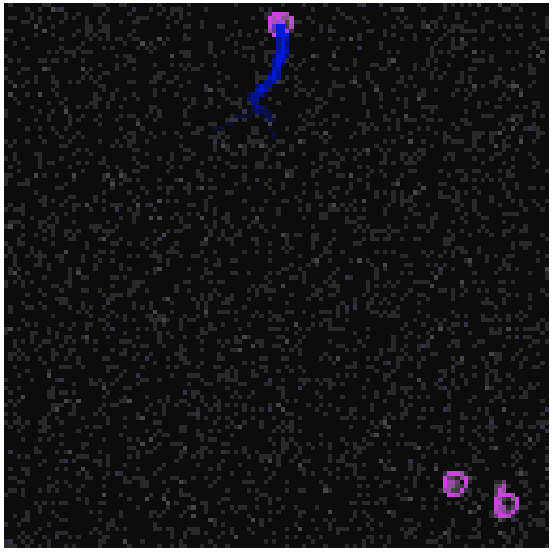
%% Getting the ImageProcessor
im_processor = im.getProcessor();

%% Converting the image…
im_array = uint8(im_processor.getFloatArray());

%% it must be flipped and rotated
im_conv = fliplr(rot90(farray,-1));
figure, imshow(im_conv);
```

TRACKING PARTICLES WITH TRACKMATE/FIJI

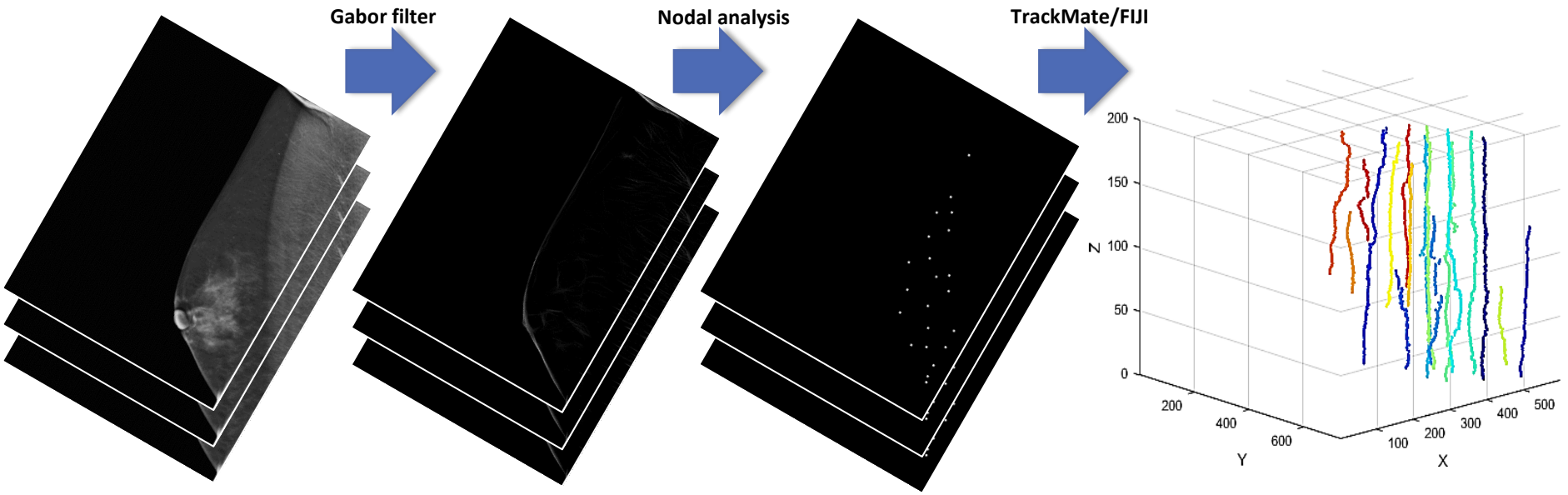
- TrackMate:
  - FIJI's plugin (shipped with it);
  - "*TrackMate provides the tools to perform single particle tracking (SPT). SPT is an image analysis challenge where the goal is to segment and follow over time some labelled, spot-like structures.*"[1]



[1] TackMate web page: http://imagej.net/TrackMate

TRACKMATE IN MAMMOGRAPHY IMAGES

- Goal of the project: identify Architectural Distortion in **DBT** images;
- DBT stands for **Digital Breast Tomosynthesis**;
- DBT is an evolution of digital mammography;
- The pipeline established by us is:



FINAL COMMENTS

- ImageJ is a powerful tool to use with all images:
  - Specially medical/biomedical images;

- The integration of ImageJ & MATLAB is very simple (in theory) but:
  - MATLAB is a proprietary software;
  - ImageJ is not well documented:
    - It is still an working in progress…

- Memory can be your nightmare!
  - Pay attention to loading unloading data;
  - `pack`, command from MATLAB, couldn't give the expected result;
  - ImageJ, MATLAB, Java Virtual Machine…



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