



***Integrity ★ Service ★ Excellence***

# ***Tutorial*** **FIJI (FIJI is ImageJ)**

**Michael Uchic**  
**AFRL/RXCM**



# Outline



- Introduction
- Overview of FIJI/ImageJ Functionality
  - Emphasis on filters and techniques useful for serial sectioning data post-processing
- Examples
- Q & A



# Introduction



- FIJI (and ImageJ) = Image processing package
  - GUI enables quick prototyping
  - FIJI has lots of plug-ins to assist with 3D data analysis
- **Open source** (GNU) – built off of ImageJ2 (BSD)
  - Java
  - Actively developed
- Close collaboration with other SciJava/open microscopy tools



ImageJ2



Bio-Formats



OME



µManager



KNIME



# Reasons to consider using FIJI/ImageJ



- Core code is robust; comparatively fast execution
- FIJI has very effective filter suite for data post-processing, esp. for common 3D tasks
  - Extensive suite of scripts/plugin developed by scientific community
- Macro language enables rapid construction of automation workflows
- Wide proliferation enables sharing/verification of workflow
- Open source enables insight into algorithm construction



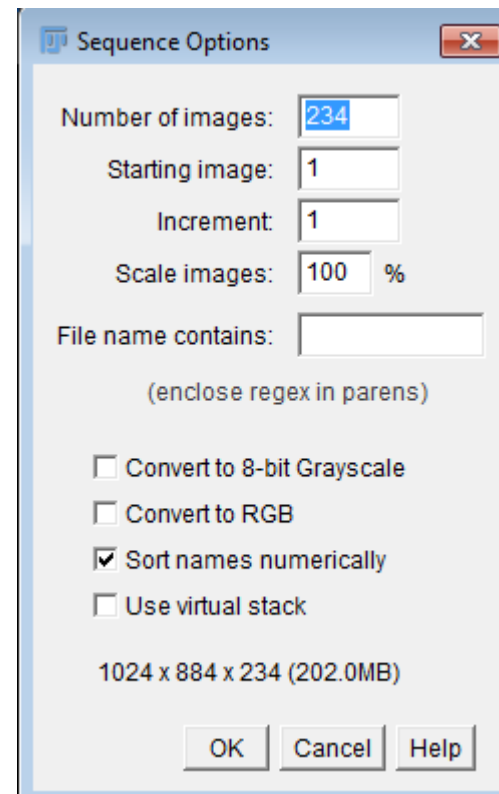
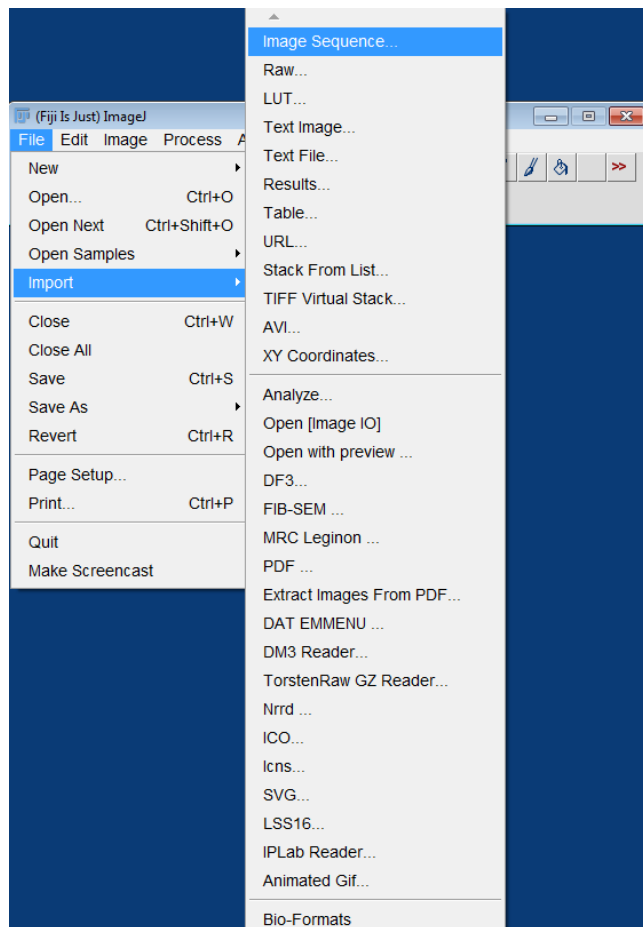
# Common Operations for 3D Data



- Importing Image Stacks
- Crop/Resizing
- Image Restoration
  - 3D Kernel Operations
- Histogram Adjustment
- Applying Threshold
- Interactive Stack and 3D Viewing



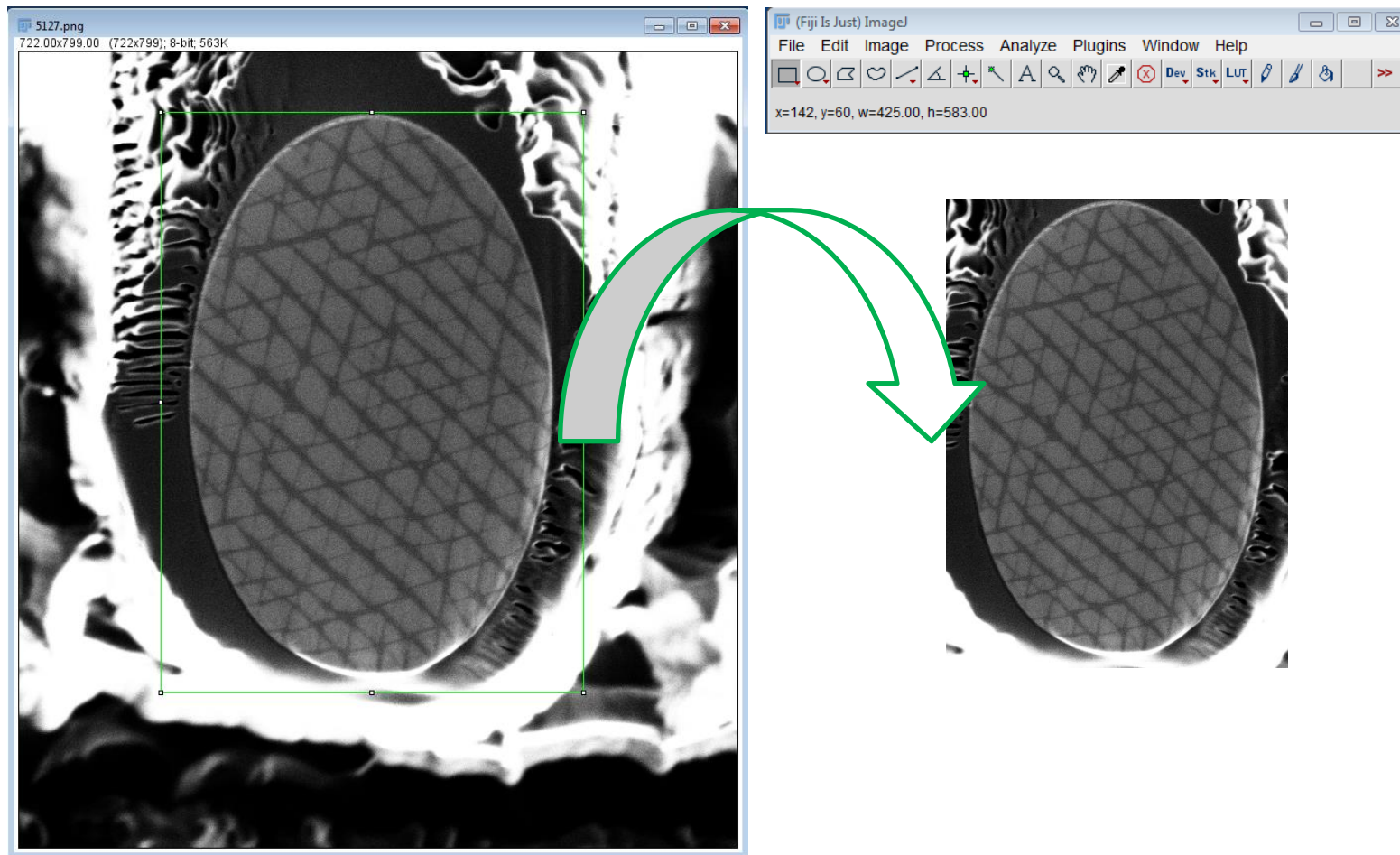
# Importing Image Stacks



Helpful to have numeric ordering of image stack



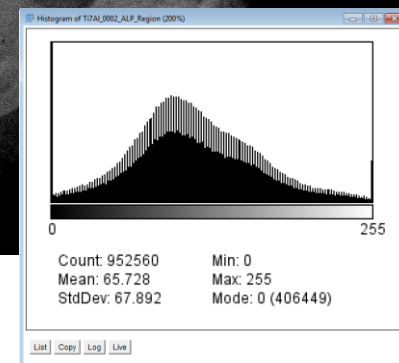
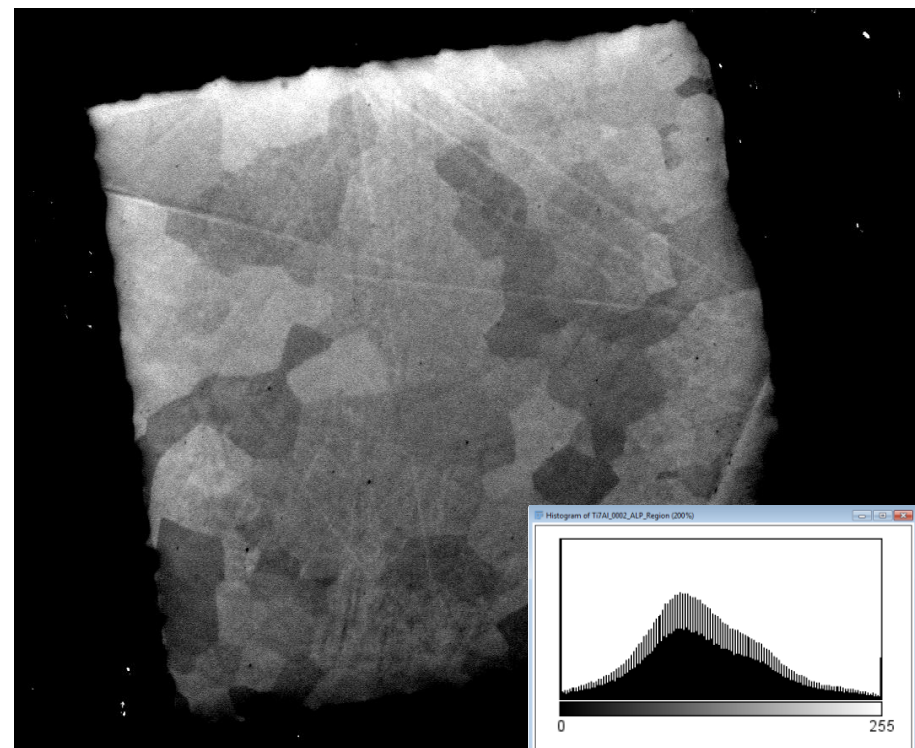
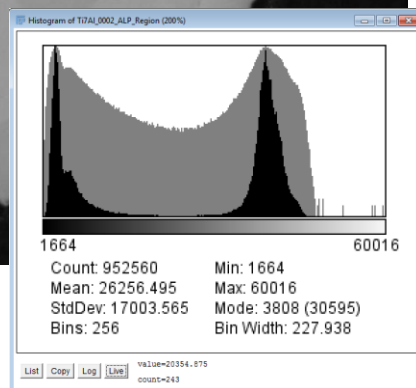
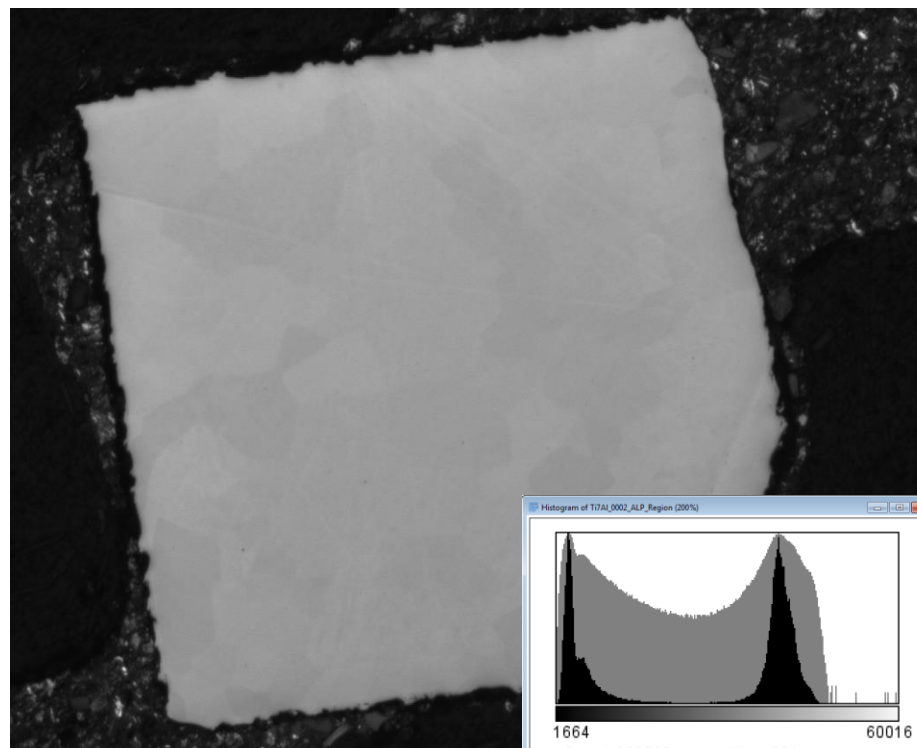
# Crop/Resizing



Works on stacks as well as individual images



# Histogram Adjustment

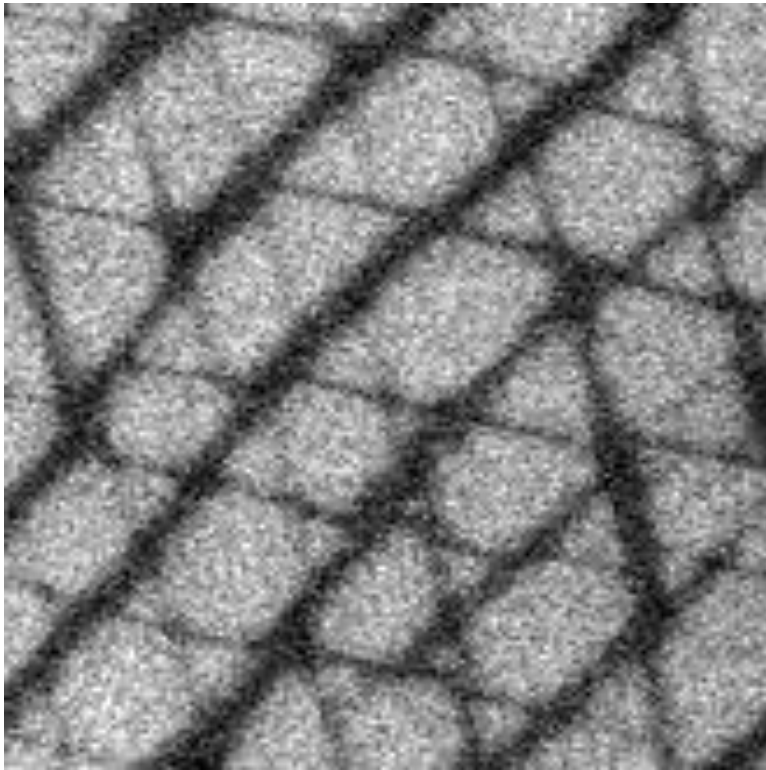


Works on stacks as well as individual images

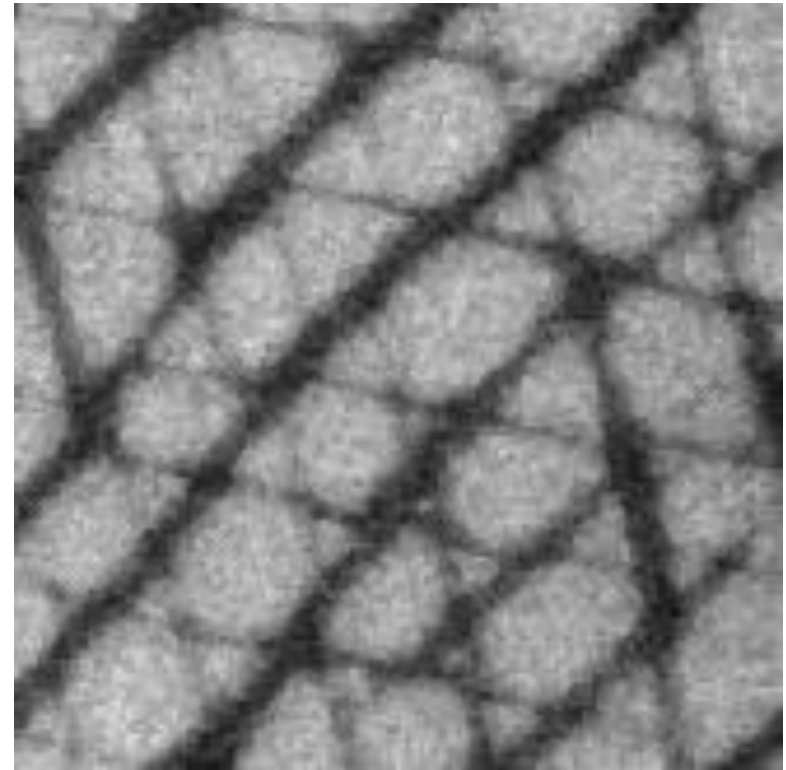




# Image Restoration



Original Image

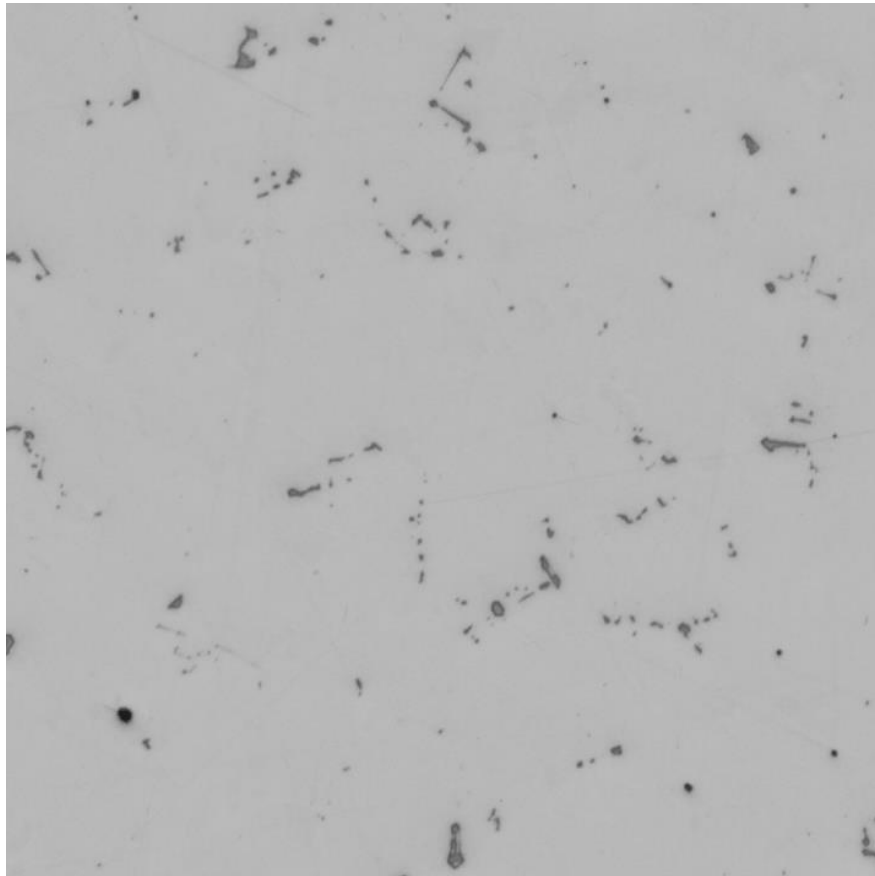


3D Median (3 x 3 x 3 pixel kernel)

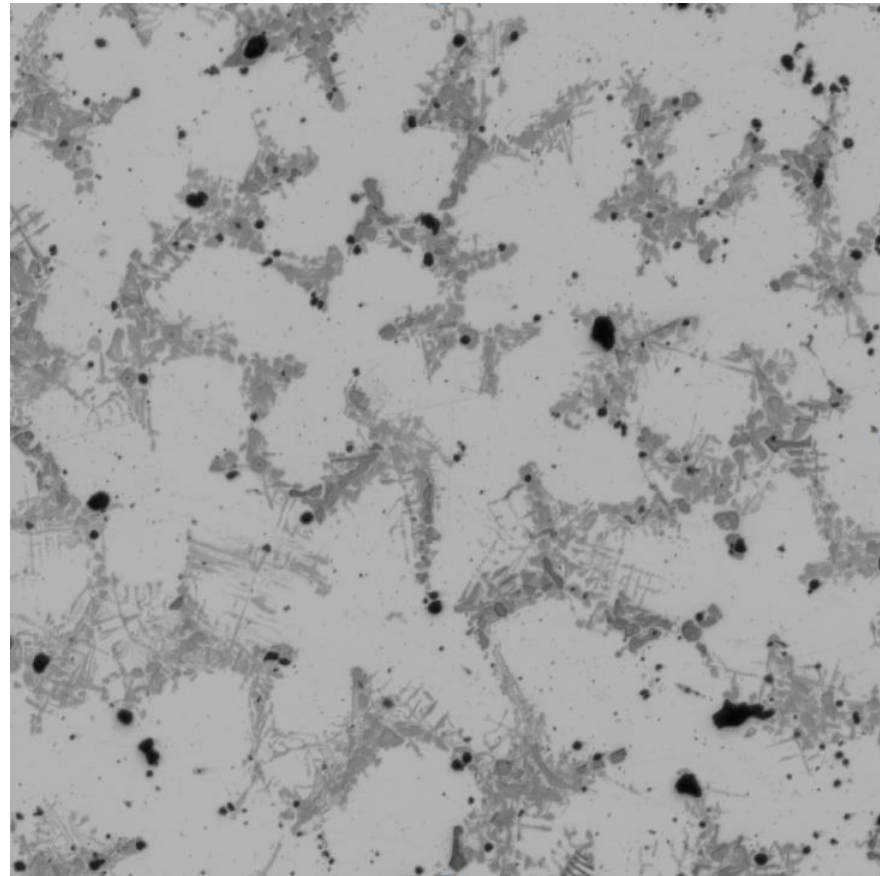
3D kernel operations can be readily performed



# Z-stack projection



Single image from sectioning experiment



MIN Z-projection thru 300 sections

Effective for feature enhancement, removing some artifacts



# Image Stitching

[http://fiji.sc/Grid/Collection\\_stitching](http://fiji.sc/Grid/Collection_stitching)



- Reconstruct big image stacks—arbitrary spacing
- Pair-wise registration to sub-pixel resolution (FFT)
- Global optimization scheme to minimize propagating alignment errors

$$\arg \min_{T_{VF}} \sum_{A \in V \setminus \{F\}} \left( \sum_{B \in V \setminus \{A\}} \|\vec{t}_{BF} - \vec{t}_{AF} - \vec{p}_{AB}\|^2 \right)$$

- Fusing options (blending); new tile coordinates also output
- S. Preibisch, S. Saalfeld, P. Tomancak (2009) "Globally optimal stitching of tiled 3D microscopic image acquisitions", *Bioinformatics*, **25**(11):1463-1465.



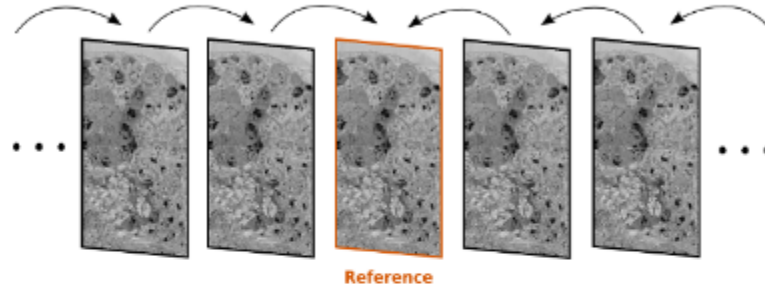
# Stack Registration

[http://fiji.sc/Register\\_Virtual\\_Stack\\_Slices](http://fiji.sc/Register_Virtual_Stack_Slices)



## Register Virtual Stack Slices

QR for this page



This plugin takes a sequence of image slices stored in a folder, and delivers a list of registered image slices (with enlarged canvas). One of the images in the sequence can be selected by the user as reference and it will remain intact.

The plugin can perform 6 types of image registration techniques:

- Translation
- Rigid (translation + rotation)
- Similarity (translation + rotation + isotropic scaling)
- Affine
- Elastic (via [bUnwarpJ](#) with cubic B-splines)
- Moving least squares

All models are aided by automatically extracted [SIFT features](#).

### Register Virtual Stack Slices (Fiji)

Author	Albert Cardona ( <a href="mailto:acardona@ini.phys.ethz.ch">acardona@ini.phys.ethz.ch</a> ), Ignacio Arganda-Carreras and Stephan Saalfeld
Maintainer	Ignacio Arganda-Carreras ( <a href="mailto:iarganda@mit.edu">iarganda@mit.edu</a> )
Source	<a href="#">on GitHub</a>
Initial release	2008
Latest version	2.0.0, September 17 <sup>th</sup> , 2014
Development status	stable, active
Category	Registration

Use of SIFT (keypoint detection): Scale & Rotation Invariant Matching



# Macro

[http://fiji.sc/Introduction\\_into\\_Macro\\_Programming](http://fiji.sc/Introduction_into_Macro_Programming)



## Why Macros?

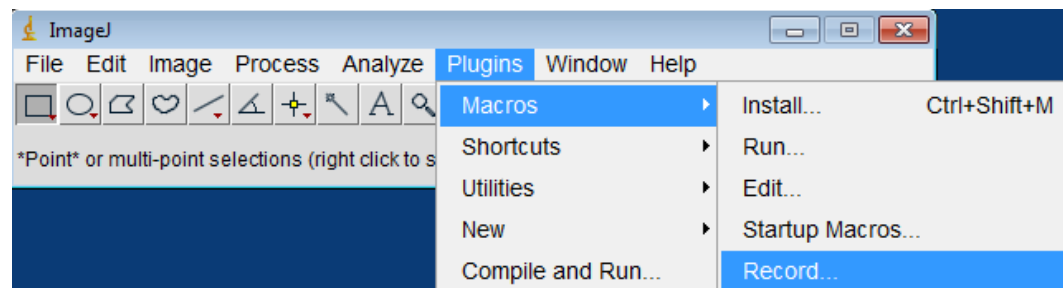
Macros can be used to

- automate repetitive tasks
- document what you did
- share common procedures
- add tools to the toolbar
- add keyboard shortcuts



Please be aware that there are several other available [scripting](#) languages that are more powerful than macros, too! See the sidebar on the right.

- Record/Edit/Run options simplify macro construction





# Macro

[http://fiji.sc/Introduction\\_into\\_Macro\\_Programming](http://fiji.sc/Introduction_into_Macro_Programming)



- Example of Macro for Stitching Serial Sectioning Data

```
1 data_path="C:\\Users\\"
2 for (i=0;i<101;z++) {
3     open(data_path+"Original_Data\\"+i+".tif");
4     run("Canvas Size...", "width=1000 height=1000 position=Center zero");
5     saveAs("Tiff", data_path+"Cropped_Data\\"+i+".tif");
6     close();
7 }
8
```



# Citing SciJava Projects

## <http://imagej.net/Citing>



Project	Citation	Google Scholar
ImageJ	Schneider, C. A.; Rasband, W. S. & Eliceiri, K. W. (2012), " <a href="#">NIH Image to ImageJ: 25 years of image analysis</a> ", <i>Nature methods</i> <b>9(7)</b> : 671-675, <a href="#">PMID 22930834</a> .	<a href="#">on Google Scholar</a>
Fiji	Schindelin, J.; Arganda-Carreras, I. & Frise, E. et al. (2012), " <a href="#">Fiji: an open-source platform for biological-image analysis</a> ", <i>Nature methods</i> <b>9(7)</b> : 676-682, <a href="#">PMID 22743772</a> .	<a href="#">on Google Scholar</a>
ImgLib2	Pietzsch, T.; Preibisch, S. & Tomancak, P. et al. (2012), " <a href="#">ImgLib2—generic image processing in Java</a> ", <i>Bioinformatics</i> <b>28(22)</b> : 3009-3011, <a href="#">PMID 22962343</a> .	<a href="#">on Google Scholar</a>
ImageJ2	Rueden, C., Schindelin, J., Hiner, M., DeZonia, B., Kamentsky, L. & Eliceiri, K. (2015). ImageJ2 [Software]. <a href="http://imagej.net/">http://imagej.net/</a>	-
SciJava Common	Rueden, C., Schindelin, J., Hiner, M. & Eliceiri, K. (2015). SciJava Common [Software]. <a href="http://scijava.org/">http://scijava.org/</a>	-
SCIFIO	Hiner, M., Rueden, C. & Eliceiri, K. (2015). SCIFIO [Software]. <a href="http://scif.io/">http://scif.io/</a>	-
ImageJ OPS	Rueden, C., Dietz, C., Horn, M., Schindelin, J., Northan, B., Berthold, M. & Eliceiri, K. (2015). ImageJ OPS [Software]. <a href="http://imagej.net/OPS">http://imagej.net/OPS</a>	-
Other	Browse the <a href="#">list of citable software</a> and <a href="#">publications using Fiji</a> for additional citation information.	-





# Helpful Web Resources



- FIJI  
<http://fiji.sc/Category:Tutorials>
- ImageJ Documentation Page  
<http://rsbweb.nih.gov/ij/docs/index.html>
- University of Chicago Integrated Microscopy Core ImageJ Site (C. Labno)  
<https://digital.bsd.uchicago.edu/ImagejandFijiHelp.html>





# Summary



- Open-source, robust software for image processing
- Plug-ins very good for key 3D tasks (stitching, alignment)
- Macro language simplifies task automation