VSI Reader ActionBar Installation & Use Cases

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BIOP, EPFL

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Summary

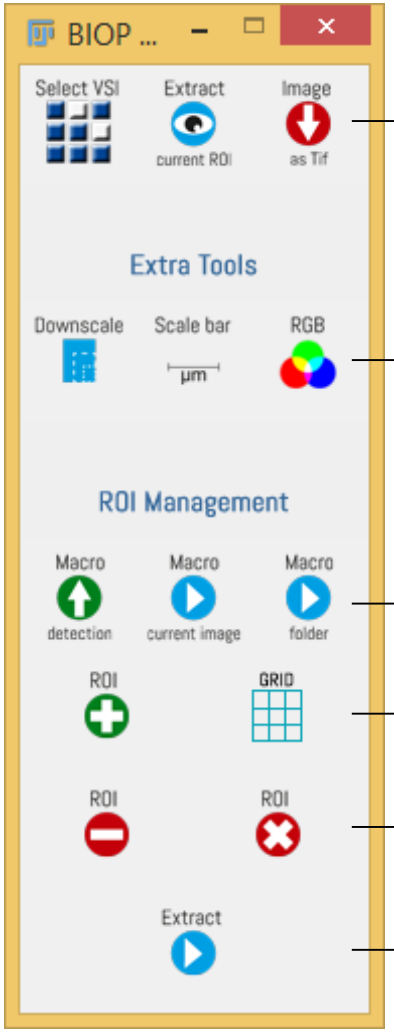
- Installation
- Opening an Image
- Extracting ROIs Manually
- Defining Multiple ROIs
  - Manually
  - Automatically
  - As a Grid
- Batch Extract ROIs

Description

Fast tiling microscopes are becoming commonplace but often rely on closed-source and closed-format systems to access the data.

This was the case with the Olympus Virtual Slide Scanner microscope. We needed a way to open and process the large images (>100k pixels) using the software in Fiji.

The result is a simple ActionBar that simplifies the most common tasks required when opening and dealing with these large datasets: ROI management and batch-processing.



Basic Functions
Open VSI, Extract ROI & Save As Tiff

Extra Tools
Downscale, Add Scalebar & Convert to RGB

ROI Tools (External Macros)
Load & Apply custom ROI detection macros

ROI Management 1
Add Manually Generated ROI & Create Grid based on ROI

Roi Management 2
Delete Last ROI & Clear All ROIs

Launch Extraction of ROIs

Note:
You will need Jerome Mutterer's excellent ActionBar Plugin if you're going to use the VSI Reader.

Link: http://imagejdocu.tudor.lu/doku.php?id=plugin:utilities:action_bar:start

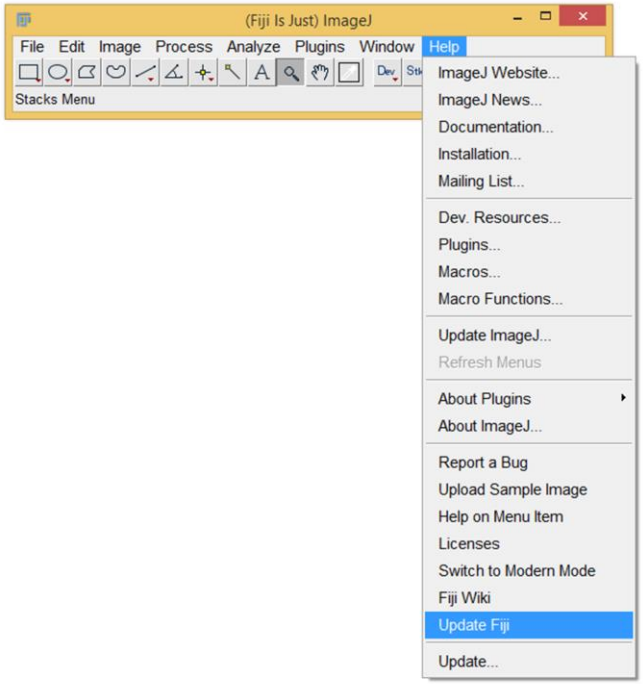
Install VSI Reader ActionBar

You can install the VSI Reader via our Fiji Update Site (PTBIOP).

Link: http://fiji.sc/List_of_update_sites

Alternatively you can download it from the BIOP Website.

Link: http://biop.epfl.ch/TOOL_VSI_Reader.html



Start Update

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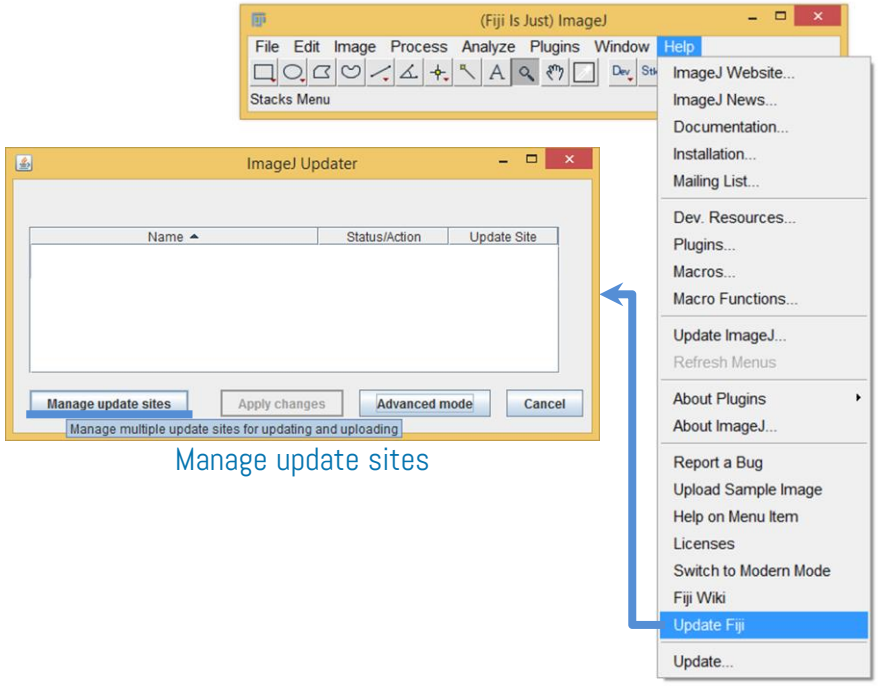

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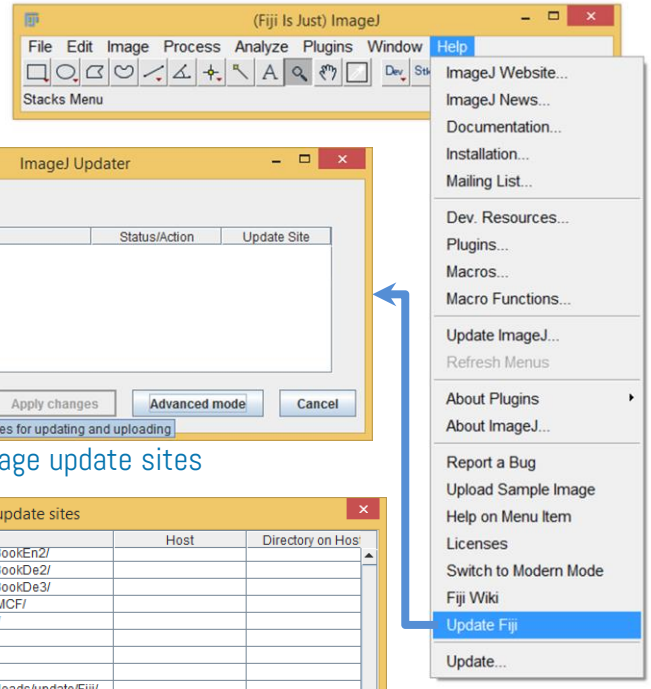

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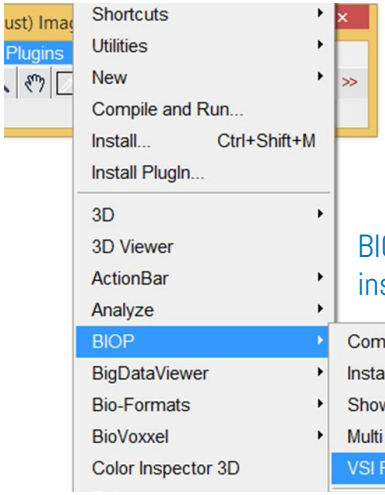
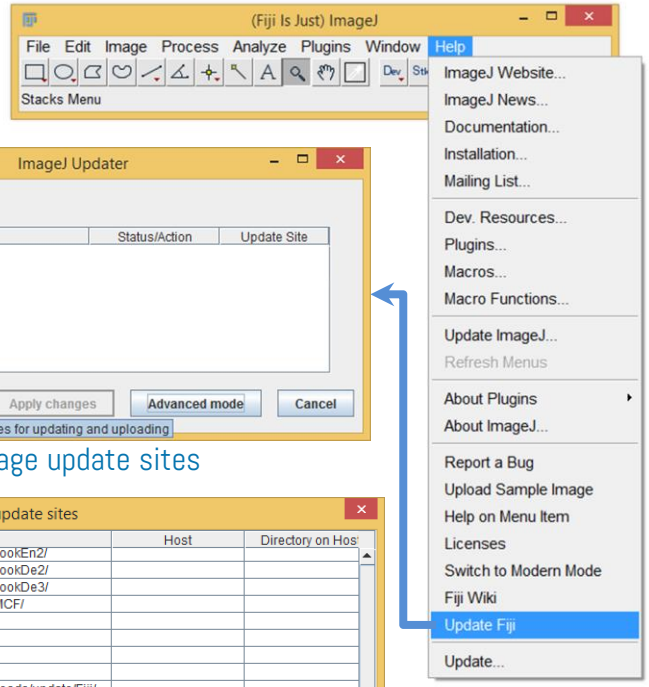


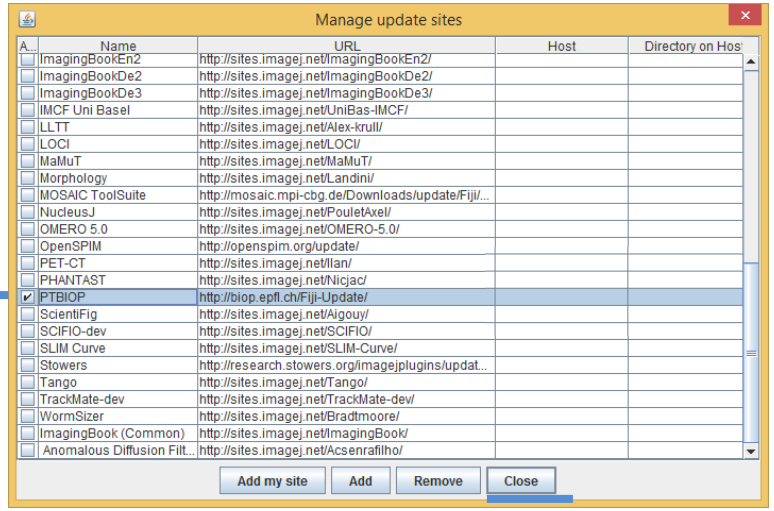
BIOP plugins are now installed

Restart FIJI



Start Update

Manage update sites



Select 'PTBIOP' and Close

Note:
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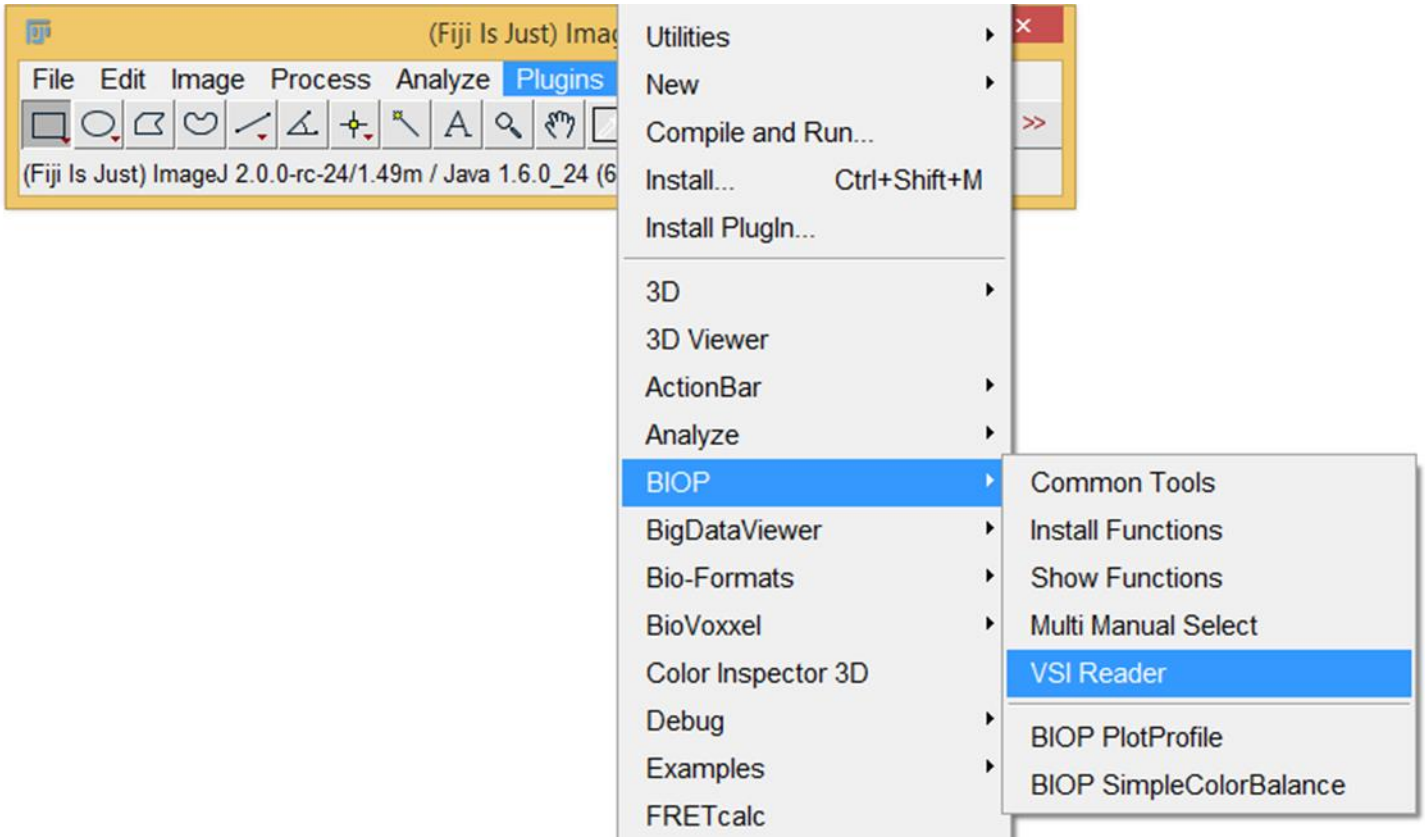
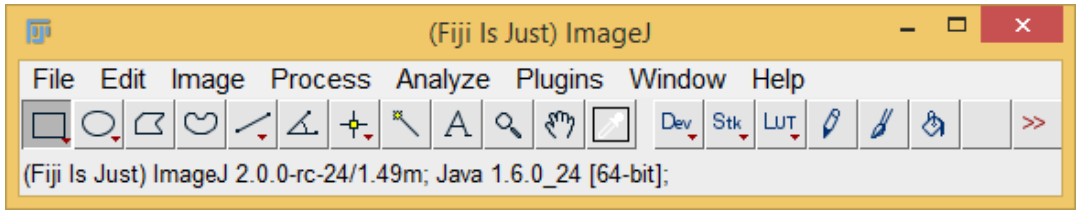
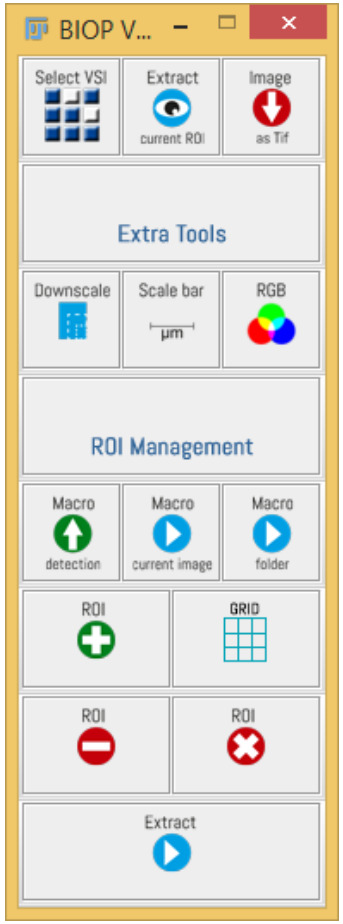
Link: http://imagejdocu.tudor.lu/doku.php?id=plugin:utilities:action_bar:start

Start VSI Reader

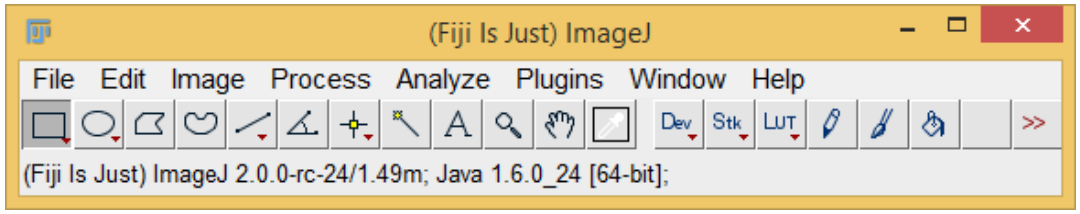


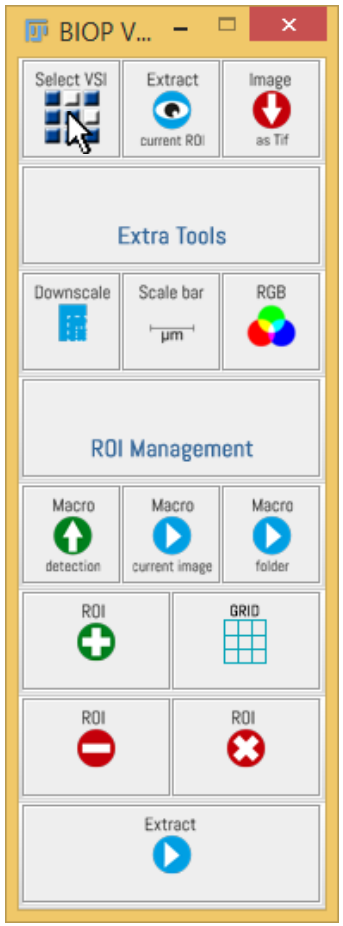
Start VSI Reader



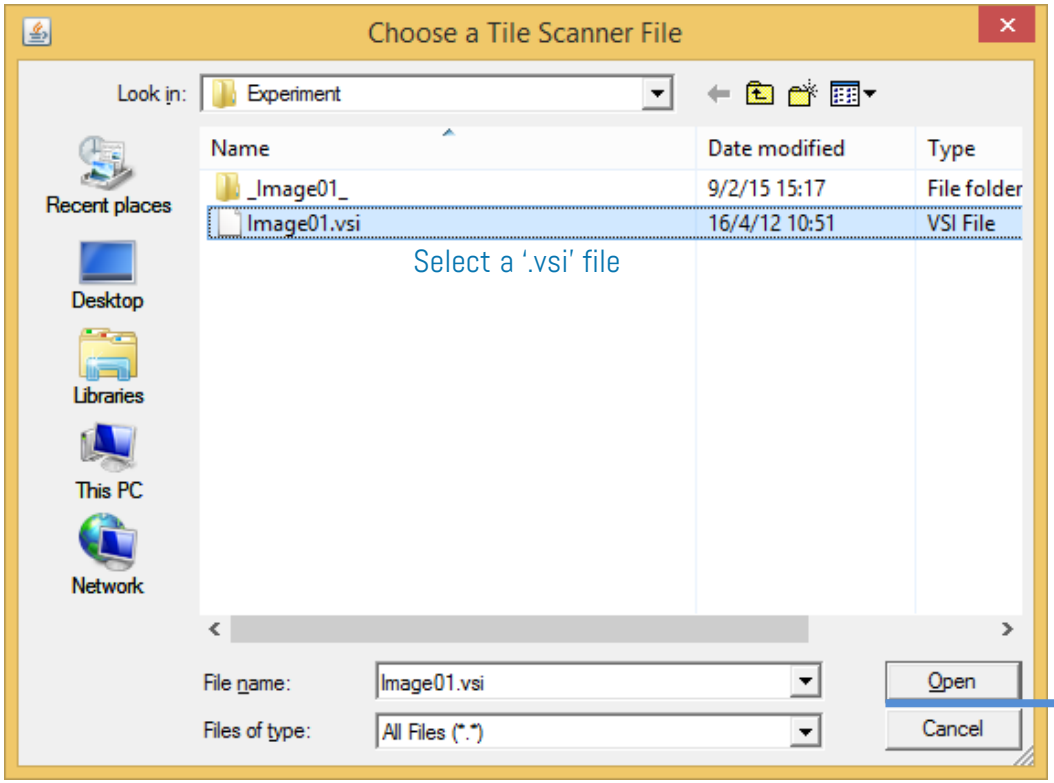


Open a VSI File 1/4



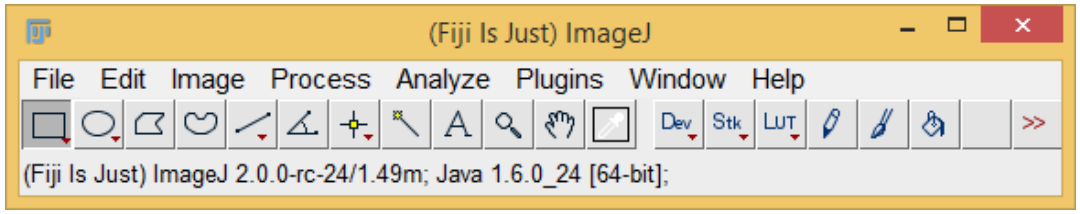


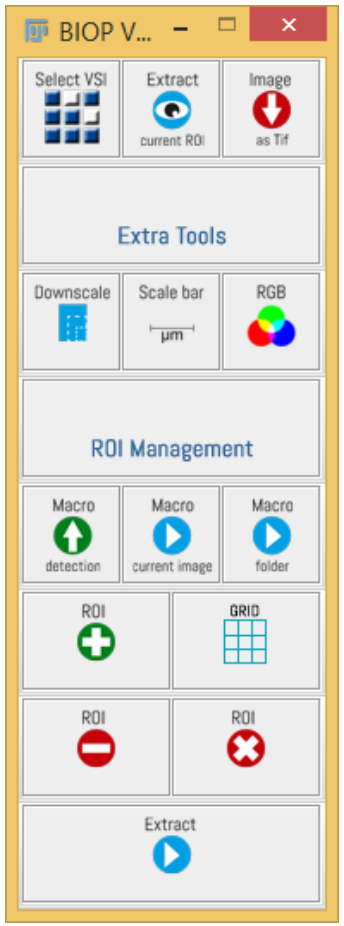
File Prompt pops-up



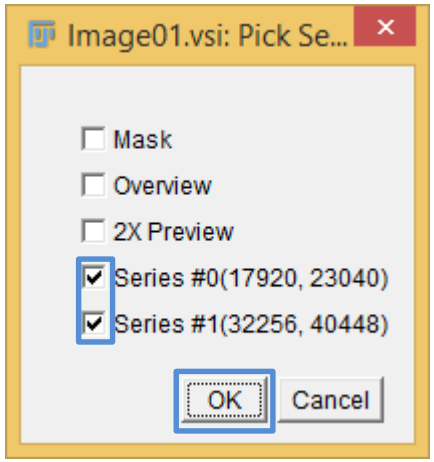
And Open

Open a VSI File 2/4





A new window pops-up

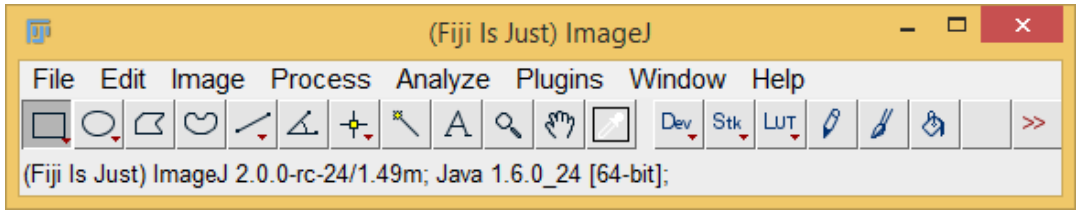


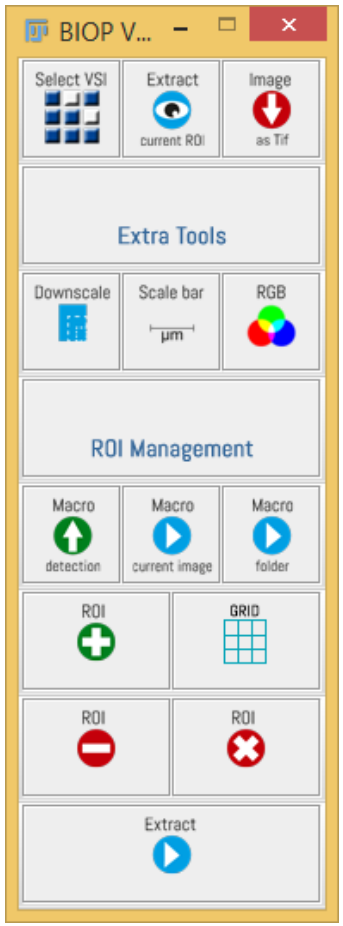
Select the serie(s) you would like to visualize

And OK

(x,y) values are the size of the entire image in pixels

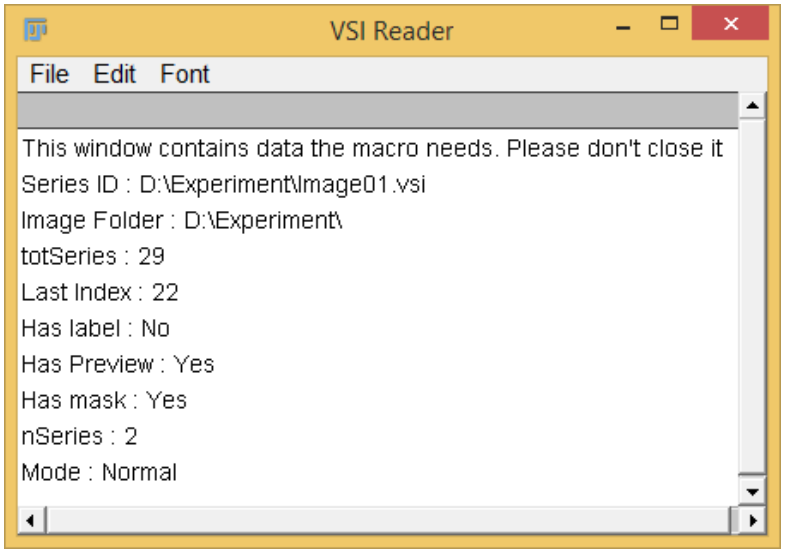
Open a VSI File 3/4





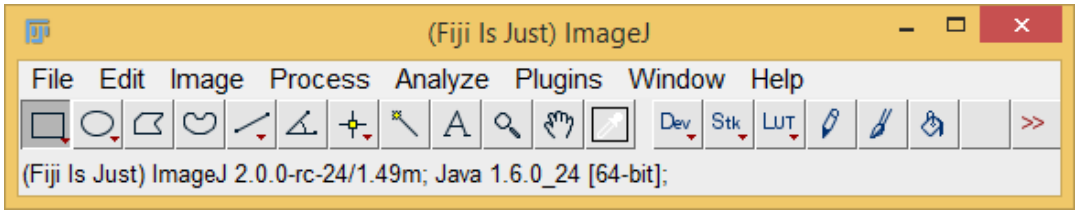
Reduced version (Thumbnail) appears for each series

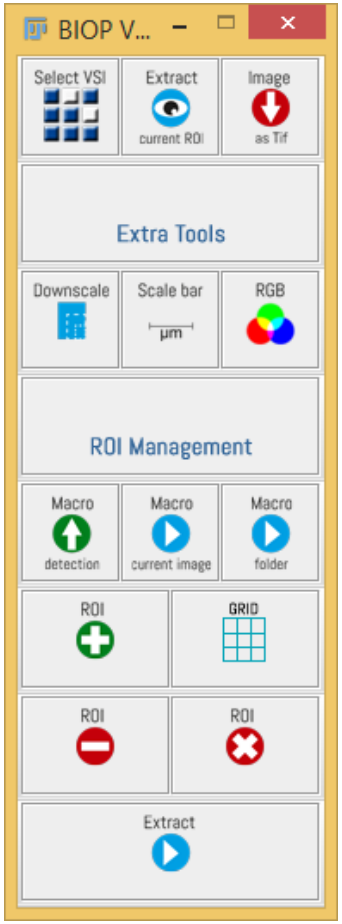
And a parameters window that contains information on the VSI File

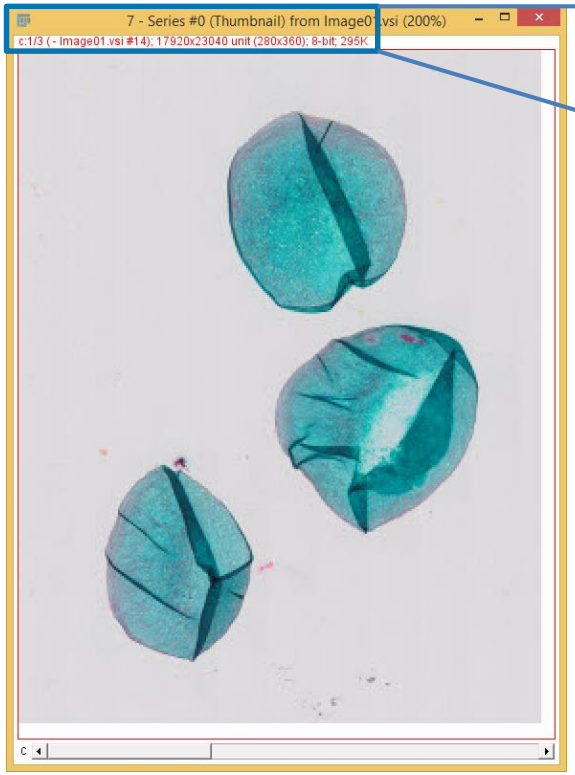


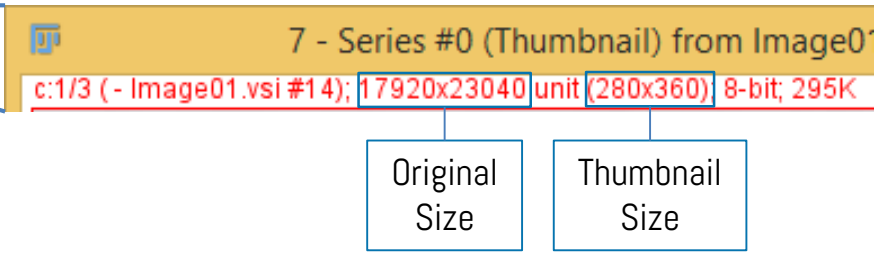
Please, do not close this window, the macro needs information to process the images

Open a VSI File 4/4

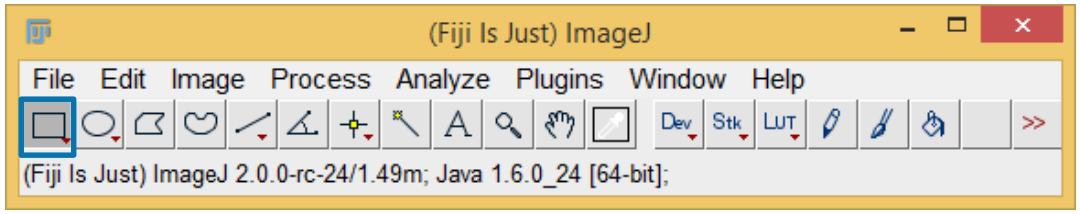


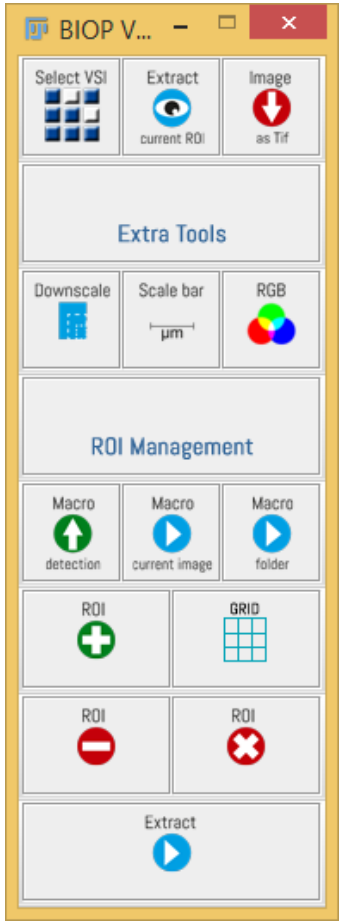


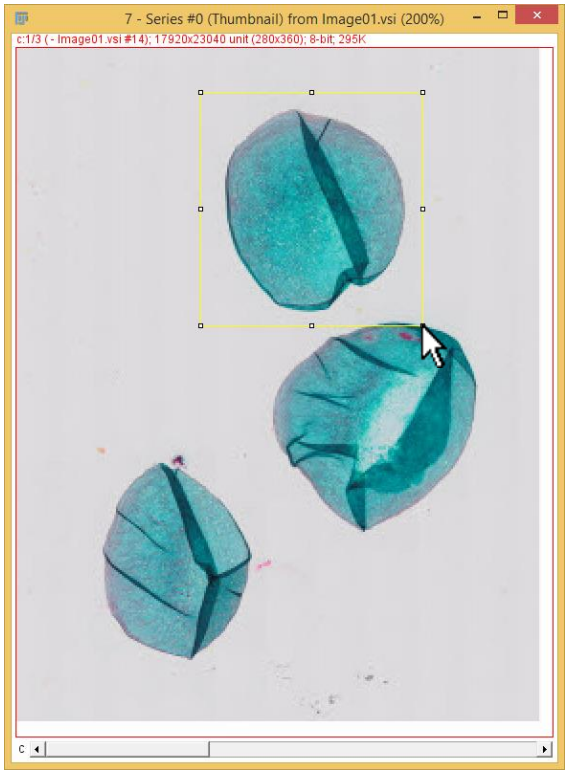




Extract ROIs 1/4

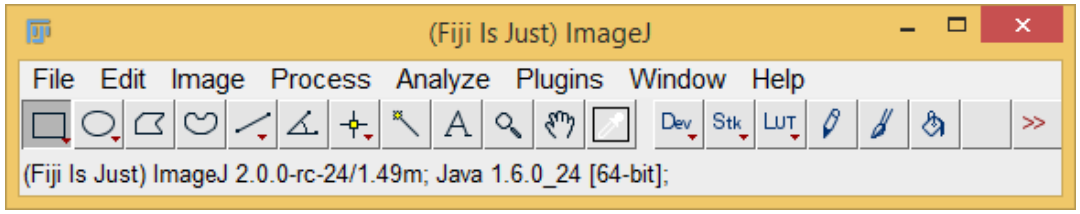


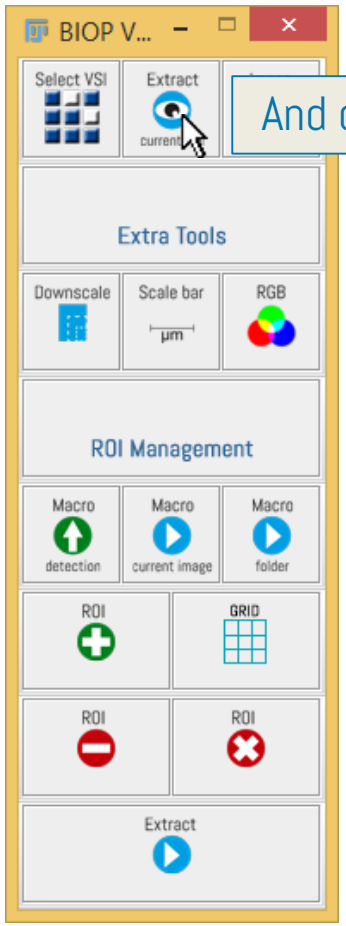




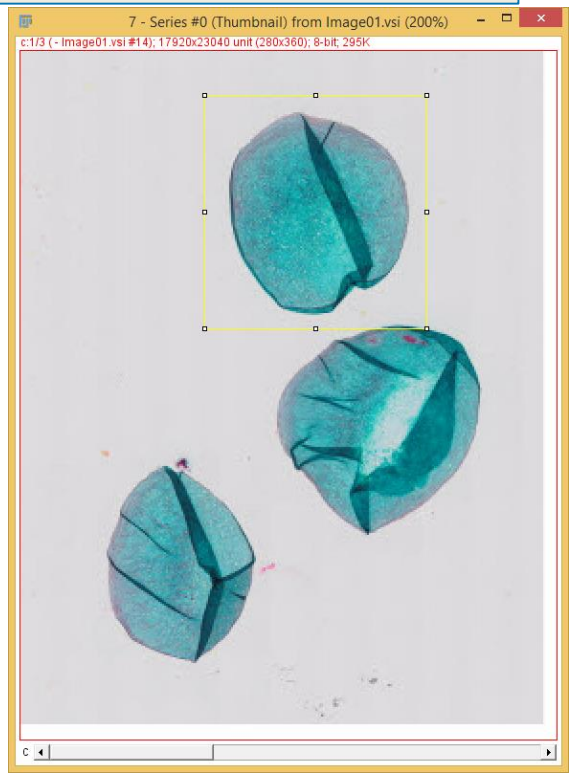
If you define a ROI using the Rectangular Selection tool...

Extract ROIs 2/4

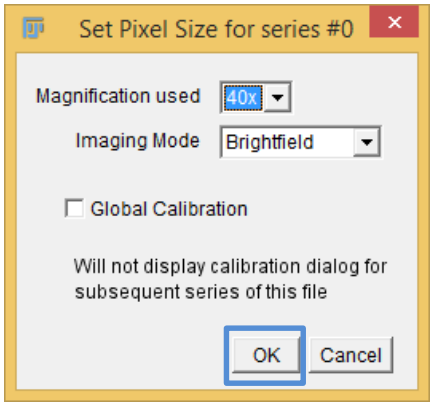




And click on "Extract Current ROI"



A window pops-up asking for calibration information



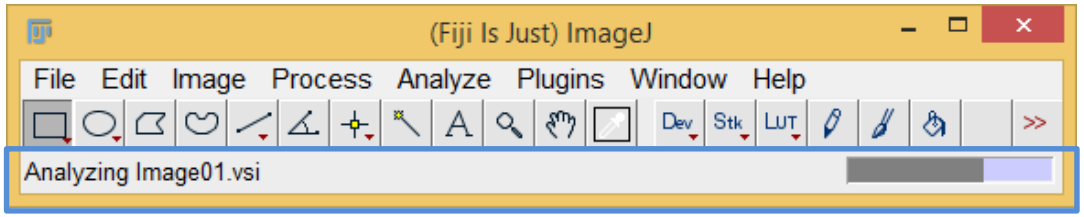
Select:
Correct Objective
Correct camera

Should not be ticked if different magnifications are used

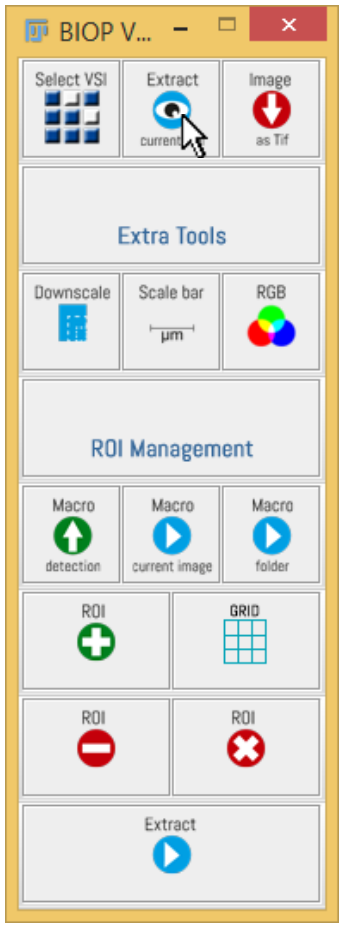
And OK

NB: Informations will be stored within the "Parameters" window, so you don't have to specify them for each new ROI in the series.

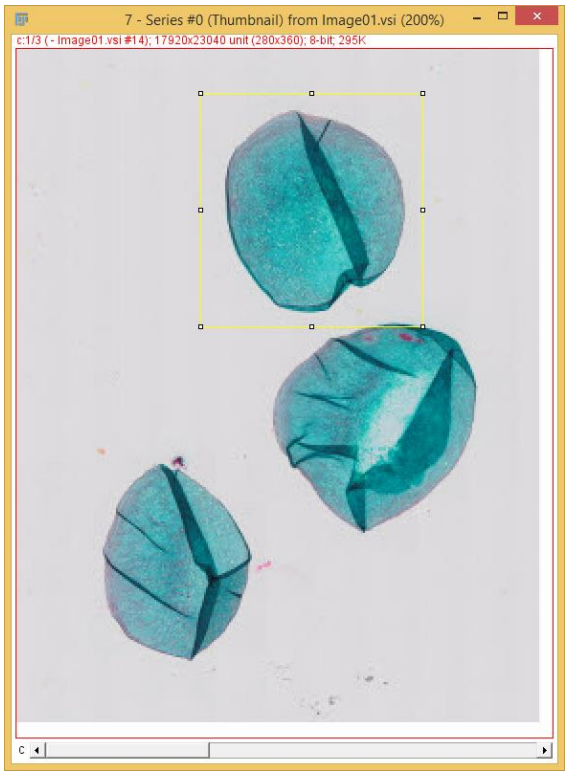
Extract ROIs 3/4

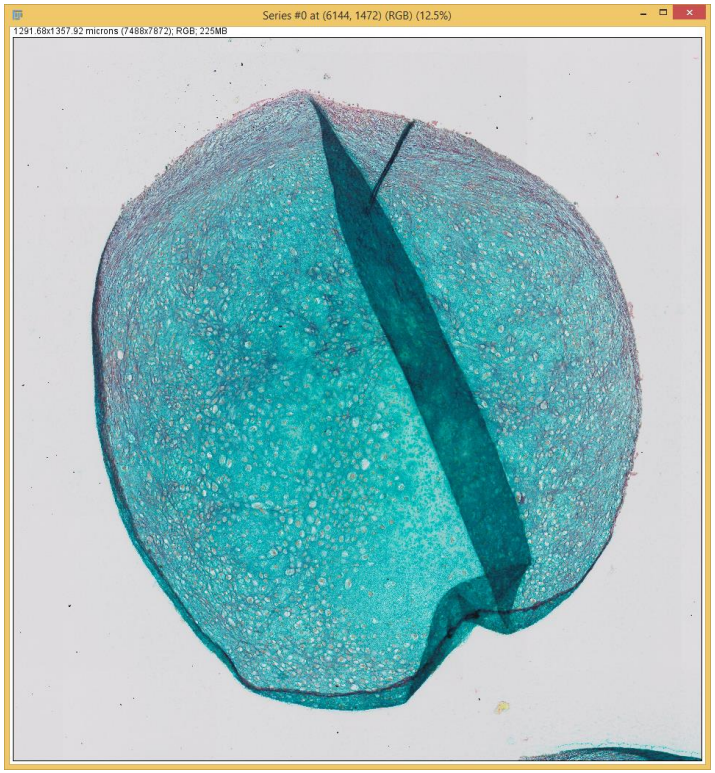


ImageJ loads the raw pixel data...



...and the full size region is extracted

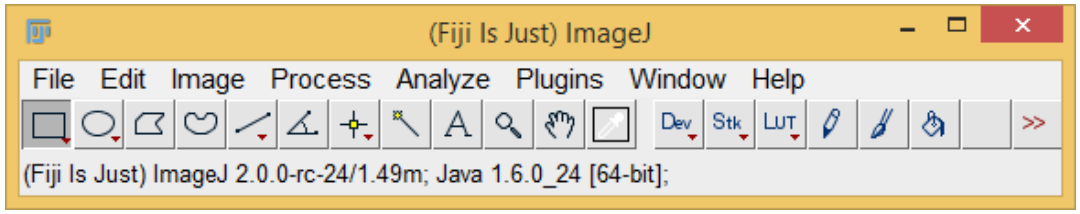


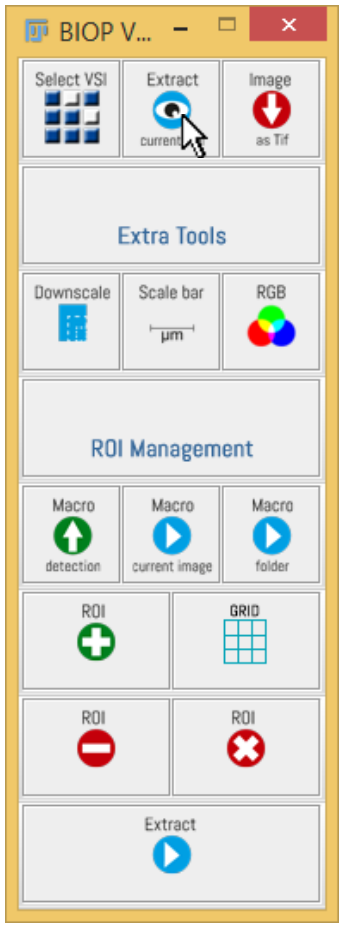


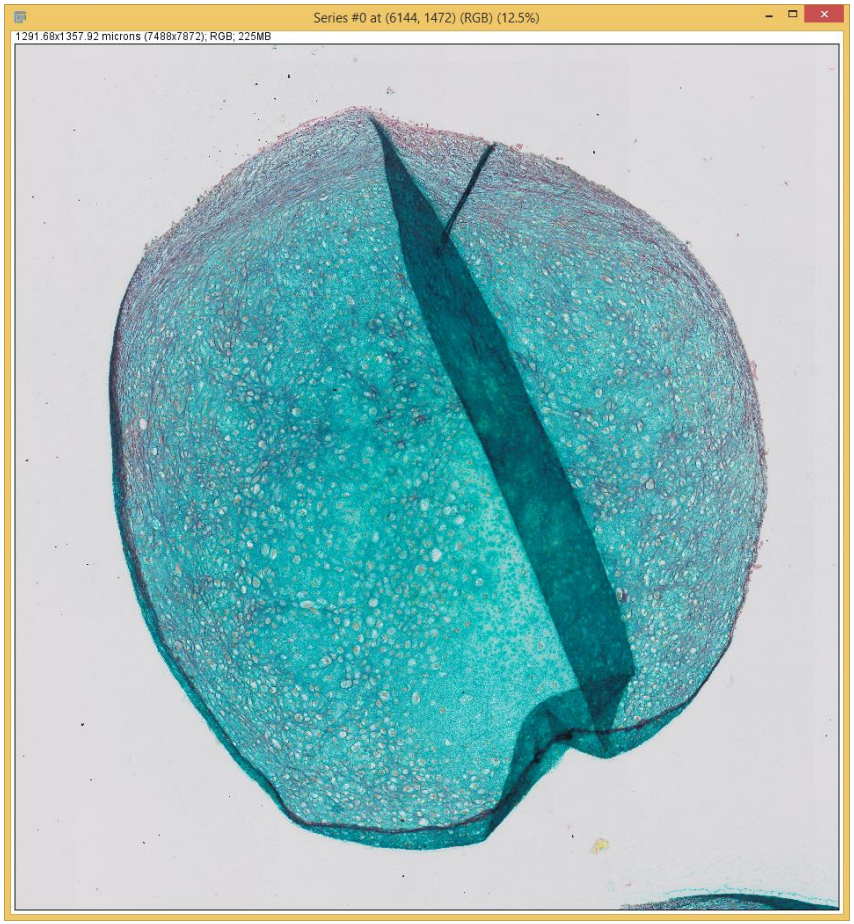
The unit is now 'microns' and the image is **RGB**.

If you specified "Imaging Mode" as **Fluorescence** Then you would get a Composite Image

Extract ROIs 3/4







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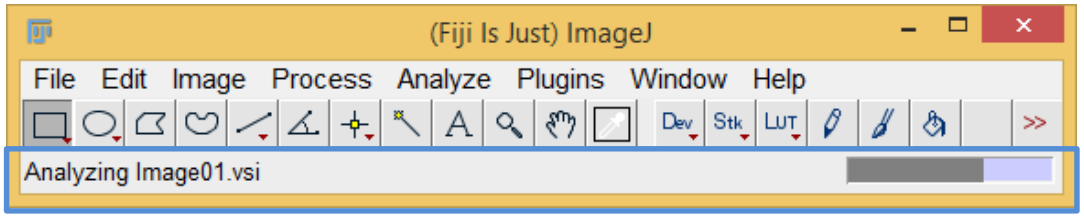
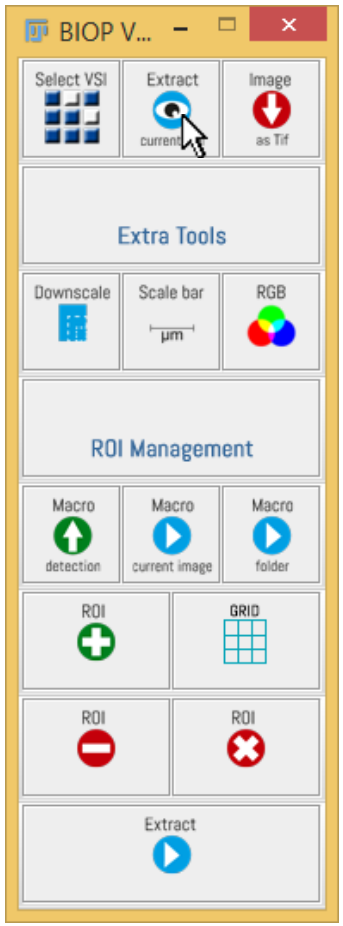
and the image is **RGB**.

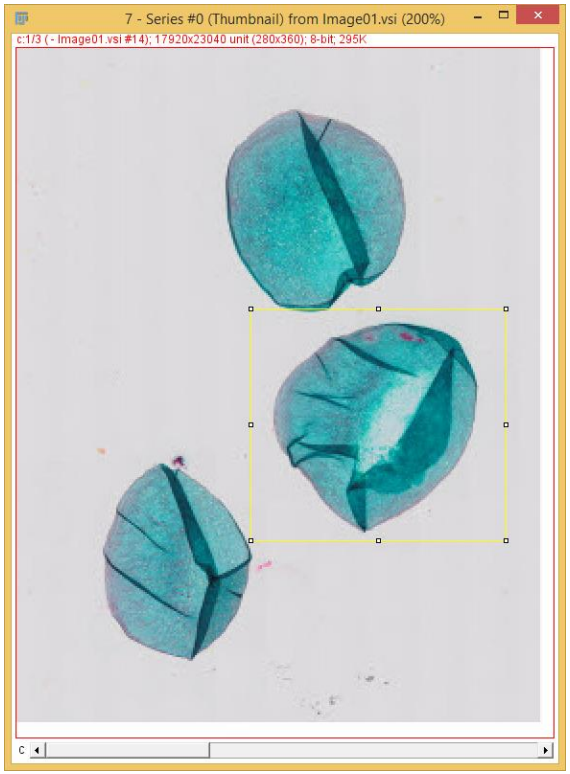
If you specified
"Imaging Mode" as **Fluorescence**
Then you would get a **Composite Image**

Extract ROIs 4/4

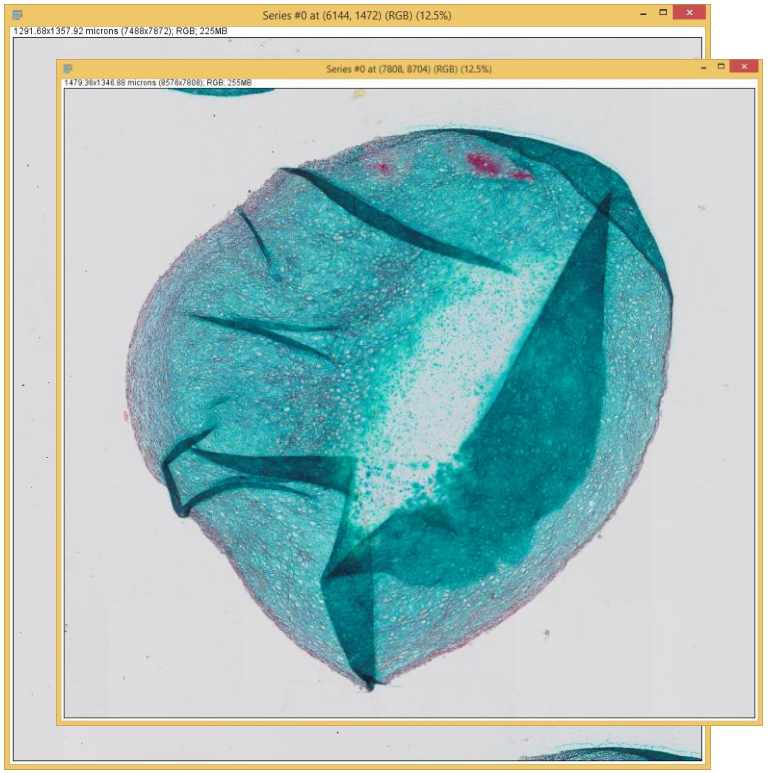




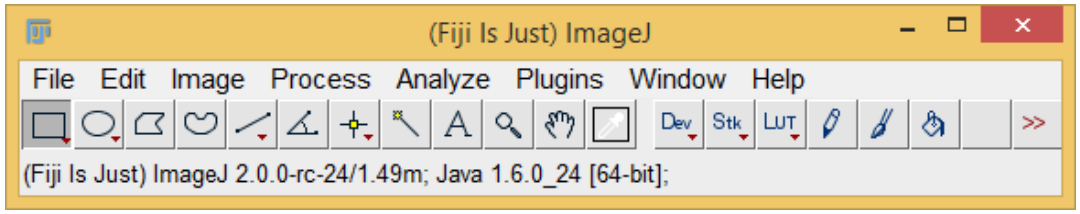
If you define a new ROI and extract

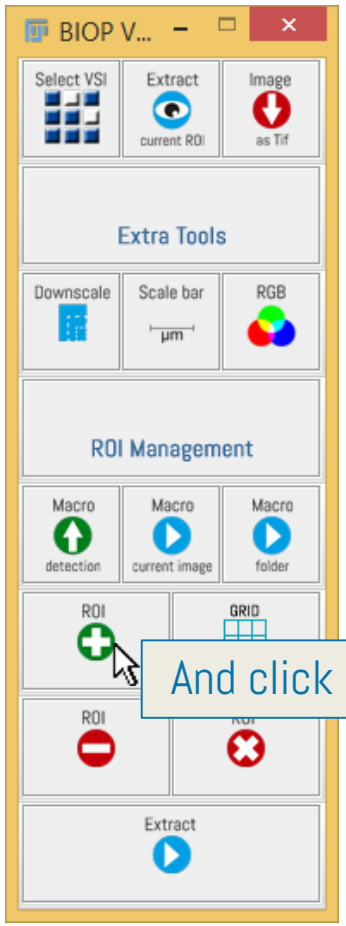


A new image pops-up



Manual Define Multiple ROIs 1/3

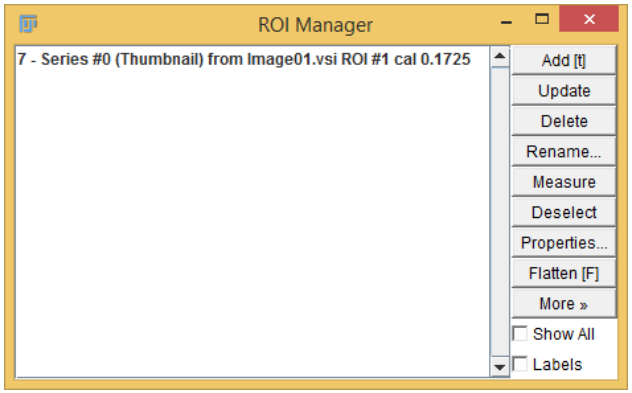




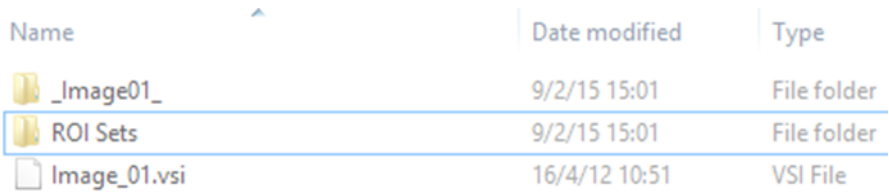
Draw a ROI

And click on "Add to ROI manager"

The ROI is now in the ROI Manager

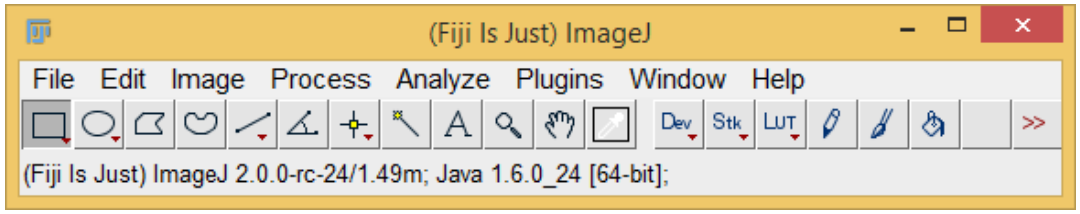


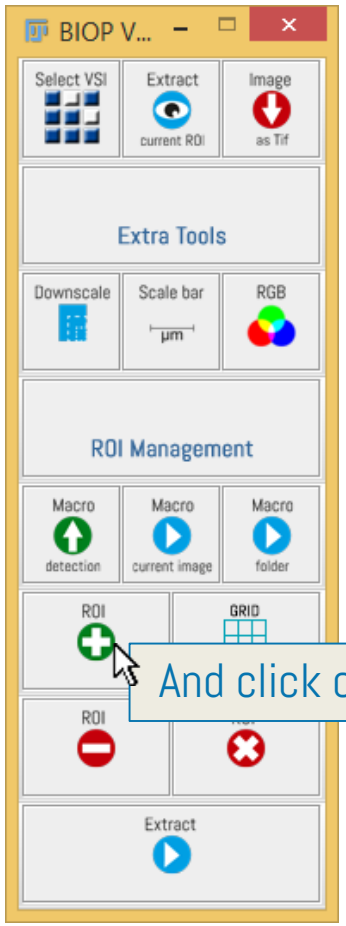
Informations (objective, imaging mode) have been already specified



Each Time you add a ROI, the ROIset is automatically saved in the ROI Sets folder

Manual Define Multiple ROIs 2/3

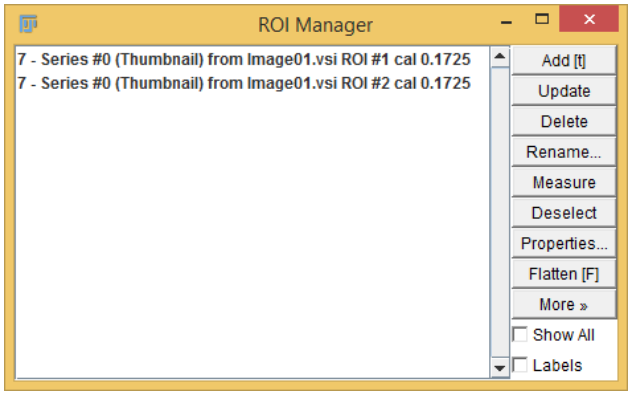




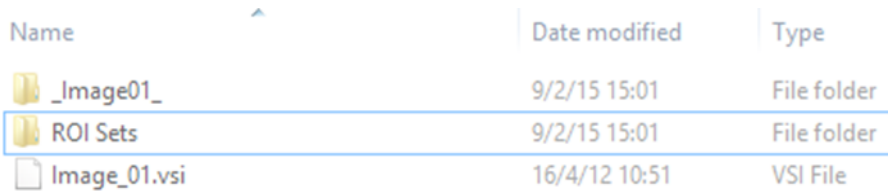
And click on "Add to ROI manager"

If you draw another ROI

The new ROI is appended

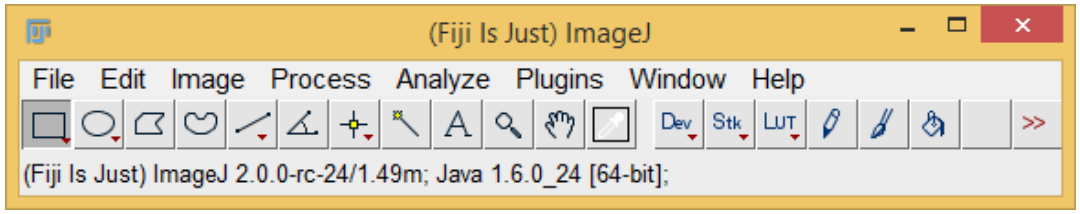


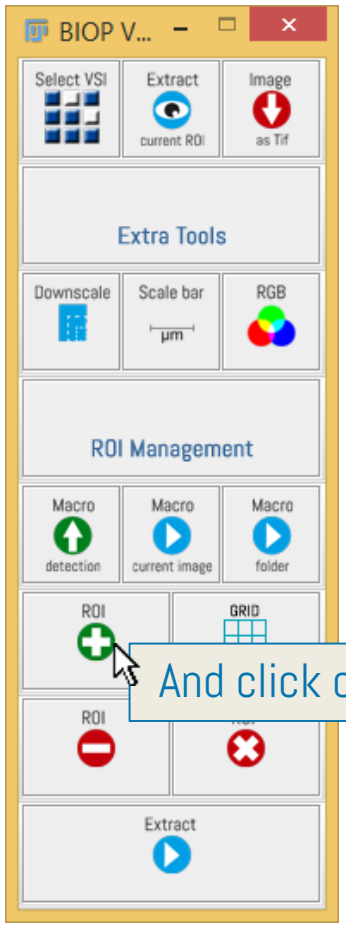
Informations (objective, imaging mode) have been already specified



Each Time you add a ROI, the ROIset is automatically saved in the ROI Sets folder

Manual Define Multiple ROIs 3/3

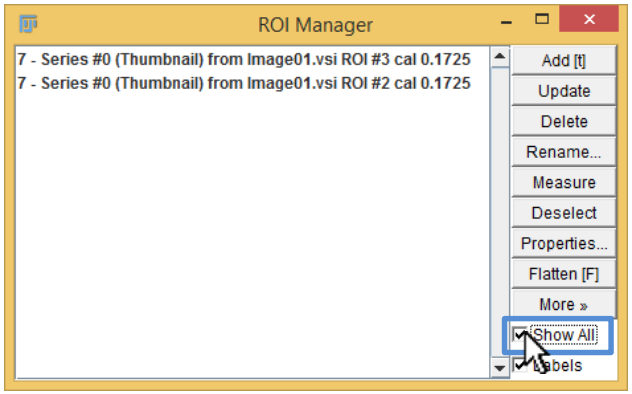




And click on "Add to ROI manager"

If you draw another ROI

The new ROI is appended



Select 'Show All' to see all the ROIs

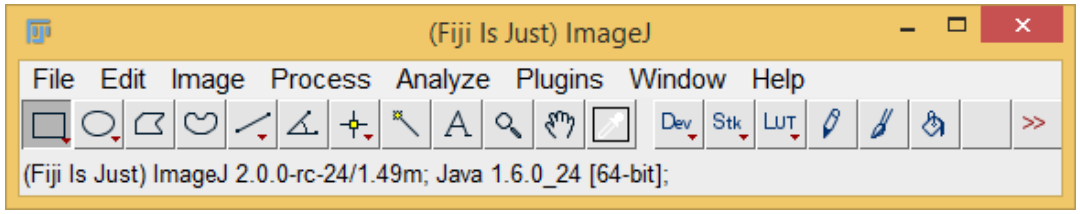
Because the ROI set is automatically saved, you can do this for:

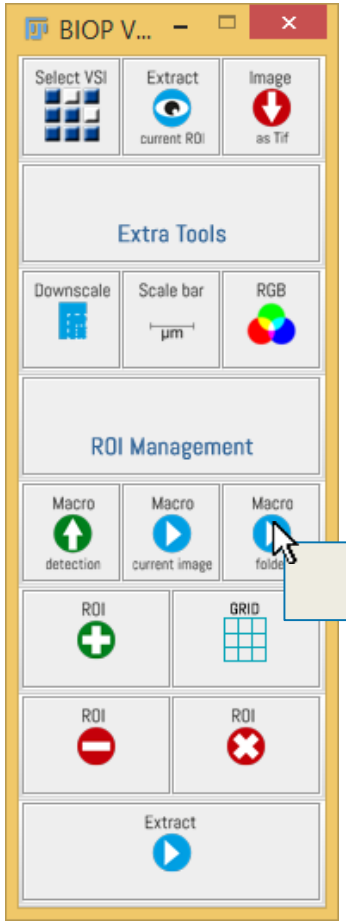
- Any number of ROIs in a Series
- Any number of Series per VSI File
- Any number of VSI Files

The only condition is that all VSI files be in the same folder

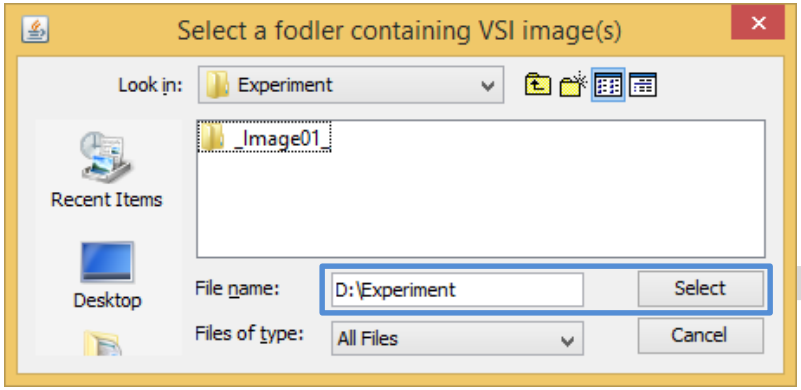
Automatic Define Multiple ROIs 1/2

SIMPLE MODE

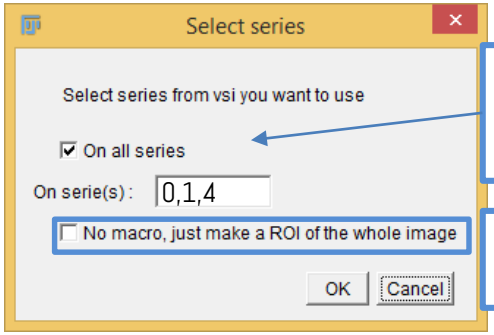




Select the folder where the VSI files are located



Click on "Batch Define ROIs"

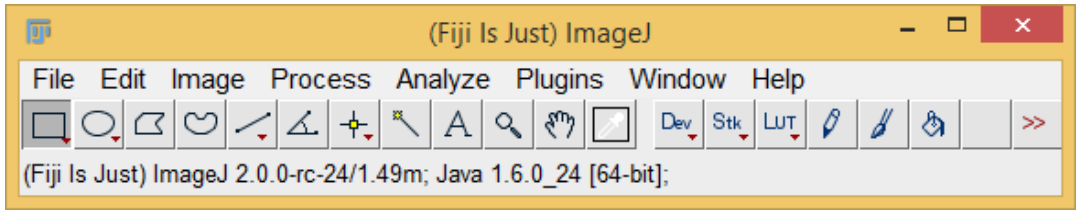


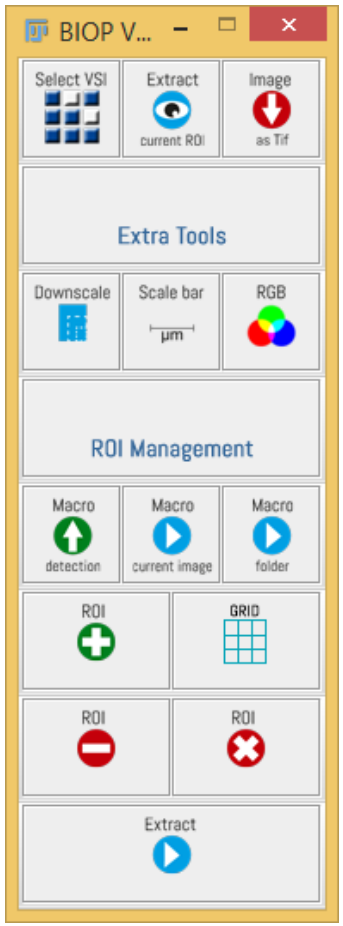
Select either all series or the series you want to extract in each VSI file, separated by a comma

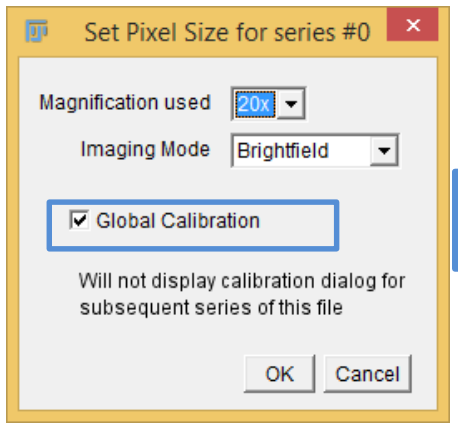
Select to use the whole image for each series.

Automatic Define Multiple ROIs 2/2

SIMPLE MODE

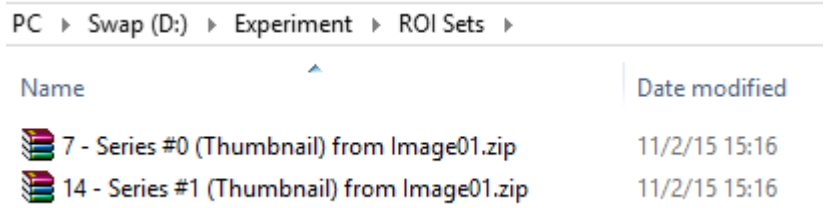




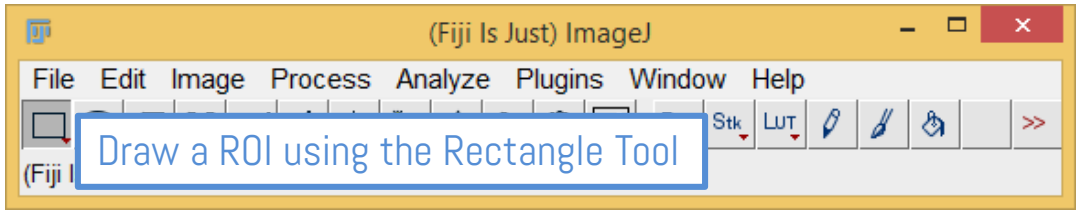


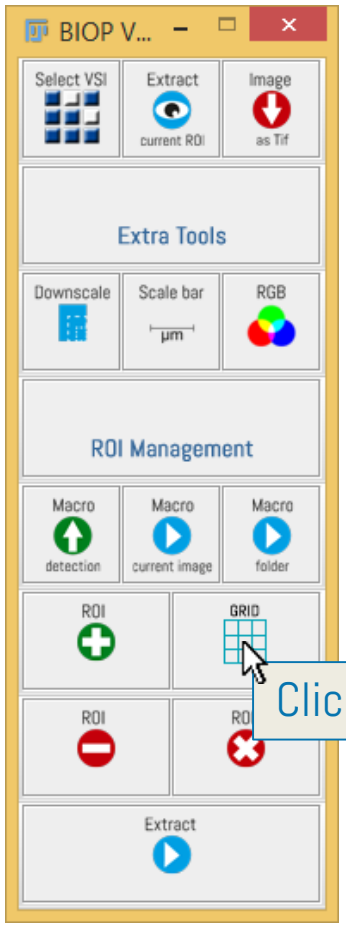
Select Global Calibration to not be prompted for each series.

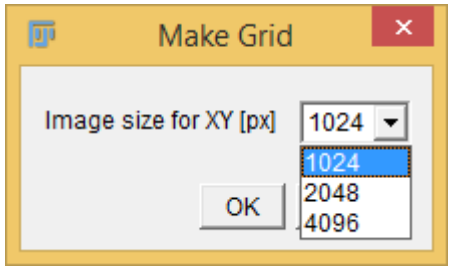
A ROI Set is saved for each series



GRID Define Multiple ROIs

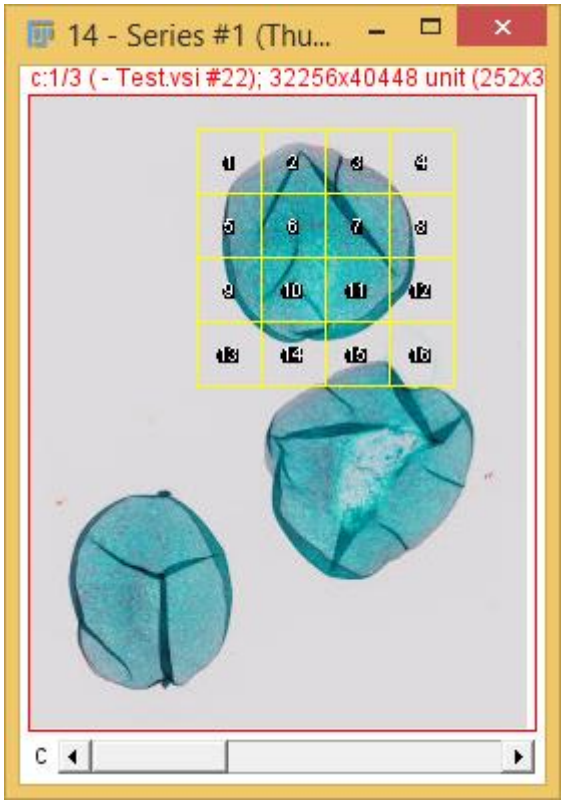






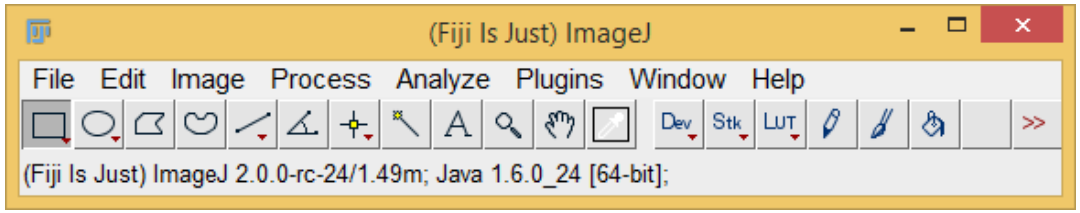
Select the size of the Individual images

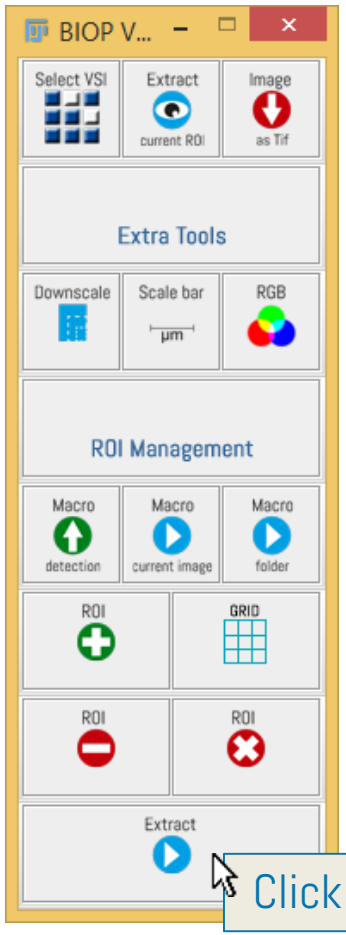
Click on 'Make Grid From ROI'

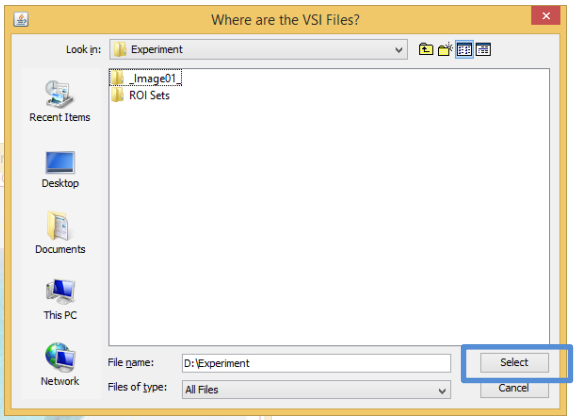


The ROIs are created and saved automatically

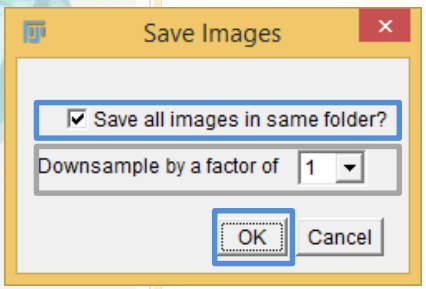
Extracting Multiple ROIs 1/2







You are prompted for the folder containing the VSI files (**And the ROI Sets folder**)

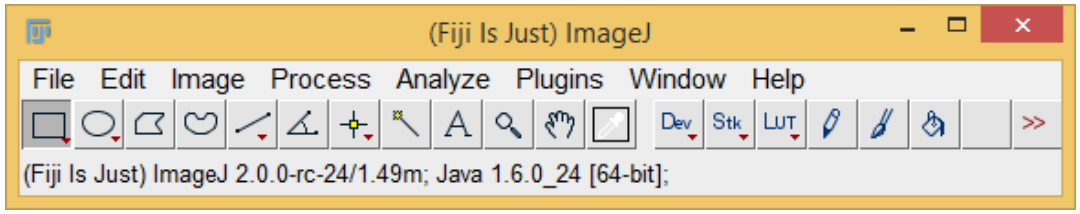


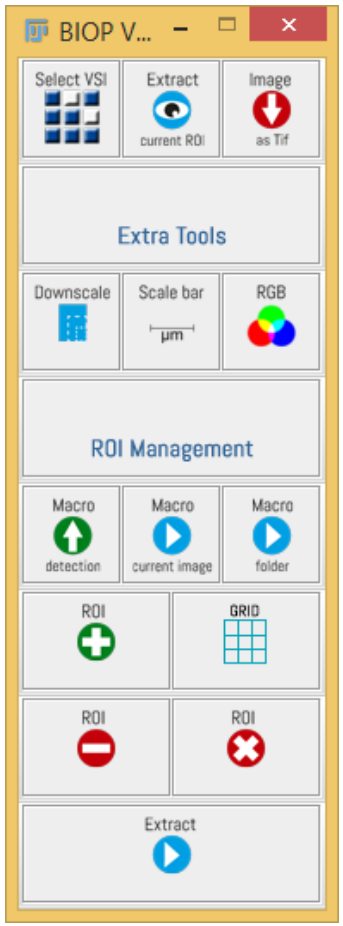
You can save all images in the same folder or in one folder per VSI file.

You can also downsample the images to speed up extraction

Click on "Process ROIs for extraction"

Extracting Multiple ROIs 2/2





Within the folder containing the orignal image

Name	Type
Image01	File folder
Image01.vsi	Visual Studio Community Content Installer File

A new folder 'Extracted' is created

Name	Type
Image01	File folder
Image01.vsi	Visual Studio Community Content Installer File
Extracted	File folder

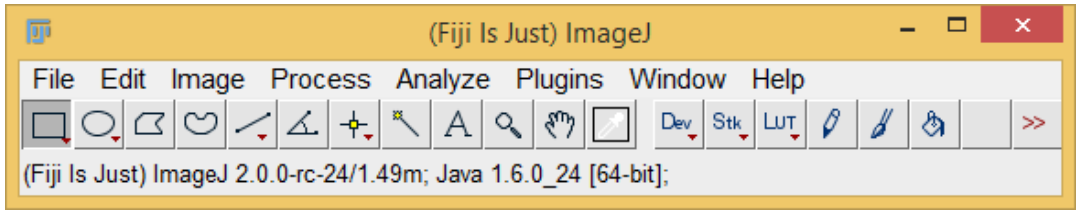
To save extracted ROI

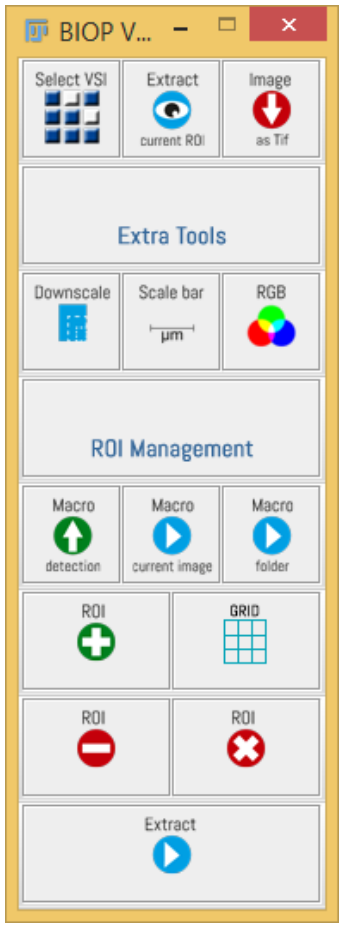
As a 'tif' file

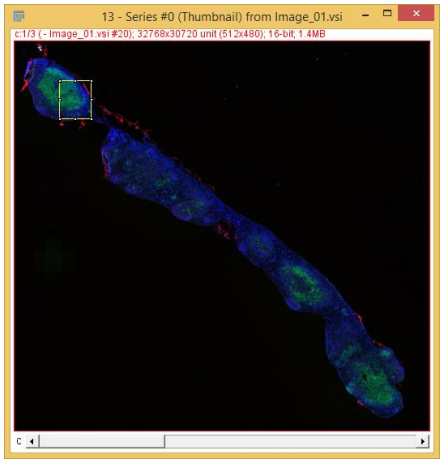
Image01.vsi_Series_0_ROI_1_Scaling_2_RGB.tif

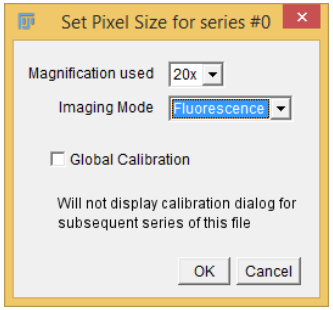
The VSI reader saves Brightfield images as RGB and Fluorescence images as composite TIFFs

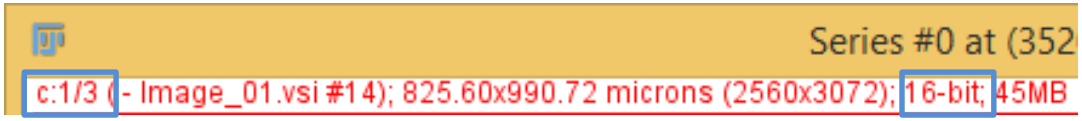
Fluorescent Acquisition Example











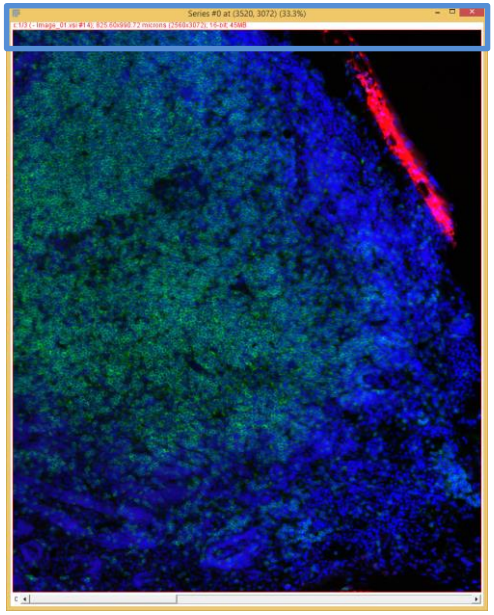


Image is a **3 Channel 16-bit Composite** and will be saved as such

End

- More is in preparation
  - Custom macros for defining ROIs
- Contact : mail at Olivier and Romain

- **Acknowledgements**
  - Anthony Grognuz, Unité de Thérapie Cellulaire, CHUV
    - **Widefield Dataset**
  - Dr. Lalit Kumar Dubey, Harris Lab, EPFL
    - **Fluorescent Dataset**

    The Fluorescent images should strictly not be used for any other purpose from you/or by any one in your organisation with out a written approval from Dr Lalit Kumar Dubey