



Image J/Fiji: Una herramienta imprescindible para estudios biológicos

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27-9-2018



Detalles técnicos del taller

- Información importante para realizar el taller:

1. WIFI:

Usuario: jsl@invitados.ugr.es

Clave: 18UGR++JSL

2. Example Images:

sl.ugr.es/taller_fiji

Detalles técnicos del taller

- Información importante para realizar el taller:
 1. WIFI:
Usuario: jsl@invitados.ugr.es
Clave: 18UGR++JSL
 2. Example Images:
sl.ugr.es/taller_fiji
- ¿Está el programa ImageJ o Fiji instalado en vuestros ordenadores?

<https://imagej.net/Fiji/Downloads>

Download Image J/Fiji:

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https://www.google.com/search?source=hp&ei=totxV 110% Suchen

Google Suchen Anmelden

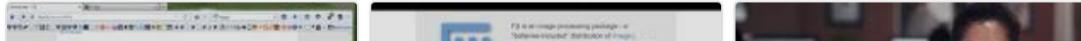
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Ungefähr 171.000.000 Ergebnisse (0,42 Sekunden)

Fiji/Downloads - ImageJ
<https://imagej.net/Fiji/Downloads> ▾ Diese Seite übersetzen
30.05.2017 - Fiji is a distribution of ImageJ which includes many useful plugins contributed by the community. ... Download Fiji for your OS ~ Yada.png.
[Fiji/Downloads - ImageJ](#) · [Frequently Asked Questions](#) · [Index of /fiji](#)

Fiji - ImageJ
<https://imagej.net/Fiji> ▾ Diese Seite übersetzen
22.03.2017 - Fiji is an image processing package—a "batteries-included" distribution of ImageJ, bundling a lot of ... Download Fiji for your OS ~ Yada.png.

Videos



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https://imagej.net/Fiji/Downloads

Suchen

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IMAGEJ

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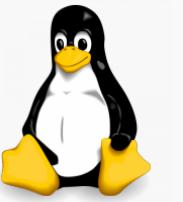
Search

Page Discussion View source History

Fiji/DOWNLOADS

Fiji is a distribution of ImageJ which includes many useful plugins contributed by the community.

~ Download Fiji for your OS ~

 64-bit	 macOS	 64-bit
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Other downloads

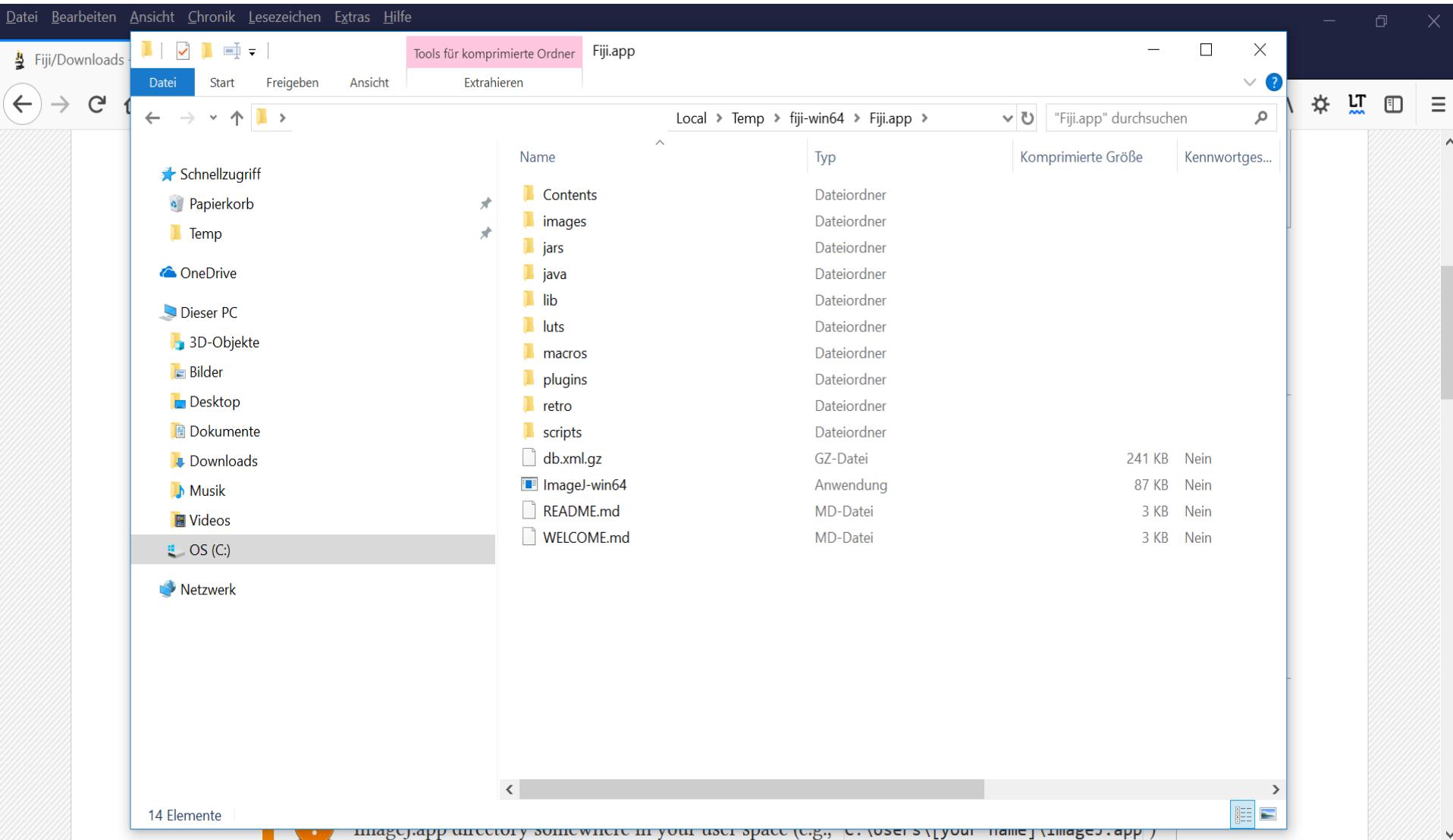
 32-bit	 No JRE	 32-bit
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Contents [hide]

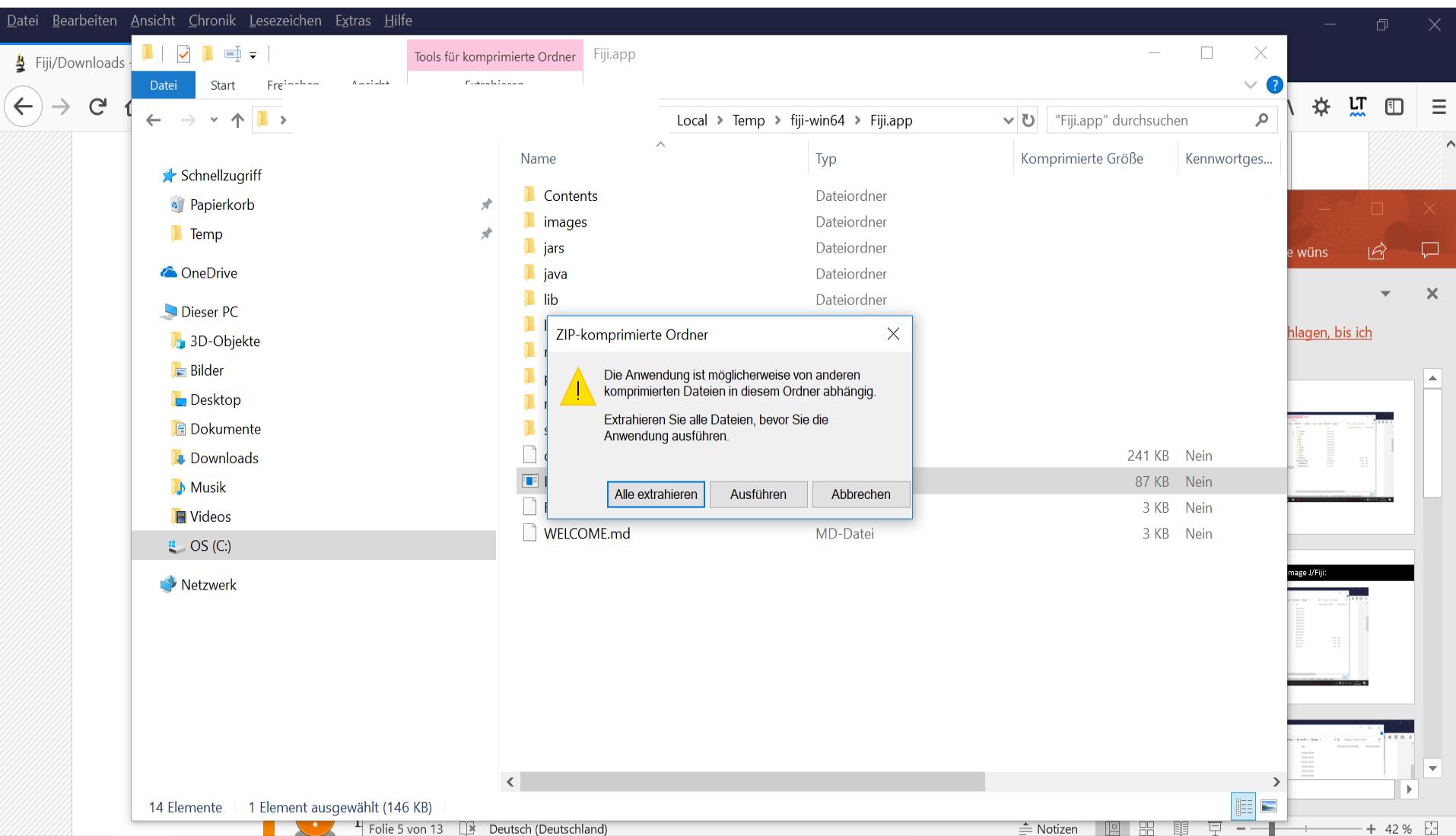
- 1 System requirements
- 2 Installation
- 3 Troubleshooting
- 4 Source code
- 5 Other downloads
 - 5.1 Life-Line Fiji versions
 - 5.1.1 Java 8
 - 5.1.2 Java 6
 - 5.2 See also

To find out if 32 bit or 64 bit version, check in Start menu: Configuración de PC → PC y dispositivos → Información PC

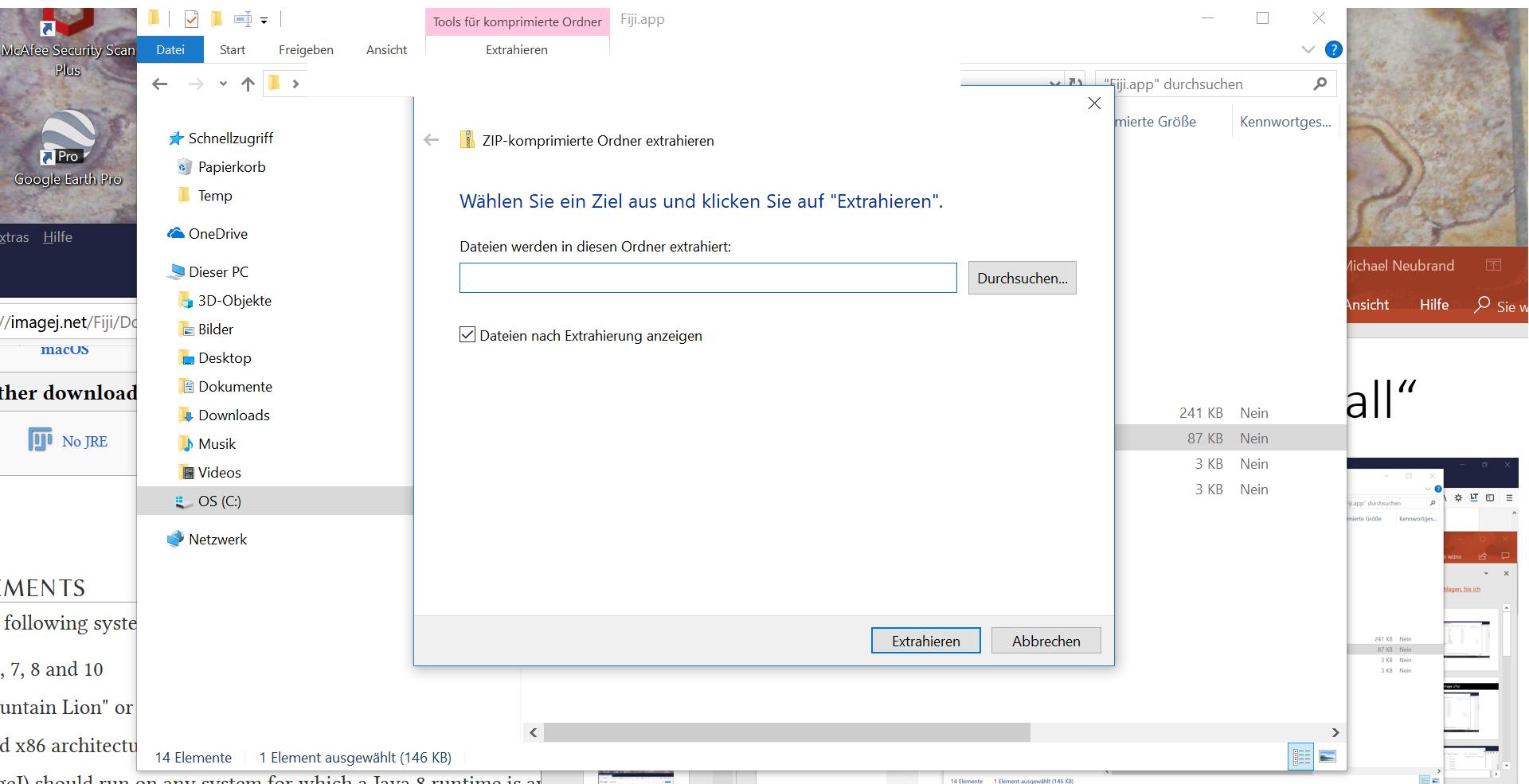
Click on ImageJ-win64



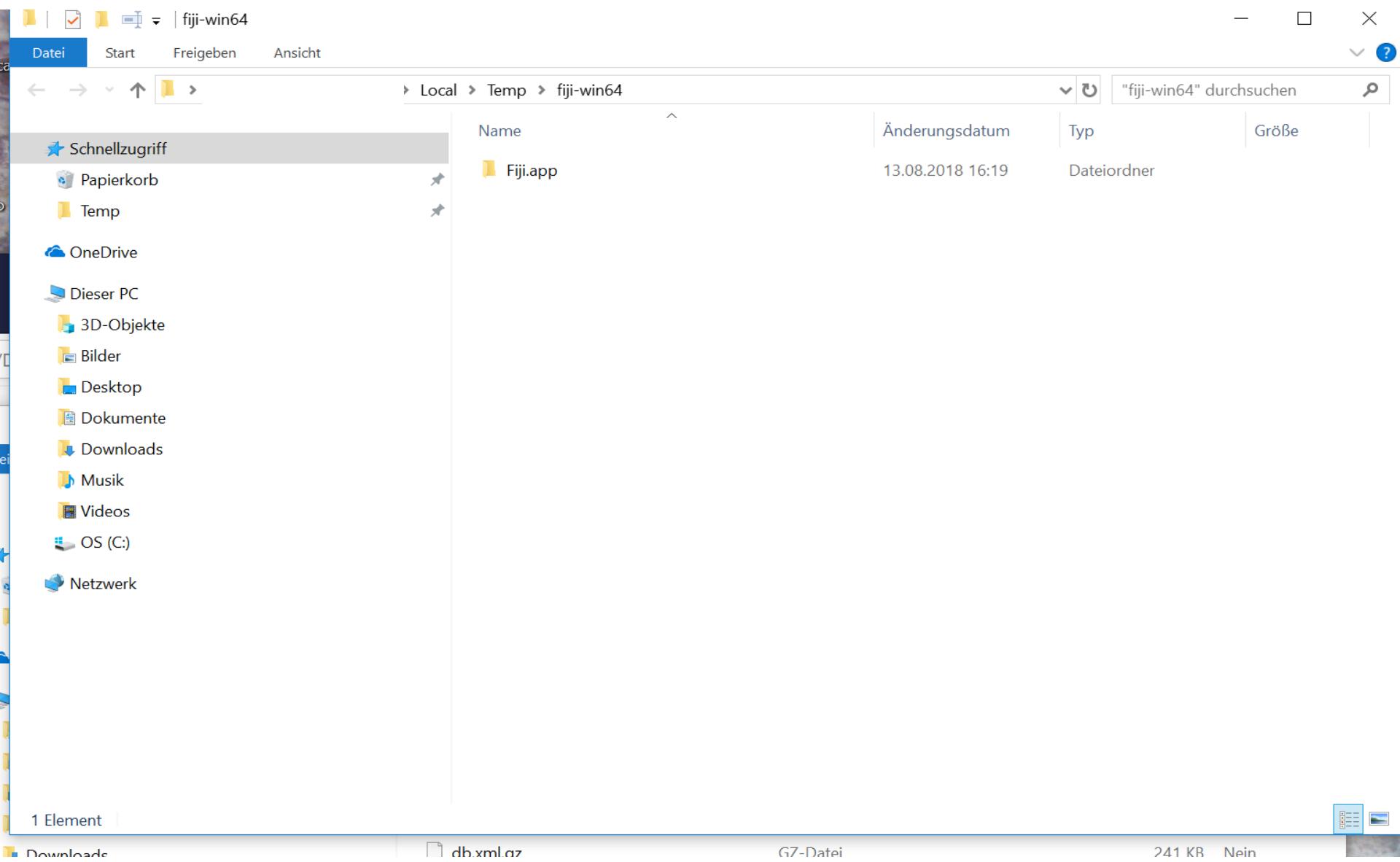
Click on extract all



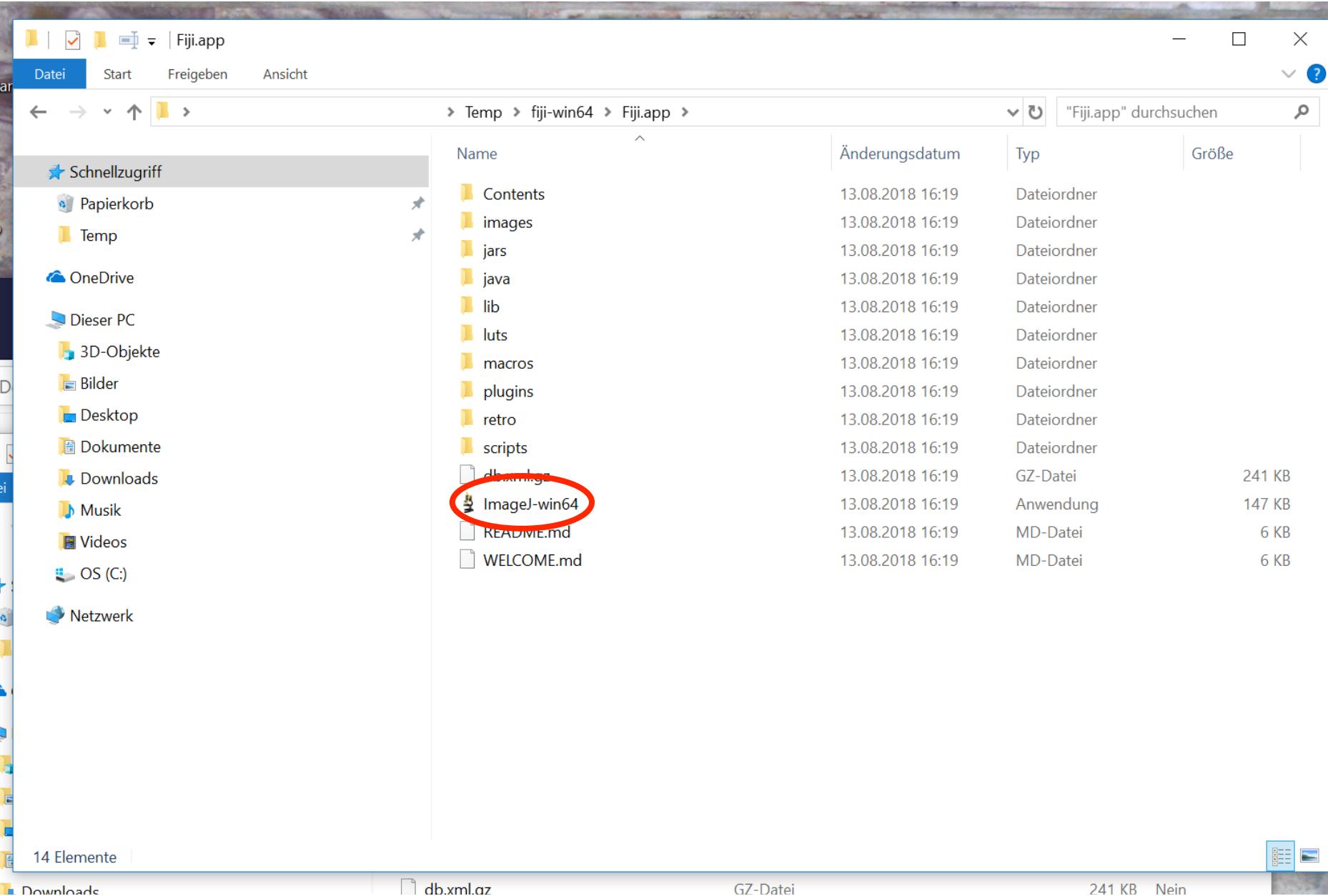
Choose a folder and click on extract all



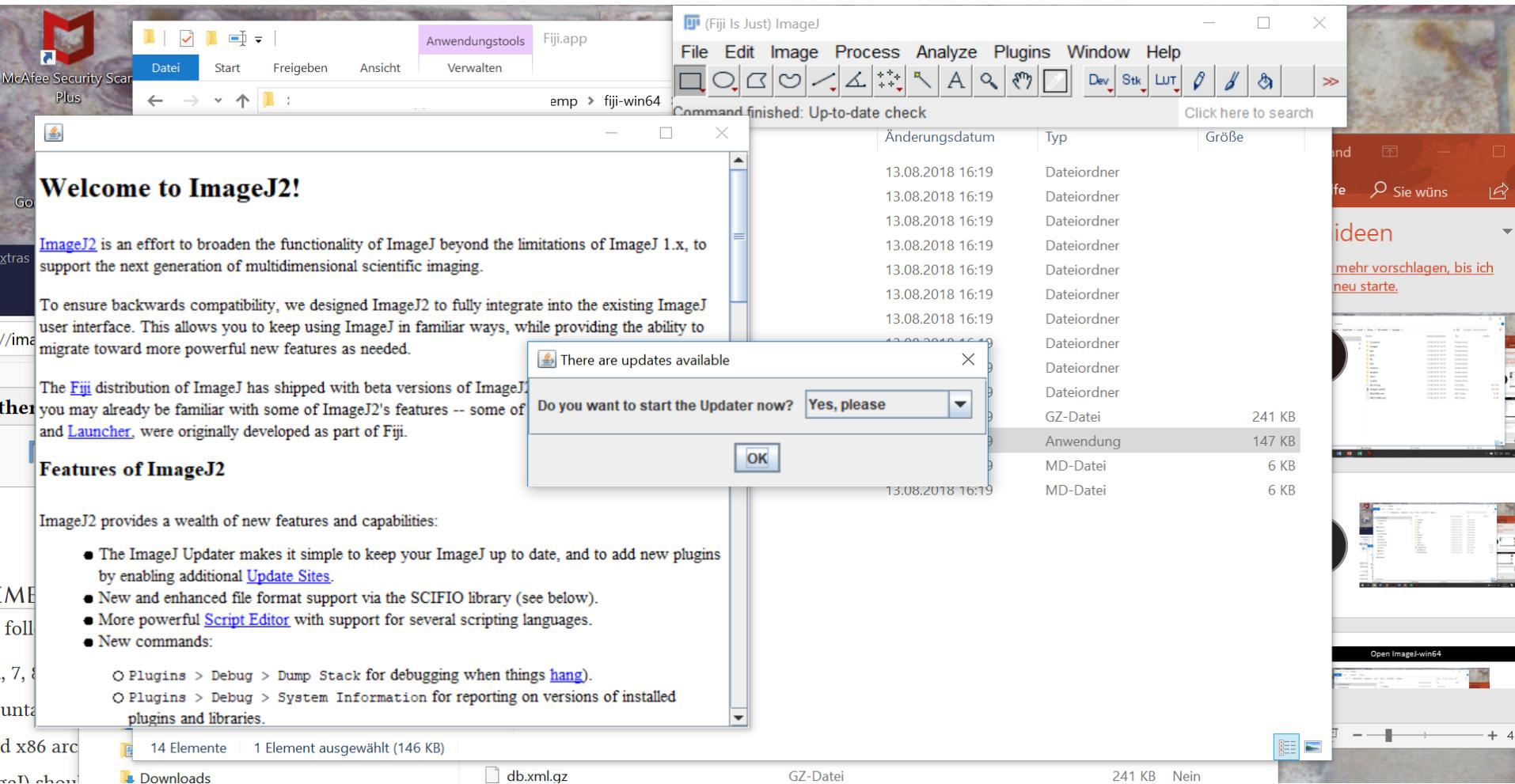
Open Fiji.app folder



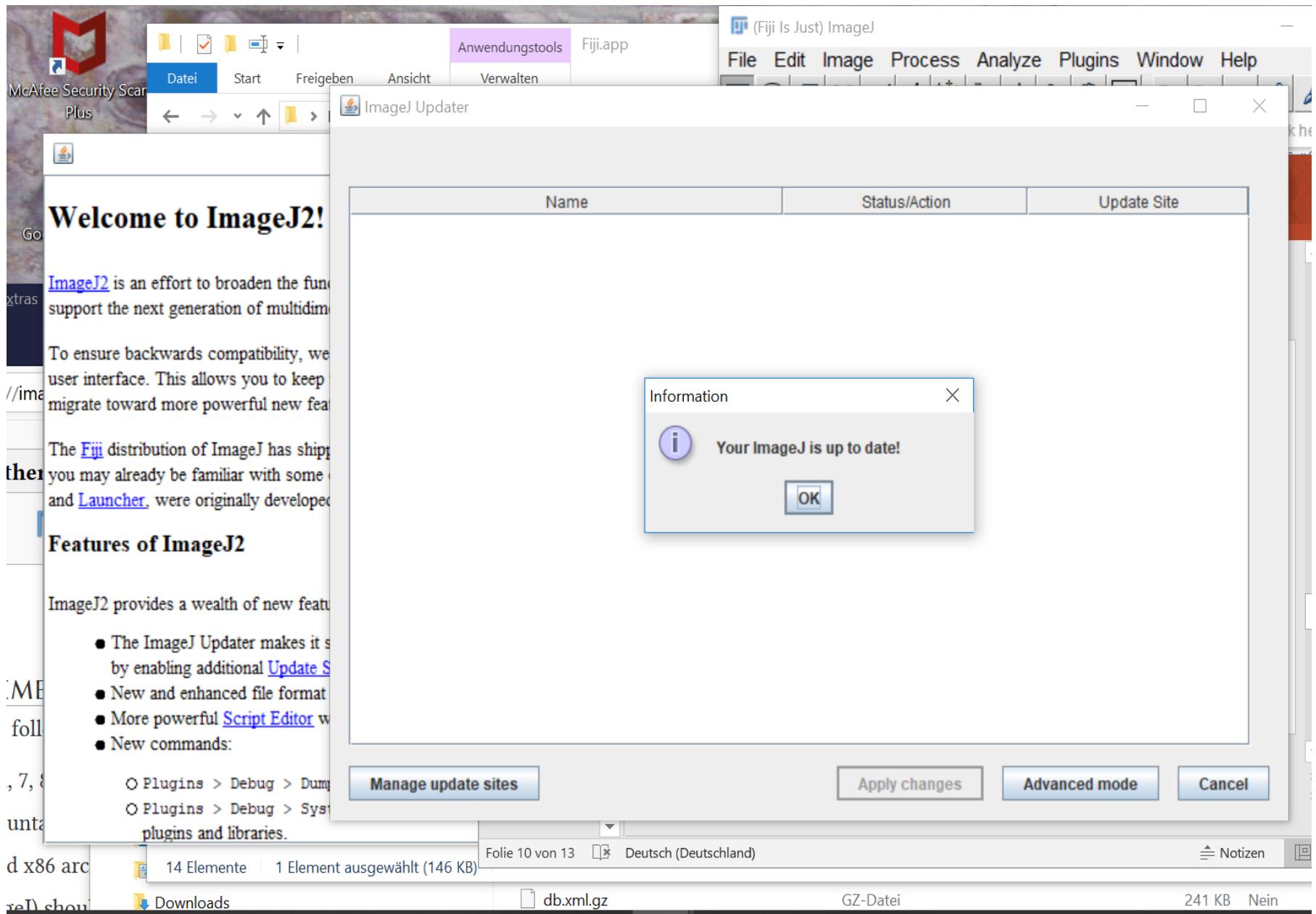
Open ImageJ-win64



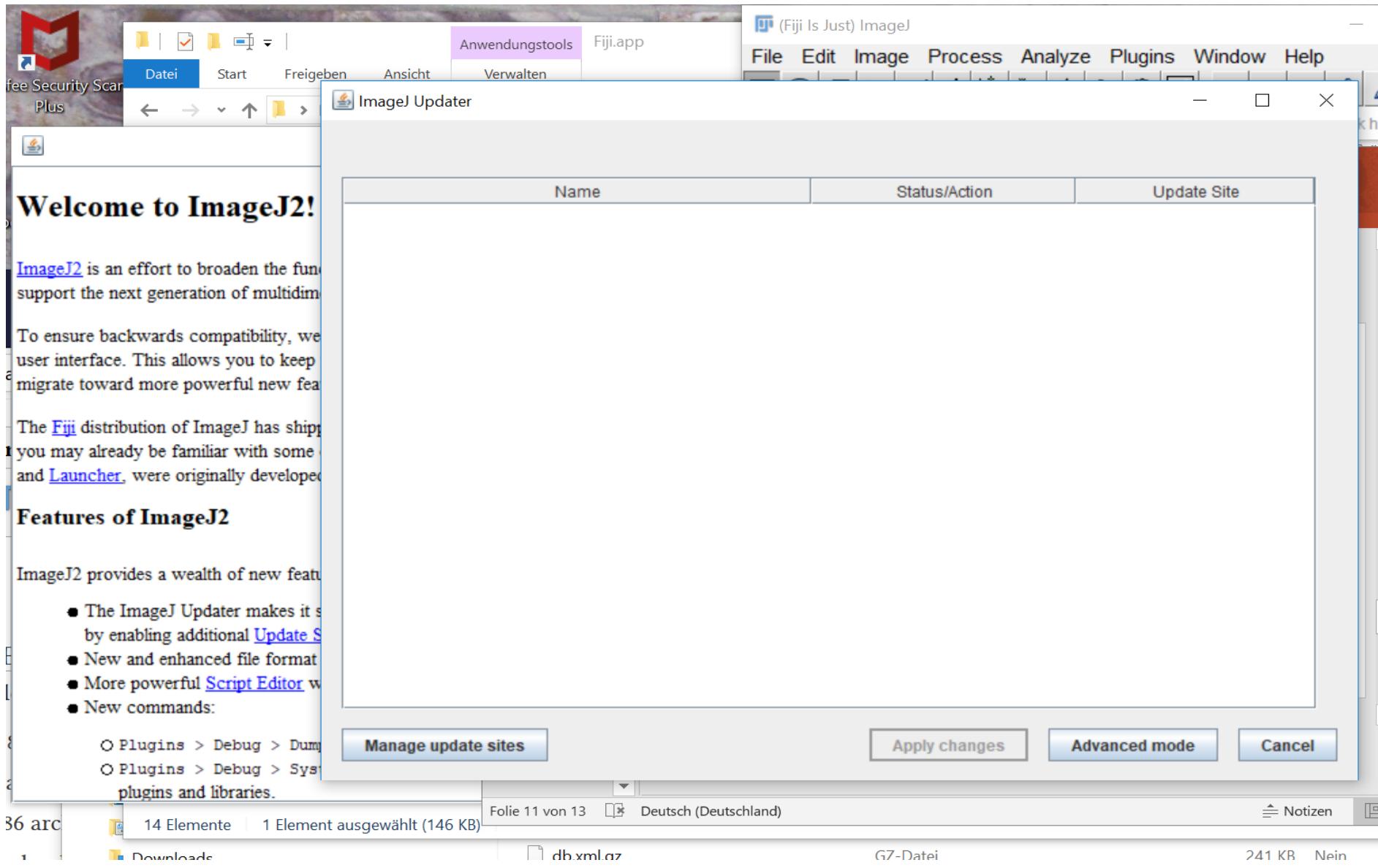
Click on OK for updates



Click on OK



Close Update- and Information-window



Objetivos del taller

- Bióloga con más de 15 años de experiencia en ImageJ
→ Taller muy práctico, desde punto de vista de un usuario
- Desde herramientas simples (que podéis combinar luego a vuestras necesidades) hasta crear un macro
- Idioma: Castellano
(Diapositivas para la introducción en castellano, durante el taller en inglés, porque el software es en inglés)



**Fiji
Is
Just
ImageJ(2)**

Image J/Fiji: ¿Qué es?

W ImageJ - Wikipedia, la enciclopedia libre

Veronica

Es seguro | https://es.wikipedia.org/wiki/ImageJ

No has accedido Discusión Contribuciones Crear una cuenta Acceder

Artículo Discusión Leer Editar Ver historial Buscar en Wikipedia

ImageJ

ImageJ es un programa de procesamiento de imagen digital de dominio público programado en Java desarrollado en el National Institutes of Health.

1 ImageJ fue diseñado con una arquitectura abierta que proporciona extensibilidad vía plugins Java y macros (macroinstrucciones) grabables. 2 Se pueden desarrollar plugins de escaneo personalizado, análisis y procesamiento usando el editor incluido en ImageJ y un compilador Java. Los plug-ins escritos por usuarios hacen posible resolver muchos problemas de procesado y análisis de imágenes, desde imágenes en vivo de las células en tres dimensiones, 3 procesado de imágenes radiológicas, 4 comparaciones de múltiples datos de sistema de imagen 5 hasta sistemas automáticos de hematología. 6 La arquitectura de plugins y entorno de desarrollo integrados de ImageJ lo han convertido en una plataforma popular para enseñar procesamiento de imagen. 7 8

ImageJ puede ejecutarse en un applet en línea, como aplicación ejecutable, o en cualquier computadora con Máquina virtual Java 5 o superior. Hay también distribuciones descargables para Microsoft Windows, Mac OS, Mac OS X, Linux, y Sharp Zaurus PDA. El código fuente de ImageJ está disponible gratuitamente. 9

El desarrollador principal del proyecto, Wayne Rasband, está en el Research Services Branch del National Institute of Mental Health.

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The screenshot shows the Spanish Wikipedia page for ImageJ. The main content area describes ImageJ as an open-source digital image processing program developed by the National Institutes of Health. It highlights its modular architecture, Java programming, and extensive user-developed plugins for various applications like fluorescence microscopy, cell analysis, and radiology. The sidebar includes links to the main page, talk page, edit, history, and search functions, along with a sidebar menu for navigating the site.

ImageJ

http://rsb.info.nih.gov/ij/

Captura de ImageJ

Información general

Desarrollador(es) National Institutes of Health

Última versión estable 1.50i 26 de marzo de 2016 (2 años, 5 meses y 22 días)

Género procesamiento de imagen digital

This screenshot displays the ImageJ software interface. At the top, it shows the URL http://rsb.info.nih.gov/ij/. Below the URL is a toolbar with various icons for image processing. The main window contains several panels: one showing a fluorescence micrograph with red and green channels; another showing a grayscale image of a tissue sample; a third showing a brain scan; and a fourth showing a gel electrophoresis pattern. Below these images are two line graphs. The bottom section is titled "Información general" and provides details about the developer (National Institutes of Health), the latest stable version (1.50i), and the date it was released (March 26, 2016). The category listed is "procesamiento de imagen digital".

„Procesamiento de imagen digital de dominio público, desarrollado en el NIH“

„...resolver muchos problemas de procesado de análisis de imágenes...“

- Imágenes de fluorescencia y campo claro
- Células in vivo
- Imágenes radiológicas
- Sistemas automáticos de hematología

Image J/Fiji: ¿Qué es?



The screenshot shows the Spanish Wikipedia page for "ImageJ". The page title is "ImageJ". The content discusses ImageJ as an open-source digital image processing program developed by the National Institutes of Health. It highlights its extensibility through Java plugins and macros, its use in fluorescence and brightfield microscopy, cell analysis, radiological imaging, and hematological systems. The page also notes its availability as a Java applet or executable, and its support for various operating systems. A sidebar on the left provides links to other Wikipedia pages and site navigation.

„Procesamiento de imagen digital de dominio público, desarrollado en el NIH“

„...resolver muchos problemas de procesado de análisis de imágenes...“

- Imágenes de fluorescencia y campo claro
- Células in vivo
- Imágenes radiológicas
- Sistemas automáticos de hematología

¿Para qué?

Convertir imágenes en valores numéricos, objetivos, estadísticamente relevantes → imprescindible para publicar en revistas de alto impacto

Important tips to use Image J/Fiji (or any other image analysis program)

The better the pictures, the easier the image analysis

- Optimize sample preparation, for example low background and good signal to noise ratio
- Take high quality images:
 - uncompressed
 - in tif format (not jpg!)
 - black and white
 - at the highest bit which allows your microscope (8 bit = 256 grey levels, 16 bit = 65536 grey levels). 12 or 16 bit images are not visible on conventional picture viewing program.

Important tips to use Image J/Fiji (or any other image analysis program)

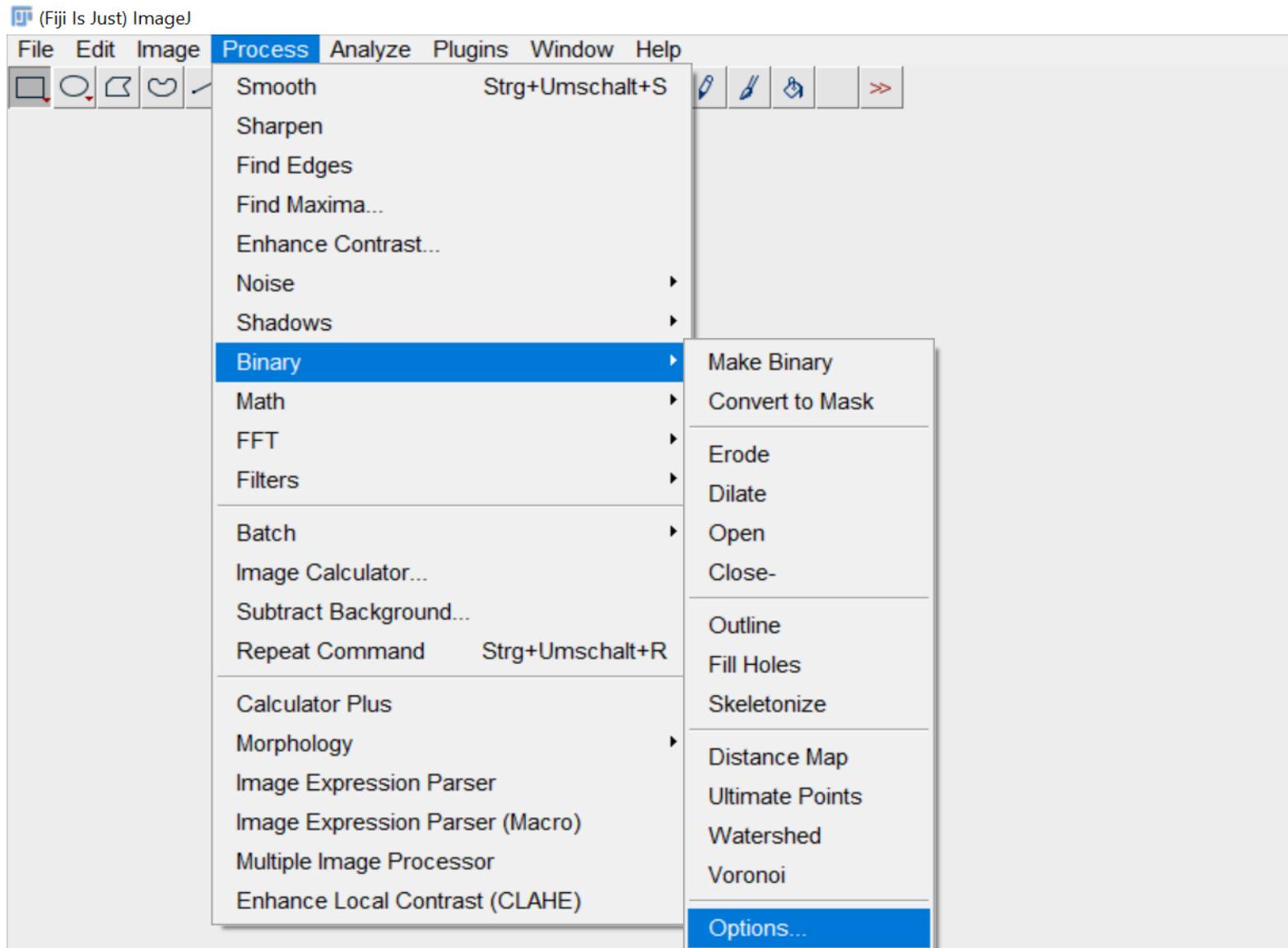
**Don't take all the pictures and
then try to analyze them!**

- Once microscope setting and images are optimized, take 2 or 3 photos.
- Always use a copy of your original image.
- Analyze these photos in Image J.
- Change microscope settings according to the needs of image analysis.
- Repeat this procedure until everything is optimized!
- Then take all pictures of your experiment (at the same settings!) and analyze them.

Workshop Overview

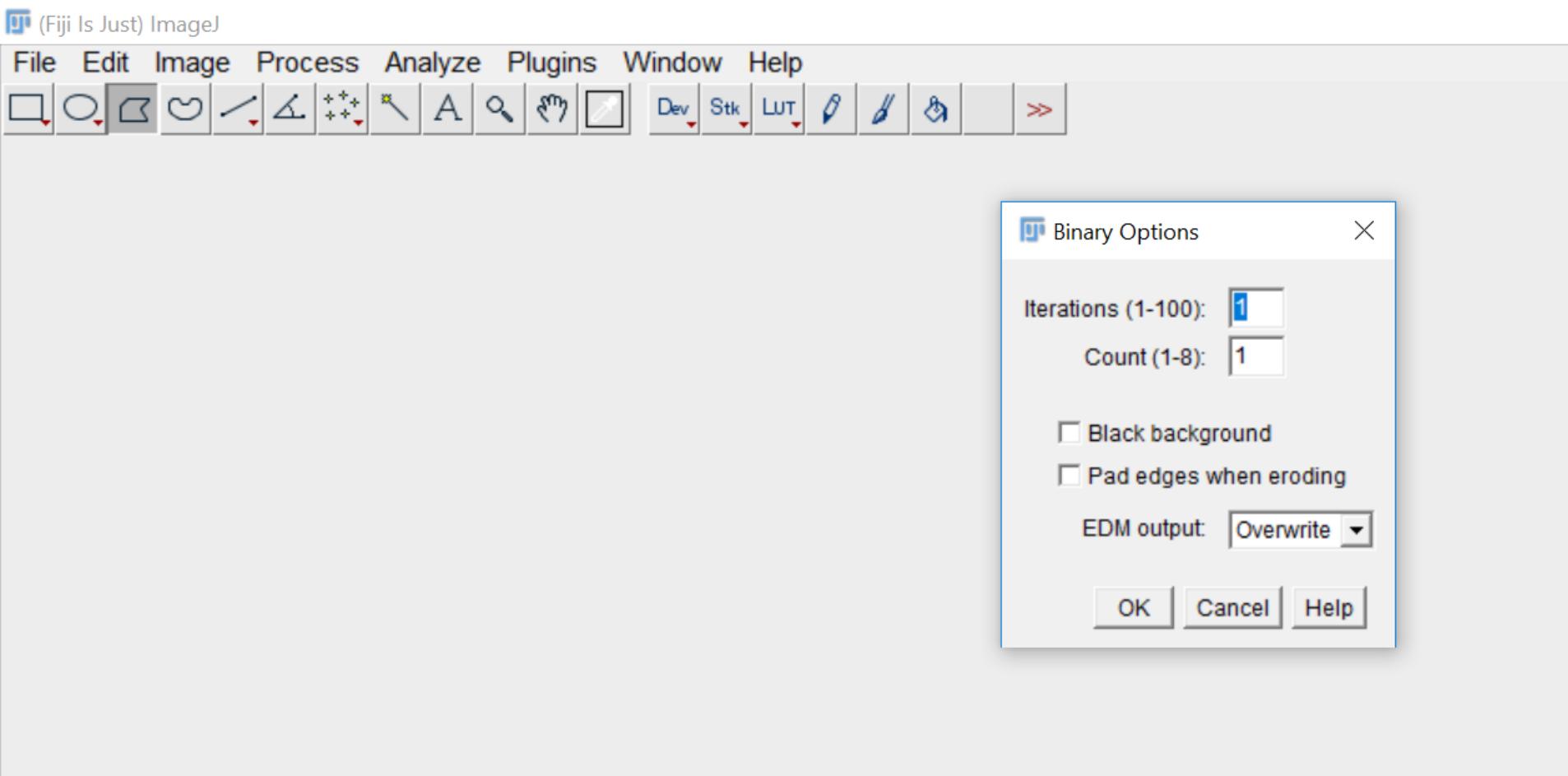
- Examples:
 - Counting Particles of Different Sizes
 - Measuring Fluorescence Intensities
- Using a Macro
 - Wound Healing Macro
 - Determination of Cell Shape
- Creating a Macro
 - Basic steps
- Practical Session with Example Images

After Installation check in *Process*: Click on *Binary* → *Options*



Multi-point or point (right click to switch; double click to configure)

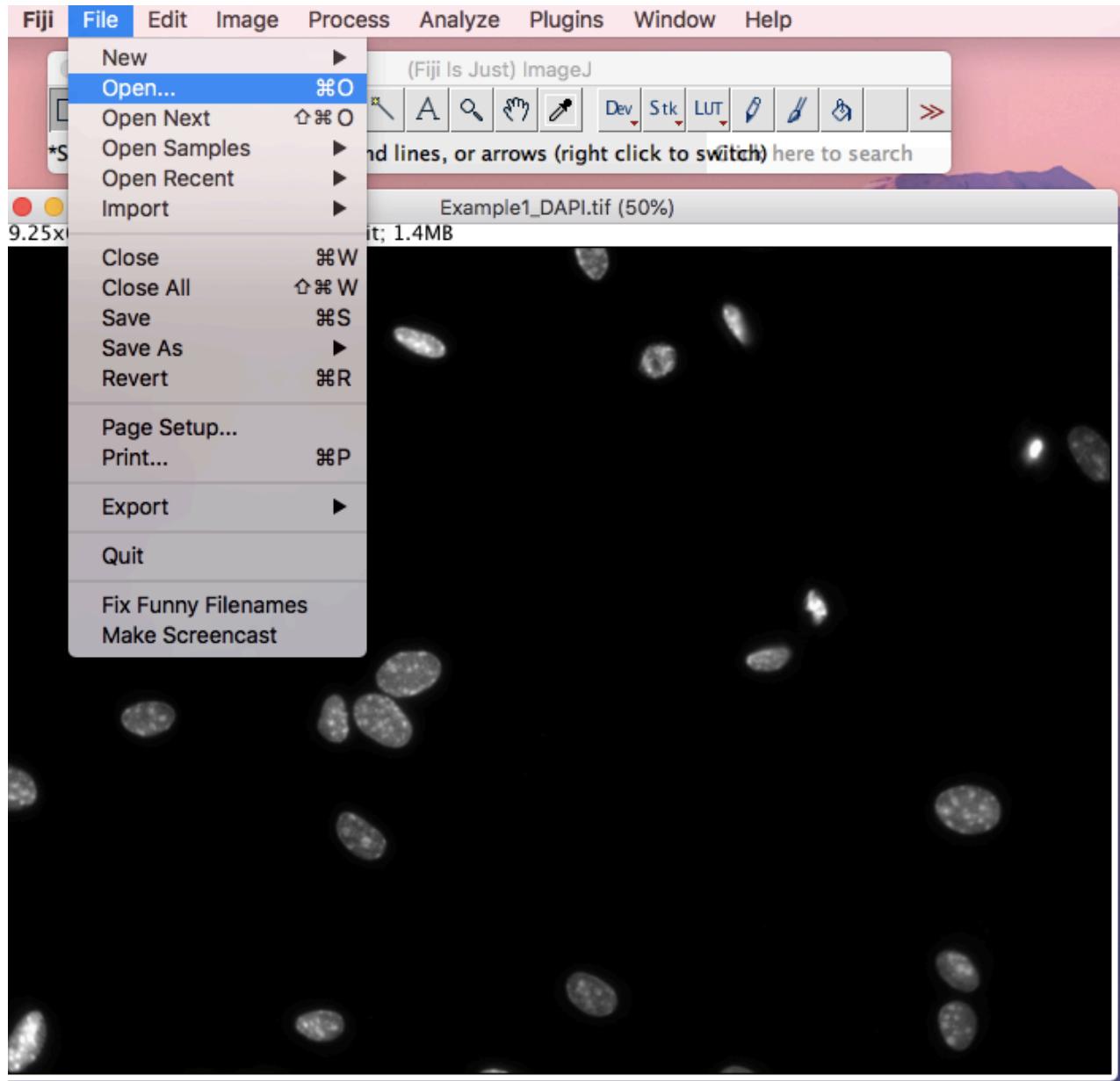
Copy these settings:



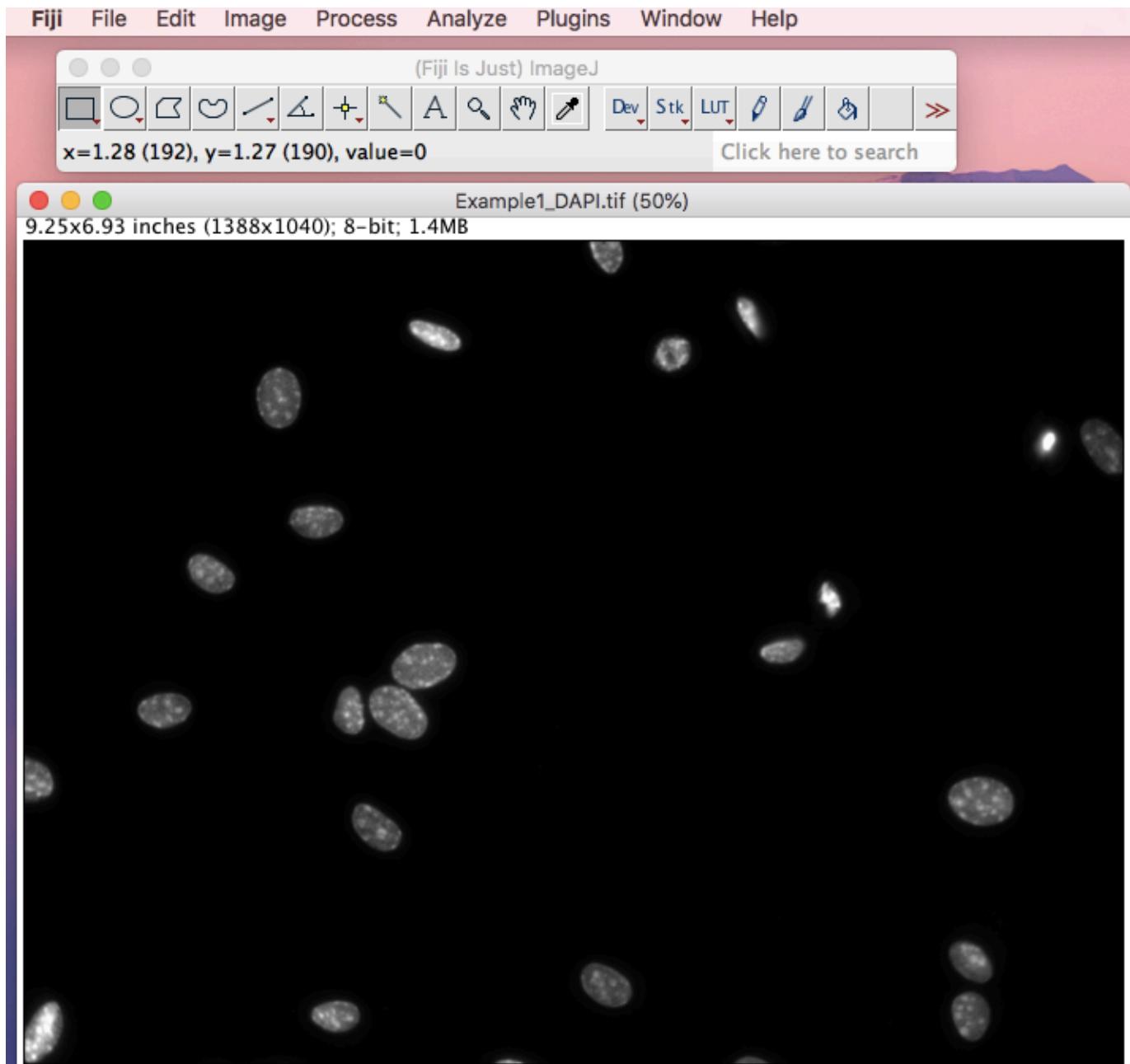
Workshop Overview

- Examples:
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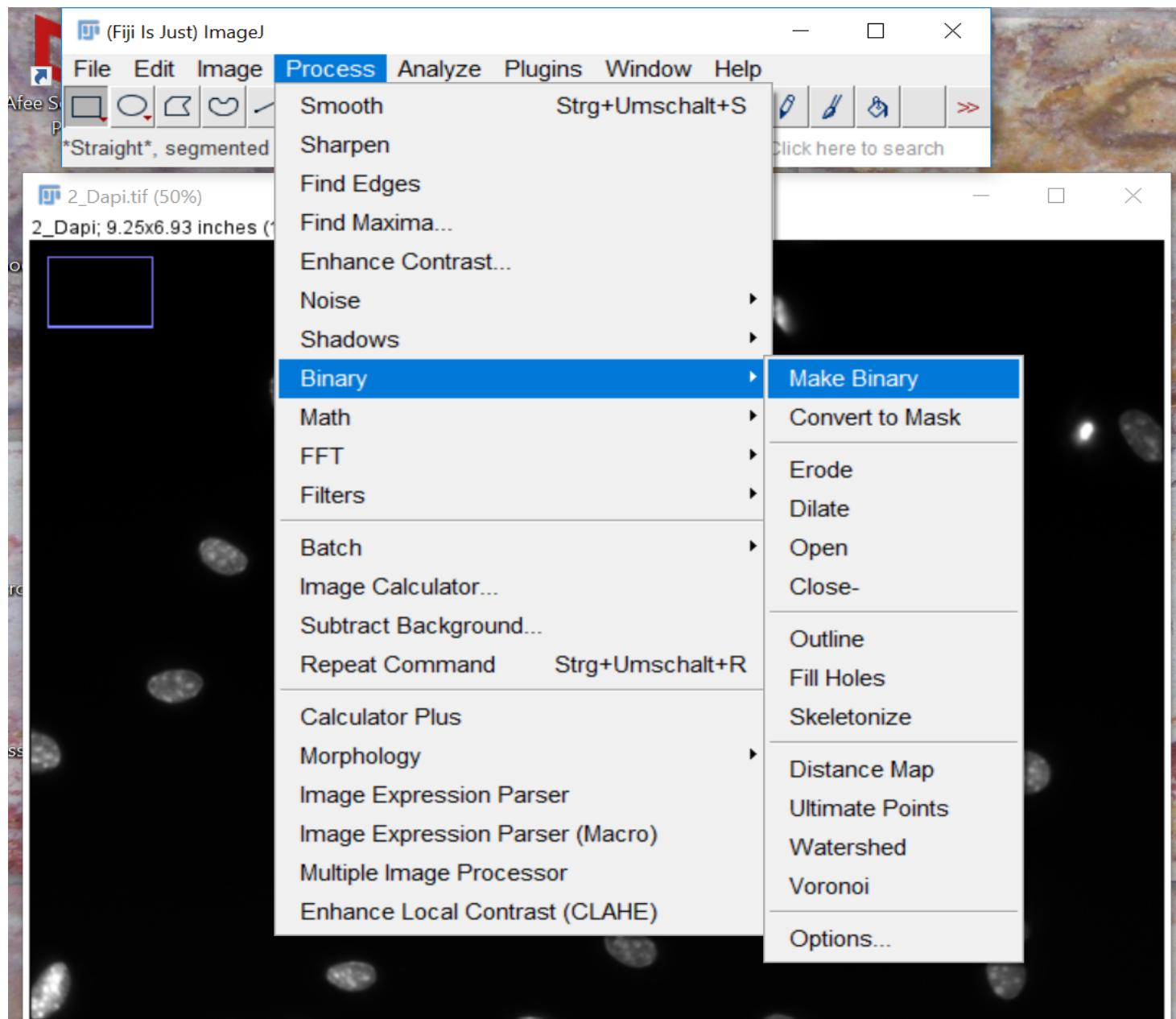
In folder Count open image Example1_DAPI



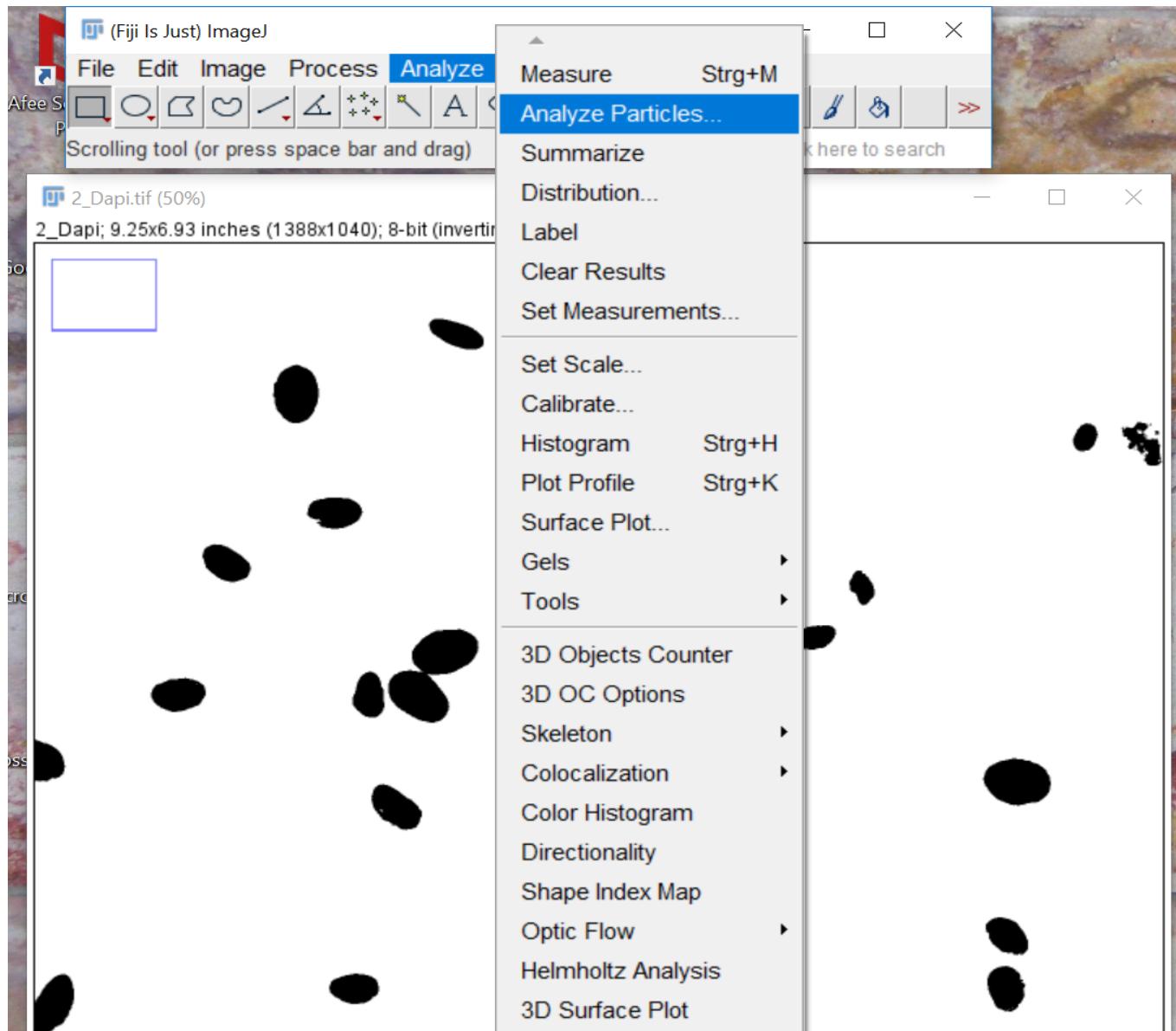
How many nuclei are in the image?



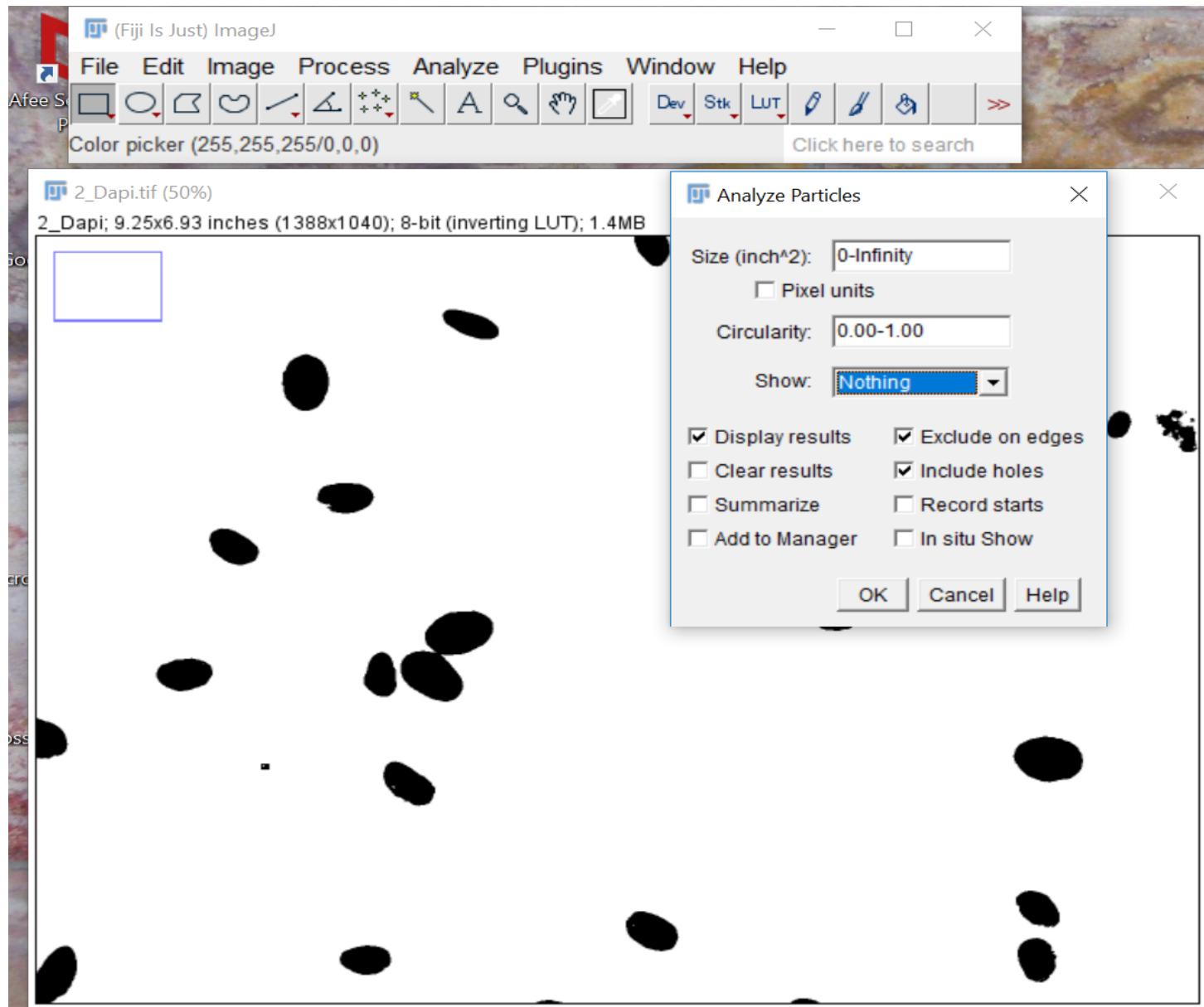
Make binary image



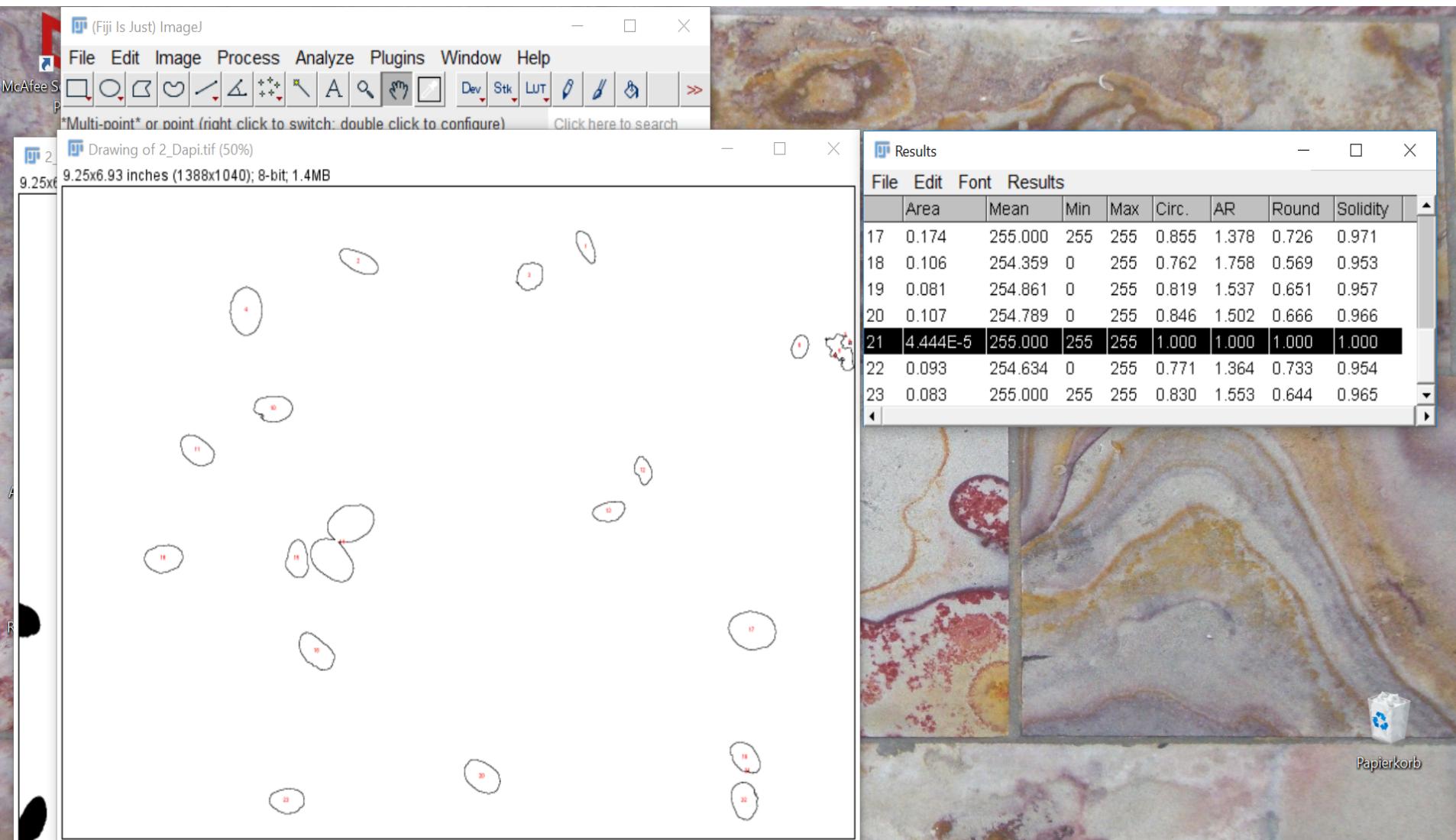
Click on Analyze → Analyze Particles



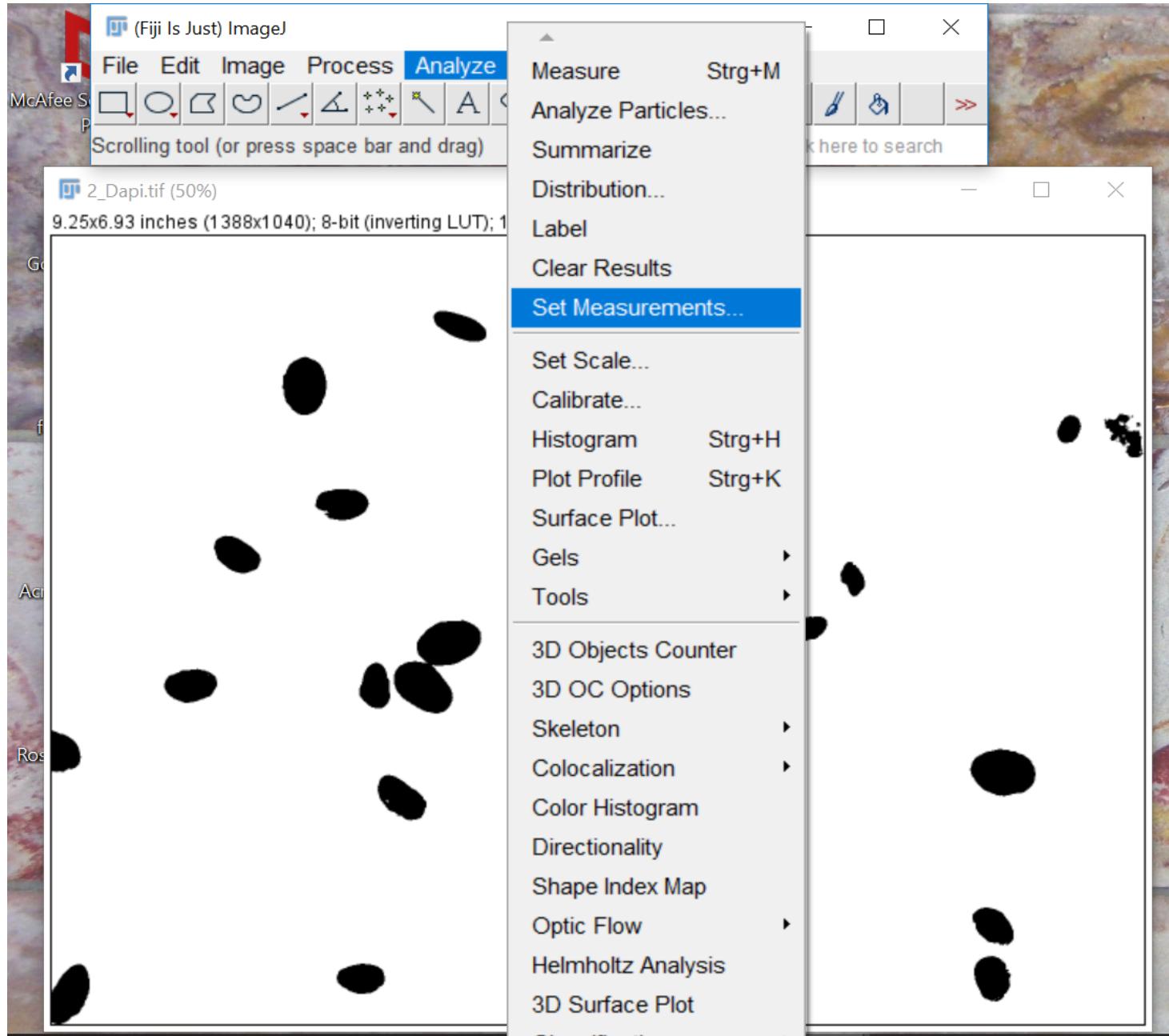
Change show *nothing* to show *outlines* and copy other parameters. Click on OK.



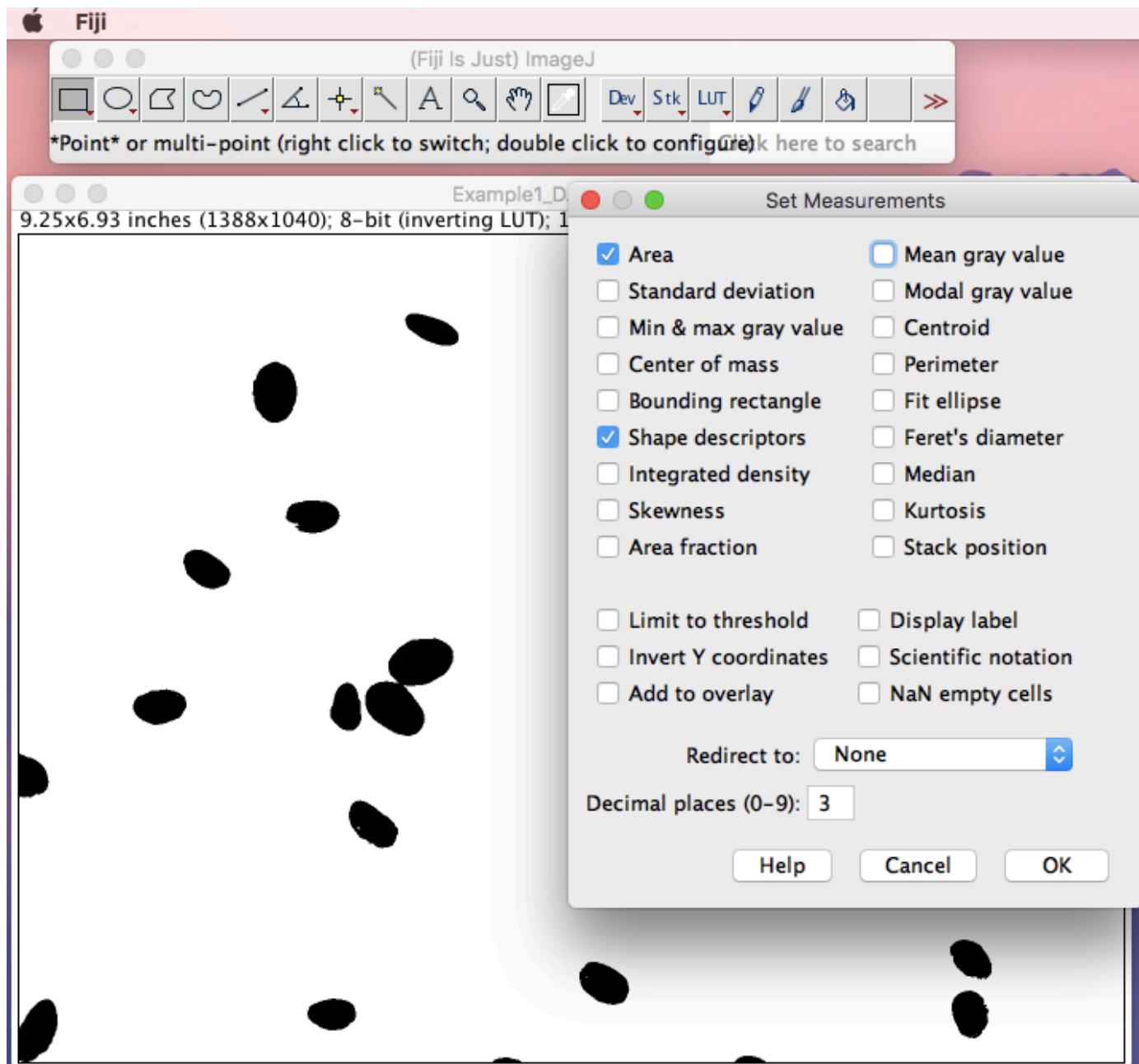
Results correspond to outlines of particles



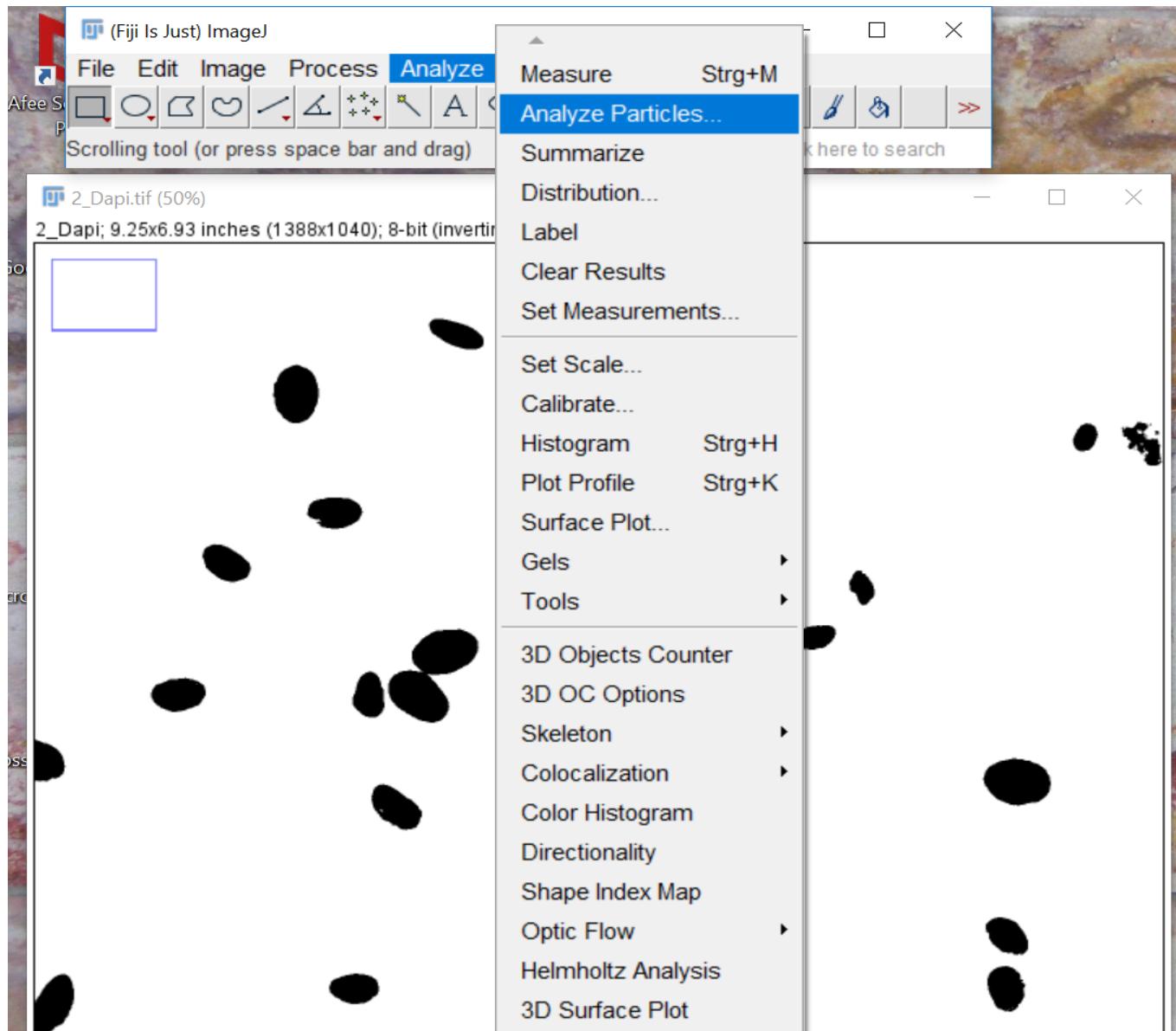
Change the parameters of *Results*



Choose area and shape descriptions



Click on Analyze → Analyze Particles



Optimize the selection of the particles by size and circularity

Finder Archivo Edición Visualización Ir Ventana Ayuda

(Fiji Is Just) ImageJ

(Fiji Is Just) ImageJ 2.0.0-rc-68/1.52g; Java 1.8.0_172 [64-bit]; Click here to search

Example1_DAPI.tif (50%)

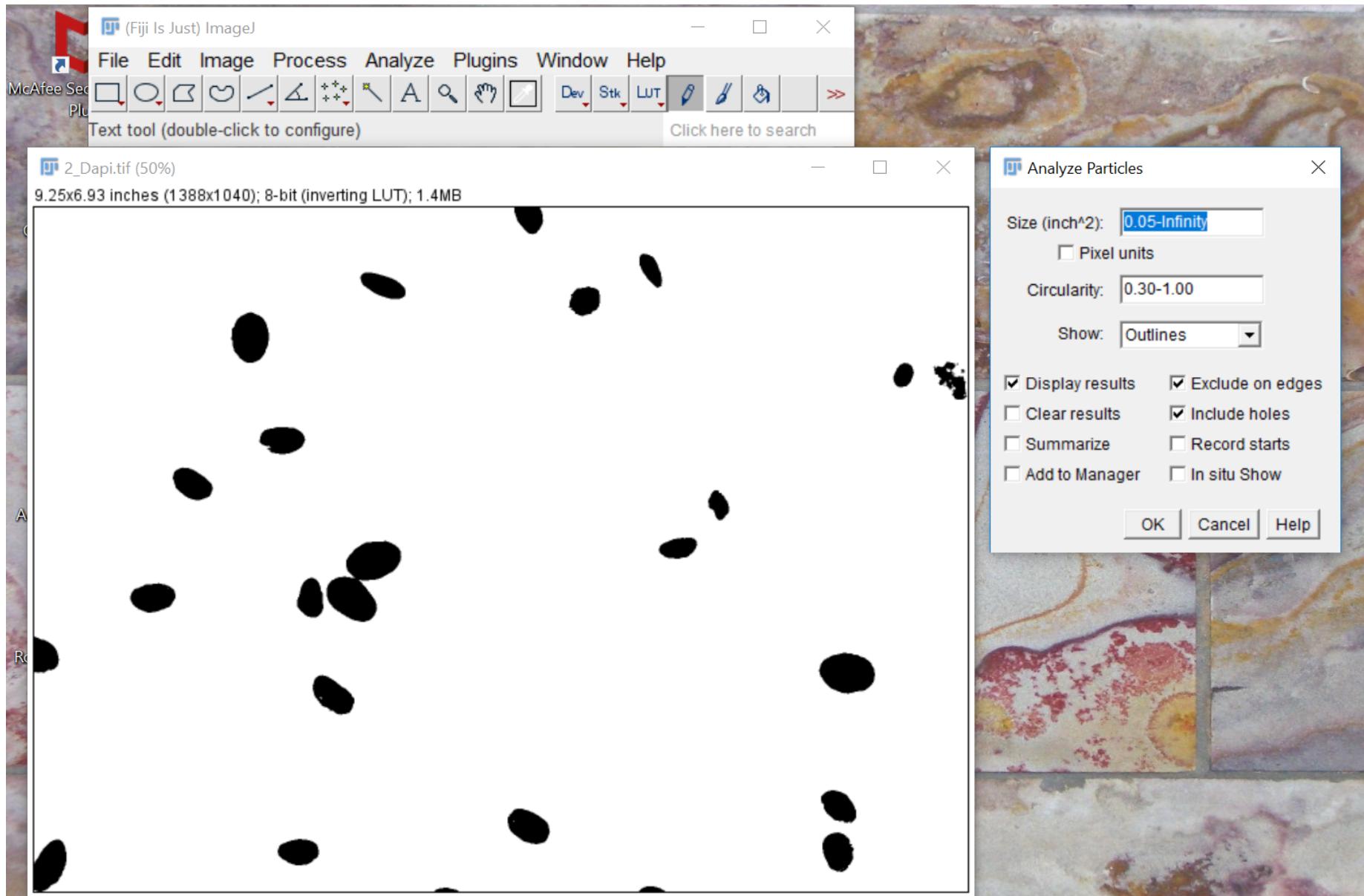
9.25x6.93 inches (1388x1040); 8-bit (inverting LUT); 1.4MB

ROI Manager

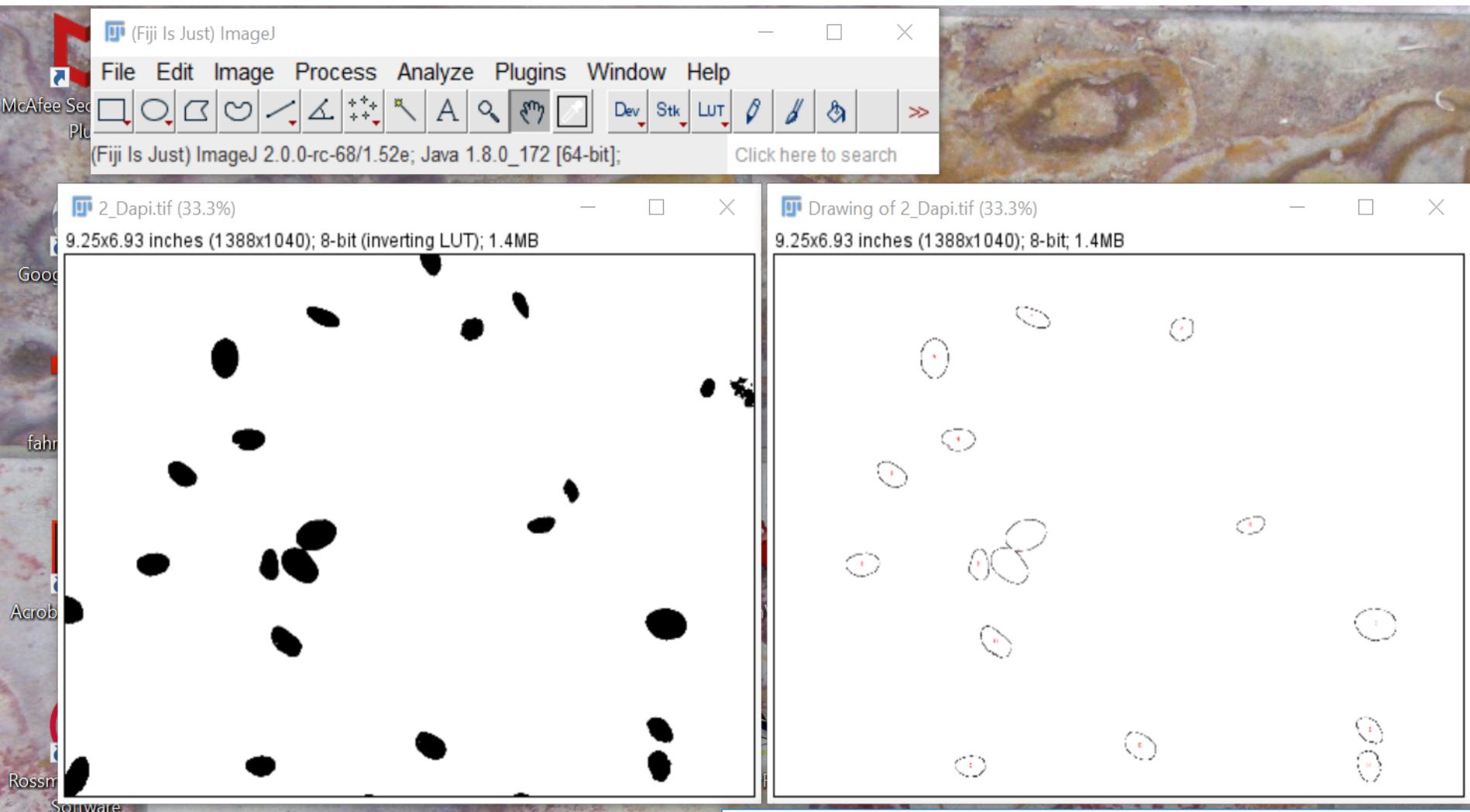
Area	Circ.	AR	Round	Solidity	
1	0.047	0.686	2.311	0.433	0.947
2	0.078	0.705	2.369	0.422	0.965
3	0.069	0.828	1.158	0.863	0.946
4	0.147	0.858	1.376	0.727	0.969
5	0.061	0.278	2.196	0.455	0.726
6	0.037	0.847	1.394	0.718	0.948
7	2.222E-4	1.000	1.553	0.644	0.909
8	9.333E-4	0.880	1.108	0.903	0.875
9	8.889E-5	0.785	2.646	0.378	0.667
10	0.095	0.704	1.618	0.618	0.956
11	0.091	0.827	1.568	0.638	0.963
12	0.041	0.765	1.662	0.602	0.919
13	0.061	0.777	1.880	0.532	0.958
14	0.328	0.453	1.935	0.517	0.819
15	0.079	0.825	1.594	0.627	0.961
16	0.101	0.824	1.565	0.639	0.963

Add [t]
Update
Delete
Rename...
Measure
Deselect
Properties...
Flatten [F]
More »
 Show All
 Labels

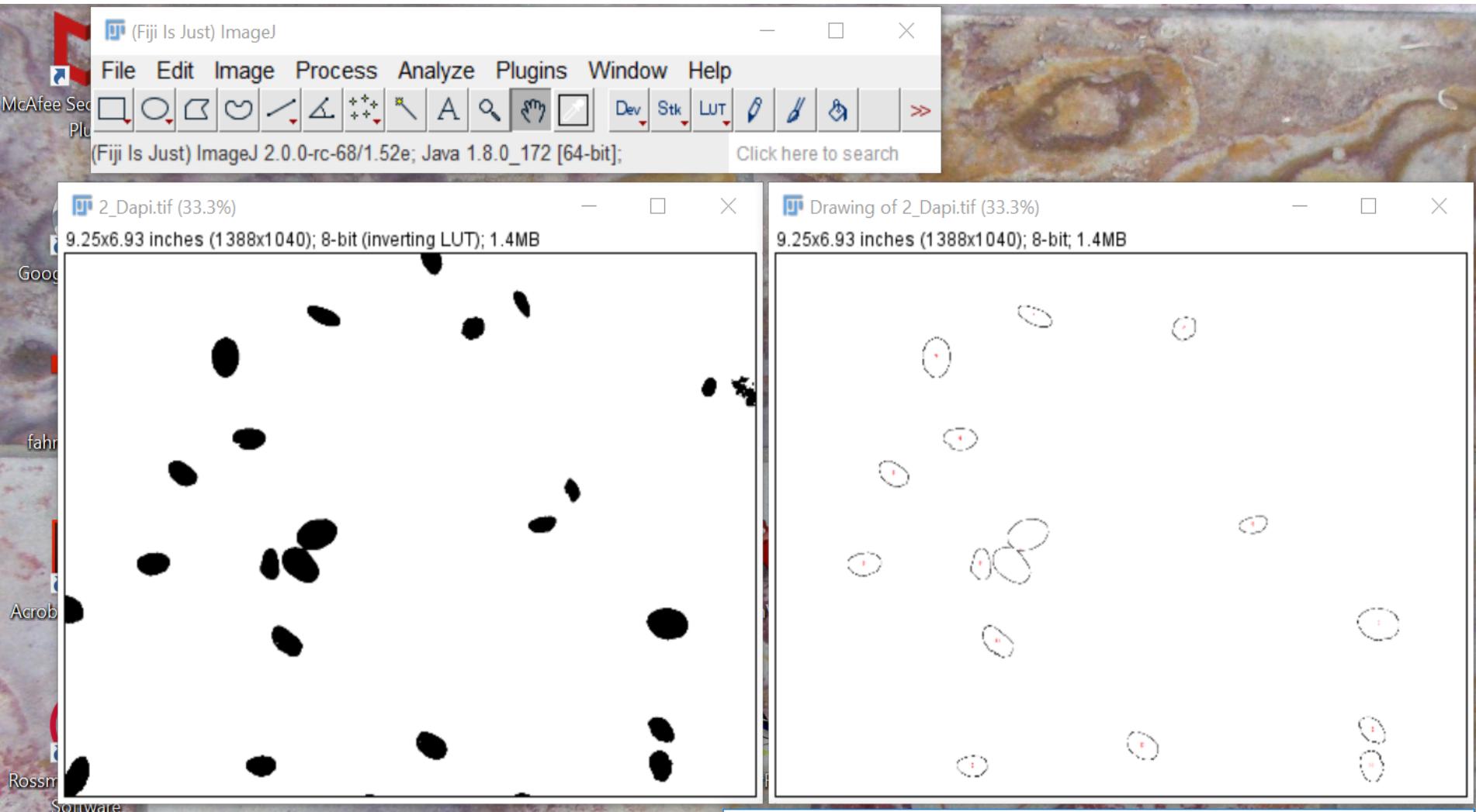
Optimize the selection of the particles by size and circularity



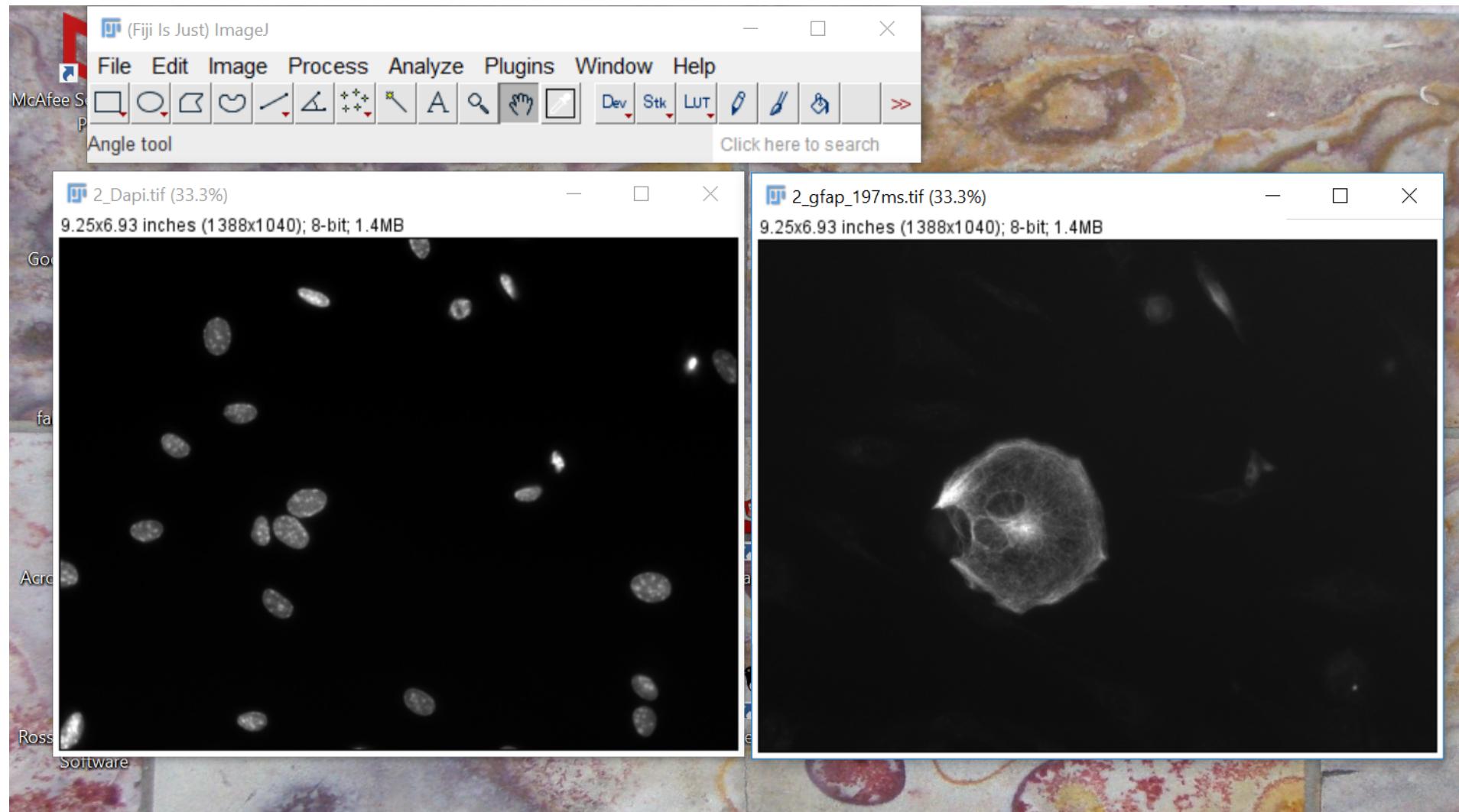
After optimization the selection of nuclei is more accurate



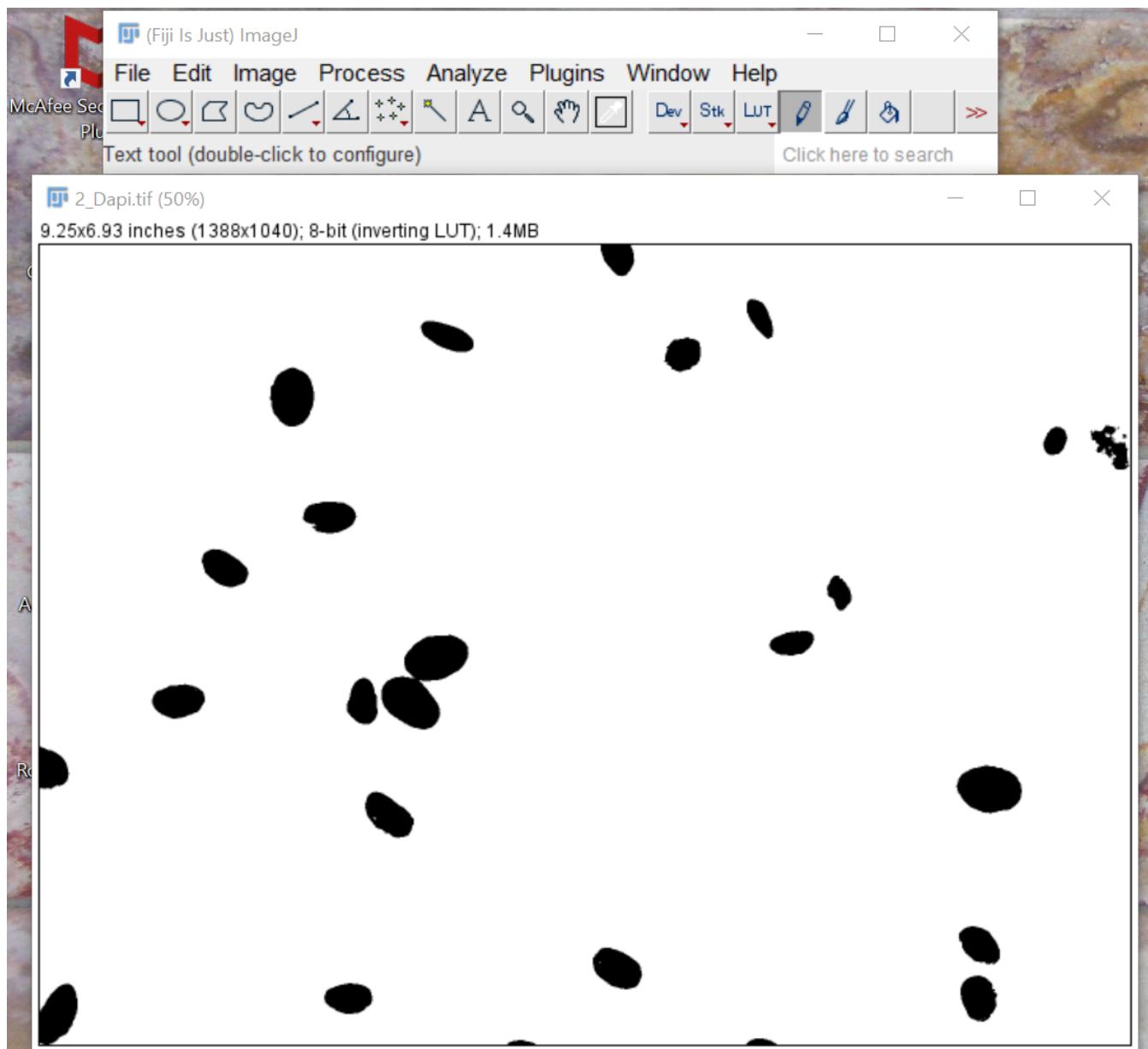
What to do with not classifiable structures?



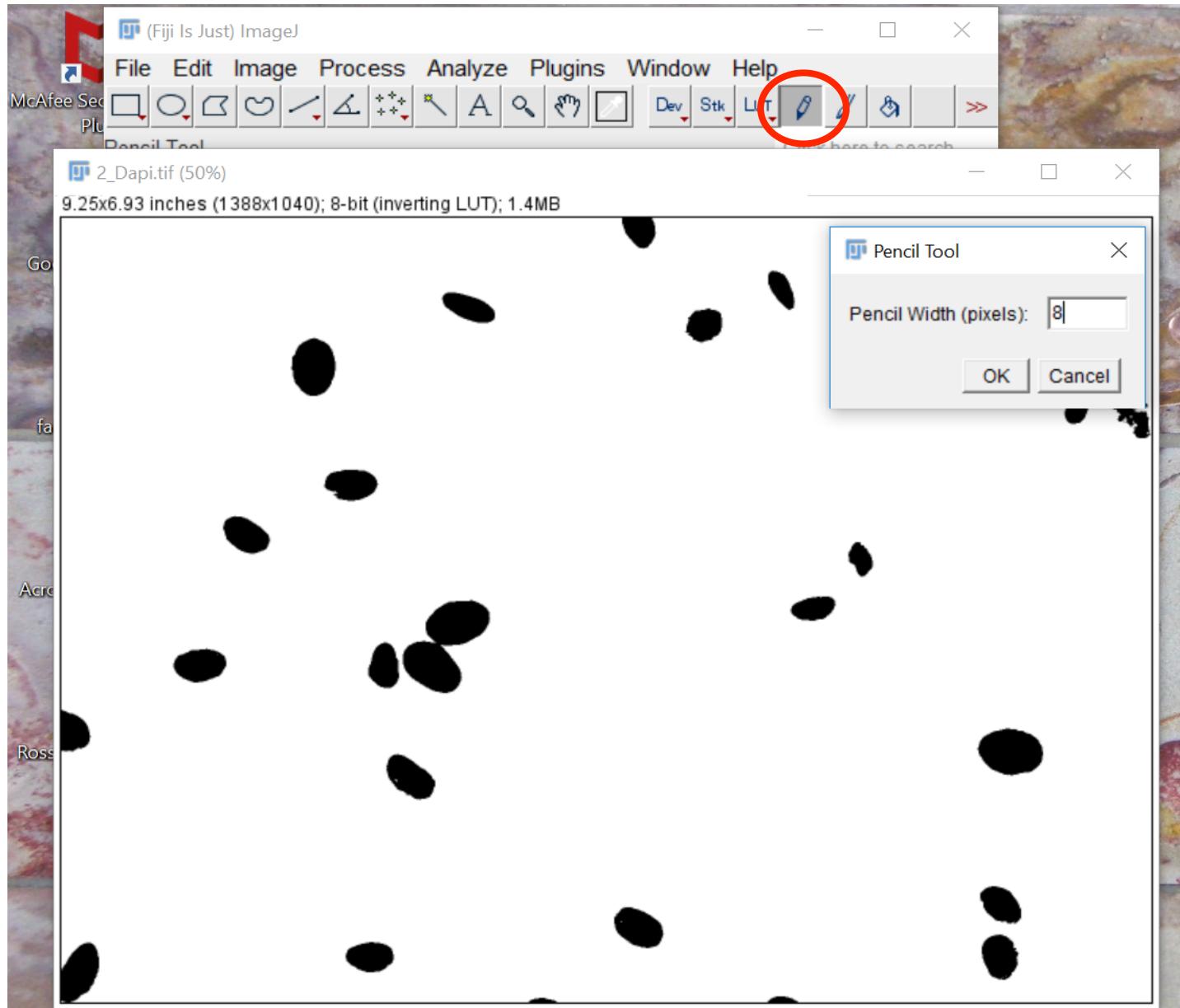
We check by eye the biology!



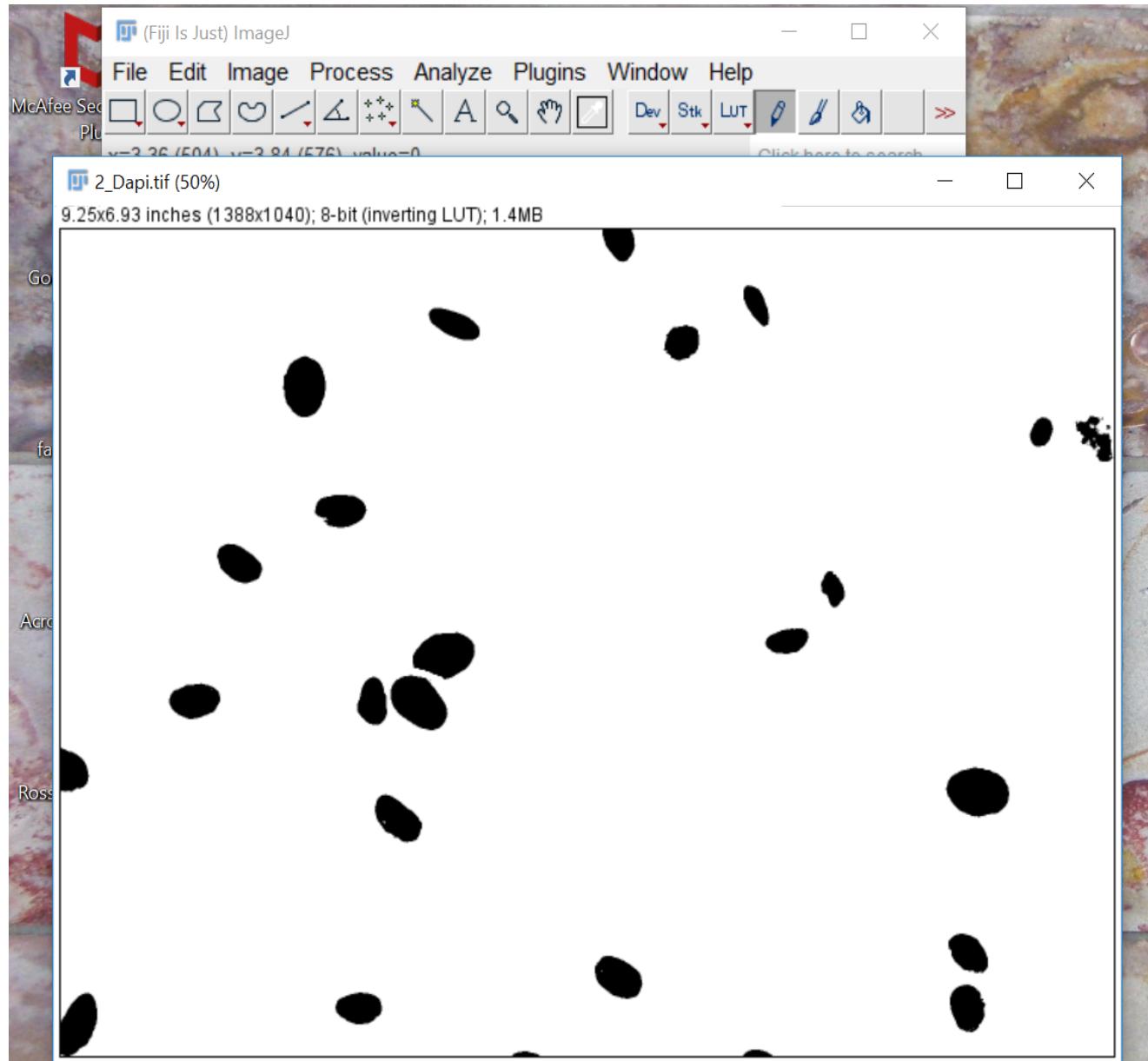
How to separate two structures?



By using the pencil tool. Adjust the width by double click on icon (Width: 8 pixels)



After using the pencil tool click on *Analyze particles*



Check mask and results

(Fiji Is Just) ImageJ

x=5.08 (762), y=1.27 (190), value=255

Click here to search

Drawing of Example1_DAPI.tif (50%)

9.25x6.93 inches (1388x1040); 8-bit; 1.4MB

The image shows a Fiji window titled "Drawing of Example1_DAPI.tif (50%)". The canvas displays a white background with several red-outlined elliptical regions, each containing a small red number from 1 to 16. The numbers are distributed as follows: 1 (top center), 2 (center right), 3 (left), 4 (center left), 5 (bottom left), 6 (center bottom), 7 (middle left), 8 (middle right), 9 (top right), 10 (bottom left), 11 (center right), 12 (bottom center), 13 (bottom right), 14 (bottom center), 15 (bottom right), and 16 (bottom left). The top menu bar includes icons for selection tools, a magnifying glass, a search bar, and various image processing options like Dev, Stk, LUT, and ROI.

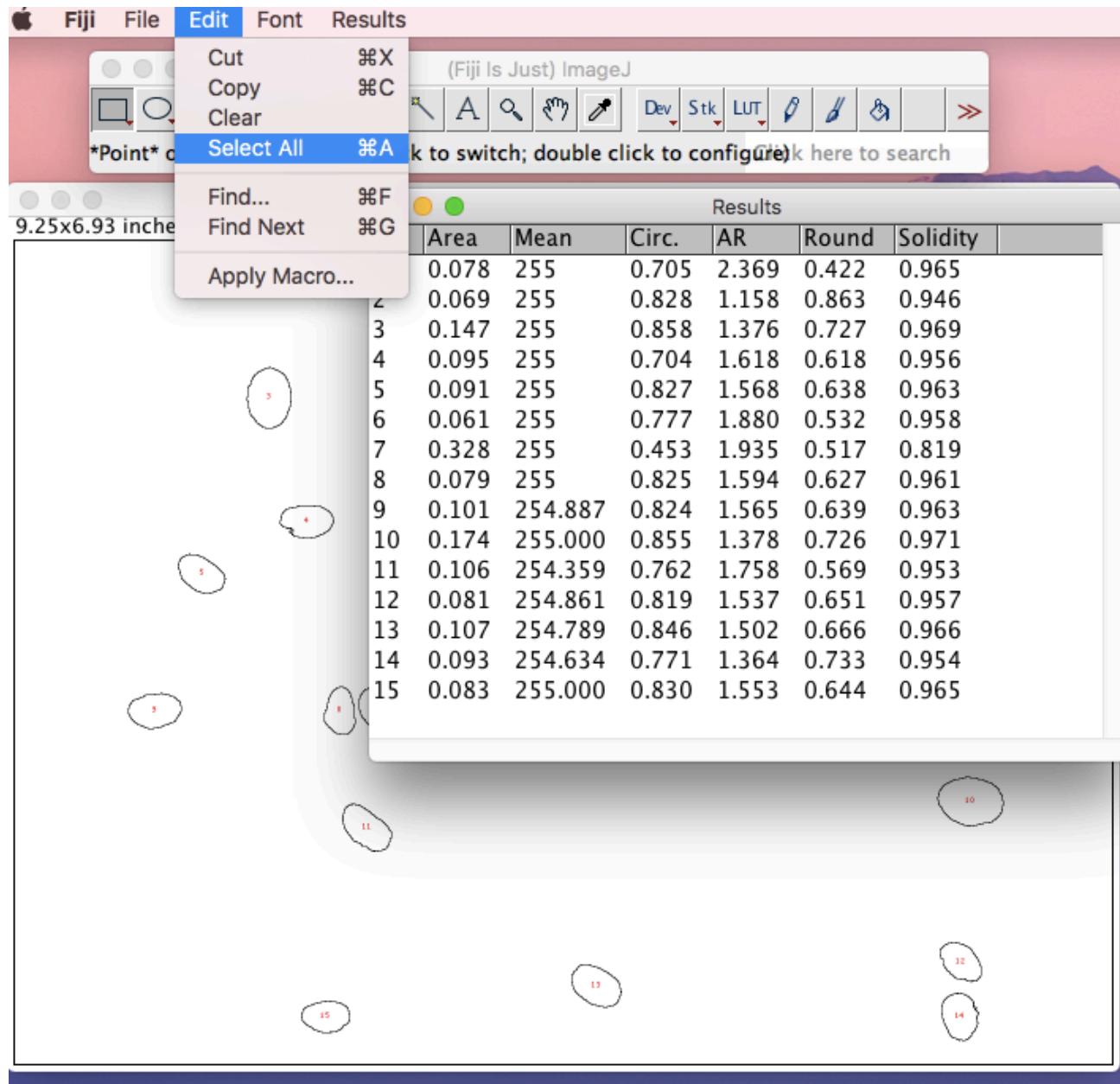
	Area	Mean	Circ.	AR	Round	Solid
1	0.078	255	0.705	2.369	0.422	0.969
2	0.069	255	0.828	1.158	0.863	0.946
3	0.147	255	0.858	1.376	0.727	0.969
4	0.095	255	0.704	1.618	0.618	0.956
5	0.091	255	0.827	1.568	0.638	0.963
6	0.061	255	0.777	1.880	0.532	0.958
7	0.151	255	0.802	1.465	0.683	0.950
8	0.151	255	0.795	1.690	0.592	0.948
9	0.079	255	0.825	1.594	0.627	0.961
10	0.101	254.887	0.824	1.565	0.639	0.963
11	0.174	255.000	0.855	1.378	0.726	0.971
12	0.106	254.359	0.762	1.758	0.569	0.953
13	0.081	254.861	0.819	1.537	0.651	0.951
14	0.107	254.789	0.846	1.502	0.666	0.966
15	0.093	254.634	0.771	1.364	0.733	0.954
16	0.083	255.000	0.830	1.553	0.644	0.969

ROI Manager

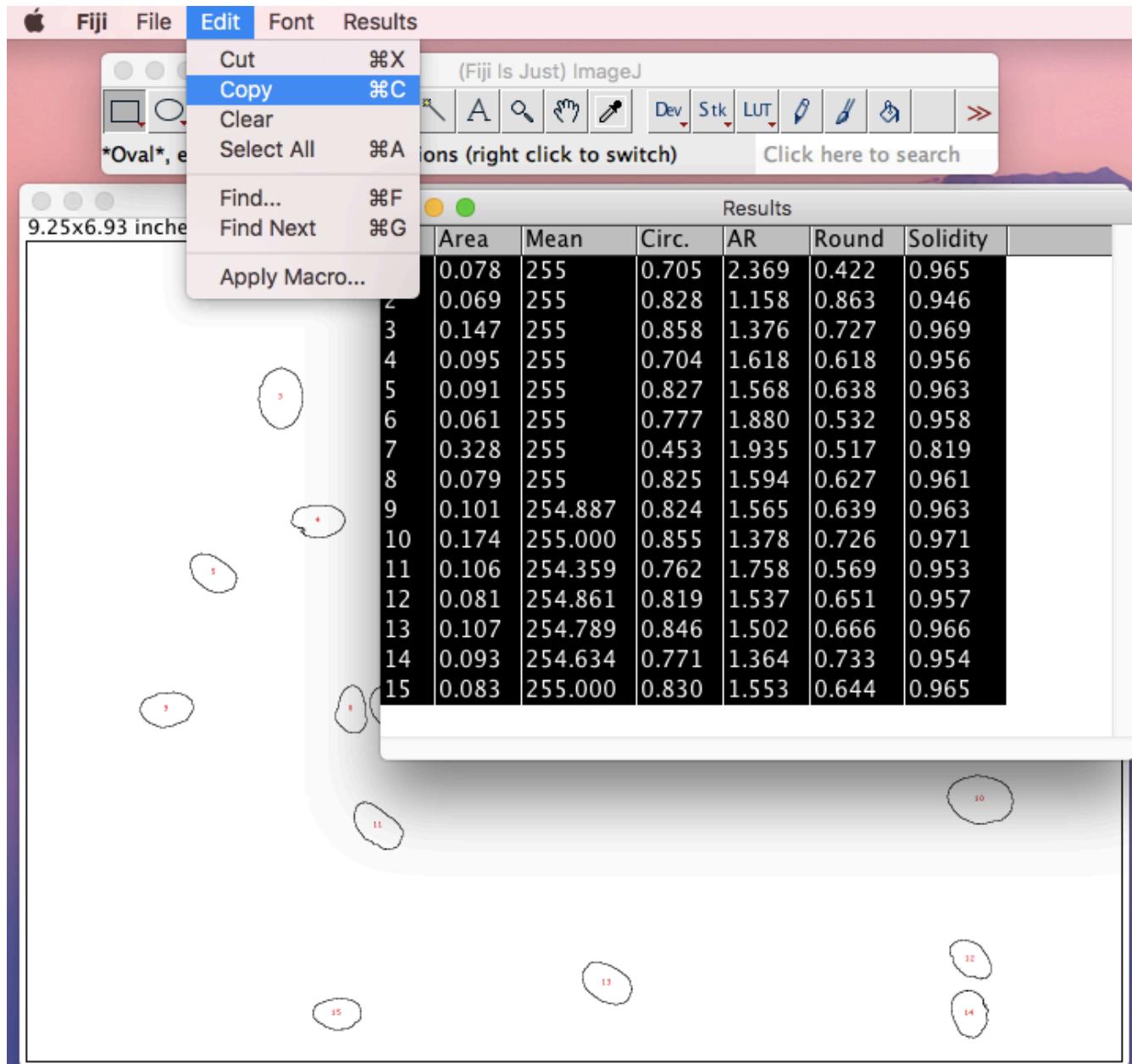
- 0001-0119
- 0002-0142
- 0003-0197
- 0004-0354
- 0005-0420**
- 0006-0518
- 0007-0536
- 0008-0595
- 0009-0593
- 0010-0593
- 0011-0708
- 0012-0741
- 0013-0911
- 0014-0941
- 0015-0980

Add [t]
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 Delete
 Rename...
 Measure
 Deselect
 Properties...
 Flatten [F]
 More »
 Show All
 Labels

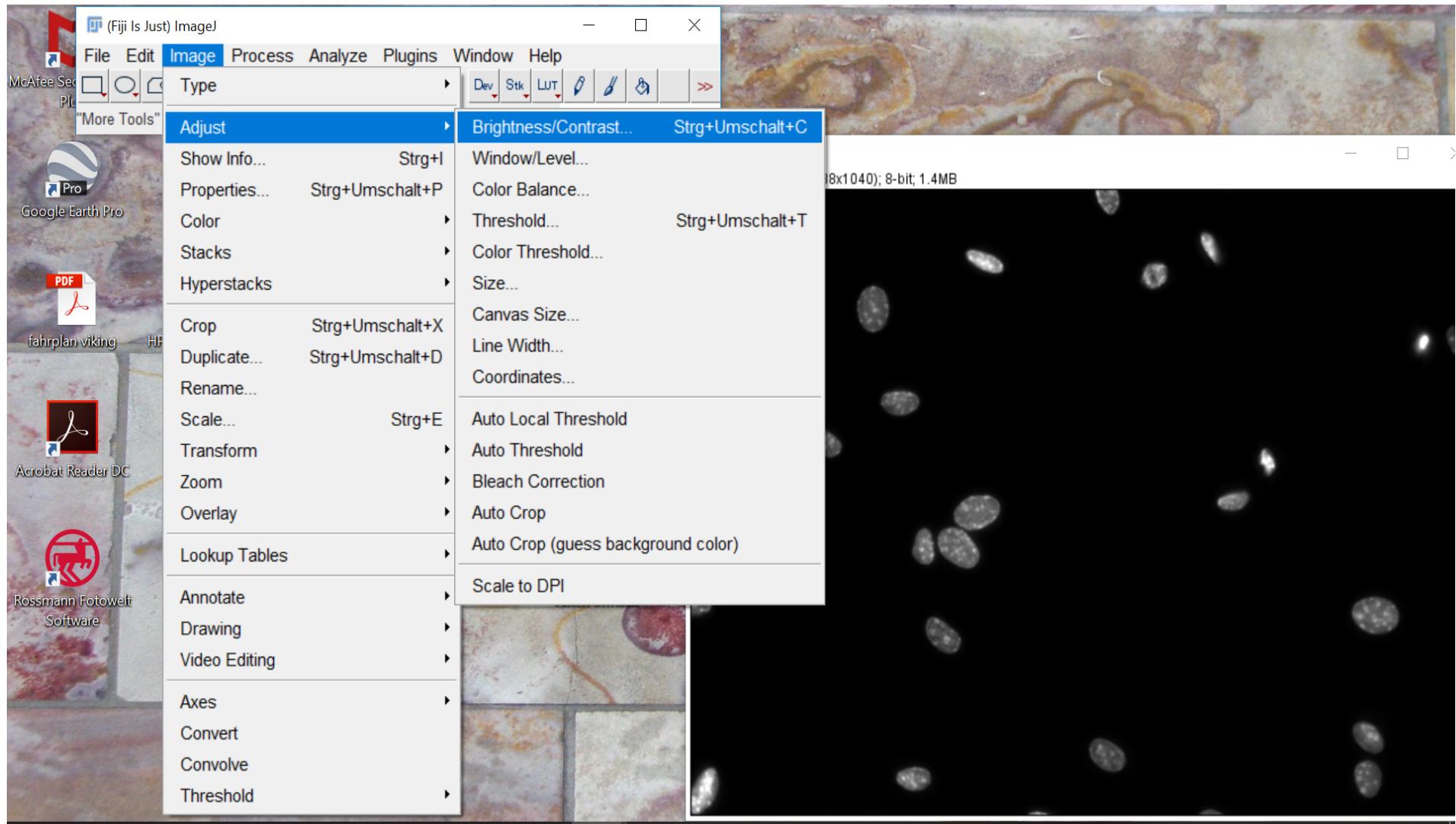
Edit → Select all to copy the data into Excel



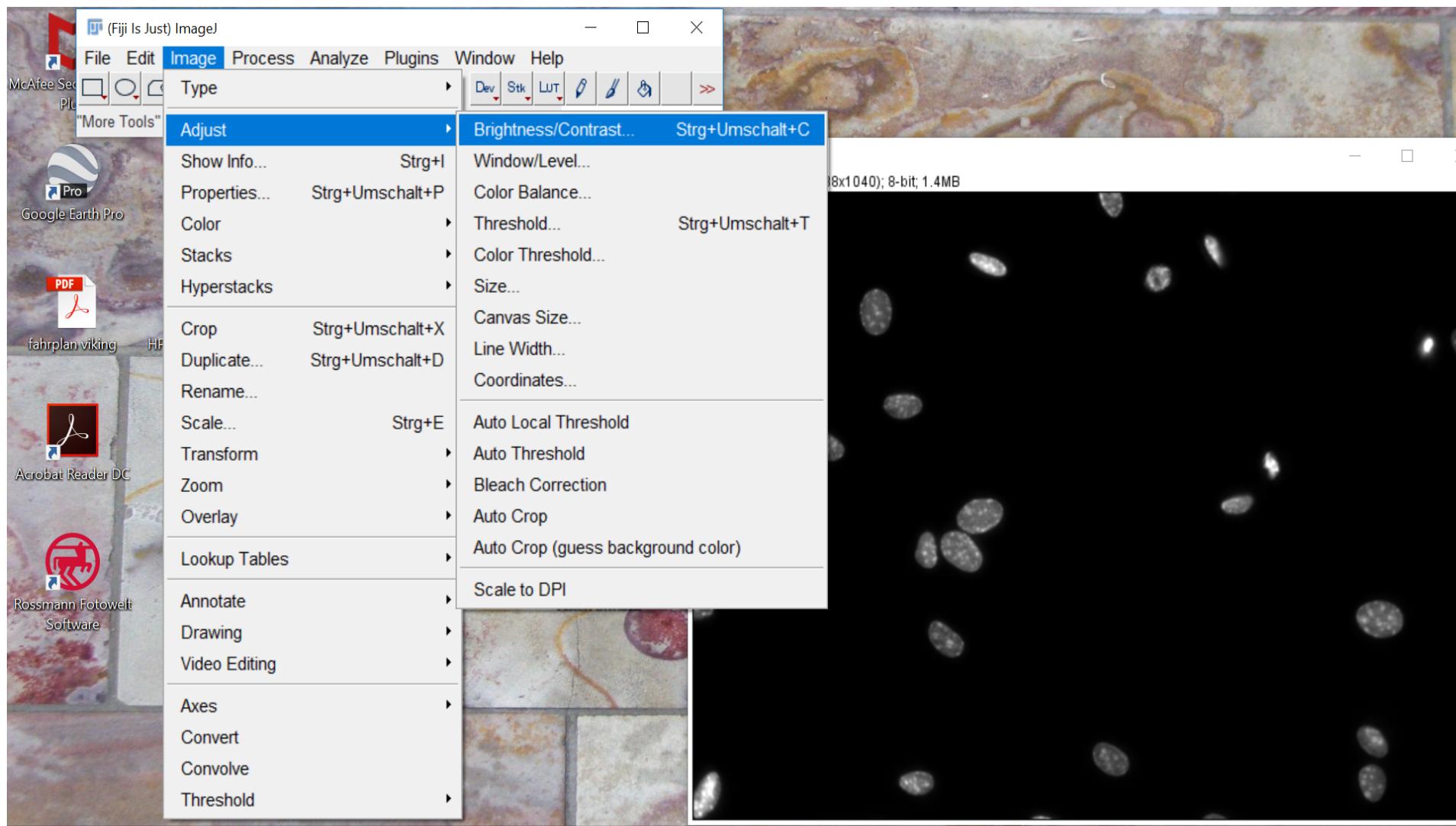
Edit → Click on Copy



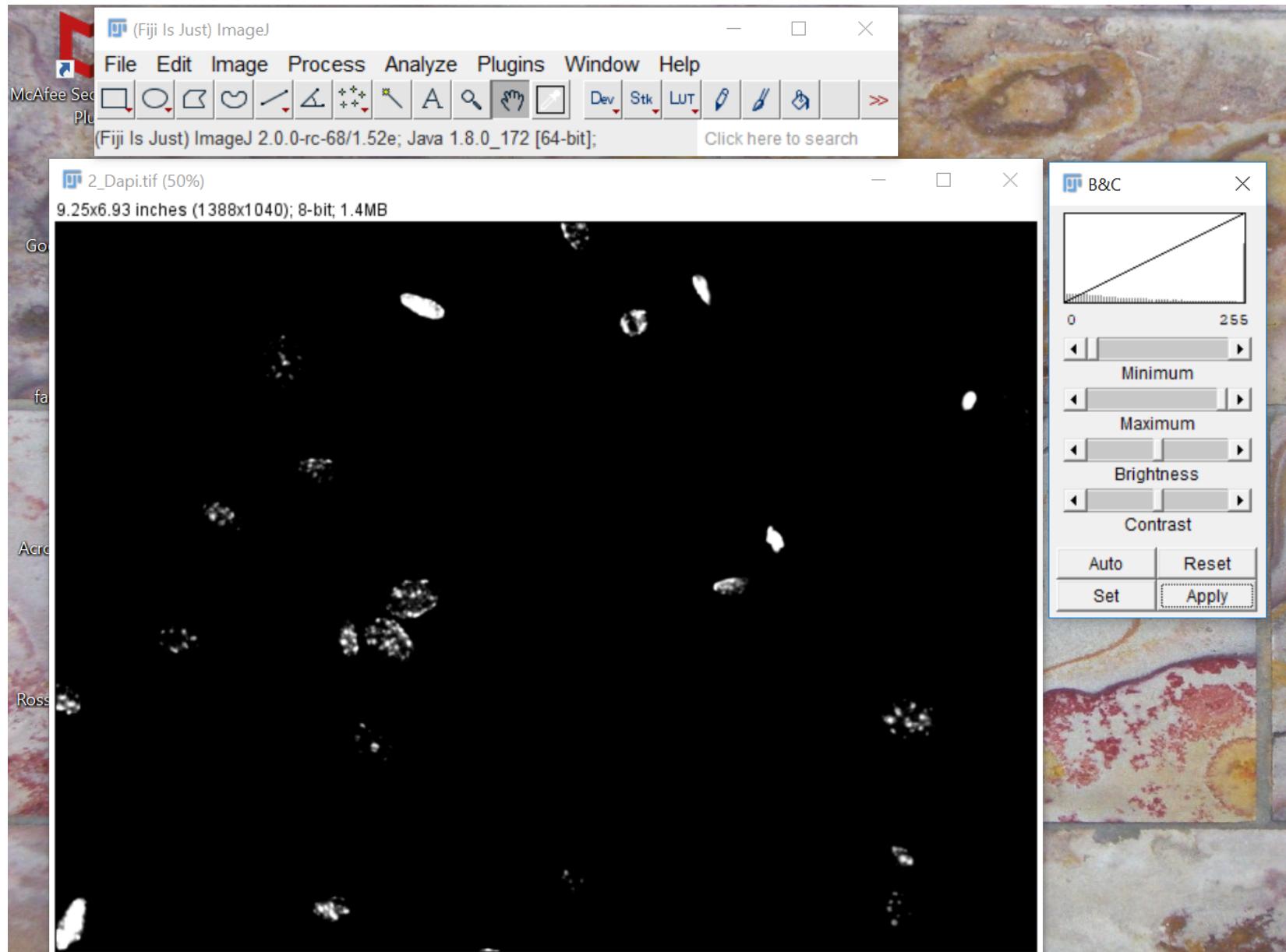
Count particles of a different size, e.g. nuclear speckles



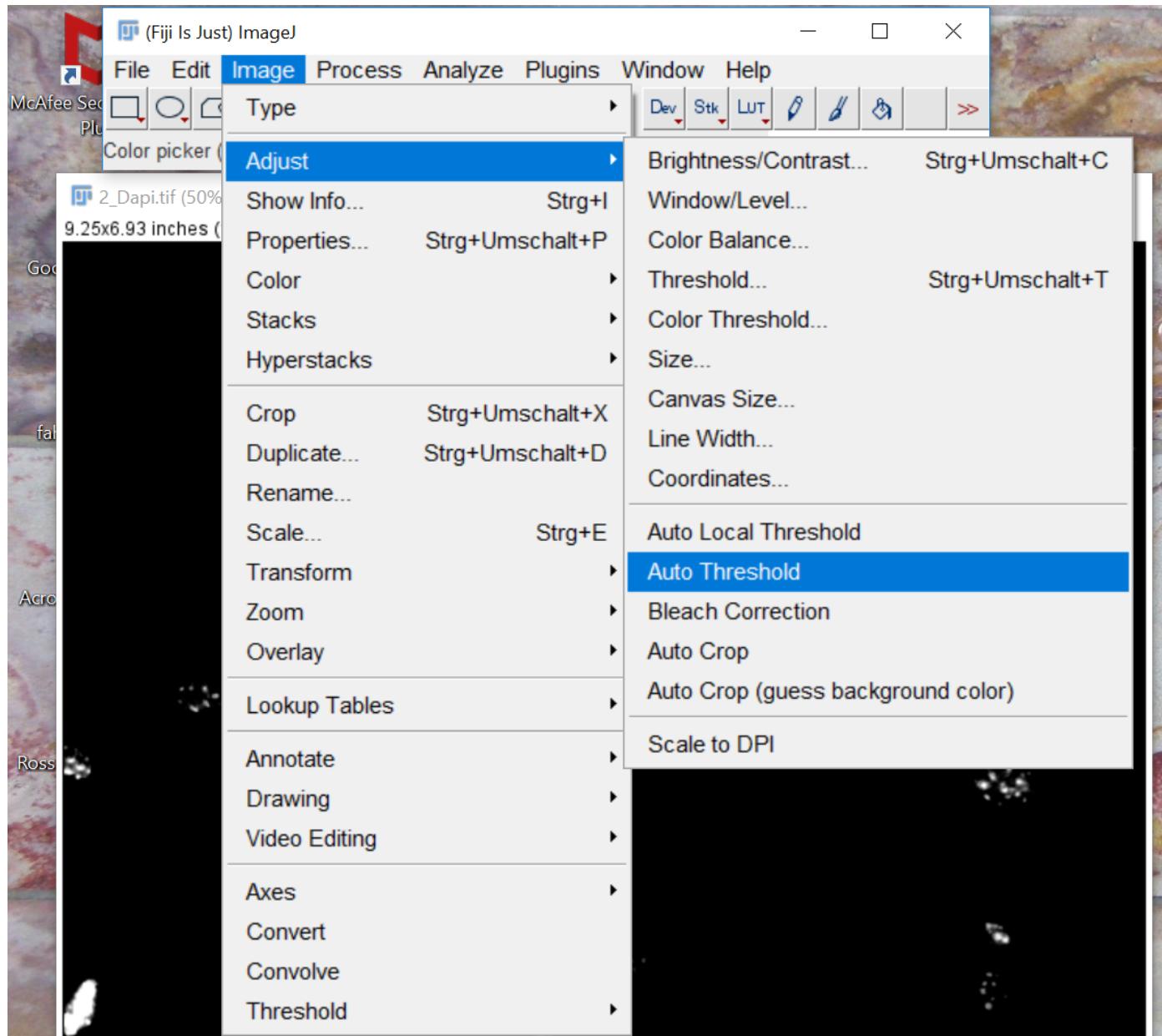
Change contrast to detect nuclear speckles



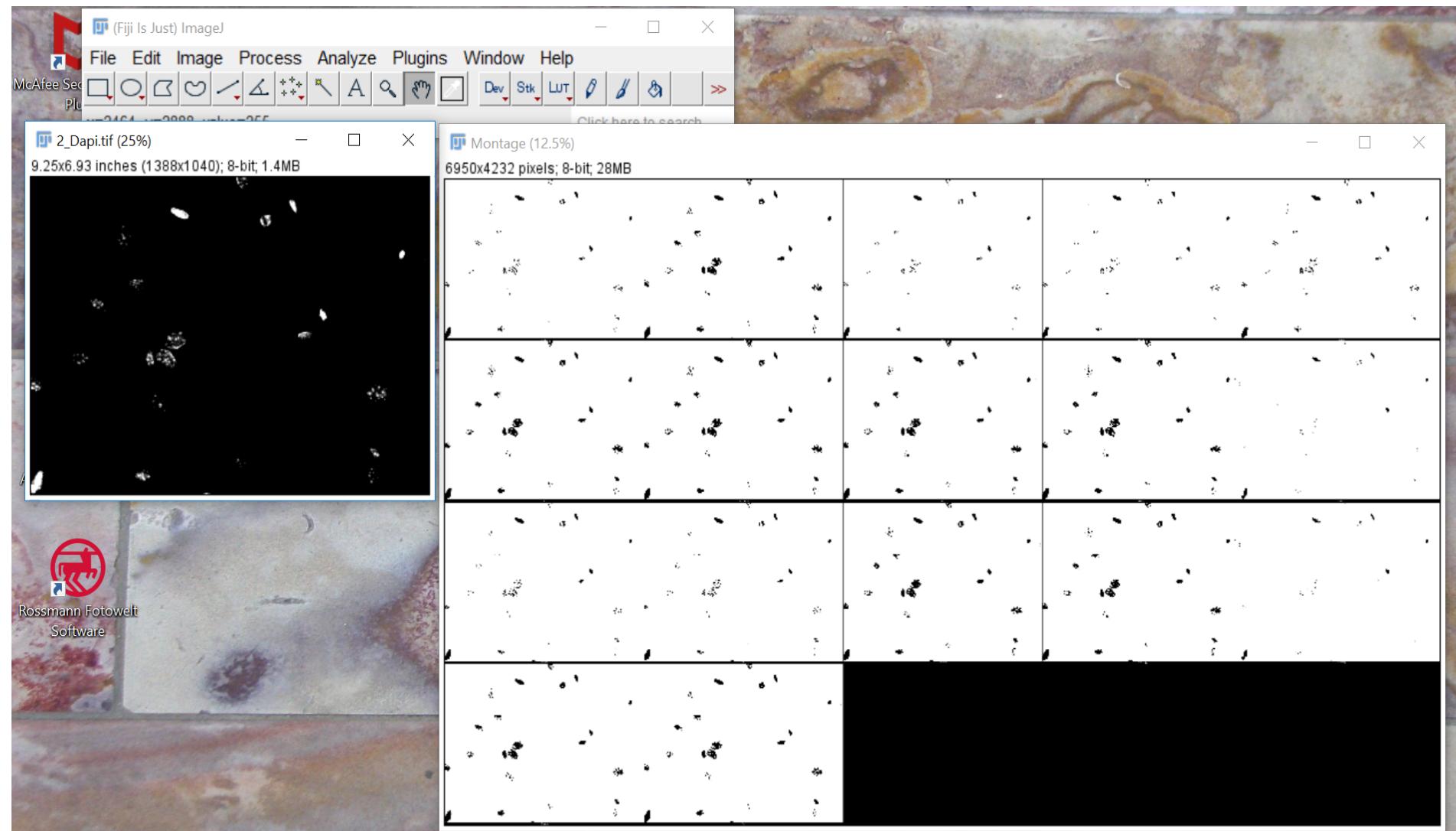
Enhanced contrast, but better: Optimize image acquisition



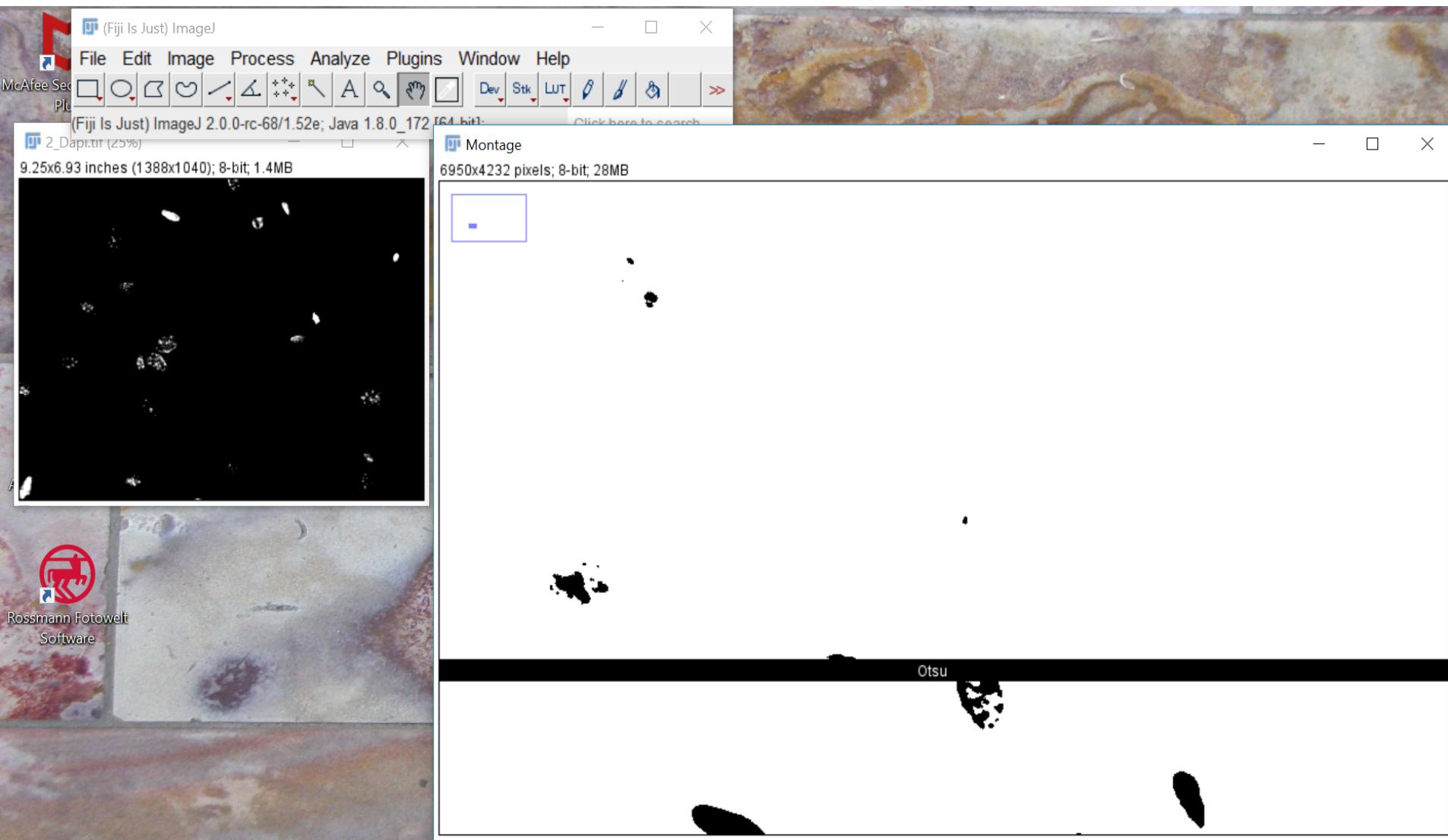
Use Auto threshold to determine best binary mask.



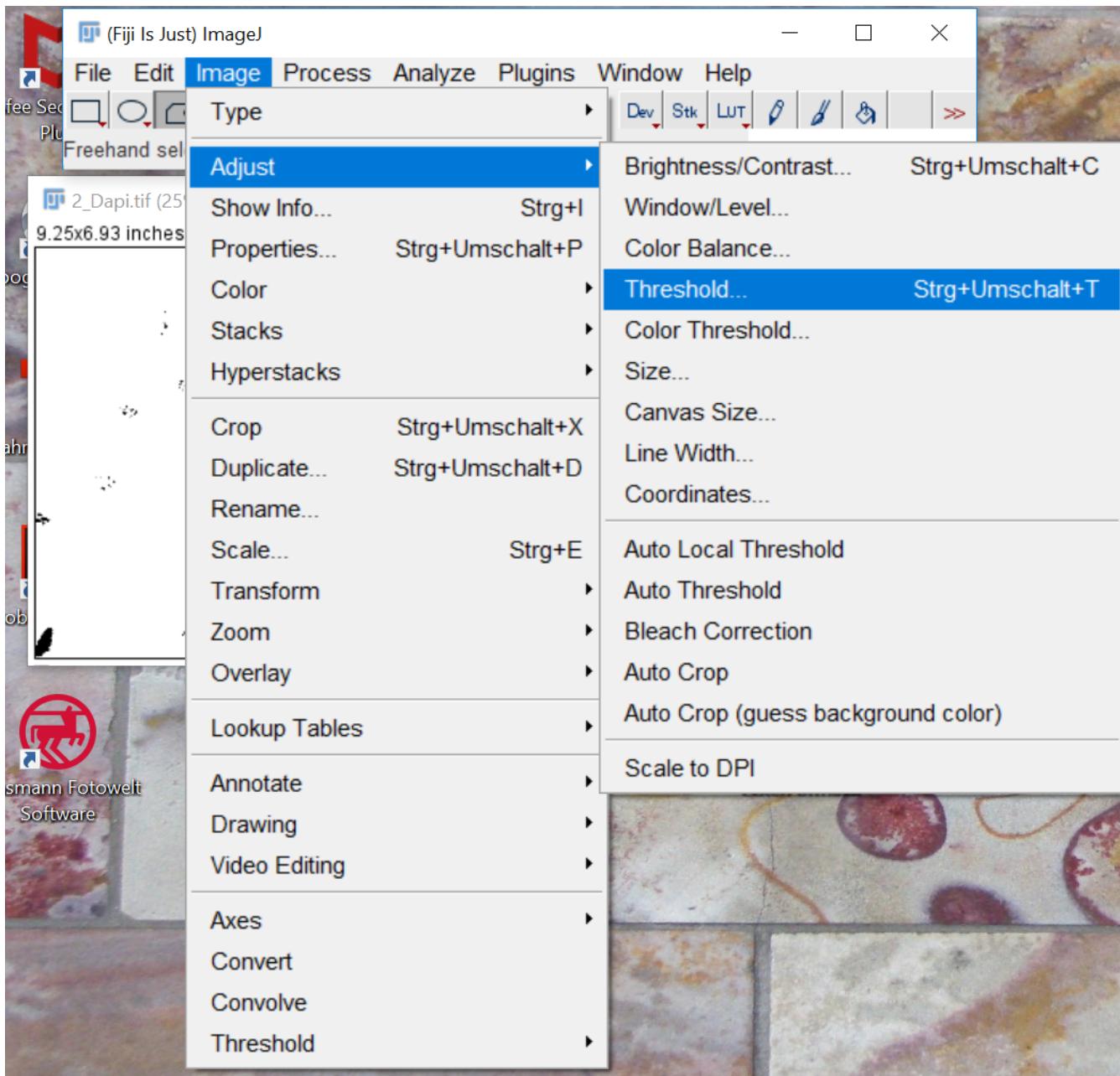
Choose best auto threshold algorithm



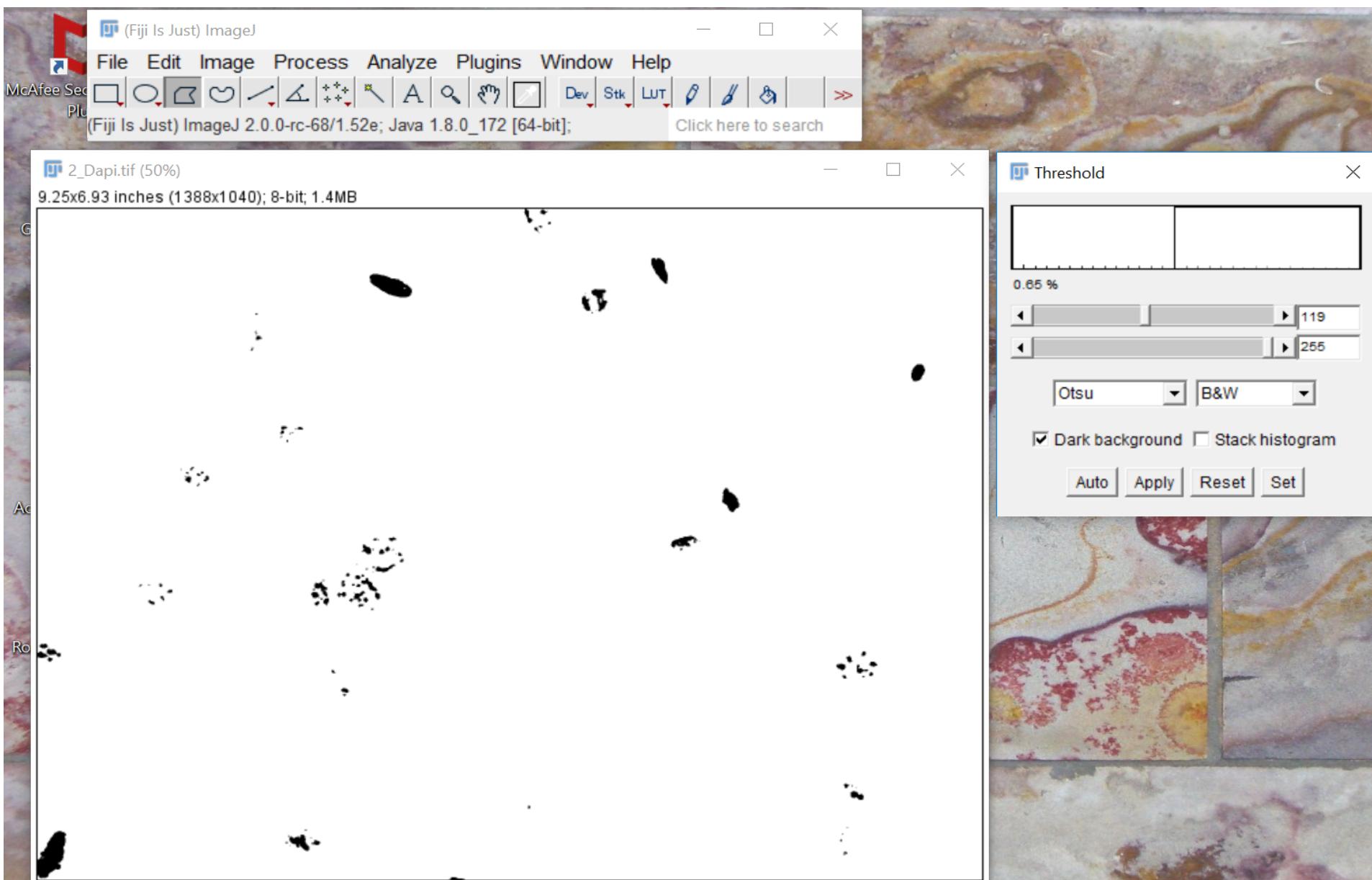
The algorithm “otsu” reflects best the image



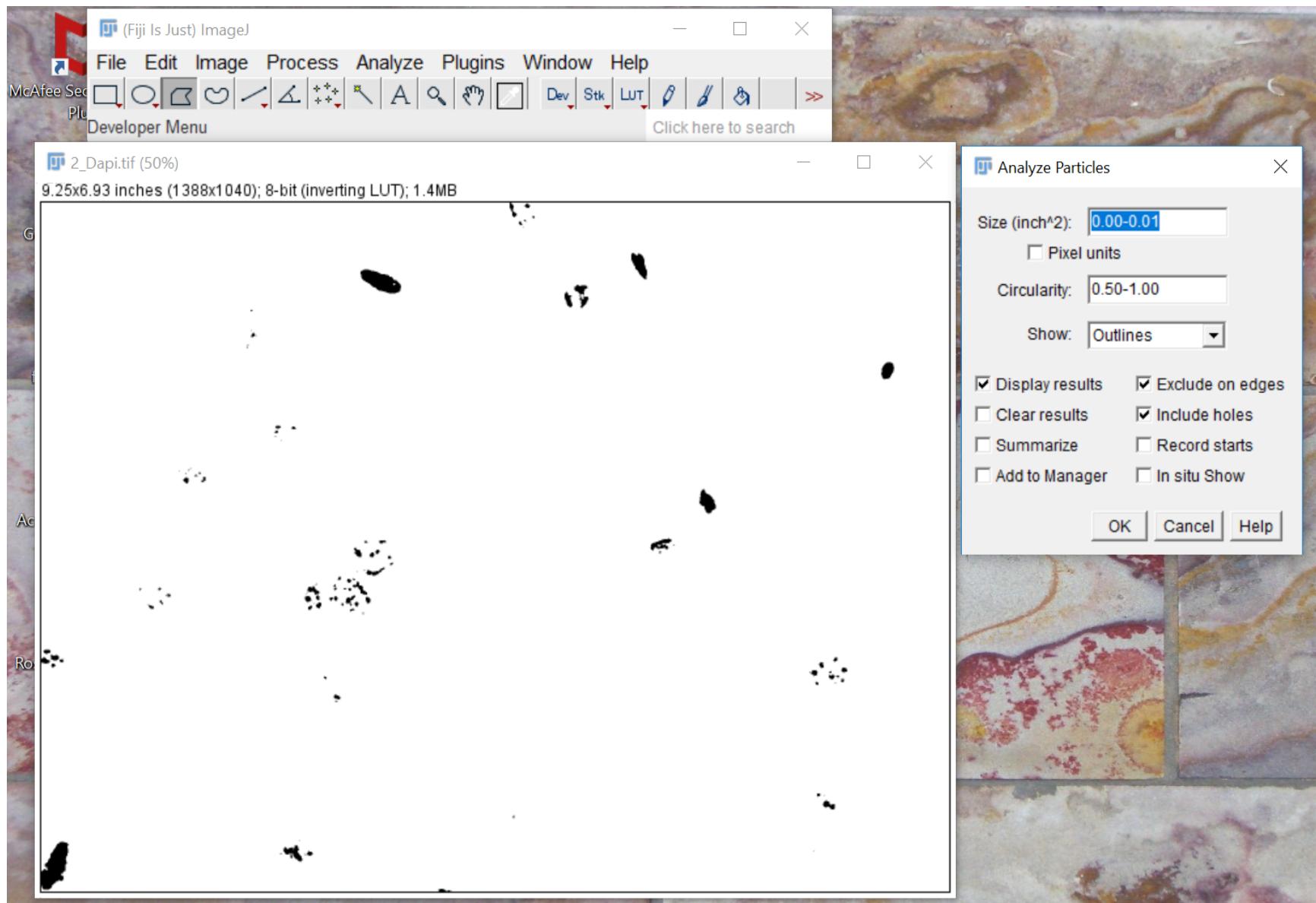
Set threshold “otsu”



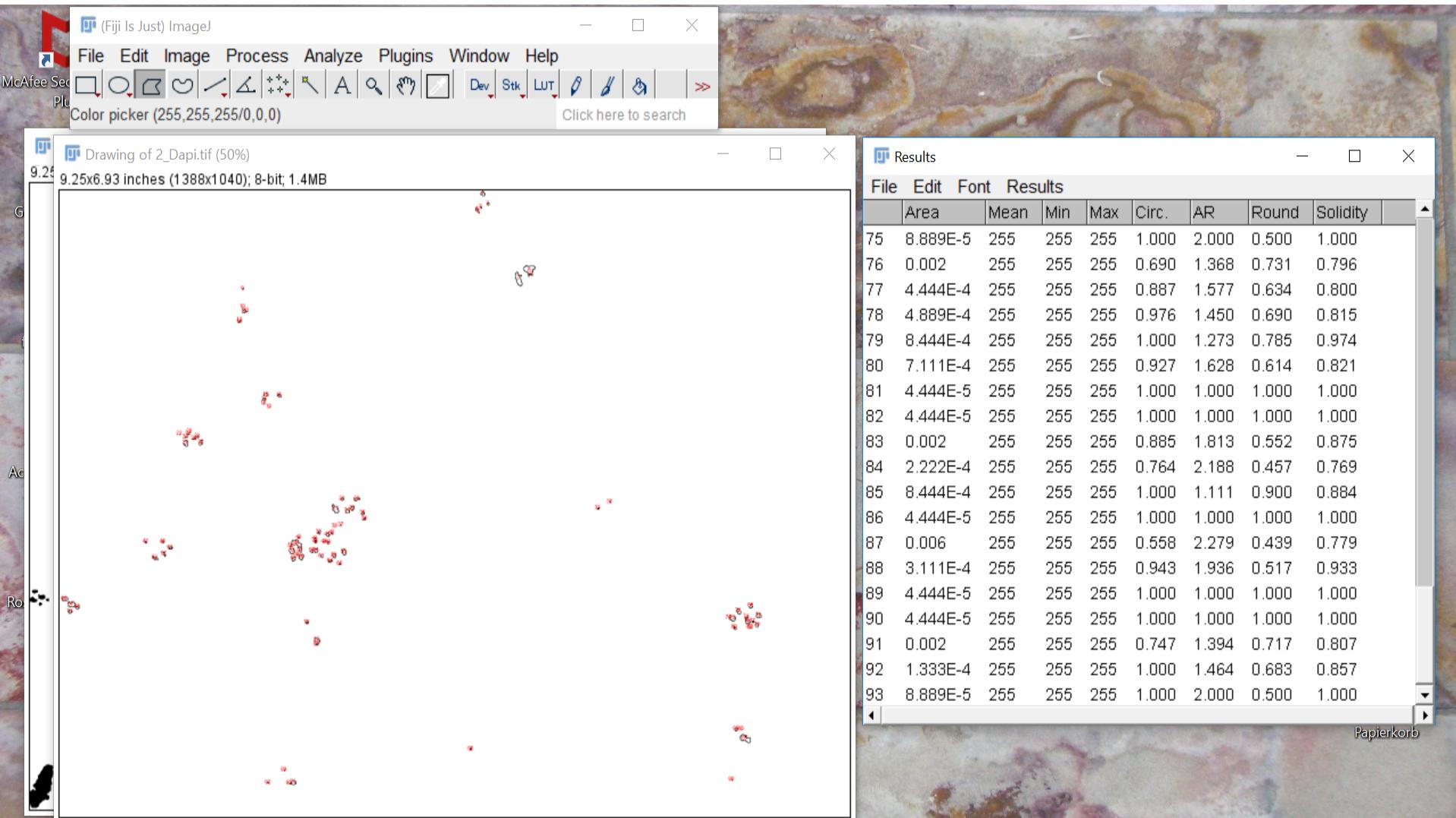
Choose “Otsu” as threshold algorithm and check *Dark background*



Go to Analyze particles and change parameters



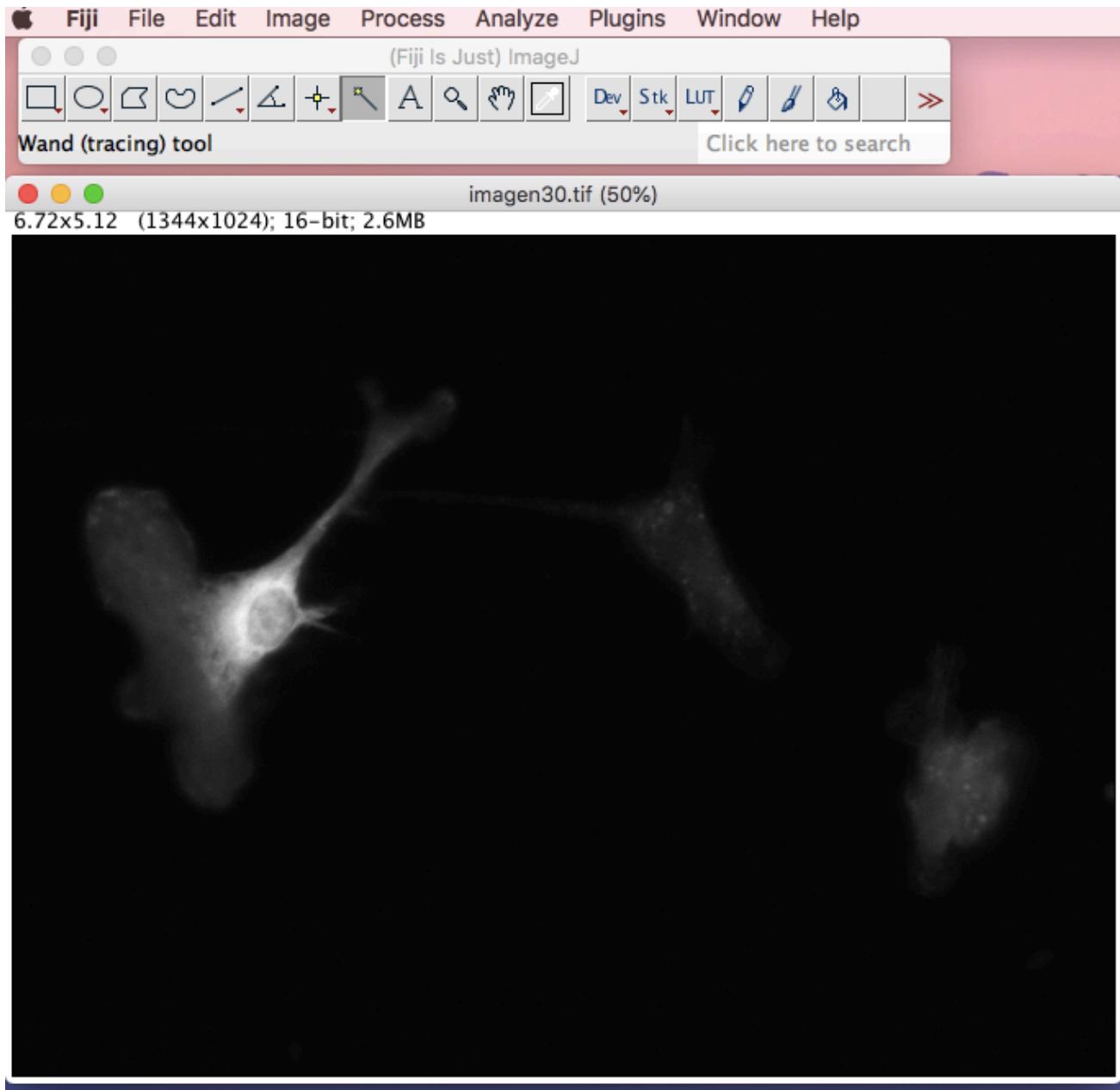
Get the results and copy and paste them into excel



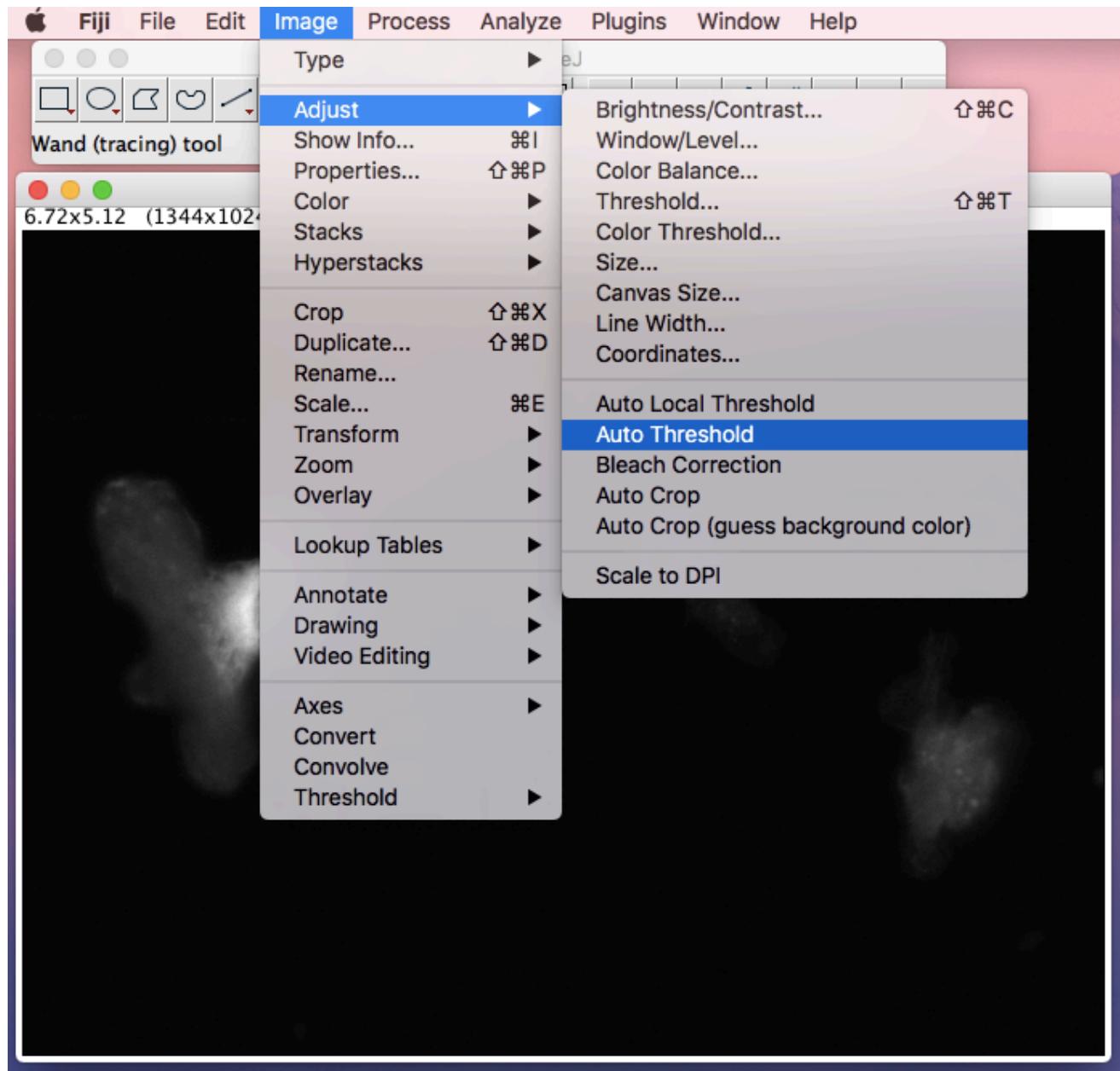
Workshop Overview

- **Examples:**
 - Counting Particles of Different Sizes
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 - Basic steps
- Practical Session with Example Images

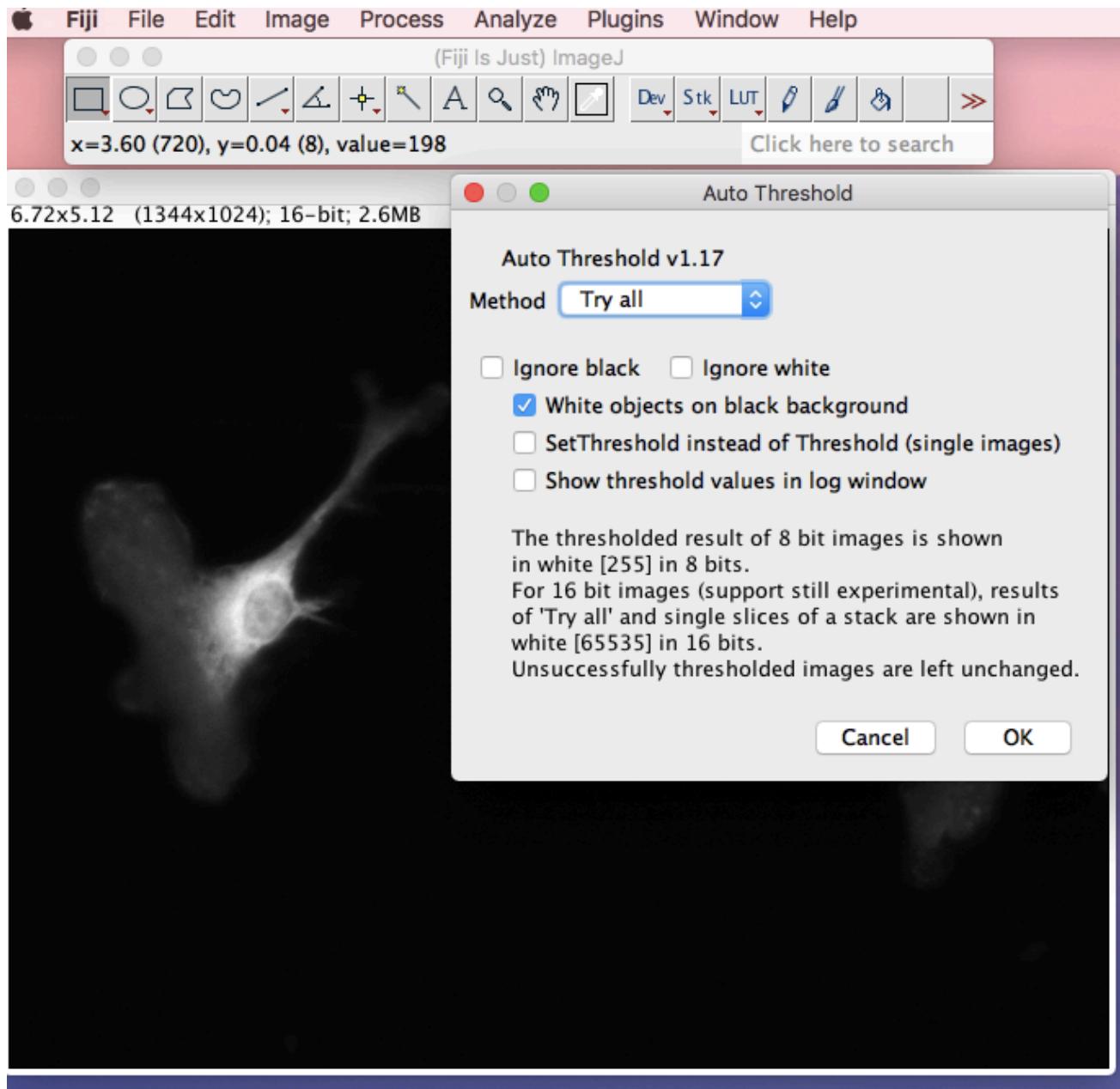
Open imagen30 from Fluorescence folder



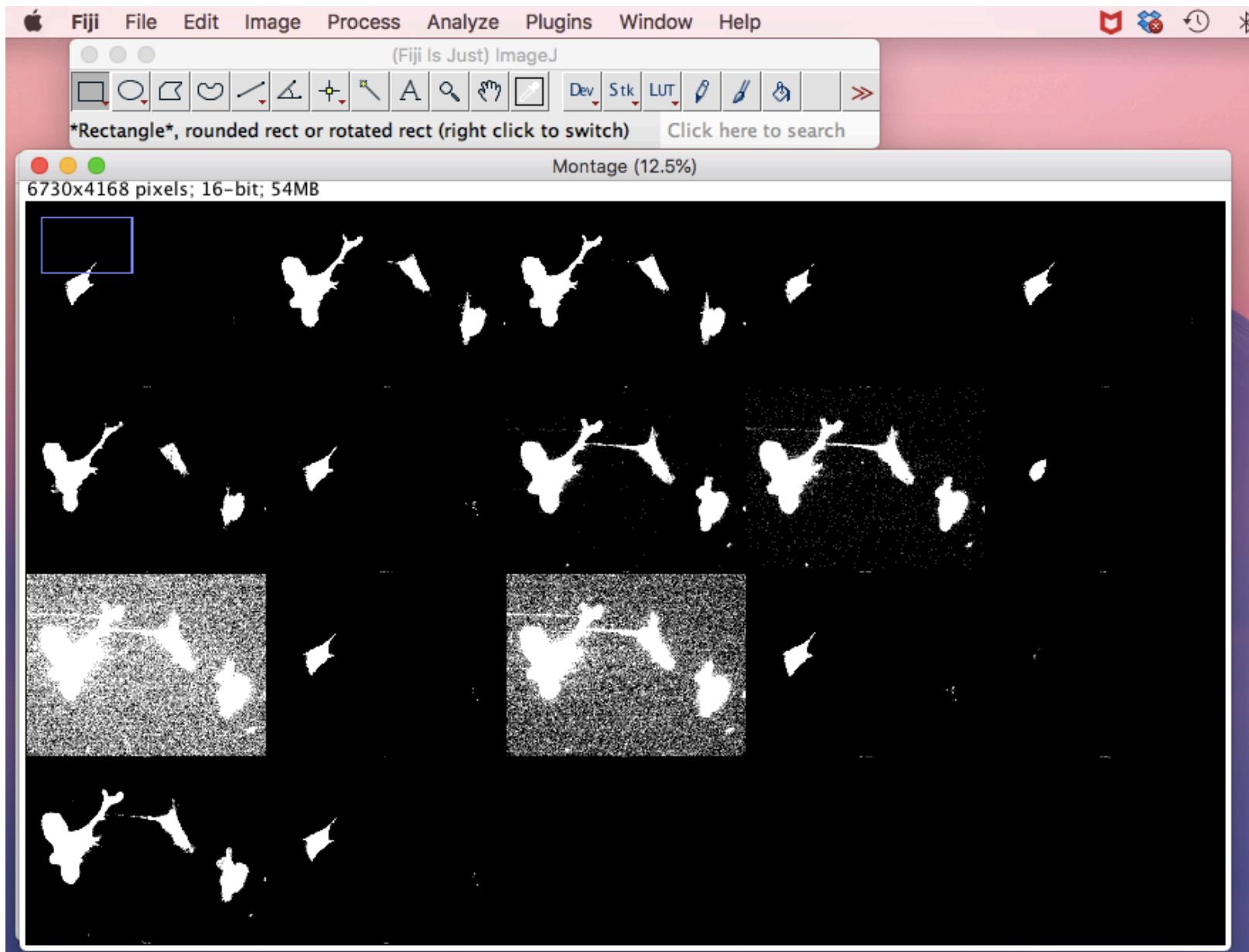
Create binary image to define the cell surroundings



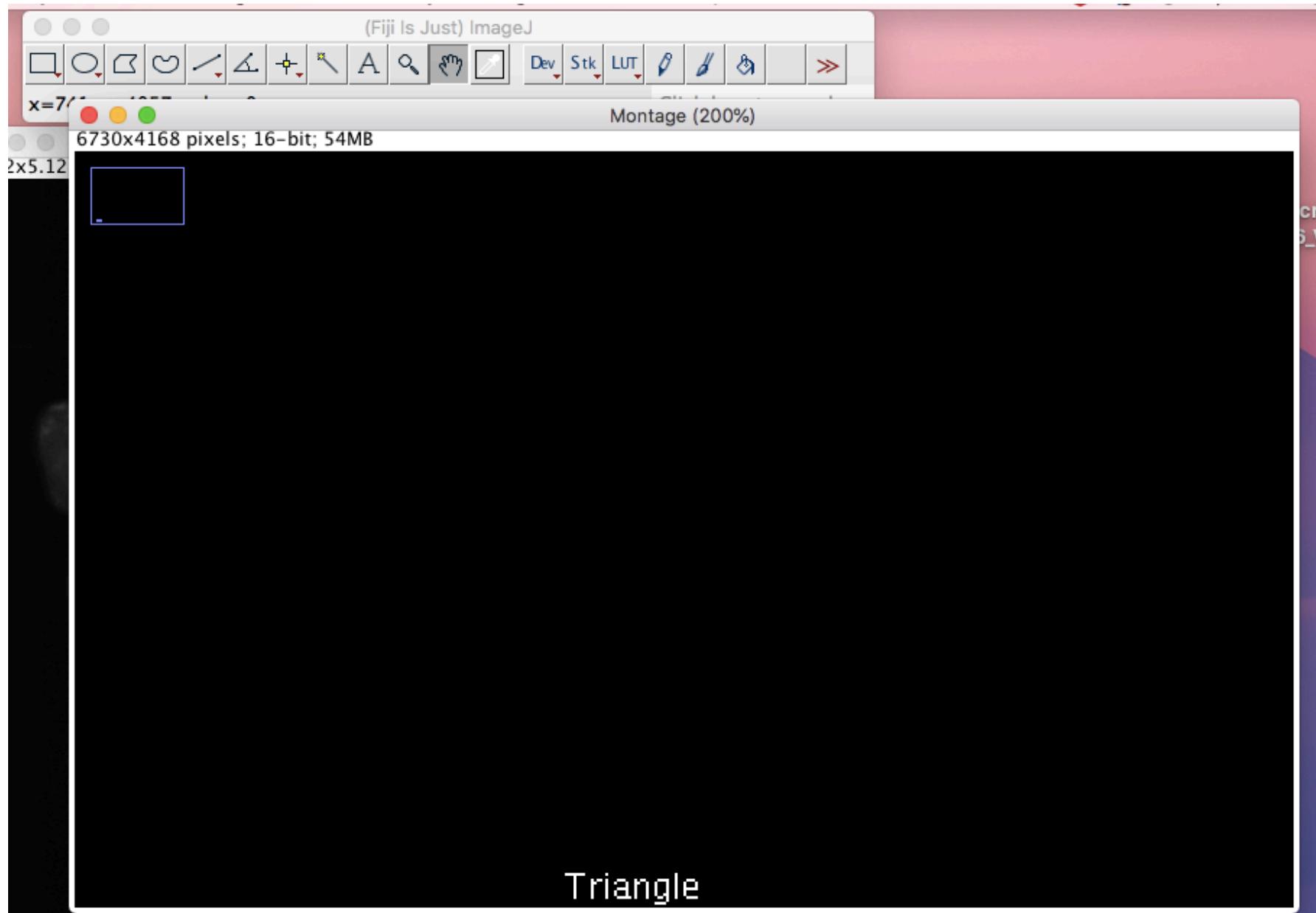
Make binary mask and check white objects



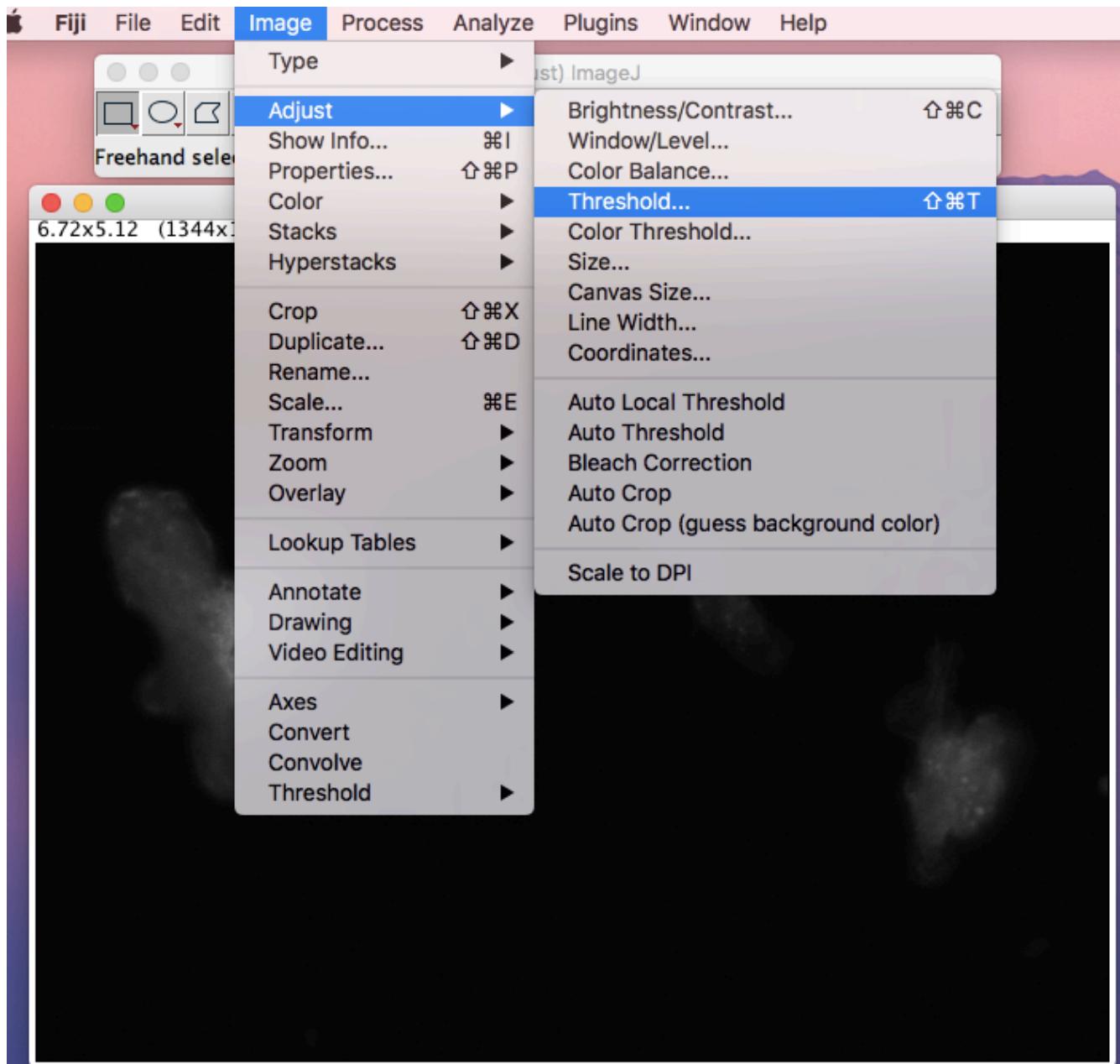
Choose threshold algorithm which resembles best original image



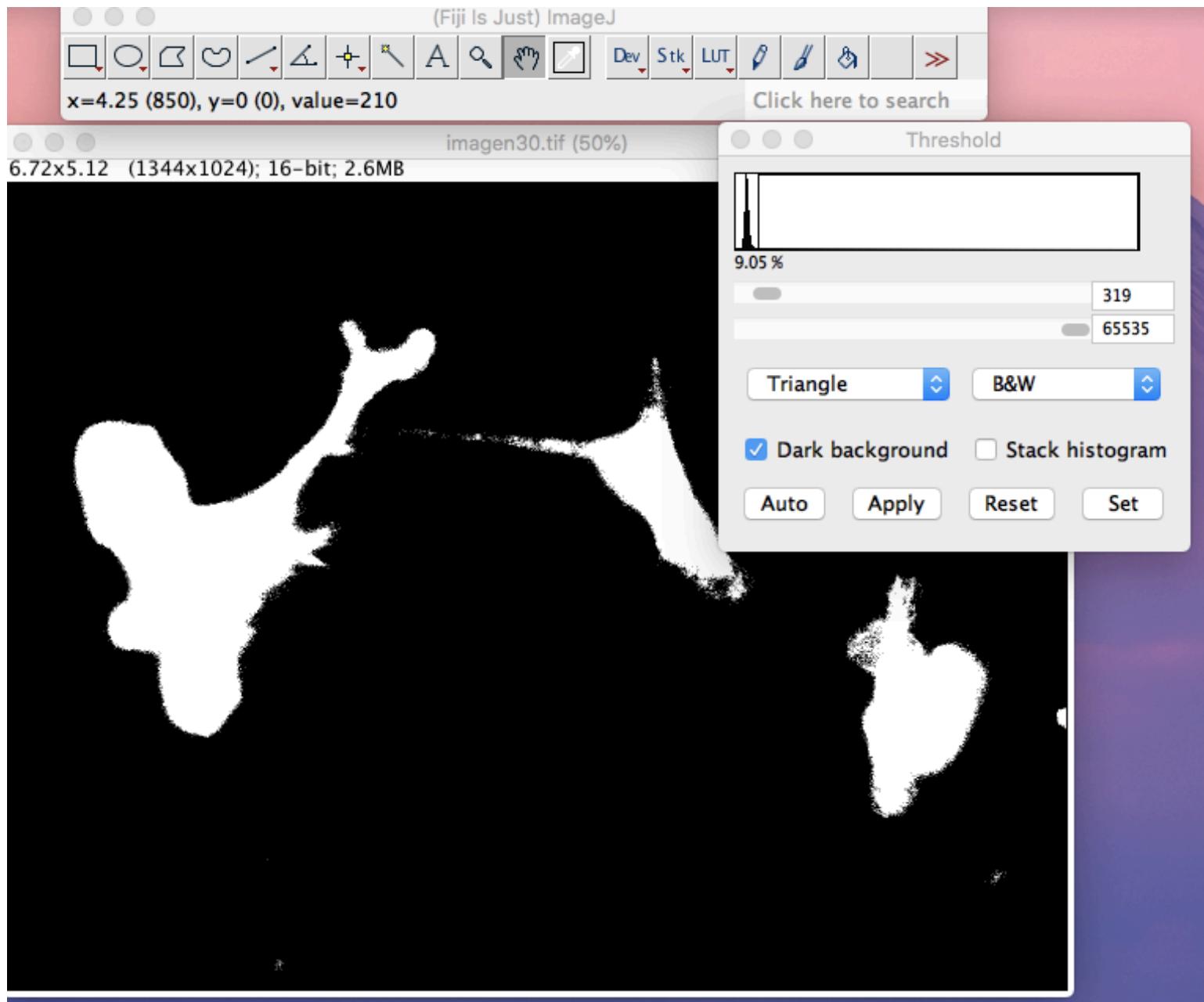
Choose the algorithm Triangle



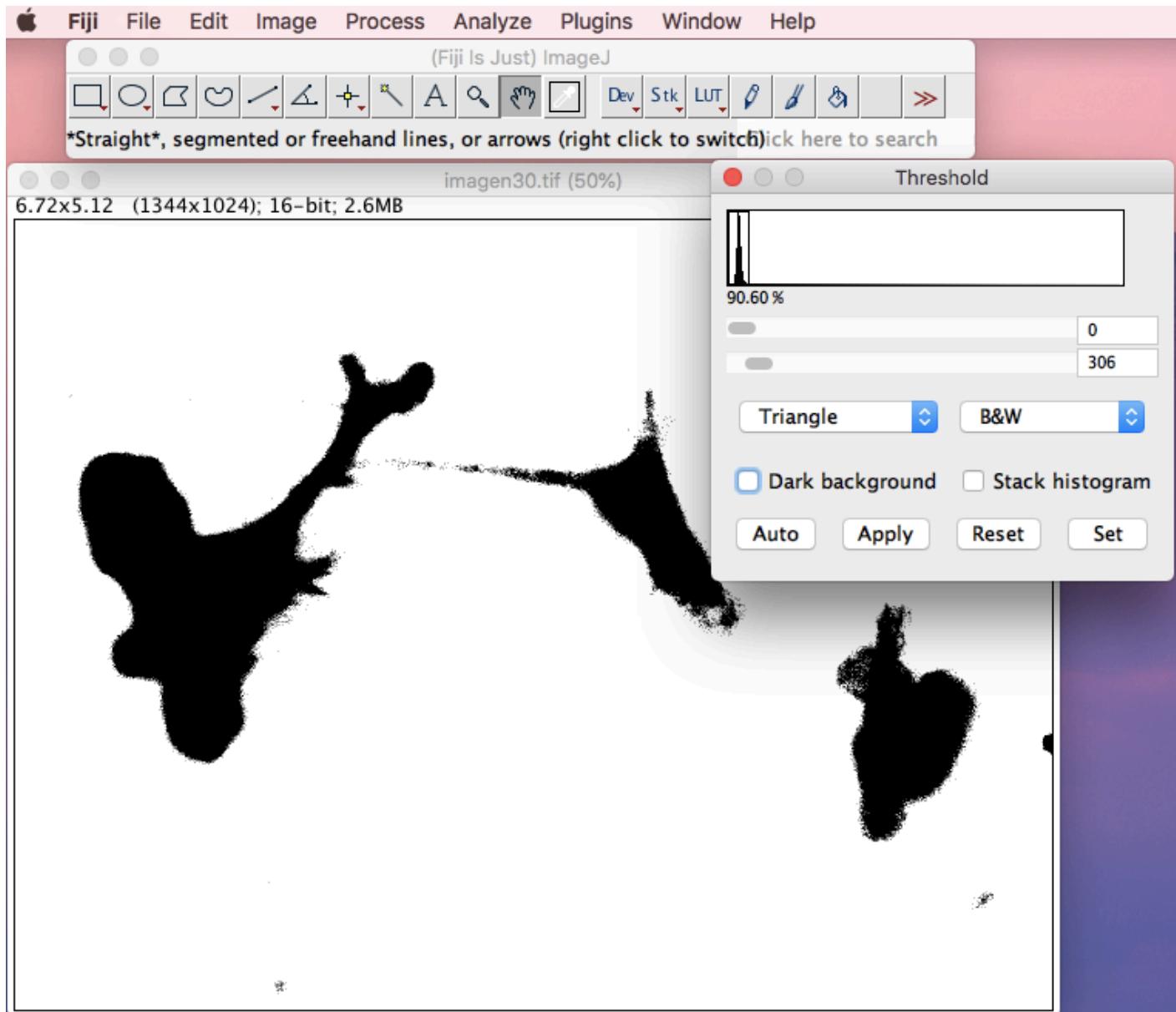
Apply Triangle threshold



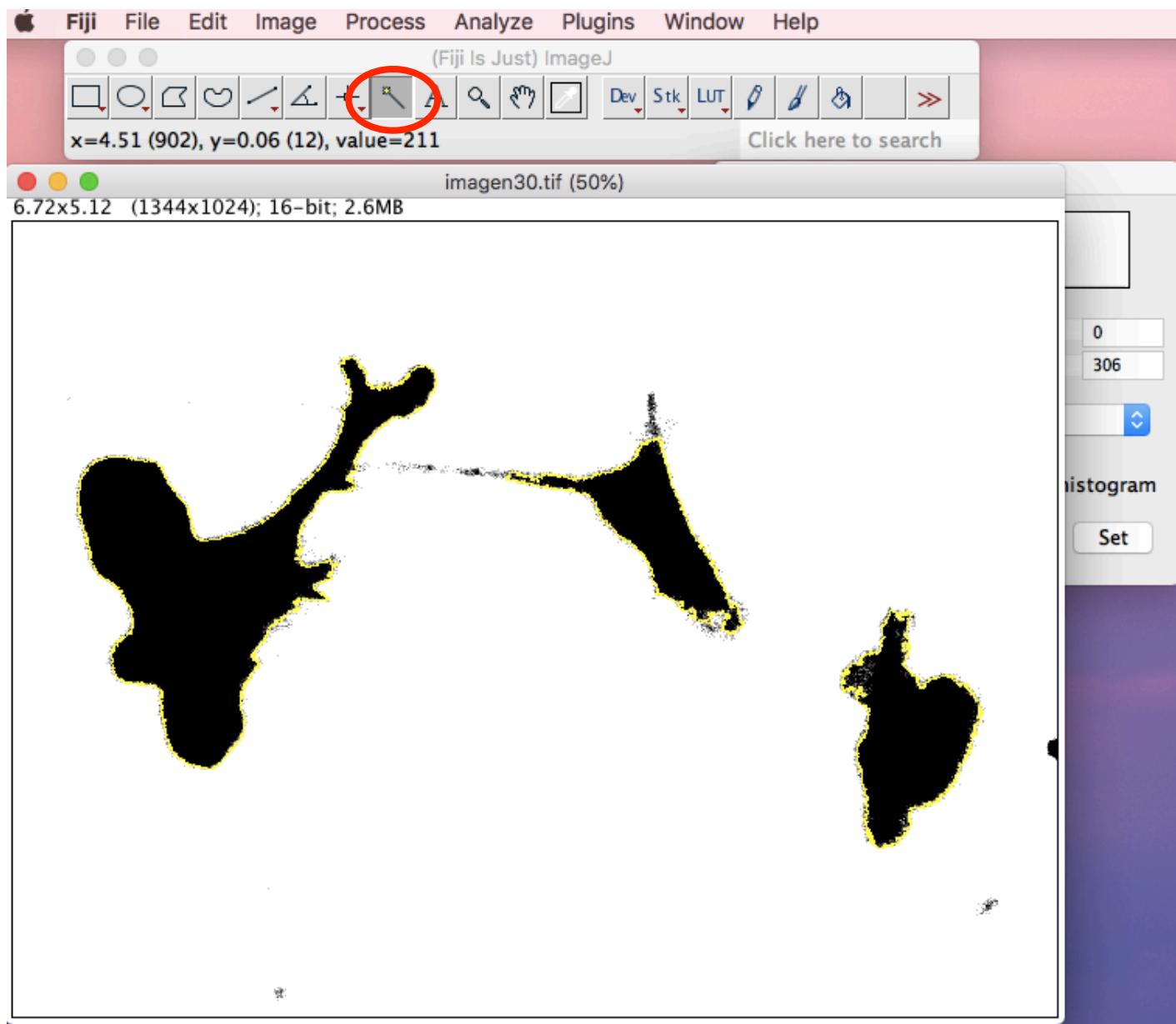
Apply Triangle threshold



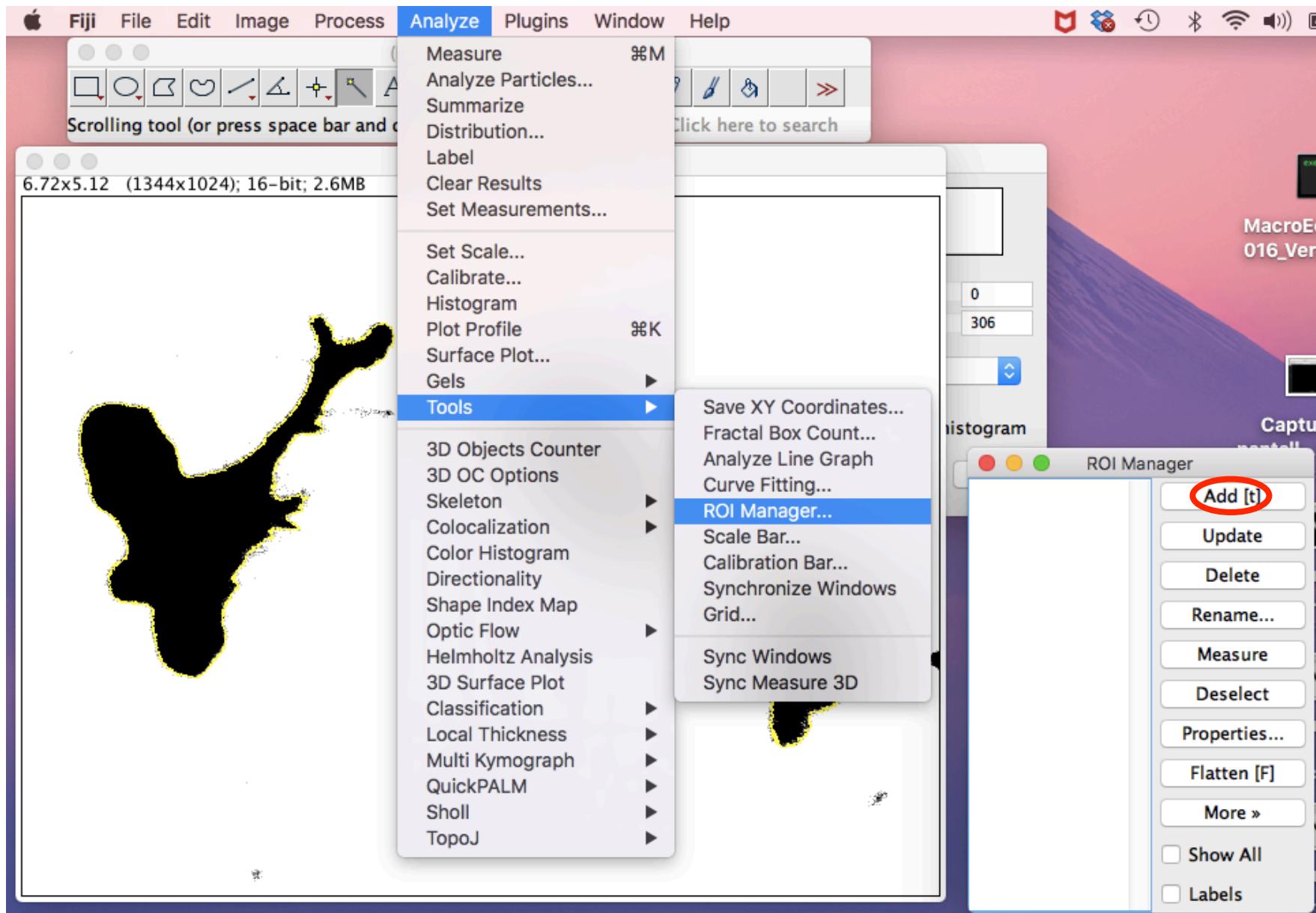
If you want to change to white background, leave *Dark background* unchecked



Use the wand tracing tool to mark outlines of the cell.
By pressing Shift + *wand tracing tool*, you label all cells.

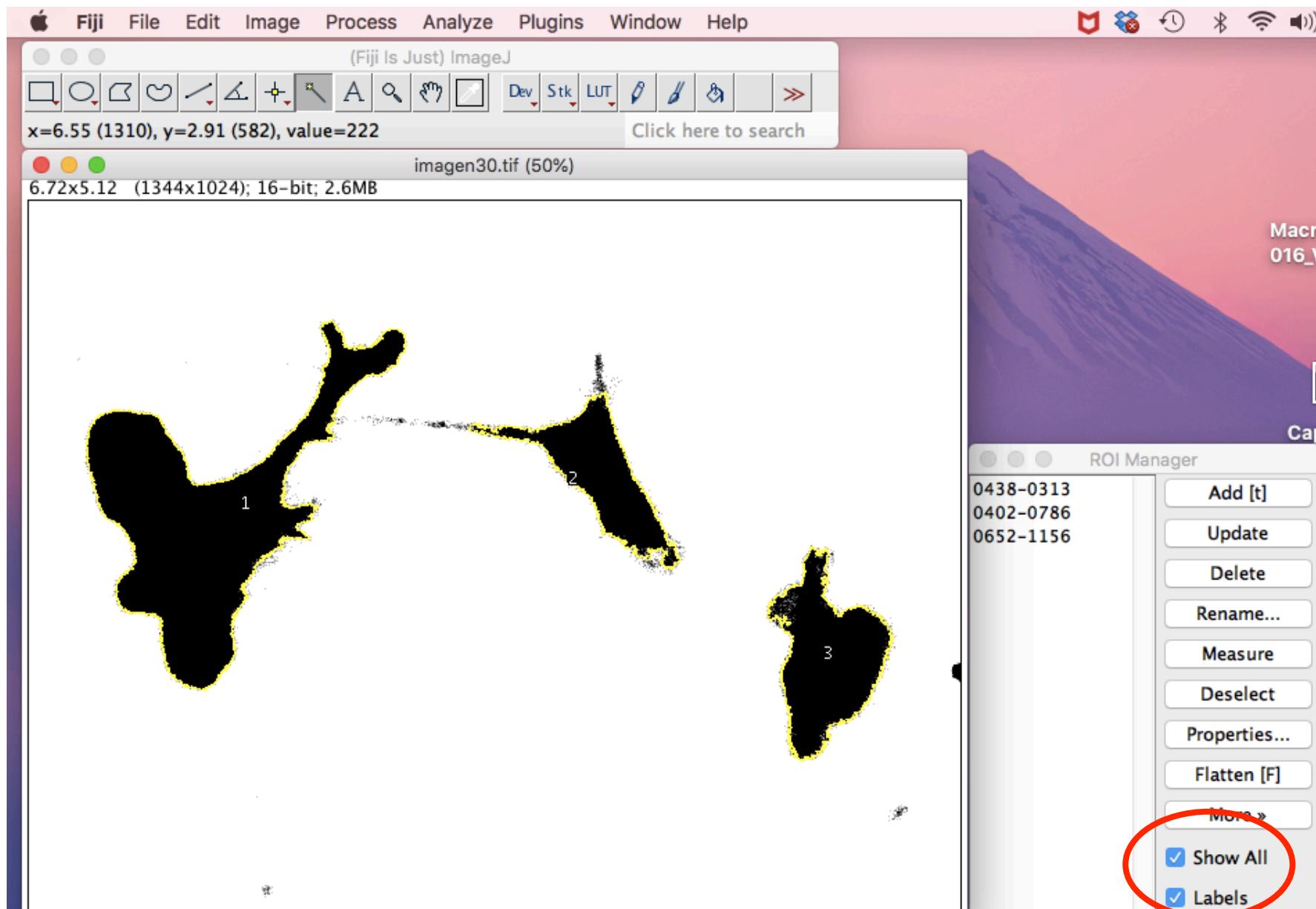


Use the ROI (Region of Interest) manager and Add cell surroundings

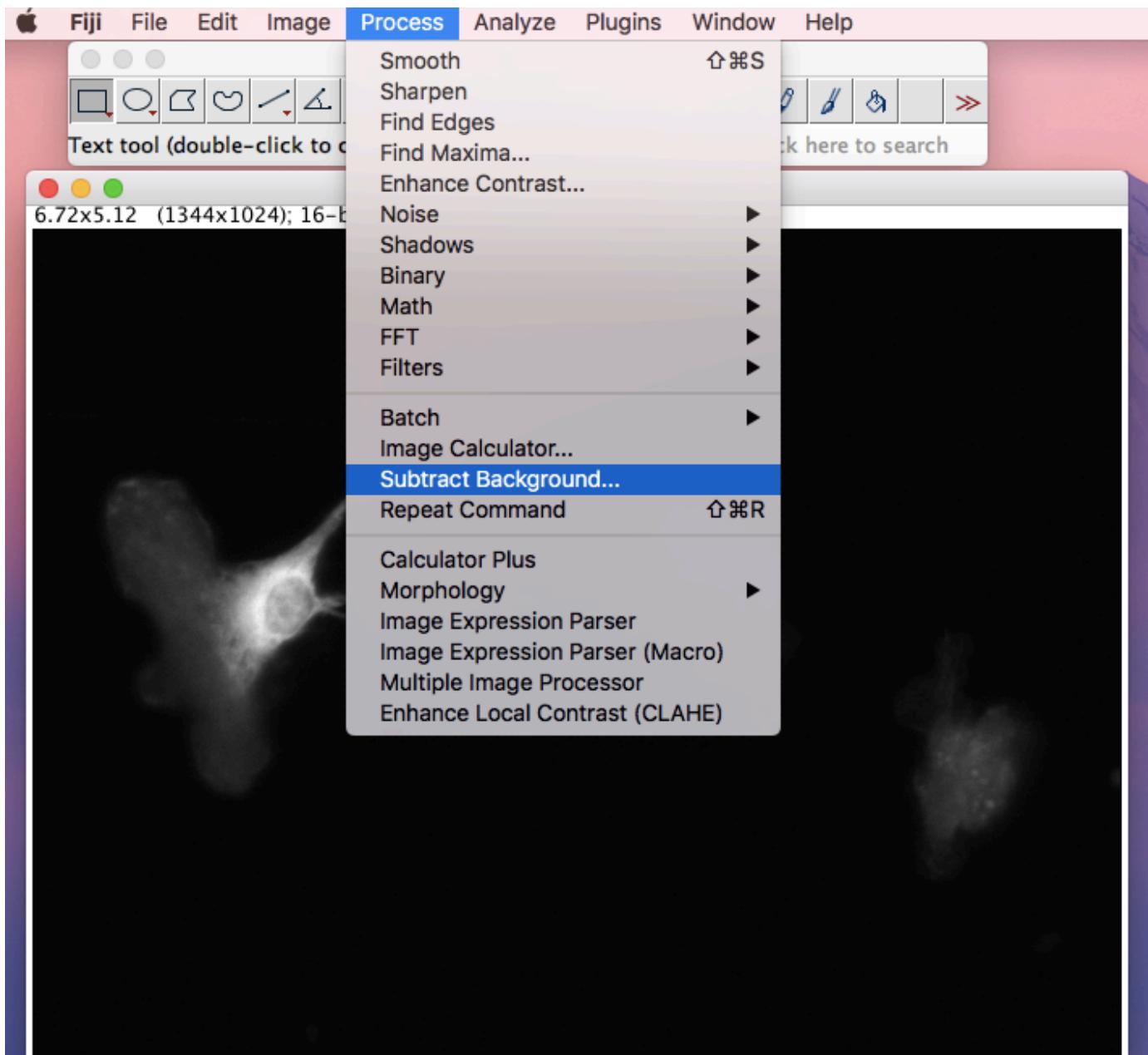


Check Show all and Labels.

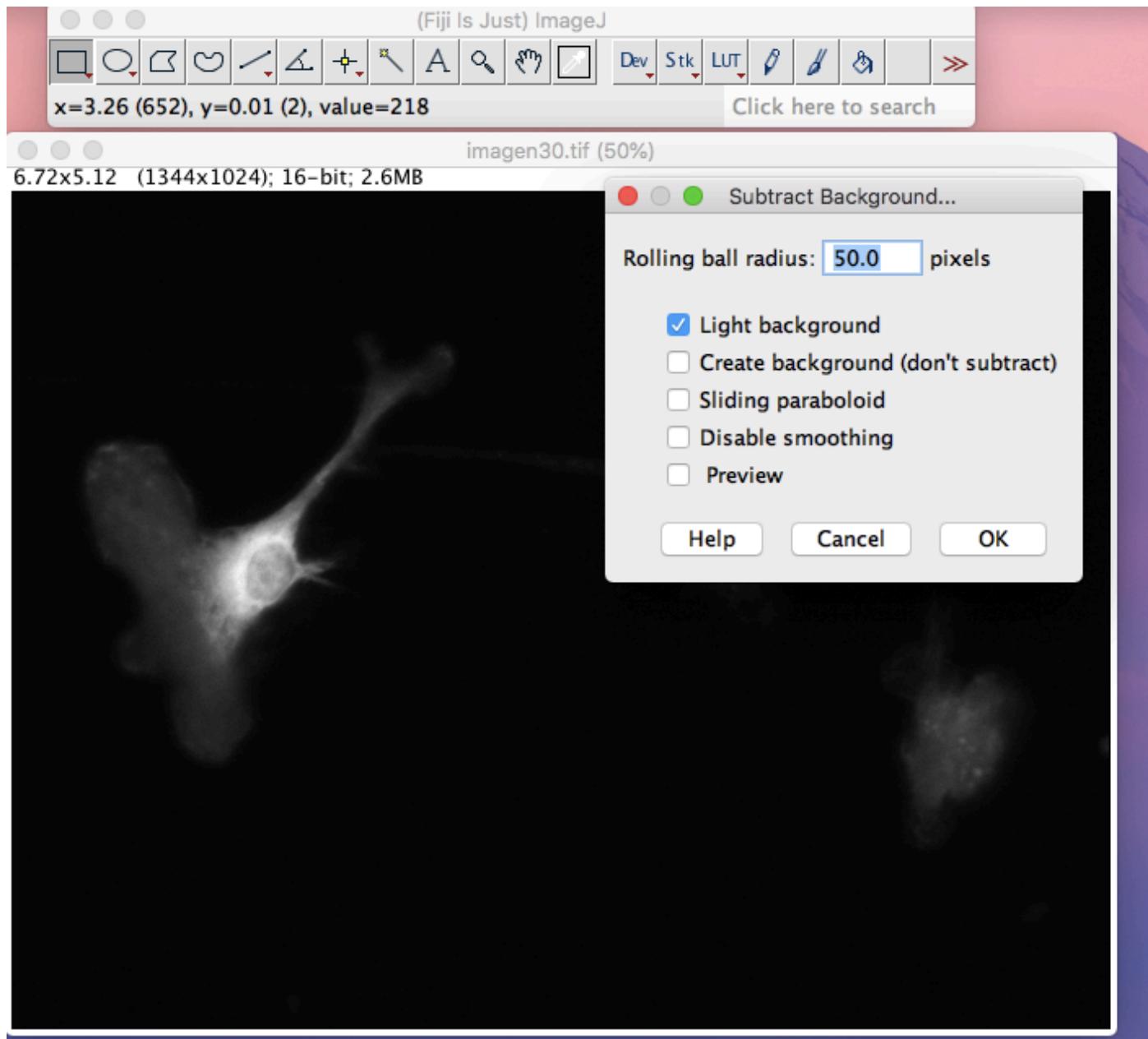
Close binary image and open original Image (Imagen30)



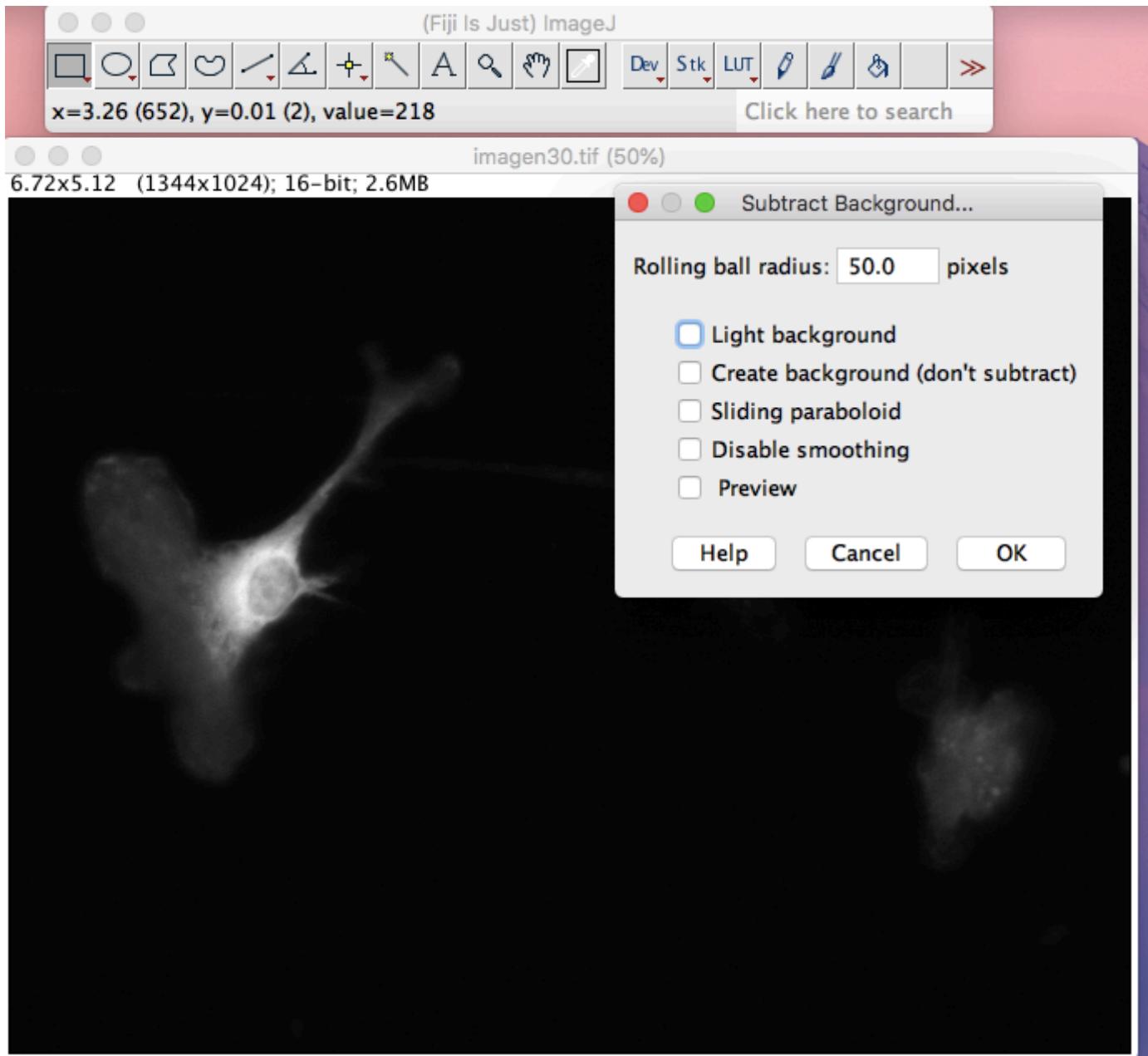
Subtract background



Subtract background

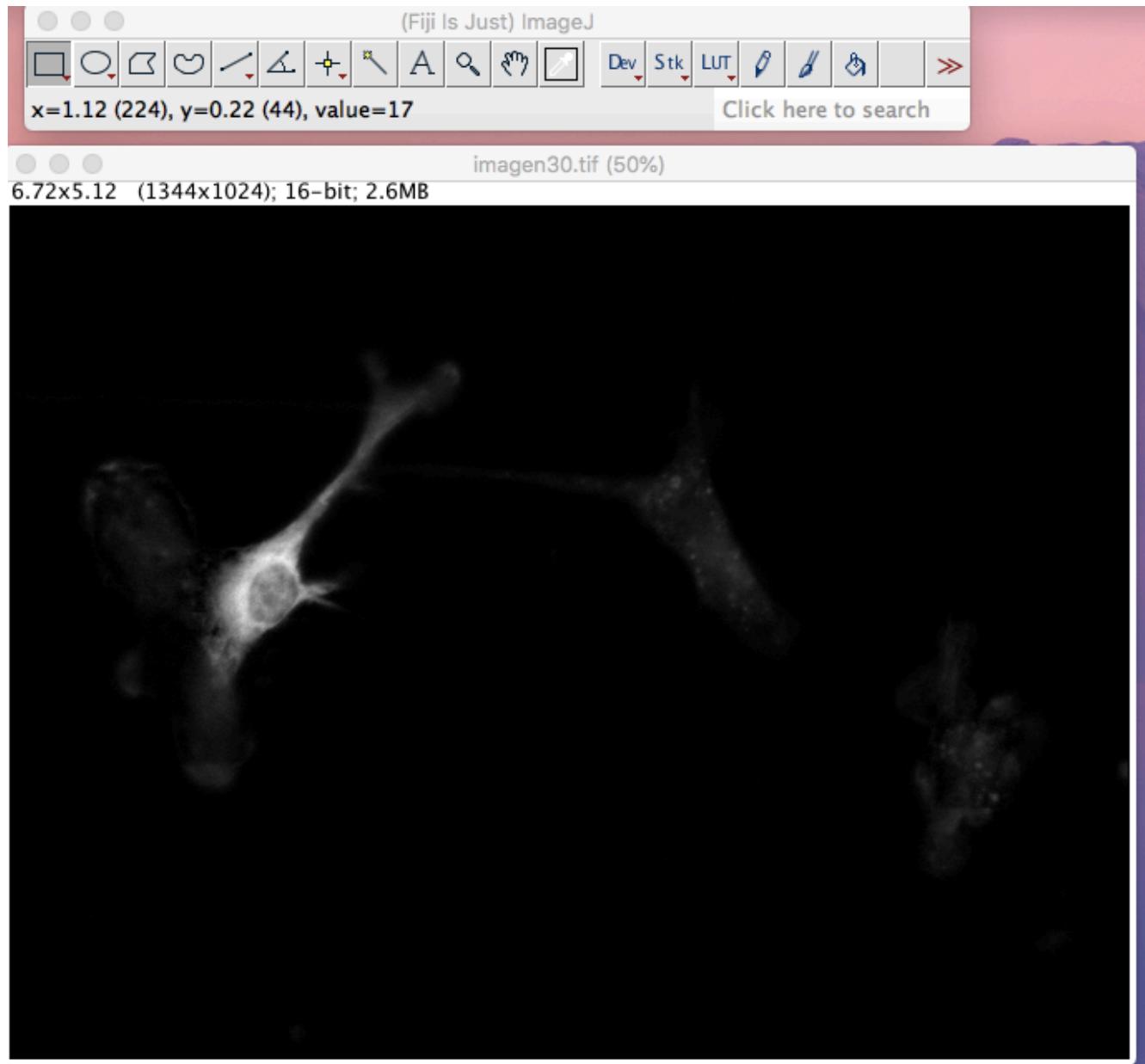


Subtract background and uncheck *Light background*

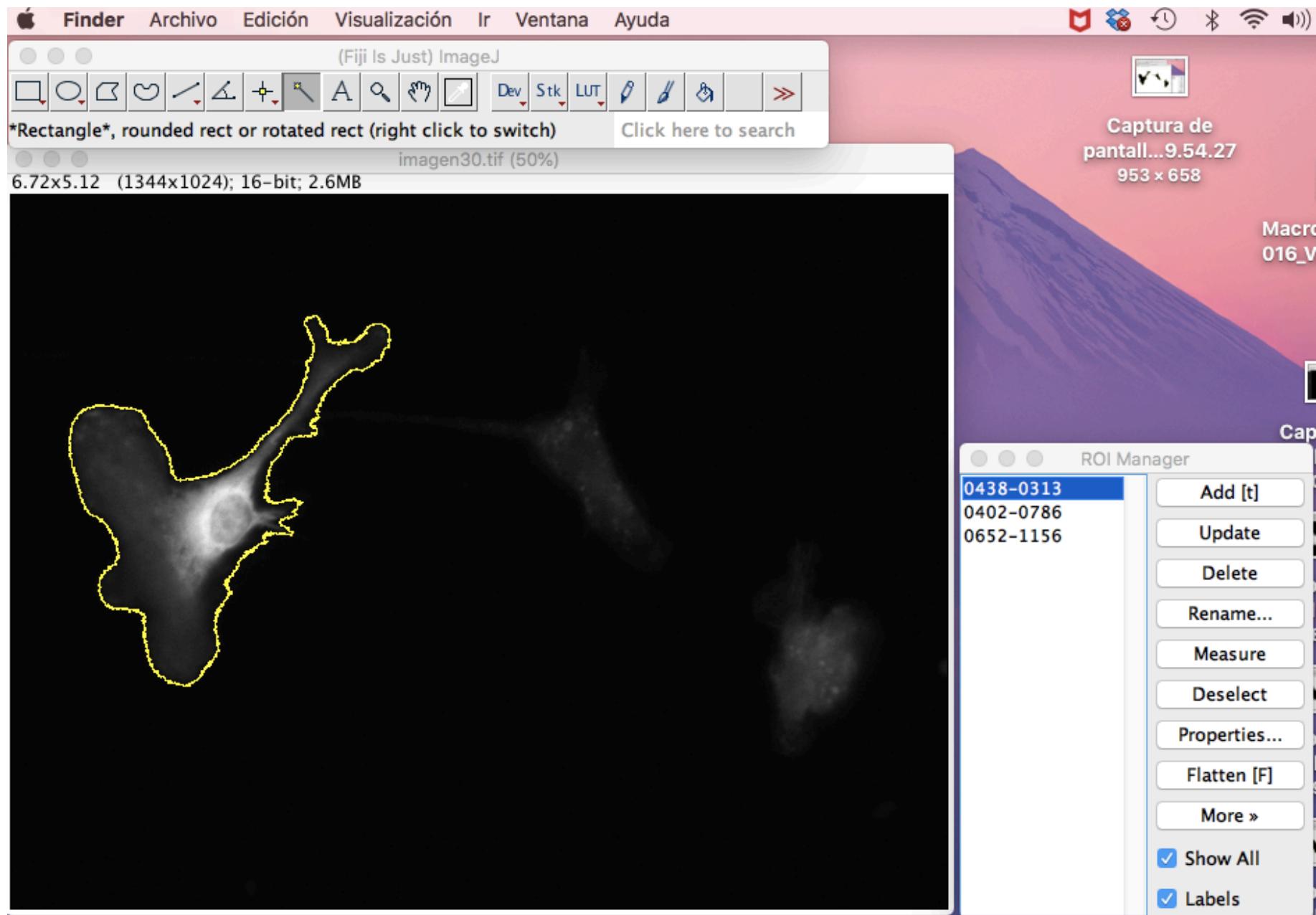


Background subtracted

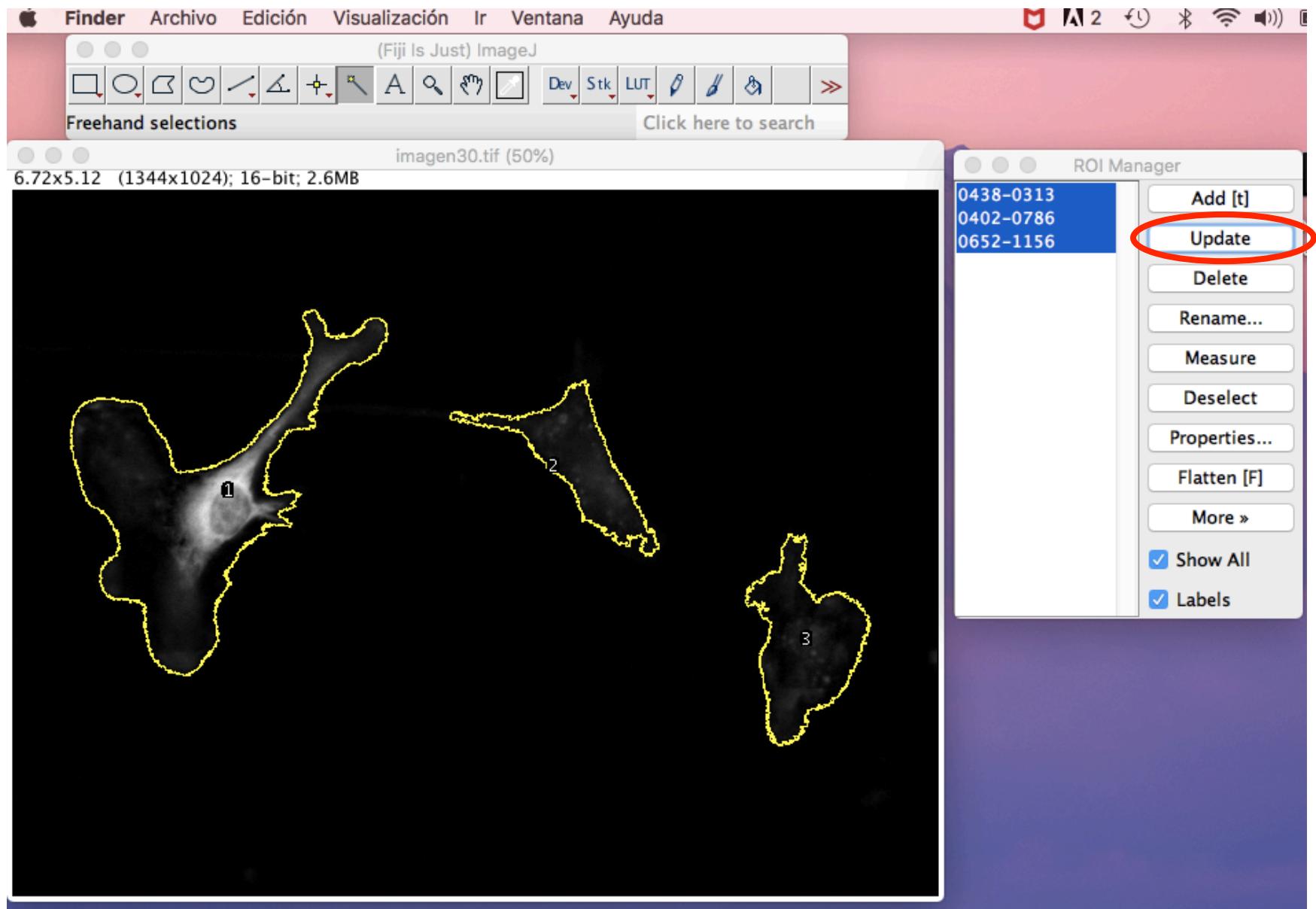
Better: Background subtraction manually



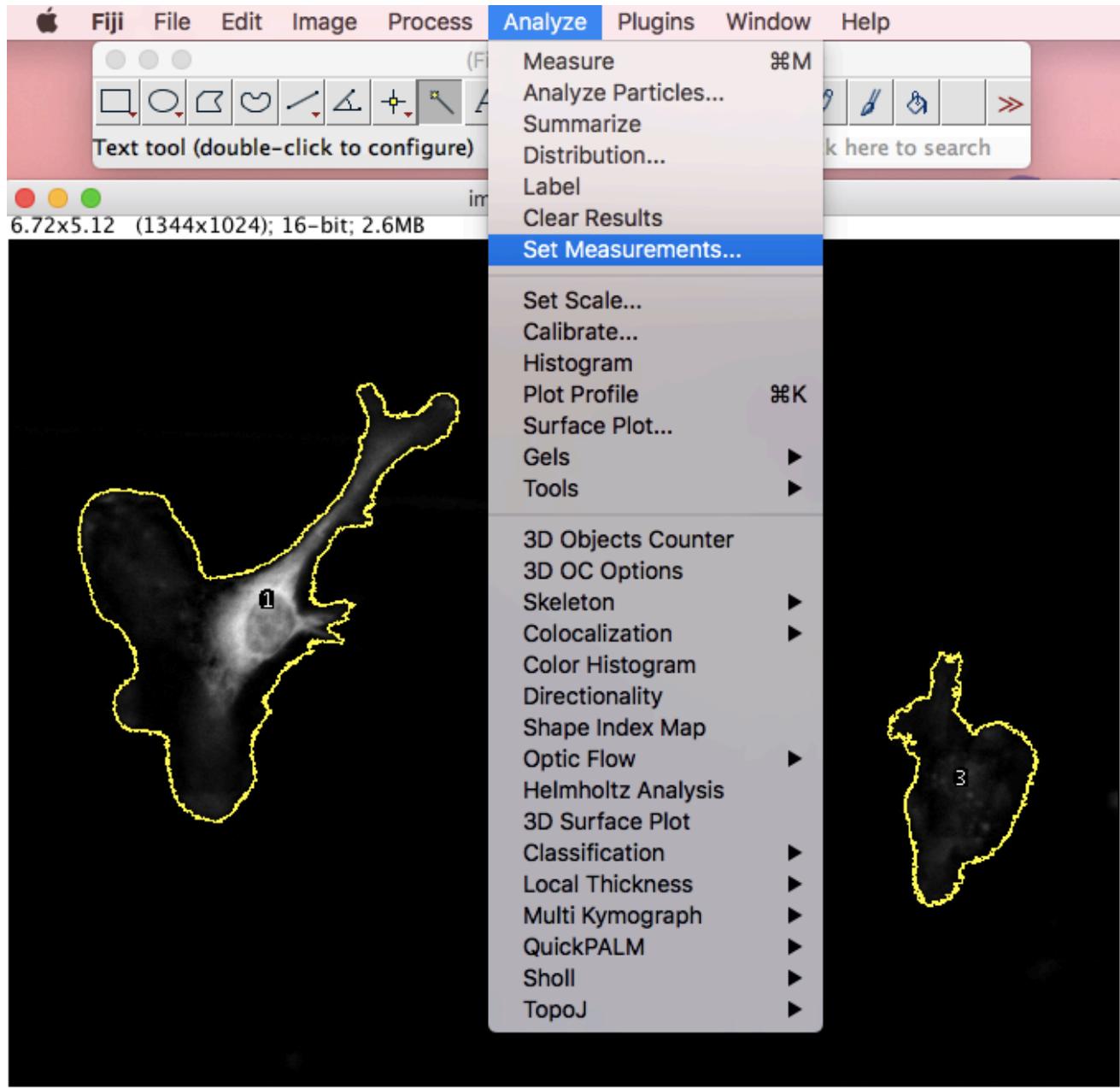
Select ROIs in ROI manager



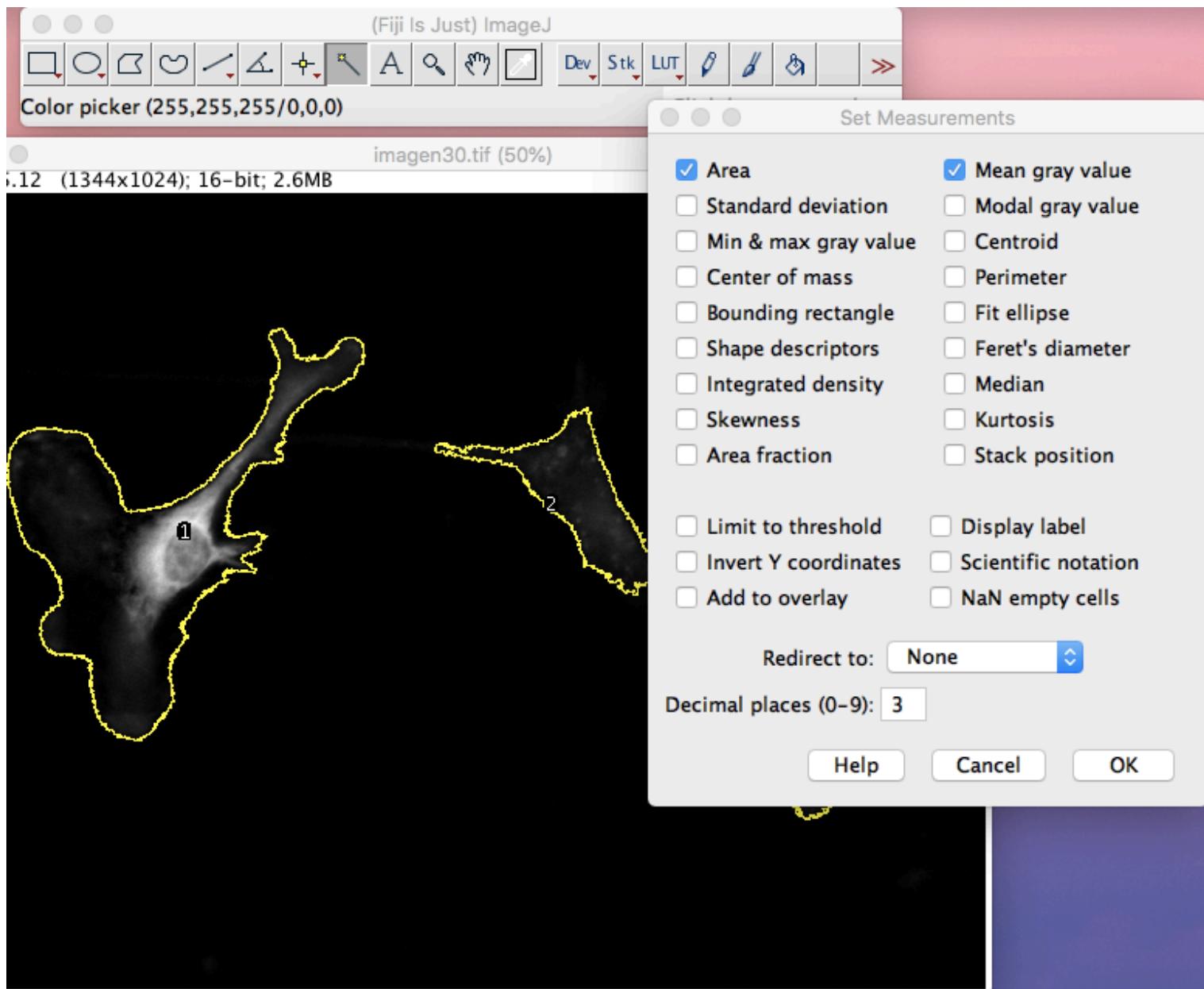
By selecting all ROIs and clicking on *Update all ROIs* become visible



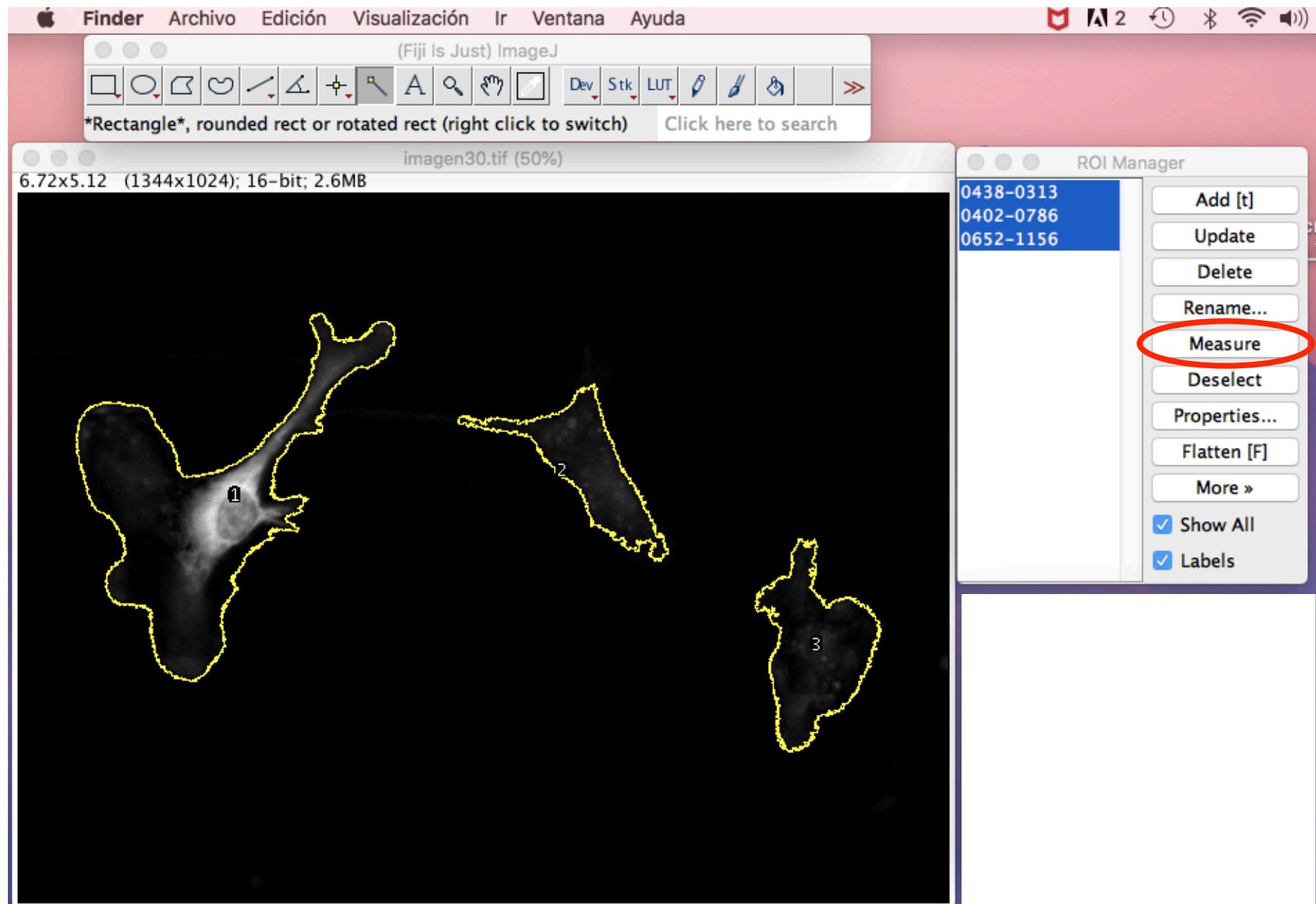
Set measurements to measure fluorescence intensity



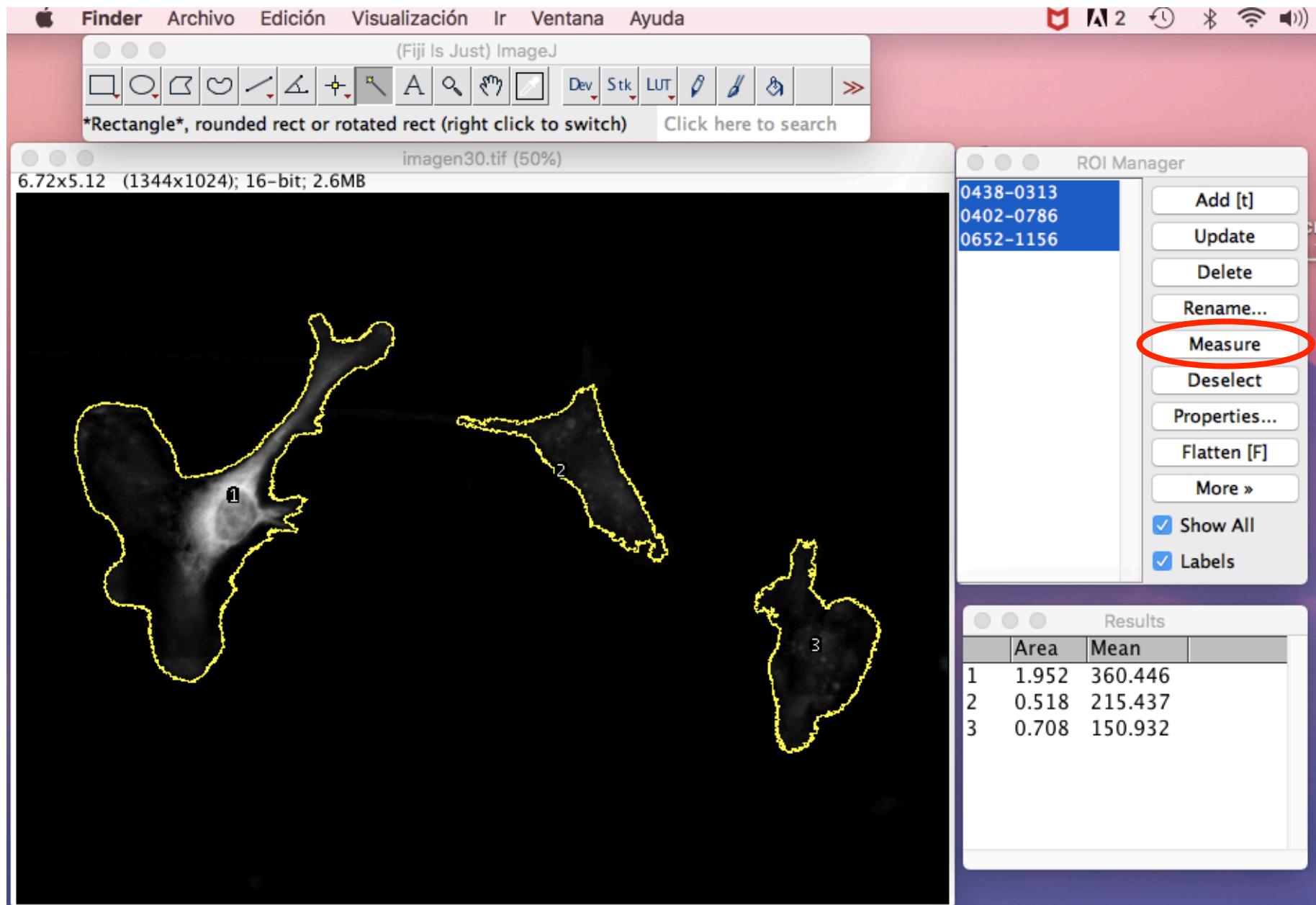
Check Mean grey value and click on OK



Click on Measure in ROI manager



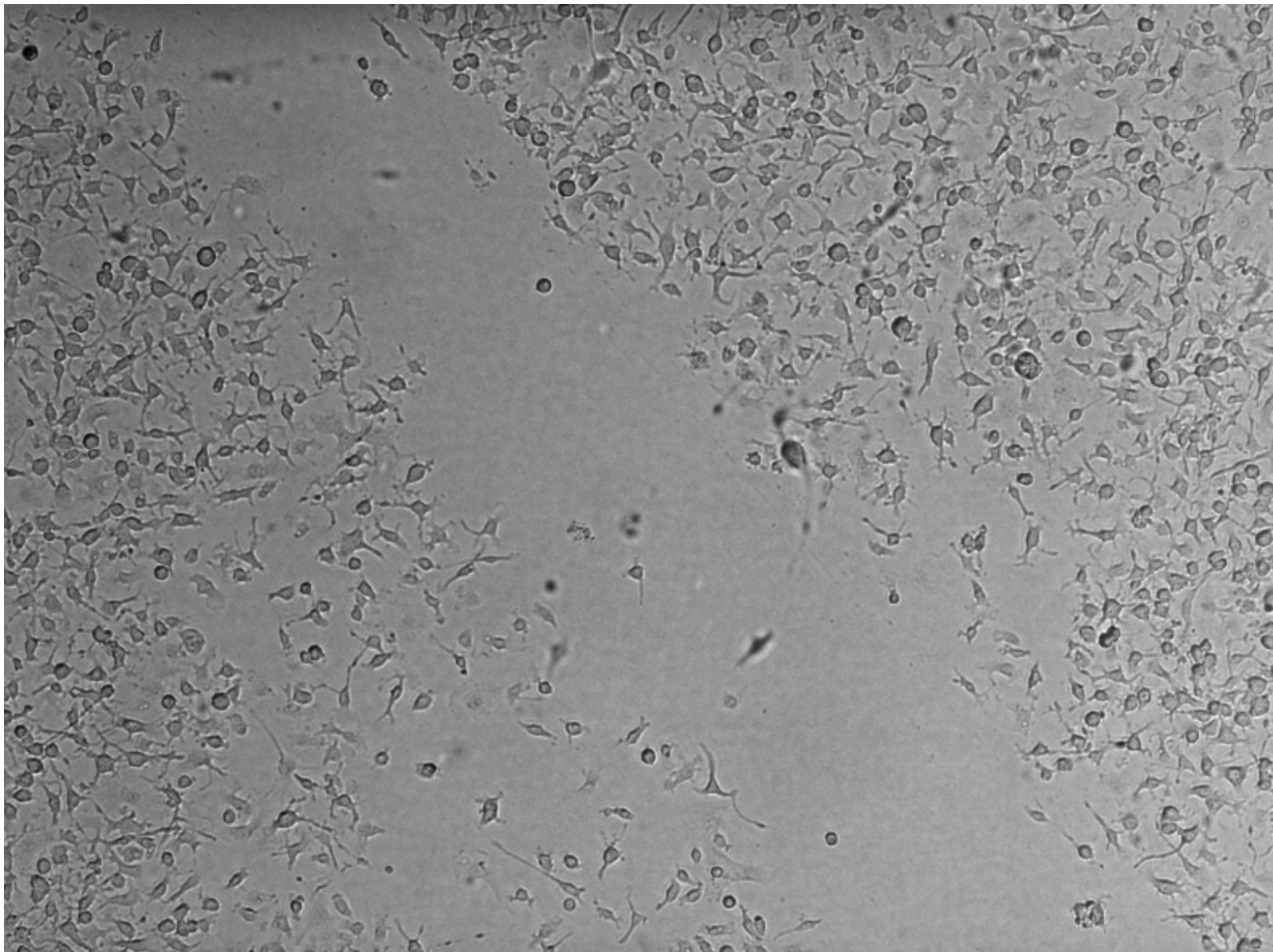
Click on Measure in ROI manager



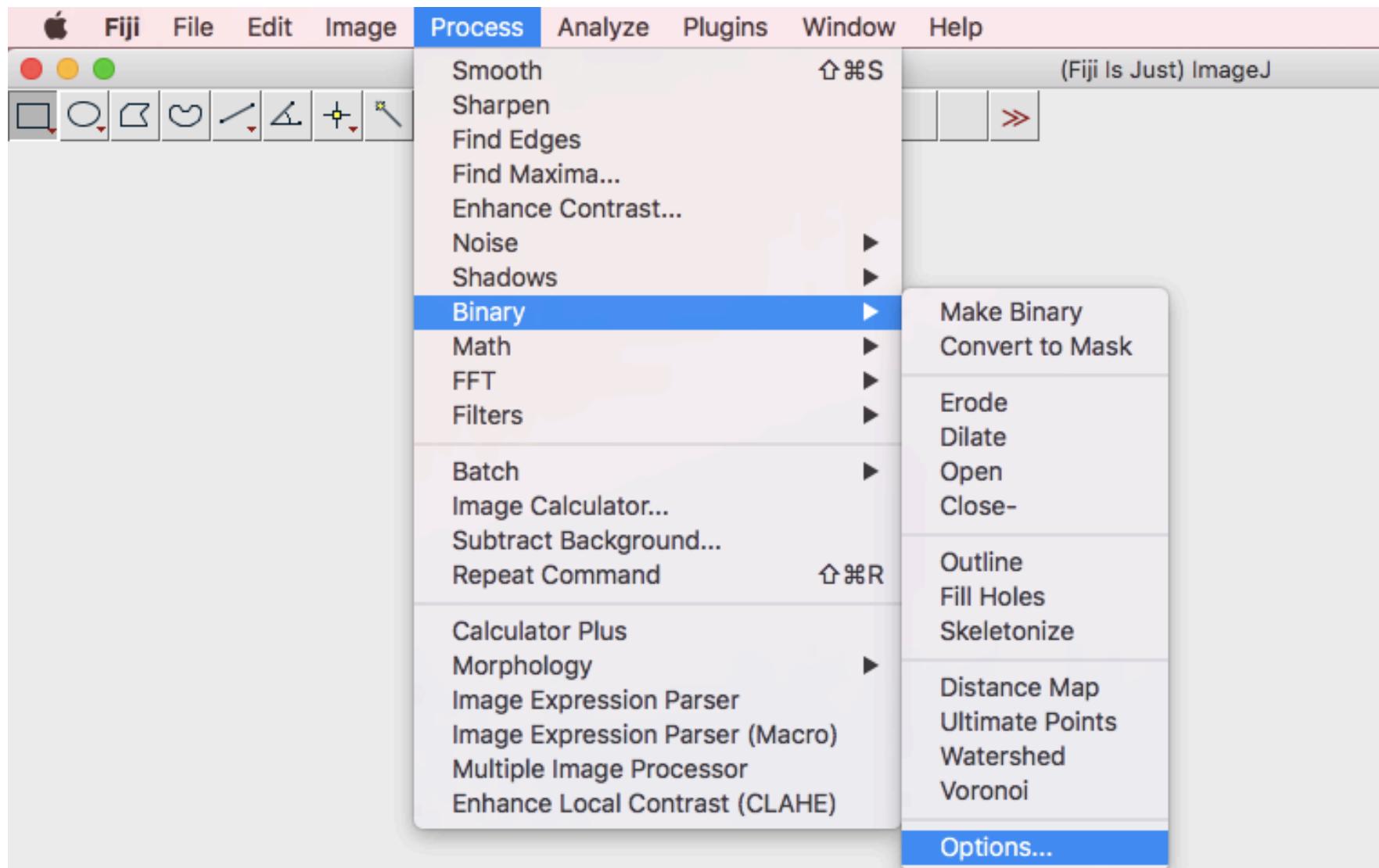
Workshop Overview

- Examples:
 - Counting Particles of Different Sizes
 - Measuring Fluorescence Intensities
- Using a Macro
 - **Wound Healing Macro**
 - Determination of Cell Shape
- Creating a Macro
 - Basic steps
- Practical Session with Example Images

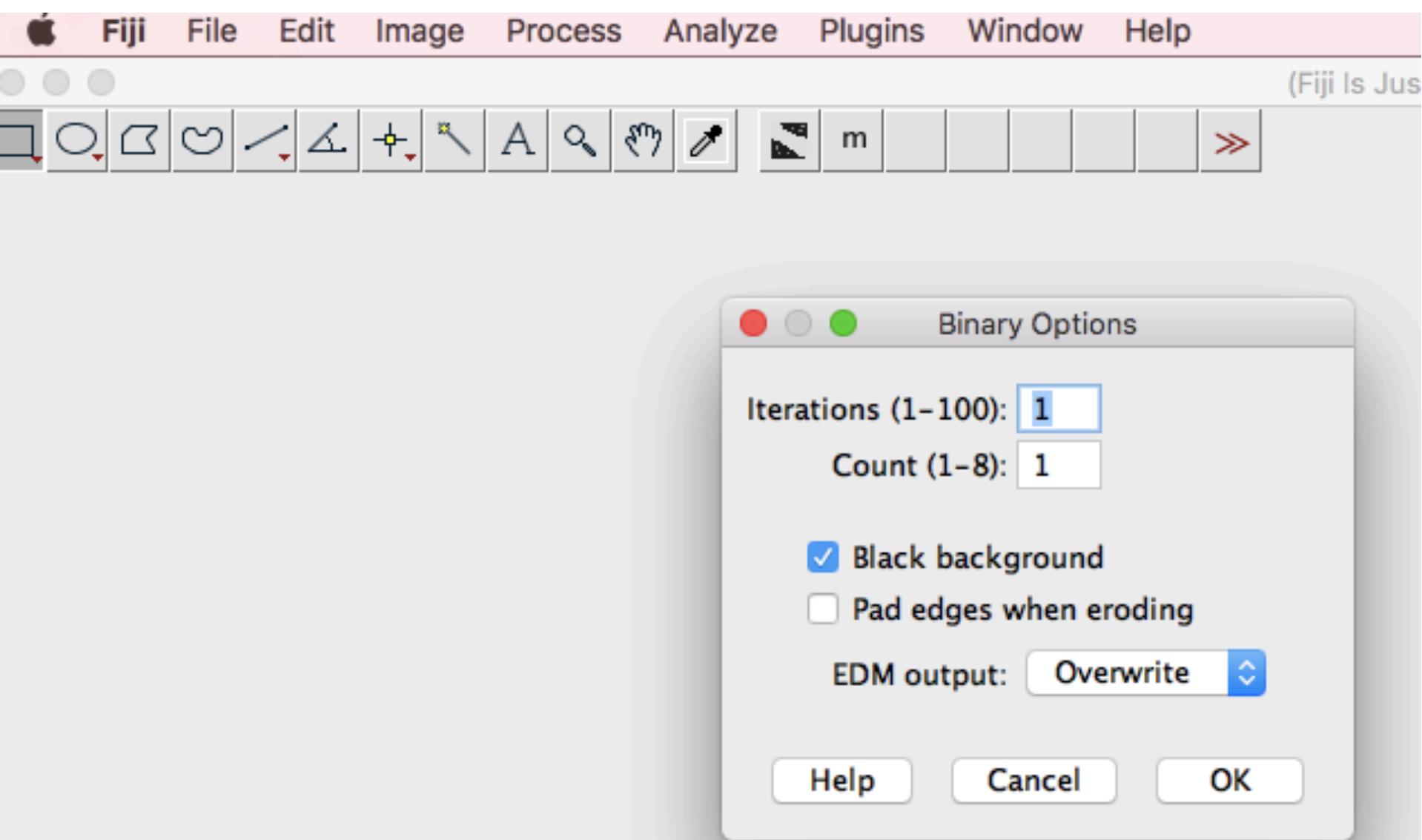
Wound Healing Macro to measure scratch width in a bright field image



Check the option *Black background*



Check the option *Black background*



Download Wound Healing Macro

A screenshot of a Google search results page. The search bar at the top contains the query "imagej wound healing macro". Below the search bar, the Google logo is displayed. A search card is visible, showing the query again and two suggestions: "imagej wound healing macro" and "imagej wound healing macro". At the bottom of the search card are three buttons: "Google Search", "I'm Feeling Lucky", and "Learn more". Below the search card, there is a link to "Report inappropriate predictions". The status bar at the bottom of the browser window shows "Germany" and various navigation icons.

Google

Suchen

Gmail Images

V

imagej wound healing macro

imagej wound healing macro

Google Search I'm Feeling Lucky Learn more

Report inappropriate predictions

Germany

Advertising Business About Privacy Terms Settings

Click on Wound Healing Tool

imagej wound healing macro

imagej wound healing macro

All Images Videos Shopping News More Settings Tools

About 25.800 results (0,39 seconds)

Wound Healing Tool - ImageJ-macros - MRI's Redmine
dev.mri.cnrs.fr/projects/imagej-macros/wiki/Wound_Healing_Tool ▾
To install the tools, drag the link MRI_Wound_Healing_Tool.ijm to the ImageJ launcher window, save it under macros/toolsets in the ImageJ installation and restart ImageJ. Select the "MRI Wound Healing Tool" toolset from the >> button of the ImageJ launcher.

Wound Healing Coherency Tool - ImageJ-macros - MRI's Redmine
dev.mri.cnrs.fr/projects/imagej-macros/wiki/Wound_Healing_Coherency_Tool ▾
Wound Healing Coherency Tool. The Wound Healing Coherency Tool can be used to analyze scratch assays. It measures the area of a wound in a cellular tissue on a stack of images representing a time-series.

Any of you know how to use MRI Wound Healing tool of ImageJ...
https://www.researchgate.net.../Any_of_you_know_how_to_use_MRI_Wound_Healing...
Feb 23, 2017 - Any of you know how to use MRI Wound Healing tool of ImageJ to ... http://dev.mri.cnrs.fr/projects/imagej-macros/wiki/Wound_Healing_Tool.

Follow the instruction for installation

Wound Healing Tool - ImageJ- X +

dev.mri.cnrs.fr/projects/imagej-macros/wiki/Wound_Healing_Tool

Suchen

Home Projects Help Sign in Register

Search:

ImageJ-macros

Overview Activity Roadmap Issues News Documents **Wiki** Files Repository

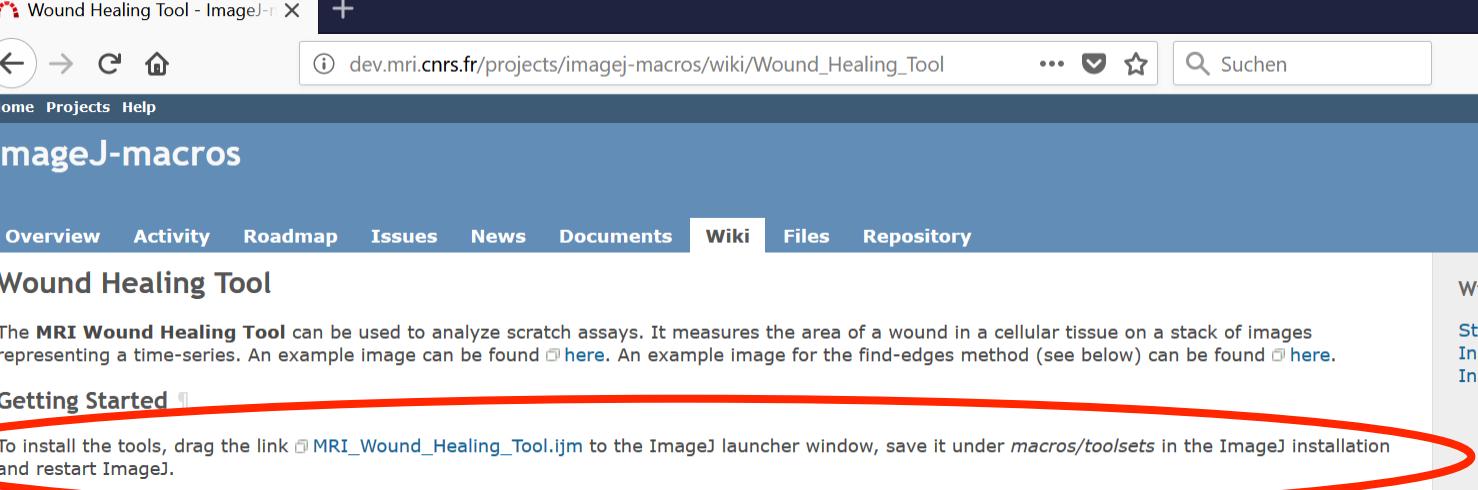
Wound Healing Tool

The **MRI Wound Healing Tool** can be used to analyze scratch assays. It measures the area of a wound in a cellular tissue on a stack of images representing a time-series. An example image can be found [here](#). An example image for the find-edges method (see below) can be found [here](#).

Getting Started

To install the tools, drag the link [MRI_Wound_Healing_Tool.ijm](#) to the ImageJ launcher window, save it under *macros/toolsets* in the ImageJ installation and restart ImageJ.

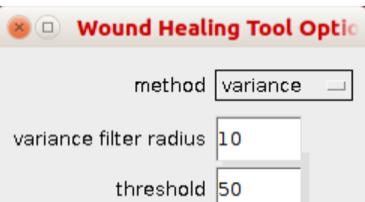
Select the "MRI Wound Healing Tool" toolset from the >> button of the ImageJ launcher.



the first button opens this help-page.
the **m** button starts the measurement on the active stack

Options

By right-clicking on the **m** button you can open the options dialog.



Drag the link into the Fiji menu

The screenshot shows a web browser displaying the [ImageJ-macros](#) website. The main content is the **Wound Healing Tool** page. At the top, there is a navigation bar with links for Overview, Activity, Roadmap, Issues, News, Documents, Wiki (which is currently selected), Files, and Repository. Below the navigation bar, there is a section titled "Wound Healing Tool" with a brief description of the tool's purpose and how to install it. A "Getting Started" section follows, with instructions on how to select the toolset from the ImageJ launcher. To the right of the main content, the source code for the "MRI_Wound_Healing_Tool.ijm" macro is displayed in a code editor. The code includes comments explaining the tool's functionality and its creation by Volker Baeker.

```
/*
 * Wound Healing Tool
 * Collaborators:
 *   Nathalie Cahuzac, CNRS, IGMM, SP
 *   Virginie Georget, MRI-CRB
 *
 * Measure the area of a wound in a cell
 * of images representing a time-series
 *
 * (c) 2010-2017, INSERM
 * written by Volker Baeker at Montpellier
 */

var helpURL = "http://dev.mri.cnrs.fr/wiki/ir
var varianceFilterRadius = 10;
var threshold = 50;
var radiusOpen = 4;
var minSize = 10000;
var methods = newArray("variance", "find
var method = "variance";
var measureInPixelUnits = false;

macro "Unused Tool - C037" {}

macro "MRI Wound Healing Tool Help Act
run('URL...', 'url='+helpURL);
```

Once opened the macro, save it as instructed

The screenshot shows the ImageJ software interface. On the left, there is a documentation page for the "MRI Wound Healing Tool". The page includes a toolbar at the top, a search bar, and a navigation menu with tabs like Overview, Activity, Roadmap, Issues, News, Documents, Wiki (which is currently selected), Files, and Repository. Below the tabs, there are sections for "Wound Healing Tool" and "Getting Started". In the "Getting Started" section, there is a list of instructions and a screenshot of the ImageJ launcher window showing the "Wound Healing Tool" toolset. On the right, a separate window titled "MRI_Wound_Healing_Tool" is open, showing the "File" menu with "Save As..." highlighted. The main content area of this window displays the Java script code of the macro:

```
var helpURL = "http://dev.mri.cnrs.fr/wiki/ir";
var varianceFilterRadius = 10;
var threshold = 50;
var radiusOpen = 4;
var minSize = 10000;
var methods = newArray("variance", "findEdges");
var method = "variance";
var measureInPixelUnits = false;

macro "Unused Tool - C037" {}

macro "MRI Wound Healing Tool Help Action" {
    run('URL...', 'url=' + helpURL);
}
```

Save it under *macros* → *toolsets* and restart Fiji

The screenshot shows the Fiji software interface. On the left, a 'Save As...' dialog is open, showing the file path 'Speichern in: toolsets'. The 'Dateityp:' dropdown is set to 'Alle Dateien (*.*)'. Below the dialog, two bullet points are listed:

- the first button opens this help-page.
- the **m** button starts the measurement on the active stack

On the right, the Fiji menu bar is visible with 'File', 'Edit', 'Font', 'Examples', 'Macros', and 'Debug' options. A code editor window titled 'MRI_Wound_Healing_Tool...' is open, displaying Java script code for the Wound Healing Tool. The code includes comments about the tool's purpose, collaborators, and copyright information, along with variable declarations for helpURL, varianceFilterRadius, threshold, radiusOpen, minSize, methods, method, and measureInPixelUnits. It also contains macros for 'Unused Tool - C037' and 'MRI Wound Healing Tool Help Action'.

```
/*
 * Wound Healing Tool
 * Collaborators:
 *   Nathalie Cahuzac, CNRS, IGMM, SP
 *   Virginie Georget, MRI-CRB
 */
/*
 * Measure the area of a wound in a cell
 * of a wound in a cellular tissue on a stack of images
 * the find-edges method (see below) can be found here.
 */

// in the Fiji Options dialog, save it under macros/toolsets in the ImageJ installation

var helpURL = "http://dev.mri.cnrs.fr/wiki/ir";
var varianceFilterRadius = 10;
var threshold = 50;
var radiusOpen = 4;
var minSize = 10000;
var methods = newArray("variance", "find");
var method = "variance";
var measureInPixelUnits = false;

macro "Unused Tool - C037" {}

macro "MRI Wound Healing Tool Help Action"
    run('URL...', 'url=' + helpURL);

```

Open the MRI_Wound_Healing_Tool by clicking on >> button

The screenshot shows the ImageJ launcher window. At the top, there's a toolbar with various icons and a search bar labeled "Suchen". Below the toolbar is a menu bar with File, Edit, Image, Process, Analyze, Plugins, Window, and Help. A sub-menu "More Tools" is open under the Plugins menu, listing several toolsets: StartupMacros*, Clear Custom Tools, Drawing Tools, Lookup Tables, MRI_Wound_Healing_Tool (which is highlighted in blue), Arrow, Brush, Command Finder, Developer Menu, Flood Filler, LUT Menu, Overlay Brush, Pencil, Pixel Inspector, Selection Rotator, Smooth Wand, Spray Can, and Stacks Menu. To the left of the launcher, a browser window titled "ImageJ-macros" is visible, displaying information about the "Wound Healing Tool".

(Fiji Is Just) ImageJ

File Edit Image Process Analyze Plugins Window Help

"More Tools" menu (switch toolsets or add tools)

Click here to search

Home

ImageJ-macros

Overview Activity Roadmap Issues News Documents Wiki Files

Wound Healing Tool

The **MRI Wound Healing Tool** can be used to analyze scratch assays. It measures the area of representing a time-series. An example image can be found [here](#). An example image for the f

Getting Started

To install the tools, drag the link [MRI_Wound_Healing_Tool.ijm](#) to the ImageJ launcher window and restart ImageJ.

Select the "MRI Wound Healing Tool" toolset from the >> button of the ImageJ launcher.

- the first button opens this help-page.
- the **m** button starts the measurement on the active stack

Options

By right-clicking on the **m** button you can open the options dialog.

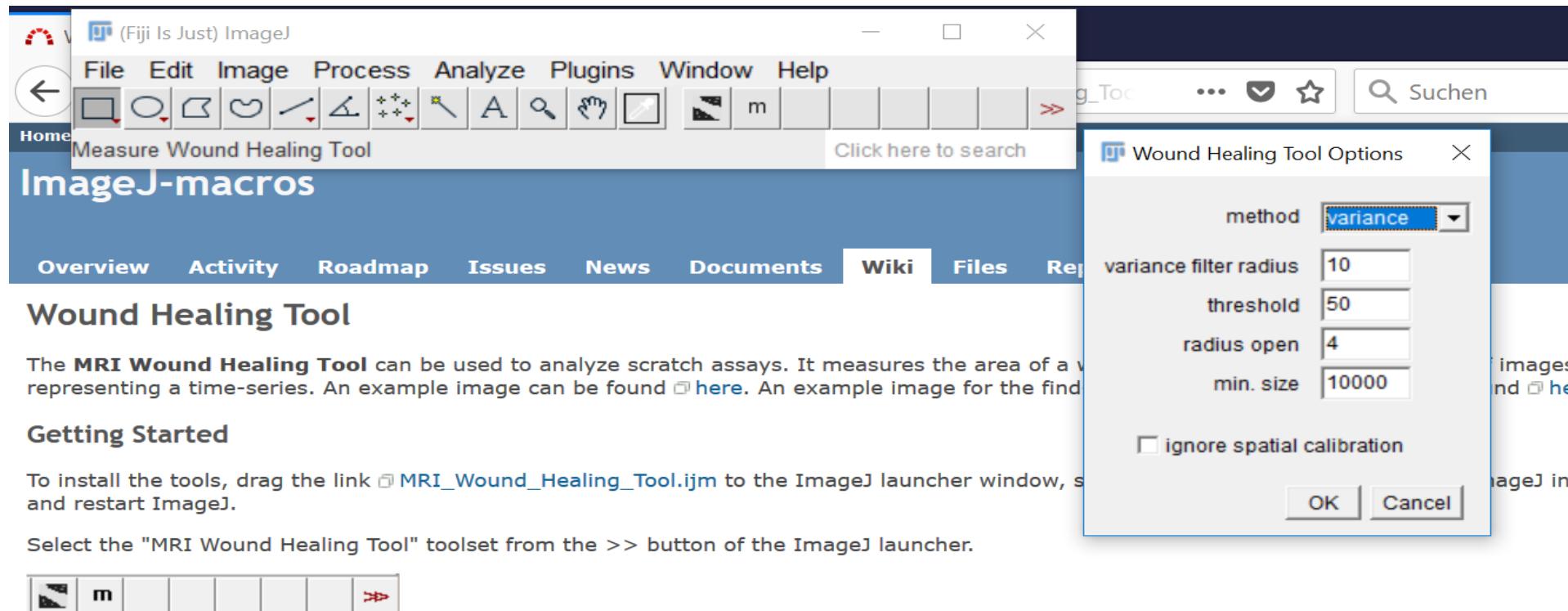
Wound Healing Tool Options

method variance

variance filter radius 10

threshold 50

By right-clicking on the *m*-button open the options dialog



- the first button opens this help-page.
- the **m** button starts the measurement on the active stack

Options

By right-clicking on the **m** button you can open the options dialog.



These parameters have to be optimized for every experiment

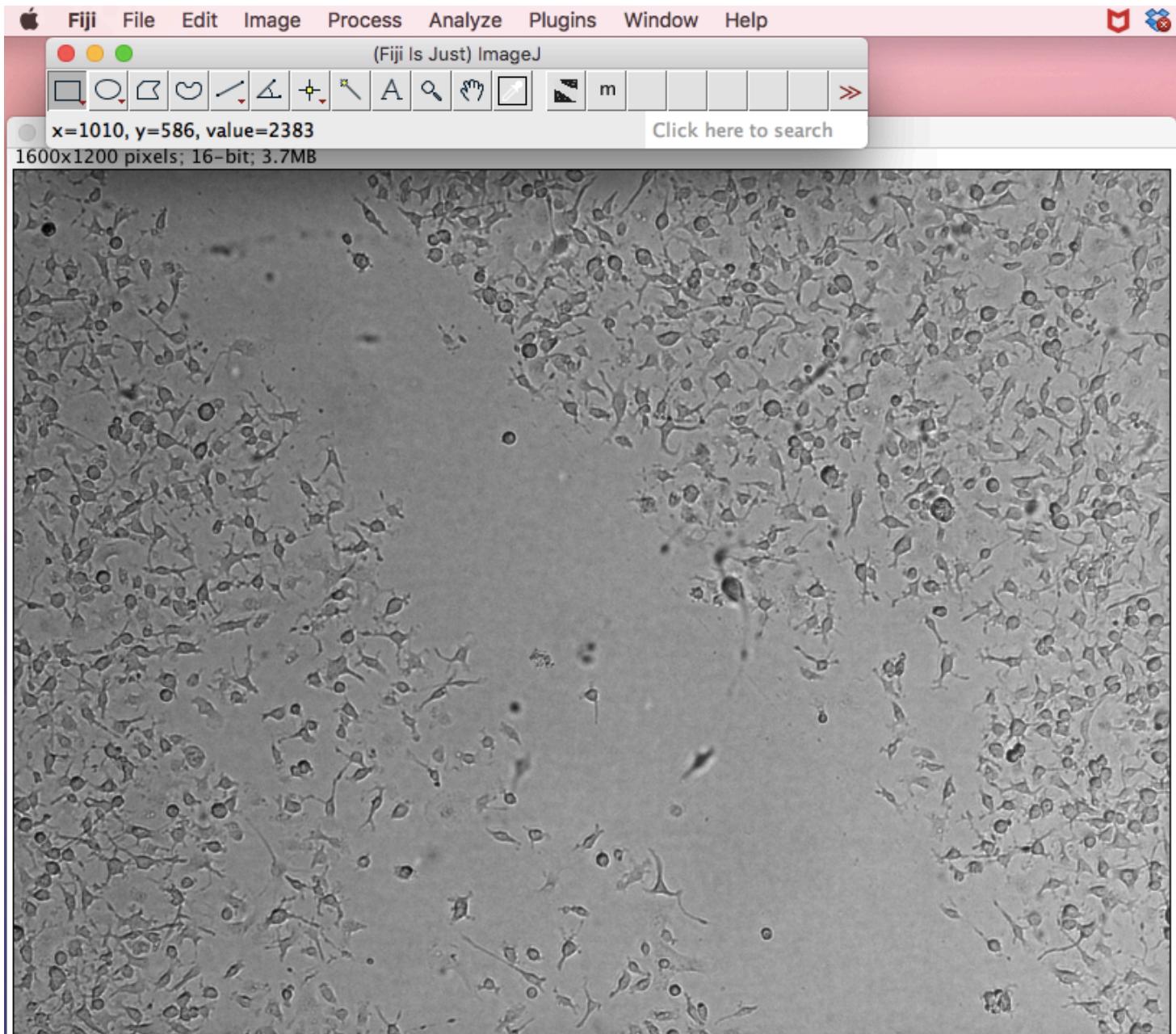
The image shows the Fiji software interface. At the top is a menu bar with options: File, Edit, Image, Process, Analyze, Plugins, Window, and Help. Below the menu is a toolbar with various icons for image processing. In the center, a dialog box titled "Wound Healing Tool Options" is open. The dialog contains the following settings:

Parameter	Value
method	variance
variance filter radius	40
threshold	50
radius open	1
min. size	26000

Below the settings is a checkbox labeled "ignore spatial calibration". At the bottom right of the dialog are "Cancel" and "OK" buttons.

For our example images we set these values:

Open image Image_31503_ikBa in Wound folder



Click on the *m*-button to run the macro

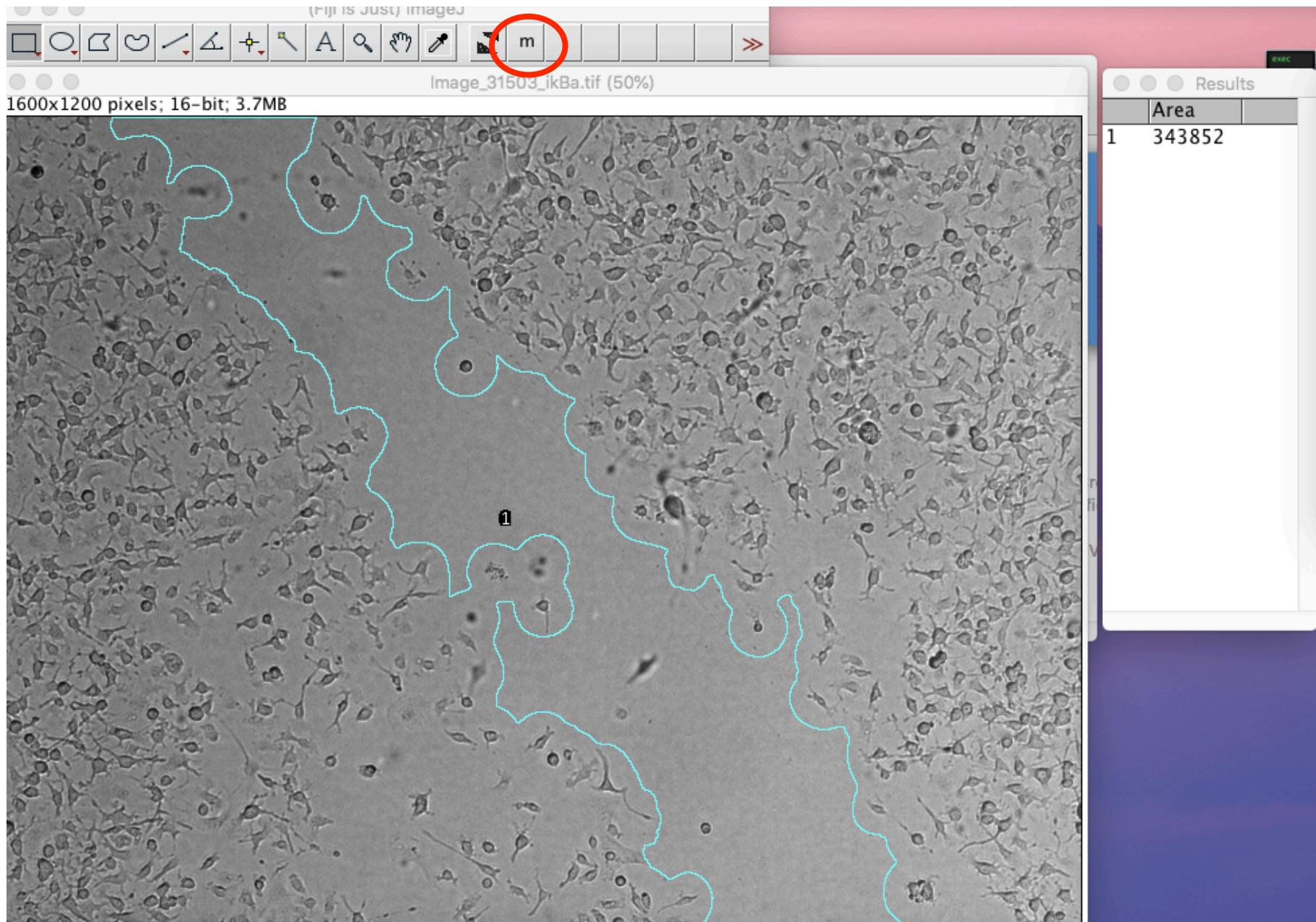
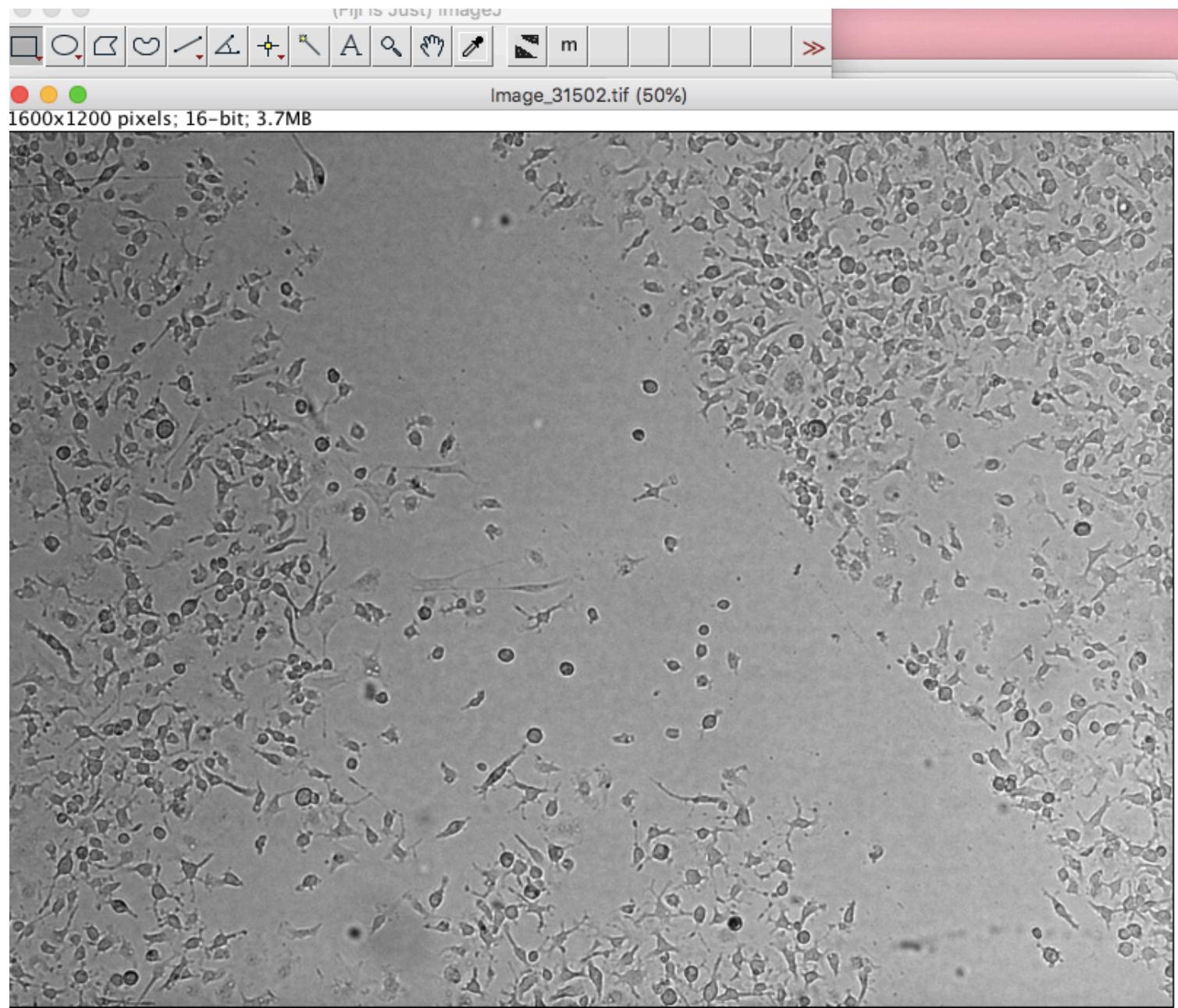
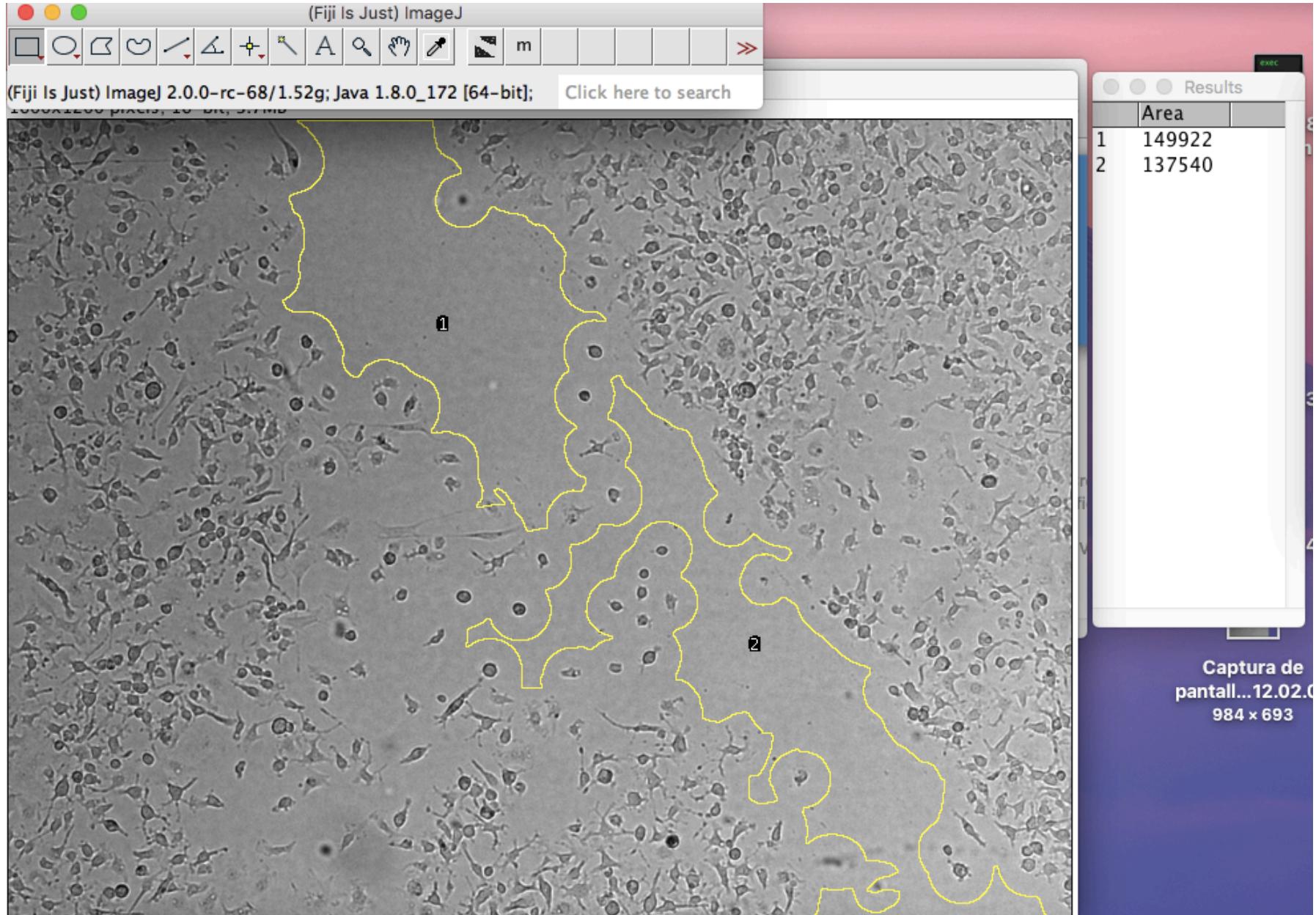


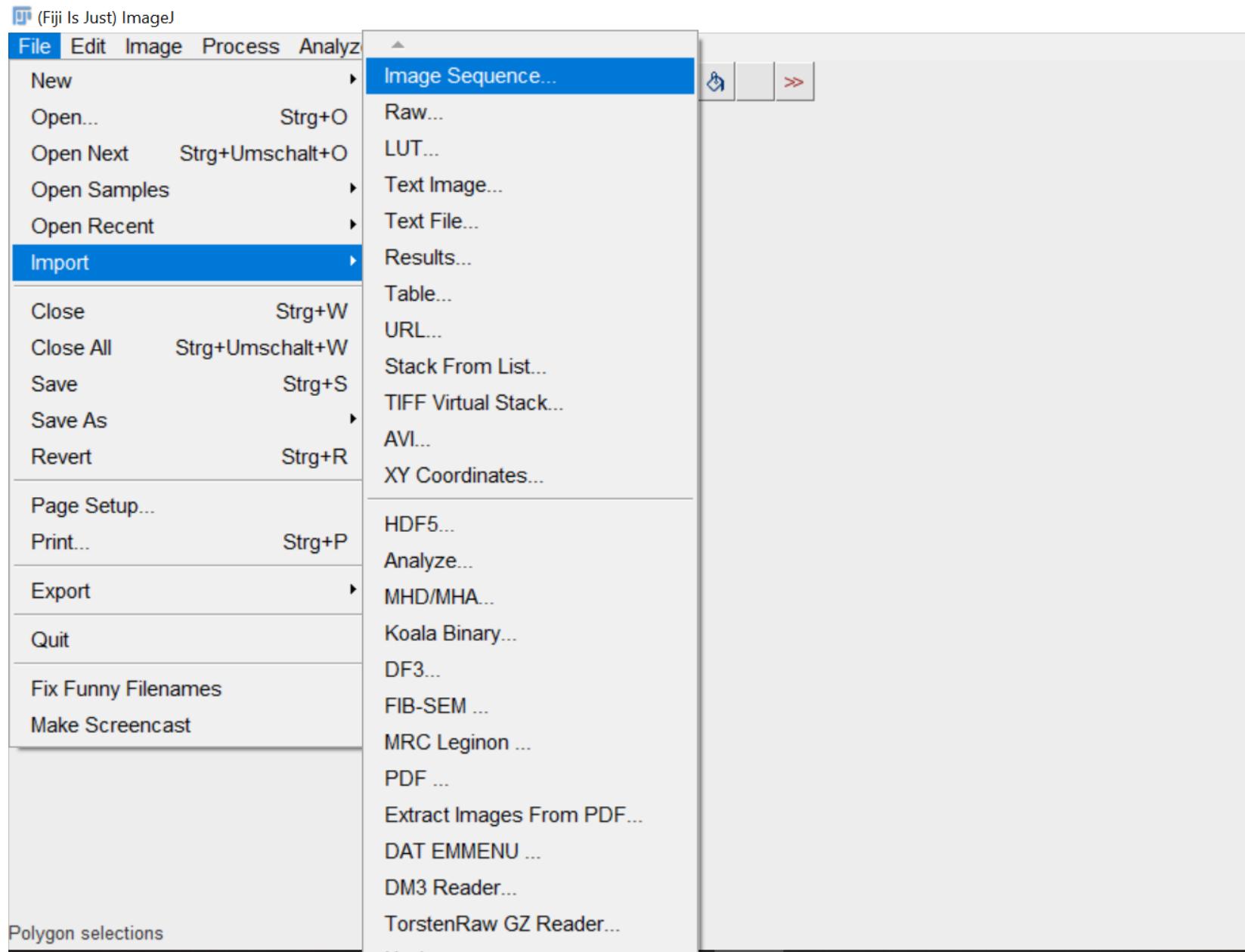
Image with cells in scratch???



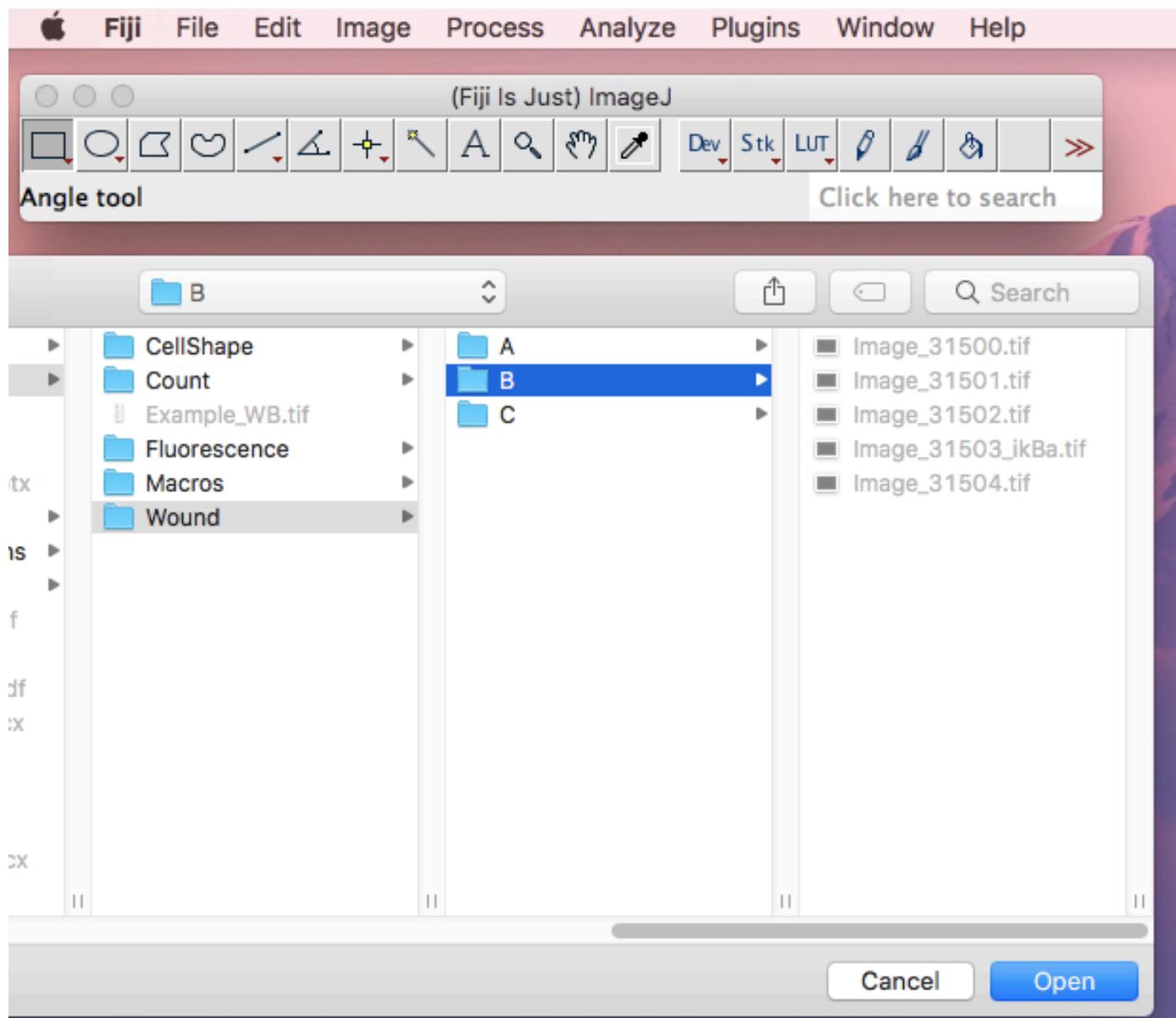
Macro calculates 2 areas, which can be summed up



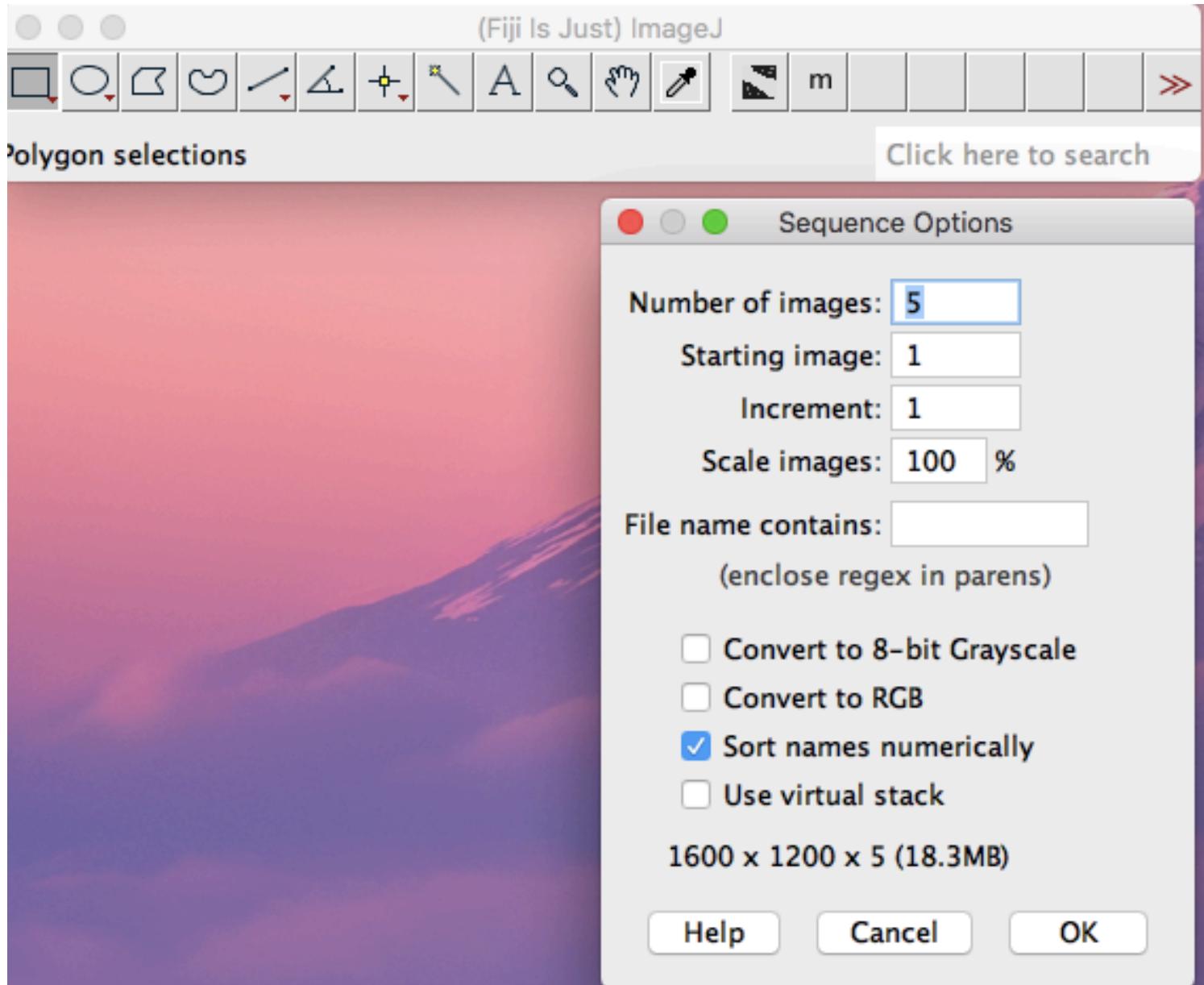
Import *Image Sequence*



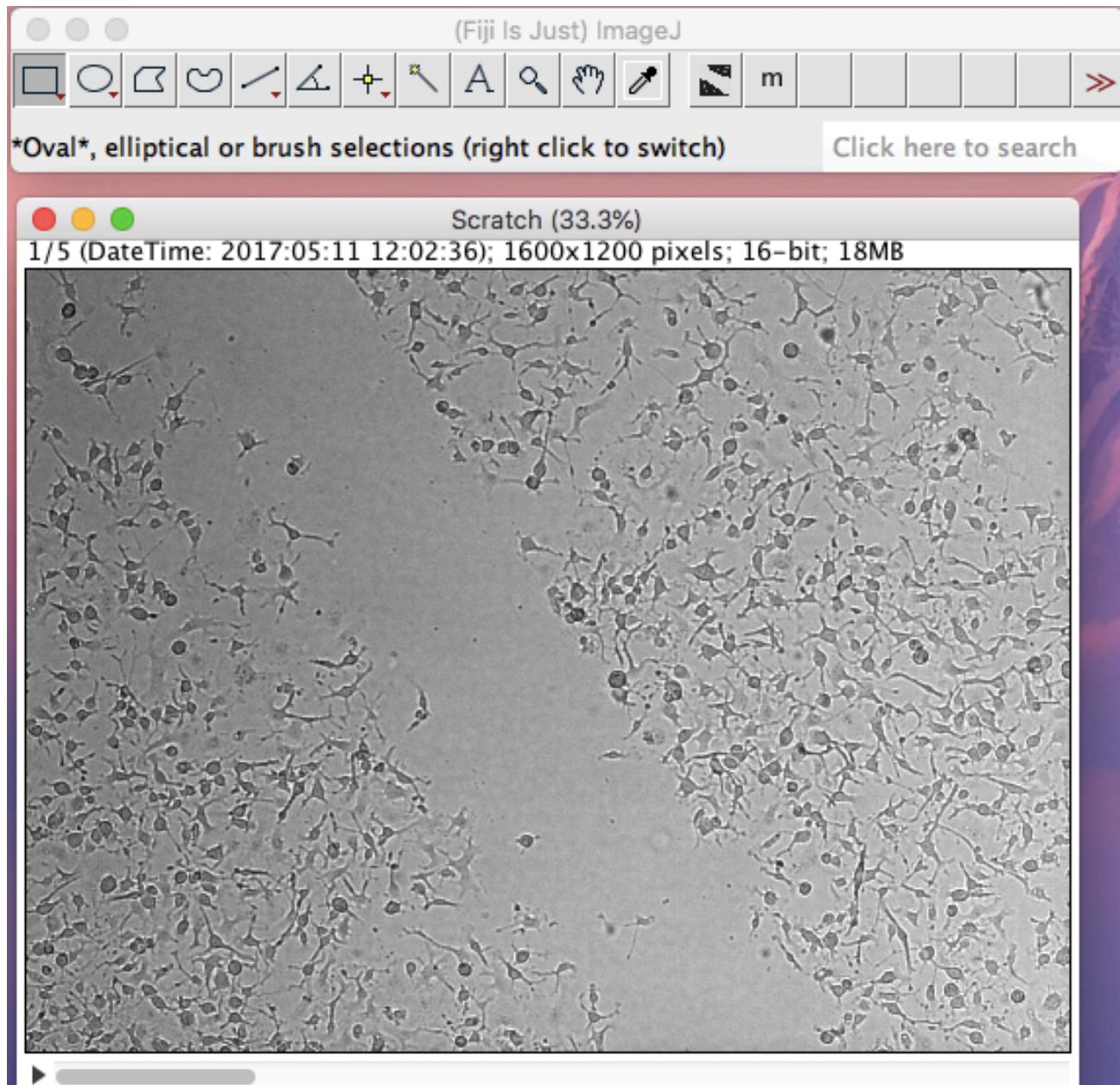
Import image sequence in Wound → B. Click on Open



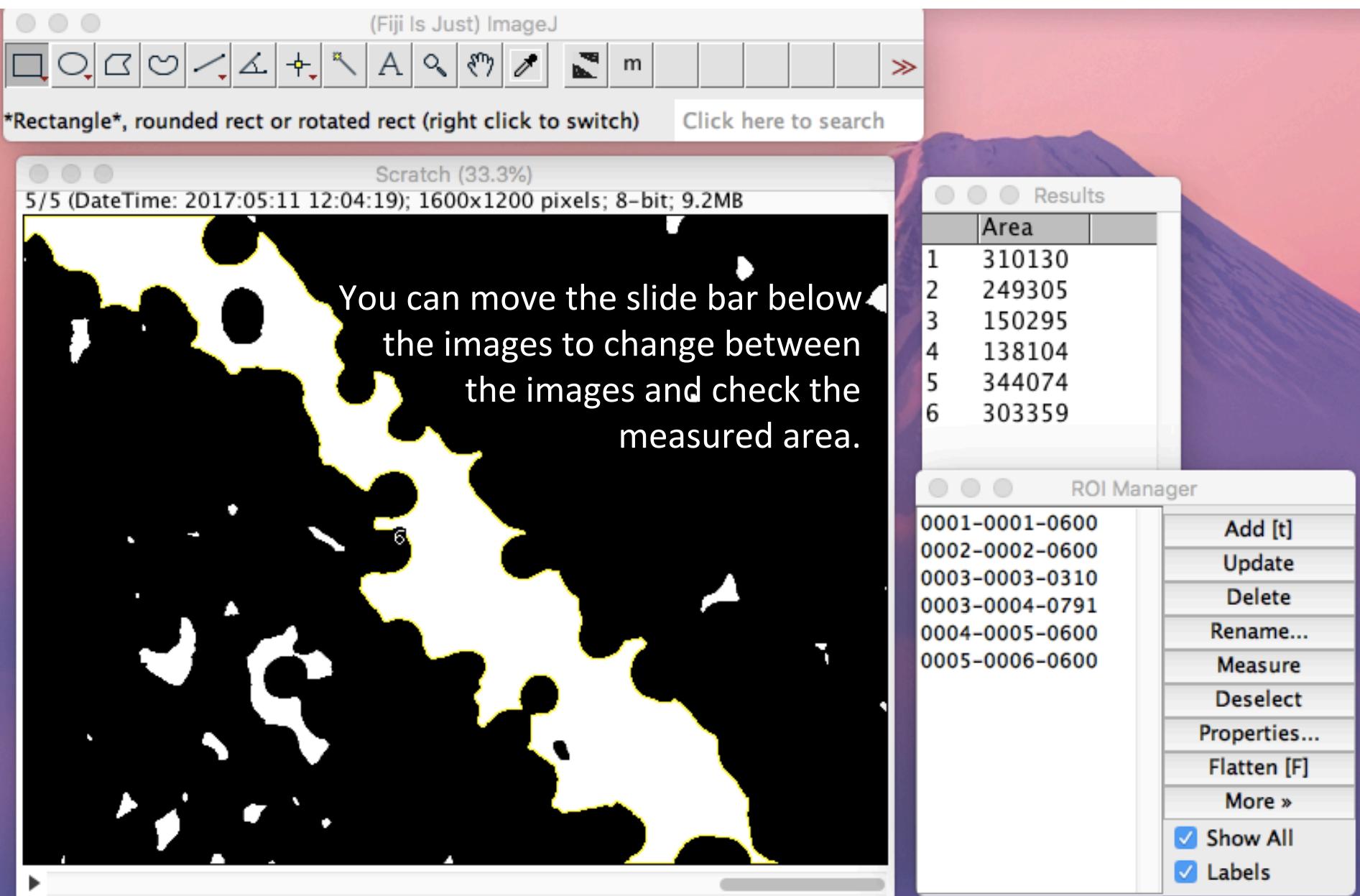
Click on OK for Sequence Options



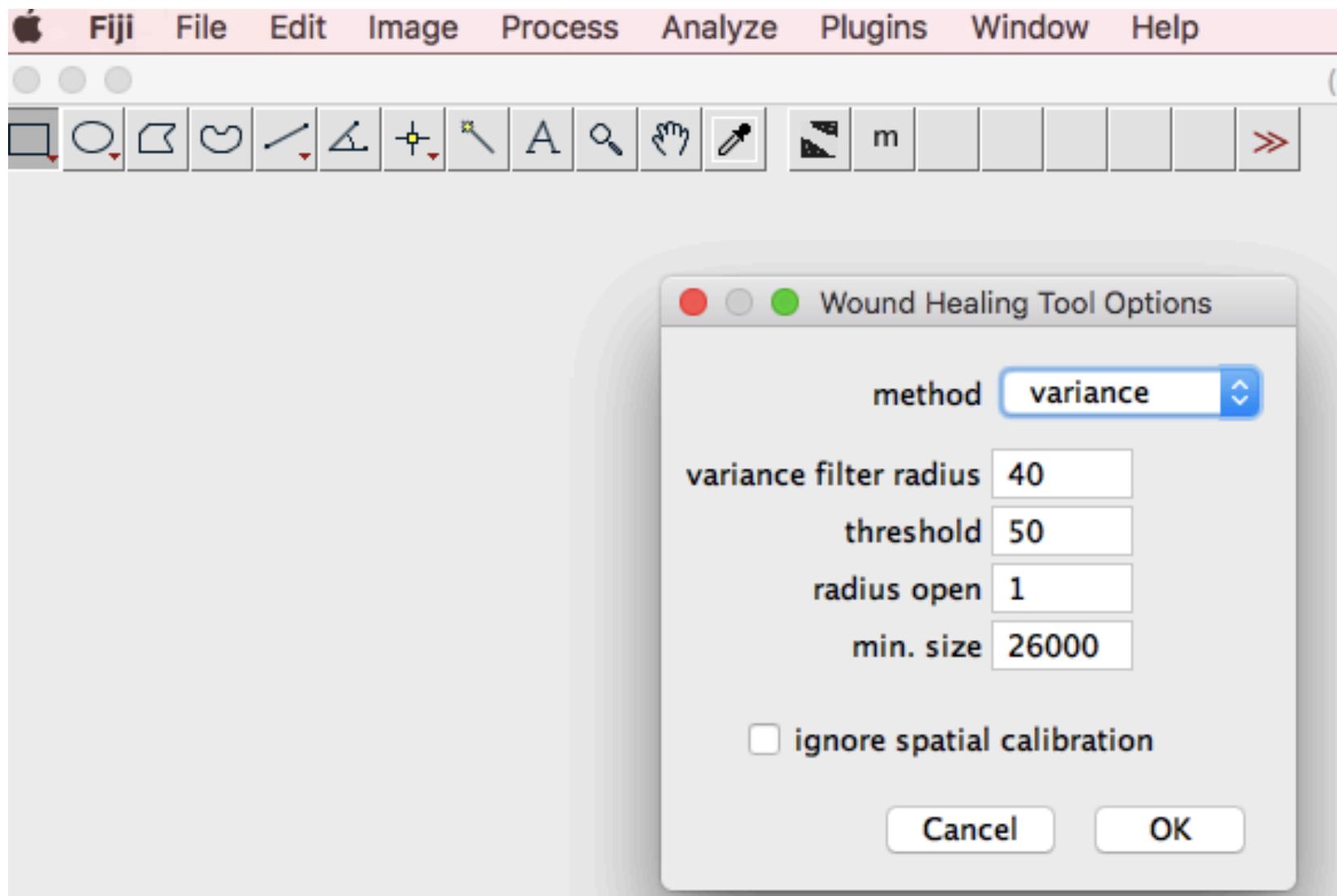
5 images are open and run macro on all of them by clicking on the *m*-button



In results all areas come up, which can be summed up



Recommendation for the practical session:
change parameters and see how the outcome is affected



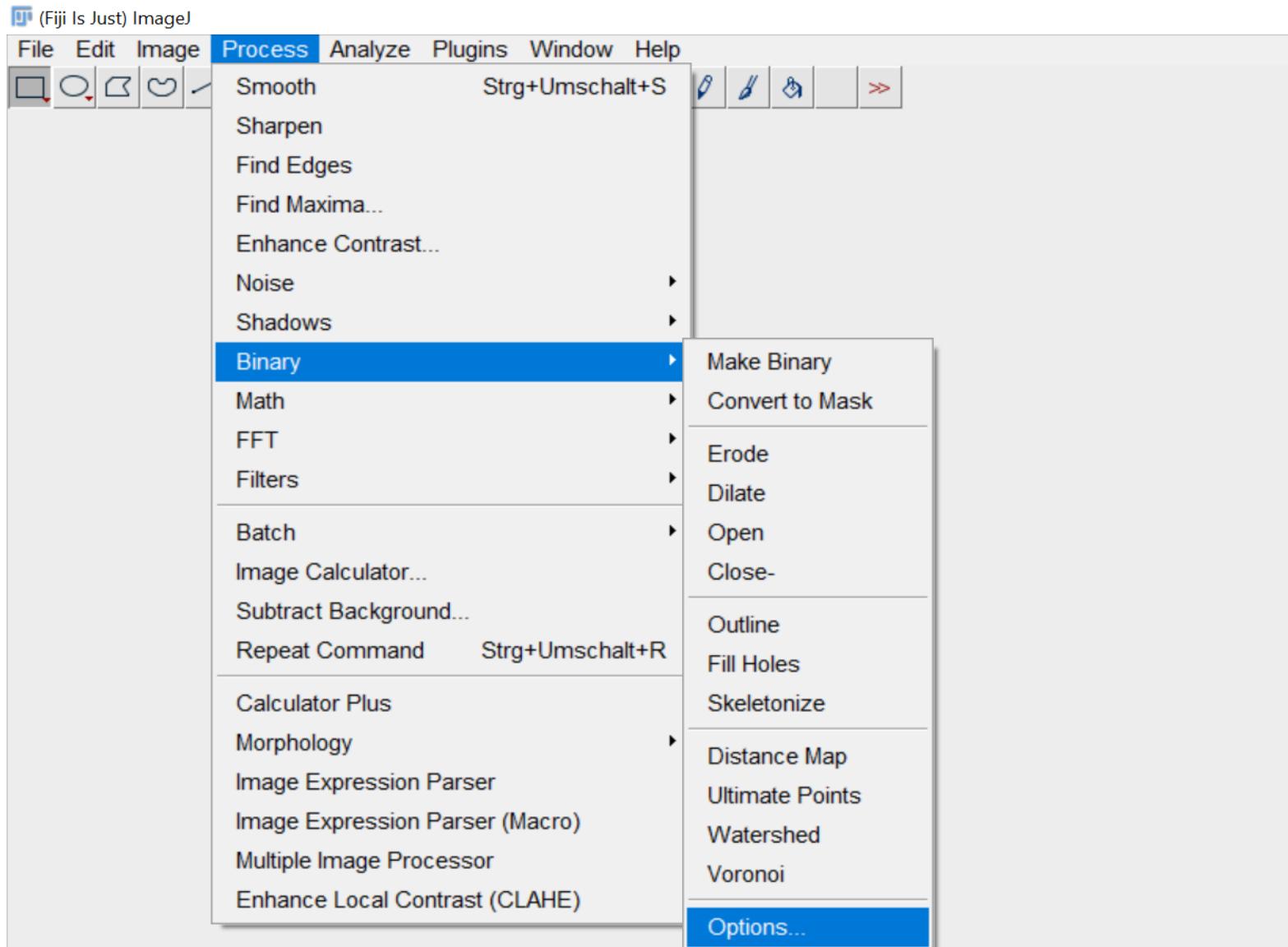
Workshop Overview

- Examples:
 - Counting Particles of Different Sizes
 - Measuring Fluorescence Intensities
- **Using a Macro**
 - Wound Healing Macro
 - **Determination of Cell Shape**
- Creating a Macro
 - Basic steps
- Practical Session with Example Images

Determination of Cell Shape, incl. circularity (values 0 – 1)



Check again in *Process*: Click on *Binary* → *Options*

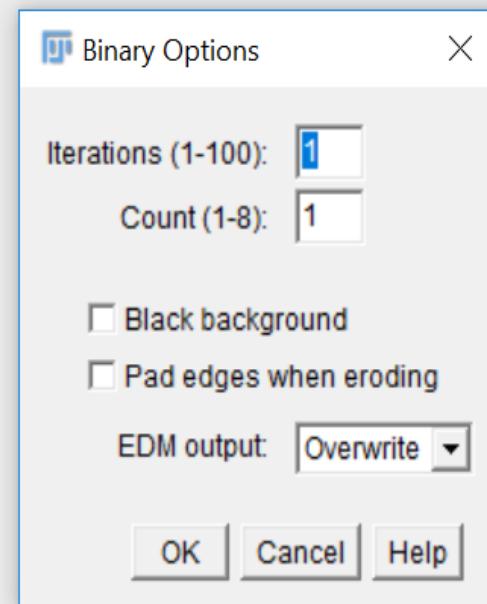


Multi-point or point (right click to switch; double click to configure)

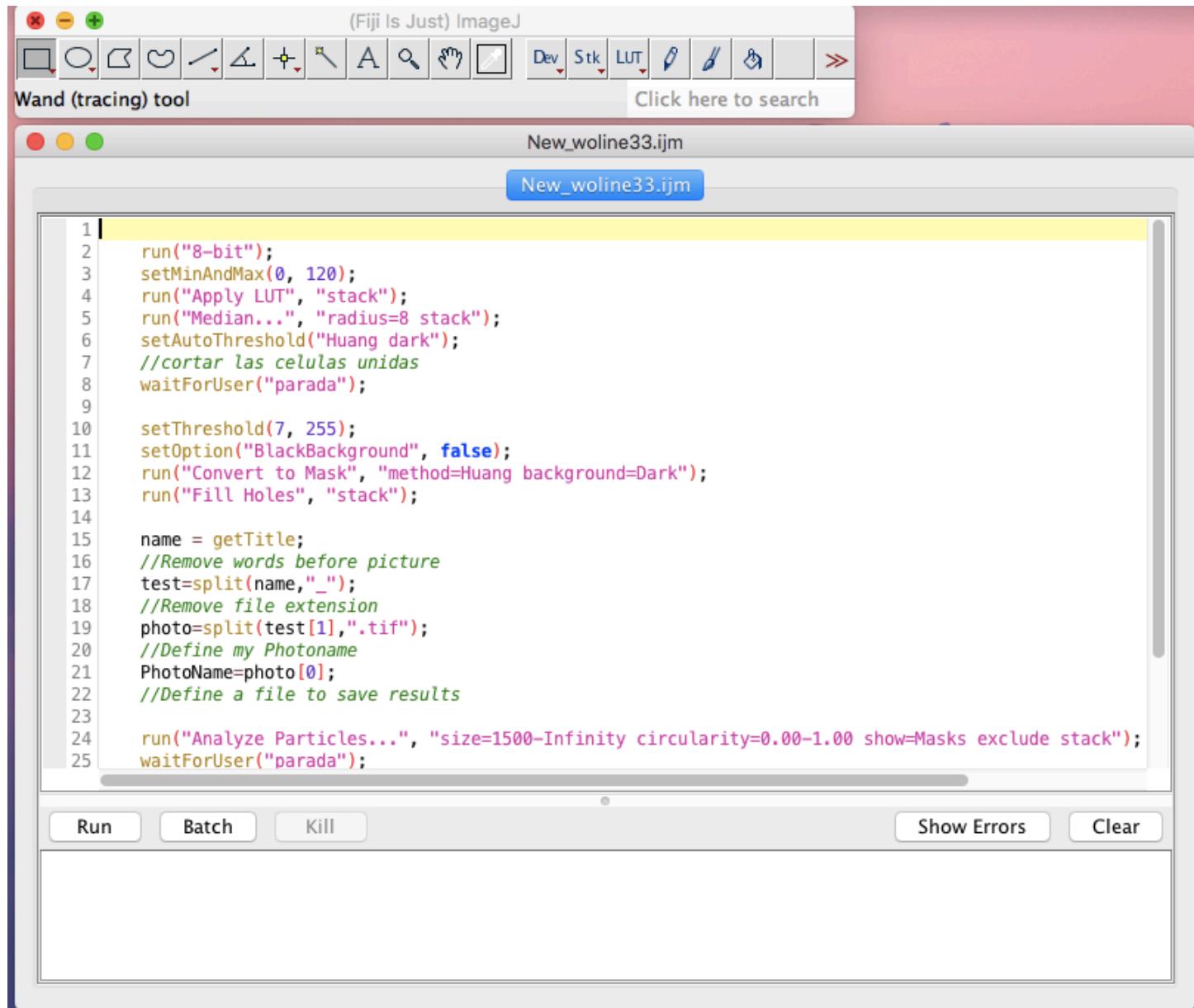
Copy these settings:

(Fiji Is Just) ImageJ

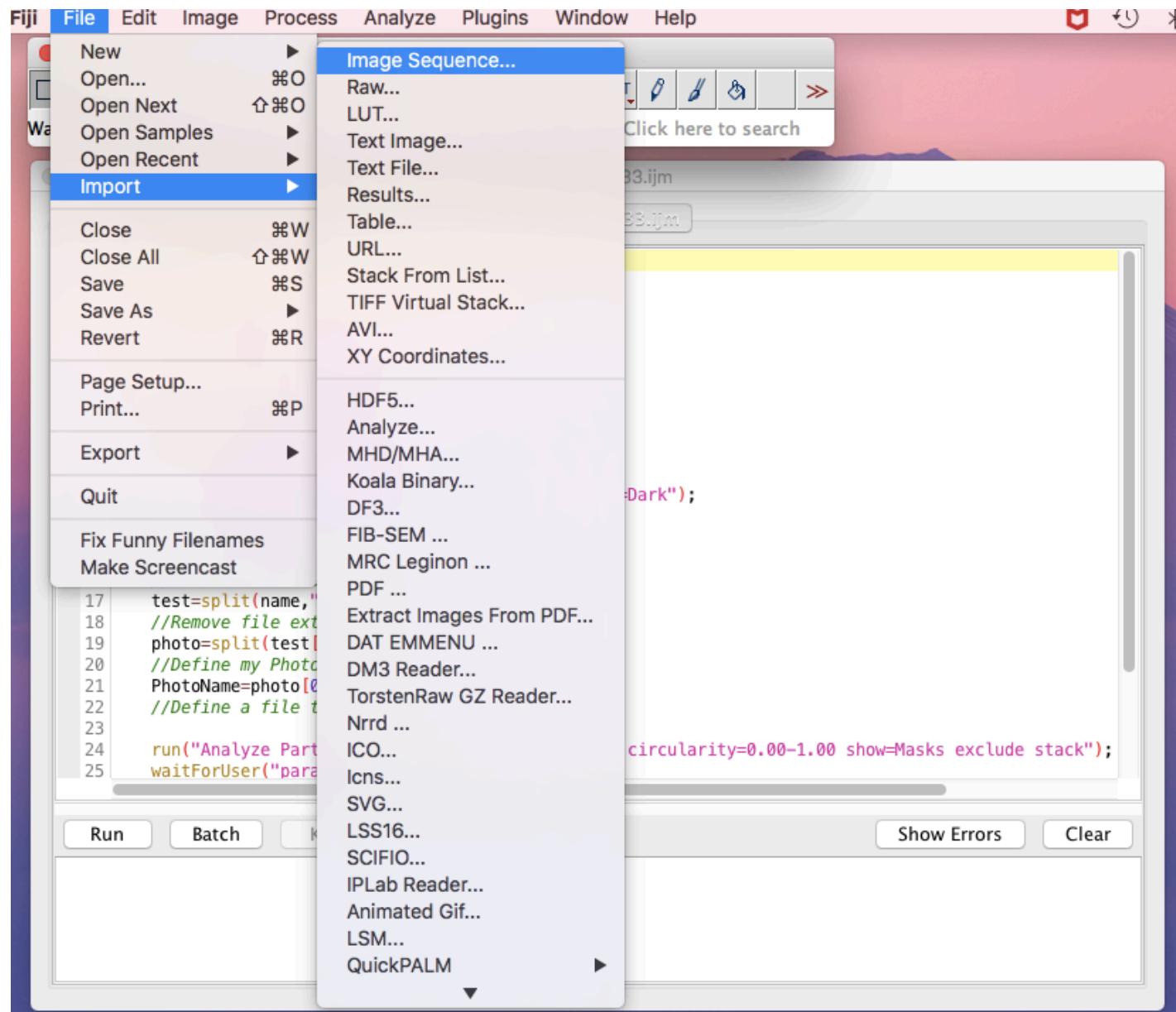
File Edit Image Process Analyze Plugins Window Help



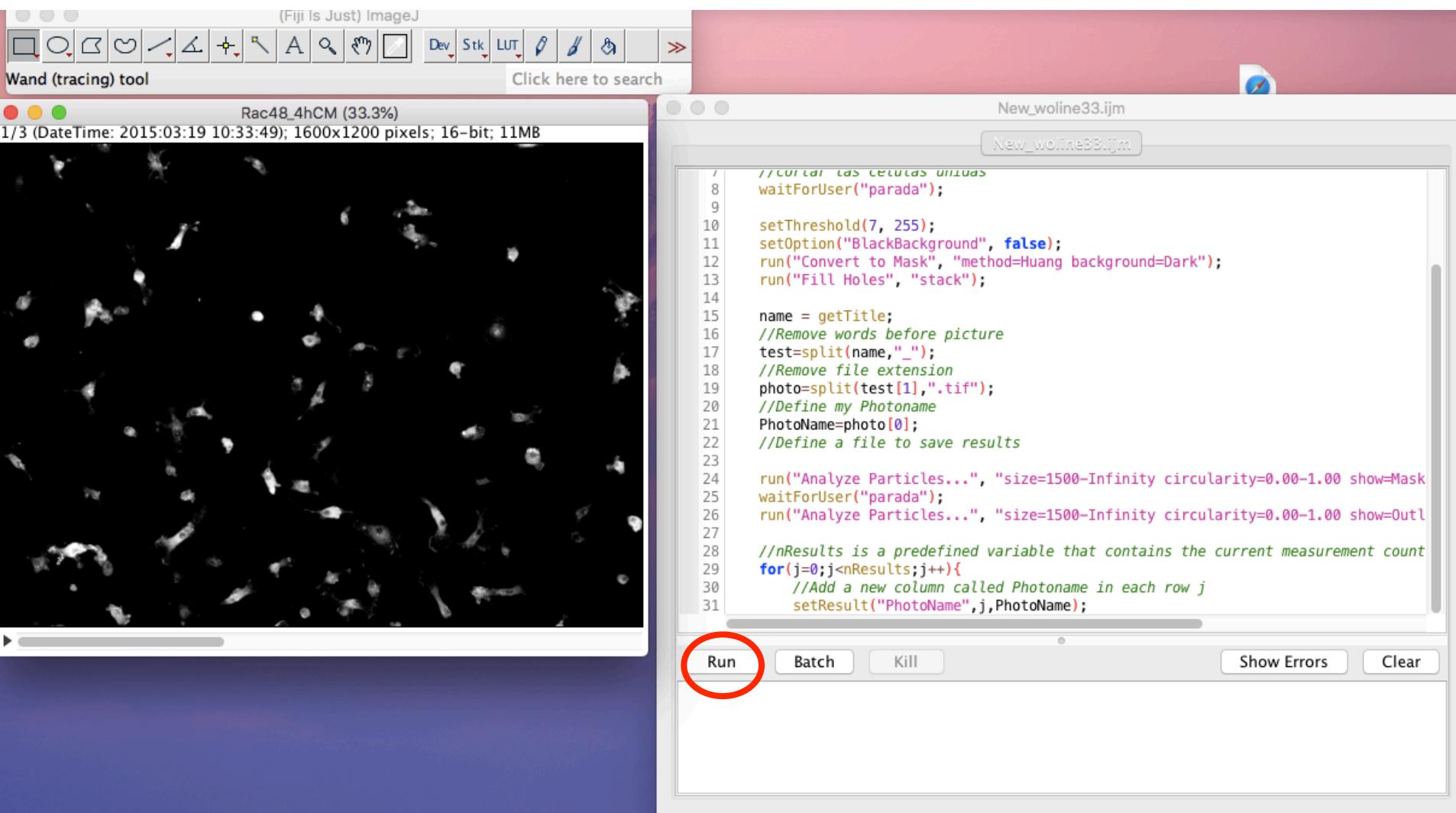
Open the custom-made macro by dragging the file new_woline33PC.ijm into the toolbar



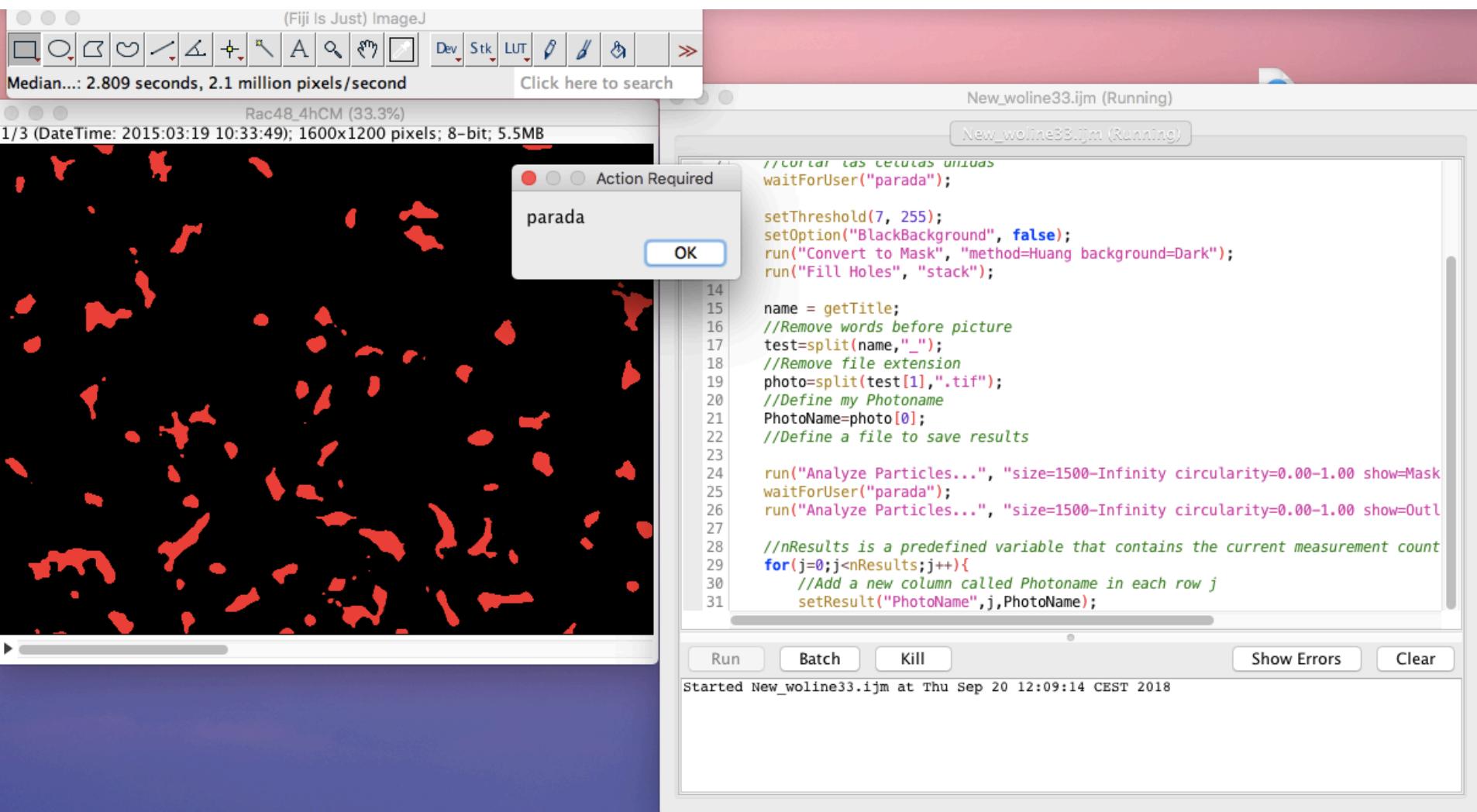
Open folder with images by *Import Image Sequence* Select folder Cell Shape → 48_4h



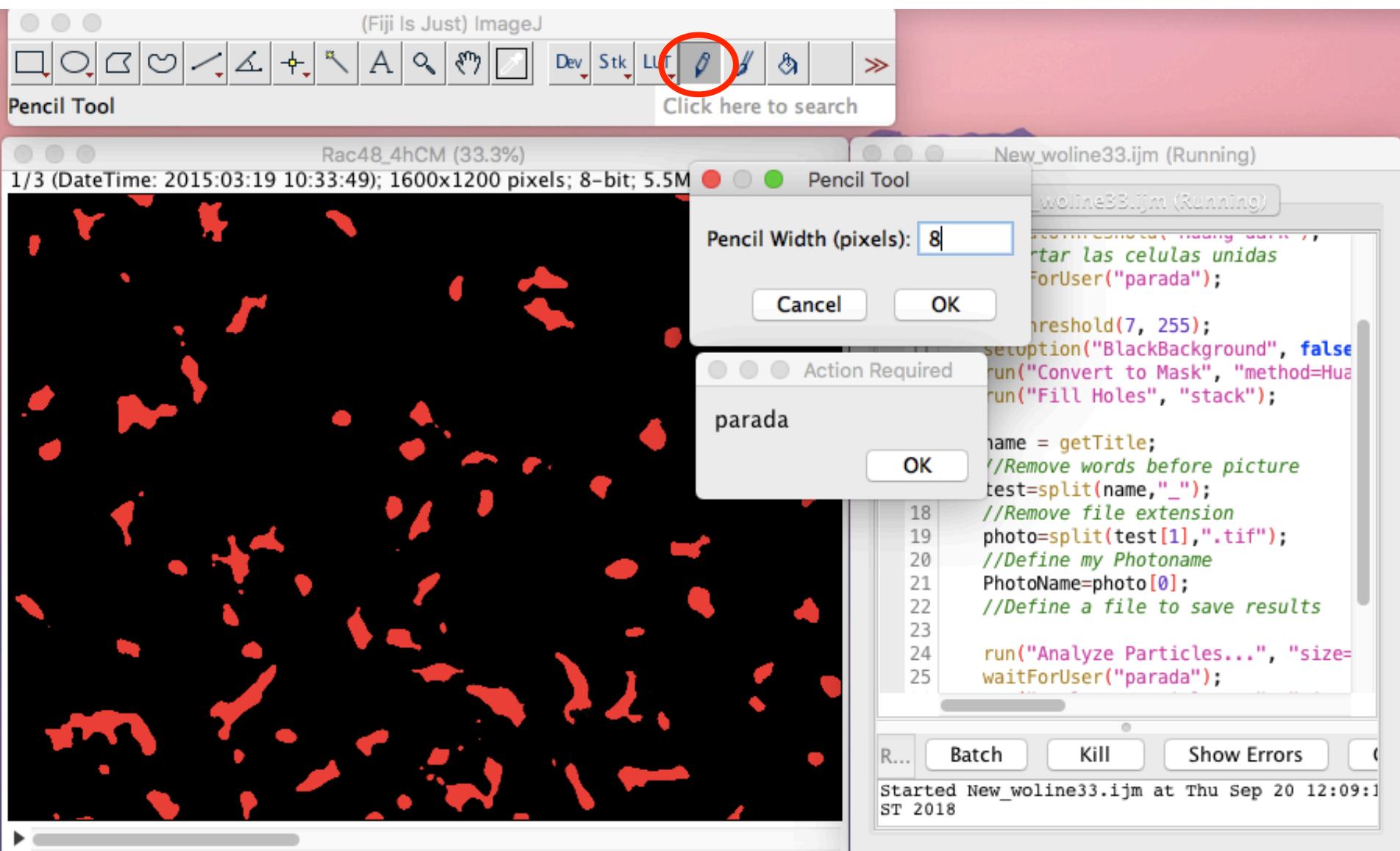
Start macro by clicking on Run



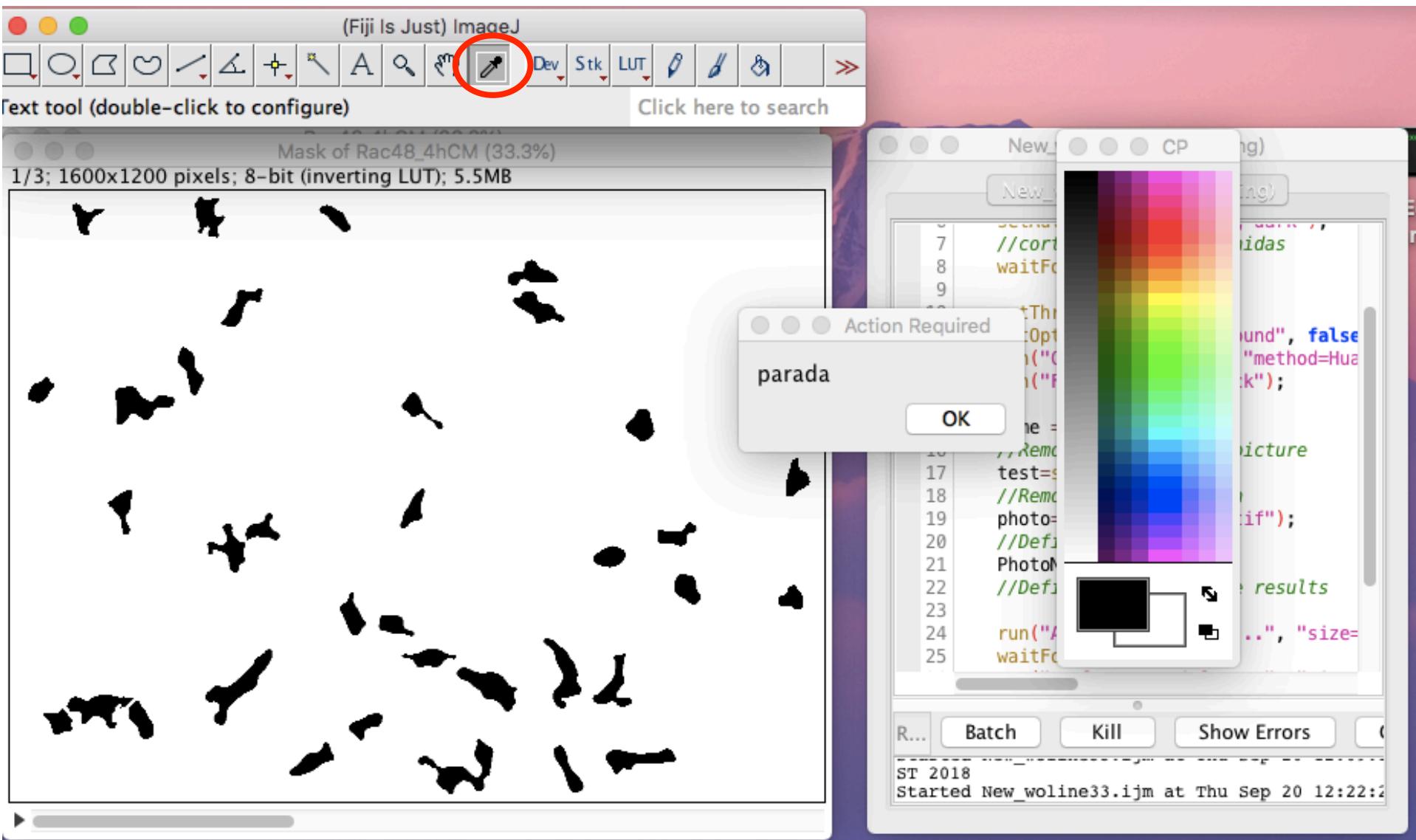
Parada: Separate cells by using the pencil tool



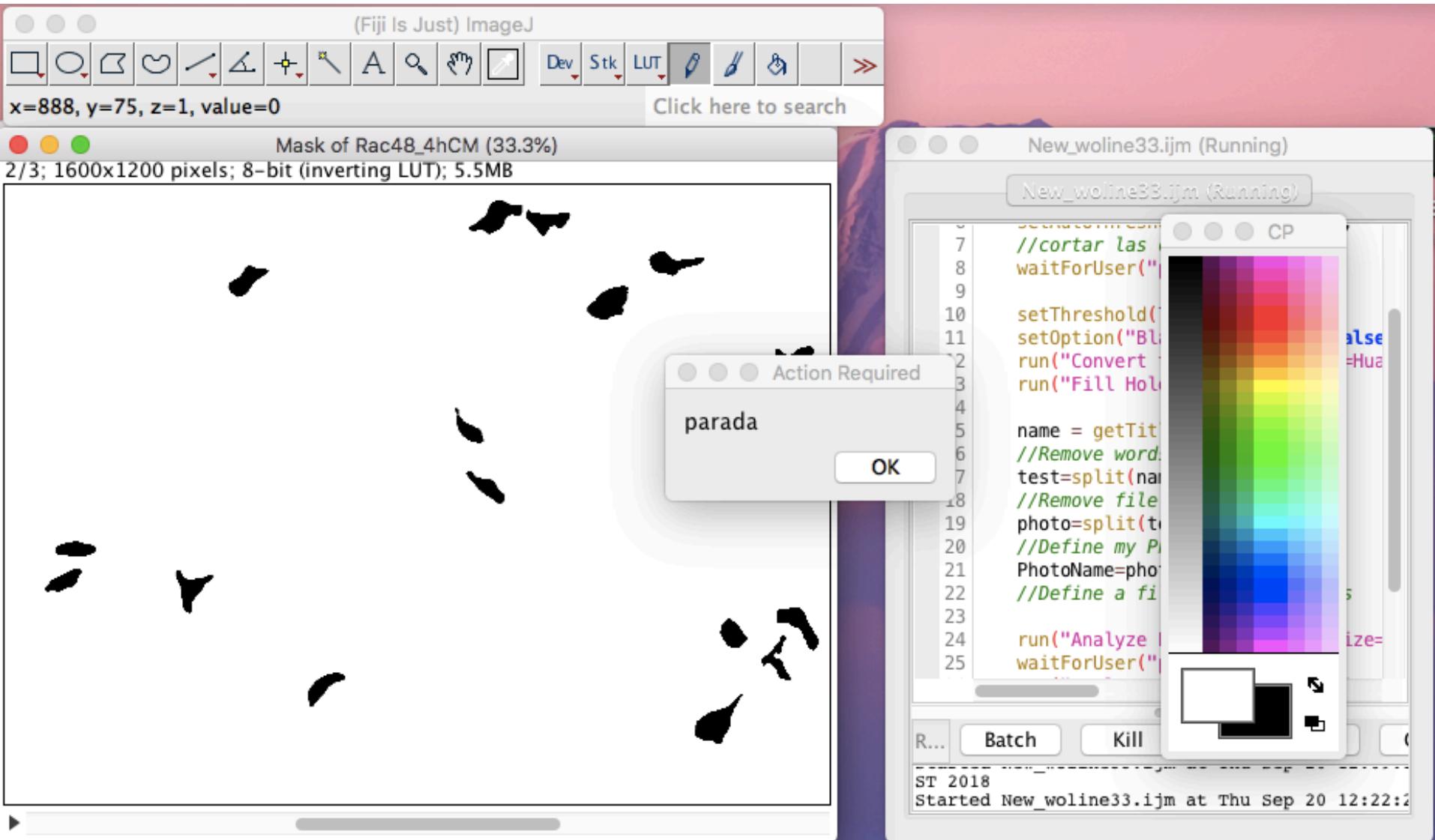
Parada: Separate cells by using the pencil tool (width: 8 pixels) on all three images, then click OK



Use Color picker to change between black and white pencil tool



Parada: Separate cells by using the pencil tool on all three images, then click OK



Get results for all cells in three images

The screenshot shows the Fiji software interface. On the left, a viewer displays a stack of images titled "Drawing of Mask of Rac48_4hCM (33.3%)". Below the viewer is a horizontal slider bar. On the right, there are two panels: a "Results" table and an "ROI Manager" panel.

Results Panel:

	Area	Circ.	AR	Round	Solidity	PhotoName
1	2755	0.385	1.677	0.596	0.723	4hCM
2	1647	0.432	1.143	0.875	0.677	0
3	1529	0.596	2.785	0.359	0.900	0
4	2548	0.463	2.314	0.432	0.784	0
5	2387	0.395	2.973	0.336	0.716	0
6	3053	0.511	2.431	0.411	0.813	0
7	2275	0.504	3.225	0.310	0.815	0
8	1610	0.765	1.645	0.608	0.928	0
9	4703	0.447	1.883	0.531	0.755	0
10	1735	0.360	2.660	0.376	0.659	0
11	2380	0.802	1.187	0.842	0.938	0
12	2103	0.582	1.579	0.633	0.859	0
13	1780	0.502	2.755	0.363	0.853	0
14	2000	0.451	2.156	0.464	0.745	0
15	1790	0.459	1.422	0.703	0.697	0
16	1897	0.483	2.311	0.433	0.732	0
17	2931	0.327	1.417	0.706	0.612	0
18	1926	0.803	1.628	0.614	0.962	0
19	2122	0.796	1.549	0.646	0.938	0
20	1524	0.717	1.094	0.914	0.885	0
21	2156	0.564	2.160	0.462	0.822	0

ROI Manager Panel:

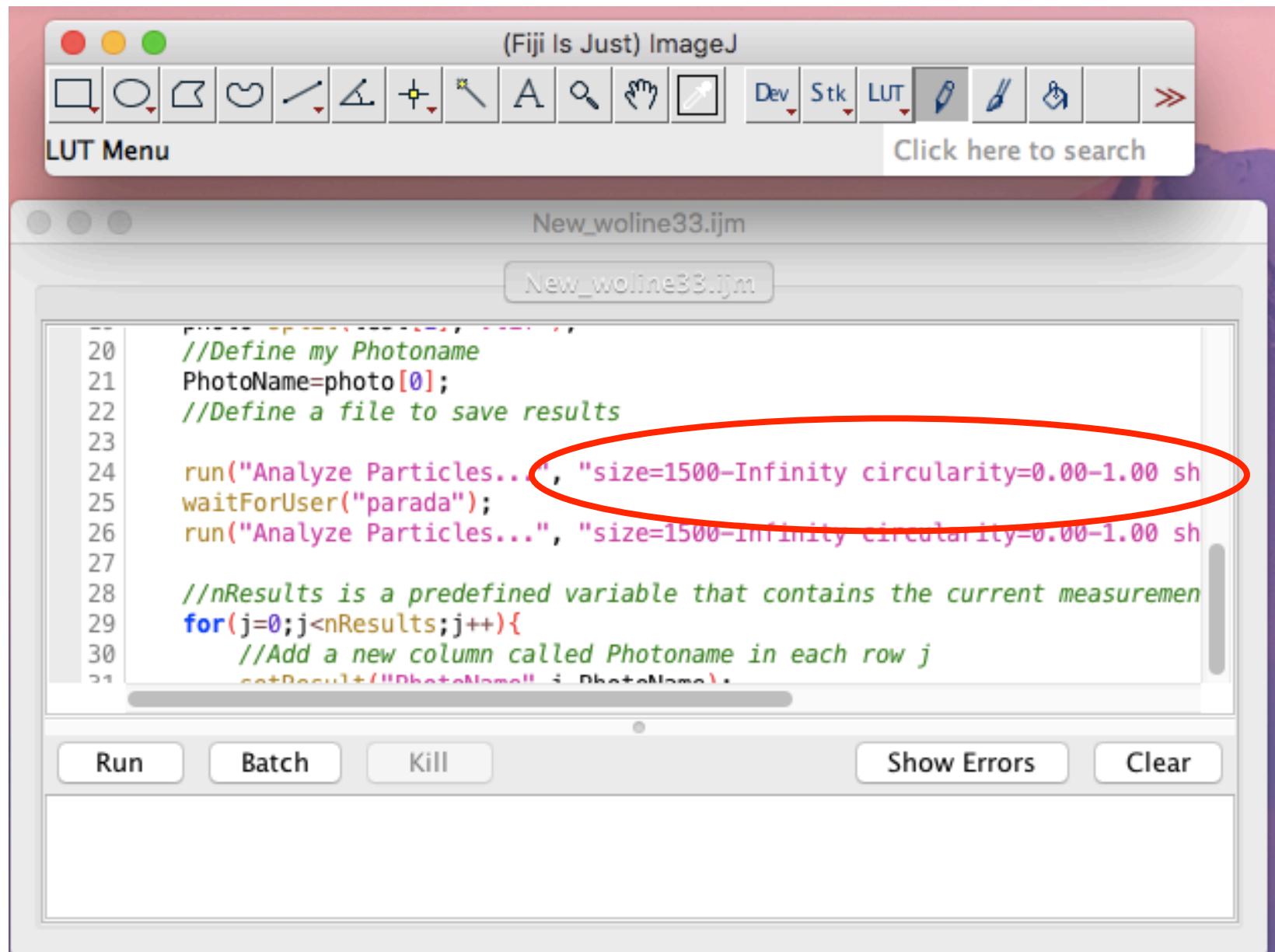
- 0001-0019-0783
- 0001-0020-0798
- 0001-0021-0831
- 0001-0022-0854
- 0001-0023-0948
- 0001-0024-0917
- 0001-0025-0975
- 0001-0026-0961

Contextual Menu (visible on the right):

- Add [t]
- Update
- Delete
- Rename...
- Measure
- Deselect
- Properties...
- Flatten [F]
- More »
- Show All
- Labels

Check your results by moving the slide bar below the images to switch between images.

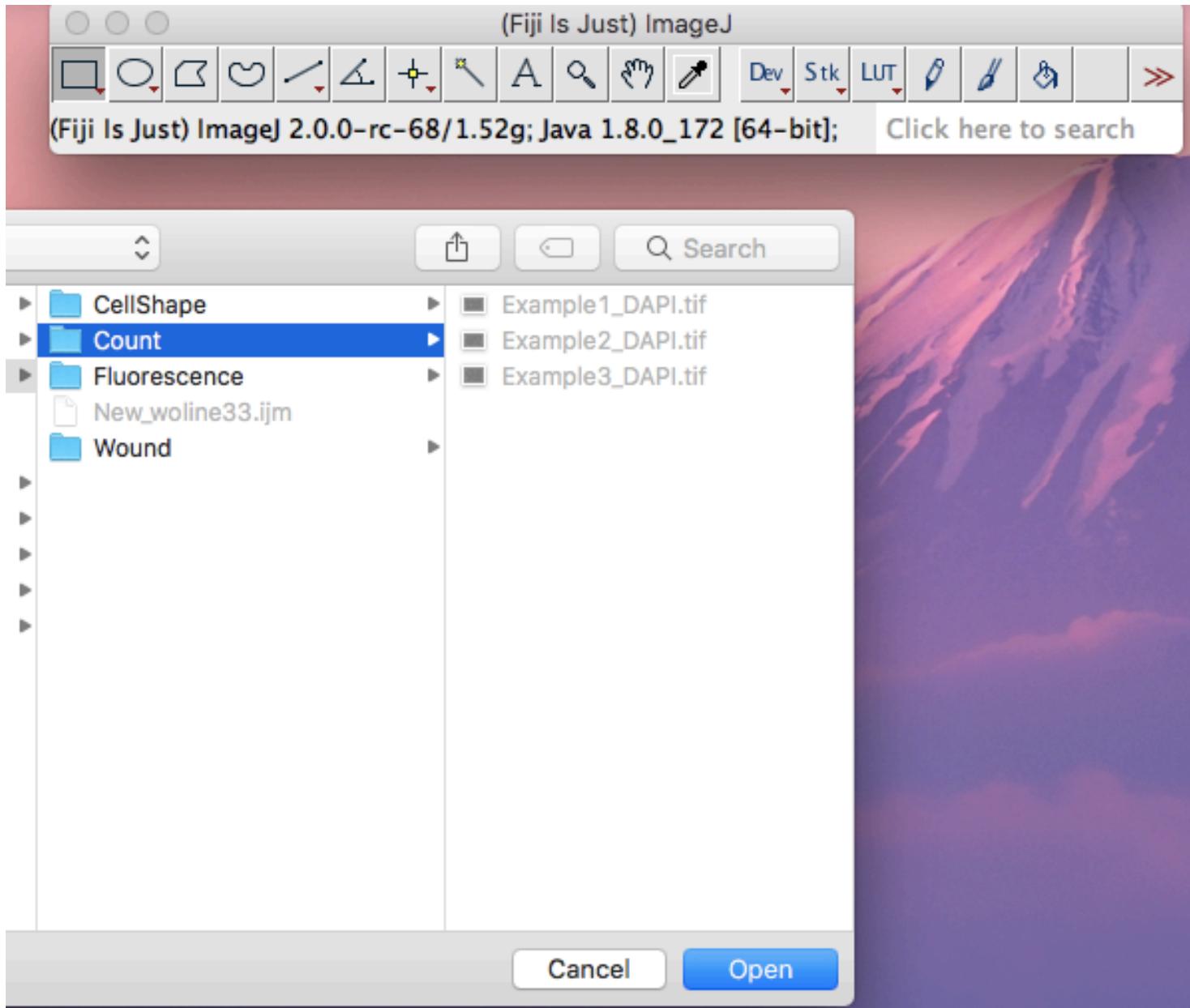
Change parameters by directly overwriting them in the macro file



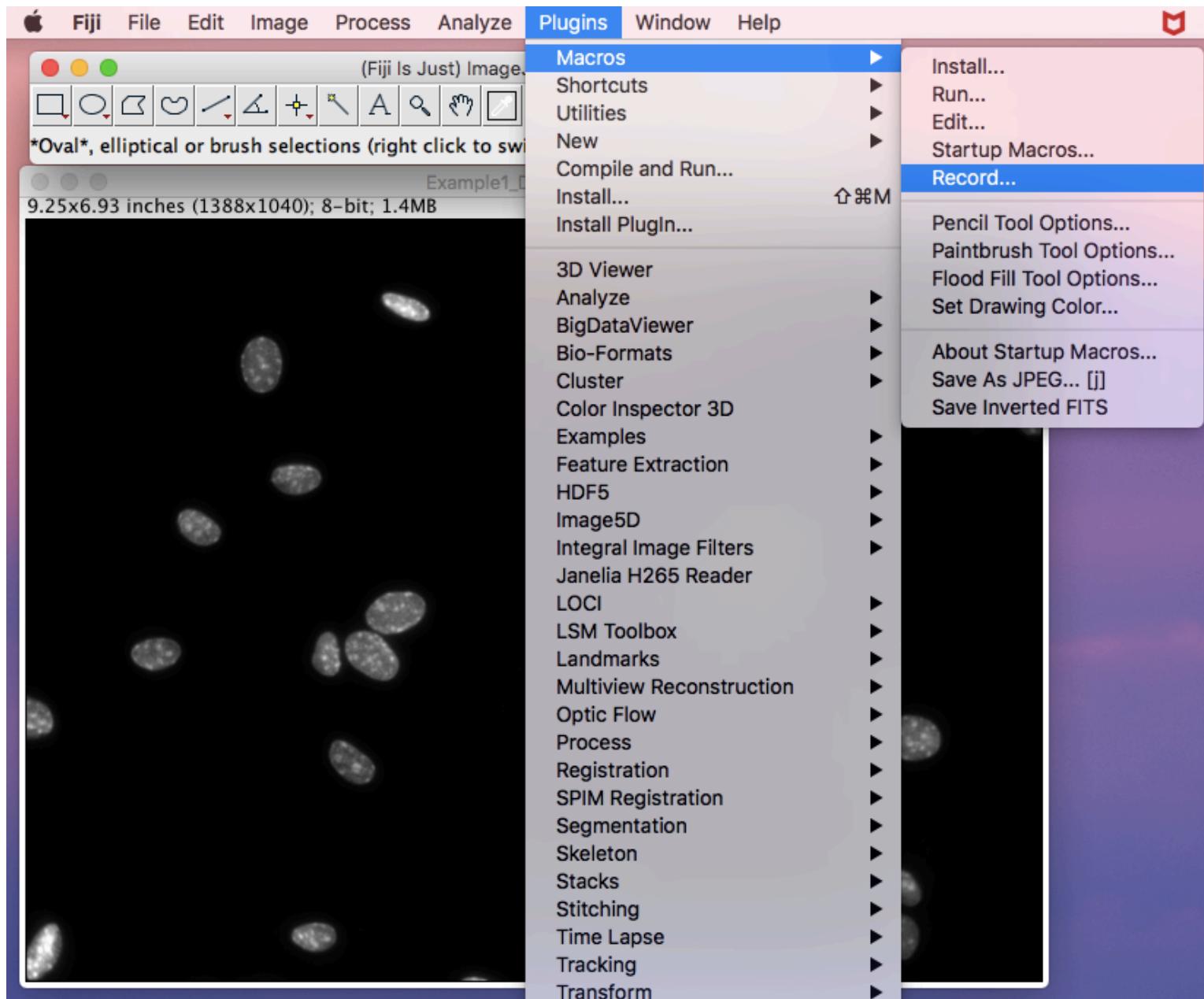
Workshop Overview

- Examples:
 - Counting Particles of Different Sizes
 - Measuring Fluorescence Intensities
- Using a Macro
 - Wound Healing Macro
 - Determination of Cell Shape
- **Creating a Macro**
 - Basic steps
- Practical Session with Example Images

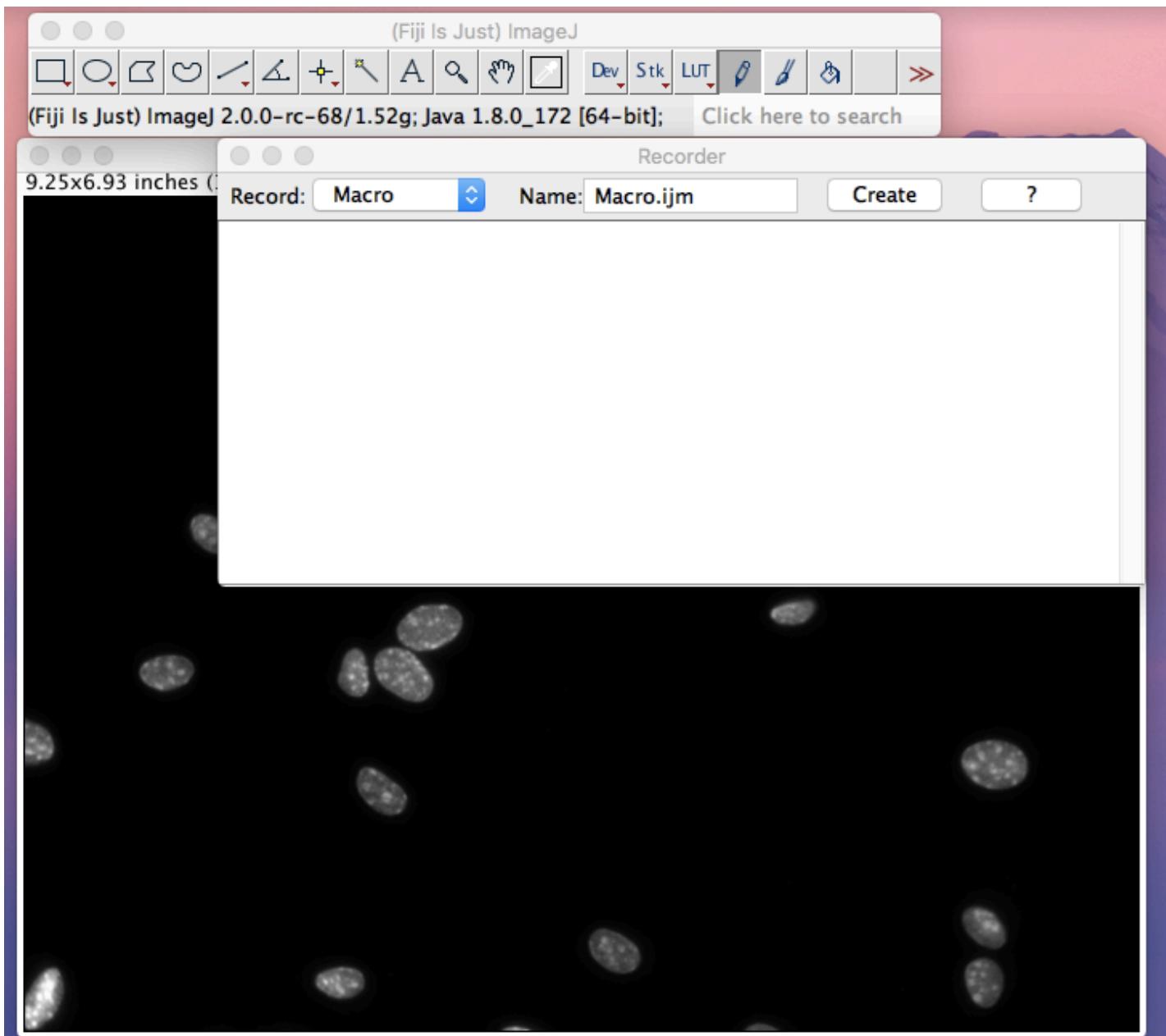
Import Image Sequence “Count” to create a macro counting nuclei



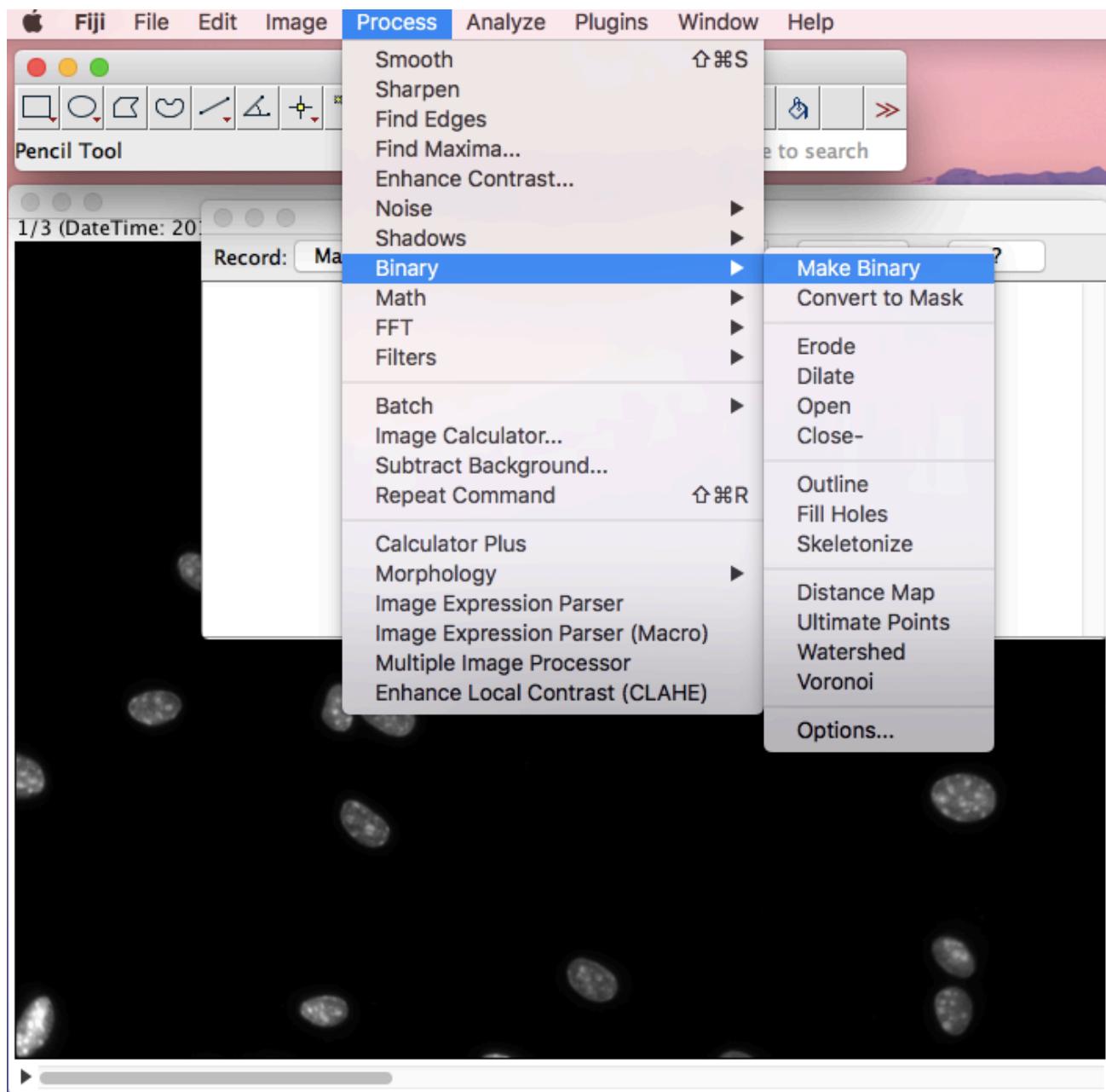
Click on *Plugins* → *Macros* → *Record*



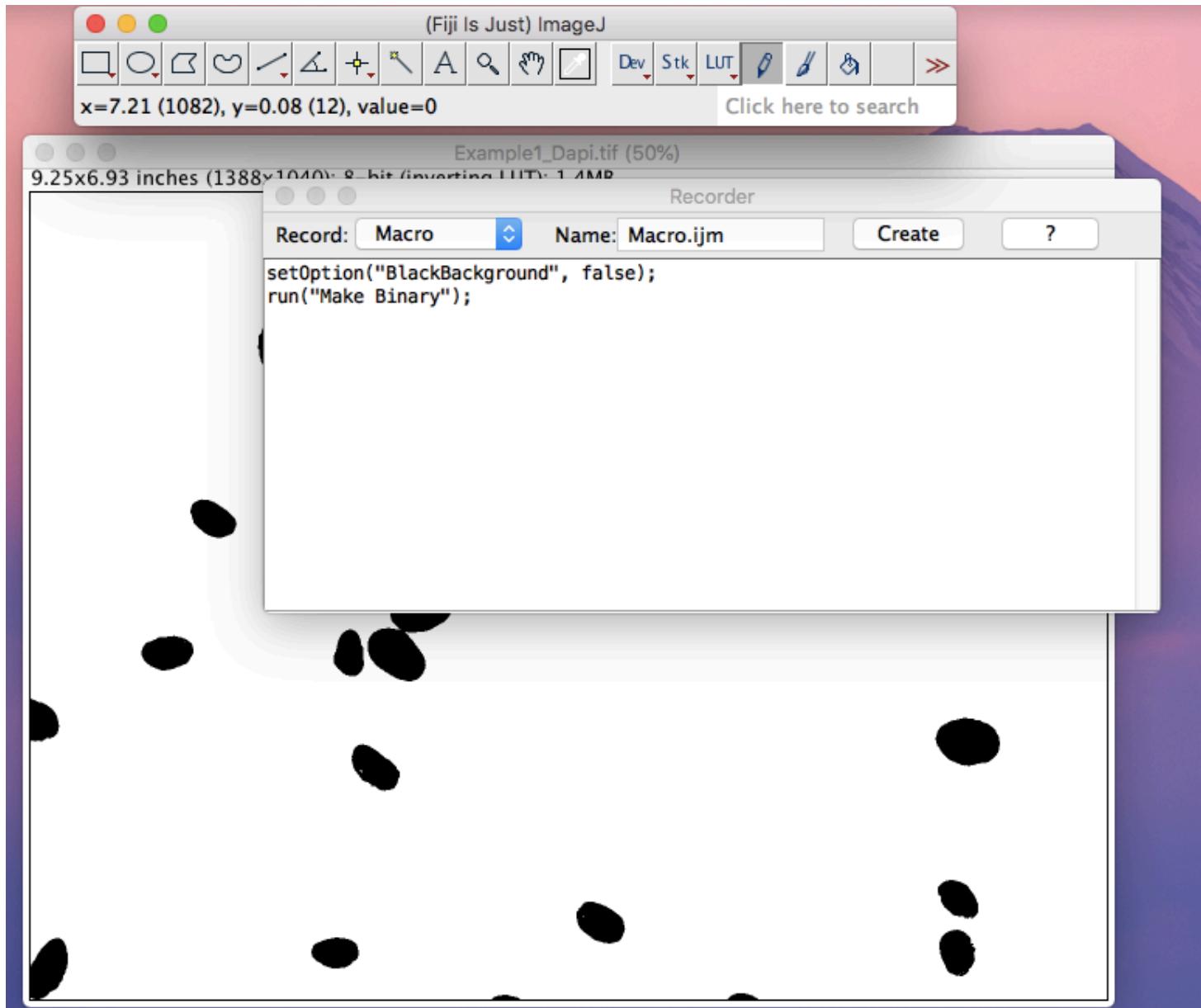
Now, all commands will be automatically recorded



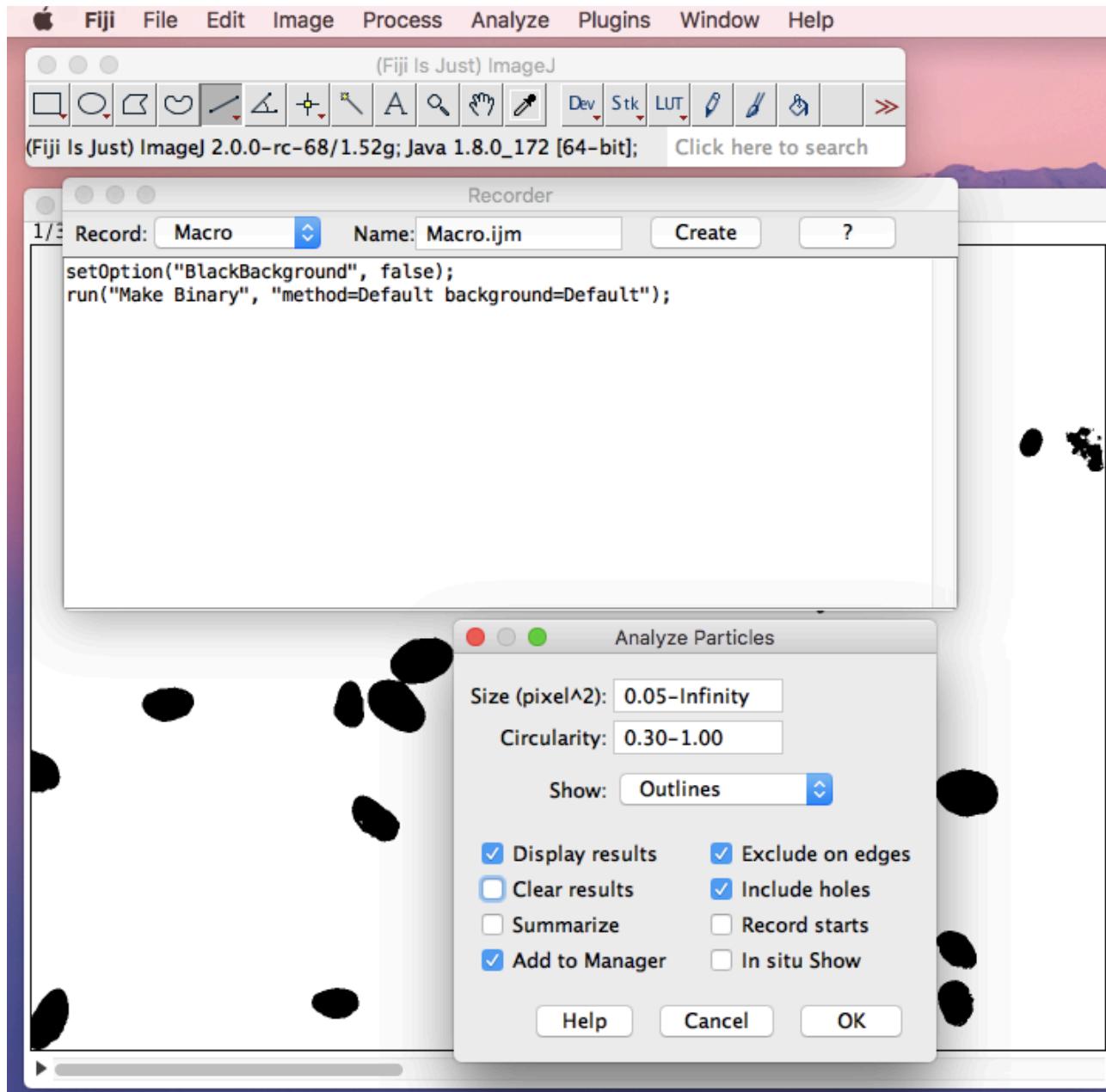
1st step: *Make Binary*



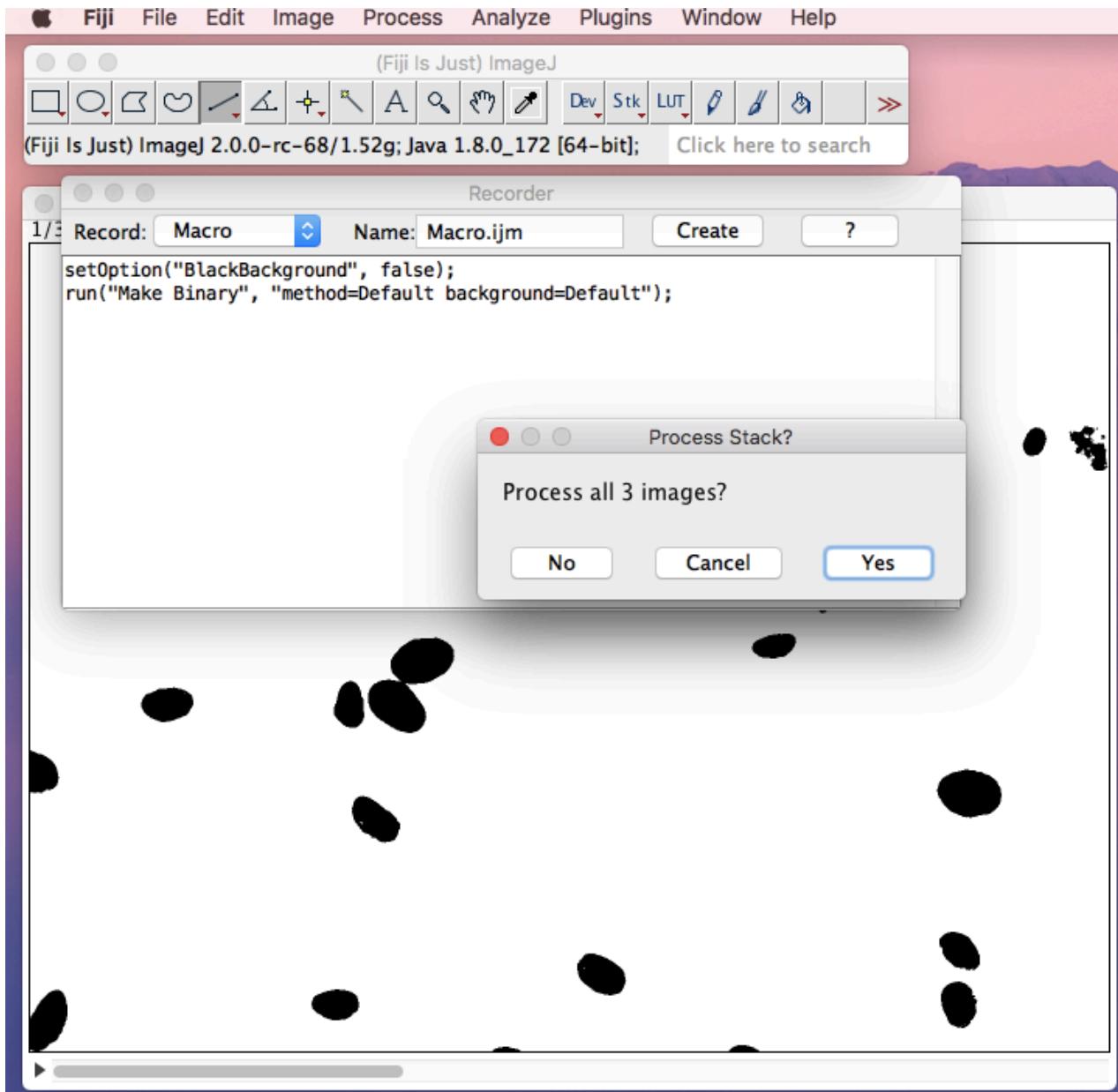
1st step: *make binary image*



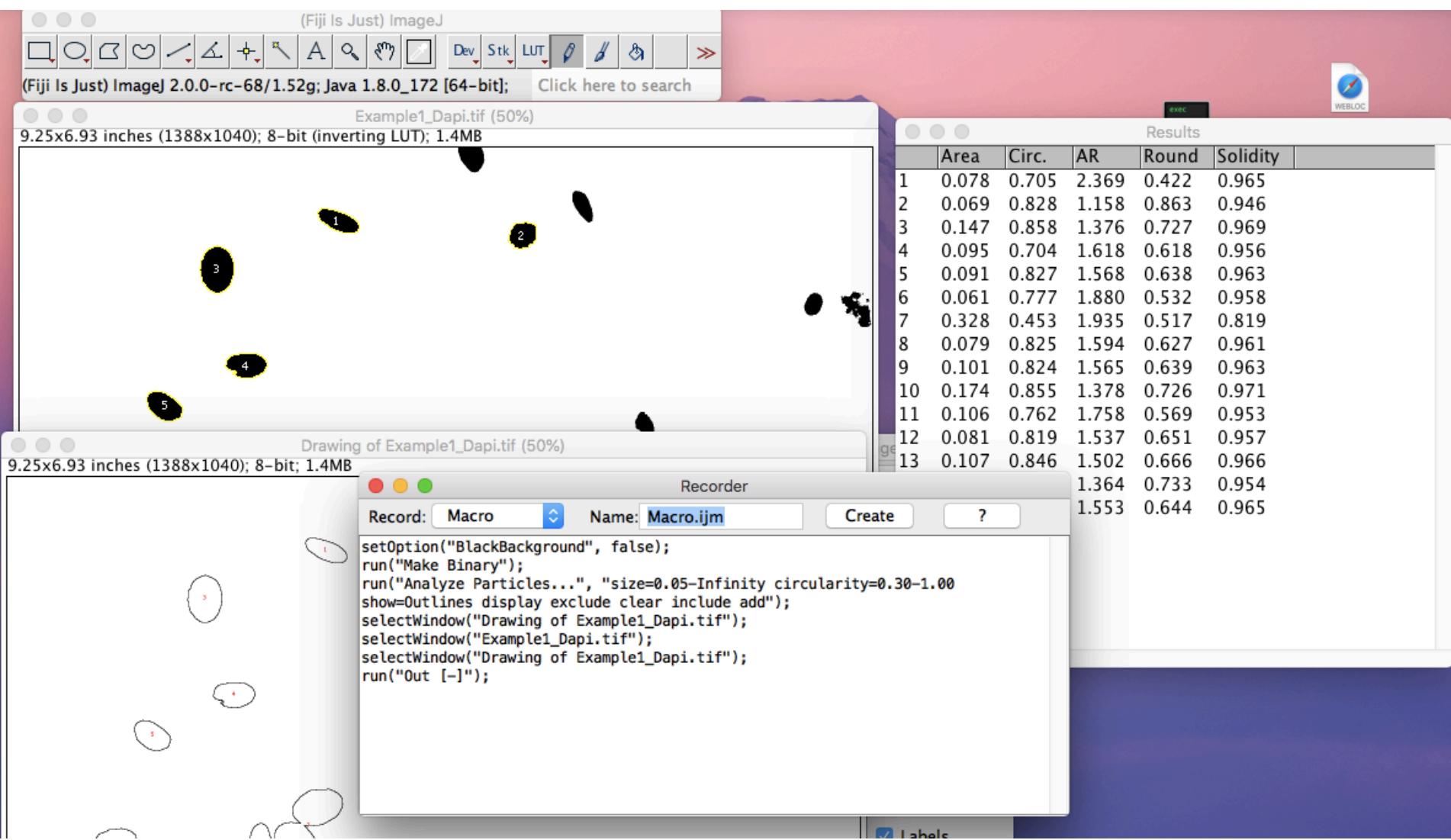
2nd step: *Analyze Particles*, set parameters and click OK



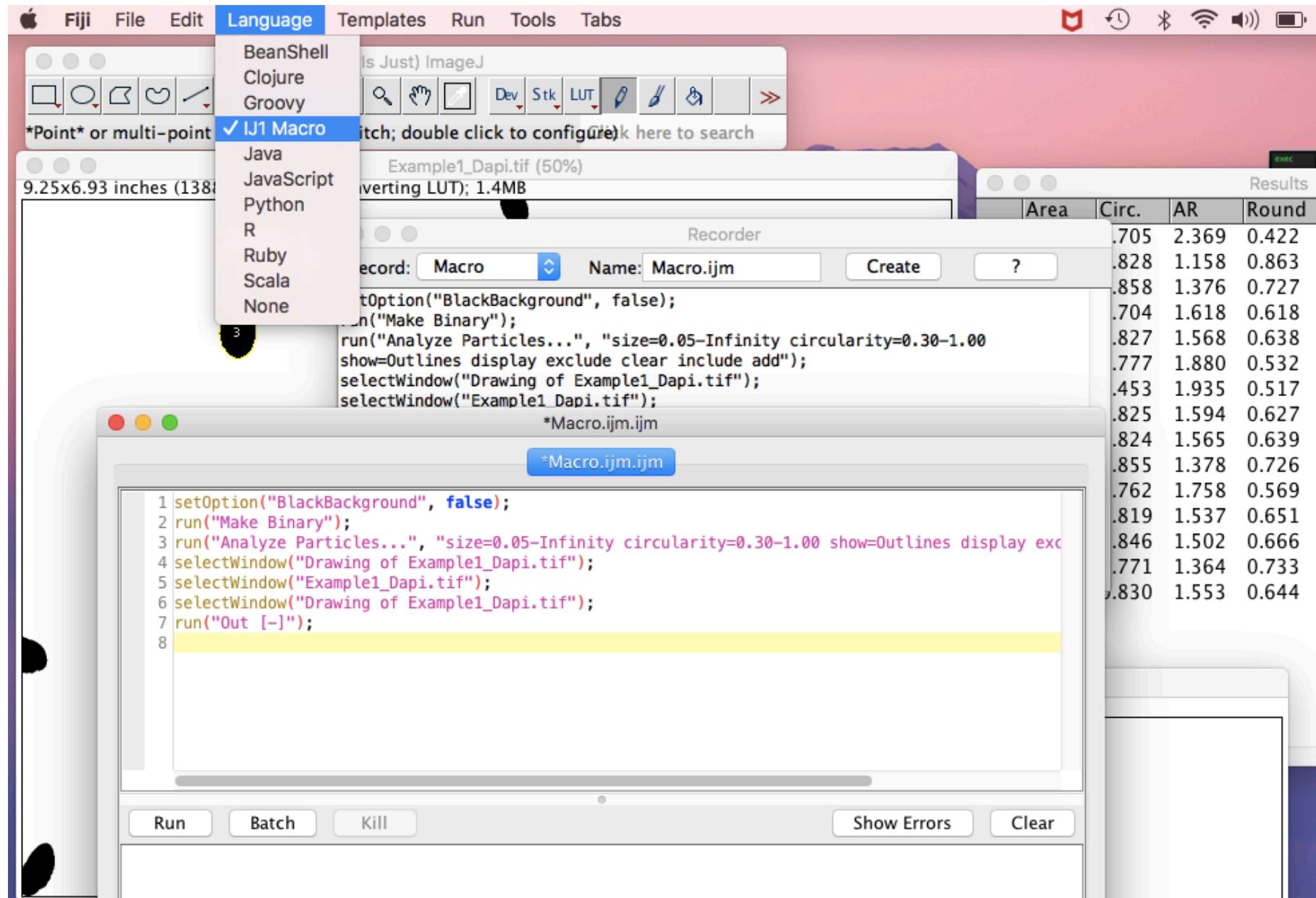
2nd step: click YES to process all images



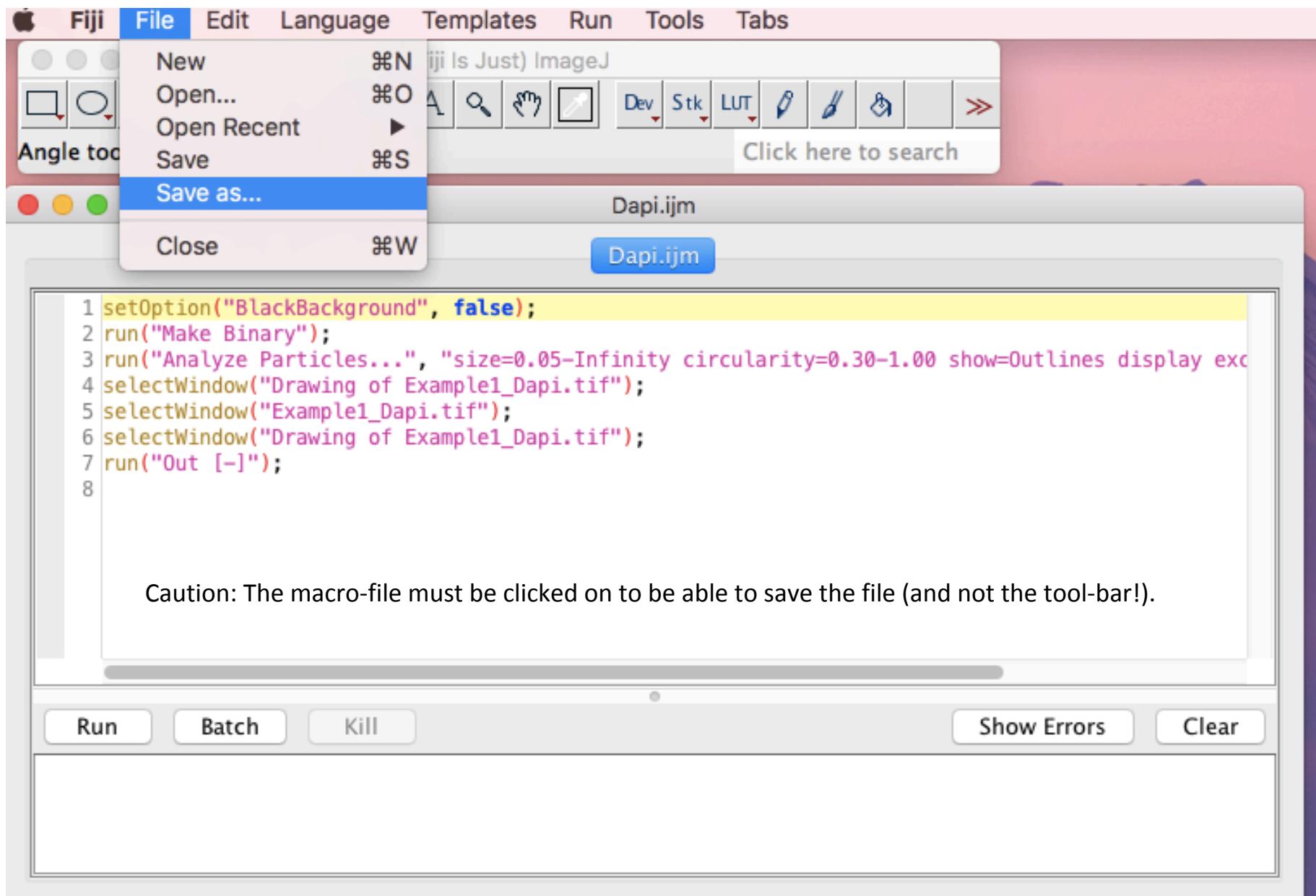
Finish all commands you want to introduce in your macro and click on Create



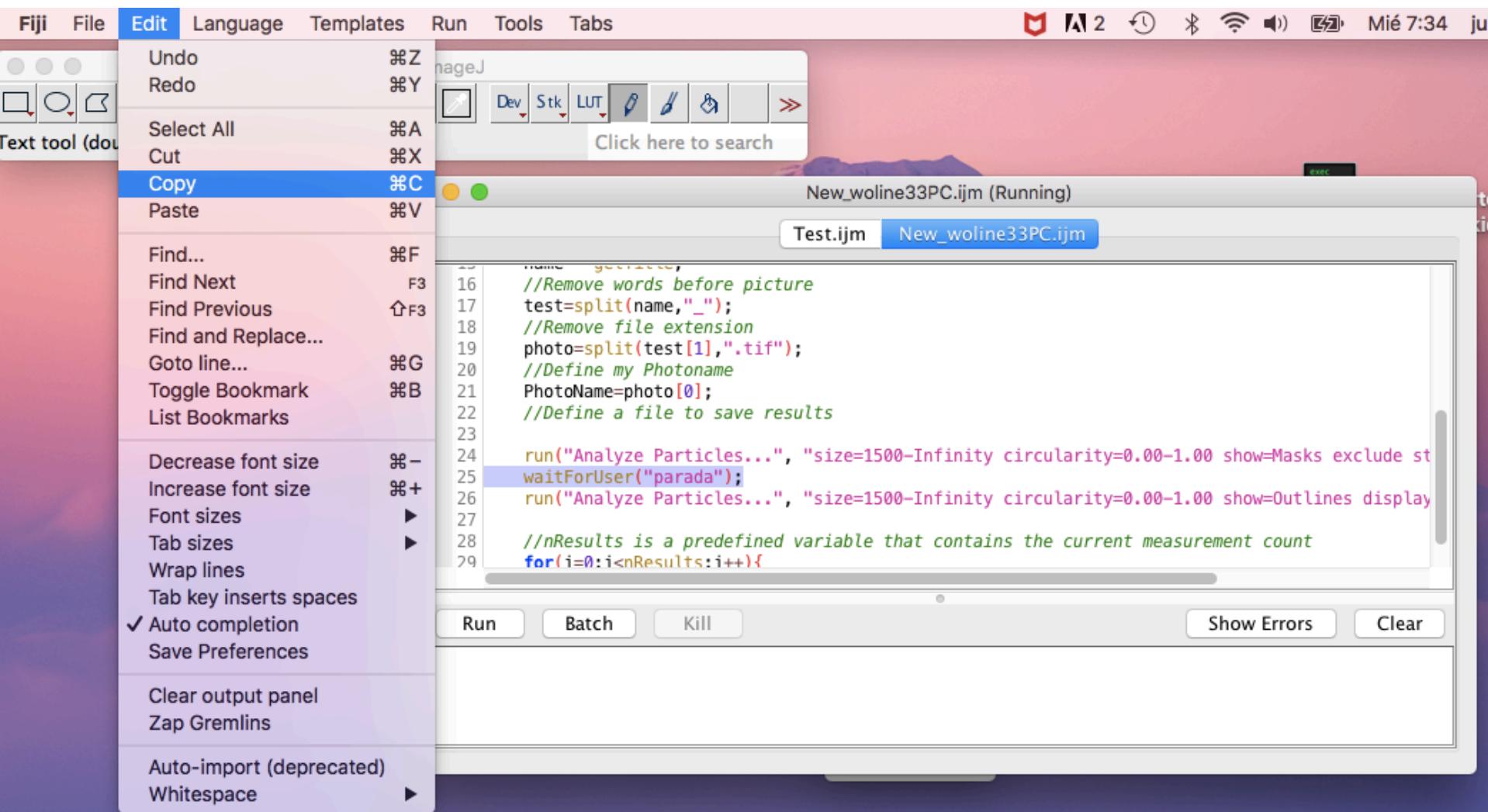
Make sure the *Language* is *IJ1 Macro*



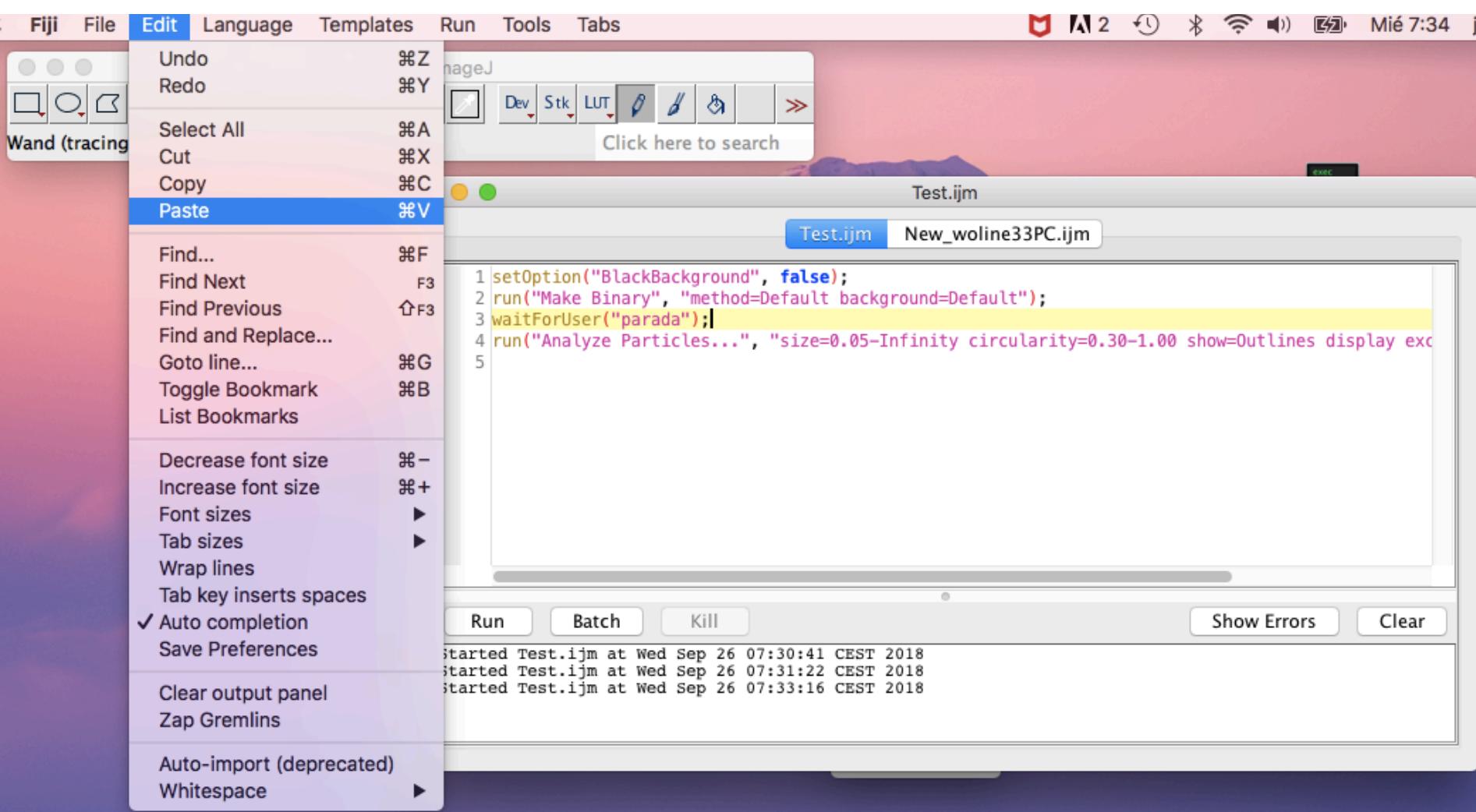
Save it with the file extension .ijm



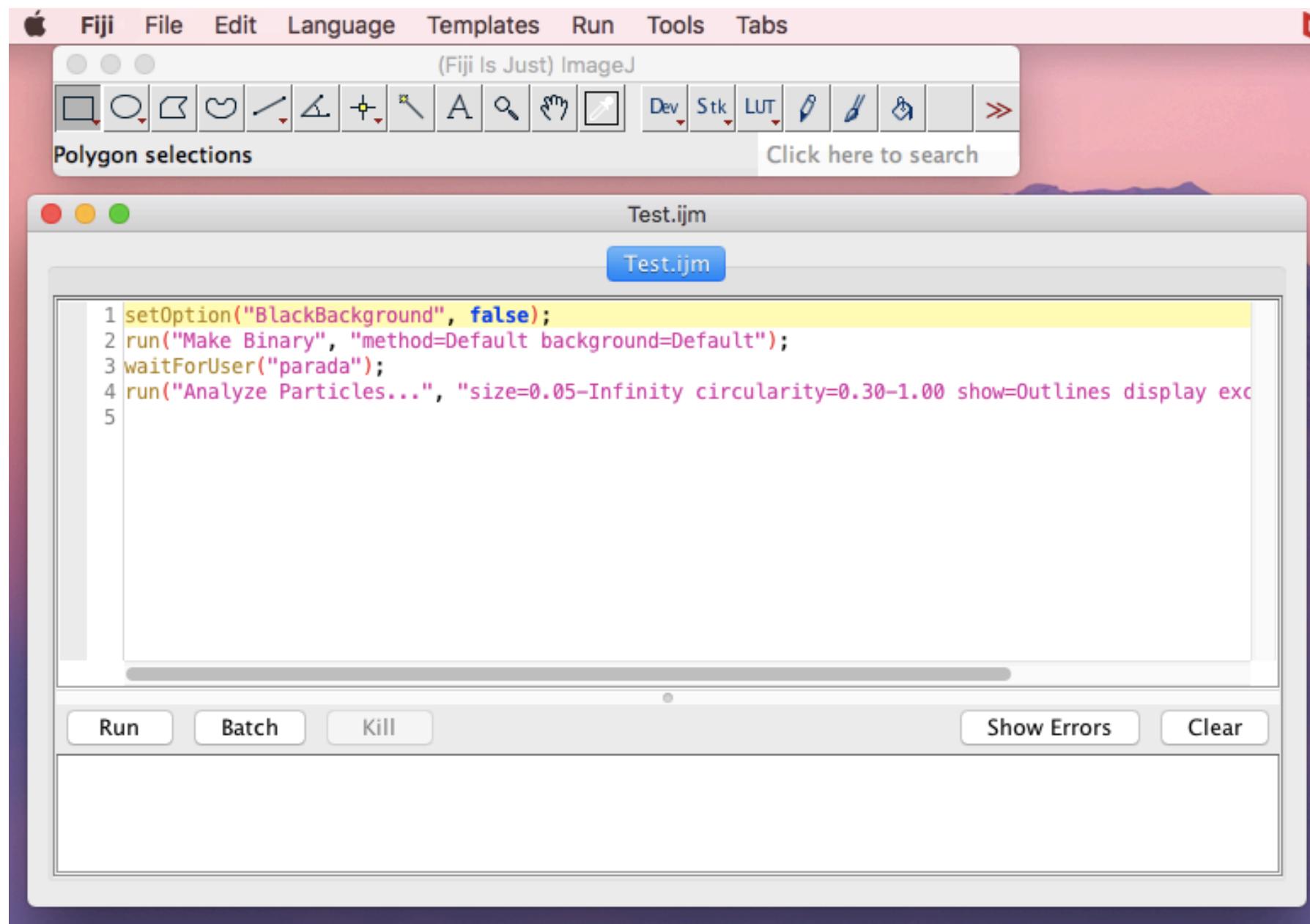
Modifications can be inserted by copy and paste



Modifications can be inserted by copy and paste



Save and close the macro. Try it in the Practical Session.



Workshop Overview

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Practical Session with Example Images

- Creating a Macro
- Examples:
 - Counting Particles of Different sizes (Count)
 - Measuring Fluorescence Intensities (Fluorescence)
- Using a Macro
 - Wound Healing Macro (Wound)
 - Determination of Cell Shape (CellShape)

THANK YOU VERY MUCH FOR YOUR ATTENTION!

Dr. Jenny Campos, Dr. Eduardo Andres (both IPBLN-CSIC) and



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