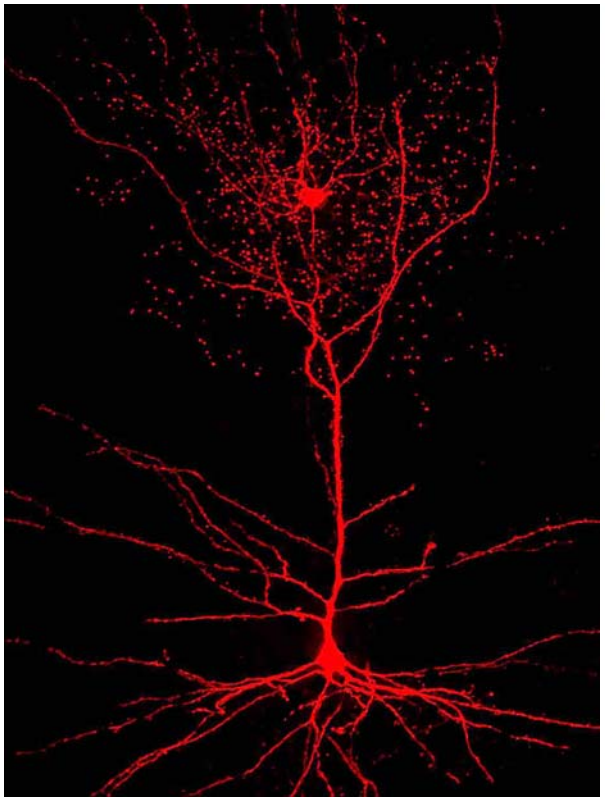
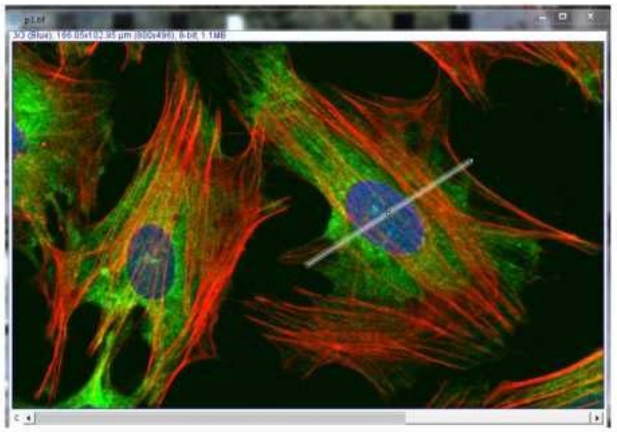
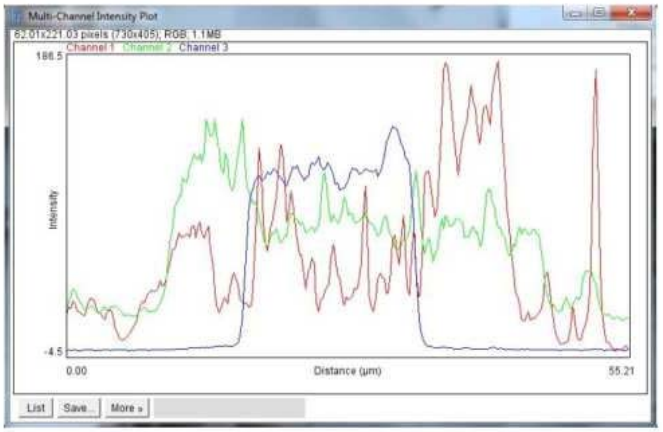
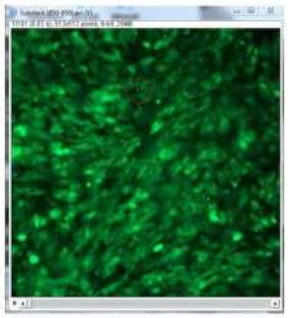
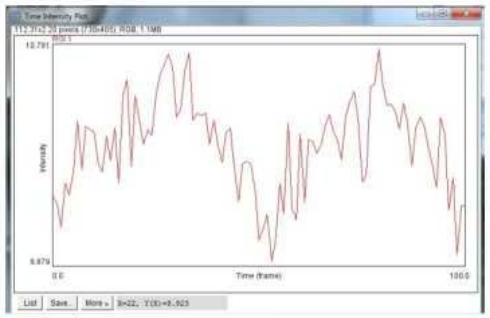
Quantitative data analysis











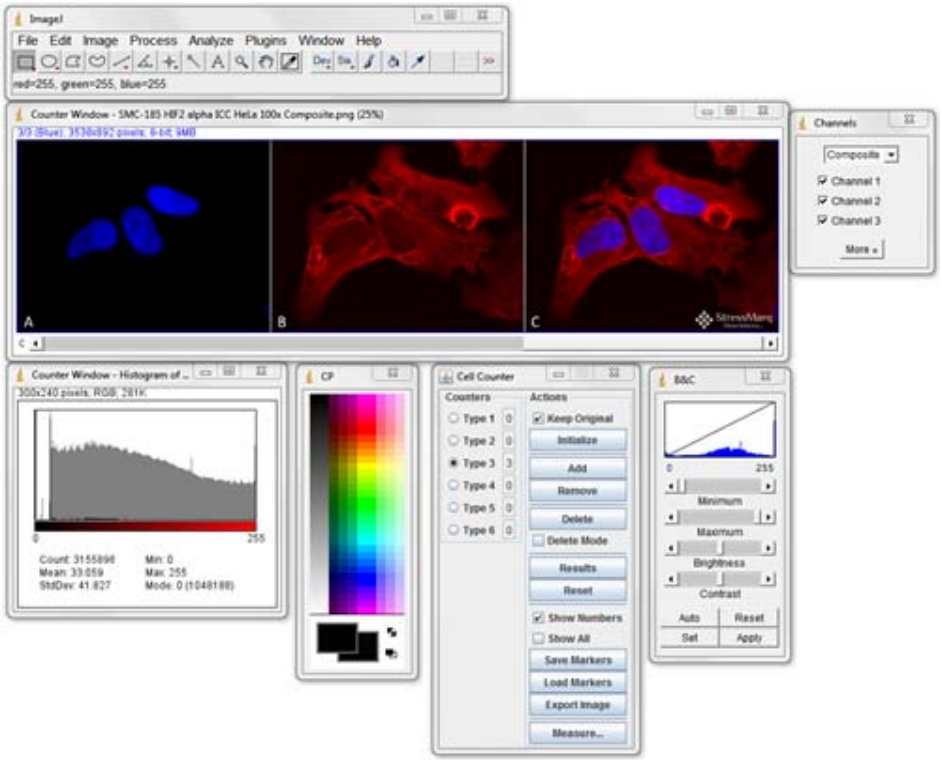
3/4/2022

Why?

- Example

Image quantification software

- IN Carta Image Analysis Software (Molecular devices)
- Nikon Image Analysis Software
- Image-Pro Plus
- Free Image Analysis Software



Fiji (ImageJ2)

Fiji

Fiji is an image processing package—a "batteries-included" distribution of ImageJ2, bundling a lot of plugins which facilitate scientific image analysis.

- **For users** - Fiji is easy to install and has an automatic update function, bundles a lot of plugins and offers comprehensive documentation.
- **For developers** - Fiji is an open source project hosted in a Git version control repository, with access to the source code of all internals, libraries and plugins, and eases the development and scripting of plugins.

Downloads

~ Download Fiji for your OS ~

64-bit

macOS

64-bit

Other downloads

32-bit | No JRE | 32-bit

See the Fiji downloads page for Life-Line versions, etc.

License

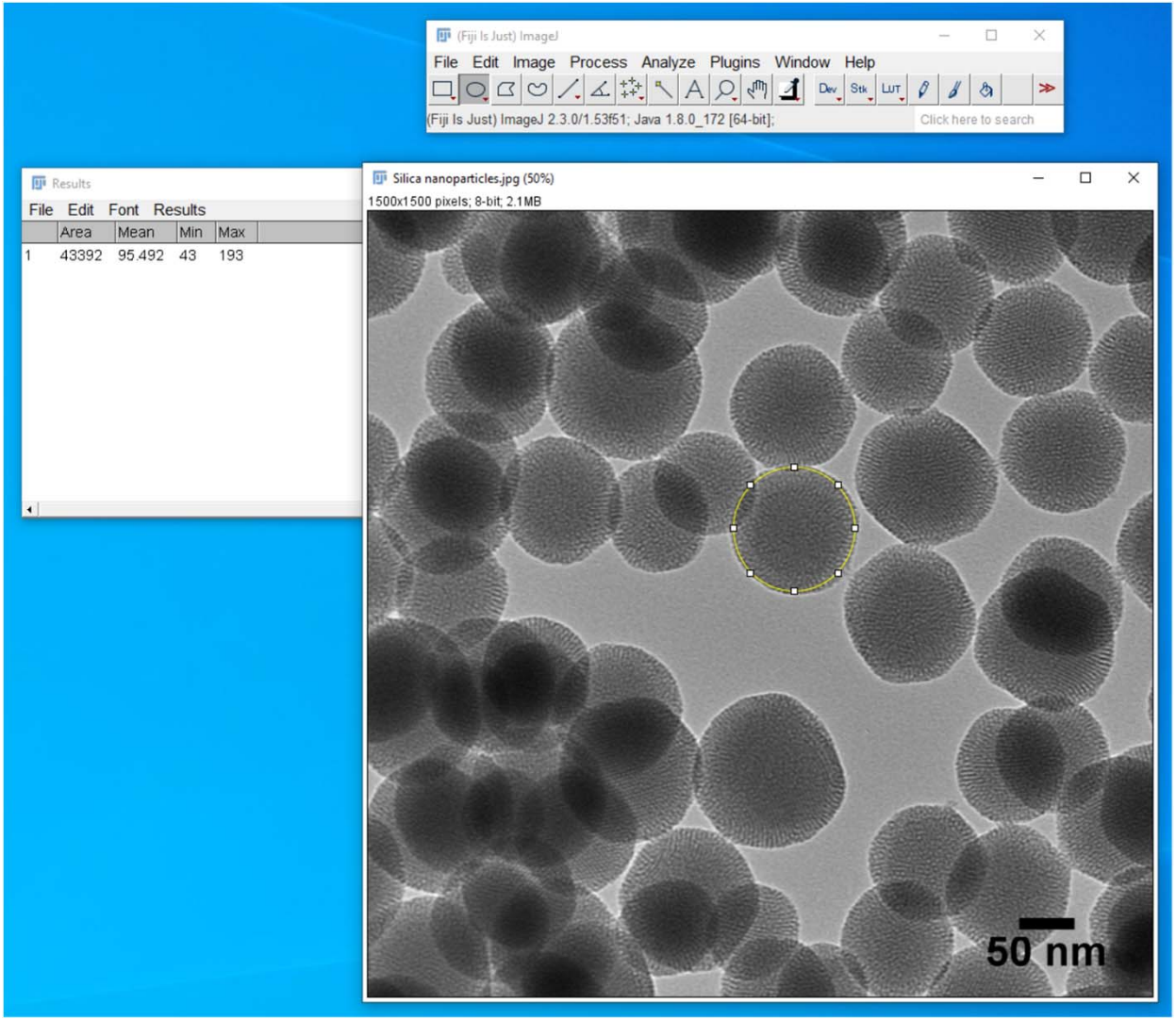
Fiji is released as open source under the GNU General Public License.

Fiji builds on top of the ImageJ2 core, which is licensed under the permissive BSD 2-Clause license.

Fiji also includes the original ImageJ, which is free of copyright.

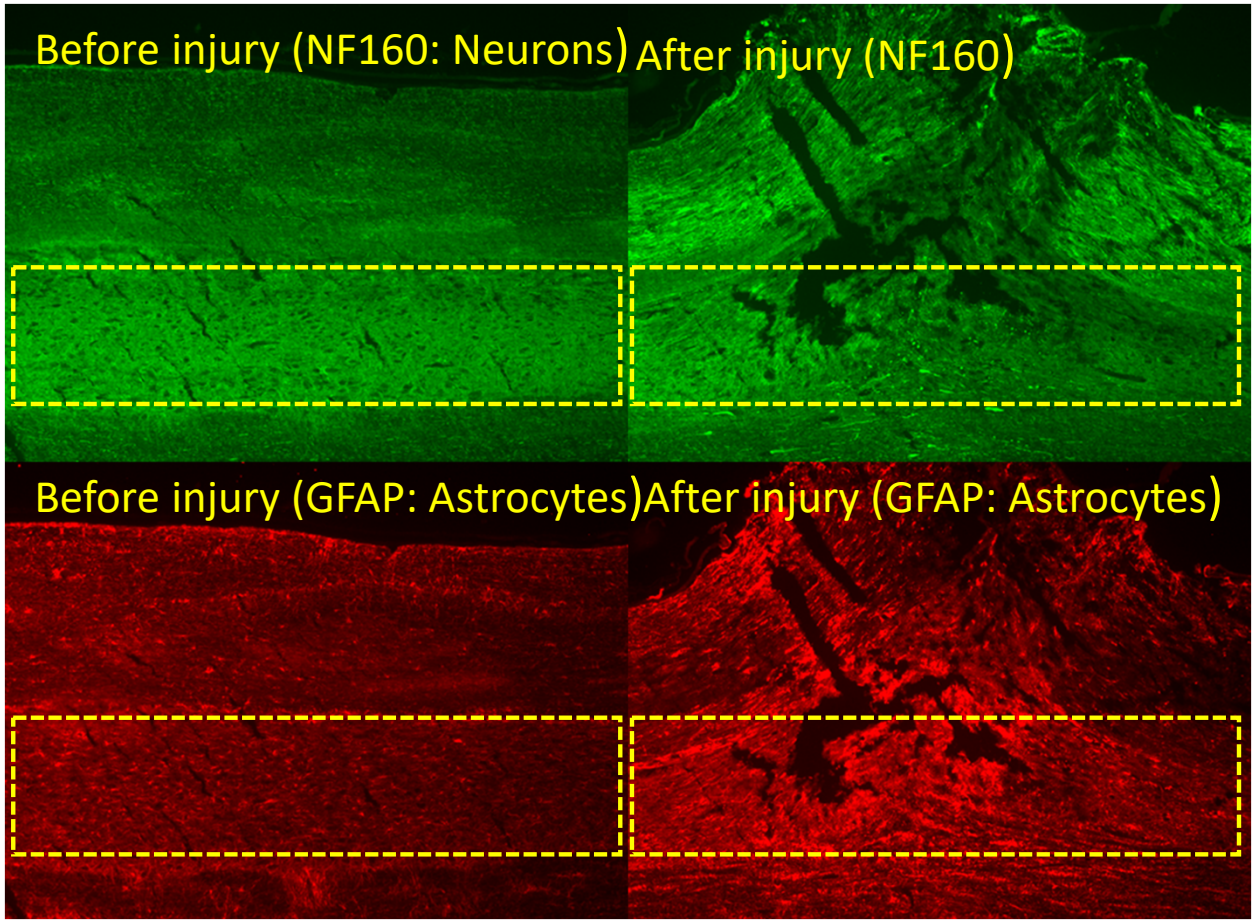
Plugins and other components have their own licenses.

Task 1. Basic measurement and Scale set



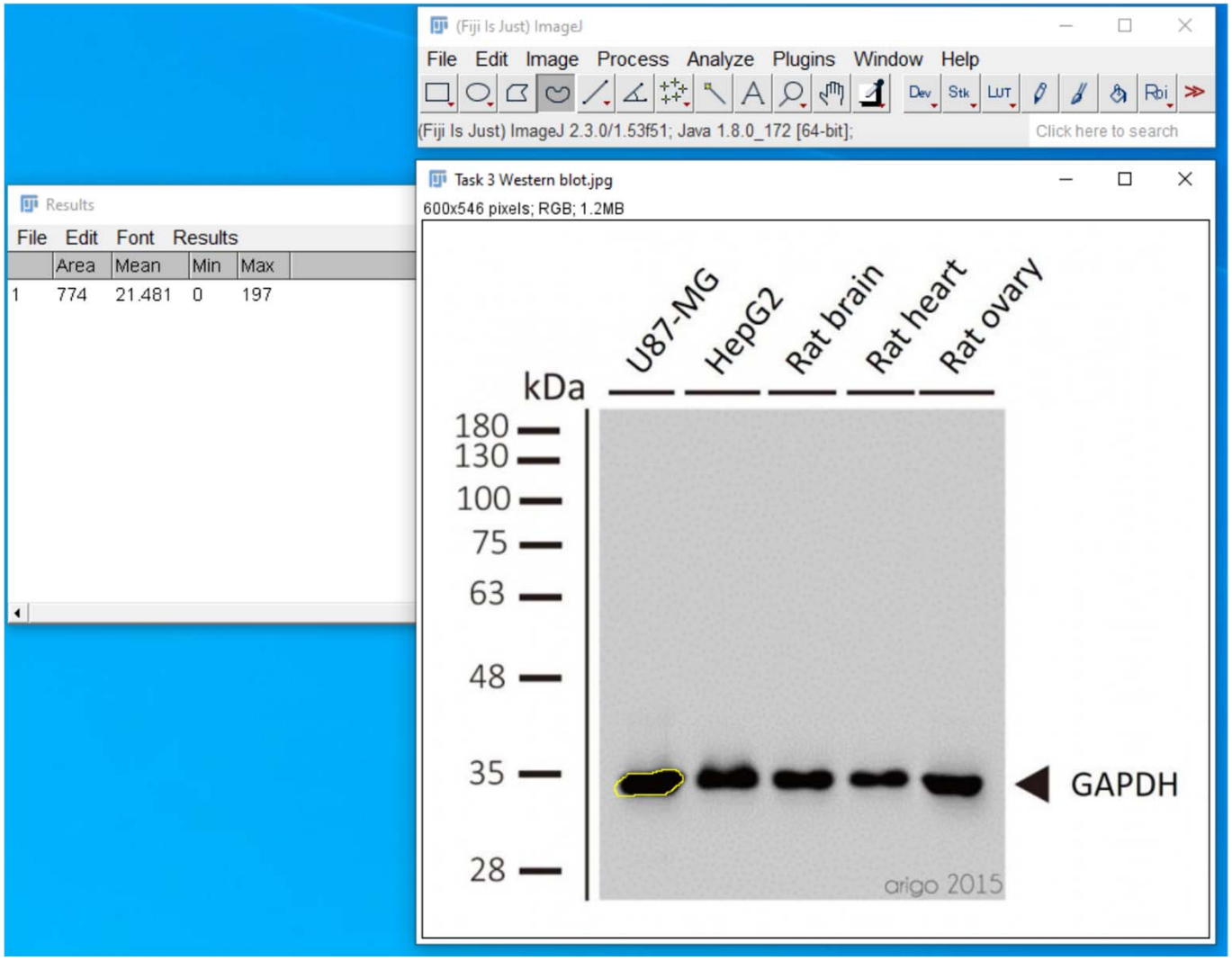
1. Set Scale
2. Measure Diameter, Area, and Distance between Nanoparticles (n=10)

Task 2. Fluorescent intensity using plot profile



1. Draw a Line within yellow boxed tissue region (gray matter)
2. Measure the Fluorescent intensity using plot profile (n=10 for each condition)
3. Quantitatively compare the intensity of each marker (NF160 for neurons and GFAP for astrocytes) between before and after injury
4. Statistically compare the results using P value (T-test): Significant difference between before and after injury?

Task 3. Protein intensity (Western blot)

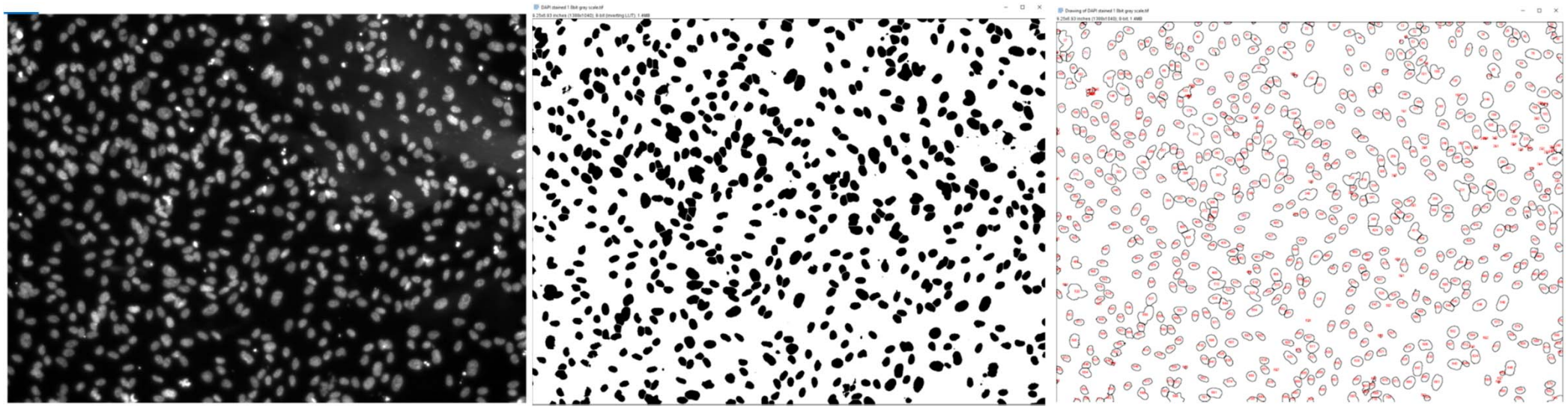


Quantitatively compare the protein intensity (GAPDH) of each cell/tissue.

1. Select “Area of Interest”
2. Measure “Integrated density”

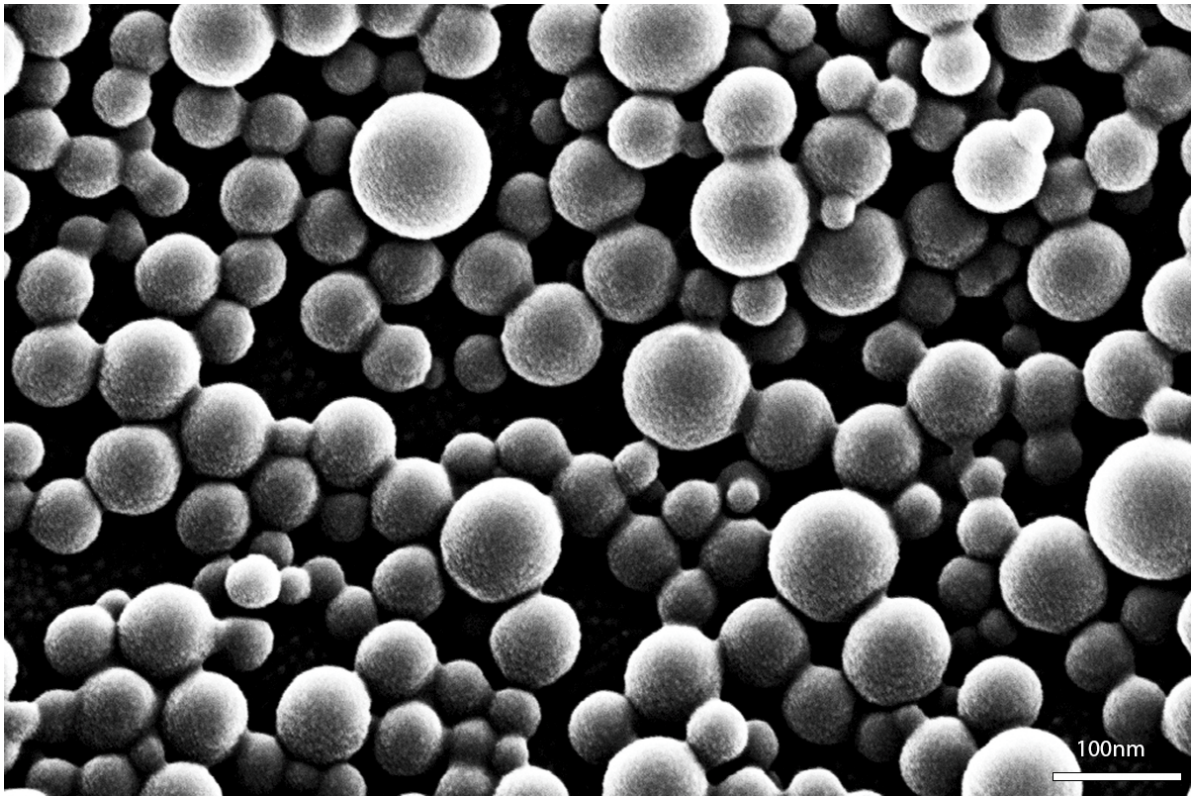
*Integrated density = Sum of Values of Pixels (similar to Area Under Curve)

Task 4. Counting Cells using a “Analyze Particle”



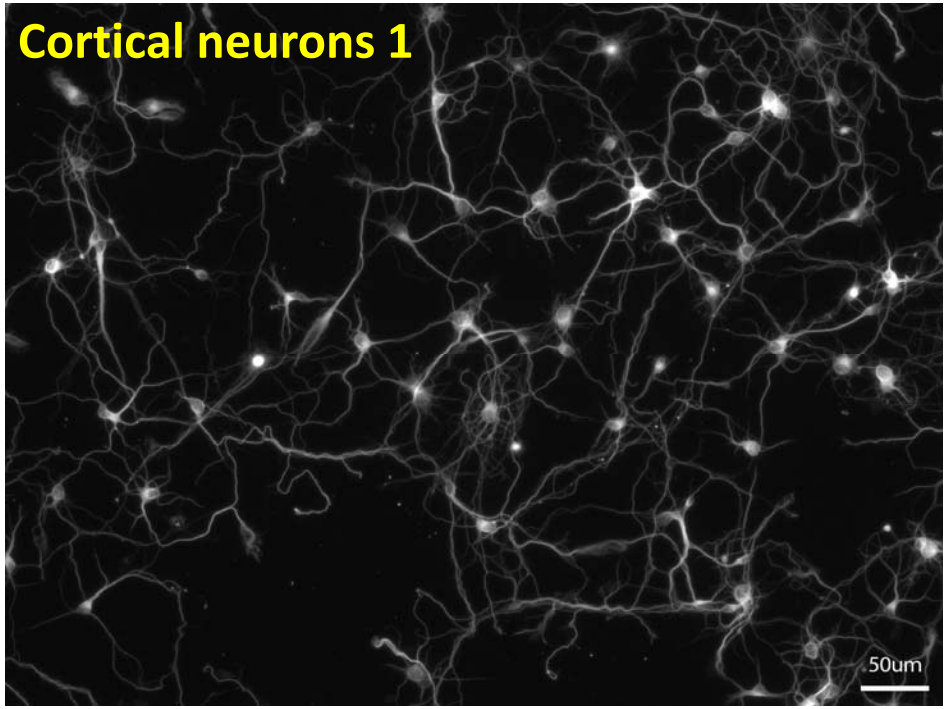
1. Drop “Task4 DAPI stained 1 8bit gray scale.tif”
2. Select the cells using a Threshold (click “dark background”)
3. Make it as a Binary image (under Process; Binary)
4. Apply “Watershed” for cell segmentation (under Process; Binary)
5. Count cells using a “Analyze Particle (click “outlines)

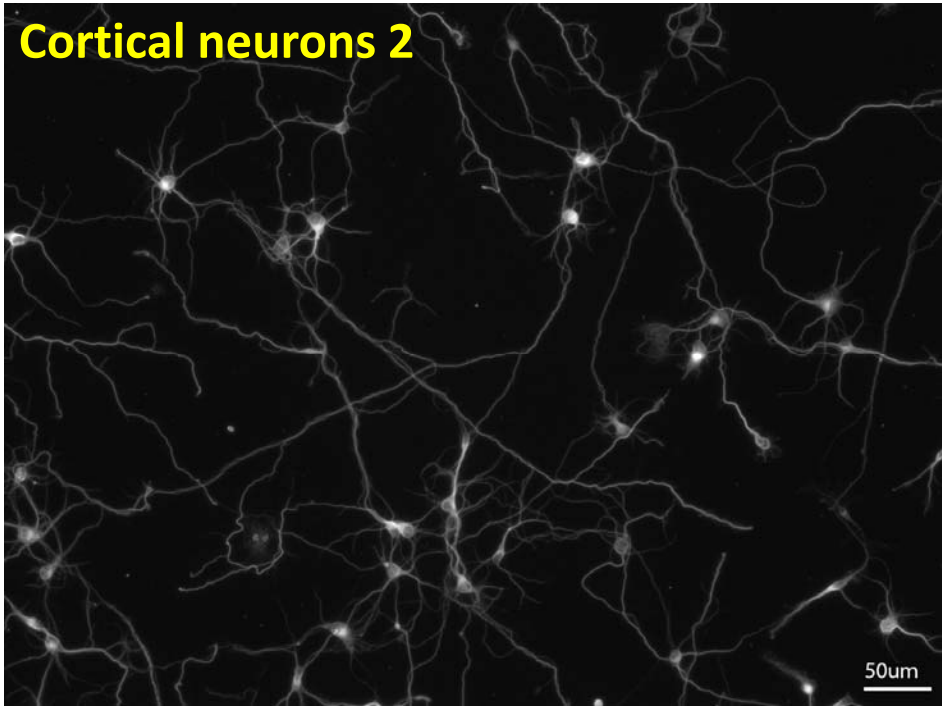
Task 5. Nanoparticle size distribution analysis



1. Drop “Task5 Nanoparticle size distribution.jpg”
2. Set Scale
3. Select the nanoparticles using a Threshold (click “dark background”)
4. Apply “Watershed” for segmentation (under Process; Binary)
5. Count the nanoparticles using a “Analyze Particle (click “outlines)
6. Save the results in Excel for size distribution analysis

Task 6. Neurite tracking

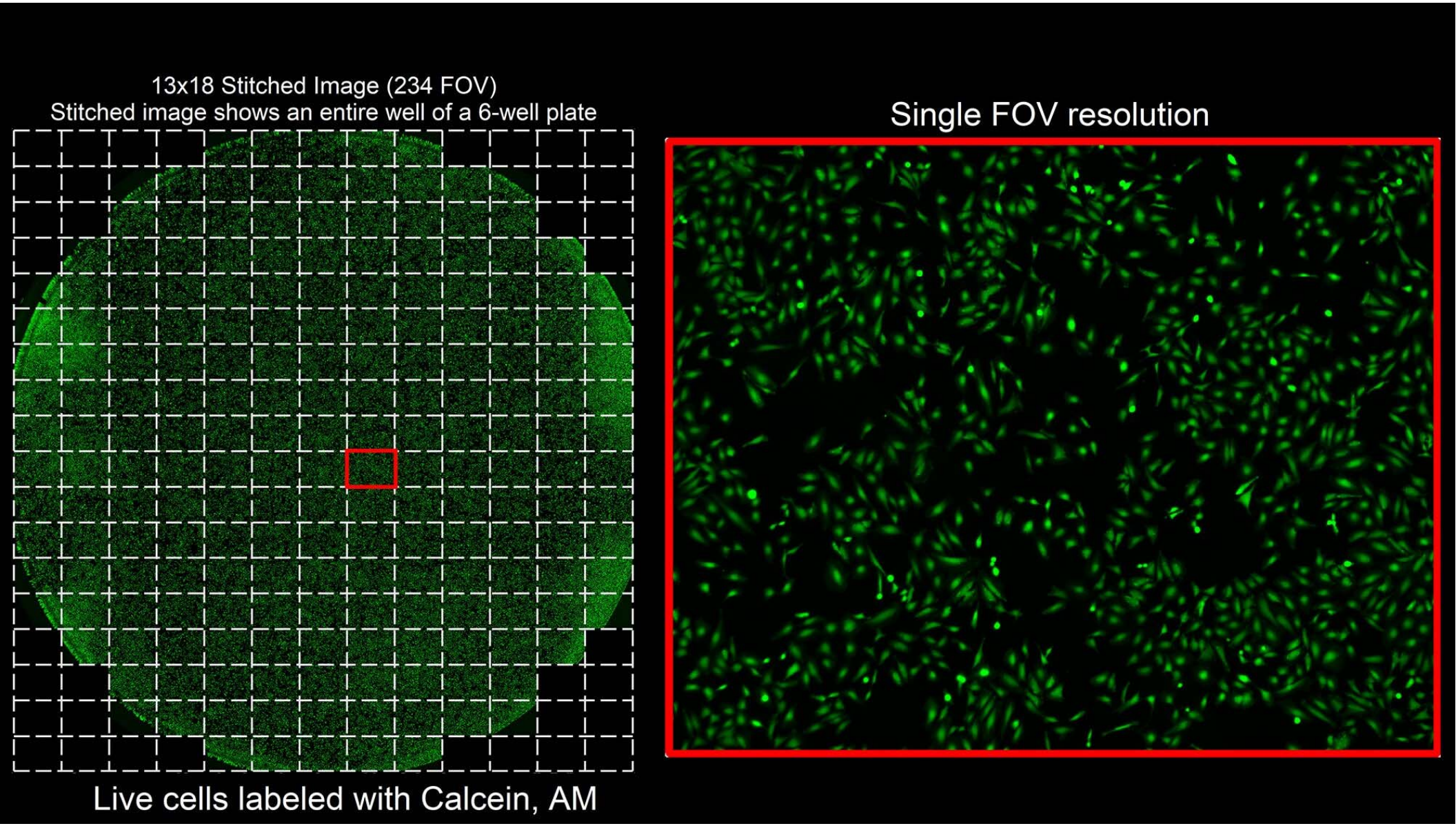




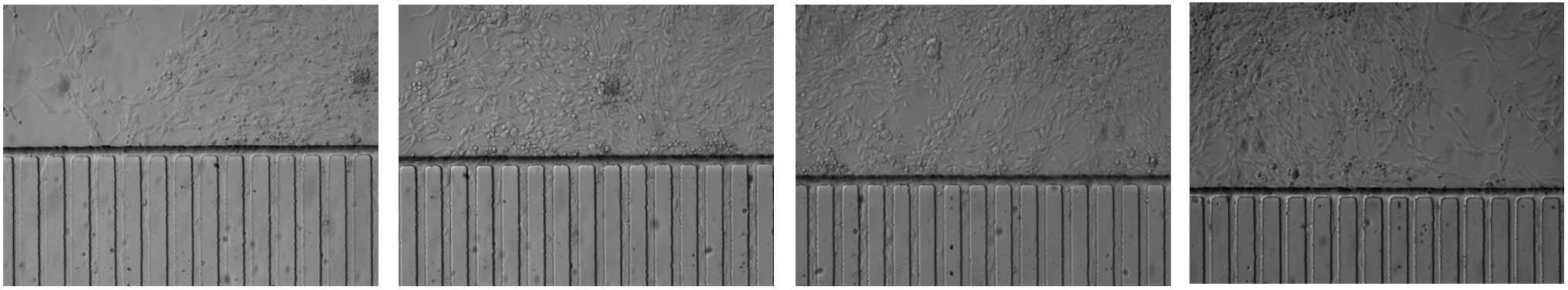
Quantitatively compare the neurite outgrowth between Cortical neurons 1 and Cortical neurons 2

1. Set Scale
2. Select the neurites using a Threshold (click “dark background”)
3. Measure

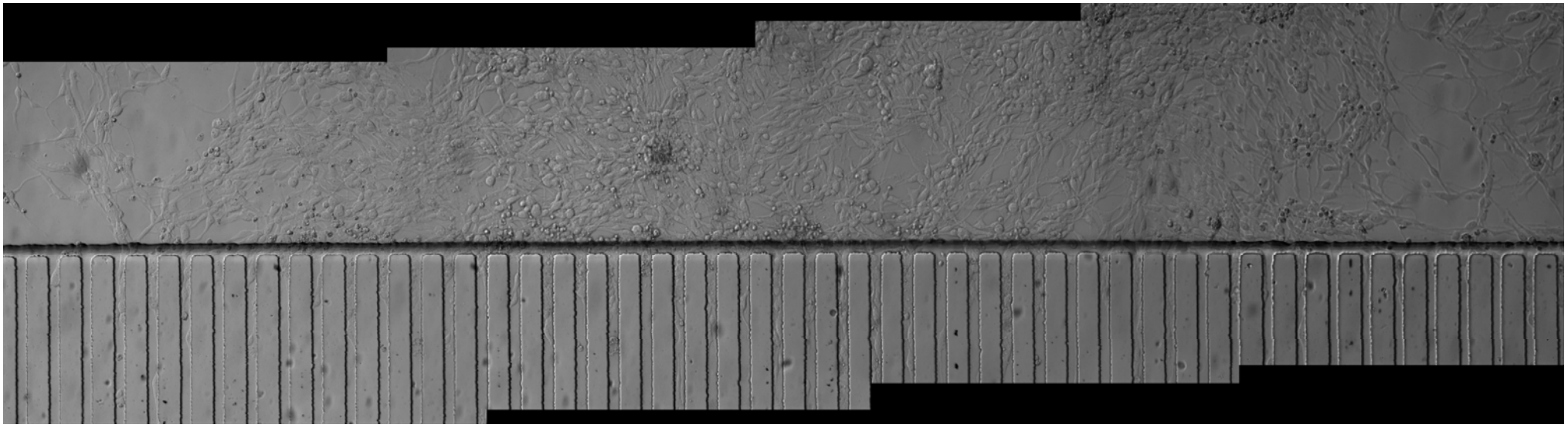
Task 7. Stitching



Tile 4 Tile 3 Tile 2 Tile 1



Stitched image



Stitch four tiles (Tiles 1-4) using a Grid/Collection Stitching (under Plugins-Stitching)

File names for tiles: **Task 7 Tile {ii}.tif**