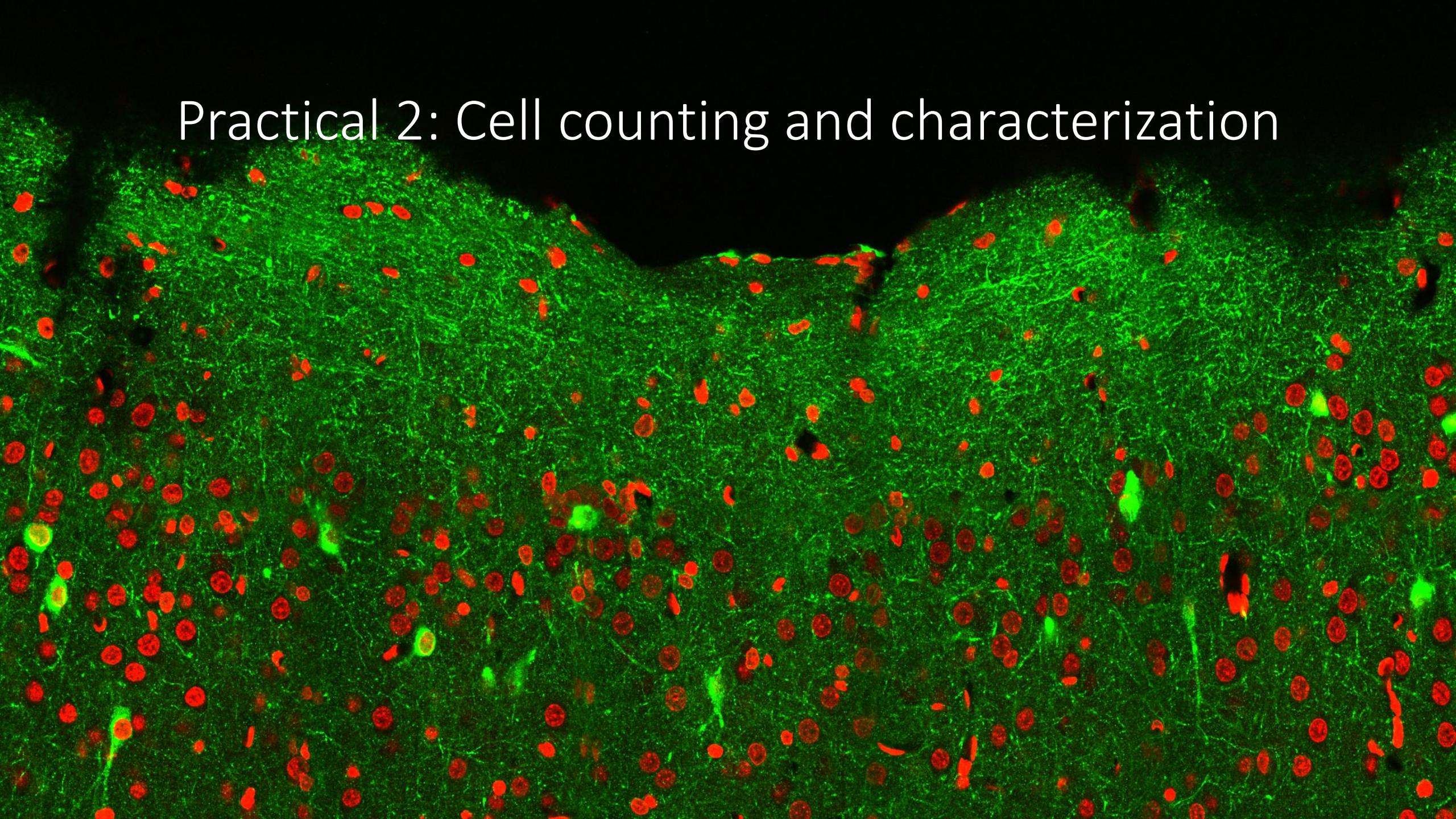
FIJI hands-on workshop

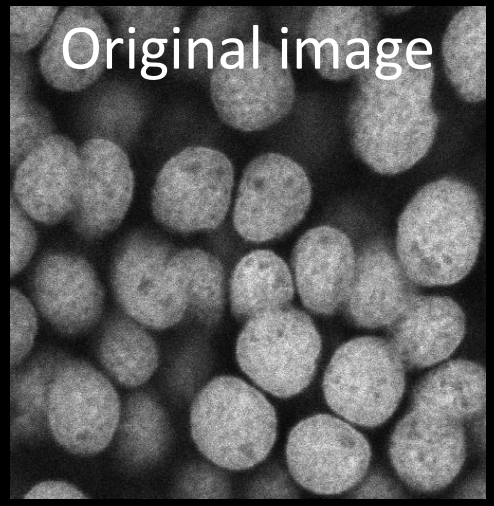
- Practical 1: Basics of FIJI ImageJ
- Practical 2: Cell counting and characterization
- Practical 3: Simple macros

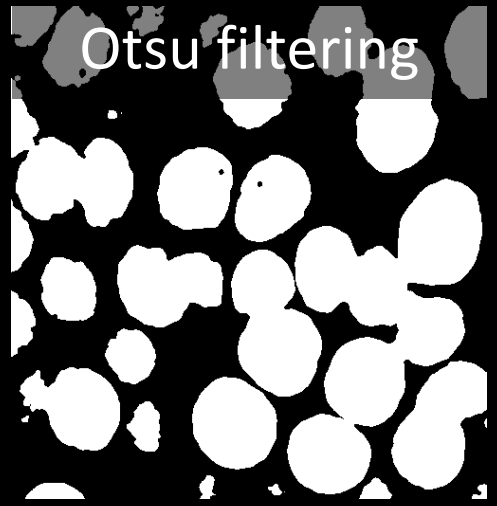
Material available from:

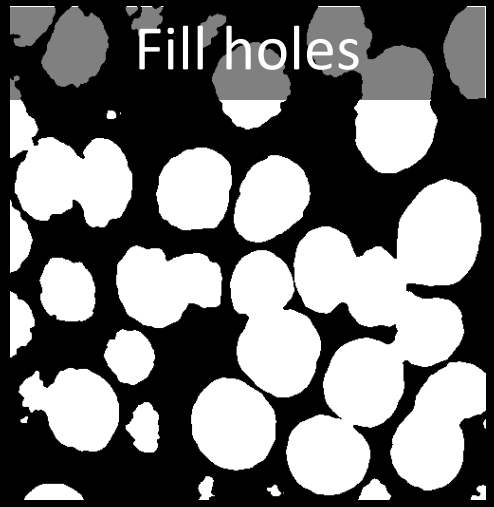
https://wiki.helsinki.fi/display/biu/FIJI+workshop

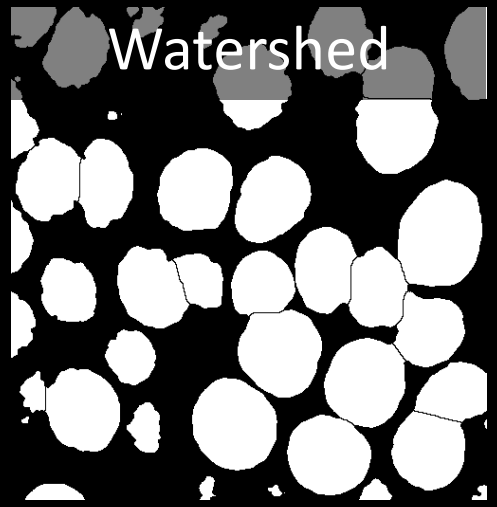
Practical 2: Cell counting and characterization

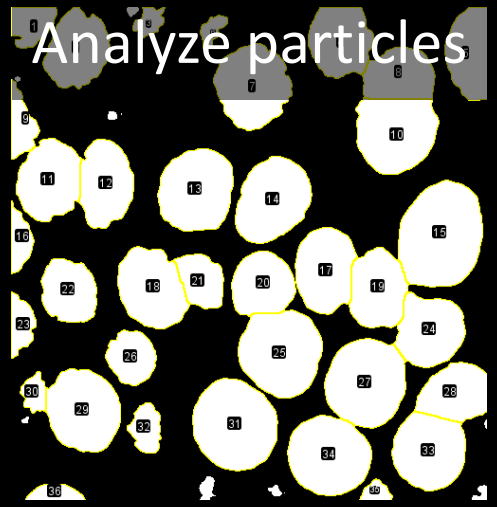




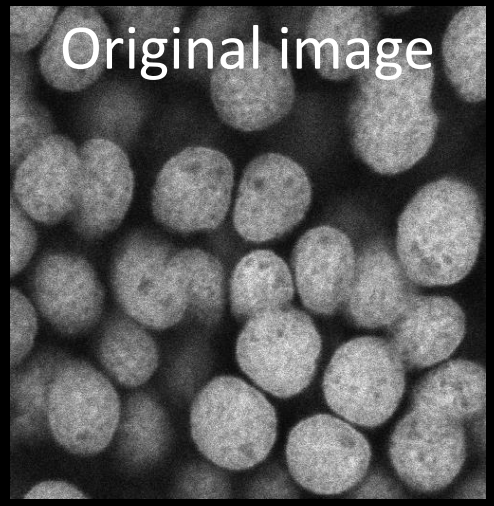


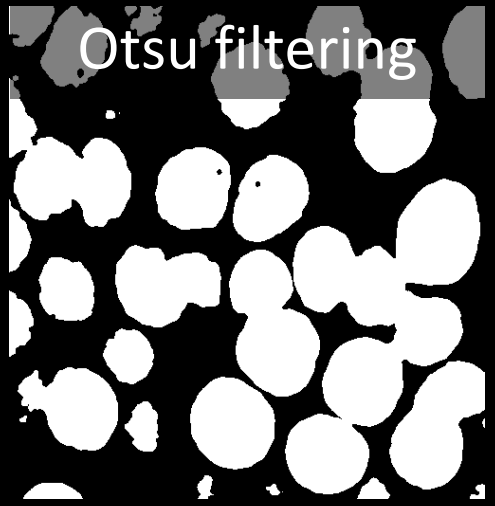






ACQUISITION → PRE-PROCESSING → SEGMENTATION → POST-PROCESSING → ANALYSIS





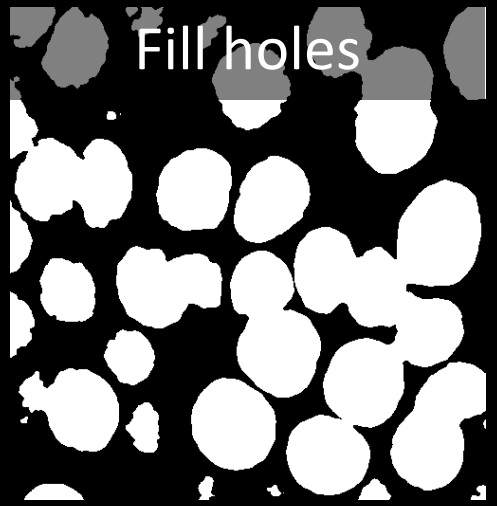
File → Open

NucleiDAPIconfocal.png

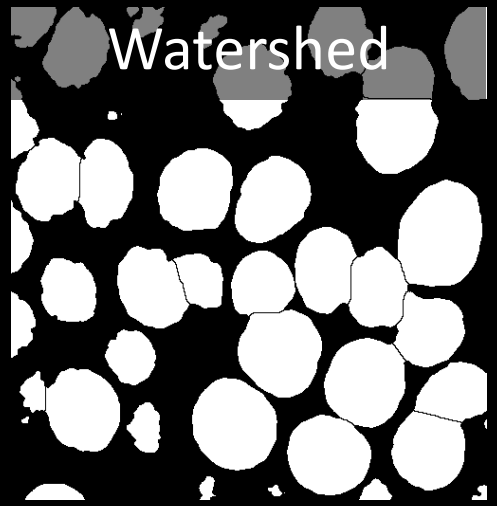
Process → Filters → Gaussian Blur…

Image → Adjust → Threshold

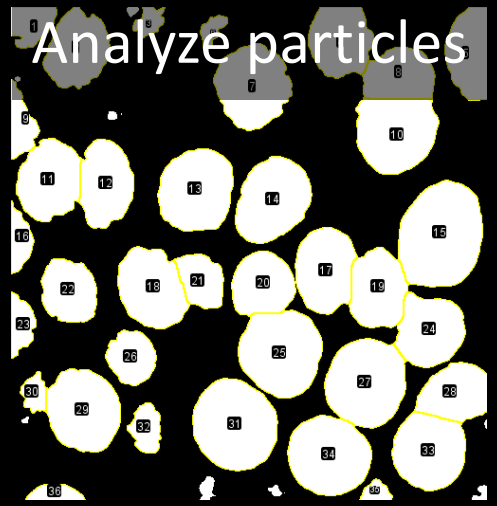
Selection color: Red
Dark background
Method: Try different



Process → Binary → Fill Holes



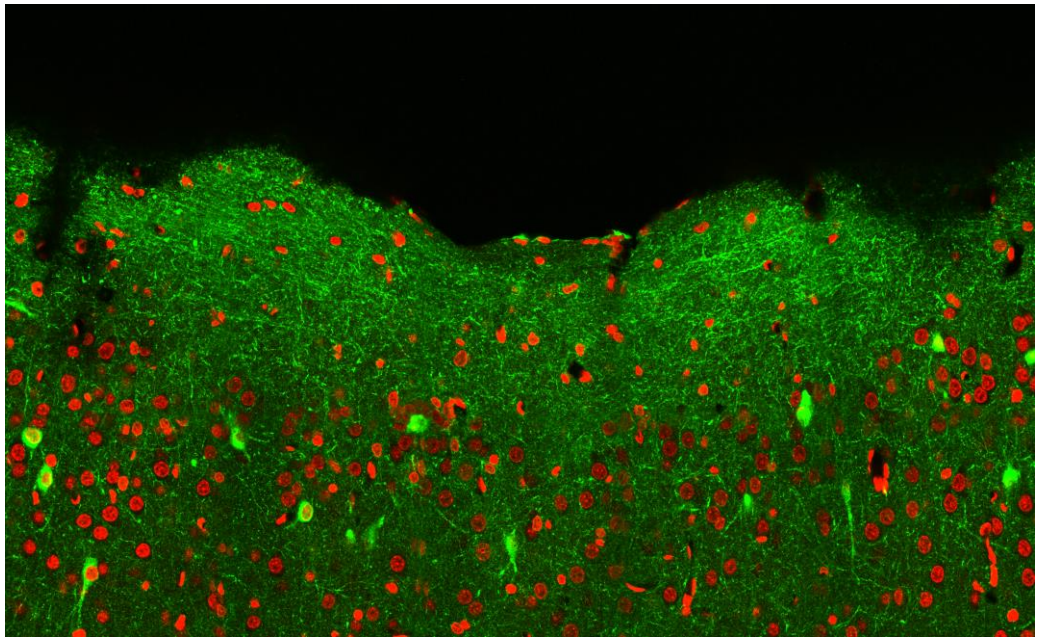
Process → Binary → Watershed



Analyze → Analyze Particles…

Practical 2: Cell counting and characterization

- Dataset:
  - 2-channel fluorescence measurement:
    - Channel 1 = DAPI labelling of nuclei (red)
    - Channel 2 = Immunostaining of Protein-X (green)
  - 5x3 tile-scanned images stitched

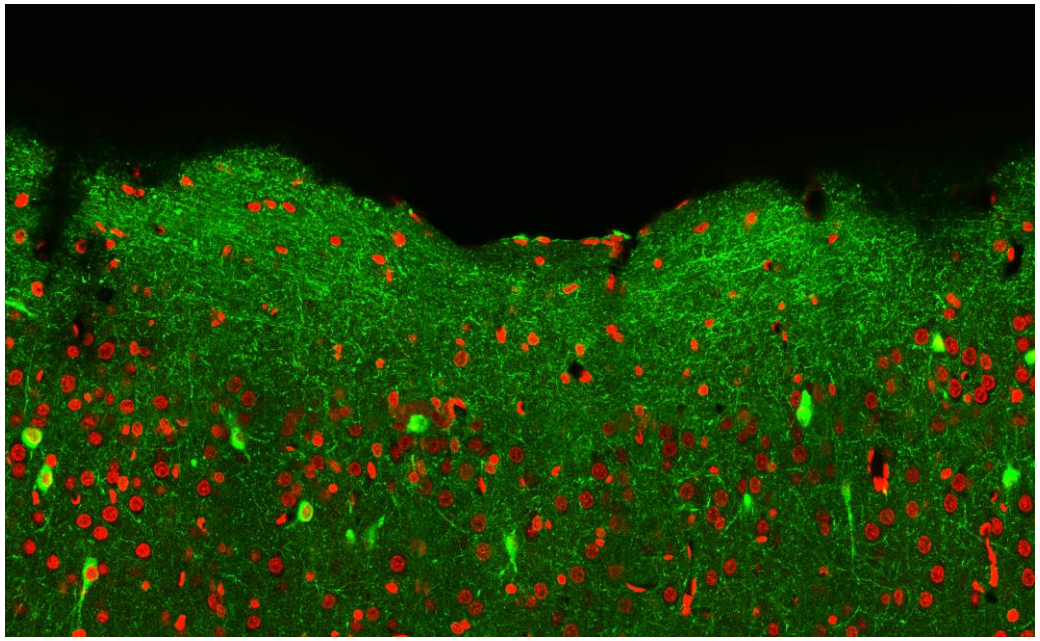


- Task:
  - Detect the nuclei and measure green intensities in nuclei area

Practical 2: Cell counting and characterization

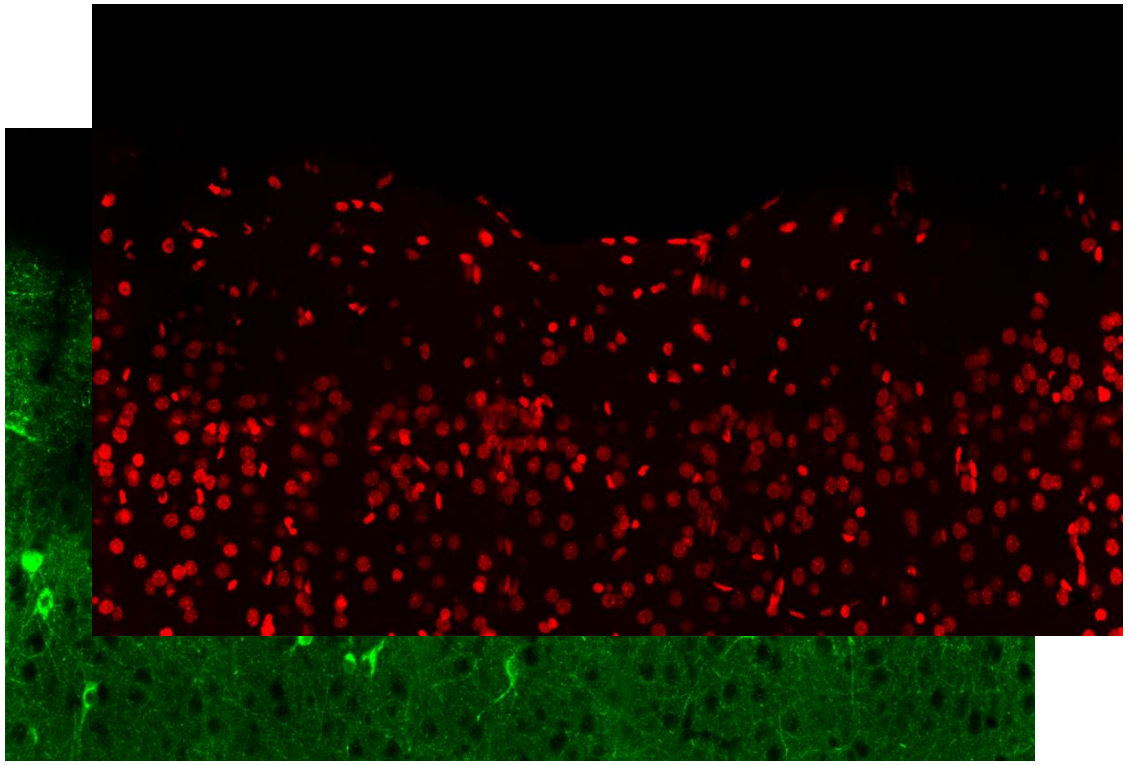
- Task:
  - **Input**: *Stitched_Corr_PosA.tif*
  - Detect the nuclei on channel 1 (red)
  - Measure intensities within the nuclei in channel 2 (green)

- Workflow in brief:
  - Filtering
  - Thresholding
  - Analyze Particles
  - Measure



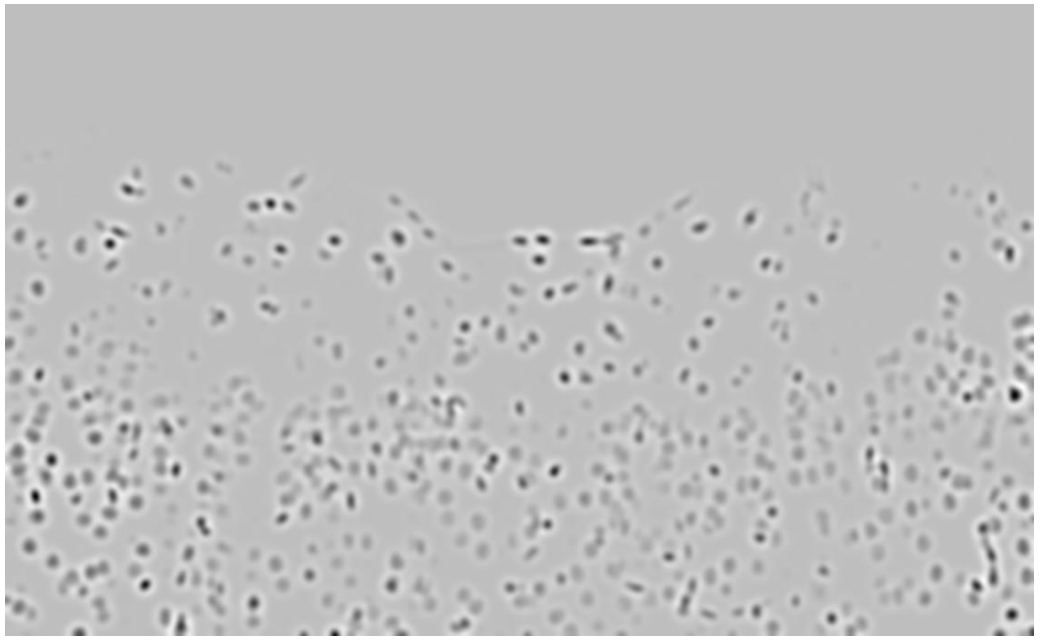
Practical 2: Cell counting and characterization

1. Split channels
2. Select channel 1
   i. Try to segment by setting threshold
3. Filter using “FeatureJ Laplacian“
4. Create a mask by thresholding
5. Watershed



Practical 2: Cell counting and characterization

1. Split channels
2. Select channel 1
3. Filter using “FeatureJ Laplacian“
   i. Use smoothing scale 9
   ii. Laplacian filter is good for detecting changes in intensity
   iii. What is Laplacian filter doing?
4. Create a mask by thresholding
5. Watershed



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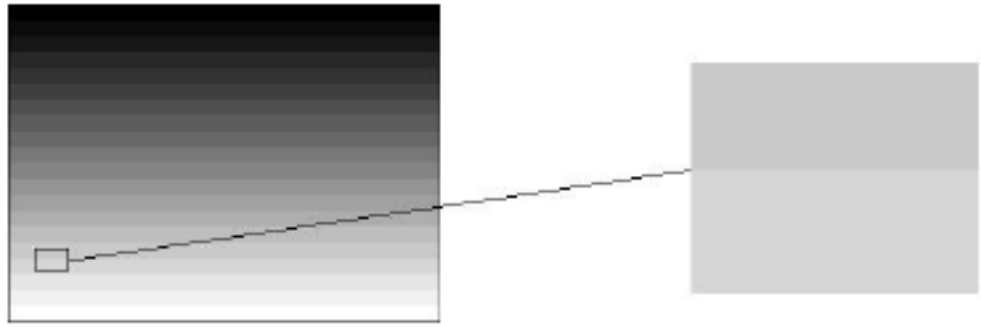
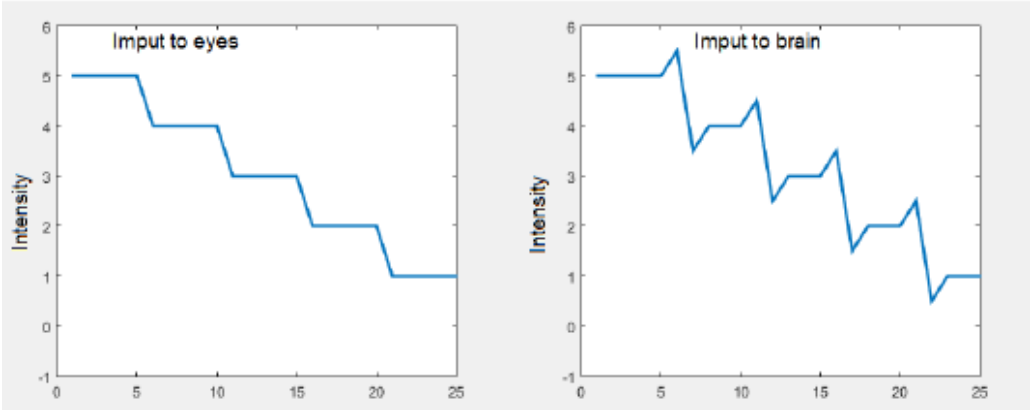
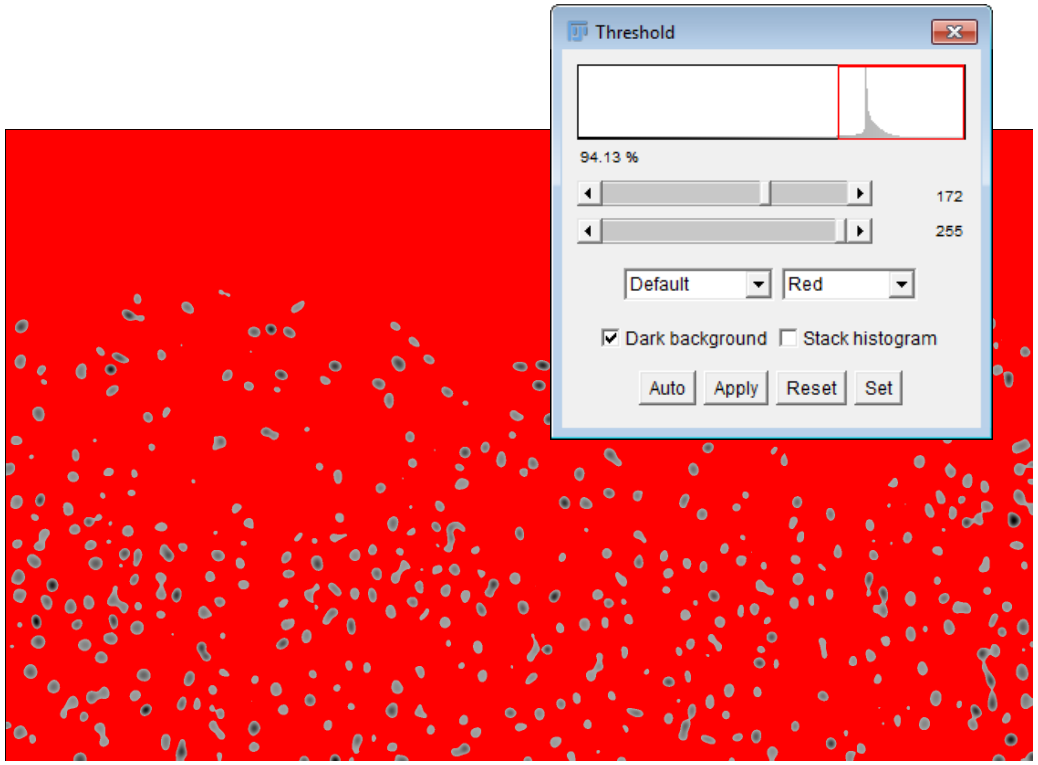




Practical 2: Cell counting and characterization

1. Split channels
2. Select channel 1
3. Filter using “FeatureJ Laplacian“
4. Create a mask by thresholding
    i. Image -> adjust -> Threshold…
        a. Select “Default” threshold filter
        b. Select “Red”
        c. Untick “Dark Background”
        d. Apply
        e. Select “Convert to Mask”
    ii. (Process -> Binary -> Convert to mask)
5. Watershed



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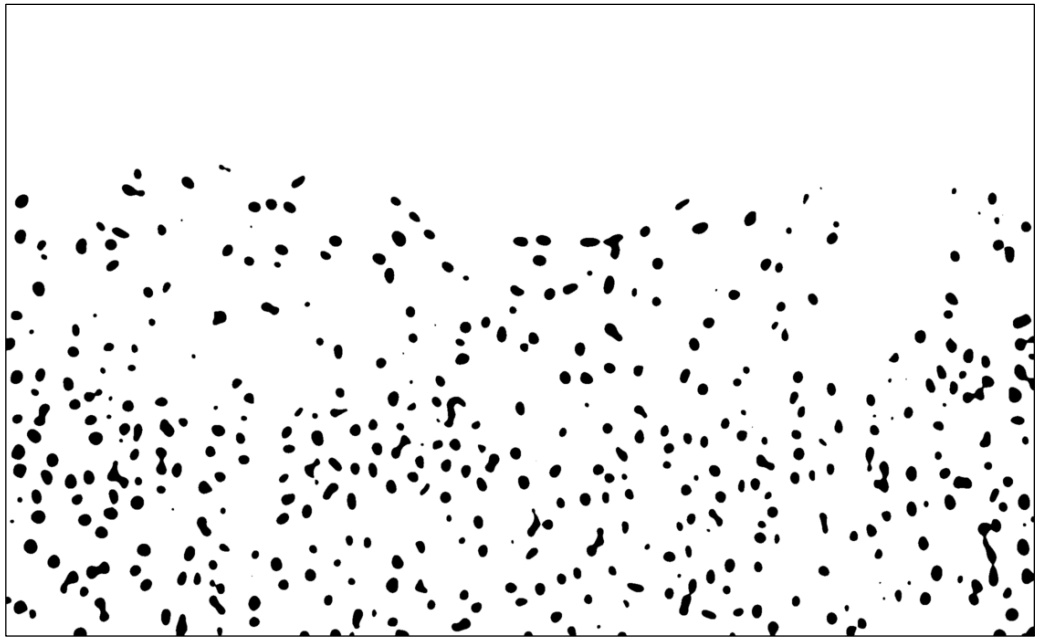
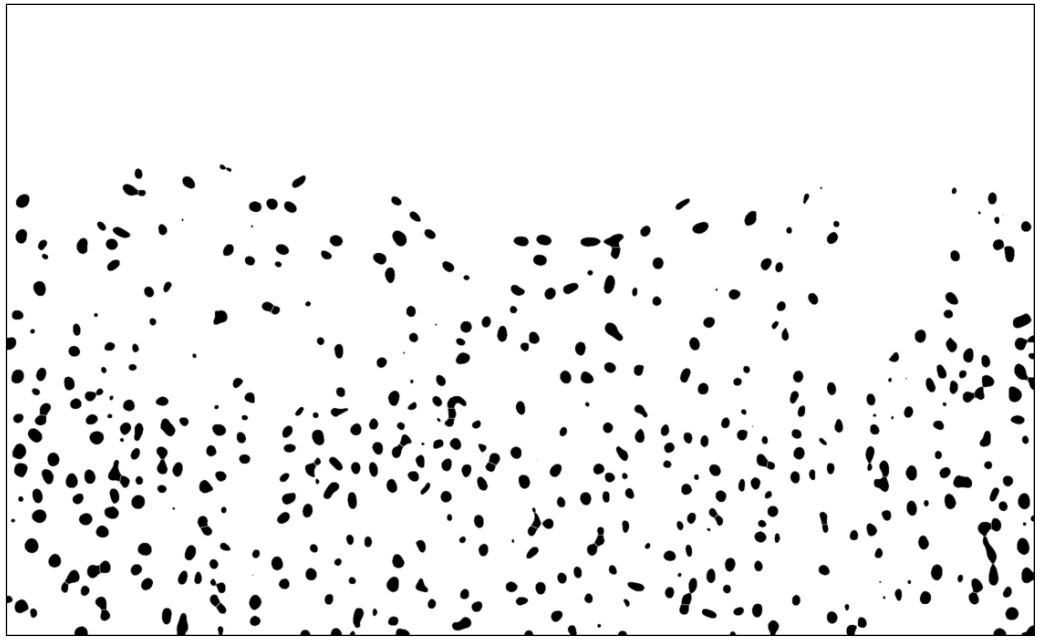


Practical 2: Cell counting and characterization

5. Watershed
6. Analyze Particles...
    i. Size 250-2000
    ii. Clear results
    iii. Add to Manager
    iv. In ROI Manager: Deselect all
7. Measure on Channel 2
    i. Select Ch 2 image
    ii. In ROI Manager
        a. Show all; without labels
        b. Measure



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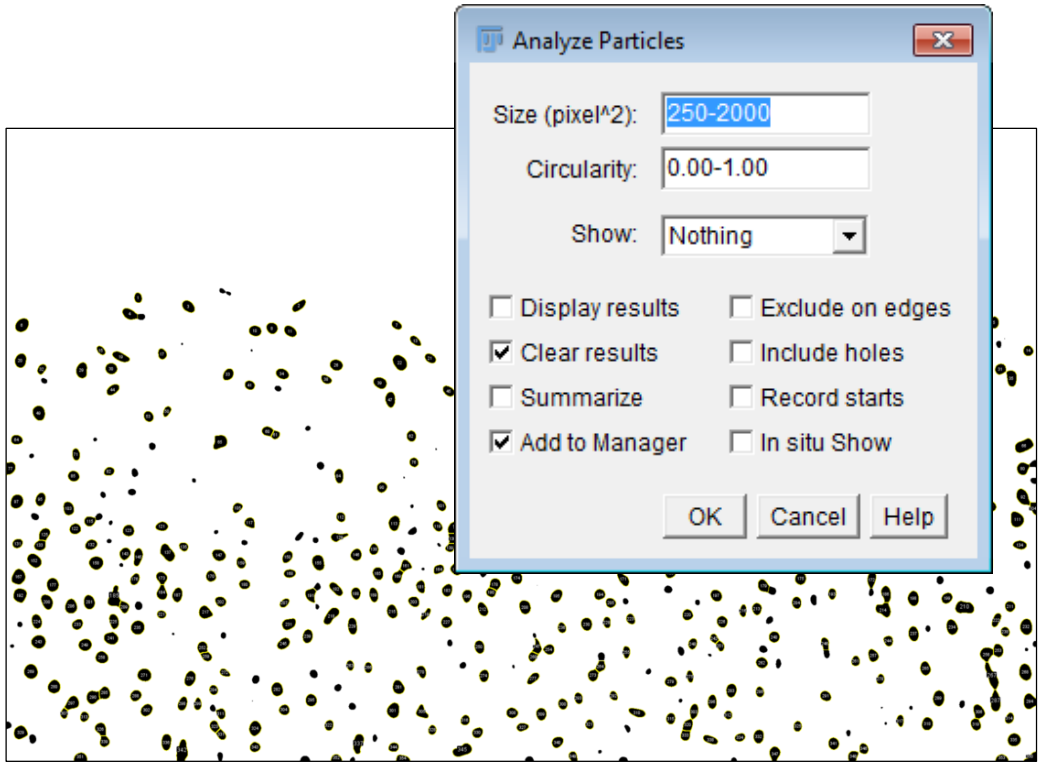

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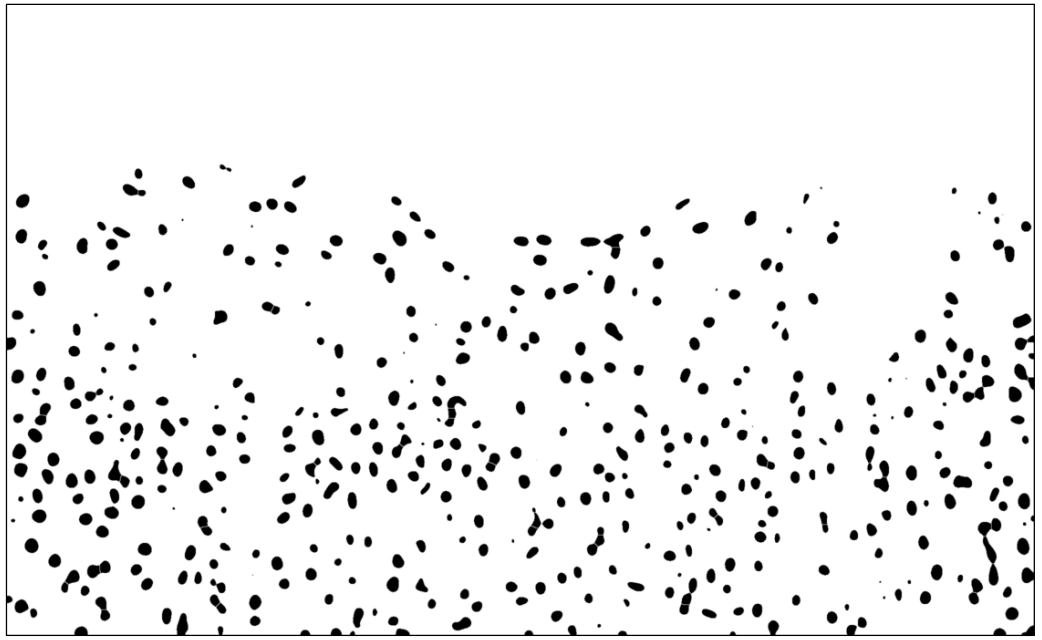

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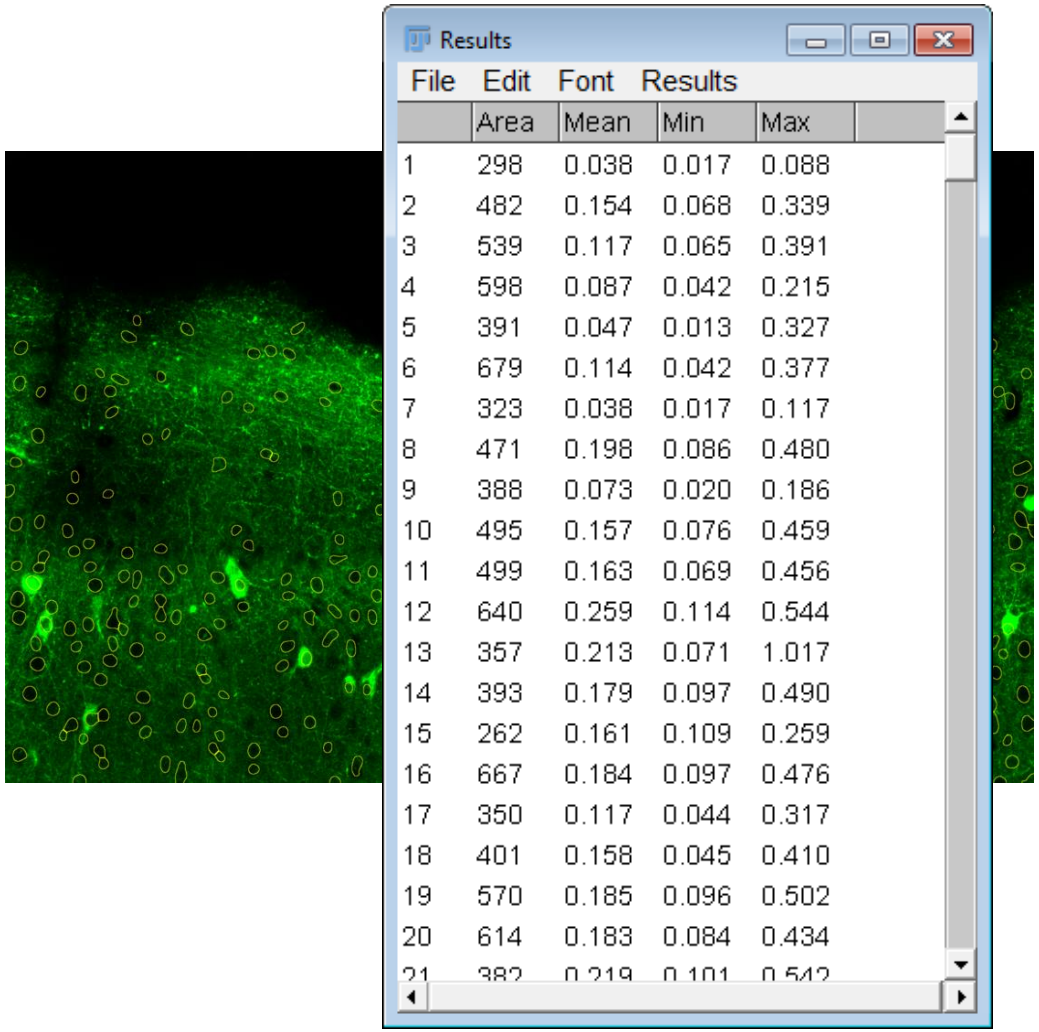

Practical 2: Cell counting and characterization

9. Analysis
   i. Save results as .csv and import in Excel
   ii. Choose a threshold value (e.g. 0.300) for positive cells
   iii. Compare the value in the column *Mean* with your threshold value
      a. Create a column *Positive Cell* and set the value to 0 or 1 for each cell
   iv. Calculate the sum of the column *Positive Cell*