

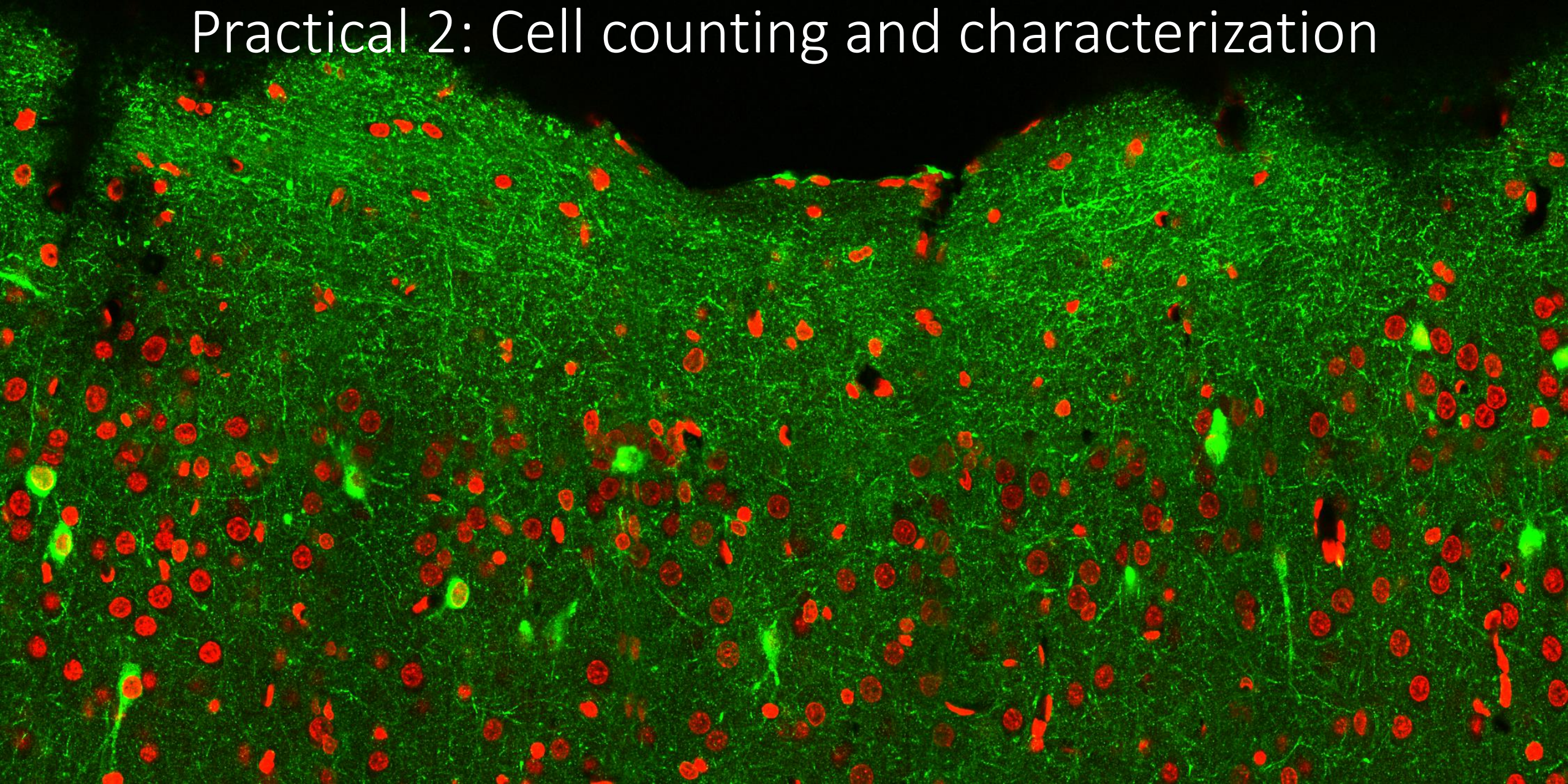
FIJI hands-on workshop

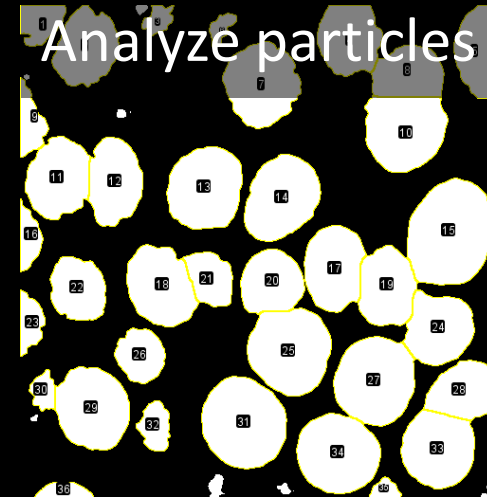
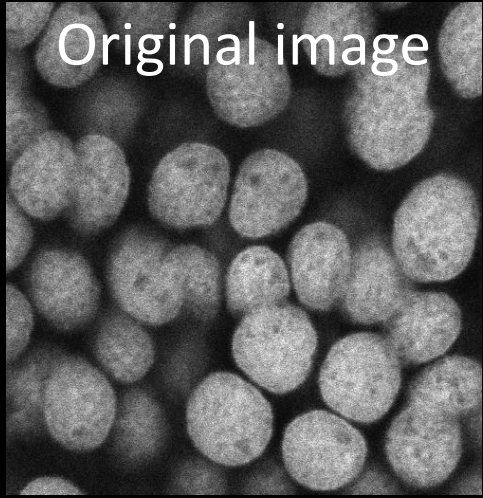
- Practical 1: Basics of FIJI ImageJ
- **Practical 2: Cell counting and characterization**
- Practical 3: Simple macros

Material available from:

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

Practical 2: Cell counting and characterization





ACQUISITION



PRE-PROCESSING



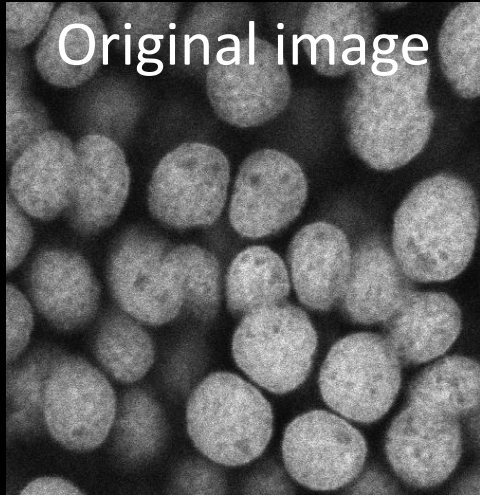
SEGMENTATION



POST-PROCESSING



ANALYSIS



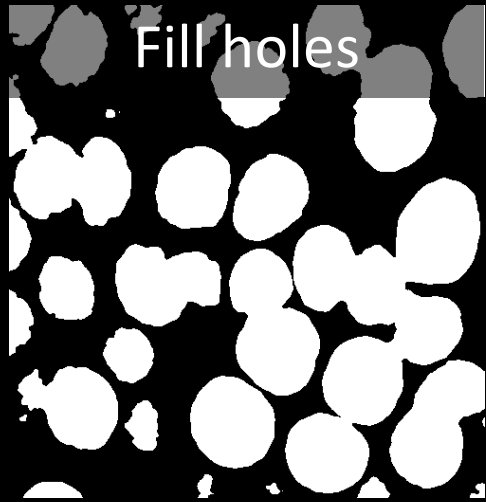
File → Open

NucleiDAPIconfocal.png

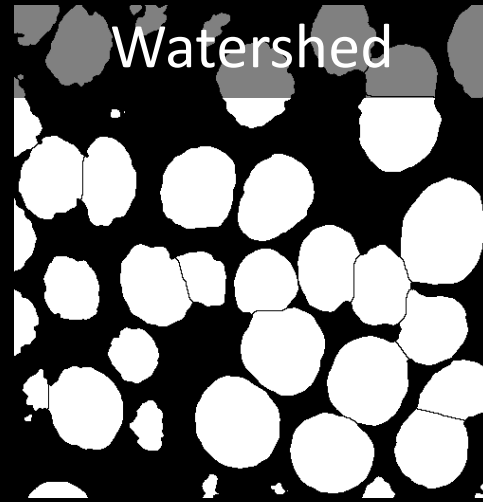
Process → Filters →
Gaussian Blur...

Image → Adjust →
Threshold

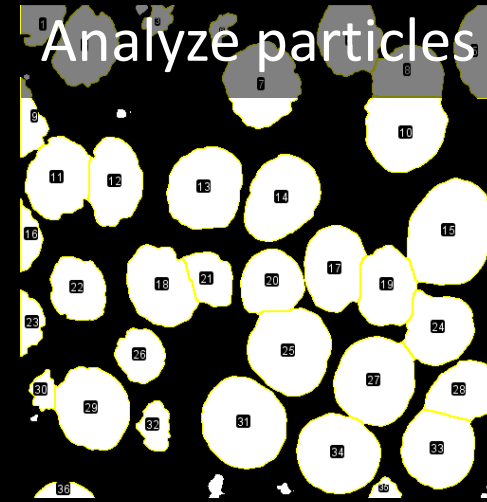
Selection color: Red
Dark background
Method: Try different



Process → Binary → Fill
Holes



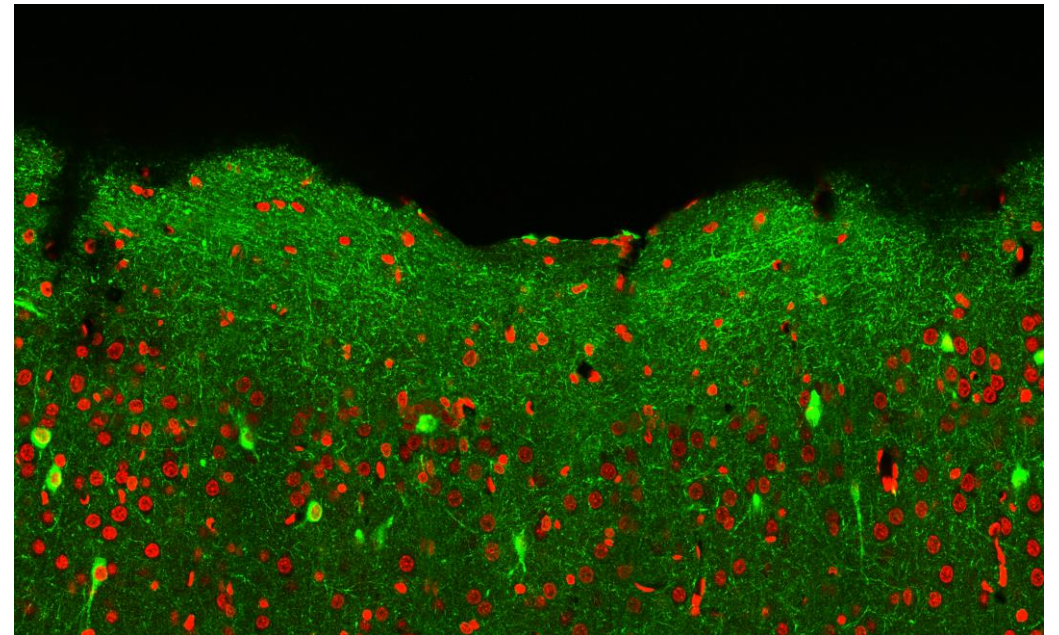
Process → Binary →
Watershed



Analyze → Analyze
Particles...

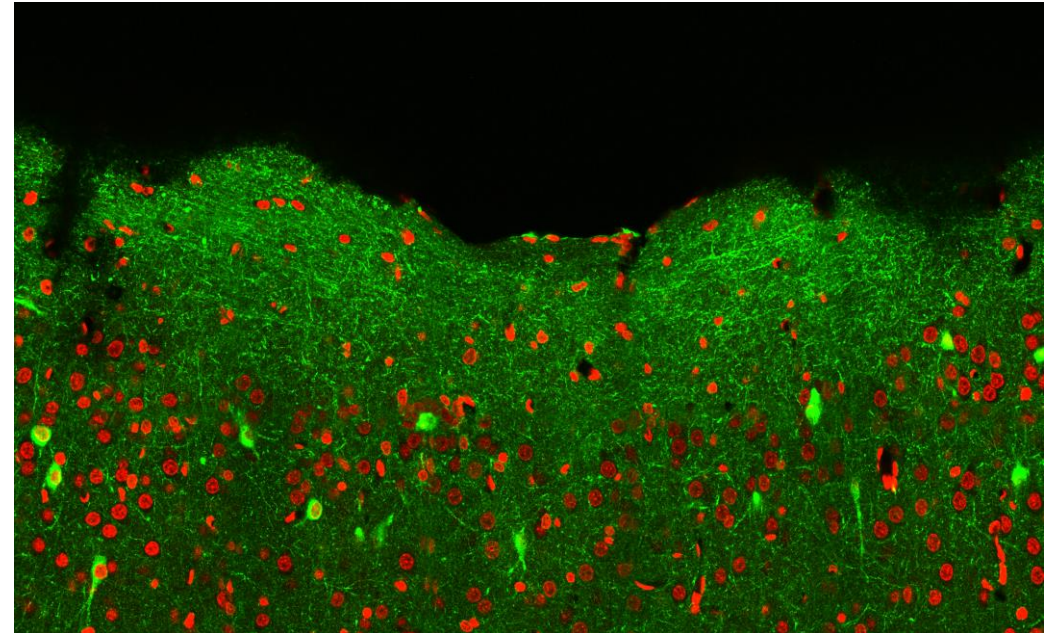
Practical 2: Cell counting and characterization

- 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



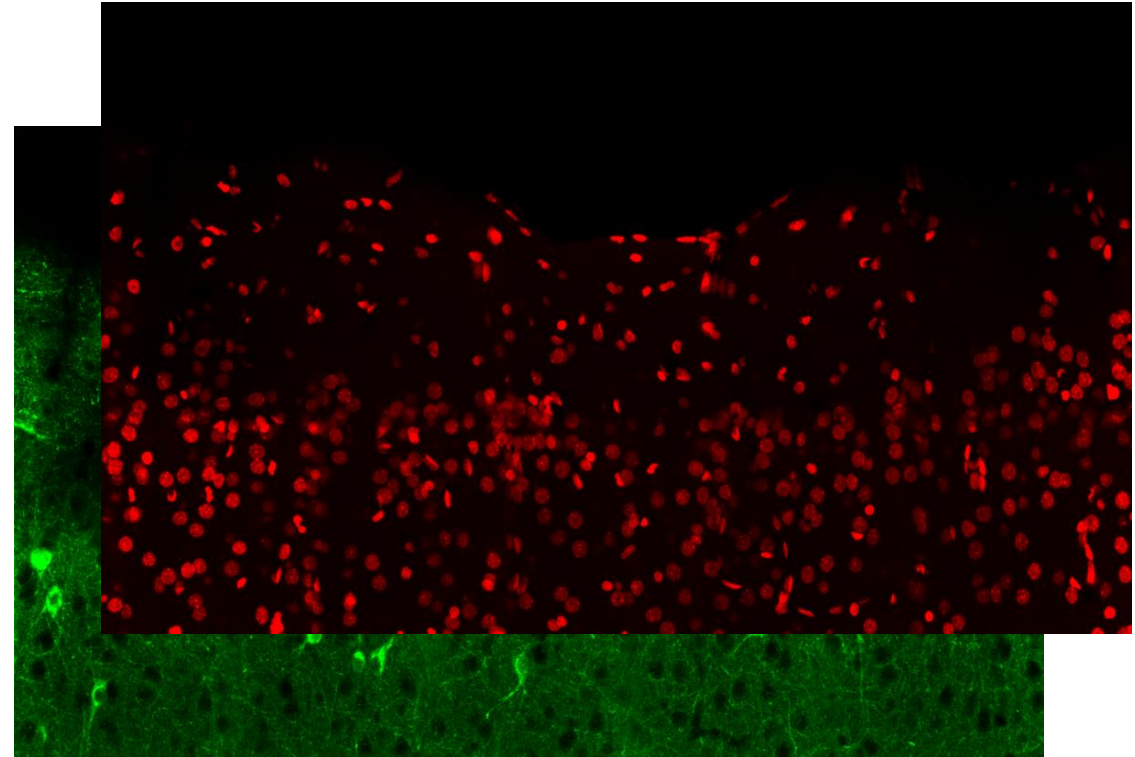
Practical 2: Cell counting and characterization

- 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



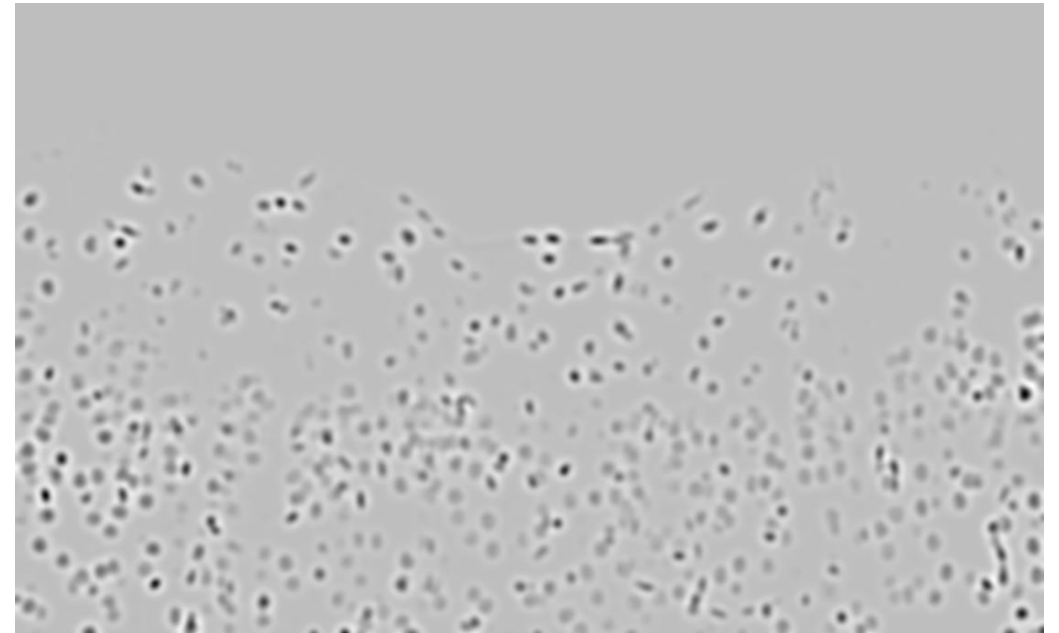
Practical 2: Cell counting and characterization

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



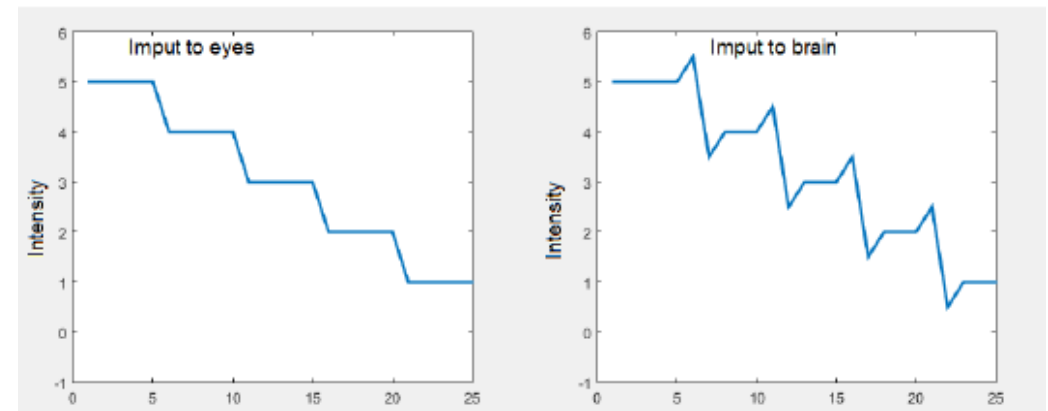
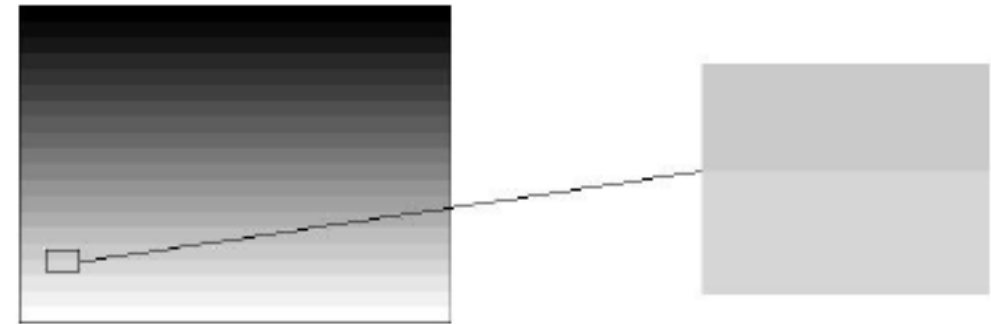
Practical 2: Cell counting and characterization

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



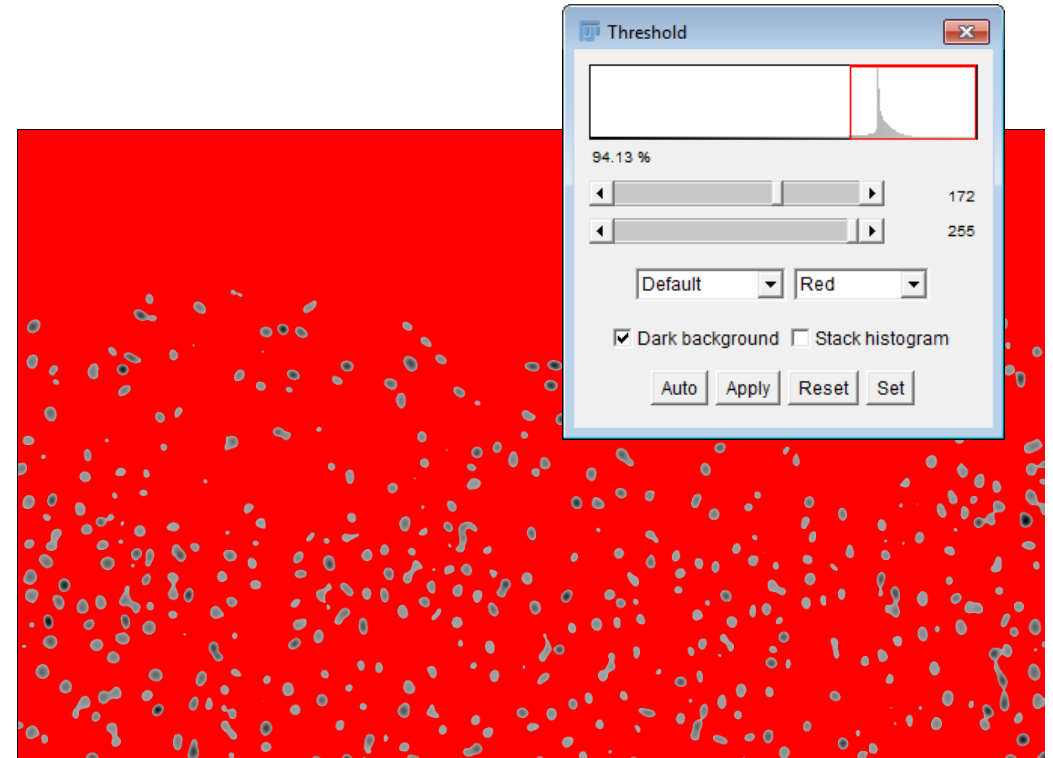
Practical 2: Cell counting and characterization

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



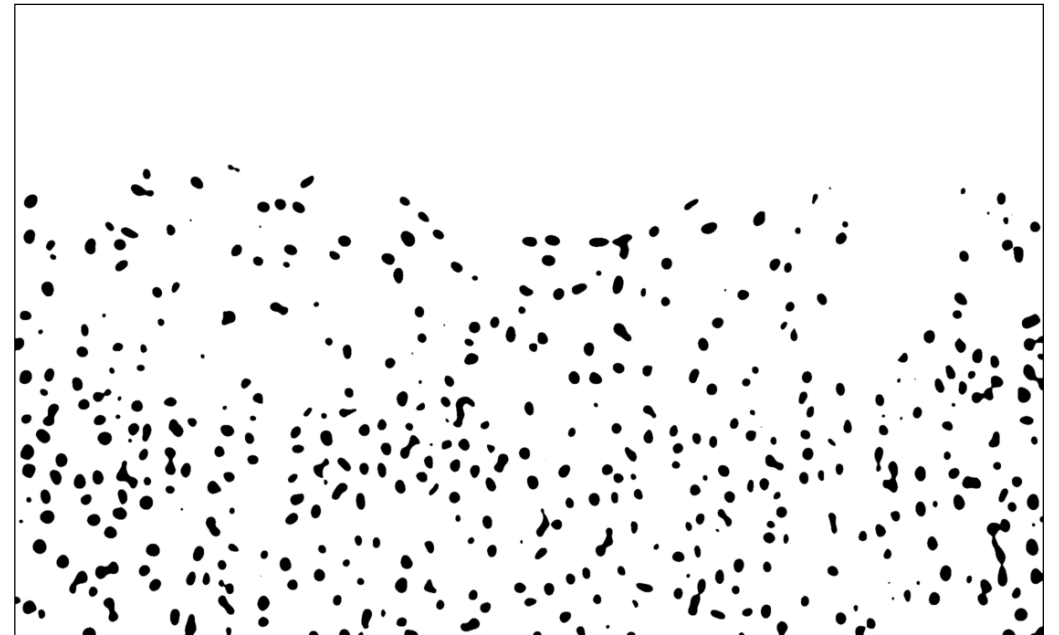
Practical 2: Cell counting and characterization

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



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...
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Practical 2: Cell counting and characterization

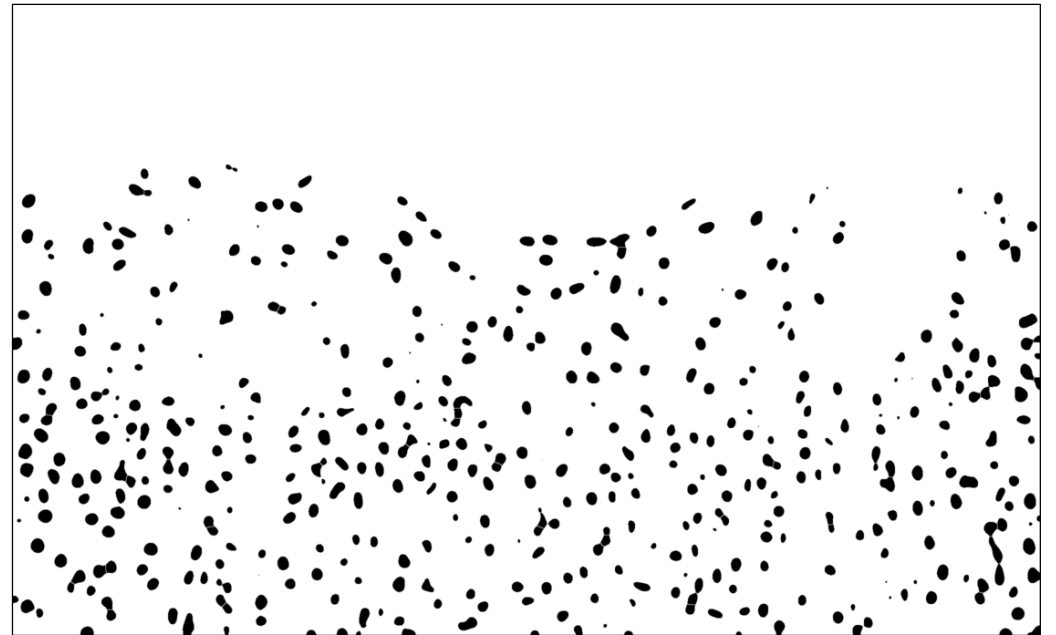
5. Watershed

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



Practical 2: Cell counting and characterization

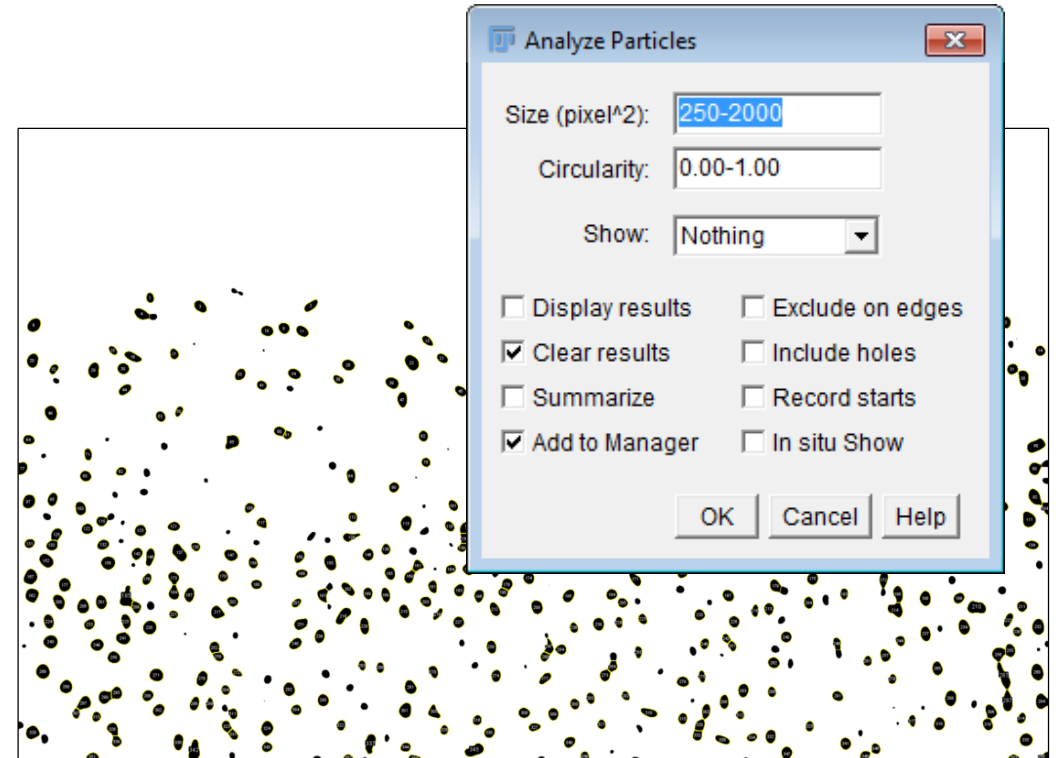
5. Watershed

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Practical 2: Cell counting and characterization

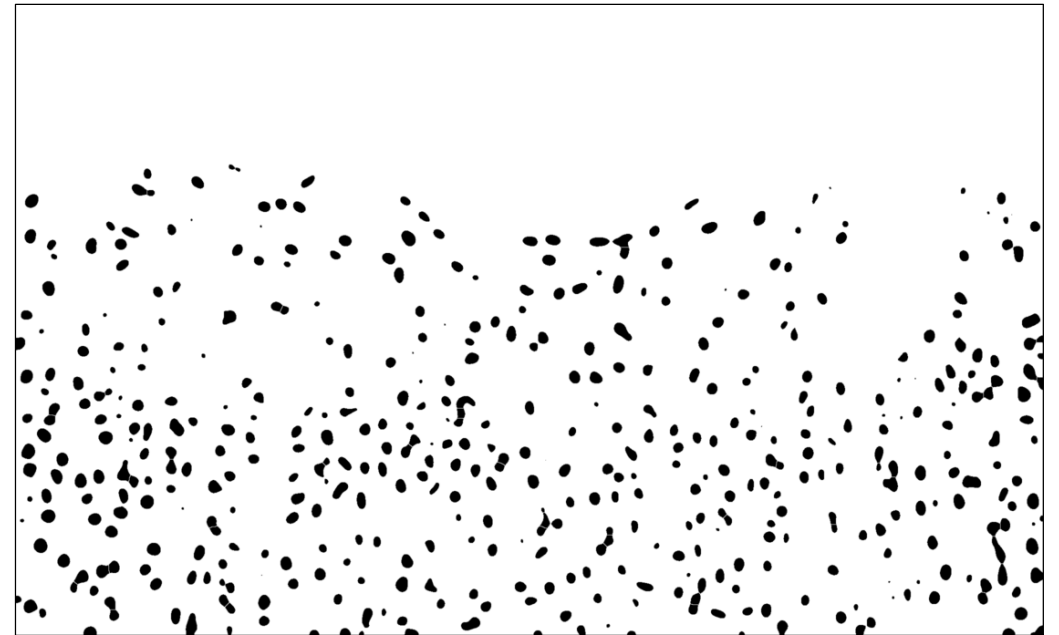
5. Watershed

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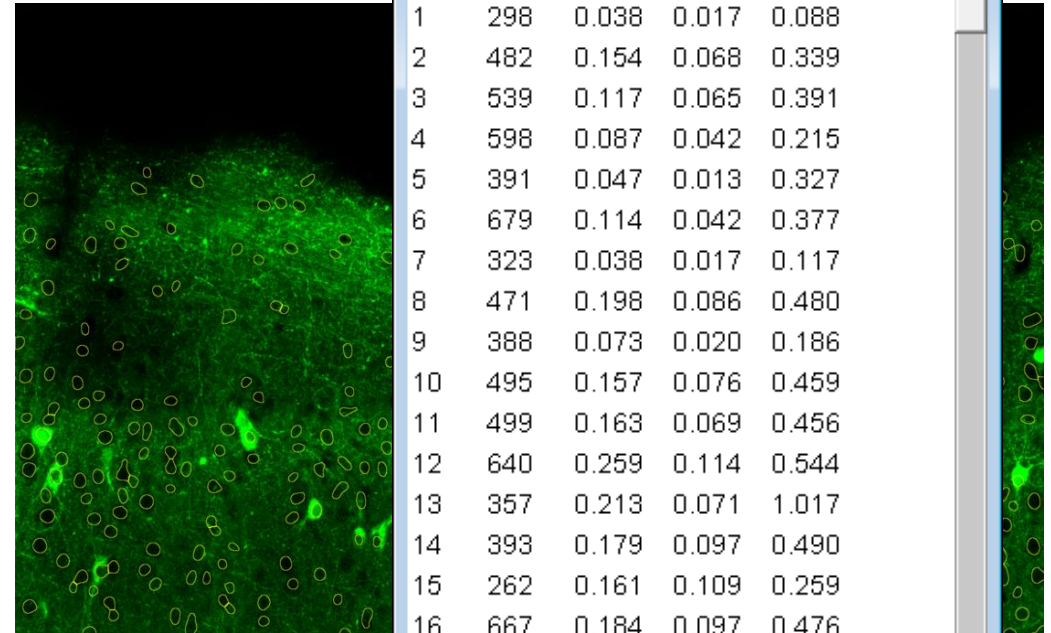
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Practical 2: Cell counting and characterization

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Results				
File	Edit	Font	Results	
	Area	Mean	Min	Max
1	298	0.038	0.017	0.088
2	482	0.154	0.068	0.339
3	539	0.117	0.065	0.391
4	598	0.087	0.042	0.215
5	391	0.047	0.013	0.327
6	679	0.114	0.042	0.377
7	323	0.038	0.017	0.117
8	471	0.198	0.086	0.480
9	388	0.073	0.020	0.186
10	495	0.157	0.076	0.459
11	499	0.163	0.069	0.456
12	640	0.259	0.114	0.544
13	357	0.213	0.071	1.017
14	393	0.179	0.097	0.490
15	262	0.161	0.109	0.259
16	667	0.184	0.097	0.476
17	350	0.117	0.044	0.317
18	401	0.158	0.045	0.410
19	570	0.185	0.096	0.502
20	614	0.183	0.084	0.434
21	382	0.219	0.101	0.512

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*

