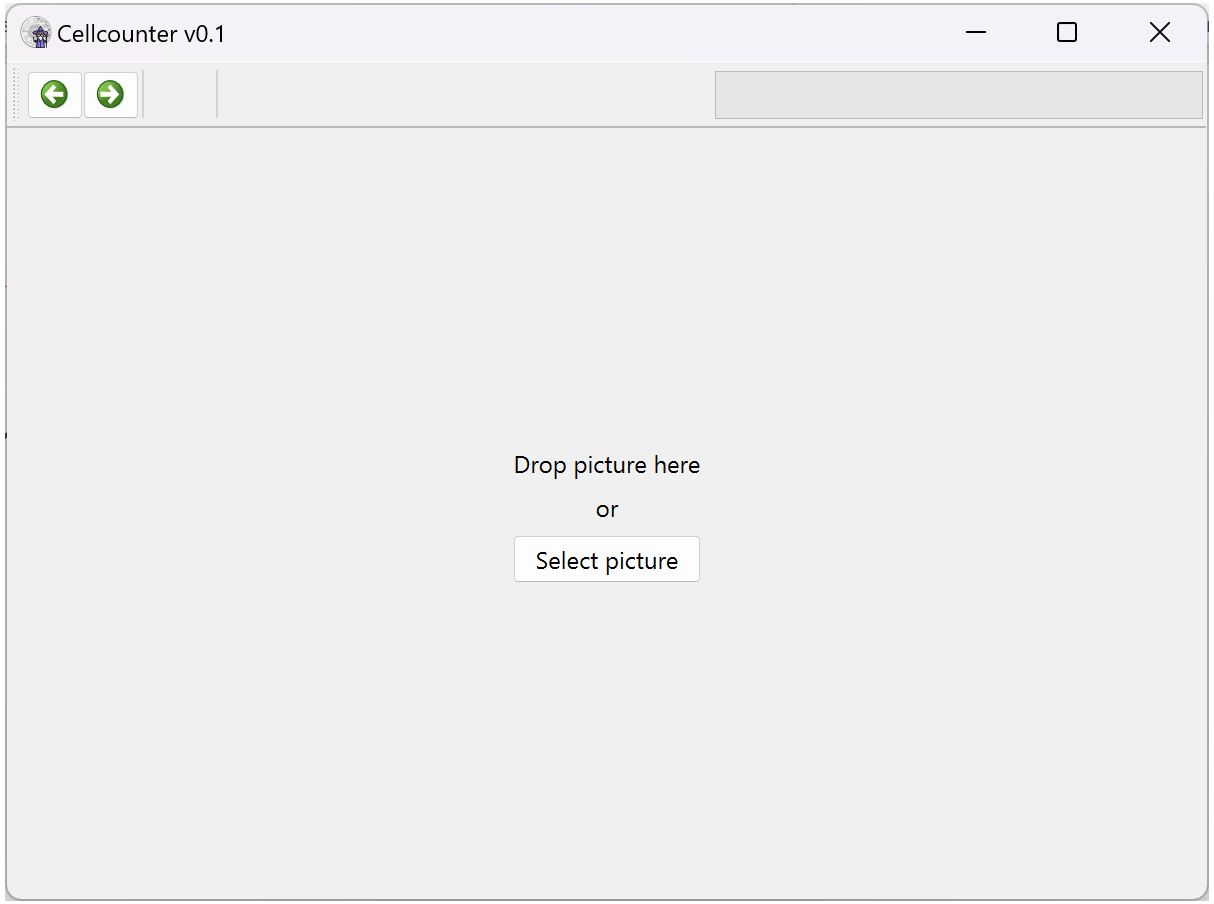

Cellcounter v0.1
Drop picture here
or
Select picture

Step 1: Select files

- Drag your image onto the window or select the image from the file selector menu
- If you want to analyze multiple images, start with the very first image in your folders
- After analyzing one image, you can use the arrows to jump to next

Cellcounter v0.1

C:/Users/domin/Documents/PEG_Hydroponics_Cross_sections/1M01 - Annotation 3/1M01_Default_Extended.tif

Image correction

Cell detection

Staining

ROI

Counting

Manual correction

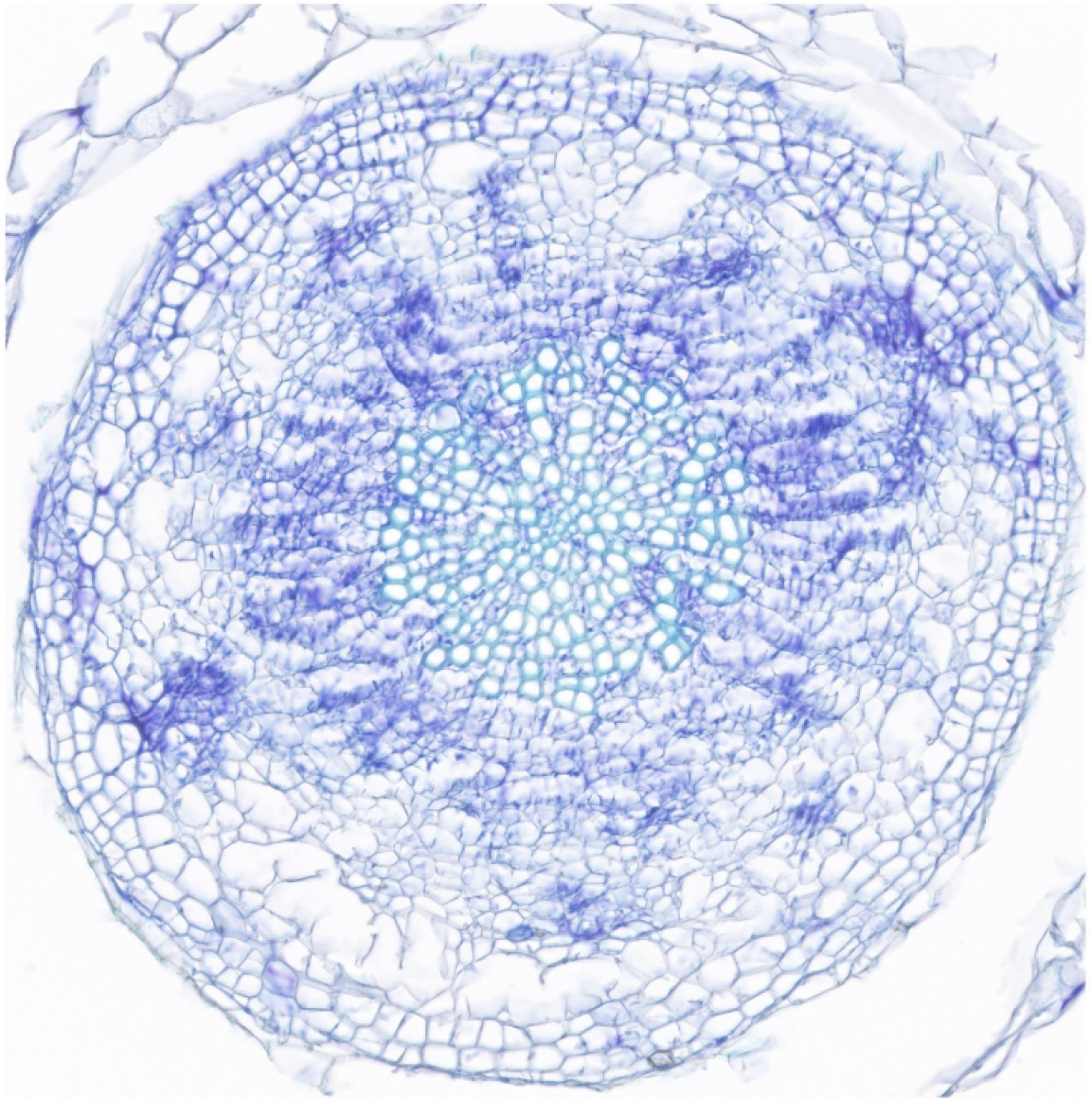
Alpha 1

Beta 0

Manual correction

Autocorrect

Autocorrect



16:41
21.06.2023

Step 2: Color correction

- This step tries to bring all images on the same contrast and brightness levels
- Press the „Autocorrect“ button
- You can change the correction by setting the alpha and beta values and pressing „Manual correction“
- Alpha corresponds to the contrast, beta to the brightness

Cellcounter v0.1

C:/Users/domin/Documents/PEG_Hydroponics_Cross_sections/1M01 - Annotation 3/1M01_Default_Extended.tif

Image correction

Cell detection

Staining

ROI

Counting

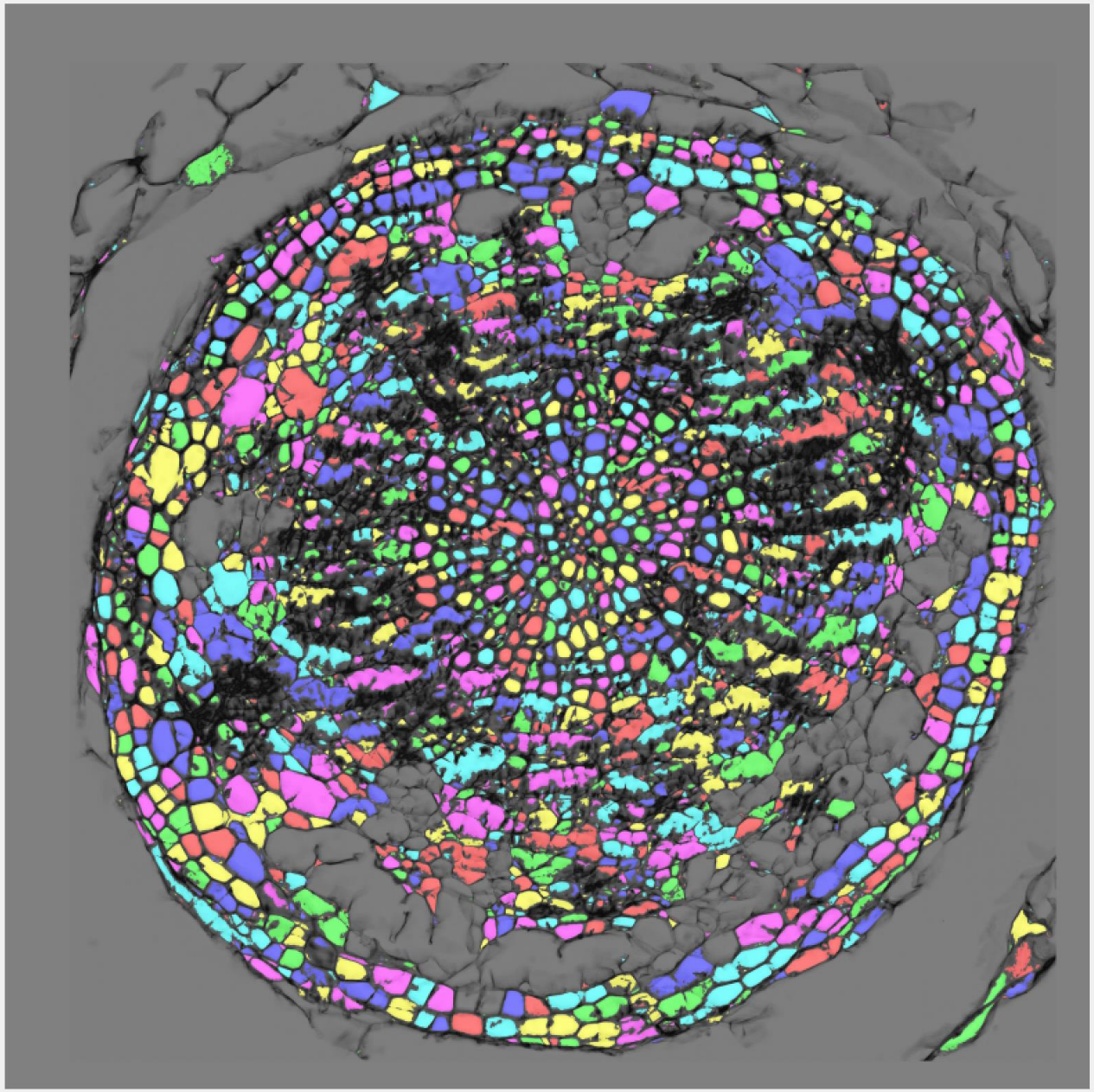
Cell detection

Threshold 200

Lower Size Cutoff 20

Upper Size Cutoff 99.5

Detect cells



16:43
21.06.2023

Step 3: Detect cells

- This step detects all cell walls in the image, without differentiating between xylem or other cells
- Start the detection with the preselected settings and change them if the detection is not to your expectation
- *Threshold:* The brightness threshold between cell walls and cell body. With higher values, the cell walls will be detected thicker, with lower values some cells walls might get overlooked
- *Lower/higher size cutoff:* Only cells with areas in between these percentiles will be used. Used to cut off pixel size cell artefacts and huge areas that are wrongly detected as cell

Cellcounter v0.1

C:/Users/domin/Documents/PEG_Hydroponics_Cross_sections/1M01 - Annotation 3/1M01_Default_Extended.tif

Image correction

Cell detection

Staining

ROI

Counting

Color thresholds

Lower Blue Threshold 80

Upper Blue Threshold 110

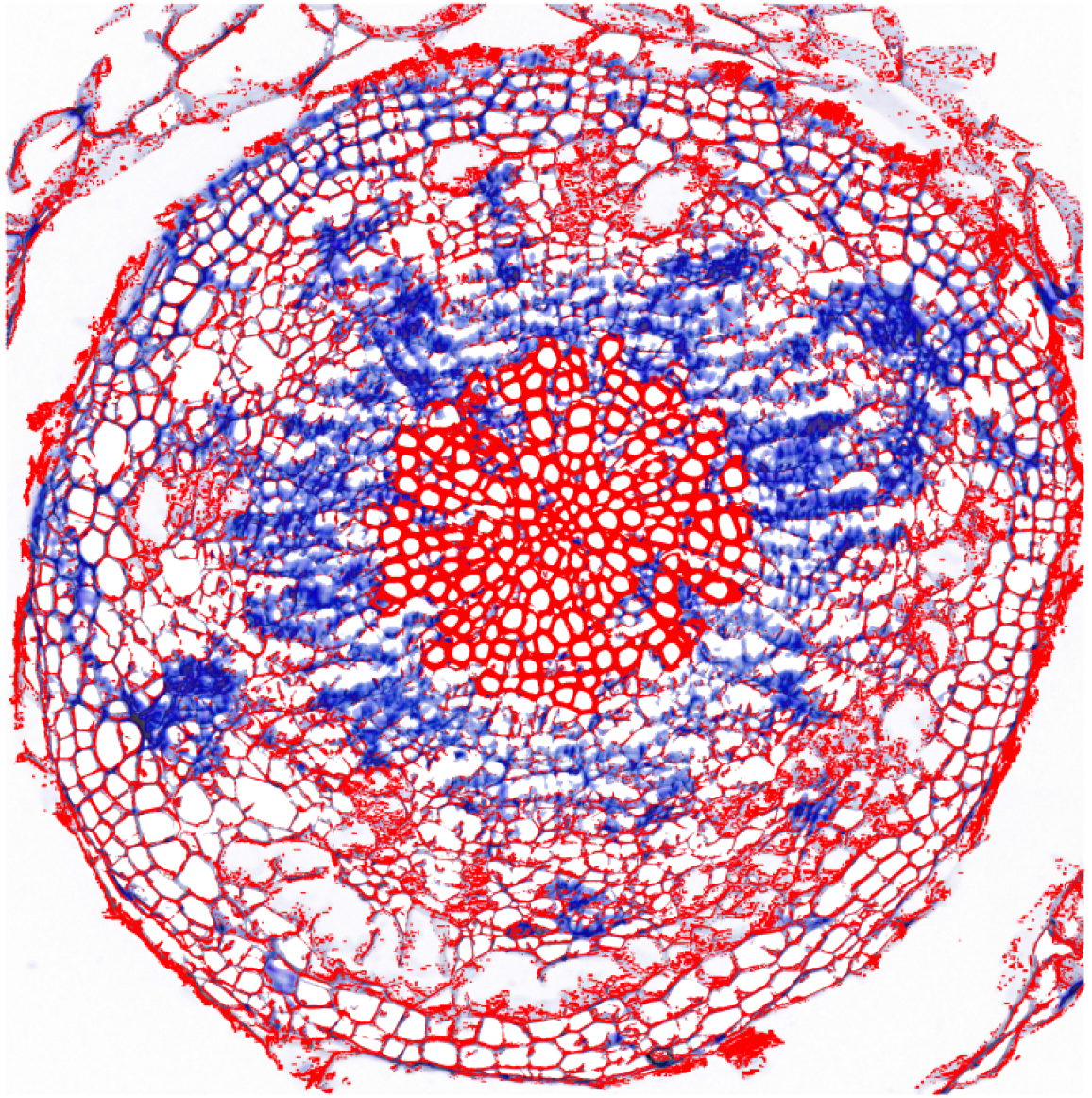
Minimum Saturation 10

Minimum Brightness 160

Erosion 1

Dilation 2

Detect staining



16:44
21.06.2023

Step 4: Xylem vessel cell wall detection

- *Lower/Upper blue threshold:* Range of blue that is detected
- *Minimum saturation/brightness*
- *Erosion:* Removes smaller insular dots
- *Dilation:* Inflates connected areas

Cellcounter v0.1

C:/Users/domin/Documents/PEG_Hydroponics_Cross_sections/1M01 - Annotation 3/1M01_Default_Extended.tif

Image correction

Cell detection

Staining

ROI

Counting

Automatic cambium search

Erosion 1.0

Blur 70.0

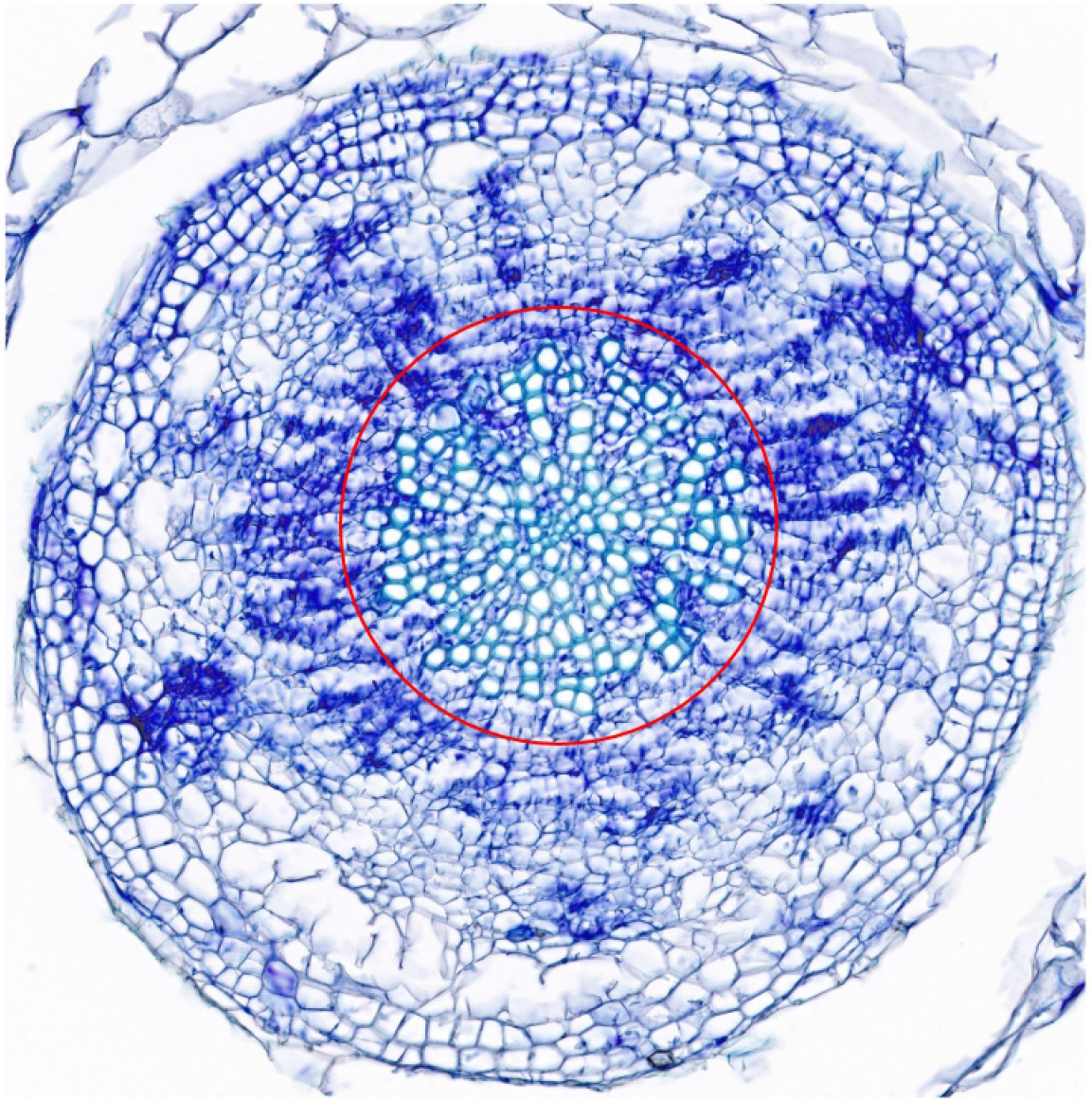
Cell threshold 132.0

Cambium cutoff 0.4

Auto detect Cambium

Manual cambium selection

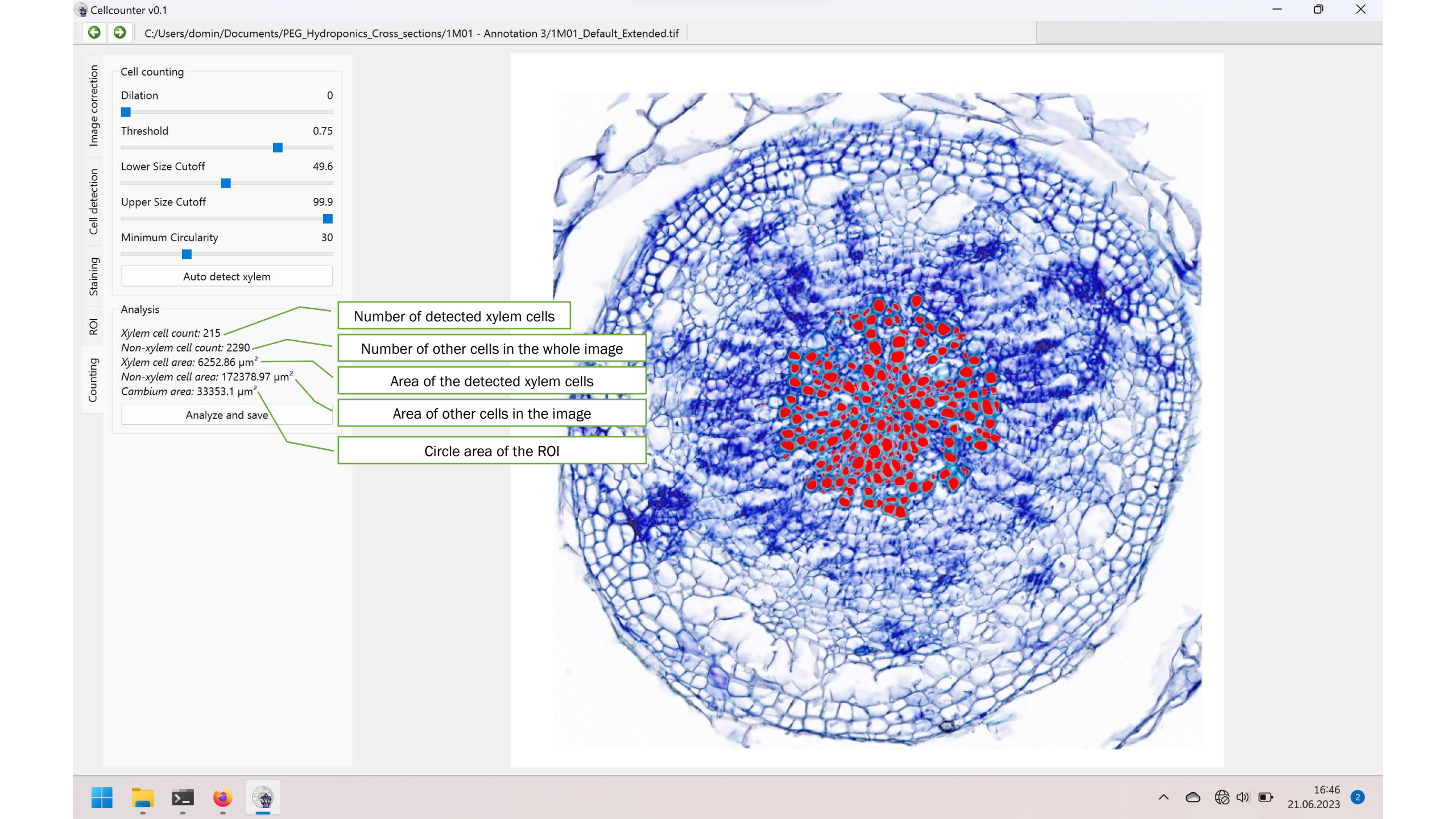
Start



16:45
21.06.2023

Step 5: Detect ROI

- Currently the automatic detection is wrong for the most images, so until I fixed it you need to do it manually
- Press „Start“
  - The dialog tells you to select the center of the hypocotyl
  - First press OK, then select the center on the image
  - A second dialog tells you to select any point on the cambium
  - Same as above, first OK, then select
  - A circle should appear
  - Repeat until you like the result


Cellcounter v0.1
C:/Users/domin/Documents/PEG_Hydroponics_Cross_sections/1M01 - Annotation 3/1M01_Default_Extended.tif
Image correction
Cell detection
Staining
ROI
Counting
Cell counting
Dilation 0
Threshold 0.75
Lower Size Cutoff 49.6
Upper Size Cutoff 99.9
Minimum Circularity 30
Auto detect xylem
Analysis
Xylem cell count: 215
Non-xylem cell count: 2290
Xylem cell area: 6252.86 µm²
Non-xylem cell area: 172378.97 µm²
Cambium area: 33353.1 µm²
Analyze and save
Number of detected xylem cells
Number of other cells in the whole image
Area of the detected xylem cells
Area of other cells in the image
Circle area of the ROI
16:46
21.06.2023

Step 6: Count cells

- The algorithm works by checking which percentage of the cells outline was colored red in step 4
- *Dilation:* How far the cells should get inflated when checking their border. If cells are not detected even though the wall is red in step 4 it might help to inflate all cells by a pixel
- *Threshold:* Minimum red percentage of the cell outline to be considered xylem vessel
- *Size cutoff:* Percentile range to again filter too small and to big cells. If some smaller cells are not detected, decrease the lower cutoff
- *Minimum circularity:* 100 is perfect circle
- You can select and deselect cells via mouse. Keep in mind that you selection is resetted when running the auto detection
- When your done with the manual selection press „Analyze and save“, because the cell count is not automatically updated!