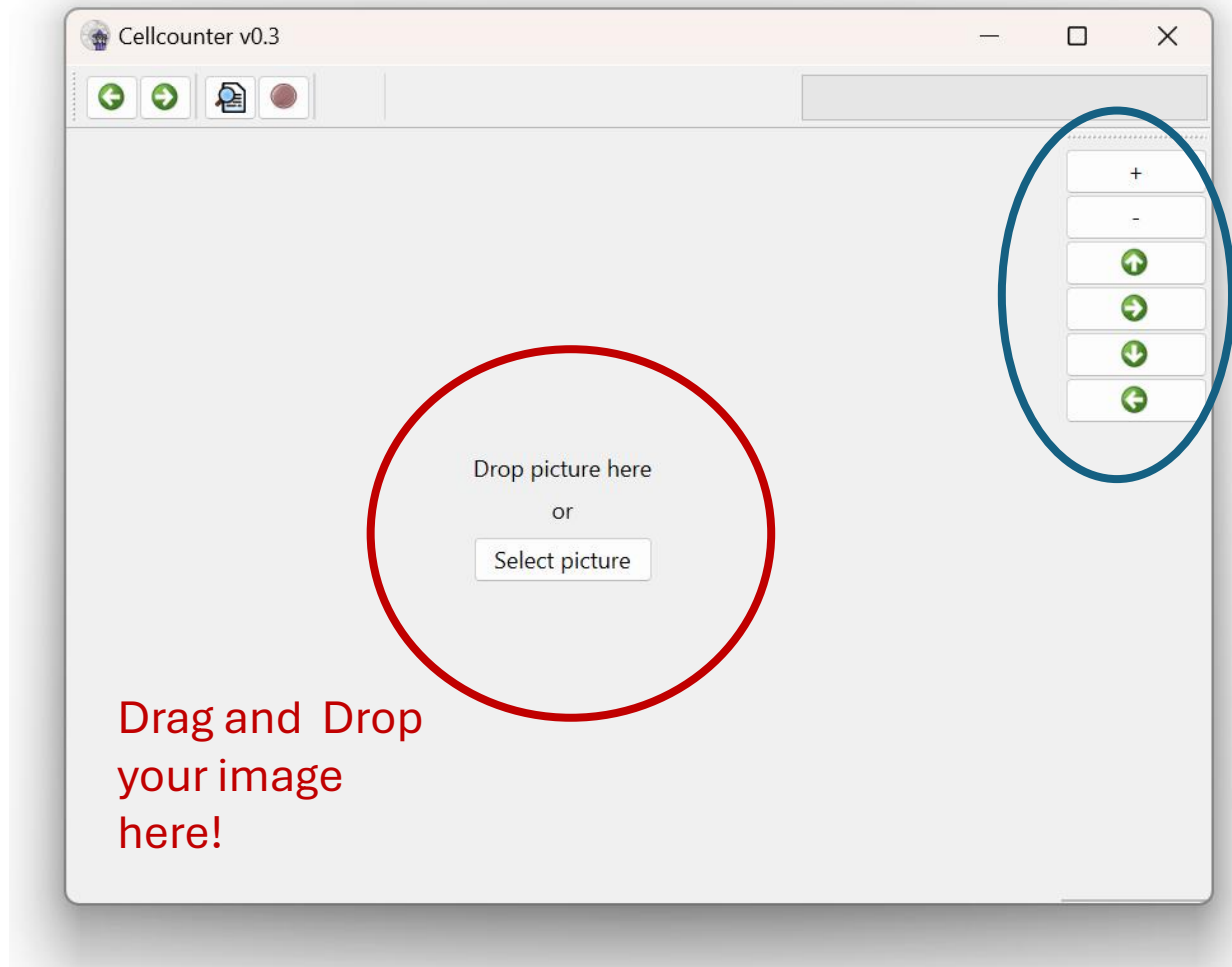


# VesselWizard – A step by step user Manual

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# 1. Drop picture into vessel wizzard



Zoom and  
Navigator  
Function:

Can be used to  
zoom in and  
navigate across  
your section

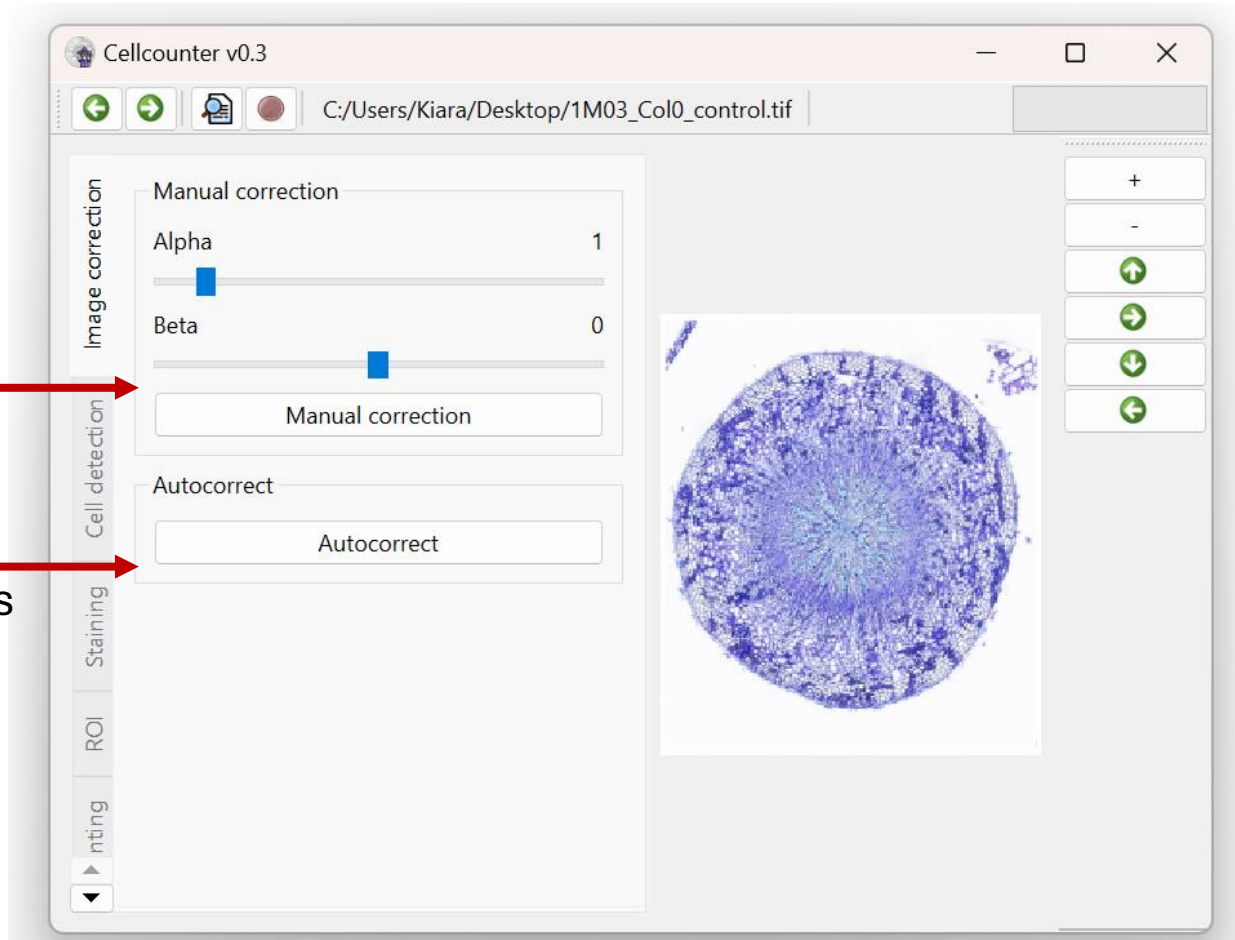
## 2. Adjust Contrast of the section

Manual correction:

- Blue shades can be adjusted manually by your self

Autocorrect:

- Adjust blue shades automatically to a value the program can work with



We suggest to use Autocorrect to adjust contrast !

After Autocorrection, the blue scale will be uniform and darker

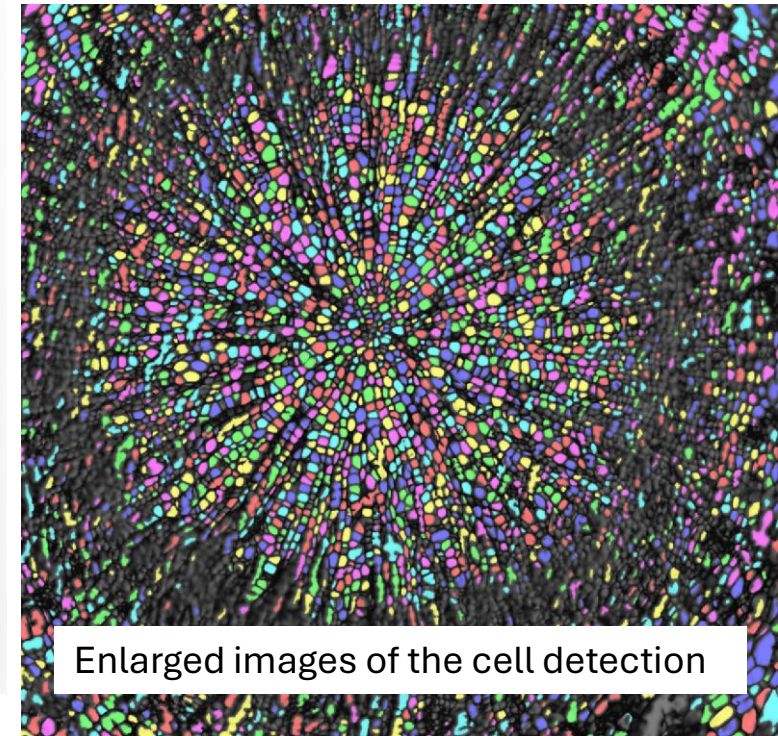
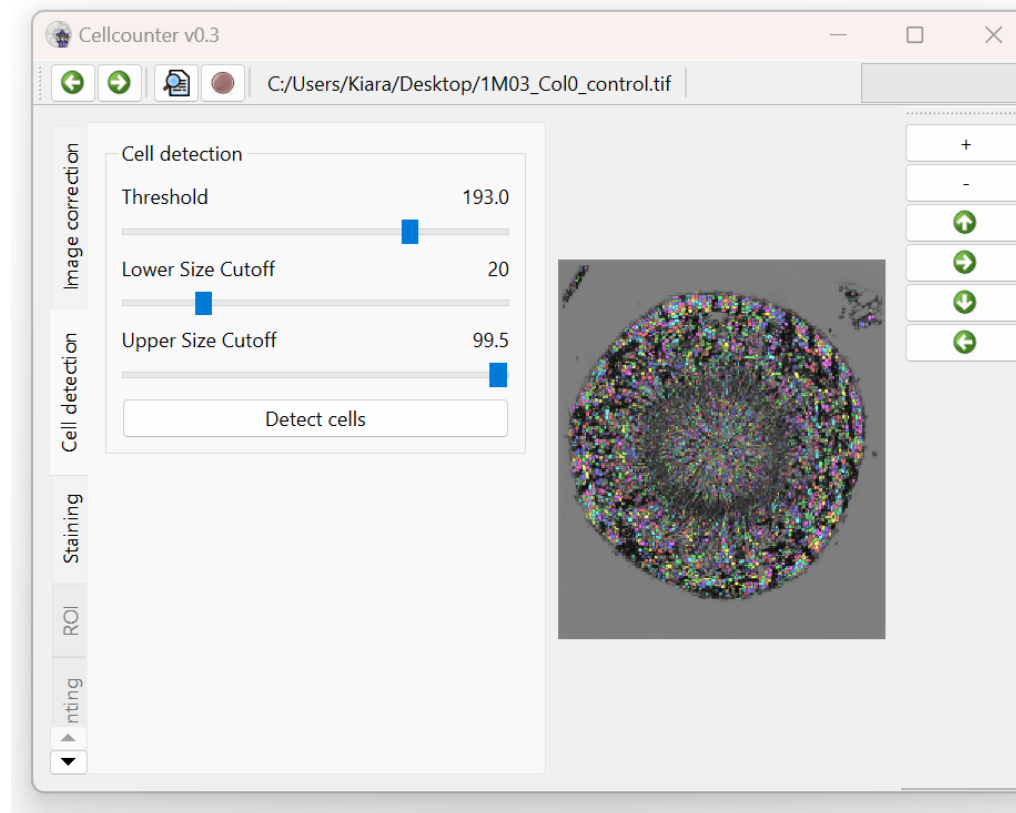
### 3. Detection of all cells in the section

Threshold:



Defines how many cells can be detected

- Lower Threshold – more cells are detected
- Higher threshold – less cells are detected

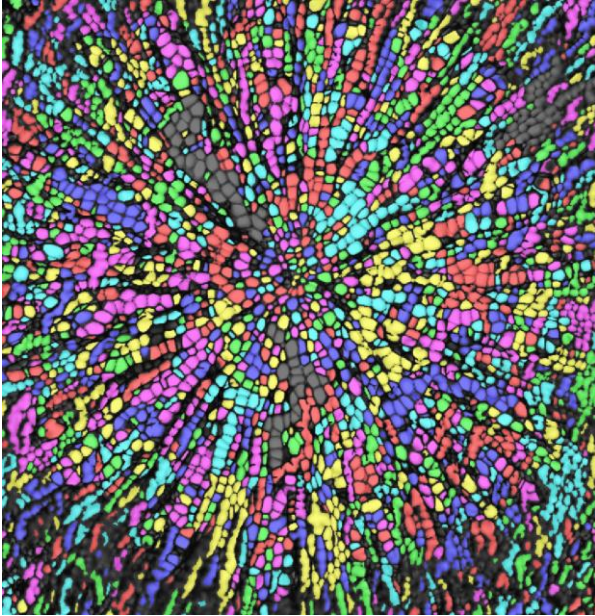


Threshold values need to be tested  
for each section  
Take care that cells are detected  
individually



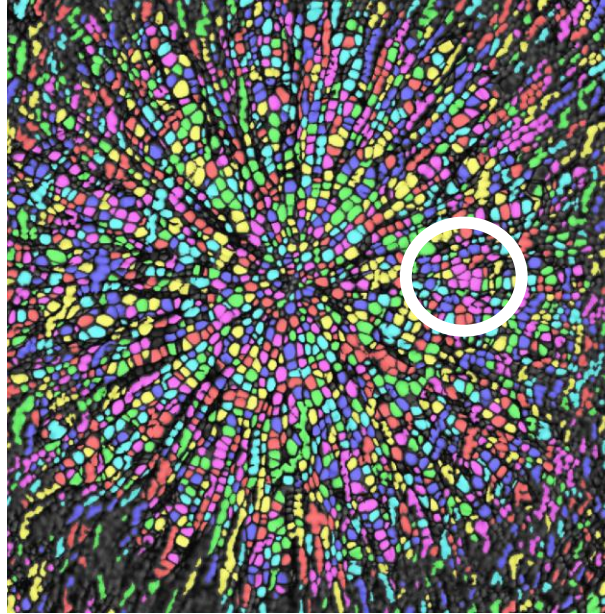
# 3. Example of good and bad image selection

Threshold 138



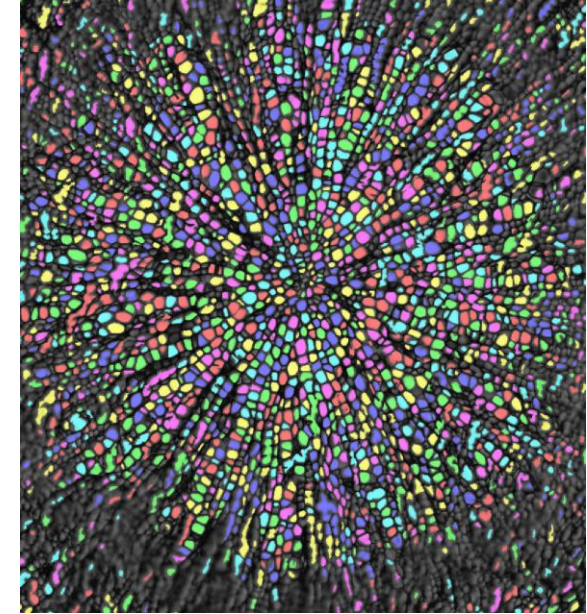
- Threshold too low
- Many cells not detected
- Many cells detected as one (cells next to each other in the same color?)

Threshold 163



- Threshold still too low
- All cells are detected
- BUT Many cells detected as one (e.g. white circle – pink cells)

Threshold 200

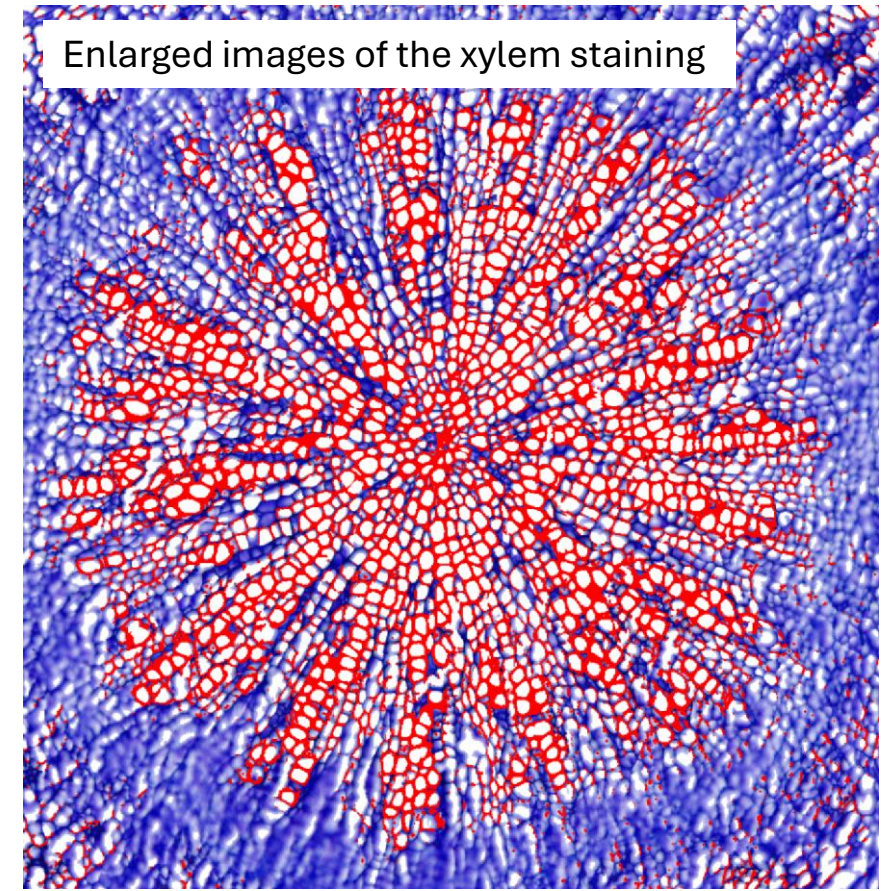
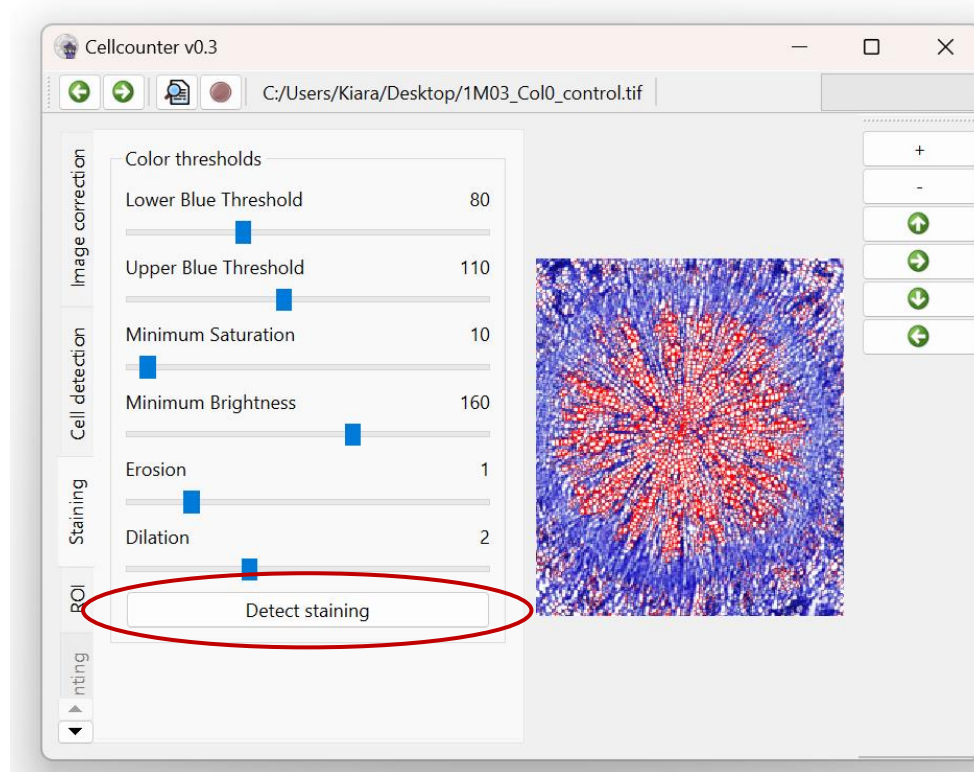


- Threshold is good
- All cells are detected
- cells are not detected detected as one

Threshold suggestion between 165 -200  
Lighter Sections need lower threshold

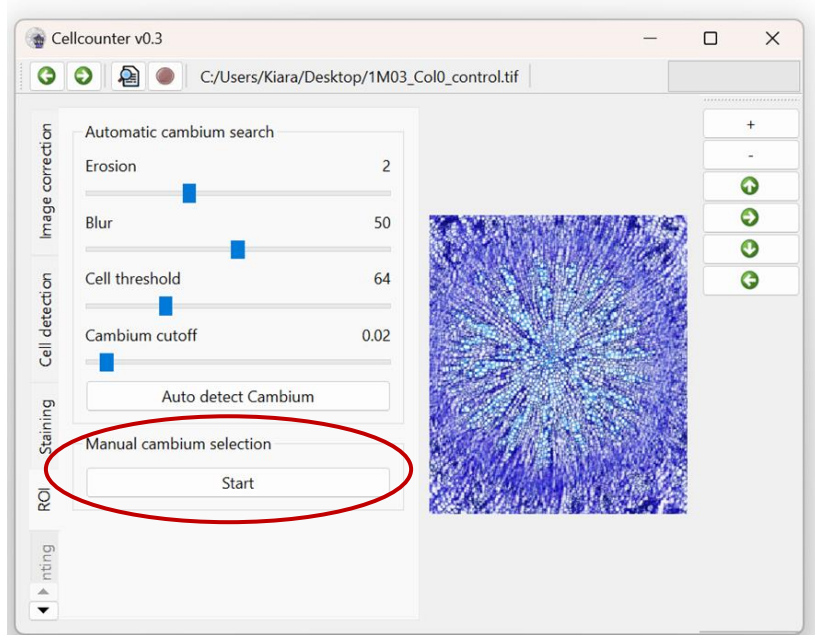


## 4. Detection of xylem staining



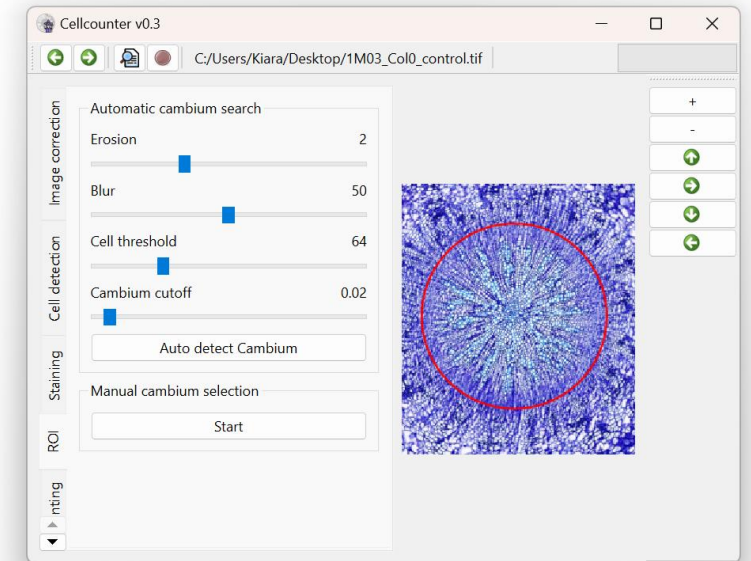
Detect Xylem Vessels –  
All Cells detected as Vessels are  
circled in red in this step

# 5. Selection of Region of interest (ROI) to count xylem vessels



Select your ROI by manual cambium selection

1. A small dialog box titled 'Step 1' with the text 'Please click on the center of the hypocotyl.' and an 'OK' button.
2. A small dialog box titled 'Step 2' with the text 'Please click on a point on the cambium.' and an 'OK' button.

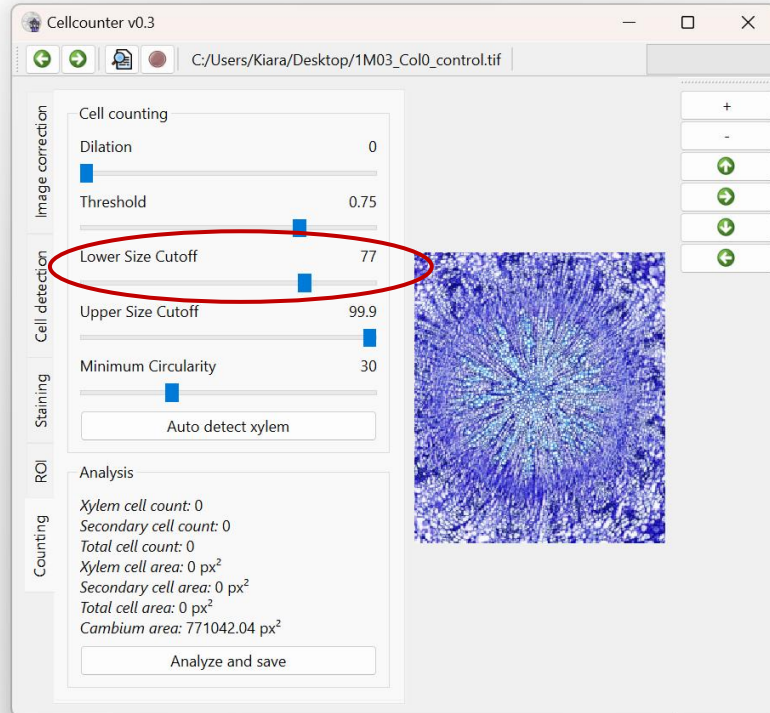


ROI has been selected (Red circle)  
In there, xylem vessels will be counted  
and missing cells can be selected in the  
next step

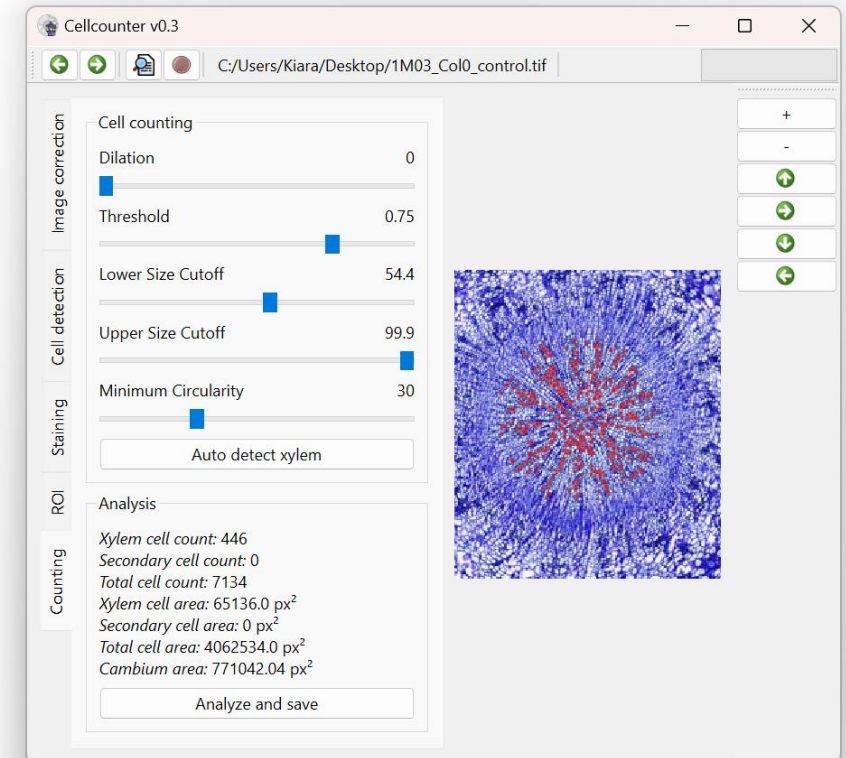
# 6. Automatic Xylem Counting

Lower Size  
Cutoff

The right lower  
Size Cut off  
needs to be  
selected that all  
cells can be  
detected



Counting  
finished

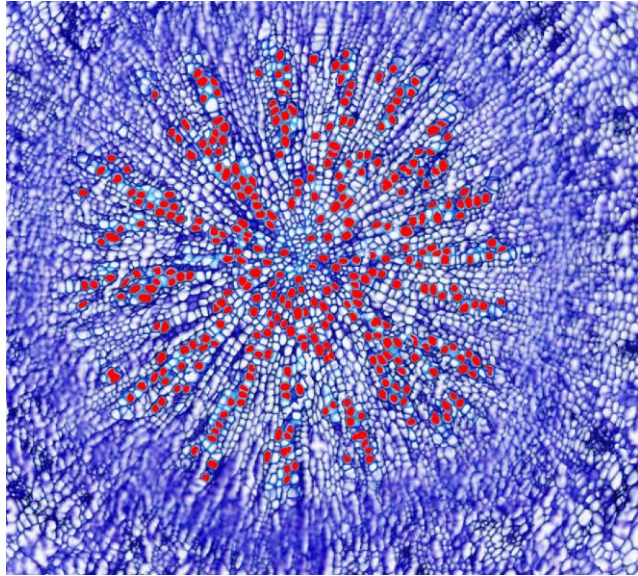


Automatically counting finished –  
manual correction if vessels were  
not detected is required



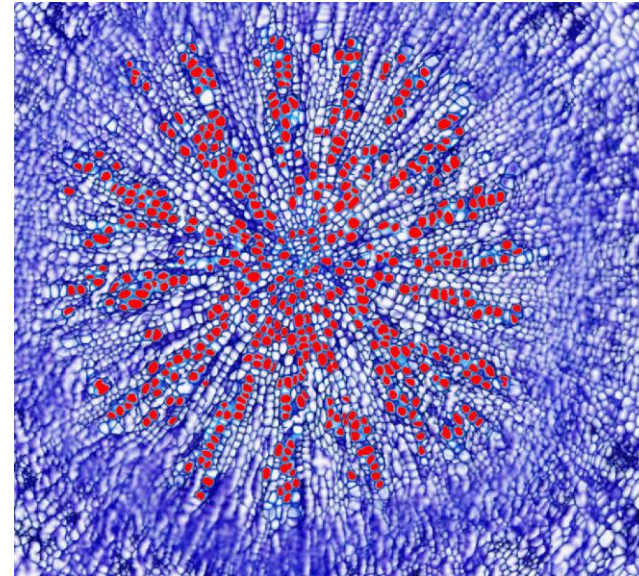
# 6.1 Xylem counting – Good Selection of Lower Size Cutoff

## Lower Size Cutoff 65



- Lower Size Cutoff too low
- Not all vessels are detected

## Lower Size Cutoff 51



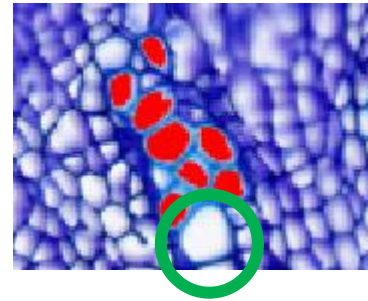
- Lower Size Cutoff good
- Most vessels are detected

We suggest using a lower size Cut off between 50 (Section with small Vessels) and 70 (Section with big vessels)

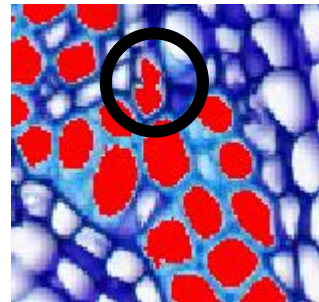
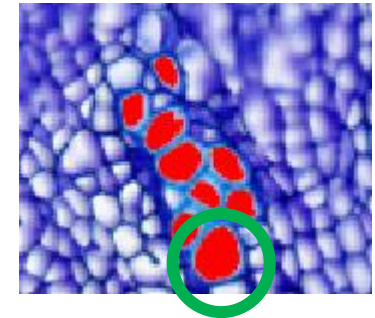
## 6.2 Manual selection of xylem vessels which were not detected automatically

### Manual Correction – What can be corrected manually?

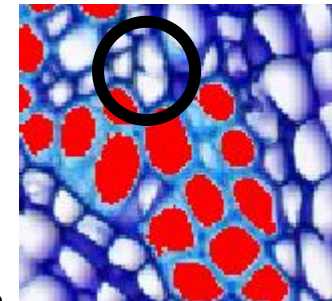
- Vessels which have not been selected – click on it to select
- Wrongly selected vessels – click on it to deselect



Click on the vessel to select it



Click on wrongly selected cell to deselect it  
*It's not a vessel, it is an parenchyma cell so deselect it*

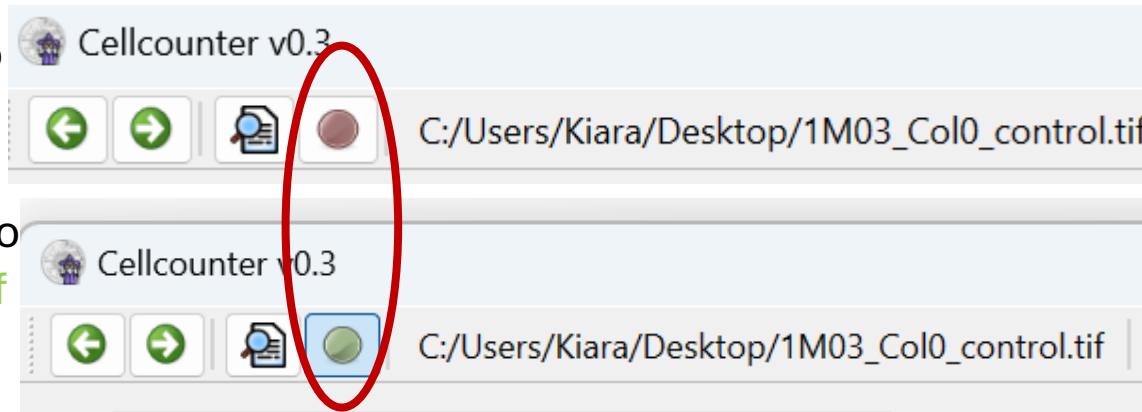





## 6.3 Selection of different vessel types

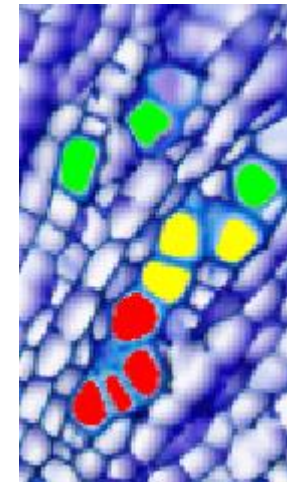
### Color Selection

Standard Selection to select **Red Vessels**

Advanced Selection to select **another type of vessels** (e.g stress related vessels)



-  Standard color for usual vessels
-  Color for special vessels
-  Color for double positive vessels  
(Can be used when the vessel is marked a red, Color selection is changed to green and the vessel is selected again)





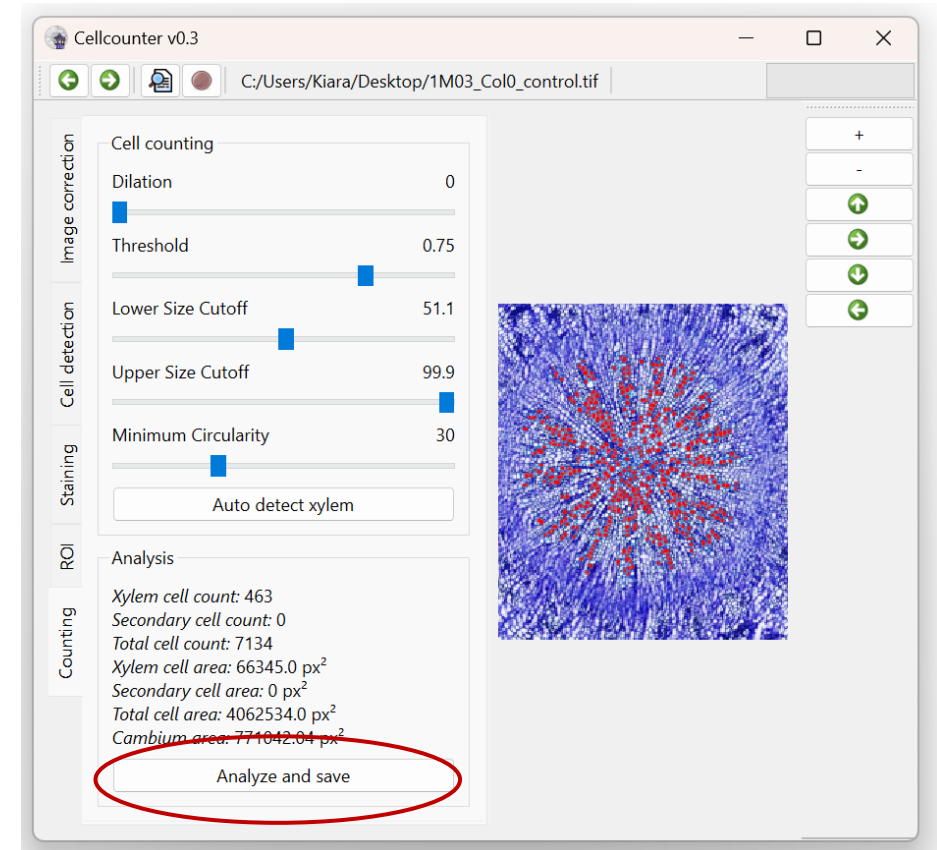
## 6. Xylem counting – Save your count

All Xylem vessel counted?

All missed vessels manually selected?

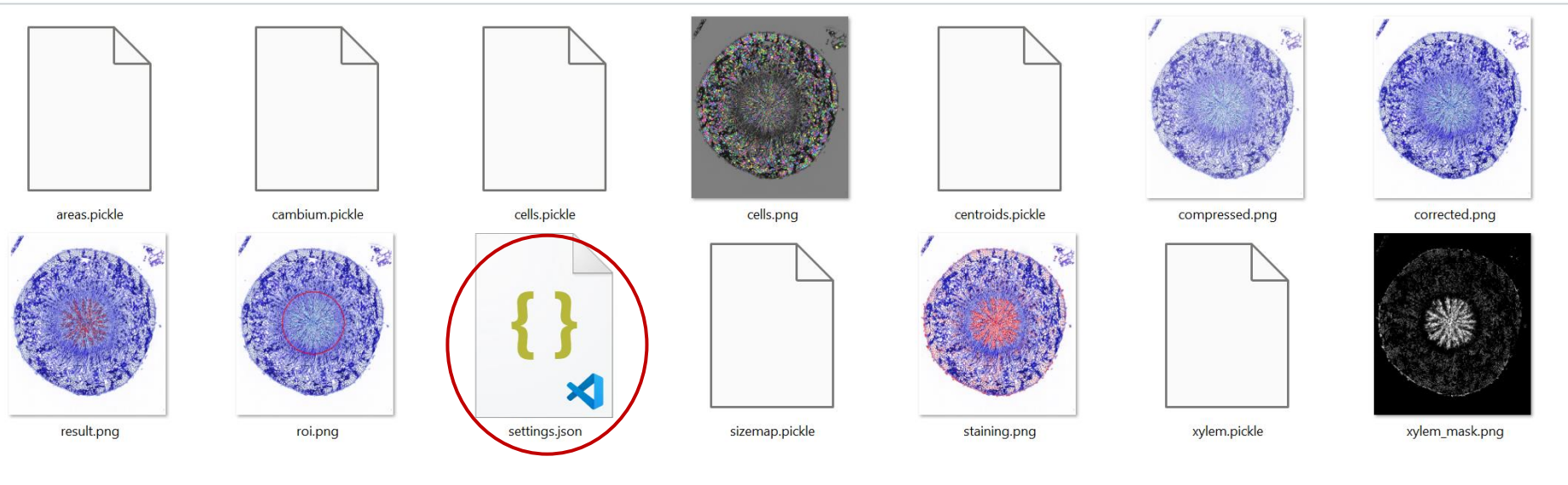
All wrongly selected cells deselected?

**Analyze and  
save your  
count!**



# 7. Metadata Folder – Documentation of all steps

All steps are saved in the metadata folder:



Settings.json file:

## 8. Read out– settings.json file for Evaluation

- All information read by the tool is saved into the settings.json file
- It contains metadata, progress in the tool and most importantly the cell counts
- The settings.json files can be read out using a batch script to produce a list of counts to use for statistics, analysis and plotting
- The batch script can be found in the evaluation folder in the Github repository
- [https://github.com/thomasgreb/Zhao-et-al\\_SL-vessels/blob/main/VesselWizard/evaluation/analyze.py](https://github.com/thomasgreb/Zhao-et-al_SL-vessels/blob/main/VesselWizard/evaluation/analyze.py)



settings.json