## VesselWizard

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#### **System requirements**

- Tested and used on Windows 10 or 11 (using the provided installer)
- Other OS may work when running from source on your own risk
- No other requirements

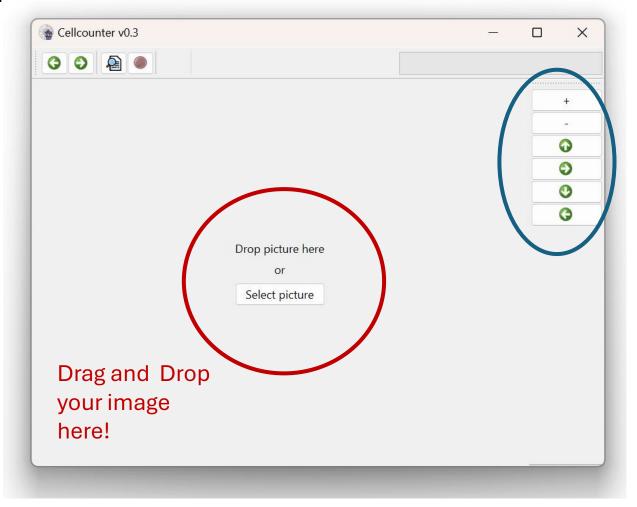
#### Installation guide

- Follow <u>this link</u> to download the installer
- Run the installer and follow the steps in the install wizard
- After the installation, VesselWizard should appear in your Start menu or on the desktop
- The installation takes about 2 minutes

For a Demo and instructions on how to use the program follow the next pages of this document. Analyzing one image takes about 2 minutes.

You can download demo images to test the program from this link.

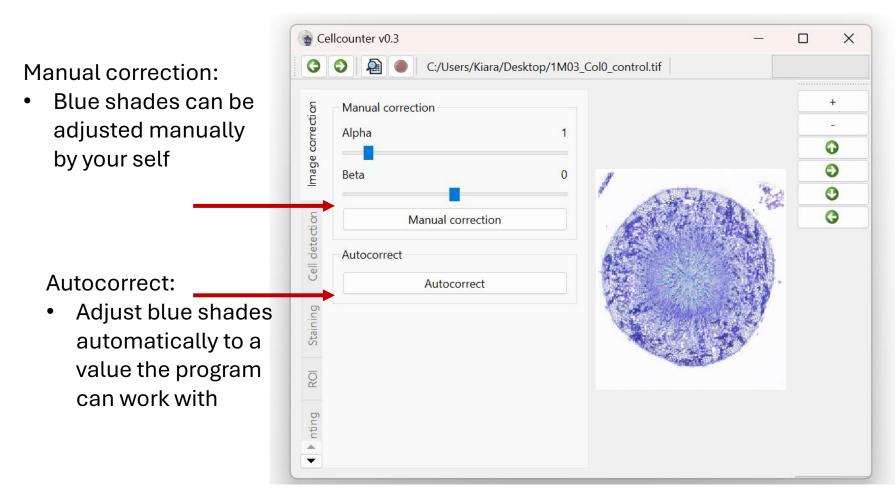
### 1. Drop picture into vessel wizzard



Zoom and Navigator Function:

Can be used to zoom in and navigate across your section

### 2. Adjust Contrast of the section



We suggest to use
Autocorrect to adjust
contrast

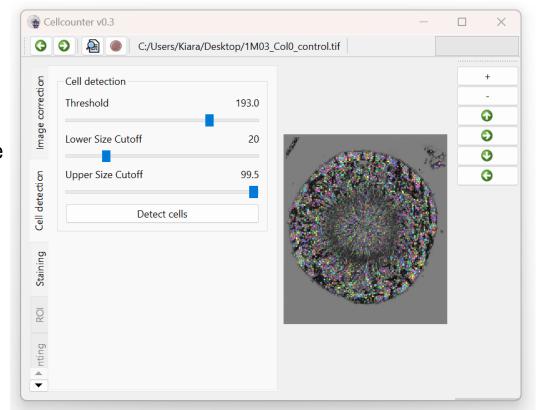
After Autocorrection, the blue scale will be uniform and darker

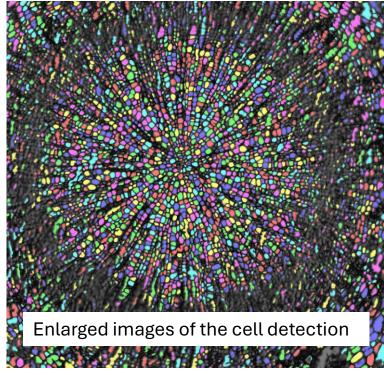
### 3. Detection of all cells in the section

Treshold:

Defines how many cells can be detected

- Lower Treshold more cells are detected
- Higher treshold less cells are detected

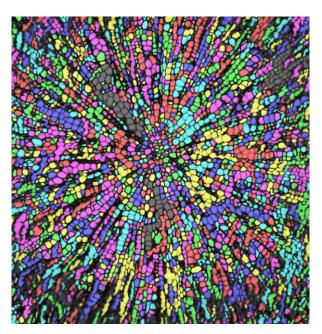




Treshold values need to be tested for each section
Take care that cells are detected individually

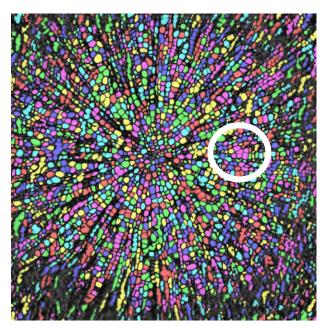
## 3. Example of good and bad image selection

### **Treshold 138**



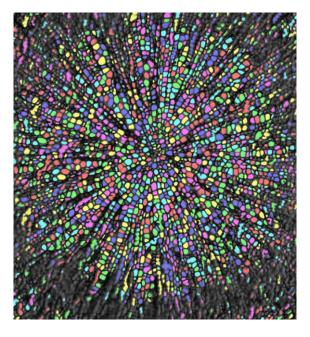
- Treshold to low
- Many cells not detected
- Many cells detected as one (cells next to each other in the same color?

### Treshold 163



- Treshold still to low
- All cells are detected
- BUT Many cells detected as one (e.g white circule – pink cells)

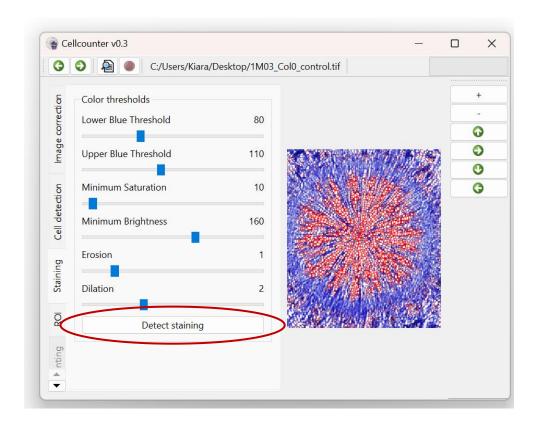
### Treshold 200



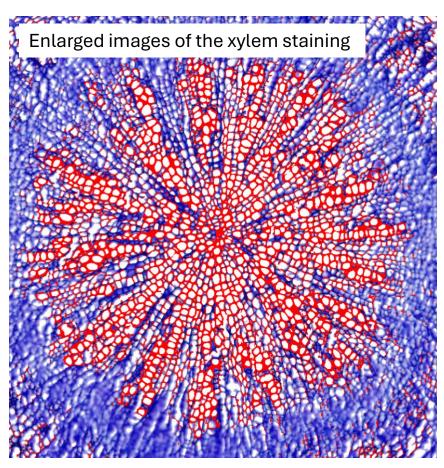
- Treshold is good
- All cells are detected
- cells are not detected detected as one

Treshold suggestion between 165 -200 Lighter Sections need lower treshold

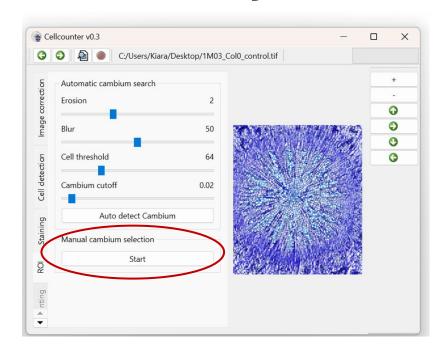
## 4. Detection of xylem staining



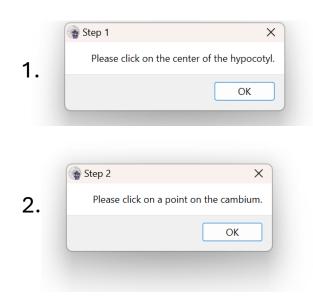
Detect Xylem Vessels – All Cells detected as Vessels are circled in red in this step

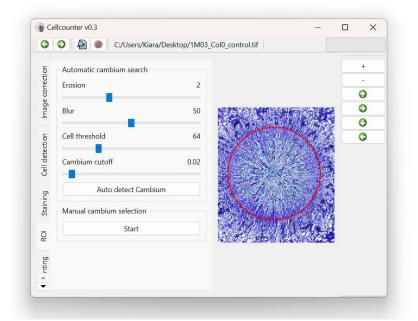


# 5. Selection of Region of Interest (ROI) to count xylem vessels



Select your ROI by manual cambium selection



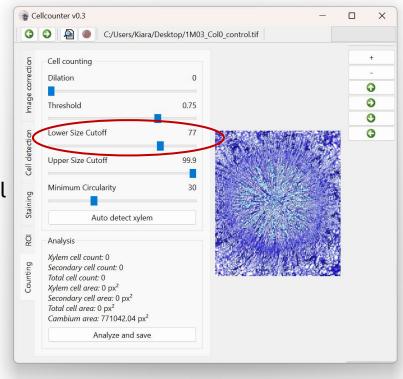


ROI has been selected (Red circle)
In there, xylem vessels will be counted
and missing cells can be selected in the
next step

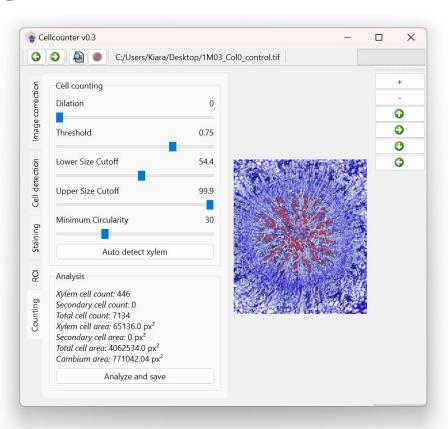
## 6. Automatic Xylem Counting

Lower Size Cutoff

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



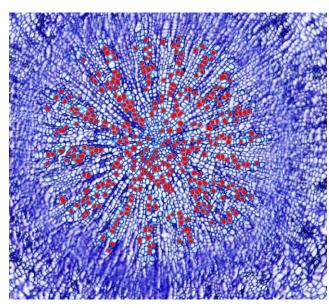
Counting finished



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

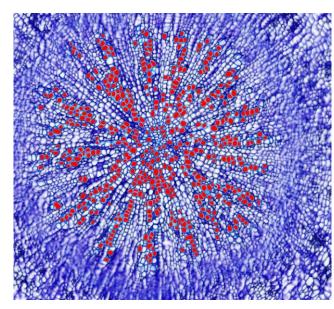
## 6.1 Xylem counting – Good Selection of Lower Size Cutoff

### **Lower Size Cutoff 65**



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

### **Lower Size Cutoff 51**



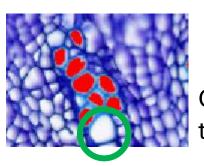
- Lower Size Cutoff good
- Most vessels are detected

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

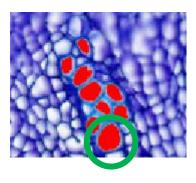
# 6.2 Manual selection of xylem vessels which were not detected automatically

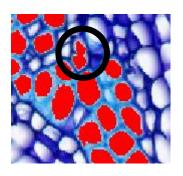
## Manual Correction – What can be corrected manually?

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

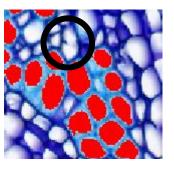


Click on the vessel to select it





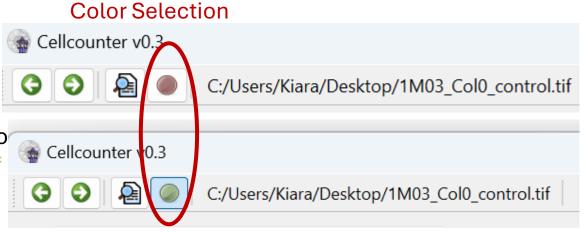
Click on wrongly selected cell to deselect it It's not a vessel, it is an parencyma cell so deselect it



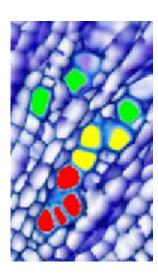
### 6.3 Selection of different vessel types

Standard Selection to select Red Vessels

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



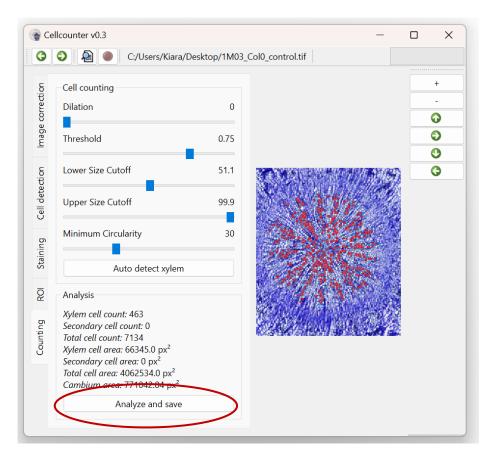
- Standard color for usual vessels
- Color for special vessels
- Color for double positive vessels (Can be used when the vessel is marked a red, Color selection is changed to green and the vessel is selected again)



## 6. Xylem counting – Save your count

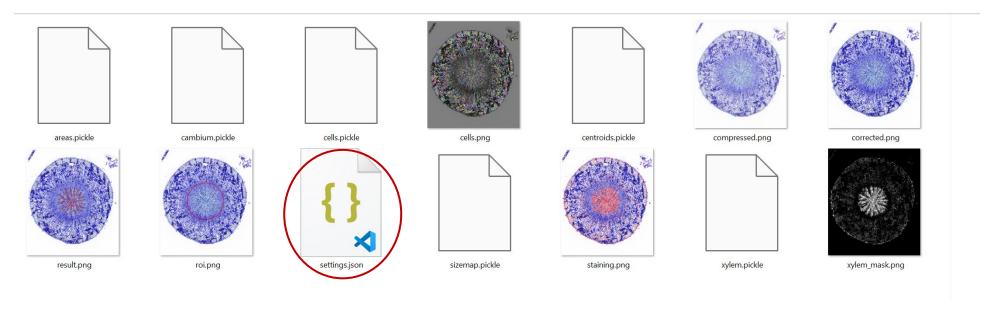
All Xylem vessel counted?
All missed vessels manually selected?
All wrongly selected cells deselected?

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\_SLvessels/blob/main/VesselWizard/evaluation/analyze.py

