

# Do the plant pollen presentation schedules fit pollinator diurnal activity?

## BACKSTORY

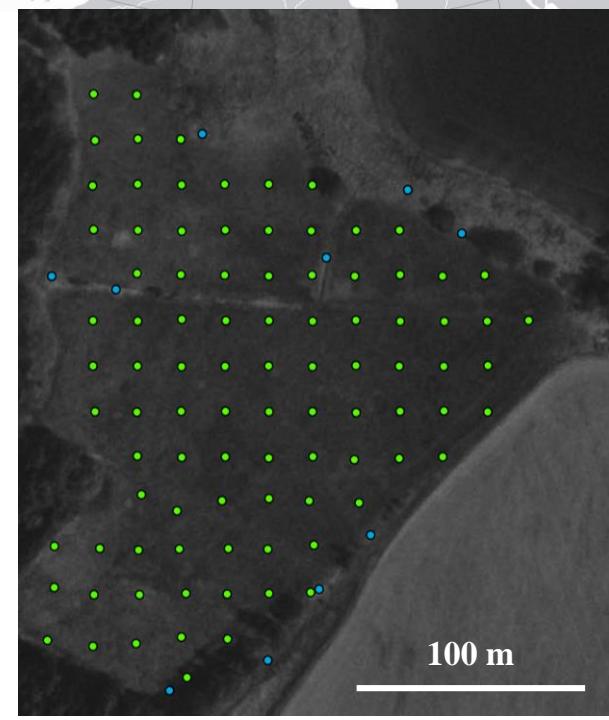
Jakub Štenc, Lukáš Jánošík, Eva Matoušková, Jirka Hadrava,  
Michael Mikát and Zdeněk Janovský

Dept. of Botany, Charles University in Prague, Czech Republic

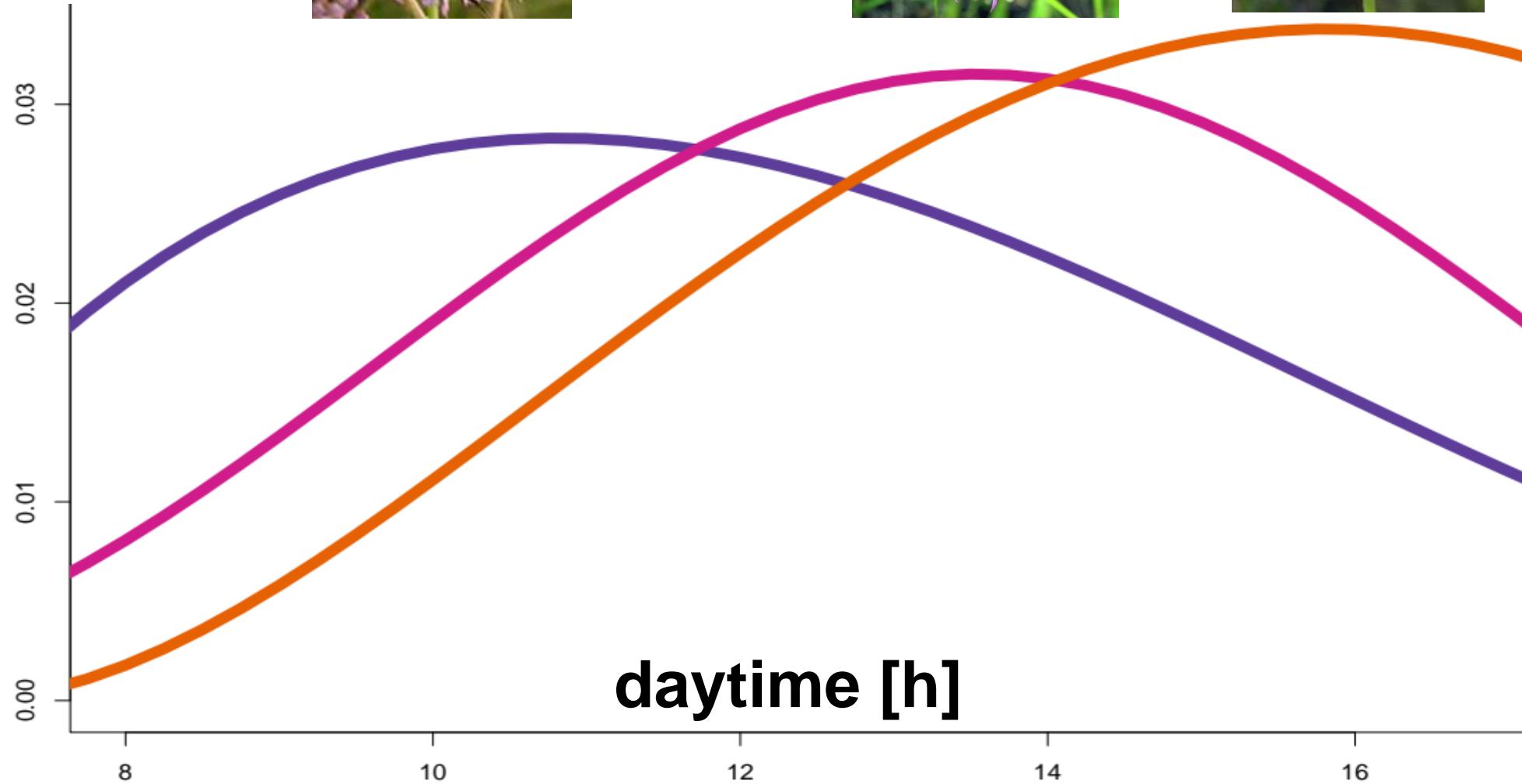


**long time ago in a meadow far far away...**

# Pollinator diurnal visitation pattern



**pollinator density**



*Succisa pratensis*  
*pratensis*



*Centaurea jacea*



*Trifolium hybridum*



# Questions

1) Do plants adjust pollen release to pollinator activity?

# Study species



*Centaurea jacea*



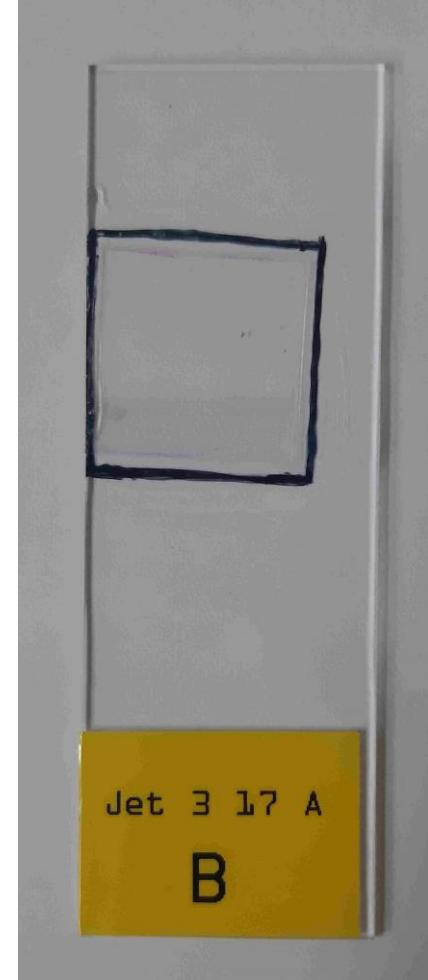
*Trifolium hybridum*

- Important forage plants at the site
- Partly sharing pollinators



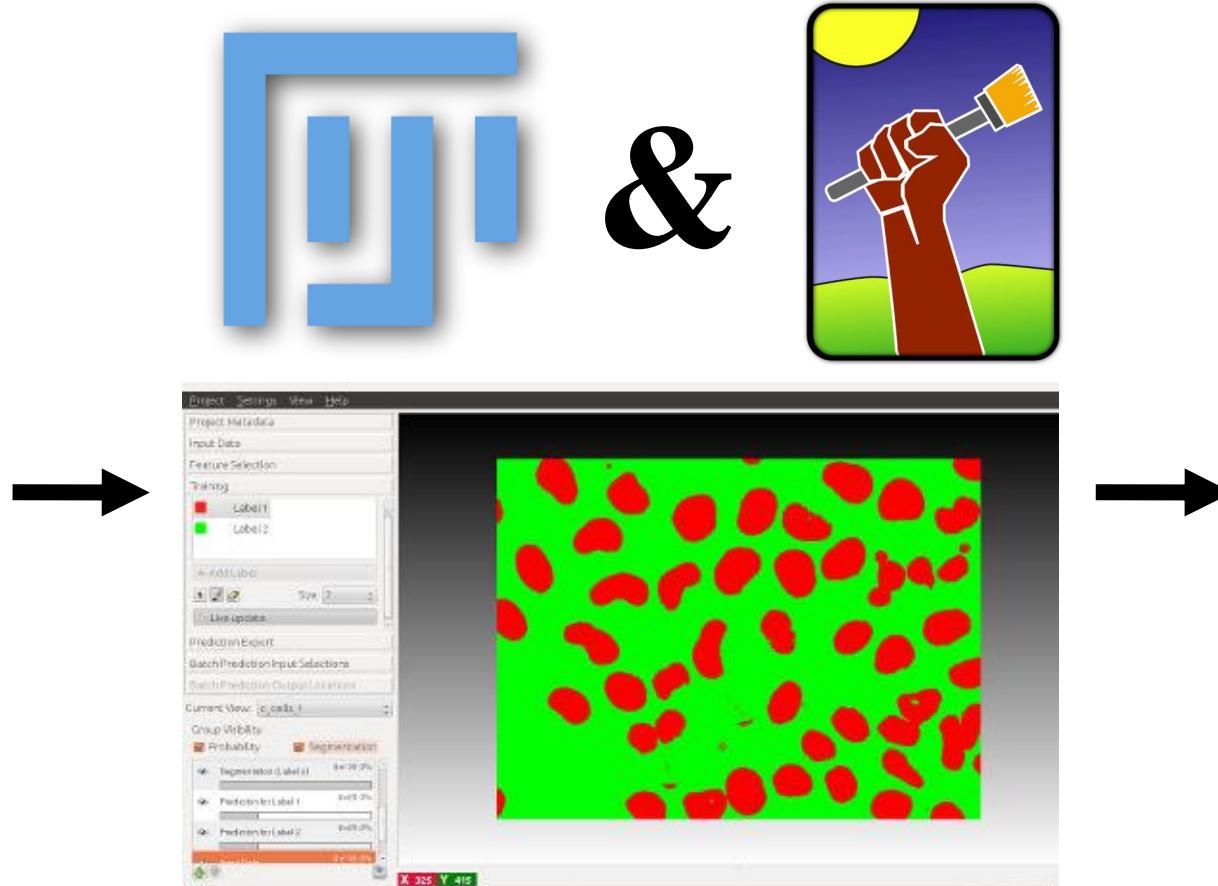
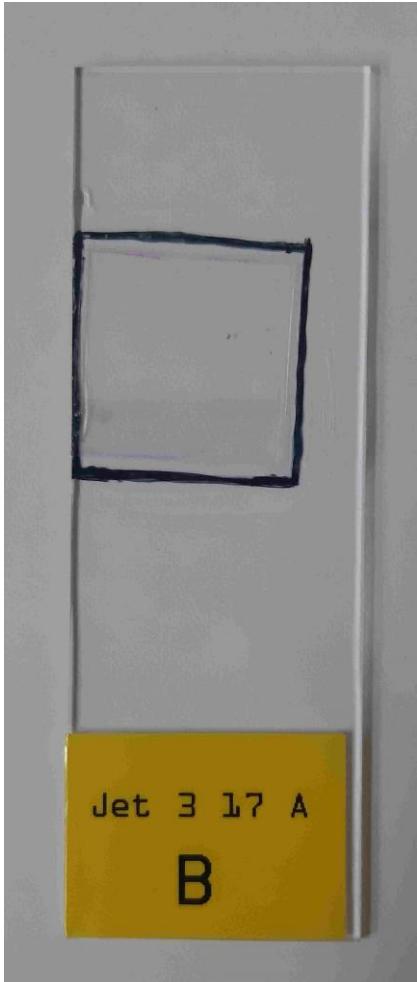
*Succisa pratensis*

# Pollen presentation schedule



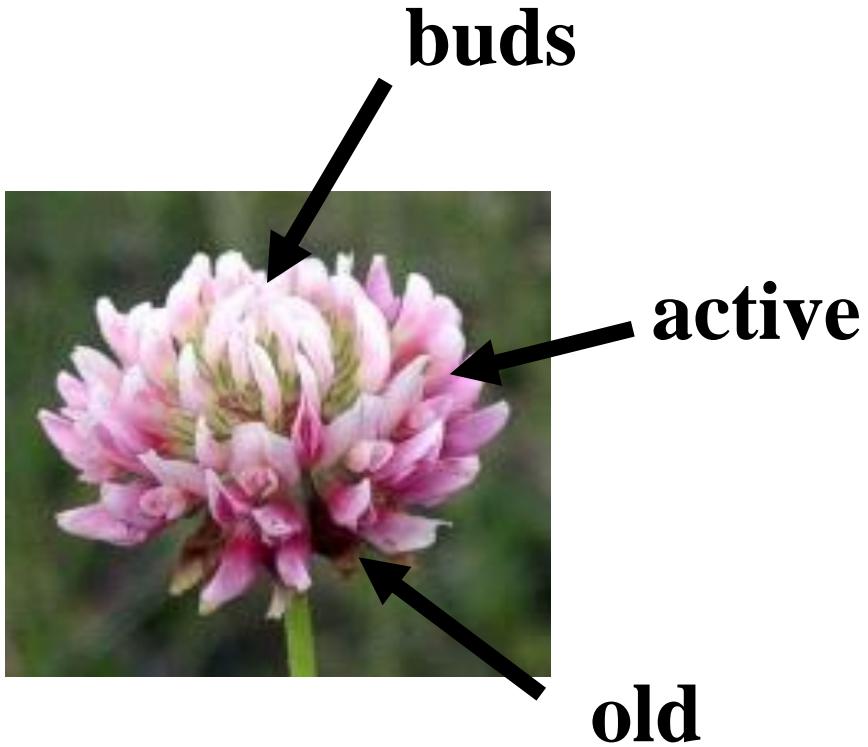
- 3 species, 2 days, 10 hours of sampling
- **1350** samples

# Pollen presentation schedule

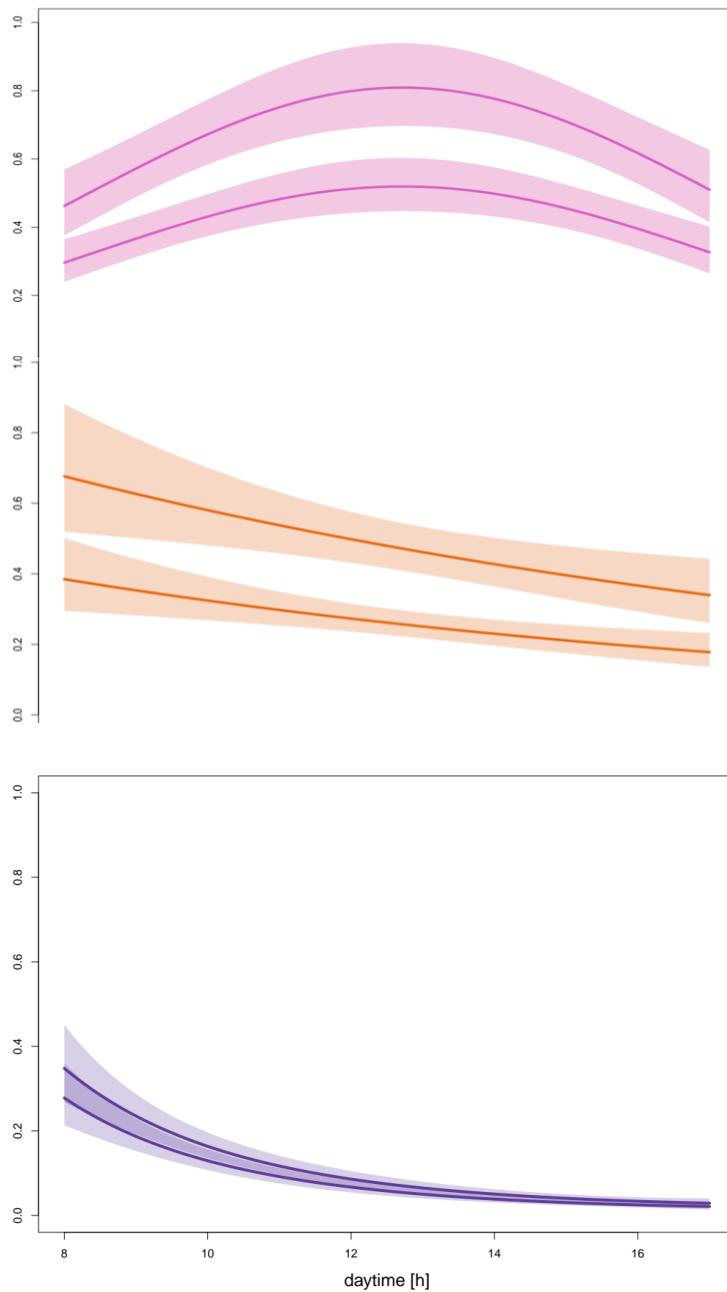


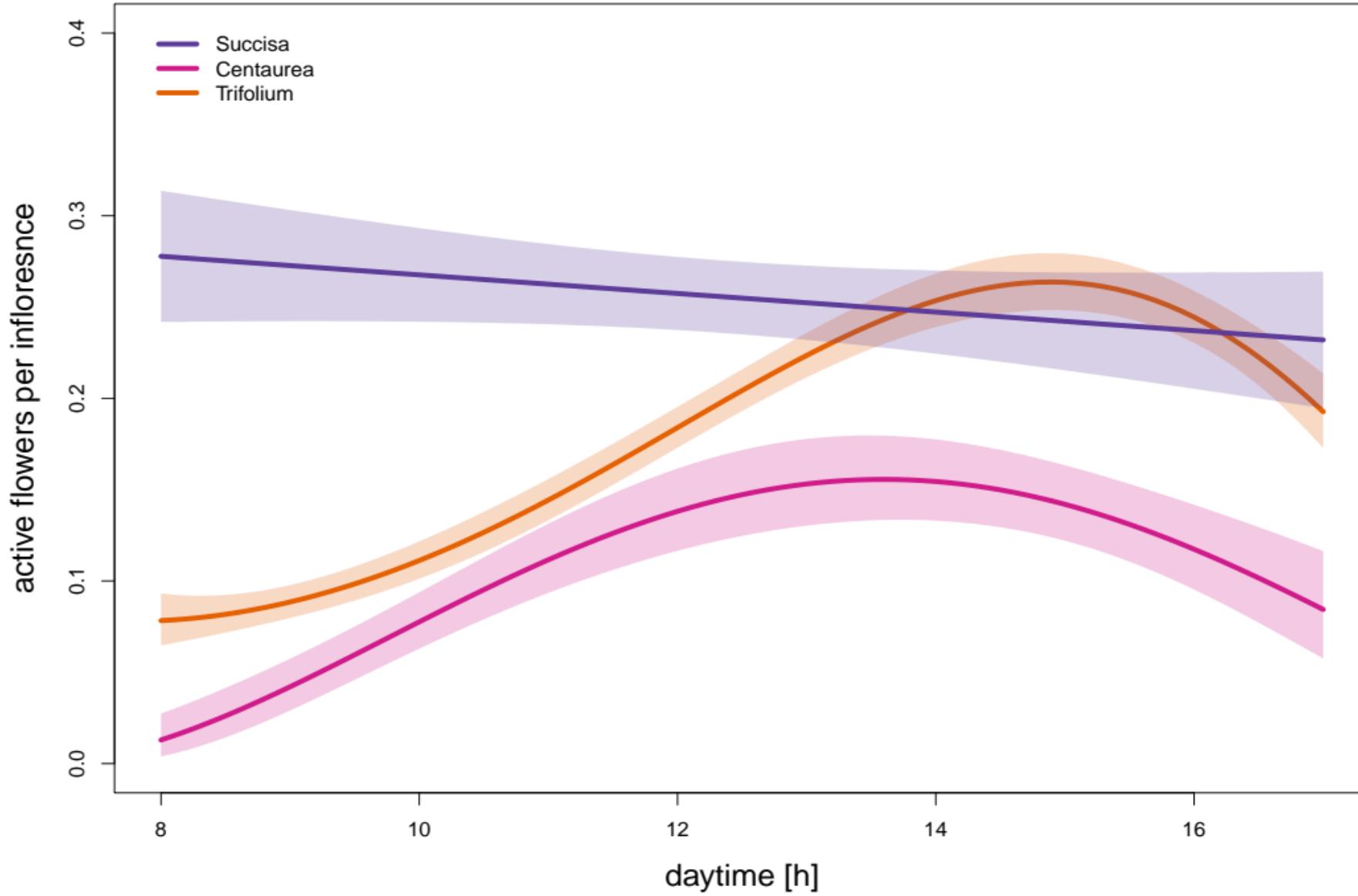
- pollen counted automatically by image analysis

# Flowering schedule

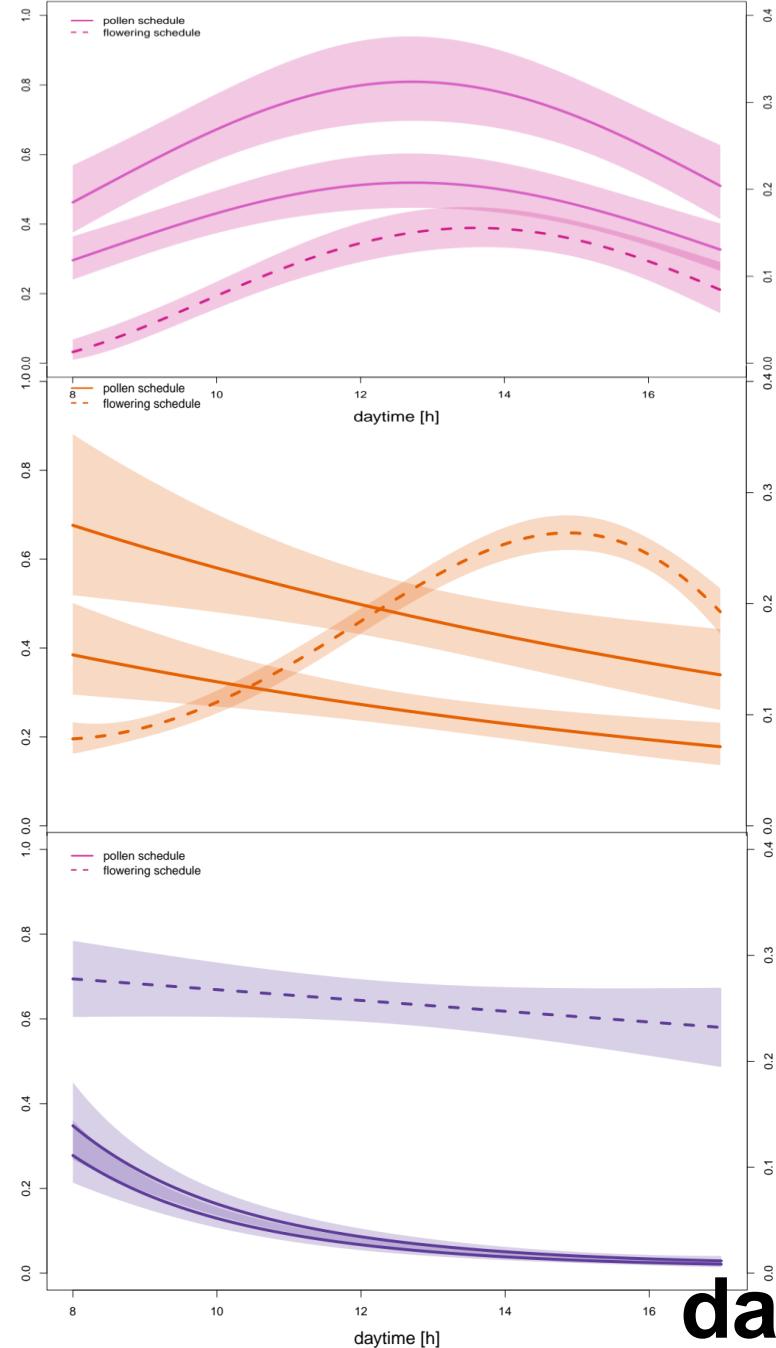


# Pollen presentation schedule





# Pollen available per flower



- *Centaurea* releases pollen gradually in open flower

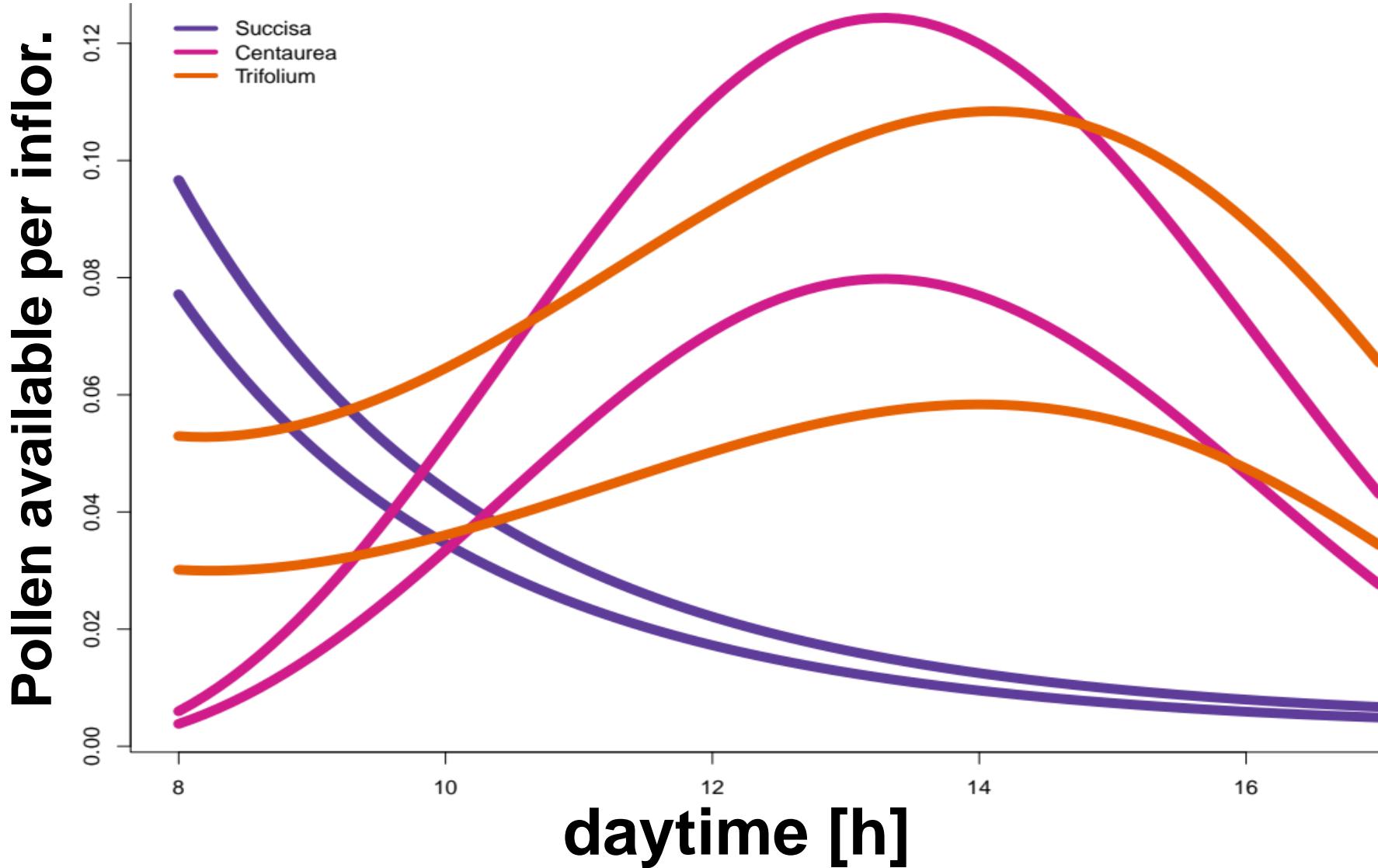


- *Trifolium* releases all pollen at once in closed flower

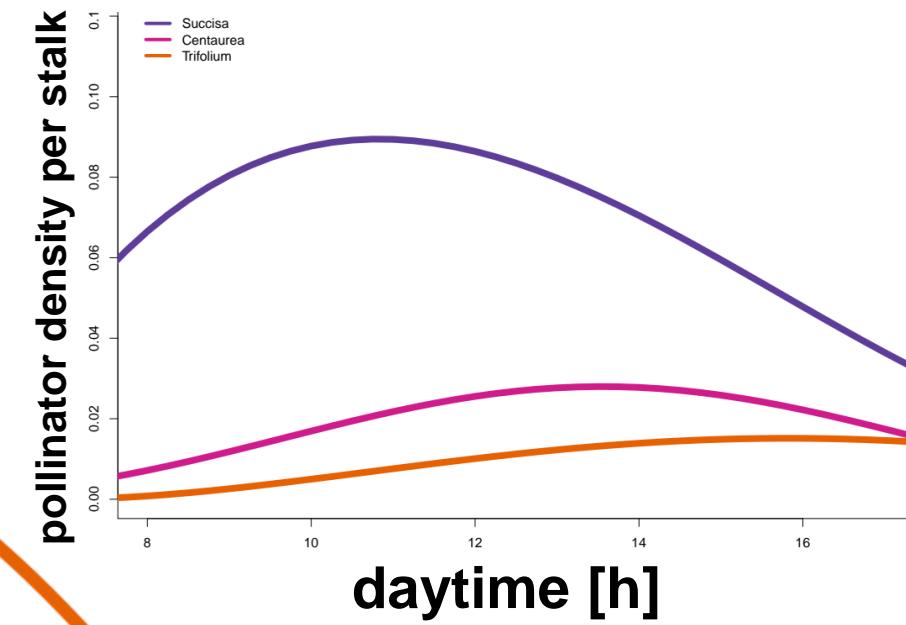
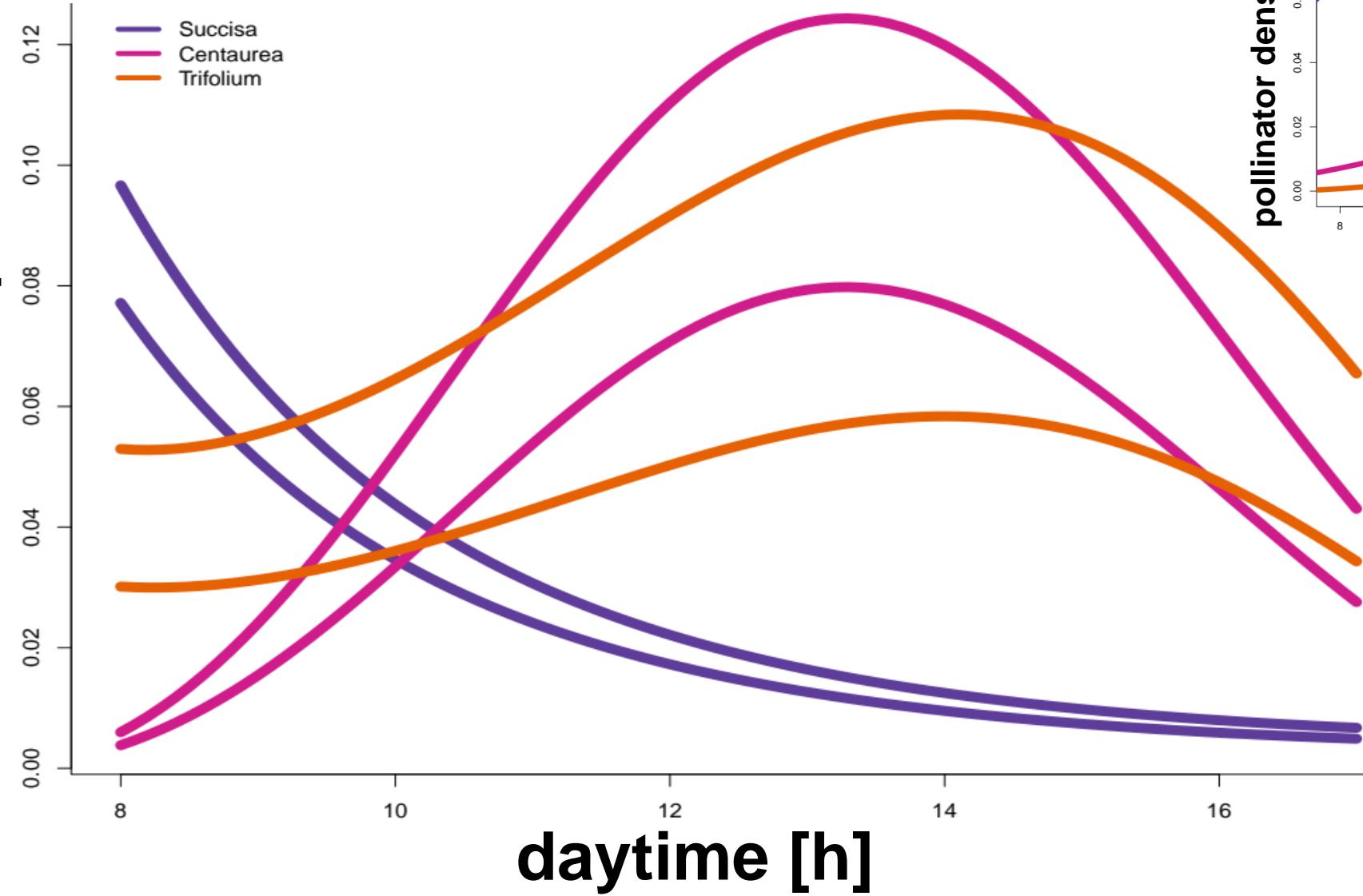


- *Succisa* releases openly all pollen at once

# Pollen presentation schedule



Pollen available per inflor.



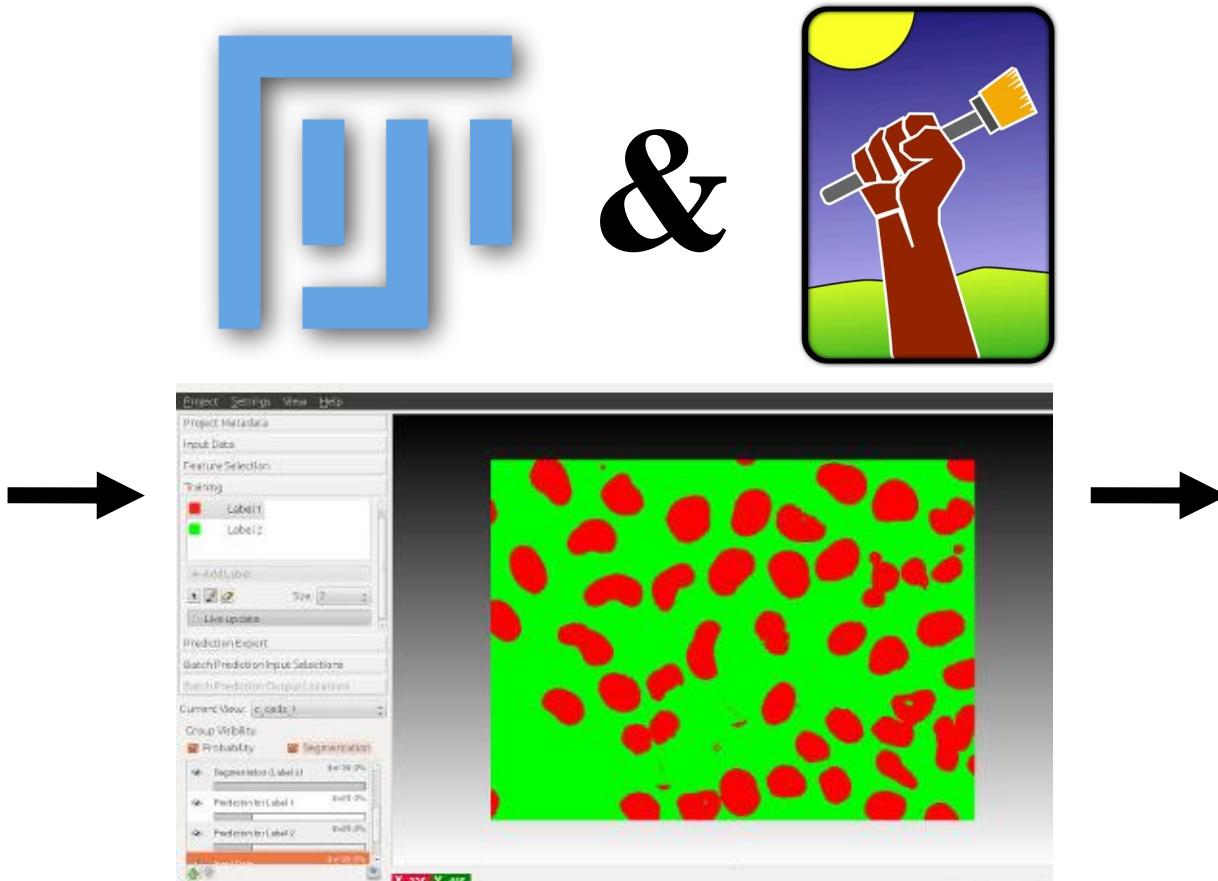
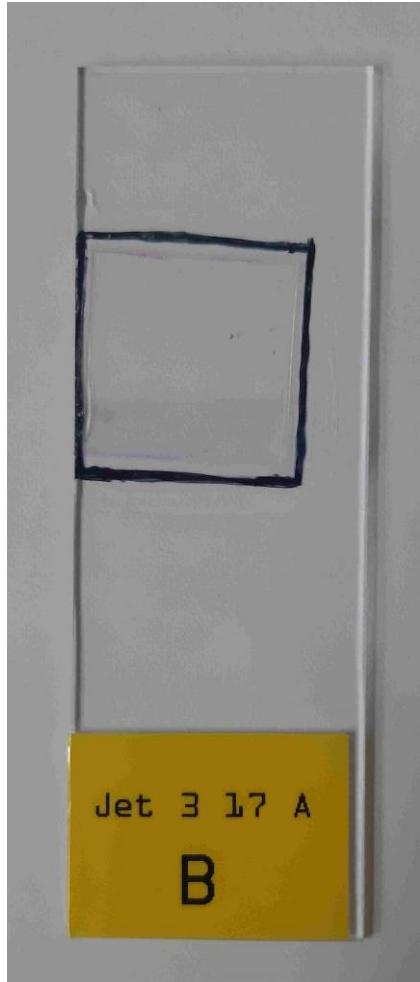
# Conclusions

- Peaks of available pollen roughly correspond with peaks in pollinator activity.
- It seems that hoverfly pollen loads track pollen availability on flowers with delay
- Can plants avoid competition for pollinators by differentiating their pollen presentation schedules?

# Conclusions

- Peaks of average pollen availability roughly correspond with peaks in pollinator activity.
- It seems that hoverfly pollen visitation lags behind pollen availability on flowers with delay
- Can plants avoid competition for pollinators by differentiating their pollen presentation schedules?

# How to count pollen?



Automatically by image analysis!

# How to count pollen?

1. Sampling
2. Scanning
3. Ilastic pixel and object classification
4. Counting and saving the results.
5. Label reading

# 1. Sampling



# 1. Sampling

- 5x5 flowers per sample in 2 ml solution
- => to obtain reasonable concentration
- Fuchsin stain (specifically staining pollen)



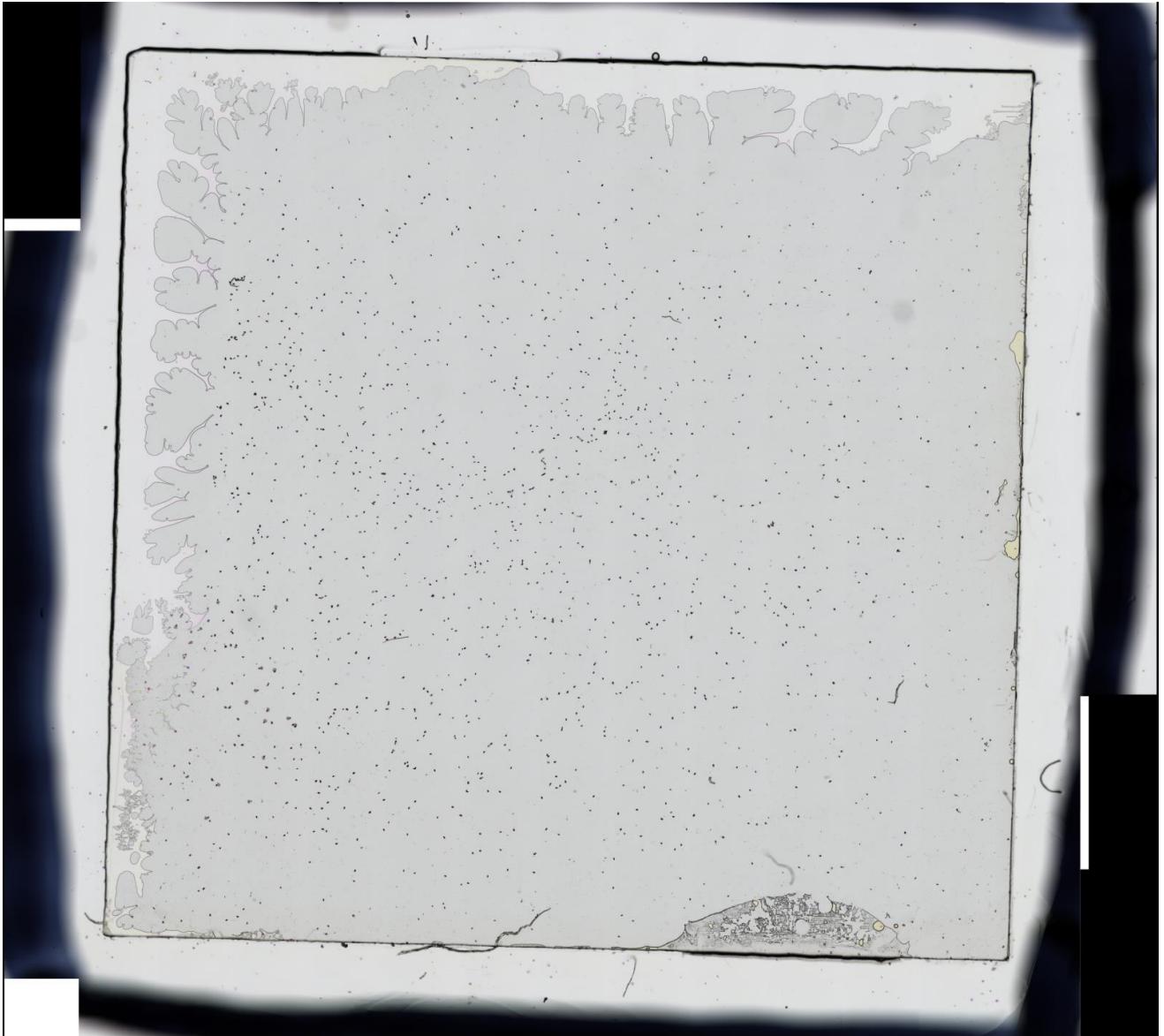
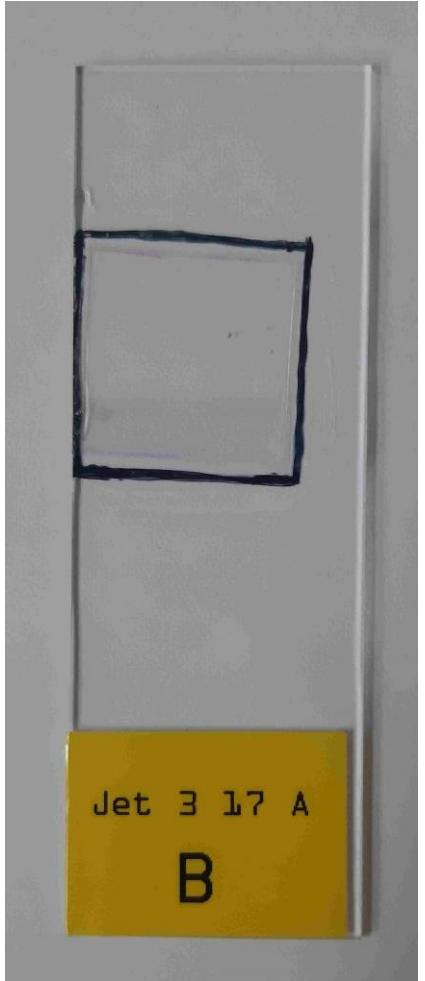
# 1. Sampling

- Sonication, vortexing and pipetted asap
- 5 µl per slide, 3 slides per vial
- => to see the procedure error





## 2. Scanning



## 2. Scanning



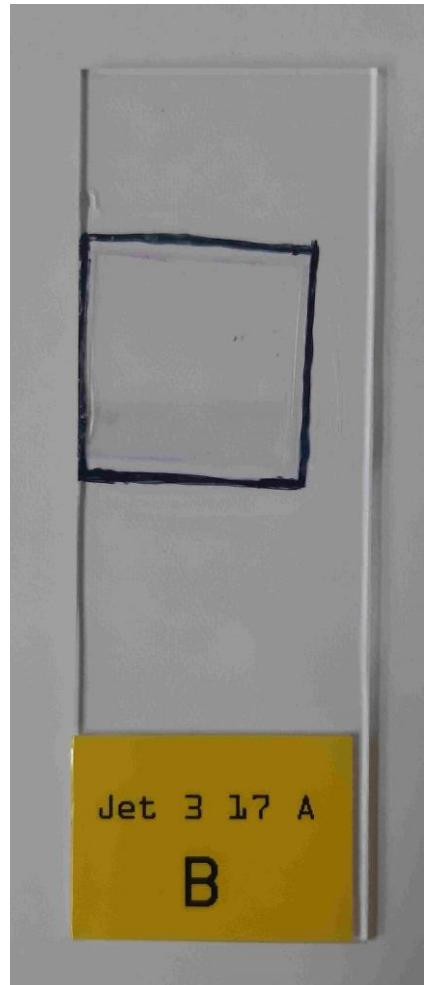
Axiostar machine



Axiostar software



# 2. Scanning



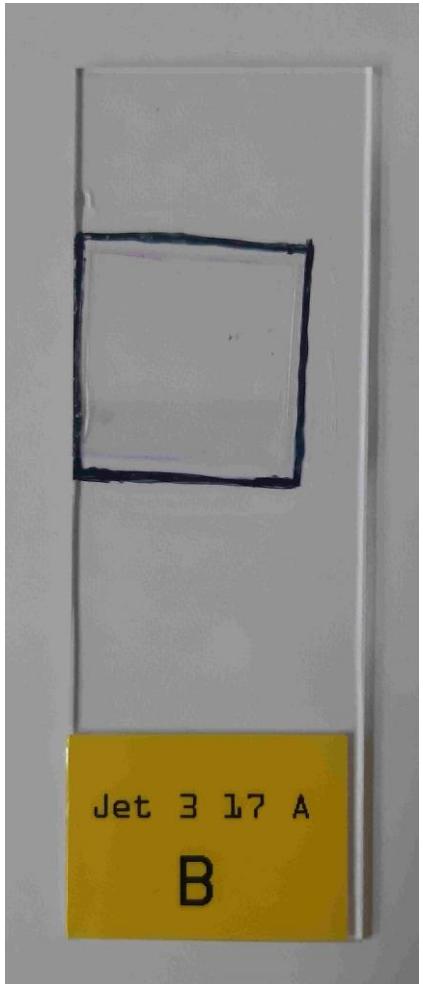
Axiostack machine



Tray for 4 slides  
Capacity about 60 slides

# 2. Scanning

Axioscan software



A screenshot of the Axioscan software interface. The main window displays a list of slides to be scanned across four trays. The trays are labeled "Tray Position: 1", "Tray Position: 2", "Tray Position: 3", and "Tray Position: 4", each containing 4 slides. The software interface includes a toolbar at the top with various icons, a menu bar, and a sidebar on the right showing "Images and Documents" and "ZEN Connect" sections.

**File Edit View Acquisition Graphics Macro Tools Window Help**

**Design Dark Workspace**

**Scan Locate Acquisition Processing Analysis**

**Stop Start Scan**

**Axio Scan**

1	To Scan: 4/4
2	To Scan: 4/4
3	To Scan: 4/4
4	To Scan: 4/4
5	To Scan: 4/4
6	To Scan: 4/4
7	To Scan: 4/4
8	To Scan: 2/2
9	
10	
11	To Scan: 2/2
12	To Scan: 1/1 Previewing
13	
14	To Scan: 4/4
15	To Scan: 4/4
16	To Scan: 2/2
17	
18	
19	To Scan: 3/3
20	To Scan: 4/4
21	To Scan: 4/4
22	To Scan: 4/4
23	To Scan: 3/3
24	To Scan: 4/4
25	To Scan: 4/4
<b>Total:</b>	69 slides to scan

**System Overview:**

- Working State: Processing
- Door Status: closed **Open**
- Stage Position: Prescan2

**Naming Definition**

**Storage Location**

**Acquisition Monitoring**

**Magazine** 2020\_11\_27\_Morphisto...as.czi\* 20200903\_RS013\_AJB0...00.czi\* 20200903\_RS013\_AJB02...001.czi\*

Select Scan Profile used for all slides with status new **DefaultScanProfile** **Smart Profile Selection...**

**Mark All Unmark All Default Order Skip Slide Expand All Collapse All**

**Tray Position: 1 | Type: Scan1x3**

Scan	Slide Position	Slide Overview	Scan Profile	Scan Status	Image Name	Name Assignment
✓	1		DefaultScanProfile	00m 40s Preview Done	07m 22s	Reset to: 2021_02_26_RecognizedCode Automatic
✓	2		DefaultScanProfile	00m 05s Preview Done	07m 22s	Reset to: 2021_02_26_RecognizedCode-1 Automatic
✓	3		DefaultScanProfile	00m 50s Preview Done	07m 22s	Reset to: 2021_02_26_RecognizedCode-2 Automatic
✓	4		DefaultScanProfile	00m 03s Preview Done	07m 22s	Reset to: 2021_02_26_RecognizedCode-3 Automatic

**Tray Position: 2 | Type: Scan1x3**

Scan	Slide Position	Slide Overview	Scan Profile	Scan Status	Image Name	Name Assignment
✓	1		DefaultScanProfile	00m 29s Preview Done	07m 22s	Reset to: 2021_02_26_RecognizedCode-4 Automatic
✓	2		DefaultScanProfile	00m 03s Preview Done	07m 22s	Reset to: 2021_02_26_RecognizedCode-5 Automatic
✓	3		DefaultScanProfile	00m 03s Preview Done	07m 22s	Reset to: 2021_02_26_RecognizedCode-6 Automatic
✓	4		DefaultScanProfile	00m 03s input required	11m 46s	Reset to: 2021_02_26_RecognizedCode-7 Automatic

**Tray Position: 3 | Type: Scan1x3**

Scan	Slide Position	Slide Overview	Scan Profile	Scan Status	Image Name	Name Assignment
✓	1		DefaultScanProfile	00m 34s Preview Done	07m 22s	Reset to: 2021_02_26_RecognizedCode-8 Automatic
✓	2		DefaultScanProfile	00m 03s Preview Done	07m 22s	Reset to: 2021_02_26_RecognizedCode-9 Automatic
✓	3		DefaultScanProfile	00m 03s Preview Done	07m 22s	Reset to: 2021_02_26_RecognizedCode-10 Automatic
✓	4		DefaultScanProfile	00m 03s Preview Done	07m 22s	Reset to: 2021_02_26_RecognizedCode-11 Automatic

**Tray Position: 4 | Type: Scan1x3**

Scan	Slide Position	Slide Overview	Scan Profile	Scan Status	Image Name	Name Assignment
✓	1		DefaultScanProfile	00m 29s input required	11m 46s	Reset to: 2021_02_26_RecognizedCode-12 Automatic

**Images and Documents**

Container 1

- 2020\_11\_27\_Mor...as.czi\* C 17.11 GB / 17.17 GB
- 20200903\_RS013...00.czi\* C 18.79 GB / 23.2 GB
- 20200903\_RS013...001.czi C 17 GB / 2.55 GB

ZEN Connect Regions

File Edit View Acquisition Graphics Macro Tools Window Help

Workspace Zoom | Reset

Scan Locate Acquisition Processing Analysis

**Start Scan**

**Axio Scan**

- 1 To Scan: 4/4
- 2 To Scan: 4/4
- 3 To Scan: 4/4
- 4 To Scan: 4/4
- 5 To Scan: 4/4
- 6 To Scan: 4/4
- 7 To Scan: 4/4
- 8 To Scan: 2/2
- 9
- 10
- 11 To Scan: 2/2
- 12 To Scan: 1/1 Previewing
- 13
- 14 To Scan: 4/4
- 15 To Scan: 4/4
- 16 To Scan: 2/2
- 17
- 18
- 19 To Scan: 3/3
- 20 To Scan: 4/4
- 21 To Scan: 4/4
- 22 To Scan: 4/4
- 23 To Scan: 3/3
- 24 To Scan: 4/4
- 25 To Scan: 4/4

Total: 69 slides to scan

**System Overview:**

- Working State: Processing
- Door Status: closed Open
- Stage Position: Prescan2

**Naming Definition**

**Storage Location**

**Acquisition Monitoring**

Magazine 2020\_11\_27\_Morphisto...as.czi\* 20200903\_RS013\_AJB0...00.czi\* 20200903\_RS013\_AJB02...001.czi\*

Select Scan Profile used for all slides with status new DefaultScanProfile Smart Profile Selection...

Mark All Unmark All Default Order Skip Slide Expand All Collapse All

**Tray Position: 1 | Type: Scan1x3**

Scan	Slide Position	Slide Overview
<input checked="" type="checkbox"/>	1	
<input checked="" type="checkbox"/>	2	
<input checked="" type="checkbox"/>	3	
<input checked="" type="checkbox"/>	4	

**Tray Position: 2 | Type: Scan1x3**

Scan	Slide Position	Slide Overview
<input checked="" type="checkbox"/>	1	
<input checked="" type="checkbox"/>	2	
<input checked="" type="checkbox"/>	3	
<input checked="" type="checkbox"/>	4	

**Tray Position: 3 | Type: Scan1x3**

Scan	Slide Position	Slide Overview
<input checked="" type="checkbox"/>	1	
<input checked="" type="checkbox"/>	2	
<input checked="" type="checkbox"/>	3	
<input checked="" type="checkbox"/>	4	

**Tray Position: 4 | Type: Scan1x3**

Scan	Slide Position	Slide Overview	Scan Profile	Scan Status	Image Name	Name Assignment
<input checked="" type="checkbox"/>	1		DefaultScanProfile	00m 29s	input required	11m 46s

DefaultScanProfile 00m 03s Preview Done 07m 22s Reset to: 2021\_02\_26\_RecognizedCode-11 Automatic

Image Name Name Assignment

- 021\_02\_26\_RecognizedCode Automatic
- 021\_02\_26\_RecognizedCode-1 Automatic
- 021\_02\_26\_RecognizedCode-2 Automatic
- 021\_02\_26\_RecognizedCode-3 Automatic
- 021\_02\_26\_RecognizedCode-4 Automatic
- 021\_02\_26\_RecognizedCode-5 Automatic
- 021\_02\_26\_RecognizedCode-6 Automatic
- 021\_02\_26\_RecognizedCode-7 Automatic
- 021\_02\_26\_RecognizedCode-8 Automatic
- 021\_02\_26\_RecognizedCode-9 Automatic
- 021\_02\_26\_RecognizedCode-10 Automatic
- 021\_02\_26\_RecognizedCode-11 Automatic
- 021\_02\_26\_RecognizedCode-12 Automatic

Container 1

2020\_11\_27\_Mor...as.czi\* \*\*\*\*\* C 17.11 GB / 17.17 GB

20200903\_RS013...00.czi\* C 18.79 GB / 23.2 GB

20200903\_RS013...001.czi\* C 17 GB / 2.55 GB

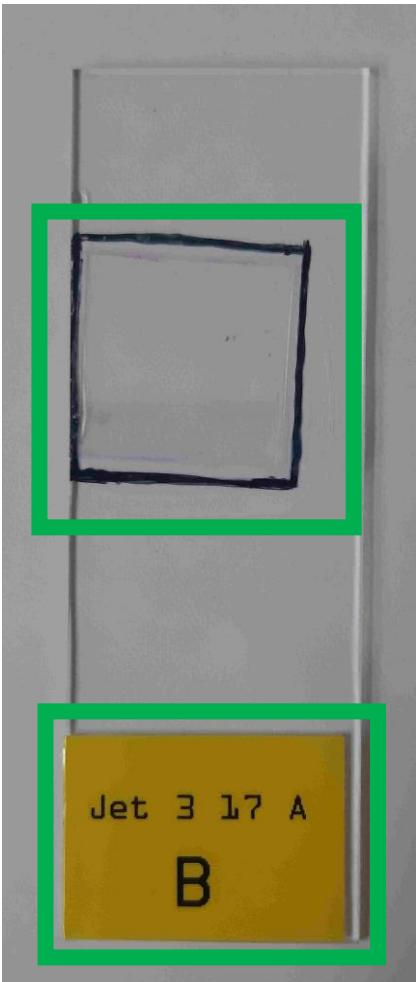
ZEN Connect Regions

Software-Guide

# ZEN 2012 SP2

## ZEN slidescan module

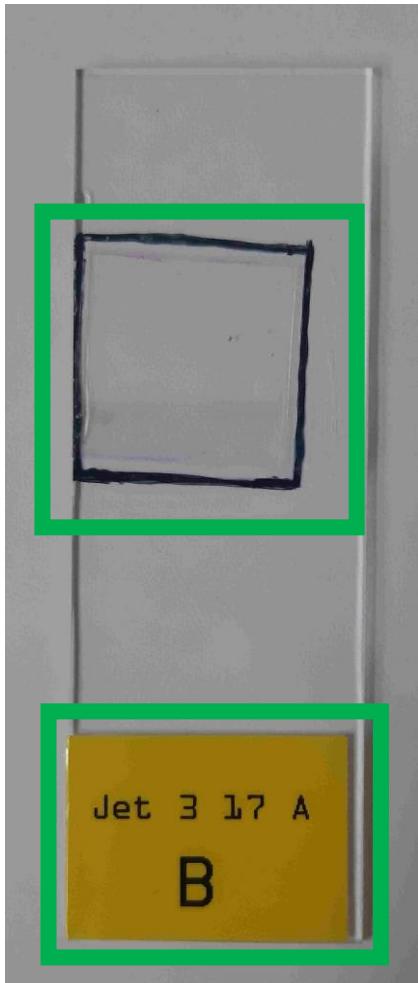
## 2. Scanning



Scan

Label

## 2. Scanning

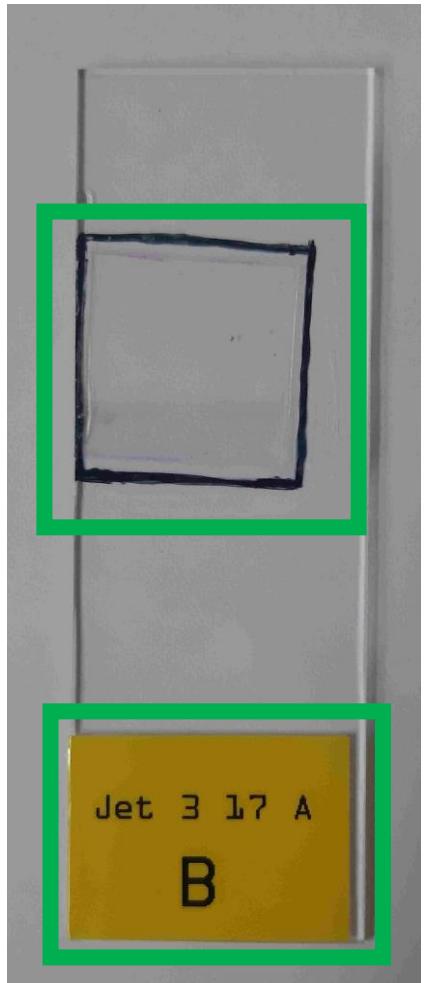


Scan  
Label

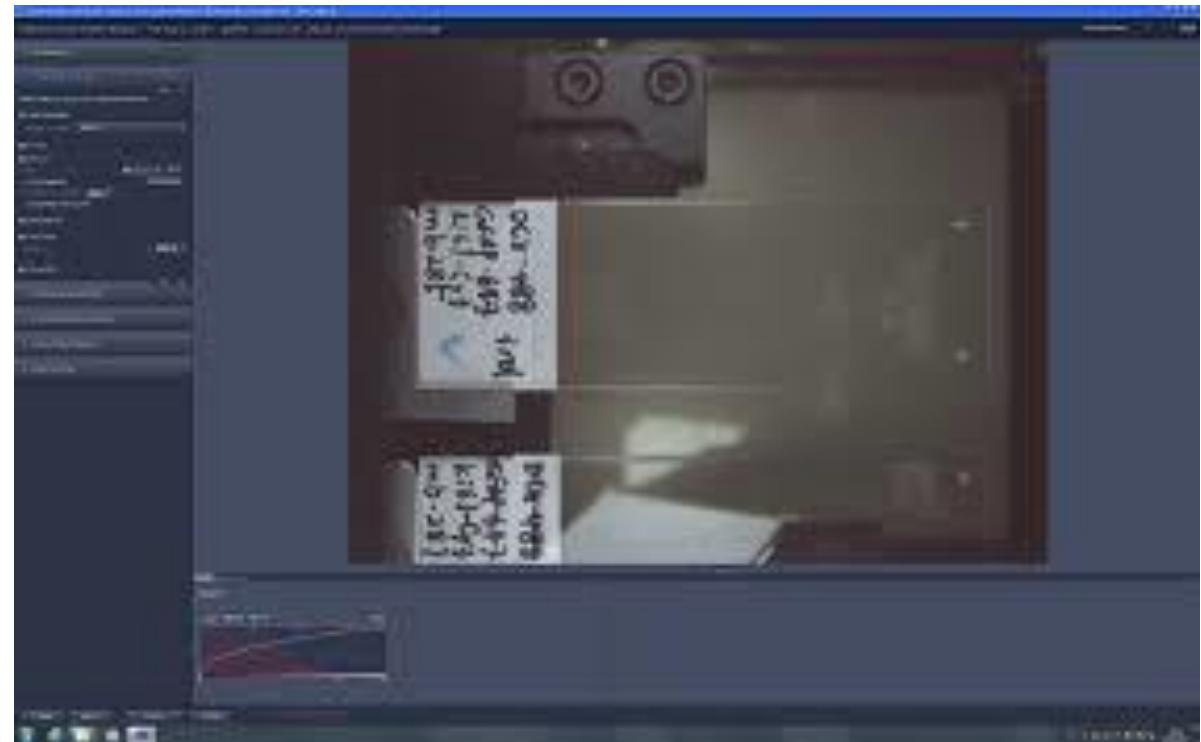
QR code:  
possible automatization



## 2. Scanning

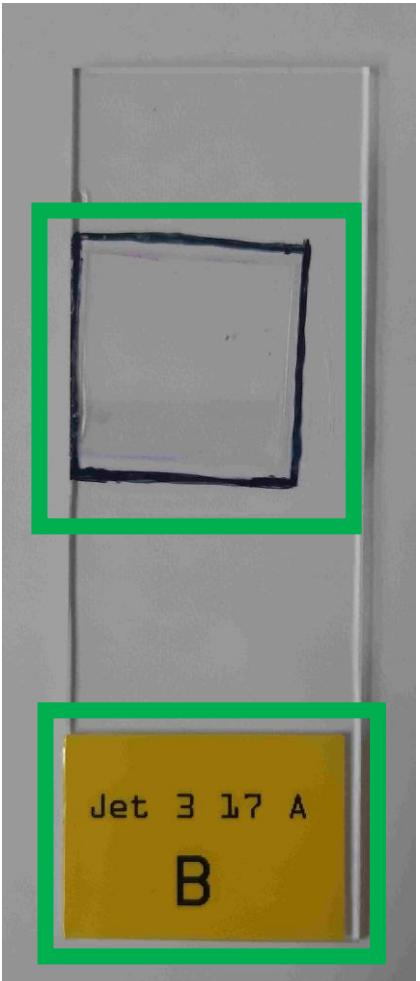


Scan  
Label

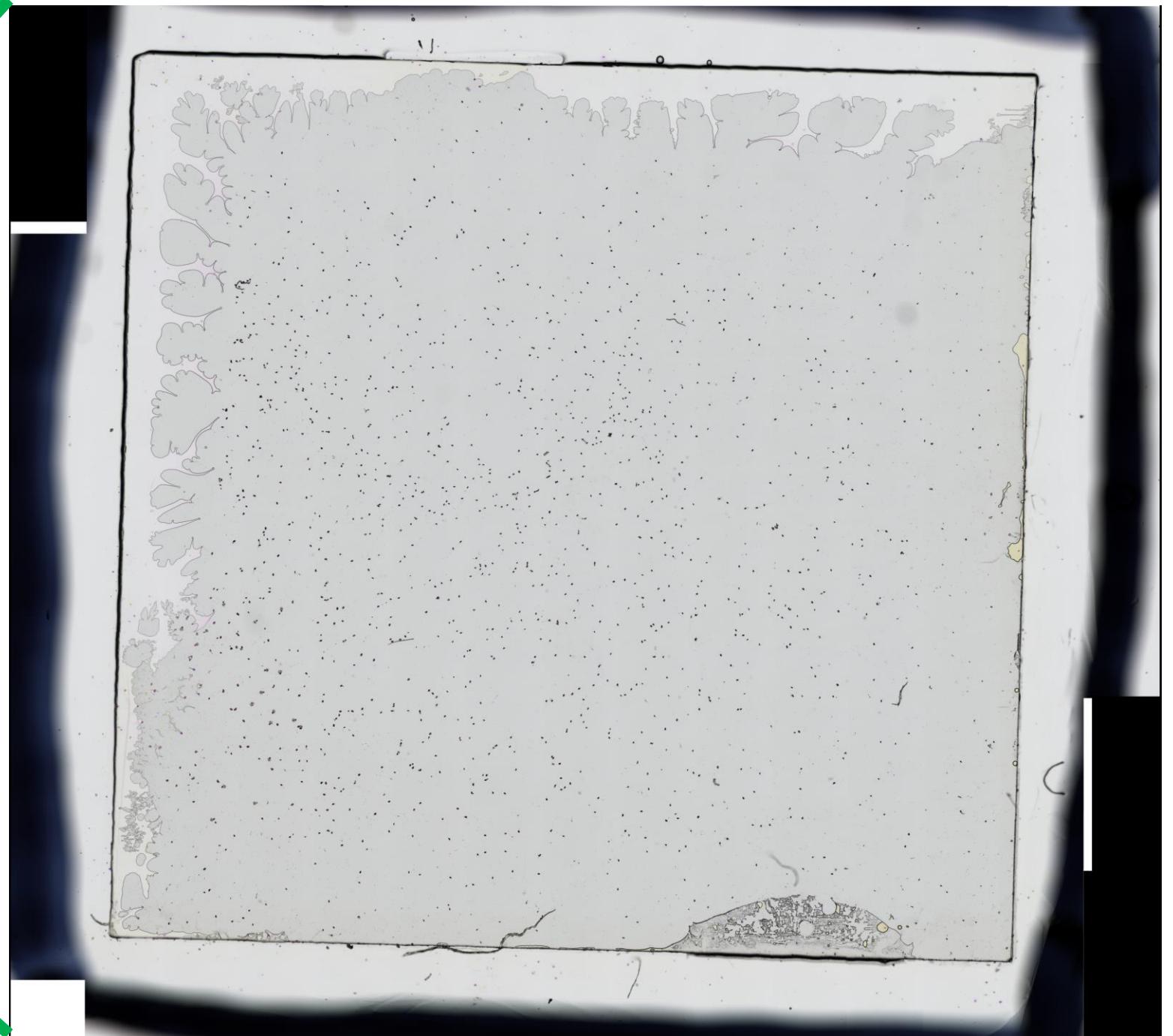
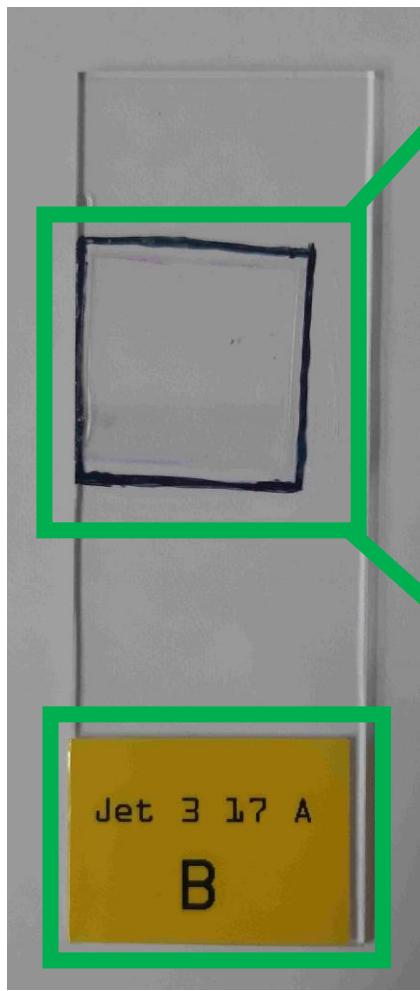


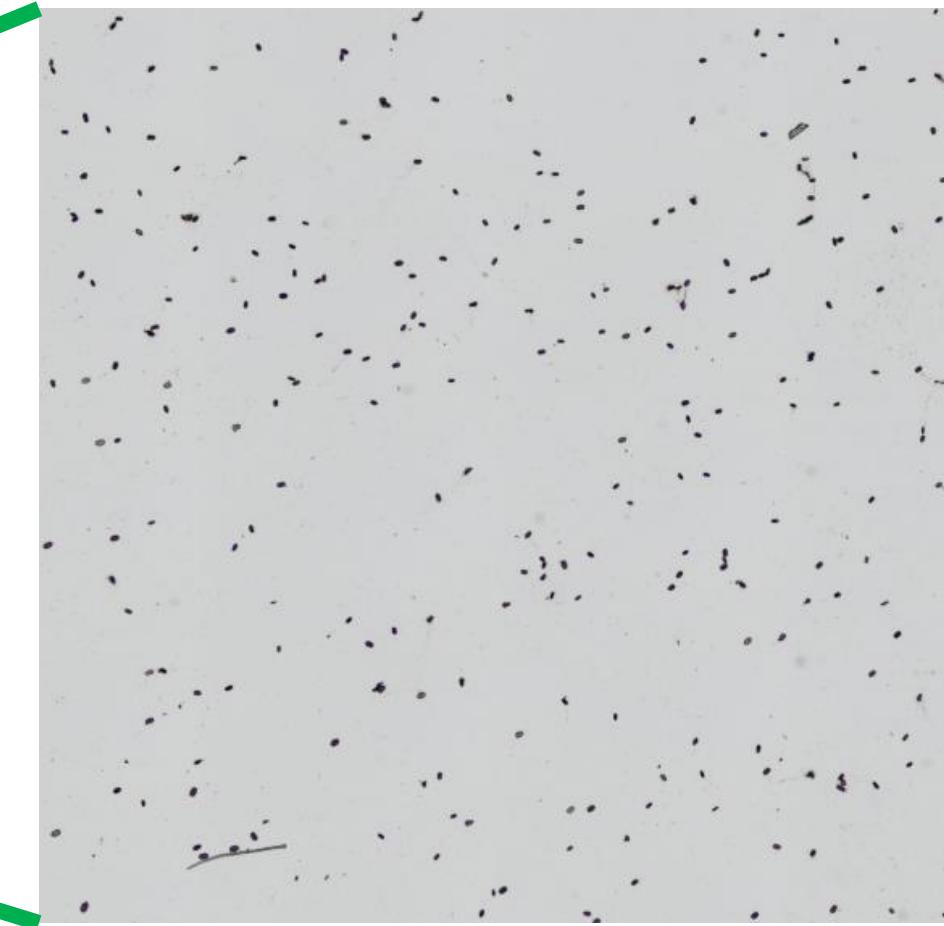
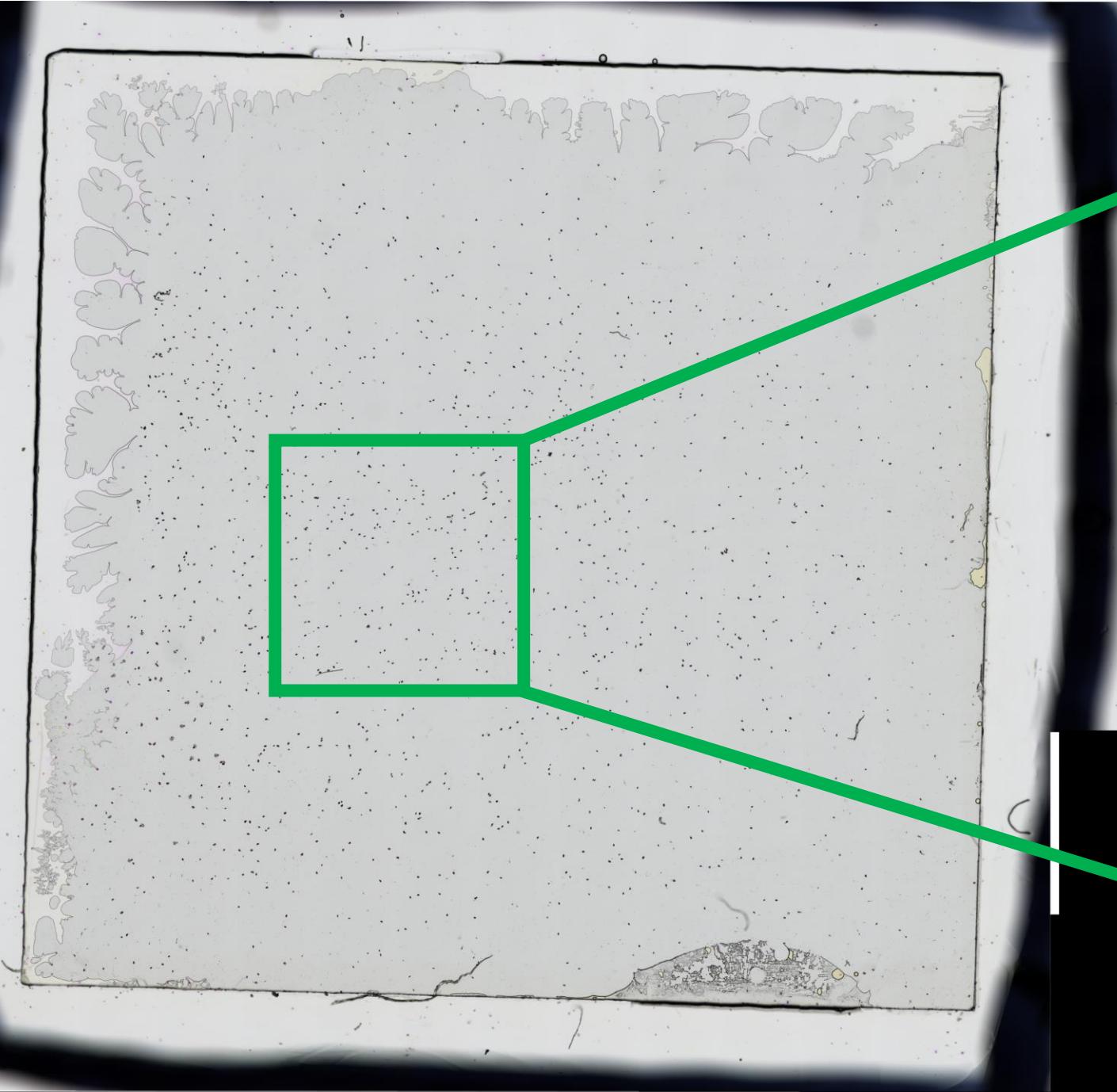
## 2. Scanning

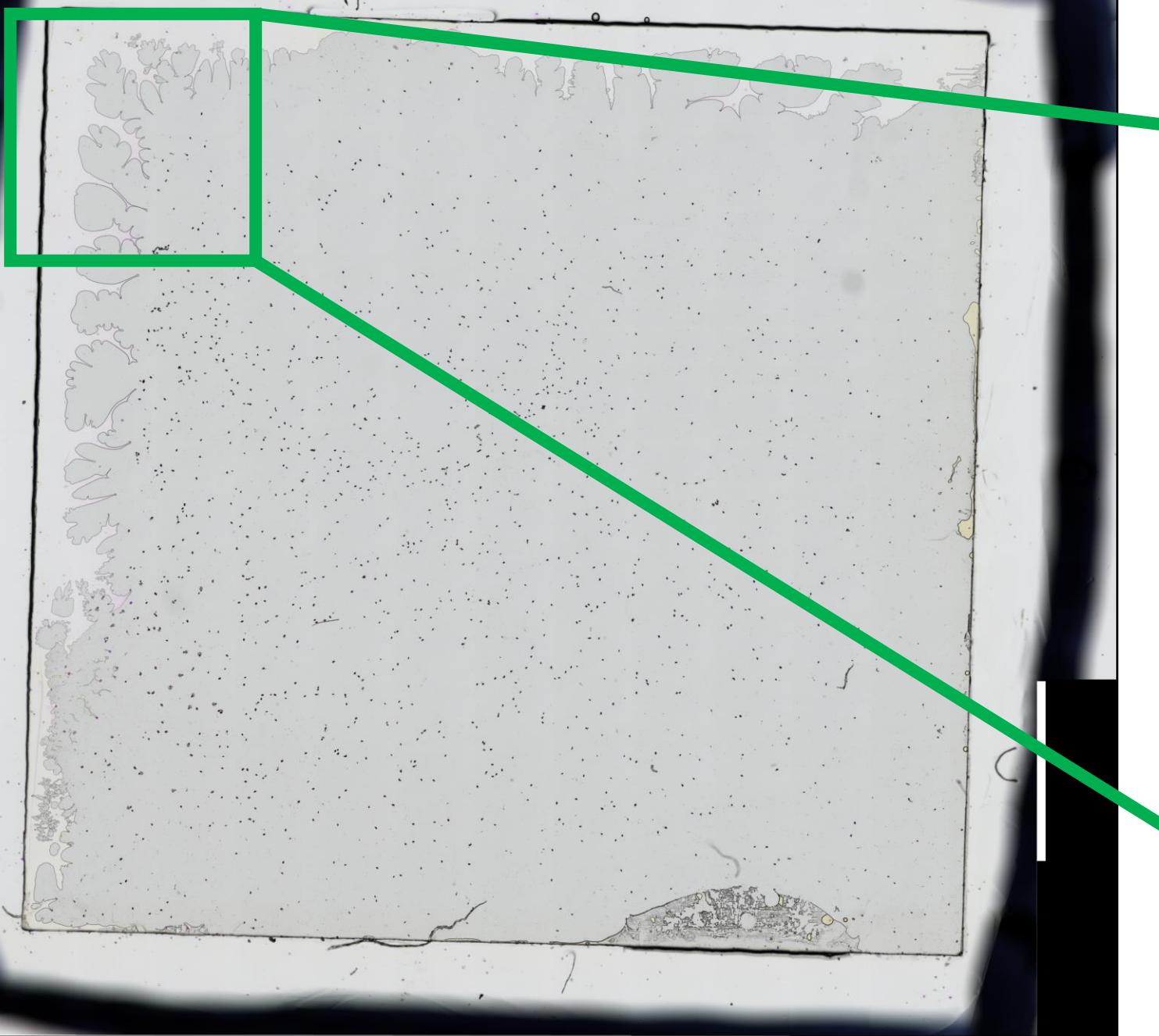
1. Needs to find the area of scanning - marker
2. Needs to set the focusing method and precision  
=> trade-off between precision and time

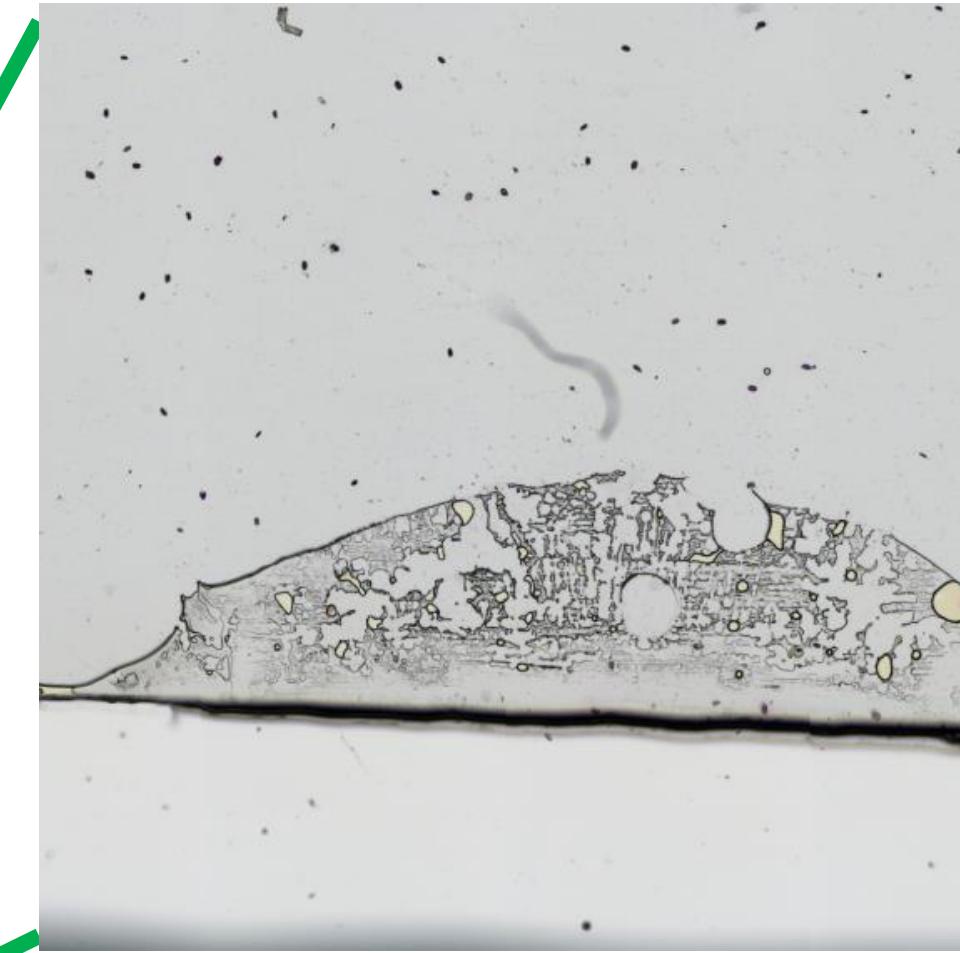
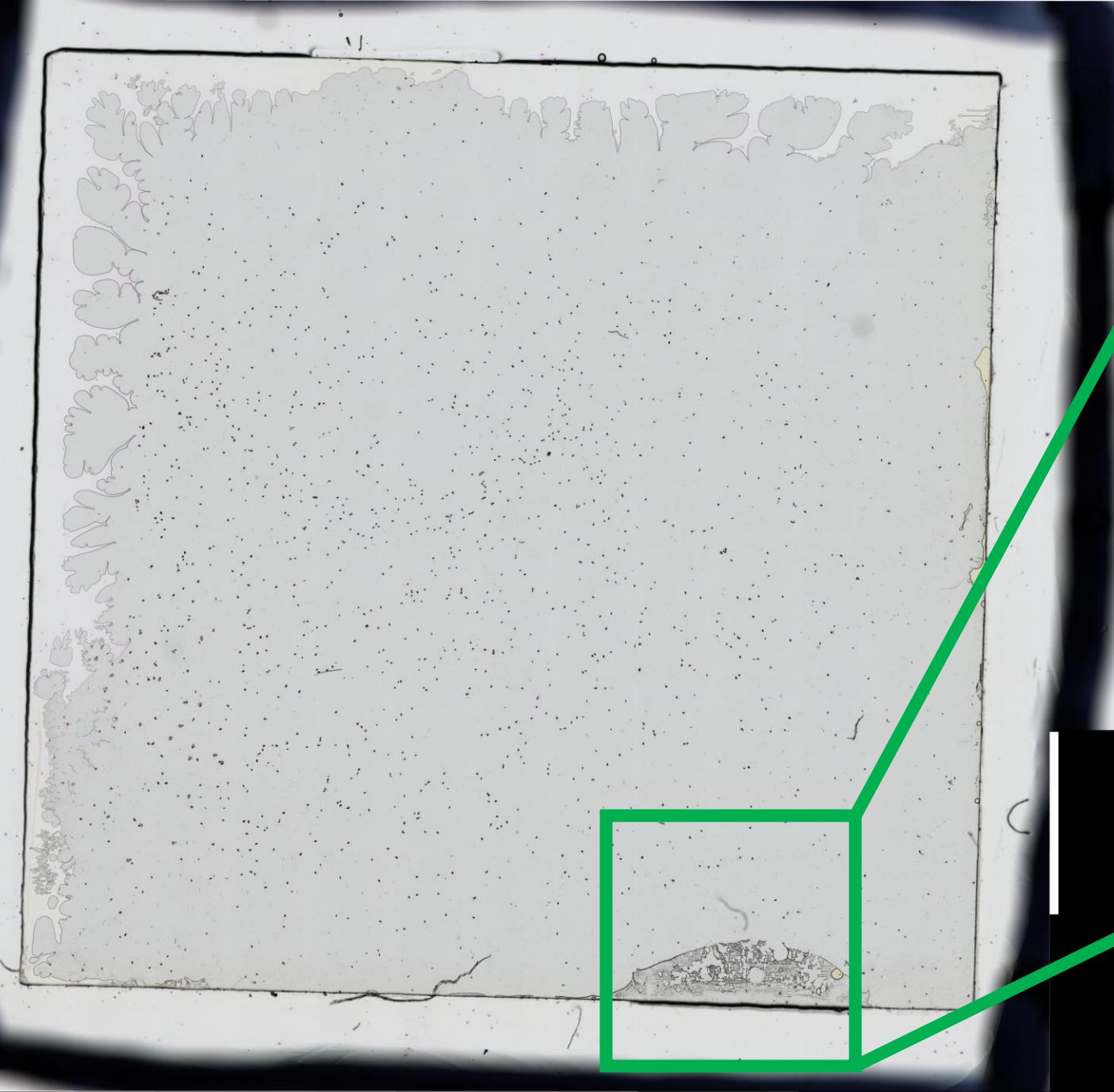


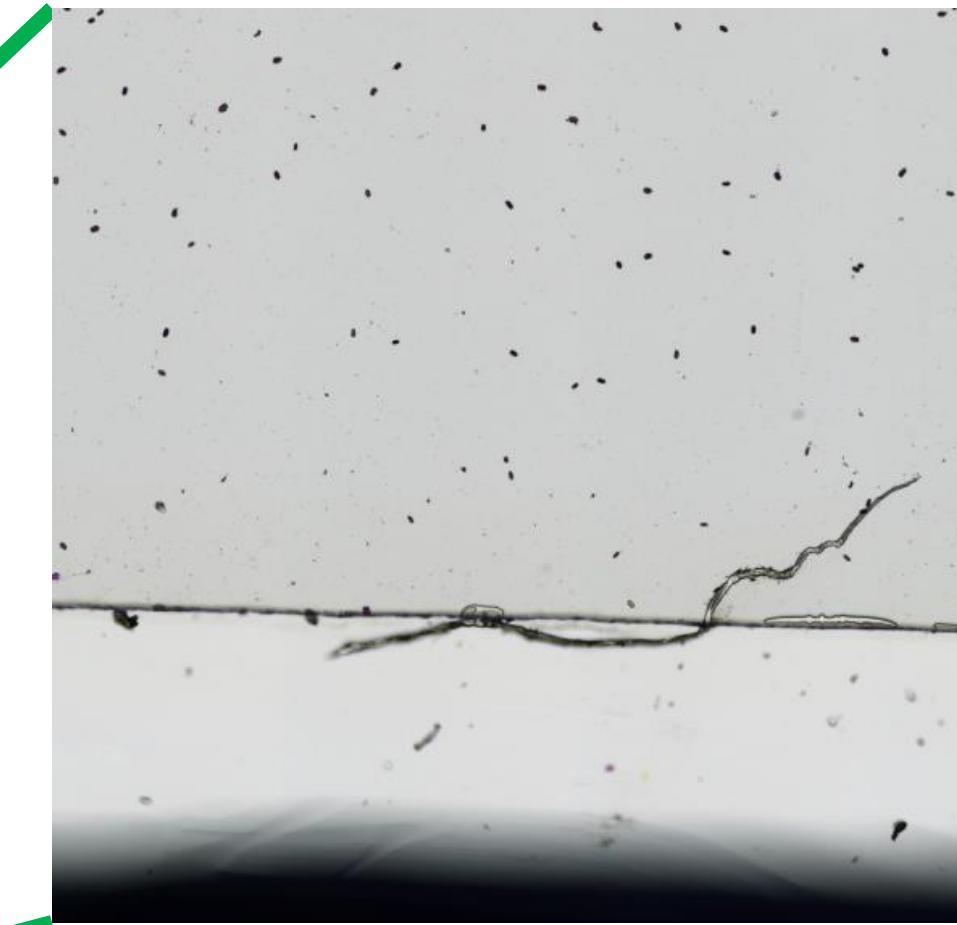
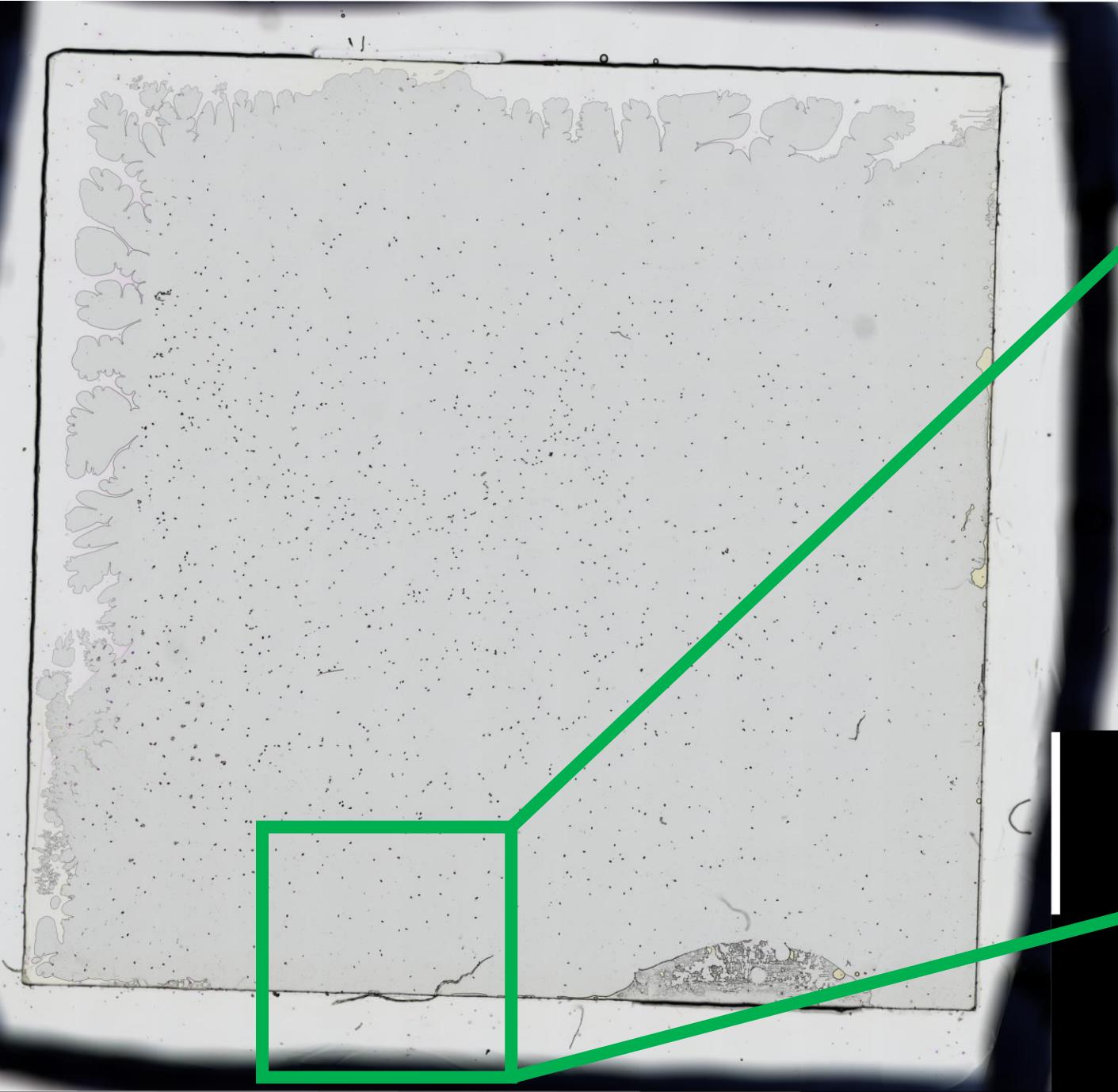
# Scan



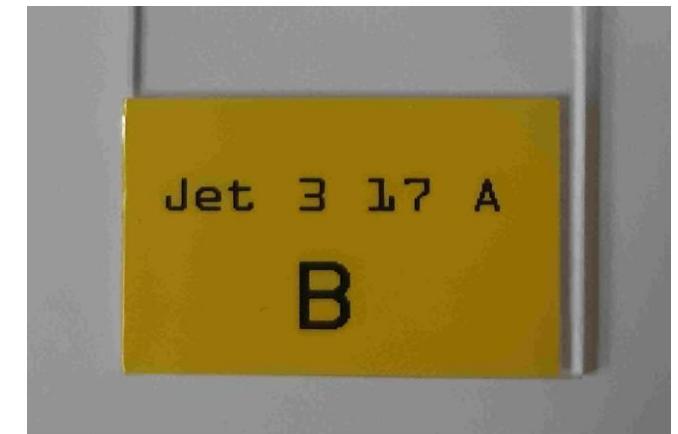
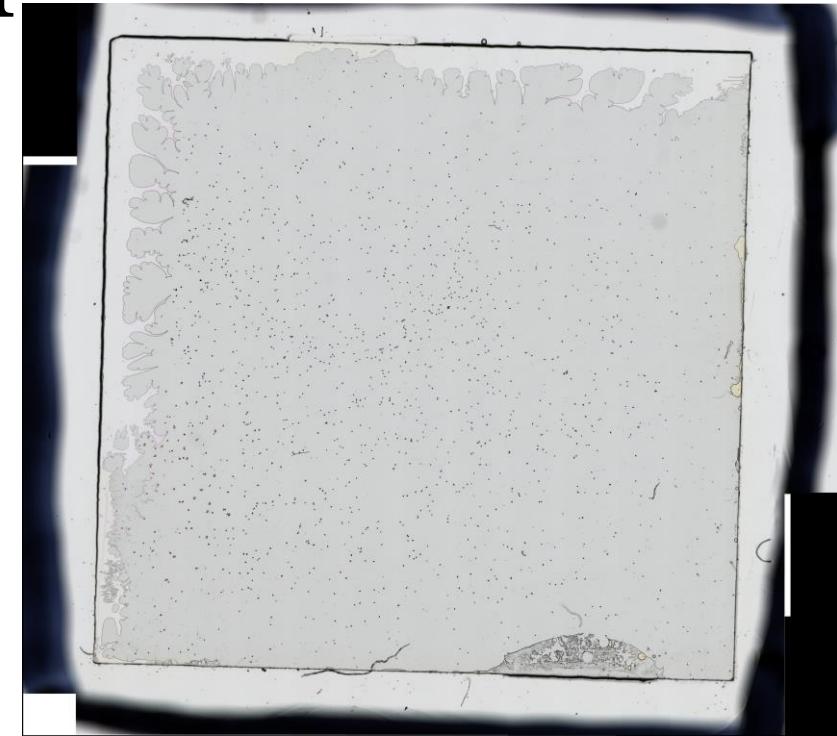
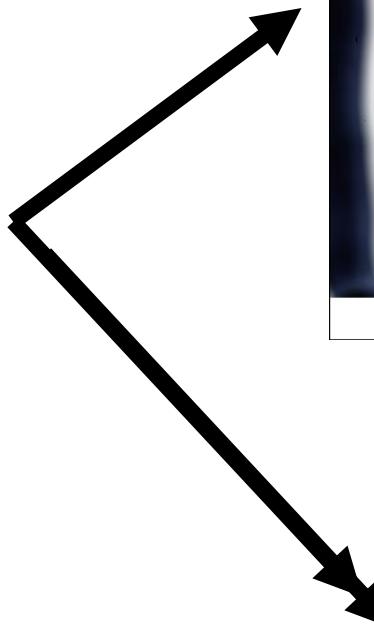
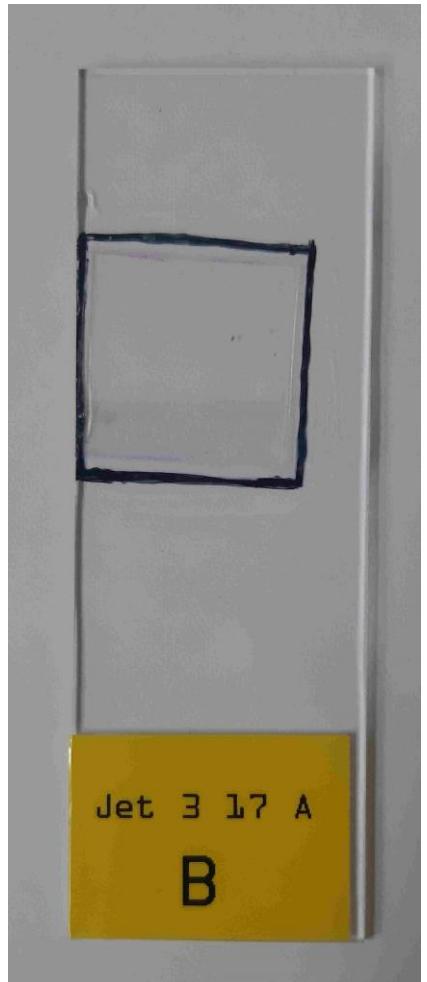






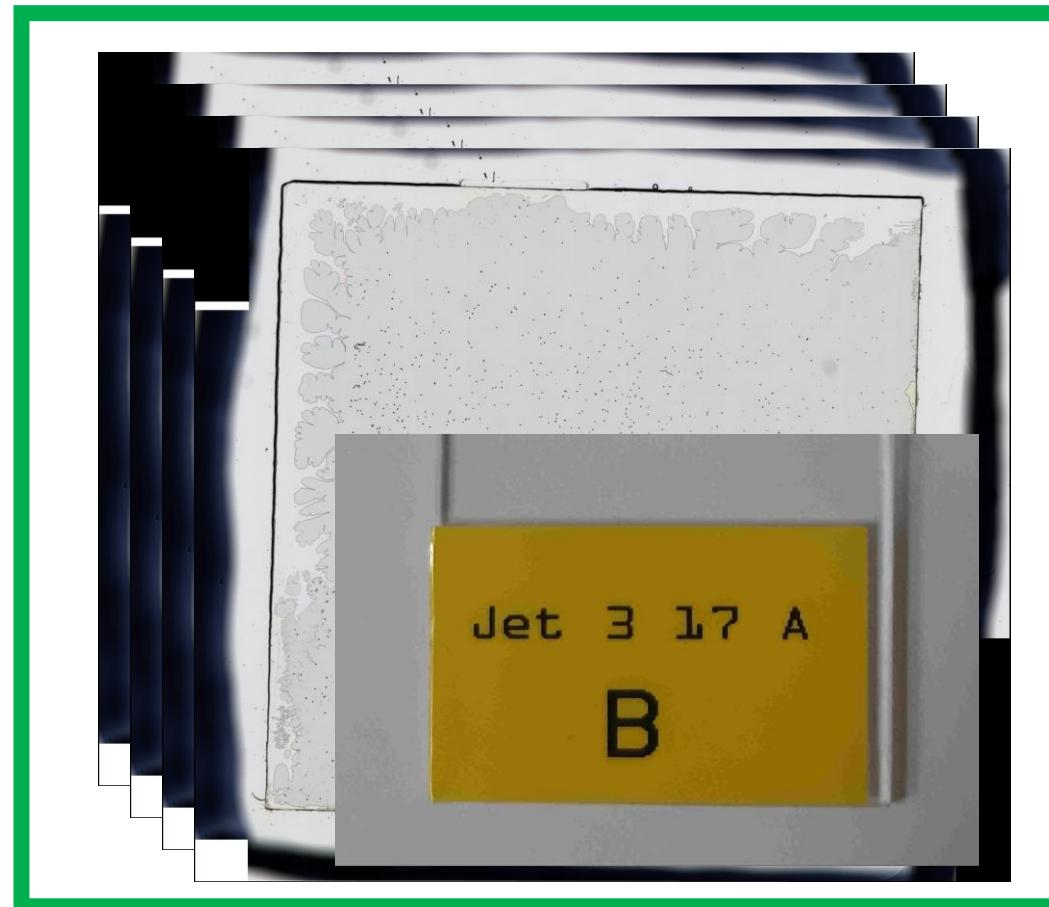


## 2. Scanned picture preparation

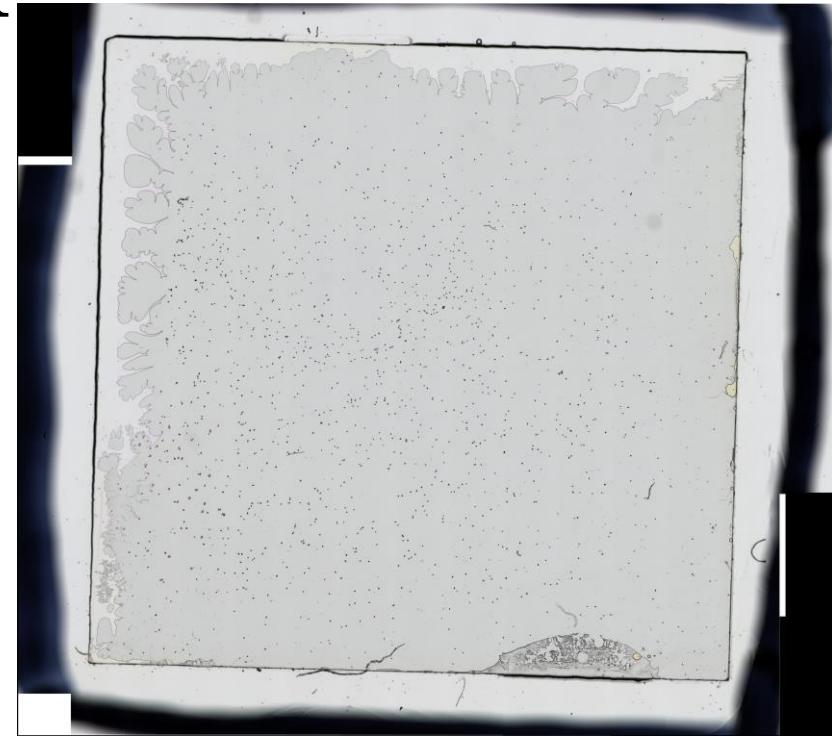
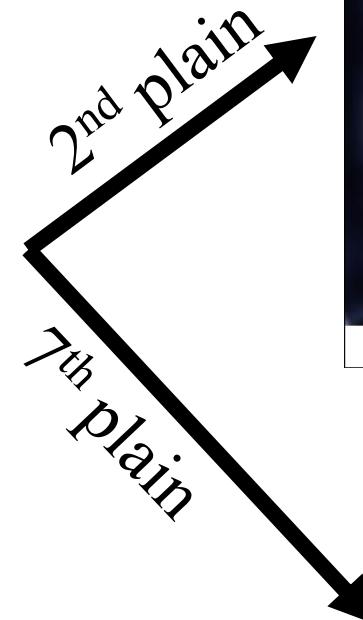


## 2. Scanned picture preparation

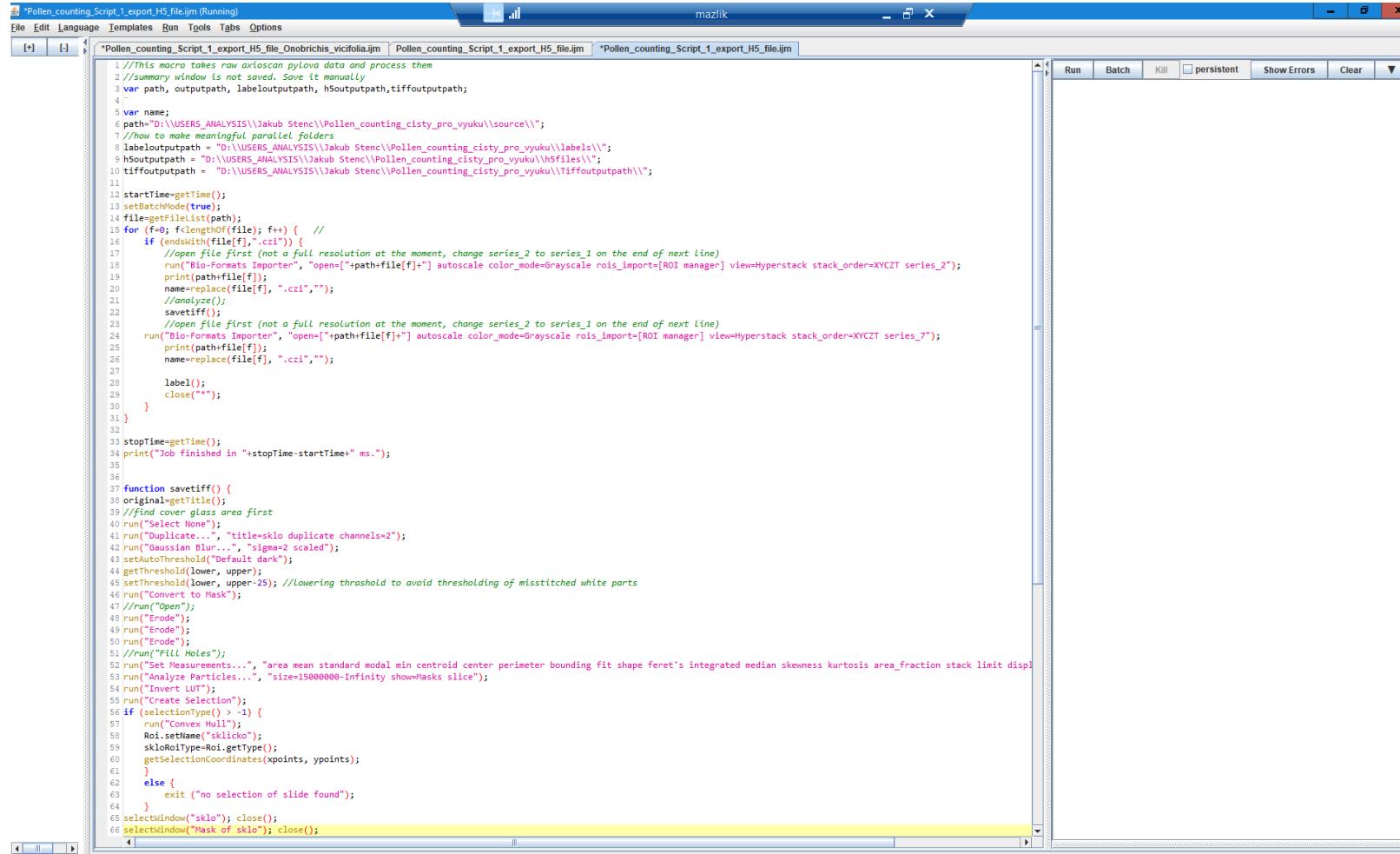
Scan in CZI format => hierarchical structure => we want to



## 2. Scanned picture preparation



# 2. Scanned picture preparation



The screenshot shows a software interface with a title bar 'mazlik'. The main window contains a script editor with tabs for 'Pollen\_counting\_Script\_1\_export\_H5\_file.ijm' (Running) and 'Pollen\_counting\_Script\_1\_export\_H5\_file.ijm'. The code in the editor is a macro script for ImageJ, specifically for pollen counting. It includes functions for opening files, processing them using Bio-Formats Importer, saving as H5 and TIFF files, and saving labels as jpg files. The script also handles ROI management and various image processing steps like thresholding and noise reduction.

```
/*Pollen_counting_Script_1_export_H5_file.ijm (Running)
File Edit Language Templates Run Tools Tabs Options
[*] Pollen_counting_Script_1_export_H5_file.ijm Pollen_counting_Script_1_export_H5_file.ijm *Pollen_counting_Script_1_export_H5_file.ijm
1 //This macro takes raw axelson ptylova data and process them
2 //summary window is not saved. Save it manually
3 var path, outputpath, labeloutputpath, h5outputpath, tiffoutputpath;
4 /*
5 var name;
6 path="D:\\USERS_ANALYSIS\\Jakub Stenc\\Pollen_counting_cisty_pro_vyuku\\source\\";
7 //how to make meaningful parallel folders
8 labeloutputpath = "D:\\USERS_ANALYSIS\\Jakub Stenc\\Pollen_counting_cisty_pro_vyuku\\labels\\";
9 h5outputpath = "D:\\USERS_ANALYSIS\\Jakub Stenc\\Pollen_counting_cisty_pro_vyuku\\h5files\\";
10 tiffoutputpath = "D:\\USERS_ANALYSIS\\Jakub Stenc\\Pollen_counting_cisty_pro_vyuku\\tiffoutputpath\\";
11
12 startTime=getTime();
13 setBatchMode(true);
14 fileGetFileList(path);
15 for (f=0; f<lengthOfFile(); f++) { ///
16   if (endsWithOfFile(f, ".czi")) {
17     //open file first (not a full resolution at the moment, change series_2 to series_1 on the end of next line)
18     run("Bio-Formats Importer", "open["+path+file[f]+"] autoscale color_mode=Grayscale rois_import=[ROI manager] view=Hyperstack stack_order=XZYCT series_2");
19     print(path+file[f]);
20     name=replace(file[f], ".czi","");
21     //analyze();
22     saveTiff();
23     //open file first (not a full resolution at the moment, change series_2 to series_1 on the end of next line)
24     run("Bio-Formats Importer", "open["+path+file[f]+"] autoscale color_mode=Grayscale rois_import=[ROI manager] view=Hyperstack stack_order=XZYCT series_7");
25     print(path+file[f]);
26     name=replace(file[f], ".czi","");
27
28     label();
29     close("");
30   }
31 }
32
33 stopTime=getTime();
34 print("Job finished in "+stopTime-startTime+" ms.");
35
36
37 function saveTiff() {
38   original getTitle();
39   //find cover glass area first
40   run("Select None");
41   run("Duplicate...", "title=sklo duplicate channels=2");
42   run("Gaussian Blur...", "sigma=2 scaled");
43   setAutoThreshold("Default dark");
44   getThreshold(lower, upper);
45   setThreshold(lower, upper-25); //lowering threshold to avoid thresholding of misstitched white parts
46   run("Convert to Mask");
47   //run("Open");
48   run("Erode");
49   run("Erode");
50   run("Erode");
51   //run("Fill Holes");
52   run("Set Measurements...", "area mean standard modal min centroid center perimeter bounding fit shape feret's integrated median skewness kurtosis area_fraction stack limit disp");
53   run("Analyze Particles...", "size=15000000-Infinity show=Masks slice");
54   run("Invert LUT");
55   run("Create Selection");
56   if (selectionType() > -1) {
57     run("Convex Hull");
58     Roi.setname("sklicko");
59     sklicko=Roi.getType();
60     getSelectionCoordinate(xpoints, ypoints);
61   }
62   else {
63     exit ("no selection of slide found");
64   }
65   selectWindow("sklo"); close();
66 selectWindow("Mask of sklo"); close();
```

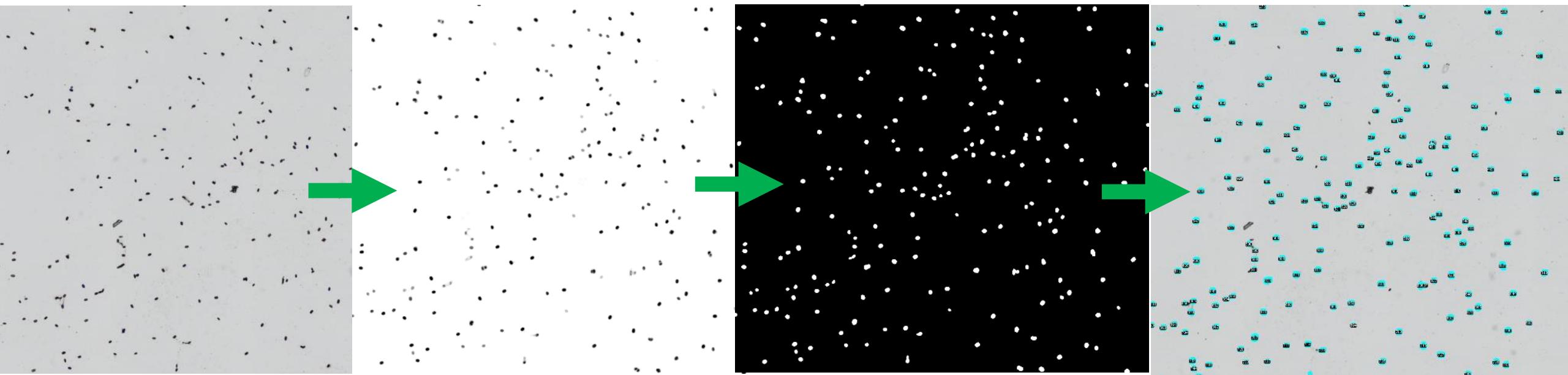
1. Open CZI file
2. Save one plane as H5 file
3. Save one plane as TIFF file
4. Save label as jpg file

And this for all files



### 3. Illastik

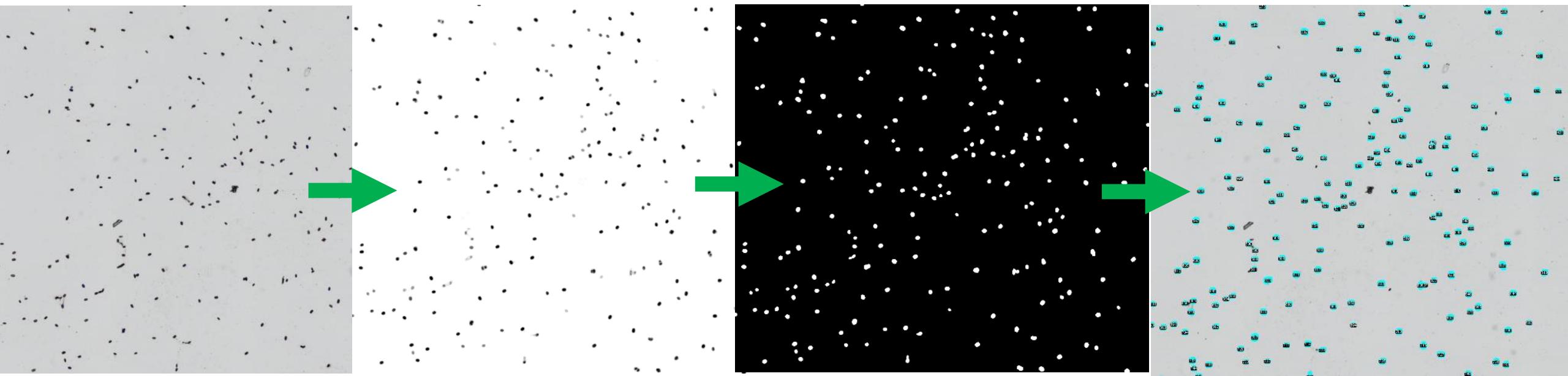
Pixel classification => Object clasification



### 3. Illastik



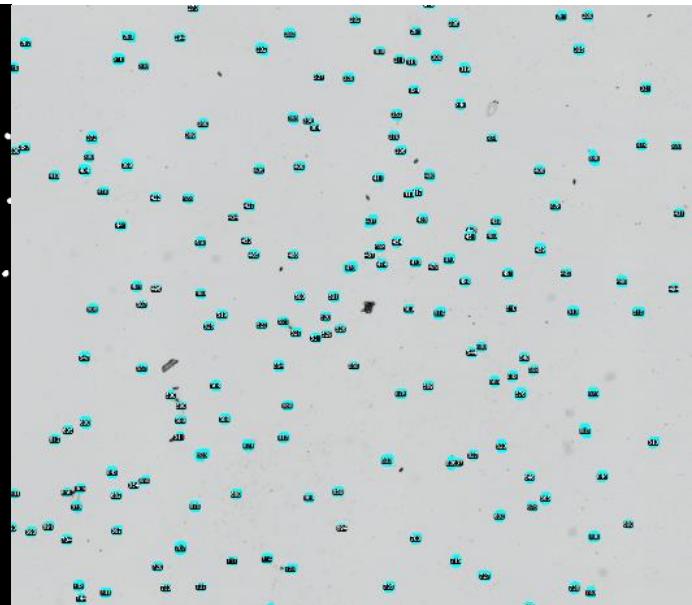
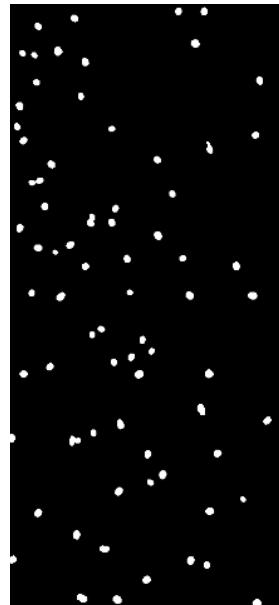
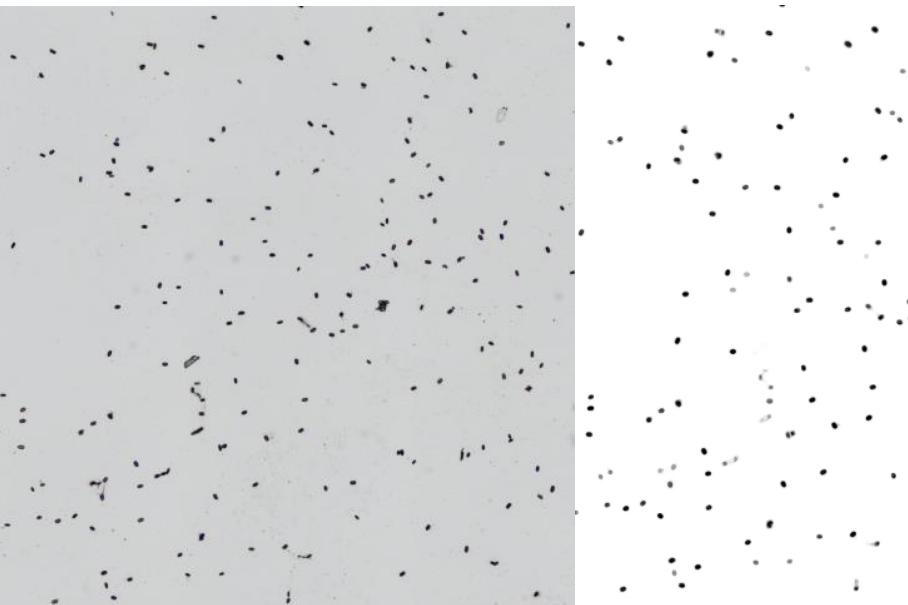
Raw picture => Pixel classification => Object classification => postprocess in FIJI



### 3. Illastik



Raw picture => Pixel classification => Object classification => postprocess in FIJI

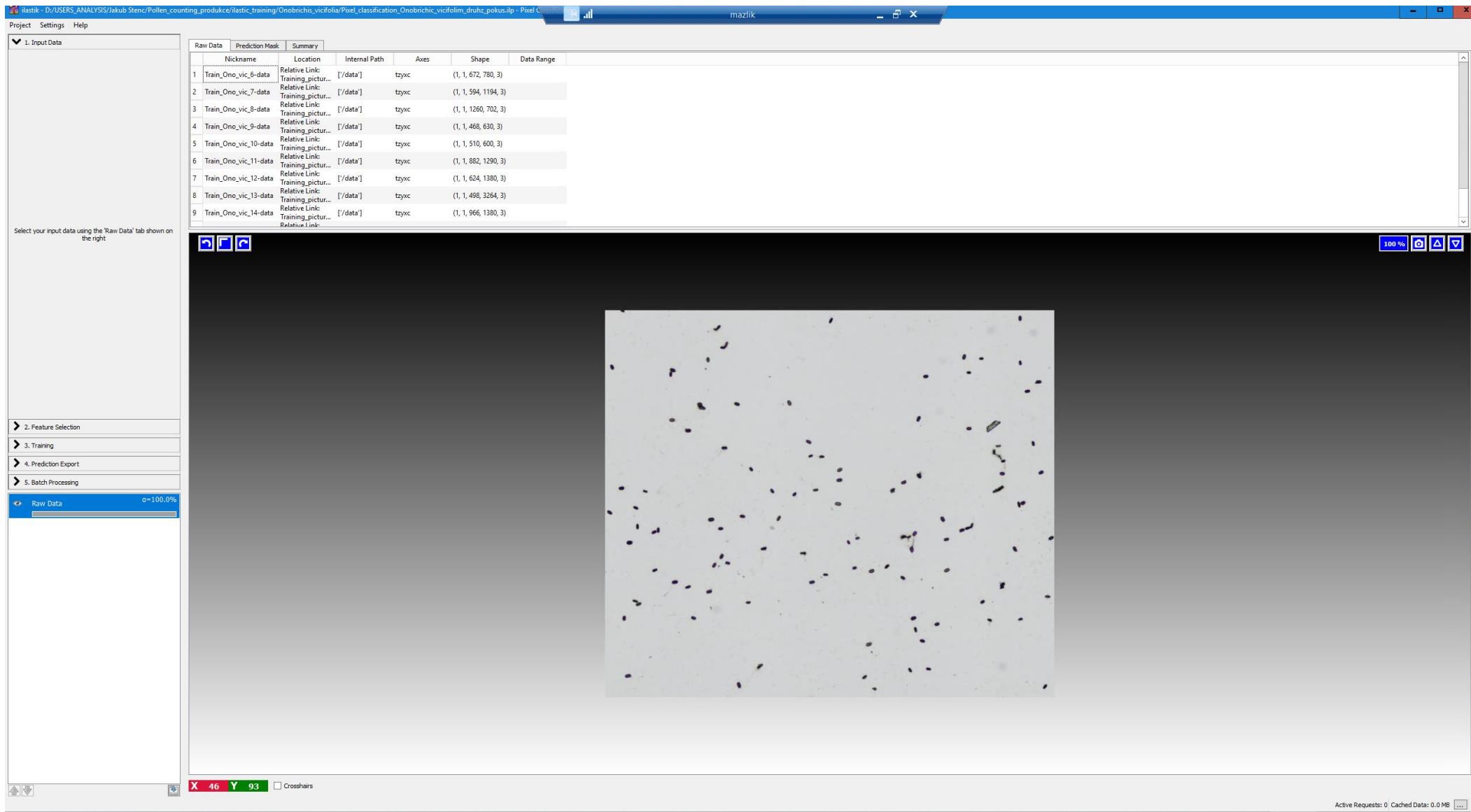


# 3. Ilastik

## Pixel classification

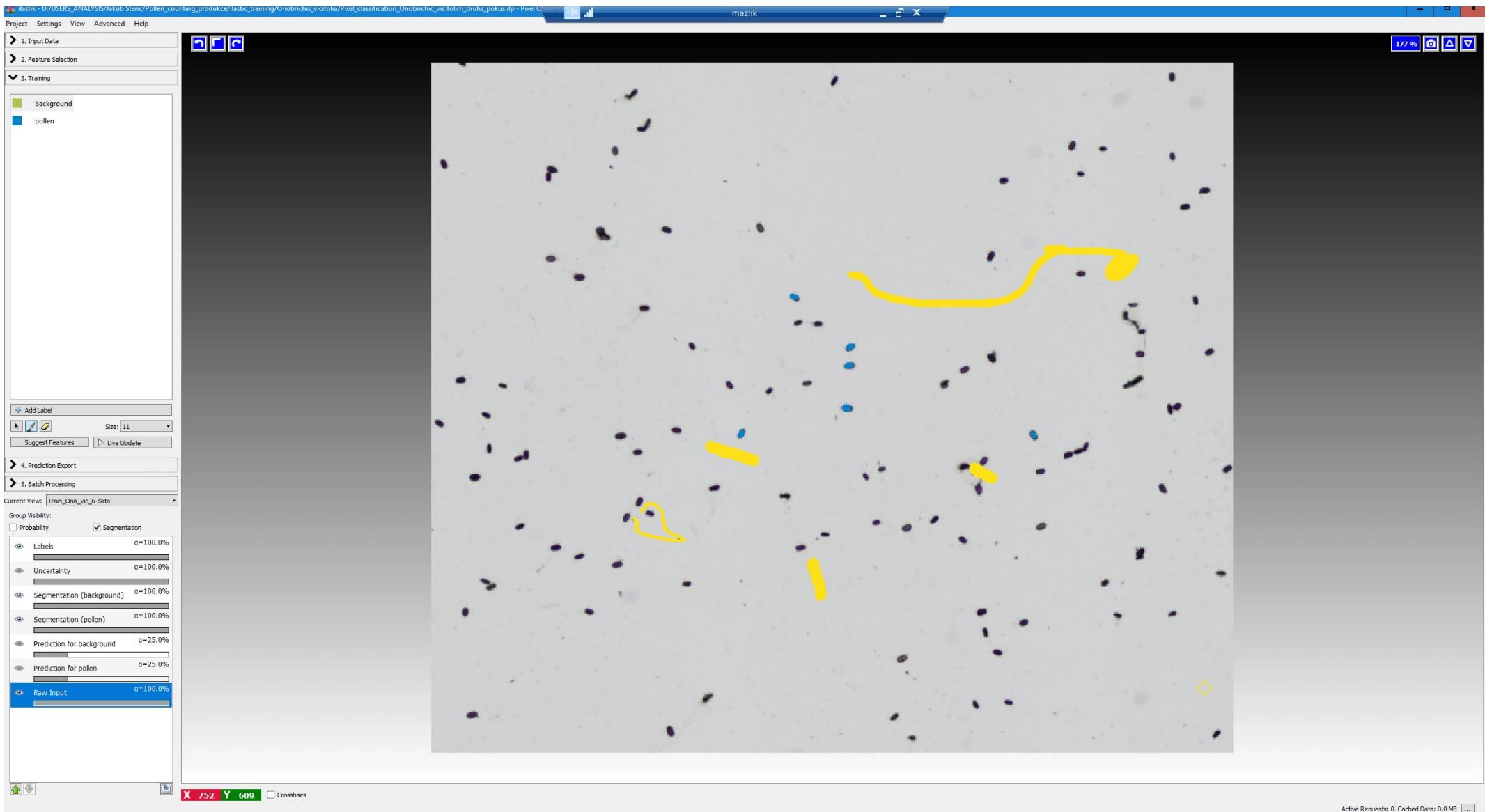
# 3. Ilastik

## Pixel classification



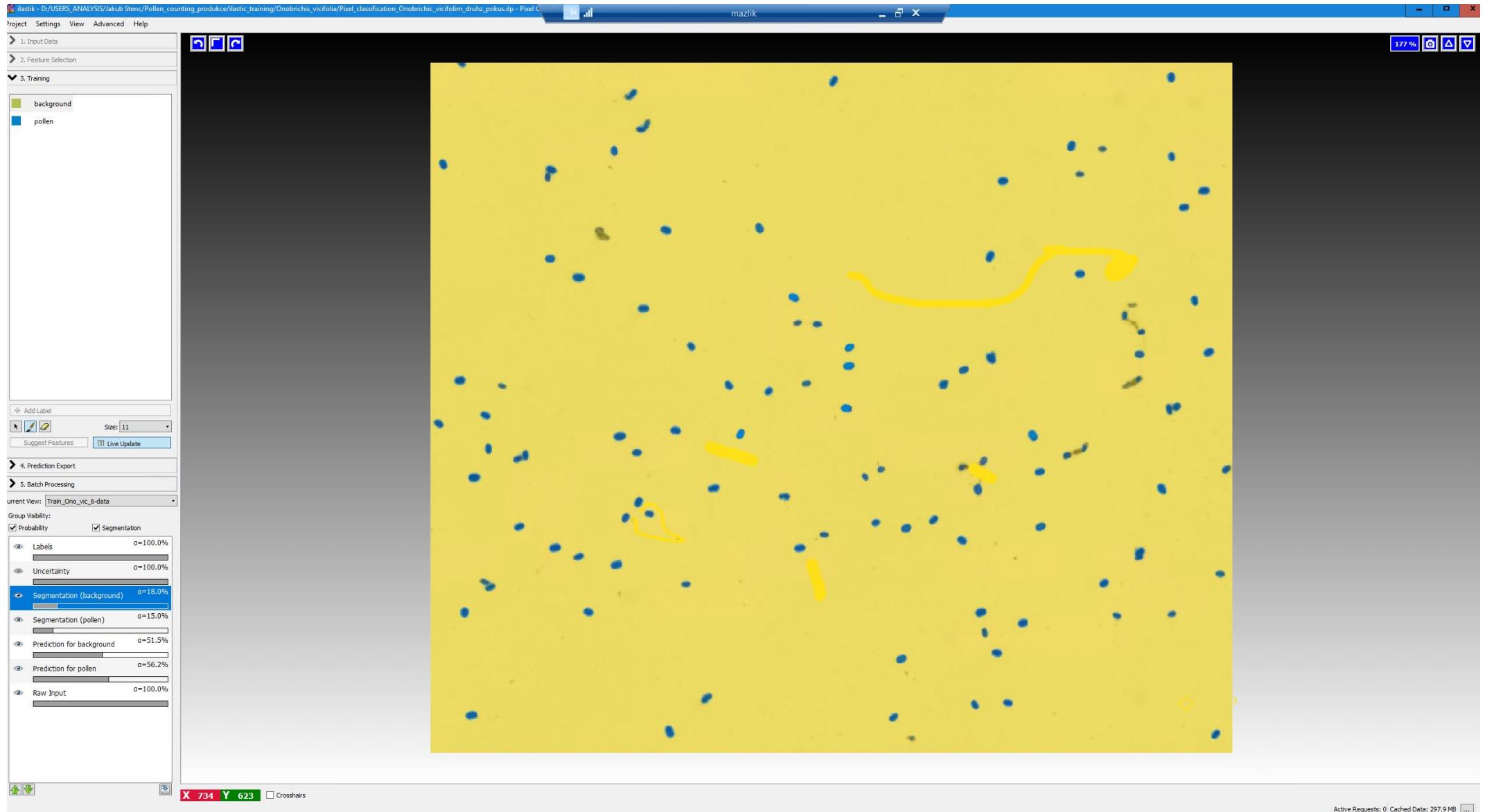
# 3. Ilastik

## Pixel classification



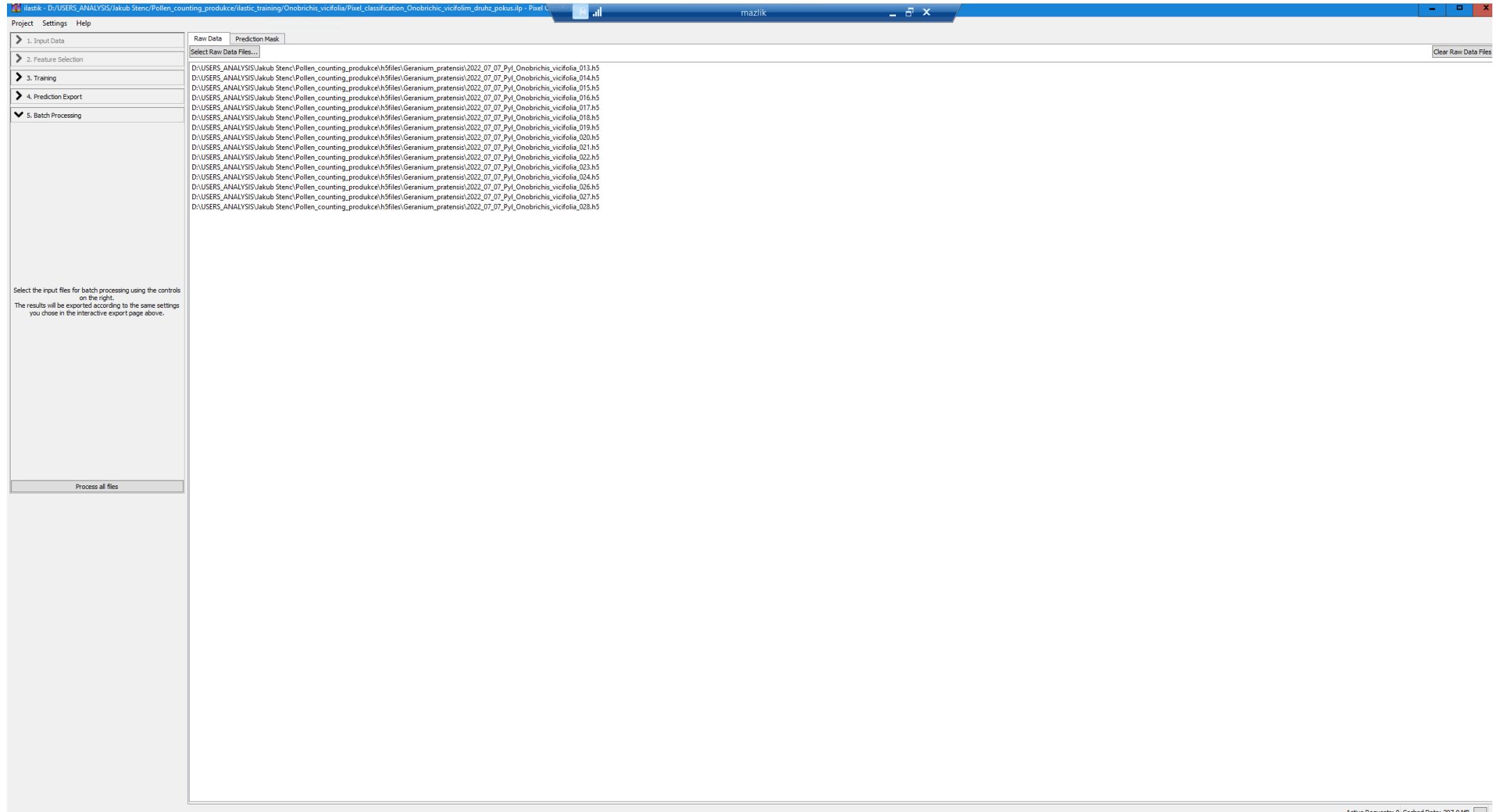
# 3. Ilastik

## Pixel classification



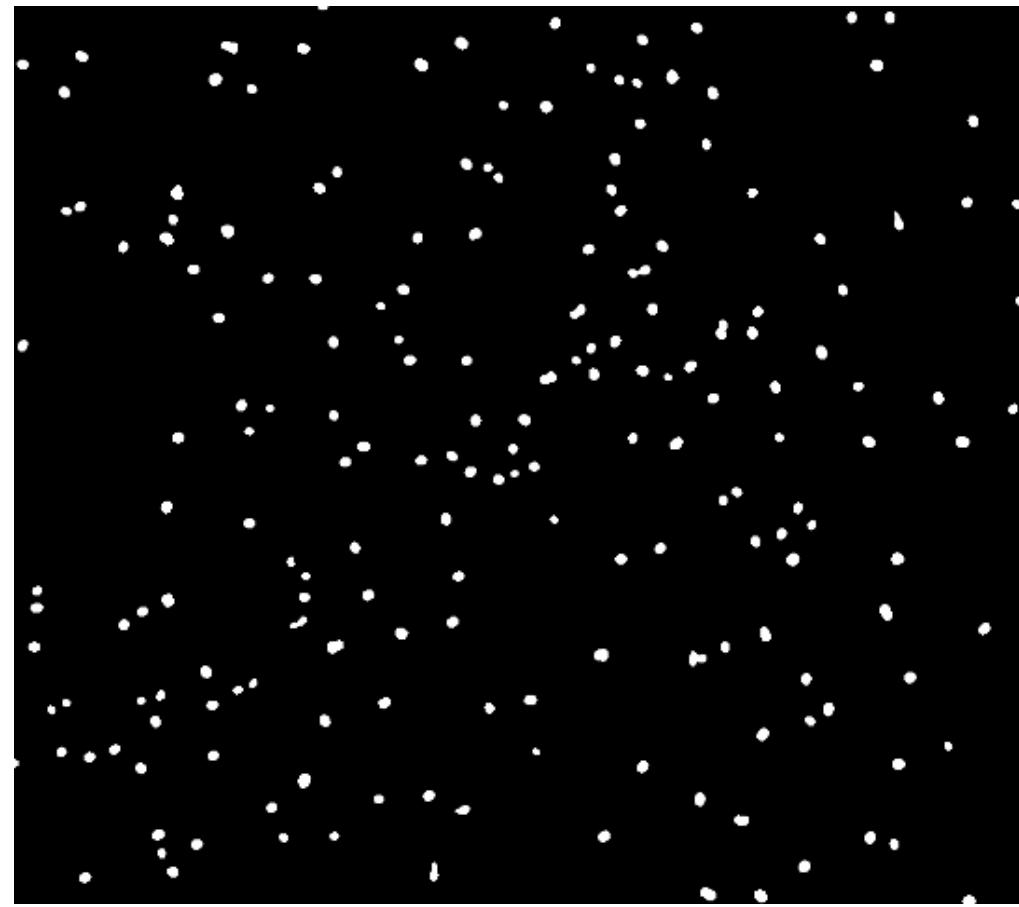
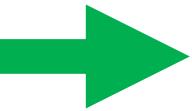
# 3. Ilastik

## Pixel classification



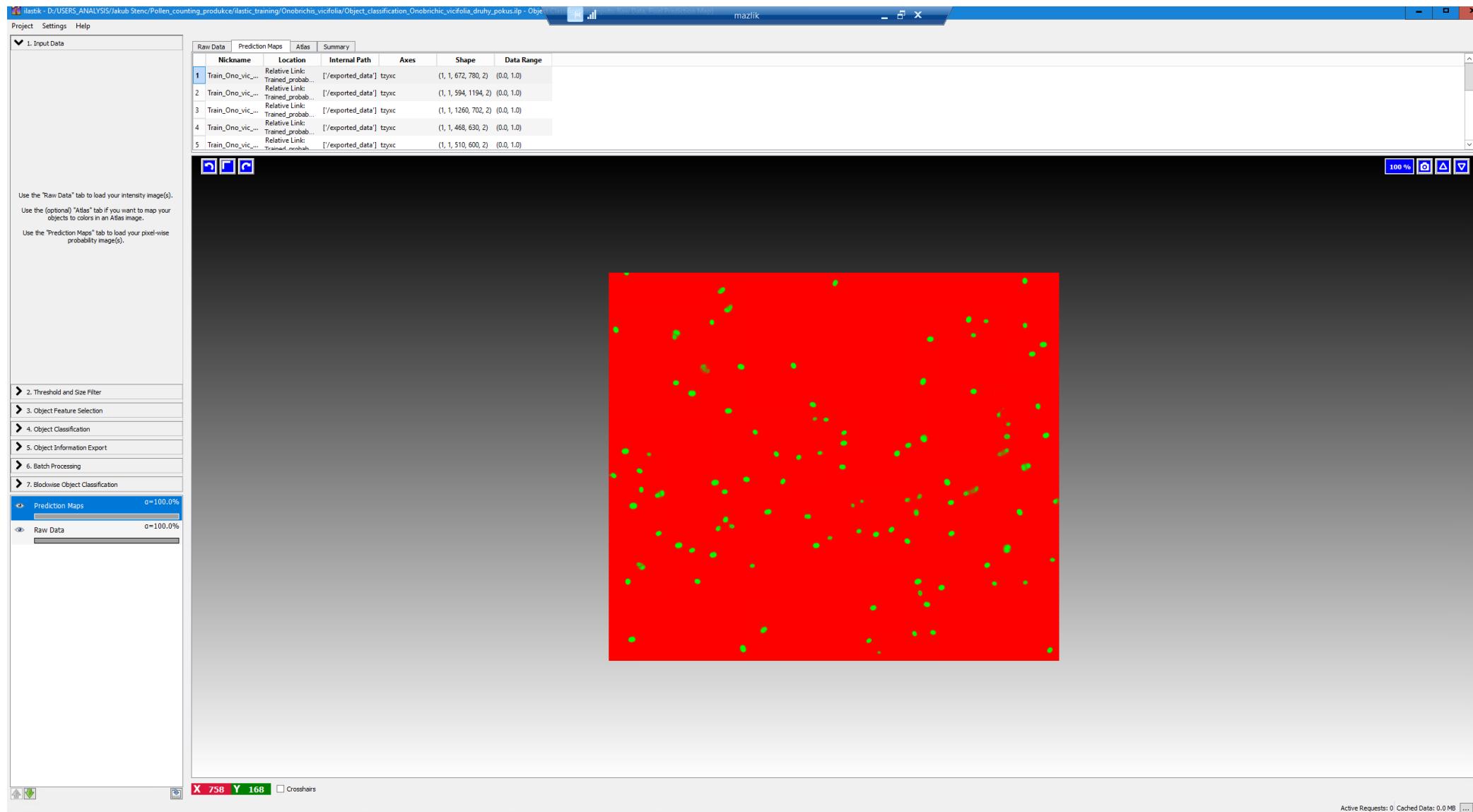
### 3. Illastik

Object classification



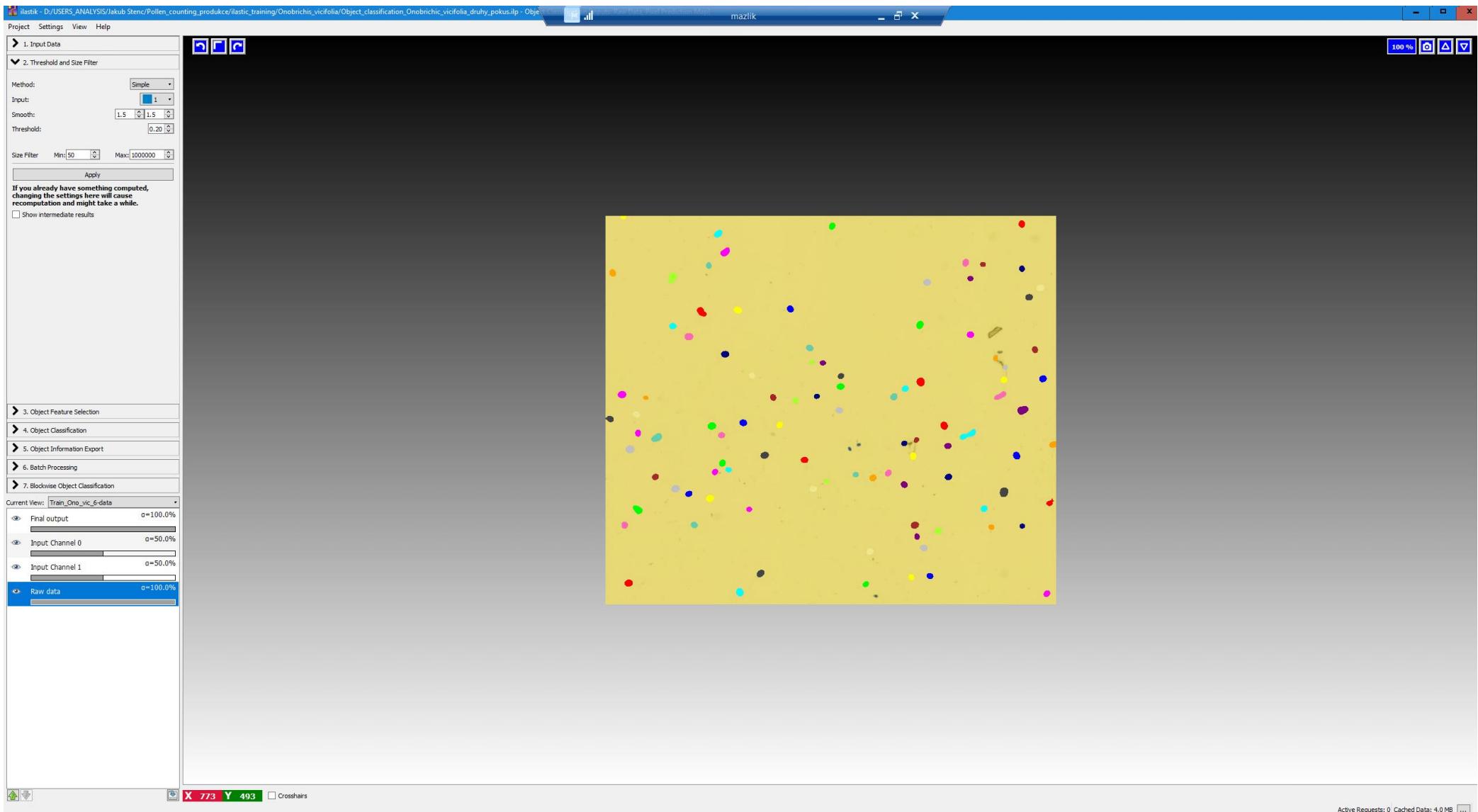
# 3. Ilastik

## Object classification



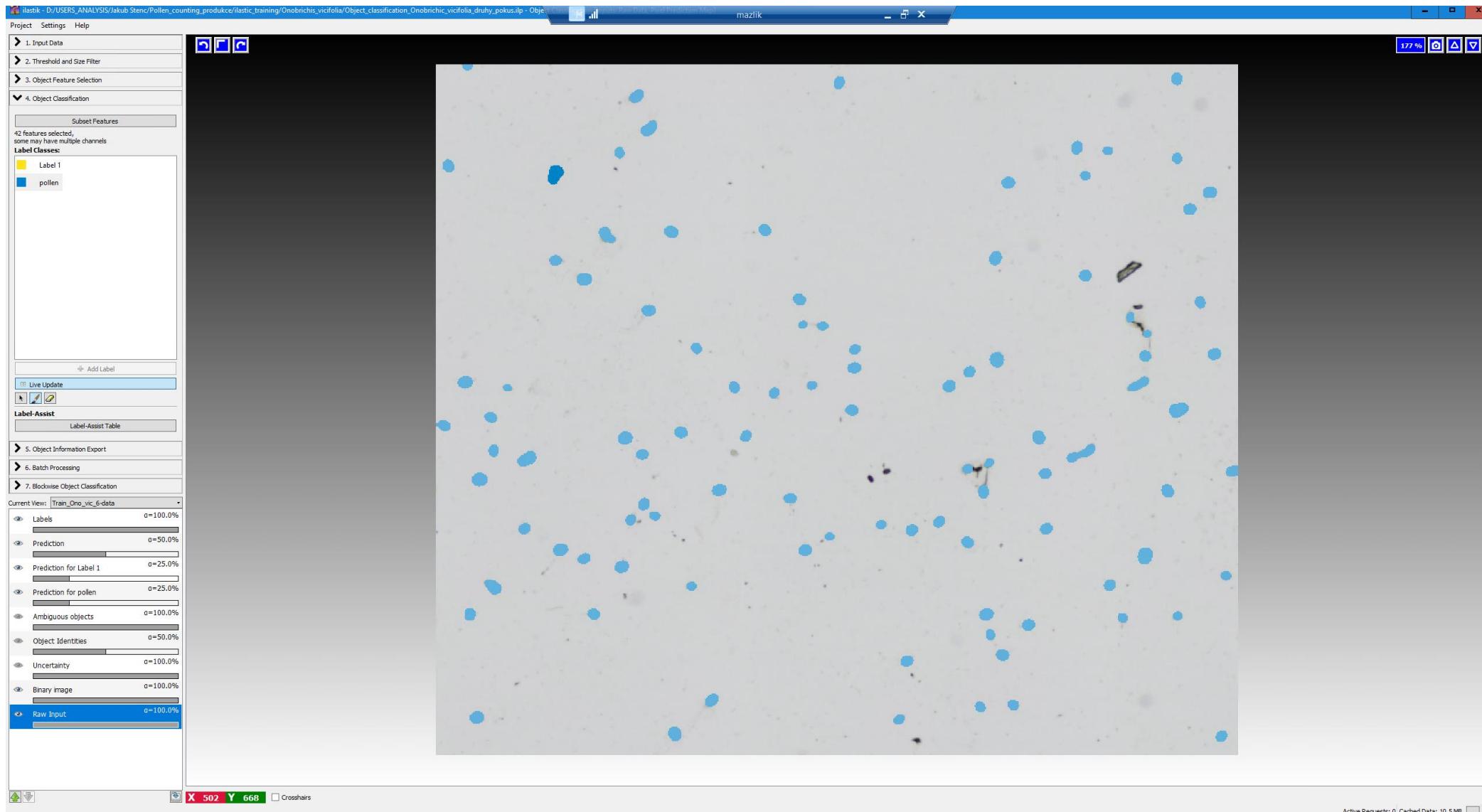
# 3. Ilastik

## Object classification



# 3. Ilastik

## Object classification





# 4. Counting

## FIJI script

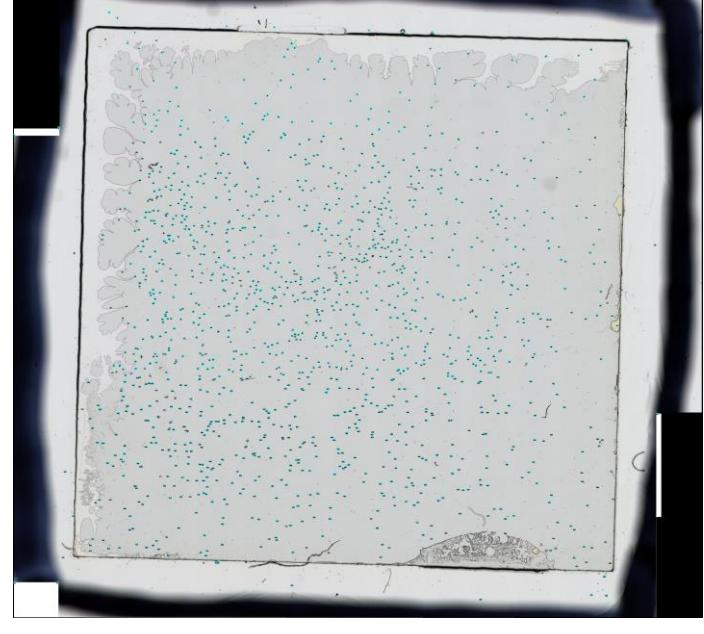
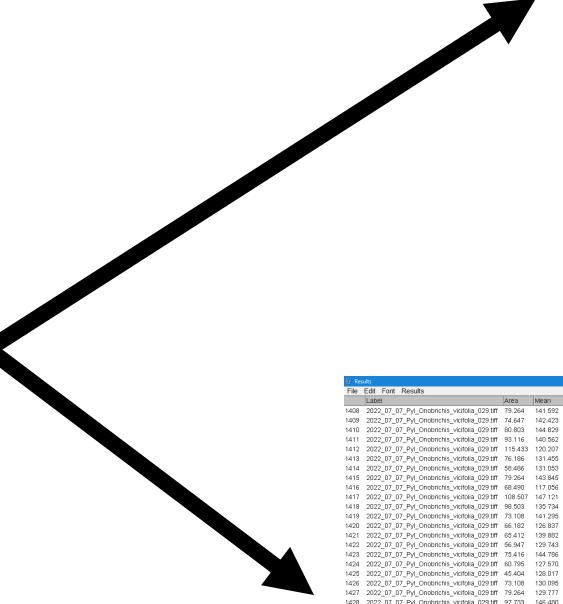
```
*Pollen_counting_Script_1_export_H5_file_Onobrichis_vicifolia.ijm | Pollen_counting_Script_1_export_H5.ijm | *Pollen_counting_Script_1_export_H5_file.ijm | Pollen_counting_Script_2_counting_pollen_after_ilasting_Onobrichis_vicifolia.ijm |
```

```
1
2
3
4 //This macro takes raw axioscan pylova data and process them
5 //summary window is not saved. Save it manually
6 var path, outputpath, labeloutputpath, h5outputpath,dataDir, dataDir2, fileList, fileList2, pollenoutputpath, dataDirTiff, tiffoutputpath,dataDirPollen,roioutputpath;
7
8
9 var name//=replace(file, ".h5", "")/;
10 var sizeMin=50;
11 var sizeMax=500000;
12 var circMin=0.50;
13
14
15 //how to make meaningful parallel folders
16
17 outputpath="D:\\USERS_ANALYSIS\\Jakub Stenc\\Pollen_counting_produke\\results\\Onobrichis_vicifolia\\";
18 roioutputpath="D:\\USERS_ANALYSIS\\Jakub Stenc\\Pollen_counting_produke\\roi\\Onobrichis_vicifolia\\";
19 dataDir = "D:\\USERS_ANALYSIS\\Jakub Stenc\\Pollen_counting_produke\\objectclass\\Onobrichis_vicifolia\\";
20 dataDirTiff="D:\\USERS_ANALYSIS\\Jakub Stenc\\Pollen_counting_produke\\tiffoutputpath\\Onobrichis_vicifolia\\";
21 tiffoutputpath="D:\\USERS_ANALYSIS\\Jakub Stenc\\Pollen_counting_produke\\tiff\\con_roi\\Onobrichis_vicifolia\\";
22 dataDirPollen="D:\\USERS_ANALYSIS\\Jakub Stenc\\Pollen_counting_produke\\pollen\\Onobrichis_vicifolia\\";
23
24 setBatchMode(true);
25
26
27 fileList = getFileList(dataDir);
28 fileList2 = getFileList(dataDirTiff);
29
30 for (f=0; f<lengthOf(fileList); f++){//*
31 if (endsWith(fileList[f],".h5")) {
32 // import image from the HS
33   fileName = dataDir+fileList[f];
34   importArgs = "select["+fileName+"] datasetname=exported_data axisorder=tzyxc";
35   run("Import HDF5", importArgs);
36   name=replace(fileList[f], ".h5","");
37 }
38 //
39
40 filename2=dataDirTiff+fileList2[f];
41
42 // import image from the HS
43 fileName2 = dataDirTiff+fileList2[f];
44 run("Bio-formats", "open["+fileName2+"] autoscale color_mode=Default rois_import=[ROI manager] view=Hyperstack stack_order=XYCZY");
45
46 name2=replace(fileList2[f], ".tiff","");
47
48
49
50 selectWindow(fileName+ "\\exported_data");
51 doCountPollen();
52 selectWindow(name2);
53 selectWindow(name2+".tiff");
54 selectImage(name2+".tiff");
55 doCropPollen();
56
57 close("");
58
59
60
61
62 }
63
64 // Main function / counting pollen and
65
66 function doCountPollen() {
67
68 dotsMask=getTitle();
69 selectWindow(dotsMask);
70
71
72 //tAutoThreshold("Default dark");
73
74 run("Convert to Mask");
75 run("Fill Holes");
76 run("Watershed");
77
78 run("Threshold...");
```

1. Open H5 file
2. Filter objects by size and roundness
3. Measure objects
4. Save results
5. Project roi and save roi projection

And this for all files

# 4. Counting FIJI script



Results		mazlik																										AR		Round	Solidity							
File	Edit	Font	Results	Label	Area	Mean	StdDev	Mode	Min	Max	X	Y	XM	YM	Perim	BX	BY	Width	Height	Major	Minor	Angle	Circ.	Feret	IntDen	Median	Skew	Kurt	%Area	RawIntDen	Slice	FeretX	FeretY	FeretAngle	MinFeret	AR	Round	Solidity
1408	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	79.264	141.592	58.896	204	40	207	3871.453	3655.081	3871.468	3655.108	36.627	3866.007	3650.205	10.527	9.650	10.958	9.210	162.428	0.742	13.416	11223.175	164	-0.461	-1.364	100	14584.000	1	4407	4164	153.435	11.000	1.190	0.841	0.920	
1409	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	74.647	142.423	62.160	204	29	207	1899.614	3655.753	1899.645	3655.773	35.213	1894.843	3651.082	9.650	9.650	10.635	8.937	45.259	0.757	13.038	10631.388	167	-0.533	-1.325	100	13815.000	1	2160	4171	32.471	10.607	1.190	0.840	0.937	
1410	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	80.803	144.829	56.204	203	53	204	1151.589	3668.464	1151.579	3668.475	37.213	1146.556	3663.364	9.650	10.527	10.950	9.396	121.959	0.733	13.454	11702.607	162	-0.371	-1.566	100	15207.000	1	1308	4177	131.987	11.000	1.165	0.858	0.929	
1411	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	93.116	140.562	58.835	203	49	207	2528.846	3669.225	2528.800	3669.207	40.042	2523.826	3663.364	9.650	12.281	12.195	9.722	91.076	0.730	14.560	13088.573	162	-0.292	-1.616	100	17008.000	1	2880	4176	105.945	11.000	1.254	0.797	0.924	
1412	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	115.433	120.207	71.992	201	22	205	832.626	3673.019	832.611	3672.957	44.870	827.239	3666.873	11.404	13.159	13.758	10.683	62.173	0.720	16.140	13875.827	133	-0.164	-1.654	100	18031.000	1	945	4194	52.431	12.969	1.288	0.776	0.932	
1413	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	76.186	131.455	67.465	202	29	204	811.675	3682.428	811.588	3682.415	35.456	806.186	3677.399	10.527	9.650	10.815	8.969	145.942	0.762	13.038	10014.975	154	-0.378	-1.542	100	13014.000	1	920	4194	147.529	10.607	1.206	0.829	0.921	
1414	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	58.486	131.058	56.385	195	26	200	3896.133	3681.403	3896.150	3681.403	31.213	3891.447	3677.399	9.650	7.895	9.418	7.907	155.501	0.754	11.402	7664.757	146	-0.408	-1.252	100	9960.000	1	4438	4192	127.875	9.000	1.191	0.839	0.910	
1415	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	79.264	143.845	63.925	203	30	206	3004.695	3703.917	3004.639	3703.916	37.213	2999.291	3699.330	10.527	9.650	10.800	9.344	24.162	0.719	13.038	11401.711	172	-0.611	-1.286	100	14816.000	1	3420	4226	32.471	11.000	1.156	0.865	0.920	
1416	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	68.490	117.056	39.562	69	54	192	3146.932	3706.728	3146.909	3706.814	34.627	3411.595	3701.962	9.650	9.452	9.226	136.866	8.718	0.724	12.042	8017.213	110	0.376	-0.960	100	10418.000	1	3890	4222	131.634	11.000	1.024	0.976	0.899	
1417	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	108.507	147.121	60.961	203	29	208	2847.307	3717.022	2847.268	3717.032	42.870	2841.388	3710.735	11.404	12.281	12.270	11.260	99.514	0.742	14.866	15963.627	174	-0.602	-1.211	100	20744.000	1	3244	4230	109.654	13.000	1.090	0.918	0.928	
1418	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	98.503	135.734	69.230	203	26	205	1329.173	3719.507	1329.270	3719.445	41.799	1324.636	3713.366	9.650	12.281	12.779	9.814	84.008	0.708	15.233	13370.230	174	-0.474	-1.473	100	17374.000	1	1512	4247	66.801	11.000	1.302	0.768	0.938	
1419	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	73.108	141.295	54.359	198	47	206	2149.507	3725.551	2149.523	3725.456	36.827	2144.857	3719.507	8.772	11.404	11.667	9.798	70.872	0.685	14.318	10329.722	158	-0.316	-1.430	100	13423.000	1	2447	4253	65.225	9.803	1.462	0.684	0.905	
1420	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	66.182	126.837	47.274	101	48	203	1067.002	3732.115	1066.942	3732.031	33.213	1062.340	3727.402	8.772	9.650	10.028	8.403	116.658	0.754	12.207	8394.294	122	0.120	-1.337	100	10908.000	1	1212	4250	124.992	10.000	1.193	0.838	0.920	
1421	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	65.412	139.882	60.886	203	43	205	1825.360	3734.910	1825.338	3734.841	33.213	1821.155	3730.034	8.772	9.650	9.593	8.682	94.217	0.745	11.705	9149.996	162	-0.404	-1.479	100	11890.000	1	2079	4252	109.983	10.000	1.105	0.905	0.909	
1422	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	56.947	129.743	56.500	56	37	205	3751.277	3742.173	3751.256	3742.145	32.042	3745.824	3737.929	10.527	7.895	10.392	6.977	159.525	0.697	12.649	7388.487	129	-0.153	-1.489	100	9601.000	1	4270	4264	161.565	8.854	1.489	0.671	0.886	
1423	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	75.416	144.786	49.535	201	56	204	2903.466	3743.989	2903.539	3743.973	35.799	2899.286	3738.806	8.772	10.527	10.799	8.892	95.232	0.739	13.000	10919.201	153	-0.232	-1.529	100	14189.000	1	3307	4262	112.620	10.000	1.215	0.823	0.933	
1424	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	60.795	127.570	42.407	93	58	203	554.212	3744.053	554.016	3744.216	32.042	549.154	3739.684	9.650	8.772	9.376	8.256	140.772	0.744	11.402	7755.565	121	0.259	-1.101	100	10078.000	1	629	4263	127.875	9.899	1.136	0.880	0.898	
1425	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	45.404	128.017	58.773	165	18	202	1779.858	3745.505	1779.854	3745.449	27.213	1775.538	3741.438	7.895	7.895	7.689	7.519	92.786	0.770	9.487	5812.441	139	-0.483	-1.128	100	7553.000	1	2028	4265	108.435	9.000	1.023	0.978	0.894	
1426	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	73.108	130.095	58.346	39	36	204	1830.698	3752.136	1830.725	3752.144	35.213	1826.418	3746.702	8.772	10.527	10.776	8.638	106.067	0.741	13.038	9510.917	138	-0.202	-1.476	100	12359.000	1	2083	4272	122.471	10.000	1.247	0.802	0.922	
1427	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	79.264	129.773	48.989	83	58	203	626.781	3754.533	626.800	3754.517	36.627	621.965	3749.333	9.650	10.527	11.937	8.454	125.363	0.742	14.422	10286.627	121	0.153	-1.506	100	13367.000	1	710	4274	123.690	10.286	1.412	0.708	0.932	
1428	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	97.733	146.784	61.726	203	45	205	997.891	3757.854	997.942	3757.830	40.284	992.161	3751.965	11.404	11.404	12.130	10.259	42.325	0.757	14.422	14316.012	180	-0.557	-1.419	100	18603.000	1	1131	4287	33.690	12.021	1.182	0.846	0.927	
1429	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	56.947	122.568	34.846	134	54	194	4112.547	3766.001	4112.573	3765.978	30.971	4108.125	3761.615	7.895	8.772	9.209	7.873	90.000	0.746	11.180	6979.854	128	0.087	-0.782	100	9070.000	1	4686	4288	116.565	9.000	1.170	0.855	0.937	
1430	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	117.742	140.660	61.221	201	36	205	859.160	3782.367	859.101	3782.337	45.297	855.557	3775.651	11.404	10.527	10.802	10.133	101.133	0.708	16.492	16561.570	168	-0.414	-1.546	100	21521.000	1	977	4304	104.036	12.944	1.285	0.778	0.913	
1431	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	87.729	139.658	60.703	205	41	205	2564.672	3783.677	2564.738	3783.670	38.070	2558.916	3788.282	11.404	10.527	11.799	9.467	19.630	0.730	14.318	12252.068	164	-0.326	-1.602	100	15921.000	1	2917	4316	24.775	11.384	1.246	0.802	0.908	
1432	2022	07	07_Pyl_Onobruchis_vicifolia_029.tif	119.281	124.129	41.065	116	42	198	1452.846	3784.884	1452.922	3784.986	44.627</td																								

Results				mazlik																															
File	Edit	Font	Results																																
Label	Area	Mean	StdDev	Mode	Min	Max	X	Y	XM	YM	Perim.	BX	BY	Width	Height	Major	Minor	Angle	Circ.	Feret	IntDen	Median	Skew	Kurt	%Area	RawIntDen	Slice	FeretX	FeretY	FeretAngle	MinFeret	AR	Round	Solidity	
1408	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	79.264	141.592	58.896	204	40	207	3871.453	3655.081	3871.468	3655.108	36.627	3866.007	3650.205	10.527	9.650	10.958	9.210	162.428	0.742	13.416	11223.175	164	-0.461	-1.364	100	14584.000	1	4407	4164	153.435	11.000	1.190	0.841	0.920
1409	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	74.647	142.423	62.160	204	29	207	1899.614	3655.753	1899.645	3655.773	35.213	1894.843	3651.082	9.650	9.650	10.635	8.937	45.259	0.757	13.038	10631.388	167	-0.533	-1.325	100	13815.000	1	2160	4171	32.471	10.607	1.190	0.840	0.937
1410	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	80.803	144.829	56.204	203	53	204	1151.589	3668.464	1151.579	3668.475	37.213	1146.556	3663.364	9.650	10.527	10.950	9.396	121.959	0.733	13.454	11702.607	162	-0.371	-1.566	100	15207.000	1	1308	4177	131.987	11.000	1.165	0.858	0.929
1411	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	93.116	140.562	58.835	203	49	207	2528.846	3669.225	2528.800	3669.207	40.042	2523.826	3663.364	9.650	12.281	12.195	9.722	91.076	0.730	14.560	13088.573	162	-0.292	-1.616	100	17008.000	1	2880	4176	105.945	11.000	1.254	0.797	0.924
1412	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	115.433	120.207	71.992	201	22	205	832.626	3673.019	832.611	3672.957	13.758	10.683	62.173	0.720	16.401	13875.827	133	-0.164	-1.654	100	18031.000	1	945	4194	52.431	12.969	1.288	0.776	0.932					
1413	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	76.186	131.455	67.465	202	29	204	811.675	3682.428	811.500	3682.428	13.758	10.693	145.942	0.762	13.038	10014.975	154	-0.378	-1.542	100	13014.000	1	920	4194	147.529	10.607	1.206	0.829	0.921					
1414	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	58.486	131.058	56.385	195	26	200	3896.133	3681.400	3896.133	3681.400	13.750	10.601	145.942	0.754	11.402	7664.757	146	-0.408	-1.252	100	9960.000	1	4438	4192	127.875	9.000	1.191	0.839	0.910					
1415	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	79.264	143.845	63.925	203	30	206	3004.695	3730.034	1821.155	3730.034	8.772	9.650	9.593	8.682	94.217	0.719	13.038	11401.711	172	-0.611	-1.286	100	14816.000	1	3420	4226	32.471	11.000	1.156	0.865	0.920			
1416	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	68.490	117.056	39.526	69	54	192	3416.400	3743.973	35.799	2899.286	3738.806	8.772	10.527	10.393	9.697	159.525	0.691	12.042	8017.213	110	0.376	-0.960	100	10418.000	1	3890	4222	131.634	11.000	1.024	0.976	0.899		
1417	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	108.507	147.121	60.961	203	29	208	98.503	135.734	69.230	203	26	3730.973	15963.627	174	-0.602	-1.211	100	20744.000	1	3244	4230	109.654	13.000	1.090	0.918	0.928								
1418	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	98.503	135.734	69.230	203	26	3730.973	15963.627	13370.230	174	-0.474	-1.473	100	17374.000	1	1512	4247	66.801	11.000	1.302	0.768	0.938													
1419	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	73.108	141.295	54.359	198	47	201	3730.973	15963.627	1329.722	158	-0.316	-1.430	100	13423.000	1	2447	4253	65.225	9.803	1.462	0.684	0.905												
1420	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	66.182	126.837	47.274	101	10	101	3730.973	15963.627	1329.722	158	-0.120	-1.337	100	10908.000	1	1212	4250	124.992	10.000	1.193	0.838	0.920												
1421	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	65.412	139.882	60.886	205	20	206	3730.973	15963.627	1329.722	158	-0.120	-1.337	100	11890.000	1	2079	4252	109.983	10.000	1.105	0.905	0.909												
1422	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	56.947	129.743	56.500	195	14	195	3730.973	15963.627	1329.722	158	-0.404	-1.479	100	11890.000	1	2079	4252	109.983	10.000	1.105	0.905	0.909												
1423	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	75.416	144.786	49.537	198	47	201	3730.973	15963.627	1329.722	158	-0.153	-1.489	100	9601.000	1	4270	4264	161.565	8.854	1.489	0.671	0.866												
1424	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	60.795	127.570	42	196	16	196	3730.973	15963.627	1329.722	158	-0.232	-1.529	100	14189.000	1	3307	4262	112.620	10.000	1.215	0.823	0.933												
1425	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	45.404	128.017	5	196	14	196	3730.973	15963.627	1329.722	158	-0.270	-1.483	100	7553.000	1	2028	4265	108.435	9.000	1.023	0.978	0.894												
1426	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	73.108	130.095	60.886	205	20	206	3730.973	15963.627	1329.722	158	-0.202	-1.476	100	12359.000	1	2083	4272	122.471	10.000	1.247	0.802	0.922												
1427	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	79.264	129.777	56.500	195	14	195	3730.973	15963.627	1329.722	158	-0.153	-1.506	100	13367.000	1	710	4274	123.690	10.000	1.286	1.412	0.708	0.932											
1428	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	97.733	146.480	51.500	198	47	201	3730.973	15963.627	1329.722	158	-0.232	-1.519	100	10806.600	1	1316.012	4287	33.690	12.021	1.182	0.846	0.927												
1429	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	56.947	122.500	42	196	16	196	3730.973	15963.627	1329.722	158	-0.270	-1.511	100	9070.000	1	4686	4288	116.565	9.000	1.170	0.855	0.937												
1430	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	117.742	140.6	50	198	47	201	3730.973	15963.627	1329.722	158	-0.14	-1.546	100	21521.000	1	977	4304	104.036	12.944	1.285	0.778	0.913												
1431	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	87.729	139.600	50	198	47	201	3730.973	15963.627	1329.722	158	-0.168	-1.602	100	15921.000	1	2917	4316	24.775	11.384	1.246	0.802	0.908												
1432	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	119.281	124.100	54.359	198	47	201	3730.973	15963.627	1329.722	158	-0.18	-1.055	100	19240.000	1	1649	4311	150.255	13.000	1.138	0.879	0.951												
1433	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	117.742	140.000	51.500	198	47	201	3730.973	15963.627	1329.722	158	-0.157	-1.584	100	21551.000	1	1103	4314	145.305	13.000	1.186	0.843	0.933												
1434	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	54.638	111.000	42	196	16	196	3730.973	15963.627	1329.722	158	-0.127	-1.744	100	7881.000	1	608	4317	146.310	9.000	1.055	0.948	0.947												
1435	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	76.955	140.100	50	198	47	201	3730.973	15963.627	1329.722	158	-0.167	-1.500	100	14013.000	1	1959	4326	126.027	10.607	1.301	0.769	0.922												
1436	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	75.416	141.100	51.500	198	47	201	3730.973	15963.627	1329.722	158	-0.143	-1.285	100	16392.000	1	4513	4328	161.565	11.000	1.137	0.879	0.912												
1437	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	105.429	143.100	51.500	198	47	201	3730.973	15963.627	1329.722	158	-0.09	-1.332	100	19618.000	1	1051	4340	31.608	12.522	1.234	0.811	0.926												
1438	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	92.346	144.833	51.500	198	47	201	3730.973	15963.627	1329.722	158	-0.147	-1.386	100	17380.000	1	843	4346	81.870	11.000	1.168	0.856	0.916												
1439	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	59.256	135.948	51.500	198	47	201	3730.973	15963.627	1329.722	158	-0.161	-1.450	100	10468.000	1	2318	4339	19.983	9.000	1.224	0.817	0.917												
1440	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	75.416	143.235	51.500	198	47	201	3730.973	15963.627	1329.722	158	-0.102	-1.520	100	14037.000	1	2971	4335	141.340	10.000	1.167	0.857	0.933												
1441	2022_07_07_Pyl_Onobruchis_vicifolia_029.tif	55.408	128.972	50	198	47	201	3730.973	15963.627	1329.722	158	-0.076	-1.428	100	9286.000	1	4578	4334	106.699	9.899	1.003	0.997	0.900												
1442	2022_07_07_Pyl																																		

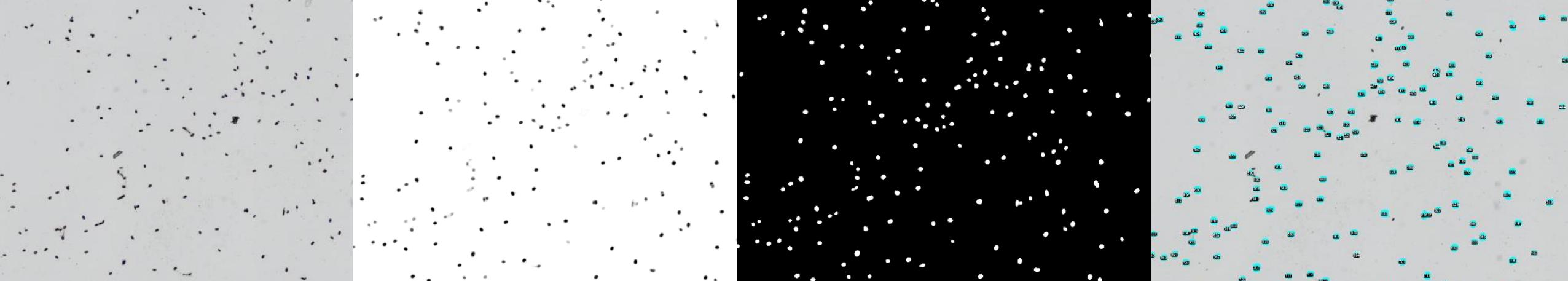
# 5. Label reading

A screenshot of the RStudio interface. The left pane shows R code for reading images, performing OCR, and writing results to a CSV file. The right pane shows a preview of the extracted text "Onovic" and "23". The bottom pane shows the command line output of the R script.

```
library("tesseract")
library("magick")
eng <- tesseract("eng")
path_label <- "C:\\Users\\jakub\\ownCloud\\R\\Pollen_production\\Onobrichis_vicifolia\\labels\\"
list_labels <- data.frame(original_name = list.files(path_label), label_name=NA)
numbers <- tesseract(options = list(tessedit_char_whitelist = "Onovic 0123456789"))
for (i in list_labels$original_name) {
  i <- "2022_07_07_Pyl_Onobrichis_vicifolia_060_label.jpg"
  input <- image_read(paste(path_label, list_labels["row.names(list_labels)[list_labels$original_name==i,]"),
  ), , $original_name, sep=""), strip = TRUE) %>%
  image_resize("996x") %>%
  image_convert(type = 'Grayscale') %>%
  image_trim(fuzz = 40)
  text<-ocr(input, engine = numbers))
  list_labels[list_labels$original_name == i]$label_name <-text}
list_labels$label_name<-gsub("\\n", "_", list_labels$label_name)
write.table(list_labels, "C:\\Users\\jakub\\ownCloud\\R\\Pollen_production\\Onobrichis_vicifolia\\labelby.xls")
read.table( "C:\\Users\\jakub\\ownCloud\\R\\Pollen_production\\Onobrichis_vicifolia\\labelby.xls")
path<- "C:\\Users\\jakub\\ownCloud\\R\\Pollen_production\\Onobrichis_vicifolia\\results\\"
potato<-list.files(path= path)
res_ults <- data.frame(potato)
for (i in c(1:length(potato))) {
  temporal_potato <- read.table(paste(path, "/", potato[i], sep = ""), header = T)
  length(temporal_potato$X)
  res_ults$pollen[i] <-length(temporal_potato$X)}
res_ults
write.csv(res_ults, "pollen_results.csv")
read.csv("pollen_results.csv")
```

Console Background XLS

```
1 JPEG 900 654 Gray FALSE 0 72x72
# ... with abbreviated variable names `colorspace`,
# ... `filesize`
```



# Thank you for attention

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