

## *Agriculture*

Semi-automated ground truth segmentation and phenotyping of plant structures using k-means clustering of Eigen-colors (kmSeg)

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### **Supplementary Information**

# Algorithm S1. Performance test of clustering methods on synthetic data.

```
% begin of the MATLAB code

% clean workspace
clear all; close all;

% loop over 100 iteration:
% by each iteration generate a bi-Gaussian distribution with a increasing number of points: 400, 800,... 40000for i=1:100

% define the number of points in each cluster starting with 200 (in both clusters - 400)
N=i*200;

% set random generator to default for better reproducibility
rng('default');

% generate a 2D bi-Gaussian (i.e. two-cluster) distribution of points

% first cluster
pd1x = makedist('Normal','mu',-2,'sigma',1); X1 = random(pd1x,N,1);
pd1y = makedist('Normal','mu',-2,'sigma',1); Y1 = random(pd1y,N,1);

% second cluster
pd2x = makedist('Normal','mu',2,'sigma',1); X2 = random(pd2x,N,1);
pd2y = makedist('Normal','mu',2,'sigma',1); Y2 = random(pd2y,N,1);

% merge both distributions together for subsequent clustering
X=[X1;X2];
Y=[Y1;Y2];
data=[];
data(:,1) = X(:);
data(:,2) = Y(:);

% total number of points in the cumulative distribution
L=size(data,1);

%%%%%%%%%%%% perform k-means clustering

% start timer
tic

% perform k-means clustering of data
[idx] = kmeans(data,2);

% stop timer
t1 = toc;

% save the number of points and calculation time for visualization of results
performance1(i,1) = L;
performance1(i,2) = t1;

%%%%%%%%%%%% perform spectral clustering

% start timer
tic

% perform spectral clustering of data
[idx] = spectralcluster(data,2);

% stop timer
t2 = toc;

% save the number of points and calculation time for visualization of results
performance2(i,1) = L;
performance2(i,2) = t2;
```

```
% perform hierarchical clustering
```

```
% start timer
```

```
tic
```

```
% perform hierarchical clustering of data
```

```
[idx] = clusterdata(data,2);
```

```
% stop timer
```

```
t3 = toc;
```

```
% save the number of points and calculation time for visualization of results
```

```
performance3(i,1) = L;
```

```
performance3(i,2) = t3;
```

```
% print output for every iteration
```

```
fprintf('i=%d\tL=%d\tkm=%3f s.\tsc=%3f s.\thc=%3f s.\n',i,L,t1,t2,t3);
```

```
end % end of the for-loop
```

```
% plot calculation time of k-means, spectral and hierarchical clustering as a function of the number of points
```

```
figuresubplot(1,2,1)
```

```
plot(X,Y,'b.')
xlabel('X');
```

```
ylabel('Y');
```

```
subplot(1,2,2)
```

```
plot(performance1(:,1),performance1(:,2),'b-','LineWidth',2)
```

```
hold on
```

```
plot(performance2(:,1),performance2(:,2),'g-','LineWidth',2)
```

```
hold on
```

```
plot(performance3(:,1),performance3(:,2),'r-','LineWidth',2)
```

```
hold off
```

```
xlabel('Number of points')
```

```
ylabel('Calculation time, sec.')
```

```
legend('k-means','spectral','hierarchical','Location','northwest')
```

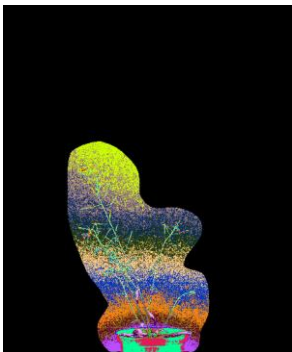
```
% end of the MATLAB code
```

Figure S1. Example of arabidopsis image segmentation (VIS, side view).

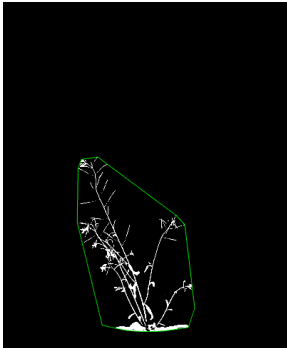
Original image



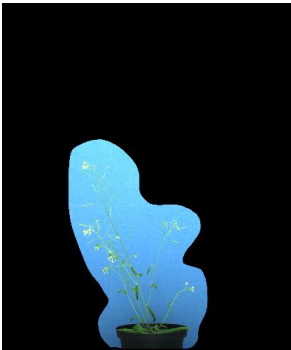
All 25 k-means color classes



Binary image & convex hull



ROI after „clean outside“



11 selected plant color classes



Segmented image



Overview of all and selected plant k-means color classes

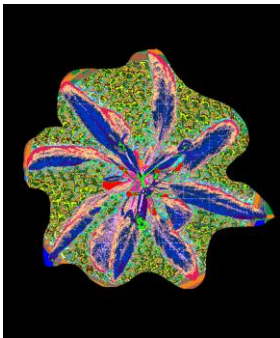


Figure S2. Example of arabidopsis image segmentation (VIS, top view).

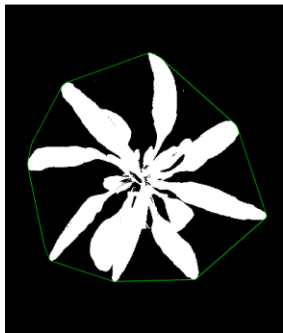
Original image



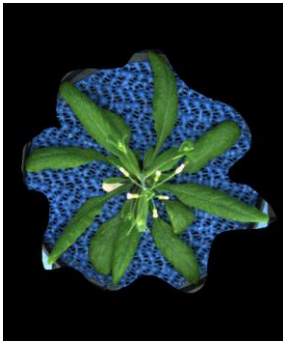
All 25 k-means color classes



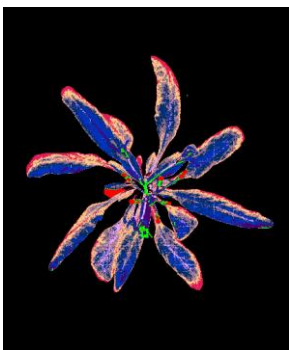
Binary image & convex hull



ROI after „clean outside“



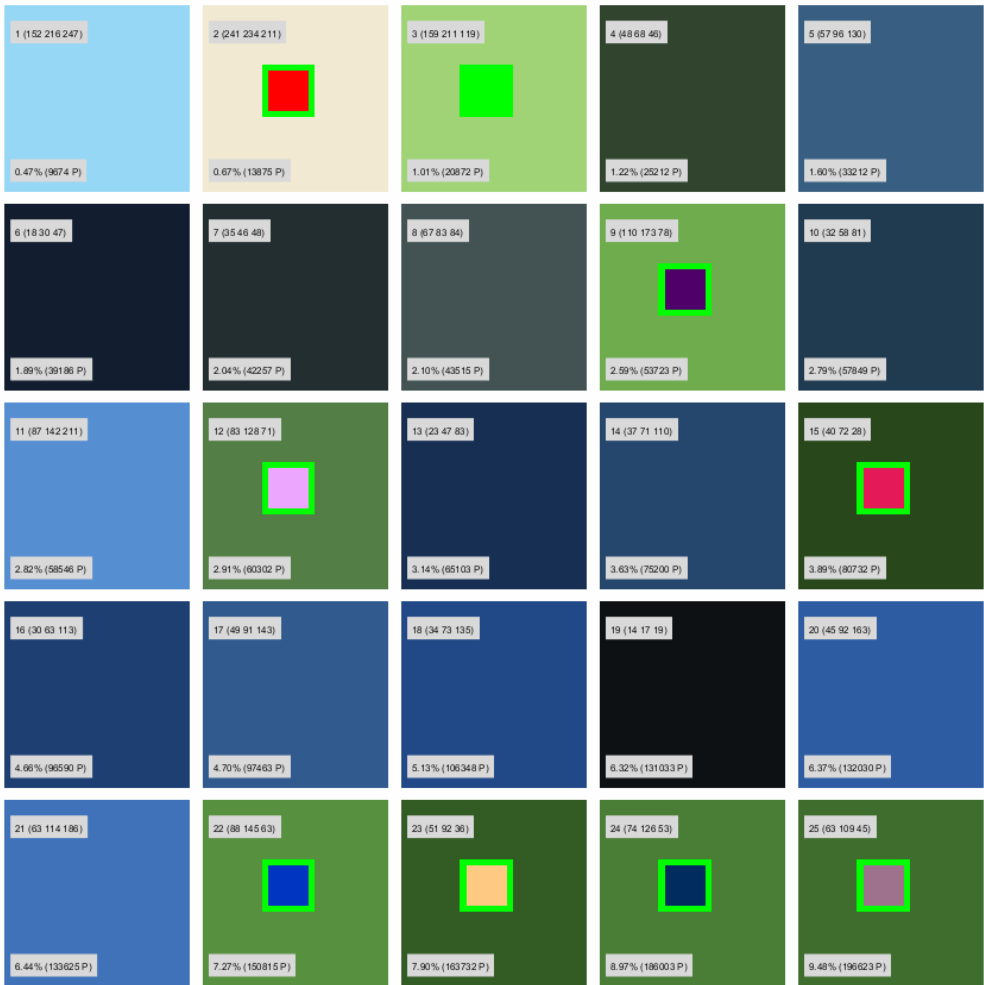
9 selected plant color classes



Segmented image



Overview of all and selected plant k-means color classes

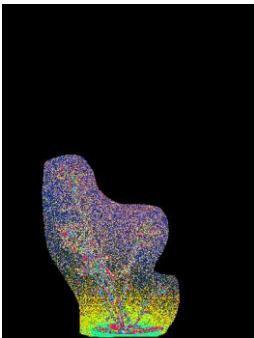


**Figure S3. Example of arabidopsis image segmentation (FLUO, side view).**

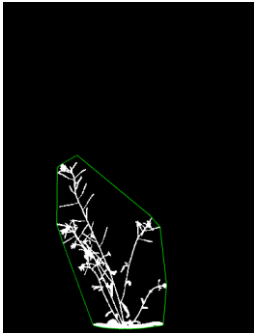
Original image



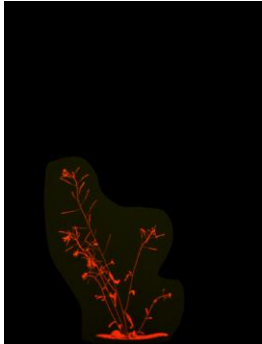
All 25 k-means color classes



Binary image & convex hull



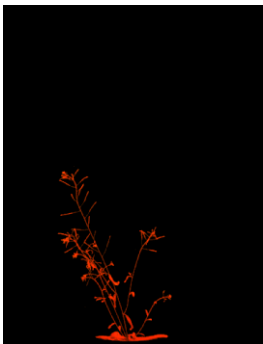
ROI after „clean outside“



12 selected plant color classes



Segmented image



Overview of all and selected plant k-means color classes

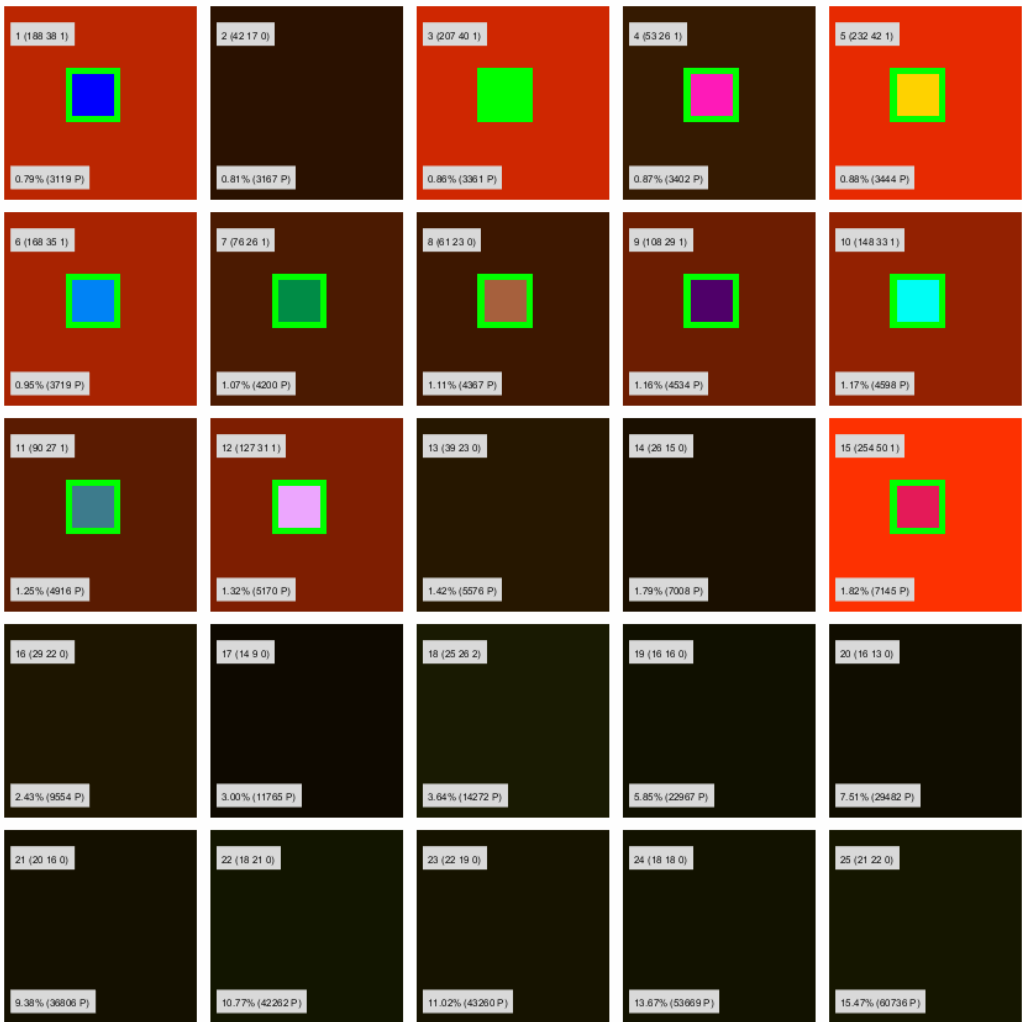
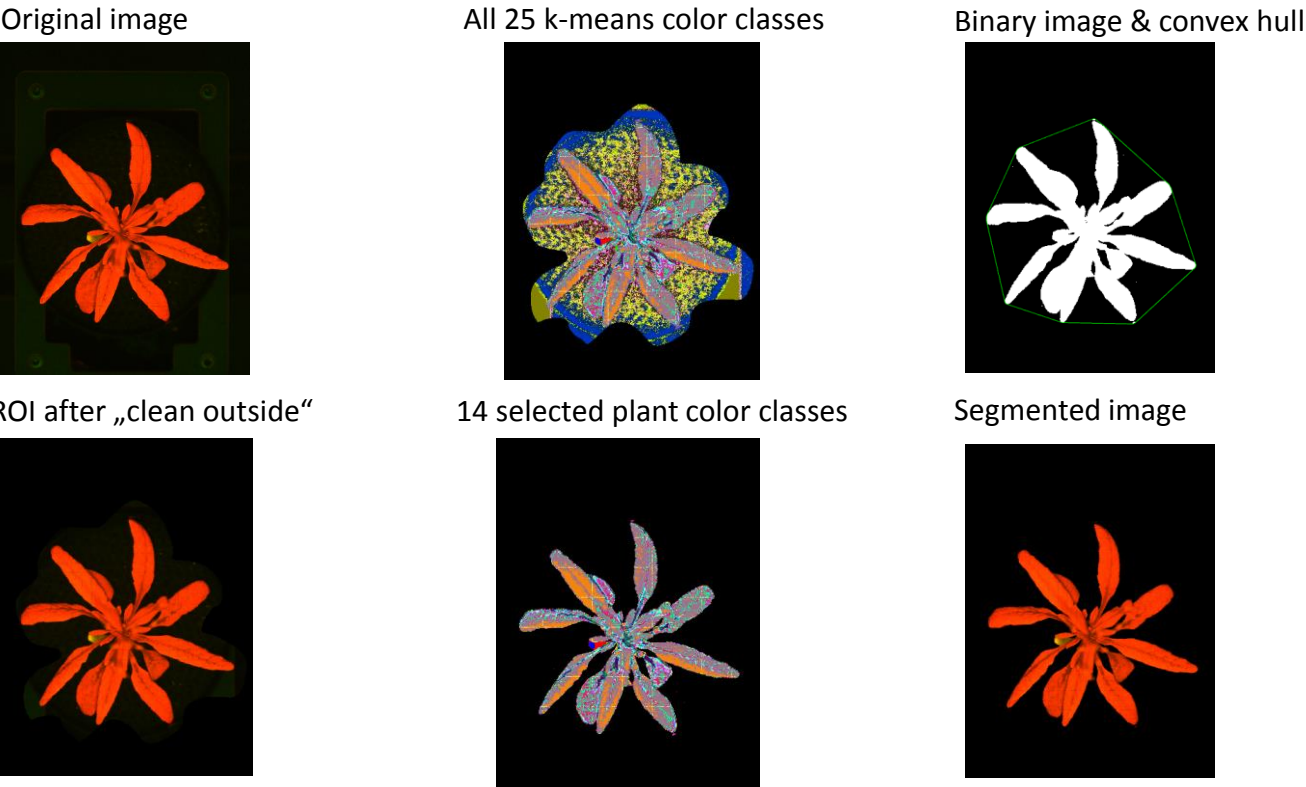


Figure S4. Example of arabidopsis image segmentation (FLUO, top view).



Overview of all and selected plant k-means color classes

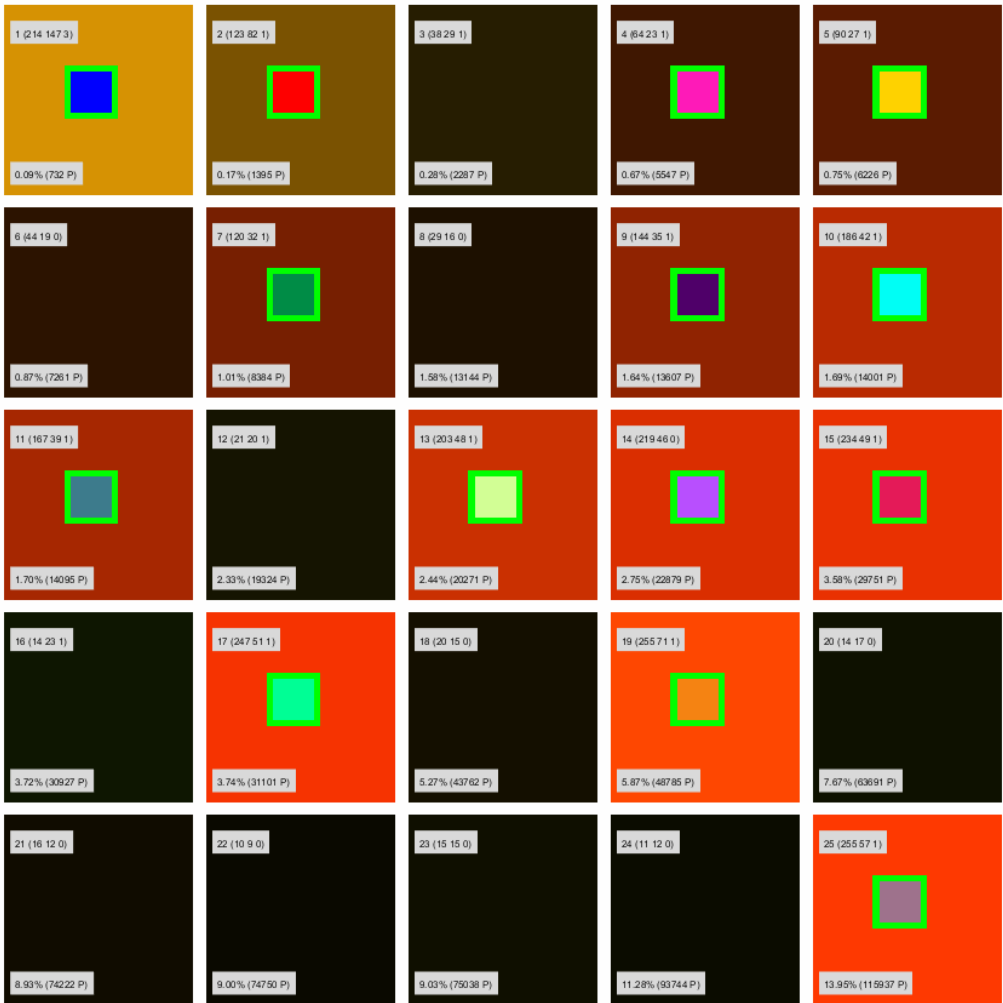
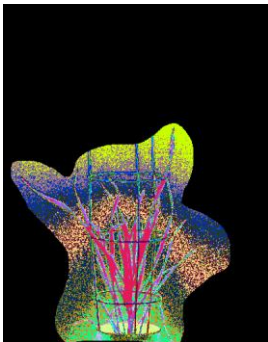


Figure S5. Example of barley image segmentation (VIS, side view).

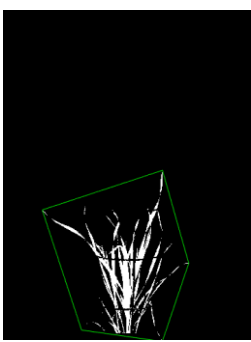
Original image



All 25 k-means color classes



Binary image & convex hull



ROI after „clean outside“



4 selected plant color classes



Segmented image



Overview of all and selected plant k-means color classes

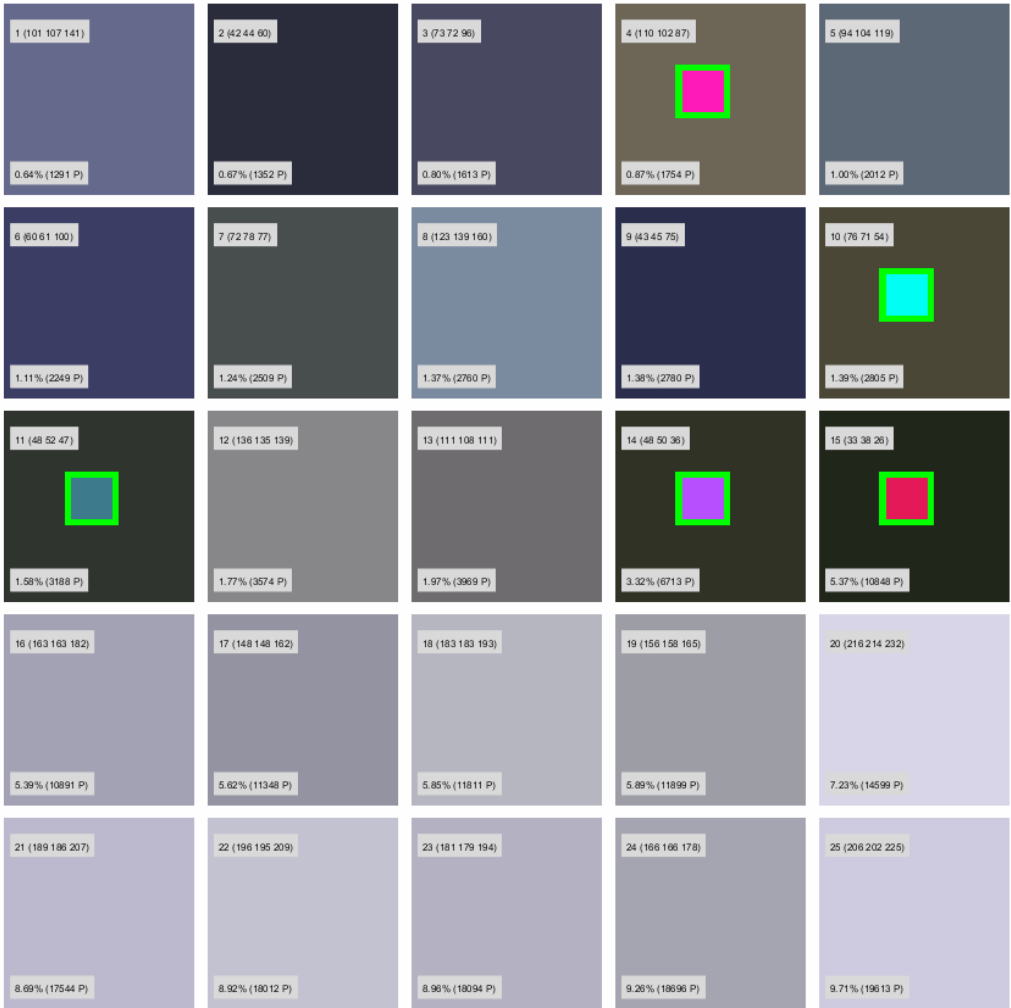
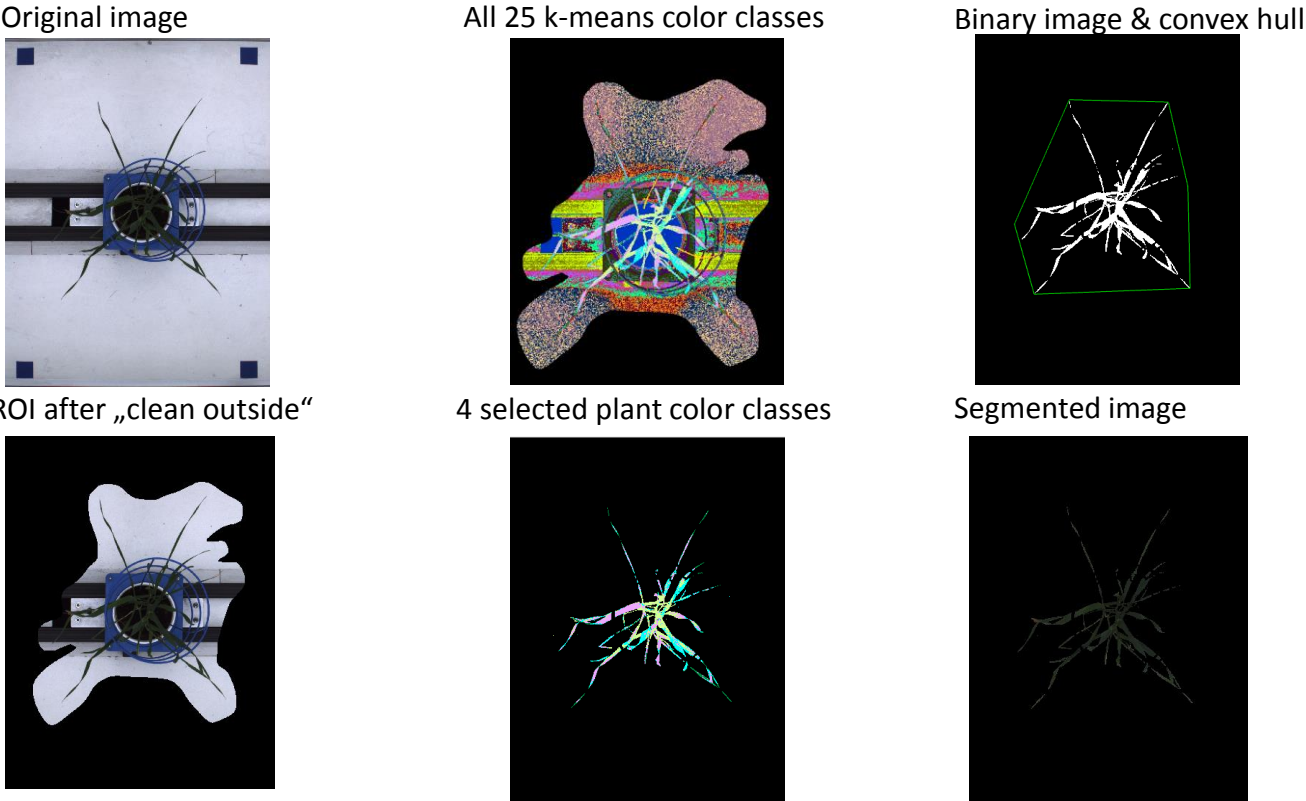




Figure S6. Example of barley image segmentation (VIS, top view).



Overview of all and selected plant k-means color classes

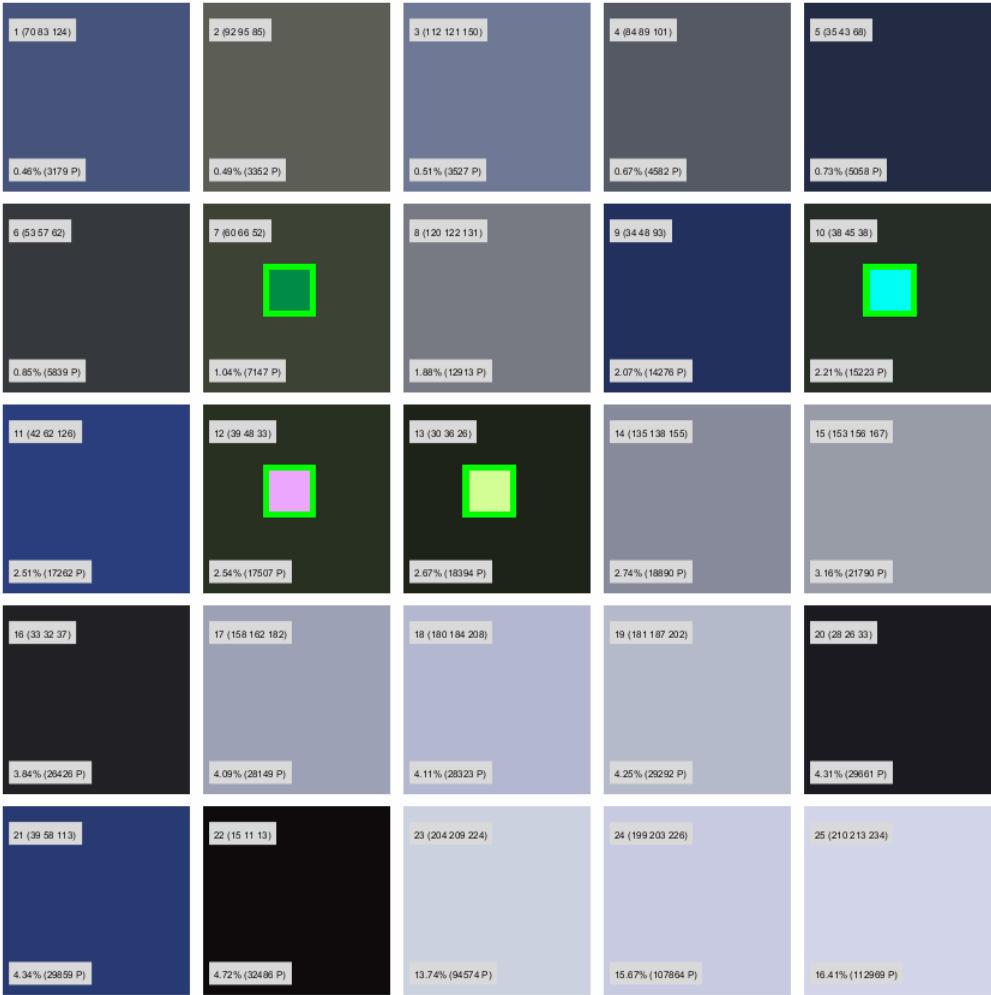
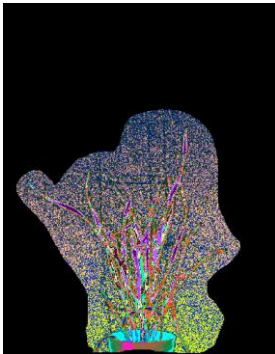


Figure S7. Example of barley image segmentation (FLUO, side view).

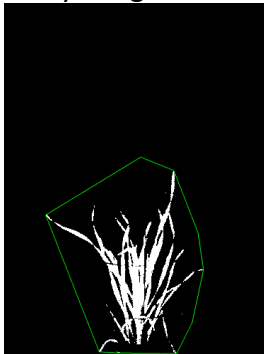
Original image



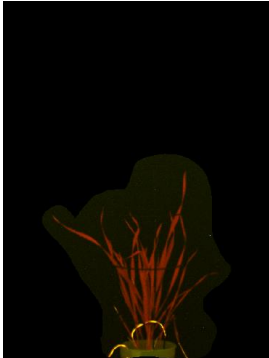
All 25 k-means color classes



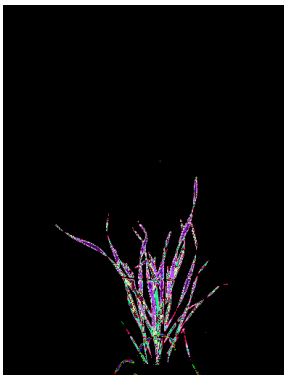
Binary image & convex hull



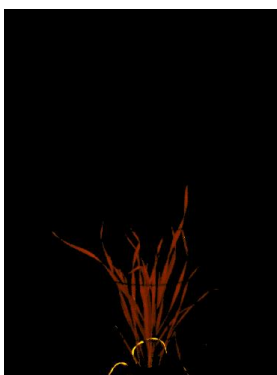
ROI after „clean outside“



10 selected plant color classes



Segmented image



Overview of all and selected plant k-means color classes

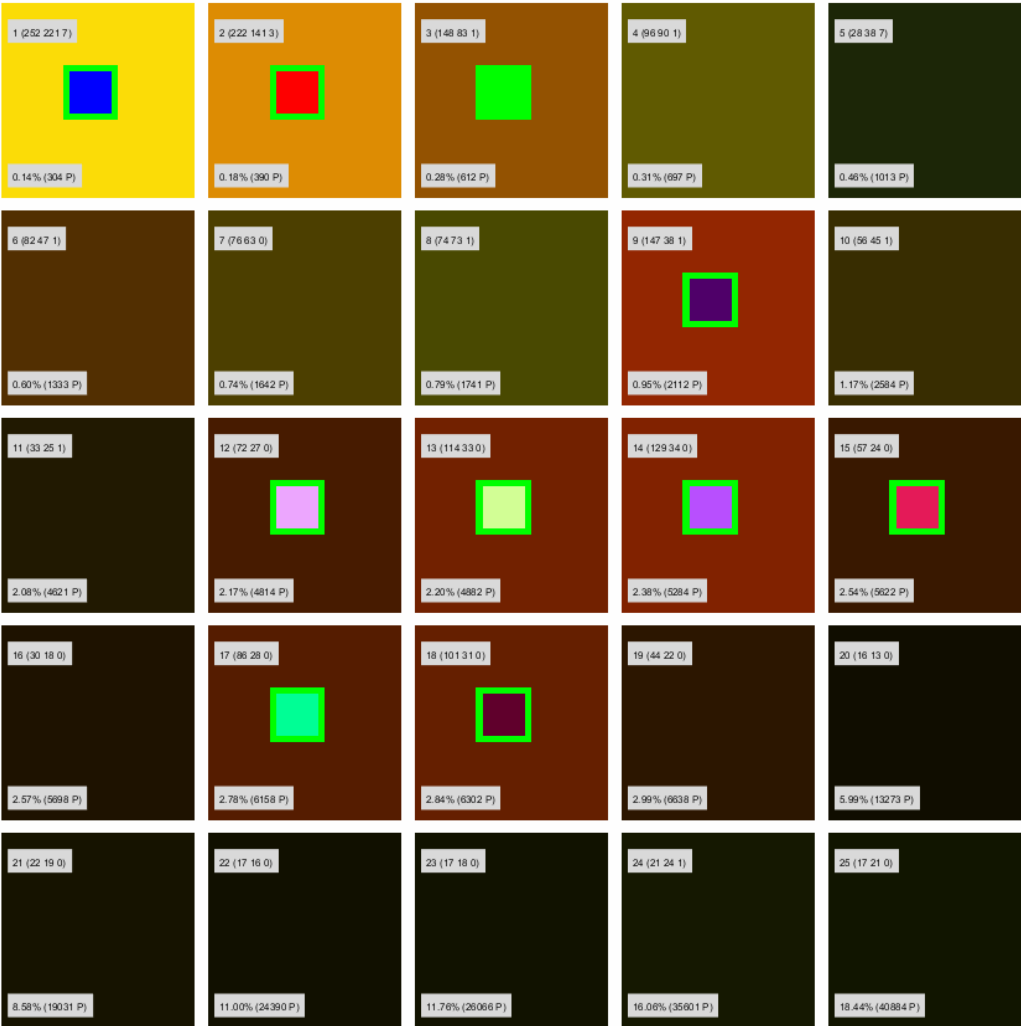
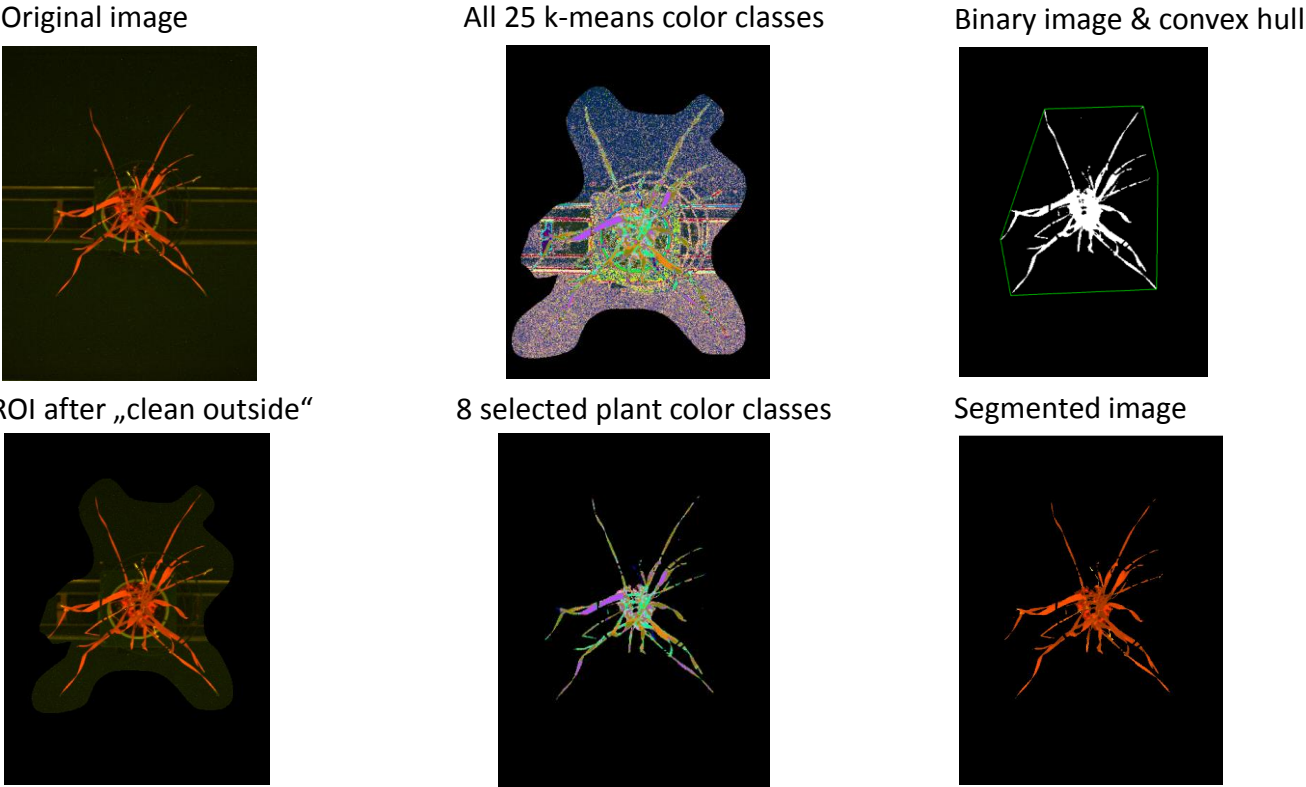


Figure S8. Example of barley image segmentation (FLUO, top view).



Overview of all and selected plant k-means color classes

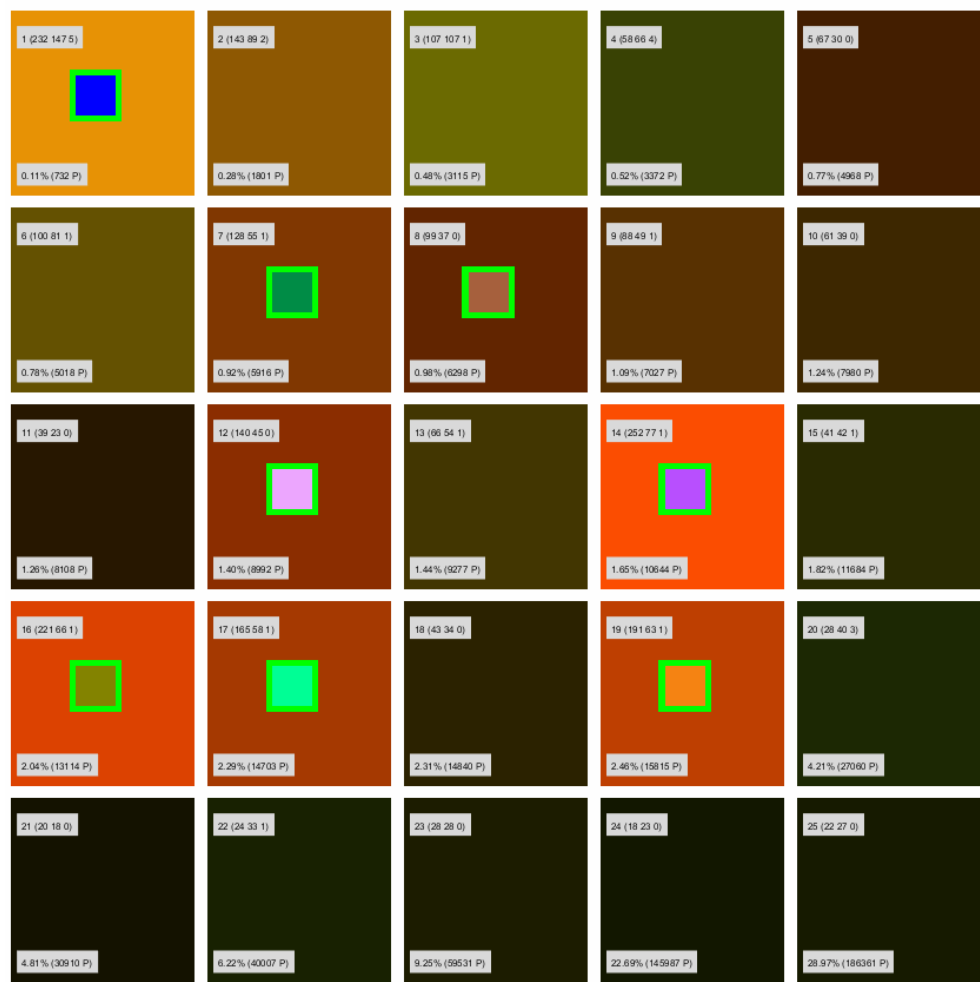
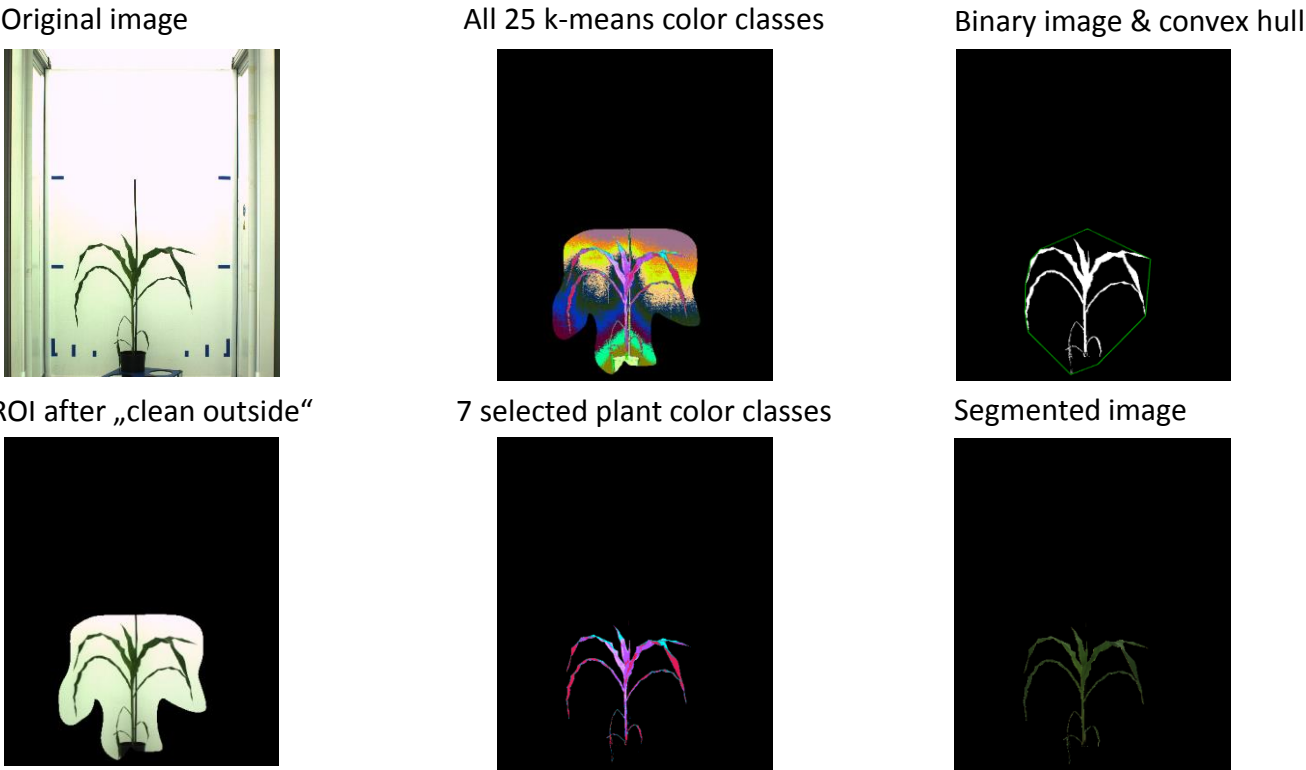


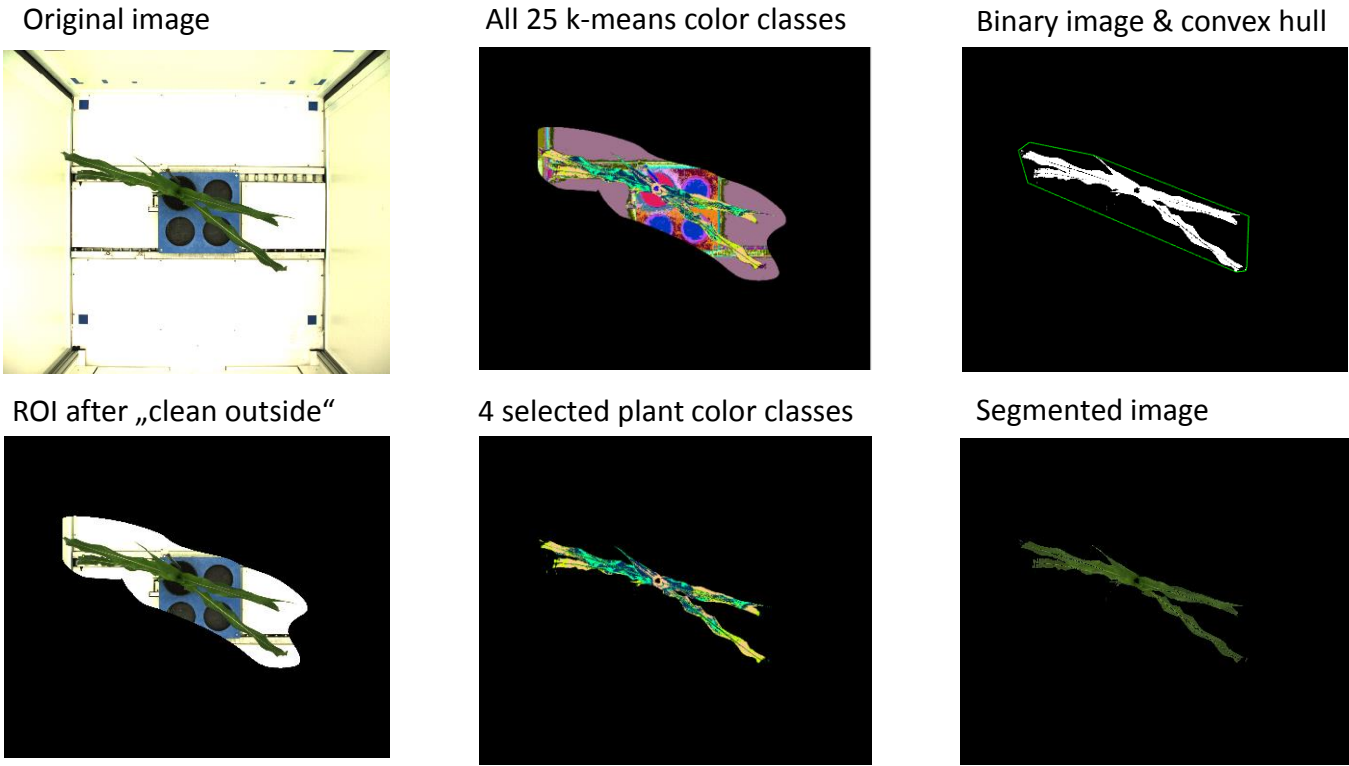
Figure S9. Example of maize image segmentation (VIS, side view).



Overview of all and selected plant k-means color classes



Figure S10. Example of maize image segmentation (VIS, top view).



Overview of all and selected plant k-means color classes

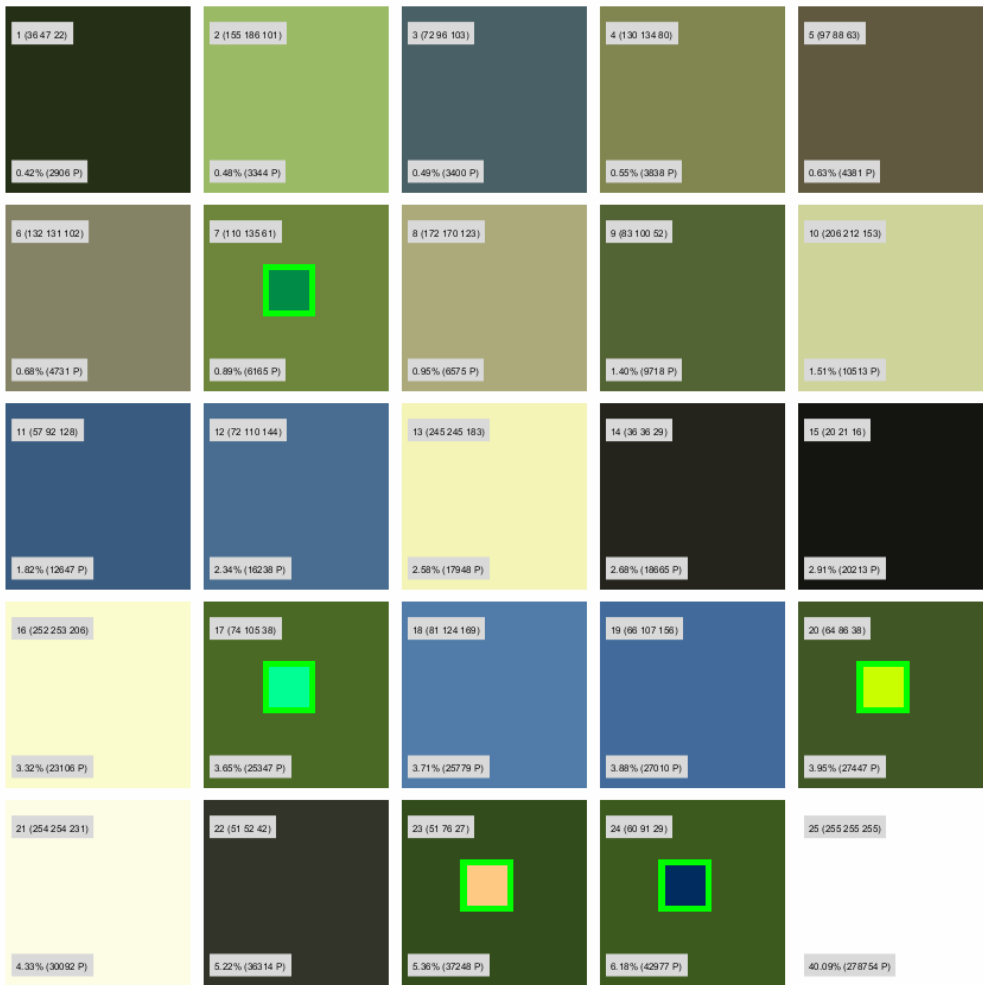
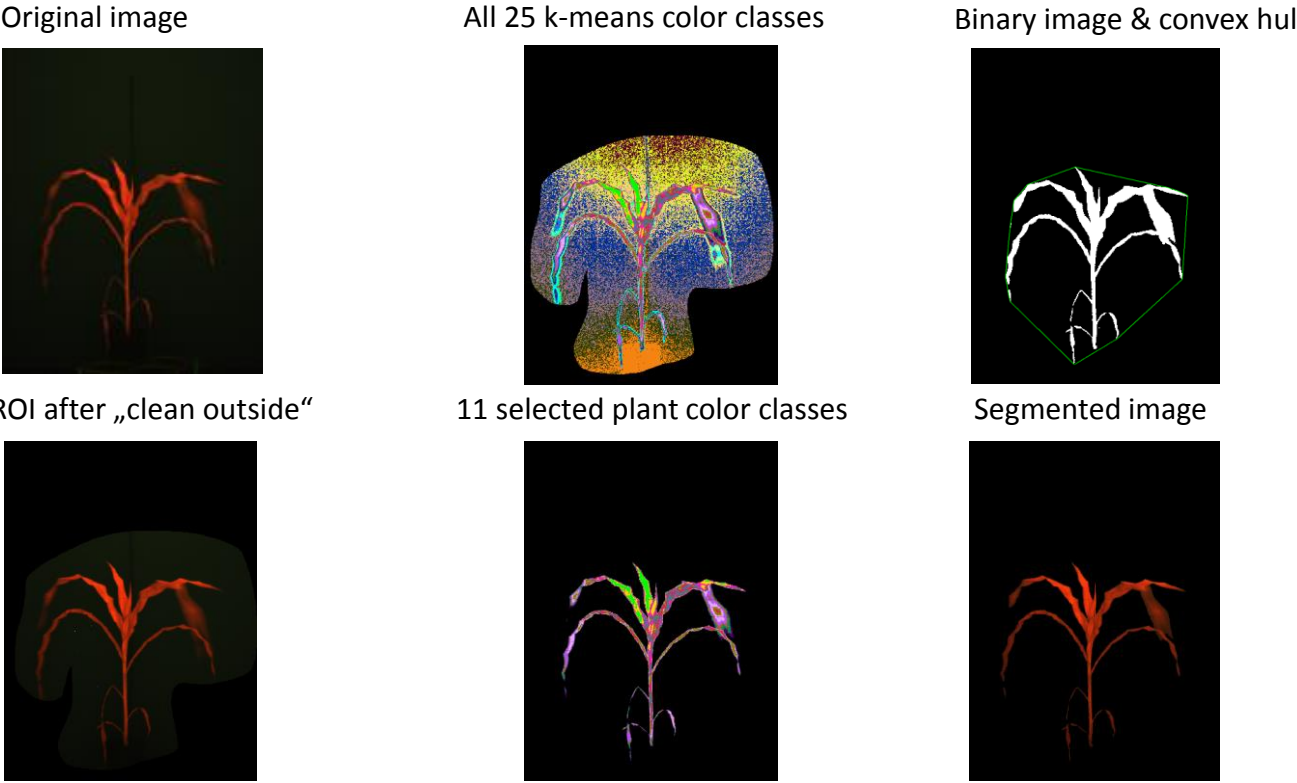


Figure S11. Example of maize image segmentation (FLUO, side view).



Overview of all and selected plant k-means color classes

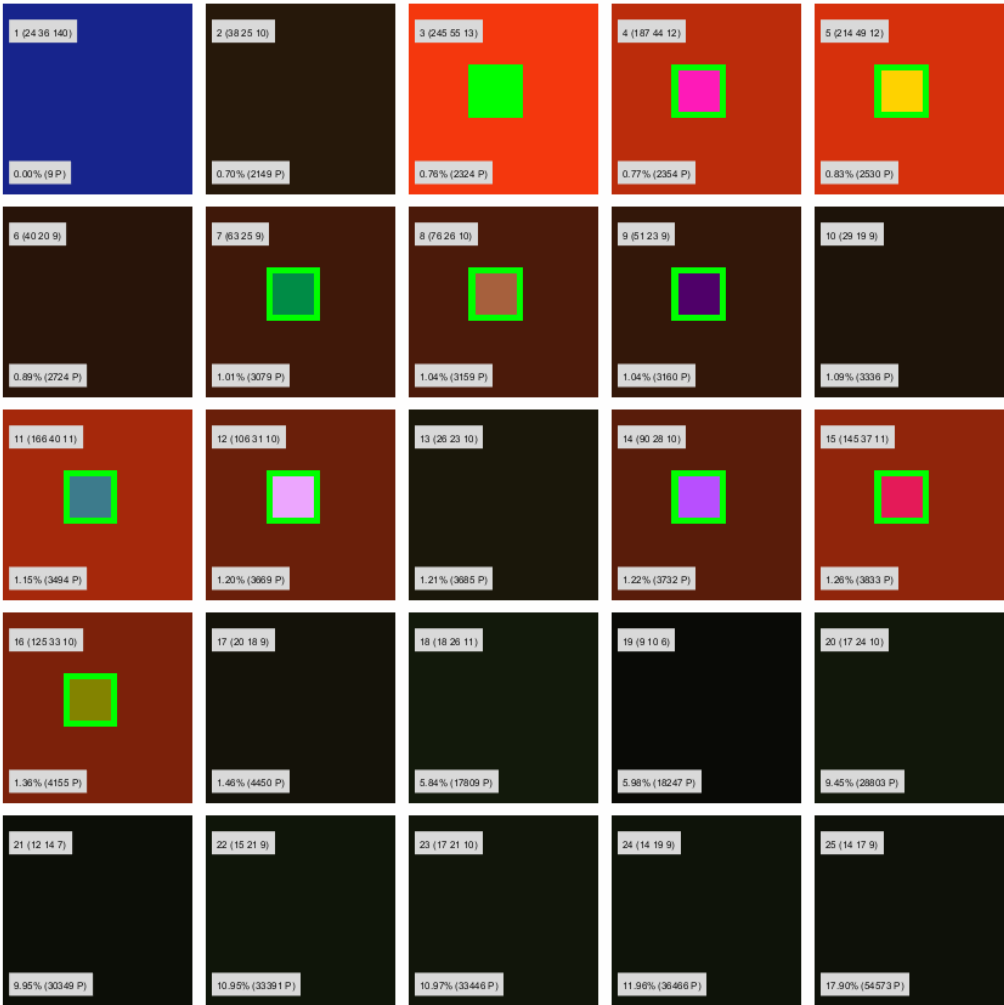
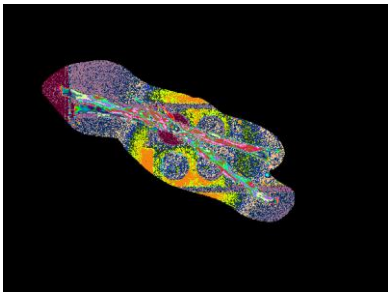


Figure S12. Example of maize image segmentation (FLUO, top view).

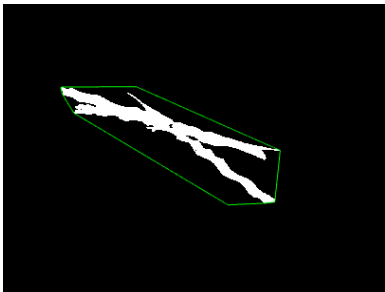
Original image



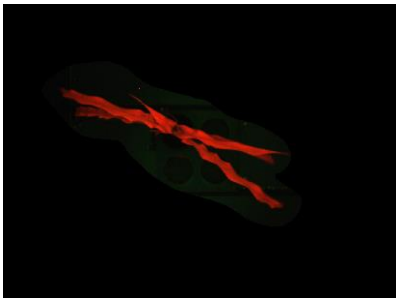
All 25 k-means color classes



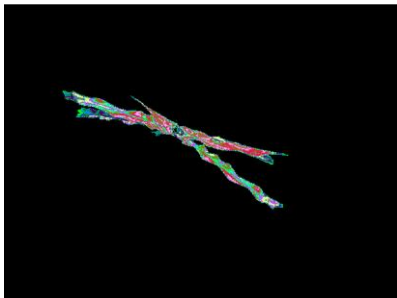
Binary image & convex hull



ROI after „clean outside“



12 selected plant color classes



Segmented image



Overview of all and selected plant k-means color classes

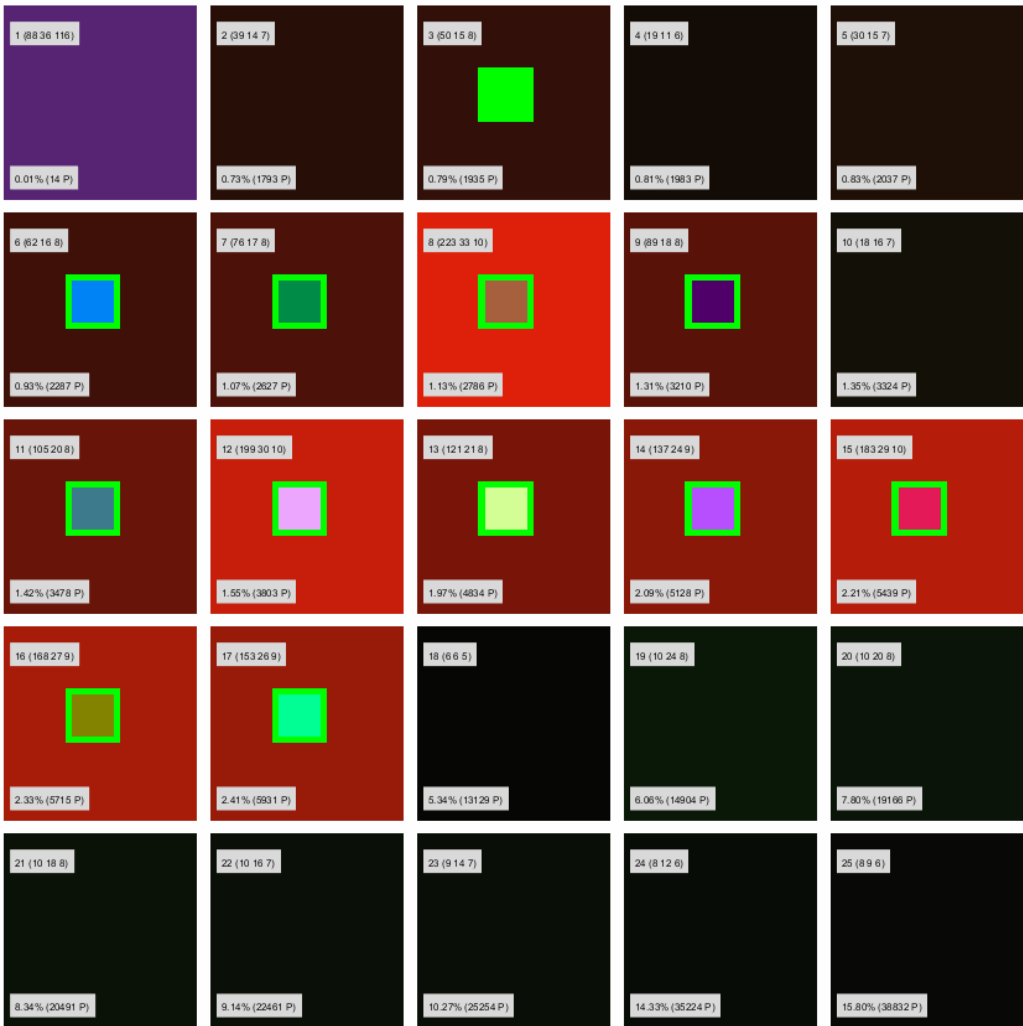


Figure S13. Example of segmentation of leaf speckles (RGB).

Original image



Segmented image



Overview of all and selected k-means color classes



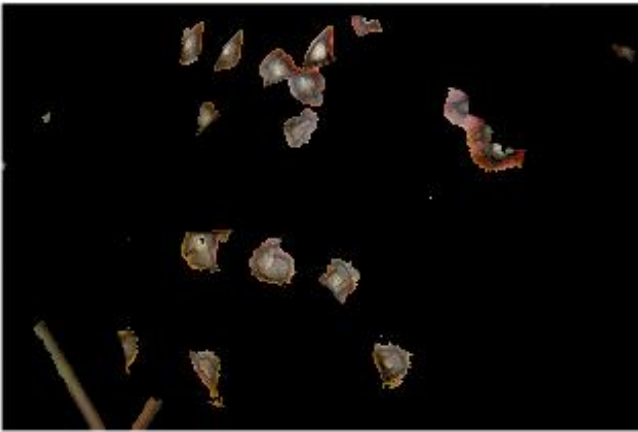


**Figure S14. Example of segmentation of leaf speckles (RGB).**

Original image



Segmented image



Overview of all and selected k-means color classes

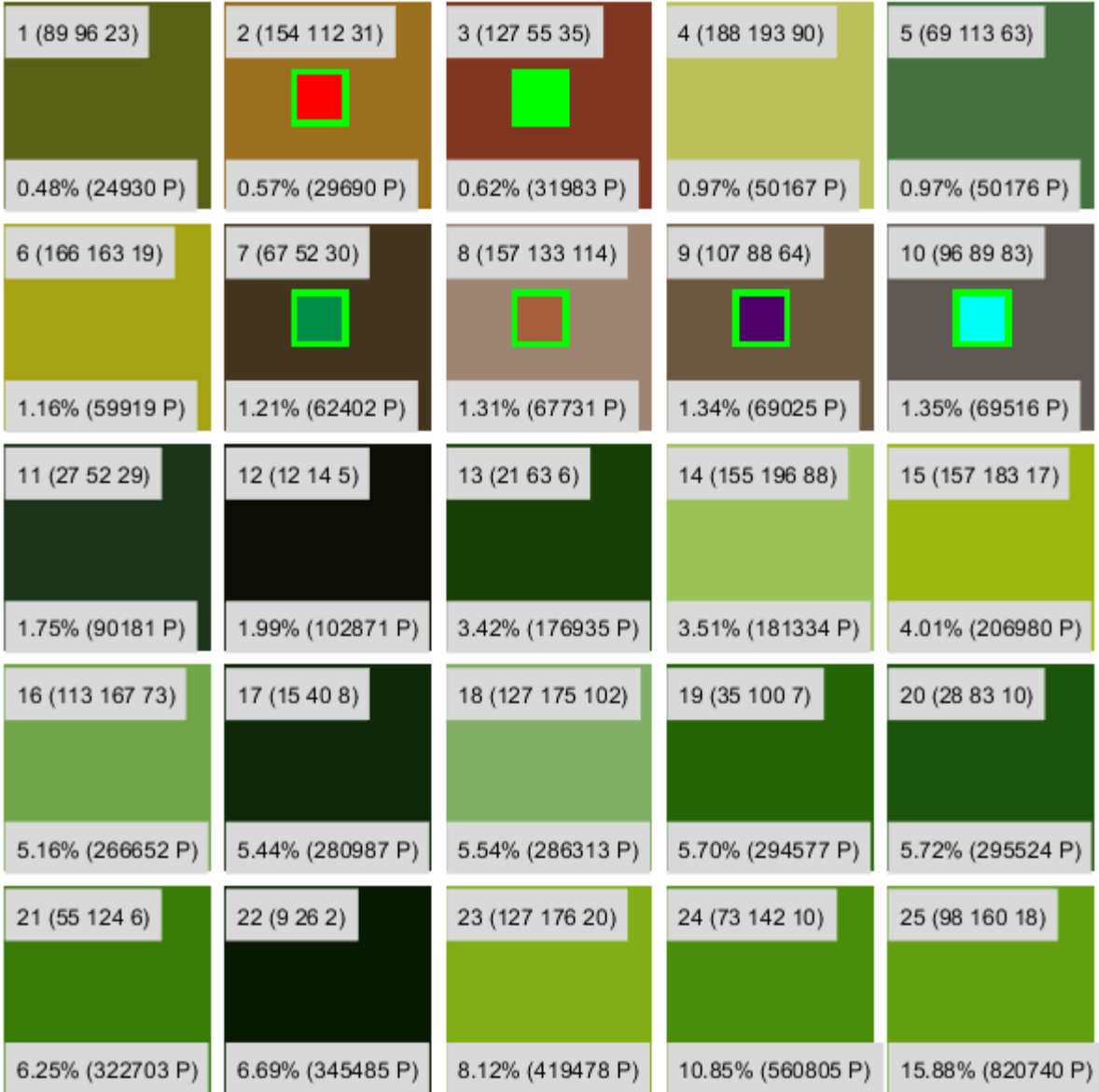
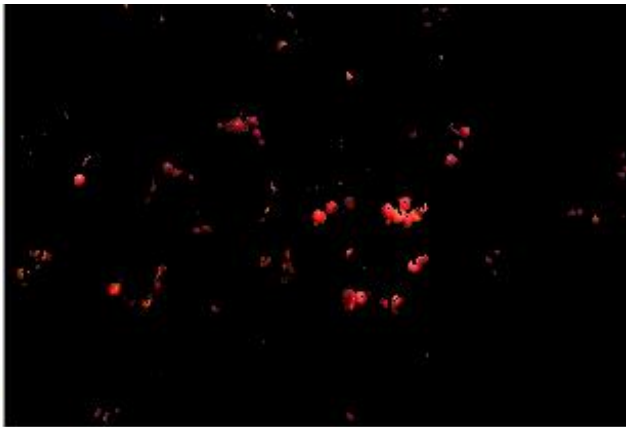


Figure S15. Example of segmentation of cherry tree image (RGB).

Original image



Segmented image



Overview of all and selected k-means color classes

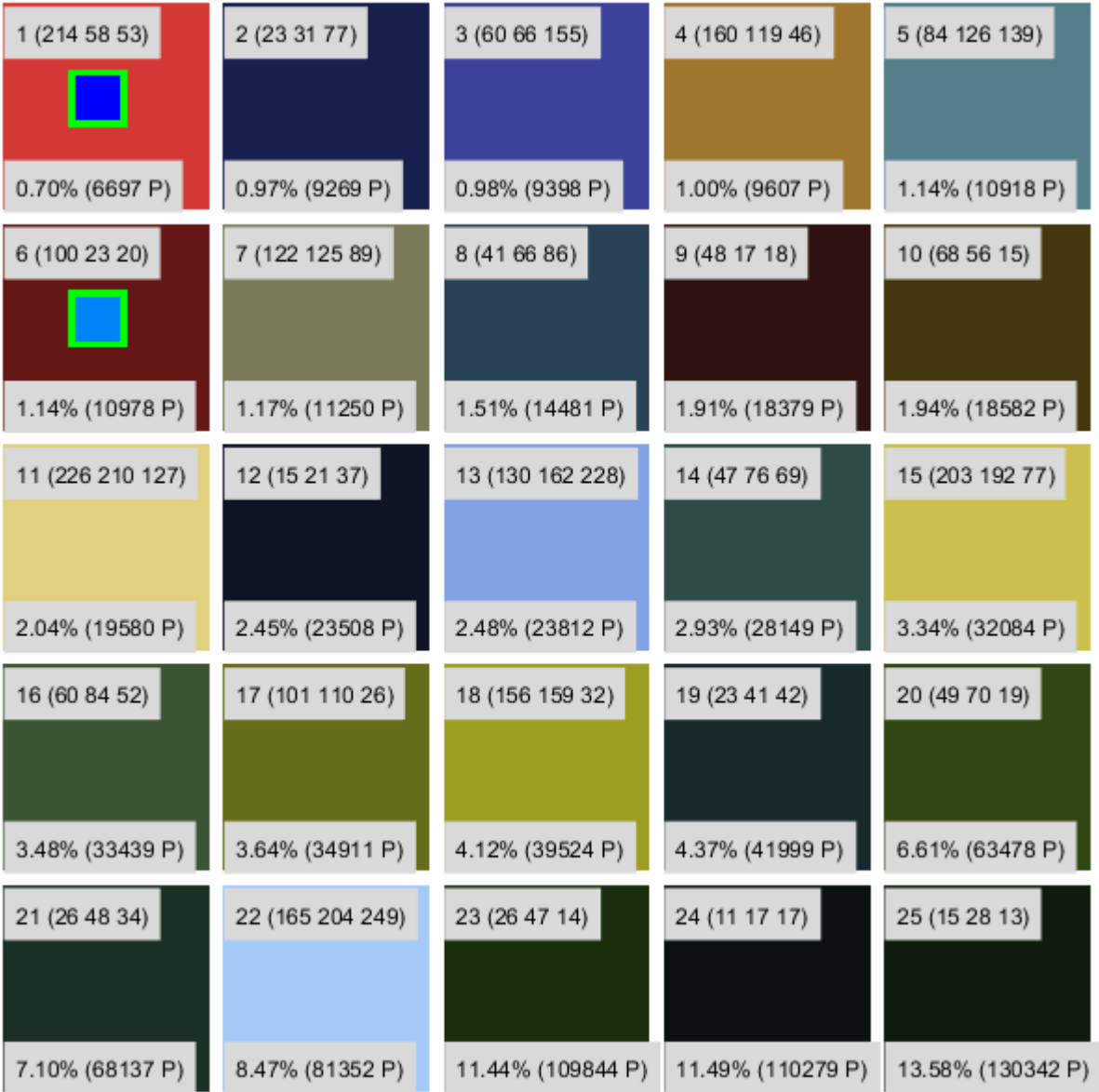
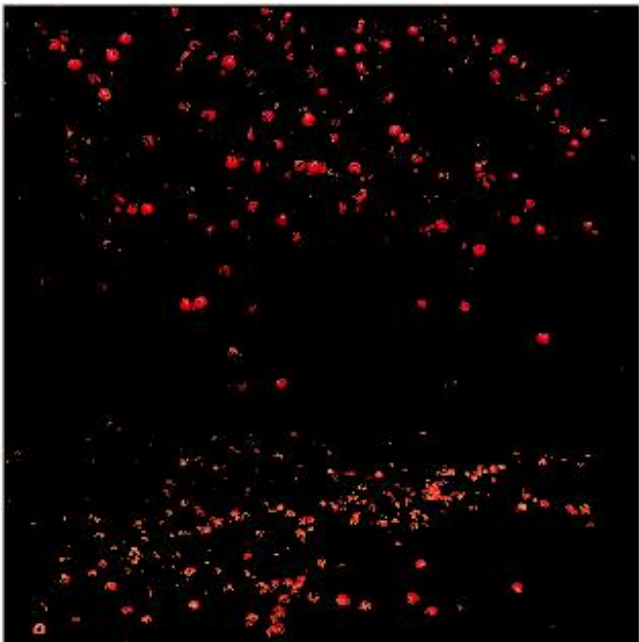


Figure S16. Example of segmentation of apple tree image (RGB).

Original image



Segmented image



Overview of all and selected k-means color classes

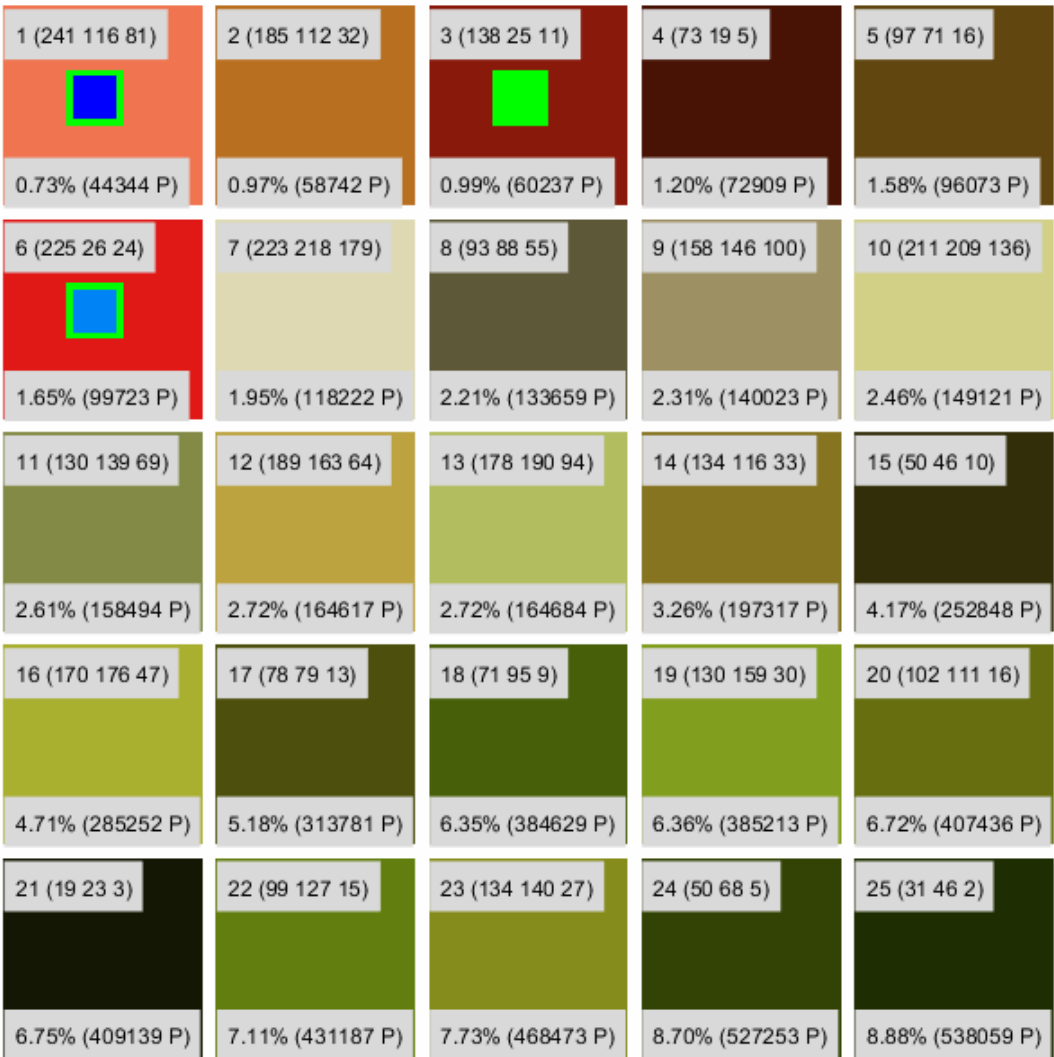
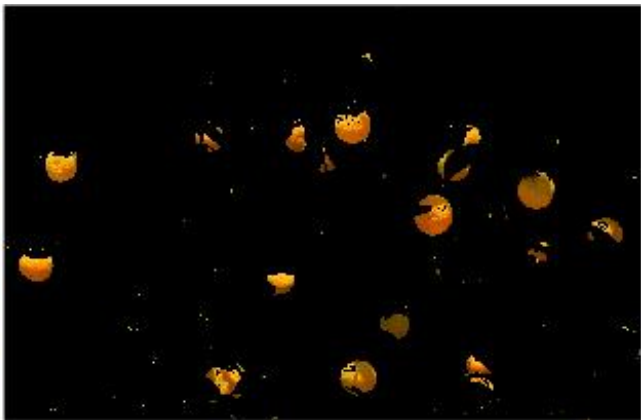


Figure S17. Example of segmentation of orange tree image (RGB).

Original image



Segmented image



Overview of all and selected k-means color classes

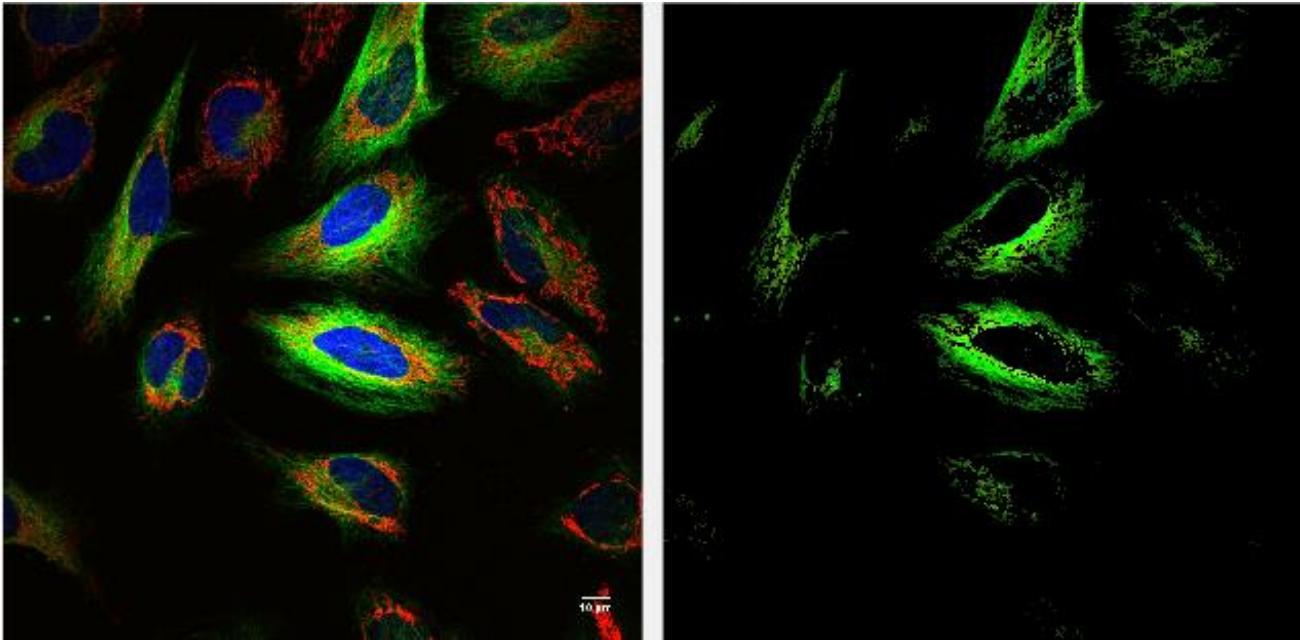




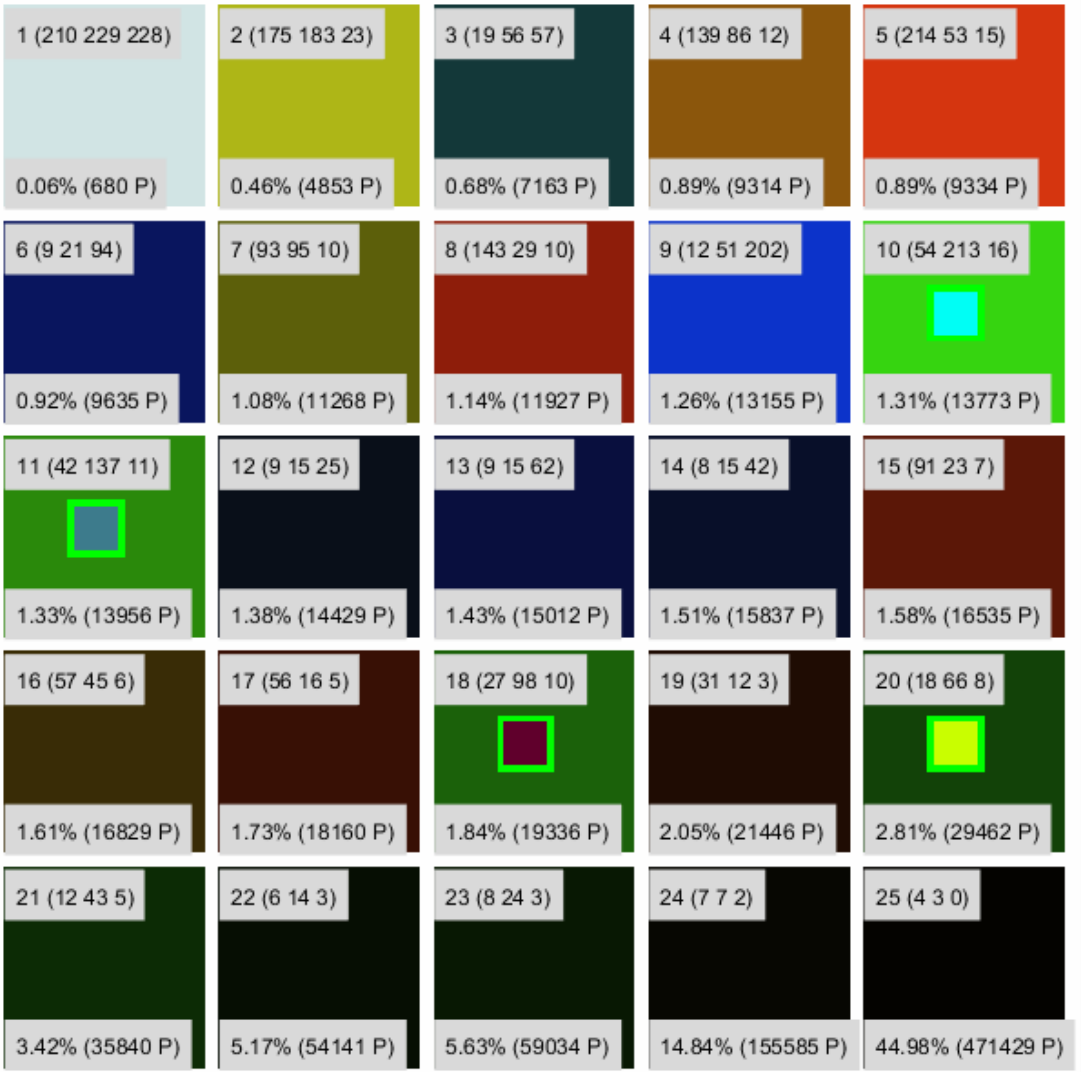
**Figure S18. Example of microscopic image segmentation (RGB of CLSM).**

Original image

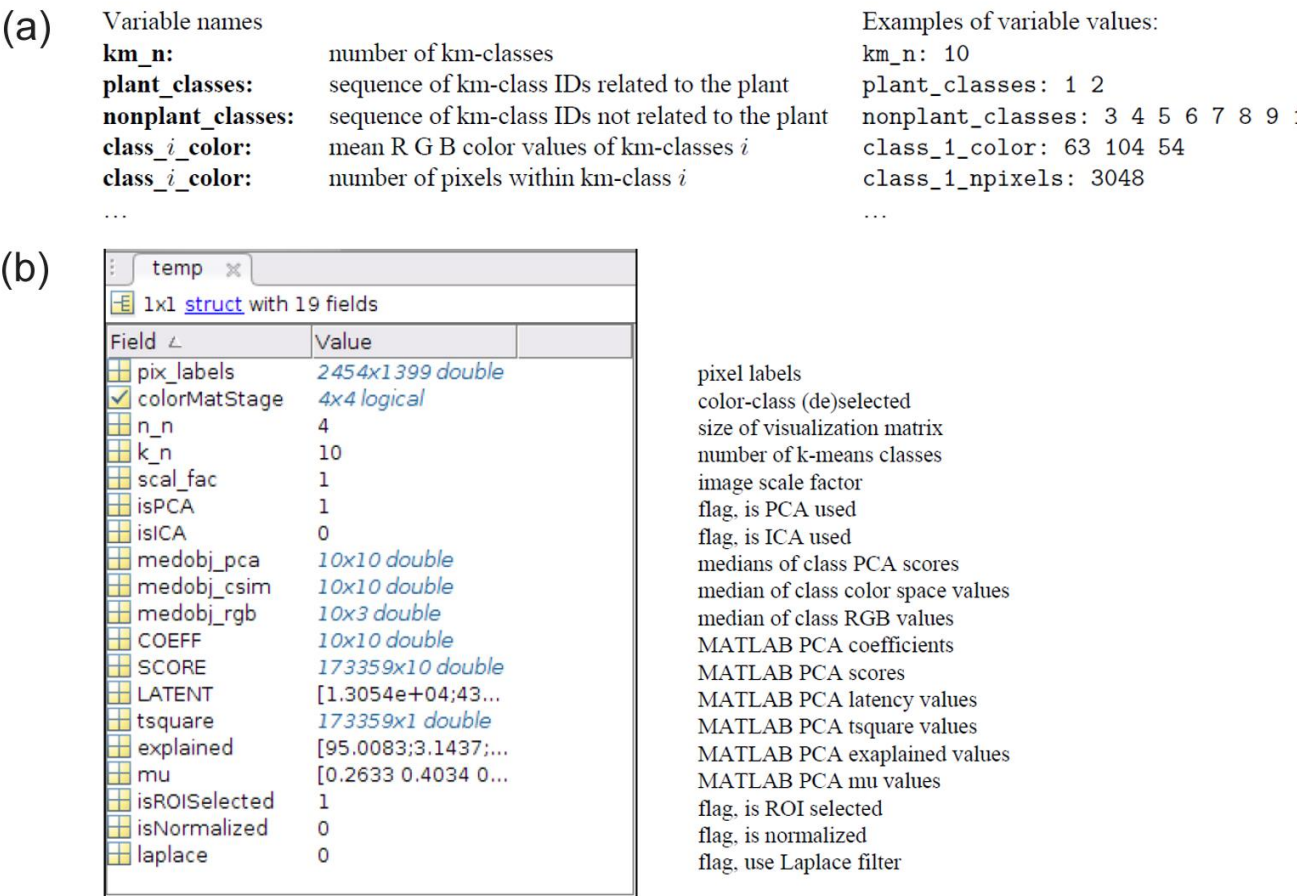
Segmented green channel image



Overview of all and selected k-means color classes



**Figure S19. Examples of ASCII (a) and MATLAB workspace (b) output files generated by the kmSeg tool for every segmented image.**



**Table S1. Overview of phenotypic traits automatically calculated using kmSeg.**

Trait name	Comments
ImageName	Full path and name of the analyzed image
Shoot Area	Number of segmented plant pixels
BBX area	Area of the bounding box of segmented image (in pixels <sup>2</sup> )
Shoot Area to BBX Area	Ratio between the shoot area and bbx area
BBX Height	Height of segmented plant (in pixels)
BBX Width	Width of segmented plant (in pixels)
Mean X	Mean coordinate of plant pixels projected onto the horizontal axis (X)
Stdev X	Stdev of coordinates of plant pixels projected onto the horizontal axis (X)
Mean Y	Mean coordinate of plant pixels projected onto the horizontal axis (Y)
Stdev Y	Stdev of coordinates of plant pixels projected onto the horizontal axis (Y)
CH Area	Area of segmented convex hull (in pixels <sup>2</sup> )
CH Area to BBX Area	Ratio between the area of the convex hull and bbx area
Shoot Area to CH Area	Ratio between the plant area and area of convex hull
R mean	Mean red intensity of segmented plant
R stdev	Stdev of red intensity of segmented plant
G mean	Mean green intensity of segmented plant
G stdev	Stdev of green intensity of segmented plant
B mean	Mean blue intensity of segmented plant
B stdev	Stdev of blue intensity of segmented plant
H mean	Mean H color intensity (HSV space)
H stdev	Stdev of H color intensity (HSV space)
S mean	Mean S color intensity (HSV space)
S stdev	Stdev of S color intensity (HSV space)
V mean	Mean V color intensity (HSV space)
V stdev	Stdev of V color intensity (HSV space)
L mean	Mean L color intensity (Lab space)
L stdev	Stdev of L color intensity (Lab space)
a mean	Mean a color intensity (Lab space)
a stdev	Stdev of a color intensity (Lab space)
b mean	Mean b color intensity (Lab space)
b stdev	Stdev of b color intensity (Lab space)
hsy_H mean	Mean H color intensity (HNY space)
hsy_H stdev	Stdev of H color intensity (HNY space)
hsy_S mean	Mean N color intensity (HNY space)
hsy_S stdev	Stdev of N color intensity (HNY space)
hsy_Y mean	Mean Y color intensity (HNY space)
hsy_Y stdev	Stdev of Y color intensity (HNY space)