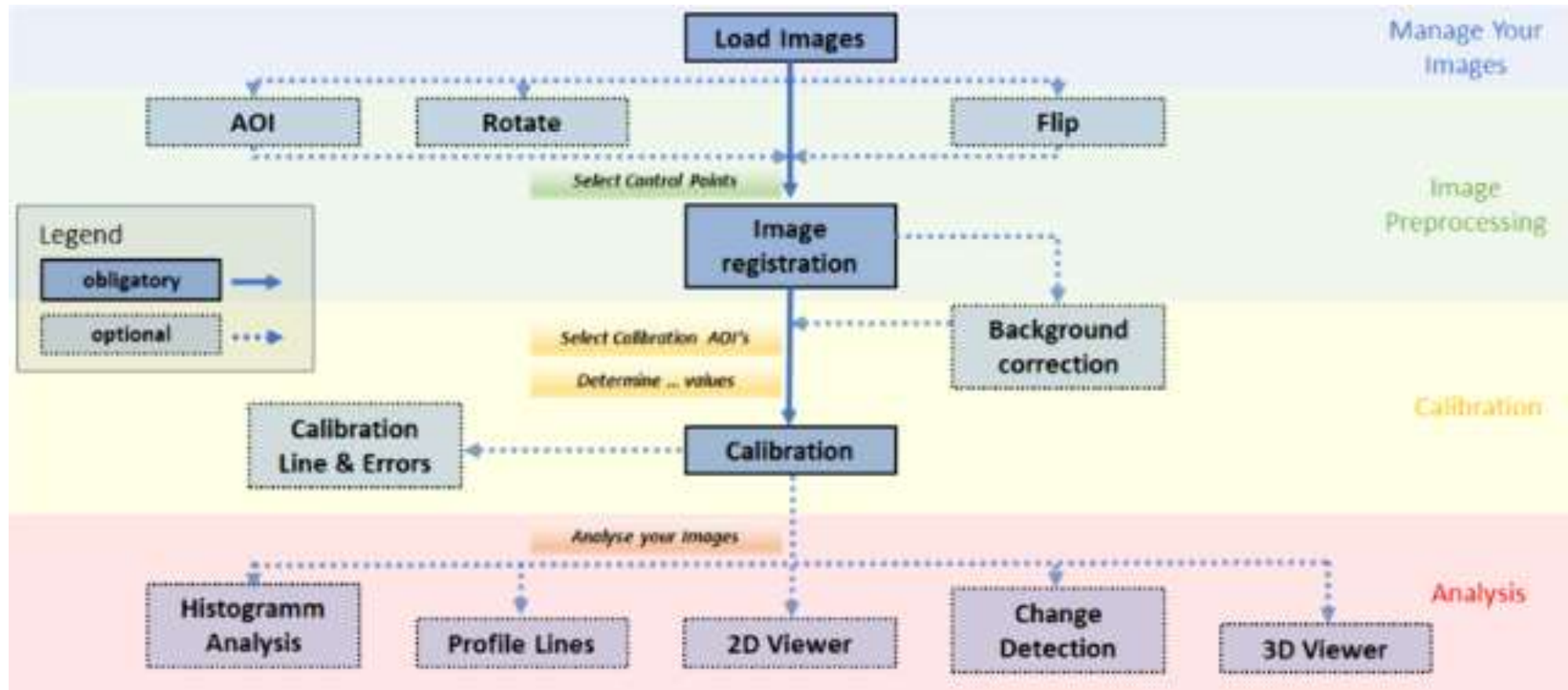


Manual Root-O-Mat

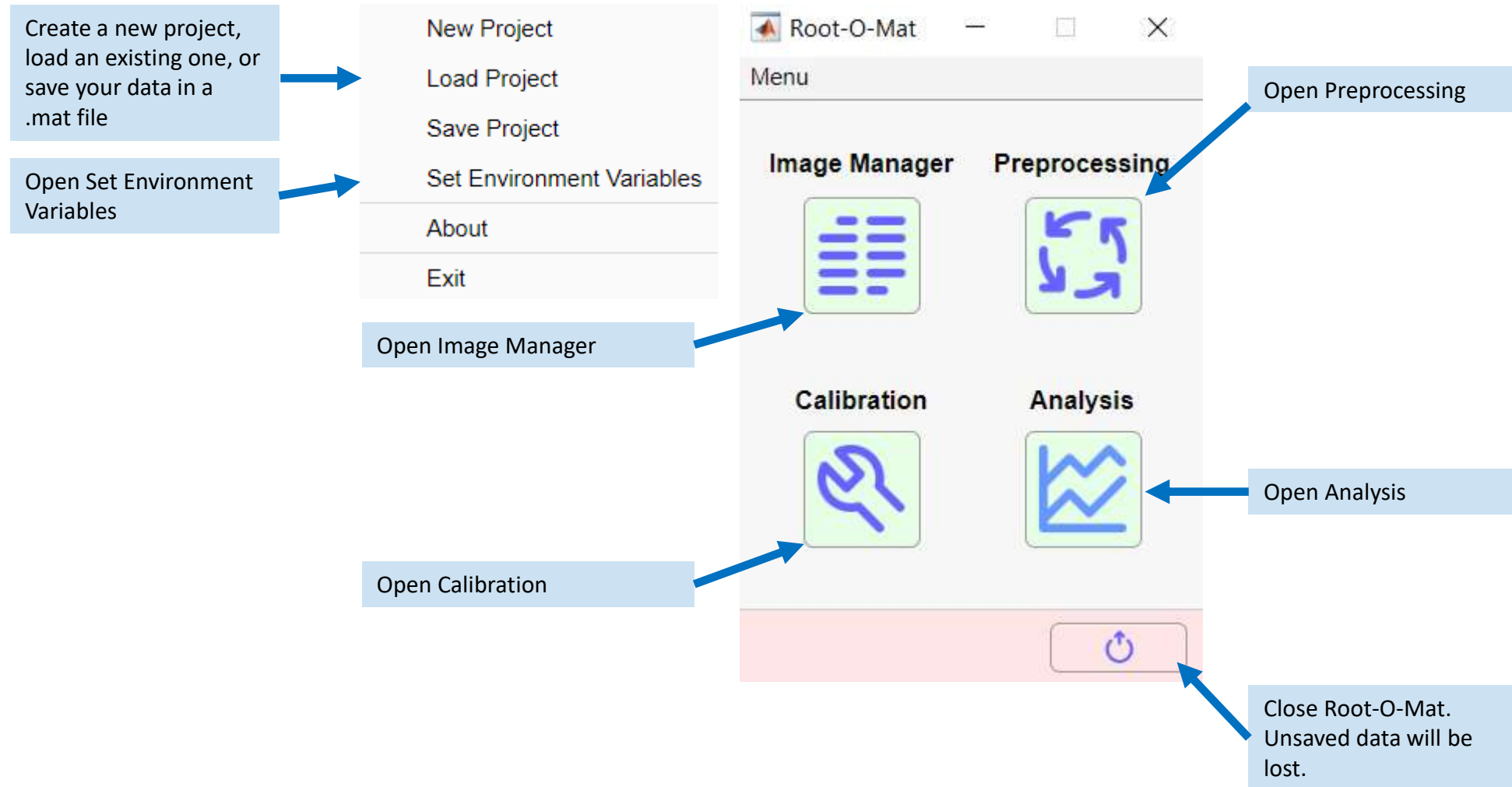
Release 1.0

Jan Tegtmeier, Sebastian Loeppmann





<https://doi.org/10.1016/j.soilbio.2021.108236>



Set Environment Variables

The Search Directory will be used to navigate to the selected folder directly when opening image data

Open the „select search directory“ dialog

Set Environment Variables

Search Path
C:\

Target Directory
C:\

The Target Directory is the default path when saving the results

Select search directory

« Seagate Expansion Drive (D:) > Root-o-Mat

« Root-o-Mat » durchsuchen

Name	Änderungsdatum	Typ	Größe
Zymo	02.01.2021 09:47	Dateiordner	

Ordner: Zymo

Ordner auswählen Abbrechen

Manage your Images

The „+“ - button creates a new image slot, the „-“ – button deletes the selected one. Use the „Duplicate“-button to copy a slot including all data

The Image List shows all image slots including their resolution. Images are shown in the Image Preview. Multi-selection is possible by pressing SHIFT

Load image data into the selected image slot

Set an Alias for the image slot selected in the Image List

Opens Image Viewer

The Image Preview shows the image selected in the Image List. 8-bit input images will be displayed in grayscale, and 64-bit result images with colormap

Open Export Data

Save changes and quit Image Manager

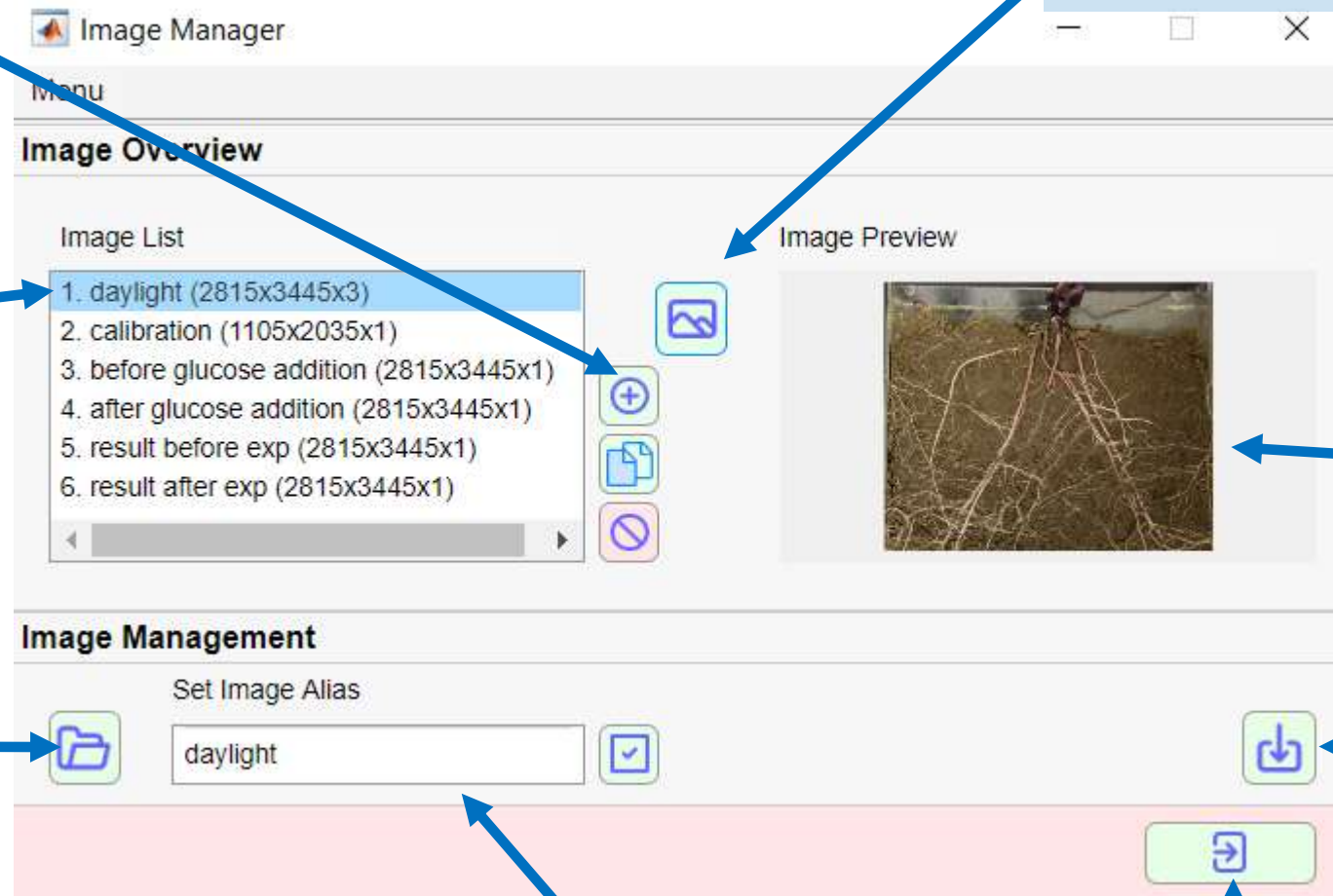


Image Preprocessing and Image Registration

Preprocessing and Referencing

Menu

Image Overview

1. daylight (2815x3445x3)
2. calibration (1105x2035x1)
3. before glucose addition (2815x3445x1)
4. after glucose addition (2815x3445x1)
5. result before exp (2815x3445x1)
6. result after exp (2815x3445x1)

Image Preview

Image Preprocessing

Rotate: 90°, 180°, 270°
Flip: Vertical, Horizontal, Crop

Conversion to Grayscale

Blue Channel
Histogram Analysis

Register Images

Select Input Image: before glucose addition
Select Reference Image: daylight
Select Method: Nonreflective similarity

Control Point Selection

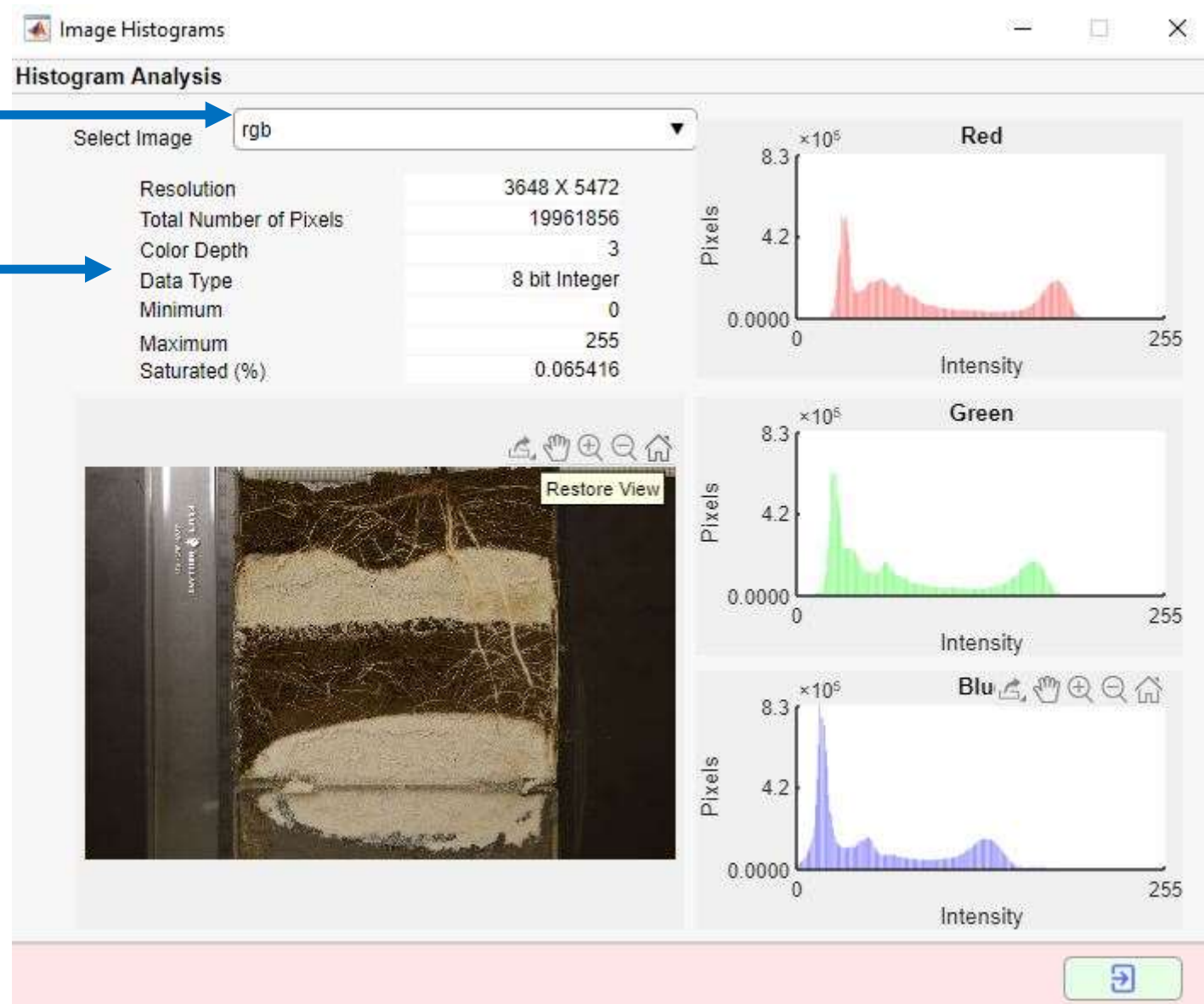
	X1	Y1	X2	Y2	
1	376.37	1694.63	512.38	1884.67	<input type="checkbox"/>
2	0	0	0	0	<input type="checkbox"/>

Manual Root-O-Mat - Jan Tegtmeier & Sebastian Loepmann

Annotations:

- Crop image
- Flips the image upside down or left-right
- Rotates the image by 90° left or right, or 180°
- Select the Input Image and the Reference Image
- Add a new control point or delete the last one in the list. Note, that there is a minimum of control points required depending on the registration method selected
- Select method for grayscale conversion
- Converts all images selected in Image List to grayscale using the method selected above. Undo the changes using the Reset-Button
- Open Histogram Analysis
- Select method for image registration. Minimum number of control points for Non-reflective similarity: 2, Affine: 3, Projective: 4
- Click on the checkbox to select a pair of control points in the input and reference image
- After image registration is performed, a new window with an overlay of the registration result and the input image opens. The original image data in the input image slot will be changed.

Image Histogram Analysis



Select your image for analysis

Basic image information are shown here

The histograms of the selected image. R/G/B components will be shown separately.

Calibration

Select AOI from image for background correction

Select AOI from the selected image to calculate the mean grayvalue for background correction

Apply Background Correction.

List of calibrations

Set an Alias for the selected calibration

Select the model used for calibration

Show the goodness of fit

Show the calibration curve

Shows the calibration formula

Performs curve fitting

The screenshot shows the 'Calibration' software interface. It includes a 'Menu' bar, an 'Image Overview' section with an 'Image List' and 'Image Preview', a 'Background Correction' section with 'Select AOI from Image' and 'Mean DN' controls, an 'AOI Selection' section with a point count and selection box, a 'Manage Calibrations' section with a list and alias setting, a 'Calculate Calibration' section with a model selector and 'Goodness of Fit'/'Calibration Curve' buttons, a 'Calibration Values' table, and a 'Calculate Results' section with 'Select Image' and 'Save Result' options. A footer contains the text 'Manual Root-O-Mat - Jan Tegtmeier & Sebastian Loepmann'.

pmol/cm ² /h	DN
0	56.7784
250	148.1935
625	202.8736
875	225.3074
1375	243.9669

Select the number of calibration points

Start AOI selection. Select the calibration image in the image list first.

Enter your calculated calibration values in the first column of the table. The second column will be filled out with the mean gray values automatically when the AOI selection has been performed

Enter the unit for x- and y-axis (will be used for labeling)

Select the zymograph to process the calculation for the resulting image

Check the „Save Result“ box and enter an Alias to make the result available in the Image List

Calculate your result

Draw a polygon for each calibration concentration



Left click to enter a new vertex. After closing a polygon with the last vertex, right click into the polygon area and select „finish“ from the menu to finish polygon. Note, that this process will repeat several times according to the number of AOIs selected in the calibration menu. You are able to delete a single vertex, however do not delete the last one. If so, you need to close the window and start AOI selection again

The mean gray value of the pixels will be shown after finishing the polygon

Analysis

Change detection: Select input images from the drop down menu

Compare images by the frequency distribution analysis

Automatic Clustering using Otsu method. Select the input images and the number of classes first

Show clustered image #1. The same thresholds are used for image #2. The Compare Button shows the changes, Statistics compares the percentage of total image area by class

Compare profile lines from two images. Select an optional background image to draw on

Compare polygons from two images. Select an optional background image to draw on

Show the difference image

Export the histogram data

Select the percentage of total image area to be classified as Hot- or Coldspots

Use the slider or the text field to enter the threshold manually

Select the image to analyse and start the clustering using the threshold above.

Start drawing a Profile Line

Start drawing AOIs

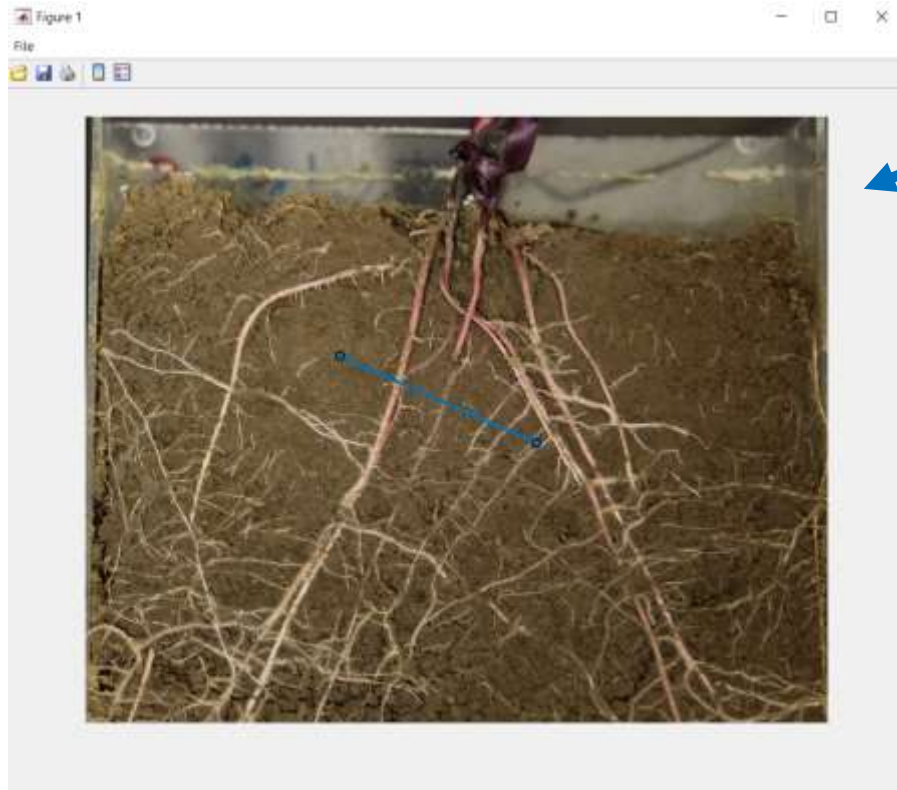
Show AOIs and Statistics

The screenshot shows the 'Analyse Results' window with the following sections and callouts:

- Change Detection:** Callout: 'Change detection: Select input images from the drop down menu'. Includes 'Select Initial State Image' (result before exp) and 'Select Final State Image' (result after exp). Callout: 'Show the difference image' points to the play button.
- Histogram:** Callout: 'Compare images by the frequency distribution analysis'. Includes 'Select Image' (before glucose addition) and 'Select Image for Comparison' (after glucose addition). Callout: 'Export the histogram data' points to the download icon.
- Clustering / Hot Spot Detection:** Callout: 'Automatic Clustering using Otsu method. Select the input images and the number of classes first' points to 'Select Image #1' (result before exp) and 'No. of Classes' (4). Callout: 'Show clustered image #1. The same thresholds are used for image #2. The Compare Button shows the changes, Statistics compares the percentage of total image area by class' points to '#1', '#2', 'Compare', and 'Statistics' buttons. Callout: 'Select the percentage of total image area to be classified as Hot- or Coldspots' points to the percentage selection buttons (0.01, 0.025, 0.1, 0.05, 0.25, 0.5). Callout: 'Use the slider or the text field to enter the threshold manually' points to the slider (min 7, max 946). Callout: 'Select the image to analyse and start the clustering using the threshold above.' points to 'result before exp' and the play button.
- Draw Profile Line:** Callout: 'Compare profile lines from two images. Select an optional background image to draw on' points to 'Select Image #1' (result before exp), 'Select Image #2' (result after exp), and 'Select Background Image' (daylight). Callout: 'Start drawing a Profile Line' points to the play button.
- AOI statistics:** Callout: 'Compare polygons from two images. Select an optional background image to draw on' points to 'Select Image #1' (before glucose addition), 'Select Image #2' (after glucose addition), and 'Select Background Image' (daylight). Callout: 'Start drawing AOIs' points to the polygon drawing icon. Callout: 'Show AOIs and Statistics' points to 'Show AOIs' and 'Statistics' buttons. The 'No. of AOIs' is shown as 3.

Manual Root-O-Mat - Jan Tegtmeier & Sebastian Loepmann

Draw a profile line into the image



Start drawing the profile line by left click into the image. Hold the mouse button until you reach the endpoint. The plot window (right) will open automatically

