

AI Analysis of Minirhizotron Imagery Using RootDetector: A Step-by-Step Guide

Introduction

RootDetector is a U-Net-based computer vision tool designed for the automated segmentation and analysis of minirhizotron imagery. It facilitates the detection of roots and foreign objects (such as insulation tape) that may obstruct soil and root systems in the images. RootDetector comes with several base models that can be retrained and adapted to increase accuracy when analyzing specific datasets. While the retraining settings are limited, they are expedient. RootDetector can be controlled remotely via command prompts.

Step 1: Install Minirhizotron Tubes

Refer to informative publications such as Rewald & Ephrath (2013) for installation guidance. Be aware that installation is invasive and will disturb the soil structure. Depending on the environmental dynamics, you may need to wait several months to years for roots to regrow and establish near the tubes (Johnson et al., 2001; Joslin & Wolfe, 1999). From our experience, installing tubes in the summer and starting observations in the following growing season is effective.

Step 2: Scanning

Before initiating the scanning process, ensure that the images captured are of high quality to facilitate precise automated analysis later.

- **Inspect the Tube:** Use a flashlight to check for condensation, water droplets and dirt inside the tube. If present, clean the interior carefully.
- **Cleaning:** Use a long pole with a padded end wrapped in a microfiber cloth to wipe the interior without scratching the tube. Remove all moisture to prevent interference with image clarity.
- **Stabilize the Tube:** While cleaning, stabilize the tube to avoid any movement, as even slight shifts can distort the position and disturb growth.
- **Weather Considerations:** Weather conditions can significantly affect image quality. Moisture might condense on the outside of the tube within the soil under warm, wet conditions, complicating the scanning process. Variations in soil moisture due to weather changes like rain or drought can alter the contrast in the images, potentially affecting analysis. Therefore, where feasible, conduct scans under similar weather conditions to maintain consistency in image quality.

Step 3: Create Evaluation Data

3.1 Selecting Evaluation Images

Set aside several images for evaluation purposes; these images must be exempt for model training. Ensure the chosen images represent the variety of conditions within the entire dataset. While we achieved good results with 10 images, using more images can increase the informative

value of the evaluation and may affect the outcome (Peters et al., 2025). Since the evaluation images serve as ground truth (i.e., ideal analysis), annotation must be conducted with utmost care and precision.

To reduce the effort while maintaining representativeness, you may choose to crop smaller sections from the selected images. We suggest cropping 1000×1000 pixel sections from random locations within each image. This can be achieved using the R code provided at the end of this section.

3.2 Annotate Evaluation Data

While we chose GIMP for its freeware availability and achieved good results, any other modern image editing software can be used for annotation; however, this guide will cover the process using GIMP.

Getting Started with GIMP

If you've never used GIMP before, don't worry—this section provides detailed instructions to guide you through the annotation process.

Download and Install GIMP:

1. **Download GIMP:** Visit the official GIMP website and download the latest version suitable for your operating system.
2. **Install GIMP:** Run the installer and follow the on-screen instructions to install GIMP on your computer.

Annotating Images in GIMP

Open Your Image

1. **Launch GIMP:** Open GIMP from your applications menu.
2. **Open the Image:**
 - Go to **File > Open**.
 - Navigate to the folder containing your evaluation image or cropped section.
 - Select the image and click **Open**.

Set Up the Workspace

1. **Understanding the Interface:**
 - **Layers Panel:** Usually on the right side, shows all layers in your image (Fig. 1).
 - **Toolbox:** Located on the left side, contains tools for editing (Fig. 2).
 - If the Layers Panel is not visible, go to **Windows > Dockable Dialogs > Layers**.
2. **Create New Layers:**
 - **Soil Layer:**
 - In the Layers Panel, click the **Create a New Layer** icon (Fig. 1, **a**).

- In the dialog:
 - **Layer Name:** Enter Soil.
 - **Layer Fill Type:** Choose Transparency.
 - Click **OK**.
- **Roots Layer:**
 - Repeat the above steps to create another new layer.
 - **Layer Name:** Enter Roots.
 - **Layer Fill Type:** Choose Transparency.
 - Click **OK**.
- 3. **Organize Layers:**
 - Ensure the **Roots** layer is above the **Soil** layer.
 - The order can be changed by dragging and dropping layers in the Layers Panel.

Fill the Soil Layer

1. **Select the Soil Layer:**
 - Click on the **Soil** layer in the Layers Panel to activate it (**b**).
 - The active layer is highlighted.
2. **Select All:**
 - Go to **Select > All** or press **Ctrl + A**.
3. **Choose Black Color:**
 - In the Toolbox, find the **Foreground/Background Color** swatches (Fig. 2, **d**)
 - Click on the **Foreground Color** (top square).
 - In the **Change Foreground Color** dialog:
 - Set the color to pure black (**HTML notation** 000000).
 - Click **OK**.
4. **Fill the Layer:**
 - Select the **Bucket Fill Tool** (Fig. 2, **e**) from the Toolbox (icon looks like a spilling paint bucket).
 - In the **Tool Options** (below the Toolbox), ensure:
 - **Fill Type:** FG color fill.
 - **Affected Area:** Fill whole selection.
 - Click anywhere on the image canvas to fill the selected area with black.

5. **Deselect All:**

- Go to **Select > None** or press **Ctrl + Shift + A**.

6. **Hide the Soil Layer:**

- In the Layers Panel, click the **eye icon (c)** next to the **Soil** layer to hide it.
- This allows you to see the image underneath while annotating.

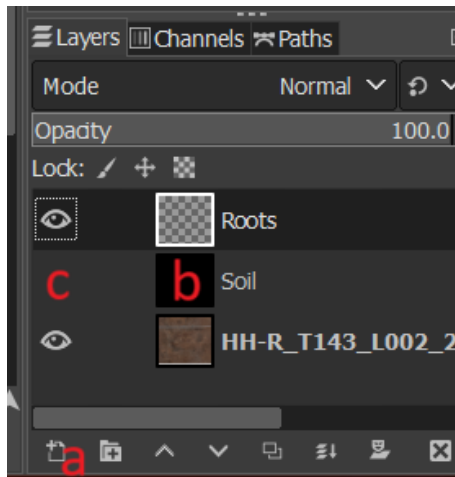


Figure 1: Layer Panel

Annotate Roots

1. **Select the Roots Layer:**

- Click on the **Roots** layer in the Layers Panel to activate it.

2. **Choose White Color:**

- Click on the **Foreground Color** swatch in the Toolbox (**d**).
- Set the color to pure white (**HTML notation FFFFFFFF**).
- Click **OK**.

3. **Select Annotation Tools:**

- **Free Select Tool (f):**
 - Icon looks like a rope lasso.
 - Use this to draw freehand selections around root structures or create polygonal selections by clicking to set anchor points.
- **Pencil and Brush Tools (g, h):**

- The icon for these tools may change appearance depending on which one was used last; it might look like a pencil or a paintbrush. Best for drawing precise, hard-edged lines.
- **Accessing the Tools:**
 - **Method 1:** Click and hold the tool icon in the Toolbox until a submenu appears, then select either the **Pencil Tool** or the **Brush Tool**.
 - **Method 2:** Right-click on the tool icon to bring up the selection menu.
- **Pencil Tool:**
 - Best for drawing precise, hard-edged lines.
 - Ideal for detailed work requiring exact edges.
- **Brush Tool:**
 - Good for broader strokes.
 - Adjust the **Hardness** setting to 100% for crisp edges, which is important for accurate annotations.

4. Configure Tool Options (i):

- **For the Free Select Tool:**
 - **In Tool Options:**
 - Uncheck **Feather Edges**.
 - Uncheck **Antialiasing**.
- **For the Pencil and Brush Tools:**
 - **In Tool Options:**
 - Set **Hardness** to **100%**.
 - Choose an appropriate **Brush Size** (e.g., 3 pixels) for the level of detail you need.

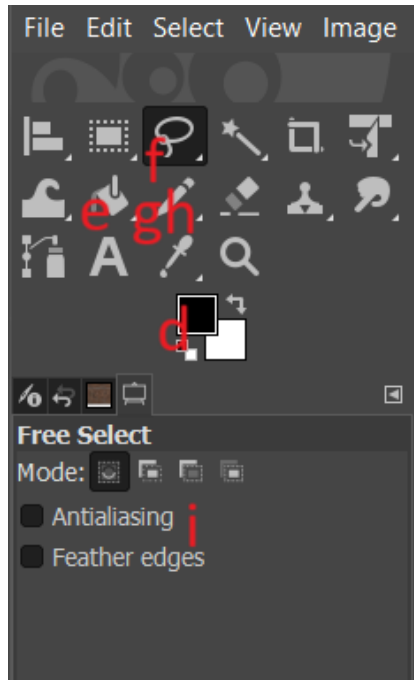


Figure 2: Toolbox and -settings

5. Annotate the Roots:

Make sure, the Roots layer is active when annotating!

- **Using the Free Select Tool:**
 - Click and drag to outline a root or click to set anchors.
 - Close the selection by connecting back to the starting point or double-clicking.
 - Once the selection is closed, fill it:
 - Select the **Bucket Fill Tool (e)**.
 - Ensure **Fill Type** is set to FG color fill.
 - Click inside the selection to fill it with white.
 - Deselect by going to **Select > None** or by pressing **Shift+Ctrl+A**.
- **Using the Pencil or Brush Tool (g, h):**
 - Simply draw over the root structures.
 - Ensure you are drawing on the **Roots** layer.
 - Zoom in for precision:
 - Use **View > Zoom** or press + and – keys.
 - Alternatively, hold **Ctrl** and use the **Mouse Wheel**.

6. Ensure Sharp Edges:

- Blurred edges can affect the model's training.
- If you notice blurry annotations, check that:
 - **Antialiasing** is turned off.
 - **Brush Hardness** is at 100%.
 - **Feather Edges** is disabled.

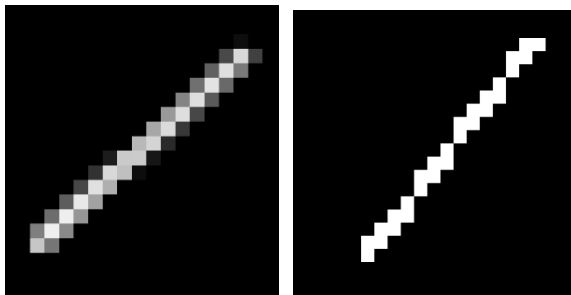


Figure 3: left side: blurry, faulty annotation; right side: correct annotation

Annotation Guidelines

- **What to Annotate:**
 - **Include:** Only structures you can confidently identify as roots.
 - **Exclude:** Soil particles, shadows, or any ambiguous structures.
- **Handling Complex Areas:**
 - **Densely Packed Roots:**
 - Leave at least a **1-pixel gap** between distinct roots (Fig. 4).
 - This helps the model distinguish separate root structures.
 - **Overlapping Roots:**
 - For roots crossing over each other, maintain gaps similar to drawing bridges in maps (Fig. 5).
 - Leave a **1-pixel gap** where one root passes behind another.
 - **Blurred or Out-of-Focus Roots:**
 - Annotate only up to where the root is clearly visible (Fig. 6).
 - Do not guess or extrapolate beyond what is discernible.
 - **Partially Obstructed Roots:**

- If a root is partially covered or obstructed, only annotate the visible sections (Fig. 7).
- **Zooming and Panning:**
 - Regularly zoom in for detailed work and zoom out to maintain context.
 - Pan around the image by holding the **Spacebar** and dragging with the mouse.



Figure 4

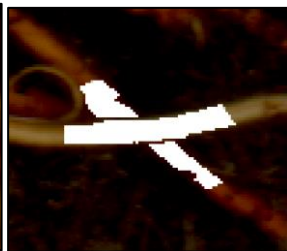


Figure 5



Figure 6

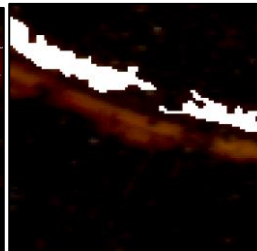


Figure 7

Saving Your Work

1. Save the GIMP Project File:

- Go to **File > Save As**.
- The default format is .xcf, which preserves layers and allows you to resume work later.
- Name the file appropriately (e.g., image1_annotation.xcf).
- Save frequently to prevent data loss.

2. Prepare for Exporting:

- **Show the Soil Layer:**
 - In the Layers Panel, click the **eye icon** next to the **Soil** layer to make it visible.
- **Check Layer Order:**
 - Ensure the **Soil** layer is below the **Roots** layer.

3. Verify the Final Image:

- The image should now display white root structures on a black background.
- No other colors or grayscale should be present.

4. Export the Annotated Image:

- Go to **File > Export As....**

- In the dialog:
 - The filename must remain the same as that of the original scan aside from the file extension.
 - Change the filename extension to .png (e.g., image1_annotation.png).
 - Click **Export**.
- In the **Export Image as PNG** dialog:
 - Uncheck **Save background color** and **Save color values from transparent pixels** to reduce file size.
 - Click **Export**.

5. Organize Annotations:

- Save all annotated .png files in a separate folder (e.g., Annotations) to avoid confusion.

R Code for Random Cropping of 1000×1000 Pixel Sections:

```
library(magick)

input_folder <- "path/to/folder"
output_folder <- file.path(input_folder, "cropped")
dir.create(output_folder, showWarnings = FALSE)

image_files <- list.files(input_folder, pattern = "\\.(jpg|jpeg|png|tiff|bmp)$",
full.names = TRUE, ignore.case = TRUE)

for (image_path in image_files) {
  img <- image_read(image_path)
  info <- image_info(img)
  if (info$width >= 1000 && info$height >= 1000) {
    x_offset <- sample(0:(info$width - 1000), 1)
    y_offset <- sample(0:(info$height - 1000), 1)
    cropped_img <- image_crop(img, geometry_area(1000, 1000, x_offset, y_offset))
    image_write(cropped_img, path = file.path(output_folder,
paste0(tools::file_path_sans_ext(basename(image_path)), ".png")), format = "png")
  }
}
```

Step 4: Evaluate Model Performance

1. **Start RootDetector:** Run the main.bat executable in the RootDetector main directory.

2. Load Evaluation Images:

- Go to **Files > Load Input Folder** and select the folder with the evaluation images or cropped sections.

3. Select Model:

- Under **Settings > Root Detection Model**, select a model of interest.
- Click **Save**.

4. Disable Detection of foreign objects

5. Process Images:

- Click **Process All** and wait for the processing to finish.
- Once completed, click **Download All** and save the predictions.zip file.

Evaluation Using Command Prompt:

1. Open Command Prompt:

- Click the Windows symbol and search for cmd.
- Open the Command Prompt application.

2. Navigate to RootDetector Directory:

- Type the following command, replacing the path with the actual path to your RootDetector directory:

```
cd C:\path\to\RootDetector
```

- Press **Enter**.

3. Initiate Evaluation:

- Run the following command:

```
main.bat --evaluate --predictions=path\to\predictions.zip --  
annotations=path\to\annotations\*.png
```

- Replace path\to\predictions.zip with the actual path to your predictions.zip.
- Replace path\to\annotations*.png with the path to your annotated .png files.

- Example:

```
main.bat --evaluate --  
predictions=C:\Users\user1\Desktop\Data\Predictions\predictions.zip --  
annotations=C:\Users\user1\Desktop\Data\Annotations\*.png
```

- Press **Enter** and wait for the process to finish.

Results:

- The results are automatically saved as output.zip in the RootDetector main directory.

- output.zip contains:
 - A folder for each evaluation image, named after the image.
 - Each folder includes an errormap.png (Figure 8).
 - **Green pixels:** Correctly identified roots (true positives).
 - **Red pixels:** Incorrectly identified as roots (false positives).
 - **Blue pixels:** Roots missed by the model (false negatives).
 - A statistics.csv file with detailed performance metrics.

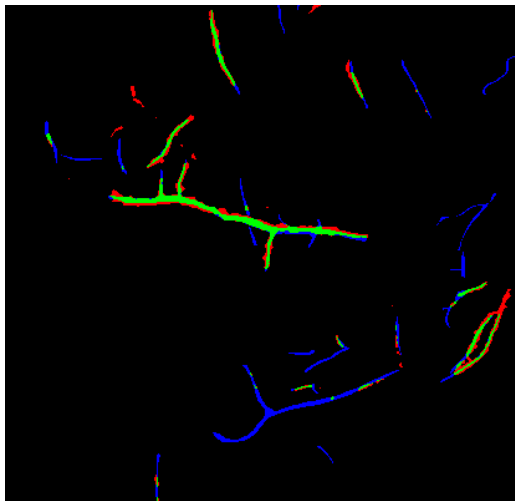


Figure 8: errormap.png

	A	B	C	D	E	F	G	H	
1	#Filename	True Positives	False Positive	False Negative	IoU	Precision	Recall	F1	
2	1194x604x219	805	1023	154	0.41	0.44	0.84	0.58	
3	1448x450x244	10075	11368	8574	0.34	0.47	0.54	0.5	
4									
5									

Figure 9: statistics.csv Table

Understanding statistics.csv:

- **Columns:**
 - **A:** Filename
 - **B:** True Positives (number of pixels correctly identified as roots)
 - **C:** False Positives (number of pixels incorrectly identified as roots)
 - **D:** False Negatives (number of root pixels missed by the model)
 - **E:** IoU (Intersection over Union—compares predicted to actual areas)
 - **F:** Precision (ratio of correctly identified roots to all items identified as roots)

- **G**: Recall (ratio of correctly identified roots to all actual roots in the image)
- **H**: F1 Score (harmonic mean of precision and recall, Eq. 1)

$$F_1 = \frac{2}{\text{recall}^{-1} + \text{precision}^{-1}}$$

Equation 1: Harmonic Mean (F1)

The F1 Score is widely used to gauge the accuracy of models in computer vision tasks by combining precision and recall into a single metric.

Calculating Final F1 Score:

- Compute the average F1 score across all evaluation images.
- In our experience:
 - For complex field data, an F1 score of at least 0.5 proved sufficient.
 - For controlled greenhouse experiments with less complexity, an F1 score of at least 0.65 was acceptable.
- Defining a suitable F1 score is ultimately up to you and depends on your experiment's requirements.
- It is advisable to consider the evaluation predictions and their respective error maps, as well as additional sample predictions from the rest of the dataset, to visually assess how closely the model's analyses match your ideal.
- Keep in mind that achieving higher accuracy requires exponentially more effort and training data.

Step 5: Create Training Images

If you determine that the model requires further training and adaptation to your experimental data, proceed with the annotation of training images. This is done in the same manner as described in **Step 3.2**, although it is advisable to use uncropped images. Start with 5 to 10 images that represent the variety of conditions within the entire dataset.

Step 6: Retrain Selected Model

1. **Prepare for Retraining:**
 - Open RootDetector and go to **Settings > Root Detection Model**.
 - Select the base model to retrain.
2. **Load Images and Annotations:**
 - Go to **Files > Load Input Images** and load the unedited scans into RootDetector.
 - Assign the respective annotations via **Files > Load Annotations**.
 - Images successfully paired with their annotations will appear bold.
3. **Configure Training Settings:**

- Navigate to the **Training** module.
- Set the following parameters:
 - **Model Type (1):** Choose **Root Detection**.
 - **Learning Rate (2):** Controls how much the model changes with each training step.
 - For smaller training sets, a lower value (e.g., 0.0001) is advisable to avoid overfitting.
 - **Number of Epochs (3):** Number of times the entire dataset is passed through the model.
 - For smaller training sets, a value of 10 is recommended.
 - **Starting Point of Retraining (4):** Displays the base model you selected.
 - **Number of Training Files (5):** Indicates the number of scans and annotation pairs.
- **Suggested Training Settings:**

Number of Training Images	Learning Rate	Number of Epochs
10	0.0001	10
25	0.0005	20
50	0.001	50

The screenshot shows the 'Training' tab of the RootDetector software. It includes a 'Model Type' dropdown menu set to 'Root Detection'. Under the 'Hyperparameters' section, the 'Learning rate' is set to 0.0001 and the 'Number of epochs' is set to 10. There is a text box for 'Starting point for retraining' with the value '2022-04-19_028a_WM' and another text box for 'Number of training files' with the value '39'. At the bottom, there is a 'Start Training' button.

Figure 10: Training Settings

4. Start Training:

- Click **Start Training**.
- Training may take several hours, depending on the settings and your computer's performance.
- Ensure that the computer does not enter sleep or energy-saving mode during training (Power Options Menu in Windows).

5. Save the New Model:

- After training, you will be prompted to save the new model with a name of your choice.
- Use a naming convention that allows for assessment of training with different settings.
 - Example: exp_10_LR00001_EP10_YYYYMMDD
 - exp: Experiment identifier
 - 10: Number of training images used
 - LR00001: Learning rate settings
 - EP10: Number of epochs
 - YYYYMMDD: Date of training

6. Evaluate Training Success:

- **Perform Frequent Evaluations:**
 - After training, assess the model's performance using the evaluation process described in **Step 4**.
 - Evaluate metrics such as the F1 Score to determine the model's accuracy.
- **Adjust Training as Necessary:**
 - If the model does not meet your accuracy requirements:
 - **Alter Training Variables:**
 - Modify the learning rate, number of epochs, or other settings.
 - Experiment with different configurations to improve performance.
 - **Increase Training Data:**
 - Annotate additional images to expand your training dataset.
 - A larger and more diverse dataset can enhance the model's ability to generalize.
- **Iterative Process:**
 - Recognize that model training is often iterative.
 - Be prepared to retrain the model multiple times, adjusting settings and data, to achieve optimal results.

Attention:

- **Avoid Overfitting:**
 - Retraining an already retrained model using the same training data can lead to overfitting.
 - Always use the original base model for each retraining iteration.
 - Ensure your training data is diverse and representative of the entire dataset.
- **Continuous Improvement:**
 - Regularly evaluate the model's performance during training.
 - Use evaluation results to guide adjustments in training settings or data augmentation.
 - Consider documenting different training configurations and their outcomes to track what works best for your data.

Step 7: Analyze Data

Once you are satisfied with the model's accuracy, proceed to analyze your dataset.

1. Load Dataset:

- Go to **Files > Load Input Folder** and select your dataset directory.
 - All subdirectories will be loaded as well.
- **Attention:** If the whole dataset cannot be loaded successfully, you may need to process your dataset in batches due to memory constraints.

2. Select Model and Settings:

- Under **Settings > Root Detection Model**, select the most accurate model.
- **Exclusion Mask:**
 - Decide whether to activate automatic detection of occlusions.
 - If activated, you will be prompted to select the most accurate Exclusion Mask model (see **Retraining Exclusion Mask Model**).
- **Skip Processing if Too Many Roots Detected:**
 - Allows for automatic skipping of images with too many roots to prevent memory overflow.
 - A value of around 200000 is recommended but may vary depending on your computer's performance.
- **Enable Processing on the GPU:**
 - Allows processing on your graphics processing unit (GPU) rather than the central processing unit (CPU).
 - Advisable if your machine has a high-end graphics card to speed up processing.
 - Should be turned off for machines without a dedicated graphics card.
- Click **Save** after configuring settings.

3. Process Data:

- Click **Process All**.
- Processing may take several hours.
- Ensure that the computer does not enter sleep or energy-saving mode.
- Successfully analyzed images will appear bold.

4. Download Results:

- Once processing is complete, click **Download All**.
- The results.zip file will be saved in your default download directory.

- results.zip contains:
 - A folder for each analyzed image, featuring:
 - Black-and-white segmentation images.
 - Skeletonized versions (roots reduced to 1-pixel width).
 - A statistics.csv file with detailed analysis of the respective image.
 - A main statistics.csv file providing detailed information about all processed images (Tab. 2).

	A	B	C	D	E	F	G	H	I
1	Filename	# root pixels	# background	# mask pixels	# skeleton pixels	# skeleton pixels (<3px wid	# skeleton pixels (3-7px widt	# skeleton pixels (>7px width)	Kimura length;
2	AD_T046_L00	90613	5705537	0	13721	6928	6162	631	15048;
3	AD_T046_L00	133331	5662819	0	27111	20593	5193	1325	30150;
4	AD_T047_L00	46465	5749685	0	11978	9698	2219	61	12978;
5	AD_T047_L00	40150	5756000	0	14417	13816	601	0	15542;
6	AD_T048_L00	21339	5774811	0	4499	3152	1312	35	4910;
7	AD_T050_L00	32538	5763612	0	10324	9802	522	0	10972;
8	AD_T052_L00	237208	5558942	0	76471	72178	4247	46	83756;
9	AD_T053_L00	81362	5714788	0	26348	24271	2077	0	28417;
10	AD_T054_L00	201774	5594376	0	66769	63356	3278	135	71796;
11	AD_T054_L00	5621	5790529	0	1419	1208	211	0	1501;

Table 2: Detailed analysis found in results.zip > statistics.csv

Understanding the Main statistics.csv:

- **Columns:**
 - **A:** Filename
 - **B:** Number of Root Pixels (pixels identified as roots)
 - **C:** Number of Background Pixels (pixels identified as soil)
 - **D:** Number of Mask Pixels (pixels identified by the Exclusion Mask Model as occlusions; always 0 if not activated)
 - **E:** Number of Skeleton Pixels (root pixels after skeletonization)
 - **F:** Skeleton Pixels (<3 px width) (count of pixels from roots originally thinner than 3 pixels)
 - **G:** Skeleton Pixels (3–7 px width) (count of pixels from roots originally between 3 and 7 pixels wide)
 - **H:** Skeleton Pixels (>7 px width) (count of pixels from roots originally wider than 7 pixels)
 - **I:** Kimura Length (estimate of total root length calculated by summing the lengths of root segments based on pixel connectivity, as described by Kimura et al. (1999). Dividing the displayed value by 10 yields total root length in millimeters.)

Exclusion Mask Model

The Exclusion Mask Model allows for automatic detection of foreign objects occluding the soil and root system, enabling accurate analysis of root length per visible area. It is advisable to retrain this model before use.

Steps:

1. Annotation:

- **Annotate Occlusions:**
 - Instead of annotating root structures in white, annotate occlusions (e.g., insulation tape, stones, debris) in red.
- **Using GIMP:**
 - Open the image in GIMP.
 - Create a new layer named Occlusions.
 - Ensure the Occlusions layer is above the and Soil layer.
 - Select the **Foreground Color** and set it to pure red (**HTML notation:** FF0000).
 - Use the **Free Select Tool** to mark the occluded areas.
 - Annotation can be coarse; precise edges are less critical. Instead focus on creating larger quantities of training data to ensure successful training (Fig. 11).



Figure 11: suggested Exclusion Mask Annotation

- **Export Annotations:**
 - Export the annotated image as a .png file, following the same steps as before.

2. Training:

- Follow the same procedure as in **Step 6**, but in the **Training** module:
 - Set **Model Type** to **Exclusion Mask**.
 - Load the images and their corresponding occlusion annotations.
- **Training Settings:**
 - Use a higher number of training images if possible, to improve accuracy.
 - Adjust the learning rate and epochs as needed.

- **Evaluation:**
 - Although it is generally possible to calculate F1 (as described in Step 4), sampling some scans from your dataset and evaluating them visually is often sufficient.

Root Tracking Module

The experimental Root Tracking Module allows for the assessment of root turnover over time.
MORE
WORK
NEEDED

Troubleshooting

- **Problem:** Input images won't load correctly.
 - **Solution:** Ensure that the images are in the correct file format. RootDetector requires .tiff, .tif, .jpeg, or .png files for analysis or training bases and .png files for annotations.
- **Problem:** The processing stops due to too many roots detected.
 - **Solution:** Increase the value in the **Skip Processing if Too Many Roots Detected** setting or process the dataset in smaller batches.
- **Problem:** The computer slows down or crashes during processing.
 - **Solution:** Ensure that your computer meets the minimum system requirements and close unnecessary applications to free up resources.
- **Problem:** The model's accuracy is unsatisfactory.
 - **Solution:** Retrain the model using more training images or adjust the training parameters (e.g., learning rate, number of epochs).
- **Problem:** Annotations appear blurry or have unintended shades.
 - **Solution:** In GIMP, make sure that all tools used for annotation have **Antialiasing** turned off and **Hardness** set to **100%**. Ensure that you are using pure black (000000), pure white (FFFFFF), or pure red (FF0000) as needed.
- **Problem:** RootDetector cannot find the images or annotations.
 - **Solution:** Verify that the file paths are correct and that the filenames of images and their corresponding annotations match exactly.

References

- **Johnson, M. G., Tingey, D. T., Phillips, D. L., & Storm, M. J. (2001).** Advances in Minirhizotron Technology for Measuring Root Dynamics. *Plant and Soil*, 235, 103–112.
- **Joslin, J. D., & Wolfe, M. H. (1999).** Disturbances During Minirhizotron Installation Can Affect Root Observation Data. *Soil Science Society of America Journal*, 63(1), 213–217.
- **Kimura, K., Kikuchi, S., & Yamasaki, S.-I. (1999).** Accurate Root Length Measurement by Image Analysis. *Plant and Soil*, 216(1–2), 117–127. <https://doi.org/10.1023/A:1004778925316>
- **Peters, J., et al. (2025).**
- **Rewald, B., & Ephrath, J. E. (2013).** Minirhizotron Installation Techniques: A Review. *Agronomy Journal*, 105(1), 14–22.