LEUPHANA UNIVERSITY OF LÜNEBURG

DFG Deutsche Forschungsgemeinschaft

Using (mini)rhizotrons to analyse root distribution: experimental set-up, image acquisition and analysis pipelines

Benjamin Delory

Benjamin.Delory@leuphana.de

@BenjaminDelory

www.benjamindelory.com

Content of the workshop

Part I: Using rhizoboxes to analyse vertical root distribution in controlled experiments

- Experimental set-up
- Image acquisition
- Image analysis pipeline (image segmentation and feature extraction)

Part II: Using minirhizotrons to analyse vertical root distribution in the field

- Installation of minirhizotrons
- Image acquisition
- Image analysis pipeline (image segmentation and feature extraction with RhizoVision Explorer)
- Data exploration and analysis

A research question to guide us in this workshop

Does the order of arrival of plant species during assembly of grassland communities affect the vertical distribution of roots in the soil?

Part I: Using rhizoboxes to analyse vertical root distribution in controlled experiments

What are rhizoboxes (or “root boxes”)?

- **Soil-filled container equipped with a transparent window**
- Used to non-destructively monitor root growth and root system development over time
- Pros:
  - Customable design (height/width/depth, construction material, etc.)
  - Easy to replicate
  - Compatible with several imaging techniques to study rhizosphere processes (planar optodes, zymography, etc.)
- Cons:
  - Constrained root growth
  - Difficult to track roots at the individual or species level in multispecies communities

Freschet et al, 2021. *New Phytologist*, 232, 973-1122 (link)

Our experimental set-up

White polystyrene plate
(avoid overheating)

Plastic container
(56.5×36.5 ×31.5 cm³, L×W×H)

Soil-filled rhizobox
(26.6×2×58 cm³, L×W×H)

Black plastic plate
(prevent root exposure to light)

Alonso-Crespo et al, 2022. *Oikos*, e08886 (link)

Image acquisition

Computer (connected to a Canon EOS 5D Mark III)

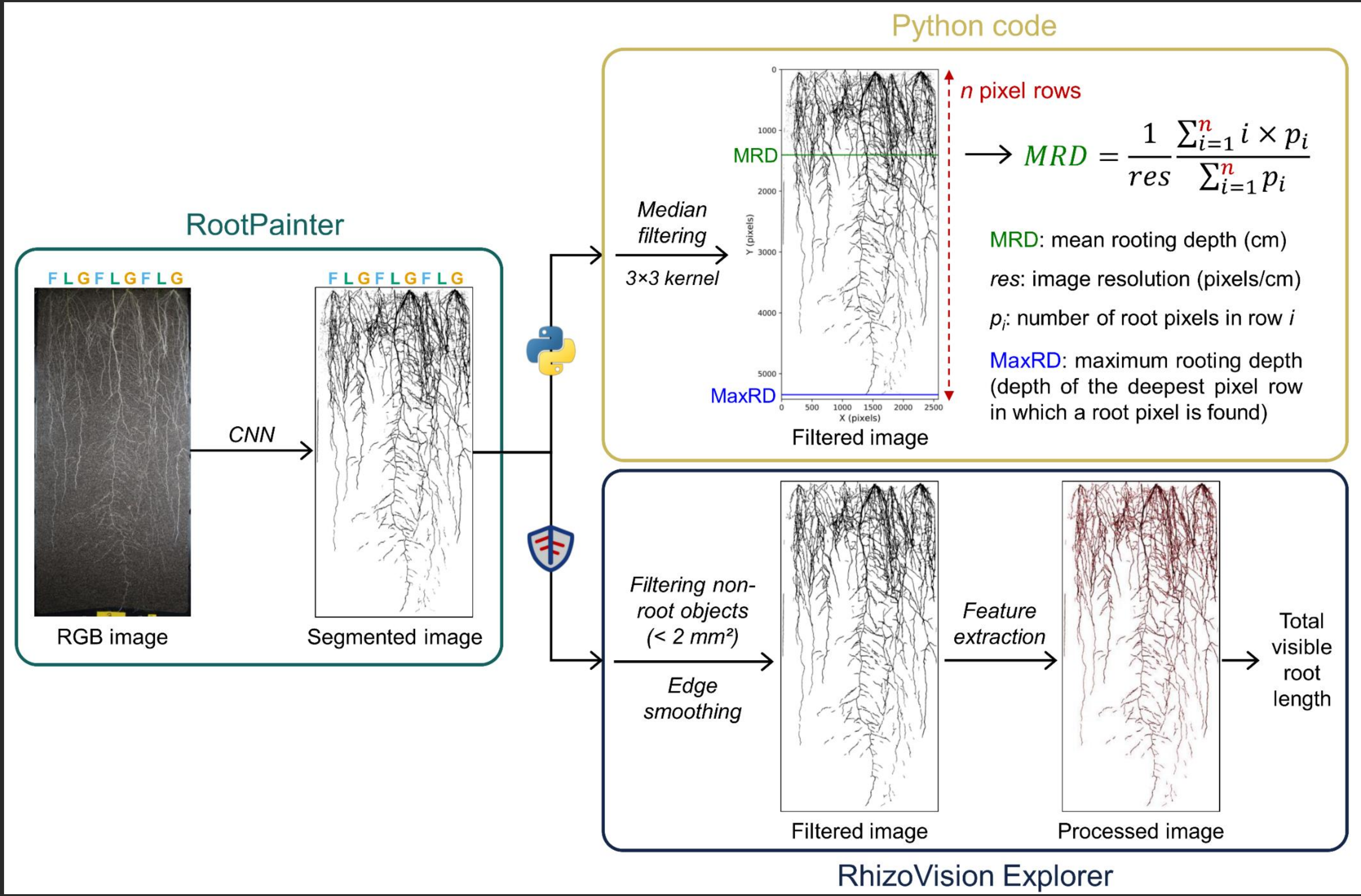
Soil-filled rhizobox (26.6×2×58 cm³, L×W×H)

Camera holder

LED tube (4300 K, 60 cm)

Alonso-Crespo et al, 2022. *Oikos*, e08886 (link)

Image analysis pipeline

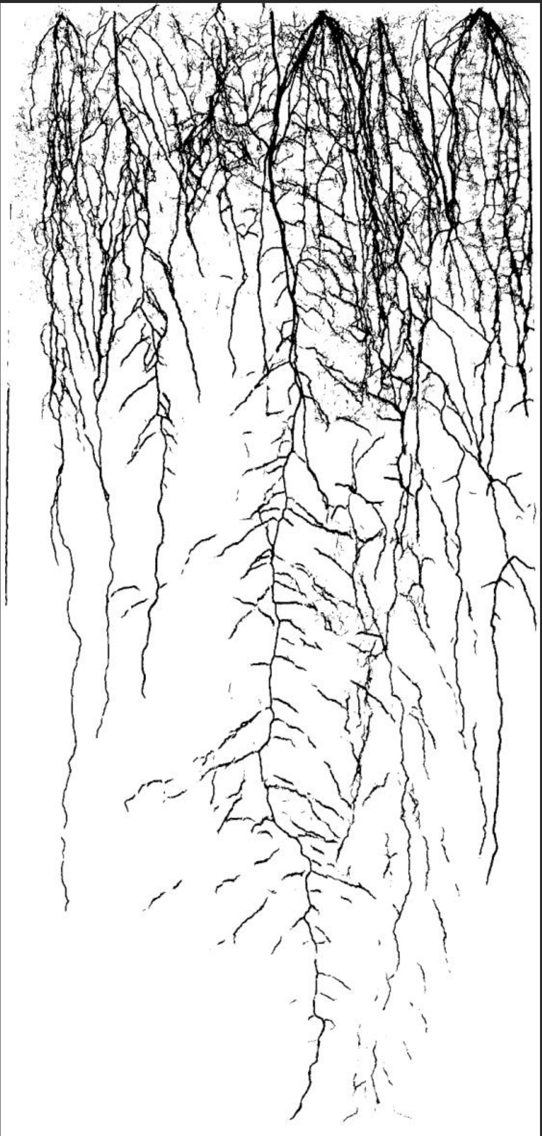


Alonso-Crespo et al, 2022. *Oikos*, e08886 ([link])

Image analysis pipeline – Step 1: image segmentation with RootPainter

RGB image

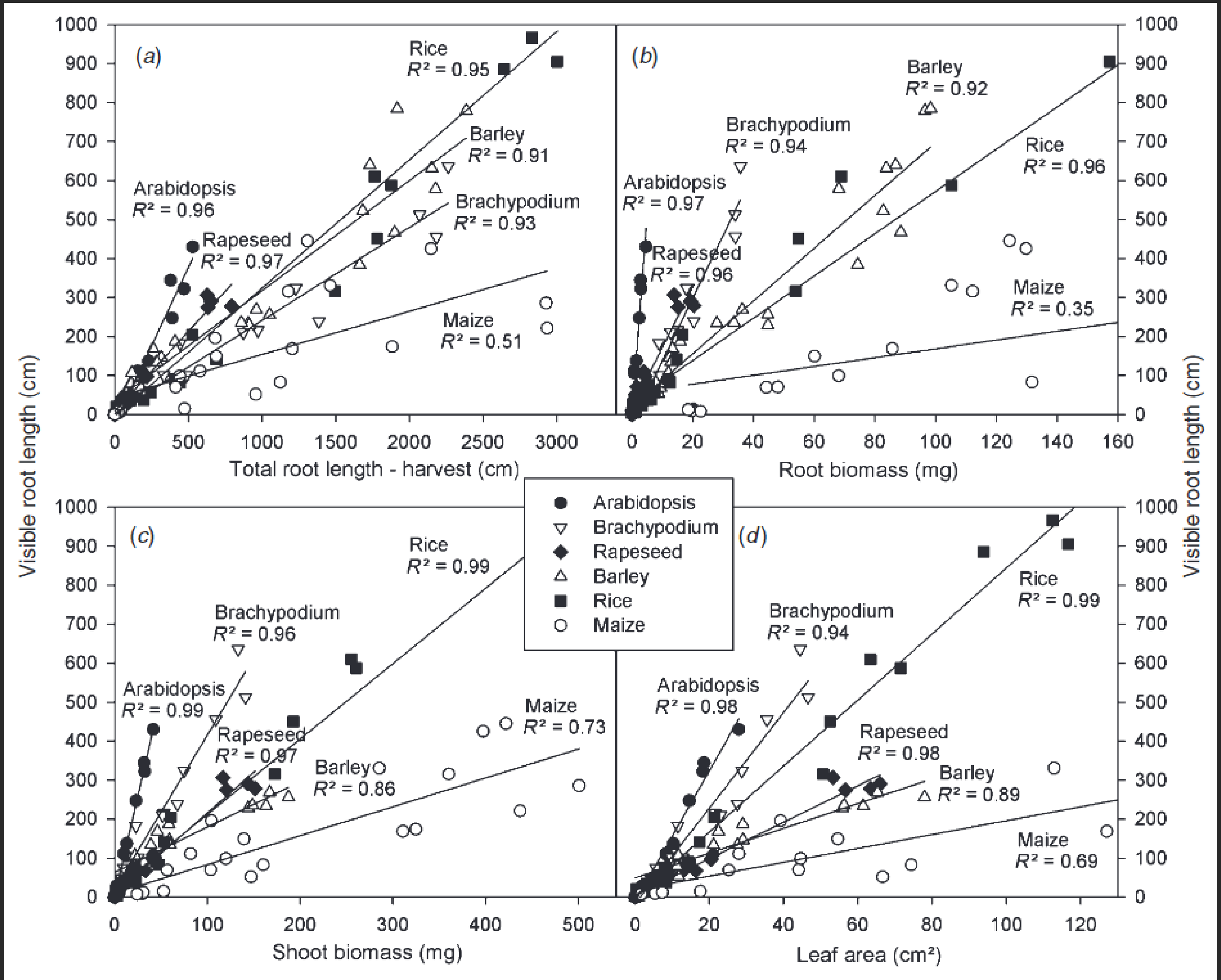
Segmented image



Link to the PhenomeForce workshop of Abraham Smith

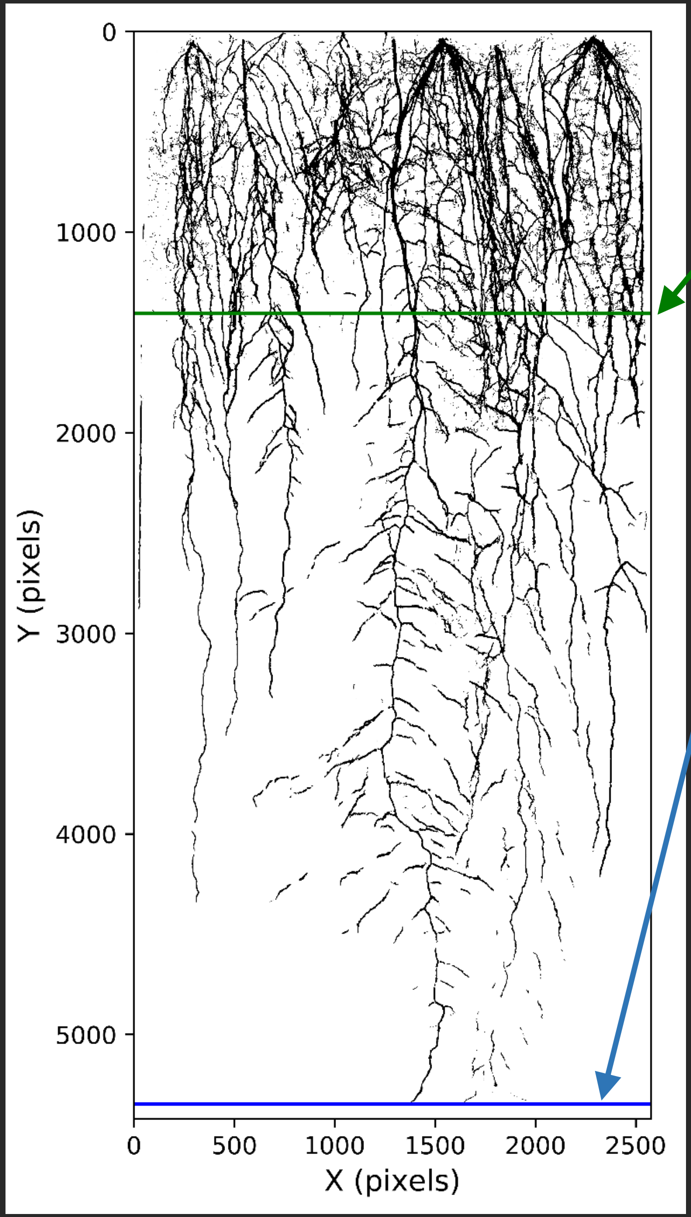
Alonso-Crespo et al, 2022. *Oikos*, e08886 (link); Smith et al, 2022. *New Phytologist*, 236, 774-791 (link).

Visible and total root lengths are correlated



Nagel et al, 2012. *Functional Plant Biology*, 39, 891-904 (link)

Image analysis pipeline – Step 2: feature extraction



Mean rooting depth (MRD): depth value above which 50% of the root pixels are located

Maximum rooting depth (MaxRD): depth of the deepest pixel row in which a root pixel is found

D.X: depth value above which X% of the root pixels are located.

Deep root fraction (DRF): proportion of root pixels located in the lower X% of the segmented/filtered image [0,1]

Root surface area (RSA): total area of root pixels in the segmented/filtered image

Relative root surface area (RRSA): proportion of root pixels in the segmented/filtered image [0,1]

Our image analysis pipeline is available as an R package

POEMdemo

R package with functions to process segmented (mini)rhizotron images. This R package was used for the Phenome Force workshop "Using (mini)rhizotrons to analyse root distribution: experimental set-up, image acquisition and analysis pipelines" (October 28, 2022).

Using Python with RStudio and reticulate

Some functions of this R package use Python code. In order to be able to use the package, you will have to install a base version of Python, create and activate your Python environment, install Python packages in your environment, as well as install and configure reticulate to use Python.

All these steps are described in the following video tutorial:

https://docs.rstudio.com/tutorials/user/using-python-with-rstudio-and-reticulate/

Required Python packages: numpy, PIL, pandas, scipy, shutil, os

Install the R package POEMdemo

To install the package, run this code in your R console:

```
install.packages("devtools")
devtools::install_github("POEMexperiment/POEMdemo")
```

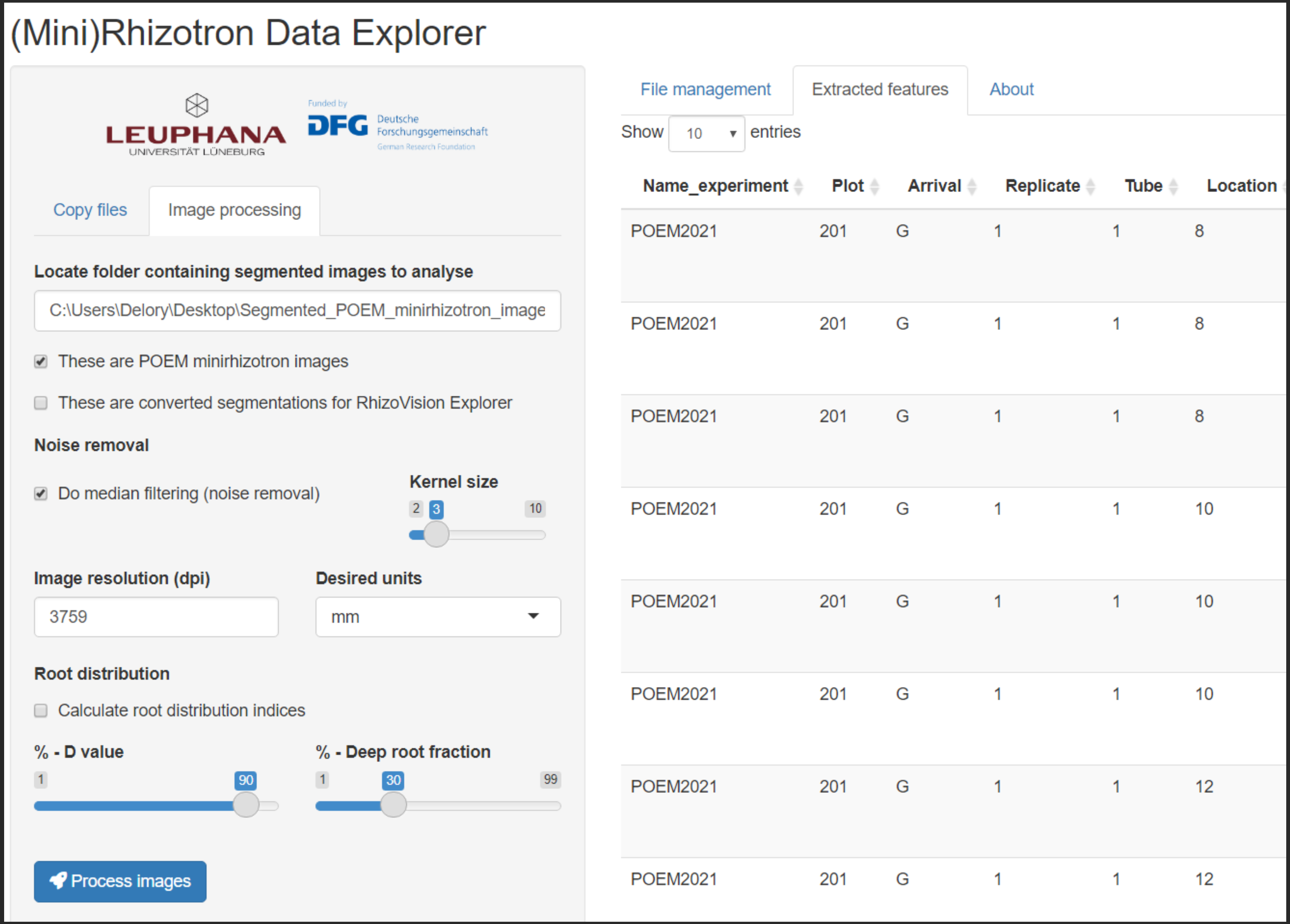
Run the rhizotron explorer (shiny app)

```
POEMdemo::rhizotron_explorer()
```

Our R package (POEMdemo) is available on GitHub

https://github.com/POEMexperiment/POEMdemo

(Mini)Rhizotron Data Explorer



Shiny app to automate feature extraction from segmented rhizobox or minirhizotron images

Uses both R and Python

Computes root distribution indices (mean and max rooting depth, D values, deep root fraction, etc.)

Let’s try it!

October 22, 2022

Dataset Open Access

Rhizobox images for RootPainter demo

Inés M. Alonso-Crespo; Vicky M. Temperton; Benjamin M. Delory

This dataset consists of 35 rhizobox images. The experimental set up and image analysis pipeline have been described in the following paper:

Alonso-Crespo IM, Weidlich EWA, Temperton VM, Delory BM (2022). Assembly history modulates vertical root distribution in a grassland experiment, Oikos, e08886.

Root images were acquired using a digital camera (Canon EOS 5D Mark III) equipped with a 28 mm lens (Canon EF 28mm f/2.8) and connected to a computer. In addition to the camera and its associated holder, our image acquisition system consists of a metallic frame holding the rhizobox vertically and two LED tubes (4300 K, 60 cm length) positioned laterally (raking lighting) to provide uniform lighting conditions over the entire height of a rhizobox. The camera, the camera holder, the LED tubes, and the frame holding the rhizobox were installed inside a closed box whose internal walls were entirely covered with dark fabric. The camera faced each rhizobox directly and the camera lens was located 54 cm from the surface of the transparent front window. Images (3,840×5,760 pixels, width×height; resolution: 94.8 pixels/cm or ~240 dpi) were acquired using our camera’s remote live view and shooting option before being saved on a computer.

Preview

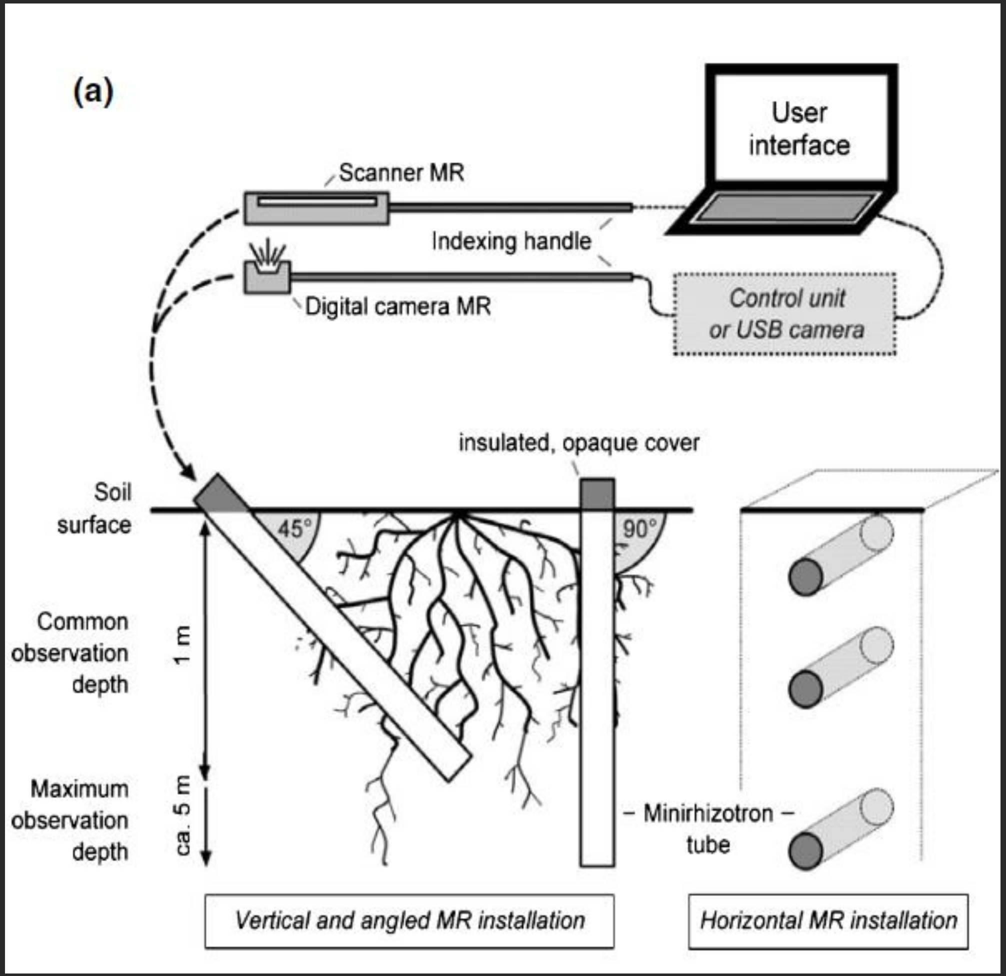
Files (327.3 MB)

Name	Size	
Rhizobox_images_RootPainterDemo.zip md5:d6eb80d018602119225eb591c0bb0ad8	320.4 MB	Preview Download
Segmented_rhizobox_images_RootPainterDemo.zip md5:e4ad7a52dfc2bbd2f7bad4f8240e1e0a	7.0 MB	Preview Download

A dataset consisting of unprocessed and segmented rhizobox images is available on Zenodo.

Part II: Using minirhizotrons to analyse vertical root distribution in the field

What are minirhizotron systems?



- **Minirhizotron systems rely on closed transparent tubes inserted into the soil**
- Used to non-destructively monitor root growth, root dynamics (turnover), root distribution and biotic interactions
- Pros:
  - Customable design (length, construction material, imaging system, etc.)
  - Easy to replicate
  - Can be installed vertically, horizontally or angled
- Cons:
  - Expensive
  - Limited viewing area
  - Data acquisition is time consuming, especially if a high spatio-temporal resolution is required
  - Difficult to track roots at the individual or species level in multispecies communities

Freschet et al, 2021. *New Phytologist*, 232, 973-1122 (link); Defrenne et al, 2021. *Plants People Planet*, 3, 640-652 (link)

Installing minirhizotrons in the field

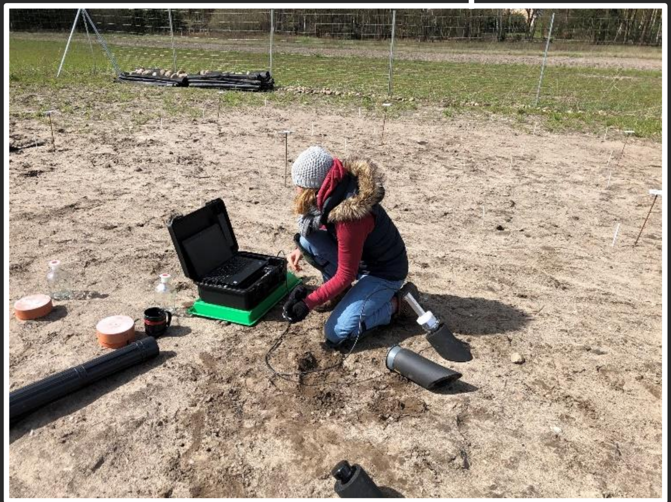
Plan ahead and give it time!

October 2020 — November 2020 — April 2021

Minirhizotron installation

6 months

Experiment and image acquisition start



Installing minirhizotrons in the field

<u>Step 1</u>: Position and level the support to take a soil core at the right angle

Installing minirhizotrons in the field

Installing minirhizotrons in the field

Installing minirhizotrons in the field

Tube protection from light and weather (insulation)

Sowing of the plots (6 months after minirhizotron installation)

Image acquisition

Minirhizotron camera (MS-190, Vienna Scientific Instruments, resolution: ~2500 dpi, image size < 4 cm²).
Website: https://www.vienna-scientific.com/products/minirhizotron-systems/manual/

CI-600/602 scanner (CID Bio-Science, resolution: up to 1200 dpi, image size ~ 388 cm²).
Website: https://cid-inc.com/plant-science-tools/root-measurement-with-minirhizotron/ci-602-narrow-gauge-root-imager/

Image acquisition

JOURNAL ARTICLE ACCEPTED MANUSCRIPT

High Frequency Root Dynamics: Sampling And Interpretation Using Replicated Robotic Minirhizotrons

Richard Nair, Martin Strube, Martin Hertel, Olaf Kolle, Victor Rolo, Mirco Migliavacca

Journal of Experimental Botany, erac427, https://doi.org/10.1093/jxb/erac427

Published: 23 October 2022 **Article history** ▾

RESEARCH ARTICLE

Plants People Planet PPP Open Access

High-resolution minirhizotrons advance our understanding of root-fungal dynamics in an experimentally warmed peatland

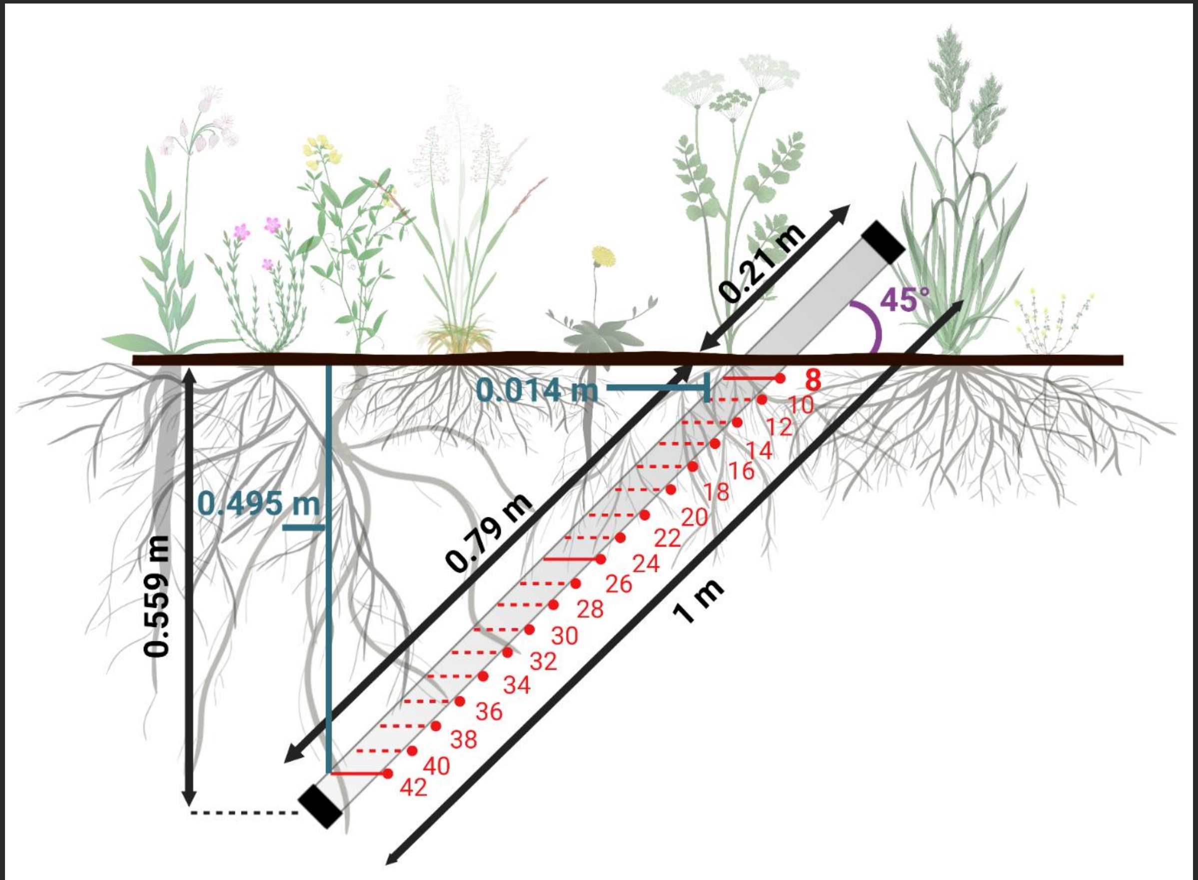
Camille E. Defrenne[1] | Joanne Childs[1] | Christopher W. Fernandez[2] | Michael Taggart[3] | W. Robert Nettles[1] | Michael F. Allen[3] | Paul J. Hanson[1] | Colleen M. Iversen[1]

Choosing the image acquisition system that suits your needs

Some **factors to consider**:

- Research question (What will you use the images for?)
- Root size
- Image size
- Image quality (resolution)
- Image acquisition time
- Level of automation
- Robustness
- Autonomy (energy supply)
- Cost (€€€)

Image acquisition in the field



Protocol for image acquisition in our field experiment

Image acquisition using the VSI MS-190 camera system

Taking a peak into the soil using minirhizotrons

27/04/2021 | 23/06/2021 | 04/08/2021 | 18/10/2021 | 14/02/2022

Soil depth

7.1 cm

21.2 cm

35.4 cm

49.5 cm

1 cm

Camera: VSI MS-190

PMMA (acrylic) tubes (diameter: 6 cm; wall thickness: 3 mm)

18 images/tube (1 image every 2.8 cm)

Images taken every 2 weeks from April to September, and once a month from October to March

12,960 images/year

Challenging situations for image analysis

Adjacent roots

Root hairs and fungal hyphae

Scratches

Water drops

Image analysis pipeline

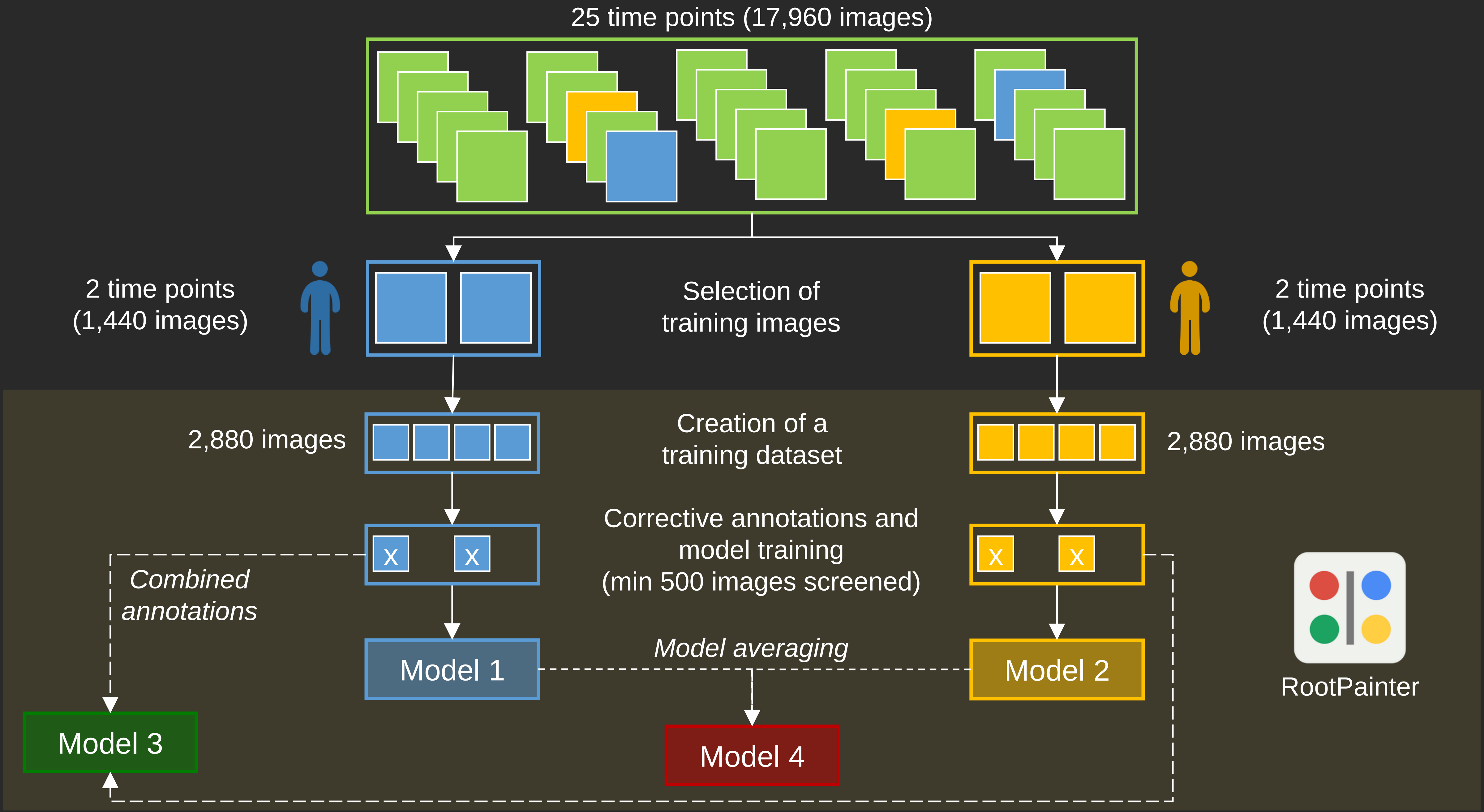
Original image

Image segmentation with RootPainter

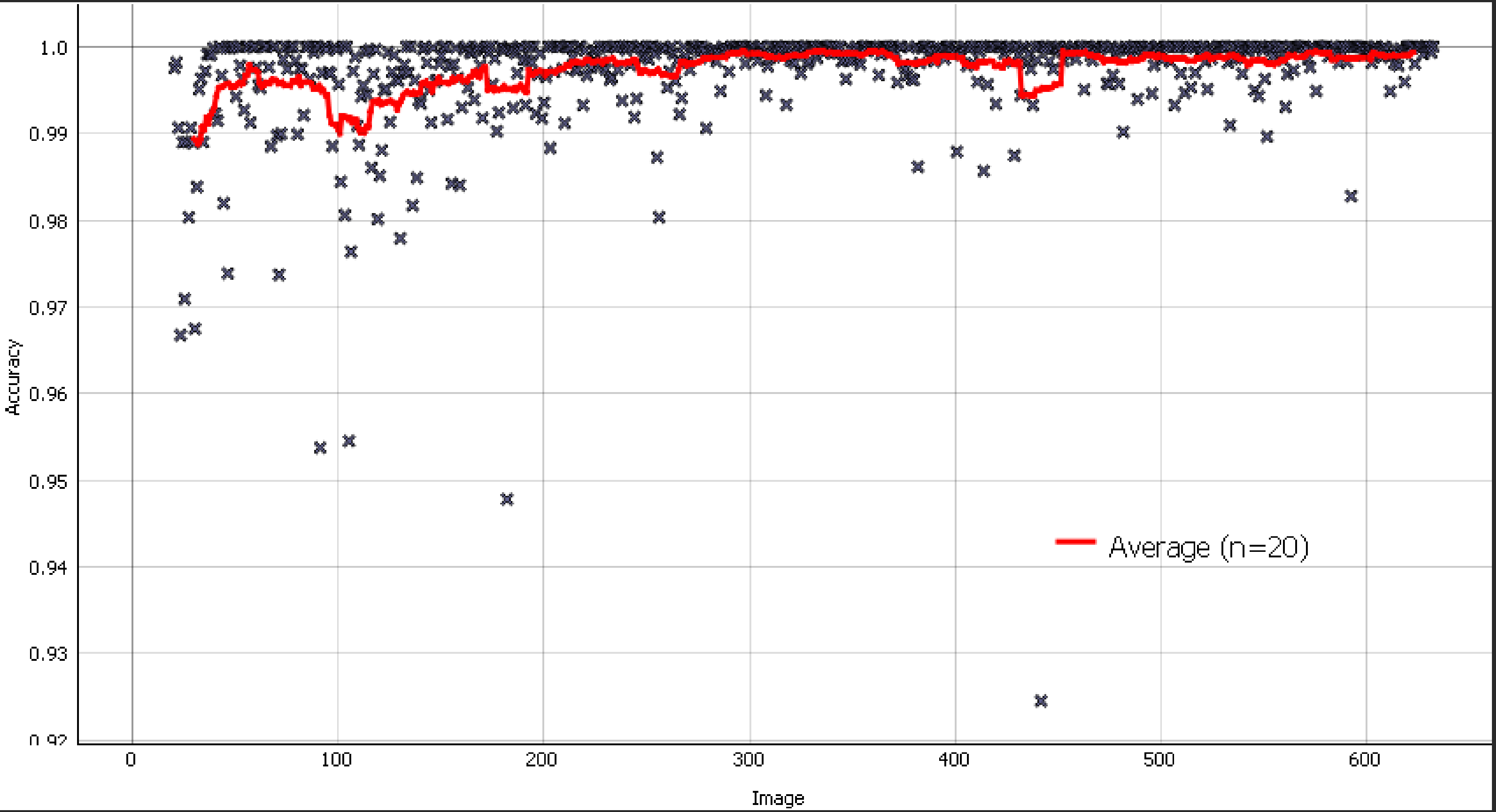
Feature extraction with RhizoVision Explorer

Smith et al, 2022. *New Phytologist*, 236, 774-791 (link); Seethepalli et al, 2021. *AoB Plants*, 13, 6 (link); Bauer et al, 2022. *Plant Phenomics* (link)

Our strategy for image segmentation

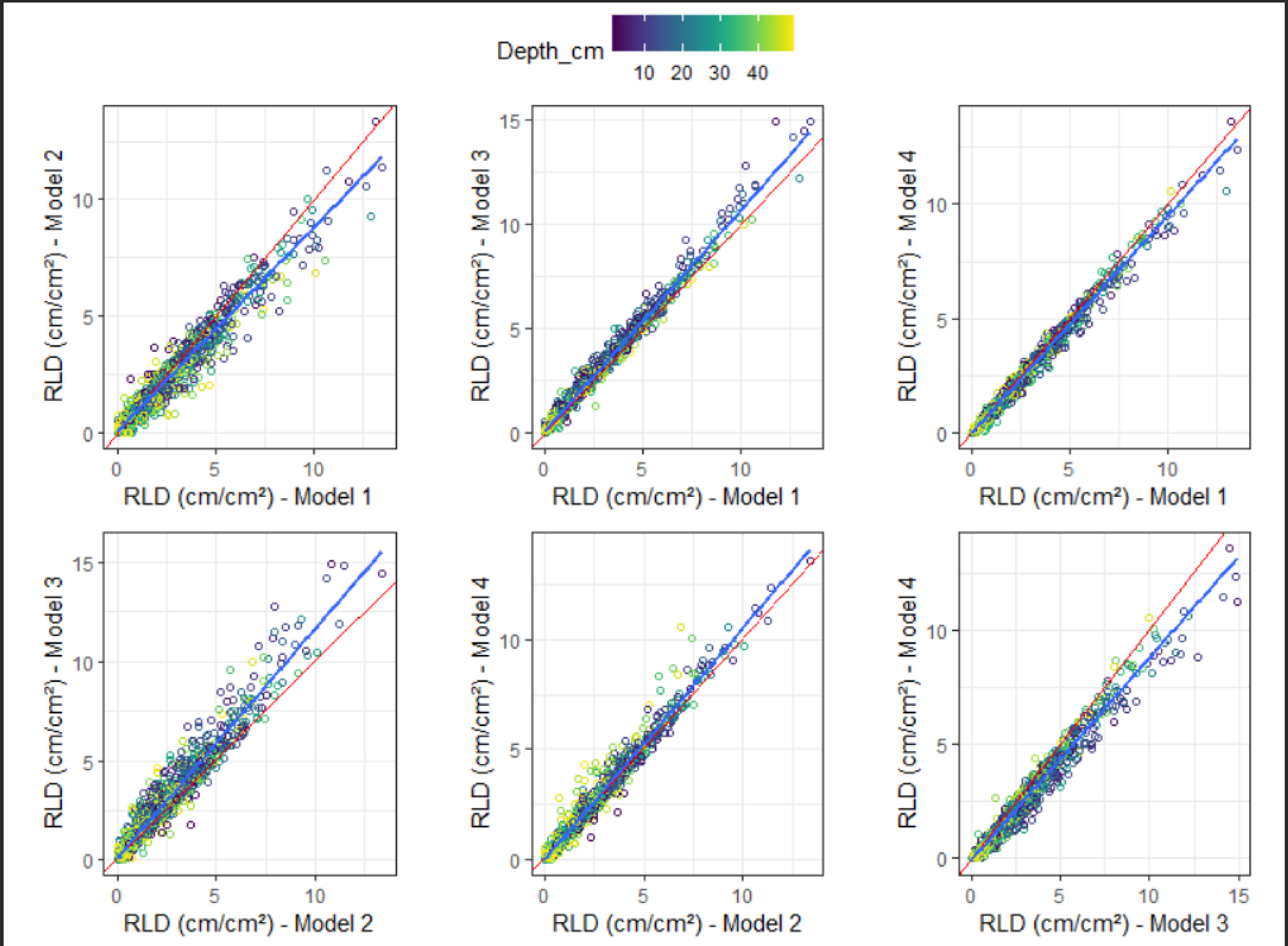


Our strategy for image segmentation



Accuracy of Model 1 for each of the images segmented as part of the interactive segmentation procedure in order of annotation. Accuracy ranges from 0 to 1 and is the ratio of pixels that were predicted correctly to the total pixels in the image (Smith et al, 2022. *New Phytologist*).

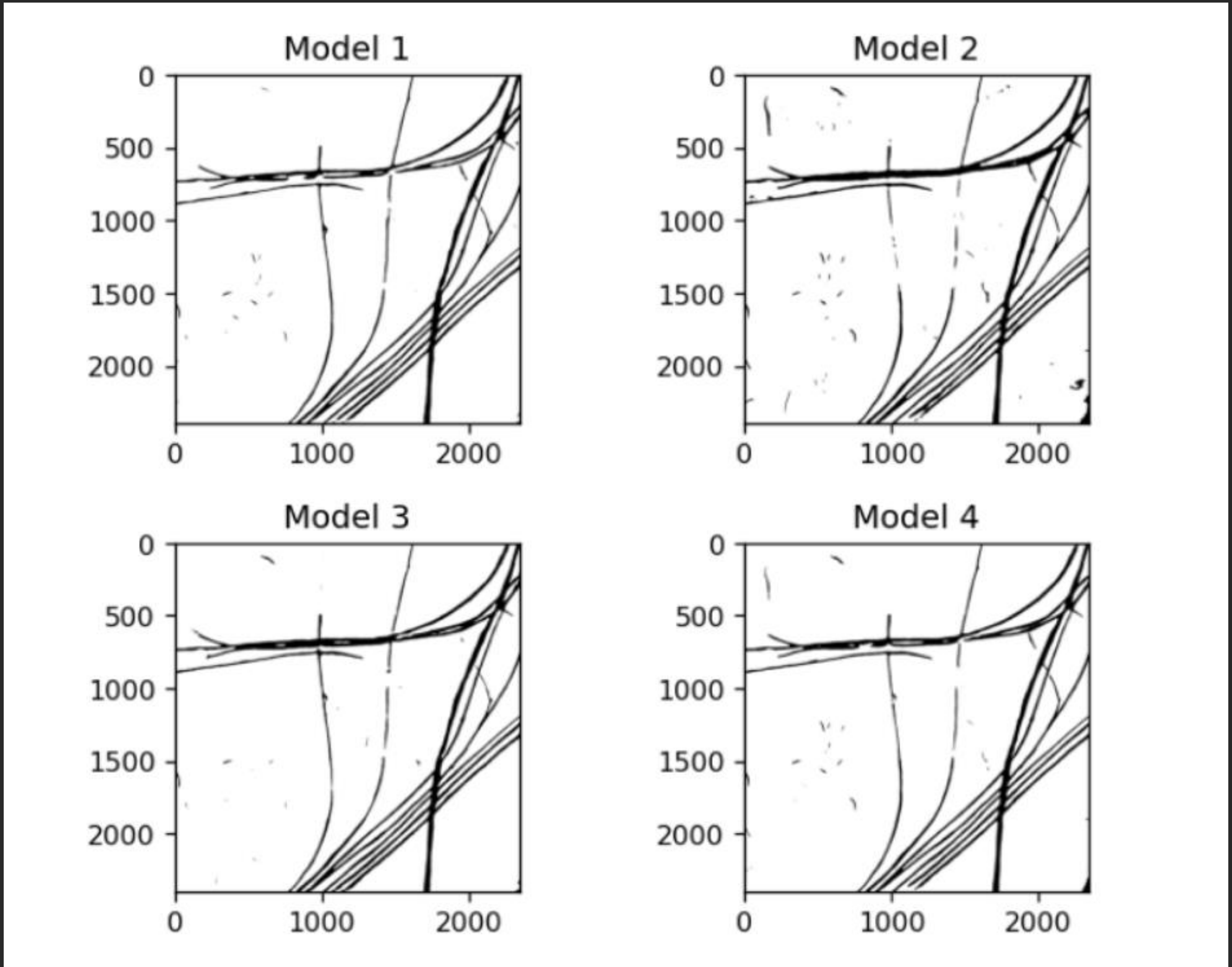
Our strategy for image segmentation



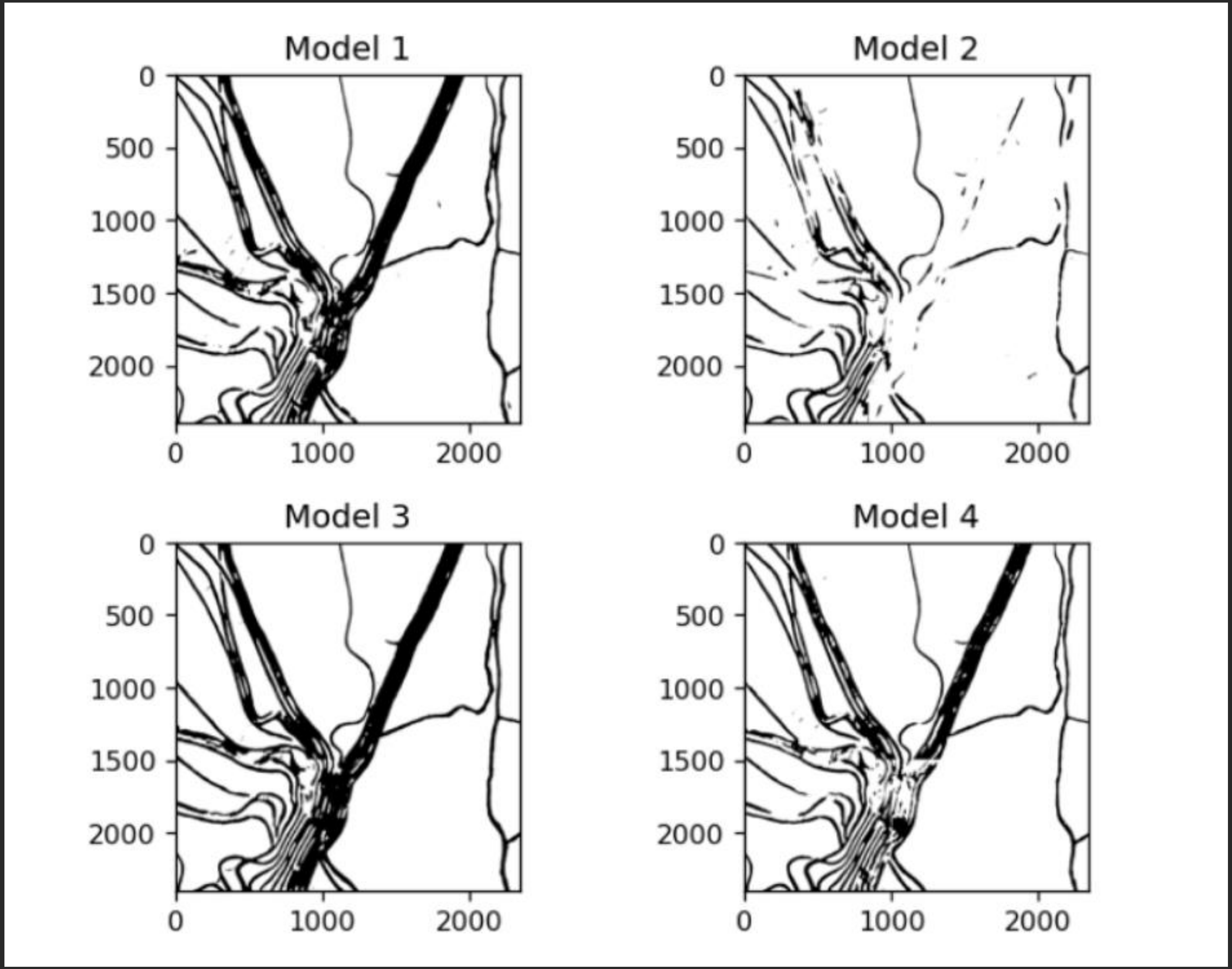
Correlation and agreement between four RootPainter models used for image segmentation (n=1,440; new images not used for model training)

RLD is the root length density in an image

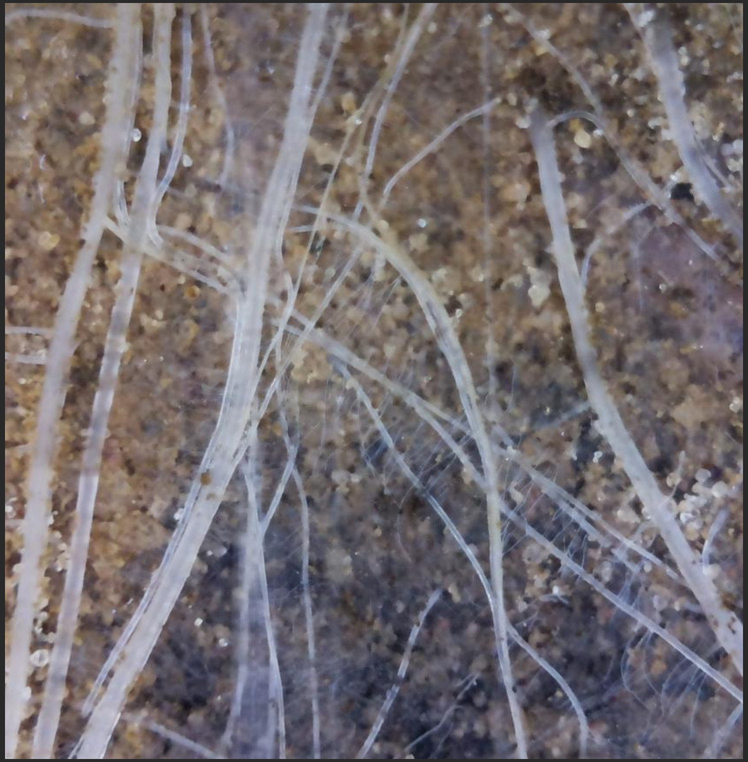
Our strategy for image segmentation

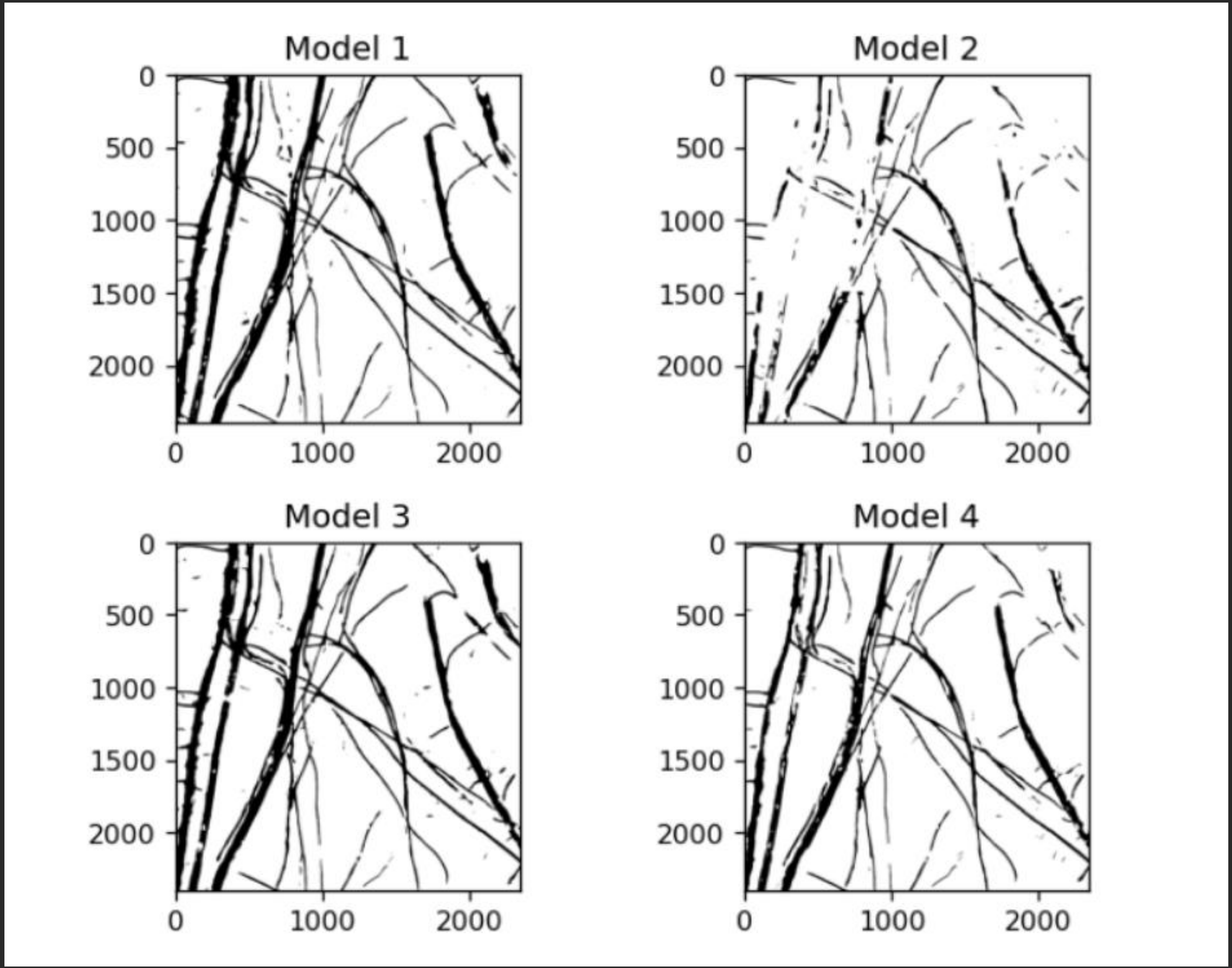


Our strategy for image segmentation

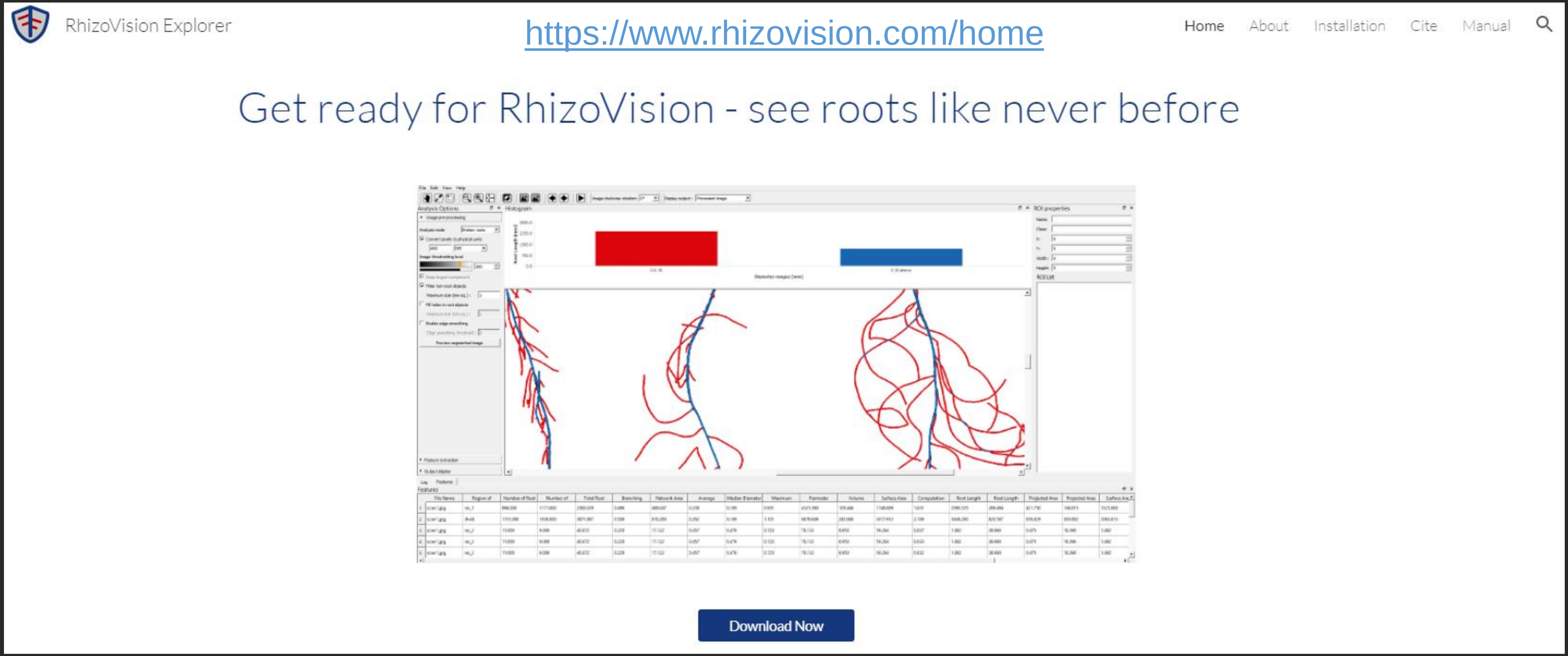


Our strategy for image segmentation





Feature extraction with RhizoVision Explorer



Link to the [PhenomeForce workshop of Dr Larry York]

Seethepalli et al, 2021. *AoB Plants*, 13, 6 ([link])

Let's try it!

October 22, 2022

Dataset Open Access

Minirhizotron images for RootPainter demo

Inés M. Alonso-Crespo; Vicky M. Temperton; Benjamin M. Delory

This dataset consists of 100 minirhizotron images (2340 x 2400 pixels; resolution: 148 px/mm) taken with the manual UHD minirhizotron camera (VSI-BARTZ MS-190) from Vienna Scientific Instruments.

The images were acquired in a grassland field experiment (POEM experiment) in which the order of arrival of three plant functional groups (forbs, grasses and legumes) was manipulated. In each plot, two root observation tubes were installed at a 45° angle six months before the start of the experiment (i.e. the first sowing event on April 13, 2021). Along each tube, images are taken at 18 different depths (from 1.4 to 49.5 cm) twice a month from April to September and once a month from October to March.

The POEM experiment is funded by the German Research Foundation.

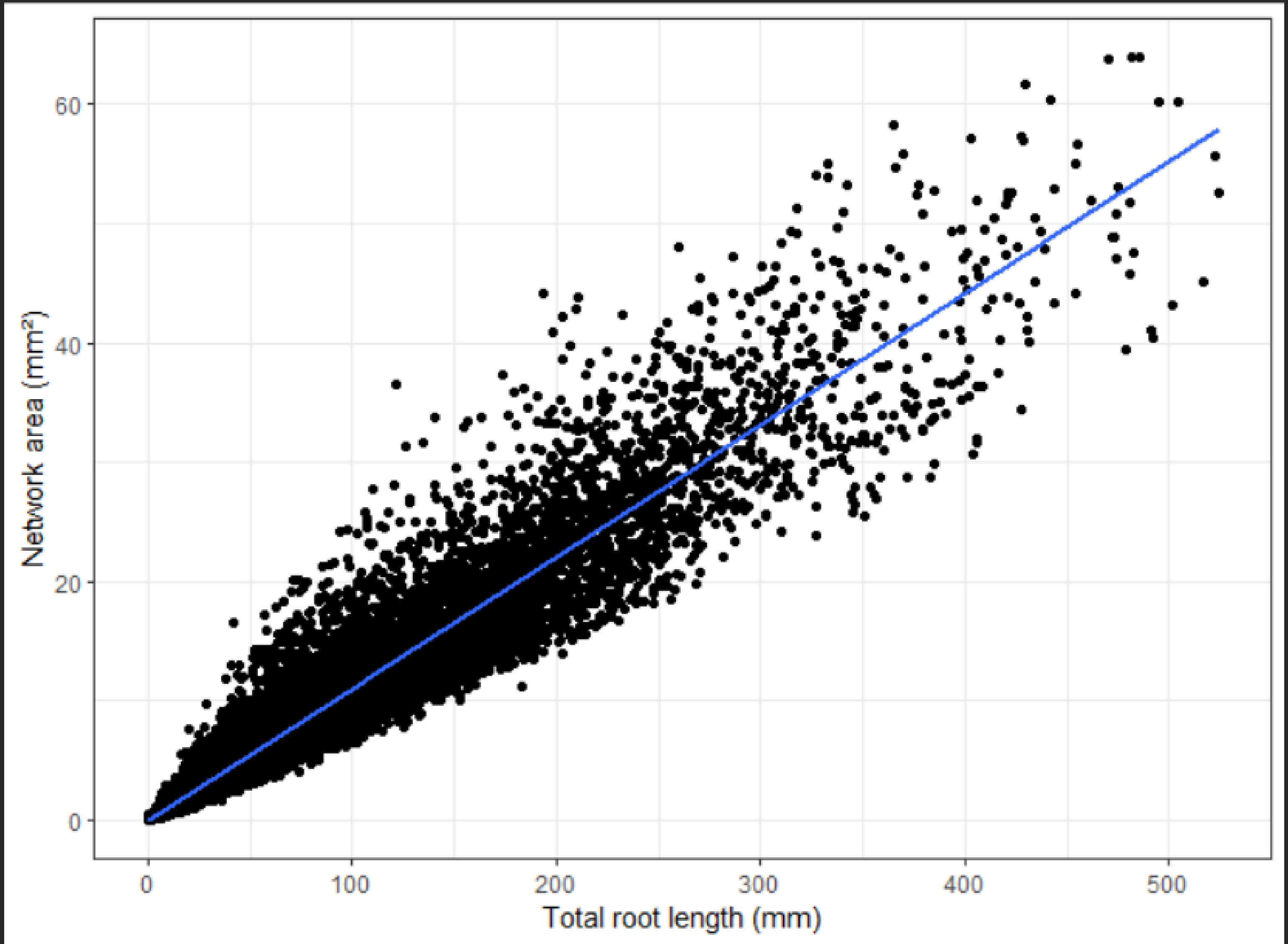
Preview

Files (1.0 GB)

Name	Size	
BenjaminDelory/Minirhizotron_images_RootPainterDemo-0.2.0.zip md5:6aae726ae6f203d8bf40d86da934172c	1.0 GB	Preview Download
Segmented_RVE_minirhizotron_images_RootPainterDemo.zip md5:35a0dda12db6ebb034946615d0f4be00	3.2 MB	Preview Download
Training_data_RootPainterDemo.zip md5:e6cb873cbc83df5e070dcb96a9a72c8d	16.4 MB	Preview Download

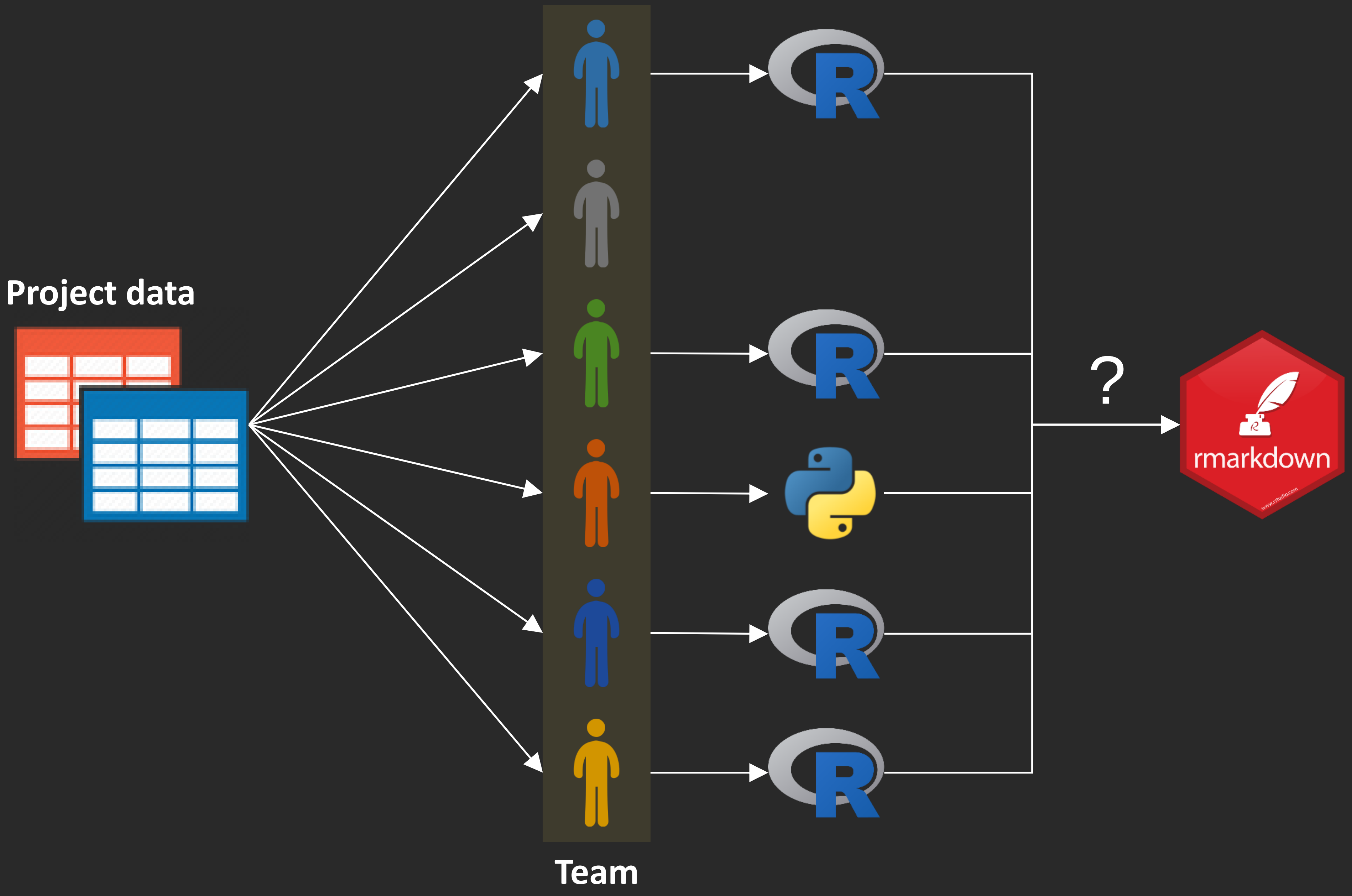
A dataset consisting of unprocessed and segmented minirhizotron images is available on Zenodo.

Data exploration and analysis



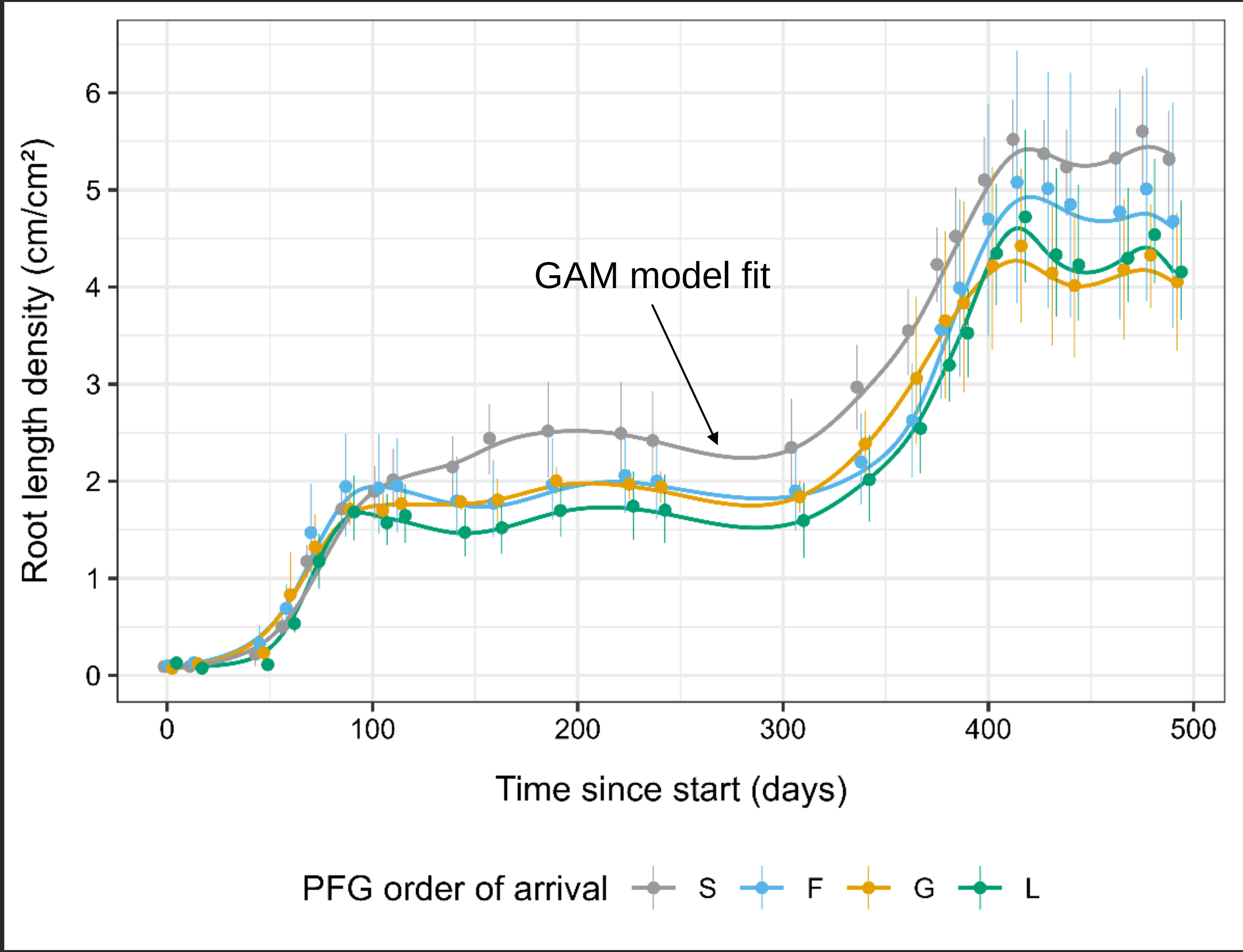
Correlation between the total root length and network area measured with RhizoVision Explorer (n=17,960)

Data exploration and analysis



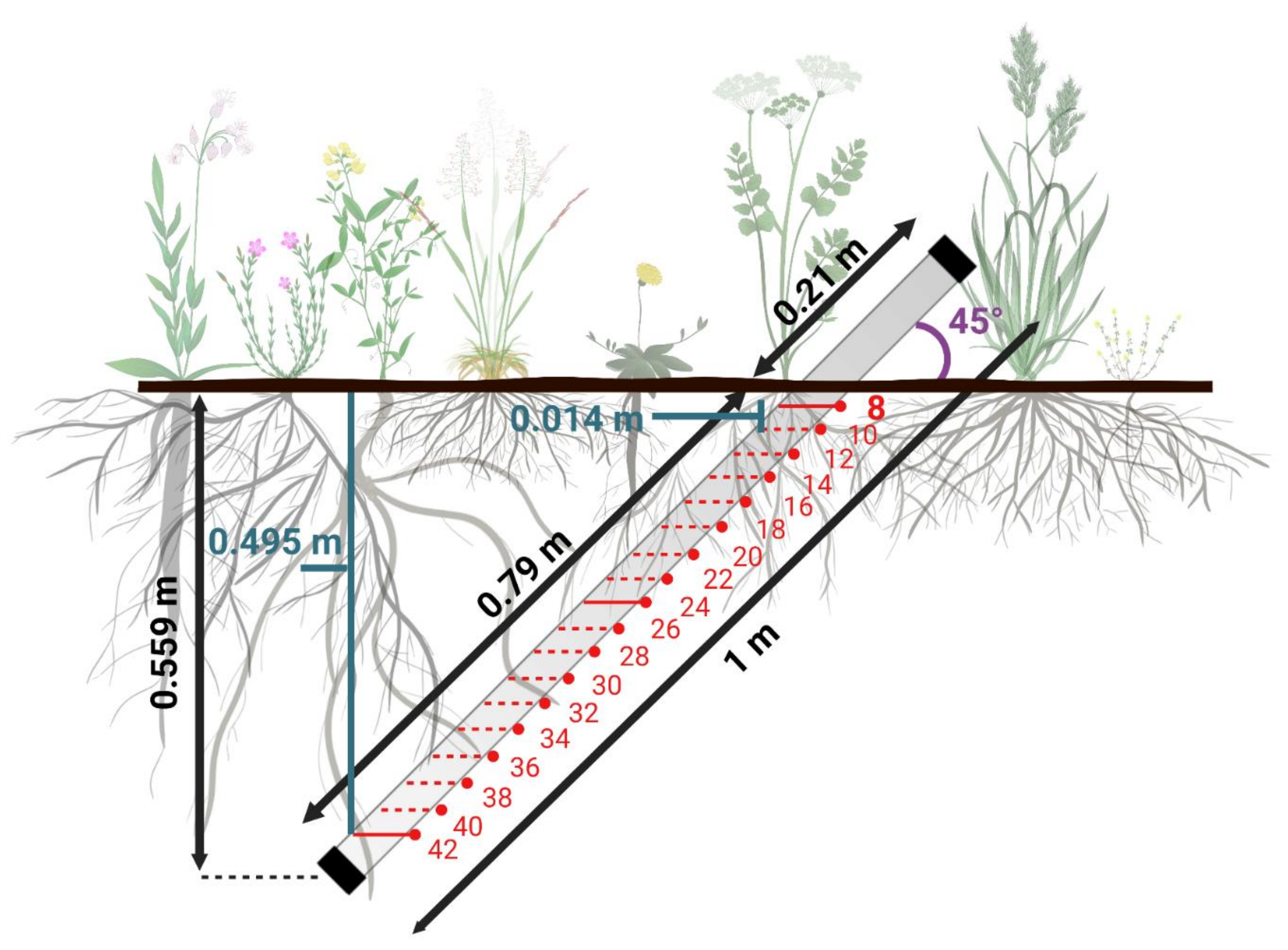
Standardisation is key to make code chunks written by different team members compatible

Data exploration and analysis



Alonso-Crespo et al, *in prep*

Estimating the mean rooting depth of plant communities



$$MRD = \frac{\sum_{i=1}^{n} d_i \times RLD_i}{\sum_{i=1}^{n} RLD_i}$$

MRD is the mean rooting depth (cm)

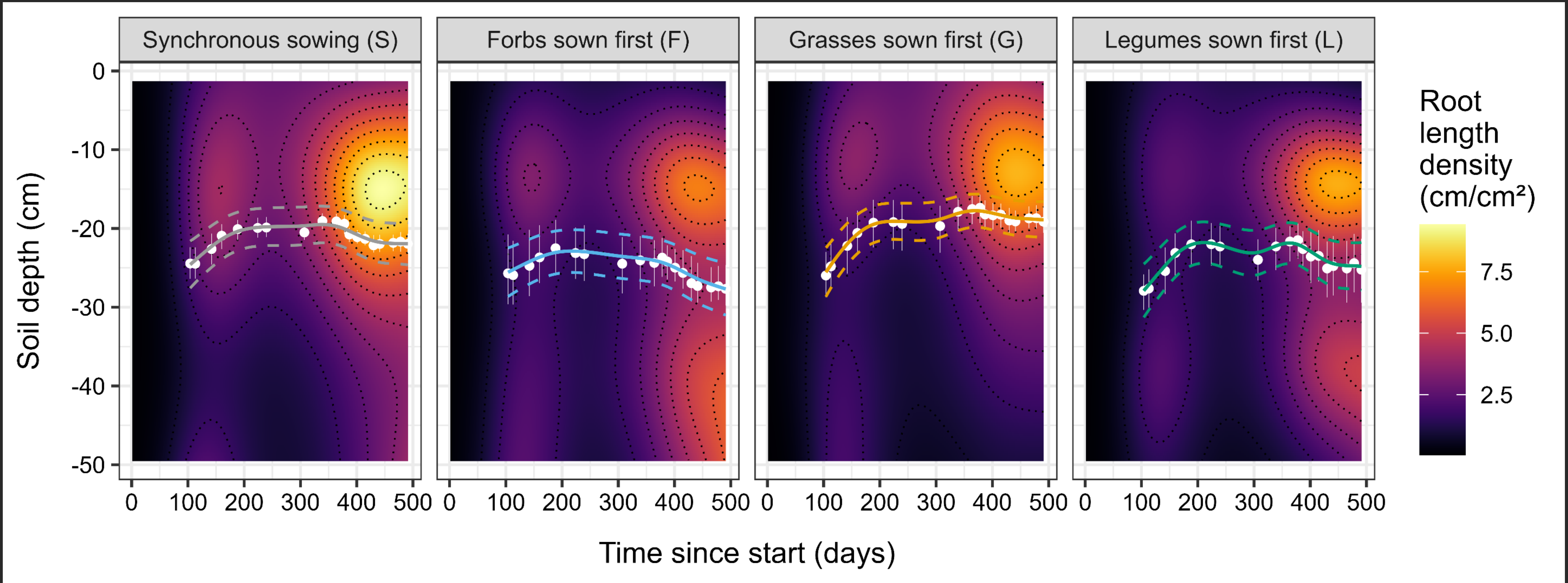
n is the number of locations along a tube at which an image was taken

d_i is the soil depth at location *i* (cm)

RLD_i is the root length density measured at location *i* (the same approach can be used for RSA)

Alonso-Crespo et al, *in prep*

Data exploration and analysis



Alonso-Crespo et al, *in prep*

Limitations of this approach

- **Tracking of the birth/death of individual roots**, which is important to estimate root lifespan and turnover, is difficult with the approach shown in this presentation. The manual analysis of some minirhizotron images using other software tools such as RootFly (Zeng et al, 2007. *New Phytologist* [link]) or RhizoTrak (Möller et al, 2022. *Plant and Soil* [link]) may be needed.

- Root length and biomass distribution results obtained with minirhizotron images should be validated using soil coring.

- **Distinguishing roots of different species** is still very difficult (using morphological criteria), especially in species-rich communities

- **Image acquisition is a time consuming process**, especially at a high spatio-temporal resolution. The development of affordable robotic minirhizotrons is an important step forward (Nair et al, 2022. *Journal of Experimental Botany* [link]).

Further reading

Rewald B, Ephrath JE. 2013. Minirhizotron Techniques. In: Eshel A, Beeckman T, eds. Plant roots: The hidden half. Boca Raton, FL, USA: CRC Press, 42.1–42.16.

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Minirhizotron Techniques

Boris Rewald
University of Natural Resources and Life Sciences

Jhonathan E. Ephrath
Ben-Gurion University of the Negev

I. Introduction

Special techniques are required to investigate root systems since they are hidden in the soil. Traditionally, destructive techniques like coring, trenching, and excavating have been used to access roots in situ. More recently, nondestructive techniques including rhizotrons and minirhizotrons (MRs) were developed in order to allow direct and repeated observations of the roots within the rhizosphere.

Installations using transparent "walls" to study roots in soil are termed rhizotrons (Böhm 1979). Walk-in rhizotron facilities or smaller-sized rhizotron chambers can also be used as lysimeters, and they may also include sensors that monitor soil conditions (Karnok and Kucharski 1982; Pan et al. 2001; Meier and Leuschner 2008). However, large rhizotrons have several disadvantages, with setup and operational cost being the primary ones; therefore, a very limited number of these facilities were built worldwide. In need for continuous nondestructive measurements of root traits in agricultural, silvicultural, and pristine ecosystems, the MR system was developed and has ever since gained wide acceptance. While glass plates ("root windows") have been used since the early 1900s (e.g., McDougall 1916), the MR concept was originally proposed by Bates (1937). In a work on fruit trees, he designed observation trenches in form of a walled chamber fitted with "root windows." However, what can probably be considered the first study with MRs as we know them today, using transparent tubes and an imaging device, was conducted by Waddington (1971).

MRs have helped improve our understanding of root systems, for example, in respect of standing stock, root production and longevity, root–parasite and root–hyphae interactions, and root phenology and distribution (e.g., Upchurch and Ritchie 1983; McMichael and Taylor 1987; Aerts et al. 1992; Hendrick and Pregitzer 1992; Hooker et al. 1995; Kosola et al. 1995; Eissenstat et al. 2000; Treseder et al. 2005; Vargas and Allen 2008; Ephrath and Eizenberg 2010).

Although reviews have previously discussed how to install the MR tubes and how to collect and use the obtained images (Taylor 1987; Box 1996; Hendrick and Pregitzer 1996a,b; Majdi 1996; Johnson et al. 2001; Mainiero 2006; McMichael and Zak 2006), there is an ongoing need to point out the proper use and possible pitfalls of MR systems to new users and to promote "good practice" standards. This chapter addresses five specific topics: (1) installation of MR observation tubes (MR-OTs), (2) MR image capturing systems, (3) image acquisition and analysis, (4) application of the MR technique, and (5) an outlook on recent and future developments, which could extend the range of applications of this technique.

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The power of Open Science

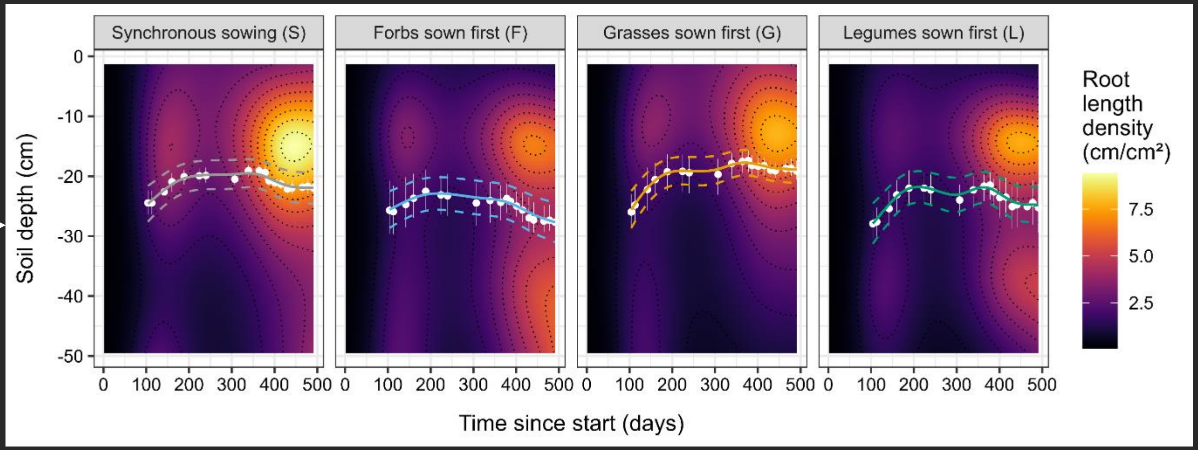
https://github.com/Abe404/root_painter

Abraham Smith

https://www.rhizovision.com/

Dr Anand Seethepalli

Dr Larry York



Acknowledgements

LEUPHANA
UNIVERSITY OF LÜNEBURG

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Emanuela Weidlich
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Linde Köhne
…
And many student helpers!

Thank you!

To contact me:
Benjamin.Delory@leuphana.de