IMPERIAL COLLEGE LONDON

REPORT OF PROJECT 3

# Computing angles of plant root tips

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*A thesis submitted in fulfillment of the requirements*
*for the degree of MSc Bioinformatics and Theoretical Systems Biology*

*in the*

Department of Life Sciences

August 15, 2018

Word count: INSERT #WORDS HERE

*"If you thought that science was certain – well, that is just an error on your part."*

Richard Feynman

IMPERIAL COLLEGE LONDON

# *Abstract*

Department of Life Sciences

MSc Bioinformatics and Theoretical Systems Biology

**Computing angles of plant root tips**

by Felicia BURTSCHER

Plant morphology studies the form and structure of plants and how these develop over time under different circumstances.
A not so frequently studied but interesting phenomenon in plant morphology is electrotropism, ie the response of a plant to an electric field, where biologists are interested in the curvature of root tip. Unlike gravitropism [DO I HAVE TO EXPLAIN IT HERE?] there exist to our knowledge no tools specifically designed to assist biologists in studying this effect, most importantly the angle resulting from the curving of root tips. This is especially relevant as the experiment setup is more complex and the images tend to be more error-prone, which is often the reason why standard gravitropism study tools fail and biologists compute angle resulting from the curved root tip manually.

Here we present a novel and stand-alone software for image processing developed in MATLAB optimised for noise-intensive electrotropism images. Unlike when doing the angle computation to measure the curvature of a root tip manually, our tool ensures a standardised version of the angle computation. On top of it, we developed a user-friendly graphical user interface (GUI) that will help the user in processing the images and compute the angles in a standardised and controlled fashion.

We use a data set of high-throughput time-lapse images of Arabidopsis roots from 5 experiments with 4-6 roots each containing between 32 and 36 images over a period of approximately 5 hours produced by the Plant Morphology lab at Imperial College London, we evaluate our results by comparing it to previously manually computed angles. [INCLUDE RESULTS A LA 70% of the images could be found to be reproduced]

AIMS METHODS RESULTS CONCLUSION

CITE SOME KEY NUMERICAL RESULTS RATHER THAN JUST GENERALITIES

# *Acknowledgements*

This project was conducted under the supervision of Dr Giovanni Sena, whom I thank for his advice and guidance. Thanks also to the members of the Plant Morphogenesis Laboratory at Imperial College London, especially Nick Oliver, for their help and expertise from the biological side as well as Suhail A Islam for fixing technical issues and his incredible patience. Finally, thanks to Prof Michael PH Stumpf, Prof Michael Sternberg and others involved in conducting and overseeing the MSc in Bioinformatics and Theoretical Systems Biology at Imperial College London.

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# List of Abbreviations

| | | |
|---|---|---|
| **GUI** | **G**raphical **U**ser **I**nterface | |
| **ie** | *latin* **i**d **e**st | that is |
| **LAH** | **L**ist **A**bbreviations **H**ere | |
| **WSF** | **W**hat (it) **S**tands **F**or | |

# Chapter 1

# Introduction

RELEVANCE AND TOPICALITY OF THE AIMS AND OBJECTIVES AND MY CONTRIBUTION TO THE RESEARCH. HOW HAS THE PROJECT ADVANCED THE FIELD?

## 1.1 Biological background

An important biological phenomenon studied by plant morphologists is *tropism* [ADD TO GLOSSARY], which is used to indicate the turning movement of a biological organism, here of a plant, when exposed to different environmental simuli [INSERT REFERENCE]. Usually the stimulus involved is added to the name, eg *phototropism* as a reaction to sunlight; it can be either *positive*, ie towards the stimulus, or *negative*, ie away from the stimulus. The most frequently observed and best studied tropism in *gravitropism*, which describes the process of how plants grow as a response to gravity. It was firstly scientifically documented by Charles Darwin [INSERT REFERENCE] and can be observed in higher and many lower plants as well as other organisms [INSERT REFERENCE]: Roots show *positive gravitropism*, ie they grow in the direction of the gravitational pull whereas stems grow in the opposite direction. An easy experiment to do is to lay a potted plant onto its side; over time the stem will begin to turn upwards and thus show negative gravitropism.
A far less studied process is *electrotropism* which describes the growth or movement of a plant when exposed to an electric field and which was the tropism under study in this project.

A high-overview explanation of the experiment setup and data collection can be found in section [INSERT REFERENCE TO SECTION METHOD].

## 1.2 Literature review

The majority of traditional root development bioassays only consider a small number of points [REFEREENCE PERRY ET AL, 2001 AND MAIN ROOTTRACE PAPER]. They are informative in terms of long-term effects on root growth; however, small and temporary changes can not be captured [REFERENCE MAIN ROOTTRACE]. Recently developed tools have considered a higher number of time points which allows a better study of how the growth process develps and the plant's responses [REFERENCE MAIN ROOTTRACE; ISHIKAWA AND EVANS, 1997; VAN DER WEELE ER AL, 2003; CHAVARRIA-KRAUSER ET AL, 2007; MILLER ET AL, 2007].

Image sequences can contain a representative "snapshot" description of a plant's developmental stage; also they can be generated at high speed [REFERENCE TO EXPERIMENT SETUP IN CHAPTER ....]. From manual time-lapse photography [REFERENCE VAN DER LAAN, 1934; MICHENER, 1938] to measure lengths of seedlings after applying different external stimuli, digital camera technology has improved and low digital storage cost has become available which has made it comparatively easy to collect large, time-stamped digital image data sets monitoring root growth [REFERENCE MAIN ROOTTRACE]. Once the root growth changes have been extracted from the imge data, one can correlate their timing with the impact of different external signals including hormonal and environmental on processes such as cell division and cell expansion [REFERENCE MAIN ROOTTRACE]. This will help to understand root development in general.

Analysing image data manually, however, is time-consuming, very subjective and thus error-prone [REFERENCE MAIN ROOTTRACE]. When the analysis is done "by eye", it becomes difficult to reproduce measurements as they are not standardised by any automatised approach and subject to human bias. Also, subtle phenotypes, ssuch as a delay in the response, might be missed [REFERENCE ROOTTRACE].

Different groups have developed tools for gravitropism and have shown the power of automatised image-analysis techniques compared to manual methods. An overview was created in figure [INSERT REFERENCE HERE].

## 1.3 Motivation

The work described here is motivated by mainly three factors: definition and standardisation of the so far manually computed angle, flexibility and user-friendliness in the pre-processing step, and adaptability to standard consumer cameras.

### 1.3.1 RootTrace

RootTrace [INSERT REFERENCE] has been developed to measure root lengths across time serie image data; biologists have also used it to measuer highly curved roots. A graphical user interface (GUI) implemented within the RootTrace framework makes it easy for the user to handle. However, this tool has failed on our image data set [INSERT REFERENCE HERE], probably due to the high noise level found in electrotropism images compared to gravitropism images.

### 1.3.2 PlantCV

COULD HAVE USED ONE OF THESE APPRAOCHES, BUT STARTED FROM SCRATCH. BASED ON THIS DATA SET.

However,

Electopism much much harder since photos noisier.

# Chapter 2

# Methods

## 2.1 Image processing

Image processing pools together a lot of different domains including physics (optics), signal processing and pattern recognition/ Machine Learning (ML) to ultimately feed computer to understand how do interpret images and make decisions based on them.

combines optics and signal processing and is often used in computer vision.

1. Image aquisition
2. Image preprocessing
3. Image segmentation
4. Image representation and description – dep on image that you are studying
5. Image understanding
6. Results (output)

### 2.1.1 What is a digital image?

We operate on digital (discrete) images:

- Sample the 2D space on a regular grid
- Quantise each sample, ie round to nearest integer

If our samples are $\Delta$ apart, we can write this as:

$$f(x, y) = Quantize f(\Delta x, \Delta y)$$

The image can now be represented as a matrix of integer values:

[INSERT EXAMPLE PICTURE HERE]

### 2.1.2 Image processing operation

An image processing operation typically defines a new image $g$ in terms of an existing image $f$.

- Transform the range of $f$

$$g(x, y) = t(f(x, y))$$

- Transform the domain of $f$

$$g(x, y) = f(t_x(x, y), t_y(x, y))$$

### 2.1.3 Key stages in digital image processing

[INSERT FLOW CHART HERE]

Problem Domain – here plant morphology

- Image Aquisition
- Image Enhancement – contrasting images so human eye can best see things, manipulate values of pixels so you can best caracterise and structure them
- Morphological Processing – growing and thinning of pixels, eg fingerpringt medical operations
- Image Segmentation – separating parts of the image such as features and object you want to look at, distribution of pixel values
- Representation & Description – based on quantised space that you're working in. What's realised in that digital output? Two ways to describe features being observed in that pixel space. Inverting etc
- Object Recognition – see example iris data set. cluster to identify different objects.

uter & Machine Vision – feeds into computer vision and ML

[Robotics & AI], [Deep Learning]

feature space that you're working in on top of your labels. put in our own bias and how we train model.

A lot of noise in the image makes it grainy. We can apply interpolation, ie smoothing the image based on its nn. bi-cubic interpolation. Smoothes some of the details as well as a side effect, but especially the noise. – important for edge detection later

## 2.2 Matlab

As a language we chose Matlab as it has a well-documented image processing toolbox with a very good documentation. Alternative languages are Python and Julia.

ImageJ

Avizo

## 2.3 Workflow

Generic image processing workflow:

However, every single case is different.

Skeletonisation

fill in holes

[INSERT FLOWCHART WITH SINGLE STEPS]

## 2.4 Pre-processing – getting the skeleton

The initial problem of coming up with a "good" definition by comparing different angle and curvature definition shifted. The focus was now on the pre-processing, the extracting the skeleton. However, we will still present some possible definitions of angles and curvature, also not implemented in the current version of the tool.

## 2.5 Computing the angle

Once you defined the lines, it is straight-forward to compute the angle.

Eg using Hough transform from Matlab Image Processing Toolbox to detect lines in an image not applicable here as highly pixled image.

We refer to figure [INSERT REFERENCE HERE] to how the angle was computed in previous approaches.

However, in the software described here the main goal was to emulate the angle that has so far been computed manually. Approaches like computing the Gaussian mean curvature, definitions of different, possibly more robust methods of computing and angle are mentioned here, but has not been incorporated in our final tool.

### 2.5.1 Using the position of the tip of the root – more in detail

INSERT FIGURE OF WHAT ANGLE WE CALCULATE AND WHAT IS THE ANGLE OF INTEREST. USER-FRIENDLY: EASILY SWITCH BETWEEN THE TWO OF THEM.

We find the angle between the two lines (point of highest mean curvture OR manually click point of highest curvature and tip and point of highest mean curvature and equally distant point/ pixel on root).

There are other ways such as finding vectors on the lines and using their dot products. However, we can use simple, basic trigonometric methods as shown in figure ....

We would then compute the angles of the two lines to the x-axis in radians mode by

$$\pi - |\tan^{-1}(\frac{x(2)-x(1)}{y(2)-y(1)}) - \tan^{-1}(\frac{z(2)-z(1)}{y(2)-y(1)})|$$

or

$$180^\circ - |\tan^{-1}(\frac{x(2)-x(1)}{y(2)-y(1)}) - \tan^{-1}(\frac{z(2)-z(1)}{y(2)-y(1)})|$$

if we wanted it in degrees mode.

The single steps are shown in figure .... [PSEUDOCODE]

1. Find the slope of each line.
2. Find the inclination of each line using $\alpha = \tan^{-1} m$, where $\alpha$ is the angle of inclination, $m$ is the slope.
3. Take the difference of these two angles.

4. Handle the case where this difference is not an acute, ie less than 90 degrees, angle. If we get a negative angle, we take its absolute value. We want to find an acute angle, so if we calculated an obtuse angle, oe greater than 90 degrees, we just subtract the value from pi radians or 180 degrees to get the acute values.

### 2.5.2 Using the angle of the tangents to the points that are x points/pixels away from the point of highest local curvature

### 2.5.3 INSERT VARIOUS OTHER IDEAS OF ANGLE COMPUTATION – even if not implemented

# Chapter 3

# Results

PROVIDE A HIGH-LEVEL REVIEW, NOT LOTS OF NUMBERS

REPRODUCABILITY OF WORK, ie make sure reader could redo it

The following section of this report will explain the tool briefly from a technical perspective and show how to use the tool in practice. Additionally, we present an example of the tool in use.

The code is open-source and publically available on [INSERT GITHUB REFERENCE HERE]; all previous versions can be found on [INSERT GITHUB REFERENCE HERE].

## 3.1 Key components

Figure [INSERT REFERENCE HERE] explains the key components of this image-analysis software tool to address the problem of highly noisy electrotropism consumer camera images of Arabidopsis roots and a standardised way of computing the angle for the curved root tip.

This tool takes the form of a MATLAB program with a graphical user interface.

## 3.2 Elaborate pre-processing tool for skeletonisation

high functionality, reiterated process

## 3.3 Optimisation: Graphical User Interface (GUI)

- User interaction

- Error messages if user does not enter right values.

INCLUDE FIGURES HERE.

## 3.4 Different ways of curvature and angle measuring

What was implemented as we found that this approach worked best on these data and this resolution.

## 3.5 Standardised and automated version of angle computation

## 3.6 Comparing the automated calculated angles with the manually calculated angles

Two approaches: 1. Converted into gray channel images. 2.

## 3.7 Learning LaTeX

### 3.7.1 A (not so short) Introduction to LaTeX

### 3.7.2 A Short Math Guide for LaTeX

### 3.7.3 Common LaTeX Math Symbols

### 3.7.4 LaTeX on a Mac

## 3.8 Getting Started with this Template

### 3.8.1 About this Template

# Chapter 4

# Discussion

PUT RESULTS IN BROADER PICTURE

## 4.1 Challenges

### 4.1.1 Low contrast

There is a very low contrast between the background and the roots, so that one could hardly recognise the roots on some images [INSERT EXAMPLE IMAGE HERE]. Inverting the image was not enough. We intensified the contrast by ... / making the background darker, we increased the brightness. We used further filtering so that the difference between two pixels is more pronounced. It was very much a trial & error process.

Classify only things without structure as noise. Noise was subtle/ hiding and could only be seen on filtered images.

Many objects of no interest which can be not

[INSERT 4 EXAMPLES HOW DIFFICULT DATA WAS]

### 4.1.2 Objects interferring with objet of interest

Eg specs not so much of an issue as separate from the root. Issue when it is connected.

### 4.1.3 Skeletonisation

Get rid of loop.

How to go along the curve?

### 4.1.4 Scalability

A lot of paramter tuning to single images. Work with single images to tune.

### 4.1.5 Reproducability

angle computation yes, but still variability in pre-processing step

### 4.1.6 Problems

A lot of trial & error / hand-picking.

### 4.1.7 High user-interaction

Might be reduced with better-quality images, however very flexible.

## 4.2 Suggestions for future data acquisition

### 4.2.1 More focus on images

### 4.2.2 Resolution

Better camera, less waste of resolution

## 4.3 Broader application of this tool

This tool can be reused for many purposes, it is not restricted to root detection. Might also be used for easier problems like gravitropism.

## 4.4 Further work

Here in the first version of the tool, the main goal was to standardise the angle computation; if in the future a method for handling the different noise pattern in the images was efficiently handled which require less user input, it would be desireable to automate the whole angle computation.

Use on better-quality images in the future.

# Chapter 5

# Conclusion

WHAT, WHY, WHY ADVANTAGE, WHAT WAS CHALLENGE?

Here we present a novel and stand-alone image-analysis-based software tool developed in MATLAB optimised for noise-intensive electrotropism images that is able to compute the curvature and angle at the root tip in a standardised fashion with a user-friendly and very flexible pre-processing step to extract the skeleton of the root from possibly very noise image data sets. It offers the possibility to extend the analysis by including different angle definitions to capture the curvature of the plant root and compare them. Also, the software tool is not limited to compute the angle of root tips but due to the flexibility of the pre-processing step can be used for any other curved or polynomial-like structure.

The software has been designed using an extensive amount of different filtering techniques optimised on the image data set described in this work [INCLUDE REFERENCE TO SECTION] and can therefore be used to work with standard images fro costumer digital cameras.

Automated image capturing as well as the design of the software presented here both aim to reduce the time-consuming process of the biologist quantifying the root tip curvature manually but more importantly, it standardises the computations and makes the results reproducible and comparable over a large amount of data.

This will contribute to understand highly complex and poorly-understood phenomenons like eletrotropism in plants and possibly other tropisms, as well as plant growth in general.

Hope to be able to standardise it in the future.

# Appendix A

# Data

The data was taken by a ... camera using a raspberry Pi. Insert details regarding data collection.

# Appendix B

# Frequently Asked Questions

## B.1 How do I change the colors of links?

The color of links can be changed to your liking using:

`\hypersetup{urlcolor=red}`, or

`\hypersetup{citecolor=green}`, or

`\hypersetup{allcolor=blue}`.

If you want to completely hide the links, you can use:

`\hypersetup{allcolors=.}`, or even better:

`\hypersetup{hidelinks}`.

If you want to have obvious links in the PDF but not the printed text, use:

`\hypersetup{colorlinks=false}`.