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President's Doctoral Scholar Award

BBSRC

ALFRED: a novel image analysis application to inform mathematical modelling of microtubule networks in nerve cells

Beatriz Costa-Gomes, Nuno Nobre, André Voelzmann, Matthias Heil, Andreas Prokop

25th March 2019

How did I get here?

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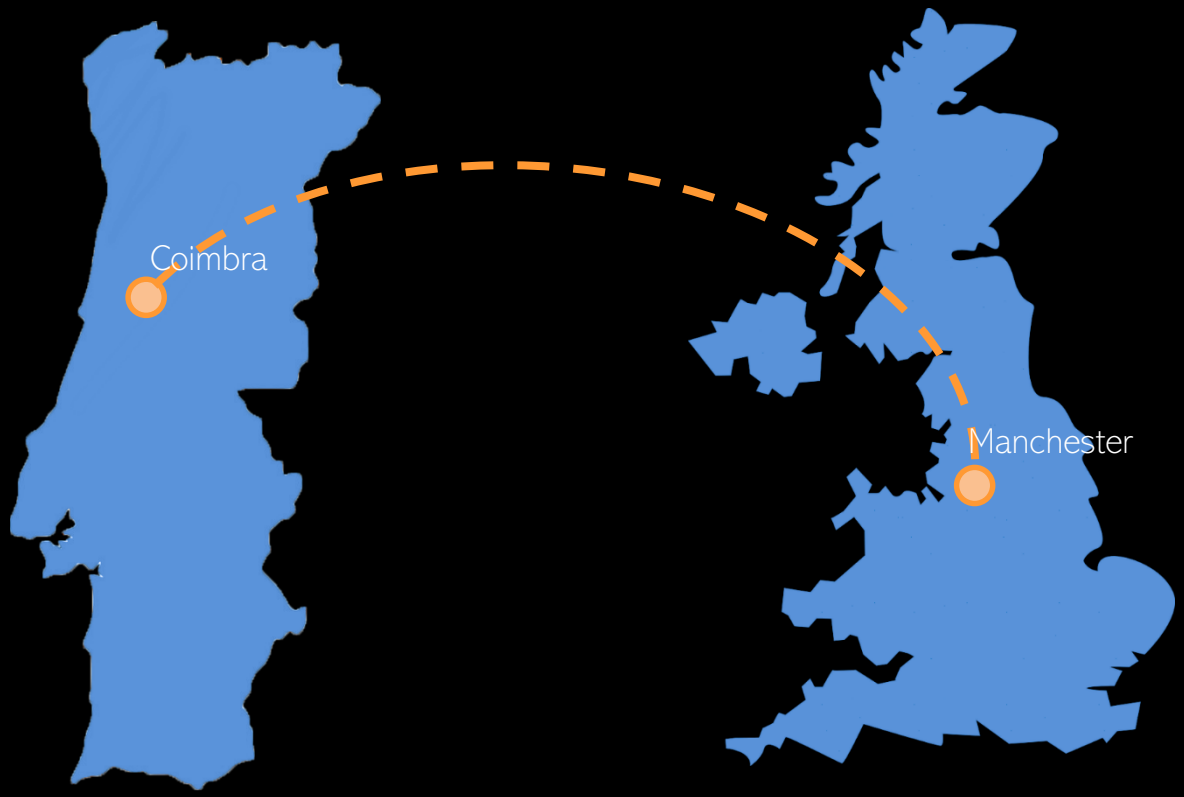
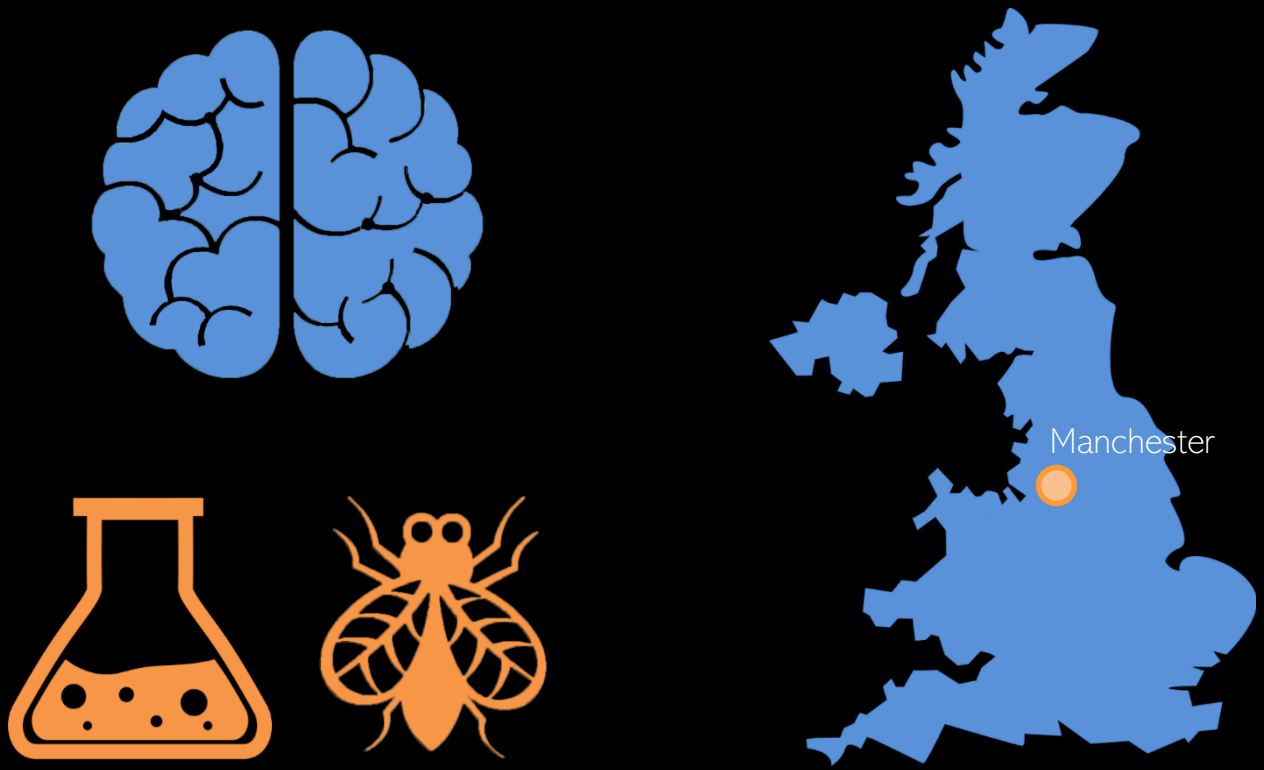


*not to scale

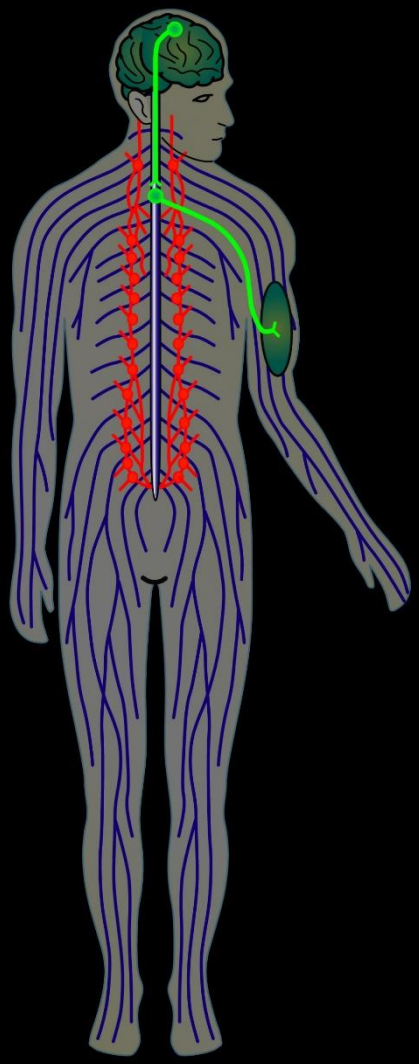
How did I get here?



How did I get here?

What am I actually working on?

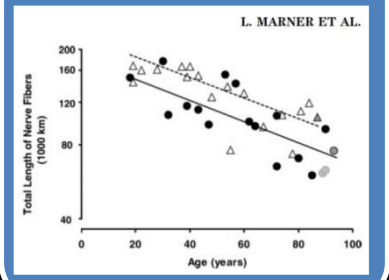
Axons are key to nervous system function



Axons: slender processes of neurons that wire the body

The delicate structures have to survive for a lifetime

We lose up to 50% of axons during ageing



Marner et al., *J. Comparative Neurology* 456 (2003)

We also lose them in many neurodegenerative diseases such as dementia or Alzheimer's Disease

Adalbert & Coleman, *Neuropathol Appl Neurobiol* 39 (2012)

Microtubules (MTs) are key to axon formation and maintenance

Axonal Microtubules

Continuous parallel bundles of MTs form the...

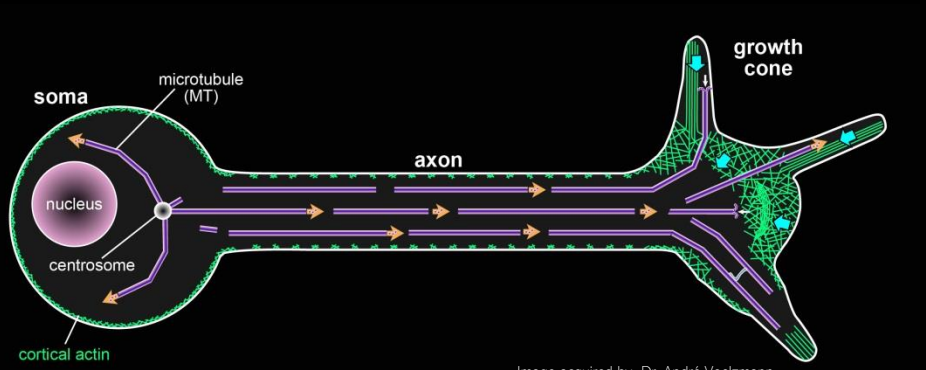
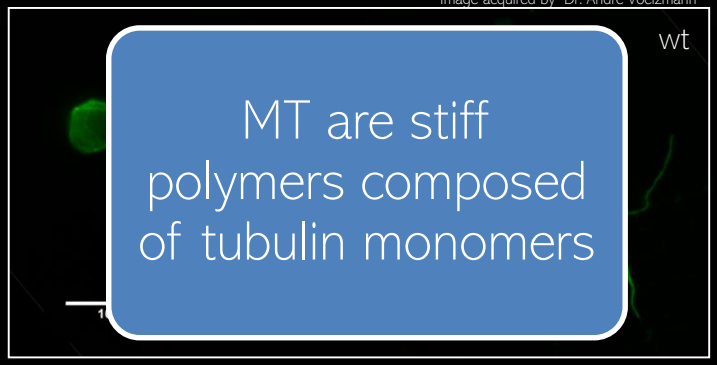
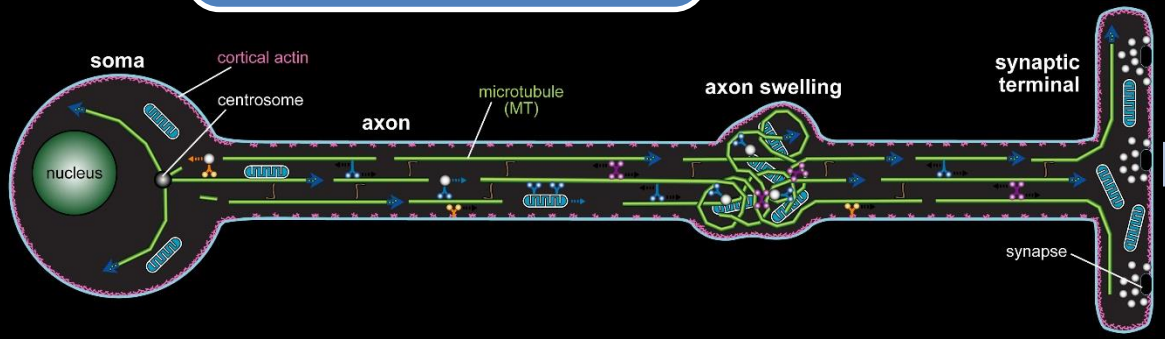


Image acquired by Dr. André Voelzmann



MT are stiff polymers composed of tubulin monomers

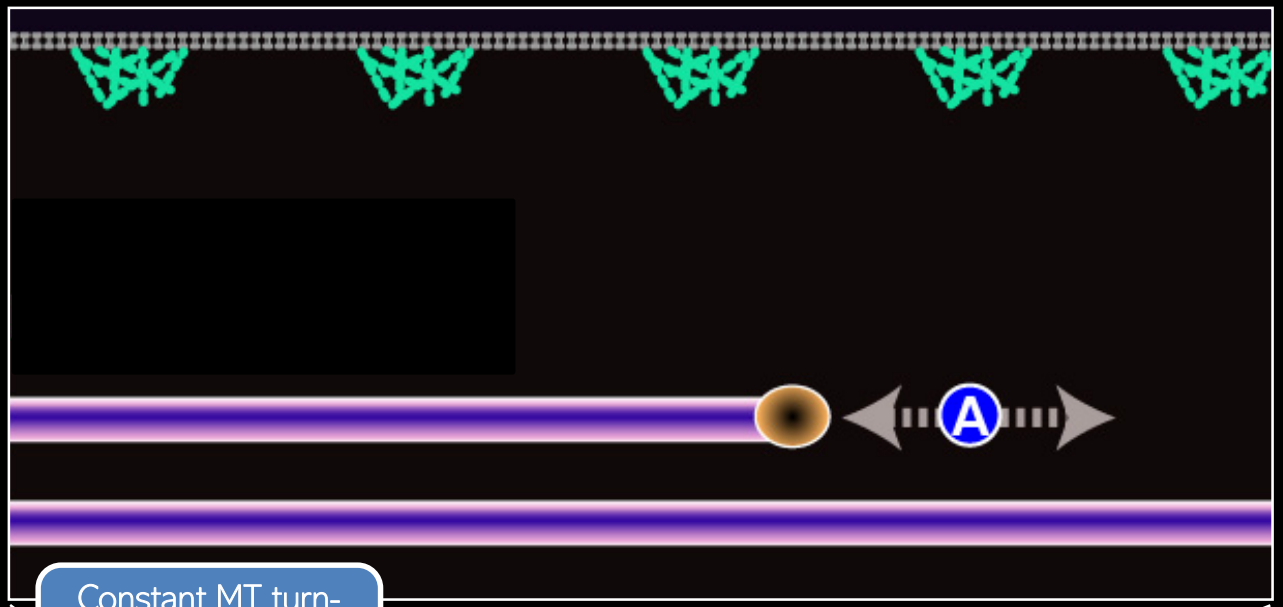
...structural backbones and life-sustaining transport highways



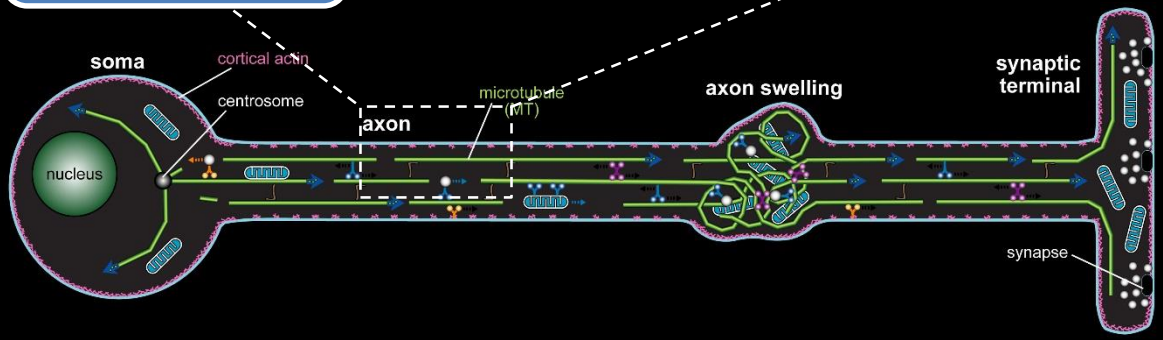
Ageing or in degeneration:

axon swellings with MT disorganisation

Local Axon Homeostasis

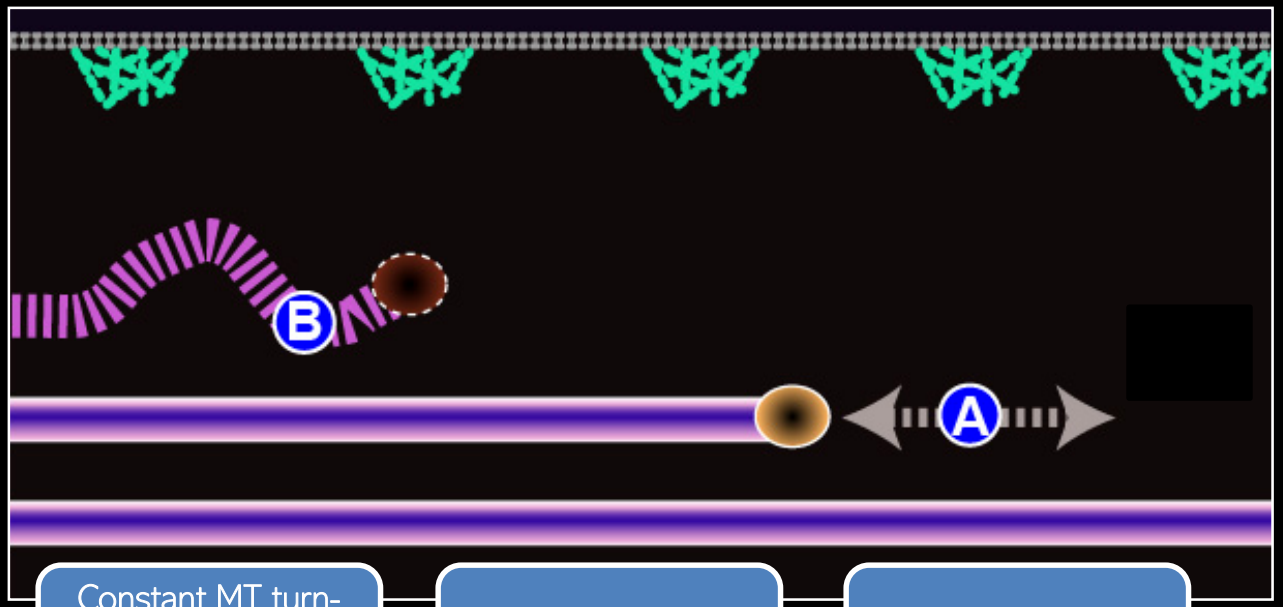


Constant MT turn-over (polymerisation, depolymerisation, severing)



Voelzmann et al., *Brain Res Bulletin* 126 (2016), 226ff..

Local Axon Homeostasis



Constant MT turn-over (polymerisation, depolymerisation, severing)

By default, MTs in axons are disorganised

MT-regulators 'tame' MTs into axonal bundles

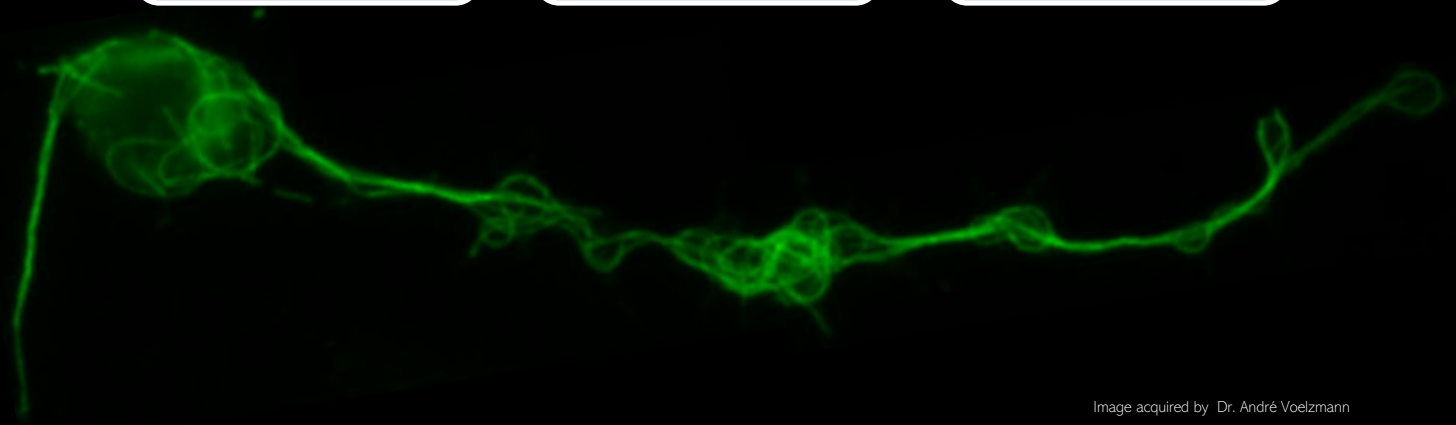
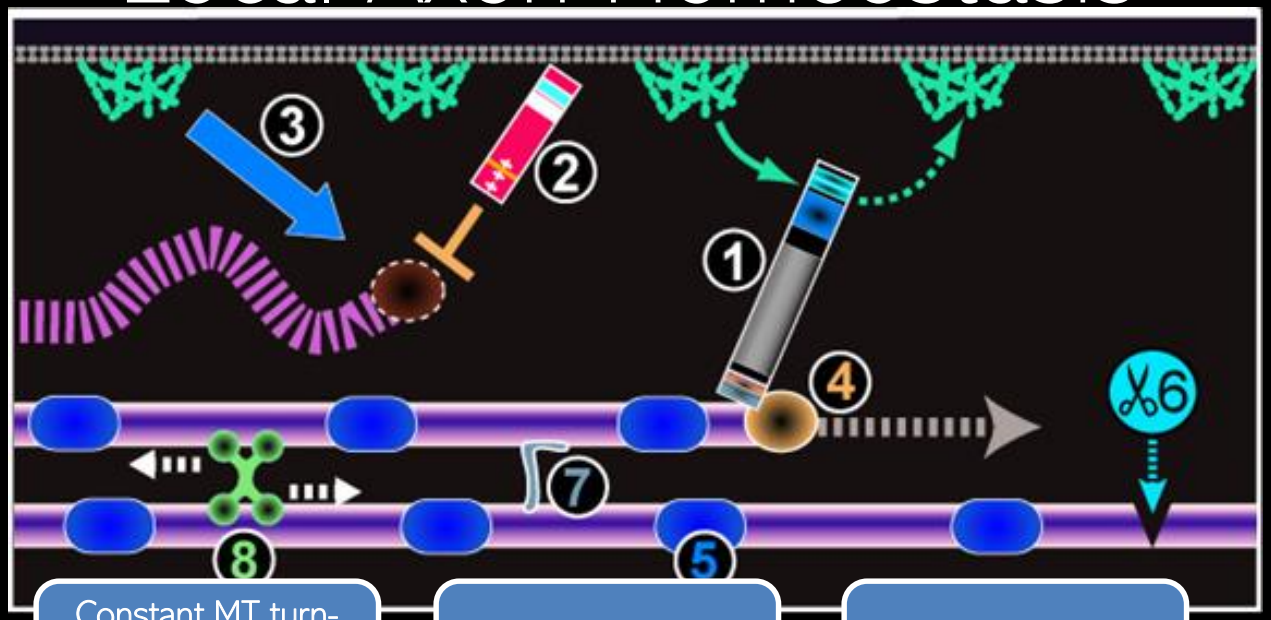


Image acquired by Dr. André Voelzmann

Voelzmann et al., *Brain Res Bulletin* 126 (2016), 226ff..

Local Axon Homeostasis



Constant MT turn-over (polymerisation, depolymerisation, severing)

By default, MTs in axons are disorganised

MT-regulators 'tame' MTs into axonal bundles

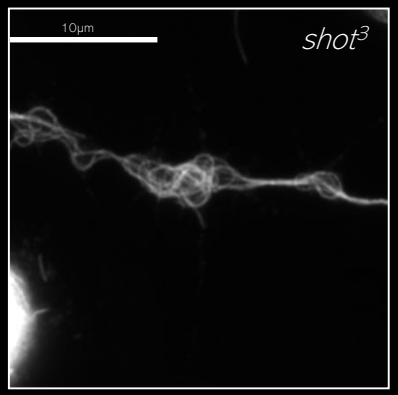
Guidance of MTs polymerisation parallel to other structural components (actin)

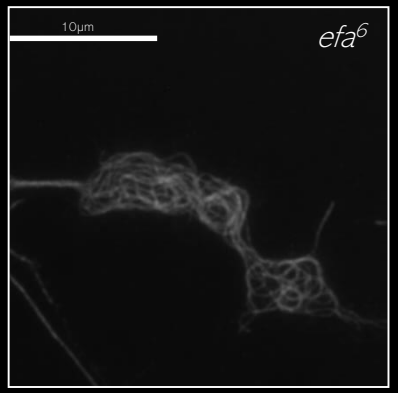
Elimination of off-track MTs

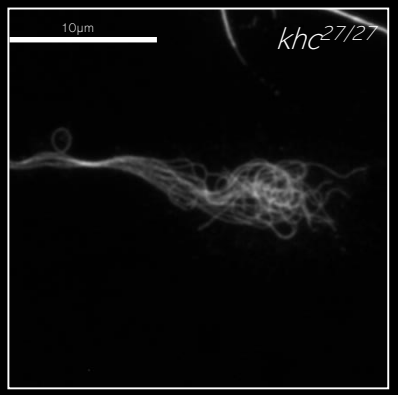
And More

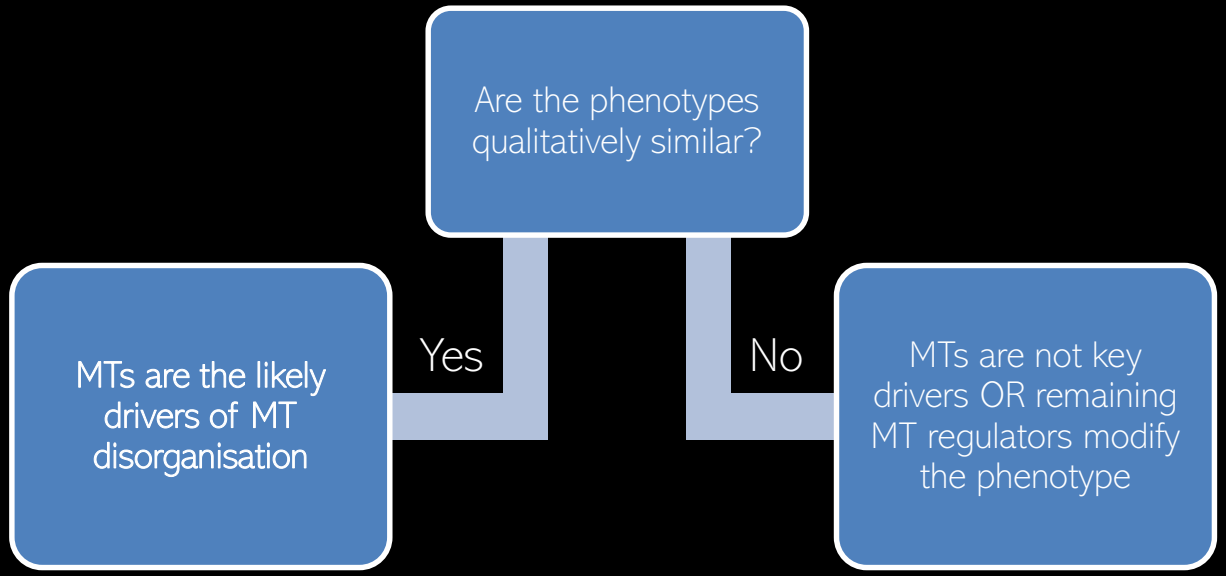
Voelzmann et al., *Brain Res Bulletin* 126 (2016), 226ff..

Are MTs the culprits of axon swellings?



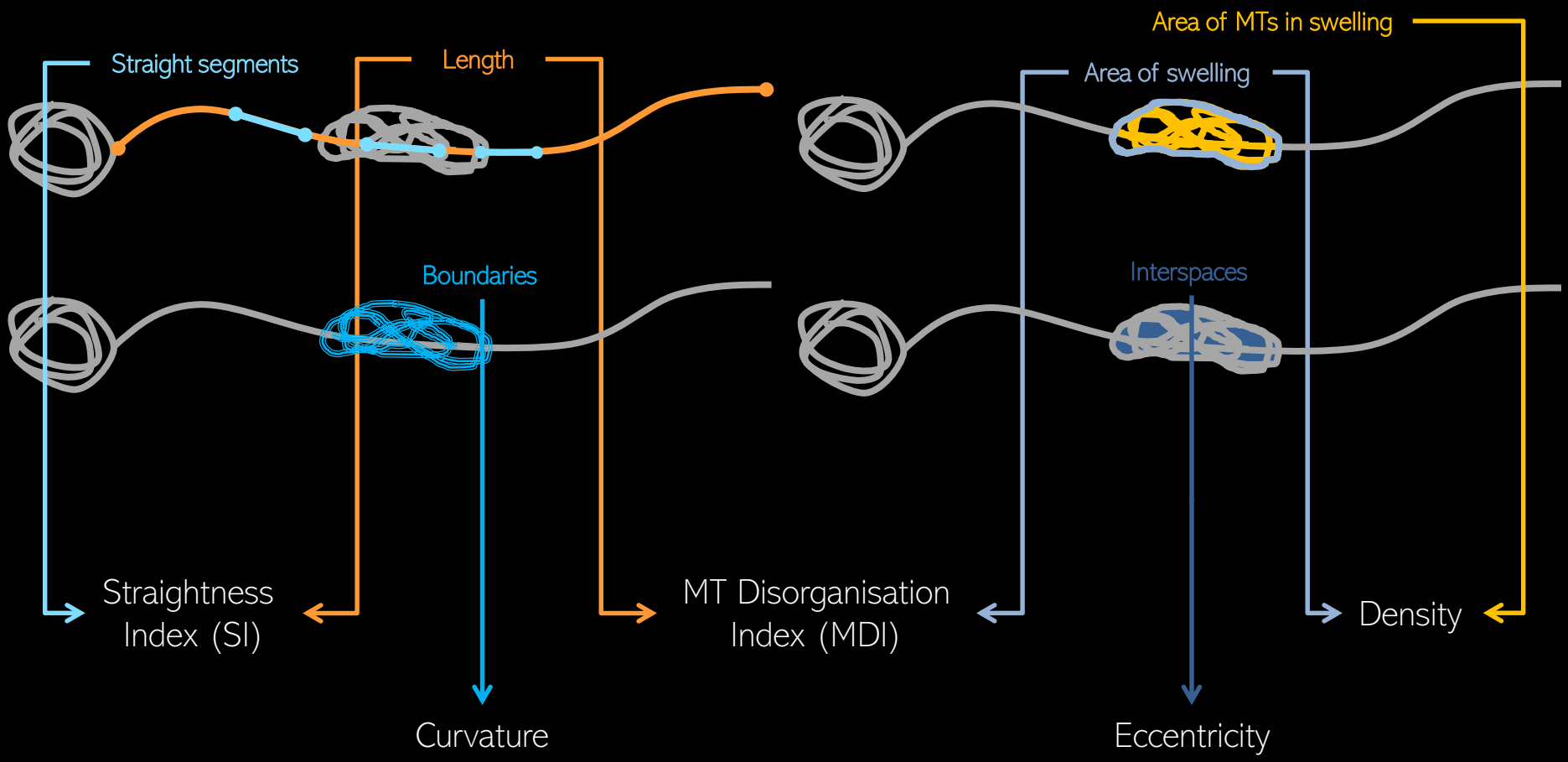






Images acquired by Dr. André Voelzmann

What am I measuring?



$$\text{SI} = \frac{\text{sum of length of n longest segments}}{\text{length}}$$

$$\text{MDI} = \frac{\text{area of swelling}}{\text{length}}$$

$$\text{Density} = \frac{\text{area of MT in swelling}}{\text{area of swelling}}$$

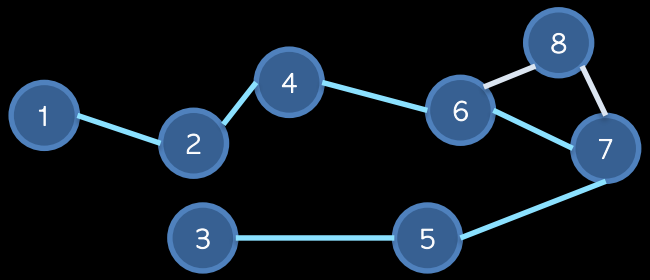
Curvature = fitting on the boundaries

Eccentricity = circularity of interspaces

But some of these values are not readily obtained. How do we get them?

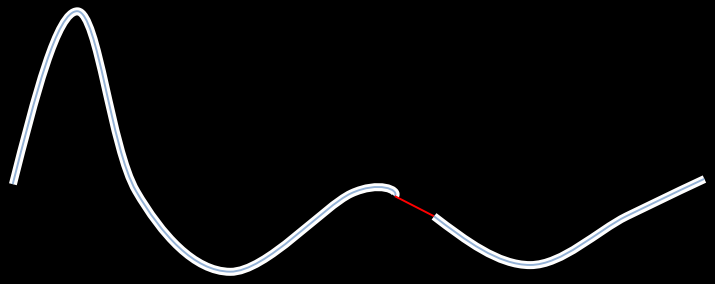
Length

Length: Algorithm that tries to find the shortest connected path between two selected nodes in a graph (e.g. 1 to 3)



Each point of the skeletonised image becomes a node in the graph

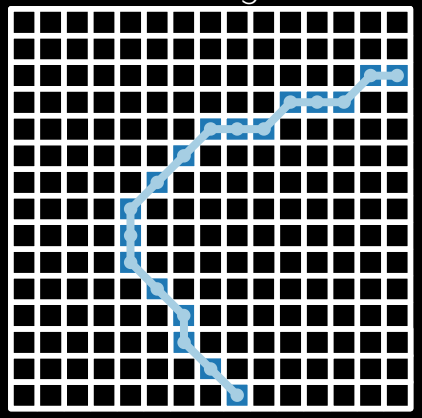
Problem: bridging artefactual gaps in real images



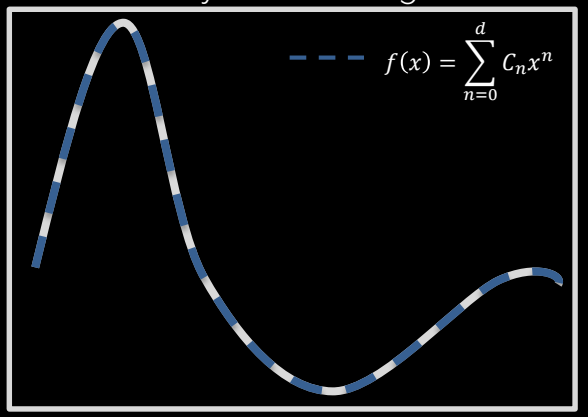
If there's a gap, it defaults to straight line

Curvature

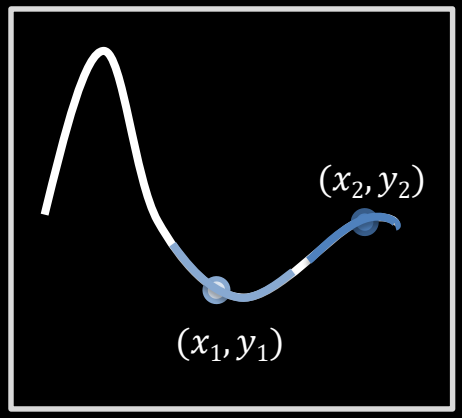
Pixel angles



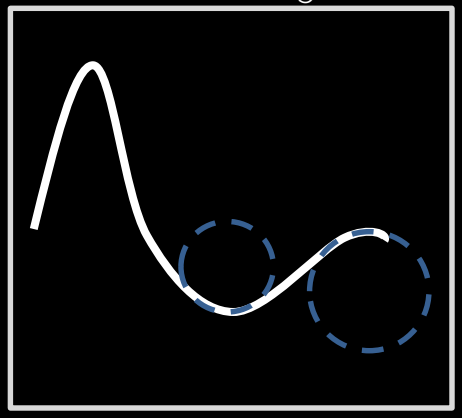
Polynomial fitting



Gaussian Filters



Pattern fitting

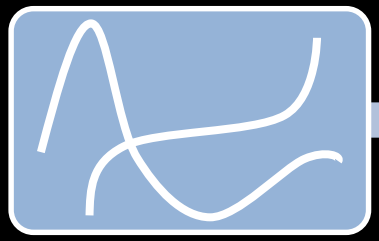


Curvature

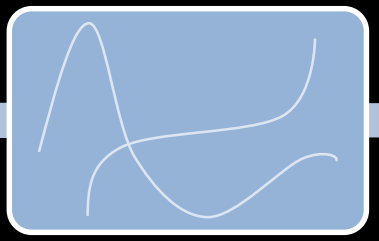
$\kappa(t)$ is obtained using a parametrisation of the skeleton of the image

$$\kappa(t) = \frac{1}{R(t)}$$

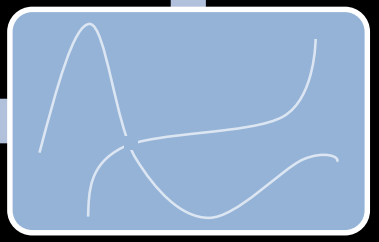
$$\kappa(t) = \frac{|x'(t)y''(t) - y'(t)x''(t)|}{(x'(t)^2 + y'(t)^2)^{3/2}}$$



Retrieve the skeleton, a pixel-wide contour of the image



Remove any junctions that the skeleton might have



Compute the derivatives needed for curvature

Gaussian Filters applied to all points in the boundary

Fit a Fourier Series of one term to the boundaries

Obtain curvature and radius (1/curvature)

Curvature

Fourier Transform

Fit a Fourier Series of one term to each of the lines

First order Fourier series:

$$f(x) = a_0 + a_1 \cos(wx) + b_1 \sin(wx)$$

Time complexity: O(N)
Space complexity: O(N)
On average, is *much* smaller.

Gaussian Filter

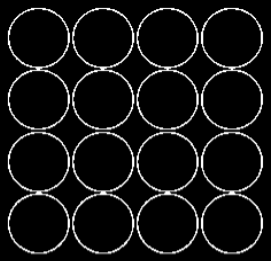
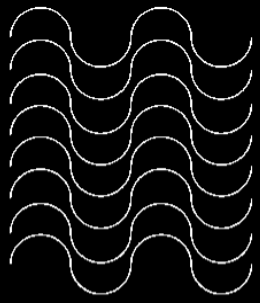
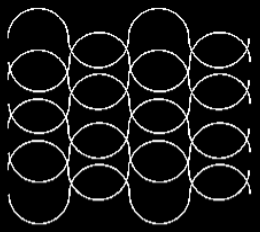
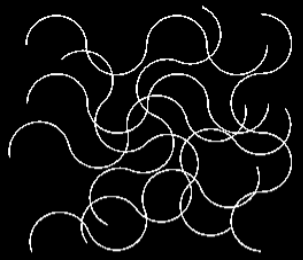
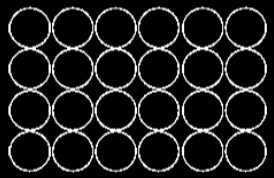
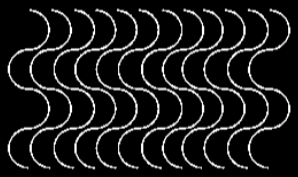
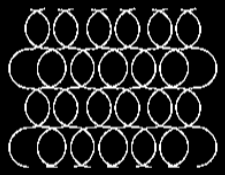
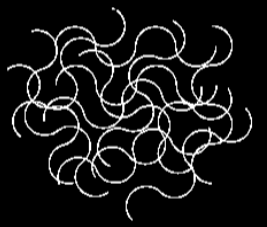
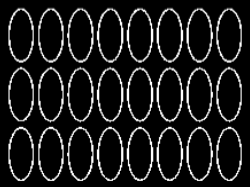
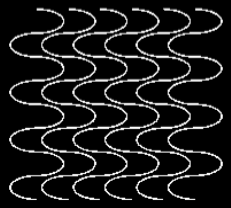
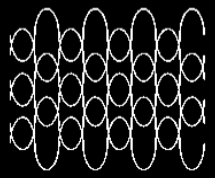
Window of a certain size centred in one point, for all the points in the image

Point to point multiplication and final sum reduction produces the desired derivatives

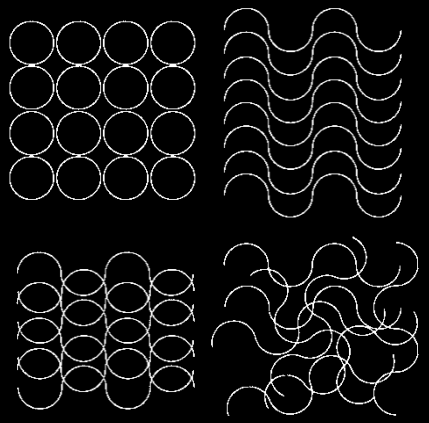
Time complexity: O(N)
Space complexity: O(N)

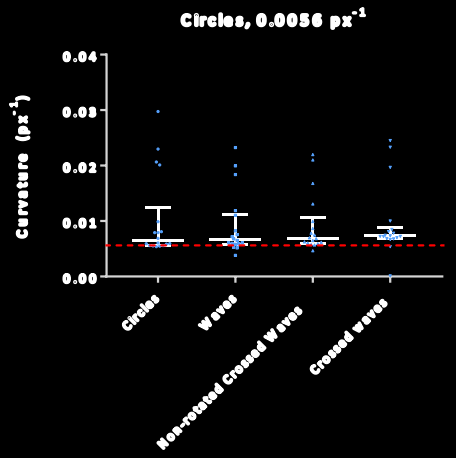
N: number of points in the image
Big-O notation: worst case scenario

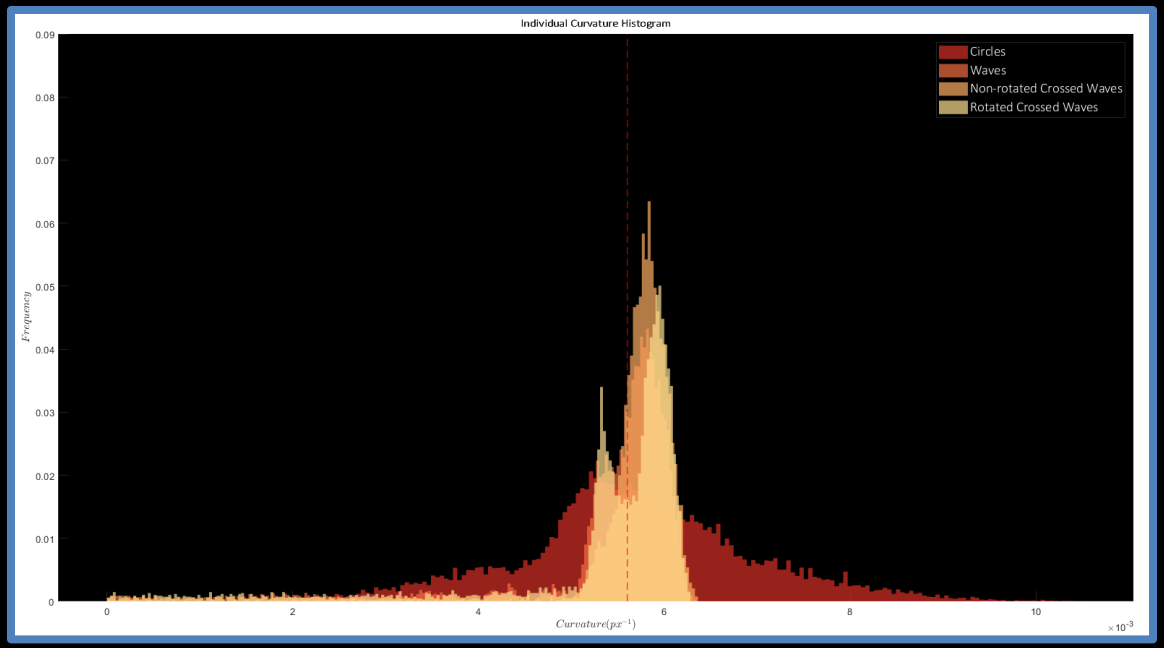
Curvature

	Separated	Waves	Non Rotated Crossed Waves	Rotated Crossed Waves
Circles Curvature 0.0056px^{-1}				
Circles Curvature 0.0085px^{-1}				
Ellipses Curvature 0.0064px^{-1} and 0.0140px^{-1}				

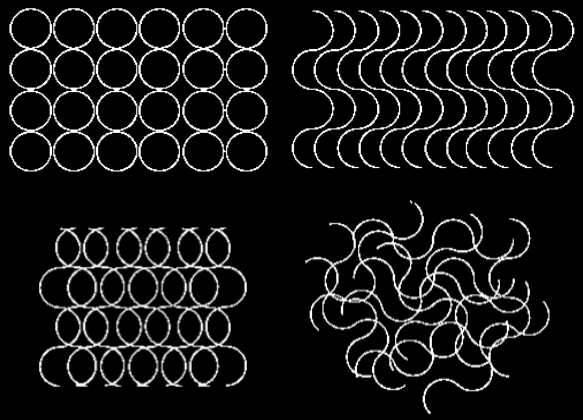
Curvature

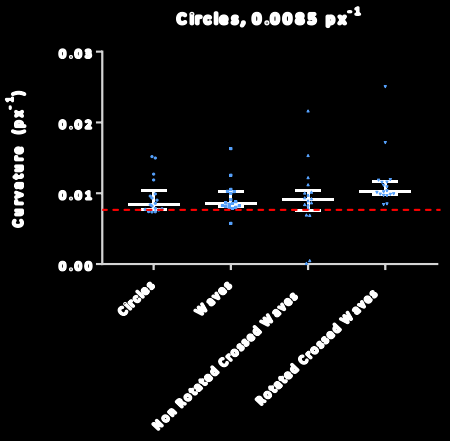


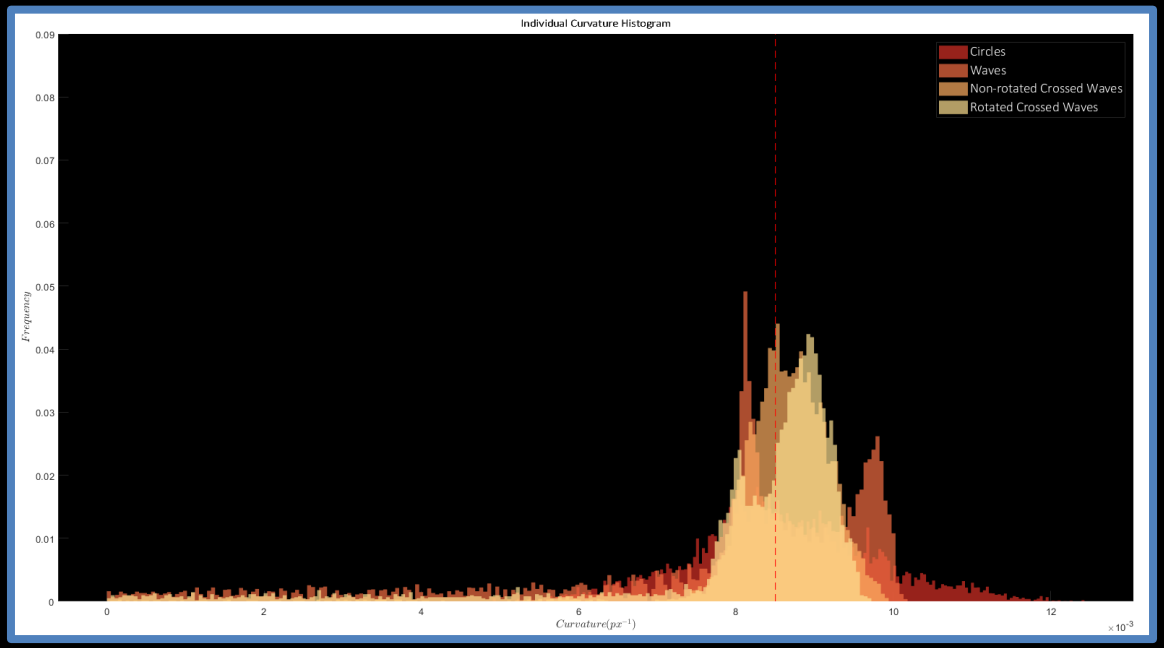

Circles, 0.0056 px-1
Curvature (px-1)
0.04
0.03
0.02
0.01
0.00
Circles
Waves
Non-rotated Crossed Waves
Crossed waves


Individual Curvature Histogram
Circles
Waves
Non-rotated Crossed Waves
Rotated Crossed Waves
Frequency
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
2
4
6
8
10
Curvature(px⁻¹)
×10⁻³

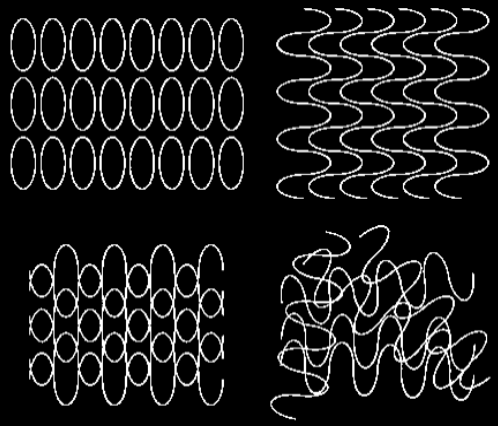
Curvature

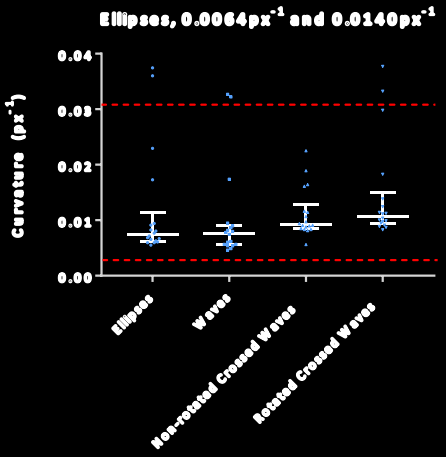


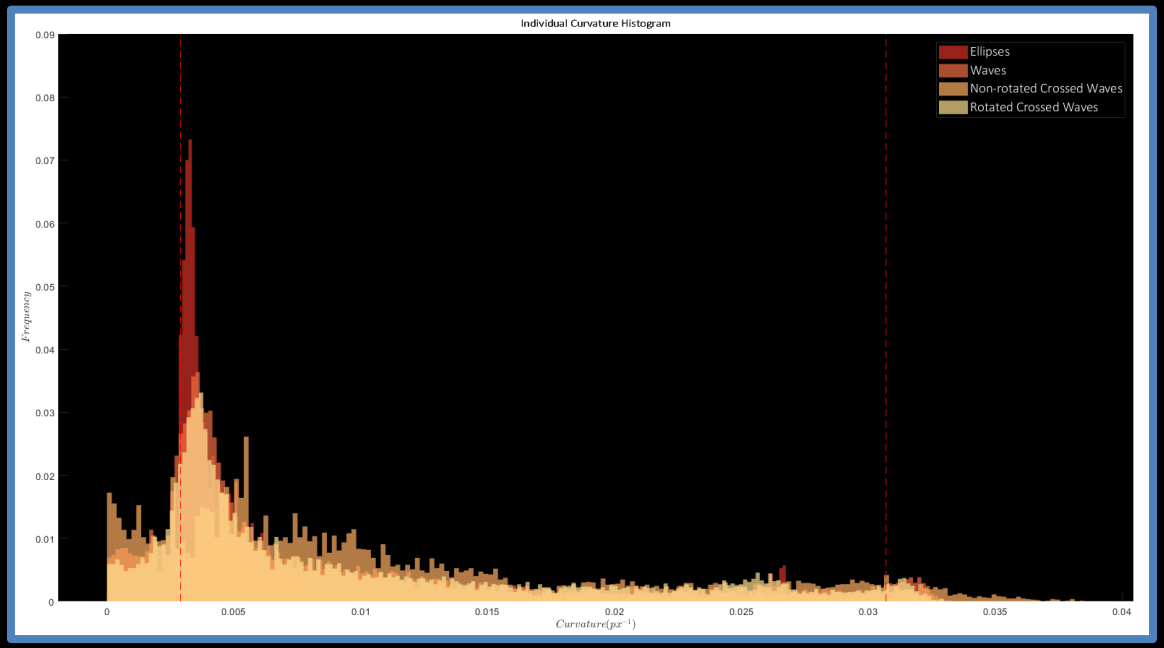

Circles, 0.0085 px⁻¹
Curvature (px⁻¹)
0.03
0.02
0.01
0.00
Circles
Waves
Non Rotated Crossed Waves
Rotated Crossed Waves


Individual Curvature Histogram
Circles
Waves
Non-rotated Crossed Waves
Rotated Crossed Waves
Frequency
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
2
4
6
8
10
12
Curvature(px⁻¹)
×10⁻³

Curvature




Ellipses, 0.0064px⁻¹ and 0.0140px⁻¹
Curvature (px⁻¹)
0.04
0.03
0.02
0.01
0.00
Ellipses
Waves
Non-rotated Crossed Waves
Rotated Crossed Waves


Individual Curvature Histogram
Ellipses
Waves
Non-rotated Crossed Waves
Rotated Crossed Waves
Frequency
Curvature(px⁻¹)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0.005
0.015
0.025
0.035

Curvature

Fourier Transform

Good for course grained analysis and general curvature of the image. Faster and occupies less space.

Doesn't allow a detailed analysis, works better if the image has a constant curvature.

Gaussian Filter

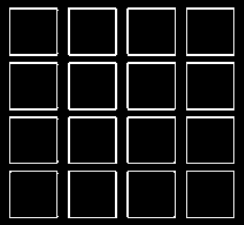
Good for fine grained analysis, highly used in image analysis.

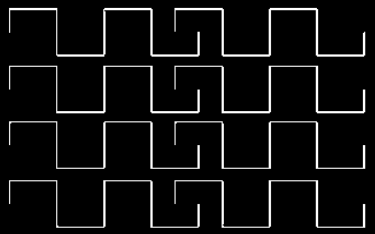
Depends on resolution, although it can be bypassed. Takes longer and occupies more space.

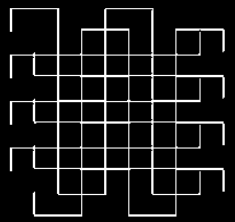
The best solution lies somewhere in the middle:
Using one to tune the other!

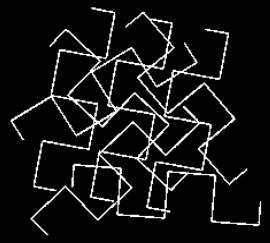
Curvature for Straightness?

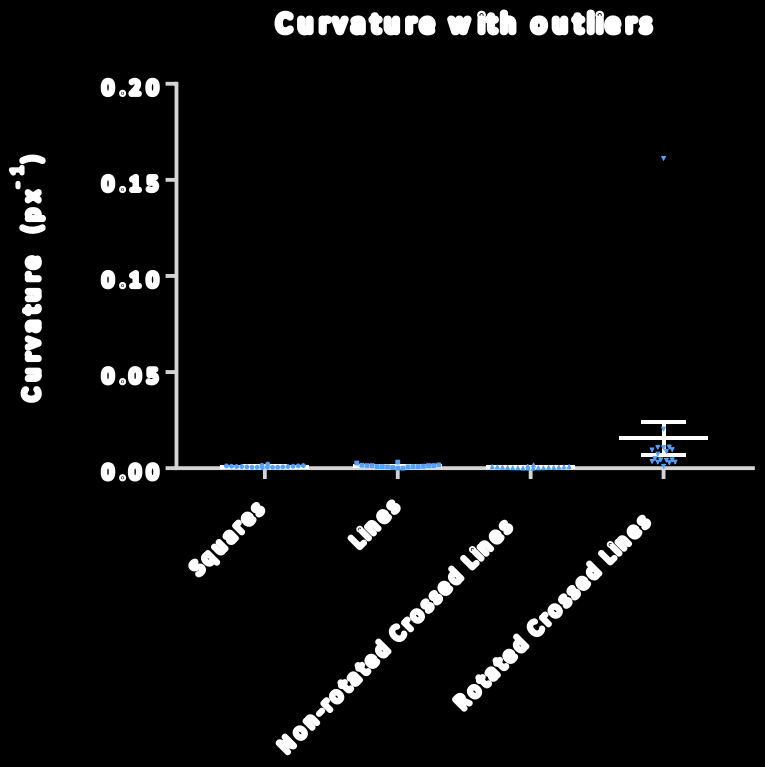
Squares
"Curvature"
0.0144px^{-1}

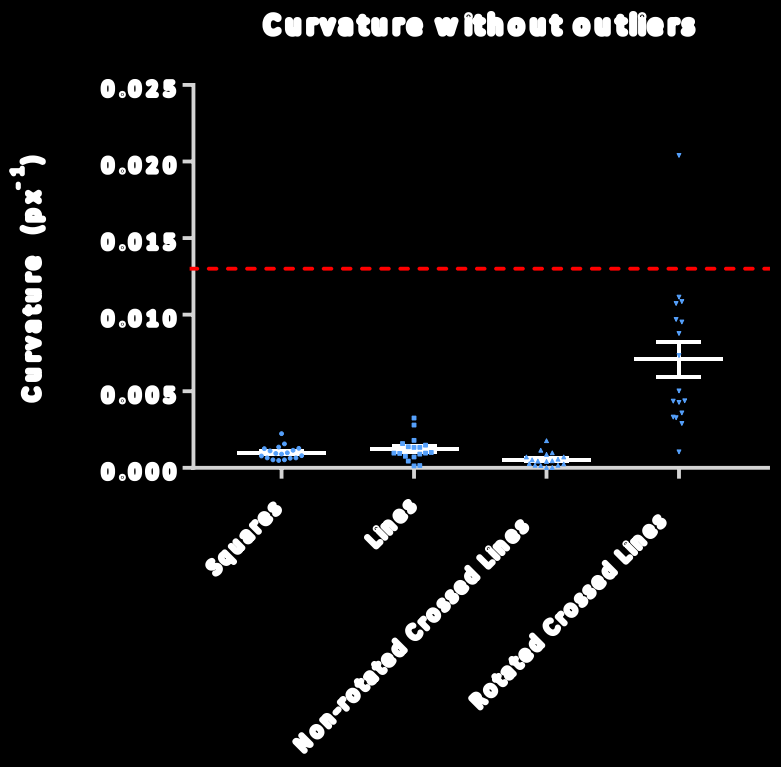






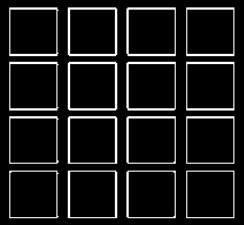


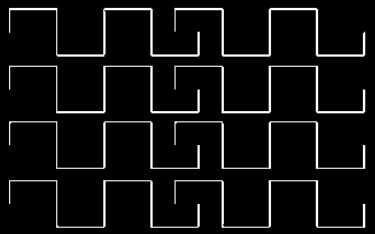


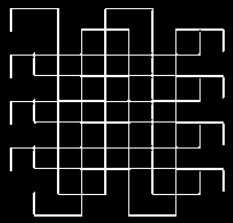


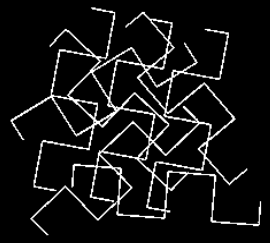
Curvature for Straightness?

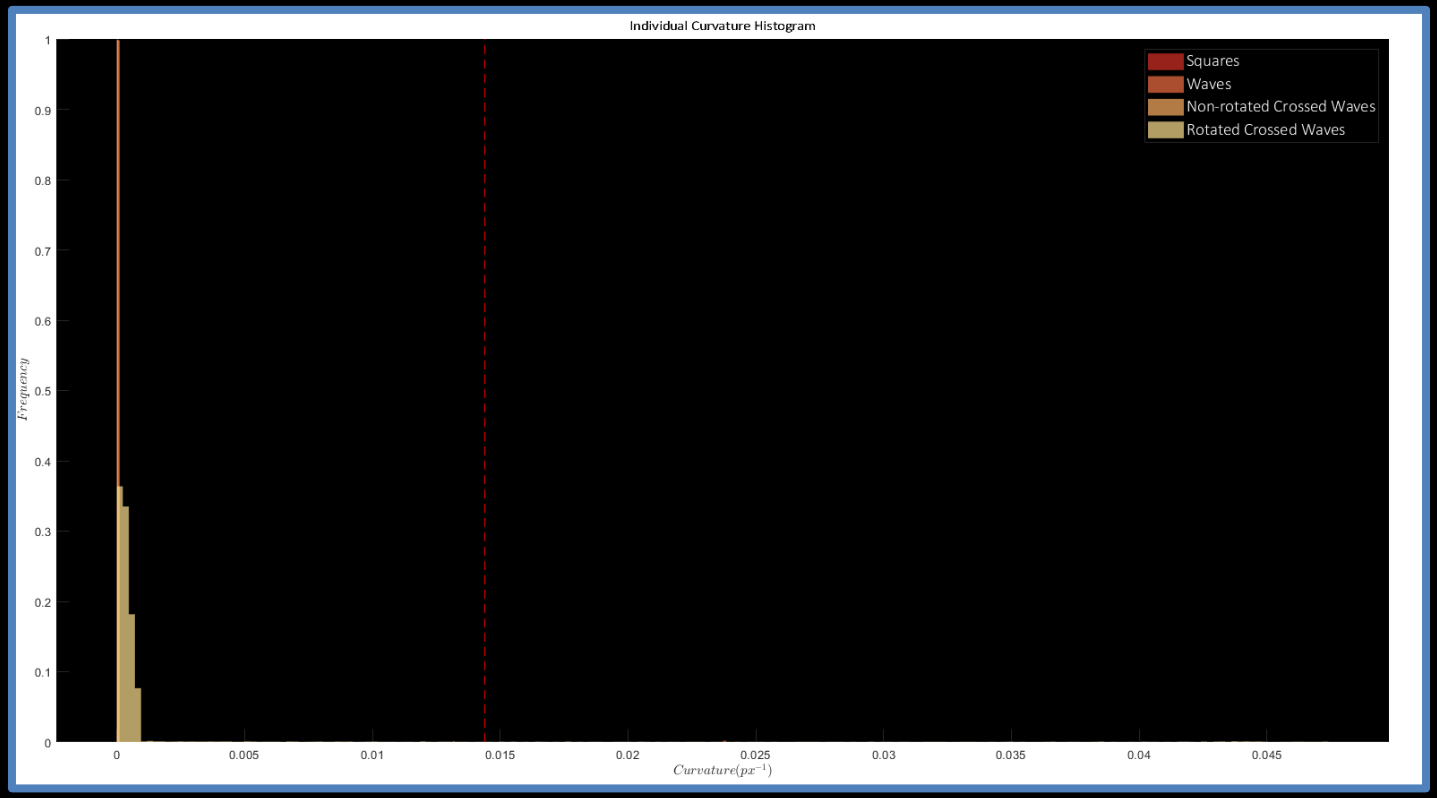
Squares
"Curvature"
0.0144px^{-1}







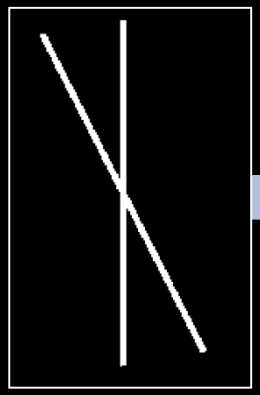




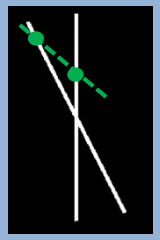
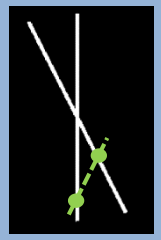
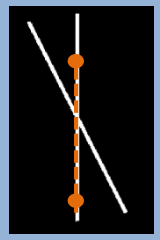
Straight Segments

Using Hough transform - the parameter space of ρ (distance of the line to the origin) and θ (angle to x-axis).

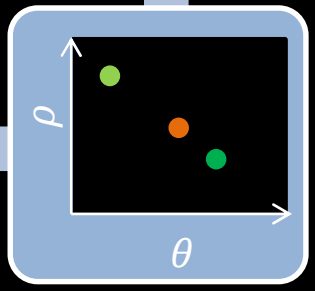
$$\rho = x \cos\theta + y \sin\theta$$



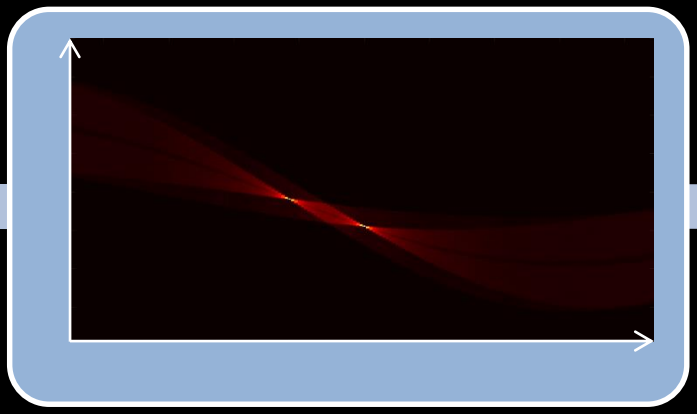
Take any two points in an image and plot the straight line between them.

Calculate both ρ and θ and plot them



Do this for all the pairs of points

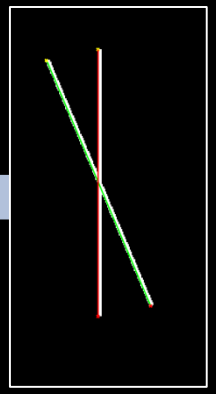


The ones on the same line are going to give more emphasis on the ρ and θ of the real straight lines

Straight Segments

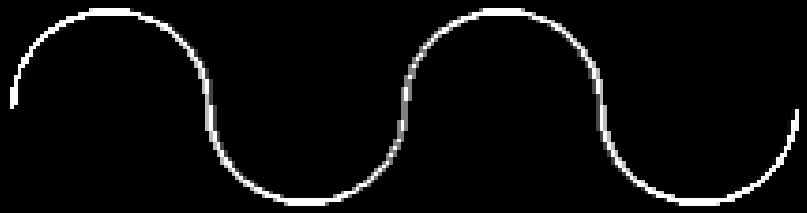
Retrieve the brightest points from the transform

Plot the resulting lines on the image

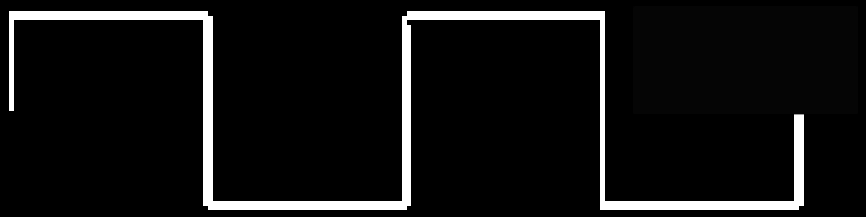


On the real image, extract the various straight segments along axons.

Straight Segments



Number of segments: 22
Straightness Index: 18.43%



Number of segments: 10
Straightness Index: 97.72%

ALFRED

Advanced Labelling, Fitting, Recognition
& Enhancement of Data

Initial image processing and creation of a skeleton, from any type of biological image format

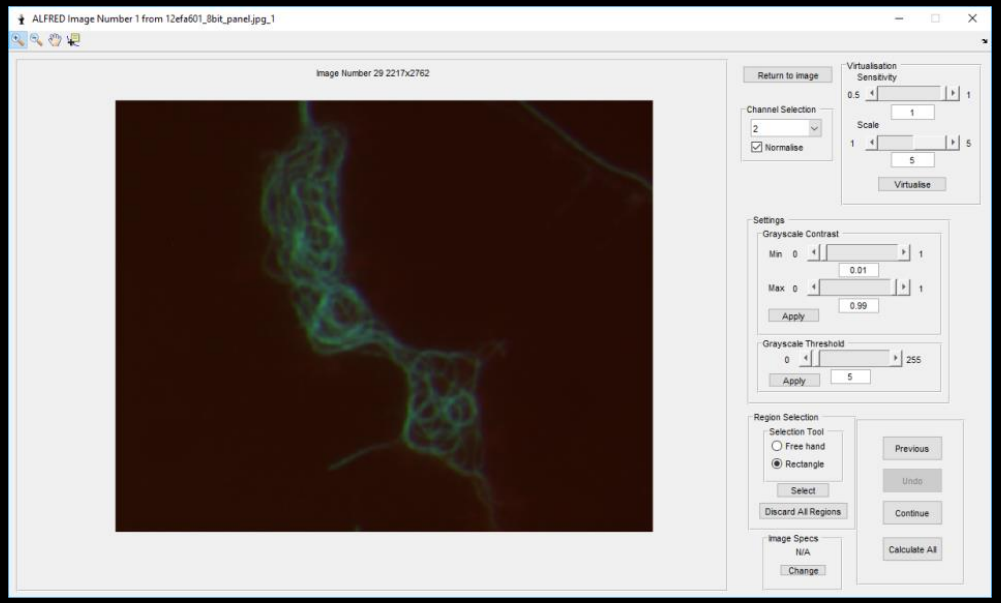
Recognises both **MTs** & **disorganised regions**

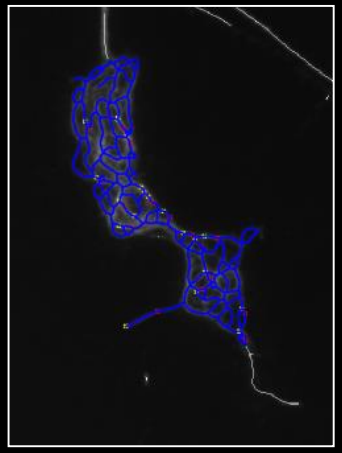
User-friendly implementation on MATLAB of the previous methods combined with existing ones

User Manual: Online Documentation and User Guide for the software

ALFRED

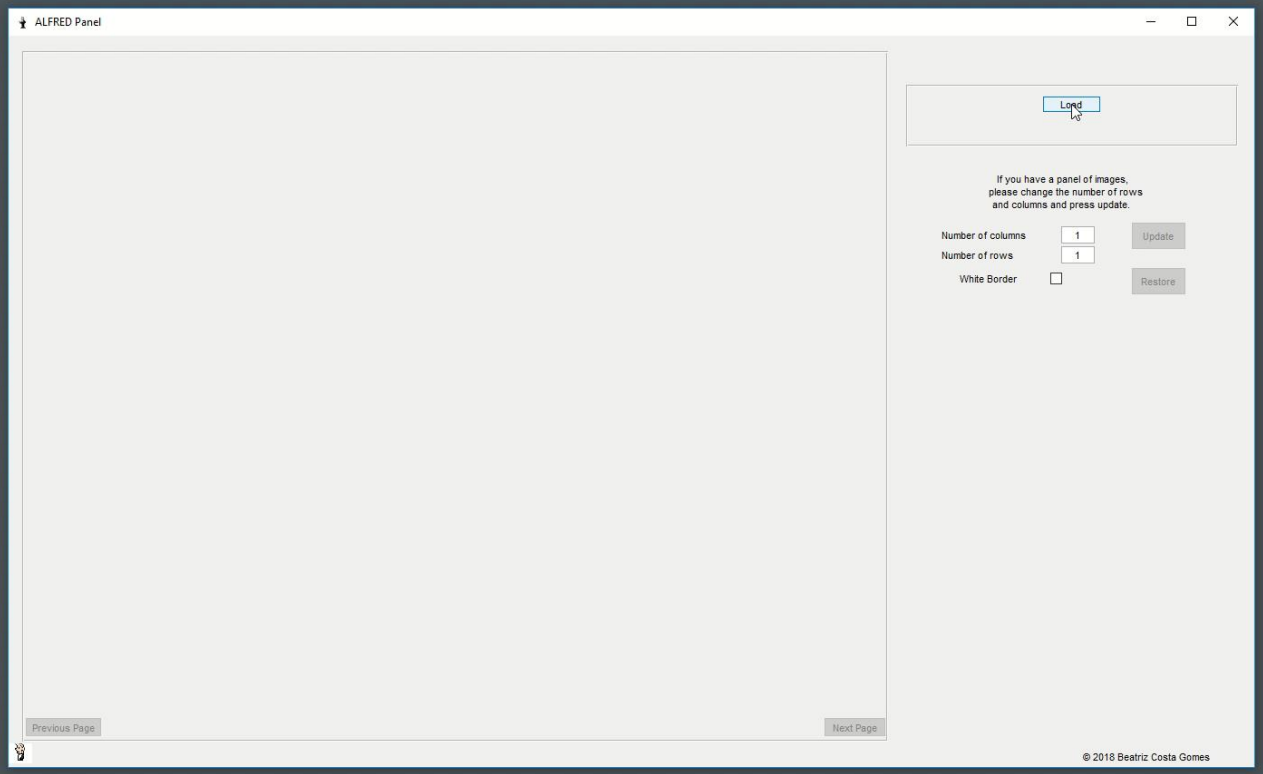
Advanced Labelling, Fitting, Recognition & Enhancement of Data





ALFRED

Advanced Labelling, Fitting, Recognition
& Enhancement of Data



The Future of ALFRED

Further validate the software

- Comparison against different softwares
- Different types of cells & different image acquisition methods
- Compare to human classification and image extraction

ALFRED and curvature algorithm paper

The Future of ALFRED

Machine Learning:

Use an unsupervised method to divide known phenotypes into different groups and run new images through to see how they are classified.

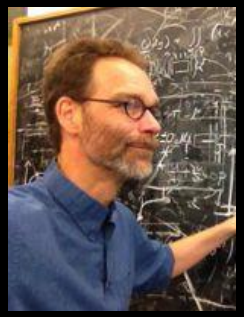
Understand biological value of paramenters

Biological analysis with ALFRED paper

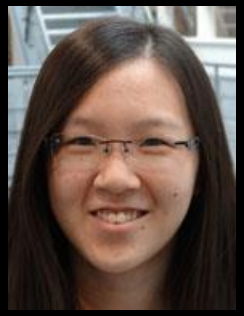
Nuno Nobre
Dr. André Voelzmann



Supervisors:
Prof. Andreas Prokop
Prof. Matthias Heil
Dr. Simon Pearce


Dr. Ines Hahn
Dr. Yu Ting Liew
Dr. Cristina Melero
Jill Parkin

Special thanks to Rita Carvalho for the ALFRED logo.

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