

Using image processing to quantify biologically relevant information in fluorescence microscopy images

Student: Hale-Seda Radoykova

Supervisor: Dr Siân Culley

Location: Randall Centre of Cell & Molecular Biophysics,
King's College London

Accurately detecting and measuring structures in fluorescence microscopy images is important yet challenging. One of the interests of the Culley lab is to develop image analysis techniques to help researchers with these tasks. The aim of this summer project was to acquire images of fluorescently labelled tubulin in *Schizosaccharomyces pombe* cells and to produce an image processing pipeline adapted to quantifying the microtubules in these images. One approach to detecting these linear structures was based on thresholding and skeletonisation, using plugins available in Fiji/ImageJ. This workflow yielded generally satisfactory results in the simplest case but was prone to creating artefacts and to loss of information. Crucially, this method could not cope with intersecting lines. As a more powerful alternative, the linear Hough transform was used to detect straight lines. The implementation in Python was efficient and overcame some of the drawbacks of the previous method. However, it required significant post-processing to faithfully detect multiple lines in images. Unfortunately, it was only feasible to parametrise simpler instances of straight lines, and the method could not be extended to e.g., curved lines. Deep learning approaches could be an exciting front for future work on this problem.

Research question

How can image processing tools be used to quantify the linear structures present in fluorescence

microscopy images of labelled tubulin in *Schizosaccharomyces pombe* (fission yeast)?

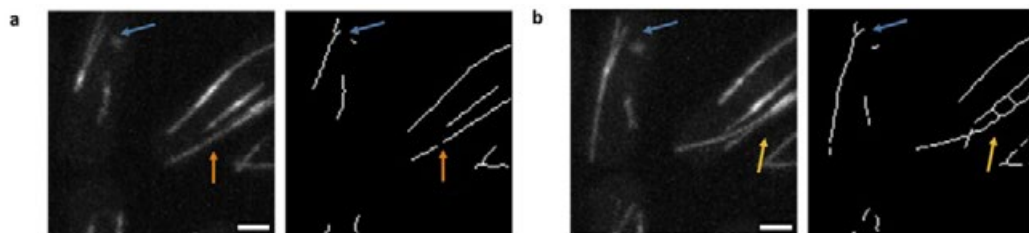


Figure 1. Artefacts resulting from the thresholding and skeletonisation process in two consecutive time frames of the same field of view. (a) Inhomogeneous lines could be interrupted (orange); (b) parallel lines could be blended together (yellow); intersecting lines cannot be skeletonised and labelled faithfully (blue). All scale bars = 2 μm .

Aim

Develop a coherent image processing pipeline suited to *S. pombe* data.

How did you address the aim?

Wet lab work

The wet lab component of the project included culturing and maintaining *S. pombe* strains endogenously expressing sfGFP-Atb2. We were able to acquire some widefield fluorescence microscopy images.

Complementary to this, our collaborators at the Dey lab (EMBL, Heidelberg) kindly provided several stacks of time-series maximum-projected confocal microscopy images with a wide field of view containing numerous *S. pombe* cells in the exponential growth phase (typically 190-210 cells per image). The bundles of microtubules in these cells looked like lines which could be parametrised.

Computational methods

The first part of the computational method implemented a simple skeletonisation pipeline using ImageJ/Fiji [1]. Manually selected 128x128 ROIs (regions of interest) were copied and sorted into several categories for further processing. ImageJ macro scripts were used to apply the same line detection procedure to each ROI: a small-radius Gaussian filter, auto-thresholding using the “triangle” algorithm, and erosion of the binarised image, followed by skeletonisation. This yielded binary images with a one-pixel wide outline of

the detected structures which could be measured within ImageJ.

One parametrised approach for straight lines is using the linear Hough transform. The transform represents each point (x_i, y_i) in Cartesian coordinate space as pair of parameters (θ_i, ρ_i) in Hough space [2]. Straight lines were parametrised using polar coordinates, as $m = \pm\infty$ for lines of the form $y = mx + c$. The linear Hough transform was implemented in Python, and output was refined using libraries, such as scikit-image [3]. The algorithm was modified to detect lines based on pixel intensity weights from raw non-binary data.

What did you find out?

Fiji pipeline

The pipeline in Fiji/ImageJ had the advantage of being relatively simple to implement and automate. It worked relatively well for detecting single straight and curved lines with consistent signal. Line endpoints and length were easy to obtain directly from the binary images.

However, with thresholding and skeletonisation techniques, there were multiple ways of obtaining satisfactory results by tuning one or more parameters. Intrinsic image heterogeneity led to loss of information along the image periphery. It could also break up lines with inhomogeneous intensities (Figure 1a, orange).

Skeletonisation was prone to creating artefacts; for example, parallel lines were sometimes blended together (Figure 1b, yellow). One significant

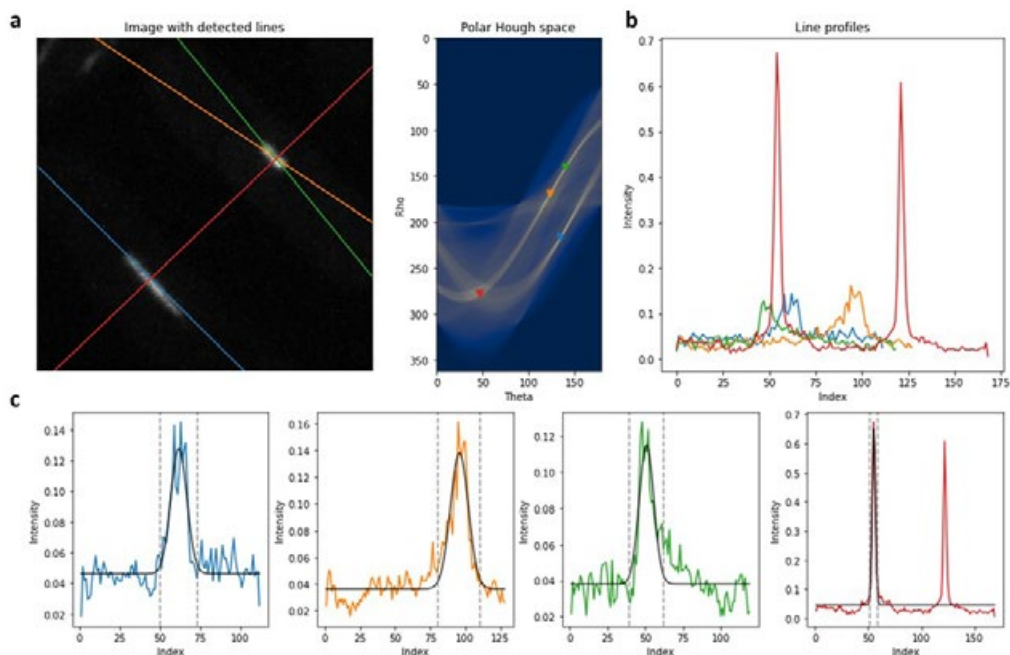


Figure 2. Lines detected by linear Hough transform. (a) Raw image with detected lines and their corresponding peaks in polar parameter space; (b) the intensity profiles of the detected lines; (c) fitted Gaussian curves (in black) and their full width (in grey).

disadvantage for downstream processing was the constraint that intersecting lines could not be labelled as separate objects (Figure 1, blue).

Hough transform

The linear Hough transform detected straight lines in unprocessed raw and binarised data alike. Working with raw data had the advantage of not losing information to processing. This workflow was able to detect interesting lines without being sensitive to interrupted lines or lower contrast.

The disadvantages of this method were related to the postprocessing of the detected lines. A local peak detector was used to identify multiple lines in Hough space; this required manual thresholding. In raw images, noise was sometimes picked up as a line by the local maximum detection algorithm, and multiple lines around the same true line were reported (Figure 2a). These had to be curated by fitting Gaussian curves to the line intensity profiles (Figure 2b). Parameters extracted from the Gaussian fits could be used to estimate the positions of line endpoints (Figure 2c).

The Hough transform could be used for simpler cases; however, handling exceptions and artefacts detected by the algorithm quickly became laborious. Extending the linear Hough transform to fit curved lines of a generalised form was not trivial and was not practical to implement here.

Future work

As the parametrised method for microtubule quantification in *S. pombe* fluorescent microscopy images could only quantify the simplest instances, future work could build upon this by covering the more complex scenarios and work in the full field of view. One intriguing possibility would be building a convolutional neural network (CNN) based on the popular U-net architecture [4]. Exciting previous work on the two-dimensional problem of nucleus segmentation has extended the U-net pixel-to-pixel mapping to pixel-to-shape mapping for instance segmentation based on star-convex objects [5] or splines [6]. Finding a suitable equivalent for predicting lines would be an exciting challenge.

Conclusion

This summer project feeds into the broader aims of the Culley lab to quantify structures in *S. pombe* and other experimental systems. Segmenting geometrically constrained objects is non-trivial and can have important implications for biological data analysis. For example, having a pipeline which accurately quantifies microtubule number, length, or curvature could enable researchers to make cross-condition comparison of microtubule properties in different experimental settings. This would be impossible or unreliable with a pipeline sensitive to noise or tending to produce artefacts.

What did you learn from participating in this project?

I thoroughly enjoyed working on my summer project at the Culley lab! On the one hand, it helped me consolidate my wet lab skills while also complementing my computational skillset with image processing and fluorescence microscopy image analysis techniques. I familiarised myself with Fiji/ImageJ macros and gained programming experience in using Python for image processing, analysis, and visualisation. It was also rewarding to persevere through multiple challenges both in the lab, trying to keep the yeast cultures happy, and in debugging my code. Decidedly, the highlight of the entire project for me was the moment in the microscopy room when we finally saw the fluorescently labelled structures moving in our living yeast cells. This has been an unmatched learning experience, and I am very grateful to Dr Culley and the RMS for making it possible!

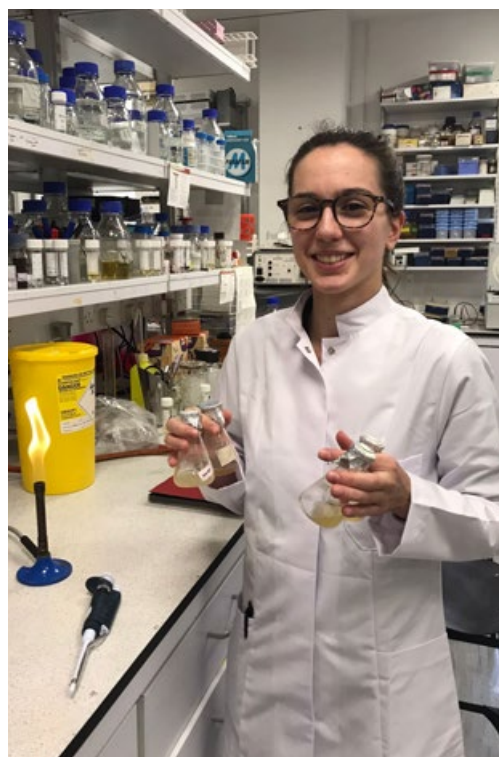
How has this project affected your long-term goals?

In the immediate future, I am beyond excited to learn and do more fluorescence microscopy for my integrated master's project next year. The aim will be to use super-resolution microscopy techniques to

give a quantitative comparison of actin remodelling in CAR-T cells vs native T cells. With the skills gained over the summer, I feel much more prepared and inspired for the upcoming year.

Working with Dr Culley has been an absolute pleasure! We are currently looking into short-term grant opportunities, as I would love to return to her group as a research assistant in the near future.

Finally, I am grateful for this wonderful opportunity, as not only did I meet inspiring researchers this summer, but I believe that quantitative microscopy might be the field to shape my postgraduate studies and career too.



Hale-Seda Radoykova.

References

- [1] Schneider, C., Rasband, W. and Eliceiri, K. NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* 9, 671–675 (2012). <https://doi.org/10.1038/nmeth.2089>.
- [2] Ballard, D.L. Generalizing the Hough transform to detect arbitrary shapes. *Pattern*