Image processing and analysis methods for the detection of islets in Mesolens images of whole mouse pancreas specimens

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Project location: Carried out remotely

The aim of this project was to develop an image processing and analysis pipeline to identify and quantify islets in confocal fluorescence Mesolens images of whole mouse pancreas specimens.

This project was carried out remotely and was entirely computational, using image datasets produced by Ms Katrina Wesencraft at the University of Strathclyde. The majority of the work was undertaken using FIJI [1], an image processing and analysis programme, using various plugins. One of the key plugins used, CLAHE [2], is a tool that breaks the image into multiple small sections, and increases the contrast within each, resulting in a locally contrasted image as opposed to carrying out the procedure on the whole image, and therefore overly contrast-adjusting some regions. Alternative plugins such as Trackmate [3], a plugin designed for particle detection and tracking, had some success for islet detection as this sought out intensely fluorescent areas of a circular shape. The in-built FIJI functions could also be used for islet detection, as once an intensity threshold was applied to the data, it was possible to use the "Analyse particles" function to detect circular shapes within images, based upon an approximate area.

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Findings

Compared to the raw datasets, the images processed using CLAHE provided clearer discrimination between islets and the rest of the pancreas tissue. Whilst this was an improvement and provided a clearer, brighter set of images, there was still the persistent issue where the islets within the pancreas, which are visible as bright spots, were not always clear initially and each image had a different level of brightness. This was combatted through enhancing the contrast within the dataset and using the histogram of the stack, such that for all images, the brightest spot in each had the same grey value and no singular image would be exceptionally brighter or darker than the others within the stack. Once the parameters had been set, this process was able to run automatically. Fig. 1 demonstrates the difference between one of the original images and the same slice after editing.

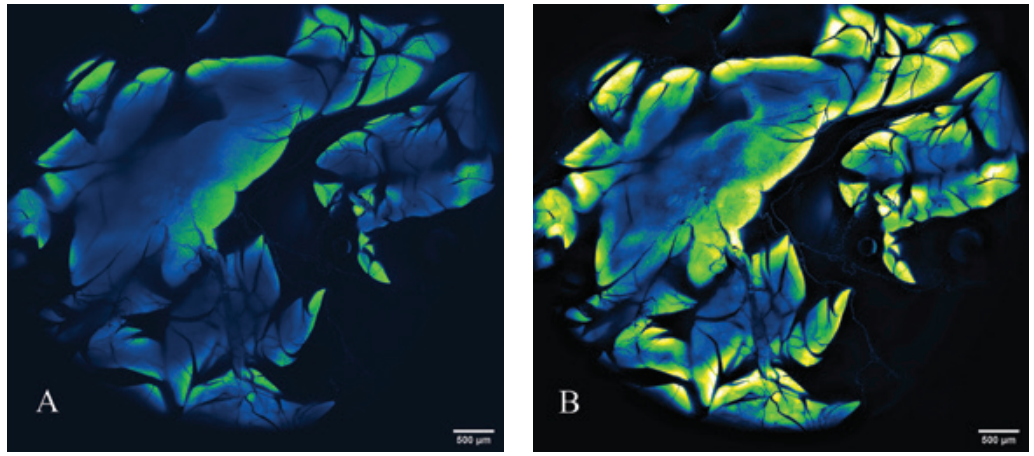


Figure 1. A comparison between an original image (A), and the altered image, after using CLAHE, and enhanced contrast (B).

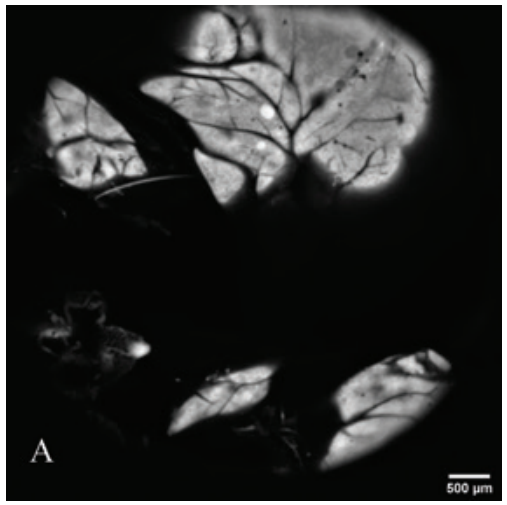
Automatically detecting the islets proved to be more difficult as islets vary in size from 50 to 500 microns and the differences in size and intensity meant that not all could be identified. Additionally, many methods that I tried often picked up false positives, detecting spots where there were none.

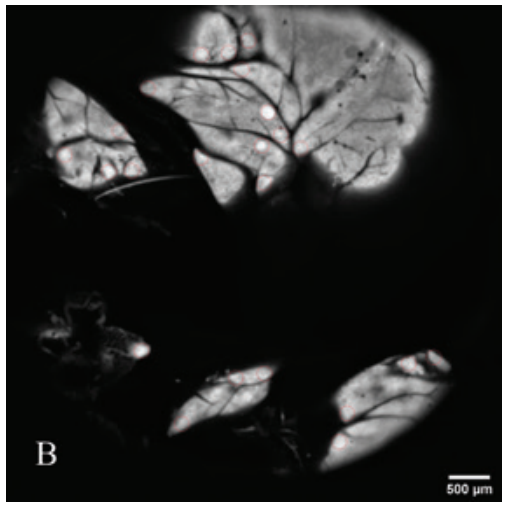
For example, the Trackmate plugin was able to highlight many islets throughout the images: by setting an approximate diameter range, it automatically detected any spots of that size. However, as shown in Fig.2, it captured a lot of false positives, where the image was brighter but where there were not islets. Another more successful method was using the FIJI 'Analyse particle' tool as discussed above and seen again in Fig. 2. This captured some areas of high intensity but was able to disregard them based on their non-circular shape. Unfortunately, the restrictions in size range required to eliminate false detections also meant that many smaller islets were being missed. Whilst these methods both had some level of success and provided an automated method of detection, they both relied on parameters being set each time based on the individual dataset.

On top of using automatic detection methods, I also hand annotated a selection of the images, following the appearance and development of islets throughout the pancreas, by circling each clearly visible spot with an editing tool. This acted as a comparison for the automatic detection methods, but it took significantly longer to perform. Manual annotation took around 1 hour, to analyse only 41 images, compared to the almost instantaneous analysis of 178 images using the 'Analyse particle' function.

Learning from participation in project

Before undertaking this internship, despite having previously used coding languages such as MATLAB, to develop figures, and read image data, I had no strong experience in image processing or image analysis. This project has allowed me to not only develop these analysis skills, learning the basics of creating and using macros to analyse images, and becoming comfortable with using programmes such as ImageJ, but it has also allowed me to refine my own broader computational skills along with a general understanding of this area,





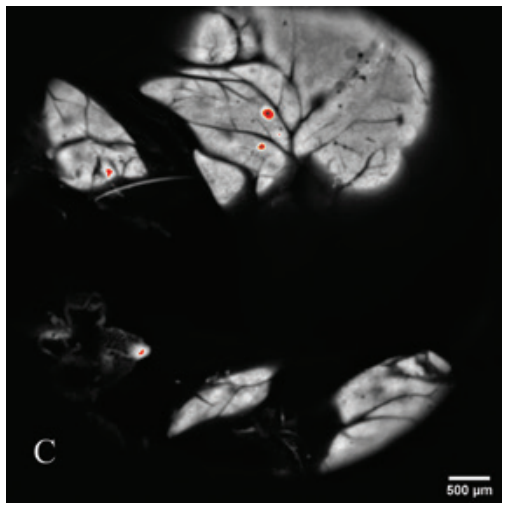


Figure 2: The original image (A), the image analysed using Trackmate, with islets identified by red boundaries (B) and the results using 'Analyse particles' function, with islets highlighted in red (C).

despite it not being an area covered within my course curriculum.

I've discovered on a personal level that I enjoy the opportunity to work on a project, and to undertake work that requires a level of self-management, tackling a problem from start to finish, and the research and work required to do so. Whilst also gaining the opportunity to work collaboratively with my project supervisors, who's expertise and advice was invaluable, and I can't thank them enough for giving me the opportunity to work with them on this. I also thank the Royal Microscopical Society for funding this project so that I could gain this invaluable experience.

Impact on long-term goals

Completing this project has sparked my keen interest in microscopy, and its applications, not only within physics (despite my background there), but within other areas of research. It has also encouraged me to start looking at career opportunities in this area. Undertaking this internship has given me an insight into some of the practicalities of a research career. I am interested in pursuing my newly discovered interest in microscopy, whether that be through continuous learning within employment after graduating, or in an ideal situation, I would love to undertake a PhD. A PhD would allow me to further develop my knowledge and expertise, whilst contributing to the exciting and diverse field of light microscopy.

[1} Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., … Cardona, A. (2012). Fiji: an open-source platform for biological-image analysis. Nature Methods, 9(7), 676–682.

[2} Zuiderveld, K. (1994) Contrast limited adaptive histogram equalization. Graphics gems IV, pp. 474–485. Academic Press Professional, Inc, San Diego, CA, USA

[3} Tinevez, J.-Y., Perry, N., Schindelin, J., Hoopes, G. M., Reynolds, G. D., Laplantine, E., … Eliceiri, K. W. (2017). Trackmate: An open and extensible platform for single-particle tracking. Methods, *115*, 80–90.



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