

Studying the dynamics of platelet actin nodules using TIRF microscopy and image analysis

RMS 2020 Summer Studentship Report: Poppy Cairney, University of Southampton

What was the aim of your project?

To study the dynamics of platelet actin nodules using TIRF microscopy and image analysis, in order to investigate the effects of different inhibitors on nodule dynamics.

How did you address the aim?

Due to the coronavirus pandemic I was unable to go to the lab to complete my project. Instead, I spent some time learning about TIRF microscopy, before Dr Thomas shared some previously captured stacks of images with me. Throughout the project I have learnt how to use the software Fiji (ImageJ) and its plugin, Trackmate, to analyse the dynamics of platelet actin nodules. This involved understanding how to process the raw data images to prepare them for tracking and understanding what the various parameters of the image processing were doing and how they affected the data output. At the start of the project, the tracking process took much longer than it did towards the end of the project, as the weeks went on, I found myself becoming much faster at setting up the plugin and gathering data needed. I analysed control platelets and platelets inhibited with two inhibitors of Rho family GTPases, Rhosin and EHT. I focused on comparing track duration (s) and mean track speed for individual nodules from the groups of platelets (control and inhibited). Using Excel, I plotted histograms of this data and was able to make statistical comparisons between the data sets.

What did you find out?

Treatment of platelets with EHT or Rhosin increased nodule track duration but had no effect on speed. The dynamics of platelet actin nodules changed dramatically through treatment with inhibitors. Visibly I was able to notice a difference in the pattern of spreading that the platelets displayed in the control compared to the treated platelets. For example, the EHT treated platelets had much longer filopodia (figure 1d) and the Rhosin platelets appeared to have more actin nodules than controls but had smaller filopodia. Using Trackmate, I was able to quantify differences in nodule dynamics due to the effects of the two inhibitors; both inhibitors on average caused an increase of nodule lifetime. The mean lifetime (s) of nodules in control platelets was 32.75 seconds, compared to 43.76 seconds in EHT treated platelets and 45.88s in Rhosin treated platelets (figure 1c).

What did you learn from participating in this project?

Over the course of the project I learnt a lot about TIRF microscopy, in the first week of the project Dr Thomas and I had zoom meetings to enable me to learn more about TIRF microscopy specifically. I read papers on how TIRF microscopes function and various types of TIRF microscopy that can be done (different expenses have different outcomes) as this was a type of microscopy I had never come across before. I also have learnt much more about platelets; platelets always come up across various modules

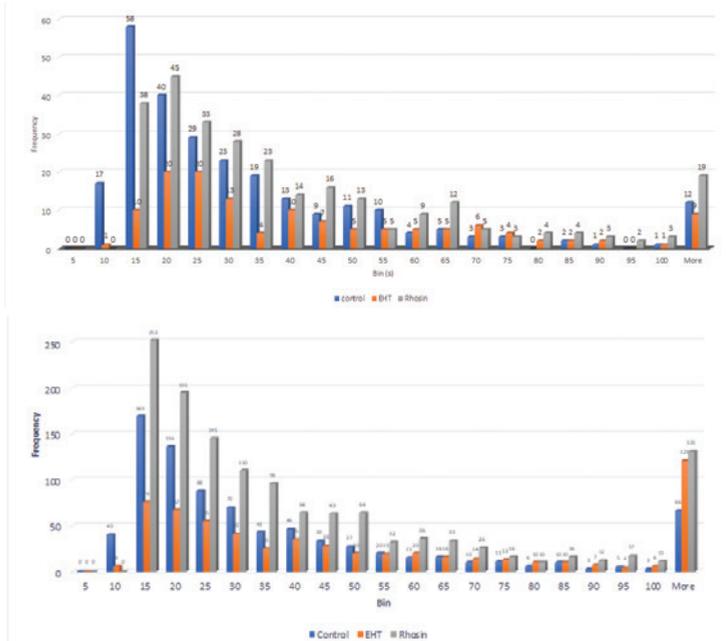
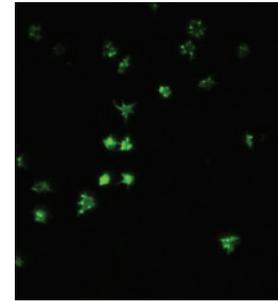
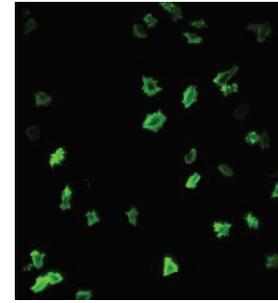


Figure 1- (a) Control platelets (DMSO first-1001) image (taken at end of movie) enhanced brightness using Fiji software. (b) Histogram comparison of nodule track duration(s) (within the first 300s of movie) of each treatment of platelets, control (blue), EHT (orange), Rhosin (grey). (c) Histogram comparison of nodule track duration(s) (whole movie length) of each treatment of platelets, control (blue), EHT (orange), Rhosin (grey). (d) EHT treated platelets (Lifeact-GFP EHT-2) image (taken at end of movie) enhanced brightness using Fiji software.

at university, but we have never solely looked into them before, so it has been eye opening to study them in such detail and see how they spread out. I learnt new skills through using Fiji and Microsoft Excel for analysis and using add-in tools to make histograms with the data. I will continue to use these skills when using Excel from now on, as I have learnt how to present data better for comparisons. I have also learnt skills in tracking images, and how altering small parameters can have a large effect on the outcome data.

How has this project affected your long-term goals?

From doing this project during the coronavirus pandemic, I unfortunately did not get to use the microscopes myself, but I would say this has given me a bigger interest in TIRF microscopy to get the full picture behind the stacks of images I analysed. As I spent a week of my project researching this type of microscopy, I am excited at the possibility of using one in the future to understand how it works, this project has opened my eyes beyond the microscopy

I have used so far through university (basic light microscopy). It has been very satisfactory to get such specific data on each actin nodule from the platelets selected, and to do this analysis on having come from a larger picture of all the platelets in the sample spreading out. I do want to progress onto a PhD after my degree and hope to use many more microscopy techniques before then, starting with my lab project in my final year at university.



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