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Exploring neutrophil behaviour in a zebrafish model of inflam- mation through the generation of novel parameters using MatLab algorithms

K. M. Henry,* S. A. Renshaw* & C. C. Reyes-Aldasoro

*Centre for Developmental and Biomedical Genetics, University of Sheffield, Sheffield, UK, Biomedical Engineering School of Engineering and Informatics, University of Sussex, Sussex, UK

Purpose/Objective: Tracking of immune cells is key to understanding their behaviour during inflammation. Current software available for tracking of immune cells is limited. The aim of this study was to develop a MatLab package of segmentation and tracking algorithms to apply in the tracking of neutrophils, fluorescently labelled in our zebrafish model [1]. The development of algorithms in MatLab allows us to explore parameters not available in other software packages such as directionality of neutrophil movement and neutrophil behaviour in- side and outside of a wound region.

Materials and methods: Tail fin transection was performed on Tg(mpx:GFP) zebrafish (3 dpf) which were imaged on a spinning disk confocal from 1 h post injury (hpi) to 7 hpi. Images were exported from Volocity^{TM} and analysed using MatLab m-files written for the tracking of immune cells. This is a fully automated analysis, after the user defines the initial thresholds based on fluorescent intensity of the images.

Results: Neutrophils from injured embryos had a lower meandering ratio and a greater speed than neutrophils tracked in uninjured embryos (meandering ratio 0.24 ± 0.03 versus 0.42 ± 0.05, $P = 0.003$; speed 4.03 ± 0.32 versus 1.31 ± 0.21 pixels/frame, $P < 0.0001$, $n = 3$), however there was no significant difference in total distance travelled (166.9 ± 17.4 versus 210.1 ± 30.28 pixels, $P > 0.05$). In injured embryos with a defined wound region, the oriented velocity towards the wound was 0.31 ± 0.24 pixels/frame. Once within the wound region, the oriented velocity of neutrophil tracks was -0.39 ± 0.32 pixels/frame; indicating that while the neutrophils travel at a similar speed, they are now travelling away from the wound. The 'in wound ratio' was 0.91 ± 0.04, indicating that once neutrophils enter the wound region they tend to stay, in the timeframe studied. The 'leave wound ratio' was 0.37 ± 0.03, a measure of the rate at which neutrophils move away from the site of injury once they have entered the wound region.

Conclusions: Using these algorithms, we can analyse the behaviour of immune cells in a more detailed way. In addition to previously available parameters such as meandering ratio and speed, more complex parameters such as velocity towards or away from a wound region and a measurement of how neutrophils behave while in a wound region are available. Combining this novel tracking technology with established assays in our laboratory will enable the further dissection of neutrophil fate following an inflammatory stimulus.

[1] K. M. Henry, L. Pase, C. F. Ramos-Lopez, G. J. Lieschke, S. A. Renshaw, and C. C. Reyes-Aldasoro, 'PhagoSight: an open-source MATLAB® package for the analysis of fluorescent neutrophil and macrophage migration in a zebrafish model', *PloS One*, vol. 8, no. 8, p. e72636, 2013.