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

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**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 inside 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 Velocity<sup>TM</sup> 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  $0.24 \pm 0.03$  versus  $0.42 \pm 0.05$ ,  $P = 0.003$ ; speed  $4.03 \pm 0.32$  versus  $1.31 \pm 0.21$  pixels/frame,  $P < 0.0001$ ,  $n = 3$ ), however there was no significant difference in total distance travelled ( $166.9 \pm 17.4$  versus  $210.1 \pm 30.28$  pixels,  $P > 0.05$ ). In injured embryos with a defined wound region, the oriented velocity towards the wound was  $0.31 \pm 0.24$  pixels/frame. Once within the wound region, the oriented velocity of neutrophil tracks was  $-0.39 \pm 0.32$  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  $0.91 \pm 0.04$ , indicating that once neutrophils enter the wound region they tend to stay, in the timeframe studied. The 'leave wound ratio' was  $0.37 \pm 0.03$ , 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.