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## RED BLOOD CELL TRACKING AND VELOCITY MEASUREMENT WITH A KEYHOLE MODEL OF MOVEMENT

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A tracking algorithm is proposed to measure the velocity of red blood cells (RBC) in intravital microscopy of tissue microvessels. Intravital microscopy was carried out on tumours growing in transparent dorsal skin flap 'window chambers' in unanaesthetized mice. Fluorescently labelled RBCs (25  $\mu$ g of DiI was used per 50  $\mu$ l of packed red blood cells) were injected into a cannulated tail vein for tracking.

The tracking algorithm is based on a keyhole model that describes the probable movement of a segmented cell between contiguous frames in a video sequence. When a history of movements exists, past, present and a predicted landing position define two regions of probability with a keyhole shape. Pre-processing segments cells from background. The keyhole is used to determine if cells in contiguous frames should be linked to form tracks and also as a post-processing tool to join split tracks and discard links that could have been formed due to noise or uncertainty. Outliers are removed based on the distribution of the average velocities of the tracks. The algorithm presents several advantages over traditional methods such as kymographs or particle image velocimetry: manual intervention is restricted to the thresholding, many vessels can be analyzed simultaneously, the algorithm is robust to noise and a wealth of statistical measures can be obtained. Average velocities of 2 tumours were  $207 \pm 155$  [ $\mu$ m/s] (mean $\pm$ std) with a range 15-797 [ $\mu$ m/s], and  $86 \pm 60$  [ $\mu$ m/s] with a range 5-300 [ $\mu$ m/s] respectively, which are consistent with the literature. Validation against a manual method is in progress.

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