Pushing the envelope: Novel zebrafish models for investigating neutrophil nuclear plasticity in vivo.

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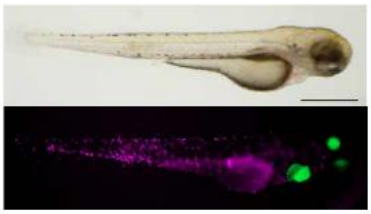
Nuclear biomechanics are essential for cellular function; hence aberrant nuclear structure underpins several diverse diseases. Neutrophils provide an unrivalled opportunity to study the relationship between nuclear structure and cellular function. Lamin intermediate filaments form the nuclear lamina, a structural meshwork supporting the nucleus and known to affect nuclear stiffness. Lamin down-regulation is associated with tumourigenesis and genetic laminopathies, but also neutrophil maturation and rapid immune migration. Neutrophils uniquely have low LaminA/C, but high LaminB2 and LaminB receptor (LBR) expression. We hypothesise that this distinctive nuclear envelope composition directly influences the function of the unique multi-lobulated nuclei characteristic of these highly migratory and plastic leukocytes. To investigate how nuclear structure influences neutrophil shape, flexibility and function, we generated compound transgenic zebrafish reporter lines labelling the neutrophil nuclear envelope in green (mpx:eGFP-lamin), chromatin in blue (H2B-cerulean) and cytoplasm in red (mCherry) to precisely display neutrophil nuclear plasticity by 4D *in vivo* imaging.

Confocal and lattice-lightsheet microscopy has displayed neutrophil migration *in vivo* at unprecedented resolution. Morphometric analyses have been used to quantify nuclear plasticity in migrating cells. Confocal imaging of >100 migrating neutrophils has demonstrated extraordinary nuclear flexibility and identified a role for nuclear lobulation. Computational analysis has been applied to distil the complex cellular and nuclear shapes, speeds, and positions observed into quantitative measures of neutrophil nuclear plasticity. In addition to confocal imaging, one of Australia's pioneer lattice lightsheet microscopes has been used at Monash Micro Imaging to successfully image neutrophil and their nuclei (**Fig. 1**). Images reveal new cellular and nuclear morphologies that could not previously be resolved *in vivo*, as neutrophils' fast migratory speed (up to 13m/min) presents a significant spatiotemporal resolution challenge. Dramatic changes in nuclear shape support the hypothesis that flexible, laminA/C-deficient neutrophil nuclei assist their mobility. As part of the visitor program at HHMI Advanced Imaging Centre (April 2018), further lattice lightsheet imaging of zebrafish neutrophil nuclei will be completed to address this hypothesis. Lastly, novel zebrafish mutants deficient in laminA/C, *laminB1*, *laminB2* and *lbr* are being examined to assess how perturbation of nuclear envelope composition affects neutrophil function. As Lamin B receptor (*LBR*) mutations in humans cause neutrophil hypo-lobulation (the Pelger-Huët anomaly), an *lbr* zebrafish mutant is predicted to alter neutrophil nuclear lobulation and hence directly assess its impact on cell function. Preliminary electron microscopy (EM) ultrastructure analysis suggests *lamin* mutants alter neutrophil nuclear shape, with further EM and functional *in vivo* studies to be conducted in coming months. Overall, these data provide the most detailed descriptive and functional analyses of neutrophil nuclear dynamics to date, informing both nuclear and leukocyte biology.



Csf3 mRNA injected 3dpf larvae

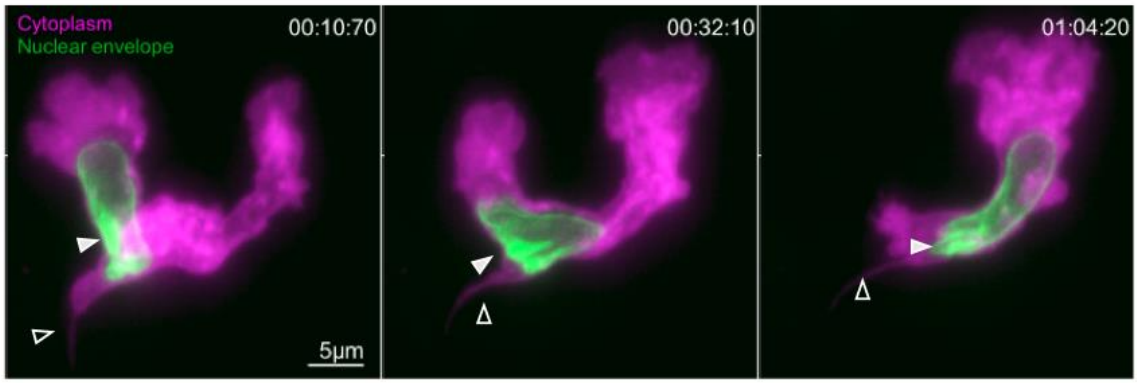


Figure. 1. A maximum projection timelapse image of a neutrophil (magenta) and its nucleus (green) captured *in vivo* using lattice lightsheet microscopy (bottom panel). The cell is migrating from left to right, in the tail of a transgenic zebrafish larvae (top panel). Filled arrows indicate nuclear lobulation/ruffling, whilst empty arrows indicate a thin cytoplasmic structure at the rear of the cell, the uropod. Imaged every 10.7 sec, every second timepoint is shown. Timescale is min:sec:msec. 151 z slices, z interval = 0.5 µm.