

MULTICOLOR 3D STED IMAGING OF NEUTROPHIL EXTRACELLULAR TRAPS

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Neutrophils are the main cell type involved in the early phase of inflammatory responses to bacterial infections and tissue injury. They are characterized by the typical multi-lobular organization of the nuclear chromatin and the presence of different types of cytoplasmic granules, containing antimicrobial peptides and inflammatory mediators, which are freely released into the extracellular environment upon cell activation[1]. In addition to this, neutrophils have been described to trap and kill bacteria also via generation of neutrophil extracellular traps (NETs), sticky filament-like structures made of de-condensed chromatin and granule-derived proteases, in a suicidal cell-death mechanism known as NETosis[2].

The production of NETs *in vitro* is commonly visualized by conventional wide-field fluorescence microscopy. As a result, their detailed molecular structure is still poorly understood and mainly relies on electron microscopy data derived from extensively treated (and possibly altered) samples[2, 3]. Therefore, optical nanoscopy is a powerful tool to further elucidate how these structures are generated, organized and behave under physiological and/or pathological conditions in a viable environment.

To this extent, we have applied multicolor STED microscopy (on a Leica SP8 3X) to human neutrophils freshly isolated from whole blood and stimulated with phorbol 12-myristate 13-acetate (PMA), a well-known chemical inducer of *in vitro* NETosis[4]. By exploiting a combination of viable fluorescent dyes and fluorochrome-labeled antibodies, we have analyzed the morphology, size and distribution of several cellular components (namely DNA, specific granule proteins and receptors as well as other organelles, such as the cytoskeleton and mitochondria) at different time points after PMA stimulation. Moreover, we have started to characterize the fate of NETs in a dynamic setting mimicking arterial flow conditions, which will be relevant for future studies aimed at understanding the possible function of these structures in cardiovascular disease.

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3. Fuchs, T.A., et al., "Novel cell death program leads to neutrophil extracellular traps". *J Cell Biol*, **176**(2), 231-41 (2007).
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