Label-free characterization of red blood cells using advanced optical techniques

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The interaction between femtosecond laser pulses and hemoglobin molecules, both in intact erythrocytes and in isolated form, was explored as a label-free strategy for erythrocytes imaging and characterization. It was observed that intrinsic fluorescent photoproducts are generated following the interaction of femtosecond laser pulses with hemoglobin [1]. Through systematic optimization of Two photon (TPEF) laser exposure parameters (excitation wavelength was set on 730nm, laser power was around 20mW), photostable and fluorescent molecular species were reproducibly formed and analyzed using TPEF and single-photon fluorescence microscopy, as well as UV-VIS absorption spectroscopy [2]. These photoproducts are formed during hemoglobin photodegradation, probably driven by porphyrin structure without necessity of Fe^{2+} ion presence [2]. Oxidative modifications, particularly through hydrogen peroxide or TBHP (tert-butyl hydroperoxide) treatment, significantly enhanced fluorescence emission intensity.

Spatially controlled formation of these photoproducts was achieved using TPEF microscopy on hemoglobin thin films, consequently enabling subcellular mapping of hemoglobin in intact erythrocytes [3]. The resulting fluorophore exhibited prolonged photostability and was successfully applied for selective *in situ* labeling of erythrocytes in whole blood as well [2], allowing visualization of hemoglobin distribution patterns without exogenous dyes.

Given the high stability of the fluorescent hemoglobin photoproduct formed by 730 nm femtosecond laser pulses, we were motivated to explore additional optics-based techniques to differentiate between healthy and altered erythrocyte subpopulations Flow cytometry revealed changes in fluorescence and light scattering (FSC/SSC) under induced oxidative stress, reflecting alterations in erythrocyte subpopulation morphology and internal composition [4]. It also confirmed corresponding impairments in erythrocytes deformability-an essential property for microcirculatory function, which was previously assessed by ektacytometry. These biophysical insights were further validated on erythrocytes from individuals with diabetes [5], confirming the diagnostic potential of the proposed label free approach.

This study demonstrates that integrating label-free nonlinear optical imaging with mechanical phenotyping enables precise, non-invasive assessment of hemoglobin dynamics and erythrocyte physiology, advancing diagnostics and research on hemoglobinopathies and erythrocyte-related diseases.

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