Invited

MULTIMODAL DYNAMIC AND STRUCTURAL IMAGING OF ERYTHROCYTES AND BLOOD CAPILLARIES

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Abstract. Several optical techniques were used to make a complex study of various parameters related to the fundamental properties of erythrocytes, aggregation and deformation, by means of imaging and measurement. Diffuse light scattering, laser diffractometry, and dual- and multiple channel laser tweezers were applied in the experiments *in vitro*. Computerized capillaroscopy and laser two-photon lifetime imaging microscopy were used for imaging the structure of the terminal capillaries and papillary dermis in the fingernail bed and the inner forearm area *in vivo.*

The ability of the erythrocytes to perform their functions is mostly due to their two intrinsic properties: reversible erythrocyte aggregation (EA) and erythrocyte deformability (ED). The former one optimizes the interaction of the moving cells with the walls in the vessels of various sizes, while the latter one enables the erythrocytes to squeeze through the terminal capillaries, given that the diameters of the capillaries are smaller than those of the cells in some parts of the body. The dynamic processes of EA and ED are tightly connected with the structural changes of the cells and the terminal capillaries. Both EA and ED were given a lot of attention especially during the last several decades. Many techniques were designed and applied to assess the mechanisms of EA and ED and their dependences on blood content and changes at various diseases. The same is true in relation to the terminal vessels and the surrounding tissues where the gas and liquid exchange with blood takes place. However many important issues are still to be studied.

In this work, we combined several optical techniques to perform a complex study of various parameters related to EA and ED by means of imaging and measurement. We used: 1) diffuse light scattering; 2) laser diffractometry; 3) dual- and multiple channel laser tweezers, also combined with microfluidics,. The above mentioned imaging and measurement experiments were performed *in vitro*. Also we used: 4) computerized capillaroscopy and 5) laser fluorescence lifetime imaging microscopy (FLIM) for imaging the terminal capillaries and papillary dermis in the fingernail bed and the inner forearm area *in vivo.*

Imaging of a pair of individual erythrocytes trapped by laser tweezers in the process of their interaction allowed us to measure the range of the interaction forces in dependence of their interaction surface area, velocity of relative movement and the proteins content in the suspending medium. We showed that the aggregating and disaggregating forces are significantly different from each other. The studies of the cells interaction kinetics in model solutions revealed a significant importance of the synergy of the contributions of different proteins or other blood components, especially for initiating the spontaneous aggregation. The diffraction imaging of erythrocyte suspensions at different shear stresses allowed to quantitatively estimate the first several moments of the erythrocyte deformability distributions characteristic of various diseases. Using FLIM of endogenous compounds allowed us to assess the molecular structure of internal and external regions of skin capillaries. It was shown that the capillaries are characterized by a fast fluorescence decay, which is originated from the blood cells and blood plasma. Using the SHG signal, FLIM segmentation was performed, which provided for spatial localization and fluorescence decay parameters distribution of collagen I and elastin in the dermal papillae. It was demonstrated that the lifetime distribution was different for the inner area of dermal papillae around the capillary loop that was suggested to be due to collagen III.

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References

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