**Discovering abnormal erythrocyte membranes - optical approaches**

M. Matić1, D. Pavlović2, M. Radmilovic2, M. Rabasovic2, V. Ilić3, A. Krmpot2, I. Drvenica3

*1 Institute of Oncology and Radiology of Serbia, 2 Institute of Physics, Belgrade, Serbia*

*3 Institute for Medical Research, University of Belgrade*

e-mail: milica.matic1210@gmail.com

Due to their complex physiological role, erythrocytes have naturally very elastic membranes, however, extremely susceptible to various endogenous and exogenous factors. Therefore, it has been speculated that abnormalities in erythrocyte membrane deformability and shape can be seen as an early sign of some acute and chronic pathological states/diseases [1,2]. In the project HEMMAGINERO [3], we are exploring whether optical methods, ektacytometry, and Two-Photon Excitation Fluorescence (TPEF) microscopy, can be used as potential diagnostics tools in identifying any changes in the shape/deformability of erythrocytes. Using ektacytometry (RheoScan D-300, RheoMeditech Inc., South Korea) we calculate the cell deformability from the intensity pattern of the laser light which is scattered by a suspension of red blood cells exposed to shear stress [4]. Our previous research already demonstrated that in-house TPEF microscopy set-up is an effective tool for label- and fixation -free imaging of erythrocytes and their membranes [5], based on a peculiar feature of hemoglobin to produce a fluorescent molecule upon interaction with ultrashort laser pulses [6,7].

 In the first phase of the project, we have used blood from healthy volunteer donors and *in vitro* made environments that simulate different conditions to which erythrocytes can be exposed in pathological processes (hyper- and hypo-osmolarity; acidosis, alkalosis). The obtained data on erythrocyte morphology by TPEF and erythrocytes deformability by ektacytometry are correlated with the results of routinely used biochemical tests for oxidative stress assessment, and mechanical and osmotic fragility indices.

 Our results show that both ektacytometry and TPEF microscopy are sensitive and reliable in determining that membranes of erythrocytes have suffered under non-ideal (meaning non-physiological) conditions of the *in vitro* environment. Further investigation is needed to conclude the precision of these optics methods in discovering abnormal erythrocyte membranes in actual patients’ blood.

 Funding: Project HEMMAGINERO No 6066079 from Program PROMIS, Science Fund of the Republic of Serbia

REFERENCES:

1. H. Chen, W. Yunpeng, C. Shaoxi, et al. Clin. Hemorheol. Microcirc. 16, 2 (1996).
2. S. Shin, Y. H. Ku, J.X. Ho, et al. Clin. Hemorheol. Microcirc*.* 36, 3 (2007).
3. <http://www.hemmaginero.rs/hemmaginero.html>
4. A.E.O. Finkelstein. Design and evaluation of a new diagnostic instrument for osmotic gradient ektacytometrie. PhD Thesis, Université Paris-Est (2017).
5. [K.S. Bukara](https://www.spiedigitallibrary.org/profile/notfound?author=Katarina_Bukara), [S.Z. Jovanić](https://www.spiedigitallibrary.org/profile/Svetlana.Jovanic-287493), I.T. Drvenica, et al. Mapping of hemoglobin in erythrocytes and erythrocyte ghosts using two photon excitation fluorescence microscopy J. Biomed. Optics 22(2), 026003 (2017).
6. W. Zheng, D. Li, Y. Zeng, et al. Two-photon excited hemoglobin fluorescence, Biomed. Opt. Express 2, 71-79 (2011).
7. E.A. Shirshin, B.P Yakimov, S.A. Rodionovet al. Formation of hemoglobin photoproduct is responsible for two-photon and single photon-excited fluorescence of red blood cells. Laser Phys. Lett. 15, 075604 (2018).