# STSM REPORT

**STSM Application number:** COST-STSM-ECOST-STSM-BM1205-190514-044689

**STSM Grantee:** Alexey Popov

**STSM title:** Blood diagnostics at cellular level using laser tweezers

**Home Institution:** University of Oulu

**Host Institution:** University of Saarbrücken

**STSM period:** 10 January 2017 to 25 January 2017

**STSM purpose**
Establishing collaboration between the Finnish and German laboratories in the area of biophotonics. In particular, for applications of novel optical instruments for improved cancer detection and treatment via nanoparticulate anticancer drugs and assessment of their effect on blood rheological properties.

**Description of the work carried out during the STSM**
One of the aspects of the cancer diagnostics and treatment is usage of a variety of nanoparticles. These particles are routinely injected into blood stream and eventually accumulate in tumors, if properly labelled. However, almost no attention is paid to the fates of the particles in the blood and their interaction with blood constituents, in particular, with red blood cells. During this STSM, experimental research to elucidate peculiarities of interaction between red blood cells and titanium dioxide ($TiO_2$) nanoparticles was performed. Such particles show promise for cancer treatment, especially when used in combination with anticancer drugs such as doxorubicin.

An in house-made holographic optical tweezers setup based on the inverted microscope was used to measure red blood cell interaction (Fig. 1). Multiple optical traps formed using a laser beam from a Nd:YAG laser are focused with a large numerical aperture oil immersion objective. The positions of the traps are controlled independently within the focal plane of the objective. Visual control of the trapped objects is done in a transmission configuration using a CCD camera. The fluorescence excitation and registration is possible using a mercury lamp and appropriate filters.

The advantage of using optical tweezers as a measurement tool is its capability to measure red blood cells interaction forces with the sub-pN accuracy when they are trapped by laser beams. This allows us to avoid direct contact with the cells, thus reducing probability of their damage and contamination and, as a result, preventing change of their shape due to change in pH, residual chemicals etc.

Before the actual experiments, the optical tweezers were calibrated by introduction of shear stress to the trapped red blood cells and finding the moment when the cell escapes from the trap. At this point the viscous drag force equals the trapping force.

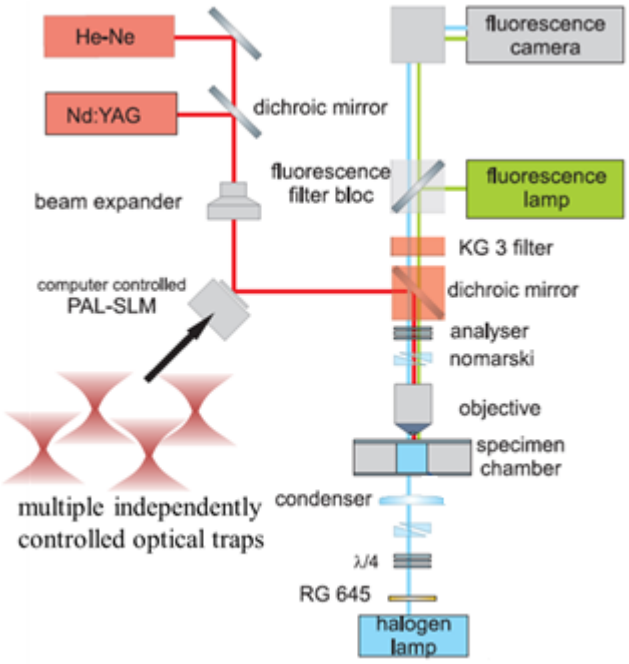
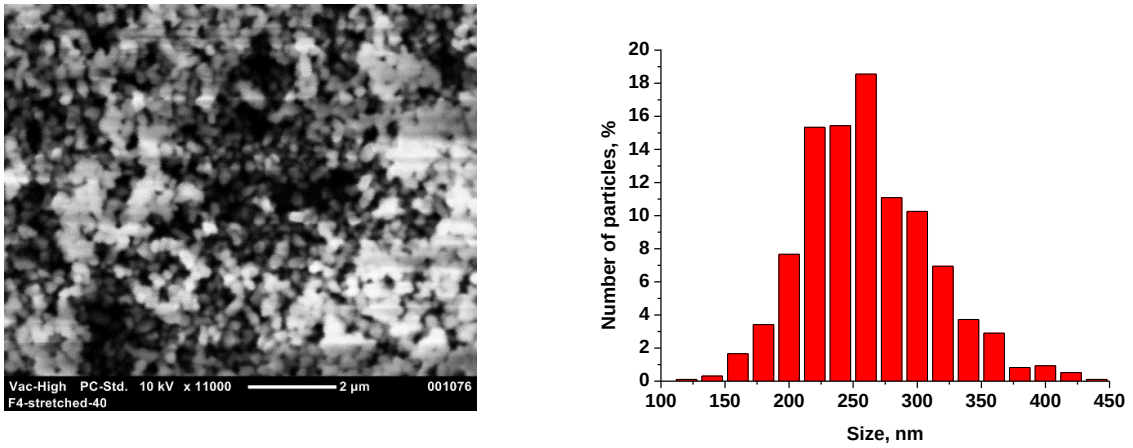



**Fig. 1.** Schematic layout of the holographic optical tweezers setup for measuring the red blood cells interaction force. The HeNe laser is used for adjustment.

Autologous blood plasma used in the experiments, was obtained from blood samples by centrifugation. Red blood cells were incubated with 0.01% $TiO_2$ nanoparticles (Kemira, Finland) suspended in phosphate buffer saline (PBS) for 3h. Non-incubated red blood cells were used as a control. An electronics micrograph and the corresponding size distribution of the particles are depicted in Fig. 2.




(a) (b)

**Fig. 2.** SEM photographs of $TiO_2$ nanoparticles (a) and the corresponding size distribution (b).

Aggregation forces between multiple pairs of red blood cells incubated with $TiO_2$ particles were measured using blood from two healthy donors. We found that the forces were substantially (more than 3 times) increased compare to the control, for the both donors. The difference was statistically significant (Fig. 3).

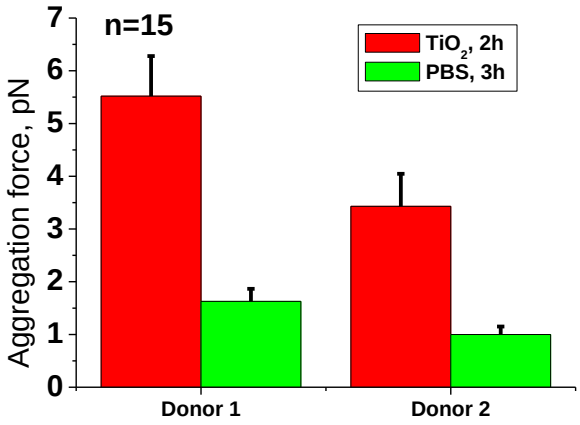
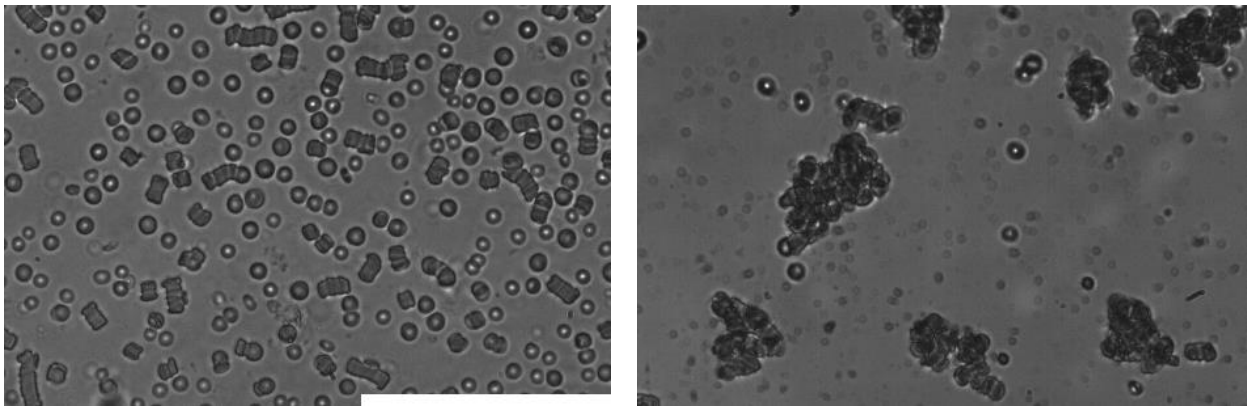



**Fig. 3.** Measured aggregation forces between pairs of red blood cells incubated with $TiO_2$ particles for 2 h and in control (incubated in phosphate buffer saline, PBS).

As an illustration, optical microscopy images were captured by a conventional microscope (Fig. 4). They indicate a drastic difference between aggregation patterns of the red blood cells. The cells in control formed coin-like aggregates, so called 'rouleaux' (Fig. 4a), while the $TiO_2$-treated cells formed ugly agglomerates of much larger sizes (Fig. 4b). This confirms the results obtained with the trapping experiments and gives logical explanation to the measured increase in interaction forces.



(a) (b)

**Fig. 4.** Optical microscopy images of red blood cells incubated in phosphate buffer saline (PBS) and with $TiO_2$ nanoparticles for 2 h. Scale bar: 100 μm.

In addition, during the research stay, the applicant gave a talk at the laboratory seminar about the scientific activities of the Finnish laboratory in the area of biophotonics and nanotechnology.

Blood was attracted particular attention due to possibility for studying it at the level of single cells (by optical tweezers), ensemble level (by Doppler optical coherence tomography in biotissue-mimicking phantoms) and tissue level (by hyperspectral imaging), thus using complementary expertise of the both partner laboratories.

We discussed collaboration in frames of Erasmus+ student and staff exchange program; invitation of Prof. Wagner and Mr. Francois Yaya to the University of Oulu for teaching a short course for doctoral students and for a short-term research, respectively.

**Description of the main results obtained**

Absolute values of aggregation forces between multiple pairs of red blood cells incubated with $TiO_2$ nanoparticles were measured. Compared to the control, the forces increased significantly - 3.4 times. This indicates the increased aggregation ability of the nanoparticles and can be used for setting concentration limits for drug testing. On the others side, this proved the usability of the novel optical tool – optical tweezers - for assessment of altered interaction between red blood cells.

**Mutual benefits for the Home and Host institutions**

Besides the research results, possible bilateral and multilateral project calls were identified – H2020 and Academy of Finland and DFG. Student and staff exchange will also be a promising option, including joint Master's and Doctoral degrees programs. Master's program in Biophotonics being developed in the University of Oulu (Finland) will serve as a solid basis.

**Future collaboration with the Host institution (if applicable)**

The Host (University of Saarbrücken) might host researchers from the Finnish laboratory for short-term visits. The effect of the same nanoparticles will be measured on different blood samples both in Finland and Germany, thus providing evidence of the repeatability of the results. In the future, the research can result in setting standards for toxicological assessment of innovative nanotechnological products for cancer treatment.

Multiple meetings at International conferences will be held: in particular, Optics and Photonics Days conference in Finland (29-31 May, 2017) is the first event of this kind: http://www.photonics.fi/opd2017

**Foreseen journal publications or conference presentations expected to result from the STSM (if applicable)**

Depending on the experimental results, we consider different options ranging from conference proceedings to journal articles in the area of biophotonics and nanotechnology (e.g. Journal of Biophotonics, Journal of Biomedical Optics, Biomedical Optics Express, Physics in Medicine and Biology, Small, Nano Letters etc.).

**STSM outcome form**

| STSM application number | Home institution & country | Host institution & country | BM1205 WG | Objective of the collaboration | Results of the collaboration |
|---|---|---|---|---|---|
| COST-STSM-ECOST-STSM-BM1205-100117-080235 | Optoelectronics and Measurement Techniques Laboratory, Faculty of Information Technology and Electrical Engineering, University of Oulu, 90014, Finland | Laboratory "Dynamic of Fluids", Institute of Experimental Physics, Campus E26, University of Saarbrucken, 66123 Saarbrücken, Germany | WG4 | Blood diagnostics at cellular level using laser tweezers | We were able to measure the changes of aggregation forces between red blood cells in presence of nanoparticles potentially applicable for design of anti-cancer drugs. |

I acknowledge that the described short-term scientific mission has been successfully carried out in the conditions here specified, and prospects for future collaboration are clearly visible.

Saarbrücken, Germany, 25 January 2017

Professor Christian Wagner

Host

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