Final report on the project NN114692

The grant no. NN114692 was supporting an international cooperative work financed by the ERA-NET and the Concert Japan programs; its title was "FEmtosecond laser Advanced manufacturing for Ship-In-a-Bottle Labon-chips Enrichment" or FEASIBLE. In the consortium the other two members were a Japanese research group (Dr. Koji Sugioka, RIKEN Center for Advanced Photonics – Wako) and an Italian one (Dr. Rebeca Martinez Vazquez, Institute for Photonics and Nanotechnologies, National Research Council – Milan). The goal of the project was the integration of femtosecond laser-based additive and subtractive microfabrication technologies into a single fabrication process to create advanced microfluidic Lab-On-a-Chip (LOC) systems. The role of the Japanese and Italian members was to develop the subtractive technologies and to create task-specific LOC systems with them. Our main task was to develop and fabricate polymer microstructures with additive technologies to be used in the LOC systems primarily in biological applications. The ultimate goal of the project was of course, the combination of the two methodologies.

The femtosecond laser-based additive technology in our case meant the technique of two-photon polymerization (TPP). Shortly, with this technique we create complex 3D microstructures out of photo-curable polymer material with a highly focused laser beam of near-infrared wavelength. The most important feature of the technique is that one can create arbitrarily-shaped structures with it having details of about 200nm, and the structures can also be tailored to the research task in hand. Our task in the project can be divided into two sub-tasks: in the first, we had to improve the quality of the fabricated structures through the optimization of the polymerizing beam, and to fabricate them in a parallel manner with multiplied beams; in the second we had to add extra functions to the structures through the modification of their surface in order for them to use as sensors or to interact with biological matter. It is imperative to note that the first task is based on the application of an active optical element in the process of TPP, a Spatial Light Modulator or SLM. The SLM can modify the phase of the polymerizing laser beam and therefore it can, for instance multiply the number of focal spots in the sample at will.

During the project we developed a method to correct for the beam distortion that arises during TPP when the polymerizing beam is focused into a thick layer of photoresist. In order to successfully integrate the polymer microstructures into the LOC systems, we often had to illuminate the sample through a thick photoresist layer that fills up the microfluidic channel. In such cases because of the large focusing angle of the beam the position of the focal spot shifts relative to the desired position and its shape distorts. We corrected for the shift and the spherical aberration-type distortion using the SLM. The correction was carried out by pre-compensating the phase-front distortion, characteristic to the spherical aberration, with the SLM; this means that we reflected the beam off the surface of the SLM during which it modified the phase of the beam to be the negative of that of the aberration. With this method we could decrease the focal shift from 10% to 2% (Figure 1(a)) and to practically eliminate the focal spot distortion (Figure 1(b)-(c)). This work was published in 2017 in the journal Micromachines.

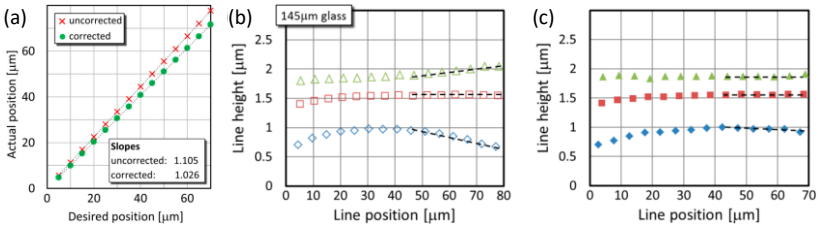

Figure 1: Improvement of focal position shift (a) and correction of focal shape distortion during polymerization through thick photoresist layer (b, c).

Using the TPP correction described above we developed Whispering Gallery mode detectors that can be inserted into LOC systems. The detectors are consisting of a light guide and of a disk-shaped resonator positioned close to each other. Because of their small (<500nm) distance, the light that is driven into the light guide couples into the resonator at well-defined wavelengths (for practical reasons around 1550nm) and a smaller intensity is measured at the other end of the light guide. When an analyte binds to the surface of the resonator it changes its effective refractive index that results in the shift of the coupling wavelengths that can be easily detected. The

quality of the device can be characterized with the Q-factor (that is the ratio of the operating wavelength and the bandwidth of the coupling band; $\lambda/\Delta\lambda$), the useful value of which lies in the range of 10^3-10^5. During our work we could increase the Q-factor with the enlargement of the diameter of the resonator to 65μm (presently it is a technical limit), with the decrease of the light guide and resonator to zero, and with the modification of their relative position, which means a single point of attachment as shown in the insert of Figure 2(a); presently our highest achieved Q-factor is $2x10^4$ (Figure 2(a)), which means that device can be efficiently applied in an experiment.

In the next step the optimized-shaped resonators were polymerized inside a microfluidic chip made by our Italian partner. The chip consisted of a microfluidic channel for liquid and two channels perpendicular to it to insert an optical fiber into each; these optical fibers are used to couple the measuring light into the light guide part of the polymerized detector that is inside the microfluidic channel (see the arrangement in Figure 2(b)). The positioning of the detector inside the channel was crucial since the two ends of its light guide must be aligned precisely in 3D to the centers of the adjacent fiber channels for efficient light coupling in and out (Figure 2(b)). According to the preliminary measurements of our Italian partner the positioning was correct and the light could be coupled in and out of the light guide and the typical coupling spectrum was observable. Although the Q-factor was smaller than what is expected, the shift of the coupling bands was evident when the resonator was immersed into media of different refractive index (Figure 2(c)), so the concept proved to be viable. As the last step of this project, we worked on the functionalization of the surface of the resonators which enables them to specifically detect a selected protein in the fluid around it. This concept was checked by coating the resonators with biotin molecule which is a ligand of the protein streptavidin, and which can bind to this protein strongly and specifically; we applied this approach and procedure in earlier published works. Preliminary results indicate the protein binding to the resonator but it must be confirmed with further experiments; we are currently working in the direction of increasing the protein binding efficiency.

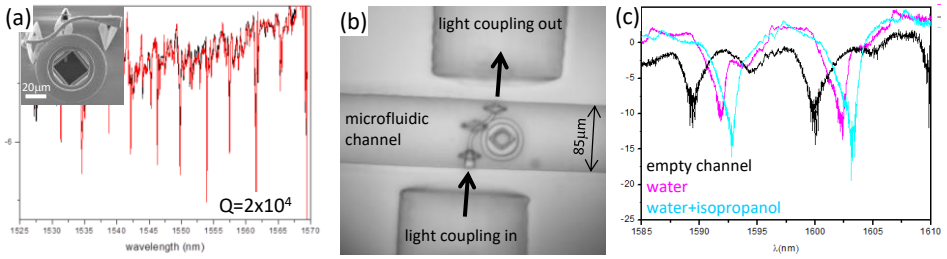

Figure 2: Coupling spectrum of a WG resonator with optimized shape (a); insert: top-view SEM image of the resonator. WG resonator fabricated inside a microfluidic channel (b). Primary results showing the shift of the coupling wavelength when the WG is surrounded with liquid of different refractive indices (c).

We cooperate with the Japanese member of the consortium in the field of cell migration exploiting the possibilities what a polymer microstructure-enhanced LOC system can provide; again, the microfluidic chip was fabricated by our partner and we polymerized its functional micro parts inside the channels. This project studies how cancer cells can transmigrate very narrow physical enclosures; the conclusions can provide further information on the metastasis of the cancer cells. The Japanese group prepared microfluidic chips that consist of 4 reservoirs connected with channels of about 140x60μm cross section (Figure 3(a)). The lower 2 reservoirs are used to infuse the system with cells, while the upper two to add various chemicals, such as chemo-attractant to the system. These two regions were separated by microchannels and a separating wall that we fabricated with TPP (insert in Figure 3(a)). The microchannels had an opening of less than 1μm width; the two regions could only communicate through them, meaning that the only way chemicals and the cells could get to the other region is through the channels. When cells are cultivated in the first, lower region, and chemo-attractant is added to the other one, the cells are provoked to migrate through the channel to the upper region. However, in order to accomplish this, the cells must change their shape considerably due to the very narrow opening; this observation of this change was the very motivation of our joint research. Our share of the cooperative work was to develop a method to

fabricate the extra narrow channels and a substantially stable separation wall. Eventually we managed to prepare channels of 0.75μm width, 2.5μm height and 6-21μm length (Figure 3(b)), surrounded by a tall separation wall of ~3μm thickness in between the two regions inside the LOC system. With fluorescent staining we verified that the two regions can only exchange liquid through the channels, and that the concentration of the chemo-attractant is nearly unchanged for several hours. The main finding of the experiments conducted with live cells was that they can only migrate through the channels with considerable change of shape or even with transient disintegration of cell organelles (Figure 3(c)). This micro device is the first of its kind that was fabricated to study migration of live cells through physical openings narrower than 1μm. It is unique also in terms of using the hybrid subtractive-additive technology for this purpose. A manuscript describing the fabrication technique and its application in cell research is under preparation.

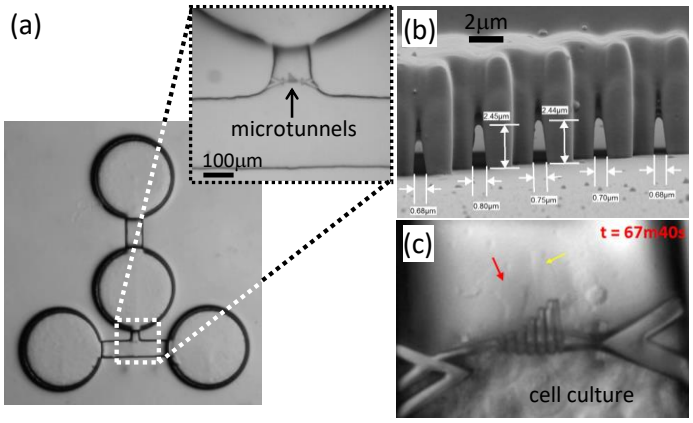

Figure 3: LOC chip to study cell migration (a); insert: localization of the microchannels in between the two functional regions. SEM image of ~0.75μm wide channels (b). Snapshot of a cell migration experiment; the two arrows are pointing to two cells already on the other side (c).

Another important result of the project is the parallel preparation of TPP microstructures with multiplied laser focal spots to be used in microfluidic channels. This preparation method significantly increases the number of polymerized structures in a given amount of time. It requires the use of an SLM, similarly to the focal spot correction, but now this device is used to multiply the single original laser beam and create the focal spots at pre-defined positions in the sample. The number and arrangement of the focal spot are determined by the available laser power and the size of the structures to be prepared. We used this technique to make two kinds of structures as described below.

The first structure was made as a mobile detector unit for surface-enhanced Raman spectroscopy (SERS) and was actuated with an optical tweezers setup in a microfluidic channel. This work was in cooperation with the Slovakian group of dr. Pavol Miskovski in Kosice at the P. J. Safarik University, who provided the microfluidic channel, the Raman setup and the optical tweezers. In our group, we designed and prepared a linear tool that has three spherical parts, two of which is for the optical trapping and the third probe part was for the detection (Figure 4(a)). The key step in the preparation was the coating of the third spheroid with 80nm silver nanoparticles (NP) which, being plasmonic particles, enhance the impinging Raman excitation field and thereby increase the detectable Raman spectra of the analyte with several orders of magnitude. The microtools were made with a quadrupled focal spot arrangement, where the four structures were made side-by-side at the same time for efficient silver NP coating. The polymer structures, still on the substrate were immersed in a silver nitrate solution, and their probe parts were illuminated with a line-shaped laser focal spot formed by a cylindrical lens. The green 532nm light reduced the silver ions into silver atoms that formed the NPs on the surface of the

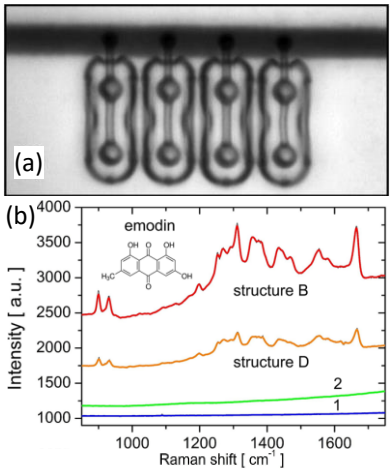

Figure 4: Optical microscopic image of the polymer microtools made with parallel TPP and coated partially with silver NPs (a). SERS spectra of emodin measured with two kinds of structures (red and orange plots) and without the structures (green and blue) (b).

polymer structures. The applicability of these structures in SERS measurements were demonstrated by detecting micromolar concentration of emodin, an anti-cancer drug in a microfluidic channel (Figure 4(b)). It was also shown that these structures can be effectively actuated by the optical tweezers. These results were published in 2015 in the journal Langmuir.

The second kind of structure that we developed during the project and prepared with parallel TPP is to be used in single cell studies. Specifically, the structure was designed to enhance the optical manipulation of single cells. Optical tweezers has been long used for direct cell manipulation where the laser focus is trapping the cell itself. However, complex polymer tools acting as intermediate objects between the traps and the cells can provide more stable trapping, precise positioning and can eliminate photodamage often associated with live cell trapping. We polymerized and functionalized special-shaped microtools (Figure 5(a)) that have three spherical parts that can be trapped with the optical tweezers and a disk-shaped one to which the cell can attach (Figure 5(b)). We demonstrated that the cells can be freely manipulated with this indirect trapping method with 6 degrees of freedom; they can be held stably with ~125nm fluctuation (Figure 5(c)) and translated with tens of μm/s speed. The cell attachment was enabled with the surface functionalization of the structures with the protein streptavidin and the cells were biotinated; the strong streptavidin-biotin binding resulted in fast (<5s) attachment of the cells. Our results demonstrating cell maneuverability were published in 2016 in Biomedical Optics Express and in the conference proceeding of SPIE.

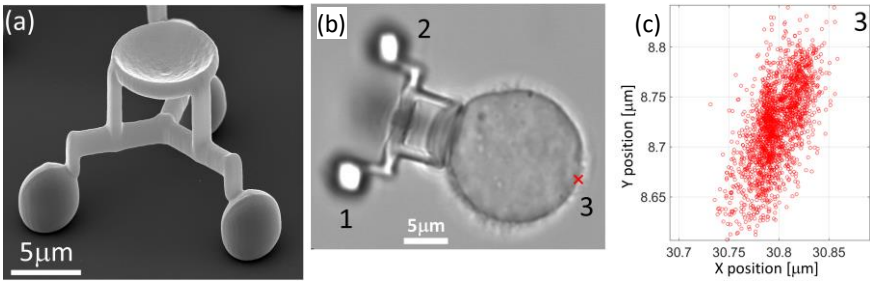

Figure 5: SEM image of the polymer cell manipulator structure (a). Optical microscopic image of a cell attached to a manipulator structure as held by the optical tweezers (b). Fluctuation of point 3 on panel (b) showing the positional stability of the trapped cell (c).

Another outcome of the three-side cooperation will be a review paper written by each consortium member and entitled “Three-dimensional femtosecond laser processing for lab-on-a-chip applications”; it was submitted to the journal Nanophotonics for publication. This paper introduces the schemes of the undeformative, subtractive, and additive 3D femtosecond laser processes of transparent materials such as glasses and photoresists for the fabrication of functional LOCs and presents the newest applications of these techniques.

Deviation from the original schedules of the project:

The original closing date of the project was September 30, 2016, which was modified to September 30, 2017 with an approval from NKFIH.

Personnel joining the project:

Sándor Valkai and Zsófia Hoyk, researchers joined the project and were partly paid from it; their joining was approved.

András Buzás, researcher and Bence Horváth, student also joined the project with an approval; they were not paid from its budget.

Larger deviation from the original budget:

In June, 2017 2.24M HUF was directed from the line 3.2 Consumables to lines 1.7 (Per diem), 3.1 (Travel, conferences) and 3.3 (Miscellaneous costs) with approval. It was needed to cover the travel expenses of Lorand Kelemen and Pal Ormos to a project meeting to Japan and to international conferences, as well as other costs such as express mails and instrument use, maintenance and repair. It did not affect the success of the project.