How to integrate a micropipette into a closed microfluidic system: absorption spectra of an optically trapped erythrocyte

Ahmed Alrifaiy1,2,* and Kerstin Ramser1,2

1Department of Computer Science, Electrical and Space engineering, Luleå University of Technology, SE-971 87 Luleå, Sweden
2Centre for Biomedical Engineering and Physics, Luleå University of Technology and Umeå University, Luleå and Umeå, Sweden

*ahmed.alrifaiy@ltu.se

Abstract: We present a new concept of integrating a micropipette within a closed microfluidic system equipped with optical tweezers and a UV-Vis spectrometer. A single red blood cell (RBC) was optically trapped and steered in three dimensions towards a micropipette that was integrated in the microfluidic system. Different oxygenation states of the RBC, triggered by altering the oxygen content in the microchannels through a pump system, were optically monitored by a UV-Vis spectrometer. The built setup is aimed to act as a multifunctional system where the biochemical content and the electrophysiological reaction of a single cell can be monitored simultaneously. The system can be used for other applications like single cell sorting, in vitro fertilization or electrophysiological experiments with precise environmental control of the gas-, and chemical content.

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OCIS codes: (350.4855) Optical tweezers or optical manipulation; (170.3880) Medical and biological imaging; (300.1030) Absorption; (280.2490) Flow diagnostics; (220.4000) Microstructure fabrication; (110.0180) Microscopy.

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1. Introduction

The development of new approaches to investigate mechanisms in living cells under in vivo like conditions is of highest importance. Desirably, measurements should be carried out under well-controlled physiological conditions, i.e., different oxygen levels and addition/removal of biochemical substances or nutrients. Many single cell studies are hitherto carried out on microscopes where they are studied by optical techniques such as optical spectroscopy, patch-clamp-, and time resolved techniques. Recently, much attention has been paid to functional systems that include optical tweezers [1] and microfluidic system [2]. These systems have released innovative approaches for basic and applied research for diverse biological studies of single cells [3].

Optical tweezers, an increasingly important tool in biophysics and cell biology, utilize light to trap and manipulate small particle in three dimensions. In a typical setup of optical tweezers, a stable trap is achieved by a strongly focused laser beam through a high-numerical aperture (NA) microscope objective. The phenomenon of using the momentum of light to manipulate particles was first experimentally demonstrated on atoms [4]. Shortly thereafter biological cells were manipulated using infrared lasers [5]. The optical tweezers are widely used in many applications like cell transport and separation [6], manipulation of biological cells [7], sample cell analysis by mass spectrometry [8], DNA analysis [9] and other applications for clinical diagnostics [10].

Microfluidic systems typically consist of a structure of channels with diameters ranging between 10 and 1000 µm with a µl/s flow of solvents. They can easily be designed individually for each experiment and have proven to give unsurpassed control over the flow and thus enable fast environmental changes [11,12]. One benefit of the systems is that they can be made of transparent materials such as rubber silica, Plexiglas or glass and as a consequence they can easily be implemented onto microscopes to be combined with suitable read-out techniques. Especially Plexiglas PMMA (Poly-methyl methacrylate) microchips have gained popularity in various biological and medical applications [13]. They are less expensive than glass microchips [14] and the complex development process of the lithographical
microchips can be avoided [15]. The main advantage of using PMMA based microfluidic chamber is the impermeability to air, gases and other properties like low toxicity, optical transparency, thermal stability and they are easy to manufacture [16]. They have been applied to produce micro-mixers [17], polymerase chain reaction microchips (PCR) [18], microfluidic reactors [19] and capillary electrophoresis microchips (CE) [20].

Recently, many new concepts of nanofluidic delivery platforms, including integrated nanofluidic probes, have been developed. For instance, the so called Nanofountain probe (NFP) [21] is a new promising technique based on Dip-Pen Nanolithography (DPN). These nanofluidic probes consist of nano-channels which are used for direct nanoliter-delivery of specific bioactive solutions through a conical tip with a nano-pore attached with sub-cellular precision to a single cell while the cellular response can be imaged simultaneously. However, the impact of the nano-probes breaking the membrane of the cell, the biocompatibility of the nanomaterials, and the impact of bio-electronic integration need further investigations before the technique can be applied for electrophysiological studies [22]. Despite of the promising prospect of the NFPs, the patch clamp technique combined with microfluidic microchips that incorporate microfluidic channels, micro pumps and micro valves are well established alternatives that can be employed directly.

The so called lab-on-a-chips facilitate a variety of biological applications. The significant benefits are that minimal amount of reagents and analytes are used and that they can function as portable clinical diagnostic devices, i.e. time-consuming laboratory analysis procedures can be reduced. The great impact of these systems is shown in studies of manipulated biological cells in environment-controlled analytical systems [23,24].

Despite of all the progresses that have been achieved by combining optical tweezers with microfluidic systems one great challenge has, to our knowledge, not been conquered yet. How can a micro-pipette be integrated within a closed movable system? In many applications such as cell sorting, in-vitro fertilization or patch-clamp experiments the biological cell has to be brought in close contact to a micro-pipette. Usually a precise and electronically steered 3D manipulation device is moved towards the cell under an open space on the microscope. Hence the possibility of applying closed microfluidic technique is unfeasible. Here we demonstrate a new concept. By integrating optical tweezers, the cell can be steered in 3D towards the micropipette that is integrated in the microfluidic system.

The fabrication of the microfluidic chamber presented here bases on PMMA, and is performed by a CNC (Computer Numerical Control) Circuit Board milling machine. A patch clamp micropipette was included within the gastight microfluidic chamber. To prove the principle, a single RBC was optically trapped and manipulated within the microchannels towards the integrated micropipette. The UV-Vis absorption spectra in the oxy and deoxy-state of the RBC were acquired under controlled conditions created by the micro flow. The experimental work presents a microfluidic chamber with an integrated micropipette combined with optical tweezers and optical spectroscopy that acts as multifunctional system for various biomedical and electrophysiological applications with complete environmental control.

2. Material and Methods

The main parts of the experimental setup, as seen in Fig. 1, are mounted on an inverted optical microscope (IX 71, Olympus, Japan) placed on a vibration-isolated table (Technical Manufacturing Corporation, TMC, USA). The techniques used in this work are presented below.

Preparation of RBC and Solutions

Fresh RBCs were prepared from blood taken from a healthy volunteer. 0.05 ml blood was diluted in 2 ml solution of Phosphate-Buffered Saline (PBS), pH 7.4, temperature 23°C. The oxygen-free solution was prepared by 20mg Natriumdithionit, Na₂O₄S₂ (Sigma-Aldrich, #147965 - $15.00 USD Received 23 May 2011; revised 30 Jun 2011; accepted 14 Jul 2011; published 20 Jul 2011 (C) 2011 OSA 1 August 2011 / Vol. 2, No. 8 / BIOMEDICAL OPTICS EXPRESS 2301
USA), dissolved in 4ml (PBS) solution. The solutions were prepared in Petri dishes and sucked into the gastight syringes of the pump system.

**Optical Trapping of Single RBC**

The optical tweezers build upon an IR-diode laser (IQ1A, Power Technology, USA), operating at 808nm with an average power of 200 mW. The wavelength of the laser was chosen to minimize heating and photodamage of the sample [25]. The laser beam was first expanded by two convex positive lenses of 50 and 250 mm, mounted on two XYZ-translation stages (Thorlabs, USA), and steered by mirrors (Thorlabs, USA) into the microscope. The laser beam was then guided to the objective throughout a dichroic mirror (750-dcspxr, Chroma Technology, USA). The expanded beam overfilled the back focal plane of the oil immersion objective (100x, 1.4 NA, Olympus, Japan) to obtain good trap stiffness. The trap stiffness was estimated qualitatively. Visual observations showed a strong and stable trap while the optically trapped red blood cells were exposed to a high flow rate of fluid (up to 20µl/s) through the channels and while imposing a force on the trapped cell by moving the stage.

The positioning of the optically trapped red blood cell was enabled by keeping the trapped cell still while moving the microfluidic chip including the micropipette mounted on the nanometer-precision XYZ translation stage of the microscope. This allowed the microfluidic chamber including the micropipette to move in micro-precision in 3 dimensions while the position of the optical trap was fixed and always positioned in the center of the field of view.

![Inverted microscope diagram](image)

**Fig. 1.** Inverted microscope that incorporates the following techniques: Gastight lab-on-a-chip with an integrated micropipette coupled to a pump system, optical tweezers for 3D steering of the single cells comprising of an IR laser, a beam expander, mirrors and a dichroic mirror and an IR blocking filter to block the IR laser. UV-Vis spectrometer with an integrated optical fiber to record the oxygenation states of the RBC, CCD camera to monitor the trapping dynamics of the cells within the micro-channel system.
Optical Spectroscopy

The oxygenation state of a single optically trapped RBC was monitored by acquiring the absorption spectra by an UV-Vis spectrometer (Ocean Optics, HR4000, USA) in the following way: The visible light from the halogen lamp of the microscope illuminated the sample. The transmitted light that had passed through the sample was collected by the microscope objective and guided through the dichroic mirror. Thereafter it was divided by a beam-splitter to the CCD (20%) and to the optical fiber (80%) situated outside the right-hand side-port of the microscope. The optical fiber was connected to the UV-Vis spectrometer. The optical fiber, with a core size of diameter of 50 µm, was mounted on a 3D translation stage (Thorlabs USA). It was precisely aligned by moving the translation stage in the XYZ directions to guide the transmitted light from the microscope objective to the center of the fiber. The optical fiber facilitated the alignment of the optical path considerably and enabled the monitoring of the absorption spectrum of a single red blood cell.

To test the alignment of the spectrometer, a RBC within the microchannel was steered to the center of the field of view, Fig. 2(B) position 1, and an absorption spectrum was taken, Fig. 2(A). The spectrum shows the typical oxygenated state of the RBC with two maxima at 542 nm and 578 nm [26]. Thereafter, the center of the field of view of the microscope was positioned in a “RBC-free” environment, Fig. 2(B) position 2. A second absorption spectrum was taken to prove that no signals from RBCs were present, Fig. 2(C). The absorption spectra shown in this paper were binomially fitted by data-analyzing software program (Igor Pro).

![Image](https://example.com/image.png)

Fig. 2. (A) UV-Vis absorption oxygenation spectrum of the single RBC, (B) The center of the fiber was moved from position 1 (single RBC) to position 2 (cell-free zone), (C) UV-Vis absorption spectrum of the cell-free zone.

Microfluidic System

The manufacturing process of the closed microfluidic chamber was performed by a CNC Circuit Board milling Machine. The desired T-shaped microchannel were initially designed by a software program (QCAD) and transferred onto a slab of PMMA by the CNC machine. The channels were then sealed between two cover slips by an UV-curable adhesive material with low viscosity and high optical transparency (EPO-TEK OG603, Epoxy Technology, USA). It classified to USP Class VI biocompatibility standards that meet the requirements for medical application. The microfluidic chamber was equipped with two inlets and one outlet adjacent to the microchannels and connected by gas-tight PEEK tubing (ScanTec, Sweden) to the pump system (neMESYS, Cetoni, Germany). The pump system was used for the infusion of cells and solutions with varying oxygen content.

The procedure of integrating the patch clamp micropipette started by CNC drilling of a hole that extended at 45 degrees from the upper edge of the microfluidic channel to the intersection zone within the T-shape microfluidic channel. The upper edge of the hole was threaded to fit a fine hollow screw (JR-5508-5, VICI Jour, Switzerland). The micropipette was inserted through the hollow screw and the drilled hole into the microfluidic channel, while the tip of the pipette was monitored visually in the microfluidic channel. To ensure an airtight seal around the drilled hole, the diameter of the hole was fitted precisely to the outer diameter of the micropipette. Additionally, a gas-tight fitting (PEEK, JR-55003-5, VICI Jour,
Switzerland) was used to make an airtight seal between the hollow screw and the hole. The hollow screw was used to fine-position the tip of the micropipette to the RBC in the microchannel, as seen in Fig. 3(A) and 3(B).

![Diagram](image)

**Fig. 3.** (A) Schematic of the gastight microfluidic chamber including the patch clamp micropipette and inlets to be connected to a pump system. (B) The micropipette was fitted within the microfluidic chamber in a gastight surrounding.

3. Experimental Results and Discussions

The closed microfluidic chamber with the integrated micropipette was placed on the microscope stage and connected the pump system for latter insertion of RBCs and for the variation of the oxygenation state of the solution. The micropipette was carefully inserted through the hollow screw into the microfluidic chamber while the tip of the pipette entering the intersection-zone within the T-shape microfluidic channel was monitored visually. The cells were inserted to the microfluidic channel by the pump system in a low flow rate of 1 µl/s. By the optical tweezers, a single RBC within the microchannel was optically trapped and steered through the microfluidic channel to the tip of the pipette. Simultaneously the trapping dynamics were recorded in real time with the CCD camera, see Fig. 4. The micropipette was then precisely adjusted by the hollow screw to attach to the membrane of the trapped cell.

The absorption spectra of the trapped cell were then acquired and monitored by the UV-Vis spectrometer under environmental variations by the pump system. Three absorption spectra of the trapped RBC in the oxy-, deoxy-, and oxy states were acquired as shown in Fig. 5.
At the beginning of the experiment (t = 0), the absorption spectrum was acquired in the oxygenated state, Fig. 5(A). The spectrum showed peaks at 540 nm and 576 nm, which is typical for the oxy-spectrum of red blood cells [21]. The trapped RBC was then deoxygenated by 1 minute exposure to the oxygen-free solution. After 59s, the absorption spectrum of the RBC was gradually transformed to the deoxygenated state, showing the typical peak at 560nm [21], see Fig. 5(B). The spectrum was transformed back to the oxygenated state after 21 minutes, Fig. 5(C).

Fig. 4. (A) Single optically trapped RBC, here the micropipette is situated above the RBC, (B) RBC in the same optical plane as the micropipette. (B) The RBC in contact with the patch clamp micropipette.

Fig. 5. Absorption spectra of the trapped RBC in contact with micropipette in the microfluidic system in (A) oxygenated state (B) deoxygenated state and (C) re-oxygenated state.

The microfluidic chamber was designed to perform electrophysiological investigations of single cells with reliable control of the environment. The presented microfluidic chamber can be modified for various electrophysiological techniques. Using CNC circuit board machines to design and create the microfluidic channel on various materials is beneficial due to the low cost and the high efficiency.

A number of improvements will be carried out on the presented prototype. The microfluidic channels will be coated with UV curable epoxy to create an optical transparency similar to glass microchips. The fine screw that is used to integrate the micropipette will be replaced with a build-in tiny 3D micromanipulator. The process of transporting the cell through the pump system to the microfluidic chamber will be replaced by a new design where the cell will be prepared directly in the microfluidic chamber. The cells will be transported to the microchannel by applying a negative pressure through developed micro valves. This is to minimize the stress that the cells experience during transport through long tubing and to ensure that the cells will not stick onto the inner wall of the tubing.

4. Conclusions

Hereby we have presented a new way of integrating a micro-pipette into a closed microfluidic system with an integrated micro-pipette. The closed lab-on-a-chip offers the possibility to analyze individual cells under environmental control with a minimum of oxygen-diffusion into the microchannels during the measurements. As a proof of principle, the oxygenation sequence of a single RBC was studied at the tip of the integrated patch clamp micropipette.

The microfluidic chamber was designed to act as a multifunctional system for simultaneous and high-throughput experiments. The future aim is to perform patch clamp experiments for electrophysiological investigations while simultaneously monitoring the biochemical composition of the sample by optical spectroscopy. The simplicity and stability of the microfluidic system has excellent potential to enable high volume production of
scalable microchips for various biomedical applications. The subsequently ambition is to use this system as a mini laboratory that has benefits in cell sorting, pharmaceutical, patch clamp, and fertilization experiments where the gaseous and the biochemical content is of importance.

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