Preliminary Studies on Flow Assisted Propagation of Fluorescent Microbeads in Microfluidic Channels for Molecular Communication Systems

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Abstract. High throughput microfluidic devices coupled with optical detection systems bring several advantages to study molecular communication (MC) by mimicking capillary vessels and arterioles. Motivated by this, we present an MC platform using fluorescence polystyrene (PS) beads as messenger molecules to transfer encoded information in microfluidic channels via flow induced diffusion. To this end, we couple multiple production and analysis techniques to construct and characterize our micro scale MC system. PS microbeads are introduced into microchannels via programmable syringe pumps serving as transmitters, while the received signal is recorded by inverted fluorescence microscope. Time lapsed images of microparticles are presented as they move across diffusion channels.

Keywords: Nanonetworking · Microfluidic channel · Flow assisted propagation

1 Introduction

Molecular communication (MC) based systems use chemical signals, molecules, or colloidal particles to transmit and receive information between devices. In case the chosen device scale is in nanometric range, they are called nanomachines which have tremendous capabilities that make them potential candidates for many different applications, such as catalysis, sensing, electronics, magnetics, photonics, and biomedicine.

Capabilities of the nanomachines can be further extended in case these tiny machines possess a communication link in between helping them to communicate and cooperate to perform complex tasks. Yet, to be able to build such a nanonetwork, one must imagine

Y. Chen et al. (Eds.): BICT 2020, LNICST 329, pp. 294–302, 2020.
https://doi.org/10.1007/978-3-030-57115-3_26

outside the box from conventional communication systems that are restricted in the nano scale. Inspired by the nature, molecular communication, already being used in unicellular or multicellular organisms, is a very good alternative to such problems.

In MC networks, encoded information is transmitted from the first nanomachine/transmitter through a diffusion channel to the second nanomachine/receiver (Fig. 1). When the propagating signal is received, the encoded information is decoded by the receiver in the form of received signal. The encoded information may be in the form of concentration, number of molecules/particle [1, 2], absorption, or fluorescence intensity, etc.

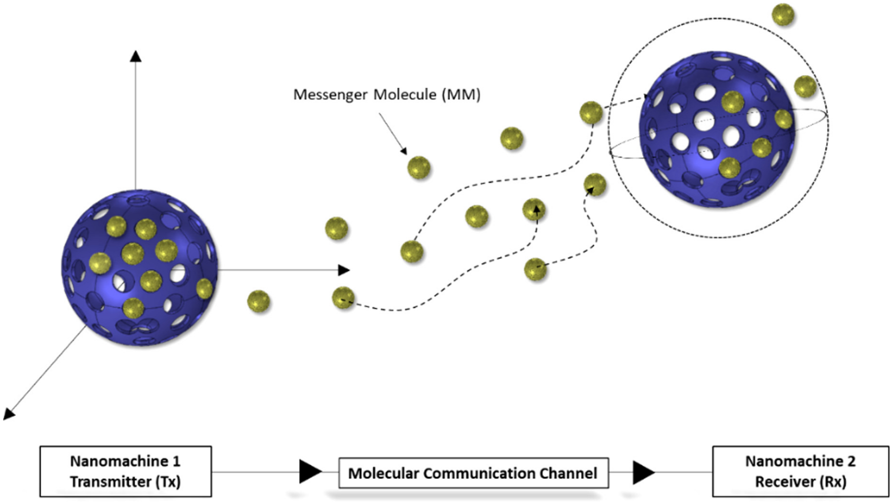

Fig. 1. A schematic representation of molecular communication.

Since most of the existing studies on micro/nano scale MC are mainly theoretical and generally assuming mathematical channel models to work on modulation problems, concrete experimental data is needed besides simulations to verify the proposed ideas [3, 4]. Microfluidics is a field that studies and manipulate small amount of fluids in channels with length and diameters are in micrometer scale provides an environment to conduct chemical and biological experiments in microscale, and it can be used to conduct MC experiments [5]. To be able to support theoretical and simulation-based studies in MC networks, in this work, we have realized a **microfluidic based flow assisted testbed** using fluorescent polystyrene (PS) microbeads which is widely used in biological tracing, in vivo imaging and fluid mechanics among other applications. Using the proposed testbed, our main aim is to create flow-assisted micro-scale and vessel-like environment, to perform a comprehensive study on flow assisted networks and to construct a building block for theoretical studies performed on MC.

2 Materials and Methods

Schematic representation of the proposed testbed design and methodology is given in Fig. 2. The experimental approach includes four main steps, including laser ablation-based microfabrication, channel surface characterization by multiple analysis techniques (atomic force microscopy (AFM), stylus profiler, scanning electron microscopy), testbed construction/data collection, and video processing.

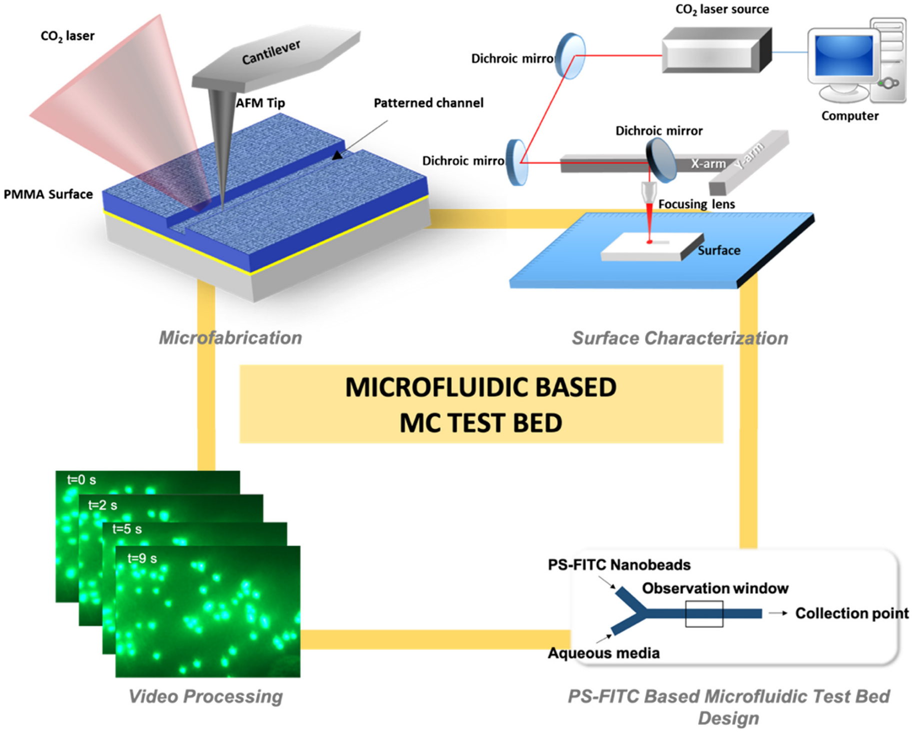

Fig. 2. A schematic representation of microfluidic based MC testbed construction.

2.1 Microfabrication

Initial step of microfabrication is the layout design of the desired microchannels according to the chosen communication scenario with a CAD tool (AutoCad Software) [6]. Layout pattern is converted to correct vector data by using the proprietary software of the direct laser writer (VersaLaser Software). We have employed VL-200 model VersaLaser CO$_2$ laser at a wavelength of 10.6 µm and source power of 30 W [7]. Fabrication of microchannels were performed with laser ablation. This method is based on removal of certain quantity of material from a solid surface by irradiating the surface consecutively with a focused laser beam. Under continuous irradiation, the material reaches its transition temperature by the heat generated and afterwards it evaporates [8].

With this method, it is possible to obtain channel profiles with variable surface waviness and depth. We have used different laser parameters (power and scan speed) as well as the distance of focal plane from the surface (z-position). Commercial 2-mm thick Poly-methyl methacrylate (PMMA) sheets of 20 cm-by-30 cm were used during engraving experiments. Channel length kept constant during experiments and is 20 mm. All experiments included single passes of the laser beam on the engraved channel. The distance between the surface of the substrate and the lens of the laser is kept constant during power/speed analysis experiments.

The main goal of the fabrication was to achieve reduced surface roughness for microchannels by heat exposure of a separate PMMA sheet at 80 °C for 3–5 min prior to laser ablation process. Later on, channels of the samples were engraved at 60% power with scanning speed of 25% (125 cm/s) and compared with the samples were no pre-treatment was applied. Inlets and outlets of the samples were engraved at 100% power with scanning speed of 1%, acquiring a more focused laser beam to perforate the PMMA.

2.2 Channel Characterization

For surface characterization, initial analyses were performed by using optical microscope. Microchannel images were acquired using Seiwa PS-888 high magnification microscope at various focal lengths to achieve depth of field (DOF) analysis. To reconstruct the 3D surface of micro channels, acquired images were analyzed using ImageJ software extended DOF analysis with focus stacking mode [9].

Additionally, surface roughness and depth of the channels were estimated by a Dektak XT stylus profiler (Bruker, Lifesci, Bogazici University). Recorded data were processed by using Vision 64 software [10].

Commercial Bruker Atomic Force Microscopy (AFM) system was also employed to compare the surface roughness values obtained through Profiler. Scanning electron microscopy (SEM) images were acquired by using Philips XL30 Environmental Scanning Electron Microscope at 5 kV.

2.3 PS-FITC (Fluorescein Isothiocyanate) Based Microfluidic Testbed

A schematic illustration of our testbed design is shown in Fig. 2. Mainly, aqueous solution of fluorescence PS micro-beads with 10 μm size and blank aqueous media were introduced into microchannels by using micro syringe pump at flow rate of 5 μL/min. Recordings were acquired at bright field mode with 1636 pixels × 1088 pixels resolution and fluorescent imaging mode by using Nikon Ti2-E inverted fluorescence microscope equipped with Nikon DS-Ri2 detector (100 frames/s) the schematic illustration is shown in Fig. 3.

2.4 Manual and Automated Time Lapsed Image Analysis

We used manual tracking plugin for ImageJ software in order to acquire (x,y) coordinates of a single bead in stacked images. The tracking plugin provides (x,y) coordinates as well as velocity and distance achieved between two frames. For automated particle recognition and counting, a MATLAB based image processing algorithm has been used [11].

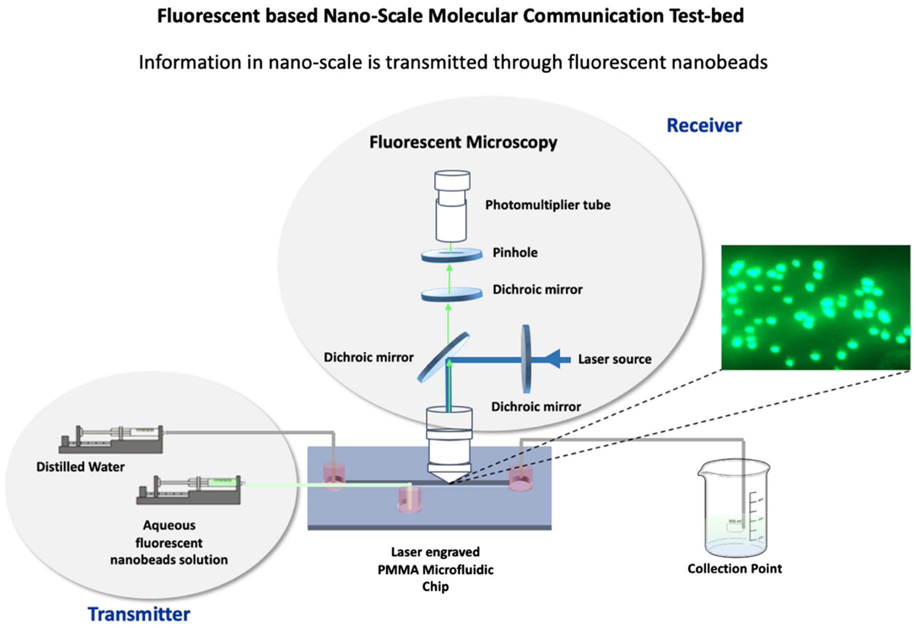

Fig. 3. A schematic representation of fluorescent microscope and straight channel testbed organization with representation of receiver and transmitter structures.

3 Experimental Results

Depth and smoothness are important parameters for the microchannels. Surface roughness and depth of the channel greatly affects the flow type and the behavior of the liquid. Finding optimum channel characteristics (depth and smoothness) is significant for maintaining laminar flow that allows flowing without mixing of multiple layers of fluid next to each other. This feature of laminar flow provides to mimic cells and their environment [12]. To find optimum channel characteristics, initial tests were performed by changing the main parameters in CO_2 laser, power (3–30 W) and scanning speed (125–500 cm/s) to ascertain the impact of the laser parameters on surface topography. While keeping the scanning speed constant, during engraving process the cross section of the micro channel is determined by the intensity within the focused laser beam and the thermal diffusivity of the substrate [8]. PMMA is a thermally stable material with low diffusivity, setting laser beam intensity as the main parameter for channel cross section determination.

Analysis of ablated channel profiler results indicates a direct correlation between channel depth and laser power for different speed values. With increasing laser power, beam can easily penetrate into the substrate to engrave deeper microchannels ranging from 15 µm to 715 µm (Fig. 4a).

Additionally, channel smoothness was found to correlate positively with increased laser power at low scanning speed (25%). This is due to the fact that the cross section of the channel is directly tied to the shape of the intensity distribution in laser beam at constant focal distance generating a top-hat structured beam shape at high power levels.

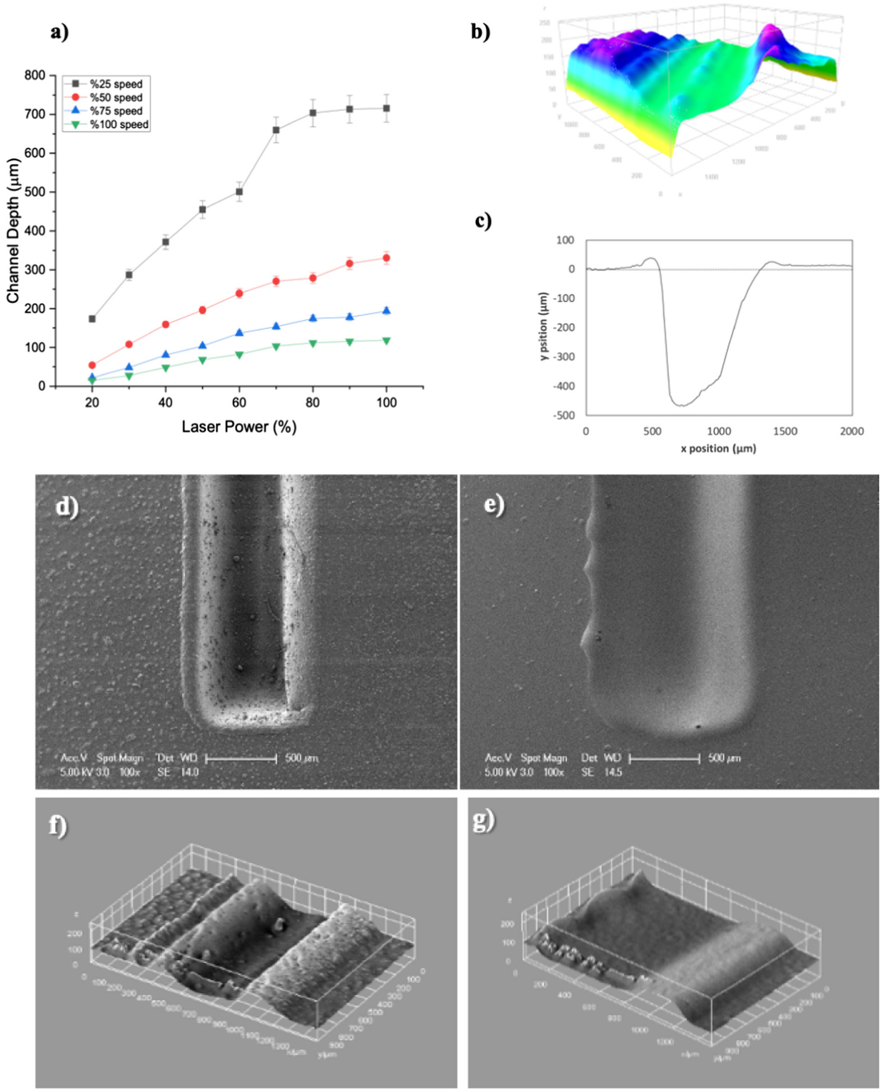

Fig. 4. a) Effect of laser power on channel depth of engraved microchannels at varying scan speeds; b) Reconstructed 3D topography surface mapping of microfluidic channel obtained at 60% laser power (18 W) and 125 cm/s scan speed where z axis is between 0 to 250 µm, y axis is between 0 to 1000 µm and x axis is between 0 to 1400 µm; c) 2D channel profile of microfluidic channel presented in b. engraved microchannels at 60% laser power and 25% scan speed; d) Without any pre-heating; e) pre-heating up to 80 °C; (f) (g) 3D surface plots constructed from DOF images of the microchannels presented in d and f.

For MC experiments, optimum conditions were chosen as 60% laser power (18 W) and 125 cm/s scan speed. Profiler results indicate that, at given parameters, channel depth is 470 µm and width is 506 µm (Fig. 4c).

Inspired by the work of Huang et al. [13] to reduce the roughness in microfluidic channels during engraving, microchannel fabrication procedure was repeated with a PMMA sheet exposed to heat treatment at 80°C. SEM images presented in Fig. 4d-e, demonstrate that pre-heating approach clearly ameliorates the surface roughness while decreasing channel depth. This is due to the fact that pre-heating reduces the amount of dissolved gas in the polymer induced during production. During the ablation process, trapped gas can escape from the ablated area right before re-solidification of the polymer.

Next, microfluidic flow experiments were performed with chips obtained without pre-heating applied. Namely, a typical time sequential image of PS microbeads retrieved from video recording given in [14], flowing in such microfluidic channels and recorded in bright field mode, is given in Fig. 5. Diffusion of particles is ensured by laminar flow from injection point (Tx) to receiver (Rx) marked with red rectangle. Each bead is labelled in the recording, and reception was done by counting the particles as they move across the receiver zone.

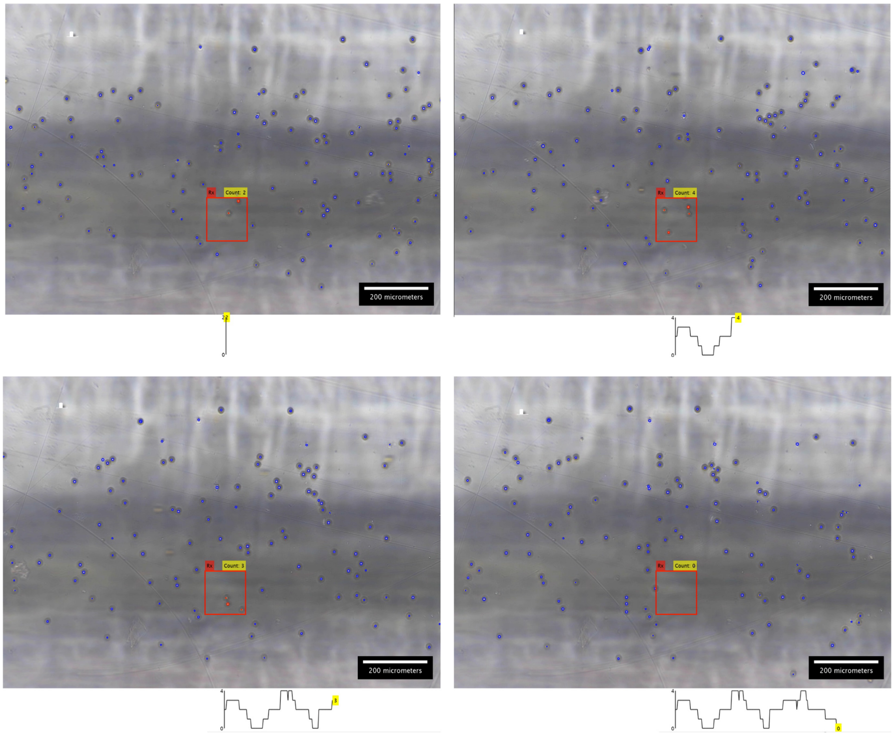

Fig. 5. Processed time resolved sequential images of PS microbead flow at magnification 20 k and pixel resolution of 1636 × 1088

4 Conclusion

In this paper, we have presented a microparticle based MC system using laser micro engraved channels. PS microbeads were released into microchannels through externally induced laminar flow. Each microbead labelled as information carriers was detected by microscopic observation. Using laser ablation based microfabrication has simplified the process compared to other techniques for microfluidic chip production such as photolithography, which is a slow and expensive technique that requires high maintenance cost due to the need for a clean room environment or an alternative wet etching technique where the material is removed by using large amounts of etchant chemicals, which might again be costly [15].

Most of the time, computerized simulations remain conceptual and build up theoretical knowledge, however not practical in real life situations. This study is a bridge between computerized simulations and real-life applications. The PMMA microfluidic chip was used in order to mimic the blood vessel structure that is not fully absorbent nor reflective so that the simulations are better aligned with practice. Therefore, the results of this study will allow us to develop channel models that will be applicable to real life problems with the help of validation through testbeds.

The preliminary data we obtained in this experimental testbed will constitute a body of information that the nanonetworking society does not possess. It is a proof-of-concept testbed that supports signal detection in micro-scale by using both bright field and fluorescence modes. Our preliminary results demonstrate that, to build a chemical signal-based communication testbed, multi-disciplinary thinking and collaboration are crucial requirements.

Acknowledgement. The authors acknowledge the financial support from BAP project fund by Bogazici University through the research project 15101. This work has been supported by the Turkish Directorate of Strategy and Budget under the TAM Project number DPT2007K120610.

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