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1 INTEGRATING OPTICAL WAVEGUIDES IN ELECTROWETTING-ON-DIELECTRIC DIGITAL MICROFLUIDIC CHIPS

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ABSTRACT

Readout through a microscope-based setup is one of the most prevalent optical detection methods used in current digital (i.e. droplet-based) electrowetting-on-dielectric lab-on-chip devices. The integration of waveguides on such devices allows further size and complexity reduction for absorption, fluorescence and surface plasmon resonance based detection mechanisms. In this paper, we show a straightforward method to integrate multimode polymer optical waveguides on an EWOD device. A proof-of-concept system is presented that allows droplets to be transported by EWOD actuation to a gap between two opposing waveguides down to 300 micrometer in size, and engulf the integrated waveguides for transmission measurements. The system is validated using a gold nanoparticle based model assay.

1. INTRODUCTION

Today, droplet manipulations based on electrowetting-on-dielectric (EWOD) have been implemented in a variety of digital lab-on-a-chip prototypes. Applications run on these devices include bio-assays such as immunoassays [1], proteomics [2], PCR [3] and cell cultures [4,5] and chemical synthesis applications [6,7]. In order to allow biomolecule detection, many sensing mechanisms including impedance spectroscopy [8], quartz microbalances [9] and optical detection have already been presented.

Optical detection is commonly used on EWOD-based lab-on-a-chip systems, typically based on a microscope-based optical readout of fluorescence [4, 10], absorption [11] or surface plasmon resonance (SPR) [12]. Recently, mounting of ring resonators [13,14] in a digital lab-on-chip was demonstrated as well, allowing label-free sensing with an evanescent field. Up to know, optical readout was mostly done by using a microscope camera or, in the case of resonators, by mounting waveguide components resting on a separate substrate on the EWOD lab-on-chip.

By integrating optical waveguides directly on the EWOD device's substrate, the need for focusing optics can be avoided and system size and complexity can be further reduced. Possible waveguide configurations include two opposing waveguides for transmission measurements, a single waveguide with gold-covered and functionalized tip penetrating the droplet for SPR measurements or an orthogonal configuration for fluorescence measurements (figure 1).

In this paper we present a proof-of-concept system that demonstrates, for the first time to our knowledge, the direct integration of optical waveguides on the substrate of an EWOD lab-on-chip. The fabrication method is discussed, concentrating on the configuration comprising two opposing waveguides for transmission measurements. Next, the compatibility between droplet manipulation and light coupling is studied. Finally, the proof-of-concept system is validated using a gold nanoparticle (Au NP) based model system.

2. MATERIALS AND METHODS

2.1 EWOD device design and fabrication

A standard process was used to create the top and bottom plates of the EWOD device on glass substrates. On the lower substrate, a 100 nm chromium layer is deposited by sputtering and patterned lithographically (Figure 2) in order to create electrodes, wires and contact pads. Subsequently, a 3.5 μm thick Parylene-C layer and a 200 nm thick Teflon-AF layer were deposited via chemical vapor deposition and spincoating, respectively.

The upper plate of the digital microfluidic device was fabricated by spincoating Teflon-AF on ITO covered glass slides to a thickness of 200 nm. Tape with a thickness of 80 μm was used as spacer between top and bottom plates. EWOD actuation was performed by applying ~ 130 V_{DC} actuation voltages to the electrodes. Further manufacturing details can be found elsewhere [15].

2.2 Waveguide design

The optical waveguide consisted of a core layer, surrounded by a cladding of lower refractive index in order to create total internal reflection. In this work, EpocoreTM and EpocladTM (Micro Resist Technology GmbH, Germany) were used as materials for respectively the core and cladding of the waveguides. These epoxies are similar to SU-8, but have a better transmission in the near infrared and visual wavelength range [16]. They consist of an epoxy monomer and an UV sensitive crosslinking agent dissolved in an organic solvent, and are applied to the surface by spin coating. Their refractive index at a wavelength of 700 nm is 1.594 and 1.585, respectively [17].

EWOD devices were designed for transmission measurements, having pairs of directly opposing waveguides each (Figure 2) positioned over the center of 1.1 mm wide EWOD actuation electrodes. Gaps between opposing waveguides were varied from 100 to 1100 micrometer in steps of 100 micrometer. The total width of the waveguide on and near the actuation electrodes was 150 micrometer. This width was sufficient to ensure that the core was surrounded by cladding while still being small enough to cause reasonably low flow resistance to the passing of droplets.

The waveguide core was 62.5 micrometer thick, targeting multimode propagation. Top and bottom cladding layers were designed to be 45 micrometer thick. The use of single mode waveguides was considered as the profile of the light cone exiting the waveguide would then not

depend on the distribution of the light over the different propagation modes. This would improve repeatability when a light source would be re-connected to the system. However, in case this would be problematic an even distribution over the propagation modes can also be obtained using a mode scrambler. In this application, multimode waveguides were selected because of the following advantages: (1) the ability to straightforward coupling to the 125 micrometer diameter multimode optical fibers used for connecting the system to an external light source and spectrometer, without a misalignment of a few micrometer being problematic. (2) As the core diameter of multimode waveguides is an order of magnitude larger compared to the core diameter of single-mode waveguides, the former waveguides can be positioned further apart without having to rely on integrated lenses to achieve sufficient light coupling. (3) Their thickness matches well the thicknesses typically used for the spacer that separates the top and bottom plate of an EWOD system. Therefore, they can serve a second function as a mechanical spacer between top and bottom plate of the EWOD device. (4) The cost of the optical components is lower."

At the distal end of the waveguides, trenches were foreseen in the lower cladding layer to allow positioning optical fibers that connect the chip to an external light source and spectrometer (Figure 2).

2.3 Waveguide fabrication

The waveguide structures were built on the lower substrate using a three-mask process based on contact UV lithography. First, in order to improve its wettability the Teflon-AF layer was activated by a short reactive ion etch (6 inch plate diameter, 3 sec at a pressure of 100 mTorr, 60 W and an oxygen flow of 32 sccm). The relation between the speed of rotation used during spin coating and the resulting layer thickness (i.e. spin curve) of Epoclad on such an activated substrate was determined experimentally and are shown in figure 3.

From this figure it is clear that the lower Epoclad layer should be spin coated at 3000 rpm for 60 seconds to reach the target thickness. After spin coating, the layer was soft baked (50°C for 10 minutes and at 120°C for 2 hours), UV exposed (550 mJ/cm² broadband UV, measured by h-line probe) and post exposure baked (5 minutes at 50 °C, ramping up to 110°C at a rate of 5°C/min, holding at 110 °C and cooling down to room temperature). Between temperature steps, a ramp of 5 °C/min was used.

Then an Epocore layer is spun at 1100 rpm for 60 seconds to reach a layer thickness of 62 µm. The Epocore layer was soft baked at 85 °C for 20 minutes, exposed at 550 mJ/cm² and post exposure baked for 5 minutes at 50 °C and 8 minutes at 85°C. Then, the lower cladding and the core layer were developed simultaneously in propylene glycol ether acetate (PGMEA).

When the second cladding layer is then deposited, the substrate is very uneven due to the presence of tall waveguide cores. As Epoclad was observed to level significantly after spin coating, a top cladding layer of a much higher average thickness had to be deposited in order to reach the projected 32.5 micrometers on top of the core layer.

As there is great variability in the final layer thickness for low spin speed, two consecutive layers were spun at 2000 rpm with a 15 minute soft bake at 110 °C in between. After this, the same fabrication parameters as for the first cladding layer were used.

Finally, the wafer was heated to 200 °C on a hotplate for five minutes. This further crosslinked the waveguide materials and restored full hydrophobicity of the Teflon AF layer.

2.4 Test setup

Droplet transport was tested using the same electronic driver board, LabVIEW software and microscope and camera setup as used in previous work [15]. Water droplets were actuated using a DC-voltage of 130 V, an activation time of 300 ms, and a relaxation time of 40 ms. A broadband halogen lamp based light source (Ocean Optics HL-2000 Vivo) and spectrometer (Ocean Optics USB4000) were connected to the EWOD device using 62.5 μm core multimode optical fiber patchcords (OZ Optics MMJ-33-62.5/125-0.9-3 fiber patchcords). One end of the patchcords was stripped and cleaved, and aligned with the waveguides using a micrometer screw based setup. Glycerol was put between waveguide and fiber for the purpose of index matching. With optimal settings (electric dark correction, 3.8 ms integration time, averaging over 100 samples) a dynamic range of 12000 could be achieved with the spectrometer.

For the droplet transport experiments, demineralized water was used. For further testing, p.a. purity NaCl and gold nanoparticles with an average diameter of 20 nm were used. The latter were fabricated as follows[18-19]: 100 μL of a 10 % (w/v) gold-salt solution (HAuCl_4 , Acros Organics, Belgium) was added to 100 mL of water. The solution was placed under a heating mantle and coupled to a reflux installation (cooling unit at 4° C). As soon as the solution started to boil, 3.6 mL of a 1 wt% citrate solution was added to the solution. After approximately 15 minutes, the color was stable and the solution was cooled down. The quality of the synthesis was checked by measuring the characteristic adsorption band using a UV-VIS spectrometer (Shimadzu, UV-PC1800).

3. RESULTS AND DISCUSSION

3.1 Droplet transport

In a first experiment, droplet transport to and from a gap between opposing waveguides was tested. The droplets were directed towards the gap between the integrated waveguides, where they stayed for the actual measurement. Afterwards they were transported towards the other end of the chip (Figure 4). Droplet movement was monitored and recorded with a light microscope and the experiment was repeated for different gap sizes. It was possible to perform the intended operation for gap sizes down to 300 micrometers in the setup used. At lower gap sizes, droplets could not squeeze through the gap between the waveguides. This rendered efficient droplet transport over waveguides spaced less than 300 μm apart impossible.

3.2 Transmission measurements on a model assay

Gold nanoparticle aggregation assays have attracted considerable interest because aggregation of these particles is accompanied by a surface plasmon shift that can easily be detected in the visual spectrum [20]. NP aggregation has a clear effect on the absorbance spectrum, both on the position of the peak of the spectrum as on the absorbance photons over a broad spectral band. These aggregation assays have already been extensively implemented for DNA, protein and small molecule detection [21-22]. Gold Nanoparticles with a mean diameter below 100 nm can

form very stable dispersions. The Au NP are stabilized by citric acid which is both used as a reductant and a stabilizing agent during Au NP synthesis. Citric acid gives the gold nanoparticles a net negative charge, making the particles repulsive for each other. Therefore aggregation of the Au NP can easily be induced by adding ions that screen these charges. Such ions can be supplied by adding a salt such as NaCl, of which the concentration then modulates attraction/repulsion between the Au nanoparticles. Thus, measuring the transmission intensity or spectrum variation of a nanoparticle dispersion as a function of the salt concentration is a good model for more complicated nanoparticle-based bio-assays, such as those based on the aggregation of for example DNA-functionalized nanoparticles induced by binding DNA aptamers. For these measurements, waveguides spaced 1000 micrometer apart were used. A 1 μ L droplet of the gold nanoparticle dispersion was pipetted on the chip and transported to the gap between opposing waveguides by EWOD actuation. After determining the transmission spectrum, a 1 μ L droplet of 4 M NaCl solution was transported on the chip towards the first droplet containing the nanoparticle dispersion and merged with that droplet.

After this operation, a clear 10% signal increase in the peak of the transmission spectrum could be observed (Figure 5). Using the same methodology with drops of increasing salt concentrations, aggregation of Au NP at different salt concentrations was induced and the effect on the average transmission in the 600-900 nm band was monitored. The results of these measurements are plotted in figure 6. The gradual increase in transmission in the Au NP – saline mixture as a function of the increase of the ion concentration is clearly visible.

A disadvantage of the waveguide material used in this work, i.e. the rather limited transmission of light shorter than 550 nm in wavelength, is also visible on figure 5. To further illustrate the useful frequency band the transmission spectrum of the waveguide - water droplet - waveguide system was determined. In order to obtain the transmittance of this system as a function of wavelength, the spectrum of the light source was compared with the spectrum received from the system with a drop of demineralized water between the waveguides. The result, normalized to the maximum transmittance at 1000 nm, is shown on figure 7.

The behavior of the transmittance limits the use of the system in e.g. common green or blue fluorescence-based applications. As detailed in earlier work [16], the transmission band obtained with the Epocore/Epoclad system is already expanded significantly from the infrared to the visual compared to the case of SU-8 waveguides. Still, clearly there is room for improvement of this aspect in further work.

Nevertheless, these results clearly demonstrate that the system shown in this work can monitor the transmission spectrum of nanoparticle dispersions over a practical wavelength range, offering great perspective for performing fully automated on-chip DNA or protein detection with Au NP aggregation assays on a highly integrated digital lab on chip. It is obvious that the design of the proof-of-principle system can be readily extended to allow more advanced assays, droplet manipulations and optical detection techniques such as SPR to be integrated.

The system as presented is already significantly more compact than the microscope-based setup used in our earlier work. No focusing optics are required. The two external optical components remaining are the light source and the miniature spectrometer, easily connected by optical fibers. The former can be readily miniaturized, while several types of chip-sized microspectrometers have already been presented [23]. Therefore, the optical waveguide-based configuration presented opens the way towards further miniaturization of flexible, EWOD-based microanalysis systems in a very compact package.

4. CONCLUSION

In summary, we have developed a process that allows the straightforward fabrication of integrated polymer optical waveguides on EWOD lab-on-chip devices using a three mask lithographic process, aiming at increased system miniaturization and complexity reduction. A fabricated proof-of-concept device allows for transmission spectrum measurements by transporting droplets automatically by EWOD actuation to a position where they are penetrated by two opposing waveguides, provided those waveguides are at least 300 micrometer apart, for the reported digital chip design parameters.

The integrated optics and the automated liquid handling functionality of this device were put to the test on a model system. By measuring of the transmission spectrum of an aggregating gold nanoparticle dispersion at increasing salt concentrations the applicability to gold nanoparticle based assays was demonstrated. These results prove that this system allows for precise measurement of the transmission spectrum in Au NP assays with minimal manual handling. In future work, we will further explore these devices for performing on-chip detection of DNA by using sandwich assays where target DNA molecules cross-link several gold nanoparticles by specific hybridization.

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2 Authors' Vitae

Frederik Ceyssens was born in Sint-Truiden, Belgium, in 1980. In 2003 he received the degree of M.S. in Electrical Engineering from the Katholieke Universiteit Leuven, Belgium. The subject of his MS thesis (done at Imec, Belgium) was the design a network-on-chip for efficient multicore communication. After that, he became research assistant at MICAS, investigating

novel microfabrication (MEMS) technologies such as SU-8 and their applications in integrated optics and sensors.

In 2009, he obtained the PhD degree. He is currently working as a research fellow of the FWO, researching novel brain-computer interfaces in a collaboration between KU Leuven and the Gasthuisberg university hospital.

Daan Witters was born in Herk-de-Stad, Belgium, in 1986. He received his B.S. and M.S. in bio-engineering from the KU Leuven, Leuven, Belgium in 2009. His master thesis was in the field of enzymatic assays performed on microfluidic systems. He started his PhD at KU Leuven in 2009 and his

research subjects concern the design and application of digital microfluidic systems for performing high-throughput bio-assays and miniaturized materials synthesis.

Karel Knez (1986) was born in Lommel, Belgium. He first obtained a B.S. in bio-medical science from the UHasselt (Belgium) and in 2009 a M.S. in bio-medical science from the KU Leuven (Belgium). At IMEC

(Inter-university Electronics Centre, Leuven, Belgium) he made a master thesis on the implementation of gold nano particles in bioassays. In 2009 he started his PhD at KU Leuven, his research is focused on the development of new Surface Plasmon Resonance based biosensors for sensitive detection and identification of micro-organisms.

Thom van Grimbergen was born in 1988 and obtained his Master's degree in Biomedical engineering from KU Leuven in 2012. That year, he also won the Imec master thesis award for his thesis "Integrating optics in digital lab-on-a-chip technology". He is currently pursuing a career in management, working towards an MBA degree.

Jeroen Lammertyn was born in Leuven in 1974. He obtained his degree of Master of Science in Bioscience Engineering and Chemistry (summa cum laude) from KU Leuven in 1997. In 2001, he obtained his PhD degree from the same university. He also holds a master's degree in biostatistics (UHasselt 2003, summa cum laude). He was a researcher at Penn State University before becoming head of the MeBioS biosensor group at KU Leuven. His research activities are framed in the supradisciplinary field of bionanotechnology and focus on development of novel bio-molecular detection concepts for integration in miniaturised analysis systems. Jeroen Lammertyn is professor at the Katholieke Universiteit Leuven, Belgium, since 2005.

Robert Puers was born in Antwerpen, Belgium, 1953, and grew up in Ghent. He received his Master of Electrical Engineering at the KU Leuven in 1977, where he obtained his Ph.D. in 1986. From 1980 he was employed as a Research Assistant at the ESAT laboratory at KU Leuven. In 1986 he became director of the clean room facilities there. He pioneered research on micromachining, MEMS and packaging in Flanders, mainly for applications in implantable biomedical monitoring. Recently, microfluidics

and MOEMS based on silicon and polymers have been added to his research. Robert Puers is professor at the Katholieke Universiteit Leuven, Belgium, since 1991.

3 List of Figures

Figure 1: Left: Waveguide configurations for used to transmission measurements in a droplet transported by EWOD actuation as demonstrated in this work. Legend: S: glass substrate, e: EWOD electrode, i: Parylene C + Teflon AF insulating layer, w: waveguides. Center and right: possible alternative configurations for fluorescence and SPR measurements , respectively. These are not part of the proof-of-concept presented here.

Figure 2: Layout of EWOD test device, showing actuation electrodes (gray) and waveguides (orange). Inset A: (1) actuation electrode (2) waveguide cladding layers (3) waveguide core. Inset B: waveguide-optical fiber coupling structure. (4) insertion path of optical fiber into coupling structure. C-E: further cross sections

Figure 3: Measured spin curve of Epoclad on plasma activated Teflon AF. Error bars denote +/- one standard deviation. It is clear spin speeds larger than 1000 rpm have to be employed in order to improve the obtainable tolerances.

Figure 4: Droplet transport to and from with the gap between two opposing waveguides. The actuation electrodes are clearly visible. As the waveguides are hardly visible against the transparent background, their contours are encircled in blue in the top left picture. With the

parameters used, a minimum gap size of 300 micrometer was required in order for the droplets to be able to squeeze through.

Figure 5: Transmission spectrum for a 1 μ L droplet of a gold nanoparticle dispersion (NP 1) and of a gold nanoparticle dispersion mixed with a 1 μ L droplet of 4 M saline (Mix 1). A 10% increase in transmission can be seen as the salt screens the charged nanoparticles, causing aggregation.

Figure 6: calibration curve of the gold nanoparticle model assay as a function of NaCl concentration. Error bars denote $\pm$ one standard deviation. Transmission shown is average transmission in 600-900 nm band.

Figure 7: Measured transmittance of the waveguide - water droplet - waveguide system, normalized at the 1000 nm wavelength. The system is of limited use below the 550 nm wavelength.