Multi-Channel Droplet Generator with Integrated Optics to Enhance Fluorescence Detection

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INTRODUCTION

Microfluidic platforms for in-vitro diagnostic applications are emerging from research applications into broader commercial acceptance, delivering on the benefits of reduced reagent costs and relocating testing from larger laboratories to remote testing facilities, e.g., Point-of-Care/Need. One factor restraining the growth of these microfluidic applications centers on the premise that the smaller liquid volumes utilized (micro-, nano- and pico-liters) often do not provide a sufficient and detectable amount of the target entity to produce a reproducible signal for a result. Techniques such as digital droplet-PCR and fluorescence-activated cell sorting (FACS) on a flow cytometry platform demonstrate that while it is mechanically feasible to miniaturize the sample size, the challenge of measuring low levels of signal and discriminating signal from background remains.

SUMMARY

We present a case study describing the design, fabrication, and application of a hybrid microfluidic-optical device that integrates an array of diffractive lenses into an injection-molded microfluidic droplet generator device. As a microfluidic feature, droplet generators can create single or parallel channel streams of single cell suspensions or oil/aqueous droplets containing cells, reagents or beads used as a tool for research and diagnostic testing. One example of this is the emerging use of digital-droplet polymerase chain reaction (dd-PCR) techniques for molecular diagnostics in which fluorophores are used as a means of optical detection.

The benefits of an integrated lens within such a device enable:

- Improved detection efficiency - collecting and amplifying low-level optical signals (e.g. fluorescence) with an enhanced signal/noise ratio (SNR) and capable of magnifying micron-scale elements,
- Increasing throughput - allowing designs that accommodate massive parallelization for sample manipulation/delivery and optical detection, e.g., up to 10,000 droplets per second,
- Reducing overall cost - using low cost, reproducible injection molding techniques to create features in consumable parts that substitute for complex and costly optical detection elements as part of a larger system.

OPTICAL DESIGN CONSIDERATIONS

Many fluid handling and processing methods found in clinical laboratory systems can be designed and integrated as molded features into LOAC devices - sample preparation, volume control and metering, transfer, mixing, and partitioning. However, there are few examples of molded features used for optical applications - elements used for imaging, signal focusing / attenuation / intensification.

As an example, flow cytometry is a widely used platform for cellular analysis for which LOAC microfluidic platforms have similar functionality. Flow cytometry measures characteristics of individual cells flowing single file in a stream of fluid through a detection zone. Microfluidics can align and move single cells or oil/aqueous droplets in a similar manner based on the microchannel dimensions. In the case of flow cytometry, the fluorescently labeled cells reach an interrogation point intersected by a laser. Emitted light from the labeled cells is given off in all directions and is collected via lenses directing the light to a series of filters and dichromic mirrors that isolate wavelength bands. A "good" lens provides spatial resolution, allowing the collection of light from the interrogation point and avoids collecting light from other regions a small distance away.

A similar application of this type using an injection-molded microfluidic device with integrated optical features was presented (1) and highlighted the unique technological capabilities of Technicolor Precision BioDevices (Technicolor). A 16-channel fluorescent droplet generator chip designed and fabricated by Technicolor achieved an 8-fold signal intensification detected through a diffractive lens (Fig. 2-4). Notably, the device improved the detection efficiency of low-level fluorescent biomarkers in addition to multiplexing 16 detection channels within a single device as described by Schonbrun, et. al.(2)

MICROFLUIDICS CHIP DESIGN AND FABRICATION

A three-layer hybrid microfluidic-optical droplet generator in a microscope slide format was designed and fabricated from Topas® COC 8007 (Polyplastics Co., Ltd.); a thermo polymer chosen for its low autofluorescence characteristics. The thermally bonded three-layer architecture chip design comprised a microfluidic channel manifold integrated with an optical layer with array of Fresnel zone plate micro lenses diffractive lenses (Figure 1).

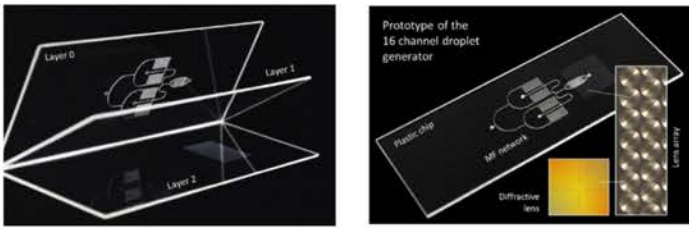

Fig. 1. Three-layer hybrid microfluidic-optical chip: (a) as fabricated in three separate layers, (b) photo of the prototype comprising the 16-channel droplet generator and array of diffractive micro lenses aligned with the channel path.

Channel Base - Layer 0

The design for the droplet generator with a 16-channel detection area was created in a microscope slide form factor from CNC micromachined acrylic/PMMA. A molding tool was created and the resultant droplet generator manifold was fabricated on an Arburg Allrounder injection molding machine.

Separation layer - Layer 1

A separation layer was inserted between the channel base and the lens array as a physical offset to facilitate the focal depth of this specific optical design. The layer can be a molded slab or high optical quality commercially available COC film.

Lens Array - Layer 2

The lens array cover slide was created using Technicolor's proprietary laser beam recording, electroformed into a mold insert using techniques similar to those used for the channel base and fabricated on an Arburg Allrounder injection molding machine.

Micro lens array characteristics: Fresnel zone, diameter 600 m, Numerical Aperture: NA = 0.58 {corresponding to D=600, f=800, n=1.54}

ASSEMBLY OF THE DEVICE

The three-layer device was assembled using low temperature thermal fusion bonding techniques to prevent deformation of the features, aligning the channel and lens array to maximize the optical pathway - positioning the lens over the channel area to interrogate the signal from the fluorescent droplets (Figure 2).

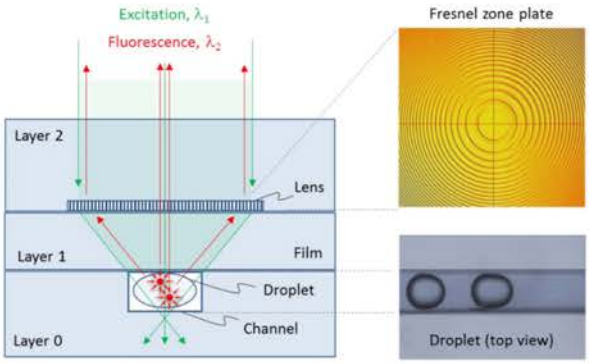

Fig. 2. Chip architecture: (a) sketch of the light focusing mechanism in the cross-sectional view of the chip, (b) microscopy image of the diffractive micro lens molded on the bottom surface of the cover layer, (c) top-view microscopy image of the channel with droplets.

METHOD AND OBSERVATIONS

The hybrid droplet generator chip assembly was created with a Fluigent Flow EZ pressure pump and characterized using a fluorescent microscope (Nikon Eclipse TE2000-U) with 5X and 2X objectives and observed under bright field and fluorescent illumination conditions. Two fluorescent markers, Resorufin CAS #635-78-9 (Tokyo Chemical Industry Co., Ltd.) and Fluorescein sodium salt CAS #518-47-8 (HiMedia Laboratories Pvt., Ltd.) were formulated in water and combined with mineral oil (Alfa Aesar Ward hill, MA CAS: 8042-47-5) and Span 80 surfactant in the chip creating droplets of about 1 nl size at a rate of 24 drop / second, passing these droplets through a 2:1 combiner junction and branching into the 16 parallel microfluidic channels for signal intensification by the micro lens array and detection (Figure 3).

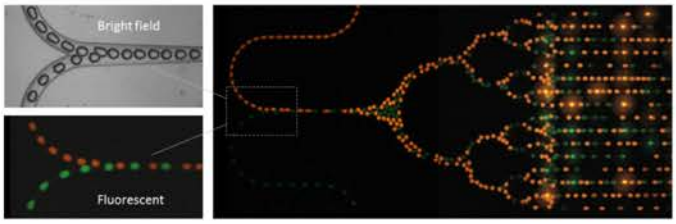

Fig. 3. Hybrid microfluidic chip channels: (left to right) 2:1 microfluidic combiner with droplets doped with two types of fluorescent markers emitting light at 521nm (green) and 580nm (orange), the 1:16 microfluidic divider and the chip detection zone as seen by the fluorescent microscope. The enhanced brightness of some droplets in the detection zones is due to the lensing effect of the micro lens array.

Data collected demonstrate an 8-fold (9.0 dB) signal intensification between the channel zones aligned with the micro lens array compared to no array. It was also observed that the Signal to Noise Ratio was improved 150-fold (21.8 dB) with the lens intensified signal compared to background (Figure 4).

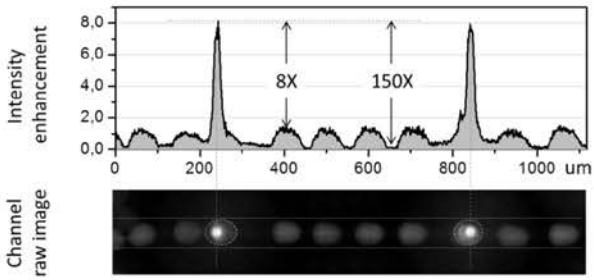

Fig. 4. Experimental data: (a) Light intensity profile along the channel normalized by the average light intensity for droplets without lensing effect, (b) raw image data of a single microfluidic channel with multiple droplets passing through the detection area comprising two micro lenses focused at the center of the channel.

FUTURE DIRECTIONS

The integration of optical elements into microfluidics has been documented in numerous research publications but has only been recently introduced as an option for large-scale manufacturing of microfluidic consumables.

New hybrid solutions are expected to emerge as applications demonstrate advantages beyond existing

configurations such as improving detection efficiency, increasing throughput and reducing overall cost of the consumable and device. Examples of possible configurations include:

- Integrated optics – i) enhanced light/matter interaction due to additional light confinement; ii) improved signal-to-noise ratio (SNR); iii) improved alignment of the light source for excitation or imaging readouts,

- In-plane solutions – i) allowing integrated light source and/or optical waveguide-based architectures; ii) improved imaging techniques and direct imaging,

- Out-of-plane solutions based on refractive or diffractive micro lens arrays adjacent microfluidic channel chips – i) massive parallelization (e.g., one lens per channel with any number of channels possible, limited only by the field of view of the imaging device); ii) creating a large detection zone addressing multiple parallel channels; iii) enhancing signal-to-noise ratio due to effective collimation of the excitation light and/or collection of a larger portion of fluorescent light.

Technicolor Precision BioDevices, clean room and fabrication facilities.

CITATIONS

1) "Integrating Optical Detection Technology into Microfluidic Consumables", Presentation by Technicolor Precision BioDevices LOAC World Congress, San Diego, Oct. 1-3, 2018.

2) E. Schonbrun, S.S. Gortih, D. Schaak, "Microfabricated multiple field of view imaging flow cytometry", Lab on a Chip, Issue 2, January 21, 2012.

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