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Standardized bio-opto-fluidic chip technology using channel only process

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Abstract

The integration of optical waveguides within biological assay chips seems to be an interesting solution to increase their functionality as it will allow local optical probing of the samples. However, the techniques proposed to build such chips are generally complex requiring multiple steps to fabricate waveguides and channels, and at the same time losing the capacity to use cost-effective fabrication technique like injection moulding. We propose a techniques where the waveguides are actually built as empty channel in the optical chip and later filled with special liquid to be used as waveguide. In this way, the chip only necessitates the fabrication of channels, some carrying the biological samples and others used as waveguides. To simplify further the fabrication, we developed a standard holder that allows to address the issue of filling the waveguide channel with the special liquid and at the same time provide connection for the sample testing. The chip was fabricated in PDMS and it was tested together with the standard holder using different liquids. Although the optical loss is still high due to scattering, this type of system was able to easily detect the bubbles appearing in a bi-phasic flow, opening the way for further improvements in the technology.

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Keywords: Micro-fluidic; Opto-fluidic; Channels; Waveguide; Liquid core

1. Introduction

It appears more and more clearly that systematic biological analysis conducted by general practitioner, as needed to help diagnosis, can only become cost-effective, and thus widespread, by using a standard bio-chip technology. Such system should allow a series of complex tests to be conducted on patient tissue or fluid sample using a disposable sample holder. Often, the analysis of biological sample is based on optical techniques like fluorescence, or spectral absorption. However, these techniques generally use wide field measurement that preclude local detection of biological measurands. To allow multiple measurements on the sample inside the same chip a viable solution seems to provide local optical illumination and detection on the chip. This will require the integration of lightguides inside the bio-chip itself to channel the light from different sources and towards different type of detectors (Fig. 1).

This thrust is one of the reasons that spurred a recent effort at integrating lightguides inside bio-chip as part of the rapidly developing field of opto-fluidics [1,2]. But these techniques have in general different issues. Often the fabrication of the bio-chip becomes much more complex, requiring multiple extra steps and/or multiple materials [1,3,4]. Actually, lightguide are usually based on total internal reflection (TIR) phenomena, which appears for certain incidence angle at the interface between two materials of different refractive indices. Hence the opto-fluidic chip have to use two different materials requiring multiple step process that would prevent the fabrication of the chip using cheap techniques like injection moulding or hot embossing

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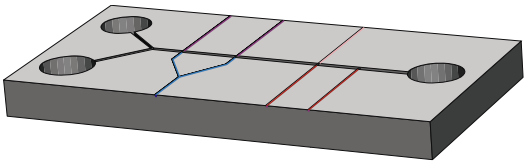

Fig. 1. A bio-chip with integrated waveguides to perform multiple optical measurement (absorption at different wavelength, fluorescence, counting, size determination).

of polymer substrate. In other cases [5,6] to overcome these issues, the structure uses a single material – and use air as the cladding to obtain TIR and guide the light. However, such waveguides are characterised by a strong contrast in the index of refraction between the core and the cladding and consequently suffer from high loss and show a large numerical aperture (NA) resulting in poor coupling efficiency with optical fibre [7,8]. Another approach has been to use fully fluidic waveguides using two liquids [1] with different index of refraction. If this approach allows to control precisely the index contrast, it also requires a complex 3D circuitry, again making fabrication harder. It is also possible to directly integrate optical fibres in the fluidic chip [2,9,10], however this approach makes the packaging more complex and would probably push the cost too high for disposable chips.

2. Principle and fabrication process

To overcome the issues we perceive in the existing techniques, we propose a fabrication process that uses a single material, requires only the patterning of a series of channel in the polymer substrate and allows controlling the difference of index of refraction between the core and the cladding of the waveguide. Actually when we fabricate the chip we build two different types of channel that, at time of use, will have two different functions. On the one side, some channels will be used for the transport of the biological sample in a liquid and in the other side, channels filled with a special liquid with a refractive index larger than the substrate, that will be used as waveguides (Fig. 2). To allow light transmission measurement, the waveguide channels are placed on both side of the sample channel, with a narrow solid wall between them to avoid leak of the waveguide fluid into the sample. We note that the waveguide channel need to be completely filled and to avoid air bubble being trapped we placed narrow filling and venting channels connected to reservoir at each ends of the channel.

The possibility to simply change the liquid core index of refraction is unique and could be used to produce on the same chip various waveguide with different properties. For example, the illuminating waveguide which will need to be coupled to external light source with optical fibre could have a small numerical aperture (NA) to improve fibre coupling efficiency. However, the receiving waveguide placed after the sample channel, could be made with a larger numerical aperture to allow collecting some of the light that is refracted by the sample and would otherwise be lost. Actually we have

$$\mathrm{NA} = \sqrt{n_{\mathrm{core}}^2 - n_{\mathrm{cladding}}^2}$$

and, for example, with the available refractive index liquids from Cargille–Sacher Laboratories Inc., USA, it will be relatively easy to obtain NA in the range 0.1–0.7 (larger value are possible with liquid with high index of refraction – up to 2.2 – but these would be more difficult to handle as they are toxic and their stability may be a concern).

To demonstrate the technique we build prototypes based on two popular techniques, SU8 photolithography and PDMS moulding, but it is understood that the final goal is to use injection moulding with polycarbonate, in a process similar to what is used for CD-ROM fabrication [11]. The prototype fabrication processes shown in Fig. 3 are relatively standard and require only few explanations.

The SU8 chip is based on a multilayer SU8 process where an exposed and cross-linked base layer of SU8 is covered with another layer which is then patterned with the channels. The base layer is needed so that the optical field in the lightguide does not come in contact with the silicon substrate which absorbs light in the visible range. The SU8 process had to be optimised by reducing the chip surface in order to minimise the stress in the layer and the resulting cracks in the walls of the channels.

The PDMS process is even simpler, as the mould patterned with standard photolithography in AZ9260 is transferred to silicon by DRIE and later replicated by casting

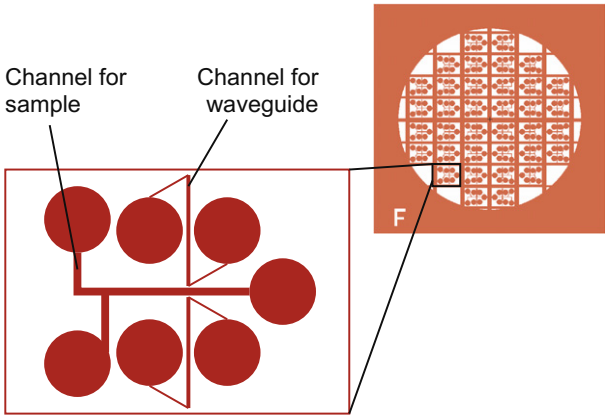

Fig. 2. Typical layout of the chip.

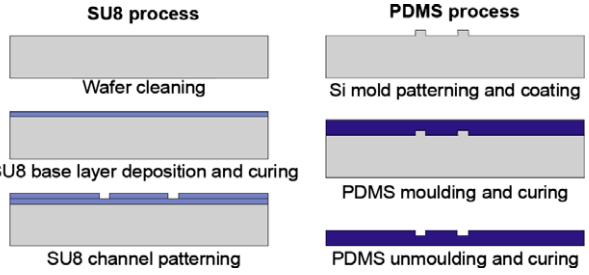

Fig. 3. Standard channel fabrication process for bio-opto-fluidic chip.

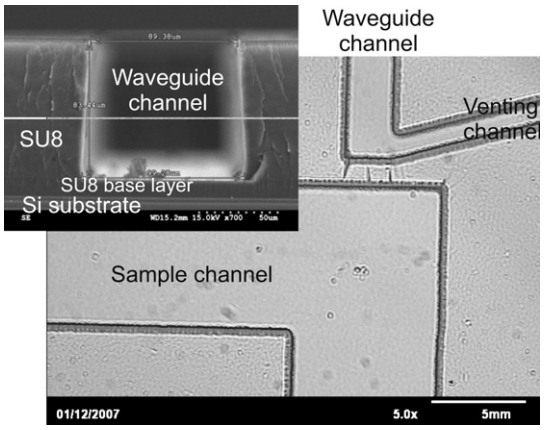

Fig. 4. SEM view of fabricated channels in SU8.

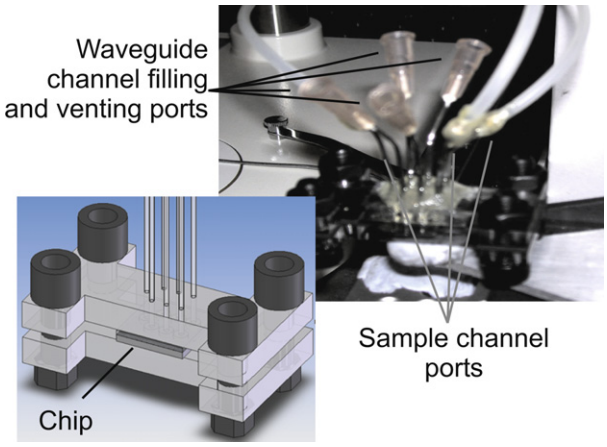

Fig. 5. CAD drawing and view of the standard holder with fluidic interfaces.

and curing PDMS. At the end of the DRIE etch, the mould is treated with a longer C_4F_8 cycle that leaves an anti-adhesion film helping unmould the PDMS after curing. This film is relatively durable and has been seen to last for several moulding/unmoulding operations.

In the fabricated chips (Fig. 4), the lightguide channel are designed for multimode operation to couple with optical fiber with about 100 μm diameter core. Thus their cross-section are roughly square with width and depth of 90 μm, while the sample channels are wider but of course of equal depth.

3. Standard chip holder

The two processes used for the fabrication (and eventually the injection molding process with polycarbonate) leave 'open' chips (see Fig. 4) that need to be closed to allow pressure driven flow of liquid without spillage. Instead of using a permanent bonding process to seal the channel, we designed a standard chip holder, shown in Fig. 5, that provides fluidic connection to the chip and at the same time closes the channels on the top. The I-shape holder is cut with a laser cutting tool (Universal M-300 Laser Platform from Universal Laser Systems Inc.) in a few minutes in 6 mm thick sheet of PMMA. Its shape provides clamping at the corners and the narrow part allows access to the lightguide on the chip. Syringe needles (with the sharp tip removed) are then inserted into the holes in the holder top plate and sealed in place with epoxy glue. It is important to ensure that the needles do not protrude below the holder top plate, as they would damage the chips. Although we designed different versions of the chip, the standard chip holder connects to all of them as the positions of the reservoir have been standardised in our chip layouts (cf. Fig. 2).

The chip is maintained water tight by clamping the chip in the holder using four screws at the edge of the holder. The SU8 material being relatively stiff (Young's modulus 4 GPa similar to polycarbonate which is about 2.4 GPa) to obtain a good seal we had to introduce a softer material (kapton or PDMS, which has a Young's modulus two order of magnitude lower) between the chip and the PMMA holder (Fig. 5). The soft seal could effectively compensates any unevenness of the chip surface. The PDMS seal could also be patterned by molding with a profile matching the channel layout that make the seal tighter by increasing the local pressure at the channel side. With the PDMS chip fabrication this additional sealing layer was not necessary.

After closing the holder we inject through the waveguide filling ports a liquid with a refractive index slightly higher than the substrate refractive index. We used liquids from Cargille–Sacher Laboratories Inc., USA, which provides stable and colourless liquid with index of refraction between 1.4 and 1.7. Knowing that the refractive index (around 600 nm) of cross-linked SU8 is about 1.59, we used a liquid from the series A with index of refraction 1.61. For the PDMS chip, we used a liquid with a lower index of refraction that matched better the index of the PDMS. However, the targeted difference of index has to be kept larger in that case (0.2), as the refractive index of PDMS is very sensitive to the curing condition and the ratio between the resin and the curing agent, and could hardly be kept constant between different process runs.

4. Testing

We verify the functionality of the chip by first testing the propagation of the light into the waveguide. We couple the light of a compact He–Ne laser into a long lightguide (15 mm) with a 20X microscope objective and observe with a camera placed above the chip and at the end of the waveguide. To record the top image of the chip, the chip is left open without the top plate of the holder. To inject the light with the microscope objective into the waveguide, we focus it slightly within the chips where the liquid core waveguide is starting. Actually, for fluid core waveguides, the waveguide can not emerge at the chips edge as it would result in spillage and we placed the waveguide end about 50 μm behind the chip edge. For a 80 μm wide core, the resulting

diffraction loss are very minimal – and clearly much smaller than those resulting from the propagation across the 300 μm wide sample channel. The light propagation in the waveguide is largely multimode as the inset showing the waveguide field in Fig. 6 clearly shows. Using the image from above we plot the evolution of the scattered light intensity over the propagation distance. In this way we are able to estimate the propagation loss at about 3 dB/cm at 600 nm. This figure is rather high and inspection of the channel sidewalls, trace back most of this figure to side wall unevenness. Additionally the refractive index liquid seems to contain scattering centres, which could be due to precipitate in the solution or impurities during manipulation. Nano-filtering of the fluid may be needed to overcome this last issue, if the waveguide loss is a critical parameter. In this preliminary study we did not try to test the possibility to use multi-NA waveguides, as described earlier, as their advantages would probably be overshadowed by the existing imperfections in this non-optimised structure.

One of the chip we have build is designed to allow simple testing by allowing to pass bubbles between the illuminating and receiving waveguides [10]. We injected two immiscible fluids (water and air or oil) using a syringe pump through a T junction, resulting in the apparition of bubbles in the bi-phasic flow. The bubbles length can be controlled by adjusting the ratio between the two flows by varying the syringes diameter. The pressure driven flow in the sample channels drives the bubbles between the illuminating and collecting waveguide (inset of Fig. 7). If the light, coupled in the device with a fibre, is collected in a similar way on the other side of the sample channel and directed toward a photodector, we observe the typical intensity variation shown in Fig. 7. On the chip, at the point where the light enters the sample channel we observe a bright spot, showing that diffraction due to micro-roughness is also present at the side wall of the sample channel, confirming the cause root of the high propagation loss observed earlier.

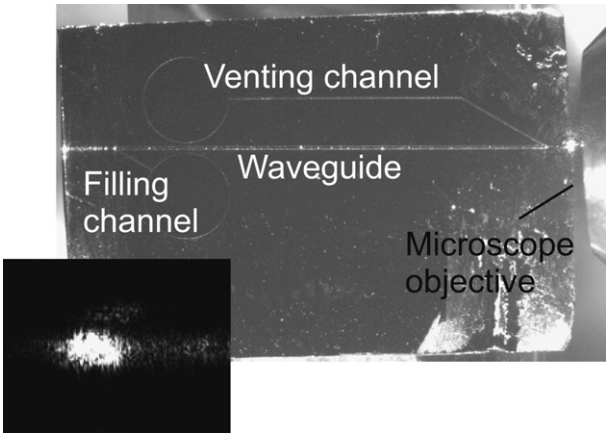

Fig. 6. Propagation in a long liquid lightguide with SU8 cladding (inset: waveguide field).

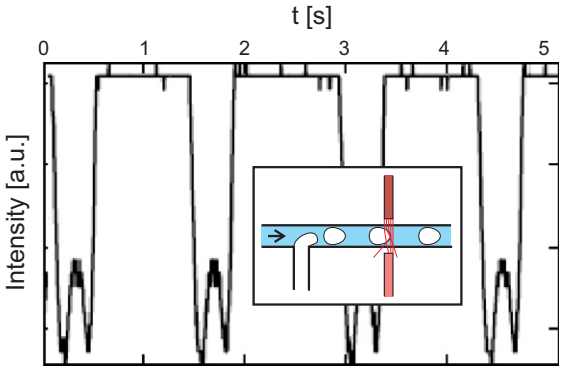

Fig. 7. Light intensity variation from the optical fibre upon passing of bubbles in the sample channel (inset: principle of bi-phasic flow experiment).

The fluctuation of the intensity is linked with the refraction occurring at the bubble interface between the two fluids of different refractive index as shown schematically in the inset of Fig. 7. Moreover, the two fluids present different absorption coefficient that will also modify the intensity of the light collected by the receiving fibre when there is a bubble passing between the waveguides. We shall note that this type of sensor is quite sensitive as the difference in the intensity at the beginning and at the end of the bubble can be traced back to the difference of radius of curvature appearing at the receding and advancing end of the bubble. In this way, this simple sensor could be used to measure the size of the bubble (in absolute units if the flow rate is known), the direction of the flow or the frequency of bubble generation [10].

In summary, we have seen that the preliminary results of our channel only fabrication process for bio-opto-fluidic chips are very promising. Our next target will be to improve the standard chip holder by simplifying the closing operation using a clamp instead of the set of screws. The chip fabrication process will also be improved so that we can use this new process to fabricate bio-chips with multiple local detection capabilities for complex physico- and bio-analysis.

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