

1. Materials and methods used in the fabrication process

Norland optical adhesive NOA61 was purchased from Unifibre s.r.l. Milan (Italy). Photopolymerization was carried out in a normal laboratory room under ambient light conditions. NOA61 monomer liquid (viscosity 300 cps at 25°C) has an absorption edge in the range 320-380 nm centered around 365 nm. As UV light source a 100 Watt mercury vapor UV flood lamp (Spectronics SB-100P) was used. This lamp is optimised for emission at 365 nm and produces a luminous flux of 6000 $\mu\text{W}/\text{cm}^2$ at 15 cm distance. In Fig.1 we show the polymerized layer thickness as a function of exposure time for two different lamp to sample distances. The results were obtained by patterning a set of lines of width 1 mm and separation 6 mm using 2 mm thick spacers. The thickness of the patterns scales logarithmically with the exposure time in qualitative agreement with theoretical prediction [1]. The maximum thickness that we could polymerize with NOA61 thiolene adhesive was about 1,5 mm. The minimum thickness that could reliably be achieved using PDMS as an anti-stick surface was about 100 μm . When patterns were formed between two surfaces using spacer thickness D we have chosen "partial curing" times about three times longer than the time needed for the polymerization front to reach distance D. Partial curing was done at a lamp-sample distance of 50 cm. Exposure time for "hard-curing" was the same as the partial curing time but the lamp-sample distance was decreased to 22 cm corresponding to a 3,7 fold increase of exposure dose. According to the datasheet of the SB100P flood lamp its intensity scales with distance L from the lamp as $1/L^{1,6}$. To avoid back-reflection of the UV light that traverses the sample during photopolymerization a UV absorbing film (UV Sun-blocker, Unisol, Padua, Italy) was layed down beneath the bottom glass plate.

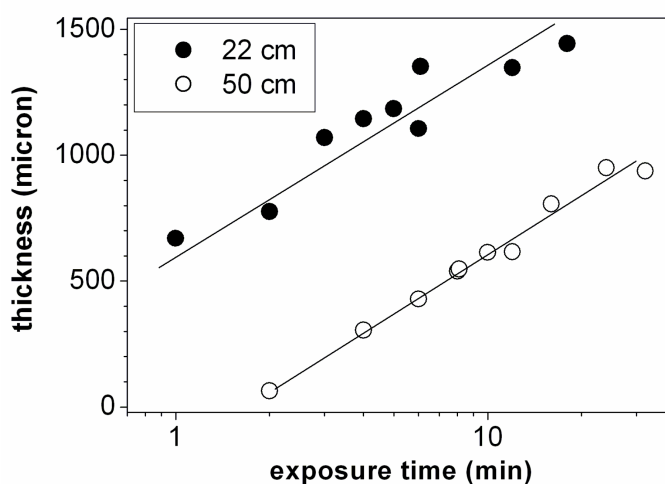


Fig.1 solidified NOA61 layer thickness during front polymerization versus exposure time for two lamp-sample distances. Lines are guides to the eye.

Photomasks used were printed at a resolution of 4000 dpi on photographic films by a local photoplottting/image-setting service company (Q&B Grafiche, Mestrino, Italy) and were available 24 hours after sending pdf files with the desired mask design, produced by CANVAS software. In many cases, to reduce the turn-around time, at the expense of resolution, masks were laser printed (600 dpi) onto transparencies and multiple transparencies were stacked to increase the optical density of the masks.

PE sheets used were bought from stores that sell them to wrap book covers. The PE sheets were bonded to a glass plate by lamination with water, acting as a reversible glue. Lamination was done by hand using a microscope glass slide wrapped with clean-room cloth as a tool.

A PDMS sheet (400 μm thick) was prepared on a glass plate by sandwiching degassed Sylgard 184 (Dow Corning) PDMS between two glass plates one of which had a PE sheet attached. Curing was done during 24 hours at room temperature. Etanol and acetone used to rinse thiolene were obtained from Aldrich. Glass substrates on which microfluidic chips were fabricated were microscope glass slides (Menzel Glaeser). Prior to use the slides were cleaned with etanol and acetone and then in a UV-ozone cleaner (Jelight Inc. 42-220 model).

Besides PDMS sheets also other "anti-stick" surfaces were explored such as a PE sheet laminated onto a glass plate or a glass plate coated with a hydrophobic self-assembled monolayer of octadecyl-tri-chloro-silane (OTS). While with these surfaces greater thickness control ($\pm 10 \mu\text{m}$) and smaller layer thicknesses (down to 25 μm thickness) were achievable compared to the used PDMS sheet these surfaces were more difficult to separate from the partially cured thiolene patterns and thus were not further investigated.

To fabricate on-chip laminated tube connectors 1 mm thick NOA61 cylindrical rings of outer diameter (O.D.) 4 mm and inner diameter (I.D.) 1,5 mm were defined photolithographically on a PE sheet and were laminated shortly after hard curing of the last layer of the chip. After hardcuring and peeling off the PE sheet a second set of identical rings was patterned, aligned and laminated to the first. After 30 min hardcuring the PE sheet was peeled away. “Barbed” pressure-fitting “male” connectors obtained are shown in Fig.2a,b. These male connectors could be fitted to “female” connectors (Fig.2c) assembled separately by using pieces of concentric PE (1,5mm O.D.) / silicone (4 mm O.D.) / PVC (6 mm O.D.) tubings. The connectors withstood fluid pressures up to 3 bar and could be reconnected several times thanks to the toughness and good adhesion of the thiolene polymer.

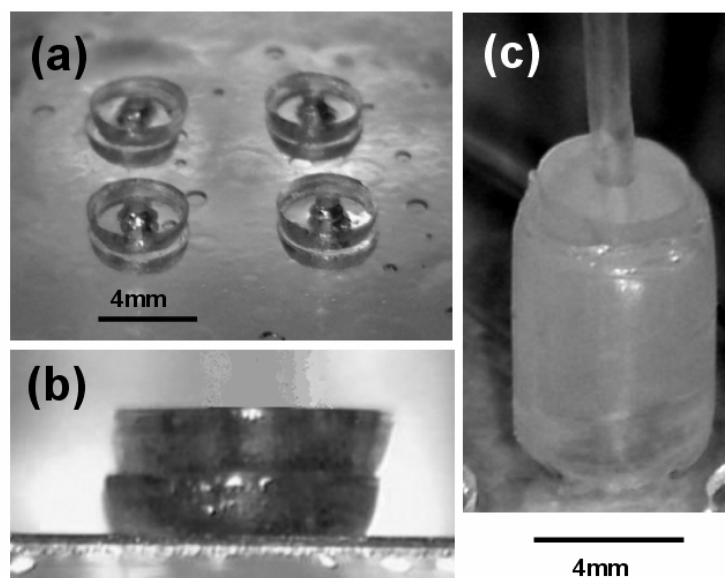


Fig.2 (a,b) laminated bi-layer “barbed” male connectors, (c) matching “female” connector, assembled by using different types of tubes.

2. Design of 3x3 combinatorial synthesis chip

In Fig.3 is shown the schematic layout of the fabricated combinatorial synthesis chip. The chip was designed to perform 3x3 combinatorial synthesis of azodyes by a two step reactions that has been described by some of us in ref. [2]. In this reaction, three different aromatic amines (reagents A,B,C) are firstly reacted with sodium nitrite (reagent D) to form the corresponding diazonium salts. Then, these products are combinatorially reacted with three different aromatic phenols (reagents E,F,G) to yield nine different azo-dyes. Preliminary tests showed the feasibility of the synthesis of a single dye in a glass/thiolene chip [3].

In the layout of the chip reagents A,B,C are delivered through fluid access holes a,b,c in layer 5 and are fed to fluidic reservoirs in layer 4 positioned at the entrance of three serpentine channels numbered 1,2,3. Reagent D is fed through fluid access hole d and is distributed by channels in layer 2 and vias in layer 3 to encounter reagents A,B,C in the mentioned fluidic reservoirs. Distribution channels in layer 2 are designed in such a way that path lengths for reagent D towards the three reservoirs are the same. This guarantees equal flow rates if liquid D is driven by a constant pressure source. Mixing is done in serpentine channels 1,2,3 and products are then fed to a 3x3 matrix of fluidic reservoirs where they encounter reagents E,F,G. The latter are fed through fluid access holes e,f,g and distributed by channels in layer 2, passing fluidic vias in layer 3. To guarantee equal flows, once again all distribution channels are designed to have the same path lengths. The combinatorial reactions then occur in 9 serpentine channels and products are recovered through 9 outlet holes in layer 5.

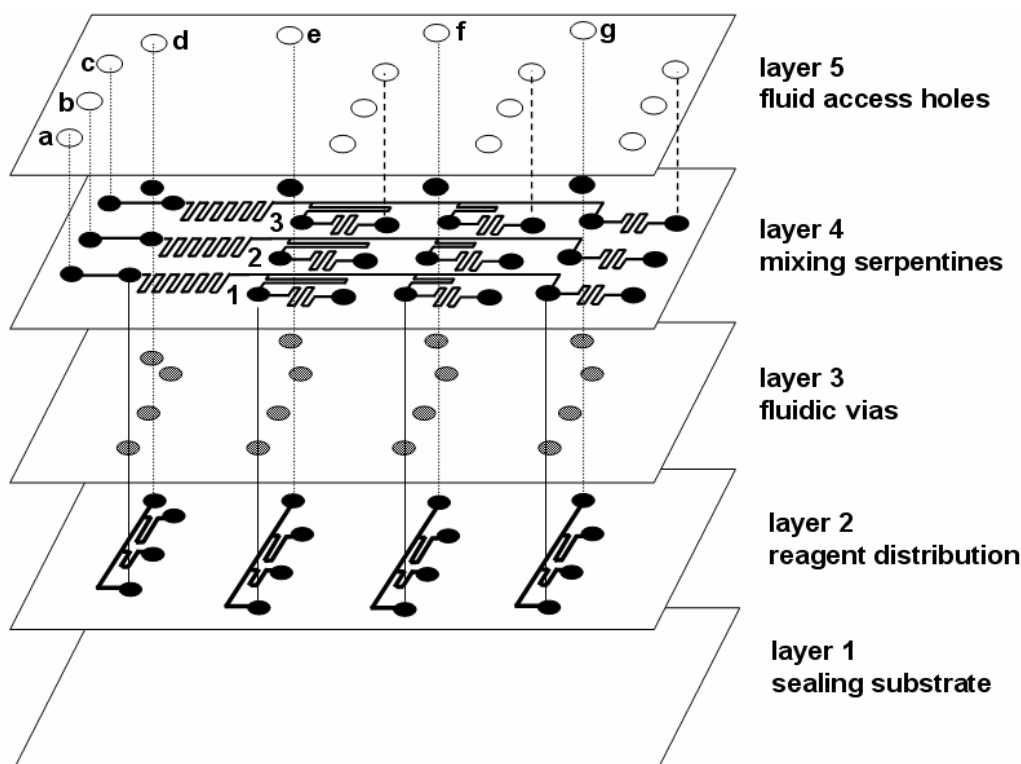


Fig.3 schematic layout of combinatorial synthesis chip

3. pH measurements with the bead array sensor

Sensor beads of diameter 300 μm functionalised with various azo-dyes are manually introduced into the cavities of the array with a toothpick under a microscope. pH measurements are performed by flowing a buffer solution (composed of CH_3COONa 0.04 M, H_3BO_3 0.04 M, NaH_2PO_4 0.04 M, Na_2SO_4 0.1 M, adding HCl 3M and NaOH 2M to adjust pH) through the chip using a peristaltic pump (Gilson MINIPULS 3) at a flow rate of 1-2 mL/min and monitoring the color of the beads at different time intervals using a commercial scanner .

azo-dye head groups used are

- #1: 2-((4-(2-hydroxyethylsulfonyl)-phenyl)diazenyl)-4-methoxyphenol
- #2: 2-((4-(2-hydroxyethylsulfonyl) phenyl)diazenyl)-3,6-dimethylphenol
- #3: 4-((4-(2-hydroxyethyl-sulfonyl)phenyl)diazenyl)-2,6-dimethylphenol
- #4: 4-((4-(2-hydroxyethylsulfonyl)-phenyl)-diazenyl)naphthalen-1-ol
- #5: 5-((4-(2-hydroxyethylsulfonyl)phenyl)diazenyl)quinolin-8-ol

The pK values of these dyes can be found in the electronic supplementary information of ref. [2].

References

- [1] J. T. Cabral, S.D. Hudson, C. Harrison, and J.F. Douglas, Langmuir , 2004, **20**, 10020
- [2] T. Carofiglio, C. Fregonese, G.J. Mohr, F., Rastrelli, U. Tonellato, Tetrahedron 2006, **62** , 1502.
- [3] T. Carofiglio, private communication.