Supplementary Material (ESI) for Lab on a Chip

Microfluidic Probe: A new tool for integrating microfluidic environments and electronic wafer probing – *Supplementary Information*

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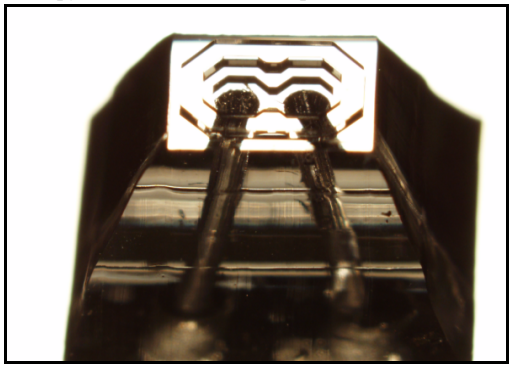
Microfluidic probe fabrication

The microfluidic probe assembly consists of three separate parts: an elastomeric tip, a polystyrene support layer and attached tubing, and a reusable metal support arm for attachment to a micropositioner. The patterning of the elastomeric tip varies depending on the substrate type and will be described below for each type.

Microfluidic Probe for planar substrates

A master mold was prepared on a 4" silicon wafer using SU-8 2150 photoresist. In order to pattern the gasket as well as the recessed channel, we used a two layer process. First SU-8 was spun and baked to yield a 70-100 micron thick layer that was exposed on an EVG 620 contact mask aligner with a 350 nm low-pass filter to leave a recessed area corresponding to the gasket. Without developing the resist, a second 100-500um layer was spun, baked and exposed to yield a raised mesa corresponding to the recessed channel. Both layers were simultaneously developed to yield the two layer structure shown in Figure 2a of the technical note. The mold was treated with trichloromethylsilane vapor prior to casting to aid the release of the elastomer from the mold. Sylgard 184 (Corning) polydimethylsiloxane (PDMS) mixed at a ratio of 10 parts base to 1 part curing agent was used to make a casting of the master mold in a 5mm thick layer. After removal from the mold, the PDMS was cut by razor into truncated pyramids as shown in the photo below.



The polystyrene (PS) support layers were 1 centimeter squares cut from standard disposable petri dishes using a heated razor blade. Holes were drilled a few millimeters apart with a drill press to accept 28-gauge PTFE tubing for the fluid inlet and outlet. We also tried to fabricate the support layers from glass, which allowed us to make autoclavable probe tips; however, the cutting and drilling were significantly more difficult and led us to use polystyrene instead.

The PDMS tips were permanently bonded to the PS (or glass) support layers by exposing both pieces to a low-power oxygen plasma for a few seconds, pressing them together and heating to 70°C for 1 hour. After bonding, holes were cored through the PDMS with a modified syringe needle to connect the channels or gaskets to the holes in the polystyrene. The PTFE tubing was inserted into the holes in the polystyrene and secured using two-part epoxy or uncured PDMS.

The metal support arms were machined from 1/16" thick cold rolled steel and attached to an MC Systems micropositioner. The PDMS/PS/Tubing assembly was attached to the metal support arm using cyanoacrylate glue to facilitate removal of the disposable portion of the device from the reusable support arm.

Microfluidic probe for substrates with buried channels

The master mold was prepared in an identical fashion to the previous mold except that only the first layer of SU-8 was used. The devices were assembled in the same manner as the previously described devices.

Microfluidic probe for substrates with channel walls

The PDMS for these devices required no molding and was simply cast on a bare silicon wafer. For the flat bottomed probes, the holes were positioned so as to be contained within the channel walls defined on the substrate. This is visible in the attached video depicting the use of the microfluidic probe. The probes were assembled as before.

Substrate Preparation

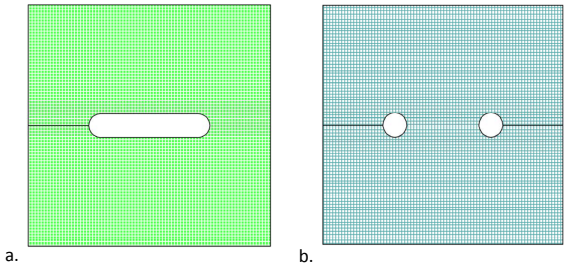
Planar Substrates

The planar substrates used for testing were clean glass or silicon wafers with no additional preparation.

Substrates with buried channels

The buried channels were prepared in SU8 on glass or silicon wafers using a multiple exposure method. SU-8 2150 was spun and baked to yield a 350-600 micron thick layer on a clean silicon wafer. The resist was exposed through a mask corresponding to the walls of the channel (green region in the figure below). The 350 nm LP filter was used for this exposure. The wafer was post-exposure baked at 95°C just long enough for the latent image to appear. A second exposure was performed at a low dose through a mask corresponding to the ceiling of the channels (blue region in figure below), without using the low-pass filter. Because of the high absorption of the deep UV radiation by the SU-8, it is possible for the top 50-100 mcicrons of SU-8 to be exposed without the underlying resist being exposed. The resist is then fully baked and developed as usual leaving a buried

channel in the SU-8. Ultrasonication of the developer solution was required to increase the development rate.



Substrates with channel walls formed by ridges

These substrates were silicon or glass wafers. Ridges, 100-500 microns high, were patterned in SU-8 2150 corresponding to the walls of the channels. The width of the ridges was typically 100 um. An example is shown in the technical note in Figure 2f.

Sensing Measurement Details

Nanowire device preparation

Silicon nanowire field-effect sensors were prepared from silicon-on-insulator wafers by top-down fabrication using a method similar to that described in reference 2. The surface of the wafer was passivated with a 2um thick SU-8 layer to insulate the electrodes from the buffer solutions and windows patterned over the channels nanowire FET devices. For the pH sensing measurements, the native oxide on the silicon surface provided sufficient electrical insulation to keep the leakage current well below the device current and no additional surface functionalization was needed.

Experimental details

0.1X phosphate buffered saline solutions were titrated with hydrochloric acid or sodium hydroxide to yield pH 6.0 to 8.0 in 0.5 pH unit increments. The nanowire device was biased into the highest current portion of the subthreshold region by applying a voltage to a metal pseudo-reference electrode in the solution to provide maximum sensitivity to surface charge. The source drain bias was fixed at 1V. Each solution was forced through the channel for 5 seconds and then allowed to sit while the current stabilized.

Patterning Details

Probe fabrication

The PDMS portion of the patterning probes was formed in two separate layers which were then plasma bonded after patterning and cutting. The bottom layer contained channels and through-holes for fluidic access as shown in Figure 5 of the technical note and was cast from a master mold patterned with a two layer SU-8 process. First a 50 micron thick layer of SU-8 2035 was patterned to form mesas corresponding to the channels to be used for surface patterning. A second much thicker layer of SU-8 2150 was used to pattern pillars corresponding to the through-holes. A 100-200 um thick layer of PDMS was spun onto the mold and cured at 70C. The pillars were high enough to protrude from the spun-on layer of PDMS ensuring that the holes went all the way through the layer when it was separated from the mold.

A second mold for the upper layer of PDMS was patterned with 75um thick SU-8 ridges corresponding to a set of fan-out channels. These channels were designed to connect to the through-holes in the lower PDMS layer on one end. The second end of each fan-out channel was terminated with a reservoir large enough to accept a coring-needle. A 5 mm thick PDMS layer was cast from this mold and cut to size.

The upper PDMS layer was permanently plasma bonded to a PS support layer with one hole drilled for each fan-out channel. Holes were cored through the PDMS to connect the fan-out channels to the holes in the PS. The lower PDMS layer was aligned to the upper layer so the through-holes mated to the ends of the fan-out channels as shown in figure 5c and permanently plasma bonded. The assembly was fitted with PTFE tubing as before and mounted on a metal support arm.

Experimental details

The substrates for testing the patterning probe were glass slides cleaned with piranha solution (3:1 H_2SO_4 : H_2O_2) before use and dehydrated by vacuum desiccation. The slides were treated with 3-aminopropyltriethoxysilane (1% by volume in anhydrous toluene) in a nitrogen glove-box for at least 1 hour to yield an amine-terminated surface. The substrates were rinsed with fresh toluene and dried with a nitrogen stream. The presence of the APTES monolayer was confirmed by contact angle measurement.

The patterning probe was positioned on the surface of the APTES treated substrate using the micromanipulator. Fluorescein isothiocyanate (FITC) in sodium bicarbonate buffer at pH 8.5 was flowed through each channel for 60 seconds. The channels were then flushed with fresh buffer to remove unbound FITC and cleared with a nitrogen stream before removing the patterning probe from the surface of the substrate. The substrate was imaged using the green channel of a Nikon epifluorescence microscope.

Videos

We have included brief videos of each type of fluidic probe in use. Each video is recorded using a lens positioned below a clear glass substrate to give a point of view looking up at the probe. The videos show the probe being brought into contact with the substrate and then show the introduction and clearing of various dyed solutions. Videos are included for a planar substrate, a substrate with a buried channel and substrate with SU-8 ridges forming channel walls.