Hollow Atomic Force Microscopy Probes for Nanoscale Dispensing of Liquids

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ABSTRACT

To enable the printing of nanometer sized droplets with volumes in the femto and attoliter range and sub-micron droplet spacing, a nanoscale dispenser (NADIS) based on an atomic force microscopy probe has been developed and microfabricated. The probe consists of a cantilever with a hollow core, which is connected to a reservoir located in the chip. The hollow cantilever acts as a microfluidic channel that connects the reservoir to the dispensing tip located at the free end of the cantilever. The tip possesses an opening at its apex with a typical size of 200 nm, realized by focus ion beam milling. The transfer of liquid from the tip opening to the surface occurs by contacting the probe tip with the sample surface and is driven by capillary pressure alone. To overcome the serial manner of writing by using a single probe, arrays of hollow AFM probes were fabricated. The feasibility to dispense droplets in parallel has been demonstrated.

Keywords: AFM, nanopatterning, microfluidic spotting, microarray

1 INTRODUCTION

The demand for specific tools intended for the deposition of small amount of material on a nanoscale at predefined locations is continuously increasing. Several dispensing tools for liquids, such as ink-jet or pin-spotting heads have been developed. They are currently widely used for various applications, such as for writing microarrays (or biochips) used in proteomics to determine the presence and/or amount of proteins in biological samples. However, such printing tools have some limitation regarding the volume of deposited material (sub-picoliters and above) [1] and the dimension of the spotted dots (typically 10 to 100 µm). Decreasing both the volume of the deposited liquid and the spot size is an important issue regarding an economic use of the dispensed liquid. Furthermore, microarrays with a higher spot-density need less biological analyte to perform an affinity assay.

Nanoscale dispensing (NADIS) is a versatile method developed to deposit small amounts of material on a substrate. The method is based on the atomic force microscope (AFM) technology. In NADIS, the dispensing method uses specifically fabricated probes. Like AFM probes, the NADIS probes are made of a flexible cantilever with a sharp tip. The tip is hollow, and a small aperture is located at the apex of the tip. The volume inside the hollow tip is filled with liquid. Due to capillarity, the liquid doesn't flow through the aperture by gravity alone. Once a NADIS probe in brought in contact with the sample, the liquid will wet the sample surface, and after withdrawal of the probe, a small volume of liquid will remain on the sample (see Figure 1). The volume of the remaining droplets can be as small as a few tens of zeptoliters (1e-21 liter), depending on several parameters such as tip aperture size [2]. Since the NADIS probe is driven by a standard AFM instrument, the droplets can be dispensed with a high spatial accuracy. The NADIS technology allows thus to deposit on a predefined location on-demand single ultrasmall droplets.

Figure 1 Conceptual sketch of nanoscale dispensing (NADIS). A specifically designed AFM probe containing liquid in its apertured tip is brought in contact with the sample surface. During the withdrawal of the probe, a small droplet remains on the sample surface.

In the first generation NADIS probes described above, the liquid was loaded directly on the cantilever. Due to the small amount of loaded liquid, in ambient environment the dispensing was limited to liquid with low volatility, such as glycerol or tetraethylene glycol. If such a first generation NADIS probe is loaded with water, the water evaporates within a few seconds. However, the aim is to use NADIS for a large variety of liquids, especially like water-based solution used as buffer for biological molecules.

In order to overcome this evaporation, a new generation of NADIS probes has been developed and microfabricated. The second generation NADIS probes are made of a hollow cantilever, with large reservoir located in the chip body. This allows first an easier loading due to the larger size of the reservoir, and second permits also to load a larger volume. The hollow core inside the cantilever acts as microfluidic channel connecting the reservoir to the tip. A schematic sketch is shown in Figure 2. The location of the reservoir on the chip rather than on the cantilever enables

furthermore the integration of a microfluidic system connected to the chip.

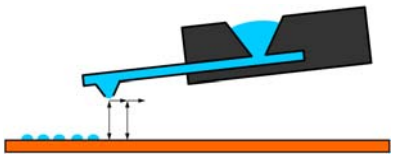

Figure 2: In the second generation of NADIS probe, the reservoir is located in the chip body, and the liquid is driven towards the tip by a microfluidic channel inside the cantilever (sketch not to scale).

The applications of the NADIS probes are various, going from the patterning of a surface with biomolecules or nanoparticles suspended in the dispensed liquid [3], to the local modification of responsive surfaces induced by the presence of the liquid [4].

2 MICROFABRICATION

2.1 Microfabrication process

Whereas the first generation NADIS probes were based on a commercially available cantilever, the second generation NADIS probes are entirely microfabricated using CSEM facilities. The microfabrication relies on the thermal fusion bonding of two pre-processed silicon (Si) wafers. Such a process allows the fabrication of hollow structures without using a sacrificial layer. The process is depicted in Figure 3.

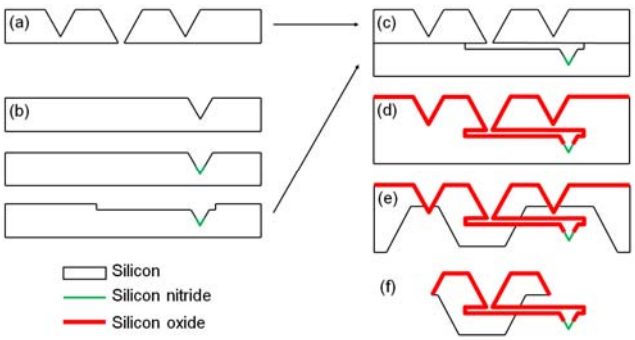

Figure 3: Process flow for the microfabrication of NADIS probes with hollow cantilevers.

(a) A first Si wafer is structured by standard photolithography and anisotropic KOH etching in order to create both, the reservoirs and a rectangular shaped V-groove that defines the future chip. (b) A second silicon wafer is processed to etch the pyramidal shaped pits by wet etching. A 100 nm thin silicon nitride (Si_3N_4) layer is than deposited on the wafer, and structured by dry etching in order to remain only at the bottom of the pits. The microfluidic channels are processed by dry etching. (c) The two pre-structured wafers are cleaned, brought together and aligned. (d) The two wafers are bonded by thermal fusion bonding and a 1 µm silicon dioxide (SiO_2) layer is grown by thermal oxidation on the exposed Si surfaces. (e) The hollow cantilever is released by wet etching. The depth of the etching is adjusted to release partly the peripheral V-groove. To perform the wet etching, the wafer was placed in a single-side etching chuck so that the cantilever side with the reservoirs was not exposed to the KOH. This avoids also an exposition of the microfluidic channels to the etching liquid. (f) The chip is extracted from the finished wafer. The released probes were then coated with a metallic layer (Au + Cr as adhesion layer) on both cantilever side. The back side metallization increases the reflectivity for the AFM laser beam (used to detect the cantilever deflection), whereas the tip side metallization avoids charging effects during the next and final step. Finally, the tip aperture is opened using focused ion beam (FIB) milling.

The hollow cantilever is made of SiO_2, and the tip apex is made of Si_3N_4. The presence of Si_3N_4 at the tip is motivated by the aim to deposit ultrasmall droplets, which requires first a small tip aperture, and thus a thin tip wall (the Si_3N_4 layer is much thinner than the SiO_2 layer), and second a sharp tip apex. A tip made by a SiO_2 layer grown thermally would present a blunt tip apex, which would increase the wetted area during the deposition of the droplets (see Figure 4).

Figure 4: Compared to a thick and blunt SiO_2 tip apex, a thin and sharp Si_3N_4 tip apex is better suited for the deposition of ultrasmall droplets.

2.2 Design

Several types of probes were fabricated, including cantilevers of different lengths, single- and double-beam cantilevers, single cantilevers and one-dimensional cantilever arrays.

Cantilevers with lengths up to 500 µm were designed. The longer hollow cantilevers, despite their area moment of inertia which is much larger than for plane cantilevers, have an estimated spring constant similar to a contact-mode plain cantilever.

A NADIS probe with a single-beam cantilever is connected to one reservoir. But NADIS probes having a U-shaped two-beam cantilever structure, with one beam connected to an inlet reservoir and the other beam to an outlet reservoir, were also designed. Such double-beam cantilever structure would allow to rinse them by flushing a liquid from the inlet reservoir to the outlet reservoir.

And finally, NADIS probe arrays have been designed. Single- and double-beam levers were considered for the NADIS cantilever arrays. All cantilevers in an array can be

connected to one (single-beam cantilever) or two (double-beam cantilever) reservoirs, so that all dispense the same liquid. An example of such a design is shown in Figure 5. To allow the delivery of a different liquid from each cantilever within one array, each cantilever has to be connected to specific reservoirs via independent fluidic networks, one for each cantilever. Each single-beam cantilever is connected to its own reservoir, and each double-beam cantilever is connected to two own reservoirs, the inlet reservoir and the outlet reservoir. The number of reservoir integrated on the chip is therefore directly proportional to the number of cantilever, and has thus a direct influence on the chip size (see Figure 6).

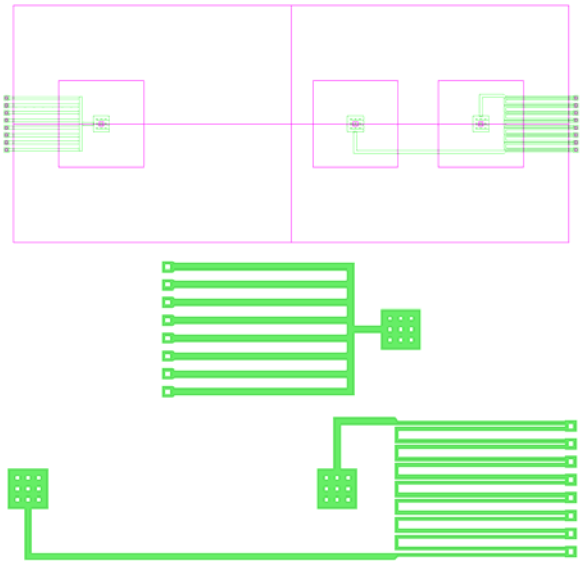

Figure 5: Top: chip design containing two 8x1 arrays of NADIS probes, one with 8 single-beam cantilevers connected to one reservoir (left), and one with 8 double-beam cantilevers connected to an inlet and one outlet reservoir (right). The chip size is 1.6 mm by 3.8 mm. Bottom: detail of both microfluidic networks used in the chip depicted at the top.

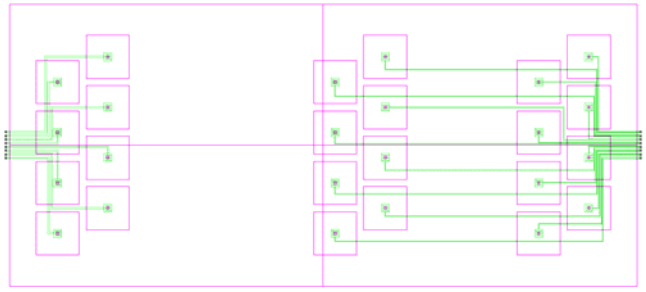

Figure 6: Chip design containing two 8x1 arrays of NADIS probes, one with 8 single-beam cantilevers (left), and one with 8 double-beam cantilevers (right), with a dedicated microfluidic network for each cantilever. The chip size is 3.8 mm by 8.5 mm.

3 RESULTS AND DISCUSSION

3.1 Microfabricated probes

Figure 7 represents scanning electron microscope (SEM) micrographs of the wafer containing the reservoirs and the V-grooves before the thermal fusion bonding. This wafer corresponds to the step (a) in Figure 3. Some examples of microfabricated probe arrays are shown in Figure 8, and Figure 9 depicts tip apertures made by FIB milling.

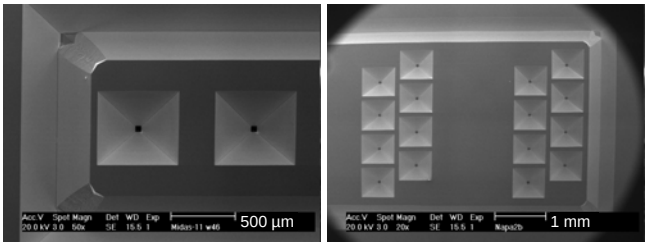

Figure 7: SEM micrographs of the wafer containing the reservoirs.

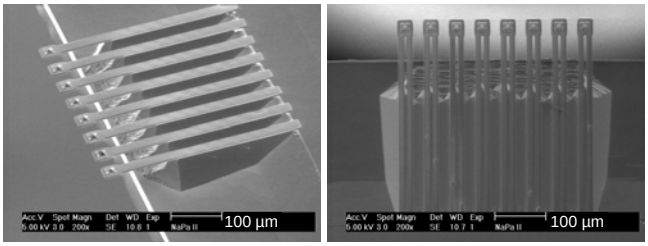

Figure 8: NADIS array with short cantilevers (SEM micrographs).

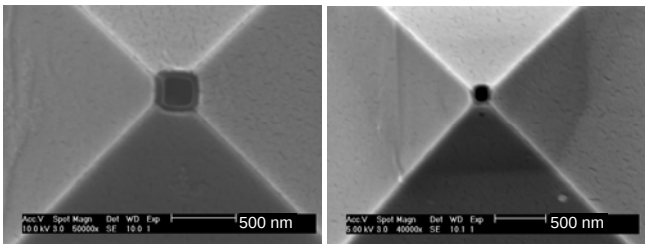

Figure 9: SEM micrographs of two different apertures milled by focused ion beam at the Si_3N_4 tip apex.

During microfabrication, one observation made was that during the drying following the last rinsing, capillary forces bent the long and flexible cantilevers towards the wafer with exceeding forces, resulting in a rupture of the longer cantilever. This phenomenon occurred as well for the single cantilevers as for the cantilever arrays. Currently, investigations are ongoing to avoid this problem. One of the investigated ways was to replace the last wet etching with dry etching, which turned out to be successful for the release (see Figure 10). Unfortunately, the dry etching

removed also the thin Si_3N_4 tip. However, a correction to eliminate this problem has been implemented in the new ongoing microfabrication run.

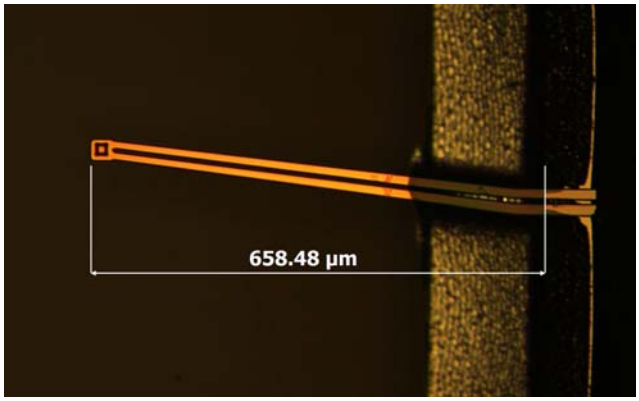

Figure 10: Optical image of a long cantilever released by dry etching. The dimension corresponds to the release length.

3.2 Experimental results

All nanoscale dispensing experiments were performed in ambient environment on standard AFMs (Veeco, Digital Instruments, Dimension 3100 and MultiMode).

The filling of the hollow cantilever was characterized by measuring the cantilever resonance frequency. A filled cantilever, due to the presence of the additional mass of the liquid, has a resonance frequency which is shifted to a lower value compared to the empty cantilever.

The transfer of liquid is characterized by measuring two successive deflection-distance curves. During the first probe-sample contact, a droplet is deposited on the surface. In the deflection-distance curve describing the second contact, a snap-in position far from the surface is recognizable in the extending curve. The snap-in is due to the fact that, when the tip enters in contact with the previously deposited droplet, a capillary force bends the cantilever towards the sample surface. The distance between the snap-in position and the sample surface corresponds approximatively to the height of the droplet deposited during the first contact. The presence of such snap-in positions describes thus a successful transfer of liquid. An example with glycerol is given in Figure 11. However, such characterization could not be demonstrated with water, mainly due to the almost instantaneous evaporation of the deposited droplet.

Examples of depositions using the hollow NADIS cantilevers are depicted in Figure 12. For those experiments, NADIS probes released by dry etching, i.e. without Si_3N_4 tips, were used, resulting in droplets with large dimensions due to the large probe aperture. The difference in droplet dimensions between both pictures is due to a difference in sample surface wettability (hydrophilic on the left hand side, and hydrophobic on the right hand side)

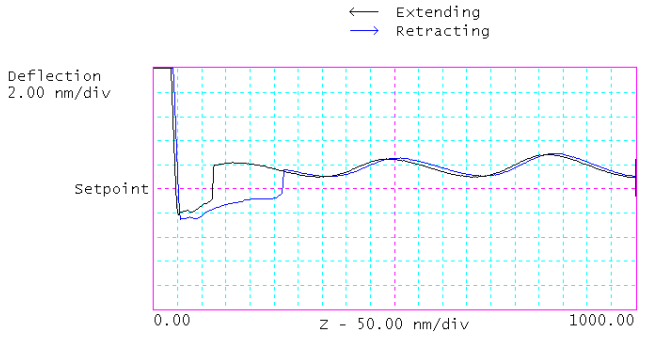

Figure 11: Typical deflection-distance curve that characterizes the transfer of liquid. Note the snap-in position at ~70 nm from the sample surface

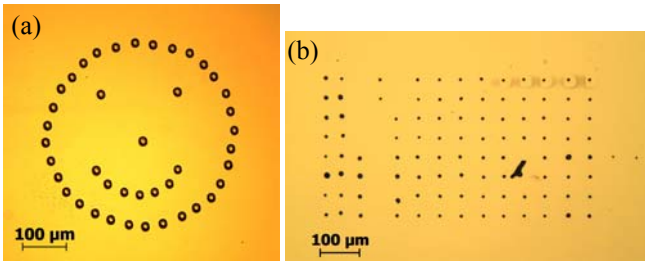

Figure 12: Optical images of an array of dispensed glycerol droplets (a) deposited with a single probe, (b) deposited with a probe array of 8 NADIS cantilever

4 ACKNOWLEDGMENTS

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