

Surface modification and modulation in microstructures: controlling protein adsorption, monolayer desorption and micro-self-assembly

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Abstract

The surface-to-volume ratio increases with decreasing scale, thus, controlling and changing the surface properties of microstructures can be a powerful tool in the design, fabrication and use of microsystems. This paper overviews several recent projects that utilize the modulation of surfaces from hydrophobic to hydrophilic and vice versa, or from protein adsorbing to non-fouling, with applications in biomedical microdevices and self-assembling microelectromechanical systems.

(Some figures in this article are in colour only in the electronic version)

1. Introduction

Careful design and control of surface properties can be of great benefit in engineered microsystems. The laws of scaling entail that with decreasing size, effects related to surface area (e.g., capillarity, electrostatic charge, adsorption of molecular layers) increasingly dominate effects related to volume (e.g., gravity, inertia). This is the well-known cube–square law, which relates the cubic scaling of volume to the quadratic scaling of surfaces; one of its consequences is the necessary existence of a ‘crossover point’ where the surface effect starts to overtake the volume effect. This crossover point is typically seen at scales of just below 1 mm [1].

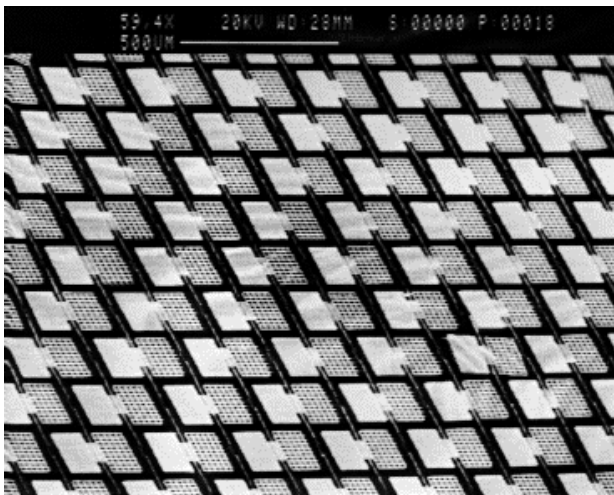
Thus, a systematic study of surface properties at the microscale, combined with methods for controlling and modifying them, has been the topic of extensive recent research. In this paper, we investigate this field in greater detail, spanning a range of research activities from microelectromechanical ‘smart surfaces’, to micropatterned ‘programmable chemistry’ that controls wettability and bio-fouling, to self-assembling microsystems.

The remainder of this paper is organized as follows: section 2 begins with earlier work on ‘smart surfaces’

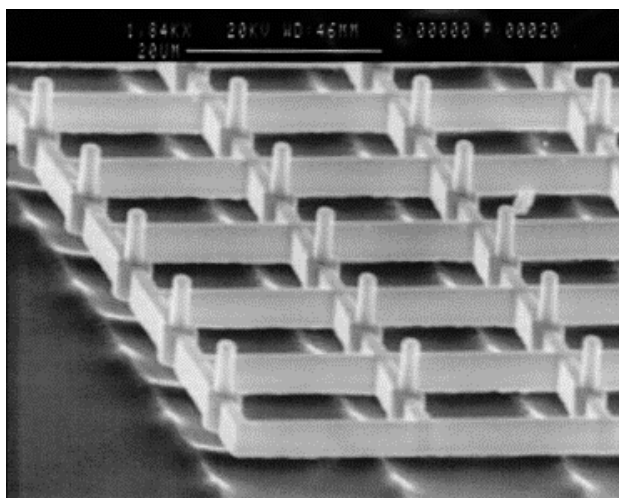
comprising arrays of microactuators, which are employed for micropositioning tasks. Section 3 investigates assembly at the microscale, describing massively parallel approaches for self-assembly based on capillary forces. In section 4, we discuss surface modifications to control hydrophobicity and protein adsorption, which has various applications in microassembly and biomedical microdevices. We conclude this paper with a summary and an outlook on open problems and future work.

2. Ciliary microsystems

During the last decade, several research groups have studied arrayed microactuators [2–22], obtaining their inspiration from biological cilia and distributed robotic conveyor systems. While these systems differ somewhat from the central theme of this paper (MEMS cilia are discrete mechanical devices, while here we want to focus on continuous properties of surfaces), there are still good reasons to review this earlier work: first, these systems represent a different kind of ‘smart surface’ able to interact with objects in contact in a controlled, programmable fashion. Second, cilia arrays have given rise to a general theory of massively parallel,



(a)



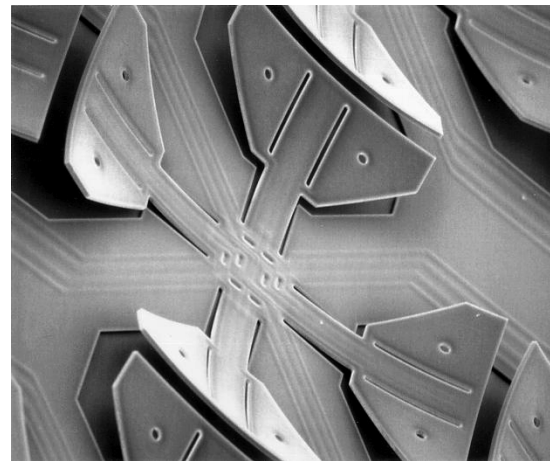
(b)

Figure 1. (a) Array of single crystal silicon microactuators. Individual actuator size is $240 \mu\text{m} \times 180 \mu\text{m}$. (b) Actuator detail with suspended silicon structures (spacing between posts: $20 \mu\text{m}$) (figures from [11]).

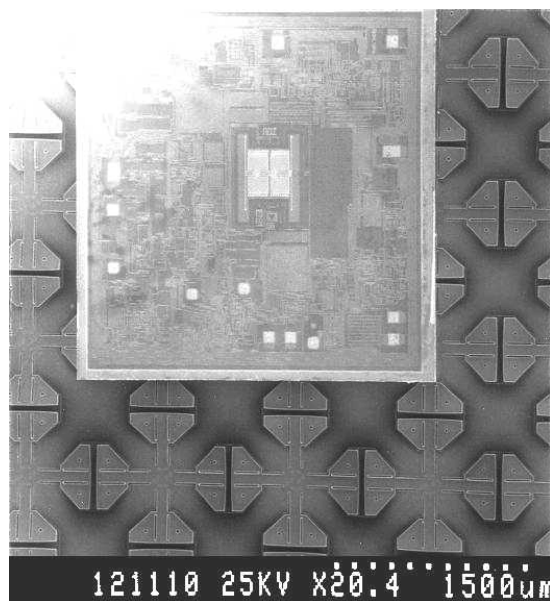
distributed manipulation [18], which is directly applicable to self-assembling systems. Here, we briefly review some of our work on microactuator arrays and summarize important theoretical results on micromanipulation and microassembly.

Actuator arrays built in single crystal silicon were introduced in the early 1990s [7, 11]. Several thousand actuators were integrated in a dense layout on a single chip (figure 1). These arrays were able to move small chiplets placed on top of them; however, the small range of motion (approximately $5 \mu\text{m}$) and limited force of each individual actuator rendered them less effective for general micromanipulation tasks.

Suh *et al* [15, 19] built several kinds of thermal bimorph polyimide microcilia, which were successfully used to position and orient millimeter-sized objects (figure 2) under optical and scanning electron microscopes [18, 23]. Employed upside-down, these cilia arrays also enabled the first ‘walking’ microrobot with full three degrees of freedom [24].



(a)



(b)

Figure 2. (a) ‘Motion pixel’ made of four polyimide microcilia (total size $1.1 \text{ mm} \times 1.1 \text{ mm}$). (b) Cilia array transporting an ADXL50 chip (size about $3 \text{ mm} \times 3 \text{ mm}$) (figures from [15] by Suh).

Coordinating hundreds or thousands of individual cilia to achieve a micropositioning task has been the topic of extensive research in robotics [18, 25, 26], which is beyond the scope of this paper. Here, we simply present some key findings that will be useful in our further discussion.

Modeling of microactuator arrays is conveniently accomplished with force vector fields, which assign a lateral force to each point in the plane. For example, figure 3 shows such a force vector field and its effect on two objects that are placed on top of it.

Even simple fields consisting only of two separate regions with constant force vectors are sufficient to position an object without any sensor feedback. Figure 3 shows the underlying principle: the lateral force on an object in contact integrates over its contact area. The resultant force for the left part is a larger force pointing towards the right. The resultant force

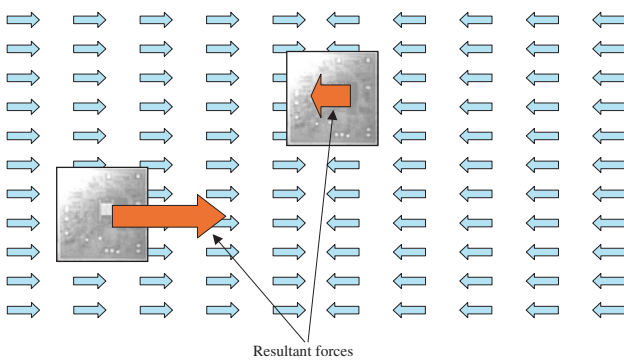


Figure 3. Concept of a programmable force field (PFF) for positioning of microparts. The force acting on the part is obtained by integrating the PFF over its surface area. A microactuator array could be used to generate this PFF.

for the right part, however, is a smaller force towards the left, since its contact area encompasses regions of forces pointing to the left as well as to the right. It is easy to see that there exist placements where the part is in equilibrium. These equilibria are the final rest positions for an object that is placed at an arbitrary initial position.

We can thus think of microactuator arrays as ‘programmable force fields’ (PFFs). It has been shown that PFFs can efficiently perform a broad range of micromanipulation tasks such as positioning, orienting and sorting [18, 25]. In addition, these general results on PFFs are very useful to devise strategies for massively parallel microassembly. The next section will investigate microassembly in greater detail.

3. Micro-self-assembly

Recent developments in the area of microfabrication techniques offer the opportunity to create a large variety

of functional microdevices (e.g., chemical, electrical, mechanical and optical transducers). Practical applications require integration of such devices into compact and robust microsystems. Monolithic integration often faces problems of material and process incompatibilities of different functionalities. Ideally, one would like to build each functional subsystem with optimized materials and processes, and then assemble them into a complex microsystem. Researchers have been investigating the adoption of macroscale ‘pick and place’ methods to assemble micro- or even nanoscale components [27–29]. One major concern in these approaches is the ‘sticking effect’ between assembly manipulators and components, due to electrostatic, van der Waals or surface tension forces [1]. On the other hand, new assembly approaches have emerged by taking advantage of this sticking effect: Whitesides and co-workers first demonstrated capillary-force-driven assembly of a simple circuit [30]; Srinivasan *et al* extended the capillary-force-driven strategy to assemble identical microscopic parts onto a single substrate [31]. In this section, we describe a novel approach based on capillary action to achieve multiple-batch assembly and bonding of microcomponents onto a substrate. In addition, electroplating as a post assembly process is used to establish electrical connections for assembled components in a parallel manner [32–35].

3.1. Self-assembly principles

In our assembly method, capillary force is exploited to drive the assembly, and the force is created by a hydrocarbon-based lubricant between hydrophobic surfaces as in [30, 31]. A schematic illustration of our assembly process is shown in figure 4. We fabricate a silicon substrate with gold patterns as destined binding sites for assembly of parts (figure 4(a)). To activate the binding sites on the substrate, a hydrophobic alkanethiol ($\text{CH}_3(\text{CH}_2)_n\text{SH}$) self-assembled monolayer (SAM) is adsorbed on hydrophilic gold patterns. For each batch of microparts, only desired binding sites on the substrate are activated by selectively deactivating other binding sites. In the deactivation process, electrochemical

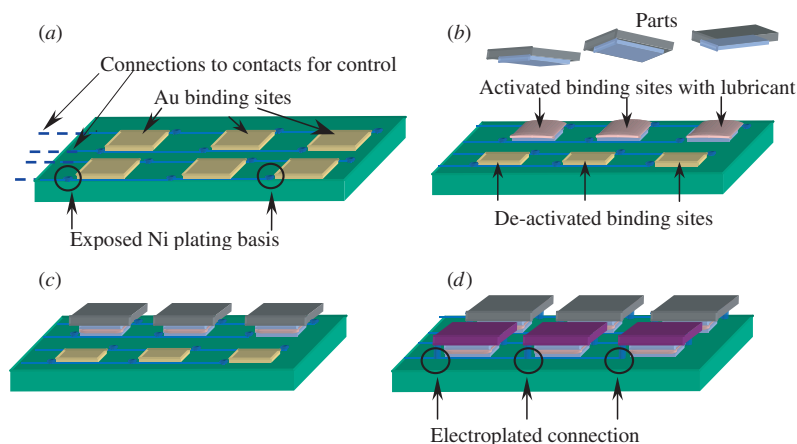


Figure 4. Schematic flow of multi-batch capillary-force-driven self-assembly. (a) A fabricated substrate for assembly with gold binding sites and nickel plating basis. (b) A substrate prepared for first batch assembly. The substrate is immersed in water with lubricant wetting exclusively the activated binding sites. (c) First batch assembly. (d) By repeating the assembly process, the second batch of components is assembled. Electroplating is performed afterwards to establish electrical connections (figure from [35] by Xiong).

reductive desorption of the SAM, i.e., $\text{CH}_3(\text{CH}_2)_n\text{SAu} + e^- \rightarrow \text{Au} + \text{CH}_3(\text{CH}_2)_n\text{S}^-$ [36, 37] is performed (figure 4(b)). Thus we can control the assembly not to occur in the gold regions where the SAM desorption has taken place. To create the driving force, we apply a lubricant to the substrate prior to assembly. Next, the substrate is immersed in water and the lubricant forms droplets exclusively on the activated binding sites. After the parts are transferred into water, the parts with a hydrophobic side are attracted and aligned to the binding sites on the substrate.

The lubricant can be cured by heat and permanently bonds the part to the substrate (figure 4(c)). The SAM adsorption, desorption and assembly steps can be repeated for multiple batches of parts assembly. Finally, electrical connections can be established between the assembled parts and the substrate by electroplating (figure 4(d)).

3.2. Experiments and results

To demonstrate the assembly, we use two different kinds of components: silicon diced chips (1 mm \times 1 mm) with one gold-coated side and commercial light emitting diodes (LEDs). Destined substrates for assembly are specifically designed for different kinds of components. For the square silicon chips, electrically isolated gold stripes are patterned in a lift-off process on a silicon substrate with a thermal oxide layer of approximately 4000 Å. A passivation layer of silicon nitride is patterned by photolithography and reactive ion etching (RIE) until gold squares (1 mm \times 1 mm) as the binding sites are exposed. The substrate for LED assembly has an additional metallization layer of 350 Å Cr/Ni, which is patterned as the basis for electroplating prior to the Cr/Au layer deposition. To activate the binding sites for assembly, a SAM is adsorbed on all the gold binding sites after having been immersed in ethanolic dodecanethiol solution for several hours. For multi-batch assembly, desired binding sites for the first batch assembly remain hydrophobic, while other binding sites are deactivated and transformed to hydrophilic via an electrochemical SAM desorption process [32].

To prepare for the assembly, a hydrocarbon lubricant composed of 97 wt% triethyleneglycol dimethacrylate (Sigma) as crosslinker and 3 wt% benzoyl peroxide (Sigma) as thermal initiator is spread on the whole substrate. The substrate is then immersed in water. The lubricant wets only the activated binding sites in water. When parts with hydrophobic binding sites are added in water, the parts are attracted and self-aligned to the binding sites on the substrate simultaneously. The lubricant is then polymerized by heating the substrate at 80 °C for 30 min, which permanently bonds the parts to the substrate. Figure 5(a) shows assembly results from one batch of silicon test chips. A second assembly is performed by repeating SAM formation and the assembly steps. A two-batch assembly is demonstrated in figure 5(b). After the assembly, electrical connections between the parts and the substrate are established by electroplating. We use LEDs to demonstrate both assembly and electroplating methods. Figure 6(a) is an example of an assembled LED. Figure 6(b) is an assembled LED with electrical connection to the substrate, and the LED is activated by potential bias applied to the contacts on the substrate.

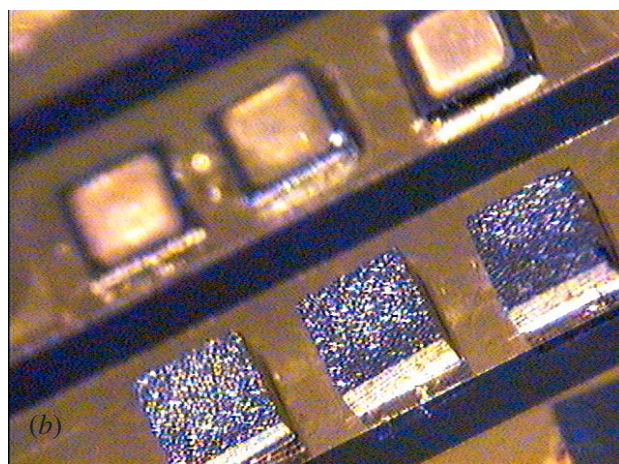
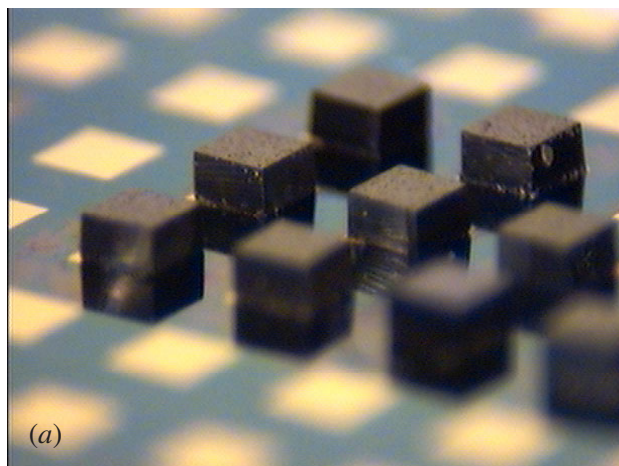


Figure 5. Assembly results. (a) An assembly result of one batch diced silicon chips. (b) A two-batch assembly result (figure from [32, 33] by Hanein and Xiong).

3.3. Binding site design

Binding site design is important for assembly, since optimal binding site design can provide for higher alignment accuracy, higher assembly yield, shorter assembly time and uniqueness of assembly orientation. When using commercial off-the-shelf parts such as LEDs with given design as shown in figure 7(a), a straightforward binding site design would simply use the same pattern as the part. However, it was shown that such a design will not always be optimal and misalignment may occur during assembly [38]. To improve assembly, we custom design the binding site and plating basis geometry using a software simulation tool [33, 38].

The simulation tool is based on a simple surface energy model of our self-assembly system. It uses the observation that self-assembly and self-alignment is the result of minimization of interfacial energy between the lubricant meniscus and the aqueous environment. As described in [33, 38], the interfacial energy is directly proportional to the exposed hydrophobic surfaces in water. In a first-order approximation, the surface energy W can be represented in terms of the hydrophobic regions on the part and the substrate, denoted by P and S respectively, as long as the lubricant bridge height is small

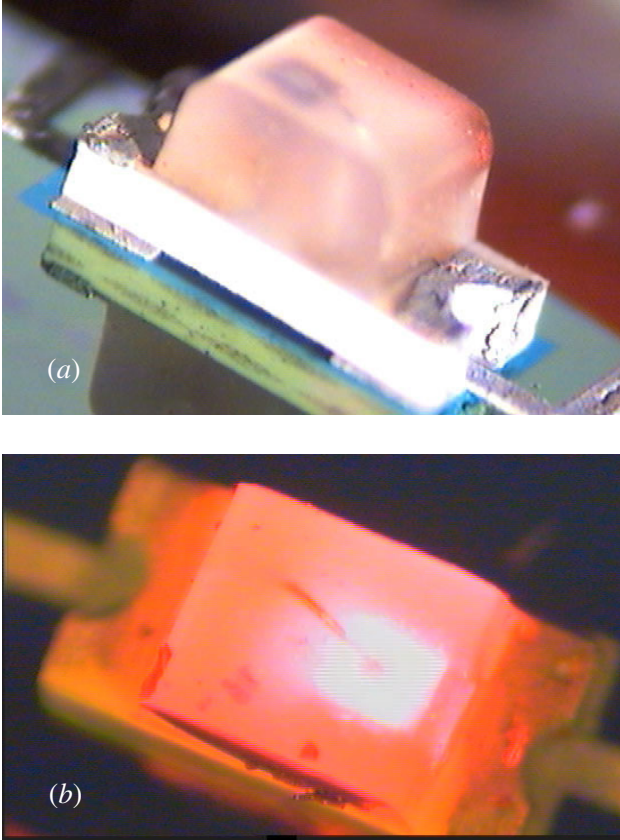


Figure 6. (a) An assembled LED. (b) An assembled LED with electrical connections to the substrate. The LED is activated by applying potential bias to the contacts on the substrate (figures by Xiong).

compared to the binding site length or width:

$$W = \gamma(|S| + |P| - 2|S \cap P|). \quad (1)$$

Here γ is the interfacial energy coefficient, and $|S|$ and $|P|$ denote the surface area of a region S and P , respectively. Since the terms $|S|$ and $|P|$ in equation (1) are constants, W is directly proportional to $-|S \cap P|$: the negated overlap area between S and P . A concise and rigorous derivation of this model is presented in [33].

It is worth noting that the gradient of the surface energy, ∇W , represents a vector field that provides the driving force for the self-assembly process. Thus, the results obtained for programmable force fields (PFFs) as summarized in section 2 can be applied here. This is particularly interesting since ∇W can be modulated during the assembly process with the desorption of hydrophobic SAMs.

To briefly describe the implementation of the simulation tool, the overlap area called $A(x, y, \theta)$ is computed with respect to three parameters, representing the relative location (x, y) and orientation θ of P to S . For a given orientation θ , the value A can be calculated efficiently by two-dimensional convolution of P with S . To characterize the overlap area as a function of rotation angle, calculations are iterated for discretized θ values in the range of 0° – 360° .

Therefore, for a binding site design, we use two plots to show the simulation results: translation and rotation. In the

translation result, the overlap ratio, which is the ratio of the overlap area to part binding site area $|S \cap P|/|P|$, is plotted as a function of relative location (x, y) of P to S . In the rotation result, each point in the plot corresponds to a maximum overlap ratio with respect to a given orientation θ .

Ideally, assembly configurations should possess a unique global energy minimum, which, according to our model, would correspond to a unique maximum of area overlap as a function of (x, y, θ) . With the given symmetric LED binding site design shown in figure 7(a), such a unique maximum is impossible to achieve. Figures 7(b) and (e) show two different binding site designs, and the design in figure 7(b) is simply a copy of the LED binding site design. Figures 7(c) and (d) are the translation and rotation simulation results for design (b), and figures 4(f) and (g) are the simulation results for design (e). Figure 4(b) shows multiple local maxima for binding site design (b) at 0° , while figure 4(e) shows only one maximum for design (e). Figures 4(c) and (f) show that design (b) has multiple maxima at various orientations, while design (e) has only two maxima for 0° and 180° (there must be two maxima because the LED design is symmetric). These simulation results indicate that design (d) is preferable, as it exhibits only two maxima in overlap.

3.4. Discussion

In this section, we have presented a self-assembly protocol as well as a modeling tool for design and simulation of capillary-force-driven self-assembly. Parallel self-assembly has been demonstrated with multiple batches of different microparts, and also with commercial-off-the-shelf LEDs. As an enabling technology, this approach offers the prospect of efficient integration of complex microsystems from a range of different microcomponents. Currently, we are applying this approach to construct hybrid complex systems such as microfluidic devices. By expanding and modifying the current assembly processes, we are able to assemble the piezoelectric actuating elements with precise placement and establish electrical connections through post-bonding electroplating in order to construct hybrid piezoelectric micropumps.

With the emergence of more and more diverse processes, materials, and devices for microsystems, we believe micro-self-assembly will be a key technique for system integration. The self-assembly protocol demonstrated in this section provides a feasible solution to overcome the incompatibility between system components and helps to achieve optimal materials and designs for individual components. It is anticipated that this technique can be applied to a wide range of components for the integration and packaging of complex systems.

Furthermore, this technique motivates us to investigate surface-force-driven self-assembly in more general terms. Exploiting the hydrophobic effect of SAMs in an aqueous solution is just one of many possible approaches. In the following section, we present another technique to modify surface properties at the microscale, by integrating a ‘smart polymer’ into MEMS processes. This provides not only a method for controlling hydrophobicity simply with an

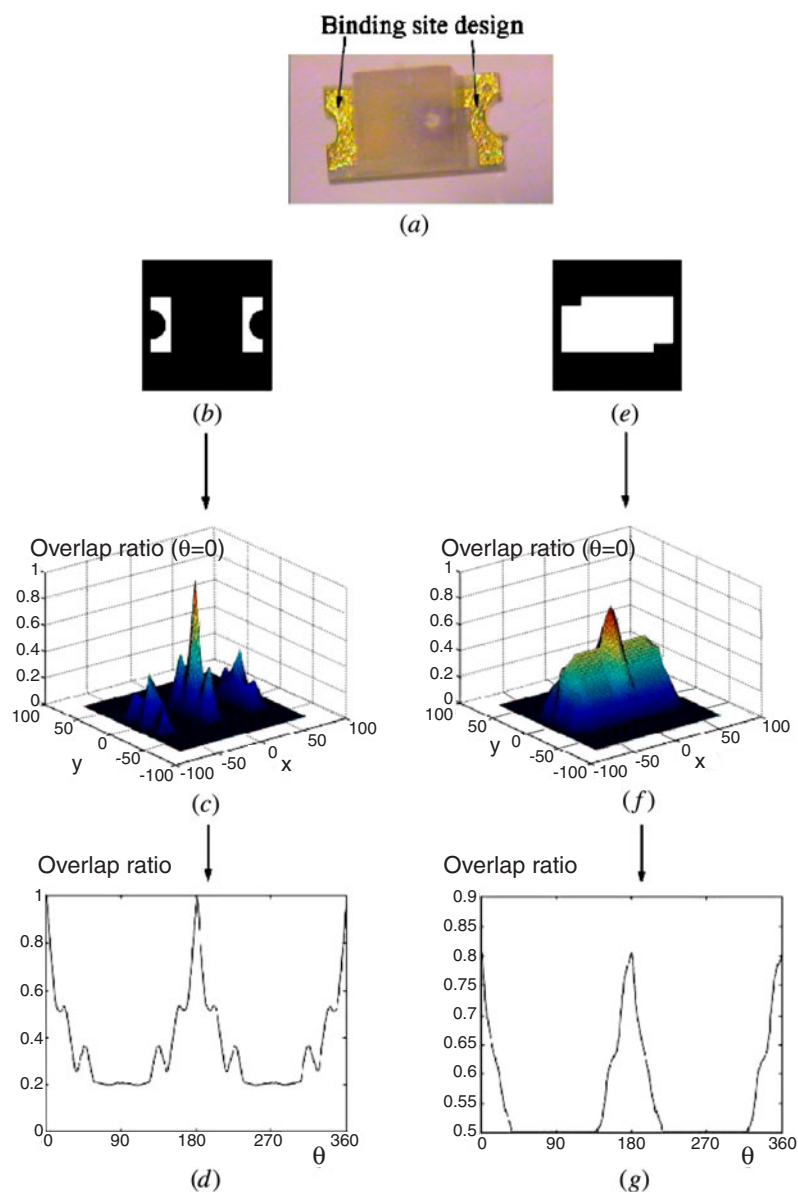


Figure 7. (a) A commercial LED with given binding site design. (b) An intuitive design for the substrate binding site is the same as the binding site pattern of the LED. (c) Translation and (d) rotation simulations for the design in (b). (e) Another binding pattern design for LED assembly, with the openings on the top-left and bottom-right corners for placing the electroplating bases. (f) Translation and (g) rotation simulations for the design shown in (e) (figures by Xiong).

electrical signal, but it can also be used to steer the adsorption of proteins and cells on micropatterned surfaces.

4. Programmable surfaces with ‘smart polymers’

Surface chemistry can be exploited to control a wide range of phenomena such as hydrophobicity and bio-fouling, i.e., the tendency of biological substances (proteins, cells, bio-films, etc) to attach to exposed surfaces. Over the past decades, a wide range of materials have been developed and studied, such as poly(ethylene glycol) (PEG) or poly(ethylene oxide) (PEO) [39, 40] especially for applications in biomedical devices and implants. Poly-N-isopropylacryl-amide (pNIPAM) is another particularly interesting candidate polymer material to realize programmable surface chemistry. Among its properties is a

transition from hydrophilic and non-fouling behavior at room temperature to hydrophobic and fouling behavior above its lower critical solution temperature (LCST). In addition, its LCST of 32 °C is close to body temperature and thus suitable for protein treatment. These properties are due to the reversible formation and cleavage of the hydrogen bonds between NH or C=O groups and surrounding water molecules with changing temperature [41].

A plasma polymerized form of pNIPAM (ppNIPAM) has been developed at the University of Washington that demonstrates the same favorable properties as the bulk material [42]. In this section, we show how vapor-phase deposited ppNIPAM on microfabricated heater arrays offers yet another opportunity for controlling the surface chemistry at the microscale.

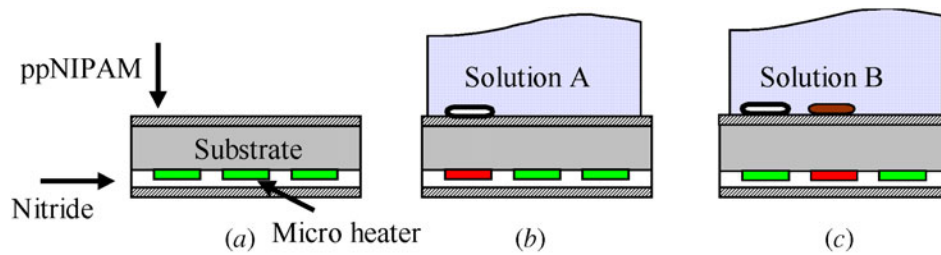


Figure 8. Schematic description of ppNIPAM devices. (a) Devices consist of microheaters on a glass slide coated with ppNIPAM. (b) Active heater (dark) turns the surface hydrophobic and fouling. Selective protein adsorption occurs exclusively on heated areas. Protein adsorption takes place on the top side of the substrate. (c) A second adsorption step with different protein solution on a different heater (figure from [43] by Wang).

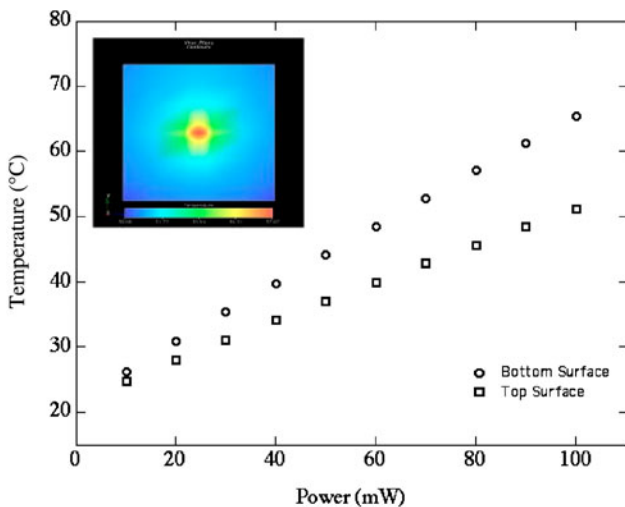


Figure 9. Simulated center temperature of top and bottom surfaces versus power (using CoventorTM). Inset: temperature profile of the top surface for 50 mW. Data from actual devices agree with values predicted for the bottom surface temperature (figure from [43] by Shastry and Wang).

4.1. Experimental setup

In this section we present a recently developed method to pattern proteins using programmable surfaces consisting of ppNIPAM [43]. Figure 8 shows the principles of our method.

The microfabrication process consists of CrAuCr heaters (thickness 150 nm, area $1 \text{ mm} \times 0.8 \text{ mm}$ and $40 \mu\text{m}$ wide lines) deposited on a glass substrate (thickness $180 \mu\text{m}$). The heaters are passivated with sputtered silicon nitride (400 nm) and the entire device is then treated with ppNIPAM in a plasma deposition process [42]. This process is particularly suited for MEMS applications as it ensures very high surface coverage, excellent adhesion and good non-fouling properties at room temperature [44]. CoventorTM, a fully integrated finite element simulation package, is used to simulate the electro-thermal properties of the designed heaters (figure 9). Temperature sensitive paint is used to characterize the heating profile.

When exposed to protein solution, proteins are adsorbed exclusively at heated areas. These protein patches remain immobilized even after the heater is turned off and the temperature is dropped below the LCST. Repeated adsorption

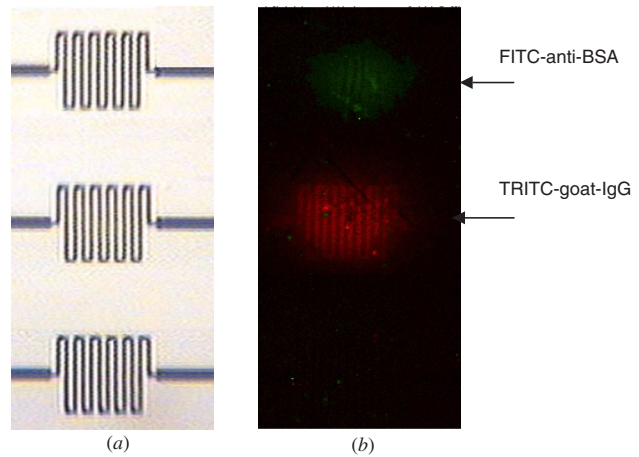


Figure 10. (a) Microheater array: three resistive CrAuCr heaters, thickness 150 nm, linewidth $40 \mu\text{m}$, area approximately 1 mm^2 . (b) FITC-anti-BSA pattern adsorbed on top heater. (c) TRITC-goat-IgG pattern adsorbed on center heater. All figures are taken from the same substrate at different wavelengths, after the two protein adsorptions (figures from [43] by Cheng and Wang).

steps form arrays of protein patches such as those required for protein chips.

4.2. Experimental results

A fabricated microheater array is shown in figure 10(a). Immunoglobulin G (IgG) test results are shown in figures 10(b) and (c). The microheater chip was first incubated with fluorescein isothiocyanate (FITC)-anti-BSA for 30 min with the upper heater turned on (94 mW). The heater was then turned off and the chip was incubated with tetramethylrhodamine isothiocyanate (TRITC)-goat-IgG with the middle heater turned on for another 30 min. The two fluorescence images (figures 10(b) and (c)) were taken from the same chip at two different wavelengths. Two patches of proteins can be clearly identified on the device surface. The second protein batch did not adhere to areas already covered with anti-BSA.

Recent work has extended this approach to controlled, localized immobilization of biological cells on thermal-responsive polymer controlled by a microheater array [45]. This technique offers a new approach to realize biochips by

using programmable surface chemistry devices. The change in hydrophobicity of NIPAM in aqueous solution in response to relatively small changes in temperature also suggests many other uses, such as valves for microfluid channels.

4.3. Discussion

The technique described in this section offers a new approach to realize programmable surface chemistry devices by using microheater arrays to control the properties of a ppNIPAM coating. Controlled protein and cell adsorption in several batches has been demonstrated, and could be applied to create larger arrays for protein chips. The performance of these devices with multiple proteins, with cells and for additional MEMS and bioMEMS applications is currently being investigated.

A major advantage of the presented technique is the ability to perform the entire process in a wet environment, which is critical to maintain the integrity of sensitive proteins during the patterning processes. Additional major advantages are the simple setup and the low power consumption.

In addition to the fouling/non-fouling change, ppNIPAM also exhibits a hydrophobic/hydrophilic change in aqueous environment in response to relatively small changes in temperature, which suggests many other uses. In particular, we believe that a combination of the micro-self-assembly techniques described in section 3 and programmable ppNIPAM surface chemistry of this section can produce a new generation of self-assembling systems that takes advantage of this simple technique for programmable surface chemistry.

5. Conclusions

This paper has overviewed several projects on microfabricated devices by attempting to show a common theme: that the control of surface properties is of central importance in the design and use of microdevices. Various techniques already exist to tune and program surfaces at the microscale: by actuation with ciliary microarrays, by electrochemical modulation of hydrophobicity and the deliberate use of capillary forces, or by integration of temperature-responsive polymers into MEMS devices. However, these are just a few of the many possibilities that open up in this rapidly expanding field. While various groups have reported initial successes, many questions remain; here, we list just a few fundamental ones.

We now know that surface effects dominate volume effects at the microscale, and that this can be exploited to create novel devices and manufacturing techniques that would not be possible at larger scales. However, as we scale further down toward nanometer dimensions, will the laws of scaling continue to work in our favor? For example, the capillary-force-driven self-assembly may only work well as long as the micropart is significantly larger than the lubricant droplet. Otherwise, good alignment can no longer be expected. Thus, nanoscale parts may self-assemble better by exploiting closer range interactions such as van der Waals forces or recombinant oligonucleotides.

What are the fundamental principles that describe and limit self-assembly? Recent work has shown an intricate relationship between assembly yield and alignment accuracy not only with surface chemistry, but also with three-dimensional part design, binding site density and the specifics of part delivery. What is needed is a comprehensive model for the dynamics of micro-self-assembly.

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