Published in final edited form as:

Lab Chip. 2009 November 21; 9(22): 3268–3274. doi:10.1039/b912876j.

Electric field directed assembly of high-density microbead arrays[†]

Kristopher D. Barbee^a, Alexander P. Hsiao^a, Michael J. Heller^{a,b}, and Xiaohua Huang^{*,a}

^aDepartment of Bioengineering, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA, 92093-0412, USA

^bDepartment of NanoEngineering, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA, 92093-0412, USA

Abstract

We report a method for rapid, electric field directed assembly of high-density protein-conjugated microbead arrays. Photolithography is used to fabricate an array of micron to sub-micron-scale wells in an epoxy-based photoresist on a silicon wafer coated with a thin gold film, which serves as the primary electrode. A thin gasket is used to form a microfluidic chamber between the wafer and a glass coverslip coated with indium-tin oxide, which serves as the counter electrode. Streptavidin-conjugated microbeads suspended in a low conductance buffer are introduced into the chamber and directed into the wells *via* electrophoresis by applying a series of low voltage electrical pulses across the electrodes. Hundreds of millions of microbeads can be permanently assembled on these arrays in as little as 30 seconds and the process can be monitored in real time using epifluorescence microscopy. The binding of the microbeads to the gold film is robust and occurs through electrochemically induced gold-protein interactions, which allows excess beads to be washed away or recycled. The well and bead sizes are chosen such that only one bead can be captured in each well. Filling efficiencies greater than 99.9% have been demonstrated across wafer-scale arrays with densities as high as 69 million beads per cm². Potential applications for this technology include the assembly of DNA arrays for high-throughput genome sequencing and antibody arrays for proteomic studies. Following array assembly, this device may also be used to enhance the concentration-dependent processes of various assays through the accelerated transport of molecules using electric fields.

Introduction

Microbead-based platforms have become a popular technology for many high-throughput biological assays such as genotyping,¹ DNA sequencing,² and protein detection³ due to the ease in which they enable multiplexing and miniaturization. Microbeads have been captured or assembled onto various surfaces *via* evaporation,^{4–6} gravity,⁷ centrifugation,⁸ and magnetic^{9,10} and electric fields.^{11–18} While all these methods have been successfully utilized, each has some limitations. For instance, controlled evaporation, or dewetting, can take hours to assemble large arrays on microfabricated templates.⁵ In addition, achieving sufficient filling efficiencies with sub-micron particles may require multiple aliquots and highly concentrated microbead suspensions.¹⁹ Gravity-dependent assembly methods can also be relatively slow

[†]Electronic supplementary information (ESI) available: Video clip of electric field directed assembly of a microbead array. See DOI: 10.1039/b912876j

© The Royal Society of Chemistry 2009

x2huang@ucsd.edu; Tel: +1 (858) 822-2155.

and often result in lower packing efficiencies.⁷ Centrifugation-based approaches face similar issues and cannot be easily automated.⁸ We recently reported a method for the rapid assembly of superparamagnetic microbeads into arrays with near perfect order using a magnetic field.¹⁰ However, it may be difficult to scale due to the limited availability of uniform and monodisperse sub-micron magnetic beads.

Methods employing electric field directed assembly on microfabricated templates offer certain advantages in that they can be fast, automatable, scalable, and used to assemble non-magnetic particles. These types of platforms also have the potential to accelerate *via* an electric field various diffusion-limited processes such as DNA hybridization²⁰ and antibody-antigen binding.²¹ Many of the reported electric-field-based methods are often used to direct the assembly of microbeads or nanoparticles into colloidal crystals or clusters with little control over their number, order or position. A few others have demonstrated more control over microbead position and order.^{14–16} However, their methods might be difficult to scale or they may not be compatible with microfluidics, biological assays and real-time epifluorescence microscopy.

We have developed a device and process that utilizes an electric field to direct the assembly of high-density arrays of protein-conjugated microbeads in a rapid, automatable and scalable fashion. Our method, unlike those previously reported, can be used to assemble wafer-scale arrays of individual microbeads with near perfect order. The microfabrication process and the fluidic device are illustrated in Fig. 1. A high-density array of wells in an epoxy-based photoresist is fabricated on a silicon wafer coated with a gold film that serves as the primary electrode (Fig. 1A). The counter electrode consists of a glass coverslip coated with indium-tin oxide (ITO), which serves as the counter electrode. A flow cell is formed by sandwiching a thin adhesive silicone gasket that contains a cutout of a flow channel between the wafer and the coverslip (Fig. 1B). As illustrated in Fig. 2, a series of low voltage electrical pulses is applied to the electrodes. The negatively charged, streptavidin-coated microbeads are directed into the wells by electrophoresis. The microbeads are permanently captured within the wells through electrochemically-induced binding between the gold and streptavidin. Using this approach, we have demonstrated that hundreds of millions of 0.5 μm and 1 μm microbeads can be captured in a rapid, efficient and ordered manner. The diameter of the wells in the photolithographically-defined templates can be easily adjusted to the desired bead size, ensuring that each well can accommodate only one microbead. This spatial control supports higher imaging efficiencies for demanding applications such as genome sequencing by reducing the total number of pixels required to image each microbead.^{10,22} Our assembly method is also simple and practical in that it utilizes low frequency, direct current (DC) pulses applied across a flow cell that is suitable for biological assays using epifluorescence microscopy.

Materials and methods

Device fabrication

The general procedure for fabricating an array of wells on a silicon wafer coated with a thin oxide layer and metal films is illustrated in Fig. 1A. The silicon wafers (100 mm) were cleaned in a 3:1 mixture of 98% H_2SO_4 :30% H_2O_2 at 85 °C for 15 min and then rinsed extensively with de-ionized water (dH_2O). (CAUTION: H_2SO_4 - H_2O_2 mixtures are extremely dangerous and should be handled with care.) The wafers were then dipped in a buffered oxide etch (6:1 of 40% NH_4F :49% HF) for 30 s and rinsed with dH_2O . (CAUTION: HF is extremely dangerous and should be handled with care). The wafers were blown dry with nitrogen and coated with a 200–300 nm layer of silicon dioxide using a plasma-enhanced chemical vapor deposition (PECVD) system (Plasmalab, Oxford Instruments). Oxide deposition was conducted at 350 °C and 20 W RF using 710 sccm N_2O and 170 sccm SiH_4 at 1 Torr.

Titanium and gold films were deposited on the oxide-coated wafers using a Discovery 18 sputter system (Denton Vacuum) or VES2550 electron-beam evaporation system (Temescal). The deposition chambers were typically evacuated to a base pressure of 9×10^{-7} Torr or less. Sputtered-coated titanium and gold films were deposited at 200 W DC in Ar at 3.0×10^{-3} Torr and 36 sccm. Evaporated Ti and Au films were deposited at 0.1–0.2 nm s⁻¹. The titanium layer, which serves as an adhesion layer between the oxide and the gold films, was approximately 30 nm thick. Gold film thicknesses ranged from 300–400 nm.

Following metallization, SU-8 2000.5, an epoxy-based, negative-tone photoresist (Microchem Corp.), was applied directly to the gold film by spin-coating at 2000 rpm for 30 s. After baking on a hotplate at 95 °C for 1 min, the wafer was patterned *via* i-line photolithography using a chrome on quartz photomask on a GCA Autostep 200 stepper system equipped with an Olympus 2145/0.45 NA reduction lens. With an intensity of ~ 475 mW cm⁻², typical exposure times ranged from 0.29 s for ~ 0.6 μ m features to 0.415 s for ~ 1.2 μ m features. The exposed wafers were baked at 95 °C for 1 min, developed for 2 min in SU-8 developer (Microchem Corp.), then rinsed with isopropyl alcohol and dried with nitrogen.

ITO deposition on glass coverslips

Using a custom-built PTFE rack, $50 \times 75 \times 0.170$ mm³ glass coverslips (Erie Scientific) were washed in batch mode in a 2% solution of Micro-90 detergent (Cole-Parmer) and rinsed extensively with dH₂O. The coverslips were soaked in acetone and then methanol with sonication for at least 30 min each. The coverslips were further cleaned in a 1:1:5 mixture of 30% H₂O₂:30% NH₄OH:H₂O at 85 °C for 1 hour and then in a 3:1 mixture of 98% H₂SO₄:30% H₂O₂ at 85 °C for 1 hour. The coverslips were rinsed extensively in dH₂O and dried in an oven at 110 °C for 30 min.

The ITO films were deposited on the coverslips using an ATC Orion 8 sputter system (AJA International). The deposition chamber was evacuated to a base pressure of 5×10^{-7} Torr or less and the substrates were heated to 325 °C. Sputtering was performed at 325 W RF and 2×10^{-3} Torr for 10 min with argon and oxygen flow rates of 20 and 0.1 sccm, respectively. The ITO films were characterized using a FPP-100 four-point probe system (Veeco) and a Lambda 20 UV-Vis spectrometer (Perkin Elmer). Typical values for the sheet resistance were 12–15 Ω . Typical values for the optical transmittance were 90–95% over a range of 450–800 nm. Prior to device assembly, the coverslips were diced into 8–10 mm $\times$ 50 mm strips using a diamond scribing tool.

Device assembly

After completion of the fabrication process, the wafers were covered with a protective layer of Shipley Megaposit SPR220-7.0 photoresist (Rohm and Haas) by spin-coating at 2000 rpm for 30 s and then baked on a hotplate at 115 °C for 5 min. Holes were drilled through the wafer in an automated fashion using a 1.0 mm diamond-coated drill bit (C. R. Laurence Co.) and a high-speed rotary tool mounted to a computer numerical control milling machine (PCNC 1100, Tormach). The wafers were then diced by hand into 12 mm-wide pieces using a scribing tool and the resist was stripped by soaking in acetone for 2 min. Each die was then rinsed with isopropyl alcohol and dried with nitrogen. Prior to use, the dice were further cleaned in a Technics PEII-B plasma system at 100 W and 3.00×10^{-1} Torr O₂ for 3 min and then rinsed with dH₂O. The final thickness of the SU-8 layer is estimated to be about 200 nm. After drying with nitrogen, the dice were fixed *via* double-coated acrylic tape (DC-UHB10FA-C, J. V. Converting Company, Inc.) to a custom-built aluminium plate, which contains ports for fluidic connections and fits securely within the aperture of our microscope stage (Fig. 1B).

The channels that form the flow cells within the device were designed in a computer-aided design program and cut out of a ~110 μm -thick, double-coated silicone tape (No. 702, Scapa Group) using a cutting plotter (CC200-20, Graphtec Corp.). Typical channel dimensions ranged from 2–6 mm in width by 26–32 mm in length. The tape containing the channel cutout was then aligned and fixed to the wafer. Narrow strips of copper tape ~30 μm thick (Cat. No. 77802, Electron Microscopy Sciences) were attached to both ends of the counter electrode and then wrapped around the ends and onto the backside of the coverslip. The coverslip was then attached to the wafer with the silicone tape to create the fluidic chamber. Electrical connections to both the gold film and the copper tape on the coverslip were made by attaching additional pieces of copper tape, each soldered to an insulated copper wire. An exploded view of the complete device is shown in Fig. 1B.

Electric field directed assembly of microbead arrays

Streptavidin-coated, fluorescent polystyrene microbeads with diameters of 0.5 μm and 1 μm (CPO1F/7066 and CP01F/7677, Bangs Laboratories, Inc.) were diluted to 0.1%–0.2% solids in a low conductance buffer (4.5 mM tris(hydroxymethyl) amino-methane, 4.5 mM boric acid, and 0.02% Triton X-100, pH 8.6, with a conductance of 61 $\mu\text{S cm}^{-1}$) (LCB). The microbead suspensions were washed 3 times with LCB. Prior to each buffer exchange, the suspensions were centrifuged for 6 min at 3000 g and then re-suspended by vortexing for ~30 sec. Suspensions were sonicated by placing them in an ultrasonic water bath for 5–10 min immediately prior to use.

The chambers were connected to a syringe pump (Cavro XR Rocket, Tecan Group, Ltd.) using 1/16" OD $\times$ 0.03" ID Teflon tubing, 1/4–28 Upchurch (Idex Corp.) and 062 MINSTAC fittings (The Lee Co.) and washed with LCB. The microbead suspensions were introduced and the total circuit resistance was measured with a digital multimeter (2010, Keithley Instruments, Inc.). Typical values ranged from ~100–200 k Ω . A function generator (33220A, Agilent Technologies, Inc.) was used to apply a series of 1 Hz, 3.0 V DC pulses with a 10% duty cycle for 30–60 s either continuously or in 10–15 s intervals with 2 min periods between each interval. The electrical waveforms were monitored using an oscilloscope (TDS 224B, Tektronix, Inc.). Following the assembly process, excess microbeads were washed away with LCB at a flow rate of 16–40 $\mu\text{L s}^{-1}$. An illustration of the assembly process is shown in Fig. 2.

Fluorescence imaging

Real-time imaging of the assembly process was performed on an epifluorescence microscope (DM LFSA, Leica Microsystems, Inc.) with a 40 $\times$ /0.55 NA objective, and a CCD camera (ORCAER, 1024 $\times$ 1344, 6.45 $\times$ 6.45 μm^2 pixels, Hamamatsu Photonics). Excitation light was from an Osram 100W HBO mercury arc lamp. The images and movies were recorded with SimplePCI software (Hamamatsu Photonics). Image analysis and background subtraction was performed with Image J.²³

SEM imaging

To disassemble the chamber and release the substrate for SEM imaging, the entire device was submersed in liquid nitrogen. The low temperature simplified the release of the wafer from the aluminium plate and the coverslip from the wafer. The diced samples were then coated for 30 s with chromium or iridium at 130 mA in Ar using a K575X sputter tool (Emitech). The images were obtained with a Phillips XL30 environmental SEM operating at 10 kV in high-vacuum mode. The aluminium plate is prepared for further use by soaking it in acetone, which aids in the removal of the acrylic tape.

Results and discussion

Rapid assembly of microbead arrays

We have demonstrated the ability to rapidly assemble high-density arrays of protein-conjugated microbeads on photolithographically-defined templates using an electric field. Standard thin film deposition and photolithography techniques are used in the fabrication process. The microbead assembly process utilizes a function generator to deliver 3.0 V DC pulses at a frequency of 1 Hz and a duty cycle of 10%. Fig. 3A and 3B shows the fluorescence and SEM images of a typical array of 1 μm beads assembled into $\sim 1 \mu\text{m}$ wells at a pitch of 2.4 μm . As can be seen, arrays with near perfect order can be assembled with filling efficiencies as high as 99.9%. Using this approach, we have also demonstrated the ability to assemble 1 μm (Fig. 3C) and 0.5 μm beads (Fig. 3D) on arrays with densities approaching 69 million microbeads per cm^2 . A movie of a typical assembly process is available as ESI.[†]

Defect rates in the assembled arrays are typically less than a few percent. Common defects include unfilled wells and microbead doublets (Fig. 4A). For example, in Fig. 3A only 5 wells remain unfilled, which corresponds to a filling efficiency of 99.9%. The variation in fluorescence intensity observed in Fig. 3A is due to the presence of doublets as well as considerable differences in microbead size as seen in Fig. 4. There are approximately 0.2% wells containing smaller, less visible microbeads and 1.5% wells containing brighter doublets or abnormally large microbeads. We found that some doublets and larger aggregates were often present in the stock and LCB microbead suspensions. Therefore, the doublets observed in the arrays may not be caused by the assembly process.

Materials considerations

Gold was chosen for the electrode upon which the microbeads were assembled primarily due to its affinity towards proteins. As described elsewhere, proteins can be adsorbed directly onto gold surfaces, with²⁴ or without²⁵ the use of an electric field. By exploiting this phenomenon, we are able to capture permanently streptavidin-coated microbeads onto the gold electrodes without any surface modifications. The binding of the microbeads is sufficient to withstand both stringent washes and the application of a reverse bias of 1.5 V DC. It is even more astounding that the assembled arrays are able to survive the liquid nitrogen treatment and the SEM imaging process. The use of platinum and ITO for the primary electrode was also investigated. However, we found that the microbeads were less likely to bind to these materials under our assembly conditions.

The choice of materials used for the counter electrodes is subject to other design constraints, with an emphasis on imaging. ITO-coated coverslips were used as counter electrodes to provide a suitable window through which optical imaging of the arrays can be performed. However, ITO films are not very robust and can be damaged easily. Therefore, it is imperative that the assembly conditions are compatible with this material. By using a low conductance buffer and minimizing the strength and duration of the electrical pulses, no obvious damage was found to occur to the ITO films.

The material used to fabricate the arrays of wells needs to meet several criteria. It must be a robust dielectric with good adhesion to gold, compatible with sub-micron photolithography and able to withstand the potentially damaging by-products of electrolysis. Positive resists such as Shipley S1805 were initially used to simplify the fabrication process. Although suitable as dielectrics, these materials were prone to delamination after prolonged exposure to the

[†]Electronic supplementary information (ESI) available: Video clip of electric field directed assembly of a microbead array. See DOI: 10.1039/b912876j

assembly conditions. In contrast, the epoxy-based negative photoresist, SU-8 2000.5, was able to withstand electrophoretic conditions well beyond those required for assembly. SU-8 2000.5 is also suitable for sub-micron photolithography, albeit with some minor defects that can be attributed to the resolution limit of the stepper system. Despite the apparent advantages of SU-8, compatibility issues may arise if this device is to be used as a platform for biological assays. In such cases, silicon dioxide or silicon nitride films may be employed. However, the use of such materials will require a more involved fabrication process.

Flow cells were created using a thin, double-coated silicone tape. Using a cutting plotter, this material can be quickly cut into various shapes and sizes. This allows channels to be created much more easily than through conventional microfabrication processes. The tape backing is made of polyester and the adhesive is silicone-based. This makes it suitable as an electrical insulator and stable in aqueous environments. Since the tape is only 110 μm thick, high field strengths can be achieved within the device at relatively low voltages. The shallow chamber height also enables the use of microscope objectives with high numerical apertures, which tend to have relatively short working distances.

The buffer system, tris(hydroxymethyl) aminomethane and boric acid (Tris-borate), was chosen for its low conductivity and its pH value. At a concentration of 4.5 mM, this solution has a conductivity of only 61 $\mu\text{S cm}^{-1}$ and a pH value of 8.6, yet still provides adequate buffering capacity under the electrophoretic assembly conditions. With an isoelectric point of ~ 5 , streptavidin is negatively charged and thus the streptavidin-conjugated microbeads are also negatively charged in this buffer.²⁶ In contrast, phosphate and bicarbonate buffering systems with similar concentrations and pH values have much higher conductivity values. With the low conductivity of the Tris-borate buffer, the microbeads can be pulled rapidly toward the electrode while the current is kept at a minimum. However, we found that the binding of the microbeads was less efficient after they were stored in such a buffer for more than one week.

The types of the microbeads that can be assembled and captured onto the gold surface depend on their surface properties, especially the charges and the nature of the chemical functionality. The streptavidin-coated microbeads were used for two reasons. First, the streptavidin molecules can serve as the functional groups for further attachment of biotinylated molecules or other moieties through the strong biotin-streptavidin affinity binding. Second, we found that under the conditions used, streptavidin-coated microbeads could be assembled and captured with ease. The electrochemically-induced binding of microbeads to the gold surface is probably due to the dative bonding between the chemical groups on the protein and gold, and other interactions such as electrostatic and van der Waals interactions.^{24,25,27} The binding of the microbeads to the gold surface is robust in that the assembled arrays can withstand the prolonged application of a bias of -1.5 V DC and harsh conditions such as exposure to liquid nitrogen, sputter coating and SEM imaging. We have also experimented with 0.5 μm and 1 μm carboxylate-modified polystyrene microbeads. Even though these microbeads could be pulled into the wells using an electric field, permanent binding was rarely observed. This suggests that electrochemically induced binding of the carboxylate groups on the microbeads to the gold surface is less efficient under our conditions.

Assembly conditions

Various electric field conditions were examined to optimize the microbead transport and assembly process. With our electrode configuration, AC dielectrophoresis was found to be an inadequate means for assembly, even at 20 Vpp over a wide range of frequencies. However, a DC potential difference of only ~ 2.0 V was required to observe appreciable electrophoretic migration of the negatively charged, streptavidin-coated microbeads towards the positive electrode. Potentials up to ~ 2.8 V DC, even when applied for extended periods of time (>30 s), did not result in a significant degree of microbead binding. However, potentials at or above

~3.0 V DC with durations as short as 50 ms enabled rapid and permanent microbead binding. In our device, a 3.0 V potential corresponds to an electric field strength of $\sim 270 \text{ V cm}^{-1}$, although stronger fields are likely to exist around the edges of the wells.²⁸ Potential differences greater than ~3.5 V DC, when applied continuously for more than 30 seconds, resulted in damage to the ITO and substantial gas evolution due to the electrolysis of water.

To minimize the possibility of bubble formation and damage to the electrodes, photoresist or microbeads, we used low frequency (1 Hz), 3.0 V DC pulses with a short, 10% duty cycle. These conditions were sufficient for rapid, directed assembly with a minimal amount of time in which the substrate and microbeads were subjected to the field. The low-frequency pulsing of the field also helped reduce the lateral microbead aggregation seen at higher frequencies or with continuously applied potentials. Frequencies lower than 1 Hz resulted in less efficient assembly as the microbeads have more time to diffuse away from the surface between pulses. Aggregation of the microbeads, which appeared to hinder their ability to be captured efficiently, was further reduced by applying the electric field in two to three 15-second intervals with a two-minute pause in between each interval. Although the electric field is generally applied for a total of 30–45 pulses, the majority of the wells will have captured a microbead within the first 20–30 pulses. The additional time is usually spent filling the remaining 10–15% of the wells. The drop in the assembly rate can be attributed to both the depletion of microbeads from the suspension and the formation of aggregates near the surface of the array.^{12,13,29,30} Some microbeads are dislodged during the washing step. This is likely due to irregularities in the microbead population or inadequate contact and binding with the gold surface.

The microbead concentration is chosen such that the suspension contains approximately 2–4 times as many microbeads as the number of wells in the chamber. Higher concentrations tend to lead to field-induced aggregation. For the smaller $0.5 \mu\text{m}$ beads, we could not achieve 100% filling with a single batch of the microbead suspension. Even though there were plenty of microbeads left in the suspension, the assembly process did not proceed any further after about 15 pulses. This was probably due to buffer depletion because the assembly process could be resumed to complete the filling of the wells by using an additional batch of microbeads.

Following the assembly process, the integrity of the streptavidin on the microbeads was verified by introducing into the chamber a solution containing biotinylated, fluorophore-labeled oligonucleotides. After a 60 min incubation, the chamber was washed and the microbeads were imaged. A comparison of the images before and after the incubation period revealed a substantial gain in the fluorescence signal on the microbeads, indicating that an ample amount of streptavidin was still intact and functional.

Assembly can still be performed using wells that are smaller than the microbeads as long as the wells are not too deep and the pitch is large enough to prevent contact between the microbeads. As is observed in Fig. 3C, the microbeads are better aligned under such a condition. If the wells are hexagonally packed and the microbeads are appropriately sized, it is very likely that the microbeads can be assembled into an array with the highest achievable packing density. However, if the microbeads are much smaller than the wells, the microbead alignment may be compromised to some degree (Fig. 4C).

As compared to magnetic assembly methods,^{9,10} the choices of the microbeads or particles available for electric field directed assembly are much greater since microbeads with magnetic properties are not required. In principle, any protein-conjugated microbeads can be used. The size of the wells that can be fabricated is only limited by the resolution of the photolithography system and perhaps by the types of photoresist used. Therefore, our method offers great flexibility and scalability for the rapid assembly of microbead arrays with various patterns, densities and pitches.

Conclusions

In summary, we have demonstrated the ability to use electric fields to direct the rapid assembly of arrays of 0.5 and 1 μm protein-conjugated microbeads on photolithographically defined templates. Standard microfabrication procedures are used to generate wafer-scale arrays of wells on gold in a robust, epoxy-based photoresist. Hundreds of millions of microbeads can be assembled within these wells in 30–45 seconds by applying low-voltage, low-frequency DC electrical pulses. Each well contains only one microbead and filling rates as high as 99.9% are easily achieved with minimal defects. Array assembly takes place within a microfluidic device that is compatible with real-time epifluorescence imaging. The methods presented here may be applied to colloidal lithography,^{31,32} micro- and nano-fabrication, and the assembly of arrays of microbeads conjugated to biomolecules such as antibodies and DNA for use in high-throughput assays. In addition, the use of such a platform may provide a means of accelerating diffusion-limited assays by actively concentrating molecules of interest *via* an electric field.^{20,21}

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported in part by grants from the NIH/NHGRI (R21HG003587, R21HG004130 and 1R01HG005096) and the NSF under a CAREER award to X. H. (BES-0547193). A portion of this work was done in the UCSB nanofabrication facility, part of the NSF funded NNIN network. We thank Dr. Brian Thibeault, Dr. Adam Abrahamsen, and Mike Silva for training and technical support at UCSB. Part of this work was also performed in the Nano3 facility at CalIT² at UCSD. We thank Larry Grissom, Dr. Bernd Fruhberger, Ryan Anderson and Dr. Maribel Montero for training and technical support at Nano3.

References

1. Gunderson KL. *Methods Mol. Biol* 2009;529:197–213. [PubMed: 19381978]
2. Cloonan N, Forrest ARR, Kolle G, Gardiner BBA, Faulkner GJ, Brown MK, Taylor DF, Steptoe AL, Wani S, Bethel G, Robertson AJ, Perkins AC, Bruce SJ, Lee CC, Ranade SS, Peckham HE, Manning JM, McKernan KJ, Grimmond SM. *Nat. Methods* 2008;5:613–619. [PubMed: 18516046]
3. Blicharz TM, Siqueira WL, Helmerhorst EJ, Oppenheim FG, Wexler PJ, Little FF, Walt DR. *Anal. Chem* 2009;81:2106–2114. [PubMed: 19192965]
4. Ferguson JA, Steemers FJ, Walt DR. *Anal. Chem* 2000;72:5618–5624. [PubMed: 11101240]
5. Yin Y, Lu Y, Gates B, Xia Y. *J. Am. Chem. Soc* 2001;123:8718–8729. [PubMed: 11535076]
6. Cui Y, Bjork MT, Liddle JA, Sonnichsen C, Boussert B, Alivisatos AP. *Nano Lett* 2004;4:1093–1098.
7. Shendure J, Porreca GJ, Reppas NB, Lin X, McCutcheon JP, Rosenbaum AM, Wang MD, Zhang K, Mitra RD, Church GM. *Science* 2005;309:1728–1732. [PubMed: 16081699]
8. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Irzyk GP, Jando SC, Alenquer ML, Jarvie TP, Jirage KB, Kim JB, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, Volkmer GA, Wang SH, Wang Y, Weiner MP, Yu P, Begley RF, Rothberg JM. *Nature* 2005;437:376–380. [PubMed: 16056220]
9. Wen W, Wang N, Zheng DW, Chen C, Tu KN. *J. Mater. Res* 1999;14:1186–1189.
10. Barbee KD, Huang X. *Anal. Chem* 2008;80:2149–2154. [PubMed: 18260655]
11. Richetti P, Prost J, Barois P. *J. Phys. Lett* 1984;45:1137–1143.
12. Trau M, Saville DA, Aksay IA. *Science* 1996;272:706–709. [PubMed: 8662565]
13. Yeh S-R, Seul M, Shraiman BI. *Nature* 1997;386:57–59.

14. Chiou PY, Ohta AT, Wu MC. *Nature* 2005;436:370–372. [PubMed: 16034413]
15. Winkleman A, Gates BD, McCarty LS, Whitesides GM. *Adv. Mater* 2005;17:1507–1511.
16. Dziomkina NV, Hempenius MA, Vancso GJ. *Adv. Mater* 2005;17:237–240.
17. Dehlinger DA, Sullivan BD, Esener S, Heller MJ. *Nano Lett* 2008;8:4053–4060. [PubMed: 18837562]
18. Hwang H, Park JK. *Lab Chip* 2009;9:199–206. [PubMed: 19107274]
19. Tam JM, Song L, Walt DR. *Biosens. Bioelectron* 2009;24:2488–2493. [PubMed: 19195871]
20. Sosnowski RG, Tu E, Butler WF, O'Connell JP, Heller MJ. *Proc. Natl. Acad. Sci. U. S. A* 1997;94:1119–1123. [PubMed: 9037016]
21. Harper JC, Polsky R, Wheeler DR, Dirk SM, Brozik SM. *Langmuir* 2007;23:8285–8287. [PubMed: 17602680]
22. Shendure J, Ji H. *Nat. Biotechnol* 2008;26:1135–1145. [PubMed: 18846087]
23. Abramoff MD, Magelhaes PJ, Ram SJ. *Biophotonics Int* 2004;11:36–42.
24. Moulton SE, Barisci JN, Bath A, Stella R, Wallace GG. *J. Colloid Interface Sci* 2003;261:312–319. [PubMed: 16256536]
25. De Roe C, Courtoy PJ, Baudhuin P. *J. Histochem. Cytochem* 1987;35:1191–1198. [PubMed: 3655323]
26. Sivagnanam V, Sayah A, Vandevyver C, Gijs MAM. *Sens. Actuators, B* 2008;132:361–367.
27. Gu Z, Huang S, Chen Y. *Angew. Chem., Int. Ed* 2009;48:952–955.
28. Cordovez B, Psaltis D, Erickson D. *Appl. Phys. Lett* 2007;90:024102.
29. Giersig M, Mulvaney P. *Langmuir* 1993;9:3408–3413.
30. Bohmer M. *Langmuir* 1996;12:5747–5750.
31. Fischer UC, Zingsheim HP. *J. Vac. Sci. Technol* 1981;19:881–885.
32. Haginoya C, Ishibashi M, Koike K. *Appl. Phys. Lett* 1997;71:2934–2936.

Fig. 1. Device fabrication and assembly. (A) Fabrication of an array of microwells on a silicon wafer. (B) Exploded view of the device. a: ITO-coated glass coverslip; b: silicone gaskets with flow channels; c: silicon wafer with a high-density array of wells in photoresist on a thin layer of gold; d: double-coated adhesive gasket to couple the wafer to the stage insert; and e: microscope stage insert with fluidic ports.

Fig. 2.

Electric field directed assembly of arrays. A cross-sectional view of a small portion of the flow cell is illustrated. By applying a series of low-voltage electrical pulses, the protein-conjugated microbeads are directed into the wells *via* electrophoresis and captured onto the gold surface through electrochemically-induced interactions between the gold and proteins. The counter electrode consists of an ITO-coated glass coverslip. The flow cell is formed between the glass coverslip and the patterned wafer using a thin, double-coated adhesive silicone tape. Drawings are not to scale.

Fig. 3. High-density microbead arrays assembled using an electric field. (A) Fluorescence micrograph and (B) SEM image of small portions from the same assembled array of 1 μm streptavidin-conjugated polystyrene microbeads at a 2.4 μm pitch. This full-frame fluorescence micrograph was acquired with a 40 $\times$ /0.55 NA objective and a Hamamatsu ORCA-ER CCD camera. (C) SEM image of a small portion of an array of 1 μm beads assembled into $\sim$ 0.8 μm wells at a pitch of 1.2 μm . (D) SEM image of a small portion of an array of $\sim$ 0.5 μm beads assembled into $\sim$ 0.6 μm wells at a pitch of 1.2 μm .

Fig. 4. Microbead doublets, size variation and alignment. (A) An SEM image with a microbead doublet in one well. (B) An SEM image showing the size variation among the 1 μm beads. (C) An SEM image of 0.5 μm beads assembled into over-sized wells. The microbead positioning and well occupancy rates may be compromised if the wells are significantly larger than the microbeads.