Electronic Supplementary Material (ESI) for Analyst

Laser-Based Directed Release of Array Elements for Efficient Collection into Targeted Microwells

Supplemental Information

Nicholas C. Dobes,[a] Rahul Dhopeshwarkar,[a] W. Hampton Henley,[a] J. Michael Ramsey,[a,b] Christopher E. Sims,[a] Nancy L. Allbritton*[a,b]

[a]Department of Chemistry, University of North Carolina, Chapel Hill, NC 27599

[b]Department of Biomedical Engineering, University of North Carolina, Chapel Hill, NC 27599 and North Carolina State University, Raleigh, NC 27695

*Corresponding Author. E-mail: nlallbri@unc.edu; Fax: (919) 962-2388; Tel: (919) 966-2291

Supplemental Experimental Information

Fabrication of the Microwell Master

Microwell masters were fabricated in a similar fashion to the pallet arrays with some modifications. First, a base layer of 1002F photoresist was spin-coated, flood exposed (1500 mJ), developed, and hard baked on 75 mm × 50 mm × 1 mm standard glass microscope slides. This base layer was then plasma treated for 2 min. The treated base layer was spin-coated with two additional layers of 1002F with a 1-1.5 h bake in a 95°C oven (Fisher Scientific, Dubuque, IA) and a 2 min plasma treatment after each coating. A mask outlining addressable 450 μm × 450 μm (L × W) square structures with 50 μm gaps was used to define the microwell-array master. The photoresist-coated slide was subjected to UV exposure (1000 mJ, Oriel, Newport, Inc., Stratford, CT). Exposure was followed by a 10 min bake in a 95°C oven and then 30 min on a rotary shaker in 1002F developer (1-methoxy-2-propyl acetate, Sigma-Aldrich, St. Louis, MO). Masters were rinsed with 2-propanol (VWR, West Chester, PA), blown dry with nitrogen, and placed on a hotplate at 95 °C for 10 min, followed by 120 min at 120 °C. Masters were silanized as previously described to ease mold release.[1]

Fabrication of the PDMS Microwell Array

Microwell arrays composed of 400 wells (450 μm × 450 μm × 300 μm [L × W × D]) were fabricated by soft lithography. The master was placed in a 100-mm square Petri dish (Fisher Scientific, Dubuque, IA) and covered with a thin layer (2 mm) of degassed PDMS followed by exposure to a vacuum (Vacubrand oil-free diaphragm vacuum pump, Fisher Scientific, Dubuque, IA) for 1 h. An

additional 2 mm layer of PDMS was then added and placed under vacuum for 1 h. Multiple PDMS layers were utilized to minimize overflow during vacuum treatment. After vacuum exposure, the PDMS was left to cure overnight at room temp on a level surface, followed by a 2 h bake in a 70°C oven (Fisher Scientific, Dubuque, IA). The microwell array was peeled from the master, plasma oxidized, sterilized and coated with fibronectin, and then mounted to the rotating stage described below.

Array Disk/Rotary Stage Fabrication

Due to the lack of correspondence per array in the number of pallets (10,000) to microwells (400) limiting the number of cells that could be collected individually in any one experiment, a rotary system was designed to hold a series of microwell arrays that could be sequentially positioned under a pallet array as release and collection was performed. Solid models for components were designed using computer aided machining software (BOBCAD/CAM, Clearwater, FL) and fabricated in acrylic plastic (McMaster Carr, Chicago, IL) using a MicroMill DSLS 3000 computer-numeric-controlled (CNC) machine (MicroProto Systems, Chandler, AZ) controlled with ArtSoft Mach3 software (Newfangled Solutions LLC, Fayette, ME). A 9-cm-diameter acrylic disk with a 1.5-cm-diameter hole in the center was cut from a 1/8” acrylic sheet. An aluminum rotational stage insert was custom fabricated. This stage was comprised of a 12 cm × 12 cm × 0.8 cm (L × W × H) base with a 9.5-cm-diameter bored center. A 3-mm deep relief was cut extending 1 cm from the bored center. An 11.5-cm-o.d., 9.5-mm-i.d., 0.3-cm-thick washer was cut with geared teeth on its outer edge. The teeth mated with a rotational gear placed in one corner of the base. A 1-mm-deep relief was cut on the inner circumference of this washer, extending 2 mm into the washer. To hold the acrylic disk in place, an additional 10-cm-o.d., 8.8-cm-i.d, 0.3-cm-thick washer was cut with a 1-mm-deep relief cut on the inner circumference of this washer, extending 2

mm into the washer (mechanical schematic, Fig. S1). A circular base, referred to as the array disk, was fabricated to hold multiple microwell arrays, as it would allow ready positioning of multiple arrays (Fig. 2C). In order to access sequential microwell arrays, the entire base was designed to be rotated by manually turning the rotational gear.

Pallet Array Holder Fabrication

To mount and position the pallet array above a microwell array, a custom holder was designed. A 10.5-cm acrylic disk with a square 1.2-cm hole halfway between the center and edge was CNC machined from a ¼" acrylic sheet. A 2-mm-deep relief was cut into one side of the disk to allow the addition of PDMS. A 2-mm-wide lip was left around the edges of the disk to contain the PDMS. Two aluminum "L" braces were then fabricated. One brace was attached to the acrylic disk. The other brace was bolted to a miniature 3-axis manipulator (Melles Griot, Albuquerque, NM). The two braces were bolted together to complete the assembly. The brace attached to the acrylic disk was slotted to enable variable positioning of the disk relative to the manipulator. A mount was added to the manipulator and a rail was added to the microscope stage for solid mounting of the device (mechanical schematic, Fig. S2).

Cell Culture

All cell lines were cultured in DMEM (Mediatech Inc., Manassas, VA) supplemented with 10% FBS (Atlanta Biologicals, Lawrenceville, GA) and 1% Pen/Strep (5000 U/mL Penicillin, 5000 U/mL Streptomycin) (Invitrogen, Carlsbad, CA) in 25 cm^2 culture flasks (Corning Inc., Corning, NY). Cells

were cultured in a 37°C incubator (5% CO_2, 95% humidity). Cell suspensions were obtained from cells enzymatically lifted from culture flasks using 2.5 mL 0.25% trypsin (Invitrogen, Carlsbad, CA). Cells were pelleted in a centrifuge (Eppendorf, Hamburg, Germany) and resuspended in 1 mL culture media. Cells were then counted with the aid of a hemocytometer (Fisher Scientific, Dubuque, IA) and diluted with culture media to the desired density.

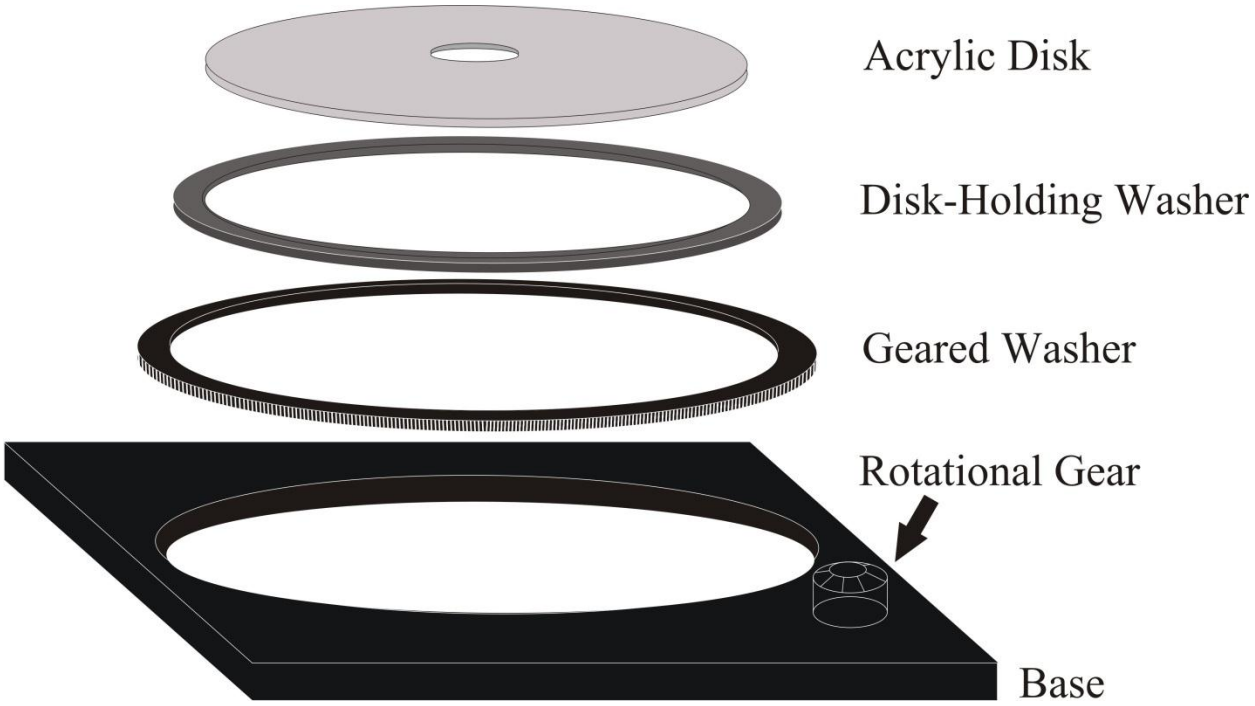

Fig. S1. Exploded schematic view of the microscope stage components for mounting the microwell arrays.

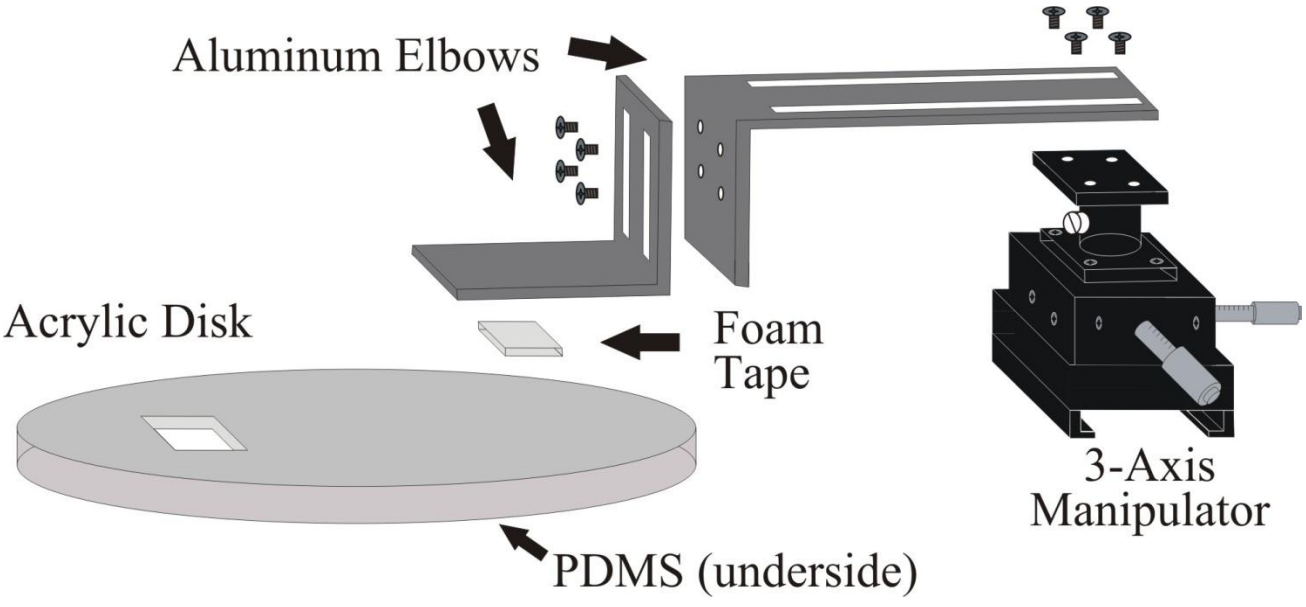

Fig. S2. Exploded schematic view of the microscope stage components for mounting the pallet arrays.

1. Y. Wang, C. E. Sims, P. Marc, M. Bachman, G. P. Li and N. L. Allbritton, *Langmuir*, 2006, **22**, 8257-8262.