Supplementary Information for

Droplet-Scale Estrogen Assays in Breast Tissue, Blood, and Serum

Supplementary Methods

Cleanroom reagents and supplies

Shipley S1811 photoresist and MF321 developer from Rohm and Haas, AZ300T photoresist stripper from AZ Electronic Materials, parylene C dimer from Specialty Coating Systems, Teflon-AF from DuPont, solid chromium from Kurt J. Lesker Canada, CR-4 chromium etchant from Cyantek, hexamethyldisilazane (HMDS) from Shin-Etsu MicroSi, concentrated sulfuric acid and hydrogen peroxide (30%) from Fisher Scientific Canada, Fluorinert FC-40 from Sigma, and SU-8 and SU-8 developer from MicroChem.

Device fabrication

Digital microfluidic devices were fabricated in the University of Toronto Emerging Communications Technology Institute (ECTI) clean room facility, using transparency photomasks printed at City Graphics. Glass wafers (Howard Glass Co. Inc.) were cleaned in piranha solution (a 3/1 v/v mixture of sulfuric acid/hydrogen peroxide) for 10 min, and coated with chromium (150 nm) by electron beam deposition (BOC Edwards). After rinsing (acetone, methanol, DI water) and baking on a hot plate (115°C, 5 min), substrates were primed by spin-coating HMDS (3000 rpm, 30 s) and then spin-coating Shipley S1811 photoresist (3000 rpm, 30 s). Substrates were baked on a hot plate (100 °C, 2 min) and exposed (35.5 mW/cm^2, 4 s) through a transparency photomask using a Karl Suss MA6 mask aligner. Then substrates were developed (MF321 developer, 3 min) and postbaked on a hot plate (100 °C, 1 min). After photolithography, exposed chromium was etched (CR-4, 2 min) and the remaining photoresist was stripped by sonicating in AZ300T (5 min).

After forming electrodes and cleaning in piranha solution (30 s), a photoresist wall was formed, using methods similar to those reported by Moon et al. (*S1*). Briefly, substrates were spin-coated with SU-8-25 (500 rpm, 5 s, then 1000 rpm, 30 s), baked on a hotplate (65°C, 5 min, then 95°C, 15 min), and then exposed to UV light (35.5 W/cm^2, 7 s). After baking (65°C, 1 min, then 95°C, 4 min), and developing in SU-8 developer, substrates were coated with 2 µm of parylene-C and 100 nm of Teflon-AF. Parylene-C was applied using a vapor deposition instrument (Specialty Coating Systems) and Teflon-AF was spin-coated (1% by weight in Fluorinert FC-40, 1000 rpm, 1 min) followed by baking on a hot plate (160°C, 10 min). The polymer coatings were removed from contact pads by gentle scraping with a scalpel to facilitate electrical contact for droplet actuation. In addition to patterned devices, unpatterned indium-tin

oxide (ITO) coated glass substrates (Delta Technologies Ltd) were coated with Teflon-AF using the conditions described above, to serve as the top plate on assembled devices (as described below).

Device operation

The device design included three input reservoir electrodes (3.5 x 3.5 mm) for the raw sample, lysing solvent, and polar extraction solvent, respectively, and a fourth reservoir electrode for collection of the processed sample. Actuation electrodes (1.5 mm x 1.5 mm with a 40 μm inter-electrode gap) formed a path linking the input reservoirs, which passed through a fifth reservoir (delineated by a photoresist wall) containing non-polar extraction solvent. Devices were assembled with an unpatterned ITO–glass top plate and a patterned bottom plate separated by a spacer formed from one or two pieces of double-sided tape (90 or 180 μm thick). Thus, depending on the spacer thickness, reservoir volumes were ~1.1 or 2.2 μl, and unit droplets (covering a single actuation electrode) were ~200 or 400 nl. A single spacer was used to process standard solutions of estradiol, while a double spacer was used for blood, serum, and tissue. Droplets were sandwiched between the two plates and actuated by applying AC potentials (18 kHz, 100 V) between the top electrode (ground) and sequential electrodes on the bottom plate via the exposed contact pads. Droplet motion was monitored by a CCD camera mated to an imaging lens positioned over the top of the device.

As described in the main text, the methods reported here required the manipulation of droplets of fluids that have a wide range of characteristics (surface tension, conductivity, etc.) by DMF. In developing the methods, we found that almost all of the reagents required for the procedure, including methanol, acetone, dichloromethane, and aqueous buffers, were actuatable. According to Chatterjee et al. (*S2*) the only liquids that are not actuatable by DMF are those with negligible conductivity and/or dipole moment; in the current work, we observed this to be the case for isooctane. The non-actuatable nature of isooctane was useful for estrogen extraction, as it facilitated manipulation of droplets of methanol inside of a (non-actuated) pool of isooctane. This process is demonstrated in the accompanying supplementary movie file.

Supplementary Figure 1S

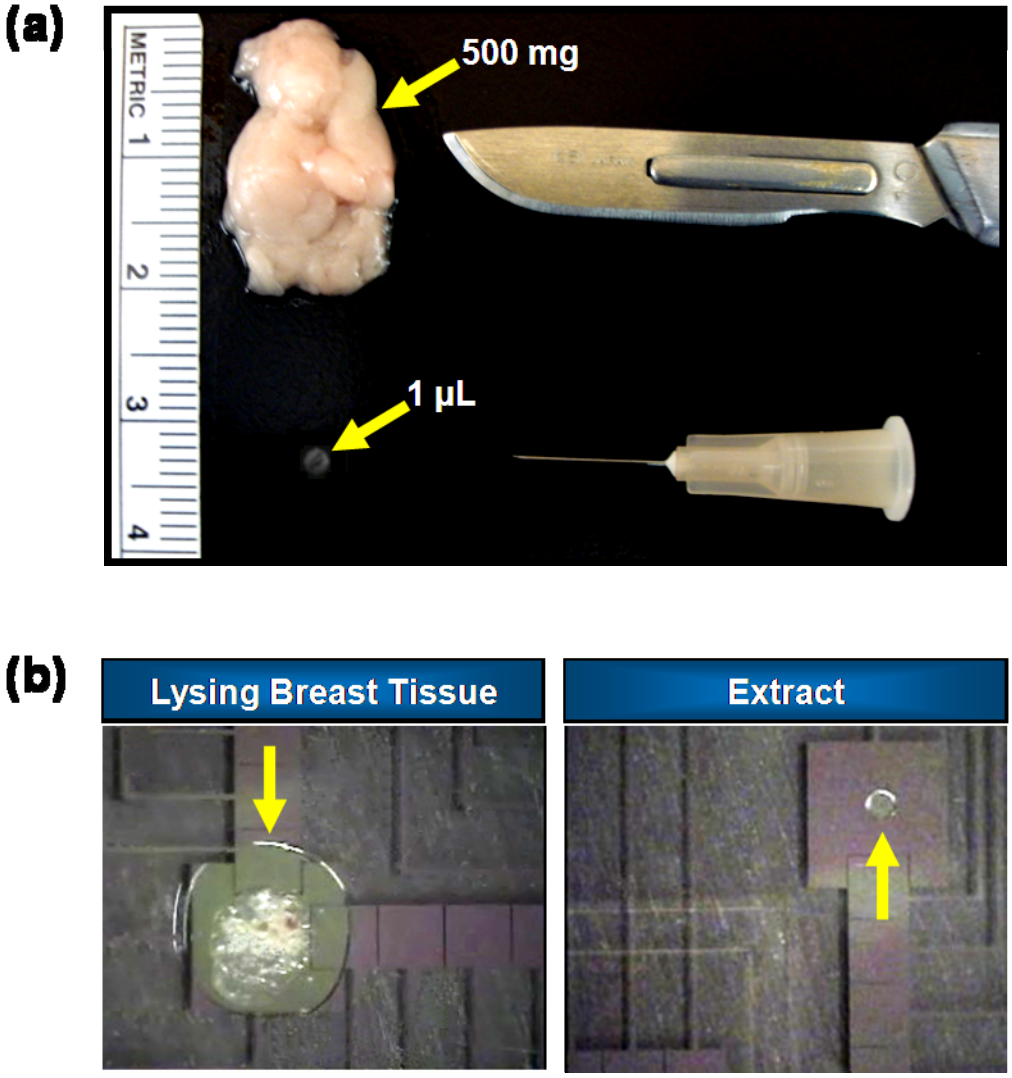

Figure 1S: Reduction in Sample Volume. (a) Picture of 500 mg of breast tissue collected by incisional biopsy (representing the sample size required for conventional analysis methods) compared to a 1 µl microaspirate collected using a 30 ga. needle (representing the sample size used with the new method). **(b)** Pictures of breast tissue microaspirate (1 µl) being lysed on the DMF device and of the dried estrogen extracted from it.

Supplementary References

S1. H. Moon, A. R. Wheeler, R. L. Garrell, J. A. Loo, C. J. Kim, An integrated digital microfluidic chip for multiplexed proteomic sample preparation and analysis by MALDI-MS. *Lab Chip* **6**, 1213-1219 (2006).

S2. D. Chatterjee, B. Hetayothin, A. R. Wheeler, D. J. King, R. L. Garrell, Droplet-based microfluidics with nonaqueous solvents and solutions. *Lab Chip* **6**, 199-206 (2006)