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Quantitative Detection of Bioassays with a Low-Cost Image-Sensor Array for Integrated Microsystems

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Experimental Section

Imaging hardware. Contact imaging of the various microfluidic devices was performed using an Aptina MT9P031I12STC CMOS image sensor (Aptina Imaging Corporation, San Jose, CA). The sensor was implemented using development kit MT9P031I12STCD and controlled using DevSuite interface software, both from Aptina. Collimated light was obtained by coupling an Edmund Industrial Optics 5X Beam Expander (Barrington, NJ) to a Fiber-Lite Series 180 High Intensity Illuminator (Dolan-Jenner Industries, Inc., Boxborough, MA).

Digital microfluidic array. The fluid handling microelectrode array was fabricated using standard microlithographic wet etch processing from thin film Au/Ti (2500 Å/500 Å) on 1 mm-thick Pyrex substrates. The upper fluidics layer was laser-machined in-house (VersaLASER, Universal Laser Systems, Inc., Scottsdale, AZ) from cast acrylic sheet (Acrylite GP, Evonik Cyro, Parsippany, NJ). The device layers were bonded using acrylic pressure sensitive adhesive transfer tape (467MP, 3M, St. Paul, MN). Electrical interconnects were constructed using standard 1 mm-pitch surface mount board connectors (SEI series, Samtec, Inc., New Albany, IN) and stock flat flex cables (Parlex USA, Methuen, MA). Array energization and droplet manipulation were achieved using custom hardware and a LabVIEW Software interface (National Instruments, Austin, TX). Construction of similar devices and droplet manipulation by dielectrophoresis is described in detail elsewhere.^[1;2]

Colorimetric assays. Fluidic microchannels were cast (Dow Corning Sylgard 184 Silicone Elastomer) using a SU-8 on silicon wafer negative mold (200 μ m x 20 μ m). Each demolded channel was sealed against a round glass coverslip (Product 26022, 18 mm-diameter round glass No. 1 coverslip, approximately 130–170 μ m-thick, Ted Pella, Inc., Redding, CA). Eosin Y solution (M10660, Chroma-Gesellschaft, Schmid & Co., Stuttgart, Germany) was serially diluted in deionized water. The absorbance of each of the resulting eosin solutions was determined from analysis of red (600 nm) and green (530 nm) pixel intensity values as quantified with the image sensor during exposure of the contact image. Data for each point is the average ± s.d. from a >50 pixel region of interest in the center of each channel.

Glucose (GO) Assay Kit GAGO-20 (Sigma-Aldrich, St. Louis, MO) was used according to the manufacturer's suggested protocol. The glucose standard reactions were loaded into 1.3 mm depth hybriwells (S-24733, Invitrogen Molecular Probes, San Diego, CA) affixed to round glass coverslips and contact imaged on the sensor. The resulting pixel intensity values were measured to determine absorbance of the colorimetric product (oxidized o-Dianisidine) as a function of glucose concentration. Data for each point is the mean intensity value obtained from a 2025 pixel (approximately 100 μ m x 100 μ m) region of interest. Intensity values were normalized to the highest value.

Bioluminescent assay. A microwell imaging cartridge for chemiluminescence measurements was constructed by laser-cutting 1 mm diameter wells into black polyester sheet material. The sheet was then mounted to a coverslip using 3M pressure sensitive acrylic adhesive transfer tape 467MP. Kinase-Glo Plus Luminescent Kinase Assay (V3771, Promega Corporation, Madison, WI) was used according to the manufacturer's protocol. ATP (A2383, Sigma-Aldrich) was serially diluted into kinase buffer (40 mM Tris-HCl pH 7.5, 20 mM MgCl₂, 0.1 mg/mL BSA). Kinase-Glo assay reactions were loaded into the array of microwells and the array was placed directly on top of the sensor chip for contact imaging. A simple opaque cover was used to protect the sensor from stray light. The relative chemiluminescence (in arbitrary units) of each reaction was then determined from analysis of the blue (450 nm) pixel intensity values as quantified with the image sensor during exposure (200 ms) of the contact image. Data shown for each point is the average \pm s.d. from 100 pixel region in the center of each well.

References

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