1-MILLION DROPLET ARRAY FOR HIGH-DYNAMIC-RANGE DIGITAL MICROFLUIDICS

A.C. Hatch[1], J.S. Fisher[1] and A.P. Lee*[1]
[1]*University of California-Irvine, USA*

ABSTRACT

Digital biology droplet reactors are useful as chemical and bio-chemical reactors discretizing reagents into picoliter to nanoliter sample volumes for individual analysis of cells, organisms, or molecules. Presented is a single droplet microfluidic device which generates 1 million densely packed, monodisperse, 50 picoliter droplets in a high density 3-Dimensional self-assembled sphere packing configuration combined with a 21-megapixel resolution and 15 cm^2 field of view fluorescence imaging setup to perform quantitative digital biology analysis. Compared to previous works, this high density digital biology reactor array yields a twofold increase in real-time monitoring reactor number, thereby simultaneously increasing throughput and dynamic range.

KEYWORDS: Droplet Microfluidics, Digital Biology, Sphere Packing, Self Assembly, High Dynamic Range,

INTRODUCTION

MicroTAS systems continuously strive to provide more information and function in ever smaller packages, as demonstrated in the growth of on-chip micro-arrays and micro-reactors for mass parallel processing. On-chip micro-wells and droplet-reactors have gained attention in the field of "digital" microfluidics for applications like "single cell" analysis and "single molecule" DNA detection [1]. Unfortunately, the dynamic range of digital microfluidic assays is lagging far behind its analog counterparts by three to four orders of magnitude due to the lack of on-chip compartmentalized reactors and limited throughput [2]. As seen in Figure 1 taken from a review by Mark et al. [3], high-density arrays lose as much as 15-50% percent of the imaging area to dead space between reactor sites resulting in a less than optimal density of digital reactors per reaction area. We present a methodology to incorporate 1 million "digital" droplet reactors on-chip, in optimally arranged 3-Dimensional (3-D) droplet packing configurations, to yield high-density 2-Dimensional (2-D) imaging arrays for high-throughput imaging and long-term visualization without complicated optical detection schemes, such as confocal microscopy, or off-chip processing for thermocycling. We have further extended dynamic-range by (1), increasing on-chip droplet-reactor density to reduce the space requirements for imaging, and (2), increase optical imaging area and resolution to capture 1 million droplets in a single snapshot for digital quantification.

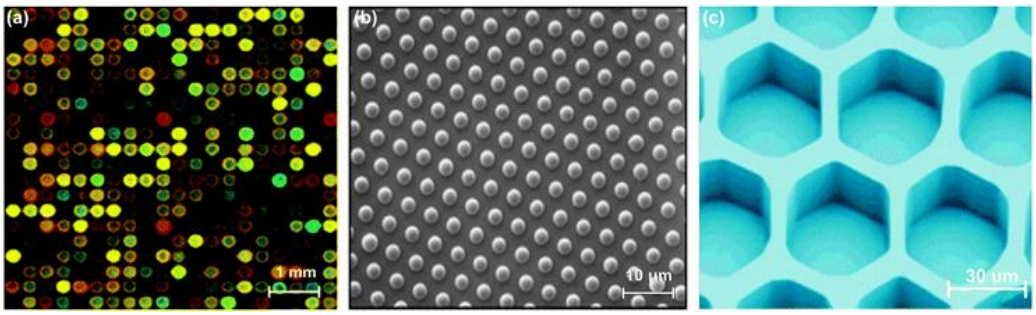

Figure 1: Images of typical micro-array, and micro-well pattern designs published in a review by Mark et al.[3]. Note a 15-50% spatial imaging area lost to inter-reactor spacing.

THEORY

We propose a means to increase digital reactor density by controlling the vertical dimension of microfluidic chambers to predictably manipulate the self-assembly of droplets into a high-density, 2-D reactor-array for optimal imaging. Figure 2 illustrates packing configurations determined from a simple sphere packing model for 3-D chamber Height to Droplet Diameter Ratio's (HDDR's) between 1-1.82 which yields a 2-fold increase in droplet density per unit area for a given droplet diameter. Table 1 summarizes calculations for corresponding HDDR values demonstrating percent changes in droplet packing density, image area coverage, and droplet area overlap. The second area of development is to increase the imaging area while maintaining imaging resolution for individual digital-droplet detection. This was accomplished using lower magnification imaging techniques off microscope compensated by a higher resolution imaging sensor to adequately resolve individual droplet reactors with a desired 20 pixels per droplet minimum.

Table 1: Chamber Height to Droplet Diameter Ratio (HDDR) calculations

HDDR	*Drops per Unit Hexagonal Area*	*Increase in Density (%)*	*Area Coverage (%)*	*Droplet Overlap (%)*
1	*1*	*0*	*90.7*	*0*
1.45	*1.17*	*17*	*97.4*	*16.5*
1.7	*1.73*	*73*	*100*	*72.6*
1.82	*2*	*100*	*97.6*	*92.4*

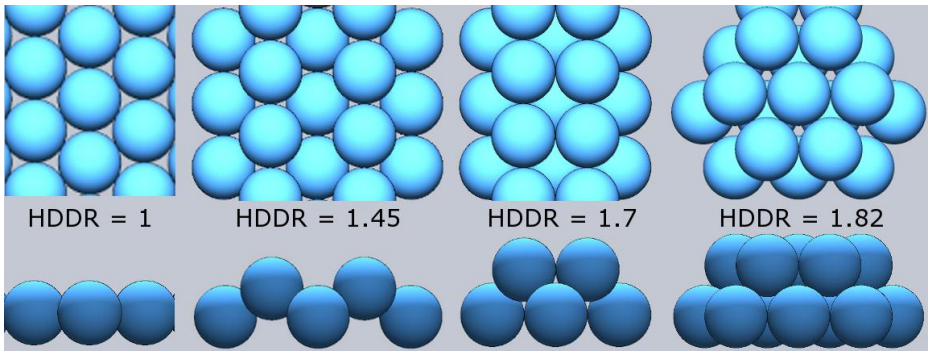

Figure 2: Illustration of maximum density packing configurations for HDDR values ranging from 1 to 1.82 using a simple sphere packing model. a) HDDR=1 results in single layer hexagonal packing, b) HDDR=1.45 results in mixed height loose square packing, c) HDDR=1.7 results in double layer close square packing, and d) HDDR=1.82 results in double layer close hexagonal packing.

EXPERIMENTAL

Microfluidic devices were fabricated using traditional soft-lithography processing however an additional glass slide was bonded to the topside of the PDMS parts to reduce chamber deformation resulting from thermal expansion and hydrostatic pressure. Several droplet microfluidic devices with varying HDDR values were fabricated using feature heights ranging from 46 µm tall to 84 µm tall to study and compare droplet packing configurations for each HDDR value. A large 128 or 256 droplet splitter device capable of generating and holding 1-million droplet reactors in an 82 µm high 11cm^2 chamber area was used to achieve the high dynamic range 1-million droplet reactor devices. Heavy mineral oil with 3% EM90 and 0.05% Triton X-100 was used as the continuous phase.

Digital PCR was performed to demonstrate single molecule DNA quantification using typical PCR protocols with the addition of 0.1 mg/mL BSA concentration to help reduce sample adsorption to tubing and PDMS surfaces as well as an additional droplet stabilizing surfactant. A PCR dilution series of a known concentration of 240 bp DNA strand sequences and a GFP labeled primer/probe pair was used to determine and compare the digital dynamic range of the device for end-point digital PCR quantification.

A large field of view fluorescence imaging device was fabricated using a relatively low cost commercial Canon 5D 21-Megapixel digital camera with 100mm macro lens to image 8.6 cm^2 to 17 cm^2 areas at 1x to 0.5x magnification values respectively. Fluorescence excitation and imaging was accomplished using a Semrock GFP filter set with a 25 mm excitation filter and a 35 mm emission filter combined with a blue LED illumination source.

Fluorescence images were processed in ImageJ and/or a custom Matlab program to perform a threshold binarization of the images followed by a watershed isolation and shape analysis of bright fluorescent spots to detect and quantify the total number of amplified fluorescent droplets present in the image.

RESULTS AND DISCUSSION

The performance of the varying HDDR driven 3-D colloidal droplet configurations resulting in high density 2D imaging arrays are demonstrated using fluorescence imaging of polymerase chain reaction (PCR) amplified droplets as in Figure 3.

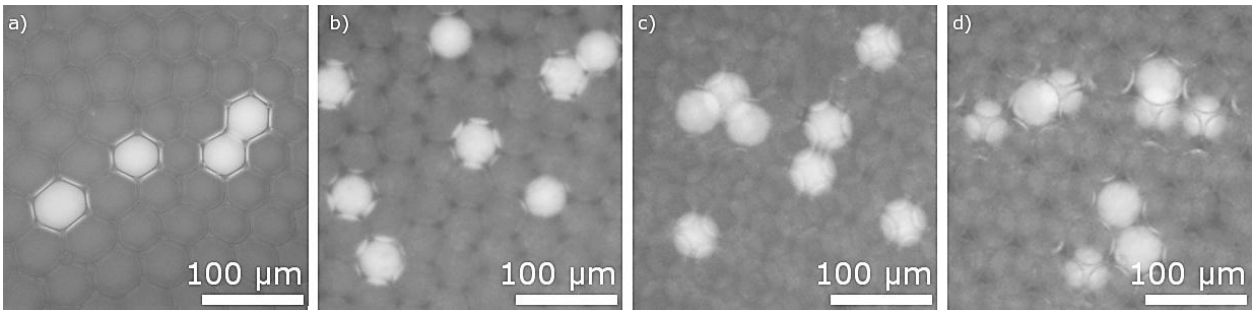

Figure 3: Digital microfluidic images with HDDR values similar to those illustrated in previously. a) HDDR=1, single layer hexagonal packing, zero overlap of droplets, note the density is slightly higher than simple sphere packing model due to deformation of droplets. b) HDDR=~1.45, loose square packing of droplets, ~17% increase in density. c) HDDR=1.7, close square packing of droplets resulting in ~73% droplet overlap and ~73% increase in packing density. d) HDDR=1.82, double layer hexagonal packing with ~93 % overlap of droplets and ~100% increase in droplet density. Despite high percentages of droplet overlap in multilayer configuration, individual droplets can still be detected using automated image processing algorithms and commercial software.

Large field of view bright field and fluorescence images of 1 million 50 picoliter droplet reactors in an 11 cm^2 chamber are shown in Figure 4. This system design can achieve up to a 20-40 pixels/droplet resolution regardless of droplet diameter given the appropriate HDDR, imaging magnification, and fluorescence excitation to match. A dynamic range of close to five orders of magnitude is demonstrated in Figure 5 for a concentration range of 20-500,000 DNA strands per reaction.

a) b)

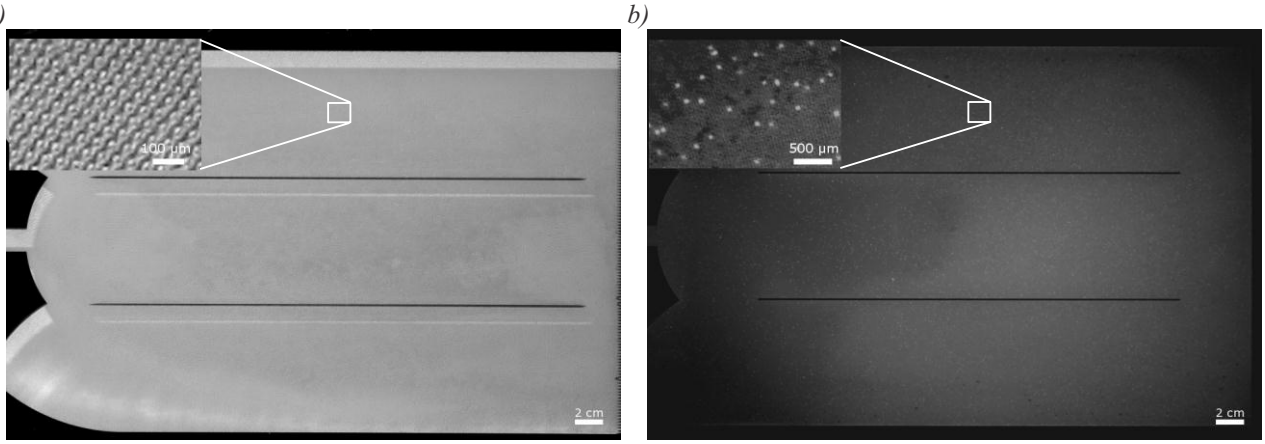

Figure 4: grayscale image and magnified insets of microfluidic chamber containing 1 million droplets in 11 cm^2 chamber (scale bar: 2 cm) a) immediately after droplet generation (scale bar: 100 µm in inset) and b) fluorescence image post 40 PCR cycles for digital PCR quantification. (scale bar: 500 µm in inset).

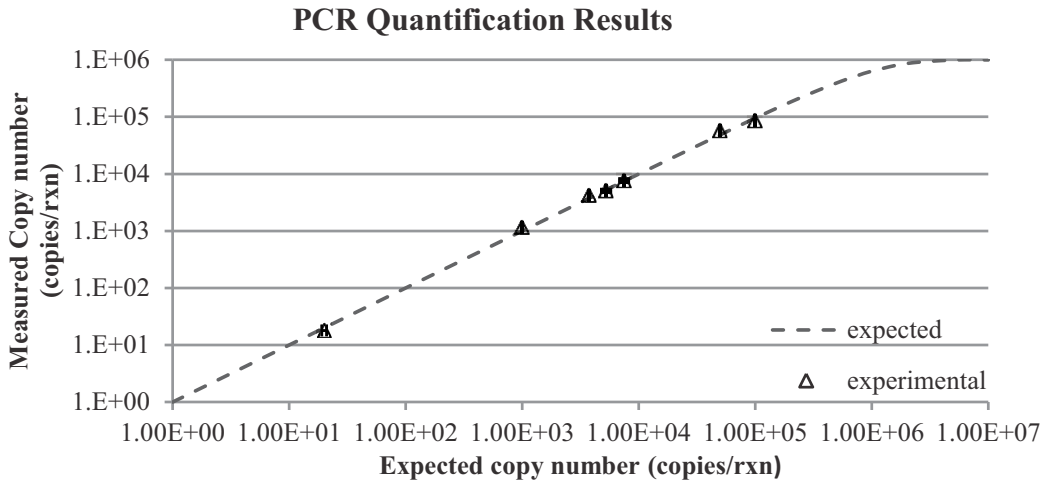

Figure 5: High Dynamic Range Digital PCR experimental results containing digital PCR dilutions were performed using 240 bp DNA sequences at concentrations ranging from 1x10^1-1x10^5 copies per 50 µL reaction, amplified 30 cycles, imaged with 5s fluorescence image on a 21-megapixel digital camera then processed to quantify the total copy number present per reaction.

CONCLUSION

Compared to previous works, this high density digital biology reactor array yields a twofold increase in real-time monitoring reactor number, thereby simultaneously increasing throughput and dynamic range. The novelty of this 3-Dimensional droplet-reactor patterning and imaging method will have a profound impact toward very-large-scale-integration (VLSI) digital microfluidics.

ACKNOWLEDGEMENTS

Authors would like to thank Beckman Coulter and DARPA MF3 center for providing funding and expertise and colleagues Rob Lin, and Armando Tovar for input, advice, and feedback on topics related to the project.

REFERENCES

[1] E. Brouzes, M. Medkova, N. Savenelli, D. Marran, M. Twardowski, J.B. Hutchison, J.M. Rothberg, D.R. Link, N. Perrimon, and M.L. Samuels, "Droplet microfluidic technology for single-cell high-throughput screening," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, Aug. 2009, pp. 14195–14200.

[2] E.A. Ottesen, W.H. Jong, S.R. Quake, and J.R. Leadbetter, "Microfluidic digital PCR enables multigene analysis of individual environmental bacteria," *Science*, vol. 314, 2006, pp. 1464–1467.

[3] D. Mark, S. Haeberle, G. Roth, F.V. Stetten, and R. Zengerle, "Microfluidic lab-on-a-chip platforms: requirements, characteristics and applications.," *Chemical Society Reviews*, vol. 39, 2010, pp. 1153-1182.

CONTACT

*Abraham P. Lee, tel: +1-949-824-9691; aplee@uci.edu