Development of transparent microwell arrays for optical monitoring and dissection of microbial communities

Michelle Halsted[a], *Paige Briggs*[b], *Ryan Hansen*[c], Andrea.C. Timm[d], Dayrl P. Briggs[e], Scott. T. Retterer[a,d,e]
[a]*The Bredesen Center, University of Tennessee, Knoxville, TN 37996*
[b]*Chemical Engineering, The University of Alabama, Tuscaloosa, AL 35487*
[c]*Chemical Engineering, Kansas State University, Manhattan, KS 66506*
[d]*Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831*
[e]*Center for Nanophase Material Sciences, ORNL, Oak Ridge, TN 37831*
halstedmc@ornl.gov

Microbial communities are incredibly complex systems that dramatically and ubiquitously influence our lives. They help to shape our climate and environment, impact agriculture, drive business and have a tremendous bearing on healthcare and physical security. Recent work has demonstrated the use of silicon based microwell arrays, combined with parylene lift-off techniques, to perform both deterministic and stochastic assembly of microbial communities *en masse*. This enables the high-throughput screening of microbial communities for their response to growth in confined environments under different conditions. Here, the improvement of the microwell platform, through the creation of transparent microwell arrays, while retaining the ability to use parylene lift-off to construct communities, is described. The transparent microwells allow continuous, high resolution, confocal imaging of communities *in situ*, and ultimately facilitates laser microscope dissection of 'successful' communities, providing genetic materials for compositional and functional analysis. Different methods for creating transparent microwell arrays, including quartz etching, were explored. However, the stability of the parylene lift-off layer during etching, limited etch rates of the quartz substrate, and the roughness of the etched surfaces proved to be significant limitations. Ultimately, an 'etchless' process, enabled by encapsulating SU-8 in a low temperature atomic layer deposited silicon dioxide, was successful.

'Etchless' microwell arrays were created by first patterning SU-8 negative photoepoxy resist on 4" glass substrates or individual cover slips as shown in Figure 1(left). Well depth was controlled by the thickness of the spin-coated SU-8 and was nominally 20 microns. By design, microwell diameters were varied in diameter from 5 to 100 microns. Individual chips were diced from the wafer and then coated with a layer of silicon dioxide using atomic layer deposition carried out at 150^{o}C. Adequate thickness of the ALD silicon dioxide layer proved critical to enabling facile peeling of the subsequently deposited parylene films during use. Parylene was deposited and patterned using an aligned negative-tone photoresist mask and aggressive oxygen plasma etch. Holes in the masking layer were slightly larger than the well to allow complete removal of the parylene from the microwell (Figure 1, right).

Transparent microwell arrays with parylene masks for dry lift-off are being used to stochastically assemble microbial communities in hopes of developing a clearer understanding of the role that confinement and community composition play in community survival under different environmental conditions. The use of high resolution laser confocal microscopy and laser microscope dissection to characterize communities within these optically accessible microarrays will be discussed.

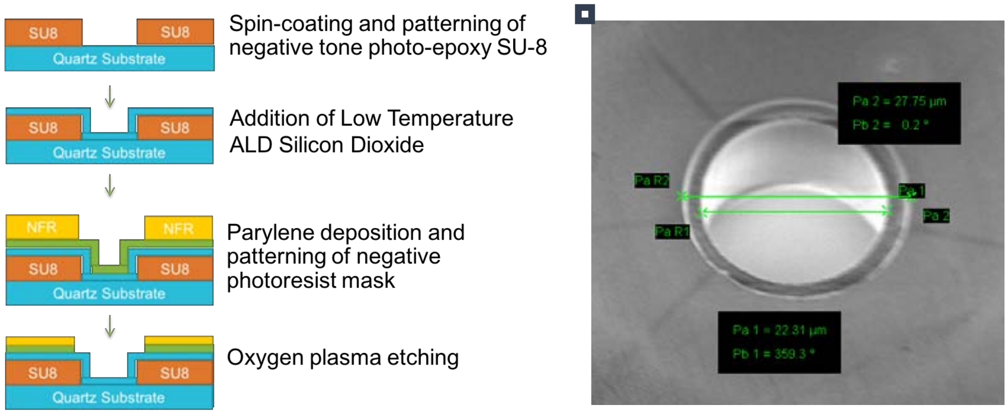

Figure 1: (left) A schematic representation of the 'etchless' microwell fabrication process. (right) Scanning electron micrograph of an 'etchless' microwell showing the slightly oversized parylene stencils patterned on a ~20 micron deep SU-8 microwell.