Microfluidic Chip Fabrication Using CNF Facilities

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Primary CNF Tools Used: Heidelberg DWL2000, GCA AS200 i-line Stepper, Oxford PECVD, Oxford 81 RIE

Abstract:

This project aimed to enable long-term fluorescence microscopy imaging of Shewanella oneidensis interactions with CdSe quantum dots by immobilizing motile bacteria physically within a channel instead of through chemical binding. To achieve this, we designed a custom "mother machine" microfluidic device composed of parallel microchannels branching from a main fluid channel. These side channels were aimed to be dimensionally matched to the diameter of individual bacteria, allowing for their physical confinement while maintaining media exchange, thus facilitating continuous observation over time. Fabrication of the device required high-resolution soft lithography using polydimethylsiloxane (PDMS) cast from a silicon mold. The Cornell NanoScale Science and Technology Facility (CNF) was essential for the creation of this master silicon mold. We used the Heidelberg DWL2000 laser writer for mask patterning, followed by photolithographic processing with the GCA AS200 i-line Stepper. Feature development and refinement were carried out using the Oxford PECVD for surface passivation and the Oxford 81 RIE system for precise etching. This platform aimed to allow us to resolve real-time nanoscale interactions between fluorescent nanoparticles and live bacteria, offering new insight into quantum dot– microbe dynamics. The machinery at the CNF facility was required to obtain the small diameter (below 0.7 μm) of the channel features, which was not attainable using our equipment or any commercial equipment.

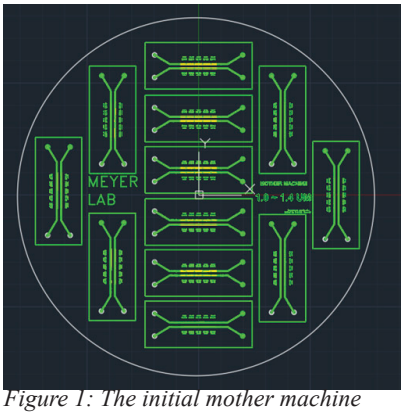

Figure 1: The initial mother machine chip design featuring sharp rectangular channels.

Summary of Research:

This microfluidics chip was designed to address the challenge of imaging interactions between motile Shewanella oneidensis bacteria 1,2 and CdSe quantum dots, which are used in our broader research on nanoparticle-based catalysis. Traditional imaging platforms require timescales too long to monitor these highly motile cells over time, so we engineered an ultraminiaturized "mother machine" to trap individual bacteria in channels that are significantly smaller than those used in previous designs3—pushing the limits of soft lithography resolution.

The design of the microfluidic chip centered on creating a high-resolution "mother machine" with sub-micron precision, featuring narrow side channels approximately 0.7 μm wide branching off a main flow channel approximately 25 μm wide. The chip was constructed from PDMS cast on a silicon mold, with SU-8 photoresist features forming the mold's surface relief. The layout was designed in KLayout software with extensive support from CNF staff, particularly Garry Bordonaro and Aaron Windsor, who provided critical guidance on optimizing the design for photolithography and transitioning from square to rounded channel geometries. This design adjustment, made between Figure 1 and Figure 2, significantly improved fluid dynamics by reducing backflow and enhancing media exchange across the confined bacteria. Fabrication at CNF involved multiple cleanroom steps, including photomask writing with the Heidelberg DWL2000, mask alignment and exposure using the GCA AS200 i-line Stepper, and multilayer etching using the Oxford 81 RIE system. Due to the chip's extremely fine features, the etch process required a two-step

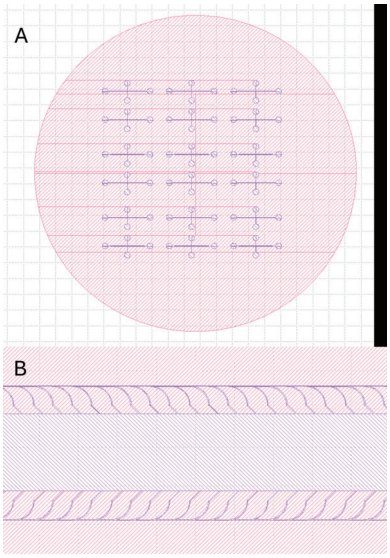

Figure 2: The mother machine design revised with the assistance of CNF staff featuring curved channels. Part A shows the full chip design and part B shows a closer view of the main channel and branching channels.

approach developed with the help of Jeremy Clark, who also provided expertise on the Oxford PECVD system for wafer preparation. Extensive cleanroom training and tool-specific instruction were essential to successfully executing this complex fabrication process.

To test the chip, PDMS was cast onto the fabricated silicon master and cured to form the microfluidic structure, which was then plasma treated and bonded to a glass coverslip to seal the channels. Fluidic testing involved introducing S. oneidensis cells into the device using a syringe pump to establish controlled flow through the microchannels. This functional testing revealed two key issues: first, the side channels were not sufficiently narrow to trap individual bacteria effectively, as shown in Figure 3; and second, leakage occurred at higher flow rates, suggesting inadequate bonding or minor defects at the PDMS–glass interface. These outcomes highlighted the need for tighter feature tolerances and improved sealing, prompting a redesign of the channel dimensions and further optimization of the fabrication protocol.

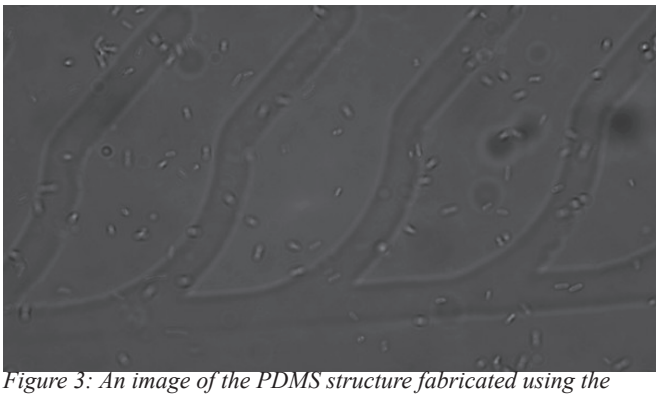

Figure 3: An image of the PDMS structure fabricated using the mask. Bacteria can be observed outside of the microchannels due to leakage at higher flow rates, and the side channels were slightly too large to trap the bacteria.

Conclusions and Future Steps:

Although the initial version of the microfluidic chip did not fully meet the functional requirements for long-term bacterial confinement, the fabrication process validated several critical aspects of the design and demonstrated the capabilities of CNF's advanced lithographic and etching tools. The pattern transfer from the KLayout design to the silicon mold using the Heidelberg DWL2000 and GCA AS200 i-line Stepper was highly successful, yielding clean and reproducible features at both the sub-micron and tens-of-micron scale. The two-step etching process developed with guidance from CNF staff, particularly Jeremy Clark, allowed for precise control over feature depths, which is essential for the multi-scale structure of the chip. While the final PDMS device exhibited some leakage at higher flow rates and did not achieve full bacterial trapping due to overly large side channels, these outcomes provided direct feedback that will inform future improvements.

The ability to prototype a custom-designed, high-resolution microfluidic device at this level would not have been possible without access to CNF's advanced photolithography and etching infrastructure, nor without the expert technical support provided throughout the process. This project has laid the groundwork for developing a next- generation mother machine capable of precisely trapping S. oneidensis for extended imaging of nanoparticle–microbe interactions. Moving forward, refined designs will incorporate narrower channel geometries and optimized bonding techniques to address current limitations. At this point no publications have been drafted.

References:

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