

## Fabrication of In-Plane Nanofluidic Devices in Glass Substrates by Flood Gun Assisted FIB Milling

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Focused ion beam (FIB) milling is well-suited for fabrication of in-plane nanofluidic devices because the nanochannels can be arranged in any two-dimensional format, confined to the nanoscale in both lateral dimensions, i.e., channel depth and width, and directly integrated with microfluidic channels. To construct the devices, we first machine V-shaped microchannels into a glass substrate by conventional lithography and etching. Nanochannels are then formed in the substrate to span the microchannels. To mill nanochannels directly into glass substrates, we use a flood gun to bathe the substrate surface with electrons to minimize surface charging. Use of a flood gun circumvents the need to coat the substrate with a conductive metal film. The micro- and nanochannels are sealed by thermally annealing a cover plate to the substrate. The nanochannels are designed with either a single pore or multiple pores in series for resistive-pulse sensing of hepatitis B virus capsids. Multiple nanopores in series permit sensing each capsid multiple times to obtain both relative size and charge information.