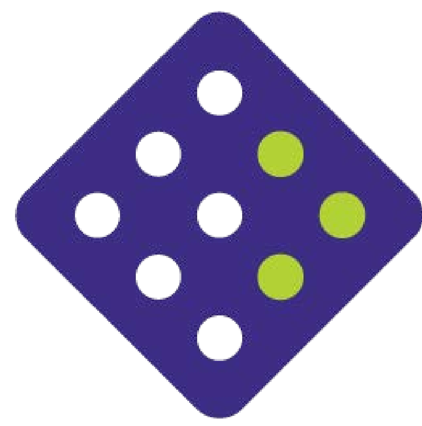


µSiM-EV: Silicon Membrane-Enabled Microfluidics for Simplified Single Extracellular Vesicle Visualization

SiMPore Inc., West Henrietta, NY 14586 • www.simpore.com • info@simpore.com

© SiMPore Inc. ALL RIGHTS RESERVED.

Overview

- The µSiM-EV enables imaged-based analysis of individual EVs.
- Works with EVs prepared by ultracentrifugation, size-exclusion chromatography, or filtration from a variety of biofluids.
- Requires simple pipette-driven loading and conventional epifluorescent microscopy, with no specialized equipment needed.

µSiM-EV Concept

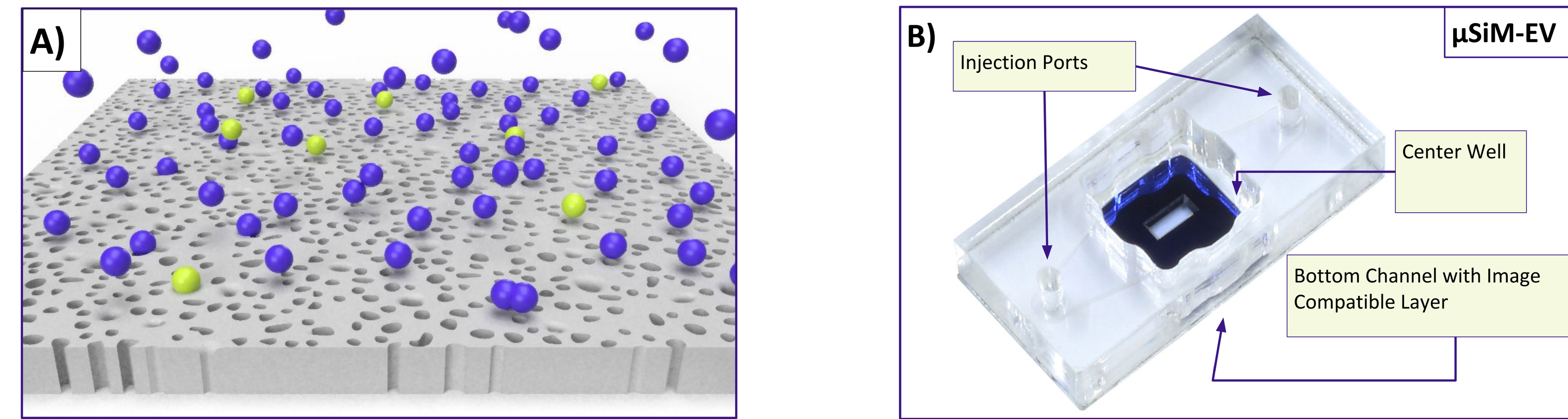


Figure 1: The EV Visualization Concept. A) Representative nanomembrane with EV-sized pores that capture individual fluorescent affinity-tagged EVs; fluorescent color indicates EVs positively labeled with affinity tags. B) Microfluidic device enabled by a silicon membrane for EV visualization ("The µSiM-EV"), with two injection ports, center well, and bottom channel with imaging-compatible layer.

Fluorescent Bead Visualization

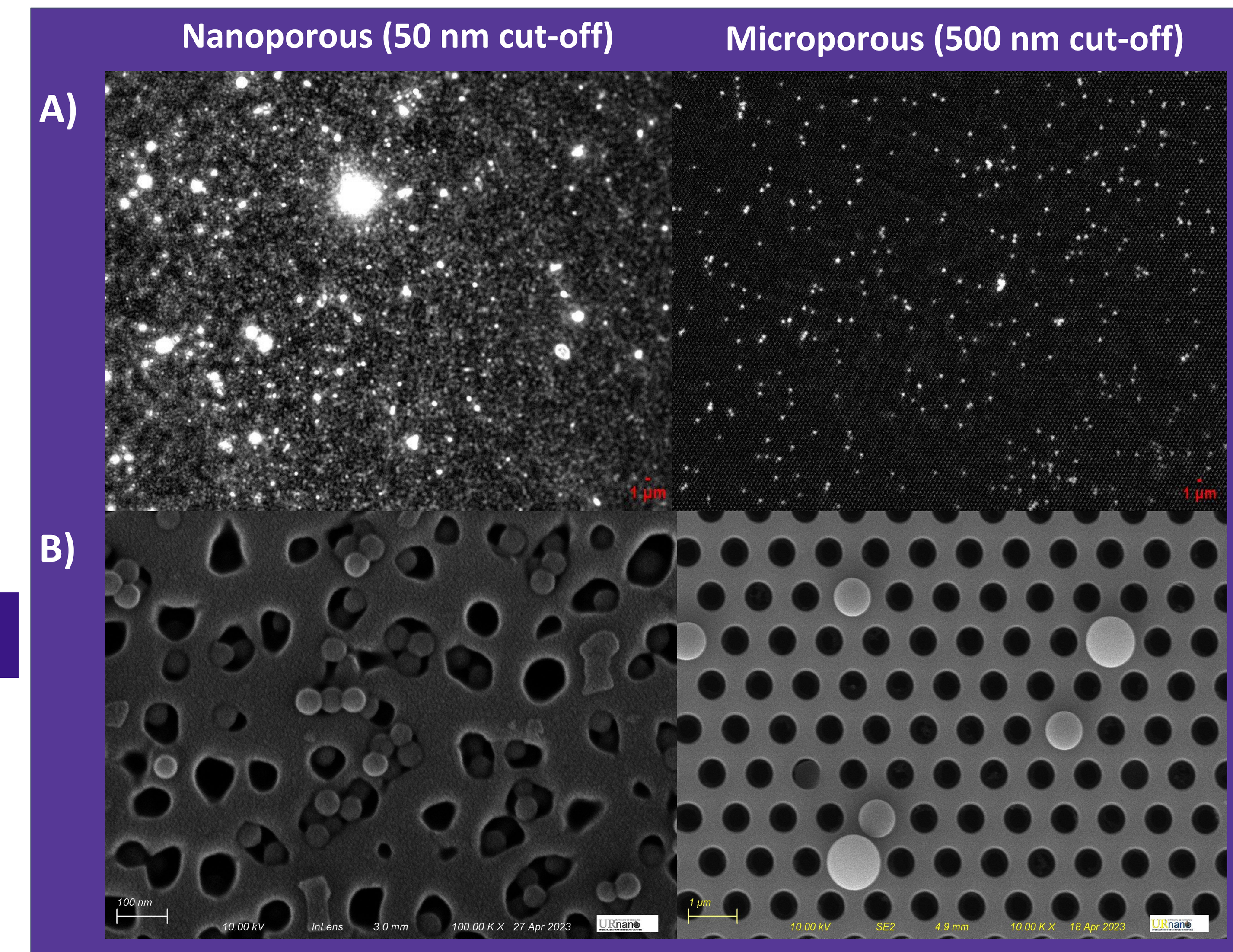


Figure 4: Imaging via Fluorescence and Electron Microscopy. A) Fluorescent polystyrene beads (45 nm Nile Red on the nanoporous membrane, 830 nm Jade Green on the microporous membrane) were injected into the µSiM-EV and imaged via fluorescence microscopy. B) The same µSiM-EV units as in A) were prepared for scanning electron microscopy (SEM) by removal of the bottom layer and depositing ~7 nm Au, then imaged via SEM.

Visualize via Antibody Staining

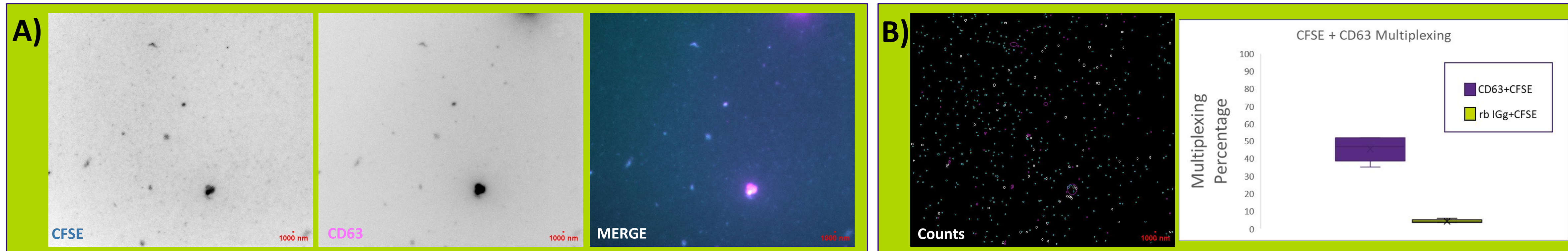


Figure 2: Immunolabeling Analysis by µSiM-EV of EVs Prepared by Dual Mode Chromatography. Human plasma (0.5 mL) was applied to a column comprising 10 mL of Sepharose CL-4B and 2 mL FractoGel-sulfate A) CFSE (cyan) and anti-CD63 (magenta) fluorescent micrographs are shown via 1% representative fields-of-view from the µSiM-EV's membrane. B) Particle counts are rendered from ImageJ ComDet using 5 px max separation, 3 px/500 nm max particle size, and 3 intensity threshold. Images in A obtained from a Zeiss Z1 epifluorescent microscope. 40X objective.

Count EVs

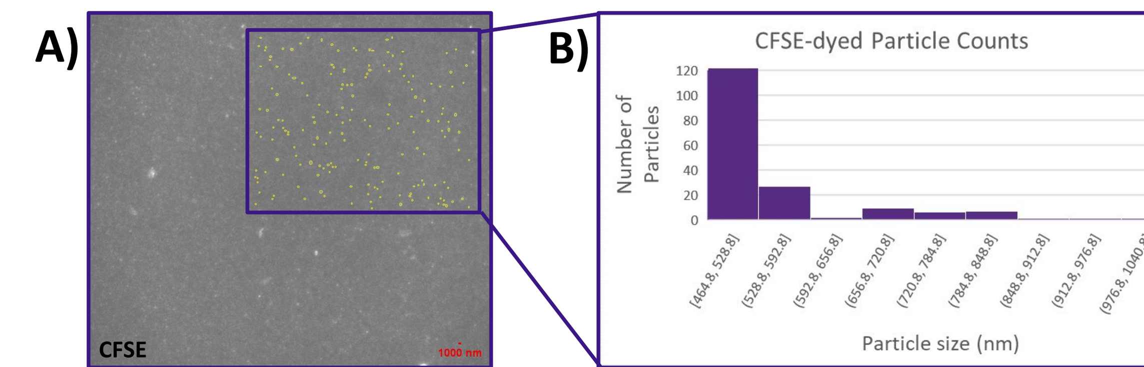
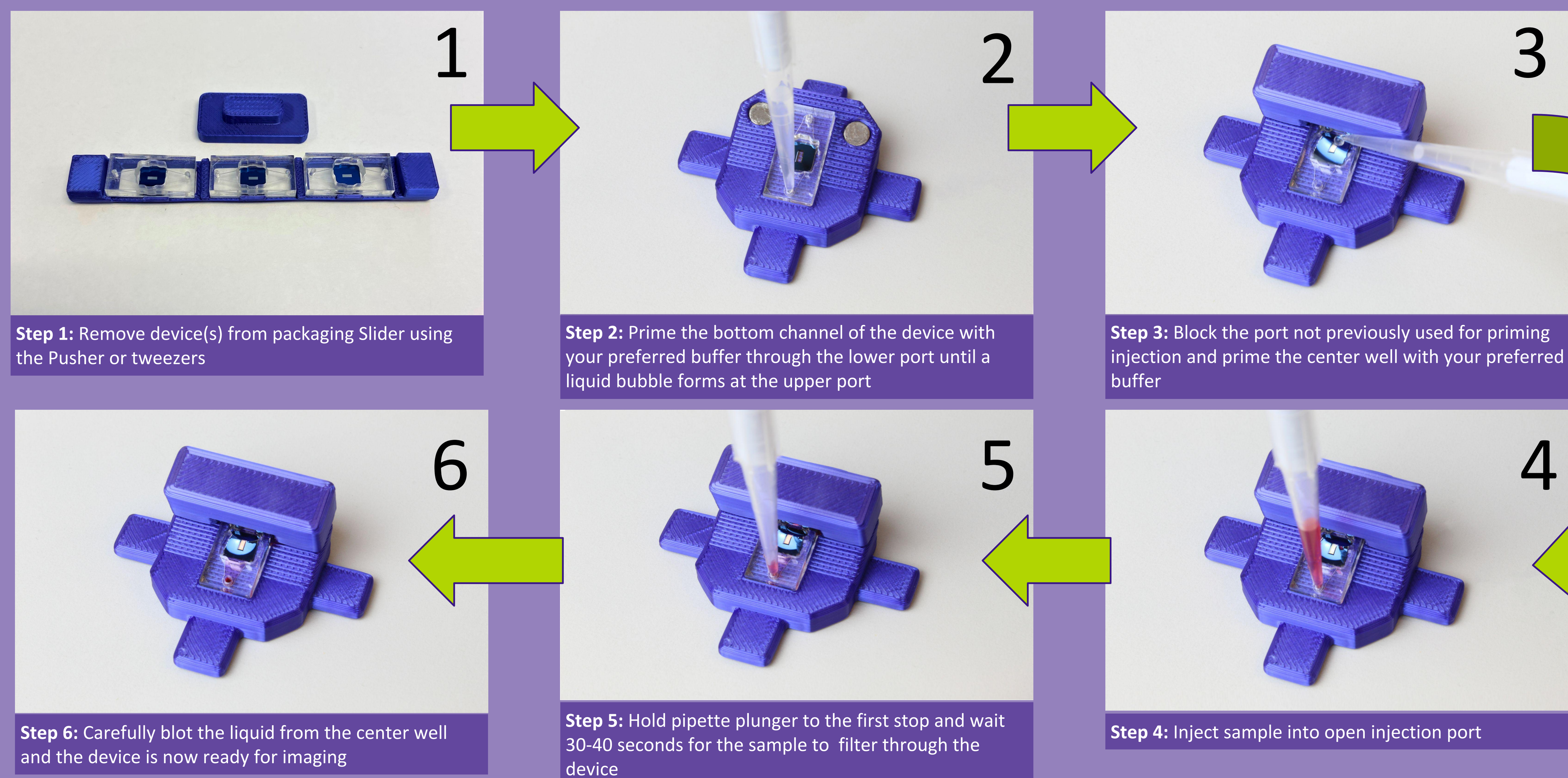


Figure 3: Count EVs prepared by Dual Mode chromatography on the µSiM-EV. Prepared EVs, as in Figure 2, were labeled with the amine-reactive pan-label CFSE. A) CFSE fluorescent micrograph B) Particle counts of CFSE-positive particles counted by ImageJ ComDet using 5 px max separation, 3 px /500 nm max particle size, and 3 intensity threshold.

EV Visualization Workflow

Injecting the Device



Step 1: Remove device(s) from packaging Slider using the Pusher or tweezers

Step 2: Prime the bottom channel of the device with your preferred buffer through the lower port until a liquid bubble forms at the upper port

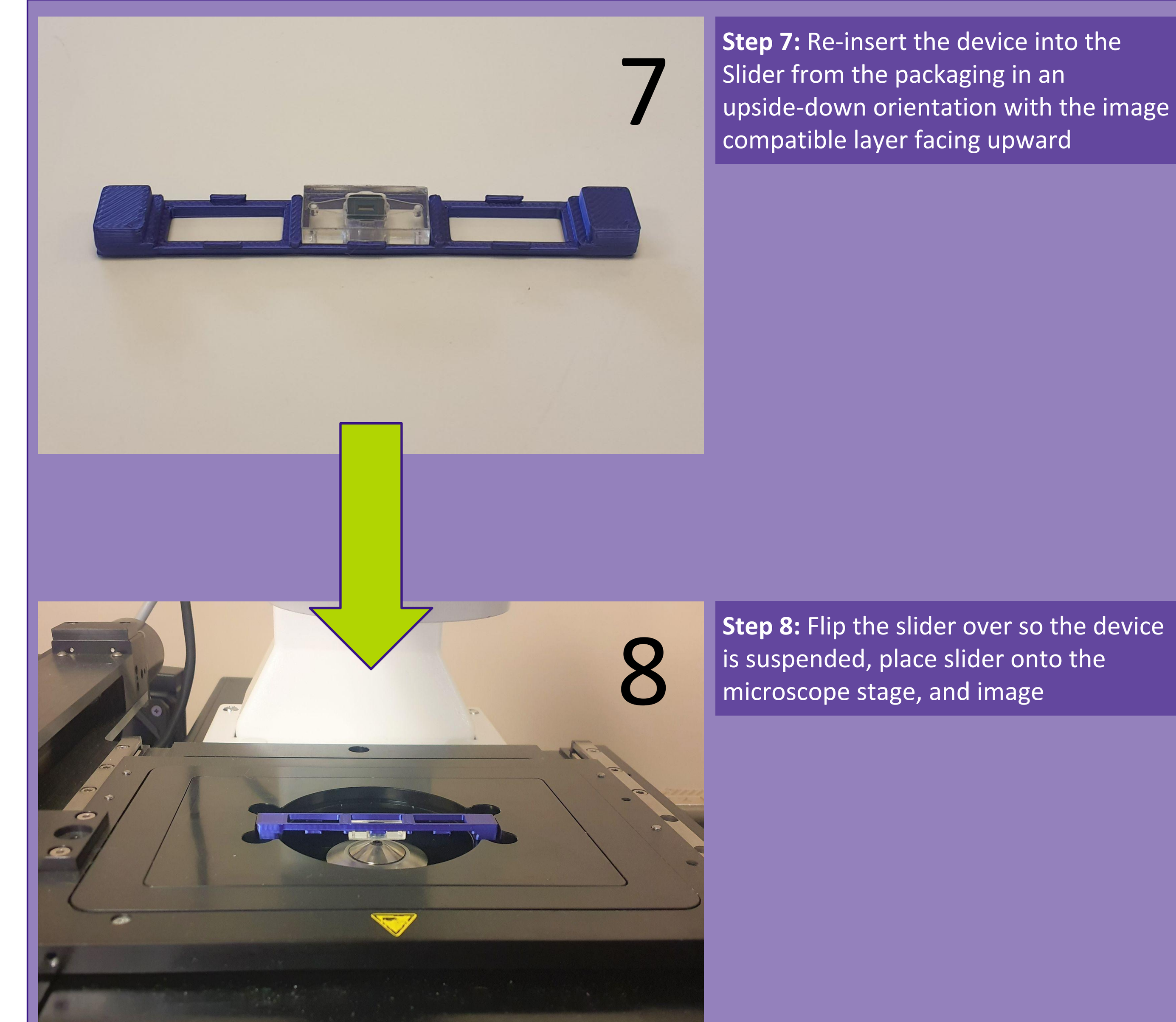
Step 3: Block the port not previously used for priming injection and prime the center well with your preferred buffer

Step 4: Inject sample into open injection port

Step 5: Hold pipette plunger to the first stop and wait 30-40 seconds for the sample to filter through the device

Step 6: Carefully blot the liquid from the center well and the device is now ready for imaging

Imaging the Device



Step 7: Re-insert the device into the Slider from the packaging in an upside-down orientation with the image compatible layer facing upward

Step 8: Flip the slider over so the device is suspended, place slider onto the microscope stage, and image

Visit us at Booth #113!

SCAN ME!
To save 10% on your first order