

# Supplementary Information

## Volumeless reagent delivery: a liquid handling method for adding reagents to microscale droplets without increasing volume

Duane S. Juang<sup>a</sup>, Joshua M. Lang<sup>b</sup> and David J. Beebe<sup>\*a,c</sup>

<sup>a</sup> Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, WI 53705, USA.

<sup>b</sup> Department of Medicine, Wisconsin Institutes for Medical Research, University of Wisconsin-Madison, Madison, WI 53705, USA

<sup>c</sup> Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, WI 53705, USA.

\*Corresponding author: [djbeebe@wisc.edu](mailto:djbeebe@wisc.edu)

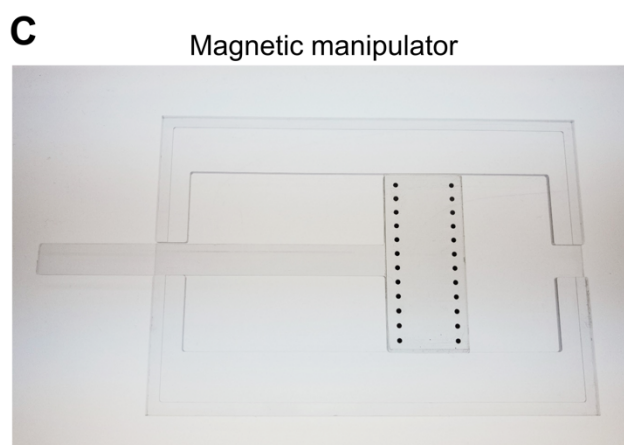
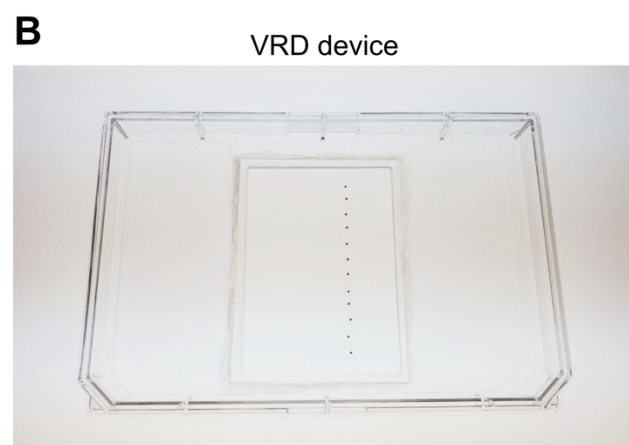
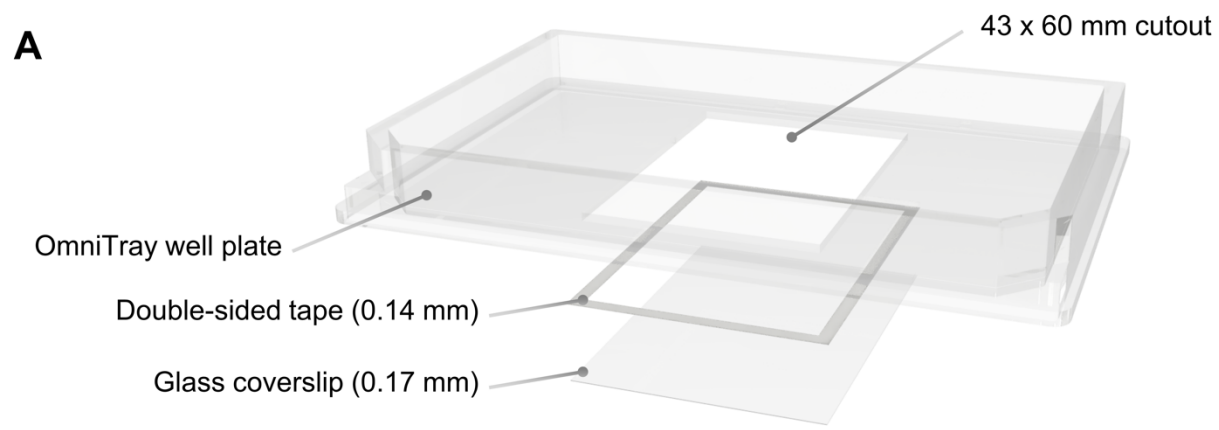
### Table of contents:

**Fig. S1** (A) Exploded view of the VRD device constructed by cutting out a 43 x 60 mm rectangular hole on the bottom of an OmniTray well plate, followed by attaching a glass coverslip to the bottom surface of the plate via double-sided tape. (B) Image of the device for performing VRD (modified from a commercial OmniTray well plate) with dried spots of food dye and (C) the magnetic manipulator for performing PMP and droplet actuation ..... S-2

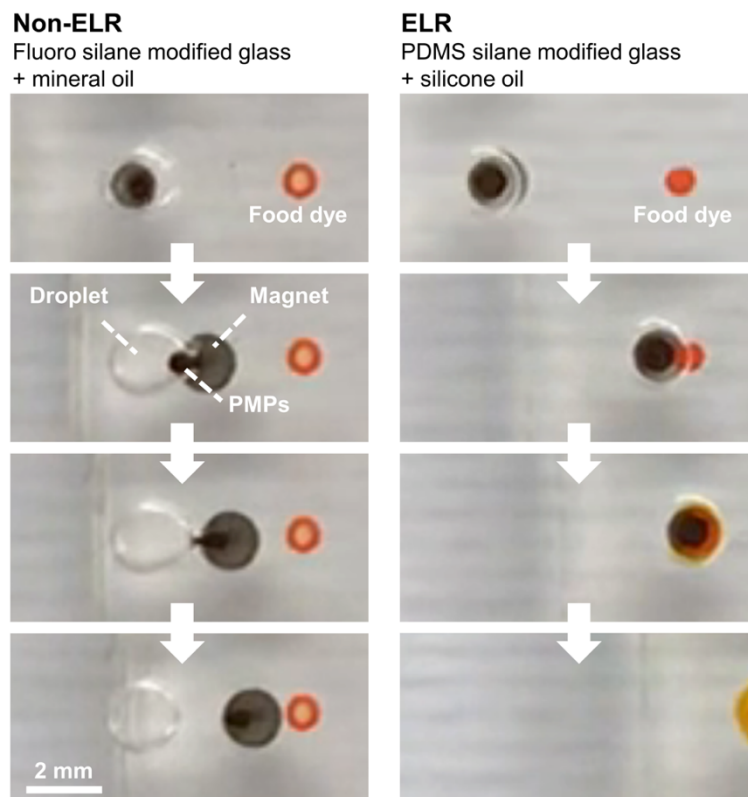
**Fig. S2** ELR (accomplished via a PDMS silane modified glass surface with silicone oil) enables better droplet manipulation via PMPs compared to a non-ELR system (fluoro silane modified glass with mineral oil)..... S-3

**Fig. S3** Protein (IgG) recovery with VRD. (A) Fluorescence microscope images of dried Alexa Fluor 488 IgG spots before (left) and after (right) VRD reconstitution. Scale bar: 2 mm. (B) Quantified fluorescence of the Alexa Fluor 488 IgG spots from panel (A) images before and after VRD. Error bars denote the standard deviation from 3 technical replicates. NS: Not significant, calculated using 2-tailed Student's t-test..... S-3

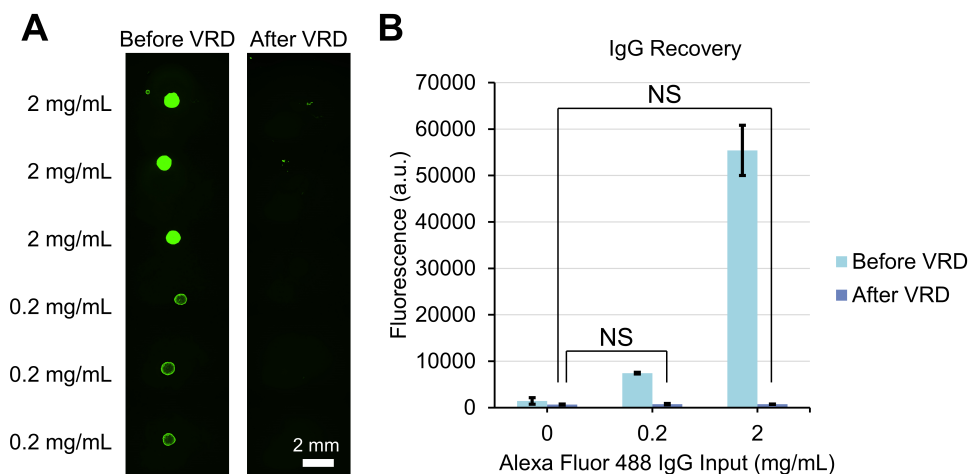
**Fig. S4** Bacterial growth-induced biofouling (droplet spreading) of non-ELR surface (fluoro silane modified glass with mineral oil) compared to no fouling/spreading observed on an ELR surface (PDMS silane modified glass with silicone oil) after a 24 h culture ..... S-4



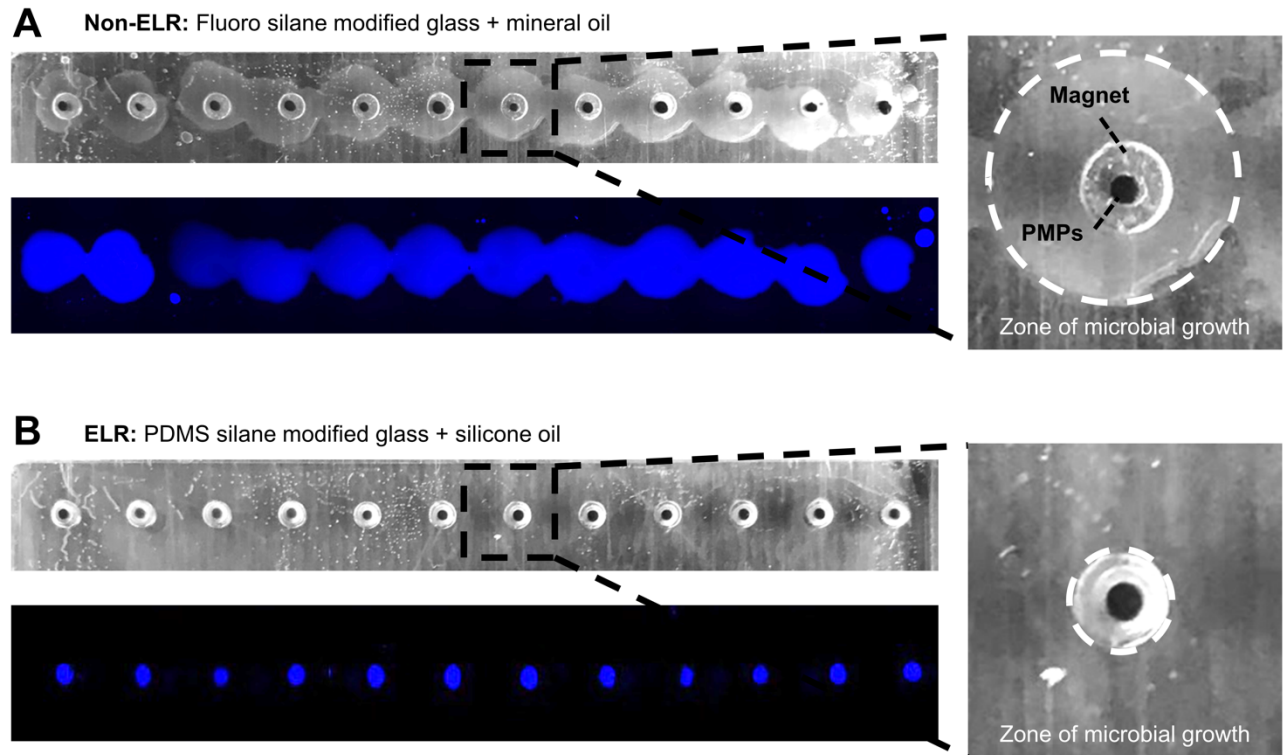
**Fig. S1** (A) Exploded view of the VRD device constructed by cutting out a 43 x 60 mm rectangular hole on the bottom of an OmniTray well plate, followed by attaching a glass coverslip to the bottom surface of the plate via double-sided tape. (B) Image of the device for performing VRD (modified from a commercial OmniTray well plate) with dried spots of food dye and (C) the magnetic manipulator for performing PMP and droplet actuation.



**Fig. S2** ELR (accomplished via a PDMS silane modified glass surface with silicone oil) enables better droplet manipulation via PMPs compared to a non-ELR system (fluoro silane modified glass with mineral oil). Scale bar: 2 mm.



**Fig. S3** Protein (IgG) recovery with VRD. (A) Fluorescence microscope images of dried Alexa Fluor 488 IgG spots before (left) and after (right) VRD reconstitution. Scale bar: 2 mm. (B) Quantified fluorescence of the Alexa Fluor 488 IgG spots from panel (A) images before and after VRD. Error bars denote the standard deviation from 3 technical replicates. NS: Not significant, calculated using 2-tailed Student's t-test.



**Fig. S4** Bacterial growth-induced biofouling (droplet spreading) of non-ELR surface (fluoro silane modified glass with mineral oil) compared to no fouling/spreading observed on an ELR surface (PDMS silane modified glass with silicone oil) after a 24 h culture. Bacteria used: *P. aeruginosa* CFP (strain PA01).