Electronic Supplementary Information for

# Microscale impeller pump for recirculating flow in organs-on-chip and microreactors

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#### **Contents:**

- CAD files for device designs (Separate file)
- CAD file for impeller design (Separate file)
- Supplemental Methods
- Supplemental Figures

#### I. CAD files for device designs

The files (Autocad dwg. extension) contain the designs for devices with both 0.5 mm channels and 1 mm channels. It is provided separately.

#### II. CAD file for impeller design

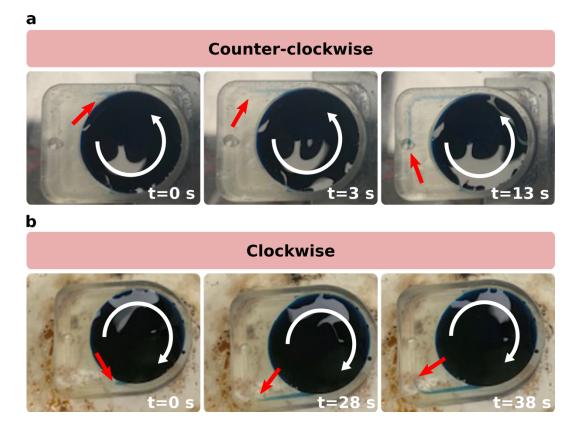
The file (Autocad dwg. extension) contains the design for the impeller piece. It is provided separately.

### III. Supplemental Methods

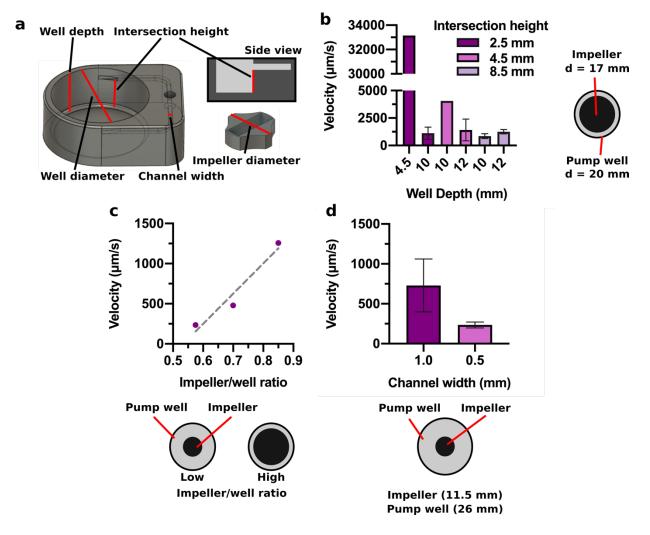
#### Initial velocity quantification through fluorescent bead tracking

The printed devices were filled with 1x PBS, and fluorescent beads (6 µm, Fluoresbrite YG Microspheres, Polysciences, Inc., PA, USA) were inserted into the pump well. Images within the channels were collected with an AxioZoom macroscope (Carl Zeiss Microscopy, Germany) with an Axiocam 506 Mono camera and a filter cube for GFP (Zeiss filter set #38) as the beads moved over time. The images were analyzed using MATLAB CellTracker to determine an average bead velocity. This method was difficult to use under higher fluid velocities due to limited camera speed, so the food coloring front method was used after device optimization.

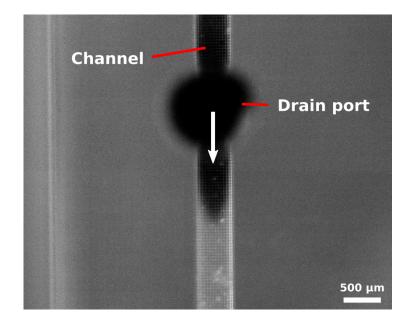
## **IV.** Supplemental Figures



*Figure S1. Fluid flow direction based off direction of impeller rotation*. *Time-lapse images of recirculating fluid flow where the impeller rotated (a) counter-clockwise and (b) clockwise (white arrow). Blue dye was inserted into the pump well, and over time, the dye exited the well and traveled through the channel (dye front marked by red arrows). (a) Counter-clockwise impeller rotation was driven by the external pump platform (6.05 V). (b) Clockwise impeller rotation was driven by a hot plate (high stirring speed, 8). Channel width was 1 mm.* 



**Figure S2.** Optimization of device parameters. Using the fluorescent bead method, the velocity across various device parameters was measured. (a) Schematic of the device and impeller piece showing the well depth, well diameter, channel-well intersection height, and impeller diameter. (b-e) Experimentally-measured velocity in a water-filled device at 5.42 V when changing specific dimensions of the pump well, impeller, or microchannel. (b) Velocity as a function of well depth and intersection height, with well diameter (20 mm), channel width (1 mm), and impeller diameter (17 mm) held constant. (c) Velocity as a function of impeller/well area ratio, with channel width (1 mm), well depth (12 mm), and channel-well intersection height (8.5 mm) held constant. (d) Velocity as a function of channel dimensions, with channel-well intersection height (8.5 mm), well depth (12 mm), well diameter (26 mm), and impeller diameter (11.5 mm) held constant. The bars represent mean  $\pm$  standard deviation (n = 4).



**Figure S3. Parabolic fluid flow.** An image of the channel within the device that was pre-filled with water and injected with blue dye as the impeller rotated (7.47 V, 700  $\mu$ m/s). The direction of fluid flow is marked with a white arrow. The dye moved through the 0.5 mm channel with a parabolic flow profile. Image collected with a Zeiss AxioZoom macroscope with a Axiocam 506 Mono camera.

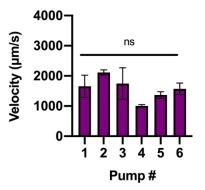
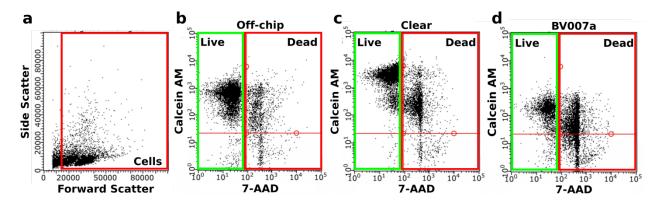


Figure S4. Velocity variation between replicates of the external pump platform. Using the food coloring front method, variations in fluid velocity across 6 different copies of the external pump platform were measured at a fixed voltage of 5.6 V. The same microdevice and impeller were used across all velocity measurements. Channel width was 1 mm. The bars represent an average velocity (n = 2) and the error bars show standard deviation. Results were compared using a one-way ANOVA, ns indicates p > 0.07.



**Figure S5. Representative flow cytometry gating for viability assays.** Primary splenocytes were cultured on-chip for 4 hrs without impeller rotation and analyzed using flow cytometry. The splenocytes were (a) gated on scatter and singlets (not shown) and (b-d) analyzed for intensity of Calcein AM and 7-AAD, for (b) live cells cultured off-chip, (c) cells cultured in a Clear chip, and (d) cells cultured in a BV007a chip. Jurkat T cells were gated similarly.