Electronic Supplementary Material (ESI) for Lab on a Chip.


# Supplementary Information

# Zeta potential characterization in commercially available microfluidic chips

Jonathan Cottet[a], Josephine O. Oshodi[ab], Jesse Yebouet[a], Andrea Leang[a], Ariel. L. Furst[bc] and Cullen R. Buie[a]

[a] Department of Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA

[b] Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA, USA

[c] Center for Environmental Health Sciences, Massachusetts Institute of Technology, Cambridge, MA, USA

Correspondences should be addressed to the following author:

Cullen R. Buie (PhD)

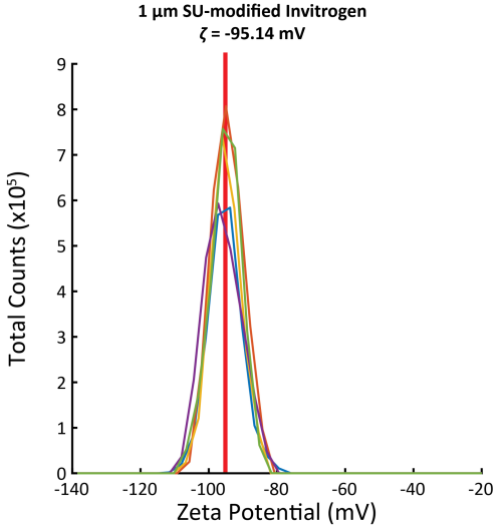
Email: crb@mit.edu

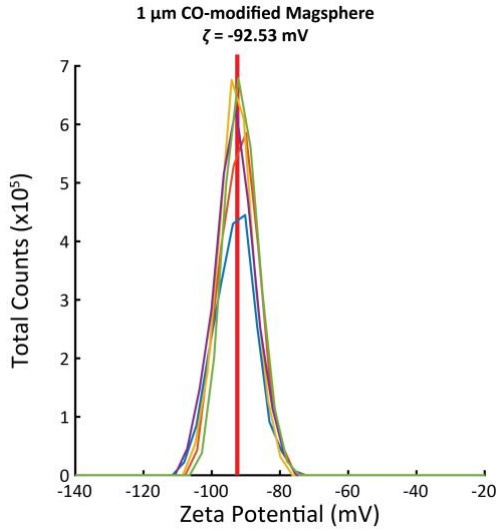
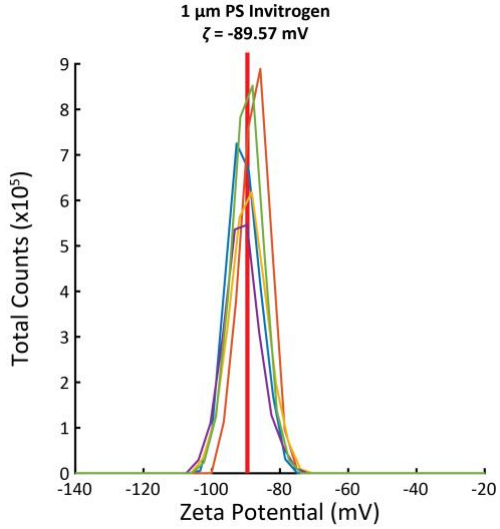
## Content

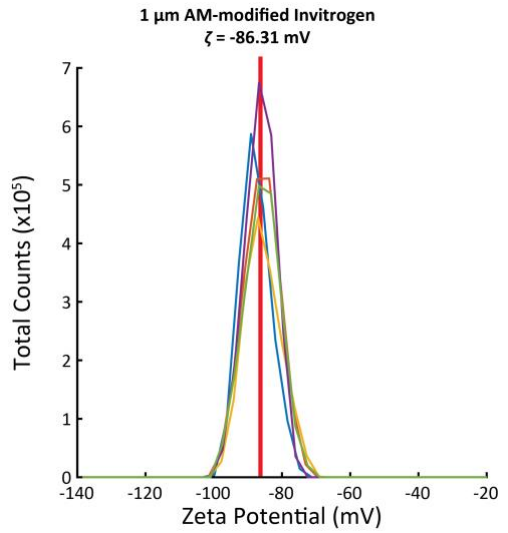
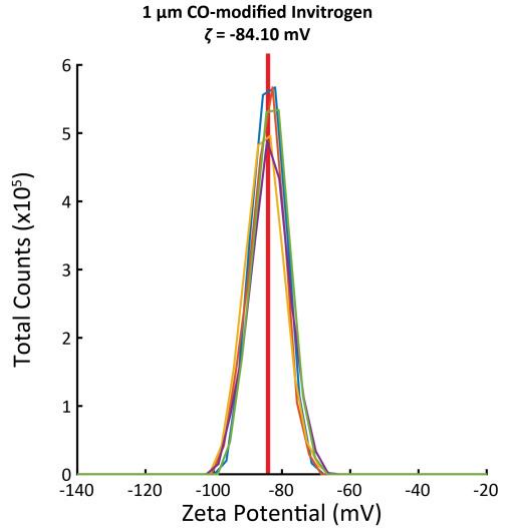
# 1. Microparticle information determined with the Zetasizer Ultra

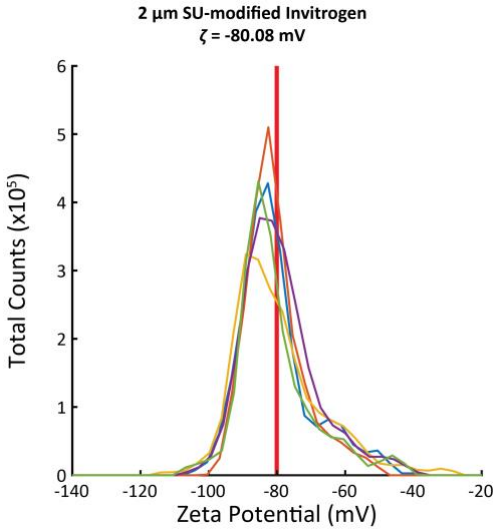
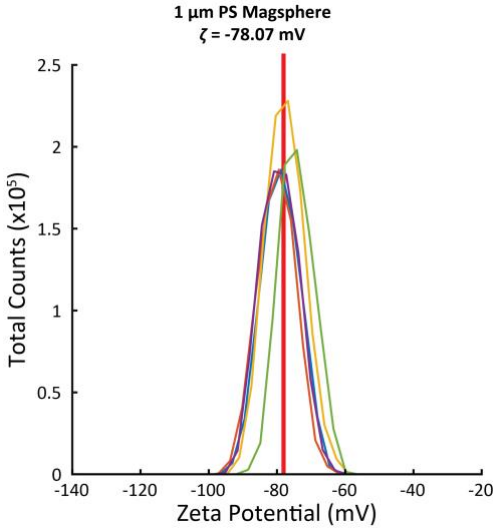
**Table S1:** Microparticle Information determined in this study in 10 mM HEPES with the ZetaSizer Ultra (Malvern). PS: polystyrene, SU: Sulfate, CO: Carboxylate, AM: Amine. (**Bold**: All particle suspension concentrations are at $10^8$ particles/ml except the 1 µm AM-modified particles from Magsphere which is at $10^9$ particles/ml).

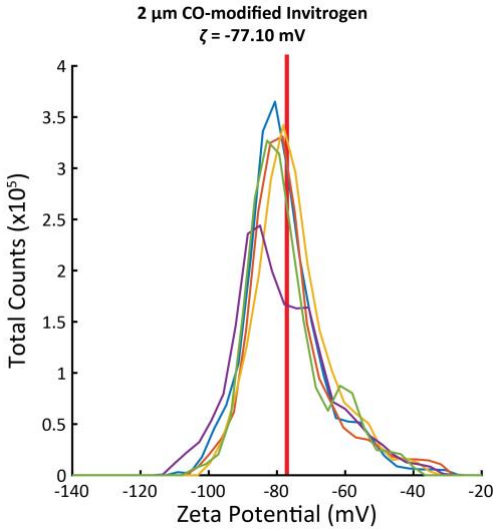
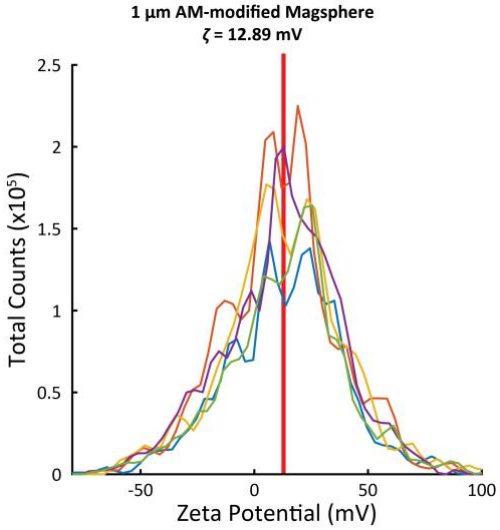
For each sample, 5 measurements were acquired successively as illustrated with the 5 distributions in the table. The mean of the 5 means, Zeta Potential Mean, is illustrated by the red vertical line, while the Zeta Potential std corresponds to the standard deviation of the 5 means. The Zeta Deviation shows how broad this deviation is around the mean and corresponds to the half-width at half the maximum amplitude of the distribution. The Zeta Deviation Mean correspond to the mean of the 5 Zeta Deviations and Zeta Deviation std corresponds to the standard deviation of the 5 Zeta Deviations.

| Particle diameter (µm) | Manufacturer | Catalog number | Surface functionalization | Zeta Potential Mean (mV) | Zeta Potential std (mV) | Zeta Deviation Mean (mV) | Zeta Deviation std (mV) | Distribution |
|---|---|---|---|---|---|---|---|---|
| 1.00 | Invitrogen | F8852 | SU-modified | -95.14 | 0.52 | 4.76 | 0.42 |  |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1.10 | Magsphere | CAYF001UM | CO-Modified | -92.53 | 0.87 | 5.47 | 0.46 |  |
| 0.99 | Invitrogen | F13081 | PS | -89.57 | 1.49 | 4.81 | 0.48 |  |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 1.00 | Invitrogen | F8765 | AM-modified | -86.32 | 0.83 | 4.95 | 0.41 |  |
| 0.99 | Invitrogen | F8823 | CO-modified | -84.10 | 0.74 | 5.30 | 0.36 |  |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 2.00 | Invitrogen | F8853 | SU-modified | -80.08 | 0.58 | 10.96 | 1.58 |  |
| 1.10 | Magsphere | PSYF001UM | PS | -78.07 | 2.26 | 5.44 | 0.21 |  |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 2.10 | Invitrogen | F8827 | CO-modified | -77.10 | 1.29 | 12.04 | 1.10 |  |
| **1.10** | **Magsphere** | **AMYF001UM** | **AM-modified** | **12.89** | **0.91** | **25.81** | **0.61** |  |

# 2. Electrokinetic characterization results

**Table S2:** Microparticle information determined in this study in 10 mM HEPES with the developed microfluidic electrokinetic characterization method. (*: All particle suspension concentrations are at $10^8$ particles/ml except the 1 µm AM-modified particles from Magsphere which is 3 $10^9$ particles/ml).

| | | | | Electrokinetics | |
|---|---|---|---|---|---|
| Particle diameter (µm) | Manufacturer | Catalog number | Surface functionalization | Mean Electrokinetic velocity Slope ($\mu m/s^2$) | Electrokinetic velocity Slope std ($\mu m/s^2$) |
| 1.00 | Invitrogen | F8852 | SU-modified | 2.40 | 0.011 |
| 1.10 | Magsphere | CAYF001UM | CO-modified | 1.78 | 0.025 |
| 0.99 | Invitrogen | F13081 | PS | 2.44 | 0.039 |
| 1.00 | Invitrogen | F8765 | AM-modified | 2.09 | 0.017 |
| 0.99 | Invitrogen | F8823 | CO-modified | 2.02 | 0.018 |
| 2.00 | Invitrogen | F8853 | SU-modified | 2.09 | 0.083 |
| 1.10 | Magsphere | PSYF001UM | PS | 1.90 | 0.016 |
| 2.10 | Invitrogen | F8827 | CO-modified | 1.95 | 0.078 |
| 1.10 | Magsphere | AMYF001UM | AM-modified | -0.28 | 0.011 |

# 3. Reservoir caps modification procedure

Materials:

- 200 µl reservoirs with top Mini Luer interface (Fluidic 934, Microfluidic ChipShop)
- Male Mini Luer fluid connector Single (Fluidic 331, PP, Microfluidic ChipShop)
- 250 µl pipette tips
- Razor Blade
- 18 G 1 ½ in. long needle (305196, BD Plastics)
- 16 G 1 in. long needle (305197, BD Plastics)
- 50 ml Luer Lock plastic syringe
- Epoxy glue
- Cutting mat
- 5-minute 2-part epoxy glue (Gorilla)
- Tweezer
- Microfluidic chip with Luer interface (Microfluidic ChipShop)
- Chip handling frame (Microfluidic ChipShop)

Pipette tip cutting

- Cut the pipet tip end to be 20 mm long (along the circle already present on the 250 µl pipette tip) above a cutting mat (Figure S1a).
- Remove 3 mm of the end of the 200 µl pipet tip with the razor blade. The final length of the pipette tip should be 17 mm (Figure S1b).

Hole in the caps

- Mount the 18 G needle on the Syringe.
- Close the reservoir cap and flip it to have the Mini Luer interface facing the cutting mat.
- Insert the 18 G needle from the bottom hole and press to make a hole on the side of the cap. (Figure S1c and S1d).
- Remove the 18 G needle and mount the 16 G needle on the Syringe.
- Open the caps and enlarge the hole in the caps with the 16 G needle (Figure S1e)
- Check that the cut pipette tip can enter inside the hole of approximately 1 mm. Enlarge the hole if necessary.

Gluing

- Place a chip with Luer Interface on the Chip Handling Frame.
- Insert the reservoir (with the hole on the cap) on the Luer Interface.
- Close the cap and insert the Male Mini Luer fluid connector Single on the cap Mini Luer interface. Check that the cut pipette tip can enter inside the hole of approximately 1 mm. Enlarge the hole if necessary.
- Mix the two parts of the epoxy glue.
- Place the pipette tip cut on a tweezer to facilitate manipulation.
- Carefully place a bit of glue around the pipette tip end.
- Insert the pipette tip inside the hole in the caps. The glue should be now touching both the pipette tip and the caps. (Figure S1f).
- Let the Epoxy solidify for 5 min.

Final steps:

- Once the glue solidified, cut the cap from the reservoir, and set it aside. Discard the reservoir part.
- Repeat this procedure to obtain a hole on the other side of another cap.

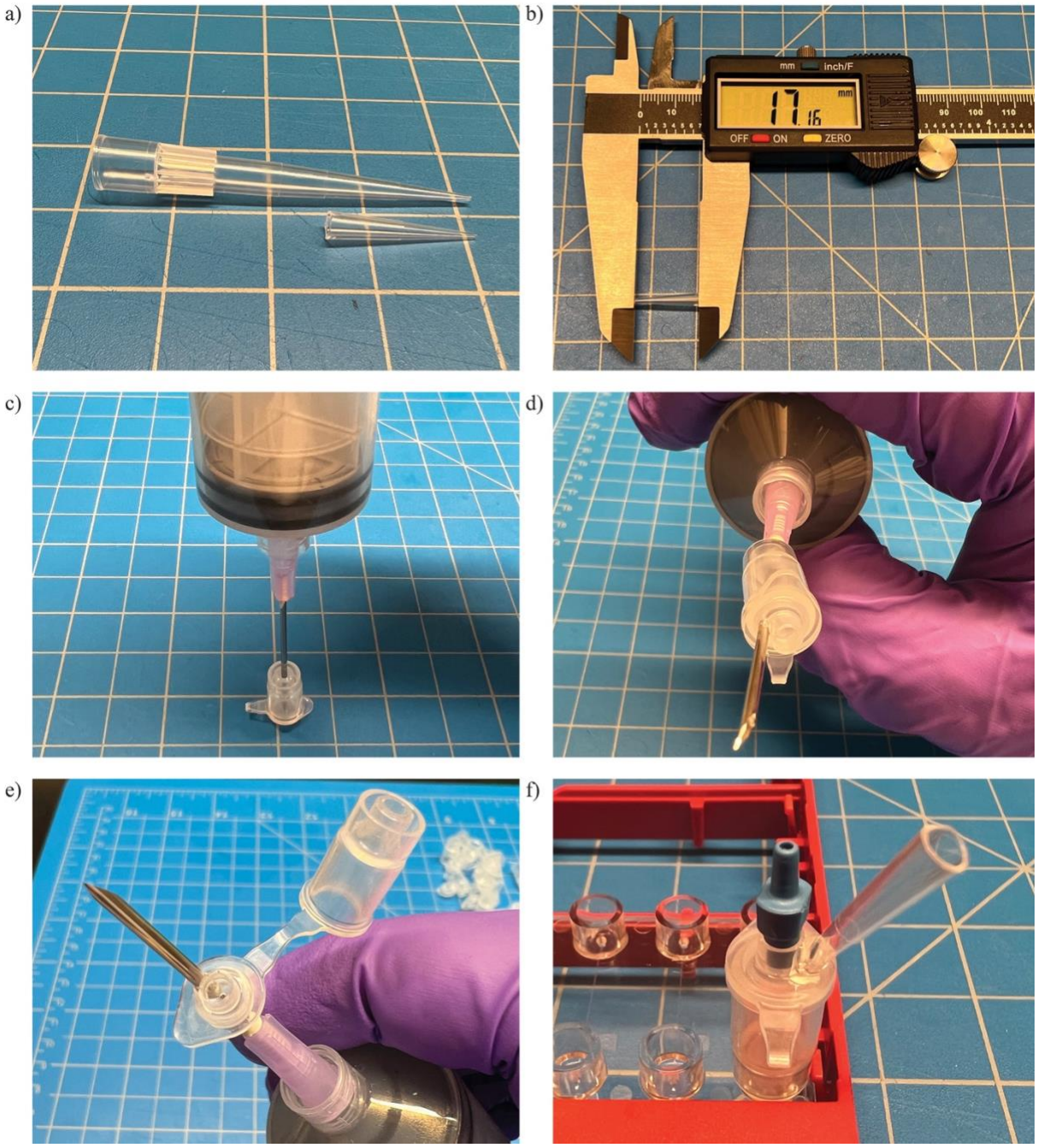



Figure S1: (a) to (i) Modification of the 200 µl reservoir caps. (a) 250 µl pipette tips before and after cutting the upper part of the tip. (b) Pipette tip length after cutting the upper and lower part of the tip. (c) Making of the hole in the cap through the bottom hole with the 18 G needle and (d) corresponding result. (e) Enlargement of the hole with the 16 G needle. (f) Final result after gluing.

# 4. Outlier removal

For each frame, the velocities of individual particles were quantified utilizing Fiji and the TrackMate plugin, supplemented by several statistical parameters: the median, the first quartile (***Q1***, or the 25th percentile), the third quartile (***Q3***, or the 75th percentile), and the interquartile range (***IQR***, defined as ***Q3*** – ***Q1***), as depicted in Figure S2. Outliers were identified by employing the '*isoutlier*' function in MATLAB, designating values less than (***Q1*** - 1.5***IQR)*** and greater than ***(Q3*** + *1.5***IQR**) as outliers. These outliers generally represent erroneously labelled velocities within the PTV dataset and were consequently excluded from the analysis.

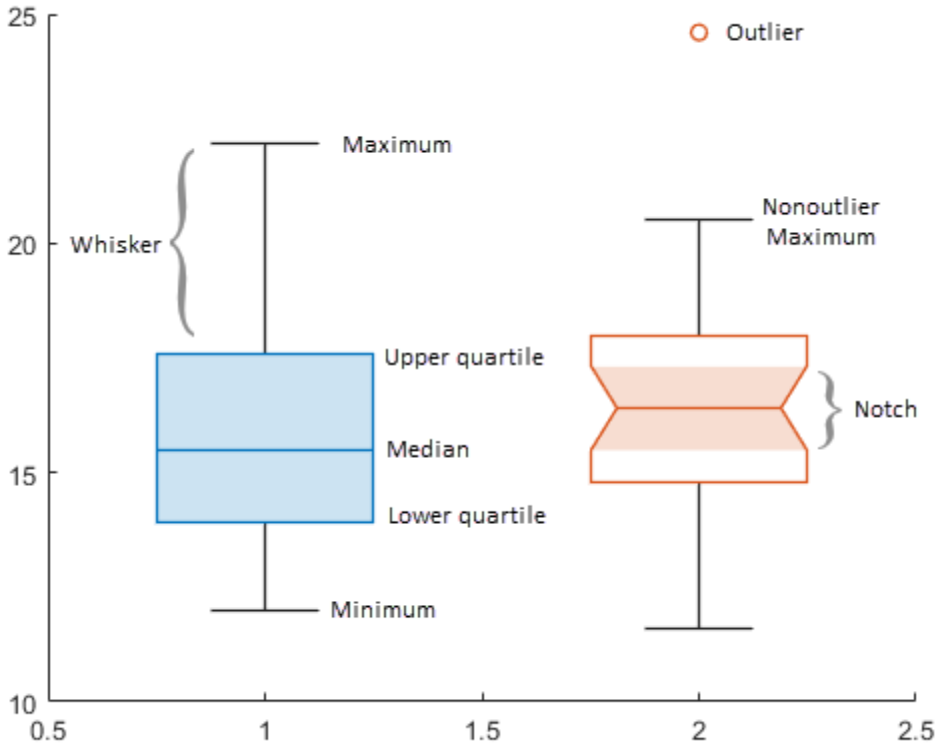



Figure S2: Illustration of a Matlab boxplot with the median, the upper quartile and lower quartile as well as an outlier. Image from
https://www.mathworks.com/help/matlab/ref/boxchart.html

# 5. Experimental flow chart

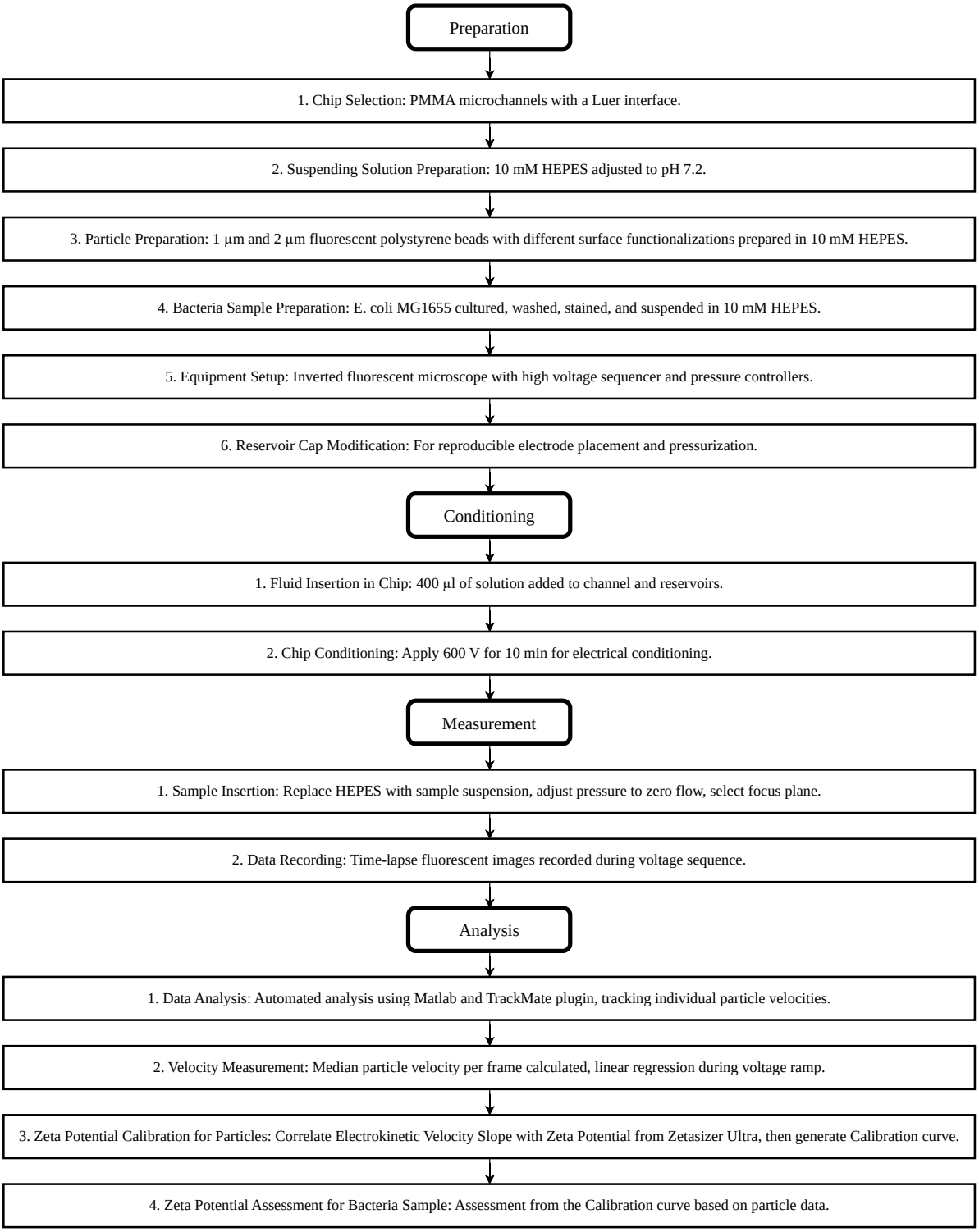



Figure S3: Experimental flow chart illustrating all the steps for the Preparation, Conditioning, Measurement and Analysis processes for a Bacteria Sample.