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## Supplemental Information

# Passive and Active Microrheology of the Intestinal Fluid of the Larval Zebrafish

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# Supplementary Material: Figures and Video Captions



## Supplementary Figures

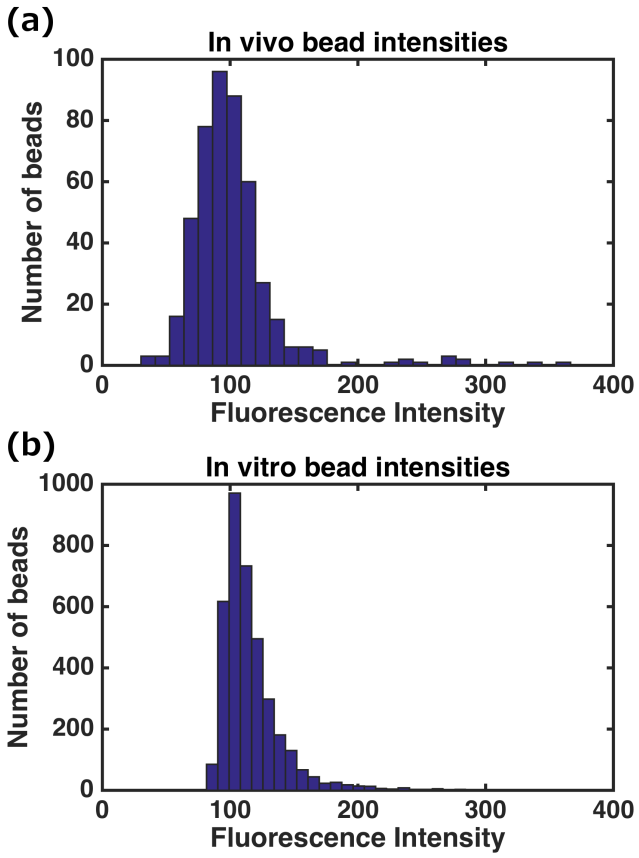


**Figure S1.** Histograms of particle fluorescence intensities for passive microrheology probes, normalized by background fluorescence levels, for (a) particles in a larval zebrafish gut, and (b) particles in 1/10x phosphate buffered saline. Both show a peak around 100 coefficient, indicating similar sizes in both contexts.

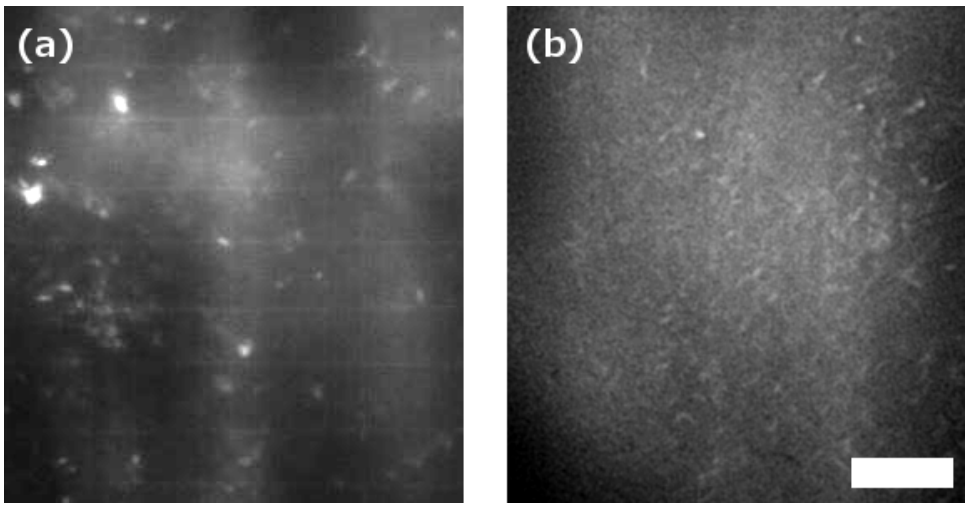


**Figure S2.** Light sheet fluorescence images of (a) passive tracer particles and (b) *Vibrio* ZWU0020 bacteria in the same region of a larval zebrafish gut. A movie of the tracer dynamics in this region is provided as Supplementary Video [X]. Both particles and bacteria sample the same space; there is no apparent sign of segregation. Scale bar: 10 microns.

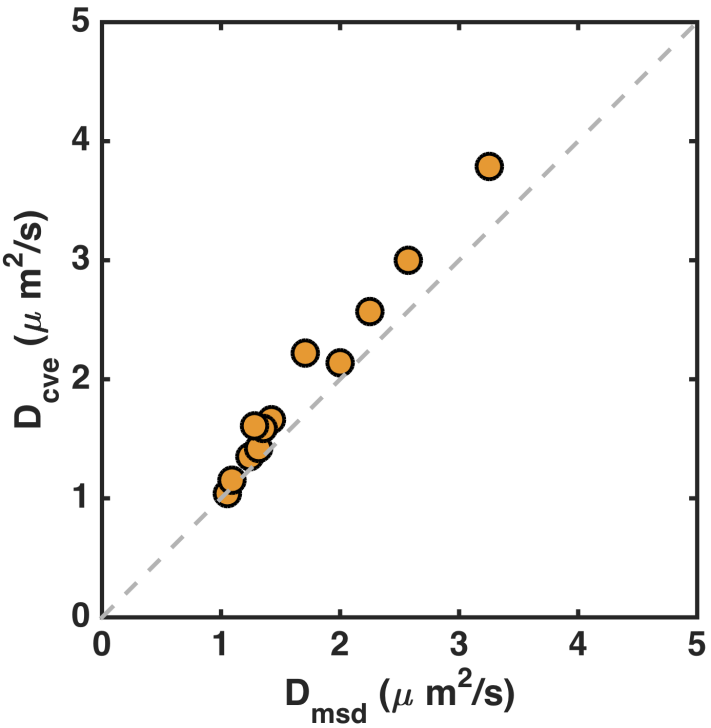


**Figure S3.** Diffusion coefficients calculated with a covariance-based estimator $D_{cve}$ and with mean-square-displacement analysis $D_{msd}$, as described in Methods. Each point is the average for all tracked particles within one fish. The dashed line indicates equality (i.e. $D_{cve} = D_{msd}$).

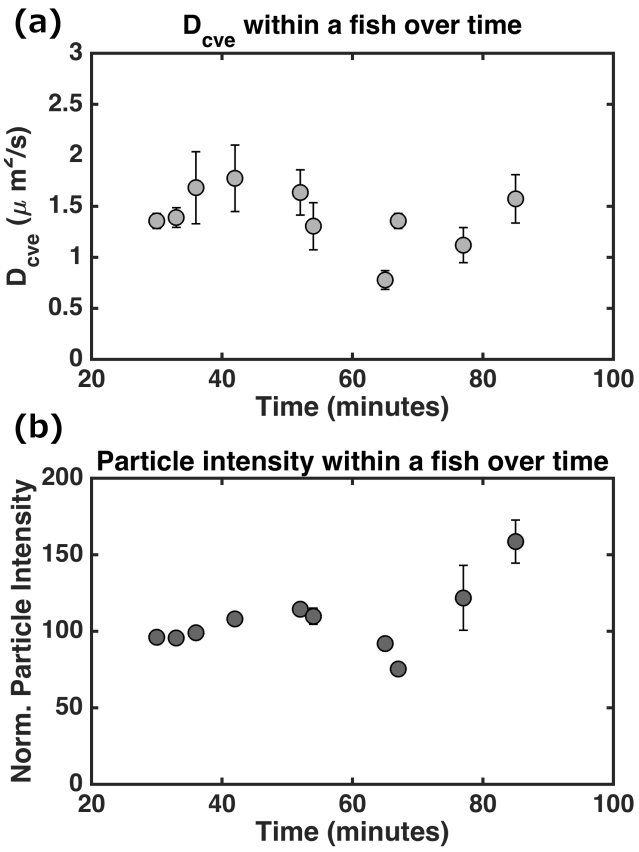


**Figure S4.** (a) Diffusion coefficients from passive microrheology, examining particles within the same germ-free larval zebrafish over a period of approximately one hour. Each point is the average of values from 100-500 particle tracks; error bars indicate one standard deviation. (b) Fluorescence intensities for the same particles over time, normalized by the background mucus autofluorescence level. Both the diffusion coefficient and the particle intensity are roughly constant over time, implying minimal particle aggregation.

## Supplementary Video Captions

**Supplementary Video 1:** Passive motion of fluorescent nanoparticles in the anterior bulb of the intestine of a 5 dpf zebrafish, captured with light sheet fluorescence microscopy.

**Supplementary Video 2:** Passive motion of fluorescent nanoparticles in the anterior bulb of the intestine of a 5 dpf zebrafish, with crypt-like invaginations indicated. Supplementary Figure 2 shows a subset of a still frame of this movie, together with an image of bacteria in the same region.

**Supplementary Video 3:** Large-scale coordinated motion of intestinal contents, including fluorescent nanoparticle probes, driven by a peristaltic contraction. These contractions occur roughly twice per minute.

**Supplementary Video 4:** Light sheet fluorescence movie of a region of a 5 dpf larval intestine in which a dense, shed bolus of mucus containing discrete particles is evident. Note that the bolus is not adhered to the epithelial wall. The embedded particles show little motion, in contrast to free particles elsewhere in the gut.

**Supplementary Video 5:** Magnetic ellipsoid in the intestinal bulb of a 5 dpf zebrafish larva being driven by an oscillating magnetic field with a chirped frequency (from 0.1 Hz to 95 Hz), as described in Methods.