

Electronic Supplementary Information

Movie M1: This clip demonstrates that the CASA plugin is able to track sperm cells. In this video, the flow was $33 \mu\text{m/s}$ from left to right. Recording and playback at 30 fps.

Movie M2: This short clip shows VCL, VAP and VSL being overlaid on a sperm cell tracked by CASA. Recording and playback at 30 fps.

Movie M3: This movie shows the flow reversal experiment and tracks one sperm cell as it reverses the flow. Such encounters are difficult to capture fully because they need a large field of view. Recording and playback at 30 fps.

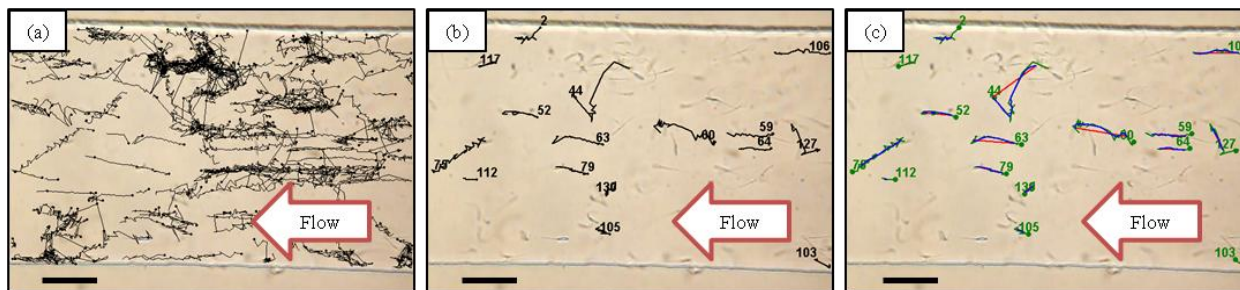


Figure S1 Sperm tracks as captured by CASA plugin in Image-J. **a**) raw unnumbered sperm tracks. Actual position of sperm centroids, tracked for 90 frames (3 seconds). Solid circles present track start **b**) Numbered tracks – a select few only are shown for clarity. **c**) VCL (curvilinear velocity) track in green, VAP (average path velocity) track in blue, VSL (straight line velocity) track in red. Scale bars $50 \mu\text{m}$.

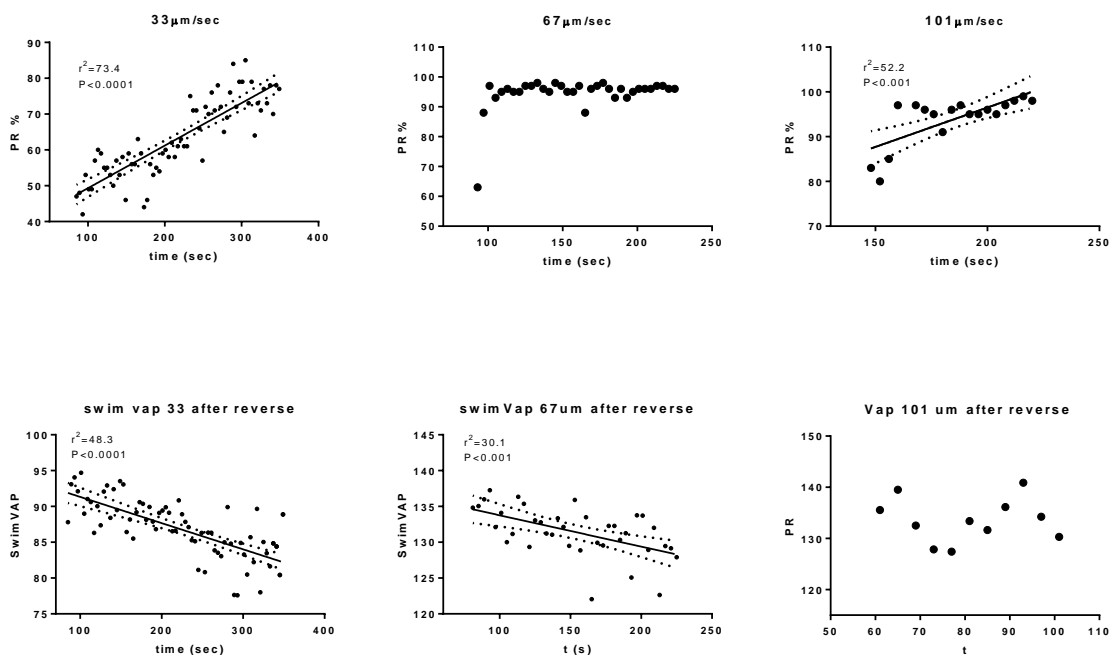


Figure S2 Linear regression was used to show that PR% return to the original PR% linearly with time for flow velocity $33 \mu\text{m/s}$. Average SwimVAP spiked momentarily at flow reversal then reduced linearly with time for flow velocities $33 \mu\text{m/s}$ and $67 \mu\text{m/s}$.