

S1 Figure: Active microrheology measurements of water viscosity measured for different probe sizes and different oscillation amplitudes. Measurement techniques and instrumentation were identical to those shown in Figure 3 and described in Methods. As shown the measured viscosity of water is largely independent of probe size and oscillation amplitude as expected for a Newtonian fluid. Thus, the marked dependence on these parameters in mucus reflects the lengthscale-dependent rheology and structure of the mucus rather than any systematic instrumentation or measurement errors. Error bars also show that our instrument/measurement error is  $\sim 2.6\%$  which is well below the measured  $\sim 20\%$  range among different data trials in mucus.