Calibration Methods for *in vivo* Microrheology with Rotational Optical Tweezers

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Studies of mechanical properties within living cells can provide unique information about biological functions. One such function, macropinocytosis, is a cellular mechanism involving the internalisation of extracellular fluids into vesicles called macropinosomes and is implicated in a number of crucial cell-specific roles [1]. The dynamic microrheology of macropinosomes could provide novel insight into its many functions, however, its limited accessibility, micro-litre volume, and highly dynamic nature has prevented quantitative measurements. Carefully calibrated Rotational Optical Tweezers (ROTs) provided a unique method to perform dynamic microrheology of these vesicles, leading to the first shear viscosity measurement of macropinosomes.

ROTs involves the transfer of angular momentum from a highly focused trapping beam to a probe particle, generating pNm of torque enabling microrheometry measurements. Specifically, we consider a birefringent vaterite microsphere that is trapped and rotated using circularly polarised light [2].

This measurement requires knowledge of the trapping power, the radius of the microsphere, and change in the degree of circular polarisation. The change in polarisation can be directly measured from the relative proportions of left and right circularly polarised light. The trapping power and the radius of the microsphere are more difficult to measure exactly and require novel calibration methods.

The trapping power can be measured using a photodiode detector prior to the trap and determining a calibration constant as well as losses in the intermediary optical components. This constant was determined by exploiting an alternative measurement of torque with linearly polarised light that is independent of power. This involved monitoring the angular orientation of the probe and employing maximum likelihood estimation.

Viscosity is inversely proportional to the radius cubed of the trapped microsphere and is the largest contributor to uncertainty in microviscometry measurements. We detail an empirical method that involves comparing the location of diffraction fringes to those from a variety of similarly sized reference vaterites. This reduced the uncertainty from $> 10\%$ to 5%, which is a significant improvement when using this method for in situ microrheology of a complex environment.

[1] J. L. Stow, Y. Hung and A. A. Wall, *Current Opinion in Cell Biology* **65**, 131-140 (2020).

[2] A. I. Bishop, T. A. Nieminen, N. R. Heckenberg and H. Rubinsztein-Dunlop, *Physical Review Letters* **92** (19), 198104 (2004).