Stokes: Hydrodynamics of cytoplasm flow in the alga *Chara australis*[1].

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OBJECTIVE

To characterize the nature of hydrodynamic flow in a giant algal cell.

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INTRODUCTION

Drag is a force that opposes the motion of an object. To a good approximation for a sphere, the drag force is given by

$$F_{Drag} = \beta DV + \gamma D^2 V^2$$

Where V is the velocity of the object, D is its diameter and β and γ are coefficients dependent on the medium (Taylor J. 2005). For objects in daily life, the quadratic term ($F_{quad} = \gamma D^2 V^2$) dominates as $D^2 >> D$, however for smaller and smaller objects, D^2 and D become on the same order of magnitude where the linear term ($F_{lin} = \beta DV$) for the drag contributes significantly and may even be dominate over the quadratic. A metric to determine the significance of the drag terms is the Reynold's number (Re), which, to order of magnitude approximation is

$$\mathrm{Re} \approx \left(\frac{F_{quad}}{F_{lin}} \right) = \frac{\gamma DV}{\beta}$$

(Taylor, 2005)

At the level of a cell and organelles, $Re \sim 10^{-4}$ which indicates that the linear drag dominates. If the sphere is assumed to be migrating through a medium of constant viscosity (η), the linear term can be re-cast as

$$F_{lin} = F_{drag} = 6\pi\eta aV$$

Where a is the particle radius (Dusenbery, 2009).

To explore the low Re environment, we used Chara, a large fresh-water alga found in Ontario (Lew et al, 2011). Under low magnification Chara can show cytoplasmic streaming of macroscopic particles at speeds as large as 50 μm/sec (Williams, 1975). The motor protein providing this motion is myosin, which hydrolyzes ATP to slide along an actin filament, pulling its cargo along with it (Ito et al, 2007). The cell's cytoplasm has a helical structure wrapped around a vacuole. Areas called the zones of indifference arise where streaming can be seen in both directions and is marked by a chloroplast free region (Drew, 1926).

The question we wanted to explore was whether the molecular motors cause significant drag of surrounding cytoplasm. At low Reynolds number, this is not expected to occur, but it might if the density and activity of the molecular motors are high enough to entrain surrounding cytoplasm. To address this question, we injected small silicon oil droplets into the cytoplasm and measured their movement relative to cytoplasmic streaming in the cell.

Methods

Pressure Probe Micropipette Fabrication. To obtain a wide aperture micropipette, borosilicate capillaries (1.00 mm OD, 0.58 mm ID, with internal filament) were double pulled. The first pull —at a high heat— stretched the capillary to create a small diameter 'pinch' in the glass. The second pull at lower heat (and no selenoid pull) created a large aperture tip (about 1–1.5 micron) suitable for pressure measurements. A plug of silicon oil (1.5 centistokes) was added to the back of the micropipette with a fine bore syringe needle. After the oil had migrated and filled the pipette tip (2–5 minutes), the rest of the pipette was filled with the silicon oil. Care was taken to ensure no air bubbles were left in the capillary. The pipette was then mounted in the pressure probe (Cross and Lew, 2013).

Chara Preparation and Impalement. An intermodal Chara cell suspended in distilled water. Using masking tape, the nodal cell was anchored at its two end points to the cover and distilled water was added to cover the cell. The dish was then placed in an upright microscope under a 4x objective. A holding pipette on a micromanipulator was placed alongside the cell opposite the injection site to prevent the Chara cell from moving. A pressure probe on a micromanipulator was placed beside the Chara cell and was slowly pressed into the cell until it punctured. A silicon oil sphere of variant sizes was injected into the cytoplasm (Figure 1). Using a Infinity-2 camera controlled by Lumenera software, the silicon oil and the cytoplasmic particles could be followed in the cell.

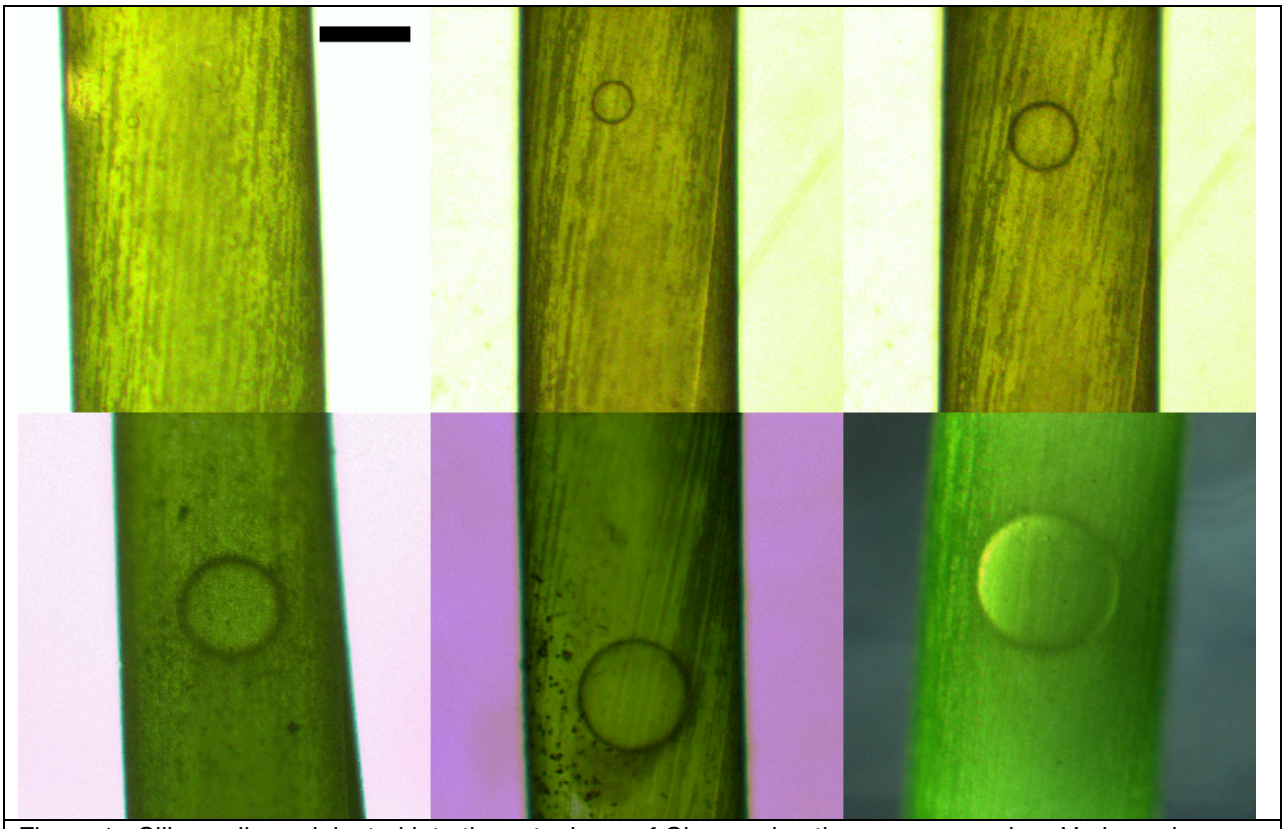

Figure 1: Silicon oil was injected into the cytoplasm of Chara using the pressure probe. Various sizes were used. From left to right, top to bottom, the sizes of the oil droplets are 30, 131, 210, 323 and 480 microns and the scale bar is 300 microns.

Confocal. In a culture dish, 2 whorl cells from Chara were suspended in ~ 4mL of distilled μL of MitoFluoGreen dye in DMSO was added and then the dish was enclosed in a black sack to prevent photo bleaching. The cells were incubated with the dye for 4 hours at room temperature. On a slide, petroleum jelly was used to create a rectangle 20 by 40 mm. A cell was then placed in the center and 1 mL of distilled water added to the slide. A cover slip placed on top. A confocal microscope with a 60x oil immersion objective was used to view the cells. Dual fluorescent images were made, one at 546 nm for autofluorescence of the chloroplast and a second at 485 nm for the MitoFluoGreen dye. Image processing was completed in ImageJ (Rasband, 2013).

RESULTS

Silicon Oil Streaming. Due to the nature of impaling the Chara, cytoplasmic streaming may or may not have been occurring during the silicon oil injection. When cytoplasmic streaming resumed, the silicon oil also began to migrate, most of the time in the direction of the cytoplasm. In one experiment, the movement was retrograde motion and in four experiments, there was no movement of the oil at all (data not shown). For the trials that showed anterograde motion, the silicon oil's velocity is plotted versus its radius in figure 2. Cytoplasm streaming occurs at various rates, so we correlated the oil droplet velocity with those of nearby cytoplasmic particles (figure 3). The oil would terminate its migration at the zone of indifference, with one of two possible processes: 1) it would rotate, centered on the indifferent zone (figure 4), or 2) migrate across the boundary and travel along in the opposite direction until it migrated across the indifferent zone again to continue the process indefinitely (figure 5).

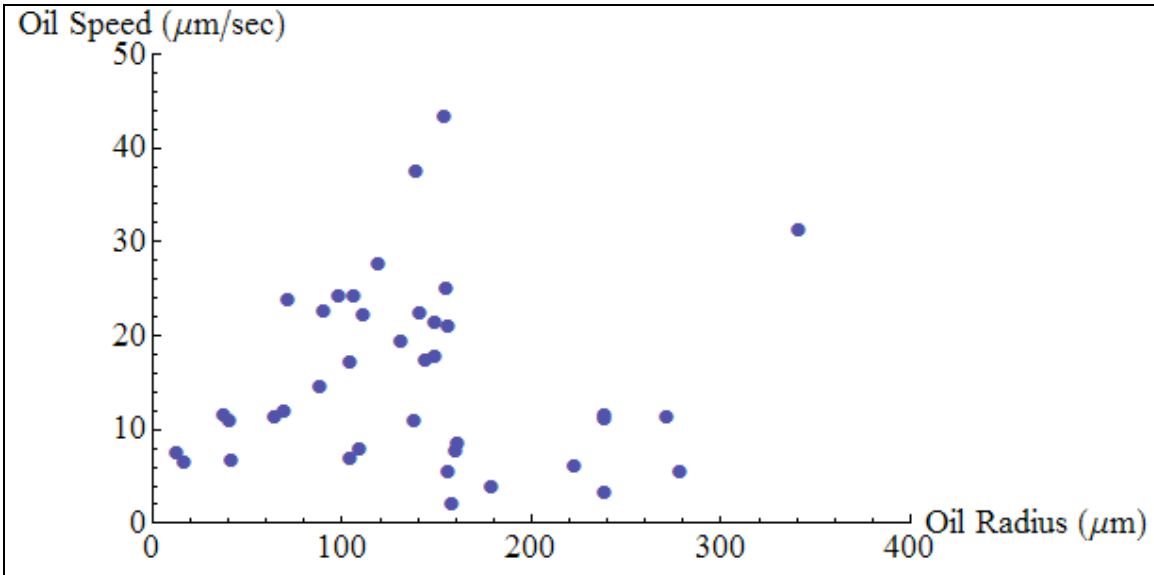

Figure 2: The speed of the oil droplet relative to its radius (n=39) shows no discernible pattern. Cytoplasm speeds vary between cells and likely contribute significantly to the scatter.

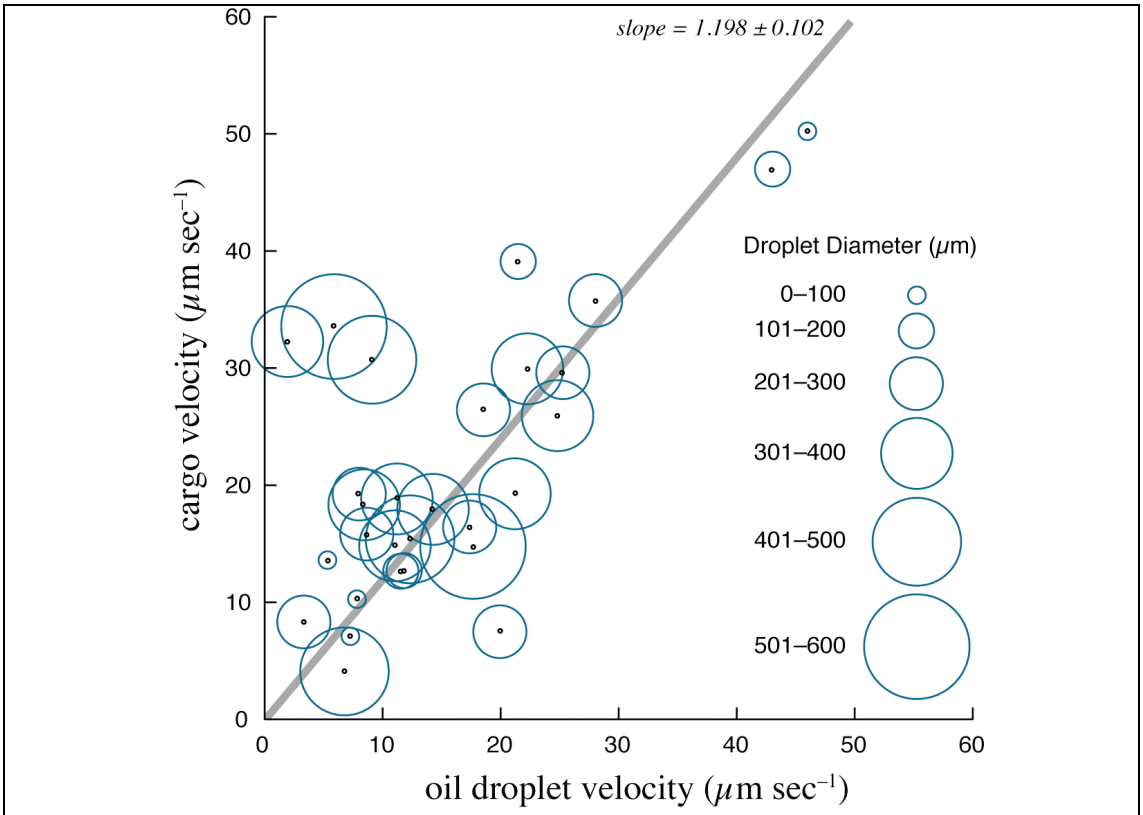

Figure 3: The cytoplasm speed and the oil speed appear to show a linear relation. The slope ± SE (from Mathematica) was 1.2 ± 0.1. Thus the cytoplasm may move slightly faster than the oil droplets. Oil droplet sizes are also shown.

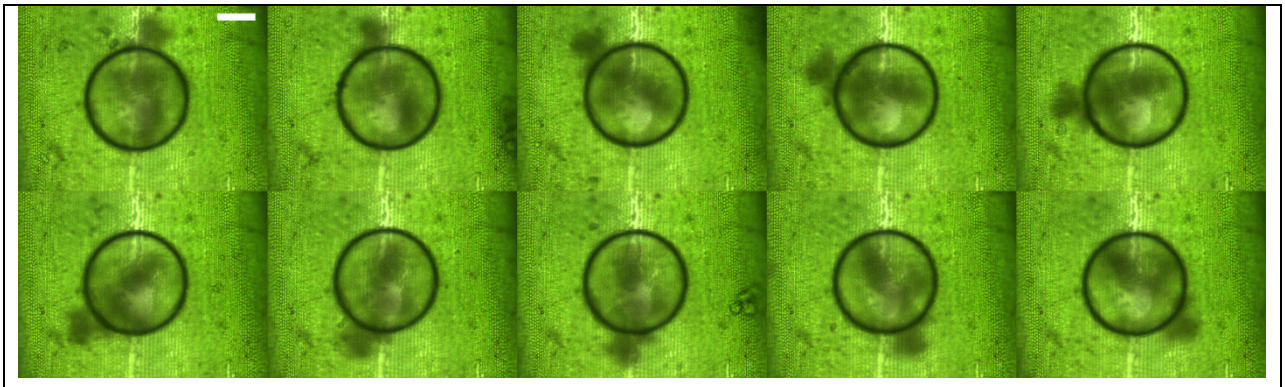

Figure 4: The silicon oil caught at the indifferent zone (the white, chloroplast free line in the center of the images). Rotation can be observed by the movement of the debris inside the droplet. The droplet also appears to bind debris externally and carry it across the indifferent zone. The scale bar is 100 microns, the time intervals are 5 seconds.

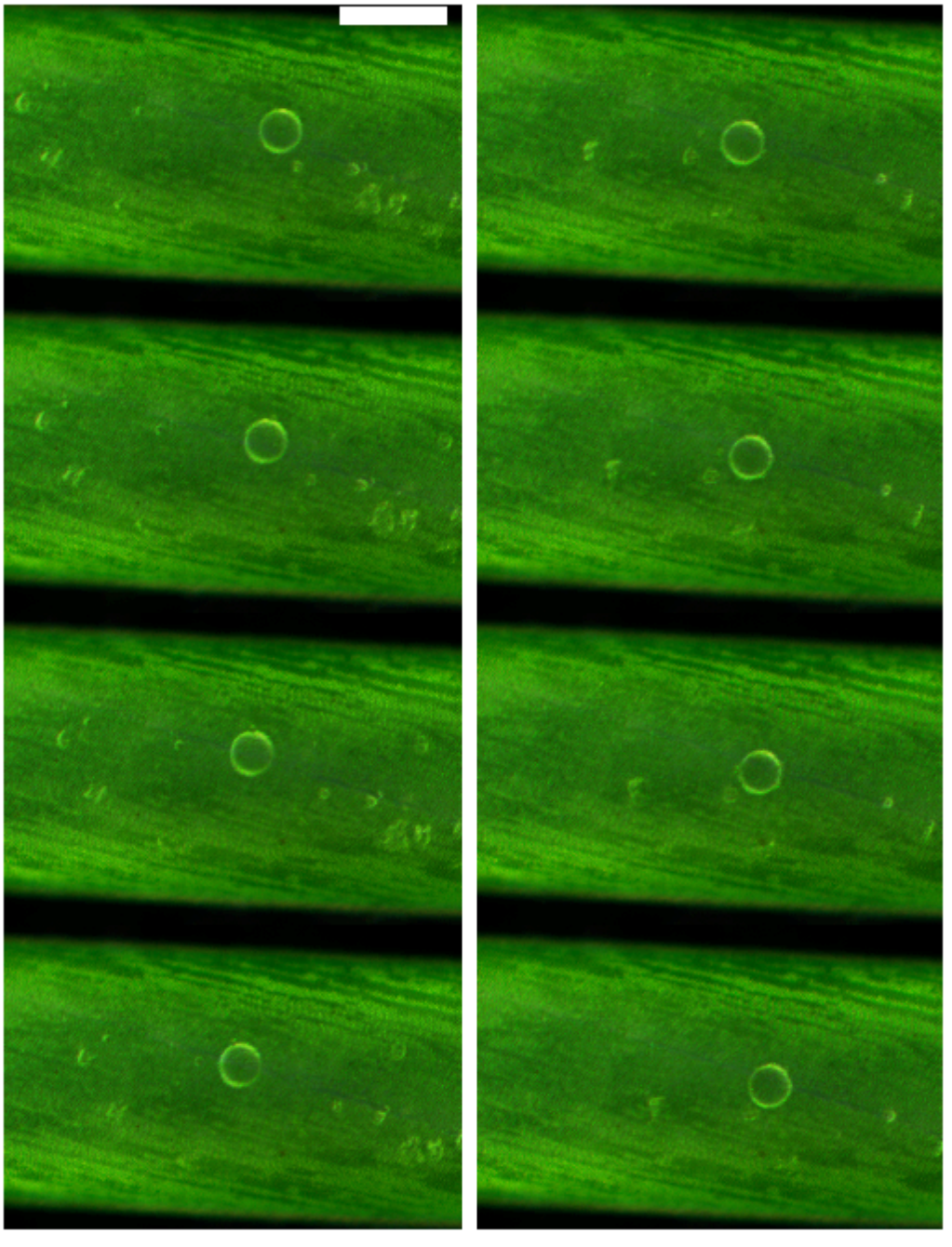

Figure 5: The oil bubble can be seen to traverse the indifferent zone and migrate in the opposite direction. The scale bar is 300 microns, the time intervals are 2 seconds.

Confocal Microscopy. During the dual imaging of chloroplast and mitochondria we found a close association of the mitochondria with the chloroplast. The mitochondria appear to embed between the chloroplast in filamentous structure near the cell surface (figure 6). Beneath the cell

surface, mitochondria were observed to move very quickly, transported along with larger particles due to cytoplasmic streaming (data not shown).

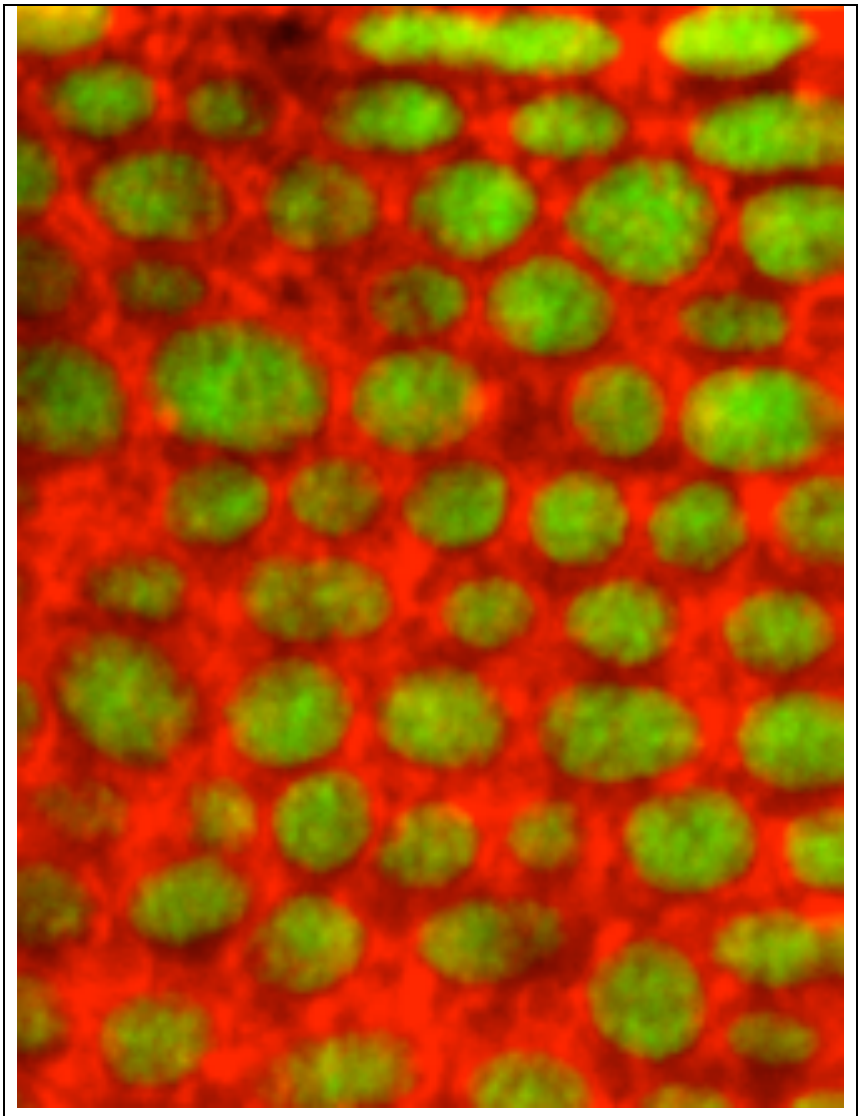

Figure 6: Overlay of the mitochondria and chloroplast fluorescent images in the stationary layer just within the surface of the Chara cell. Mitochondria were false coloured red and chloroplasts green. Deeper within the cell, mitochondria movement could be observed as streaks, due to their fast movement because of cytoplasmic streaming.

DISCUSSION

Assuming that Chara applies a near identical and constant force on the silicon oil, we expect an inverse relation of the speed of the oil and its radius according to equation 3. However, the lack of any clear pattern in figure 2 indicates that the force exerted varies between cells and may even temporally vary as the silicon oil was found to speed up and slow down as it migrated. A more robust measure is how the oil varies with respect to the cytoplasm by tracking a large macroscopic particle in it relative to the oil. The line of best fit in figure 3 shows that the speeds are close to a one-to-one ratio indicating that the silicon oil is moving in concert with the cytoplasm and independently of size. This is an unexpected result for a low Reynold's number environment, since objects moving through solution (the cytoplasmic particles being transported by myosin) are not expected to drag the solution along with them. If the fluid was stationary we would also expect the oil be stationary as well. Verchot-Lubicz et al (2010) provide an explanation, suggesting the large size of the cargo dragged by the myosin motor protein causes a significant entrainment of fluid along with it that drags the cytoplasm along. This must be a strong enough effect to move oil droplets that far exceed the size of cargo dragged by myosin motors, possibly because the density of motors with their cargo is so high.

An intuitive explanation can be provided for the mitochondrial positioning at the surface ell where chloroplasts form a stationary layer. The chloroplasts are sources for oxygen and sugar which the mitochondria intake to provide the cell with ATP as required. This appears to be a common theme among photosynthetic organisms as a tight positional coupling of the mitochondria and chloroplast has been found in diatoms (Prihoda et al, 2012) as well as Arabidopsis thaliana (Bhushan, et al 2003). Beneath the cell surface, mitochondria —much smaller than the oil droplets— move along with other particles in the cytoplasmic stream.

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