The budding yeast *Saccharomyces cerevisiae* is a useful model for studying mitotic microtubule (MT) specialisation because it comprises functionally equivalent structures and proteins to vertebrates. Its mitotic spindle has an old spindle pole body (SPB) and a newly synthesised one. The old SPB synthesises long astral microtubules that probe the bud cortex [2][4][6]. This interaction relies on the MT +end tracking protein (+TIP) Kar9. Kar9 is unusual among +TIPs because it follows the shrinking MT tip in addition to the growing one. This may be due to its ability to form a liquid-like condensate in vitro [5]. However, it is unknown if this remains true in vivo. In order to determine this, I will examine the behaviour of Kar9 in the following two scenarios: 1) when viscous drag acts on Kar9 during diffusive or directed movement and 2) when two Kar9 clusters appear to fuse into one.

The Elyra 7 is a state-of-the-art microscopy system and one of the first of its kind in Canada. Building on the principles of structured illumination microscopy (SIM), the Elyra 7 is equipped with Lattice SIM^2, which allows us to resolve structures down to 60 nm – far beyond the diffraction limit of conventional microscopy – and at much higher speeds than other super-resolution microscopy techniques, such as STED. Using this system, we hope to capture the shape of fluorescently-labelled Kar9 through the course of its dynamic movements during spindle positioning. However, the Elyra 7 system is not without fault. As with other super-resolution microscopy techniques, SIM^2 is prone to creating artefacts. For the purposes of my project, I will examine different methods to resolve this caveat and select the optimal one for our purpose to analyse the shape of Kar9. One possible idea is to train a classifier to recognize the “background” with the created artefacts from the Kar9 regions of interest.

The behaviour of phase-separated droplets in viscous media has been well characterised theoretically and experimentally in non-biological experiments. In particular, we are concerned about the way droplets tend to deform both in response to viscous drag as a result of movement through intracellular fluid and during fusion events in living cells. When kar9 droplets move through the intracellular fluid, we expect the viscosity and pressure resulting from the flow of the surrounding fluid to deform the drop, while the interfacial tension of the droplet resists deformation and tries to maintain spherical shape [7]. Thus, we would expect higher viscous forces on the droplet when it is moving at higher velocities. I will correlate the 2D shape of Kar9 droplets with its 3D velocity with respect to the SPB. Kar9 is hypothesised to deform by elongating in the direction of the viscous drag, where the faster it is moving, the more it will deform. On the other hand, when two droplets fuse, the aspect ratio of the fused droplet is expected to decrease exponentially when it is plotted against time. This characteristic fusion dynamic has been previously confirmed in biological structures in vivo [1]. Specifically, once two droplets touch, they form an energetically unfavourable dumbbell shape. The surface tension drives the fused droplet to return to a spherical shape. The time this process takes in a Newtonian fluid is called the characteristic time, τ, given by $\tau \approx (\eta/\gamma) \cdot l$, where η is the drop viscosity, γ is the surface tension, and l is the characteristic length of the drops [3]. I will analyse the shape of the droplets during fusion events and graph the characteristic time against the characteristic length. The data is hypothesised to show a linear trend. Thus, a linear fit of the data should approximate the ratio of viscosity and the surface tension of the droplet. Previously, this type of shape analysis has only been achieved at the micrometre scale over minutes. Shape analysis at the time and size scale of Kar9 droplets, which are about 250 nm and fuse within seconds, will require new and sophisticated computational methods to resolve.

This project will combine important concepts in biology, physics, and computer science. I will analyse cutting-edge, brand new data from a unique microscopy system and quantitatively measure the shape of Kar9 to characterise its physical properties during spindle positioning.

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