

- Imaging, manipulating and measuring HeLa cells
- Amy Raper
- Chengxi Zhu
- Advanced Imaging Centre, Cambridge

Lay Summary

Cell membrane tension is an important mechanical property of a cell that fluctuates, affecting processes like cell division. Tether force is a direct readout of membrane tension. This project looked at HeLa cells using confocal microscopy, combined with the use of optical tweezers, in order to assess the most accurate imaging modality to optimise the measurement of membrane tension for biologists. Experiments were performed under both brightfield and fluorescence microscopy for comparison.

Optical tweezers use a focused laser to trap and manipulate beads, allowing forces at the piconewton scale to be measured. Beads were brought into contact with the cells and used to pull membrane tethers. The force required for stable tether formation was calculated by bead displacement (from the trap centre), and by trap stiffness (determined through active calibration when a force was applied).

Overall, this project allowed quality comparison of accuracy between imaging methods when measuring tether forces. Results showed that Fluorescence provided more precise localisation for position detection, while Brightfield exhibited higher fluctuations and higher standard deviation. By knowing fluorescence provides a truer force reading than brightfield and is therefore more reliable, these findings bridge physics and cell biology, and could potentially offer better insights into how tension regulates biological processes.

Aim

Using optical tweezers and trapped beads to pull membrane tethers from cells, this 6 week study aimed to image, manipulate and measure HeLa cells, specifically to assess membrane tension, and evaluate the precision and accuracy of imaging modality by comparing brightfield and fluorescence microscopy.

How the aim was addressed

When a bead is suspended in a medium, it goes through random fluctuations called brownian motion. So for context, the beads were first observed in brownian motion, using the confocal spinning disk microscope (Figure 1). Then, to address the aim, the optical tweezers were calibrated using an active stiffness method with 2 μm yellow-green carboxylate microspheres. Beads were optically trapped and subjected to controlled stage movements at increasing speeds (1–5 $\mu\text{m/s}$) to apply known viscous drag forces. Laser power was incrementally increased from ~16–72mW. Beads were moved 1–5 $\mu\text{m/s}$ in both the X and Y directions to measure direction-dependent displacement (Δx).

Using the Faxén-corrected Stokes drag equation to calculate the applied force (f), and Hooke's Law ($k=f/\Delta x$), the trap stiffness (k) was determined from the bead's displacement (Δx). This calibration is critical for the subsequent membrane tether experiments, as it allows bead displacement to be accurately converted into force values.

Throughout the project, HeLa cells were cultured and split every two days to prevent over-confluence and maintain healthy dividing cells for imaging and manipulation. Non-synchronised cells were used: non labelled for brightfield and Caax mCherry cells for fluorescence.

For imaging, HeLa cells were plated (50 μ L of a 1:10 diluted culture + 2 mL T25 media) and incubated overnight to promote attachment to the plate. Imaging media was added prior to acquisition to maintain physiological conditions, promote clarity and minimize phototoxicity.

To assess membrane tension, membrane tethers were pulled from the HeLa cells. The beads were coated in Concanavalin A (200 μ g/mL) to enable anchoring via glycoproteins, and added to the imaging plates. Once the beads were brought into contact with cells for 10-30 seconds, the optical trap was moved away using incrementally increasing laser powers to pull a stable membrane tether from the cell surface.

To calculate the tether force, displacement from the centre of the bead (Δx), and stiffness (k) from the specific laser powers during calibration were used.

This process was done with both brightfield and fluorescence, to compare.

Results

Figure 2 and 3 represent the tether pulling assay, where a stable tether was formed. It was ultimately concluded that the average standard deviation for Membrane Tether Force (pN) in Brightfield was 1.521, whilst in Fluorescence it was 0.498 (Table 1), leading to the declaration that Fluorescence mode is more precise in comparison to Brightfield.

Fluorescence has a smaller average Standard Deviation of Tether Force and exhibits less fluctuations, with a smaller fluctuation percentage (Fig. 4). This means it is a more reliable reading of tether force. The beads localisation precision is higher, and therefore Fluorescence position detection is more accurate than Brightfield; Fluorescence has a higher signal to noise ratio. Biologists can leverage this precision in measuring membrane tension to explore complex biological questions, with more confidence. E.g. high-accuracy tracking of tension dynamics in cell division. Overall, this is a really important development that can ensure a level of certainty in further applications.

What did I learn from participating in this project?

As a Biomedical science student, this summer studentship, though daunting at first, has allowed me to gain invaluable experience in a completely new field for me. This project opened the doors to Biophysics, and taught me indispensable lessons in microscopy, experimental planning and data analysis - skills will serve me well throughout the rest of my academic career.

Using new principles in microscopy allowed me to calibrate bead stiffness, and use this to accurately measure membrane tension. Combining both theoretical and experimental understanding to do this was an enjoyable learning experience, and getting the chance to work with optical tweezers so often to compare imaging modes was extremely rewarding. Working with software such as Fiji and Metamorph to track bead displacement helped me develop strong image analysis skills. I also gained gratifying and valuable experience in HeLa cell culture, including splitting and plating, which was essential for maintaining healthy cells throughout the experiments.

This studentship has given me a solid foundation in quantitative live-cell imaging and biophysical force measurement. Being involved in the full experimental process, from culturing and bead preparation to image acquisition and force calculation, significantly deepened my understanding of the workflow and improved my confidence in microscopy-based research, which I can now translate into future projects.

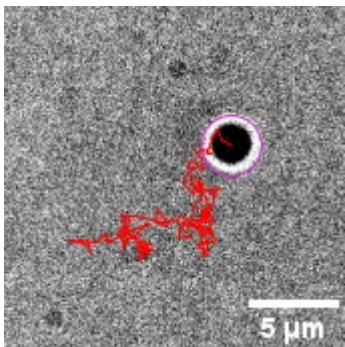
How has this affected my long term goals?

This project has broadened my perspective on the potential career paths open to me. While I am still exploring whether a PhD is the right next step, working with optical tweezers and advanced microscopy techniques has certainly made further research an appealing prospect. I particularly enjoyed learning how imaging can be applied to answer complex biophysical questions, and this experience has deepened my appreciation for the role of microscopy in cutting-edge biomedical research.

I remain passionate about fields where scientific understanding can be translated into meaningful real-world impact. This studentship has shown me that microscopy and quantitative imaging could remain an important part of my future, whether through further academic research, collaborative projects, or science communication. Regardless of the path I ultimately take, the technical skills, analytical mindset, and curiosity I have developed through this project will continue to influence my career journey.

Figure 1

A



B

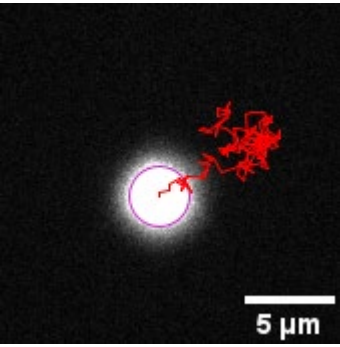


Table 1

Tether Force (pN)			
Brightfield (SD)	mA	Fluorescence (SD)	mA
1.072764	1500	0.367579	1550
1.923117	1550	0.420511	1600
0.745945	1550	0.446622	1600
1.743269	1500	0.537434	1700
1.98181	1550	0.690635	1750
1.660819	1750	0.527311	1800
Average	1.521287	0.498349	

Table 1. Standard deviation of tether force (SD) are compared between two imaging modalities: brightfield and fluorescence, and the laser power they were measured at. Tether force was measured from membrane tethers of HeLa cells

Figure 2

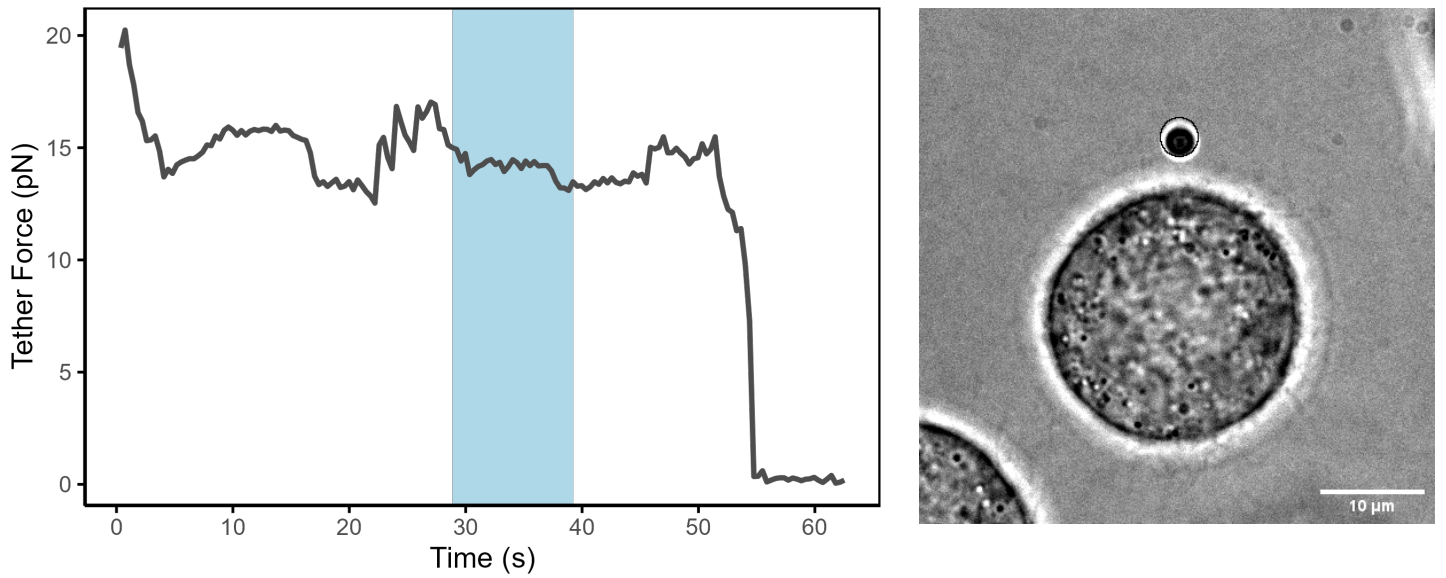


Figure 2. (A) Representative time trace of tether force (pN) measured from the tether pulling assay with a WT HeLa cell in a mitotic state at ~ 38.86 mW laser power in Brightfield mode. Blue highlight represents the first stable tether shown in (B). The peaks represent pulling trap away to form tether. In (B) the bead is trapped by the optical tweezer, and is displaced $\sim 0.5 \mu\text{m}$ from the trap centre, shown by the black circle. The displacement for a significant period of time represents a stable membrane tether formation.

Figure 3

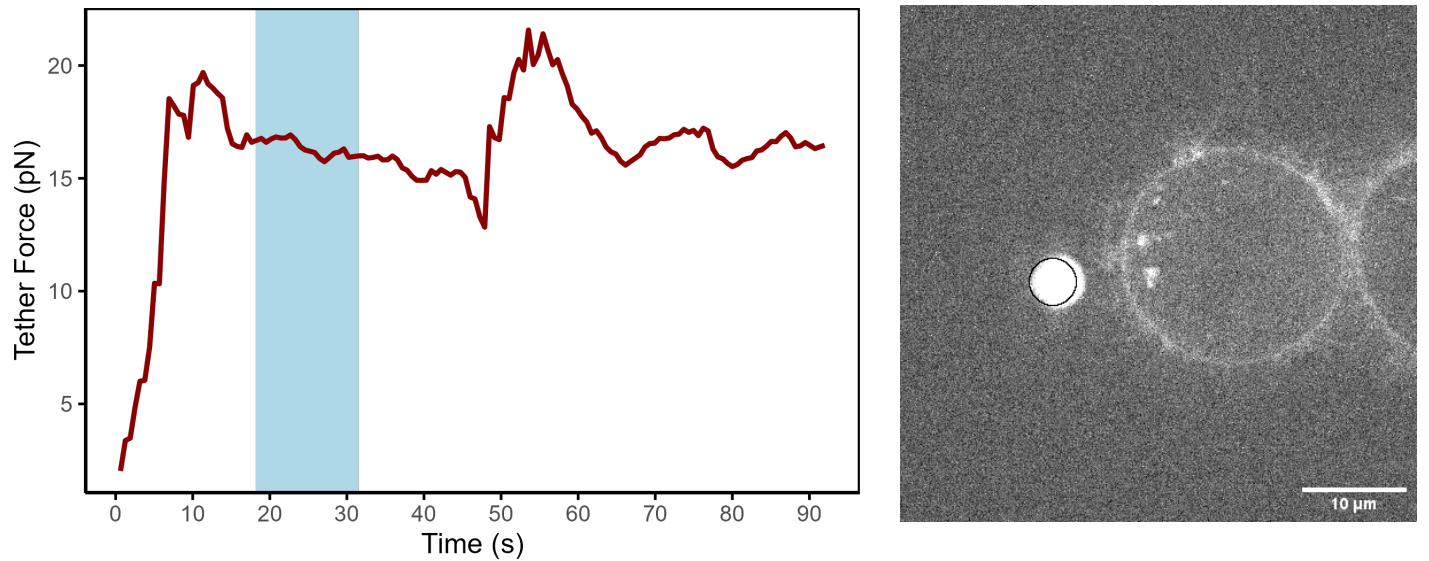
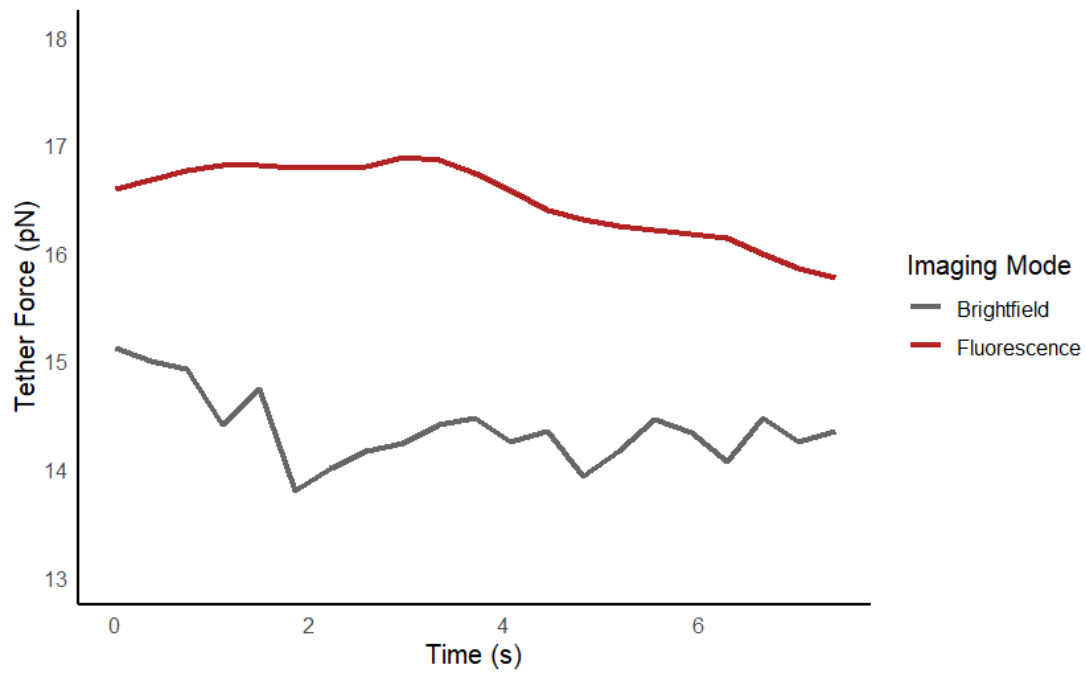


Figure 3. (A) Representative time trace of tether force (pN) measured from the tether pulling assay with a Caax mCherry HeLa cell in a mitotic state at ~ 38.86 mW laser power in Fluorescence mode. Blue highlight represents the first stable tether shown in (B). In (B) the bead is trapped by the optical tweezer, and is displaced $\sim 0.62 \mu\text{m}$ from the trap centre, shown by the black circle. The displacement for a significant period of time represents a stable membrane tether formation

Figure 4

a



b

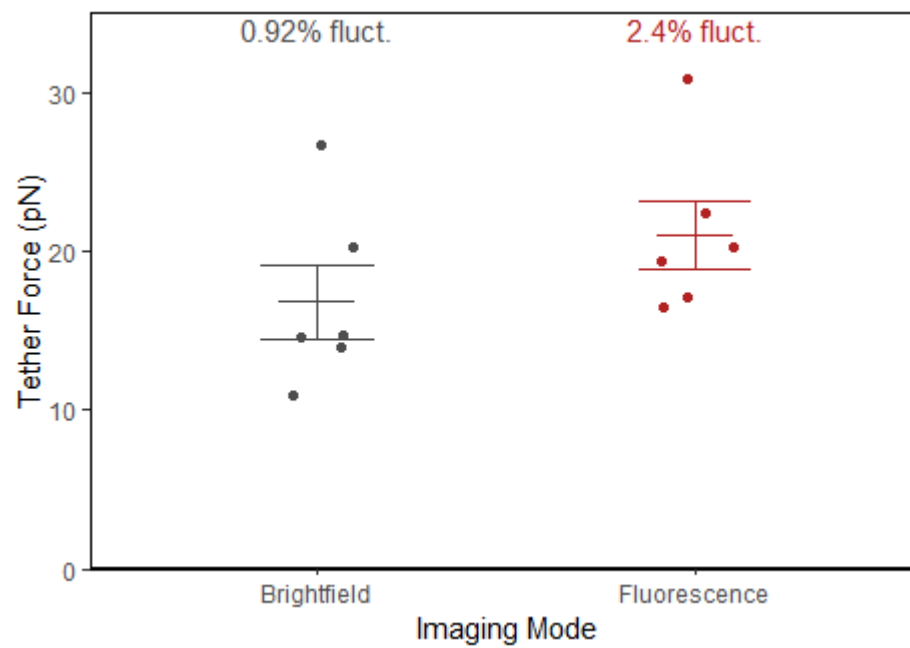
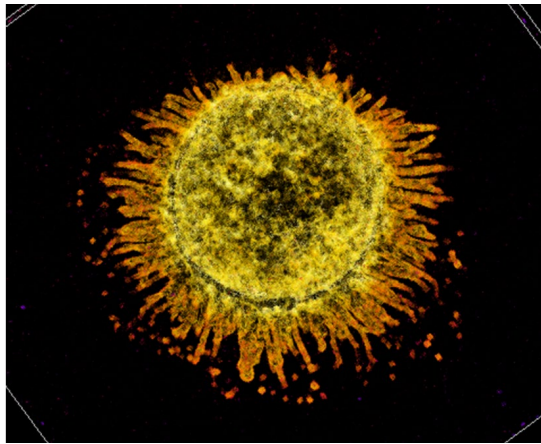
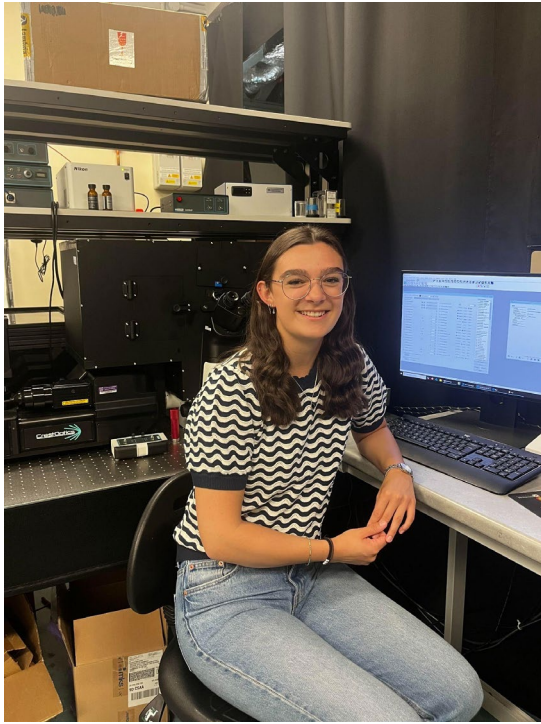


Figure 3. (A) Representative time trace of tether force (pN) from the stable membrane tether in Figure 1 and Figure 2, respectively (shown by blue highlight). Brightfield imaging is in grey (SD 0.745945) whilst Fluorescence is in red (SD 0.367579) (B) Tether force in Brightfield and Fluorescence, error bars indicate means $\pm$ standard error. Fluctuation percentages are displayed above.

Picture of me



HeLa cell