

Title of Project: Expanding our knowledge on Expansion microscopy: Visualising Cancer cell markers and Cell-in cell structures in H1299 Cells

Name of Student: Charlotte Todd

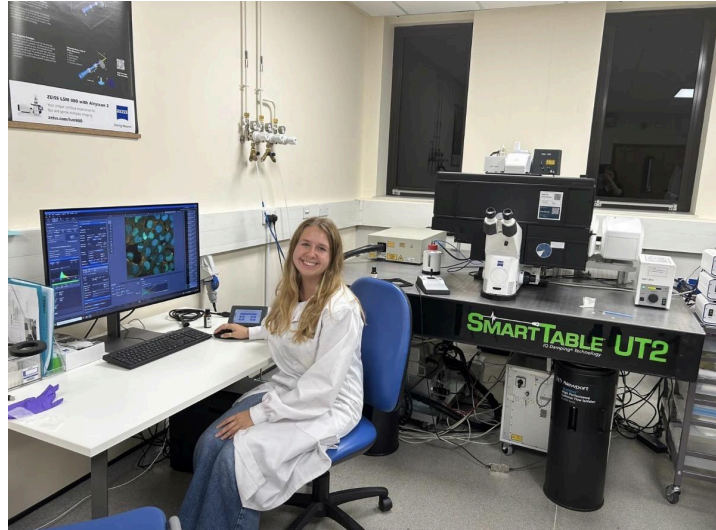
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Project Location: University of Durham Biosciences Department, Durham, UK

Lay Summary

Light microscopy is a fundamental technique utilised throughout many scientific disciplines, however these microscopes cannot resolve structures below about 200 nanometers, a value called the diffraction limit. Expansion microscopy (ExM) is a developing technique that offers a way to overcome this limit by embedding cell samples into a hydrogel that can then be expanded using water. This physical enlargement of the cell allows for increased resolution of small structures while maintaining relative shape and position within the cell. This project utilises this technique to study mutant p53 H1299 cancer cells including expression of various cancer markers and how they interact with neighbouring cells. Increased imaging depth and resolution enables researchers to study cancer cell structures without needing expensive imaging equipment.

Photograph:



Aims

The aims of this project were to use ExM to:

- Investigate cancer cell marker p53 expression in a mutant H1299 cell line
- Examine cell-in-cell structures associated with tumour growth and assess expression of Sox2 and ALDH1A1
- Quantify nuclear area pre-ExM and post-ExM

Methods

To address these aims Expansion microscopy was applied to H1299 mutant p53 cells and performed in 5 stages: fixation, labelling/anchoring, gelation, homogenisation and expansion. H1299 cells grown on glass coverslips were fixed, washed in PBS, blocked and then cancer markers (Sox2, p53 and ALDH1A1) were labelled with fluorescent antibodies and nuclei stained with DAPI. Glutaraldehyde was used for cross-linking to preserve structure then cells were washed in PBS. Gelation solution (monomer, APS and TEMED) was pipetted onto 1cm x 1cm wells and coverslips placed cell-side down on top. After 30 mins polymerisation, gels were incubated in proteinase K digestion buffer at 37°C, trimmed and expanded in distilled water (changed every 30 min) to ~4x size. Expanded cells were cut, re-stained with Hoechst, mounted in ibidi dishes, and imaged using a Zeiss Apoptome and Confocal 980. Image processing was then performed in Fiji.

Results

P53 Overexpression in H1299 mutant cells

First localisation and expression of the tumour suppressor protein, P53 was assessed using an anti-p53 antibody (green). ExM was undergone on a co-culture of H1299 p53 KO and P53-R273H mutants and it could be determined that the antibody was successfully preserved during the ExM process. P53 overexpression was observed in specific cells within the co-culture confirming p53-R273H cells were present and suggesting enhanced cancer cell invasion of other cells (Fig 1). By using expansion microscopy it was possible to distinguish p53 being associated with microtubules (shown by white dotted box) and being transported towards the nucleus, as microtubules are roughly 25nm in diameter they are very close to the diffraction limit so ExM is a valuable technique to visualise this.

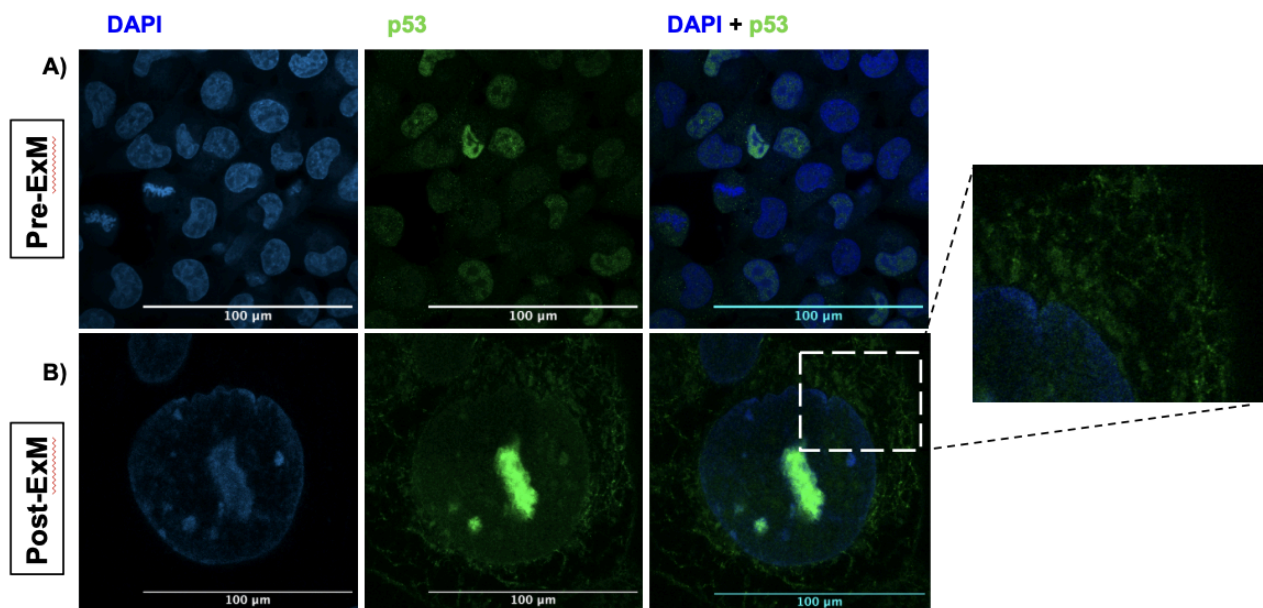


Figure 1. P53 expression in H1299 cells Pre-expansion (A) and Post-expansion (B). H1299 p53 KO and P53-R273H mutants were co-cultured and stained with DAPI (Blue) and anti-P53 (Green) 1:100 (A), 1:50 (B). Images were taken on 980 Confocal with scale bar = 100 μm.

Expression of Sox-2 and ALDH1A1 in Cell-In-Cell Structures

Cell-in-cell (CIC) structures (indicated by white arrows) were identified within the p53 KO and P53-R273H mutant co-culture. These structures showed overexpression of the cancer cell markers Sox-2 and ALDH1A1 linked to tumour progression.

Pre-expansion images identified CICs but had limited resolution making exact localisation of the markers difficult. Post-ExM, Sox2 expression was observed in engulfed cell nuclei at a higher clarity at the same magnification (Figure 2). The ALDH1A1 signal also appeared sharper and localisation could be seen within the cytoplasm, consistent with its interactions with cellular pathways and nuclear and actin boundaries were also much clearer (Figure 3). This demonstrates the ability of ExM to enhance cancer-associated marker signals enabling assessment of their localisation within cell structures without requiring highly expensive techniques.

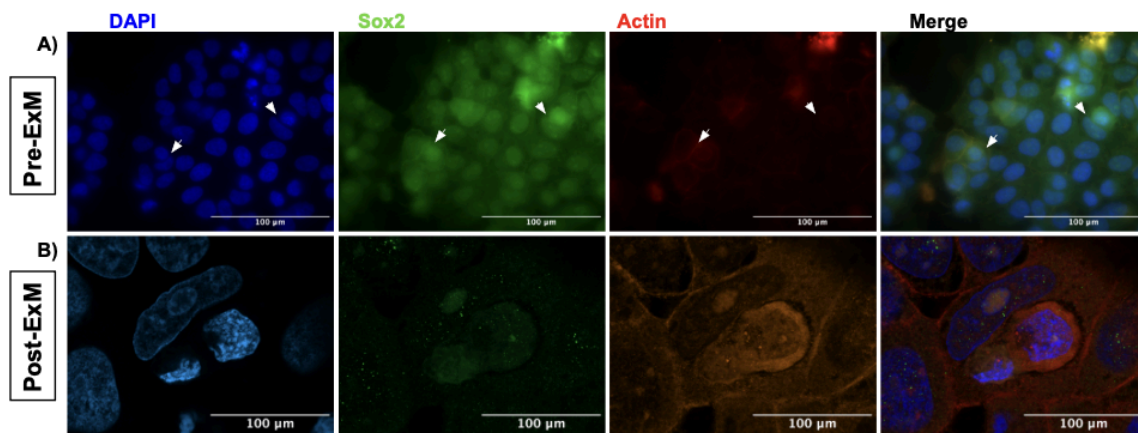


Figure 2. Sox2 expression in H1299 cells Pre-expansion (A) and Post-expansion (B). H1299 p53 KO and P53-R273H mutants were co-cultured and stained with DAPI (Blue), anti-Sox2 (Green) and Actin (Red) 1:100 (A), 1:50 (B). White arrows indicate Cell-in-cell structures. Images were taken on Zeiss Apotome x63 (A) and 980 Confocal x63 (B) with scale bar = 100 µm.

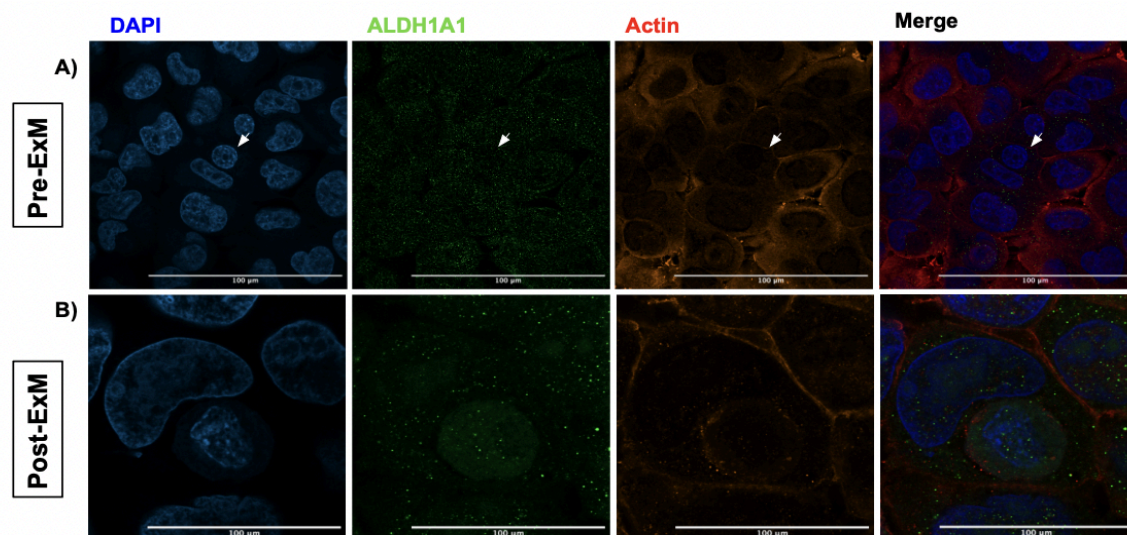


Figure 3. ALDH1A1 expression in H1299 cells Pre-expansion (A) and Post-expansion (B). H1299 p53 KO and P53-R273H mutants were co-cultured and stained with DAPI (Blue), anti-ALDH1A1 (Green) and Actin (Red) 1:100 (A), 1:50 (B). White arrows indicate Cell-in-cell structures. Images were taken on Zeiss 980 Confocal x63 with scale bar = 100 µm.

Quantifying Nuclear Area Pre and Post-ExM

To evaluate the expansion process, mean nuclear area was measured in H1299 cells before and after ExM using FIJI. Mean nuclear area increased from $188\mu\text{m}^2$ pre-ExM to $2648\mu\text{m}^2$ post-ExM, demonstrating an approximately 14-fold increase in area (Figure 3). Significance was confirmed with a T-Test where $p < 0.001$. However Post-ExM measurements also exhibited greater variability, shown by standard deviation error bars, suggesting expansion is not entirely uniform across all nuclei and while enlargement is obvious this has to be taken into account for analysis.

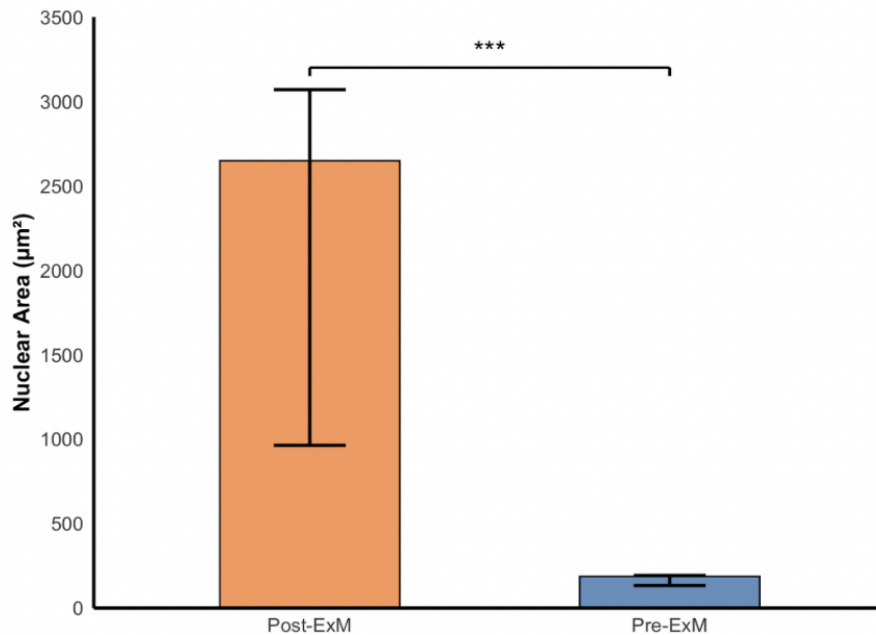


Figure 3. Nuclear area measurements pre- and post-expansion microscopy. Bar plots represent the mean nuclear area of H1299 cells calculated using Fiji. Error bars show standard deviation (SD) of nuclear area measurements (Pre-ExM $n = 100$, Post-ExM $n = 18$). T-test was undergone with *** indicating $p < 0.001$.

What did you learn from participating in this project

Through this project I received hands-on training on intricate microscopes such as the Zeiss Apotome and Confocal 980 which has enhanced my knowledge of microscopy. Using Zen software, I learned how to produce high quality publishable images using LSM plus and Airyscan as well as how to create Z stacks, 3D images and orthogonal views. I also understand the role of key components such as beam splitters and laser power and had practice at troubleshooting common technical problems which may occur during imaging. I've developed skills in expansion microscopy including preparing hydrogels that can crosslink to cell samples and expand in water to enlarge cell structures. I also have a deeper understanding of how to pair primary and secondary antibodies for optimal immunofluorescence staining and how to aliquot antibodies for maintenance. Outside the laboratory I've also gained insight into inter-disciplinary communication and how different areas of research come together to achieve shared goals.

How has this project affected your long-term goals? (Will you do a PhD? Or what career path will you choose? Will you aim to do microscopy in the future?)

This project has cemented my decision to pursue postgraduate study and undergo a research masters, as I've enjoyed the hands-on aspect of my studentship and the independence of leading my own project. I am also more open to doing a future PhD as the research field has been more social and supportive than I expected. I've developed a strong interest in microscopy and found it fascinating to visualise microscopic structures within cells. As imaging is so vital within science I will definitely be incorporating these skills into my future career as I have a strong interest in neuroscience which utilises lots of microscopy techniques. I am also excited to follow the development of ExM as a technique and see what other research fields it can provide valuable data for.