

Assessing Microscopy Methods to Study Localisation of Coilin and SMN proteins in Mammalian cells

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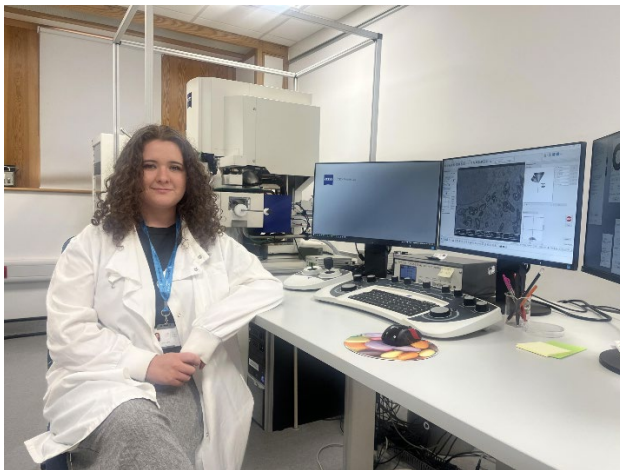
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Project Location: Babraham Institute, Cambridge

Lay Summary

Membraneless organelles (MLOs) are present in eukaryotic cells, providing specialised compartments for cellular processes. MLOs contain proteins and nucleic acids and are phase-separated from their immediate surroundings. The proteins Coilin and SMN are present in MLO Cajal bodies in the nucleus and cytoplasm of mammalian cells. Observations of these Cajal bodies and specifically how SMN and Coilin are co-localised could bring rise to further investigation into signalling interactions, as well as providing insight into the performance of different microscopy techniques in observing MLOs

Using PANC1 cells as the mammalian cell line, fluorescently labelled using immunostaining techniques to identify Coilin and SMN for imaging. My research focused on assessing various imaging techniques to gain insight into which would be most informative in future experiments involving Cajal bodies. My key findings were that SIM microscopy is best suited for distinguishing the proteins in the MLOs separately, and CLEM resulted in an interesting overlap between Coilin fluorescence signalling and electron density in the nucleolus.



Aim

The project aimed to assess the wide range of confocal microscopy, Structured Illumination Microscopy (SIM), and Correlative Light and Electron Microscopy (CLEM) to provide an overview of how effective these microscopic techniques are at illustrating the co-localisation and interactions of Coilin and SMN within cells.

Addressing the aim

To address the aim, a variety of microscopy techniques were used, including laser scanning confocal microscopy, spinning disc confocal microscopy, structured illumination microscopy (SIM), and standard fluorescence microscopy, to assess the capabilities of light microscopy

in showing the distinguishable spatial relevance of protein shape in the co-localisation of Coilin and SMN using Imaris software for image processing and to produce deconvoluted images. Additionally, electron microscopy, coupled with the CLEM strategy, was used, which involved imaging the same cell using both light and electron microscopy. This approach enabled the combination of molecular specificity from laser scanning confocal microscopy and high-resolution ultrastructure from electron microscopy, allowing for high-resolution imaging and precise localisation of cellular components. To prepare the samples for imaging, PANC1 cells were cultured and plated on chamber slides. They were then fixed with 4% PFA and immunostained to label Coilin and SMN fluorescently. Western blots were then performed to confirm the presence of these proteins. For the CLEM strategy, PANC1 cells were seeded on gridded coverslips to facilitate the same cell imaging. Electron microscopy samples were immunostained and embedded in hard resin.

What did you find out?

Light Microscopy results:

As seen in Figure 1, the presence of proteins in the nucleus of PANC1 cells is shown. Blue represents SMN, and red represents Coilin. The resolving power of the microscopes and processing differ, affecting the ability to distinguish the spatial relevance of the fluorescent signals corresponding to the proteins.

The more advanced SIM system reveals that the signals for each protein are significantly more distinct, indicating that SIM is the most suitable method for further investigation of the spatial interactions of these proteins. Using super-resolution systems and optimisation to reduce light diffraction allowed for the distinction between proteins, which is best seen in SIM.

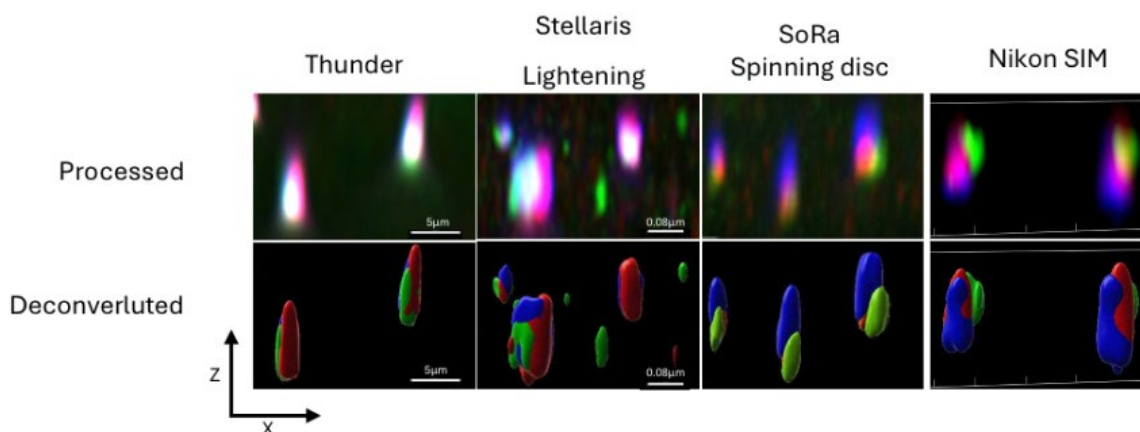


Figure 1. Top panels z-stack 3D reconstruction of four different light microscopes using standard fluorescence microscopy, laser scanning confocal, spinning disc confocal, structured illumination microscopy (SIM) with their highest resolving processing. Bottom panels show a deconvoluted representation of the fluorescent signals.

CLEM results:

Figure 2 shows the electron microscopy (STEM) imaging of the hard resin-embedded, immunostained samples, which revealed significant ultrastructural disruptions attributable to the permeabilisation process and the use of TET. Specifically, the puncturing of lipid membranes facilitated immunostaining but concurrently compromised cellular integrity. Ultrastructural analysis demonstrated extensive damage across multiple organelles:

mitochondria exhibited rupture and disintegration, the endoplasmic reticulum appeared ruptured, and there were notable tears in the nuclear membrane. Also, the bilayer structure of the nuclear membrane was not visible due to the significant disruption caused by the immunostaining protocol. Interestingly, endosomal structures remained intact. However, it is essential to acknowledge that this damage is only detrimental for experiments where the proteins of interest are expected to localise at the mitochondria or the ER. This underscores the critical advantage of electron microscopy in revealing ultrastructural details that are not discernible through fluorescence microscopy alone. While light microscopy provides valuable localisation of fluorescent signals, it does not capture the extent of cellular disruption induced during sample preparation, emphasising the importance of EM in assessing cellular integrity and structural preservation.

In Figure 3, the overlap of the fluorescent signalling and ultrastructural results from the same cell is shown, indicating a strong correlation between Coilin fluorescent signals and areas of higher electron density in the nucleolus, suggesting a potential function or purpose for Coilin to localise in the nucleolus.

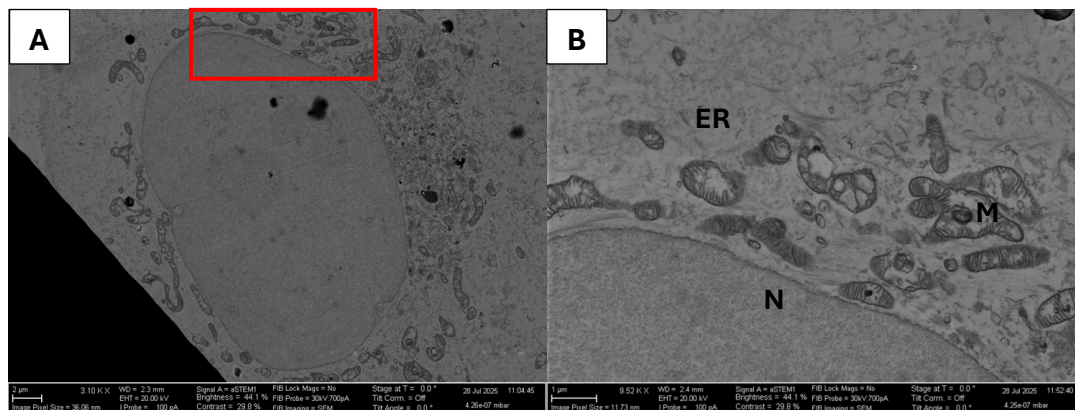


Figure 2. Electron microscopy (STEM) imaging of the hard resin-embedded, immunostained samples, depicting the disrupted ultrastructure. A) overview of whole cell B) zoom in on nuclear membrane and membrane-bound organelles in cytoplasm (ER = endoplasmic reticulum, M = mitochondria, N = nucleus)

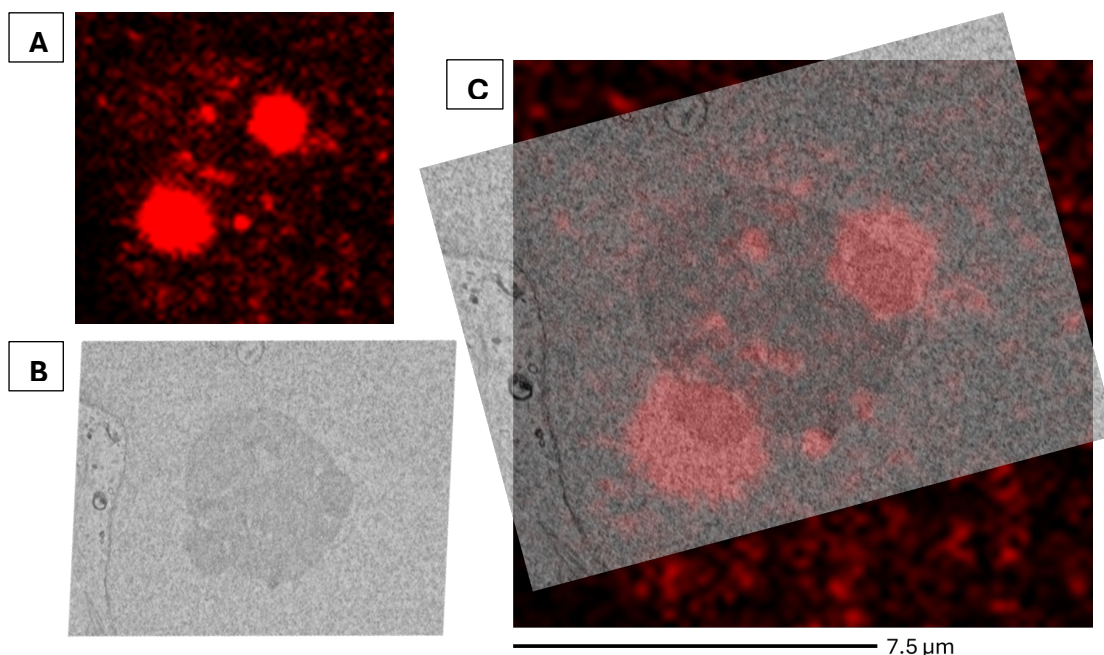


Figure 3. Results from CLEM strategy for same cell imaging A) laser confocal imaging of coilin fluorescent signalling. B) Electron microscopy image of structures overlapping the nucleolus. C) overlapped images showing corresponds with structure and fluorescence in the nucleolus

Concluding that the co-localisation of Coilin and SMN was seen throughout, with little deviation from each other. Using the CLEM strategy, it was concluded that the correlation between the structures found in the nucleolus and Coilin signalling was directly correlated. Future work could include a true comparison of light microscopes using gridded coverslips to perform the same cell imaging, as well as improving the CLEM strategy by incorporating live cell imaging and FIB-SEM.

What did you learn?

Participating in this project allowed me to develop a comprehensive understanding of various microscopy techniques and their applications. I particularly enjoyed learning how to optimise imaging conditions to enhance localisation images. The project taught me how to effectively combine light and electron microscopy, enabling detailed analysis of both fluorescence and ultrastructural features within the same cells. I gained practical skills in image overlay and alignment, which are crucial for correlating data from different microscopy modalities. I also learnt the whole process of sample preparation, including tissue culturing, immunostaining techniques, and western blotting, to produce viable cells for imaging. Additionally, I learned about the importance of precise sample preparation and technical adjustments to improve image quality, as well as the various sample fixations required for light and electron microscopes. I gained skills in processing image data using Z-stacks to form 3D reconstructions and deconvoluted images of the fluorescent signals. Overall, this experience deepened my knowledge of microscopy and imaging techniques, and it highlighted how integrated approaches can provide a more complete understanding of cellular structures.

How does the project impact your future aspirations?

Before undertaking this project, I had considered pursuing a PhD, but I was uncertain about whether I wanted microscopy to be a significant part of the PhD I would pursue. Completing this project has deepened my understanding of the research environment and provided valuable insight into how the biological industry operates, reinforcing my long-term goal of pursuing a PhD. The experience has expanded my skills with various microscopy techniques, giving me a broader appreciation for imaging technologies that I would not have gained solely through an undergraduate degree. This exposure has strengthened my passion for microscopy and imaging, and I am now eager to apply for a PhD program with a focus on advanced imaging techniques.