Charlotte Pain

Charlotte Pain is a Leverhulme Trust-funded Early Career Research Fellow working in the Plant Endomembrane group at Oxford Brookes University, UK. Her research is focused on developing image analysis methods that enable quantification of the plant endoplasmic reticulum and identification of novel ER shaping proteins.

Tell us about your background. How did you first become interested in image analysis and the endoplasmic reticulum?

The endoplasmic reticulum is one of the most beautiful, complex and dynamic organelles in plant cells. Its unique structure, spanning the entire cell just below the cell membrane, is fully contiguous and constantly changing, making it a unique challenge for image analysis.

What are you working on currently?

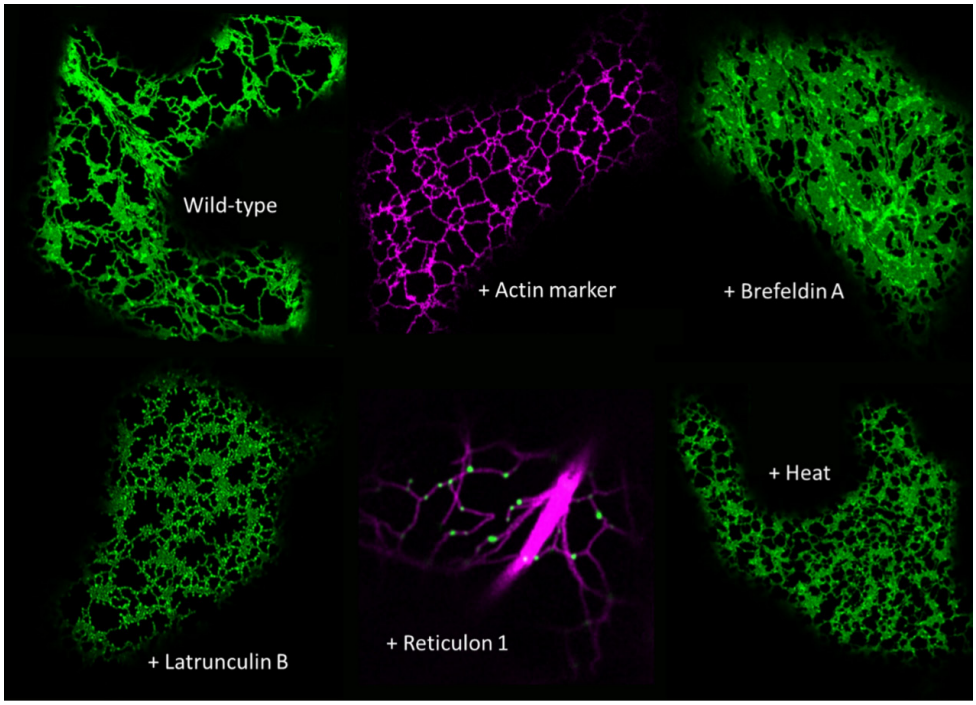
Currently, my research focuses on investigating novel ER morphogens and understanding the mechanisms that determine ER shape. Additionally, using known ER morphogens, I am exploring the functional roles of different ER structures and investigating the different ER structures that we see in plant development and during plant stress responses. To achieve this, I am using a variety of both traditional and newly-developed assays to investigate the function of the plant ER.

What does a typical day look like for you?

A typical day for me has a heavy focus on microscopy work, with sample preparation in the morning, followed by microscopy in the afternoon, with time set aside for image analysis and processing. Our lab frequently employs *Agrobacterium*-mediated transformation of tobacco leaves, which requires culturing transgenic *Agrobacterium tumefacians*, and injecting them into mature tobacco leaves after transferring them to an infiltration buffer at the correct density. At the Oxford Brookes University Bioimaging unit, we have access to a range of advanced equipment, including Zeiss 880 and 800 laser scanning confocal microscopes. Depending on the requirements of the experiment, I will use one of these machines in the afternoon to collect data. Image analysis is performed using various software tools such as FIJI, or AnalyzER which was developed as part of my PhD.

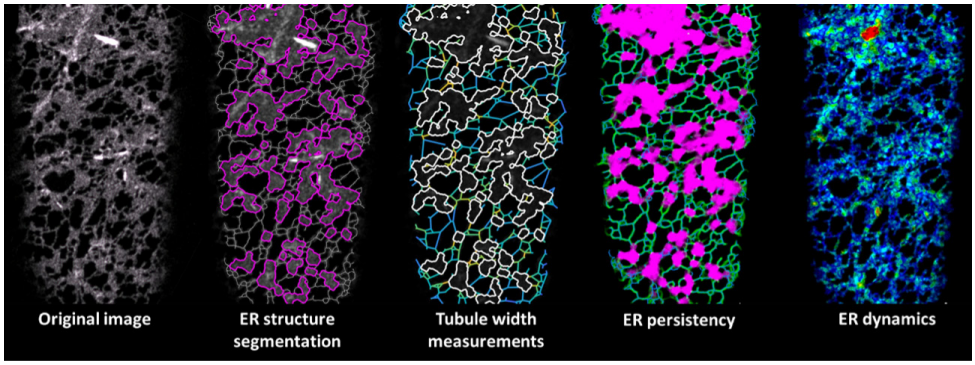
What do you most enjoy about your work?

I find a lot of enjoyment in my work, for several reasons. Firstly, every day is different and brings new challenges, which keeps things exciting. Additionally, I am fortunate to work in a supportive environment with colleagues who are always willing to lend a hand.



Above: The endoplasmic reticulum in tobacco leaf epidermal cells, marked with a fluorophore-HDEL. The 'wild-type' ER structure is shown in comparison to ER structure modified by co-expression with an actin marker, pharmacological treatments including brefeldin A and latrunculin B, co-expression with the ER morphogen Reticulon 1, and after heat shock.

Left: Example analysis of ER structure and dynamics in an *Arabidopsis thaliana* root. From left to right: the original image, identification of ER cisternae (magenta), graphical representation of the cisternae and tubules making up the ER, a persistency analysis, and optical flow analysis of ER dynamics.



What do you find most challenging?

One of the biggest challenges of working with microscopy is that the technology can be complex and finicky, and it can be difficult to troubleshoot technical issues when they arise. Microscopy equipment requires careful maintenance and calibration to ensure that it is functioning correctly, and technical problems can be time-consuming and frustrating to resolve.

What are you hoping to work on in the future?

In the future, I hope to continue with my current work while also exploring opportunities to develop novel assays for live cell imaging of dynamic cellular events, such as protein production and transport through the plant secretory pathway. This may involve the use of recently developed optical techniques, including Focused Ion Beam–Scanning Electron Microscopy and single molecule tracking, but also making use of recent developments in fluorophore technology, such as optogenetics, irreversibly photoconvertible fluorophores, and new protein tags designed for correlative light and electron microscopy. By pursuing these avenues, I aim to gain a deeper understanding of plant cell biology and to contribute to the advancement of the field.

> "The endoplasmic reticulum is one of the most beautiful, complex and dynamic organelles in plant cells"

What advice would you give to aspiring scientists in this area?

Collaboration is key. There are many incredible scientists out there, each of whom brings their own skills, knowledge and ways of doing things to the team. From these people you can learn new things about your own processes as well as gain a new perspective. Science should be a collaborative process and finding a supportive network of scientists makes all the challenges a lot easier. Be patient and persistent, research in plant cell biology can be challenging and time-consuming. Aspiring scientists should be prepared to put in the hard work, stay focused on their goals, and be patient when results don't come as quickly as they would like.

Who are your scientific heroes?

I look up to individuals who embody a combination of intellect, hard work and kindness. I am fortunate to have crossed paths with several people who possess these qualities, but one person who had a profound impact on my scientific career was my PhD supervisor, Professor Chris Hawes. Chris was an exceptional mentor who not only possessed a great intellect but also had a great sense of humour, making our interactions both productive and enjoyable. His contributions to the field of plant cell biology have been invaluable, particularly in championing the use of microscopy in this field. Chris's intelligence, humour and dedication to his research and students made him an exemplary scientific leader and a role model for aspiring scientists.

Selected Publications from SEB journals

Pain C, Kriechbaumer V. 2019. Defining the dance: quantification and classification of ER dynamics. Journal of Experimental Botany 71, 1757-1762.