

# Microscopy & Microtechniques

## Lasers Reveal the Secrets of Plant Cell Walls

Scientists working at STFC's Central Laser Facility (CLF) have found that the cell walls of plants play a more important role in regulating plant proteins than previously thought and this new research has the potential to lead to more disease-resistant crops.

The research, led by Dr Joseph McKenna from Oxford Brookes University, found that cell walls control a surprising number of plant functions via proteins within the cell. Cell walls act to protect the cell and provide its scaffolding. The research demonstrates that manipulating the cell walls could result in crops with higher yields or with a greater resistance to disease.



*Octopus imaging facility*

Dr McKenna, Research fellow in Bioimaging at the University's Department of Biological and Medical Sciences, said: "The investment in state-of-the-art microscopy systems at Oxford Brookes allows us to see structures in a way which we wouldn't have believed possible 10 years ago. This combined with the extreme resolving power available at the Central Laser Facility allows us to monitor the movement of individual proteins, the machines of life, within the cell membrane."



*Dr Joe McKenna*

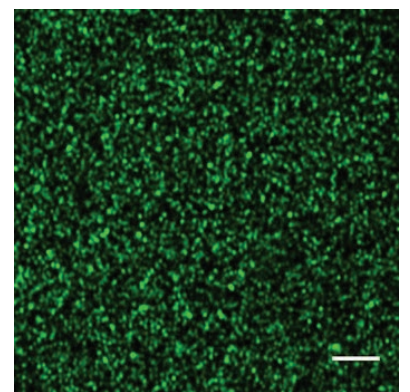
"For centuries light microscopy has been limited to a fixed resolution by a law of physics. This meant we could not accurately characterise structures using a fluorescent microscope which were under 200nm in size. Therefore, we were unable to even see these nanodomains within the membrane. However, over the last few decades a number of techniques have been developed which allow us to navigate our way past this resolution limit and characterise even smaller structures. The investment at Oxford Brookes allowed the renovation of a new centre for bioimaging and the purchase of two Zeiss Airyscan confocal microscopes, enabling us to see the nanodomains and characterise them in the plant membrane."

The Bioimaging unit at Oxford Brookes is also a Zeiss labs@location partner, meaning we have a close relationship with Carl Zeiss in using the latest in imaging technologies. Airyscan imaging allows us to see down to 120nm resolution. However, while fantastic at imaging the nanodomains, we needed an imaging technology which could allow images to be taken much faster and allow us to determine the speed of their movement.

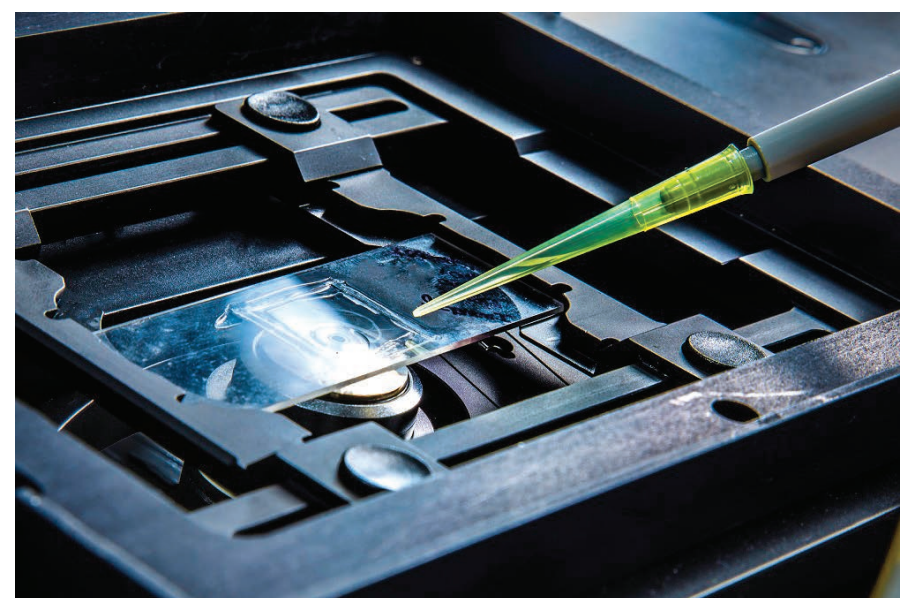
For that, we turned to our colleagues and collaborators at the CLF and a technique which allowed us to image individual molecules moving through the membrane. This was called Total Internal Reflection (TIRF) single particle imaging and tracking. The image acquisition and analysis pipeline available at CLF made this work possible and allowed us to determine the speed with which these nanodomains moved within the membrane. The combination of the imaging facilities available at Oxford Brookes and the CLF and our long term collaboration made this work possible."

The team of cell biologists at Oxford Brookes University used high-resolution microscopy methods to investigate the interaction between proteins and cell walls in plants.

One of these proteins moves hormones between cells, helping determine how the plant will grow. Another lets plants respond to external threats, for example by detecting the presence of damaging micro-organisms, such as viruses, and activating the plant's innate immune system.



*Nano Domain taken by Brookes confocal*



*Octopus pipette*

Dr Dan Rolfe, Lead Data Scientist at Octopus said: "This is a great example of how multidisciplinary research is needed to address key challenges for humanity. A combination of advanced microscopy and analysis techniques originally translated from astronomy and applied to gain insights into human cancers has been applied in this plant research. And it has revealed a new understanding of plant biology which has important implications for food security."

He added: "With the Octopus imaging cluster at CLF, it is possible to perform single molecule tracking where a thin layer of the surface of the cell in question is illuminated with the laser to image individual molecules or small numbers of molecules. This allows researchers to look at the paths molecules take in cells - how molecules move and if they get "stuck" or move freely. These capabilities, originally developed in Octopus to understand molecular interactions critical to the development and treatment of cancers, and incorporating analysis methods from astronomy, can now be used to further research in plant science, especially the dynamics of proteins in plants. Laser microscopy is useful because it can reveal in fine detail behaviours of specific molecules through time."

Professor Stan Botchway is a senior scientist at CLF and STFC Biomedical Network Lead. He said: "In this particular research, the research team combined the Octopus total internal reflection fluorescence (TIRF) single molecule microscope with a custom Bayesian analysis - a statistical procedure that extracts distribution based on the observed population - to track the single protein molecules. TIRF is an excellent method to image very thin samples because it significantly reduces the out of focus signal, which normally degrades the microscopy signal. This is usually a problem with trying to detect single separated molecules such as the proteins being investigated here. Although TIRF is mostly used for mammalian cells, the group have shown its applicability to plant research, which is unique."

In the future, the research team aims to investigate how the properties of cell walls could be adjusted to alter how the proteins function, giving crops greater protection against pathogens and creating hardier plants.

Professor John Runions of Oxford Brookes' Department of Biological and Medical Sciences said: "We can't create new farmland so our only recourse is to make plants that produce food for us and our animals more efficiently."



This research was published in the Proceedings of the National Academy of Sciences doi: 10.1073/pnas.1819077116

The Octopus imaging cluster uses multicolour light sources to combine multiple beams, colours and timings. It is used to investigate major challenges in the life sciences.