The Imaging Core facility in the UCD Conway Institute of Biomolecular & Biomedical Research, Dublin

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The Imaging Core facility in the UCD Conway Institute in Dublin boasts an array of instruments and techniques, as well as expertise, and has been facilitating research in its home of University College Dublin (UCD) and amongst the wider research community in Ireland for many years.

The UCD Conway Institute of Biomolecular & Biomedical Research explores the fundamental mechanisms of health and disease to discover and develop preventative strategies and novel diagnostic and therapeutic solutions. Situated on the 300-acre Belfield Campus of University College Dublin, the largest university in Ireland, the Conway Institute brings together over 450 researchers from all over the university and its associated teaching hospitals.

The institute is located in a purpose-built facility that opened in September 2003 and was named after the

The atrium garden and cafe in UCD Conway Institute are a light-filled meeting point for researchers, staff and graduate students in the 11,500 sqm building

first professor of biochemistry in UCD, Professor Edward J. Conway, who took up this position in 1932 and was the first Irish-based scientist to be awarded Fellowship of the Royal Society.

Today, the institute is proud of its core technologies programme which offers access to a suite of genomics, proteomics, research pathology, cytometry and imaging technologies. This allows bespoke project design and analysis across several experimental platforms. The Imaging Core facility delivers comprehensive solutions to challenging research questions for academic partners and industrial partners.

Imaging capabilities

The institute's Imaging Core facility focuses on providing cutting edge imaging and know-how to researchers in UCD, other Irish research institutes and industry. The integrated suite of instruments spans all aspects of light and electron microscopy as well as sample preparation and image processing and analysis. They work with a full gamut of biomedical researchers on diverse applications, from retinal disease to clinical viral diagnostics, from nanoparticle-cell interaction to toxicity and food quality control.

The core team's technical expertise assists in the widest possible range of applications for microscopy, in advancing the development of new applications and new imaging technologies. The facility opened in 2008 and consolidated imaging into one centre. It does not conduct its own research programme, but instead is on a mission to serve. "*The core facility is made to serve the scientific community: principal investigators, postdocs and students. It is so they do not waste their time studying the technology, but come here to resolve their scientific questions. We have the know-how*," explains Director Dr Dimitri Scholz, whose role blends his passion for science, microscopes and working with people.

Dimitri studied biology at Moscow National University in Russia before completing a PhD in cell biology at the National Medical Academy in Moscow. He then took up a postdoc position at the Max-Planck Institute for Experimental Cardiology, before moving to the role of senior scientist at the Kerchhoff Clinic in Germany. He then became

Our Tecnai20 transmission electron microscope

assistant professor at the Medical University of South Carolina, US, where he studied intracellular trafficking of macromolecules in heart cell muscle. "*Microscopy was my major and favourite application and I was always helping colleagues plan and work experiments in microscopy*," he recalls. "*I decided to make my hobby my profession by joining UCD to establish the Imaging Core in 2008*." His team in UCD has imaged an impressive range of materials, from cancer cells to cheese and sausage, and from nematode worms to prehistoric teeth and fruit flies.

The Imaging Core facility has two transmission electron microscopes (TEM) and one scanning electron microscope (SEM). It also houses two laser scanning confocal microscopes – one of which is a spinning disc microscope – and five epi-fluorescent microscopes that are equipped for live cell imaging. One high-end transmission light microscope, a couple of self-made systems and the image analysis suite complete the line-up. The TEM is especially popular for oncology, cardiology, genetics and nanoparticle research areas and is used for gold standard identification of virus samples from the National Virus Reference Laboratory, which is situated on the UCD campus. "*SEM is used a lot by the biological department, but also in palaeontology and geology*," says Dimitri. "*One of the weirdest samples I looked at using an electron microscope was bacterial remnants on the teeth of prehistoric humans.*"

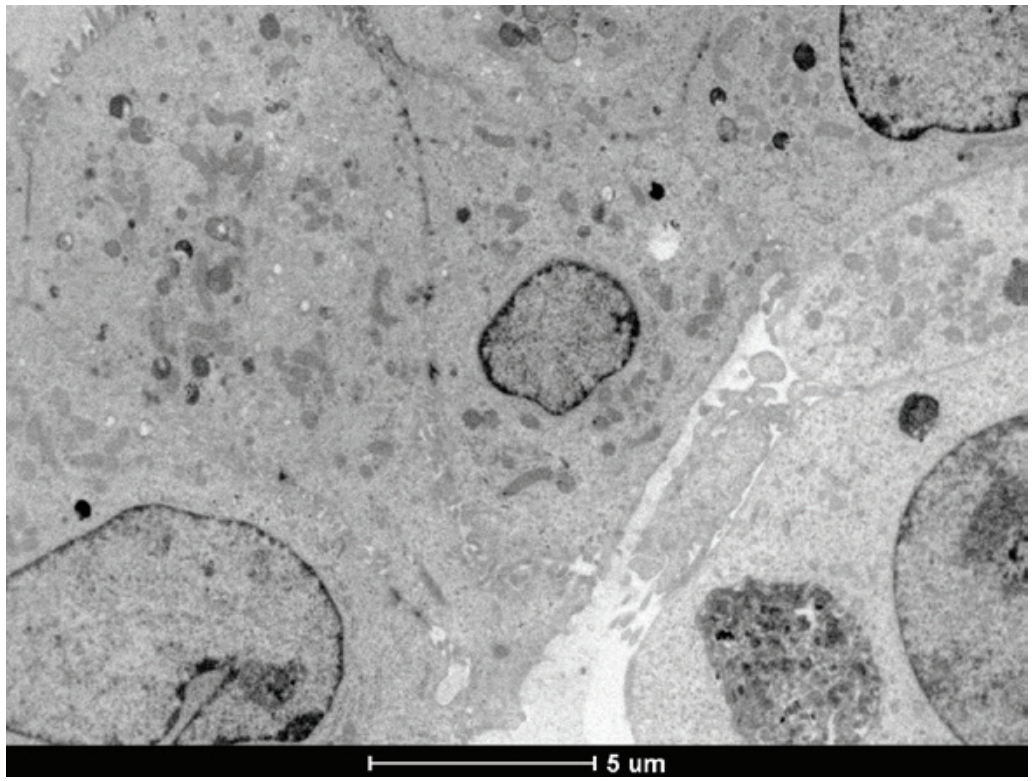
The facility also houses a high pressure freezer for chemical-free tissue fixation in electron microscopy, which is ideal for many of the biological samples

that are distorted with chemical fixatives. *Drosophila larvae*, for example, has a hard cuticle that impedes chemical fixation and so the best approach is to freeze it rapidly using liquid nitrogen under high pressure. This strategy is also popular with those looking to image the nematode *C. elegans* and plant tissues.

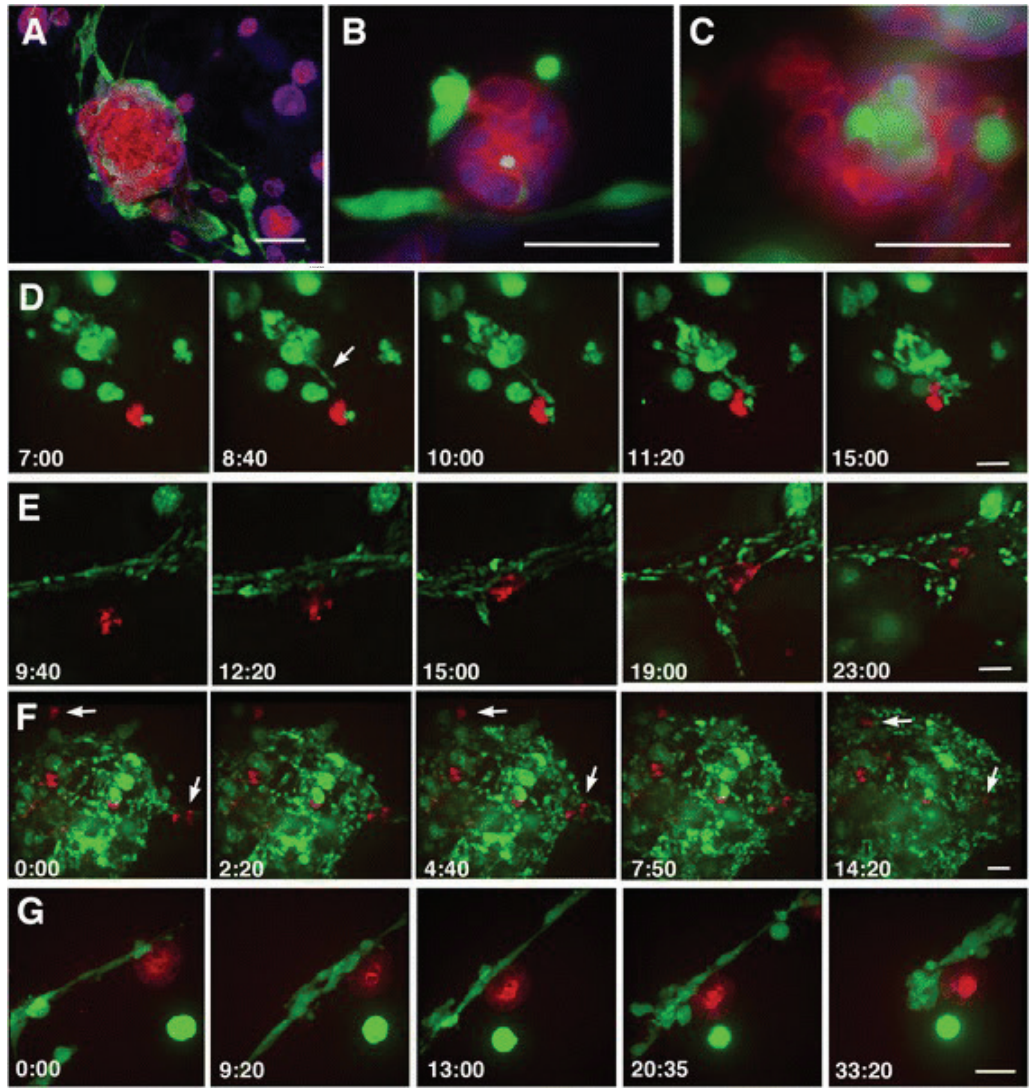
The facility views its live cell imaging as one of two points of excellence. Live imaging demands that physiological conditions are maintained as cells or a living organism are imaged. In one recent paper, the facility pioneered a new technique for watching cancer cell interactions over time (Ivers *et al.* (2014)). The tumour microenvironment has a strong influence on cancer cells and conventional 2-D culture systems obscure true cancer cell dynamics. This is of interest because the effect of normal cells on cancer cells can reveal mechanisms of self-defence against cancer. To reveal the interaction of cancer and normal epithelial cells, breast cancer labelled with GFP and epithelial cells labelled with cherry fluorescent protein were co-cultured. Time-lapse epi-fluorescent microscopy revealed previously unseen cell behaviour.

The imaging showed that normal epithelial cells were engulfed and destroyed by the cancer cells when outnumbered, but not when there was a relatively large population of normal epithelial cells. "*This is an important paper and probably one of my favourites*," says Dimitri. Another fruitful use of epi-fluorescent microscopy involved a collaboration with Maynooth University, which tracked the entry and accumulation of an antibiotic in bacteria in a study of antibiotic resistance. Recently, the team assisted a cancer surgeon from the Royal College of Surgeons in Dublin to analyse cancer biopsies using epi-fluorescent microscopy.

A second point of excellence is the unit's biomedical electron microscopy, which is much utilised in oncology, dermatology, nano-biology and the biology of model organisms such as nematodes, insects and zebrafish.



Non-cancer epithelial cells (upper left) and cancer cells (bottom right) under transmission electron microscope. (Ivers et al. (2014) with permission of Cancer Cell International



Dynamic interaction of the non-cancer cells (red) with cancer cells (green) in a 3D culture. (Ivers et al. (2014) with permission of Cancer Cell International

However, the Imaging Core facility is also combining its expertise to develop a new point of excellence: soft x-ray tomography (SXT). The term soft arises because each photon is less energetic than in classical x-rays, but it is a hot new microscopy technique for labs focused on disease research and drug discovery. It is being pioneered by a spin-out company from UCD, SiriusXT, which developed the first commercial lab-scale SXT microscope of its kind in the world. Resolution lies between light and electron microscopy, but it generates 3-D images of, for example, cells without the sectioning required in TEM. The team at the Imaging Core facility has assisted in its development.

Expertise

The team in the Conway Institute cooperates with Irish research centres beyond UCD, including CRANN in Trinity College Dublin, a material science centre with separate know-how and which offers access to high resolution microscopy. This is useful for UCD researchers too and the Imaging Core facility often cooperates with CRANN scientists. "*We've a synergistic relationship*," Dimitri explains.

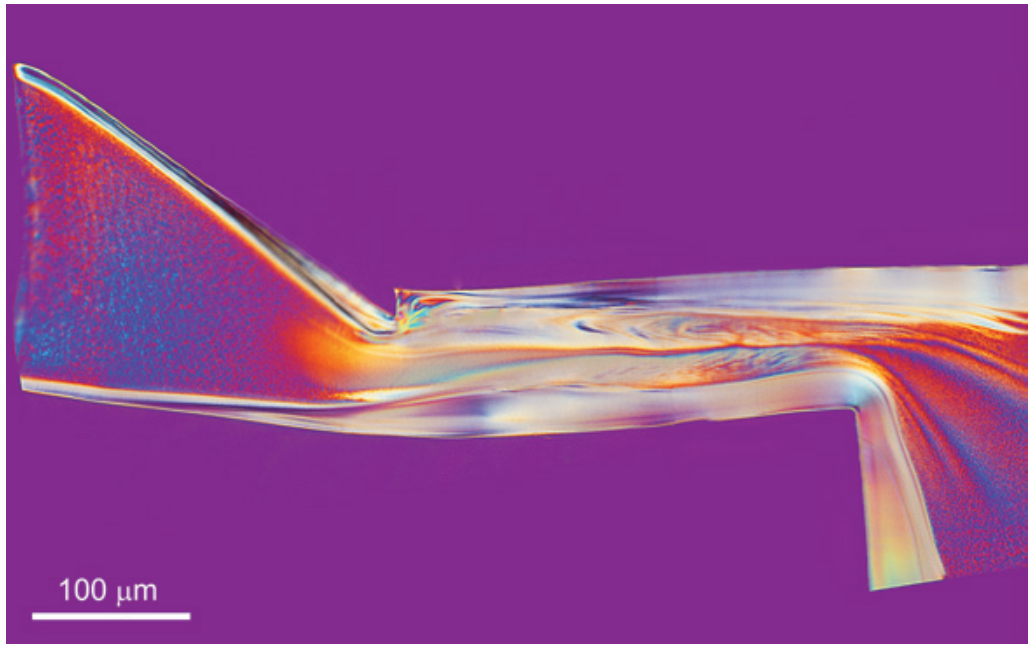
"*They often send us samples for sectioning, because this is something we have expertise in*." The UCD team recently sectioned materials such as polymers and steel for CRANN, using its ultramicrotome to cut ultra-thin sections with a diamond knife. These are then imaged under their high-resolution microscope.

Advice and guidance are major perks that the experienced staff at the Imaging Core facility can offer to researchers and others who come to the imaging facility. An R&D project supported by the state agency Enterprise Ireland, for example, sought to develop a field microscope for farmers to detect parasites in farm animal waste. "*The challenge was not detecting the parasite. That was straight forward. The challenge was to make this fast and easy and quantitative,*" Dimitri explains. The product is being taken towards commercialisation by Irish company CF Pharma.

The image team provides expert advice on translating a scientific problem in imaging into practical research plans and supporting users until the point of publication. By adopting a problem-solving approach, clients are guided towards a microscopy solution that works for their research – simply, accurately and quickly. The assistance offered gives the imaging team the greatest job satisfaction. A truly diverse range of materials are imaged; for example, commercial experiments and imaging were carried out on cheese and sausage meat for Teagasc, the state agency in charge of agriculture and food research and development.

A boon for biomedical researchers is the experience and training that the imaging team of Dimitri Scholz and Tiina O'Neill and Niamh Stephens offer. "*My decision to add electron microscopy was probably one of my best scientific decisions,*" says Associate Professor Oliver Blaque, a molecular biologist who uses the *C. elegans* nematode to study cilia and cilia-related diseases. "*Tiina has sectioned and imaged worms for us for the last ten years, but the core facility has also been excellent in helping to train staff*." Four research assistants over the last decade have been trained in electron microscopy on *C. elegans* and Prof. Blaque's lab is now well known in the field for its

'When Art meets Science'. Fly head image courtesy of Karl Gaff



Melting plastic in polarised light

capabilities here. He has also used the fluorescence recovery after photobleaching (FRAP) technique to understand the dynamics of molecules in cilia.

In addition the core team harbours expertise in immuno-labelling for light- and electron microscopy; multi-dimensional advanced data analysis; and contemporary approaches such as photo-conversion, total internal reflectance fluorescence (TIRF), fluorescence resonance energy transfer (FRET), fluorescent lifetime imaging microscopy (FLIM), single plane illumination microscopy (SPIM) and super-resolution microscopy.

The core team provide fee-for-play imaging services, but keeps prices competitive and only charges the equivalent of maintenance costs, rather than capital costs.

Staff Profile: Dimitri Scholz

Role: Director of Biological Imaging

Qualifications: PhD, Dr Sci in cell biology

Joined the team: 2008

Microscopy speciality: Light- and electron microscopy of all kinds

Favourite microscope and why: Live cell imaging, because we can study processes as they happen in nature. Also, correlative microscopy. When light microscopy is followed by electron microscopy to study same structure with high resolution.

Favourite publication and why: Dynamic and influential interaction of cancer cells with normal epithelial cells in 3D culture (Ivers *et al.* (2014)). This study used all modalities of microscopy available in our facility: multi-dimensional live imaging ("normal" 3D imaging over time (4th dimension), in two colours (5th dimension), from the multiple fields of view (6th dimension); electron microscopy; and advanced image analysis.

Proudest moment at work: When I advise a user to choose a particular method that he or she did not suspect exists and this is successful. This has happened more and more as I gained experience.

Aspiration for future direction of facility: For the imaging care facility to became *de facto* the all-UCD facility because we serve users from all UCD schools and beyond. I hope we can transform the facility into an all-Ireland platform. We will continue developing the new modality of microscopy: the soft X-ray microscopy. We hope to implement more of the super-resolution fluorescent microscopy. We are housing elmi2018 and hope to be inspired by the great ideas of colleagues from all over the Europe.

Best advice you received as a scientist: Build on your strengths!

Favourite pastimes: Hiking, biking, travelling.

Staff profile: Niamh Stephens

Role: Technical Officer

Qualifications: Degree and PhD in Microbiology

Joined the team: 2017

Microscopy speciality: A mix of EM and fluorescent microscopy.

Favourite microscope and why: For EM it's the FEI Tecnai 120 because it images biological samples well. For LM it's the Zeiss AxioImager M1 as it is easy to use and provides good quality fluorescent images for a variety of samples.

Favourite publication and why: I don't have a favourite. The best paper is the one most relevant and helpful to what I am working on at the time. A recent interesting one would be: Correlative super-resolution fluorescence and electron microscopy using conventional fluorescent proteins *in vacuo* (Peddie *et al.* (2017)).

Proudest moment at work: As a microbiologist, I was quite proud of the first time I imaged bacteria under the electron microscope.

Aspiration for future direction of facility: Investment in newer and updated technologies to keep up with the ever-changing technological advances.

Best advice you received as a scientist: To bounce ideas off other scientists and to always remember what you're doing and why you're doing it.

Favourite pastimes: Going to concerts and cycling.

Staff profile: Tiina O'Neill

Role: Senior Technical Officer

Qualifications: MSc

Joined the team: Joined the Conway Institute's Electron Microscopy laboratory in 2006, which became part of the Imaging Core facility in 2008.

Microscopy speciality: TEM

Favourite microscope and why: FEI Tecnai G2 20, I use it a lot to image nanoparticles and it's good for tomography too. It's one of the most reliable microscopes we have.

Favourite publication and why: There's too many, but a recent one that I found really interesting was: 3D correlative light and electron microscopy of cultured cells using serial blockface scanning electron microscopy (Russell *et al.* (2017)).

Proudest moment at work: The first time I successfully managed to immuno-gold label cryosections (Tokuyasu method). With this labelling technique, we managed to locate the FPR2/ ALX receptors in untreated and treated cells.

Aspiration for future direction of facility: I'd hope we have the opportunity to invest in some new equipment like serial block face SEM that would allow us to expand our range of services.

Best advice you received as a scientist: Negative result is a result too.

Favourite pastimes: Enjoying good food and wine with my family and friends.

User testimonial from Professor David Brayden, UCD

Oral drug formulations contain ingredients other than the active drugs. Recipes in preclinical research for delivering poorly absorbed molecules can include intestinal absorption enhancers. These are agents whose mission is to temporarily and reversibly increase the permeability of the small intestine and allow an active drug to be absorbed into the body that would otherwise have to be injected. This enhanced permeability can be especially important for peptides, proteins, RNA and other active compounds that do not cross the gut wall easily.

At the UCD Conway Institute, Professor David Brayden is interested in several enhancers in clinical trials that aim to improve the delivery of oral peptides, including insulin and Glucagon-like-1 analogues for treatment of Type II diabetes. One such agent is sodium caprate, a medium chain fatty acid found in milk. However, its surfactant-like action causes mild but reversible damage to epithelial cells in the intestine. In his lab, David is working to understand the time and concentration dependent actions of sodium caprate on intestinal epithelial cells, and the recovery mechanisms used to repair the gut barrier after the job is done.

'*The Core staff provide the highest level of expertise in microscopic techniques. They not only helped with study design by ensuring that the chosen microscopy was the correct option but they also outlined several other novel and established techniques that could help in answering the proposed scientific questions and progress the research,*" says David.

"*We used high content analysis microscopic techniques to examine behaviour of cultured live human intestinal cells in response to caprate using different concentrations over time. Fluorescent agents light up the cells as different parameters change – the size of the nucleus, cell calcium levels, the epithelial membrane's electric potential. These are all clues to what happens to cells that are stressed, so we get information about sub-lethal events that give us mechanistic information.*"

Case Study

Of worms and men: Imaging Cilia

Up to 20 years ago, scientists thought most cilia on mammalian cells were akin to the human appendix, a mere remnant of evolution. Motile cilia have obvious functions in cell and fluid movement; for example, in the respiratory tract they beat in a rhythmic wave to clear dirt and mucus. Apart from sensory perception (smell, taste, light), almost nothing was known about the functions of most non-motile cilia, found on most cell types.

Today, these hair-like cell appendages are recognised as crucial cogs in cell-to-cell communication, especially during embryonic tissue development. Indeed, defects in primary cilia are responsible for a wide range of diseases – or 'ciliopathies' – characterised by cystic kidneys, blindness, bone abnormalities, nervous system detects, amongst others.

Associate Professor Oliver Blacque at the UCD Conway Institute is investigating the various molecular mechanisms that establish and maintain the ciliary membrane, including the barriers at the base of cilium that compartmentalises the organelle from the cell proper. He uses the relatively simple *C. elegans* nematode as a model (60 of its 959 cells have cilia) because a lot of blueprint cilium biology is shared between worms and humans.

In a recent paper, his lab's work describes new genes that control the levels of a ciliopathy protein (polycystin 2) at the ciliary membrane (Scheidel *et al.* (2018)). His lab relied on the core imaging facility's transmission electron microscopy technology, coupled with sample fixation via high pressure freezing, to observe what happens to the ciliary membrane and polycystin-2 vesicles when these genes are knocked out. "For our conclusions, high-pressure freezing fixation rather than standard chemical fixation was essential to preserve ciliary vesicle and membrane integrity," Oliver explains.

In another study, his group investigated various ciliopathy protein molecules that act as 'gatekeepers' to enable membrane diffusion barriers at the ciliary base (Lambacher *et al.* (2016)). Using spinning disk confocal microscopy, combined with fluorescence recovery after photobleaching (FRAP), they found that the ciliopathy proteins represent fixed, non-moving components of the barrier. Furthermore, super-resolution (STED) microscopy of live worms revealed how ciliopathy proteins arrange themselves in a series of concentric rings. "They look like slinky molecules and somehow this arrangement provides barrier properties," Oliver explains.

The core unit's instruments and expertise play an essential role in the research. "The facility allows us to push the boundaries of imaging in living worms," he explains. "It also provides wonderful training for new lab members to become experts in technically challenging techniques like electron microscopy."

For more information about the Imaging Core facility go to www.ucd.ie/conway/research/coretechnologies/imagingcore

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