



# The Electron Microscopy Science Technology Platform at the Francis Crick Institute

*Lucy Collinson*

**The Francis Crick Institute is a biomedical discovery institute dedicated to understanding the fundamental biology underlying health and disease. Its work is helping to understand why disease develops and to translate discoveries into new ways to prevent, diagnose and treat illnesses such as cancer, heart disease, stroke, infections, and neurodegenerative diseases.**

An independent organisation, its founding partners are the Medical Research Council (MRC), Cancer Research UK, Wellcome, University College London, Imperial College London and King's College London. The Crick was formed in 2015, with many of the Crick's scientists joining from two 'parent' institutes, the MRC's National Institute for Medical Research and Cancer Research UK's London Research Institute, and in 2016 it moved into a brand new state-of-the-art building in central London which brings together 1500 scientists and support staff working collaboratively across disciplines, making it the biggest biomedical research facility under a single roof in Europe.



## Building the Crick

- The total floor space is the size of 17.5 football fields
- There are over 1,500 rooms (twice as many as Buckingham Palace)
- There are over 100 km of mains power cables installed (equivalent to the distance from London to Southampton)
- There are over 800 solar panels on the roof
- The Crick was at one point the biggest single building construction project in the UK

## Facility design

There are 14 Science Technology Platforms (STPs) at the Crick, which concentrate expertise and advanced equipment into teams that are accessible to all Crick researchers. Electron microscopes are housed in two of the STPs – Electron Microscopy (EM) and Structural Biology. The process of designing the Crick EM facilities began back in 2008, only a few months after the land had been acquired and the project officially announced. The challenge was to build a facility capable of holding high-end imaging equipment, in a central London site surrounded by tube lines, roads, and the local, regional and international trains at St Pancras station. The site, an old railway storage yard to the west of St Pancras station and north of the British Library, was surveyed to identify the optimal position for the

facility. A sweet-spot for physical vibration, acoustic vibration and electro-magnetic fields was identified at the southwest corner of the site in the lower basement, almost thirty metres underground.

A team of experts in electron microscopy, NMR, advanced light microscopy, vibration control and electromagnetic field control worked together with the construction team, engineers and architects over the next 8 years to deliver the project. This interaction was key to the success of the project, and included frequent on-site visits to monitor the complex build.

The main construction phase finished in 2013 and a two-year period of fitting-out began. This phase of the build included installation of measures to attenuate electromagnetic fields and vibrations.

Each microscope room is a six-sided shielded box, with walls that contain complex metallic layers to attenuate DC fields, and an active cancellation system to attenuate AC fields. Under each microscope is a concrete platform, cast in place, and supported by air springs that remove environmental vibration to <1 Hz. Each room has tight control of air quality, airflow, temperature stability and humidity, all of which are monitored through a complex building management system with 27,000 individual monitoring points. In case of power outages, the entire imaging suite is supported by a dedicated uninterruptible power supply, which was recently tested and proved invaluable during a power failure at the local electricity sub-station.

The time, effort, teamwork and expertise that went into the project delivered an impressive suite of rooms tailored to running sensitive high-resolution imaging experiments on a wide array of high-end instrumentation.

## Instrumentation

The Structural Biology STP holds two FEI Titan Krios transmission EMs (TEMs), for studying macromolecules and frozen hydrated cells. The EM STP holds a range of microscopes that enable us to study samples across scales, from single molecules to whole model organisms. These include two benchtop scanning EMs (SEMs), two TEMs, three SEMs, a Focused Ion Beam SEM and a microCT system. Each of these systems has additional specialised functions:

- The Phenom-World DelPhi benchtop SEM has an integrated fluorescence microscope for correlative imaging
- The FEI Twin 120 kV TEM has a cryo stage for screening vitrified macromolecular samples prior to imaging on 200 kV and 300 kV TEMs
- The FEI BioTwin 120 kV TEM has an integrated iCorr fluorescence microscope for correlative microscopy
- The FEI Quanta SEM has a Delmic SECOM integrated super-resolution fluorescence microscope for high accuracy correlative microscopy
- The Zeiss Sigma and Merlin SEMs have Gatan 3View stages for volume EM
- The Zeiss Crossbeam 540 FIB SEM is used for volume EM and also has a Leica cryo-stage for cryo-electron tomography sample preparation
- The Zeiss Versa 510 microCT has Atlas 5 software for 3D correlative imaging

## Science and Technology Development

The EM STP collaborates with Crick researchers on ~80 projects at any one time. Some examples of our recent work are:

- Studies of the role of RAD51 paralogs in repair of DNA damage, processes that are involved breast and ovarian cancer, with Simon Boulton's lab using TEM (1, 2)
- Studies of *Mycobacterium tuberculosis* infecting human lymphatic endothelial cells and



macrophages with Max Gutierrez's lab, using 3D correlative light and electron microscopy (CLEM) (3-5)

- Studies of B cell responses in the human immune system, with Facundo Batista's lab, using SEM and volume EM (6-8)

The EM STP also develops new workflows. Some examples of our recent work are:

- Development of sample preparation for integrated light and electron microscopy (9-11)
- Development of a workflow for 3D CLEM using SBF SEM, used to study tuberculosis, HIV and cancer (5, 12)
- Development of cryo-correlative imaging of vitrified whole cells using synchrotron radiation (13-16)

## Staff

The EM STP team consists of nine postdoctoral scientists: seven are electron microscopists and two have a background in physics, optics and image analysis. They are...

### Name: Lucy Collinson

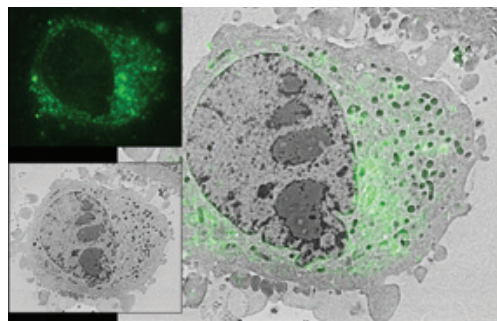
**Role:** Head of EM STP and Microscopy Prototyping



**Qualifications:** Degree and PhD in Microbiology, post doc in Cell Biology

**Joined the team:** 2006

**Microscopy speciality:** 3D correlative microscopy



Lucy's favourite image: Green fluorescent protein labelled lipids, localised to membranes in HeLa cells, imaged by integrated light and electron microscopy.

**Favourite microscope:** SEM, TEM, SBF SEM, FIB SEM, microCT – basically whichever one I'm standing in front of at the time....

**Favourite publication and why:** The last one: 'UltraLM and miniLM: Locator tools for smart tracking of fluorescent cells in correlative light and electron microscopy' {Brama, 2016 #832}, because it involves microscope hacking, and cuts across biology, microscopy, physics, optics, coding and engineering.

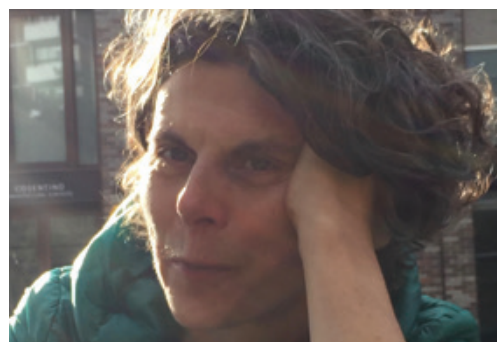
**If you weren't a scientist, what would you be and why:** If I could get paid for something I love but am not particularly good at, then a surfer!

**What's the best advice you've been given:** There's no such thing as a stupid question

**What three items would you take to a desert island:** Surfboard, insect repellent, luxury yacht

### Name: Raffaella Carzaniga (known as Raffa)

**Role:** Deputy Head of the EM STP



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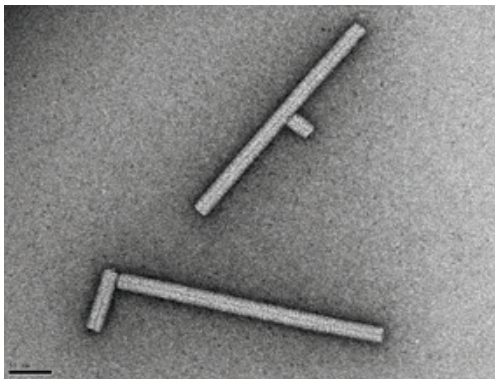
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Raffa's favourite image: Negative staining of tobacco mosaic virus, imaged by transmission EM.

**Qualification:** PhD in Cell Biology

**Joined the team:** 2012

**Microscopy speciality:** Jack of all trades

**Favourite microscope:** My first magnifying glass

**Favourite publication and why:** I do not have one – I like all of them

**If you weren't a scientist, what would you be and why:** A Buddhist nun

**What's the best advice you've been given:** Better to be a small fish in a big pond than a big fish in a small pond

**What three items would you take to a desert island:** A solar panel, a cable and my iPhone

**Name: Christopher Peddie**

**Role:** Principle Laboratory Research Scientist



**Qualifications:** MBiochem (Hons), PhD in neuroscience, post-docs in neuroscience and in peripheral nerve injury models

**Joined the team:** 2011

**Microscopy speciality:** CLEM, ILSEM, SBF SEM, FIB SEM

**Favourite microscope:** Zeiss Crossbeam 540 (new, shiny and extremely capable), and the venerable Jeol 1010

**Favourite publication and why:** 'Acute manipulation of diacylglycerol reveals roles in nuclear envelope assembly & endoplasmic reticulum morphology' (17) – one of my first projects after joining the team, a steep learning curve, and an interesting group of people to work with



Christopher's favourite image: FIBing the moon' – digging trenches in resin for 3D imaging of cells using Focused Ion Beam SEM.

**If you weren't a scientist, what would you be and why:** Probably I'd focus on something more hands on like mechanical engineering since I seem to have a particular aptitude for that type of work and I find it interesting, but in another time and place (and with a bit more brilliance up top) I'd have really liked to be a veterinary surgeon. Or a helicopter pilot.

**What's the best advice you've been given:** Controls are a prerequisite, question the dogma, and if you *really* want to find all the typos, read it backwards

**What three items would you take to a desert island:** Telescope, comfortable armchair, good whisky

**Name: Anne Weston**

**Role:** Senior Laboratory Research Scientist



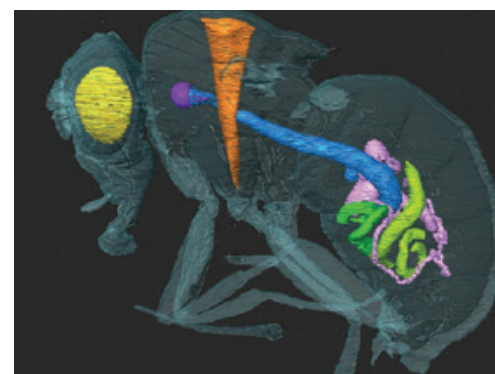
**Qualifications:** Degree in Zoology, PhD in Bioinformatics

**Joined the team:** 2003

**Microscopy speciality:** Multi-disciplinary but probably more likely to be associated with SEM and MicroCT

**Favourite microscope:** SEM

**Favourite publication and why:** 'Imaging transient blood vessel fusion events in zebrafish by correlative volume electron microscopy' (18) – even though I wasn't involved in this publication I like it because it was really the start of the whole volume EM interest in our lab and that is a technique which I particularly enjoy



Anne's favourite image: 3D reconstruction of the fruit fly, image by microCT.

**If you weren't a scientist, what would you be and why:** A vet because it combines science with my love of animals

**What's the best advice you've been given:** The advice given to me during my PhD viva where one of the examiners suggested it would be a good idea for me to get back into a career in electron microscopy (!!!)

**What three items would you take to a desert island:** If we are talking purely material things then sun cream so that I don't burn to a crisp, my kindle (fully charged of course) and a camera (with plenty of spare batteries) – this one is a bit of a cheat as it would be dual purpose in that I could use it to take photos but it would also have all my favourite photos of my family still stored in its memory!

**Name: Matt Russell**

**Role:** Senior Laboratory Research Scientist

**Qualifications:** MA/MSci in Biochemistry, PhD in Cell Biology

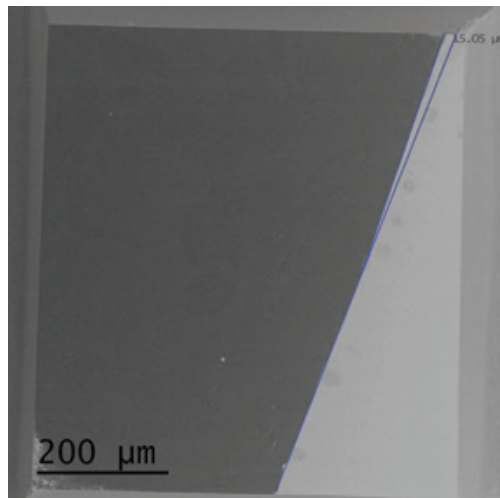


**Joined the team:** 2013

**Microscopy speciality:** 3D correlative light and electron microscopy

**Favourite microscope:** Philips EM400

**Favourite publication and why:** 'Mycobacterium tuberculosis replicates within necrotic human macrophages' (3). Because it used



Matt's favourite image: Revealing resin-embedded cells under a platinum coat for correlative 3D EM using Serial Block Face SEM.

techniques for correlating LM, serial block face SEM, and TEM that we'd predicted could be useful in an earlier methods paper

**If you weren't a scientist, what would you be and why:** Journalist, because I've always wanted to try to understand the truth about things

**What's the best advice you've been given:** Do something that really interests you

**What three items would you take to a desert island:** My wife, my cast iron skillet, and a stereo microscope

## Name: Marie-Charlotte Domart



**Role:** Senior Laboratory Research Scientist

**Qualifications:** Degree in Cell Biology and Physiology, Masters in Oncology, PhD in Biochemistry and Molecular Biology, Postdoc in Cell Biology

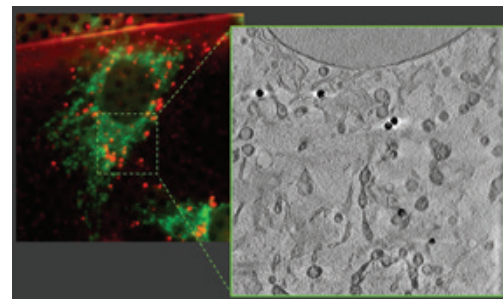
**Joined the team:** September 2013

**Microscopy speciality:** Correlative light and soft X-ray or electron microscopy

**Favourite microscope:** Light microscope: Zeiss LSM 710 inverted confocal microscope. Electron microscope: FEI Tecnai G2 Spirit BioTWIN with Gatan Orius CCD camera

**Favourite publication and why:** Without a doubt my postdoc paper, 'Acute manipulation of diacylglycerol reveals roles in nuclear envelope assembly & endoplasmic reticulum morphology' (17) as this work not only marks the start of my collaboration with Lucy and Chris, but also introduced me to CLEM, which led me to join the team!!

**If you weren't a scientist, what would you be and why:** Pastry chef! I love cakes and the precision needed to make visually perfect cakes (picturing a French patisserie window...)



Marie-Charlotte's favourite image: 3D correlative imaging of vitrified cells using cryo-soft X-ray tomography (with Liz Duke and colleagues (Diamond Light Source) and Eva Perriero and colleagues (ALBA synchrotron)).

**What's the best advice you've been given:** Eat cake and carry on!

**What three items would you take to a desert island:** a machete, a pot and a surf board!

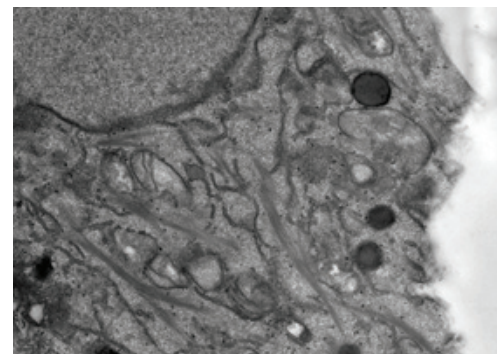


## Name: Julia Konig

**Role:** Senior Laboratory Research Scientist

**Qualifications:** PhD in Cell Biology

**Joined the team:** 2016



Julia's favourite image: Microwave-processed cell, embedded for transmission EM in only two hours.

**Microscopy speciality:** High pressure freezing and electron tomography

**Favourite microscope:** FEI Tecnai G2 Spirit BioTWIN

**Favourite publication and why:** 'Membrane remodeling during embryonic abscission in *Caenorhabditis elegans*' (19). Because nothing is better than combining live-cell imaging with high resolution ultrastructure

**If you weren't a scientist, what would you be and why:** A fashion designer, because I like to make my own clothes

**What's the best advice you've been given:** Relax!

**What three items would you take to a desert island:** Crisps, Crisps, Crisps

## Name: Martin Jones

**Role:** Deputy Head of Microscopy Prototyping

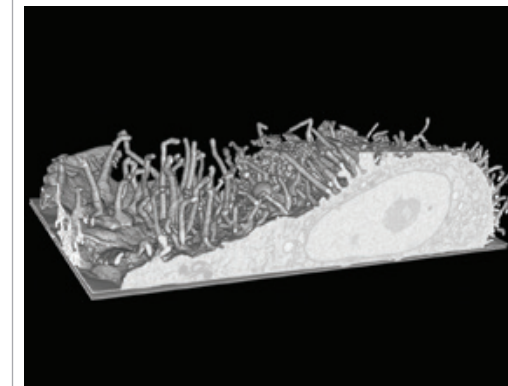


**Qualifications:** Undergraduate degree in Physics with Electronics and Optoelectronics, MSc in Evolutionary and Adaptive Systems, DPhil in Experimental Atomic Physics (Quantum Information)

**Joined the team:** 2014, although I've been interacting with the team since I saw Bram Koster give a talk on CLEM in 2012 and started thinking about building in situ CLEM systems.

**Microscopy speciality:** Image analysis and microscope hardware development

**Favourite microscope:** I don't really use them myself. The FIB SEM is definitely impressive. Also the smartphone microscope platforms that we use for outreach work (and are used by various Cancer Research UK outreach teams around the country now)



Martin's favourite image: Automated reconstruction of the surface of a HeLa cell from Focused Ion Beam SEM data.

**Favourite publication and why:** 'UltraLM and miniLM: Locator tools for smart tracking of fluorescent cells in correlative light and electron microscopy' (11), which is the realisation of an idea I had and randomly pitched to Lucy, the development of the project is why I ended up in the EM STP

**If you weren't a scientist, what would you be and why:** Teacher. Before my degree I was more or less planning to go into teaching, I think it's one of the most important but unfortunately underappreciated jobs. During my DPhil and physics postdocs I taught various courses and labs and really enjoyed the challenge of having to explain things in

different ways for different students, it really makes sure you understand it yourself!

**What's the best advice you've been given:** My old history teacher at school was worried that I was too shy and couldn't hold conversations very well, so he told me: whenever someone asks you a question, think of what their follow up question might be and try to answer that too, before they ask. I think this approach works very well in science too, following a line of reasoning along to its logical conclusion is important

**What three items would you take to a desert island:** MP3 player with all my music, Crocodile Dundee knife and some sort of tent/shelter

## Name: Lizzy Brama

**Role:** Senior Laboratory Research Scientist

**Qualifications:** Degree in Physics, PhD in experimental quantum optics

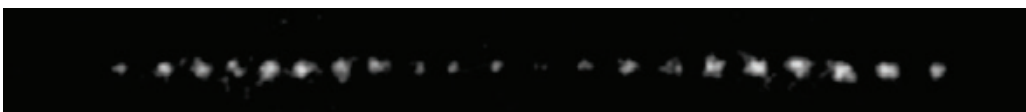


**Joined the team:** 2014

**Microscopy speciality:** Building them

**Favourite microscope:** Till Photonics iMic, more people ought to have bought them

**Favourite publication and why:** 'UltraLM



Lizzy's favourite image: Cavity-induced power broadening ions.

and miniLM: Locator tools for smart tracking of fluorescent cells in correlative light and electron microscopy' (11), because it was my first paper where I felt like I knew what I was talking about

**If you weren't a scientist, what would you be and why:** A carpenter, or a baker, or an architect, or a pro triathlete. Because I like making things, buildings, and riding my bike

**What's the best advice you've been given:** If it's not ok, it's not the end

**What three items would you take to a desert island:** A boat to get away, food and water for the journey home.

## Future Directions

Now that EM image acquisition is becoming more automated, one of the biggest challenges in our work is handling and analysing the huge amounts of data we produce. Volume EM techniques, including SBF SEM and FIB SEM, can turn out thousands of images in just a few days. Two approaches we are pursuing to deal with the data deluge are:

### 1) Smart data acquisition

Correlative microscopy may be seen as a form of smart data acquisition, in that cells of interest are identified using one imaging modality (e.g. fluorescence microscopy) and then high resolution images are collected using a second imaging modality (e.g. electron microscopy). Introducing miniaturised fluorescence microscopes into volume EMs will allow us to track fluorescent cells on-the-fly during automated data acquisition runs, so that we only collect images from the cells and tissues of interest. Miniaturisation is required due to the extremely tight space within the SEM chamber in these systems. Our prototype fluorescence microscope, the miniLM, is only 2.8 mm in diameter (11).

## 2) Automated image analysis

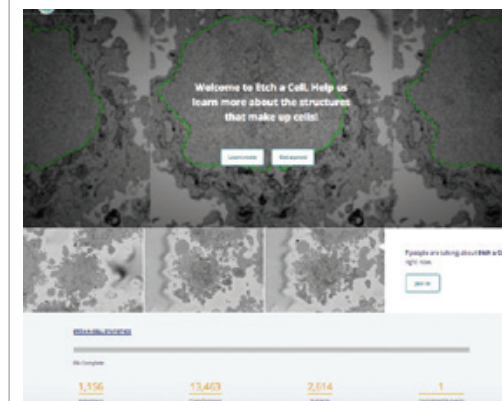
Development of automated image analysis algorithms for EM is challenging. Many cell membranes have similar grey values in EM images, and so simple histogram thresholding often gives a poor result when trying to select a subset of cell organelles. Manual annotation (segmentation) of organelles is usually required to create accurate 3D models, but the process is extremely time-consuming. Machine learning techniques are starting to deliver semi-automated image analysis algorithms, particularly in the field of connectomics. However, machine learning requires large amounts of 'ground truth' training data, which is lacking in EM because of the time taken for expert manual segmentation.



Fiona Hanson/Francis Crick Institute

To deal with this problem, we are harnessing the brute force power of Citizen Science to produce ground truth segmentations. Our project 'Etch a Cell' ([www.zooniverse.org/projects/h-spier/etch-a-cell](http://www.zooniverse.org/projects/h-spier/etch-a-cell)) is hosted by the Zooniverse platform, which has more than a million volunteer citizen scientists working on projects from the arts to ecology, and from space to medicine. Etch a Cell asks citizen scientists to draw around cell organelles, starting with the nuclear envelope. Within two

weeks of launch, Etch a Cell had more than 1000 volunteers and more than 13,000 nuclear envelope segmentations. The first average models of nuclear envelopes are now being made and analysed, and



will be used to train computers via deep learning algorithms. Once you have finished reading *infocus*, we hope you will grab a cup of tea, and stop by the Etch a Cell website to add a few segmentations of your own...

## Further Information

If you would like to visit us and hear more about the impact of EM on biomedical research, you can register for our Crick EM Opening Symposium. The symposium will take place on July 12<sup>th</sup> and 13<sup>th</sup> 2017, and will feature talks from international experts on EM imaging across scales, from molecules to whole organisms. The symposium will be accompanied by a day of workshops on July 14<sup>th</sup>. Registration can be found at [www.rms.org.uk/crick-symposium-2017](http://www.rms.org.uk/crick-symposium-2017).

**Website:** [www.crick.ac.uk/research/science-technology-platforms/electron-microscopy](http://www.crick.ac.uk/research/science-technology-platforms/electron-microscopy)

**Twitter:** @EM\_STP and @EtchACell

**Email:** [lucy.collinson@crick.ac.uk](mailto:lucy.collinson@crick.ac.uk)

You can view more information about the Francis Crick Institute on the RMS Facilities Database at [www.rms.org.uk/facilities-database](http://www.rms.org.uk/facilities-database). Further information about Etch a Cell is available at [www.zooniverse.org/projects/h-spier/etch-a-cell](http://www.zooniverse.org/projects/h-spier/etch-a-cell).

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