Spinning Disk Super-Resolution Microscopy – Bringing Super-Resolution in Focus for the Cell Biologist.

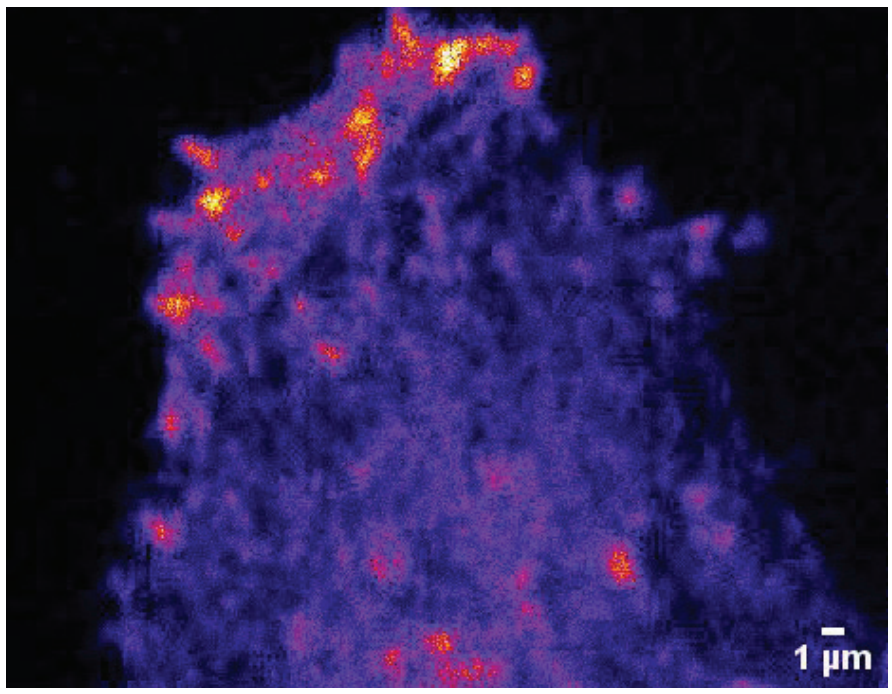
Ann P. Wheeler, Blizard Institute, Barts and the Royal London School of Medicine and Dentistry, Queen Mary University London, UK: Neveen A. Hosny and Martin M. Knight, Institute of Bioengineering, School of Engineering and Materials Science, Queen Mary University London, UK.

Email: a.p.wheeler@qmul.ac.uk Website: www.icms.qmul.ac.uk/imaging/index.html

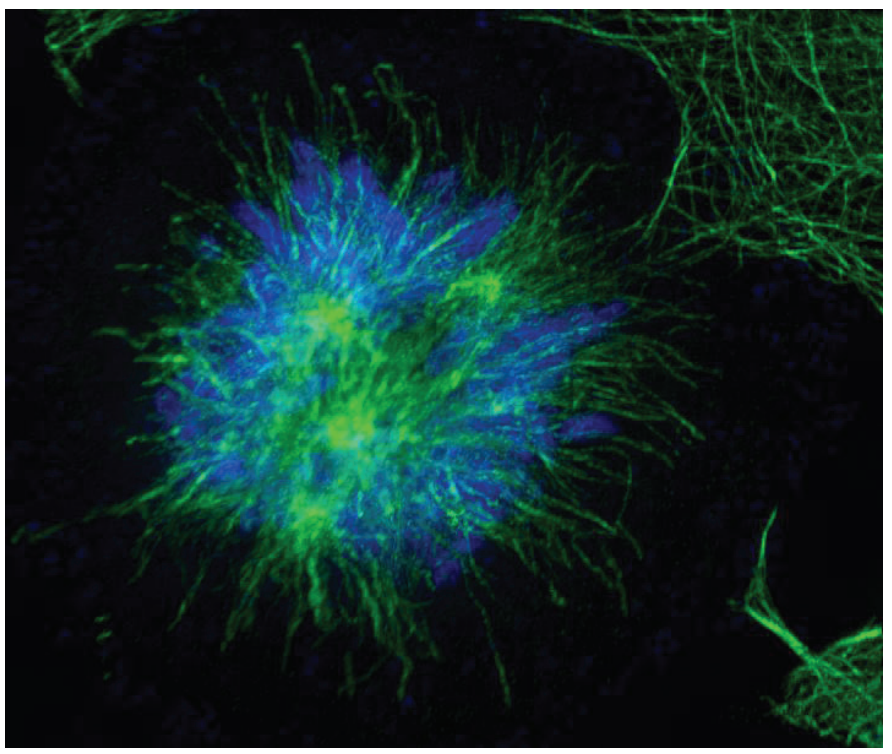
A team from QMUL, headed by Dr Ann Wheeler and Prof Martin Knight , has recently taken on this challenge successfully by generating a new microscopy technology, Spinning disk super-resolution imaging. This work was published in October of 2013 in Plos ONE: www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0074604

The nucleus is the control centre of the cell and is well known for being the organelle which determines the fate of cells and their behaviour. However how the components in the nucleus interact together to determine fate is really difficult to see. This is because structures in the nucleus can be very small, often less than 100 nm in size. Unfortunately even the best light microscope can only image structures clearly larger than 200 nm. To make matters worse the nucleus is very dense and scatters light making it difficult to obtain a focussed image. Similarly the primary cilium is a narrow hair-like cellular projection, only 200nm in diameter. This makes it very difficult to visualise the spatial and temporal protein dynamics within the cilium and which are believed to control many aspects of cell function.

A team from the Advanced light microscopy facility, at the Blizard Institute in QMUL (BALM), headed by Dr Ann Wheeler, has recently taken on these challenges by successfully generating a new microscopy technology, Spinning disk super-resolution imaging. "We were motivated to do this because Professor Martin Knight and Dr John Connelly both work on different aspects of how the cells' environment regulate cell behaviour through changes in the structure and proteome of cellular organelles. Dr John Connelly, from the Blizard Institute QMUL, has recently shown that a cells' fate can be determined by controlling how a cell spreads out as it attaches to a surface. Dr Martin Knight, from the School of Engineering and Materials Science at QMUL, looks at how the primary cilium senses changes in the environment and causes changes in cell behaviour. Both of these projects needed a technique which would be able to visualise cellular structures nanometres in size," said Dr Ann Wheeler.



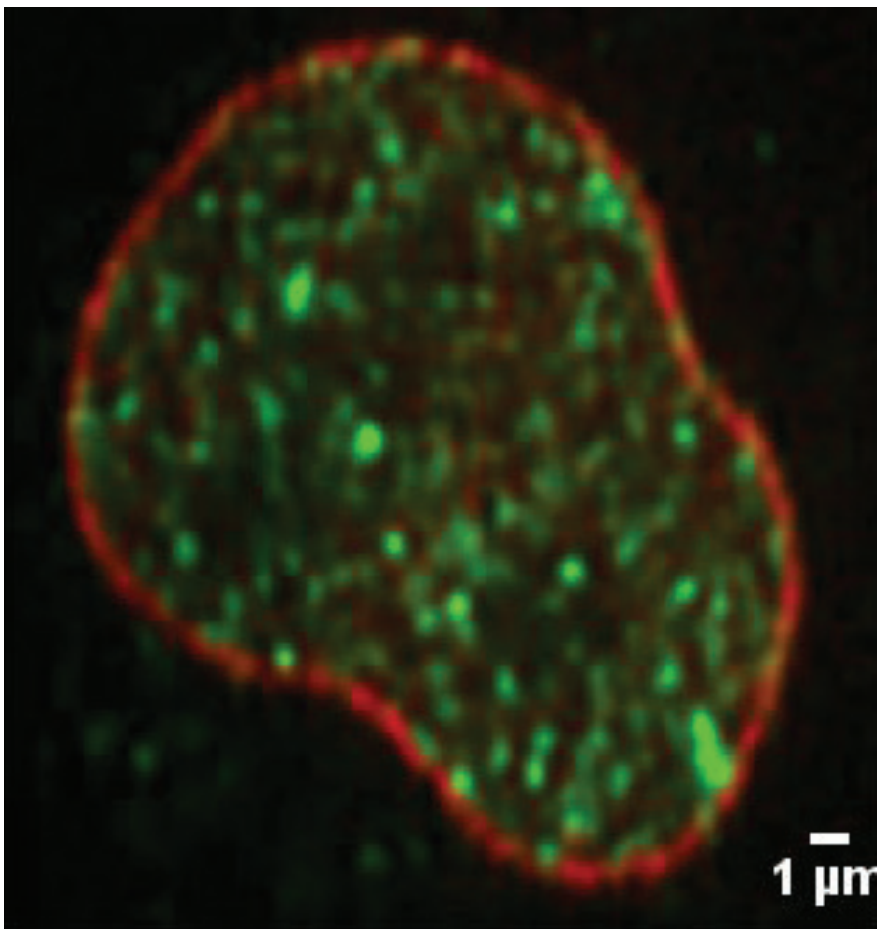
2 ColEosActin Super-Resolution - This shows the organisation of Actin, which is a protein needed to give the cell structure, at the edge of the cell. The cell edge is highly dynamic and so actin can be enriched in specific domains here and SDSI maps at high resolution the structure of these actin domains.



Microtubules - This image shows the organisation of DNA and Microtubules in a cell which is about to divide. Microtubules are thin filaments which generate the force needed to separate the Chromosomes during cell division.

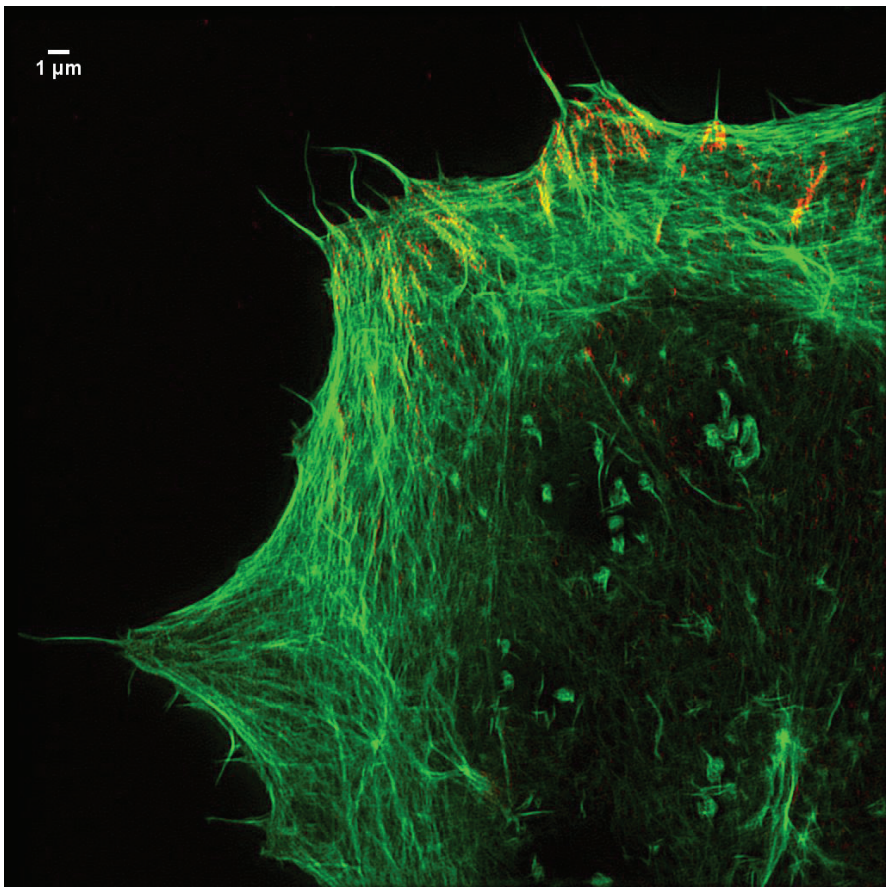
Recent advances in optical physics have led to the development of a new set of light microscopy methodologies which allow the resolution limit of light microscopy to be broken. These microscopy methodologies are called super-resolution imaging and allow visualisation of structures as small as 20 nm. Although, there are limitations to these methodologies; some require complex microscopy setups which are very costly, others can obtain amazing improvements in resolution for structures which are no more than 200 nm away from the coverslip. However, neither of these solutions were appropriate for the Knight or Connelly lab research because the parts of the cell they needed to image were more than 200 nm away from the coverslip, in the middle of the cell. As the UK was in a recession at the beginning of the project, causing a squeeze on research budgets, a new way forwards was needed.

To solve these problems a new type of super-resolution microscopy was developed by Dr Ann Wheeler and Dr Neveen Hosny a postdoctoral research associate. With support from the EPSRC QMUL Discipline bridging fund, together they developed a low cost, easy to use, super-resolution microscope which was capable of imaging the nucleus and other organelles in the centre of the cell. The technology relies on the use of a spinning disk confocal microscope. Spinning disk microscopy technology, coincidentally, was originally brought to the west by another QMUL microscopist, Professor Alan Boyde.



Nuclear Membrane and Chromatin - Here we show the membrane around the nucleus in Red and organisation of Chromatin, which packages up DNA in green in the nucleus at super-resolution. This image shows that there are regions of Chromatin which specifically interact with the nuclear membrane and the very fine structure of the chromatin in the cell.

Spinning disk super-resolution relies on a set or technologies called Photoactivated Localization Microscopy (PALM) / Stochastic Optical Reconstruction Microscopy (STORM). Both of these technologies make use of fluorescent probes which can be switched on and off using light or chemicals in the sample environment. The PALM/STORM probes each individually blink on and off randomly. Localisation of each random blink allows a very detailed map of a structure which has been labelled by the probes to be built up. The more images of random blinks that can be acquired the better the resolution of the output image. This is where the spinning disk comes into its own. It is able to collect many focussed images of PALM/STORM dye blinks from anywhere in the cell including the nucleus. More excitingly we found that it was possible to use more than one PALM/STORM dye at a time on the spinning disk. This meant that we were able to find out how two separate components in the nucleus were interacting with one another, which is a useful tool in investigations of stem cell fate specification.



Focal Adhesions: This image shows the interaction between actin (green), which gives the cell structure and paxillin which is a protein involved in cell adhesion (red). The super-resolution image gives unprecedented detail of structure of the interactions between these two proteins.

Having collected all of this data it was important to decide on the best way to build up the super-resolution image map of structures we had imaged. The image processing for PALM/STORM is a new and evolving field; powerful statistical analysis of the PALM/STORM dye blinks is needed for generation of a robust super-resolution image. Several of these alogrithms were compared to find out which was the most suitable for the spinning disk super-resolution microscopy technique. Since spinning disk microscopy acquires images slightly differently from other PALM/STORM methods and images parts of the cell no other microscopes can reach we expected slightly different results from those others had seem. Happily we were able to achieve an image resolution with our current setup of 80nm. This is around threefold improvement on standard confocal microscopy. It allowed us to see interactions between chromatin and the nuclear membrane and small changes in the arrangement of the cytoskeleton which occur when the cells physical environment is altered.

One of the major advances of the technique is, following the precision engineering the spinning disk system is very straight-forwards for cell biologists to use. Once all the important components are attached the only really important thing is to ensure the system is stabilised at a standard temperature. This means that the technique can be used by anyone with a bit of microscopy experience. "Creating an easy to use super-resolution microscopy technique with broad application was one of the major aims of this project," said Dr Ann Wheeler. "As head of the light microscopy facility I see all sorts of different interesting Biomedical research every week. Although stem cell research is vitally important it's great to have generated a technique which can be used in applications ranging from the organisation of the bacterial membrane, to HIV viral restriction in infection and cancer cell invasion. Using spinning disk microscope we have truly created a technology that opens a window on structure and organisation in the centre of the cell.

The group picture l-r is Dr Ann Wheeler, Dr Neveen Hosny and Professor Martin Knight

Read, Share and Comment on this Article, visit: www.labmate-online.com/articles

New Raman Microscope Enables High-Resolution Materials Analysis

Materials scientists, engineers, and academic researchers can accelerate their research and understand materials in applications ranging from pharmaceutical formulation and life sciences to semiconductor manufacturing and geology using a new Raman imaging microscope. The microscope is so easy to operate that scientists of all abilities can simply walk up and use it to produce stunning chemical images without learning a new technique.

Designed to quickly reveal molecular structure, chemical composition and sample morphology, the **Thermo Scientific** DXRxi Raman imaging microscope can provide new insights, identify defects and confirm product quality with a high degree of confidence. By employing the image-centric software interface, users can quickly profile materials through information-rich chemical images.

Instant visual feedback and instinctive image-driven control separates this instrument from other Raman microscopes. The DXRxi microscope can analyse large areas, providing microscopic detail in just seconds. Organisations with multiple disciplines can leverage the simplicity and approachability of the DXRxi microscope to realise an immediate impact in research output.

"The DXRxi microscope enables scientists to find the needle in a haystack quickly," said Ryan Kershner, Product Manager, Raman Spectroscopy, Thermo Fisher Scientific. "Because this high-powered microscope is so simple to operate, students and expert microscopists can rapidly collect data and answer complex questions in a variety of fields, from biological tissue to carbon nanotube research."

The DXRxi microscope offers the following features: new Thermo Scientific OMNICxi image-centric software provides visually driven data acquisition and intuitive sample targeting and parameter optimisation; automated alignment and calibration saves time and frustration; near-instant visual chemical profiling requires no spectroscopic expertise to interpret; ability to analyse large samples quickly.

For More Info, email: 29007pr@reply-direct.com