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FIRST PERSON

First person – Xian Hu

First Person is a series of interviews with the first authors of a selection of papers published in Journal of Cell Science, helping early-career researchers promote themselves alongside their papers. Xian Hu is first author on 'Micro-stepping extended focus reduces photobleaching and preserves structured illumination super-resolution features', published in JCS. Xian is a Postdoc in the lab of Oddmund Bakke at the Department of Biosciences University of Oslo, Norway, investigating the link between intracellular vesicular trafficking and mechano-sensing by using state-of-the-art bioimaging and image-processing technologies.

Xian Hu

How would you explain the main findings of your paper in lay terms?

When imaging living cells with specific fluorescent markers, the central problem is that the cells are harmed by the high-intensity light from the microscope and that fluorescence is limited as the labels decay, a process called bleaching. The original intent of the work presented in this paper is to add a simple inexpensive modality to spinning disk confocal microscopes, so that we can image the entire cell and use at least 10× less light than the default 3D imaging.

By synchronizing the imaging with the movement of the sectioning, using the built-in piezo drives of our microscopes, we were able to maintain confocality and record an image of the entire cell in one sweep. As a result, the cells can either be observed 10× more frequently or 10× longer. This improvement does not come 'for free' as the approach 'loses' the Z-resolution. For many applications, this information is not needed, and an added value is that we also save computer storage – which is another limitation for extended 3D imaging.

The key discovery came when we noticed that different sweep modes produce slightly different outcomes, even though they travelled the same path – one of them allowed for super-resolution imaging. We show this for a couple of examples, such as recording of endosomes in cells and actin dynamics, but the method is universal.

Were there any specific challenges associated with this project? If so, how did you overcome them?

This invention was a practical bi-product of an ongoing imaging project and I struggled with the detection of fluorescent molecules on fast moving endosomes. I was using a great iSIM spinning disk for recording live cells in 3D but suffered from the lack of speed and fluorophore bleaching. I was thinking that, maybe, I should have used a point scanner where I could have opened up the pinhole, which allows me to trade some of the confocality with a thicker optical section. But then the imaging speed of such a system will become a problem.

I started to speculate on how I could reach a thick section using the spinning disc microscope. With my background, it then seemed obvious that the fast-moving piezo stage will achieve this, as long as we could synchronize the imaging with the stage movement. Together with my collaborator Felix Margadant, a specialist in practical optics, software and electrical engineering, we made an early prototype and moved on from there.

The idea and the implementation itself were straightforward, and the images are easy to interpret as long as you know what you are looking for and where to look. To narrow down the biological research experience I accumulated over years to a simple imaging 'trick' was the most-challenging part of the project.

When doing the research, did you have a particular result or 'eureka' moment that has stuck with you?

The 'real' eureka moment came after we made a simple small gadget to synchronize the control of the stage and the camera exposure, and it worked on the first attempt. It did maintain confocality; great, but predictable! But when we tried to optimize the stage movement to optimize speed, frame rate and projection quality while keeping the artifacts low, we suddenly realized that the Nyquist limit for motion-artifact suppression was not good enough to implement SIM imaging. The needed tweak was to separate movement and exposure. The same night, we then obtained the first super-resolving projections.

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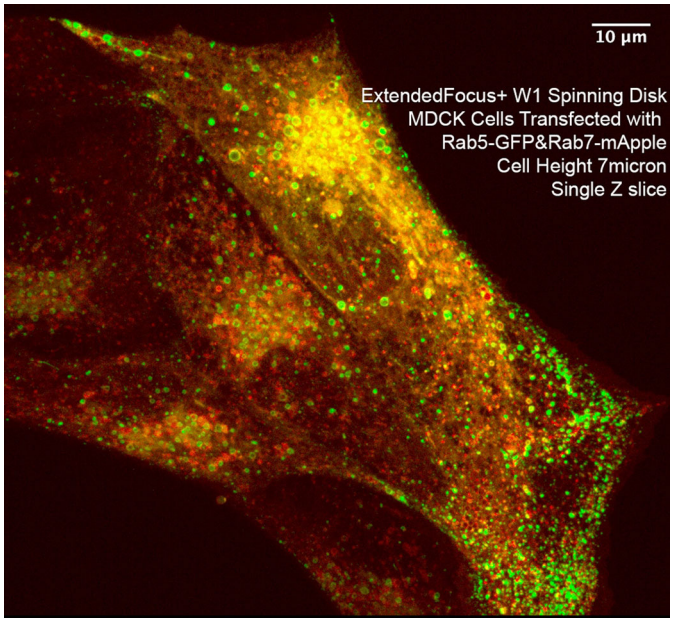

The fluorescence image of a 3D cell captured by taking a single Z-slice at high resolution and in focus.

Why did you choose Journal of Cell Science for your paper?

In the beginning, I thought that this could be published in a more technical journal as they usually require less time. I was eager to go on with my endosome maturation project. However, fortunately, I was invited to present my work at an EMBO Endocytosis Meeting in Ischia and – after giving my presentation – to my surprise, the audience were more curious about the method I had used, rather than my then preliminary biological results. At one of the dinners in the conference, I was introduced to Dr Petra Gross, Senior editor of JCS, who suggested that such a method would be of interest to the readers of JCS, if we made it accessible to cell biologists… Wow, that was great positive feedback. We did, indeed, submit to JCS and, after excellent thorough feedback from the monitoring editor and reviewers, we performed more experiments and resubmitted an improved version that is now published.

Have you had any significant mentors who have helped you beyond supervision in the lab? How was their guidance special?

James Binfield Pawley (we called him Jim) from the University of Wisconsin-Madison, and all the other faculty at Jim's University of British Columbia summer microscopy courses. My first application to Jim's course was approved by Jim but rejected by my department. It was Jim who didn't give up on me when I told him I couldn't make it. After he found out that funding was the problem, he issued me a scholarship to attend his course as a student – and that changed my life. After my first course as a student, I became a regular staff member of Jim's course until he retired in 2013. I have never been a pure biologist; I love bioimaging too much and I love to help fellow colleagues to set up their imaging experiments. With the knowledge and friendship I gained from Jim's course, I built the microscopy core of the MechanoBiology Institute of Singapore from scratch before I left for my PhD programme. As a postdoc, I joined a EuroBioimaging facility in Oslo headed by Prof Oddmund Bakke, where I got my own project within intracellular trafficking combined with core facility work. I initiated the NorMIC microscopy workshops in Oslo in the hope that it will one day become the continuation of Jim's course. I think the late James Pawley is the person that has affected my life most significantly and I never got to thank him enough for this.

What motivated you to pursue a career in science, and what have been the most interesting moments on the path that led you to where you are now?

I was lucky enough to be the founding microscopy facility manager of the Mechanobiology Institute (MBI), Singapore for the first two years. I was very happy and proud during that time. To see the microscopy core being built up from an empty new building. But, slowly, I started to feel some resistance. We had some of the most-advanced light microscopes at that time – the Zeiss Elyra NSTORM, NSIM, etc., but it was relatively difficult to convince biologists to switch to the more-daring new technologies. I did organize workshops, helped them to run the imaging experiments, but the adoption to new technology was still not as fast as I hoped it would be. Besides, I started to feel a glass ceiling on many occasions as I did not have a PhD but performed all kinds of advanced imaging experiments for mentions in the acknowledgements. So, after two years, when the MBI microscopy core had grown to a decent size, I left the facility for a PhD position, with a project to elucidate the link between forces and molecular motion of talin, a question that required all my background in advanced imaging.

Who are your role models in science? Why?

I had two PhD supervisors, Michael Sheetz and Alexander Bershasky. They are both great scientists and have very different styles of addressing scientific questions, sometimes complementary. With their mentoring, I was shown how great research can be done in different ways. They have both played an important role in shaping me to become the scientist I am today.

What's next for you?

My dream job is to become a combination of microscopy manager and research scientist, as I equally like to work with microscopes/interact with users, and work on projects of my own. But such jobs are rare. I will complete the two manuscripts in hand as soon as possible and submit grant applications to follow up my ideas. I do not have any direct plans to leave academia, but competition is rather stiff in the field and I would give myself another one or two years to try.

Tell us something interesting about yourself that wouldn't be on your CV

Felix Margadant is both my collaborator and husband. We met in Jim's course. He specializes in image processing/building microscopes etc. I'm a biologist who's interested in optimizing microscopes and using them to study biology. Our interests match and our skills complement each other. We have been working together for many years on various projects. He was elected to be the acting microscopy manager for MBI ever since I went on to pursue my PhD. People call me mostly by the name Edna; EF is not only the name of our joint invention, it is also the initials of our first names. James Pawley attended our wedding in my hometown, the now very famous city of Wuhan.

Reference

Hu, X., Jalal, S., Sheetz, M., Bakke, O. and Margadant, F. (2020). Microstepping Extended Focus reduces photobleaching and preserves structured illumination super-resolution features. *J. Cell Sci.* **133**, jcs240796. doi:10.1242/jcs.240796