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FIRST PERSON

First person – Kerstin Hinterndorfer and Felix Mikus

First Person is a series of interviews with the first authors of a selection of papers published in Journal of Cell Science, helping researchers promote themselves alongside their papers. Kerstin Hinterndorfer and Felix Mikus are co-first authors on 'Ultrastructure expansion microscopy reveals the cellular architecture of budding and fission yeast', published in JCS. Kerstin is a PhD student in the lab of Robbie Loewith at University of Geneva, Switzerland, working on TOR signaling and expansion microscopy in yeast. Felix is a predoctoral fellow in the lab of Dr Gautam Dey in Heidelberg, Germany, investigating how nuclear pore complex dynamics regulate nuclear remodeling and proteostasis in closed and semi-closed mitosis.

Kerstin Hinterndorfer

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

F.M.: In microscopy, the ability to resolve objects on the nanoscale is often the limiting factor in studying intracellular structures. To improve on this, one can use imaging setups with modified optics that break the theoretical resolution limit for visible light – so-called super resolution microscopes. These systems are, however, highly specialised, tricky to use and quite expensive. Expansion microscopy (ExM) provides an attractive complement to optical and computational super-resolution techniques. In ExM, samples are enlarged by anchoring them in a gel that can be swollen in water. The straightforward physical expansion of the sample allows for the visualisation of protein complexes and subcellular structures using conventional microscopes accessible to most research labs around the world. Here, we set out to optimise ExM to work reliably in model yeasts. Relying on a robust strategy to digest the yeast cell wall, our protocol results in a four-fold isotropic expansion in both budding and fission yeast cells. To test the possibilities this opens up, we used it to study small intracellular structures and, for validation, compare it to data gained using different, less accessible and often low-throughput microscopy methods. These structures were the gateways connecting the nucleus to the rest of the cell, nuclear pore complexes (NPCs), the density of which needs to be maintained throughout cellular and nuclear growth. Using ultrastructure-ExM (U-ExM) we were able to count the pores at all the different stages of the fission yeast life cycle. We further show that changing the way of fixing cells drastically influences the morphology of intracellular compartments and the detection of proteins by specific antibodies, with cryo-fixation proving superior to chemical fixation.

K.H.: Cells behave as tiny machines, themselves made up of thousands of intricate cogs and widgets which, owing to their small size, are difficult to observe, and are challenging for our efforts to understand how cells work. Particularly frustrating is the fact that the sizes of many of these miniscule cellular devices are just below the resolution limit of easy-to-use light microscopes. To circumvent this obstacle, we developed a process wherein we could physically expand *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* cells. *S. cerevisiae* and *S. pombe* are simple yeasts that have been used by generations of cell biologists to dissect conserved aspects of cell function. In other words, what one learns from studying yeast almost always reveals new information regarding the function of our own cells. The major benefit of our method is that it is easy to implement and thus can be directly used by the scientific community.

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

F.M.: Tubulin immunostaining proved to be a major roadblock on the fission yeast side of the project. In yeast, since tagging endogenous proteins with either affinity or fluorescent tags is so routine, specific antibodies are rare (especially those optimised for immunofluorescence). Of a variety of tubulin antibodies that we tested, only one worked, and that barely – we even had trouble observing mitotic spindles (many microtubules bundled together!). Switching to cryofixation of cells provided the major breakthrough.

K.H.: Yeasts have a rigid cell wall (to protect them from their often harsh environment) and this must be removed before the cells could be expanded. Cell wall removal is easy, but we first needed to find conditions that would 'fix' the material inside the cell prior to cell wall removal. This took a lot of trial-and-error experiments before we found a protocol that allowed us to remove the cell wall without compromising the composition of the cell interior.

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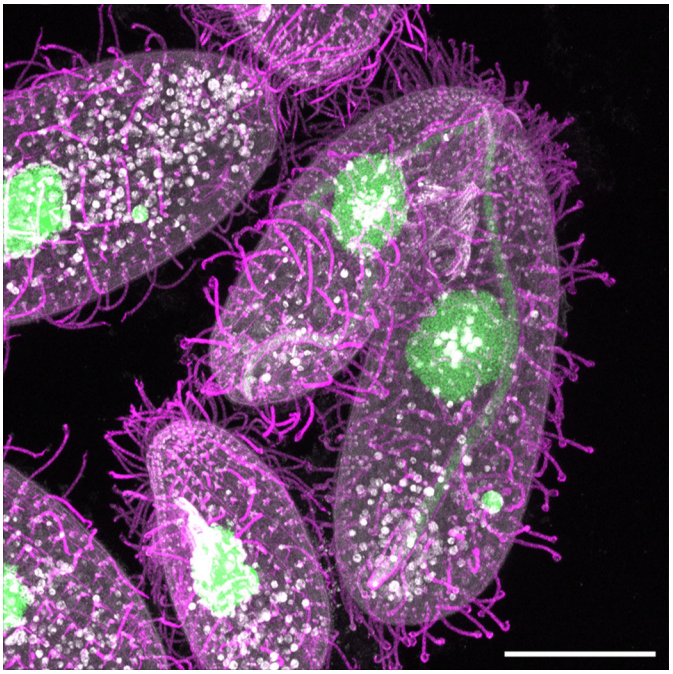
Felix Mikus



Aside from in yeasts, U-ExM can also be used for example for *Tetrahymena thermophila*. Here, meiotic remodelling of the micronucleus can be appreciated. Tubulin is shown in magenta, DNA in green, and NHS ester pan-labelling in grey. Scale bar: 20 µm (corrected for the expansion factor). *Tetrahymena* kindly provided by Emine Ali.

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

F.M.: Maybe not a eureka moment, but when I first saw properly expanded cells with the pan-labelling (a general protein stain that shows 'protein density' in a similar way to contrasting in electron microscopy), I was really giddy and must have shown it to everyone I met that day! Saying that samples expand four-fold really does not capture how much larger they actually do become and the amount of subcellular structures one can all of a sudden image!

K.H.: I was working on yeast expansion for my master thesis project in the Chemical Biology master program at the University of Geneva, so much of this work provided my first experiences using these techniques and I had many moments where I was absolutely fascinated by my results. My favorite moment though was realizing the level of amazing detail we could capture for the duplication of a particular cellular widget – the spindle pole body. Although this process had been biochemically described previously, we were the first to actually observe it by light microscopy. The realization that we made something previously invisible, visible, was absolutely incredible, since now this technique can be applied to other questions that cannot be assessed by alternative methods. Beyond the actual work in the lab, I loved how the paper writing process brought the two stories of *S. cerevisiae* and *S. pombe* together to generate this bigger story of yeast expansion.

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

F.M.: JCS has a broad audience of cell biologists, many working on organisms with cell walls, that could benefit from this protocol. The Tools and Recourses section seemed like a great fit for this type of manuscript.

K.H.: The possibility to submit our story as a JCS 'Tools and Resources' article was a great opportunity for us. I found many interesting, high-quality publications that were relevant for my research in Journal of Cell Science, which is why we decided this would be the best journal to share this new technique with as many other labs who could benefit from it as much as possible.

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

F.M.: There have been many people that have supported me, including supervisors along the way and my family and friends. Gautam Dey, my PhD supervisor and co-supervisor of this project, is definitely one of them! He took the risk of hiring me as his first lab member during a pandemic, without the chance of in-person interviews! Throughout the last two years I have learned a ton from him scientifically, but also the importance of networking, being engaged in the scientific community and the general approach to science as a whole! Somehow, he also found a way of leaving me plenty of room for collaboration and side projects while still keeping me on track!

K.H.: The program coordinator and director of the Chemical Biology master program were really amazing! Of all the amazing help they provided for all of us while getting settled in Geneva, I was most fascinated by how they managed to create a sense of community, collaboration and support between the students joining the program, creating a starting point for our professional networks and some very strong friendships. We were also immediately welcomed into the existing community of labs associated with this MSc program, which also had the same amazing spirit. Now that I am working full time in the lab, I am incredibly grateful for several amazing post-docs and other PhD students with whom I am working, who uphold this same spirit and are always supporting each other, be it in science, paperwork or personal issues.

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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?

F.M.: I have always had an interest in science and nature, and practical courses, internships and lab rotations focused and reinforced this general interest. There have been many interesting points throughout my undergraduate studies, but the degree of exposure to different research topics and the (often direct) involvement in them during my PhD has been very exciting! The upcoming 'traversing European coastlines' (TREC) expedition – a highly collaborative mission to sample the biodiversity along the European coast from the Arctic circle down to the Mediterranean – will be a highlight and allow us to apply U-ExM to a lot of species never before imaged at this resolution, some maybe never before!

K.H.: We had some lab classes and talks from scientists at my high school and I loved these so much that I knew that this is what I needed to pursue after graduation. I really liked this idea of trying to understand the world around us, uncovering truths that were not known before, and I was absolutely fascinated by the sheer complexity of life during our first biochemistry classes. I remember being the only one in my class who actually enjoyed studying the TCA cycle. During my Bachelor thesis project, I witnessed an actual discovery; in fact, the PI of the lab would not tell me and made me figure it out by myself from the data we generated during this project. The moment I understood that I was the second person in the world who ever knew this was amazing, and I knew I needed to stay in science.

Who are your role models in science? Why?

F.M.: I do not currently have just a single person, but for me, people working to improve science in all its aspects (e.g. publishing, equality) are those I look up to even if I may not always agree with all their approaches completely.

K.H.: I consider many of the people I worked with those past three years as role models. I saw many amazing scientists using their time and skills to think outside the box do a bit more than just their job and in many cases I saw this consistent dedication to always strive to make things better ending up making a big difference.

What's next for you?

F.M.: Luckily, I still have some time left in my PhD! Afterwards, I am planning on staying in academic research for a post-doctoral position, but have not decided on a specific focus.

K.H.: To finish optimizing cryo-ExM and finally jumping into some open questions about TOR signalling that we could not answer so far because of the 200 nm resolution limit of conventional light microscopy.

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

F.M.: For some years, I have compiled some of my favourite microscopy images of the year into a calendar as a Christmas gift for my mum, and I think this year I might have enough images to pick this up again.

K.H.: I might have fallen in love with yeast a bit too much since I started working in the lab. The summer between finishing my masters and starting my PhD I tried making currant berry wine at home for the first time and I have loved doing this ever since. I completely failed at elderflower wine, but my next project will be homemade apple cider.

Reference

Hinterndorfer, K., Laporte, M. H., Mikus, F., Tafur, L., Bourgoint, C., Prouteau, M., Dey, G., Loewith, R., Guichard, P. and Hamel, V. (2022). Ultrastructure expansion microscopy reveals the cellular architecture of budding and fission yeast. *J. Cell Sci.* **135**, jcs260240. doi:10.1242/jcs.260240