

EMBO Practical Course

Ultrastructure expansion microscopy: from cells to tissue

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This year's U-ExM course brought together 16 participants from a wide range of nationalities, biological backgrounds and career stages, ranging from PhD students to research group leaders. The biennially conducted workshop was characterized by vivid discussions and intensive, genuinely hands-on sessions that allowed all participants to gain practical experience according to their individual needs.

The common aim of the many existing ExM protocols is to increase resolution in light microscopy up to the super-resolution range, without the need for expensive and highly sophisticated microscopy systems or sample preparation steps, as required for STED or EM. The principle of ExM is conceptually straightforward: a biological sample is anchored to a polymer, then digested or denatured, and subsequently expanded by adding water, which leads to isotropic swelling of the gel. Since the sample is linked to the polymer, it follows this expansion. In this way, the distance between individual points of interest increases, allowing researchers to overcome the diffraction limit that otherwise restricts optical resolution.

ExM can be used to visualize dendritic spine and synaptic cleft organization in neurons, microtubule and mitotic spindle architecture, nucleic acids, as well as membrane-bound organelles, to name only some prominent examples. However, the protocol has to be chosen carefully, depending on both the structure of interest and the sample size (see Figure 1).

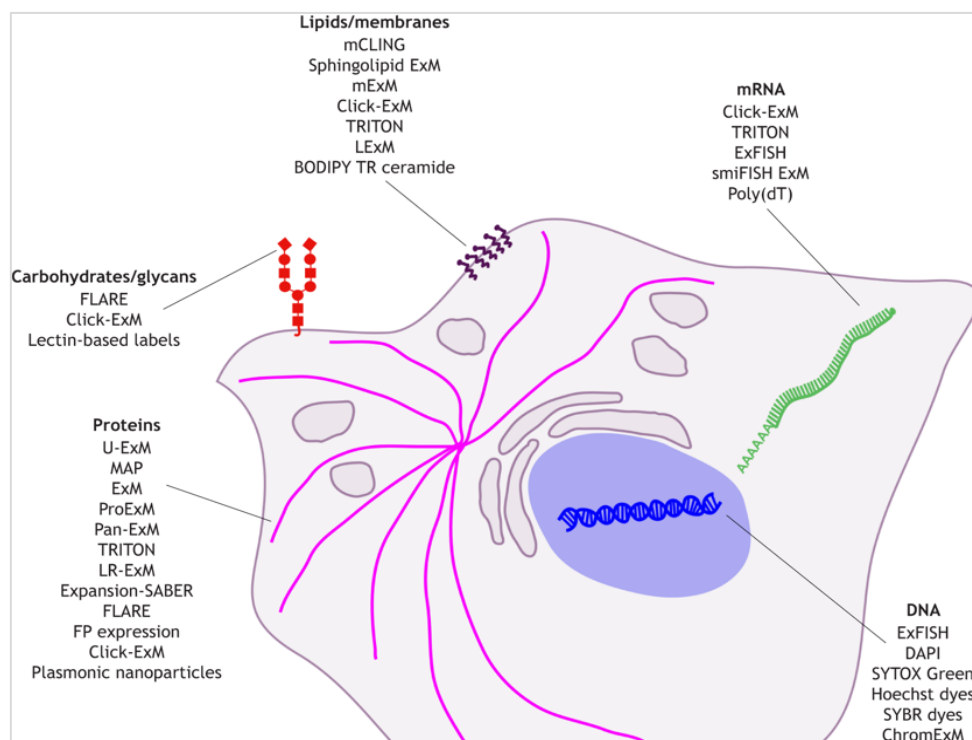


Fig. 1: Overview of labeling methods and dyes targeting specific cellular components used in ExM (Hümpfer, Thielborn and Ewers, 2024).

I was taught the classical Expansion Microscopy protocol, based on Chen et al. 2015 and Tillberg et al. 2016, two years ago and have followed it routinely ever since. In the ExM field, there is a common tendency to stick to the protocol one knows best and has successfully adapted to the desired sample type, according to the saying “never change a running system.” This makes sense, since every ExM

workflow requires optimization for the respective sample. The same applied to me, until I was introduced to U-ExM during this workshop in Heidelberg.

We started the week with an introductory session in which the outline of the course and the principles of U-ExM were explained, and in which we introduced ourselves to one another. Immediately afterwards, we began the wet-lab work and prepared different samples, ranging from single cells to tissue sections, according to the U-ExM protocol. On the third and fourth days, we had the chance to image our samples at the Advanced Light Microscopy Facility of EMBL, using Leica confocal systems, such as the Stellaris, as well as the Viventis light-sheet system, guided by Leica experts. On day five, we dived into image visualization with Blender, a GPL-licensed open-source software, and an EMBL-designed Blender plug-in from the Banterle group, which represents a valuable free alternative to commercial visualization software such as Arivis, IMARIS or Aivia.

The workshop provided many impulses to rethink our own currently used protocols and to question whether, where and how we might need to change, adapt or fine-tune them. In particular, the discussions with other participants about their technical struggles and practical solutions were extremely helpful. Many unpublished handling tips were openly shared by the instructors, which was one of the most valuable aspects of the course. Overall, the workshop gave me the feeling of being welcomed into the ExM community and of gaining connections I can turn to in case of practical problems or doubts, even after the course.

What was also striking to me was the mixture of career stages among the participants. For the PhD students among us, this was especially valuable, because it gave insight not only into different scientific career paths and job opportunities, but also into what is required to build up an independent research profile and, eventually, one's own research group.

From my perspective, ExM is still relatively unknown, even though its implementation *per se* is quite straightforward. The truly challenging part, which can take many months to achieve, is the fine-tuning of the protocol to one's own specific needs. In this sense, the workshop served as an important starting point: on the one hand, it helped to spread awareness of this method, and on the other hand, it provided practical advice, troubleshooting strategies and a network that can help overcome the often cumbersome process of protocol adaptation.

My own work is rooted in the neural stem cell field, using hiPSC-derived cortical organoids. For this type of model system, ExM and U-ExM offer a particularly exciting opportunity to visualize structures that are difficult to access with conventional light microscopy, such as the mitotic spindle. This is highly relevant for my project, as mitotic spindle architecture and orientation play an important role in stem cell division and maintenance. So far, I have applied ExM together with LSFM to depict the overall architecture of cortical organoids. However, my interest has increasingly moved towards the orientation of the mitotic spindle with respect to the apical surface of the ventricular zone in neural rosettes, of which around 400 can be distributed throughout a cortical organoid, depending on the applied differentiation protocol.

With the standard ExM protocol, I was so far not able to reliably image the mitotic spindle, most likely because Proteinase K digestion may compromise microtubule preservation or tubulin signal detection. Thanks to the U-ExM course, I now have both practical tips and a protocol at hand that may allow me to approach these structures more successfully. This makes the course directly relevant for my PhD project and, more broadly, for organoid research, where visualizing subcellular structures within complex three-dimensional tissues remains technically challenging.

Overall, I am very grateful that I was able to attend this insightful workshop thanks to the GSCN Travel Award. The course challenged me to critically rethink my own protocols, gave me concrete ideas for adapting ExM to my research questions, and left me highly motivated to test the learned U-ExM protocol adaptations in my own organoid work.