A novel selective plane illumination microscope to study mouse pre-implantation development

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Selective plane illumination microscopy is a technique where optical sectioning is achieved by specifically illuminating the focal plane with a thin sheet of excitation light. Its main advantages compared to a confocal microscope are reduced light dose and therefore lower photo-toxicity, as well as high imaging speed. Although various configurations of selective plane illumination microscopes have been developed none of them is compatible with the stringent culture conditions required for robust long-term in vitro culture of mouse pre-implantation embryos.

To overcome this limitation we have designed and built a truly inverted selective plane illumination microscope. In our setup the specimen is held in a custom-designed holder with a transparent bottom made of a material with a refractive index matched to that of the immersion medium. The sample is completely isolated from the immersion medium and the objectives and it can be conveniently mounted without embedding in agarose. Multiple embryos can be placed adjacent to each other for high-throughput multi-position imaging.

Using this microscope we have succeeded to image mouse pre-implantation embryos for three days from the one cell stage up to the 64 cell stage, with spatial and temporal resolution sufficient for automated nuclei tracking. We have reconstructed the early embryonic lineages of several embryos and used this comprehensive dataset to identify the onset of lineage segregation with good statistical power. Furthermore, our inverted selective plane illumination microscope is well suited for imaging other photosensitive samples requiring high-throughput 3D imaging and challenging culture conditions.