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Iterative Direct Expansion Microscopy

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Abstract:
While dense biomolecule-rich structures (such as synapses) support diverse biological functionsof importance in brain computation and pathology, visualization tags such as antibodies often cannot access biomolecules of interest in those crowded environments. Thus, visualization and deciphering of the nanoscale organization of such compact structures remains difficult. Here, we report a technology that not only allows nanoscale resolution imaging of intact tissues in ascalable way, but also offers a novel protein de-crowding effect allowing access of tags to individual biomolecules within densely packed structures, otherwise inaccessible.
This technology is the next generation of expansion microscopy, our technique for overcoming the diffraction limit of optical microscopy through physical magnification of biological specimens (Science (2015) 347(6221):543-548). While, in the original expansion microscopy(ExM) process, about 4.5x linear expansion and hence, a resolution of 60 nm (300 (diffractionlimit) / 4.5 (expansion factor)) was achieved, we recently demonstrated that, by iterating the expansion process (iExM), it is possible to obtain higher expansion factors (~20x) and higher resolution (300/20 ~15 nm) (Nature Methods (2017) 14, 593-599). However, in iExM, the biomolecules themselves were not retained during the process, but instead DNA strands were used to encode relative location information, which requires staining before expansion withDNA-conjugated antibodies.
Our new technology, which we call iterative direct expansion microscopy (idExM), not only allows iterative rounds of expansion leading to high expansion factors (up to 100 fold) and thus,excellent super resolution (<10 nm resolution) on ordinary microscopes, it retains the biomolecules throughout the whole process. Thus, the tags can be brought in to label the biomolecules after the expansion, when they are de-crowded from each other and more accessible to tags such as antibodies. We are now using idExM to visualize nanoscale details of highly complex and compact architectures in intact brain circuits.
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