NEXT GENERATION EXPANSION MICROSCOPY TOWARDS WHOLE TISSUE NANOSCALE IMAGING AND HIGHLY MULTIPLEX NANOSCOPY

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In modern biology, diffraction-limited microscopy is a powerful tool to observe microscopic structures and processes of biological specimens. However, diffraction-limited microscopy is unable to resolve nanoscale configurations of biomolecules below the diffraction limit, which severely limited its capability of analyzing intricate and subtle biological/pathological changes. Recently, Expansion Microscopy (ExM) has emerged as a ground-breaking new principle for scalable, nanoscale optical imaging of biological specimens[1–3]. Rather than optically magnify samples, ExM works by embedding biological tissue into a water-swellable polyelectrolyte hydrogel, enzymatically homogenizing them, and then isotropically expanding the tissue-hydrogel physically in pure water. Typical ExM protocols expand tissues by ~100 folds in volume, thus enabling nanoscale optical imaging with resolution ~60 nm using diffraction-limited microscopes. However, most ExM methods cannot process thick tissue and contain an enzymatic digestion step that destroys most of endogenous tissue proteins, obscuring highly multiplex imaging.

To address this, our lab has developed the next generation ExM methods utilizing a mechanically robust, biomolecule retention, water-swellable polymer and an enzyme-free homogenization approach (**Fig. 1A**). The new tools allow isotropic expansion of thick tissue and whole organism by up to ~10 fold in each dimension and by up to ~1000 fold in volume in pure water. In combination with reflectance confocal imaging system, we performed large volume, nanoscale imaging of the mouse neuromuscular junction, the human optic nerve, and the whole 5dpf zebrafish embryo (**Fig. 1BC**). In addition, the next generation ExM enables expansion of a wide range of human tissues while conserving most of endogenous proteins and carbohydrates even after 6 rounds of serial imaging, paving the way to highly multiplex nanoscale imaging of tissue samples with only commercially available reagents (**Fig. 1D**).

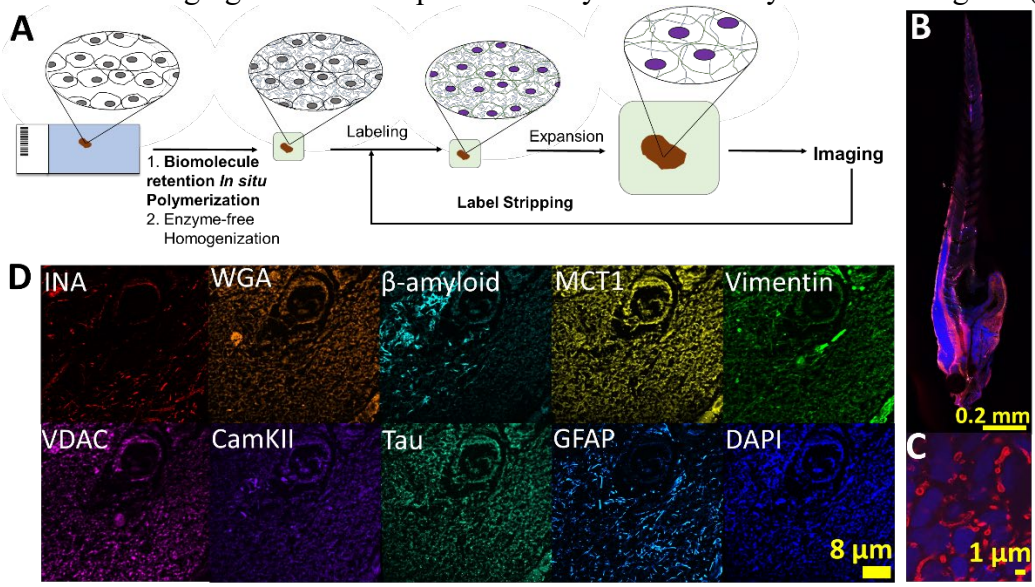

Figure 1. (**A**) Schematic of the next generation ExM work flow. (**B**) 4× expanded 5dpf zebrafish in PBS. (B: DAPI; R: Tom20). (**C**) Zoomed image of (**B**). (**D**) 10-colored image of 5× expanded human Alzheimer's brain tissue section in 1/10 PBS.

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