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Tissue clearing and imaging using CLARITY and Ce3D

Until recently, imaging deep into intact organs using fluorescence microscopy posed a significant challenge. Imaging depth for conventional one-photon excitation microscopy is limited to a few tens of microns due to appreciable light scattering and absorbance in dense, turbid tissue. This limited penetration depth is not significantly improved even with the use of genetically encoded fluorescent reporters or the incorporation of molecular labeling methodologies of targets expressed throughout the tissue. The recent advent of tissue clearing methods has enabled optical transparency of whole intact organs. Utilizing high-speed light-sheet microscopy on these cleared samples provides full, volumetric visualization with cellular resolution.

Here, we present a combination of two clearing techniques, namely CLARITY and Ce3D, for imaging a diverse set of intact tissues. We first exploit the active removal of lipids out of the sample using an externally applied electric field, and then clear the sample passively using Ce3D chemistry. This results in a more optically transparent tissue, allowing for deeper 3-D imaging using a commercially available light-sheet microscope. To this end, we have successfully applied this technique to image several intact adult mouse organs, such as the brain, heart, kidney and liver. We also present the image-processing pipeline which, includes the generation of large seamless image datasets, preliminary analyses, and image visualizations.

Affiliation

Pfizer

Terms and Conditions

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Primary author(s) : Dr ASANO, Shoh (Pfizer); Dr SONG, LouJin (Pfizer); Dr ROTH FLACH, Rachel (Pfizer); Dr HALES, Katherine (Pfizer)

Presenter(s) : Dr ASANO, Shoh (Pfizer); Dr HALES, Katherine (Pfizer)

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