

## Don't be a Scatterbrain: Tissue Clearing Techniques and Considerations for Brain and Body

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Tissue clearing has come of age in the last few years to become an accessible way for scientists to process large tissues for whole mount imaging. In general, tissue clearing techniques are focused on the goals of reducing scatter and absorption of photons to render naturally opaque tissues optically transparent. The broad array of tissue types and the need for compatibility with diverse imaging probes and evolving imaging technologies has yielded dozens of published protocols. These techniques range from the simple and broadly applicable to the technologically complex and tissue specific. The choice of a clearing technique, or whether to use clearing at all, has a dramatic influence on experimental design. Among these considerations are the choice of molecular targets, the spectra used for detection, the resolution required during imaging, the specific measurements to be taken during analysis, and the imaging system to be used. In addition, it is crucial to consider that imaging of whole cleared tissue is an exercise in patience. Clearing and staining protocols can often require weeks or months to complete. Imaging can require days. The volume of data collected can comprise many terabytes, and the analysis of those datasets may require specialized software and high-end computers. Researchers considering whole mount cleared tissue imaging will find opportunities to examine questions that would be inaccessible using conventional methods. Here we will outline the various techniques available to researchers, offer tips for getting started, and discuss considerations for designing an experiment.