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Fixative-eXchange (FX)-seq: a platform for transcriptomics analysis of PFA-Fixed and FFPE Samples

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Single-cell transcriptomic profiling of fixed tissues holds immense potential to bridge molecular biology with structural and clinical insights. Paraformaldehyde (PFA)-fixed samples provide essential spatial context in neuroscience, while clinical formalin-fixed paraffin-embedded (FFPE) archives offer access to annotated pathological specimens and patient outcomes. Yet, this promise has remained largely unrealized due to fixation-induced damage that severely compromises reverse transcription (RT) efficiency, limiting current protocols to fresh or frozen tissues.

Here, we introduce Fixative-eXchange sequencing (FX-seq), a robust and scalable method for single-nucleus RNA sequencing from PFA-fixed and FFPE-treated specimens. FX-seq integrates two key innovations: (1) an organo-catalyst that removes PFA crosslinks under mild conditions to improve in situ RT yield, and (2) a regiospecific platinum-based crosslinker that prevents RNA leakage without inhibiting RT. Using FX-seq, we obtained high-quality transcriptomic data from PFA-fixed mouse brain and FFPE-preserved human cancer tissues, demonstrating its broad utility.

By leveraging the intrinsic stability of fixed samples, FX-seq enables efficient multiplexed profiling across multiple timepoints and subjects. This flexibility supports diverse applications, including single-nucleus spatial transcriptomics, multiplexed tissue analysis, and high-resolution snRNA-seq from small anatomical subregions. FX-seq thus establishes a powerful framework for unlocking transcriptomic information from archival and structurally preserved specimens across both basic and clinical research domains.