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Expansion Pathology: Nanoscale Imaging of Clinical Specimens with Optical Microscopy

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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[1,2]. 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[1,2].

We further developed a clinically optimized form of ExM that supports nanoscale imaging of human tissue specimens fixed with formalin, embedded in paraffin, stained with hematoxylin and eosin, and/or fresh frozen. The method, called expansion pathology (ExPath), transforms clinical samples into an ExM-compatible state, then utilizes an ExM protocol with protein anchoring and mechanical homogenization steps optimized for clinical samples[3,4] (**Fig.1**) ExPath enables ~60-nm-resolution imaging of diverse biomolecules in intact tissues using conventional diffraction-limited microscopes and standard antibody and fluorescent DNA in situ hybridization reagents. We use ExPath for optical diagnosis of kidney minimal-change disease, a process that previously required electron microscopy, and we demonstrate high-fidelity computational discrimination between early breast neoplastic lesions for which pathologists often disagree in classification. We also reported a new, fast variant, rapid expansion pathology, that can be performed on < 5-µm-thick tissue sections, taking < 4 h with immunostained tissue sections and < 8 h with unstained specimens[5] (**Fig. 2**). In contrast, the conventional expansion pathology, can be completed in ~1 d with immunostained tissue sections and 2 d with unstained specimens. Both ExPath versions require only inexpensive, commercially available reagents and hardware commonly found in a routine pathology laboratory, thus these new techniques may enable the routine use of nanoscale imaging in pathology and clinical research.

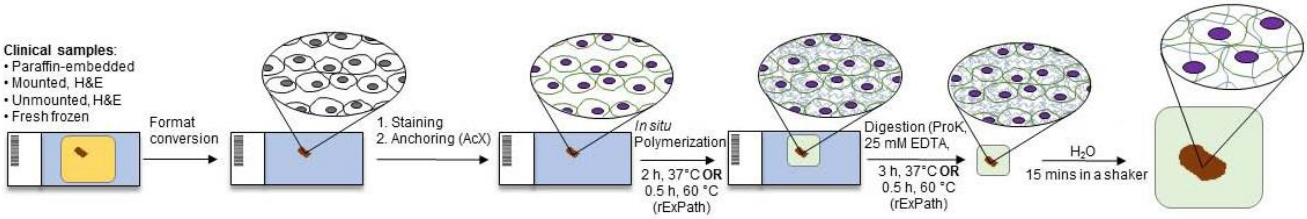

Figure 1. Schematic of ExPath/rExPath workflow. Adapted from Ref. 5.

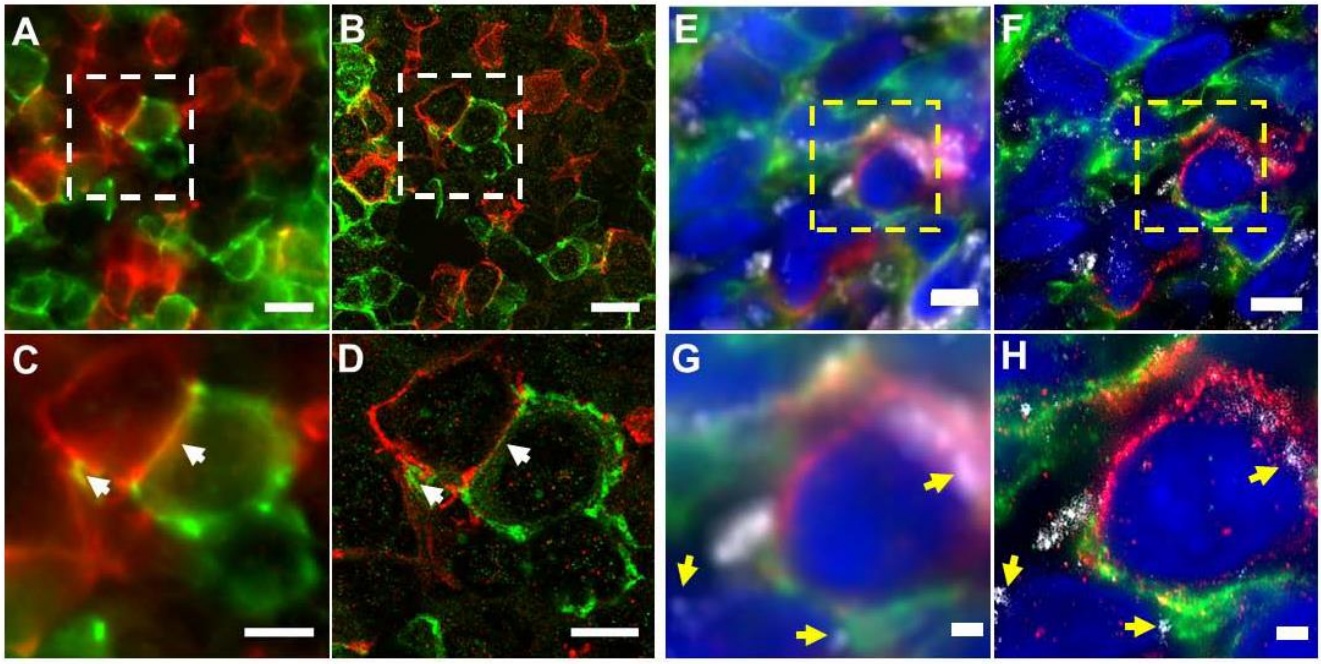

Figure 2. Rapid ExPath imaging of lymph node specimens from patients. (A) Pre-expansion image of a normal human lymph node specimen acquired with a spinning disk confocal microscope. Green, IgD; Red, CD8. (B) rExPath image of the specimen of A acquired with the same confocal microscope. Expansion factor: 4.0. (C and D) Fields of view zoomed into the corresponding areas outlined by a dashed white box in A and B, respectively. White arrows indicate examples of pre-expansion overlapped IgD and CD8 patterns being resolved after expansion. (E) Pre-expansion image of a human lymph node specimen with HIV acquired with a wide-field fluorescence microscope. Green, CD8; Red, PD-1; Grey, p24; Blue, DAPI. (F) rExPath image of the specimen of E acquired with the same microscope. Expansion factor: 4.58. (G and H) Corresponding fields of view zoomed into the areas outlined by a dashed yellow box in E and F, respectively. Yellow arrows indicate examples of p24 being localized with sub-diffraction limit precision. Scale bar (biological scale): (A and B) 5 μm; (C and D) 1 μm; (E and F) 10 μm; (G and H) 2 μm. Adapted from Ref. 5.

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