Nanoscale Imaging of Biomolecules Using Molecule Anchorable Gel-enabled Nanoscale In-situ Fluorescence Microscopy

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A functional and integrative understanding of a biological system requires precise knowledge of the spatial arrangement of components across length scales, from tissue-level organization to individual biomolecules at the nanoscale. Unfortunately, most methods for nanoscale imaging require expensive hardware and extensive expertise. Researchers can circumvent these challenges by physically and isotropically magnifying preserved biological specimens embedded in a cross-linked water-swellable hydrogel, in a technique termed expansion microscopy (ExM) [1]. ExM is a powerful imaging strategy that offers a low-cost solution for nanoimaging with conventional microscopes that has been adapted to many varying needs of biological research, such as the imaging of multiple biomolecule classes [2-5] and fixation methods [6]. However, current ExM protocols require prior treatment with specialized reactive anchoring chemicals to link specific labels and biomolecule classes to the gel. In addition, most techniques reportedly use strong Proteinase K to digest endogenous epitopes to enable expansion and are limited by using mechanically fragile gel formulas to expand specimens by at most 4.5× linearly. Protocols designed for larger expansion have been developed, but have not yet been demonstrated beyond cultured cells and brain tissue sections [7-9].

An ideal ExM protocol would: (1) be easy to use; (2) provide 10-fold or greater expansion with minimal distortion; (3) be capable of conserving a comprehensive array of biomolecule classes that can be labeled after expansion; and (4) be simultaneously compatible with a broad range of tissue-types (including mechanically tough tissues such as kidney) and fixation methods. To date, no documented ExM method can achieve all these features. Here, we present Molecule Anchorable Gel-enabled Nanoscale *In-situ* Fluorescence MicroscopY (MAGNIFY), a new variant of ExM that meets all the requirements above. MAGNIFY uses a new hydrogel formula, including methacrolein as the sole anchoring agent, that retains a spectrum of biomolecules, thus eliminating the need for a separate, molecule-specific anchoring step. When homogenized with a hot denaturant-rich solution, MAGNIFY can retain proteins, nucleic acids, and lipids within the gel matrix, allowing for post-expansion labeling in a broad range of biological specimens. Additionally, we show that MAGNIFY can expand conventionally preserved tissues by up to ~11-fold, resulting in an effective resolution of ~25 nm for an ~280-nm diffraction-limited 1.15 NA objective lens (~280/11). We also demonstrate that when

combined with Super-resolution Optical Fluctuation Imaging (SOFI) [10], a computational post-processing method that relies on the independent temporal fluctuations of fluorophores to distinguish emitters, MAGNIFY-SOFI can achieve ~13 nm lateral effective resolution when using the same 1.15 NA objective lens on a conventional spinning disk confocal microscope [11, 12].

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