Super-resolved Expansion Microscopy

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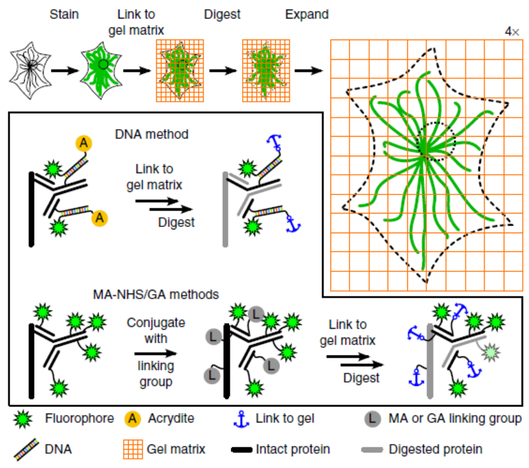
ABSTRACT Super-resolution fluorescence microscopy is a powerful imaging technique that overcome the diffraction limit by means of optics and spectroscopy. Expansion microscopy has also recently emerged as a novel technique improving resolution, but dependent on physically expanded sample linked to swellable polymer matrix. The enlarged sample allows for going over the resolution limit under conventional diffraction-limited fluorescence microscopy. Combining the two methods (i.e. super-resolution imaging of expanded samples) may result in sub-10-nm resolution. However, the enlarged sample volume reduces labeling density and it makes difficult to apply expanded samples to super-resolution microscopy. To overcome the challenge, we first designed the biotin-avidin complex to amplify fluorescence intensity in expanded sample. The densely labeled fluorophores can boost the signal intensity. And then we observed subcellular structures with DNA points accumulation for imaging in nanoscale topography (DNA-PAINT), which is a method that utilizes transient hybridization of two short complementary DNA strands as a modality of localized-based super-resolution microscopy. In this study, we demonstrate the efficiency of amplified signals quantitatively and enhanced the spatial resolution from the combination of super-resolution microscopy and expansion microscopy.

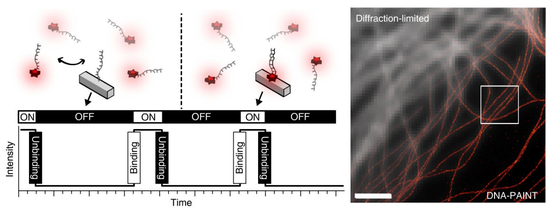
KEYWORDS: Expansion Microscopy, Signal Amplification, DNA points accumulation for imaging in nanoscale topography (DNA-PAINT), Stochastic Optical Reconstruction Microscopy (STORM)

❖ Introduction

Expansion Microscopy



DNA-PAINT



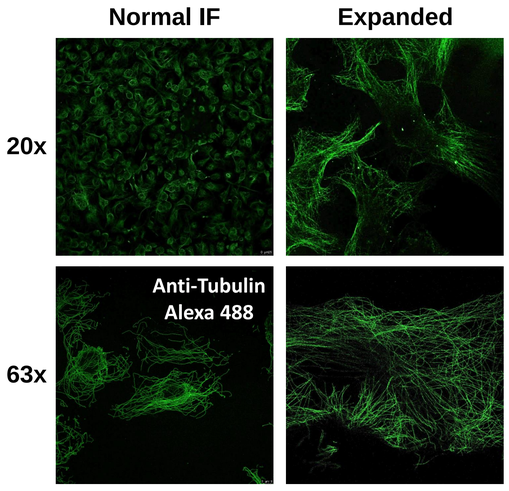
Immunofluorescence imaging is commonly for molecular-specific imaging in various biological samples. However, conventional fluorescence microscopes have limitation in resolution due to optical diffraction. Super-resolution microscopes overcomes the resolution limit, but the instrumentation is complicated and the probe choice is limited. To overcome these problems, Boyden and coworkers invented **Expansion microscopy (ExM).** Expansion microscopy, which uses physically enlarged samples embedded in a swellable polymer matrix, surpass the resolution limit with conventional microscopes.

Among variety of super-resolution microscopy, we applied two techniques based on single-molecule localization and temporal separation of fluorophores via stochastic switching between dark state and bright state. To induce stochastic switching of fluorophores, **Stochastic optical reconstruction microscopy (STORM)** uses photoswitchable fluorophores and **DNA points accumulation for imaging in nanoscale topography (DNA-PAINT)** utilizes transient hybridization of short DNA oligos. The freely diffusing dye-labeled (imager) strands bind to the target (docking) strands.

When combined with super-resolution fluorescence microscopy, ExM may open new windows to achieve the ultimate resolution of a few nanometers.

Chozinsky et al. *Nature Method* (2016)
Schnitzbauer et al. *Nature Protocol* (2017)

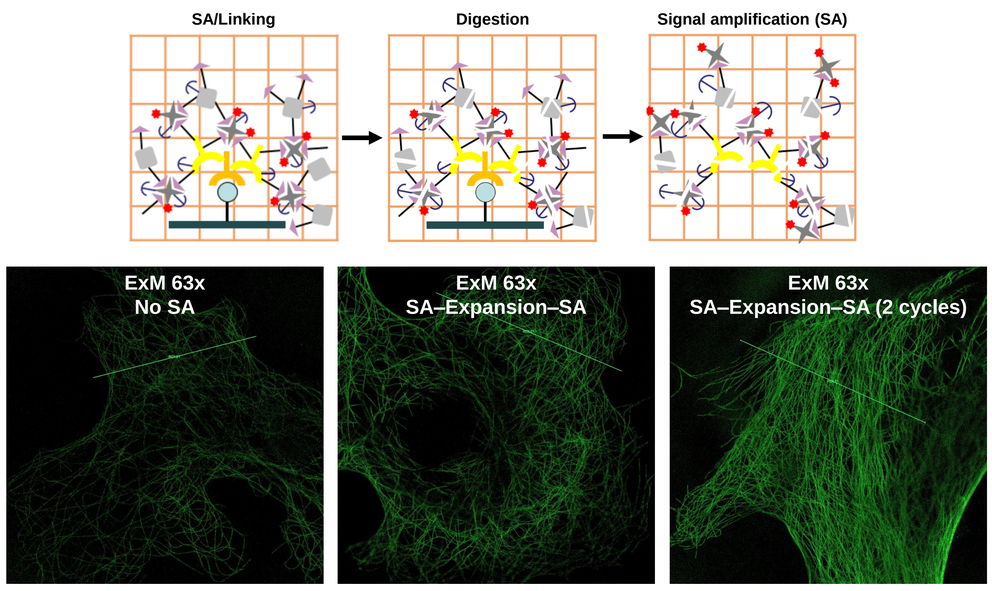
❖ Pre- and Post-expansion Images



- Based on the method introduced by Chozinsky et al, we magnified the sample volume to ~4 times after expansion.
- After staining of cultured cells with a standard immunofluorescence protocol, a crosslinking agent (glutaldehyde) was used to link the antibody conjugated with fluorophores to the polymer gel. Then, protease enzyme digested cytoskeletons in the cells, and the polymer is expanded through dialysis.
- We successfully expanded the specimen more than 4 times as in the published papers. As in the previous ExM reports, we could observe the fine details in the images of expanded specimens more clearly that were ambiguous in the images of the unexpanded specimens.

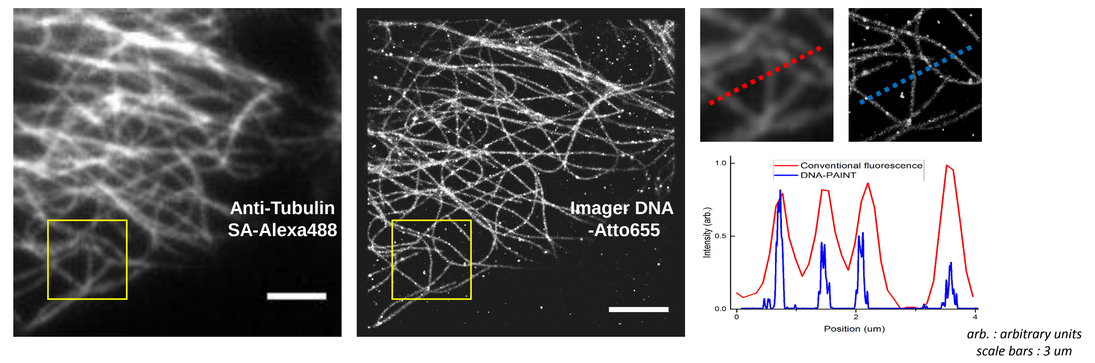
Chozinsky et al. *Nature Method* (2016)

❖ Expansion microscopy with signal amplification



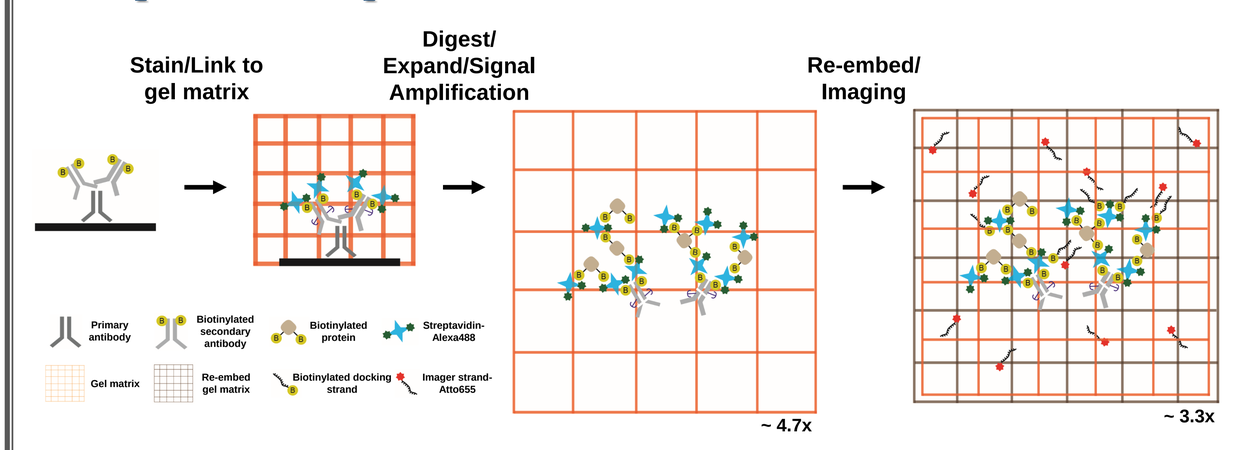
- Although ExM can improve the resolution through physical expansion, it also expands the distance between fluorophores, resulting in lower labeling density that makes it hard to be directly applied to super-resolution microscopy. Hence, we tried to find labeling methods for increasing the labeling density of ExM. Since antibodies are used in ExM, signal amplification (SA) via biotin/avidin interaction can be readily implemented to ExM for improvement of labeling density.
- We put the SA process before/after gelation, and repeated the process for multiple cycles to compare the amplification factor of specimen for each cycle.
- When we compare the relative fluorescence intensities, we observe about three times increase in fluorescence after 1 cycle amplification process. After 2 cycles of SA, the fluorescence intensity was 4 times increased from the non-amplified signal.

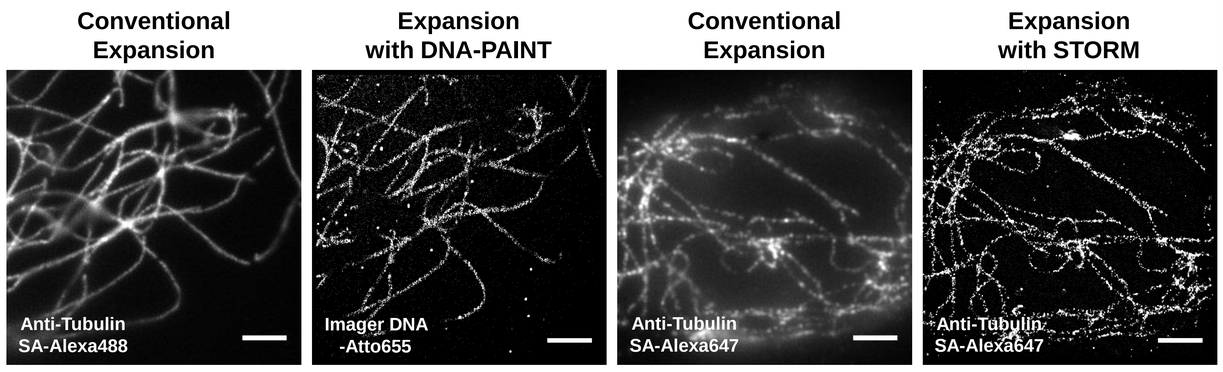
❖ DNA-PAINT Image



- Microtubules in a Cos7 cell are sequentially labeled with anti-tubulin antibody, biotinylated secondary antibody, streptavidin-Alexa488 and then biotinylated docking DNA strand.
- DNA-PAINT images were obtained using imager DNA strand conjugated to Atto655 fluorophore and complimentary to the docking strand.
- The DNA-PAINT images clearly demonstrates the resolution improvement as reported.

❖ Expanded sample with DNA-PAINT/STORM





- Microtubules stained with anti-tubulin, biotinylated secondary antibody and streptavidin-Alexa488 are physically expanded. Then, the biotinylated docking strand binds to streptavidin.
- The sample is re-embedded in non-swellable gel for preventing swelling or shrinking of the sample.
- DNA-PAINT images of the expanded sample were obtained by using transient binding between docking strands and their complementary imager strands conjugated to Atto655.
- For Stochastic optical reconstruction microscopy (STORM), the microtubules are stained with anti-tubulin and biotinylated secondary antibody. Then, the expanded sample is re-embedded before staining with streptavidin-Alexa647. The sample labeled with streptavidin-Alexa647 was imaged in STORM imaging buffer containing 100mM mercaptoethyl amine.
- After the sample is re-embedded in non-swellable gel, the imaging condition for DNA-PAINT and STORM is compatible with the expanded gel samples in ExM and does not change the volumes of expanded gels.

❖ Conclusion and Future work

- Expansion microscopy improves the spatial resolution with physically expanded samples, however suffers from the reduction of fluorescence intensity due to elongated distances between fluorophores. We used biotin-avidin interaction to amplify fluorescence intensity.
- DNA-PAINT improves the spatial resolution by localizing fluorescent DNA oligos transiently binding to their complementary strand.
- We found imaging condition compatible both for expansion microscopy, DNA-PAINT and STORM.
- Since ExM expands the sample volume by ~4 times and DNA-PAINT resolution is ~20 nm, we expect to achieve final resolution of ~5 nm.
- To achieve final resolution of 5 nm, the distance between fluorophores should be 2.5 nm or less according to Nyquist criterion. Thus, we will increase the labeling density of ExM-PAINT/STORM via signal amplification of the expanded sample using cyclic labeling of biotin-avidin.
- We are going to use biotinylated primary antibody, nanobody and peptide tag to reduce the distances between fluorescent probes and their target molecule to accurately probe the underlying structure with the 5-nm resolution.