# Highly Bright Expansion Microscopy

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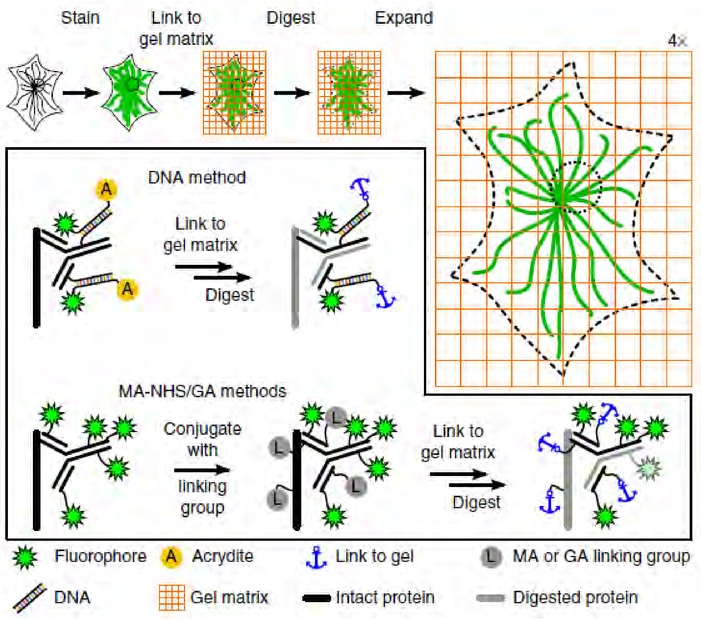
## ❖ Expansion microscopy

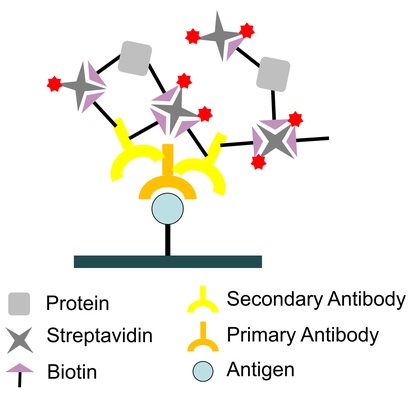



Chozinsky et al. *Nature Method* (2016)

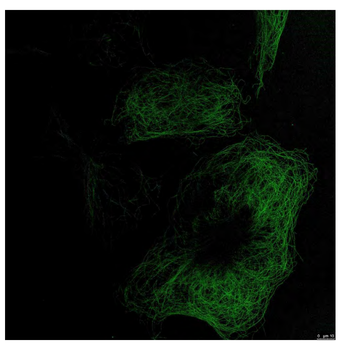
It is highly challenging to visualize the fine structure of cell, clearly. Fluorescence imaging, using imunohistorical technique, is broadly used by many biologists for its verified efficiency and manageable property. However, traditional scanning based microscopes have limitation for sample magnification with high resolution. Even though we use other super-resolution microscopes, it is awkward to deal with instruments and needs specialized conditions. To overcome this problems, Boyden and coworkers reports expansion microscopy (ExM).

Expansion microscopy, which uses physically enlarged samples embedded in a swellable polymer matrix, surpass the diffraction limit in spatial resolution with conventional microscopes. When combined with super-resolution fluorescence microscopy, ExM may open new windows to achieve the ultimate resolution of a few nanometers. However, expanded samples in ExM have lower labeling density that makes it hard to be directly applied to super-resolution microscopy. Here we introduce a labeling method that increases the labeling density of ExM by using the tyramide signal amplification and/or the biotin/avidin interaction. We are trying to amplify the fluorescence intensity of expanded samples, and quantitatively examined the amplification efficiencies with confocal microscopy. With this approach, ExM can provide additional four- or five-fold increment of spatial resolution in super-resolution microscopy to visualize the ultrastructures with single-digit resolution.

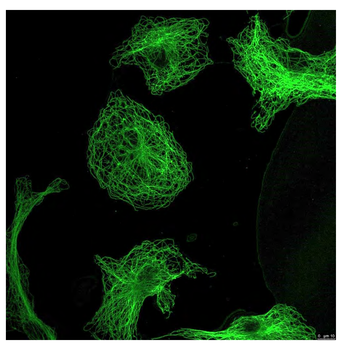
## ❖ Signal amplification using biotin/avidin interaction



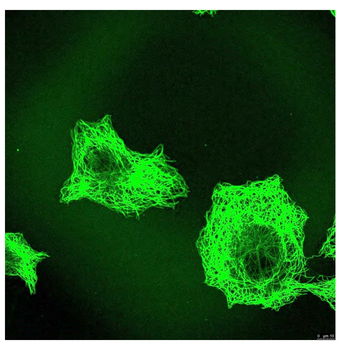

- Avidin and other biotin-binding proteins, including streptavidin, have the ability to bind up to four biotin molecules. The bound streptavidin can offer other binding sites for biotins. This repeated binding process can make fluorescence intensity stronger when using fluorophore-conjugated streptavidin.
- Signal amplification (SA) sequence: (1) Primary Ab, (2) Secondary Ab (biotinylated), (3) Streptavidin-Alexa488, (4) BSA (biotinylated) or Secondary Ab (biotinylated), (5) Streptavidin-Alexa488 (1 cycle = steps 4 & 5)
- After SA, we can find brighter signal than regular immunostaining using biotinylated secondary antibody. The image obtained after 2 cycles of SA process shows more amplified fluorescence than the image from 1 cycle SA.



**No SA**



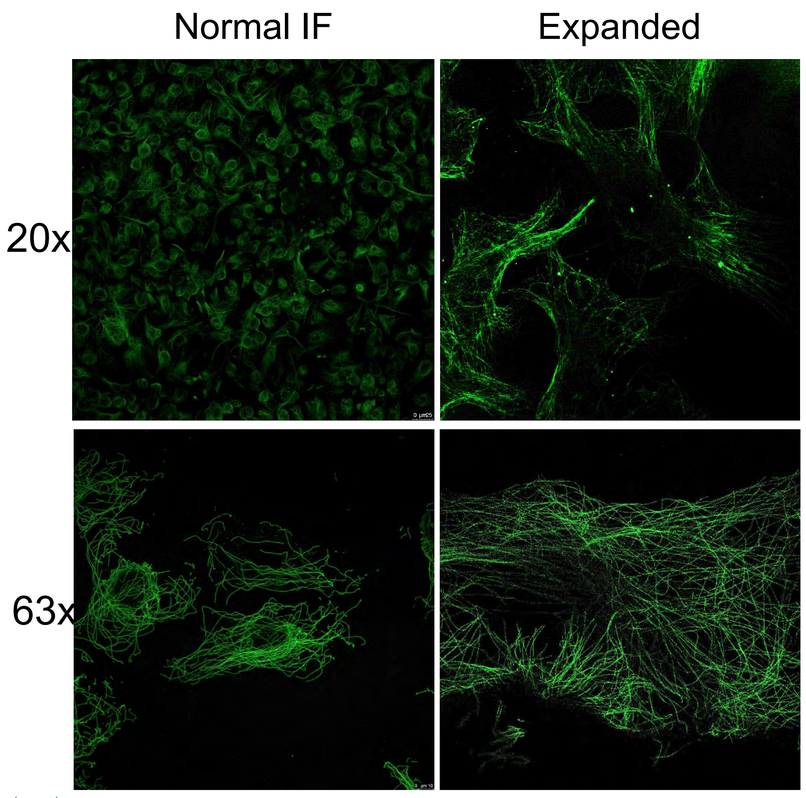
**1 cycle of SA**



**2 cycles of SA**

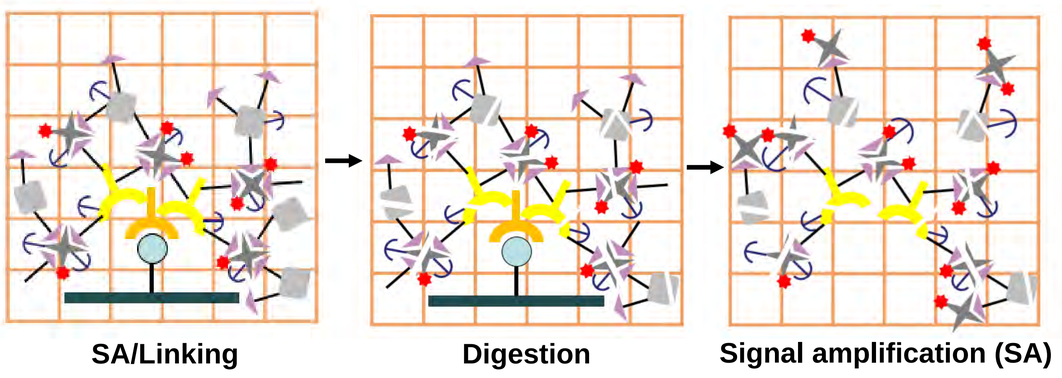
## ❖ Pre- and Post-expansion Images

- In the previous works, the sample volume can be magnified to 4~4.2 times after expansion process.
- After the immunostaining of a specimen with polymer-linkable probes, crosslinking agent such as glutaldehyde or methacrylic acid N-hydroxysuccinimidyl ester (MA-NHS) can link the probes to the polymer gel. Then, protease digests proteins in the specimen, and the polymer is expanded through dialysis.
- We successfully expanded the specimen more than 4 times as in the published papers. As with the original 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.

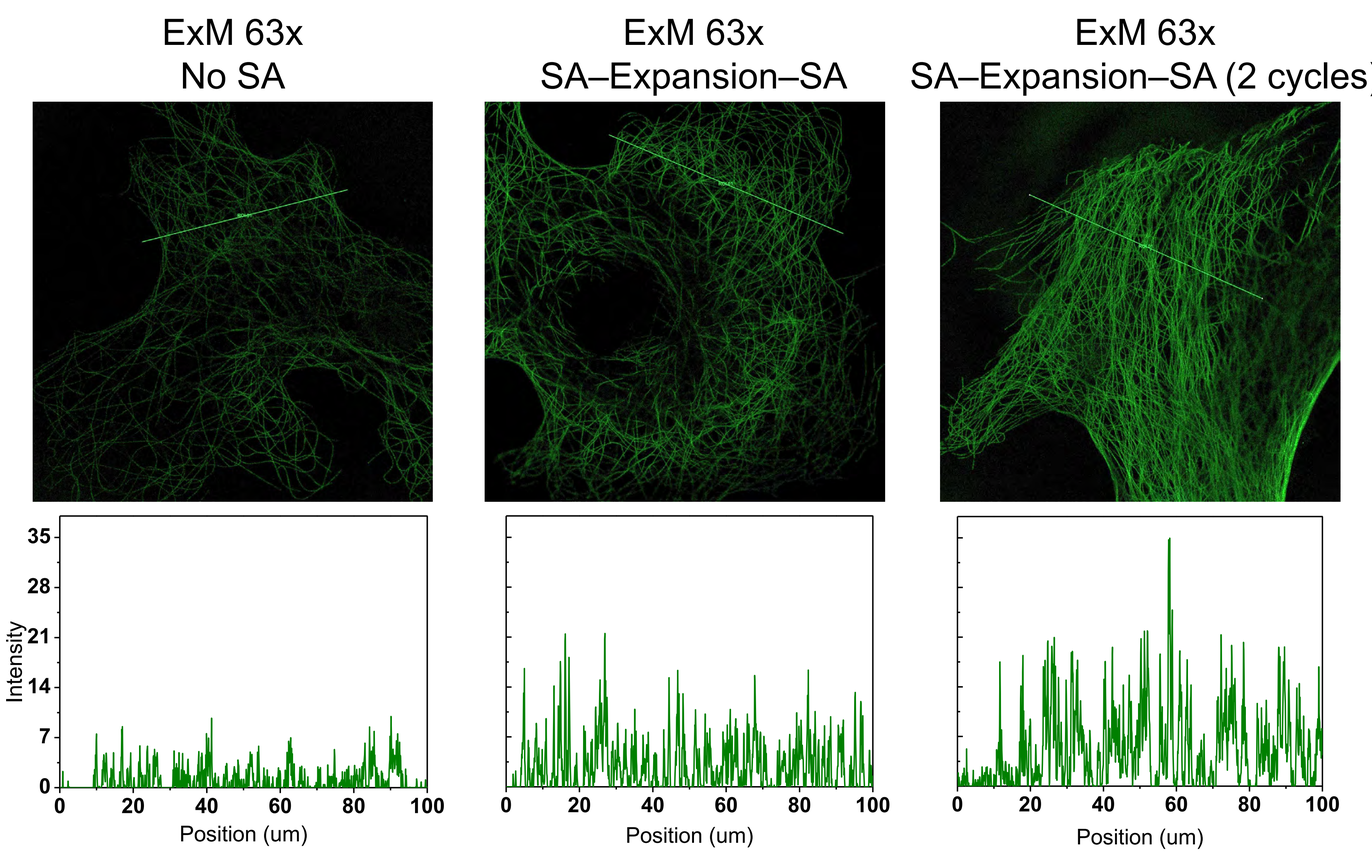



Chozinsky et al. *Nature Method* (2016), Chen et al. *Science* (2015)

## ❖ Expansion microscopy with signal amplification via biotin/avidin interaction




- Although ExM can improve the resolution through physical magnification, it results 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 went on the repetitive process to compare the amplification factor of specimen in 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.




## ❖ Future work

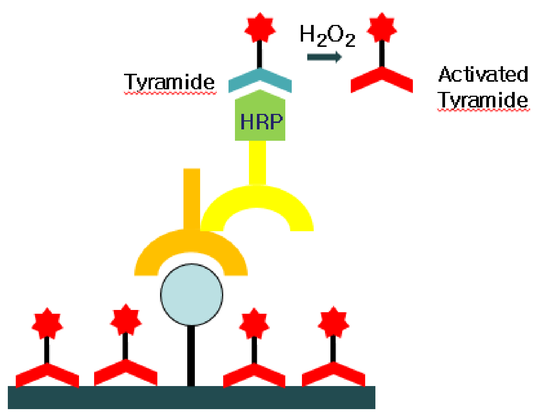
### Super-resolution fluorescence microscopy of highly bright, expanded samples

The highly bright expanded samples will be imaged with super-resolution fluorescence microscopy such as STORM (stochastic optical reconstruction microscopy). Since ExM expands the sample volume by ~4 times, STORM resolution of ~20 nm will be improved to ~5 nm.

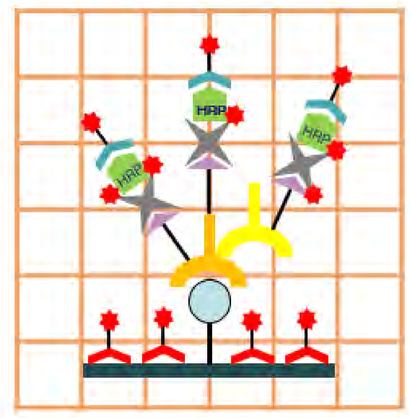
High labeling density is required to achieve the 5-nm resolution. It is because labeling density limits the final spatial resolution according to the Nyquist-Shannon sampling theorem. Therefore, fluorophores should be placed every 2.5-nm or less in order to achieve 5-nm resolution.

### Expansion microscopy combined with tyramide signal amplification (TSA)

- Tyramide signal amplification (TSA) is an enzyme-mediated detection method that utilizes the catalytic activity of horseradish peroxidase (HRP) to generate high-density labeling of a target protein.
- We are going to use TSA for higher intensity of signal, using HRP-conjugated streptavidin. Since the ExM experiment has protease digestion step, HRP-conjubated streptavidin will be added in the SA step after digestion.
- Generally, TSA increases the sensitivity of signal and reduces background interference. Also, STORM resolution is dependent on labeling density. In these reason, we anticipate a better resolution for STORM with higher labeling density of ExM combined with signal amplification via biotin/avidin as well as TSA.




**Tyramide signal amplification (TSA)**




**Expansion with TSA**

Kevin et al. *Tyramide signal amplification strategies for fluorescence labeling* (2008)