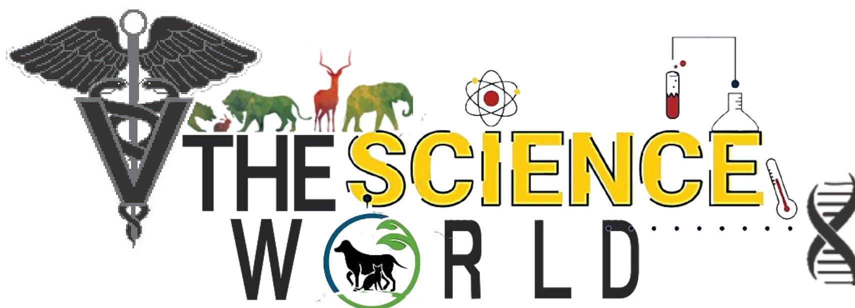

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Popular Article

Expansion Microscopy-Application and Advantages

Archana Choudhary, *[1] Satish[2] and Kanika Yadav[3]
[1]Department of Veterinary Clinic Complex, Arawali Veterinary College, Sikar,332403 Rajasthan India
[2]Department of Veterinary Gynaecology, Arawali Veterinary College, Sikar-332403 Rajasthan India
[3]PhD Scholar, Division of Veterinary Surgery and Radiology
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Introduction

In optical microscopy, fine structural details are resolved by using refraction to magnify images of a specimen. The development of super-resolution microscopy is one of the most revolutionary breakthroughs in molecular and cellular biology in the 21st century. Super-resolution microscopy has provided researchers with powerful new means to overcome the physical diffraction limit. Expansion microscopy (ExM) is an emerging technology initially developed in the Boyden Laboratory that overcomes limitations of optical super-resolution techniques by physically expanding tissue samples with a water-swellable polymer (Chen et al., 2015) by 4–5× linearly in each dimension. This allows super-resolution imaging of biological specimens with reagents and hardware that most biological laboratories already have access to. In addition, the fully expanded tissue-gel is more than 98% water by volume, thus rendering the specimen transparent and dramatically reducing optical aberrations even deep into tissue.

Definition

Expansion Microscopy is a method to magnify physically a specimen with preserved ultrastructure. OR Expansion microscopy (ExM) is a sample preparation tool for biological samples that allows investigators to identify small structures by expanding them using a polymer system. (Markoff, 2015). OR Expansion microscopy (ExM) is a recently invented technology that uses swellable charged polymers, synthesized densely and with appropriate

topology throughout a preserved biological specimen, to physically magnify the specimen 100-fold in volume, or more, in an isotropic fashion (Chen et al., 2015).

History

In 2015, Chen et al., all of MIT first described expansion microscopy as a method to enhance microscopy resolution by swelling a sample rather than using stronger microscopy equipment. Since then, the use of ExM has continued to grow. Due to the recency of development, there is limited information on applications. However, the most common modern use is in biological samples. In 2016, several papers were published detailing workarounds for ExM's traditional limitation of labeling probes. These changes proposed a way to use ExM with conventional microscopy probes, allowing it to be used more widely. In 2016, these new labeling methods were applied to allow fluorescence microscopy of RNA molecules, which in turn led to a new spatially precise in situ sequencing method, namely ExSeq (expansion sequencing), in 2021.ExM is a rapidly growing field, and there is hope that many biological mysteries will be elucidated using ExM techniques within coming years.

Purpose

Traditional light microscopy has limits of resolution that prevent it from reliably distinguishing small structures that are important to biological function and must instead be imaged by a higher-resolution technique, such as electron microscopy. For example, synaptic vesicles are 40- 50 nanometers in diameter, which is below the commonly quoted resolution limit of 200 nanometers for light microscopy. Expansion microscopy solves this problem by expanding the underlying tissue sample Fig 1 .

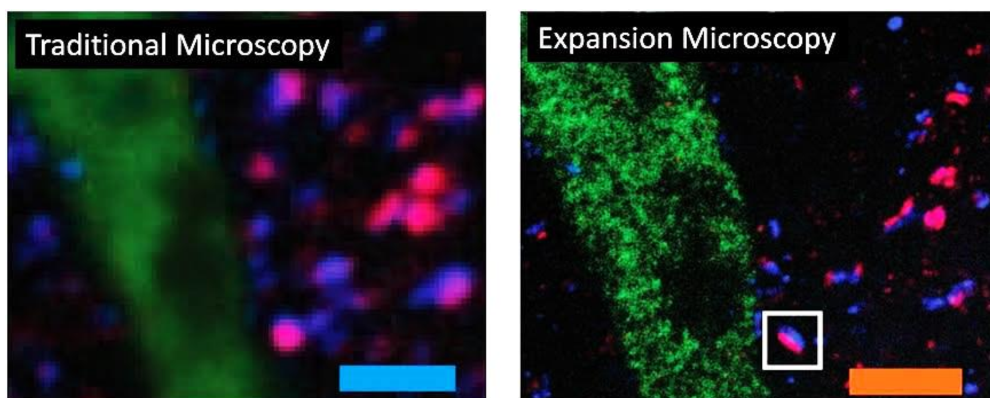

Figure 1 Traditional microscopy and Expansion microscopy

Principle

A swellable hydrogel is synthesized inside a tissue slice and then expanded. During the expansion, two biomolecules (red dots, Fig. 2) inside the tissue slice move apart. If the two biomolecules were 100-nm apart before the expansion, then conventional diffraction limited

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microscopy with a resolution of 250 nm cannot resolve these two biomolecules. However, the two biomolecules would be clearly resolved with the same microscopy after 3-fold expansion, which makes the distance between the two molecules 300 nm.

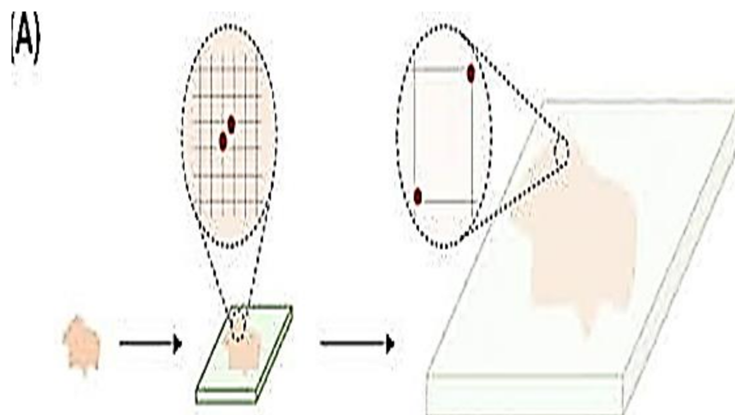
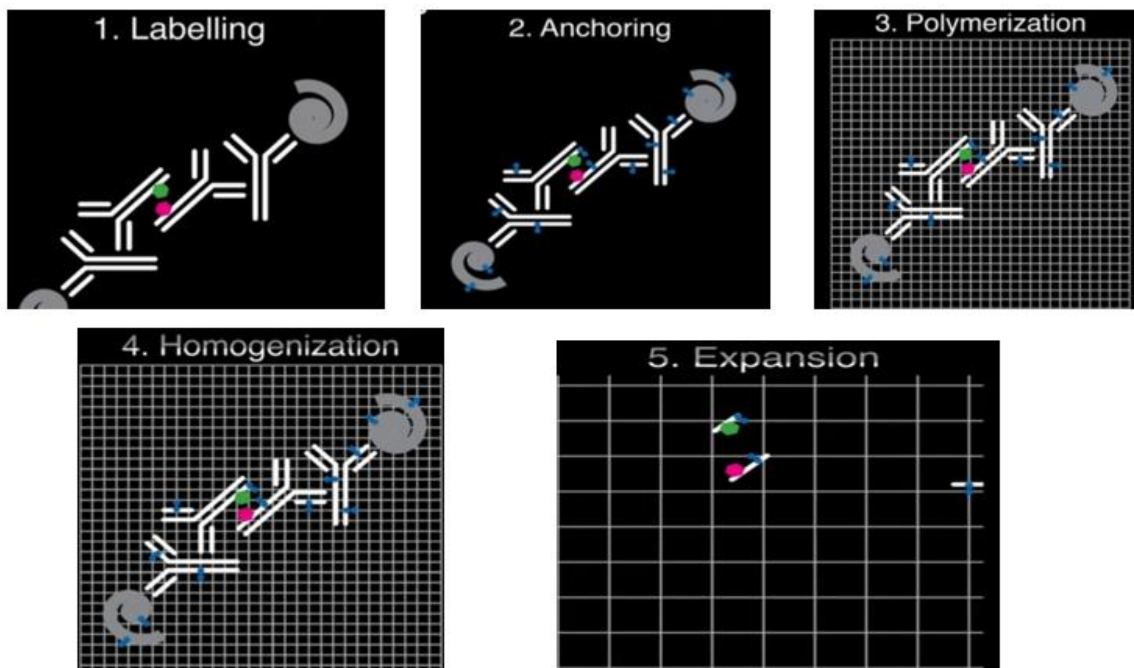


Figure 2 During the expansion, two biomolecules (red dots)

Expansion microscopy is a multistep process. The sequence of steps is:

1. Labelling
2. Anchoring
3. Gelation
4. Homogenization
5. Expansion



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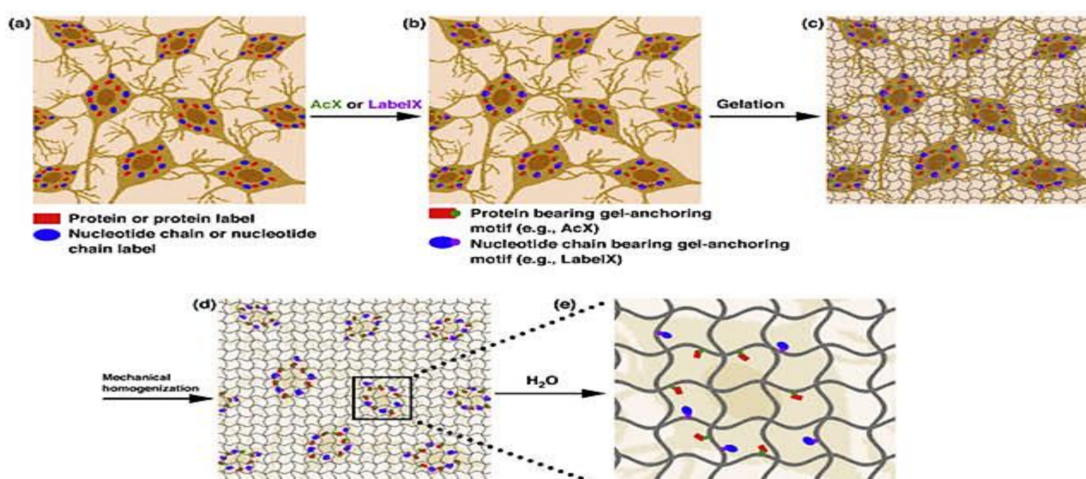
Fixed samples are first labeled with probes.

Next, the specimen is treated with compounds that bind to key biomolecules or labels (Ex: Acrydite , AcX , MA-NHS).It is done so they can be tethered to the hydrogel polymer chains synthesized in the next step.If this step fails, the gel will not expand uniformly because the cell will try together and prevent expansion. The digestion solution breaks down the proteins and allows expansion. A hydrogel made of closely spaced, densely cross-linked, highly charged monomers is then polymerized evenly throughout the cells or tissue, intercalating between and around the biomolecules or labels. Sodium acrylate, a monomer used to produce superabsorbent materials along with the comonomer acrylamide and the cross-linker N-N′-methylenebisacrylamide. After triggering free radical polymerization with ammonium persulfate (APS) initiator and tetramethylethylenediamine (TEMED) accelerator. Then the embedded specimen goes through a homogenization step involving denaturation and/or digestion of structural molecules. It can be done enzymatically (often with Proteinase K) or mechanically (i.e., softening the specimen by disrupting key protein-protein interactions that are not needed). If this step fails, the gel will not expand uniformly because the cell will try together and prevent expansion. The digestion solution breaks down the proteins and allows expansion. Following digestion, the sample is washed with low-salt buffer or pure water which pulls out all of the salt. This causes the sample to physically get bigger. Expansion causes the gel to be physically expanded in all directions, which causes the fluorophores that are attached to the gel to expand as well.

Variants of Expansion microscopy

1. **Protein retention Expansion microscopy (proExM)**
   - It is a simple, yet powerful, variant of ExM, in which proteins are anchored to the swellable polymer generated during the ExM process via a commercially available small molecule (which we call AcX for short).
   - AcX binds to amines on proteins and simultaneously to the polymer matrix.



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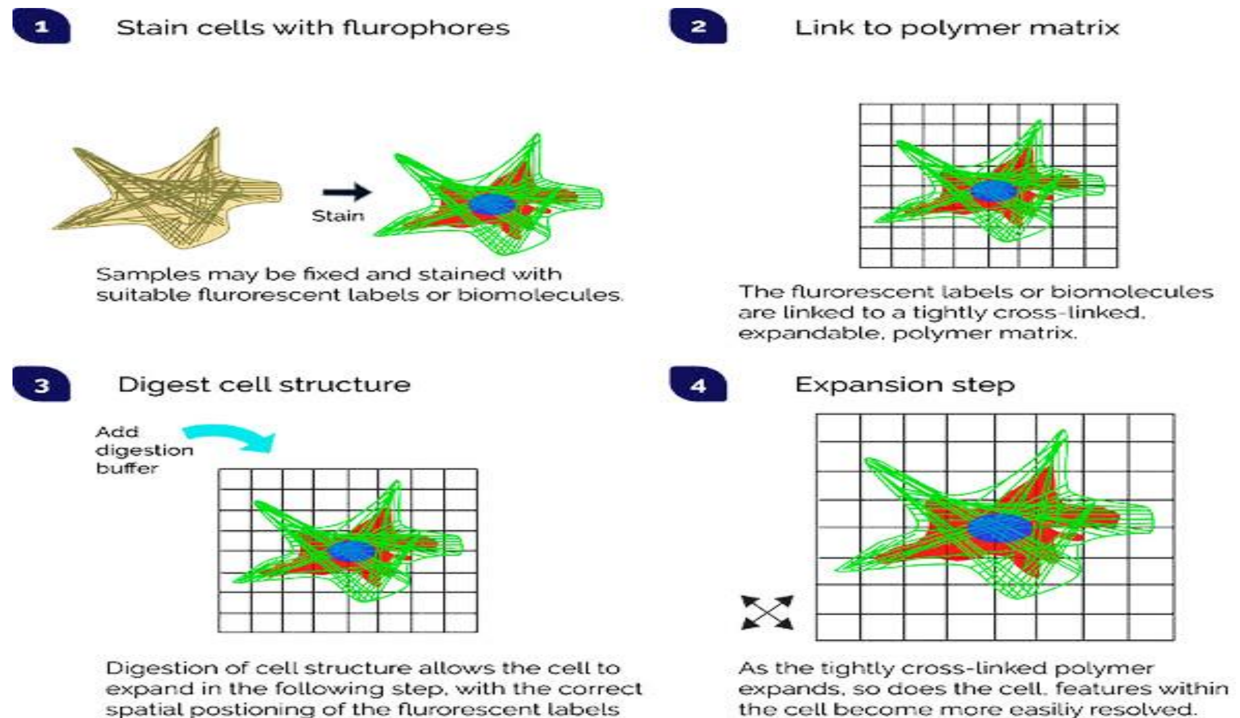
2. **Expansion Fluorescence in Situ Hybridization (ExFISH)**

- It is a version of ExM in which RNAs in a biological sample are retained during the ExM process.
- In ExFISH, RNAs are covalently anchored to the hydrogel with a small molecule that we call Label X (and which is made by mixing two commercially available reagents) that binds to guanine and also to the hydrogel.
- By applying the protein-anchoring reagent (AcX) simultaneously, you can anchor both RNAs and proteins to the hydrogel for dual protein/RNA visualization.
- For imaging, RNAs can be labeled with FISH probes after expansion.



3. **Iterative ExM (iExM)**

- It is a process where a biological specimen is first expanded by ExM, then a second swellable gel is formed in the space opened up by the first expansion, and then the sample is expanded a second time.
- This double expansion process results in a linear expansion factor of about ~4.5 × 4.5 = 20× and an effective resolution of ~25 nm after two rounds of expansion (larger than the expected ~300 nm/20 = 15 nm due to the size of the antibodies and DNA linkers).
- iExM is sufficient for resolving proteins within synapses in 3D, as well as very fine parts of neurons (such as dendritic spine necks).

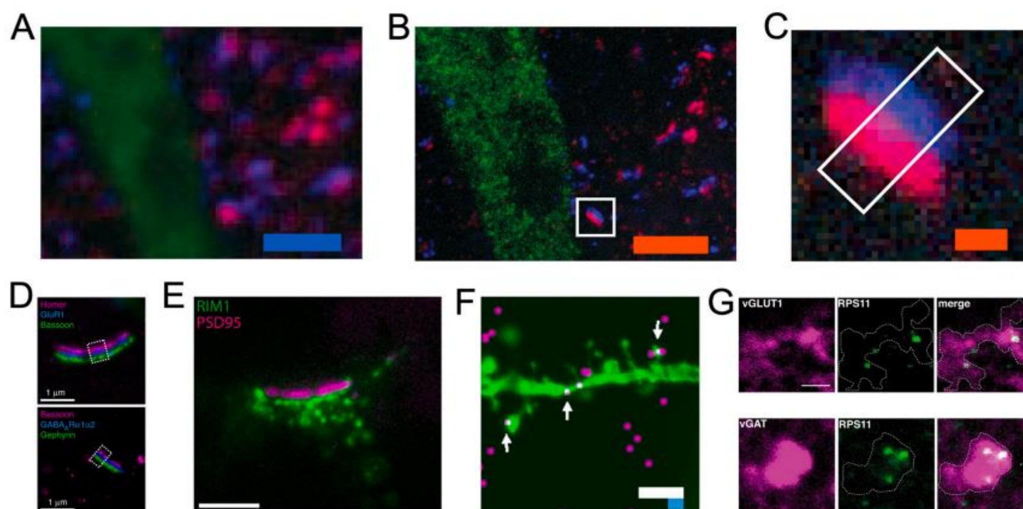
Applications

Disease diagnosis

- This process can be used for optical diagnosis of kidney minimal-change disease, early breast neoplastic lesions and to spot the difference of normal human tissue specimens to cancer tissue specimens, enabling a routine use of clinical research.
- This imaging reveals sub-diffraction limit sized features of the intermediate filament's keratin and vimentin, critical in the epithelial mesenchymal transition, cancer progression and initiation of metastasis.

Neuroscience

- ExM magnifies biological specimens such as brain circuits and allows them to be more easily mapped.
- Biomolecules, such as proteins and nucleic acids, are anchored to the polymer, which is then swelled in order to expand the biomolecules. Due to the increased distance between the biomolecules, ordinary microscopes can then perform nanoscale resolution imaging.
- Through the use of ExM technique, neuroscientists can more easily map images of synapses, cells, and neural circuits.



Advantages

One key advantage is that it also allows investigators to stain for and visualize particular molecules in the sample, such as specific proteins or RNA to identify their density and distribution in relation to the biological structures of interest. The most beneficial principle of expansion microscopy is that it requires no specialized equipment.

Conclusions and future directions

Expansion microscopy is a unique approach to super resolution imaging, enabling rapid and easy nanoscale imaging of specimens in 3D. Samples are optically clear which reduces the effects of diffraction and scatter. This enables greater imaging depth with minimal introduction of optical aberrations. Multicolor applications of ExM are possible with minimal constraints on fluorophore choice. Recent innovation of this technique has extended the range of biomolecules and labelling approaches compatible with it. However, with all of these advances its incompatibility with live cell imaging, remains. It has been suggested that higher expansion factors and therefore resolution may be achieved and would supersede other super resolution techniques. If a unified protocol could be developed, imaging of DNA, RNA, proteins and lipids may be combined to reveal organization of heterogeneous complexes.

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