

# Evaluation of direct grafting strategies in Expansion Microscopy

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**Introduction:** Expansion microscopy, introduced in 2015, enables nanoscale imaging on a conventional microscope via physically and isotropically expanding specimens, achieving ~70 nm resolution.

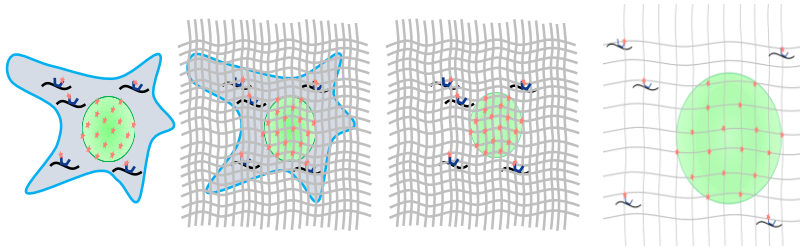


Fig. 1. The concept of expansion microscopy

**Issues:** 1. Poor signal retention;  
2. Time-consuming

**Methods:** Trifunctional linkers (TRITON): allowing simultaneous targeting, labeling and grafting of biomolecules.

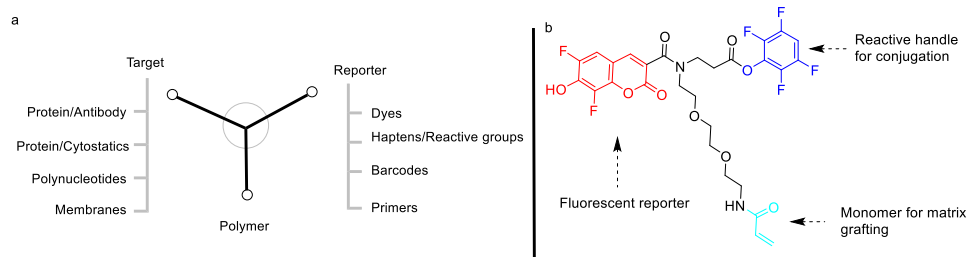
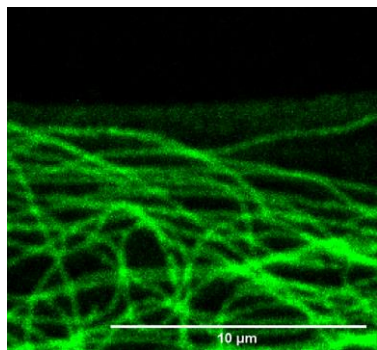


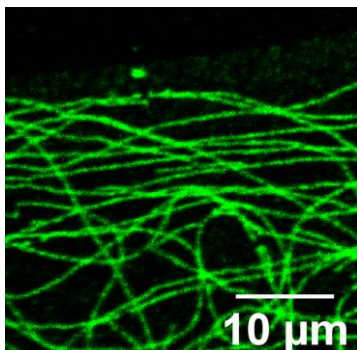
Fig. 2. The concept of TRITON

## Results:

### 1. Direct immunostaining method (microtubule)

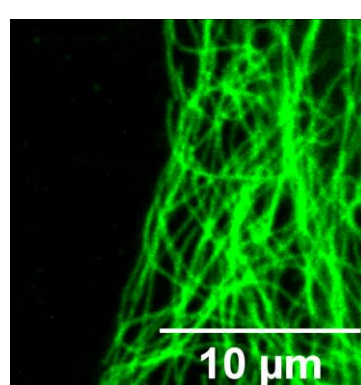


X4, Pre-image

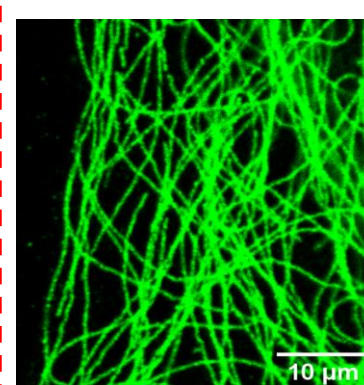


X4, Post-image

### 2-1. Indirect immunostaining method (microtubule)

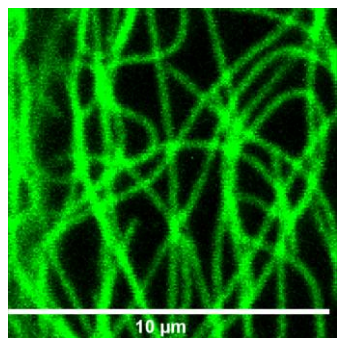


X4, Pre-image

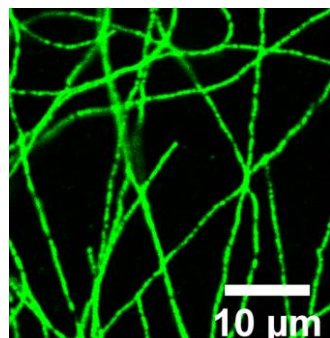


X4, Post-image

### 2-2. Indirect immunostaining method (microtubule)

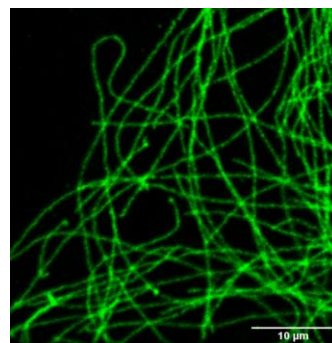


X10, Pre-image

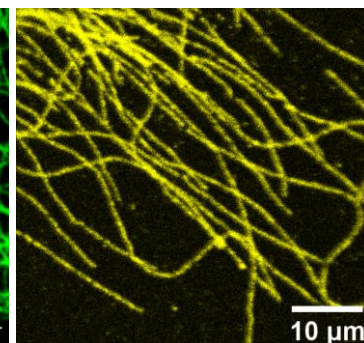


X10, Post-image

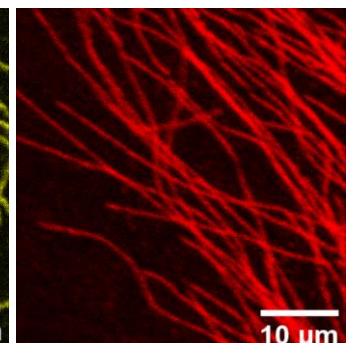
### 3. Post-gelation labeling (microtubule)



Atto 488

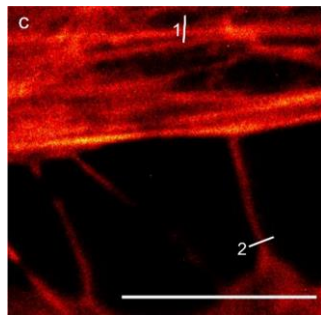


Cy 3

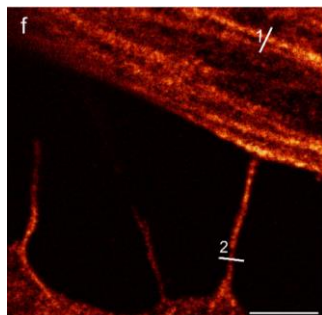


Cy 5

#### 4. Direct cytoskeleton staining (Actin)

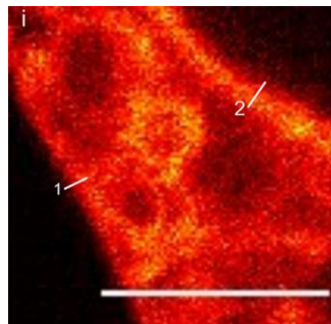


X4, Pre-image

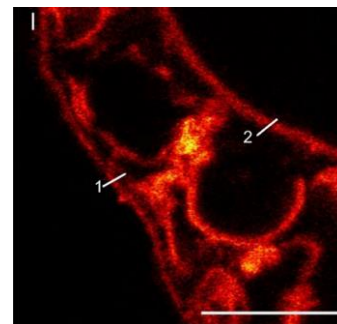


X4, Post-image

#### 5. Membrane staining



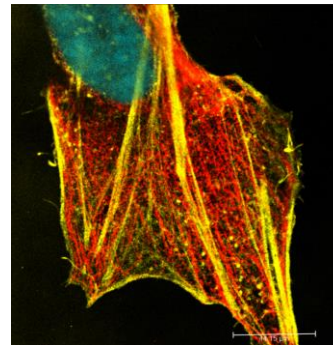
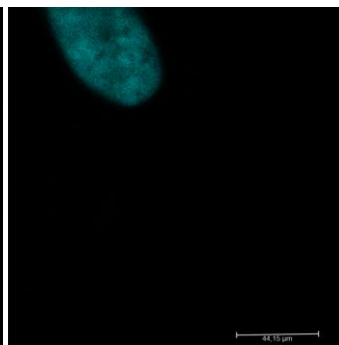
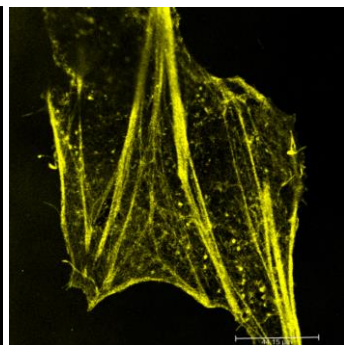
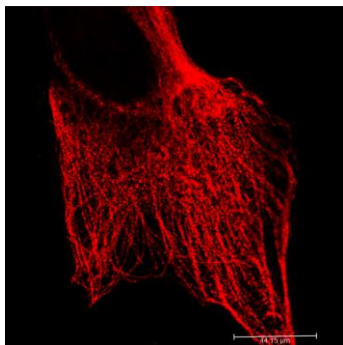
X4, Pre-image



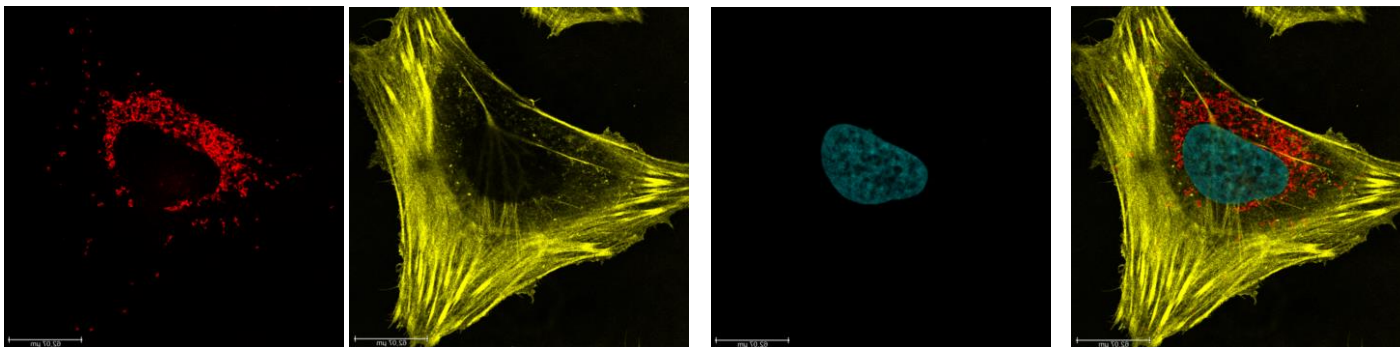
X4, Post-image

#### 6. Multi-color staining

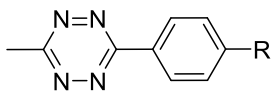
a. post-image: microtubule, actin and nucleus



## b. post-image: mitochondria, actin and nucleus



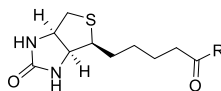
The portfolio of TRITON for post-gelation labeling



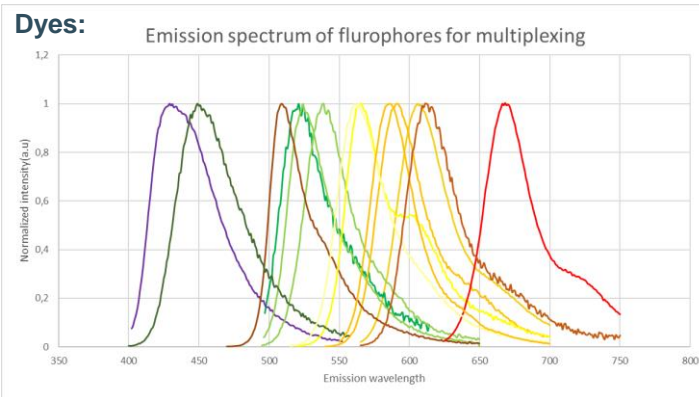
Tetrazine



Azide



Biotin



## Conclusions:

**a.** Targeting, labeling and grafting of biomolecules in one step; **b.** Allowing different labeling strategies and targeting biomolecules; **c.** Be compatible with x4 and x10 ExM; **d.** Achieving a resolution of 46 nm in x10 ExM.

**Ref:** Wen, Gang, et al. "Evaluation of Direct Grafting Strategies via Trivalent Anchoring for Enabling Lipid Membrane and Cytoskeleton Staining in Expansion Microscopy." *ACS nano* (2020).