

# Ultrastructure of Plasmodesmata by Electron Tomography



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Brocard



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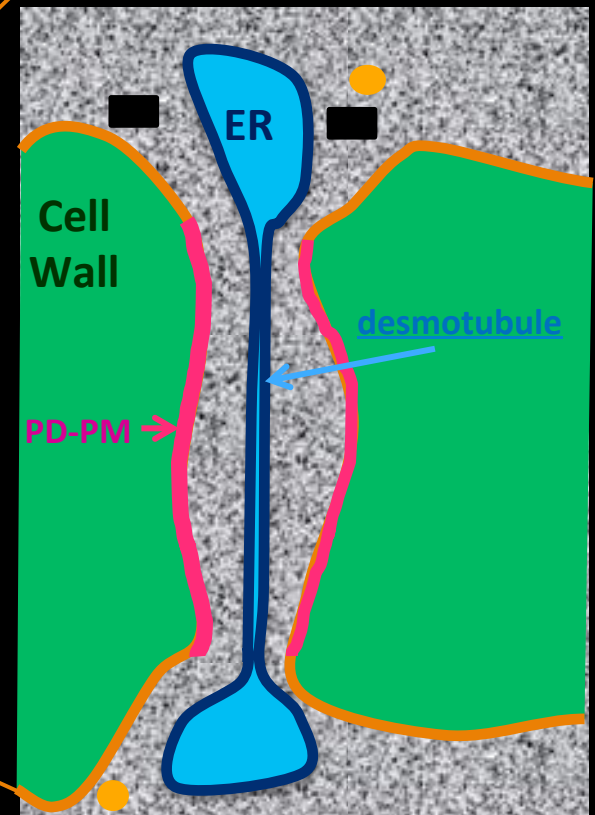
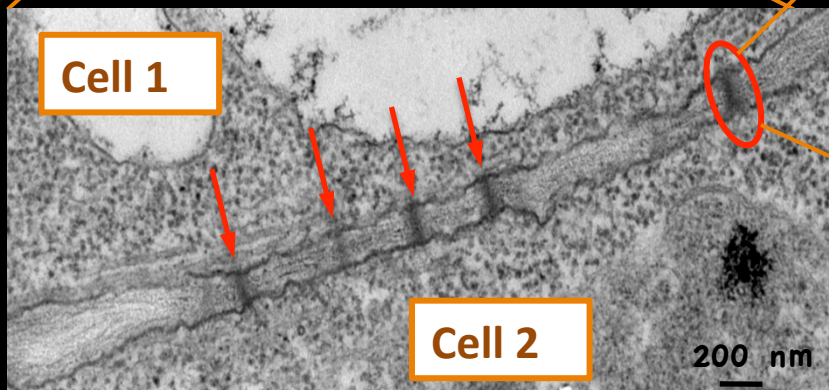
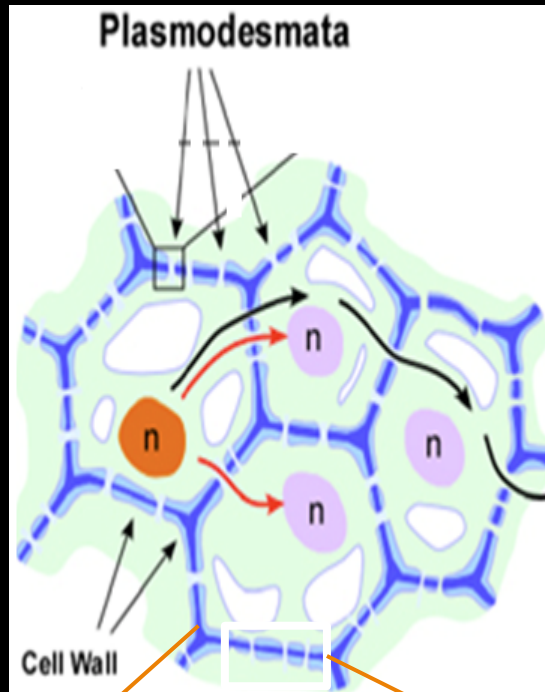


Emmanuelle  
Bayer

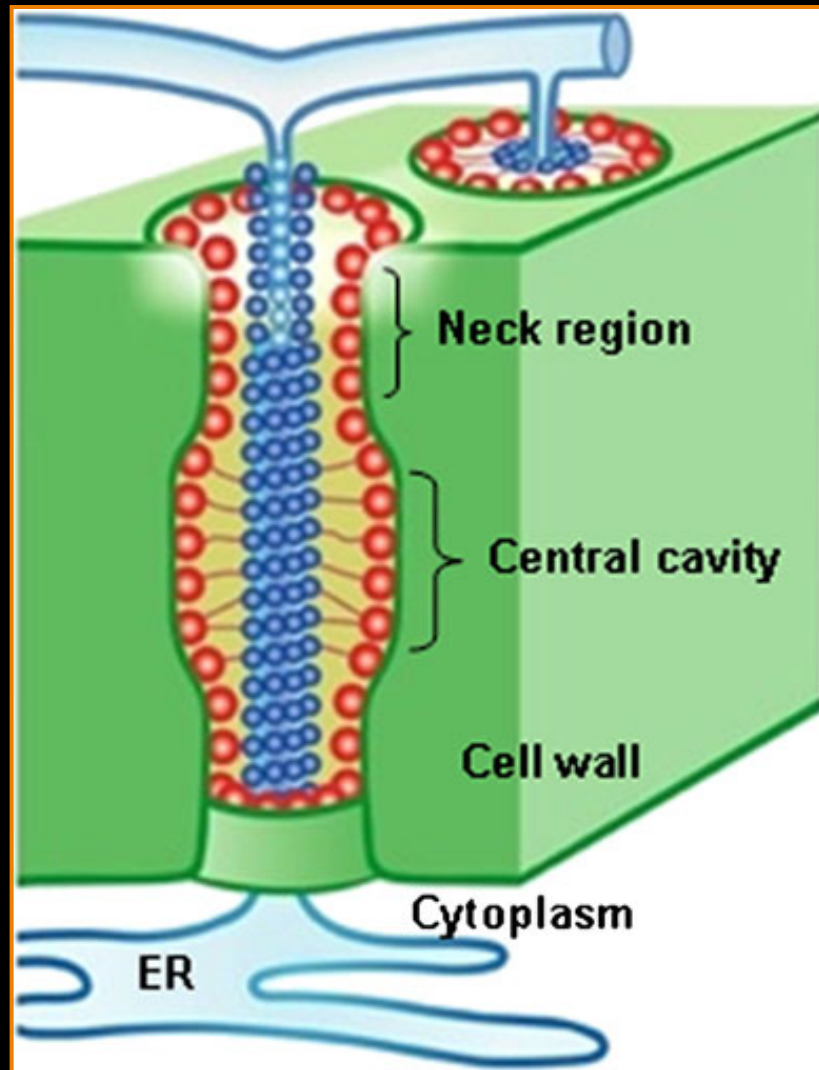


# Plasmodesmata are channels connecting plant cells

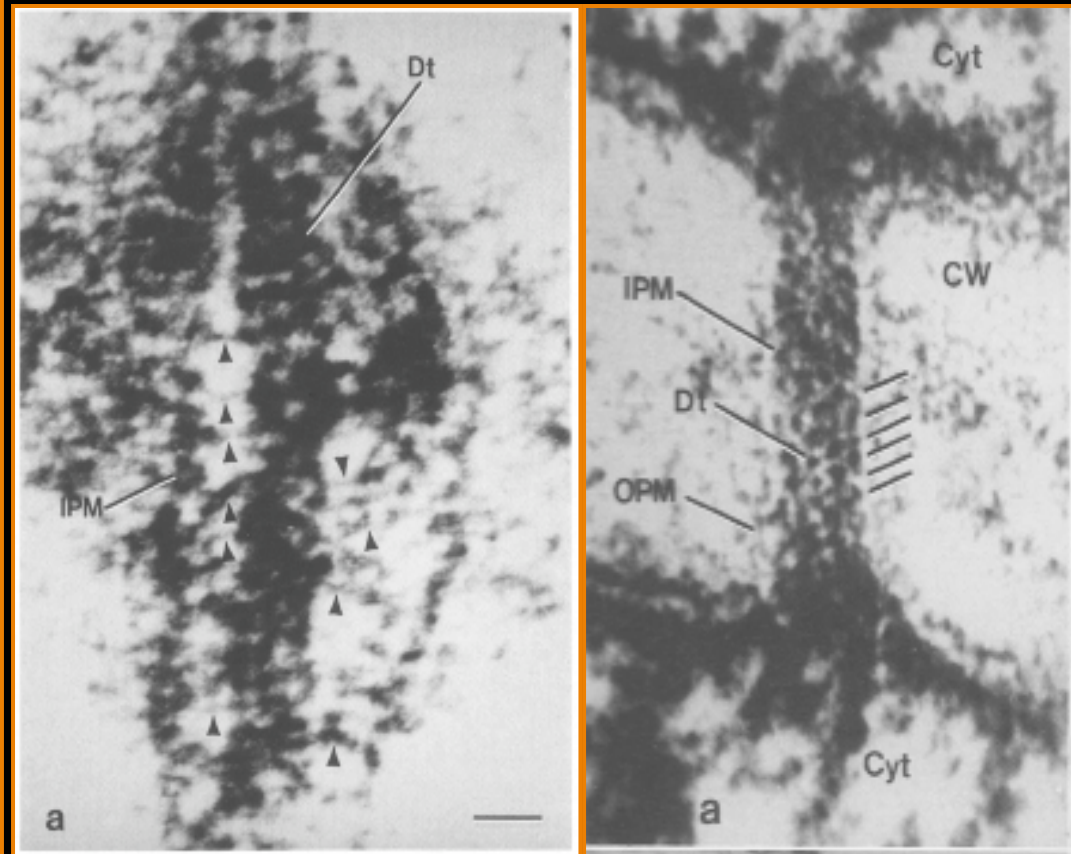
- ▶ Key elements of cell-to-cell communication
- ▶ Implicated in several key processes
- ▶ Dynamic structures



# The ultrastructure of plasmodesmata



Bell et Oparka, 2011

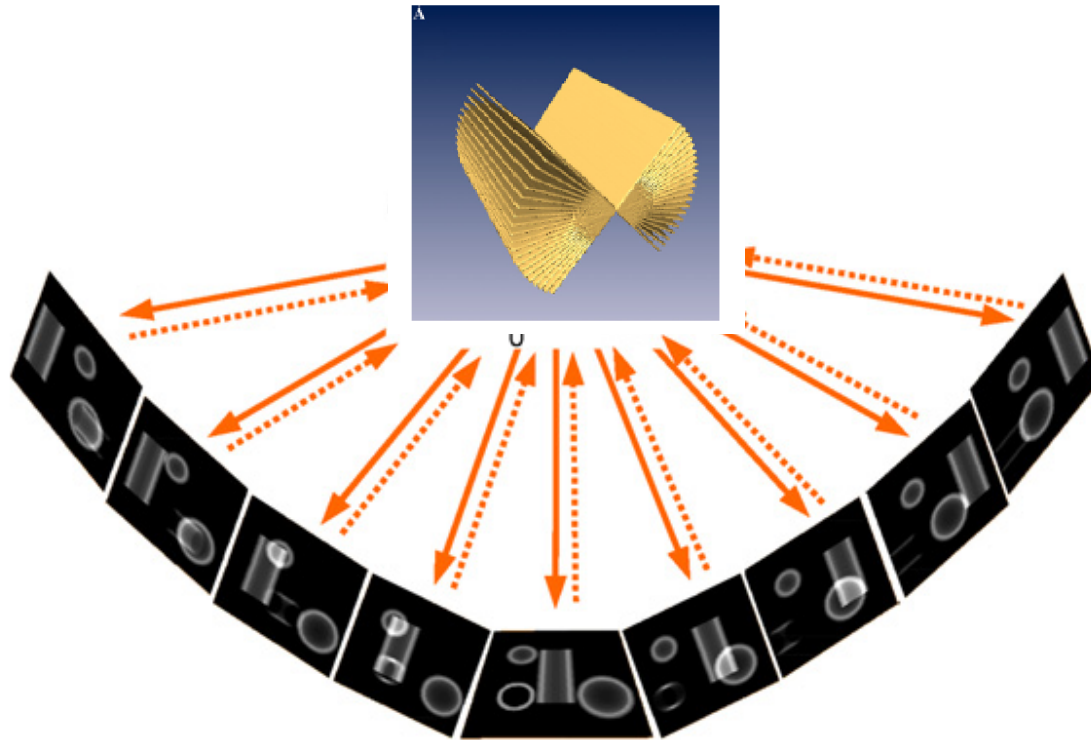


Ding et al., 1992

# Electron Tomography

(B)

Faisceau  
d'électrons



M. Bárcena and A J. Koster 2009

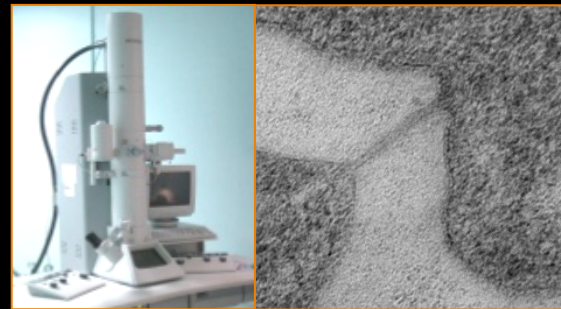


# Different Steps are required for ET

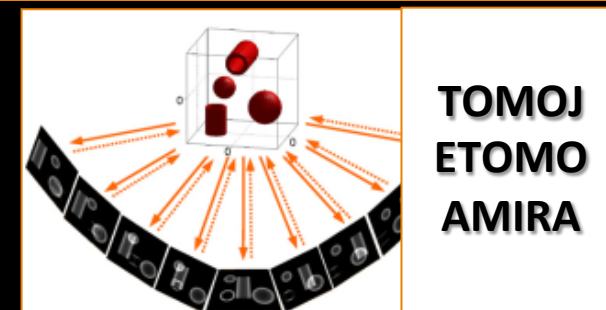
## 1. Sample Preparation: cryo-methods



## 2. Acquisition

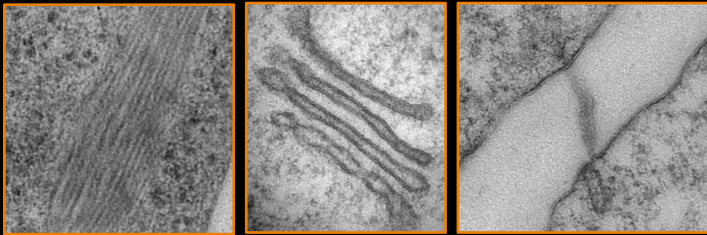


## 3. Alignment/Back-projection=3D view



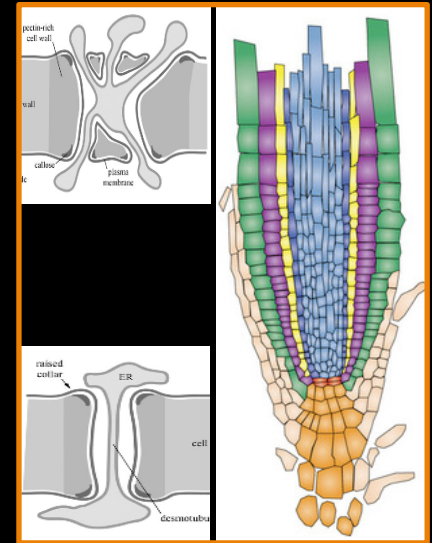
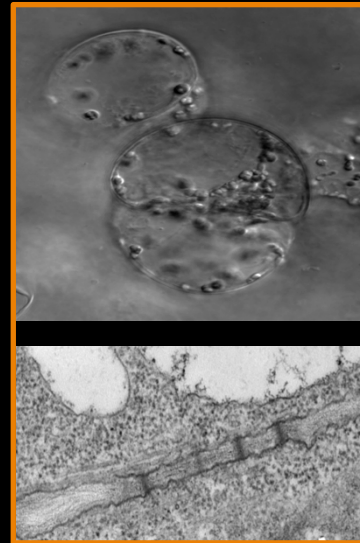
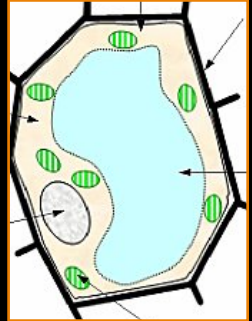
# Sample Preparation

- Best preservation is obtained by **HPF** because:
  - instantanetly
  - no pH and Osmolarity artefact
- ➔ Membrane and cytoskeleton preservations are optimized
- **Optimized Freeze Substitution**
- ➔ Appropriate staining



# Materials

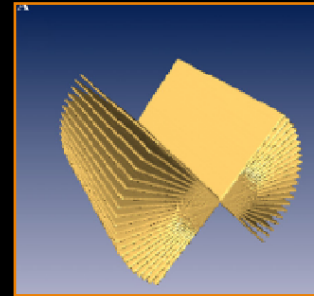
- Plant cell specificities:
  - vacuole
  - cell Wall
  - large size
- Cells in suspensions and roots instead of Leaves



# Acquisition

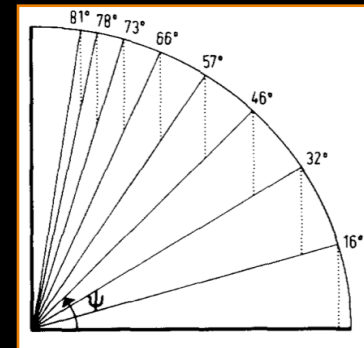
- TEM alignment, eucentric position

- Max tilt range:  $+70^{\circ} \rightarrow -70^{\circ}$



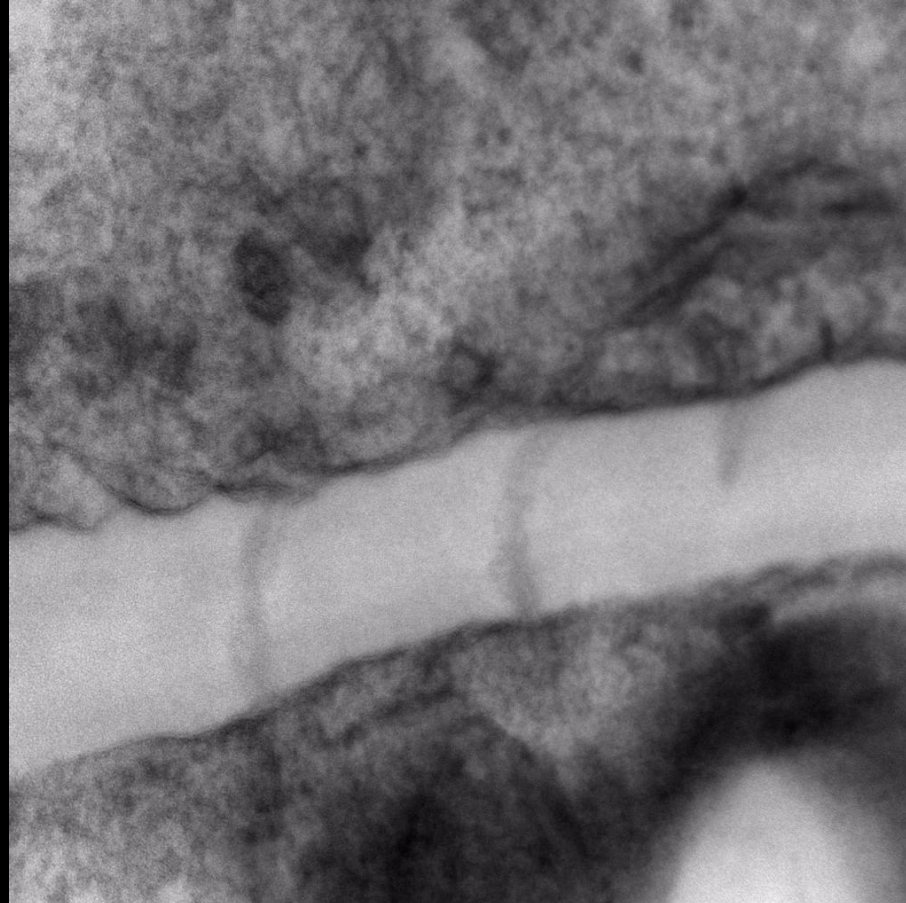
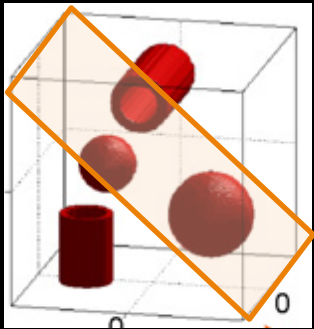
- Sections thickness

- **Linear tilt** or **Saxton tilt scheme**



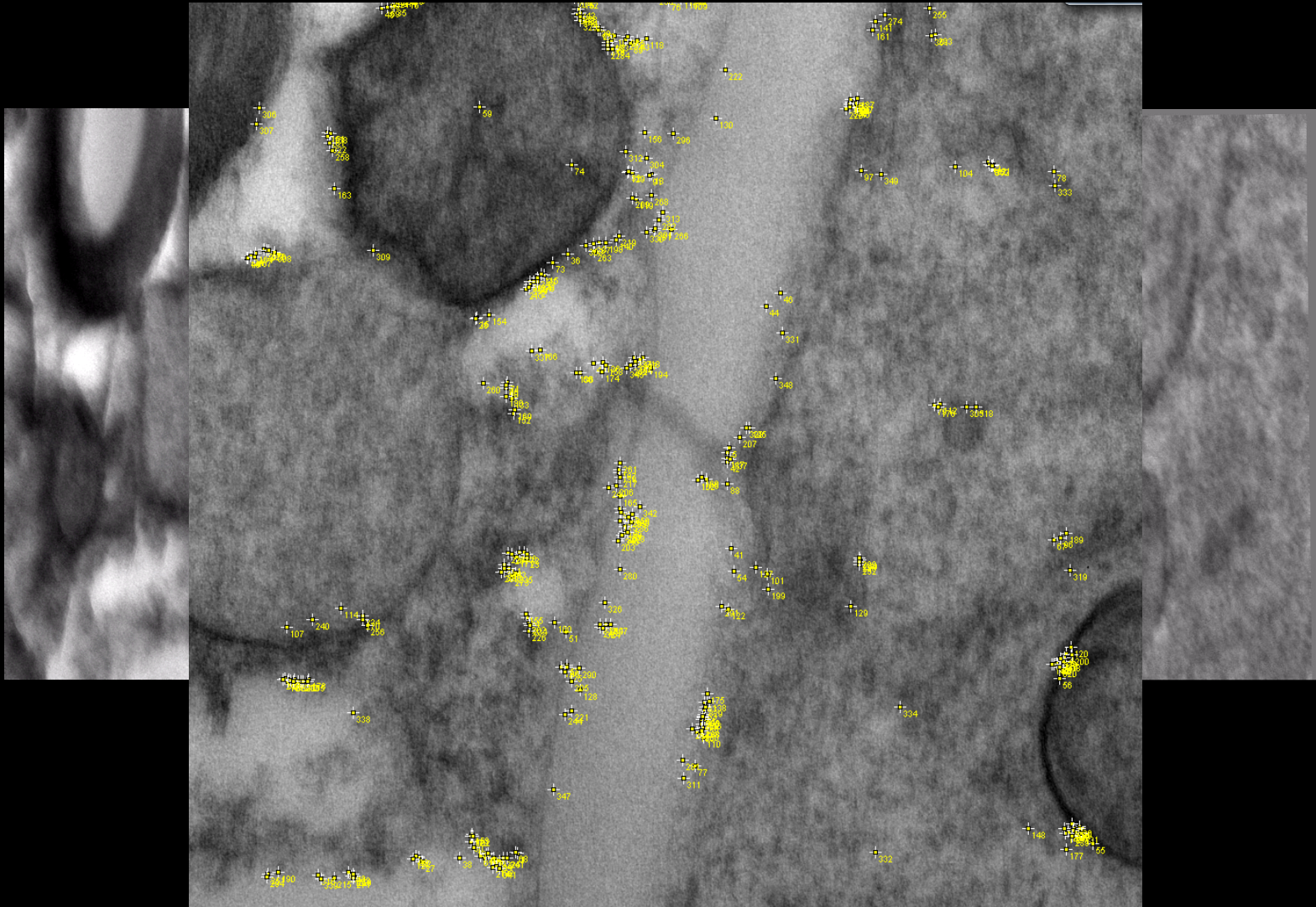
➔ Resolution =  $f(\pi \cdot \text{thickness} / \text{projection number})$

# Acquisition

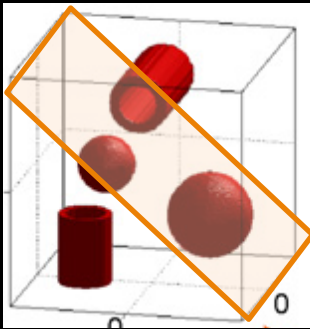




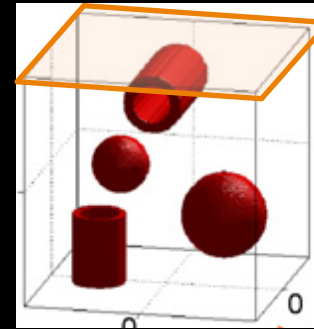
# Alignement



# Back-Projection

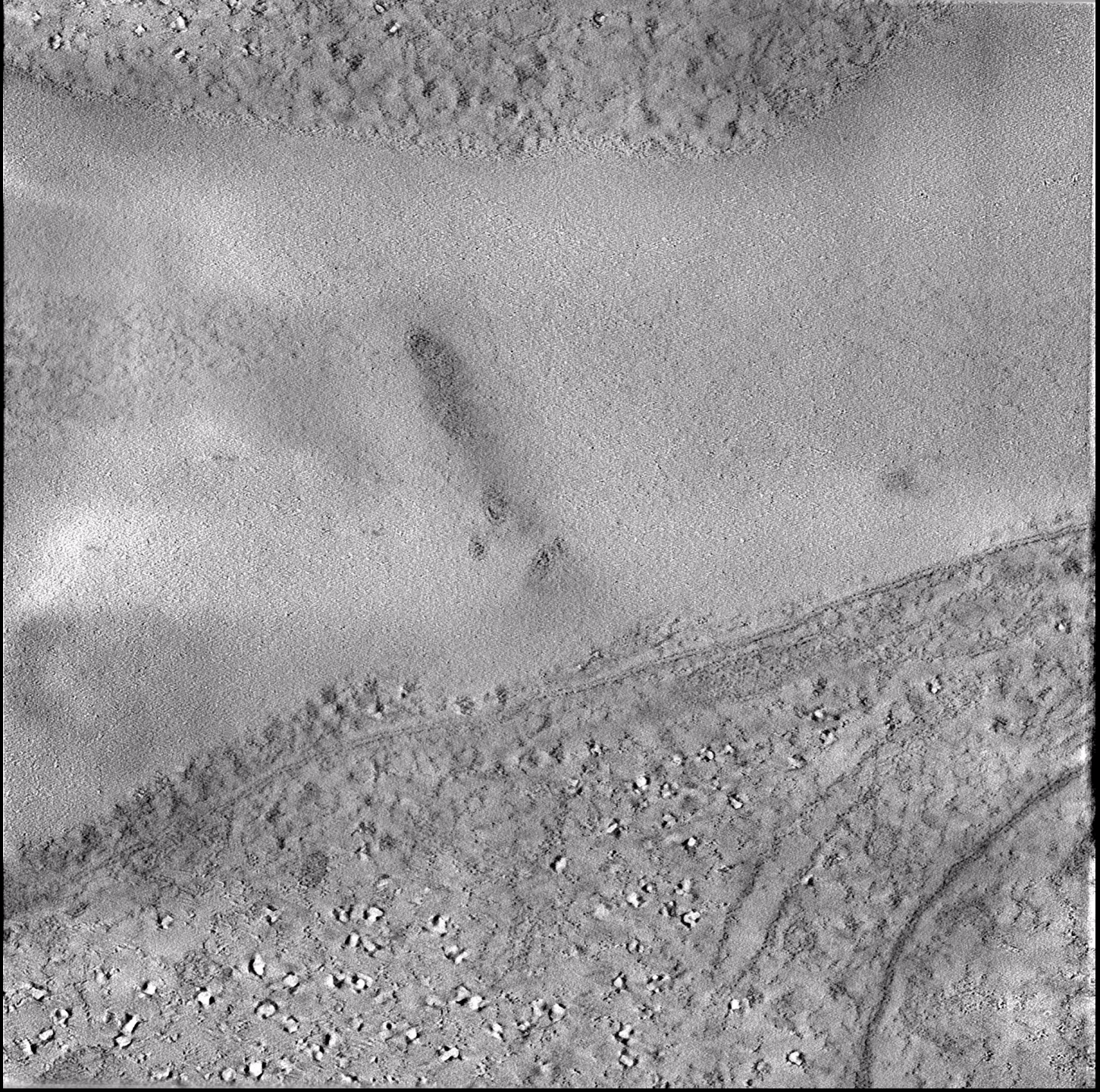
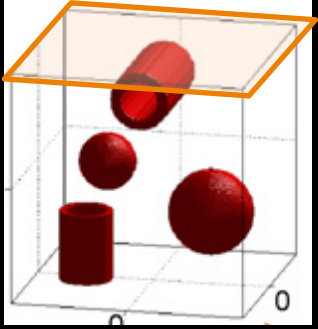


$(x, y, \theta)$

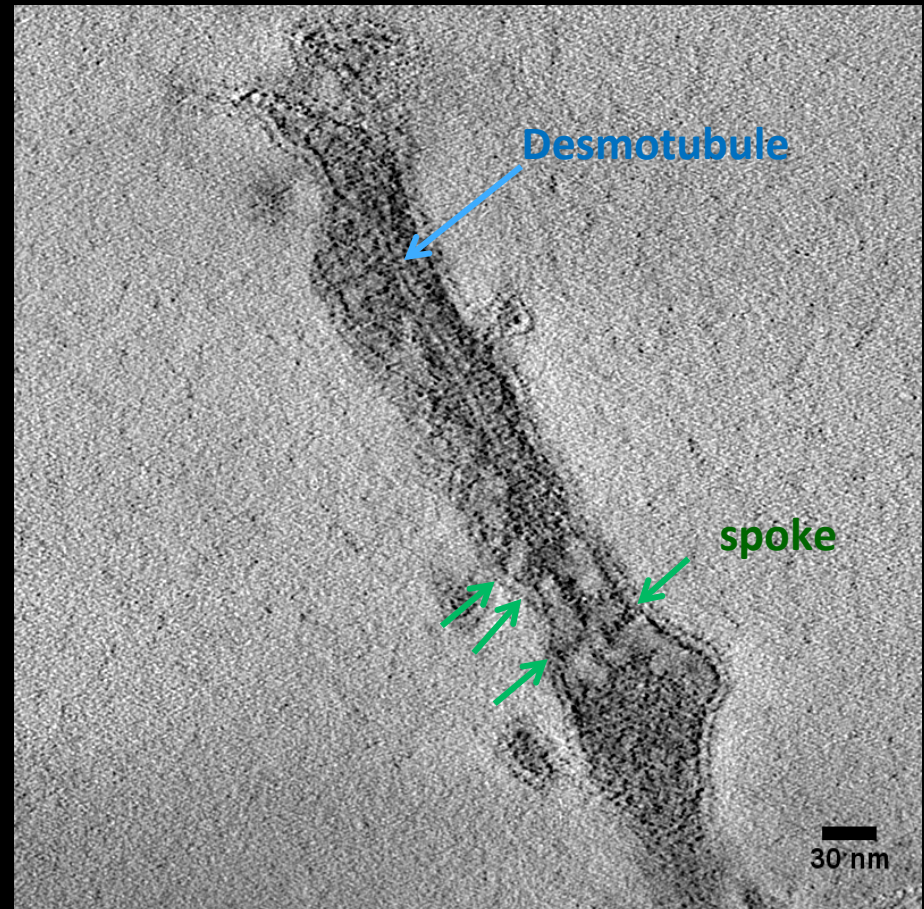
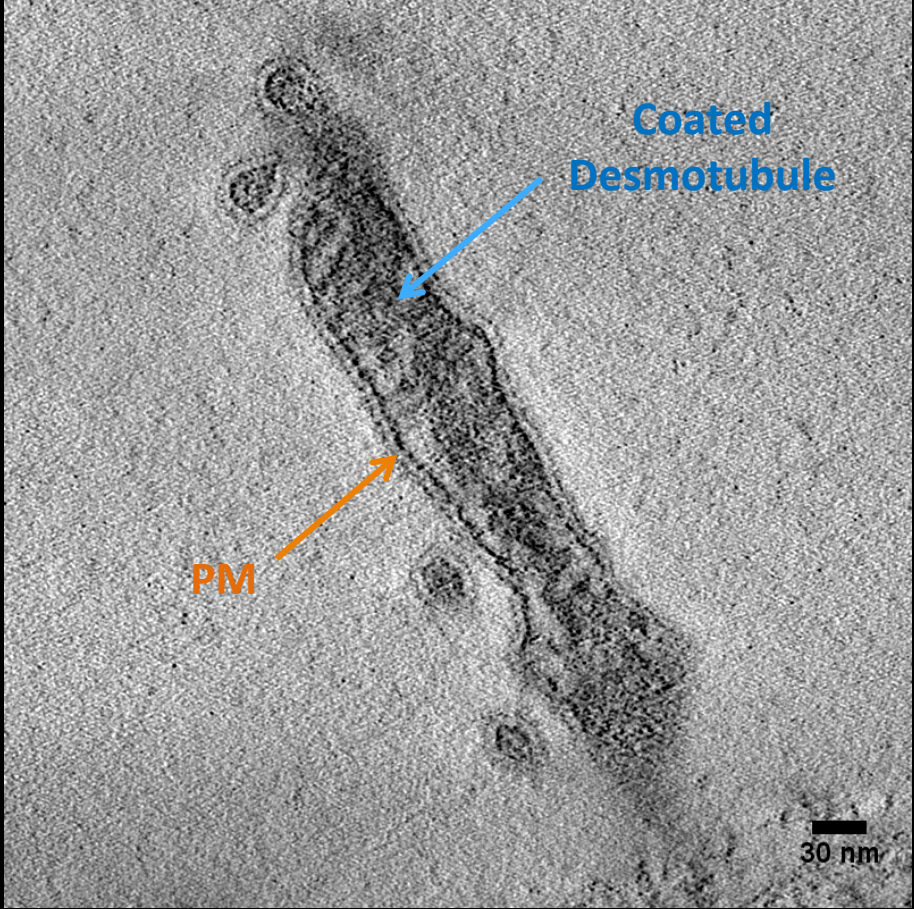


$(x, y, z)$





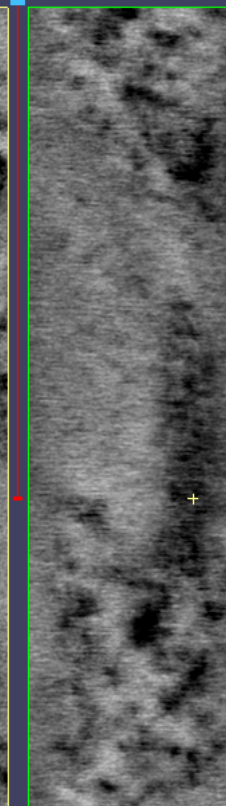
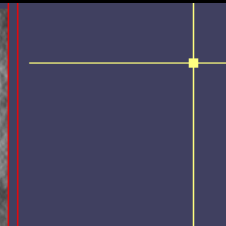
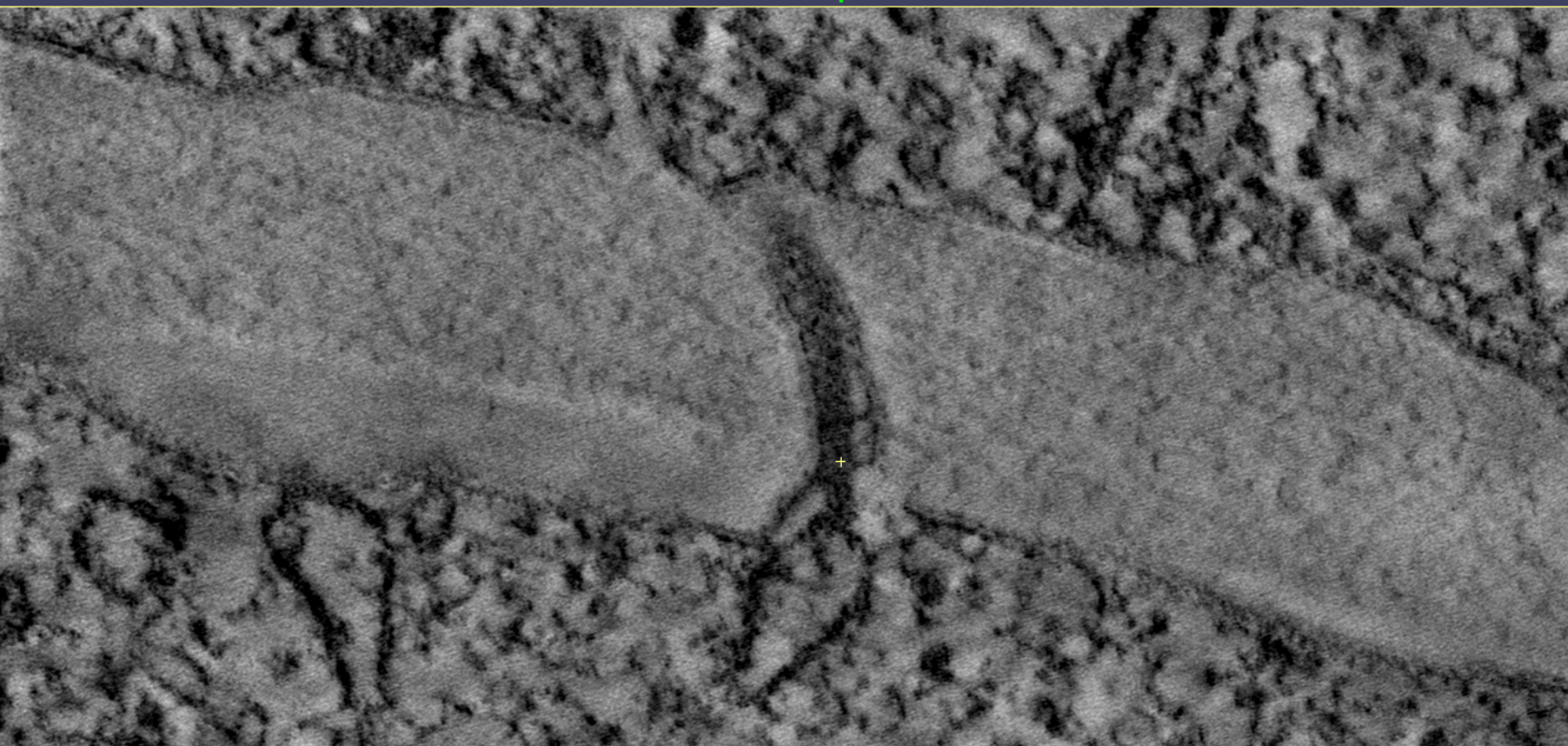
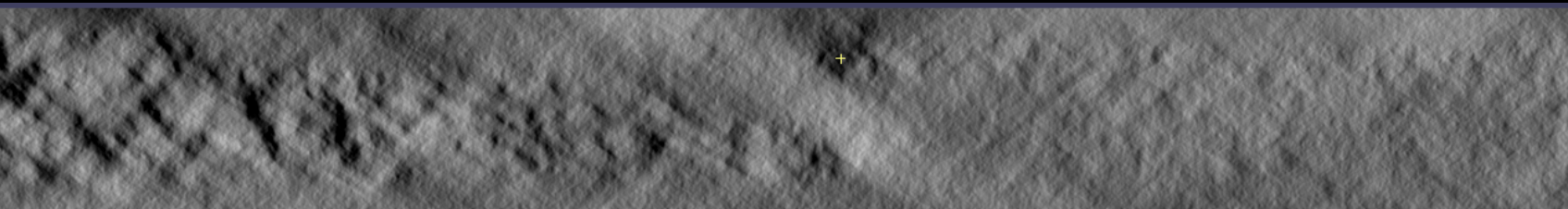




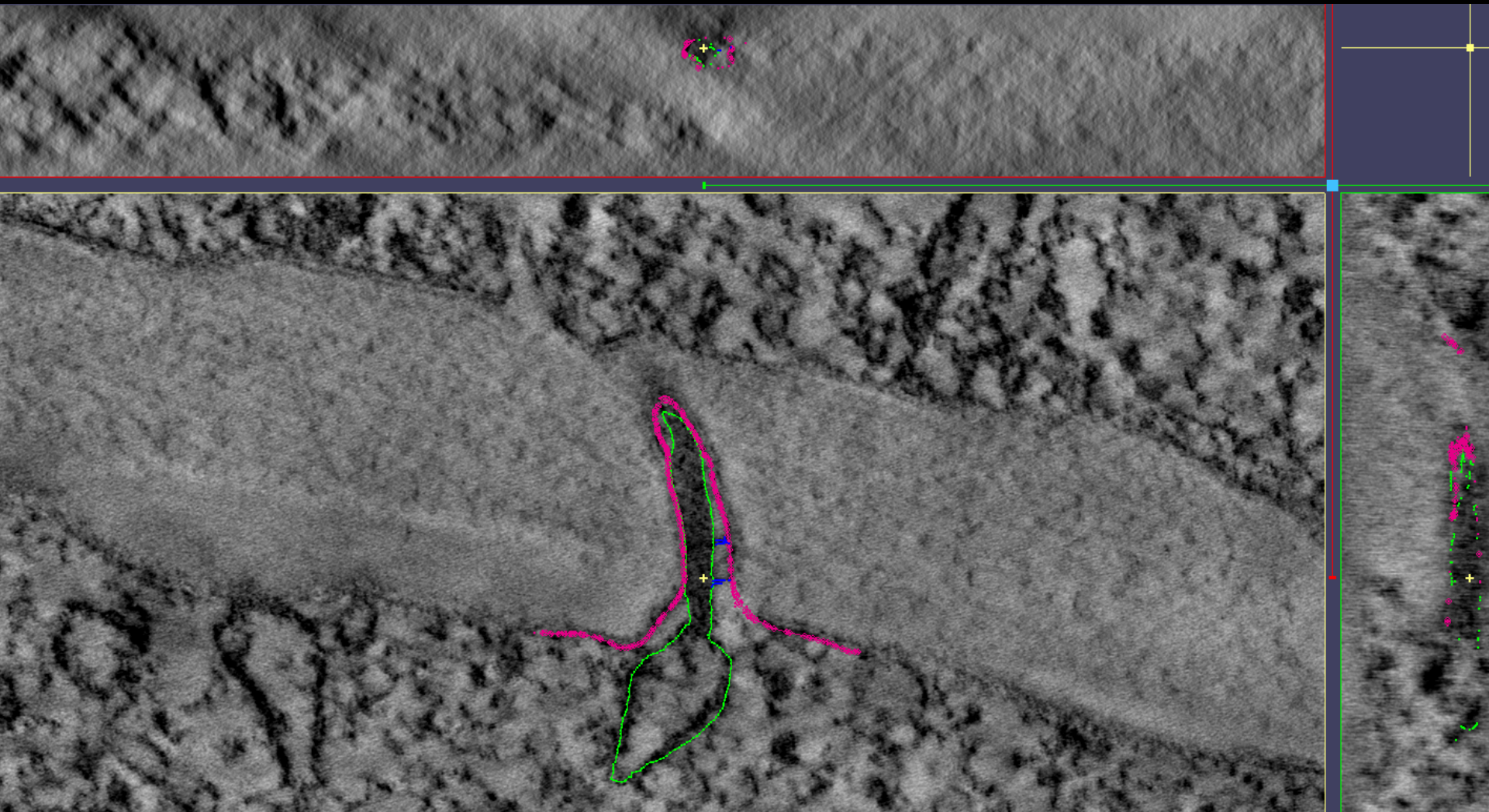
*sections of reconstructed tomogram*



# 3D-View

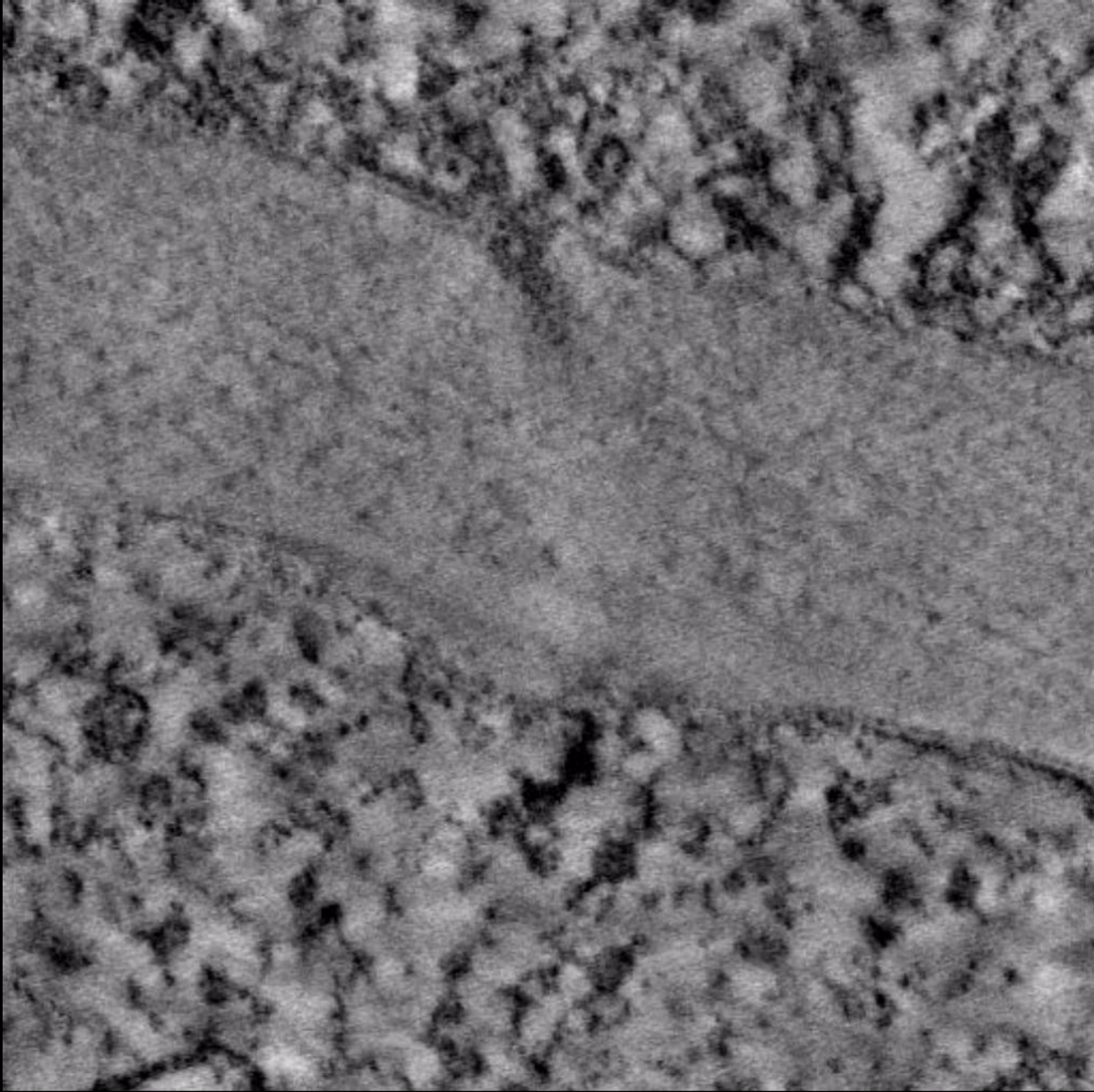


# Segmentation

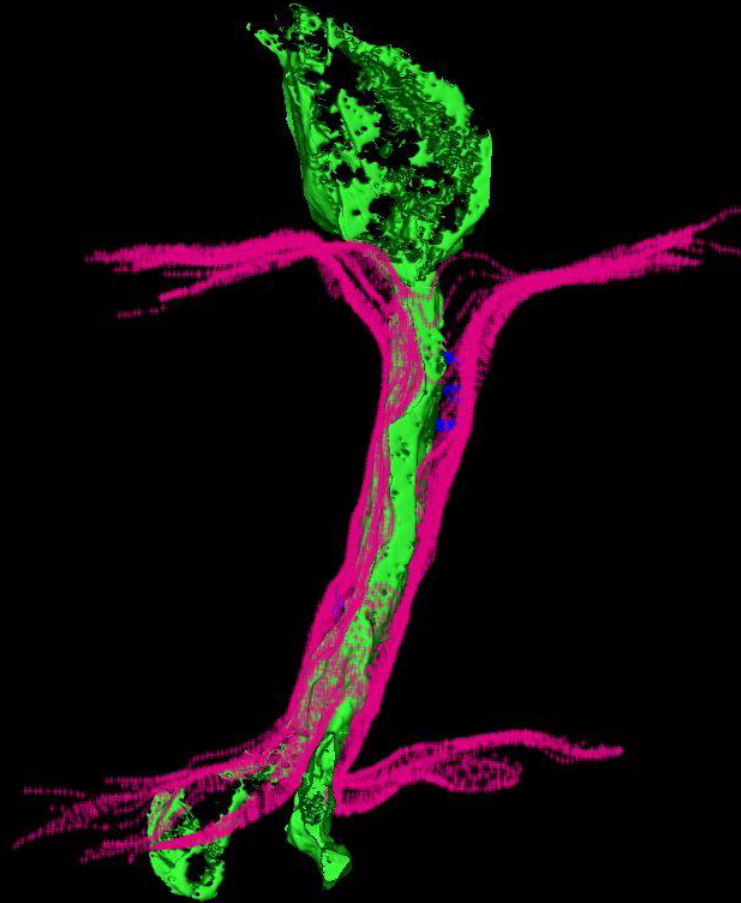




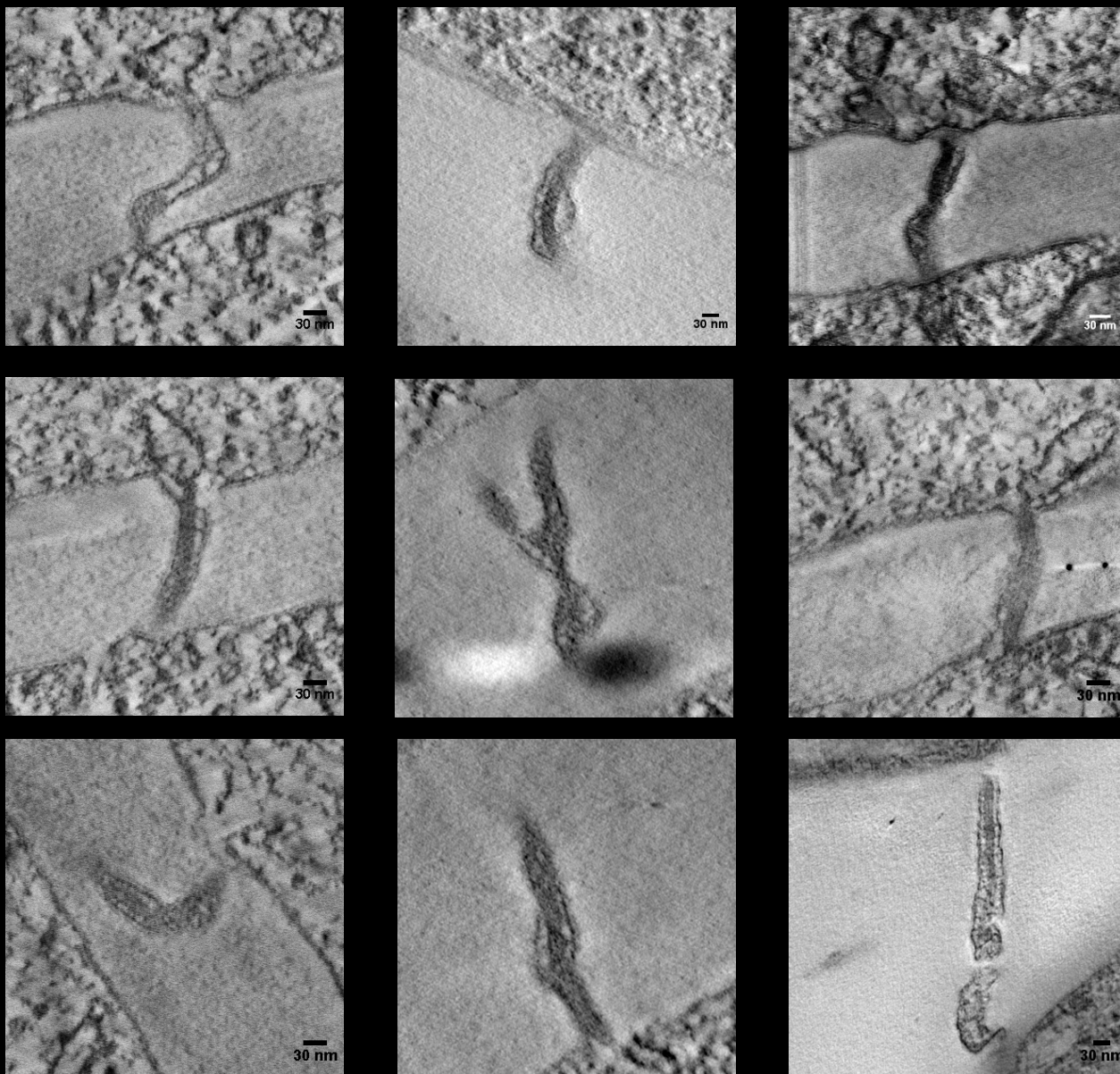
# 3D-View



# 3D-View



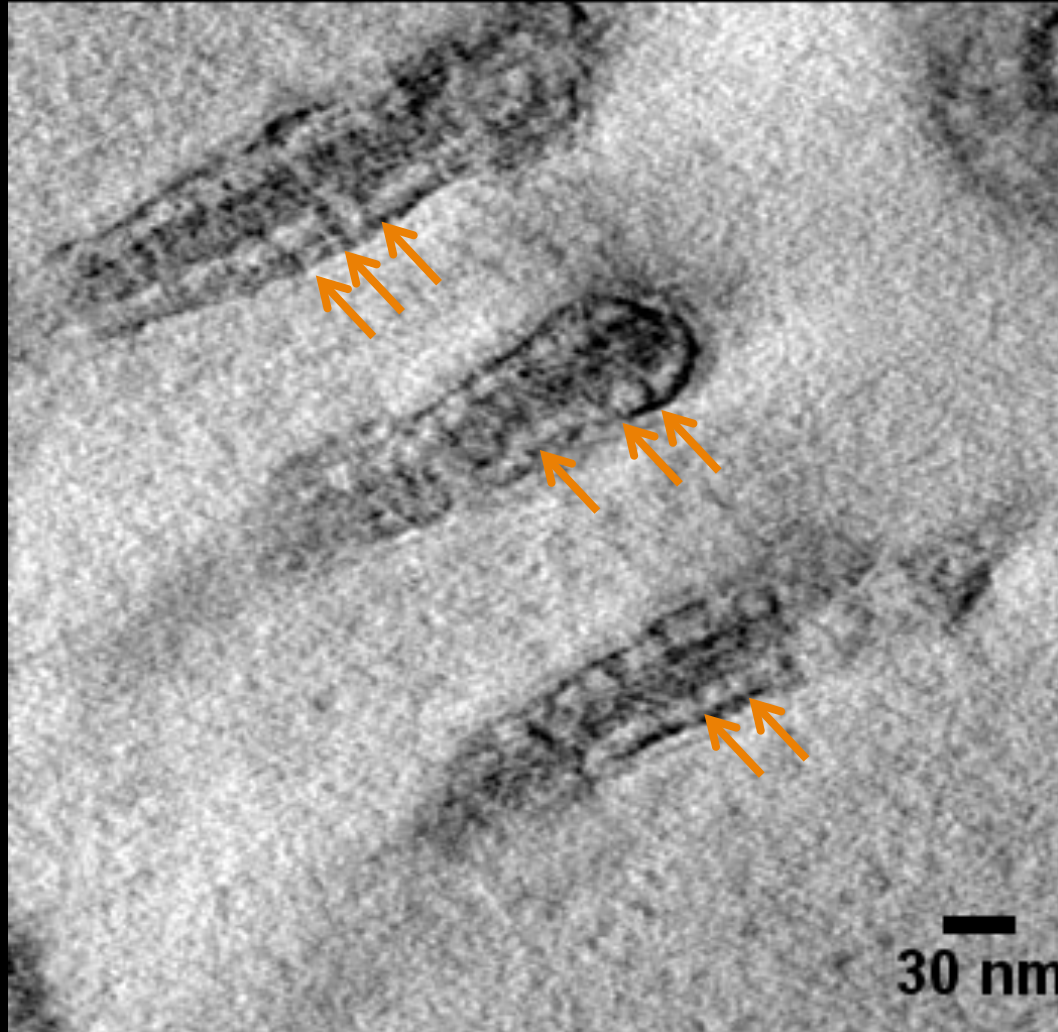




—  
60 nm

*sections of reconstructed tomogram*

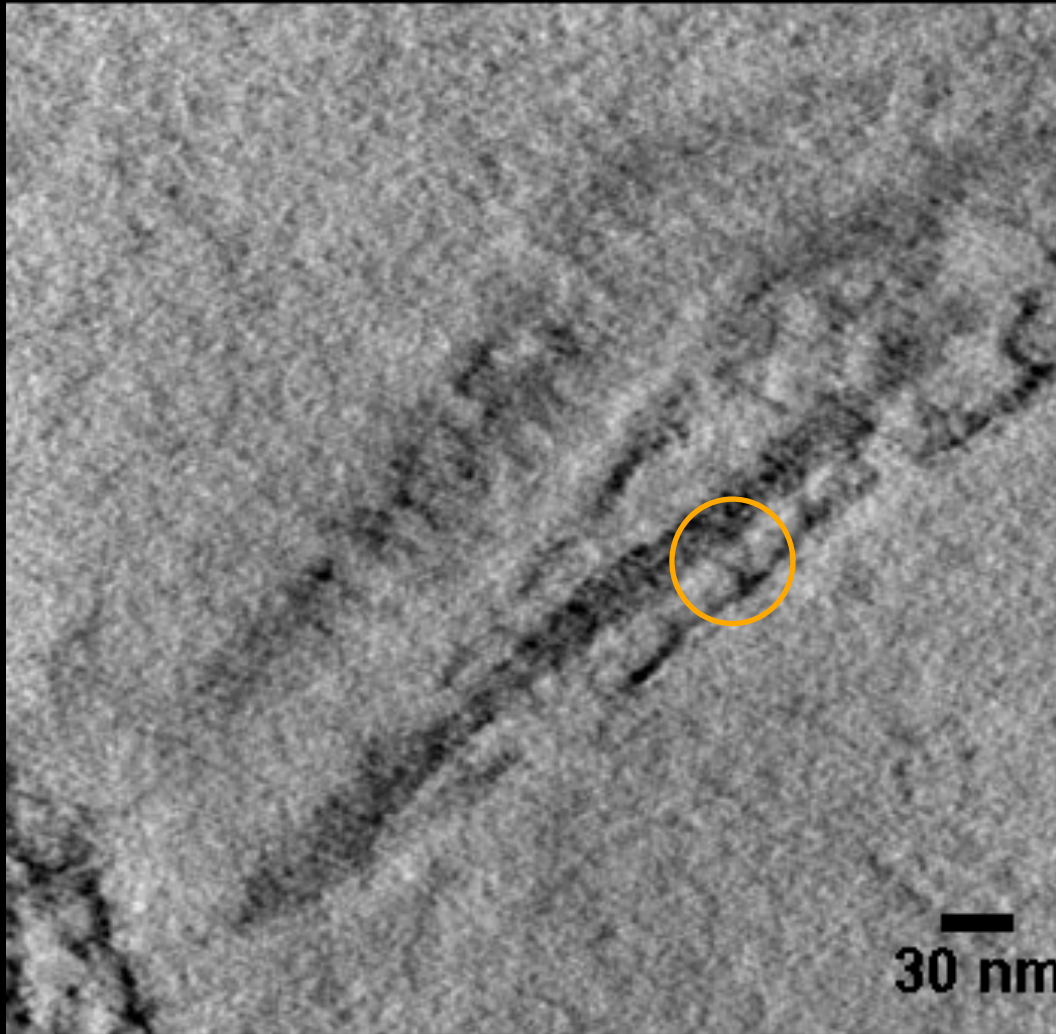
# Dt-PM Spokes



*sections of reconstructed tomogram*

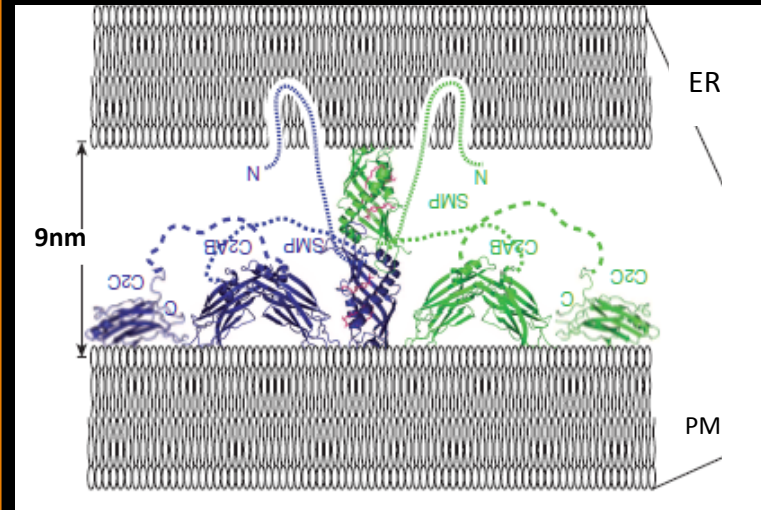


# Dt-PM Spokes

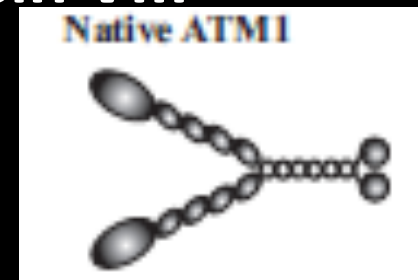


sections of reconstructed tomogram

## SYTA (Schauder et al., 2014)

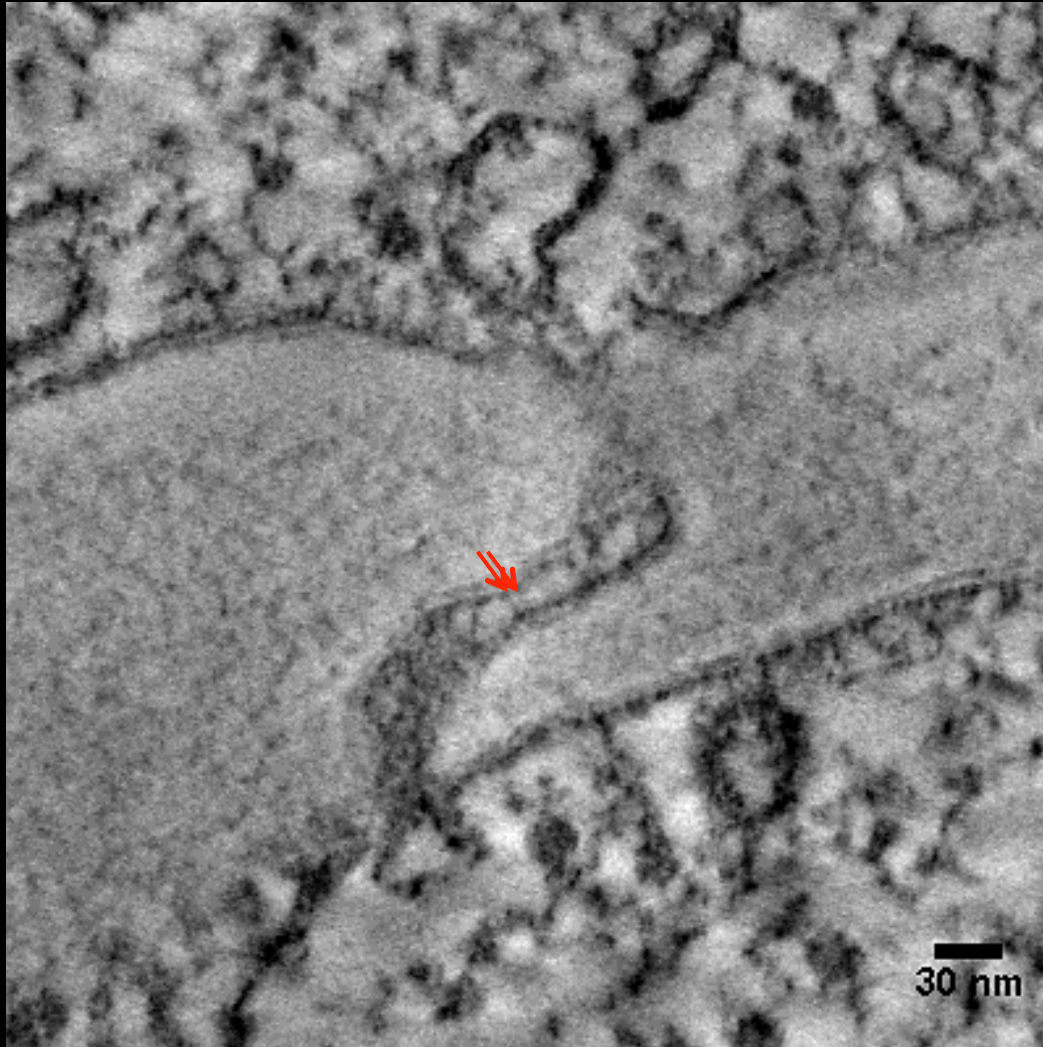


## Myosin VIII (Haraguchi et al., 2014)



Plasmodesmata proteome

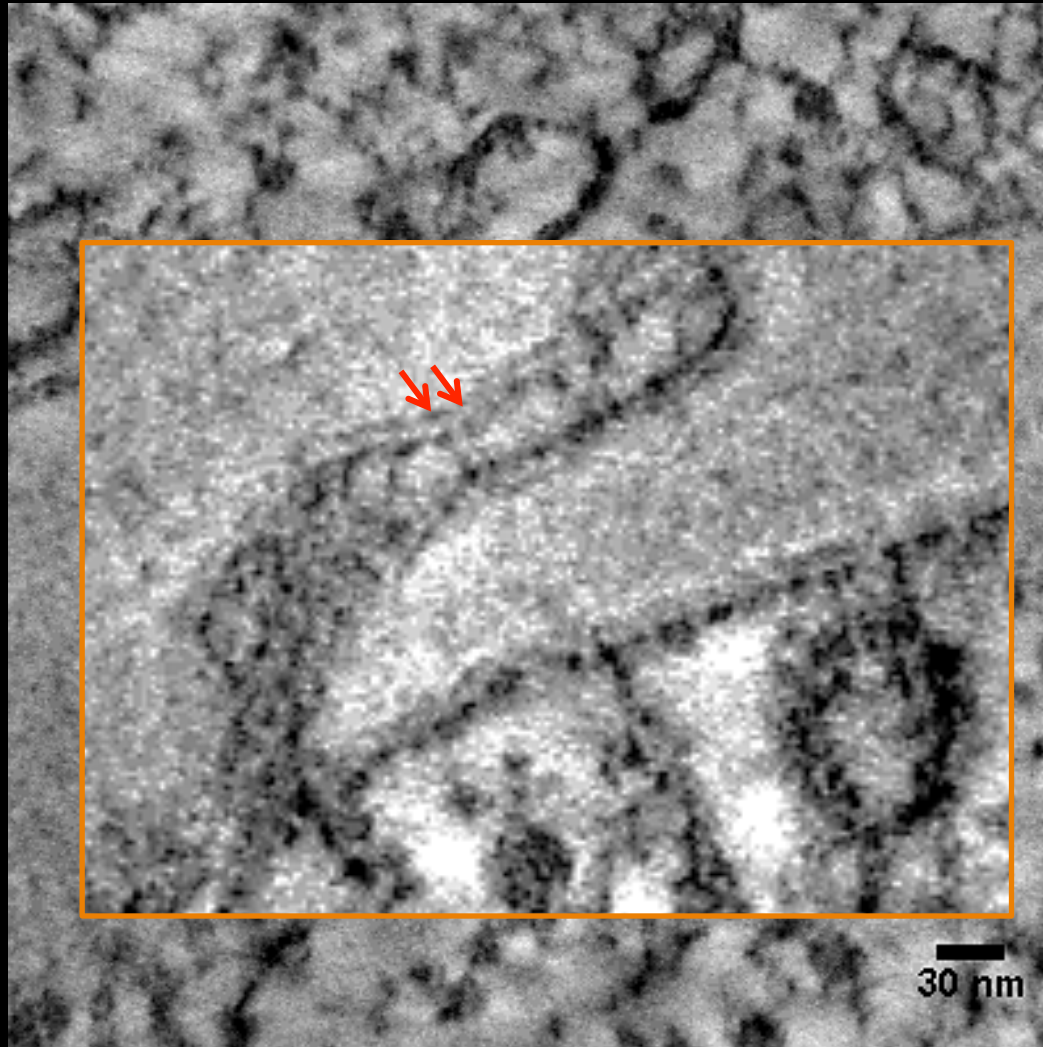
# PM-PM spokes



*sections of reconstructed tomogram*



# PM-PM spokes



*sections of reconstructed tomogram*

**BIC**

Bordeaux Imaging Center



**Thanks for your attention**



Laboratoire de  
 **BIOGENÈSE MEMBRANAIRE**



UNIVERSITÉ  
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SEGALÉN