

Cryo-electron tomography and volume averaging of axonemes

Thomas Heuser

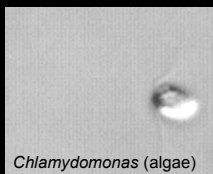
Nicastro Lab, Brandeis University

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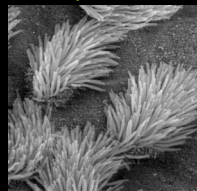
Outline

- Background: cilia, flagella & the Dynein Regulatory Complex
- Techniques: cryo-electron tomography and volume averaging
- Structural comparison of wild type and mutants
- Summary

Cilia and flagella: organelles for motility and sensing



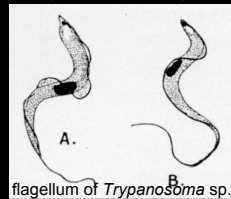
Chlamydomonas (algae)



Cilia in humans
(Ibáñez-Tallón et al. 2003)

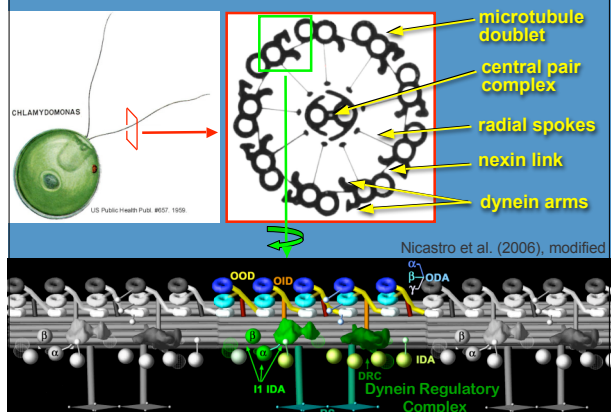


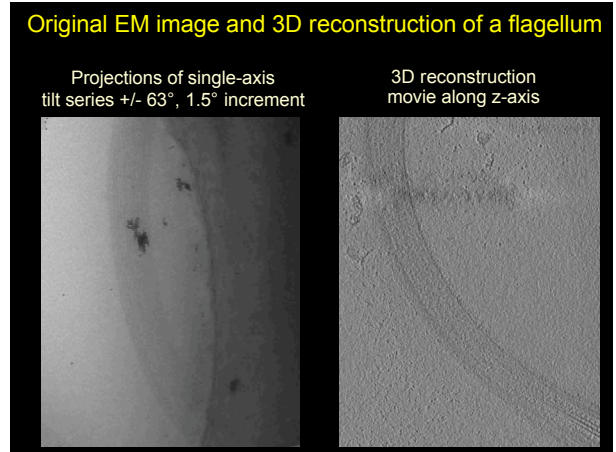
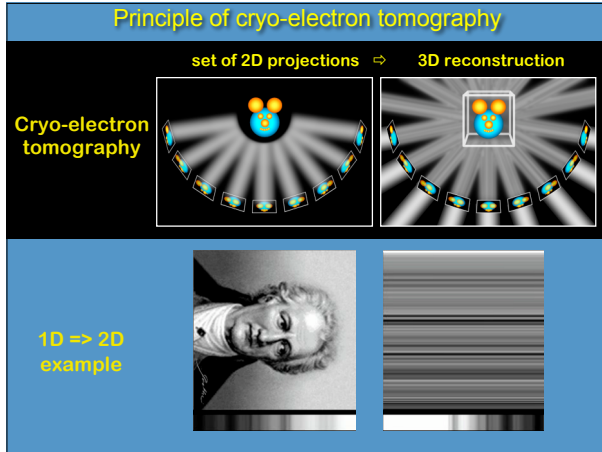
Sea urchin sperm



flagellum of *Trypanosoma* sp.

Axoneme structure





Technical details 1

Tilt series recording:

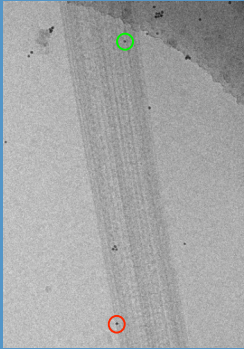
- Tecnaí F30
- Accelerating voltage: 300 kV
- Defocus: -6 to -8 μm
- Tilting increment: 1-2 degree
- Tilting range: -65 to +65 degree (-70 to +70)
- Accumulative dose: ~ 80 to 100 $\text{e}/\text{\AA}^2$
- Acquisition software: SerialEM

Technical details 2

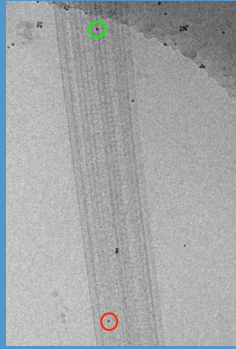
Tomogram reconstruction:

- Fiducial (10 nm Au) based using IMOD

Technical details 2

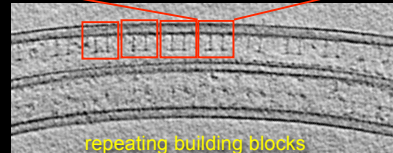
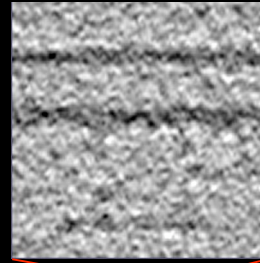


Slice 20 (~ -23°)



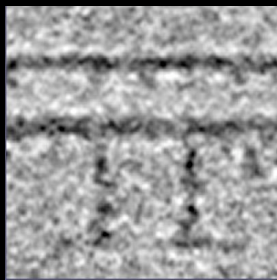
Slice 40 (~ +17°)

Noise problem: Highly repetitive structure allows for averaging



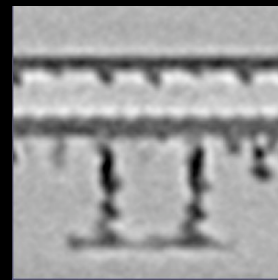
repeating building blocks

Noise problem: Highly repetitive structure allows for averaging



5 repeat units

Noise problem: Highly repetitive structure allows for averaging



720 repeat units

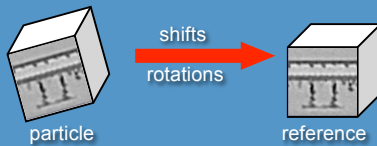
Technical details 3

Volume averaging:

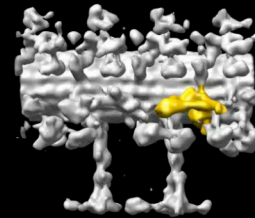
PEET (Particle Estimation for Electron Tomography)
3D averaging technique

Cross correlation based comparison between a particle and a reference by scanning the Euler angles. Correct rotations are determined by highest correlation coefficient. Correct shifts are obtained from the cross correlation peak.

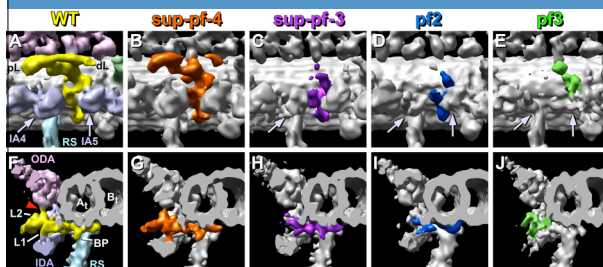
→ allows determination of all six parameters (shifts + rotations)



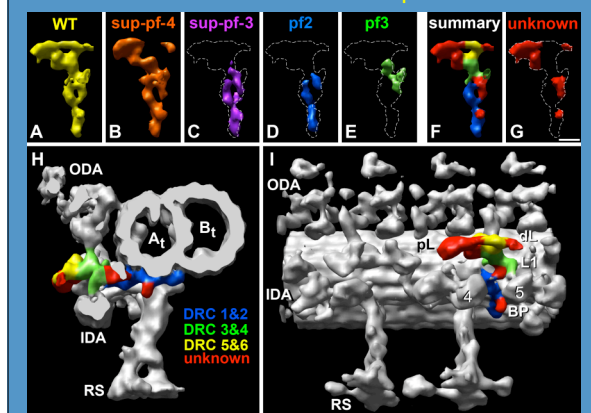
Isosurface rendering of the averaged wildtype axoneme



WT – *drc* mutant comparison



Localization of DRC components



Summary

Cilia and flagella are highly conserved sensing and motility organelles and contain multiple copies of a building block

The dynein regulatory complex (DRC) is a key regulator for dynein activity and axoneme motility

Techniques: Cryo-electron tomography and volume averaging

- 1.) Tilt series recording
- 2.) Fiducial based tomogram reconstruction using IMOD
- 3.) Identifying repeating building blocks in the tomogram
- 4.) 3D alignment and averaging using PEET (Particle Estimation for Electron Tomography)

Structural comparison of wild type and mutants allows localization of DRC components

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