RELION-5-Based Pipeline for Cryo-ET: Structural Analysis of Cilia

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Abstract

Cilia are a motile organelle that are present in a wide variety of organisms. Motile cilia commonly display a highly ordered structure that shows a nine-fold symmetry of the microtubule doublet and its associated proteins, mantled by a membrane (Fig.1). The outer dynein arms (ODA) are the main protein for force generation during ciliary beating. They exhibit a 24 nm periodic repeat. Other proteins such as inner dynein arms (IDA) and the radial spokes (RS) exhibit a 96 nm periodic repeat. Humans express multiple cell types that are ciliated, such as lung epithelial cells (Fig. 2). If cilia are defective, this is most often due to a congenital disease termed primary ciliary dyskinesia (PCD). Defective cilia in lungs cause accumulation of mucus, causing chronic infection. This eventually leads to scarred lung tissue. Current diagnostic techniques are laborious and subsequent treatment is poor. CryoET is a promising technique to resolve structural detail of PCD patient cilia, in order to better understand the underlying defects, especially if genetic analysis did not yield any information. Subtomogram averaging within Relion5[1] can be used to generate higher resolution structures for mechano-structural analysis of patient samples.

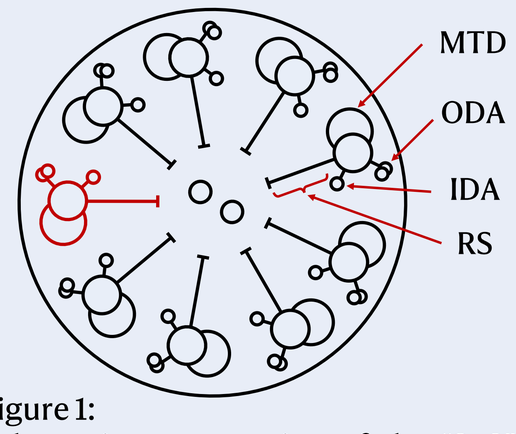


Figure 1:
Schematic cross section of the “9+2” periodic structure. Diameter: 250 nm

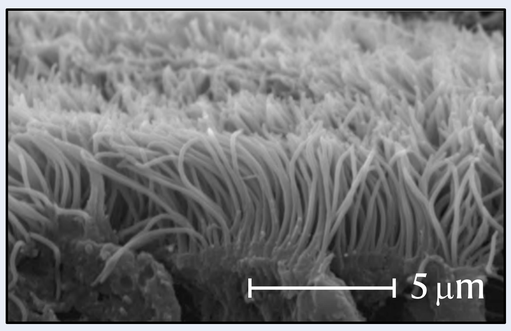
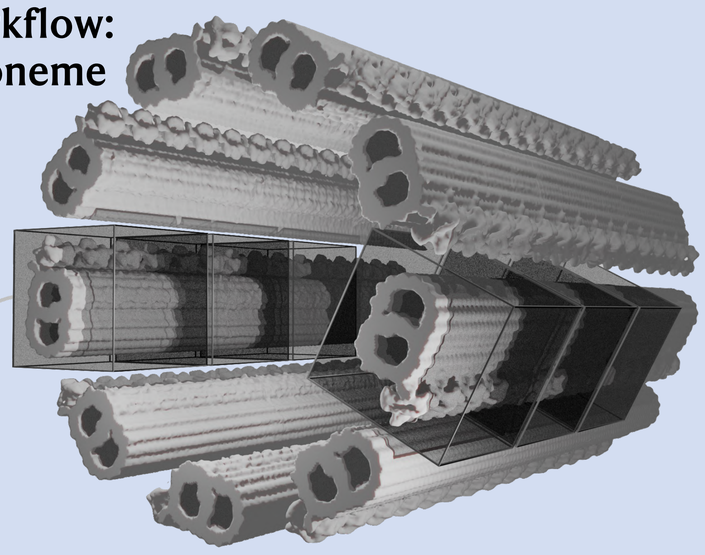


Figure 2:
SEM micrograph of multi-ciliated cells from a human lung trachea epithelium (healthy)[2]

Workflow: Axoneme



[Preprocessed denoised tomograms]

crYOLO picked particles

Manually picked particles

Convert COORDS_TRACED_FID via coords2star.py to a warp compatible .star file

Import and extract COORDS_TRACED in Relion5 at bin = 6 (pixel size: 27.06, box size: 54)

Combine .star files via CombinePriors.py

45248 particles

Visualize particles in Relion5

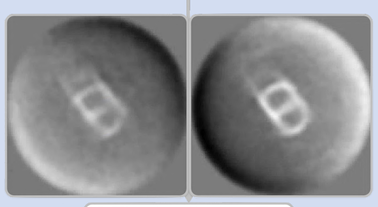
Absolute grey scale reference

Initial centering of particles

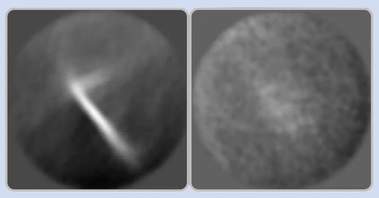
Re-extract at bin3 (pixel size: 13.53, box size: 54)

Classify into 30 classes, no alignment

medium good and good classes

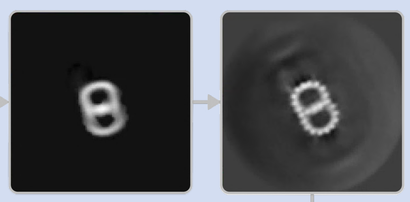


Trash particles

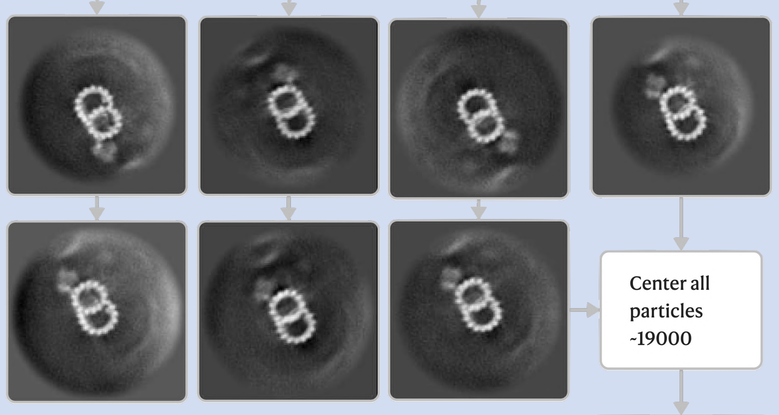


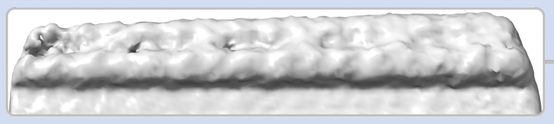
44117 particles

Rough alignment with a tight mask

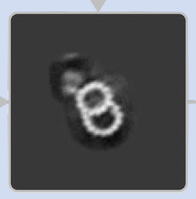


Classification

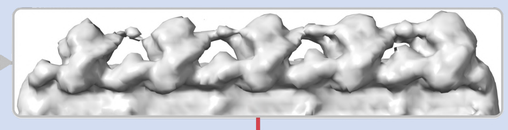




Center all particles ~19000



Align periodicity



Multidimarray getImage error

3D Refine at bin2

Working .star: ~5000 particles

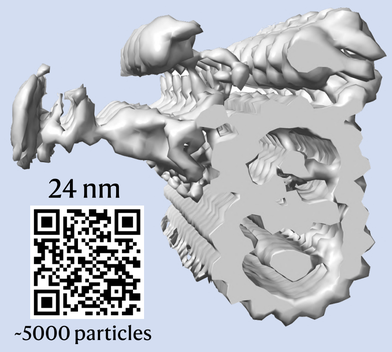
3D refine bin2

Remove duplicates

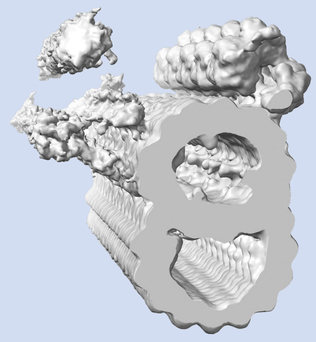
3D refine bin2

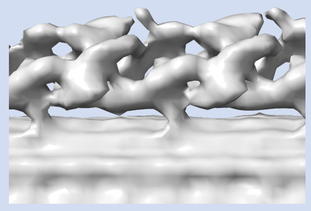
Final Resolution: ~19.5 Å (No PP); 23.5 Å (PP)

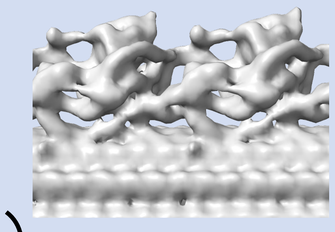
Tomo refinement



~5000 particles



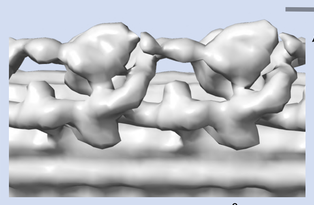




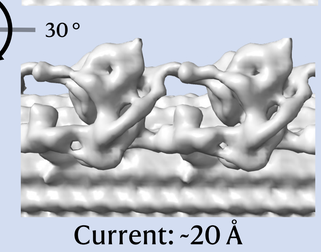
30 °

ODA

~2000 particles



Previous: ~35 Å



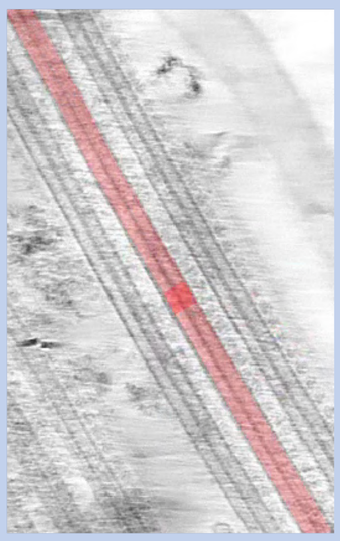
Current: ~20 Å

Particle picking: crYOLO[3]

Box size	40
Low_Pass_Cutoff	0.3
Early	50
Threshold	0.05
Filament mode	Yes
Box distance	12
Tomography mode	Yes
Tracing memory	8
Tracing_min_length	5

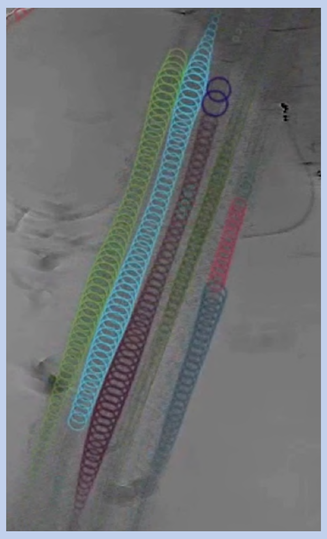
20 tomograms were manually picked as trainings data input; The polarity of the filament is not recognized in the output; Manual addition of coords2star.py priors to Relion5 .star file (.py script)

Training:



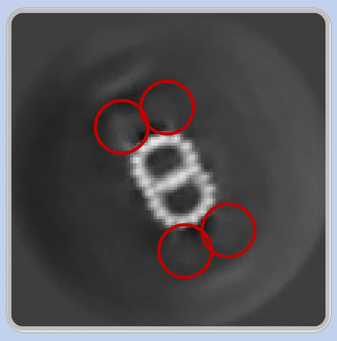
.cbox file format in trainings data annotation:
X, Y and Z coordinates, filament ID, box size (X, Y and Z)

Prediction:

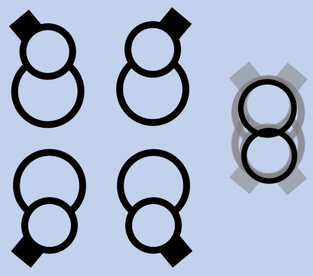


crYOLO particles predicted via filament mode. Some filaments are predicted in a fragmented fashion

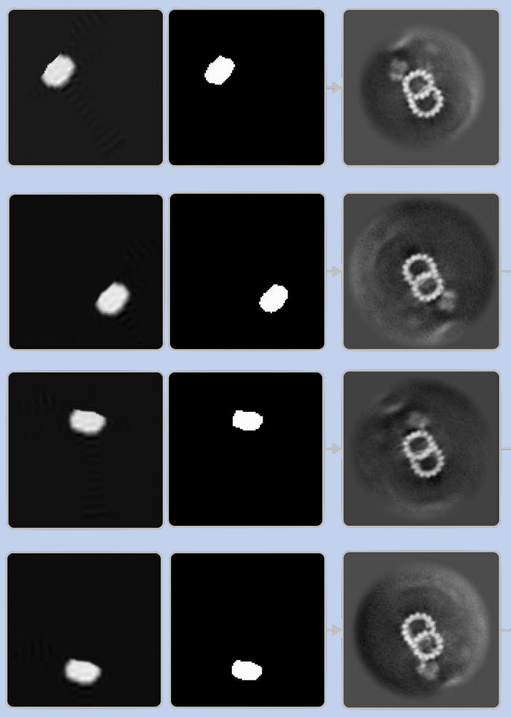
Classification:



Bin3: Pixel size: 13.53



Schematic of misalignment



.star file modification only causes problems at alignment steps performed **after** re-extraction of subtomograms at lower binning. Particle reconstruction at lower binning still works

rot angle: + 180

rot + tilt angle: ×(-1)
rot angle: - 55

rot + tilt angle: ×(-1)
rot angle: + 125

Error: Multidimarray getImage: n larger than NSIZE

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Thank you

ScopeM - Greta Marie Assmann - Leo Luo - Aristea Anna Leventi - Roger Benoit - Huy Bui - Chao Chi - Ricardo Righetto - Sjors Scheres

Sources:
[1]Alister Burt et al. “An image processing pipeline for electron cryo-tomography in RELION-5”. In: *FEBS Open Bio 2024* doi: 10.1002/2211-5463.13873
[2]Gudis D, Zhao K-Q, Cohen NA. Acquired Cilia Dysfunction in Chronic Rhinosinusitis. In: *American Journal of Rhinology & Allergy*. 2012;26(1):1-6. doi: 10.2500/arja.2012.26.3716
[3]Thorsten Wagner et al. “SPHIRE-crYOLO is a fast and accurate fully automated particle picker for cryo-EM”. In: *Communications Biology 2019* doi: 10.1038/s42003-019-0437-z