

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

Tamino Cairoli<sup>1</sup>, Charlotte de Ceuninck van Capelle<sup>1,2</sup>, Pavel Afanasyev<sup>1</sup>, Takashi Ishikawa<sup>1,2</sup>

<sup>1</sup>ETH Zurich, <sup>2</sup> Paul Scherrer Institute

## 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<sup>1</sup> can be used to generate higher resolution structures for mechano-structural analysis of patient samples.

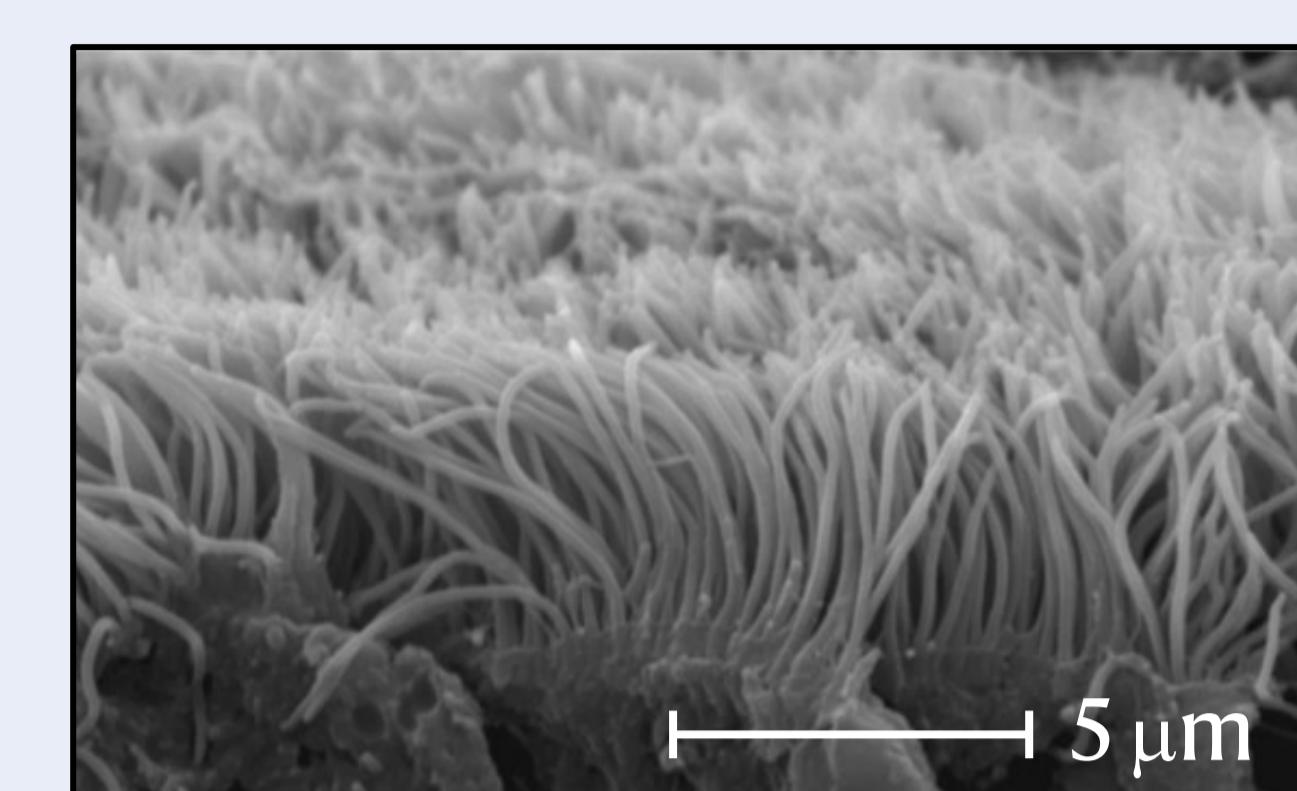
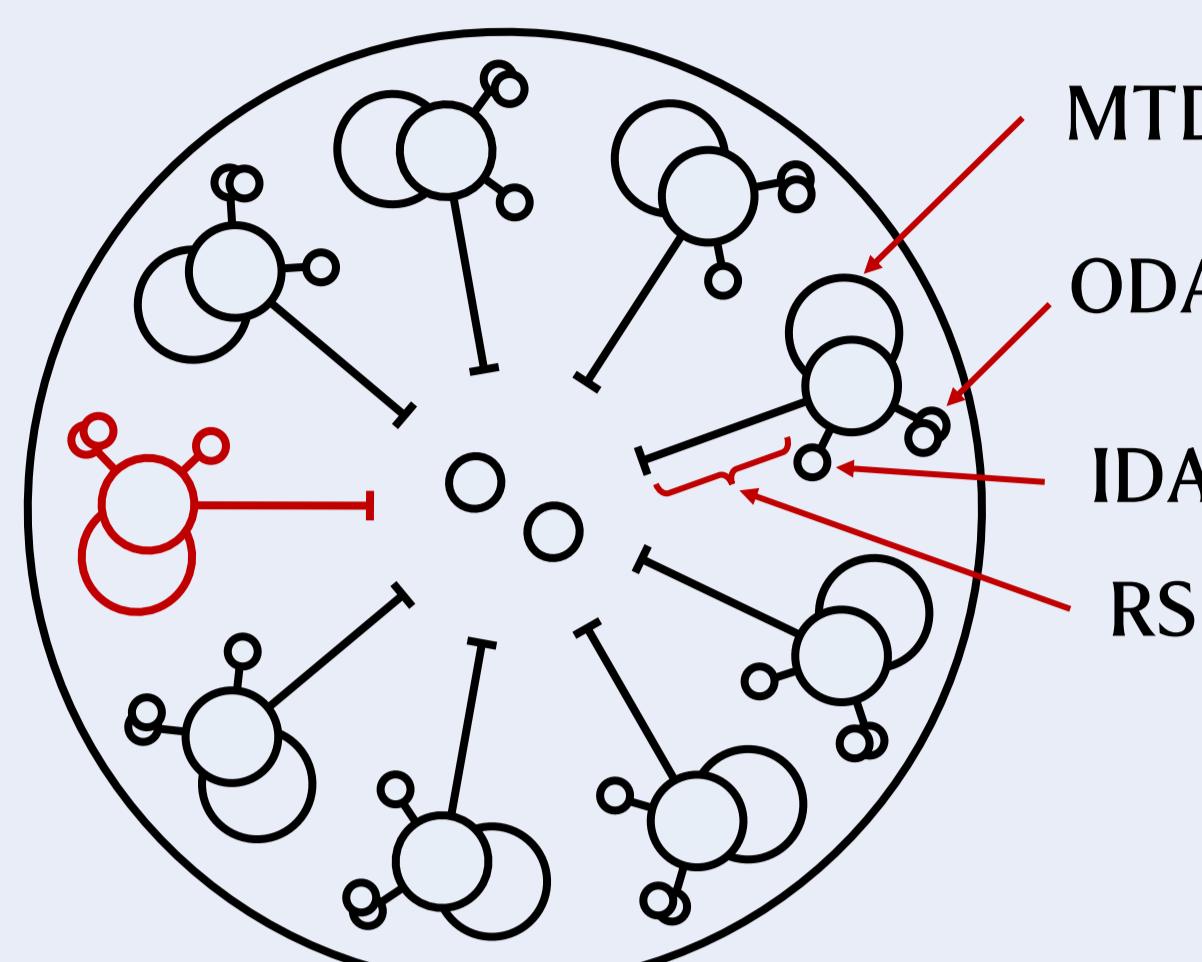
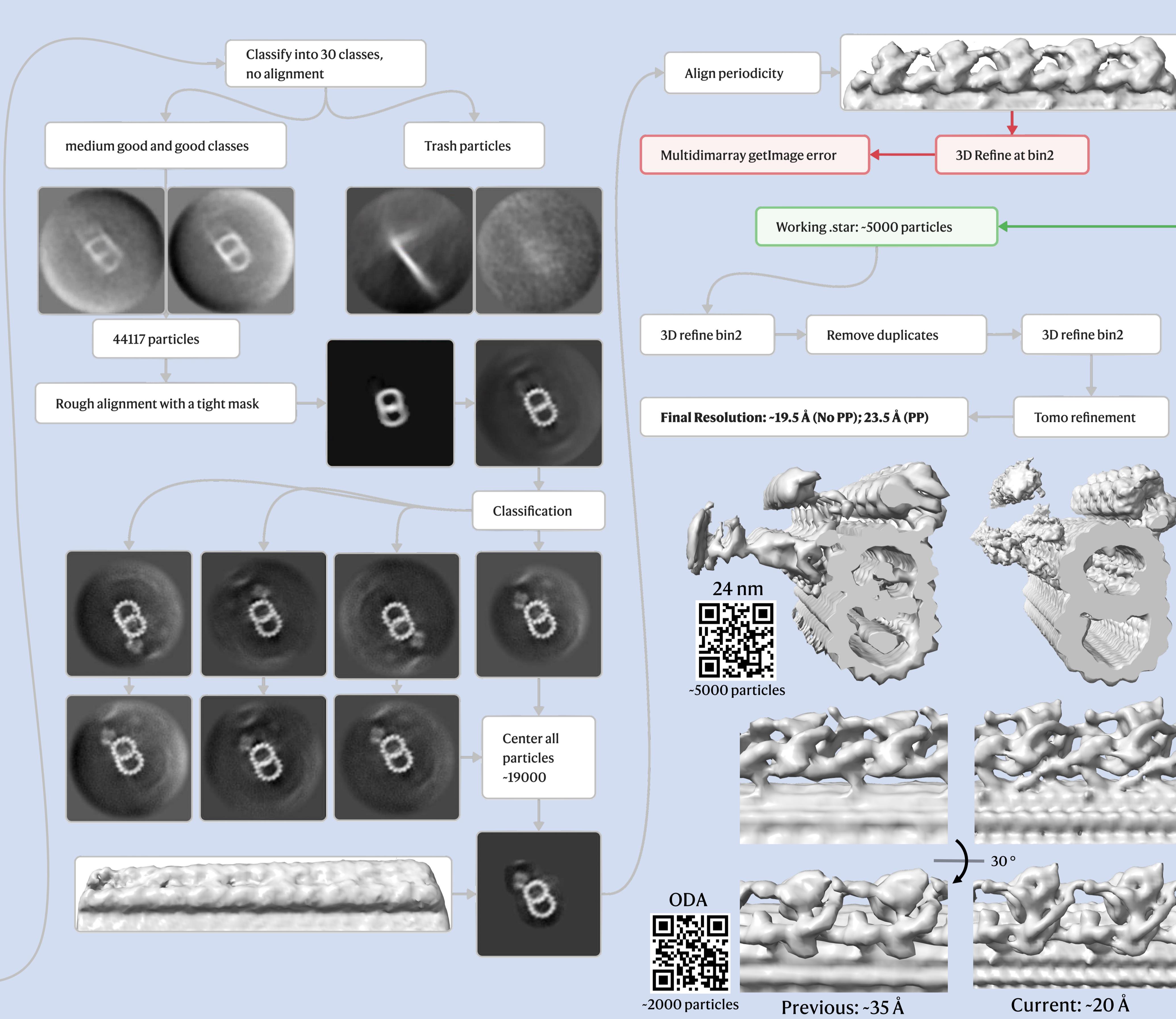
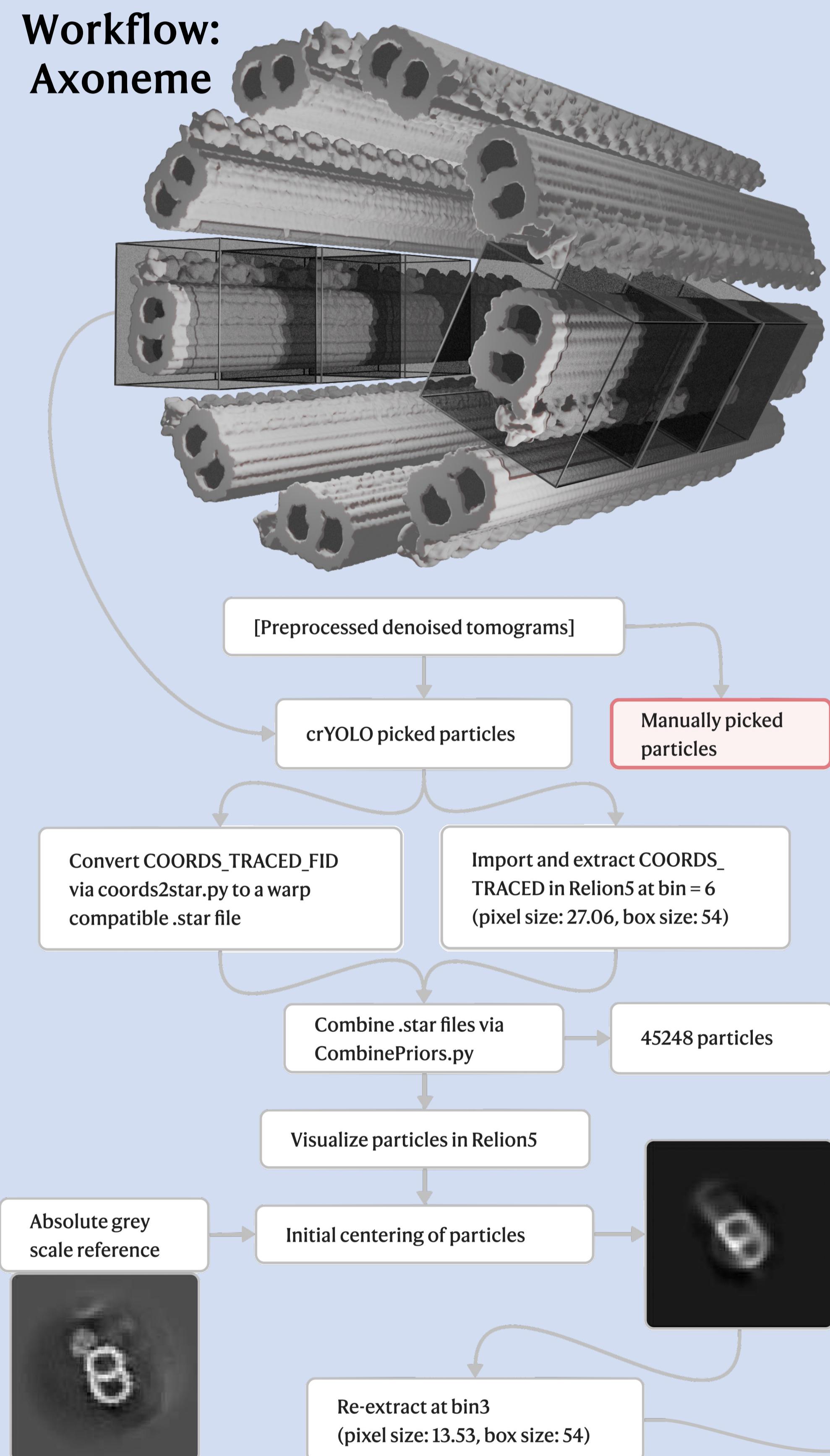


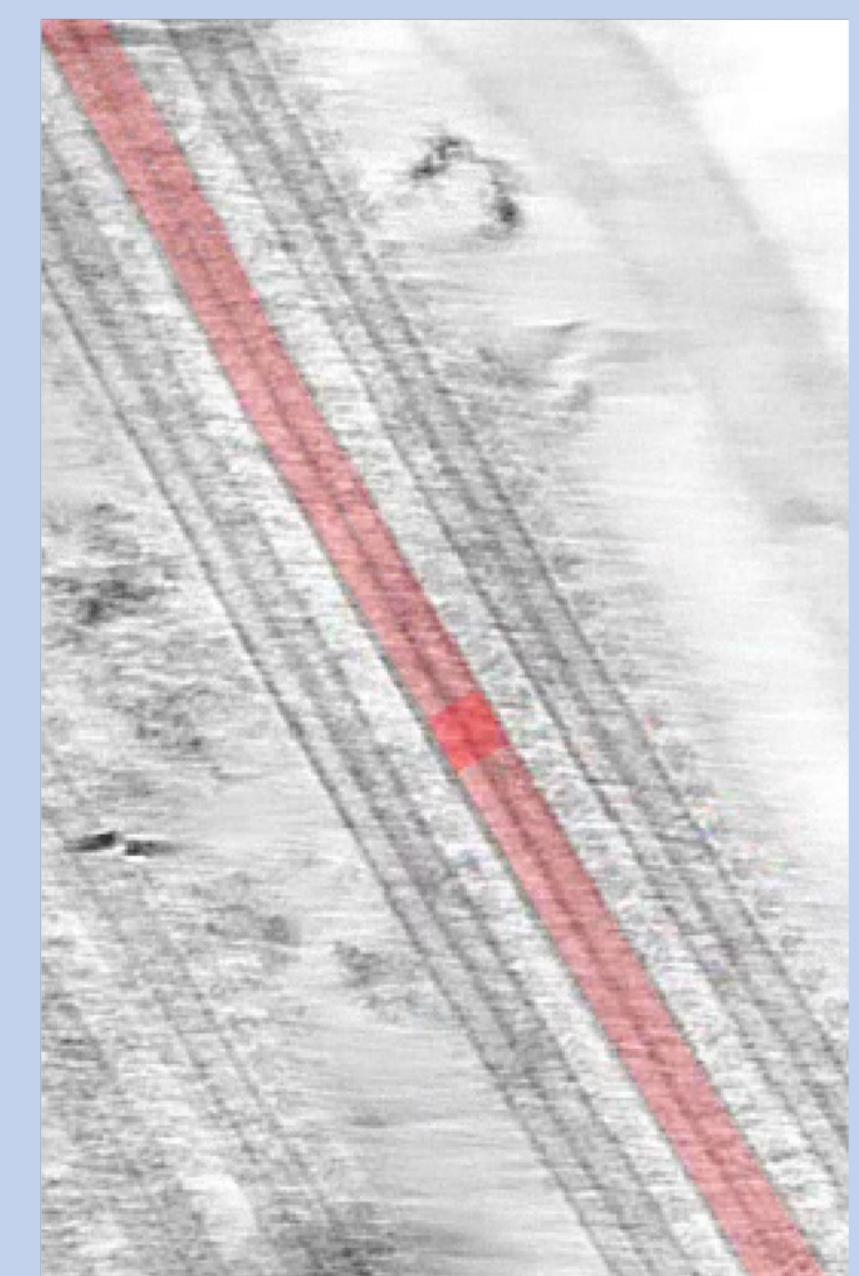
Figure 1:  
Schematic cross section of the "9+2" periodic structure. Diameter: 250 nm

Figure 2:  
SEM micrograph of multi-ciliated cells from a human lung trachea epithelium (healthy)<sup>2</sup>

## Workflow: Axoneme



## Particle picking: crYOLO<sup>3</sup>

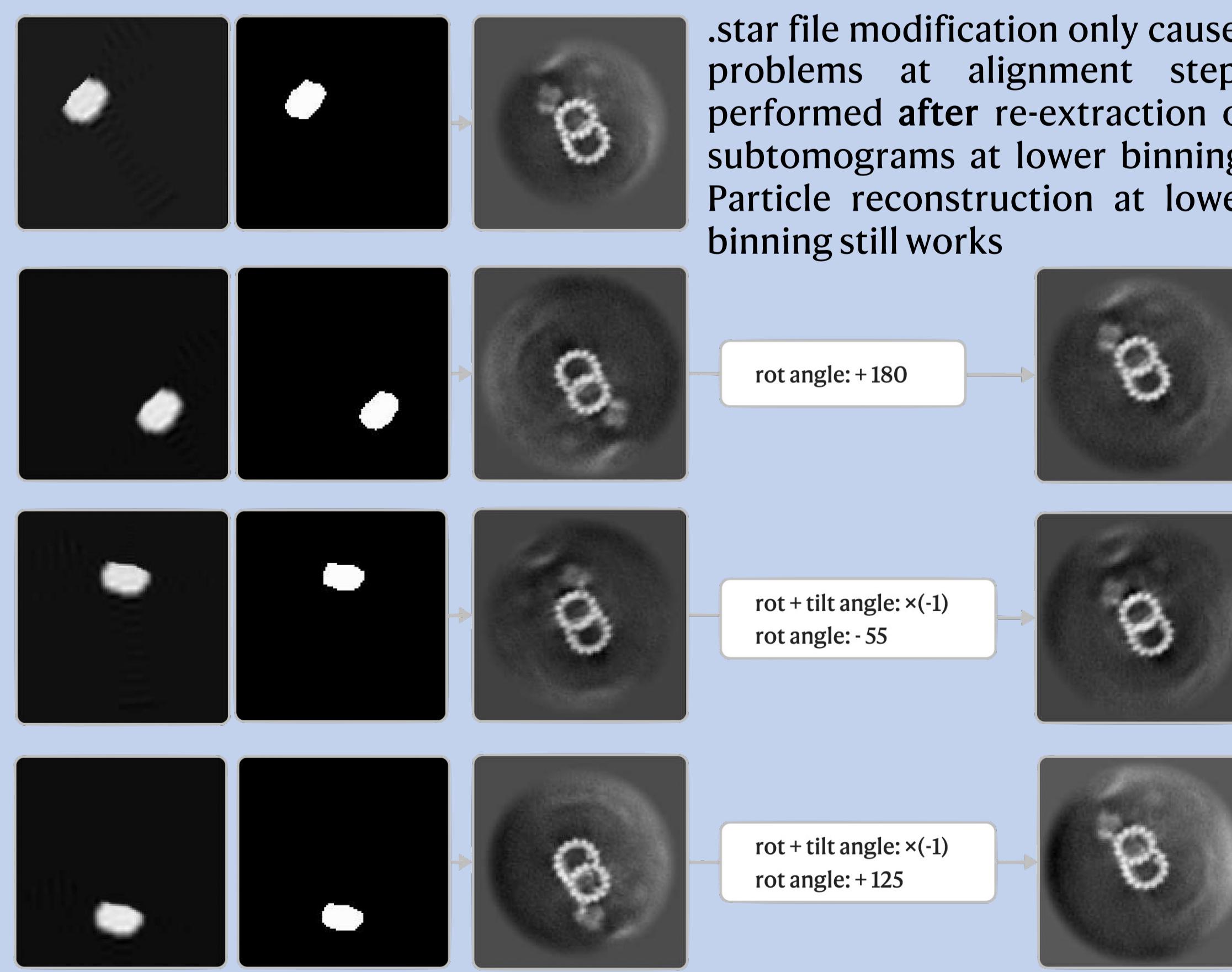
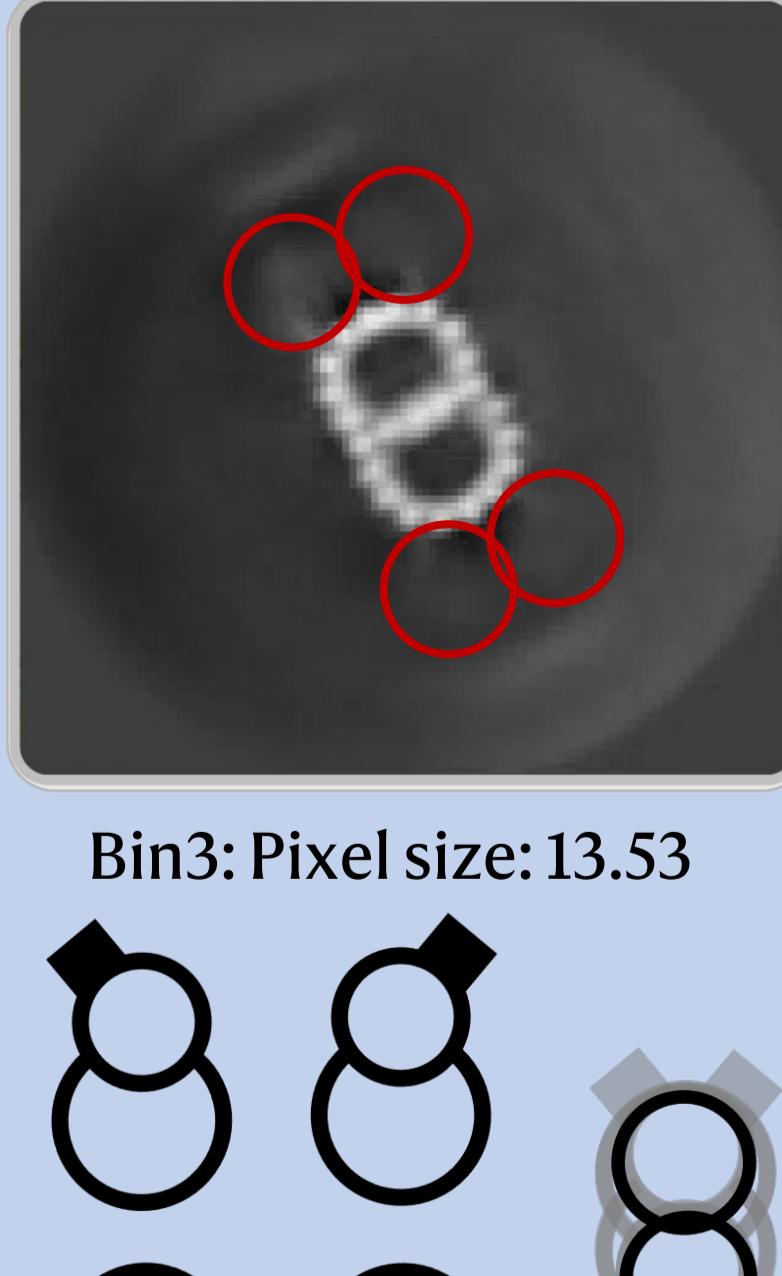


### Training:



### Prediction:

### Classification:



.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

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)

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

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

Bin3: Pixel size: 13.53  
Schematic of misalignment

Sources:  
<sup>1</sup>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  
<sup>2</sup>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  
<sup>3</sup>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

## Contact information

e-Mail: [tcairoli@student.ethz.ch](mailto:tcairoli@student.ethz.ch)  
tel.: +41 78 644 74 00

LinkedIn: tamino-cairoli

## Thank you

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