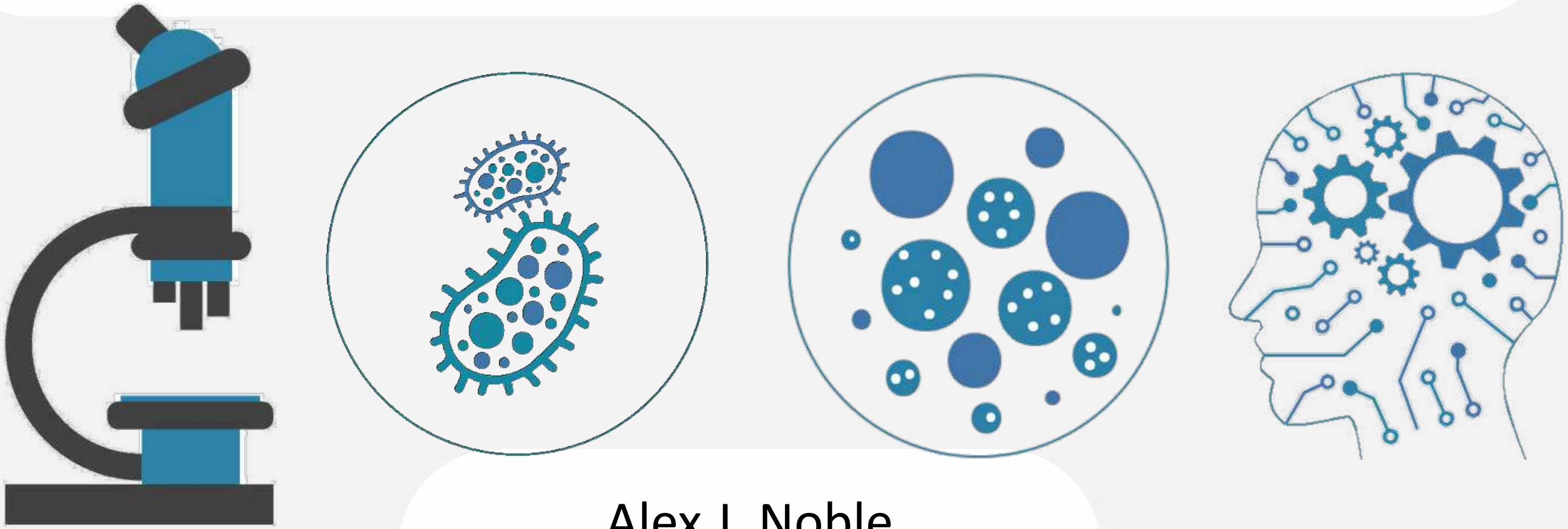
A survey of cryoET software and workflows



Alex J. Noble

CryoEM Course – Tomo!

3-6-24

Today's goals

You should:

- Become familiar with capabilities of existing cryoET software
- Gain realistic expectations for cryoET projects

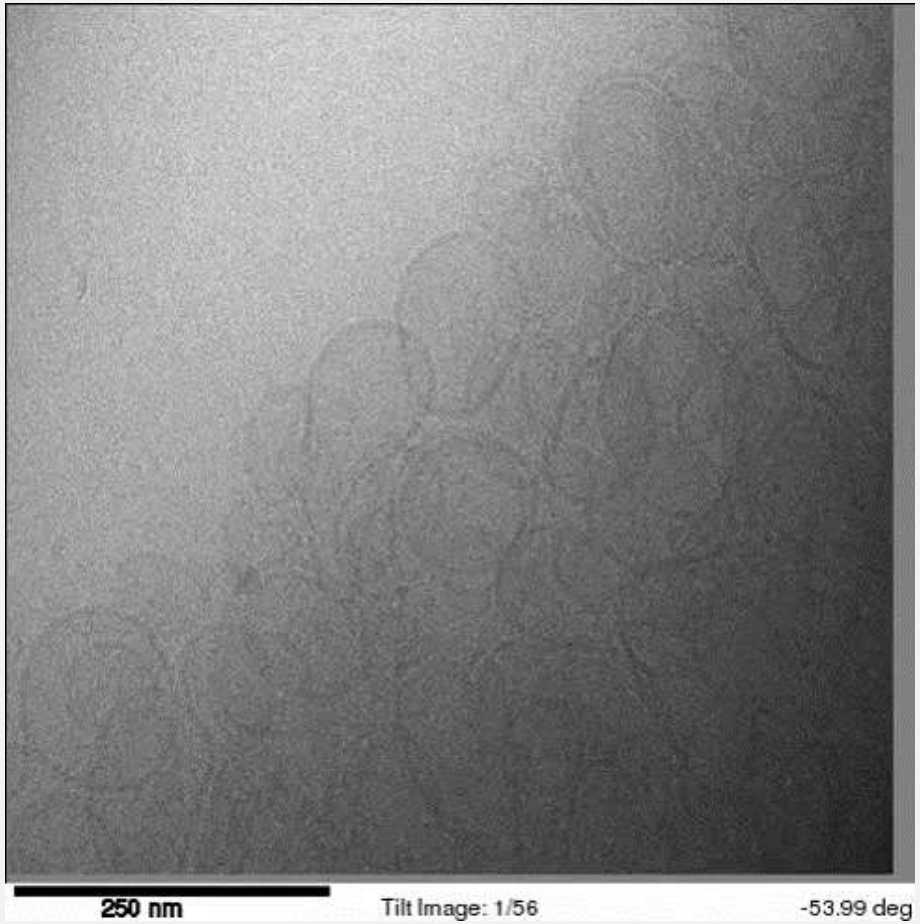
Background

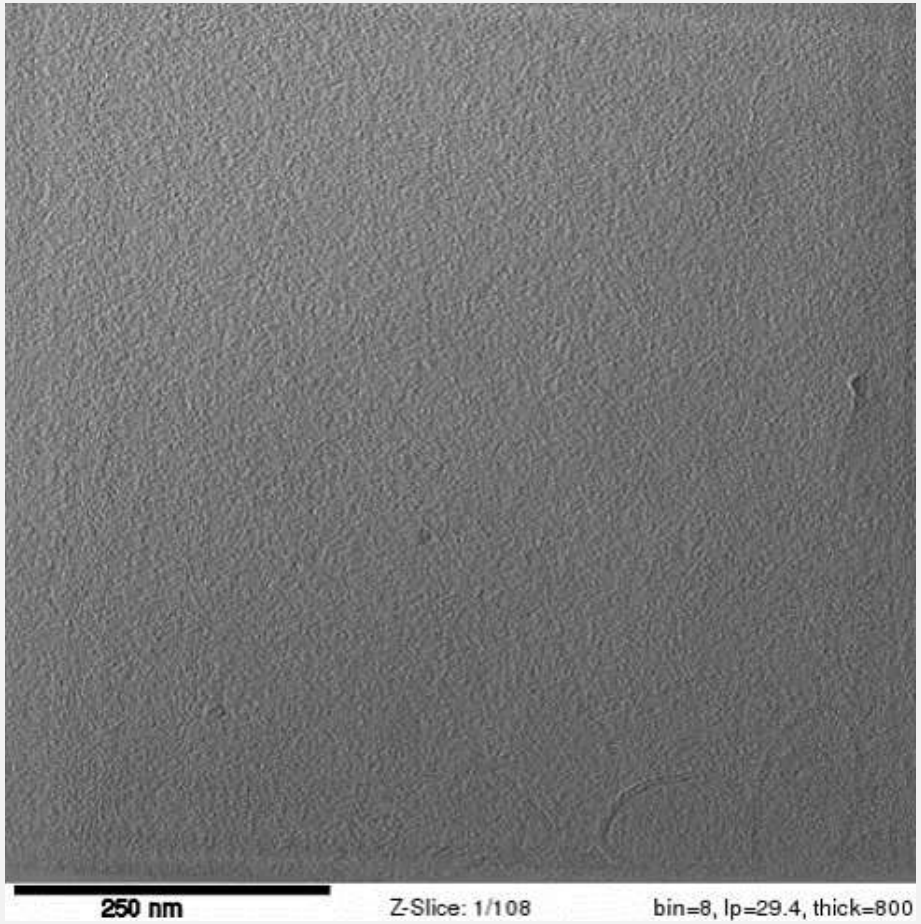
Learned last time from Wei Dai:

Background

Learned last time from Wei Dai:

- Tilt-series > align > tomogram

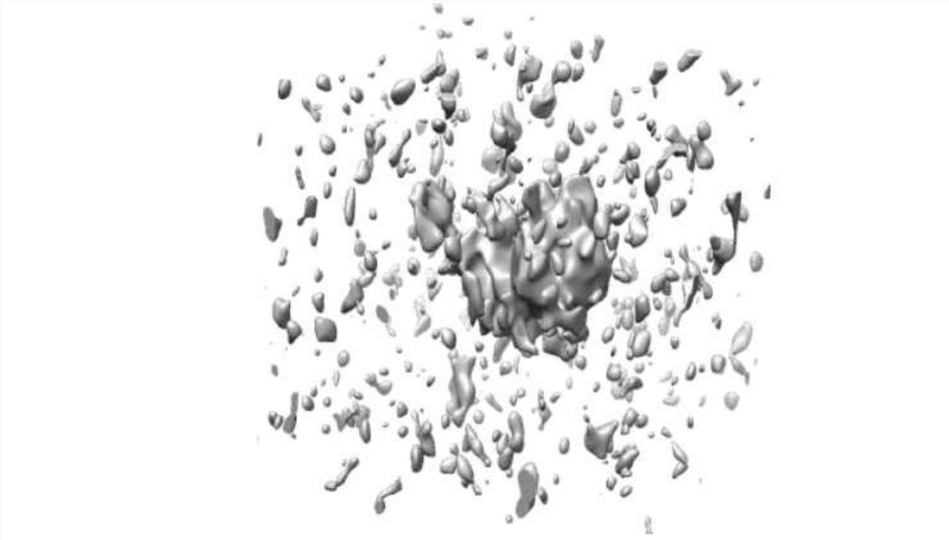


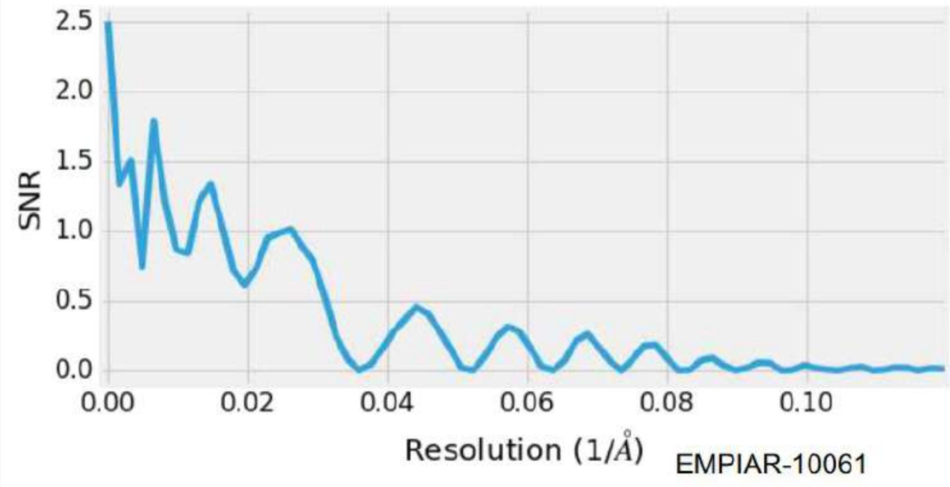


Background

Learned last time from Wei Dai:

- Tilt-series > align > tomogram
  - Beam damage & low SNR





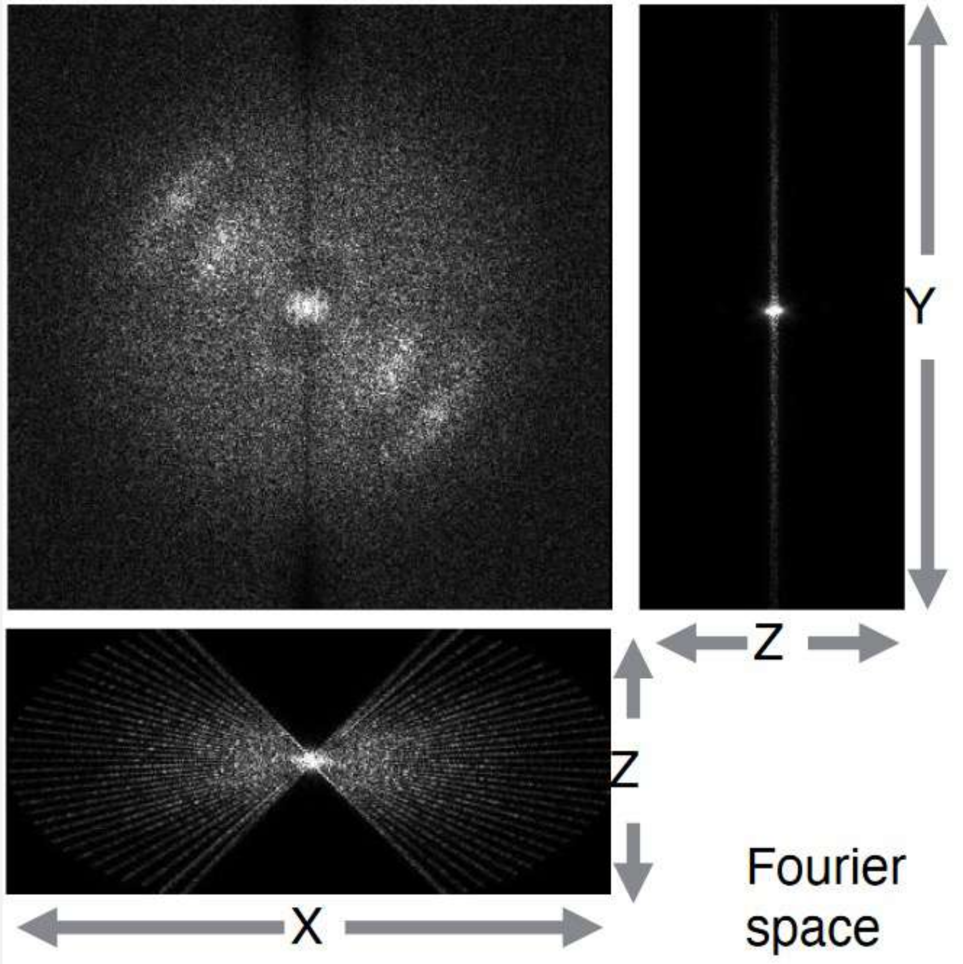
EMPIAR-10061

Background

Learned last time from Wei Dai:

- Tilt-series > align > tomogram
  - Beam damage & low SNR
  - Missing wedge

Chen et al., 2017



Background

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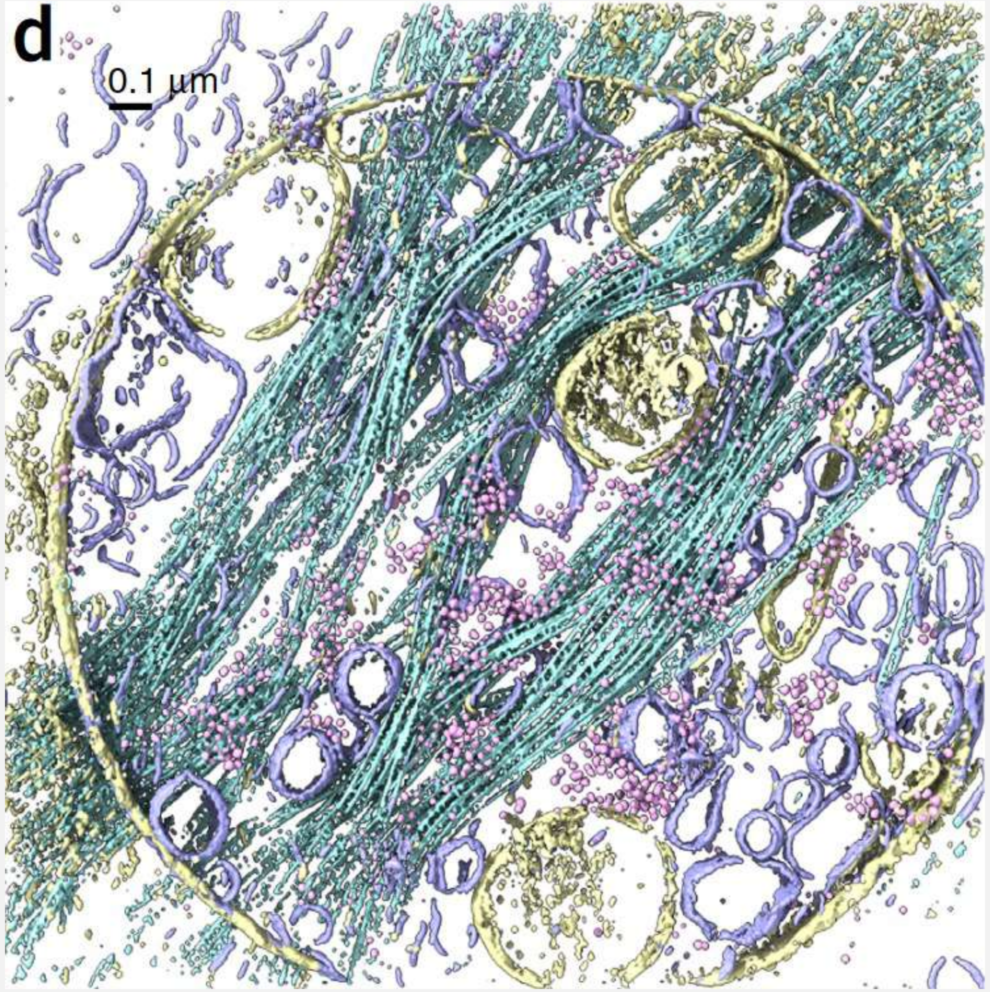
- Tilt-series > align > tomogram
  - Beam damage & low SNR
  - Missing wedge
  - Segmentation

Chen et al., 2017



Background

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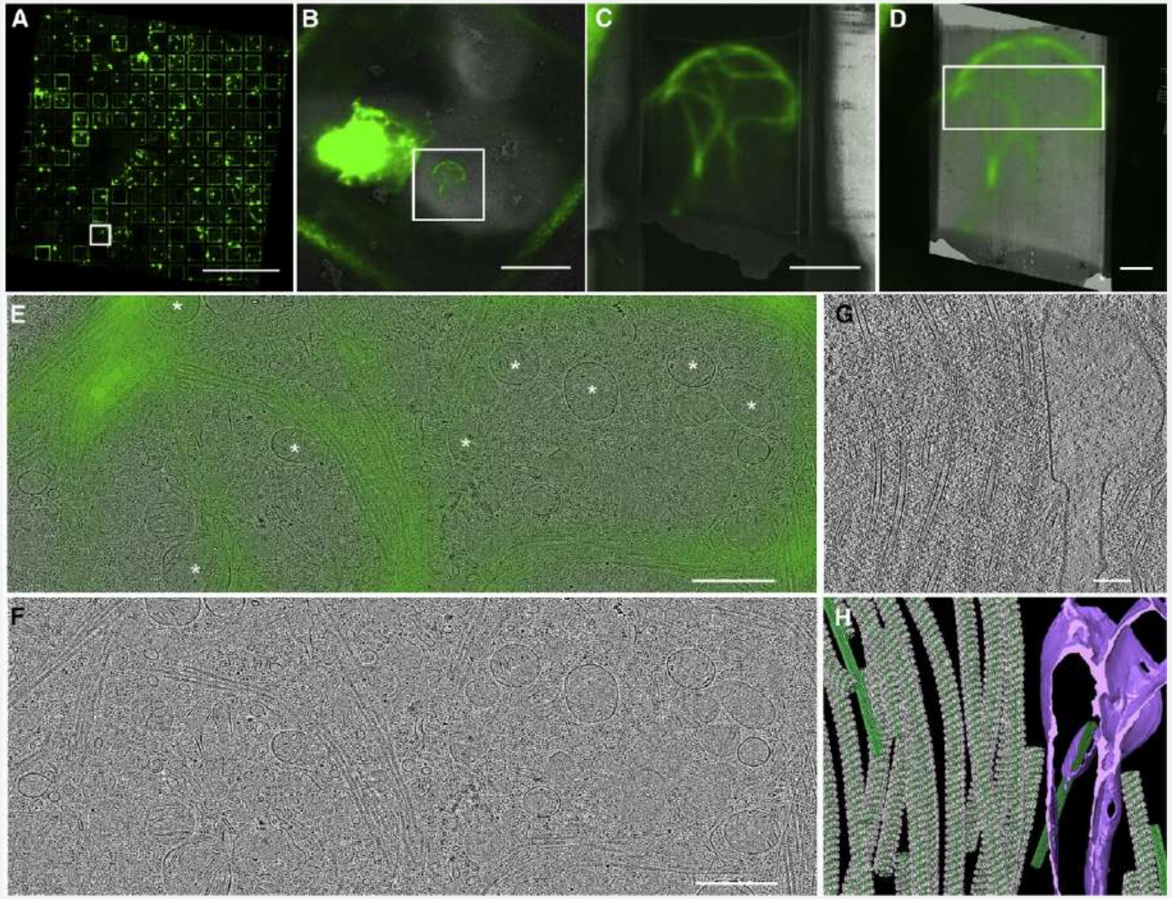
- Tilt-series > align > tomogram
  - Beam damage & low SNR
  - Missing wedge
  - Segmentation
- Visualize in (thin) cells

Watanabe et al., 2020



Background

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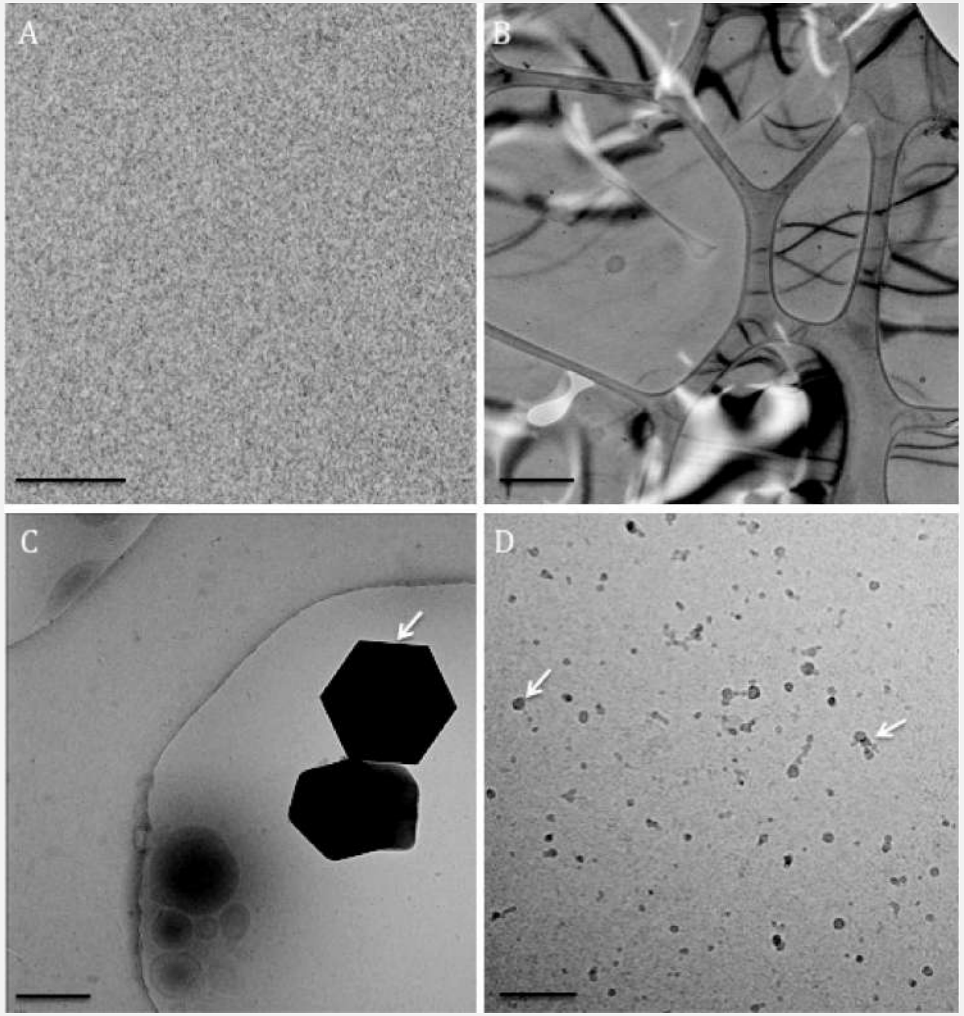
  - Missing wedge
  - Segmentation
- Visualize in (thin) cells
- Good/bad ice

Thompson et al., 2016



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  - Missing wedge
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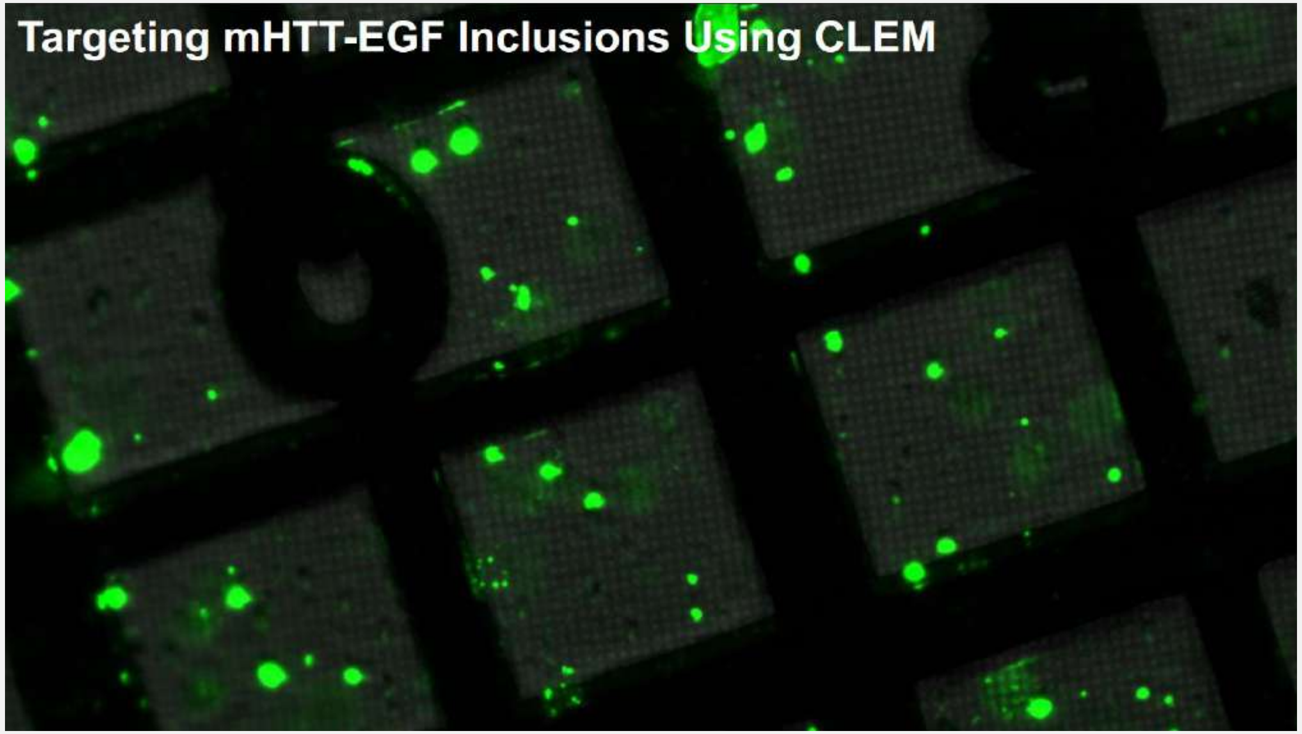
- Visualize in (thin) cells
- Good/bad ice
- CLEM, labeling



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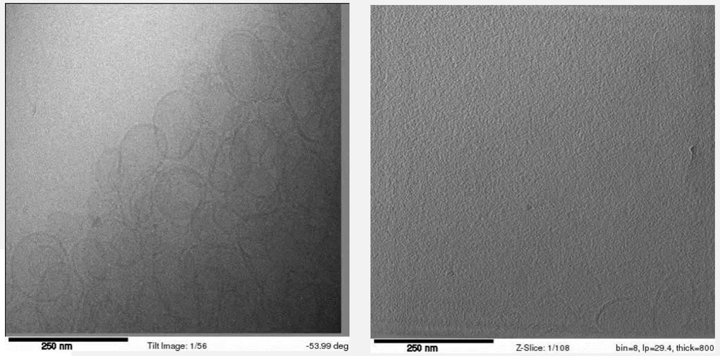
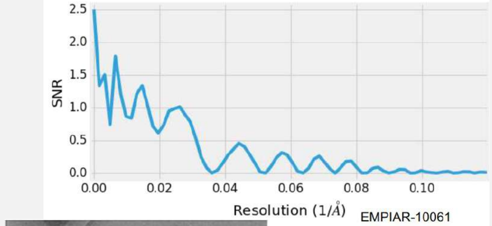
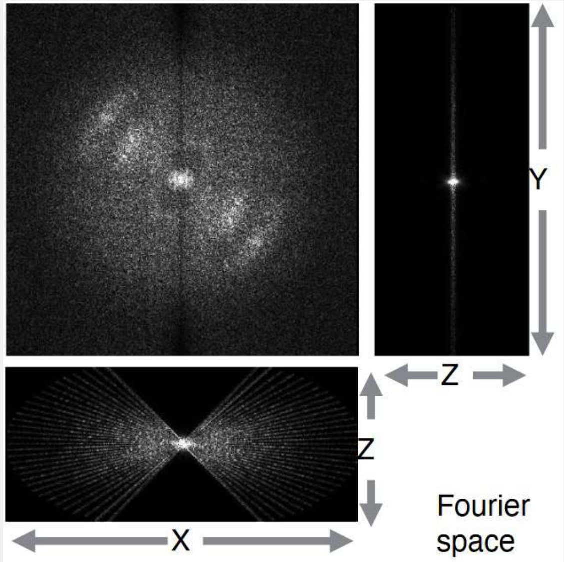
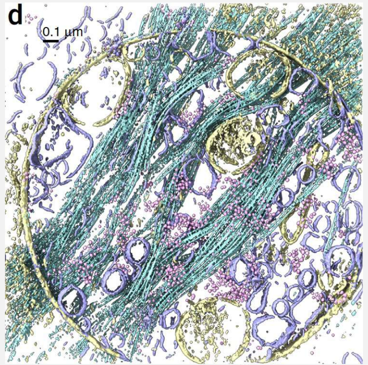
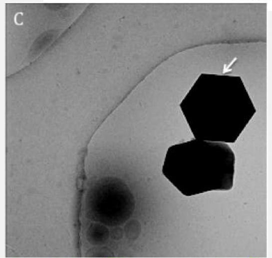
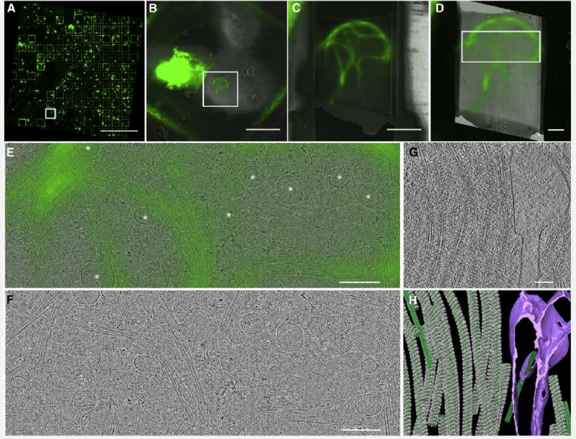


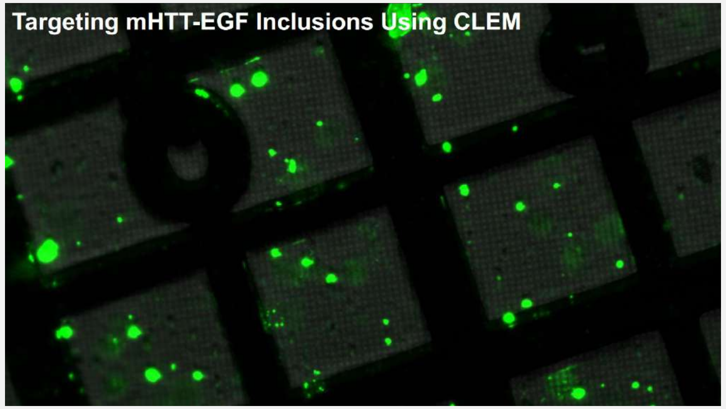










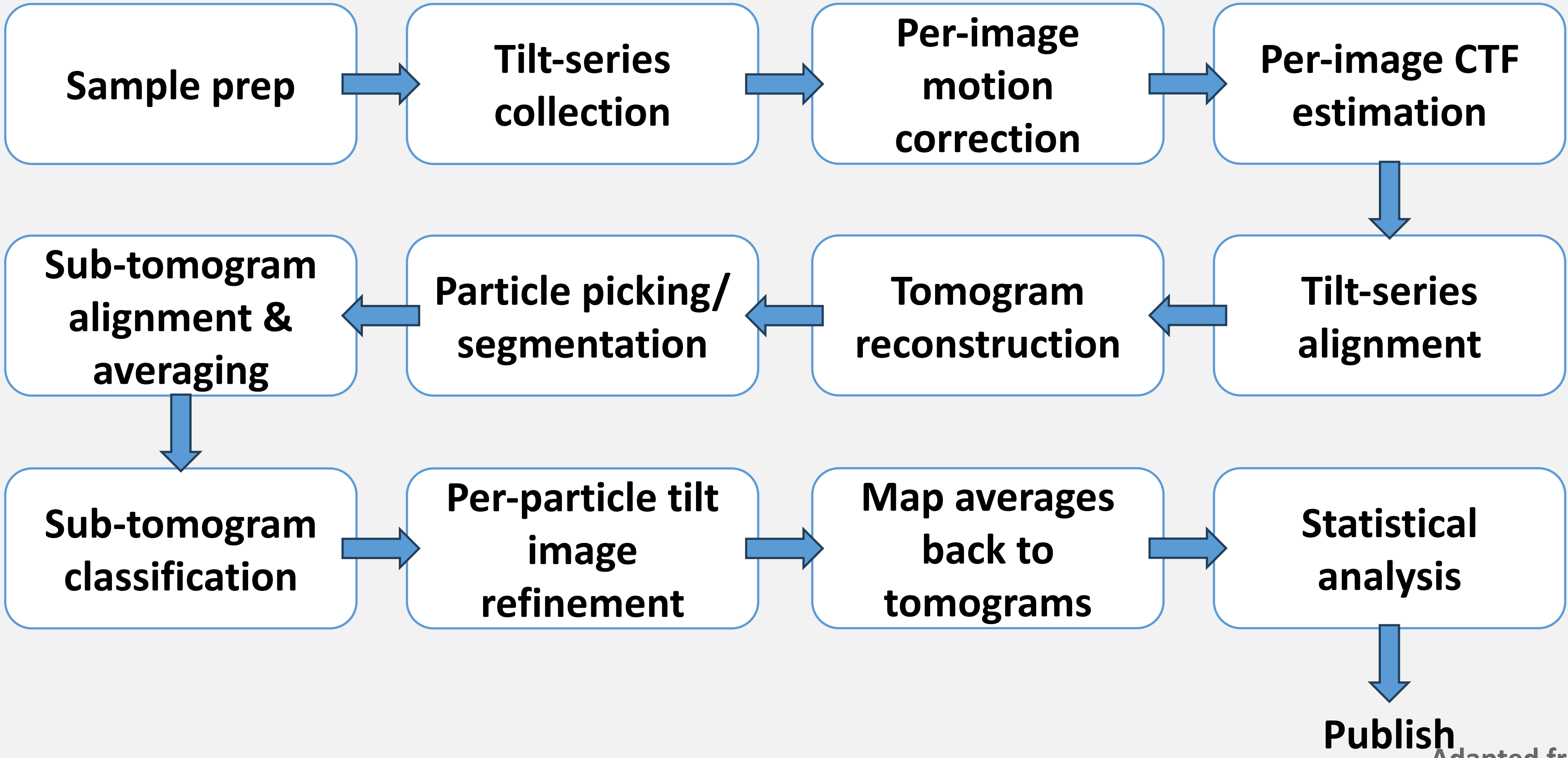


Today’s plan

Today’s plan

- Analyze software in every step of the workflow

Typical workflow

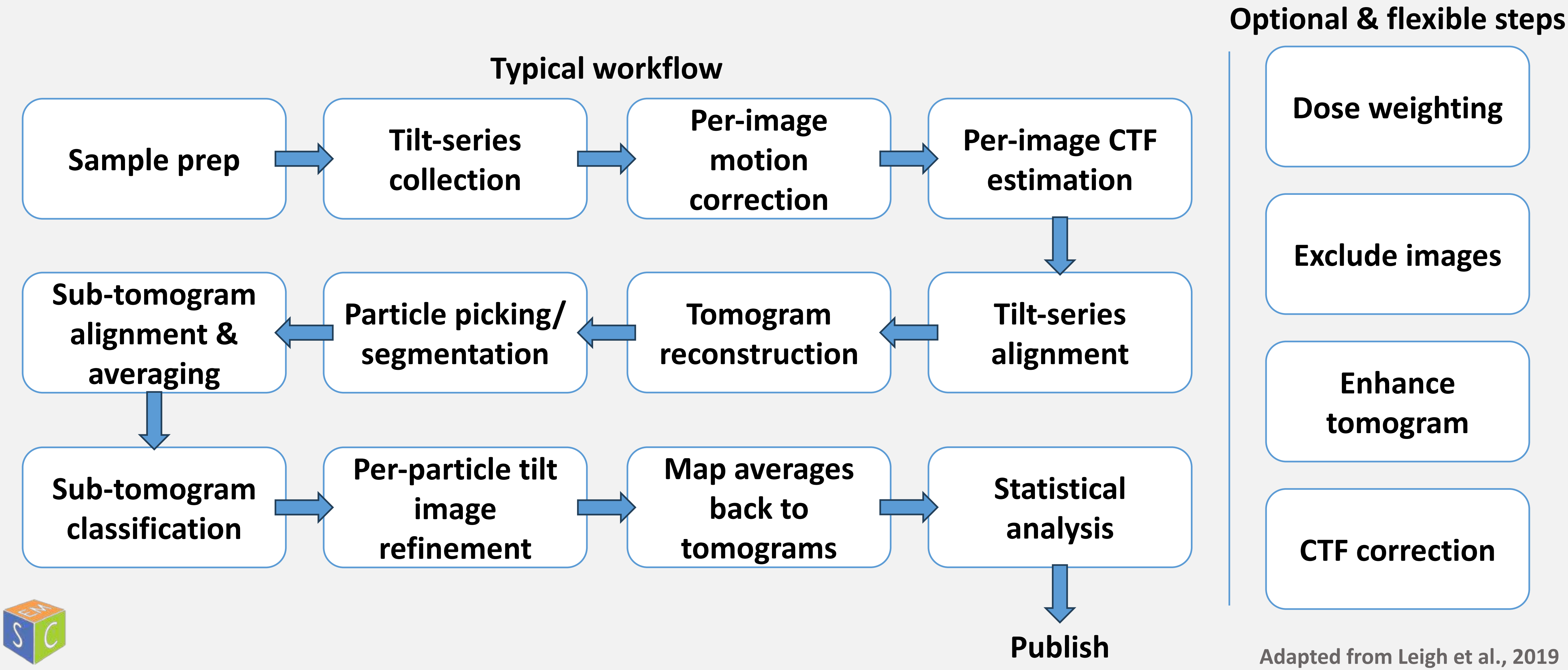


Sample prep
Tilt-series collection
Per-image motion correction
Per-image CTF estimation
Tilt-series alignment
Tomogram reconstruction
Particle picking/ segmentation
Sub-tomogram alignment & averaging
Sub-tomogram classification
Per-particle tilt image refinement
Map averages back to tomograms
Statistical analysis
Publish

Adapted from Leigh et al., 2019

Today's plan

- Analyze software in every step of the workflow



Optional & flexible steps

Typical workflow

Sample prep

Tilt-series collection

Per-image motion correction

Per-image CTF estimation

Tilt-series alignment

Tomogram reconstruction

Particle picking/ segmentation

Sub-tomogram alignment & averaging

Sub-tomogram classification

Per-particle tilt image refinement

Map averages back to tomograms

Statistical analysis

Publish

Dose weighting

Exclude images

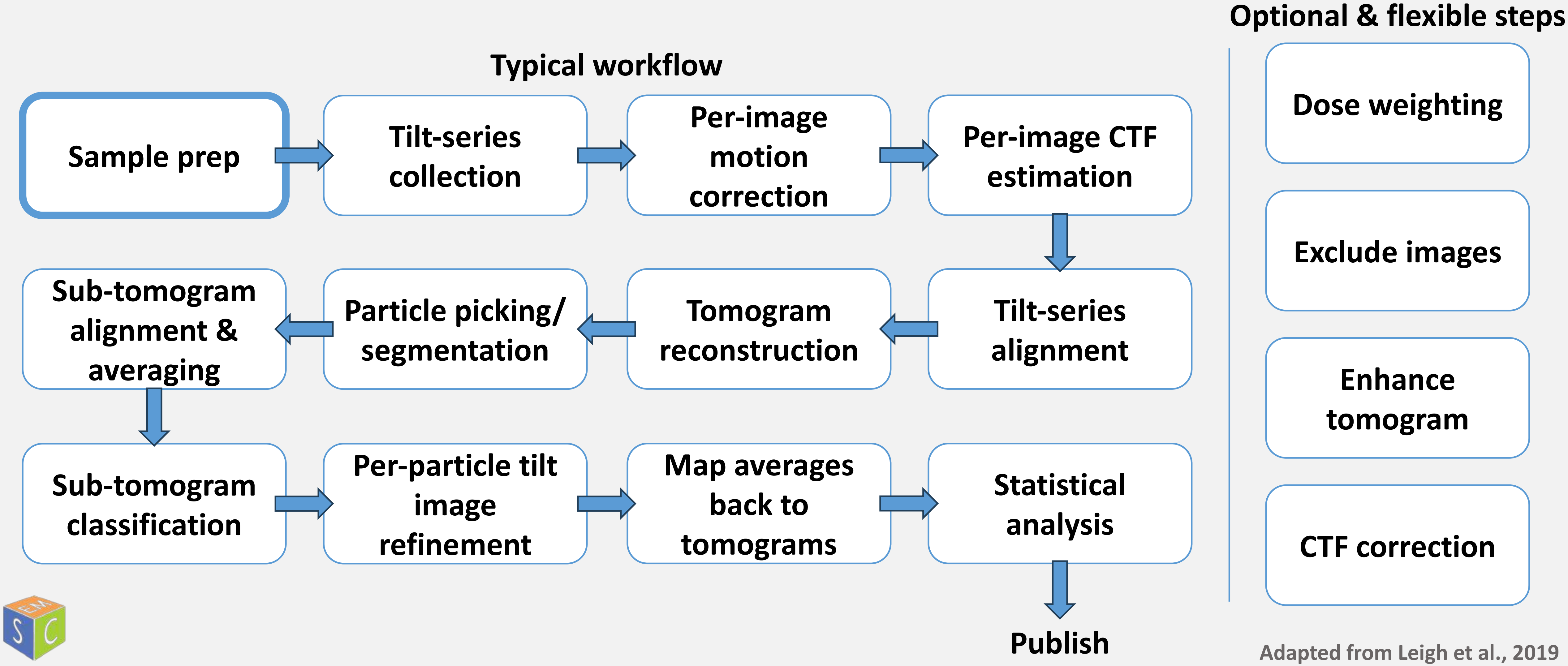
Enhance tomogram

CTF correction

Adapted from Leigh et al., 2019

Today's plan

- Analyze software in every step of the workflow



Typical workflow

Sample prep

Tilt-series collection

Per-image motion correction

Per-image CTF estimation

Tilt-series alignment

Tomogram reconstruction

Particle picking/ segmentation

Sub-tomogram alignment & averaging

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Statistical analysis

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Optional & flexible steps

Dose weighting

Exclude images

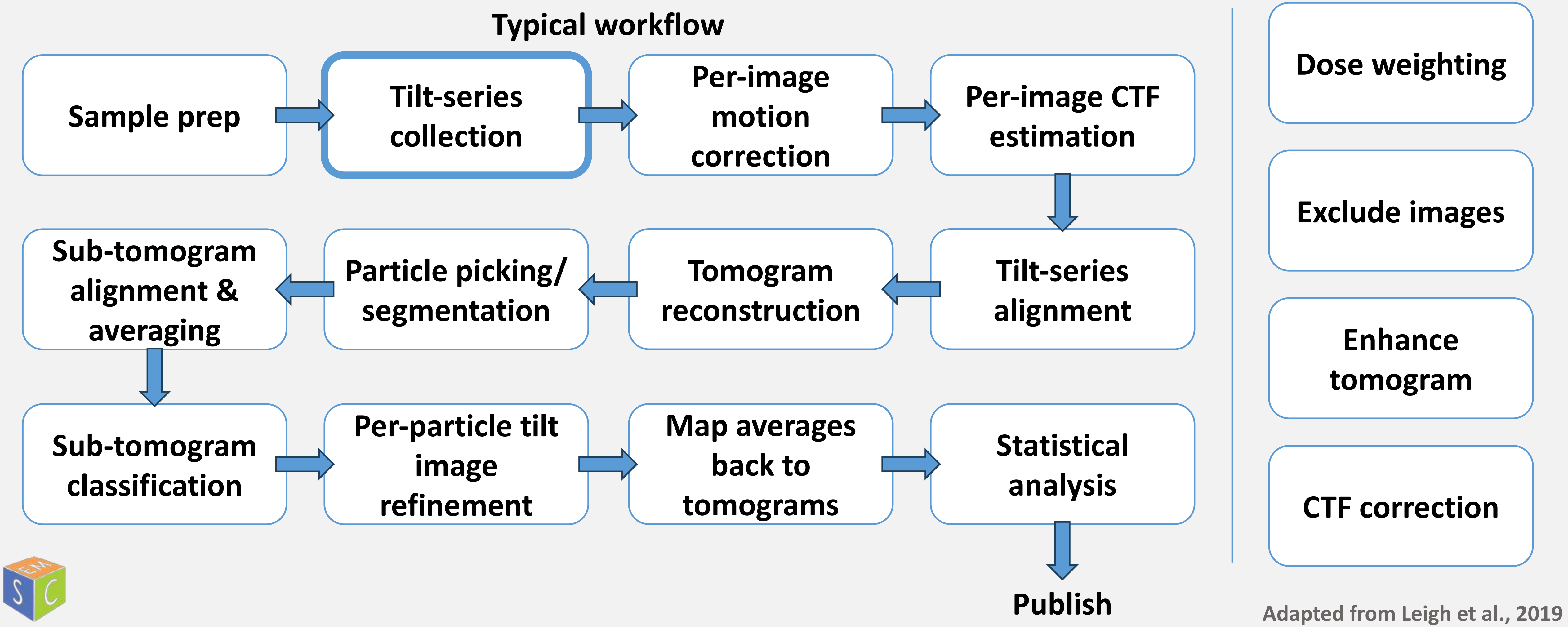
Enhance tomogram

CTF correction

Adapted from Leigh et al., 2019

Today's plan

- Analyze software in every step of the workflow



Optional & flexible steps

- Dose weighting
- Exclude images
- Enhance tomogram
- CTF correction

Typical workflow

Sample prep → Tilt-series collection → Per-image motion correction → Per-image CTF estimation → Tilt-series alignment → Tomogram reconstruction → Particle picking/ segmentation → Sub-tomogram alignment & averaging → Sub-tomogram classification → Per-particle tilt image refinement → Map averages back to tomograms → Statistical analysis → Publish

Adapted from Leigh et al., 2019

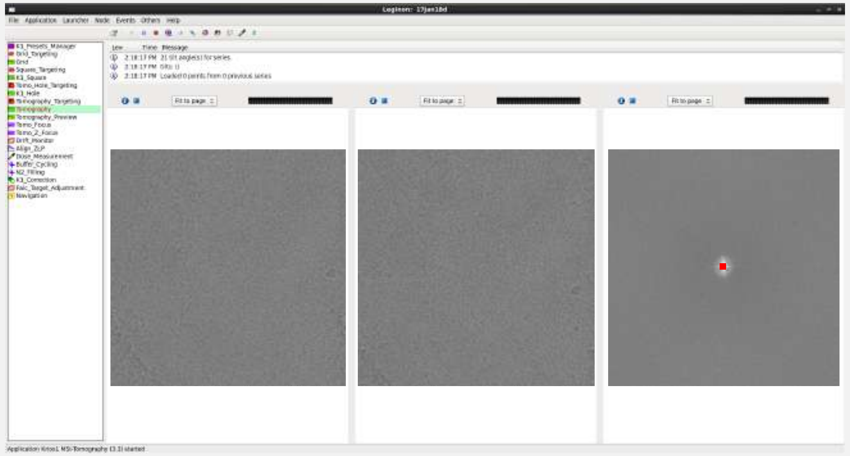
Software for **Tilt-series collection**

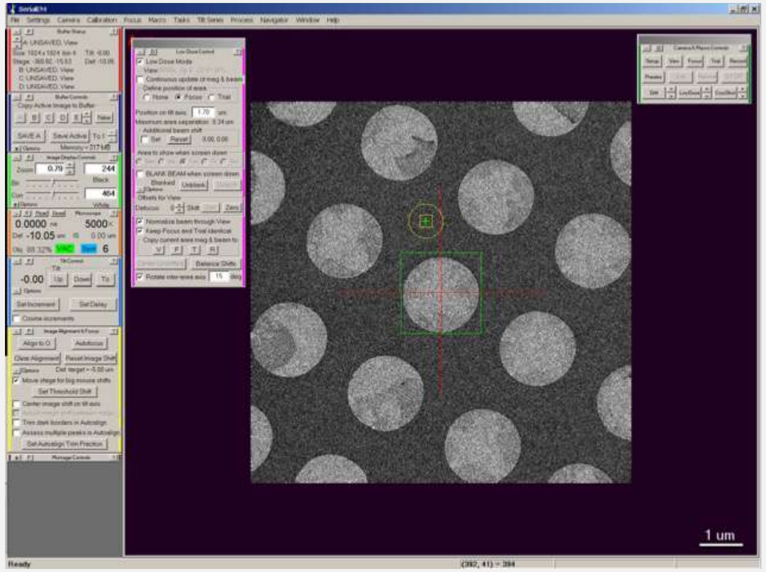
What to look for:

- Accurate tracking: <10% error
- Dose-symmetric collection
- Minimal frame drift: <3 angstroms
- Collection speed: Range is 2-30 minutes per tilt-series

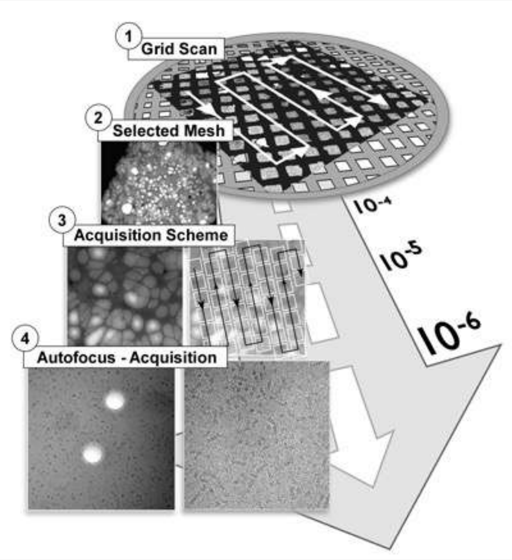
Software for **Tilt-series collection**

Leginon



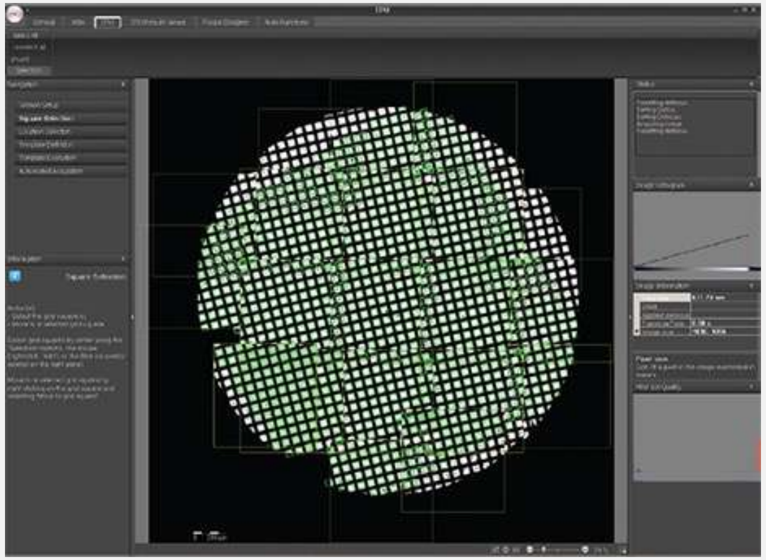


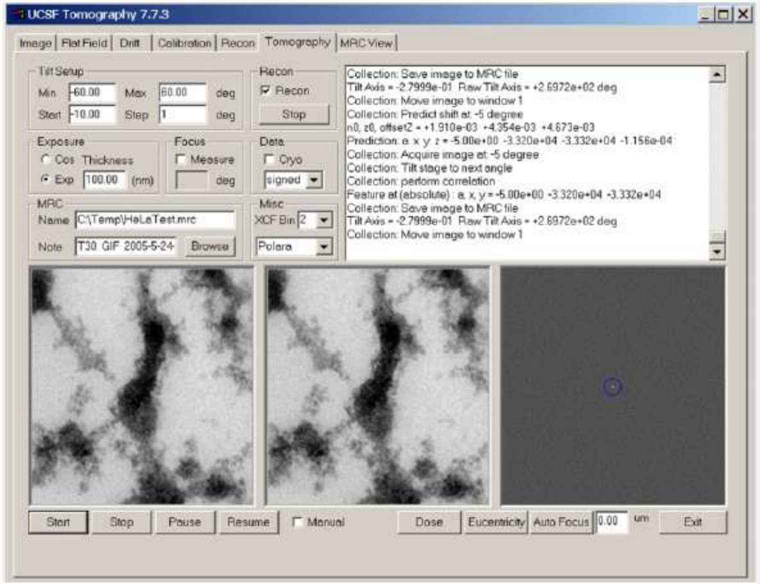
SerialEM



TOM Toolbox

EPU





Tilt Setup
Min -60.00 Max 60.00 deg
Start -10.00 Step 1 deg

Recon
☑ Recon

Exposure
○ Cos Thickness
● Exp 100.00 (nm)

Focus
☐ Measure
deg

Data
☐ Cryo
signed

MRC
Name C:\Temp\HeLaTest.mrc
Note T30 GIF 2005-5-24

Misc
XCF Bin 2
Polara

```
Collection: Save image to MRC file
Tilt Axis = -2.7999e-01  Raw Tilt Axis = +2.6972e+02 deg
Collection: Move image to window 1
Collection: Predict shift at -5 degree
n0, z0, offsetZ = +1.910e-03  +4.354e-03  +4.673e-03
Prediction: a x y z = -5.00e+00 -3.320e+04 -3.332e+04 -1.156e-04
Collection: Acquire image at -5 degree
Collection: Tilt stage to next angle
Collection: perform correlation
Feature at (absolute) : a, x, y = -5.00e+00 -3.320e+04 -3.332e+04
Collection: Save image to MRC file
Tilt Axis = -2.7999e-01  Raw Tilt Axis = +2.6972e+02 deg
Collection: Move image to window 1
```

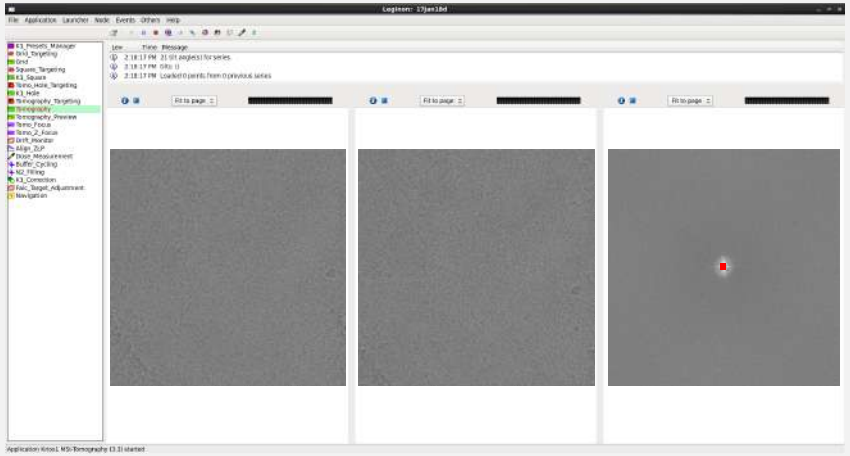
0.00 um

UCSF Tomography

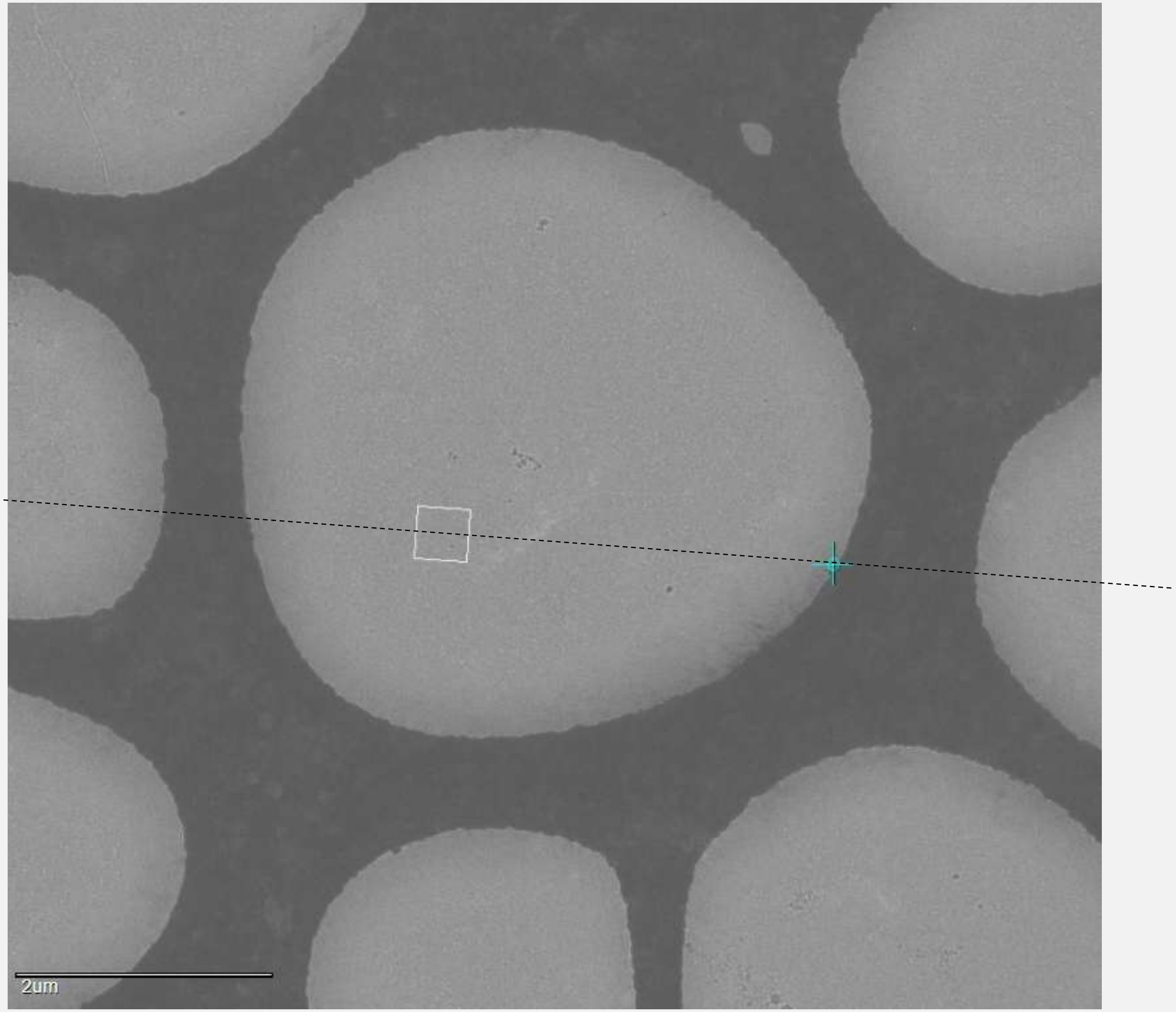
Software for

Tilt-series collection

Leginon



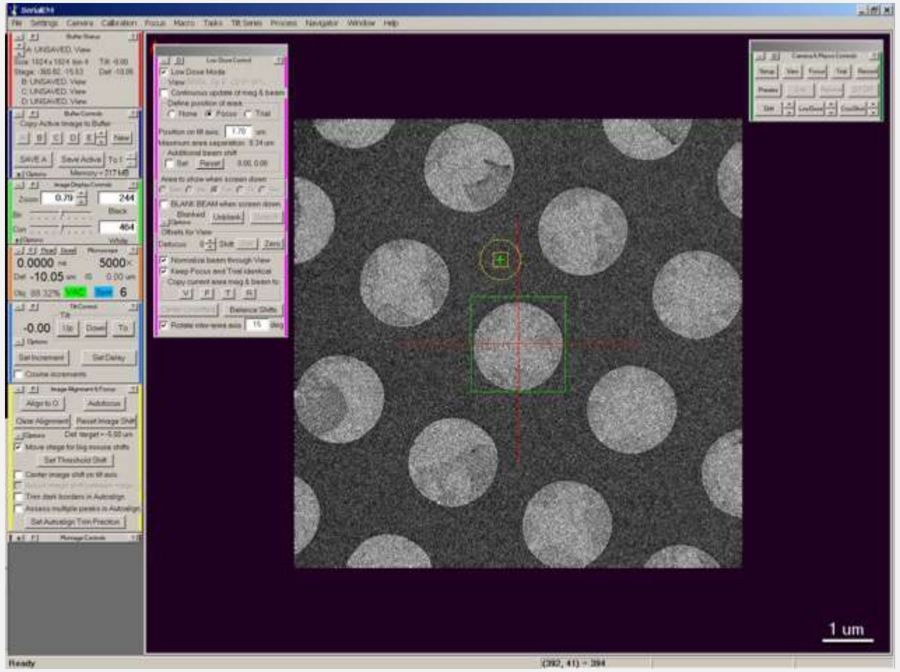
- Semi-automated targeting
- Predictive collection
- Auto multi-grid atlas
- Need to know tilt axis
- ~15 minutes per tilt-series



Suloway et al., 2009

Software for Tilt-series collection

SerialEM

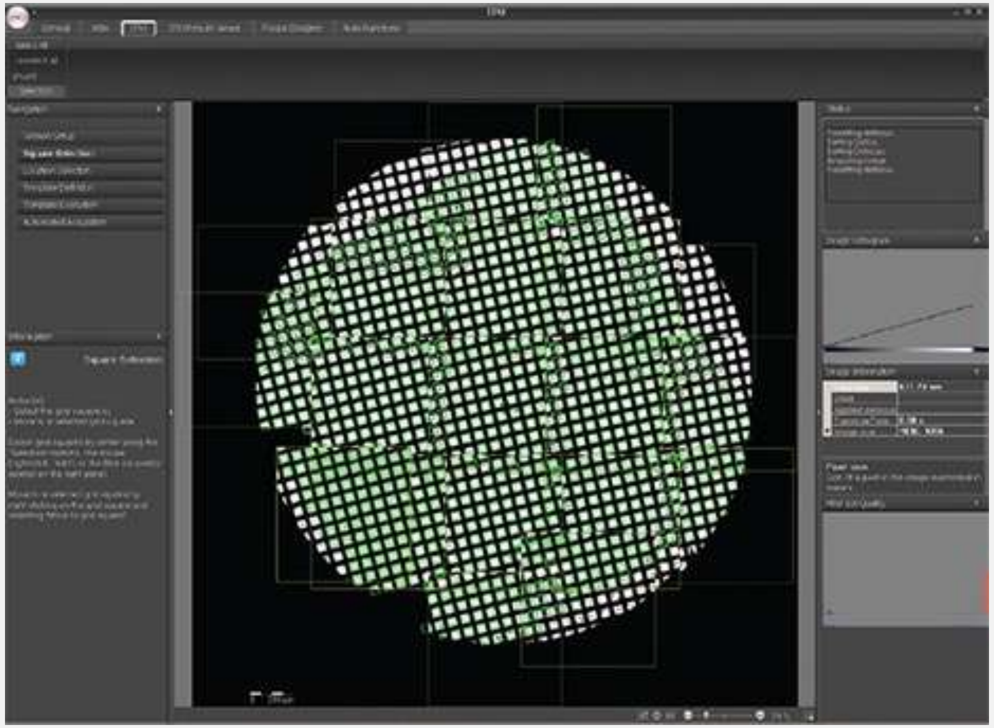


Mastronarde et al., 2003, 2005

- Fully customizable collection with scripting
- Existing scripts allow for very accurate tracking
- 15+ minutes per tilt-series
- Learning curve

Software for Tilt-series collection

EPU Tomo 5

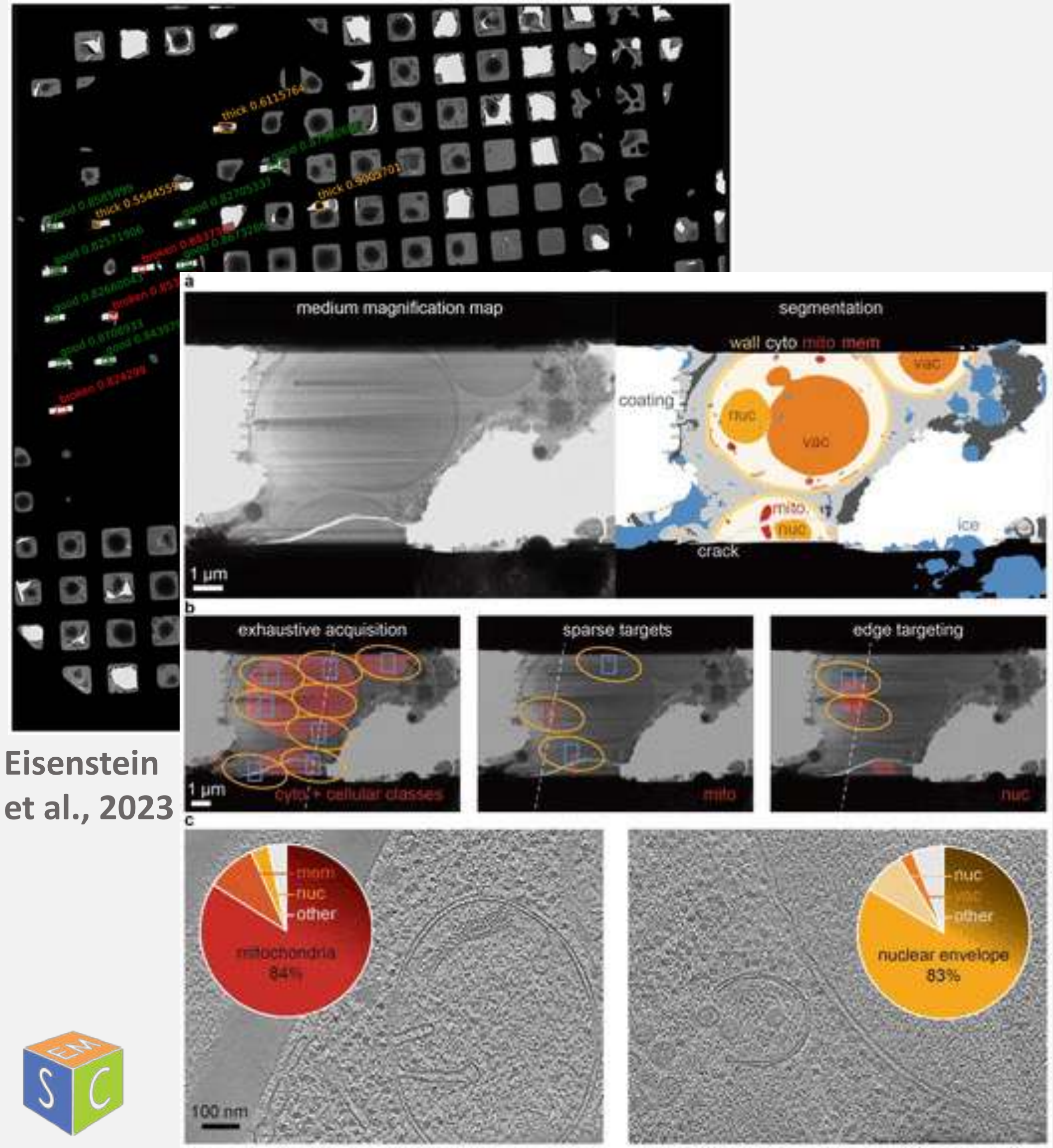


- Multi-site batch collection
- Auto multi-grid atlas
- Tomo Live – tomogram reconstruction and analysis in near-real time
- Commercial software

https://www.thermofisher.com/us/en/home/electron-microscopy/products/software-em-3d-vis/tomography-software.html

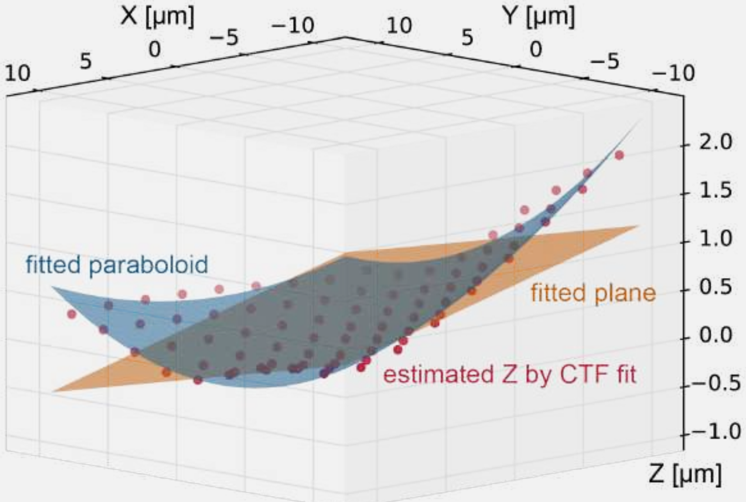
Software for Tilt-series collection

SPACE-Tomo



Eisenstein et al., 2023

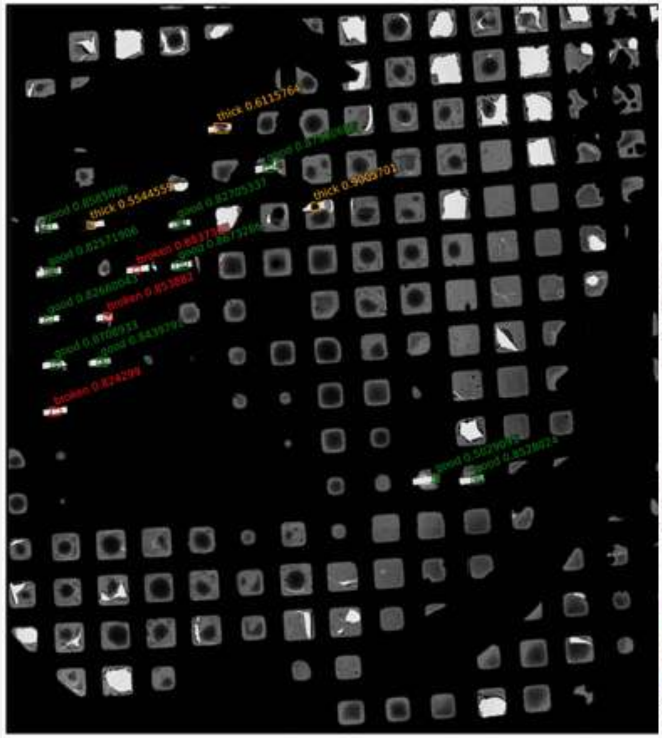
- Built on SerialEM
- Auto grid and lamellae targeting
  - Uses machine learning
- Parallel collection
- 2+ minutes per tilt-series

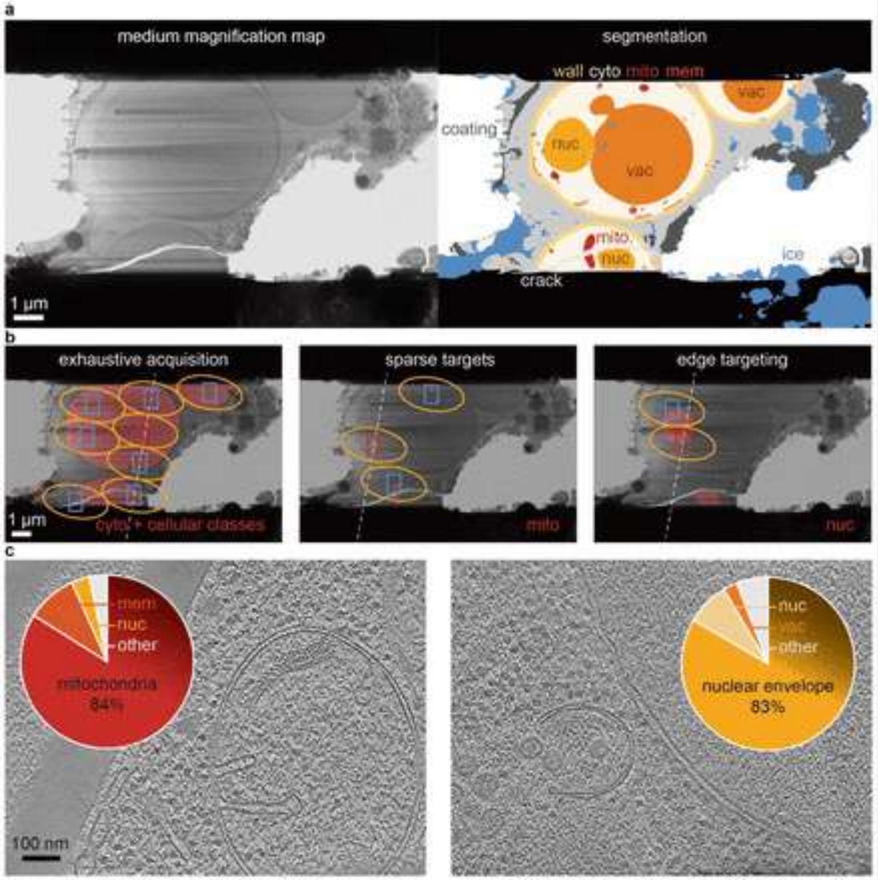


Software for **Tilt-series collection**

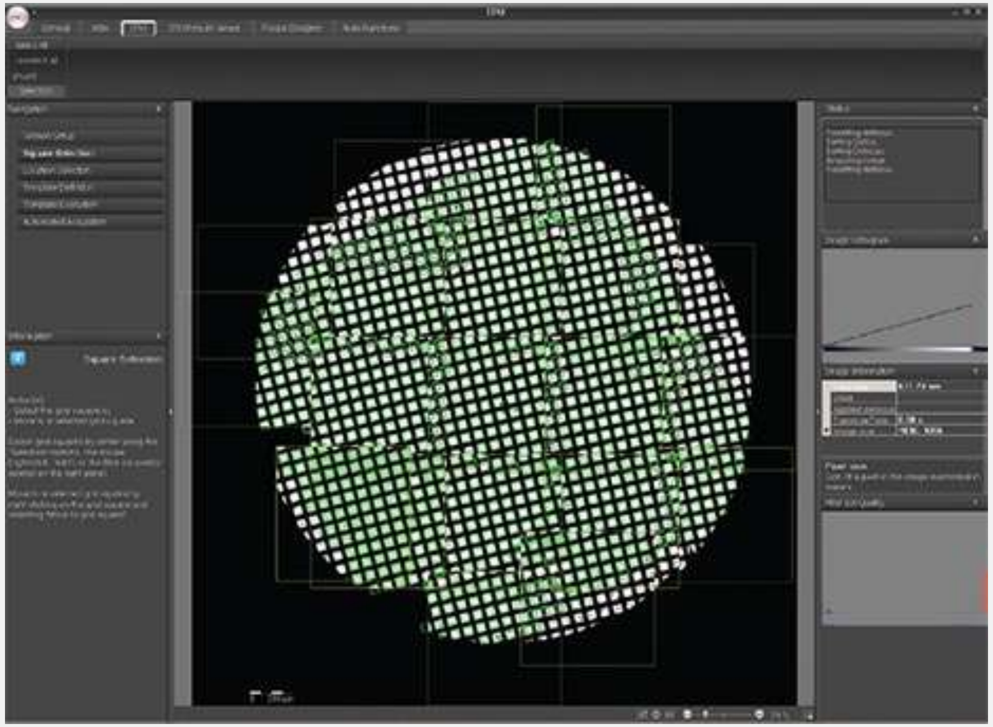
- Best workflows are (S)PACE-Tomo and EPU Tomo 5

SPACE-Tomo

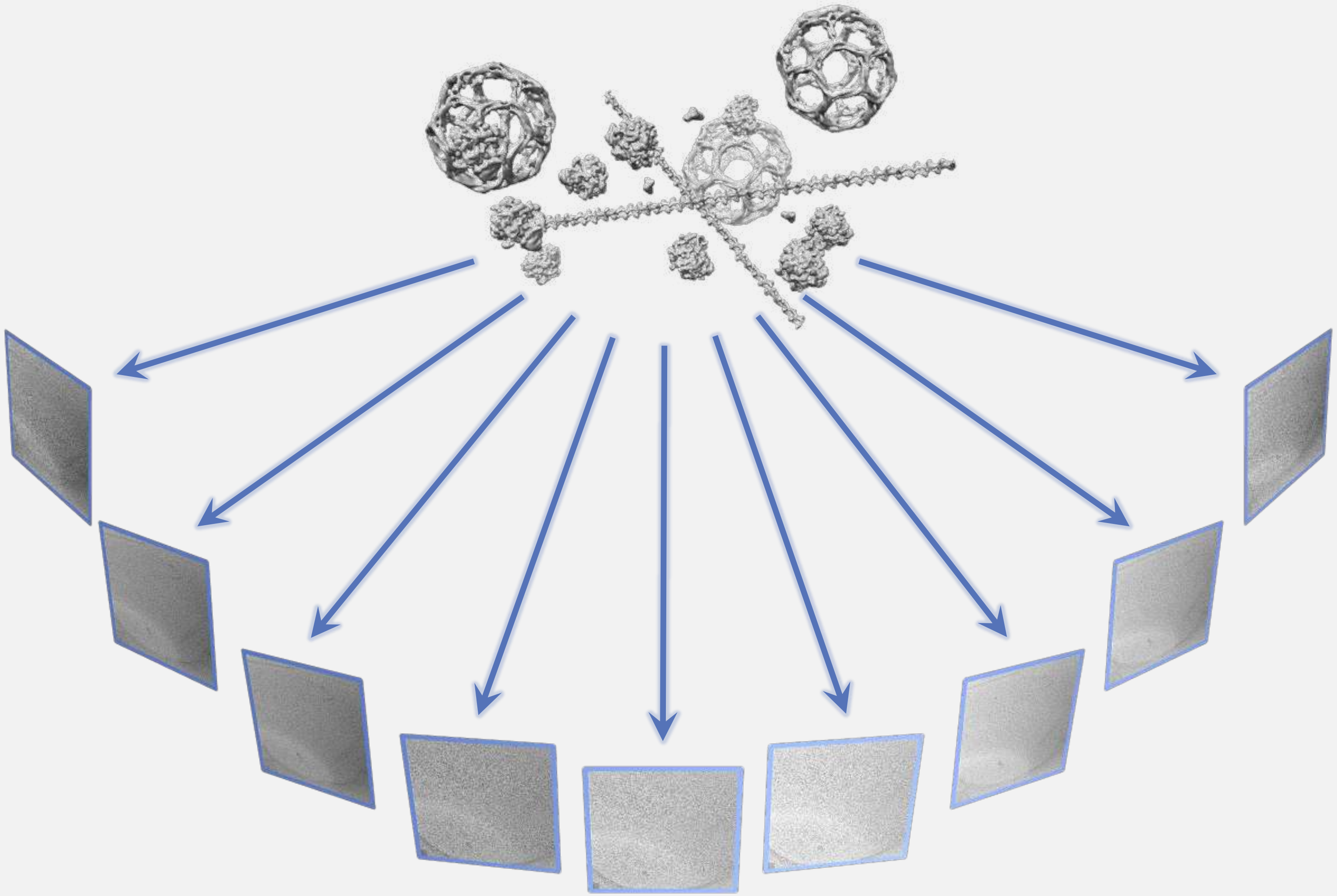




EPU Tomo 5



Now you have a tilt-series of frames



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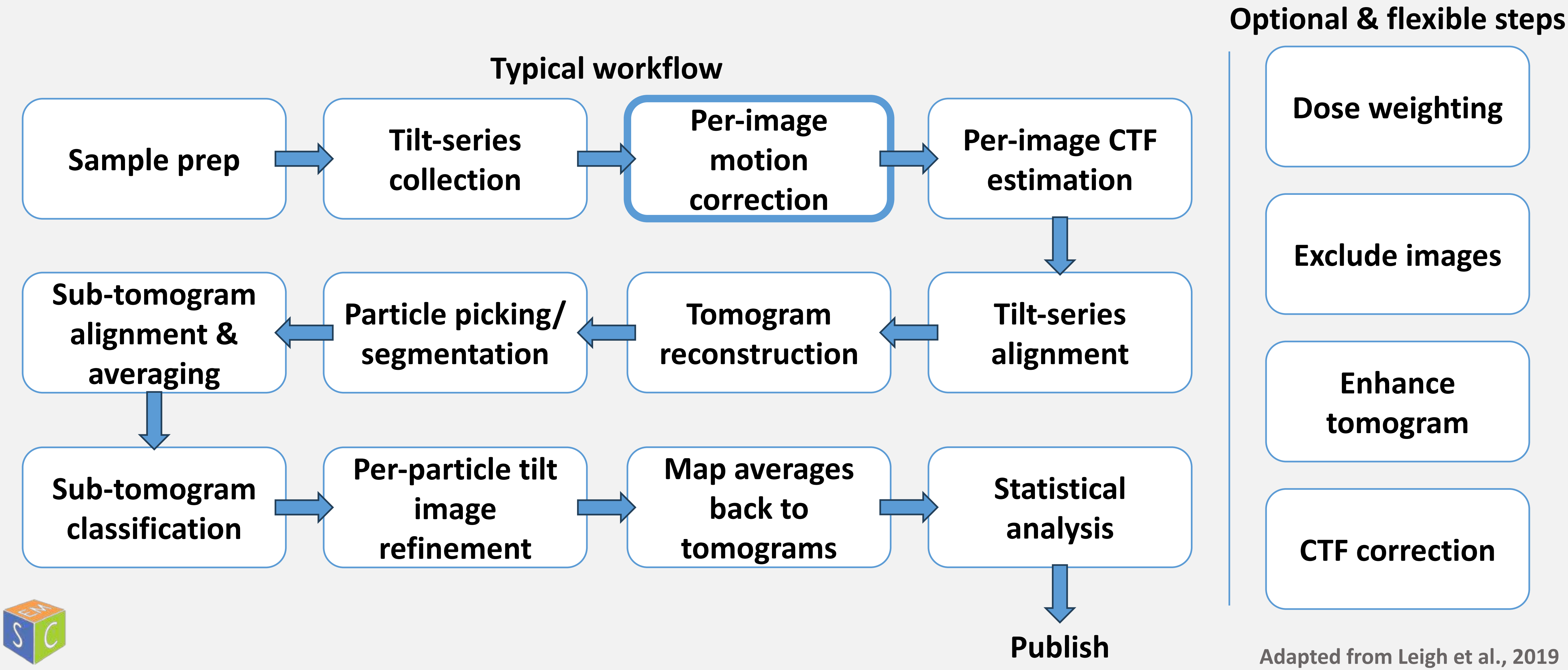
- Analyze software in every step of the workflow



Optional & flexible steps

Typical workflow

Sample prep

Tilt-series collection

Per-image motion correction

Per-image CTF estimation

Tilt-series alignment

Tomogram reconstruction

Particle picking/ segmentation

Sub-tomogram alignment & averaging

Sub-tomogram classification

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Map averages back to tomograms

Statistical analysis

Publish

Dose weighting

Exclude images

Enhance tomogram

CTF correction

Adapted from Leigh et al., 2019

Software for Per-image motion correction

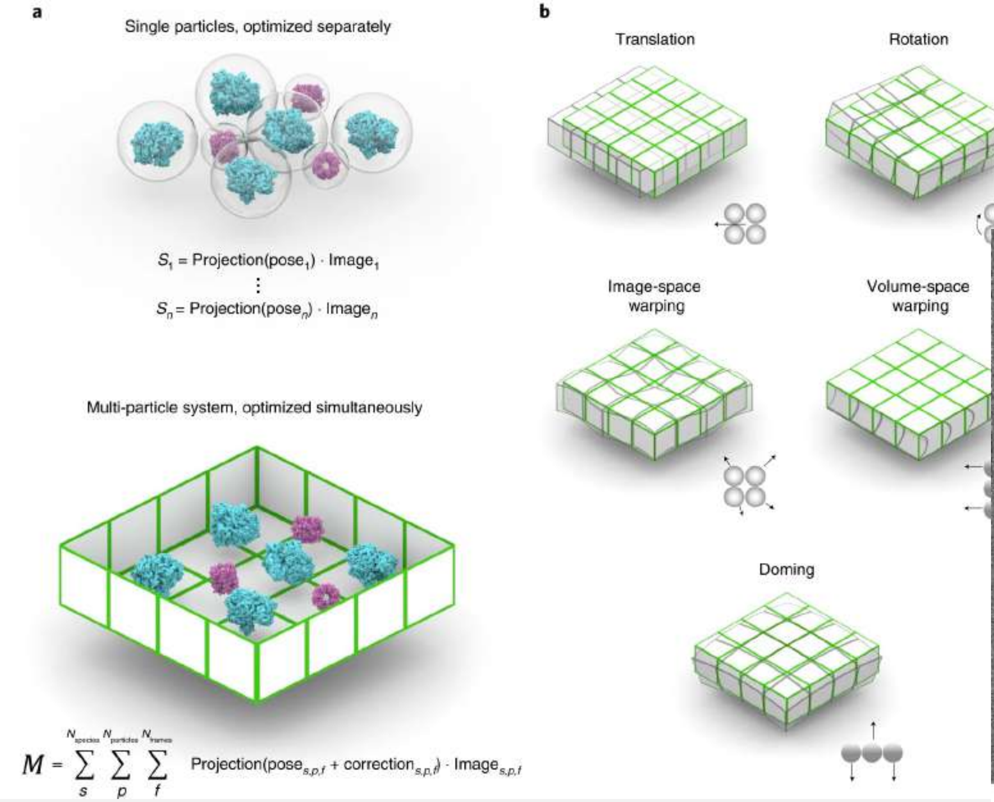
What to look for:

- Full-image and local frame alignment
- Many options for controlling parameters
  - Dose weighting
  - Ignore first frames
- Able to process low-dose cryoET images
- GPU support

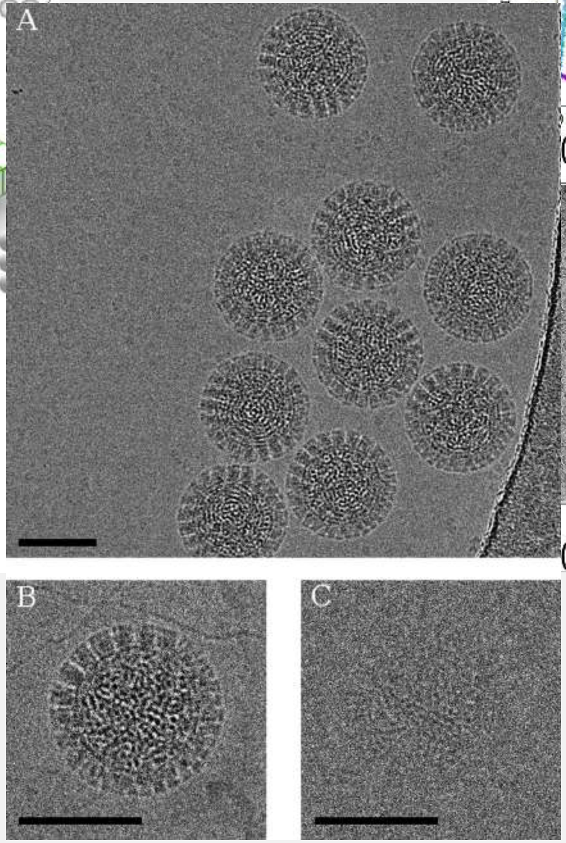
Software for

Per-image motion correction

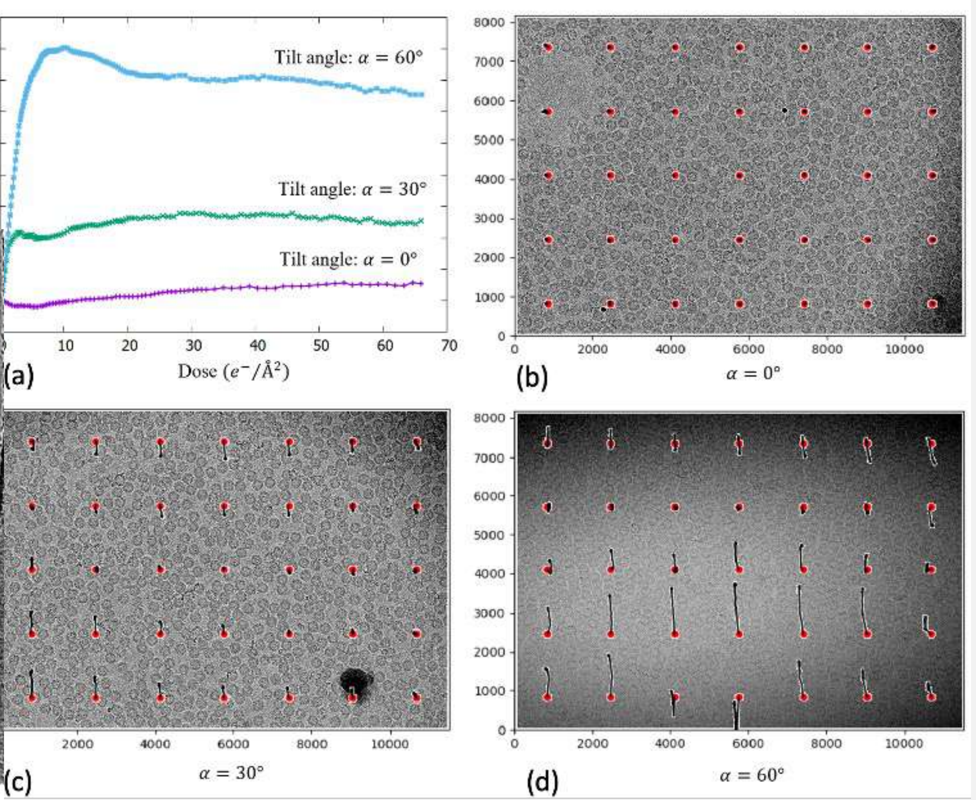
Warp



Unblur



Motioncor2/3



Software for Per-image motion correction

All:

- Global and local
- Near real-time with collection
- Dose weighting

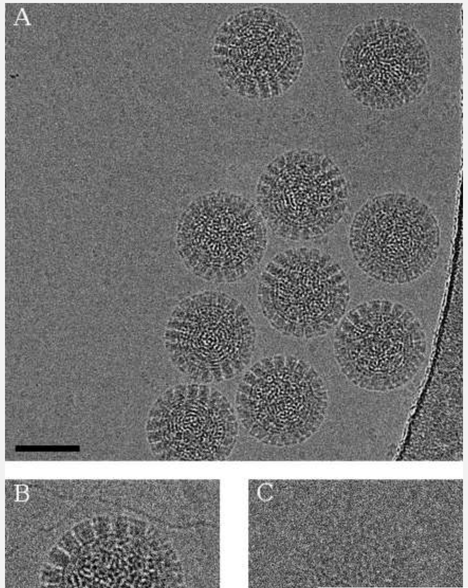
Motioncor & Warp:

- GPU
- CTF estimation

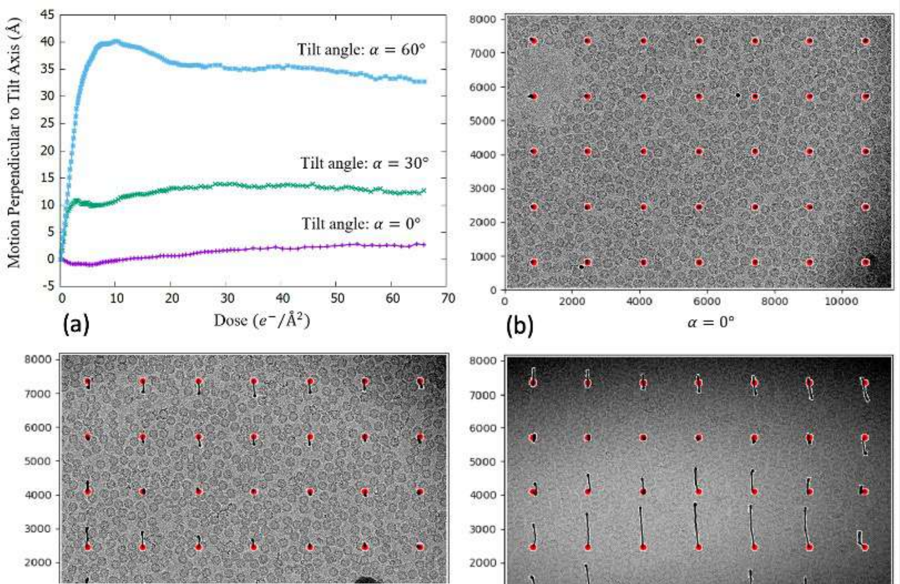
Warp:

- 3D modeling

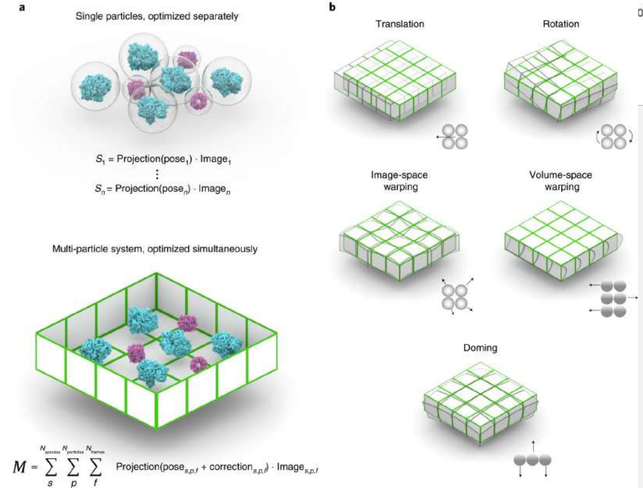
Unblur



Motioncor2/3



Warp



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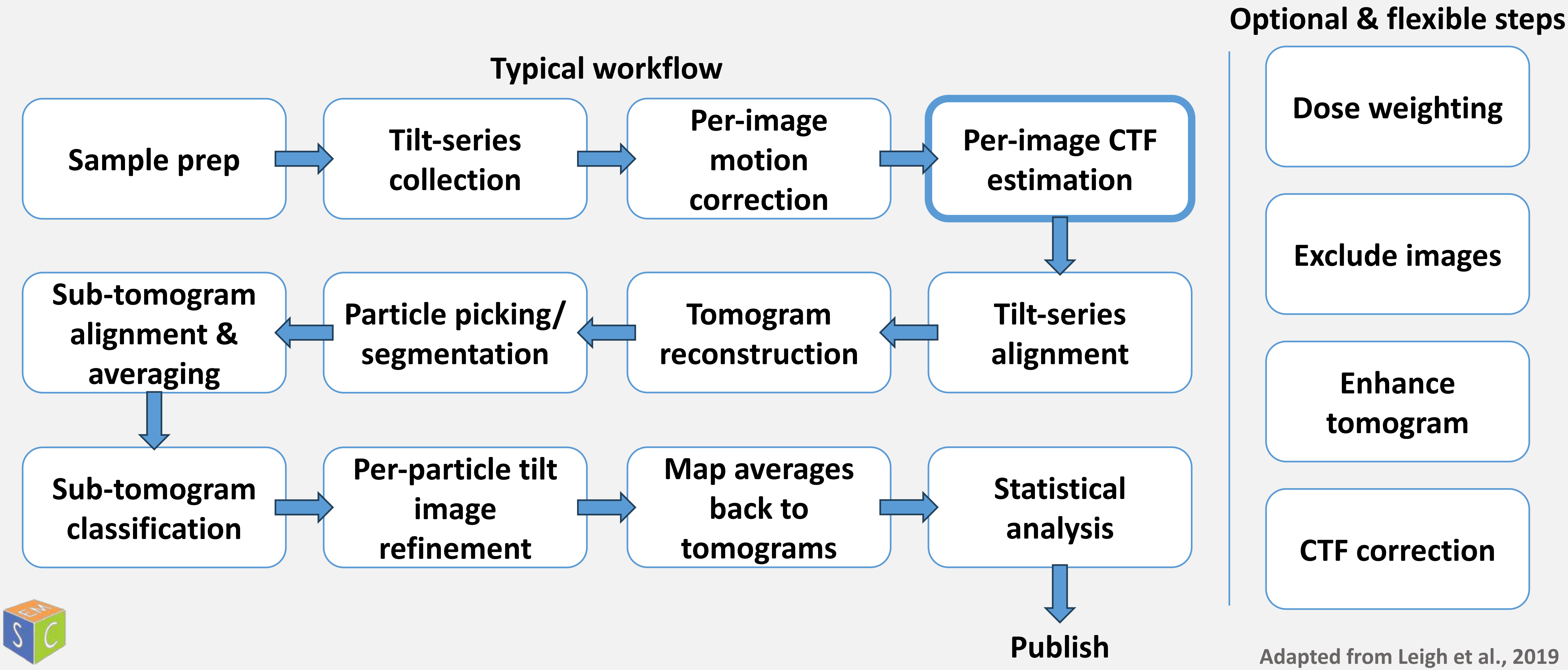
- Analyze software in every step of the workflow



Typical workflow

- Sample prep
- Tilt-series collection
- Per-image motion correction
- Per-image CTF estimation
- Tilt-series alignment
- Tomogram reconstruction
- Particle picking/ segmentation
- Sub-tomogram alignment & averaging
- Sub-tomogram classification
- Per-particle tilt image refinement
- Map averages back to tomograms
- Statistical analysis
- Publish

Optional & flexible steps

- Dose weighting
- Exclude images
- Enhance tomogram
- CTF correction

Adapted from Leigh et al., 2019

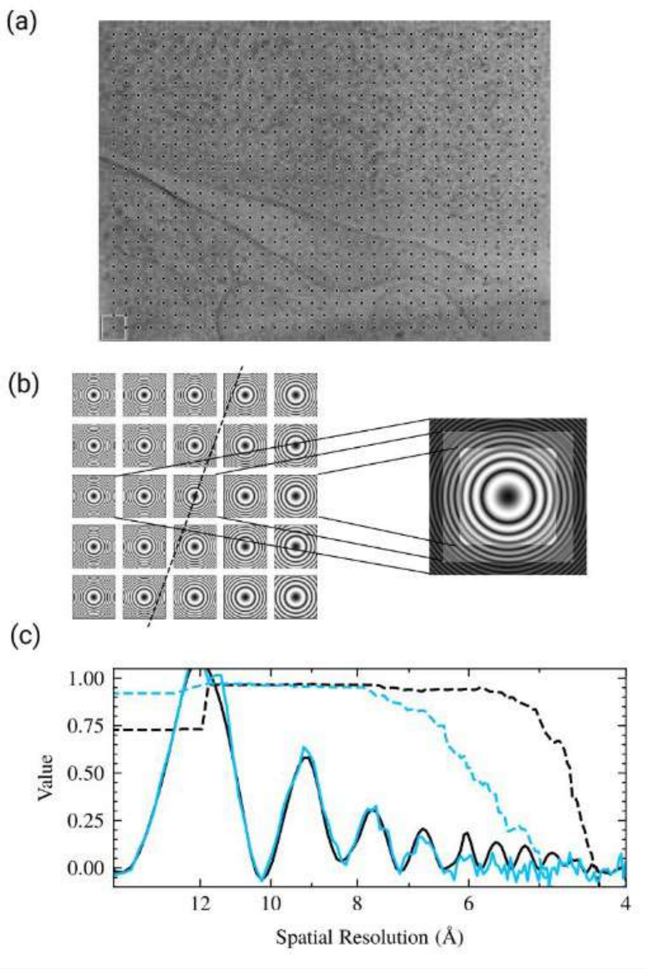
Software for Per-image CTF estimation

What to look for:

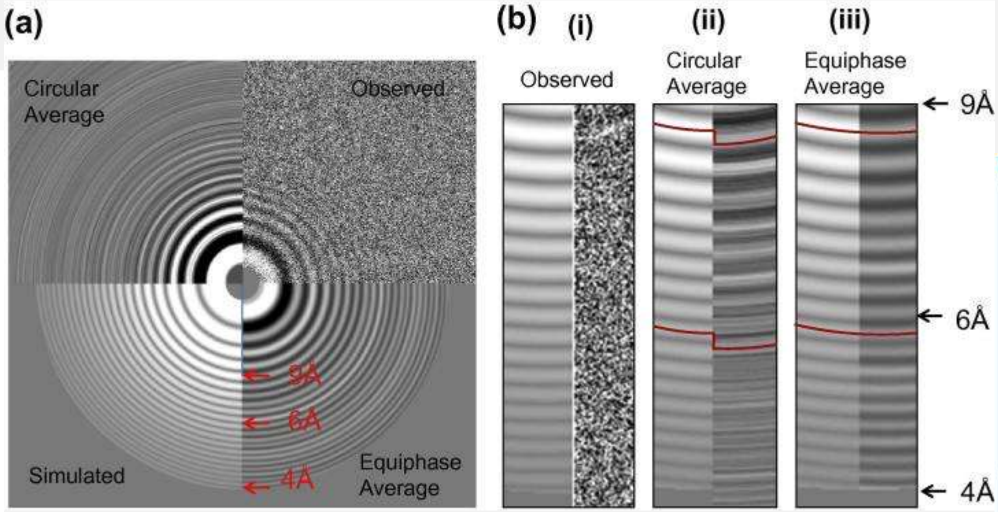
- Accurate high-resolution estimation (3-4 angstroms)
- Local CTF estimation (defocus gradient)
- Refines based on whole tilt-series

Software for Per-image CTF estimation

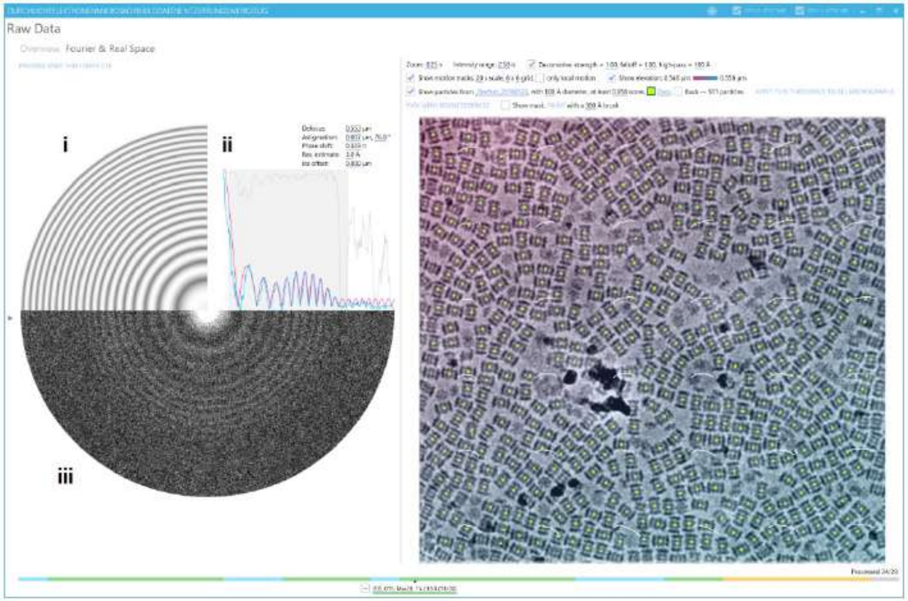
CTFFIND 4/5

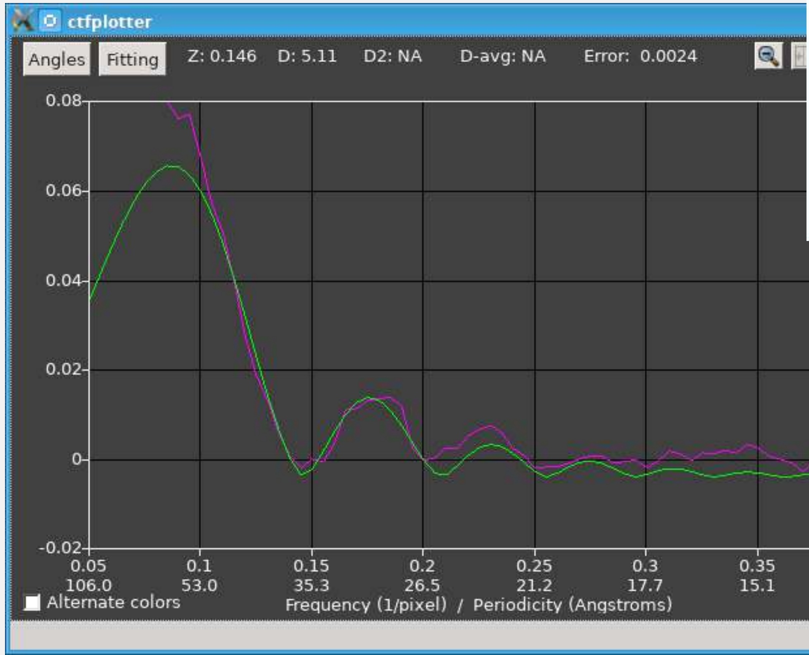


GCTF



Warp





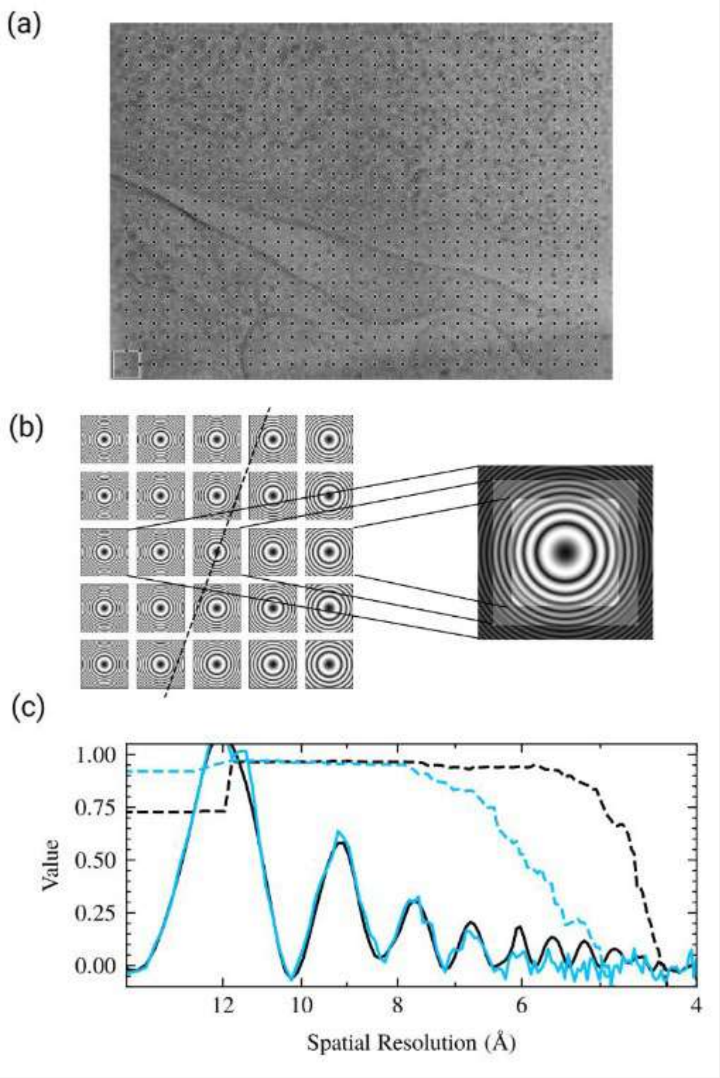
Z: 0.146 D: 5.11 D2: NA D-avg: NA Error: 0.0024

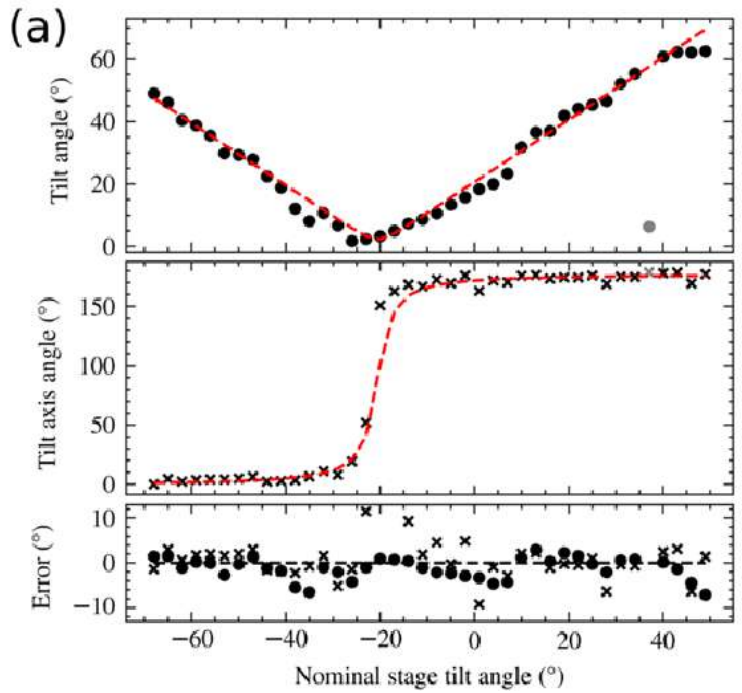
CTFPlotter

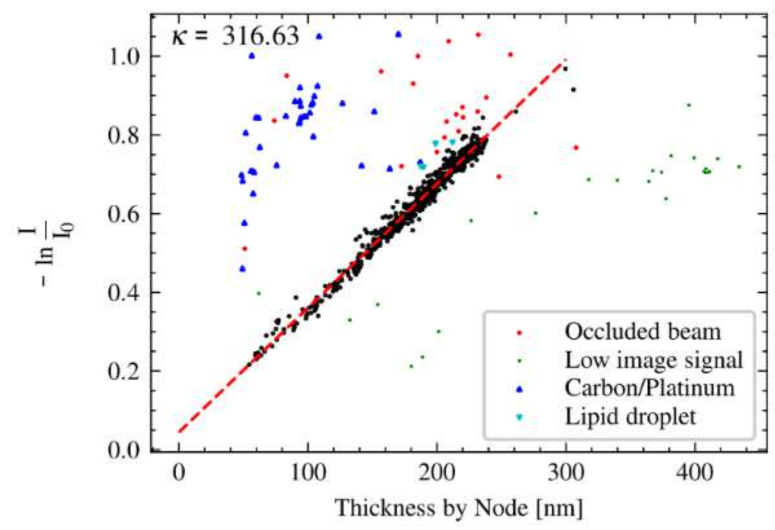
Software for

Per-image CTF estimation

CTFFIND 4/5







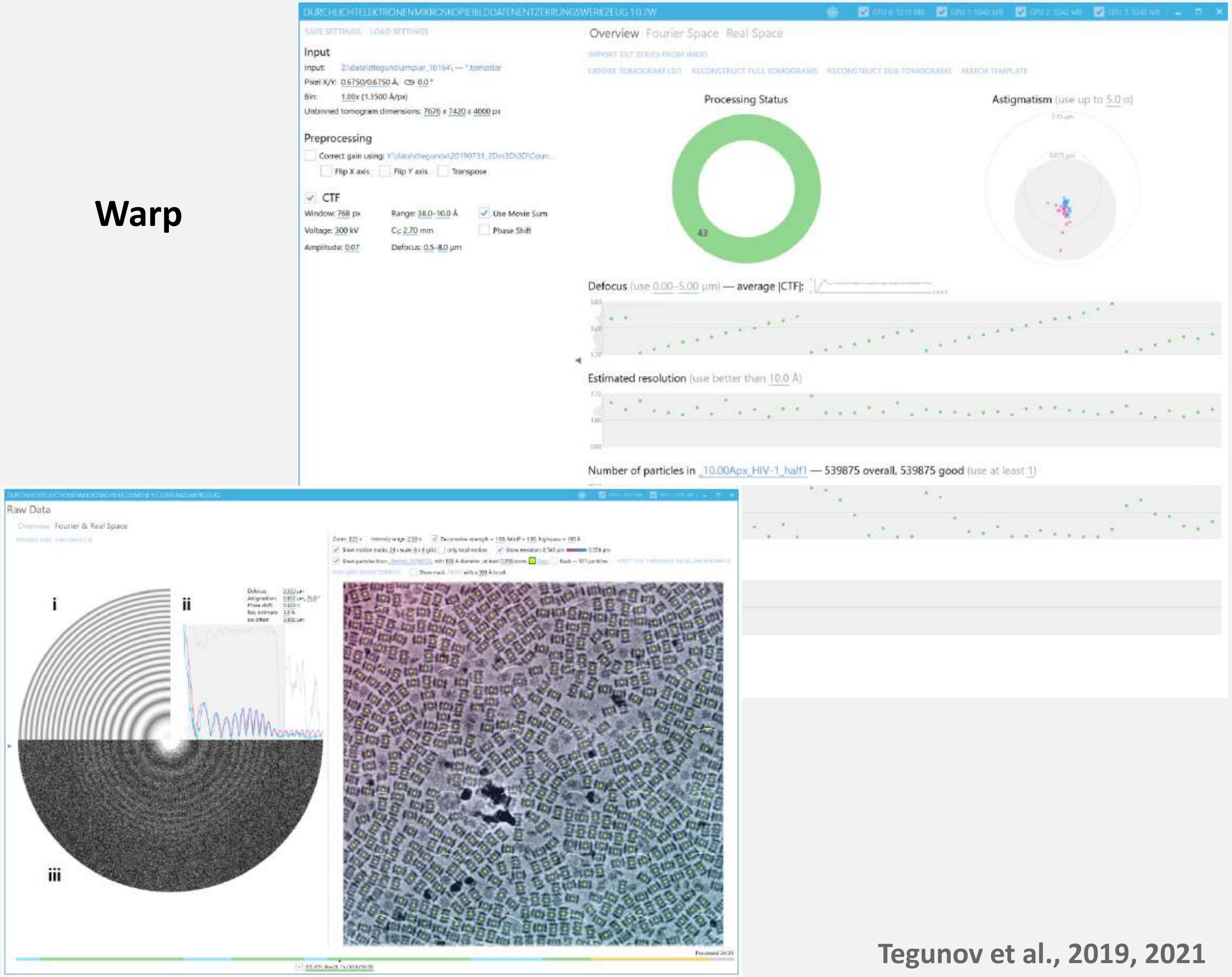
- Local CTF estimation
- Estimates tilt axis angle per image
- Estimates local ice thickness
- Corrects for CTF

Elferich et al., 2023

Software for **Per-image CTF estimation**

- Local CTF estimation
- Estimates tilt axis angle per image
- Determines handedness
- Refines based on whole tilt-series
- Corrects for CTF
- Local CTF refinement

Warp



DURCHLICHTELEKTRONENMIKROSKOPIEBILDDATENENTZERRUNGSWERKZEUG 1.0.7W

Input

Input: Z:\data\dtegunov\empiar_10164\ — *.tomostar

Pixel X/Y: 0.6750/0.6750 Å, 0.0 °

Bin: 1.00x (1.3500 Å/px)

Unbinned tomogram dimensions: 7676 x 7420 x 4000 px

Preprocessing

☐ Correct gain using: Y:\data\dtegunov\20190731_2Dvs3D\3D\Coun...

☐ Flip X axis ☐ Flip Y axis ☐ Transpose

☑ CTF

Window: 768 px

Range: 38.0–10.0 Å

☑ Use Movie Sum

Voltage: 300 kV

Cs: 2.70 mm

☐ Phase Shift

Amplitude: 0.07

Defocus: 0.5–8.0 µm

Astigmatism (use up to 5.0 σ)

Defocus (use 0.00–5.00 µm) — average |CTF|:

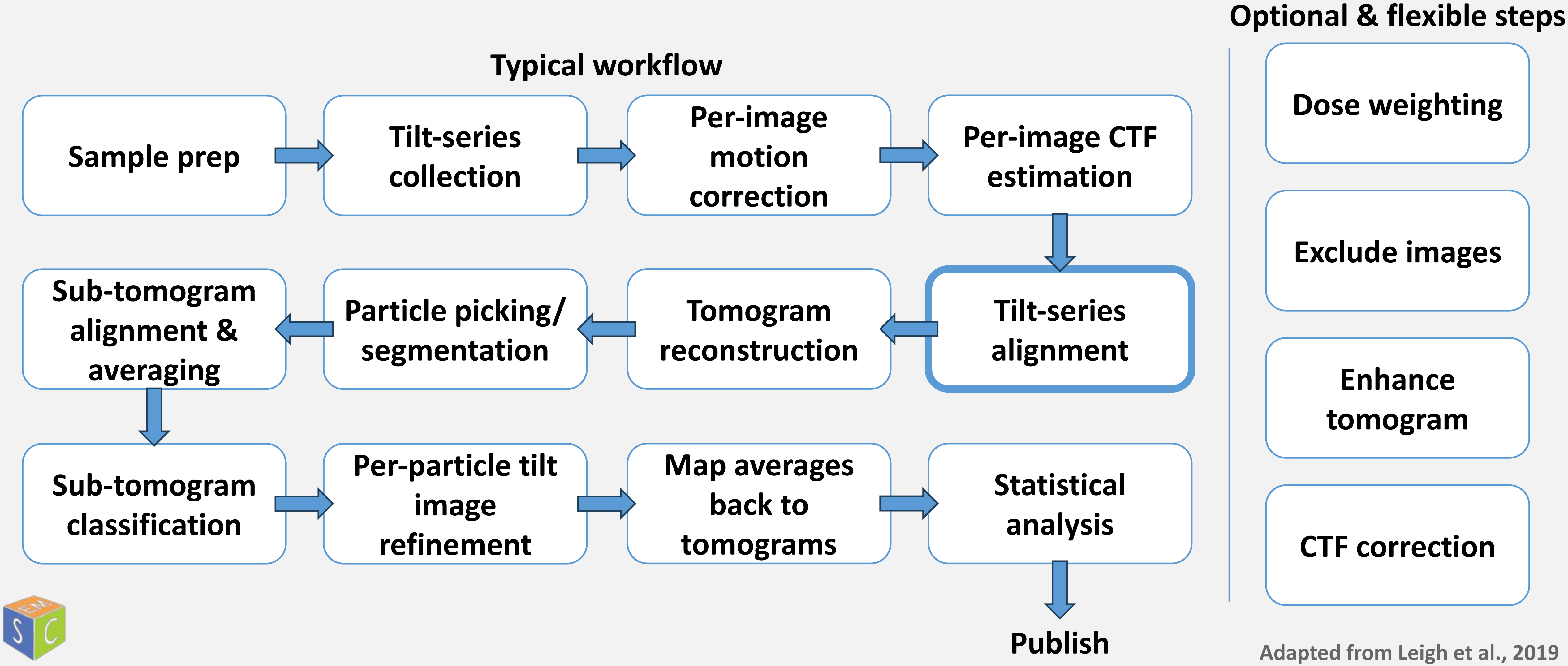
Estimated resolution (use better than 10.0 Å)

Number of particles in _10.00Apx_HIV-1_half1 — 539875 overall, 539875 good (use at least 1)

Tegunov et al., 2019, 2021

Today's plan

- Analyze software in every step of the workflow



Typical workflow

Sample prep

Tilt-series collection

Per-image motion correction

Per-image CTF estimation

Tilt-series alignment

Tomogram reconstruction

Particle picking/ segmentation

Sub-tomogram alignment & averaging

Sub-tomogram classification

Per-particle tilt image refinement

Map averages back to tomograms

Statistical analysis

Publish

Optional & flexible steps

Dose weighting

Exclude images

Enhance tomogram

CTF correction

Adapted from Leigh et al., 2019

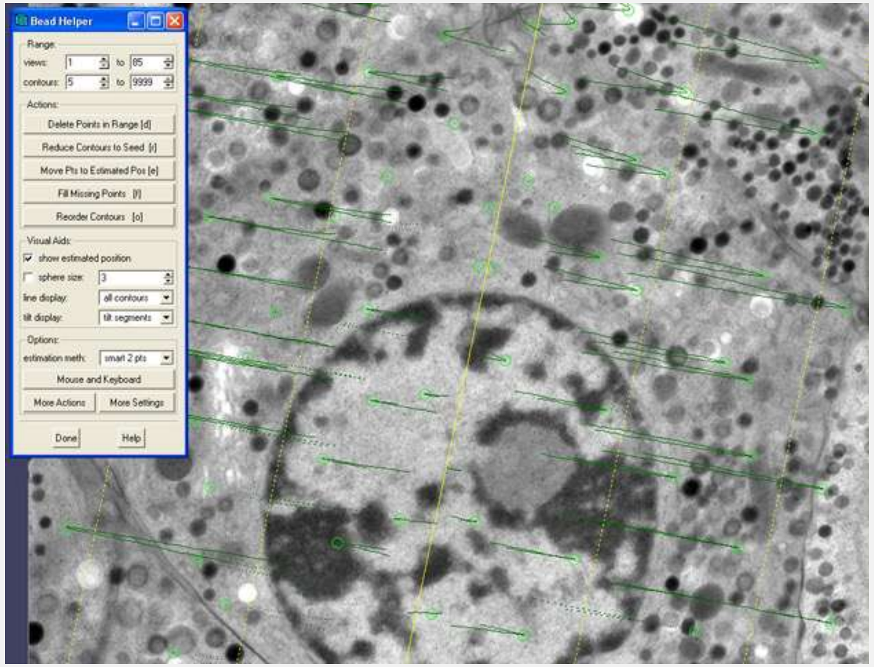
Software for Tilt-series alignment

What to look for:

- Robustness and accuracy for your sample
- Number of parameters that need to be played with
- Speed – can it keep up with data collection?

Software for Tilt-series alignment

IMOD



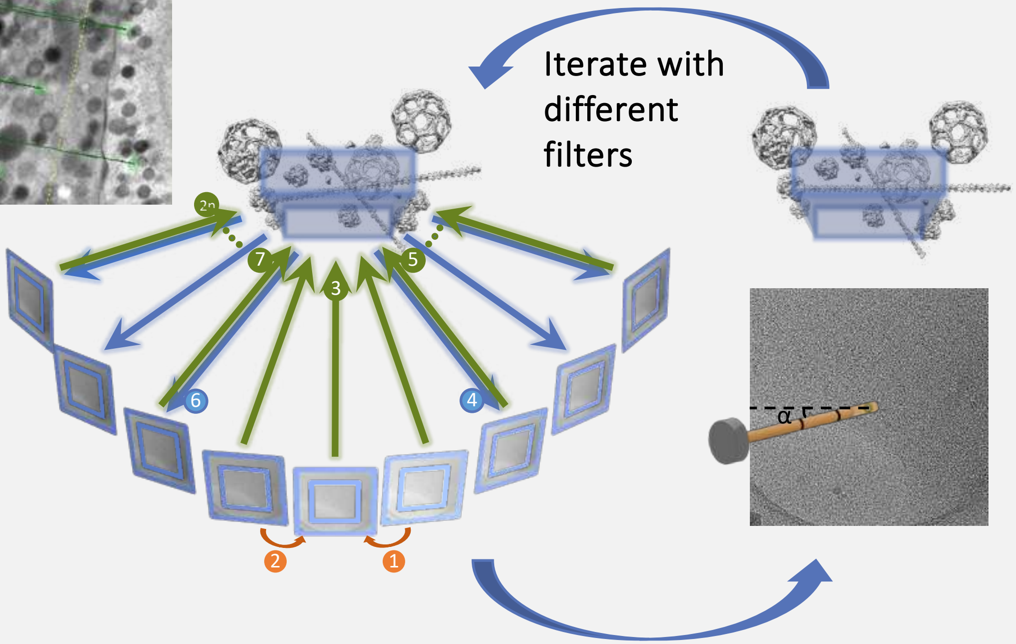
Range:
views: 1 to 85
contours: 5 to 9999

Actions:

Visual Aids:
☑ show estimated position
☐ sphere size: 3
line display: all contours
tilt display: tilt segments

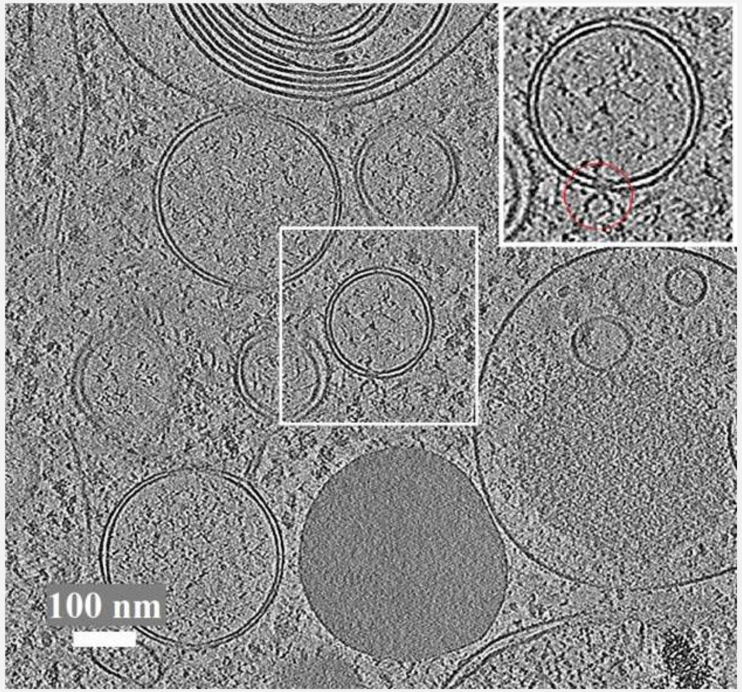
Options:
estimation meth: smart 2 pts

Protomo



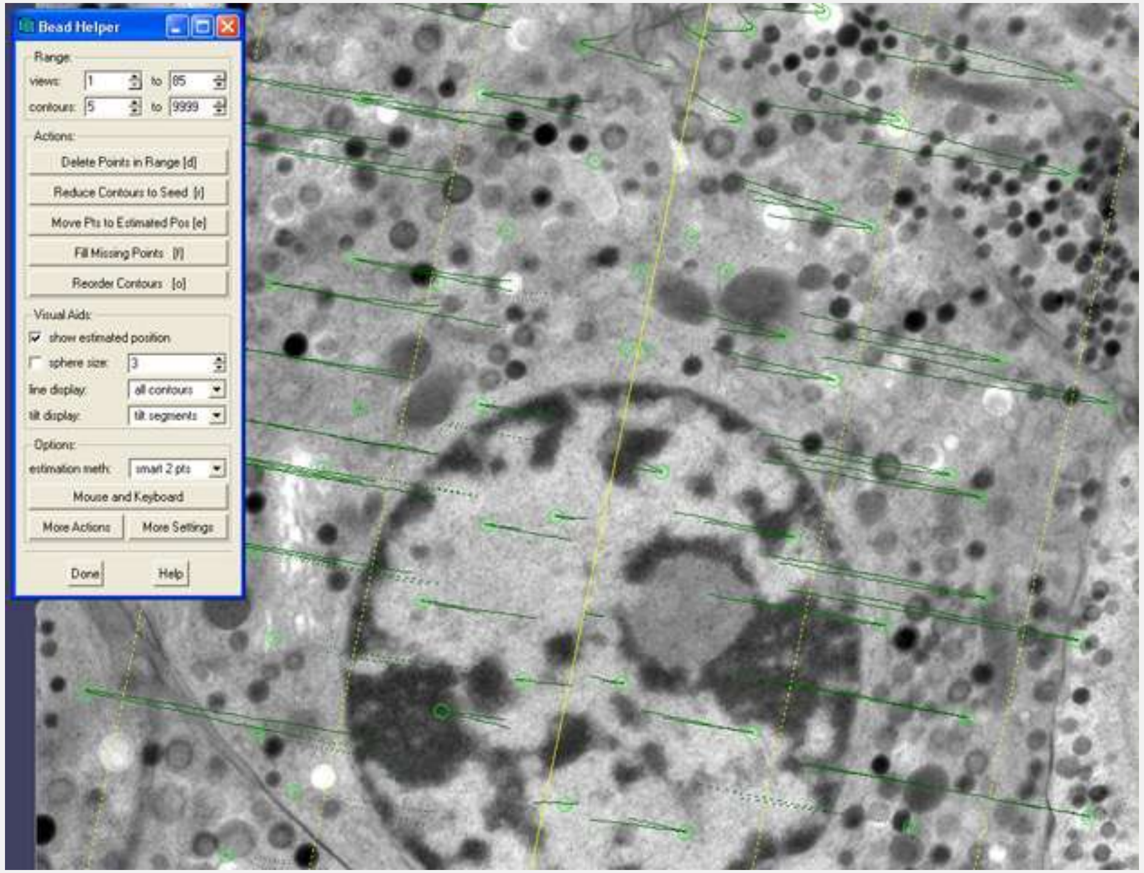
Iterate with different filters

Aretomo



Software for Tilt-series alignment

IMOD (and other gold bead tracking software)



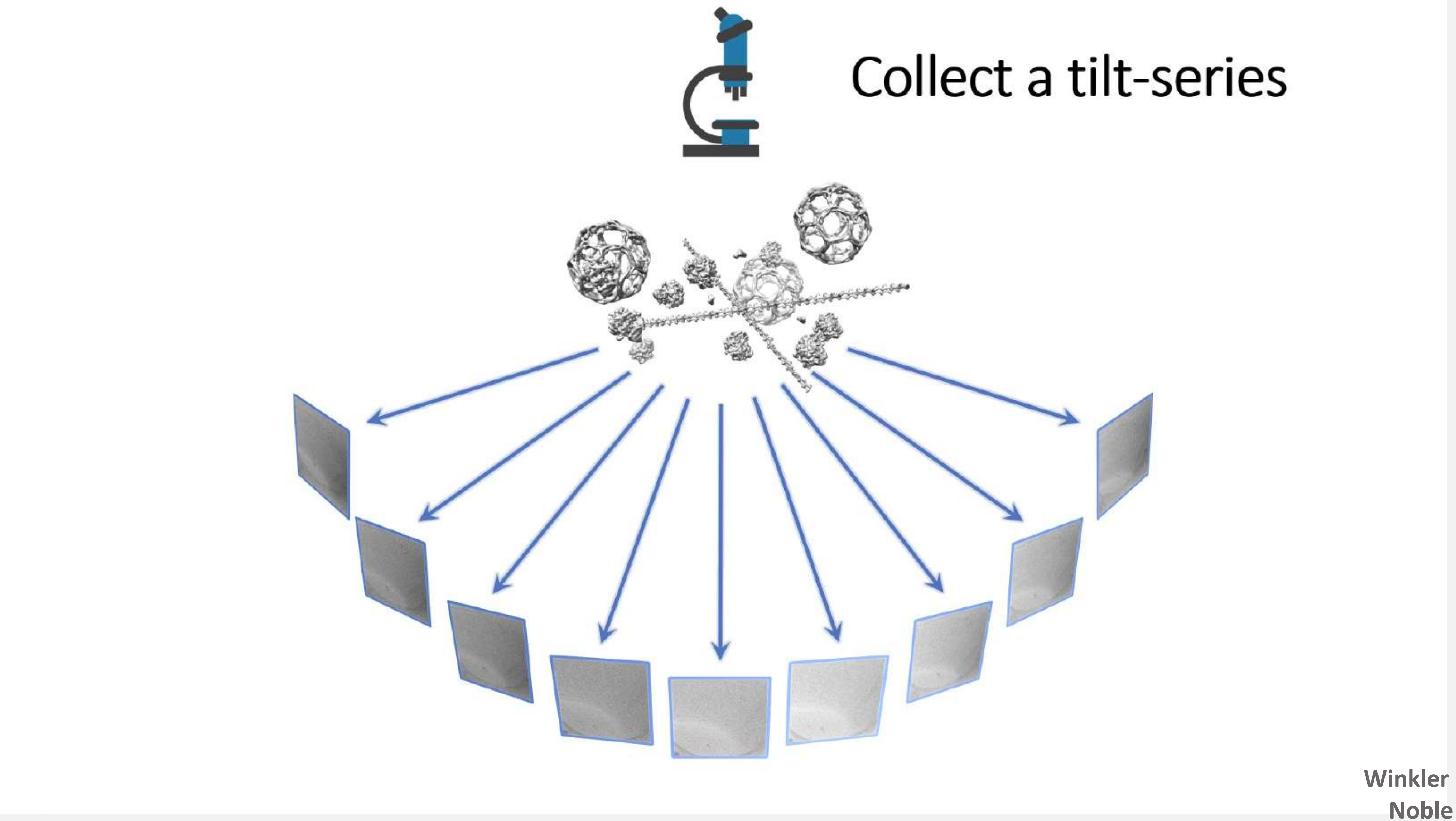
views: 1 to 85
contours: 5 to 9999
☑ show estimated position
☐ sphere size: 3
line display: all contours
tilt display: tilt segments
estimation meth: smart 2 pts

David Mastronarde

- Requires a sufficient number of well-behaved gold beads
  - Sample prep optimization
- Semi-automated in IMOD
- Automated in other workflows

Software for Tilt-series alignment

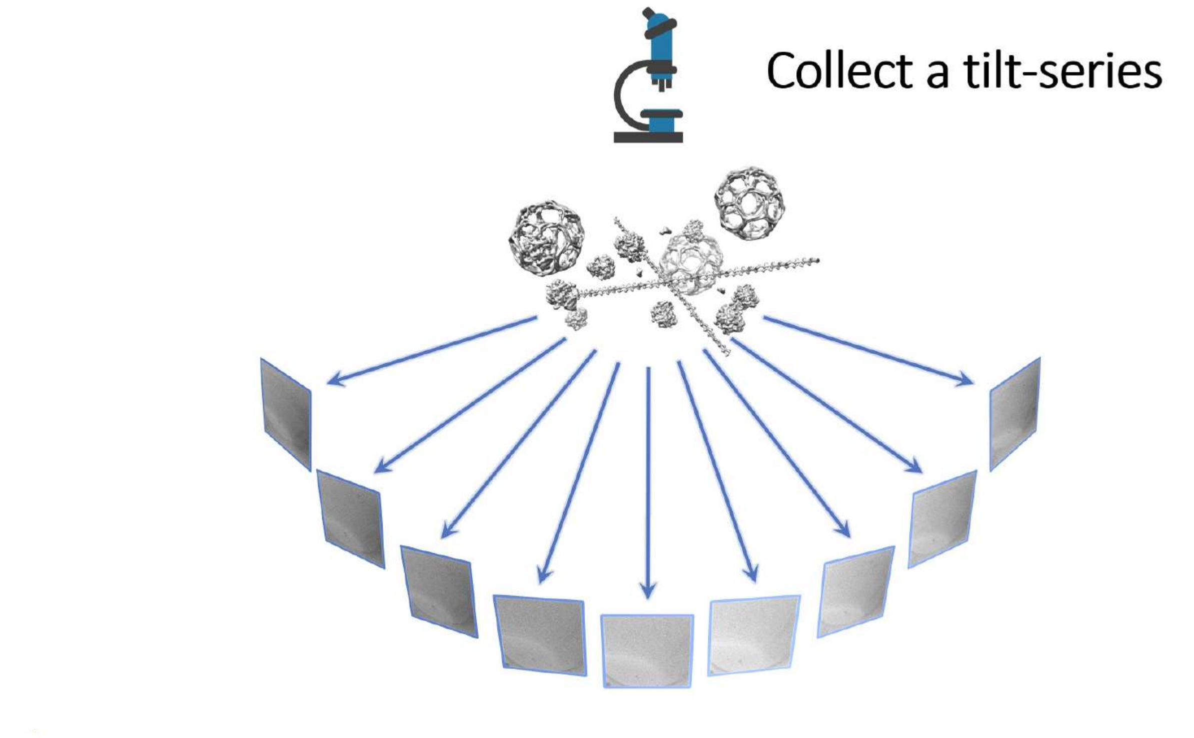
Protomo



Winkler & Taylor, 2006
Noble & Stagg, 2015

Software for **Tilt-series alignment**

Protomo

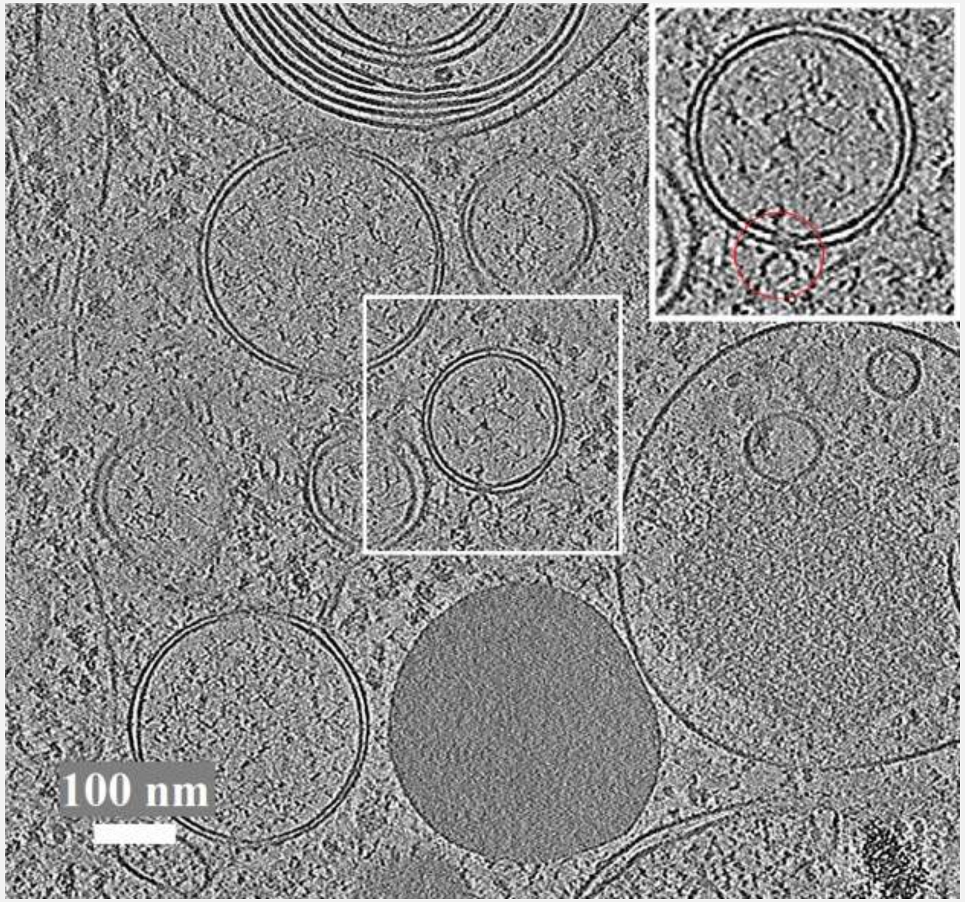


- No gold beads
- One of the most accurate methods
- Slow – hours to days
- Only thing it doesn’t refine are nominal tilt angles

Winkler & Taylor, 2006
Noble & Stagg, 2015

Software for Tilt-series alignment

Aretomo



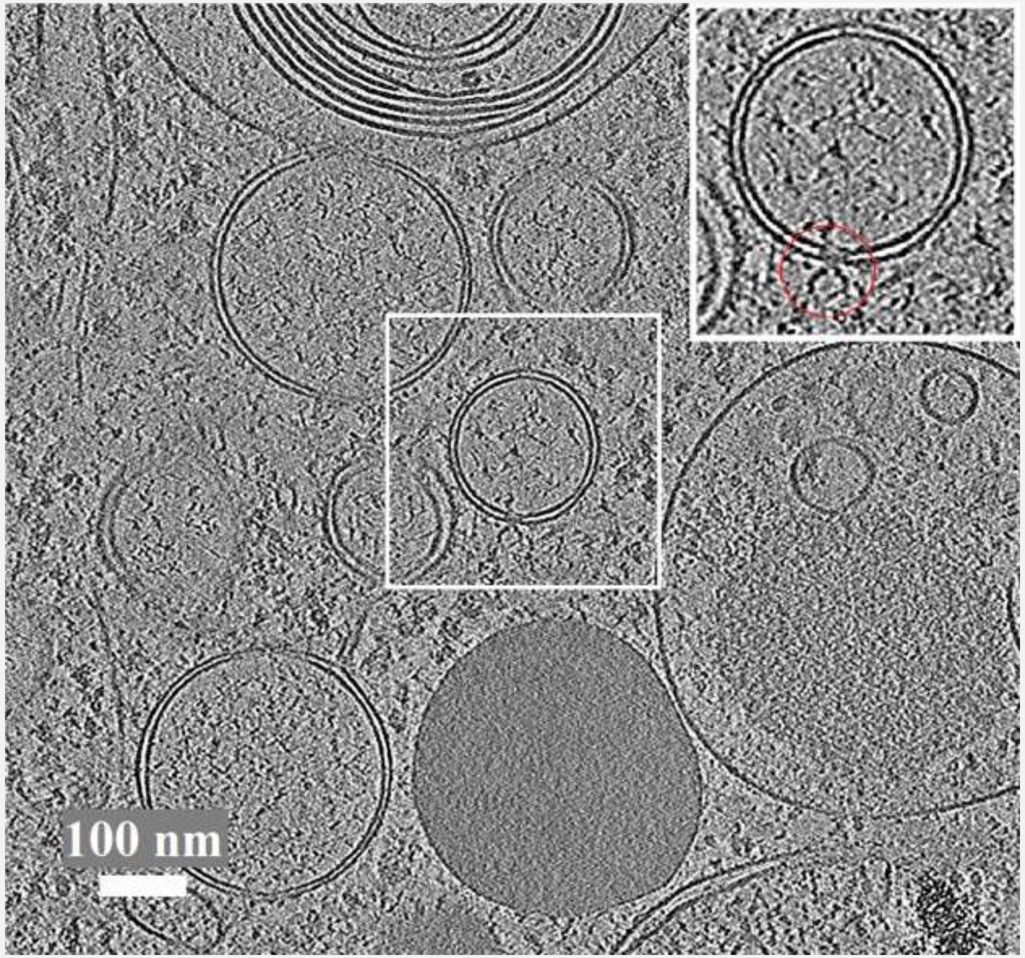
Zheng et al., 2022

- No gold beads
- Similar method as Protomo
- Local alignment
- Aretomo2 includes CTF estimation
- Fast – a couple minutes

Software for

Tilt-series alignment

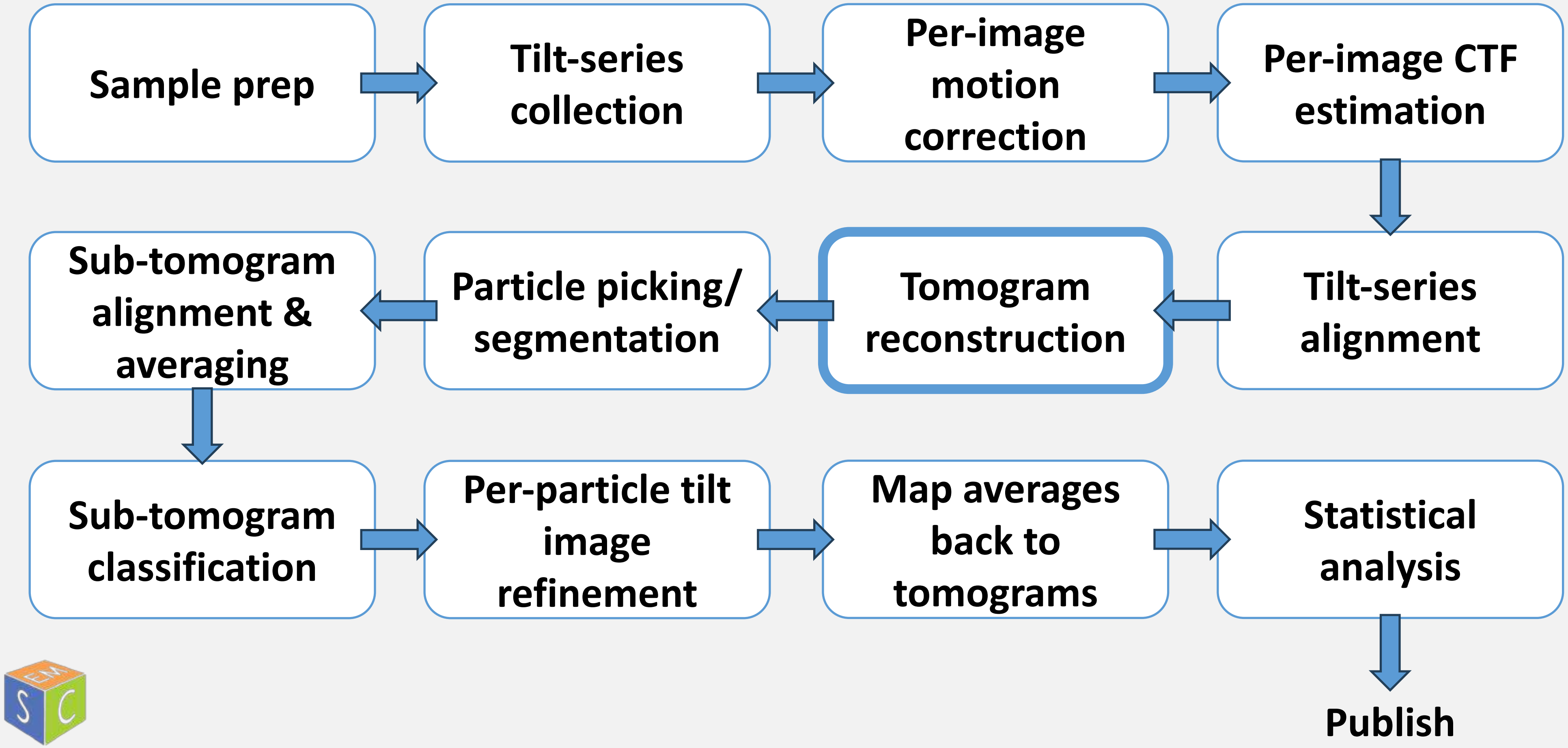
- Best workflow is Aretomo



Today's plan

- Analyze software in every step of the workflow

Typical workflow



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Exclude images

Enhance tomogram

CTF correction

Adapted from Leigh et al., 2019

Software for **Tomogram reconstruction**

What to look for:

- Fidelity to original data
- How missing wedge is treated
- Is the resulting tomogram useful for visualization/segmentation or sub-tmogram processing?

Software for Tomogram reconstruction

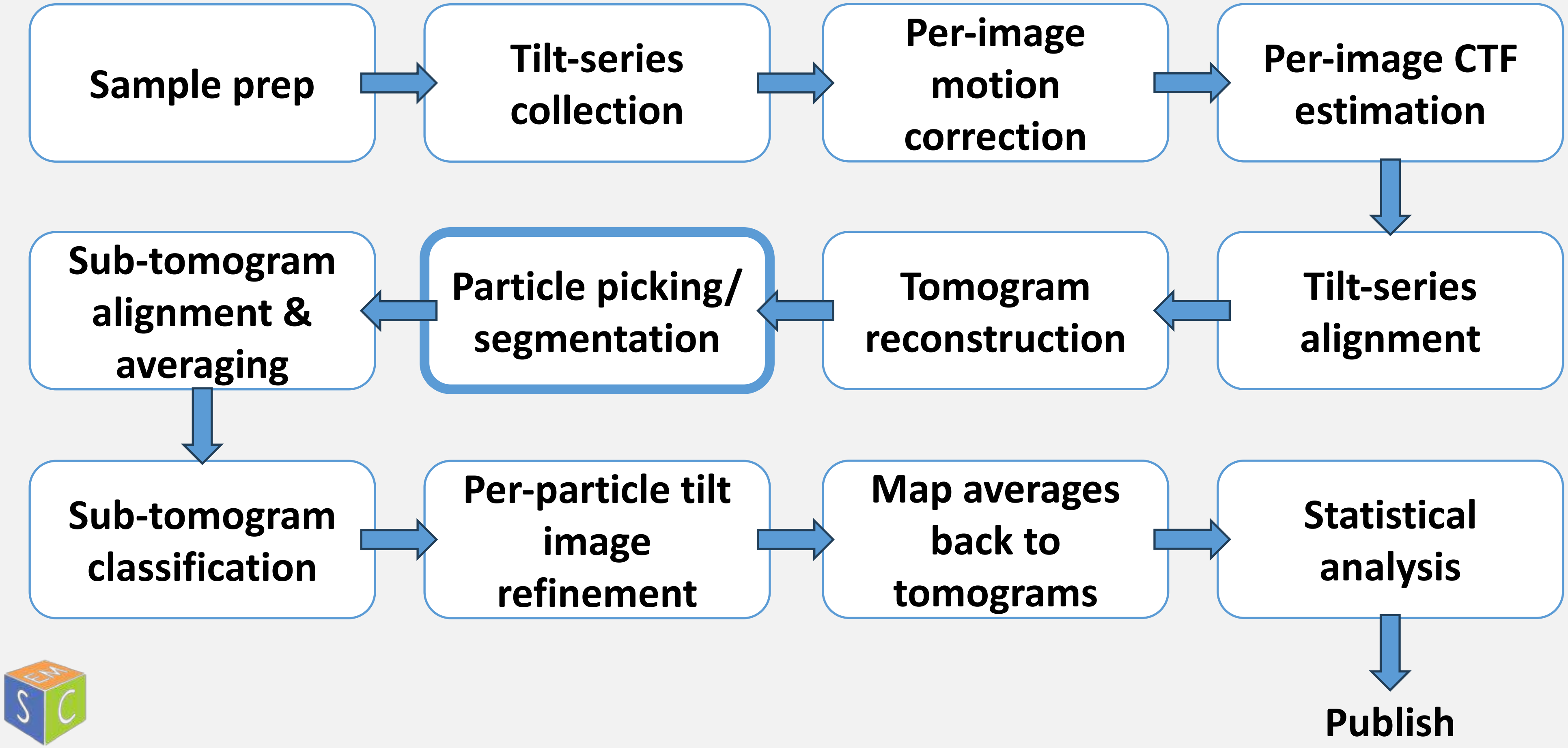
Many, many algorithms and software:

- Algorithms: Weighted Back-Projection (WBP), SIRT, ART, SART, FIRT, MBIR, …
- IMOD
- Aretomo
- Protomo
- Warp
- TomoAlign
- Tomo3D
- EMAN2

Today's plan

- Analyze software in every step of the workflow

Typical workflow



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Adapted from Leigh et al., 2019

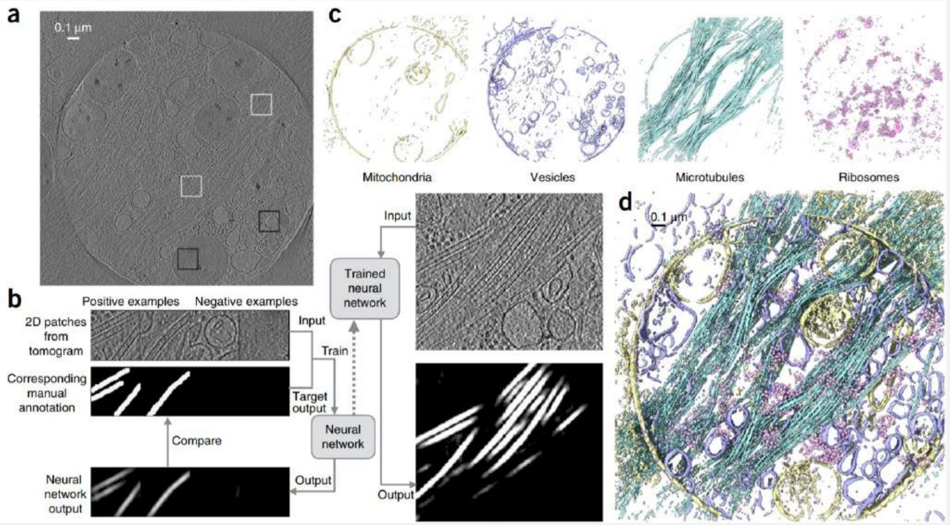
Software for **Particle picking/ segmentation**

What to look for:

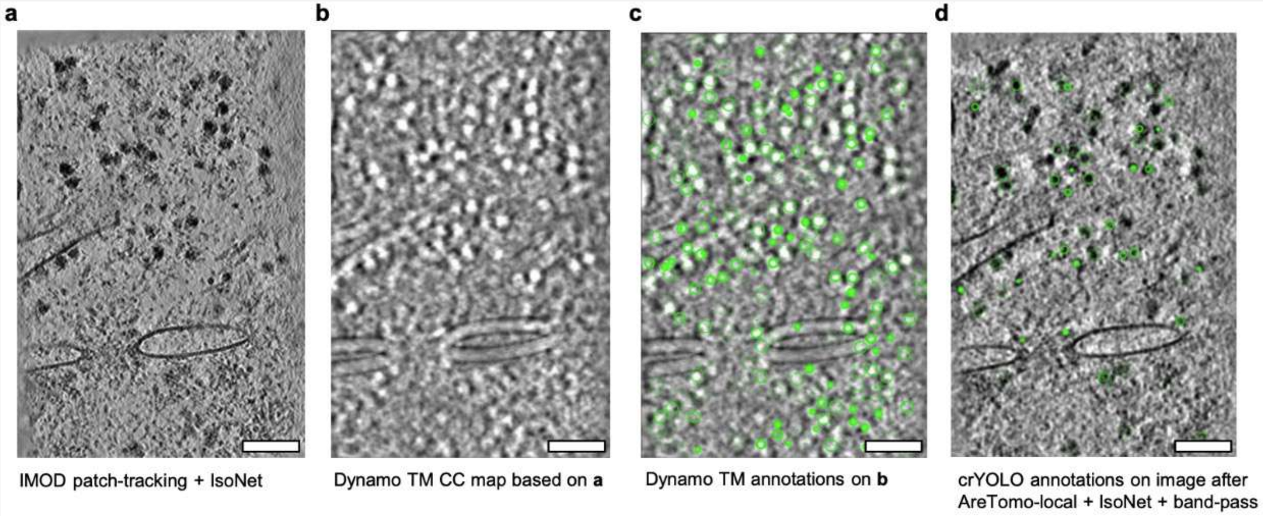
- Are my objects of interest picked?
- How much error is there?
- Reproducibility
- Is there an iterative workflow (e.g. retraining)?

Software for **Particle picking/ segmentation**

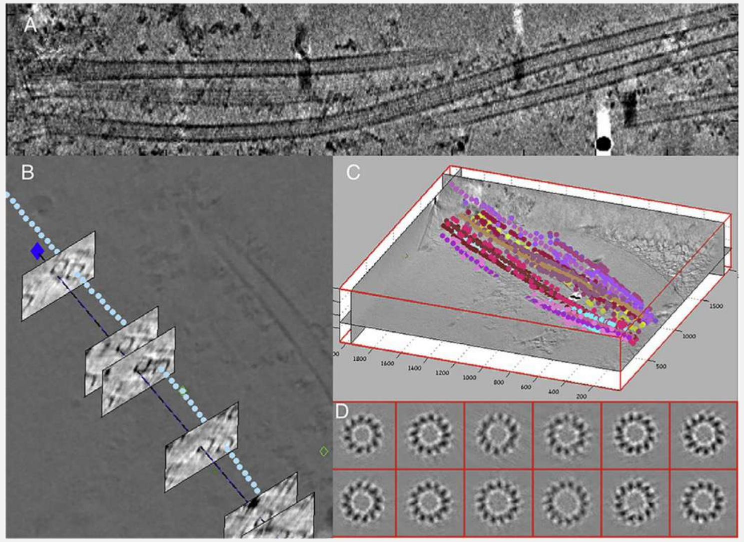
EMAN2



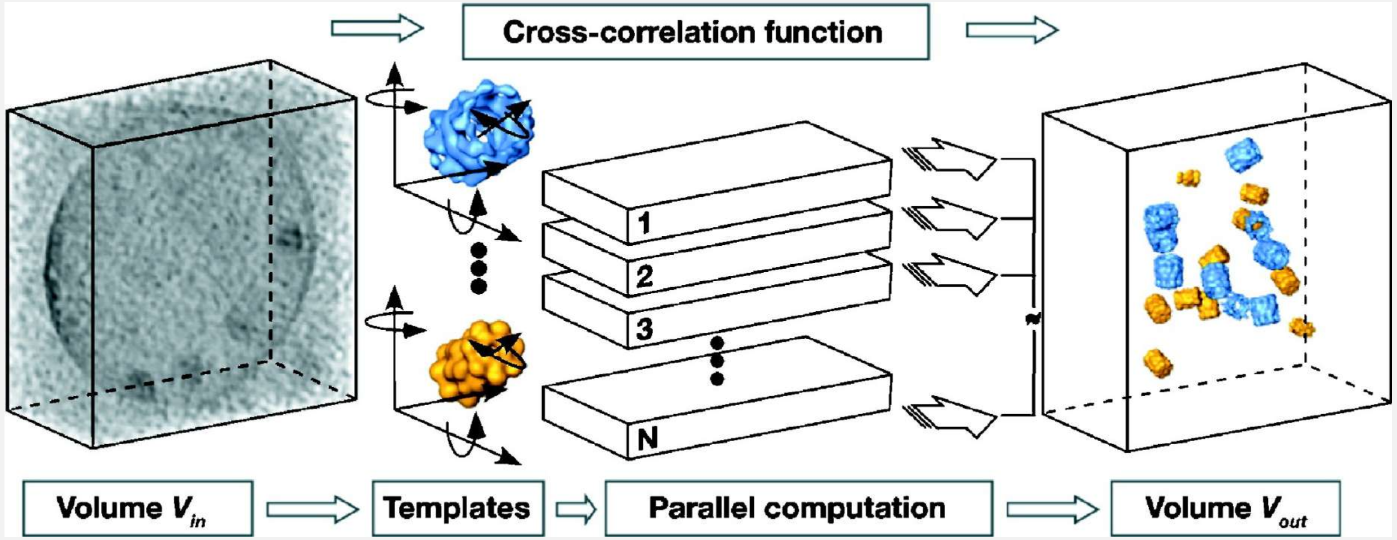
CrYOLO



Dynamo

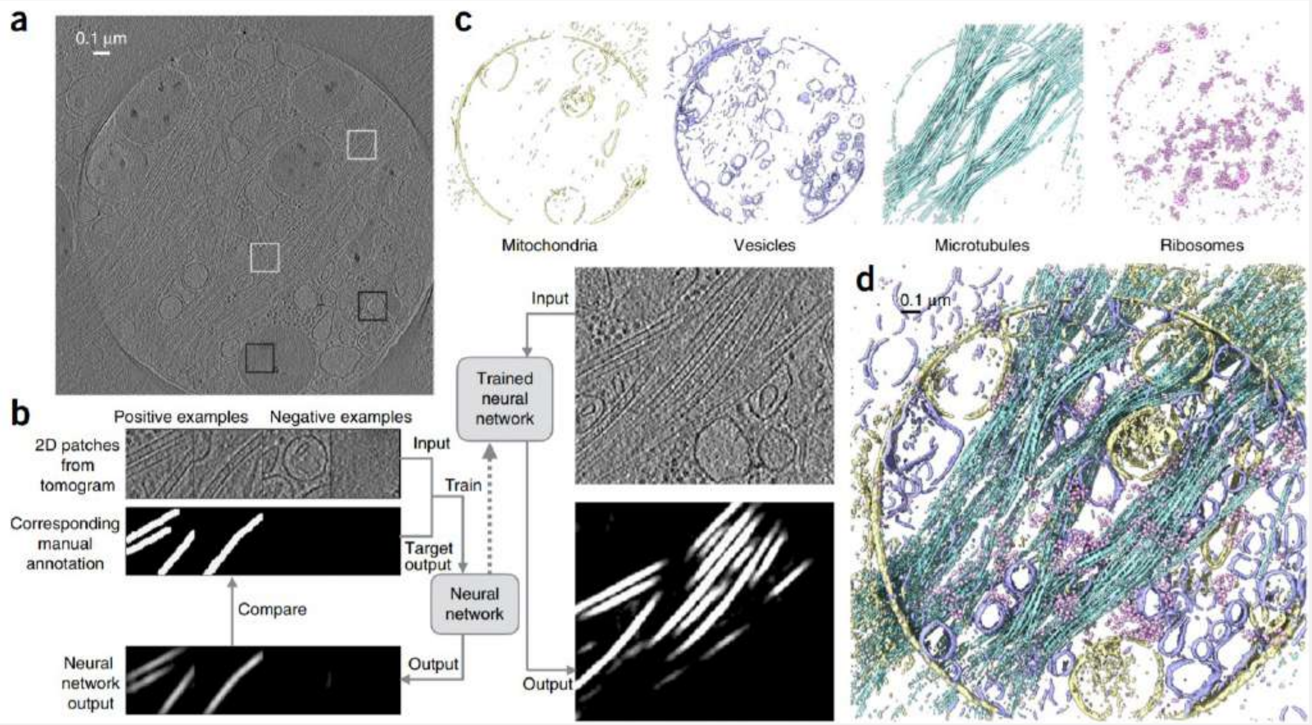


Template matching



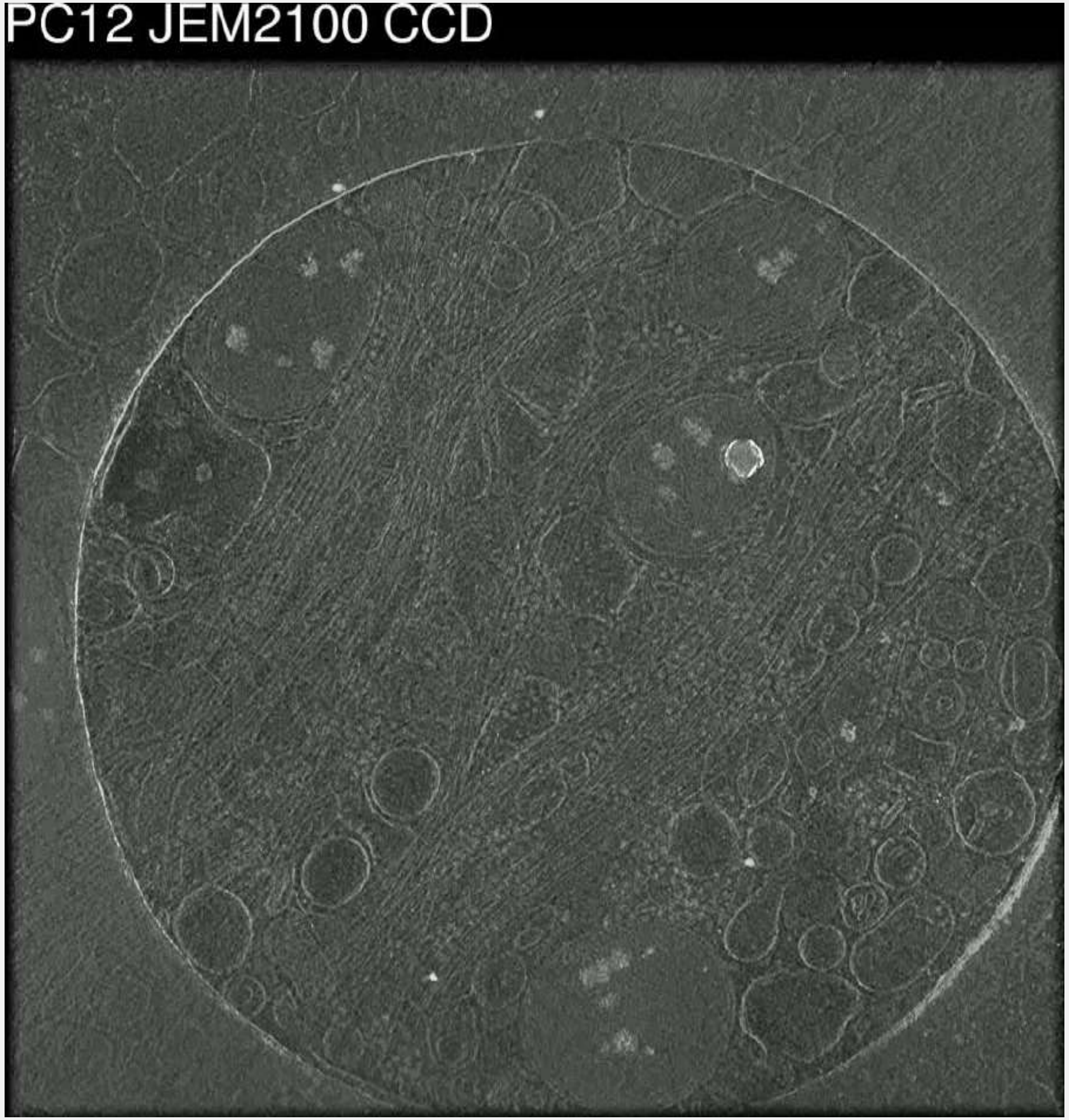
Software for Particle picking/ segmentation

EMAN2



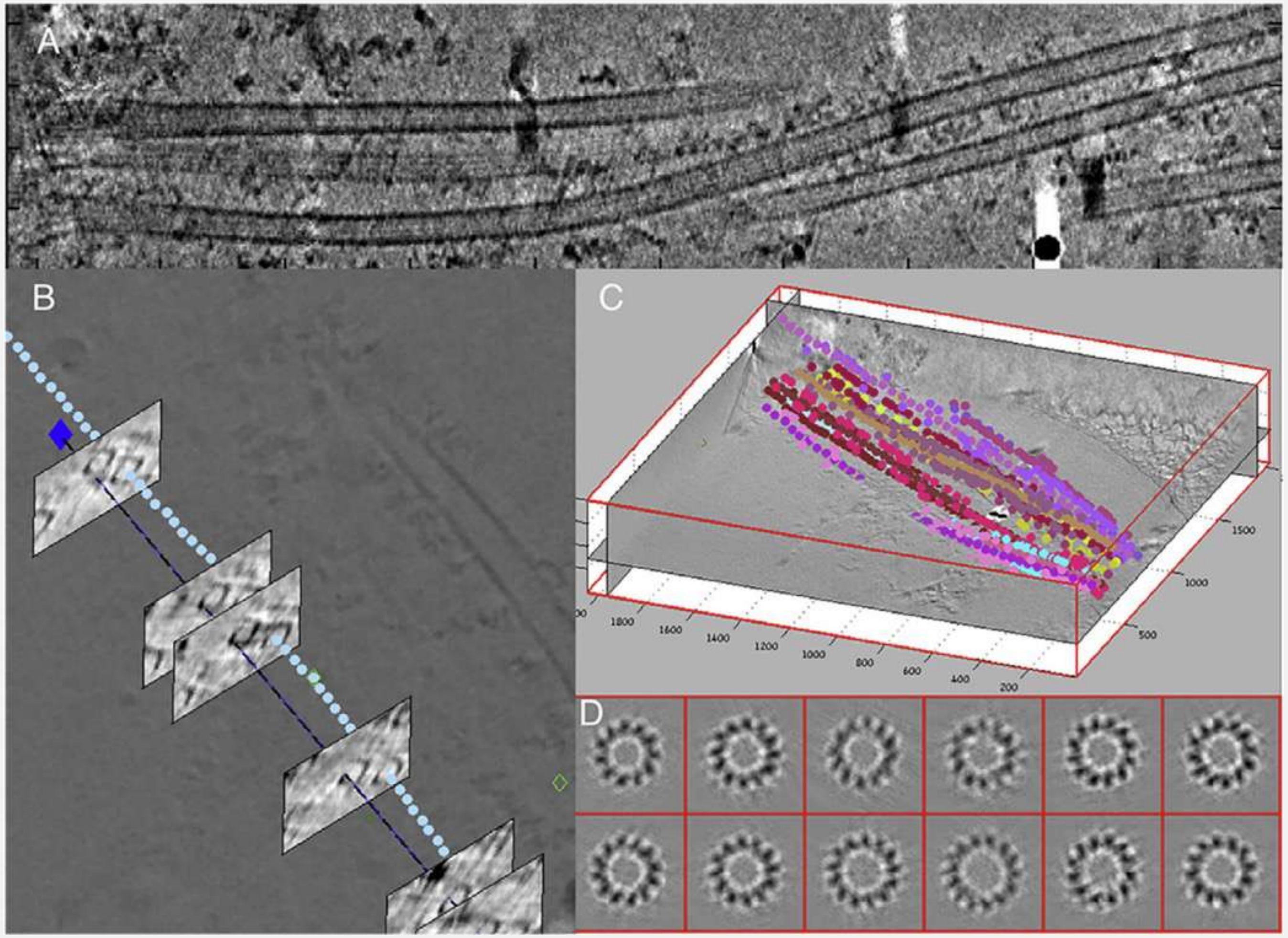
- Machine learning
- Train with positives and negatives

Chen et al., 2017



Software for Particle picking/ segmentation

Dynamo

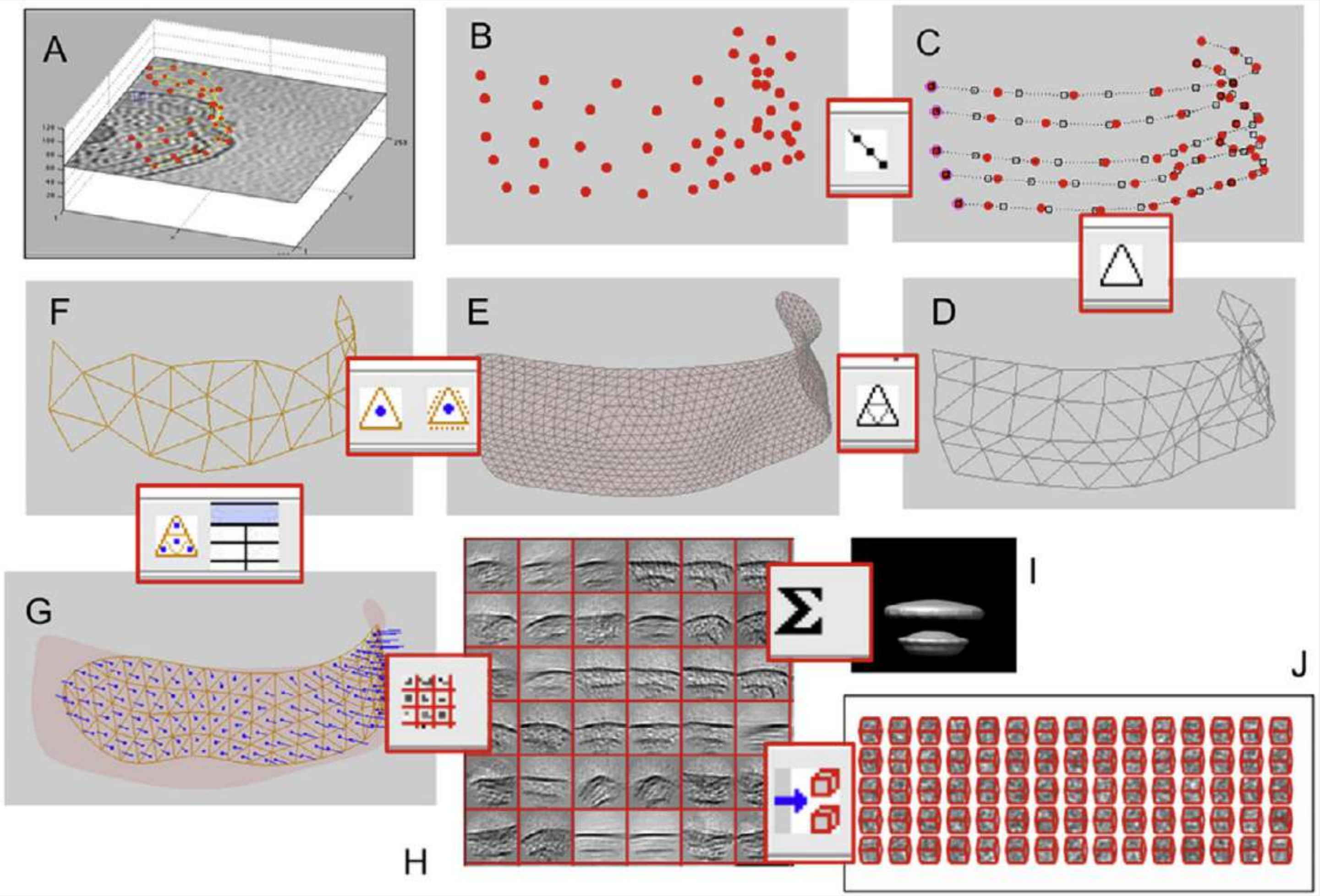


- Semi-automated
- Backbone, helical, and circumferential picking
- Helical symmetry determination

Castaño-Díez et al., 2012, 2017, 1018, 2021

Software for Particle picking/ segmentation

Dynamo

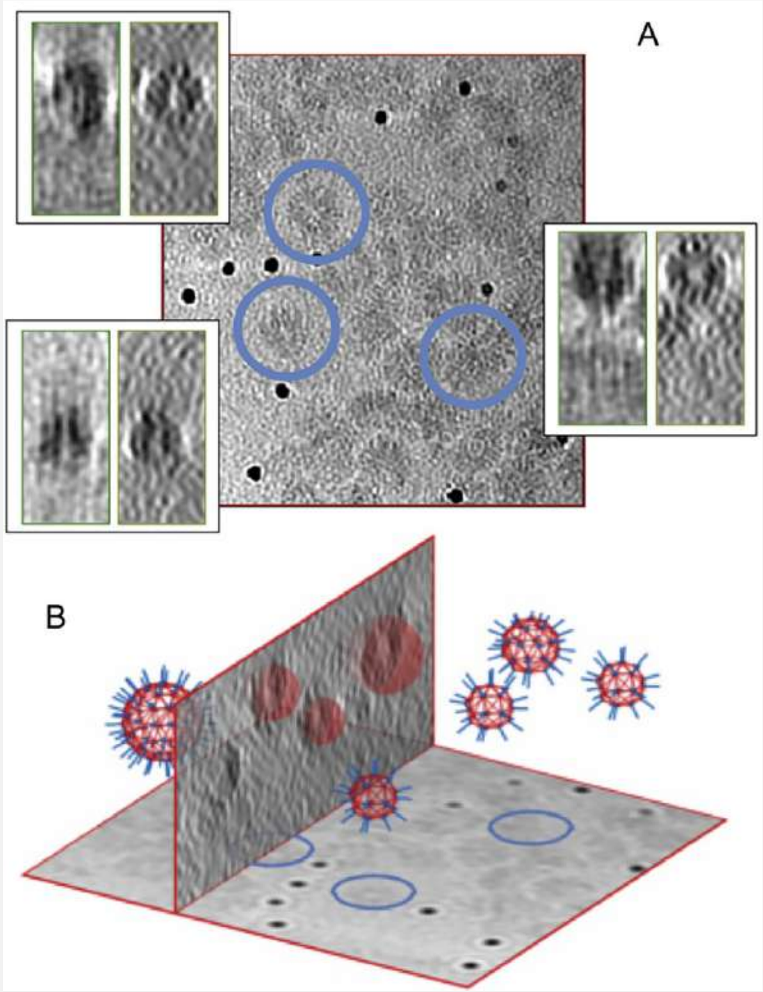


- Create meshes to pick on any shape membrane

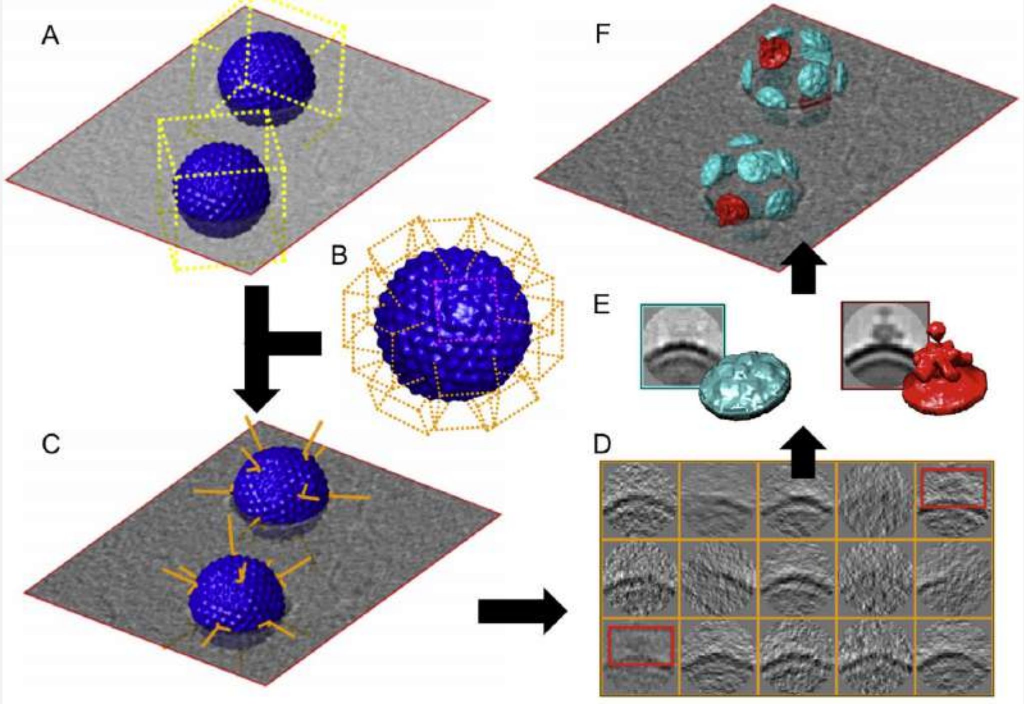
Castaño-Díez et al., 2012, 2017, 1018, 2021

Software for Particle picking/ segmentation

Dynamo



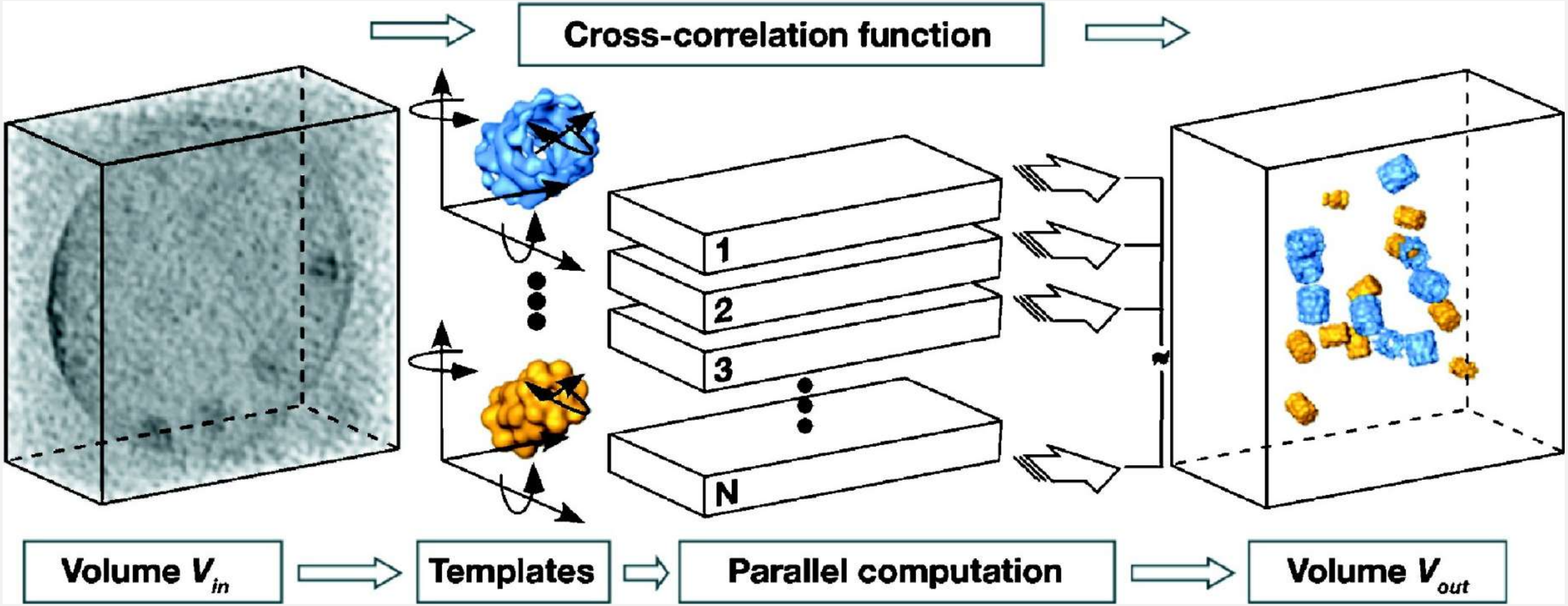
- Pick vesicles and pick around vesicles



Castaño-Díez et al., 2012, 2017, 1018, 2021

Software for Particle picking/ segmentation

Template matching

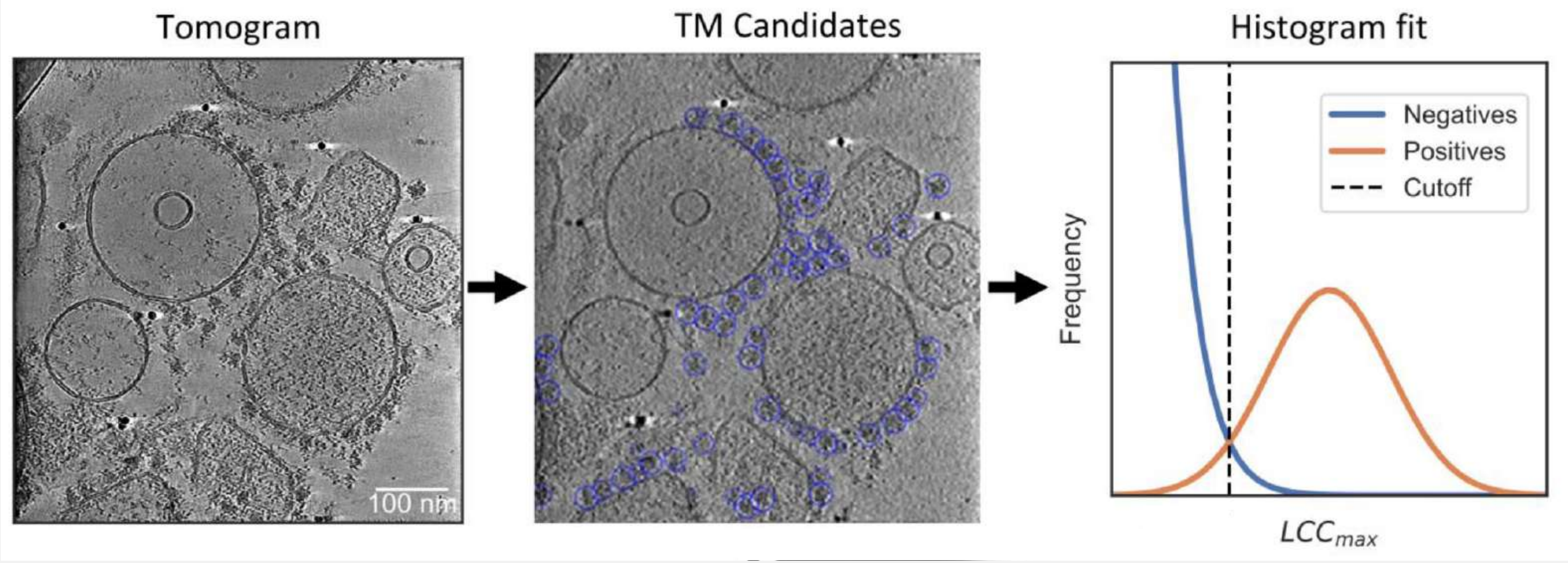


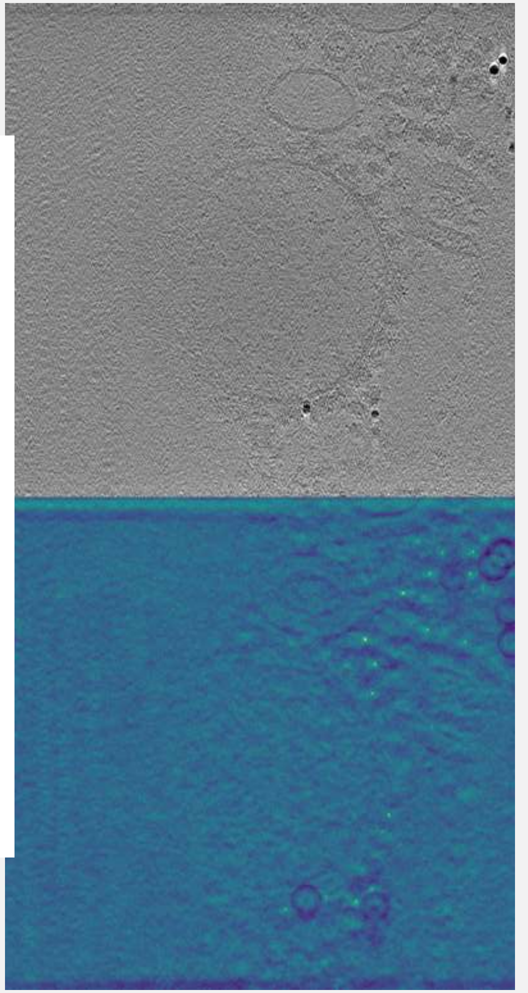
- Pick based on size and shape

Lucic et al., 2005

Software for Particle picking/ segmentation

Template matching in PyTOM



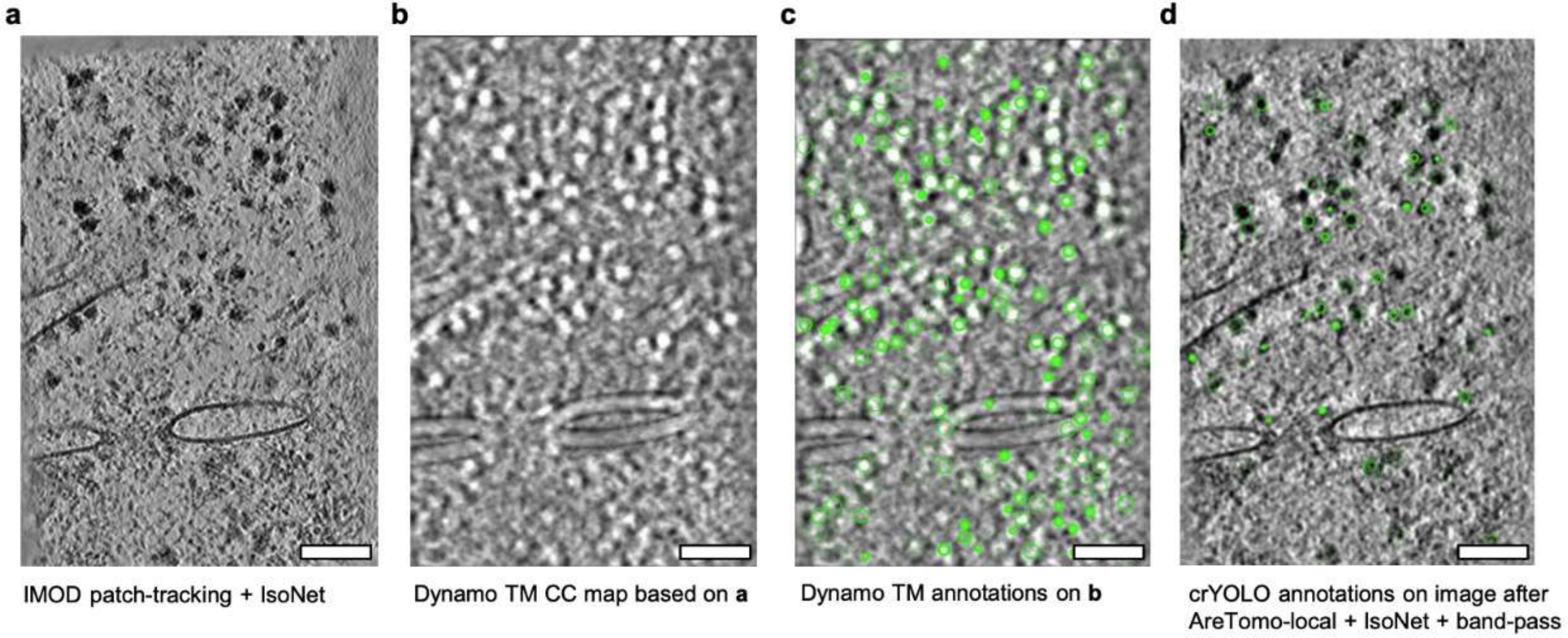


- GPU-accelerated, refines positions and angles

Chaillet et al., 2023

Software for Particle picking/ segmentation

CrYOLO



IMOD patch-tracking + IsoNet

Dynamo TM CC map based on **a**

Dynamo TM annotations on **b**

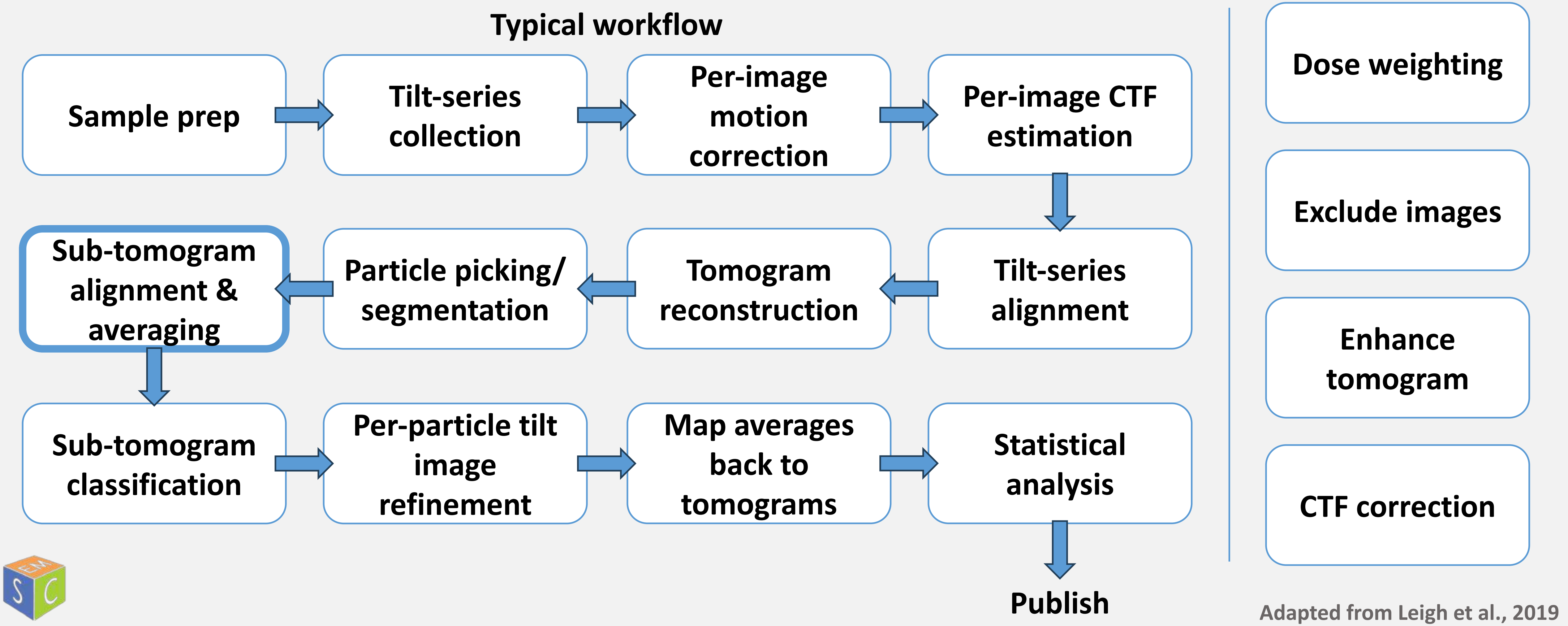
crYOLO annotations on image after AreTomo-local + IsoNet + band-pass

- Machine learning
  - Requires many positive and negative labels

Wagner et al., 2019
Balyschew et al., 2023

Today's plan

- Analyze software in every step of the workflow



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Exclude images

Enhance tomogram

CTF correction

Adapted from Leigh et al., 2019

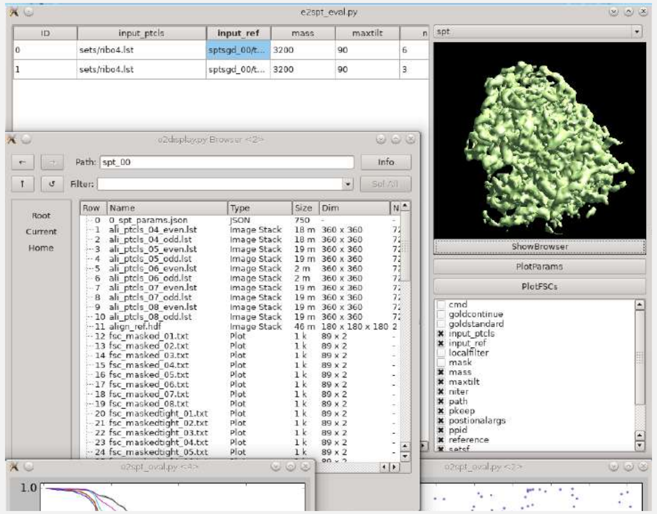
Software for Sub-tomogram alignment & averaging

What to look for:

- Is it aligning in 3D or 2D?
  - If 3D, can it be moved to 2D easily?
- Is the angular search reasonable? (most are not automated like in cryoEM)
- Pay attention to binning – start with very binned data
- Use GPUs if you can

Software for Sub-tomogram alignment & averaging

EMAN2



ID	input_ptcls	input_ref	mass	maxtilt	n
0	sets/ribo4.lst	sptsgd_00/t...	3200	90	6
1	sets/ribo4.lst	sptsgd_00/t...	3200	90	3

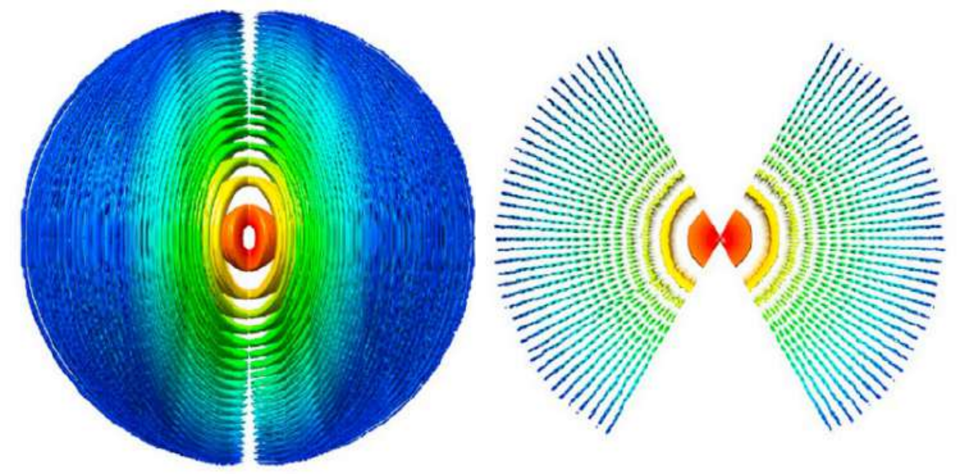
Path: spt_00

Filter:

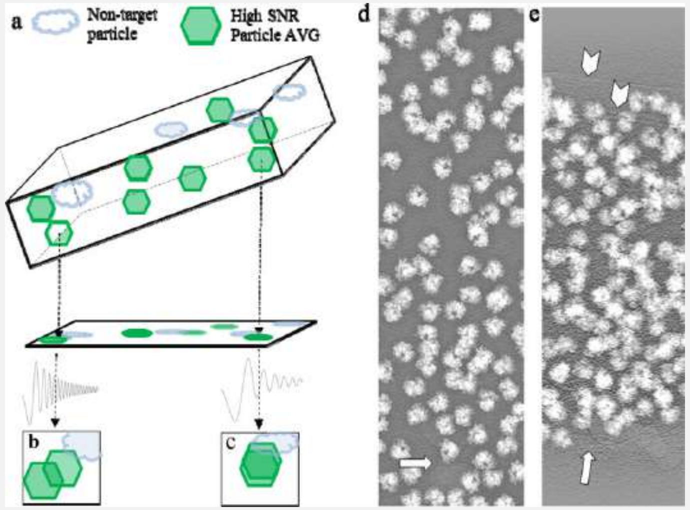
Row	Name	Type	Size	Dim	N
0	0_spt_params.json	JSON	750	-	-
1	ali_ptcls_04_even.lst	Image Stack	18 m	360 x 360	72
2	ali_ptcls_04_odd.lst	Image Stack	18 m	360 x 360	72
3	ali_ptcls_05_even.lst	Image Stack	19 m	360 x 360	72
4	ali_ptcls_05_odd.lst	Image Stack	19 m	360 x 360	72
5	ali_ptcls_06_even.lst	Image Stack	2 m	360 x 360	72
6	ali_ptcls_06_odd.lst	Image Stack	2 m	360 x 360	72
7	ali_ptcls_07_even.lst	Image Stack	19 m	360 x 360	72
8	ali_ptcls_07_odd.lst	Image Stack	19 m	360 x 360	72
9	ali_ptcls_08_even.lst	Image Stack	19 m	360 x 360	72
10	ali_ptcls_08_odd.lst	Image Stack	19 m	360 x 360	72
11	align_ref.hdf	Image Stack	46 m	180 x 180 x 180	2
12	fsc_masked_01.txt	Plot	1 k	89 x 2	-
13	fsc_masked_02.txt	Plot	1 k	89 x 2	-
14	fsc_masked_03.txt	Plot	1 k	89 x 2	-
15	fsc_masked_04.txt	Plot	1 k	89 x 2	-
16	fsc_masked_05.txt	Plot	1 k	89 x 2	-
17	fsc_masked_06.txt	Plot	1 k	89 x 2	-
18	fsc_masked_07.txt	Plot	1 k	89 x 2	-
19	fsc_masked_08.txt	Plot	1 k	89 x 2	-
20	fsc_maskedtight_01.txt	Plot	1 k	89 x 2	-
21	fsc_maskedtight_02.txt	Plot	1 k	89 x 2	-
22	fsc_maskedtight_03.txt	Plot	1 k	89 x 2	-
23	fsc_maskedtight_04.txt	Plot	1 k	89 x 2	-
24	fsc_maskedtight_05.txt	Plot	1 k	89 x 2	-

- ☐ cmd
- ☐ goldcontinue
- ☐ goldstandard
- ☒ input_ptcls
- ☒ input_ref
- ☐ localfilter
- ☐ mask
- ☒ mass
- ☒ maxtilt
- ☒ niter
- ☒ path
- ☒ pkeep
- ☒ postionalargs
- ☒ ppid
- ☒ reference
- ☒ setsf

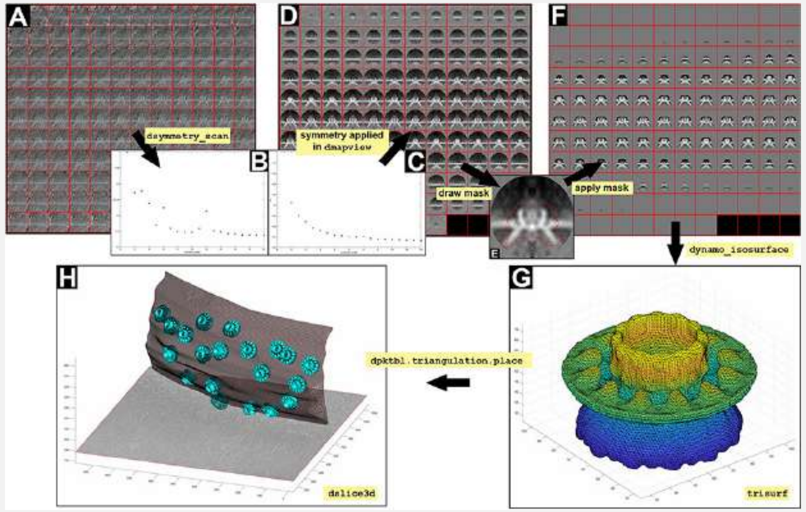
Relion



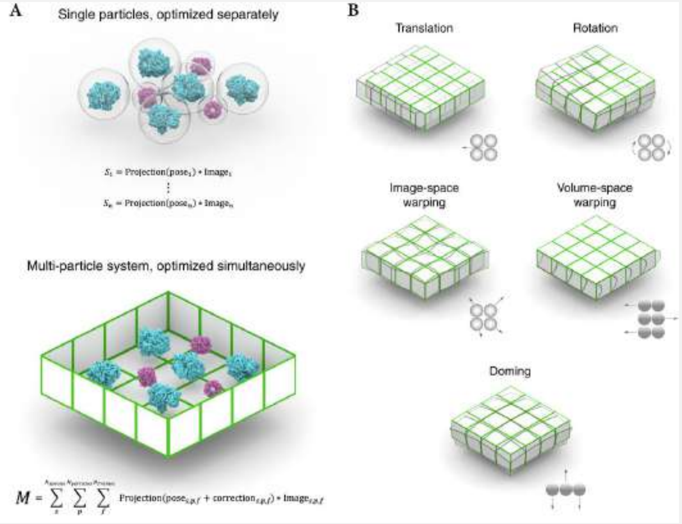
EMClarity



Dynamo



Warp



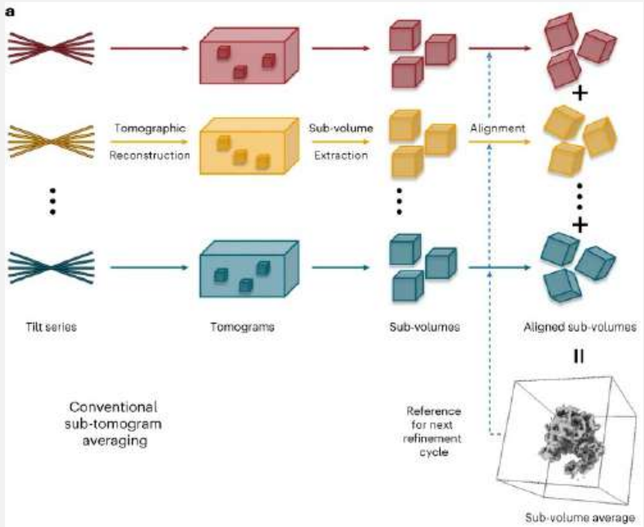
$$S_1 = \text{Projection}(\text{pose}_1) * \text{Image}_1$$

$$\vdots$$

$$S_n = \text{Projection}(\text{pose}_n) * \text{Image}_n$$

$$M = \sum_{s}^{N_{species}} \sum_{p}^{N_{particles}} \sum_{f}^{N_{frames}} \text{Projection}(\text{pose}_{s,p,f} + \text{correction}_{s,p,f}) * \text{Image}_{s,p,f}$$

NextPYP



Software for Sub-tomogram alignment & averaging

- 3D alignment: Can’t refine image angles
- 2D alignment: Can refine image angles – higher resolution
- Common workflow: Start with 3D alignment and high binning (account for missing wedges properly), then go to 2D refinement

Today's plan

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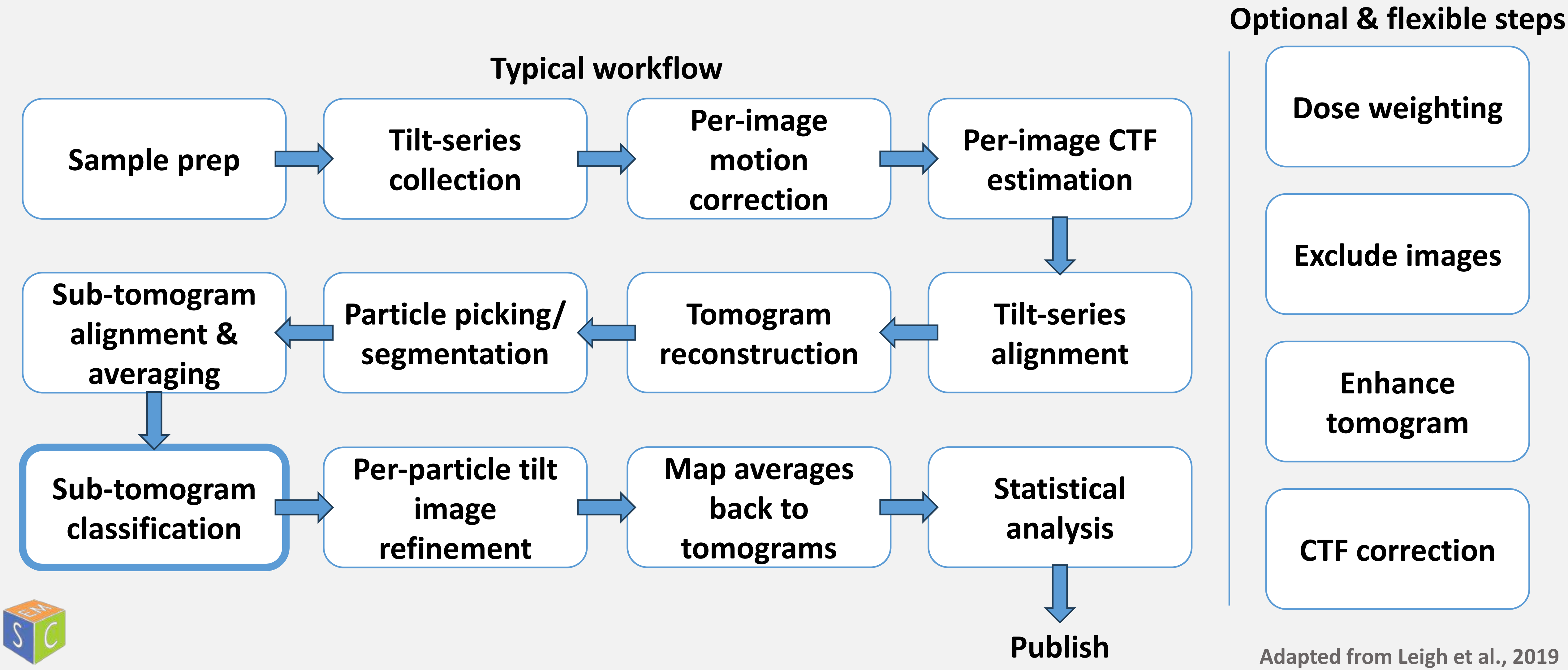

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CTF correction

Adapted from Leigh et al., 2019

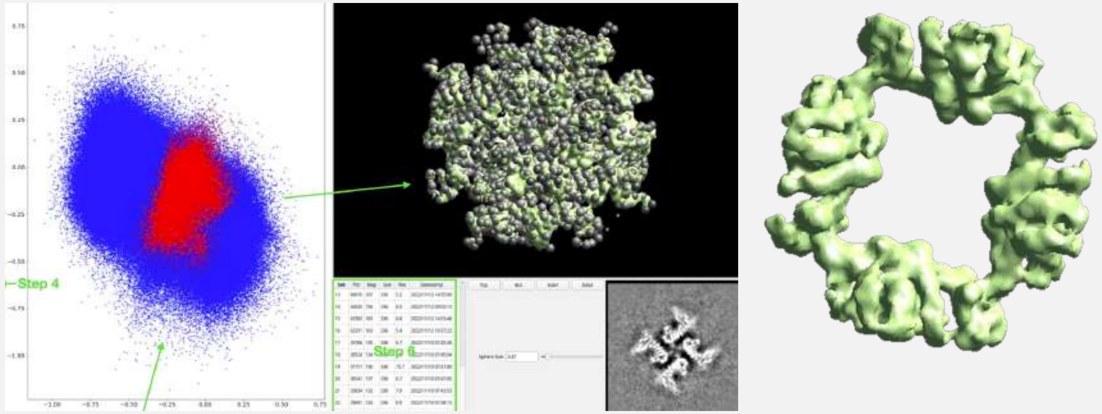
Software for Sub-tomogram classification

What to look for:

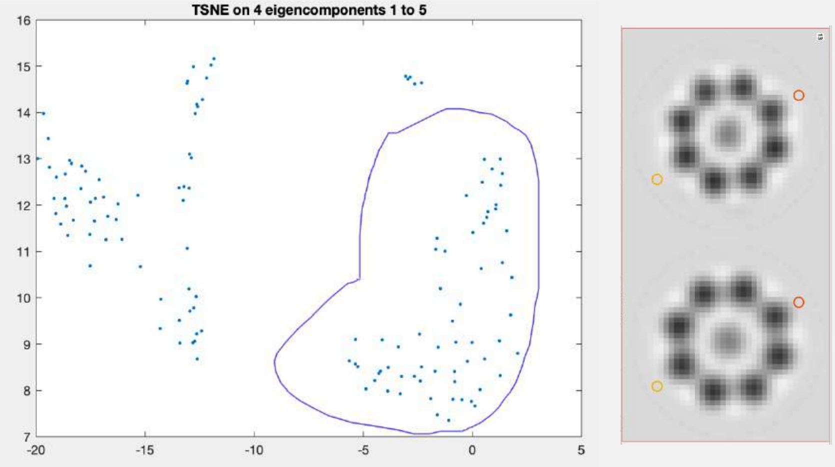
- Can the software separate real features?
- Watch out not to classify by missing wedge or defocus or ice thickness
- Speed is often an issue – 3D data takes long to process

Software for Sub-tomogram classification

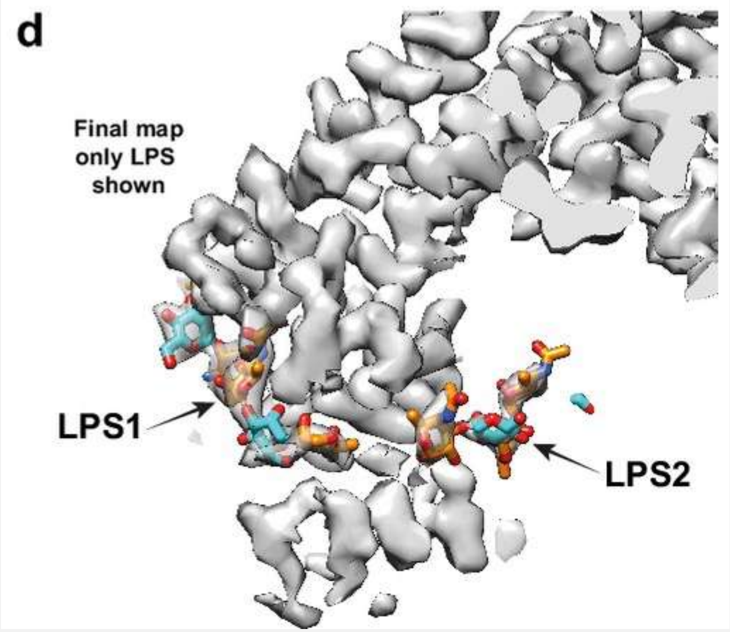
EMAN2



Dynamo



Relion



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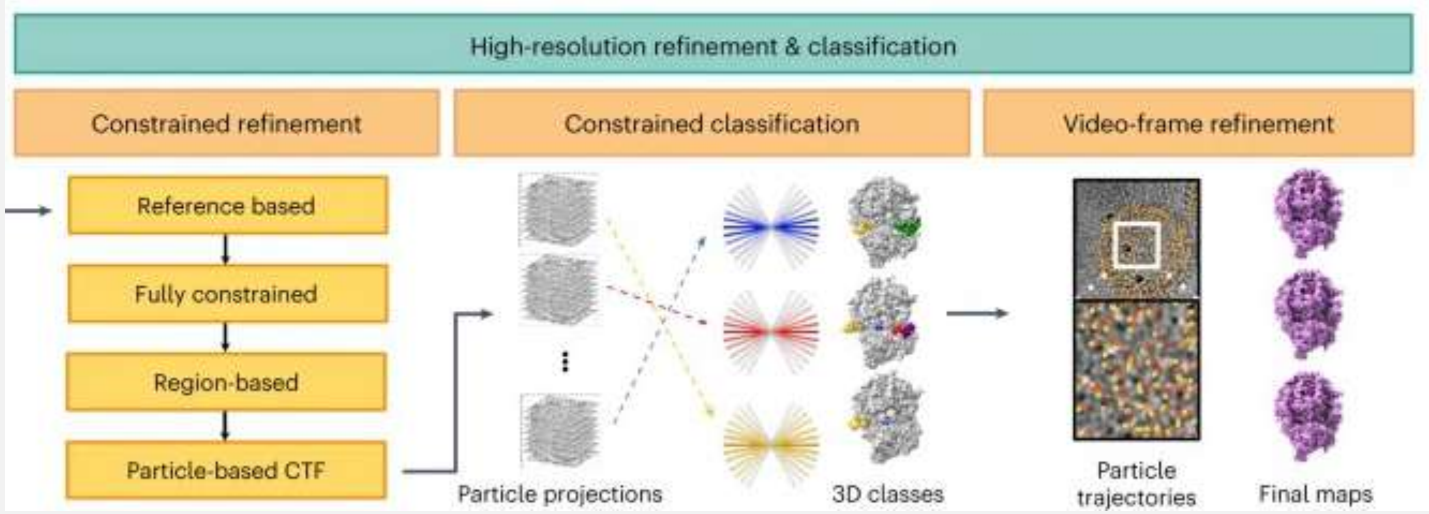


High-resolution refinement & classification

Constrained refinement

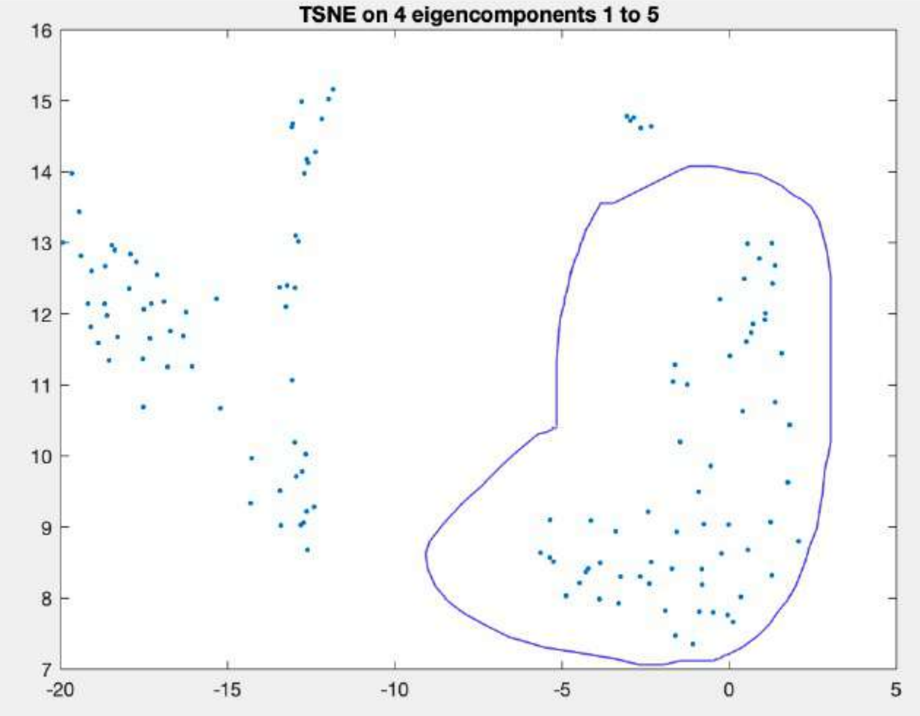
- Reference based
- Fully constrained
- Region-based
- Particle-based CTF

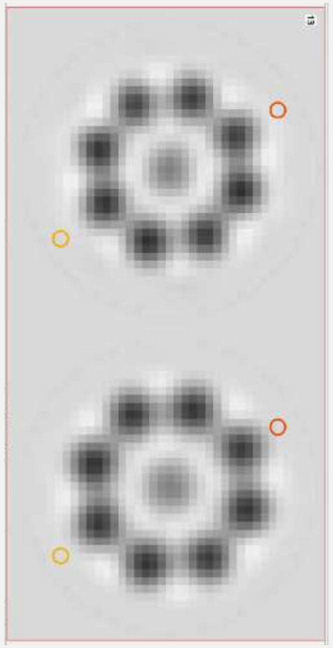
Constrained classification

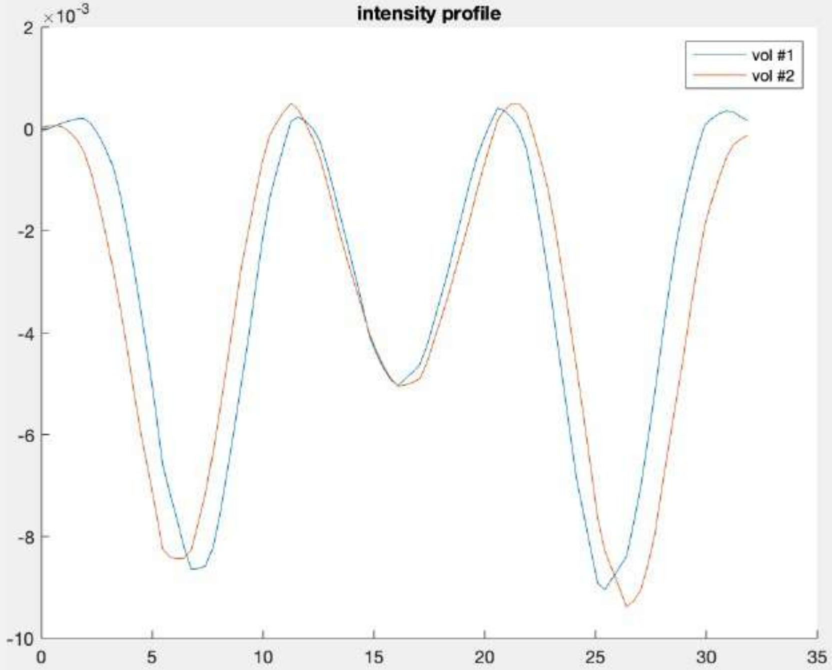
Video-frame refinement

Software for Sub-tomogram classification

Dynamo



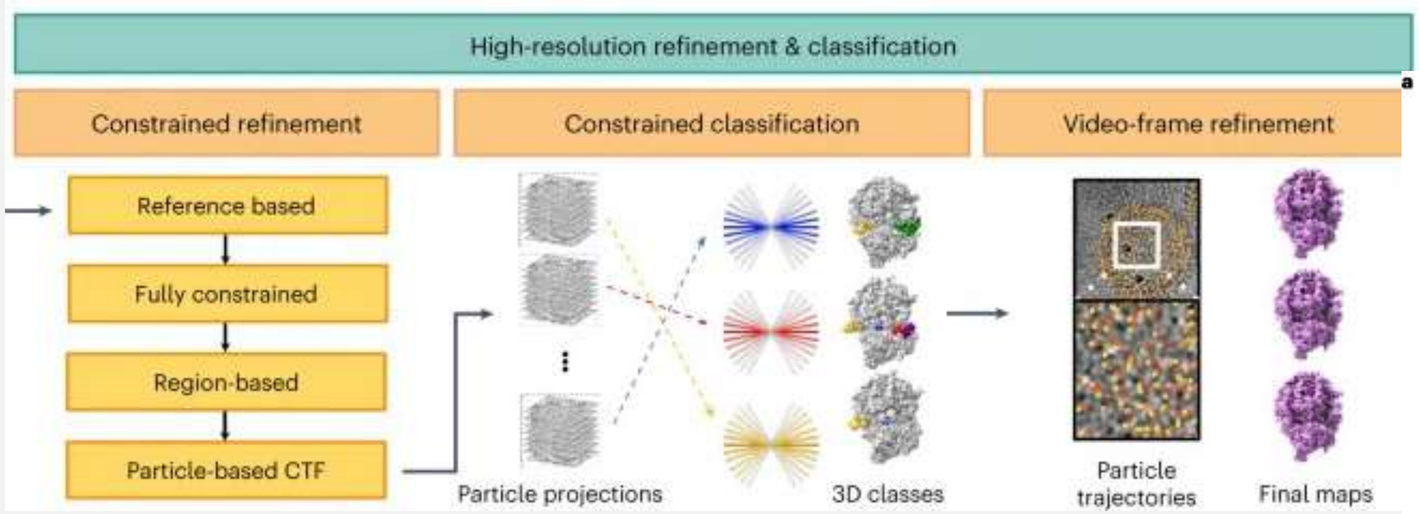


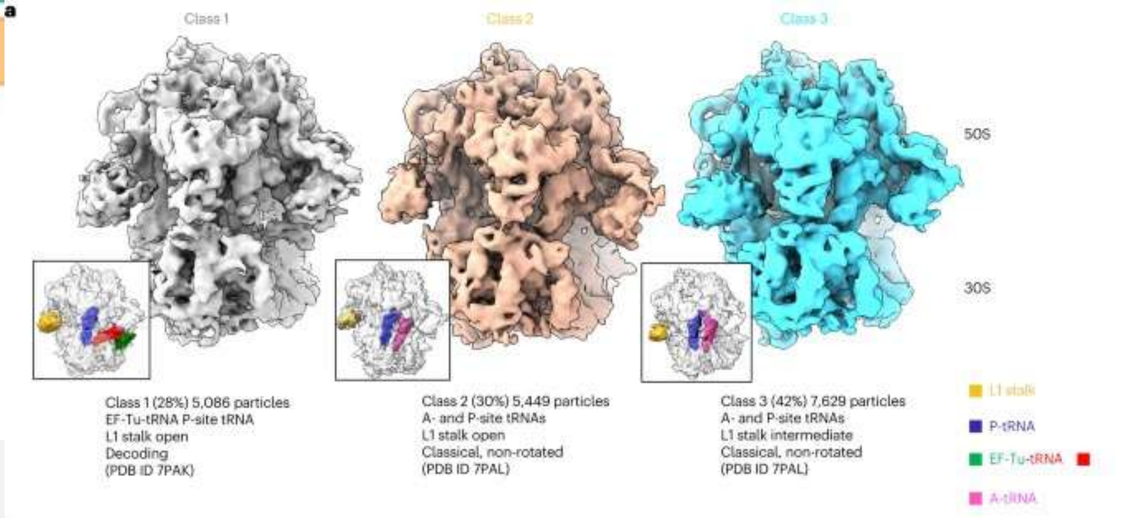


- 3D classification using PCA and k-means clustering
- Easy to ignore missing wedge classification

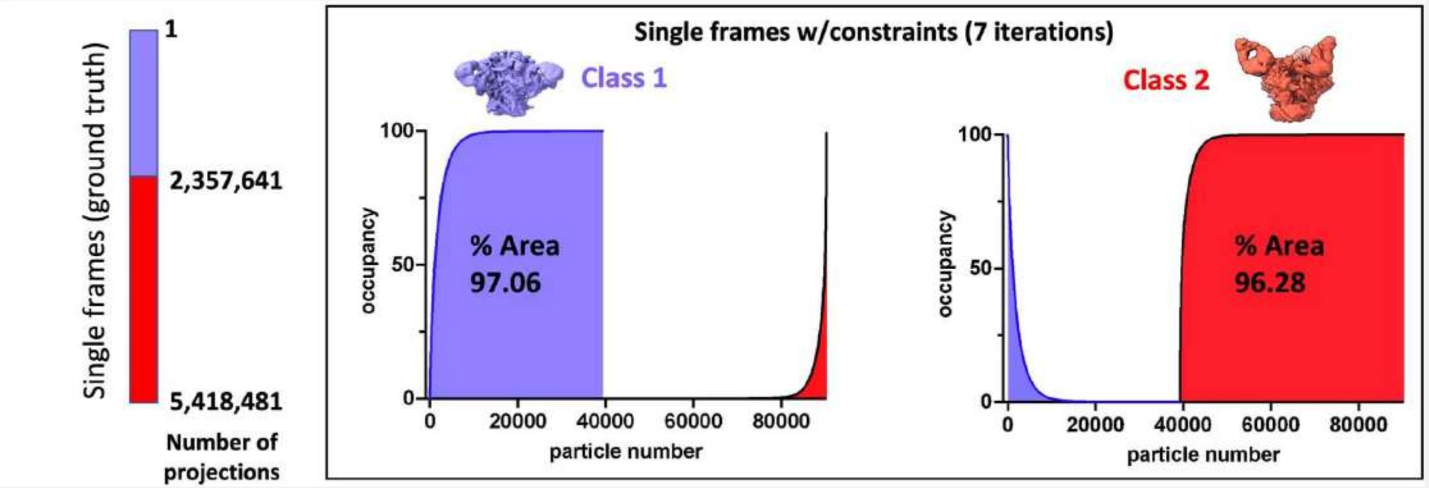
Software for Sub-tomogram classification

NextPYP





- Classification based on 2D particle images and frames



Liu et al., 2023

Today's plan

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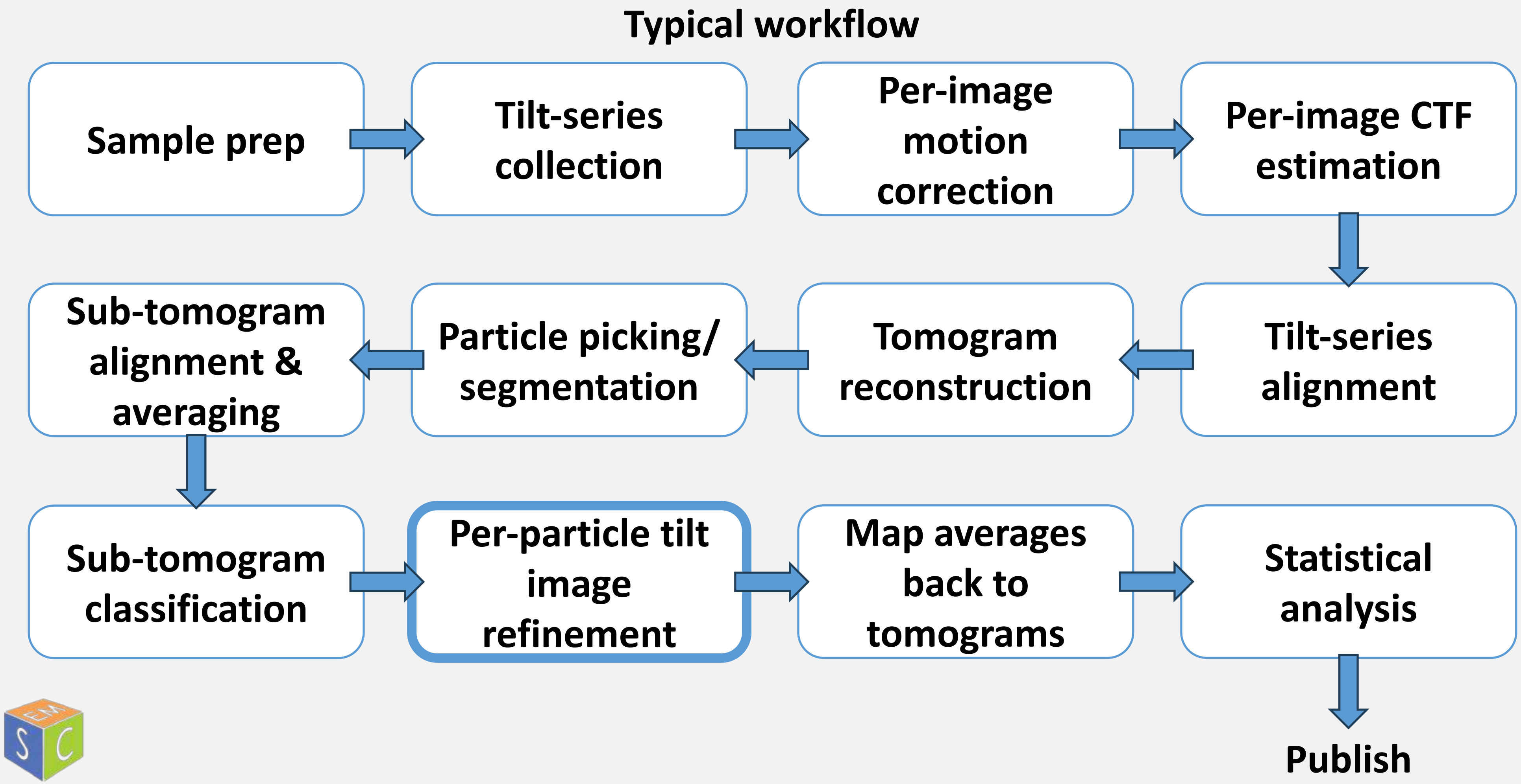


Typical workflow

Sample prep → Tilt-series collection → Per-image motion correction → Per-image CTF estimation → Tilt-series alignment → Tomogram reconstruction → Particle picking/ segmentation → Sub-tomogram alignment & averaging → Sub-tomogram classification → Per-particle tilt image refinement → Map averages back to tomograms → Statistical analysis → Publish

Optional & flexible steps

- Dose weighting
- Exclude images
- Enhance tomogram
- CTF correction

Adapted from Leigh et al., 2019

Software for Per-particle tilt image refinement

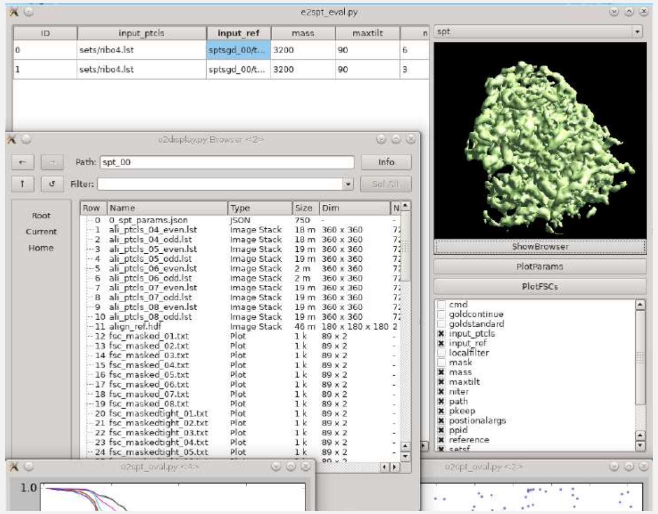
What to look for:

- Can you remove high tilts?
- Does the software feed back into the rest of your workflow well?
- Can you refine frames and other high-resolution corrections if needed?

Software for

Per-particle tilt image refinement

EMAN2



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e2display.py Browser <2>

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4	ali_ptcls_05_odd.lst	Image Stack	19 m	360 x 360	7
5	ali_ptcls_06_even.lst	Image Stack	2 m	360 x 360	7
6	ali_ptcls_06_odd.lst	Image Stack	2 m	360 x 360	7
7	ali_ptcls_07_even.lst	Image Stack	19 m	360 x 360	7
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9	ali_ptcls_08_even.lst	Image Stack	19 m	360 x 360	7
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12	fsc_masked_01.txt	Plot	1 k	89 x 2	-
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14	fsc_masked_03.txt	Plot	1 k	89 x 2	-
15	fsc_masked_04.txt	Plot	1 k	89 x 2	-
16	fsc_masked_05.txt	Plot	1 k	89 x 2	-
17	fsc_masked_06.txt	Plot	1 k	89 x 2	-
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21	fsc_maskedtight_02.txt	Plot	1 k	89 x 2	-
22	fsc_maskedtight_03.txt	Plot	1 k	89 x 2	-
23	fsc_maskedtight_04.txt	Plot	1 k	89 x 2	-
24	fsc_maskedtight_05.txt	Plot	1 k	89 x 2	-

☐ cmd
☐ goldcontinue
☐ goldstandard
☒ input_ptcls
☒ input_ref
☐ localfilter
☐ mask
☒ mass
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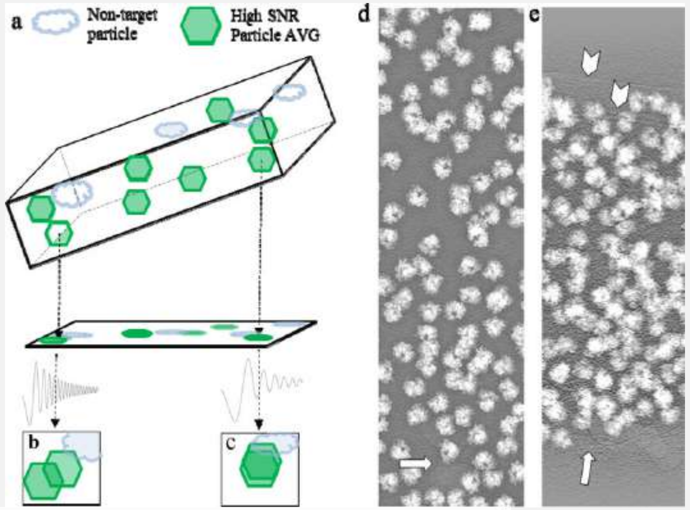
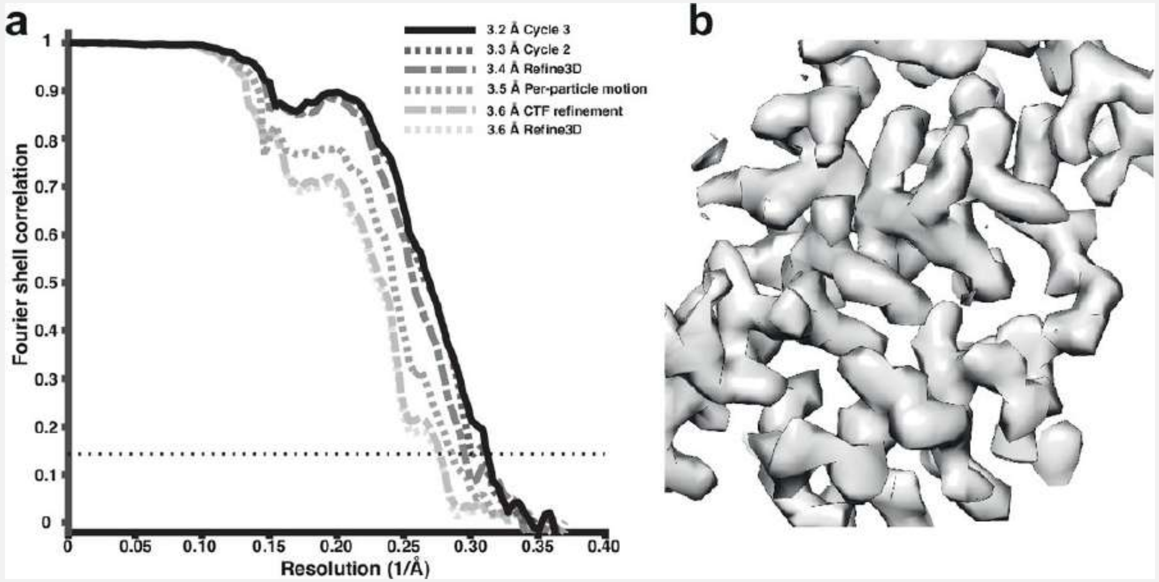
☒ postionalargs
☒ ppid
☒ reference
☒ setsf

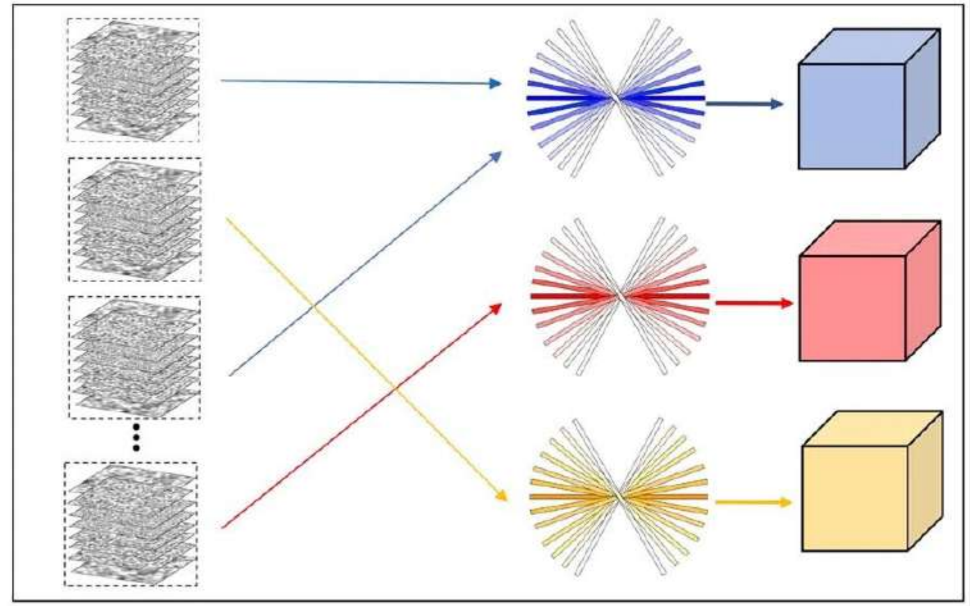
EMClarity



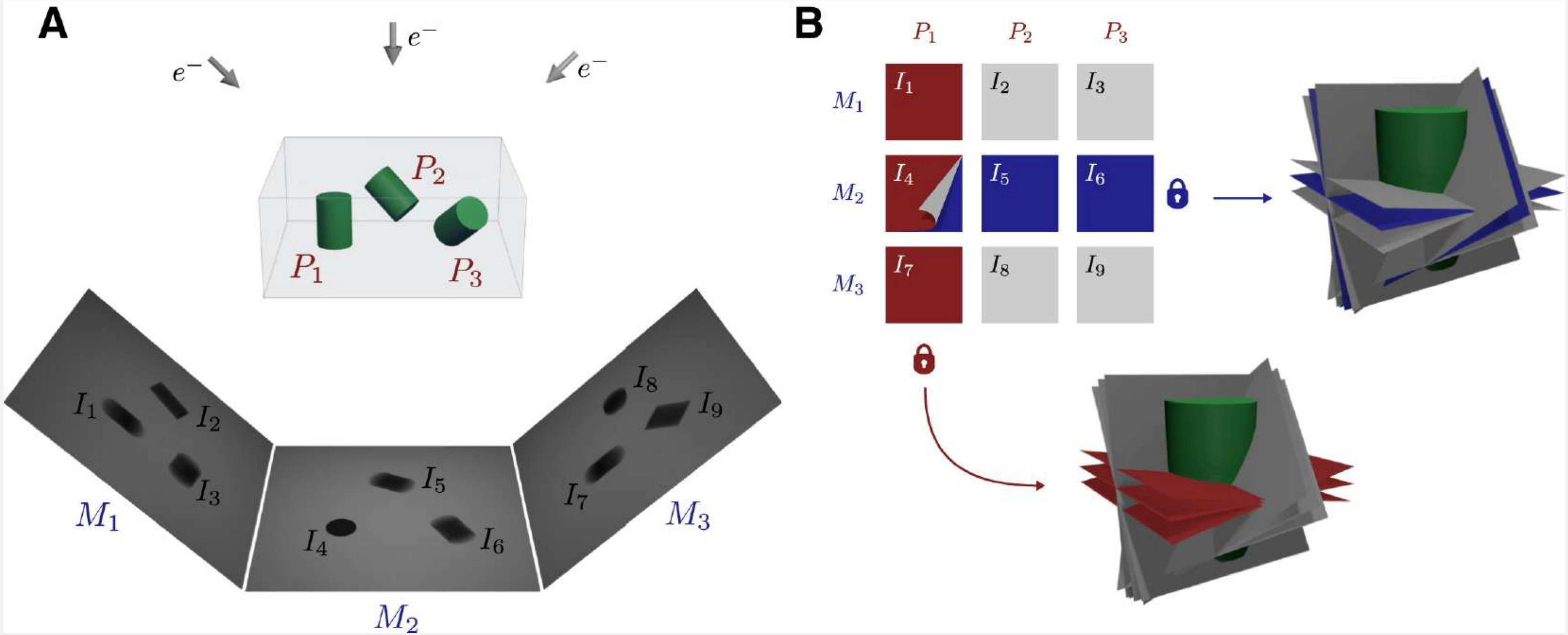
Relion



NextPYP



Software for Per-particle tilt image refinement



All work on a basic principle:

- CryoET is really cryoEM with **many slices per particle**

Bartesaghi et al., 2012

Today’s plan

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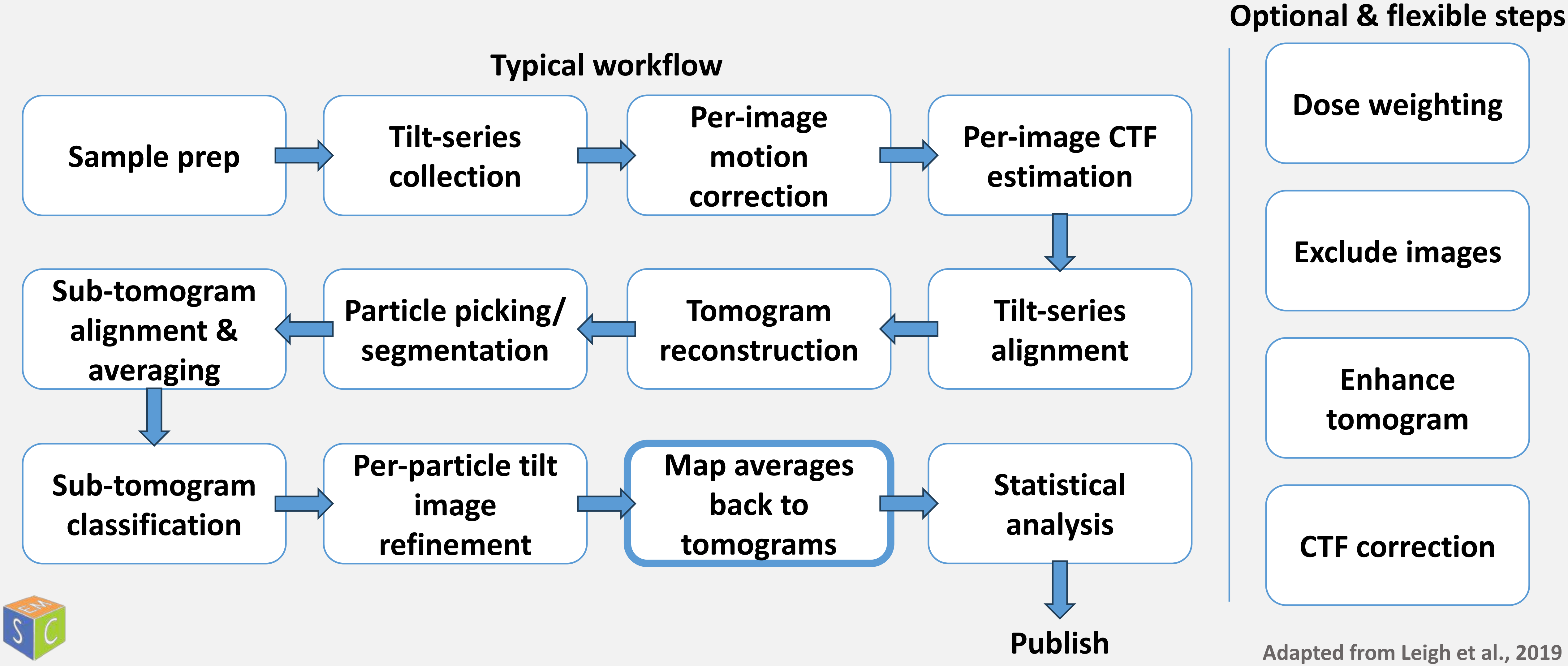

Typical workflow

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Optional & flexible steps

Dose weighting

Exclude images

Enhance tomogram

CTF correction

Adapted from Leigh et al., 2019

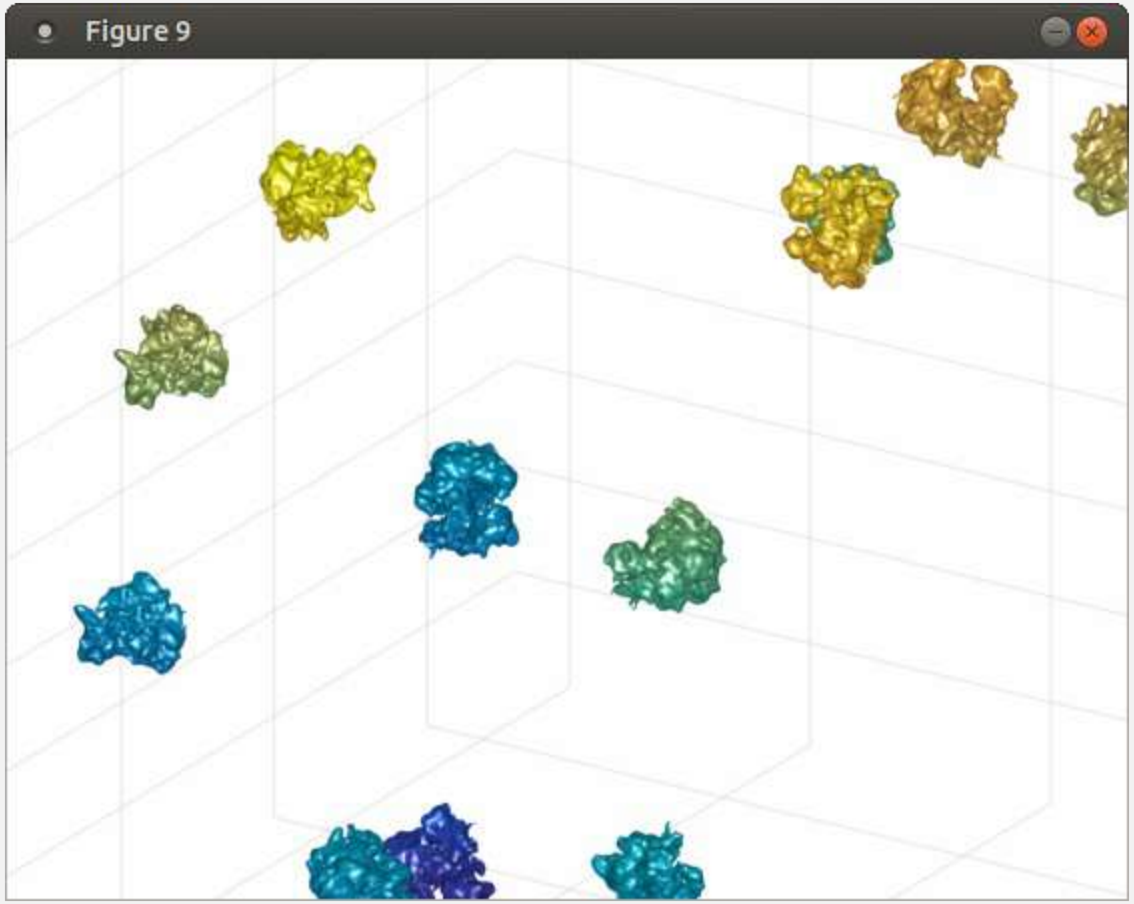
Software for Map averages back to tomograms

What to look for:

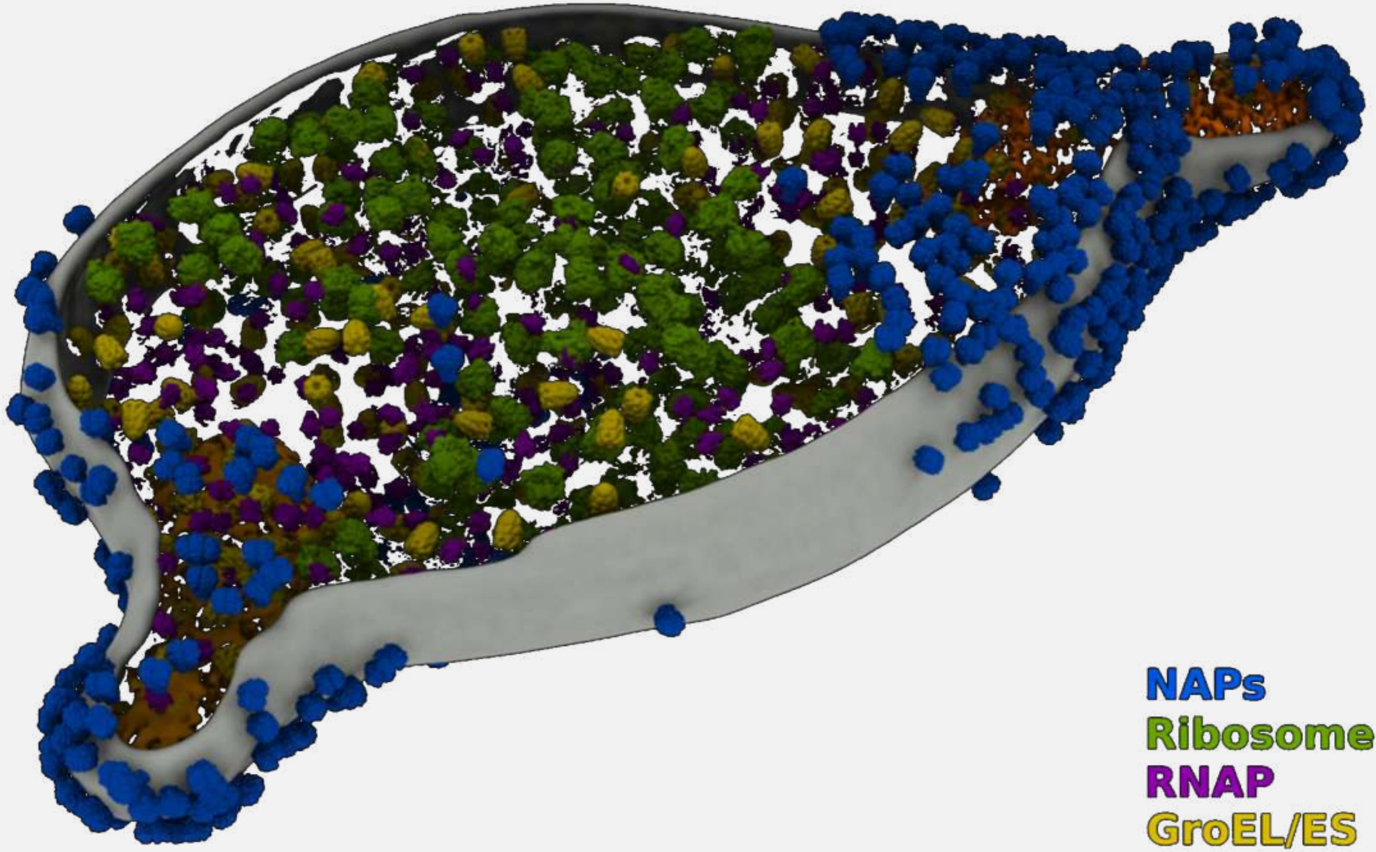
- Does it place it into an existing scene or make a new tomogram?
- Is it overlayed on the original tomogram?
- Can you map your classes back easily?
- This is for visual analysis, so it should be user-friendly

Software for Map averages back to tomograms

Dynamo



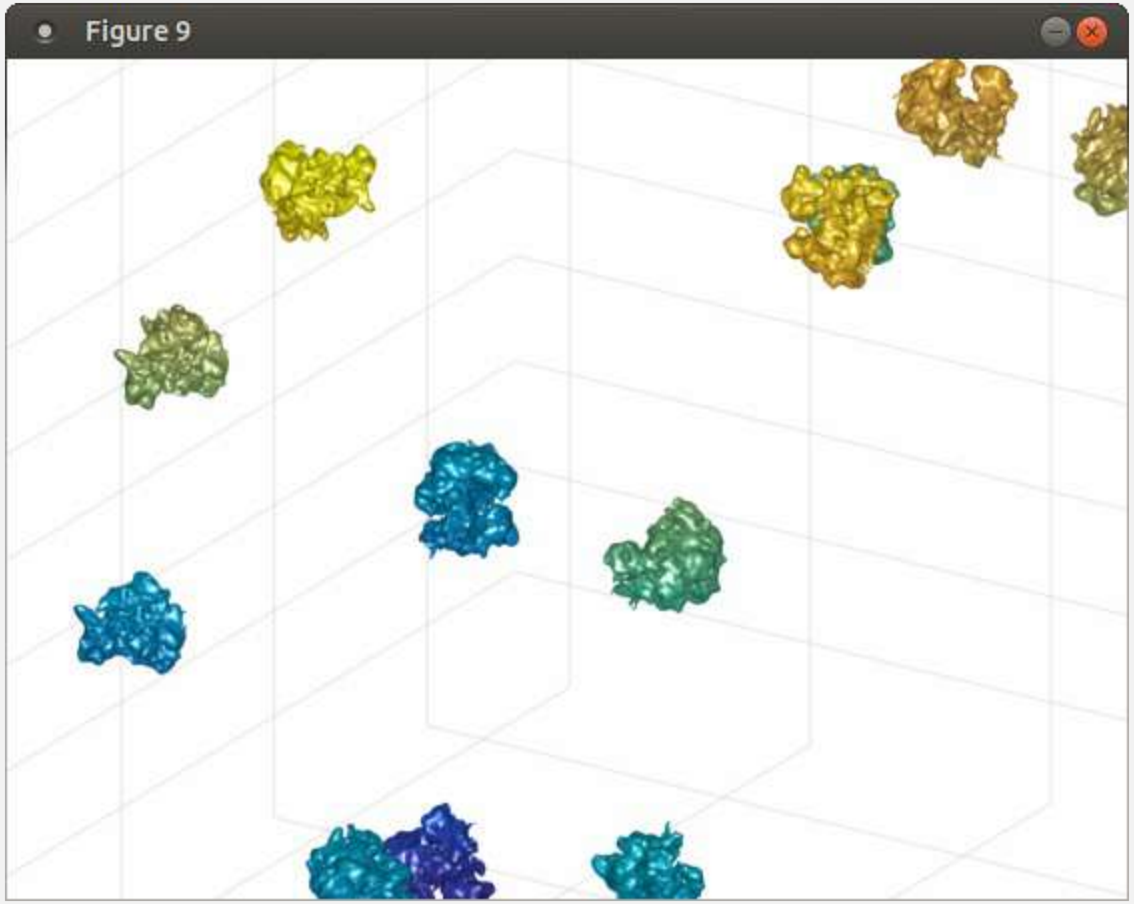
ArtiaX



Software for

Map averages back to tomograms

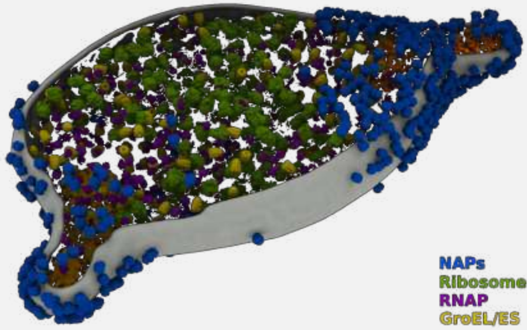
Dynamo



dynamo-em.org

- Takes a Dynamo .tbl alignment file with classes and re-builds a fake tomogram with only those objects
- Can open the fake tomogram in normal viewing software (Dynamo, 3dmod, ChimeraX, EMAN2)
- Scriptable in Matlab

Software for Map averages back to tomograms



ArtiaX

- Takes alignment/ segmentation files from various software, imports the averages in a point cloud in ChimeraX
- Only viewable in ChimeraX
- Can overlay with the tomogram
- Can manipulate objects in real-time and in VR
- Scriptable in ChimeraX

Ermel et al., 2022

Today's plan

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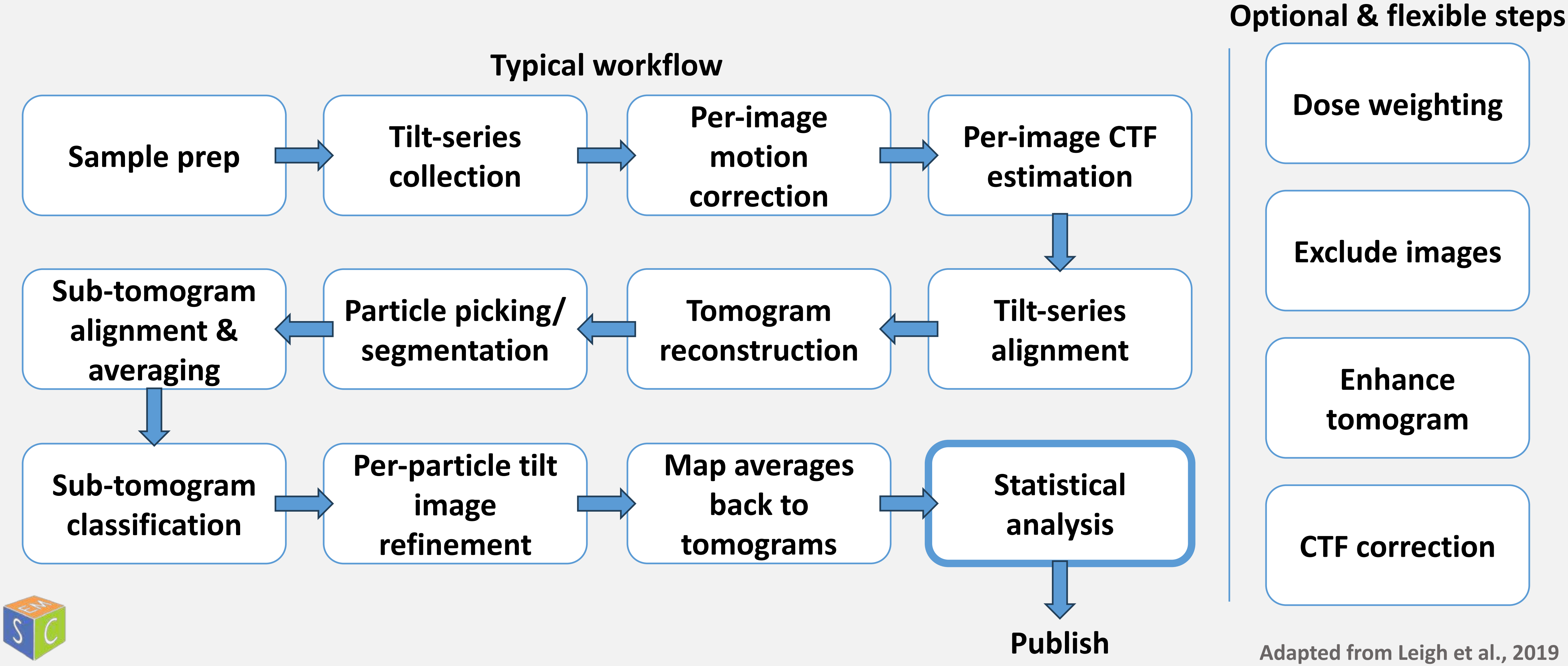

Typical workflow

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Optional & flexible steps

- Dose weighting
- Exclude images
- Enhance tomogram
- CTF correction

Adapted from Leigh et al., 2019

Software for Statistical analysis

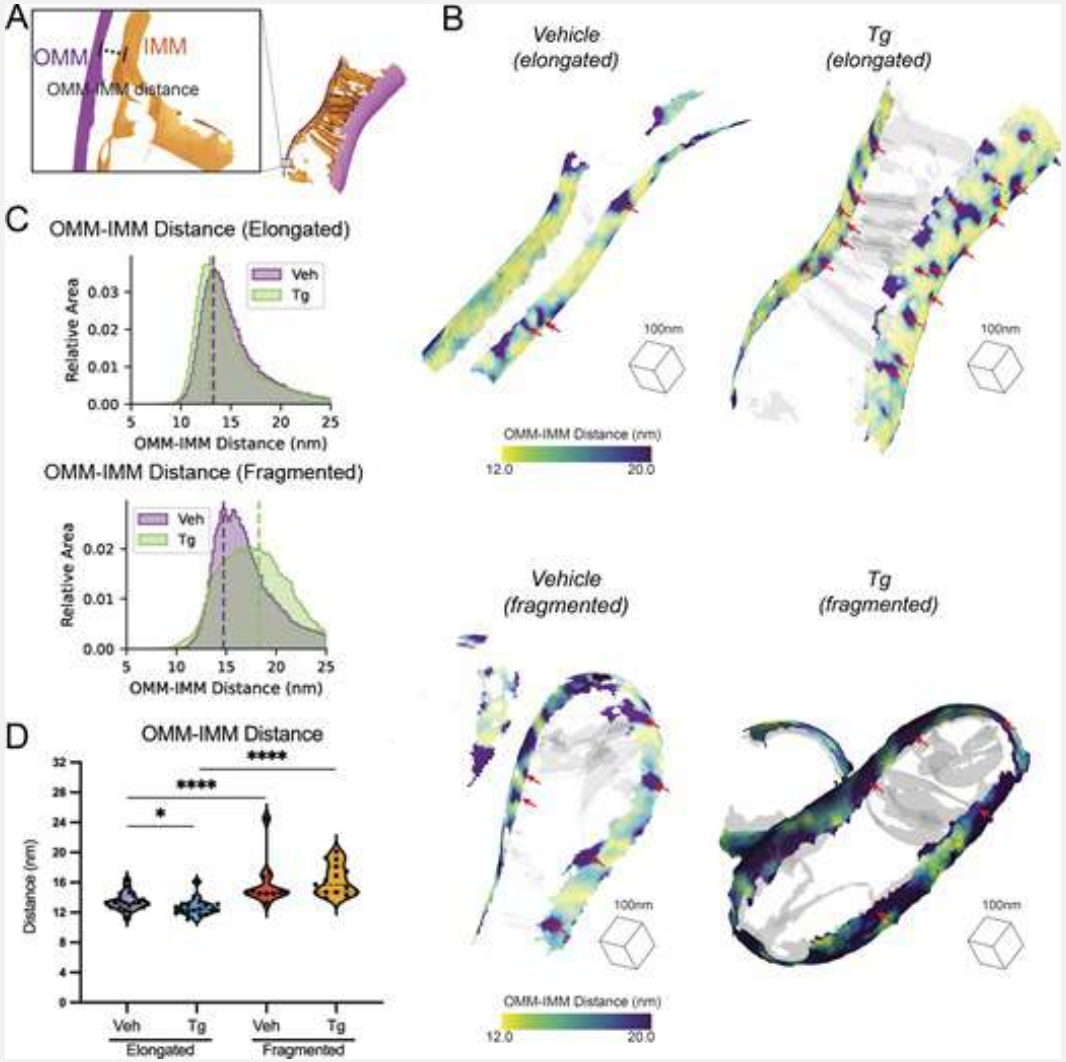
What to look for:

- Use the standard scientific method
  - Does all of your data support your hypothesis?
  - Perform proper statistics

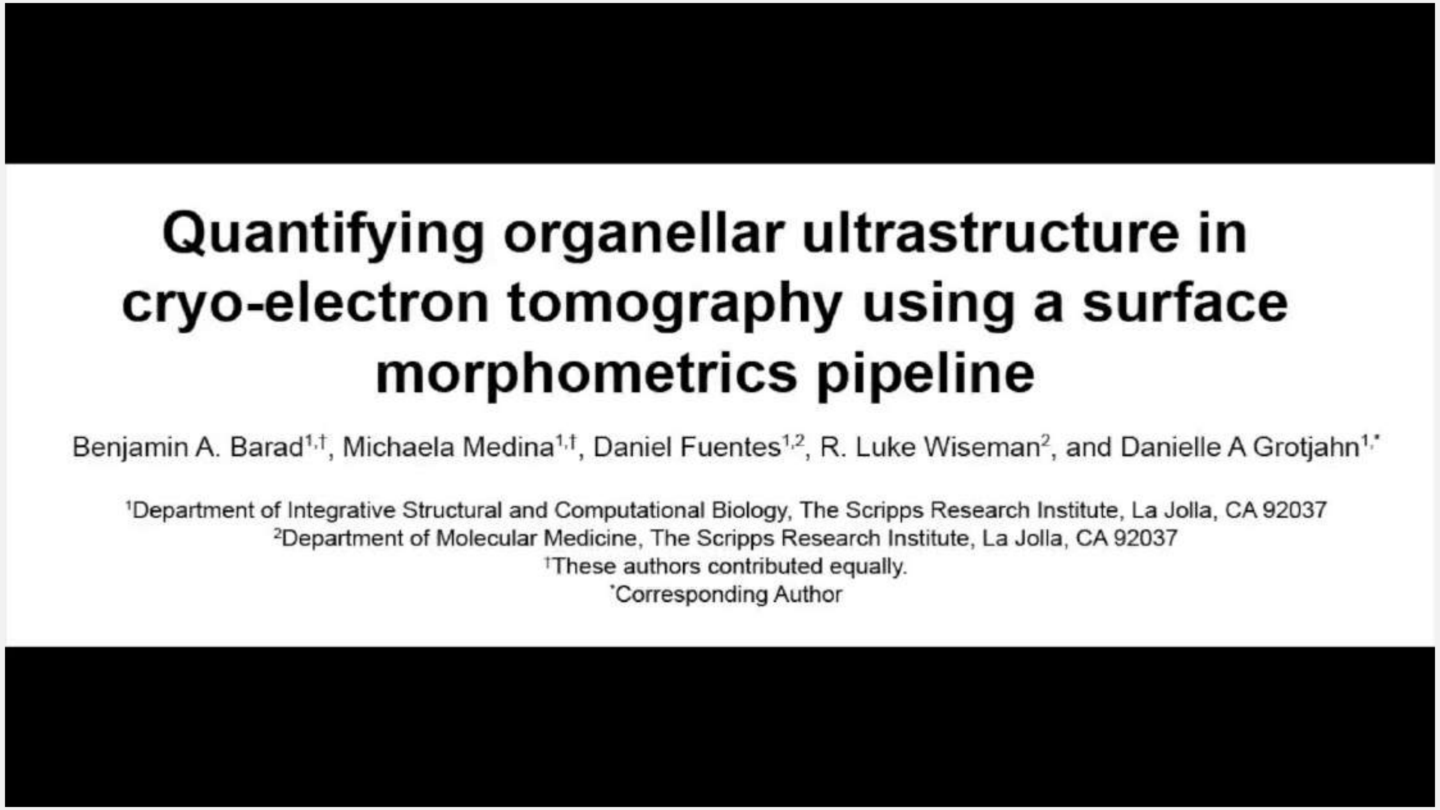
Software for **Statistical analysis**

calculator.exe

Surface Morphometrics



Software for **Statistical analysis**

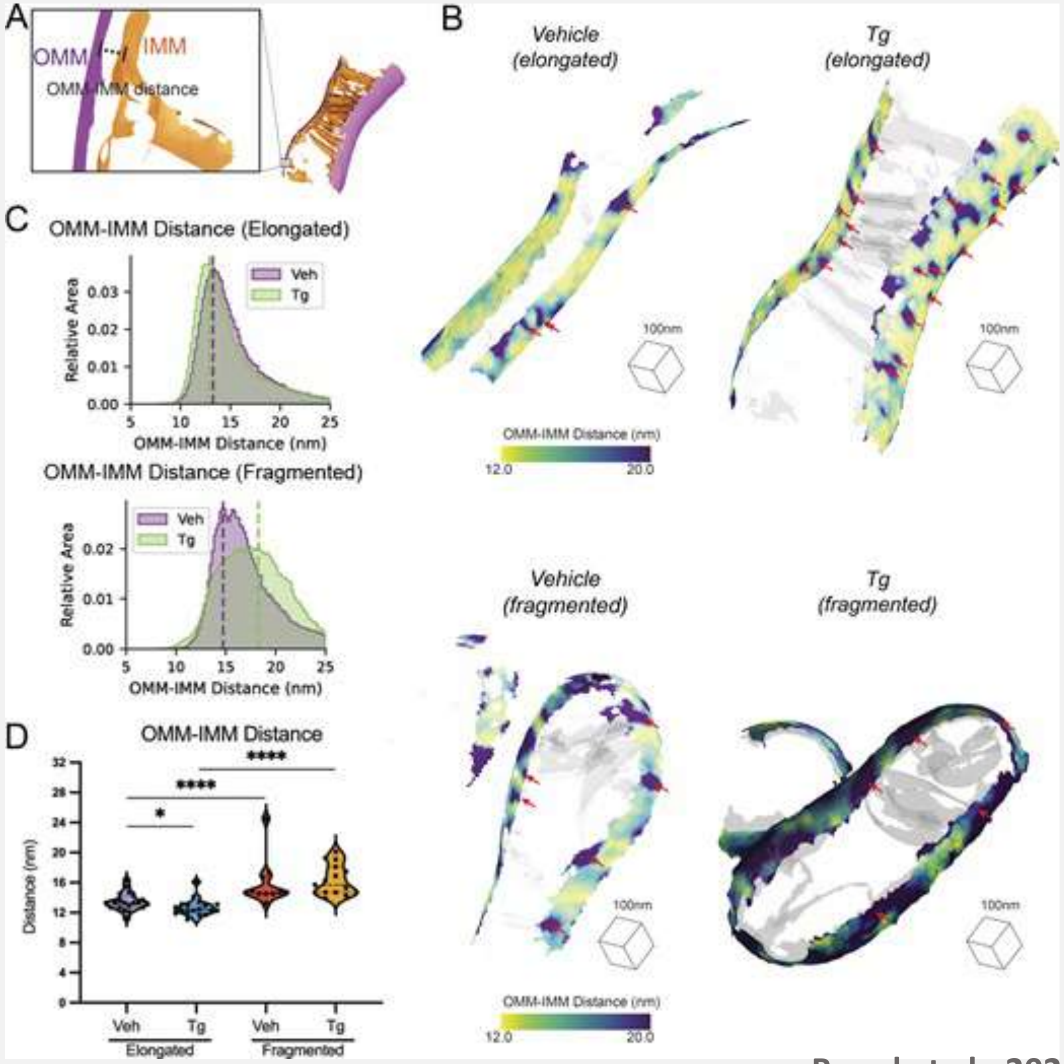


Quantifying organellar ultrastructure in cryo-electron tomography using a surface morphometrics pipeline

Benjamin A. Barad[1,†], Michaela Medina[1,†], Daniel Fuentes[1,2], R. Luke Wiseman[2], and Danielle A Grotjahn[1,*]

[1]Department of Integrative Structural and Computational Biology, The Scripps Research Institute, La Jolla, CA 92037
[2]Department of Molecular Medicine, The Scripps Research Institute, La Jolla, CA 92037
[†]These authors contributed equally.
[*]Corresponding Author

Surface Morphometrics

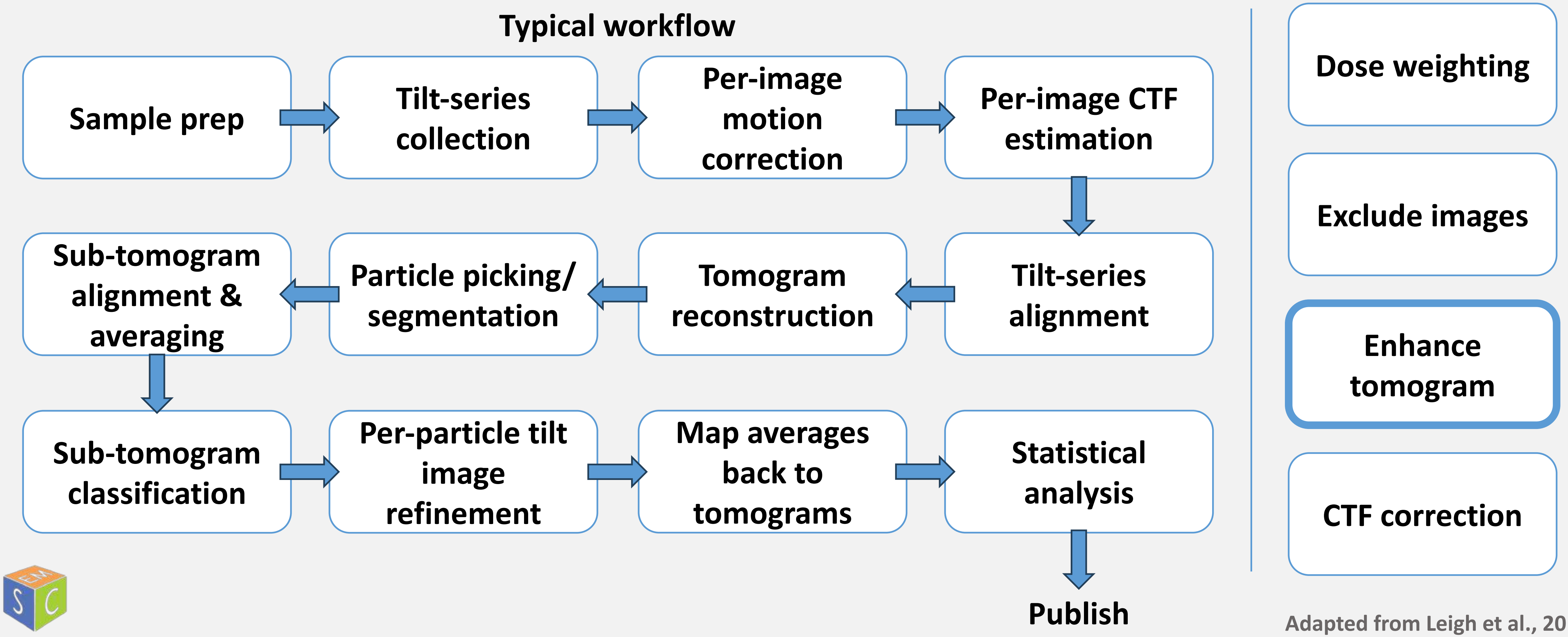


- Helps model membranes
- Helps perform statistics on membrane spacings

Barad et al., 2023

Today's plan

- Analyze software in every step of the workflow



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Particle picking/ segmentation
Sub-tomogram alignment & averaging
Sub-tomogram classification
Per-particle tilt image refinement
Map averages back to tomograms
Statistical analysis
Publish

Optional & flexible steps

Dose weighting
Exclude images
Enhance tomogram
CTF correction

Adapted from Leigh et al., 2019

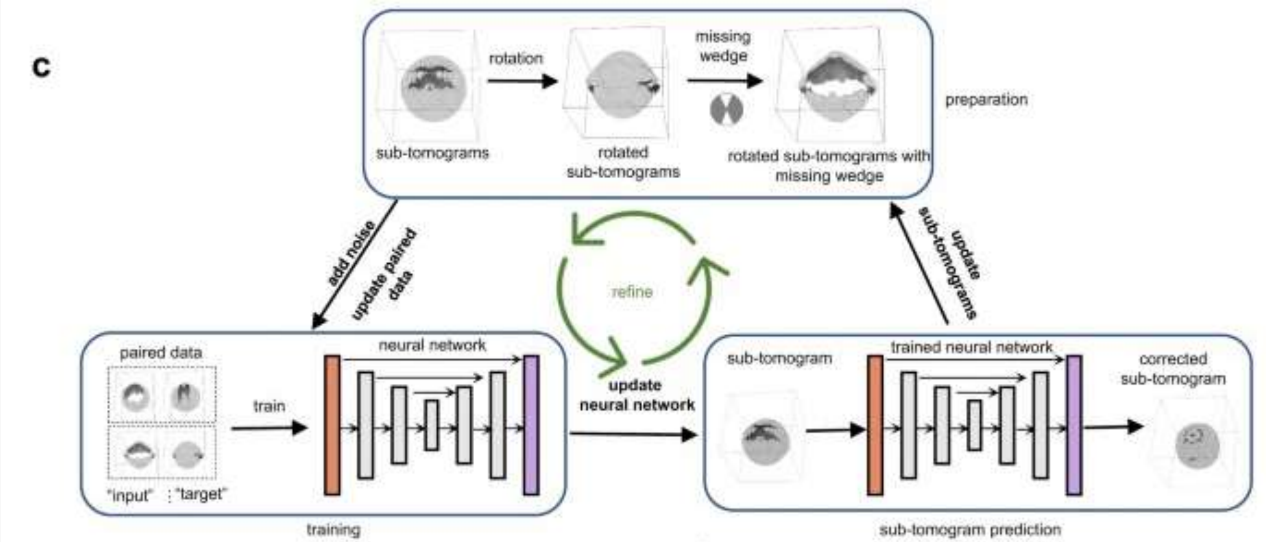
Software for Enhance tomogram

What to look for:

- Is it just bandpass filtering?
- If it’s machine learning, watch out for hallucinations
  - Do not use enhanced tomograms for downstream processing

Software for Enhance tomogram

IsoNet



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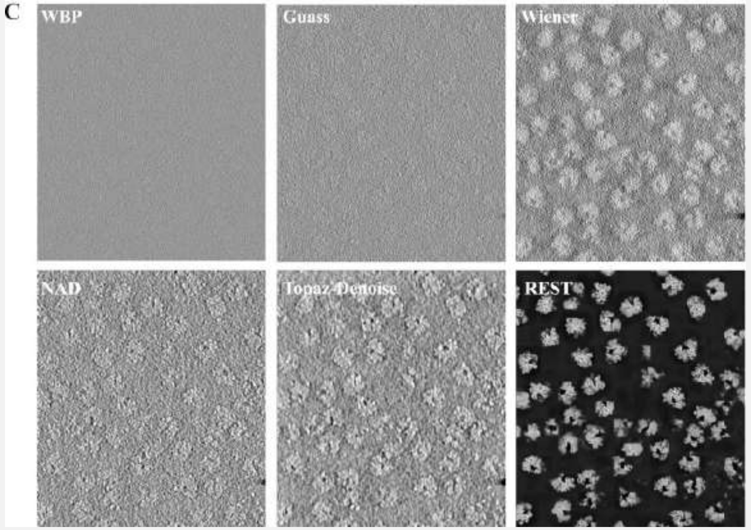
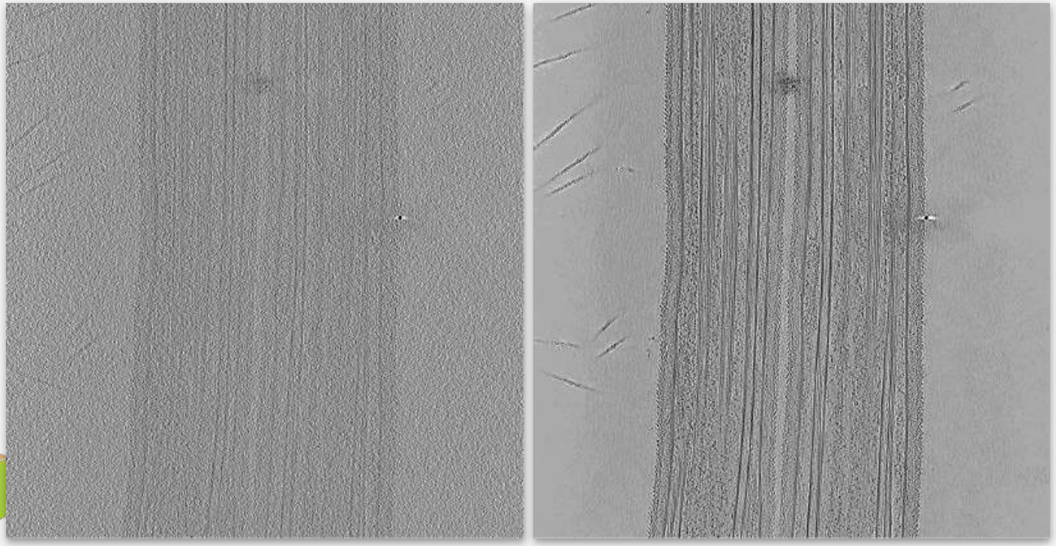
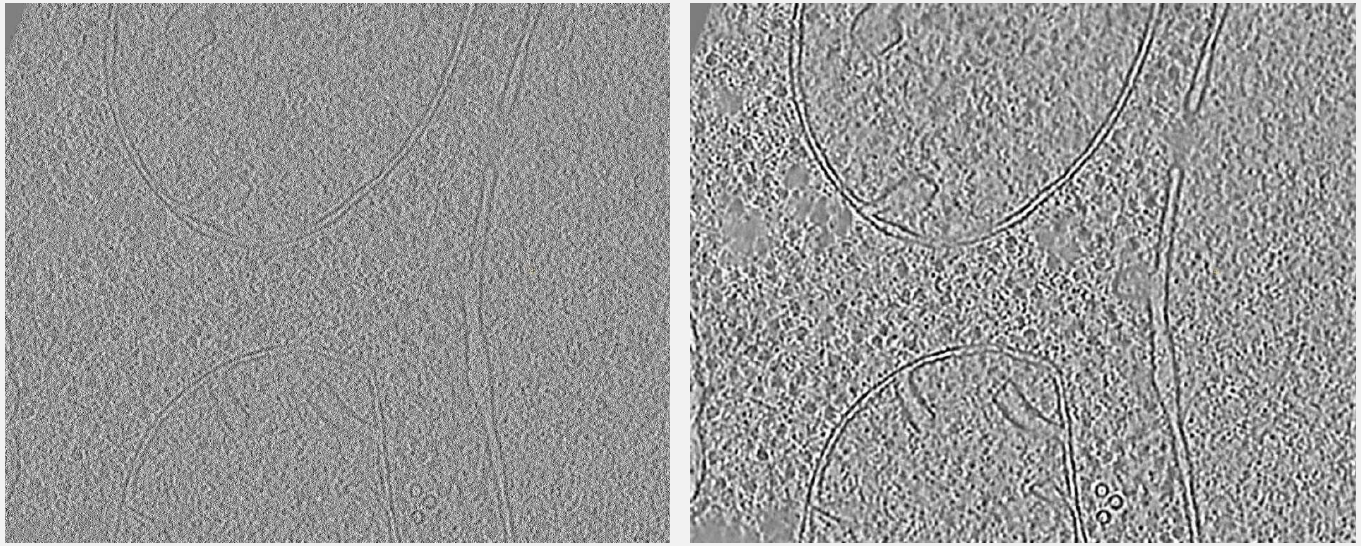


Cryo-CARE

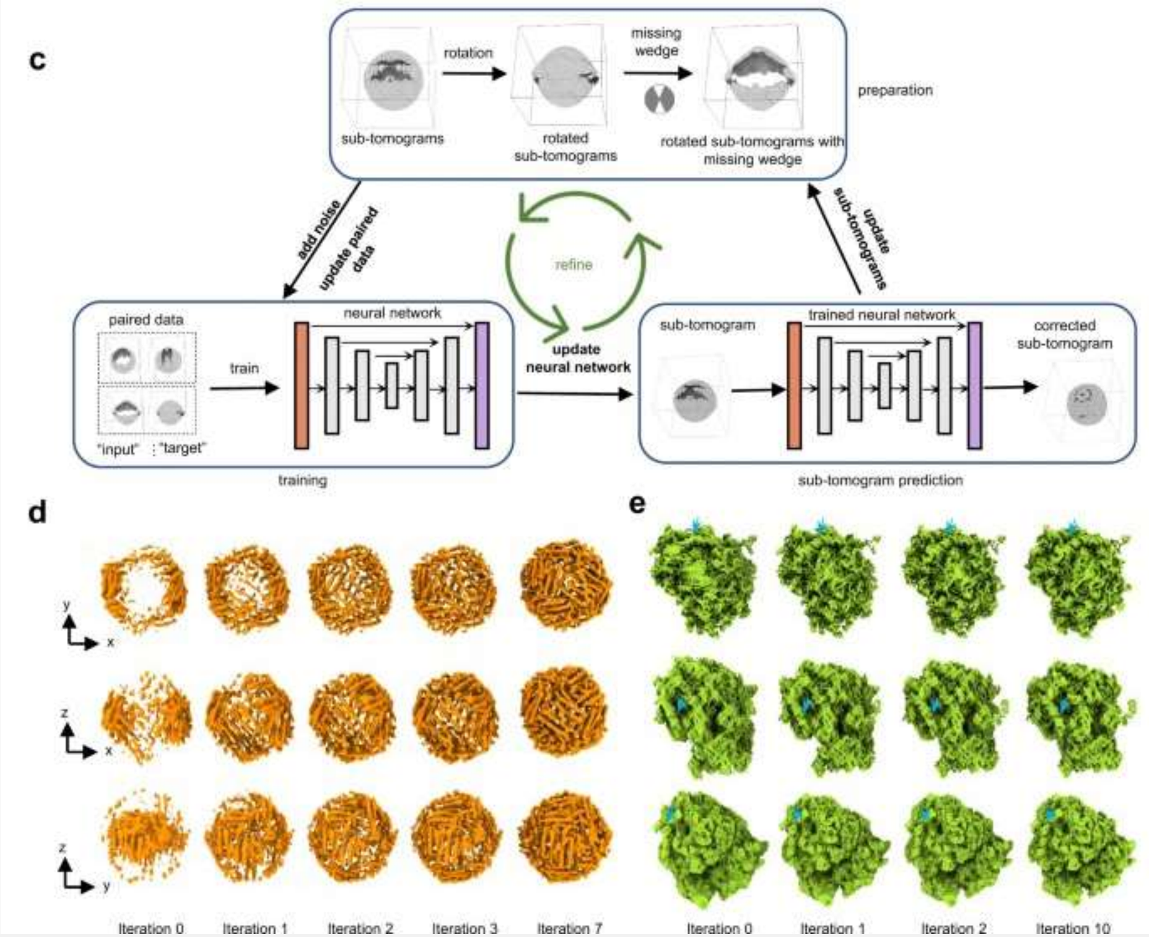


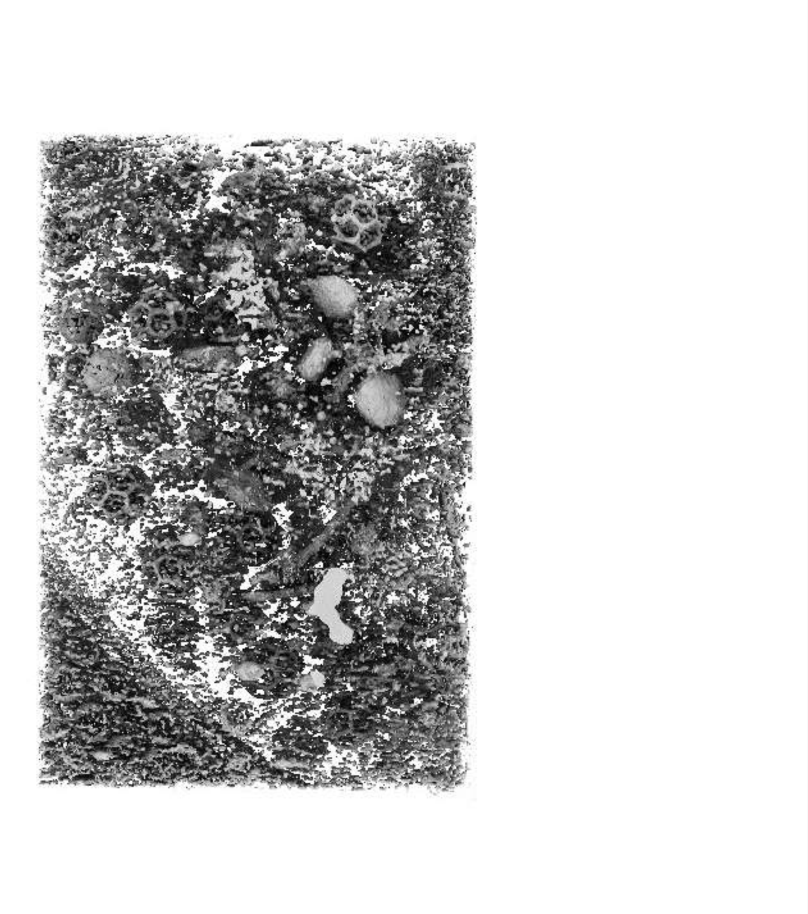
Topaz-Denoise



Software for Enhance tomogram

IsoNet





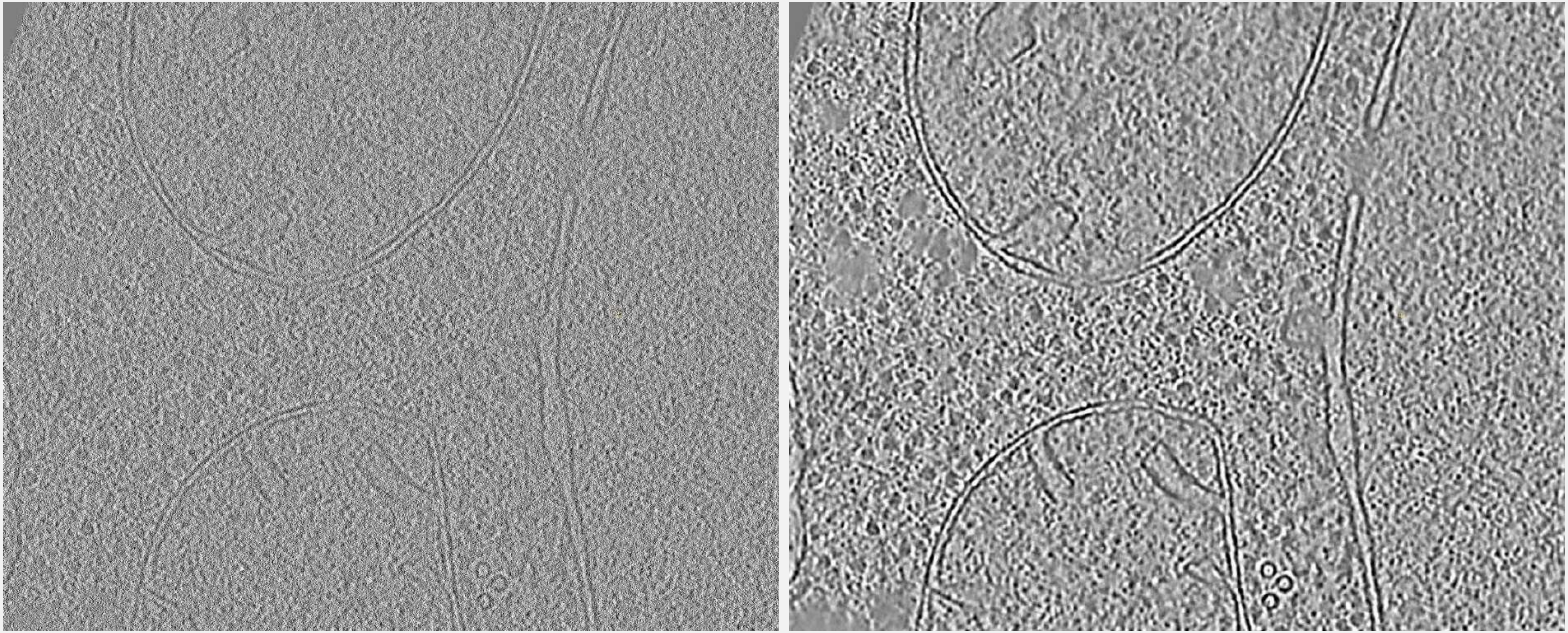
- Machine learning to fill in missing wedge – be careful!
- Additional filtering and contrast enhancement

Liu et al., 2022

Software for

Enhance tomogram

Topaz-Denoise



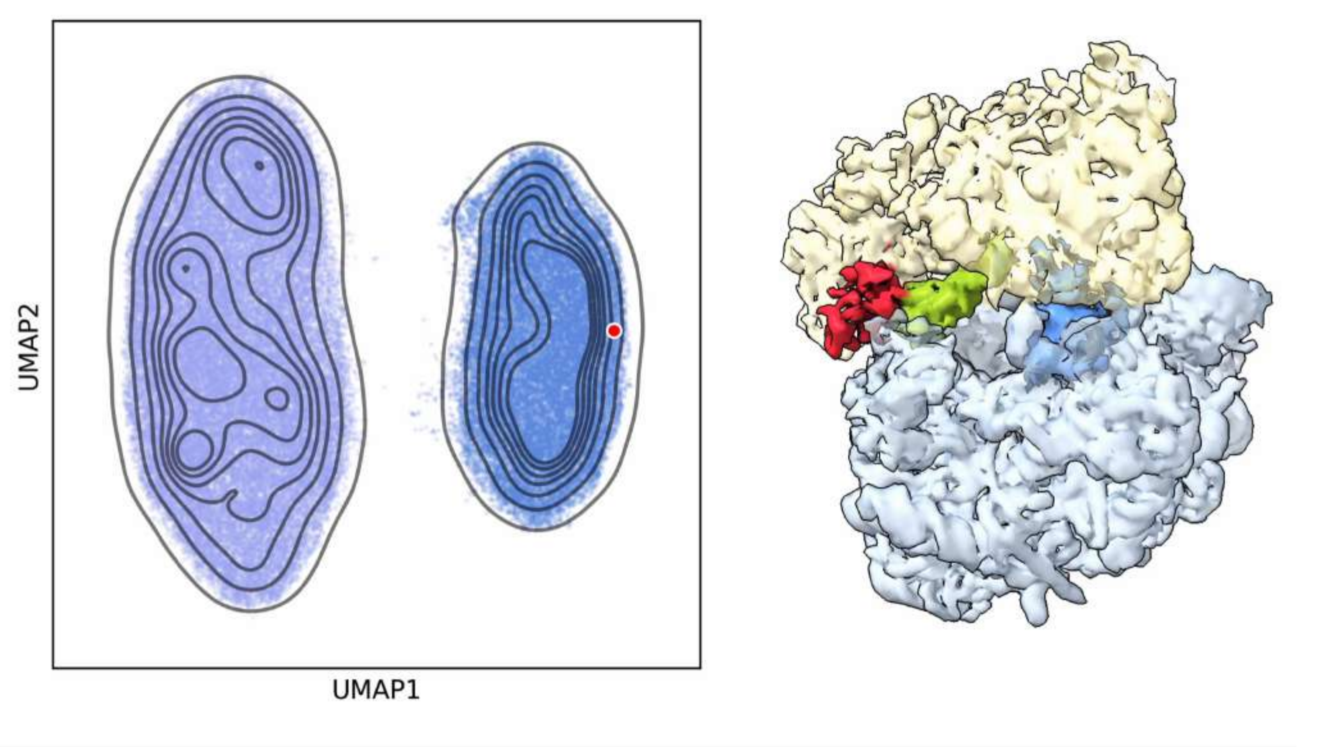
- Machine learning noise2noise to learn+remove cryoET noise

Bepler et al., 2020

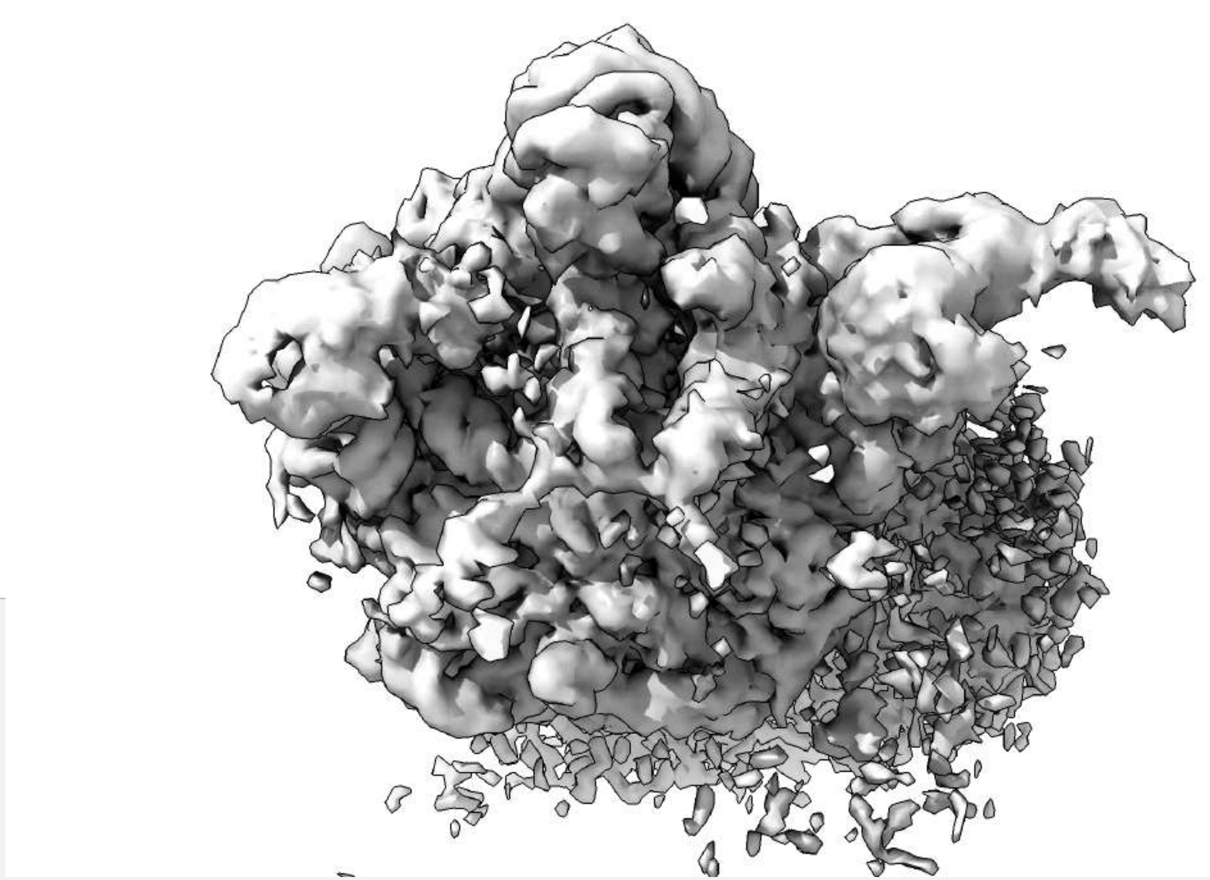
Additional tools

- Flexibility analysis

CryoDRGN-ET



TomoDRGN



- Machine learning VAEs to detangle flexibility

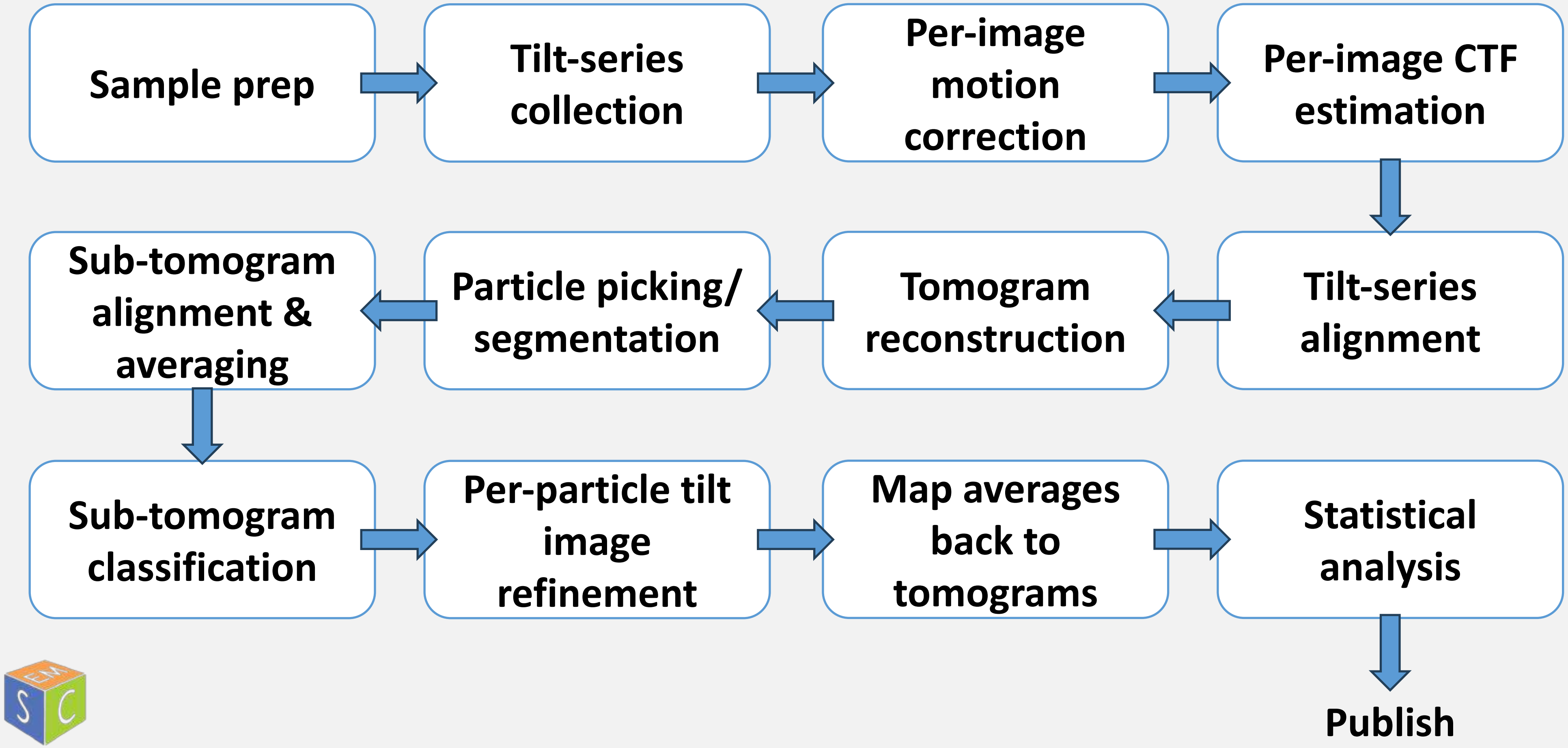
Additional tools

Additional tools

- Perseverance
- Coding skills
    - I recommend understanding terminal and Python
    - Necessary for scripting between software and analyzing results
    - Use GPT to help learn coding and to help code

Multi-step packages

What softwares provide chunks of the workflow?



Typical workflow

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5. Tilt-series alignment
6. Tomogram reconstruction
7. Particle picking/ segmentation
8. Sub-tomogram alignment & averaging
9. Sub-tomogram classification
10. Per-particle tilt image refinement
11. Map averages back to tomograms
12. Statistical analysis

Publish

Optional & flexible steps

- Dose weighting
- Exclude images
- Enhance tomogram
- CTF correction

Adapted from Leigh et al., 2019

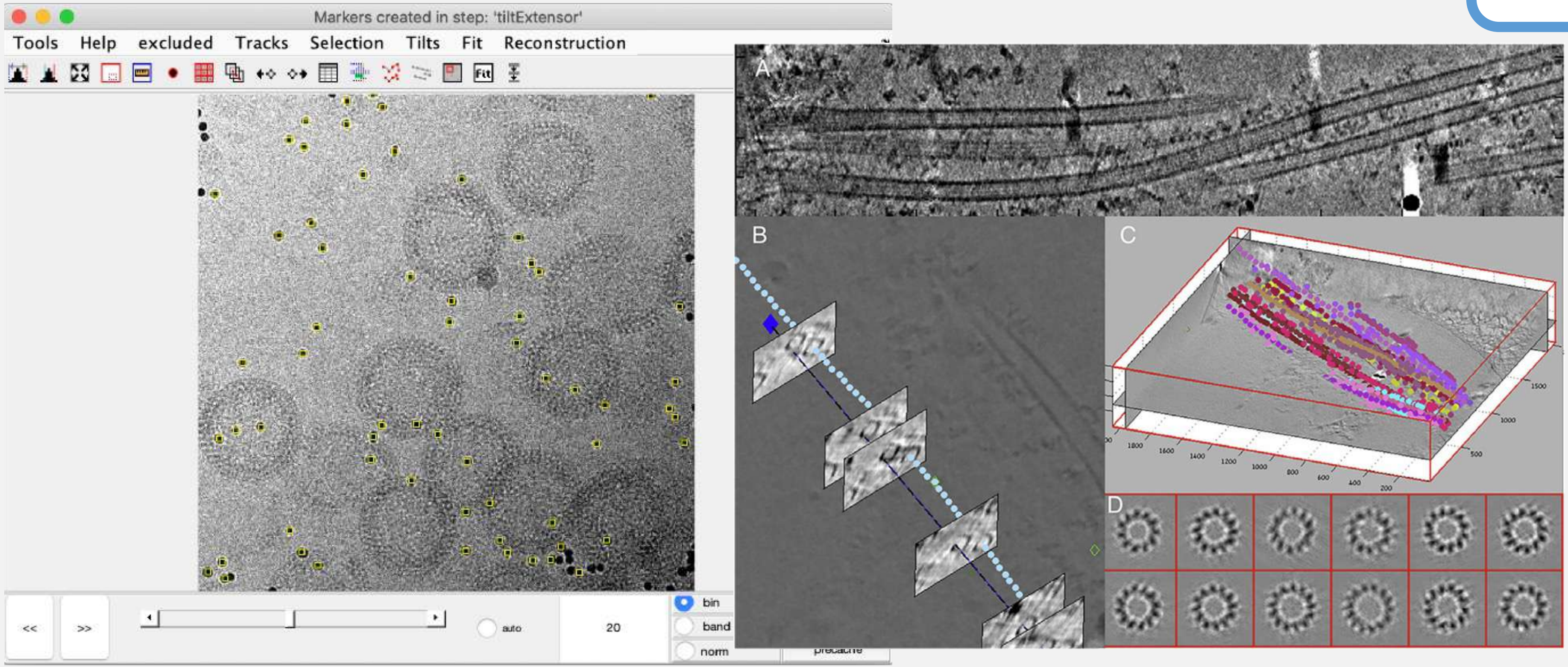
Multi-step packages

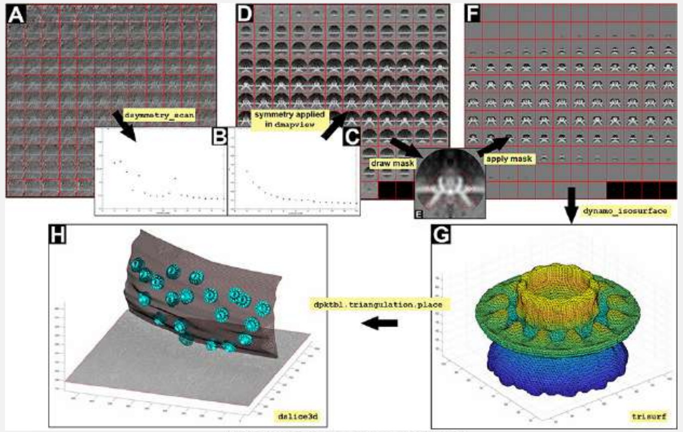
What softwares provide chunks of the workflow?

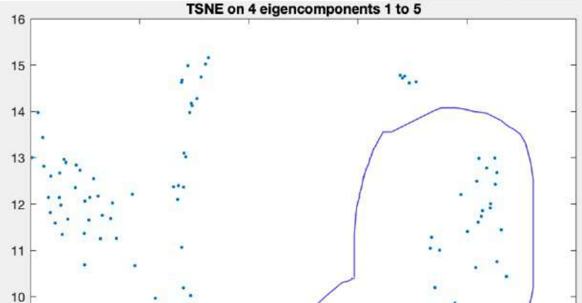
- Dynamo – Tomogram bookkeeping, segmentation, 3D alignment & classification, no CTF
- EMAN2 – Most of the workflow with some limitations
- TomoBEAR – Workflow mostly automated with some limitations
- Relion 4/5 – Most of the workflow (missing tomogram bookkeeping)
- teamtomo.org workflow – Relion 3, Warp, Dynamo, requires Windows+Linux, scripting
- NextPYP – Most of the workflow with some limitations, nice GUI

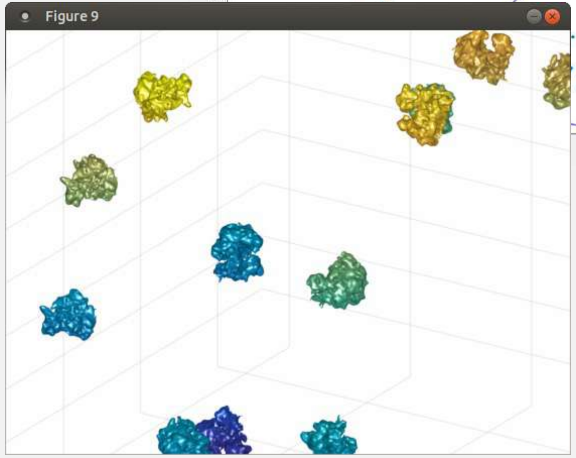
Multi-step packages

Dynamo







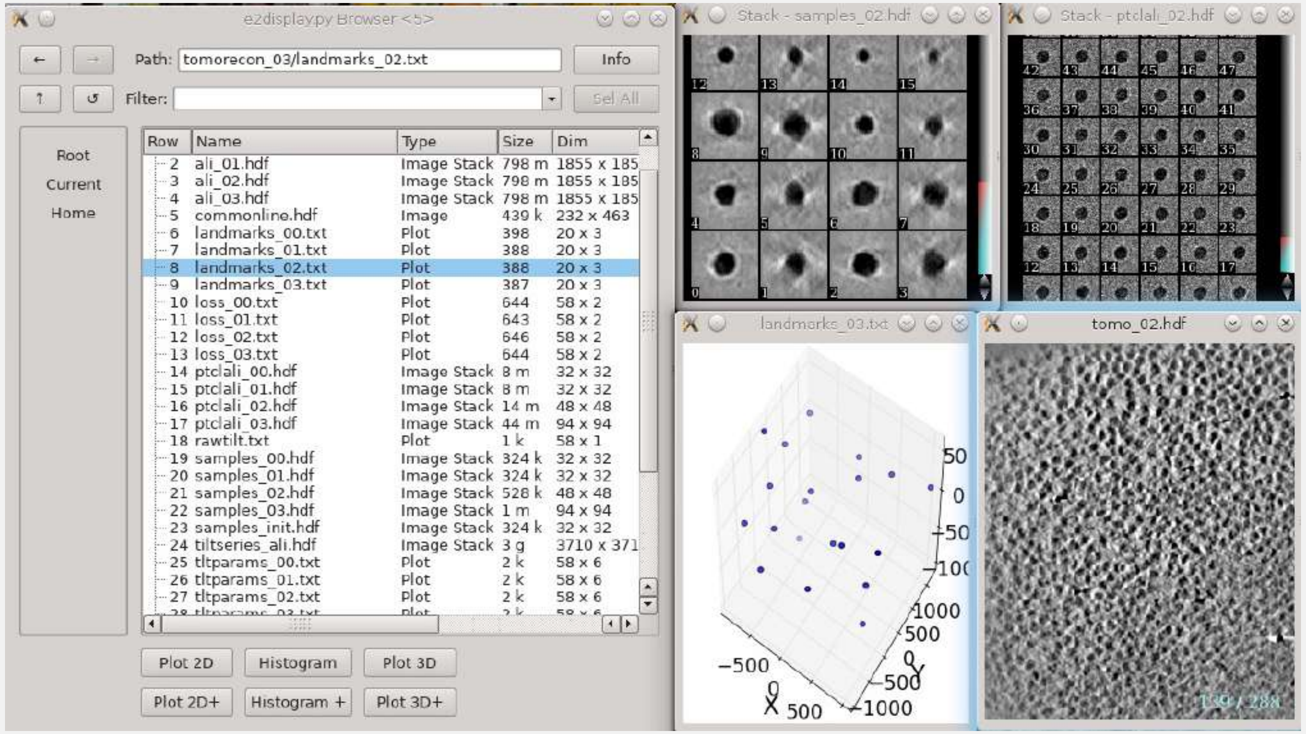


Tilt-series alignment, semi-automated picking/segmentation, 3D sub-tomogram alignment & classification, map to tomograms

- Full tutorials at dynamo-em.org

Multi-step packages

EMAN2



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8	landmarks_02.txt	Plot	388	20 x 3
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12	loss_02.txt	Plot	646	58 x 2
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20	samples_01.hdf	Image Stack	324 k	32 x 32
21	samples_02.hdf	Image Stack	528 k	48 x 48
22	samples_03.hdf	Image Stack	1 m	94 x 94
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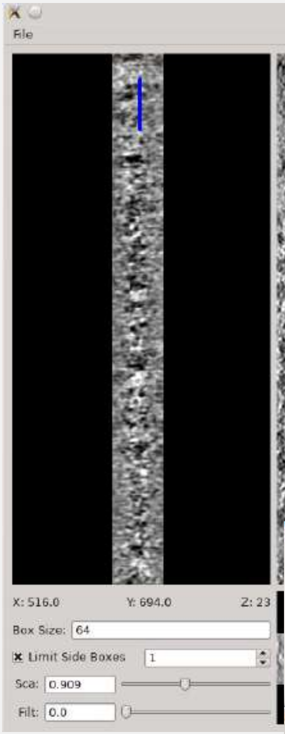

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☒ Limit Side Boxes 1

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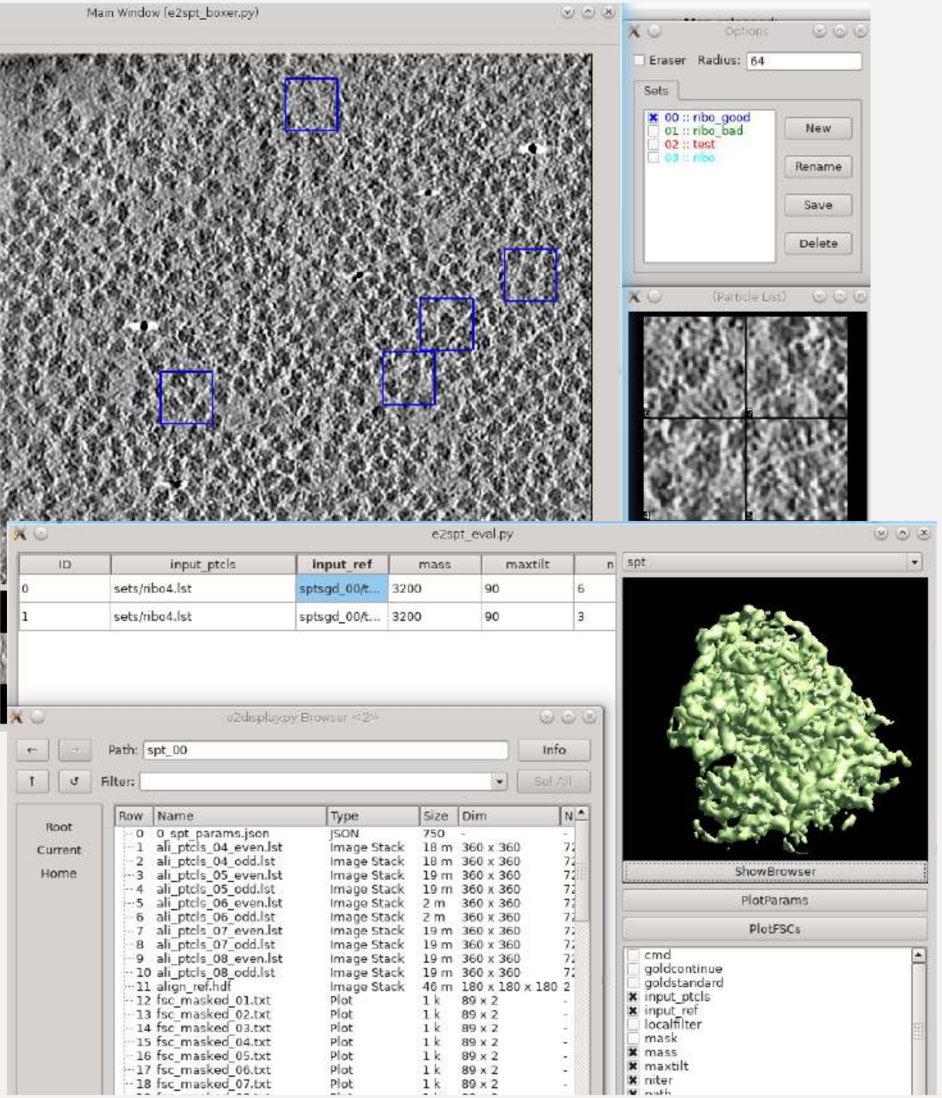

☐ Eraser Radius: 64

☒ 00 :: ribo_good
☐ 01 :: ribo_bad
☐ 02 :: test
☐ 03 :: ribo

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1	sets/ribo4.lst	sptsgd_00/t...	3200	90	3

☐ cmd
☐ goldcontinue
☐ goldstandard
☒ input_ptcls
☒ input_ref
☐ localfilter
☐ mask
☒ mass
☒ maxtilt
☒ niter

Path: spt_00

Filter:

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2	ali_ptcls_04_odd.lst	Image Stack	18 m	360 x 360	7
3	ali_ptcls_05_even.lst	Image Stack	19 m	360 x 360	7
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11	align_ref.hdf	Image Stack	46 m	180 x 180 x 180	2
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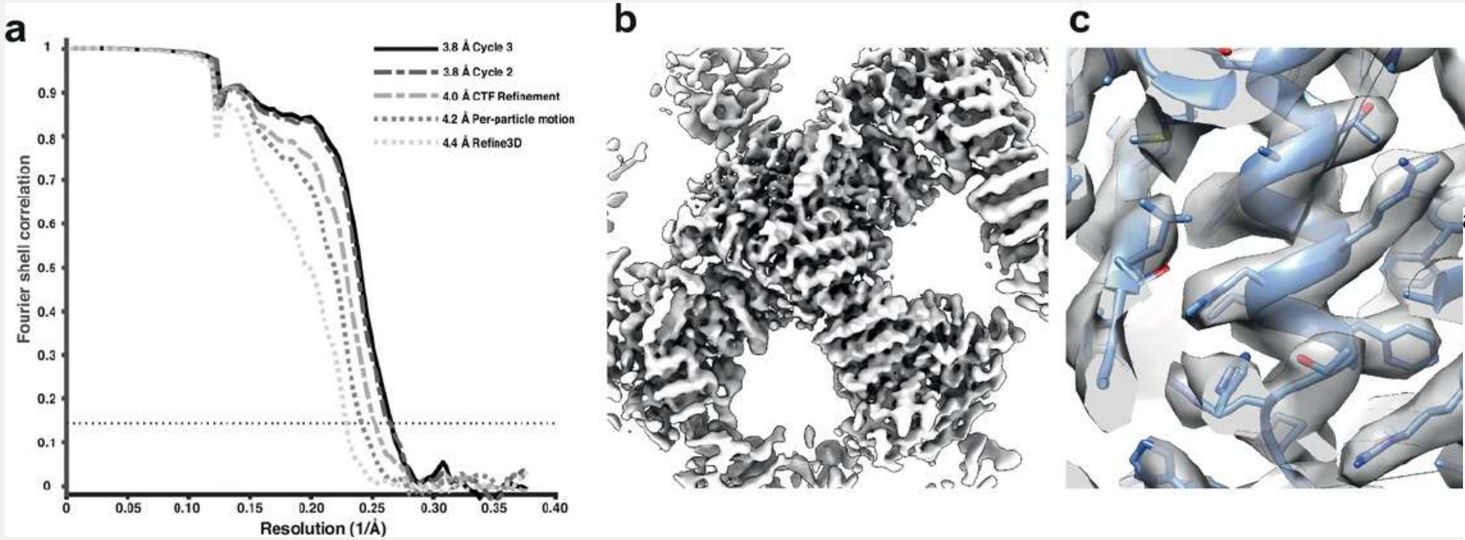
14	fsc_masked_03.txt	Plot	1 k	89 x 2	-
15	fsc_masked_04.txt	Plot	1 k	89 x 2	-
16	fsc_masked_05.txt	Plot	1 k	89 x 2	-
17	fsc_masked_06.txt	Plot	1 k	89 x 2	-
18	fsc_masked_07.txt	Plot	1 k	89 x 2	-

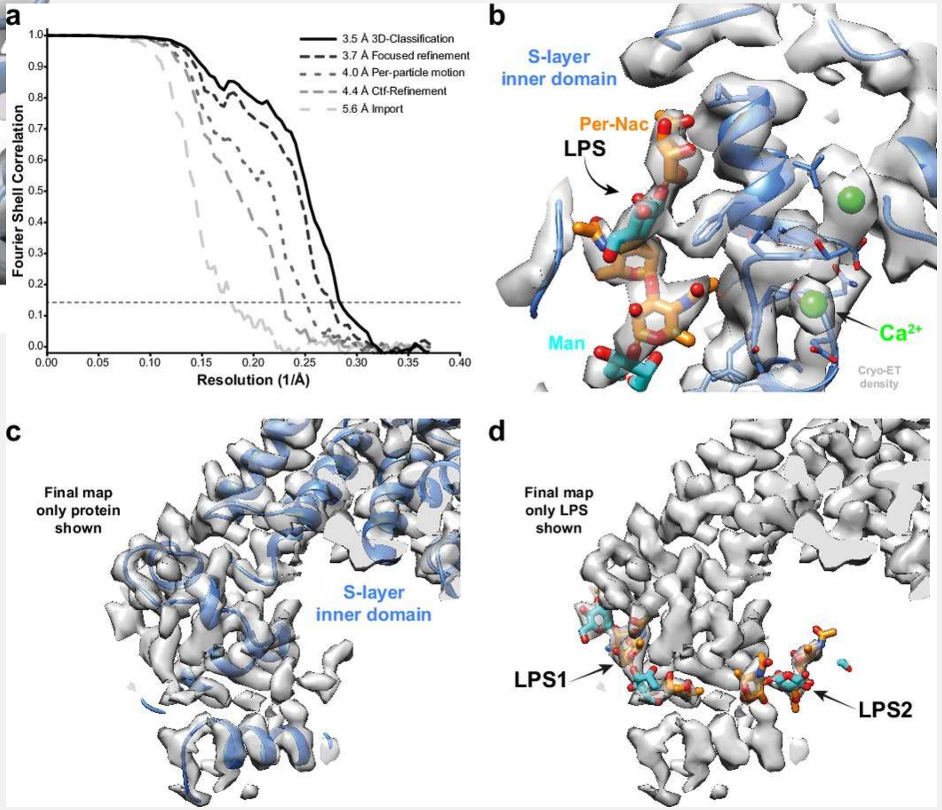
Tilt-series alignment, reconstruction, CTF, machine learning picking/segmentation, sub-tilt refinement

- Full tutorials at blake.bcm.edu/emanwiki/EMAN2/e2tomo

Multi-step packages

Relion4/5



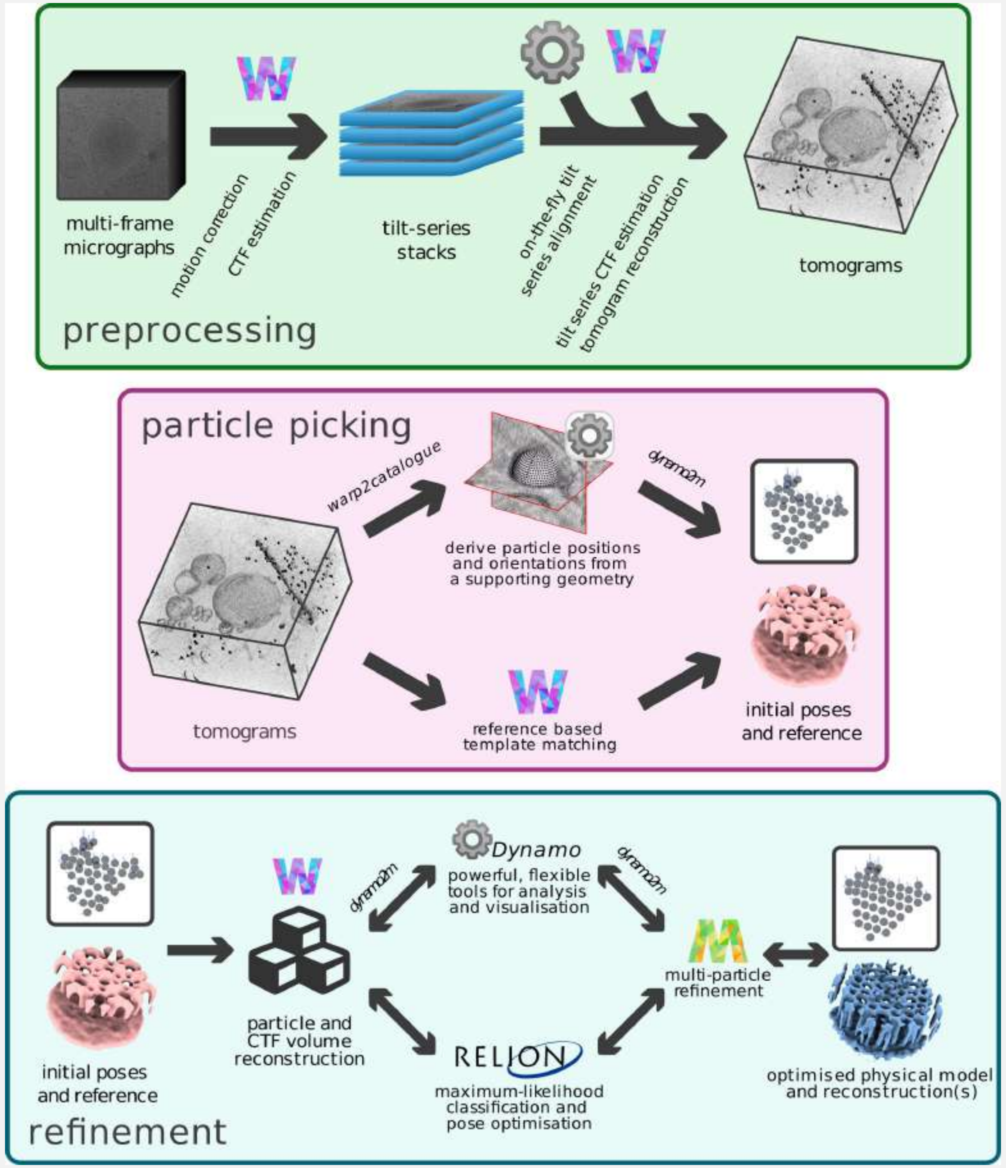


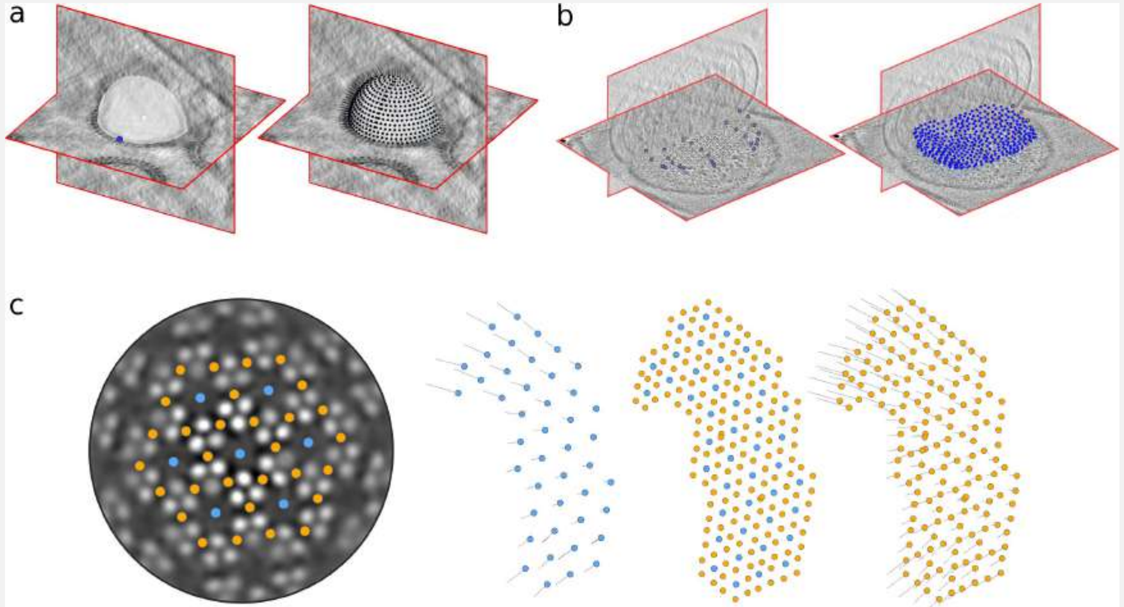
3D alignment, 2D sub-tilt refinement, CTF refinement, Bayesian 3D classification, most other Relion tools

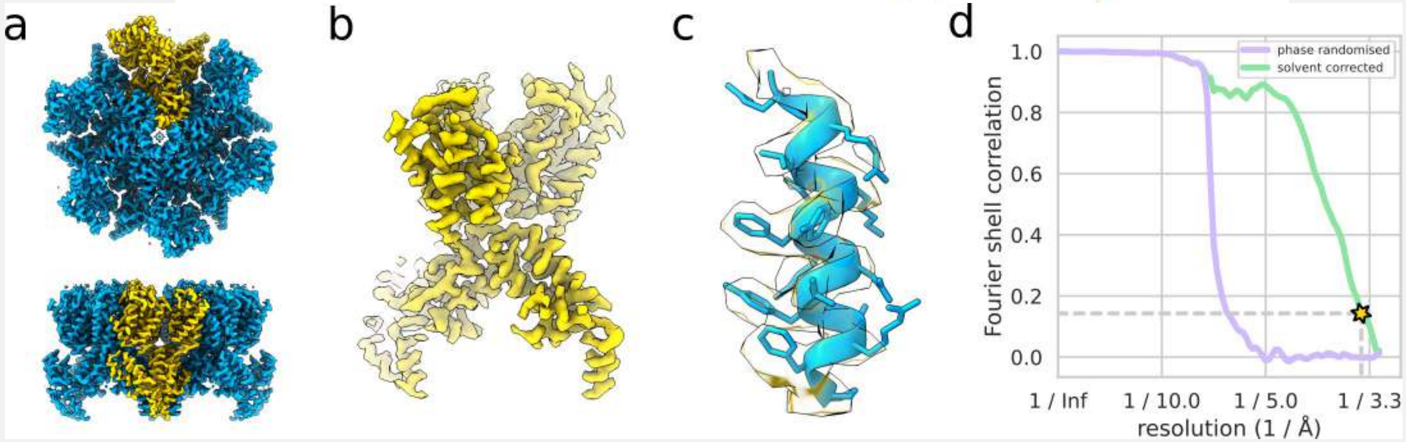
- Full tutorials at relion.readthedocs.io

Multi-step packages

teamtomo.org





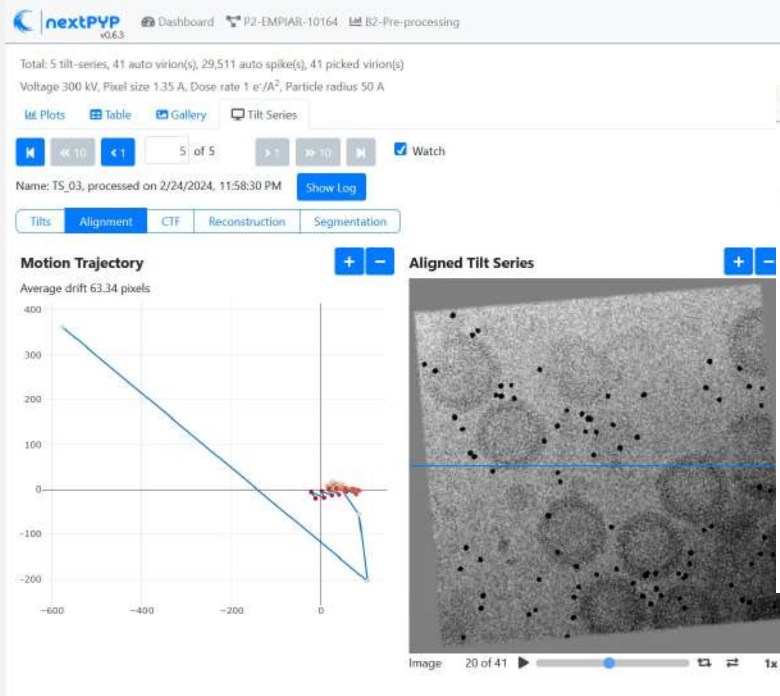


- Full tutorial at teamtomo.org

Burt et al., 2021

Multi-step packages

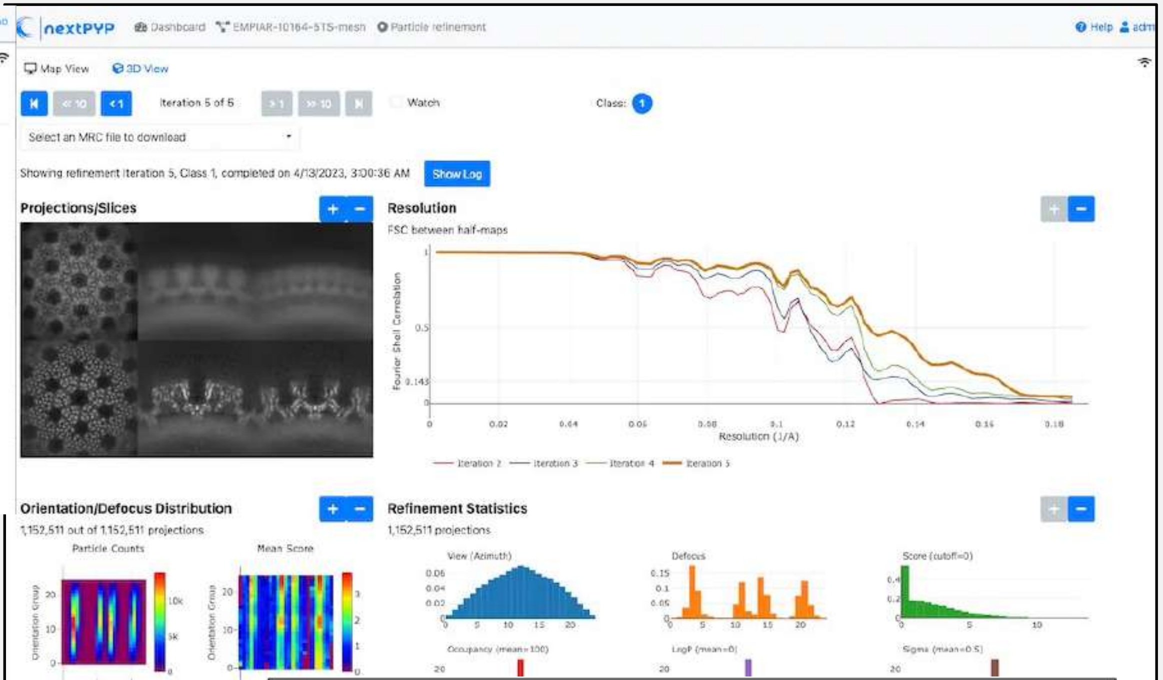
NextPYP


Total: 5 tilt-series, 41 auto virion(s), 29,511 auto spike(s), 41 picked virion(s)
Voltage 300 kV, Pixel size 1.35 A, Dose rate 1 e⁻/A^2, Particle radius 50 A

Name: TS_03, processed on 2/24/2024, 11:58:30 PM

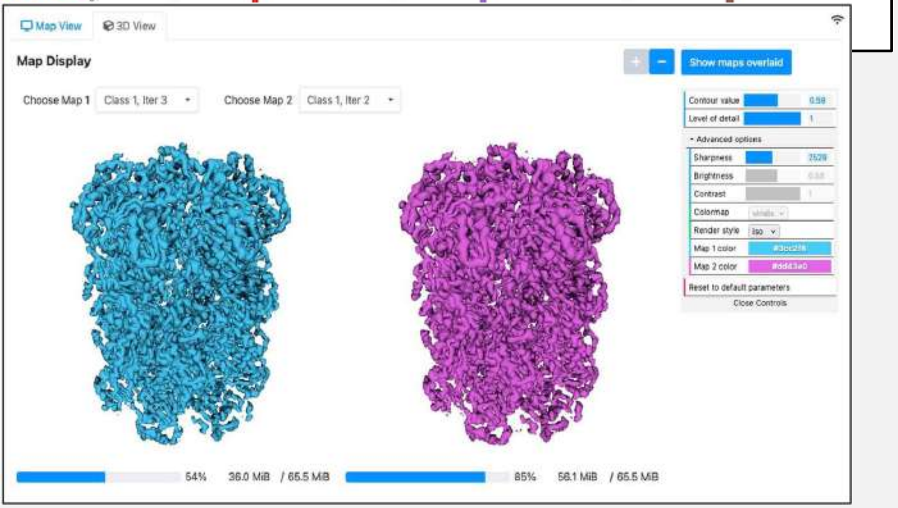
Average drift 63.34 pixels


Total: 5 tilt-series, 41 auto virion(s), 29,511 auto spike(s), 41 picked virion(s)
Voltage 300 kV, Pixel size 1.35 A, Dose rate 1 e⁻/A^2, Particle radius 50 A


Showing refinement iteration 5, Class 1, completed on 4/13/2023, 3:00:36 AM

1,152,511 out of 1,152,511 projections

1,152,511 projections


Choose Map 1: Class 1, Iter 3
Choose Map 2: Class 1, Iter 2
Contour value: 0.59
Level of detail: 1
Render style: iso
Map 1 color: #3cc2f8
Map 2 color: #dd43a0
54% 36.0 MiB / 65.5 MiB
85% 56.1 MiB / 65.5 MiB

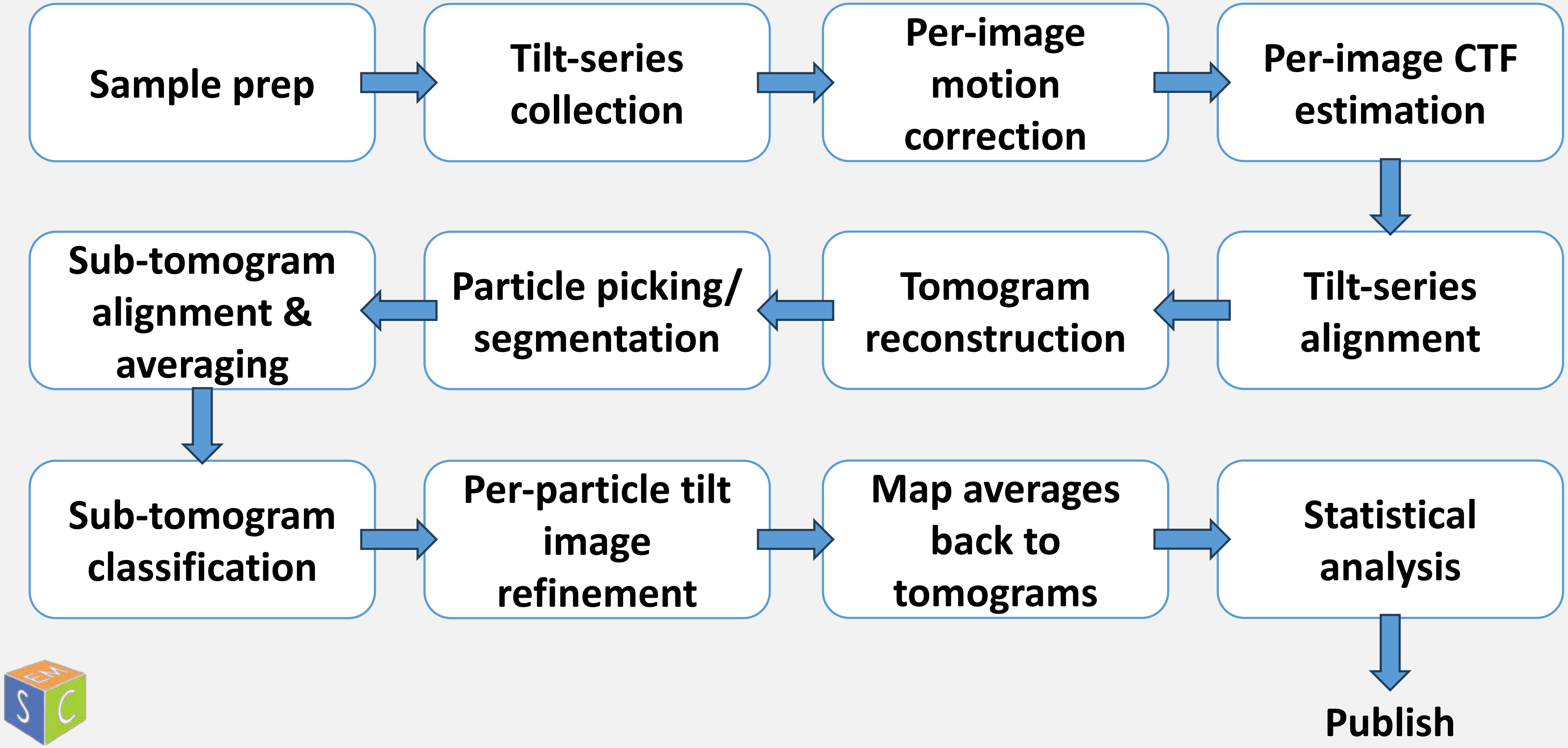
Frame+tilt-series alignment, CTF, reconstruction, denoise, machine learning picking/segmentation, sub-tilt refinement, map to tomogram (ArtiaX)

- Full tutorials at nextpyp.app

Today’s plan

- Analyze software in every step of the workflow

Typical workflow



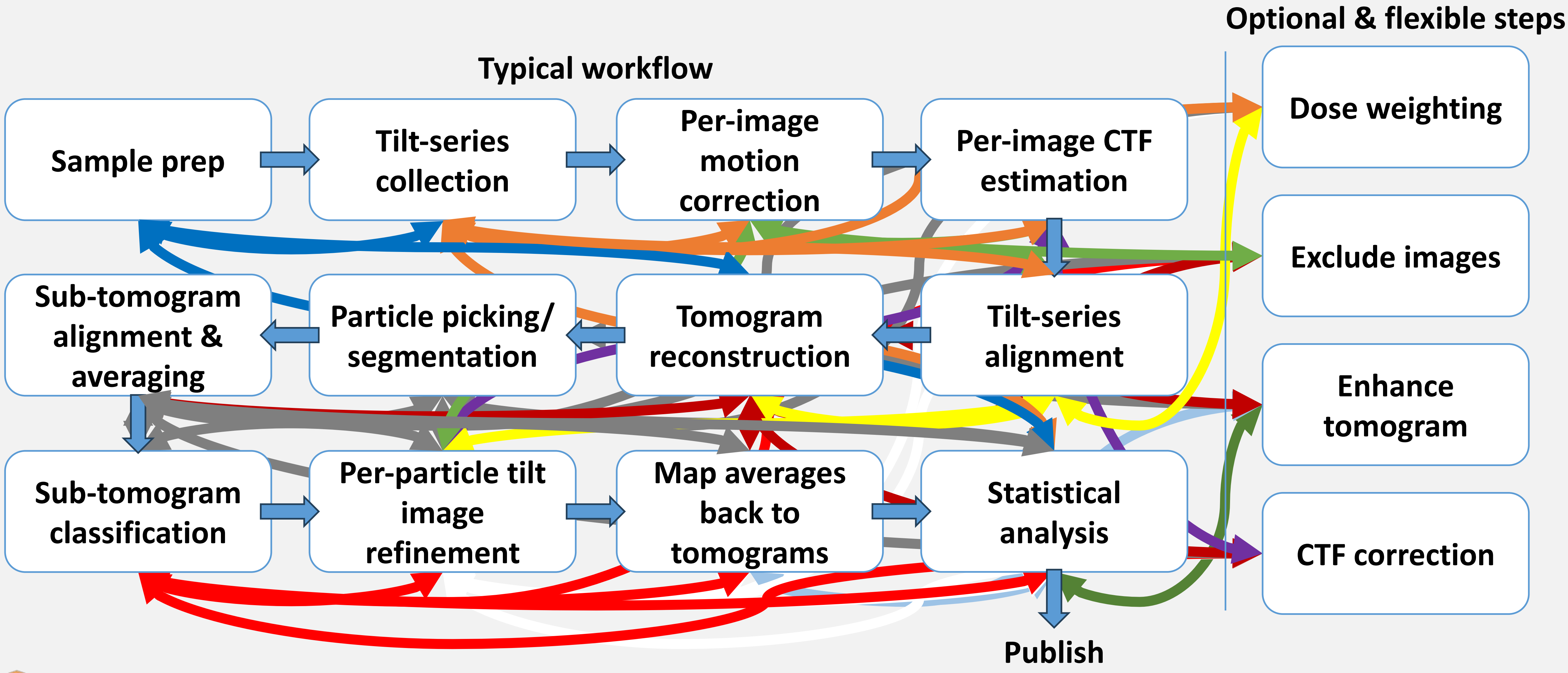
Sample prep → Tilt-series collection → Per-image motion correction → Per-image CTF estimation → Tilt-series alignment → Tomogram reconstruction → Particle picking/ segmentation → Sub-tomogram alignment & averaging → Sub-tomogram classification → Per-particle tilt image refinement → Map averages back to tomograms → Statistical analysis → Publish

Optional & flexible steps

- Dose weighting
- Exclude images
- Enhance tomogram
- CTF correction

Adapted from Leigh et al., 2019

Real workflow



Optional & flexible steps

Typical workflow

Sample prep

Tilt-series collection

Per-image motion correction

Per-image CTF estimation

Sub-tomogram alignment & averaging

Particle picking/ segmentation

Tomogram reconstruction

Tilt-series alignment

Sub-tomogram classification

Per-particle tilt image refinement

Map averages back to tomograms

Statistical analysis

Publish

Dose weighting

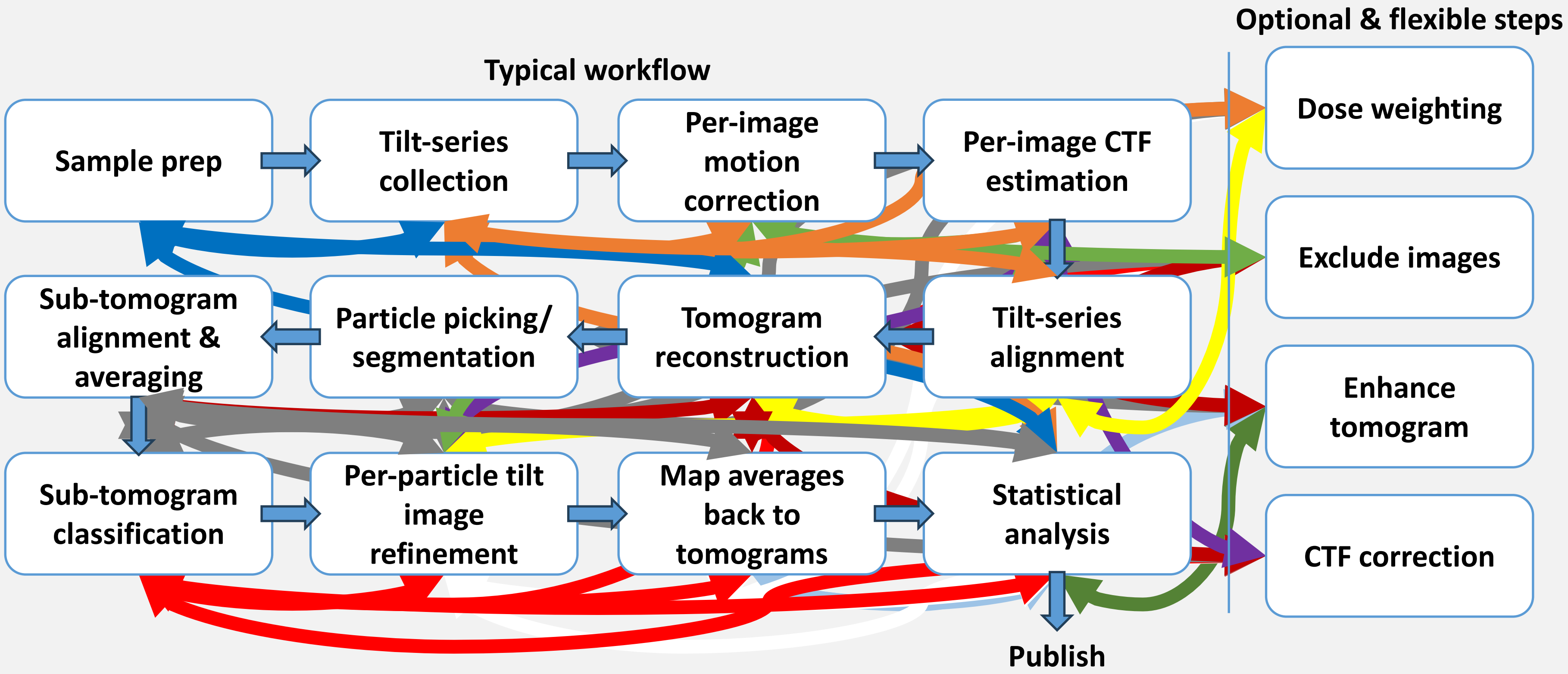
Exclude images

Enhance tomogram

CTF correction

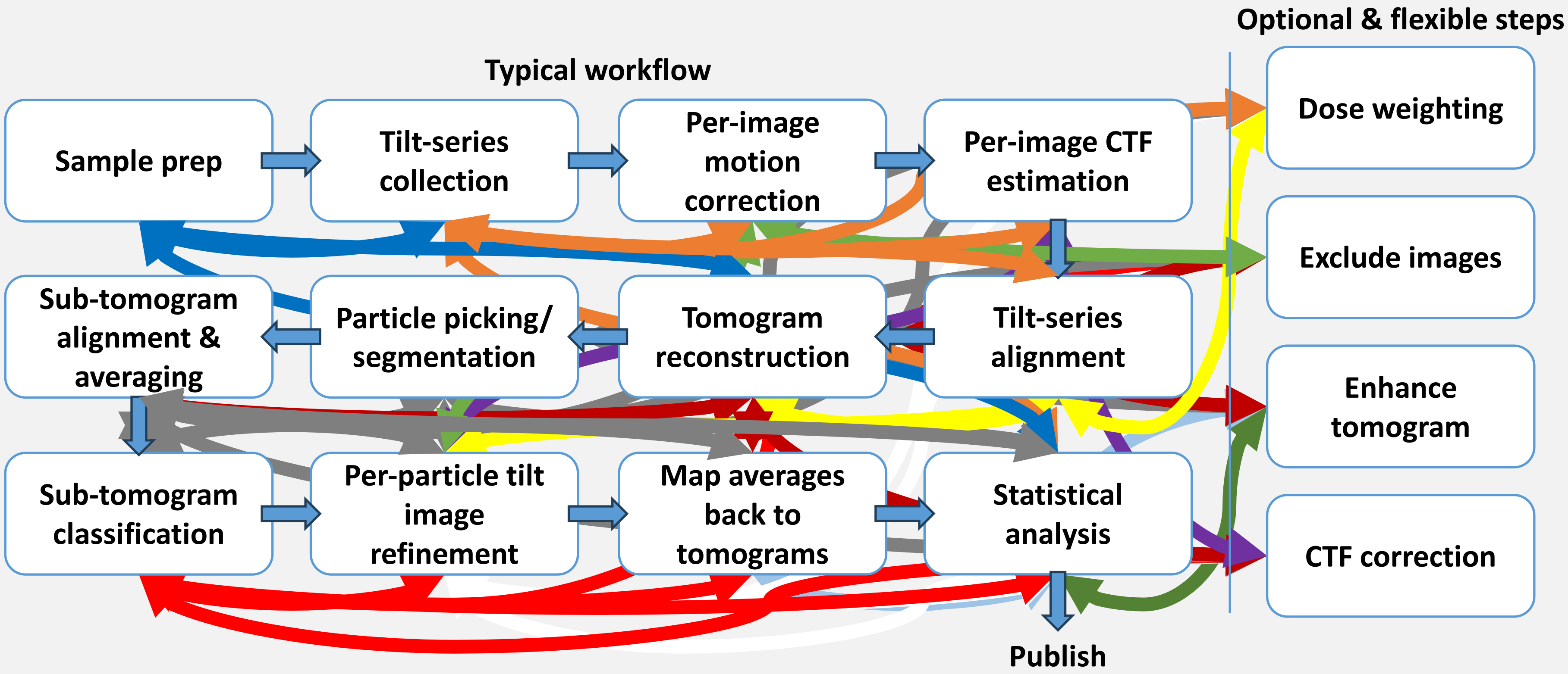
Adapted from Leigh et al., 2019

Real workflow



- Most cryoET projects take **years**
- Most projects target **large proteins** that are **everywhere**
- There are **as many** cryoET software **workflows as** there are cryoET **labs**

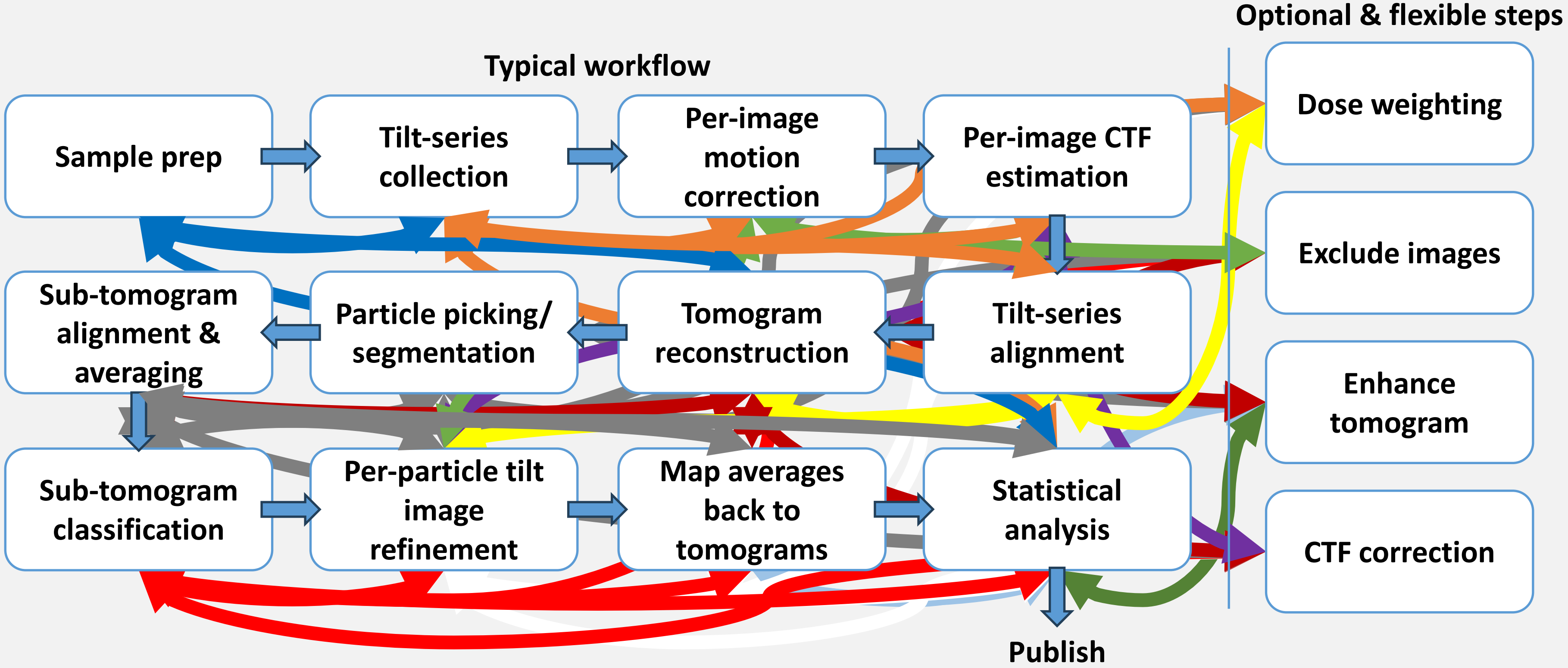
Real workflow



Main areas that require improvement:

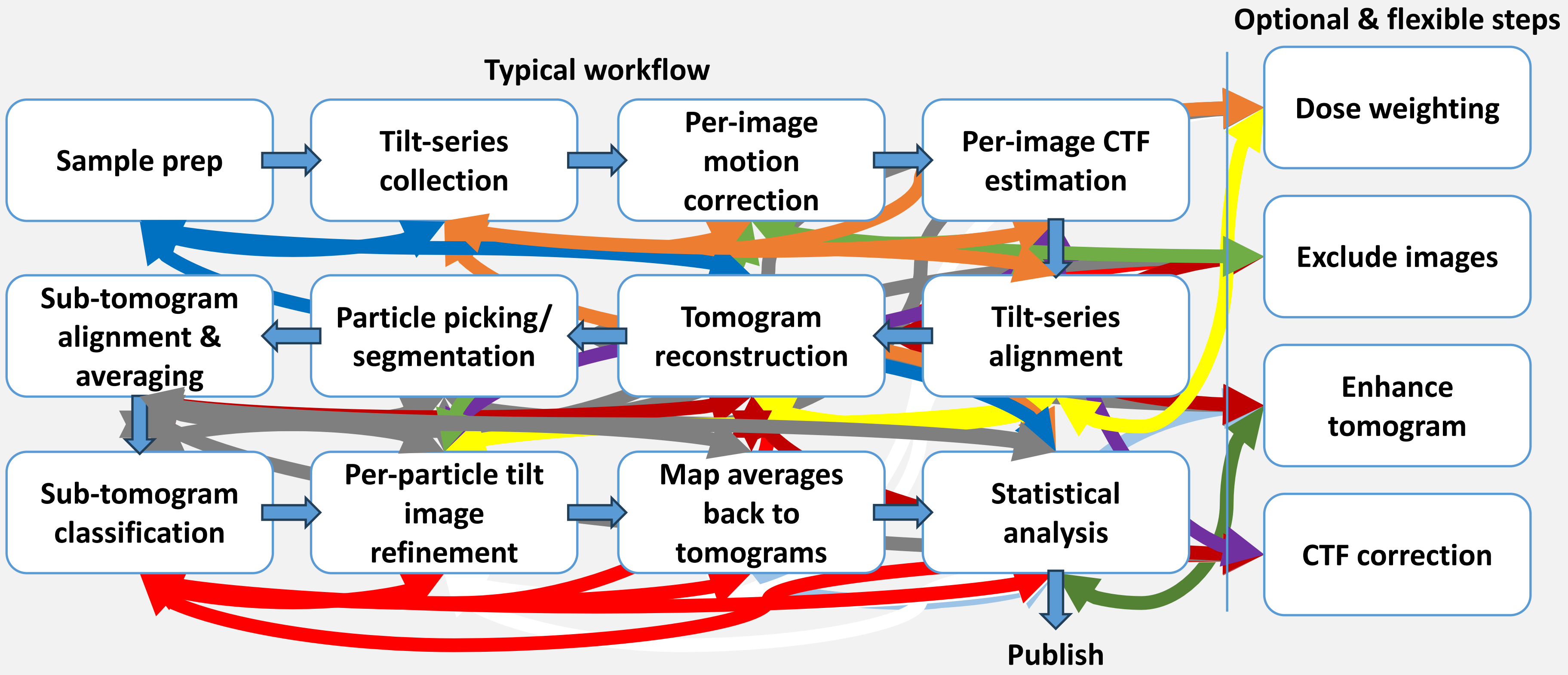
- Automated object identification & classification
- How to deal with overlapping proteins
- Fully automated workflows

Real workflow



- Many **softwares** are **missing** from this presentation
- **Workshops** exist and take **1-5 days** to learn large portions of the workflow
- The **best way** to learn is to **work on a cryoET project!**

Real workflow



Optional & flexible steps

Typical workflow

Sample prep

Tilt-series collection

Per-image motion correction

Per-image CTF estimation

Sub-tomogram alignment & averaging

Particle picking/ segmentation

Tomogram reconstruction

Tilt-series alignment

Sub-tomogram classification

Per-particle tilt image refinement

Map averages back to tomograms

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Dose weighting

Exclude images

Enhance tomogram

CTF correction

Last points:

- Always **look at your data** at every step to make sure it makes sense
- CryoET can be complicated, but it’s worth it to see **native biology**=)

Thank you!

Questions/ Discussion?