# Current Practices, Better Options

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# Optimization

- Reducing processing time
  - Computation
  - Manual processing
- Reducing (human-based) errors in processing
- Reducing amount of (low-quality) data
- Assuring robustness w.r.t. sample

# Reducing Computation Time

- Good SW development
  - Fast, robust, and sustainable
  - Properly tested
  - Easy to parallelize if possible
  - Open-source

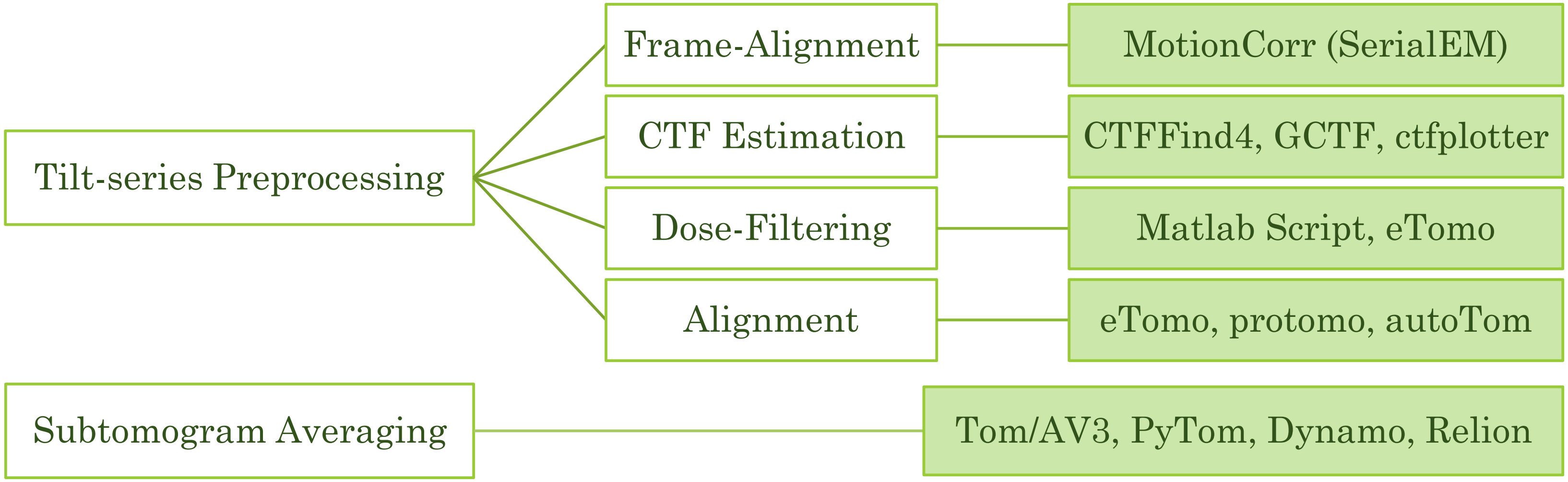
Subtomogram averaging

| Box Size | Matlab | C++ double precision | C++ single precision | GPU |
|---|---|---|---|---|
| 36 | 1m 11s | 0m 41s | 0m 30s | 0m 10s |
| 72 | 8m 46s | 6m 04s | 3m 47s | 0m 46s |
| 144 | 85m 48s | 65m 11s | 38m 47s | 6m 21s |
| 288 | 805m 17s | 558m 55s | 423m 04s | 47m 33s |

- Times are for 1 iteration of 100 subtomograms and 100 rotations
- GPU is version **not** at all optimized

# Reducing Errors in Processing

- Most caused by switching among different SW



Tilt-series Preprocessing

Frame-Alignment

MotionCorr (SerialEM)

CTF Estimation

CTFFind4, GCTF, ctfplotter

Dose-Filtering

Matlab Script, eTomo

Alignment

eTomo, protomo, autoTom

Subtomogram Averaging

Tom/AV3, PyTom, Dynamo, Relion

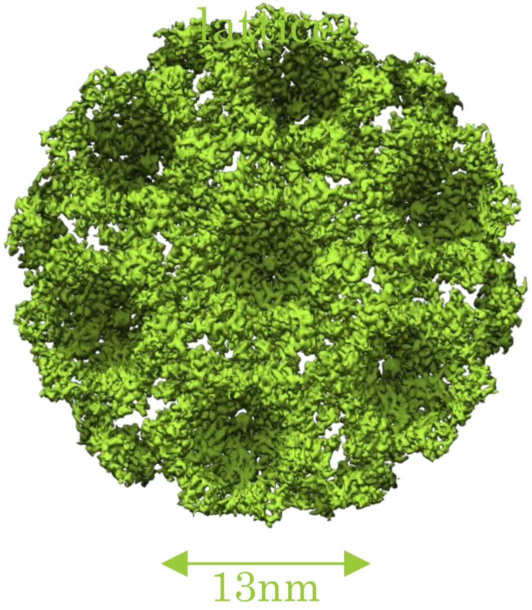
- Automate the transitions as much as possible
- Use SW providing a “complete” pipeline – emClarity, Warp
- **Always check results after each step**

# Reducing Amount of Data

- In tomography less is often more
  - Prefer quality over quantity
  - Less data is easier to process, especially if some manual steps are required
- Starting with positions on a grid map
  - Improve your choice during the acquisition
  - Go back to a grid map after your processed a whole dataset
- Remove low-quality data in each step
  - Bad tilts, hard-to-align tomograms, bad particles etc.
  - Tools/scripts to facilitate analysis of the data

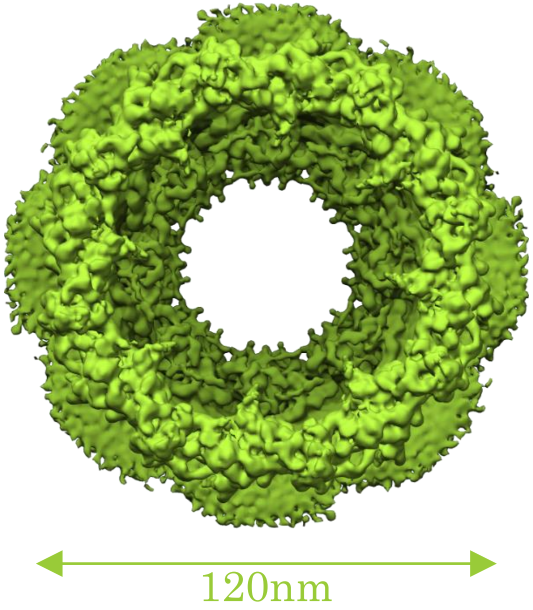
# Robustness w.r.t. Sample

## Immature HIV-1 CA-SP1 lattice



- Pixel size: 1.35Å
- Sample thickness: ~160nm
- Particles per tomogram: ~9 VLPs (~350 subtomograms per VLP)
- Symmetry: 6 fold
- Best reported resolution: 3.1Å

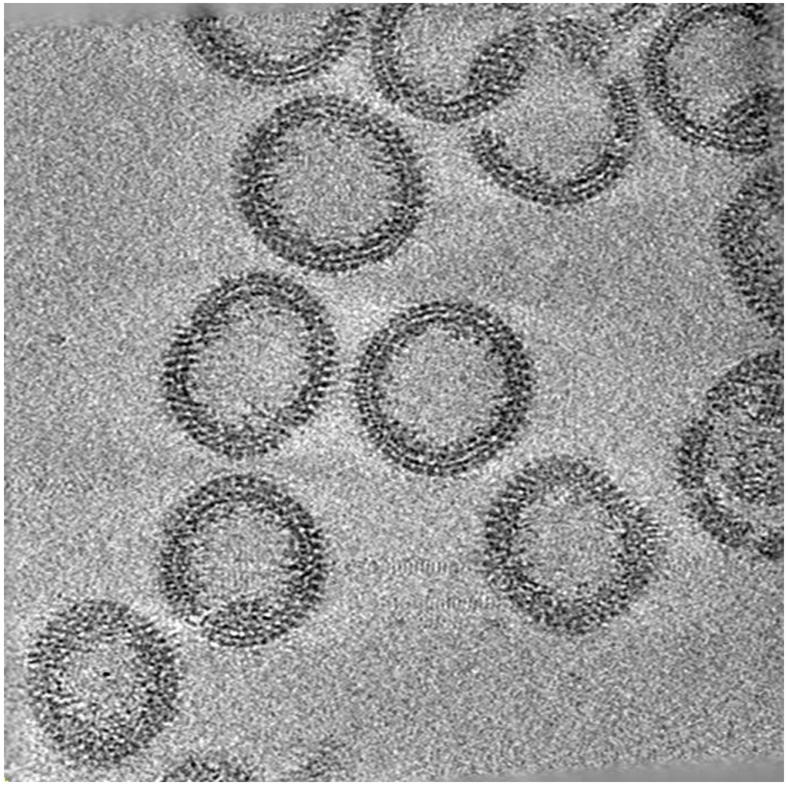
## Human Nuclear Pore Complex (NPC)



- Pixel size: 3.35Å
- Sample thickness: ~450nm
- Particles per tomogram: 0-14 NPCs per tomogram
- Symmetry: 8 fold
- Best reported resolution: ~20Å

# Robustness w.r.t. Sample

Immature HIV-1 CA-SP1 lattice



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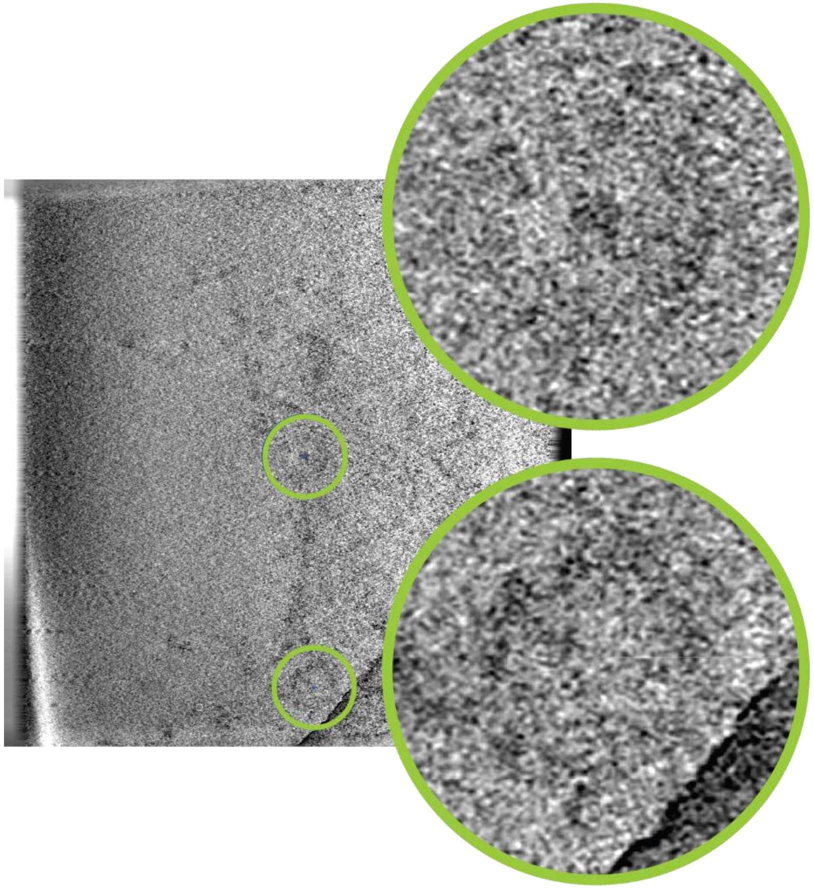

# Robustness w.r.t. Sample

| HIV | | NPC |
|---|---|---|
| ~30min per TS<br>+5 hours setup | Tilt-Series Acquisition<br>⚙ | ~40min per TS<br>+6 hours setup |
| ~1h per TS | Tilt-Series Preprocessing<br>⚙ ↑↑↑ | ~1h per TS |
| ~20-60 min per TS<br>Max 10 TS a day | Tilt-Series Alignment<br>✋ | ~20-60 min per TS<br>Max 10 TS a day |
| ~60min per reconstruction | Tomogram Reconstruction<br>⚙ ↑↑↑ | ~90min per reconstruction |
| ~10min per tomogram | Particle Picking<br>✋ | ~15min per tomogram<br>Max 20 tomogram a day |
| Up to 1 week | Subtomogram Averaging<br>⚙ ↑↑↑ | Weeks |
| 2-3 weeks | 100 tomograms | 8-10 weeks |

# Robustness w.r.t. Sample

## HIV

- Tilt-Series Alignment
  - Few fiducials but can be automatically tracked
  - Problem is the precision
  - Can be overcome with local alignments based on subtomogram positions (emClarity, Warp)

- Particle Picking
  - Manual picking using geometry is fast
  - Template matching works as well (emClarity, Warp?)

## NPC

- Tilt-Series Alignment
  - Poor fiducial distribution
  - Problem is choosing "good" fiducials and track them (low SNR at high tilts)
  - Low SNR prevents (for now) successful use of local alignments approach

- Particle Picking
  - Manual picking is demanding and requires experience
  - Template matching does not work – easier to pick manually than clean the many false positive
  - Maybe deep-learning approaches might help here

# SW with a “Complete” Pipeline

- emClarity
  - Very good results (HIV: 3.1Å)
  - Improvement of tomogram alignment based on subtomograms
  - Interesting classification method
  - Requires Imod and Chimera
  - Some parts still missing
  - Written in Matlab and uses GPU
  - Not flexible / modular
  - Hard-coded settings
  - So far rather user-unfriendly

- Warp for SA
  - Also very good results (HIV: 3.3Å)
  - Improvement of tomogram alignment based on subtomograms
  - Self-contained
  - Written in C#
  - GUI
  - Not explored: parameters, modularity

# Summary

- Currently the processing is not robust w.r.t. samples
  - For difficult samples the automatization remains challenging
- Some samples can be processed efficiently and with little manual intervention
  - Optimize your sample as much as possible
  - Take your time during acquisition
    - Careful and experienced setup of positions
    - Prefer data quality over acquisition speed
  - Do not lose control over your data
- Share your data, parameters, and experience to help improve current SW and benchmark different approaches