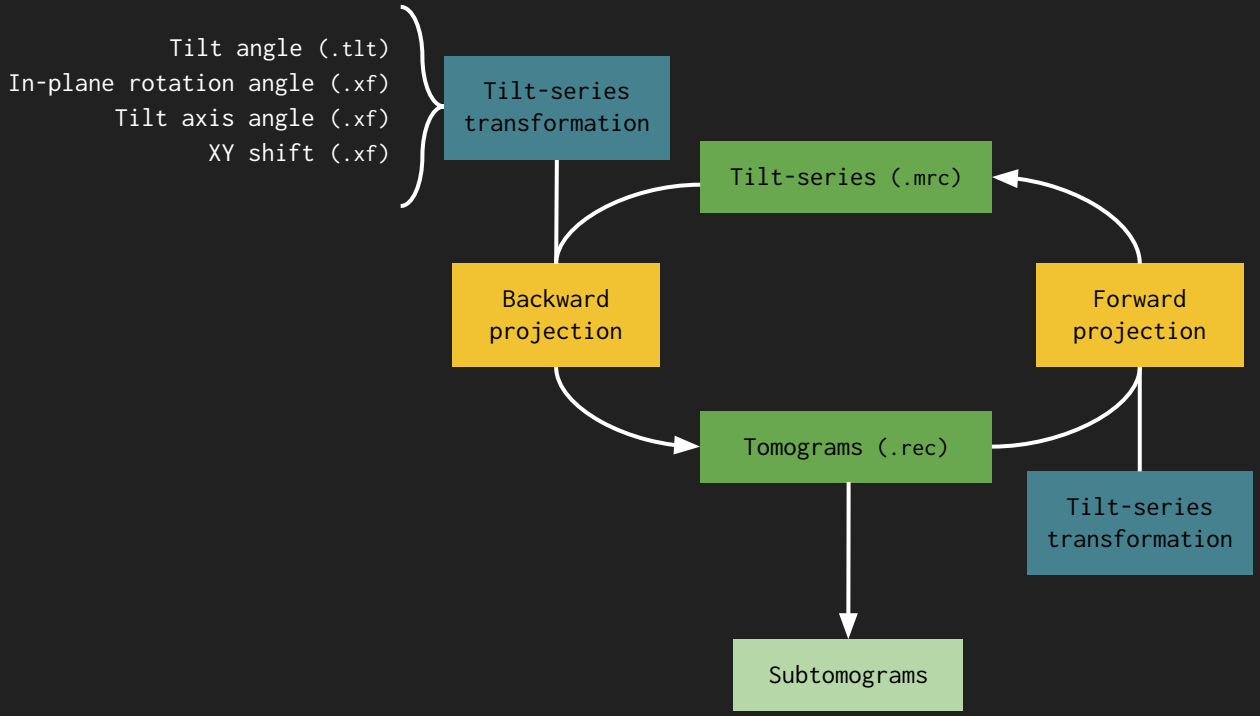
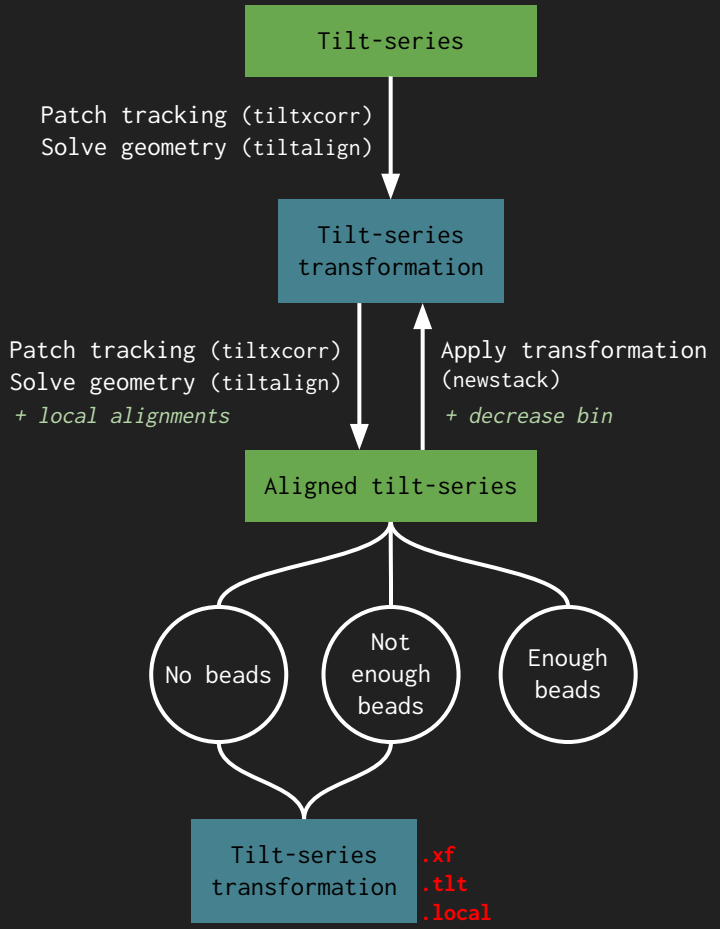
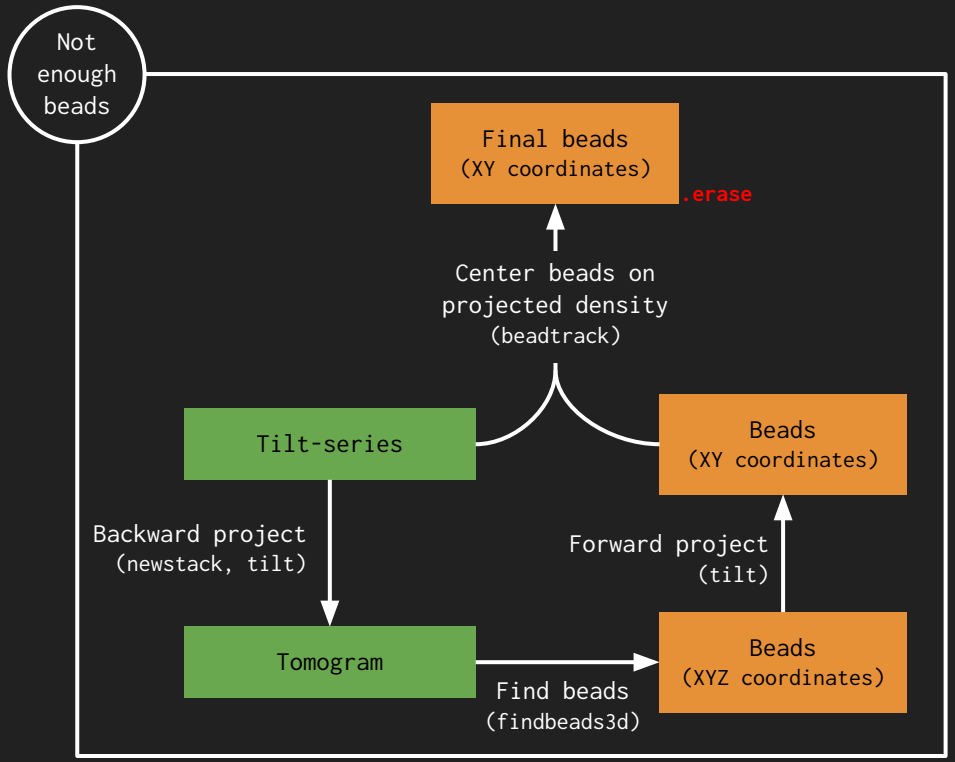
emClarity practicals
eBIC-STRUBI
24 Nov 2021

Tilt-series alignment

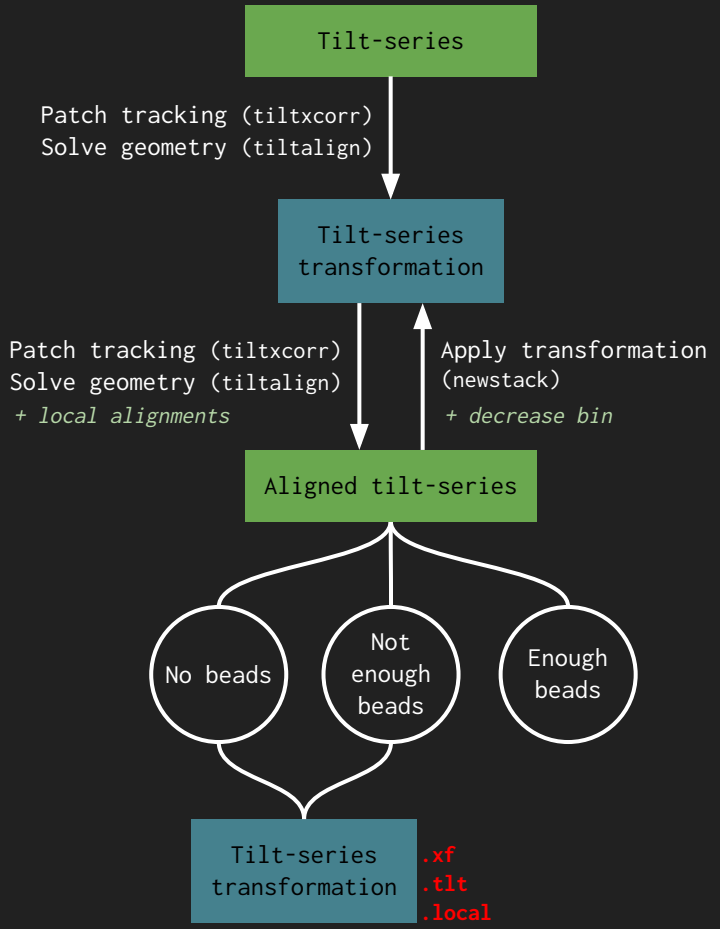


autoAlign


Tilt-series
Patch tracking (tiltxcorr)
Solve geometry (tiltalign)
Tilt-series transformation
Patch tracking (tiltxcorr)
Solve geometry (tiltalign)
+ local alignments
Apply transformation (newstack)
+ decrease bin
Aligned tilt-series
No beads
Not enough beads
Enough beads
Tilt-series transformation
.xf
.tlt
.local


Not enough beads
Final beads (XY coordinates)
.erase
Center beads on projected density (beadtrack)
Tilt-series
Beads (XY coordinates)
Backward project (newstack, tilt)
Forward project (tilt)
Tomogram
Beads (XYZ coordinates)
Find beads (findbeads3d)

autoAlign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.xf
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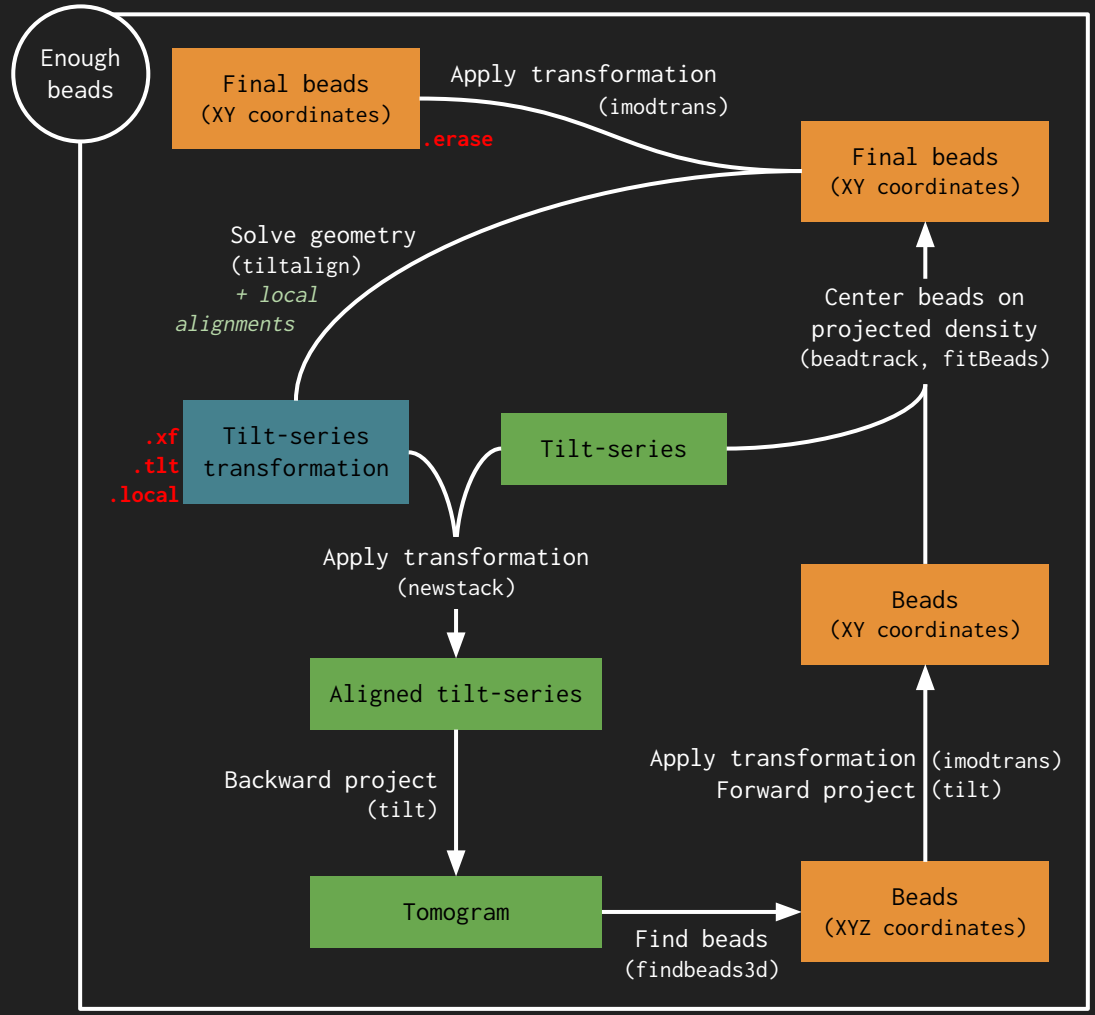
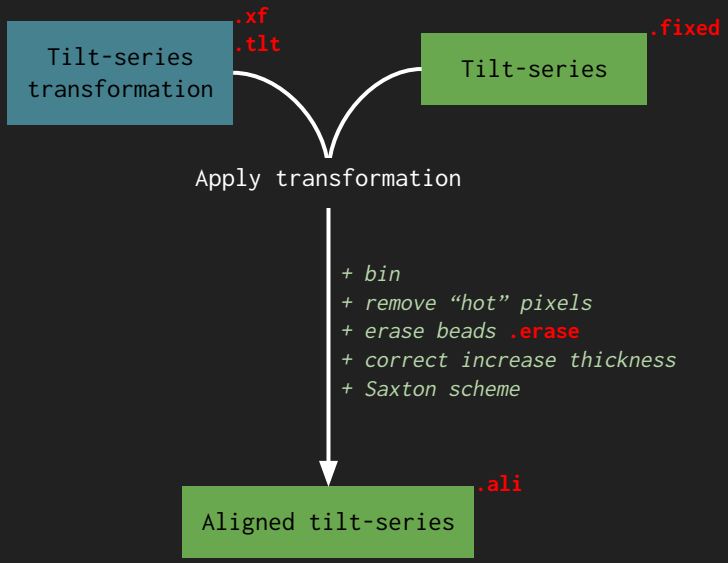

Enough beads
Final beads (XY coordinates)
Apply transformation (imodtrans)
.erase
Final beads (XY coordinates)
Solve geometry (tiltalign)
+ local alignments
Center beads on projected density (beadtrack, fitBeads)
.xf
.tlt
.local
Tilt-series transformation
Tilt-series
Apply transformation (newstack)
Beads (XY coordinates)
Aligned tilt-series
Backward project (tilt)
Apply transformation (imodtrans)
Forward project (tilt)
Tomogram
Find beads (findbeads3d)
Beads (XYZ coordinates)

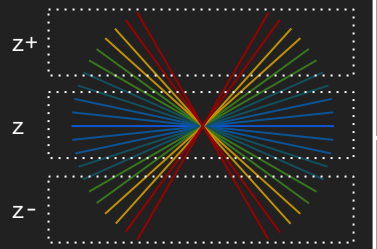
ctf estimate



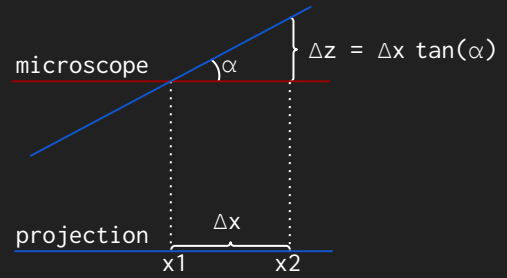
ctf estimate

Aligned tilt-series .ali

- Get average defocus
  - Compute 1D average PS at z
  - Solve defocus _psRadial_1.pdf



- Gradient check
  - Compute 1D average PS at z- _psRadial_1.pdf
  - Compute 1D average PS at z+ _psRadial_3.pdf
  - Check defocus: z- < z < z+



- Get astigmatic defocus for each view
  - Divide image in tiles
  - Get Δz and deduce stretching factor
  - Stretch/shrink the PS of the tiles
  - Add tiles together _ali1-PS.mrc
  - CTFFIND _ali1_ctf.tlt

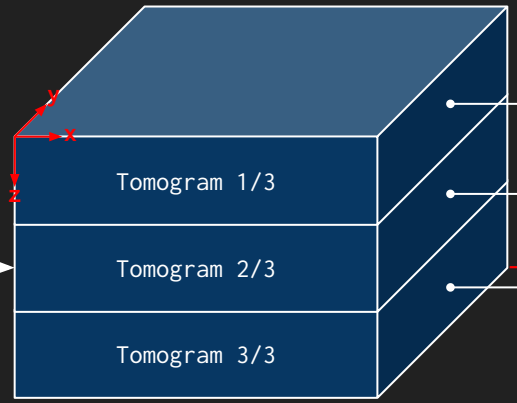
ctf 3d

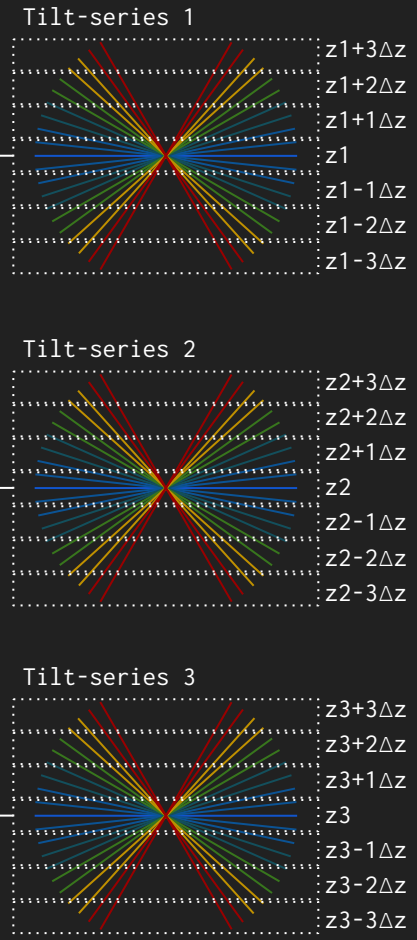
Aligned tilt-series .ali

Resample

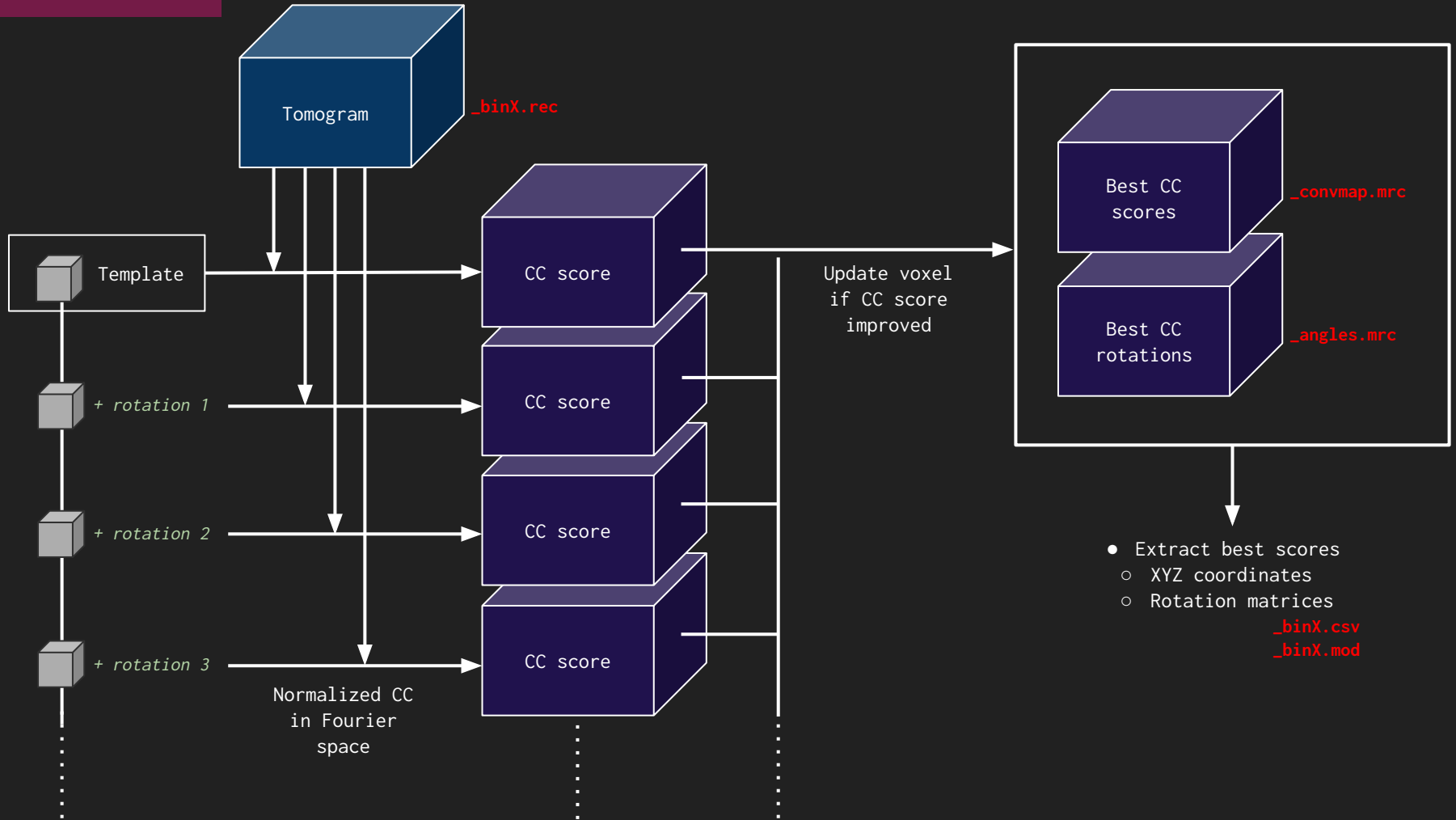
Aligned tilt-series _binX.fixed

- Compute nb sections
  - Thickness of subregion
  - Sampling rate
  - Target resolution
- Prepare tilt-series:
  - Apply exposure filter
  - CTF multiply strips
- Weighted-backproject each section (tilt, trimvol) .tlt, .local
- Stack the sections into one tomogram (newstack)

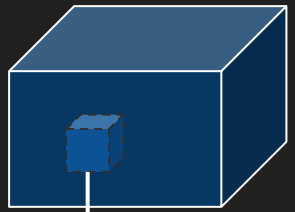




templateSearch

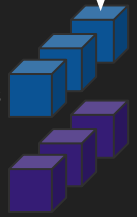


avg



_binX.rec

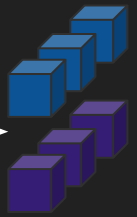
Extraction
+ masking



Subtomograms

Sampling functions

+ symmetry



Microscope reference frame

Particle transformations

Reconstruction reference frame

Sum all particles

+ CC weighting

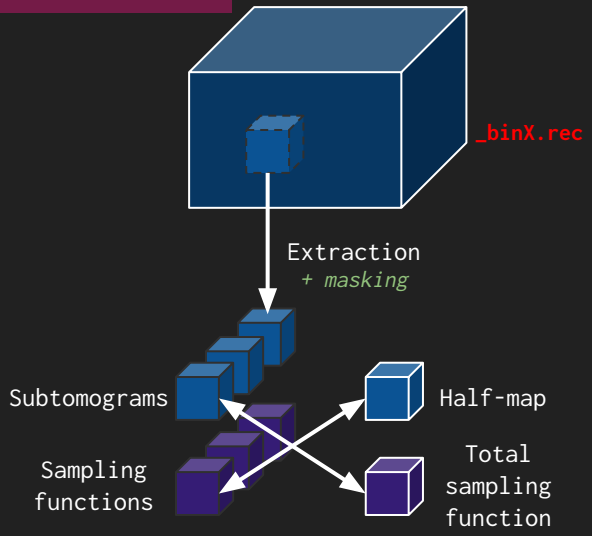
_NoWgt.mrc

_Wgt.mrc

.mrc

- Volume-normalized SPA Wiener filter: correct for the CTF amplitudes and find the optimal denoising filter
  - Correct for the amplitudes using the total sampling function
  - Appropriately mask the CTF corrected half-maps
  - Estimate SSNR: Compute the FSC and frequency cutoffs
  - Add contribution of b-factor and MTF

alignRaw



- Calculate translation between P and R:
  - Transform R and its sampling function into the microscope reference frame
  - Mask with `particleRadius`
  - Compute the CCC map
  - Mask with `Peak_mRadius` further restricting shift
  - Extract the XYZ coordinates of the peak's COM

- Calculate the CCC between P and R:
  - Transform P and its sampling function into the reconstruction reference frame
  - Mask with `Ali_mRadius`
  - Compute the CCC score

Current particle transformation <=> Get initial CCC score → Find best particle transformation → Set best rotation → Refine best particle transformation → Set best rotation → Calculate final translation <=> Updated particle transformation

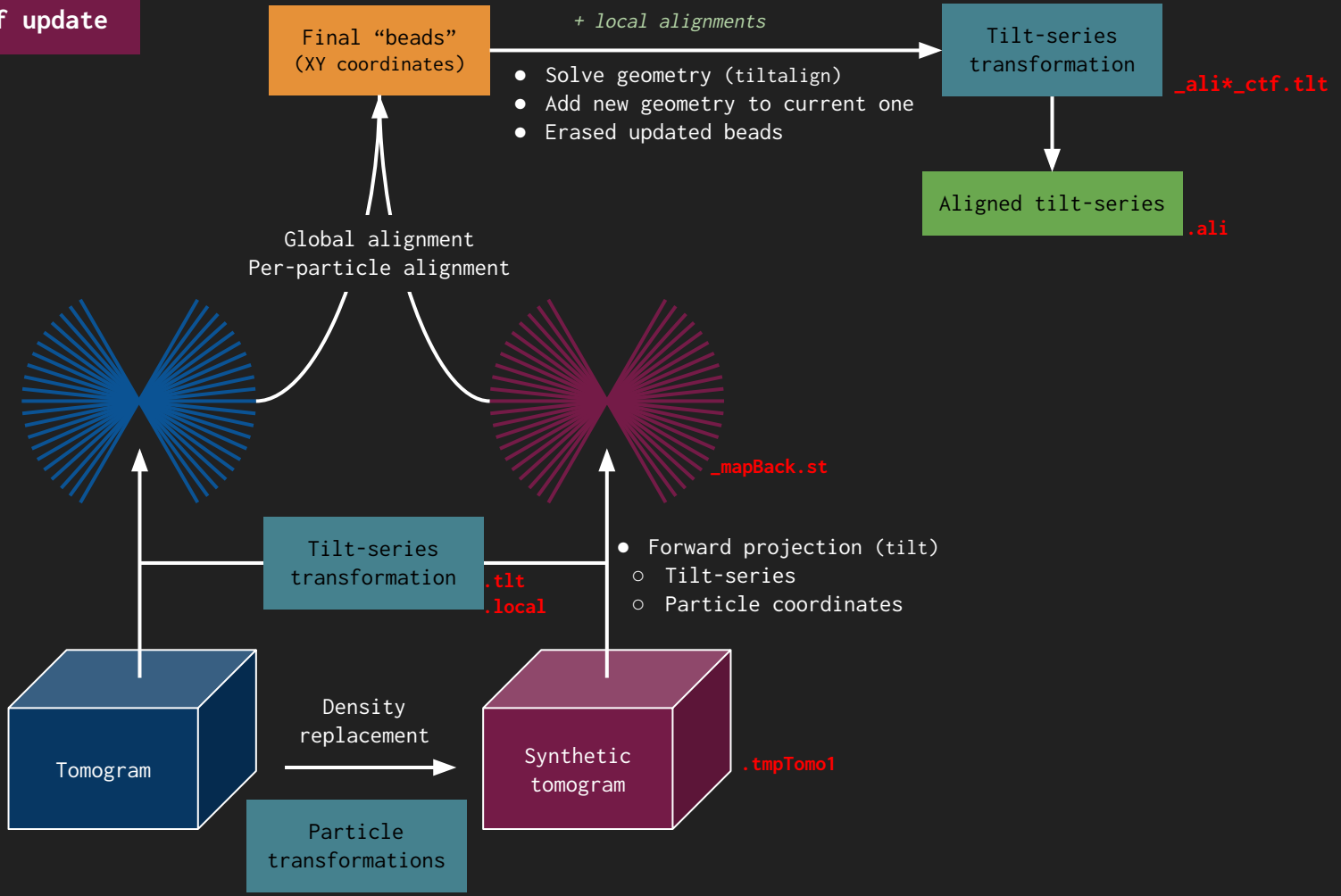
- 3D rotation
- XYZ shift

- Calculate translation
- Calculate CCC score

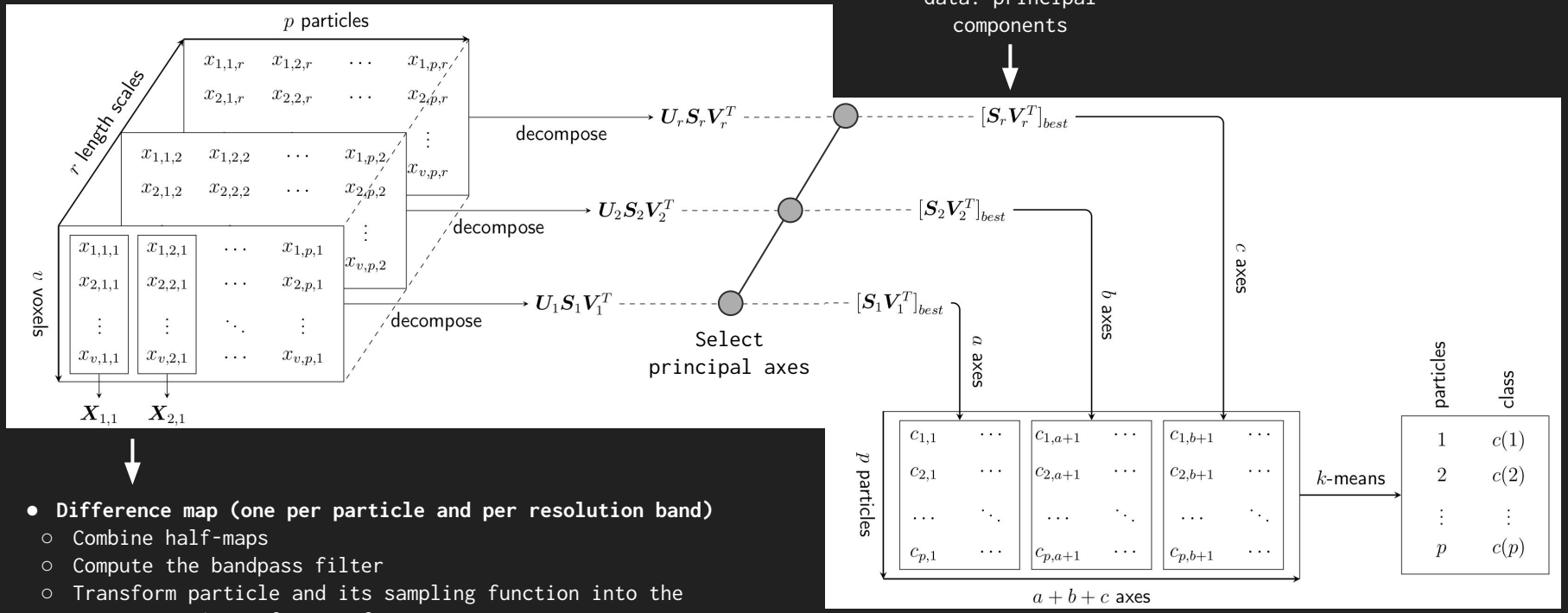
- Calculate translation
- Calculate CCC score

- 3D rotation
- XYZ shift
- CCC score

tomoCPR, ctf update



pca, cluster



- **Difference map (one per particle and per resolution band)**
  - Combine half-maps
  - Compute the bandpass filter
  - Transform particle and its sampling function into the reconstruction reference frame
  - Multiply reference with particle's sampling function, subtract particle and apply bandpass
  - Extract the voxels within the molecular mask