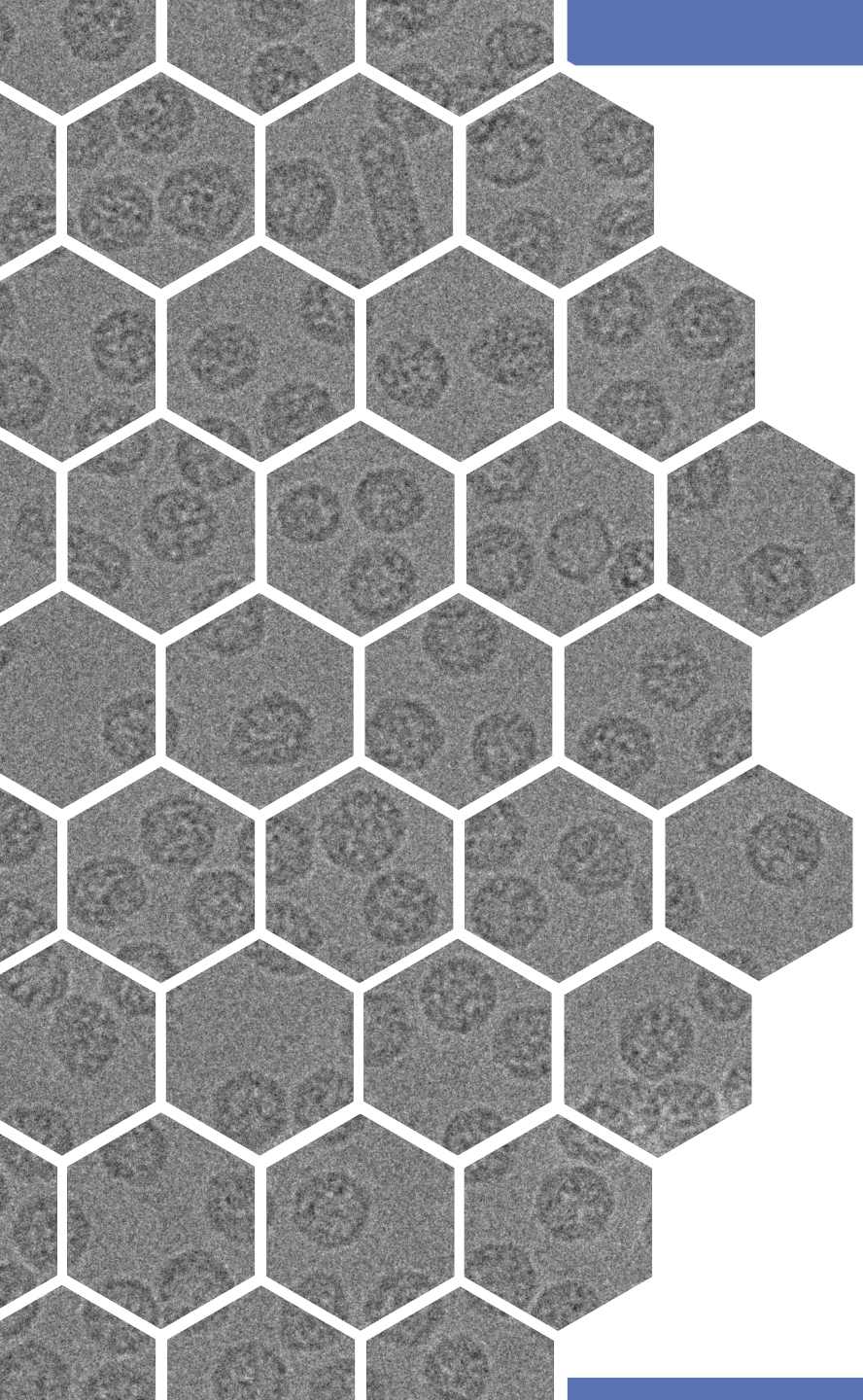


CryoET data acquisition

Misha Kopylov

NYSBC
Tomography shortcourse
2025

Tomography state of the field

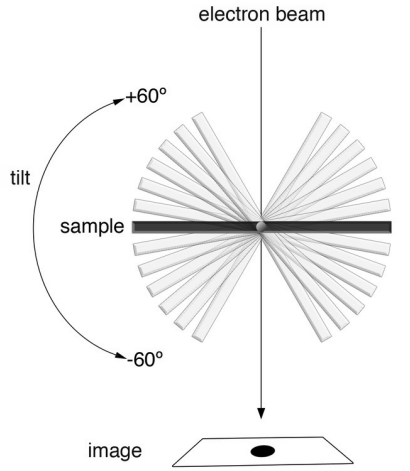
1. Tomography sample preparation **is finally figured out** and **everyone agrees on the best practices**

2. Tomography data acquisition settings and platforms **are fully unified** and **“standard” settings can be applied for 100% of the sessions**

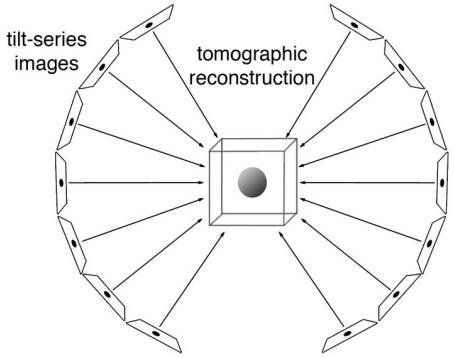
3. Tomography data processing starting from image formats, coordinate system and ending with sub-volume refinement software **are all coordinated** and applied universally **making data processing a breeze**

4. Happy April Fools Day!

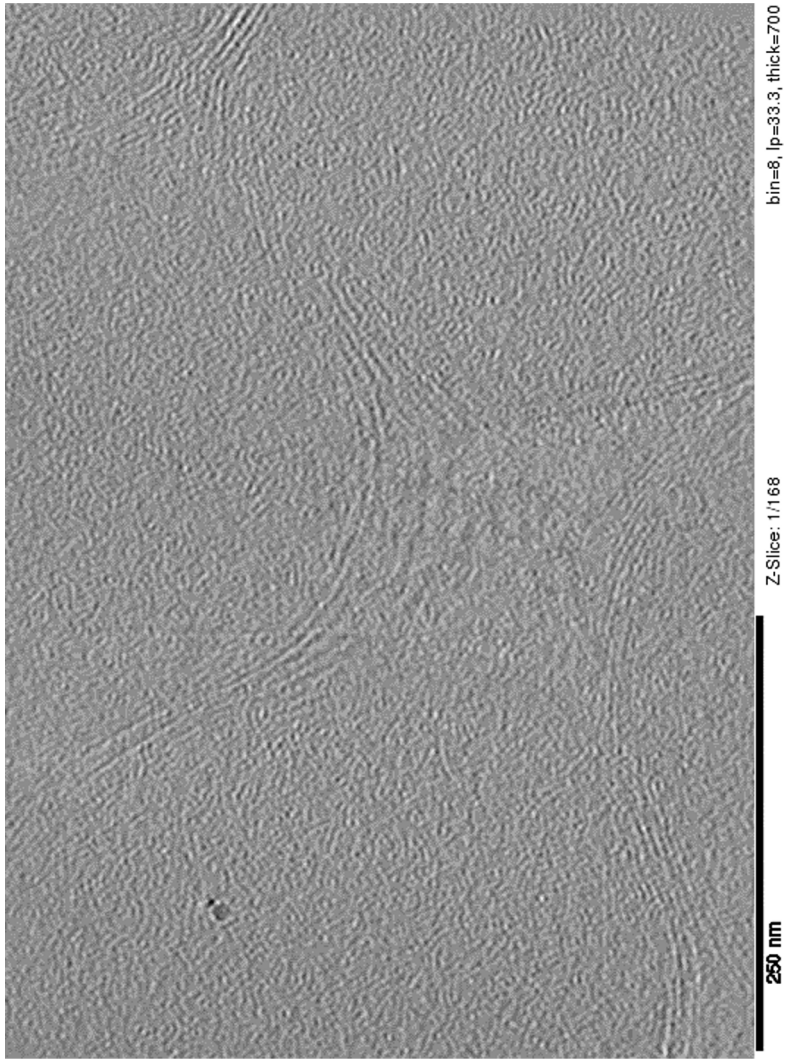
Tomography: principle



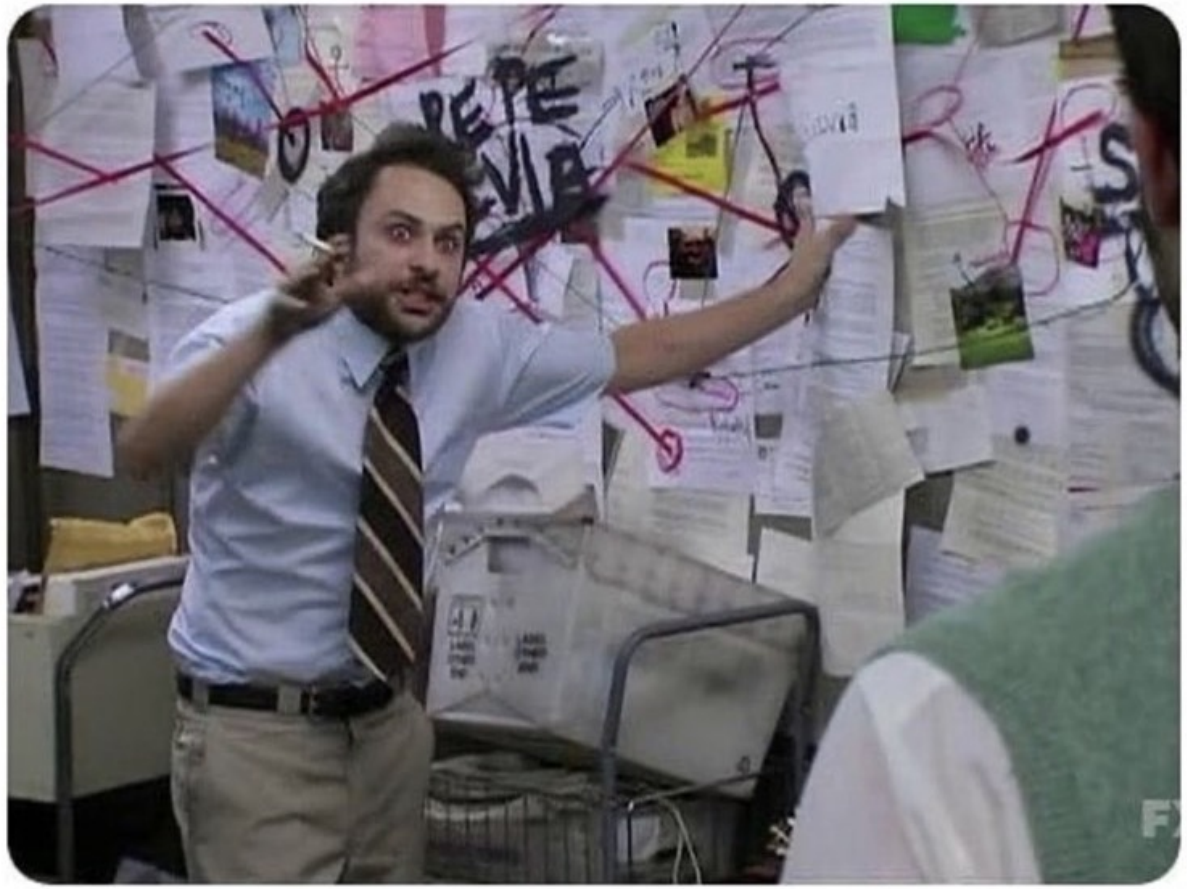
Acquire tilt series



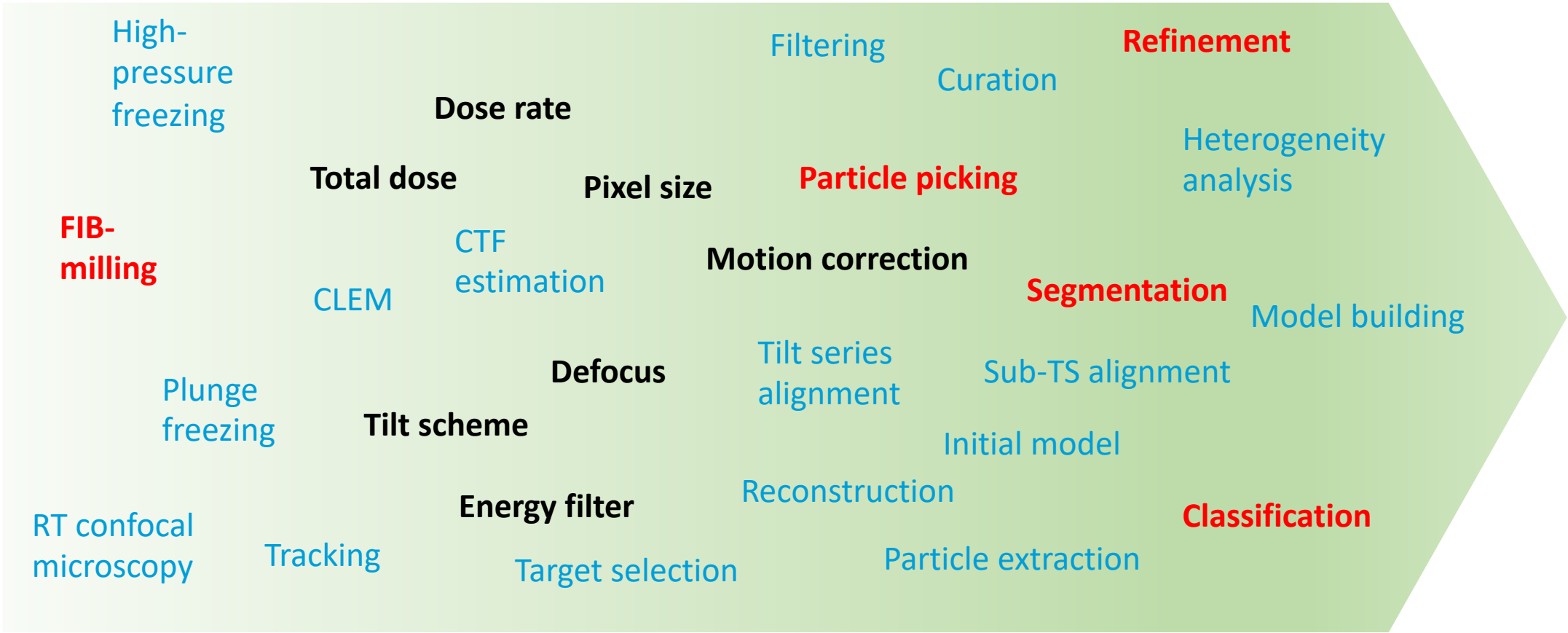
Align frames, align tilt series, reconstruct



Tomography pipeline



Tomography pipeline



Acquisition parameters

Hardware setup:
energy filter, magnification, dose, beam size, defocus

Session setup:
tilt scheme, navigation, tilt range, targeting

Choice of hardware

TFS Krios G4
300 kV

TFS Krios G3i
300 kV

JEOL Cryoarm
300 kV

TFS Glacios
200 kV

JEOL Cryoarm
200 kV

At 300 kV electron's mean free path is 320 nm

At 200 kV MFP = 260 nm

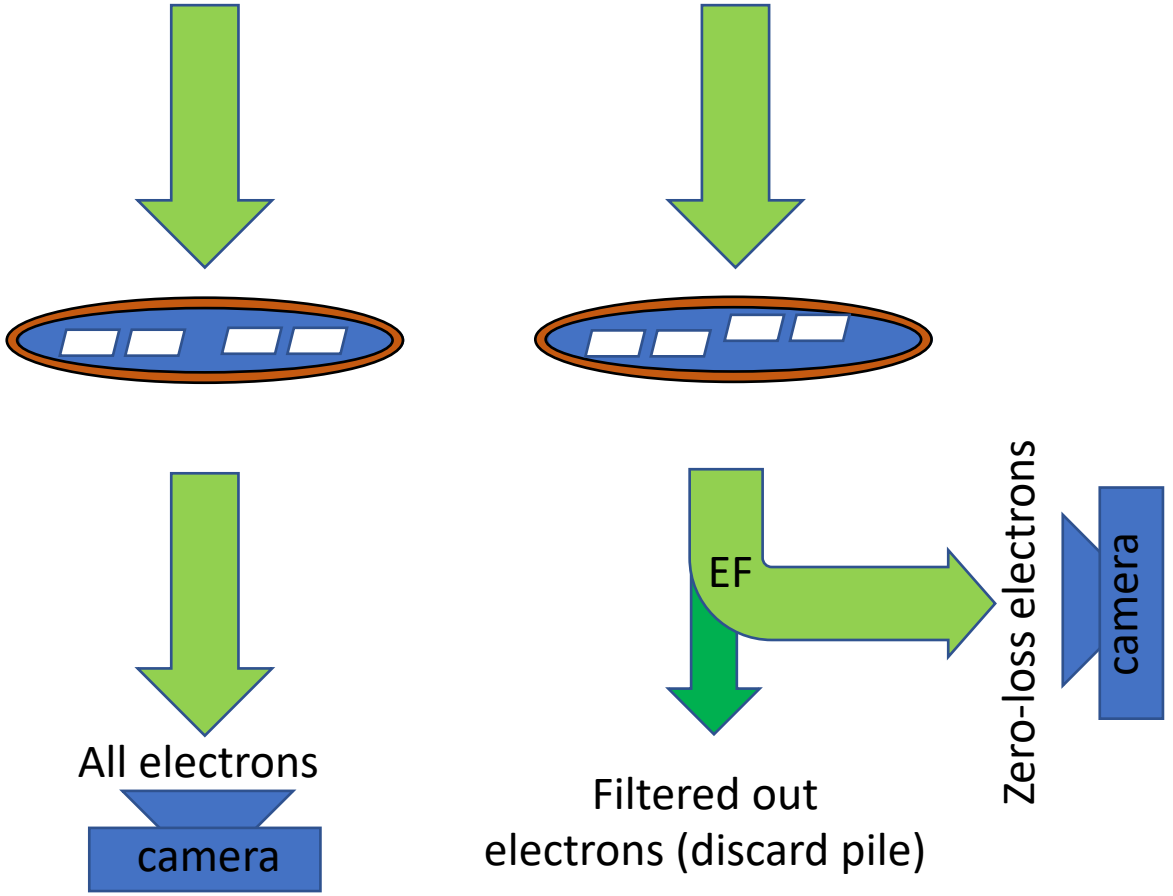
Hardware setup: energy filter

TFS Krios G4
300 kV

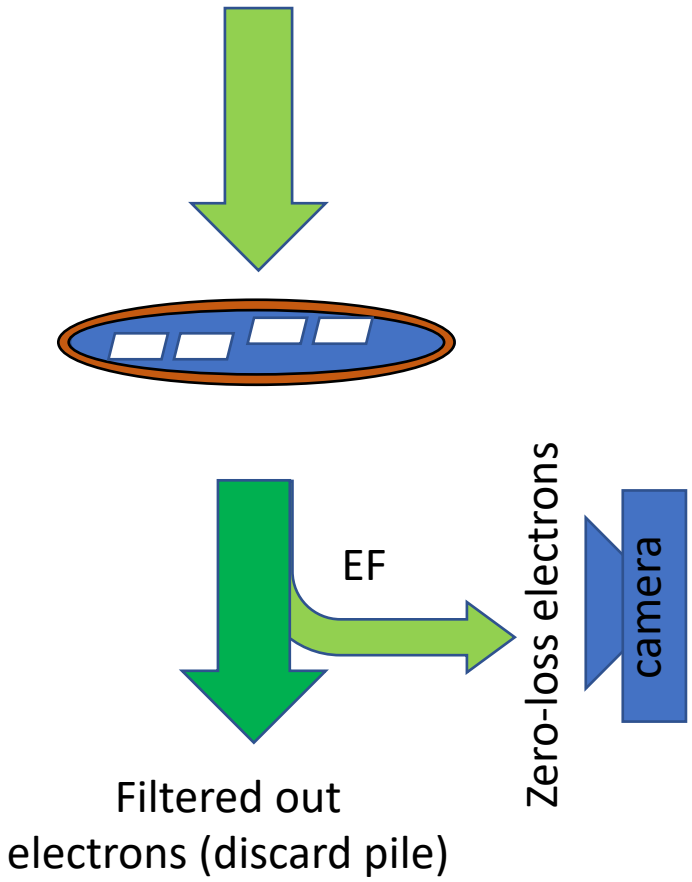
Falcon 4i
Selectris energy filter

TFS Krios G3i
300 kV

Gatan K3
Bioquantum energy filter



Hardware setup: energy filter



Precision of filtering out inelastically scattered electrons is set by the energy filter slit width

The narrower the EF filtering range, the better the contrast

In thicker ice and/or at higher tilts the effective dose rate on the camera can drop to 10% due to inelastic scattering. This WILL cause gain artifacts

Hardware setup: pixel size

K3 sensor is 5760 x 4092 H pixels

Pixel size directly influences:
- Highest theoretical resolution
- Field of view

Pixel size indirectly influences:
- Tracking accuracy
- Beam settings

1.3 Å/px	2.2 Å/px	3.6 Å/px
FOV 0.8x0.5 µm 0.4 µm^2	FOV 1.3x0.9 µm 1.1 µm^2	FOV 2.1x1.5 µm 3.1 µm^2

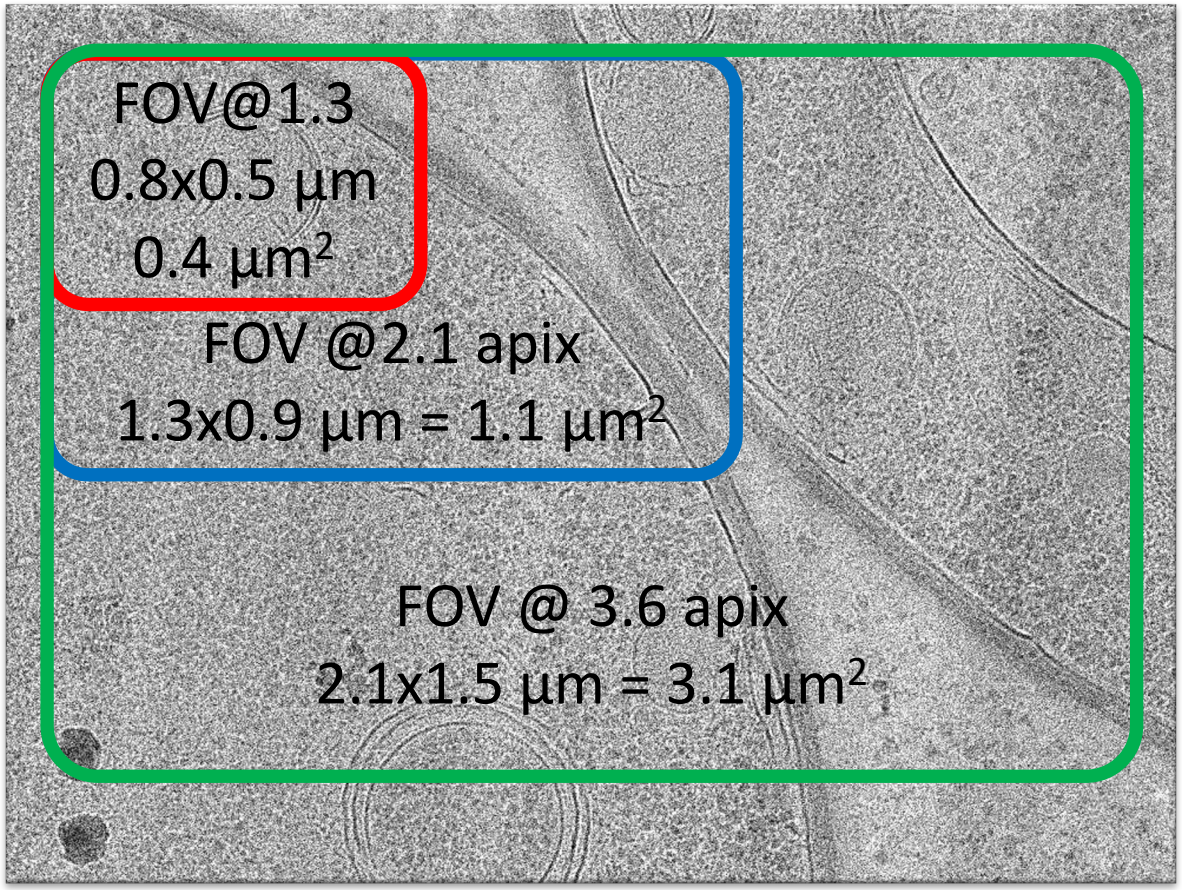
Hardware setup: pixel size

Pixel size directly influences:

- Highest theoretical resolution
- Field of view

Pixel size indirectly influences:

- Tracking accuracy
- Beam settings



Hardware setup: electron dose

Dose per second per pixel on the detector level
For K3 16-32 $e^-/Å^2/s$
This will change with ice thickness change

Dose rate (flux)
$e^-/Å^2/s$

Dose per series
$e^-/Å^2$

Total accumulated dose per specimen area for the tilt series – depends on desired resolution and sample tolerance.
Usually, **80-150 $e^-/Å^2$**

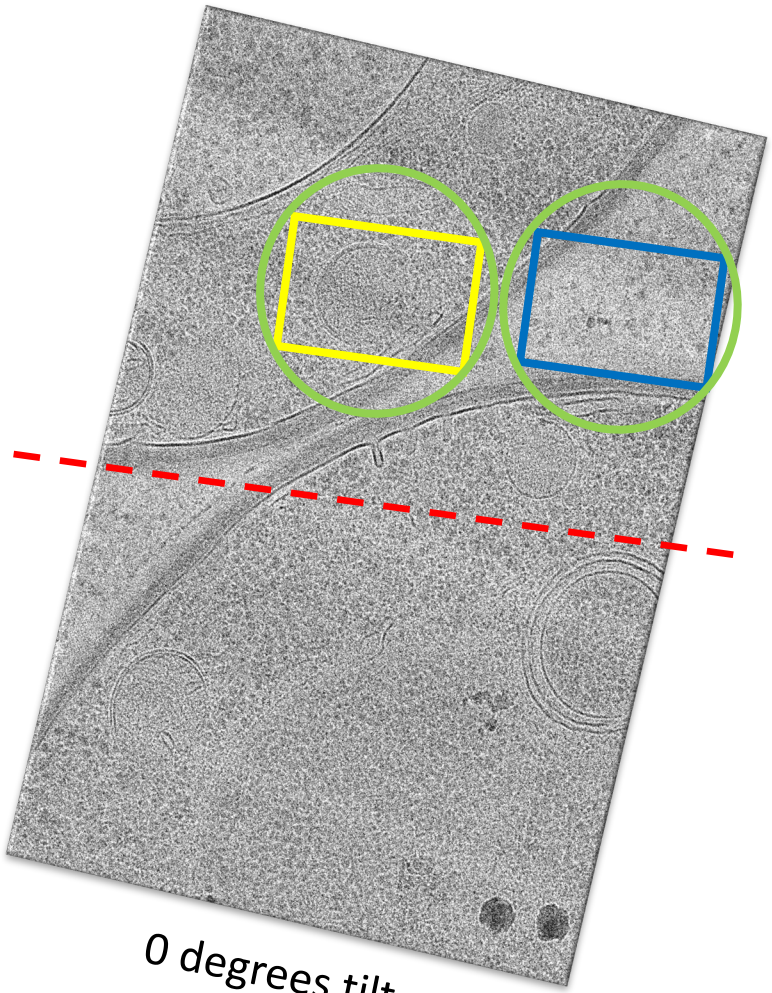
Single-tilt dose per specimen area – depends on total dose per series and acquisition strategy
Usually 2-5 $e^-/Å^2$

Dose per tilt image
$e^-/Å^2$

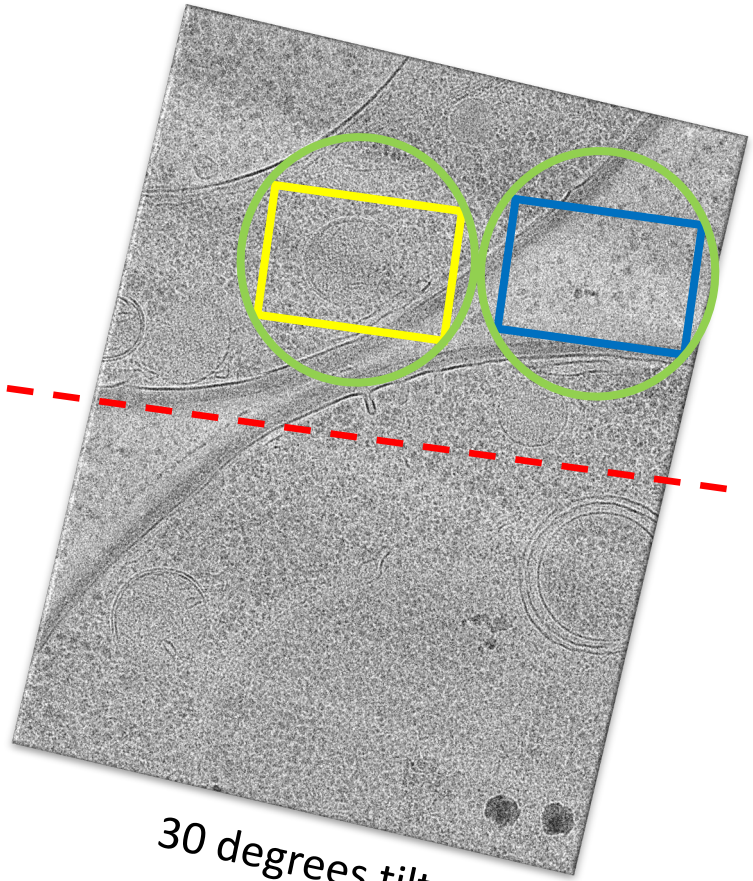
Dose per frame
$e^-/Å^2$

Dose for a single frame for motion correction – depends on the camera speed and exposure time.
Usually **0.2-0.5 $e^-/Å^2$**

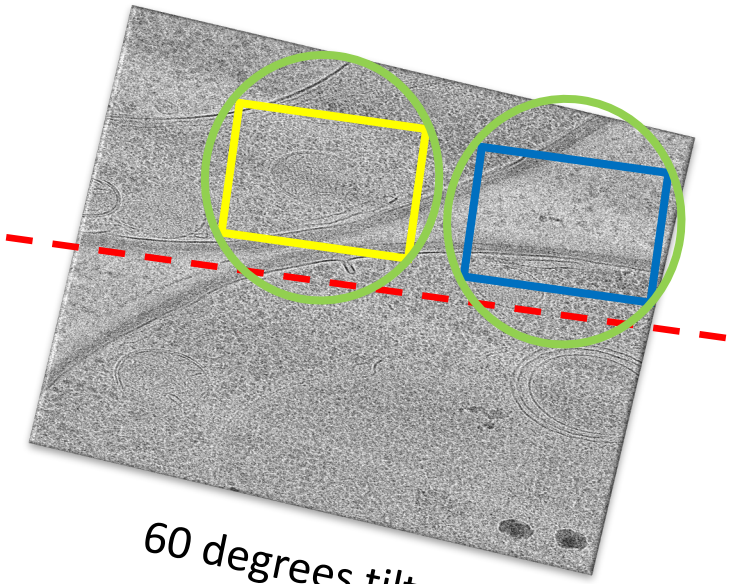
Hardware setup: beam size



0 degrees tilt



30 degrees tilt

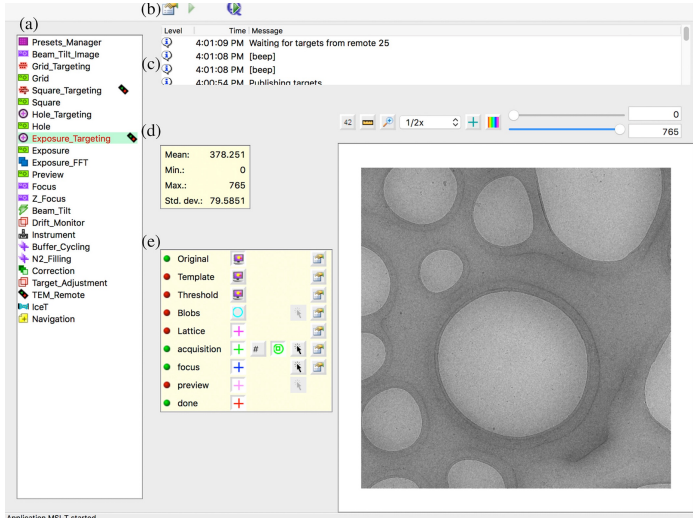


60 degrees tilt

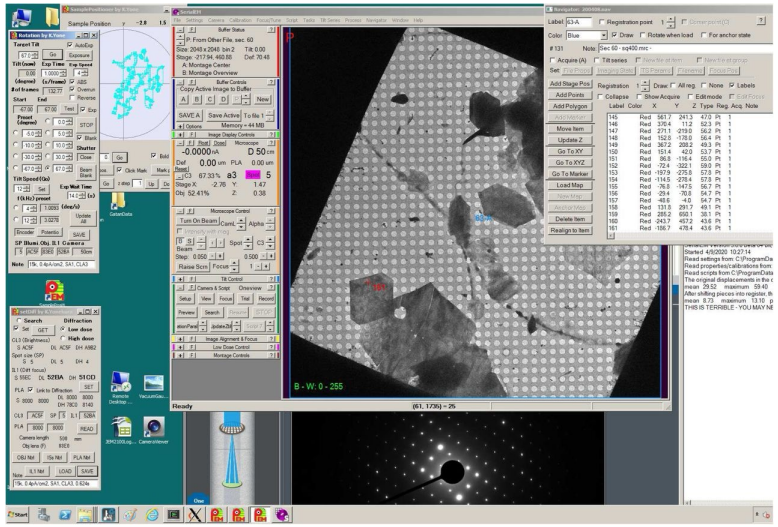
Software setup: choices!

TFS Tomo5

Leginon



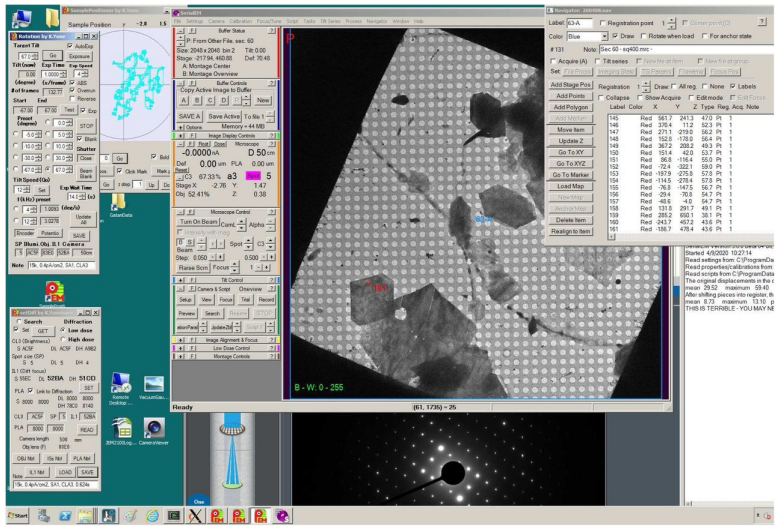
SerialEM



Software setup: dilemma

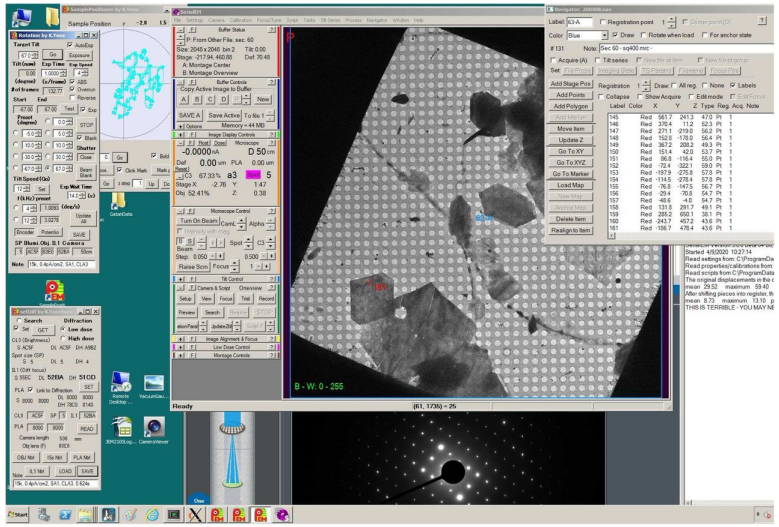
TFS Tomo5

SerialEM



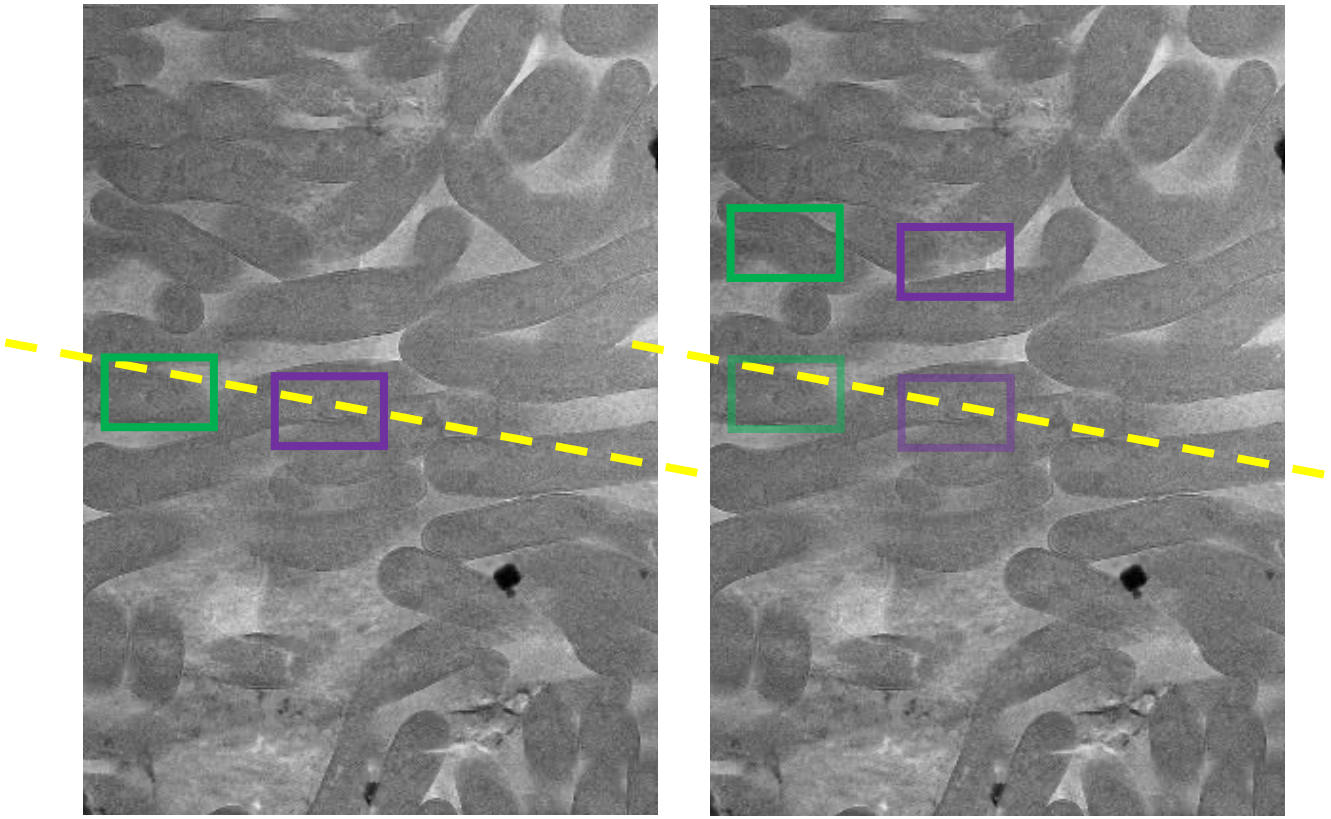
Software setup: choice

SerialEM + (S)PACE



Software setup: navigation

Stage shift: “Classic”



Stage shift to every target, at least ½ of the area is wasted

Image shift: (S)PACE

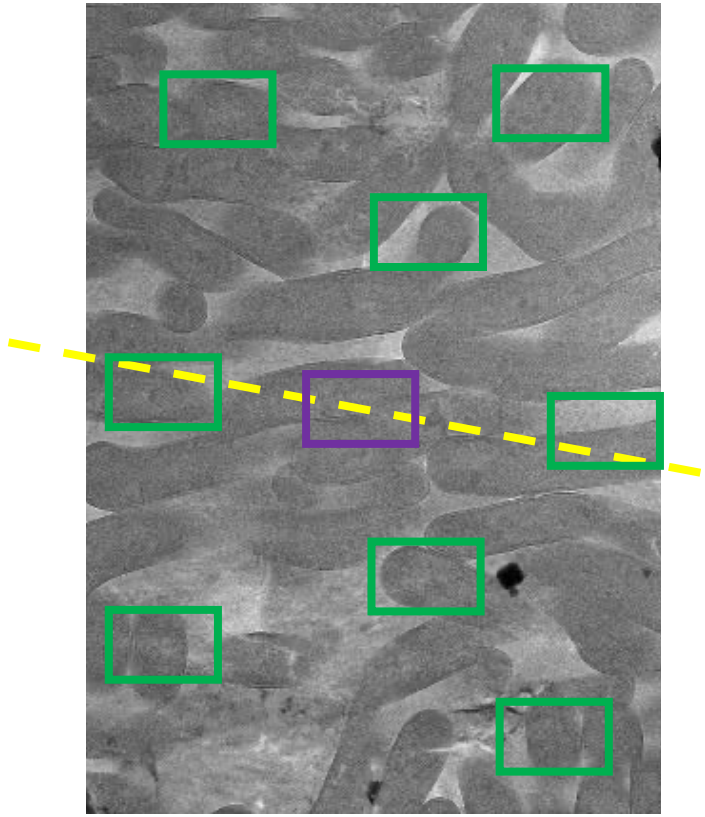


Image shift in all directions

Software setup: navigation

Stage shift
”Classic”

Easy to setup, fast targeting, slow acquisition:
~15 min/TS, half of the usable area is destroyed due to tracking

Record tracking image, adjust image shift, record acquisition target
Next tilt angle

Record tracking image, adjust image shift, record acquisition target
…

Move to the **next target**

Image shift
(S)PACE

Takes time to get things setup properly, slow but very precise targeting, blazing fast acquisition (depending on the number of targets)

Record tracking image, adjust image shifts for targets in a group, image shift and acquire all targets in a group
Next tilt angle

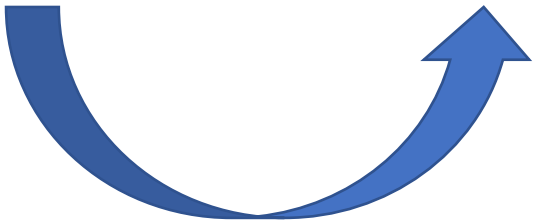
Record tracking image, adjust image shifts for targets in a group, Image shift and acquire all targets in a group
…

Move to the next **group of targets**

Software setup: tilt scheme

Swing

-45 -42 -39 -36 -33 … 0 … 33 36 39 42 45



Probably best possible tracking, fast.
May be useful for lamellae milled
at very sharp angles

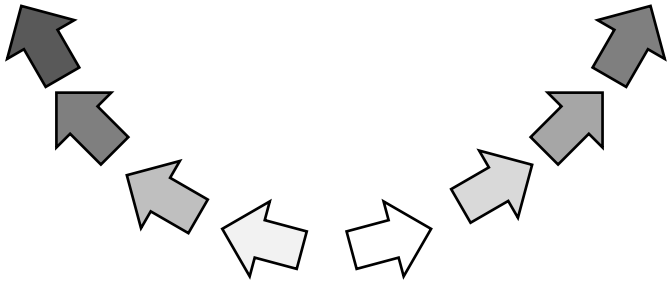
Bi-directional

0 3 6 9 … 42 45 0 -3 -6 -9 … -42 -45

Good tracking and speed.
Requires “anchor” to return to
the same zero position
Can be started at an arbitrary angle

Dose-symmetric

0 3 6 -3 -6 9 12 -9 -12 … 42 45 -42 -45



Best resolution: lower tilt electrons get
the best sample
Much slower especially at higher angles
and tracking can be an issue

Software setup: tilt range

Patterned grids

-60° to 60°

Start at zero stage tilt
(for flat grids it's also zero specimen tilt)

3 degrees increment

Lamella

-40° to 60°

Start at a sample pretilt: 20 degrees
(for lamella it's the milling angle)

3 degrees increment

Typical setup for data collection on a lamella

- 300 kV scope
- Fringe free illumination
- Energy slit 15 eV
- 24 $e^-/Å^2/s$ dose rate
- 100 µm objective aperture

- SerialEM 4.2 + (S)PACE
- Dose-symmetric tilt scheme
- 3 degrees tilt increment

- 100 $e^-/Å^2$ TS total dose
- 3 $e^-/Å^2$ per tilt
- 10 frames per tilt
- 2 Å/px
- **2-4 µm defocus range**

?