NeCEN

The need for speed at a big facility

Workshop on Challenges for High Speed Cryo Electron Tomography
NYSBC, 28 Nov 2018

Christoph Diebolder, PhD
NeCEN, Leiden University

Christoph Diebolder

- Sr. EM Scientist at NeCEN
- Tasks: Operation and maintenance of cryo lab, cryo TEMs, user training

NeCEN

- **Ne**therlands **C**enter for **E**lectron **N**anoscopy, Leiden University
- Open access cryoEM facility serving the dutch and international cryoEM community
- Main Service: SP/ TOMO data acquisition on Titan Krios

The need for speed at a big facility

[How preliminary real-time automoatic, fiducialless reconstructions, within a couple of minutes of ending a tilt series would save lots of beam time]

...

?

[We encourage presenters to address the questions posed below. Presentations should not be limited to the presenter's own work. We do **not** expect presenters to have all the **answers** and we encourage **plenty of questions and discussion** both during and after the talks]

The need for speed at a big facility

[How preliminary real-time automoatic, fiducialless reconstructions, within a couple of minutes of ending a tilt series would save lots of beam time]

- Clearly, saving beam time would save lots of money (several k$/day on a Titan Krios) and shorten the waiting queue (up to months for dedicated machine)
- Inefficient use of beam time isn’t just an issue at big(ger) facilities but also as small facilities and reseach groups

Introduction NeCEN

Krios 1

Optimized instrument for tomography. Key features:

- S-FEG 300 keV
- C-Twin pole piece
- STEM package
- FEI Volta phase plate
- Gatan energy filter with K2 Summit DED
- Gatan Orius CCD camera
- EFTEM package
- Falcon II DED
- EPU, Tomo4, Serial EM, UCSF Tomo
- Automated dual-axis batch tomography

Krios 2

Optimized instrument for single particle acquisition. Key features:

- X-FEG high-brightness gun 300 keV
- C-Twin pole piece
- Cs corrector
- STEM package
- Gatan Orius CCD camera
- Falcon 3 Electron Counting DED
- EPU automated data collection for SPA
- Automated dual-axis batch tomography

The need for speed at any facility

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The need for speed at any facility ...doing cryoET

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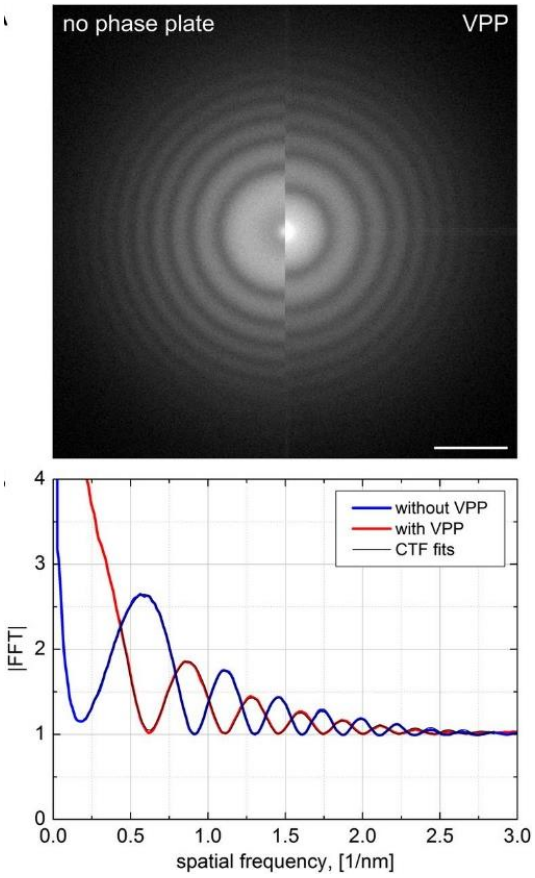
- Rapidly increasing demand in cryoET due to recent developments in SP-tomography and in-situ structural biology

Some developments impacting CryoET

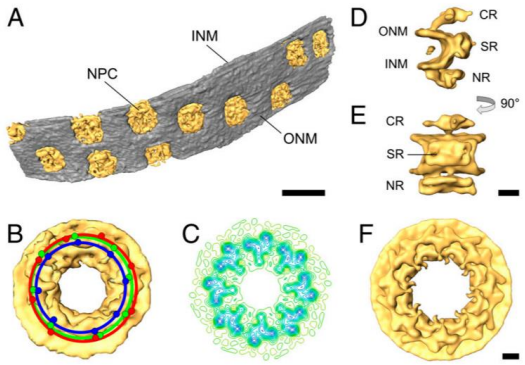
Phase plates



Danev et al.,
PNAS (2014)

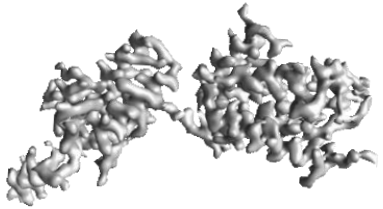
Lamella FIB milling

ThermoFisher
SCIENTIFIC

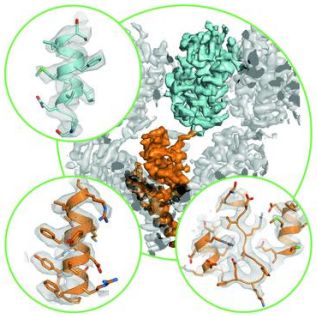


Rigort et al.,
PNAS (2012)

High resolution sub tomogram averaging



Hymes & Zhang,
Nat Methods (2018)



Turonova et al.,
JSB (2018)

[How *preliminary real-time automoatic, fiducialless reconstructions, within a couple of minutes of ending a tilt* series would save lots of beam time]

- Is “automatic” reconstruction needed?

  Yes, definately! (e.g. Using IMODs batchruntomo, Mastronarde & Held, J Struct Biol, 2017)

- Why “fiducialless”?

  Not Necessarily (e.g. APPION-Protomo, Noble & Stagg, JSB 2015)

- Why “preliminarily”, in “a couple of minutes” and “real time”?

Pro fiducialless

-sometimes ficudials can’t be used and/or sample prep is complicated (FIB lamellae..)

-fiducials might move independently from sample

-fiducials might introduce artefacts

-possibly more robust for automation (?)

(see e.g. APPION-Protomo, Noble & Stagg, J Struct Biol 2015)

Con fiducialless

-fiducial can have additional functions (e.g. ProteinA-gold for immuno gold labelling, quantum dots for CLEM etc.)

-fiducials often give better alignments at cryo conditions

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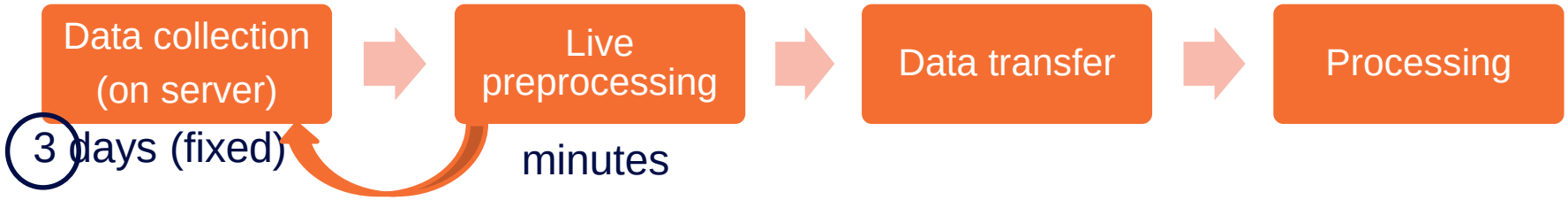
- Why “preliminarily”, in “a couple of minutes” and “real time”?

Single particle 4 years ago

Single particle 2 years ago



Single particle now

How to improve tilt series acquisition based on live preprocessing?

- Which parameters to monitor?
- How “preliminary” should the reconstruction be?
- How to estimate the quality of a tilt series / tomogram??

Can tomogram reconstruction (and sub tomogram averaging) keep up with the speed of data collection (e.g. one tilt series per minute)?

Acknowledgements

NeCEN Team

- Dr. Ludovic Renault – *Head of Facility*
- Ms. Susanne Roodhuyzen – *Operations Manager*
- Dr. Christoph Diebolder – *Senior EM scientist*
- Dr. Rebecca Dillard – *EM scientist*
- Mr. Bart Alewijnse – *Computer Engineer*

Power Users

- Briegel lab (UL – IBL)
- Koster Lab (LUMC)
- Förster Lab (UU)
- Thermo Fisher Scientific

1 open vacancy – EM scientist

I: www.NeCEN.nl E:necen@biology.leidenuniv.nl T: @NeCEN_2p0

On-the-fly pre-processing and monitoring

Data pre-processing can be automatic and live during data collection
Tailored to NeCEN's operators

Transfer
10-20 sec @
1GB/sec

"pre-processing" server

Pre-processing server 1 (GPU, 60TB)

Import movies

Align movies

MotionCor, MotionCor2
10-30sec

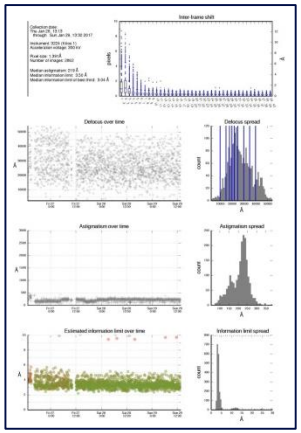
CTF estimation

gCTF,
CTFFIND4
15sec

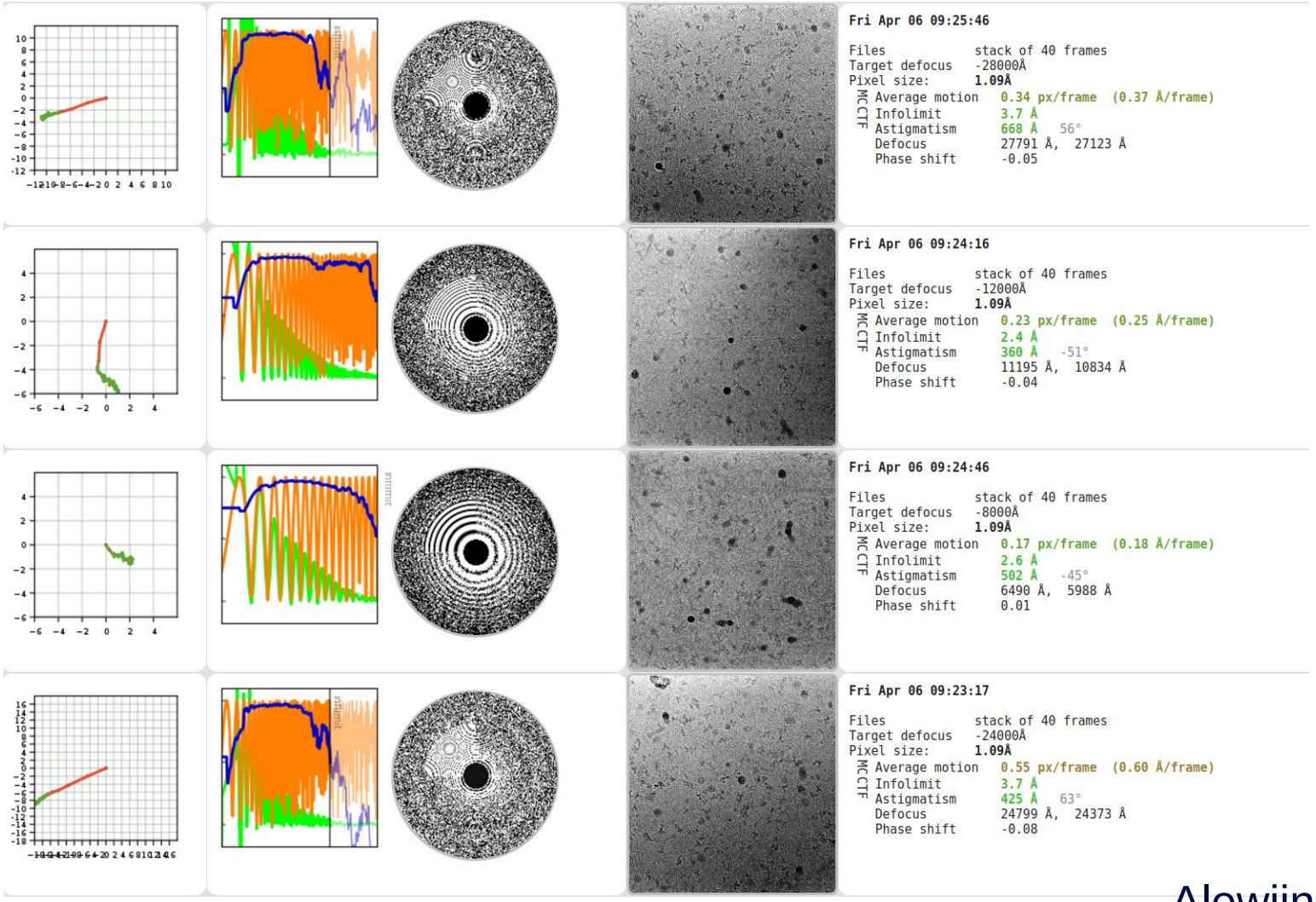
Acquisition
approx. 10-30sec, 1.5 GB stack,

Live feedback during data acquisition

Delivery of data acquisition quality report (drift, defocus range, etc)



Alewijnse et al.,
JSB (2017)



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Particle Picking?

gAutomatch, xmipp,
RELION?

2D classification ?

RELION, CL2D-xmipp?

Fully automated 3D reconstructions?

RELION, cryoSPARC?

Maskell, Renault et al., 2015, *Nature*

Alewijnse et al., JSB (2017)