NeCEN

Netherlands Centre of Electron Nanoscopy

User Base and Access to the facilities

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NeCEN
Leiden University

Introduction to NeCEN

What is NeCEN?

Netherlands Center of Electron Nanoscopy (NeCEN)

NeCEN is a centralized open access facility dedicated to high resolution cryo-electron microscopy for life science applications

NeCEN offers research institutes and companies access to highly advanced cryo-transmission electron microscopy infrastructure and related services

NeCEN opened its doors in 2012 and was restructured in 2016.

www.necen.nl

Historical perspective

2006 National initiative to create a centre of excellence

It was recognized that the financial resources required to sustain the upcoming generation of 300 kV cryoEM instruments would be too large for one single university in the Netherlands

2010 Ten Dutch universities involved with cryoEM joined forces

Resources were provided by the universities and the national resource foundation (NWO) to purchase two instruments

2012 NeCEN opened its doors as an international facility

The Open Access Facility was managed by two cryo EM experts

2016 NeCEN restructured and expanded it services

The Open Access facility is managed by a team of five persons

Research and development is carried out in collaborations

NeCEN and the Dutch financial system to support science

In 2009 the vision for NeCEN that was formulated earlier (in 2006) was downscaled due to the global financial crisis of 2008. This crisis had a significant impact on the budgets for Science in the Netherlands.

Funding the Science derives from the **Dutch Research Council** (taxes). There are no other funding sources (e.g. Welcome, HHMI, Private).

The organization and access to NeCEN is tailored to the Dutch situation. NeCEN aims to be structured as a cost-effective resource.

Pay-for-Usage is the standard mode of access. This mode generates funds to (partly) support the required Maintenance, Upgrades and Personnel to provide a sustainable cutting-edge resource.

NeCEN Mission

Be a center of excellence in high resolution cryo-electron microscopy for life science applications

- Provide access to cutting-edge cryoEM technology and expertise
- Cutting edge: implies ongoing upgrades of instrumentation
- Expertise: implies more than mere instrumentation

Two modes of access for research institutes and companies

- Open access - pay for usage - various price models
- Excellence-science based access – free of costs

Offer education and training

- National, international, academic and industrial users

Cryo Electron Microscopy groups in the Netherlands

University of

Groningen
Nijmegen
Utrecht
Amsterdam
Leiden
Delft
Eindhoven
Maastricht

FEI/Thermo Fisher (Eindhoven)

NeCEN stakeholders

The Dutch cryo Electron Microscopy community

Leiden University Faculty of Science

Leiden University Medical Center

Dutch Research Council

FEI Company / Thermo Fisher

NeCEN Team


Roman Koning – LUMC operator / Correlative light and electron tomography
Ludo Renault – Facility manager / Single particle Analysis (SPA)
Chris Diebolder– Operator / SPA / CET
Susanne Roodhuijzen – Program manager / Office
Julio Ortiz – Operator / SPA / CET / FIB milling
Bart Alewijnse
System manager / Processing
NeCEN

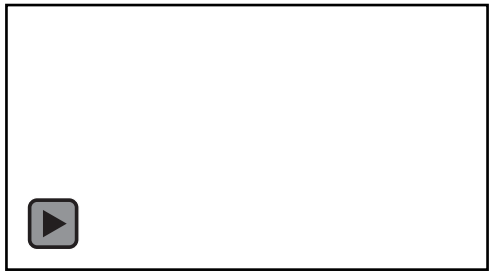
Services

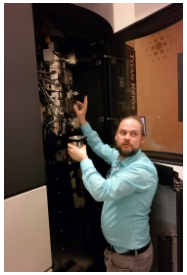
Services

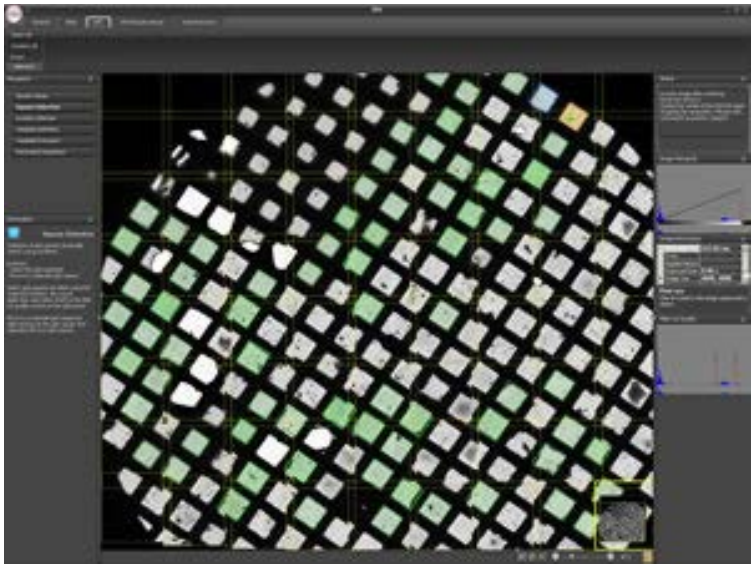
- Sample preparation and quality assessment
- Data collection
- Data processing
- Training and education
- Research and development

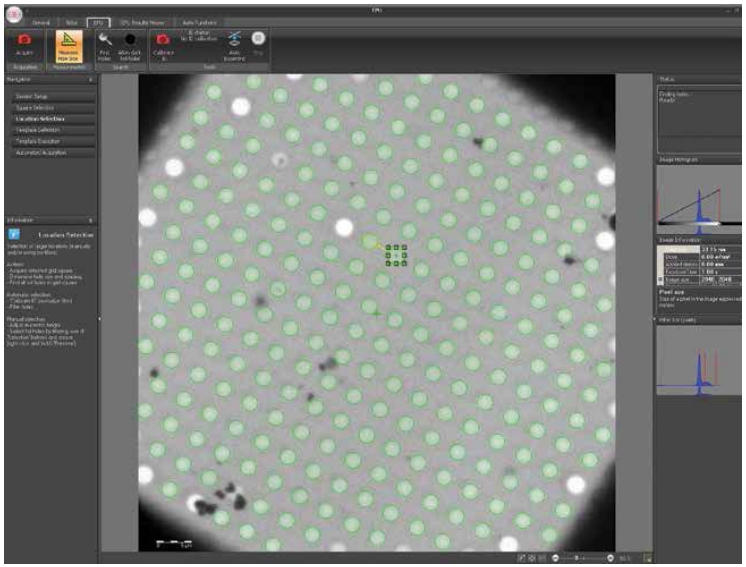
Sample preparation and quality assessment

- Sample freezing with an FEI Vitrobot
- Screening of freezing conditions for optimization sample prep









Data collection

Titan 1

- FEI Falcon 2
- GIF with Gatan K2 Summit
- FEI Volta phase plate
- STEM Detector

Titan 2

- XFEG High Brightness Gun
- Cs Corrector
- FEI Falcon 3 – Counting mode
- STEM Detector

Expanding data collection services

Talos L120C

- Screening
- Helping users to optimize samples for the Krios

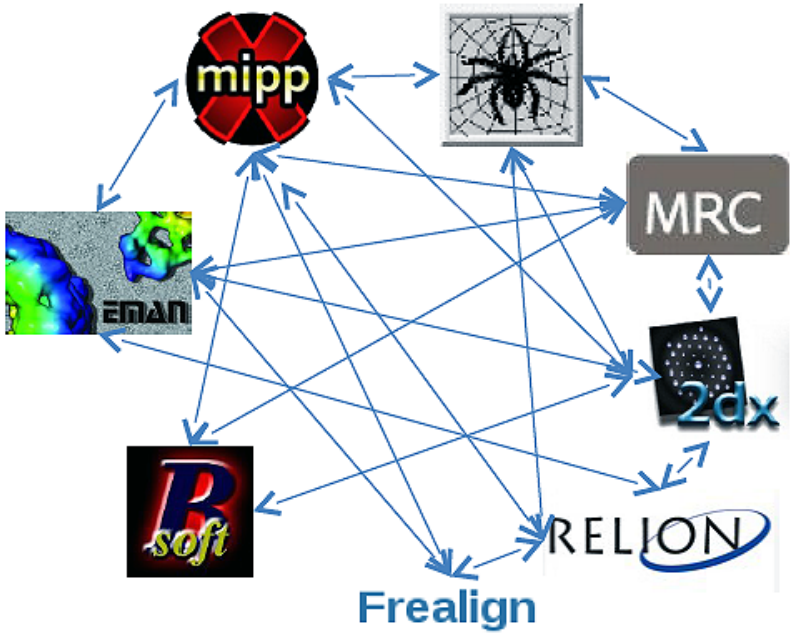
Scios dual-beam (End 2017)

- Preparing lamellae for cryo-electron tomography

Data processing

Image processing in Scipion

- Automated particle picking
- 2d classification
- Initial model generation
- 3d classification
- 3d refinement



Scipion principles

- Integrate EM software packages to be used in the same project
- Full project traceability, improving reproducibility
- Execute complete workflows in an automated manner
- Easy to install and use
- Easy to extend with new protocols

CNB
CENTRO NACIONAL DE BIOTECNOLOGIA

Group Carazo

Traning and education

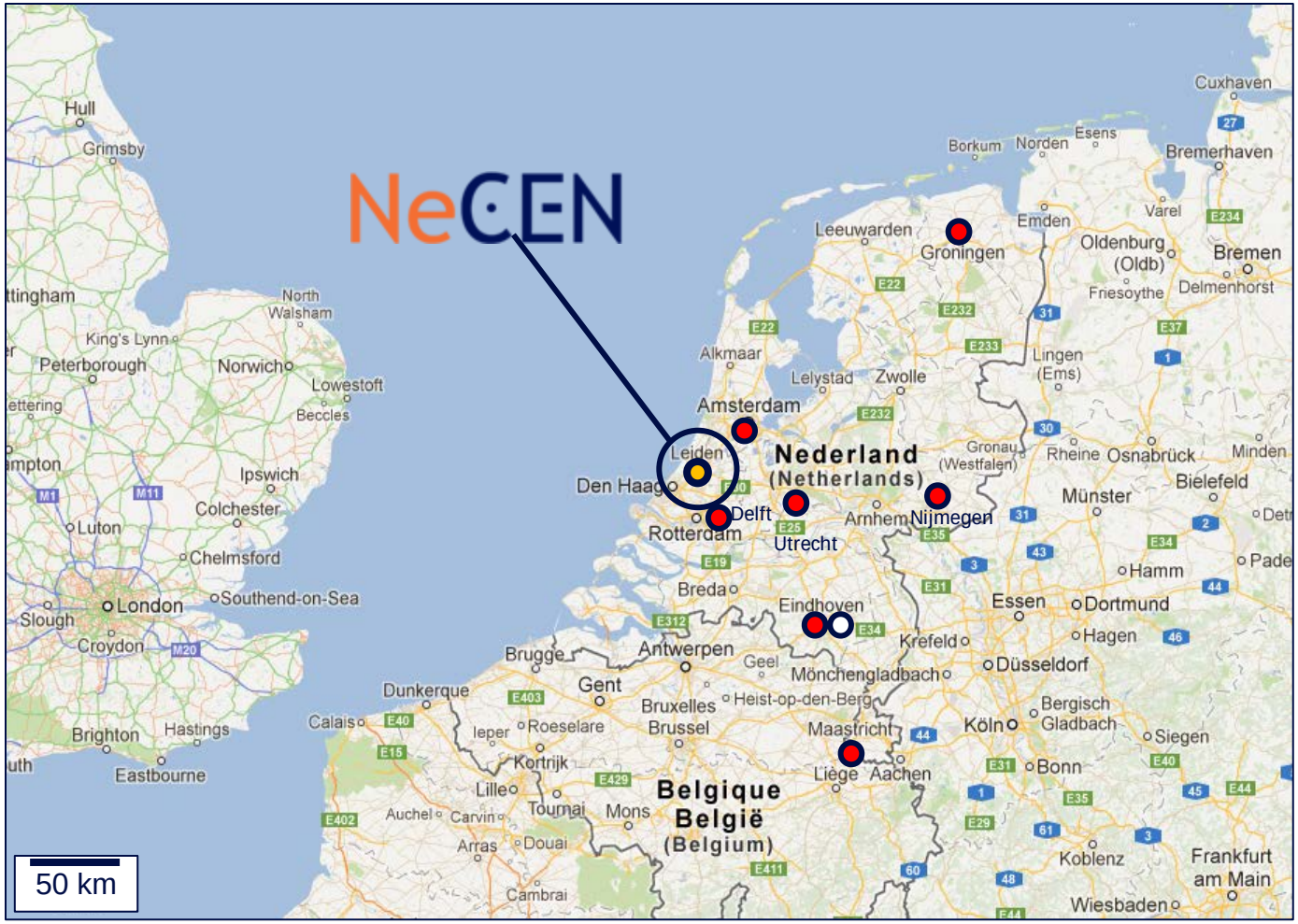
Trainings and education on the various aspects of cryoEM

- Specimen preparation, data collection and processing
- *via* scientific collaborations
- *via* hands-on workshops
- *via* hands-on courses

NEMI: Electron Microscopy groups in the Netherlands

University of
Groningen
Nijmegen
Utrecht
Amsterdam
Leiden
Delft
Eindhoven
Maastricht
FEI/Thermo Fischer

Briegel lab
Förster lab
Peters lab
Koster lab
Oostergetel lab
Klumperman lab
Gerritsen lab
Van Heel lab
Engel lab
Kruit lab
Joost lab
Hoogenboom lab
Sommerdijk lab
Van Nifrik lab



STW Enabling new technology
nanonextnl innovating with micro and nanotechnology
FOM
REAL NANO
NWO
ThermoFisher SCIENTIFIC

Users and Access

Targeted user group

NeCEN targets users that have **optimized their specimen preparation elsewhere**, e.g. in collaboration with their local cryo EM resources using the scientific environment required for the biological question at hand.

Access modes

Open-Access Mode

- Pay-per-usage
- Application by submitting a form
- Evaluation based upon technical merits
- Acceptance by the NeCEN team using a score-sheet

Reviewed-Access Mode

- Free of cost *via* European large scale infrastructure projects (Instruct, iNext)
- Application by submitting a form
- Evaluation based upon scientific merits first, next on technical feasibility
- Acceptance by an external Review Board (Instruct, iNext) using a score-sheet

www.necen.nl

NeCEN

ABOUT NECEN | SERVICES | USER ACCESS | MICROSCOPES | PEOPLE | RESEARCH PROJECTS

A facility for electron microscopy with two of the most advanced **cryo-transmission electron microscopes** available world-wide

about NeCEN | send in an application

OUR SERVICES

ELECTRON MICROSCOPY

NeCEN is an open access facility for high resolution cryo electron microscopy of biological samples. Two state of the art Titan Krios transmission electron microscopes allow cost efficient automated data collection for our customers.

SAMPLE PREPARATION

Flash freezing enables preservation of biological samples in a vitrified, close to native state. The cryo lab at NeCEN is equipped with all instrumentation needed to prepare the sample it takes to collect atomic resolution data.

DATA PROCESSING

No high resolution structure without data processing. The experienced operators at NeCEN help you on site to get most out of your data using state of the art hardware and software.

COURSES

Want to know it all? Intense training courses at NeCEN will make you a cryo-EM expert!
We are seeking applicants to participate in the **Cryo-EM School** to be hold on January 2017

Read more

Access for Data collection

Access time units

- Per day (24 h), most often 2-4 days per project
- Current waiting time between application and data collection 2-3 months

Operation of the microscope

- A NeCEN operator, exceptions only with NeCEN guidance

Remote access

- The user can be on-site or send in cryo-prepared grids
- Full remote control is possible (selected users, currently only two)

Delivered to the customers

- Hard-disk with data (option is download). Data is removed from NeCEN
- Detailed report on the acquired data statistics (monitored on-the-fly)

NeCEN Project form and Score sheet

In short the technical requirements

How feasible is the proposed project?

Does the sample meet the safety requirements (BSL)?

Are the particles not too small?

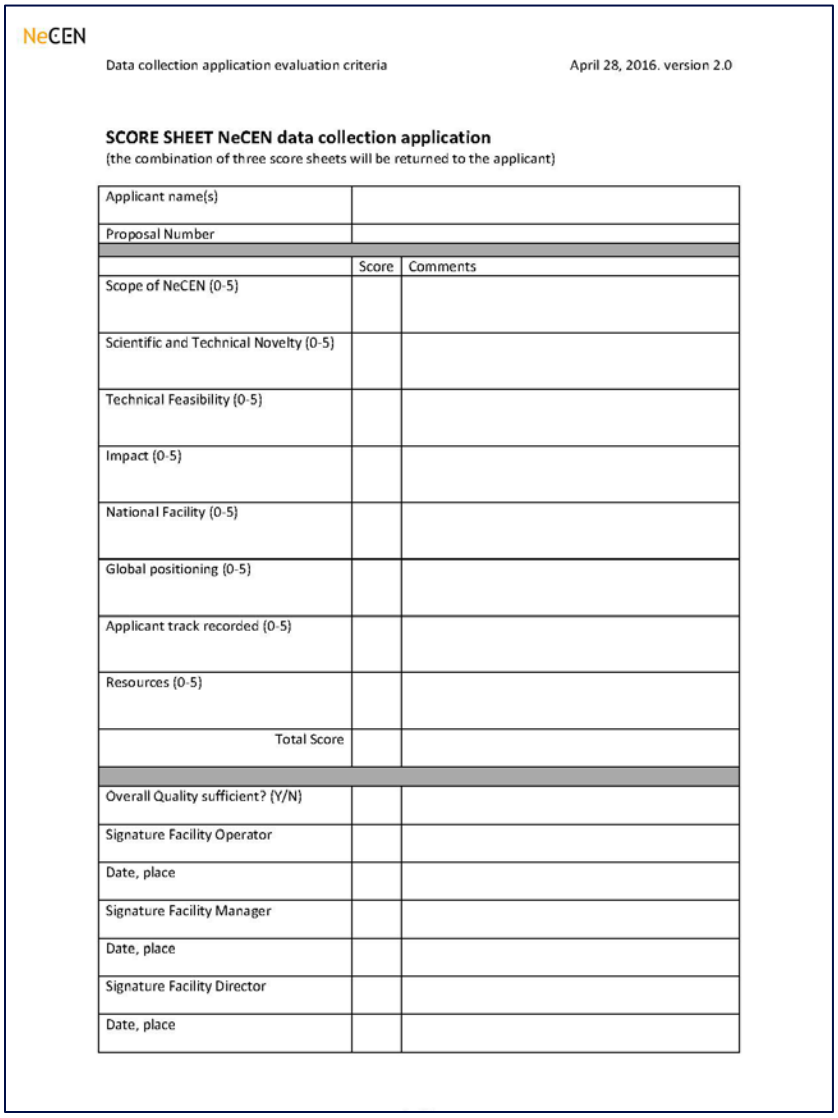
Sufficient information on homogeneity (2D classification, low resolution reconstruction)?

On doubt: the applicant is contacted and given advise and or to obtain more information.

On start of data collection the sample may appear to be significantly different from the supporting data in the application.

The applicant is contacted, possibly leading to extensive screening for good areas and/or preparing for new cryo-grids at NeCEN.

Costs count as data collection per day.



NeCEN

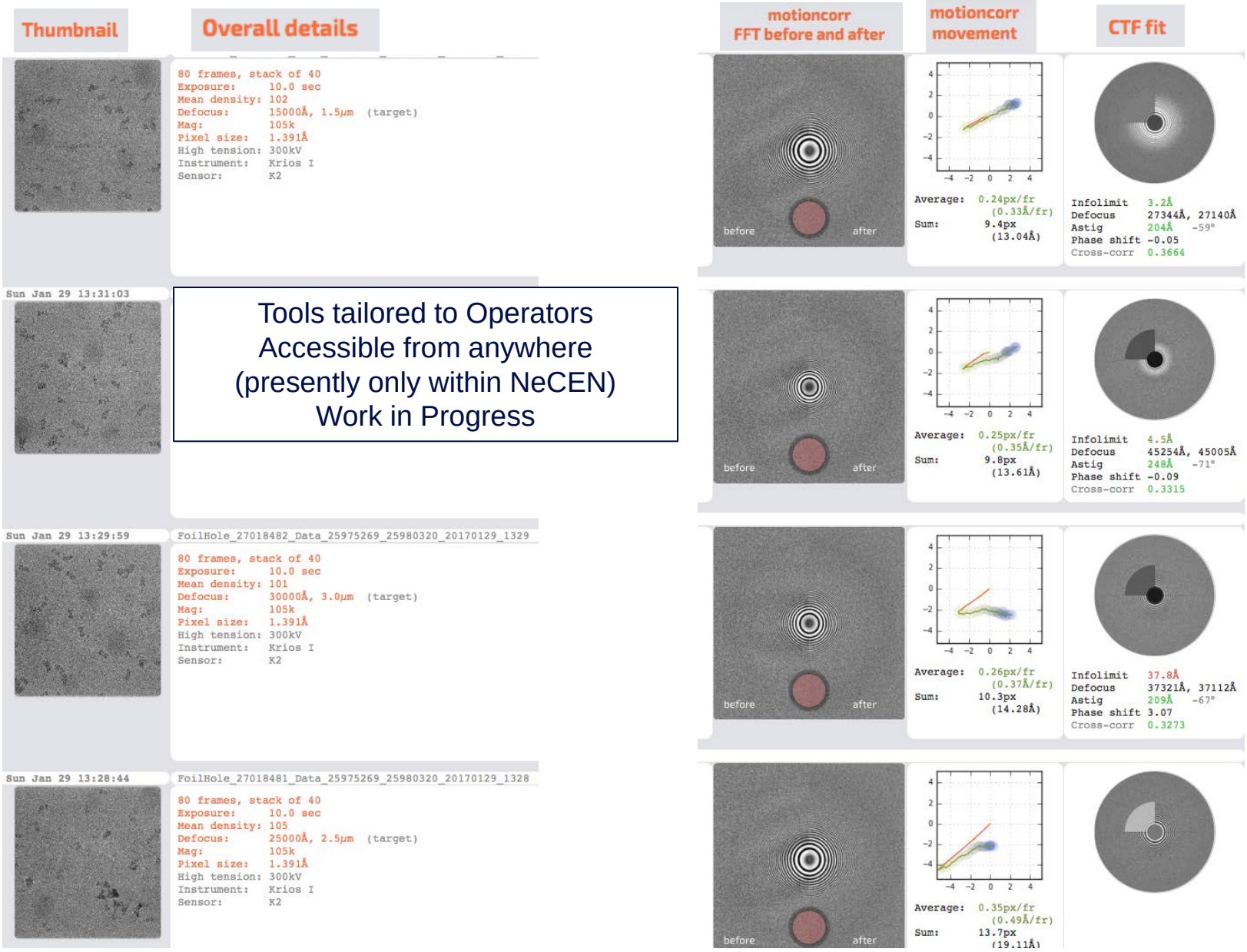
Data collection application evaluation criteria April 28, 2016. version 2.0

SCORE SHEET NeCEN data collection application

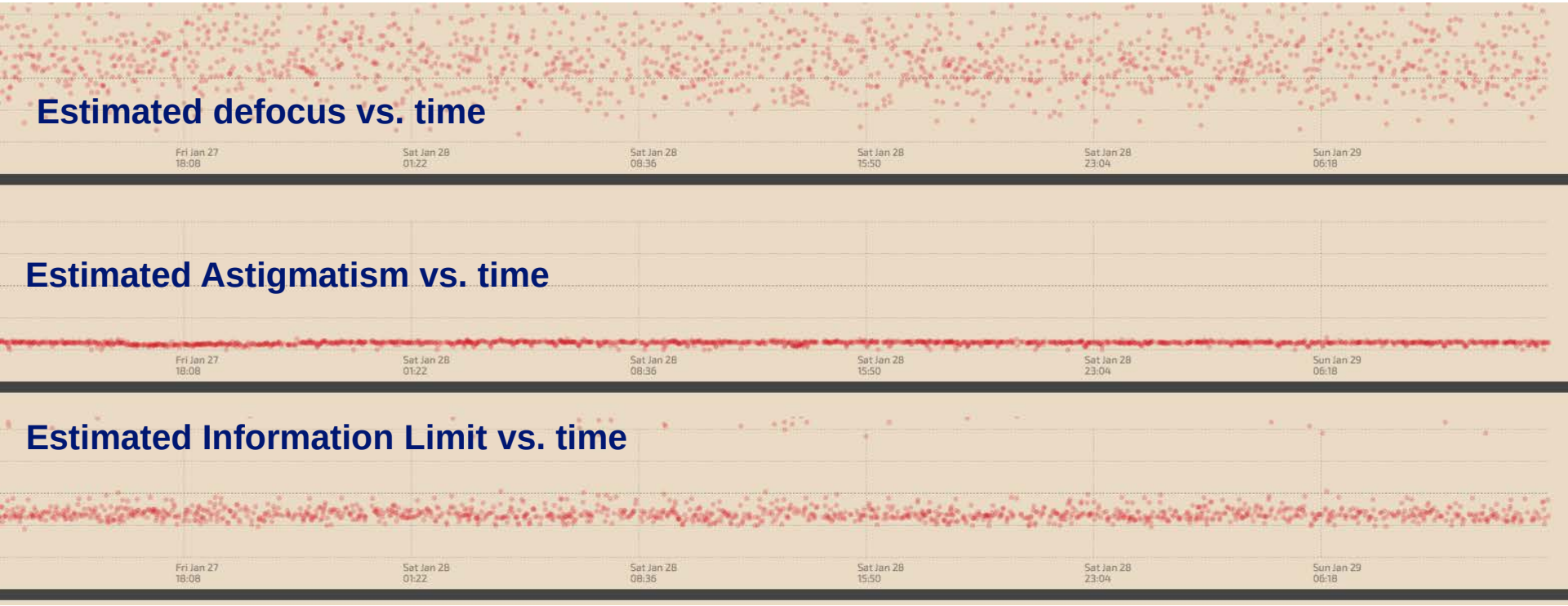
(the combination of three score sheets will be returned to the applicant)

Applicant name(s)		
Proposal Number		
	Score	Comments
Scope of NeCEN (0-5)		
Scientific and Technical Novelty (0-5)		
Technical Feasibility (0-5)		
Impact (0-5)		
National Facility (0-5)		
Global positioning (0-5)		
Applicant track recorded (0-5)		
Resources (0-5)		
Total Score		
Overall Quality sufficient? (Y/N)		
Signature Facility Operator		
Date, place		
Signature Facility Manager		
Date, place		
Signature Facility Director		
Date, place		

On-the-fly pre-processing and monitoring



On-the-fly pre-processing and monitoring



If any of the values seem abnormal then the operators can pause and fix the problem(s) to collect the best quality data.
Meta-data of all data sets is kept to compare reports over time.

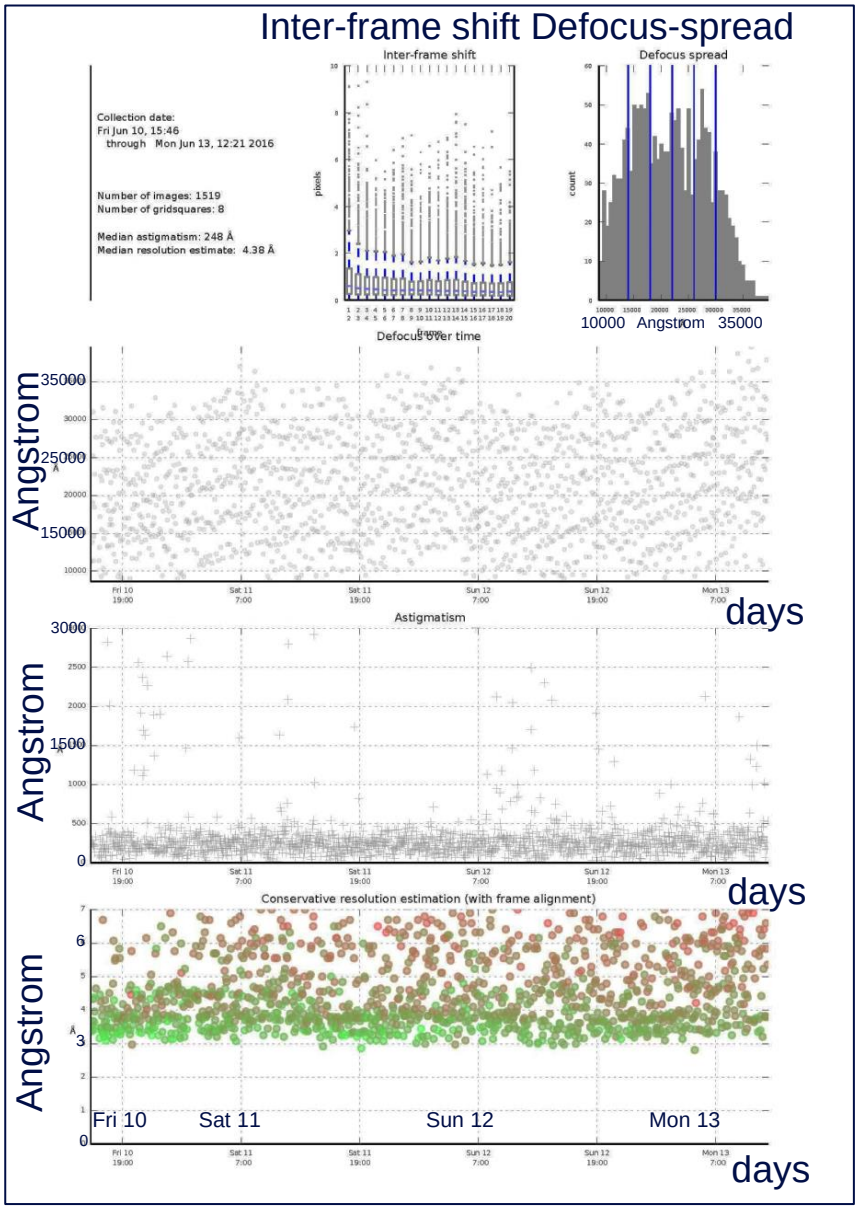
Report on quality collected data for users

Collection data/time
Number of images
Number of grid squares
Median astigmatism
Median resolution estimate

Defocus over time

Astigmatism over time

Information limit estimation including frame alignment



Report on quality collected data for users

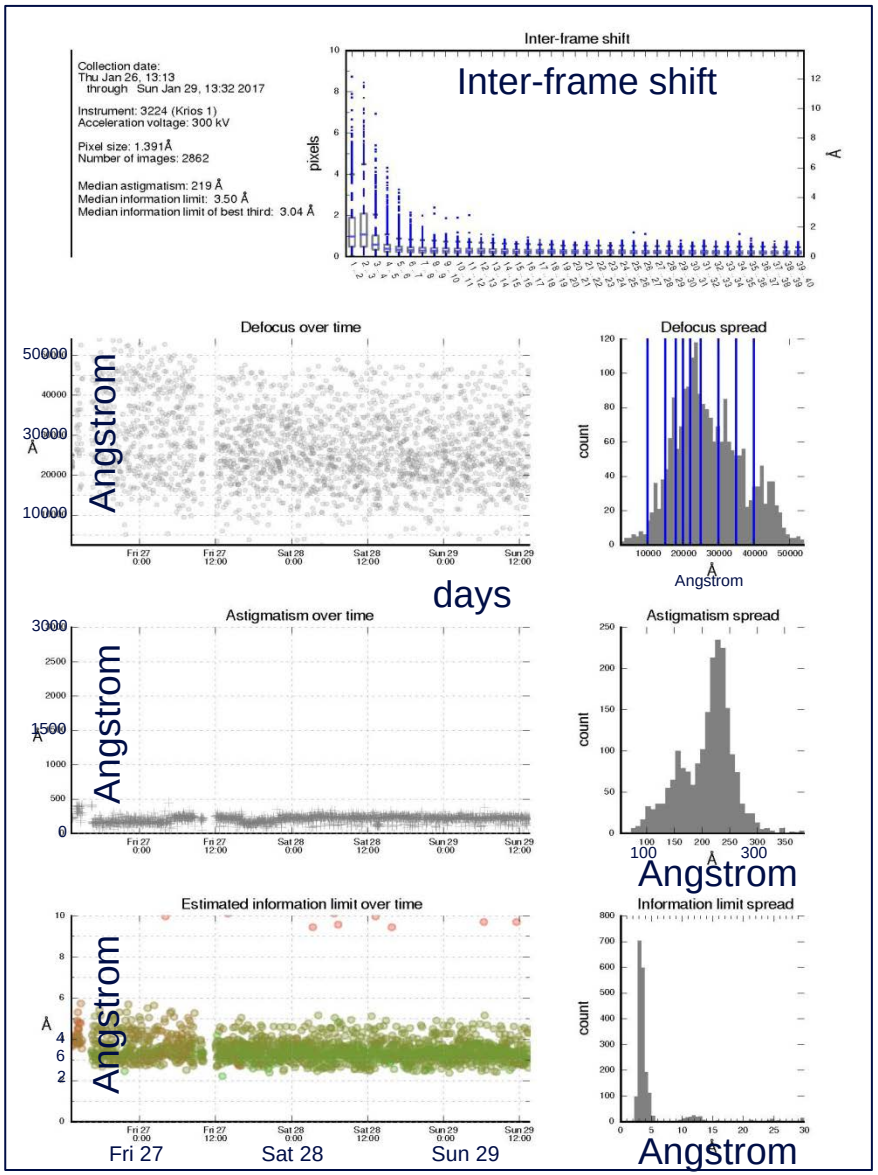
Collection data/time
Number of images
Number of grid squares
Median astigmatism
Median resolution estimate

Defocus over time

Astigmatism over time

Information limit estimation including frame alignment

Continuous carbon on lacey-grids



Open-Access mode

Open Access

Academic

- About 94% of all users (June-Nov 2016)
- Pay-per-usage. Pricing is per day
- Mostly per project (2-4 days)
- Mostly from established cryoEM labs
- About 17% from the Netherlands, 83% from abroad (95% Europe)

Industrial

- About 6% of all users (June-Nov 2016)
- Pay-per-usage. Pricing is per day
- Mostly several projects combined into a larger package
- Mostly from less established cryoEM experts

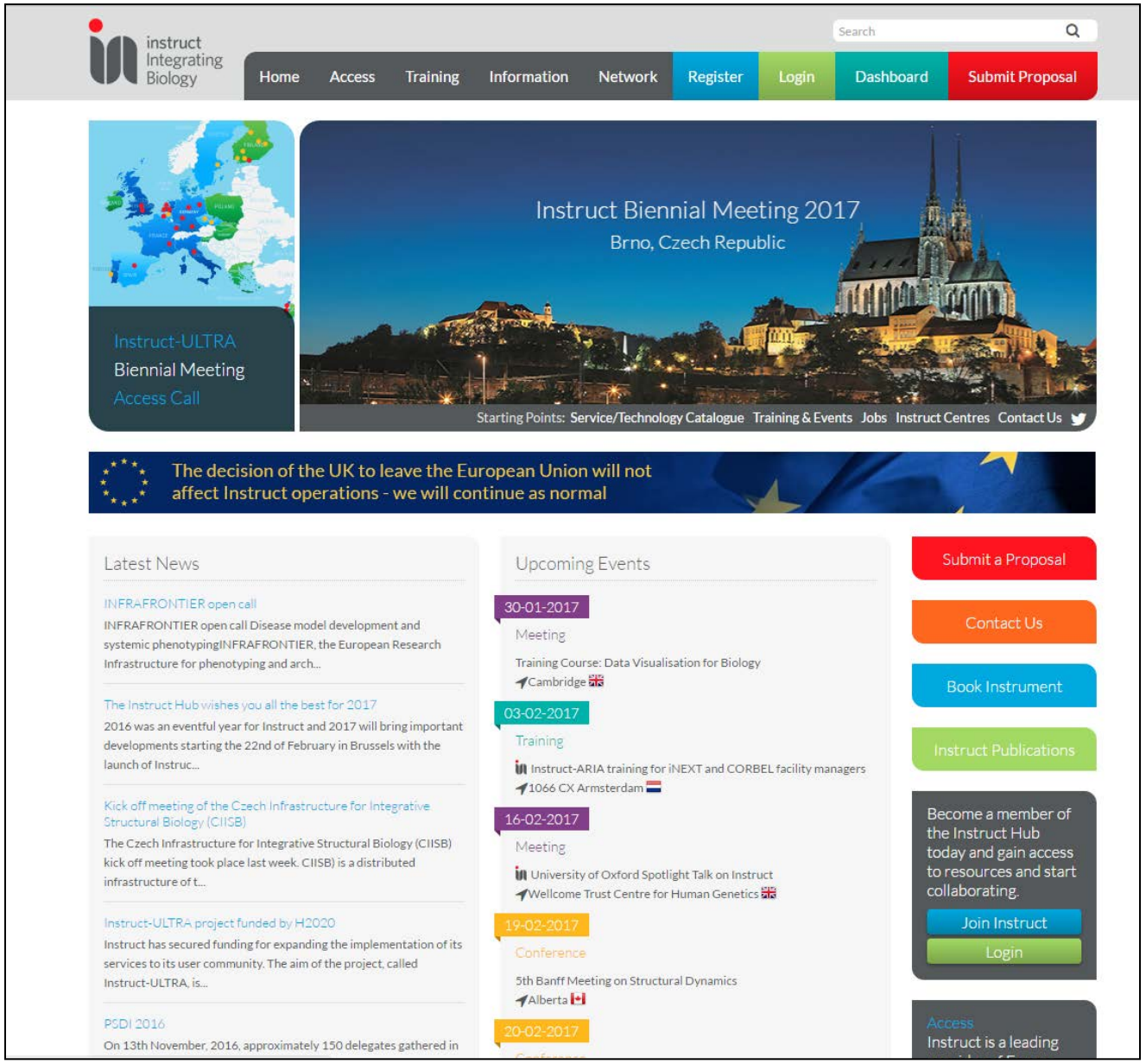
Reviewed-Access mode

Reviewed-Access

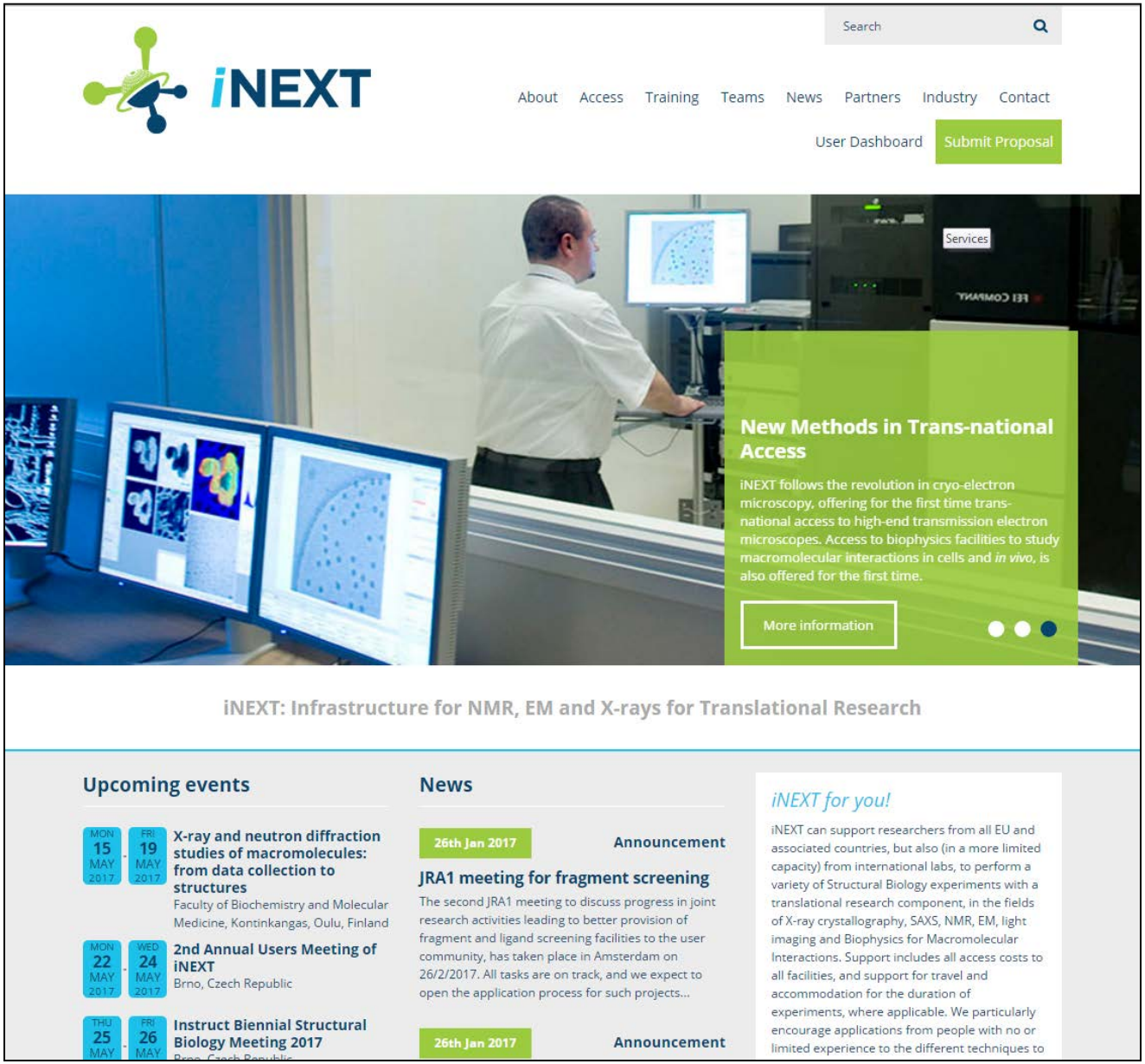
About 22% of all users

- Acceptance is reviewed by an external (European) committee
  - Instruct or iNext
- Procedure similar to the Open-Access procedure
  - A form with questions that are scored on a score-sheet
  - Emphasis in the Reviewed access procedure is Scientific Excellence
- Free of user-costs
- Time-unit is per day
- Mostly from less established cryoEM labs but from well established structural/cell biology labs

www.structuralbiology.eu



www.inext-eu.org



Netherlands Centre for Electron Nanoscopy

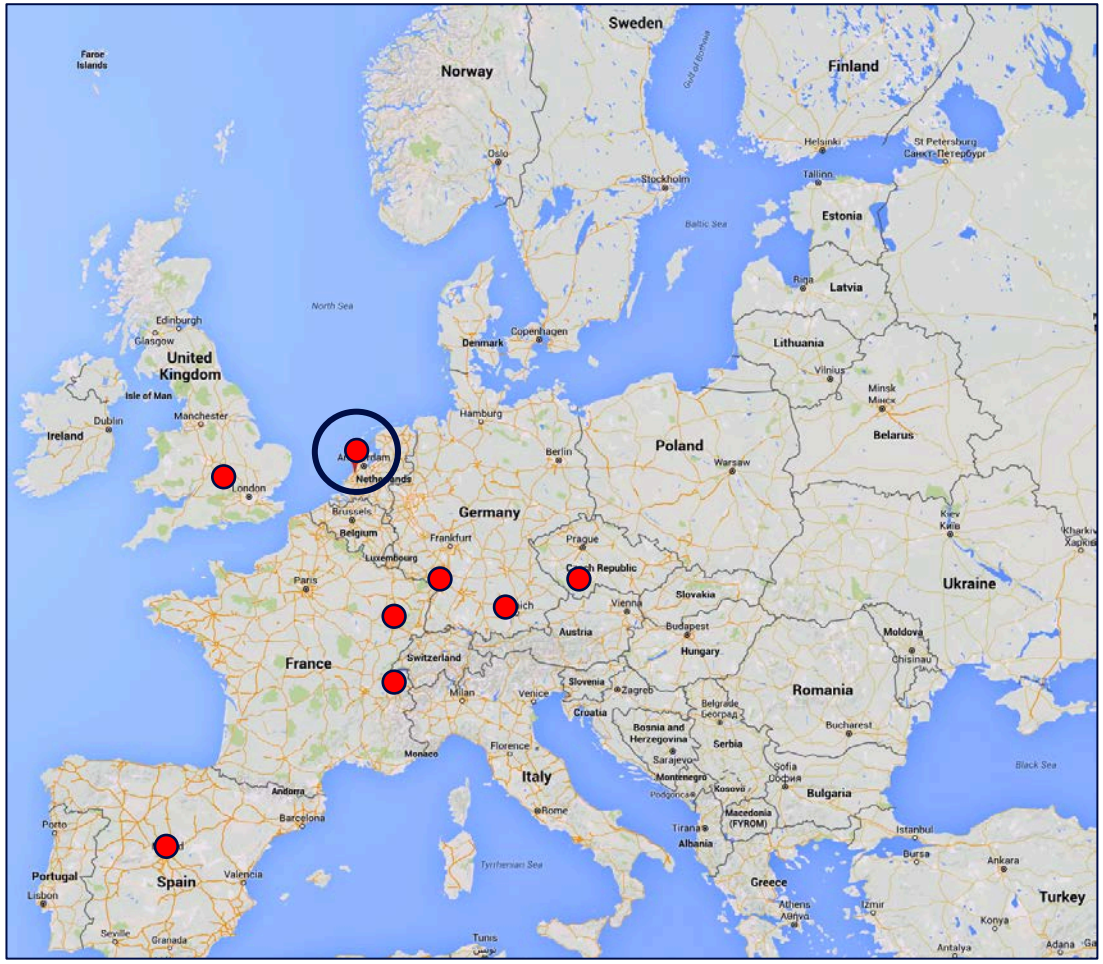
NeCEN embedded in European infrastructure networks

Instruct

iNext

One of several sites providing Electron Microscopy services

- Diamond (Oxford)
- EMBL (Heidelberg)
- CEITEC (Brno)
- MPI (Martinsried)
- IGBMC (Strassbourg)
- CNRS (Grenoble)
- Weizmann (Rehovot)
- CNB (Madrid)



Access and Upgrades

Access and Upgrades

State-of-the-art requires on-going process of updates and upgrades. During the last 5 years considerable upgrades were necessary.

- Detectors: Falcon 1, 2, 3, K2, K3, Phase Plate, Movies, Counting
- Most likely many other hardware-related upgrades necessary in the future

This implies less data collection time on the most advanced instruments

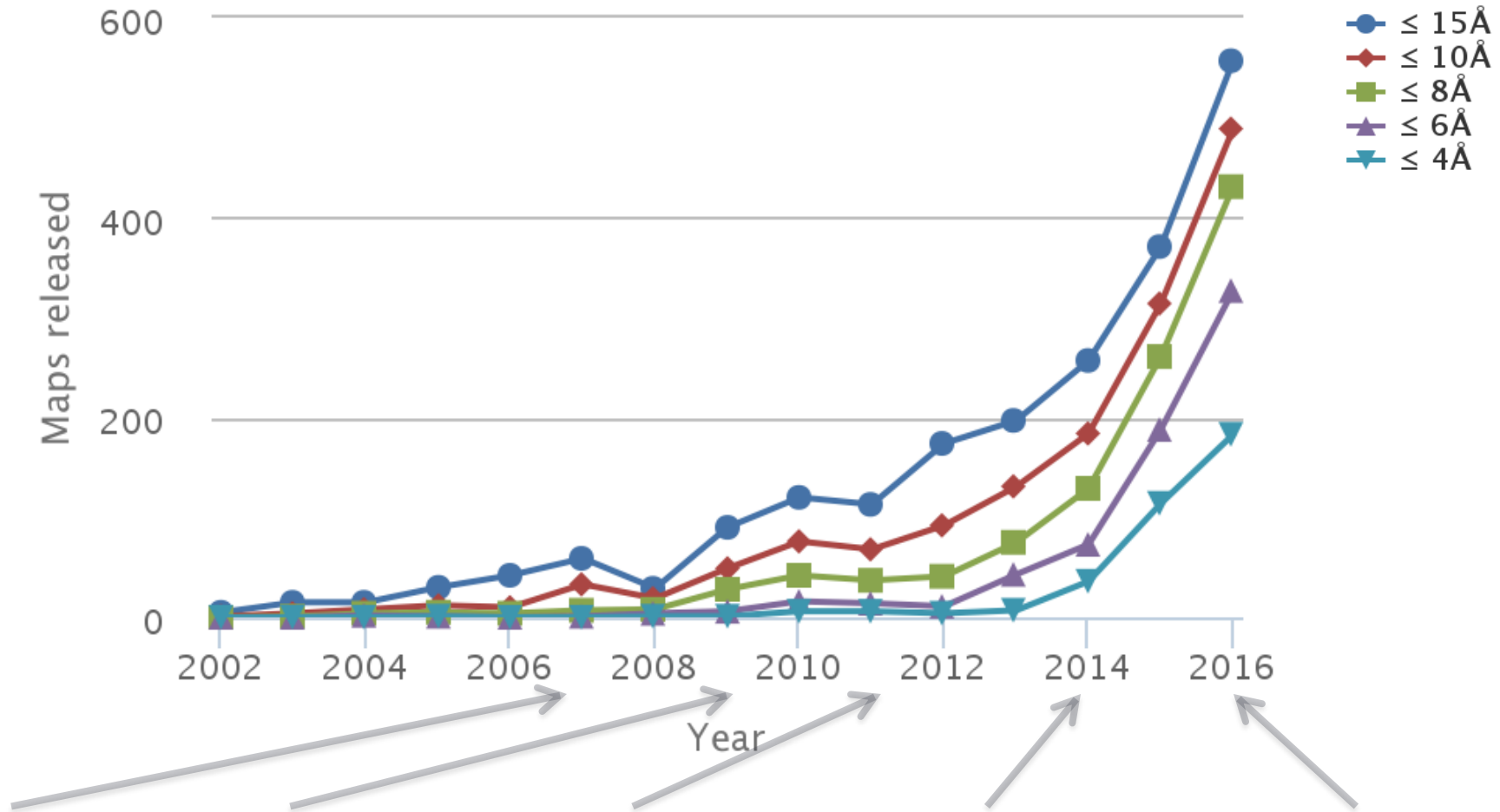
- Not only upgrades of specific hardware, but also on related accessories, software for data collection and processing.

At NeCEN, on average, about 20% of microscope access time had to be dedicated to hardware upgrades and updates.

In order to remain cutting-edge a dedicated microscopy it is a necessity to be able to provide reliable and robust access to data collection. This has impact on the pricing and on the number of instruments to be housed.

EMDataBank: Maps achieving a certain level of resolution

https://www.ebi.ac.uk/pdbe/emdb/statistics_num_res.html/



TITAN KRIOS release date

Mid-2009, appearance of the 1st generation DED from FEI (Falcon 1)

The first K2 detectors became available at the end of 2011

The 2nd generation of DED (Falcon 2) collecting movies and associated software

The 3rd generation of DED (Falcon 3) with improved DQE and GPU accelerated software

Concluding remarks

Summary

- *How are users selected/ deselected?*
  - *Open-access: rank on technical feasibility*
  - *Reviewed-access: scientific impact*
- *Who are your users (EM, X-ray, other)?*
  - *Established EM labs, most X-ray groups work with a local cryoEM group*
- *What is the experience level?*
  - *Moderate to high*
- *What do the users want to do?*
  - *Majority: Just collect data*
  - *For help with specimen preparation we refer to other labs*
  - *For help with processing we offer collaboration*
  - *We do not have had requests for interpretation*
- *Do users need to come to your facility or is remote access possible?*
  - *Most users will visit one day. For subsequent data collection they send specimen.*
  - *Remote monitoring is possible. For only NeCEN-linked users we allow Remote Control.*
- *Do users pay for microscope time or for other use of the facility?*
  - *Most users opt for Open-Access (70%), 30% will follow the Review-Access route*
  - *Current waiting time after submission somewhere between 2 and 3 months.*