

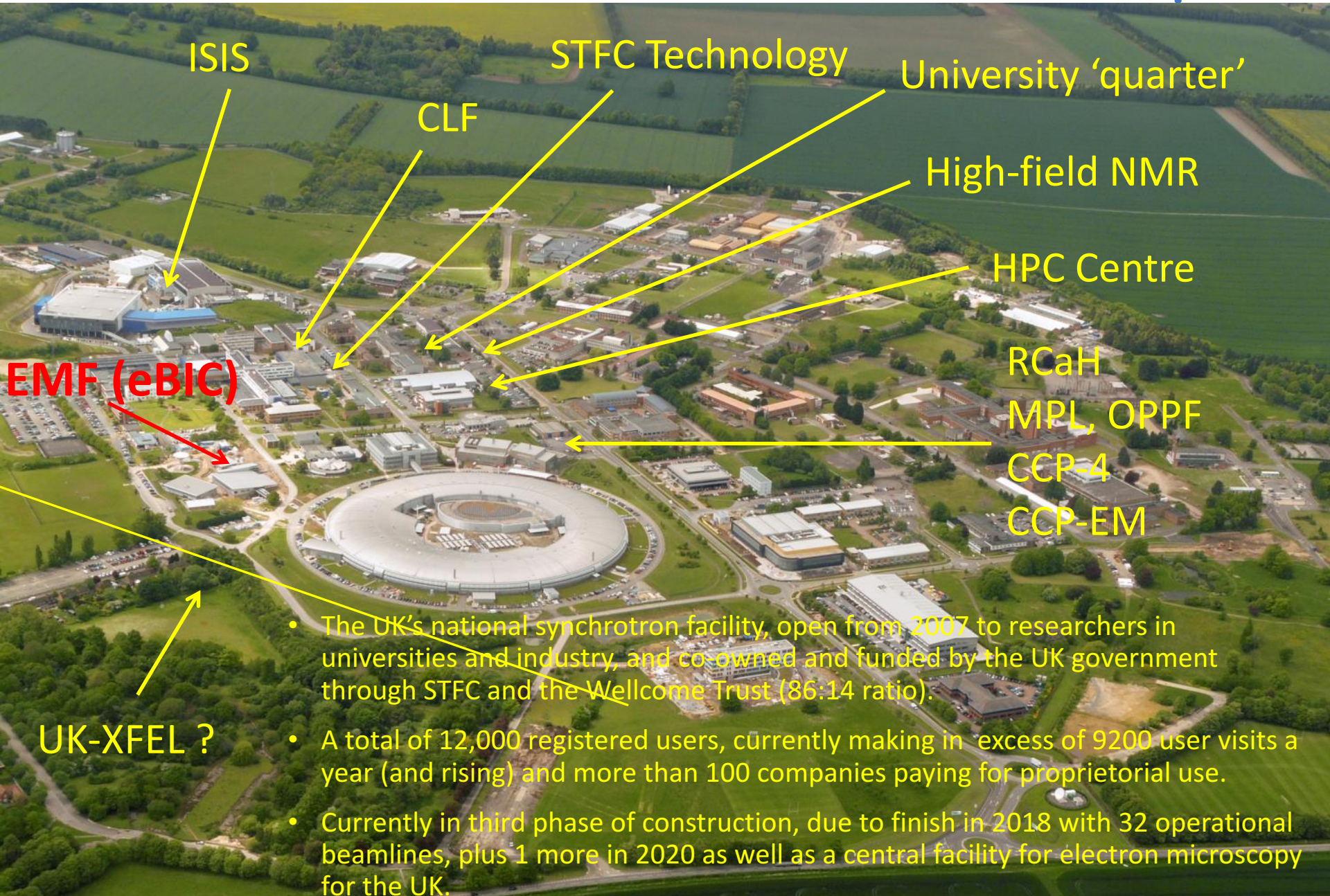
eBIC: The Electron Bio-Imaging Centre At Diamond Light Source

New York Structural Biology Centre

6/2/17

Alistair Siebert

Diamond & Harwell Research Campus



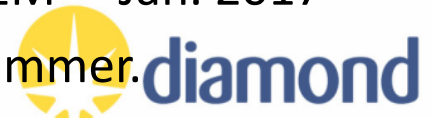
EMF (eBIC)

UK-XFEL ?

- The UK's national synchrotron facility, open from 2007 to researchers in universities and industry, and co-owned and funded by the UK government through STFC and the Wellcome Trust (86:14 ratio).
- A total of 12,000 registered users, currently making in excess of 9200 user visits a year (and rising) and more than 100 companies paying for proprietorial use.
- Currently in third phase of construction, due to finish in 2018 with 32 operational beamlines, plus 1 more in 2020 as well as a central facility for electron microscopy for the UK.

eBIC Aims

- The UK National Centre for cryo-EM:
 - Free-at-the-point-of-access to state-of-the art facilities.
 - Peer reviewed application process.
 - Beamline-like 24/7 operation supported by expert staff to facilitate intensive external user program.
- Cutting-edge in-house research program under eBIC director - Peijun Zhang.
- Foster the development of integrated structural biology in the UK, linking with other developments, including CCP-EM, EMDB and iNEXT.
- Training courses to bring in structural and cell biologists:
 - “Advanced Data Collection For High Resolution Cryo-EM” – Sept. 2016
 - 1st iNEXT FEI sponsored “Sample Preparation For Cryo-EM” - Jan. 2017
 - FEI sponsored (and x10 oversubscribed!) - #2 this summer.



User Access (80% of Microscope Time)

- **1) Rapid access:** 48 hr allocation, peer reviewed every 3 months (preferred international access route).
 - Typically top 30-50% of applications are selected.
 - Currently ~3 month turn-around → 6 weeks....
- **2) Block Allocation Group (BAG) access:** Multiple sessions to research group consortia.
 - Deadline every 6 months for a 2 year time period (PRP assess every 6 months).
 - Currently 7 BAGs - 115 days/6 months *i.e.* 50% of total time allocated over Krios I and II.
 - BAGs vary from 5-15 PIs.
- **3) Proprietary/Industrial access:** <10% of total user time but increasing demand...
- **Acceptance criteria:** Based on scientific excellence, subject to standard Diamond T&Cs, notably that the work should be published. **Requires evidence of suitable cryo-EM samples.** Free at the point of use (for all Academic users). Industrial and non UK use capped at 30%.
- **Travel and subsistence:** Covered for UK users (normally 1-2 people per visit). Accommodation at onsite guest house or nearby.
- **iNEXT** (EU H2020 I3 type grant) provides funded access to European users.



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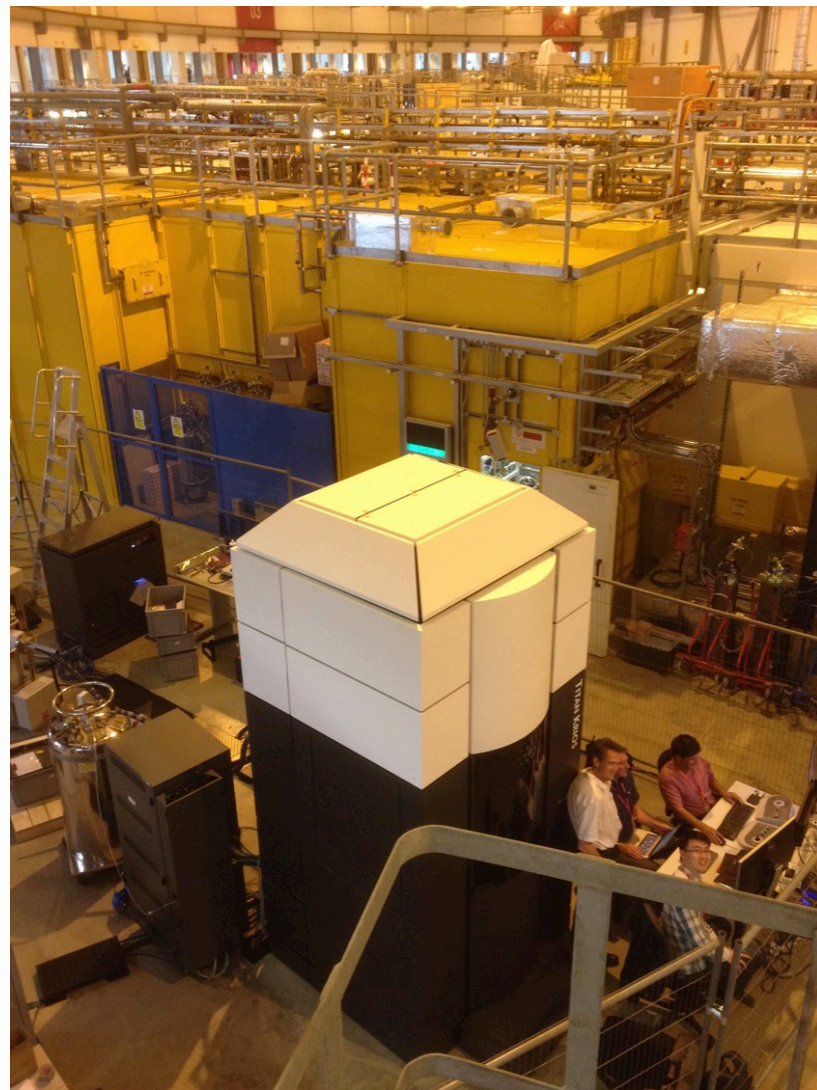
Electron Microscopes: Krios I



Falcon II→III Direct Electron Detector 6/17.
Quantum K2 - pioneering EPU integration with FEI.
Volta Phase plate...

Installation: 8/5-5/6 (2015)
Cryo data: 16/6
Acceptance: Friday 26/6
First external users: Monday 29/6

(Huge demand for EM time...(!)...
Remodel new building for more microscopes...)



Electron Microscopes: Krios II



Also on the synchrotron experimental hall floor...

Equivalent specification to Krios I

First external users 7/16

Some initial issues, full user program now running

Moving autumn 2017 to purpose-built EM facility

EMF: eBIC, ePSIC & I14



I14 Hard X-ray Nanoprobe Beamline:

Absorption and fluorescence spectroscopy

Ptychography (scanning diffraction imaging) and coherent imaging

ePSIC:

Complementary UHR EM centre for the Physical Sciences

Collaboration between DLS, Oxford University, Johnson Matthey and JEOL

External user program commencing Spring 2017

JEOL ARM 300F for <50 pm resolution

JEOL ARM 200F for chemical analysis with environmental cells <100 pm



New eBIC Facility

- Sample preparation, loading and general labs. + multiple rooms for smaller microscopes
- Initially constructed with two large rooms for two Titan Krios
- Now remodelled to house four Krios



Microscopes: Krios III



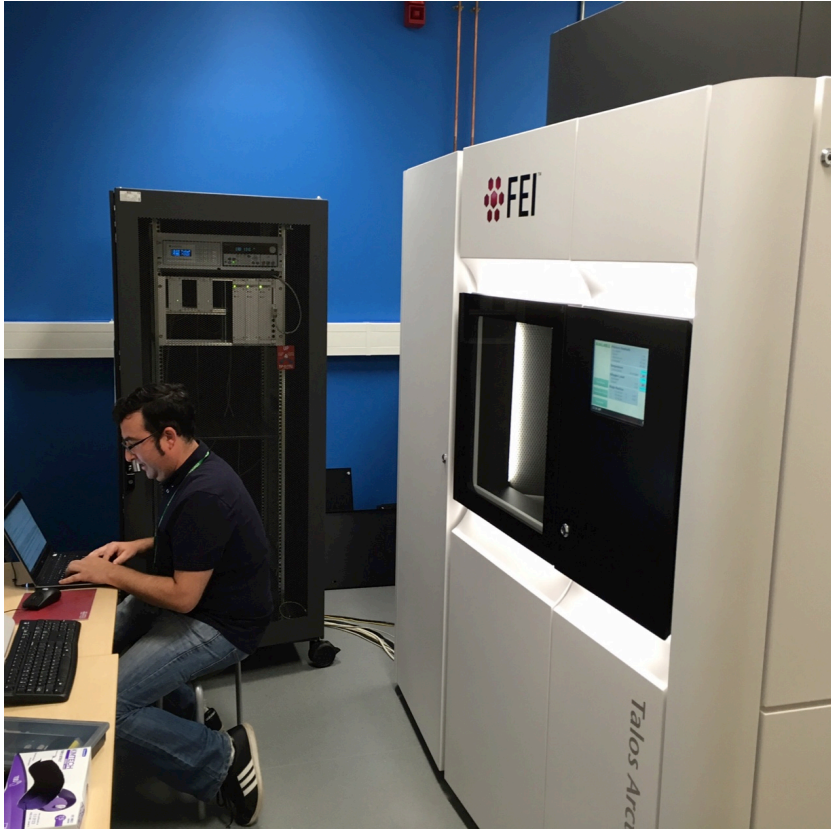
Installation commenced 7/1/17.

Specification equivalent to Krios I & II (+ Falcon III)

Krios IV (G3) CWAT 8/3 - delivery ~20/3

Krios II moves in Autumn 2017, Krios I?

Microscopes: Talos Arctica and Scios FIB-SEM



Falcon II → III 12/17 & Ceta camera
Volta phase plate
Installation complete 10/16
Training and in-house testing ongoing



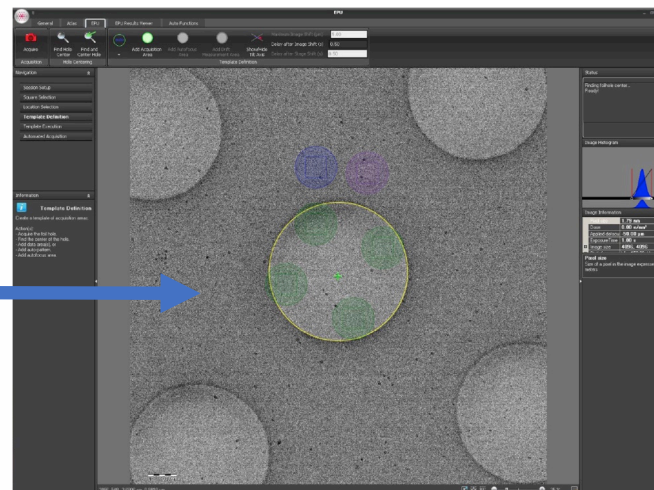
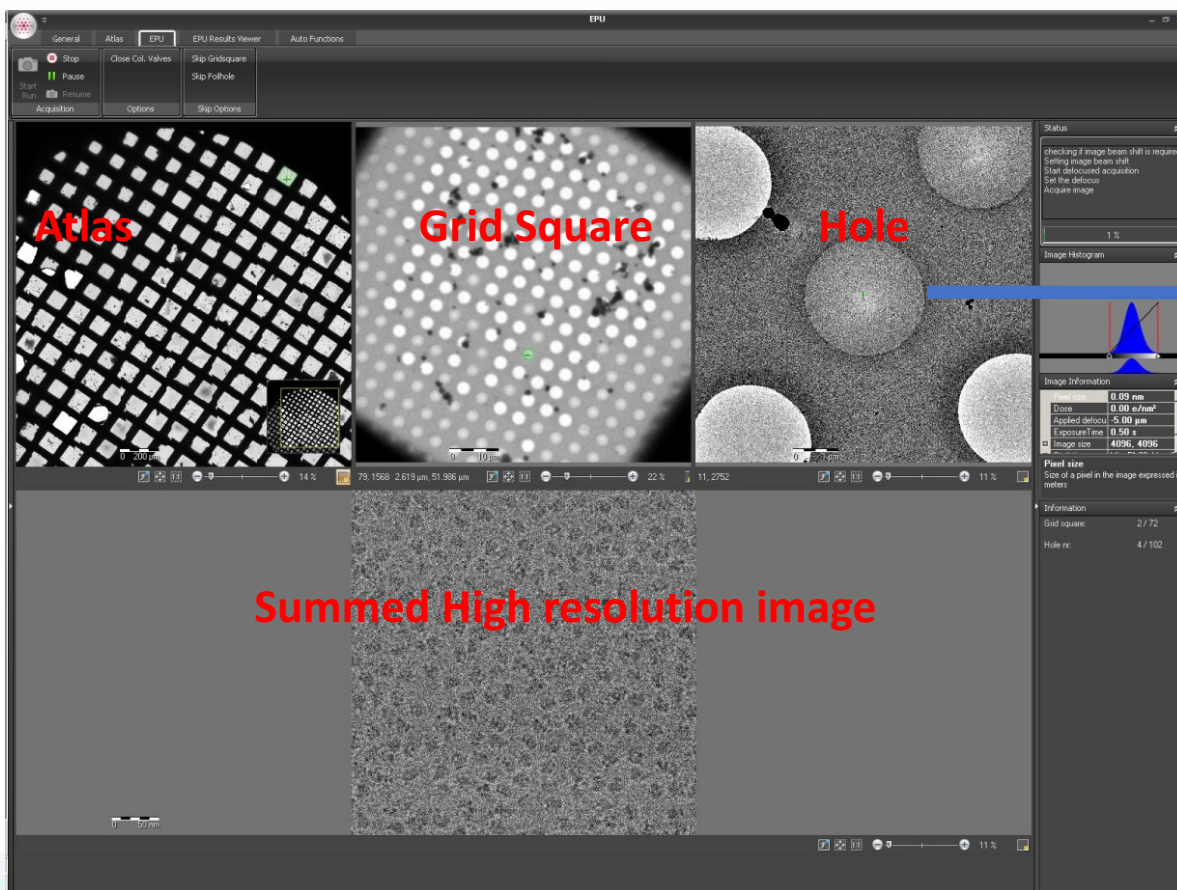
Prototype MPI Martinsried cryo stage
Platinum GIS
Quorum sample loading system → FEI ...
Moved from Oxford 1/17
Commissioning now



A Typical Krios User Day

- Session starts at 9 - 9.30: (most people are early!)
- 9.15 - 10.15: Load grids into the auto grid cartridges and the Krios cassette
- 10.20 - 10.30: Load cassette into the Krios
- 10.30 – 12: Survey grids to determine the best grid
- 12 – 12.45: Collect grid atlas image
- 12.45 – 3: Setup EPU, microscope alignments, detector gain references *etc.*
- 3 – 4: Check images, trigger OTF processing, assess CTF *etc.*
- Automatic collection for ~40 hours
- New EPU version emails eBIC and 24 hr staff (EHCs) + EHC walk around every ~4 hrs with 24hr eBIC on-call support.

EPU – Automated Image Acquisition



Setup the template for image acquisition: **Focus**, **Drift Measurement** and **Exposure(s)** area. This template will be automatically repeated for every selected hole.

Images provided by Sonja Welsch (FEI)

Automated SP data collection pioneered by NRAMM

Also FEI tomo 4



EM Data Flow

- Data capture at full rate on both direct electron detectors (17 fps for Falcon II or 40 fps for the K2)
- Falcon II data rate ~50-100 movies/hr ~2Tb/ 48 hr session
- eBIC was the first facility to have the Quantum-K2 Summit detector integrated with EPU ~25-75 movies/ hr ~ 2.4 Tb/ 48 hr session
- All data are directly written to our high-speed central computing/storage facility and immediately available for to users for on-the-fly processing and archiving
- All data are archived to tape and stored for the lifetime of the medium
- Diamond CPU & GPU clusters available to external users
- On-the-fly processing with Scipion → MotionCorr2, Relion, XMIPP, EMAN2 *etc.*

Automated Processing Pipeline

- Dedicated user workstations available in eBIC user suite
- Scipion (scipion.cnb.csic.es/m/home) allows users to align movie frames, estimate defocus values, pick, align and 2D classify particles (users can upload templates *etc.*)
 - Now with headless processing with workflow templates...
- We recommend users (at least) verify CTFFIND output to check the requested defocus range is correct.
- Tomography pipeline to stack and motion correct movies. IMOD based batchruntomo implementation in progress...

Getting your data home

- Bring USB HDDs for on-the-fly data copy during acquisition (>5 Tb)
- FTP/Globus tested at 1 GB/s from SLAC and DESY.
 - Requires dedicated portal, online instructions...



Data & User Statistics

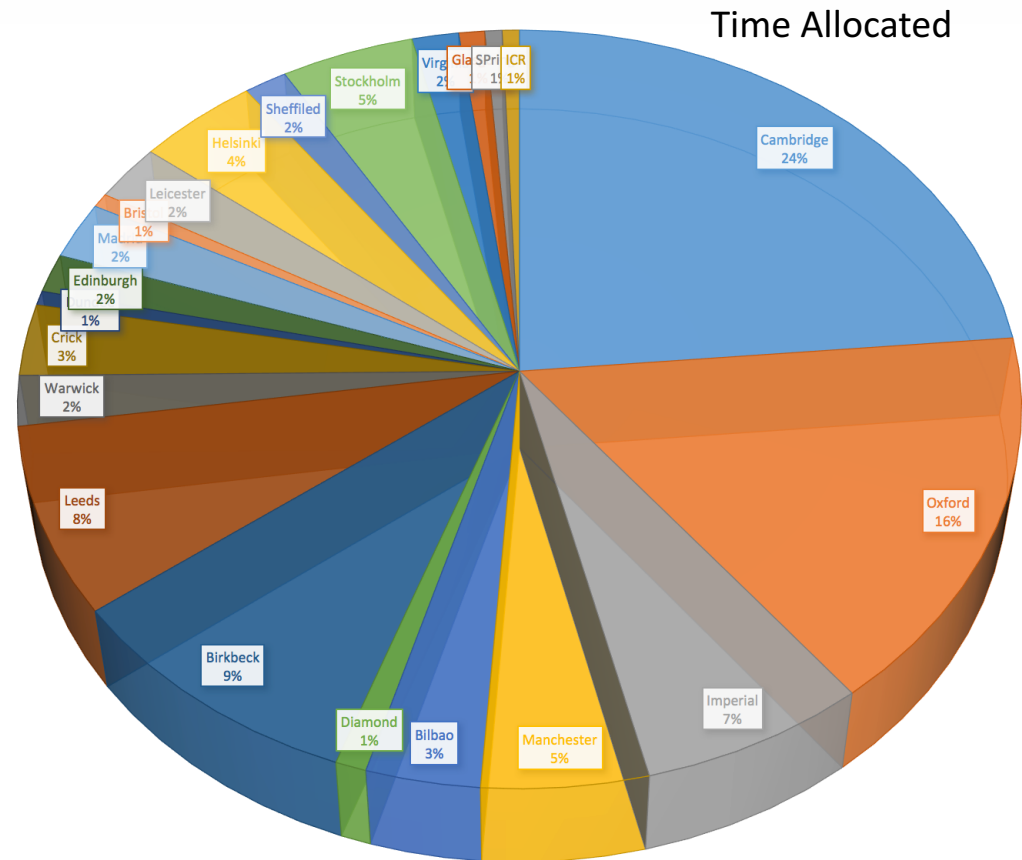
As of the 23/11/16:

152 external visits, 69 unique groups.

543 users, 223 unique.

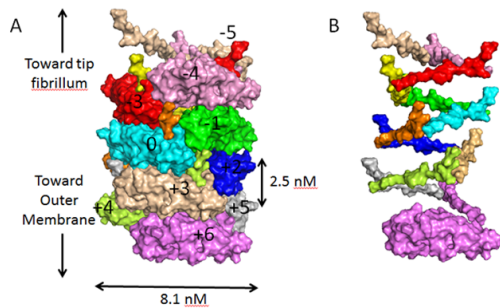
450 Tb of data generated.
(but see Alun's slide → OTF processing!)

Krios I first year of operation delivered 220 days of external user time – 40% over projected.

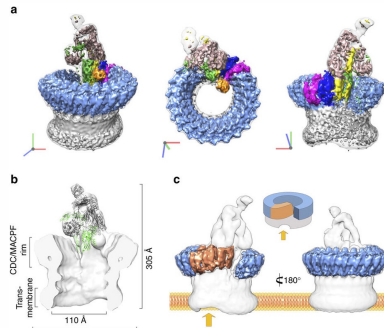


*14 groups from Cambridge, 10 groups from Oxford, 7 groups from Birkbeck, 5 groups from Imperial, 4 groups from Manchester and Bilbao, 3 groups from Leeds and the Crick,, 2 groups from Edinburgh and Dundee, 1 Group from Diamond, Warwick, Madrid, Bristol, Leicester, Helsinki, Sheffield, Stockholm, Virginia, SPri8, ICR

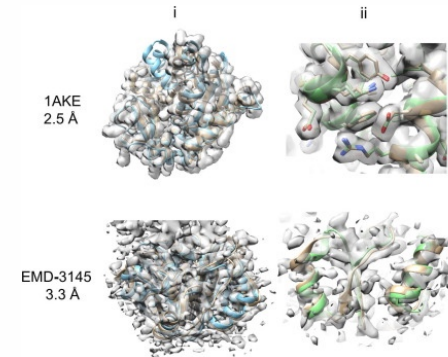
Some Highlights



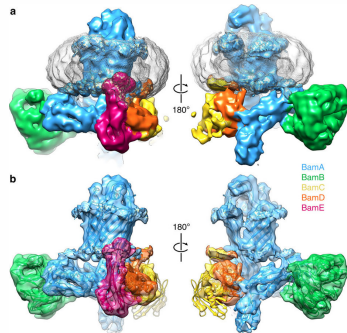
Hospenthal *et al. Cell* 2016



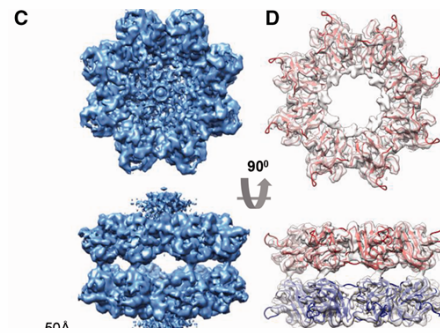
Serna *et al. Nat Commun* 2016



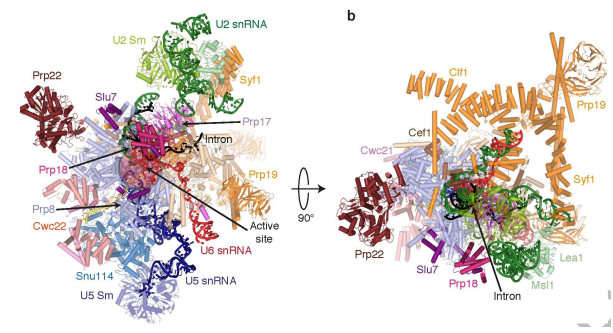
Joseph *et al. Methods* 2016



Ladanza *et al. Nat Commun* 2016



Ramsay *et al. Human Mol Gen* 2016



Fica *et al. Nature* 2017

- Also aware of multiple reconstructions approaching 3Å and three at greater than 3Å resolution. Many in preparation/review...
- Industrial visits promising too....
- Further details see:
<http://www.diamond.ac.uk/Science/Integrated-facilities/eBIC.html>

People

eBIC appointments:

Peijun Zhang (Director)

Dan Clare

Alistair Siebert

Corey Hecksel

Diamond Light Source staff:

Jean Lane, Alun Ashton, Kevin Savage,

Nick Rees, Michelle Bennett,

Alison Roblin, the EHC team *et al.*

FEI installation and application support team:

Alan Boswell, Alex Buzduga, Sonja Welsch, Felix de Haas, Sacha de Carlo, Alberto Gonzalez *et al.*

Gatan Quantum K2 Installations:

Liam Spillane.

eBIC management:

Dave Stuart, Kay Grünewald, Helen Saibil & Martin Walsh

eBIC opening symposium 24/4/17 – apply online.

