Establishing a cryo-EM laboratory

David Bhella

EMBO practical course in image processing for cryo-EM

Summary

- Obtaining funding
- Planning and building your laboratory space
- Procurement
- Installation and acceptance testing

- The Scottish Centre for Macromolecular Imaging
  - How did we do the above?
  - Experience of the JEOL CryoARM 300

Budgeting - what do I need?

- Microscopes
- Rooms for the microscopes
- Support equipment
- A laboratory for the support equipment
- Up front purchase of service/maintenance
- Consumables
- Computing/Data storage

- People

Microscopes

- Screening (£250k)
- Feeder (£1-2M)
- Data collection (£3.5-4.5M)

Ancillary equipment

- Glow Discharge - (£6k-13k)
- Coater - (£20k-£30k)
- Vitrification Robot - (£60k)
- Grid storage - (£6k)

- Consumables (£10-20k pa)
  - Ethane
  - Liquid Nitrogen
  - Specialist EM grids
  - C-clips/autogrids

Computing resources

- Data storage
  - 1PB DIY ~ £40-60k
  - 1PB Enterprise ~£100-150k
- Archive/Backup
- Computing
  - GPU Workstation
    - Consumer ~£5k
    - Enterprise ~£10k
  - GPU Cluster > £100k

Build costs

People

Budget for feeder site

SCMI
The Scottish Consortium for Macromolecular Imaging

- Microscope
  - side entry 200 kV with DDD £1200k
- Ancillary equipment and consumables £150k
- Build costs
  - Paint the walls, air handling and power £250k
- Computing
  - Workstations £20k
- People
  - 2 FTE 5 years £600k

- Total £2220k

Budget - high end

SCMI
The Scottish Consortium for Macromolecular Imaging

- Microscope
  - 300 keV automated, energy filter DDD £4000k
- Ancillary equipment and consumables £160k
- Build costs
  - Room refit, air-handling, power, control room £500-1000k
- Computing
  - Storage array and backup £500k
  - Cluster £200k
- People
  - 2x FTE 5 years £600k

- Total <u>£6460k</u>

Obtaining funding

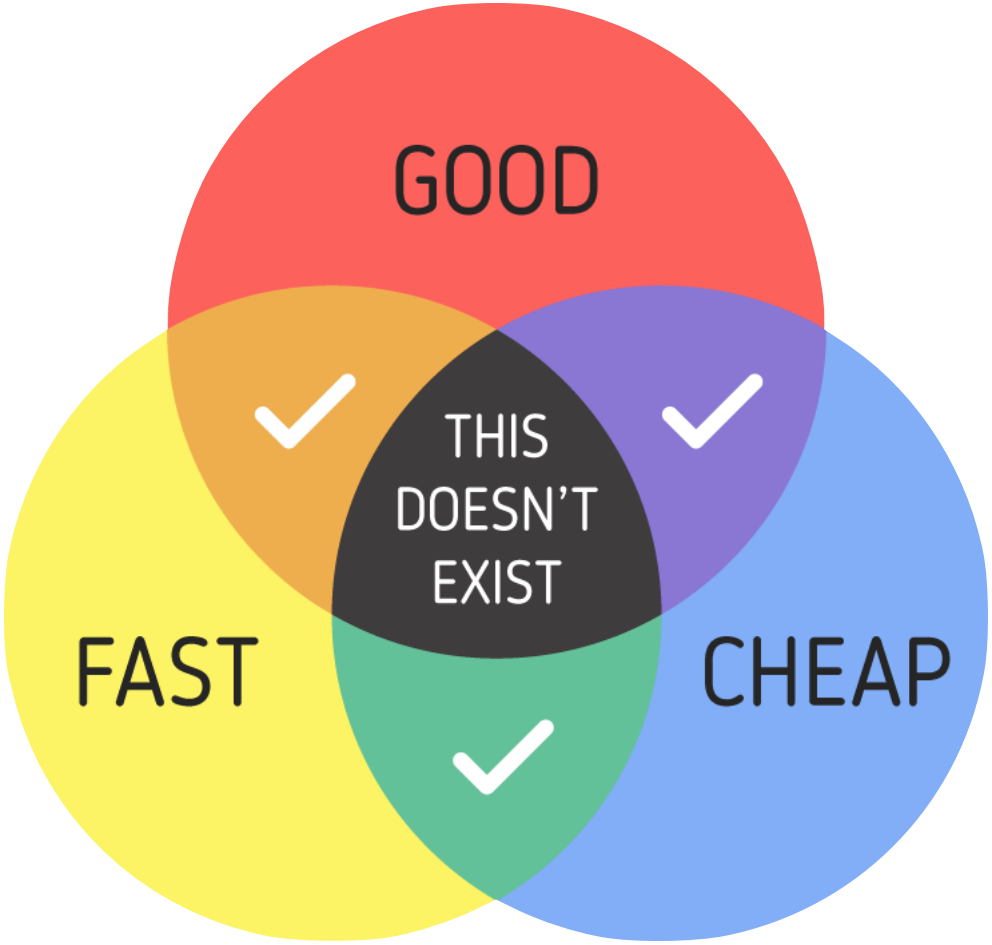
SCMI
The Scottish Consortium for Macromolecular Imaging

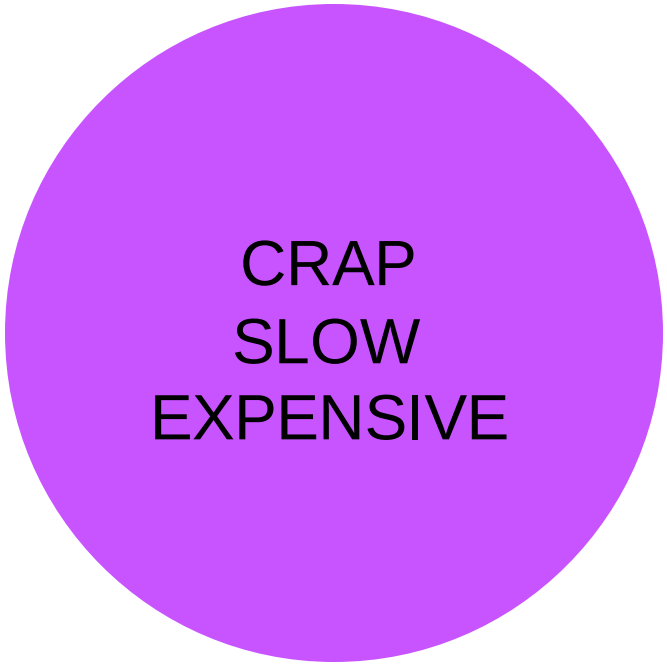
Planning your laboratory space

Building your laboratory space

- Floor
- Air-handling/cooling
- Power
- EM field mitigation
- Noise mitigation
- Lighting

Working with University estates

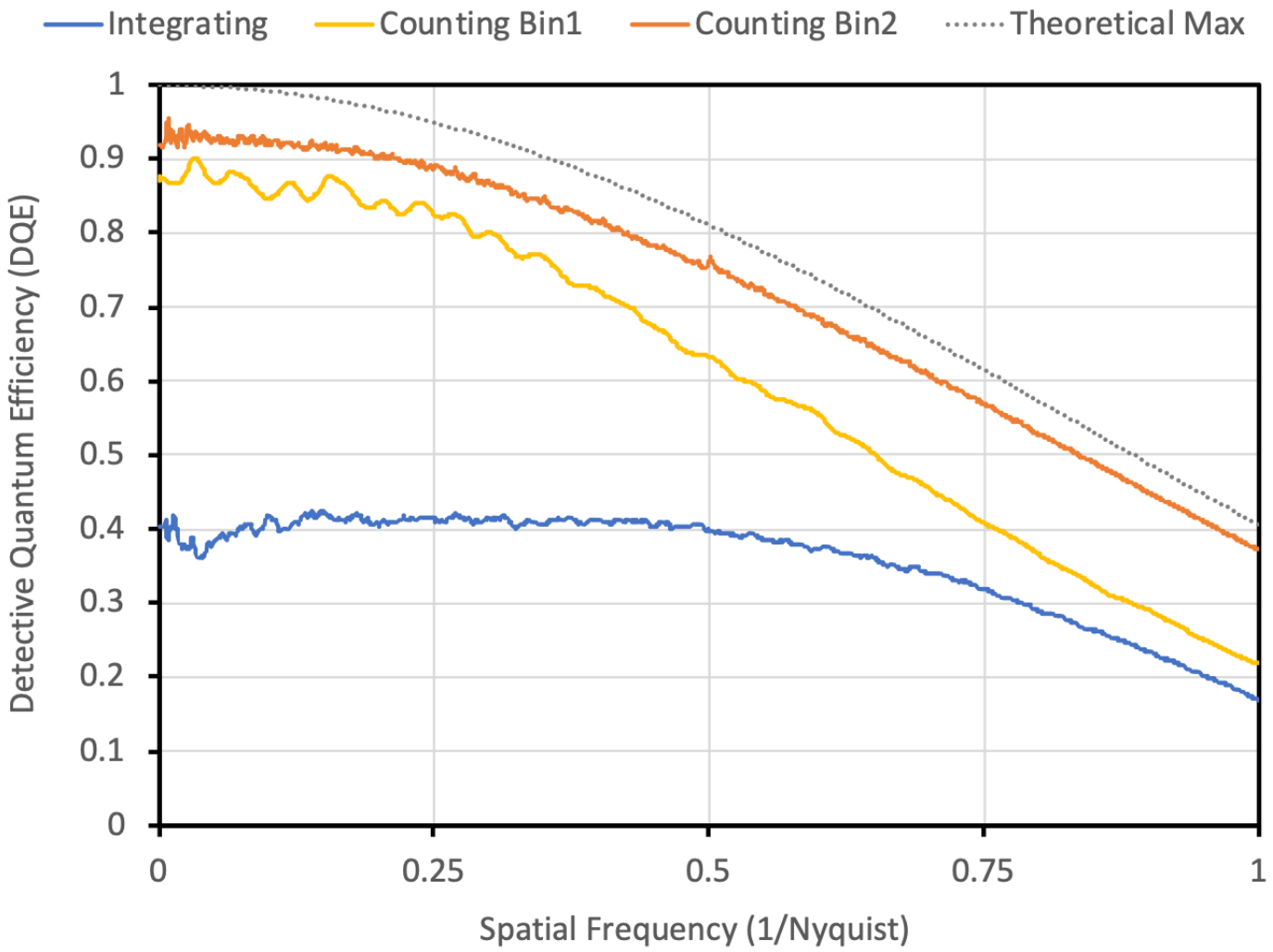




Procurement

- Microscope specification
  - Voltage
  - Gun properties
  - Stage
    - Side entry or autoloader
    - Drift rates
  - Vacuum system
  - Phase plates
  - Energy filter
  - STEM
- Detector specification
  - DQE at 0, 0.5 and 0.8
  - Frame rates
  - Dimensions
  - Counting and linear

Direct Electron DE64



Installation day!

Acceptance tests

Project time-line

0 3 6 9 12

Grant writing

Grant review

Procurement

Delivery lead time

Securing support

Insomnia

Planning/Building

18 21 24 27 30

Column build

Applications/software

Acceptance Sign-off

The Scottish Centre of Macromolecular Imaging

A case study

Wellcome call for CryoEM infrastructure

SCMI
The Scottish Consortium for Macromolecular Imaging

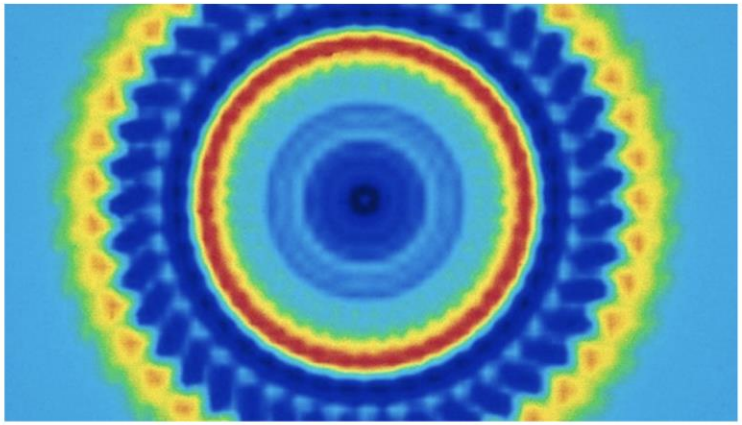
News | 2 March 2017

We're investing £20m in cutting-edge cryo microscopy

We've awarded £20 million for cryo-electron microscopy equipment to support world-leading structural biologists across the UK.

Share this page:

Genetics, genomics and molecular biology



Credit: Helen Saibil, Wellcome Images

A bacterial pore-forming toxin captured by cryo-electron microscopy. The dark blue ring is the protein which attacks host membranes.

Cryo-EM is transforming areas of science essential for improving health, from seeing how drugs get into cells or visualising the atomic structure of a virus to aid vaccine development. This funding will allow scientists to address important biomedical questions that were simply unanswerable a few years ago.

The funded research groups are:

- Professor Gideon Davies, University of York

Contacts +

Latest news

News | 17 January 2019

Researchers can now transfer Wellcome grants outside the UK

News | 14 January 2019

2018 was a flagship year for Innovations – and 2019 is equally promising

SCMI funders

SCMI
The Scottish Consortium for Macromolecular Imaging

MRC Medical Research Council

Scottish Funding Council
Promoting further and higher education

SULSA
Scottish Universities Life Sciences Alliance

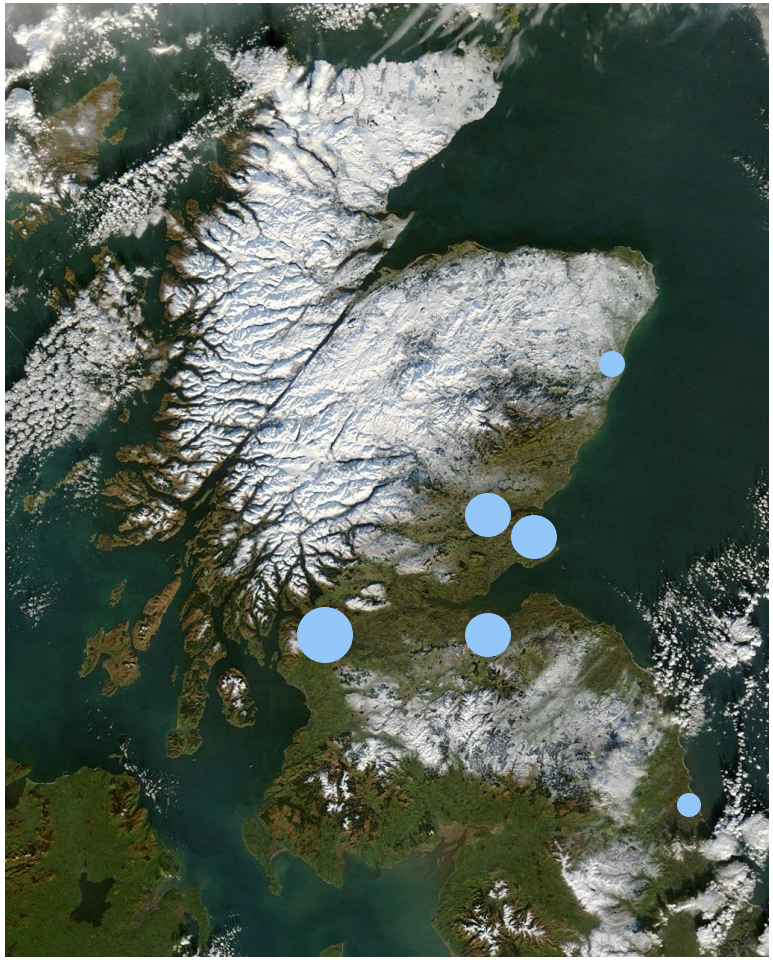
University of Glasgow
VIA VERITAS VITA

THE UNIVERSITY of EDINBURGH

University of St Andrews

University of Dundee

A network for structural biology research



- SCMI is envisaged as a hub and spoke network
  - 200 keV FEG feeder instruments at each partner institution
    - Glasgow - JEOL F2 Cryo
    - Edinburgh - FEI Tecnai F20
    - Dundee - JEOL 2200

Sustainability - recoverable costs

- Maintenance
  - ~£150k per annum
  - First five years included in purchase
- Consumables
  - Liquid nitrogen, power and services
- Data management - IT investment
  - £200k every two years.

Sustainability

- Projected recoverable costs £299k pa

- Subscription rates - based on projected uptime of 255 days pa
  - Tier 1 - £50k pa - 23 sessions (2 or 3 days)
  - Tier 2 - £28k pa - 13 sessions
  - Tier 3 - paid per access academic SULSA £3400/session
  - Tier 4 - paid per access academic non-SULSA £4080/session

  - Glasgow and Edinburgh are signed up for 5 years of tier one access
  - Dundee and St. Andrew's signed up for 5 years of tier two access

Sustainability - cash flow model

SCMI
The Scottish Consortium for Macromolecular Imaging

Capital Investment Proposal
Wellcome Trust CryoEM Facility
Business Plan

Discount Factor	0 1.00	1 0.95	2 0.90	3 0.86	4 0.81	5 0.77	6 0.74	7 0.70	8 0.66	9 0.63	10 0.60
1. Capital Investment Requirements											
Capital Costs £K	**Year 0**	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Year 6**	**Year 7**	**Year 8**	**Year 9**	**Year 10**
Cryo-EM Purchase	(4,300)										
Installation Costs	(230)										
IT Infrastructure Costs	(200)			(200)			(220)				(266)
Total Capital Expenditure £K	**(4,730)**	**0**	**0**	**(200)**	**0**	**0**	**(220)**	**0**	**0**	**0**	**(266)**
Capital Grants / Donations £K											
Wellcome Trust Capital Funding	4,000										
SFC Capital Funding	100										
MRC Capital Funding	400										
St Andrews	75										
Edinburgh	100										
Dundee	25	25	25	25	25						
Glasgow	200										
Sulsa Contribution	60										
Total Capital Grants / Donations £K	**4,960**	**25**	**25**	**25**	**25**	**0**	**0**	**0**	**0**	**0**	**0**
Net Capital Expenditure	**230**	**25**	**25**	**(175)**	**25**	**0**	**(220)**	**0**	**0**	**0**	**(266)**
2. Cashflow Impact on Income & Expenditure											
	Year 0	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Year 6**	**Year 7**	**Year 8**	**Year 9**	**Year 10**
Incremental Income											
University of Glasgow 1FTE contribution		57	59	61	63	64	65	66	67	68	69
Beatson Institute of Cancer Research 1 FTE contribution		38	39	40	41	42	43	44	45	46	47
Income from Gold Subscription Partners to cover running costs (per Billing Appendix)		150	150	150	150	150	150	150	150	150	150
Income from Silver Subscription Partners to cover running costs (per Billing Appendix)		28	28	28	28	28	28	28	28	28	29
Income from Bronze Subscriptions (per Billing Appendix - build up from 25% to 75% capacity over 4 years)		35	49	70	105	105	105	105	105	105	105
Total Incremental Income £K	**0**	**308**	**325**	**349**	**387**	**389**	**391**	**393**	**395**	**397**	**400**
Incremental Costs											
Lab Manager (Grade 8)		(57)	(59)	(61)	(63)	(64)	(65)	(66)	(67)	(68)	(69)
Research Technician (Grade 6)		(38)	(39)	(40)	(41)	(42)	(43)	(44)	(45)	(46)	(47)
Maintenance Contracts							(755)				(831)
Consumables		(60)	(61)	(61)	(62)	(62)	(63)	(64)	(64)	(65)	(66)
Training Costs		(10)	(5)	(10)	(5)	(10)	(5)	(10)	(5)	(10)	(5)
Scientific Advisory Group Costs			(5)		(5)		(5)		(5)		(5)
Total Incremental Costs £K	**0**	**(165)**	**(169)**	**(172)**	**(176)**	**(179)**	**(936)**	**(184)**	**(187)**	**(189)**	**(1,022)**
Net Surplus / (Deficit)	**0**	**143**	**156**	**177**	**211**	**211**	**(545)**	**209**	**209**	**208**	**(623)**
3. DISCOUNTED CASHFLOW	**Year 0**	**Year 1**	**Year 2**	**Year 3**	**Year 4**	**Year 5**	**Year 6**	**Year 7**	**Year 8**	**Year 9**	**Year 10**
TOTAL PROJECT CASHFLOW £K	**230**	**168**	**181**	**2**	**236**	**211**	**(765)**	**209**	**209**	**208**	**(889)**
TOTAL CUMULATIVE PROJECT CASHFLOW £K	**230**	**398**	**579**	**581**	**817**	**1,028**	**263**	**472**	**681**	**889**	**0**

JEOL JEM Z300

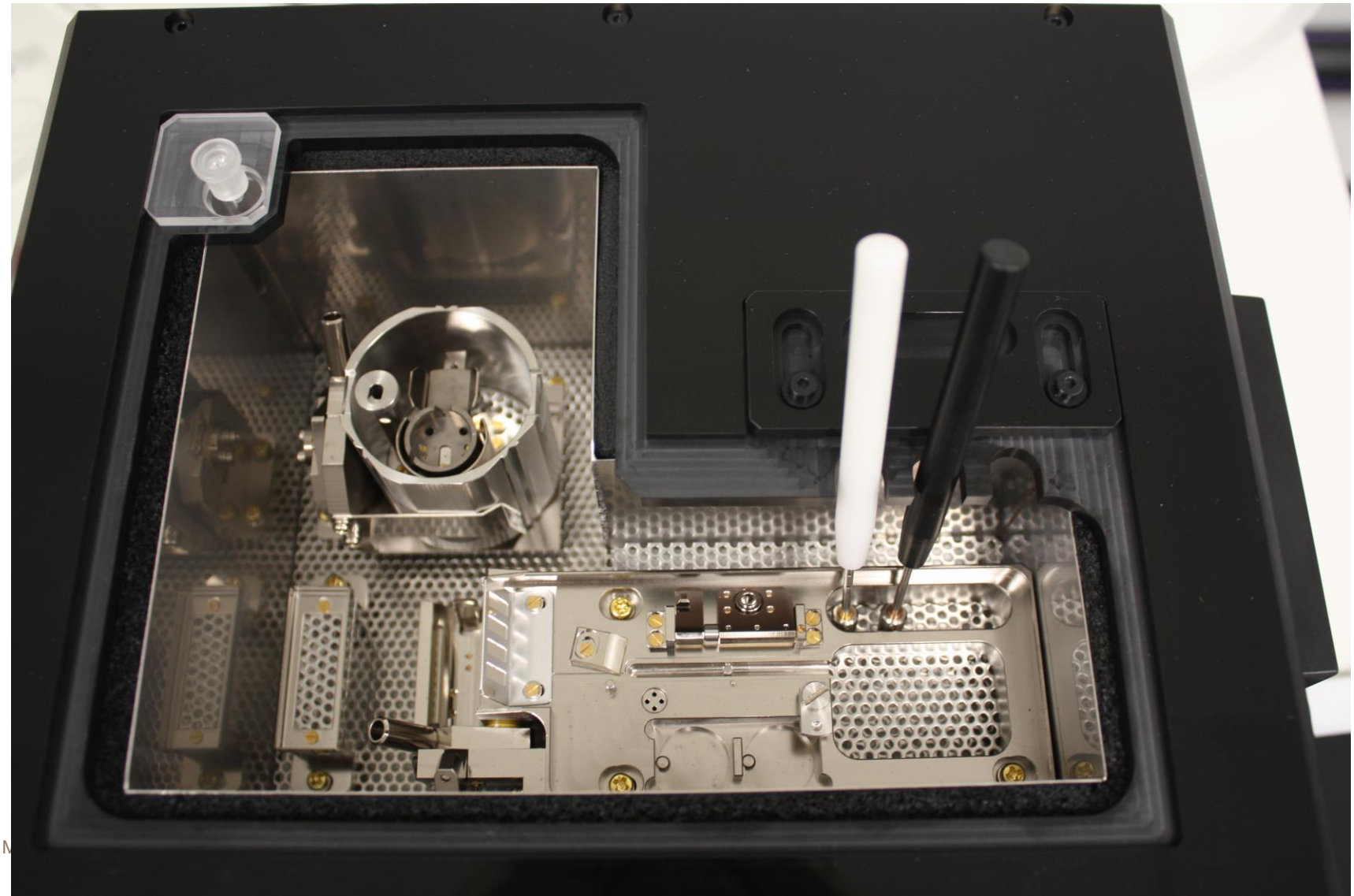
- *"CryoARM 300"*
- 12 slot specimen autoloader
- Automation
- Energy filter
- Phase plate
- Cold field emission gun
- STEM
- Direct Electron DE64

JEOL JEM F200

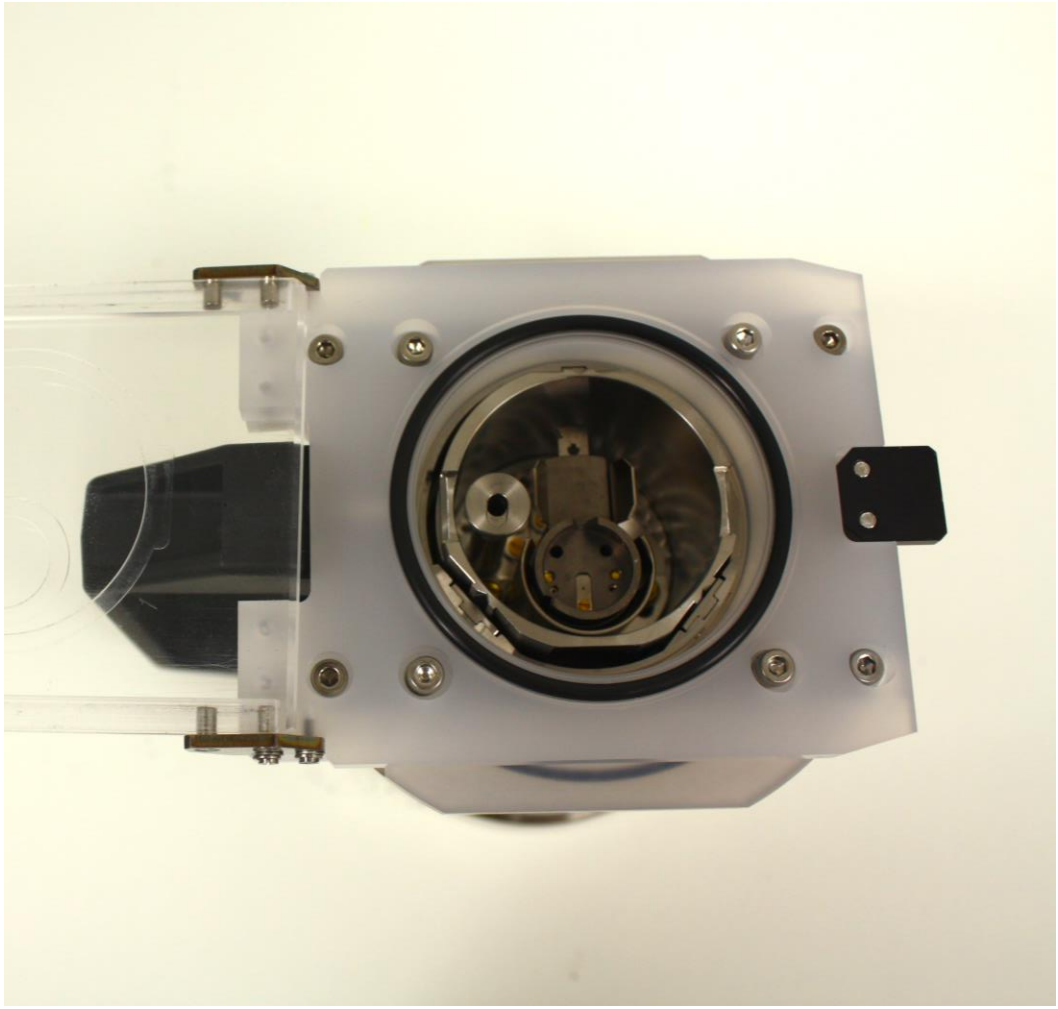
- *“F2-Cryo”*
- Automation
- Phase plate
- Cold field emission gun
- ARM lenses
- STEM
- Direct Electron DE20

Cartridge and loading station

SCMI
The Scottish Consortium for Macromolecular Imaging

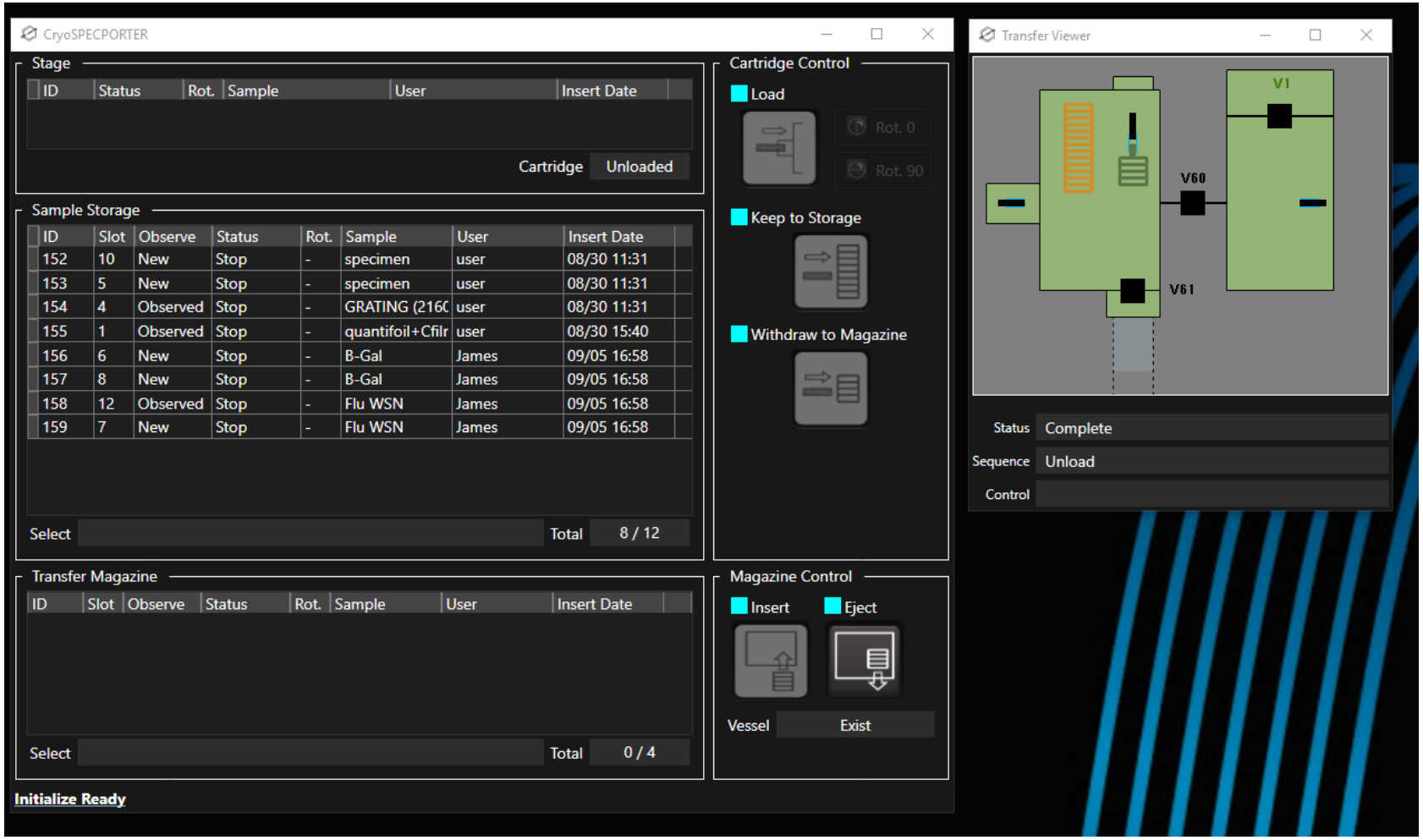


Specimen loading

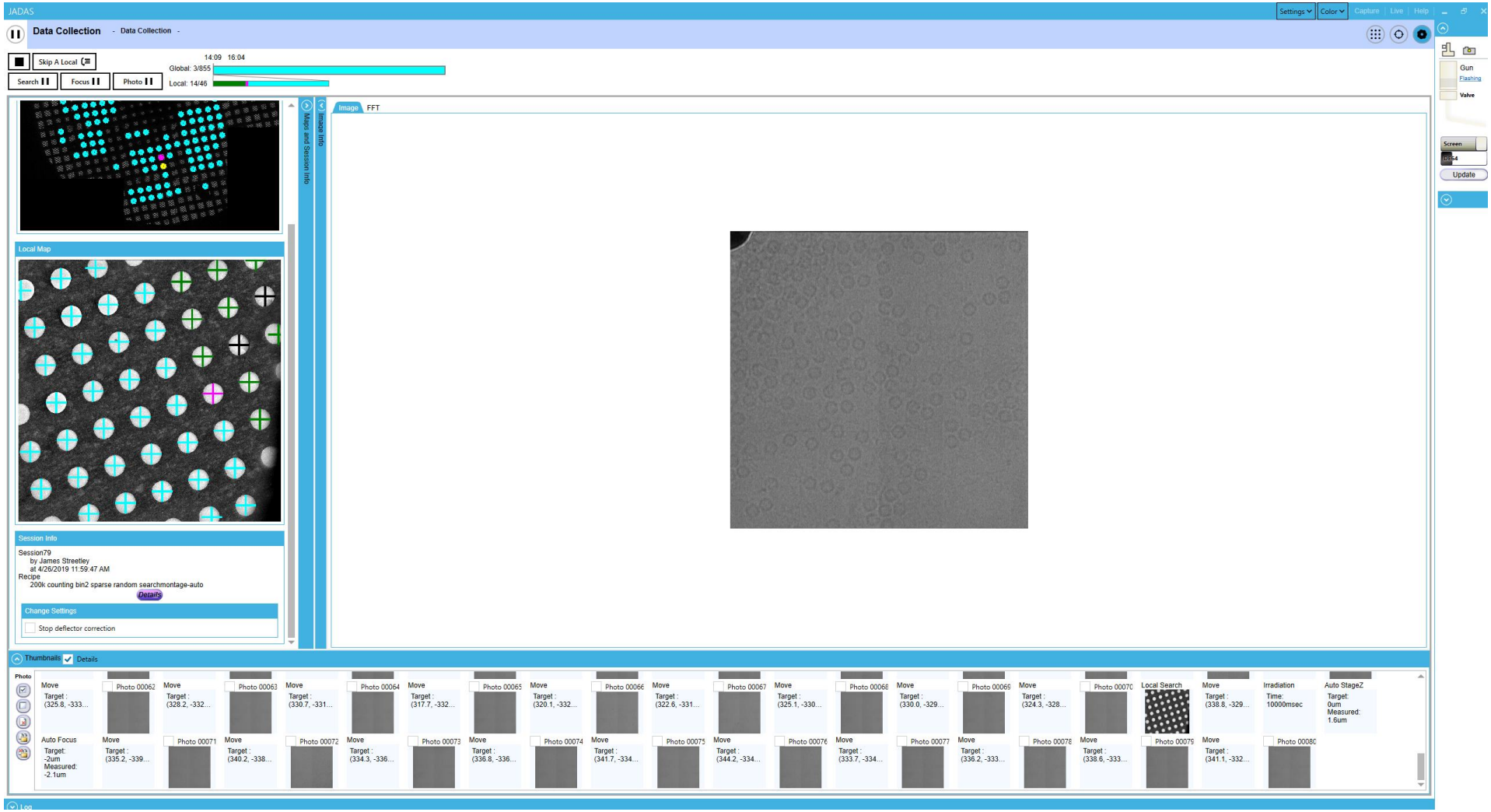


Specimen loading

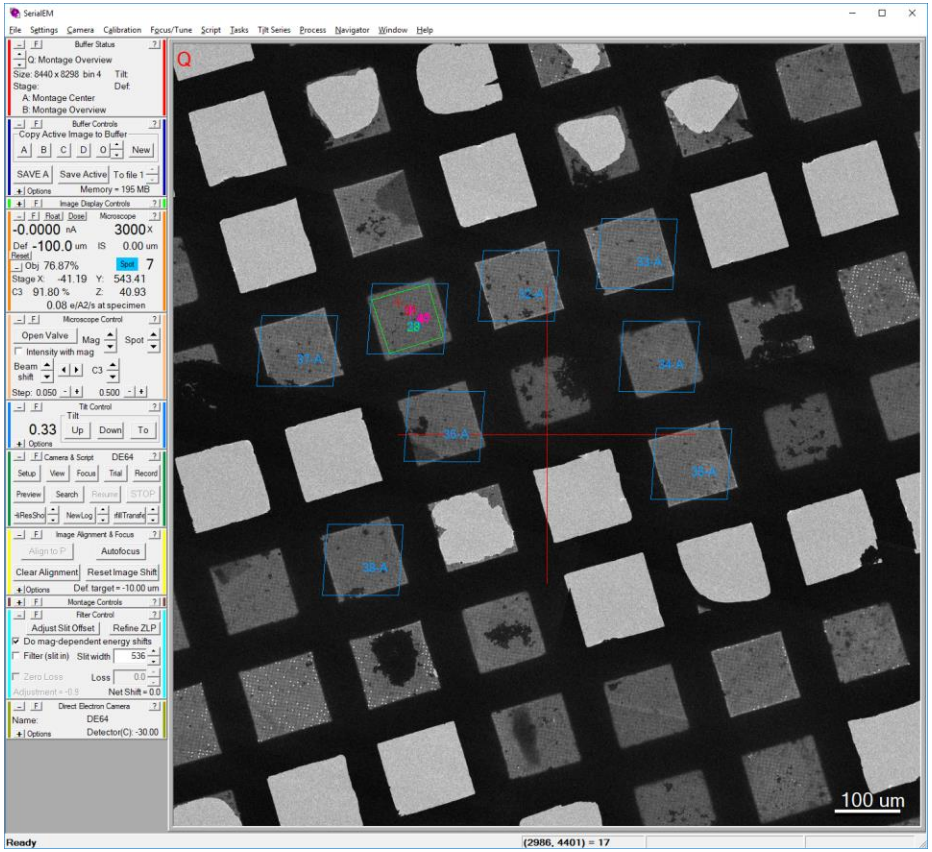
Flexible autoloader

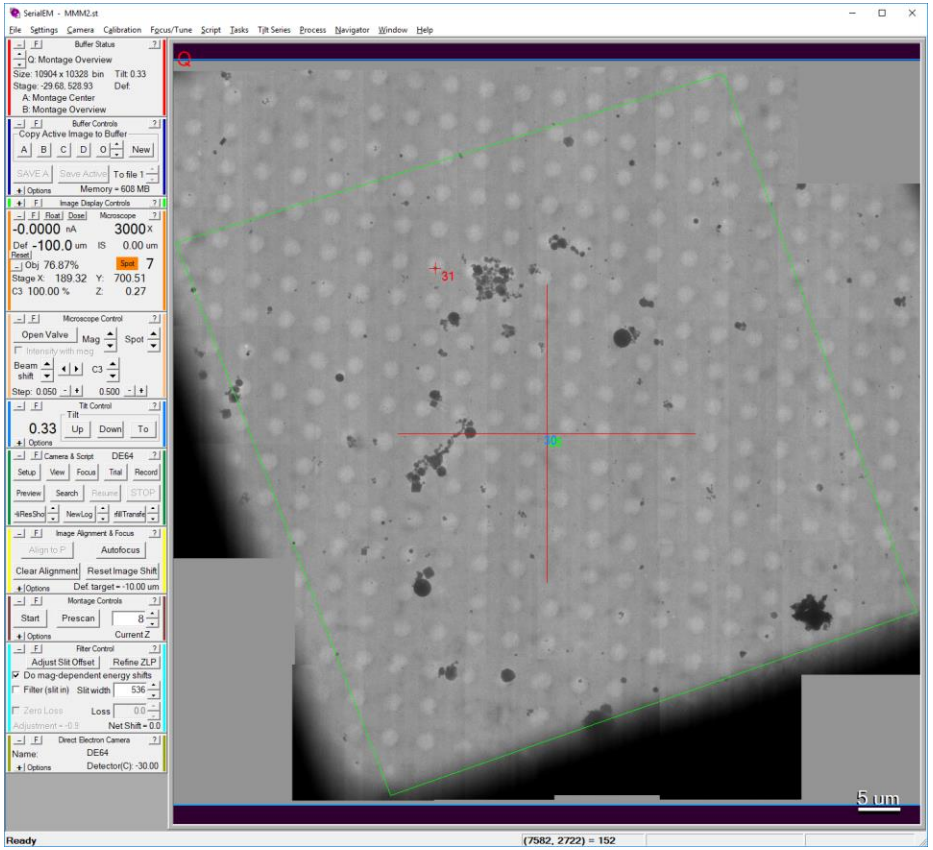


Automation - JADAS



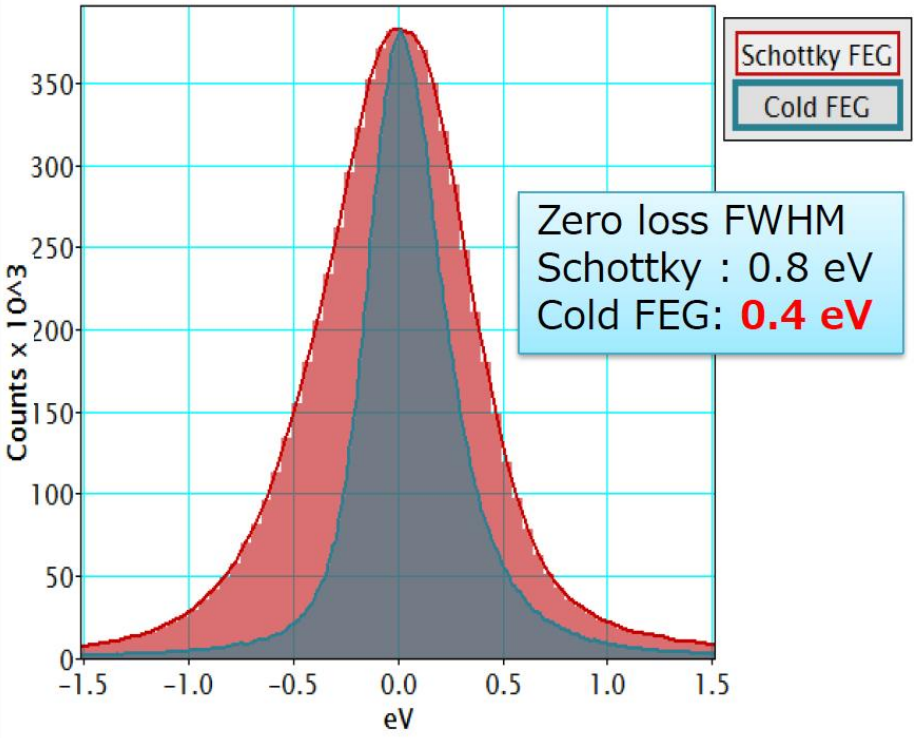
Automation - SerialEM



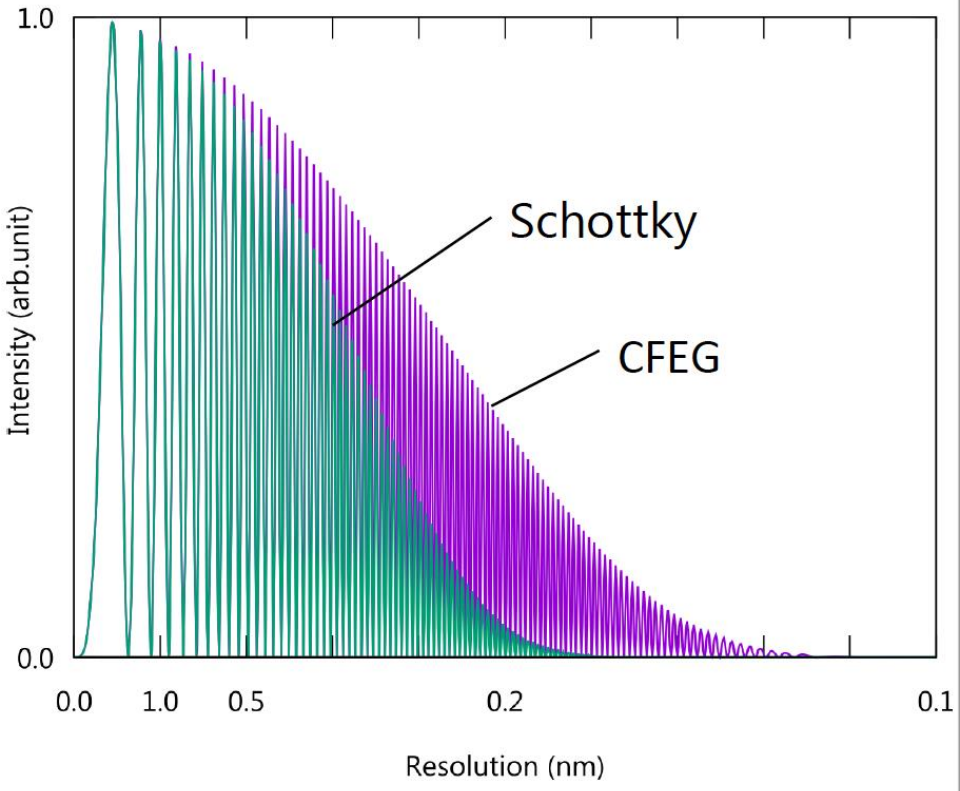


Cold FEG emitter

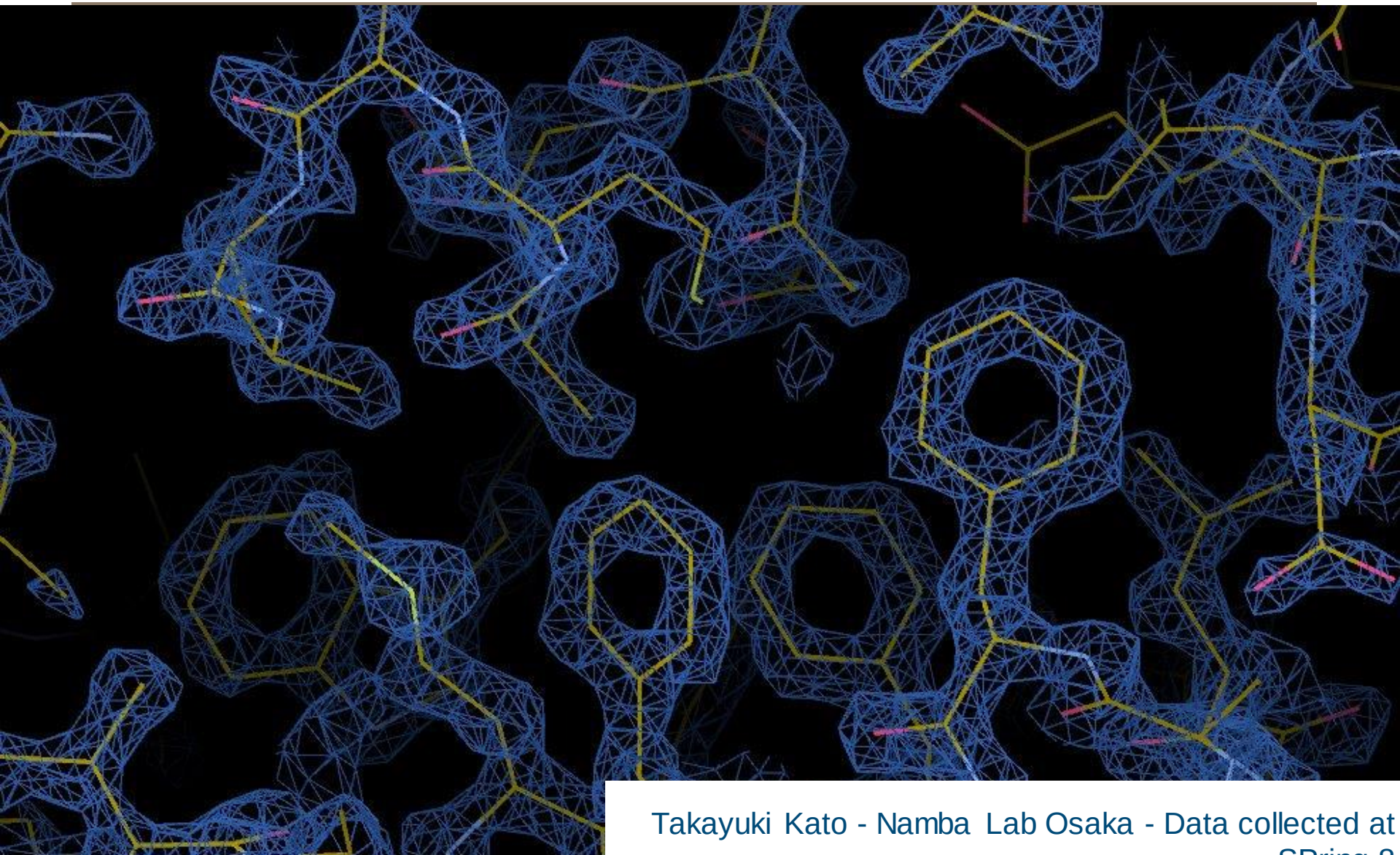
Energy spread of CFEG



Contrast transfer function



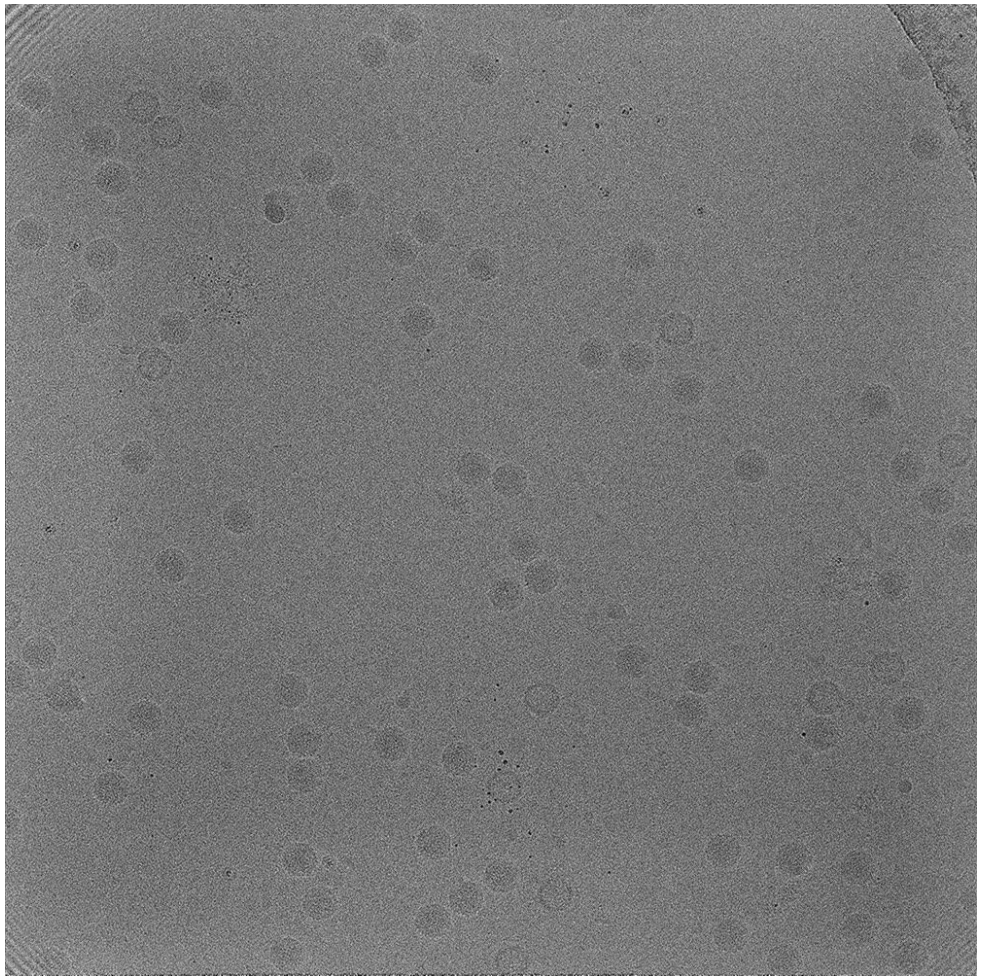
Record-breaking apoferesolution



Takayuki Kato - Namba Lab Osaka - Data collected at

CryoARM first data

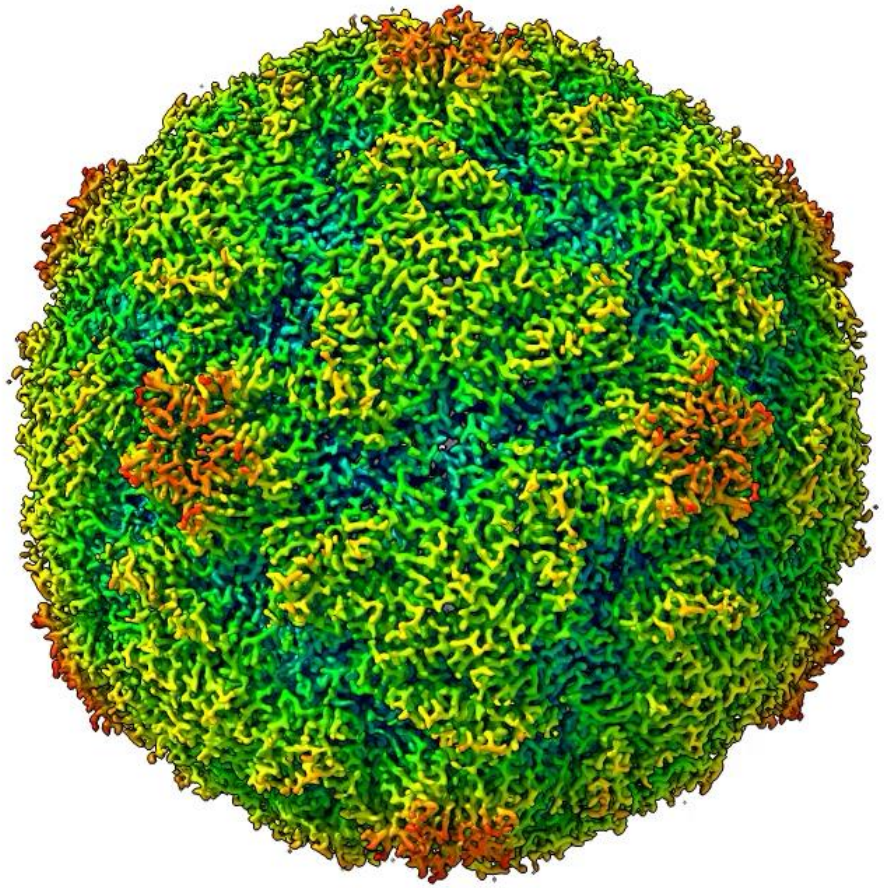
Rhinovirus A16



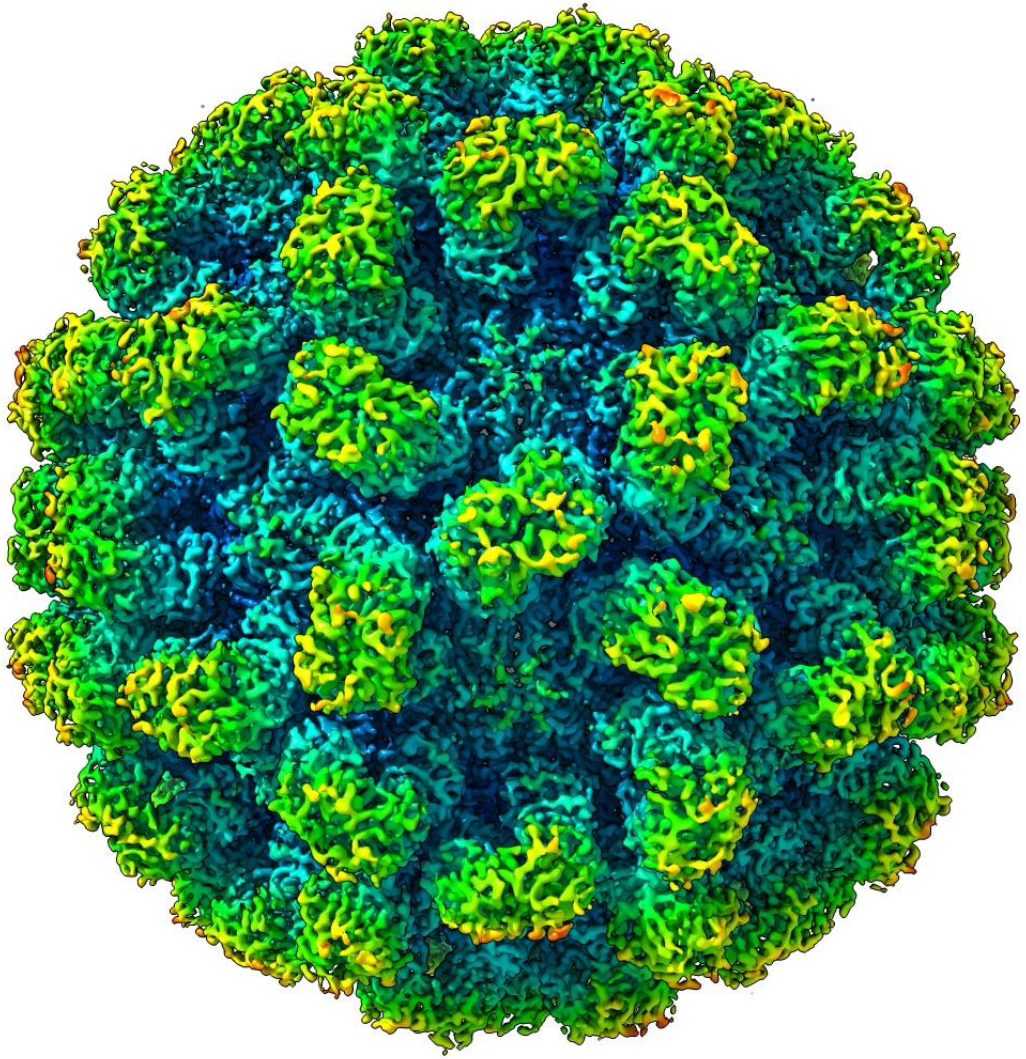
- Total dose ~80 e/Å^2
- Linear 8k x 8k
- 60kx mag (0.996Å/pixel)
- 25 fps
- 2 second exposure

Rhinovirus A16

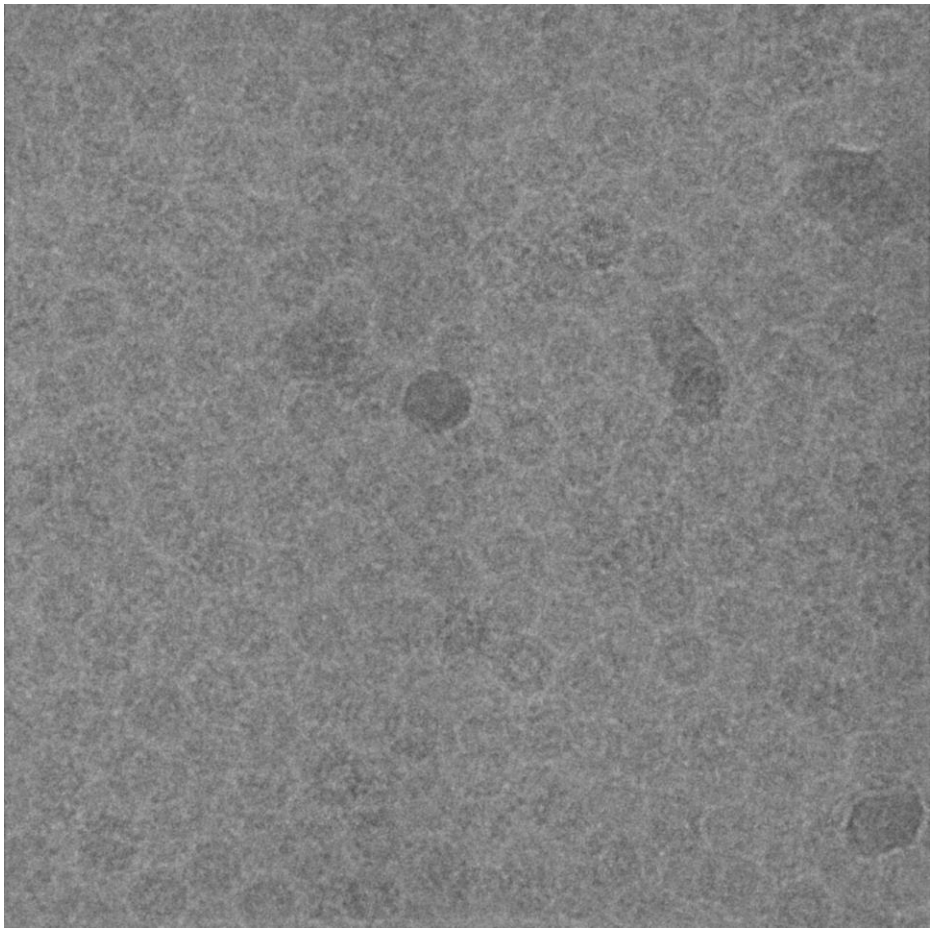
SCMI
The Scottish Consortium for Macromolecular Imaging



Vesivirus 2117



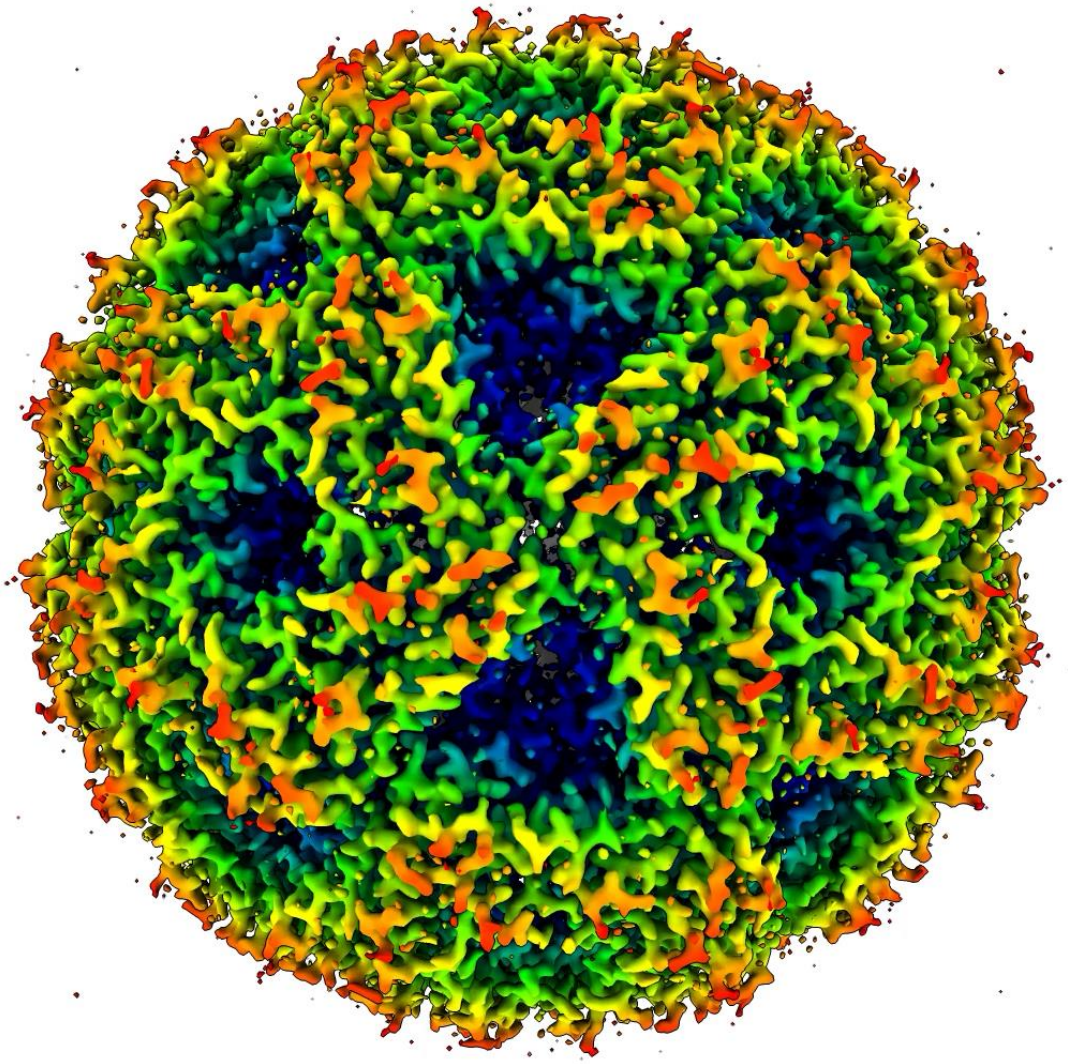
Lumazine Synthase



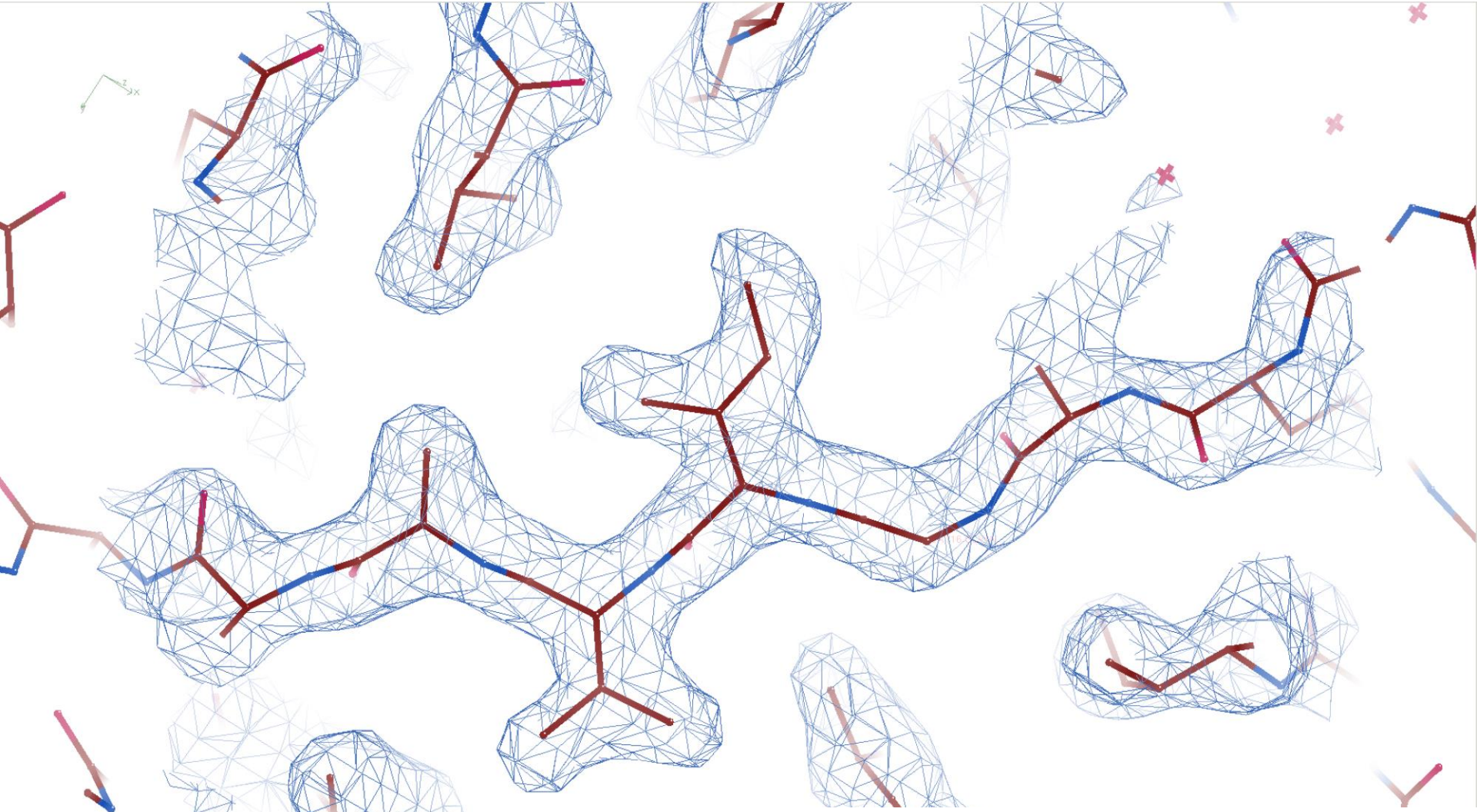
- Total dose 69 e/Å^2
- Bin x2 Counting
- 200kx mag (0.597Å/pixel)
- 141 fps
- 10 second exposure
- 41 frames per movie
- 35 counted frames integrated per frame
- Resolution limit 1.78Å (GCTF)

Lumazine Synthase

SCMI
The Scottish Consortium for Macromolecular Imaging

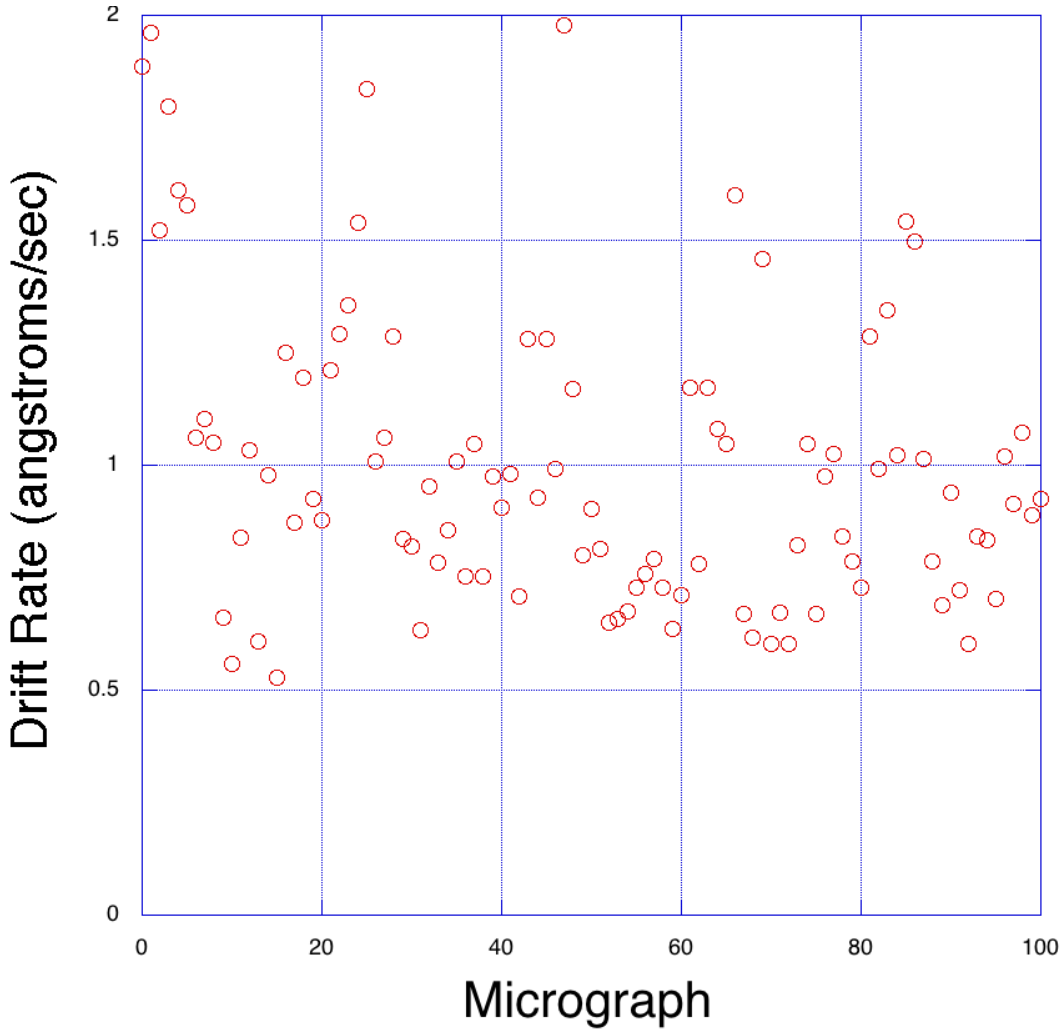


Lumazine Synthase

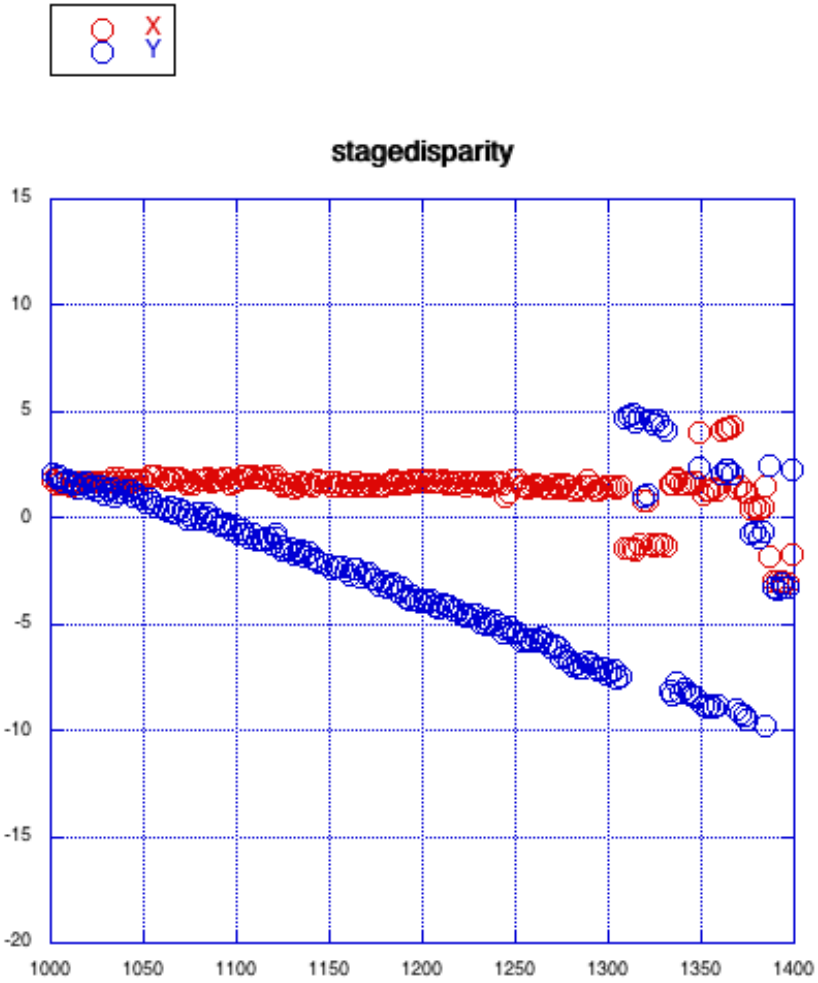


PDB 1HQK - Zhang et al. 2001 JMB 306 (5) pp1099-1114

Performance metrics - drift



Performance metrics - stage disparity



Summary

- CryoARM 300 selected on grounds of novel technologies and innovative design
- CryoARM 300 installation started April 2018
- Software setup and hardware/software troubleshooting has taken time
- Workflow development and hardware troubleshooting ongoing
- SCMI moved to service delivery 1st August 2019

Acknowledgements

- SCMI
  - James Streetley
  - Mairi Clarke
- Rhinovirus
  - Jens Madsen (University of Southampton)
  - Mariam Haider
- Vesivirus 2117
  - Ian Goodfellow (University of Cambridge)
  - Ed Emmott (University of Cambridge)
- Lumazine Synthase
  - Arvind Patel
  - Vanessa Cowton
  - Sarah Cole