The essentials of a cryoEM lab

What do you need and what can you borrow?
How do you assess a new specimen?
How do you service your equipment?
How do you validate your equipment?

Joaquin Ortega

Department of Anatomy and
Cell Biology

McGill

NRAMM Workshop. November 2017

What do you need and what can you borrow?

Possible scenarios for a new Assistant Professor in cryo-EM:

1-Coming to an institution with a tradition in cryo-EM and a stablished cryo-EM core facility

2-Coming to an institution with no tradition in cryo-EM and no cryo-EM core facility

Coming to an institution with a stablished cryo-EM facility

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Search

About FEMR Access Instrumentation Resources Research Publications People Contact Location

Welcome to the Website of the Facility for Electron Microscopy Research at McGill University.

The Facility for Electron Microscopy Research (FEMR) is a world-class, open access electron microscopy facility at McGill University. FEMR offers a comprehensive range of electron microscopy (EM) resources, expertise and services for both routine and advanced sample preparation, electron microscopy imaging, and analysis of biological matter, hydrated and beam-sensitive materials, and ambient temperature materials.

FEMR builds on a long and successful history of supporting researchers throughout the region and beyond (see History). FEMR plays an essential role in maintaining McGill at the leading edge of multidisciplinary research, education and training in the life, materials and physical sciences. The success of FEMR is the result of the combination of scientific expertise, experienced technical staff and well-maintained, state-of-the-art research infrastructure. This infrastructure facilitates both cutting-edge and more traditional tools in EM research.

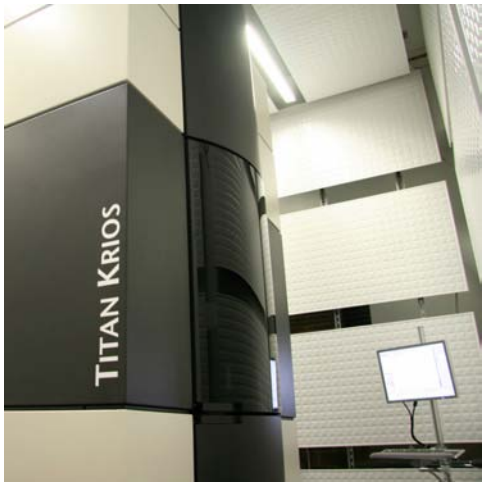
Access to the research infrastructure at FEMR begins with a one-to-one consultation with staff who will provide guidance and advice on the application of electron microscopy to your research project.

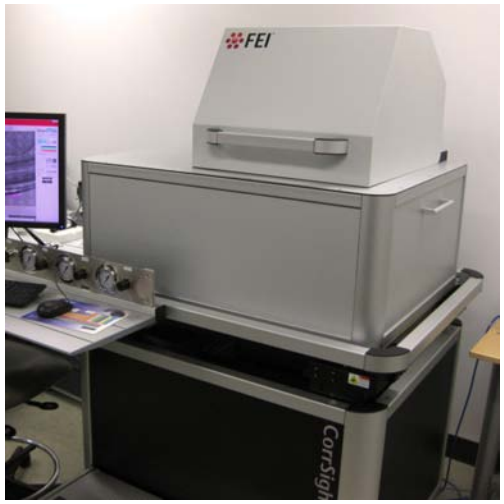
Operation of the FEMR has only been possible through the financial support of Canada Foundation for Innovation (CFI), NanoQuebec-Quebec Nanotechnology Infrastructure (NQ-QNI), Canadian Institutes for Health Research (CIHR), Natural Science & Engineering Research Centres (NSERC) of Canada, Fonds de recherche Santé Québec, and McGill University.

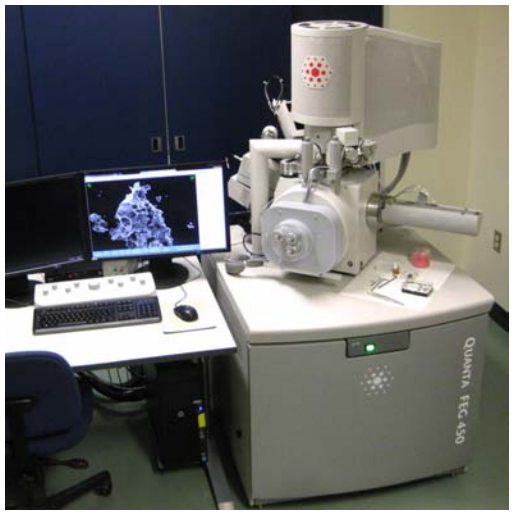
Coming to an institution with a stablished cryo-EM facility

Get to know the instruments available in your facility

Get to know what instruments are useful for your research







Coming to an institution with a stablished cryo-EM facility

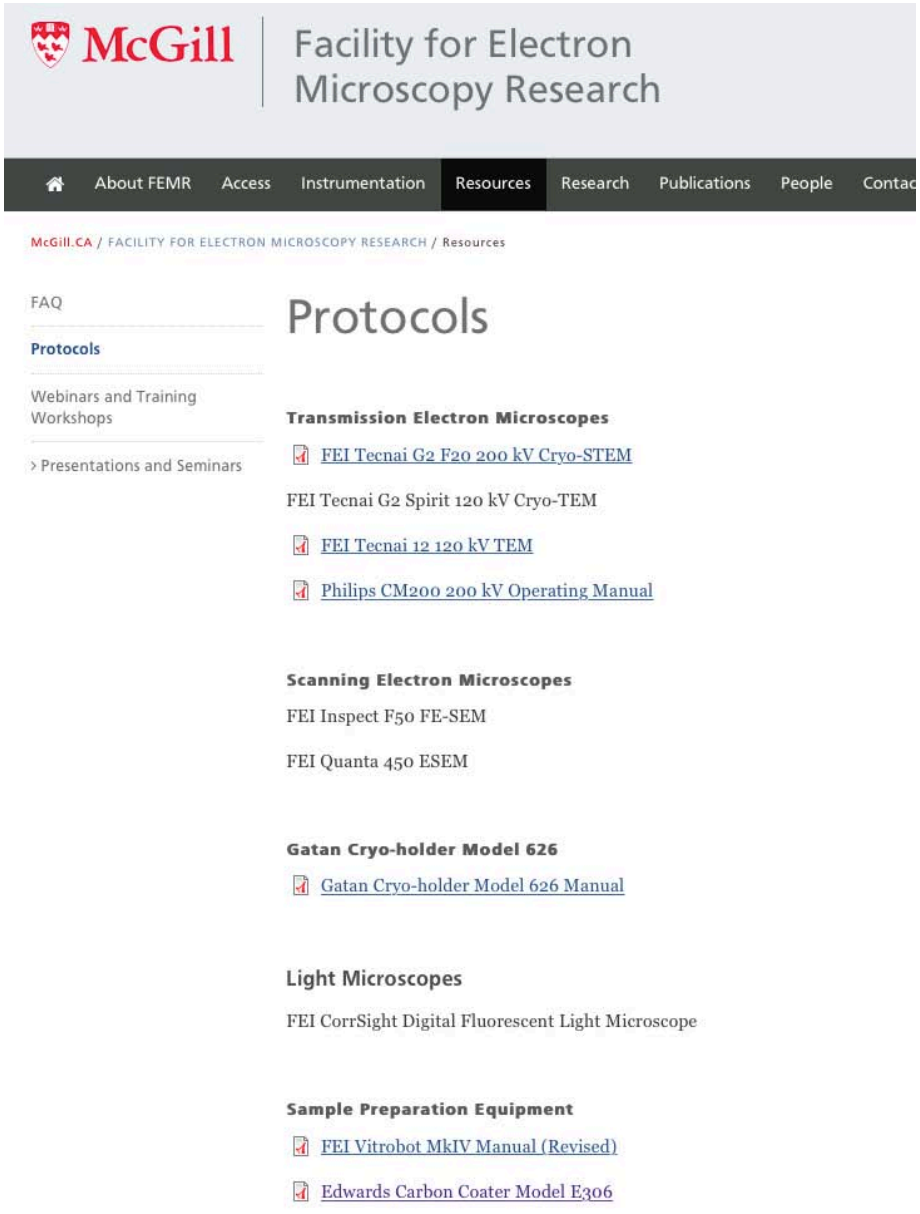
Learn how to use the microscopes. Does the facility has a training program?

Table 1 - Schedule of General Training Sessions

Week	Instrument	Instructor	Building/Room
1	FEI Tecnai 12 - operation, sample loading, negative staining	Jeannie Mui	Strathcona Anatomy & Dentistry Building, Room B30
1	FEI Inspect F50 FE-SEM and Leica ACE600 Sputter Coater	David Liu	W.H. Wong Building, Room 0360; Strathcona Anatomy & Dentistry Building, Room B25
2	Tecnai G2 F20 Cryo-STEM and Gatan Cryo-holder Model 626	Kaustuv Basu/Jeannie Mui	Strathcona Anatomy & Dentistry Building, Room B31
2	Philips CM200 TEM	David Liu	Otto Maas Chemistry Building, Room 215
3	FEI Tecani G2 F20 Cryo-STEM - operation	Kaustuv Basu/Jeannie Mui	Strathcona Anatomy & Dentistry Building, Room B31
4	FEI Quanta 450 ESEM	David Liu	W.H. Wong Building, Room 0310
4	FEI Vitrobot Mk IV	Kaustuv Basu	Strathcona Anatomy & Dentistry Building, Room 147

Coming to an institution with a stablished cryo-EM facility

Use the training resources provided by the facility. Does the facility provide protocols?



Coming to an institution with a stablished cryo-EM facility

How do you access the microscopes? Do they have an scheduler?

<Print Preview> <Simple View>

Building
Otto Maass Chemistry Building
Rutherford Physics Building
SADB - Sample Preparation
Strathcona Anatomy & Dentistry
Wong Building

Microscope:
FEI CorrSight DFLM
Helios Nanolab DualBeam
Tecnai 12 120 kV TEM
Tecnai G2 F20 Cryo-STEM
Tecnai G2 Spirit 120 kV
Titan Krios Cryo-STEM

September 2017

S	M	T	W	T	F	S
					1	2
3	4	5	6	7	8	9
10	11	12	13	14	15	16
17	18	19	20	21	22	23
24	25	26	27	28	29	30

October 2017

S	M	T	W	T	F	S
1	2	3	4	5	6	7
8	9	10	11	12	13	14
15	16	17	18	19	20	21
22	23	24	25	26	27	28
29	30	31				

November 2017

S	M	T	W	T	F	S
			1	2	3	4
5	6	7	8	9	10	11
12	13	14	15	16	17	18
19	20	21	22	23	24	25
26	27	28	29	30		

Strathcona Anatomy & Dentistry - Tecnai 12 120 kV TEM

<< Go To Week Before Go To This Week Go To Week After >>

Time:	Sun Oct 22	Mon Oct 23	Tue Oct 24	Wed Oct 25	Thu Oct 26	Fri Oct 27	Sat Oct 28
00:00	S Kelly Sears		S Kelly Sears				
01:00	"		"				
02:00	"		"				
03:00	"		"				
04:00	"		"				
05:00	"		"				
06:00	"		"				
07:00	"		"				
08:00	"		"				
09:00	"		"		Tanzila Wasi (04-2018)	Ximena Zottig (03-2016)	
10:00	"	Jeannie Mui	"	Mathew Sebastiao (07-2016)	"	"	
11:00	"	"	"	"	"	Regiana de Oliveira (06-2017)	
12:00	"	"	"	Regiana de Oliveira (06-2017)		"	
13:00	"	Isabelle Rouiller	"	"		"	
14:00	"	Regiana de Oliveira (06-2017)	Isabelle Rouiller	Yao Shen (04-2017)	Regiana de Oliveira (06-2017)	Camila de Britto Para de Aragao (11-2015)	
15:00	"	"	"	"	"	"	
16:00	"	"		Jeannie Mui	"	"	
17:00	"			"	Yao Shen (04-2017)		
18:00	"				"		
19:00	"						
20:00	"						
21:00	"						
22:00	"						
23:00	"						

Coming to an institution with a stablished cryo-EM facility

Does any of the microscopes has any special access requirements? Krios?

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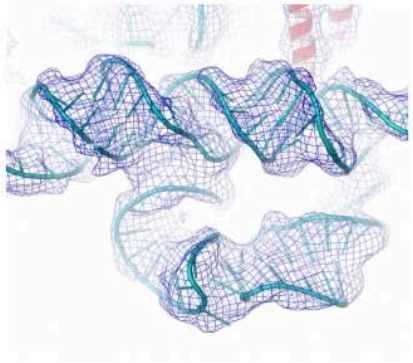
- Starting Your Research Project
- › Training
- ˅ Access to FEI Titan Krios Cryo-STEM
  - Project Waiting List for Titan Krios
- Access to FEI Helios Nanolab 660 DualBeam
- Project Form
- Project Form - FEI Titan Krios Cryo-STEM
- Scheduler
- Afterhours Building Access
- Internal Responsibility System

Accessing the FEI Titan Krios Cryo-STEM



Access to Titan Krios Cryo-TEM

The Titan Krios Cryo-STEM is a high-throughput instrument capable of collecting large volumes of data in an autonomous manner. It is, however, an extremely expensive instrument to operate and in high-demand by research groups throughout the region and beyond.

To maximize effective access and throughput of this instrument, FEMR has a strict policy of screening procedures and quality checks before any sample may be loaded into the Krios.

The first steps to accessing the Titan Krios cryo-STEM is, as for any other project, to complete and submit the standard Project Form and Project Form for Cryo-EM and attend an Assessment Interview. Once the project has been identified as appropriate for this instrument, submission of samples will follow the procedure for cryo grid quality control for loading into the Krios.

Procedure for Cryo Grid Quality Control

No samples may be loaded into the Krios without being previously screened by negative-stain TEM and cryo-EM.

Negative-stain TEM is the conventional method of staining samples on a TEM grid with uranyl acetate or uranyl formate and imaging in the FEI Tecnai 12 or FEI Tecnai Spirit 120 kV TEM.

How do you asses a new specimen?

Does any of the microscopes has any special access requirements? Krios?

Negative staining screening

Cryo-EM grid screening

High-resolution data collection

Can I load my samples on the Krios?

Coming to an institution with a stablished cryo-EM facility

Very important… get to know the people running the facility…

Kaustuv Basu
FEMR Krios operator

And be nice to them…

Coming to an institution with no cryo-EM facility

As a new Assistant Professor have reasonable expectations…

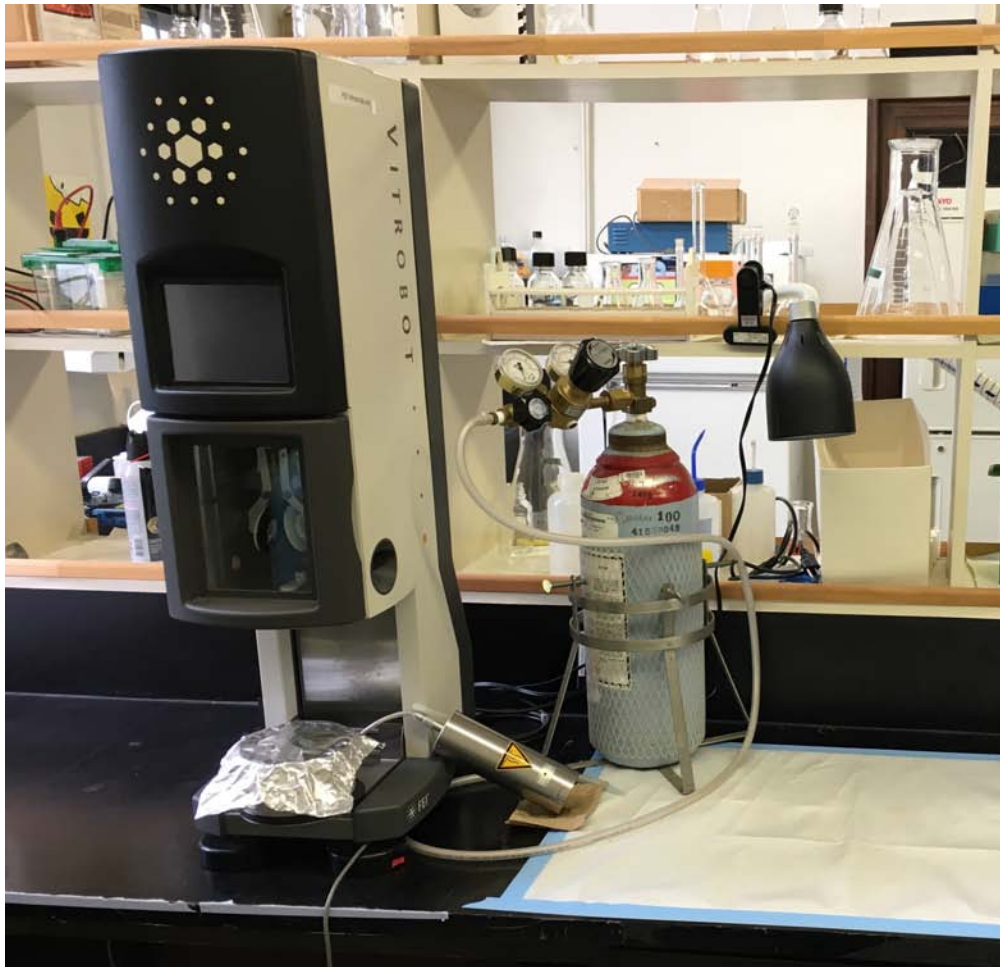
What if an Arctica for screening and a Krios for data collection is not on your recruitment package?…

What do you need to move your research program forward in cryo-EM? What are the essentials?

Coming to an institution with no cryo-EM facility

Equipment for sample preparation

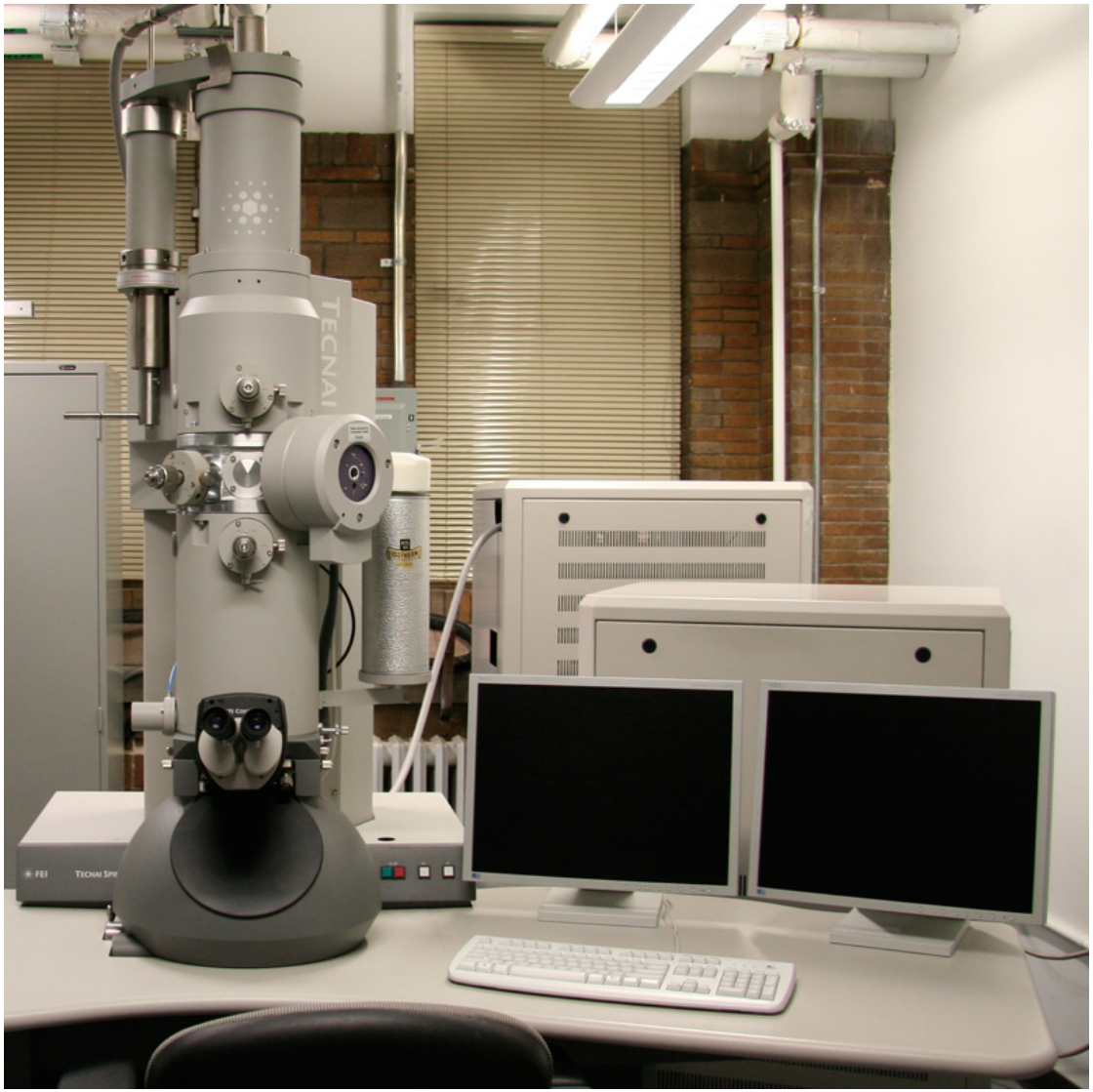
Carbon coater with glow discharge



Vitrobot for specimen vitrification

Coming to an institution with no cryo-EM facility

Screening electron microscope for cryo-EM



FEI Tecnai Spirit with Gatan Ultrascan 4000 CCD at FEMR…

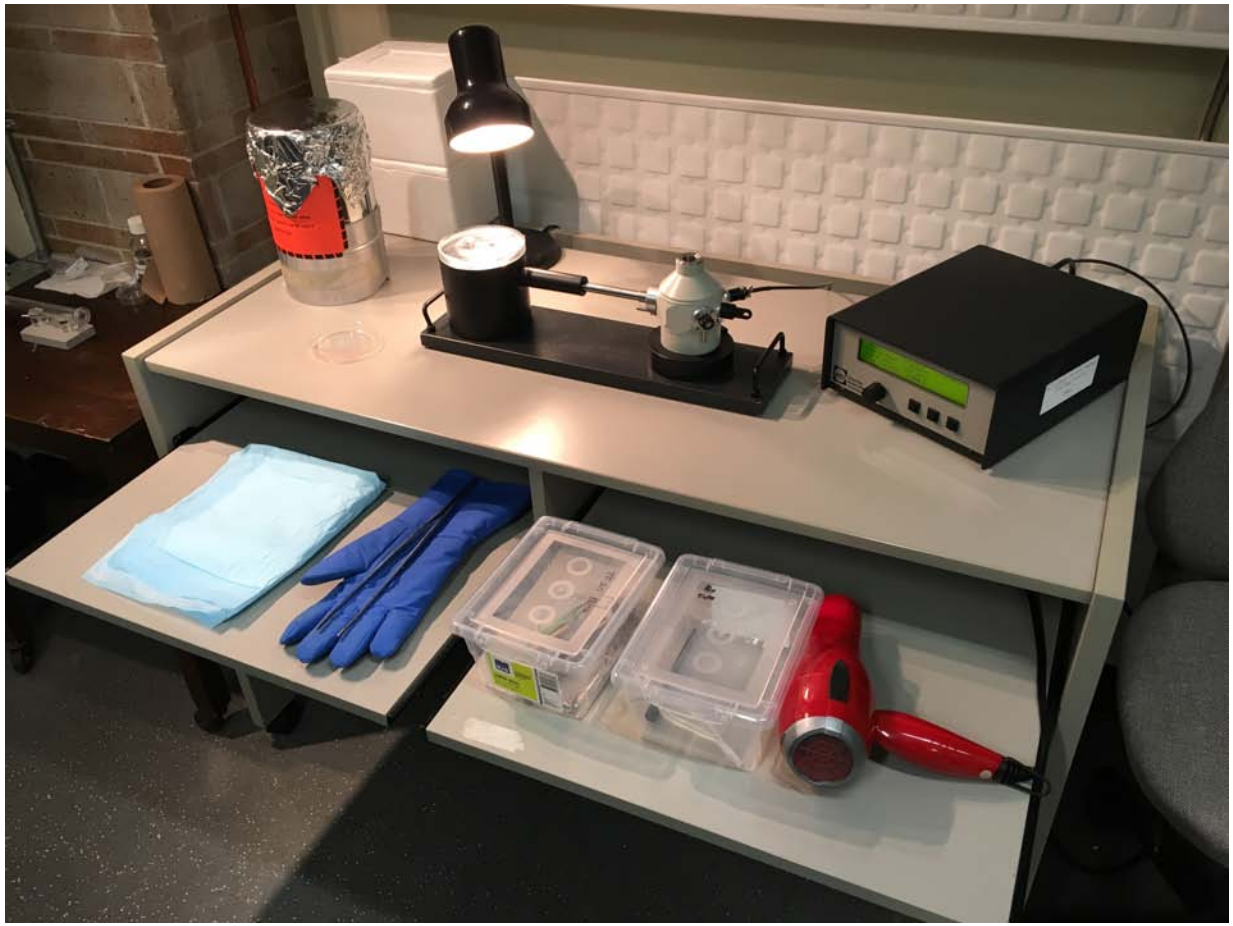
Coming to an institution with no cryo-EM facility

Screening electron microscope for cryo-EM

FEI Tecnai F20 with Gatan Ultrascan 4000 CCD at FEMR

Coming to an institution with no cryo-EM facility

Cryoholder for sample transfer to the microscope



Cryoholder and controller box

Pumping station for cryo-holder

Coming to an institution with no cryo-EM facility

But how do you access a Krios for high-resolution data collection?

In the US and Europe you may have access to National Resources:

- NRAMM, NCI-supported National Cryo-EM Facility (NCEF), Diamond

In other countries there are core EM facilities where you can access to a Krios by paying a fee-for-use:

- NeCEM in the Netherlands
- FEMR at McGill (Canada)

Get guaranteed access to these facilities as part of your recruitment package

How do you service your equipment?

You must have your microscopes under service contract

If you have multiple instruments from the same vendor you can negotiate discounts

What about your DED? Do you need a service contract as well?

Strategies to minimize downtime on the microscopes: Have dedicated operators for high end instruments (Krios, FIB), train well your users.

If possible have two of those items that could constitute a bottleneck if they break: cryo-holder

How do you validate your equipment?

Important to have performance evaluation and quality checks on your system.

Microscopes gets aligned by staff once a week and alignments are saved

Acquire an information limit image using a standard gold/carbon cross-grating grid (every week or after service or if microscope went down).

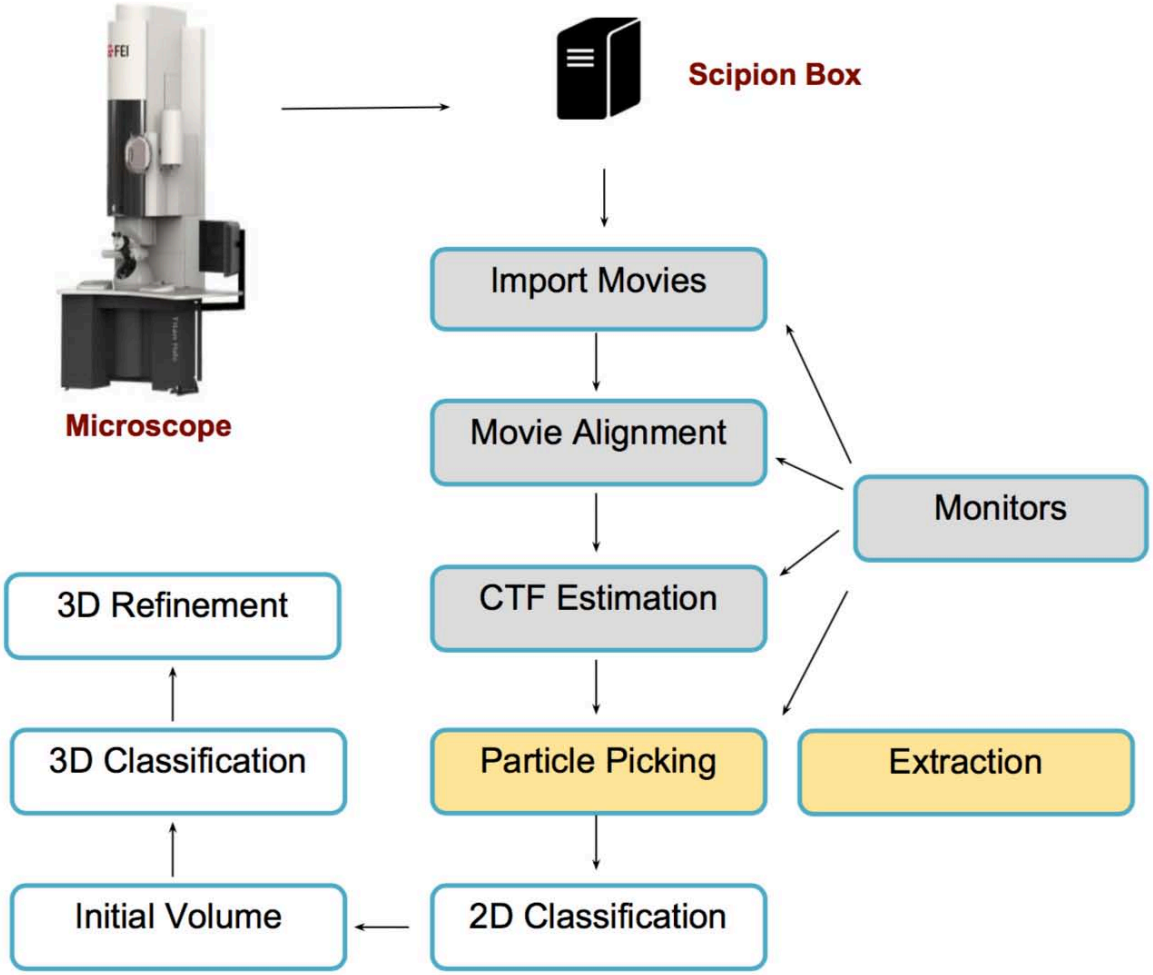
Evaluating the DQE of the camera (twice a year).

Obtain a 3D reconstruction of a 20S proteasome using standardized data collection and processing procedures. Ensures performance of the microscope + DED + processing pipeline (Once year).

Results of these test should be tracked over time and correlated with modifications or repairs.

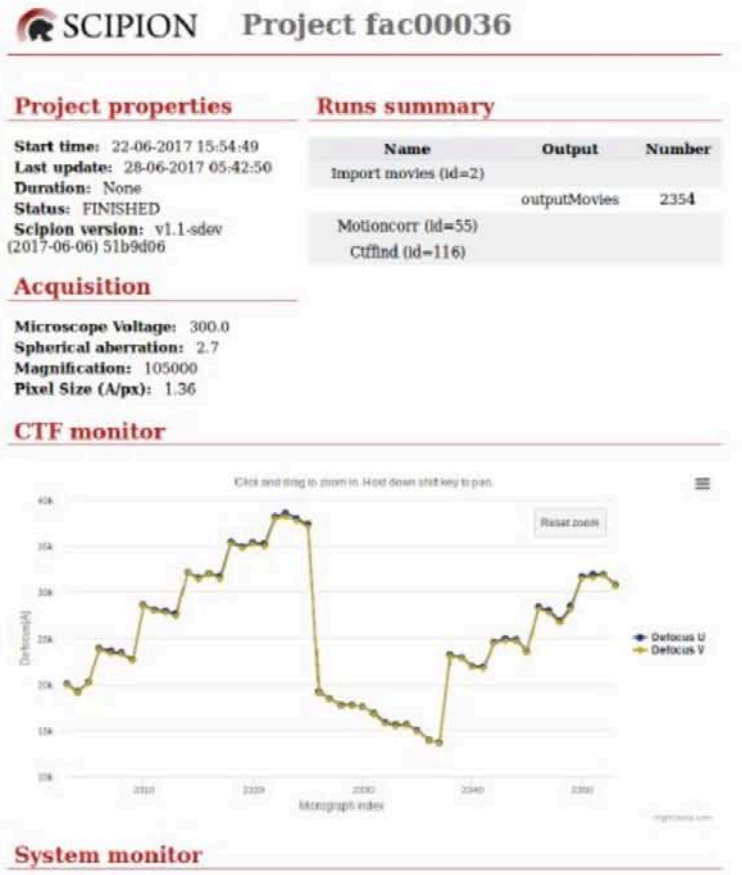
How do you validate your equipment?

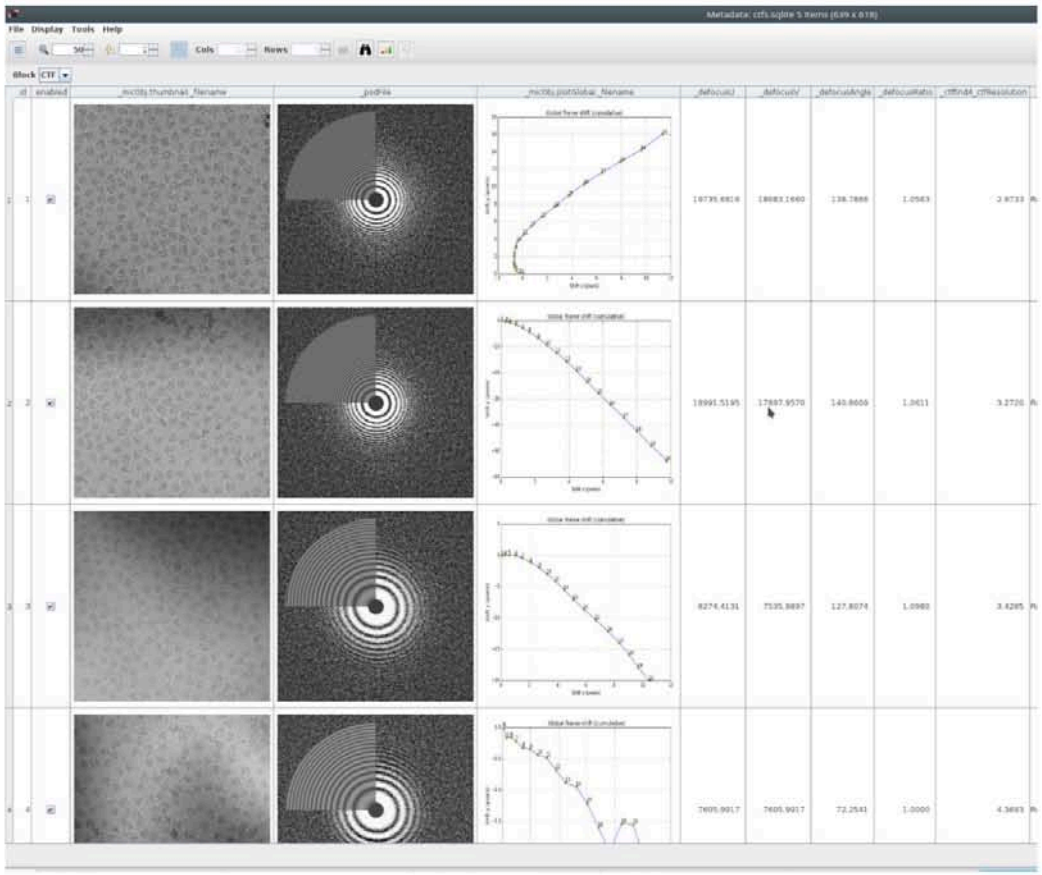
Streaming systems enable efficient use of microscope time and constantly evaluate the performance of the instruments



From C.O. Sorzano

How do you validate your equipment?





From de la Rosa Trevin

Thank you for your attention!