

Using automation to help your EM imaging



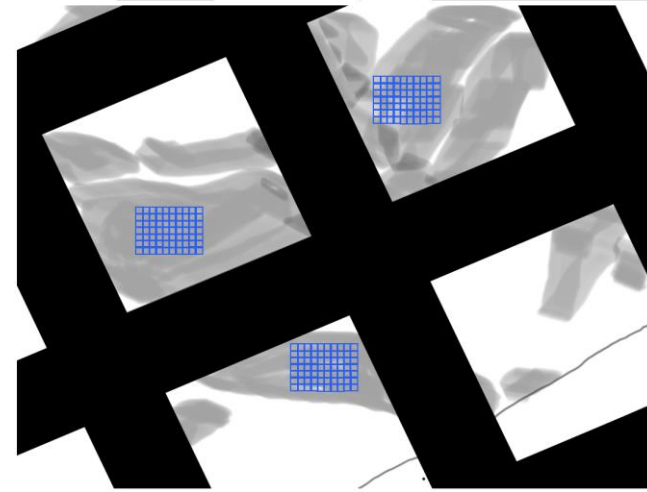
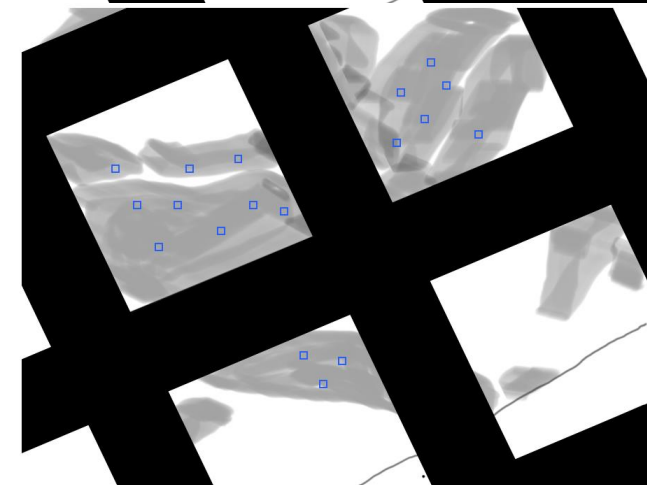
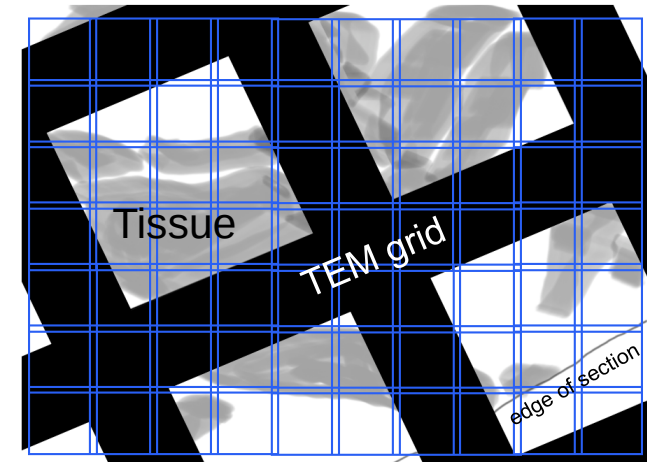
Matt Russell, CUI at KCL
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Overview

- Intro to automation of tasks in electron microscopy
 - So you can see what's possible
 - How to do it
- You may not need any of this!
- Other methods still have their place, eg Toluidine blue staining
- Pick and choose automation as needed
- Automation can be done with existing standard equipment

Tasks to automate in EM imaging

- Mapping and navigation
 - Low mag montage
 - Where is my section/sample?
 - Where are the regions of interest (ROIs)?
 - Overlay correlative LM map for CLEM
 - Share map with pathologist
- Imaging of multiple features
 - Higher mag multi-ROI
- Imaging of a large feature
 - Higher mag tile scans
- Imaging of lots of large features
 - Multi-ROI tile scans
- Volume imaging. For example;
 - Batch TEM tomography,
 - Serial imaging (TEM, SEM)



Software for EM automation – various!

- **SerialEM**

- Mastronarde Group
- Open source
- TEM

- **LLP/Shotmeister**

- JEOL/System in Frontier
- TEM

- **SEMography Recorder**

- JEOL/System in Frontier
- SEM

- **MAPS**

- Thermofisher Scientific
- TEM, SEM

- **Atlas 5**

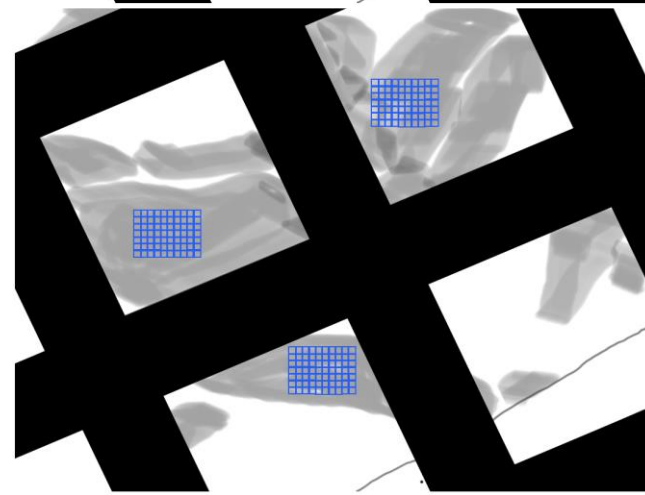
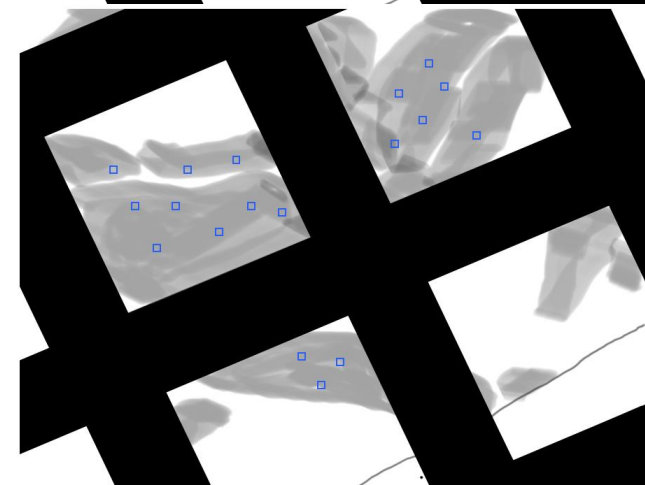
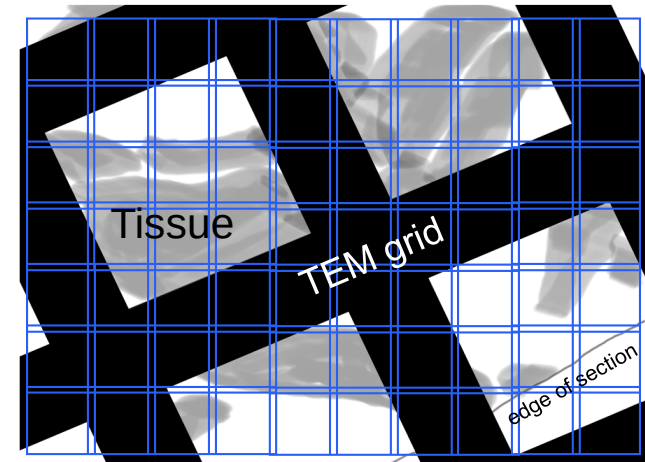
- Zeiss
- SEM

- **AZtec**

- Oxford Instruments
- TEM, SEM

Tasks to automate in EM imaging

- Various software environments are available
- Similar processes needed
 - Mapping and navigation
 - Cross-correlation to track and refind ROIs
 - Autofocus
 - Alignment (with different Mag/LM/etc.)
 - Tile stitching
- Similar workflows possible across these software environments
- I'll illustrate these with a few examples
 - You don't have to use the software I used!



TEM automation example; human skeletal muscle



The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

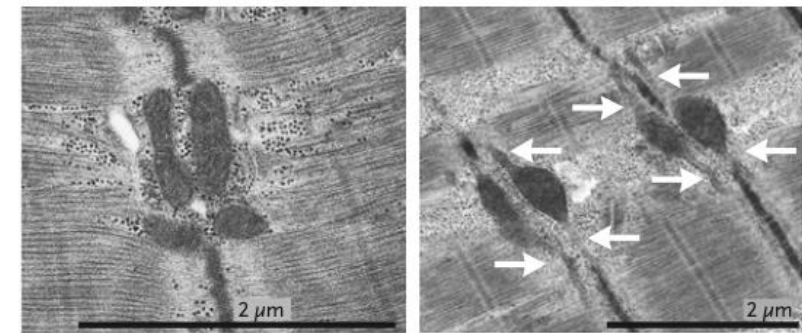
Effects of Germline VHL Deficiency on Growth, Metabolism, and Mitochondria

Silverio Perrotta, M.D., Domenico Roberti, M.D., Ph.D., Debora Bencivenga, Ph.D., Paola Corsetto, Ph.D., Katie A. O'Brien, Ph.D., Martina Caiazza, M.S., Emanuela Stampone, Ph.D., Leanne Allison, B.Sc., Roland A. Fleck, Ph.D., Saverio Scianguetta, Ph.D., Immacolata Tartaglione, M.D., Ph.D., Peter A. Robbins, D.Phil., Maddalena Casale, M.D., Ph.D., James A. West, Ph.D., Clara Franzini-Armstrong, Ph.D., Julian L. Griffin, D.Phil., Angela M. Rizzo, Ph.D., Antonio A. Sinisi, M.D., Andrew J. Murray, D.Phil., Adriana Borriello, Ph.D., Federico Formenti, D.Phil., Ph.D., and Fulvio Della Ragione, M.D.

SUMMARY

Mutations in *VHL*, which encodes von Hippel–Lindau tumor suppressor (VHL), are associated with divergent diseases. We describe a patient with marked erythrocytosis and prominent mitochondrial alterations associated with a severe germline VHL deficiency due to homozygosity for a novel synonymous mutation (c.222C→A, p.V74V). The condition is characterized by early systemic onset and differs from Chuvash polycythemia (c.598C→T) in that it is associated with a strongly reduced growth rate, persistent hypoglycemia, and limited exercise capacity. We report changes in gene expression that reprogram carbohydrate and lipid metabolism, impair muscle mitochondrial respiratory function, and uncouple oxygen consumption from ATP production. Moreover, we identified unusual intermitochondrial connecting ducts. Our findings add unexpected information on the importance of the VHL–hypoxia-inducible factor (HIF) axis to human phenotypes. (Funded by Associazione Italiana Ricerca sul Cancro and others.)

B Electron Microscopy of the Vastus Lateralis



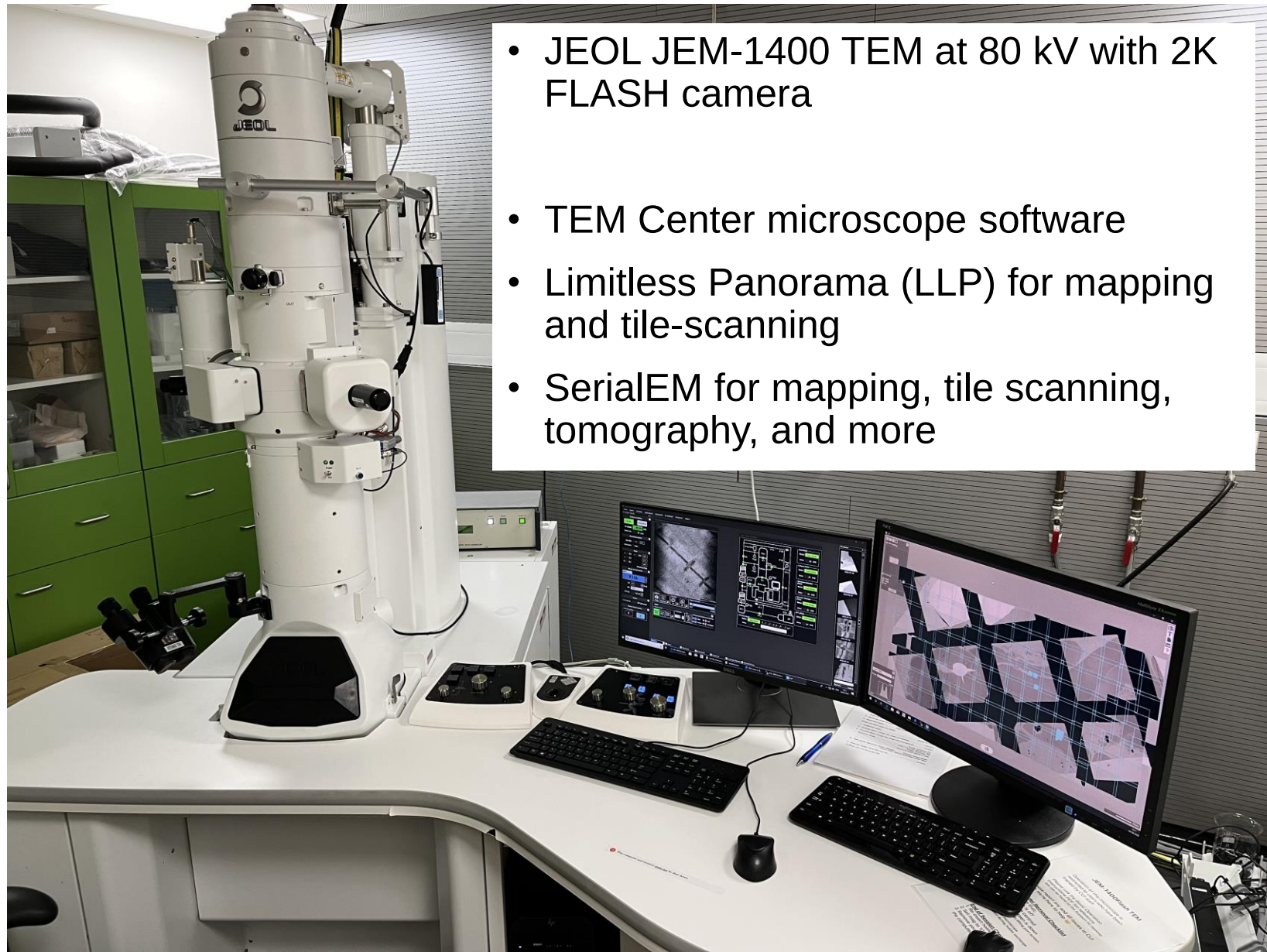
CTR

III

Figure 3. Effects of Low Levels of VHL on Mitochondrial Respiratory Activity.

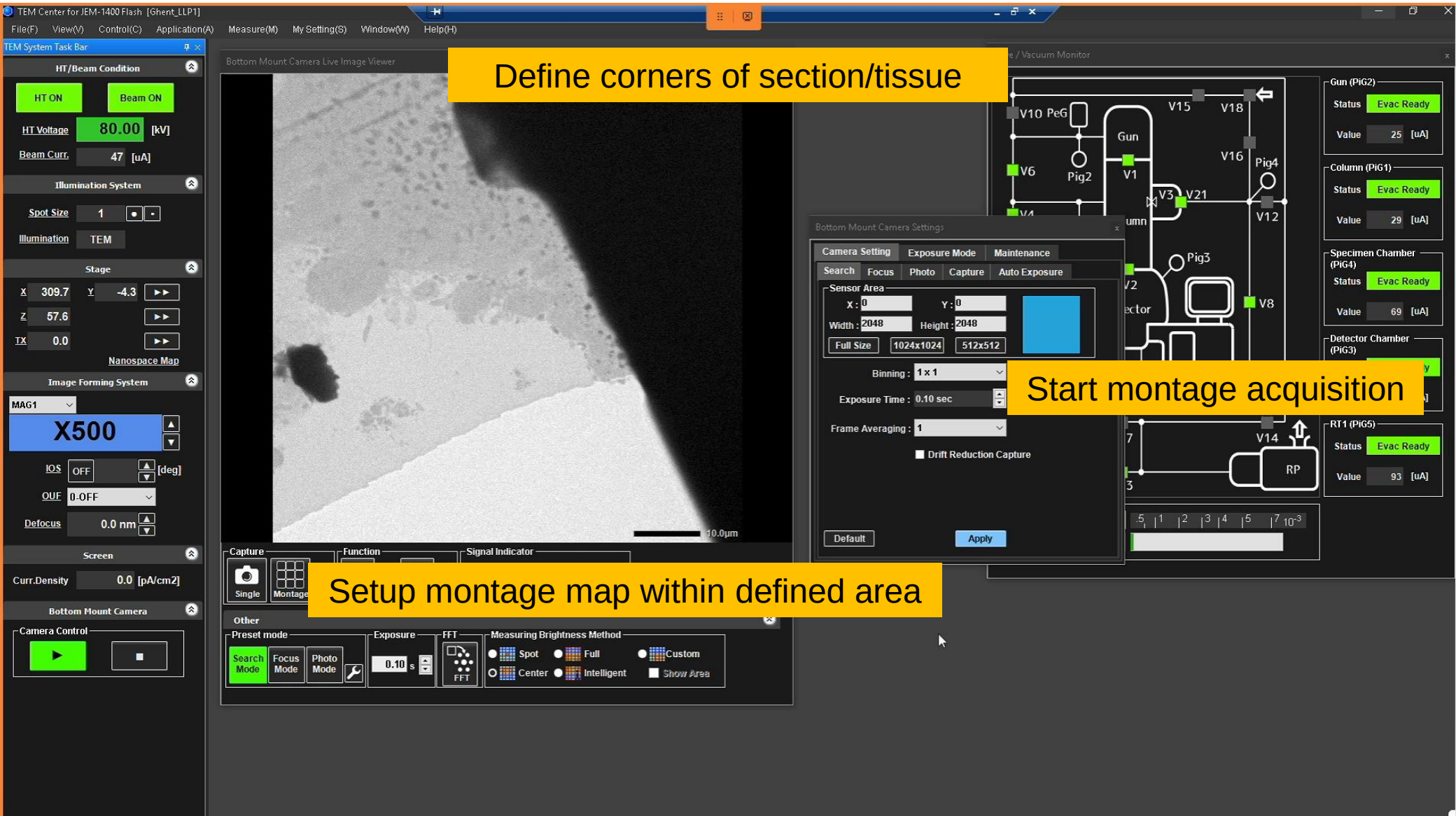
Panel A shows a functional evaluation of vastus lateralis biopsy specimens; on the left, the mitochondrial respiration rates are corrected for the wet mass of muscle and include LEAK state respiration (i.e., oxygen consumption in the absence of ATP synthesis) with malate and octanoyl carnitine (OctM_L); oxidative phosphorylation (OXPHOS)–state respiration with malate and octanoyl carnitine (OctM_p); OXPHOS–state respiration through the N pathway by complex I with glutamate and malate (GM_p); OXPHOS–state respiration through NS pathways by complex I and II with glutamate, succinate, and malate (GMS_p); and electron transport chain capacity by titration of the mitochondrial uncoupler FCCP (GMS_E). On the right, the respiratory states are instead corrected for GMS_E . Data are the means obtained from duplicate experiments on the same muscle-biopsy specimen in both a control and the patient. Differences are shown above the bars as percentages. Panel B shows a transmission electron microscopy analyses of vastus lateralis–biopsy specimens from a control (CTR) and the patient (III). The image on the right shows mitochondrial extensions (arrows). These extensions are larger than T tubules and are located at the same level as mitochondria bodies, on either side of the Z line and in close proximity to it. They provide direct continuity between mitochondria and are not associated with a triad.

TEM automation; human skeletal muscle



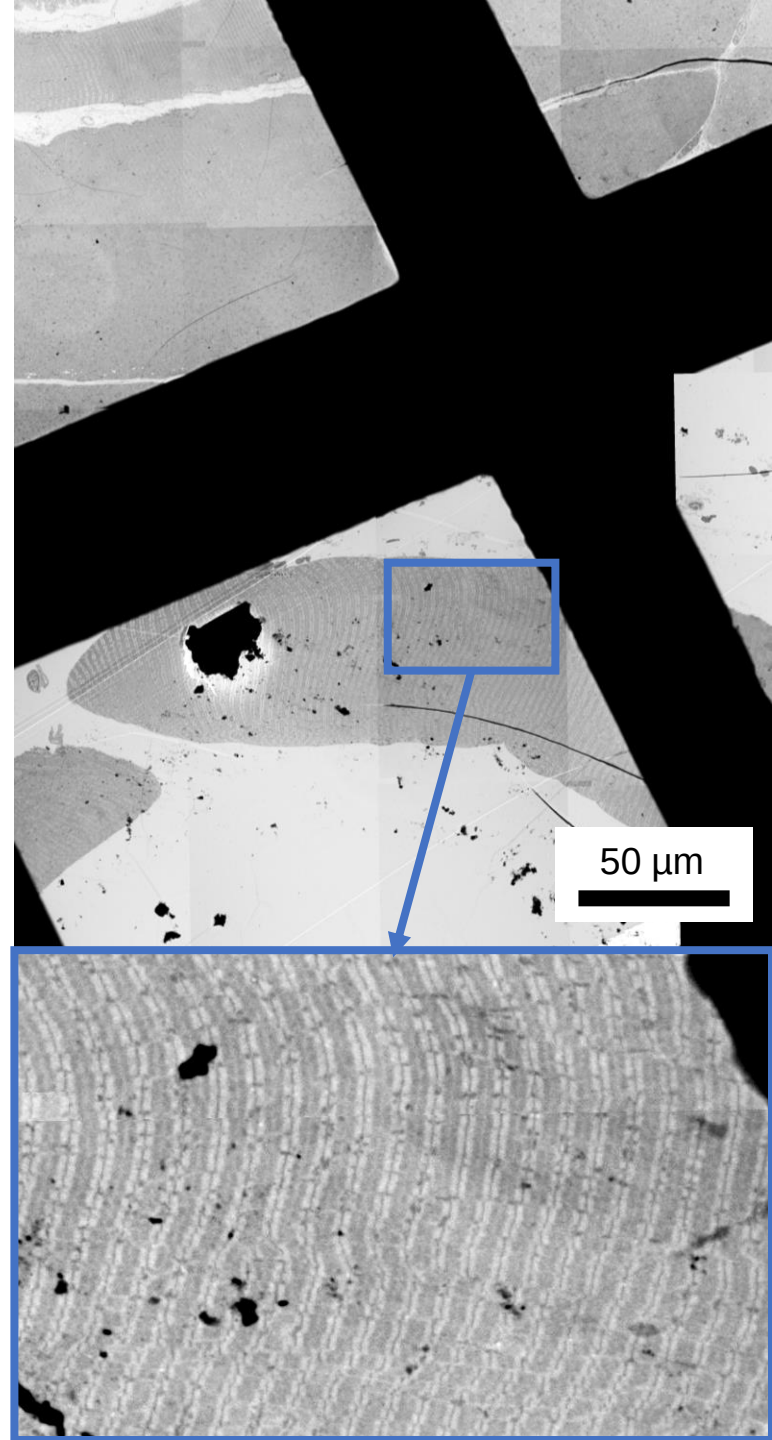
- JEOL JEM-1400 TEM at 80 kV with 2K FLASH camera
- TEM Center microscope software
- Limitless Panorama (LLP) for mapping and tile-scanning
- SerialEM for mapping, tile scanning, tomography, and more

Limitless Panorama (LLP); 500X montage map



500X montage map

- Mapping and navigation
 - Low mag tile scan
 - Section/sample overview
 - Regions of interest (ROIs)
 - Overlay correlative map (eg IHC of adjacent section, FM, etc.)
 - Share with pathologist and multidisciplinary team, eg, to decide on high mag ROIs



TFS MAPS – align LM to low mag EM for CLEM

The screenshot displays the Thermo Scientific Maps 3.30 software interface. The main window shows a large TEM image of a hexagonal lattice structure. A green rectangular region of interest is highlighted. Three alignment points are marked: a blue circle on the left, a green circle at the top right, and a pink circle at the bottom right. A cyan line connects the blue and green points, and a magenta line connects the green and pink points. A scale bar at the bottom right indicates 10 nm. The left sidebar shows the project tree with 'test_fluorescence' selected, and the 'Fluorescence_data' folder expanded, showing 'Composite_FM_RFI_3stack.ome', 'TEM_data', 'Tile set 2600x (stitched)', and 'Overview 84x (stitched)'. The bottom left shows the 'Tile set 2600x (stitched)' metadata: 9089 px x 10318 px, 1.1 pm x 1.1 pm, and 10 nm x 11.35 nm. The bottom right shows the 'Workflow: Alignment Wizard' panel with a preview of the alignment points and a 'Multi-Point Alignment' button. The bottom status bar shows 'No tile selected', 'JOB CONTROL', 'RUN', and 'Data Exchange Velox TEM'.

Aligning layers with 3 point method

Workflow: Alignment Wizard

The viewer below displays the data that will be moved. Select distinct alignment points by right mouse click and then choose "Place Point". Next find the same features in the viewer to the left and place the points in the same way. Use the "Snapshot" feature to acquire more images if needed.

Points 1 2 3 Link Rotation

Stage Axes

Mirror Image

Multi-Point Alignment

10 nm

51.6°

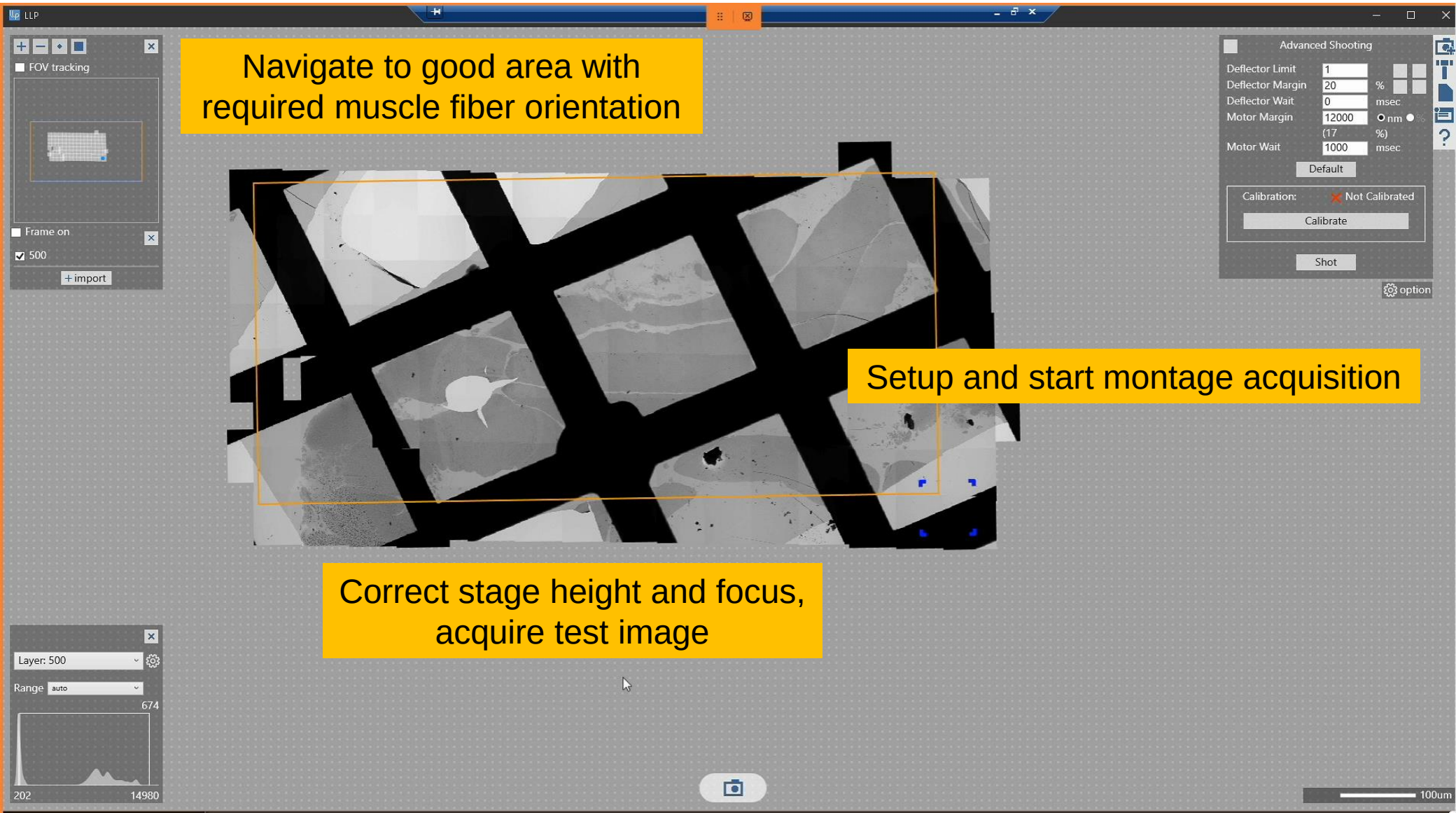
1 mm

359.8°

Diego Cordero

10:58 AM 12/10/2024

LLP; montage at 15,000X



LLP; viewing 15,000X montages



LLP – TEM montaging for quantitation

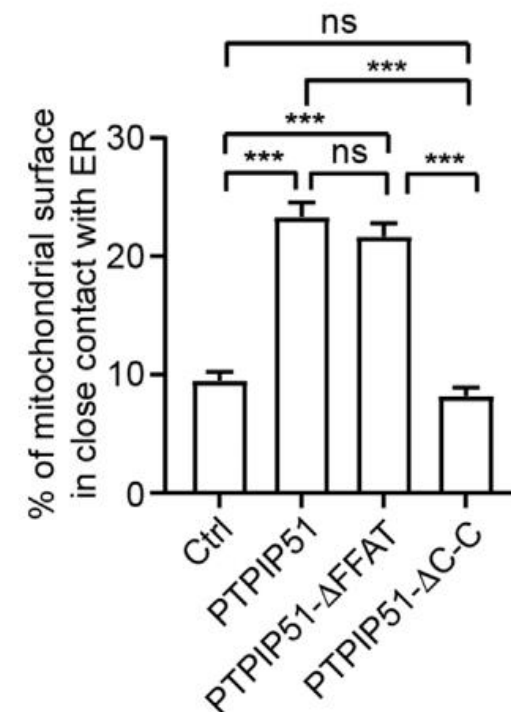
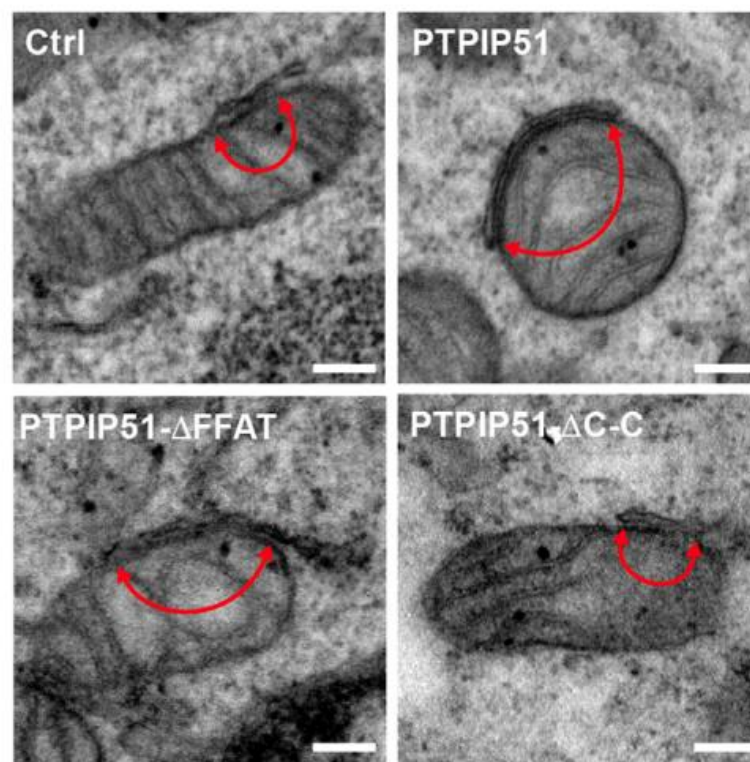
The PTPIP51 coiled-coil domain is important in VAPB binding, formation of ER-mitochondria contacts and IP3 receptor delivery of Ca^{2+} to mitochondria

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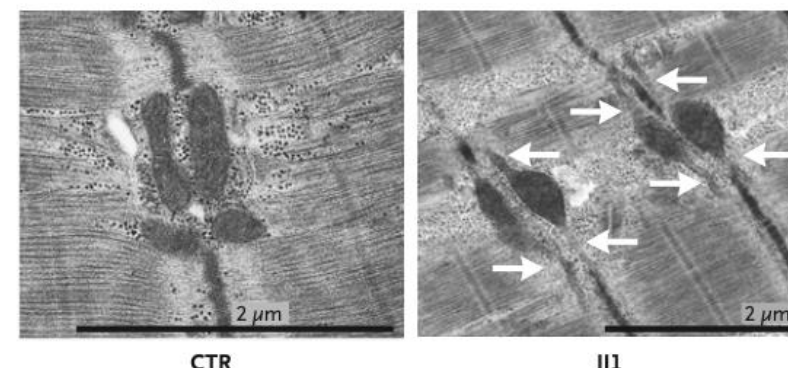
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BRIEF REPORT

Effects of Germline VHL Deficiency on Growth, Metabolism, and Mitochondria

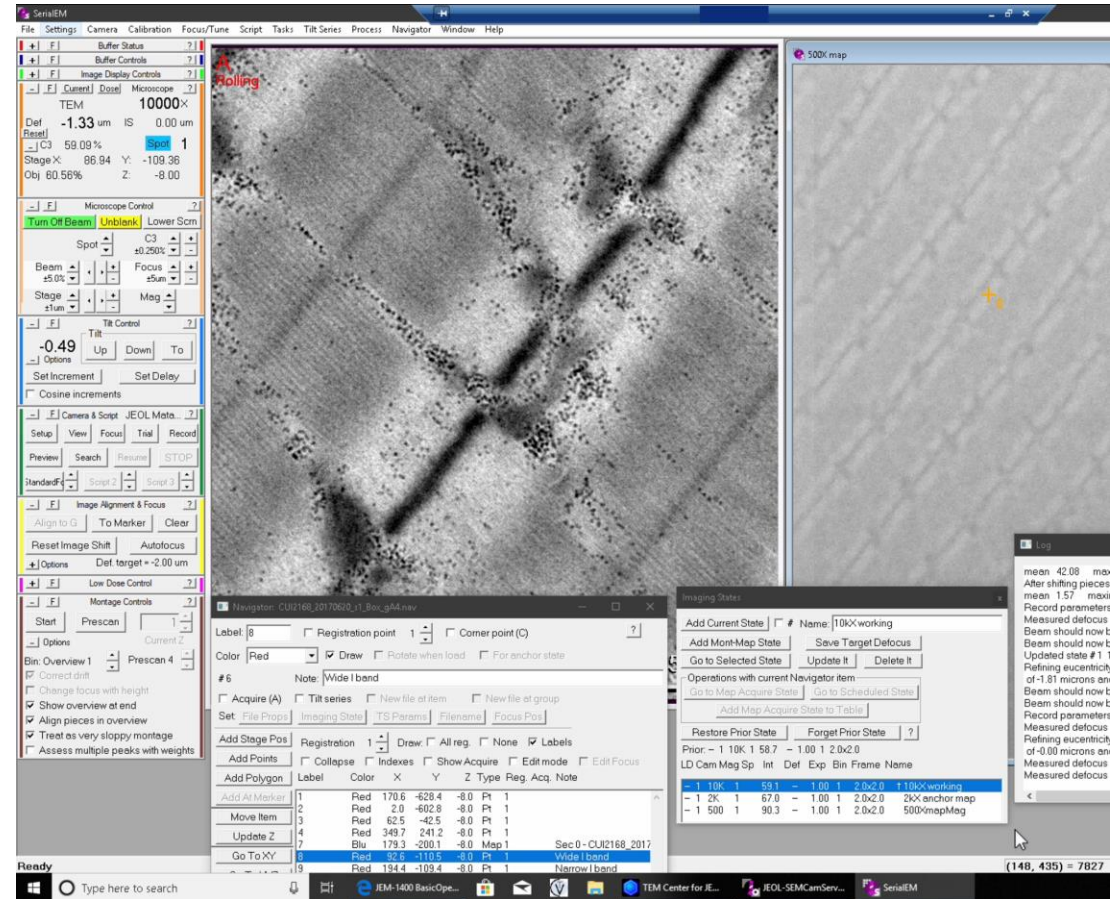
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B Electron Microscopy of the Vastus Lateralis



SerialEM – TEM automation

- Initially developed for tilt series acquisition
- Now lots of functions, including automated imaging
- Navigator
 - Stores list of 'Items' with;
 - ...stage position
 - ...action(s) to perform
- Highly customizable
 - Scripting and Python code integration



SerialEM Batch Imaging

- Define ROIs;
 - 10kX Map (working)
 - 2kX Anchor Map
- Check ROI automation; Realign to Item
- Set up batch imaging parameters
 - Montage setup
 - Tilt series setup
 - Acquire at items

SerialEM; 500X montage map

SerialEM - CUI2168_20170620_s1_Box_gA4_500Xmap.mrc

File Settings Camera Calibration Focus/Tune Script Tasks Tilt Series Process Navigator Window Help

Buffer Status Buffer Controls Image Display Controls

TEM 500X

Def 0.00 um IS 0.00 um

Reset

C3 90.28% Spot 1

Stage X: 176.31 Y: -194.79

Obj 60.57% Z: -9.59

Microscope Control

Turn Off Beam Unblank Lower Scrm

Spot C3 ±0.250%

Beam ±5.0% Focus ±5um

Stage ±1um Mag

Tilt Control

Tilt -0.04 Up Down To

Options

Set Increment Set Delay

Cosine increments

Camera & Script JEOL Mata...

Setup View Focus Trial Record

Montage Search Resume STOP

Standard Script 2 Script 3

Image Alignment & Focus

Align to G To Marker Clear

Reset Image Shift Autofocus

Options Def. target = -1.20 um

Low Dose Control

Montage Controls

Start Prescan 1

Options Current Z

Bin: Overview 1 Prescan 4

Correct drift

Change focus with height

Show overview at end

Align pieces in overview

Treat as very sloppy montage

Assess multiple peaks with weights

B Rolling

100 um

Log

Updated state #1 10kX working with current imaging state

Record parameters set from state #2 2kX anchor map

Record parameters set from state #3 500XmapMag

Record parameters set from state #1 10kX working

Running Z height to optimal focus distance routine

The return value from SetHexOLC was 0

The return value from SetHexOLC was 0

'Standard Focus' set

Measuring required Z height adjustment

Measured defocus = 2.64 microns drift = 1.13 nm/sec

Last defocus in autofocus: 2.64 um

Moved stage Z by -2.639548 um

Checking accuracy after Z movement

Measured defocus = 0.84 microns drift = 0.31 nm/sec

Last defocus in autofocus: 0.84 um

Z height is correct within +/- 2 um

Record parameters set from state #3 500XmapMag

The original displacements in the overlap zones have

mean 42.08 maximum 152.26 pixels.

After shifting pieces into register, the displacements have

mean 1.57 maximum 4.33 pixels.

Navigator

Label: 7 Registration point 1 Corner point (C)

Color Blue Draw Rotate when load For anchor state

#5 Note: Sec 0 - CUI2168_20170620_s1_Box_gA4_500Xmap.mrc

Acquire (A) Tilt series New file at item New file at group

Set: File Props Imaging State TS Params Filename Focus Pos

Add Stage Pos Registration 1 Draw All reg None Labels

Add Points Collapse Indexes Show Acquire Edit mode Edit Focus

Add Polygon

Label	Color	X	Y	Z	Type	Reg.	Acq.	Note
1	Red	170.6	-628.4	-9.6	Pt	1	C	
2	Red	2.0	-602.8	-9.6	Pt	1	C	
3	Red	62.5	-42.5	-9.6	Pt	1	C	
4	Red	349.7	241.2	-9.6	Pt	1	C	
7	Blue	176.3	-194.7	-9.6	Map	1		Sec 0 - CUI2168_20170620_s1_Box_gA4_500Xmap.mrc

Go To XY

Go To XYZ

Go To Marker

Load Map

New Map

Anchor Map

Delete Item

Realign to Item

Imaging States

Add Current State # Name: 500XmapMag

Add Mont-Map State Save Target Defocus

Go to Selected State Update It Delete It

Operations with current Navigator item

Go to Map Acquire State Go to Scheduled State

Add Map Acquire State to Table

Restore Prior State Forget Prior State

Ready

Type here to search

JEM-1400 BasicOp...

TEM Center for JE...

JEOL-SEMCamServ...

SerialEM - CUI216...

(594, 1667) = 8225

16:47

29/05/2025

SerialEM; Align 500X to 10,000X – Shift to Marker

SerialEM - CUI2168_20170620_s1_Box_gA4_500Xmap.mrc

File Settings Camera Calibration Focus/Tune Script Tasks Tilt Series Process Navigator Window Help

Buffer Status Buffer Controls Image Display Controls

Current Dose Microscope

TEM 10000X

Def 6.02 um IS 0.00 um

C3 59.09% Spot 1

Stage X: 115.54 Y: -134.42

Obj 60.63% Z: -7.97

Microscope Control

Turn Off Beam Unblank Lower Scrn

Spot C3 ±0.250%

Beam ±5.0% Focus ±5um

Stage ±1um Mag

Tilt Control

0.01

Up Down To

Set Increment Set Delay

Cosine increments

Camera & Script JEOL Matio...

Setup View Focus Trial Record

Montage Search Resume STOP

Standard Script 2 Script 3

Image Alignment & Focus

Align to G To Marker Clear

Reset Image Shift Autofocus

Options Def. target = -1.20 um

Low Dose Control

Montage Controls

Start Prescan

Options Current Z

Bin: Overview 1 Prescan 4

Correct drift

Change focus with height

Show overview at end

Align pieces in overview

Treat as very sloppy montage

Assess multiple peaks with weights

Rolling

Place marker at same feature in 10,000X, Shift to Marker

500X map

Find feature in both 500X and 10,000X, Add at Marker in 500X another feature

Navigator: CUI2168_20170620_s1_Box_gA4.nav

Label: 10 Registration point 1 Corner point (C)

Color Red Draw Rotate when load For anchor state

#8 Note:

Acquire (A) Tilt series New file at item New file at group

Set: File Props Imaging State TS Params Filename Focus Pos

Add Stage Pos Registration 1 Draw: All reg. None Labels

Add Points Collapse Indexes Show Acquire Edit mode Edit Focus

Add Polygon

Label	Color	X	Y	Z	Type	Reg.	Acq.	Note
1	Red	170.6	-628.4	-8.0	Pt	1		
2	Red	2.0	-602.8	-8.0	Pt	1		
3	Red	62.5	-42.5	-8.0	Pt	1		
4	Red	349.7	241.2	-8.0	Pt	1		
7	Blu	176.3	-194.7	-8.0	Map	1		Sec 0 - CUI2168_2017
8	Red	89.7	-105.0	-8.0	Pt	1		Wide I band
9	Red	191.4	-103.9	-8.0	Pt	1		Narrow I band
10	Red	112.3	-118.5	-8.0	Pt	1		

Move Item

Update Z

Go To XY

Go To XYZ

Go To Marker

Load Piece

New Map

Anchor Map

Delete Item

Realign to Item

Imaging States

Add Current State # Name: 10kXworking

Add Mont-Map State Save Target Defocus

Go to Selected State Update It Delete It

Operations with current Navigator item

Go to Map Acquire State Go to Scheduled State

Add Map Acquire State to Table

Restore Prior State Forget Prior State

Prior: - 1 10K 1 58.7 - 1.00 1 2.0x2.0

LD	Cam	Mag	Sp	Int	Def	Exp	Bin	Frame	Name
- 1	10K	1	59.1	-	1.00	1	2.0x2.0	↑	10kXworking
- 1	2K	1	67.0	-	1.00	1	2.0x2.0		2kX anchor map
- 1	500	1	90.3	-	1.00	1	2.0x2.0		500XmapMag

Log

Last defocus in autofocus: 2.64 um

Moved stage Z by -2.639548 um

Checking accuracy after Z movement

Measured defocus = 0.84 microns drift = 0.31 nm/sec

Last defocus in autofocus: 0.84 um

Z height is correct within +/- 2 um

Record parameters set from state # 3 500XmapMag

The original displacements in the overlap zones have

mean 42.08 maximum 152.26 pixels

After shifting pieces into register, the displacements have

mean 1.57 maximum 4.33 pixels

Record parameters set from state # 1 10kXworking

Measured defocus = -7.22 microns changed by 6.02 to target drift = 0.29 nm/sec

SerialEM; Check ROI automation; Realign to Item

SerialEM - CUI2168_20170620_s1_Box_gA4_batchmaps.mrc

File Settings Camera Calibration Focus/Tune Script Tasks Tilt Series Process Navigator Window Help

Buffer Status Buffer Controls Image Display Controls

Current Dose Microscope Control

TEM 10000X

Def -3.22 um IS 0.51 um

Reset C3 59.09% Spot 1

Stage X: 87.06 Y: -109.14 Z: -8.00

Obj 60.54%

Microscope Control

Turn Off Beam Unblank Lower Scrn

Spot C3 ±0.250%

Beam ±5.0% Focus ±5um

Stage ±1um Mag

Tilt Control

-0.49

Up Down To

Set Increment Set Delay

Cosine increments

Camera & Script JEOL Mats...

Setup View Focus Trial Record

Preview Search Resume STOP

Standard Script 2 Script 3

Image Alignment & Focus

Align to G To Marker Clear

Reset Image Shift Autofocus

Options Def. target = -2.00 um

Low Dose Control

Montage Controls

Start Prescan

Options Current Z

Bin: Overview 1 Prescan 4

Correct drift

Change focus with height

Show overview at end

Align pieces in overview

Treat as very sloppy montage

Assess multiple peaks with weights

Realign to Item:
SerialEM uses Anchor Map
and ROI map to find ROI
and adjust to correct position

Open ROI Map in
window for reference

Navigation: CUI2168_20170620_s1_Box_gA4.nav

Label: 11 Registration point 1 Corner point (C)

Color Blue Draw Rotate when load For anchor state

#9 Note: [Sec 0 - CUI2168_20170620_s1_Box_gA4_batchmaps.mrc]

Acquire (A) Tilt series New file at item New file at group

Set File Props Imaging State TS Params Filename Focus Pos

Add Stage Pos Registration 1 Draw All reg None Labels

Add Points Collapse Indexes Show Acquire Edit mode Edit Focus

Add Polygon Label Color X Y Z Type Reg Acq Note

Label	Color	X	Y	Z	Type	Reg	Acq	Note
1	Red	170.6	-628.4	-8.0	Pt	1		
2	Red	2.0	-602.8	-8.0	Pt	1		
3	Red	62.5	-42.5	-8.0	Pt	1		
4	Red	349.7	241.2	-8.0	Pt	1		
7	Blu	179.3	-200.1	-8.0	Map	1		Sec 0 - CUI2168_2017
8	Red	92.6	-110.5	-8.0	Pt	1		Wide I band
9	Red	194.4	-109.4	-8.0	Pt	1		Narrow I band
10	Red	115.3	-122.0	-8.0	Pt	1		
11	Blu	87.1	-109.1	-8.0	Map	1		Sec 0 - CUI2168_2017
12	Blu	87.1	-109.1	-8.0	Anc	1		Sec 1 - CUI2168_2017
13	Blu	193.3	-113.3	-8.0	Map	1		Sec 2 - CUI2168_2017
14	Blu	193.3	-113.3	-8.0	Map	1		Anchor - Sec 3 - CUI2

Go To XY

Go To XYZ

Go To Marker

Load Map

New Map

Anchor Map

Delete Item

Realign to Item

Record parameters set from state #1 10KX working
Measured defocus = 5.40 microns changed by -7.40 to target drift = 0.17 nm/sec
Refining eucentricity: Fit to 8 positions gives tilt axis Z displacement
of -0.00 microns and lateral displacement of -12.53 microns
Measured defocus = -1.67 microns drift = 0.02 nm/sec
Measured defocus = -2.04 microns changed by 0.04 to target drift = 3.47 nm/sec
Record parameters set from state #2 2KX anchor map
Backlash adjustment to stage coordinates is 0.13, 0.22
The real map is now in Buffer C - be careful to mark points only on that!
Record parameters set from state #1 10KX working
Measured defocus = -0.12 microns changed by -1.88 to target drift = 0.60 nm/sec
Backlash adjustment to stage coordinates is -0.20, 0.49
The real map is now in Buffer C - be careful to mark points only on that!
The two images correlate most strongly assuming a shrinkage of -0.0%
Disparity in stage position after aligning to center of map frame: -0.68-0.34
Aligning to target with image shift equivalent to stage shift: -0.68-0.34
Disparity in stage position after aligning to map item itself: -0.37-0.36 (align shift 0.330)
Finished aligning to item in 2 rounds

Ready

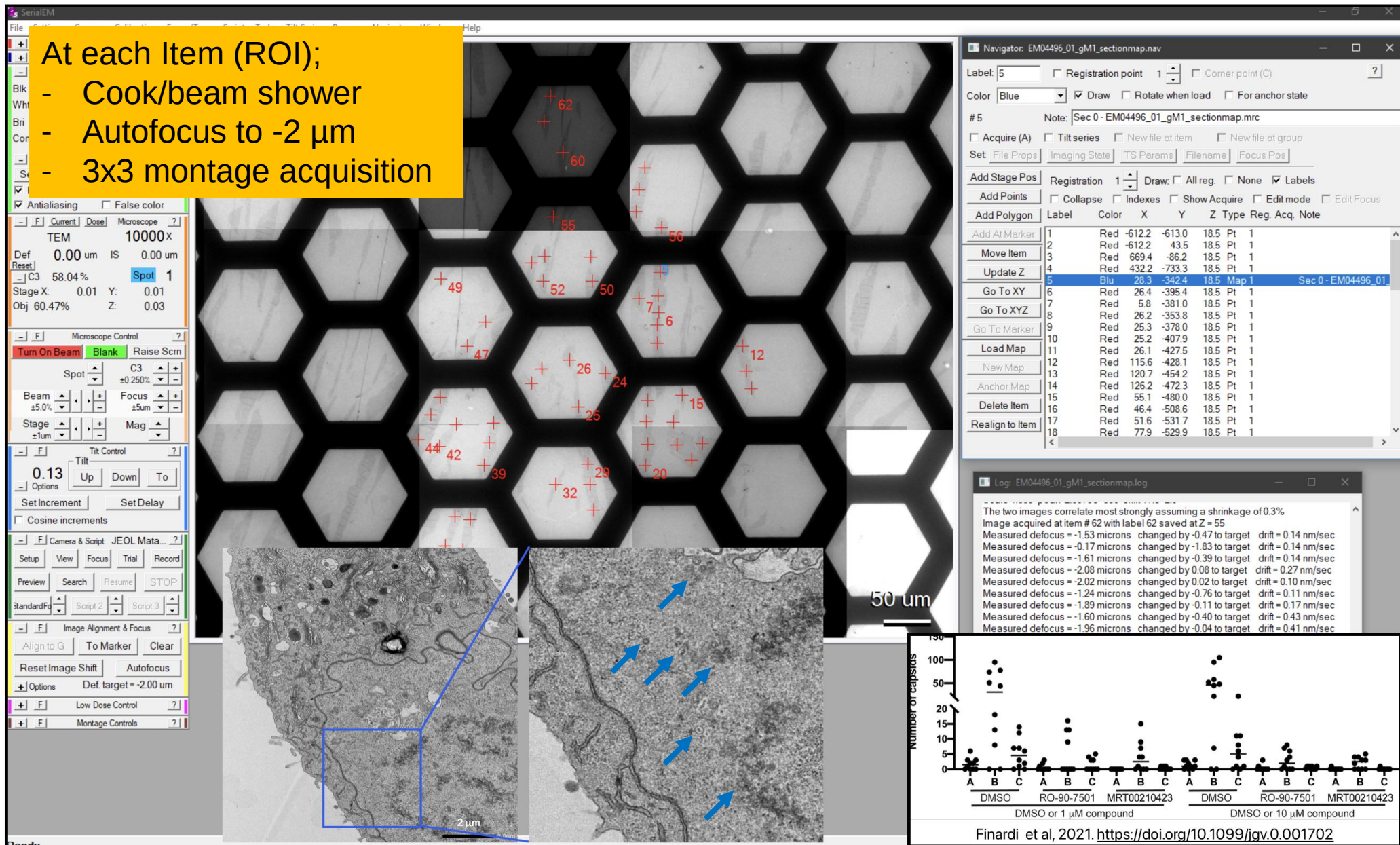
(680, 1008) = 5424

10:51 30/05/2025

SerialEM; Automated ROI imaging for quantitation

At each Item (ROI);

- Cook/beam shower
- Autofocus to $-2\ \mu\text{m}$
- 3x3 montage acquisition



SerialEM; Automated batch dual axis tomography

File Settings Camera Calibration Focus/Tune Script Tasks Tilt Series Process Navigator Window Help

Buffer Status

Buffer Controls

Image Display Controls

Current Dose Microscope

TEM 10000X

Def -3.22 um IS 0.00 um

Reset

C3 59.09% Spot 1

Stage X 115.20 Y -121.89

Obj 60.54% Z -8.00

Microscope Control

Turn Off Beam Blank Raise Scrn

Spot C3 ±0.250%

Beam ±5.0% Focus ±5um

Stage ±1um Mag

Tilt Control

Tilt -0.49

Options Up Down To

Set Increment Set Delay

Cosine increments

Camera & Script JEOL Mata...

Setup View Focus Trial Record

Preview Search Resume STOP

Standard Script 2 Script 3

Image Alignment & Focus

Align to G To Marker Clear

Reset Image Shift Autofocus

Options Def. target = -2.00 um

Low Dose Control

Start Prescan 1

Options Current Z

Bin: Overview 1 Prescan 4

Correct drift

Change focus with height

Show overview at end

Align pieces in overview

Treat as very sloppy montage

Assess multiple peaks with weights

Rolling

500K map

Navigator: CUI2168_20170620_s1_Box_gA4.nav

Label: 13 Registration point 1 Corner point (C)

Color Blue Draw Rotate when load For anchor state

#11 Note: Sec 2 - CUI2168_20170620_s1_Box_gA4_batchmaps.mrc

Acquire (A) Tilt series New file at item New file at group

Set File Props Imaging State TS Params Filename Focus Pos (CUI2168_20170620_s1_

Add Stage Pos Registration 1 Draw: All reg. None Labels

Add Points Collapse Indexes Show Acquire Edit mode Edit Focus

Add Polygon Label Color X Y Z Type Reg. Acq. Note

Add At Marker

Move Item

Update Z

Go To XY

Go To XYZ

Go To Marker

Load Map

New Map

Anchor Map

Delete Item

Realign to Item

1	Red	170.6	-628.4	-8.0	Pt	1	
2	Red	2.0	-602.8	-8.0	Pt	1	
3	Red	62.5	-42.5	-8.0	Pt	1	
4	Red	349.7	241.2	-8.0	Pt	1	
7	Blu	179.3	-200.1	-8.0	Map	1	Sec 0 - CUI2168_201706
8	Red	92.6	-110.5	-8.0	Pt	1	Wide I band
9	Red	194.4	-109.4	-8.0	Pt	1	Narrow I band
10	Red	115.3	-122.0	-8.0	Pt	1	
11	Blu	87.1	-109.1	-8.0	Map	1	TS Sec 0 - CUI2168_201706
12	Blu	87.1	-109.1	-8.0	Anc	1	Sec 1 - CUI2168_201706
13	Blu	193.3	-113.3	-8.0	Map	1	TS Sec 2 - CUI2168_201706
14	Blu	193.3	-113.3	-8.0	Map	1	Anchor - Sec 3 - CUI2168

Imaging States

Add Current State

Add Mont-Map State

Go to Selected State

Operations with current

Add Map Acquire

Restore Prior State Forget Prior State

Prior: - 1 10K 1 58.3 - 1.00 1 2.0x2.0

LD Cam Mag Sp Int Def Exp Bin Frame Name

- 1	2K	1	67.0	- 1.00	1	2.0x2.0	2kX anchor map
- 1	500	1	90.3	- 1.00	1	2.0x2.0	500XmapMag

Parameters for: Mapping Final Data

Primary Action

Acquire and save image or montage

Make Navigator map

Skip saving to file

Acquire multiple Records Setup

Acquire tilt series

Run script SetStandardFocus

Primary Action-related

Cycle defocus target in autofocus, drift wait & TS

From -2 to -1 um in 10 steps

Adjust beam tilt to compensate for image shift

Use map hole vectors for shifts (no adjustment)

Acquire non-montage images in Low Dose with

Record View Search

Task-related options

Do hybrid Realign to item/Align to Template

Realign to scaled map with P P C R C V C S

Relax stage after final movement

Skip Z in: Initial move & Realign Run at nearest

Skip stage move to item if possible

General options

Do subset from index 1 to 12

No message box when errors occur

Turn off filament at end

Retract cameras at end

Send email at end

Run script at end SetStandardFocus

Hide unselected options

Save Parameters to File Read in Parameters

GO Postpone Cancel

Tasks before or after Primary

Coma-free Alignment Every item Setup

Correct Astigmatism Every item Setup

Rough Eucentricity Every item Setup

Eucentricity by Focus Every item Setup

Autocenter Beam Every item Setup

Realign to Item Every item Setup

Cook Specimen Every item Setup

Fine Eucentricity Every item Setup

Align to Template Every item Setup

Wait for Drift Every item Setup

Autofocus Every item Setup

Run Script before Action Every item Setup

Script to run: SetStandardFocus

Primary Action (Tilt Series) occurs here

Run Script after Action Every item, after

Script to run: SetStandardFocus

Hole Finder & Combiner Every item, after Setup

General controls for task: Run Script before Action

How often to run task

Every 1 items

Only at start of group

Only at end of group

Every 15 minutes

When moved > 40 um

Run after Primary Action

Run at nearest item with label note starting with

Hide unused tasks Restore Default Order

Position in sequence

Move Up

Move Down

Aligning to target with image shift equivalent to stage shift -0.68-0.34

isparity in stage position after aligning to map item itself. -0.37-0.36 (align shift 0.33 0

nished aligning to item in 2 rounds

record parameters set from state #1 10KX working

Ready

Type here to search

JEM-1400 BasicOpe...

TEM Center for JE...

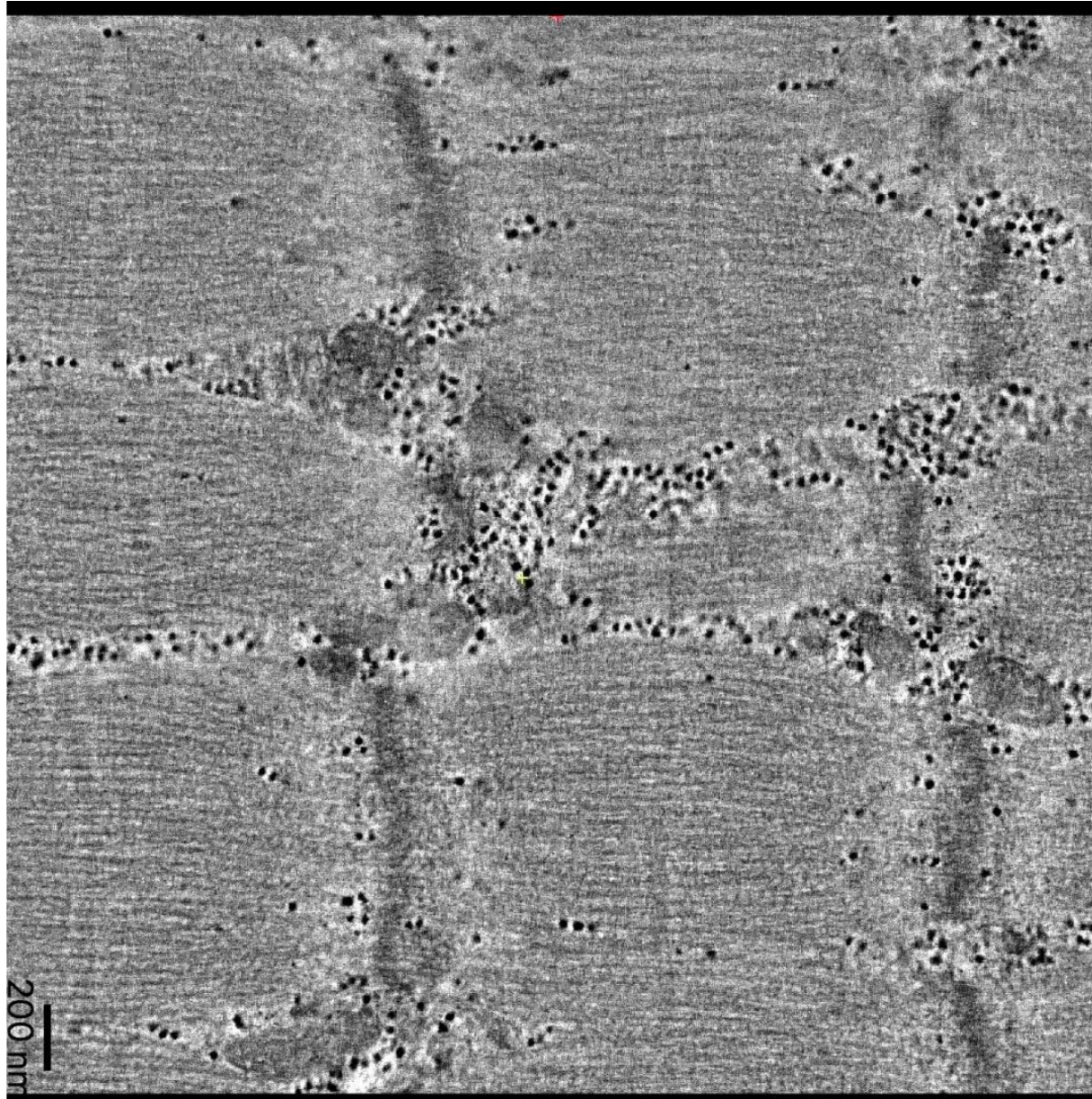
JEOL-SEM CamServ...

SerialEM

(3835, 8479) = 200

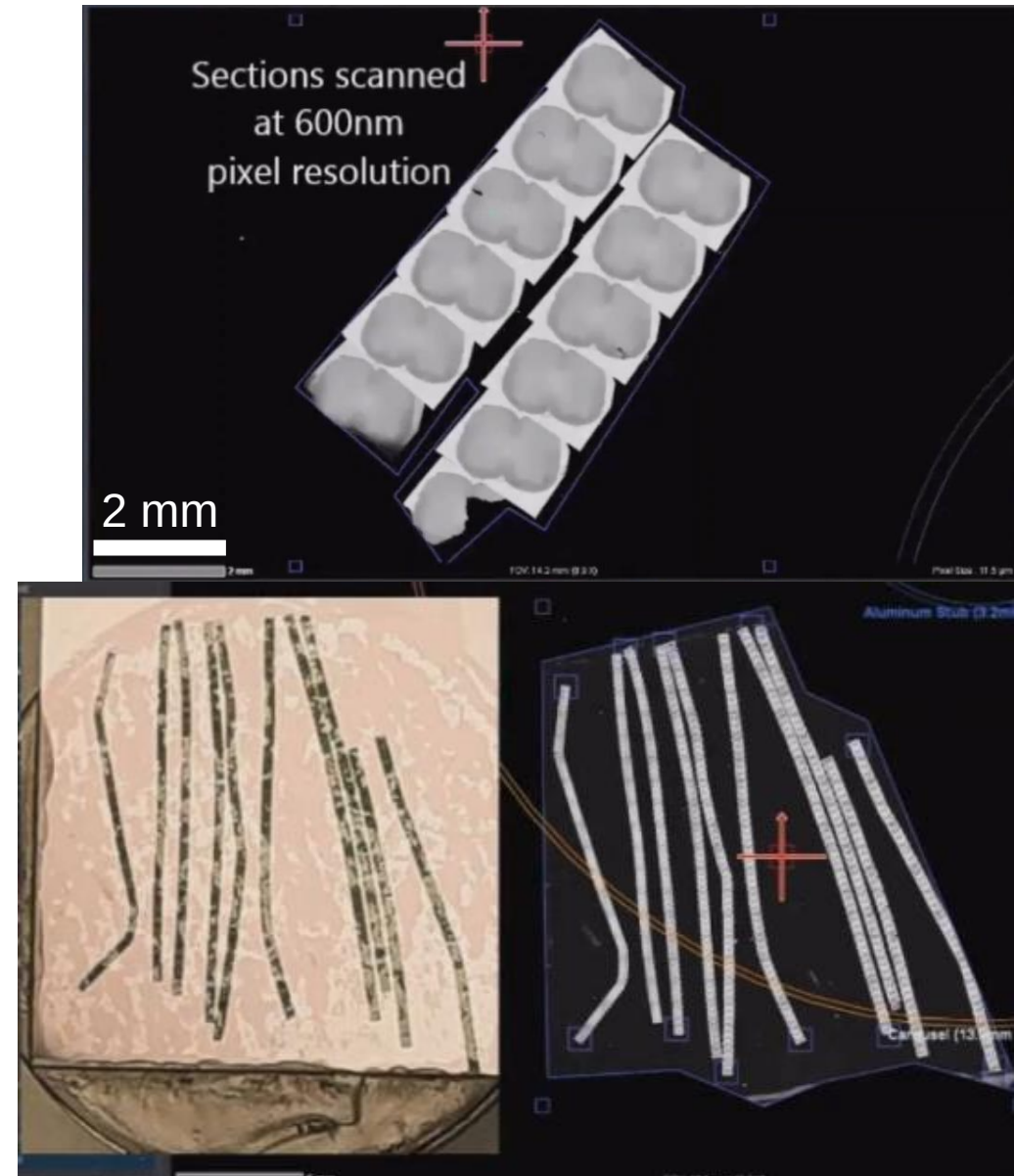
11:03 30/05/2025

Automated batch dual axis tomography with SerialEM



Zeiss Atlas 5 for SEM of large areas & array tomography

- Map section ribbons
- Define sections
- Define ROI in a section
- Acquire montage
- Propagate ROI to other sections and acquire serial montages



Mapping and imaging sections by SEM

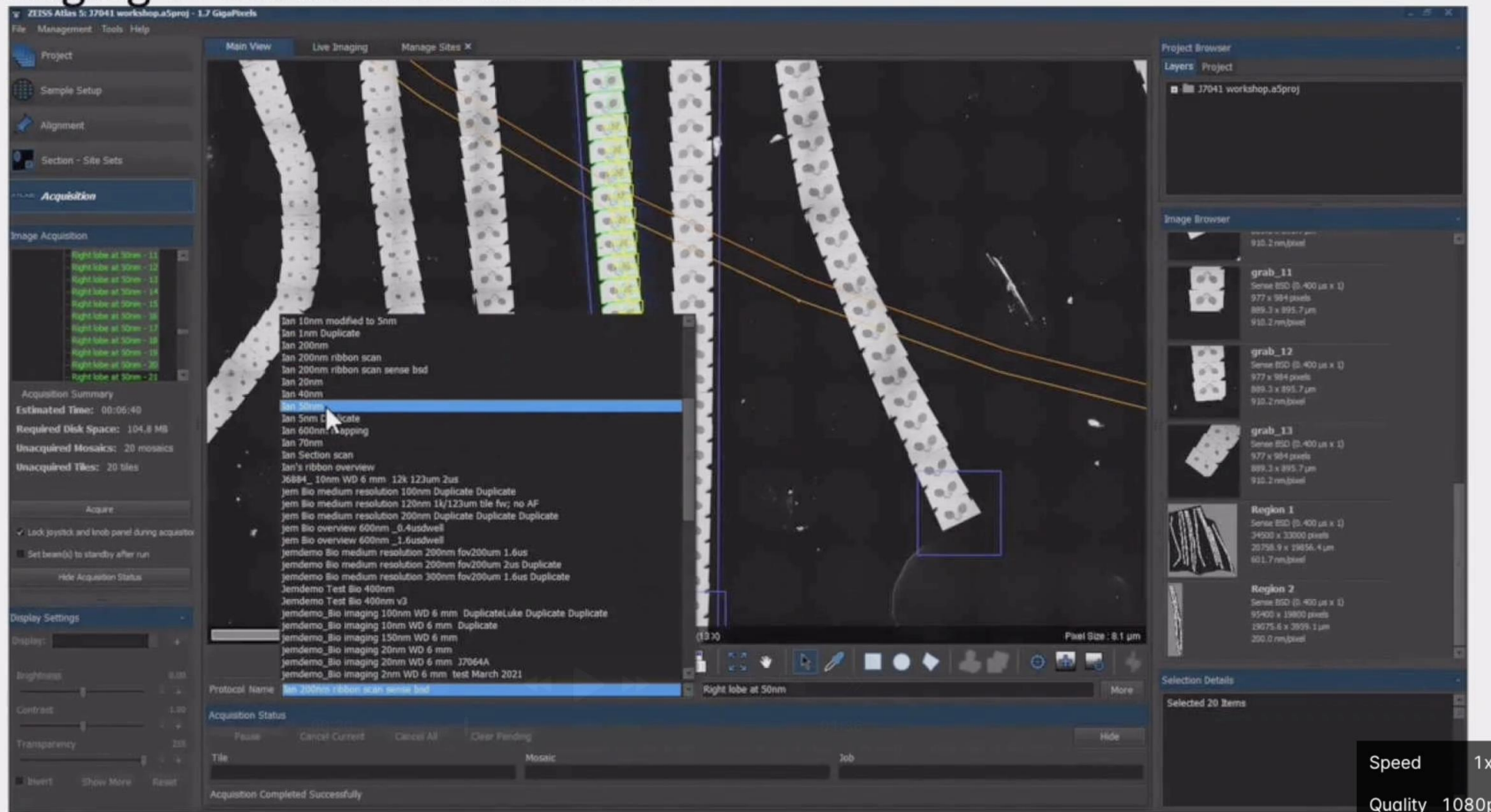
ZEISS

Laboratory for Molecular Cell Biology

UCL

Basic BSD imaging of sections on the SEM

Automated imaging
using ATLAS 5



Speed 1x

Quality 1080p

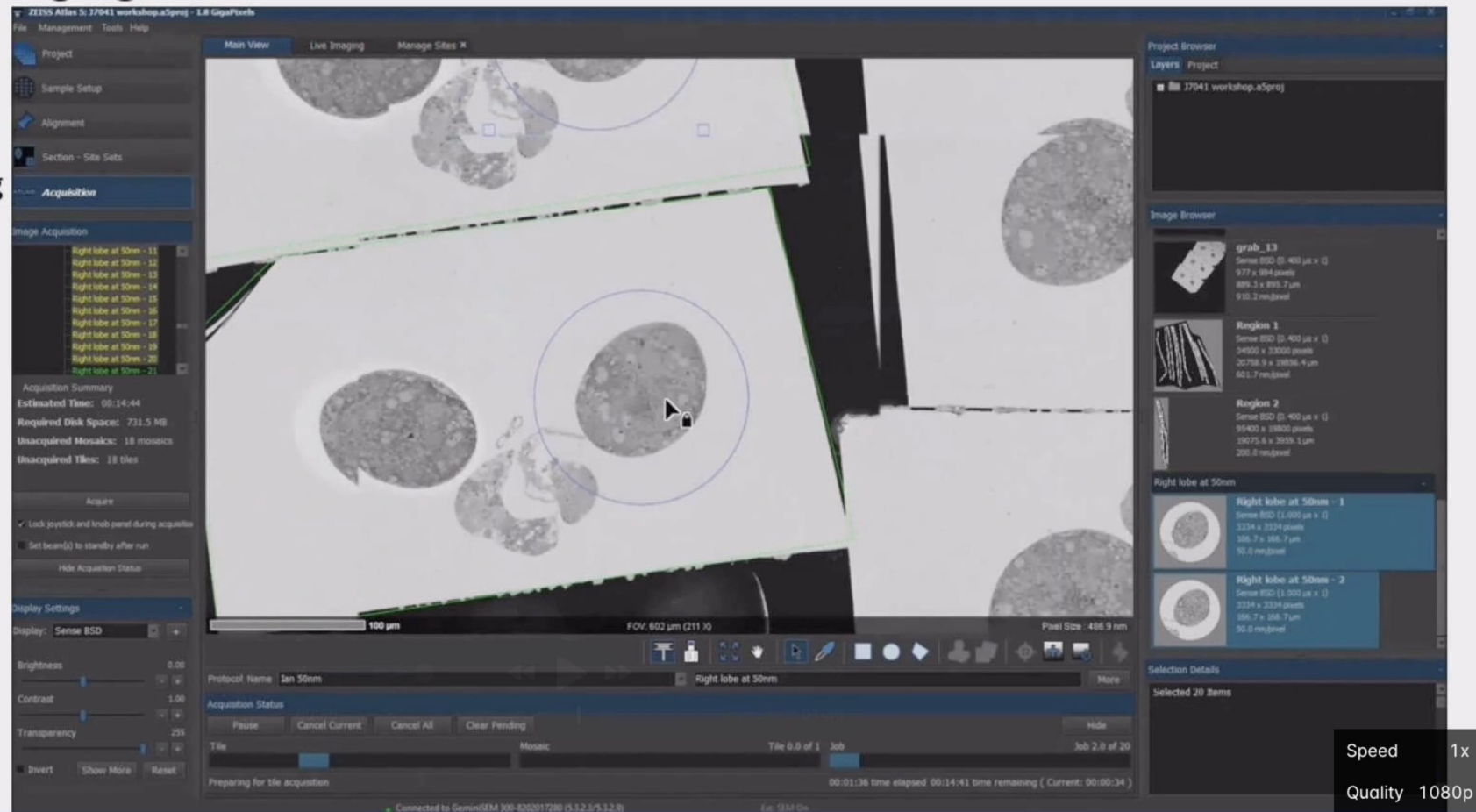
Defining ROI in sections

ZEISS

Laboratory for Molecular Cell Biology 

Basic BSD imaging of sections on the SEM

Refining, repeating
and reimaging using
ATLAS 5



Ian White - M&M 2022 Presentation

Getting sensitive with the SEM: High resolution backscatter detection on sections in ultrastructural cell biology.

<https://zeiss.wistia.com/medias/4t2g22k5ft>

Mapping and imaging sections by SEM; Mouse spinal cord

ZEISS

Laboratory for Molecular Cell Biology



Case Study 2: Very large fields of view at high resolution

Mouse spinal cord:

Sample courtesy of:

Amanda Pocratsky
Institute of Neurology
UCL

TEM Imaging

Half a cord only just fits in a 1mm x 2mm slot
grid, so half the sample is trimmed away
destructively already.

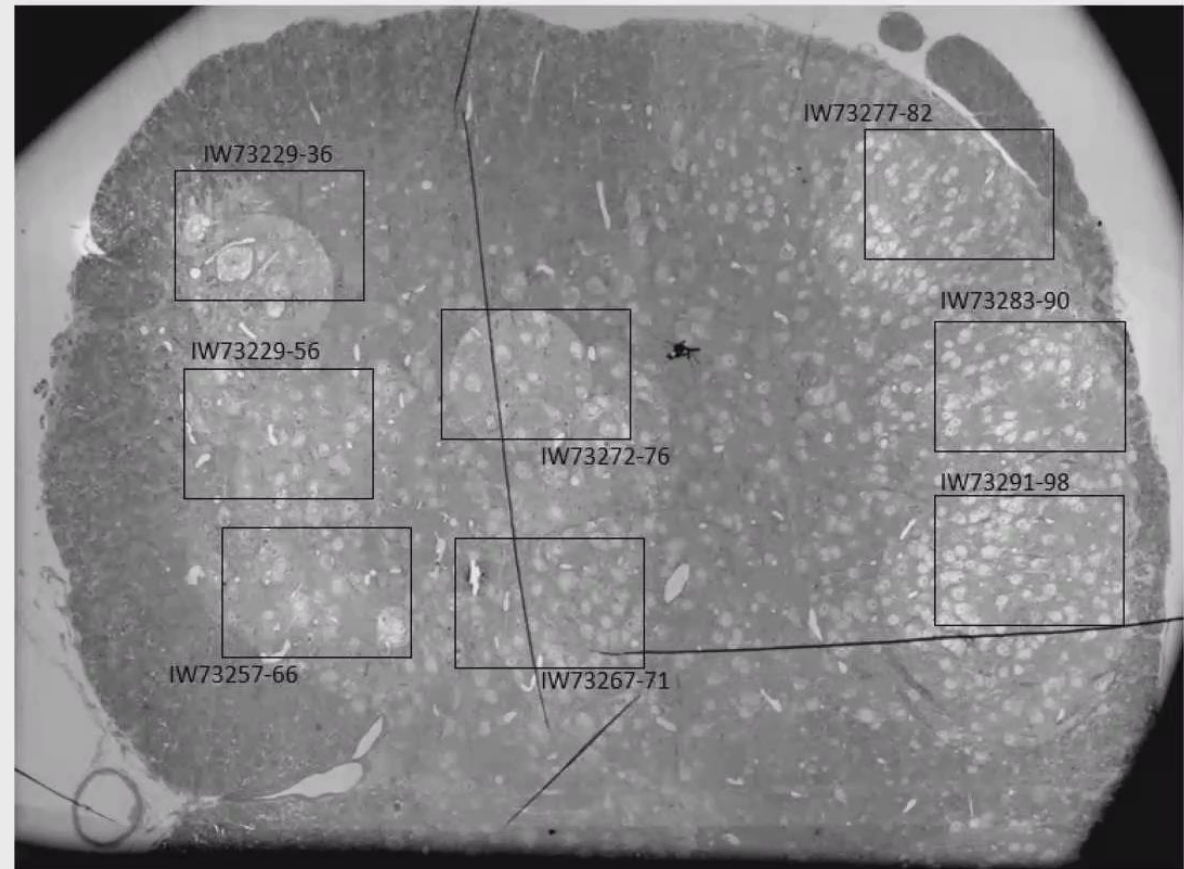
Low power imaging

Higher power

Even higher power to see nuclear membranes!

Mapping to show where ROI are situated in
cord

No chance for serial or 3D if required



Conclusions

- TEM
 - 500X map
 - Can see tubule, vessel, subregion of organ, orientation
 - Correlative approaches;
 - Overlay tol blue, FM, or IHC on adjacent section
 - Quantitation
 - Mitochondria phenotype
 - Virus load
 - Context
 - 2D and 3D
- SEM
 - No grid bars
 - Better chance of finding rare disease feature eg Lewy bodies
 - Can see entire biopsy, not destroying patient material
 - Share data with pathologist and/or multidisciplinary team consultation
 - Interactive

Acknowledgements

- Staff at CUI at KCL
 - Leanne Allison
- Federico Formenti at KCL
 - Silverio Perrotta (University of Naples)
- Eudri Venter, JEOL UK
- Diego Cordero, Thermofisher Scientific
- David Mastronarde, CU Boulder
- EM STP, The Francis Crick Institute
- Blair Strang Lab at City St George's, London
- LMCB at UCL
 - Ian White
- Zeiss

TEM automation example; multi-ROI 15,000X montages

The screenshot displays the TEM System Task Bar interface. On the left, the 'HT/Beam Condition' panel shows 'HT ON' and 'Beam ON' buttons, with 'HT Voltage' at 80.00 [kV] and 'Beam Curr.' at 46 [uA]. The 'Illumination System' panel shows 'Spot Size' at 1 and 'Illumination' set to TEM. The 'Stage' panel shows X, Y, and Z coordinates. The 'Image Forming System' panel shows 'MAG1' set to X15k. The 'Screen' panel shows 'Curr. Density' at 0.8 [pA/cm]. The 'Bottom Mount Camera' panel shows 'Camera Control' buttons. The main window displays a 'Bottom Mount Camera Live Image Viewer' showing a grayscale image of a sample. A 'Capture' panel shows 'Single' and 'Montage' buttons. A 'Preset mode' panel shows 'Search Mode' selected. An 'Exposure' panel shows '0.10 s'. An 'FFT' panel shows 'Spot', 'Full', 'Center', and 'Intelli' options. A 'Measuring Brightness Method' panel shows a histogram with values 3029, 9353, and 11457. A 'Layer: 15000' dropdown is visible. A 'Range: auto' dropdown is visible. A 'Start acquisition' button is at the bottom right. A yellow arrow points to the 'Start acquisition' button. A yellow box contains the text 'Start Macro' to set multi-ROI acquisition positions. Another yellow box contains the text 'Find feature to estimate offset between 500X and 15kX'. A third yellow box contains the text 'Start acquisition'.

TEM System Task Bar

Bottom Mount Camera Live Image Viewer

HT/Beam Condition

HT ON Beam ON

HT Voltage 80.00 [kV]

Beam Curr. 46 [uA]

Illumination System

Spot Size 1

Illumination TEM

Stage

X -70.6 Y 76.3

Z 60.8

IX 0.0

Nanospace M

Image Forming System

MAG1

X15k

IOS OFF

QUE 0.0FF

Defocus 0.0 nm

Screen

Curr. Density 0.8 [pA/cm]

Bottom Mount Camera

Camera Control

Capture

Single Montage

Other

Preset mode

Search Mode Focus Mode Photo Mode

Exposure 0.10 s

FFT

Measuring Brightness Method

Spot Full Center Intelli

Layer: 15000

Range: auto

9353

3029 11457

Start acquisition

Start Macro' to set multi-ROI acquisition positions

Find feature to estimate offset between 500X and 15kX

SerialEM; Define ROIs; Maps and Anchor Maps

At each ROI, Save an 'ROI' Map (10kX) and an Anchor Map (2000X)

First iteration sets conditions for automated Anchor Map acquisition

SerialEM - CUI2168_20170620_s1_Box_gA4_batchmaps.mrc

File Settings Camera Calibration Focus/Tune Script Tasks Tilt Series Process Navigator Window Help

Buffer Status
Buffer Controls
Image Display Controls

Current Dose Microscope
TEM 2000X
Def -1.33 um IS 0.24 um
Spot 1
Stage X: 86.86 Y: -109.25
Obj 60.56% Z: -8.00

Microscope Control
Turn Off Beam Unblank Lower Scm
Spot C3 ±0.250%
Beam ±5.0% Focus ±5um
Stage ±1um Mag

Tilt Control
Tilt -0.49
Up Down To
Set Increment Set Delay
Cosine increments

Camera & Script JEOL Mako...
Setup View Focus Trial Record
Preview Search Resume STOP
Standard Script 2 Script 3

Image Alignment & Focus
Align to G To Marker Clear
Reset Image Shift Autofocus
Options Def. target = -2.00 um

Low Dose Control
Start Prescan
Options Current Z

Montage Controls
Start Prescan
Options Current Z
Bin: Overview 1 Prescan 4
Correct drift
Change focus with height
Show overview at end
Align pieces in overview
Treat as very sloppy montage
Assess multiple peaks with weights

Navigator: CUI2168_20170620_s1_Box_gA4.nav

Label: 12 Registration point 1 Corner point (C)
Color Blue Draw Rotate when load For anchor state
#10 Note: Sec 1 - CUI2168_20170620_s1_Box_gA4_batchmaps.mrc
Acquire (A) Tilt series New file at item New file at group
Set: File Props Imaging State TS Params Filename Focus Pos

Add Stage Pos Registration 1 Draw All reg None Labels
Add Points Collapse Indexes Show Acquire Edit mode Edit Focus
Add Polygon Label Color X Y Z Type Reg. Acq. Note

Label	Color	X	Y	Z	Type	Reg.	Acq.	Note
1	Red	170.6	-628.4	-8.0	Pt	1		
2	Red	2.0	-602.8	-8.0	Pt	1		
3	Red	62.5	-42.5	-8.0	Pt	1		
4	Red	349.7	241.2	-8.0	Pt	1		
7	Blu	179.3	-200.1	-8.0	Map	1		Sec 0 - CUI2168_2017
8	Red	92.6	-110.5	-8.0	Pt	1		
9	Red	194.4	-109.4	-8.0	Pt	1		
10	Red	115.3	-122.0	-8.0	Pt	1		
11	Blu	87.1	-109.1	-8.0	Map	1		Sec 0 - CUI2168_2017
12	Blu	87.1	-109.1	-8.0	Anc	1		Sec 1 - CUI2168_2017

Imaging States

Add Current State # Name: 2kX anchor map
Add Mont-Map State Save Target Defocus
Go to Selected State Update It Delete It
Operations with current Navigator item
Go to Map Acquire State Go to Scheduled State
Add Map Acquire State to Table
Restore Prior State Forget Prior State ?

Prior: - 1 10K 1 58.7 - 1.00 1 2.0x2.0
LD Cam Mag Sp Int Def Exp Bin Frame Name
- 1 10K 1 59.1 - 1.00 1 2.0x2.0 10kX working
- 1 2K 1 67.0 - 1.00 1 2.0x2.0 2kX anchor map
- 1 500 1 90.3 - 1.00 1 2.0x2.0 500XmapMag

Log

Record parameters set from state #1 10kX working
Measured defocus = -7.22 microns changed by 6.02 to target drift = 0.29 nm/sec
Beam should now be turning OFF
Beam should now be turning ON
Updated state #1 10kX working with current imaging state
Refining eucentricity: Fit to 8 positions gives tilt axis Z displacement
of -1.81 microns and lateral displacement of -12.53 microns
Beam should now be turning OFF
Beam should now be turning ON
Record parameters set from state #1 10kX working
Measured defocus = 5.40 microns changed by -7.40 to target drift = 0.17 nm/sec
Refining eucentricity: Fit to 8 positions gives tilt axis Z displacement
of -0.00 microns and lateral displacement of -12.53 microns
Measured defocus = -1.67 microns drift = 0.02 nm/sec
Measured defocus = -2.04 microns changed by 0.04 to target drift = 3.47 nm/sec
Record parameters set from state #2 2kX anchor map
Backlash adjustment to stage coordinates is 0.13, 0.22
The real map is now in Buffer C - be careful to mark points only on that!

Ready

Type here to search

JEM-1400 BasicOpe... TEM Center for JE... JEOL-SEM CamServ... SerialEM - CUI216...

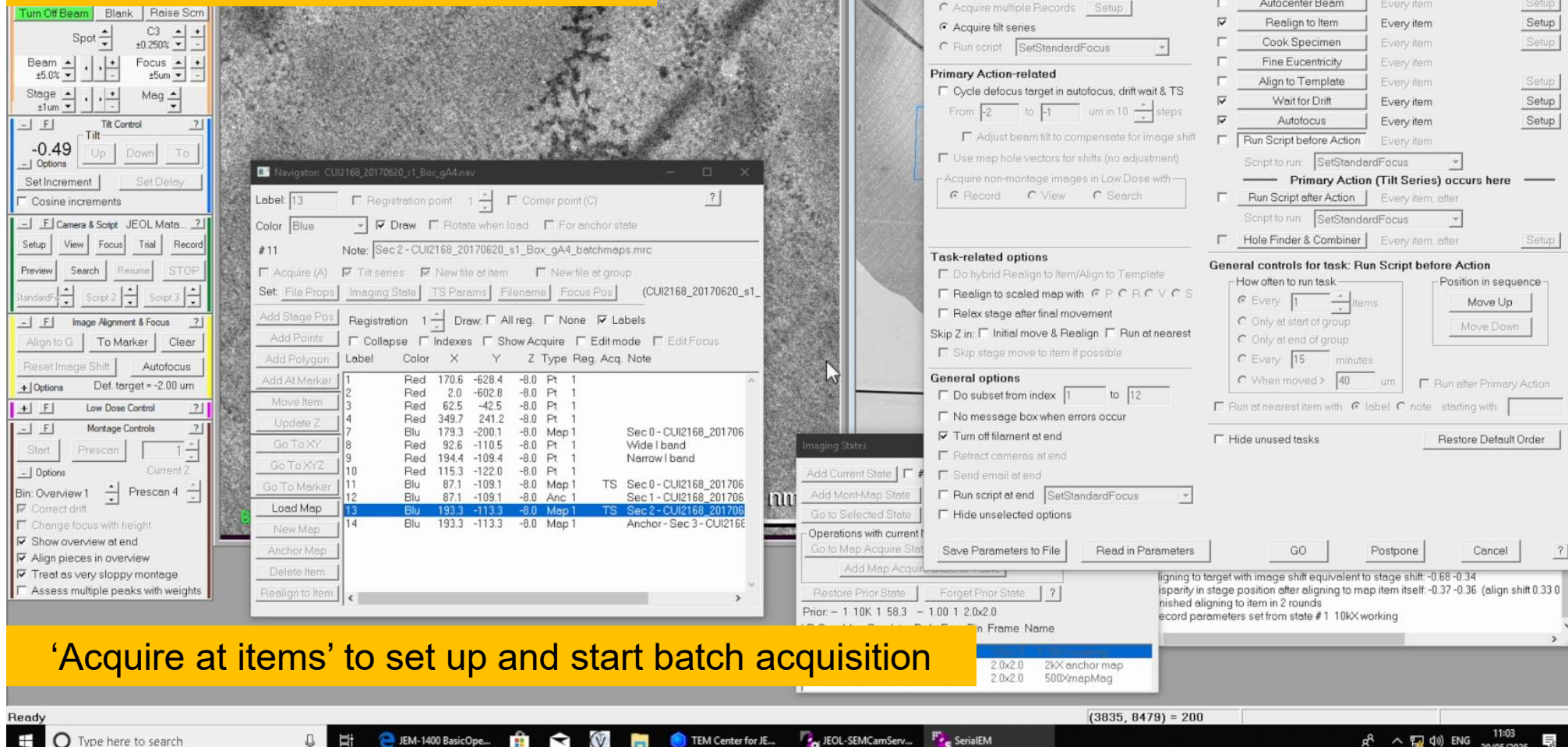
(1341, 1169) = 190

10:43 30/05/2025

SerialEM; Automated batch dual axis tomography

Select 'Tilt series' for first ROI item,
and setup tilt series parameters

Select 'Tilt series' for remaining ROI
items; Setup is copied from first



The screenshot displays the SerialEM software interface, which is used for automated batch dual axis tomography. The interface is divided into several panels:

- Left Panel (Instrument Controls):** Contains controls for the electron microscope, including 'Turn Off Beam', 'Blank', 'Raise Scm', 'Spot', 'Beam', 'Focus', 'Stage', 'Mag', 'Tilt Control', 'Options', 'Set Increment', 'Set Delay', 'Cosine increments', 'Camera & Script', 'JEOL Meta...', 'Setup', 'View', 'Focus', 'Trial', 'Record', 'Preview', 'Search', 'Resume', 'STOP', 'StandardF', 'Script 2', 'Script 3', 'Image Alignment & Focus', 'Align to G', 'To Marker', 'Clear', 'Reset Image Shift', 'Autofocus', 'Def. target = -2.00 um', 'Low Dose Control', 'Start', 'Prescan', 'Current Z', 'Bin: Overview 1', 'Prescan 4', 'Correct drift', 'Change focus with height', 'Show overview at end', 'Align pieces in overview', 'Treat as very sloppy montage', and 'Assess multiple peaks with weights'.
- Navigation Window (CUI2168_20170620_s1_Box_gA4.nrv):** Displays a list of items for acquisition. The table below shows the items:

Label	Color	X	Y	Z	Type	Reg.	Acq.	Note
1	Red	170.6	-628.4	-8.0	Pt	1		
2	Red	2.0	-602.8	-8.0	Pt	1		
3	Red	62.5	-42.5	-8.0	Pt	1		
4	Red	349.7	241.2	-8.0	Pt	1		
7	Blu	179.3	-200.1	-8.0	Map	1		Sec 0 - CUI2168_201706
8	Red	92.6	-110.5	-8.0	Pt	1		Wide l band
9	Red	194.4	-109.4	-8.0	Pt	1		Narrow l band
10	Red	115.3	-122.0	-8.0	Pt	1		
11	Blu	87.1	-109.1	-8.0	Map	1		TS Sec 0 - CUI2168_201706
12	Blu	87.1	-109.1	-8.0	Anc	1		Sec 1 - CUI2168_201706
13	Blu	193.3	-113.3	-8.0	Map	1		TS Sec 2 - CUI2168_201706
14	Blu	193.3	-113.3	-8.0	Map	1		Anchor - Sec 3 - CUI2168

- Parameters Window (Mapping/Final Data):** Shows the 'Primary Action' and 'Primary Action-related' settings. The 'Primary Action' is set to 'Acquire tilt series'. The 'Primary Action-related' settings include 'Cycle defocus target in autofocus, drift wait & TS' and 'Adjust beam tilt to compensate for image shift'.
- Tasks before or after Primary:** A list of tasks to be performed before or after the primary action, including 'Come-free Alignment', 'Correct Astigmatism', 'Rough Eucentricity', 'Eucentricity by Focus', 'Autocenter Beam', 'Realign to Item', 'Cook Specimen', 'Fine Eucentricity', 'Align to Template', 'Wait for Drift', 'Autofocus', and 'Run Script before Action'.
- General controls for task: Run Script before Action:** Includes settings for 'How often to run task' (Every 1 items), 'Position in sequence' (Move Up, Move Down), and 'Run after Primary Action'.

The bottom of the interface shows the 'Ready' status and the 'Type here to search' bar. The taskbar at the bottom includes icons for JEM-1400 BasicOpe..., TEM Center for JE..., JEOL-SEM CamServ..., and SerialEM.

'Acquire at items' to set up and start batch acquisition

SerialEM ROI screening schizonts for quantitation of food vacuole morphology

The role of *Plasmodium* V-ATPase in vacuolar physiology and antimalarial drug uptake

Arne Alder^{a,b,c} , Cecilia P. Sanchez^d , Matthew R. G. Russell^{e,f} , Lucy M. Collinson^e , Michael Lanzer^d, Michael J. Blackman^{g,h} , Tim-Wolf Gilberger^{a,b,c}, and Joachim M. Matz^{a,b,g,1} 

PNAS 2023 Vol. 120 No. 30 e2306420120

<https://doi.org/10.1073/pnas.2306420120>

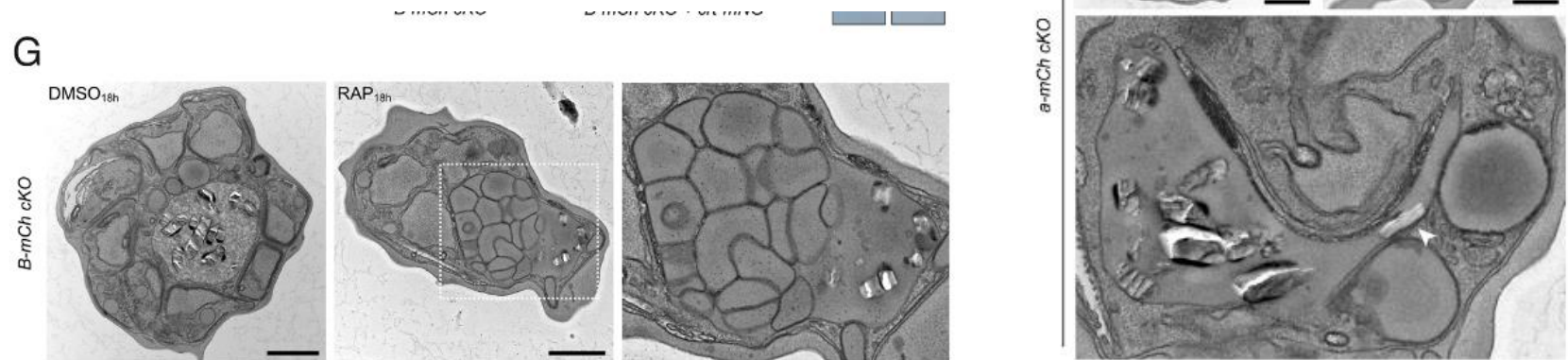


Fig. 3. The absence of V-ATPase subunit B impairs intravacuolar hemoglobin release and heme sequestration. (A–C) DV swelling and reduced hemozoin motility upon loss of subunit B. *B-mCh cKO* parasites expressing CRT-mNG were treated with DMSO/RAP from 18 h post invasion onward and imaged live at the schizont stage. (A) Representative fluorescence micrographs. DNA, Hoechst 33342. (B) Fraction of parasites displaying hemozoin motility. $n = 5$. (C) CRT-mNG-delineated area. $n = 180$ parasites from three experiments. Individual and mean values (bars); paired t test. (D and E) The swollen DV of subunit B-deficient parasites contains native hemoglobin. *B-mCh cKO* parasites were (D) visualized by Giemsa staining or (E) released by saponin and subjected to western blot. Parental B11 parasites were further treated with protease inhibitor F64 (21.7 μ M). Hb, hemoglobin α . (F) Western blot of *B-mCh cKO* parasites expressing PM2-mNG. The mNG signal is predominantly associated with a band of ~28 kDa, corresponding to mNG alone. Unprocessed PM2-mNG (~78 kDa) was not detected. (G) Transmission electron micrographs of *B-mCh cKO* parasites. Note the accumulation of intra-DV vesicles (zoomed region, dashed white frame). In 9% of DMSO_{18h}- ($n = 32$) and 84% of RAP_{18h}-treated parasites ($n = 51$), the hemozoin-containing DV matrix displayed an electron density comparable to that of the RBC cytoplasm. (H)

Mapping and imaging sections by SEM

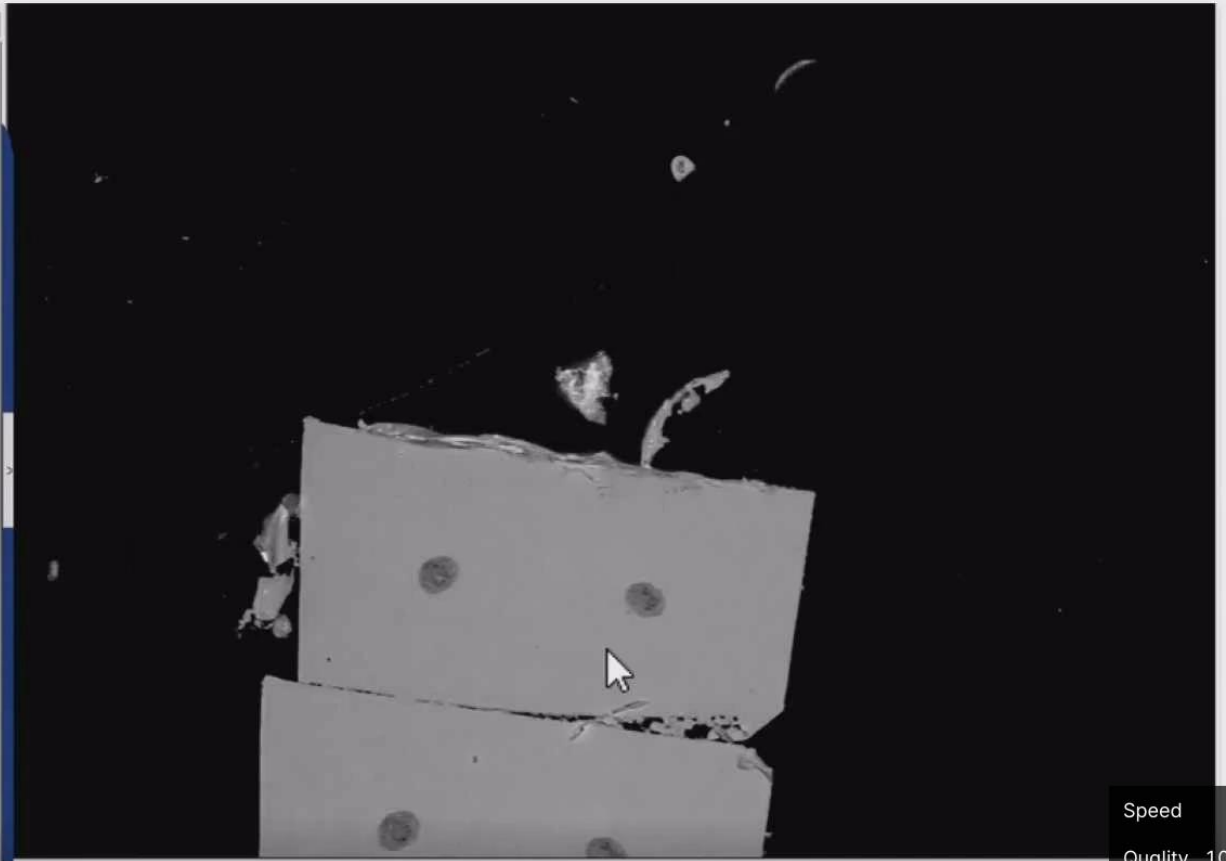
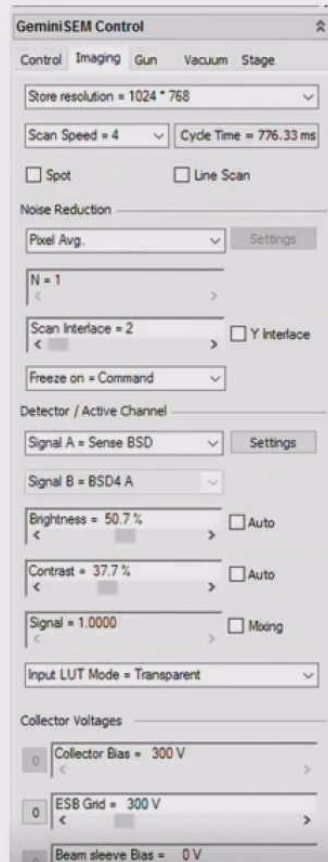
ZEISS

Laboratory for Molecular Cell Biology

UCL

Basic BSD imaging of sections on the SEM

First section images using SmartSEM



Speed 1x

Quality 1080p