OMB Number: 4040-0001
Expiration Date: 11/30/2025

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R) **Use Current Standard SF 424 Form**

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

○ Pre-application ● Application ○ Changed/Corrected Application

4.a. Federal Identifier

b. Agency Routing Number

2. DATE SUBMITTED | **Application Identifier**

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION **UEI*:** [redacted]

Legal Name*: [redacted]
Department: [redacted]
Division:
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]

Person to be contacted on matters involving this application
Prefix: First Name*: [redacted] Middle Name: [redacted] Last Name*: [redacted] Suffix:
Position/Title: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email: [redacted]

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** [redacted]

7. TYPE OF APPLICANT* X: Other (specify)

Other (Specify): Private, non-profit, state-related, educ inst

Small Business Organization Type ○ Women Owned ○ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

● New ○ Resubmission
○ Renewal ○ Continuation ○ Revision

If Revision, mark appropriate box(es).

○ A. Increase Award ○ B. Decrease Award ○ C. Increase Duration
○ D. Decrease Duration ○ E. Other *(specify)* :

Is this application being submitted to other agencies?* ○Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
JEOL JSM-IT700HRLA Scanning Electron Microscope

12. PROPOSED PROJECT
Start Date* 02/01/2024
Ending Date* 01/31/2025

13. CONGRESSIONAL DISTRICTS OF APPLICANT
[redacted]

Use Current PAR Opportunity Number

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: First Name*: [redacted] Middle Name: [redacted] Last Name*: [redacted] Suffix:

Position/Title: [redacted]
Organization Name*: [redacted]
Department: [redacted]
Division: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$319,200.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$319,200.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:

DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR

○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name*: [redacted] Middle Name: [redacted] Last Name*: [redacted] Suffix:

Position/Title*: [redacted]
Organization Name*: [redacted]
Department: [redacted]
Division:
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
ZIP / Postal Code*: [redacted]
Phone Number*: [redacted] Fax Number: Email*: [redacted]

Signature of Authorized Representative* [redacted]

Date Signed* 05/23/2023

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:

424 R&R and PHS-398 Specific
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OMB Number: 4040-0010
Expiration Date: 11/30/2025

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: [redacted]
UEI: [redacted]
Street1*: [redacted]
Street2: [redacted]
City*: [redacted]
County:
State*: [redacted]
Province:
Country*: [redacted]
Zip / Postal Code*: [redacted]
Project/Performance Site Congressional District*: [redacted]

Additional Location(s) File Name:

Funding Opportunity Number: PAR-22-080. Received Date: 2023-05-23T10:12:57.000-04:00

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ○ **Yes** ● **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ○ Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ○ **Yes** ● **No**

6.a. If yes, identify countries:

6.b. Optional Explanation:

	Filename
7. Project Summary/Abstract*	[redacted]
8. Project Narrative*	[redacted]
9. Bibliography & References Cited	[redacted]
10.Facilities & Other Resources	
11.Equipment	[redacted]
12. Other Attachments	[redacted]

Funding Opportunity Number: PAR-22-080. Received Date: 2023-05-23T10:12:57.000-04:00

This request is for funds to purchase a JEOL JSM-IT700HRLA Scanning Electron Microscope (SEM) with EDS. The instrument will be housed within the [redacted] large, centralized imaging facility serving the needs of NIH supported researchers across our campus and at other local institutions. The mandate of this core facility is to provide access to and training for a full range of light and electron microscopy platforms, image analysis, and morphometric technologies to research groups within the school of medicine and elsewhere across campus.

SEM is an essential service provided by the center. The current microscope within the facility is a 22-year-old JEOL 6335F SEM. This microscope has been in constant service since it was purchased in 2001, using funds provided by the S10 mechanism [redacted]. During its life cycle it has been used between 1000-2000 hours each year for NIH supported research and has been kept under service contract throughout this entire time.

The primary reason for submitting this proposal is to replace this microscope because the manufacturer (JEOL) will no longer support or service the system (letter included) due to a lack of available parts or expertise in the field service team to service the device. All service support will end in April 2024.

A second reason for this application for a new instrument is to improve and expand the SEM capabilities of the core to meet the needs of our current NIH funded biomedical researchers. SEM technology has advanced significantly over what was available 22 years ago, and the current system lacks the resolution, speed, and sensitivity of a current instrument partly because of technologic advances but also general decreases in performance due to age (even with continuous maintenance). To put this in context, the system was originally supplied with a polaroid camera back, and the most recent update possible for the computer system (from Windows XP to Windows 7) was a decade ago.

Finally, as is described throughout the proposal, the analytic capabilities of SEM (using EDS) are completely absent from our current system. We have a significant and growing group of NIH funded users who have truly fundamental needs for this analytic functionality and have made a compelling case to bring this technology to the center.

Together we feel that these reasons present a very strong and compelling case to replace and upgrade our current SEM with a contemporary device.

The Scanning electron microscope (SEM) is an essential tool to study tissue structure and local elemental components in tissues and cells. The SEM available within the [redacted] is over 20 years old and will no longer be supported by the manufacturer and needs to be retired. This together with a defined need for more sophisticated analytical imaging modalites (EDS) by our NIH funded user population is driving this request to place a contemporary high quality SEM in the [redacted] to allow practical application of SEM imaging modalities tools to the largest number of NIH funded investigators here at the [redacted].

Bibliography and references cited

These are included in the individual project descriptions

Equipment:

This request is for funds to purchcase a JEOL JSM IT700 HRLA scanning electron microscope

Specific system components in the quote.

The total cost of the instrument with accessories and freight is $319,200. A detailed breakdown of the costs of the specific requested parts on the microscope is shown on page 1 of the quote. Pages 3-5 list the specific features of each item. Essentially the line items we are requesting are:

Line 1: JSM-IT700HRLA: Low Vacuum Scanning Electron Microscope

This is the Core instrument, with a FEG source (discussed above) and both high and low vacuum modes.

Cost is $213,000

Line 2: and 3: Airlock chamber and chamber camera

This microscope will be placed in a multi-user facility serving users with a very wide range of interests and experience. We have found over the last 20+ years that having airlock protection and in-column cameras are extremely valuable and in reality, essential for less experienced users or to minimize the likelihood of damage to the system.

Cost is $29,300

Line 4: EDS detector.

This is a significant addition to the cost of the instrument, however it is the most commonly requested addition by our larger SEM user group as is detailed in projects and in the instrumentation plan it brings significant analytic capabilities to the device which are important to meet the needs of our NIH supported user base.

Cost is $60,400

Line 5: and 6: SF-SEM supporter, and utility plugin

This software is essential for the CLEM approaches we are developing in the [redacted] and which are considered important for the future utility of the instrument.

Cost is $12,000



Instrument:
JSM-IT700HRLA
Low Vacuum Scanning Electron Microscope

Prepared for:
[redacted]

[redacted]

Bid No.: ***JSM-iT700HRLA***

JEOL Contact:

Bill Powell
Senior Sales Manager
wpowell@jeol.com
978-764-2397
Sales Administration Support:
SalesAdmin@jeol.com
978-535-5900

JEOL Solutions for Innovation

JEOL USA, Inc.
11 Dearborn Rd, Peabody, MA 01960
T 978-535-5900 • F 978-536-2221 • www.jeolusa.com

JSM-IT700HRLA

Quotation

11 Dearborn Rd Peabody, MA 01960 Phone: 978-535-5900 Fax: 978-536-2221

[redacted]

Quotation No.: SQC011854_4

Quotation Date: 4/26/2023

Quoted Price Valid for 30 Days

Line	Qty	Description	Ext. Price
1	1	JSM-IT700HRLA: Low Vacuum Scanning Electron Microscope	$213,000.00
2	1	MP-91140LLC-IT7: Airlock Chamber, 127mm Dia x 45mm H	$23,900.00
3	1	MP-94321CS: JEOL Chamber Scope	$5,400.00
4	1	EX-74212U4L2Q: JEOL JED-2300 Dry SDD EDS Detector, 30 mm	$60,400.00
5	1	SF-SEM_SUPPORTER: SYSTEM IN FRONTIER, INC. SEM SUPPORTER SOFTWARE	$10,000.00
6	1	SF-SEM-ZOOM: Software Utility Plug-in	$3,000.00

Sub Total Amount: **$315,700.00**

Freight:	**$3,500.00**
Total Amount:	**$319,200.00**

All Prices are in US Dollars.

Optionally Available (May replace above item(s) or be added to system):

Line	Qty	Description	Ext. Price
1	1	MP-94370LSED: LOW VACUUM SECONDARY ELECTRON DETECTOR	$10,400.00

Please send your Purchase Order to salesadmin@jeol.com

Freight Terms:	FCA: DESTINATION, LOAD DOCK
Estimated Delivery:	Estimate Delivery TBD
Payment Terms:	90% Net 30 days after Delivery 10% Net 30 days after Acceptance
Acceptable forms of Payment:	Check, Wire or ACH

Basic Terms & Conditions:

Prices quoted do not include any applicable sales and/or use taxes.

JEOL manufactured equipment is guaranteed against defects in workmanship and component failure for a period of one year from the date of customer's first use of the instrument. In the event that installation and/or acceptance is delayed by the Customer but not by JEOL, the warranty period will begin 60 days after the agreed upon delivery date. Under this warranty policy the labor necessary to repair the instrument and all parts (except consumables) are provided by JEOL at no cost to the Customer. JEOL reserves the right to repair or replace components at their sole discretion. The warranty does not cover components which have been damaged through misuse or accident on the part of the buyer nor does it include components which have been modified by the customer.

Third Party electrical/safety inspection, if required, would add to the promised delivery schedule, and the cost of inspections and any required physical modifications to the instrument would be borne by the buyer.

JEOL USA will hold the instrument for up to 30 days past the agreed delivery date. In the event that the customer's intended room is not ready, JEOL will deliver the instrument to an appropriate storage or temporary facility provided by the customer.

JEOL USA, Inc. Budgetary Quotation

JEOL USA, Inc.
salesadmin@jeol.com

Line	Qty	Detailed Description	Ext. Price
1	1	**JSM-IT700HRLA: Low Vacuum Scanning Electron Microscope** JSM-IT700HR – An analytical, high resolution, thermal Schottky type field emission scanning electron microscope. Includes both high and low vacuum modes of operation. A. BASIC PERFORMANCE: 1. Resolution: 1.0nm at 20kV, 3nm at 1kV (HV) 1.8nm at 15kV (LV) 2. Analytical Resolution: 3.0nm at 15kV and 3nA 3. Accelerating Voltage: 500V to 30kV 4. Beam Current Range: 1pA to ≥300nA 5. Magnification: X5 to X600,000 (print 128mm x 96mm) X14 to X1,679,499 (display 358mm x 269mm) 6. Low Vacuum Pressure: 10Pa to 150Pa B. ELECTRON OPTICS: 1. Schottky-type, in-lens thermal field emission gun 2. Probe current range from several pA to ≥300nA 3. Automated gun isolation valve 4. 4-stage adjustable objective lens aperture strip 5. Aperture Angle Control Lens (ACL) 6. Electromagnetic beam blanking, automatic after image acquisition C. DETECTORS: 1. Everhart Thornley type secondary electron detector 2. High sensitivity, multi-segment, solid state backscattered electron detector 3. JEOL fully embedded energy dispersive micro analysis system with Peltier cooled silicon drift detector (SDD) D. SCANNING SYSTEM: 1. High resolution digital acquisition up to 5120 x 3840 pixels 2. Advanced scan modes for charge reduction 3. Software includes: multiple live imaging modes, live image capture as AVI file, measurement & annotation, image processing, pseudo color image, eco mode 4. Zeromag for seamless transition from optical to SEM image 5. Automated montage (image stitching), includes large area EDS map 6. Interface for external scan control and data exchange 7. Operation panel for control of most common SEM functions E. IMAGE PROCESSING 1. Gamma, brightness/contrast, invert, binary coding, pseudo color (standard and custom), filtering 2. On screen measurement and annotation 3. Image archiving and database management F. STAGE AND SPECIMEN CHAMBER: 1. Large analytical chamber with 11+ ports 2. 5-axis, asynchronous, motorized, mechanically eucentric stage with following travel: X= 125mm, Y= 100mm, Z= 5-80mm	**$213,000.00**

Line	Qty	Detailed Description	Ext. Price
		continuous, T=-10° to +90°, R=360° 3. Stage Navigation System – top mount color camera, captures sample image for point and click sample navigation. 4. Specimen holder kit (32mm and 51 mm holders) G. VACUUM SYSTEM: 1. Fully automated vacuum sequencing 2. Pneumatically controlled valving 3. TMP, SIP (x2) and RP 4. Ion pump battery backup system 5. Operating pressure up to 150Pa in LV mode H. COMPUTER AND SOFTWARE: 1. PC with Windows 10, 64 bit OS, Touch Panel LCD Display (24") 2. JSM-IT700HR SEM control software with embedded Smile View™ Lab central data management software 3. Microsoft Office, Home and Business I. Installation and proof of performance in customer's laboratory J. One year parts and labor warranty (begins with customer's first use of instrument) K. User's course at JEOL, including tuition and supplies for one person. Valid for attendance within one year of new instrument installation L. One set of operator manuals	
2	1	**MP-91140LLC-IT7: Airlock Chamber, 127mm Dia x 45mm H** The load lock chamber attaches to the SEM chamber door for sample exchange without venting the chamber. The maximum sample size is: 127mm (d) x 45mm (h)	**$23,900.00**
3	1	**MP-94321CS: JEOL Chamber Scope** Specimen Chamber Scope is a high resolution CMOS camera which enables real time observation inside the specimen chamber. Fully embedded with SEM software interface.	**$5,400.00**
4	1	**EX-74212U4L2Q: JEOL JED-2300 Dry SDD EDS Detector, 30 mm** JEOL Energy Dispersive Microanalysis System with Peltier cooled Silicon Drift Detector (SDD) and EDS software. This system includes all of the hardware and software components for multi-point analysis, image capture, hyper spectral mapping, line scan, large area montage, drift correction, elemental quantification and reporting. HARDWARE: 1. Dry SDD (30mm^2), Peltier cooled, ultra-thin film window for detection from Be to U. Guaranteed energy resolution (Mn-Kα FWHM): 129 eV. 2. Digital pulse processor and power supply 3. EDS integration kit for connecting to SEM SOFTWARE: 1. Fully embedded with SEM for integrated management of image and analysis data with graphic display of image and analysis	**$60,400.00**

Line	Qty	Detailed Description	Ext. Price
		positions. 2. Full area, partial area, multi point/area, line scan collection 3. Automatic qualitative and quantitative analysis (ZAF or Phi-Rho-Z) with manual override 4. Active, hyper spectral mapping (up to 4096 x 3072 pixels), quantitative mapping. Reconstruction of element distribution during live acquisition or stored data. Real time filter and net count map. 5. Montage – automated large area montage (image and map). Serial map of multiple areas. 6. Drift correction 7. Visual Peak ID (VID) – synthetic spectrum based on elements identified 8. Help Guide – step by step help guide to data collection 9. Report generation with SmileView™ Lab, Export to Microsoft® Word or PowerPoint. 10. Can support dual EDS detectors	
5	1	**SF-SEM_SUPPORTER: SYSTEM IN FRONTIER, INC. SEM SUPPORTER SOFTWARE** SEM Supporter Software from System In Frontier, Inc. This software provides a means for sample navigation in an SEM from an optical image, SEM image or user defined templates. SEM Supporter automates SEM image collection from one or more locations, can automatically create photo montages and provides a means of image overlay and image analysis*. Recommended to be installed on a separate PC from SEM GUI. *Image analysis through optional add on modules	**$10,000.00**
6	1	**SF-SEM-ZOOM: Software Utility Plug-in**	**$3,000.00**

Line	Qty	Optionally Available Detailed Description (May replace above item(s) or be added to system)	Ext. Price
1	1	**MP-94370LSED: LOW VACUUM SECONDARY ELECTRON DETECTOR** Low vacuum secondary electron detector allows collection of secondary electrons in low vacuum mode.	**$10,400.00**

Letter from [redacted] at JEOL confirming termination of support for our current SEM

JEOL USA, INC.
11 Dearborn Road • P.O. Box 6043 • Peabody, Massachusetts 01961-6043
Tel: 978.535.5900 • Fax: 978.536.2205

NOTICE: End of service level agreements for the JSM-6330F

June 1, 2022

Dear [redacted]

I would like to thank you for being a valuable customer of JEOL. I hope your JSM-6330F has performed well over the years. Being such an old instrument, we have been continually trying our best to keep up support for critical parts. As such after April 1st, 2024, we will no longer be able to offer a new service level agreement for your instrument. We will continue to offer labor to support the instrument as long as we have the personnel available to do so.

Best Regards,

[redacted]

Service Sales and Business Development Manager

Letter from [redacted] at JEOL USA describing service contract costs for the requested microscope

Date: 5/2/2023
[redacted]

JSM-IT700HRLA
Service Level Agreement

[redacted]

Contract Type:
PREMIER SERVICE CONTRACT

Valid until:
3/31/2024

Service contract questions:
[redacted]
CONTRACT ADMINISTRATOR
978-536-2311
[redacted]

Service requests:
[redacted]
DISTRICT MANAGER
800-501-5365
[redacted]

JEOL
Solutions for Innovation

JEOL USA, Inc.
11 Dearborn Road
Peabody, MA 01960
Tel: 978-535-5900
Fax: 978-536-2314
www.jeolusa.com

[redacted]

Covered Items

Model	Description	Annual List Price	Term Net Price
386634	JSM-IT700HRLA ANALYTICAL SEM 386634/813022304	$24,817.98	$24,073.44
813007488	MP-94320CS CHAMBERSCOPE	$527.99	$512.15
813013879	EX-74600U4L2Q DRY SD30 DETECTOR UNIT	$4,661.86	$4,522.00
813007496	MP-94330SNS STAGE NAVIGATION SYST	$527.99	$512.15
813007615	MP-94340BEIW BACKSCATTERED DETECTOR	$593.38	$575.58
1 year of contract coverage if purchased with a new instrument. Offer expires on 3/31/2024	**System Total Price (USD):**	$31,129.20	$30,195.32

This service level agreement covers only items listed in the Covered Items table.
If you have a question about a particular or missing item please contact your salesperson for clarification.

Benefits:

- Dedicated account manager
- Factory trained engineers
- Telephone support
- Call history tracking
- Unlimited emergency visits
- Service quality guarantee
- Up to two preventive maintenance visits
- Repair parts included
- Average response time of one working day
- Apertures and scintillators included
- 20% discount on parts purchased from our parts department

Applicable terms and conditions:
PREMIER EO TERMS AND CONDITIONS US V3 2018

JEOL Terms and Conditions Web Site:
http://www.jeolusa.com/SERVICE-SUPPORT/Terms-and-Conditions

JEOL USA Inc. is an Affirmative Action/EEO Employer.
This quote contains confidential pricing and coverage information for the use of the Client.
Distribution or disclosure to others is prohibited.

Email from [redacted] showing multi-instrument discounts

Service contract multi-instrument discount

RE: service contract costs

[redacted]

Start your reply all with: Okay, thank you! Got it, thanks! Ok, thanks. Feedback

[redacted]

Premier: $31,129.20 is List pricing. With the multi-instrument discount for three instruments should be around $27,068. (net)

Kind Regards,

[redacted]

[redacted]

Justification of Need

This request for a **JEOL JSM-IT700HRLA** Scanning Electron Microscope (SEM) with secondary electron, backscattered electron, and EDS) Detectors.

Description of requested device

The cost, robustness, performance, and ease of use of scanning electron microscopes has changed dramatically over the last several years, such that current machines, which satisfy almost all needs for biomedical researchers, are significantly less expensive than our previously purchased equipment. In this application we have restricted our request to a device that offers the flexibility and ease of use suitable for the large user cohort we routinely work with, but that also offers sufficient resolution and sensitivity for current and future projects.

Competition

We have evaluated similar platforms including the Thermo-Fisher Apreo, and devices from Zeiss, Tescan, and Hitachi. There are multiple fundamental, but mostly practical, reasons for requesting the JEOL instrument:

- Our positive experience with the JOEL SEM currently in house. The current SEM (JEOL 6335F) has been extremely reliable and while it does not offer the resolution and performance of the newer generation of SEMs, it has shown relatively little deterioration in performance since it was purchased.
- Service availability. The consistent performance of the device we are replacing is partly because the JEOL service team is located in the [redacted] area, so service turnaround time is generally immediate (within 24-48 hours) and commonly on the day of request.
- Multi-instrument service contract discounts. Less important, but still fiscally relevant, is that we have two other JEOL electron microscopes. Both are current TEMs: a JEOL 1400 PLUS and a JEOL 1400 Flash (both acquired through the S10 mechanism ([redacted])). The latter grant supported purchase of a JEOL 1011 which was replaced with the JEOL 1400 Flash with an insurance claim due to a flood in 2020. Because of this, we can decrease maintenance costs with a significant (15%) multi-instrument discount ([redacted]) and our annual maintenance contract is thereby reduced from $31,129 to $27,068. Importantly there will also be a concurrent reduction (to 15%) in the maintenance contract costs for our two TEMs
- Technology development. As detailed both in the description of need, and in the individual project descriptions for the device, we are developing novel CLEM approaches which allow large area light microscopy scans to be correlated to the same positional loci in XYZ in both confocal and Backscattered SEM imaging approaches. The integration of the technologies relies on a seamless transition of sample loci/fiducials from the confocal images to the SEM low magnification scan. This has been done and is working well between the Nikon confocal platform and the JEOL JSM 7800 SEM (on loan from JEOL). The software developed will also work with the requested device and programs developed by JEOL specifically for this approach are included in this quote.

There are also multiple reasons we are requesting this device which are not specific to this device but are important when considering our user base and are significant when placing a delicate complex microscope in a multi-user facility such as the [redacted]

- It is an “all in one system”. essentially all the features that are important to its use in a multi-user facility are included in the base instrument: secondary, backscattered and EDS detectors working at high or low vacuum are included as well as a Color Navigation Camera to look at sample position in real time.
- It is a Field Emission Gun-SEM. The current system in the [redacted] also has a field emission source which has been extremely reliable and has failed only once in its entire 22-year lifetime (though was replaced once as warranted by the service team).
- The high/low vacuum switch is automated and easy to use (no aperture changing).

- Its resolution performance is exactly what we need for our user base:1nm at 20kV and 3.0nm at 1kV in high vacuum mode, but perhaps more importantly 3nm at 15kV in general use conditions.
- Alignment is very simple and highly automated, including corrections for focus, astigmatism and brightness/contrast. This is a boon in a multi-user, predominantly graduate student/post-doctoral fellow oriented environment and certainly makes training far simpler and more effective for the users.
- Image montaging is automated which is essential for the future uses planned for this SEM.
- We do not need to use a water chiller to reach the resolution specifications, we do have enough chilled water supply to cool all the current electron microscopes, but there is a concern that it is working at close to maximum since we upgraded one of our older TEMs, a JEOL1011 to a JEOL1400 Flash.

Specific system components in the quote.

Line 1: JSM-IT700HRLA: Low Vacuum Scanning Electron Microscope

This is the Core instrument, with a FEG source (discussed above) and both high and low vacuum modes.

Line 2: and 3: Airlock chamber and chamber camera

This microscope will be placed in a multi-user facility serving users with a very wide range of interests and experience. We have found over the last 20+ years that having airlock protection and in-column cameras are extremely valuable and in reality, essential for less experienced users or to minimize the likelihood of damage to the system.

Line 4: EDS detector.

This is a significant addition to the cost of the instrument ($60,000) however it is the most commonly requested addition by our larger SEM user group. It allows transitioning a commonly descriptive technology into an analytic platform and has been specifically requested by all projects in the McGowan Institute for Regenerative Medicine part of the application ([redacted] (working on behalf other users at [redacted]), [redacted]). Given the constant requests for this platform, we feel that it is an important addition to the instrument which will expand its future, long term utility enormously as well as benefit current NIH-supported users.

Line 5: and 6: “SF-SEM” supporter, and utility plugin

This software platform is a third party addition sold by JEOL which is essential for our CLEM approaches. Before we integrated the software into our current device, we had to manually calibrate and adjust position, rotation, orientation, etc. between the LM and EM platforms. This navigation toolset facilitates the transition of coordinates seamlessly using overlays and image corrections between the two imaging modalities.

Other instrumentation on the [redacted] Campus

Currently there are five SEM's on the [redacted] campus. Two of them are in the [redacted] These are an Apreo SEM with EDAX/EDS and EBSD and a Zeiss Sigma 500 VP. The use of these devices is essentially saturated and realistically not available to researchers within the NIH supported community in the Medical School for routine use (see letter from [redacted] who runs that facility). A third, a JEOL JSM-6390LV is in the department of biological sciences and is also unavailable to users outside the biological sciences department (see letter from [redacted], manager of the [redacted]). The other two are within the [redacted] One of these (a JEOL JSM7800F) is on a temporary loan from JEOL specifically for development of new correlative light/EM approaches (CLEM). This machine is under the management of the PI of this grant ([redacted]) and is used exclusively by a single senior staff member within the [redacted] specifically for the CLEM project. We expect this device to be returned to JEOL within the next year and need to transition this work to another device (the machine requested here). The final machine, also in the [redacted] is a JEOL JSM 6335F, this is the only SEM generally available to NIH funded researchers within the School of Medicine. The instrument was funded through the S10 mechanism 22 years ago ([redacted]) and has been in constant service and under service contract since it was installed in 2001. Unfortunately, even with multiple upgrades including new detectors, a new computer and operating systems and associated hardware, JEOL will no longer offer a maintenance contract for this microscope due to a lack of critical parts and engineer expertise starting in April 2024 (See Letter from [redacted], Service Sales Manager at JEOL). We will retire this machine if this grant is funded.

Use of the current SEM.

Within the [redacted], electron microscopies are essential and frequently used technologies that are part of the larger repertoire of optical methods used to support NIH supported research. SEM modalities that are heavily used in the [redacted] are secondary electron detection to define tissue or cell structure at high resolution, and backscattered electron detection for immuno-localization of proteins on cell surfaces, or for "TEM like confirmation" of tissue structure at high resolution as an SEM correlate of light microscopy. Furthermore, there is a compelling number of requests to integrate analytical approaches such as EDS by individual users which is not currently available within the [redacted]



Figure 1: mandrel-less polymeric technology generates microfiber bundles with tunable structure for sutures or tissue surrogates used in tendon and ligament repair (Adv Healthc Mater. 2022 Jul;11)

Currently the use of SEM within the [redacted] falls into two clusters. The first cluster is comprised of "long term heavy users". These are scientists who are dependent on the power of the SEM approach to answer structural questions which are beyond the resolution of conventional or even super-resolution microscopy approaches, but which are also refractile to thin sectioning TEM approaches. For example, measuring the number and size of fenestrae in liver sinusoidal endothelial cells (see the [redacted] and the [redacted] users), or assessment of the cell shape and surface organization of cytoskeleton in 3D in relation to tissue components (see the [redacted] project). For a second population of users the use of scanning electron microscopy is essential but episodic; in other words, investigators absolutely need to use the technology to rationalize an hypothesis, to define presence, absence, shape or number of structures in a sample, or to investigate the quality or structure of a material at high resolution in three dimensions (for example see **Figure 1**) but on an occasional basis. Importantly though, these studies are nearly always an integral part of a larger experimental series. Because of this, in our environment, which is driven primarily by NIH supported research themes, a significant population of users of our SEM have occasional but intense need for the technology, and as such the traditional extended project descriptions typical of an S10 grant are less apparent. To expand on this Table 1 below shows the use of SEM by NIH funded users over the last 4 years. As can be seen in

the table below, there is a large cohort of users who use smaller amounts of time, for example [redacted] is absolutely depended on SEM to demonstrate the quality and uniformity of the microfiber bundles generated by the mandrel-less spinning technology shown in Figure 1. This was a single bout of very heavy activity over several weeks. Other research groups use the device in a more consistent way (**see Table 1**) with hundreds of hours logged over the entire period (2019-2023).

PI Name	JSM 6335F SEM 2019-2023	JSM-7800 SEM 2021-2023	PI Total	[redacted]	130	0	130
[redacted]	95	0	95	[redacted]	42	0	42
[redacted]	158.5	8	166.5	[redacted]	14	0	14
[redacted]	10	0	10	[redacted]	27	0	27
[redacted]	175	3.5	178.5	[redacted]	3	3.5	6.5
[redacted]	121.5	0	121.5	[redacted]	24.5	0	24.5
[redacted]	48	0	48	[redacted]	69	0	69
[redacted]	6	0	6	[redacted]	23	9	32
[redacted]	87.5	2	89.5	[redacted]	4	0	4
[redacted]	1	184	185	[redacted]	0	167.5	167.5
[redacted]	18	0	18	[redacted]	520	5	525
[redacted]	208	0	208	[redacted]	30.5	0	30.5
[redacted]	16	0	16	[redacted]	0	111.5	111.5
[redacted]	3	0	3	[redacted]	12	4	16
[redacted]	3	0	3	[redacted]	6	0	6
[redacted]	3.5	0	3.5	[redacted]	71	0	71
[redacted]	0	9	9	[redacted]	40	0	40
[redacted]	123	0	123	[redacted]	6	0	6
[redacted]	0	8	8	[redacted]	13	0	13
[redacted]	0	148	148	[redacted]	630	0	630
[redacted]	25.5	0	25.5	[redacted]	864	135	999
[redacted]	13	0	13	[redacted]	12	0	12
[redacted]	14.5	0	14.5	[redacted]	0	74.5	74.5
[redacted]	8.5	0	8.5	[redacted]	4	0	4
[redacted]	9	4.5	13.5	[redacted]	14.5	0	14.5
[redacted]	8.5	12.5	21	**Total Hours**	**3654**	**889.5**	**4554.5**

Table 1. Note: All users had NIH funding at the time of use of the SEMs, also use of the JSM 7800 is only for the last 2 years.

A third developing theme within our environment is the development of novel correlative light/EM approaches (CLEM) which are becoming heavily used by federally supported users at our institution. Use of these technologies is becoming more continuous and consistent as we refine the tools and the workflow. The CLEM approaches within the application (essentially all the use of the JSM 7800) are based upon approaches developed within the [redacted] that allow correlative imaging between large area confocal microscopy and high-resolution backscattered electron SEM ultrastructure. Using this approach, we are able to define individual subcellular structures in large areas (up to a 12mm coverslip: 113 mm^2 area) in 3D and then find the same structures in 3D using SEM as a high-resolution EM correlate. Results from the method are starting to appear in high impact journals as described in the CLEM component. When we initially developed this technique, we were using our current JSM 6335 device for the SEM component of the imaging, unfortunately acquiring images at high resolution was quite challenging and as a result we were sending samples to our JEOL colleagues in [redacted] for image tiling. To ease the workload for JEOL based investigators, a JSM 7800 was placed on temporary loan within the [redacted] for the last 2 years. Currently the approach is gaining significant traction in our local environment, but our JEOL colleagues are expecting the return of the loaner instrument. Fortunately, the requested device uses similar

detector technology as our current SEM, and in fact has twice the scanning speed which means that we will be able to seamlessly transfer the CLEM projects from the loaner JSM 7800 to this new microscope.

Requests for analytical approaches

When the instrument we are retiring was purchased, analytical approaches using SEM were not considered to be a critical need by the [redacted] steering committee. However, this need has changed dramatically as investigations using SEM have become increasingly quantitative. Users from the [redacted] group are tightly integrated into the fabric of the [redacted] and use a continuum of imaging approaches (light and EM) provided by the [redacted] and all have needs for EDS. On our campus, these approaches are only possible using the devices available within the [redacted], however as detailed in the letter from [redacted], these machines are at saturated and our cadre of users often need to wait weeks to get time to analyze their samples, hence their request to integrate the method into the instrument we are requesting here.

In summary, taken together, because of the lack of other SEMs on campus generally available to NIH supported investigators, the importance of the device to a large number of research programs, the growing interest in large area backscatter scanning/CLEM approaches, a clear need for integrated analytical tools and the imperative to retire the microscope we have within the [redacted] there is an essential need to replace our current SEM instrument with the requested device.

Defining Accessible User Time (AUT)

The use and access to instrumentation within the [redacted] is carefully controlled and is detailed at length in the management plan. However, we do our best to maintain a truly “open door” philosophy such that we can get maximal use from all our microscopes. Essentially users fall into three groups: “Expert”, “Trained” and “Novice”. Expert users have been through extensive training and have over 50 hours of logged usage time on instruments. Trained users have been through a multipart training program, though have yet to log significant use hours or have only sporadic need for devices. Novice users are still undergoing training.

Obviously, the training program varies between instruments. For example, a simple upright light microscope has only a brief training period to ensure that devices are protected, computer interfaces are learned, and the user can perform basic alignments ([redacted]) etc. All advanced imaging approaches (both light and electron) demand much longer and in-depth training with time committed to didactic coursework in the case of graduate students and fellows.

Electron microscopy has its own set of challenges, TEM may involve months of training before users can reliably cut sections or be trusted with diamond knives. SEM on the other hand has generally relatively simple preparation steps, dehydration, mounting, coating and perhaps polishing of samples, all of which are relatively automated or simple. However, in both TEM and SEM modalities the instruments themselves are relatively fragile and events such as inadvertently cracking the vacuum can lead to significant downtime as the system restarts and reaches operating condition once more. Therefore, it is important to protect the device from damage and to ensure users are aware of the potential problems that can occur. Because of this, we have implemented established management and supervision plans for all our electron and optical systems. These will also be adhered to for this device.

Only “Expert” users are permitted unsupervised 24/7 access. They have been trained in SEM alignment and can correct minor issues in beam quality.

“Trained” users will work with [redacted], to set up experiments but once imaging conditions are established, users will be allowed to work in an unsupervised fashion. This approach has worked very well for our other electron microscopes. These users are not allowed to use systems after usual work hours and while they can change samples they are not permitted to change fundamental system settings such as apertures, and primary beam alignments.

“Novice” users will only be allowed to work in a supervised way. Supervision will be by [redacted]. Generally, these users have one of the two staff sitting with them until the sample is actively being scanned. They can move the sample around, focus, change magnification and take pictures, but we ask

them not to correct even simple astigmatism or alignment issues. As they are only at the microscope during the working day their use can be readily supervised and managed.

This stratified approach is currently in place and works well for all our advanced systems including the electron microscope platforms. The TEMs are used between 1500 and 1800 hours/year. The current SEM (the JEOL JSM 6335 we are retiring) is currently used around 800-900 hours/year and we expect the system requested here to match this level of use immediately. We anticipate that the addition of the EDS capability will increase usage to 1500 hrs/year and we expect the system will absorb 350+ hours of CLEM imaging a year, with a total of 1,850 total hours predicted for first year. Given the extra capabilities (EDS), resolution, and ease of use of the requested device we expect use to hover around 1800-1950 hours/year which we consider to be close to the maximum AUT. Downtime for maintenance on this device should be similar to our other electron microscopes, which is currently about one-two weeks per year The AUT could rise as the absolute level of instrument use after hours is entirely dependent on the number of users who have reached "Expert User" level.

Technical Expertise

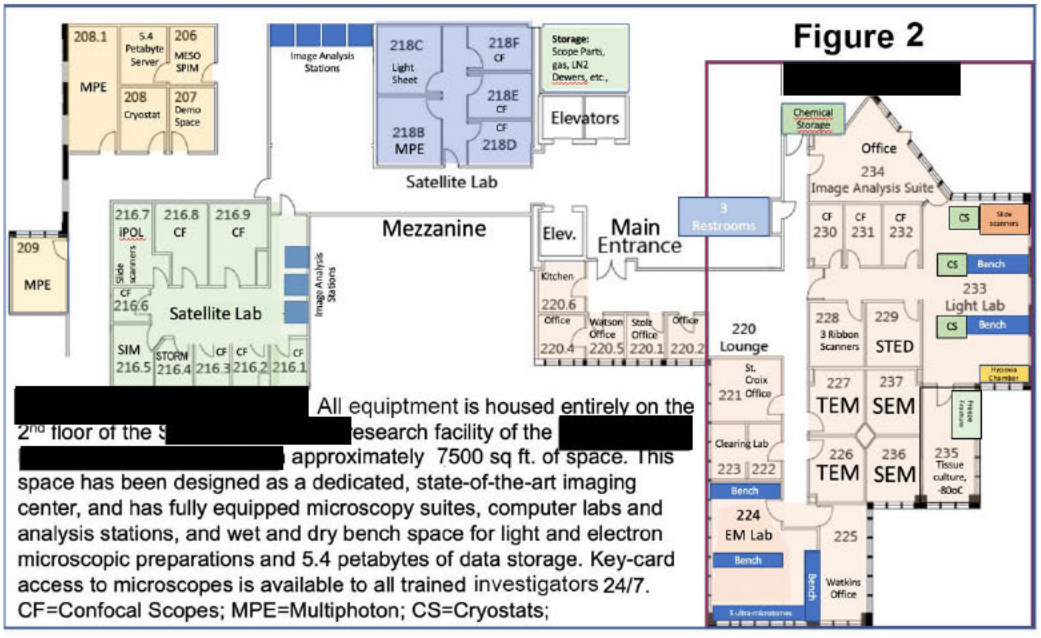
A. Description of the [redacted]

As Microscopy evolved within the rubric of modern biomedical science to become a primary investigative method, there has been a concurrent and dramatic increase in the cost and complexity of imaging systems. Further the expertise needed to optimize the use of these tools and techniques in modern biomedical research has become very specialized. The [redacted] was formed 31 years ago to meet this challenge and provide a "cutting edge" resource able and equipped to address any microscopic research needs from the very simple, to the most complex for the larger NIH funded medical research community at the [redacted]. Since its inception the Center has become an integral component of the medical research community, as described below.

Facilities: The space allotted to the [redacted] is an entire floor of the [redacted] This exclusive location allows us to manage user access, with approved users being able to access the space via elevator using a validated keycard. All imaging suites are also controlled by keycards, and individual rooms by controlled keypads. Because of this, we can limit access to specific instruments as needed. The space has dedicated microscope rooms for each of the light and electron microscopic technologies and an extensive image analysis suite. Sufficient wet lab space is available for multiple investigators to perform concurrent experiments.

The Center has two "core" system areas (Satellite Labs [redacted] which currently house 20+ microscopes including multiple confocal, widefield, live cell, multiphoton, TIRF and super-resolution microscopes. If this application is successful, the requested instrument will be housed in a dedicated room in this space (Room [redacted]), currently housing the JSM 6335 which will be retired.

Figure 2



[redacted] All equiptment is housed entirely on the 2nd floor of the S[redacted]esearch facility of the [redacted] approximately 7500 sq ft. of space. This space has been designed as a dedicated, state-of-the-art imaging center, and has fully equipped microscopy suites, computer labs and analysis stations, and wet and dry bench space for light and electron microscopic preparations and 5.4 petabytes of data storage. Key-card access to microscopes is available to all trained investigators 24/7. CF=Confocal Scopes; MPE=Multiphoton; CS=Cryostats;

B. Leadership and Staffing of the Center

There is significant technical and intellectual expertise in the use of all microscopy modalities (light and electron) within the CBI. The following details the staff of the center, and in particular highlights expertise in Electron microscopy.

The Director: [redacted] is a distinguished

professor with tenure within the faculty of the Medical School. His primary function is to direct the operation of the [redacted] He reports directly to the steering committee. Within the [redacted] is: 1. A consultant to the user groups to provide help in the design of experiments using microscopy, and in the execution of more complex methodologies; 2. Responsible for the education of users at all levels to ensure the application of microscopic technique is optimized. He has directed courses nationally (Quantitative Fluorescence Microscopy, in [redacted] which is ongoing), internationally ([redacted]); and has assisted in courses at [redacted] and the light sheet microscopy course), and in [redacted]. He has been an author on more than 750 peer reviewed papers which include microscopy-based methods as fundamental tools.

Associate Directors: [redacted]s an independently funded (PHS) tenured full professor in the Department of Cell Biology and secondary appointment in Pathology. [redacted] has an active research program focusing on liver disease as the Director of the [redacted] and serves as an Executive Committee Member for the [redacted] Her roles are to 1: supervise the daily running of the electron microscopy aspects of the facility including management and training of the technical specialists; and 2: consult with user groups who wish to use the EM facilities within the [redacted] will be the faculty primarily responsible for this system.

[redacted] is an independently funded (PHS) tenured full professor in the Department of Cell Biology. [redacted] focuses her research activities on the application of optical microscopy to the study of living systems, specifically combining free radical imaging, FRET, confocal, multiphoton and physiologic measurements in live model systems, tissues and cells.

[redacted] is an independently funded tenure stream assistant professor in the Department of Cell Biology. His research program is directed to development and application of image acquisition and analysis/processing applications for massive data structures (multi-terabyte volumes).

Technical Specialists: The technical base consists of 14 post-doctoral fellows and staff, all trained microscopists, 3 of whom work exclusively on the use and application of Electron microscopy. [redacted] Level IV research specialist) manages the EM facility along with [redacted] (Level IV research specialist) and [redacted] (Level IV research specialist). Together these staff have aggregate experience in the use of Electron microscopy for conventional non-cryo EM totaling over 70 years. Each is a truly expert electron microscopist. Furthermore, we have a staff of 2 undergraduate research assistants who provide general lab maintenance for the light and EM lab.

These specific staff ([redacted]) are responsible for the processing and experimental manipulation of materials for microscopy (including tissue/cell, fixation, sectioning, immuno-gold labeling, and SEM and TEM microscopy). They assist users directly in the application of electron microscopy technique, though equally they perform complete procedures for users who are not sufficiently experienced to perform their own experiments. They are also responsible for the day-to-day running of the facility, including management of microscope usage, microscope maintenance, bookkeeping, solution preparation, etc.

Technical Expertise in Scanning Electron Microscopy: [redacted] will be the faculty responsible for managing the SEM. She has been responsible for the other SEM system in the [redacted] since its purchase. She has published multiple articles involving EM methods and her input ensures a seamless transition from experimental conception through design and execution. [redacted] will be assisted by [redacted] as needed. We have found this collaborative interaction particularly useful when designing CLEM experiments which may demand high level expertise in preparation for both LM and SEM.

The device will be managed on a day-to-day basis by a single highly expert [redacted] technician, [redacted] M.S. who with 15+ years of EM experience). [redacted] will be responsible for all training to ensure consistency of knowledge. He trains users, attends experimental planning sessions, makes sure that systems are functioning properly and checks the systems daily for cleanliness, and functionality, he is an essential member of the EM imaging team. We will also ensure that one of the other EM microscopy specialists within

the [redacted] M.S., (a level IV technician with more than 30 years imaging experience) is able to manage and supervise all EM activity in [redacted] absence.

Administrative assistance: The primary administrative responsibilities are the monthly billing of charge-back users, processing of [redacted] purchase requisitions and other general administrative duties. This duty is performed by a senior research specialist ([redacted]) in conjunction with the business manager for the [redacted] ([redacted]).

Activity of the Center: During the last year (Spring 2022-Spring 2023) the Center has had collaborative and/or service interactions with over 320 NIH funded research teams. Unfortunately, expressing this information in tabular form expands this section beyond the page limit. Furthermore, we have collaborated with 23 unfunded investigators to generate new data for grant submission (for example [redacted] a junior tenure stream faculty who is included in this application). This activity has resulted in submission and/or publication of over 400 peer reviewed manuscripts in the last 5 years, of which almost 200 were collaborative with [redacted] faculty. Again, this table exceeds the limits of this section of the proposal.

Research Projects:

This application is being submitted for three specific reasons. The first and most critical need is to provide a replacement for the current microscope such that essential SEM services can continue to be provided to NIH supported researchers across our entire campus. The second reason is to expand our capabilities to provide analytic tools (EDS). The third reason is to allow continued technology developments in the [redacted] for correlative light and SEM approaches. We have divided the descriptions of research projects into groups which include "individual user" projects and three thematic clusters: 1. The [redacted] Liver Research Center, 2. The [redacted] Institute for Regenerative Medicine, 3. CLEM projects and ongoing CLEM developments within the [redacted].

Individual User Projects

We have discussed the use of SEM at our institution earlier in this application. To repeat, the majority of use is episodic and intense and used to support and further expand results from other optical or molecular biologic/biochemical technologies. Some core users make more consistent use of the technology, and we have chosen projects from two investigators to illustrate this activity. [redacted] and [redacted] are both senior, well-funded investigators in the departments of Medicine and Pharmacology respectively, both are heavy users of the current device and are planning to continue to make significant use of the requested system in the future.

[redacted] **– Redox Signaling in the Liver Microcirculation. Grant numbers:** R35HL[redacted], R01HL[redacted], R01HL[redacted].

Liver sinusoidal endothelial cells (SECs) are a highly specialized cell type that permits rapid, passive diffusional exchange of molecules and particulates between the blood and hepatocytes through transcellular structural pores called fenestrae.[1] In humans without liver pathology, fenestrae are "holes" in the cell averaging ~50-100 nm in diameter and function to sieve circulating nutrients, lipids, and lipoproteins for normal liver metabolism.[2] While larger fenestrations can be seen by super-resolution light microscopy approaches like STED (~70 nm resolution) or SIM (100-120 nm resolution), it is not possible to resolve the smaller structures, or clustered pores, using any optical approach. SEM is an essential method for obtaining accurate measurements of number, size and shape of these structures.

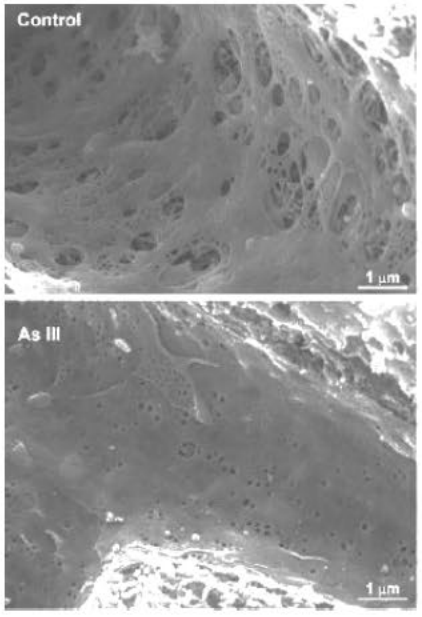


Fig. 1. Scanning electron micrographs of mouse livers from mice treated exposed to arsenic. Images were published in [redacted]

Early in development, SECs differentiate to lose many biomarkers of normal endothelium, such as junctional expression of platelet endothelial cell adhesion molecule-1 (CD31) and a basement membrane containing the matrix protein laminin-1.[3] In the setting of liver injury and disease, SECs have the capacity to undergo defenestration and form tight junctions limiting trans-endothelial cell transport, a process called capillarization.[4,5] Capillarization precedes vascular remodeling of other liver vessels, such as the hepatic arteriole, causing blood flow shunting, vascular channel formation, and subsequent liver fibrosis.[4,6] Animal models have been used to demonstrate that liver capillarization affects the systemic vasculature by decreasing liver metabolism of lipids, lipoproteins, and glucose to promote atherogenesis in response to environmental stresses and aging.[7,8] The [redacted] group has shown than regulation of the capillarization process involves reduction-oxidation (redox) signaling.[9,10] To study the capillarization process *in vivo* and *in vitro*, the "gold standard" technique is scanning electron microscopy (SEM) as shown in **Figure 1 and 2** from [redacted] published work.[9,10] Images captured using SEM can be analyzed to calculate the porosity of SECs in animal models and cells. As part of his ongoing NHLBI funded

studies (R35[redacted] aims to define how impaired redox signaling can impact SEC function and the capillarization process. Specifically, their program will define how the family of cytochrome b5 reductases (CYB5Rs)[11], which are known enzymes to modulate redox balance, control SEC phenotype in cardiovascular disease (i.e. hypertension, atherosclerosis) and hematological diseases (i.e. sickle cell disease).

There is significant concern that without continued and consistent access to SEM, NIH funded projects from his group will be impacted dramatically. For example, one of his grants (HL[redacted]) has a central goal to define the impact of SEC expressed CYB5Rs on capillarization in the setting of cardiovascular and hematological disease. They are proposing to study novel murine CYB5Rs knockout models to determine whether impaired redox signaling drives SEC dedifferentiation and capillarization in models of hypertension, atherosclerosis and sickle cell disease. In each facet of this project, they will use SEM to extract data on the shape, porosity and health of the endothelium as shown in Figures 1 and 2 above. A second goal is to determine if acute challenge with drivers of cardiovascular and hematological disease modulate SEC dedifferentiation and defenestration.

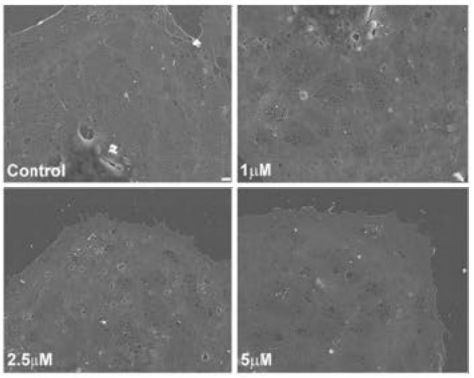


Fig. 2. Scanning electron micrographs of mouse livers from mice treated exposed to arsenic. Images were published in [redacted].

[redacted]

[redacted]: **Project 1: Studies of apical junctional complex dynamics during bladder filling and voiding: Grant numbers:** 5R01DK[redacted], 5P30DK[redacted], 5R01DK[redacted]

The epithelial apical junctional complex (AJC) is comprised of the tight junction, the adherens junction, desmosomes, and an associated actomyosin cytoskeleton (**Fig. 1A**). Integral to AJC function is the requirement that it maintain its continuity in the face of external mechanical forces that accompany normal physiological functions such as breathing, eating, and urination. How this is accomplished is poorly understood. To explore these events, [redacted] group are studying the bladder umbrella cell, which forms the outermost layer of the stratified bladder urothelium, which lines the renal pelvis, ureters, bladder, and proximal urethra. The umbrella cell AJC is visible by SEM (see inset **Fig. 1B**). Umbrella cells form a high resistance barrier that accommodates bladder filling by flattening but after voiding they return to a more compact state. Their studies to date reveal that the AJC of umbrella cells expands during bladder filling and contracts upon voiding [1, 2]. Filling-induced expansion of the AJC depends on the actin cytoskeleton (formin-mediated polymerization in particular) and Rab13-dependent trafficking events (but not Rab11A- or Rab8A-dependent). They surmise that RAB13 promotes delivery of AJC cargoes (e.g., claudins) to the expanding junctional ring. His group has also observed that within 5 min of voiding there is dynamin-dependent endocytosis, as well as RhoA- and non-muscle myosin IIA-dependent contraction of the AJR-associated actin ring. This returns the AJC to its pre-filled state.

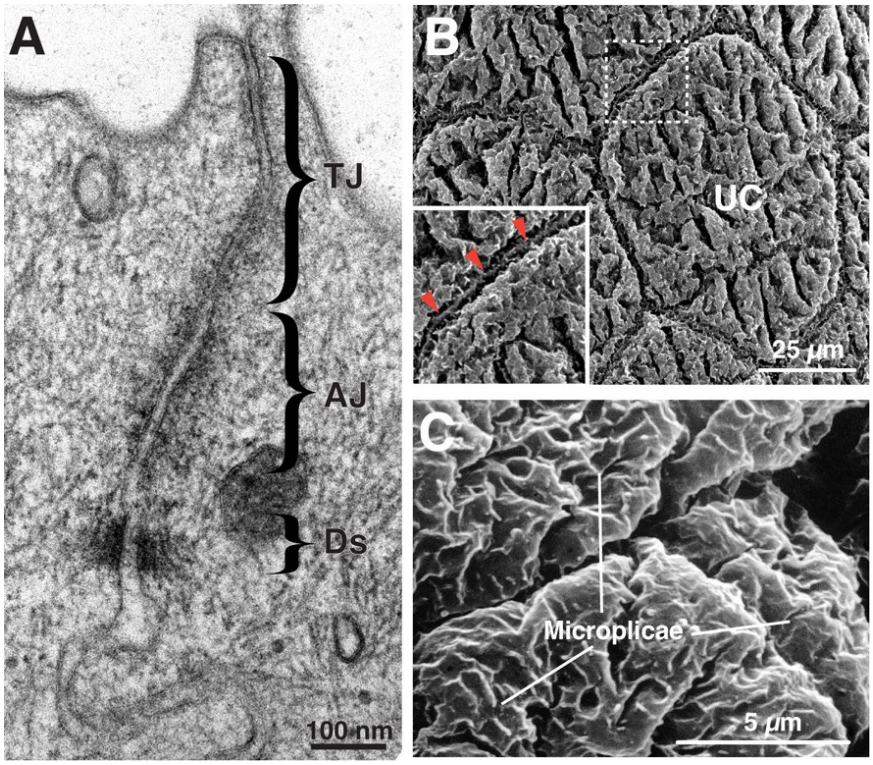


Figure 1. Ultrastructure of the umbrella cell. (A) TEM of apical junctional complex between two adjacent umbrella cells. Legend: TJ, tight junction; AJ, adherens junction; Ds, desmosome. (B) SEM of surface-exposed umbrella cells (UC). Inset: The AJC, viewed from above, is marked with red arrowheads. (C) High magnification view of umbrella cell apical surface. Microplicae (surface ridges) or marked.

Their current studies are focused on understanding the AJC-associated cytoskeleton, because it organizes the AJC, it responds to and generates cellular forces, and disrupting the actin cytoskeleton in particular causes defects in adhesion and paracellular permeability [3-5]. Despite the well-documented association between the actomyosin network and the AJC, there are surprisingly few studies that have carefully analyzed how these elements are organized with respect to one another. Most studies have employed cultured cells and focused on the actin cytoskeleton associated with the adherens junction [6]. For example, in cultured Madin-Darby Canine Kidney cells, punctate non-muscle myosin IIB (NMMIIB) staining overlaps considerably with a continuous ring of F-actin at the adherens junction [7]. Thus, an important goal of their studies is to understand the organization of the umbrella cell AJC and to decipher the role of the cytoskeleton in umbrella cell AJC function.

When examined by confocal microscopy, the actin cytoskeleton between adjacent umbrella cells appears as a thin continuous ring with no structural information apparent. However, using techniques originally developed to reveal the ultrastructure of the cytoskeleton of glass-grown cultured cells [8], the [redacted] group are now able to visualize the cytoskeleton of detergent-extracted, native umbrella cells using SEM (**Fig. 2**). While presumptive short actin filaments are visible using this technique (see inset in **Fig. 2B**), at the moment

the instrument we are retiring does not have the resolution of the requested device even with carefully managed preparations to better understand the nature and organization of these filaments. This is mostly due to 20+ year old design and loss of performance due to age. Furthermore, the [redacted] group plan on decorating the actin filaments with myosin S1 fragments, as well as performing immunogold labeling to detect actin-associated proteins including alpha-actinin and NMMIIA (the predominant non-muscle myosin expressed in umbrella cells). Detection of gold critically depends on the back-scatter detector we have requested. In addition to the actin cytoskeleton, they are also exploring the elaborate intermediate filament cytoskeleton (comprised of cytokeratins) that interfaces with the AJC. While current models of the AJC incorporate radial cytokeratins emanating from desmosomes, the newest evidence indicates that there is also a circumferential band of cytokeratins that surrounds the AJC [9]. However, there are limited insights into how this circumferential band is organized and its relationship to the actin cytoskeleton. They are using multiple other light and EM approaches and preliminary confocal microscopy studies indicate that the cytokeratin cytoskeleton is closely apposed to the actin cytoskeleton and may be tethered by plectin, a large structural protein that can bind both actin and cytokeratins (**Fig. 2C**). Equally preliminary EM studies further support a close continuity between the actin and the cytokeratin cytoskeleton (**Fig. 2B**). To better understand this organization, they will again make use of immunogold labeling coupled with SEM to probe the AJC cytoskeleton and the relative expression and location of actin, plectin, cytokeratins, and other proteins associated with desmosomes including desmoplakin.

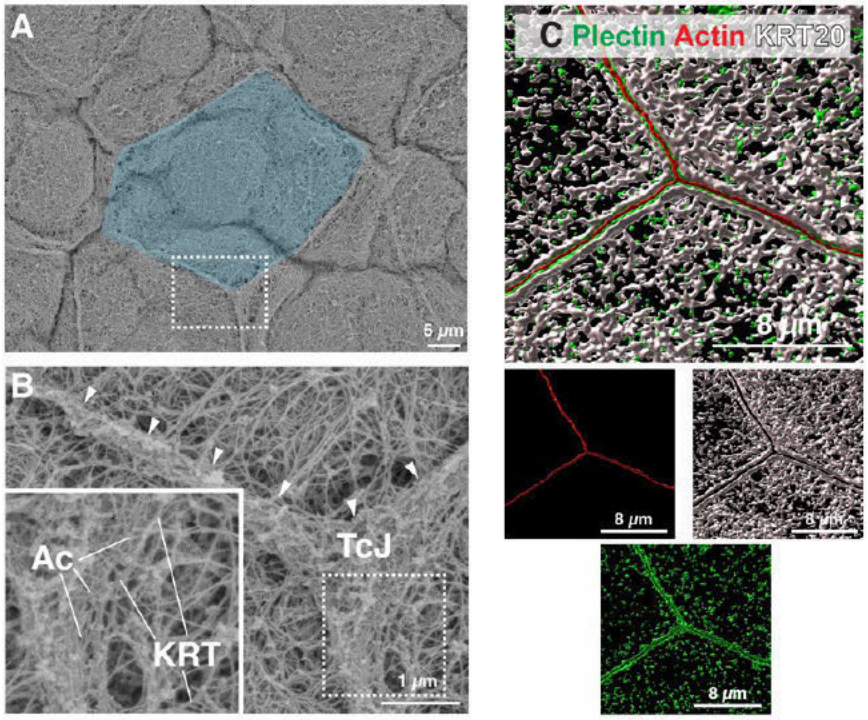


Fig. 2. Organization of the umbrella cell cytoskeleton. (A-B) SEM of detergent-extracted umbrella cell cytoskeleton. Boxed region in A is magnified in panel B, and boxed region in B is magnified in the inset. The position of the AJC is marked with arrowheads. Legend: TcJ, tricellular junction (region of contact between three umbrella cells); Ac, actin; KRT, keratin intermediate filaments. (C) Distribution of plectin, actin, and KRT20 in the umbrella cell AJC.

[redacted] **Project 2: Organization of fibroblasts and the extracellular matrix in the bladder lamina propria**. The [redacted] group are also using SEM to understand the organization of the connective tissues in the bladder wall. As an example, they alkaline-macerated mouse bladders (which removes all cellular and ECM components but those comprised of fibrillar collage), fractured the extracted bladders, and used SEM to explore the overall organization of the fibrillar collagen in the bladder wall (**Fig. 3**)[10]. Using these techniques, this team will further define the regions of the mouse bladder that undergo fibrosis when mice are subjected to partial bladder outlet obstruction, a treatment that results in an enlarged, fibrotic bladder. They will also macerate bladders with 8N HCl, followed by treatment with collagenase, which removes the extracellular matrix, leaving behind the cellular components [11]. This will allow the team to explore the organization of the fibroblasts that populate the connective tissue and their expression of surface markers. Given the size of the bladders (on the order of several millimeters), the large area-scanning feature of the requested SEM will be ideal for obtaining a complete picture of the fibroblasts and other cells that populate the bladder wall.

Thematically Clustered Projects:

Commonly SEM use becomes thematically essential and is heavily used by a large cohort of users within a single research focus. The [redacted] are two of these groups. Each has fundamental research themes that can only be addressed using SEM approaches and are best presented as a project cluster. The two clusters we have chosen include a group that has investigators that use SEM in a consistent manner [redacted] the other (the [redacted]) is a cluster that has a fundamental need for SEM but uses it in an episodic intense way. All investigators (except one, [redacted]) are federally (NIH/PHS) funded independent faculty.

[redacted]

The [redacted] is a meta organization within the [redacted] that brings together researchers, fellows and students at all levels and in most science and engineering programs across the campus. It was established over 20 years ago by the [redacted] and [redacted]. The Institute serves as a single base of operations for the University's leading scientists and clinical faculty working to develop tissue engineering, cellular therapies, and artificial and biohybrid organ devices. The major thematic goals within the Institute build on pre-existing strengths in its faculty around the concept that the sum of the parts is far greater than the individual, and it has been genuinely successful with substantial funding from the NIH, DOD and NSF (about $14,000,000 each year) as well as other philanthropic organizations and private donors. The major efforts within [redacted] are in Tissue Engineering and Biomaterials, Cellular Therapeutics (principally capitalizing on stem cell biology) and the development and implementation of Medical Devices and Artificial Organs.

Thematic readouts of success for this group are commonly refractile to standard biochemical or molecular biologic approaches as the performance measures depend on substrates or devices that are manmade. Because of this there is a tremendous reliance on microscopy characterize the device or its therapeutic efficacy. The PI of this proposal and the associate director of the [redacted] are both members of [redacted] and have enjoyed challenging and successful interactions with its faculty since inception.

The majority of [redacted] faculty use resources with the [redacted] and over 50% of this group make use of our current SEM. There are myriad NIH supported minor users from this group all of whom make episodic use of SEM. In this application we have focused on a small cluster of consistent heavy users, with a variety of projects. Importantly, all these investigators have specifically requested EDS capabilities to be included in the funding request.

[redacted] ***Ph.D.***

Project Title: *Biomaterials at the Neural tissue interface*

Grant numbers 5U01NS[redacted]; 5R01NS[redacted];

[redacted] research is generally focused at the biomaterial/neural tissue interface. SEM is an indispensable tool for studying the micro/nano devices at high magnification, characterizing the surface morphology of various biomaterials (topography, roughness, porosity), investigating the interaction between cells and device surface and understanding the failure mode of implantable neural electrodes. The use by her team is continuous and extensive and SEM data commonly features in publications[2-19].

Exemplars of her use of SEM spans both clinical and basic science though nearly always with a clinical relevance. For example, her team is working on developing brain-computer interfaces to restore movement for people living with paralysis due to injury or disease (5). Although the therapeutic potential is great, long-term stability of the interface is critical for widespread clinical implementation. While many factors can affect recording and stimulation performance including electrode material stability and host tissue reaction, these factors have not been investigated in human implants.

In the clinical study shown in **Figure 1**, [redacted] to characterize the material integrity and biological tissue encapsulation via explant analysis in an effort to identify factors that influence electrophysiological performance. She examined a total of six Utah arrays explanted from two human participants involved in intracortical BCI studies. Two platinum (Pt) arrays were implanted for 980 days in one participant (P1) and two Pt and two iridium oxide (IrOx) arrays were implanted for 182 days in the second participant (P2). The recording quality followed a similar trend in all six arrays with an initial increase in peak-to-peak voltage during the first 30–40 days and gradual decline thereafter in P1. Scanning electron microscopy was used to assess material degradation. There is tremendous excitement in her group about the possibility of adding EDS to the system we are requesting. Essentially, while her team is very dependent on the SEMs within the [redacted] they are unable to perform elemental analysis concurrent with other SEM investigations.

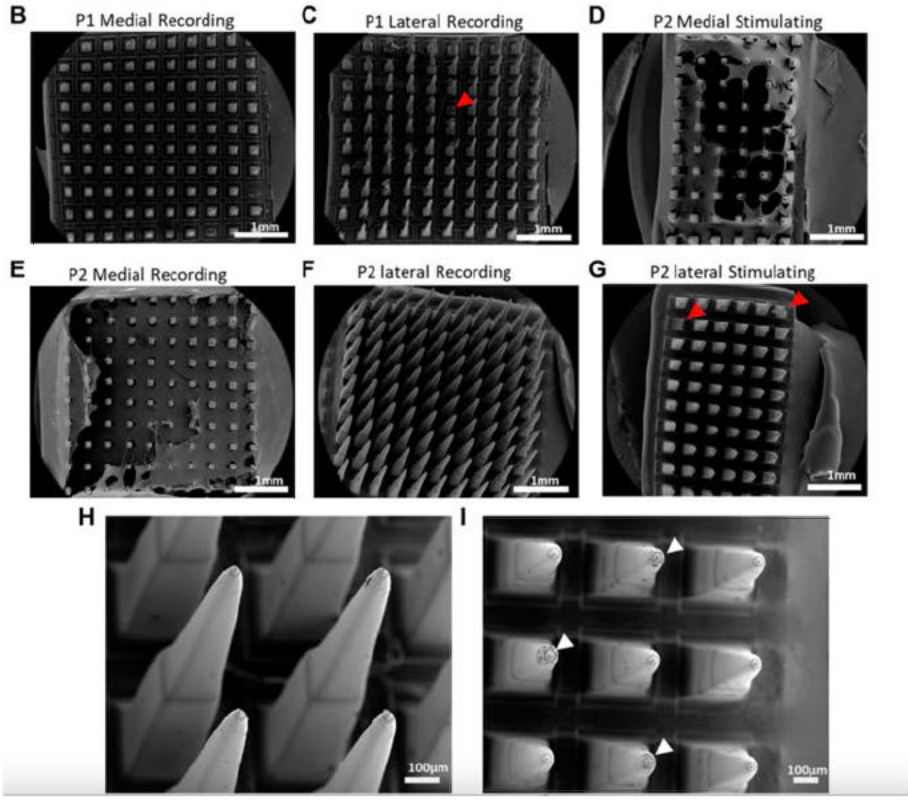


Figure 1. Explanted Multielectrode arrays from human brain After months to years of Brain computer Interface Study. (B,C) Recording arrays implanted into P1 after enzymatic treatment. (D) Medial stimulating array implanted into P2. (E,F) Medial and lateral recording arrays implanted into P2. (G) Lateral stimulating array implanted into P2. (H,I)Higher magnification images of recording array in (F) and stimulating arrays in (G), respectively. White arrows indicate electrodes which were used for stimulation. Red arrows indicate representative electrodes which were excluded from analysis.

The need for the EDS technology is highlighted in a recent article from her group in iScience (2) which detailed elemental investigations using EDS allowed characterization of implanted electrodes. There is no doubt that *in vivo* sensing of neurotransmitters has provided valuable insight into both healthy and diseased brain, however, chronically implanted Ag/AgCl reference electrodes suffer from degradation, resulting in errors in the potential at the working electrode. The group described a simple, effective way to protect *in vivo* sensing measurements from reference polarization with a replaceable subcutaneously implanted reference using SEM to compare changes in surface morphology and composition of chronically implanted Ag/AgCl electrodes in mice. Postmortem histology revealed extensive cell death and gliosis in the surrounding tissue. As accurate reference potentials are critical to *in vivo* electrochemistry applications, this simple technique can improve a wide and diverse assortment of *in vivo* preparations, shown in **Figure 2.** This work was done partially using the system available within the [redacted] and partially with the systems in the [redacted] the latter only because of the lack of EDS capabilities on the systems in the [redacted] Notably all the other microscopy technologies used in her group (histology, confocal microscopy) are performed using the instrumentation in the [redacted] There are continued requests to enable EDS within the system in the [redacted] however it is simply too old to do this in any practical way, but it is an important addition to the system requested in this application.

CLEM approaches using SEM

Development and Use of Correlative Light – Electron Microscopy Approaches using Scanning Electron Microscopy

Investigators :

[redacted] 5P30CA[redacted] 5P01HL[redacted] 5R01DK[redacted] R01GM1[redacted]; R01CA[redacted].

Within the CBI we make extensive use of fluorescence microscopy (FM), to provide insight into mesoscopic structure of biological tissues and cells. Importantly though, even when using super-resolution approaches (Structured Illumination Microscopy (SIM), Stimulated Emission Depletion Microscopy (STED) or stochastic optical resolution microscopy (STORM)) the ultimate resolution of the optical methods, though being extended beyond the traditional diffraction limit, is still limited to about the 20 to 50 nm range[1]. Also, as these approaches rely on positional reporters of molecular identity the fundamental structural information of the sample is not seen in the super-resolution image. All these approaches are available within the [redacted] and while we have extensive expertise in the advantages and limitations of each approach, we are commonly unable to resolve structures of interest within their structural context. Standard TEM certainly allows sub nanometer level resolution, but generally suffers from a relatively small field of view – essentially the grid diameter 1-2mm[2]. The lateral spatial limit can be avoided using Scanning EM which when operated in backscattered mode has essentially an unlimited lateral spatial field of view (only limited by the motion limits of the sample chamber) with nanometer resolution. Ultimately, we wanted to develop methods that allow generation of structural images which tissue structure information with nanometer resolution that could be correlated with the distribution information from light microscopy. But more importantly with the added advantage of being able to specifically select events by light microscopy from across large fields of view and then interrogate the specific chosen loci by EM. Essentially the fluorescence microscope provides large scale cellular dynamics information but lacks high resolution, whereas EM shows dramatically improved resolution but has been lacking in terms of large-scale analysis. The correlative approach described here[2] seeks to first identify the areas of interest in FM, followed by a high-resolution investigation by EM, within the same specimen such that rare events can be studied, easily and rapidly.

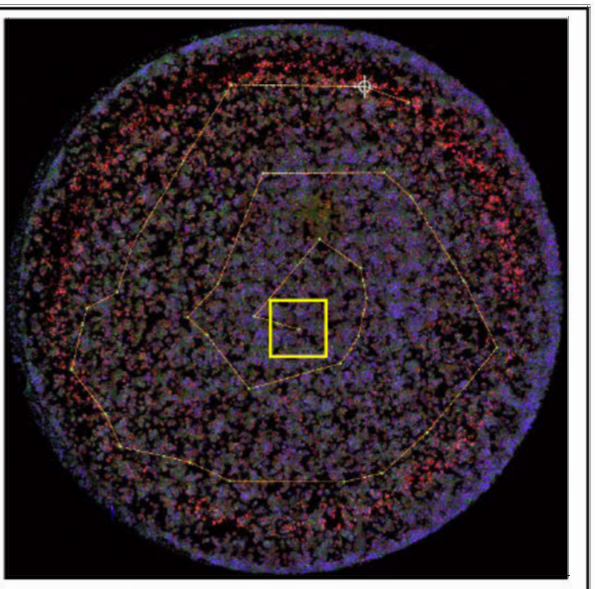


Figure 1. Large area confocal reconstruction with ROIs of stained cells on a Met-Tek plate selected for subsequent SEM analysis

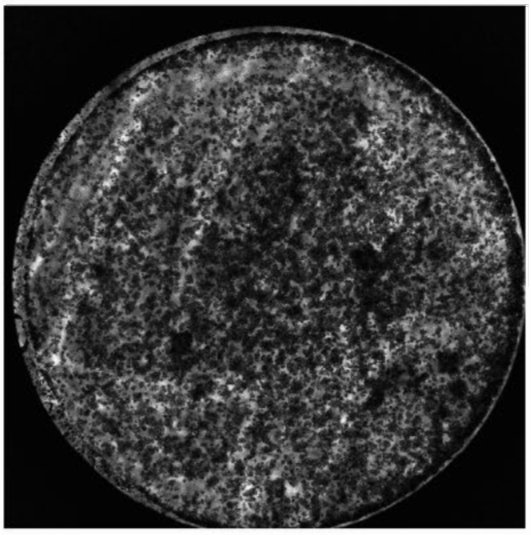


Figure 2. A low magnification backscatter SEM image of cells shown in Figure 1

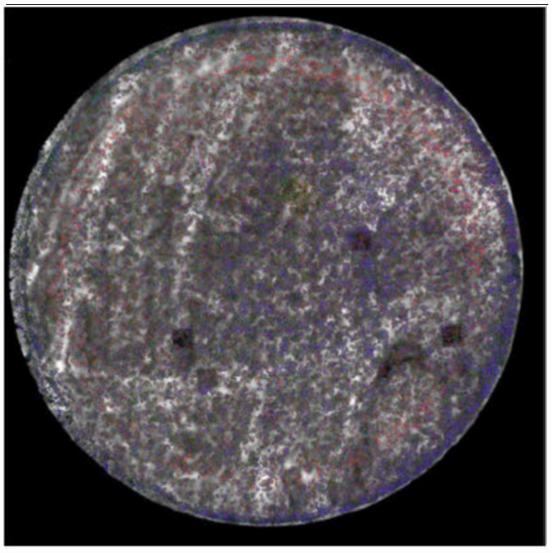


Figure 3. Overlay of confocal LM and backscatter SEM scans.

Collecting large area data sets using medium resolution in 3D using confocal microscopy is relatively simple. (**Figure 1**). One can then select specific areas within the sample and collect higher resolution 3D volumes with subcellular information (**Figure 2**). The samples can then be enbloc stained with heavy metals and embedded for EM. The innovation in this approach is developing approaches that allow the same locations to be found in both the light and EM modalities. To do this rather than using grids for example to define fiducials we actually use the sample itself as the fiducial. The prepared EM sample is then scanned at low magnification and superposed on the LM montage (**Figure 3,4**). We have developed software approaches which allow us to correct for rotation/shrinkage etc and then superpose the light and EM data sets on each other and more importantly transfer the positional coordinates from the light microscope images to the EM sample.

A second problem we solved in this approach was finding the location of individual structures in the Z axis. Standard serial block face sectioning or milling approaches do not work for large area samples however we have developed polishing approaches and devices that allow relatively precise removal of material from the block (in 100 nm steps) such that essentially serial sectioning can be performed, this is shown in **Figure 5**.

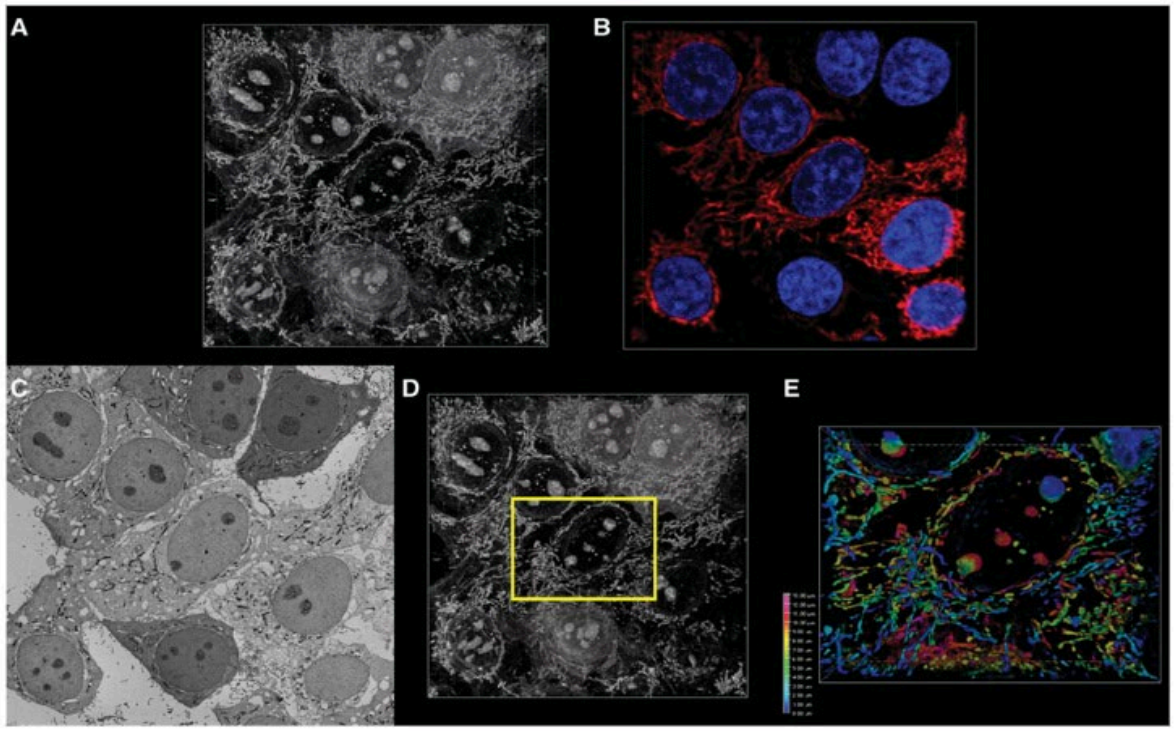


Figure 4. Correlative light and EM imaging of cell culture specimen. A. 3D z-stack of SEM images, inverted to show cell structures (white). B. 3D z-stack of FM images showing Tom20 (red) and nuclei (blue). C. Single-plane SEM image. D. 3D view of reconstructed SEM images, inverted and skewed to highlight mitochondrial structure. E. Depth-coded ROI outlined in D.

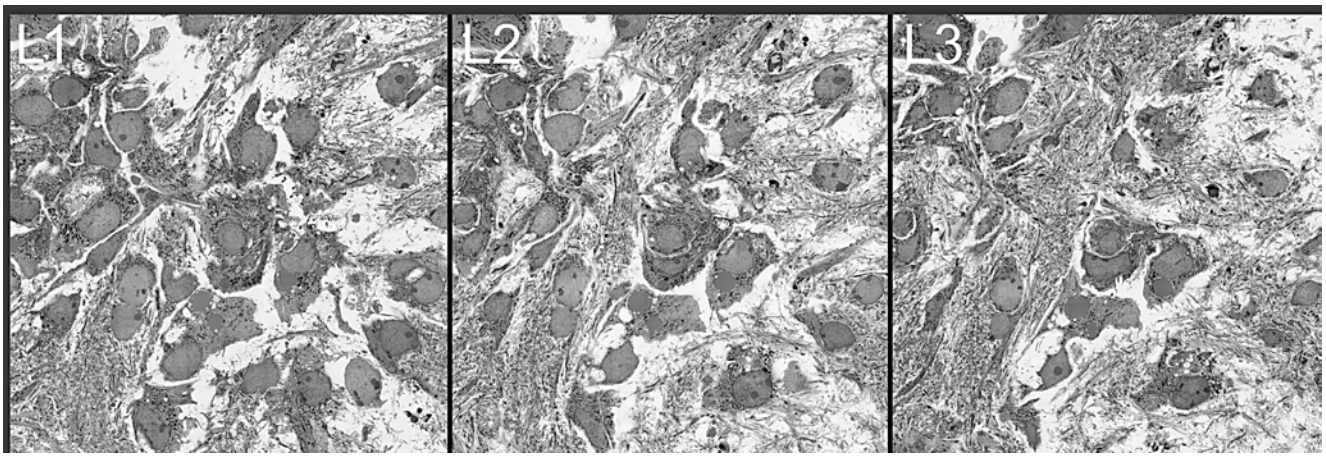


Figure 5. Backscattered images of sequentially polished tissue culture cells. polished block faces, 500nm separation

Summary Tables:

In the first year we expect the AUT for this microscope to be 1850 hours

Research Projects of Major users

Name	Grant number	Project Title	grant start date	grant end date	Usage in hours	%age of total AUT
[redacted]	R35HL[redacted]	[redacted]tudies in diovascular	1/1/2022	31/12/2028	150	8%
	R01HL[redacted]	[redacted]	1/12/2020	11/30/2023		
	R01HL[redacted]	[redacted]	1/5/2021	2/28/2025		
[redacted]	5R01DK[redacted]	[redacted]	9/6/2021	6/30/2026	150	8%
	5P30DK[redacted]	[redacted]	1/9/2008	7/31/2023		
	5R01DK[redacted]	[redacted]	1/8/2019	5/31/2024		
[redacted]	5U01NS[redacted]	[redacted]	9/30/2019	8/31/2023	200	11%
	5R01NS[redacted]	[redacted]	1/8/2019	5/31/2024		
[redacted]	5R01 DE[redacted]	[redacted]	9/14/2020	8/31/2025	125	7%
[redacted]	5U24DE[redacted]	[redacted]	1/5/2020	4/30/2025	100	5%
[redacted]	NIH 1 DP2GM[redacted]	[redacted]	9/8/2022	8/31/2025	200	11%
[redacted]	5P30DK[redacted]	[redacted]	1/7/2019	3/31/2024	150	8%
	3R01DK[redacted]	[redacted]	1/1/2004	5/31/2024		
[redacted]	5P30DK[redacted]	[redacted]	7/1/2019	3/30/2024	200	11%
[redacted]	5K01DK[redacted]	[redacted]	7/15/2020	4/30/2025	100	5%
[redacted]	5R01DK[redacted]	[redacted]	9/20/2019	5/31/2024	50	3%
[redacted]	5P30CA[redacted]	[redacted]	9/10/1997	7/31/2025	200	11%
	5P01HL[redacted]	[redacted]	1/3/2014	4/30/2024		
				Totals	**1625**	**88%**

Research projects of other users

Name	Grant number	Project Title	grant start date	grant end date	usage in hours	%age of total AUT
[redacted]	5R01DK[redacted]	[redacted]	7/23/2019	6/30/2024	75	4%
[redacted]	R01CA[redacted]	[redacted]	1/1/2001	8/31/2024	75	4%
[redacted]		[redacted]			75	4%
				Totals	**225**	**12%**

Usage in annual hours by major and minor users

We predict that AUT will be 1850 hours for the first year of use of this microscope 1625 of these hours or 88% of the total AUT this use will be by heavy users with NIH funding and 8% or 150 hours by other NIH funded users. In total this is 96% of or 1775 hours of the AUT

Usage of specific Features

Secondary electron detection is used by all users except those only using the device for CLEM approaches. EDS use is focused to the bio-engineering cohort, the backscattered detector is used by CLEM users and those who will use immunolabelling approaches with gold probes.

Name	Secondary detector	Backscattered detector	EDS
[redacted]	X	X	
	X	X	
	X	X	X
	X	X	X
	X		X
	X		X
	X		
	X	X	
	X		
	X		
		X	
		X	
		X	
	X		X

Administration (Organizational/Management Plan)

A. Organizational Plan

I. Steering Committee.

The grant will be administered by the steering committee of the [redacted]. To ensure that the [redacted] meets the experimental goals of all the user groups a steering committee has been established. While not all departments who make use of the [redacted] are represented on the committee, a single individual from each of a cross-section of the departments making major use of the [redacted] are included, as well as the director of the [redacted] and a senior administrative representative. The function of this committee is to make major policy decisions for the [redacted] such as issues of cost recovery, project priority, educational programs; and establish guidelines for the shared use of instrumentation, including electron microscopes, light microscopes, confocal microscopes, image analysis equipment and microtomes. Note that this steering committee is not for this instrument but for the [redacted] as a whole. [redacted] or his designee will represent the financial commitments made by the institution to support this instrument are maintained in the unlikely event of a financial shortfall within the Center (see attached letter).

- [redacted] Vice Provost for Research
- [redacted] Interim Chairman, Department of Immunology
- [redacted] Chairman, Department of Cell Biology (Chair of committee)
- [redacted] Associate Director [redacted] Professor Cell Biology
- [redacted] Associate Director [redacted] Professor of Cell Biology
- [redacted] Head Administrator, [redacted] and Dept of Cell Biology
- [redacted] Director, [redacted] and Distinguished Professor of Cell Biology

Once notice that the grant has been awarded is received, JEOL USA will be notified, and the microscope will be ordered.

The space for the microscope has been designed and is specifically equipped for this microscope and is currently housing the same type of microscope which is being retired. The room, #236 within the [redacted] is 12 ft by 13 ft and has been built according to guidelines described by the steering committee, and is equipped in terms of ventilation, power, chilled water hook-ups and networking to house the requested device.

Following receipt and installation of the microscope, JEOL USA, and the director of the [redacted] will ensure that the microscope is in optimal working order and able to produce images at the specified tolerances using all the observation modes described. The director of the [redacted] will then report to the steering committee who will review the results obtained by the director and JEOL USA. If satisfactory, they will approve payment to the vendors.

The director of the [redacted] will initiate an education program on the use of the microscope to optimize its use in the research environment within the [redacted] as this device duplicates much of the functionality of a current device we are certain that this program can be rapidly implemented.

[redacted], the staff member within the [redacted] responsible for SEM imaging as well as [redacted] will be trained directly by JEOL specialists on instrument operation and routine maintenance. Overall oversight of all EM services within the [redacted] is under the supervision of [redacted]. Direct supervision of the microscope will be handled by [redacted], supported by [redacted]. Both staff have extensive experience with the current SEM and are the designated trainers for this system and are partially salaried to manage and maintain core instrumentation (see below).

The steering committee will meet to define priorities for the various projects which have significant need for the instrumentation to assure equal access to the machine by all user groups. The submission of this application was driven by the steering committee of the [redacted] in response to the absolute and clear recognition that the current device needs to be retired because of the lack of service support, and the fact that we are not able to provide quality SEM time using sampling modalities beyond simple secondary electron imaging, to an established and growing community of imaging scientists all of whom need access

to advanced SEM approaches Thus the steering committee see this as an opportunity in that we will be able to give a larger PHS supported group access to both conventional secondary and backscattered electron imaging and more analytic SEM approaches such as EDS.

At the end of the grant period, the director of the [redacted] will submit annual detailed reports to NIH describing users, applications, and the value of the instrumentation to the research community within the [redacted] medical school. Following expiration of the one-year warranty period for the microscope, the instrumentation will be placed under service contract with JEOL USA.

II. Monitoring use of the requested instrumentation

Training:

To ensure the use of all instrumentation (including the microscope for which funding is requested here) is optimized, and to protect instrumentation from abuse, all users must show significant competence in the operation of the machinery prior to being allowed unsupervised use of the equipment. Training in the use of the SEM microscopy, theory and practice will be provided, both in the form of formal courses given by [redacted] as well as hands on training on the system by [redacted]. Prior to a satisfactory demonstration of technical competence, all instrumentation use is supervised or performed by [redacted] The formal courses are “Multiparametric Microscopic Imaging” and “Imaging in Cell Biology”. These are 3 credit graduate level courses offered annually by the Department of Cell Biology. It is not necessary to take these didactic courses before using the microscope, however each user must go through sequential training sessions. Clearly in the case of graduate school training, this also includes several training sessions on the sample preparation methodologies. In the case of faculty or users who wish to do their own microscopy, training is directed specifically to the use of individual microscopes. This is normally two 3 hour sessions and is accompanied by a lab manual developed in house by Core leaders for the instrument. In the case of the SEM the first session integrates sample preparation, critical point drying, coating technology and also serves as a first introduction onto sample entry (though we do not let beginners change samples), focus, control, automated functionality, image storage and recall (how to use our servers). The second session focuses on optimizing image quality and perfecting sample preparation as well as identifying problems (charging issues, over or under-coating samples etc). During the first several sessions of use, users may not turn devices on or off, or manipulate anything other than the most basic of parameters. We have developed this highly conservative strategy because of the ease of damaging the system or retrieving lost samples inside the sample chamber. All users must receive primary training directly from [redacted] staff and faculty and never second hand from [redacted] users. Following the training sessions users are supervised until it is felt that they can use systems independently. This is a stacked training approach, learn and prove competence using very basic skills then learn and prove competence on more advanced microscope technology. Users can only use the microscopes during the working day for the first 10 sessions or 50 hours of use, after this period and following specific instruction on how to startup systems they are allowed to book after hours time (ID privileges/card key access are changed). Users in this group are considered “Expert” and will have logged at least 50 hours on the specific system they wish to use (see discussion of AUT earlier for further breakdown of user categories). Booking weekend time (24/7) reservation is generally reserved for users who can genuinely demonstrate a need for weekend time, this includes residents/fellows who cannot find sufficient time away from clinical responsibilities during the work week. So far this allotment of time and training has worked flawlessly. As all users generally want to use the latest and greatest technology, controlling access through education and discussion has been extremely valuable. This interventionist approach is performed by the faculty in the [redacted] with the PI of the user lab and the effected students/fellows etc. The goal is to choose the appropriate technology and to build rational expectations for the experiments. In the case of this particular instrument and EM microscopy in general, nearly all experiments start with light microscopy, commonly standard confocal or large areas scanning and then moving to TEM or SEM. Once it is clear that a SEM approach is ideal, we develop an integrated experimental approach which commonly uses both light and electron microscopy approaches and in fact it is the experimental integration of these two approaches and the “hunt” for rare events that led to the development of the novel CLEM technologies that form part of this application.

Reserving instrument time

We already have a cadre of users who will probably use substantial time on the microscope. However, there are always transitions in the user pool, and as new users come on board they will be trained as described above. The process for reserving microscopes is simple and is common to all instrumentation within the [redacted] Reservations may be scheduled one month in advance for a maximum of three time blocks of 3 hours each week (9 hours total). If the user is more than 15 minutes late for a reserved session, it is forfeited unless we receive a phone call alerting us to their delay. Reservations for all instrumentation are made using the internet, using an online reservations calendar for each instrument. This method not only allows remote reservation of instrument time but also effective and easy billing and measurement of instrument usage. All unreserved time is available on an ad hoc basis. Use of the facility, including all instrumentation outside normal working hours, will be possible though limited to those users fully trained in instrument use (i.e. users can only book time once they are trained, otherwise the trainer must book the time for them, until they become competent on that particular instrument). To gain access to the unit during these times, users must have their ID cards listed with security such that they can gain access through the electronically locked door to the Core Imaging Facility, following a specific request to the staff of the [redacted] who are responsible for letting the security staff know who is allowed access.

The use of the system will be somewhat nuanced by the need to provide support for the CLEM approaches which will take extended scanning time. We expect the majority of the scanning for this approach to be carried out by [redacted] who will be managing the microscope. He has been responsible for using the JSM 7800 we have had on loan. Fortunately, most of the scanning for this type of imaging is highly automated. Following a rapid low magnification scan (which takes about 1 hour), the correlates between the LM and EM images are generated (again about an hour) and then high resolution stitched EM images are generated. This latter part of the process is the slowest aspect of the workflow. The solution that we have tested and works well is to start the CLEM imaging in the late afternoon (around 3pm) and then run the high resolution scanning overnight. It is likely that this will be done two or three days a week at current workloads. Importantly overnight scanning does not take time away from conventional daytime use and should expand the AUT. On all the other microscopes within the Center that are used for long-term overnight imaging (live cell, slide scanning, large volume light sheet data collection) we use a remote computer management software (Splashtop) to monitor progress. We have many seats (20) for this package and use it routinely. We have been using and will continue to use this application to manage the SEM work as needed.

Reporting use of the SEM in publications:

The [redacted] has received multiple S10 grants and has established procedures to encourage investigators to cite the grant in publications. The first and most obvious is signage (there is a sign requesting that investigators cite the award on the doors to microscope rooms that have S10 funded equipment in them and on the splash screen of the computer running the system). The second and most effective is during usage reporting each year when preparing the AUR the PI of the grant sends an email to all users from the prior year (easily gathered from our reservation system) asking if they have published any microscopy figures using systems that were supported by the S10, and if they were to ensure that they have cited the grant. This often demands retroactive correction of papers, but it is effective.

Using Biohazards/Human Samples/Vertebrate animals etc

The [redacted] has an established and approved set of biohazard protocols in place (detailed at the end of this application). As the majority of the samples used for SEM are heavily fixed there are few concerns however we expect all users to follow and be cognizant of these protocols which are published on the Center website.

III. Prioritization of application.

All trained users will have equal priority (assuming they are from a PHS funded laboratory) regardless of the absolute level of PHS funding. Funded investigators will take priority over unfunded investigators who are developing preliminary data for PHS submission. Beyond this, however, our prime goal is to provide

funded [redacted] scientists with microscope resources and time to perform investigations as easily and quickly as possible. We generally feel that the use of SEM becomes inefficient after 3 hours of continuous use, thus sequential bookings are discouraged. However, those who wish to work for long periods are asked to take an afternoon/evening session to maximize the number of users who can use the microscope. We expect there will be little or no problems with prioritization (we have had none in the past), however, the steering committee will be called upon to resolve conflicts if needed.

B. Financial Plan

I. Fiscal management of the [redacted]

Funding the [redacted] The [redacted] does not work as a traditional fee for service facility and most of the costs needed to run the center come from grants held by Center faculty or in collaboration with colleagues. The primary sources of funding are listed below.

Institutional Support: [redacted] is a tenured distinguished professor within the [redacted] Medical School, other faculty are tenured full or tenure stream assistant professors as such their salaries are guaranteed by the institution, though all are expected to and are successful in generating a large proportion of their salary through PHS funding mechanisms. Currently, all [redacted] faculty cover between 75% and 85% of salary on grants, the rest is provided by the institution.

Programmatic PHS support: The [redacted] is funded to a large degree through multiple programmatic PHS grants, in which the [redacted] is listed as a core resource for the grant. There are multiple currently funded program grants including; "Cancer Center support Grant" (PI [redacted] P30 CA[redacted]), "[redacted] Center for HIV protein interactions ([redacted] PI [redacted] 5P50GM[redacted]), Cardiolipin as a Novel Mediator of Acute Lung Injury. ([redacted] P01 HL[redacted]), [redacted] Liver Center (PI [redacted] 5P30DK[redacted]), the [redacted] (PI [redacted] 5P30DK[redacted]) and the Brain Imaging Library (PI [redacted] 1R24 MH[redacted]). We also have three other P30 grants in submission with the Dental School and Children's hospital, a U54 grant to expand kidney imaging capabilities and a U01 grant to develop massive imaging approaches for brain nano architecture.

PHS funding of the [redacted]: Apart from the individual funding going to [redacted], these consist of continual heavy users who share components of external funding with the faculty of the center. This is part of the fabric of the center and users contribute dependent on use. Many of these interactions are close, productive traditional collaborations where the Center faculty are actively involved in the research and the interactions are closely associated with their scientific interests. However, there are a large cadre of individuals who just need access to microscopes and have a minor commitment to the center from their grants (a few thousand dollars/year), Historically the PHS funded users of the microscope we are retiring fall into this category with funding commitments commensurate with use, we expect this to continue with the requested instrument.

Occasional chargeback users: This income is derived from occasional users within the medical research area; users are billed at an hourly rate (depending on instrument type) for technical specialist, or equipment time. These users are generally PHS supported investigators who either have no resources budgeted for microscopy or have insufficient need for microscopy to become "heavy users". Importantly this is very rarely implemented as the majority of users fund the Center through their grants.

IV: Costs for maintenance and running of the requested instrumentation.

While the [redacted] has a balanced budget it has a specific cost recovery account which pays for the upkeep, staffing and costs of running instrumentation within the core facility. The cost model is specifically limited to the use of instrumentation in the core and is a small fraction of the total center budget (less than 20% ($421,212 in 2023 with total funding from grants in 2023 for [redacted] at $3,102,340). The services are divided into two categories: simple ($20/hour) (basic light microscopy, microtomy) and advanced ($40/hour) (confocal, live cell, multiphoton, super-resolution, TEM, SEM). The cost recovery includes service contracts for instrumentation which we feel needs this level of support (electron microscopes, select light microscopes), costs for service visits and parts not covered by service

contract (laser replacement, software updates, machine shop costs). Some components which cannot easily be purchased on grants for core equipment (such as EMCCD cameras) are purchased and amortized over a multi-year period.

Staffing salaries are also included, specifically with respect to this application staff serving the EM microscopy core. 40% of [redacted] salary is covered by this account as well as salaries for similarly skilled staff in the EM component of the core. This allows staff to fulfill the service mission of the core and to ensure the workspaces are clean, the microscopes are cared for properly and users are appropriately trained. If this grant is funded, we will include 15% of [redacted] salary into the core as he will oversee the use of the microscope. The funds needed for this account are derived via the various funding sources described above either with a designated line item on NIH grants (microscope use service) or by direct billing recovery.

The first year costs for costs for this instrument will be relatively minor as tabulated below, as there are no service contract costs during the first year while the system is under warranty. However, we predict that 15% of the total time that [redacted] dedicates to core function and 20% of [redacted] salary will be necessary to manage the new device (based upon current time spent with our other EM systems).

Costs for running the SEM in first year

Staff Name	Salary/benefits from Core	Fraction for this device (15% of total)
[redacted] (15% total Salary)	$13,472	$13,472
[redacted] (50% salary)	$16,500	$2,475 (15% of core fraction)
Total		$15,947

Beyond the salary costs described in the table above we do not anticipate any other costs needed to run or install the system as the room it will be placed in is already designed and functioning as a microscope room with appropriate cooling, electrical supply and networking.

After the first year of operation this microscope will be placed under service contract. The retail cost for the service contract is $31,129, with multi-instrument discounts this drops to $27,068 (see email from [redacted] senior sales manager, JEOL). It is not possible to get accurate year-over-year increases in service costs but we can include salary increases. Overall, the usage cost escalates as shown in the table below, this allows a 3% staff salary increase each year.

While use of this system will be a mix of “expert” and supervised use we expect use levels to be similar to our current SEM system plus added use time partly due to including the CLEM approach in the system use and added capabilities systems. Effectively by year 5 our running costs will have risen to $45016/annum or 1125 user hours at our current user rate of $40/hour to cover costs. Given the activity of our current systems this should not be a problem (see Financial Plan for [redacted] above). Importantly though, as described throughout the application and in the letter from the Vice Provost for Research ([redacted]) if there is a shortfall in funding, operating costs will be covered using institutional funds for the lifetime of the instrument.

	Warranty	**Year 2**	**Year 3**	**Year 4**	**Year 5**
JEOL SEM microscope	1 year	$27,068	$27,068	$27,068	$27,068
Staff salaries	$15,947	$16,425	$16,918	$17,425	$17,948
Total	**$15,947**	**$43,493**	**$43,968**	**$44,493**	**$45,016**
AUT needed at @$40/hr	**399**	**1087**	**1099**	**1112**	**1125**

Annual service costs

Institutional Commitment (startup funding and space commitments to the [redacted].

The [redacted] opened its doors as a centralized imaging facility 31 years ago. At this time the Dean's office of the Medical School awarded the [redacted] $550,000 in start-up funding to purchase core and ancillary support equipment, to pay technicians salaries for an initial two-year period and to refurbish lab space. To ensure optimal performance and direction of the facility, a salaried, tenured faculty position was established within the Medical School for [redacted].

Subsequently following successful S10 submissions and a continued expansion in funding and utility the institution has provided approximately 7,500 sq. ft. of high-quality dedicated research space. This space has been built specifically to house the [redacted] with appropriate power, air handling, and water supplies throughout.

As can be seen, the Center continues to be well supported by the numerous NIH grants (programmatic and otherwise). Chargeback income supports the remaining budgetary needs of the center. However, Institutional leadership ([redacted]) has guaranteed that this instrument will continue to be supported with regard to service contracts and other costs if there is any shortfall in funding for the Center. This is described fully in the attached letter of support from [redacted].

Overall Benefit

As discussed at length throughout this proposal, the acquisition of this SEM is considered imperative in ensuring the continued functionality of the [redacted] as an Advanced Imaging Facility. This is reinforced by evidence of activity (publications), interactions with NIH funded scientists. The microscope requested here cannot be funded in a facile way by any other funding mechanism. Overall, scanning electron microscopy has become an absolutely essential component of modern microscope-based imaging and without the requested microscope requested here the capabilities of the [redacted] and its ability to serve the research community in which it thrives will be diminished considerably.

The summary table from [redacted] details the heavy use of all the devices acquired through the S10 program at the [redacted] in the last 5 years and essentially highlight the overall benefit of this centralized facility to the institution as a whole. Most importantly with regard to this submission, all of the machines acquired by the [redacted] are very heavily used and well maintained.

Letters of support.

Letter defining institutional Commitment from [redacted], Senior Vice Chancellor for Research.
Letter from [redacted], JEOL USA stating that service support for the current system in the [redacted] will end in spring 2023.
Letter from [redacted], head of the Nanoscience Center, stating that their systems are at saturation usage.
Letter from [redacted], head of the Biology department imaging facility stating that their systems are not available to users outside of their department.
Letter from the [redacted], JEOL USA defining annual maintenance contract costs for the requested instrument
Email from [redacted], JEOL USA, senior sales representative defining annual multi-instrument maintenance contract discounts
Letter from [redacted], director of the [redacted] expressing support for this application.

Protection for Human Subjects

N/A

Inclusion of Women, Minorities and Children

N/A

Vertebrate Animals
N/A
Biohazards

The following details the Biohazard protocols in place in the [redacted]. This includes details for cellular and animal work and has been approved by the University BioSafety Office.

1. All groups and laboratories using live animals for microscopy must have an approved IACUC protocol prior to bringing living animals to the [redacted] Rooms and microscopes that will be used for live animal work must be approved as animal outside use areas and must be appropriately equipped. It is essential that you consult with [redacted] while preparing to submit your IACUC protocol describing proposed work in the [redacted]

2. All groups and laboratories using recombinant or synthetic nucleic acids for microscopy must have an approved IBC protocol describing the work prior to bringing samples to the [redacted] It is essential that you consult with [redacted] prior to bringing recombinant or synthetic nucleic acid materials into the [redacted]

3. All users working with known or potential human pathogens or other potentially biohazardous agents must comply with the [redacted] Biosafety Guidelines (EH&S Guideline Number 05-003: [redacted]) in addition to all requirements of these [redacted] specific shared facility guidelines.

a. For work associated with an approved IACUC protocol for which an EH&S risk assessment has been generated, a copy of the EH&S approved risk assessment, which describes biological agent-specific risks as well as proper work practices, decontamination methods, and/or specialized personal protective equipment must be provided to [redacted] prior to beginning work.

b. Work with biological hazards not described in an IACUC protocol must be registered with EH&S via an agent registration workbook [redacted] At the time of EH&S registration user groups shall request generation of an EH&S risk assessment, which describes biological agent-specific risks as well as proper work practices, decontamination methods, and/or specialized personal protective equipment. A copy of the approved EH&S risk assessment must be provided to [redacted] prior to beginning work.

4. No eating, drinking, or applying cosmetics in the microscope rooms ever.

5. Read and understand the EH&S risk assessment for your pathogen(s) and notify [redacted] of your intention to use potentially pathogenic samples before beginning work in the facility.

6. Biohazard signage must be posted on the microscope room door indicating the biological agent in use and the appropriate biosafety level to be used while working with the agent. The EH&S risk assessment shall indicate the IBC or EH&S approved biosafety level to be used with each specific agent.

7. Sample preparation requirements:

a. The sample should be prepared in your lab, sealed with parafilm, surface decontaminated with an appropriate EPA-registered disinfectant, and carried to the Microscopy Core Facility in a sealed, secondary container labeled with a biohazard symbol (e.g., Tupperware-like container) in accordance with EH&S Guidelines 03-017, Specimen Transport Between Research Facilities. The secondary container should be disinfected with an appropriate EPA-registered disinfectant prior to leaving your laboratory.

b. Upon entering the Microscopy Core Facility, decontaminate the outside of your sample using the EPA-registered disinfecting wipes (quaternary ammonium compound) provided by the [redacted] and follow with a 70% ethanol wipe down. These EPA-registered disinfecting wipes are provided at all microscope work stations

d. If you wish to perform experiments on material using the [redacted] culture facilities it is critical that these experiments are approved by [redacted] faculty and performed only within designated culture facilities identified within the [redacted] This includes using incubators with labeled shelves, which will be designated by [redacted] staff.

e All work with biological agents with the potential to produce aerosols should be carried out inside a biosafety cabinet, and all microscope slides, dishes, etc. will be surface-decontaminated with an appropriate EPA-registered disinfectant (e.g. quaternary ammonium compound wipes or vesphene) prior to removal from the biosafety cabinet and transport to the microscope. ,

8. No gloves on the keyboards, computer mice, or any common equipment. It is important to note that, because samples are fixed and/or sealed and have been surface decontaminated twice, handling samples with gloves is unnecessary. **If you touch common equipment with a gloved hand, you must immediately decontaminate the equipment**. Handling common equipment with contaminated gloves places all users of the facility at risk for exposure. If samples require BSL-2 handling practices, either don gloves when required to manipulate sample and remove gloves and sanitize hands prior to touching common equipment or use the One Glove Rule to mitigate contamination of common equipment. Plastic film is available in the Microscopy Core to cover the keyboard to make cleanup easier, and should be used when samples require BSL-2 handling practices. Hand sanitation dispensers are present in all open lab areas and in the corridors inside the imaging suites these should be used *ad libidum.*

9. Report any spills, drips, splatters, or any potential contamination of the equipment to a [redacted] Technical Director **immediately**. Post a warning sign that the equipment cannot be used until fully cleaned and decontaminated. The Technical Director will coordinate clean up procedures. EH&S is available for consultation in case of large spills, or in situations where sensitive equipment may need to be decontaminated [redacted]

10. When you are finished with your work, wipe down the microscope, workstation (keyboard and mouse), and other work areas with the disinfecting wipes (quaternary ammonium compound) provided by the Core Facility and follow with 70% ethanol wipe down. Clean the objectives with lens paper and 70% ethanol and follow with Sparkle Glass Cleaner.

11. After imaging, cells may be fixed in 2% paraformaldehyde in the designated biosafety cabinet. The cells may then be stained for other specific proteins as appropriate and imaged again. Once cells are fixed the samples are no longer biohazardous. If cells/samples will not be fixed, the cells should be treated with an appropriate EPA-registered disinfectant (e.g. 1 part Clorox bleach to 9 parts liquid sample volume) for an appropriate time (e.g. 20 minutes for Clorox bleach), the liquid removed to the appropriate liquid waste container or disposed via sink and the vessel discarded in the University's biohazard waste stream.

12. Training requirements. All personnel must complete the following training prior to working in the [redacted] and as part of their general workplace training within the University.

EHS Training

- Blood Borne Pathogen Training (annually)
- Chemical Hygiene Training (every three years)
- Instructions for completion of EH&S training may be found here: [redacted]

IACUC Training

- Fundamental of Laboratory Animal Research Training (general session and specific animal session as it pertains to research)
- For additional information regarding required IACUC training modules contact the Research Conduct and Compliance Office.

Other University Training

- Human Subjects Research Module (as it pertains to research)
- This training module is available through the CME program in [redacted]

OMB Number: 4040-0001
Expiration Date: 11/30/2025

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator				
Prefix:	First Name*: [redacted]	Middle Name: [redacted]	Last Name*: [redacted]	Suffix:
Position/Title*: [redacted]				
Organization Name*: [redacted]				
Department: [redacted]				
Division: [redacted]				
Street1*: [redacted]				
Street2: [redacted]				
City*: [redacted]				
County:				
State*: [redacted]				
Province:				
Country*: [redacted]				
Zip / Postal Code*: [redacted]				
Phone Number*: [redacted]		Fax Number:		
E-Mail*: [redacted]				
Credential, e.g., agency login: [redacted]				
Project Role*: PD/PI		Other Project Role Category:		
Degree Type:		Degree Year:		
Attach Biographical Sketch*:	File Name: [redacted]			
Attach Current & Pending Support:	File Name: [redacted]			

Funding Opportunity Number: PAR-22-080 . Received Date: 2023-05-23T10:12:57.000-04:00

OMB No. 0925-0001 and 0925-0002 (Rev. 10/2021 Approved Through 09/30/2024)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: [redacted]

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Distinguished Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Start Date MM/YYYY	Completion Date MM/YYYY	FIELD OF STUDY
[redacted]	BSc	09/76	06/79	Zoology
[redacted]	PhD	10/79	01/83	Neurobiology
[redacted]	NA	02/83	09/85	Postdoctoral Fellow
[redacted]	NA	09/85	09/86	Postdoctoral Fellow

A. Personal Statement

Optical Microscopy has formed the core of my research career since my graduate training in England. Subsequently at the Pasteur and at the Dana Farber I honed these skills and had independent and collaborative interactions using imaging as a tool with a specific focus on the optical microscopy of cancer and immunotherapeutics. When I joined the [redacted] 29 years ago, my mandate was to build and develop a large imaging center serving the larger community, and to pursue my own independent research goals. Since then I have developed the [redacted] to a center employing 4 faculty and over 20 staff. We build, test, and use cutting edge optical tools for all types of research microscopic imaging in cells, tissues and animals from the single molecule to the whole animal, the goal being to build highly flexible, maximally effective imaging solutions, to be used by academic researchers. In fact a major focus of my career and of the Center is to develop, train and imbue researchers at all levels (undergraduate, student, post-doc and faculty) with a solid understanding (both theoretical and practical) of the power of microscopy. As a professor of Cell Biology a major focus of my research has been to develop, build, and apply computer aided microscopes and analysis tools for imaging subcellular events at all levels of resolution within fixed and living systems. These include high speed Total internal Reflection Fluorescence microscopes able to image at 100 frames/second, high speed confocal systems able to collect multicolor 3D stacks in the second timeframe and other prototype confocal systems able to scan very large tissue sections with submicron resolution at very high speed. My achievements in this field had led to my promotion to Distinguished Professor in 2014. Most recently we have been developing a novel Correlative Light and Electron Microscopy which allows selection of structures from large volume 3D confocal imaging for subsequent downstream imaging by SEM. The major advantage of the approach is being able to select rare events in 3D by light microscopy and being able to pinpoint them automatically in the EM volume. These feature within this application and are gaining significant traction in the NIH funded work by multiple investigators. I have been using EM as a central part of my work since I was a graduate student and have published multiple high impact papers using the approach (for example see section 1 of the scientific contributions). SEM is an essential extension of these approaches and I am excited to submit this grant application to support ongoing efforts within the [redacted].

B. Positions, Scientific Appointments, and Honors

2023 Canadian Foundation for Innovation Chair of MAC panel, American Cancer Center (ACS) Council member, NIH 1 panel (2023/10 ZCA1 TCRB-Q (O1) R, Chair of panel

2022	NIH 4 (Chair of 1) panels American Cancer Society (ACS) council member, BioImaging North America(BINA) executive board; CFSERC Reviewer
2021	NIH 5 (Chair of 3) panels, American Cancer Society (ACS) council member, BioImaging North America(BINA) executive board
2020	NIH 3 panels CFI 2 panels, ACS council BINA executive board
2019	Singapore SERC 1 panel, NIH 3 panels (Chair of 2) NSF 1 panel, CFI MSIR 1 panel Chair
2018	ACS, 2 panels (Chair of both), NIH 2 panels, Israeli ministry of Science 1 panel
2017	ACS, 3 panels (Chair of all), CFI, MAC panel July, (Chair), NIH 4D Nucleome (2 panels), Dutch Research council.
2016	ACS, 2 panels (Chair of both), CFI, SEP panel July, (Chair), NIH P41 structural biology center NYC(Chair),
2015	Canadian foundation for Innovation MAC panel (Chair), ACS CCRE Panel Jan and June (Chair) NIH panels on "the 4D nucleome" (Co-Chair), ZAT1-HS19, Panelist, EM S10 panel Chair and 2 other NIH panels
2014 – pres.	Distinguished Professor Cell Biology, [redacted]
2014	ACS Study Section, Atlanta, GA, Jan -21st-22nd, 2014 (Chair of Panel) NIH Study section High End Instrumentation, March 18th (Chair of Panel) ACS Study Section, Atlanta, GA, June -26th - 27th (Chair of Panel) and 1 other NIH panel
2013	American Cancer Society Review Panel Jan 22-23th (Chair of Panel) NIH study section S10 Electron Optics July 30th (Chair of Panel) and 7 other NIH panels
2012	NIH study section SBIRs March 15 (Chair of Panel) ACS Study Section, Atlanta GA, June 20th-21st (Chair of Panel) and 7 other NIH panels
2011	Chair or co-chair of multiple SBIR panels and sat on 5 other NIH panels
2010	CFI Toronto Canada January March 17th Chair of Panel NIH Study Section S10's November 17th-19th (Chair of Panel) 5 other NIH and ACS panels
2009	CFI : Chair of Panel,Toronto Canada January 28th-30t, chaired 3 panels and 7 other NIH panels as Reviewer
2004 – pres.	Vice Chair, Department of Cell Biology and Physiology, [redacted]
2004- 2008	MI Panel, Standing Member
2002 – pres.	Professor Department of Immunology (secondary appt) [redacted]
2001 – pres.	Professor, Department of Cell Biology and Physiology, [redacted]
1997 – 00	Associate Professor, Department of Cell Biology and Physiology, [redacted]
1993 – 96	Assistant Professor, Cell Biology and Physiology, [redacted]
1991 – pres.	Director, Center for Biologic Imaging, [redacted]
1991 – 93	Assistant Professor, Neurobiology, Anatomy and Cell Science, [redacted]
1986 – 91	Research Associate I,II, Dana-Farber Cancer Institute, Harvard Medical School, Boston,

Honors

2014- pres.	Distinguished Professor [redacted]
2009	Raines Professor, University of Western Australia
2005	Excellence in Education Award, [redacted].
2003- pres.	Fellow, Royal College of Pathologists
2000	Honorary Doctorate, University of Umea, Sweden
1999	Raine Travelling Fellow, University of Western Australia, Perth, Australia
1996-03	Member, Royal College of Pathologists (London)

C. Contributions to Science

1. Muscular Dystrophy: My early research career focused exclusively on the use of optical methodologies to dissect the disease process of muscular dystrophy. This X-linked disease affects about 1 in 3000 boys and prior to the discovery of dystrophin in the late 1980's there was no possibility of pre-natal or definitive pathologic diagnosis. While the first few years of my career focused on the basic biology of the muscle regenerative pre-cursor (the satellite cell) during my postdoctoral period at the Dana Farber Cancer Institute I became closely involved with [redacted] research team and contributed to multiple articles describing the localization and expression of dystrophin in various tissues. These studies made it possible to suggest putative functions for Dystrophin and products (antibodies etc) developed by the team allowed diagnostic tools to be developed.

a) [redacted]

2. **Imaging the Immune system/Imaging Cancer:**

Since my postdoctoral fellowship at the Dana Farber within Harvard Medical School I have had a continued interest in imaging cancer, in fact I have published over 100 collaborative papers involving cancer or some aspect of tumor biology. I have been involved in the [redacted] Cancer Center since my arrival here and have been continuously funded to perform imaging studies of cancer for over 30 years. These studies have initially used electron microscopic methods but over the last 25 years have grown to focus primarily on imaging Immunotherapeutic approaches, and most specifically the basic cell biologic processes leading to communication within and between cellular components of the immune system. This expansion of my interest in cancer imaging has fascinated me since I first studied cellular interaction between antigen presenting cells and particular antigens in the early 90's. The process of cellular uptake of antigen from first exposure in the skin for example, trafficking to the lymph node and then processing the antigen such that appropriate signals are sent to other members of the innate and adaptive system is a complex process and represents a true challenge for the microscopist. Initially our studies focused on the process of antigen uptake and presentation. However, subsequently we demonstrated and published that the interactions between dendritic cells could lead to signaling along small tubular interconnections (tunneling nanotubes). This discovery and other fundamental investigations of how dendritic cells and other antigen presenting cells pick up and process material have been a thematic constant in my research for the last 25 years. Generally we have taken a lead role in these investigations as they are very amenable to microscopy.

a) [redacted]

3. Probe Development:

Beyond our thematic scientific efforts, another major funded research theme of my group has been to develop imaging tools to collect useful data from novel fluorescent probes based upon the interaction between a non-fluorescent genetically encoded protein which binds a non-fluorescent dye molecule with high avidity which then becomes extremely brightly fluorescent. This probe technology was funded through the Networks and Pathway program (I was a co-director of the original research team involved in this program led by [redacted] has presented some unique imaging problems and opportunities. Initially it was necessary to extend the living system imaging platforms to work robustly and rapidly in the near IR range in wide-field, evanescent and confocal modalities. However, more recently with increased sophistication in the dye technologies and with renewed federal funding it has become important to measure multiple colors simultaneously in a ratiometric way and to develop 3 dimensional analysis tools to probe and reconstruct the datasets. Essentially as these probes only fluoresce when bound to the target (optically silent genetically encoded protein) they may be targeted to specific tissues or subcellular organelles. Novel chemistries currently allow accurate quantitation of pH, and are able to detect, quantify free radicals, and now locally deliver these species (see this proposal). My role in all these studies has been to develop the imaging platform for each technology. This includes the integration of software and hardware and analysis tools to generate useful quantitative data. Some of the more recent high impact papers are below

a) [redacted]

4. Cutting Edge Imaging:

As part of our ongoing research themes we are currently developing various technologies these include high speed approaches (both *in vivo* and *ex vivo*), imaging at 1000's fps, multicolor TIRF approaches, fast large area imaging approaches, correlative methods (for both EM and Mass Spectrometry) and various image analysis approaches needed to dissect motion from relative motion, the bibliography below includes significant contributions published in collaboration with this team in the last year.

a) [redacted]

Complete List of Published Work in MyBibliography:

[redacted]

Scopus H-Index 124, Google H-Index 149 lifetime, 74 since 2016

[redacted]

Commons ID: [redacted]

Other Support-Project/Proposal

Title: Cancer Center Support Grant
Major Goals: The Center, which forms the Cell and Tissue imaging core within this proposal performs microscopic analyses services for members of the [redacted] research community, from the traditional to the cutting edge using a full complement of microscope technologies.
Status of Support: Active
Project Number: P30 CA[redacted]
Name of PD/PI: [redacted]
Source of Support: NCI
Primary Place of Performance: [redacted]
Project/Proposal Start and End Date: 07/01/20-07/31/25
Total Award Amount: $13,590,517

Year (YYYY)	Person Months (##.##)
2023	1.20 calendar
2024	1.20 calendar

Title: Mechanisms of Immune Dysfunction After Trauma and Surgical Sepsis
Major Goals: This is a continued effort with [redacted], we have been working together for over 25 years and I provide support with all microscopy efforts in his continued studies.
Status of Support: Active
Project Number: R35 GM[redacted]
Name of PD/PI: [redacted]
Source of Support: NIGMS
Primary Place of Performance: [redacted]
Project/Proposal Start and End Date: 06/01/18-05/31/23
Total Award Amount: $2,907,214

Year (YYYY)	Person Months (##.##)
2023	0.18 calendar

Title: Molecular Mechanisms of Eastern Equine Encephalitis Virus Pathogenesis
Major Goals: We will provide deep tissue imaging (8mm) confocal reconstructions of fixed brains using novel ribbon scanning confocal tools of brains infected with restricted use (level 3) virus.
Status of Support: Active
Project Number: R01 A[redacted]
Name of PD/PI: [redacted]
Source of Support: NIAID
Primary Place of Performance: [redacted]
Project/Proposal Start and End Date: 06/01/18-05/31/23
Total Award Amount: $2,500,000

Year (YYYY)	Person Months (##.##)
2023	012 calendar

OVERLAP: There are no overlaps in research projects. Several current grants will be ending before 12/23 which will decrease [redacted] effort by ~1.2 CM. Additionally, his effort on P30 CA[redacted], P30 DK[redacted] R35 ES[redacted] and R01 CA[redacted], will be reduced by 2% if [redacted] effort on funded applications reaches 100%. In these proposals his effort is primarily administrative such that small reductions will not impact the utility of the Center to the user group.

I, PD/PI or other senior/key personnel, certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with Public Health Services terms and conditions if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements

[redacted]

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OMB Number: 0925-0001

Expiration Date: 01/31/2026

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ○ Yes ● No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

○ Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period	*Anticipated Amount ($)	*Source(s)

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3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Human Fetal Tissue Section

*Does the proposed project involve human fetal tissue obtained from elective abortions? ○ Yes ● No

If "yes" then provide the HFT Compliance Assurance

If "yes" then provide the HFT Sample IRB Consent Form

5. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ○ No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

6. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

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