FIVE-YEAR PLAN FOR ELECTRON MICROSCOPY

UNIVERSITY OF SOUTHERN CALIFORNIA

CENTER FOR ELECTRON MICROSCOPY AND MICROANALYSIS (CEMMA)

As one of the top 25 research universities in the country, USC requires nano-scale imaging resources that will support a diverse range of research needs. At present, many of our leading researchers must farm out key assays, which slows progress and exposes our most venturesome projects to outsider interests.
This document outlines a vision for CEMMA that promotes collaborative research between the USC Dornsife College of Letters, Arts and Sciences and the USC Viterbi School of Engineering.

This document contains an overview and draft budget that covers imaging needs for the physical and life sciences for a 12-month period and 5-year period.

Contents

I. OVERALL PLAN

MISSION

To provide nano-scale imaging and microanalysis support to the research community

Goals

1. Construct and run a state-of-the-art shared nano-scale imaging and microanalysis core center, housing modern high-resolution imaging and microanalysis tools that can be applied to the diverse on-going research being conducted at the USC and in the surrounding research community.

2. Attract a solid user base that will apply the nano-imaging capabilities provided by CEMMA for project development and for developing successful applications for grants.

3. Utilize existing technical skills and imaging tools to develop projects that incorporate nano-imaging approaches.

Achieving these goals

1. A successful nano-imaging center will require a combination of dedicated users and dedicated tools available for use.

2. The dilemma faced in achieving these goals is that attracting users will require dedicated tools, but successful equipment grant applications will require a team of dedicated users.

3. Use currently available technical skills and resources to build a user base.

The Plan for Attracting Users

1. Establish faculty collaborations.

2. Train students (e.g. the MASC 535L class on transmission electron microscopy)

3. Provide expertise and recharge rates attractive to users.

CURRENT STATUS

CEMMA invites faculty, staff and students from the Physical Sciences and Life Sciences to make use of the shared resources to assist in their projects.

Currently, researchers from the Physical Sciences make up the majority of the users. There are few users from the Life Sciences.

II. CURRENT CAPABILITIES

Life Sciences
Specimen Preparation Laboratories – Initial Status

1. Available Resources

A laboratory containing the following:
Three ultramicrotomes only one machine is fully operational.
Eight diamond knives – one had an identifier and was returned to the owner.
Two old, upright light microscopes
Two hot plates

An empty laboratory.
This space was completely empty with bench-tops contaminated with unknown substances that could not be removed using detergent or acetone. The space will be used for specimen preparation.

To make the space safe and attractive for students the benches were painted with epoxy paint. The laboratory was equipped with the following:

Basic laboratory equipment:
- pH meter
- Balance
- Bench-top centrifuge
- Refrigerator
- -20°C Freezer
- Automatic pipettes
- Mixers
- Inverted light microscope
- Basic seating
- Simple CCD camera for teaching
- Film-casting apparatus for specimen grids

Advanced Equipment:
Cryo-box with indirect UV illumination for low temperature polymerization of acrylic resins. Specimens embedded in these resins will be used for immunocytochemistry.

Consumables:
- Storage boxes
- Embedding molds
- Embedding resins
- Chemical crosslinking agents
- Buffers and other processing chemicals
- Storage and reagent bottles
- Bacterial growth media
- Colloidal gold probes

2. Available Specimen Preparation Capabilities

Routine morphology

A very basic need for life scientists is to be able to image specimens in the TEM and SEM. This need is only partially addressed by CEMMA. Specimens can be chemically cross-linked and dehydrated for TEM or SEM morphological examination. Dehydrated specimens in ethanol can be critical point dried for SEM examination. Ultrathin sections for TEM examination can be produced using the available ultramicrotome.

Immunocytochemistry

Immunocytochemistry can be performed on thawed, thin cryo-sections of chemically cross-linked specimens using the cryo-attachment available for the ultramicrotome. Immunocytochemistry can also be performed on sections of acrylic resin embedded specimens. CEMMA is able to perform resin embedding using the progressive lowering of temperature (PLT) technique.

Physical Sciences
Specimen Preparation Laboratories – Initial Status

1. Initial Resources

Three stereo microscopes for sample holder examination
One low vacuum sputter coater
One low vacuum carbon coater
Support computer for offline data analysis

2. Purchased Resources

<u>Computer or Software:</u>

- Hardware upgrades for EDAX computer
- Electron diffraction analysis software for support computer
- XPS analysis software for support computer

<u>Equipment:</u>

- High-resolution optical microscope for sample inspection
- Flange and electronics for EBIC adaptation to SEM
- Flange for cathodoluminescence experiments in the SEM
- Desiccators for samples and associated vacuum pumps
- Machined parts and electronics for micro-indenter in SEM
- Holder blades for *ex situ* TEM experiments
- Tools for debugging problems with TEM image drift
- TEOS for insulator deposition in FIB
- Calibration sample for the XPS
- Pumping station for cryo-holder

<u>Facilities Upgrade:</u>

- Sound insulation for TEM
- Fans for air circulation in TEM room

3. Resources Available on Campus

UV cleaner in chemistry department

HSC cryo holder
Oscilloscopes
Ammeters
Source-measurement units
Stress-strain *in situ* station

4. Upgraded Resources

TEAM software on support computer

Drift correction upgrade on SEMs

Upgrades for many computers to make them compatible with modern software

SUMMARY

Specimen preparation capabilities are basic, but it is possible to produce specimens that can be imaged in both the SEMs and TEM.

Our imaging is limited in both the SEM and TEM by sample preparation methods. Both instruments are underperforming due to instabilities in the rooms such as the air conditioning system (which fluctuates on and off), AC magnetic fields, and spikes in the power. The instabilities in the TEM room are being subdued by carefully identifying their sources and fixing them.

We have no dedicated sample cleaning equipment. We currently borrow time and resources from the chemistry department to clean samples. A sample that takes minutes to clean with a plasma cleaner takes hours with the resources we can borrow (if it even cleans).

III. FISCAL YEAR 14/15

OVERVIEW

Specimen preparation capabilities currently available in CEMMA are basic, but it is possible to produce specimens that can be imaged in both an SEM and a TEM.

In order to attract users, CEMMA must be able to handle most aspects of specimen preparation and imaging available for the physical sciences and life sciences.

The environment must be user-friendly to attract students.

Specimen preparation depends on rapid processing so basic laboratory functions must be available on-site. A stock of reagents, chemicals and equipment that are specific to electron microscopy preparation must be available for students to test preparation methods.

Users will be encouraged to purchase their own stocks of chemicals and other supplies once they become sufficiently comfortable using these imaging approaches.

Life Sciences

The specimen preparation laboratories will be prepared for multi-user activity.

All imaging equipment and specimen preparation protocols will be tested for functionality.

Faculty collaborations will be established.

Lectures will be presented to the different departments and schools within USC.

Students will be encouraged to train in, and use electron microscopy labs for specimen preparation.

Specimens from diverse life science fields will be prepared and imaged using current imaging tools within CEMMA.

A search for funds to purchase essential specimen preparation equipment will be established.

Physical Sciences

We cannot compete with larger centers and national laboratories for image resolution. However, *in situ* microscopy equipment is worth more, dollar-for-dollar, than a state-of-the-art high resolution TEM optics. By investing in spectroscopy and *in situ* microscopy we can provide a better support to our researches at a lower cost.

The specimen preparation tools will be purchased and prepared for multi-user activity.

Faculty collaborations will be established.

Lectures will be presented to the different departments and schools within USC and to neighboring schools such as UCLA, UC Irvine, and Caltech.

Students will be encouraged to train in, and use electron microscopy labs for specimen preparation.

A search for funds to purchase essential specimen preparation equipment will be established.

IV. Fiscal Year 14/15 Budget

Equipment

(Highest to Lowest Priority)

All equipment requested for fiscal year 14/15 would be placed in the CEM building. Please see Instrument Space Assignment section.

Life Sciences

Transmission Electron Microscope	$750,000
Refrigerated centrifuge	$ 20,000
Dewar flasks for liquid nitrogen handling & storage	$ 8,700
Sub-Total	$778,700

Operations

High Vacuum Sputter Coater	$ 35,000
High Vacuum Carbon/Metal Evaporator	$ 40,000
Glow Discharge System	$ 15,000
Card Readers (5)	$ 10,000
Student Work Station Computer (2)	$ 6,000
Sub-Total	$106,000

Physical Sciences

XEI Plasma Cleaner	$ 35,000
Spicer Field Cancellation System for the TEM	$ 35,000
Spicer Field Cancellation System for the SEM	$ 35,000
Hummingbird Biasing Holder	$ 40,000
Gatan Upgrade for Quantum GIF	$ 60,000
Gatan Precision Ion Polishing System II	$ 125,000
Gatan Precision Etching Coating System	$ 85,000
Gatan Dimple Grinder	$ 30,000
Gatan Double Tilt Heating Holder	$ 80,000
Gatan Double Tilt Holder w/ Faraday Cup	$ 70,000
Gatan Double Tilt Cryo Holder	$ 60,000
Protochips Poseidon 500 Electrochemistry Holder	$ 130,000
JEOL ADF Detector	$ 40,000
Fischione 2020 Tomography Holder	$ 22,000
Gatan Tomography Software for DM	$ 70,000
Gatan Vacuum Transfer Holder	$ 55,000
Gatan EDS Control Upgrade for DM	$ 30,000
Sub-Total	$1,002,000

TOTAL EQUIPMENT	**$1,886,700**

MATERIALS AND SUPPLIES

Life Sciences

Laboratory	
PC-based laptop computer for operating the CCD camera	$ 1,500
Stereo microscope for dissection	$ 2,500
60°C vacuum oven for resin polymerization	$ 3,400
Small rotary pump for vacuum oven	$ 2,400
High Pressure Freezer	
Specimen holders for high-pressure freezer	$ 2,500
Flat specimen carriers, various sizes (400)	$ 1,400
Membrane carriers, various sizes (400)	$ 1,400
Sapphire discs (100)	$ 750
Aclar foils	$ 500
Freeze Substitution	
Reagent baths	$ 600
Flat embedding inserts	$ 200
Flow-through inserts	$ 600
Flow-through rings	$ 350
Reagents	
Embedding resins	$ 2,800
Fixing and processing chemicals	$ 4,000
Stains	$ 2,000
Specimen Preparation	
Glass slides, grids, blades, molds	$ 3,000
Immunoreagents	$ 4,000
Growth media	$ 3,000
Diamond Knives (x2)	$ 6,000
Outside Equipment usage	$10,200
Sub-Total	$52,300

Operations

Replacement Computer for the 7001 FSEM	$11,000
Replacement Computer for the 4500 FIB	$11,000
XPS Ion Depth Profile Sputtering System (Refurbished)	$11,000
Chemicals	$ 1,000
Liquid Nitrogen	$ 5,000
Lab supplies	$11,000
Sub-Total	$50,000

Physical Sciences

Noise suppression equipment and electronics	$ 5,000
UPSs for vital TEM components	$ 5,000

Source-measure unit	$ 5,000
Oscilloscope	$ 5,000
Picoammeter	$ 2,000
Vacuum parts for HCS Cryo Holder	$ 2,000
Specimen Grids	$ 3,000
PC Upgrades for Cryo Holder controller computer	$ 2,000
Electronics parts for *in situ* biasing experiments	$ 3,000
Sub-Total	$32,000

TOTAL SUPPLIES **$134,300**

INSTRUMENT MAINTENANCE AGREEMENT (IMA)

JEOL 7001 FSEM	$ 36,000
EDAX EDS/EBSD	$ 20,000
JEOL 4500 FIB	$ 62,000
EDAX EDS	$ 15,000
JEOL 6610 SEM	$ 14,000
EDAX EDS	$ 12,000
JEOL 2100F TEM/STEM and Gatan camera/GIF	$113,000
EDAX EDS	$ 17,000
Kratos XPS	$ 33,000
GE DeltaVision OMX *	$ 0
Sub-Total	$322,000

TOTAL IMA **$322,000**

* Under manufacturer's warranty

STAFFING

Life Sciences

Two research assistant positions	$ 28,008
One full-time technical position	
Salary	$ 40,000
Fringe	$ 13,400
Sub-Total	$ 81,408

Operations

Three existing research assistant positions	$ 42,012
DeltaVision OMX Staff Position (40%)	
Salary	$ 25,000
Fringe	$ 8,375
One existing student worker (50%)	$ 18,000
Three Existing Full Time Staff (Salaries and Fringe)	$ 403,880
Sub-Total	$497,267

Physical Sciences

One research assistant positions	$ 14,004
One full-time technical position	
Salary	$ 40,000
Fringe	$ 13,400
Sub-Total	$ 67,408

TOTAL STAFFING $646,083

TRAVEL

Professional development	$ 15,000
Outside analysis	$ 12,000
Sub-Total	$ 27,000

TOTAL TRAVEL $ 27,000

UNIVERSITY SERVICES

Pressurized gas	
Nitrogen	$ 5,000
Argon	$ 1,000
Oxygen	$ 1,000
Liquid carbon dioxide	$ 1,000
Facilities Improvement	$ 10,000
Data connection	$ 2,000
Sub-Total	$ 20,000

TOTAL UNIVERSITY SERVICES $ 20,000

V. Fiscal Year 14/15 Budget Justification

Equipment

All equipment requested for fiscal year 14/15 would be placed in the CEM building. Please see Instrument Space Assignment section.

Life Sciences

Transmission Electron Microscope: Including a **transmission electron microscope** in the list of urgently-needed equipment for CEMMA was a carefully considered decision. A well-chosen general use TEM is an important addition to a nano-imaging center. It will provide rapid screening of diverse specimens, will be easy to use and will have the flexibility to support the diverse imaging needs of the community.

The JEOL JEM-2100F is the only TEM currently available for users in CEMMA. Although the TEM offers better resolution than a microscope operating at lower accelerating voltage, it is not good at producing low magnification images, or enhanced contrast of thin sections. The JEOL JEM-2100F is not ideal for imaging thin (<70nm) sections of biological material or negatively stained particles.

Refrigerated centrifuge: CEMMA is equipped with a cryo-ultramicrotome that can use used to produce thawed thin sections of chemically cross-linked biological material for immunolabeling. One of the important steps in preparing these thin sections for TEM examination is to dry them in a thin film of methyl cellulose, a water soluble plastic that is prepared by solubilisation in water. Purification is by centrifugation at low temperature and high speed. A refrigerated centrifuge is essential if cryo-ultramicrotomy is to be successfully performed. This machine will also be very important for concentrating cells into pellets and when preparing solutions of reagents.

Dewar flasks for liquid nitrogen handling & storage: Cryo-preparation of specimens requires the use of liquid nitrogen. Specimens are prepared for ultramicrotomy, for low temperature embedding and for freeze substitution using liquid nitrogen. Handling this cryogen requires specialized Dewar Flasks. Storage of frozen specimen is also an important need for all aspects of cryo-electron microscopy. Frozen specimens can be stored for sectioning or for freeze substitution and resin embedding.

Operations

High Vacuum Sputter Coater: High resolution scanning electron microscopy requires specimens to be coated with a very thin and uniform metal layer. Currently, our specimens are coated with gold, which often obliterates high-resolution detail. The high-resolution sputter coater will have a large impact on both life science and physical science research.

High Vacuum Carbon/Metal Evaporator: Scanning EM, Transmission EM and Electron Backscatter Diffraction (EBSD), requires specimens and substrates to be coated with thin films of different metals and/or carbon. For example, thin carbon films are essential for supporting specimens to be imaged in the TEM. Carbon and metal coating is important for

SEM work. For example, a carbon coating is important for detection of immunogold particles on surfaces of biological specimens using back-scatter electrons.

Glow Discharge System: Glow discharging surfaces makes them hydrophilic, a property that is important when examining particles (such as nano-material, polymersomes, lypososmes and viruses) in the TEM.

Student Work Station Computers (2): To setup additional workstation for students to access instrument data and use licensed scientific software. Which will also protect and secure major instrument computers.

Card Readers: The Card Readers will be placed at each scientific preparation unit to enable tracking of usage for the billing, security from unauthorized use, control lab safety and maintain and teach laboratory cleanliness protocol.

Physical Sciences

XEI Plasma Cleaner: TEM imaging is hindered by contamination. Plasma cleaning is an effective means of removing sample contamination. We have borrowed a UV cleaner from the chemistry department thus far. We have unreliable access to the cleaner and it only works on a few sample types. A plasma cleaner would replace the UV cleaner.

Spicer Field cancellation system for the TEM and SEM: Our TEM's and SEM's resolution is not limited by the manufactures performance specifications, but by AC magnetic field's random beam shifts. A field cancellation system would impede AC magnetic fields from influencing the TEM's and SEM's performance.

Hummingbird Biasing Holder: *In situ* biasing experiments are an important expansion to our characterization capabilities, they have been requested by many PIs, and this holder would facilitate that research.

Gatan Upgrade for Quantum GIF: This upgrade would dramatically improve our GIF's performance by increasing the spectrum acquisition speed.

Gatan Precision Ion Polishing System II: This vital piece of sample preparation equipment will allow for precision ion milling of samples using Argon instead of Gallium.

Gatan Precision Etching Coating System: This system is vital piece of sample preparation for samples that require large amounts of material to be removed.

Gatan Dimple Grinder: This equipment is for materials that are not easily milled or etched, when a large amount of material must be removed.

Gatan Double Tilt Heating Holder: *In situ* sample heating is a direct means of characterizing the high temperature properties of a material. Many experiments, such as chemical mapping of alloys as they change with temperature, can be completed using this equipment.

Gatan Double Tilt w/ Faraday Cup: An important part of materials characterization is knowing the beam current used to probe the sample. This holder is capable of determining the beam current *in situ*.

Gatan Double Tilt Cryo Holder: Radiolysis and sputter damage can be minimized by cooling the sample. This holder would provide cooling to the sample and has the ability to orient the sample along a particular zone axis.

Protochips Poseidon 500 Electrochemistry Holder: An important development for *in situ* microscopy is its use in understanding battery technology at the nano-scale. This holder can be used to study batteries *in situ*.

JEOL ADF detector: Our scanning imaging capabilities are limited to a small field of view. The detector would expand this field of view and help to provide a better signal contrast in the TEM.

Fischione 2020 Tomography Holder and Gatan Tomography Software for DM: Tomography is used to create 3D images from 2D projections acquired at different angles. This is accomplished by tilting the sample; a holder capable of tilting to high angles is required. Once the projections are acquired software is needed to reconstruct the final 3D image.

Gatan Vacuum Transfer Holder: Many samples are air sensitive. Imaging them in their working state requires a vacuum transfer holder.

Gatan EDS Control Upgrade for DM: This software would allow simultaneous acquisition for electron spectroscopy and x-ray spectroscopy.

INSTRUMENT SPACE ASSIGNMENT

Life Sciences

Transmission Electron Microscope	CEM 105
Refrigerated centrifuge	CEM 208
Dewar flasks for liquid nitrogen handling & storage	CEM 208
Microwave Processor	CEM 208
High-Pressure Freezer	CEM 104
Freeze Substitution System	CEM 104

Operations

High Vacuum Sputter Coater	CEM 103
High Vacuum Carbon/Metal Evaporator	CEM 103
Glow Discharge System	CEM 103
Student Work Station Computer (2)	CEM 101B

Physical Sciences

XEI Plasma Cleaner	CEM 106F
Spicer Field Cancellation System for the TEM	CEM 106F
Spicer Field Cancellation System for the SEM	CEM 106D

Hummingbird Biasing Holder	CEM 101
Gatan Upgrade for Quantum GIF	CEM 106F
Gatan Precision Ion Polishing System II	CEM 104
Gatan Precision Etching Coating System	CEM 104
Gatan Dimple Grinder	CEM 104
Gatan Double Tilt Heating Holder	CEM 101
Gatan Double Tilt Holder w/ Faraday Cup	CEM 101
Gatan Double Tilt Cryo Holder	CEM 101
Protochips Poseidon 500 Electrochemistry Holder	CEM 101
JEOL ADF Detector	CEM 106F
Fischione 2020 Tomography Holder	CEM 101
Gatan Tomography Software for DM	CEM 101A
Gatan Vacuum Transfer Holder	CEM 101
Gatan EDS Control Upgrade for DM	CEM 106F

MATERIALS AND SUPPLIES

Life Sciences

60°C vacuum oven: Epoxy resins are used for embedding biological material for thin sectioning. The resin holds the specimens while they are being imaged in the TEM. Epoxy resins require heat to properly polymerize. A vacuum will ensure that the liquid resin efficiently infiltrates specimens prior to polymerization. Currently the laboratory has an old oven with a defective door.

Rotary pump: A pump is required to create a vacuum in the 60°C oven.

PC-based laptop: A laptop is required to operate the small CCD camera used for teaching. The camera attaches to the eyepieces of microscope and displays an image on the screen of the computer. The CCD operating software is not stable on a MacBook Pro (my personal computer).

Stereo microscope: A simple stereo microscope is an important part of the EM lab. It assists with specimen embedding, dissection and other delicate tasks where fine control is important.

High Pressure Freezer Supplies: The high-pressure freezer is an important tool for advanced imaging in the life sciences. Specimens immobilized using this rapid freezing method have improved ultrastructure when compared with chemically cross-linked specimens. Currently such a machine is not available on-site. Access to a suitable machine is available in Pasadena. Specimens of different types are handled using dedicated specimens carriers. The carriers can be 100µm or 200µm deep and specimens can be held on flat sapphire discs or Aclar foils. Having the different specimen carriers available for users is important and will make it possible to handle the many different specimens available at USC.

Freeze Substitution Supplies: Specimens immobilized by freezing can be dehydrated and embedded in resin while still in a frozen state by using freeze substitution. Reagent baths and specialized inserts are used for processing the frozen specimens at low temperature. These consumables are used for all stages of the processing, up to and including the low temperature resin polymerization under UV light. They are thus not re-useable.

Reagents: Specialized resins, chemicals and other reagents such as heavy metal stains are not usual components of most life sciences laboratories. High volatile, toxic chemicals such as these are difficult to store in the same location as biological reagents. It is thus important to have stocks of these chemicals available in one location. Users can test protocols incorporating these chemicals, replacing them as necessary.

Specimen Preparation: Students and other users will have access to specialized specimen preparation supplies, which are not familiar to most life scientists, kept in the EM laboratory.

A central store of basic immunoreagents accessible to multiple users will standardize immunocytochemical approaches. A stock of two sizes of protein A gold (5 and 10 nm) and a variety of unconjugated bridging antibodies will provide researchers with a reliable source of immunoreagents used for antibody visualization.

In some instances, specimens will be generated that can be processed and imaged in order to test or develop preparation techniques. These specimens will require growth media.

Diamond knives are essential tools for all electron microscopists. Students will be encouraged to prepare sections using a diamond knife as soon as possible.

Operations

Replacement Computers: The two computers that currently run the FSEM and the FIB microscopes use the XP operating system, which is four generations slower than the current industry standards. Microsoft has announced they are phasing out support for their XP operating systems. Replacement computers will run the Windows 7 operating system which will create a faster and more secure environment, and will also allow the software to be upgradable in the future to the newest and most stable version.

XPS Ion Depth Profile Sputtering System (Refurbished): The Kratos XPS was donated to CEMMA in December 2013 with a non-functioning depth profiling system. This will refurbish the Ion Depth Profile system and allow use of both the destructive and the non-destructive profiling systems for materials. This refurbished unit is useful for physical science research, for example, the surface chemistry of thin films.

Physical Sciences

Noise suppression electronics: CEMMA's instruments are limited in performance by inference from their environment. AC magnetic fields, power surges in the electronics, and 60Hz noise all limit the spectroscopy and resolution. With a few simple modifications much of the noise can be suppressed.

UPSs for vital TEM components: Many electronics parts in the TEM would benefit from being supported by a UPS. They are susceptible to damage if their power fails.

Vacuum parts for HCS Cryo-Stage: The HCS cryo holder requires heating and cooling in vacuum. A small pumping station is needed to provide this vacuum.

Source-measure unit: This system is needed for biasing samples.

Oscilloscope: This is needed for measuring the noise on the high tension tank and debugging problems in the microscopes electronics.

Picoammeter: This is required for measuring the beam current from either a holder, the stage of the SEMs, or a biasing holder. For analytical work in any of our electron microscopes the beam current must be known.

Specimen Grids: A wide range of grids are needed to support the sample imaged in the TEM. The grids will be used for testing samples.

PC Upgrades for Cryo-Stage controller computer: The controller for the HCS cryo-holder requires a small computer to automate its operation.

Electronics parts for *in situ* biasing experiments: We have been able to locate collaborators who will let us borrow their holders for *in situ* experiments. The small electronics parts are needed for accessing the holder. This would allow us to acquire preliminary data to be used in writing proposals for purchasing *in situ* holders.

INSTRUMENT MAINTENANCE AGREEMENT

CEMMA is equipped with highly sophisticated analytical instrument systems. The maintenance agreements covers routine preventive maintenance, unlimited malfunction repair, phone support, computer and software support and replacement parts. The maintenance of these systems by the manufacturer is needed to ensure the instrument is returned to normal operation in the shortest time period, only the manufacturer can provide the original parts and the specific technical expertise for repairing the instrument, provide the proprietary software to operate the instrument and the necessary technology to perform diagnostic tests for assessing the potential future life.

STAFFING SUPPORT

Life Science

Research assistant position # 1:

(This is a new position)

Specializes and works with life science researchers to help design projects with traditional image and preparation techniques for specimens in the TEM such as:

- Immunocytochemistry
- Positive and negative staining
- TEM fixation and processing
- Ultrathin sectioning with the Ultramicrotome
- Support technique development and provide assistance on collaborative projects
- Setting up and giving expert demonstration for laboratory walkthrough and outreach program tours

Research assistant position # 2:
(This is a new position)
Specializes and works with life science researchers to help develop projects in 3-D tomography, Cryo EM imaging, and use of advanced techniques for preparing specimens for the TEM. Such techniques will include but not be restricted to:

- Cryo-ultramicrotomy ultrathin sectioning
- Progressive lowering of temperature and freeze substitution techniques
- Embedding protocols, acrylic resins such as the Lowicryls for immunocytochemistry
- Preparation of gold probes
- Support technique development and provide assistance on collaborative projects
- Setting up and give expert demonstration for laboratory walkthrough and outreach program tours

Life Sciences full-time technical position:
(This is a new position)
Electron microscopy for the life sciences is labor-intensive and specialized. Experience in complicated specimen preparation techniques are needed in order to be successful. This full time technical position will work under senior staff creating a consistent technical laboratory data flow and organization, which would allow the senior staff to focus on collaboration and developing of new projects with researchers. Some responsibilities would be:

- Processes biological specimens for TEM and SEM
- EM imaging, and following laboratory and safety protocols
- Collects and records image data and assists in data processing
- Maintains accurate records of all protocols, applied and equipment used
- Coordinates with users to assist with interpretation of data.
- Maintains and calibrates equipment
- Coordinates with operations director to schedule routine maintenance and basic repairs
- Maintains life sciences preparation laboratory in good order and stocked with necessary chemicals, solutions and other reagents
- Oversees safe handling and disposal of hazardous and non-hazardous materials, and maintains the laboratory in an orderly, safe, clean and operable condition
- Assists with experimental design, and application and development of novel protocols
- Understands basic laboratory safety, chemical safety and handling of blood borne pathogens
- Understands biosafety level 2 procedures

Operations
Research assistant position # 3:
(Ian McFarlane currently holds this position)
Specializes and works with researchers and users to train and instruct in the proper use of the SEM and FIB for life and physical science techniques.

- Trains in the proper use of the Critical Point Dryer
- Interprets data from EDS spectrum and mapping
- Ensures training consistency
- Certifies users

- Works with difficult non-conducting unaltered materials
- Provides expert knowledge for specialized materials like soft to hard biotic samples (bacteria to bone), natural to synthetic materials (rock strata to semiconductors) and biological specimens and other hydrated materials
- Images medical devices
- Images cross-sectional depth dependent topography using the FIB
- High resolution backscattering imaging
- Low vacuum backscattering imaging
- Setting up and giving expert demonstration for laboratory walkthrough and outreach program tours for both K-12 and undergraduate students

Research assistant position # 4:
(Yuzheng Zhang currently holds this position)
Specializes and works with researchers and users to train and instruct in the in proper use of the SEM and FIB for physical science technique.

- Ensures training consistency
- Certifies users
- Provides expert knowledge for specialized materials like nano-crystalline aluminum, SnO2 nanowire, nano-twinned copper and shale rocks
- Developed and executes FIB TEM sample lift-out process
- General maintenance of FIB such as basic trouble shooting, beam alignment and probe replacement
- Obtains crystalline orientation map using EBSD
- EBSD data acquisition of ceramics, metals & alloys
- Data post-processing using OIM analysis software
- Sets up and gives expert demonstration for laboratory walkthrough and outreach program tours

Research assistant position # 5:
(Rohan Dhall currently holds this position)
Specializes and works with researchers to help develop projects for traditional image on the SEM, TEM and STEM.

- *In situ* material manipulation and measuring
- Records and interprets electron diffraction and CBED patterns of crystalline materials
- TEM Dark field imaging
- STEM bright field and high angular dark field imaging
- TEM and STEM high resolution imaging
- EDS mapping and interpreting
- Programing with LabView, Matlab, Mathematica and Python
- Ensures training consistency
- Certifies users
- Provides expert knowledge for specialized materials like nano particles and wires, carbon nano tubes, graphene, and molybdenum disulfide.
- Setting up and give expert demonstration for laboratory walkthrough and outreach program tours.

DeltaVision OMX full-time technical position:
(Marc Green currently holds this position)
This position is currently paid from funding sources outside of CEMMA budget.
Requesting to place this 40% staff position to be funded directly from CEMMA budget.
This will allow a more accurate accounting of the costs that are incurred for the Delta Vision OMX.
This position would continue to be a 40% funded position for fiscal year 14/15 and would increase to 100% funded position for the years after.

The 3D-SIM super-resolution microscopy has only been commercialized in the last few years and expert support is required to assist investigators in interpreting data, setting up experiments for imaging and transferring existing confocal-type experimental designs into imaginable samples. New users require extensive training in both 3D-SIM theory and optimization.

- Ensures training consistency
- Aids investigators in evaluating data quality and super-resolution applicability
- Researchers will be able to quickly localize cellular structures and proteins in cells and engineered objects, looking deeper, enabling visualization of minute structures and their functions
- Imaged fine structures in bacteria with potential in fuel cell technology
- Proteins localizing to centers of DNA damage and repair with direct implication in Cancer
- Engineered polymers assembling inside cells with potential in drug delivery

One existing student worker (20 hours/week):
(Saurabh Baburao Chaure currently holds this position)
To continue to support the laboratory information database computer system:

- Networking
- Programing
- Problem solving
- Emergency bug and error removal
- Other computer related responsibilities

Physical Sciences
Research assistant position # 6:
(This is a new position)
With the acquisition of an XPS, CEMMA has expanded their characterization capabilities. An RA position would help senior staff by taking up less of their time with basic instrument training and maintenance calls. This would allow the staff to spend their time more efficiently being able to focus on writing proposals for new add-on to existing equipment.

- Ensures training consistency
- Certifies users
- Surface analysis interpretive experience
- Data processing
- Supports technique development and provides assistance on collaborative projects
- Sets up and gives expert demonstration for laboratory walkthrough and outreach program tours

Physical Science full-time technical position:
(This is a new position)
A huge assistance to the research community would be to have a technician capable of quickly making FIB prepared samples. Currently we rely on an RA to help users make these time consuming (each one can take 6 hours) samples. An RA can only work a 10-hour workweek and has to balance many other assignments. With a full time technician this would dramatically improve the number of samples made. This technician would provide support to researchers by eliminating the need to spend unnecessary time and add expert knowledge for short projects in preparing TEM samples.

- Maintains the preparation laboratory in good order and stocked with necessary chemicals and supplies
- Oversees safe handling and disposal of hazardous and non-hazardous materials
- Maintains the laboratory in an orderly, safe, clean, operable condition
- Coordinates with operations manager to schedule routine maintenance and basic repairs

TRAVEL

Professional Development: Will improve individual performance of CEMMA Staff and promote the development of knowledge and skills by attending seminars, conferences and symposia. A focus on technical education, hands-on training and networking opportunities will benefit USC researchers who are CEMMA users.

Outside analysis: This will allow travel to National Laboratories and outside core electron microscopy facilities at universities that provide advanced technologies that are not available at CEMMA. Facilities that have already accepted CEMMA's proposals for equipment support are the National Center for Electron Microscopy at Berkley and the Center for Integrated Nano-technologies at Sandia.

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Pressurized gas: For the use of pneumatic valves on major equipment and support gas for performing experiments with preparation equipment.

Facilities Improvement: For updating facilities as need for new equipment.

Data jack connection: To allow access, for each of the preparation equipment, to data card readers and lab database computer software.

VI. 5-YEAR PLAN

Teaching, and technique development over the next 5 years will focus on five major areas of interest, all of which are important for USC researchers in the physical and life sciences.

Life Sciences

A. Routine morphology and immunocytochemistry
A very basic need for life scientists is to be able to image specimens in the TEM and SEM. This need is only partially addressed by CEMMA.

Morphology and immunocytochemistry are basic approaches to the study of biological specimens. Preparation methods vary depending on the specimens under study and the targeted results. It is therefore important to have a shared imaging resource that is able to satisfy most needs. The following equipment will be important for creating a state-of-the-art nano-imaging resource at USC.

Microwave Processor
High-Pressure Freezer
Freeze Substitution System

B. Correlative light and electron microscopy
An emerging technology in the life sciences is to combine the advantages of light microscopy (e.g. imaging living specimens, time-lapse imaging, low magnification scans, 3-D reconstruction) with the power of electron microscopy (e.g high resolution imaging of subcellular details). Using CLEM, light and electron microscopy images are superimposed over one another to reveal information not accessible using individual imaging modalities.

An important prerequisite for successful correlative imaging is the necessity to immediately immobilize specimens after light microscopy imaging in preparation for electron microscopy. Rapid freezing of specimens offers a more efficient immobilization than the slower chemical crosslinking approach.

Comparing the whole cells imaged by light microscopy is technically challenging because the target cells have to be located and undergo extensive serial sectioning. Only 3-D reconstructed cells can offer true correlative imaging.

CLEM requires a distinct set of tools to work successfully that will include the following:

CO_2 Incubator and Laminar Flow Cabinet
Laser Scanning Confocal Microscope
Automated Array Tomography System
3-View Imaging with dedicated Scanning EM

C. Cryo-imaging of small particles and 3-D reconstruction
This is where suspensions of particles are imaged in a rapidly frozen, thin layer of water.

This approach will require:

A TEM working at 200 or 300kV with
- A 70 degrees tilt goniometer
- A cryo-kit inside the TEM to prevent specimen contamination
- A cryo-specimen stage
- Low-dose imaging capabilities
- Dedicated software to control image collection
- 3-D reconstruction software
- Vitribot plunge freezer
- Cryo-ultramicrotome

Physical Sciences

D. Aberration Corrected TEM and *In situ* Microscopy

A persistent problem in TEMs has been the aberrations of the objective lens that limit resolution. Over the last ten years instrumentation has been developed to correct for these aberrations and the resolution of TEMs has improved by a factor of four. The more intense beam used in aberration corrected imaging increases the signal acquired by spectrometers. These improvements are vital to characterizing 2D materials, nanowires, and nanoparticles.

In situ microscopy has also developed rapidly over the last decade. New holders allow existing TEMs access to characterization methods previously relegated to specialized microscopes. An example is the cathodoluminescent holder made by Gatan. Defect analysis and optical band-gap determination are now possible on the nanometer scale in any microscope. This characterization is important for semiconductor research at USC.

E. SEM upgrades, He Ion Microscope and FiB Microscope

Our current electron beam induced current (EBIC) apparatus is capable of characterizing samples used in prototyping, but not devices made on a wafer scale. Groups interested in comparisons between similar devices have to test them by disconnecting and reconnecting wires outside of the microscope. This time consuming task is not useful for failure analysis or statistical analysis of devices. An adaptation to the SEM can be made to allow for faster EBIC characterization of devices.

Recent developments in ion beam optics have lead to better ion beam milling. Faster and more precise milling in modern focused ion beam microscopes will lead to an expanded use of this important sample preparation technique. In addition, further use as a field emission SEM will increase SEM usage.

VII. 5 YEAR PLAN BUDGET

EQUIPMENT

All equipment requested in the 5-year plan would be placed in the USC Michelson Center for Convergent Bioscience.

A. Routine morphology and immunocytochemistry		
Microwave Processor		$ 20,000
High-Pressure Freezer		$ 300,000
Freeze Substitution System		$ 30,000
	Sub-Total	$ 350,000
B. Correlative light and electron microscopy		
CO2 Incubator and Laminar Flow Cabinet		$ 30,000
Laser Scanning Confocal Microscope		$ 500,000
Automated Array Tomography System		$ 80,000
3-View imaging with dedicated Scanning EM		$1,500,000
	Sub-Total	$2,110,000
C. Cryo-imaging of small particles and 3-D reconstruction.		
A Vitribot plunge freezer		$ 30,000
Cryo-ultramicrotome		$ 120,000
3-D Cryo TEM		$1,600,000
Double tilt cryo tomography holder		$ 200,000
	Sub-Total	$1,950,000
D. Aberration Corrected TEM and *In situ* Microscopy		
Aberration Corrected TEM		$5,000,000
Gatan Vulcan Cathodoluminescence Holder		$ 440,000
	Sub-Total	$5,440,000
E. SEM upgrades, He Ion Microscope and FIB Microscope		
EBIC probe station for SEM		$ 50,000
Helium Ion Beam Microscope		$1,600,000
FIB/STEM Crossbeam Microscope		$1,400,000
	Sub-Total	$3,050,000
	TOTAL EQUIPMENT	**$12,900,000**

INSTRUMENT MAINTENANCE AGREEMENT

Current Instrumentation:	Fiscal Year				
	14/15	15/16*	16/17*	17/18*	18/19*
JEOL 7001 FSEM	$36,000	$37,080	$38,192	$39,338	$40,518
EDAX EDS/EBSD	$20,000	$20,600	$21,218	$21,854	$22,510
JEOL 4500 FIB	$62,000	$63,860	$65,775	$67,749	$69,781
EDAX EDS	$15,000	$15,450	$15,913	$16,390	$16,882
JEOL 6610 SEM	$14,000	$14,420	$14,852	$15,298	$15,757
EDAX EDS	$12,000	$12,360	$12,730	$13,112	$13,506
JEOL 2100F Gatan Camera/GIF	$113,000	$116,390	$119,881	$123,478	$127,182
EDAX EDS	$17,000	$17,510	$18,035	$18,576	$19,133
Kratos XPS	$33,000	$33,990	$35,009	$36,059	$37,141
GE DeltaVision OMX	Warranty	Ex Warranty	Ex Warranty	$70,000	$100,000
Sub-Total	**$322,000**	**$331,660**	**$341,610**	**$421,854**	**$462,410**

* Inflation Factor - 3%

Proposed Instrumentation:

	Fiscal Year				
	14/15**	15/16**	16/17**	17/18	18/19*
Laser Scanning Confocal Microscope	N/A	N/A	N/A	$100,000	$103,000
3-View Imaging SEM	N/A	N/A	N/A	$40,000	$41,200
3-D Cryo TEM	N/A	N/A	N/A	$85,000	$87,550
Aberration Corrected TEM	N/A	N/A	N/A	$150,000	$154,500
Helium Ion Beam Microscope	N/A	N/A	N/A	$50,000	$51,500
FIB/STEM Crossbeam Microscope	N/A	N/A	N/A	$50,000	$51,500
Sub-Total	**-**	**-**	**-**	**$475,000**	**$489,250**

* Inflation Factor - 3%, ** Instrumentation proposed for USC Michelson Center

MATERIALS AND SUPPLIES

	Fiscal Year				
	14/15	15/16	16/17*	17/18*	18/19*
Life Sciences	$52,300	$30,000	$30,900	$31,827	$32,781
Operations	$50,000	$50,000	$51,500	$53,045	$54,636
Physical Sciences	$32,000	$30,000	$30,900	$31,827	$32,781
Sub-Total	**$134,300**	**$110,000**	**$113,300**	**$116,699**	**$120,200**

* Inflation Factor - 3%

STAFFING (EXISTING)

	Fiscal Year				
	14/15*	15/16*	16/17*	17/18*	18/19*
Three Existing Full Time Staff (Salaries and Fringe)	$403,880	$415,996	$428,476	$441,331	$454,570
Three existing research assistant	$42,012	$43,272	$44,571	$45,908	$47,285
One existing student worker (50%)	$18,000	$18,540	$19,096	$19,669	$20,259
Sub-Total	**$463,892**	**$477,809**	**$492,143**	**$506,907**	**$522,115**

* Inflation Factor - 3%

STAFFING (REQUESTED)

	Fiscal Year				
	14/15*	15/16* ^	16/17*	17/18*	18/19*
Three new Technicians (Salaries and Fringe)	$140,175	$160,200^	$165,006.00	$169,956.18	$175,054.87
Three new research assistant	$42,012	$43,272	$44,570.16	$45,907.26	$47,284.48
Sub-Total	**$182,191**	**$203,472**	**$209,576**	**$215,863**	**$222,339**

* Inflation Factor - 3%, ^ Includes three full-time technical position

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	Fiscal Year				
	14/15	15/16*	16/17*	17/18*	18/19*
Professional development	$15,000	$15,450	$15,913	$16,390	$16,882
Outside analysis	$12,000	$12,360	$12,730	$13,112	$13,506
Sub-Total	**$27,000**	**$27,810**	**$28,644**	**$29,504**	**$30,389**

* Inflation Factor - 3%

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	Fiscal Year				
	14/15	15/16*	16/17*	17/18*	18/19*
Pressurized gas	$8,000	$8,240	$8,487	$8,741	$9,004
Facilities Improvement	$10,000	$10,300	$10,609	$10,927	$11,255
Data connection	$2,000	$2,060	$2,121	$2,185	$2,251
Sub-Total	**$20,000**	**$20,600**	**$21,218**	**$21,855**	**$22,510**

* Inflation Factor - 3%

INSTRUMENT MOVING COST (IMC)

The list below accounts for the current major instruments in the CEM building, which are being moved to the new USC Michelson Center for Convergent Bioscience building. Expenses include an estimate for disassembling, packing and reassembling by the instrument manufacturer and rigging/moving costs.

JEOL 7001 FSEM	
Manufacturer	$ 30,000
Moving	$ 2,500
JEOL 4500 FIB	
Manufacturer	$ 32,000
Moving	$ 2,500
JEOL 6610 SEM	
Manufacturer	$ 15,000
Moving	$ 2,500
JEOL 2100F TEM/STEM	
Manufacturer	$ 80,000
Moving	$ 5,000
Kratos XPS	
Manufacturer	$ 35,000
Moving	$ 2,500
GE DeltaVision OMX	
Manufacturer	$ 50,000
Moving	$ 2,500
Sub-Total	$259,500
TOTAL IMC	**$259,500**

VIII. 5-YEAR PLAN BUDGET JUSTIFICATION

A. Routine morphology and immunocytochemistry

A **microwave processor** will assist with preparing specimens for morphology. Microwave processing is able to reduce specimen preparation times considerably (from 3-4 days to 2-3 hours). This technology is still young, so its uses in EM and light microscopy specimen preparation are still being developed. However, rapid specimen preparation is a well-developed approach. Microwave processing offers and attractive approach for students who are unable to dedicate long hours for specimen preparation.

High-Pressure Freezer. Conventional biological EM processing has the potential to damage ultrastructure by creating or removing high-resolution detail. This damage has been identified to occur both during the initial fixation (chemical cross-linking) and during specimen dehydration, required for the specimens to be embedded in resin. High pressure freezing avoids both of these artifacts by immobilizing biological specimens without altering cellular ultrastructure. This approach to specimen preparation is ideal not only for bacteria, yeast and other model systems, but also for more traditional specimens from the life sciences. The high-pressure freezer is also an important tool for correlated light and electron microscopy (CLEM; see below), because it can immediately immobilize specimens examined by a confocal microscope. This machine is useful for life science research.

Freeze Substitution System. One way of avoiding ultrastructural damage to biological specimens during processing is to dehydrate specimens in the still-frozen state, after they have been rapidly frozen in a high-pressure freezer. A freeze substitution system provides the control required to keep specimens frozen while being dehydrated and embedded in resin, and to slowly warm the specimens without ice crystal formation.

B. Correlative light and electron microscopy

For it to be successful as a center dedicated to the life sciences, CEMMA has to be equipped with a **tissue culture** capability, a **Laser Scanning Confocal Microscope** and a high-pressure freezer, all in close proximity to each other. Freshly cultured specimens are examined using the confocal and then immediately immobilized using the high-pressure freezer for subsequent EM examination. Two technical difficulties have then to be overcome. The region of interest examined by the confocal has to be located and this region has to be serially sectioned for 3-D reconstruction.

Currently two approaches for 3-D serial sectioning are available; the **Automated Array Tomography System** and the **3-View System with dedicated Scanning EM**.

The **Automated Array Tomography System (ATUM)** The ATUM (Automatic Tape UltraMicrotomy) System is a recent development that automates serial section production of resin embedded specimens. Working with an ultramicrotome and diamond knife, the ATUM controls a moving metal tape that removes serially produced sections from the water surface of the diamond knife trough. The sections on the metal tape are mounted on a silicon wafer, labeled and imaged by light microscopy. The same sections, and specific regions of interest can then be imaged in an SEM using a back-scatter electron detector. This allows for serial sections to be easily produced, immunolabeled multiple times for examination by light microscopy and produce a 3-D reconstruction of immunolabeled antigens overlaid on a high-resolution 3-D reconstruction of the specimen.

The **3-View System** combines automated serial sectioning with scanning EM. An ultramicrotome placed inside an SEM automatically shaves off thin sections from a resin-embedded block. An image of the top face of the embedded specimen is automatically recorded by the SEM, then the specimen block advances, another thin section is removed, and the block face imaged. Large volumes of embedded material can automatically be imaged using this approach to produce 3-D reconstructions for analysis. The 3-View System is fitted into a **dedicated Scanning EM,** which is able to perform high-resolution imaging of the face of the resin block. It is not practical to install this system onto a scanning EM that will be used for more conventional imaging.

3-D Reconstruction Software and Computers. The ability to produce 3-D reconstructions is an essential part of modern electron microscopy. Reconstructions of specimens offer high-resolution information of whole cells, an essential step toward Correlative Light and Electron Microscopy (CLEM). CLEM is an approach where images of specimens collected by light microscopy are combined with high-resolution images of the same regions to combine the advantages of both imaging systems.

C. Cryo-imaging of small particles and 3-D reconstruction.

Vitribot plunge freezer: The Vitribot is an automated plunge freezer that is used in combination with a cryo-TEM. It is able to reproduce ideal freezing conditions for vitrification of thin films of water.

Cryo-ultramicrotome: A modern ultramicrotome is essential for teaching, and also for preparing sections through frozen (vitrified) material. Thin, frozen sections imaged using a cryo-specimen stage and a TEM will reveal ultrastructural detail not observable using any other method.

3-D Cryo TEM:

Double tilt cryo tomography holder: One approach for producing 3-dimensional reconstructions of specimens is to collect multiple high resolution images of a specific region as it is gradually tilted in a transmission electron microscope (TEM). Real space back projection of the collected images into a 3-D reconstruction space together with the original tilt angle will yield a 3-dimensional reconstruction of the original object (a 3-D tomogram).

Physical restrictions of the TEM usually limit the tilt range to 70° in each direction, giving a 140° total tilt and not the full 180° possible (N.B. the FEI Talos TEM as a tilt range of ±90°). This limitation results in an incomplete set of projections and thus, missing structural information. This lost information, called the "missing wedge", causes elongation in the electron beam direction and other distortions.

A double tilt specimen holder allows the TEM operator to tilt the specimen under observation in two directions, getting closer to a full 360° examination. First images are collected as the specimen is tilted through the range available on the TEM. Then the specimen is turned 90° and a second full tilt series of images is collected. The two sets of collected images are back projected and superimposed on one another to create a more complete 3-D tomogram with reduced missing wedge-induced distortions."

D. Aberration Corrected TEM and *In situ* Microscopy

Aberration Corrected TEM: Elemental and chemical analysis of nano-scale materials requires electron probes that are smaller than those available by conventional transmission electron microscopy. Aberration correction allows for these small probes to be formed, thereby allowing the characterization of material's composition down to the atomic scale.

Gatan Vulcan Cathodoluminescence Holder: This holder allows for optical characterization of materials far below the spatial resolution of optical microscopes. Quantum wells, defect states, and bandgaps can now be mapped at the nanometer scale. This tool would allow all research groups pursuing improvements to optical materials to better characterize their samples, and in ways only a handful of other institutions can.

E. SEM upgrades, He Ion Microscope and FiB Microscope

EBIC probe station for SEM: Equipment that will allow for wafer scale failure analysis *in situ*. This will be used to probe new devices without having to destroy them, and will help identify failure sites.

Helium Ion Beam Microscope: Helium ion microscopy allows both a new type of imaging and milling not available with standard electron microscopy and focused ion beam milling. Compared to an electron, a helium ion is heavy and does not penetrate as far into a sample. This allows for image contrast just on the surface of the sample, with no damage to the interior, a powerful capability when looking at delicate samples such as biological samples or those made from carbon. If the intensity of ions is increased they will start to mill away the surface, but in a much more controlled way than is possible with focused gallium ion milling techniques. This scalpel-like precision is a good compliment to the bulk milling capabilities of gallium ions and has enough precision to make even fine-featured photonic structures.

FIB/STEM Cross Beam Microscope: The current FIB capabilities allows us to mill samples, but in the process we induce significant damage and leave our samples thicker than would be ideal to have. New focused ion beam optics allow better milling control and higher device throughput. Typically it takes us six hours to make a sample, and with newer, more automated systems, that time can be brought down to two hours. This tool would not only improve the quality of the samples, but also the time it takes to make them, providing more samples for TEM work.