Introduction to the Microscope Imaging Network
Foundational Core Facility

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CSU has several research core facilities

**EMERGING INNOVATIONS FACILITIES**

Provide access to resources and services in new or emerging area of research and technology. These facilities may serve a broad or focused spectrum of researchers primarily within CSU. Emerging Innovations Core Facilities may receive seed support to enable development and ensure agility of resources for new areas of research service.

**FOUNDATIONAL CORE FACILITIES**

These facilities serve a broad spectrum of researchers spanning multiple departments and colleges within CSU as well as external academic and commercial partners and represent a center of excellence for a given technology or area of expertise. Foundational Core Facilities are financially supported, in part, by the OVPR to ensure stability and quality of these important resources.

**INSTITUTIONAL CORE FACILITIES**

Represents critical physical infrastructure that supports the strategic goals of the CSU research mission. These facilities may operate as a recharge center and/or may have specific compliance regulation needs. Institutional Research Facilities are designated through a partnership between college leadership and the Vice President for Research.

**SPECIALIZED RESEARCH SERVICE FACILITIES**

Provide access to resources and services within a unit or research community. These facilities may receive one-time funding support from the OVPR to enable specific activities but are typically supported through their home unit.

https://www.research.colostate.edu/cores/
Foundational Core Facilities provide access to resources and/or services considered to be essential to the research enterprise. These facilities serve a broad spectrum of researchers spanning multiple departments and colleges within CSU as well as external academic and commercial partners. Foundational Core Facilities are financially supported, in part, by the OVPR to ensure stability and quality of these important resources.
Structure of the MIN

Facility Mission: Enable research and development programs at CSU by providing:
- expertise in microscopic imaging
- access to state of the art microscopy instrumentation
- access for new or unfunded investigators to obtain preliminary data for grant applications
- training for researchers (students, postdocs, PIs) in microscope imaging and related technologies
- demonstrations of microscope applications in formal courses

Advisory Board: Provide faculty supervision and oversight to the MIN Director, annual evaluation of the MIN Director. Aid in decisions on prioritizing requests, establishing academic programs, related to MIN equipment, and resolving issues regarding accessibility problems.
- James Bamburg, BMB (Chair) – SDC, Wide-field, All-in-one
- Randy Bartels, ECE
- Susan Bailey, ERHS
- Brad Borlee, MIP – Laser-scanning confocal
- Jennifer DeLuca, BMB (co-Chair) – TIRF
- Santiago Di Pietro, BMB
- Mercedes Gonzalez-Juarrero – Laser-scanning confocal
- Shane Hentges, BMS – Laser-scanning confocal
- Karyn Hamilton, HES
- Matt Kipper, CBE – SDC/AFM
- John Rash, BMS – TEMs
- Tim Stasevich, BMB

Instrument supervisors: Provide training, fee-for-service use, scheduling, assist with/coordinate service.
- Alisa Shaw, BMB – SDC, Keyence all-in-one
- Laurie Minamide, BMB – Wide-field microscopes
- Keith DeLuca, BMB – Total internal reflection
- Ellen Brennen-Pierce, Engineering – Confocal/AFM
- DN Rao Veeramachaneni & Suzanne Royer, BMS – TEMs
- Connie King, BMS – Laser-scanning confocal
- Grace Borlee, MIP – Laser-scanning confocal
## Instrumentation Overview

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Department</th>
<th>Location</th>
<th>Capabilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olympus spinning-disc confocal</td>
<td>BMB (CNS)</td>
<td>MRB 224</td>
<td>Autofocusing; incubator; 3D stage; 4 laser lines; holographic PB/PA/PC system; DIC; spherical aberration compensation for all objectives; Slidebook software (3I); RAID terabyte storage.</td>
</tr>
<tr>
<td>Nikon Eclipse Ti TIRF</td>
<td>BMB (CNS)</td>
<td>MRB 224</td>
<td>Autofocusing; 4 laser lines; Hg illumination source. 3D stage; incubator; wide field; N-STORM; RAID terabyte storage; Nikon Elements software.</td>
</tr>
<tr>
<td>Keyence all-in-one microscope</td>
<td>BMB (CNS)</td>
<td>MRB 224</td>
<td>3D stage; mapping of viewing area; pseudo-DIC; incubator; autofocus; low photobleaching mode; real-time overlays; structured illumination; Peltier adapter; analysis software.</td>
</tr>
<tr>
<td>Nikon wide-field microscopes</td>
<td>BMB (CNS)</td>
<td>MRB 224</td>
<td>Fluorescence; DIC; incubator; dark-field; Metamorph software.</td>
</tr>
<tr>
<td>Zeiss LSM 800 confocal</td>
<td>BMS (CVMBS)</td>
<td>A/Z W05</td>
<td>4 lasers; 3D stage; color camera, autofocusing; one GaAsP detector; Zen Blue acquisition and processing software.</td>
</tr>
<tr>
<td>JEOL-1400 electron microscope</td>
<td>BMS (CVMBS)</td>
<td>A/Z W13</td>
<td>120kV; several specimen holders with differing tilts, one compatible with LN₂; digital camera; magnification between 50x and 1,200,000x.</td>
</tr>
<tr>
<td>JEOL-2000 electron microscope</td>
<td>BMS (CVMBS)</td>
<td>A/Z W13</td>
<td>80kV to 200kV; magnification between 50x and 500,000x; film.</td>
</tr>
<tr>
<td>Zeiss LSM 510 confocal</td>
<td>MIP (CVMBS)</td>
<td>Micro B318</td>
<td>Multi-spectral analyzer; dual laser scanning system; incubator; Zeiss imaging software to capture and process images.</td>
</tr>
<tr>
<td>Olympus FV-1000 confocal</td>
<td>MIP (CVMBS)</td>
<td>RIC</td>
<td>Bright-field; DIC; up to 4 fluorophores simultaneously; 6D imaging (3D, time, wavelength, mosaic scanning); FRAP &amp; PA; colocalization.</td>
</tr>
<tr>
<td>Nikon Eclipse Ti-E confocal + atomic force</td>
<td>CBE (COE)</td>
<td>Scott 362</td>
<td>3D stage; DIC; 4 laser lines; autofocus; EMCCD camera; stage heater; MIRO and Nanomechanics software; NanoScope Analysis Software; acoustic enclosure and vibration isolation.</td>
</tr>
</tbody>
</table>

**Other hardware:** Plasma cleaner, microelectrode puller, fluorescence dissection microscope (coming soon!)
Instrumentation Grants

**Purpose:** Extend the capabilities of the MIN
- Adopt new imaging techniques (e.g., Lattice light sheet microscopy)
- Provide access to state-of-the-art equipment
- Acquire multi-use instruments that satisfy a broad range of research needs

*Example: Keyence BZ-X710 All-In-One fluorescence microscope*
- image stitching/screening
- automated quantification
- structured illumination for optical sectioning
- live-cell incubation for time lapse studies

(Fluorescence dissection microscope, TEM, etc.)

Bright field montage of Sudan Black treated slice (30 µm thick) of human hippocampus imaged on the BX-X710 with a 2X objective (30 images). Acquisition and stitching was done in less than 2 min.

Projection image of an image stack through a 30 µm section of mouse cortex showing nuclei (blue) and dendrites (Map2 stained) in layers 1 and 2 taken on the BZ-X710.
Occasionally, the necessary hardware does not exist. In that case, we design it ourselves.

**Example**: Incubation chamber for flat-sided nunc tubes.
- Enables chronic imaging of brain slices in an inverted spinning disk confocal microscope
- A custom chamber designed in the MIN maintains temperature in the tube during imaging

Other times, it’s just cheaper to build our own microscope components.

**Example**: Stage-top incubators for inverted microscopes.
- Commercial system: ~$10k (+)
- Custom-built chamber: ~$1k
  - Controls temperature, humidity, CO$_2$/air mixture
  - More flexibility to adapt to specimen mounting or microscope hardware

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**Video Article**

**Modified Roller Tube Method for Precisely Localized and Repetitive Intermittent Imaging During Long-term Culture of Brain Slices in an Enclosed System**

Benjamin B. Fixman$^1$, Isaac W. Babcock$^1$, Laurie S. Minamide$^*$$^1$, Alisa E. Shaw$^1$, Marina I. Oliveira da Silva$^{1,2}$, Avery M. Runyan$^1$, Michael T. Maloney$^{1,3}$, Jeffrey J. Field$^1$, James R. Bamburg$^1$
Purpose: To provide unfunded potential users with either training costs or access time to enable data acquisition for grant applications.
- Up to $2k of imaging/training costs
- Pending approval of the MIN Director and Advisory Board

Example: Structural dynamics of reconstituted nucleosomal arrays – Prof. Jeff Hansen’s Lab
- Preliminary data showed large aggregates of chromatin *in vitro* with unknown structure
- FRAP data collected on spinning disk confocal with Pilot Project funding
- FRAP suggested that packaged chromatin fibers in chromosomes are dynamic rather than static, and that chromatin within chromosomes may be liquid-like.
- Net result: *Successful NSF proposal*

Download the application form: [https://www.research.colostate.edu/min/pilot-projects/](https://www.research.colostate.edu/min/pilot-projects/)
**New Model:** An Interphase Chromosome is a Hierarchical Assemblage of Globules Packaged as 10-nm Fibers

- **Topologically Associating Domain (TAD)** (3C, Hi-C) ~5x10³ nucleosomes
- **Compartment** (Hi-C, microscopy) ~10⁶ nucleosomes
- **Interphase chromosome** ~10⁶ nucleosomes
- ** Territory in the nucleus**

- **Compact chromatin domain** (microscopy) ~10³ nucleosomes
- **Interdigitated polymer melt packaging of the 10-nm chromatin fiber**
- **Nucleosome**

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**Compact chromatin domain (microscopy)**

- ~10³ nucleosomes

**Interdigitated polymer melt packaging of the 10-nm chromatin fiber**

- **Nucleosome**

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**New Model:** An Interphase Chromosome is a Hierarchical Assemblage of Globules Packaged as 10-nm Fibers

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**Compact chromatin domain (microscopy)**

- ~10³ nucleosomes

**Interdigitated polymer melt packaging of the 10-nm chromatin fiber**

- **Nucleosome**
Images contain quantitative data that can be extracted and/or analyzed with a variety of image processing tools and algorithms.

**Example 1:** Determine mean cell size of micro-algae.
- User collected images in the MIN, director wrote Python code to automate cell size recording.
Image Processing & Data Analysis

Images contain quantitative data that can be extracted and/or analyzed with a variety of image processing tools and algorithms.

**Example 2:** De-noise laser-scanning images with singular value decomposition (SVD).

\[ X = U S V^* \]
Microscopy Lecture Series – Beginning Fall 2019

- Topics to include
  - Overview of MIN facilities
  - Optics primer
  - Detection of light primer
  - Specific types of microscopy (survey driven)
    - Confocal
    - Wide-field
    - Atomic force
    - Electron microscopy
    - Phase contrast
    - Super-resolution
    - Photobleaching
    - FRET, FRAP
    - Multiphoton microscopy
    - Etc.
  - Sample preparation & fluorophore selection
  - Data processing and storage

Education and Outreach Efforts
Professional Science Master’s (PSM) program in Microscope Imaging Technology

• To fill a growing need for technically trained managers of core microscope facilities (first of its kind in nation)
• Offered as IDP or SDP for many BS majors in Molecular Biosciences or Engineering
• Training in microscope design, operation, maintenance, data acquisition, analysis, storage and business operation
• Required internships, both internal within core facilities and external within industry
• Program management is combined with PSM for Biological Data Analytics for savings on operational costs

Coursework tailored to six major skill areas
1. Cell & molecular biosciences (for those in undergraduate engineering degree programs)
2. Basic engineering and optical design (for those in undergraduate cell and molecular biosciences)
3. Optical microscopy design and applications (all students)
4. Image processing software, applications & automation (all students)
5. Business management principles (all students)
6. Communication (all students)

More information: https://psm-ns-mit.colostate.edu/
Exploiting the expertise of the MIN personnel to expand our services.

- The state-of-the-art for microscopic imaging is advancing rapidly
- Many commercial microscopes do not provide the latest advancements due to the time required to engineer turn-key products
- Adapt to recent advances in the field of microscopy by adopting new techniques
- Discovering our own microscopy advances in response to the needs of the CSU research community
- New laboratory space in A/Z and donations of equipment from numerous labs to begin exploring!
- Projects in progress:
  - Spatial-coherence-gated HiLo (not Hilo) – wide-field optically sectioned fluorescence imaging
  - Fourier ptychography – brightfield and darkfield combination providing phase contrast imaging with large field of view
  - Deep-tissue nonlinear microscopy
  - Others??
Occasionally, a custom-built microscope is required

- Long lead times to make systems turnkey for commercial sales
- Need for preliminary data to write instrument acquisition or research proposals
- *Example:* second-harmonic generation (SHG) imaging in sheep gut
  - A quote for a custom-built nonlinear microscope for SHG imaging provided by the MIN secured a donation from a medical company to build the system and collect preliminary data.
Scattering of Illumination Intensity

Illumination decays exponentially with depth: \( P_b = P_0 e^{-z/l_s} \)

Gray matter: 100 µm @ 630nm

Scattering of Signal Intensity

Multiphoton vs. linear microscopy

Reduced photobleaching

1-photon vs. 2-photon

Photos by Steve Ruzin

Fluorescence from out of focus planes  Fluorescence from focal spot only

Deeper imaging (less scattering)

Gray matter: 200 µm @ 800 nm

800 nm

630 nm
SHG arises from structural protein assemblies

Signal $\sim I^2$
Contrast: collagen, muscle, membranes, etc.
Multimodal Nonlinear Imaging

TPEF (red), SHG (green), and THG (blue). Samples from Prof. Tobet's lab.
Large scale multimodal imaging at full spatial resolution

Sample from Prof. Hentges’ lab.
Takeaway messages

• The MIN (like all CSU core facilities) is here to help you advance your research
• Wide range of commercial instrumentation and image processing software
• The Pilot Project program can provide access to instrumentation for unfunded researchers
• Custom microscopy hardware
• Image processing and data analysis consulting
• Potential for custom microscope designs

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