My name is Abigail Moye and I am a fourth year Biomedical Sciences Ph.D. Candidate in the Biochemistry and Molecular Biology Program. I work in Dr. Visvanathan Ramamurthy’s laboratory at the Eye Institute.

Our lab focuses on retinal degeneration and the roles of different retinal proteins that are linked to disease. My project centers on the protein ARL2BP, mutations in which cause blindness and other cilia-related symptoms in humans. My goal is to elucidate the role ARL2BP plays in the development and maintenance of the highly modified primary cilium present in photoreceptor cells. One way we examine the length of photoreceptor cilia is by immunofluorescence. We have used the new Nikon N-SIM E structured illumination microscopy (SIM) super-resolution microscope system to get an even closer look at the localization of a few proteins involved in photoreceptor cilium structure.

These images show 3 different proteins of photoreceptor cilium: rootletin (green), acetylated tubulin (magenta) and male germ cell-associated kinase 1 (red). A maximum intensity projection of a z-stack of images is shown as a traditional widefield fluorescence image (left), compared to a processed SIM super-resolution image before (center) and after (right) deconvolution.
Featured Microscope

Nikon A1R Confocal with N-SIM E Super-Resolution

**A1R+ Resonant Scanning Confocal System**
- High-resolution galvano & high-speed resonant scanners
- High-sensitivity GaAsP PMT detectors
- 405/488/561/640 nm lasers

**N-SIM E Super-Resolution System**
- Doubles conventional optical resolution
- 488/561/640 nm lasers
- ORCA-Flash4.0 sCMOS camera

**Eclipse TiE Inverted Microscope with Perfect Focus**
- 10x, 20x, 40x oil, 60x oil, 100x oil objectives
- DIC, epifluorescence
- Motorized, encoded stage with high-speed piezo Z-drive

**Tokai Hit Stage-Top Environmental Control System**

**NIS-Elements Acquisition and Analysis Software**
Sample Requirements for SIM

Double your resolution!

N-SIM E

SIM works best on samples that are:

- Less than 7 um thick
  ![Less than 7 um thick](image)
- Bright and photostable
  ![Bright and photostable](image)
- Mounted on #1.5 coverslips
- Mounted with Prolong Gold
- Not Vectashield

Also, please note that N-SIM E requires:

- 100X magnification
- Labels excited with 488, 561, or 640nm light
- At least 1 second to acquire each image
Optical microscope resolution is restricted by the diffraction of light at the objective rear focal plane, which creates a **diffraction barrier** that dictates a maximum resolution of approximately 200 nanometers in the lateral \((x,y)\) dimension and 500 nanometers in the axial \((z)\) dimension, depending upon the objective numerical aperture and the average wavelength of illumination.

**Diffraction Barrier Equ.**

\[
d = \frac{\lambda}{2NA}.
\]

**SIM takes advantage of the Moiré Effect for super resolution**

When we image a subject that has a pattern, the image will sometimes show moiré, which is simply a new pattern that is created when two patterns overlap.

\[\Delta \sim \frac{0.6\lambda}{NA} > 250 \text{ nm}\]

The moiré fringe effect can be exploited for super resolution microscopy techniques. Utilization of high spatial frequency laser interference to illuminate sub-resolution structure within a specimen produces moiré fringes, which are captured. These moiré fringes include modulated information of the sub-resolution structure of the specimen.
Structured Illumination Microscopy (SIM) excites the sample with a line pattern of **sinusoidally alternating intensity maxima and minima with a frequency at the diffraction limit** (~250 nm). Simply, you place a grate in the light path with alternating white and black lines ~ 250 nm apart from each other.

Rotating the grate in specific, predefined angles results in a collection of raw images that provides an interference pattern between the illumination source and the sample. This interference pattern encodes subresolution structural information that is normally filtered by a conventional microscope, as it is under 250 nm in diameter. A high-resolution image is thus reconstructed by mathematical processing of raw images acquired with several directions of the patterned excitation. Typically, 2D imaging requires nine raw images (three phases along three orientations at 120°).

More simply, a grating pattern is inserted into the light path. The resulting interference pattern caused by the grid (Moiré fringes) reveal hidden spatial information on objects that are normally sub-diffraction limited (less than 250 nm in width).
The Animal Models & Imaging Facility (AMIF) recently added an IVIS SpectrumCT to our family of small animal imaging systems. This is an upgraded version of the IVIS Lumina II that we have had since the start of the AMIF in 2008.

Features of the system include:
- Integrated optical and microCT technology
- 3D optical tomography for bioluminescence & fluorescence
- Multispectral fluorescence and spectral unmixing
- Low dose and ultra fast microCT

In addition, the software is the same, user-friendly Living Image platform that current IVIS users are familiar with.

The IVIS SpectrumCT has improved fluorescent capabilities over the IVIS Lumina. Fluorescent filter options include:
- 10 narrow band excitation filters: 415-760 nm
- 18 narrow band emission filters: 490-850 nm

The IVIS Lumina II will continue to be available for use. Contact us today for more information, training for independent use or assistance with adding the IVIS SpectrumCT to your ACUC protocol.

As always, the AMIF is happy to help you with experimental planning, ACUC protocol updates and anything else mouse related.
The Imaging Facilities will soon begin using a new online system, **Agilent's iLab Operations Software**, for our equipment reservations, reporting and billing. The software is very similar to CORES, so we expect that this will be an easy transition for all of our users.

Our “Go-Live” date is tentatively scheduled for April 5. Watch your email for additional information about the switch.

**Upcoming Webinars from The Jackson Laboratory**

Jax Webinars are a great resource for new lab members or when you are interested in learning about a new model. Here are some upcoming webinars. Check out the jax.org for many more. [https://www.jax.org/education-and-learning/webinars](https://www.jax.org/education-and-learning/webinars)

- **Guidelines for Sizing Experimental Mouse Colonies**
  1PM—April 13, 2017

- **Comparing Immunodeficient Mice for Cancer, Immunity & Transplant Research**
  1PM—April 20, 2017

- **Efficient Mouse Colony Management**
  1 PM—May 4, 2017

Also be sure to look at the on-demand videos for even more resources. [https://www.jax.org/education-and-learning/on-demand-videos](https://www.jax.org/education-and-learning/on-demand-videos)
Please let us know when you publish a manuscript with data from the MIF or AMIF so that we may acknowledge your achievement in our newsletter!


Recent Publications (Cont.)

Terrell-Hall et al.


Please Remember to Acknowledge Us!

**AMIF**: “Small animal imaging and image analysis were performed in the West Virginia University Animal Models & Imaging Facility, which has been supported by the Mary Babb Randolph Cancer Center and NIH grants P20 RR016440, P30 GM103488 and S10 RR026378.”

**MIF**: “Imaging experiments and image analysis were performed in the West Virginia University Microscope Imaging Facility, which has been supported by the Mary Babb Randolph Cancer Center and NIH grants P20 RR016440, P30 GM103488 and P20 GM103434.”
The purpose of this newsletter is to inform researchers about the AMIF and MIF. We want all investigators, graduate students and staff to be knowledgeable about the equipment and resources that are available. The staff are always glad to discuss upcoming studies with investigators to best utilize the core resources available. To learn more about our facilities, please check out our websites (to the left) or contact us to speak directly with AMIF or MIF staff.