

Core Facilities Annual Report FY2012

Dr. Charles Delwiche and Amy Beaven

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Executive Summary

The Imaging Core's (IC) two confocal microscopes, a Leica SP5X and a Zeiss LSM710, saw a combined average usage of 43.5 hours per week in FY2012, which is a 26% decrease from the previous year. The Zeiss was used 223 more hours than the Leica in FY2012 (1,244 hours vs. 1021 hours, respectively). One goal of the IC in the coming year will be to increase use of the Leica by encouraging researchers to take advantage of the microscope's unique capabilities, including a motorized stage, white light laser, high speed scanner and environmental chamber.

From FY2009 through the end of FY2012, 243 different researchers were trained to independently operate the two confocal microscopes, including students taking the 2-credit course, BSCI427/CBMG688W, Principles of Microscopy. Use of the two microscopes has already resulted in 30 publications, bringing the total number of publications made possible through the use of the IC's current and previous microscopes to at least 74 (detailed below, under "Publications").

The IC strives to keep users costs at a minimum. However, due to loss of temporary subsidies associated with the acquisition of the two new microscopes, it will be necessary to increase rates in order to maintain service contracts on both systems. At the 2010 User's Meeting it was decided that rather than have a one-time large jump in costs it would be preferable to ramp up user fees to the anticipated level. A detailed analysis of the facility's finances using current and projected income and expenses shows that a 7% rate increase each year until FY2020 will be necessary to help offset the expected decrease in income due to loss of subsidies in FY2013. The new rates of \$26/hr (peak), \$23/hr (off-peak) for unassisted use of the confocals, which will go into effect on September 1, 2012, is still well below the average hourly rate of \$32.50 seen at similar institutions with equivalent instrumentation. The ultimate hourly rate for unassisted use anticipated in FY2005 is roughly \$38/hour, but this will be revised based on real income and expenses.

While the IC has grown significantly in the last few years, there remains room for further expansion. Specific recommendations include: 1) Installation of reflection suppression notch filters at major laser lines on the Leica SP5X, and the replacement of one or more PMTs with hybrid detectors. This new technology has the potential to increase sensitivity and improve image quality, particularly for live cell imaging. 2) A reliable way to backup/store image data and 3) Acquisition of advanced image analysis software.

It is also recommended that the Director of the facility attend the University of Virginia's Workshop of FRET Microscopy, March 11-16, 2013 and/or the American Society for Cell Biology meeting, December 15-19, 2012 to continue enhancing the facility's technical capacity.

With regard to the Genomics Core, it is worth noting that although CBMG pays for the salary of the Genomics Core Director, CBMG accounted for only 3.5 percent of sequencer use over the past year and only 6.8% of sequencer use over the last 2 years.

Introduction

Established in the year 2000 by the Department of Cell Biology and Molecular Genetics, the Imaging Core (IC) was designed to enhance research and education at the University by providing students and faculty with access to sophisticated light microscopes and imaging instrumentation whose purchase and maintenance costs far exceed the budgets of individual investigators. Serving as the primary resource for advanced light microscopy in the Biological Sciences at the University of Maryland, the IC carries the mission of providing state of the art light microscopy instrumentation, training users in basic and advanced light microscopy techniques and introducing the latest technology and innovations in light microscopy.

Located in room 0107 Microbiology Building, the IC facility contains 9 rooms, five of which are dedicated microscope space, a working darkroom, office space for the Director and a wet-bench lab space with fume hood. When first established, the IC contained a single confocal microscope and a deconvolution microscope. Over the years, demand for time on the instruments increased dramatically, necessitating the purchase of a second confocal in 2008. At present, the IC contains 2 state-of-the-art confocal microscopes, a deconvolution microscope, 2 fluorescence microscopes and an automatic film processor.

The Director of the IC, Amy Beaven, oversees the routine operation of the laboratory and is available during normal business hours to provide training on all equipment, guidance on experimental design, assistance with image analysis and technician-assisted microscope operation. Since taking over the IC operation in November 2005, Ms. Beaven has trained over 397 researchers from at least ten different departments in six colleges and three different campuses of the University of Maryland.

The IC is used by a diverse group of investigators, including undergraduates, graduate students, post-docs, technicians and faculty. Students enrolled in the annual 2-credit class, Principles of Microscopy, gain hands-on experience in the operation of the IC's fluorescence and Leica SP5X confocal microscope. This course has trained an average of thirteen students each year for the past ten years.

Funding for operation of the IC comes from a combination of user fees and support from the University of Maryland. The Director's salary is provided by the Department of Cell Biology and Molecular Genetics and equipment maintenance costs have at times been subsidized by the college, thereby providing even occasional users with appropriate training and access to instrumentation, while simultaneously, keeping instrument use costs low. We have found that this strategy provides exceptional opportunities for research and training, and enables our students to perform experiments with instrumentation that is at the leading edge of technology.

Facility Mission

The mission of the Imaging Core Facility, located in 0107 Microbiology Building, is to enhance research and education within the College by:

- Providing access to state-of-the-art light microscopy and imaging instrumentation.
- Offering detailed training opportunities and support in basic and advanced light microscopy techniques.
- Keeping researchers up to date with the latest technology and innovations in light microscopy.

Organizational Structure and Governance

- Director of the Facility: Amy Beaven
- Faculty supervisor: Dr. Charles Delwiche, Professor
- Advisory Committee: Dr. Charles Delwiche, Professor (CBMG) Dr. Ricardo Araneda, Assistant Professor (BIOL), Dr. Stephen Wolniak, Professor (CBMG), Dr. Antony Jose, Assistant Professor (CBMG)

Personnel

The Director of the Facility, Amy Beaven, is the only full-time staff member within the facility. She was hired in 2005 to manage the Imaging and Genomics Core facilities and was promoted to Director in 2010. Ms. Beaven received her Master's degree in Biology in 1999 and has over 10 years intensive experience in confocal imaging techniques. She is available during the hours of 8am-4:30pm to provide guidance in experimental design, training on all equipment, technician-assisted confocal operation and assistance with image analysis.

History of the Facility

Amy Beaven was hired to manage the Imaging Core Facility in November 2005. She took over for the previous director of the facility, Dr. Robert Brown, who had left the University several months previously. At this time, the facility contained both Imaging and Genomics related equipment. Instrumentation included a Zeiss LSM 510 confocal microscope (0107E), a DeltaVision deconvolution microscope (0107F), an Olympus fluorescence microscope (0107), a Bio-Rad FX Pro Plus Imager, a Konica film processor (0107A), an ABI 3730xl DNA sequencer (0107H), two ABI 3100 DNA Sequencers (0107H) and an ABI 7700 Sequence Detector Real-Time PCR machine (0107H).

Summary of changes in instrumentation since November 2005

- August 2006: A Mini Med 90 Film Processor (cost: \$3,588.00) replaced the old Konica processor. The department paid \$2,500.00 of the total cost and each of the following PIs contributed \$109: Jonathan Dinman, Jeffrey DeStefano, Kenneth Frauwirth, David Mosser, Anne Simon, Wenxia Song, Richard Stewart and Elizabeth Gantt. The developer is serviced monthly by United Medical.
- October 2006: Dr. Steve Wolniak (Interim Chair of CBMG) procured a Zeiss Axiophot fluorescence microscope for the facility following Dr. Ron Weiner's retirement. A CoolSnap EZ monochrome camera, computer workstation and Nikon Elements software (total cost: \$13,400.00) were purchased in 2007 for the microscope using CBMG funds.
- April 2007: The 7700 Sequencer Detector was replaced with a Roche LightCycler 480 Real-Time PCR machine, which was purchased through CBMG using the Bioscience Research Building capital equipment funds (and is housed in BRB; see below).
- August 2007: Due to a drop in usage, the 3100 "North" DNA sequencer was taken out of operation.
- December 2008: The instruments in 0107H MICB (two ABI 3100 DNA sequencers, the ABI 3730xl DNA Sequencer and the Roche LightCycler 480 Real-Time PCR machines) were moved to the new Genomics Core, room 2229 Bioscience Research Building.
- December 2008: The Leica SP5 X confocal microscope was installed in room 0107H MICB. This microscope was obtained by Drs. Ian Mather and Steve Wolniak via an NSF grant.
- October 2009: The LSM510 confocal microscope was dismantled to make way for the new LSM710 confocal microscope. This microscope was purchased using College Funds, authorized by Dean Norma Allewell.
- January 2009: Genomics Core Equipment: Bio-Rad CFX 96 Real-time PCR machine was purchased and placed in room 2229 BRB.
- April 2010: Genomics Core Equipment: Due to a drop in usage, the 3100 "West" DNA sequencer was taken out of operation.
- September 2010: Dr. Charles Delwiche donated a Napco CO2 incubator to the Imaging Core.
- July 2011: Genomics Core Equipment: July 2011: Both the 3100 "West" and 3100 "North" DNA sequencers were sold through Terrapin Trader.
- November 2011: A Thermo Scientific Midi 40 CO2 incubator was purchased using Imaging Core funds (\$3,194.00)

Current Imaging Core Equipment

Equipment	Location	Description	Purchase Date	In-College Rate History (Academic Year)
Zeiss LSM 710 Confocal Microscope	0107E MICB	405 diode, argon (458, 488, 514nm), 561, 633. 3 PMTs, manual stage	October 2009	2009/2010: \$15.00/hr 2010/2011: \$18.60/hr 2011/2012: \$22.00/hr
Leica SP5X Confocal Microscope	0107H MICB	405 diode, argon (458, 488, 514), WLL. 5 PMTs, automated stage, resonance scanner, environmental amber	December 2008	2008/2009: \$15.00/hr 2009/2010: \$15.75/hr 2010/2011: \$18.60/hr 2011/2012: \$22.00/hr
Deltavision Deconvolution Microscope	0107F MICB	Standard DAPI, FITC, Rhodamine filters, automated stage	1999	2008/2009: \$8.00/hr 2009/2010: \$8.00/hr 2010/2011: \$8.00/hr
Axiophot Fluorescence Microscope	0107G MICB	Standard DAPI, FITC, Rhodamine filters, CoolSnap monochrome camera, workstation with Nikon Elements	CoolSnap, Elements: July 2007	\$2.00/hr since purchase
Olympus Fluorescence Microscope	0107 MICB	Standard DAPI, FITC, Rhodamine filters	Unknown	\$2.00/hr since 2005
Mini Med 90 Film Processor	0107A MICB	Standard film processor	August 2006	\$0.00/hr since purchase
Thermo Scientific Midi 40	0107L	CO2 incubator	November 2011	\$0.00/hr since purchase

Current Genomics Core Equipment

Equipment	Location	Description	Purchase Date	In-College Rate History (Academic Year)
3730xl DNA Sequencer	2229 BRB	96 capillary DNA sequencer	June 2004	2005/2006: \$25.00/run 2006/2007: \$35.00/run 2007/2008: \$35.00/run 2008/2009: \$37.00/run 2009/2010: \$38.00/run 2010/2011: \$38.00/run 2011/2012: \$38.00/run
Roche LightCycler 480 qPCR	2229 BRB	96 and 384 well real-time PCR machine	April 2007	2007/2008: \$7.00/hr 2008/2009: \$7.50/hr 2009/2010: \$8.00/hr 2010/2011: \$8.50/hr 2011/2012: \$8.50/hr
Bio-Rad CFX96 qPCR	2229 BRB	96-well real-time PCR machine	January 2009	2009/2010: \$8.00/hr 2010/2011: \$8.50/hr 2011/2012: \$8.50/hr

Summary of Facility Usage

During FY2012, use of the Zeiss LSM710 averaged 23.9 hours per week and Leica SP5X use averaged 19.6 per week. The combined average usage of 43.5 hours per week is a decrease from previous years (58.8 hours per week in FY11 and 52.8 hour per week in FY10), with both microscopes seeing a decrease in the number of hours used.

Table 1: Zeiss LSM710 Summary Data:

Fiscal Year	Total Income	Total # Hours Used	Total hours used for UMCP courses	Total # Training Sessions
2010	\$12,370.13	803.675	0	44
2011	\$33,448.35	1762.25	0	33
2012	\$27,895.48	1244.00	0	20
Total	\$73,713.96	3809.925	0	97

Table 2: Leica SP5X Summary Data:

Fiscal Year	Total Income	Total # Hours Used	Total hours used for UMCP courses	Total # Training Sessions
2009	\$5,090.75	345.78	0	39
2010	\$18,362.80	1282.517	70.96	43
2011	\$24,290.48	1325.3	55.5	35
2012	\$21,882.08	1021.25	62.75	29
Total	\$69,626.11	3974.85	189.21	146

Table 3: Combined Microscope Data:

Fiscal Year	Total Income	Total # Hours Used	Total hours used for UMCP courses	Total # Training Sessions
2009	\$5,090.75	345.78	0.00	39
2010	\$30,732.93	2086.19	70.96	87
2011	\$57,738.83	3087.55	55.50	68
2012	\$49,777.56	2265.25	62.751	49
Total	\$143,340.07	7784.77	189.21	243

During FY2012, forty-six different laboratories from 7 different departments (AGNR, Biology, CBMG, Chem, ENGR, ENT, IBBR) and 2 off campus laboratories (Origine, University of Maryland School of Medicine) made use of the facility's confocal microscopes. CBMG accounted for 50% of the total microscope use. Please see the following page for more information about microscope use by PI and department.

Figure 1: Top Confocal Users by Department FY2012

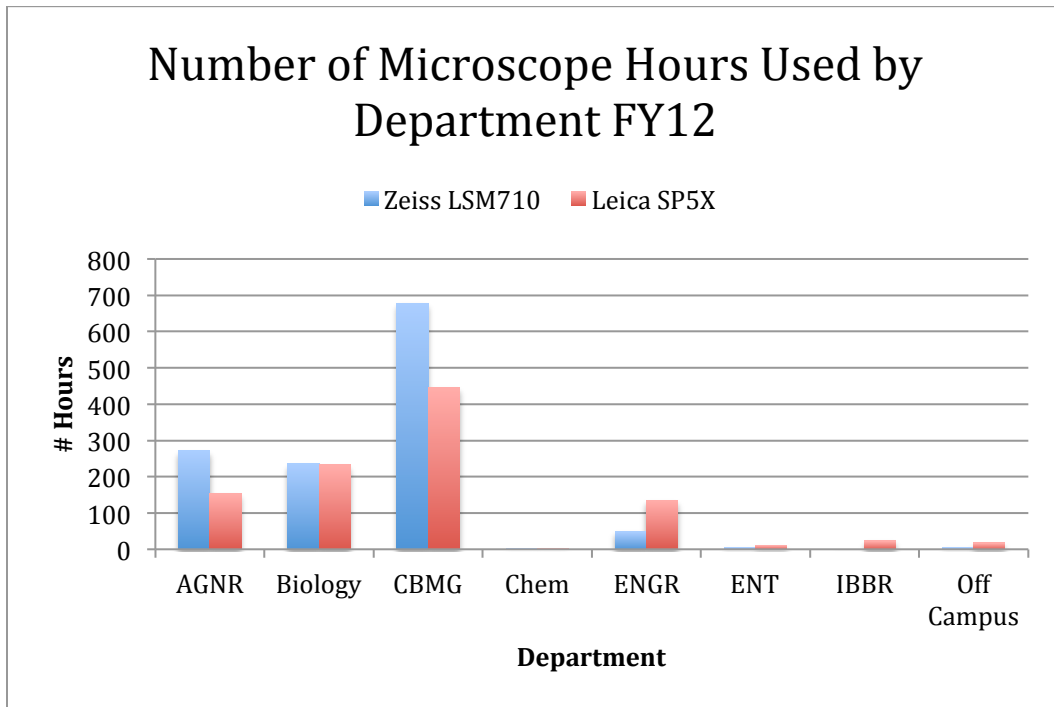
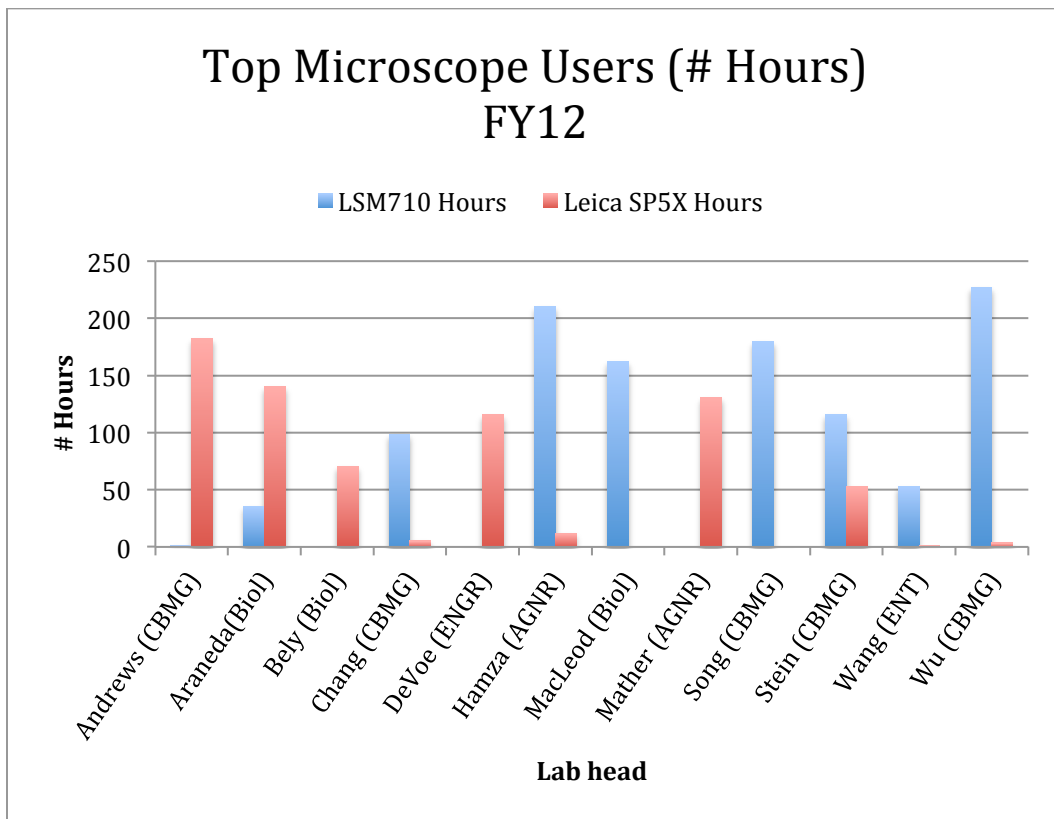


Figure 2: Top Confocal Users FY2012



Publications

Publications that entailed the use of the Zeiss LSM 510:

1. Bish, S. E., W. Song, and D.C. Stein. 2008. Quantification of bacterial invasion into host cells using a beta-lactamase reporter strain: *Neisseria gonorrhoeae* invasion into cervical epithelial cells requires bacterial viability. *Microbes Infect.* 10:1182-1191.
2. Sikes, J. M. & Bely, A. E. Radical modification of the A-P axis and the evolution of asexual reproduction in *Convolutriloba* acoels. *Evolution and Development* 10, 619-631 (2008).
3. The MHC class II-associated invariant chain interacts with the neonatal Fc gamma receptor and modulates its trafficking to endosomal/lysosomal compartments. Ye L, Liu X, Rout SN, Li Z, Yan Y, Lu L, Kamala T, Nanda NK, Song W, Samal SK, Zhu X. *J Immunol.* 2008 Aug 15;181(4):2572-85
4. Activation of the JAK/STAT-1 signaling pathway by IFN-gamma can down-regulate functional expression of the MHC class I-related neonatal Fc receptor for IgG. Liu X, Ye L, Bai Y, Mojidi H, Simister NE, Zhu X. *J Immunol.* 2008 Jul 1;181(1):449-63.
5. Identification and characterization of an alternatively spliced variant of the MHC class I-related porcine neonatal Fc receptor for IgG.
6. Ye L, Tuo W, Liu X, Simister NE, Zhu X. *Dev Comp Immunol.* 2008;32(8):966-79. NF-kappaB signaling regulates functional expression of the MHC class I-related neonatal Fc receptor for IgG via intronic binding sequences. Liu X, Ye L, Christianson GJ, Yang JQ, Roopenian DC, Zhu X. *J Immunol.* 2007 Sep 1;179(5):2999-3011
7. Thyagarajan, R., N. Arunkumar, and W. Song. 2003. Polyvalent antigens stabilize BCR surface signaling microdomains. *J. Immunol.* 170: 6099-106.
8. Onabajo, O., M. Seeley, A. Kale, B. Qualmann, M. Kessels, S-H. Tan, and W. Song. 2008. Mammalian actin-binding protein 1 regulates BCR-mediated antigen processing and presentation in response to BCR activation. *J. Immunol.* 180(10):6685-95.
9. Sharma, S., Orłowski G. and W. Song. 2009. Btk regulates BCR-mediated antigen processing and presentation by controlling the actin cytoskeleton dynamics in B cells. *J. Immunol.* 182: 329-339.
10. Dong CH, Rivarola M, Resnick JS, Maggin BD and Chang C (2008) Subcellular co-localization of Arabidopsis RTE1 and ETR1 supports a regulatory role for RTE1 in ETR1 ethylene signaling. *Plant Journal* 53(2): 275-286
11. Wenming Wang, Alessandra Devoto, John G. Turner, and Shunyuan Xiao. Expression of the Membrane-Associated Resistance Protein RPW8 Enhances Basal Defense Against Biotrophic Pathogens. *Molecular Plant-Microbe Interactions.* 2007 8:966-976
12. Wenming Wang, Xiaohua Yang, Samantha Tangchaiburana, Roland Ndeh, Jonathan E. Markham, Yoseph Tsegaye, Teresa M. Dunn, Guo-Liang Wang, Maria Bellizzi, James F. Parsons, Danielle Morrissey, Janis E. Bravo, Daniel V. Lynch, and Shunyuan Xiao. An Inositolphosphorylceramide Synthase Is Involved in Regulation of Plant Programmed Cell Death Associated with Defense In Arabidopsis. *The Plant Cell* 2008 20:3163-3179
13. Song, W., L. Ma, R. Chen, and D. C. Stein. 2000. Role of lipooligosaccharide in Opa-independent invasion of *Neisseria gonorrhoeae* into human epithelial cells. *J. Exp. Med.* 191 (6):949-60.
14. Cheng, P. C., B. K. Brown, W. Song, and S. K. Pierce. 2001. Translocation of the B cell antigen receptor into lipid rafts reveals a novel step in signaling. *J. Immunol.* 166 (6):3693-701.
15. Song, W. 2001. Signaling, actin dynamics and endocytosis. *Acta Biophysica Sinica.* 17 (1):10-18.
16. Brown, B. K., and W. Song. 2001. The actin cytoskeleton is required for the trafficking of the B cell antigen receptor to the late endosomes. *Traffic.* 2 (6):414-27.
17. Parent, B. A., X. Wang, and W. Song. 2002. Stability of the B cell antigen receptor modulates its signaling and antigen-targeting functions. *Eur. J. Immunol.* 32:1839-46.

18. Li, C., K. Siemasko, M. R. Clark, and W. Song. 2002. Cooperative interaction of Igalpha and Igbeta of the BCR regulates the kinetics and specificity of antigen targeting. *Int. Immunol.* 14:1179-91.
19. Stoddart, A., M. L. Dykstra, B. K. Brown, W. Song, S. K. Pierce, and F. M. Brodsky. 2002. Lipid Rafts Unite Signaling Cascades with Clathrin to Regulate BCR Internalization. *Immunity* 17:451-62.
20. Thompson, M. V., and Wolniak, S. M. 2008. A Plasma Membrane-Anchored Fluorescent Protein Fusion Illuminates Sieve Element Plasma Membranes in Arabidopsis and Tobacco. *Plant Physiology*, 146: 1599-1610
21. Sirichandra, C., *Gu, D. J., Hu, H.-C., Davanture, M., Lee, S., Djaoui, M., Valot, B., Zivy, M., Leung, J., Merlot, S. and Kwak, J. M. (2009) Phosphorylation of the Arabidopsis AtrbohF NADPH oxidase by OST1 protein kinase. *FEBS Letters* 483 (2009) 2982-2986
22. Jammes, F., Song, C. J., D. J. Shin, Munemasa, S., Takeda, K., Gu, D., Lee, S., Cho, D. S., Giordo, R., Garg, A., Sritubtim, S., Leonhardt, N., Ellis, E. B., Murata, Y. and Kwak, J. M. (2009) MPK9 and MPK12 are preferentially expressed in guard cells and positively regulate ROS-mediated ABA signaling. will be submitted shortly.
23. Zhang XN, Mount SM. Two alternatively spliced isoforms of the Arabidopsis thaliana SR45 protein have distinct roles during normal plant development. *Plant Physiol.* 2009 Apr 29 [PubMedID 19403727](#)
24. Rajagopal, A., Rao, A. U., Amigo, J., Tian, M., Upadhyay, S. K., Hall, C., Uhm, S., Mathew, M. K., Fleming, M. D., Paw, B. H., Krause, M. & Hamza, I. (2008). Haem homeostasis is regulated by the conserved and concerted functions of HRG-1 proteins. *Nature* 453, 1127-31
25. dos Reis Figueira A, Golem S, Goregaoker SP, Culver JN. A nuclear localization signal and a membrane association domain contribute to the cellular localization of the Tobacco mosaic virus 126-kDa replicase protein. *Virology.* 2002 Sep15;301(1):81-9. PubMed PMID: 12359448.
26. Padmanabhan MS, Goregaoker SP, Golem S, Shiferaw H, Culver JN. Interaction of the tobacco mosaic virus replicase protein with the Aux/IAA protein PAP1/IAA26 is associated with disease development. *J Virol.* 2005 Feb;79(4):2549-58. PubMed PMID: 15681455; PubMed Central PMCID: PMC546588.
27. Padmanabhan MS, Shiferaw H, Culver JN. The Tobacco mosaic virus replicase protein disrupts the localization and function of interacting Aux/IAA proteins. *Mol Plant Microbe Interact.* 2006 Aug;19(8):864-73. PubMed PMID: 16903352.
28. Dutta S., and Baehrecke E.H. (2008) Warts is required for PI3K-regulated growth arrest, autophagy and autophagic cell death in Drosophila. *Curr. Biol.* 18, 1466-1475.
29. Juhász G., Hill J.H., Yang Y., Sass M., Baehrecke E.H., Backer J.M. and Neufeld T.P. (2008) The class III PI(3)K Vps34 promotes autophagy and endocytosis but not TOR signaling in Drosophila. *J. Cell Biol.* 181, 655-666.
30. Berry D.L. and Baehrecke E.H. (2007) Growth arrest and autophagy are required for programmed salivary gland cell degradation in Drosophila. *Cell* 131, 1137-1148.
31. Martin D.N., Balgley B., Dutta S., Chen J., Cranford J., Kantartzis S., Rudnick P., DeVoe D.L., Lee C. and Baehrecke E.H. (2007) Proteomic analysis of steroid-triggered autophagic programmed cell death in Drosophila. *Cell Death and Differentiation* 14, 916-923.
32. Martin D. and Baehrecke E.H. (2004) Caspases function in autophagic programmed cell death in Drosophila. *Development* 131, 275-284.
33. M. Srinivasan and K.A. Frauwirth (2007). "Reciprocal NFAT1 and NFAT2 Nuclear Localization in CD8+ Anergic T Cells is Regulated by Suboptimal Calcium Signaling". *J. Immunol.* 179:3734-3741.
34. Structural Analysis of "Flexible" Liposomes: Implications for the Transdermal Penetration of Liposomal Structures. Oluwatosin A. Ogunsola, Margaret E. Kraeling, Sheng Zhong, Darrin J. Pochan, Robert L. Bronaugh, Srinivasa R. Raghavan to be submitted to *Langmuir*, June 2009
35. Kreczko, A., A. Goel, L. Song, and H.-K. Lee (2009) Visual deprivation decreases somatic GAD65

puncta number on layer 2/3 pyramidal neurons in mouse visual cortex. *Neural Plasticity* 2009: 415135.

36. Henry J. Adler, Elena Sanovich, Elizabeth F. Brittan-Powell, Kai Yan, and Robert J. Dooling (2008) WDR1 presence in the songbird basilar papilla. *Hearing Research* 240: 102-111.
37. Isabelle C. Noirot, Henry J. Adler, Charlotte A. Cornil, Nobuhiro Harada, Robert J. Dooling, Jacques Balthazard and Gregory F. Ball (2009) Presence of aromatase and estrogen receptor alpha in the inner ear of zebra finches. *Hearing Research*, 252 (49-55)
38. Title: Evaluation of In Vitro Penetration of Quantum Dot Nanoparticles into Human Skin Authors: M.E.K. Kraeling, O.A. Ogunsola, C.T. Sasik, N.V. Gopee, D.W. Roberts, N.J. Walker, W.W. Yu, V.L. Colvin, P.C. Howard and R.L. Bronaugh. Journal: Manuscript in preparation for *Toxicological Sciences*
39. Padmanabhan MS, Kramer SR, Wang X and Culver JN. 2008. TMV-Aux/IAA interactions: reprogramming the auxin response pathway to enhance virus infection. *J. Virol.* 82:2477-2385.
40. Culver, J.N. and Padmanabhan, M.S. 2007. Virus-induced disease: altering host physiology one interaction at a time. *Annu. Rev. Phytopathol.* 45:221-243.
41. Huang J, Tian L, Peng C, Abdou M, Wen D, Wang Y, Li S, Wang J.(2011) DPP-mediated TGFbeta signaling regulates juvenile hormone biosynthesis by activating the expression of juvenile hormone acid methyltransferase. *Development*, 138(11):2283-91.
42. Chen C, Samuel TK, Krause M, Dailey HA, and Hamza I. Heme utilization in the *Caenorhabditis elegans* hypodermal cells is facilitated by Heme Responsive Gene-2. *J Biol Chem.* 2012;287:9601-12. Epub 2012 Feb 2
43. Chanroj, S. Lu Y, Padmanaban S. Nanatani K, Uozumi N, Rao R, Sze H. Plant-specific cation/H⁺ exchanger 17 and its homologs are endomembrane K⁺ transporters with roles in protein sorting. *J. Biol Chem.* 2011 Sep 30: 286(39):3393-41. Epub 2011 Jul 27.
44. Lu, Y, Chanroj S, Zulkifi L., Johnson MA, Uozumi N, Cheung A, Sze H. Pollen tubes lacking pair of K⁺ transporters fail to target ovules in *Arabidopsis*. *Plant Cell.* 2011 Jan:23 (1)81-93. Epub 2011 Jan 14.

Publications that entailed the use of the Leica SP5 X (to date):

1. Sikes, J. M. and Bely, A. E. (2008), Radical modification of the A–P axis and the evolution of asexual reproduction in *Convolutriloba* acoels. *Evolution & Development*, 10: 619–631. doi: 10.1111/j.1525-142X.2008.00276.x
2. Sikes, J.M. and Bely, A.E. (2009), Making heads from tails: Development of a reversed anterior-posterior axis during budding in an acoel. *Devel. Biol.* 338 (1): 86-97.
3. Hou, H.Y., Heffer, A., Liu, J., Anderson, W.R., Liu, J., Bowler, T. and Pick, L. (2009) Stripy Ftz target genes are coordinately regulated by Ftz-F1. *Dev. Biol.* 335:442-453.
4. Zhang, H., Liu, J., Li, C.R., Momen, B., Kohasni, R.A. and Pick, L. (2009). A fly model for diabetes: deletion of *Drosophila* Insulin-Like peptides causes growth defects and metabolic abnormalities. *Proc. Natl. Acad. Sci. U S A.* 106:19617-22.
5. Jammes, F., Song, C. J., D. Shin, Munemasa, S., Takeda, K., Gu, D., Cho, D. S., Lee, S., Giordo, R., Sritubtim, S., Leonhardt, N., Ellis, E. B., Murata, Y. and Kwak, J. M. (2009) Two MAP kinases, MPK9 and MPK12, are preferentially expressed in guard cells and positively regulate ROS-mediated ABA signaling. *Proc. Nat'l. Acad. Sci. USA*, 106: 20520-20525.
6. Kong, D., Cho, D. S., Hu, H.-C., Li, J., Lazzaro, M., Lee, S., Jeon, B.-W., Munemasa, S., Murata, Y., Nam, H. G, Pei, Z.-M. and Kwak, J. M. (2010) *Arabidopsis* glutamate receptor homologs form Ca²⁺-permeable cation channels and contribute to Ca²⁺ uptake. Submitted.

7. Cho, D.S., Villiers, F., Kroniewicz, L., Lee, S., Zhao, J., Hirschi, K., Leonhardt, N. and Kwak, J. M. (2010) CAX1 and CAX3 contribute to the regulation of cytosolic pH and function in crosstalk between auxin and ABA in guard cells. In preparation.
8. Bely, A.E. and J.M. Sikes (2010). Latent regeneration abilities persist following recent evolutionary loss in asexual annelids. *Proceedings of the National Academy of Sciences* 107:1464-1469.
9. Flannery, A, Czibener, C. and Andrews, N.W. (2010) Palmitoylation-dependent association with CD63 targets the Ca²⁺ sensor synaptotagmin VII to lysosomes. *J. Cell Biol.* 191:599-613.
10. Zattara, E.E. and A.E. Bely (2011). Evolution of a novel developmental trajectory: fission is distinct from regeneration in the annelid *Pristina leidyi*. *Evolution & Development* 13:80-95.
11. Cortez, M., Huynh, C., Fernandes, M.C., Kennedy, K.A., Aderem, A. and Andrews, N.W. (2011) *Leishmania* promotes its own virulence by inducing expression of host CD200. *Cell Host & Microbe* 9:463-471. Highlight in *Nature Reviews Microbiology*
12. Fernandez, M.C., Cortez, M., Flannery, A.R., Tam, C., Mortara, R.A. and N.W. Andrews. (2011) *Trypanosoma cruzi* subverts the sphingomyelinase-mediated plasma membrane repair pathway for cell invasion. *J. Exp Med.* 208(5): 909-21.
13. Nunez-Parra A., Pugh V., Araneda R.C. (2011) Regulation of adult neurogenesis by behavior and age in the accessory olfactory bulb. *Mol Cell Neurosci.* 2011 May 10. [Epub ahead of print]
14. Huang J, Wang Y, Raghavan S, Feng S, Kiesewetter K, Wang J. (2011) Human down syndrome cell adhesion molecules (DSCAMs) are functionally conserved with *Drosophila* Dscam^[TM1] isoforms in controlling neurodevelopment. *Insect Biochem Mol Biol.* [Epub ahead of print]
15. Feng S, Huang J, Wang J. (2010) Loss of the Polycomb group gene polyhomeotic induces non-autonomous cell overproliferation. *EMBO Rep.* 12(2):157-63.
16. Clavio PE and KA Frauwirth (2012). Anergic CD8⁺ lymphocytes have impaired NF-κB activation with defects in p65 phosphorylation and acetylation. *J. Immunol.* 188:1213-1221.
17. Sijacic, P, Wang W and Zhongchi Liu (2011) Recessive antimorphic alleles overcome functionally redundant loci to reveal TS01 function in *Arabidopsis* flowers and meristems. *PLoS Genetics* 8(11): 31002342.
18. Kendall E, Shao C and Don L. DeVoe. Formation of asymmetric folded bilayers by lipid bubble injection in a thermoplastic microfluidic chip. Accepted to *Small*.
19. Shao, C., Kendall E and Don L. DeVoe. Electro-optical BLM chips enabling dynamic imaging of ordered lipid domains. Accepted by *Lab on a Chip*.

Publications that entailed the use of the LSM710 (to date):

1. Roy V., Smith J.I., Wang J., Stewart J.E., Bentley W.E. & Sintim H.O. (2010) Synthetic analogs tailor native AI-2 signaling across bacterial species. *Journal of the American Chemical Society*, 132 (32), pp 11141–11150
2. Montey KL, Quinlan EM. (2011) Recovery from chronic monocular deprivation following reactivation of thalamocortical plasticity by dark exposure. *Nat Commun.* 2:317.
3. He, K., A. Lee, L. Song, P. O. Kanold, and H.-K. Lee. (2011) AMPA receptor subunit GluR1 (GluA1) serine-845 site is involved in synaptic depression but not in spine shrinkage associated with chemical long-term depression. *Journal of Neurophysiology*, 105: 1897-1907.
4. Dong C.-H., Jang M., Scharein B., Malach A., Rivarola M., Liesch J., Groth G., Hwang I. and Chang C. (2010) Molecular association of the *Arabidopsis* ethylene receptor ETR1 and a regulator of ethylene signaling, RTE1. *J. Biol. Chem.* 285: 40706-40713.
5. Liu, X., Lu, L., Palaniyandi, S., Zeng, R., Gao, L.Y., Mosser, D.M., Roopenian, D.C. and X. Zhu. (2011). The neonatal FcR-mediated presentation of immune-complexed antigen is associated

- with endosomal and phagosomal pH and antigen stability in macrophages and dendritic cells. *J. Immunol.* 186(8): 4674-86.
6. Meyer, M.T., V. Roy, WE Bentley, and R. Ghodssi. (2011). Development and validation of a microfluidic reactor for biofilm monitoring via optical methods. *J. Micromech. Microeng* (21): 054023 (10pp).
 7. Driscoll MK, Albanese J, Xiong ZM, Mailman M, Losert W, and **Cao K.** (2012). A novel automated image analysis of nuclear shape: What can we learn from a prematurely aged cell? *Aging* 2012 Feb, Vol. 4, No 2
 8. Chen C, Samuel TK, Sinclair J, Dailey H and Hamza I. An intercellular heme trafficking protein delivers maternal heme to the embryo during development in *C. elegans*. *Cell.* 2011; 145:720-731
 9. Yuan X, Protchenko O, Philpott CC, and Hamza I. Topologically conserved residues direct heme transport in HRG-1-related proteins. *J Biol Chem.* 2012;287:4914-4924. Epub 2011 Dec 15.
 10. Recovery from chronic monocular deprivation following reactivation of thalamocortical plasticity by dark exposure. Montey KL, Quinlan EM. *Nature Communications* 2011;2:317
 11. Chau Huynh, Yuan, X., Miguel, D., Renberg, R., Protchenko, O., Philpott, C., Hamza, I., and Andrews, N. (2012) Heme uptake by *Leishmania amazonensis* is mediated by the transmembrane protein LHR1. *PLoS Pathogens.* 8:7:e1002795.

Outreach Activities During FY12

1. August 11, 2011: The Imaging Core hosted the Carl Zeiss Lecture Series in Imaging Technologies Workshop. Topics Included:
 - a. Imaging Basics and Confocal Microscopy
 - b. TIRF and its Applications
 - c. How Superresolution is Changing Research
2. August 30, 2011: The annual Imaging Core Users Meeting.
3. 2011 Fall Semester: Amy Beaven trained members of the class CBMG688W and BSCI472, Principles of Microscopy, to use the Axiophot fluorescence microscope and the Leica SP5X microscope.
4. 2011 Fall Semester: Amy Beaven assisted members of BSCI415 with the acquisition of confocal images.

Operating Cost Analysis

Current Data

At the end of FY2012, the Imaging Core account held a surplus of \$89,433.76. This money will be used in the current fiscal year to pay for both confocal microscope service contracts; a total of \$74,985.86.

Table 4: Total Imaging Core Facility Income and Expenses from FY09-FY12

Total Imaging Core Income (including subsidies) FY09-FY12	Total Imaging Core Expenses FY09-FY12	Net Based on Calculations	*Actual Account Balance
\$255,839.21	\$167,763.82	\$88,075.39	\$89,433.76

* The actual account balance differs from the calculated balance because the revolving account (295083) was not created on exact the date of the Leica SP5X purchase.

Table 5: Cost Breakdown: Leica SP5X

Year	Service Contract Cost	Expenses	Income	Income (subsidies)	Income - Expenses
FY2009	0	\$6,113.25	\$5,090.75	0	\$-(1,022.55)
FY2010	\$26,000.00	\$2,375.80	\$18,362.80	\$37,500.00	\$27,487.00
FY2011	\$36,075.00	\$488.25	\$24,290.00	\$37,500.00	\$25,226.75
FY2012	\$52,296.00	\$2,055.56	\$21,882.08	\$37,500.00	\$5,030.52
Total	\$114,374.00	\$11,032.86	\$69,625.63	\$112,500.00	\$56,721.77

Table 6: Cost Breakdown: LSM 710

Year	Service Contract Cost	Expenses	Income	Income (subsidies)	Income - Expenses
FY2009	0	0	0	0	0
FY2010	0	\$1,187.90	\$12,370.00	0	\$11,182.10
FY2011	\$17,730.00	\$1,231.50	\$33,448.00	0	\$14,486.50
FY2012	\$19,260.00	\$2,950.56	\$27,895.58	0	\$5,685.02
Total	\$36,990.00	\$5,369.96	\$73,713.58	0	\$31,353.62

It should be noted that the Leica SP5X confocal was heavily subsidized through FY12 (Table 5) by CBMG, VPR and IBBR. Those subsidies have now expired and user fees will need to cover the full cost of all expenses in future years.

Also note that the Leica SP5X service contract is much more costly than the Zeiss LSM710 contract (Tables 5 and 6) because it has several additional features, including an environmental chamber, high speed (resonance) scanner, white light laser and motorized stage.

Projected Cost Analysis:

If rates remain unchanged, the Imaging Core account will be in deficit by -\$96,557 (Table 7) at the end of FY20. However, if rates are raised by 7% each year through FY20, the account is projected to maintain an average balance of \$58,631 each year (Table 9), ending with a surplus of \$76,095 at the end of FY20 (Table 8). Income from the Zeiss LSM710 will help offset the loss of revenue from the Leica SP5X. These projections are based on microscope use in FY12.

Table 7: Projected Account Balance Through FY20: If Rates Unchanged

Microscope	Projected Expenses (consumables & service contracts)	Projected Income	Income-Expenses
SP5X	\$431,487	\$186,668	\$(244,819)
LSM710	\$168,400	\$227,228	\$58,828
Total	\$599,887	\$413,896	\$(185,990)
Account Balance End of FY20: \$-(96,557)			

Table 8: Projected Account Balance Through FY20: 7% Rate Increase Per Year

Microscope	Projected Expenses (consumables & service contracts)	Projected Income	Income -Expenses
SP5X	\$431,487	\$264,315	\$(167,171)
LSM710	\$168,400	\$322,234	\$153,834
Total	\$599,887	\$586,549	\$(13,337)
Account Balance End of FY20: \$76,095			

Table 9: Yearly Projected Account Balances with 7% Rate Increase Per Year

Year	Hourly Rate	End of Year Account Balance
FY13	\$23.54	\$73,247
FY14	\$25.19	\$60,658
FY15	\$26.95	\$51,920
FY16	\$28.84	\$47,300
FY17	\$30.86	\$47,089
FY18	\$33.02	\$51,594
FY19	\$35.33	\$61,145
FY20	\$37.80	\$76,095
Average yearly account balance: \$58,631		

Proposed Rate Schedules

Table 10: Proposed rate schedule for unassisted use (~7% rate increase through 2020):

Academic Year	Users w/in CMNS & AGNR (excluding VetMed)	On-campus users not affiliated with CLFS	Users Not affiliated with campus
Current Rate	\$22/hr	\$36.50/hr	\$73.20/hr
2012/2013	\$26/hr (peak) \$23/hr (off-peak)	\$39/hr	\$78/hr
2013/2014	\$25/hr	\$42/hr	\$84/hr
2014/2015	\$27/hr	\$44/hr	\$90/hr
2015/2016	\$29/hr	\$48/hr	\$96/hr
2016/2017	\$31/hr	\$51/hr	\$103/hr
2017/2018	\$33/hr	\$55/hr	\$110/hr
2018/2019	\$35/hr	\$59/hr	\$118/hr
2019/2020	\$38/hr	\$63/hr	\$126/hr

Table 11: Proposed rate schedule for assisted use:

Academic Year	Users w/in CMNS & AGNR (excluding VetMed)	On-campus users not affiliated with CLFS	Users Not affiliated with campus
Current Year	\$47.20/hr	\$59.00/hr	\$177.00/hr
All Future Years	\$50/hr	\$60/hr	\$180/hr

A thorough examination of confocal microscope rates at other institutions indicates that the facility's microscopes are priced competitively. The average rate for similar confocal microscopes at 24 institutions was \$32.50/hour. For more details, please see Table 12 on the follow page.

Table 12: Example Confocal Rates (updated July 2012)

Facility	Instrument	Hourly Rate	Additional Information
Berkeley Biological Imaging Facility	Zeiss LSM710	\$36	Training \$140/user
	Zeiss LSM510	\$28	
Cornell U Life Sciences Imaging Core	Zeiss LSM710	\$30	Training \$125/user
	Zeiss LSM510	\$25	Training \$75/hr
	Leica SP2	\$20	Training \$100/user
University of Virginia School of Medicine	Zeiss LSM510	\$39	
Iowa State University Confocal Facility	Leica SP5 X	\$31	\$1/hr increase in rate from last year
Northwestern U Biological Imaging	Leica SP2	\$30	
	Zeiss LSM510	\$40	
UMBC Imaging Facility	Leica TCS 4D	\$20	Last year: \$12/hr
Arizona State Imaging Facility	Zeiss LSM510	\$25	Training is \$46/hr
University of Illinois	Zeiss LSM700	\$28	
	Zeiss LSM710 MP	\$31	
Duke University Light Microscopy Core	LSM 510, Leica SP5, Zeiss LSM780	\$25.75	
Ohio State University Imaging Facility	Olympus FV1000	\$30	
Michigan State U Center Advanced Microscopy	Zeiss LS510 Meta	\$35	
	Olympus FV1000	\$35	
U of Washington Keck Facility	BioRad MRC600	\$39	After 6pm and lasting over 6 hours: \$23/hr
	Leica TCS SP	\$39	
Oklahoma State University	Leica SP2	\$30	
U of Georgia Ultrastructural Center	Leica SP2 MP	\$60	
UNC Medicine	Zeiss LSM510	\$39	
	Leica SP2	\$39	
University of Connecticut	Leica SP2	\$10	
Purdue U Life Science Imaging Facility	Zeiss LSM 710	\$30	
University of Minnesota Stem Cell Institute	BioRad 2000	\$50	
University of Alabama	Zeiss LSM710, Leica SP1	\$30	
Oregon State U	Zeiss LSM 510 Meta	\$17	Last year: \$15/hr
Yale School of Medicine	Zeiss LSM510, LSM710	\$39	
University of Iowa	Leica SP2	\$20	
University of Texas Austin	Leica SP2	\$40	
UMD School of Medicine	LSM 510 Meta	\$40	Training \$200/user
Average Hourly Rate		\$32.5	

Genomics Core Facility

Genomics Core Facility Income and Expenses FY2012 Only

1. Over the last two fiscal years, the Genomics Core spent approximately the same amount it collected in income from charge-backs. In FY2012, the Genomics Core spent \$8,098 more than it made. This was partially due to the purchase of a capillary array (\$4,825) in FY2012 (no array was purchased in FY2011).
2. The actual balance in the account at the end of FY2012 was \$34,429.
3. Though the facility lost money on both the 3730xl and LC480 last year, it broke even over the last 2 years. I suggest a small rate increase of \$1 more per run on the 3730xl and \$0.50 per hour on the qPCR machines.
4. It should be noted that, although CBMG pays for the salary of the Genomics Core Director, CBMG accounted for only 3.5 percent of sequencer use over the past year, and only 6.8% of sequencer use over the last 2 years.

Table 13: Genomics Core Facility Income and Expenses FY2012 Only

Instrument	Income	Consumables	Service contract	Total Expenses	Balance
3730xl	\$23,368	\$11,584.55	\$19,882	\$31,466	\$(8,098)
LC480	\$5,126	\$990	\$5,500	\$6,490	\$(1,363)
BioRad CFX96	\$4,327	\$0	\$3,700	\$3,700	\$627
Total	\$32,821	\$12,574	\$29,082	\$41,656	\$(8,835)

Table 14: Genomics Core Facility Income and Expenses from FY2011-2012

Instrument	Income	Consumables	Service contract	Total Expenses	Balance
3730xl	\$57,644	\$17,590	\$39,764	\$56,354	\$289.66
LC480	\$9,765	\$2,688	\$11,000	\$13,688	\$(3,923)
BioRad CFX96	\$9,293	\$0	\$3,700	\$3,700	\$5,593
Total	\$76,702	\$20,278	\$54,464	\$74,742	\$1,959

Figure 3: DNA Sequencer Use by Department FY2012

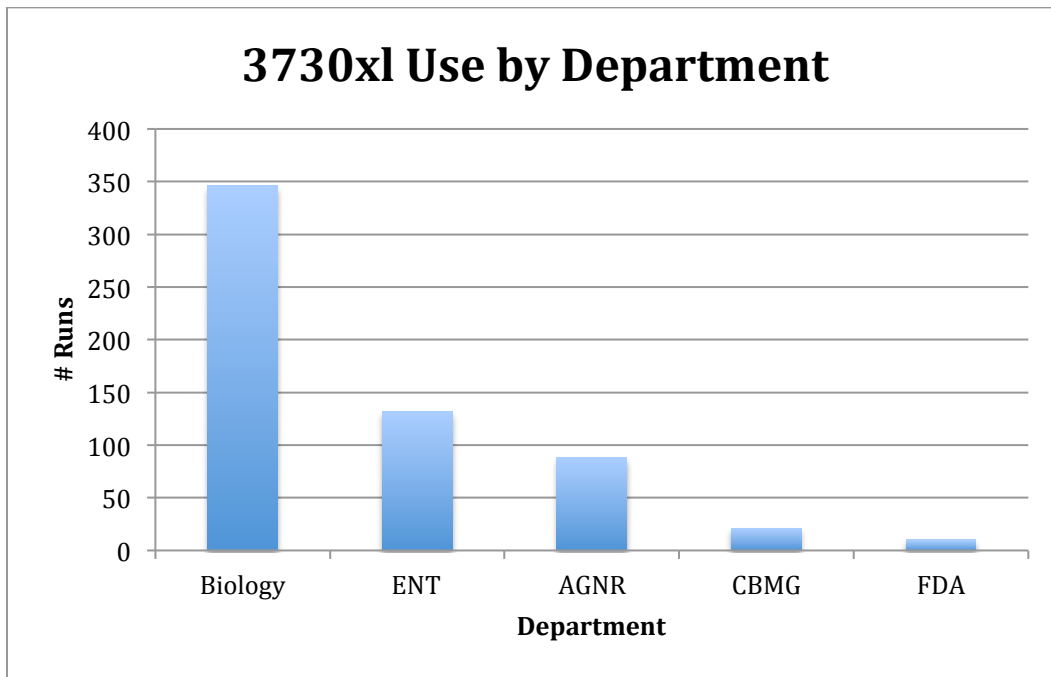
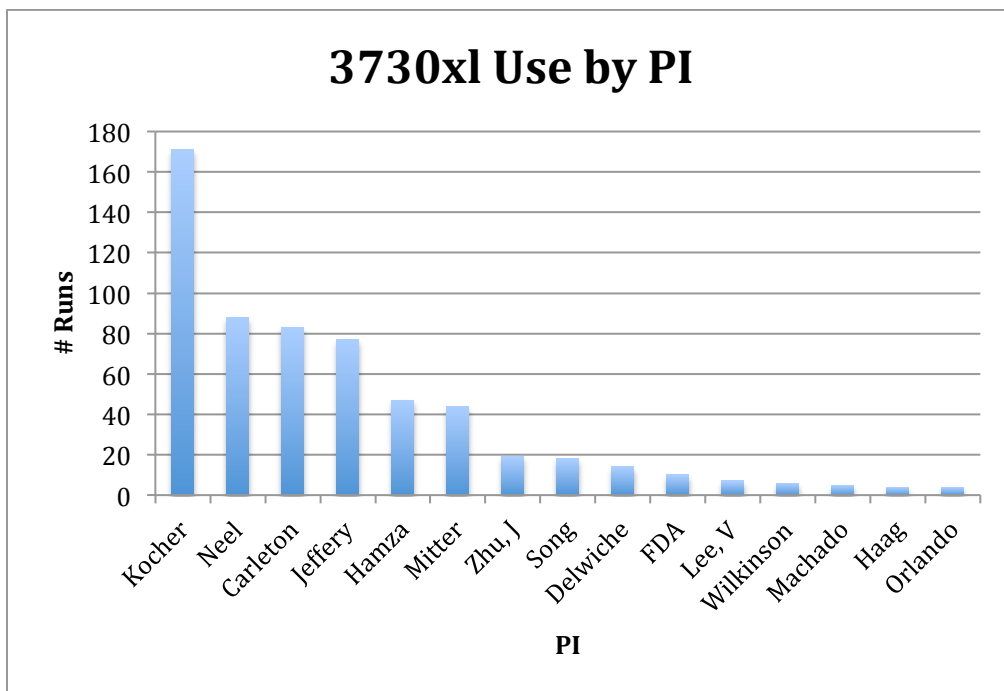


Figure 4: Top DNA Sequencer Users FY2012



Current and Proposed (in parenthesis) Imaging and Genomics Core Rate Schedule

Instrument	Users within CMNS	On-campus users not affiliated with the College	Users not affiliated with the campus
Zeiss LSM710	\$22.00/hr (\$26/hr- peak) (\$23/hr - off-peak)	\$36.50 (\$39.00)	\$73.20/hr (\$78.00/hr)
Leica SP5 X	\$22.00/hr (\$26/hr- peak) (\$23/hr - off-peak)	\$36.50 (\$39.00)	\$73.20/hr (\$78.00/hr)
DeltaVision Deconvolution	\$8.00/hr (\$8.00/hr)	\$11.00/hr (\$11.00/hr)	\$20.00/hr (\$20.00/hr)
Olympus Fluorescence	\$2.00/hr (\$2.00/hr)	\$2.00/hr (\$2.00/hr)	\$2.00/hr (\$2.00/hr)
Axiophot Fluorescence	\$2.00/hr (\$2.00/hr)	\$2.00/hr (\$2.00/hr)	\$2.00/hr (\$2.00/hr)
MiniMed Film Processor	\$0.00 (\$0.00)	\$0.00 (\$0.00)	\$0.00 (\$0.00)
ABI 3730xl South DNA Analyzer	\$38.00/run (\$39.00/run)	\$38.00/run (\$39.00/run)	\$100.00/run (\$100.00/run)
Roche LightCycler 480 Real-Time PCR	\$8.50/hr (\$9.00/hr)	\$14.00/hr (\$14.00/hr)	\$21.00/hr (\$21.00/hr)
Bio-Rad CFX 96 Real-Time PCR	\$8.50/hr (\$9.00/hr)	\$14.00/hr (\$14.00/hr)	\$21.00/hr (\$21.00/hr)