

Core Facilities Annual Report

Amy Beaven

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FY10 Annual Report for Imaging Core Facility

Executive Summary

Over the last two years, the Imaging Core (IC) acquired two brand new state-of-the-art confocal microscopes, whose total purchase price totaled almost \$1 million. The two new microscopes, a Leica SP5 X and a Zeiss LSM 710, saw combined average use of 52.8 hours per week, which is a slight increase over previous years (49 hours per week). Thirty-nine PIs from nine different departments in five Colleges and three different campuses made use of the IC's instrumentation.

Through the end of FY10, at least 122 graduates students, undergraduates, technicians, post-docs and faculty have been fully trained to independently operate the new confocal microscopes, including those who were taught through the 2-credit course, BSCI427/CBMG688W, Principles of Microscopy. Use of the two new confocals has already resulted in six publications, bringing the total number of publications made possible through the use of the IC's microscopes to at least forty-seven.

The IC strives to keep users costs to a minimum. However, an operating cost analysis of the IC using past, current and projected income and expenses, showed the IC would need to increase instrument rates by approximately 18% each year in order to cover the cost of disposables and operating expenses, while still being able to recover the bulk of the cost of service contracts. Total costs in FY11 are estimated to be \$48,455.00, though that amount will increase to \$71,516.00 in the next fiscal year. The Director's salary, which is paid for by the Department of Cell Biology and Molecular Genetics, was not included in the calculations.

While the IC has grown significantly in the last two years, there remains room for further expansion. During the last FY, the IC applied for and received funding from the Dean's to upgrade the IT infrastructure within the facility, including the purchase of an additional computer workstation for image processing and analysis, as well as hardware for increased data storage capabilities. Upon completion of the project, the IC will be prepared to expand its image processing and analysis capabilities further. Acquisition of advanced image analysis software would be desirable.

The IC would greatly benefit from the addition of several new pieces of equipment, including a CO2 incubator for keeping mammalian cell cultures on site, a laminar flow hood for cell culture and a TIRF microscope. The IC's DeltaVision deconvolution microscope is rarely used because it is outdated and obsolete. An extensive upgrade and/or new deconvolution microscope is needed.

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 280 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 confocal microscopes. This course has trained an average of ten students each year for the past ten years.

Funding for operation of the IC comes from a combination of user fees and cost-sharing 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 been subsidized by the college, thereby providing even occasional users with appropriate training and access, and 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 in basic and advanced light microscopy techniques.
- Introducing 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. Ian Mather, Professor (AGNR), Dr. Stephen Wolniak, Professor (CBMG), Dr Wenxia Song, Associate Professor (CBMG)

Personnel

The Director of the Facility, Amy Beaven, is the only full-time staff 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 8 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).

Changes in Instrumentation since November 2005

- August 2006: the Konica Film Processor was replaced with a Mini Med 90 Film Processor (cost: \$3,588.00). 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 from Dr. Ron Weiner's old laboratory. A

CoolSnap EZ monochrome camera, computer workstation and Nikon Elements software (total cost: \$13,400.00) were purchased for the microscope using departmental 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.
- 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 Dr. Ian Mather and Dr. Steve Wolniak via an NSF grant.
- October 2009: the LSM 510 confocal microscope was dismantled to make way for the new LSM 710 confocal microscope. This microscope was purchased using College Funds, authorized by Dean Norma Allewell.

Current Equipment

Zeiss LSM 710 Confocal Microscope

- Location: 0107E Microbiology Building
- Description: Purchased in October 2009. Lasers include 405 diode, Argon (458, 488, 514nm), 561 and 633. 3 PMTs, manual stage.
- Rate for the 2009/2010 Academic Year: \$15.00/hr
- Proposed rate for the 2010/2011 Academic Year: \$18.70/hr

Leica SP5 X Confocal Microscope

- Location: 0107E Microbiology Building
- Description: Purchased in December 2008. Lasers include 405 diode, Argon (458, 488, 514nm), White Light Laser. 5 PMTs, automated stage, high-speed resonance scanner, environmental chamber.
- Rate for the 2009/2010 Academic Year: \$15.75/hr
- Proposed rate for the 2010/2011 Academic Year: \$18.70/hr

DeltaVision Deconvolution Microscope

- Location: 0107E Microbiology Building
- Description: Purchased in December 2008. Lasers include 405 diode, Argon (458, 488, 514nm), White Light Laser. 5 PMTs, automated stage, high-speed resonance scanner, environmental chamber.
- Rate for the 2009/2010 Academic Year: \$8.00/hr
- Proposed rate for the 2010/2011 Academic Year: 8.00/hr

Axiophot Fluorescence Microscope

- Location: 0107K Microbiology Building
- Description: Standard DAPI, GFP and Rhodamine long pass filters, CoolSnap Monochrome camera, computer workstation with Nikon Elements software
- Rate for the 2009/2010 Academic Year: \$2.00/hr

Olympus Fluorescence Microscope

- Location: 0107 Microbiology Building
- Description: Standard GFP and Rhodamine filters.
- Rate for the 2009/2010 Academic Year: \$2.00/hr
- Proposed rate for the 2010/2011 Academic Year: \$2.00/hr

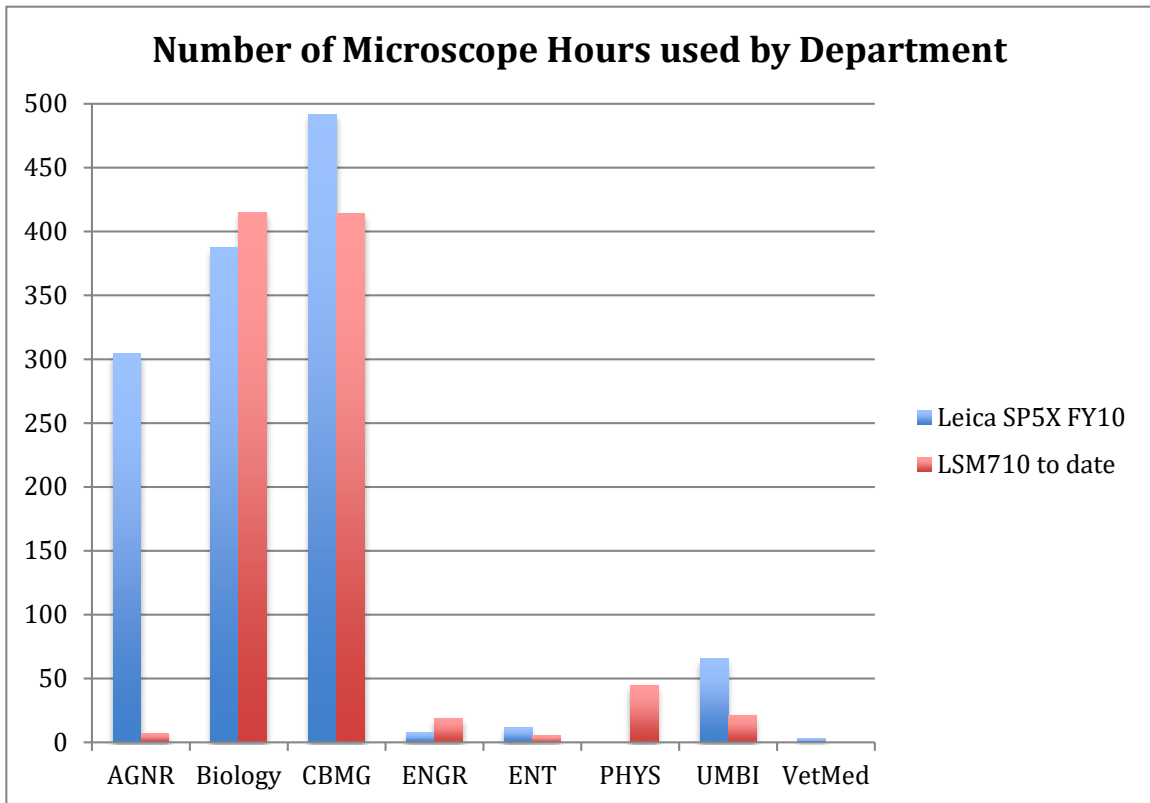
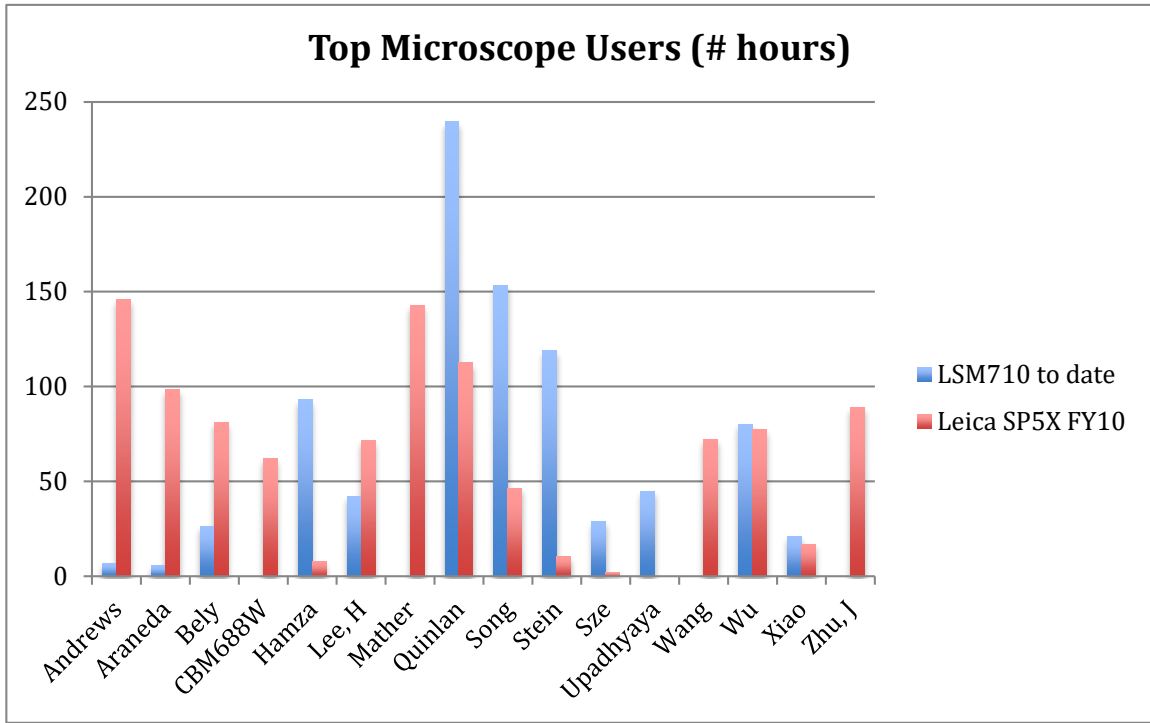
Mini Med 90 Film Processor

- Location: 0107A Microbiology Building
- Description: Standard automatic film processor.
- Rate for the 2009/2010 Academic Year: \$0.00/hr
- Proposed rate for the 2010/2011 Academic Year: \$0.00/hr

Facility Users

Number of microscope hours used by PI:

PI	Department	Leica SP5X FY10	LSM710 to date
Andrews, Norma	CBMG	146.11	6.75
Araneda, Ricardo	Biology	98.34	5.5
Bely, Alex	Biology	80.96	26.25
Bentley, William	ENGR	7.51	0
Briken, Volker	CBMG	38.49	4
Carr, Catherine	Biology	0	8.5
CBMG688W	CBMG	61.93	0
Chang, Caren	CBMG	6.05	6.25
Culver, James	UMBI	22.8	0
DelCampillo, Elena	CBMG	3.25	3.5
Delwiche, Charles	CBMG	8.4	0
El-Sayed, Najib	CBMG	14.25	10.5
Ghodssi, Reza	ENGR	0	18.5
Haag, Eric	Biology	1.92	0
Hamza, Iqbal	Biology	7.7	93
Kahn, Jason	HHMI	3	0
Keefer, Carol	ENT	0	3
Kwak, June	CBMG	13.48	0
Lamp, William	ENT	0	2
Lee, Hey-Kyoung	Biology	71.61	42
Liu, Zhongchi	CBMG	18.41	0
Mather, Ian	AGNR	142.88	0
Mosser, David	CBMG	9.25	2
Muro, Sylvia	UMBI	24.46	0
Payne, Gregory	UMBI	1.11	0
Pick, Leslie	ENT	11.5	0
Quinlan, Elizabeth	Biology	112.46	239.5
Shammam Shihab	Biology	14.25	0
Song, Wenxia	CBMG	46.33	153.25
Stein, Dan	CBMG	10.21	119
Sze, Heven	CBMG	2.21	28.75
Taneyhill, Lisa	AGNR	0	7
Thompson, Matthew	CBMG	21.85	0
Upadhyaya, Arpita	PHYS	0	44.5
Wang, Jian	AGNR	72.18	0
Wolniak, Steve	CBMG	14.06	0
Wu, Louisa	CBMG	77.46	79.93
Xiao, Shunyuan	UMBI	17	20.75
Zhu, Jian	AGNR	89.1	0
Zhu, Xiaoping	VetMed	3	0



Publications

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

1. 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.
2. 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.
3. 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.
4. 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.
5. 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.

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. Synthetic analogs tailor native AI-2 signaling across bacterial species, *Journal of the American Chemical Society (JACS)*2010, 132 (32), pp 11141–11150

Outreach Activities (FY10)

August 28, 2009: CBMG Departmental Retreat. Amy Beaven gave a 10-minute presentation about the Imaging and Genomics Core facilities.

2009 Fall Semester: Amy Beaven trained members of the class CBMG688W, Principles of Microscopy, to use the Axiophot fluorescence microscope and the Leica SP5X confocal microscope.

November 2009: The Imaging Core granted 5 hours of LSM710 microscope time to an undergraduate HHMI student so that he could finish his research project. The undergraduate student, Siva Gaddam, submitted a grant proposal to the Imaging Core which was approved by Amy Beaven and Dr. Charles Delwiche.

January 14-15, 2010: Leica Advanced Imaging Workshop. Two day workshop with seminars and hands-on confocal demonstrations. Seminars were given by Dr. Lee Peachey

(UPenn), Dr. Owen Schwartz (NIH), Geoffrey Daniels (Leica) and Linda Brown (Leica).
Topics Included: Multispectral Fluorescence Imaging, White Light Laser Applications, Optimization of Confoal Scanning Parameters, Multi-Photon Imaging, Laser Microdissection and its Applications Advanced Fluorescence and Analytical Confocal Methods.
Approximately 50 people attended the workshops, including researchers from the USDA, NIH and NIST.

Operating Cost Analysis: Imaging Core Facility

To date:

1. The Zeiss LSM 710 was installed in October 2009 and began operating on November 1, 2009. During the time period from November 1, 2009 to July 31, 2009, income collected from user fees (\$15.00/hr rate) totaled \$14,281.38. Based on average monthly income (\$1,586.00), total income for the instrument over one year is projected to be \$19,039.00. Subtracting expenses to date (\$2,727.75), net income is projected to total \$16,312. The service contract for the microscope is due on October 31, 2010 and costs \$17,730.00. Net income for the instrument over a one year period (minus expenses) is projected to cover all but ~\$1,000 of the service contract.
2. The Leica SP5 X was installed in late December 2008 and began operating on February 16, 2009. During the time period from February 16, 2009 to July 31, 2010, income collected from user fees (\$15.00/hr year 1, \$15.75/hr year 2) totaled \$25,812.11 (a total of \$975.00 in fees were waived for the class CBM688W). Based on the average monthly income during the last full year of operation (\$1,584.00), total income for the instrument is projected to be \$32,148.00 (\$25,812 + \$6,336 projected) for the 2 year period from December 2008 to December 2010. Subtracting expenses (\$5,454.00), net income for the instrument is projected to be \$26,694.00. The service contract for the microscope was paid on December 23, 2009 and cost \$26,000. The next service contract will be due on December 22, 2009 and will cost \$26,000. Over a 2 year period, the Leica SP5 X is expected to lose \$25,306.00.
3. LSM710 usage so far has averaged 102.7 hours per month (25.6 hours per week). Use of the SP5 X during the last full year of operation averaged 109.1 hours per month (27.25 hours per week).

Projected Cost Analysis:

1. Monetary loss to the facility has been alleviated thus far due to the surplus of ~\$25,000 gained from the use of the old Zeiss 510 over the course of ~10 years. Taking this into account, the facility is projected to be in deficit by ~\$1,725.00.00 by the end of the calendar year. User fees will need to be raised in order to pay for future facility expenses (estimated at \$3,000/year) and the cost of both service contracts (\$43,730.00/year).

2. The rates will need to be raised even further in the following year when we'll need to start paying for the white light laser contract cost (\$24,786) on the Leica SP5 X. Total service contract costs for both instruments will increase to \$68,516.00. Adding in yearly expenses of \$3,000 per year, the facility will need to recoup a total of \$71,516 per year. Over a 5 year period, the facility will need to recoup a total \$222,019 in user fees and service contracts costs. See the following table for details:

Year	LSM 710 contract cost	SP5 X contract cost	Projected expenses	Current projected deficit	Subsidies	Total
2010-2011	\$17,730	\$26,000	\$3,000	\$1,725.00	(\$37,500)	\$10,955
2011-2012	\$17,730	\$50,786	\$3,000	\$0	(\$37,500)	\$34,016
2012-2013	\$17,730	\$50,786	\$3,000	\$0	(\$37,500)	\$34,016
2013-2014	\$17,730	\$50,786	\$3,000	\$0	\$0	\$71,516
2014-2015	\$17,730	\$50,786	\$3,000	\$0	\$0	\$71,516
Total	\$88,650	\$229,144	\$15,000	\$1,725.00	(\$112,500)	\$222,019

3. Current in-college usage rates are: \$15.00/hr for the LSM 710 and \$15.75/hr for the Leica SP5X. By increasing rates incrementally over the next five years, the facility should be able to cover all projected expenses. Based on total expenses of \$334,519 over 5 years and an average microscope usage rate of 106 hours per month, the hourly rate would need to be increased a minimum of 18% per year.

Proposed Rate Schedule:

Academic Year	Users w/in CLFS & AGNR (excluding VetMed)	On-campus users not affiliated with CLFS	Users Not affiliated with campus
2010-2011	\$18.60/hr	\$31.00/hr	\$62.00/hr
2011-2012	\$22.00/hr	\$36.50/hr	\$73.00/hr
2012-2013	\$26.00/hr	\$43.00/hr	\$86.25/hr
2013-2014	\$30.50/hr	\$51.00/hr	\$102.00/hr
2014-2015	\$36.00/hr	\$60.00/hr	\$120.00/hr

Projected Income per year based on average usage

Year	Rate	Estimated Income for both microscopes
2010-2011	\$18.60/hr	\$47,280.00
2011-2012	\$22.00/hr	\$55,790.00
2012-2013	\$26.00/hr	\$65,833.00
2013-2014	\$30.50/hr	\$77,683.00
2014-2015	\$36.00/hr	\$91,656.00
Total		\$338,252.00

4. Alternatively, we could institute a different rate schedule for each microscope. Because the SP5 X service contracts are much higher, the rates for this microscope would have to be significantly higher than those of the LSM 710. I am hesitant to do this because it could result in less use of the SP5 X, which is a valuable asset to the facility. Service contract costs are higher for the SP5 X because this microscope has additional features not offered on the LSM710, including the white light laser, automated scanning stage, high-speed scanner and environmental chamber.
5. Please see the following table for information on rates at other University facilities.

Example Confocal rates

Facility	Instrument	Hourly Rate	Additional Information
UMD School of Medicine	LSM 510 Meta	\$40.00	Training is \$200 per person
Iowa State University Confocal Microscopy Facility	Leica SP5 X Nikon CS1	\$30.00 \$25.00	
Ohio State University Microscopy and Imaging Facility	Olympus FV1000	\$25.00	
Northwestern University Cell Imaging Center	Zeiss LSM 510 Meta	\$44.00	Training + \$27/hr
UMBC Imaging Facility	Leica SP1	\$20.00	
Duke University Light Microscopy Core Facility	LSM 510, Leica SP5	\$25 .00	Heavily subsidized by Duke Office of the Provost, School of Medicine, Cancer Center,

			NIH
Stanford University Medical Center, Cell Science Imaging Facility	Leica SP5 2 photon	\$54.00	\$280 set up fee for training, setting up computer accounts etc.
Stanford University Medical Center, Cell Science Imaging Facility	Leica SP2 confocal	\$54.00	\$280 set up fee for training, setting up computer accounts etc.
New York University School of Medicine	LSM 510, LSM 710 MP, Leica SP5	\$35 .00	Training is \$50/hr
Yale School of Medicine Stem Cell Center	Leica SP5	\$40.00	
Harvard Medical School Imaging Center	Nikon A1	\$50.00	\$200/person plus hourly rate
Cornell U Life Sciences Imaging Core	Zeiss LSM 710	\$30.00	Training Fee: \$110 per user
Oregon State U	Zeiss LSM 510	\$15.00	Training: \$120 per person
Rockefeller U	Zeiss LSM 510	\$40.00	
IUPIU	Ziess LSM 510	\$55.00	Training: \$110/hr

Genomics Core Facility

1. I propose to keep the 3730xl rates the same and increase the LC480 and BioRad CFX96 by ~5%.

Instrument	Income	Expenses	Net	Service contract	Net minus Service Contract
3730xl	\$26,330.00	\$5,882.30	\$20,447.70	\$19,882.00	\$565.70
LC480	\$6,986.00	\$1,279.25	\$5,706.75	\$5,500.00	\$206.75
CFX96	\$3,014.00	\$0.00	\$3,014.00	Under warranty until 1/22/2012	\$3,014.00

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

Instrument	Users within College of Life Sciences	On-campus users not affiliated with the College	Users not affiliated with the campus
Zeiss LSM710	\$15.00/hr (\$18.60/hr)	\$25.00/hr (\$31.00/hr)	\$50.00/hr (\$6200/hr)
Leica SP5 X	\$15.75/hr (\$18.60/hr)	\$26.25/hr (\$31.00/hr)	\$52.50/hr (\$6200/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 (\$38.00/run)	\$38.00/run (\$38.00/run)	\$100.00/run (\$100.00/run)
Roche LightCycler 480 Real-Time PCR	\$8.00/hr (\$8.50/hr)	\$13.00/hr (\$14.00/hr)	\$20.00/hr (\$21.00/hr)
Bio-Rad CFX 96 Real-Time PCR	\$8.00/hr (\$8.50/hr)	\$13.00/hr (\$14.00/hr)	\$20.00/hr (\$21.00/hr)