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LBRN projects – proposed Summer 2020



VISION and MISSION of the SPM Laboratory: To become an internationally recognized scanning photon and probe imaging laboratory. Our mission is to develop state-of the-art high spatial and temporal resolution imaging modalities and image analysis techniques. Part of our mission is to become a resource for collaborators in the state of Louisiana in order to provide high resolution and novel imaging methods and analysis techniques.

BACKGROUND: Optical microscopy and scanning probe microscopy (SPM) are among the most widely utilized techniques in scientific and engineering studies. We are developing our laboratory at Southeastern Louisiana University (SLU), the Southeastern Photon/Probe Microscopy (SPM) lab, to become an international recognized laboratory for the development of state-of-the-art imaging techniques and image analysis methods.

The SPM lab will consist of two components: (1) an image acquisition and instrumentation facility comprising laser scanning confocal microscopy and scanning probe microscopy, or atomic force microscope (AFM), and (2) an image analysis component. The imaging instrumentation will be home-built by students and capable of single molecule and single photon fluorescence sensitivity, nanometer localization, and dynamic fluorescence measurements with picosecond temporal resolution. LabView will be the interface for instrument control.

In terms of specific applications, we will address nanoscale scientific problems of significance including electronic dynamics of nanoparticles and the physics of plasmons and plasmon-enhanced photon emission from nanoparticles. We will also be a resource for fluorescence imaging for biologist and chemists. Our instrumentation will have high applicability to such fields as molecular imaging and cellular imaging.

Figure 1 shows a schematic diagram of the optical portion of the lab facility that will be developed in the fall of 2020. This is not part of this summers' project but it is provided to give context for the proposed projects this summer.

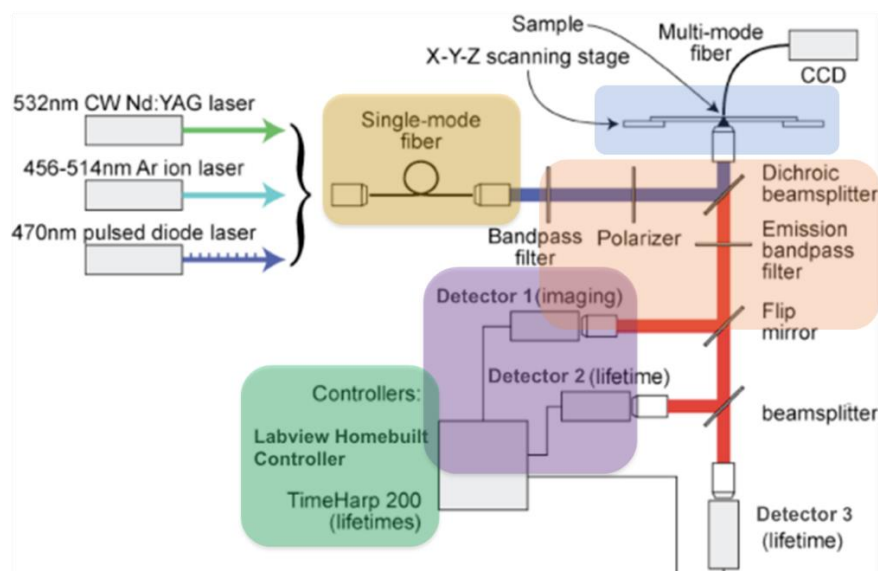


Figure 1. Schematic diagram of the laser scanning confocal microscopy instrumentation.

We have focused our previous nanoscale optical research on studying the electronic properties of quantum dots, myosin V motor protein dynamics, and the study of intracellular viscosity using fluorescence anisotropy.

Quantum dots offer advantages over conventional organic bioimaging markers in the following aspects:

- Narrower emission band providing multiple images to be acquired simultaneously with multiple labels.
- Increased photostability (absence or prolonging of photobleaching).
- Broader excitation spectrum allowing for single source excitation.
- Single molecule sensitivity via increased quantum efficiency (yield).
- Single and two photon emission control allowing for the application of well known non-linear imaging mechanisms and lifetime imaging methods.

Applications include drug delivery, theranostic agents, single molecule sensors of local biological environments/activities, and real-time *in vivo* deep tissue imaging agents. See, for example, the following references of biological applications of quantum dots:

- Nanodots Featuring Efficient FRET for Real-Time Monitoring of Drug Delivery and Two-Photon Imaging, *Adv. Mater.* 25(45) (2013) 6569-6574.
- H. Tada, H. Higuchi, T.M. Wanatabe, N. Ohuchi, In vivo real-time tracking of single quantum dots conjugated with monoclonal anti-HER2 antibody in tumors of mice, *Cancer Res.* 67(3) (2007) 1138-1144.

- Q.L. de Chermont, C. Chaneac, J. Seguin, F. Pelle, S. Maitrejean, J.P. Jolivet, D. Gourier, M. Bessodes, D. Scherman, Nanoprobes with near-infrared persistent luminescence for in vivo imaging, Proc. Natl. Acad. Sci. U.S.A. 104(22) (2007) 9266-9271.

Below are the specific GOALS AND DELIVERABLES for this summer's work (Summer 2020). Project #1 will be projects worked on by Southeastern-sponsored students. Project #2 and project #3 will be LBRN-sponsored student projects.

1. LabView control interface (work supported by Southeastern)

Figure 2 shows a version of the control interface that we (PJ Moyer lab) developed at UNC Charlotte. Two Southeastern undergraduate physics majors have begun work to develop this interface this summer. The interface provides the following features:

- x and y analog output voltages to control the piezoelectric scanner
- tip control and feedback loop for scanned probe microscopy tip control
- multiple simultaneous image acquisition channels (multi-colored fluorescence imaging), sample topography, etc.
- zoom capabilities
- stop and park over a specific point for spectroscopic analysis

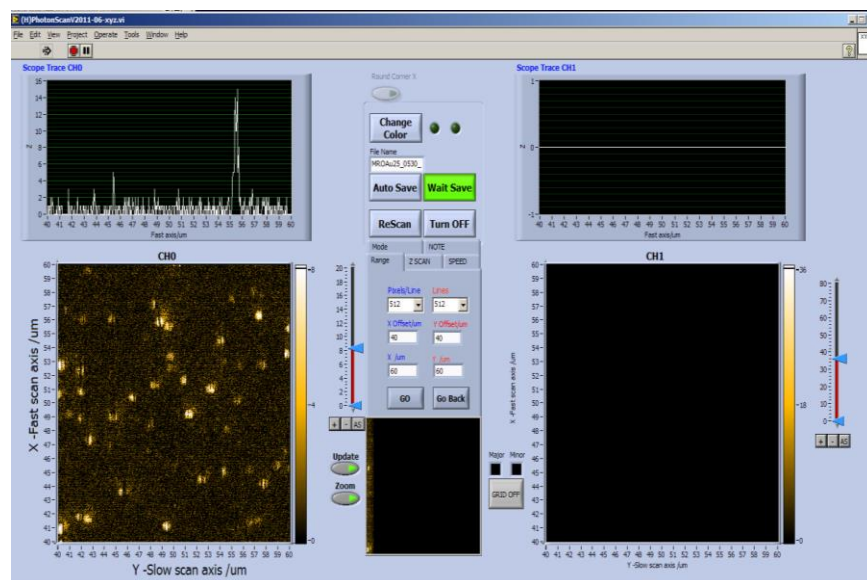


Figure 2. Interface that will be developed by Southeastern students.

DELIVERABLE – A functional interface and data acquisition program for an optical and scanning probe microscope. The interface would include feedback control for the AFM.

2. A stand-alone image deconvolution program (work supported by LBRN)

All imaging methods have a ‘blurring’ mechanism or some fundamental technical limitation to how finely the detail of a sample can be imaged. Optically, this is determined by diffraction effects which are limited by the wavelength of light being used. For SPM, the limitation is the physical size of the tip. Either way, the imaging mechanism has what is known as a point spread function (psf). The final image is a convolution of the sample itself and the point spread function. This deliverable will take the final image and deconvolve that image with the point spread function to ‘recover’ information that may have been lost in the imaging process. This program can be used on any imaging data that an external researcher would send us provided we could estimate the point spread function of their imaging method.

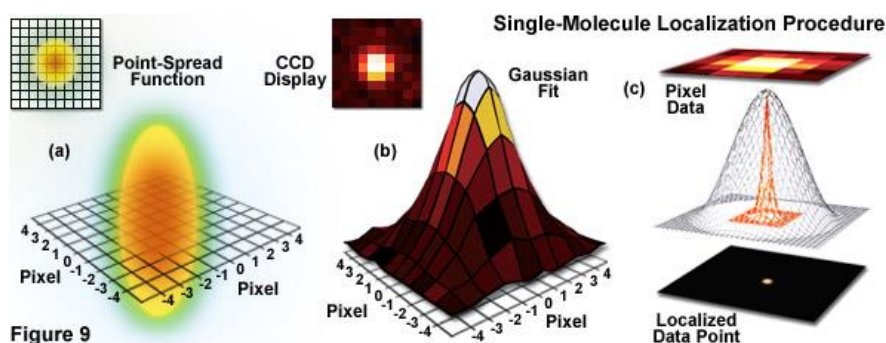


Figure 3. Depiction of the concept of a point spread function (reference <http://zeiss-campus.magnet.fsu.edu/articles/superresolution/introduction.html>)

Figure 4 shows an example of a deconvolution program we wrote and applied in our lab at UNC Charlotte. In this experiment, we used a Bessell function as the PSF. In this project, we will use a Gaussian function. The Gaussian is determined by the image of single molecule or a single quantum dot.

The molecules shown in figure 4 are localized to a position of less than 10 nm using this method. This feature has applicability to future projects via the localization of single biomolecules and tracking them on the nm scale. For example, dynamic processing myosin motor protein molecules along an actin filament can be studied under various physiological conditions. Figure 5 shows an example of previous data we acquired by myosin motor proteins (labeled with green fluorescent markers) on actin filaments (red fluorescence image). This provides an example of how this instrument and deconvolution program can study biological processes on the nanometer scale.

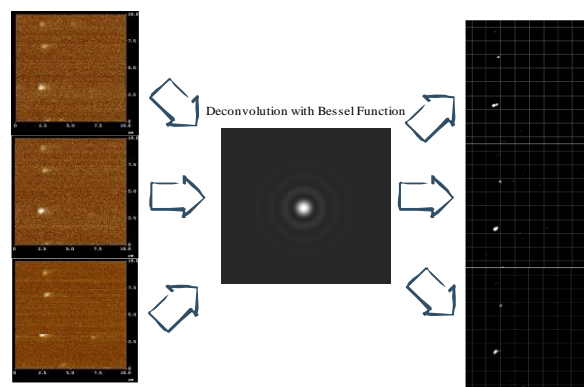


Figure 4. Demonstration of single molecule imaging and deconvolution to yield better than 4 nm localization. Images on left are raw data. Images on right result from deconvolution of raw image with a Bessell function (shown in center) to yield localization of molecules with 4 nm accuracy.

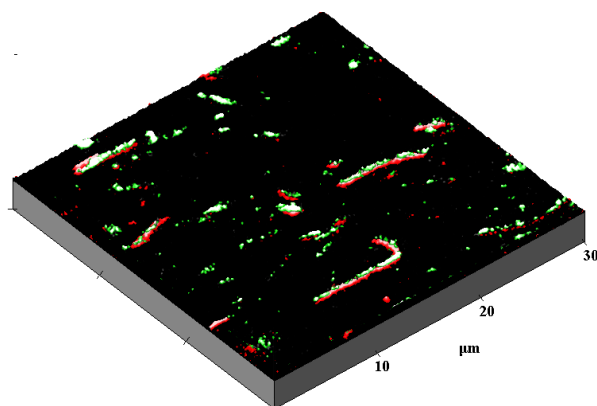


Figure 5. Myosin V motor protein imaging (green) overlapped with actin filaments (red).

DELIVERABLE – Stand-alone image deconvolution program for any image and the corresponding point spread function (PSF) for that particular imaging system.

3. Two photon imaging using fluorescence lifetime imaging microscopy (FLIM) of single quantum dots

Two photon imaging of conventional organic fluorescent molecules is a technique used commonly in bio-imaging to effectively narrow the point spread function via the nonlinear nature of the interaction of the excitation laser with the sample. The PSF is narrowed because it is the result of the square of the linear PSF. Multiplying a Gaussian function by itself significantly narrows the instrument PSF and improves the spatial resolution.

This particular project is a novel method proposed by PJM by utilizing the two-photon excitation/emission of quantum dots and the familiar FLIM method. This method has the potential to add high spatial and temporal resolution to bioimaging with the benefits of quantum dots over that of organic fluorescent molecules.

This work is based on work that was previously published by the group of PJM. It takes advantage of two-photon emission from quantum dots in the presence of plasmonic nanostructures. The work does not require plasmon coupling but it makes it much more efficient.

Figure 6 demonstrates two photon excitation/emission of single quantum dots {This work has been published in *NanoLetters*, Sharonda Johnson LeBlanc, Mason McClanahan, Marcus Jones, and Patrick J. Moyer, “Enhancement of multiphoton emission from single CdSe quantum dots coupled to gold films,” *NanoLetters*, **13**, 1662-1669 (2013)}. Figure 6 demonstrates the significant multiphoton emission using the Hanbury-Brown Twiss (HBT) geometry utilized in photon correlation spectroscopy.

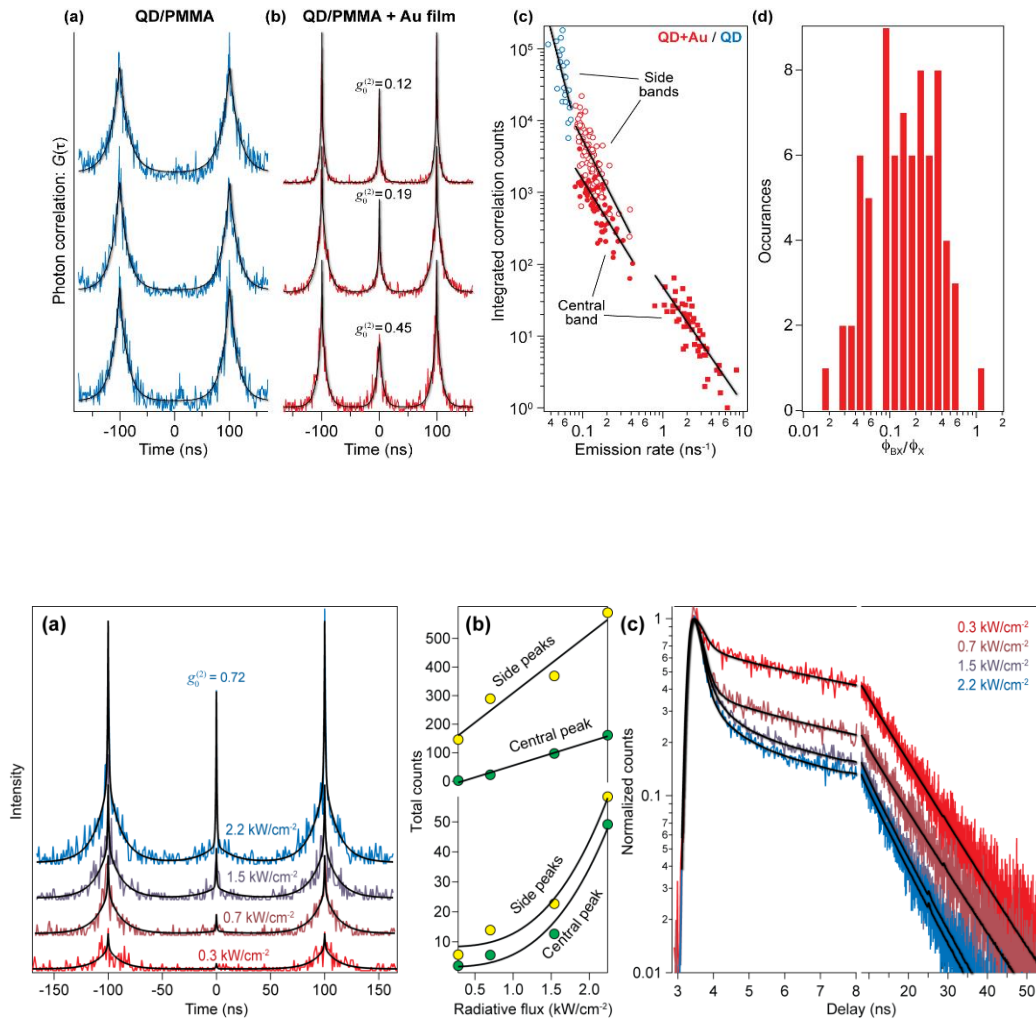


Figure 6. Two photon emission experiments as published in *NanoLetters* by the PI (Moyer). This is one calibration that will be performed during the set up stage for confirming proper instrument performance.

Figure 6 demonstrates (1) multiphoton emission of a single quantum dot (note the central peak of the photon correlation spectra), (2) the fact that we can separate out fast vs. slow lifetime components, and (3) the nonlinear nature of the fast component.

These characteristics provide an excellent opportunity for a novel high spatial and temporal bioimaging mechanism using single quantum dots.

This project will involve the simulation of the type of data presented in figure 6 as a function of xy image position, and a comparison of two images: (1) the spatial mapping of the linear single photon slow fluorescence component and (2) the spatial mapping of the nonlinear two-photon nonlinear fast fluorescence component.

We will also work on a patent study and consider the submission of a provisional patent based on this work.

DELIVERABLE – A full study and comparison of one-photon vs. two-photon imaging of single quantum dots, including applicability to biological systems. Also, we will provide a feasibility study for a patent using this technique.