Quantitative Non-Linear Optical Imaging in the Nano- Regime

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ABSTRACT

The development of functional solid state non-linear optical (NLO) systems for device applications is critical to several fields. Optical computing, laser hardening, 3-dimensional data storage and remote sensing are just a few of the areas advanced by the characterization of new NLO systems. One promising venue for the development of these technologies is the nano-/meso-scale self assembly of viable chromophores into tunable aggregates. Here we present a method by which individual aggregates can be quantitatively imaged by two photon fluorescence near field scanning optical microscopy (NSOM).

Keyword list: two-photon, near-field, TPEF, TPA, NSOM, rhodamine B

1. BACKGROUND

NSOM imaging using a single mode fiber optic cable allows for the use of a femptosecond pulsed excitation source, provided that the probe is not metalized[1]. The transition to near-field imaging is needed when attempting to optically resolve features below the Bragg diffraction limit. The resolution for near field optical imaging is related to several factors, including the probe coating, atmospheric conditions, illumination source proximity and the aperture size of the illuminating source. This resolution for a given probe can vary from the aperture size (D) to as small as D/4 and is applicable when the aperture proximity to a feature is within the Fraunhofer Distance [Equation 1].

$$d = 2D^2 / \lambda \qquad [I.]$$

Equation 1. The Fraunhofer Distance.
(d as distance between feature and aperture, D as aperture diameter, λ as wavelength of the illumination)

Two photon excited state fluorescence (TPEF) is a potential NSOM imaging mode. In this mode, illumination is provided from a pulsed laser system at a wavelength that is within the two photon absorption (TPA) spectra of the sample. The illumination is delivered to the sample from the probe and collected in the far field from the sample, along with the TPEF of the sample. The illumination is removed from the TPEF signal and a near-field fluorescence image is generated. This image will have enhanced resolution over a similar luminescence from a one photon process. This is due to the quadratic relationship the third order TPA process has to the incident flux and the uneven intensity distribution at the aperture. Typical TPEF measurements are taken using a standard reference material [Equation 2][2].

$$\delta = \delta_0 \bullet (PL / PL_0) \bullet ([C] / [C_0]) \bullet (\eta / \eta_0) \qquad [II.]$$

Equation 2. TPA Cross-Section Determination from a TPEF Standard

The use of TPEF NSOM to obtain TPA images of organic nanorods has been shown elsewhere[1]. This imaging technique allows comparison of individual nanoscale aggregates in order to correlate TPEF response to both morphology and size of the sample. Comparing the volume of a given feature, as determined by the AFM image, provides relative TPEF response within a given image frame.

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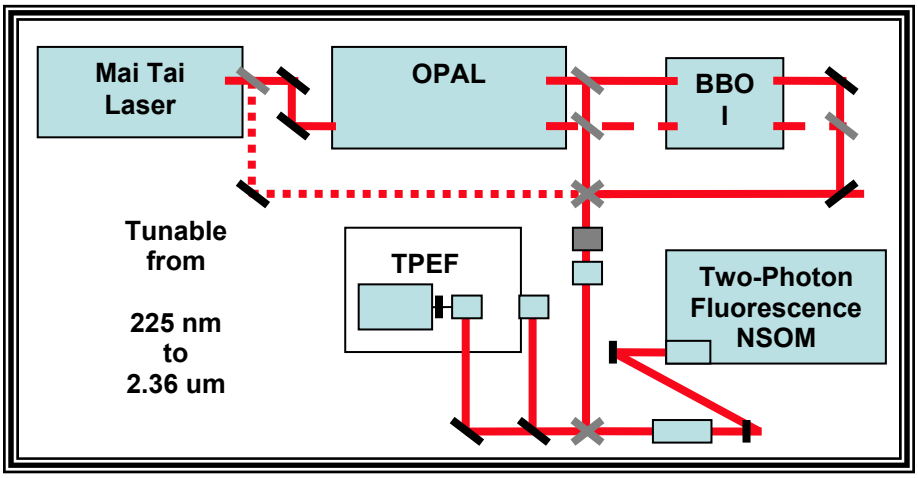
2. EXPERIMENTAL

The TPA cross sections and fluorescence were obtained by the TPEF method[2-4]. The 840 nm excitation source was obtained from a Spectra-Physics Mai Tai unit. The 1100 nm excitation source was obtained from the Mai Tai unit coupled into a Spectra-Physics OPAL temperature tunable optical parametric amplifier. The intensity was monitored by a Thor Labs DET 100A photo-voltmeter. Fluorescence was collected using a Hamamatsu R7518P PMT. AFM and TPEF NSOM images were obtained from a CDP-MoScan Near-Field Multiscope [Scheme 1]. Probes were made using S-FS single mode optical fiber from Newport Corp. Rhodamine B dye was obtained from Ciba Chemicals. Rhodamine B impregnated polystyrene spheres were obtained from Melorium Inc. and have a 0.5% wt loading. Slide deposition is done with and aspirator on freshly hewn mica slides.

Scheme 1. Experimental Set-Up for Bulk TPEF and TPEF NSOM Measurements



3. RESULTS AND DISCUSSION

There is a need to develop a method of TPEF NSOM imaging that allows for quantitative imaging. Current imaging techniques allow a relative comparison between features on a single sample slide. However, this method does not readily allow for comparison when samples, experimental conditions, or probe type differ. In order to perform quantitative TPEF measurements by NSOM, there are two avenues that can be taken. The first avenue is a determination of the true plane wave/pulse shape, flux profile, probe aperture shape, and the G-factor at the sample. A second, more functionally elegant method is to have a known standard in the field of view for the wavelength at which imaging will occur.

As there are no standards for quantitative TPEF NSOM, a standard need first be developed. There are several properties that should be targeted while attempting to develop a standard for quantitative TPEF NSOM.

a. Well characterized TPA spectra at the wavelengths of interest.
b. Encapsulation of the signaling species to give environmental robustness.
c. Viable for bulk TPEF or TPA z-scan measurements in suspension.
d. Only trivial spectral changes on transition to solid-state.
e. A commercially available product with a known formulation.
f. A variety of available sizes, application selectable for specific feature sizes.

An appropriate dye at the wavelengths of 840 nm and 1100 nm is rhodamine B. It has been used extensively in other TPA standard studies[2,5], is readily commercially available and has a readily observable TPA cross section over a broad (300+ nm) range of the Vis-NIR spectrum. Rhodamine B also is used in two photon laser scanning confocal microscopy to good effect, though not with the resolution possible by NSOM. A dye impregnated polystyrene sphere was chosen

over a dye surface labeled system to give uniform signal across the depth of the feature and to ensure that the dye would not significantly change its behavior on transition from suspension to solid state . Dye loading of 0.5% was chosen to maintain a penetration depth in excess of 1 um. Results for the TPA cross section are given in Table 1, as well as the results from two other TPA standard studies[2,5].

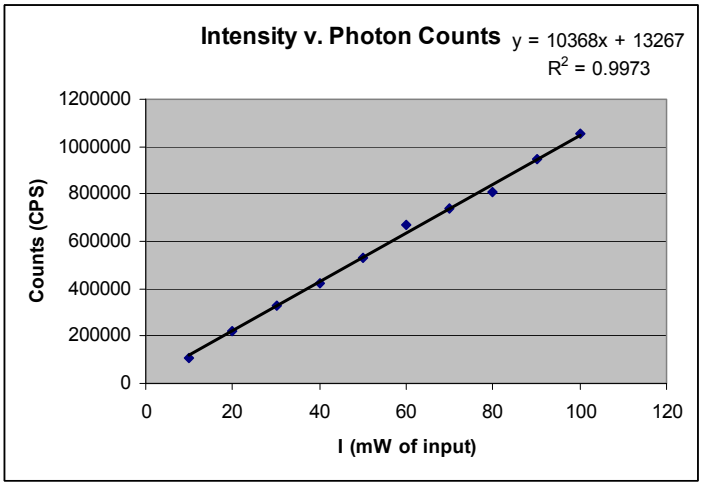
Table 1. TPEF Measured Cross Sections and Standards for Rhodamine B Systems.

Wavelength	Rh B Sphere per 600 nm sphere	Rh B Sphere per molecule	Rh B Doped PMMA Film	Rh B in Methanol	Rh B in Methanol (Webb et. al.[2])	Rh B in Methanol (Rebane et. al.[5])
840 nm	6.6 x 10^8 GM	160 GM	173 GM	169 GM	165 GM	180 GM
1100 nm	8.6 x 10^6 GM	21 GM	23 GM	28 GM	26 GM	24 GM

These measurements confirm the findings in other TPEF standard studies and provide confidence that the polymer impregnated species varies little from the solution phase dye. Determining the full sphere cross section provides a value to attribute to the total integrated value of the TPEF image of a feature.

The TPEF NSOM imaging was done intentionally without monitoring the absolute throughput of the incident beam from the probe, which is both time consuming and system-destabilizing. Instead, by locating the probe over a region without a TPEF responsive feature, it can be shown that incident intensity (mW) correlates directly with counts when the incident beam is not fully filtered. [Figure 1.]

Figure 1. Confirmation of Linear Relationship Between Input Intensity and PMT Response



Once the linearity of the probe throughput was confirmed, TPEF NSOM images were obtained using input intensities over the range measured. Figure 2 provides a comparison of a TPEF image to the simultaneously obtained AFM image, while Figure 3 presents the intensity study images performed at 840 nm. All fields of view are 5 um x 5 um at a resolution of 20 nm.

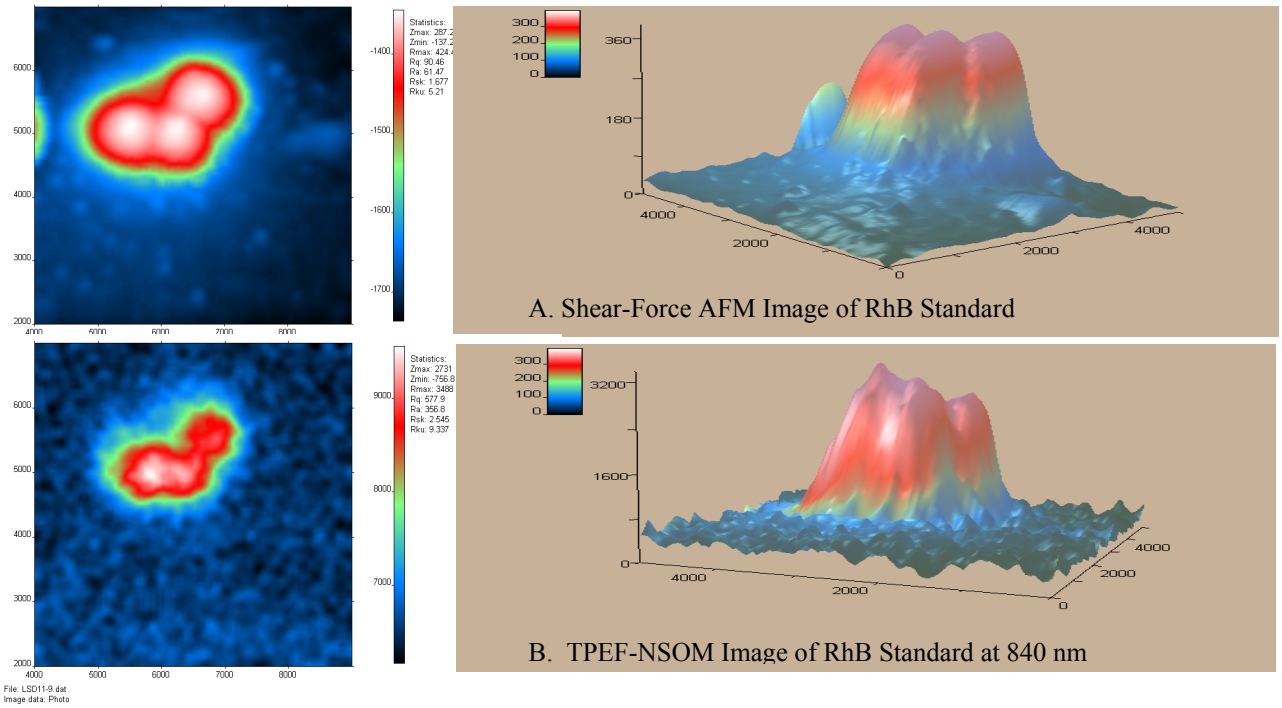


Figure 2. Representative Images of RhB Standard (2d and 3d Renderings)

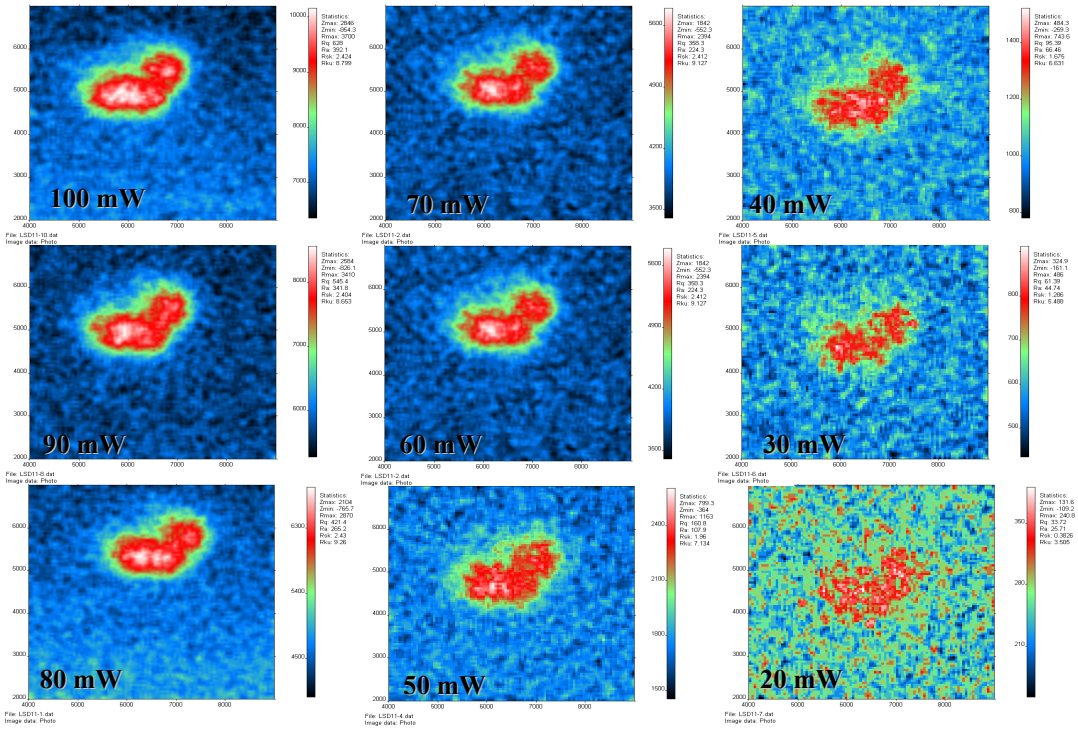


Figure 3. TPEF NSOM Images of RhB Standard Spheres with Intensity Change

In order to confirm the intensity-squared dependence of the features observed, it is necessary to remove the background counts of the non-feature region from the entire image and then integrate the counts in both x and y. The log-log plot of input power (mW) and counts (CPS), conventionally used to confirm TPA response, gives a slope very close to two [Figure 4] which confirms the viability of this method for quantitative imaging..

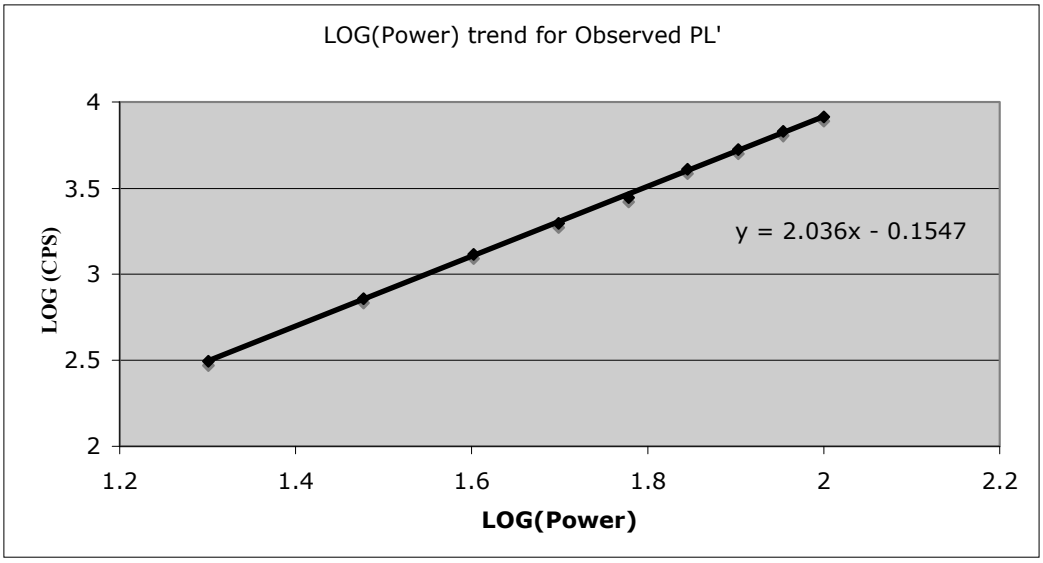


Figure 4. LOG (mW) v. LOG (CPS) for TPEF NSOM RhB Spheres Study

Additionally, a height dependence study was initiated. A gradual progression from full contact mode to a non-contact mode height of ~1.5 um in ~100 nm steps is presented in Figure 5. Though not calculated implicitly, whole feature CPS integration and broadening in the TPEF-NSOM image feature should give effective point source height and focal length. This may be useful for better defining a near field system that normally obfuscates these typical far field descriptions. Lack of peak intensity degradation during probe height changes provides an empirical display of effective penetration depth. Differing non-linearity in the feature signal and background signal allows for accurate image correction to account for intrinsic systemic NLO response within the field of view, should one be observed.

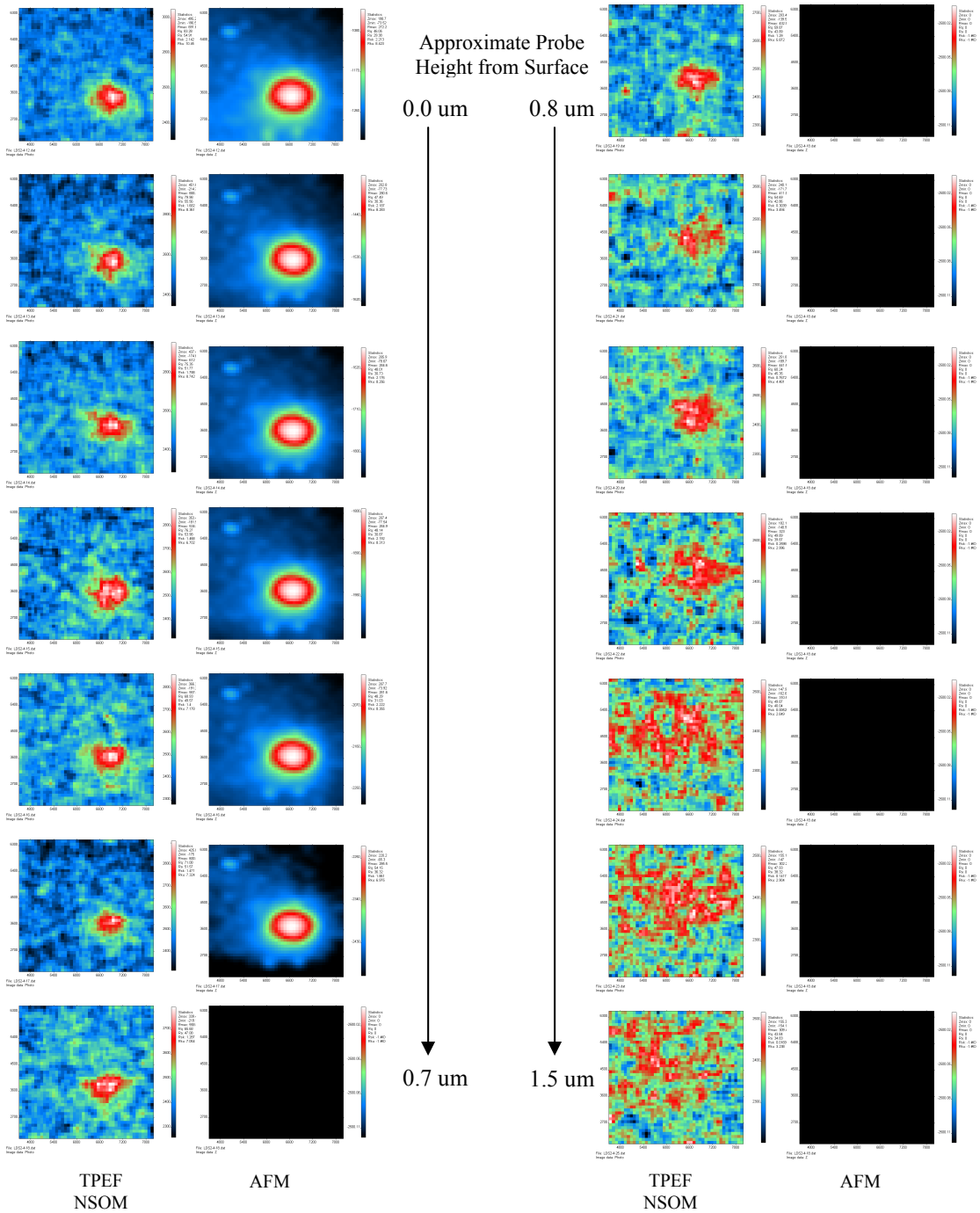


Figure 5. Height Study of RhB Spheres by TPEF NSOM with 820 nm Excitation
(Field of view is 3.5 um x 3.5 um)

4. CONCLUSIONS

Despite the many complications that arise from attempting an absolute TPA cross section measurement via NSOM, a referential method has been displayed here. A reference material is characterized for two NIR wavelengths and is confirmed to provide a quantitative NLO response. The ease of calculation and relatively fast processing time of this method should provide a fast and elegant method by which to compare nanoscale aggregates. The robustness of the system provides the possibility of imaging second harmonic generation and non-linear Ramen response in nanoscale materials.

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