

Fluorescence News

Enhanced and Localized Multiphoton Excited Fluorescence Near Metallic Silver Islands: Metallic Islands Can Increase Probe Photostability

KEY WORDS: Radiative decay engineering; metal-enhanced fluorescence, localized excitation; enhanced fluorescence; enhanced photostability.

Recently we reported that close-proximity metallic silver islands or colloids can alter the radiative decay rate, Γ_m , and/or excitation rate, E_m , of fluorophores [1–5]. We have shown that the quantum yield of low quantum yield fluorophores can be increased, with a maximum predicted increase of $1/Q_0$, (Q_0 -quantum yield in absence of metal), whereas significant increases in emission intensity from high quantum yield species, in the absence of any non-radiative rate modifications, can only be observed by substantial increases in E_m . Complimentary to our previous results and interpretations, we can now report that *enhanced* and *localized* multiphoton excitation of rhodamine B (RhB) fluorescence occurs near metallic silver islands.

The increase in fluorescence emission intensity for RhB molecules adjacent to metallic silver islands (Fig. 1) is accompanied by a reduction in lifetime, compared to that observed using 1-photon excitation. Given the high quantum yield of RhB ($Q_0 = 0.48$), these results can be explained by the metallic particles significantly increasing the E_m of the RhB molecules. Moreover, given the sample geometry (Fig. 2) and the absence of any notable increase in emission intensity using 1-photon excitation, as well the fact that the 1-photon mean lifetime remained essentially unchanged both in the presence and absence of silver, suggests that enhanced 2-photon excitation is *localized* to regions in close proximity to the silver islands.

It is informative to discuss the nature of this enhanced and localized excitation. In addition to metallic particles and/or colloids modifying a fluorophore's radiative decay rate, they are also known to increase excitation rates by concentrating the incident light [6,7]. The maxi-

imum enhancement in the incident electric field has been calculated to be a factor of 140 near appropriately sized metallic ellipsoids [8]. Because the incident intensity is the square of the incident field strength for a 1-photon process, enhancements in excitation rates by a factor of up to 2×10^4 are possible. It is this phenomenon that one can typically attribute to increases in observed apparent quantum yields near metallic particles to greater than unity. However, a much more dramatic enhancement is possible for multiphoton excitation. For a 2-photon absorption process the rate of excitation is proportional to the square of the incident intensity. This suggests that 2-photon excitation could be enhanced by a factor of 3.8×10^8 . Such an enhancement in the excitation rate is thought to provide selective excitation of fluorophores *near* to metal islands or colloids, even if the solution contains a considerable concentration of other fluorophores that could undergo 2-photon excitation at the same wavelength, but are more distant from the metals surface (Fig. 3). This interpretation is borne out by the fact that given the overwhelming excess of high quantum yield RhB in this sample geometry ($\approx 96\%$ of solution is too distant for *any* fluorophore-metal effect), the fluorescence lifetime is still shorter than that typically observed for bulk solution RhB in the absence of metal (Fig. 4).

In our opinion, enhanced and localized multiphoton excitation by metal particles may have numerous applications in the biochemical and biological applications of fluorescence. For example:

- *More Generally:* The preferential enhanced excitation of fluorophores in close proximity to metallic islands or colloids even in the presence of high concentrations of other emitting species (see Fig.

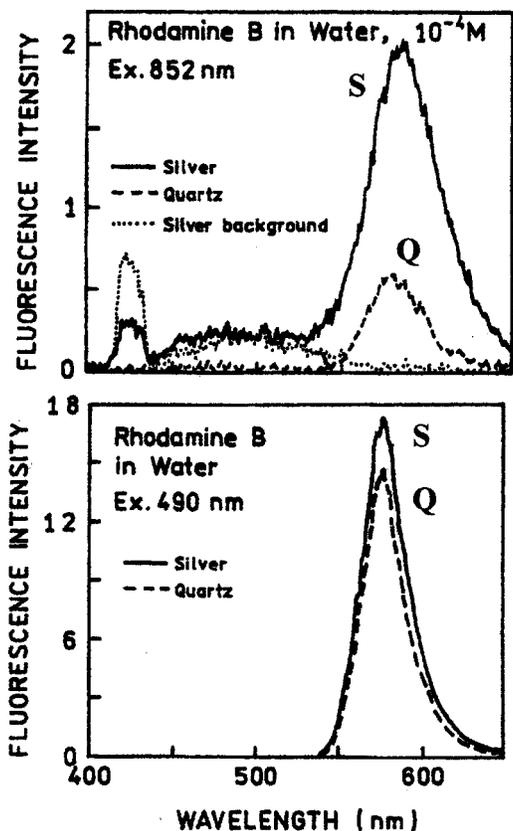


Fig. 1. (Top) Emission spectra of 10^{-4} M RhB between silver island films (S) with 2-photon excitation at 852 nm (—) from a Tsunami mode-locked Ti:sapphire laser, 80 MHz repetition rate, 90 fs pulse and about 0.5 W average power. Also shown are the emission spectra observed from uncoated quartz slides (Q), and silver islands alone without RhB. (Bottom) RhB between silver islands, S(—), or quartz plates, Q(…), with 1-photon excitation at 490 nm.

3). Even further enhancements may be possible by using low Q_0 species.

- *Specifically*: By directing metallic colloids to a cell membrane by the presence of a covalently bound antibody, one may be able to specifically



Fig. 2. Sample geometry. Two silver islanded quartz plates sandwich $\approx 1 \mu\text{m}$ RhB solution. Given that metal-fluorophore interactions are thought to extend some 200 \AA into the solution, in this sample geometry only $\approx 4\%$ of RhB molecules are within this active region. This suggests that the *true* enhancement effect in Fig. 1 is at least 25 times higher.

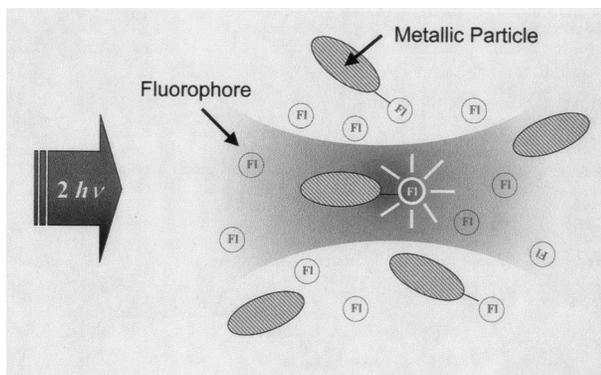


Fig. 3. Preferential multiphoton excitation of fluorophores in close proximity to metal, in the presence of free fluorophore, FI.

excite cellular autofluorescence. If the enhancement by the metallic particles is significantly large, one could imagine their use in tissues where scattering reduces the peak intensity of excitation pulses.

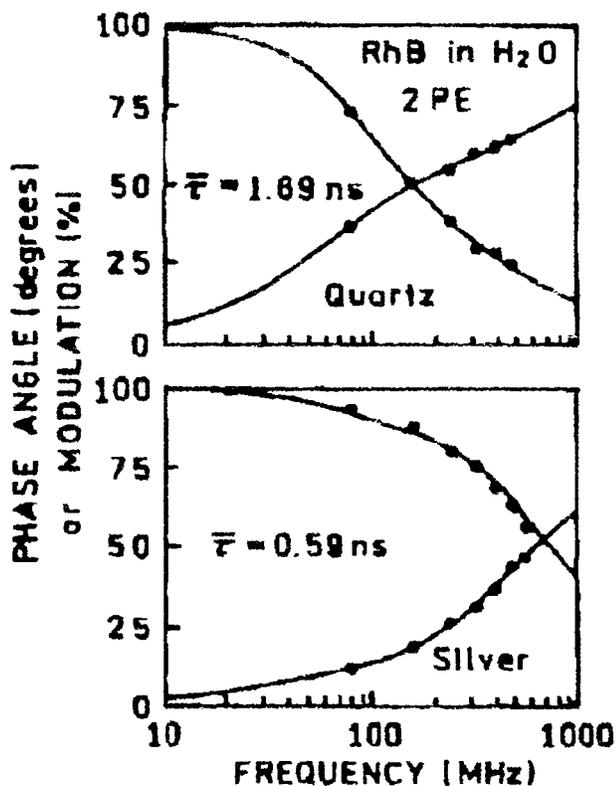


Fig. 4. Frequency domain intensity decay of RhB between quartz slides (top) and silver island films (bottom) with 2-photon excitation at 852 nm observed at 580 nm.

In the many applications of fluorescence, the photostability of fluorophores is an important consideration. This is particularly true in single molecule detection, where it has been estimated that approximately 1000 photons can be observed from a highly stable fluorophore such as a Rhodamine prior to photodecomposition [9]. Because photochemical destruction usually occurs in the excited state, a reduction in fluorescence lifetime resulting from the close proximity of metallic particles is expected to result in increased photostability. For our samples we found that for 1-photon excitation, the photostability was unaffected by the presence or absence of silver islands (Fig. 5, bottom). However for 2-photon excitation, an increased photostability was observed for RhB in the presence of silver islands (Fig. 5, top). These results are consistent with the shorter lifetime observed for RhB between the silver islands and with our interpretation that multiphoton excitation occurs preferentially near the silver islands.

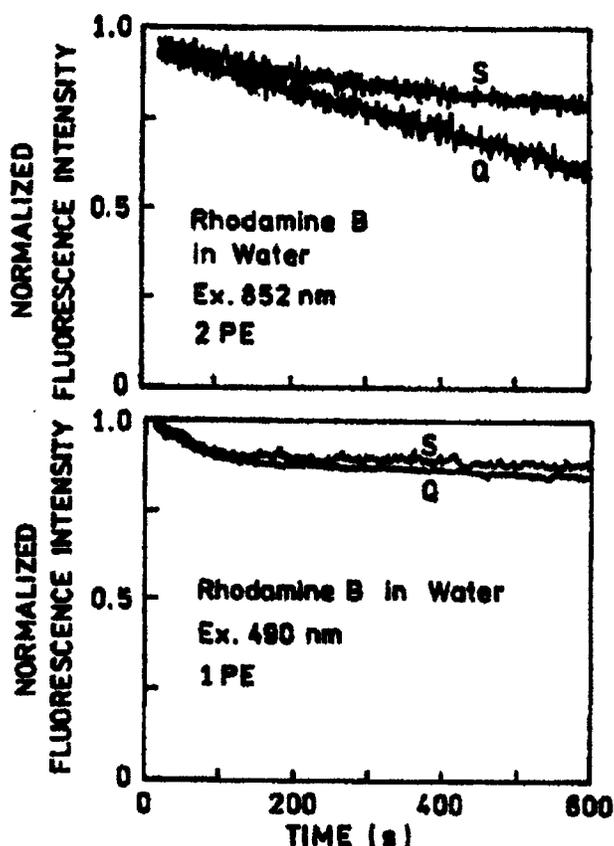


Fig. 5. Photostability of RhB between quartz slides, (Q), and silver island films, (S), with 2-photon excitation at 852 nm (top) and with 1-photon excitation at 490 nm (bottom). The 490-nm excitation was from an argon ion laser attenuated to about 10 mW. Fluorescence was observed at 580 nm.

The use of metallic structures to enhance multiphoton excitation has already been considered in apertureless near-field scanning optical microscopy [10,11]; however, to the best of our knowledge, multiphoton excitation has not been reported near metallic colloids or islands.

REFERENCES

1. J. R. Lakowicz (2001) Radiative Decay Engineering: Biophysical and Biomedical Applications, *Anal. BioChem.* **298**, 1–24.
2. J. R. Lakowicz, Y. Shen, S. D'Auria, J. Malicka, J. Fang, Z. Gryczynski, and I. Gryczynski (2002) Radiative Decay Engineering 2. Effects of silver island films on fluorescence intensity Lifetimes and Resonance energy transfer, *Anal. Biochem.* **301**, 261–277.
3. J. R. Lakowicz, I. Gryczynski, Y. B. Shen, J. Malicka, and Z. Gryczynski (2001) Intensified fluorescence, *Photonics Spectra* **35**, 96–104.
4. C. D. Geddes and J. R. Lakowicz (2002) Metal Enhanced Fluorescence, *J. Fluoresc.* In Press.
5. I. Gryczynski, J. Malika, Z. Gryczynski, C. D. Geddes, and J. R. Lakowicz (2002) The CFS engineers the intrinsic radiative decay rate of low quantum yield fluorophores, *J. Fluoresc.* **12**, 11–13.
6. C. J. Chen and R. M. Osgood (1983) Direct observation of the local field enhanced surface photochemical reactions, *Phys. Rev. Lett.* **50**, 1705–1708.
7. S. Link and M. A. El-Sayed (2000) Shape and size dependence of radiative, nonradiative and photothermal properties of gold nanocrystals, *Int. Rev. Phys. Chem.* **19**, 409–453.
8. J. Kummerlen, A. Leitner, H. Brunner, F. R. Aussenegg, and A. Wokaun (1993) Enhanced dye fluorescence over silver island films. Analysis of the distance dependence, *Mol. Phys.*, **80**, 1031–1046.
9. W. P. Ambrose, P. M. Goodwin, J. H. Jett, A. Van Orden, J. H. Werner, and R. A. Keller (1999) Single molecule fluorescence spectroscopy at ambient temperature, *Chem. Rev.* **99**, 2929–2956.
10. Y. Kawata, C. Xu, and W. Denk (1999) Feasibility of molecular-resolution fluorescence near-field microscopy using multiphoton absorption and field enhancement near a sharp tip, *J. Appl. Phys.* **85**, 1294–1301.
11. E. J. Sanchez, L. Novotny, and X. S. Xie (1999) Near-field fluorescence microscopy based on two-photon excitation with metal tips, *Phys. Rev. Lett.*, **82**, 4014–4017.

Joseph R. Lakowicz¹
 Ignacy Gryczynski¹
 Joanna Malicka¹
 Zygmunt Gryczynski¹
 Chris D. Geddes^{1,2}

ABOUT THE AUTHORS

Joseph R. Lakowicz Ph.D., is director of the Center for Fluorescence Spectroscopy at the University of Mary-

¹ Center for Fluorescence Spectroscopy, Department of Biochemistry and Molecular Biology, 725 W. Lombard St, Baltimore, Maryland 21201.

² To whom correspondence should be addressed. e-mail: chris@cfs.umbi.umd.edu

land School of Medicine in Baltimore. He has a B.S. in chemistry from LaSalle University and an M.S. and a Ph.D. in Biochemistry from the University of Illinois at Urbana. He is also the founding editor of the *Journal of Biomedical Optics* and the *Journal of Fluorescence*. He is a co-founder of the Society of Fluorescence.

Ignacy Gryczynski Ph.D., is a research professor at the University of Maryland School of Medicine. He has an M.S. and a Ph.D. in physics from the University of Gdansk Institute of Physics in Poland.

Joanna Malicka Ph.D., is a postdoctoral fellow at the Center for Fluorescence Spectroscopy. She has an M.S. and a Ph.D. in chemistry from the University of Gdansk.

Zygmunt Gryczynski Ph.D., is an associate professor in the Department of Biological Chemistry and Molecular Biology at the University of Maryland in Baltimore. He is also the assistant director of the Center for Fluorescence Spectroscopy. He has an M.S. and a Ph.D. in physics from the University of Gdansk Institute of Physics.

Chris D. Geddes Ph.D., is an assistant professor at the University of Maryland Biotechnology Center, Center for Fluorescence Spectroscopy in Baltimore. He has a B.Sc. from Lancaster University in England and a Ph.D. in physical chemistry (fluorescence spectroscopy) from the University of Wales, Swansea. He is the editor of the *Journal of Fluorescence*.