Integrated-Optic Nanoparticle Biosensor Arrays

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ABSTRACT: Under IREE support, we have established a collaboration with the MOSAIC group at the Institut Fresnel, in Marseille, France. The collaboration centers around photophysical properties of metal nanocavities. The PI spent a total of four weeks in France (mostly in Marseille, with a few days in Grenoble) while Mr. Mahdavi spent just over 14 weeks. The primary focus of Mr. Mahdavi's PhD studies is the computational analysis of light propagation through sub-wavelength nanocavities. While in Marseille, he was able to participate in experimental studies of light transmission and single-molecule fluorescence through nanocavities; these studies provide experimental results with which to compare simulation. One important result in particular is the observation of a 12x fluorescence enhancement in a gold nanocavity, compared to a factor of 6.5 in an aluminum nanocavity. Simulation results are consistent with this observation. Further experimental results were obtained with different nanocavity shapes and relevant computational studies are underway. As a result of these efforts, we anticipate submitting one or more papers combining experimental and computational results. We have begun studies of energy transfer within nanocavities; these studies will continue beyond the duration of the IREE award. We also anticipate that further researcher exchange between the two groups will occur in the future.

INTRODUCTION

The parent award is to the University of Utah.

Activities associated with the parent award include the study of fluorescence enhancement mechanisms in metallic nanocavities dispatched to form an array [1] (apertures were etched in ~100nm thick metals, with diameters ~100-250nm) Furthermore, our group has investigated the use of nanocavities as real-time biosensors [2], and the use of microfluidics to overcome mass-transport limitations in biosensors. Enhancements of 7x have been obtained using nanocavities in gold. We have developed computational models to describe the two mechanisms involved in this enhancement – enhancement in excitation intensity, and enhancement in molecular emission [3].

The MOSAIC research group at Institut Fresnel has performed more recent studies of single molecule fluorescence from isolated metal nanocavities [4, 5] and has demonstrated similar enhancements (~6.5x) within aluminum holes. This work is performed in close collaboration with Prof. Ebbesen's group at ISIS (Strasbourg, France). They have proposed a computational model for excitation enhancement [6], which well-describes their early experimental results, and suggests that the bulk of the enhancement occurs during excitation. The key question we wanted to address was the relative contribution between the excitation and emission enhancement mechanisms. Additional questions include the role of the metal, the role of the nanocavity shape, and the influence of localized surface plasmon resonances at the edges of the aperture.

Going into the collaboration, we anticipated a number of outcomes. Foremost among them was to obtain additional experimental data of enhancement factors versus nanocavity geometry with which to compare simulation. Geometrical factors include nanocavity shape and thickness. Additional factors include the effects of metal (gold or aluminum) and excitation and emission wavelengths. With these data, we plan to determine the relative contributions between excitation and emission enhancement effects. The final outcome was to establish a long-lasting collaboration between the two groups, starting with our visit to Marseille, and leading to further exchange of researchers and joint publications.

Broadly speaking, the MOSAIC group at Institut Fresnel is a group of physicists and biologists who develop novel optical approaches to study cellular architecture and biophysical processes. Of closer relevance to the travel award, they employ isolated nanometric apertures milled in a metallic film to enhance the fluorescence emission of single molecules, an effort led by Dr. Wenger. Nanoapertures possess a number of desirable properties for biophotonics, such as localization of excitation light within the nanocavities, strong isolation from emission produced by unbound species, and an apparent increase in absorption and emission yield. The simplicity of the structures and their ease of use should further expand their application towards the real-time detection and identification of a low number of molecules.

IREE travelers (Salt Lake City to Marseille):
Steve Blair - Oct. 9-21, 2006; Feb. 3-17, 2007
Farhad Mahdavi - Oct. 9 – Dec. 22, 2006; Jan. 22 – Feb. 16, 2007

RESEARCH ACTIVITIES AND ACCOMPLISHMENTS OF THE INTERNATIONAL COOPERATION

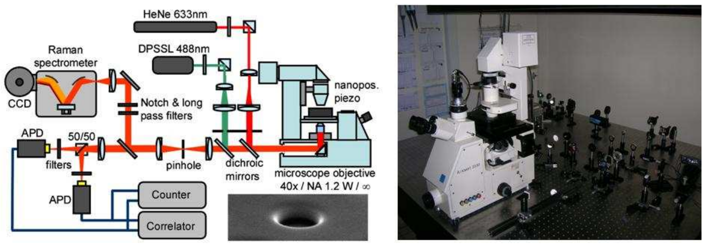

Figure 1: experimental setup for confocal fluorescence correlation spectroscopy developed in Marseille.

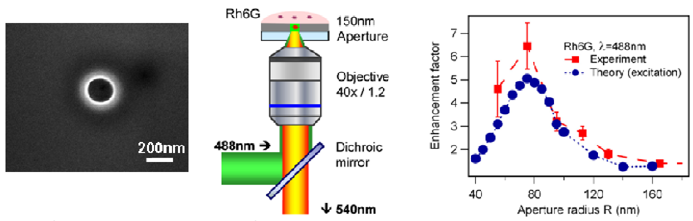

Figure 2: Scheme for the investigation of a single aperture milled in an opaque metallic film and single-molecule fluorescence enhancement factors obtained in a circular aperture.

The common thread between the groups at Utah and Institut Fresnel is the study of fluorescence enhancement in metal nanocavities. Each group has used different experimental and computational approaches, but the groups have achieved similar results. The experimental FCS setup at Institut Fresnel (Fig. 1 and 2) is the more flexible in that it detects individual molecules from a solution environment that interrogate single nanocavities, while the computational methods of Utah are more flexible in that they do not impose restrictions on the symmetry of the computational domain. Based upon these factors, the collaboration is a natural one, with benefits to both parties.

Prior to the actual visitation, Prof. Blair and Dr. Wenger communicated about the proposed research program and discussed the nanocavity samples that were needed. Based upon his on-going collaboration with Prof. Ebbesen's group in Strasbourg, Dr. Wenger was able to arrange the fabrication of nanocavity samples on gold and aluminum of various sizes and shapes. During the Prof. Blair's first visit in October 2006, we then laid out a detailed workplan to be accomplished under IREE travel support. The program began with the measurement of light transmission through individual nanocavities of different shapes, following with the measurement of fluorescence enhancement from these structures. From there, we wanted to progress to the measurement of energy transfer between two dye molecules within a nanocavity. Throughout, we wanted to support the measurements with calculations.

During Mr. Mahdavi's stay in Marseille, he was integrated into the MOSAIC group by sharing office space with the postdoc involved in the experimental efforts (Dr. Gérard) and also by close interaction with the project leader (Dr. Wenger). Mr. Mahdavi was also able to interact with the theoretical/computational group led by Prof. Evgeny Popov, which was an extraordinarily valuable experience. While he spent most of his time performing calculations according to experiments being performed, Mr. Mahdavi did assist with experimental efforts and gained better appreciation for experimental research.

The range of activities undertaken during Mr. Mahdavi's visit is far too broad to describe here, so only a few of these activities will be highlighted. One example is the optical properties of rectangular nanocavities. Light transmission measurements were performed by Ebbesen's group in Strasbourg and Mr. Mahdavi performed corresponding simulations to this published data. The results are shown in Figs. 3 and 4, where very good qualitative agreement is obtained with the experimental data displayed in the inset. The additional transmission peaks shown in the simulations are due to the differences in normalization methods, but the shifts (and changes in magnitudes) in the main transmission peaks with aspect ratio are very consistent with experiments.

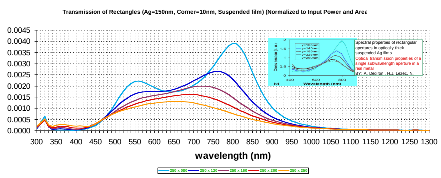

Figure 3. Computed transmission spectra through single rectangular apertures in a silver film.

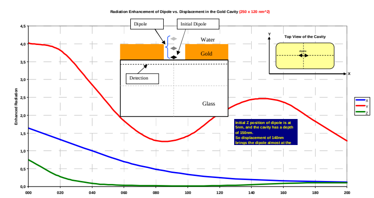

Figure 4. Computed dipolar emission enhancement within a rectangular aperture.

Some of the key new experimental results are shown in the experimental curves below, which illustrate enhancements in single-molecule fluorescence with different nanocavity shapes and different metals. The experimental results regarding the influence of the aperture shape appeared really surprising, as they tend to contradict some of the numerical computations. We are still investigating the reasons for the different behavior observed experimentally, but we presumably infer some defects with the two samples of we investigated. However, the increased enhancement obtained for nanocavities in gold is expected and explained by theory due to the greater influence of surface plasmons.

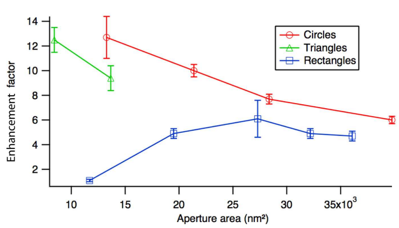

Figure 5. Influence of the aperture shape on the fluorescence enhancement

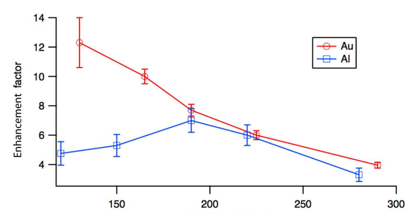

Figure 6. Influence of the metal on fluorescence enhancement for circular nanoapertures.

Finally, during the last two weeks of our visit, we initiated measurements on the effects of nanocavity confinement on fluorescence-resonance energy transfer (FRET) using samples of DNA with controlled separation between two dye molecules (Cy-3 and Alexa-648) that we designed and synthesized at Utah. Developing efficient FRET molecular systems was challenging, and the initial results within the nanoapertures were hard to interpret, but we are continuing with this effort.

Broader Impacts of the International Travel

The original scope of the parent award was to study fluorescence enhancement effects in metallic nanocavities. The additional perspectives offered by the collaboration were to extend these studies into the single-molecule regime and to interact with another group with similar interests in the photophysics and biological applications of these structures. Due to their close interaction with Prof. Ebbesen's group, our collaborators at Institut Fresnel had more ready access to samples of various metals and geometries. Two activities were initiated that expand the original scope of our research. The first was look at the effects of metal. Our group has been focused on using gold due to primarily to its advantageous chemical properties [2], whereas many other groups, including our hosts, use aluminum. Based upon the experimental measurement comparing enhancements from the two metals, we plan to submit a joint publication including the experimental results along with supporting computational results. The second activity was the study of energy transfer within nanocavities. These studies are on-going and we anticipate another joint publication describing the results. A key issue is whether nanocavity confinement simply increases the radiative emission of both dyes or results in an actual increase in Förster radius, where the latter would be of greater significance.

The IREE supported travel has fostered a longer-term interaction between the two groups. For example, Dr. Wenger is considering spending part of an upcoming sabbatical at Utah. We are also considering establishing a more regular exchange of scientists between the two groups, preferably involving PhD students from each side. In addition to this exchange of personnel, we also have significant scientific exchange planned in terms of completing and publishing results from our visit and establishing additional areas of scientific collaboration. We plan to meet again in mid-June in Dijon for Surface Plasmon Photonics 3 conference.

Mr. Mahdavi experienced life in Marseille through the aid of a friend who guided him. He discovered that Marseille was very rich in culture and history. Part of the tour included the opportunity to sample various wines. Marseille is also a good place to sample African and Mediterranean food. Mr. Mahdavi had the chance to attend French classes for international students. During the visit, he traveled to Strasburg to visit Prof. Ebbesen's group at the Université Louis Pasteur. Prof. Ebbesen published the original paper on light transmission through arrays of metal nanocavities, which created a new field of study in nanophotonics, and continues to be involved in seminal work in the field. While there, he gave a presentation about Utah and our research, and met with Dr. Jean-Marie Lehn (1987 Nobel Prize in Chemistry, whose work laid the foundation of the important field of supramolecular chemistry) as well as Prof. Ebbesen and Dr. José Dintinger, who performs most of the sample fabrication and measurement. Overall, Mr. Mahdavi felt that the experience was very positive and helpful to his PhD research and to his overall appreciation of the broader scope and application of nanophotonics.

DISCUSSION AND SUMMARY

One important scientific accomplishment was to show the significant difference between fluorescence from aluminum and gold nanocavities, which, in retrospect, should have been anticipated, but was nonetheless surprising at first, especially by the high enhancement factors and the high count rates per molecules obtained. We now have enough comparisons between experimental and computational results to suggest that there is reasonable correlation between the two, allowing us to better understand the photophysical processes involved in nanocavities. We also anticipate additional outcomes of scientific importance to materialize. For example, the relative contribution between excitation and emission enhancement mechanisms is still under study, but we expect to arrive at some general conclusions and publish the results (the emission calculations are significantly more involved than the excitation calculations, and thus take more time to complete). Finally, we have established what we expect to be a longer-term collaboration and exchange between the two groups, which is significant in its own right.

The IREE program is of high value and impact and should definitely be supported on an on-going, NSF-wide basis. Much of its value lies in the fact that this type of travel support is difficult to obtain through other sources. The award is especially valuable for CAREER awardees, and perhaps a mechanism should be implemented for new CAREER proposals to include an international collaboration component via an IREE supplement (which is granted with the parent award). It would be beneficial for the CAREER awardees themselves to be able to travel for an extended period of time.

Additional recommendations:

- it would be beneficial to include some support for a representative from the host laboratory to travel to the awardee site, perhaps within a month of completion of the awardee’s travel. This would allow for follow-up and planning for continued collaboration and exchange.
- due to the length of stay of the main traveler, providing some additional travel support for one short trip home would be nice. In our case, Mr. Mahdavi’s stay overlapped the Christmas/New Year’s holidays, so he returned during that time to be with his family (also, the Institut Fresnel was closed during that time).
- an issue we encountered was that the living allowance for the extended stay didn’t quite cover expenses, shortening the traveler’s stay somewhat. The allowable amount should be increased by about 15%, but this will strongly vary depending upon location.

ACKNOWLEDGEMENTS

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7. BRIEF BIOGRAPHIES OF RESEARCHERS

Steve Blair received the BS and MS degrees from Rose-Hulman Institute of Technology in 1991 and 1993, respectively, and the PhD degree from the University of Colorado at Boulder in 1998. In 1998, he started as an Assistant Professor with the Electrical and Computer Engineering Department at the University of Utah in Salt Lake City. Since 2005, he has been an Associate Professor. Prof. Blair's research interests include slow-light nonlinear optics, plasmonics, photonic microsystems, and microarray technology.

Farhad Mahdavi received the B.S. degree from Sharif University, Tehran, Iran, in 1986, and the M.E. degree from the University of Utah, Salt Lake City, in 2004. Since 2004, he has been a Ph.D. candidate in Electrical Engineering at the University of Utah. His research interests are plasmonics and light transport via photonic nanostructures.

Jérôme Wenger qualified from the Engineering School of Optics 'SupOptique' in 2001, and received the PhD degree in 2004 at the Institut d'Optique (Orsay-France). During his PhD, he studied quantum optics, performing new experiments on quantum cryptography and quantum communication. He then joined the MOSAIC team at the Fresnel Institute in Marseille, first as a postdoc before getting a permanent position as CNRS junior researcher in 2005. He is presently working on nanophotonics and biophotonics, developing new set-ups to enhance the optical contrast in microscopy.

Davy Gérard received his Ph.D. degree in 2004 at the Université de Bourgogne (Dijon, France). His work there was devoted to the study of optical properties of photonic and plasmonic crystals. In the following years he kept working on nanophotonics, first at the Institut d'Electronique Fondamentale (Orsay - France) and then at the Université de Franche-Comté (Besançon, France). He is now part of the Fresnel Institute, working on single-molecule fluorescence in nanoapertures.