

Nano-Biophotonics

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to the University of Exeter
as a thesis for the degree of

Doctor of Philosophy in Physics

April 2010

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Abstract

Photonic techniques are the methods of choice for probing biological systems, as they are non-invasive, non-ionising, inexpensive, and are ubiquitous. When applied to the treatment and prevention of disease and for pathology in general, biophotonics offers a means to bridge the gap between understanding of molecular structures and their role in physiological functions. There is a wide range of such techniques used in imaging, assaying, bio-sensing, optical diagnosis, each of which has limitations as well as benefits. The experiments outlined in this thesis use nanotechnology to overcome the limitations of resolution, contrast and chemical specificity with photonic techniques in biology.

The experimental work outlined in this thesis is divided over three chapters, the first of which is concerned with nanostructured metallic surfaces for use in surface enhanced Raman scattering (SERS) for protein assay applications. This chapter gives details of the methods used to produce and characterise SERS substrates using gold and silver thermally evaporated onto butterfly wing sections, together with the protocols developed for manufacturing biomimetic analogues of these naturally occurring nanostructures. The conjugation system designed to modify the metal surfaces for use in an avidin/biotin model protein binding assay is described, together with an account of the efficacy of the final assay. The results obtained show that such naturally occurring nanostructures, and their biomimetic analogues, are suitable for use as SERS substrates for wet protein binding assays. This work represents a major advance in the field of SERS assay.

The next experimental chapters describe experiments that use coherent Raman scattering (CRS) methods to probe the interactions between nanoparticles and live cell cultures, as well as provide chemically selective images of tissue samples. Chapter 6 gives an account of work undertaken to investigate gold nanoshells, novel nanoparticles comprising spherical silica cores surrounded by a layer of gold, whose plasmon resonance wavelength may be tuned by altering the shell thickness and core diameters. Gold nanoshells exhibit unique optical properties arising from this plasmon resonance, and their ability to rapidly heat under exposure to light at this resonant wavelength has been used for photodynamic therapy of tumours in mice, in one instance with white

blood used cells to carry gold nanoshells to the site of the tumour. Although previous photothermal experiments involving tumour destruction using nanoshells have given promising results, they largely ignored the interactions between single cells and the gold nanoshells. The experiments in this chapter probe these interactions, using coherent Raman scattering (CRS) microscopy.

CRS is a chemically-selective multiphoton modality that can be used to image live cells noninvasively and with excellent chemical specificity and contrast. To determine the extent to which CRS microscopy-induced heating of gold nanoshells would affect live cells, macrophage cells were induced to phagocytise gold nanoshells prior to exposure to CRS. A trypan blue vital staining technique was used to determine cell survival rates after exposure to a range of laser powers. The photoluminescent qualities of gold nanoshells were also exploited as a marker for phagocytosis, the rate of which was investigated using CRS, as a function of the concentration of two different H₂S donors in the cell medium.

The final experimental chapter investigates the ability of CRS to image organic nanoparticles within cells and tissues. Two varieties of nanoparticles and cell lines were used in this chapter: dendrimer/plasmid DNA nanoparticles with human skin cancer cells and apo lipoprotein E (APOE) conjugated to albumin nanoparticles with human brain endothelial cells. Dendrimer nanoparticles have been shown to exhibit characteristics that make them efficient potential candidates for gene therapy, since they are able to spontaneously bind to DNA and deliver it across cell membranes. Before this gene delivery vector is used in humans it is important to investigate the distribution of these nanoparticles as they pass inside cells, using a non-invasive imaging technique that provides sufficient spatial resolution and chemical selectivity. APOE-conjugated albumin nanoparticles have been found to cross the blood-brain barrier, and hence their uptake by brain endothelial cells is of great relevance for engineering drugs to treat brain disease.

The potential of SRS microscopy to image the blood vessels within the brain was investigated using slices of mouse brain. This provided clear images of the blood-brain barrier, a selectively permeable membrane comprising endothelial cells lining the blood vessels innervating the brain. The BBB effectively blocks access to over 95% of drugs

from entering the brain, targeted engineering of drugs to cross this barrier is necessary in order to treat diseases of the brain. Therefore, the images provided in these brain imaging experiments have demonstrated the potential for coherent Raman scattering microscopy in quantifying drug nanoparticle uptake within dosed animal brains.

The interaction between ApoE-albumin nanoparticles and brain endothelial cells was investigated in using coherent Raman scattering. Signal intensity from three stimulated Raman scattering microscopy images of endothelial cells exposed to nanoparticles was colocalised using the relative intensity of signal probing three Raman bands identified in spontaneous Raman scattering spectra of the nanoparticles. The pump wavelengths used were 813 nm, 821 nm and 938.9 nm and the Stokes wavelength was 1064 nm in each case. The data obtained showed that ApoE-albumin nanoparticle signal associated with the cells was overlapped by signal from CH₂ within the sample, indicating the involvement of lipid-rich structures within the cells in receptor membrane endocytosis.

Acknowledgements

Throughout my PhD I have been supported and encouraged by a large number of people without whose help and friendship the project would not have been so successful. Firstly, my thanks go to my supervisor, Julian Moger, for his enthusiasm, insight, support and hilarious anecdotes at conferences. His drive to establish Exeter University's Biomedical Physics group as an internationally acclaimed multiphoton imaging research centre has been truly inspirational and I am very grateful for the opportunity to work with him. Peter Winlove deserves a special mention, as the leader of my research group and my second supervisor. It seems there is no area of Physics in which he is not only an expert, but is also in possession of at least half a dozen relevant lewd anecdotes. His wealth of remarkably detailed knowledge is matched by his contagious enthusiasm for the topic – I blame him almost entirely for my decision to undertake this PhD!

I would also like to thank Ellen Green and Dick Ellis for their patience and wisdom while training me during my first forays into wet lab experimentation, and for loaning me innumerable books and obscure items of experimental paraphernalia. It is one thing to read about a technique in a book, but quite another to have such experienced experimentalists show you how it's really done. Andy Murray, James Parsons and Piers Andrew have all helped me get to grips with evaporating techniques and using the SEM as well as the darkfield flow cell, while Cierán Stewart assisted me with the AFM calibration of my samples – thanks guys! Thanks must also go to John Meakin and the workshop boys for all their help with the nuts-and-bolts of designing, maintaining and ordering kit. As a technical support team, they are second to none. Dave Colridge deserves my deep gratitude for enduring my conversation during lunch breaks, and for continually coming to my rescue whenever Microsoft Works shows itself to be an oxymoron.

I am deeply indebted to several collaborators for all their help. Evgeny Sirotkin, Pete Vukusic and Feodor Ogrin were excellent collaborators for the butterfly wing paper. Maria de la Fuente of the London School of Pharmacy gave me excellent training in cell culture and has gone above and beyond the call of duty to supply me with materials, including dendrimer nanoparticles, plasmid DNA and skin cancer cell lines. She has

cycled to work through snow drifts and even when ill, all to make sure my reagents would be posted on time. Thank you, Maria! Special thanks must also go to Larisa Mihoreanu and David Begley of Kings College London for their advice, good humoured support and the numerous samples they've supplied me with. Other scientists who have very kindly supplied me with samples are Alex Kendall (thank you for the LPS!) and Matt Whiteman (thank you for the NaHS and GYY4137) both of Exeter's Peninsula College of Medicine and Dentistry (PCMD).

I was very grateful to receive support from Mohit Chopra (also from PCMD) for cryogenically storing my cell lines under liquid nitrogen. Without his help in this respect, my cell culturing experiments would have been nigh on impossible and I wouldn't have had the opportunity to pretend that Martin Smith and I were characters in Jurassic Park. Thanks also to John Hale, who teamed with Martin to provide the ultimate cautionary tale to all Physics PhD students – you guys have left very big shoes to fill (metaphorically anyway). And a noose (literally).

GP forever has my gratitude for our innumerable fascinating and inspirational discussions during our mentor meetings. He has taught me many things, but one lesson in particular will stay with me forever: I shall never attempt to make grape jam in a pressure cooker. I must also give my thanks to: the motley crew of PhD students in the Biomedical Physics research group (past and present), the late-night workers, the Christmas “meeting”/Summer ball jesters, the coffee-time philosophers and the Friday pub gang, who have all helped to make my time here at Exeter very enjoyable and “educational”. Thanks to the photonics crew for welcoming me with open arms and readily proffered Rubik's cubes, iPhones and crosswords. In particular, thanks to Tom Constant for preventing my auto-defenestration. You happy now, Tom?

My family has given me a great deal of support throughout my PhD and I owe them a great debt of thanks; in some cases I also owe a great debt of money. In particular, my lovely husband Simon has been wonderfully supportive, and I thank him from the bottom of my heart. Si – without you, not only would this thesis not have been possible, but not much else that I cherish in life would be possible either. With a love that will echo through the ages, Simon, I dedicate this thesis to you!

List of Abbreviations

CARS – coherent anti-Stokes Raman scattering
CRS – coherent Raman scattering
DMEM – Dulbecco's modified Eagle's medium
DMSO – dimethyl sulphoxide
DPBS – Dulbecco's phosphate buffered saline
EDC - ethyl dimethylaminopropyl carbodiimide
EDTA - ethylenediaminetetraacetic acid
ELISA - enzyme-linked immunosorbent assays
EM – electromagnetic
FBS – foetal bovine serum
FWM – four wave mixing
GNS – gold nanoshells
LSP – localised surface plasmon
MPA – 3-mercaptopropanoic acid
NHS – N-hydroxy succinimide
NIR – near infrared
OPO – optical parametric oscillator
PBS – phosphate buffered saline
PMMA - poly(methyl methacrylate)
SAM – self assembled monolayer
SEM – scanning electron microscope
SERDS – shifted excitation Raman difference spectroscopy
SERS – surface enhanced Raman spectroscopy
SPP – surface plasmon polariton
SRG – stimulated Raman gain
SRL – stimulated Raman loss
SRS – stimulated Raman scattering
TEM – transmission electron microscope

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