

Nano-Biophotonics

Submitted by Natalie Garrett

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

Doctor of Philosophy in Physics

April 2010

This thesis is available for Library use on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgment.

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

.....

Natalie Garrett

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

Table of Contents

Nano-Biophotonics	1
Abstract.....	2
Acknowledgements.....	5
List of Abbreviations	7
Table of Contents.....	8
List of Figures	11
1. Introduction	15
1.1. The History of Photonics in Biology	15
1.2. Emerging Photonic Techniques in Biology.....	27
1.2.1. Coherent Raman Scattering	27
1.3. Thesis Aims.....	28
1.4. Overview of Thesis.....	29
2. Background and Literature Review of Raman-based Biophotonics	31
2.1. Nanotechnology in Biophotonics.....	31
2.2. General Cell Structure.....	35
2.3. Chemical Assay.....	36
2.4. Raman Scattering	39
2.5. Surface Enhanced Raman Scattering	42
2.6. SERS Substrates.....	45
2.6.1. Gold Nanoshells	48
2.7. Coherent Raman Scattering.....	51
2.8. Contribution of this Thesis to the field	56
3. Theory	57
3.1. Theory of Raman Scattering.....	57
3.2. Surface Enhanced Raman Scattering Theory.....	62
3.2.1. Electromagnetic Contribution to Surface Enhanced Scattering	62
3.2.2. Chemical Enhancement in SERS.....	64
3.3. Photoluminescence.....	65
3.4. Plasmon Wavelength Tuning in Gold Nanoshells	66
3.4.1. Dielectric Function of Particles of Gold in the Mie Regime	66
3.4.2. Absorption, Scattering and Extinction	69

3.5. Coherent Raman Scattering	70
3.5.1. CARS Theory	70
3.5.2. Stimulated Raman Scattering Theory	79
3.5.3. Application of CRS to Biophotonics	82
4. Instrumentation	83
4.1. Raman Laser Microspectrometer	83
4.2. Coherent Raman Scattering Setup.....	84
4.3. Spectrometer	91
4.4. Metal Evaporators	92
4.5. Scanning Electron Microscope.....	93
4.6. Ultraviolet Curing System	93
4.7. Cell Culture.....	94
5. Surface Enhanced Raman Scattering Using Biological & Biomimetic Nanostructures.....	95
5.1. SERS Protein Assays	95
5.2. Materials and Methods.....	98
5.2.1. Preparation of natural nanostructure substrates.....	99
5.2.2. Fabrication of biomimetic nanostructures	102
5.2.3. Imaging and Spectroscopy	102
5.2.4. Thiophenol Monolayers.....	102
5.2.5. Bioconjugation for Model “Immunoassay” System.....	103
5.3. Results.....	106
5.3.1. SEM Imaging of Substrates	106
5.3.2. Enhancement factors	108
5.3.3. Bioconjugation	111
5.3.4. Normal Raman Spectra	113
5.3.5. SERS Assay.....	114
5.4. Discussion and Conclusions	116
6. Biophotonic Applications of Gold Nanoshells.....	118
6.1. Introduction	118
6.1.1. Nanoshells in Photodynamic Therapy.....	118
6.1.2. Photoluminescence of Nanoshells.....	119
6.1.3. Nanoshells in this Thesis	120
6.2. Experimental	120
6.2.1. Manufacturing Process of Metal Nanoshells.....	120
6.2.2. Characterisation of Gold Nanoshells by SEM.....	121

6.2.3. Determining the Plasmon Characteristics of aged Nanoshells	122
6.2.4. Verifying CARS Signal/Enhancement from Gold Nanoshells	127
6.2.5. Mouse Macrophage Cell Culturing Protocols	128
6.2.6. Cell Counting and Viability Staining Methods.....	130
6.2.7. Nanoshells as Contrast for CARS Microscopy	134
6.2.8. Ablation Study.....	136
6.2.9. Monitoring the Effects of Hydrogen Sulphide on Phagocytosis using GNS Contrast	138
6.3. Discussion and Conclusions	143
7. Label-free Imaging of Organic Nanoparticles	148
7.1. Introduction	148
7.2. Experimental	151
7.2.1. Comparison of Brain Tissues with SRS and CARS.....	151
7.2.2. Brain Cell Culturing Protocols	156
7.2.3. Albumin Nanoparticles.....	156
7.2.1. Cancer Cell Culturing Protocols.....	164
7.2.2. Dendrimer Nanoparticles.....	164
7.3. Discussion and Conclusions	172
8. Conclusions and Suggestions for Further Work.....	176
8.1. SERS Using Biological and Biomimetic Nanostructures	177
8.1.1. Summary	177
8.1.2. Future Work.....	178
8.2. Biophotonic Applications of Gold Nanoshells.....	180
8.2.1. Summary	180
8.2.2. Future Work.....	182
8.3. Label-free Imaging of Organic Nanoparticles	182
8.3.1. Summary	182
8.3.2. Future Work.....	183
9. References	185
10. Publications, Conference Proceedings & Academic Achievements.....	200
Publications.....	200
Conference Proceedings	200
Academic Achievements.....	201

List of Figures

Fig. 1.1 Depth of field ranges for lenses of high and low numerical apertures [5].	18
Fig. 1.2 The anatomy of an animal cell [7]	19
Fig. 1.3 Schematic cross-sectional structure of the cell membrane [8]	20
Fig. 1.4 Endothelial cells under the microscope. Nuclei are stained blue with DAPI, microtubules are marked green by an antibody and actin filaments are labelled red with phalloidin [12].	22
Fig. 1.5 A schematic diagram of a confocal microscope [14]	23
Fig. 1.6 White light microscopy images of an animal cell. (A) was imaged using bright-field optics, (B) with phase-contrast optics and (C) was imaged with differential interference-contrast optics [15]	24
Fig. 1.7 Comparison of images produced with TEM and SEM [16]	25
Fig. 2.1 A schematic diagram of an antibody [39]	38
Fig. 2.2 Spontaneous Raman spectrum of the P22 virus, exhibiting the characteristic Raman modes typically observed in biological samples [44]	41
Fig. 2.3 Molecular energy level schematic for CARS and SRS depicting the interaction between the pump (ω_p), Stokes (ω_s) and anti-Stokes (ω_{AS}) beams. The dashed lines denote virtual states while the energy difference between the ground state ($V=0$) and first excited state ($V=1$) corresponds to a Raman resonance (Ω_R).	53
Fig. 3.1 Extinction spectra of nanoshells with varying shell thicknesses [95].	69
Fig. 3.2 The resonant, non-resonant and mixed contributions to the CARS signal as a function of detuning Δ , calculated using the equations in section 3.5.1, with a Lorentzian bandwidth Γ of 10 cm^{-1} .	74
Fig. 3.3 The overall CARS signal intensity as a function of detuning Δ , calculated using the equations in section 3.5.1, with a Lorentzian bandwidth Γ of 10 cm^{-1} .	75
Fig. 3.4 Energy level diagram for (a) spontaneous Stokes Raman scattering, (b) general four wave mixing processes, (c) SRS, (d) resonant CARS, (e) nonresonant CARS from an electronic contribution, (f) the phase matching condition in CARS. The dashed lines denote virtual vibrational states.	77
Fig. 3.5 Spectral profile for SRS, adapted from [112]	81
Fig. 4.1 The Coherent Raman scattering system at the University of Exeter.	85
Fig. 4.2 Schematic diagram of the CARS microscope [138]	86
Fig. 4.3 Schematic diagram of the optical bench	87
Fig. 4.4 Transmission profile for Chroma Z850rdc-xr long pass dichroic filter, data supplied by the manufacturers	88
Fig. 4.7 Custom-built SRS detector	91
Fig. 4.8 Transmission of the grating in CARS spectrometer, data supplied by manufacturers.	92

Fig. 5.1 Schematic diagram illustrating the formation of a self assembled monolayer (SAM) of thiophenol on a gold surface.	99
Fig. 5.2 Regions of interest on <i>Graphium weiskei</i> butterfly wings.....	101
Fig. 5.3 A schematic diagram depicting the chemical conformation of <i>gauche</i> and <i>trans</i> configurations of 3-mercaptopropanoic acid and cysteamine.	105
Fig. 5.4 SEM images of: the <i>G. weiskei</i> nano-cone arrays (A) and the biomimetic substrate (B), with nano-cone heights of 524nm peak-to-peak distances of 390nm.	107
Fig. 5.4 SEM images of green regions on <i>G. weiskei</i>	108
Fig. 5.5 SERS spectra of a thiophenol monolayer on a butterfly wing coated with 70nm silver (left) and an un-enhanced Raman spectrum of neat thiophenol in solution (right).....	109
Fig. 5.6 Enhancement factor of metalized wing nanostructures as a function of metal deposition thickness: ■ data points correspond with silver, ▲ with gold.	110
Fig. 5.7 Graph depicting SERS spectra of MPA on gold-coated butterfly wings as a function of deposition times. The red, black and blue lines correspond with deposition times of 1 hour, 2 hours and 18 hours respectively.....	112
Fig. 5.8 SERS spectra of MPA a) prior to activation with EDC/NHS, b) after activation	112
Fig. 5.9 SERS spectra of avidin bound to gold-coated wing (b) and the avidin-biotin complex bound to gold-coated wing (a).	113
Fig. 5.10 SERS spectra of metalized wing-bound avidin exposed to a range of Biotin solution concentrations.....	114
Fig. 5.11 Ratios of the heights of the peaks at 975 cm^{-1} (peak 1) and 1000 cm^{-1} (peak 2) in the SERS spectra of the avidin-biotin complex for gold-coated butterfly wings for a range of concentrations of biotin in buffer solution.....	115
Fig. 6.1 SEM images of seeded gold nanoshells (left) and fused nanoshells with incomplete shells (right)	121
Fig. 6.2 SEM image of a single, complete nanoshell. The scale bar is 200 nm.	122
Fig. 6.3 Darkfield microscopy image of gold nanoshells annealed to a glass coverslip, in air; the green and the purple box represent the flow cell experiment locations for spectra 1 and 2 respectively.....	124
Fig. 6.4 Scattering profile for GNS 1. The dashed lines denote the theoretical calculation, the solid lines are the experimental result: black is for GNS surrounded by air, red is for GNS surrounded by culture medium	125
Fig. 6.5 Scattering profile for GNS 2. The dashed lines denote the theoretical calculation, the solid lines are the experimental result: black is for GNS surrounded by air, red is for GNS surrounded by culture medium.	125
Fig. 6.6 Extinction spectrum of fresh gold nanoshells (nanoComposix)	126
Fig. 6.7 Theoretical extinction spectrum of gold nanoshells, silica core radius 60nm gold shell thickness 15nm, in phosphate buffered saline calculated based on Mie scattering theory, with the peak intensity scaled to match [130]	127

Fig. 6.8 Emission spectra of gold nanoshells under excitation from Stokes (black) and pump and Stokes simultaneously (red).....	128
Fig. 6.9 Schematic diagram outlining the Neubauer Improved haemocytometer [177]	133
Fig. 6.10 Epi- (left) and forwards- CARS (right) images of gold nanoshells suspended in agarose gel. Images were obtained using pump and Stokes wavelengths of 918 nm and 1265 nm respectively. Each image width is 260 μm	135
Fig. 6.11 CARS and white light microscopy images of macrophage cells, with and without GNS. Left to right: before exposure to CARS, CARS image and after exposure to CARS and trypan blue staining. The top row corresponds with undosed cells and the bottom row with cell exposed to GNS. The CARS excitation wavelengths were tuned to the CH resonance for the F-CARS (red) and off this resonance for the epi-CARS (blue).....	136
Fig. 6.12 Graph depicting the percentage of photothermally ablated macrophage cells as a function of laser energy at the sample (red line). The baseline mortality exhibited by both GNS-containing cells unexposed to CARS, and GNS-free cells exposed to CARS up to 35 J laser energy at the sample is also shown (blue line).....	137
Fig. 6.13 Effect of H ₂ S donors on macrophage uptake of GNS.....	140
Fig. 6.14 Forwards-CARS (grey) and epi-CARS (red) signal obtained from mouse macrophages containing gold nanoshells. The excitation wavelengths used were: $\lambda_p = 924 \text{ nm}$ $\lambda_s = 1064 \text{ nm}$ (A), $\lambda_p = 919 \text{ nm}$ $\lambda_s = 1263 \text{ nm}$ (B), $\lambda_p = 912 \text{ nm}$ $\lambda_s = 1278 \text{ nm}$ (C), $\lambda_p = 924 \text{ nm}$ $\lambda_s = 1255 \text{ nm}$ (D).....	141
Fig. 6.15 Forwards CARS (grey) overlapped with epi-CARS (red) of A431 cancer cells incubated with nanoshells and varying concentrations of GYY excited at $\lambda_p = 924 \text{ nm}$ $\lambda_s = 1255 \text{ nm}$. Concentrations of GYY for each image: A = 10 μM , B = 100 μM , C = 0.5 mM, D = 1.0 mM	142
Fig. 7.1 Schematic diagram detailing the blood-brain barrier and its transport mechanisms, adapted from [182, 183].....	149
Fig. 7.2 CARS (left) and SRS (right) images of a mouse brain perfused with FD4 fluorescein isothiocyanate-dextran, with pump and Stokes wavelengths at 816.....	152
Fig. 7.3 SRS image of an undosed, “blank” mouse brain (left), the red blood cells from which are isolated for clarity in the right image. Scale bar = 40 μm	153
Fig. 7.4 Three dimensional analysis using ImageJ Volume Viewer of a stack of images obtained with SRS of an undosed brain, imaged using pump and Stokes wavelengths of 816 nm and 1064 nm respectively. The depth of the stack is 13 μm	154
Fig. 7.5 Three dimensional rendering using ImageJ Volume Viewer, of a blood vessel in a blank mouse brain, imaged using SRS pump and stokes wavelengths of 813 nm and 1064 nm respectively. The stack depth is 24 μm	155
Fig. 7.6 Spontaneous Raman spectrum of ApoE-albumin.....	158
Fig. 7.7 Spontaneous Raman spectrum of PEG-albumin.....	158
Fig.7.8 A comparison of spontaneous Raman spectra of ApoE-albumin and PEG-albumin nanoparticles dried onto aluminium-coated microscope slides.	159

Fig. 7.9 Epi-CARS images of nanoparticles. The left-most image illustrates thresholding at 1 standard deviation above the mean pixel intensity value; the right-most image illustrates thresholding at 2 standard deviations above the mean pixel intensity value.	160
Fig. 7.10 A comparison of colocalisation parameters on images of brain endothelial cells devoid of nanoparticles. In A, the colocalisation intensity threshold was set to the mean intensity, giving false-positive nanoparticle signal. In B, the intensity threshold was set to the mean + 1 standard deviation, which effectively screened out all false positive signal. The scale bar is 40 μm	161
Fig. 7.11 CARS image of human brain endothelial cells inoculated with ApoE-albumin nanoparticles incubated for 45 minutes (left) and 15 minutes (right). Scale bars are both 40 μm	162
Fig. 7.12 CARS image of brain endothelial cells inoculated with PEG-albumin nanoparticles for 45 minute incubation (left) and 15 minute incubation (right). Scale bars are 40 μm (left) and 20 μm (right).	163
Fig. 7.13 Molecular structure of DAB16.....	165
Fig. 7.14 Spontaneous Raman spectrum of DAB, with no background subtraction	168
Fig. 7.15 Spontaneous Raman spectrum of pDNA, with no background subtraction.....	168
Fig. 7.16 Spontaneous Raman spectrum of DAB/pDNA nanoparticles, with no background subtraction	169
Fig. 7.17 SRS and CARS images of cancer cells with and without dendrimer nanoparticles. Top row: undosed cancer cell, bottom row: cancer cell dosed with a high concentration of DAB/pDNA nanoparticles (200 μL per culture dish). SRS image at 813 nm and 1064 nm (left), EPI-CARS image at the same pump and Stokes wavelengths (middle) and SRS at 921 nm and 1064 nm (right). Each image is of a sample area 30 μm across.	170
Fig. 7.18 SRS images of confluent cancer cells with dendrimer/pDNA nanoparticles at a low concentration (100 μL per dish). Scale bar = 50 μm . Top row: RGB merged images of three confluent cancer cell regions imaged at 813 nm and 1064 nm (red), 938.9 nm and 1064 nm (green) and 821 nm and 1064 nm (blue); the white pixels marked with arrows indicate signal from nanoparticles. Bottom row: greyscale images of the same three regions, imaged at 813 nm and 1064 nm, for comparison.	171