Professor Paola Borri

Challenge Grants - CH2016
Application Ref: CH160031

Title: Professor

First Name: Paola

Surname: Borri

Other Names:

Address: Cardiff University
School of Biosciences
Sir Martin Evans Building
Museum Avenue

Town: CARDIFF

Postcode: CF10 3AX

Country: United Kingdom

Nationality:

Nationality
Italian

Email Address: BorriP@Cardiff.ac.uk

Web Address: http://www.cardiff.ac.uk/people/view/81122-borri-paola

Telephone (mobile):

Telephone (work): +44 (0)29 208 79356

Fax: +44 (0) 29 208 74116

Applicant Career Summary

Statement of qualifications and career:

Qualification	Date
Professor at Cardiff University	2011
Reader at Cardiff University	2007
Senior Lecturer at Cardiff Univeristy	2004
Habilitation (Venia Legendi) from Dortmund University (Germany), Physics Department	2003
EU Marie Curie Fellow	2001
Postdoctoral Research Associate, Dortmund University (Germany), Physics Department	1999
Assistant Research Professor at the Mikroelektronik Centret and Research Center COM (Technical University of Denmark, Lyngby, Denmark.	1997
Ph.D. Florence University (Italy), Physics degree	July 1997
Laurea (M. Sc. equivalent) in Physics at Florence University (Italy), degree summa cum laude (110/110 cum laude).	March 1993

Field of Specialisation: nonlinear optics, coherent light-matter interaction, nano-photonics, bio-photonics, multiphoton microscopy, ultrafast laser spectroscopy

Outline of present research: Wolfson Research Merit Award: Shedding new light on cells with coherent multiphoton nanoscopy

Biological questions are increasingly demanding non-invasive tools to resolve biomolecules and their interactions at the smallest possible scale in space and in real-time. Coherent Antistokes Raman Scattering (CARS) microscopy has emerged in the last decade as a novel label-free technique which is able to distinguish biomolecules and cellular constituents in living cells based on their chemical composition. My research aim is to develop and demonstrate a new generation CARS microscopy technology with unprecedented sensitivity and spatial resolution.

Publications: Selected list of recent papers (from >110 published articles in internationally peer-reviewed journals since 1996). The three most significant publications within the last 5 years are indicated with an asterisk*

*Bradley, J. et al. 2016. Quantitative imaging of lipids in live mouse oocytes and early embryos using CARS microscopy. Development (10.1242/dev.129908)

*Di Napoli, C. et al. 2016. Quantitative spatio-temporal chemical profiling of individual lipid droplets by hyperspectral CARS microscopy in living human adipose-derived stem cells. Analytical Chemistry 88(7), pp. 3677-3685. (10.1021/acs.analchem.5b04468)

Karuna, A. et al. 2016. Hyperspectral volumetric coherent anti-Stokes Raman scattering microscopy: quantitative volume determination and NaCl as non-resonant standard. Journal of Raman Spectroscopy (10.1002/jrs.4876)

Masia, F. et al. 2015. Hyperspectral image analysis for CARS, SRS, and Raman data. Journal of Raman Spectroscopy 46(8), pp. 727-734. (10.1002/jrs.4729)

*Pope, I. et al. 2014. Coherent anti-Stokes Raman scattering microscopy of single nanodiamonds. Nature Nanotechnology 9(11), pp. 940-946. (10.1038/nnano.2014.210)

Di Napoli, C. et al. 2014. Chemically-specific dual/differential CARS micro-spectroscopy of saturated and unsaturated lipid droplets. Journal of Biophotonics

7(1-2), pp. 68-76. (10.1002/jbio.201200197)

Di Napoli, C. et al. 2014. Hyperspectral and differential CARS microscopy for quantitative chemical imaging in human adipocytes. Biomedical Optics Express 5 (5), pp. 1378-1390. (10.1364/BOE.5.001378)

Masia, F., Borri, P. and Langbein, W. W. 2014. Sparse sampling for fast hyperspectral coherent anti-Stokes Raman scattering imaging. Optics Express 22 (4), pp. 4021-4028. (10.1364/OE.22.004021)

Zumbusch, A., Langbein, W. W. and Borri, P. 2013. Nonlinear vibrational microscopy applied to lipid biology. Progress in Lipid Research 52(4), pp. 615-632. (10.1016/j.plipres.2013.07.003)

Li, B., Borri, P. and Langbein, W. W. 2013. Dual/differential coherent anti-Stokes Raman scattering module for multiphoton microscopes with a femtosecond Ti:sapphire oscillator. Journal of Biomedical Optics 18(6), article number: 066004. (10.1117/1.JBO.18.6.066004)

Pope, I. et al. 2013. Simultaneous hyperspectral differential-CARS, TPF and SHG microscopy with a single 5 fs Ti:Sa laser. Optics Express 21(6), pp. 7096-7106. (10.1364/OE.21.007096)

Rocha-Mendoza, I., Borri, P. and Langbein, W. W. 2013. Quadruplex CARS micro-spectroscopy. Journal of Raman Spectroscopy 44(2), pp. 255-261. (10.1002/jrs.4181)

Masia, F. et al. 2013. Quantitative chemical imaging and unsupervised analysis using hyperspectral coherent anti-Stokes Raman scattering microscopy. Analytical Chemistry 85(22), pp. 10820-10828. (10.1021/ac402303g)

Masia, F., Langbein, W. W. and Borri, P. 2012. Measurement of the dynamics of plasmons inside individual gold nanoparticles using a femtosecond phase-resolved microscope. Physical Review B: Condensed Matter and Materials Physics 85(23), pp. 235403-235413. (10.1103/PhysRevB.85.235403)

Present Employer:	Cardiff University
Present Department:	School of Biosciences
Present Position Start Date:	01/09/2011
Present Position End Date:	31/08/2031
Present Position Description:	Full Professorship, Cardiff University funded (permanent post). Wolfson Research Merit Award holder since Jan 2015 (5-years award). EPSRC Leadership Fellow 2010-2015.
Pending Applications:	Co-applicant in responsive mode application to BBSRC; ref n. BB/P007511/1 title "Lipid droplets in oocytes: shedding new light on why fats are good or bad for development." PI: Prof Karl Swann (Cardiff School of Biosciences)

Existing grants:

10/2013-09/2017 Workpackage Leader of EU Marie Curie Multi-partner ITN "FINON-Nonlinear Optical Nanoscopy" under the FP7-PEOPLE-2013-ITN call - Euro 3,470,652 (585,513 to Cardiff)

10/2014-09/2018 Principal Investigator of BBSRC iCase PhD studentship award BB/L015889/1 "Novel plasmonic nanoparticles for applications in quantitative biosensing and bioimaging" £ 94,126

08/2016-07/2019 Joint Lead Applicant in CRUK Multidisciplinary Project Award "Using CARS microscopy to realise the potential of 3D culture for personalised medicine and drug discovery" £ 403,597

Organisation

Cardiff University

Proposal

Subject: Subject Group 06: Biochemistry, structural biology and molecular cell biology / Cell biology (including molecular cell biology)

Project Title: Addressing the dietary needs of Kazakhstan: Developing low-cost omega-3 fatty acid production by microalgae using CARS microscopy

Keywords: optical microscopy, lipids, nonlinear optics, quantitative chemical imaging

Rationale & Motivation: This proposal addresses the global challenge of sustainable health and well-being and will contribute to the theme of sustainable local research and innovation capacity. We will specifically collaborate with the ODA country of Kazakhstan. Despite recent improvements in healthcare in response to Kazakhstan 2030 and other state-mandated policy reforms, Kazakhstan still lags behind on key indicators of health and economic development. The World Health Organization (WHO) health profile for Kazakhstan estimates the average life expectancy at birth to be only 65 years (men at 59 years). According to WHO, non-communicable chronic diseases accounted for 85% of all deaths in Kazakhstan, with ischaemic heart disease being the leading cause (57%). The link between poor quality of diet and cardiovascular diseases is well known. A recent report (Lancet Glob Health 2015; 3: e132–42) showed that Kazakhstan has one of the worse dietary patterns worldwide, significantly lacking consumption of healthy items including polyunsaturated fatty acids (PUFAs), and in particular omega-3s.
In this project we will address the dietary need of Kazakhstan for essential omega-3 PUFAs. In high-income developed countries, presently these are consumed mainly through oily fish such as salmon, tuna, and mackerel. Kazakhstan as a lower income continental country is severely limited in that respect. Moreover, overfishing is a growing global concern for the sustainability of the environment. Microalgae are a promising alternative to oily fish, as a primary vegetarian source of omega-3s. They are easy to grow in large quantities at low cost, and can have a high yield of PUFA accumulation. In this project, in collaboration with the faculty of Biology and Biotechnology in Al-Farabi Kazakh National University we aim to develop a new chemical method to analyse and, thus, control levels of PUFA in cultured algae. This will allow their proper commercial exploitation as sources of low cost omega-3 supplements.

Start Date: 05/12/2016

End Date: 04/12/2017

Research proposal: See attachment

Programme of work: Month 1: Receive 4 batches of purified cultures from Kazakhstan for immediate analysis, and pre-cultured microalgae to be grown in Cardiff for time-course experiments.
Months 1-4: Measure CARS chemical maps for the 4 strains and compare with 2 popular algae used for PUFA production in Europe.
Months 4-8: Monitor by CARS the lipid accumulation over time in living algae. We will elucidate the relationship between cultivation period and content and spatial localisation of specific FAs.
Month 8: Visit our lab by a researcher from our collaborators in Kazakhstan for knowledge exchange.
Month 8-12: Identify best strategies to achieve an increased omega-3 production using N and P deprivation, and temperature stress (relevant to growth in open-ponds in the summer months).
We will have weekly meetings (remote video calls) with the collaborators in Kazakhstan, and a final project meeting in month 12 to review the outcomes and discuss future developments.

Potential applications: We will use a novel non-invasive chemical analysis technique (CARS/FSC3 microscopy) to determine, and in turn control, lipid production in intact living algae for the first time. This will have a major impact in addressing the dietary need for omega-3 PUFAs, presently consumed mainly through oily fish in developed countries, and their shortfall in a lower income land-locked country such as Kazakhstan. Moreover, farmed fish need a ready supply of omega-3 PUFAs. For these reasons there is increasing global interest in the direct utilisation of algae oil both as nutraceuticals and as a supplement for farmed fish.
Notably, the application of CARS microscopy to lipid production in microalgae is a promising new development which could boost the commercial exploitation of this microscopy technology, beyond academic research. This grant will help extending the internationally leading role of our lab in the development and application of CARS/FSC3 microscopy to unmet needs in biochemistry.

Collaborations: We will collaborate with the Faculty of Biology and Biotechnology in Al-Farabi Kazakh National University (Drs. Tsurkan, Karpenyuk and Orazova). They will provide us with 4 strains of microalgae isolated from freshwater sources in Kazakhstan. They have expertise in the cultivation and study of these species and have previously collaborated with Dr Irina Guschina (IG) and Prof John Harwood (JH) from Cardiff University, on the subject of lipid analysis from microalgae (see Ref.[4] in research proposal). They will be involved throughout the project via weekly progress meetings (remote video calls), and will send a researcher to Cardiff for knowledge exchange (see Letter of Support).
JH and IG at Cardiff University School of Biosciences will provide in-house expertise on the lipid analysis of microalgae by traditional lipid extraction and chromatographic methods.
Prof Wolfgang Langbein from Cardiff University School of Physics will collaborate with Prof Borri on the CARS data analysis.

Outline of Data Management and Data Sharing Plan:	The directly acquired data will consist of 3D volumetric images and hyperspectral images (CARS intensity versus 3D spatial coordinates and wavenumbers). Typically these data sets are large (10^6-10^9 bytes). They will be stored in a standard file format (ASCII for direct data, TIFF for images) with metadata about the acquisition settings. Data will be stored on University servers. Cardiff University has recently invested over £200k in developing a resilient, replicated storage system that provides a current capacity of 200TB. Secondary users of the data will be other scientists or microscope companies. Data sharing will be implemented directly from the originators via a local web-accessible database. Sharing via a third-party will occur through publication in open access scientific journals and image databases. We will also use the publically-accessible Open Microscopy Environment (http://www.openmicroscopy.org) to organise large datasets and make them available online. Within this environment, we will be using OMERO client-server software for visualization, management and analysis of images. The University servers currently run an OMERO server. The Science Commons Open Access Data Protocol will be employed to ensure that the data can be legally integrated in public databases. We aim to release the datasets with the publication of the results. Data will be stored/archived locally for at least 10-15 years.
Use of Animals in Research:	No
Details of Animal Licence:	
Field Research Overseas -permission:	
Field Research Overseas -collection of specimens:	
Lay Report:	

In this project, we aim to address the dietary need of Kazakhstan for essential omega-3 (n-3) fatty acids (FAs). Specifically, we will employ cross-disciplinary approaches and develop a new quantitative chemical microanalysis method to analyse and, in turn, regulate the accumulation of n-3 fatty acids in several microalgal strains available in the country.
In the last decade, it has become clear that dietary polyunsaturated fatty acids (PUFAs) deficiency is linked to major diseases such as arthritis, cancer, cardio-vascular disease and dementia. It is also recognised that the ratio of dietary n-3/n-6 PUFA is important with most populations consuming less n-3 PUFAs than is advised. Essential FAs are produced by photosynthetic organisms and the long chain PUFAs recommended in most diets are produced mainly by algae. These algae are at the base of food chains and, when they are consumed by fish, their PUFAs accumulate in fish oils. This is the source of most of the n-3 PUFAs consumed in developed countries. However, the demand for fish oils is outstripping supply and the situation is currently unsustainable.
Kazakhstan is a lower income land-locked country, and its population has a poor diet with few vegetables and fish consumed. Even rice is usually white rice with the nutritious bran (and its oil) removed. Not surprisingly, life expectancy especially for men is poor (59 years). Moreover, cardio-vascular disease is very common (57% of deaths). Since low dietary n-3 PUFAs are directly linked to cardio-vascular disease there is an urgency to increase their consumed levels in the country, using low-cost available algae supplies.
In this project we will use a novel non-invasive technique (called CARS/FSC3 microscopy) to examine, and in turn control, lipid production in intact living algae for the first time. The method can quantify lipid contents and distinguish between different types of lipid. It will be used to evaluate growth conditions that give rise to increased quantities of desirable oils. Specific objectives and time-scale are: 1) Build a data-base of lipid chemical maps from CARS/FSC3 microscopy in 4 strains of microalgae available from Kazakhstan, together with 2 ‘control’ species and correlate data with standard biochemical analysis of acyl lipids (Month 1-4). 2) Monitor lipid accumulation over time on living algal cells using CARS/FSC3 time-lapse microscopy (Month 4-8). 3) Investigate nitrogen (N) and phosphorus (P) deprivation, together with temperature alteration, as stresses which change n-3 PUFA production (Month 8-12).
By identifying the best strategy for increased n-3 production in different algal species abundant in Kazakhstan, we will be able to stimulate the local economy for the future supply of good quality fats. In addition, collaboration with the Al-Farabi Kazakh National University will allow their scientists the benefit of accessing a unique chemical imaging technique pioneered in our lab, beyond state-of-the-art.

Financial Details

Financial Details:

Year	Payment type	Justification	Amount Requested
Year 1	Consumables	Chemicals for lipid extraction and gas chromatography; optical microscopy consumables	**11,000.00**
Year 1	Equipment	NA	**0.00**
Year 1	Travel UK	NA	**0.00**
Year 1	Travel International	Travel and subsistance to host in Cardiff for 1 month a researcher from Kazakhstan	**3,000.00**
Year 1	Other Expenses	Indirect and Estates costs (100% FEC)	**54,756.00**
Year 1	Research Costs	NA	**0.00**
Year 1	Research Assistant Salary	Salary for 12 months	**41,830.00**
Total			**110,586.00**

Sum requested from the Royal Society:	91269.00
Other Funding Sources:	There are no other funding sources sought or received for the proposed project
Justification of expenses:	We are requesting the salary of a post-doctoral research assistant for 12 months in order to carry out the measurements and data analysis for this project (at 80% FEC; £33464 salary plus the associated indirect and estates costs of £43805). We already have a suitable candidate available (hence no barrier to the timely start of this project). This would be Dr Lukas Payne who has a Masters of Physics with Photonics (Honours) from St Andrews University, and finished his PhD project in September 2015 at Cardiff University in Borri's lab (see attached CV). He has contributed to the latest optical microscopy technology developments of the laboratory, including CARS as well as other nonlinear microscopy techniques (resonant four-wave mixing), with emphasis on nanostructures and high spatial resolution imaging. He is ideally suited to carry out the CARS work in this project. We are also requesting £7000 for chemical consumables needed for algae culture and to carry out the lipid extraction and chromatographic work, and £4000 for CARS and optical microscopy consumables (small optomechanics and optical filters, imaging gaskets, glassware, cleaning optics tools and chemicals). Furthermore, we are requesting £3000 for travel and subsistence costs to host a researcher from our collaborators' group in Kazakhstan to visit our lab for four weeks (at month 8 of the project) in order to gain first-hand knowledge and experience of the novel methods developed in this project. We plan for this to be Dr. Yana Tsurkan who has already been to Cardiff as part of her post-graduate thesis work and, because of her prior knowledge of lipid biochemistry would rapidly be able to gain experience of CARS techniques. Beyond the lifetime of this project, we wish to sustain this knowledge exchange via programmes specifically available through the UK-Kazakhstan Newton fund (mobility grant, workshops). Total sum requested from the Royal Society (at 80% FEC research assistant salary): £91269.
Other Staff Costs:	Yes
Total Number Purchased Animals:	0
Total cost of purchased animals:	0
Total Procedure Cost:	0.00
Animals Total Cost:	0.00

Project Description

1. Aims and objectives: In this project, in collaboration with the faculty of Biology and Biotechnology in Al-Farabi Kazakh National University, we aim to address the dietary need of Kazakhstan for essential omega-3 fatty acids (FAs). Specifically, we will employ **cross-disciplinary approaches** and develop a new chemical method to analyse and, in turn, regulate the **accumulation of omega-3 fatty acids** in several **microalgal** strains available in the country.

Omega-3 FAs are known to be essential in the healthy human development (for example of the brain and the eye), and to play an important role in the prevention and treatment of a number of very important inflammatory conditions including ischemic heart disease [1,2]. To act physiologically, these FAs need to be 20C or 22C in length and most dietary advice is to consume significant quantities of such long chain polyunsaturated fatty acids (PUFAs). At present, long chain omega-3 PUFAs are provided for human consumption mainly in the form of fish oils, which is expensive for a continental country such as Kazakhstan. Moreover, overfishing is a growing global concern for the sustainability of the environment. Since the primary source of these PUFAs is microalgae, they are a most promising target for research into low-cost alternatives to oily fish. Notably, microalgae can produce high amounts of long chain PUFAs, in contrast to land plants, especially in response to different growth conditions [3].

We will investigate microalgae available from our collaborators [4], isolated from freshwater sources in Kazakhstan. Four strains, including *Oocystis rhomboideus*, will be used (chosen for their PUFA contents), and compared to two promising species already under development in Europe - the marine *Phaeodactylum tricornutum* or *Pavlova lutheri* and the freshwater *Nannochloropsis limnetica* (useful for aquaculture) [5]. At Cardiff University School of Biosciences there is long-standing expertise in studying the lipid content of microalgae (Prof John Harwood and Dr Irina Guschina), and in identifying conditions causing maximum production of important long-chain polyunsaturated omega-3 and omega-6 FAs in these species [3,6]. Complementary to this expertise, based on traditional lipid extraction where algal cells are destroyed, my laboratory has recently developed a novel tool to **image non-invasively lipids in intact living specimens** and quantify the lipid content and chemical composition with high sensitivity and spatial resolution. The technique is based on Coherent Antistokes Raman Scattering (CARS) microscopy. It uses the interaction of light with vibrating chemical bonds which produces light scattered at a different wavelength (hence colour) compared to the incident light. The wavelength shift is a direct signature of the frequency of the vibration, which in turn depends on the type of chemical bond. Although this scattering phenomenon usually produces a very weak signal, it can be enhanced when two short laser pulses are used to coherently drive the vibrations so that the scattered light from all bonds of the same type in the focal volume constructively interfere, generating CARS. In my lab, we are developing innovative CARS excitation/detection schemes beyond state-of-the-art, fully home-built including quantitative data analysis software. This technology development is the main focus of my research and the Wolfson Research Merit Award. We have shown that CARS microscopy is successful in imaging unstained lipids, and quantifying their content and chemical composition, in many living cell types, including human stem cells differentiating into adipocytes, and mouse eggs and embryos (see Publication section; note papers in high-impact journals including Nature Nanotechnology, Development, and Analytical Chemistry). Importantly, the technique provides unprecedented quantitative information on living specimens, not available with fluorescence labelling methods, since lipid stains are often non-specific, can cause artefacts and suffer from photobleaching.

In this project, we will investigate the production and accumulation of omega-3 PUFAs in living algal cells using our latest-generation CARS microscope. Preliminary data (see figure) indicate the potential of the technique to image the lipid content in two strains of algae (*Chlorella sorokiniana* and *Myrmecia bisecta*), despite the large autofluorescence background from pigments in these species. We have specifically developed a data analysis algorithm (called FSC^3) which can noise-filter and decompose the acquired images into spatially-resolved maps of concentrations of chemical components, offering the most advanced quantitative analysis tool for this type of data to date. Within this project we will use optimised measurement and data analysis conditions (illumination power, wavelength, detection filters, data processing) to achieve the best chemical selectivity for polyunsaturated fatty acids especially omega-3 PUFAs in microalgae, and maximum sensitivity against backgrounds. Specific objectives, milestones and time-scale for this project are:

O1) Build a data-base of lipid chemical maps using CARS/FSC^3 microscopy in 4 strains of microalgae available from our collaborators in Kazakhstan, together with two 'control' species and correlate data with standard biochemical analysis of acyl lipids (Month 1-4). **Milestone 1**: Total lipid content (productivity) per dry weight and specific content of the FAs of interest in different lipid classes, alongside the lipid spatial distribution, quantitatively and statistically analysed.

O2) Monitor lipid accumulation over time in living algal cells using non-invasive CARS/FSC^3 time-lapse microscopy (Month 4-8). **Milestone 2**: Establish the relationship between cultivation period, lipid content and spatial localisation of specific FAs.

O3) Investigate nitrogen (N) and phosphorus (P) deprivation, together with temperature alteration, as stresses which change omega-3 PUFA production, using CARS/FSC^3 time-lapse microscopy; **Milestone 3:** Best strategy for increased omega-3 production identified (Month 8-12).

[1] Calder, P.C.(2003) N-3 polyunsaturated fatty acids and inflammation: from molecular biology to the clinic. Lipids 38, 343-352. [2] Das, U. (2002) The lipids that matter from infant nutrition to insulin resistance. Prostagland. Leuk. Essent. Fatty Acids 67, 1-12. [3] Harwood, J.L. and Guschina, I.A. (2009) The versatility of algae and their lipid metabolism. Biochimie 91, 679-684. [4] Tsurkan, Y., Karpenyuk, T., Guschina, I. et al. (2015) Identification of newly-isolated microorganisms containing valuable polyunsaturated fatty acids. J.Biotech Res 6, 14-20. [5] Freire, I., Cortina-Burgueno, A., Grille, P. et al. (2016) Nannochloropsis limnetica: a freshwater microalga for marine aquaculture. Aquaculture 459, 124-130. [6] Guschina, I.A. and Harwood, J.L. (2006) Lipids and Lipid metabolism in eukaryotic algae. Prog Lipid Res 45, 160-186.

2. Scientific impact and potential outcomes

With this work, we aim to develop a new non-invasive method to measure, and in turn optimise, the production and accumulation of omega-3 PUFAs in lipids of several microalgal strains available in Kazakhstan. This new method will enable the quantification of important parameters previously inaccessible in living specimens, namely lipid content and chemical composition, lipid spatial distribution within algal cells, and evolution over time. The strains from Kazakhstan will be compared with two of the most popular FA producing species available in Europe, already highlighted for development as commercial sources of omega-3 PUFAs. The use of CARS/FSC^3 as a novel non-invasive technique to follow algae while they are growing offers excellent new opportunities to control yields and maximise productivity. With the current high interest in omega-3 PUFAs, this is particularly timely. We anticipate that the outputs of this project will be publishable in high impact research journals in the field of biochemistry and chemical imaging.

Beyond research papers, potential outputs will include **stimulating research and innovation in Kazakhstan through our collaborators' visiting Cardiff** (see Programme of work) and gaining first-hand knowledge and experience with the latest generation of CARS microscopy. Beyond the lifetime of this project, we wish to sustain this knowledge exchange via programmes specifically available through the **UK-Kazakhstan Newton fund** (mobility grant, workshops).

The scientific outputs from this project will be key to demonstrating important preliminary work in this new area of research, leading to future larger collaborative project proposals under the **Global Challenge Research Fund (GCRF)**, with funding from **BBSRC and EPSRC**. These Research Councils have each received directly allocated portions of the GCRF, and the BBSRC is leading a co-funding partnership to support multidisciplinary awards for Global Agriculture and Food Systems.

This project at the biochemistry / nonlinear optics / quantitative image analysis interface will provide excellent **cross-disciplinary training and career progression opportunities** for the research assistant appointed and the visiting scientist from Kazakhstan.

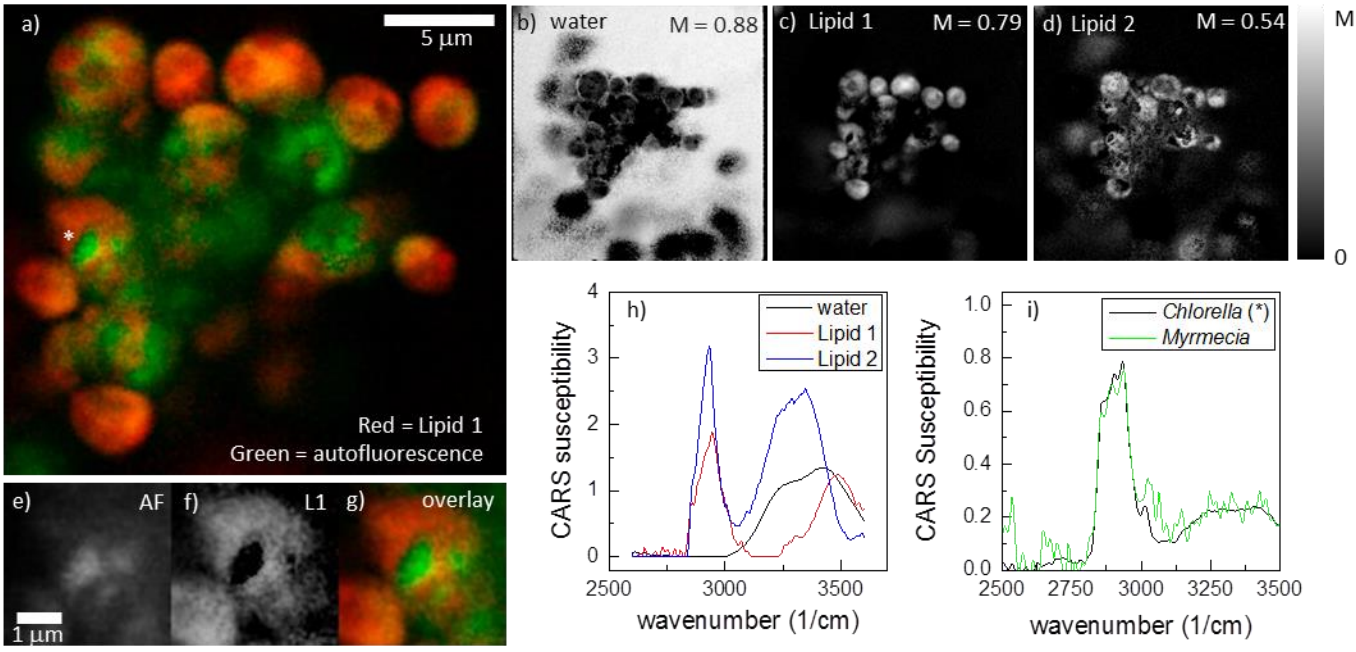

Fig.1. Hyperspectral CARS microscopy on living cells from *Chlorella sorokiniana* and *Myrmecia bisecta* after 2 weeks cultivation. a) 2D *xy* image showing an overlay of the spatially-resolved map of the lipid concentration (L1) and autofluorescence (AF) in *Chlorella sorokiniana.* b-c) Concentration maps in vol:vol units from 0 to M. Corresponding spectra suggesting two lipid types shown in (h). e-g) zoom over the region (indicated by *) of the AF and L1 maps, and corresponding overlay. i) Single-point spectra showing different lipid types in the two species, consistent with *Chlorella sorokiniana* being rich in α-linolenic acid (18:3n-3) and *Myrmecia bisecta* having a high arachidonic acid (20:4n-6) content, hence higher =CH band at 3010/cm.

Lukas M. Payne

MPhys, PhD

2940 Barndt Road, Telford, PA 18969 (US)
18 Cwrt Pen-Y-Bryn, Cardiff, Wales CF14 3LE (UK)
Cell: 215-272-0109 (US)
Cell: +44 (0)7528 590969 (UK)
Home: (215) 234-2580
Fax: (215) 234-2575
PayneL5@cardiff.ac.uk
Lpayne5@mac.com

EDUCATION

Cardiff University (School of Biosciences)
Cardiff, Wales
October 2011 – September 2015 (3.5 year **PhD** in lab of Professors Paola Borri and Wolfgang Langbein)
PhD Thesis Title: Optical extinction and coherent multiphoton micro-spectroscopy of single nanoparticles.
Thesis work included development of a novel experimental wide-field technique for the measurement of nanoparticle (NP) optical cross-sections, a novel spectroscopic extinction technique for measurements on individual NPs, four-wave mixing (FWM) imaging of metallic NPs, and coherent anti-Stokes Raman scattering (CARS) microscopy of nanodiamonds and gold NPs. The development of the wide-field technique included programming of a companion software for analysis of data. The method is capable of measurement of hundreds of NP optical cross-sections simultaneously, at the single particle level. A correlative study of metallic NP dimers using extinction spectrocopy, electron microscopy, and FWM imaging formed the initial effort in the development of an in vitro plasmon ruler using only FWM. CARS microscopy on nanodiamonds, correlated with the wide-field extinction technique, differential interference contrast (DIC) microscopy, and electron microscopy, provided proof of concept for use of non-fluorescent nanodiamonds as novel molecular markers. Some experimentation using CARS to study gold NPs coated with small molecules constituted a preliminary effort in the use of gold NPs to provide surface-enhanced CARS. This research contained a strong cross-subject component, which meant I needed to develop skills outside of my degree and comfort-zone, particular in inorganic chemistry. I have also spent over 100 hours demonstrating for undergraduates in laboratory classes.

St. Andrews University
St. Andrews, Fife, Scotland
Fall 2006 – June 2010 (Five year degree program completed in four years)
Honours Masters of Physics with Photonics
Thesis Title: Annealing Nanophotonic Waveguides to Reduce Losses
Thesis work included: annealing nanophotonic waveguides and photonic crystals, aligning a 1550nm centered laser source through the waveguides for power transmission measurements, and electron microscopy with high resolution such that waveguide surface roughness ≤30nm was visible.
Higher-level courses most pertinent to my degree classification are Photonic Applications, Biophotonics, Principles of Optics, Computational Physics, Lasers (1&2), Non-linear Optics and Optoelectronics, and Electronics, as well as others. Additional Mathematics and Biology modules outside of the physics curriculum completed.

Germantown Academy
Fort Washington, Pennsylvania
Fall 1995- Summer 2006 - (Grades 2-12)
Honours Physics, Chemistry, and Biology. Senior year Advanced Placement courses in Biology, Physics, Calculus, and French 5.
Cum Laude Society inductee

WORK EXPERIENCE

April 2016 – **Cardiff University Biophotonics Group**
I was hired as a research associate in the lab of Professors Paola Borri and Wolfgang Langbein. During this time, I was tasked with continuing work on the application of metallic NP dimers and FWM towards an in vitro plasmon ruler. This work included some further extinction spectroscopy, but also progressed the analytical aspect of the study. Furthermore, I have worked on a wider study of optical cross-sections and spectra of various species of nanoparticles using the wide-field extinction technique and extinction spectroscopy. The new NPs of interest include, silver nanocubes, carbon nanotubes, cadmium selenide nanoplatelets, quantum dots and silver NP dimers.

Lukas M. Payne

July 2015 – Sept 2015 **Cardiff University Biophotonics Group / BBI Solutions**

This was a secondment hosted by BBI Solutions and the lab of Professors Paola Borri and Wolfgang Langbein, whereby we used the wide-field optical technique to characterize the optical cross-sections of several batches of different nominal diameter gold nanoparticles. The measured cross-sections were used to determine the ensemble particle radii and associated measurement error. This data was compared to transmission electron microscopy and dynamic light scattering measurements of the ensemble particle diameters of the different particle batches. The comparison of the three methods indicated that our technique is an effective and accurate tool for the measurement of particle sizes, comparable to industry-standard techniques. It also provides optical information not obtained by the standard methods.

June 2009 - July 2009 **St. Andrews University Biophotonics Group**

Summer Internship in Biophotonics Research

Research work involved using pulsed/mode-locked lasers to destroy selected single cells, in an effort to study cell growth in a continuous single-cell sequence. Also critical to the work were mammalian cell culture techniques, single cell seeding, long-term observation, and time-lapse video recording. Laser work involved optical rig design and modification. Additional work involved Phase Contrast microscope assembly and usage. The designing and building an optical tweezers rig was begun, but time did not allow for its completion.

June 2008 - September 2008 **Merck & Co., Inc.** - West Point, Pennsylvania

Summer Internship - Vaccine Research Department. Research subject involved the *C. diffiçile* bacteria and potential vaccine targets. Responsible for running Cytotoxicity tests using flow cytometry, to confirm vaccine candidate efficacy. Conducted bead calibration for validation on flow cytometer and data analysis. Microscopic imaging version of the cytotoxicity test was also investigated.

June 2007 - September 2007 **Merck & Co., Inc.** - West Point, Pennsylvania

Summer Internship - Vaccine Research Department. Work on *C. diffiçile* toxins A & B includes fluorescence labelling, spectrophotometry, Flow Cytometry, SDS PAGE, and Mammalian Cell Culture. Laboratory techniques and research performed required literature search and the application of ideas based on the chemical and physiological properties of the toxins. By the end of the internship, an assay was developed to determine the effectiveness and nature of the vaccine. Often engaged sponsor (*C. diffiçile* vaccine group leader) in discussions based on the nature of vaccine development, and other research.

VOLUNTARY WORK

May 2005 - Spent two weeks in New Orleans helping with the reconstruction efforts after Hurricane Katrina. Work included helping in a kitchen (serving free food and cooking), landscaping, and house rebuilding/breakdown.

Other - Helped high school in various voluntary efforts.

OTHER SKILLS

Experience in LabView, Maple, Mathematica, Python, and ImageJ Macro programming.

Extensive experience with Macintosh OS X, and associated software.

Experience with Windows OS, Microsoft Word, Power Point and Excel.

Intermediate skill with spoken and written ***French***

PUBLICATIONS

1. Payne, Lukas M. Optical extinction and coherent multiphoton micro-spectroscopy of single nanoparticles. PhD Thesis, Cardiff University. 2015.
2. L. Payne, G. Zoriniants, F. Masia, K. Arkill, P. Verkade, D. Rowles, W. Langbein, and P. Borri. Optical micro-spectroscopy of single metallic nanoparticles: quantitative extinction and transient resonant four-wave mixing. *Faraday Discuss.*, 2015. DOI: 10.1039/C5FD00079C
3. I. Pope, L. Payne, G. Zoriniants, E. Thomas, O. Williams, P. Watson, W. Langbein, and P. Borri. Coherent anti-Stokes Raman scattering microscopy of single nanodiamonds. *Nature Nanotechnology*, 9(11):940-946, 2014. DOI: 10.1038/nnano.2014.210
4. L. Payne, W. Langbein, and P. Borri. Polarization-resolved extinction and scattering cross-sections of individual gold nanoparticles measured by wide-field microscopy on a large ensemble. *Applied Physics Letters*, 102(13), 2013. DOI: 10.1063/1.4800564

Relevant Conferences Talks

1. L. Payne oral presentation on publication 3 at NaNaX6 - Nanoscience with nanocrystal, Bad Hofgastein, Austria, 18-23 May, 2014.

I have given 1 more oral presentation at Photon14 (Imperial College London), and 3 poster presentations, which are here unlisted as they are only in reference to the extinction microscopy.

ҚАЗАҚСТАН РЕСПУБЛИКАСЫ
ӘЛ-ФАРАБИ АТЫНДАҒЫ
ҚАЗАҚ ҰЛТТЫҚ
УНИВЕРСИТЕТІ

БИОЛОГИЯ ЖӘНЕ
БИОТЕХНОЛОГИЯ ФАКУЛЬТЕТІ

050038, Алматы қаласы, әл-Фараби данғылы,71
Тел. 377-33-34 кос.1201

РЕСПУБЛИКА КАЗАХСТАН
КАЗАХСКИЙ НАЦИОНАЛЬНЫЙ
УНИВЕРСИТЕТ
ИМ. АЛЬ-ФАРАБИ

ФАКУЛЬТЕТ БИОЛОГИИ И
БИОТЕХНОЛОГИИ

050038, г. Алматы, проспект аль-Фараби, 71
Тел. 377-33-34 доп.1201

****

Royal Society Challenge Grants application

"Addressing the dietary needs of Kazakhstan: Developing low-cost omega-3 fatty acid production by microalgae using CARS microscopy" by Prof Paola Borri at Cardiff University

Letter of Support

We strongly support the proposed collaborative project by Prof Paola Borri with our group (Drs. Tsurkan, Karpenyuk and Orazova) at the Faculty of Biology and Biotechnology in Al-Farabi Kazakh National University.

This project will set a stepping stone in identifying the best strategy for increased omega-3 production in different algal species abundant in Kazakhstan, and will help stimulate the local economy for the future supply of good quality fats.

We are happy to **provide Prof Borri with four strains of microalgae isolated from freshwater sources in Kazakhstan**. We have expertise in the cultivation and study of these species and have previously collaborated with Dr Irina Guschina and Prof John Harwood from Cardiff University on the subject of lipid analysis from microalgae (see Tsurkan, Y., Karpenyuk, T., Guschina, I. et al. (2015) Identification of newly-isolated microorganisms containing valuable polyunsaturated fatty acids. J.Biotech Res 6, 14-20).

We look forward to be actively involved throughout the project via weekly progress meetings (remote video calls). Moreover, **we will send a researcher at month 8 of the project to Cardiff University** to gain first-hand knowledge and experience with the latest generation of CARS microscopy technology available in Prof Borri laboratory. This will be a unique opportunity for knowledge exchange, and to **discuss medium/longer term plans of introducing such technology development into our laboratories**.

Beyond the lifetime of this project, we are keen to **sustain this knowledge exchange** via programmes specifically available through the **UK-Kazakhstan Newton fund**. We are also keen to engage with our local government agencies to **push the impact of this work toward making a real advance in Kazakhstan's nutritional system**.

We very much hope that Prof Borri's application will be successful and look forward toward starting this promising and exciting collaboration

Sincerely,
Prof Tatyana Karpenyuk,
Faculty of Biology and Biotechnology
Al-Farabi Kazakh National University
5 August 2016