Biointerphases Focus Topic: Bioimaging
Room: 23 - Session BN+AS-WeA

Bioimaging
Moderator: M. Grunze, University of Heidelberg, Germany

2:00pm **BN+AS-WeA1 Label-free Non-Invasive Imaging of Live Cells by Raman Micro-Spectroscopy**, ***I. Notingher***, University of Nottingham, UK **INVITED**

Stem cells have enormous potential for cell replacement therapies in curing age-related illnesses such as Alzheimer's and Parkinson's disease, as well as diabetes and cardiovascular disorders. In addition, they are a reliable cell source in tissue engineering and stem cell seeded scaffolds could provide an unlimited supply of grafts to replace and repair diseased tissues.

However, the current conditions to derive specific cell types remain suboptimal, generally producing only low yields of the desired differentiated lineages within highly heterogeneous populations that are not suitable for clinical use due to the presence of mainly unwanted cell types. This current limitation in the delivery of validated stem cells suitable for clinical applications, highlights the immediate need for non-invasive techniques capable of phenotypic identification of live cells within highly heterogeneous populations.

Raman micro-spectroscopy (RMS) is a label-free technique which can be used for imaging of live cells. This technique combines the high chemical specificity of Raman spectroscopy with the high spatial resolution of optical microscopy to provide detailed molecular information of complex biological samples. Since RMS has only a minimal background signal from water, it allows repeated observations of viable cells maintained under physiological conditions, which is difficult by other molecular vibrational techniques.

In the first part we will focus on using RMS to detect molecular markers for individual live cardimyocytes (CMs) derived from human embryonic stem cells (hESCs). The ability to monitor and quantify these spectral markers during differentiation periods as long as 5 days is also demonstrated. The analysis of Raman spectra of hESC-derived CMs were characterised by increased signals associated to myofibrils and glycogen compared to the other differentiated cells present in the cultures. The prospects of label-free Raman activated cell sorting are also discussed.

The second part will present results on using RMS for imaging and quantifying spectral markers in neuronal stem cells (NSCs). Raman spectra of undifferentiated NSCs are compared to those of glial cells derived from NSCs, with the aim to identify molecular markers which can be used for assessing the differentiation status of the NSCs. High resolution spectral maps corresponding to nucleic acids show that NSCs are characterized by increased concentrations of cytoplasmic RNA.

These studies demonstrate that RMS represents a feasible approach for label-free non-invasive characterization of individual live cells and can be used to assess the differentiation status and the phenotypes of individual cells.

2:40pm **BN+AS-WeA3 Perfluoropentane Gas and Liquid Filled Hollow Silica Micro/Nano Spheres for Ultrasound Guided Surgery and HIFU Therapy**, ***A. Liberman***, *H.P. Martinez, Z. Wu, C.V. Barback, S.L. Blair, Y. Kono, R.F. Mattrey, W.C. Trogler, A.C. Kummel*, University of California San Diego

The reported positive margin rate from wire localized excisions of breast cancers is approximately 20-50%; however, by preoperatively injecting a radio active seed into the tumor under CT guidance, the excision rate is halved because the surgeon can constantly reorient the dissection to place the seed in the center of the specimen. Unfortunately, radioactive seed localization has several safety challenges , only single foci can be localized, and incisions are required to implant the seeds, so it is rarely employed. As a safe alternative, gas-filled hollow Fe-doped silica particles have been developed, which can be used for ultrasound-guided surgery even for multiple focii. The function of the Fe doping is to render the silica shells biodegradable. The particles are synthesized through a sol-gel method on a polystyrene template, and subsequently calcined to create hollow, rigid microspheres. The Fe-doped silica shell is derived from tetramethoxy orthosilicate (TMOS) and iron (III) ethoxide, which forms a rigid, mesoporous shell upon calcination. The microshells are filled with perfluoropentane (PFP) vapor or liquid. The flourous phase is contained within the porous shell due to its extremely low solubility in water. Considerable testing of particle functionality, signal persistence and acoustical properties have been performed in various phantoms including ultrasound gel, chicken breast, and excised human mastectomy tissue. *In vitro* studies have shown that continuous particle imaging time is up to approximately 45 minutes, and will persist for over five days. Furthermore, preliminary *in vivo* particle injection longevity studies have been performed in a rabbit model which are consistent with *in vitro* data showing signal presence even five days post injection. These silica spheres may be used a sensitizing agent in high intensity focused ultrasound (HIFU). Traditional ultrasound agents pose several potential drawbacks such as poor *in vivo* persistence (minutes) and high risk (cardiac complications) during continuous perfusion. Preliminary *in vitro* results in HIFU ablation in an agar tissue phantom model suggest that very few particles are needed in order to develop a sensitizing effect to HIFU (approx. 1-10 µg/ml particles/agar varying by particle size). A novel technique has been developed to fill the particles with perfluorocarbon liquid which vaporizes upon exposure to HIFU thereby increasing the sensitivity compared to gas filled particles.

3:00pm **BN+AS-WeA4 Differentiation of Breast Cancer Cell Lines with ToF-SIMS**, ***L.J. Gamble***, *M. Robinson*, University of Washington, *F. Morrish, D. Hockenbery*, Fred Hutchinson Cancer Research Center

Cancer is a heterogeneous malignancy that manifests itself in a variety of morphological types and clinical outcomes. Tumor metabolism plays a large role in cancer onset and progression, and its causes and effects are under intense scrutiny. Time-of-flight secondary ion mass spectrometry (ToF-SIMS) has been increasingly utilized for examining biological samples including biomaterials, cells, and tissues. The incorporation of cluster ion sources has allowed the detection of many high mass organic species that can be used to characterize biological surfaces. In combination with principal component analysis (PCA), we use ToF-SIMS to determine differences in the chemical makeup of eight different breast cancer cell lines. Four cell types are of the triple-negative (TN) phenotype, and four cell types are the luminal phenotype. Spectra have been acquired on an IONTOF TOF.SIMS V using Bi_3^+ before and after C_{60} etching. Using positively charged mass fragments, many of the cell lines can be separated from one another within a 95% confidence interval, with only two TN lines overlapping. Biological significance of the loadings peaks will be discussed with species such as diacylglycerols and cholesterol playing a role in the separation. This work is the foundation for future studies using human tumor biopsy samples that will help elucidate the link between fatty acid composition within a tumor and the potential drug resistance of that tumor.

4:00pm **BN+AS-WeA7 Biological Applications of Lipid Imaging with Cluster-TOF-SIMS and MALDI-TOF**, ***A. Brunelle***, CNRS, Institut de Chimie des Substances Naturelles (ICSN), France **INVITED**

Time-of-Flight Secondary Ion Mass Spectrometry (TOF-SIMS) using keV energy metal cluster beams as primary ions is now recognized as a powerful method for *in situ* chemical, biological and medical applications [1,2]. It opens a new field of surface imaging, particularly for biological tissue sections. Compared to the more established MALDI (Matrix Assisted Laser Desorption Ionisation) imaging approach, TOF-SIMS imaging provides the incomparable advantages of a routine micrometre scale resolution and of an easy sample preparation which does not require matrix coating of the surface. However TOF-SIMS suffers from some limitations, such as the narrow mass range, the lack of structural analysis of the species by tandem mass spectrometry, and the fact that mainly lipids are preferentially released from the biological samples.

This lecture intends to show the wealth of powerful information that can be obtained from the chemical analysis of biological surfaces, with several examples chosen among various applications such as the localization of xenobiotics, natural substances, lipid markers from genetic diseases and non-alcoholic fatty liver disease. The strengths and weaknesses of lipid imaging using TOF-SIMS and MALDI-TOF will also be compared, showing the complementarity between the two methods.

Tissue imaging using TOF-SIMS can also be associated with histology for medical diagnosis in order to correlate structural features with ion images. The possibility to use the same tissue section for both histology and mass spectrometry imaging has been tested. It is a major advantage in terms of sample preparation and precision on the histological structure localization, provided that none of the two methods disturbs the performances of the other [3].

Massive cluster ion sources of argon have recently been used for SIMS experiments [4]. These ion beams interact with surfaces with incomparable physical properties, and thus hold the promise for new opportunities such as organic depth profiling or large increases of sensitivity. Recent results will be presented showing the possibility of depth profiling in organic samples,

but also the enhancement of the sensitivity, using both massive clusters and bismuth for dual beam depth profiling.

[1] F. Benabdellah, A. Seyer, L. Quinton, D. Touboul, A. Brunelle, O. Laprévote, *Anal Bioanal Chem* 396 (2010) 151-162

[2] D. Touboul, O. Laprévote, A. Brunelle, *Curr Opin Chem Biol* 15 (2011) 725-732

[3] C. Bich, S. Vianello, V. Guérineau, D. Touboul, S. De La Porte, A. Brunelle, *Surf Interface Anal* in press DOI:10.1002/sia.4846

[4] H. Gnaser, K. Ichiki, J. Matsuo, *Rapid Commun Mass Spectrom* 26 (2012) 1-8

4:40pm **BN+AS-WeA9 ToF-SIMS Image Analysis of Mouse Diaphragm Muscle Cross-Sections**, ***D.J. Graham***, *N.P. Whitehead, S.C. Froehner, D.G. Castner*, University of Washington

Duchenne muscular dystrophy (DMD) is a common, X-linked, neuromuscular disease, caused by mutations in the dystophin gene. The absence of dystrophin in DMD patients causes progressive muscle degeneration, characterized by inflammation, fibrosis and failure of muscle regeneration. Profound muscle weakness ensues, ultimately leading to respiratory or cardiac failure, and death around the age of 20 to 30. The *mdx* mouse is a dystrophin-deficient animal model of DMD. In addition to being a model for DMD, *mdx* mice have been found to be resistant to obesity when fed a high fat diet.

In this study we used ToF-SIMS imaging to study the differences in lipid composition of skeletal muscle cross-sections of *mdx* and wild type mice. Results show differences in both the morphology of the tissue and the distribution of lipids within the tissue. For example the *mdx* mouse tissue shows a more loosely organized muscle fiber structure with fibrotic tissue formation, whereas the fibers in the wild type mice show more tightly packed muscle fibers. Understanding differences in lipid composition between these mice can hopefully provide new insight into differences in muscle lipid metabolism and help understand the metabolic pathways that protect *mdx* mice from diet-induced obesity.

5:00pm **BN+AS-WeA10 ToF-SIMS Characterisation of the Distribution and Permeation of Topically Applied Pharmaceuticals**, ***D.J. Scurr***, University of Nottingham, UK, *A. Judd,* Keele University, UK, *K. Wan,* University of Central Lancashire, UK, *J. Heylings,* Dermal Technology Laboratory Ltd., UK, *G. Moss*, Keele University, UK

Drug delivery through skin provides opportunities to reduce compliance challenges and is suited to use in environments in the developing world, e.g. micro needle patch delivery of vaccines. In this study, a widely applied chemical antiseptic, chlorhexidine is considered. This is utilised in low concentrations in topical skin treatments where permeation through the stratum corneum of the skin is of particular importance, as insufficient permeation leads to bacterial re-colonisation. Current standard practice for assessing the permeation of this, or any active pharmaceutical ingredient (API), through the upper skin layer is to firstly remove microscopic layers of pre-treated in vitro skin using adhesive tape (‘tape stripping’). The removed material is then analysed using high performance liquid chromatography (HPLC) to evaluate the API penetration. The sensitivity of HPLC often requires several tape strips to quantify the API. [1] Using this protocol, the permeation of the API is difficult to confirm and no information regarding lateral distribution is gleaned. Here, we develop a novel alternative method for assessing the permeation of this molecule within skin tissue and apply this protocol to assess the effectiveness of a permeation enhancing dendrimer pre-treatment.

In order to characterise permeation and lateral distribution of chlorhexidine through the skin, this study uses the high spatial resolution of time of flight mass secondary ion mass spectroscopy (ToF-SIMS) to analyse cross-sectioned skin. The samples are treated with chlorhexidine, cryomicrotomed and analysed by ToF-SIMS under cryo conditions. The results of these studies provide the specific localisation and real permeation depth of the chlorhexidine within the stratum corneum. Additionally, freeze dried tape stripped materials have also been analysed using ToF-SIMS as an independent cross-validation, the results from which support the observations made for the skin cross-sections. This ToF-SIMS methodology has also been applied to demonstrate the increased permeation of chlorhexidine following a dendrimer pre-treatment of the skin. In these samples the secondary ion fragments specific to the chlorhexidine structure are identified at higher intensities and are localised at increased depths within the skin tissue. This methodology which shows great promise in the development of transdermal delivery of pharmaceutics. The high lateral resolution and molecular specificity complement label based approaches such as confocal microscopy and optical approaches that can function in vivo such as stimulated Raman scattering microscopy.

[1] Wagner et al, (2002) J. Pharm. Sci., 91 (8)

5:20pm **BN+AS-WeA11 Coherent X-ray Microscopy of Vitrified Biological Samples**, ***A. Rosenhahn***, Ruhr-University Bochum, Germany, *T. Gorniak, T. Senkbeil, A. Buck, M. Beckers, M.H. Grunze*, Karlsruhe Institute of Technology, Germany

Coherent X-ray microscopy of hydrated biological samples – especially in the 'water window' at photon energies of 284–540 eV – is of tremendous interest for life sciences due to the high contrast of organic matter with respect to the aqueous background. We present recent progress in imaging of biological samples with coherent X-ray microscopy (holography and ptychography) and scanning X-ray nano/microprobe imaging. The main goal is application of these emerging techniques to the in-situ analysis of vitrified biological specimen. Ptychography uses coherent diffraction patterns at different sample positions while maintaining a fixed spatial overlap between the fields of view. By introducing this spatial redundancy to the data an additional constraint for the iterative reconstruction algorithm is achieved. This enhances the convergence of phase retrieval drastically and allows imaging of extended samples. We supplement these results with resonant ptychographic imaging at the oxygen K-edge core level resonances of test particles where both, absorption and phase shifts, revealed information about the chemical composition of the samples. First results on imaging of frozen hydrated specimen and samples in the liquid environment will be shown and discussed.

5:40pm **BN+AS-WeA12 Characterization of Nanoparticles Implanted into Tissues for Enhancement of Ion-Mobility Mass Spectrometry Surface Imaging of Sagittal Brain Sections**, *E.K. Lewis,* Ionwerks, Inc., *J.F. Moore,* MassThink, *T.F. Egan,* Ionwerks, Inc., *B. Chen, B. Brinson,* Rice University, *V.M. Womack, D. Barbacci,* Ionwerks, Inc., *R. Hauge,* Rice University, *A.S. Woods,* National Institute on Drug Abuse / IRP, ***J.A. Schultz***, Ionwerks, Inc.

Our research previously included the first demonstration of implanting nanoparticles nanometers below the surface of a biological tissue to provide a completely new method MALDI imaging. Now, we are working towards combining optical histology and molecular surface imaging. We are currently using x-ray photoelectron spectroscopy (XPS) to characterize the nanoparticle coverage, composition, and the depth nanoparticles are implanted into our biological tissue(s). Nanoparticles were implanted at energies from 2-6kV, initial XPS depth profiles demonstrated they are implanted into tissue at depths from 10-30nm. The use of nanoparticulate implantation provides a basis from which future surface imaging techniques can be developed and tested. This method is important in that not only are we preserving the optical histology, and location of lipids and peptides, but MALDI signals are increased on the order of 2-4 times over typical preparation with several orders of magnitude less matrix.

Authors Index

Bold page numbers indicate the presenter

— B —

Barbacci, D.: BN+AS-WeA12, 2
Barback, C.V.: BN+AS-WeA3, 1
Beckers, M.: BN+AS-WeA11, 2
Blair, S.L.: BN+AS-WeA3, 1
Brinson, B.: BN+AS-WeA12, 2
Brunelle, A.: BN+AS-WeA7, **1**
Buck, A.: BN+AS-WeA11, 2

— C —

Castner, D.G.: BN+AS-WeA9, 2
Chen, B.: BN+AS-WeA12, 2

— E —

Egan, T.F.: BN+AS-WeA12, 2

— F —

Froehner, S.C.: BN+AS-WeA9, 2

— G —

Gamble, L.J.: BN+AS-WeA4, **1**
Gorniak, T.: BN+AS-WeA11, 2
Graham, D.J.: BN+AS-WeA9, **2**
Grunze, M.H.: BN+AS-WeA11, 2

— H —

Hauge, R.: BN+AS-WeA12, 2
Heylings, J.: BN+AS-WeA10, 2
Hockenbery, D.: BN+AS-WeA4, 1

— J —

Judd, A.: BN+AS-WeA10, 2

— K —

Kono, Y.: BN+AS-WeA3, 1
Kummel, A.C.: BN+AS-WeA3, 1

— L —

Lewis, E.K.: BN+AS-WeA12, 2
Liberman, A.: BN+AS-WeA3, **1**

— M —

Martinez, H.P.: BN+AS-WeA3, 1
Mattrey, R.F.: BN+AS-WeA3, 1
Moore, J.F.: BN+AS-WeA12, 2
Morrish, F.: BN+AS-WeA4, 1
Moss, G.: BN+AS-WeA10, 2

— N —

Notingher, I.: BN+AS-WeA1, **1**

— R —

Robinson, M.: BN+AS-WeA4, 1
Rosenhahn, A.: BN+AS-WeA11, **2**

— S —

Schultz, J.A.: BN+AS-WeA12, **2**
Scurr, D.J.: BN+AS-WeA10, **2**
Senkbeil, T.: BN+AS-WeA11, 2

— T —

Trogler, W.C.: BN+AS-WeA3, 1

— W —

Wan, K.: BN+AS-WeA10, 2
Whitehead, N.P.: BN+AS-WeA9, 2
Womack, V.M.: BN+AS-WeA12, 2
Woods, A.S.: BN+AS-WeA12, 2
Wu, Z.: BN+AS-WeA3, 1