CAF Central Analytical Facilities
STELLENBOSCH UNIVERSITY

CELL IMAGING UNIT

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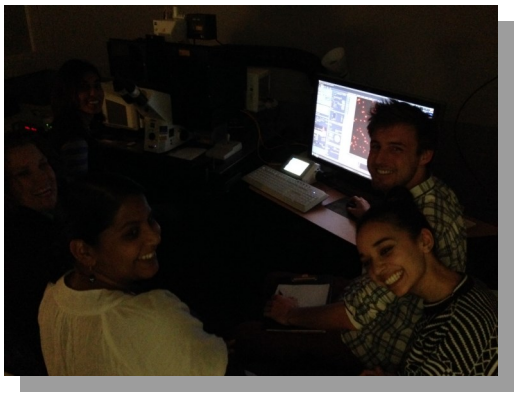
WELCOME

Welcome to the 5th issue of the CAF Cell Imaging Unit Newsletter. We have had quite a few changes this year and with the newsletter, we would like to keep you updated on the happenings in our unit. Please feel free to distribute this newsletter. We hope you enjoy this issue!

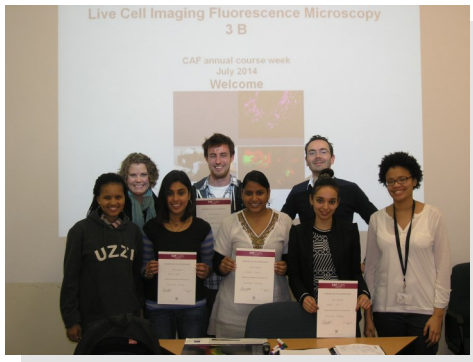
CAF MID-YEAR TRAINING COURSE 2014

In July this year, our CAF hosted its annual Mid-Year training week. In the Cell Imaging Unit, training was specifically given on our Carl Zeiss LSM Elyra imaging system, focusing specifically on confocal microscopy as well as super-resolution microscopy. This course was hosted Dr Ben Loos and Miss Rozanne Adams.



The concepts and principles in fluorescent microscopy were discussed, with focus on confocal microscopy including 3D imaging, intensity-based analysis, fluorescence resonance energy transfer (FRET) and colocalization.

The participants also received hands-on training in image acquisition using a variety of techniques on the Carl Zeiss Elyra LSM 780 confocal microscope.



We would like to thank all the staff and participants for the success of the event.

IN THIS ISSUE

RECENT ARTICLES

THE DETRIMENTAL EFFECTS OF ACUTE HYPERGLYCEMIA ON MYOCARDIAL GLUCOSE UPTAKE

Joseph D, Kimar C, Symington B, Milne R, Essop MF.

Acute hyperglycaemic (AHG) episodes have been linked to reduced glucose uptake, though the underlying mechanisms are unclear. This study hypothesized that AHG induces the production of reactive oxygen species (ROS) and upregulates the non-oxidative glucose pathway (NOGP) activation.

In this study, H9C2 cardiomyoblasts were exposed to 25 mM glucose for 24 h versus. 5.5 mM glucose controls ± modulating agents during the last hour of glucose exposure: a) antioxidant #1 for mitochondrial ROS and b) antioxidant #2 for NADPH oxidase-generated ROS, c) NOGP inhibitors. ROS levels (mitochondrial, intracellular) and glucose uptake were evaluated by flow cytometry. AHG elevated ROS, activated NOGPs and blunted glucose uptake. Transketolase activity (pentose phosphate pathway [PPP] marker) did not change. Respective 4-OHCA and DPI treatment blunted ROS production, diminished NOGP activation and normalized glucose uptake. NOGP inhibitory studies identified PKCβII as a key downstream player in lowering insulin-mediated glucose uptake. When we employed an agent (benfotiamine) known to shunt flux away from NOGPs (into PPP), it decreased ROS generation and NOGP activation, and restored glucose uptake under AHG conditions.

This study demonstrates that AHG elicits maladaptive events that function in tandem to reduce glucose uptake, and that antioxidant treatment and/or attenuation of NOGP activation pathway may limit the onset of insulin resistance.

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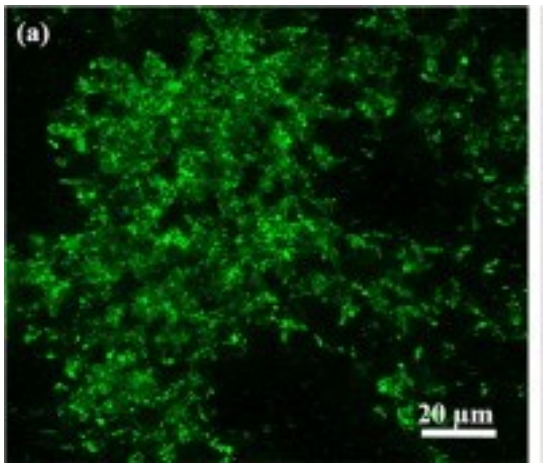
A NANOFORCE ZNO NANOWIRE-ARRAY BIOSENSOR FOR THE DETECTION AND QUANTIFICATION OF IMMUNOGLOBULINS

Neveling DP, van den Heever TS, Perold WJ, Dicks LMT.

In this study, they describe the development of a ZnO nanowire-array biosensor that able to detect immunoglobulins. Lysozyme was covalently bonded to a self-assembled monolayer (SAM) 3-mercaptopropanoic acid and linked to hydrothermally synthesized ZnO nanowires. And this was confirmed by atomic force microscopy (AFM), Fourier transform infrared (FTIR) spectroscopy. Confocal microscopy was also used to conirm the immobilization of the lysozymes. Attachment of the antibodies to immobilized lysozyme ZnO nanowire constructs displayed a piezoelectric signal, which was shown to be a result of increased voltage due to the presence of a Schottky barrier. The response of the piezoelectric nanoforce biosensor is in a linear relationship with antibody concentration ranges from 50 ng/ml to 1 µg/ml,



Fluorescence microscopy images of the biosensor surface immobilized with lysozyme.

with a limit of detection (LOD) of 102.76 ng antibodies/ml.

This technology has the promise to develop into biomolecular detection systems, allowing for the rapid detection of pathogens or disease states and providing a feasible approach to detect biomolecular interactions.

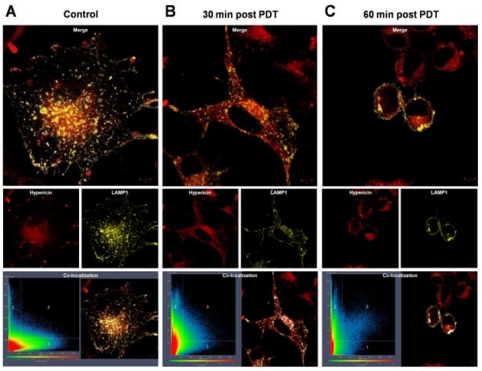
RECENT ARTICLES

St John's Wort (Hypericum perforatum L.) Photomedicine: Hypericin-Photodynamic Therapy Induces Metastatic Melanoma Cell Death

Kleemann, B, Loos B, Scriba TJ, Lang D, Davids LM.

Hypericin, an extract from St John's Wort, is a promising photosensitizer and induces tumour cell death through various mechanisms including apoptosis, necrosis and autophagy-related cell death. However, few publications explore the efficacy of the compound for melanoma treatment. The aim of this study was to investigate the response mechanisms of melanoma cells to hypericin-PDT in an *in vitro* tissue culture model.

Super-resolution structured illumination microscopy (SR-SIM) was used to visualise hypericin uptake and co-localization to the endoplasmic reticulum, mitochondria, lysosomes and melanosomes. Hypericin activation of induced a rapid, extensive change of the tubular mitochondrial network into a beaded appearance, loss of structural details of the endoplasmic reticulum and concomitant loss of hypericin co-localization. This was not found in



Hypericin-PDT induced loss of structural details of calreticulin positive structures (endoplasmic reticulum). (A) Control (hypericin-treated, sham-irradiated). (B) 30 min post PDT. (C) 60 min post PDT.

lysosomal-related organelles, suggesting that the melanoma cells may be using these intracellular organelles for hypericin-PDT resistance.

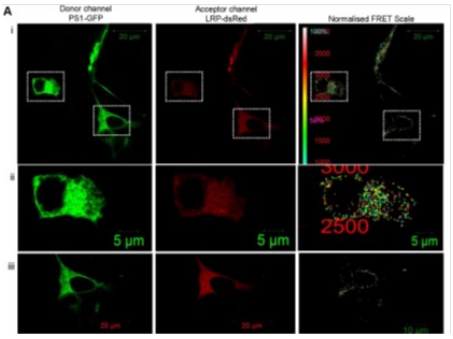
An increase in cellular granularity suggests increased pigmentation levels in response to hypericin -PDT. Pigmentation in melanoma is related to a melanocyte-specific organelle, the melanosome, which is implicated in drug trapping, chemotherapy and hypericin-PDT resistance. However, hypericin-PDT was effective in killing both unpigmented and pigmented melanoma cells through specific mechanisms. Further research is needed to shed light on these mechanisms. and/or attenuation of NOGP activation pathway may limit the onset of insulin resistance.

High Resolution Imaging Study of Interactions between the 37 kDa/67 kDa Laminin Receptor and APP, Beta-Secretase and Gamma-Secretase in Alzheimer's Disease

Jovanovic K, Loos B, Da Costa Dias B, Penny C, Weiss STF.

Alzheimer's disease (AD) is the most prevalent form of dementia in elderly. The neurodegeneration caused by the amyloid β peptide, which is produced by sequential proteolytic cleavage of the Amyloid Precursor Protein (APP) by the β– and γ-secretases. Previous publications showed that 37 kDa/67 kDa laminin receptor (LRP/LR) is involved in APP processing, but the exact mechanism remains largely unclear. This study aimed to assess whether LRP/LR interacted with APP, β- or γ-secretase.

Confocal microscopy revealed that LRP/LR showed strong co-localisation with APP, β- and γ-secretase, respectively, at various sub-cellular locations. SR-SIM showed that interactions were unlikely between LRP/LR and APP and b-secretase, respectively, while there was strong co-localisation between LRP/



FRET analysis with PS1-GFP donor and LRP-dsRed acceptor in HEK293 cells. Distinct FRET signal is detected on the normalized FRET scale (i). Scale bar: 20 mm. Enlarged view showing that energy is transferred in the cytoplasmic and plasma membrane regions of the cell. (ii) and (iii). Scale bar: 5 mm.

LR and c-secretase at this 80 nm resolution. The FRET analysis was used to determine the protein-protein interactions, with which only an interaction between LRP/LR and c secretase was observed. This finding were confirmed using FLAG co-immunoprecipitation. This proves that LRP/LR influences amyloid β shedding through direct interaction with the γ-secretase and possibly indirectly through with the β-secretase interaction.

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MSSA CONFERENCE

We would like to announce our participation in the 52nd annual MSSA conference to be held from 2-5 December 2014 at the Protea Hotel and Conference Venue, Stellenbosch. This is a special event as this is the first MSSA hosted in Stellenbosch for a long time.

We here at CAF Cell Imaging Unit look forward to this event and will be presenting some of the work we have done. Many prominent researchers and academics will also be presenting on their research. Delegates will be introduced to the latest technology in microscopy and this is also a time to socialize with old friends from other institutions and industry.

Registration has already opened and all students and researchers with an interest in microscopy are welcome to attend. Please visit www.mssa2014.co.za for more information. We hope to see you there!

STAFF DEVELOPMENTS

Mrs Lize Engelbrecht has returned from maternity leave as of the 1st September 2014. Her little boy is doing really well, although he does not allow his mom to sleep a lot!

The unit will now be running as usual. Please feel free to contact us for any enquiries on flow cytometry, confocal or wide-field microscopy, or sample preparation for TEM.

The BD/CAF Flow Cytometry Unit on the Tygerberg campus will be running as per usual and all enquiries can be forwarded to Mrs Andrea Gutschmidt.

CELL IMAGING UNIT

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