CAF Central Analytical Facilities
STELLENBOSCH UNIVERSITY

FLUORESCENT MICROSCOPY UNIT

ISSUE 7 FEB—MAY 2015

WELCOME!

Welcome to the second issue of the CAF Fluorescent Microscopy Unit Newsletter for 2015. The focus of this newsletter is to highlight the recent publications of users within this unit. We also have some changes to the online booking system we would like to make our users aware of as well as news on our recent flow cytometry training. Our goal is to keep our users informed and up to date on the current happenings in the unit and share as much of our knowledge base as possible. We hope you enjoy this issue!

IN THIS ISSUE

FLOW CYTOMETRY COURSES 2015

From March to May this year, the Fluorescence Imaging Unit hosted its three flow cytometry training courses on the BD FACSAria.

The two day courses, hosted by Mrs Lize Engelbrecht and Ms Rozanne Adams, offered both knowledge on the concepts and principles of fluorescence and single particle analysis using flow cytometry as well as practical experience on single-colour experiments, two colour experiments, compensation, particle sorting and hands on analysis of the data.

The course is structured to give the attendees background knowledge on the instrument and field, so they may be able to plan experiments efficiently and provides an understanding of the principles of

the technology and its powerful uses in the field of science. This empowers researchers to gain more data from their experiments and deliver accurate, publishable data.

With the success of the training courses, we would like to thank all the staff and participants for the attendance and hard work.

For all who are interested in training in the unit, do not hesitate to contact us. Some confocal microscopy courses are planned for the near future, but we offer training based on the needs of our clients.

INSTRUMENT UPDATE

- The FACS Aria has been repaired after being out of order for a few weeks. Please contact us for bookings.
- Zeiss offered to loan us a brand new confocal microscope for the time that our own instrument is not available due to the upgrade in Germany. After experiencing problems with the setup its final installation is now confirmed and users are welcome to book.
- Our own super-resolution microscope is expected to be shipped from Germany towards the end of August, so we hope to be up and running by early September.

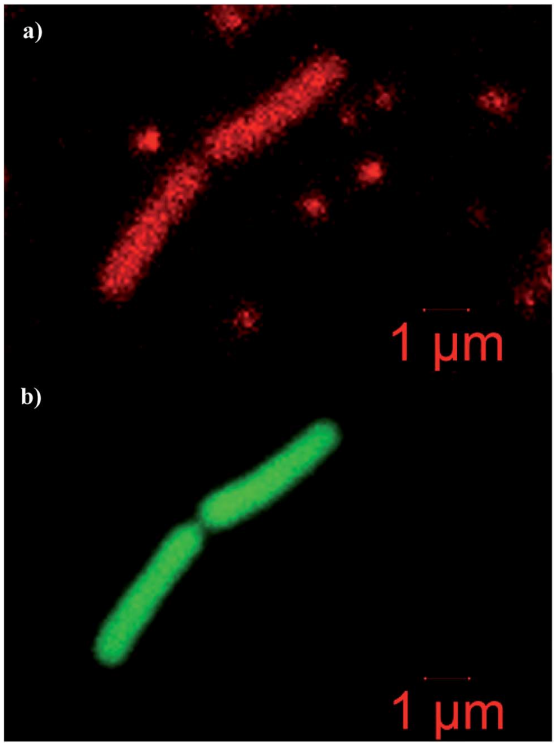
RECENT ARTICLE 1

DEVELOPMENT OF BIOCONJUGATED DYE-DOPED POLY (STYRENE-CO-MALEIMIDE) NANOPARTICLES AS A NEW BIOPROBE

A. Swanepoel, I. du Preez, T. Mahlangu, A. Chetty and B. Klumperman.

Dye-doped nanoparticles are highly sensitive labelling devices that, when functionalized with receptor molecules such as antibodies, peptides, or lipids, cluster around target cells, creating localized amplified fluorescence signals. Dye-doped nanoparticles, like quantum dots (QDs), have been extensively developed and tested for detection of bacteria but can result in potential toxicity due to leaching of heavy metal ions from the particles. Here the use of poly(styrene-co-maleimide) (PSMI) nanoparticles as a bioprobe was investigated. A hydrophobic fluorescent dye, Exalite 613, was encapsulated in the nanoparticles and the surface of these nanoparticles was functionalized with amine groups in order to facilitate attachment of anti-bacterial antibodies. These were then used to target *Escherichia coli (E. coli)* microbes.

Electron microscopy confirmed the formation of nanoparticles with an average size of 50 nm. Confocal microscopy of *E. coli* contacted with dye-loaded bioconjugated PSMI nanoparticles show that the *E. coli* present could be clearly identified. Fluorescent signals from the bound nanoparticles could be distinguished from background noise by the characteristic size and shape of the bacterial cells, and was confirmed by a SYTO 9 nuclear dye counterstain.



Confocal fluorescence microgaphs of E. coli K-12 cells labelled with (a) dye-loaded bioconjugated PSMI nanoparticles, and (b) counterstained with SYTO 9 nucleic acid stain.

The dye-loaded PSMI nanoparticles thus display the potential to be used as a bright and stable fluorescent probe that can easily be tailored to a specific target via the conjugation of suitable receptor molecules to the nanoparticle surfaces.

NEW ONLINE BOOKING SYSTEM

After testing an online booking system based on Outlook meetings, we've decided to switch over to the Quartzy system which many of you might be familiar with. This booking system is fairly intuitive and is quite transparent for the user. Many other units and laboratories also use this organized system

It requires all users of the unit to register on www.quartzy.com and then join the group CAF Fluorescent Microscopy Unit. All members are able to see the calendar of our five instruments, as well as the calendar of the three staff members.

When making a booking on an instrument you have not been trained on, please also make the same booking on the calendar of the appropriate staff member.

Lastly, after your session in our unit, note the actual time you have used the instrument on the logsheet provided next to each instrument.

RECENT ARTICLE 2

NEUTROPHIL AND MONOCYTE RESPONSES TO DOWNHILL RUNNING: INTRACELLULAR CONTENTS OF MPO, IL-6, IL-10, PSTAT3 AND SOCS3

Mari van der Vyver, Lize Engelbrecht, Carine Smith, Kathryn Myburgh.

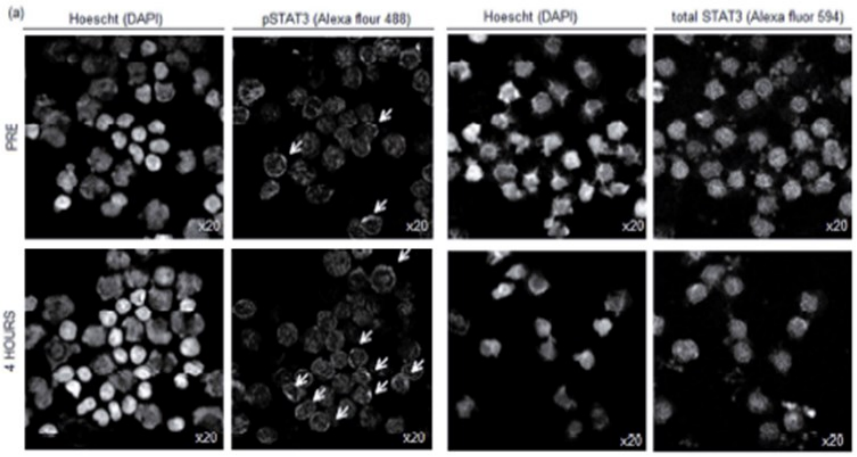
High intensity exercise result in activation of the immune system, characterised by an acute systemic cytokine response that includes both pro and anti-inflammatory markers. Elevated levels of circulating neutrophils and peripheral blood mononuclear cells (PBMCs) are also seen during the first 6 hours following exercise.

Healthy male (n=12) volunteers participated in high intensity running. Blood samples were analysed for serum concentrations of various cytokines, as well as markers of muscle damage.

One of the aims of the study was to determine whether peripheral blood mononuclear cells respond to inflammatory interleukins (ILs) by increasing intracellular signalling.

For this purpose, confocal microscopy, and subsequent fluorescent intensity analyses, was used to evaluate intracellular IL-6, IL-10, MPO and STAT3/SOCS3 levels. The biggest response to the exercise was seen at 4 hours post-injury.

Intracellular monocyte IL-10 and IL-6 decreased by 15% and 20-30% respectively, coinciding with elevations in serum IL-10, suggesting immune cell cytokine release in response to exercise. Intracellular PBMC p-STAT3 to total STAT3 ratio increased from pre-exercise to 4h, indicating that circulating monocytes are responsive to increased serum IL-6 suggesting a negative feedback loop via STAT3 signalling.



Intracellular signalling. (a) Confocal microscopy images taken at x20 magnification as representation of overall observations. Images are displayed in grey scale for improved contrast,

NEW SAFETY MEASURES IN THE UNIT

Due to the fact that we house high cost instruments, we've identified our previous safety arrangements to be lacking. Also, we realized with an increasing number of users being trained to use the instruments after hours, we have a responsibility to put measures in place, so our users can work in the unit any time of the day safely.

For this purpose, an alarm system, including panic buttons, which is linked to Campus Security has been installed. Only users who are fully trained and who notified the staff of the unit o f their planned analysis after hours, will receive an access code. This code will be changed from time to time, so make sure to notify staff every time you plan to work after hours. Emergency numbers are displayed in several places in the unit.

Also, a card reader system has been partially installed. We are waiting for the final installation, and will notify users as soon as it is in place.

We friendly request that users keep codes confidential and do not lend out cards to others for access.

We hope that our users will always feel safe and secure while working here!

RECENT ARTICLE 3

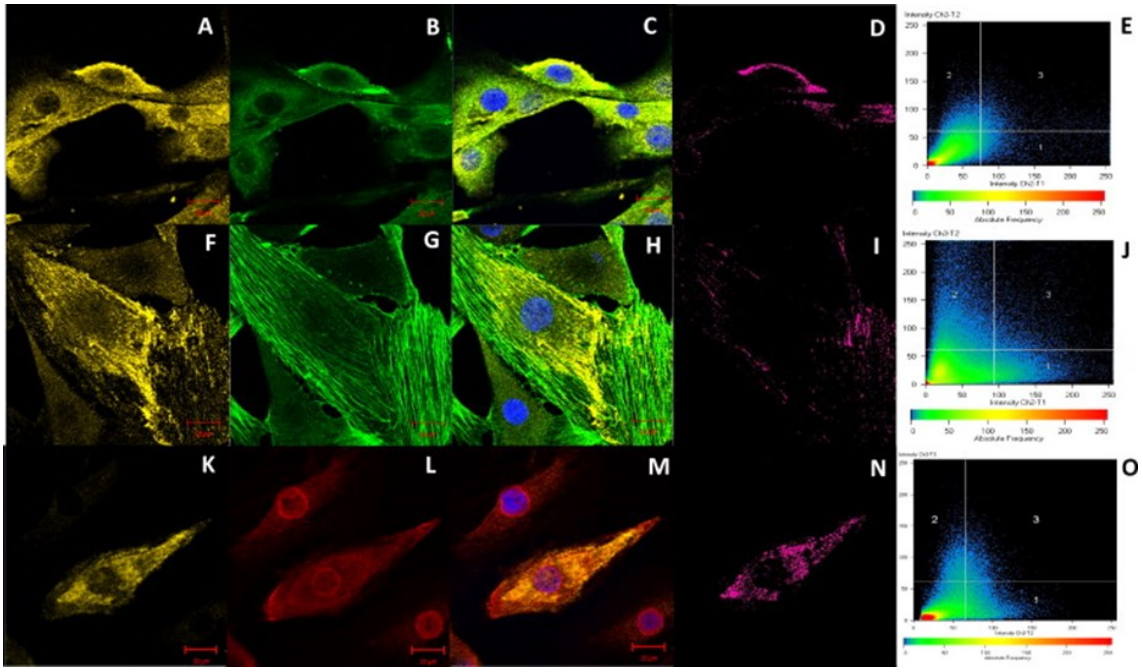


Fig. 1 Fluorescent imaging and co-localisation analysis of MyBPH and MYH7 in differentiated H9c2 cardiomyocytes. (A & F) MyBPH labelled with a Cy5 secondary antibody (Yellow). (B & G) MYH7 labelled with a Alexa Fluor 488 antibody (Green). (C & H) Overlay of images a, b and Hoechst staining of nucleus. (D & I) Co-localisation of MyBPH and MYH7 generated from merged images (Pink). (E, J) Scatter diagram generated by co-localisation analysis. (K) MyBPH labelled with Cy5 secondary antibody (Yellow). (L) UBC9 labelled with Cy3 secondary antibody (Red). (M) Overlay of images k, l and Hoechst staining of nucleus. (N) Co-localisation of MyBPH and UBC9 generated from merged images (Pink). (O) Scatter diagram generated by co-localisation analysis with quadrant three representing the degree of co-localisation. Scale bar 20 µm.

CELL IMAGING UNIT

ASCRIBING NOVEL FUNCTIONS TO THE SARCOMERIC PROTEIN, MYOSIN BINDING PROTEIN H (MYBPH) IN CARDIAC SARCOMERE CONTRACTION

Jomien Mouton, Ben Loos, Johanna C Moolman-Smook, Craig Kinnear.

Myosin binding protein H (MyBPH) is a protein of unknown function, which shares sequence and structural similarities with myosin binding protein C (cMyBPC), which is frequently implicated in hypertrophic cardiomyopathy. With the similarity between cMyBPC and MyBPH, they proposed that MyBPH, like cMyBPC, could be involved in hypertrophic cardiomyopathy pathogenesis.

The role of MyBPH and cMyBPC in cardiac cell contractility of differentiated H9c2 rat cardiomyocytes in response to β-adrenergic stress after siRNA knockdown of MyBPH and cMyBPC was analysed. Individual knockdown of either protein was found to warrant no effect on cardiac contractility, while concurrent knockdowns reduced cardiac contractility. This proves that the proteins functionally compensate for one another and are critical for cardiac contractility. Furthermore it is shown that both proteins co-localise with the autophagosomal membrane protein LC3, suggesting that both proteins are involved in autophagosomal membrane maturation processes. The results of this study ascribe novel functions to MyBPH, which may contribute to our understanding of its role in the sarcomere.

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