CAF Central Analytical Facilities
STELLENBOSCH UNIVERSITY

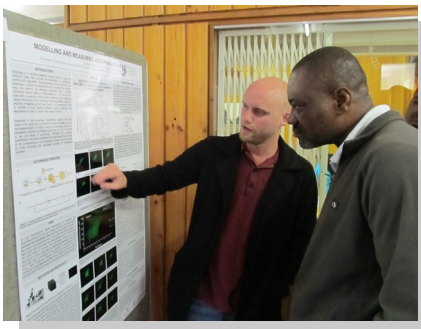
CELL IMAGING UNIT

WELCOME

Welcome to the first issue of the CAF Cell Imaging Unit Newsletter for 2014! The overall aim of our newsletter is to inform clients about the current developments within our unit. Please feel free to distribute this newsletter to any interested persons. We hope you enjoy this issue!

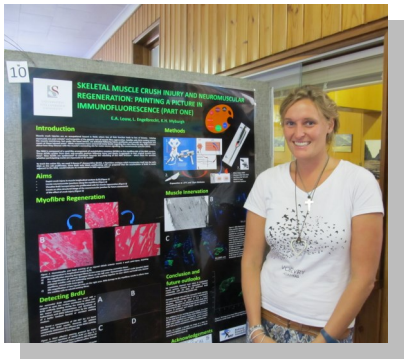
1ST ANNUAL CAF STUDENT SYMPOSIUM

This year we held the first and very successful Annual Student Symposium on the 27th March 2014, kindly sponsored by Carl Zeiss. The aim of this symposium was to provide a platform for post graduate students to present their research conducted in our analytical laboratories. This also provided the opportunity for other researchers to learn about other research technologies and build interdisciplinary relationships.



Several abstracts were submitted by various students using the different CAF units. Speakers were selected from a variety of fields. Poster were also presented at the symposium, which attracted a lot of attention from the audience present, with people interacting with the students.

Competitions were held for best presentation as well as best poster presentation. The Imaging Unit was quite well represented, with several students presenting data collected at our unit.



With the success of this event, we would like to thank all the staff, participants and contributors for the success of the event.

IN THIS ISSUE

CONFERENCE ATTENDANCES

COMBINED CONGRESS 2014

Ms Thembeka Mabiya, a MSc Student at ARC -Infruitec-Nietvoorbij and University of the Western Cape, presented a part of her MSc work at the Combined Congress 2014, held at Rhodes University.

The focus of her work was to determine the ploidy level of "FeherBesztercei", a plum root-stock recently introduced into South Africa. Flow cytometry was used to determine the ploidy level of each of the new rootstock, with known South African plum rootstocks “Marianna" (2n) and "Maridon"(3n) serving as controls.

Her experimental work clearly demonstrated that demonstrated that flow cytometry could be used to distinguish the two closely-related "Marianna" and "Maridon" rootstocks. Furthermore, "FeherBesztercei" was found to be is a hexaploid. Furthermore, to our knowledge this is the first time this method has used to distinguish the ploidy level between "Marianna" and "Maridon" rootstocks.

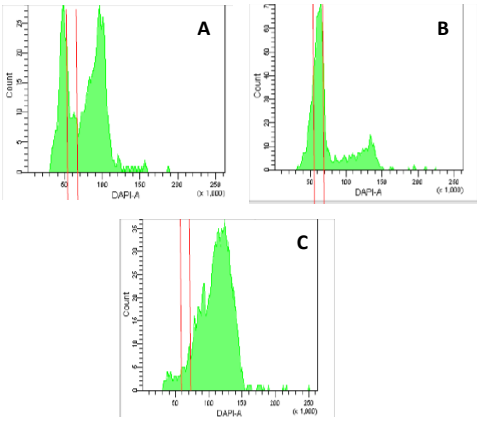


Fig 1.The flow cytometry histograms showing the different ploidy levels for each root stock. A, Diploid “Marianna”. B, triploid “Maridon” and C, ‘Hexaploid ’Feherbesztercei” peak

In conclusion, flow-cytometry was found to a suitable method to distinguish between closely related varieties that differ on chromosome ploidy level.

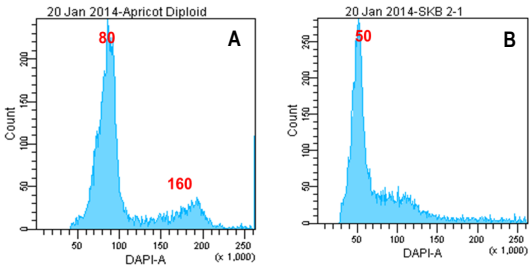
10TH SOUTH AFRICAN PLANT BREEDING SYMPOSIUM

In March 2014, Mr Mlamuli Motso, a D. Tech student from the ARC and Tshwane University of Technology, presented a part of his D. Tech. project at the SAPB Symposium in Thaba Nchu.

His work focused on estimating DNA content in *Cyclopia* (Honeybush) species. Flow cytometry was selected as his method of choice for estimating DNA content. This led to an investigative study on the optimization of flow cytometric analysis for the estimation of DNA content in honeybush species.

Several factors were found to influence his ploidy analysis. The high phenolic compounds present in his sample lead to interference of DNA binding and fluorescence emission. This lead to erroneous measurement of ploidy level. Furthermore, with these shifts of fluorescence observed, a suitable reference standard was implemented into the samples.

The quantity and quality of light may play a role in the variations of ploidy as it can result in endoreduplication. Several recommendations were made for this study. Reducing agents are needed to counteract the interference with DNA dye binding. Several other plant tissue samples have to be analysed for phenolics to determine the plant tissue to use. Nuclei extraction protocol may also have to be optimized and modified to suit the experiment.



The flow cytometry histograms depicting the shift of the standard plant peak before (A) and after (B) chopping up with a honeybush species. This depicts the inhibition of DNA dye binding by the phenols in the honeybush species.

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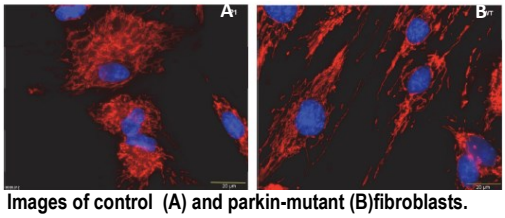
RECENT ARTICLES

Mitochondrial impairment observed in fibroblasts from South African Parkinson's disease patients with parkin mutations

van der Merwe, C., Loos, B., Swart, C., Kinnear, C., Henning , F., van der Merwe, L., Pillay K., Muller, N., Zaharie, D., Engelbrecht, L., Carr, J. and Bardien, S.

Parkinson's disease (PD) is a neurodegenerative disorder characterised by the loss of dopaminergic neurons in the substantia nigra in the midbrain. A major cause of autosomal recessive PD is loss-of-function mutations in the parkin gene. Parkin has been implicated in the maintenance of healthy mitochondria, which may be due to possible compensatory mechanisms.

The aim of this study was to determine whether South African PD patients with parkin mutations demonstrate evidence for mitochondrial dysfunction. To assess mitochondrial function, Live Cell Imaging was performed on the Olympus IX-81 microscope with human fibroblasts using Mitotracker Red and the nuclei were counterstained with Hoescht. Images were analysed using Cell^R analysis software. Although no differences in the degree of mitochondrial network branching were found in the fibroblasts, ultrastructural abnormalities were observed with Transmission Electron Microscopy, including the presence of electron-dense vacuoles. Also, decreased ATP levels were found, which are consistent with mitochondrial dysfunction were observed in the patients' fibroblasts compared to controls.



Images of control (A) and parkin-mutant (B)fibroblasts.

In conclusion, these findings suggest that parkin-null patients exhibit features of mitochondrial dysfunction. The mitochondria acts as a key role player in PD pathogenesis, and this will have important implications for the design of new and more effective therapies.

STAFF DEVELOPMENTS

Mrs Lize Engelbrecht is on maternity leave as of the 25th April 2014 and will be returning to work the 1st September 2014. Arrangements have been made with regards to the unit. The unit will be running as usual and several arrangements have been made for the next few months.

For all flow cytometry, wide-field microscopy and general enquiries, contact Ms Rozanne Adams. Confocal Microscopy enquiries can be forwarded to Ms Dumisile Lumkwana.

Dr Ben Loos will also be available on Thursdays for the assistance with project planning on confocal microscopy. He can be contacted at bloos@sun.ac.za.

The Tygerberg CAF Lite Unit will be running as per usual and all enquiries can be forwarded to Mrs Andrea Gutschmidt.

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Please feel free to contact us for any enquiries.