





# Annual Report 2017/2018 of the **Central Analytical Facilities**

### 2018 CAF committee:

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Prof Eugene Cloete Dr Therina Theron Me Malene Fouche ( Secretariat)

#### **CAF Management**

Prof Gary Stevens (Director)

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### Pls on recent equipment grant applications

Prof Johan Burger Prof Bert Klumperman Dr Ben Loos Prof Quinette Louw Prof Marena Manley Prof Kathy Myburgh Prof Marina Rautenbach Prof Gerhard Walzl Prof James Warwick Prof André van der Merwe

#### **Invited CAF Unit Managers**

Dr Marietjie Stander Mr Carel van Heerden Prof Lydia Joubert Me Fransien Kamper

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Contents

Overview
Selected articles about development within CAF
Mining the information in the CAF client database
Nuclear magnetic resonance analysis of polymer structures
Mass spectrometry applied to test for adulteration and contamination in food and beverages
X-ray CT analysis of evolutionary optimization of snake fangs
Capacity building at Electron Microscopy
Next Generation Sequencing in the field of Human Genetics

Financial Reports	
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### CAF mid-year training, June 2018

The annual CAF Training Initiative took place from 18-22 June. Seventeen different workshops were presented with 108 participants. The participants were welcomed at a special dinner on 19 June at Stias. Some of the sponsors of the workshops had displays at the dinner. More photos of the training can be viewed at the end of this report.





### Launch of the Vibrational Spectroscopy unit in March 2018

The new Vibrational Spectroscopy unit was launched on 6 March 2018. This world class facility offers hyperspectral imaging and data analysis services to students, researchers and industry clients. The purchase of the equipment was made possible by a NRF National Equipment Program grant to Prof Marena Manley (below center). Mr Stephen Dlamini (below left), represented the NRF at the launch.



# Overview

2018 is the centennial year of Stellenbosch University and it is interesting to reflect on the fact that the Central Analytical Facility has been in existence for <sup>1</sup>/<sub>5</sub> of the University's life-time.

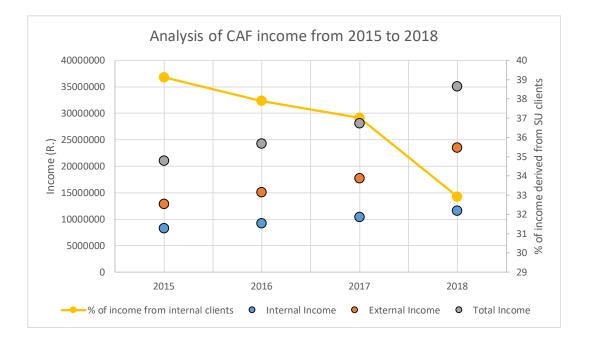
ver this time CAF has grown from an organization that was heavily dependent on financial support from the SET faculties at SU, with a handful of staff and equipped with mostly outdated and poorly functioning equipment, to an organization with 35 permanent employees, that is largely financially self-sufficient, and that manages a comprehensive array of very up-to-date and advanced analytical equipment.

I would like to congratulate CAF staff past and present, as well as the many Stellenbosch academics who have partnered with CAF in National Equipment Programme and other equipment applications, for each of their contributions to building the comprehensive research support structure that CAF has become. 2018 has seen a good consolidation of the long-term financial viability of CAF, through growth in income generated from external clients.As is clear in the graph below, income from services to internal clients has grown steadily from R8.2 million in 2015 to a projected R11.5 million in 2018. Despite this, the percentage of total CAF income derived from services to internal clients has fallen from 39% to 33% over the same period. This reflects substantial growth in income earned from services provided to both industry clients and to clients from other academic institutions. Income derived from each of these three markets is now approximately equal, making CAF relatively secure

against factors that may influence the availability of research funds at specific institutions.

Given the financial pressures and likely threats to future subsidy derived income that currently exist at all research intensive universities in South Africa, it is essential that extra analytical capacity within CAF be used to grow our external client base in industry and within the international market. CAF services are very competitively priced in an international context, yet only 8% of CAF income from external clients is currently derived from international clients. CAF needs to identify specific services to market to international academic clients, which will be favourable both from the perspective of diversifying the client base and stimulating collaborative research at Stellenbosch University.

2018 has seen relatively few new equipment acquisitions, primarialy due to the scheduling of National Equipment Programme (NEP) calls. However, the following very important developments did take place. A NEP grant to Prof Marena Manley allowed for the purchase of a range of hyperspectral imaging equipment that is housed in a new CAF unit for Vibrational Spectroscopy, managed by Dr Janine Colling (in the red dress, lower photo panel, facing page). The unit was launched in March 2018 and photographs of the event in the Department of Food Science are presented on page 4.



A grant from the Faculty of Medicine and Health Sciences, with CAF co-funding, resulted in the purchase of a new ZEISS Fluorescence Microscope. This will result in the Flouresence Microscopy Unit, managed by Lize Engelbrecht, opening a new point of service on the Tygerberg Campus.

The process of creating the Nuclear Medicine Research Initiative (NuMeRI) Node for Infection Imaging, which will be managed by CAF, at Tygerberg Hospital continues. A state-of-the art, fully digital, PET/CT scanner was purchased from Philips and is currently under construction. The approximately R19 million needed to construct the building to house the NII has been raised from a variety of sources, including a large investment from the Faculty of Medicine and Health Sciences. During 2018 a large number of NEP grants were submitted by Stellenbosch University for new equipment to be housed within CAF units. The details are provided in the table below.

The remainder of this report presents the the relevant CAF financial information and a number of interesting articles from several CAF units. These are intended to showcase specific analytical capabilities, as well as interesting developments. From a capacity building point of view, it is very encouraging to note how the Electron Microscopy Unit has trained post-graduate students to work as analysts on specific industry projects, typically after hours. This creates extra analytical capacity and brings in valuable income whilst also meeting very important needs in the South African R&D and mineral exploration sectors. In the process of performing this work, the students gain very valuable experience that most certainly enhances their future employment prospects. The amount of time these students work is carefully managed to stay within the conditions of grant for those with bursaries.

In closing, CAF continues to grow in a sustainable way, largely due to the commitment within Stellenbosch University to develop its capacity for advanced research and due to the continued investment by the South African state in advanced analytical infrastructure. Consequently, CAF is well placed to advance the scope and level of support it provides to researchers at Stellenbosch University.

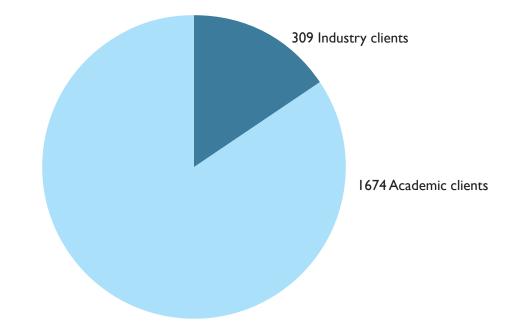
Gary Stevens Director

Principle Applicant	Equipment	CAF Unit	Requested from NRF	Contribution by Own/ Other Institution	Total Cost of Equipment
Prof. Marina Rautenbach	Mass-Directed Auto- Purification & QC system	LCMS	R6 287 870,00	R3 143 935,00	R9 431 805,00
Prof. Gerhard Walzl	Gemini 300FESEM with advanced system for automated 3D-microscopy by in situ ultramicrotomy	Electron Microscopy	R9 999 826,00	R4 999 913,00	R14 999 739,00
Prof. Bert Klumperman	Bruker AVANCE NEO 700 MHz NMR instrument with cryoprobe and autosampler	NMR	R I 0 000 000,00	R16 665 450,00	R26 665 450,00
Prof. Samantha Sampson	Amnis® ImageStream®X Mark II Imaging Flow Cytometer	Fluorescence Microscopy	R8 432 791,00	R4 216 396,00	R12 649 187,00

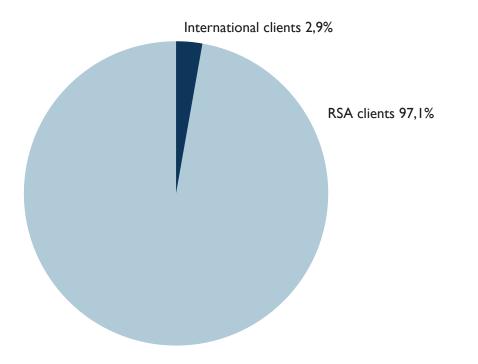
## Mining the information in the CAF client database

The online form CAF created in 2017 to collect important information from all CAF clients has produced interesting results about who exactly the CAF clients are as the graphs below show. The online system was developed because of the National Research Foundation's requirements (from 1 April 2017) that the all universities must report a comprehensive profile for each person who uses National Equipment Programme funded equipment for a period of five years after the commissioning of the equipment. The graphs below provide details about the make-up of CAF clients during 2017.

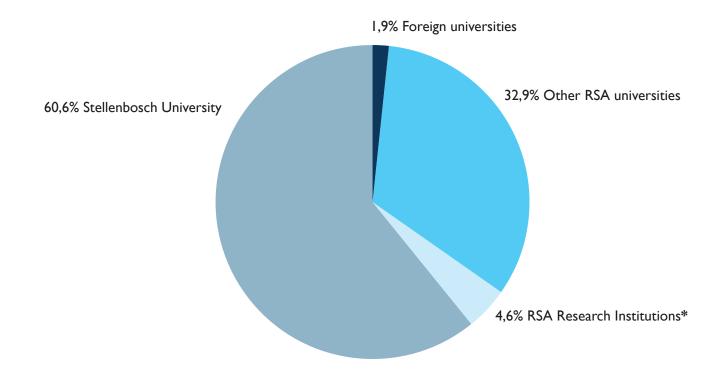
CAF academic clients relative to industry clients:



The proportion of local industry clients relative to international industry clients:

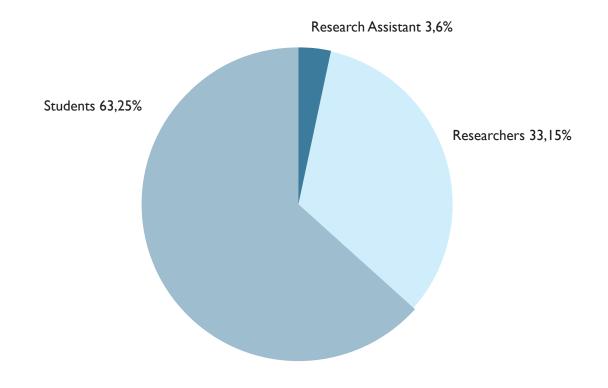


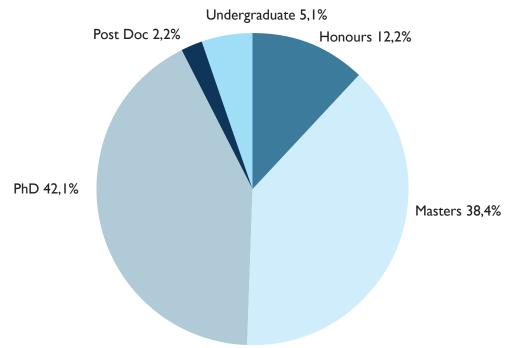
### The proportions of different kinds of academic clients:



\* Including iThemba Labs, ARC, MRC, CSIR, SA Institute for aquatic biodiversity, etc.

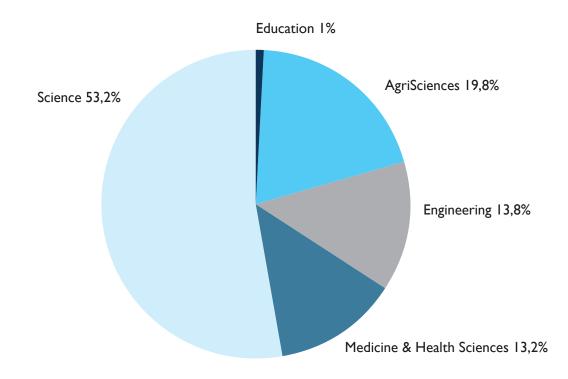
The profile of CAF academic clients:





The proportions of CAF academic clients who were students, postgraduate students and post-doctoral researches:

The proportions of Stellenbosch University student clients according to faculty:



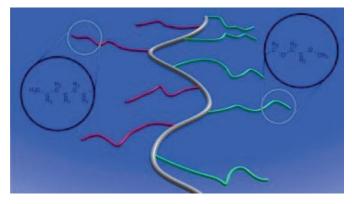
# Nuclear magnetic resonance analysis of polymer structures

### by dr Jaco Brand

In a post-polymerization modification reaction, the polymer research group of Prof Bert Klumperman and Dr Rueben Pfukwa exploited the anhydride functionality, and used it to graft poly(ethylene glycol) (PEG) side chains, by an imidization with an amineend functional PEG, forming n-BEVEMI-PEG molecular brushes. Diffusion ordered NMR spectroscopy experiments performed by the NMR unit under the leadership of Dr Jaco Brand were crucial in proving the incorporation of those PEG side chains (brushes), and purity of the resulting copolymer, by means of their differential diffusion rates observed in solution. Poly(n-butyl vinyl ether-alt-maleic anhydride) (n-BVEMAh) copolymers were readily prepared via free radical alternating copolymerization of maleic anhydride (MAh) and n-butyl vinyl ether (n-BVE).

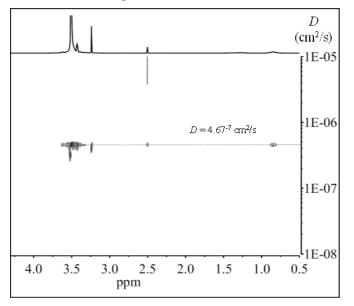
Diffusion-ordered spectroscopy (DOSY)seeks to separate the NMR signals of differently sized molecules according to their diffusion coefficient in solution. A series of spin echo spectra is measured with different pulsed field gradient strengths, and the signal decays are analysed to extract a set of diffusion coefficients with which to synthesise the diffusion domain of a DOSY spectrum. DOSY analysis thus produces two dimensional correlation maps with chemical shifts and diffusion coefficients on the horizontal and vertical axes respectively.

NMR signals of nuclei on the same molecule are correlated with the same diffusion coefficient, allowing for the differentiation of NMR signals produced by molecules of different sizes or their diffusion rates. This is a very powerful method for investigating sample purity and supramolecular interactions, which is finding increasing applications in the analysis of polymer materials. Herein the DOSY method



Cartoon representation of n-BVEMI-PEG brushes

proved to be a convenient, non-invasive and nondestructive method for qualitatively analysing polymer molecular brushes which have potential application in solvent-free coatings.



DOSY 1H NMR spectrum of n-BVEMI-PEG brush, and its diffusion rate, obtained in deuterated dimethyl sulfoxide (2.5 ppm) at 298 K.

Traditional coatings for decorative and protective purposes have largely been applied as solutions in organic solvents. Upon evaporation of the solvent and concomitant crosslinking of the binder molecules, a robust coating is formed that protects the underlying substrate from exposure to the atmosphere (oxygen, water, UV irradiation, etc). Although this technology works very well for its purpose, there is a large drawback in the evaporation of the organic solvent, which ends up in the atmosphere, causing health and environmental problems. Consequently, increasingly stringent legislation has been adopted over the years that limits the permissible amount of organic solvent in coating formulations.

A number of different methods have been developed to reduce the so-called volatile organic content (VOC) of coatings. Typical examples are the well-known waterbased coatings (latex paint) and powder coatings (often used for washing machines, refrigerators, etc). Another technology to reduce VOC is based on high-solids coatings, where the fraction of solvent is reduced while modifying the polymeric binder in such a way that the viscosity of the coating still allows its application via spraying or brush. The required modification of the binder often leads to a reduction in its mechanical properties due to a decrease in the chain length of the polymers. In previous research we had discovered that molecular brushes, despite their very large molecular weight, often behave as viscous liquids. This is quite remarkable, since usually, high molecular weight polymers are either rubbery or glassy solids. In the current research, we further explored the utilization of this peculiar property of molecular brushes.

The obtained n-BVEMI-PEG brushes are viscous liquids at room temperature, hence were termed liquid molecular brushes (LMBs). The LMBs displayed relatively low viscosities at shear rates typical for processing or application of surface coatings (1000 s-1), which is caused by their shear thinning behaviour. At low shear rates, higher viscosities were observed, which will help to avoid unwanted flow after application. Due to this rheological behaviour, these LMBs have the potential to be used as liquid binders for coating applications.

We further explored the preparation of crosslinkable molecular brush based binders. We grafted PEG12 and linoleamide side chains onto n-BVEMAh alternating copolymers.

The PEG12 side chains introduce the liquid nature, while linoleamide side chains confer oxidative crosslinking ability, although viscous liquid behaviour was only observed at low linoleamide side chain contents. Thermal crosslinking, presumably due to oxidative crosslinking, was only observed at very high temperatures, above 150 °C. However, the oxidative crosslinking could be effected at low temperatures (50 °C) in the presence of a cobalt (II) ethylhexanoate drier catalyst. The extent of drying is dependent on catalyst loading, and % linoleamide side chain functionalization. A challenge however, is that full crosslinking was only observed at very high linoleamide

side chain % functionalization, and long reaction times were required. Nevertheless, we believe that binders based on LMBs have the potential to play an important role in the development of solvent-free coatings.

This research strongly benefited from the utilization of DOSY NMR analysis for confirming the incorporation of the molecular brushes and purity of the resulting polymer.

DOSY maps recorded for the molecular brush showed that the IH NMR resonances of the PEG side chains and the molecular brush's backbone all aligned on the same horizontal line showing that they had the same translational mobility and hence were linked together. Additionally, only the diffusion signal corresponding to the brush are observed, meaning that there was no residual PEG in the molecular brush sample. In this way DOSY analysis not only helped to provide evidence for successful grafting, but also of the resulting polymer's purity. Indeed the DOSY method is fast gaining popularity as an efficient tool for detecting traces of impurities, such as unreacted reagents and by-products, in polymer materials synthesis. This type of qualitative analytical information is usually provided by traditional chromatographic methods, which, however, require longer analysis times, large amounts of organic solvents, and the use of stationary phases, which elicits vexing adsorption effects. This is not to say DOSY analysis is an alternative to chromatographic methods, but it is obviously a strongly complimentary technique, with the benefit of faster run times and less tedious application.

The ability to quickly assess different molecular component mixtures in solution, without their actual time-consuming, pricey physical chromatographic separation, has vast applicability in Chemistry, not only in the polymer field, but also in the Natural Products, Medicinal protein-ligand interactions, Food science, Beverage industry (Wine, Juice), Botany and Biochemistry research fields, to name just a few.

Reference: Mpho M. Phiri, Waled Hadasha, Rueben Pfukwa and Bert Klumperman, 2016. Synthesis and characterization of liquid molecular brush binder for coating application, European Polymer Journal, 2018, 91, 178-186.

# $Mass \ spectrometry \ applied \ to \ test \ for \ adulteration$ and contamination in food and beverages

by dr Marietjie Stander, Malcolm Taylor and Erick van Schalkwyk

In today's well-informed society people are more aware of what they consume and would like to be sure that the contents of a product correspond to its label. The food industry, like most industries suffers from the odd criminal mind that would put self-gain and the highest possible profits above the quality of their products and the well-being of their consumers.

Ver the past 14 years the Mass Spectrometry Unit at CAF has been involved in the testing of products as a result of a number of food/ notorious beverage scandals in 2007/2008 when 6 Chinese babies died and 296 000 fell ill after drinking melaminetainted infant formula. Melamine is an industrial chemical which is used to produce kitchen ware and table tops. Melamine  $(C_3H_6N_6)$  has a high nitrogen content. The industry standard method to determine protein content is to measure the nitrogen content of a sample, which makes melamine adulteration an easy and inexpensive way to artificially increase the apparent protein content of products.

Melamine was also added to gluten meal that was exported world-wide including South Africa, where it was used in pet food. A method developed for Prof Cruywagen at the Department of Meat Science, University of Stellenbosch, was used to test for melamine in hundreds of pet food products and milk formula at our laboratory. The method was later transferred to commercial labs and is still used weekly to test raw materials used in the manufacturing of pet food as well as in milk products.

The same method was used by Cruywagen et al <sup>1,2</sup> to show that when melamine is used as fertilizer, it passes through the food chain into grass and will end up in meat, milk and eggs.



Beverages like fruit juice, wine and spirits also fell prey to foul play in Europe and the East over the past decade. In 2011 di(2-ethylhexyl) phthalate (DEHP) was found in food and drinks in Taiwan after it was added as a clouding agent which affected 900 products sold by 40 000 retailers.

Phthalates are added to plastics, primarily polyvinyl chloride (PVC) as plasticizers to increase their flexibility and transparency. They are anti-androgens and have various other adverse health effects resulting in their banning in a large number of products including childrens toys and erasers.

Diet is believed to be the main source of phthalates in our bodies, with fatty foods like milk, butter and meat the major source and not water bottles like many people believe. Many studies have been conducted at our laboratory to test for phthalates, for many different clients.



It includes the testing of baby bottles, water bottles, printed paper and soft plastic toys for plasticizers including phthalates and bisphenol A.

The GC-MS laboratory has implemented a very sensitive method to test for phthalates in wine and spirits to ensure that products were not contaminated when pumping them through pipes that may contain phthalates. The LC-MS laboratory has developed a number of tests to ensure that beverages are not contaminated by preservatives like natamycin, sorbic acid, citric acid and benzoic acid, colorants and artificial sweeteners. Additionally, wine is tested for traces of Ochratoxin A, a harmful secondary metabolite of fungi growing on grapes. Fruit juice and honey are also prone to adulteration where cheap adulterated imported products is a serious threat to our own industries.

Adulteration of fruit juice can have many forms including the substitution of higher value fruits with inexpensive fruits, the addition of sugar and water to stretch the volumes and the addition of artificial sweeteners. We have developed a fast screening method for fruit juice testing that screen for a number of preservatives, colorants and artificial sweeteners and at the same time tests for marker compounds for certain fruits and test for the ratio of sugars and oligosaccharides.



Grapes for example contains roughly equal amounts of fructose and glucose and only trace amounts of sucrose. High amounts of tartaric acid are a marker for grape juice. Apples and pears contain sorbitol that is not present in grapes,

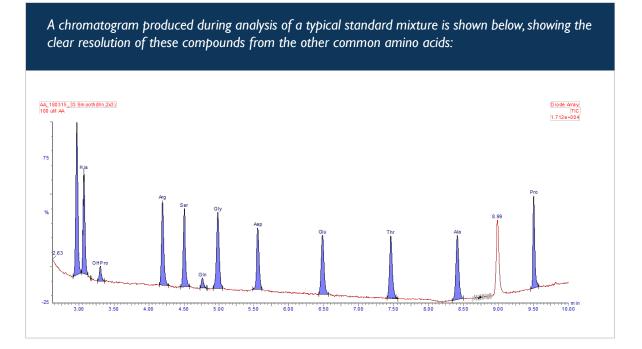
# whilst certain amino acids are more prevalent in apple juice.

The accidental or fraudulent mixing of animal products or by-products from different species is also an important concern for consumers with health or ethical concerns. Gelatin, for example, is produced by the hydrolysis of collagen, which is extracted from materials such as bones and hides of pigs and cows obtained from abattoirs, and is used as a gelling and thickening agent in a variety of food products including meat, confectionery products and water-based desserts. It is also widely used in nutriceuticals and the pharmaceutical industry.

Nearly 80% of gelatin manufactured worldwide is produced from pork by-products. In Islamic countries, however, consumption of porcine products is forbidden so alternative products such as agar agar, carrageenan and vegetable gums are often used as gelatin substitutes to avoid the health and ethical concern associated with gelatin.

However, cross-contamination between different products can occur since they are often produced in the same processing facility. It is important therefore to be able to test for either the presence of gelatin in vegetarian products, or to distinguish between porcine and bovine gelatins in Halal products.

Gelatin has a characteristic amino acid sequence, namely a repeating G-X-Y sequence, where G is glycine, X and Y are proline and hydroxyproline. Acid hydrolysis of the collagen peptides making up the gelatin leads to the presence of glycine (Gln), hydroxyproline (OHPro) and proline (Pro) amino acid residues in the acid hydrolysate. In the LC-MS laboratory at CAF, these amino acids are first derivatized and then analysed using ultraperformance liquid chromatography using UV detection.

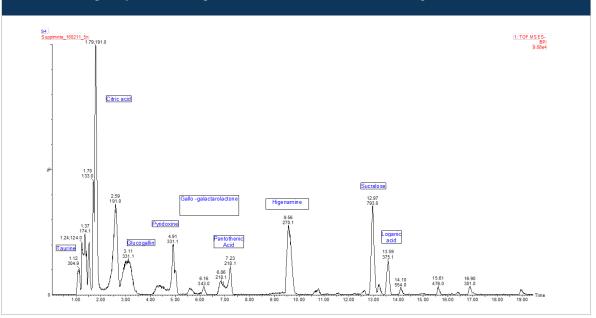


Nutritional supplements are widely used by consumers to improve their health and general well-being, and by athletes to give them an edge in performance over their competitors. Due to the high stakes in international sport, athletes are constantly being driven to perform.

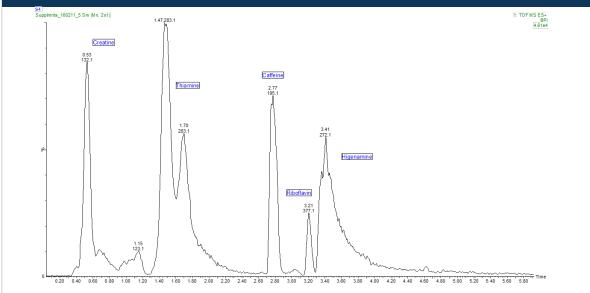
This has led to a booming global industry, worth 1 billion Euro in Germany in 2002 and US\$16.7 billion in the USA in 2000. Competition amongst manufacturers for this lucrative market has led to the addition of banned performance-enhancing substances into various nutritional supplements. An International Olympic Committee study in 2002 found 94 out of 634 (14.8%) supplements contained one or several prohormones not mentioned on the label. These prohormones are metabolized by the body into the active drug, often a banned anabolic steroid, resulting in a positive drug test with serious consequences for the athlete.

The LC-MS laboratory of CAF, together with Prof. Kathy Myburgh of the Muscle Research Group, Department of Physiology, University of Stellenbosch, has developed an untargeted screening method for banned stimulants and steroids using the Synapt G2 High-Resolution Mass Spectrometer (Waters, Milford, USA). This allows supplement samples to be screened to check that their contents correspond to their labels, and also to check for the presence of 45 compounds against a set of calibration standards.

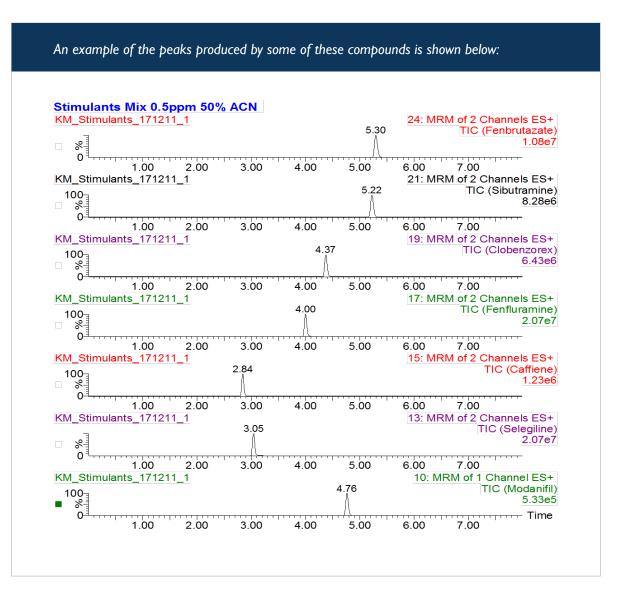
An example of an untargeted analysis of a commercially available supplement sample is shown below, illustrating the presence of high levels of creatine, taurine, caffeine, higenamine and sucralose:







A targeted tandem mass spectrometry method was also set up on the Xevo TQ-MS (Waters, Milford, USA) instrument to measure trace concentrations of these compounds.



Mass spectrometry is well suited to its emerging role in the new science of Foodomics and it is expected that the Mass Spectrometry Unit of CAF will play an increasing role in years to come, in ensuring the quality and safety of the products used by South Africa's population.

References:

<sup>[1.]</sup> Cruywagen, CW, van de Vyver WF, Stander MA (2011) Quantification of melamine absorption, deposition and excretion by sheep. Journal of Animal Science Jul;89(7):2164-9

<sup>[2.]</sup> Cruywagen, C.W., Stander, M.A., Adonis, M. and Calitz T. (2009). Hot Topic: Pathway confirmed for the transmission of melamine from feed to cow's milk. J. Dairy Sci. 2009. 92:2046-2050

X-ray CT analysis of evolutionary optimization of snake fangs

By Prof Anton du Plessis

stress [kPa]

80000 7/0000

> Mankind is often fascinated by spectacular natural structures, and sometimes we would like to learn how evolution converged to create such structures. Is the structure mechanically optimized for its basic task?

Snake fang evolution is one such interesting topic as venomous snakes provide an example of a fascinating pinnacle of the evolutionary process. Snake fangs are larger than normal teeth and are meant to deliver venom during a bite. This simple task has however evolved across all venomous species into not one but three basic shapes.

The question is: is one of these three shapes optimized mechanically while the others are not? W hat are the driving forces in the shape optimization for these fangs?

This kind of biomechanical study sheds some new light on the pressures involved in the fang's evolution. MicroCT is a 3D imaging technology which allows the viewing of internal details of objects non-destructively. Its use has grown over the last few years, and has been reviewed for its use in biological sciences [1]. Locally, this technology is available at the CT Scanner facility which provides microCT, nanoCT and 3D image analysis capabilities [2].

In the study reported in [3], the internal and external morphology of fangs were analysed in detail using nanoCT scans and biomechanical simulations were conducted using image-based simulation methods. This work comprised comparison of the results across approximately 20 fangs from different species.

The results indicate that of the various fang shapes that have evolved, none is superior to the other biomechanically – that means when one fang is longer and thinner, it has thicker walls to compensate, or when it is more curved it has a thinner venom canal, etc. The details of the morphology and the simulation details are reported in another paper [4].

An example is shown in the image above, the simulation is made by fixing the base (blue) and applying a load to the tip region (in green) – force direction indicated by the arrow. Linear elastic isotropic material properties are selected and the simulation is done using a direct finite element code based on microCT data. The resulting colours show areas of high stress as in the figure, in this case on the underside of the fang.

These simulations are not limited to biological structures and may be applied to any microCT data. It also finds application in engineering applications where the design files can be compared to the microCT data of the real parts, including rough surfaces and internal defects. The differences can then help to understand why a part fails or where the weakest point in the structure is. For more information please go to www.sun.ac.za/ctscanner

#### References:

- A. du Plessis, C. Broeckhoven, A. Guelpa, and S. G. le Roux,
  "Laboratory x-ray micro-computed tomography: A user guideline for biological samples," Gigascience, vol. 6, no. 6, 2017.
- [2] A. du Plessis, S. G. le Roux, and A. Guelpa, "The CT Scanner Facility at Stellenbosch University: An open access X-ray computed tomography laboratory," Nucl. Instruments Methods Phys. Res. Sect. B Beam Interact. with Mater. Atoms, vol. 384, 2016.
- [3] C. Broeckhoven and A. du Plessis, "Has snake fang evolution lost its bite? New insights from a structural mechanics viewpoint," Biol. Lett., 2017.
- [4] A. Du Plessis, C. Broeckhoven, and S. G. Le Roux, "Snake fangs: 3D morphological and mechanical analysis by microCT, simulation, and physical compression testing," Gigascience, vol. 7, pp. 1–8, 2018.

# Capacity building at Electron Microscopy



**Back from left to right:** Priscilla Nyakombi, Gestel Kuyler, Lydia-Marie Joubert (Unit Manager), Ian Weir, Nonkuselo Madlakana **Front from left to right:** Elrika Harmzen-Pretorius (Sr Analyst), Feziwe Mamba, Madelaine Frazenburg (Sr Analyst).

By Prof Lydia-Marie Joubert

When the EM Unit experienced a marked increase in the number of industry clients who require regular electron microscopy imaging and analysis, their workload moved into after-hour and weekend operation. Since one of the principles under which the unit runs, is to use normal office hours mainly for research applications and academic users, and restrict non-academic applications to early morning and late afternoon, they decided to recruit student researchers who are proficient in electron microscopy to support regular industry users.

urrently we have 4 MSc and I PhD students using our Zeiss MERLIN and Zeiss EVO scanning electron microscopes after hours and over weekends, for routine imaging done mainly for clients in polymer sciences who submits samples on most weekdays, and also clients from the mining industry who regularly submits large numbers of samples for quantitative elemental analysis.

Our daily courier service additionally supports sameday pickup and delivery of samples around the Cape Peninsula, after which student analysts get informed via a WhatsApp group that an analyst is needed for the evening. Students collect data and share images and analytical reports with the client via Google Drive for same-day access.

Students log their own hours at the instrument, and a minimum number of images or data points are required per hour, to earn the associated hourly fee. For payment structure, students are classified as technical research support, and obtain valuable 'real life' experience, working with non-academic clients whose deadlines and imaging requirements must be met rapidly and proficiently.

This has an additional benefit to their resume for future employment, and their own research benefits from in-depth knowledge of EM operation.

Nonkuselo Madlakana, our PhD candidate in Earth Science, mostly works on the analytical applications with Sr Analyst Madelaine Frazenburg, while Gestel Kuyler, Priscilla Nyakombi and Fesiwe Mamba (MSc Polymer Sciences) as well as lan Weir (MSc Earth Sciences) do SEM imaging under guidance of Sr Analyst Elrika Harmzen-Pretorius. EM Unit Manager Lydia-Marie Joubert runs a monthly electron microscopy training course which involves theoretical lectures and demos, followed by one-on-one instrument training using the student's own samples.

She describes the student analysts as a wonderful and dedicated group to work with, and their support services are of great value to the workflow of the EM Unit, and very much appreciated.



According to **Ian Weir,** who is finalizing his MSc in Earth Science:

"I was actually looking for an analytical job to make a supplementary income. Elrika approached me (after I completed the training course) and asked if I would be interested and I said MOST DEFINITELY. I thoroughly

enjoy analytical work and have been extremely grateful for the opportunity as a student analyst. It has made my work output from SEM imaging a lot more proficient while at the same time I am learning an invaluable skill set. It is definitely something I can see myself doing in the future!"



**Feziwe Mamba**, an MSc student in Polymer Sciences, says:

"I was motivated to apply [to become a student analyst] by the fact that I already had the basic skills required for SEM analysis. Knowing that our clients' samples are more challenging, I wanted to find a way of dealing with

such complications on my own yet meeting deadlines. I hope to gain more experience and be able to learn new skills such as spotting and correcting instrumental errors and be able to come up with new ideas to improve the analysis technique."



Priscilla Nyakombi, who is doing her MSc in Polymer Sciences, added:

"What motivated me to apply was, I wanted to learn more about the SEM instrument like how does it operate, how to get get the best images and which parameters you have to look on when using it.What I am hoping to get is more experience, since I will become

more familiar with the instrument. I am also doing a lot of electrospinning on my MSc project and am using the same instrument to study the morphology of nanofibers, so this also help me on my project as well".



**Gestel Kuyler,** another MSc student in Polymer Sciences, is excited about this opportunity and responds:

"I am fascinated by the nanoscale world! I am using scanning electron microscopy in my own nanofiber research and saw it as a valuable opportunity to learn more about the

instrument and gain operational experience. I also hope to gain industry exposure and practical SEM skills which I can apply to my own research, as well as future work. It is a great opportunity to expand your skill set and resume."



Our most senior student analyst, **Nonkuselo Madlakana,** who is finalizing her PhD in Earth Sciences, responds:

"I was always fascinated by how the microscope works, I always wanted to know the background information on how the quantitative analysis is processed by different softwares. I

dream of managing a lab in the near future, so I took this as an opportunity to learn the fundamental basics of the instrument. I have acquired a lot of experience since 2014. I did not only learn about geology, but also on how to apply the instruments on non geological samples such as biology and polymer science. I believe the experience I have acquired would help me in the near future. And I am looking forward to enhance my understanding in all the software applications that are available."

"I believe the experience I have acquired would help me in the near future. And I am looking forward to enhance my understanding in all the software applications that are available." Next Generation Sequencing in the field of

# Human Genetics

By Alvera Vorster & Carel van Heerden

The DNA sequencing unit has a long history of supporting research at Stellenbosch University, as well as other universities. Since its inception over two decades ago, the unit has supported research in the field of Human Genetics though cycle sequencing and fragment analysis.

his traditional service provision role was expanded to include next generation sequencing (NGS), following a National Equipment Program grant award to Prof Johan Burger; for the procurement of the ABI 5500xl and Ion Torrent Personal Genome Machine (PGM). With these instruments in its arsenal, the unit aimed to become a trusted provider of high quality massively parallel sequencing data.

In September 2013 the DNA sequencing unit became the first laboratory to sequence a human genome on African soil, using the 5500xl. With the 5500xl dedicated to the generation of large datasets, the PGM was employed to generate data for small genomes or sub-sets of larger genomes through fragmentation-based workflows. The introduction of the targeted sequencing technologies like AmpliSeq TM soon afterwards allowed the PGM to be employed to investigate specific areas of the human genome at a reduced cost and with an improved data delivery time.

A second NEP grant was awarded to Prof Altus Viljoen for the procurement of an Ion Torrent Proton. This platform had approximately 10x the data capacity of the PGM and led to the birth of the whole human exome (WES) sequencing service that we are currently offering. To date, the Ion Proton has generated 326 data sets – approximately 4 890 000 000 000 bp of data.

This includes more than 280 human exomes; half of which were sequenced in 2017/ 2018. These WES projects, some small scale (n = 2) and other large scale (n = 100), are co-ordinated by South Africans, to investigate the genetic determinants of a range of heritable human disorders in the local context.

These are four of the research groups in the field of Human Genetics that are currently supported by the NGS service; Cardiovascular Genetics Group at the University of Cape Town. The Cardiovascular Genetics Group (*http://www.hatter.uct.ac.za/imhotep-study-0*) aims to discover the genetic causes of inherited and sporadic heart diseases in South Africa. By studying families with rare monogenic diseases, they hope to identify the mutations and biological pathways that ultimately may be targeted to relieve symptoms and prevent sudden cardiac death in patients. In order to address this aim, they have turned to next generation sequencing, specifically whole exome sequencing (WES), as the preferred method of DNA variant detection.

During the first six months of 2018, 100 genomic samples were submitted to the DNA sequencing unit at the CAF for WES. This data that had been generated, to support the research efforts of the Cardiovascular Genetics Group, are the first exomes for congenital heart disease patients in Africa.

"I have been very impressed with how the projects were handled, from start to finish. At the start of each project I would receive an expected time of completion of the exomes and when the data would be available to me as the client (via lon Reporter and/or external hard drive). During the actual process I would receive regular updates on what stage the process was at and if any problems had been encountered. I appreciated this most of all [sic] as this allowed me to plan around the issues (not that there were many). We are still in the early stages of the data analysis but I have been very impressed with the amount of data that they generated, their data quality as well as their service delivery times; that was spot on. They have been professional, efficient and if the way they handled this project is any indication we should be having some great genetics results for heart disease soon."

– Dr Gasnat Shaboodien – Deputy Director, Cardiovascular Genetics Research Group, Hatter institute for Cardiovascular research in Africa



## The Parkinson's disease Research Group at Stellenbosch University

The Parkinson's disease (PD) Research Group at Stellenbosch University (www.sun.ac.za/parkinsons) focuses on studying the genetic basis in South African patients with PD, as well as the underlying disease mechanisms. PD is the most common neurodegenerative movement disorder and results from the loss of neuronal cells in a specific part of the brain known as the substantia nigra. Symptoms include slow movements and reflexes, involuntary trembling of the body and limbs, stiff muscles, difficulty in maintaining balance, and patients also experience various psychological manifestations.

The prevalence of PD in South Africa is not known but it is estimated to affect seven to ten million people worldwide. The advent of high-throughput NGS technologies has revolutionised the way patient's genomes can be screened for genetic defects that can lead to PD. Although these approaches are quite expensive, in the long run, they are more cost-effective than the traditional methods used in the past. Currently, NGS is used in two student research projects.

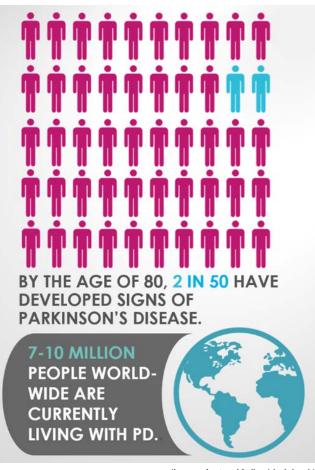
Mr Oluwafemi Oluwole is pursuing doctoral studies and his project involves the use of a commercially available targeted resequencing gene panel from Thermo Fisher Scientific. In collaboration with a team in Nigeria, PD patients from both countries are screened for genetic defects in 751 genes using a Neurological Research Gene panel on the Ion Torrent platform at CAF. Since PD has been significantly understudied in Black populations worldwide, this research is important and relevant.

To date, 33 black South African and 43 Nigerian PD patients have been sequenced and analysis of the results are underway. Preliminary analysis of data from 47 patients, show sequencing reads of good quality and after applying stringent quality criteria, a total of 2,021 variants were identified. Thirty rare variants, predicted to be pathogenic based on bioinformatic tools, were prioritised and will be studied to assess their involvement with the development of PD. These variants will be validated by direct cycle sequencing at CAF.

Ms Amokelani Mahungu is a Masters student and her project involves setting up a custom-designed gene panel for PD in order to facilitate the rapid screening of all the known PD-associated genes in a cohort of > 650PD patients. To address this aim, a 23 gene panel was designed using Agilent's SureSelect technology. This gene panel is due for evaluation during July 2018, when 16 selected PD patients will be sequenced on CAF's Ion Torrent Proton platform. Given the unique ancestry of SA populations the PD research group anticipates that novel mutations in these genes may be identified.

"In summary, the NGS service that the DNA Sequencer Unit at CAF provides has had a major impact on our research. It allows us to comprehensively screen our unique collection of DNA samples obtained from South African patients. Ultimately, this has the potential to facilitate important breakthroughs on the genetic causes, in local patients, of this debilitating and poorly understood disorder."

Prof Soraya Bardien – Principle Investigator, PD Research Group at Stellenbosch University



(Image:Amica Muller-Nedebock)

Primary Immunodeficiency Diseases Genetics Network (PIDDGEN)



DST-NRF Centre of Excellence for Biomedical Tuberculosis Research; South African Medical Research Council Centre for Tuberculosis Research; Division of Molecular Biology and Human Genetics, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town, NHLS Immunology Unit, Division Medical Microbiology, Department of Pathology, Faculty of Medicine and Health Sciences, Stellenbosch University, Cape Town.

Primary immunodeficiency diseases (PIDs) collectively represent a significant burden of disease. Recent estimates suggest that there are as many as 42,000 South African PID cases. Unfortunately the diagnosis of PIDs

in our country have been hampered, in part, by high prevalence of several infectious diseases, lack of awareness and training and, despite available clinical algorithms and basic laboratory diagnosis, the lack of transport and laboratory infrastructure for more advance immune test samples. To address these issues, the Primary Immunodeficiency Disorders Genetic Network (PIDDGEN), a multidisciplinary team of researchers and clinicians from Stellenbosch University's Faculty of Medicine and Health Sciences, have been providing molecular diagnoses to South African patients with Primary Immunodeficiencies.

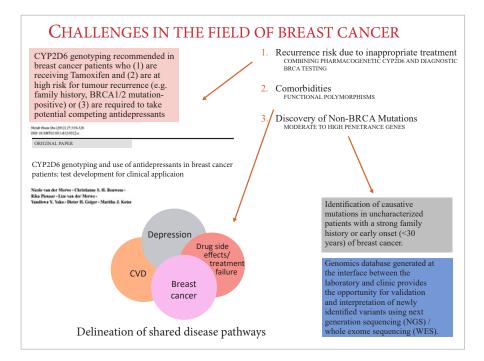
Our patients have frequently seen many doctors and were subjected to many tests and futile treatments.Their

journey through the health care system, "a diagnostic odyssey", frequently spans many years in search for an answer to the cause of their illness. PIDs, when left undiagnosed and untreated are often associated with severe morbidity and increased mortality. Quality of life for diagnosed PID patients is significantly improved compared to undiagnosed patients and there is a definite cost saving for diagnosed patients. However, for many PIDs, effective treatment is available, but since the diagnosis maybe quite challenging, several potentially treatable and serious PIDs remain undiagnosed. The PIDDGEN Program helps patients to access diagnostic genomics for previously undiagnosed diseases. All patients are carefully screened, counselled, and enrolled irrespective of financial or social background.

To facilitate molecular diagnoses, we have been using whole exome sequencing (WES) to identify the genetic mutations causing disease in each of our patients. The Stellenbosch University's Central Analytical Facility has been our preferred WES service provider for a number of years and through this partnership, we have sequenced the exomes of 41 PID patients and 52 of their family members. Of these, we were thus far able to provide a molecular diagnosis for 27 (66%) of our PID patients. This is significant given that for several PIDs, the diseasecausing mutation has a direct impact on the patient treatment.

#### **Breast Cancer Research**

Three major challenges in the field of breast cancer have been identified as research priorities. The first is the need to combine genetic testing of high-risk patients with familial breast cancer with pharmacogenetics to reduce recurrence risk in cancer survivors due to drug



failure as a consequence of anti-cancer treatment that does not match the patient's genotype. The second is the delineation of key pathways through which genes implicated in breast cancer and associated co-morbidities can serve as nutritional and drug targets across diagnostic boundaries.

The third is the discovery of genetic alterations underlying familial breast cancer not attributed to mutations in the two major tumour suppressor genes, BRCA1 and BRCA2 (see figure above).

There is currently no consensus on eligibility criteria for WES in BRCA1/2 mutation-negative familial breast cancer patients. To address these challenges, the pathology-supported genetic testing (PSGT) platform was used to develop an exome pre-screen algorithm (EPA) for selection of genetically uncharacterised patients for WES. First, diagnostic BRCA testing is offered as a routine service according to standard referral guidelines, or the chronic disease risk screen is offered to patients receiving hormone therapy, are at high risk for tumour recurrence, or are required to take potentially competing antidepressants.

In certain cases, combined diagnostic and pharmacogenetics testing is performed to explain the presence of comorbidities or predict drug response/recurrence risk. Finally, where extended mutation analysis of the entire BRCAI and 2 genes as well as the CYP2D6 gene is unable to explain breast cancer or the occurrence of drug side effects/failure, WES is performed to identify potential novel causative genes/mutations.

In a study that consisted of 164 breast cancer patients (60 Mixed Ancestry and 104 Caucasian) and 160 cancer-free controls, common genetic risk factors for cardiovascular disease (CVD) were shown to be significantly associated with earlier age (10 years on average) of breast cancer onset/diagnosis and body mass index (BMI) in patients stratified according to estrogen receptor (ER) status, after adjustment for potential confounders. Age at diagnosis/onset of breast cancer was significantly lower in patients with ER-negative versus ER-positive tumours, after adjustment for ethnicity, while BMI was significantly higher in patients with ER-positive compared to ERnegative tumours after adjustment for age, ethnicity, and a family history of cancer.

These findings contributed to the development of the EPA used to select a genetically uncharacterized family for WES and facilitated the development of a framework for WES performed alongside clinical and pathology assessments. Results supported previous findings indicating that the majority of genetically uncharacterised breast cancer cases may be caused by a combination of low-moderate penetrance mutations exerting their effect against a high-risk clinical background.

To our knowledge, this is the first study using WES to investigate the significance of folate pathway SNPs as risk reduction targets beyond BRCA1/2 in familial risk. WES preceded by PSGT facilitated the identification and clinical interpretation of genetic risk factors of relevance to both cancer development and tailored therapeutic intervention in a single test.

 Prof Maritha Kotze - Division of Anatomical Pathology at Stellenbosch University's Faculty of Medicine and Health Sciences

It is our mission to continue to provide cutting-edge sequencing services in the South African context, with the highest possible amount and quality analytical data, in the shortest possible time-frame.

# Financial Reports

### by Fransien Kamper

		January 2015 - 31 December 2015	January 2016- 31 December 2016	January 2017- 31 December 2017	Budget: January 2018 - December 2018
MS Unit	Internal invoicing	2     3 784	I 969 796	2 463 824	2 159 302
	External invoicing	4 274 813	4 754 380	5 379 758	5 880 561
	Total logbook income	6 388 598	6 724 176	7 843 582	8 039 863
	Expenses				
	Salaries	2 955 982	2 787 168	2 963 154	3 347 585
	Running costs	955 287	I 042 577	969 322	I 063 902
	Maintenance	607 627	632 923	789 232	813 781
	Travel Costs	2 380	948	36 784	7 970
	Small Equipment & KKW	12 736	91 539	24 511	38 878
	Total Expenses	4 534 012	4 555 155	4 783 002	5 272 117
FM unit	Internal invoicing	663 703	848 044	926 172	I 160 884
	External invoicing	31 493	39 136	155 292	90 082
	Total logbook income	695 195	887 180	I 081 464	I 250 967
	Expenses				
	Salaries	807 244	1 056 051	I 034 828	883 622
	Running costs	168 609	218 819	259 077	263 999
	Maintenance	41 815	38 610	16 393	156 517
	Travel Costs	31 817	9 468	7 025	0
	Small Equipment & KKW	31 430	4 686		0
	Total Expenses	1 080 915	I 327 634	3 7 323	304   38
SEM UNIT	Internal invoicing	422 687	656 850	648 946	996 662
	External invoicing	419 190	913 020	520   6	2 986 555
	Total logbook income	841 878	I 569 870	2 169 062	3 983 218
	Expenses				
	Salaries	716 526	1 049 188	I 397 948	2 010 350
	Running costs	35 925	143 704	97 459	232 670
	Maintenance	169 080	98 971	436 073	12 486
	Travel Costs	32 493	14 476	26 348	56 644
	Small Equipment & KKW	34 310	131 804	91 666	162 639
	Total Expenses	988 335	I 438 I 44	2 049 494	2 474 790

		January 2015 - 31 December 2015	January 2016- 31 December 2016	January 2017- 31 December 2017	Budget: January 2018 - December 2018
ICP & XRF UNIT	Internal invoicing	800 336	761 409	860 114	I 072 347
	External invoicing	1 390 363	I 828 224	2 230 688	3 101 831
	Total logbook income	2 190 698	2 589 633	3 090 802	4  74  78
	Expenses				
	Salaries	2 125 561	533 321	I 995 338	2 423 773
	Running costs	580 638	591 558	727 564	1 152 145
	Maintenance	140 647	232 660	216 324	802 128
	Travel Costs	29 990	95 634	20 225	133 606
	Small Equipment & KKW	15 882	4 642	116 450	0
	Total Expenses	2 892 718	2 457 816	3 075 902	4 511 652
DNA UNIT	Internal invoicing	3 058 897	3 198 595	3 805 695	3 953 251
	External invoicing	4 964 903	4 902 329	4 830 122	6 804 812
	Total logbook income	8 023 801	8 100 924	8 635 818	10 758 063
	Expenses				
	Salaries	2 261 695	2 301 652	2 440 238	3 027 319
	Running costs	4 211 622	4 382 754	4 445 734	6 728 481
	Maintenance	371 367	199 323	317 250	135 602
	Travel Costs	20 427	916	2 780	0
	Small Equipment & KKW	20 588	186 512	94 901	0
	Total Expenses	6 885 699	7 071 156	7 300 903	9 891 402
NMR UNIT	Internal invoicing	669 227	847 097	656 004	722 542
	External invoicing	478 053	500 894	967 805	791 220
	Total logbook income	I 147 279	347 99	I 623 809	1 513 763
	Expenses				
	Salaries	932 580	1 099 918	49  23	1 359 999
	Running costs	295 759	296 141	359 470	393 460
	Maintenance	40 184	63 570	7 377	25 356
	Travel Costs	27	0	0	0
	Small Equipment & KKW	0	0	33 576	0
	Total Expenses	I 269 794	I 459 629	I 549 546	I 778 8I5

		January 2015 - 31 December 2015	January 2016- 31 December 2016	January 2017- 31 December 2017	Budget: January 2018 - December 2018
CT UNIT	Internal invoicing	293 869	445 672	528 663	858 673
	External invoicing	I 200 353	1 595 162	I 886 564	3 069 092
	Total logbook income	I 494 222	2 040 834	2 415 226	3 927 765
	Expenses				
	Salaries	921 886	1 083 401	I 147 982	I 393 564
	Running costs	226 192	176 930	359 553	365 725
	Maintenance	169 200	550 312	317 000	316 464
	Travel Costs	84 462	55 697	75 088	16 244
	Small Equipment & KKW	31 127	21 406	64 287	67 290
	Total Expenses	I 432 867	I 887 746	1 963 910	2 159 287
NEURO- MECHANICS UNIT	Internal invoicing	213 844	463 998	494 473	511 313
	External invoicing	58 250	534 742	702 577	794 822
	Total logbook income	272 094	998 740	I 197 050	1 306 135
	Expenses				
	Salaries	505 769	748 201	I 225 596	475 941
	Running costs	3 2	139 797	179 398	15 409
	Maintenance	0	25 779	0	42 400
	Travel Costs	0	0	34 680	30 917
	Small Equipment & KKW	0	143 845	25 713	18 275
	Total Expenses	507 081	I 057 622	I 465 387	I 582 942
VIBRATION SPECTROSCOPY UNIT	Internal invoicing	0	0	0	105 840
	External invoicing	0	0	0	0
	Total logbook income	0	0	0	105 840
	Expenses				
	Salaries	0	0	33 924	407 619
	Running costs	0	0	0	3 778
	Maintenance	0	0	0	0
	Travel Costs	0	0	0	0
	Small Equipment & KKW	0	0	0	0
	Total Expenses	0	0	33 924	411 397

		January 2015 - 31 December 2015	January 2016- 31 December 2016	January 2017- 31 December 2017	Budget: January 2018 - December 2018
TOTAL UNITS INCOME	Total internal income	8 236 346	9 191 460	10 383 891	11 540 816
	Total external income	12 817 419	15 067 887	17 672 923	23 518 976
	Total Income: All Units	21 053 765	24 259 347	28 056 814	35 059 792
ADDITIONAL INCOME					
	Interest Received	935 186	658 894	I 050 629	485 346
	Funds Received VR(R)	1 000 000	1 000 000	750 000	750 000
	Salary Contribution VR(R)	3 171 000	3 392 970	3 596 548	3 955 164
	VAT Refund on Equipment	I 234 455	772 588	128 910	0
	TOTAL ADDITIONAL INCOME	6 340 641	5 824 452	5 526 087	5 190 510

TOTAL INCOME	27 394 406	30 083 799	33 582 901	40 250 302	
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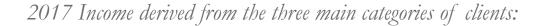
EXPENDITURE	TOTAL EXPENDITURE				
	Expenses				
	Salaries: Admin	3 0 533	I 558 230	I 827 860	1 967 315
	Salaries : Units	11 227 243	11 658 900	13 388 130	16 329 772
	Salaries: Bonus	216 378	299 718	366 750	299 326
	17% Levy (IKVK)	2 178 961	2 561 541	3 004 397	3 998 226
	Running costs (sum of units)	6 475 344	6 992 280	7 397 578	10 219 569
	Maintenance (sum of units)	I 539 920	1 842 148	2 099 649	2 304 735
	Travel Costs (sum of Units)	202 840	177 139	202 930	245 381
	Small Equipment & KKW (Sum of Units)	146 073	584 434	451 104	287 082
	CAF General Running Costs	431 663	473 143	748 646	587 200
	Travel Costs-Courier	55 684	72 556	77 797	80 000
	Development New Labs			415 719	
	Infrastructure	644 500	262 33	92 912	2 023 976
	Infrastructure NII			2 000 000	
	Equipment	1 162 848	931 176	904 483	I 300 000
	Equipment Repair fund	867 200	500 000	500 000	500 000
	CAF Vehicle Fund	0	20 000	45 000	45 000
	Loan VR(R)	540 000	540 000		
	Total normal operational costs	26 999 188	29 473 596	33 522 955	40 187 582
	Surplus per year	395 217	610 203	59 946	62 720
	CAF Overdraft (Originally 5 Rmilj Facility)	4 000 000	3 500 000	0	0

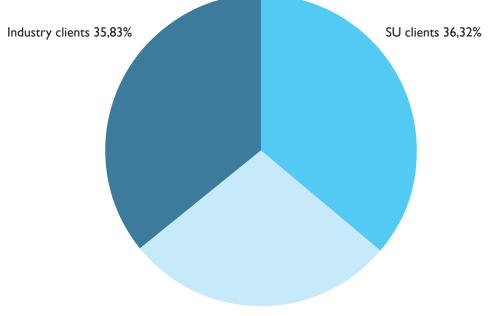
	January 2015 - 31 December 2015	January 2016- 31 December 2016	January 2017- 31 December 2017	Budget: January 2018 - December 2018
NRF EQUIPMENT FUNDING	27 106 909	25 258 792	15 527 968	0
NRF-NEP Total grants	20 004 000	17 300 000	10 237 142	
ALT/US Funds	5 000 000	6 217 076	5 127 016	
Departments/ Faculties/ VR(R) Contributions	1 002 211	647 806		
CAF Contributions	1 100 698	1 093 910	163 810	

NEP EQUIPMENT DETAILS		27 106 909	25 258 792	15 527 968
	Pegasus GCxGC-HRT-MS	8 956 372		
	ELYRA PI with 3D PALM and ELY CO2/T management component	4 115 544		
	Carl Zeiss Merlin Field Emission Scanning Electron Microscope with STEM for Correlative Microscopy	10 102 222		
	Agilent 7900 ICP-MS	3 932 772		
	Integrated real-time neurophysiological and biomechanical analysis system		9 520 169	
	Capillary Sequencer		4 074 549	
	Waters Ultra Performance Convergence Chromatograph (UPC2) connected to a Waters Xevo TQ-S MS		II 664 074	
	BD FACSMelody Cell sorter			7 380 393
	LabScanner, Prediktera Software and Via-Spec transmission access			8 147 575

FUNDS					
	Emergency Equipment Repair Fund	474 480	I 036 543	353 701	523 701
	Vehicle Replacement		59 686	108 464	153 464
	Reserve, Food Security Project	1 035 185	I 109 835	I 188 095	I 188 095
	Maintenance Fund Equipment: BD FACS Jazz sorter (2013)	I 089 483	1 168 049	1 250 413	34  53

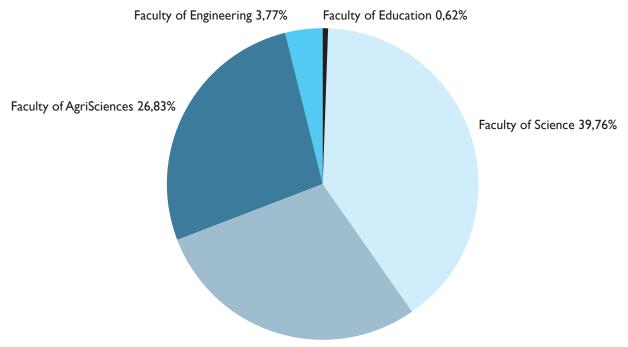
### Graphs detailing aspects of CAF income during 2017





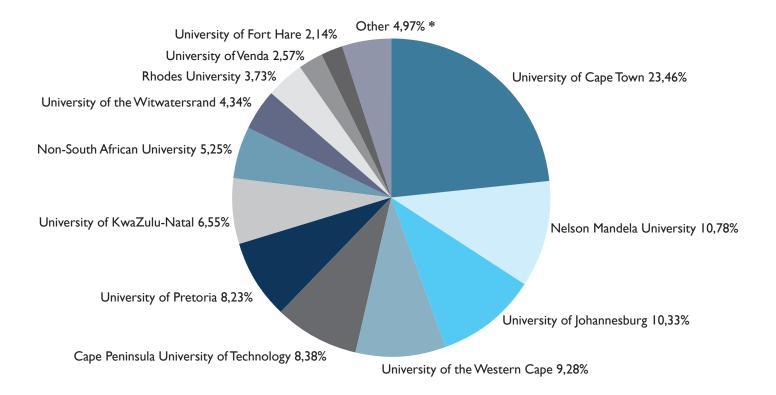
Clients from other SA universities 27,85%

### Analysis of CAF income from internal clients by faculty:



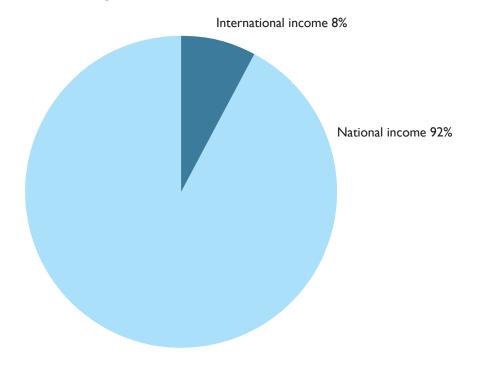
Faculty of Medicine and Health Sciences 29,01%

### Analysis of CAF income from South African external academic clients by university:



\* Other: Durban University of Technology, North-West University, UNISA, University of the Free State, Walter Sisulu University, Central University of Technology, Vaal University of Technology, University of Zululand, Tshwane University of Technology

# Analysis of the proportion of CAF income from external clients that is derived from international clients:



### CAF mid-year training participants



#### **Editorial team**

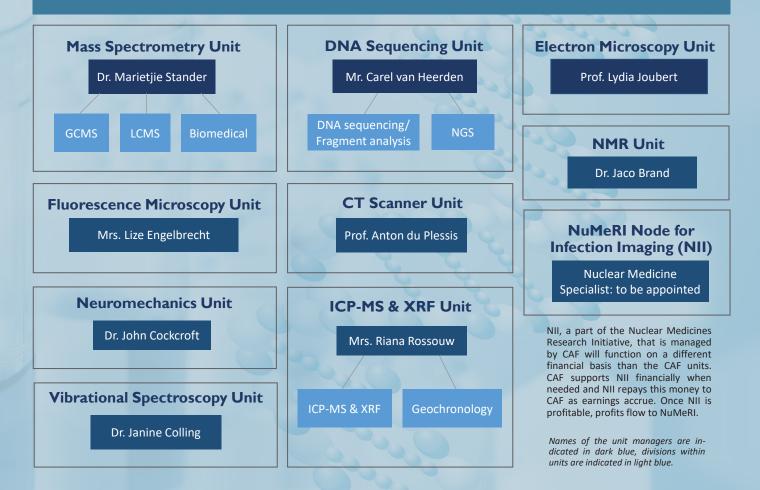
Writers: Prof Gary Stevens, Dr Jaco Brand, Dr Marietjie Stander, Prof Anton du Plessis, Prof Lydia-Marie Joubert, Alvera Vorster, Carel van Heerden, Malcolm Taylor and Erick van Schalkwyk

Compiled by: Elbie Els • Financial information: Fransien Kamper • Design & Layout: Elbie Els

CAF structure at end 2018

### CAF MANAGEMENT

### Director: Prof. Gary Stevens • Manager: Mrs. Fransien Kamper



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