CAF Central Analytical Facilities
STELLENBOSCH UNIVERSITY

# 2020/2021 Annual Report of the Central Analytical Facilities

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# Overview

Prior to the start of 2019 it would have been impossible to imagine that CAF, or indeed most Stellenbosch University environments, would be able to survive and function during the massive disruption caused by the COVID 19 pandemic. It has been both deeply humbling and hugely inspiring to witness how people and operational units have adapted their mode of working and found a way to keep SU teaching and research functioning under the most trying of circumstances. I would like to express my gratitude to all CAF staff who found ways to keep their laboratories functioning under all levels of lockdown and who found novel ways to provide analytical and administrative services whilst restricting laboratory and office access to only essential staff.

As CAF is largely self-funding, the disruption to services caused by the pandemic presented an immediate financial challenge. During the 1st half of 2020, Stellenbosch University granted CAF a financial facility which allowed us to cover our costs and to continue to operate. Total losses for 2020 were conservatively estimated at R6. 4 million (see Figure 1). Thankfully CAF was able to submit a claim against the university's interruption of business insurance which was successful and which paid out R5 million, as reflected in the financial statements at the end of this report. A request has been submitted requesting the R1.4 million loss not covered by the insurance claim to be covered by the COVID contingency fund. If this request is granted CAF will have broken even in 2020, as it stands a loss of R1.38 million has been recorded for the year.

Income and costs for the 2nd half of 2021 are more difficult to predict than is normally the case, because many CAF clients in research and industry are in the process of recovering from COVID disruption, whilst still also being affected by the ongoing pandemic. However, the financial projection for 2021 presented in this report predicts a R2.8 million loss. This is not sustainable and all efforts are currently being made to ensure that expenses do not exceed income. As indicated by the graph below, CAF expenses are normally closely matched with income. The shortfall of income in 2019, preceded the pandemic and probably reflects the effects of national policy changes relating to the way NRF post-graduate student bursaries are allocated, as well as the overall availability of research funding to South African researchers. These factors will continue to put pressure on CAF sustainability into the future. This requires both the development of innovative strategies to expand income in areas where excess capacity exists to serve industry clients in particular, and careful evaluation of the academic value to SU of the services CAF provides that have historically proved to be financially unsustainable.

The need for CAF profitability to improve is also clear if equipment funding requirements are considered. The success of SU competitive grant applications to the National Equipment Program (NEP) of the NRF has afforded the university the opportunity to purchase in execs of R30 million in large analytical equipment, on average, for each of the past 10 years. The resultant equipment base has become essential to the comprehensive analytical services that CAF provides to SU researchers. Many of these items of equipment will reach the end of their lifespans during the next 10 years and it is very unlikely that future NEP funding will provide for the replacement of this equipment. All past applications from SU to NEP for equipment replacement have failed, in contrast to our success with applications for equipment the university does not have. Some aging equipment may be replaced by different new equipment, particularly where major new analytical technology has recently been developed, but many essential services at CAF rely on mature technologies where we will require a new version of the equipment we currently manage. On average, at least R15.5 million is needed each year for the next eight years in order to meet this equipment replacement requirement (Figure 2), with the requirement for such expenditure spread over most CAF units (Figure 3). This value is higher than the amount that SU typically budgets for large equipment investment via the ALE funds and these funds must also cover co-investment to NEP applications. Thus, it is clear that CAF will need to have the capacity to fund at least a portion of these equipment replacement costs.

Prof Gary Stevens
CAF Director

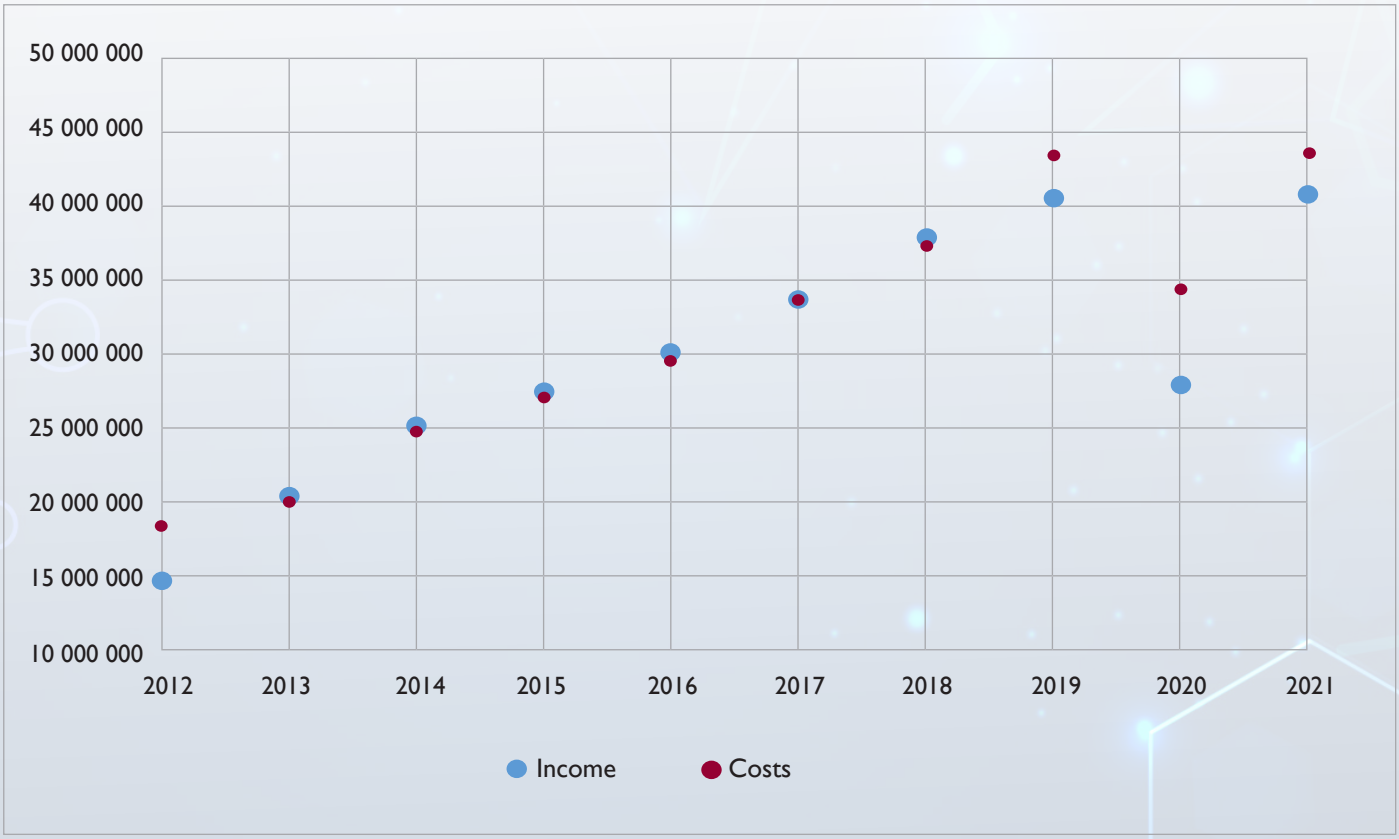



***Figure 1:*** *CAF income and costs for the period 2012 to 2021. A significant component of CAF cost is related to the purchase of expensive reagents. This component of cost scales with demand and explains the significant decrease in cost during 2020 when the COVID pandemic had the greatest impact on the volume of work flowing through CAF labs. It is important that CAF takes the steps during 2021 to reestablish the sustainable growth in services that is apparent in the information for 2013 to 2018.*

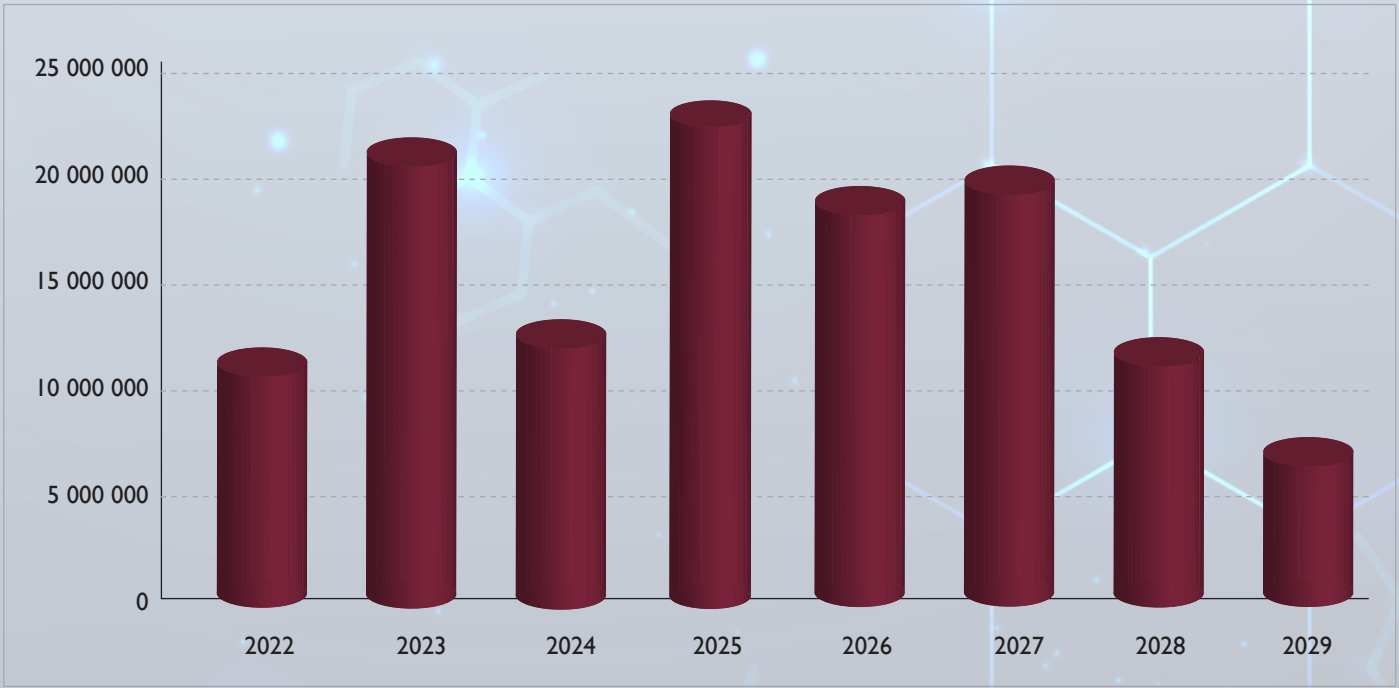



***Figure 2:*** *The predicted cost of large equipment replacement in CAF between 2022 and 2029. Only equipment over R500 000 is included. The vertical axis represents cost in South African Rand at 2021 values.*

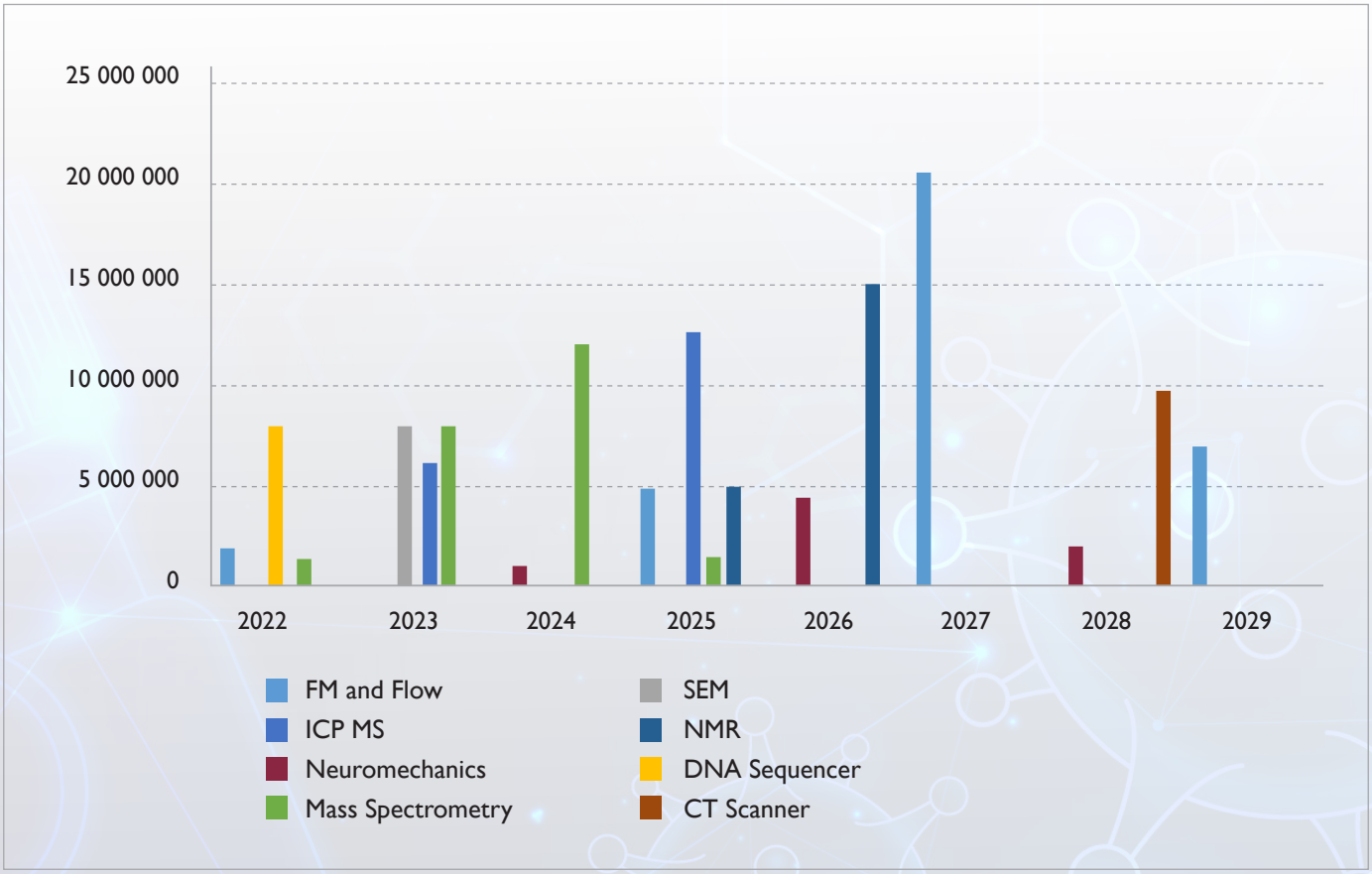



***Figure 3:*** *The information from Figure 2 expressed by CAF unit. Only equipment over R500 000 is included. The vertical axis represents cost in South African Rand at 2021 values.*

# Profile of the CAF client base

Since 2017, CAF has collected comprehensive information on the use of CAF facilities. This enables us to provide the NRF with a comprehensive profile of the use of NEP-funded equipment. Figures 4 - 8 below provide some information on the CAF client base in 2020 as well as on possible changes to the profile of CAF clients over time:

***Figure 4:*** *The number of active CAF clients from 2017 to 2020, including the percentage of industry and academic clients.*

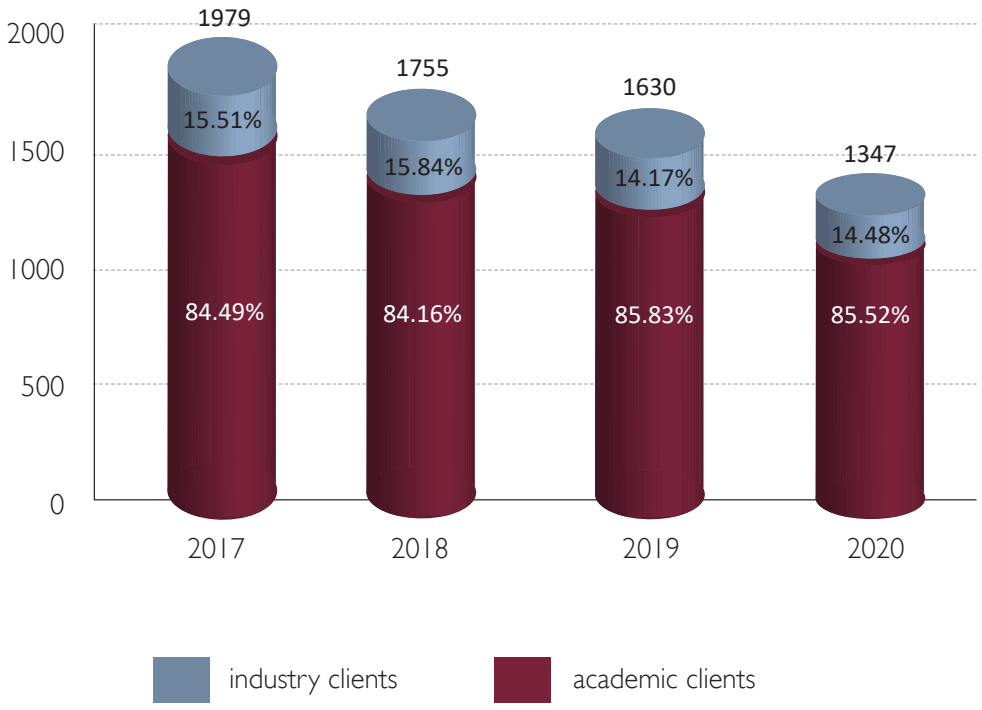



***Figure 5:*** *The subdivision of CAF academic clients according to type of institution for 2020.*

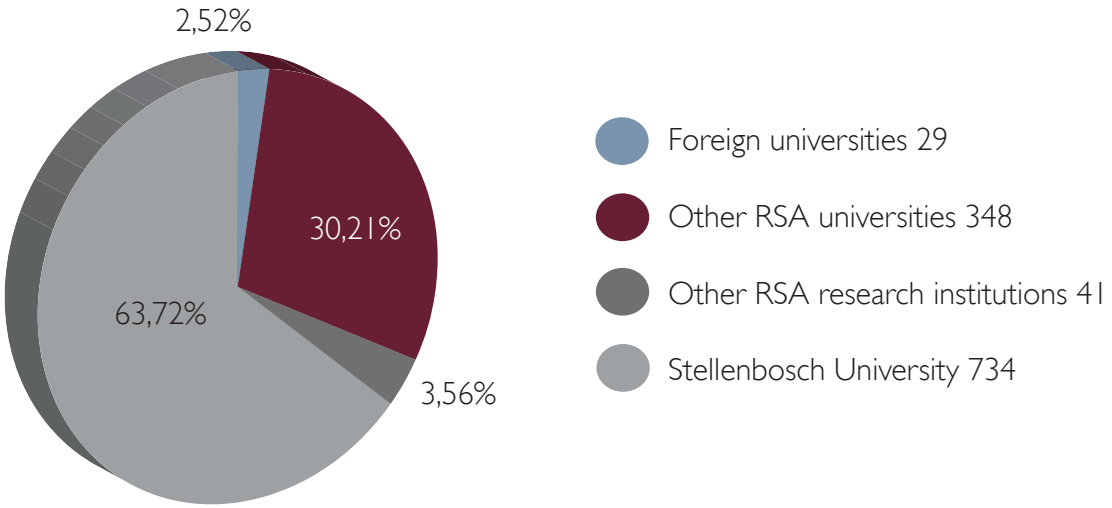


***Figure 6:*** *The profile of CAF academic clients for 2020.*

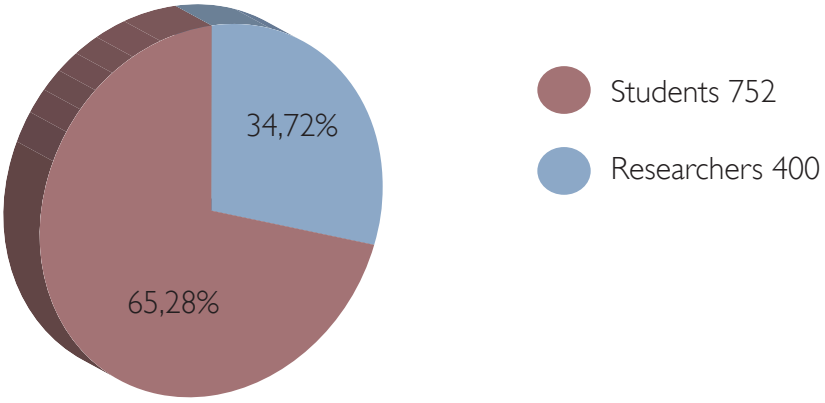



***Figure 7:*** *The subdivision according to level of study of the 65,28% students for 2020 compared with 2019.*

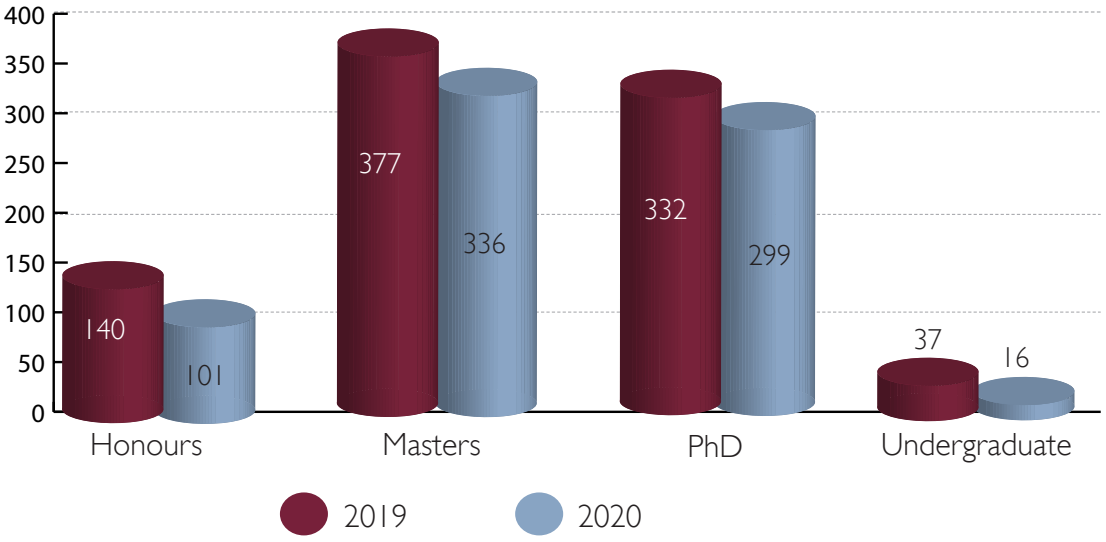
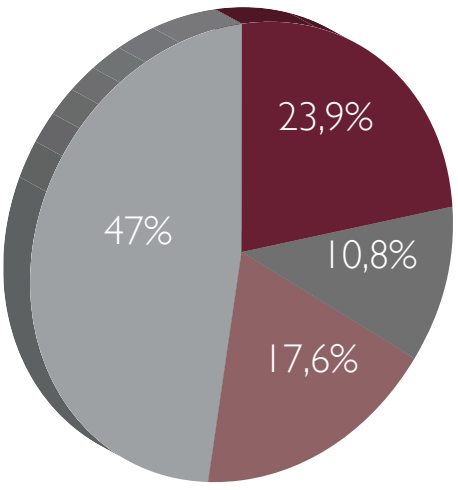



***Figure 8:*** *Stellenbosch University student clients of CAF for 2020 classified according to faculty.*




Faculty of Agrisciences 175
Faculty of Engineering 79
Faculty of Medicine & Health Sciences 129
Faculty of Science 345

Less than 1%:
School of Public Leadership 1
Faculty of Education 3
Military Science 1
Faculty Art & Social Sciences 1

# Advanced techniques at the NMR and DNA Units used to study the world's most important herbicide and the way in which weeds build up resistance to it

*By Dr Jaco Brand*

Plantago lanceolata is a plant that is native to Europe and that has been introduced to many countries of the world, where it occurs as a weed in arable fields. P. Lanceolata has typically been well controlled using broad-leaf herbicides. In 2003 Prof ALP Cairns of the Department of Agronomy found a glyphosate-resistant plantago (Plantago lanceolata L.) population located in the Robertson district of South Africa. He subsequently subjected the plant to different glyphosate dosages, and the highest dosage (7200 g a.e. $ha^{-1}$) showed no acceptable levels of control, whereas the recommended dosage rate for glyphosate is 540 g a.e. $ha^{-1}$.

In 2018, continued concerns by growers about why this was happening on their farms prompted Dr PJ Pieterse of the same department to secure funding and recruit a PhD student to investigate the mechanisms responsible for glyphosate resistance in a plantago population from the Robertson area.

With advanced and sensitive techniques available at the Central Analytical Facilities (CAF) and funding from one of the biggest herbicide manufacturers (Syngenta, UK), it was now possible to investigate the possible mechanisms of glyphosate resistance.

Glyphosate [N-(phosphonomethyl)glycine] is by far the world's most important herbicide due to its versatility and affordability [1-3].Glyphosate-resistant weed species have become very common, and they threaten glyphosate-based weed management strategies.This is because glyphosate is the world's most important herbicide, and is used worldwide to control a broad spectrum of weeds in various cropping systems. Consequently, high selection pressure from glyphosate abuse has led to the evolution of resistance to glyphosate in weeds. Glyphosate resistance was first reported in 1996 in an apple orchard. Since the development of glyphosate, more than 71 different countries have reported glyphosate resistance [1-3].

## Solid-state $^{31}P$ and $^{13}C$ NMR spectrometry

Relatively low concentrations of glyphosate in plant cells can be observed by using $^{31}P$ and $^{13}C$ NMR spectroscopy [4]. Specifically, for this study, due to the easy sample preparation, the translocation of $^{13}C_2$ enriched glyphosate in dried leaves was detectable by solid-state nuclear magnetic resonance (NMR) [5-7] at the CAF NMR laboratory. Resistant and susceptible plantago biotypes was grown in small plastic pots containing coarse gravel. A drop of $^{13}C_2$ enriched glyphosate was then applied at the middle of some of the mature fully expanded leaves [3] of both biotypes. After two days, the

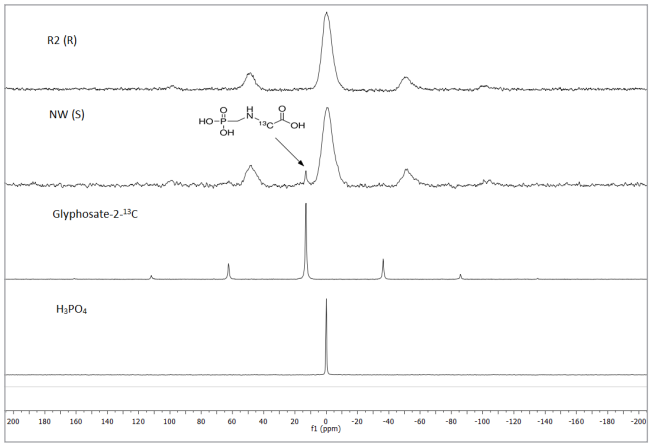



***Figure 9:*** *The $^{31}P$ NMR spectra obtained from untreated NW (S)/R2 (R) biotypes plantago (Plantago lanceolata L.) dried leaf tissue, 48 hours after glyphosate application to other leaves on the same plant. The arrow indicates glyphosate signal at 13 ppm, in the NW (S) biotype only, and identical to the glyphosate-2-$^{13}C$ (99% atom) standard's chemical shift. All $^{31}P$ spectra are referenced against orthophosphoric acid set at 0 ppm.*

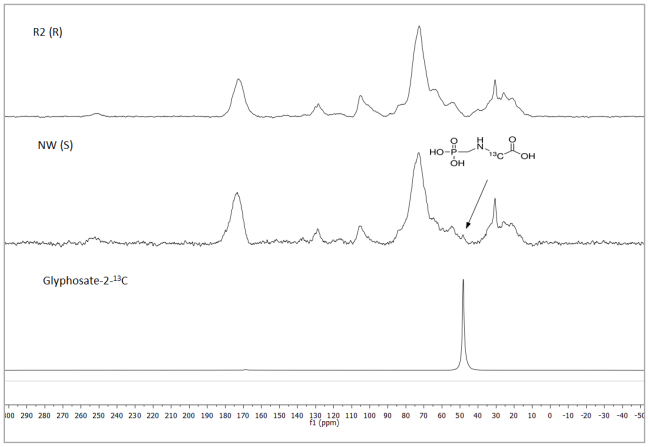



***Figure 10:*** *$^{13}C$ NMR spectra obtained from untreated NW (S)/R2 (R) biotypes plantago (Plantago lanceolata L.) dried leaf tissue, 48 h after glyphosate application to other leaves on the same plant. The arrow indicates glyphosate signal at 50 ppm, in the NW (S) biotype only, and identical to the glyphosate-2-$^{13}C$ (99% atom) standard's chemical shift.*

plants where harvested and the untreated leaves separated from the treated ones on each plant biotype. The untreated leaves were milled using a milling machine and then vacuum-dried immediately before being subjected to solid state $^{31}P$ and $^{13}C$ NMR spectrometry [8]. The spectra were acquired using an Agilent VNMRS 500 MHz two-channel solid-state NMR spectrometer. All cross-polarisation (CP) magic angle spinning (MAS) spectra were recorded with the latest VnmrJ 4.2 (Agilent Technologies Inc., Santa Clara, CA, USA) instrument software at ambient temperature.

The $^{31}P$ CP MAS NMR spectra from the untreated leaves of the *S* biotype yields a glyphosate signal at 13 ppm, at the same chemical shift as the (commercial standard) $^{13}C_2$-labeled glyphosate relative to an $H_3PO_4$ (aq) reference standard (Figure 9). This translocation of glyphosate through the plant appears to be very quick in S biotypes compared to *R* biotypes, or is perhaps absent in *R* biotypes [3] since no glyphosate signal was observed for the untreated leafs of the *R* biotype. This means that glyphosate remained at the site of application in the *R* biotype, even after 48 hours following glyphosate application. This inhibition of glyphosate translocation allows *R* biotypes to survive glyphosate treatments by inhibiting the amount of glyphosate that can spread from the treated leaves to the meristems allowing new plant growth to continue [9]; this was certainly the case in plantago *R* biotypes.

## EPSPS cDNA sequencing at the CAF DNA Unit explored

Results from the DNA sequencing showed a single point mutation in the EPSPS Syntase enzyme. This is an essential enzyme in the Shikimic acid pathway, which leads to prevention of the biosynthesis of the amino acids phenylalanine, tyrosine, and tryptophan. These point mutations very likely grants the resistance to glyphosate, together with the reduced translocation observed by the CAF NMR Unit.

## Results

This research benefited from the utilisation of solid-state NMR analysis that allowed the tracking of glyposate uptake and translocation within plants.

The results unambiguously show the first evidence of glyphosate resistance mechanisms in plantago. This is very disturbing as it threatens the world's most important weed control resource (glyphosate).

In future, other weed species besides plantago from other farms should be collected and tested for resistance. Moreover, the mechanisms of resistance of these troublesome weed species should be investigated using the methods (or potentially more sensitive future methods) available at the CAF to document the possible mechanism(s).

***Figure 11:*** *The troublesome plantago (Plantago lanceolata L.) weed that has infested many orchards and vineyards in the Western Cape Province of South Africa.*

Article published on 30 April 2021 and available online at *https://www.mdpi.com/2073-4395/11/5/884*.

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# PhD students' success at the Mass Spectrometry LC-MS Unit

*By Elbie Els*

Two PhD students under the supervision of Prof Marietjie Stander, manager of the Mass Spectrometry LC-MS Unit and Prof André de Villiers of Chemistry, finished their PhD studies successfully. Keabetswe Masike and Tlou Mosekiemang relied largely on the LC-MS facilities.

**Keabetswe Masike**

Keabetswe Masike's research project focused on characterising plant phenolic compounds based on their liquid chromatography-photodiode array-ion mobility-high resolution mass spectrometry (LC-PDA-IM-HR-MS) methods. The compounds were then characterised based on the retention time, mass spectral information (including high resolution and tandem MS data), spectroscopic data and collision cross section (CCS) value data.

Plants and plant-derived products contain a variety of phenolic compounds, with a broad range of health benefits and useful applications such as drug design. These phenolic compounds are often amplified by rearrangements, thus producing isobaric and isomeric species. The standard analytical method for the identification and characterisation of plant phenolics, LC-PDA-HR-MS, is not able to discriminate isomeric species in complex plant samples. The incorporation of ion mobility spectrometry (IMS) into LC-PDA-HR-MS workflows is being recognised as an additional orthogonal dimension of separation to HR-MS, whereby ions are separated through a drift region and filled with gas, based on their size, shape and charge. The attractive component of IMS is the determination of the collision cross section (CCS/Ω) values, which describes the unique rotationally averaged surface area of the ion as it interacts and travels through the gas-filled drift region.

Masike decided to embark on this research because the CCS as a feature can be beneficial in the development of an in-house phenolics compound library in analytical laboratories, which can help to expedite the characterisation of phenolic compounds in varying research fields, such as plant metabolomics and food science.

Four Protea plants, comprising two hybrid cultivars, black beauty (Sheila (P. magnifica × P. burchelli) cross) and limelight (P. neriifolia × P. burchelli), and two pure species (P. neriifolia and P. cynaroides) were collected from the commercial farm "FynBloem" and from the Harold Porter National Botanical Garden respectively for this study. As her research focused on optimising chromatographic and mass spectrometric (LC-MS) methods for the analyses of a range of plant metabolites, this meant that most of her PhD studies required the LC-MS lab. Some plant metabolites isomerise, making it difficult to differentiate by MS. The benefits of the Synapt G2 instrument in the LC-MS lab is the ion mobility spectrometry (IMS) capability. Thus, the integration of IMS into MS has become an appealing tool for the analyses of structurally similar metabolites.

***Figure 12:** PhD student Keabetswe Masike in the LC-MS Unit.*

**Some interesting discoveries were made:**

***Figure 13:** The flower head of a Protea plant.*

The black beard of the flower head gets its colour from anthocyanidins, the same compounds that is responsible for the colors of berries and red wine. The concentrations of these red pigments are so high that they appear black.

The post harvest problem of leaf browning that cause losses especially in the export market was studied. The species and hybrids that are more prone to blackening have differences in their phenolic profiles compared to the species and hybrids that are not, including the King Protea (P. cynaroides). Benzenetriol- and/or hydroquinone-glycoside derivatives were identified in the plants susceptible to leaf blackening and phenolic compounds with known protective properties against biotic and abiotic stressors were linked to the stems not prone to blackening.

*"Such observations serve as preliminary insights that can help accelerate plant improvement and aid in the selection of trait-specific markers in plant metabolomics."*

- K. Masike, PhD dissertation

*"The LC-MS lab staff were quite helpful. The collective knowledge regarding sample preparation, LC-MS analyses and data processing has been helpful for the progression of my research work. As most of my PhD studies involved the analysis of plant extracts using the LC-MS lab, I was able to obtain advice from the staff regarding which options were available to me regarding sample preparation, LC-MS analyses and data processing."*

Masike submitted and defended her thesis successfully. Her future plans involve being a committed researcher and an effective educator with a fascination for solving challenging problems in the biological sciences using analytical instrumentation.


***Figure 14:*** *The manifestation of leaf blackening in the cultivar 'Pink Ice' (P. susannae x P. compacta) seven days post-harvest, expressed on the lateral leaf margins.*

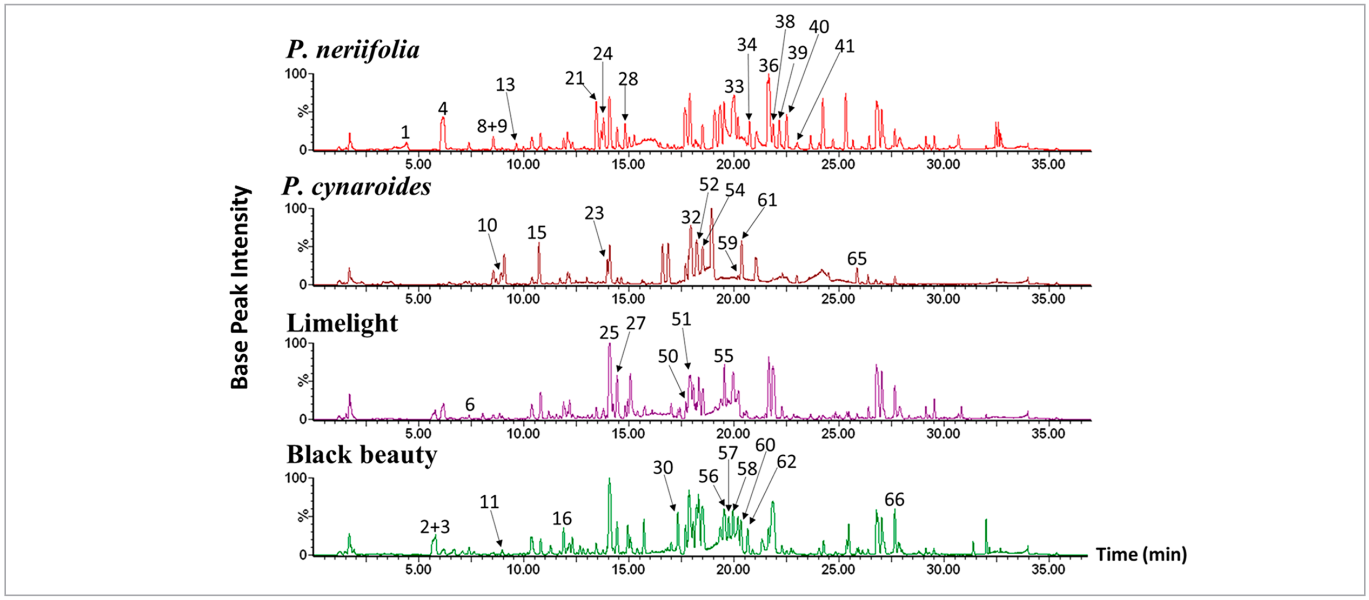



***Figure 15:*** *Stacked UHPLC-HR-MS base peak ion (BPI) chromatograms illustrating the different chromatographic profiles between extracts obtained from leaf tissues of P. neriifolia, P. cynaroides (King Protea), Limelight (P. neriifolia × P. burchelli), and Black beauty (Sheila (P. magnifica × P. burchelli) cross). Compound numbers correspond to Tables 1 and 2 in the article in The Journal of Agricultural and Food Chemistry online at https://pubs.acs.org/doi/abs/10.1021/acs.jafc.9b06361.*

## Tlou Mosekiemang

Tlou Mosekiemang was admitted to a PhD Chemistry (Environmental Analytical theme) degree and was funded by the TRECCAfrica II initiative (administered by the International Office of Stellenbosch University) and Prof AJ de Villiers.

The sole reason for the TRECCAfrica II initiative was to train staff members from a group of six universities (including Mosekiemang's current employer, the University of Botswana) to attain relevant teaching qualifications (in this case a PhD). He was nominated by the Department of Environmental Science in Botswana to undertake this training.

Mosekiemang's study focussed on chromatography and mass spectrometry through which he developed methods for the detection of antiretroviral drugs and their metabolites in wastewater. These methods required highly sensitive detection systems such as mass spectrometry. In addition, they realised that no information on antiretroviral drug metabolites was available to address this issue. They relied heavily on high-resolution mass spectrometry for structural elucidation of these unknown compounds.

Samples were collected from two municipal wastewater treatment plants with diverse demographic catchment areas and different treatment processes in the Western Cape province of South Africa. Sampling was timed to coincide with high daily inflows at each plant to maximise the chances of acquiring representative samples of the respective catchment areas.

This study demonstrated the suitability of direct injection LC-ESI-MS/MS for the simultaneous quantification of all three major therapeutic classes of antiretroviral drugs and selected metabolites in aqueous matrices. This is the first reported method for the simultaneous analysis of NNRTI, NRTI and PI antiretroviral drugs and metabolites by direct injection.

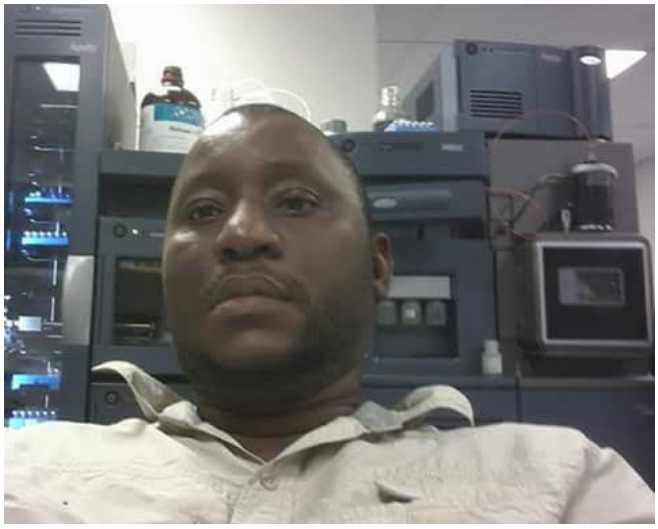


***Figure 16:*** *PhD student Tlou Mosekiemang in the LC-MS Unit.*

An interesting result in Figure 2 in Mosekiemang's article in *Chemosphere* is the difference in concentrations of drugs in waste water during the drought and the wet season. The levels were much higher in the drought, mainly due to the dillution of water in the wet season, but there are differences in the ratios of the different drugs. It was also found that different wastewater treatment techniques showed different results in their efficacies in removing these drugs with certain mostly polar drugs (lamivudine) being completely removed and other including emtricitabine and efavirenz were more persistent (*Chemosphere*).

Mosekiemang passed his dissertation and defence. He plans to continue with his teaching and research career at the University of Botswana.

*"It is very important for students and other researchers to have access to the Central Analytical Facilities – particularly researchers from less privileged universities in Southern Africa."*

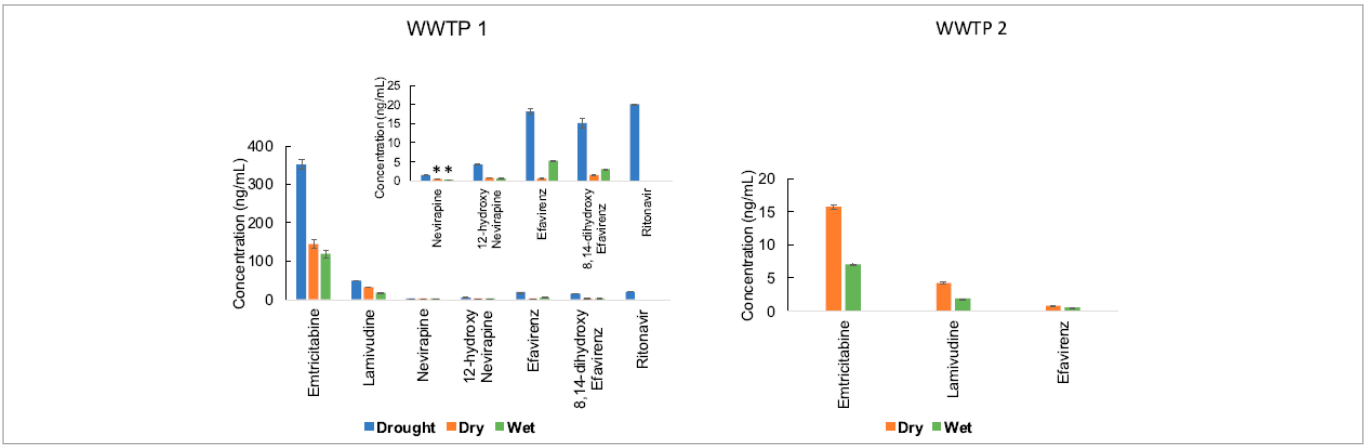



***Figure 17:*** *Comparison of seasonal effects on the occurrence of antiretroviral drugs and their metabolites in raw wastewater in the dry and wet seasons for samples collected in April and July 2016 (orange and green) and at the end of a severe drought (April 2018, blue). Error bars represent standard deviations for sample replicates (n ¼ 5). See the article in Chemosphere online at https://www.sciencedirect.com/science/article/pii/S0045653518325475.*

# Confocal microscopists and data engineers collaborate to develop a new image analysis tool

*By Lize Engelbrecht*

Confocal microscopy is one of the least invasive high-resolution imaging technologies and therefore very powerful in visualising dynamic processes in live cells. It also allows for imaging different levels of a sample and eliminating all out-of-focus light in the process for better resolution in all the dimensions. Acquiring what is called a 'Z-stack' in this way is called 'optical sectioning', and during postprocessing, these images can be reconstructed to enable the researcher to visualise the sample in three dimensions. The acquisition of a Z-stack is therefore in essence a type of virtual sectioning that can be performed repetitively. This is in contrast to the once-off physical sectioning of a sample that occurs for example when imaging with an electron microscope for three-dimensional image reconstruction.

In the Central Analytical Facilities Fluorescence Microscopy Unit, the Zeiss LSM780 ELYRA PS1 confocal microscope is equipped with an on-stage incubator that has been used extensively over the past nine years by various students following dynamic processes, such as cell migration and chemotaxis, cell stress and cell death, nuclear transportation and many more. Mitochondrial dynamics have been a specific focus of the Neuro Research Group led by Prof Ben Loos of the Department of Physiological Sciences. When the mitochondrial processes, such as fission and fusion, are impaired, it is usually one of the molecular indications for the onset of neurodegenerative disorders such as Alzheimer's or Parkinson's disease.

Mitochondria are responsible for the energy generation in the cell and are polarised across their membrane to allow electron transport. A healthy mitochondrial network is in a constant equilibrium in which fission (fragmentation) of certain parts occurs while fusion takes place in other parts of the network. This can, however, only truly be appreciated when imaging in real time. One of the functions of these processes is mitochondrial quality control. Damaged mitochondrial strands are removed and depolarised to prevent them from fusing with the network again, in preparation for their degradation. The equilibrium may change depending on the metabolic demands of the cell, and a better understanding of these dynamics and their effects is critical in providing insight into possible treatment strategies that can be applied during this very early phase of neurodegeneration.

Although three-dimensional imaging of the mitochondrial network in live cell studies is nothing new, the quantification of the fission, fusion and depolarisation processes has been limited. Under homeostatic conditions, fission and fusion occur within a five-second time frame. Up to now, consecutive images in a time lapse had to be compared manually in order to detect changes in the network morphology and to identify fission and fusion events. This is a very laborious task that few research groups would undertake. Thus far, quantification has simply not included rapid and dynamic changes in these events.

In 2015, Prof Loos started a collaboration with Prof Thomas Niesler's group at the Department of Electronic and Electrical Engineering with a view to developing more automated, high-throughput tools for three-dimensional image visualisation and analysis. As part of this collaboration and his PhD research into virtual reality-guided visualisation and quantification of microscopy data in three dimensions, Dr Rensu Theart developed the Mitochondrial Event Localiser (MEL) tool to investigate the dynamics of the three-dimensional mitochondrial network.

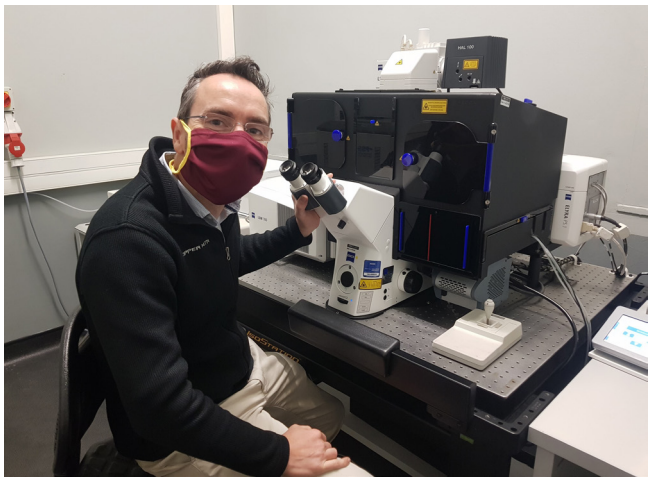


***Figure 18:*** *Prof Ben Loos in the CAF FM Unit.*

## How does the Mitochondrial Event Localiser work?

The MEL automatically pinpoints events such as fission, fusion and depolarisation in a large time-lapse dataset in which each time point is acquired as a Z-stack for three-dimensional reconstruction.

The workflow firstly requires the Z-stack data to be preprocessed to ensure the best possible quality and consistency between image frames of the time lapse. Through a process called 'hysteresis thresholding', background signal is removed from each frame, resulting in a filtered binarised image. After compensating for any slight movement of the mitochondria, arrays of these stacks are ready for automatic processing.

The prepared arrays are the input to the MEL automatic image analysis algorithm that will analyse the data and produce a list of mitochondrial event locations. It will look for potential sites of (i) fusion, whereby two smaller fragments of mitochondria form a larger single structure in a subsequent frame, (ii) fission, whereby a large mitochondrial fragment separates into two smaller fragments, and (iii) depolarisation, whereby a fragment disappears in consecutive frames due to a loss of fluorescence signal. Through a process called 'back-and-forth structure matching', many locations of these events are identified and localised in the three-dimensional time lapse. The output generated by the MEL tool consists of the input Z-stacks with superimposed colour-coded event localisations.

Since the processing steps can produce false positives, the group went even further to develop a validation tool that enables a human expert to investigate each event individually by displaying the cropped image of the event identified by the MEL tool next to binary images of the frames just before and just after the event. The frames are further super-imposed, and the changed fragments are colour coded to aid the investigator to confirm that the event has indeed been identified correctly.

***Figure 19:*** *Dr Rensu Theart*

***Figure 20:*** *Prof Thomas Niesler*

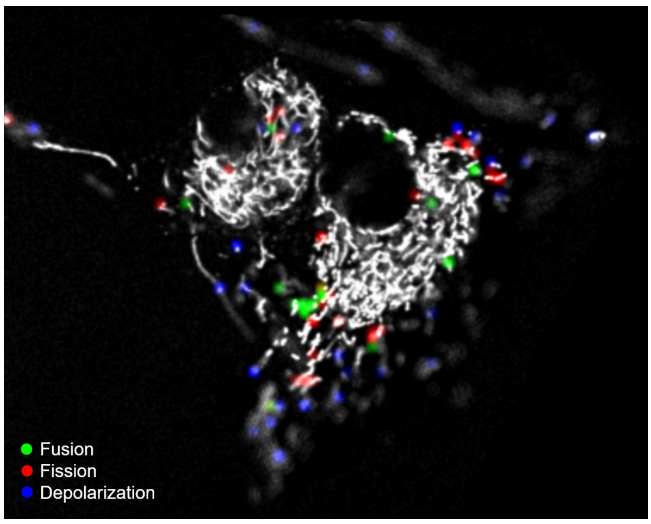



***Figure 21:*** *The output of the MEL tool shows the input Z-stack (white) with super-imposed colour-coded event locations of fusion (green), fission (red) and depolarization (blue).*

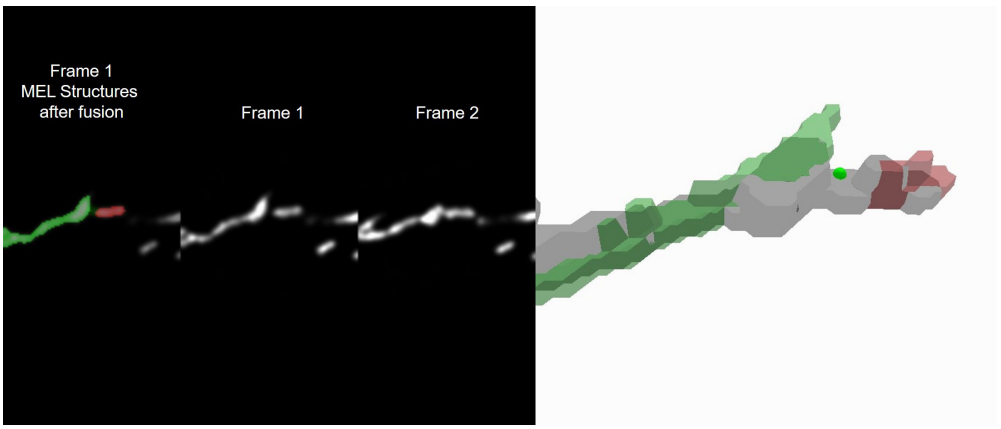



***Figure 22:*** *The mitochondrial event validation tool enables researchers to easily identify and remove false positive events identified with the MEL plugin. The left panel represents one event (cropped) identified with the MEL plugin with the two separate strands before fusion, with the next two frames representing the frame just before the event and just after the event. The final image is a 3D reconstruction of the two frames super-imposed and colour-coded.*

In a recent publication in the academic journal PLOS One, the group showed that the number of fission, fusion and depolarisation events in healthy mammalian control cells was kept in equilibrium at an average ratio of 9.3/7.2/2.3 events. However, when treated with peroxide to perturb the mitochondrial network, the balance clearly shifted towards fusion at a ratio of 15/6/3. This was observed to settle quickly to a new equilibrium (6.2/6.4/3.4) that was more comparable to the control cells.

### What can these types of results tell researchers?

The mitochondrial network and morphological changes are so dynamic that the interpretation of qualitative data and the limited quantitative data currently available is cause for debate amongst researchers. Some believe that a highly networked mitochondrial structure is indicative of improved cellular health, while a fragmented network indicates a cell that is under severe stress, which is detrimental. Others contend that a highly networked structure is only the first sign of a stress response and that fragmentation indicates increased cellular control through these adaptive mechanisms, to drive removal of dysfunctional mitochondria. Since the MEL allows tracing the development and direction of these processes, either towards fission, fusion or no change, in other words being in equilibrium, it will allow a much better understanding of this context. Hence, the truth might depend on the circumstances, and a simple observation of the network without regard to the dynamic behaviour could lead to major misinterpretations. Fission does not only separate mitochondria that are to be removed from the network but also separates mitochondria that need to be transported elsewhere in the cell, for example to the synapses in neurons. This can be a long distance, more than a meter for some neurons. In contrast, when mitochondria need to be protected against degradation, fusion may be the preferred response in the cell since material is shared and diluted, and respiration can take place across a larger network.

The accurate quantification of the relationship between fission, fusion and depolarisation in a three-dimensional cellular context will enable researchers to better describe the equilibrium under various conditions and to identify a deviation from this equilibrium and the consequent effects. This will have particular application in the study of neurodegenerative diseases and has immense potential not only to aid researchers in the development of treatments that target mitochondrial function but also to serve as a diagnostic tool for the early detection of these diseases. Coupled with powerful high-throughput platforms, the MEL could become a new standard, allowing a completely new categorisation of cells according to their fission and fusion behaviour under healthy and diseased conditions. Since the mitochondria are also the nexus of cellular fate, in other words life or death (apoptosis), this application may also be of value in the development of anticancer drugs, in which failing mitochondria are desired.

Dr Theart is in the process of converting the tool into a plugin for the globally most utilised free-imaging software, called 'FIJI/ImageJ' (available here: https://github.com/rensutheart/MEL-Fiji-Plugin), and a patent for the method has been filed under the name 'Mitochondrial event localiser (MEL) to quantitatively describe fission, fusion and depolarisation in the three-dimensional space' (South African Provisional Application No. 2020/00654). Previously, Dr Theart published a tool for improved analysis of colocalisation in the three-dimensional space. This work is an indication that the confocal microscope at Stellenbosch University is not only used by biologists and researchers to interpret data for their research but is also a crucial component in the development of image analysis strategies by our data engineers that will make a global impact. Given the trend of data science and computing, including deep learning approaches and artificial intelligence, more of such applications can be anticipated.

# Financial Reports

By Fransien Kamper

| | | 2018 | 2019 | 2020 | 2021 Budget |
|---|---|---|---|---|---|
| MS UNIT | Internal invoicing | 2 040 163 | 1 893 475 | 1 033 947 | 1 606 423 |
| | External invoicing | 5 148 560 | 6 803 566 | 5 661 872 | 8 494 000 |
| | Total logbook income | 7 188 723 | 8 697 041 | 6 695 819 | 10 100 423 |
| | Expenses | | | | |
| | Salaries | 3 708 383 | 4 202 003 | 4 645 421 | 4 527 570 |
| Note1 - all Units | ICR | 875 255 | 1 360 713 | 1 132 374 | 1 698 800 |
| | Running costs | 906 574 | 1 171 430 | 849 691 | 968 665 |
| | Maintenance | 829 955 | 905 603 | 962 537 | 595 000 |
| | Travel costs | 11 805 | 281 | | |
| | Small equipment & KKW | 70 461 | 5 952 | 21 860 | 133 983 |
| | Deferred costs | | 255 800 | | 399 996 |
| | Total expenses | 6 402 433 | 7 901 782 | 7 611 884 | 8 324 013 |
| | | | | | |
| FM UNIT | Internal invoicing | 1 261 988 | 856 494 | 573 022 | 997 826 |
| | External invoicing | 74 017 | 189 215 | 109 064 | 58 580 |
| | Total logbook income | 1 336 005 | 1 045 709 | 682 086 | 1 056 406 |
| | Expenses | | | | |
| | Salaries | 889 764 | 930 059 | 1 023 435 | 1 066 855 |
| | ICR | 12 583 | 37 843 | 21 813 | 11 716 |
| | Running costs | 313 664 | 425 804 | 92 688 | 43 989 |
| | Maintenance | 79 978 | 59 150 | 108 802 | 565 660 |
| | Travel costs | 3 674 | 6 653 | 334 | |
| | Small equipment & KKW | 36 455 | 114 407 | | |
| | Deferred costs | | 150 000 | | 150 000 |
| | Total expenses | 1 336 118 | 1 723 916 | 1 247 071 | 1 838 220 |
| | | | | | |
| SEM UNIT | Internal invoicing | 948 918 | 918 242 | 687 982 | 1 036 158 |
| | External invoicing | 2 107 221 | 732 351 | 757 935 | 1 231 337 |
| | Total logbook income | 3 056 139 | 1 650 593 | 1 445 917 | 2 267 495 |
| | Expenses | | | | |
| | Salaries | 2 100 941 | 1 684 505 | 1 389 647 | 1 581 789 |
| | ICR | 358 228 | 146 470 | 151 587 | 246 267 |
| | Running costs | 196 673 | 62 968 | 98 324 | 199 709 |
| | Maintenance | 35 975 | 93 673 | 23 065 | 103 664 |
| | Travel costs | 64 975 | 5 491 | 3 473 | |
| | Small equipment & KKW | 177 800 | 86 628 | 3 635 | 102 962 |
| | Deferred costs | | 120 000 | | 120 000 |
| | Total expenses | 2 934 592 | 2 199 735 | 1 669 731 | 2 354 391 |
| | | | | | |
| ICP & XRF UNIT | Internal invoicing | 1 045 643 | 1 005 564 | 549 250 | 738 537 |
| | External invoicing | 2 759 674 | 2 366 846 | 1 957 142 | 3 249 592 |
| | Total logbook income | 3 805 317 | 3 372 410 | 2 506 392 | 3 988 129 |
| | Expenses | | | | |
| | Salaries | 2 417 316 | 2 709 331 | 2 026 864 | 2 196 150 |
| | ICR | 469 145 | 473 369 | 391 428 | 649 918 |
| | Running costs | 857 977 | 1 005 950 | 708 254 | 682 475 |
| | Maintenance | 539 500 | 1 156 984 | 400 981 | 563 846 |
| | Travel costs | 77 034 | 62 089 | 9 182 | |
| | Small equipment & KKW | 29 597 | 66 476 | | |
| | Deferred costs | | 354 613 | | 399 998 |
| | Total expenses | 4 390 569 | 5 828 812 | 3 536 709 | 4 492 388 |

| | | 2018 | 2019 | 2020 | 2021 Budget |
|---|---|---|---|---|---|
| DNA UNIT | Internal invoicing | 4 690 289 | 3 774 647 | 2 925 162 | 3 606 443 |
| | External invoicing | 6 259 800 | 5 752 054 | 4 158 801 | 4 367 644 |
| | Total logbook income | 10 950 090 | 9 526 701 | 7 083 963 | 7 974 087 |
| | Expenses | | | | |
| | Salaries | 2 986 764 | 3 089 240 | 3 400 922 | 4 178 936 |
| | ICR | 1 064 166 | 1 150 411 | 831 760 | 873 529 |
| | Running costs | 6 669 796 | 5 604 611 | 5 531 489 | 3 937 815 |
| | Maintenance | 255 726 | 175 405 | 143 324 | 230 354 |
| | Travel costs | 774 | 831 | | |
| | Small equipment & KKW | | 51 228 | 83 118 | 82 578 |
| | Deferred costs | | 133 333 | | 285 000 |
| | Total expenses | 10 977 226 | 10 205 059 | 9 990 613 | 9 588 212 |
| | | | | | |
| NMR UNIT | Internal invoicing | 697 665 | 660 625 | 565 437 | 614 171 |
| | External invoicing | 641 179 | 910 628 | 421 555 | 815 664 |
| | Total logbook income | 1 338 844 | 1 571 254 | 986 992 | 1 429 834 |
| | Expenses | | | | |
| | Salaries | 1 342 756 | 1 429 138 | 1 217 297 | 1 824 552 |
| | ICR | 109 000 | 182 126 | 84 311 | 163 133 |
| | Running costs | 383 393 | 517 358 | 563 174 | 401 028 |
| | Maintenance | 12 678 | 48 897 | -1 236 | 2 700 |
| | Travel costs | | 2 911 | 12 | |
| | Small equipment & KKW | | | 10 427 | |
| | Deferred costs | | | | |
| | Total expenses | 1 847 827 | 2 180 430 | 1 873 985 | 2 391 413 |
| | | | | | |
| CT UNIT | Internal invoicing | 663 253 | 490 600 | 321 330 | 283 351 |
| | External invoicing | 2 764 088 | 1 551 760 | 1 303 930 | 2 490 616 |
| | Total logbook income | 3 427 341 | 2 042 360 | 1 625 260 | 2 773 967 |
| | Expenses | | | | |
| | Salaries | 1 563 400 | 1 646 964 | 1 259 025 | 1 445 954 |
| | ICR | 469 895 | 310 352 | 260 786 | 498 123 |
| | Running costs | 408 092 | 277 121 | 309 351 | 345 893 |
| | Maintenance | 313 044 | 565 225 | 156 774 | 149 006 |
| | Travel costs | 24 491 | 58 676 | 327 | 348 |
| | Small equipment & KKW | 42 057 | | 25 243 | 74 508 |
| | Deferred costs | | 341 108 | | 350 002 |
| | Total expenses | 2 820 979 | 3 199 446 | 2 011 506 | 2 863 835 |
| | | | | | |
| NEUROMECHANICS UNIT | Internal invoicing | 569 253 | 323 158 | 203 044 | 314 450 |
| | External invoicing | 826 252 | 1 069 544 | 954 667 | 1 097 215 |
| | Total logbook income | 1 395 504 | 1 392 703 | 1 157 711 | 1 411 665 |
| | Expenses | | | | |
| | Salaries | 1 475 937 | 2 060 312 | 2 107 151 | 1 455 369 |
| | ICR | 140 463 | 213 909 | 190 933 | 219 443 |
| | Running costs | 46 213 | 70 248 | 22 299 | 88 350 |
| | Maintenance | 66 010 | 43 315 | 23 802 | 37 002 |
| | Travel costs | 15 589 | 72 581 | | |
| | Small equipment & KKW | 55 196 | 48 070 | 49 449 | |
| | Deferred costs | | 68 711 | | 99 998 |
| | Total expenses | 1 658 945 | 2 577 146 | 2 393 635 | 1 900 161 |

| | | 2018 | 2019 | 2020 | 2021 Budget |
|---|---|---|---|---|---|
| VIBRATIONAL SPECTROSCOPY UNIT | Internal invoicing | 57 175 | 104 529 | 57 450 | 91 600 |
| | External invoicing | 18 264 | 44 949 | 5 250 | 3 540 |
| | Total logbook income | 75 439 | 149 478 | 62 700 | 95 140 |
| | Expenses | | | | |
| | Salaries | 407 321 | 595 708 | | |
| | ICR | 3 105 | 8 990 | 1 050 | 708 |
| | Running costs | 7 636 | 7 824 | 3 857 | 2 130 |
| | Maintenance | | | | |
| | Travel costs | | | | |
| | Small equipment & KKW | | | | |
| | Deferred costs | | 25 008 | | |
| | Total expenses | 418 062 | 637 530 | 4 907 | 2 838 |
| | | | | | |
| TOTAL UNITS INCOME | Total internal income | 11 974 346 | 10 027 336 | 6 916 624 | 9 288 959 |
| | Total external income | 20 599 056 | 19 420 912 | 15 330 215 | 21 808 187 |
| | Total income: all units | 32 573 402 | 29 448 248 | 22 246 839 | 31 097 147 |
| | | | | | |
| ADDITIONAL INCOME | | | | | |
| | Interest received | 465 843 | 1 511 454 | 353 657 | 494 762 |
| | Funds Received VR(R) | 750 000 | 750 000 | 750 000 | |
| | Salary contribution VR(R) | 3 952 335 | 4 203 342 | 4 355 720 | 4 454 290 |
| | Infrastructure NII repayment | | 2 000 000 | | |
| | US loan / ALT 2020 funds: Detector CT | | 2 321 000 | | |
| | VAT refund on equipment | | 94 451 | | |
| | Faculty contributions | | | | |
| | NII levy | | | 142 324 | 405 611 |
| | BIOGRIP 7% levy | | | | 35 364 |
| | VAT Refund on Equipment | | | | 4 111 718 |
| | TOTAL ADDITIONAL INCOME | 5 168 178 | 10 880 247 | 5 601 701 | 9 501 744 |
| | | | | | |
| TOTAL INCOME | | 37 741 580 | 40 328 495 | 27 848 540 | 40 598 891 |

| | | 2018 | 2019 | 2020 | 2021 Budget |
|---|---|---|---|---|---|
| EXPENDITURE | TOTAL EXPENDITURE | | | | |
| | Expenses | | | | |
| | Salaries | | | | |
| | Salaries: Admin | 1 983 822 | 2 184 381 | 2 355 196 | 2 470 387 |
| | Salaries: Restructure | | | | |
| | Salaries: Units | 16 892 583 | 18 347 259 | 17 069 762 | 18 277 175 |
| | Salaries: Bonus | 299 326 | | | |
| | 17% / 20% ICRR (indirect cost recovery) | 3 501 839 | 3 884 182 | 3 066 043 | 4 361 637 |
| | Running costs (sum of units) | 9 790 017 | 9 143 314 | 8 179 127 | 6 670 053 |
| | Maintenance (sum of units) | 2 132 866 | 3 048 251 | 1 818 049 | 2 247 231 |
| | Travel costs (sum of units) | 198 342 | 209 513 | 13 328 | 348 |
| | Small equipment & KKW (sum of units) | 411 566 | 372 761 | 193 732 | 394 031 |
| | Deferred costs | | 1 448 573 | | 1 804 994 |
| | CAF general running costs | 674 184 | 592 964 | 468 200 | 348 749 |
| | Students | | 342 663 | 350 338 | 269 524 |
| | Interest | | | 17 224 | 42 599 |
| | Travel costs-courier | 80 034 | 89 313 | 69 883 | 64 504 |
| | Development new labs | | 1 121 212 | | |
| | Infrastructure | 29 989 | 115 217 | 11 804 | |
| | Infrastructure NII | | | | |
| | Equipment | 608 733 | 27 648 | 77 071 | 207 833 |
| | Maintenance | | | 23 336 | |
| | Equipment repair: CT scanner | | 2 344 334 | 519 207 | |
| | Equipment repair fund | 500 000 | | | |
| | CAF vehicle fund | 45 000 | | | |
| | Equipment replacement fund | | | | 4 000 000 |
| | NMR purchase & infrastructure | | | | 1 248 031 |
| | HPC hardware | | | | 1 000 000 |
| | Total normal operational costs | 37 148 302 | 43 271 587 | 34 232 298 | 43 407 098 |
| | Surplus per year before special income | 593 278 | -2 943 092 | -6 383 758 | -2 808 207 |
| | | | | | |
| Special additional income | COVID insurance claim Note 2 | | | 5 000 000 | |
| | | | | | |
| Surplus/Shortfall | Surplus/Shortfall per year | 593 278 | -2 943 092 | -1 383 758 | -2 808 207 |
| | | | | | |
| EQUIPMENT EXPENDITURE | | | | | |
| | NRF-NEP total grants | | 23 982 455 | | 7 120 740 |
| | ALT/US funds | | 8 000 000 | | 3 560 370 |
| | Loan: 2020 ALT | | 2 643 935 | | |
| | Contributions: Faculty of Science | | 500 000 | | |
| | CAF contribution | | | | 173 001 |
| | ALT FUNDS - NMR purchase | | | | 12 552 478 |
| | Science Faculty: contribution NMR | | | | 10 000 000 |
| | Strategic funds: NMR | | | | 4 400 000 |
| | CAF contribution: NMR | | 871 213 | | 548 031 |
| | TOTAL | | 35 997 603 | | 38 354 620 |

| | | 2018 | 2019 | 2020 | 2021 Budget |
|---|---|---|---|---|---|
| EQUIPMENT DETAILS | | | | | |
| | Mass-Directed Auto Purification & QC system | | 9 431 805 | | |
| | Amnis Image StreamX MarkII Imaging Flow Cytometer | | 12 673 106 | | |
| | Gemini 300FESEM with advanced system for automated 3D | | 13 892 690 | | |
| | Spectral Flow Cytometer | | | | 10 854 111 |
| | 400mhz and 600mhz nuclear magnetic resonance | | | | 27 500 509 |
| | | | | | |
| | TOTAL | | 35 997 601 | | 38 354 620 |
| | | | | | |
| FUNDS | | | | | |
| | Emergency equipment repair | 1 582 635 | 1 688 915 | 1 078 605 | 1 090 000 |
| | Vehicle replacement | 160 930 | 239 363 | 248 948 | 248 948 |
| | Reserve, food security project | 1 201 041 | 1 214 773 | 1 221 614 | 1 221 614 |
| | Maintenance fund equipment: BD FACS Jazz sorter (2013) | 1 185 280 | 1 214 029 | 1 240 769 | 895 000 |
| | Equipment replacement | | | | 4 000 000 |
| | Deferred costs | | 1 448 573 | 1 448 500 | 3 253 494 |
| TOTAL FUNDS | | 4 129 885 | 5 805 653 | 5 238 436 | 10 709 056 |

| CAF UNITS: Financially ring-fenced DSI funded research infrastructure platform nodes | | | | | |
|---|---|---|---|---|---|
| | | 2017-2018 | 2019 | 2020 | 2021 Budget |
| NII | NODE funding | 26 315 789 | 381 396 | 3 742 420 | 1 500 000 |
| | Bridging funding | 19 000 000 | -6 831 086 | | |
| | Interest received | 2 720 338 | 1 566 713 | 282 219 | 164 000 |
| | Income | | | 869 559 | 1 692 000 |
| | Private patients | | | 939 909 | 4 102 436 |
| | Total income | 48 036 127 | -4 882 977 | 5 834 107 | 7 458 436 |
| | Expenses | | | | |
| | Salaries & running costs | 1 080 | 2 109 155 | 5 240 134 | 11 117 178 |
| | Building & equipment | | 32 213 533 | 3 122 671 | 450 000 |
| | Total expenses | 1 080 | 34 322 688 | 8 362 806 | 11 567 178 |
| | Year end balance | 48 035 047 | 8 829 383 | 6 300 684 | 2 191 943 |

| | | | 1 April 2019-31 March 2020 Year1 | 1 April 2020-31 March 2021 Year 2 | 1 April 2021-31 March 2022 Year 3 |
|---|---|---|---|---|---|
| BIOGRIP | NODE funding | | 5 842 139 | 7 480 163 | 5 967 465 |
| | Interest received | | 19 425 | 256 974 | 150 000 |
| | Income | | | 263 207 | 278 142 |
| | Total income | | 5 861 564 | 8 000 344 | 6 395 607 |
| | Expenses | | | | |
| | Salaries & running costs | | 827 288 | 2 778 684 | 3 283 152 |
| | ICR (indirect cost recovery) | | 292 107 | 391 440 | 298 373 |
| | Equipment | | 4 726 110 | 4 327 468 | 2 385 940 |
| | Total expenses | | 5 845 505 | 7 497 592 | 5 967 465 |
| | Balance for each grant period | | 16 059 | 502 752 | 428 142 |

NOTE 1: ICR costs have been shifted to reflect on the unit costs, to the revenue to which they relate.

NOTE 2: Covid Insurance claim approved - funds not yet received at 13 July 2021.

NOTE 3: BIOGRIP NODE is reported in periods as to be reported to BIOGRIP HUB
Income for Year 3 - not yet received from the HUB

# Graphs detailing aspects of CAF income during 2020

***Figure 23:*** *Percentage of income derived from the different categories of clients for 2020.*

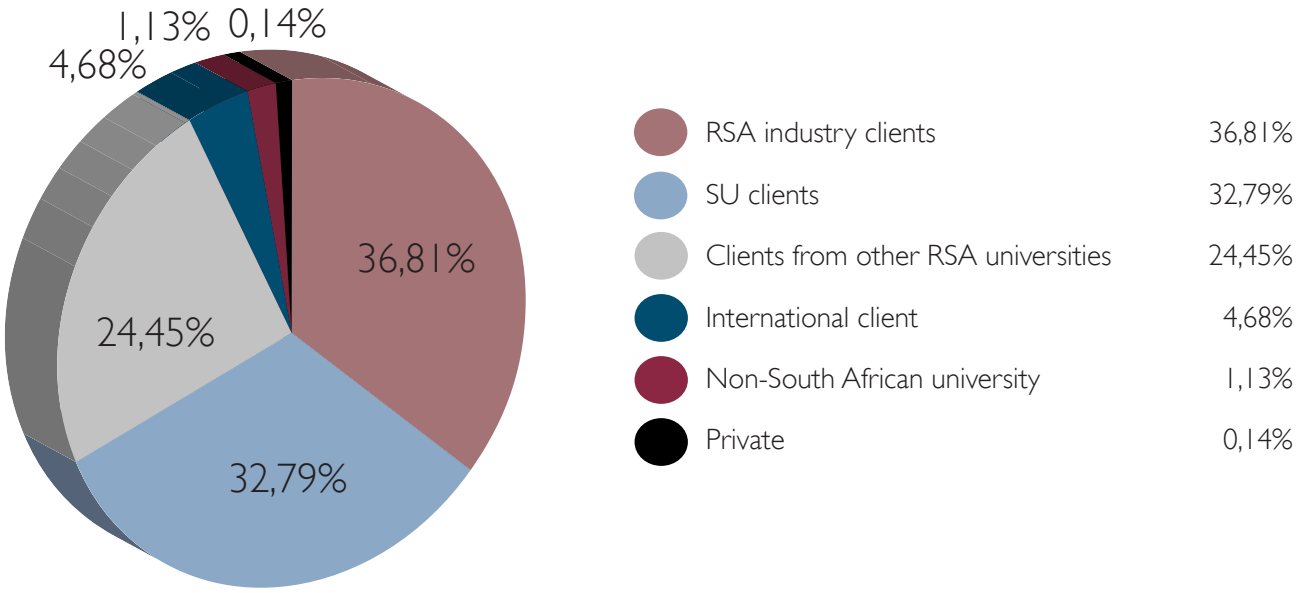



***Figure 24:*** *Analysis of percentage of CAF income for 2020 from internal clients by faculty.*

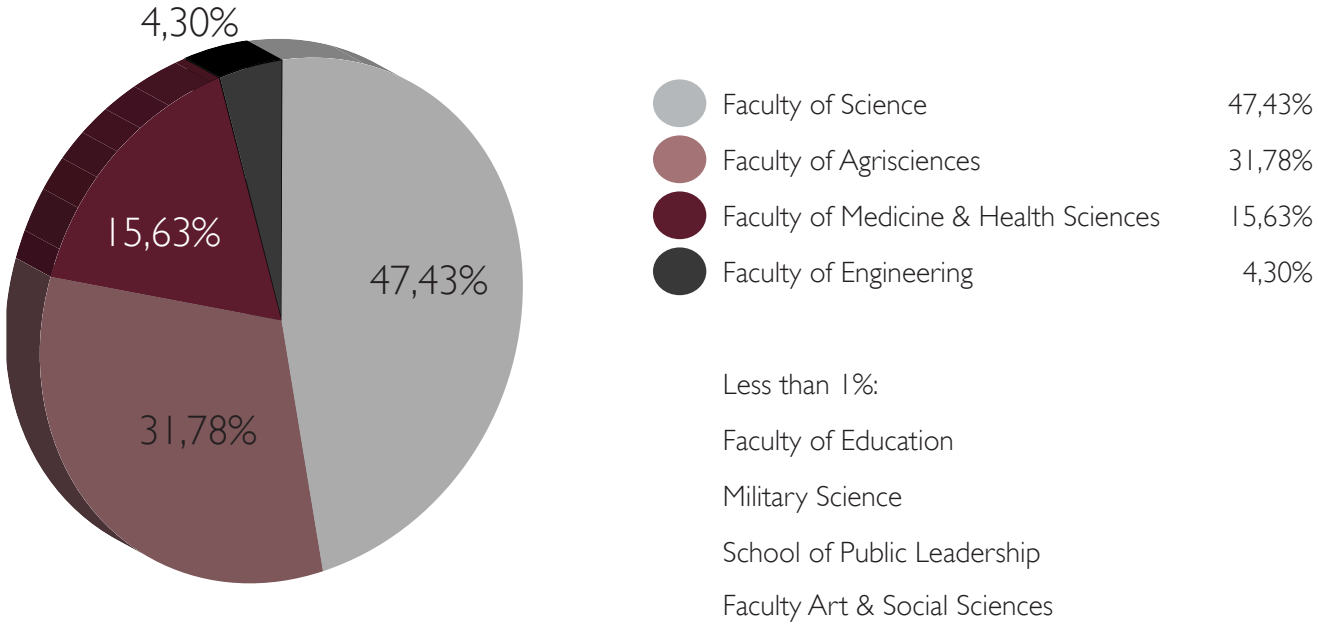
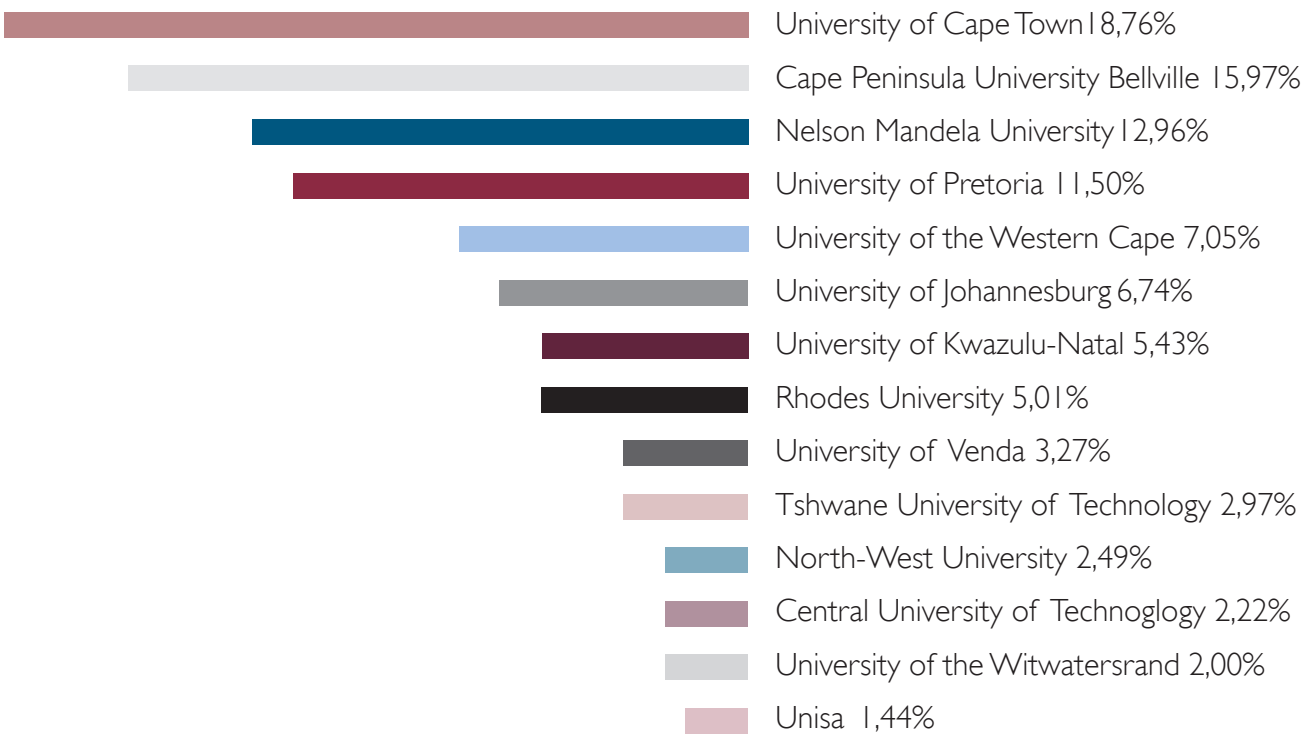
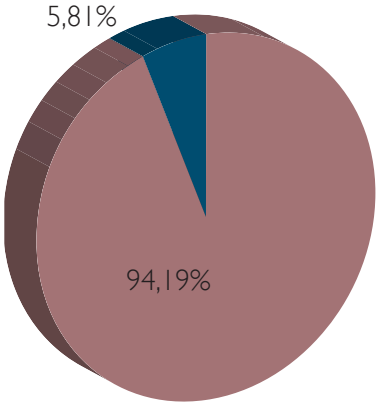


***Figure 25:*** *Analysis of CAF income for 2020 from South African external academic clients by university.*




**Less than 1%:**
University of Limpopo
University of the Free State
University of Zululand
Durban University of Technology
University of Fort Hare
Walter Sisulu University

***Figure 26:*** *Analysis of the proportion of CAF income for 2020 from external clients.*

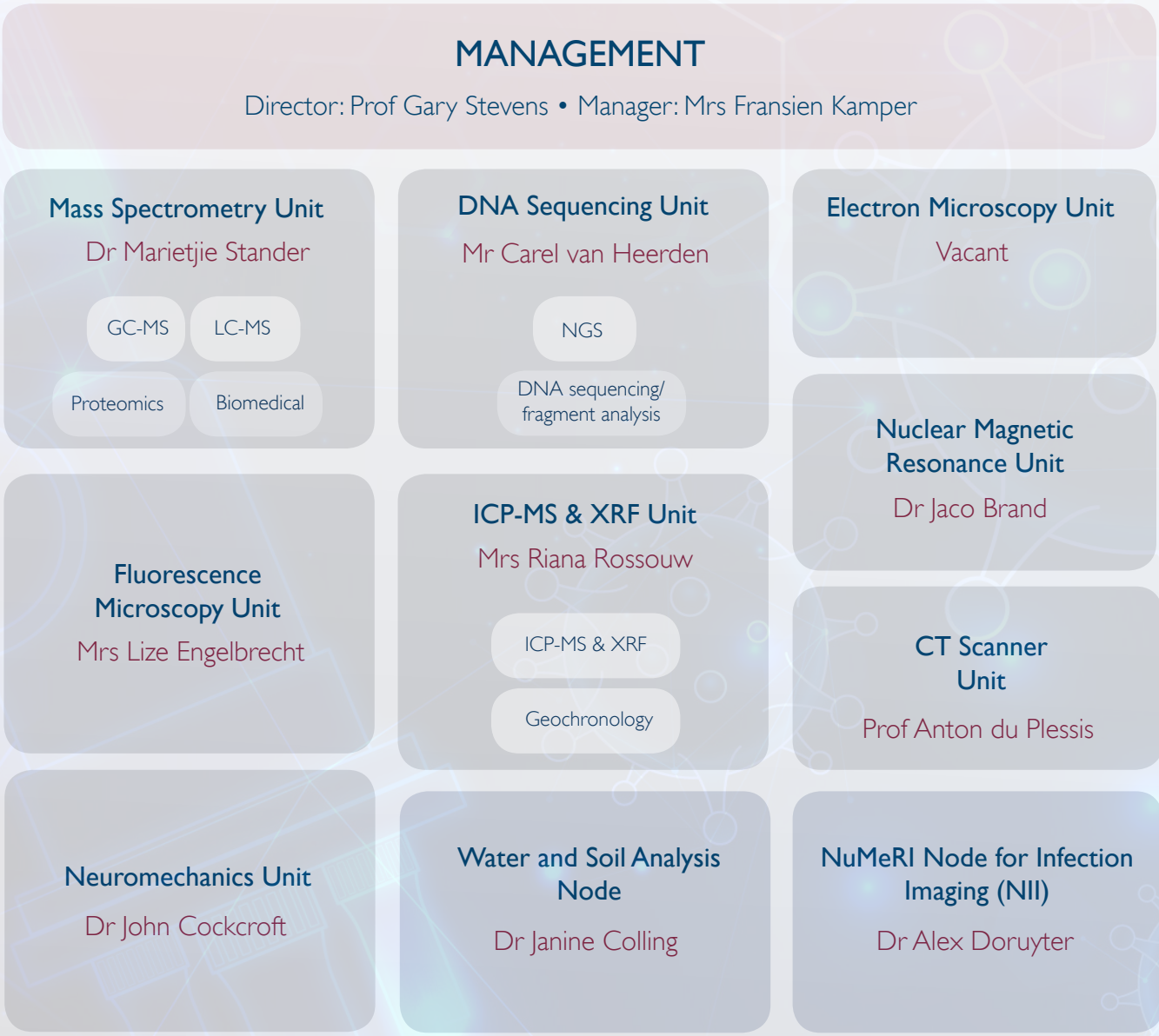



National income 94,19%

International income 5,81%

# CAF structure 2021

***Figure 27:*** *CAF structure for 2021 showing management, units and nodes.*




*Please note: Names of the unit managers are indicated in maroon and divisions within units are indicated in white blocks.*

# EDITORIAL TEAM

Writers:
Prof Gary Stevens
Dr Jaco Brand
Elbie Els
Lize Engelbrecht

Compiled by:
Elbie Els

Financial information:
Fransien Kamper

Design and layout:
Elbie Els

www.sun.ac.za/caf