CAF Central Analytical Facilities
STELLENBOSCH UNIVERSITY

2019/2020 Annual Report of the Central Analytical Facilities

SU 100 1918 · 2018

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Mr Carel van Heerden
Dr Janine Colling
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Overview

The CAF annual report, presented to the CAF Committee in the middle of each year, contains a summary of the financial data for the past year and a prediction of the financial outcomes for the current year. These predictions have typically proved to be very accurate, but this year, with the report prepared in the midst of the COVID-19 pandemic in South Africa, the financial predictions for 2020 have considerable inherent uncertainty.

During 2017 and 2018, CAF produced a small excess of income, R59 946 and R593 278 respectively, whilst in 2019 CAF realized a significant deficit of R 2 943 091. This was partly due to the fact that the ICP and CT units both suffered large component failures, with resultant high repair costs and significant laboratory downtime. In addition, the deficit was likely also due to declining levels of funding in the South African research sector in general. This is possibly reflected in the fact that CAF income from services offered remained on a upwards trend until 2018, with income from the private sector consistently representing approximately 35% of total income (***Figure 1A***). In 2019 income from all academic clients decreased significantly and the proportion of income from private sector clients increased to > 40%. This decrease in income from academic clients may be predicted to continue, if the steady year-on-year decrease in NRF funding to MSc and PhD students at SU is considered (***Figure 1B***). These changes to the environment within which we function require that CAF continue to attract a greater proportion of business from the private sector, as well as continue to benefit from Department of Science and Innovation funding for the large science infrastructure projects that have flowed from the South African Research Infrastructure Roadmap (SARIR). Several initiatives are underway to achieve both these aims and progress with the establishment of the SARIR NuMeRI and BIOGRIP nodes at SU during the review period is covered later in this report.

Figure 1A: Total CAF income, income from RSA academic clients and income from SU academic clients for 2016 - 2019 and 2020 (projected)

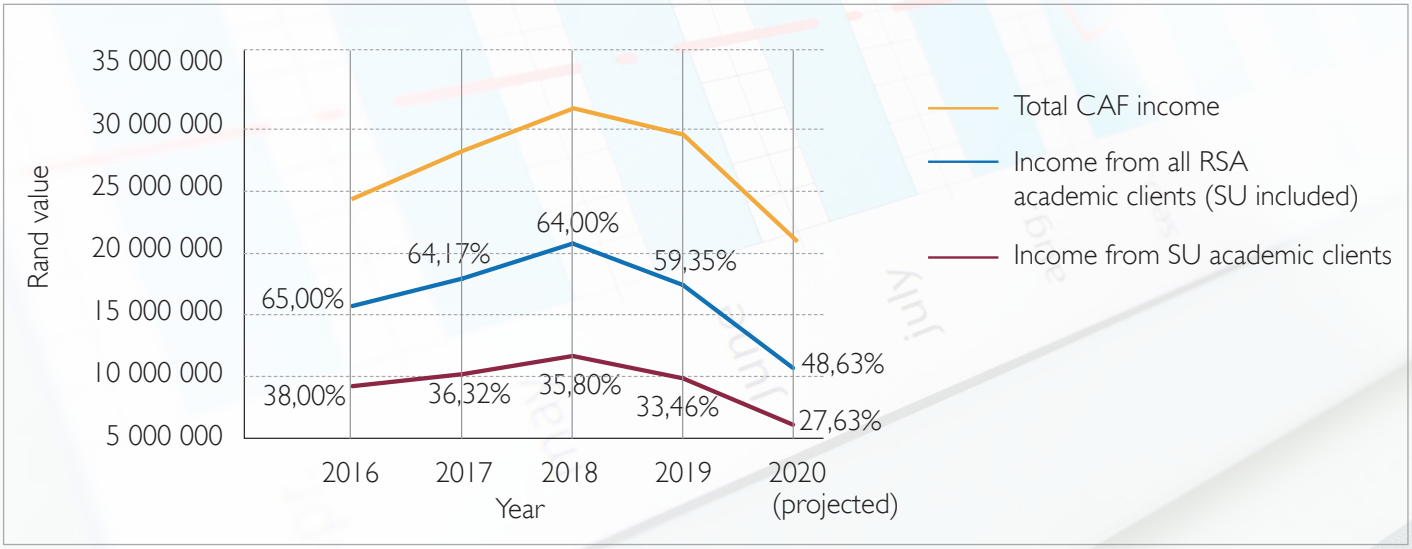


Figure 1B: New awards of NRF postgraduate bursaries at SU for 2016 - 2020

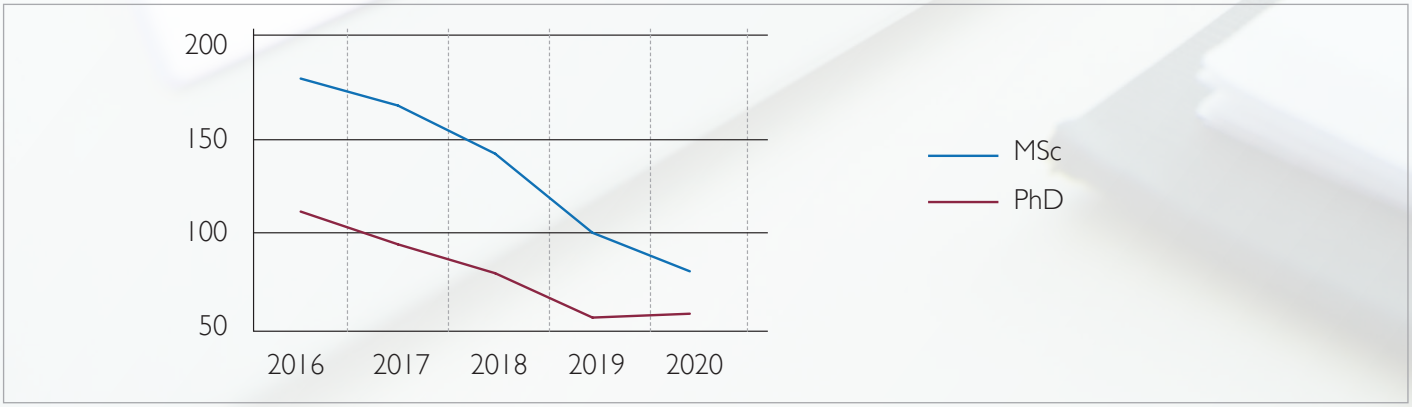
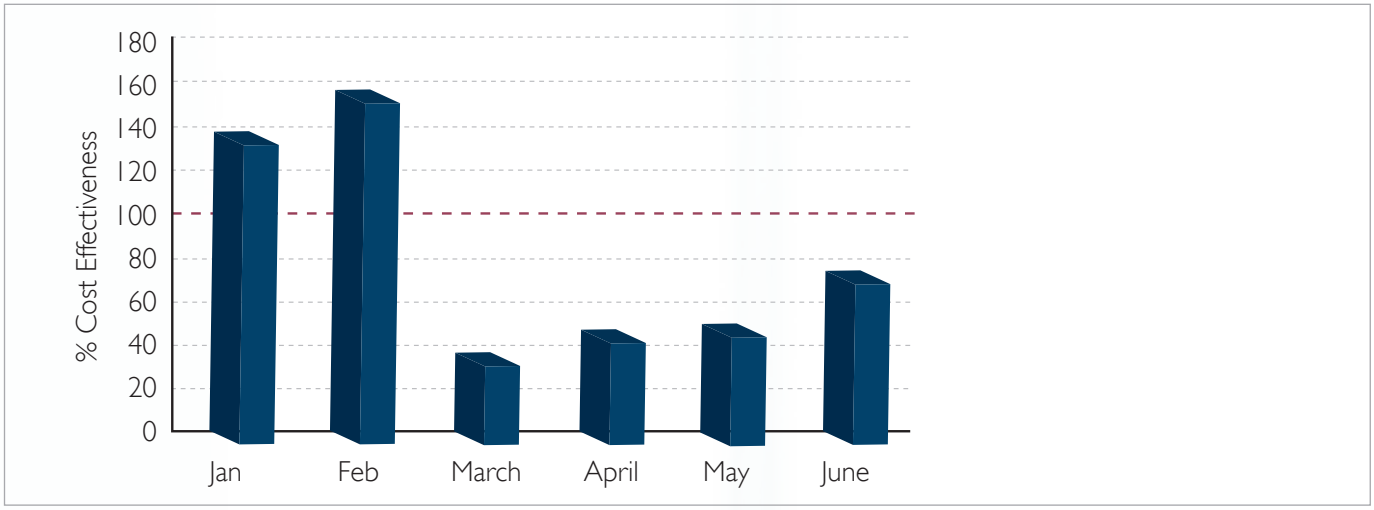


Figure 2: CAF cost effectiveness January - June 2020



As illustrated by the 2020 month-on-month cost effectiveness information for CAF (***Figure 2***), the year started exceptionally well. This good start was a result of all units performing well and the MS and DNA units both producing substantial profits. From early March, demand for services collapsed as behavior changed due to the COVID-19 pandemic and then the lockdown. Most CAF units provide some services to companies and researchers that are involved in activities that were permitted under all levels of lockdown. Consequently, plans were implemented to allow staff to work in their labs safely and most CAF staff were granted permits to assist with this essential services related work. CAF was one of the first environments at SU to return to work fully, with the necessary safety protocols in place. The commitment shown by the staff in achieving this has been humbling to behold. Activities have been remapped, work environments rearranged, staff hours staggered with different shifts and with data processing being conducted by people working from home while others run the labs. The net result is that a lot of important work was conducted during lockdown, with clients slowly returning and the cost effectiveness of CAF being restored. Most CAF services have relied on direct contact with our clients and in some cases, such as at the two microscopy units, this was central to our functioning. All CAF units have had to adapt to new ways of working that keep the clients out of the lab, despite the fact that this is in some cases substantially less productive and very challenging. Training activities have also been curtailed and are currently restricted to on-line courses and workshops. Because of these adaptations, CAF is currently in a position to support all returning postgraduate students and researchers with a full offering of analytical services.

In reflecting on the functioning of CAF since COVID-19 impacted on SU, it is important to recognize the exceptional achievements of Dr Marietjie Stander and Mr Carel van Heerden and the teams of people who report to them. Marietjie manages the MS Unit (eight staff members) which has not seen cost-effectiveness drop below 75%, despite the extreme disruption to research activities and business in South Africa and it is very likely that the MS Unit will be cost effective in July (***Figure 3A***). The DNA Sequencer Unit managed by Carel (six staff members) was more severely affected at the start of lockdown, but has already in June returned to being fully cost-effective (***Figure 3B***). The resilience and adaptability demonstrated by the teams at the MS and DNA Sequencing units is present throughout CAF. Consequently, I am confident that CAF will deliver better financial performance for 2020 than is projected in this report. Some of this improvement will come from supporting the wide range of research projects that will flow from the COVID-19 pandemic.

Figure 3A: Income/Costs for the MS Unit, January - June, 2019 and 2020

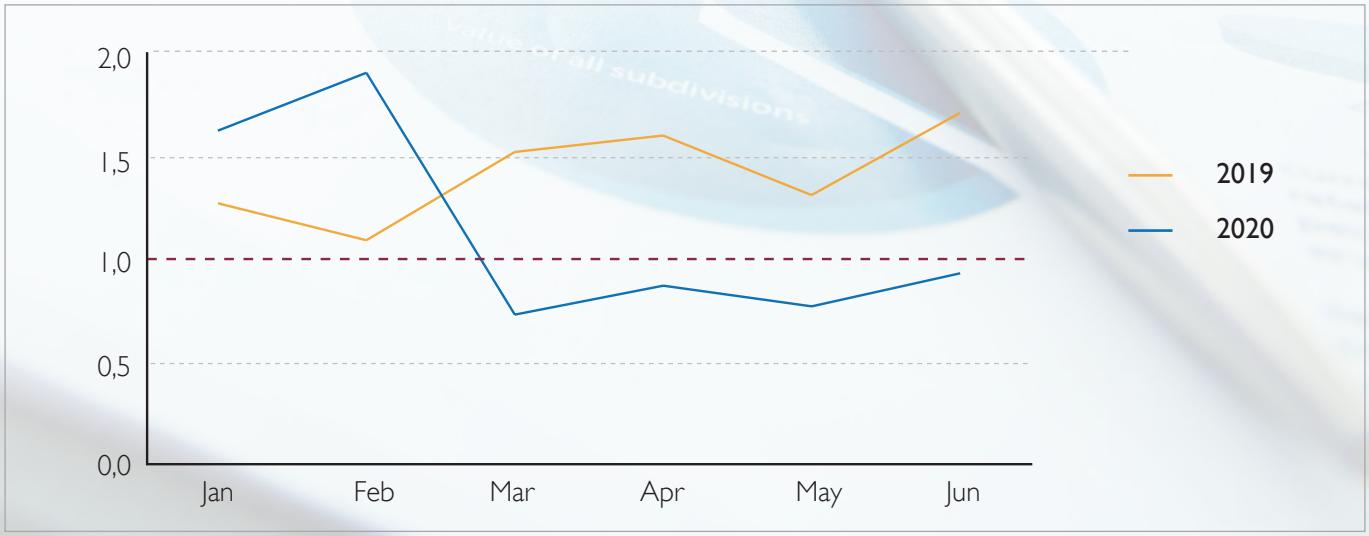
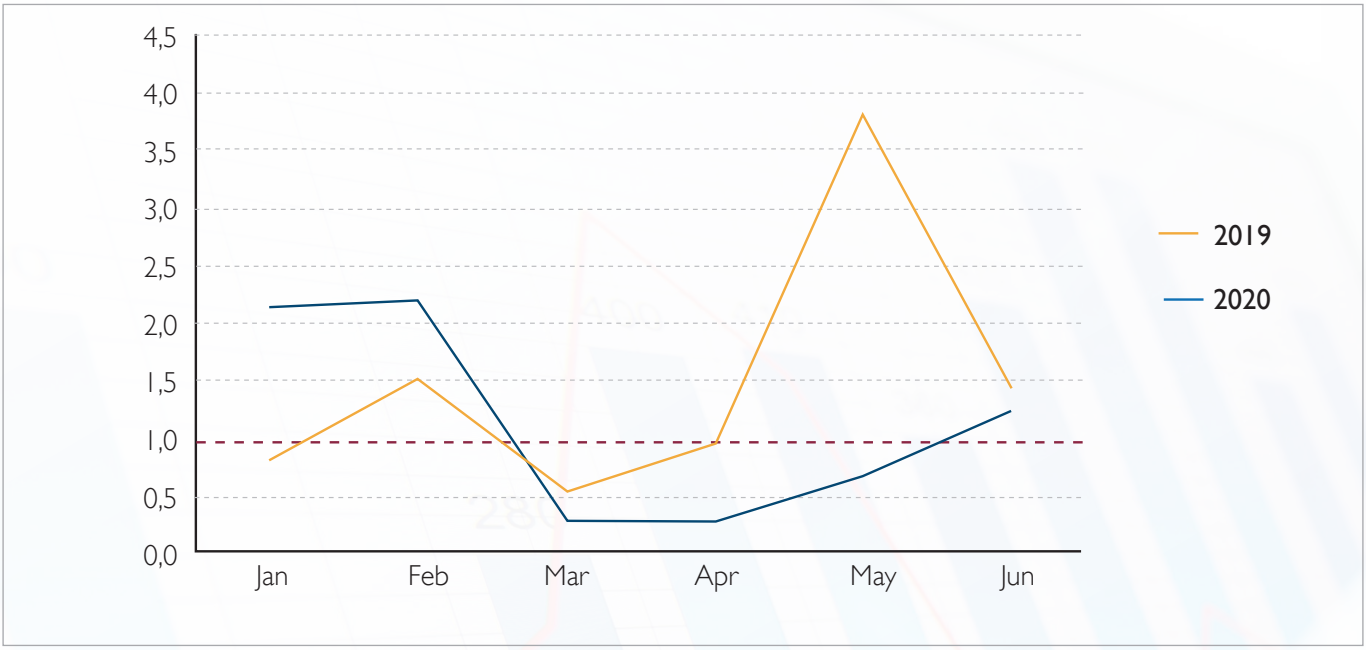


Figure 3B: Income/Costs for the DNA Unit, January - June, 2019 and 2020



The disruption to CAF activities that resulted from COVID-19 has posed a substantial challenge to CAF due to the requirement that CAF be largely self-funding. As is evident in the financial report, SU has provided CAF with a R10 million financial facility in order to ensure that CAF has the liquidity necessary to navigate the year and continue to deliver on its mission to provide the best possible analytical services in support of research at Stellenbosch University. The challenge for CAF is to adapt to the new circumstances whilst also finding all available opportunities that these present, such that we end the year in the best financial position possible. The extraordinary dedication, commitment and application shown by the CAF staff in 2020 leave me in no doubt that they will do this very well indeed.

Prof Gary Stevens
CAF Director

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 2019 as well as on possible changes to the profile of CAF clients over time:

Figure 4: The percentage of industry and academic clients for 2017, 2018 and 2019

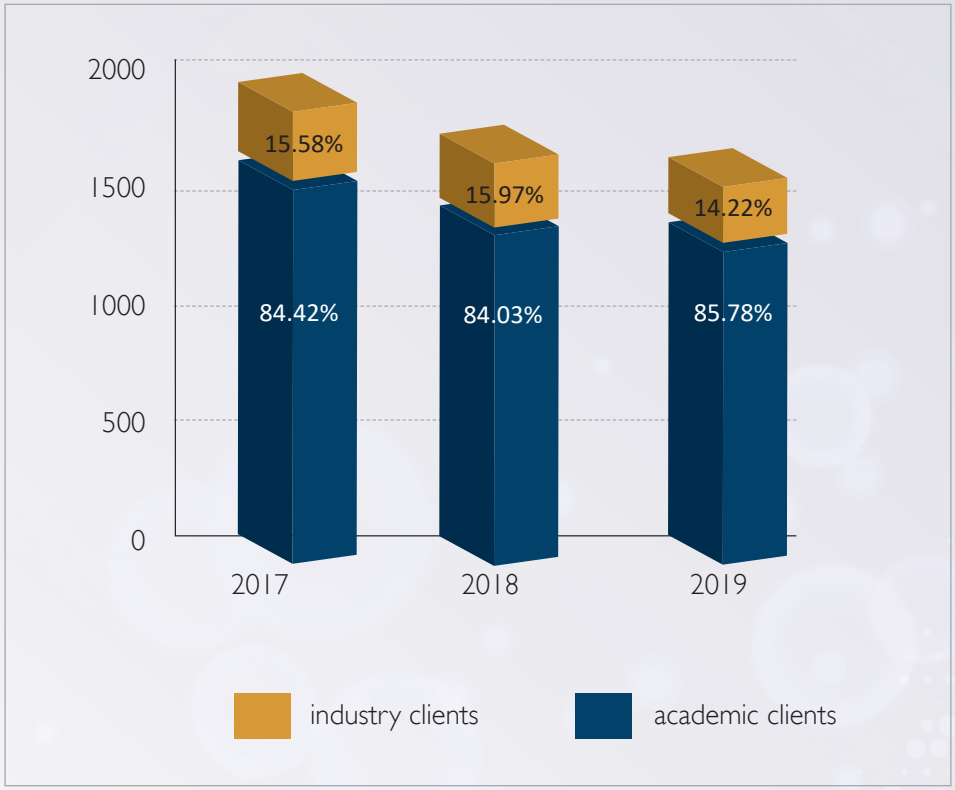


Figure 5: The proportions of different kinds of academic clients

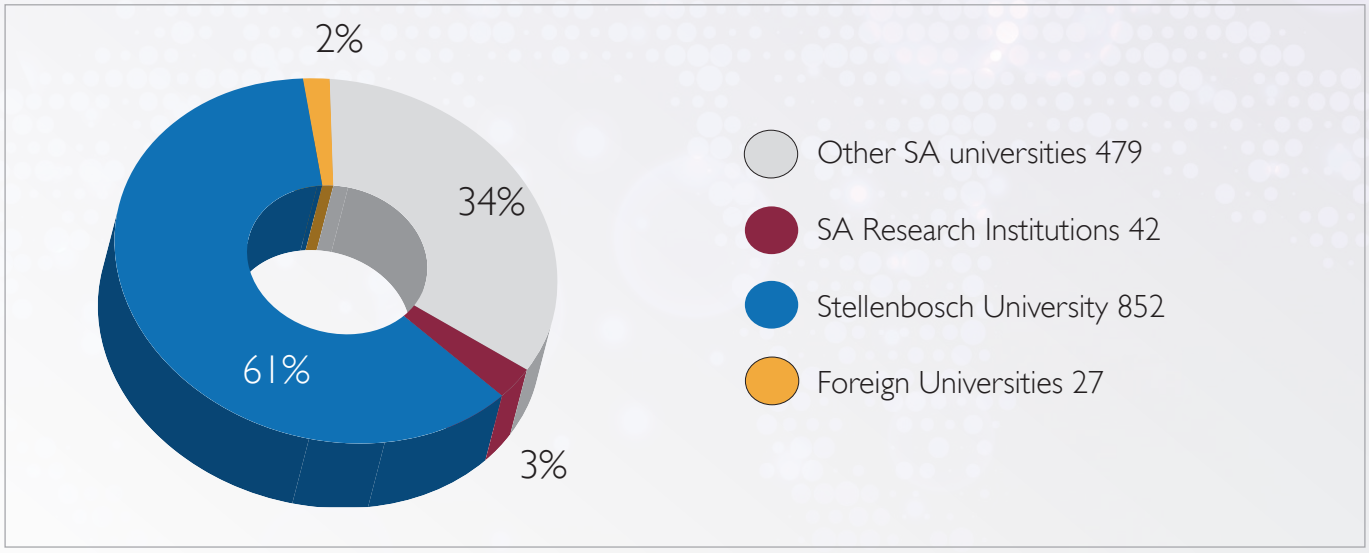


Figure 6: The profile of CAF academic clients

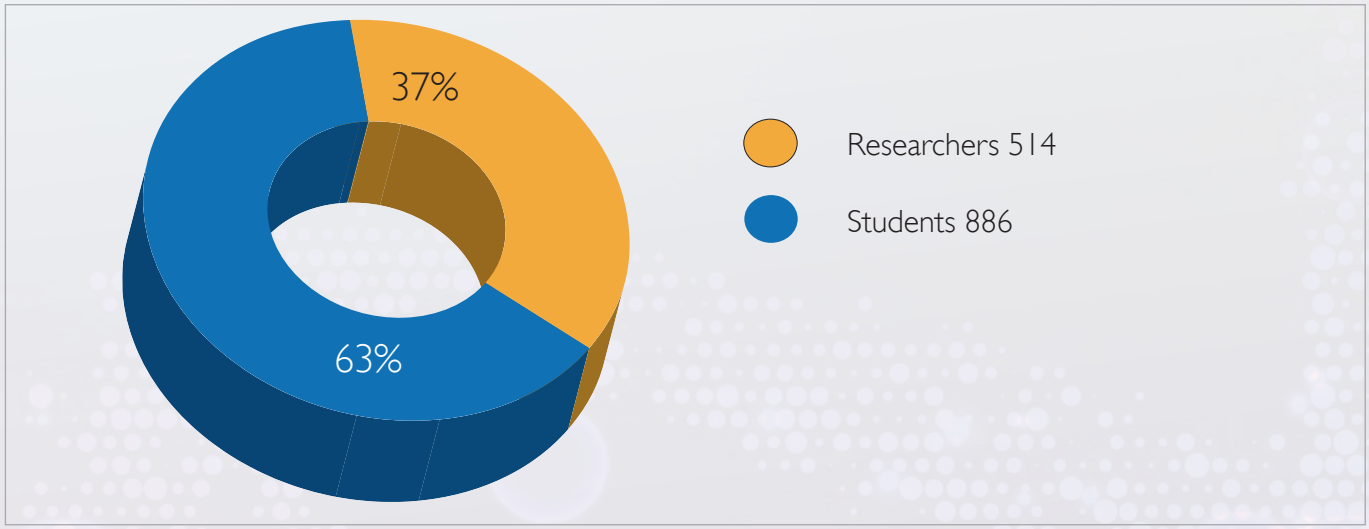


Figure 7: The subdivision of the 63% students from the previous graph

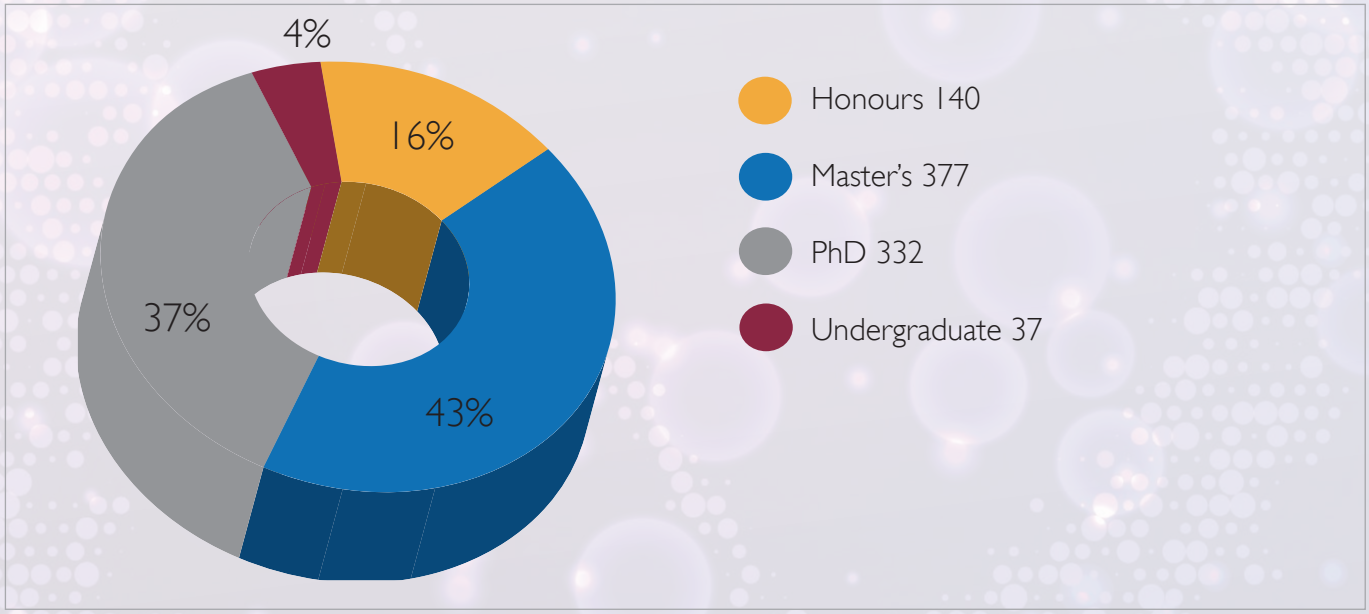
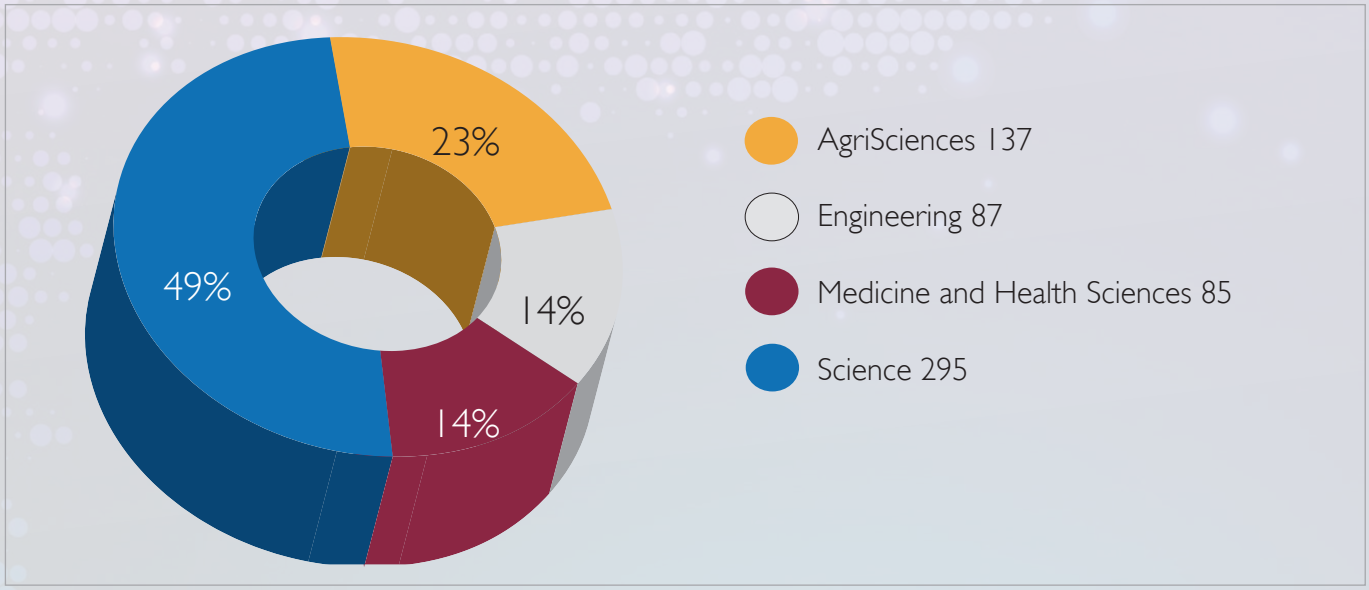


Figure 8: The proportions of Stellenbosch University student clients according to faculty



New facility for *Water and Soil Analysis*

by Dr Janine Colling

A new national facility for water and soil analysis is currently being set up at Stellenbosch University. The new facility will form one of the nodes of BIOGRIP, a national research infrastructure platform hosted by UCT and with nodes at several South African institutions. The Stellenbosch BIOGRIP Node for Water and Soil Biogeochemistry will focus on the interdisciplinary study of the chemical, physical, geological and biological processes that influence the environment.

The BIOGeochemistry Research Infrastructure Platform (BIOGRIP) is a new initiative to promote South Africa's biogeochemistry research by providing access to world class analytical facilities, various training opportunities and generating meaningful datasets by monitoring various biogeochemical environmental variables. BIOGRIP will consist of four nodes based at four universities across South Africa. Each node will focus on a different aspect of biogeochemistry including Stellenbosch University (Water and Soil Node), University of the Free State (Mineral Node), North-West University (Atmospheric Node) and the University of Cape Town (Isotope Node) (***Figure 9***).

Funding for this initiative was provided by the Department of Science and Innovation (DSI) as part of the South African Research Infrastructure Roadmap (SARIR). The main goal of SARIR is to support the development of advanced infrastructure and cutting-edge analytical facilities to promote high quality and innovative research. BIOGRIP will enable researchers to gain a deeper insight into how human activities in the past have impacted the environment and will also enable us to evaluate the impact of current practices on these areas, in the future. The study of earth and the environment was listed as one of the national research priorities and strategic goals for SARIR. Prof Sarah Fawcett (Department of Oceanography) at UCT and Prof Jodie Miller (Department of Earth Science) at SU were the co-champions of the BIOGRIP proposal. The BIOGRIP hub, which will coordinate and manage the platform, will be based at UCT with Prof Judith Sealy as the Director.

Expanding Analytical Services

Currently, a selection of water and soil analytical services are offered by CAF units such as the ICP and XRF unit (***Figure 10***).

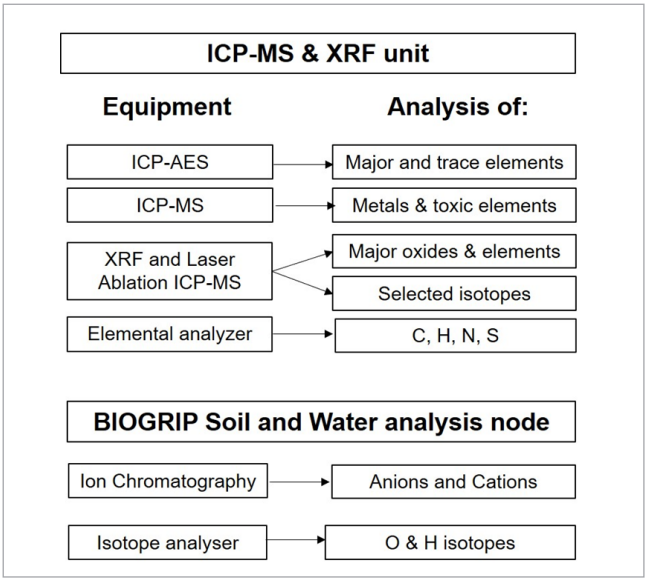


Figure 10: Overview of soil and water analytical services provided by CAF after the establishment of the BIOGRIP node.

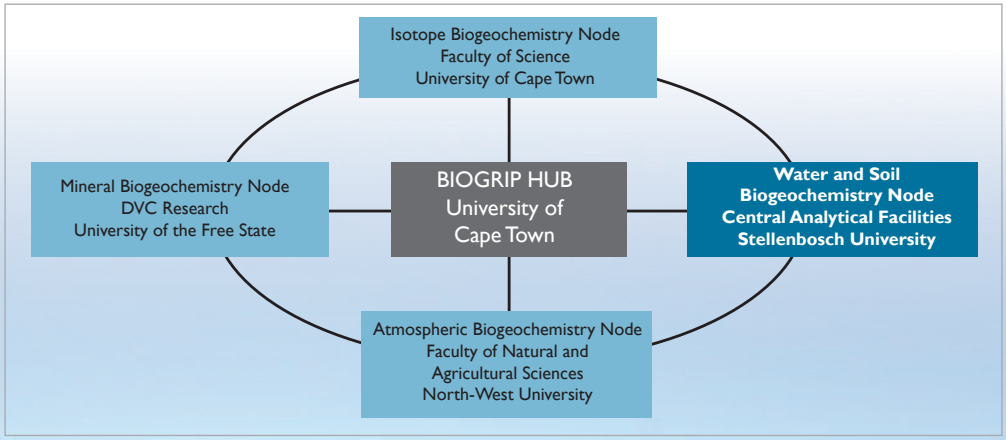


Figure 9: Thematic areas of the BIOGRIP initiative and their respective hosts.

Photo: Wikipedia

The water and soil facility will focus on providing standard analytical services and access to new state-of-the-art equipment. The unit will house an Ion Chromatography (IC) system from Metrohm, similar to the one depicted in ***Figure 11***. This instrument can be used for the quantification of both cations (Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}) and anions (F^-, Cl^-, NO_3^-, NO_2^-, SO_4^{2}) in various liquid samples. Ion Chromatography is a form of liquid chromatography, which involves separating ions and polar molecules based on their affinity to an ion exchanger. When a sample passes through the column, the ionic species adhere to the column and separate based on their size and type. By changing the column conditions, the absorbed ions dissociate, and their identity and concentration can be determined using their retention time and comparing to a standard solution. Quantification of ions is one example of routine analysis, which is regularly used to evaluate water quality for various day-to-day applications.

The unit will also feature advanced instruments for conducting the analysis of hydrogen and oxygen stable isotopes.

Figure 11: An Ion Chromatography system (Metrohm) can be used for the quantification of anions and cations (photo by Datamax https://commons.wikimedia.org/w/index.php?curid=3694084).

What are stable isotopes and why are they useful?

Isotopes are elements with the same number of protons, but a different number of neutrons in their nucleus. Some isotopes are stable, whilst the radio-active isotopes break down by emitting particles and energy (radiation) and decay according to a specific half-life. This property makes radio-isotopes useful as tracers in scientific research, medical diagnostics and for dating purposes.

Stable isotopes are also useful tracers to study various processes in a range of scientific fields. As an example, oxygen is a stable isotope which has 8 protons (***Figure 12***). Three stable isotopes for Oxygen, ^{16}O, ^{17}O, ^{18}O with 8, 9 and 10 neutrons respectively, can be found in nature. Their natural abundance varies with the lighter ^{16}O isotope being the most abundant isotope, accounting for 99.76%, whilst the heavier isotopes ^{17}O (0.038 %) and ^{18}O (0.205 %) occur in lower concentrations (Wright, 2017). Differences in the isotope behaviour result in the development of isotope fractions or ratios ($^{18}O/^{16}O$) during natural processes such as evaporation and precipitation. These unique signatures in respective sources such as rainwater, make isotopes useful tracers to explore various hydrological and meteorological processes. Analysis of complex water cycles can shed new light on the origin of water resources and assist with evaluating the sustainable use of ground water supply from aquifers.

Quantification of isotope ratios

Traditionally, isotope analysis is conducted using integrated ratio mass spectrometry (IRMS) instruments. These instruments can quantify isotope ratios with high precision and accuracy, but they also have some disadvantages (Sengupta, 2014). Analysis of oxygen and hydrogen isotope ratios in water can be challenging. The reason for this is that water samples cannot be analysed directly on the instrument because it acts like a "sticky" material. If injected into the delicate high vacuum IRMS equipment, it will remain on the surfaces and can continue to interfere with other measurements. As a result, the isotopes have to be processed and analysed separately by equilibrating the water sample using reference gasses such as CO_2, which has known isotope compositions. These gasses are then analysed to quantify the isotopes. The analysis of the ^{17}O isotope, which is present in lower abundance, is also challenging and requires the use of powerful reducing agents such as cobalt fluoride. The instruments also use ultra-pure references gasses as carrier gasses during analysis. Collectively, these factors can make isotope analysis using IRMS, a time consuming and expensive procedure.

As an alternative, isotopes can also be analysed using laser absorption spectrometry such as cavity ring down spectroscopy and off-axis integrated cavity output spectroscopy (ICOS). The node will be equipped with an ICOS triple isotopic

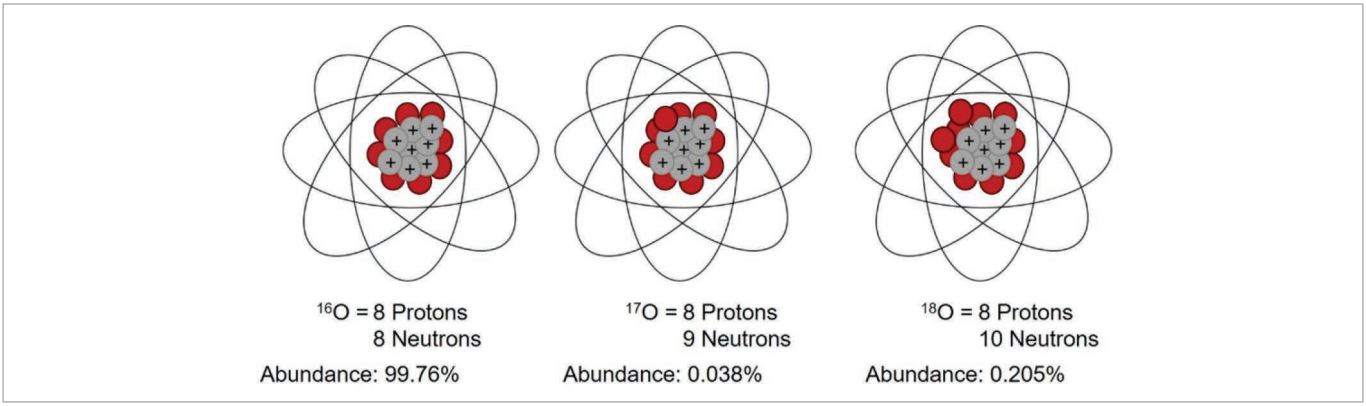


Figure 12: Stable isotopes of oxygen vary in their number of neutrons in the atom's nucleus and in the percentage abundance in which they can be found in nature.

water analyser from Los Gatos Research (Inc) (***Figure 13***). This instrument can be used for the quantification of $\delta^{2}H$, $\delta^{17}O$, $\delta^{18}O$ in fresh water and seawater samples. During analysis, the water sample is introduced as a gas into the cavity. A laser light, which is positioned off axis with respect to the cavity, shines light into the cavity, which contains highly reflective mirrors (R > 99.9 %). The light is reflected back and forth over a 1000-times creating an exceptional long (5 – 10 km) effective optical pathlength. This increases the molar absorptivity by the gas and enables quantification of low concentrations of isotopes such as ^{17}O, with high precision using global standards. The advantage of the ICOS instrument is that it is more robust and less sensitive to thermal changes and vibrations (Sengupta, 2014). It has a shorter measurement time (1 Hz), requires almost no sample preparation and all of the isotopes in the sample are analysed simultaneously. The instrument can be operated in high performance or high throughput mode depending on the precision of the analysis required (***Table 1***). The benefits of this instrument include that samples can be analysed at a reduced cost, it has a higher throughput of samples and faster turn-around time, allowing results to be reported more rapidly.

Table1: Typical precision (1σ) of analysis for the O/H isotope ICOS analyser

Isotope	High performance mode	High throughput mode
$\delta^{2}H$	0.15‰	0.4‰
$\delta^{17}O$	0.02‰	0.1‰
17O-excess	0.015‰	
$\delta^{18}O$	0.02‰	0.1‰

Analysis of isotopes offer many research opportunities and future plans include the acquisition of equipment for the analysis of carbon and nitrogen stable isotopes, transition metal stable isotopes (Cu, Fe, Zn, Cr) and radionuclides.

Various basic services for the routine testing of a range of soil and water parameters will also be available. These include the analysis of pH, EC, ions, total organic carbon (TOC) and nutrient analysis amongst others. The results from these tests can be

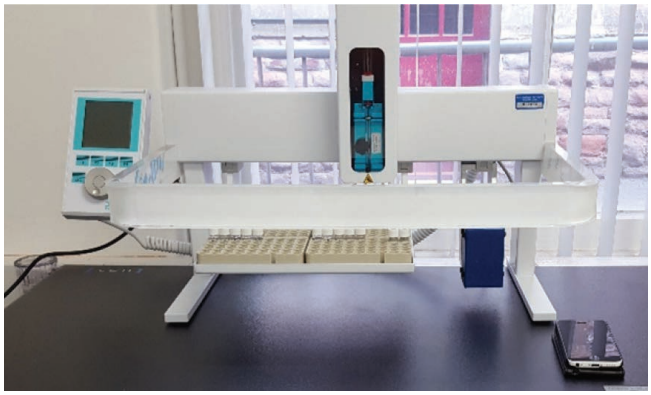


Figure 13:The integrated cavity output spectroscopy (ICOS) triple isotopic water analyser from Los Gatos Research (Inc) can be used for quantification of the $\delta^{2}H$, $\delta^{17}O$, $\delta^{18}O$ in aqueous samples (photo: Mr Lewis John).

used to evaluate if the quality of water intended for human and animal consumption complies with the latest guidelines as described by the South African National Standards (SANS 241) (DWA, 2011) and the World Health Organization (WHO). It can also enable assessing the suitability of water for use in the agricultural sector and ensuring water treatment solutions are appropriate for treating industrial effluent water. A comprehensive list of analytical services will be made available and this will be expanded as new equipment is added.

Training and research opportunities

The unit will focus on providing researchers and post-graduate students with technical support to perform research projects. Clients from higher education institutes and the private and public sector will be able to submit samples for routine analysis. Students will have the option to receive hands-on training on all instruments during various training opportunities. This will empower them with the necessary advanced skills to operate instruments, conduct experiments and to develop new analytical methods that are not currently available in South Africa.

We invite anyone interested to follow the CAF website or Facebook page or send an email to jcolling@sun.ac.za to receive updates and relevant information. We look forward to develop the new facility into an excellent training and research facility, which will enable world-class research that can compete and contribute on the global arena and advance our knowledge of biogeochemistry.

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Brilliant new instrument takes single-cell analysis to the *next level*

by Lize Engelbrecht

At the end of June 2019, a new addition to the Fluorescence Microscopy Unit's array of equipment arrived at the Stellenbosch University's Tygerberg Campus. Imagine having access to an instrument capable of taking single cell analysis by flow cytometry to the next level, by adding a visual of every cell or particle running through the system at high speed. This type of analysis is made possible by the AMNIS® ImageStreamXMk II developed by Luminex Corporation. This amazing technology is the first of its kind in Africa and funded by the NRF National Equipment Programme.

During July last year the NEP applicants, Prof Samantha Sampson from the Division of Molecular Biology and Human Genetics and Prof Carine Smith, from the Department of Physiological Sciences, senior members of their research teams and our CAF staff, Dr Dalene de Swardt and Lize Engelbrecht received training on the operation and maintenance of the equipment, as well as the workflow of data analysis in the IDEAS software by a UK-based specialist, Dr PJ Chana.

To introduce the equipment to the research community a roadshow was organised in collaboration with Biocom Biotech to raise awareness of its capabilities with presentations in Stellenbosch and Tygerberg, but also various other locations in South Africa. Furthermore, another training initiative was organised in November when a US-based specialist, Dr Owen Hughes visited South Africa. New users were introduced to the workflow of the analysis software. To gain the depth of information available in the data, the software is extremely powerful but requires learning of a new way of working with the data.

Soon after commissioning, various researchers started exploring what the AMNIS® ImageStreamXMk II has to offer. In the past year, our unit analysed and imaged samples from various research groups around the Western Cape. Marine ecologists and microbiologists who are currently studying the Southern Benguela upwelling region, particularly focussing on the picoplankton and microbial community distributions, acquired beautiful images of the diatoms found in these water samples (***Figure 15***).

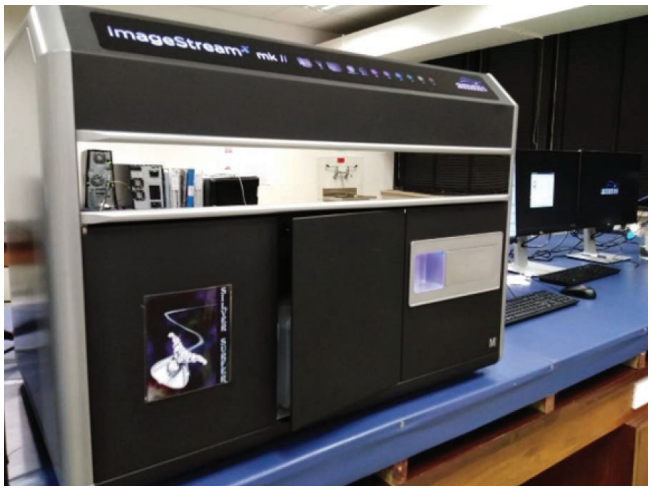


Figure 14: The new AMNIS ImageStreamX MkII in the Flow Cytometry Centre at the Tygerberg Campus, Stellenbosch University.

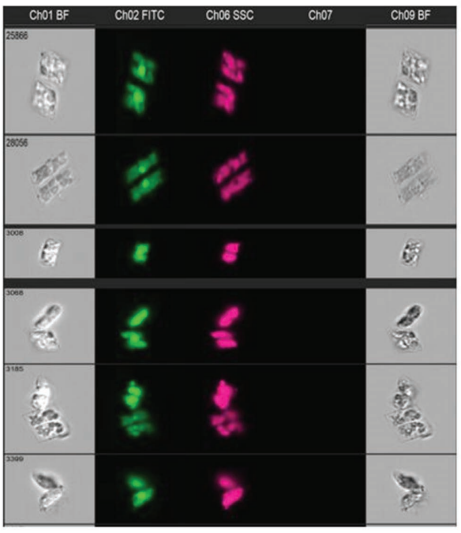


Figure 15: Diatoms found in ocean water samples from the Southern Benguela upwelling region.

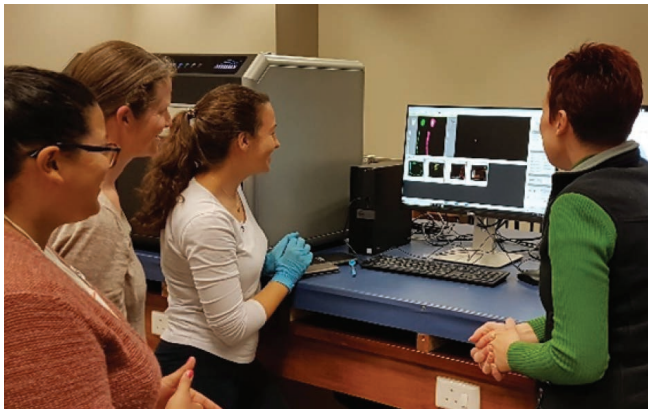


Figure 16: Dr Emma Rocke and colleagues from the University of Cape Town looking at ocean water samples with Dr de Swardt, the operator of the equipment.

Researchers from the Institute of Wine Biotechnology of Stellenbosch University investigated the cell wall properties of wine yeast and algae co-cultures from different generations for improved wine production. Private sector clients explored the capabilities of the imaging flow cytometer by looking at isolated microbial cultures from compost.

The technology can be applied to research questions from a wide range of fields. The equipment is not only beneficial for advancements in the medical sciences, such as immunology, oncology and drug discovery but also related topics such as microbiology, virology and parasitology. There are several examples in the literature where oceanography benefited from this technology. Of course, cell biologists, who rely heavily on microscopy to visualise subcellular structures and study cellular functions (such as cell signalling and cell-cell interaction, cell cycle and mitoses, internalization, co-localisation, nuclear transportation, DNA damage and repair and many other aspects) will benefit from the availability of this new technology in South Africa.

The beauty of this equipment is that it runs like a flow cytometer, ie. thousands of particles or cells in suspension can be analysed rapidly, but provides the large amount of visual information one can gain from microscopy images. At high magnification (40x and 60x objectives) acquisition of about 1200 cells per second is significantly faster than it would have been on a microscope, while at low magnification (20x objective) an acquisition speed of up to 5000 cells per second can be achieved. Apart from the increased speed of image acquisition, the software allows for investigation of many physical cellular parameters, such as texture, shape changes, localisation of molecules of interest and many more that are only available from imagery, eliminating some of the subjectivity of the researcher searching for cells of interest on a microscope before acquisition. With the growing demand in cell biology for quantification instead of qualitative reporting, especially on large data sets for statistical power, this type of acquisition allows researchers to produce datasets meeting the requirements of current modern microscopy.

This versatile instrument is equipped with seven lasers ranging in the UV range to the infrared range, allowing the user to use any fluorescence marker currently on the market. Altogether ten fluorescence channels are available to use simultaneously as well as two channels for transmitted light microscopy images.

An automated acquisition function, called ***autosampler***, where samples can be acquired unattended from a 96 well plate, allows a user to load a plate and programme the sequence of samples and run the experiment overnight. With automated cleaning and shutting down procedures, this gives the user the flexibility of running the experiment without having to be present. With all these features, the AMNIS ImageStreamX MKII allows for investigation of many different parameters at once at high speed, the use of an array of fluorochromes across the whole fluorescence spectrum and automation which allows the user to use their time more productively.

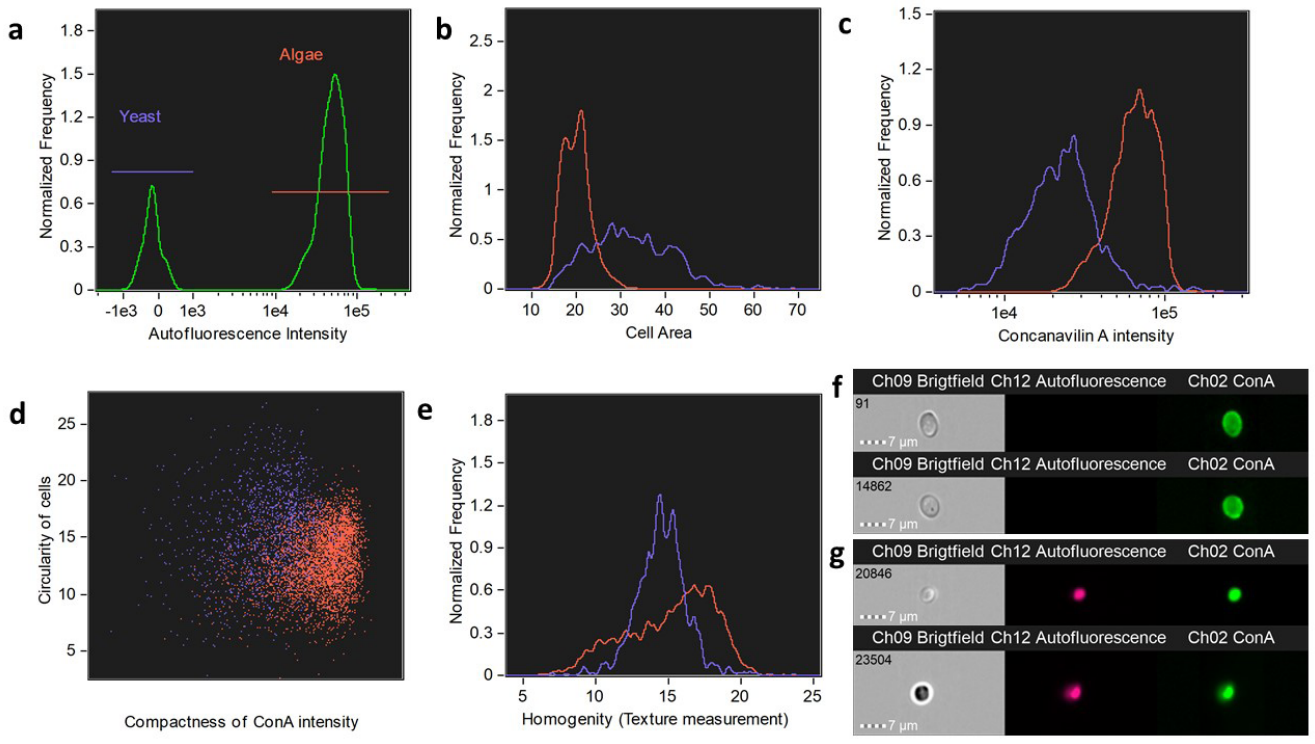


Figure 17: ***Characterising yeast and algae co-cultures. a)*** *Here yeast and algae are distinguished based on the presence of autofluorescence and various aspects of these cells are compared, including* ***b)*** *area of the cells,* ***c)*** *intensity of *ConA staining,* ***d)*** *circularity and compactness of ConA binding, and* ***e)*** *homogeneity as a measure of texture. Representative images of* ***f)*** *yeast cells and* ***g)*** *algae cells.*

*Concanavilin A (ConA) is widely used to characterize glycoproteins and other sugar-containing entities on the surface of various cells.

Prof. Carine Smith's first project using the equipment is completed and submitted for publication. She described the research as follows:

"Human primary monocytes were differentiated and polarised into M1 macrophages. Cargo to be delivered – which can be anything from live stem cells to pharmaceuticals – was simulated using latex nanobeads. These beads were coated with bacterial effectors – specifically those used by Listeria spp to facilitate their expulsion from host cells to enhance their dissemination in host organisms. They were taken up into macrophages via phagocytosis and then, as the fagosome matured and acidified, the effectors became active and facilitated cargo expulsion from the macrophages. The AMNIS was used to confirm macrophage polarisation, as well as to generate data indicating the success and efficacy of cargo expulsion (***Figure 18***). Importantly, using the AMNIS, we could

1) show that expulsion did not result in carrier cell lysis (or other abnormal cell morphology), which is an important consideration in drug delivery, where cell lysis would contribute to tissue damage and prolong recovery
2) generate high quality, statistically verifiable data.

Up to now, expulsion mechanisms of macrophages has only been demonstrated in single cells, using confocal microscopy. Here, we could not only demonstrate expulsion visually but also back it up with numerical data to show the efficacy of our intervention. Using AMNIS technology, drug delivery science can now progress from merely showing that it is possible, to calculating precise concentrations of cargo that will be delivered within specific time frames."

The next phase would be to investigate intracellular co-localisation of potential therapeutic targets in the context of neuro-inflammation.

Many students from the Division of Molecular Biology and Human Genetics have already been trained and are using the equipment to visualise intracellular Mycobacterium tuberculosis, specifically to study M. tuberculosis persisters which are resistant to current vaccines and treatments, but also to assess microbial interactions and investigating subcellular components associated with M. tuberculosis protein secretion. This might lead to novel interventions and ultimately contribute to improved public health, wellbeing and quality of life.

"These potential long-term benefits will have a particular impact in the developing world, where the dual burden of infectious and non-communicable disease is greatest. Decreasing morbidity and mortality will also promote productivity and economic growth" Prof Samantha Sampson said.

Combining the applications of flow cytometry and fluorescence microscopy has revolutionized these conventional technologies into a brilliant tool that now streamlines research that was previously very complicated to perform (e.g. nuclear translocation). Also, having both the technologies available as one, fills the important shortfall of each. Where in flow cytometry statistics are rapidly obtainable unfortunately with no imagery output possible, in microscopy images are rapidly producible but acquiring statistics is generally a time consuming and laborious task with subjective outputs.

The new and advanced technology delivers an instrument where images and statistics can be obtained in real time. This is one of the most anticipated integration platforms currently available.

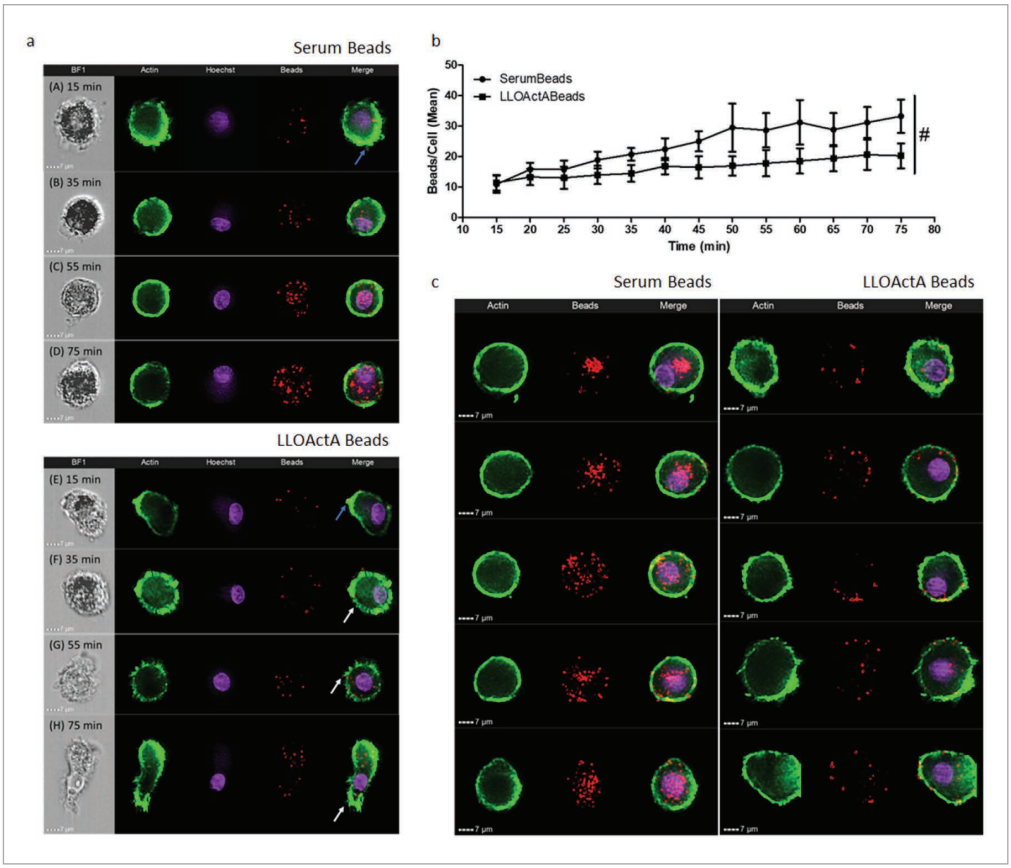


Figure 18: ***Phagocytic phases under control and effector treated conditions over time****. Macrophages populations were exposed to Serum beads or LLOActA beads for different time periods and analysed using imaging flow cytometry.*
a) *Accumulation of beads is seen under Serum bead exposure.*
b) *Average number of beads per cell given for effector treated (LLOActA beads) and control (Serum beads) over time.*
c) *Representative images of macrophages at 75 min suggest a tendency for bead distribution predominating at the periphery of cells during LLOActA bead exposure.*
Blue arrows: pseudopodia; White arrows: actin membrane spikes; BF1: first bright-field channel. Data points are means and error bars indicate SEM. Statistics: #, ANOVA main effect of treatment, $p < 0.01$

Electron Microscopy contributes to local production of ***personal protective equipment***

by Jurgen Kriel

One of the main reasons for the strict lockdown regulations in South Africa during COVID-19, was to prevent overcrowding of hospitals and provide them with precious time to prepare for the expected influx of patients as transmissions peaked. Over time, it became apparent that this preparation mainly centred around one critical element – the procurement of personal protective equipment (PPE). In light of these events, the Electron Microscopy (EM) Unit is currently providing critical EM analytical support to the Stellenbosch Nanofiber Company (SNC), which is in the process of manufacturing reusable filters for face masks.

Continuous provision of PPE to health care workers (HCWs) is of paramount importance in the fight against the COVID-19 pandemic. PPE constitutes a range of products including gloves, face shields, surgical gowns and face masks. HCWs come into contact with multiple patients per day and therefore require certified equipment to serve as an effective barrier between them and their patients. Therefore, PPE can be regarded as the barrier that not only prevents HCWs from becoming carriers of the novel coronavirus but also prevents hospitals themselves from becoming transmission 'hotspots', which can result in the closing of hospitals. Sadly, as a result of increased global demand, many countries are struggling to provide frontline HCWs with adequate PPE and South Africa is no exception.

In response to these shortages, many local manufacturers have repurposed their production lines to manufacture various forms of PPE for general public use. The difficulty with mass producing medical-grade PPE is that manufacturers must adhere to the strict International Organization for Standardization guidelines as well as be certified by government to produce medical equipment.

Figure 19: The ThermoFischer Apreo VolumeScope scanning electron microscope is the newest addition to the Electron Microscopy Unit and has been instrumental in assisting SNC to perform much-required analysis for manufacturing PPE.

SNC is a prime example of a company specialising in the commercial-scale manufacturing of advanced biomedical nanofiber materials. Nanofiber materials have extremely versatile biomedical applications, encompassing wound dressing, drug release materials and cell culture scaffolds. In response to the growing demand for PPE, SNC is currently working on the production of the most important part of medical-grade face masks, namely the filter layer. What is unique about SNC's filter layers is that they physically entrap and immobilise viral particles as opposed to conventional melt-blown polypropylene layers that electrostatically trap particles. This might seem like a small difference, but it allows for the nanofiber-based filters to be washed and reused, whereas the electrostatic properties of the polypropylene-based filters diminish with each wash.

In order to confirm whether these filter layers are capable of entrapping nanoscale particulates and to assess how robust these nanofibers are, scanning electron microscopy (SEM) analysis is required to measure the distance between fibers as well as the fiber size. This makes SEM analysis integral to the production of nanofiber-based filter layers.

The ThermoFischer Apreo VolumeScope scanning electron microscope is the newest addition to the CAF Electron Microscopy Unit and has been instrumental in assisting SNC to perform this much-required analysis. Although the main purpose of the Apreo is to function as a serial block-face microscope, capable of acquiring 3D volumetric EM datasets, it is also a very capable scanning electron microscope for general image acquisition, which makes it an extremely versatile tool.

The procurement of the Apreo was accompanied by the appointment of a new CAF staff member, Mr Jurgen Kriel. Currently finishing his PhD in Physiological Sciences at Stellenbosch University, Kriel was appointed in March 2020 to provide SEM analytical services to medical researchers on Tygerberg Campus. Although the national lockdown has put a hold on many research projects, the Apreo continued running to provide industry clients such as SNC with essential analytical services. However, these services are not provided without risk. Being near Tygerberg Hospital has its inherent dangers in a time when Tygerberg has the highest number of confirmed COVID-19 cases in the Western Cape (at the time of writing this article).

Safety guidelines are of paramount importance, not only for the safety of employees but also for that of their families. "It was quite a difficult decision to go back to work. Right before the Level 5 lockdown was imposed, my mother started with chemotherapy. As much as I wanted to help SNC, I also did not want to place my family in harm's way. My manager, Ms Madelaine Frazenburg, was very understanding and left the decision up to me. Having a vulnerable family member really puts the importance of adhering to the safety guidelines into perspective. After I decided to help SNC, I was very relieved to see how well everyone on Tygerberg Campus adhered to these guidelines" Kriel said.

Until a vaccine is developed, the demand for appropriate PPE will remain high. Being able to reuse medical-grade face masks will alleviate the financial burden on hospitals significantly. "Various tests are ongoing to demonstrate the robustness of the filters, but initial tests have already shown that we maintain filtration efficiency even after 10 cycles of submersion in boiling water for 10 minutes and air drying" Dr Megan Coates, Research and Development Manager at SNC said. SNC is currently in the process of building partnerships for further production of face masks once testing on the filters has been completed.

Figure 20: One of the filter layers (which is part of medical-grade face masks) before the SEM analysis.

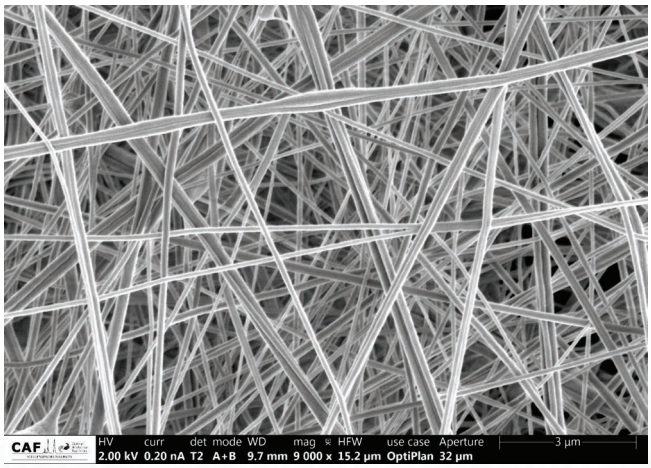
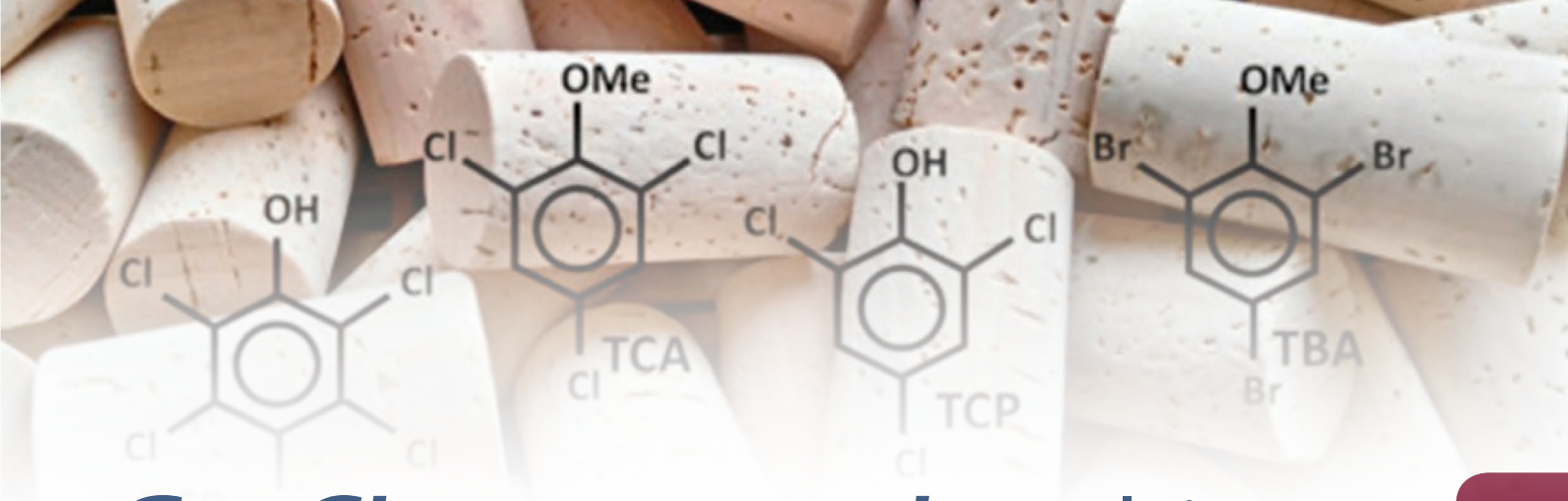


Figure 21: An image of a SEM analysis of the filter.



Gas Chromatography solving problems for the wine industry at the Mass Spectrometry Unit

by Lucky Mokwena

In 2019 the GC-MS laboratory partnered with Thalès Wine Cellar Services (Pty) Ltd as a service provider for the analysis of releasable haloanisoles and halophenols in corks, wood, wines and water.

Haloanisole contamination causes development of 'cork taint', a musty off-aroma, in affected wines. Cork taint results in significant economic loss for the wine and allied industries every year; therefore, extensive quality control procedures are necessary for the wine industry and cork production facilities to monitor levels of haloanisoles in both cork and wine products. Because of the extremely low human sensory thresholds for these compounds (~1–4 ng/L for 2,4,6-TCA in wine), highly sensitive analytical methods are needed to detect the haloanisoles at threshold concentrations or lower. The CAF GC-MS laboratory offers these capabilities.

Haloanisole determination either confirms or denies haloanisole involvement in wine contamination. In addition, it can be used to detect potential sources of contamination.

It should be noted that these molecules are extremely fragrant and that once the wine is contaminated, the process cannot possibly be reversed.

For screening purposes, group soaks of 50 corks are widely used. Group soaks allow more corks to be sampled and dramatically reduce the total number of analyses required.

The origin of haloanisoles can be attributed to the bio-degradation of 2,4,6-tricholorophenol, 2,3,4,6-tetra-chloro--phenol, pentachlorophenol and 2,4,6- tribro-mophenol, respectively, which can be found in winery environments. Several materials, including barrel oak wood and cork stoppers, may be contaminated and release these molecules into wine. Various materials including oak products (wood tanks, barrels, chips and staves) may be contaminated by haloanisoles and halophenols. Once polluted, those materials may release haloanisole and halophenol molecules into wine.

Solid-phase micro-extraction is used, and detection and quantification are performed by gas chromatographic triple quadrupole at the GC-MS laboratory. The haloanisoles and halophenols in corks are extracted by soaking with an aqueous-alcoholic solution before analysis.

What are haloanisoles?

Haloanisoles are a family of volatile chemical compounds that can contaminate wines and cause musty or mouldy aromas. Haloanisoles can contaminate a whole winemaking facility and can be introduced through contaminated water supplies or even from the vineyard.

2,4,6-TCA is the main compound responsible for cork taint in wine. The other haloanisoles (TeCA, PCA and 2,4,6-TBA) are biodegradation by-products of certain wood preservatives, with 2,4,6-TBA sometimes also originating from flame retardants.

What are halophenols?

Halophenols can also be formed when phenols present in wood/board from the decomposition of the lignin react with a source of bromine or chlorine in other areas of the winery environment.
Halophenols are the biochemical precursors of halo-anisoles:

- 2,4,6-trichlorophenol (2,4,6-TCP) → 2,4,6-TCA
- 2,3,4,6-tetrachlorophenol (TeCP) → TeCA
- 2,3,4,5,6-pentachlorophenol (PCP) → PCA
- 2,4,6-tribromophenol (2,4,6-TBP) → 2,4,6-TBA

Used to protect wood, 2,4,6-TCP, TeCP and PCP are responsible for air contamination. 2,4,6-TBP is a flame retardant recently identified in some contaminations.

Potential sources of contamination

Haloanisoles can be transferred into wine through a cellar's atmosphere or through contact with contaminated materials from tank coatings, hoses, barrels, oak chips, filter pads and closures, and additives such as bentonite. Haloanisoles are formed by the action of mould on halophenol precursors.

Until recently, chlorine bleach was widely used as a sanitiser in wineries. When it comes into contact with sources of phenol, such as wood, plastics or even grape and wine phenolics, bleach can form 2,4,6-TCP, the direct precursor of 2,4,6-TCA. Common moulds and soil bacteria transform 2,4,6-TCP into 2,4,6-TCA, which is very volatile and becomes airborne, contaminating the wine.

Sensory thresholds

Haloanisoles are ranked among the most powerful odour compounds, with odour thresholds in the low part-per-trillion range. All haloanisoles have similar odours, but their sensory impacts in wine vary with the specific compound and wine characteristics.

Reported 2,4,6-TCA thresholds in wine are typically in the range of 2 ng/L for detecting a noticeable difference and 6 ng/L for true recognition. 2,4,6-TCA levels below the 2 ng/L difference threshold can still impact a wine, usually described as 'muted' aromas and flavours.

2,4,6-TBA is virtually as powerful as 2,4,6-TCA, while TeCA is approximately three times less potent. PCA is unlikely to reach its odour threshold of 4 000 ng/L in wine but is still a useful indicator of origins of contamination.

How can haloanisole analysis in wine point to a source of contamination?

For both bulk and bottled wine, the relative concentrations of 2,4,6-TCA, TeCA, PCA and 2,4,6-TBA often suggest a possible contamination source. For example, the presence of pentachlorophenol-treated wood in the cellar would be suspected when TeCA and PCA are the predominant haloanisoles.

With bottled wines, bottle-to-bottle variability provides additional information. Significant bottle variability and the predominance of 2,4,6-TCA suggest that the corks may be the contamination source.

Analysing wine at bottling is highly recommended. It is the only way to confirm whether contamination occurred before or after bottling. If a sample taken at bottling is positive, causes of contamination in the cellar can be investigated.

GC-MS Division

The division under the management of Mr Lucky Mokwena, with a staff complement of three including Mr William Arries and Ms Lindani Kotobe, forms part of the Mass Spectrometry Unit under the leadership of Dr Marietjie Stander. The divisional laboratory is equipped with two Thermo Scientific triple quadrupole mass spectrometers and two Agilent single quadrupole mass spectrometers, including two flame ionisation detectors.

Figure 22 (from left to right): Cornea Cilliers (Thales Services (Pty) LTD), Lucky Mokwena (CAF-GCMS Lab), Jacqueline van Wyk (Thales Services (Pty) LTD) William Arries (CAF-GCMS Lab) and Lindani Kotobe (CAF-GCMS Lab).

Figure 23: Preparation of cork samples by soaking in 12% ethanol solution for 24 hours before sampling and instrumental analysis of haloanisoles.

Financial Reports

By Fransien Kamper

		January 2017-31 December 2017	January 2018-31 December 2018	January 2019-31 December 2019	2020 Projection
MS UNIT	Internal invoicing	2 463 824	2 040 163	1 893 475	1 116 525
	External invoicing	5 379 758	5 148 560	6 803 566	5 813 914
	Total income	7 843 582	7 188 723	8 697 041	6 930 439
	Expenses				
	Salaries	2 963 154	3 708 383	4 202 003	4 537 372
	Running costs	969 322	906 574	1 171 430	1 066 003
	Maintenance	789 232	829 955	905 603	467 585
	Travel costs	36 784	11 805	281	
	Small equipment & KKW	24 511	70 461	5 952	
	Deferred costs			255 800	255 800
	Total expenses	4 783 002	5 527 178	6 541 068	6 326 761
FM UNIT	Internal invoicing	926 172	1 261 988	856 494	571 444
	External invoicing	155 292	74 017	189 215	99 490
	Total income	1 081 464	1 336 005	1 045 709	670 934
	Expenses				
	Salaries	1 034 828	889 764	930 059	1 026 187
	Running costs	259 077	313 664	425 804	111 654
	Maintenance	16 393	79 978	59 150	17 975
	Travel costs	7 025	3 674	6 653	
	Small equipment & KKW		36 455	114 407	
	Deferred costs			150 000	150 000
	Total expenses	1 317 323	1 323 535	1 686 073	1 305 816
SEM UNIT	Internal invoicing	648 946	948 918	918 242	673 895
	External invoicing	1 520 116	2 107 221	732 351	493 665
	Total income	2 169 062	3 056 139	1 650 593	1 167 560
	Expenses				
	Salaries	1 397 948	2 100 941	1 684 505	1 389 026
	Running costs	97 459	196 673	62 968	89 443
	Maintenance	436 073	35 975	93 673	
	Travel costs	26 348	64 975	5 491	3 473
	Small equipment & KKW	91 666	177 800	86 628	
	Deferred costs			120 000	120 000
	Total expenses	2 049 494	2 576 365	2 053 265	1 601 942

		January 2017-31 December 2017	January 2018-31 December 2018	January 2019-31 December 2019	2020 Projection
ICP & XRF UNIT	Internal invoicing	860 114	1 045 643	1 005 564	287 802
	External invoicing	2 230 688	2 759 674	2 366 846	1 343 125
	Total income	3 090 802	3 805 317	3 372 410	1 630 927
	Expenses				
	Salaries	1 995 338	2 417 316	2 709 331	2 067 713
	Running costs	727 564	857 977	1 005 950	711 867
	Maintenance	216 324	539 500	1 156 984	129 609
	Travel costs	20 225	77 034	62 089	9 182
	Small equipment & KKW	116 450	29 597	66 476	
	Deferred costs			354 613	354 613
	Total expenses	3 075 902	3 921 424	5 355 442	3 272 984
DNA UNIT	Internal invoicing	3 805 695	4 690 289	3 774 647	2 559 571
	External invoicing	4 830 122	6 259 800	5 752 054	3 135 614
	Total income	8 635 818	10 950 090	9 526 701	5 695 184
	Expenses				
	Salaries	2 440 238	2 986 764	3 089 240	3 447 762
	Running costs	4 445 734	6 669 796	5 604 611	4 532 327
	Maintenance	317 250	255 726	175 405	43 354
	Travel costs	2 780	774	831	
	Small equipment & KKW	94 901		51 228	9 842
	Deferred costs			133 333	133 333
	Total expenses	7 300 903	9 913 060	9 054 648	8 166 618
NMR UNIT	Internal invoicing	656 004	697 665	660 625	331 547
	External invoicing	967 805	641 179	910 628	1 127 233
	Total income	1 623 809	1 338 844	1 571 254	1 458 780
	Expenses				
	Salaries	1 149 123	1 342 756	1 429 138	1 504 081
	Running costs	359 470	383 393	517 358	424 032
	Maintenance	7 377	12 678	48 897	
	Travel costs			2 911	
	Small equipment & KKW	33 576			
	Deferred costs				
	Total expenses	1 549 546	1 738 826	1 998 304	1 928 113
CT UNIT	Internal invoicing	528 663	663 253	490 600	155 110
	External invoicing	1 886 564	2 764 088	1 551 760	2 846 986
	Total income	2 415 226	3 427 341	2 042 360	3 002 096
	Expenses				
	Salaries	1 147 982	1 563 400	1 646 964	1 258 795
	Running costs	359 553	408 092	277 121	1 207 896
	Maintenance	317 000	313 044	565 225	476 283
	Travel costs	75 088	24 491	58 676	
	Small equipment & KKW	64 287	42 057		4 513
	Deferred costs			341 108	341 108
	Total expenses	1 963 910	2 351 084	2 889 094	3 288 595

		January 2017-31 December 2017	January 2018-31 December 2018	January 2019-31 December 2019	2020 Projection
NEUROMECHANICS UNIT	Internal invoicing	494 473	569 253	323 158	283 315
	External invoicing	702 577	826 252	1 069 544	1 052 862
	Total income	1 197 050	1 395 504	1 392 703	1 336 177
	Expenses				
	Salaries	1 225 596	1 475 937	2 060 312	2 149 738
	Running costs	179 398	46 213	70 248	62 769
	Maintenance		66 010	43 315	
	Travel costs	34 680	15 589	72 581	
	Small equipment & KKW	25 713	55 196	48 070	51 969
	Deferred costs			68 711	68 711
	Total expenses	1 465 387	1 658 945	2 363 237	2 333 187
VIBRATIONAL SPECTROSCOPY UNIT	Internal invoicing		57 175	104 529	114 950
	External invoicing		18 264	44 949	52 500
	Total income		75 439	149 478	167 450
	Expenses				
	Salaries	33 924	407 321	595 708	800 559
	Running costs		7 636	7 824	1 616
	Maintenance				
	Travel costs				
	Small equipment & KKW				
	Deferred costs			25 008	25 008
	Total expenses	33 924	414 957	628 540	827 183
TOTAL UNITS INCOME					
	Total internal income	10 383 891	11 974 346	10 027 336	6 094 158
	Total external income	17 672 923	20 599 056	19 420 912	15 965 388
	Total Income: All Units	28 056 814	32 573 402	29 448 248	22 059 547
ADDITIONAL INCOME					
	Interest received	1 050 629	465 843	1 511 454	187 876
	Funds received VR(R)	750 000	750 000	750 000	750 000
	Salary contribution VR(R)	3 596 548	3 952 335	4 203 342	4 422 522
	Infrastructure NII repayment			2 000 000	
	US loan / ALT 2020 Funds: Detector CT			2 321 000	
	VAT refund on equipment	128 910		94 451	
	TOTAL ADDITIONAL INCOME	5 526 087	5 168 178	10 880 247	5 360 398
TOTAL INCOME		33 582 901	37 741 580	40 328 495	27 419 945

		January 2017-31 December 2017	January 2018-31 December 2018	January 2019-31 December 2019	2020 Projection
EXPENDITURE	TOTAL EXPENDITURE				
	Salaries				
	Salaries: Admin	1 827 860	1 983 822	2 184 381	2 336 644
	Salaries: Units	13 388 130	16 892 583	18 347 259	18 181 233
	Salaries: Bonus	366 750	299 326		
	17% / 20% ICRR (Indirect cost recovery)	3 004 397	3 501 840	3 884 182	3 193 078
	Running costs (sum of units)	7 397 578	9 790 017	9 143 314	8 207 608
	Maintanance (sum of units)	2 099 649	2 132 866	3 048 251	1 134 806
	Travel costs (sum of units)	202 930	198 342	209 513	12 655
	Small equipment & KKW (sum of units)	451 104	411 566	372 761	66 324
	Deferred costs (sum of units)			1 448 573	1 448 573
	CAF general running costs	748 646	674 184	592 964	182 119
	CAF-funded post-graduate students			342 663	350 000
	Travel costs - courier	77 797	80 034	89 313	56 891
	Development of new labs	415 719		1 121 212	
	Infrastructure	92 912	29 989	115 217	11 804
	Infrastructure NII	2 000 000			
	Equipment	904 483	608 733	27 648	36 813
	Equipment repair: CT Detector			2 344 334	
	Equipment repair fund	500 000	500 000		
	CAF vehicle fund	45 000	45 000		
	Loan VR(R)				
	Total normal operational costs	33 522 955	37 148 302	43 271 586	35 218 547
	Surplus per year	59 946	593 278	-2 943 091	-7 798 602
	CAF facility				10 000 000
EQUIPMENT EXPENDITURE					
	NRF-NEP total grants	10 237 142		23 982 455	
	ALT/US funds	5 127 016		8 000 000	
	Departments, faculties, VR(R) contributions				
	Loan: 2020 ALT			2 643 935	
	Contributions: Faculty of Science			500 000	
	CAF contribution	163 810		871 213	
	TOTAL EQUIPMENT INCOME	15 527 968		35 997 603	

		January 2017-31 December 2017	January 2018-31 December 2018	January 2019-31 December 2019	2020 Projection
NEP EQUIPMENT DETAILS					
	Integrated real-time neurophysiological and biomechanical analysis system				
	Capillary Sequencer				
	Waters Ultra Performance Convergence Chromatograph (UPC2) connected to a Waters Xevo TQ-S MS				
	BD FACSMelody Cell sorter	7 380 393			
	LabScanner, Prediktera Software and Via-Spec transmission access	8 147 575			
	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	
	TOTAL NEP EQUIPMENT	15 527 968		35 997 601	

FUNDS					
	Emergency equipment repair fund	1 353 701	1 582 635	1 688 915	1 500 000
	Vehicle replacement	108 464	160 930	239 363	240 000
	Food security project	1 188 095	1 201 041	1 214 773	1 215 000
	Maintenance fund equipment: BD FACS Jazz sorter (2013)	1 250 413	1 185 280	1 214 029	1 220 000
	Deffered costs			1 448 573	2 897 146

		January 2017-31 December 2017	January 2018-31 December 2018	January 2019-31 December 2019	2020 Projection
CAF UNITS: Financially ring-fenced DSI funded research infrastructure platform nodes					
NII UNIT	Start-up funding			38 697 186	5 317 420
	Interest received			4 287 051	296 819
	Income				1 065 365
	Private patients				1 078 000
	Total income			42 984 237	7 757 603
	Expenses				
	Salaries & running costs			2 033 955	6 366 108
	Building & equipment			32 044 619	3 113 933
	Total expenses			34 078 574	9 480 041
	Year end balance			8 905 663	7 183 225
BIOGRIP	NODE funding			5 842 139	7 480 163
	Interest received			36 598	
	Internal invoicing				159 000
	External invoicing				206 700
	Total income			5 878 737	7 845 863
	Expenses				
	Salaries & running costs			480 549	2 965 173
	ICR (indirect cost recovery)			292 107	374 008
	Equipment			5 106 081	4 506 682
	Total expenses			5 878 737	7 845 863
	Year end balance			0	0

Graphs detailing aspects of CAF income during 2019

Figure 24: 2019 percentage of income derived from the four main categories of clients

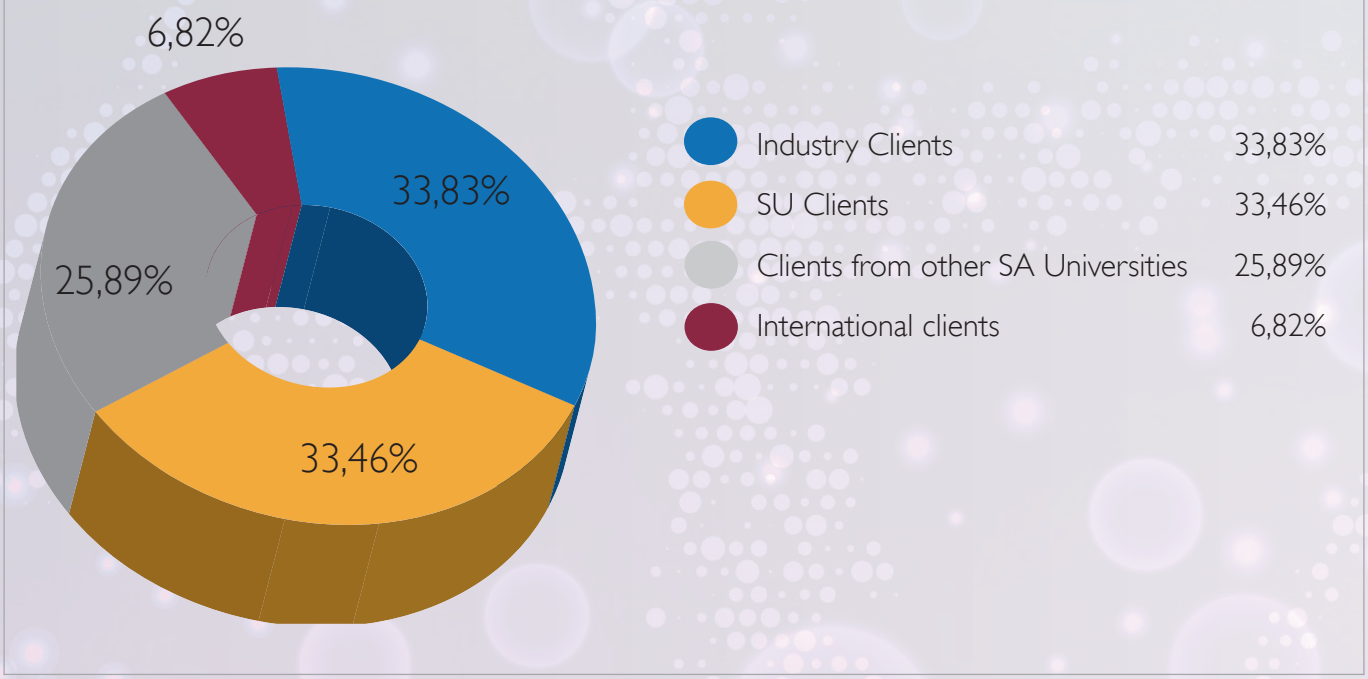


Figure 25: Analysis of percentage of CAF income from internal clients by faculty

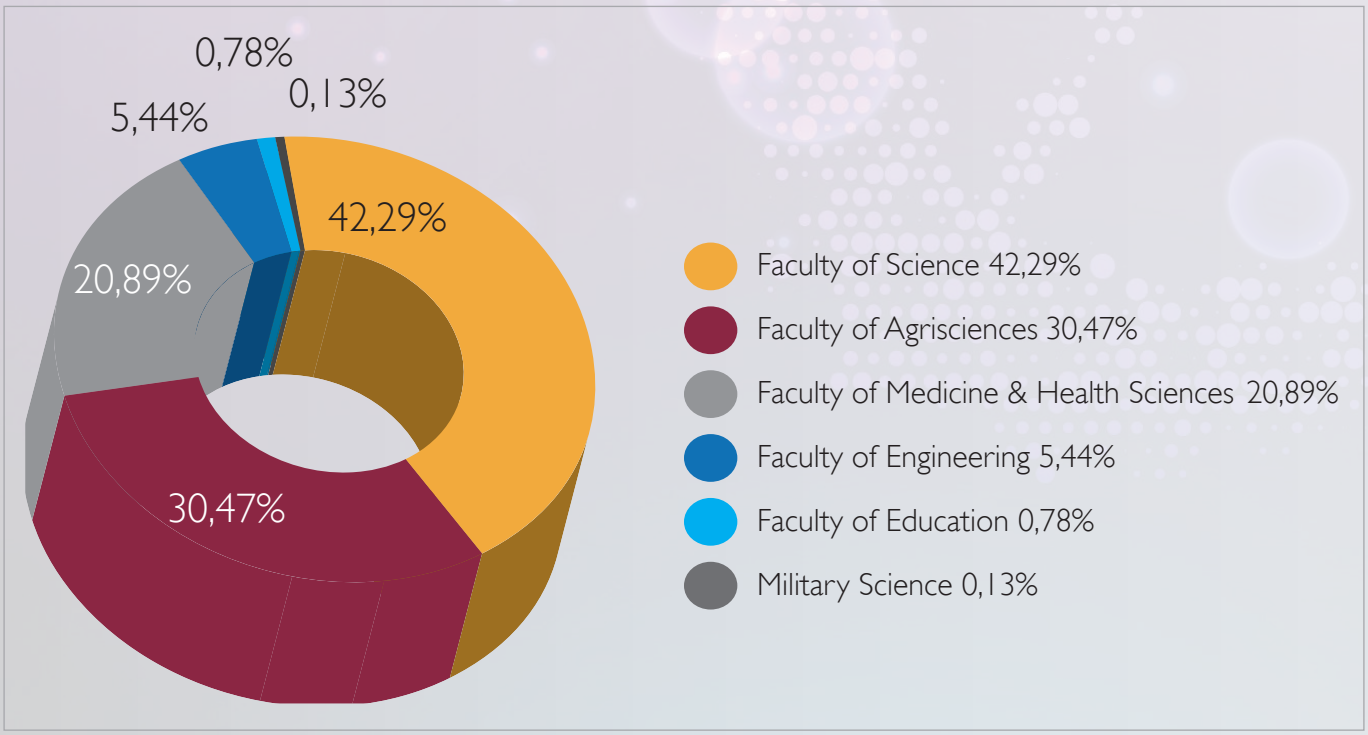
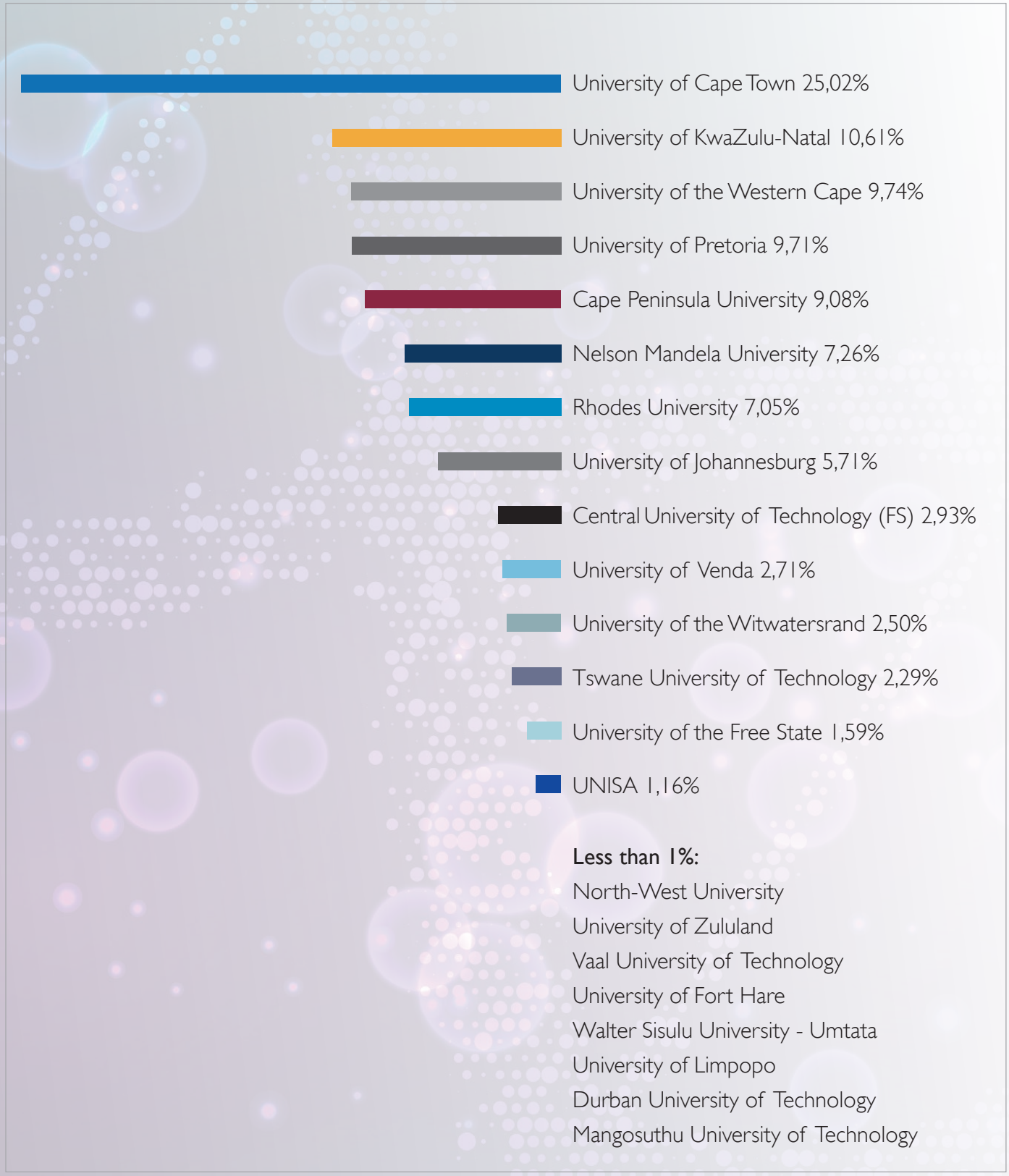


Figure 26: Analysis of CAF income from South African external academic clients by university



Graphical summary of progress towards establishing the two DSI-funded nodes within CAF

Figure 27: Node for Water and Soil Analysis

Goal/Activity	2019											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
DSI approves final BIOGRIP proposal												
Initiated and finalized contract between UCT and DSI												
UCT HUB receives 2019 funding												
Appoint unit manager (Dr J Colling)												
Goal/Activity	**2020**											
	Jan	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
Finalize contract between nodes and HUB												
SU receives BIOGRIP funding (2019 period)												
Rent CSIR laboratory space												
Launch of soil and water unit												
Equipment procurement (P), installation and training (I&T) and Analytical services offered												
- Ion Chromatography system			**P**	**P**				**I & T**	**Anion/cation analysis***			
- H/O isotope analyser			**P**	**P**					**I & T**	**Isotope analysis***		
- General water and soil analytical services								**I & T**		*** & ****		
- C isotope analyser												**P****
- Total Organic Carbon (TOC) analyser												**P****
- Automated UV spectrophotometer												**P****
- Microbial analytical equipment												**P****
Appoint support personnel												

Completed
Projected timeline to initiate and complete activity
Commencement of analytical services

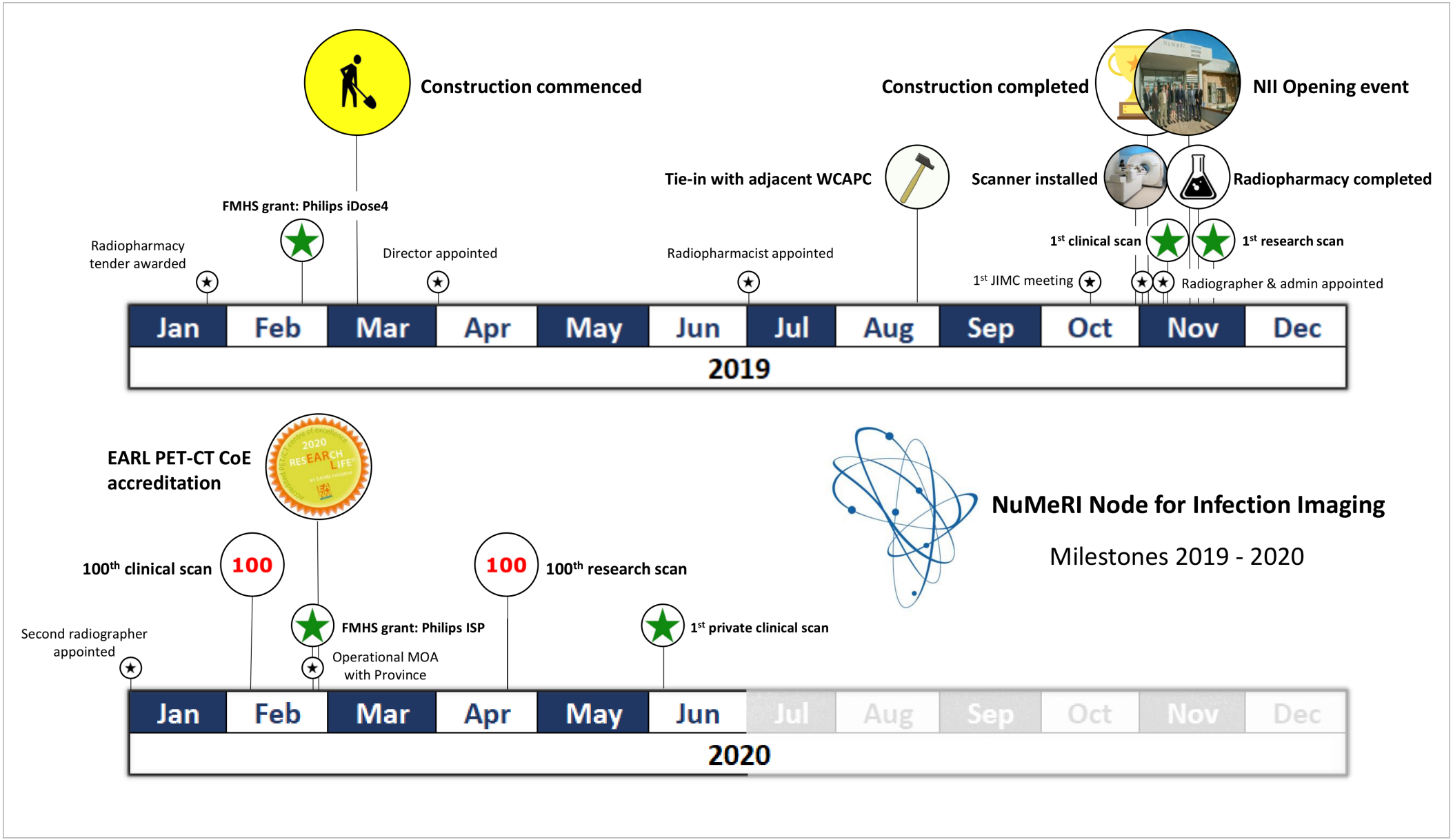
*** Services offered include:**

Anion/Cation quantification (IC)	Cl, F, Br, NO_2, NO_3, PO_4, SO_4, Na, K, Ca, Mg, NH_4
H/O isotope analysis	$\delta^{18}O$, $\delta^{17}O$, δD, ^{17}O-excess
General services	pH, Conductivity, Turbidity

**** Expanded services to be offered includes analysis of:**

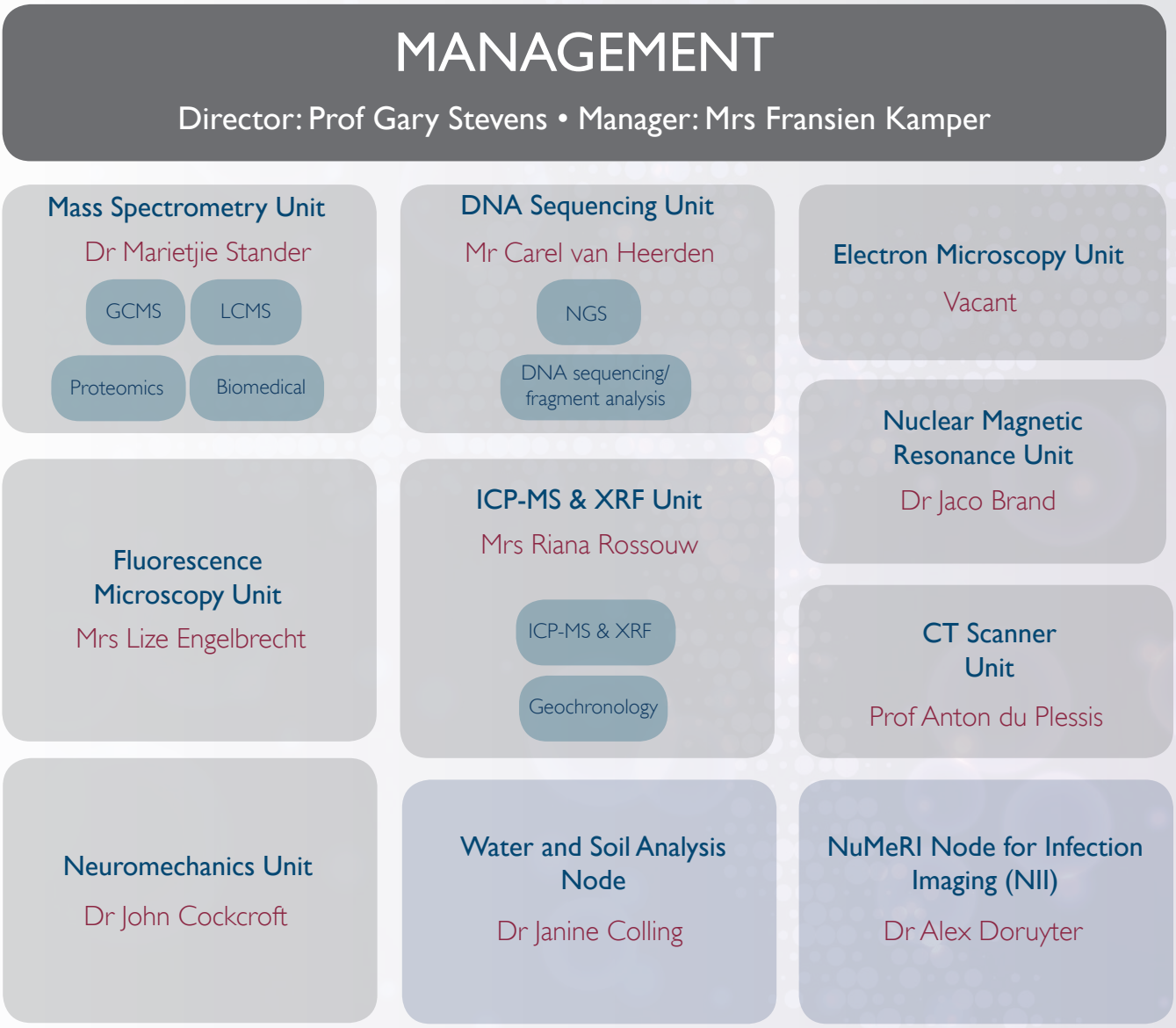
Alkalinity, colour, COD, TOC, TDS, TSS, *E. coli*, total coliforms, $^{13}C/^{12}C$ isotope analysis

Figure 28: NuMeRI Node for Infection Imaging (NII)



CAF structure 2020

Figure 29: CAF structure showing management, units and nodes.



PLEASE NOTE: Names of the unit managers are indicated in maroon and divisions within units are indicated in light blue blocks.

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