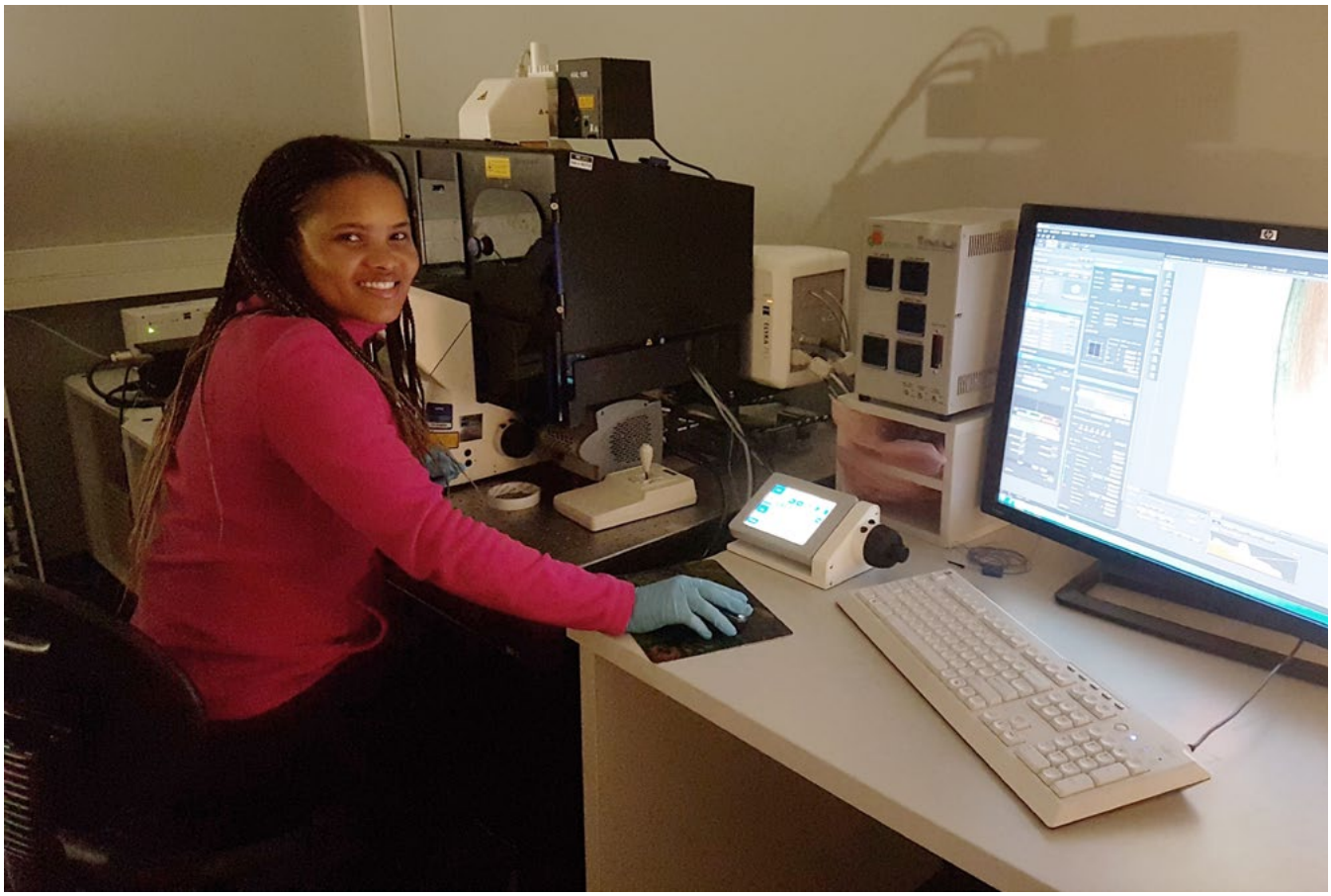
Lighting up what we cannot see

Lize Engelbrecht shares some useful applications of fluorescence microscopy

The quest to visualise the smallest of smallest components of our surroundings, in conditions closest to their natural circumstances, has been key to some of the most astonishing discoveries in the 21st century. It is especially important in the biological and medical fields to see what happens inside cells, bacteria and living organisms, not only to understand the world around us, but also to improve treatment strategies, develop new drugs and increase food quality. And with the drive to implement nanotechnology in several areas of development, the need to visualise things at nanoscale is also becoming essential.

While electron microscopy offers the best resolution – making objects as small as atoms visible – it does not allow visualisation of live cells and requires considerable sample preparation. Another technique, called fluorescence microscopy, is growing in popularity because different molecules can be viewed in different colours, often in live conditions. Various types of fluorescence microscopes, ranging from simple epifluorescence microscopes to more complicated designs such as confocal and light-sheet microscopes, are used routinely in research today.

A fluorescence microscope is a specialised type of

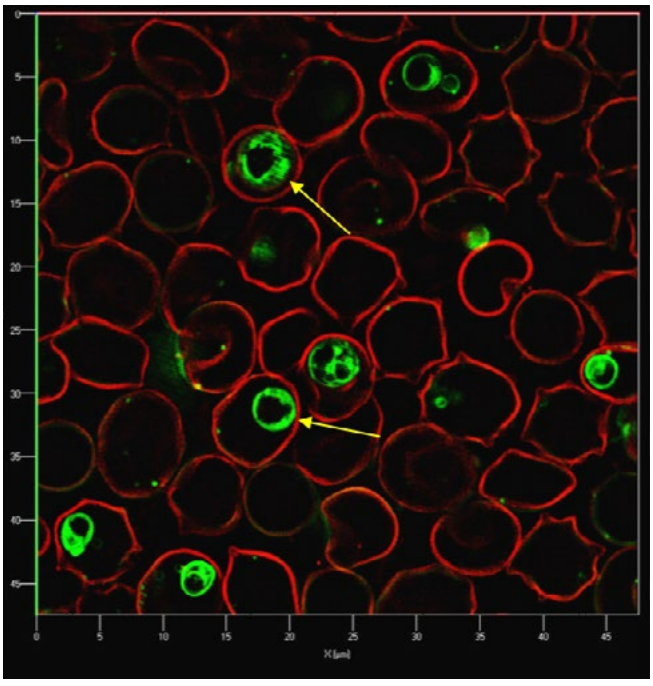


Dumisile Lumkwana, analyst in the CAF Fluorescence Microscopy Unit at Stellenbosch University, acquiring fluorescence images for students on the Zeiss LSM780 ELYRA PS1 confocal super-resolution microscope.

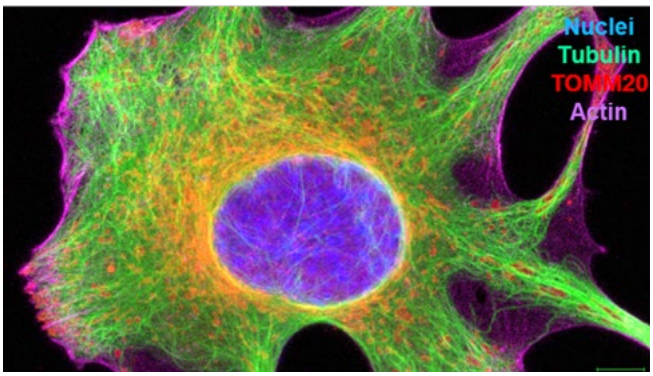
light microscope that uses fluorescence to generate an image. The specimen is illuminated with light of a specific wavelength, which is absorbed by certain molecules in the specimen, causing them to emit light of a different colour than the absorbed light. The illumination light is often from lasers in higher-end microscopes, but could also be from xenon-arc or mercury-arc lamps in the case of epifluorescence microscopes. Sometimes specimens naturally contain these fluorescent molecules ('autofluorescence'), but usually fluorescence molecules, called fluorophores or fluorochromes, need to be added to the samples to label the molecules of interest. In some cases, cells can also be genetically modified to express so-called fluorescence proteins in conjunction with the proteins of interest, to make those proteins visible.

In the Fluorescence Microscopy Unit of the Central Analytical Facilities (CAF) at Stellenbosch University, a state-of-the-art confocal super-resolution microscope, originally funded by the DST National Equipment Programme, is available to researchers, students and industry representatives to view samples from a range of sources.

The staff of the unit, Lize Engelbrecht and Dumisile Lumkwana, have imaged samples ranging from nanoparticles synthesised by polymer chemists to toothbrush bristles for dentistry. They work often with bacteria, yeast, fungi and cell cultures, but many tissue samples from human patients, animals and plants are also brought to the unit to be imaged.



Super-resolution microscopy images of the intra-erythrocytic stage (within red blood cells) of the malarial parasite, *Plasmodium falciparum*, stained with trypan blue (red) and neutral lipid-binding fluorescent dye LipidTOX (green). *© Adrienne Leussa*



Neuronal cells labelled to show the actin filament network (purple), the tubulin network (green), the mitochondrial network (red) and the cell nucleus (blue). The cytoskeleton and mitochondrial network of these cells are often compromised in Alzheimer's disease. *© Dumisile Lumkwana*

Fluorescence microscopy for a healthier society

Globally, the main use of fluorescence microscopy is for medical research. This is also the case at the CAF Fluorescence Microscopy Unit. Some of the main diseases investigated by the users of the laboratory are tuberculosis, malaria, Alzheimer's disease, diabetes mellitus and cancer.

Tuberculosis

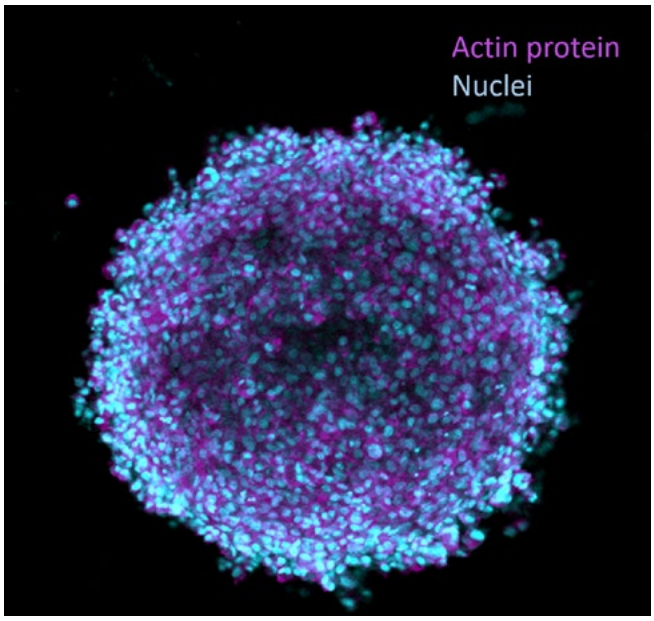
Various studies on the bacterium causing tuberculosis, *Mycobacterium tuberculosis*, incorporate confocal microscopy. Novel genes found in the TB bacteria can be deliberately modified to investigate the function of the proteins they produce. The only way to make these proteins visible for microscopy would be to label them with fluorescence, or to insert the genetic material for fluorescent proteins – such as mCherry (red) or Green Fluorescent Protein (GFP, green) – amongst these genes. The effects of treatment strategies on the bacterium itself can also be visualised, and diagnostic devices tested.

Malaria

The infection of red blood cells with malaria has also been investigated in the unit. Malaria has different stages, called ring stage, trophozoite stage and schizont stage, after which the red blood cells burst open and release merozoites that will infect new red blood cells. These stages can easily be visualised with confocal microscopy.

Alzheimer's disease

Members of the Neuro Research Group at Stellenbosch University, headed by Professor Ben Loos, use cell cultures to study the causes of Alzheimer's disease and to find better treatments to slow down disease progression. In affected people, cell degeneration occurs in parts of the brain,



A spheroid of cells representing a glioma – a tumour found in the brain or spinal cord – cultured for 72 hours. *© Jurgen Kriel*

eventually destroying memory and other important mental functions. The disease is caused by certain proteins malfunctioning, so they form plaques in the brain. Inside cells, the mitochondrial network and stability of the cytoskeleton are compromised. The cytoskeleton is made of filamentous proteins, including tubulin and actin, and these are being investigated at the Fluorescence Microscopy Unit. Brain tissue is also being studied, using a protocol called 'clarity' to make samples translucent enough for light to pass through, followed by fluorescent labelling of only the interesting structures, such as blood vessels or neurons. This could increase insight into the disruption of the neuronal network in Alzheimer's patients.

Diabetes mellitus

Blood clotting is an important process in the human body, but in people with diabetes mellitus there is a higher tendency for blood clotting to a dangerous level. Professor Resia Pretorius and her physiology students at Stellenbosch University study the nature of blood clotting in diabetes mellitus and other conditions, including Alzheimer's disease, Parkinson's disease and pregnancy. They also endeavour to find treatments that would prevent excessive blood clotting. Certain molecules in these blood clots can be labelled with fluorescent markers, enabling the researchers to study the proteins in the clots, as well as the shape and size of the clots.

Cancer

Many cancer researchers are now growing cancer tumours in 3D cultures, called spheroid cultures, to investigate their properties in more natural conditions. By staining certain components of the spheroids, one can investigate the functionality of the tumour in real time, and also determine the efficacy of different cancer treatments.

Fluorescence microscopy for sport sciences

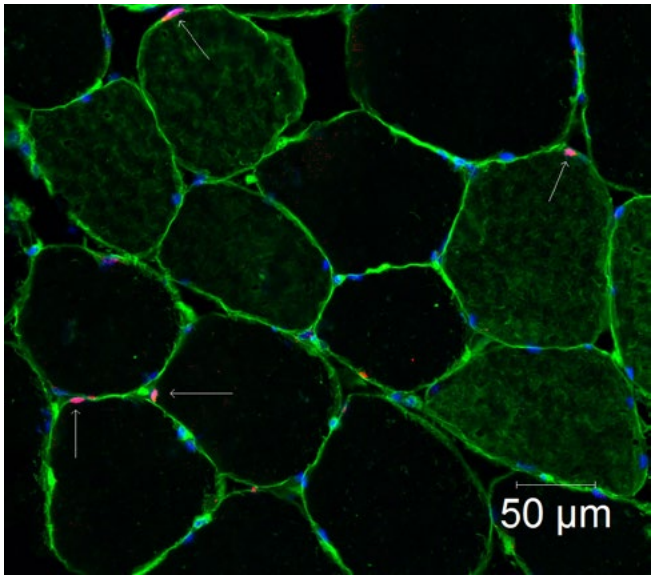
Even for sport-related research, fluorescence microscopy provides some insight, especially when studying inflammation, the regeneration of muscle after injury, and the connection between the nervous system and muscle.

Inflammation

After injury there is an invasion of inflammatory cells, which can be labelled for their specific markers. Scientists then use microscopy to follow the progress and clearing of the inflammation over time and in different conditions, especially in studies on antioxidants or anti-inflammatory medicine.

Regeneration of muscle after injury

A group of physiologists led by Professor Kathy Myburgh is using confocal microscopy in investigating the regeneration of muscle after injury. Satellite cells are small cells found amongst muscle fibres that quickly proliferate through division and move to an injury site to repair damaged fibres. They do this by fusing to the muscle or to each other to make new fibres, but there is a race between this repair process and the formation of scar tissue, which would ultimately determine how fast and how well a sportsperson could recover from such an injury. The researchers study these satellite cells to determine the conditions under which they

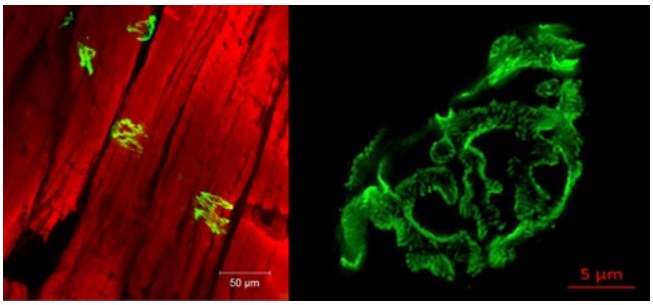


A cross-section through muscle fibres, with many nuclei (blue) localised on the periphery of each fibre (green). Small satellite cells (red) are 'on standby' to repair damage to muscle fibres after an injury. *© Cameron Sugden*

would best be able to repair the damage. Another group is attempting to develop a cellular shuttle, using inflammatory cells called macrophages as vehicles. The confocal microscope is used for long time-lapse studies to investigate the capability of macrophages to engulf other particles or cells in order to offload these contents elsewhere in the body. Perhaps one day this method could be used as a stem cell therapy to enhance recovery from injury.

Connection between neurons and muscle

The neuromuscular junction – the place on the muscle fibre where an impulse from the nervous system is transferred to the muscle – can be labelled with fluorescent-labelled bungarotoxin. This toxin is found in the venom of the Taiwanese krait snake, and usually binds to molecules of the neuromuscular junction to paralyse the snake's victim. By investigating changes in the structure of the neuromuscular junction during disease or injury, better recovery strategies and medicine can be developed.



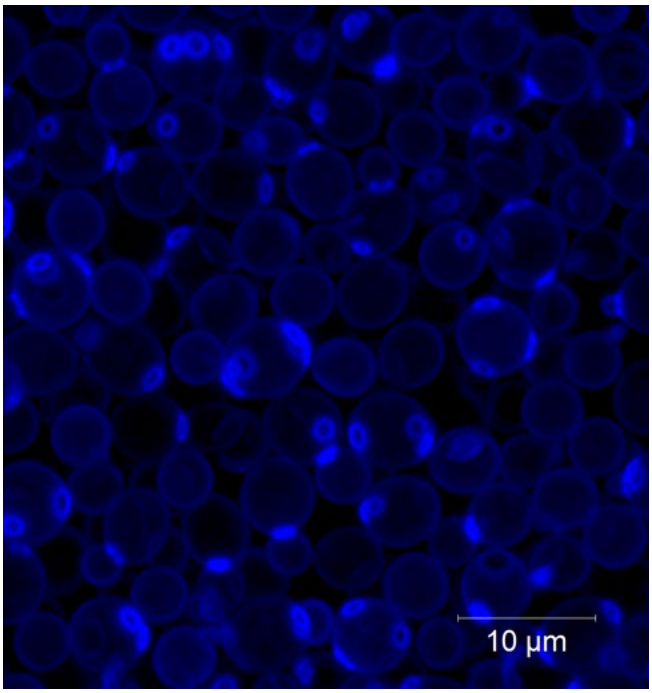
Neuromuscular junctions on muscle fibres are labelled using toxins in snake venom, tagged with green fluorescent molecules. The actin (protein) filaments of the muscle fibres are labelled using a toxin from mushrooms, tagged with red fluorescent molecules. *© Lize Engelbrecht*

Fluorescence microscopy for the market

Fluorescence microscopy is also useful for many projects conducted at Stellenbosch University to improve products for human consumption or other household use. Natural processes, such as the pollination of honeybush flowers, have been studied too.

Wine yeast

Students supervised by Professor Florian Bauer from the Institute for Wine Biotechnology use confocal microscopy to investigate wine yeast, which is important for the fermentation process. In a study to reduce wine protein haze, they found that using yeast cells with high levels of chitin in their cell walls resulted in much clearer wine. This was because the cells were binding grape chitinases, the enzymes believed to be the major proteinaceous contributor to haze formation. The researchers therefore proposed that yeast cells with high chitin levels should be investigated for use as clarifying agents.



Wine yeast cells contain chitin in their cell walls, specifically in the region of the bud scar, where cell division previously occurred. Increased levels of chitin in the cell wall are reported to reduce wine haze. *© Thulile Ndlovu*

Effect of oxidative stress on fruit quality

Fluorescent markers for oxidative stress have been used in the unit to assess storage conditions for apples that would prevent loss of quality, and also to determine whether the position of a bunch of grapes on the vine could cause different levels of oxidative stress. Similar studies have been performed on pomegranates to investigate oxidative stress after the fruit have fallen from different heights on the tree.

Wax and oily products

Another application of fluorescence microscopy is the labelling of waxy or oily types of molecules. A substance called Nile Red, for example, can be used to measure the thickness and condition of the wax layer of fruits such as pomegranates or nectarines. The dye can even be used to test products such as Vaseline or other skincare creams. In one such study, different creams were used on skin biopsies and the depth of penetration into the skin was visualised with the confocal microscope.

For more information on any of these projects, or for enquiries about fluorescence microscopy at Stellenbosch University, please contact Ms Lize Engelbrecht (lizeb@sun.ac.za, 021 808 9327, www.sun.ac.za/caf). Training and support for research projects can be provided, as well as method development in more advanced microscopy techniques.

Lize Engelbrecht completed her MSc: Physiological Sciences at Stellenbosch University in 2012, and is now Manager of the Fluorescence Microscopy Unit.