Dr Bob Asselbergh has won third prize in the Olympus Image of the Year Award 2017. His stunning image of a segmented overlay of a mouse sciatic nerve provides a glimpse of art in the field of image quantification.

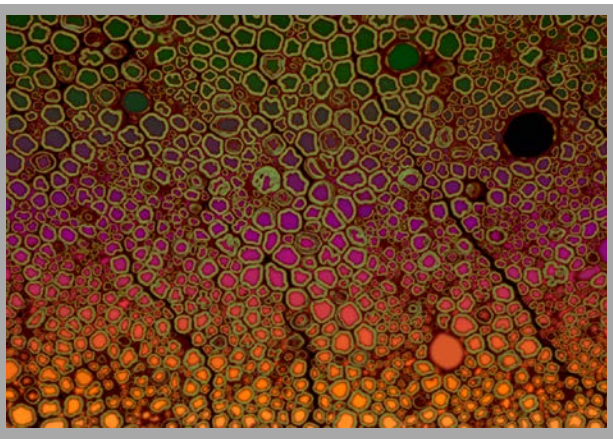
“Even if you have beautiful microscopy pictures,” Bob says, “what ends up in a paper is usually just a very small image – or the picture doesn’t get included at all.”

In his role as a research associate at the VIB at the University of Antwerp, Bob has used his expertise in microscopy and imaging to contribute to a large number of different research projects.

The image

Bob took his award-winning image as part of a study into nerve degeneration. “The image comes from a mouse model for peripheral neuropathy,” Bob says. “The mouse has a mutation, which is also present in humans where it causes Charcot–Marie–Tooth disease (CMT). In this mouse model we used histology to look at the sciatic nerves of the mouse. In the image you can see a cross section of these nerves stained with toluidine blue. It’s a normal light microscopy stain, from which I could measure the diameter of individual axons.”

What gave this image its striking colours was the automated process of segmentation. Using an ImageJ script designed to recognise individual axons, Bob was able to measure hundreds of axon diameters per image in a short space of time. With these measurements, Bob and his team have been able to generate powerful data to demonstrate small changes in average axon diameter.



One of the effects of nerve degeneration is that the average axon diameter drops. Automated image analysis techniques measure the number and size of individual axons on microscopy images. The software also gives the image its colour gradient.

As part of the segmentation, each axon gets a unique value. Bob explains: “By applying a different colour to each value you get a new image with this beautiful gradient effect. The final image is just an overlay – a merge of the colours and the original image.”

Taking the image

The VIB-UAntwerp Center for Molecular Neurology offers a range of microscopy support to different research groups. Bob comments: “In-house we have fluorescence, live-cell imaging, laser scanning confocal microscopes as well as more simple research microscopes. We also use high-content imaging, spinning disk, super resolution and electron microscopes from the microscopy facilities of the University of Antwerp and from other VIB centres across the country.”

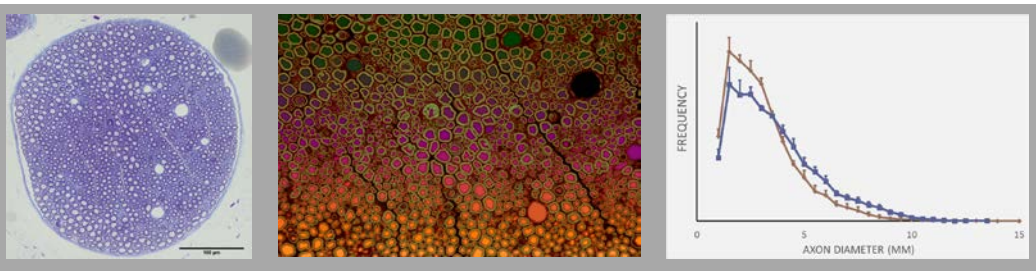
For his Image of the Year entry, Bob used the Olympus UC30 colour camera. Bob says: “It’s a good camera, the quality of the image is great. The sections we looked at are sometimes pretty large, so I took overlapping images with a manual stage and I stitched them together – but I’m very happy with the quality of the camera and with the images it produces.”

The science behind the image

The quantification of both the number and the diameter of axons provides two important parameters of research into neuropathy. Bob explains: “We found that our mutant mice have an age-dependent phenotype that resembles humans with CMT. The average axon diameter is often lower in mice with nerve degeneration, making it a suitable parameter for studying and quantifying nerve damage based on histological sections.”

“Patients with CMT,” Bob continues, “can have muscle atrophy caused by the degeneration of peripheral axons. Again, the same thing happens in the mice – they show some muscle weakness when they get older. We also see similar electrophysiological deficits in mice and in humans.”

To obtain reliable data, it was necessary to study a large number of samples and animals. Automated segmentation and analysis was essential to measure the diameter of each axon in each of the images. The results could then be plotted to summarise the measurements of many different cross sections in a clear, easy-to-understand way.



From picture to paper: A cross section of a bundle of axons (left), colours added by the segmentation software (middle) and a quantification such as the one used for publication[1]

With this quantitative information about the impact of nerve degeneration at the axon level, the question is: What can be done about CMT? Bob explains: “Research with this mouse model is continuing and it is necessary to develop a hypothesis on what could reverse nerve degeneration. The research group of

Professor Vincent Timmerman, who is leading this study, is currently running experiments to investigate the use of drugs to counter this axonal degeneration."

About Bob Asselbergh

Bob began his research career at Ghent University, graduating in 2004 with a degree in Bioscience Engineering. By studying plant–pathogen interactions during his PhD research, Bob first came into contact with microscopes and after graduating he went on to work for the microscopy core facility of the VIB in Ghent. A few years later he moved to the VIB in Antwerp to use his expertise in imaging and microscopy to support multiple research projects and provide training and assistance on all aspects of microscopy within the centre.

Reference

1. Bouhy D et al. A knock-in/knock-out mouse model of HSPB8-associated distal hereditary motor neuropathy and myopathy reveals toxic gain-of-function of mutant Hspb8. Acta Neuropathologica 2018;135(1): 131–148.

More information: www.olympus.eu/imageoftheyear