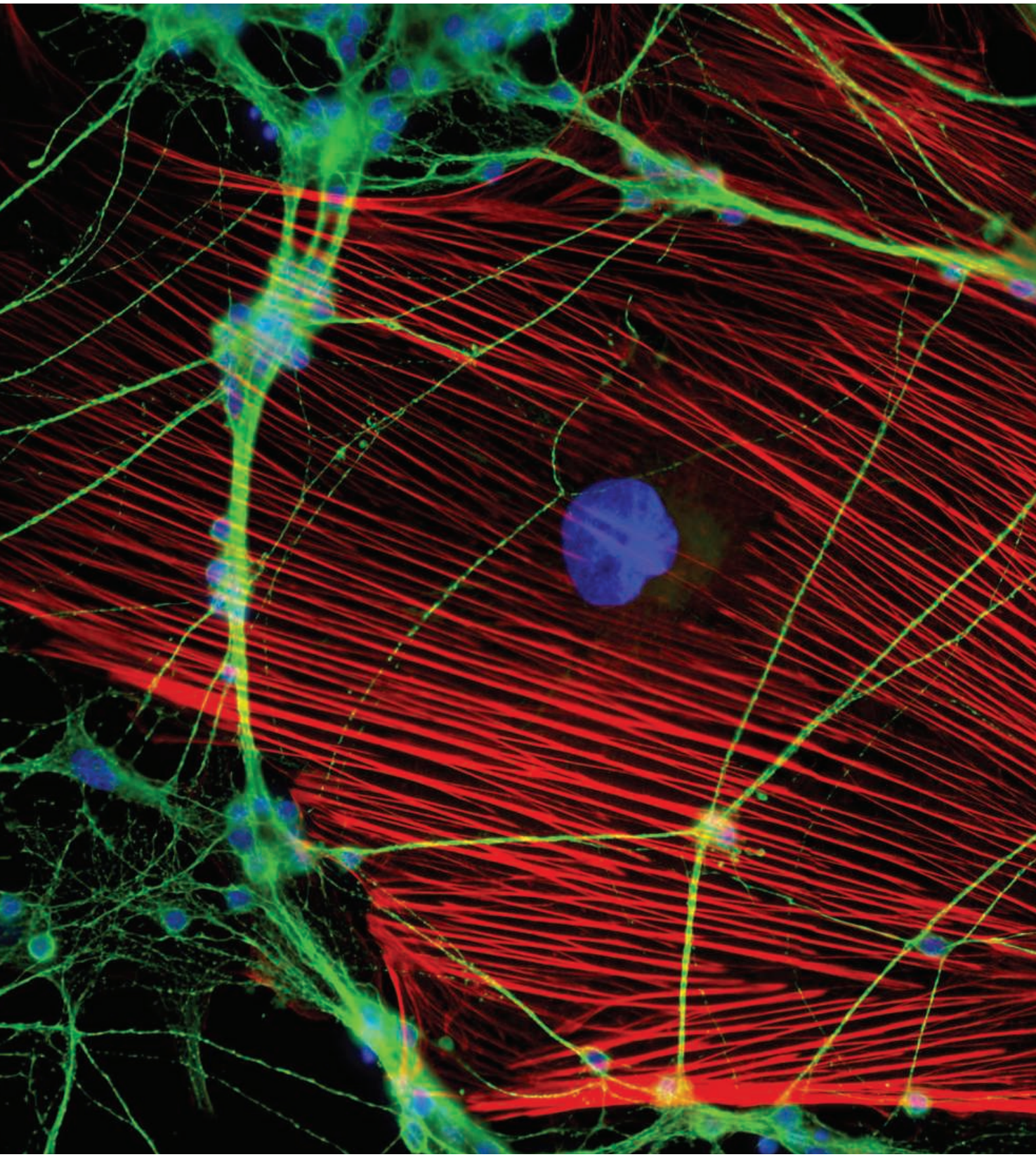
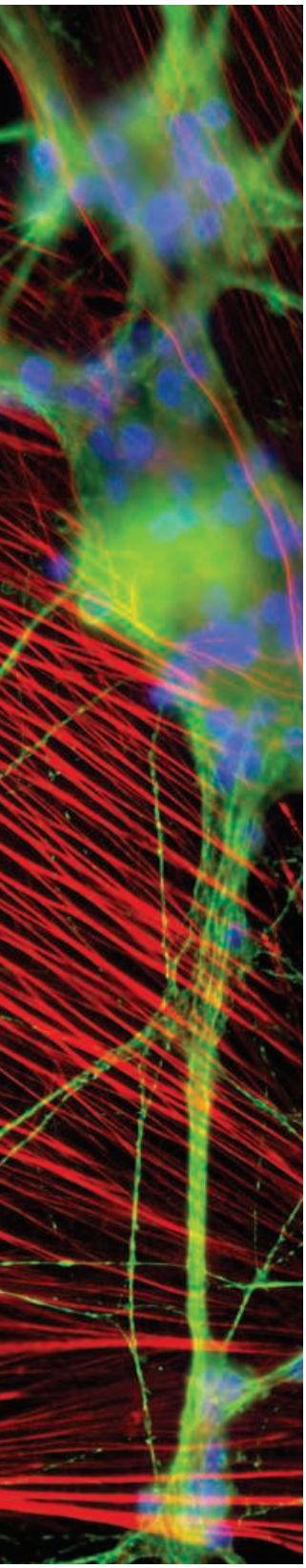




A BIOLOGICAL **REVOLUTION**

The molecular mechanisms of cells and organisms can now be viewed in unprecedented depth and detail thanks to recent advances in super-resolution microscopy. Burning biological questions about how diseases occur and much more can now be resolutely tackled.

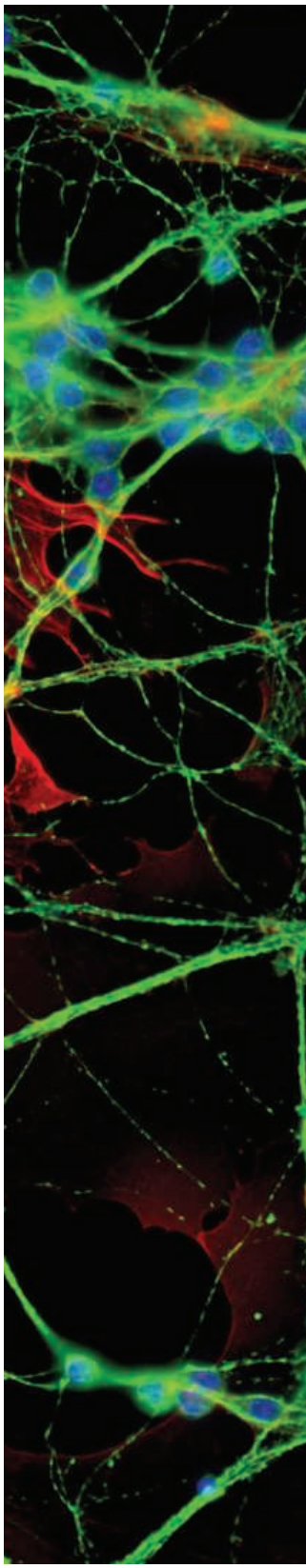
The debilitating misery of Parkinson's and the profound anguish relating to Alzheimer's disease could soon be mitigated, or even eliminated, thanks to the unified efforts of experts across several research disciplines and dramatic leaps forward in the field of microscopy.

Super-resolution microscopy is a new technology that enables scientists to view biological matter down to an unprecedented level and in razor sharp resolution. Zooming in on molecules and whole living organisms, scientists can now address questions that were inconceivable less than a decade ago.

Whereas previously it was not possible to see anything smaller than a wavelength of light, new optical super resolution microscopy techniques mean that scientists can observe cells on a nanometre scale. One nanometre is one-billionth of a metre. That's a difficult figure to comprehend, but by comparison, a standard sheet of paper is about 100,000 nanometres thick, while a strand of human DNA is 2.5 nanometres in diameter.

DNA is the building block of the genome that directs the sequences and the structures of proteins. Proteins are the molecular machines of the cells and are one of the key components controlling biological processes. There are thousands of different types within the human body, each with a specific task, location and three-dimensional structure.

Scientists are now able to manipulate DNA and modify proteins and study protein processes at the molecular level. Scientists are interested in visualising protein molecules in living cells to learn about physiology and what causes diseases. To be able to visualise living cells and single proteins the most advanced light microscopes are needed. ›



"Super-resolution microscopes are great research instruments for the fields of biology, medicine and life sciences. We are currently conducting research in the fields of diabetes and Alzheimer's disease"

Professor Theo Lasser,
Head of the Laboratory of Biomedical Optics

Step in super-resolution microscopy – a field that is rapidly expanding, especially since the Nobel Prize in Chemistry was awarded to Eric Betzig, Stefan W. Hell and William E. Moerner in 2014 for surpassing the limitations of the light microscope. The three researchers found a way to see beyond the diffraction of light and enable an enhanced structural resolution in biological and other specimens down to 10 nanometres. The field continues to grow through the alliance of research communities from different fields including chemists, biologists, physicists and engineers.

Professor Clemens Kaminski, from the Cambridge University Department of Chemical Engineering and Biotechnology, explains how super-resolution microscopy has undergone major changes in recent years allowing for the direct visualisation and analysis of subcellular structures in living cells.

"The key work that we've pioneered is to use super-resolution techniques to look at the molecular mechanisms of proteins which go wrong; proteins which start to fold and adopt shapes in which they don't function correctly, and in those shapes, they start to cluster with each other and cause diseases such as Parkinson's disease. For the first time we are able, with the help of these super-resolution methods, to see this process directly not just in the test tube, but even in the cell. And that really opens up for us a completely new way of answering questions about the molecular mechanisms of diseases such as Parkinson's and Alzheimer's disease."

In the analysis of an Alzheimer's sufferer's brain it's possible to observe chains of proteins that make hard fibrous structures but in a conventional microscope the images shown by the wave of light are very limiting. Kaminski describes these kinds of images as a blurred mess. He says: "We cannot differentiate what the molecules look like at all. But with super-resolution, now we are able to see exactly how this process of aggregation, how these molecules grow in size and cause toxic effects that cause brain cells to die – we can see this directly now for the first time."

The field is developing so quickly that the teams of people on the cutting-edge of innovation in the sector cannot wait for commercial instruments to use in their work to become available and so they are creating their own equipment.

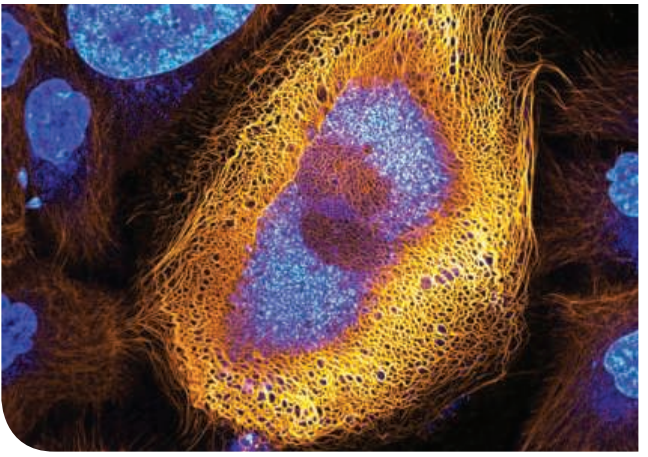
"We have to build our own instruments and we have laboratories and physicists and engineers and biologists and medics who all work side-by-side to make these developments possible," Kaminski says.

Another scientist at the forefront of the field is Professor Theo Lasser, the Head of the Laboratory of Biomedical Optics (LOB) at the École Polytechnique Fédérale de Lausanne (EPFL) – a research institute and university in Lausanne, Switzerland, that specialises in natural sciences and engineering.

Lasser and his team have moved beyond the initial limitations of only spatial resolution and developed a technique that can perform both 3D super-resolution microscopy and fast 3D phase imaging in a single instrument. Phase imaging is a technique that translates the changes in the phase of light caused by cells and their organelles into refractive index maps of the cells themselves.

Above right: Immortalized human skin cells (HaCaT keratinocytes) expressing fluorescently tagged keratin

Below: A super-resolution microscope system



Left: Professor Clemens Kaminski, from the Department of Chemical Engineering and Biotechnology, Cambridge University

The technique combines the sensitivity and high time-resolution of phase imaging with the specificity and high spatial resolution of fluorescence microscopy. The researchers also devised an algorithm to recover the phase information from a stack of images taken by a classical microscope. For the fast 3D imaging, the team custom-designed an image-splitting prism, which allows the simultaneous recording of a stack of eight displaced images. This means that the microscope can perform high-speed 3D phase imaging.

The prism is also used for super-resolution optical fluctuation imaging (SOFI). This method uses fluorescent dyes to improve 3D resolution through correlation analysis of the signal. Using this, the researchers performed 3D super-resolution imaging of stained structures in the cells, and combined it with 3D label-free phase imaging. The two techniques used together revealed images of the inner architecture, cytoskeleton and organelles in living cells across different time points.

"With microscopy and super-resolution, we can see details that we have never been able to see before. Thanks to this technology, the resolution is dramatically improved. We are continuing this journey with our SOFI technology," Lasser says.

Lasser and his team have named this new microscopy platform PRISM – Phase Retrieval Instrument with Super-resolution Microscopy. Lasser explains: "We offer PRISM as a new microscopy tool and anticipate that it will be rapidly used in the life science community to expand the scope for 3D high-speed imaging for biological investigations. We hope that it will become a regular workhorse for neuroscience and biology."

It's clearly a very complex and technical field, but in real terms, how will super-resolution microscopy change our lives?

Lasser explains: "Super-resolution microscopes are great research instruments for the fields of biology, medicine and life sciences. We are currently conducting research in the fields of diabetes and Alzheimer's disease. We even found out that intestinal bacteria facilitate Alzheimer's disease. Today, we can define these bacteria by analysing their DNA profile – the microbiome. Based on the microbiome, we are trying to see whether someone has an increased risk of developing Alzheimer's disease.

"Years ago, one could hardly imagine that this would ever be possible. Today, this DNA reading of the microbiome takes approximately one month. In our research, we can shorten this period to four to six hours, thanks to super-resolution. Based on that knowledge, it is very likely that we can track the traces of other diseases in the near future."

The mechanisms by which protein aggregation contributes to the development and progression of neurodegenerative diseases, such as Parkinson's and Alzheimer's, can now be viewed and tracked by researchers and scientists. This now presents the possibility to develop drugs to inhibit this process.

The ongoing progress is remarkable and it highlights the benefits of cross-pollination and overlapping expertise, as the advances in the super-resolution microscopy field comes from a truly interdisciplinary research community.

Professor Kaminski concludes: "It's a great time to be in this field because so much is happening so quickly and there are lots of questions out there that we still haven't been able to answer. We need to be faster even than we are now. We are only just beginning to imagine what we can do with these techniques and what we have ahead of us is a revolution in biology."