Angelika Rück visualises the dance of molecules

Angelika Rück measures the luminescence time of molecules in order to find out whether proteins are 'speaking' with each other. She hopes that she will soon be able to differentiate inflammation from tumours. Rück, who is head of microscopy at the Ulm-based ILM, has worked hard with her colleagues to make the Eselsberg-based Ulm University location one of southern Germany's leading life cell imaging centres. Rück has the ability to bring (laser) light into the darkness of living cells and, as such, is a sought-after partner of biomedical researchers and endoscope manufacturers.

The chemist's latest success is the establishment of a central research institution in cooperation with the University of Ulm. The one-million-euro "Imaging Center", 50% of which is being financed by the DFG, was actually her own idea. Angelika Rück is known for her ability to sense new trends and cutting-edge issues. The new central institution enables the ILM and the University of Ulm to work together in even better conditions than before in order to initiate new projects, and boost the reputation of the ever-expanding ILM.

Rück never imagined that she would one day be working on decay times, oscillating molecules, and FLIM and SLIM (her two personal developments). She studied physical chemistry at the University of Ulm and did her PhD on the clarification of the structure of a gas-phase molecule using laser spectroscopy. Her interest in laser spectroscopy was the reason why she chose not to work in industry (she was actually offered a job with Hoechst) and decided instead to stay in Ulm.

Where do photosensibilisers best exert their deadly effect?

Induction of apoptosis in some cells (stained blue) during photodynamic therapy.

At the Institute for Laser Technologies in Medicine, as the ILM was formerly called, the young chemist started to focus on photodynamic laser therapy. She was particularly interested in the biochemical mechanism of cell death induced by photosensibilisers. In order to investigate whether a connection existed between cell death and the effect of photosensibilisers, Rück had to use microscopic methods to investigate living cells. Fluorescence detection enabled her to work out the best position of sensibilisers and the absorption spectrum provided her with information on the energy level of these molecules. Photosensibilisers need to reach a specific energy level before they can exert their deadly effect on highly reactive oxygen molecules, explains Rück. She established an optimal phototoxicity profile (ranging from necrosis to apoptosis and including all intermediary apoptotic steps) using laser light. The work that she does now still involves the investigation of these mechanisms using biochemical and molecular biology methods.

Angelika Rück believes she would certainly also have had a great career in industry thanks to her bestselling idea, initially rejected by many, that eventually became a great success. This idea centred around the development of a laser scanning microscope with spectral resolution to detect the fluorescence spectrum of "her" photosensibilisers, something that had been rather difficult and awkward up until then.

A sense for market requirements

Rück's first funding application for a national call failed; and the manufacturers she contacted were initially also quite reluctant. However, she eventually found a regional manufacturer who was willing to back her idea and she submitted a second project proposal. This industrial partner ended up appropriating Rück's project and turning it into an award-winning bestseller. Rück and the ILM received an expensive spectrometer as a kind of consolation for the licensing fees they missed out on. The commercial success of the development also confirmed that Rück's idea had been an excellent one.

The laser scanning microscope with high spectral resolution allows the differentiation of up to eight fluorophores (fluorescent molecules), which is of key importance when dealing with biomedical issues. The entire fluorescence spectrum can be visualised with a special algorithm. Thanks to these fluorescent proteins, spectroscopic tools now allow the detection of protein interactions based on the transfer of energy from one molecule to another.

The ability to monitor protein interactions is of great importance in research into Alzheimer’s disease, for example. The interaction of two proteins plays a big role in the formation of neurodegenerative plaques, which are typical of Alzheimer’s disease. The modulation of these interacting proteins might be a potential strategy for treating the disease. In a recently completed project, Angelika Rück carried out investigations into such processes in cooperation with neurologists from Ulm University. The researchers used spectrally and time-resolved methods to show when and under which conditions two proteins interact. In addition, they developed methods that enable Alzheimer researchers to investigate living cells. In future, these investigations will be continued with animals using more sensitive microscopic methods.

Berlin acquaintance becomes a direct hit

FLIM (“fluorescence lifetime imaging”) of 5-ALA-induced porphyrins measured over a period of 4 to 8 nanoseconds.
© ILM

Angelika Rück first became interested in time-resolved methods when she got to know Wolfgang Becker, head of a small Berlin-based company, whom she met at a congress several years ago. "The best thing that ever happened to me was coming into contact with Becker's small company," said Rück. Becker's company develops and produces devices that measure the decay time of individual photons. This is done by ultrasensitive detectors that measure the photons emitted by a molecule. In the time that has passed since their first meeting, Becker and Rück have become close collaborators.

The measurement of the decay time of fluorescence provides information about the stage that tumour cells have reached and also enables inflammation to be differentiated from tumours. This has not been possible with previous methods, said Angelika Rück explaining the importance of the so-called FLIM method. Molecules in a ground electronic state are excited with laser light (laser diodes) using ultrashort laser pulses in the pico- and femtosecond range. The time a molecule requires to decay into its ground electronic state is referred to as decay time and is in the range of nanoseconds. The decay time is strongly influenced by the molecule's biochemical environment and is the sum of a broad range of processes of different and known duration. The decay time depends on pH or viscosity and enables the detection of molecular interactions and the transfer of energy. The decay time changes when the molecules start to interact with each other, explained Rück.

Diagnostics to differentiate inflammation from tumours

The decay time also differs between tumour cells from different biochemical environments. Based on the finding that such cells have different decay times, Angelika Rück plans to work with colleagues from the Department of Urology at Ulm University Hospital and an industrial partner with the aim of developing new diagnostic tools that might enable them to differentiate tumours from inflammation. While current diagnostic tools used by doctors are unable to provide this information, photodynamic diagnostics has the potential to do so using the 5-ALA (5-aminolevulinic acid) molecule that is frequently used in photodynamic diagnostics. Angelika Rück explains that inflammation generates different derivates from those resulting from tumour-related decay times, and that this difference can be detected.

Rück explains that the combination of time-resolved and spectrally-resolved laser scanning microscopy, i.e. FLIM and SLIM, has already been shown to work well. The next step will involve using this method in clinical settings. The combined method and its algorithms are still very complex, but Rück believes that combined FLIM-SLIM is able to compensate for these disadvantages as it provides “phenomenal” information. Cell culture experiments are currently being carried out in the Department of Urology at the University Hospital Ulm and the investigations will in the future also involve patients. Although FLIM-SLIM involves complex equipment, some endoscope manufacturers have already expressed their interest in the technology, said Angelika Rück.

Raman microscopy with a great future

Representation of different areas in a cell using cluster analysis that is part of spectrally resolved Raman microscopy.
© ILM

Angelika Rück has also since discovered the advantages of Raman microscopy for the research she and the ILM are carrying out. Raman microscopy is a spectroscopic technique that relies on molecular vibrations. The interaction of laser light with molecular vibrations leads to an energy shift between the incident light and the back-scattered light. This energy shift is unique to each molecule and allows the chemical identification of compounds in a sample. Spectrally resolved Raman microscopy can thus be used to visualise the vibration spectrum of individual molecule groups in cells, including proteins, lipids and DNA. Angelika Rück learned about this type of molecular imaging when she attended a seminar held by the company Witec, a Raman microscope specialist from Ulm, and Rück and Witec have since become close cooperation partners. The ILM owns a Raman microscope that differentiates tumours from their vibration bands. This "highly interesting" method is already used in cell diagnostics, but not yet in clinical settings. Rück believes that Raman microscopy, which does not involve foreign molecules, can be applied in many areas. A research project, which also involves the Reutlingen-based NMI, is focusing on detecting different stages of colon cancer using Raman microscopy.

Angelika Rück sees the new Imaging Center as the first of many highlights in her work at the ILM. This new core facility, which she is currently setting up in cooperation with Thomas Wirth, the head of the Institute of Physiological Chemistry at the University of Ulm, will enable researchers to use high-resolution scanning microscopy. The ILM and the University of Ulm will receive two new laser-scanning microscopes equipped with an ultramodern femtosecond laser. Angelika Rück is delighted with the opportunity to carry out two-photon microscopy in the not-too-distant future.

One of the microscopes will be used for cell culture work, and the other for imaging deep tissue structures, for example for investigating mouse brains or CAM membranes. The rooms for the new Imaging Center are currently being renovated and will be ready for the Imaging Center in autumn 2010. Angelika Rück is delighted that the establishment of the Imaging Center will add to the Ulm-based ILM's reputation as a top-class location for life cell imaging in the south of Germany.

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