MULTIPHOTON TOMOGRAPHY

The German PhD student Maria Göppert predicted two-photon effects in Göttingen in 1928. Her first paper was submitted Oct 28 with the title „Über die Wahrscheinlichkeit des Zusammenwirkens zweier Lichtquanten in einem Elementarakt" ("On the probability of two light quantum working together in an elementary act"). In 1930, she finished the PhD thesis under supervision of Max Born, married the American Mayer and published the thesis in 1931 under the name Maria Goeppert-Mayer. She described in these two famous papers the possibility of two photons being absorbed simultaneously at a high concentration of photons in time and space. Maria Goeppert-Mayer became the second female Nobel laureate in physics in 1963. The unit GM for the characterization of two-photon absorption cross sections (1 GM = 10^{-50} cm^4 s $photon^{-1}$) was introduced to honor her prediction of two-photon effects.

The invention of the laser as intense light source in 1960 provided the chance to prove her hypothesis. In 1961, first two-photon excited fluorescence was demonstrated by Kaiser and Garrett in laser exposed europium-doped crystals. Two red photons were simultaneously absorbed and induced a green fluorescence. The pioneer of two-photon effects Wolfgang Kaiser (born July 17, 1925 in Nürnberg) lives in Munich, Germany.

In the seventies, Sheppard and Kompfner suggested to build a non-linear laser microscope. However, it took until 1990 that the first two-photon microscope was build by Denk, Strickler, and Webb. They demonstrated two-photon fluorescence in cells using a single focused laser beam provided by a mode-locked femtosecond dye laser. Soon later, tunable NIR solid state femtosecond lasers based on titanium:sapphire became available. Winfried Denk (born Nov 12, 1957 in Munich) is now professor in Heidelberg, Germany.

The first in vivo two-photon imaging system for clinical use was introduced by König and coworkers of the JenLab GmbH company in Jena, Germany, in 2002 (patent filed in 2000, first publication in 2003. The certified multiphoton tomographs DermaInspect™ and MPTflex™ are mainly used for high-resolution imaging of human skin. Labeling is not required. The resolution is better than 400 nm lateral and 2 µm axial. Applications include melanoma detection, diagnostics of dermatological disorders, cosmetic research, skin ageing measurements as well as in situ drug monitoring. Several thousands of patients have been investigated in European countries, Japan, Australia, and California.

Multiphoton imaging is achieved by focusing femtosecond laser radiation at low picojoule pulse energy into the skin. Intrinsic fluorophores, such as elastin, melanin, flavines and reduced nicotinamide adenine dinucleotide (NADH) can be excited by the two-photon process. The excitation of these biomarkers reveals the morphologic structure of the skin. Additionally, second harmonic generation (SHG) can be induced to detect the collagen network. The most powerful advantage of multiphoton imaging is the ability to provide non-invasively superior optical tissue sectioning. There is no out-of-focus photostress and bleaching.

In 2010, the first certified clinical CARS (Coherent Antistokes Raman Spectroscopy) tomograph was introduced to the market by König and JenLab coworkers. The tomograph provides multiphoton sections based on autofluorescence and SHG as well as chemical fingerprints based on CARS signals from lipids, water, and other non-fluorescent and non-SHG-active intratissue substances.

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