

Introduction

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Since the development of nonlinear laser imaging tools, such as the two-photon technique [1] for example, many technological advancements have been made in the field of microscopy and, more generally, imaging. It took more than 60 years to move from the discovery of the two-photon interaction [2] to its exploitation in microscopy. Since the 1990s, an exponential growth of publications in the field of microscopy (Figure 1) has led to the introduction of the two-photon technique in the laboratories of many researchers worldwide.

Since the first interaction schemes, where all photons were accumulated and collected on the detector after the laser irradiation (integration mode), other kinds of investigation modes have been developed, based, for example, on the lifetime response of the fluorescent molecule (fluorescent lifetime microscopy), on the spectral behavior of fluorescence emission (multispectral two-photon emission), or on the ability of the illuminated molecule to double the frequency of the coherent excitation due to its nonlinear susceptibility (second- and third-harmonic generation microscopy).

Further developments in microscopy have led to other nonlinear interaction schemes such as coherent anti-Stoke Raman spectroscopy (CARS) [3] (Figure 2) and resonant Raman scattering [4].

The nonlinear characteristic of the interaction of pulsed light with a molecule has also led to applications that are useful in increasing the resolution below the diffraction limited barrier [5].

All these imaging tools, together with well-developed photon based technology, such as confocal microscopy, have enlarged the field of applications in biological imaging of molecules, cells, and tissues.

Consequently, the new frontier of cell biology imaging has moved from a fixed cell to a living cell with the advent of the laser and more sensitive wide-field fluorescent microscopes. The advent of confocal microscope has improved the axial resolution, while the application of multiphoton processes has finally permitted the study of cell biology in tissues and, consequently, in living organisms, as well as allowing optical manipulation [6].

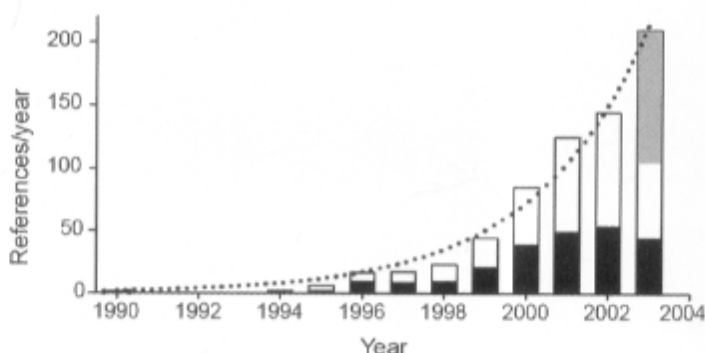


Figure 1 Publications on two-photon microscopes in recent years.

In all cases, a common approach in these methodologies was based on the same concept: labeling the cell with fluorescent probes and observing the topographical changes under some circumstances. Some important characteristics have been measured such as the compartmentalization of some molecules in the cell, their diffusive dynamics, the interaction with other partner molecules, their response to external stimuli, either pharmacological or electrical for example, and so on.

A big step forward has been the study of the nonlinear interaction of light with molecules. The unwanted effect of photobleaching (Figure 3), which generally limits the acquisition time, has turned out to be useful in many applications.

First, a relationship between the photobleaching and the photodamaging energy pathway has shown the possibility of perturbing the chemical state of species by means of a photochemical effect or by creating a plasmon field that perturbs the molecule itself.

A new method of performing cell biology was born: with respect to the "passive" method of "illuminating and observe," the laser has been used for the first time to perturb and observe the reaction of the system.

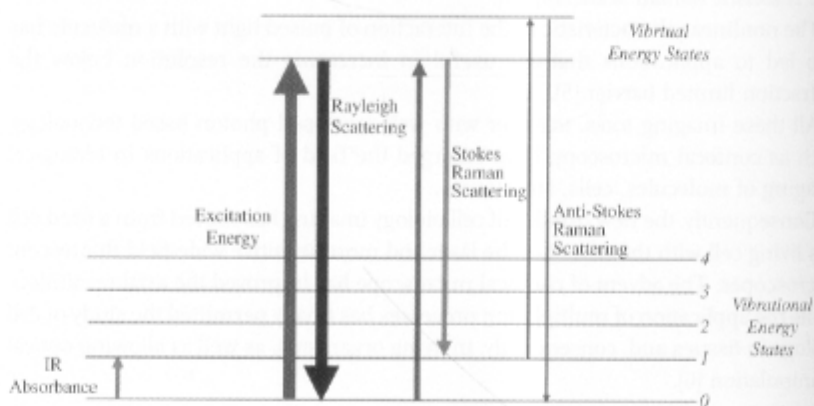


Figure 2 Raman and CARS interaction schemes on molecular energy levels.

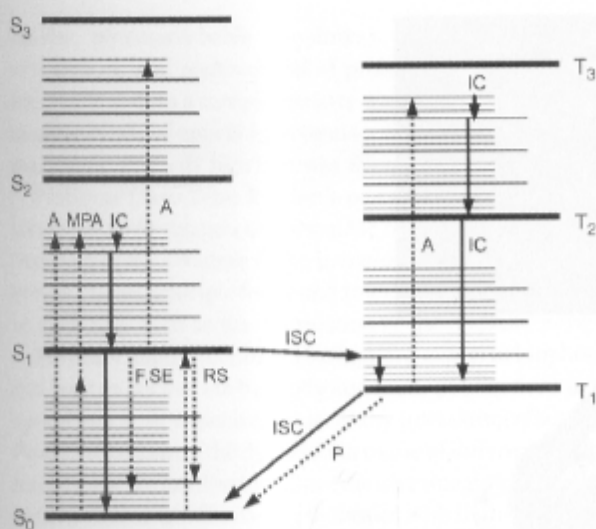


Figure 3 Energy level pathway involved in photobleaching.

This has been applied to single molecule both *in vitro* and in living cells to denaturate molecules, break or move subcellular structures, or create pores into the cell membrane.

The advent of this new cell biology method, a sort of interacting investigation scheme, has led me to gather a team of experts on the topic of "laser imaging and manipulation in cell biology."

My intention is to bring to the attention of the reader a broad panorama of applications from one-photon to multiphoton interaction schemes, from the single living cell *in vitro* to the cell in its physiological environment, like a tissue, where the laser was used to manipulate the sample.

The common aspect bridging all these arguments is based on the "photobiology" of the molecular interaction with light. Even in a tissue, all origins of macroscopic phenomena lie on a molecular base mechanism of interaction with light.

Starting from this consideration, it was easy to present a similar aspect based on a light-molecule interaction mechanism in imaging or manipulation of both cell and tissue.

Following this scheme, the first part of the book is dedicated to the basics of multiphoton imaging and nanoprocessing in cells. The second part focuses on the light-molecule interaction mechanisms. The third part broadens the field of imaging and manipulation from cell to tissue, illustrating tissue imaging applications. Finally, the fourth part describes light manipulation applications on tissues.

The invited authors have made significant contributions to these fields and to applications.

In particular, Professor Karsten Köning has been a pioneer in work on the multiphoton processing of cells [7]. He was in fact one of the first researchers to demonstrate the possibility of using multiphoton interactions to process a cell by

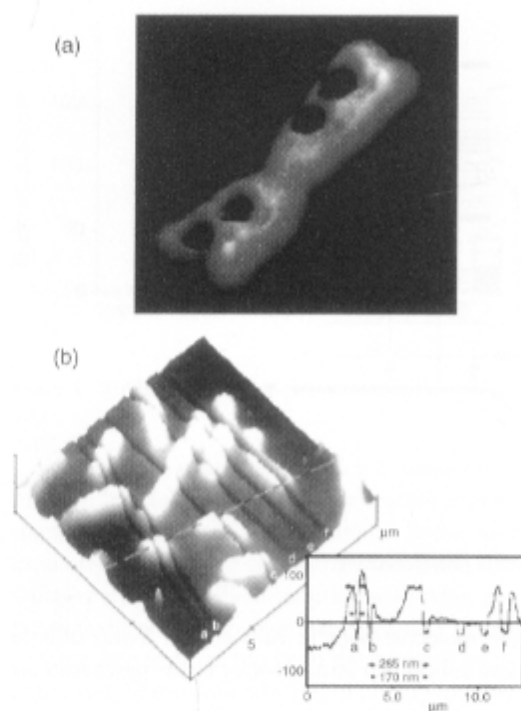


Figure 4 Femtosecond laser nanodissection of chromosomes (see Figure 2.1 and related text).

means of a photochemical effect that creates breaks in structures or pores in the membrane (Figure 4).

This mode of operation opened new kind of powerful applications, such as nano-operation of stem cells which are very useful for transfection protocols.

Dr. Gereon Hüttmann has worked for many years in the field of multiphoton imaging, mostly focusing on tissue imaging for biomedical applications. Particular attention was also devoted in both his group and that of Professor Alfred Vogel, from the same institute, to the study of photochemical effects due to the interaction of laser light with molecules. These effects form the basis of many phenomena described in this book.

Professor Daniel J. Jay has developed a method for chromophore-assisted laser inactivation (CALI) of protein function. His research is focused on proteomics, cell motility, and cancer metastasis, and he is a noted authority on protein inactivation strategies. His contribution to understanding the basis of laser–molecule interaction during optical processing of cell has been essential in revealing the range of applications also in experiments where not only breakage of structures but also selected protein inactivation was involved. This opens the way to many interesting experiments in the optical manipulation of biological processes.

Professor Andrew Woolley has carried out extensive work on photoswitching in recent years. In particular, he has worked on the photon control of peptide confor-

mation, photoswitchable crosslinker, photoswitching photocontrolling peptide area helices, and photocontrol of protein conformation and activity. These experiments allowed, in a complementary way with respect to Daniel J. Jay, the application to other kinds of optical intervention in cell physiology, where the protein was not inactivated while its function was altered.

Professor Claus Peter Richter is one of the most well-known experts in the field of laser optical stimulation of nerves. He has worked in recent years on high-resolution mapping of the cochlear nerve using optical stimulation, revealing optical stimulation as a novel principle for neural interfaces. He has demonstrated applications also in medicine, such as the stimulation of the auditory nerve.

Professor Valery Tuchin is a recognized expert in biophotonics, biomedical optics, and laser medicine. He has authored many publications in the field of the physics of optical and laser measurements. Many interesting studies have been performed by Professor Tuchin on the diffusion in tissue of different substances and nanoparticles. In particular, he has developed an extensive imaging methodology with optical clearing agents, obtaining cell imaging in tissues with higher penetration depth and contrast.

Professor Stefan Andersson Engels' group has carried out research within biomedical and pharmaceutical laser spectroscopy applications, with many studies devoted to light propagation in turbid media. In particular, he has studied the properties of photodynamic therapy (PDT), especially with respect to different photosensitizers. He has applied this research to oncology, with particular attention to the development of biomedical imaging applications devoted to both diagnosis and therapy of tumors.

Dr. Roberto Pini is an expert in the study of light propagation in biological tissues, including laser-tissue interaction and light excitation of organic chromophores for diagnostic and therapeutic purposes. He has also studied the synthesis, characterization, and functionalization of metal nanoparticles for medical use. He has conducted preclinical and clinical studies on the use of lasers and other optoelectronics devices in minimally invasive diagnostics and therapy with applications in ophthalmology, microvascular surgery, neurosurgery, and dermatology.

It is worth noting the development of technology in this field produced by industry. Many advances in technology have been made in the field of microscopy, with new tools for micro-dissection, catapulting, or even nanosurgery for cell transfection.

In parallel, strong chemical advances have been made and new molecular labels have been developed both for tagging purposes and photochemical manipulation of molecules.

Industry has also made notable advances in the field of light sources. New more powerful laser sources or incoherent light sources, like LEDs, have opened up new perspectives for applications. Particular attention to technological development, with respect to laser sources, has been devoted to the emission wavelength, pulse-width duration, repetition rate, and light power.

Regarding the structure of the book, Part 1 introduces the principles of multiphoton imaging, discussing two-photon microscopy and multiphoton tomography. Particular attention is devoted to endogenous cellular fluorophores and second-harmonic generation active biomolecule structures. This is followed by the principles

and mechanism of femtosecond laser nanoprocessing. Such methodologies will be applied to living cells, in particular stem cells, illustrating interesting applications for transfection methodologies.

A description of nanosurgery operation to other living cells and in particular to living animals follows, opening up interesting possibilities for applications in different fields, such as neuroscience.

In Part 2, we investigate light-molecule interaction mechanisms by means of basic photophysics. Particular applications are described, such as chromophore-assisted laser inactivation, photoswitches, and optical activation of neurons.

In Part 3, we move from single cell to tissue by investigating some mechanisms of light-tissue interaction, with particular attention to tissue clearing operation. This kind of bleaching process enables an increase in penetration depth and contrast in imaging the tissue.

Continuing with the theme of tissues, in Part 4 we move from imaging to manipulation methods performed by illumination. In particular, photodynamic therapy is analyzed, starting from its cell biology base. This argument is connected to previous studies at the single cell level, described in the chromophore-assisted light inactivation operation.

Regarding PDT, immunological aspects, photophysical properties, and clinical applications are described.

Finally, and still in Part 4, another light operation on tissues modality is explained: laser welding of tissues, which is depicted with particular attention to the mechanisms of laser welding operation, together with a description of different modalities of operation and examples of clinical use.

In all parts, the cell biology aspect is described and introduced in different environments and contexts. We wish to describe both imaging and manipulation in a cell biology context, demonstrating how the light interaction and operation on complex systems such as tissues can be explained by mechanisms that rely on the cell biology base.

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