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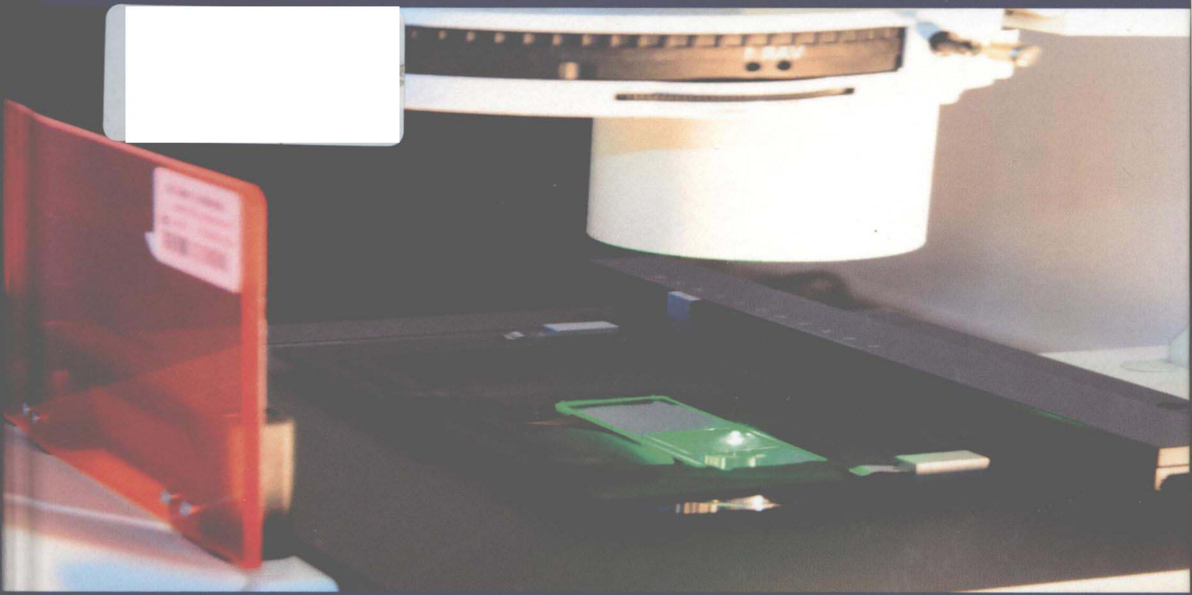

Optics in Instruments

Applications in Biology and Medicine

Edited by
Jean-Pierre Goure

iSTE

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Optics in Instruments

Preface

The optical components of instruments are either elements made by the combination of several systems in order to obtain images, for example the objectives and eyepieces when the detector is the eye, or other elements such as gratings, interferometers, etc., acting on the propagation of light in order to obtain measurement, as well as on other sources (illumination and/or modification of materials) and detectors. Signals or images in color are recorded, processed and analyzed. The understanding of how these systems work requires mastery of a certain number of key concepts ranging from the propagation of waves in different media to the interaction of light, in particular, of lasers with inorganic and organic materials. Optics is thus the basis of instruments of measurement, but it is also a source of processing instruments in particular with the help of coherent sources such as lasers.

The previous book from the same editor *Optics in Instruments* published by ISTE and John Wiley & Sons in 2011 provided the knowledge necessary for the understanding and implementation of instruments whose main elements are based on optical components and their role in technological development. The following chapters have been dealt with successively: geometrical optics, photometry, light sources, colorimetry, analysis and image processing, leisure optics including in low-level light. Finally, to illustrate all this, the last chapter dealt with the evolution from classical microscopy to the scanning tunneling microscopy.

This second book, *Optics in Instruments: Applications in Biology and Medicine* is devoted to instruments, systems and optical techniques that are amongst the most widely used in biology and medicine, as well as current developments and applications. The objective is to allow students and non-specialists in optics to better understand how physical phenomena take place in biology and medicine.

The first chapter deals with confocal laser scanning microscopy, at the basis of high-performance instruments in biological and medical analysis. It follows Chapter 9 of the previous book mentioned above. Confocal microscopy is a method of optical microscopy which has generated much enthusiasm within the community of researchers in biology. A system of confocal scanning laser microscopy is a costly and complex system. The authors provide the basis for understanding the general principle of this method as well as the main elements of current systems. Finally, they point out the recent advances which have enabled us to achieve exceptional optical resolution.

Chapter 2 concerns flow cytometry, the first devices of which were marketed 40 years ago and which allow us to measure, at high-speed and simultaneously, the parameters of a cell flowing in front of one or more laser beams. It allows the analysis and/or sorting of cell subpopulations and applies to animal or vegetable cells, fixed or living, and promotes increasingly accurate and powerful quantimetric approaches to cell life. This tool is located at the crossroads of skills in physics, optics, chemistry, biochemistry, computer science and biology. The author describes the principle and basic elements of cytometers and gives their applications. These devices work routinely for systematic analysis.

Optical coherence tomography (OCT) is a contactless technique of optical imaging using infrared light. The measurement of the travel time of light waves reflected or backscattered by the internal structures of an object allows us to determine at which depths they are located. We obtain images in two or three dimensions of a semi-transparent object by scanning the light beam sent to the object. Chapter 3 is devoted to some elements of optical coherence tomography, a promising technology that enables an in-depth analysis of tissue with excellent resolution.

Everyone knows the boom that lasers brought to instruments and in particular to optical instruments and techniques. One of those areas where their intervention is very appreciable is the medical sector, where they are used for analysis and care. Their efficiency no longer needs to be demonstrated and their impact is growing. Therapeutic applications of lasers are discussed in Chapter 4. Optical phenomena brought into play in the interaction between light and biological tissue, the physico-chemical processes at the origin of different effects and mechanisms of action, are described. To illustrate these, the description of some current therapeutic applications of lasers is provided.

Guided optics concerns the propagation of electromagnetic waves in dielectric structures (optical fibers and integrated optics) or at the boundary between these structures and a metallic layer. The propagation modes in these structures led to numerous applications in telecommunications and instrumentation (microsensors). At the interface between a metallic medium and a transparent medium such as glass, the surface plasmon modes propagate which can be used for analysis and for the creation of biosensors. Chapter 5 thus deals with plasmonics.

Jean-Pierre GOURE
March 2013

Introduction

Optics is a science which covers a very large domain and is experiencing indisputable growth. Major progress is significantly due to the arrival of lasers. After having long been limited to the visible spectrum and to traditional applications of images, the arrival of these new light sources, as well as technical progress, opened up almost all areas of the medical sector and biology. Optics is the part of physics that deals with light phenomena and systems using or emitting light. The latter is of a wave nature (phenomena of interferences, diffraction, polarization) but also of a corpuscular nature (the notion of photons, quantum optics, etc.). It thus belongs to the electromagnetic waves covering the whole spectrum of frequencies (i.e. wavelengths) from γ-rays to radio waves through to X-rays, ultraviolet, visible and infrared. Regarding this book, devoted to applications of optical instruments for biology and medicine, we will consider only the spectral range stretching from UV to IR.

Associated with mechanics, electronics and computer science, optics allowed the development of a considerable number of instruments, whose optical component or methodology is often the essential part of the system. The oldest devices are mainly systems whose role is to magnify objects and bring them closer. Historically, optical instruments used in medicine and biology first led to better observations of objects with small dimensions and the correction of defects of vision. It was thus possible to increase the capacities of natural performing sensors like the eyes. These are optical systems providing a real image of the object on the retina. The analysis and processing of the image received within this detection system takes place in the brain.

We are in constant contact with optical instruments that produce images, such as the original classical microscope. The accuracy of these systems is due to the intrinsic performance of the components that constitute them, but also to the quality and precision of the mechanical assemblies as well as the electronic operating system and computer processing of images or signals. In current systems, the image is not necessarily visual but more often recorded on a detection system and then processed and analyzed. It is no longer just a planar representation of visible objects, but also objects illuminated with other domains of wavelengths (IR, UV) and sometimes with spatial reconstitution by holography and interferometry, or by assemblies of sequential planes.

In addition to imaging systems, many measuring and control devices have emerged based on physical phenomena. Wave and particle aspects of light have non-negligible effects, which are taken advantage of. A great number of instruments utilize techniques based on interferometry with different types of interferometer (Michelson, Mac Zenhder or Sagnac), on diffraction for spectrometric analysis with the help of gratings, or on absorption or scattering. It involves the notions of spatial and temporal coherence of the beams and therefore of the sources. The goal is greater accuracy and reproducibility of results in routinely used systems.

Finally equipment has been designed to be used in medical treatment, based in particular either on the interaction between light and the human body or on the observation and collection of samples.

Modern instruments are complex and result, as was pointed out above, in the convergence of several sciences (optics, mechanics, electronics, computing) to which must be added mathematics (image analysis and processing). Examples of instruments used are numerous. They can be classified into several categories depending upon their purpose. They can be used:

– to form images on the eye (visual inspection of the object by endoscopy);

– to form images on a detection system with an array of photodetectors;

– to provide signals for analysis and control;

– to process materials. In these cases, which are now very numerous, the light beam from these devices is used for shaping inorganic or organic materials (polymerization);

– for medical treatment. The light beam acts as a tool for treatment.

Several technological advances have contributed to this growth.

I.1. The development of new sources of photons

The role of light sources strongly depends upon the purpose of the instrument. The source is often an external one, for example when we need to analyze a material by spectroscopy or absorption. It may sometimes be inside the system, for example in the case of an interferometer, or be the main part of the device, in the case of laser therapy.

Among sources of small size, light-emitting and super-luminescent diodes (LEDs) are used for lighting. They are used directly, possibly collimated, to supply microsensors (biosensors) or through the intermediary of a fiber bundle (illuminating the microscope stages). The control of the electroluminescence of organic materials has enabled the creation of organic light-emitting diodes (OLED) with applications in visualization.

UV radiation from certain sources such as excimer lasers is used as an antibacterial agent. In hospitals, the cold light of the sources and the associated optics illuminates the operating rooms without the accumulation of heat.

In dentistry the sources emitting in the blue or near-UV allow the polymerization of resins (a photochemical reaction called photo-polymerization).

The rapid development in recent years of lasers for medical purposes has allowed a huge step forward. To the power of these sources and their coherence and to the possibility of modulating them with a very high repetition rate, must now be added the diversity of the wavelengths emitted. Fundamental research undertaken at the quantum level has allowed quite considerable advances in the field of quantum boxes, which allow the production of blue LEDs. Doped fiber lasers also take their place in research and instrumentation.

The quality of laser radiation (low divergence, monochromaticity, spatial and temporal coherence, the possibility of ultra-short pulses and high repetition rates) led to its use in measurements in laboratories or in analysis departments.

The trend observed in the spatial domain where the dimensions of components are becoming increasingly smaller and smaller is reflected in the time domain. Pulses of some lasers are in the order of femtoseconds and still tend to decrease. The shortening of the pulses and their increase in energy has led to multiple applications and therefore lasers are at the basis of much equipment. In this book several technologies and types of equipment will be described: flow cytometry is a technique which allows measuring simultaneously, cell by cell, several parameters of fluorescence and scattering of the incident light (the fluochromes being excited by lasers); confocal scanning laser microscopy, which has made possible notable advances in accuracy, as well as multiphotonic confocal microscopy; optical coherence tomography (OCT) based on interferometry, which allows depth measurements. All these types of equipment are efficient, but their cost and maintenance is high. They also require trained personnel.

The chemical composition is obtained by different optical methods: absorption, scattering, classical spectrometry or even RAMAN spectrometry, etc. The detection of the characteristic wavelengths emitted by atoms and molecules under certain conditions allows us to identify them thanks to a diffraction grating spectrometer. The intensity of the emission can lead to the measurement of their concentration. In absorption, a broad-spectrum source is placed in front of the absorbent material. The transmitted light is analyzed and allows the same identification. Measurements by optical methods often without contact are not destructive when energy is low and in domains compatible with living organisms.

However, when energy is high, or for certain wavelengths, the effect can be destructive and can cause burns (UV, IR) or the destruction of cells (γ- and X-rays). By contrast, these effects, properly controlled, can be used to perform treatments (IR or UV laser). Lasers thus have numerous applications in medicine, not only in measurement or diagnosis, but also in treatment: ophthalmology (retinal detachment, etc.), dermatology (treatment of skin diseases), rheumatology, etc. They are involved in microsurgery for eliminating kidney stones, relieving blocked arteries, reducing cancerous tumors, rejoining tissues, etc., and in plastic surgery where the demand is very high.

The laser is the instrument of research in many scientific fields taking advantage of its power and/or of shorter and shorter pulse durations.

I.2. The miniaturization of systems

The size of most objects used in general is reducing and optics has not escaped this law. The arrival of microsystems has been aimed at the miniaturization of elements necessary for an instrument. The study of micro-opto-electromechanical systems (MOEMS) resulting from the combination of optics, electronics and mechanics has led to many applications. The production of such components is based on the use of techniques derived from microelectronics for mass production, i.e. in thousands of copies.

Guided optics (fiber and planar waveguides) finds its place in medical instruments. One important area is that of endoscopy which allows us to see images with systems which are minimally invasive but effective. Applications include all examinations in the human body by natural pathways (fibroscopy). The image is captured at the end of the endoscope through a small lens which forms an image on a straight fiber bundle transmitting this image to an eyepiece or an array of detectors. A second fiber brings the light required for illumination of the examined part. In a fibroscope – a variety of flexible endoscopes designed to explore certain tubes or body cavities, such as the esophagus, bronchi, duodenum, common bile duct, colon, bladder, etc. – a metallic wire can rotate the lens for the purpose of visual examination. Fibroscopy also allows the removal of samples of mucosa. The endoscope is then equipped with surgical instruments: pliers for grasping and removing a foreign body within a cavity of an organism for analysis, scissors, a basket for removing calculus, string and diathermy handles (to remove polyps). Tests were made with a third fiber supplying energy from a light source adapted for the treatment.

Many studies concern fiber microsensors or integrated optics microsensors (biosensors or optodes). The fiber can transmit the data from a microsensor placed at the end (fiber probes) or is itself the sensor by modification of light propagation inside the fiber, when this is subjected to stress from the external environment. In optical guided waves, plasmonics use the modification of the propagation of light waves at the interface between a medium such as silica and a conductive material for detection and measurement.

Following on from microtechnology, nano-optics and biophotonics are becoming the subjects of research in development with applications in various fields in medicine and biology. Very important advances have naturally been made towards nanotechnology. Studies focus on objects or structures with nanometric size or on nanoparticles. Optics is itself involved in the design of biochips.

The study of smaller and smaller objects has modified the way of obtaining an image from the classical microscope to the scanning tunneling microscope or to confocal microscopy. Near-field microscopes, where the probe is a tapered optical fiber, allow us to have an "image" of the material surface within a few nanometers.

I.3. The development of materials

The area that affects most people, and is most well known to the general public, is the area of sight. The development of ophthalmology is at the origin of a large medical sector (treatment of eye diseases and correction of vision problems) and of a wide industrial market. Important technological improvements have emerged over the last 50 years. Optometrists are able to make prescription glasses with high complexity to correct all known defects (myopia, hyperopia, presbyopia, etc.). Glasses with adapted lenses (that is to say progressive lenses) can correct multiple defects simultaneously. In addition, with the arrival of femtosecond lasers, we know how to work with the cornea and improve vision. The fitting of lenses, made of a specific material and with an adapted focal length, allows us to replace the crystalline lens which has become gradually opaque (cataract surgery) and thus restore correct vision.

I.4. Optics, information carrier

Optical recording and reading has been enabled due to this field. Light can, on a single digital disk, record and read hundreds of millions of pieces of information. In telecommunications this has contributed to the rapid and phenomenal boom of exchanges: high speed communication systems whose physical medium is a single mode optical fiber in which infrared pulses are transmitted. This allows the exchange of very large amounts of data and images between centers of measurement and characterization and laboratories or between centers of measurement and doctors. Monitoring remote medical patients using networks of sensors and biosensors has become possible with FTTH (Fiber To The Home) where the fiber comes to the subscriber.

I.5. The contribution of mathematics: analysis and image processing

In the case of images obtained on a detection system and intended to be viewed or interpreted by humans, the characterization of a color, its representation, and its restitution are very important. This is the object of colorimetry. Moreover, as these images are degraded for many reasons, adaptive optical techniques, and image analysis and processing can significantly improve them.

In this book, some current and significant developments of optics in instruments and technologies used in medicine and biology are presented. Naturally, there are other interesting areas that we have not mentioned above. For example, many studies concern the applications of holography. A hologram should allow us to reconstruct three-dimensional images of bones or organs of the human body. The doctor can also better identify breaks or tumors and know which actions to perform for successful surgery. Medical holography is still in its infancy, but shows promise for a bright future.