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Aims and Scope

Chemical sensors and biosensors are becoming more and more indispensable tools in life science, medicine, chemistry and biotechnology. The series covers exciting sensor-related aspects of chemistry, biochemistry, thin film and interface techniques, physics, including opto-electronics, measurement sciences and signal processing. The single volumes of the series focus on selected topics and will be edited by selected volume editors. The *Springer Series on Chemical Sensors and Biosensors* aims to publish state-of-the-art articles that can serve as invaluable tools for both practitioners and researchers active in this highly interdisciplinary field. The carefully edited collection of papers in each volume will give continuous inspiration for new research and will point to existing new trends and brand new applications.

Preface

The detection and identification of biomolecules represents an ever growing part of analytics that influences our lives in various fields, e.g., the diagnosis of genetic or acute diseases, and forensic identification or the monitoring of food safety regarding infections or genetically modified contributions.

Although these and more applications have been enabled by the progress in both molecular biology and detection technology in the past, there is no standard use yet directly at the various points of interest (on-site). Instead, rarely these analytical techniques are applied on a routine base, and when applied they are conducted in specialized laboratories. In order to fully explore their potential socioeconomic impact, they would have to be applied routinely and preferably at (or near) on-site. Then the information provided would not be delayed and could be used immediately for a swift response.

There are practical obstacles hindering such on-site methods. The technology used would have to be robust and self-contained, so that users with minimal training could also use them as well as understand the results. On the other hand, contrary to the high-end, high-throughput analytics in specialized laboratories, the equipment costs would have to be significantly decreased. These two points represent probably the main factors still limiting a wider application. Moreover, miniaturization that decreases the required sample volume (which assists in increasing sensitivity) and thereby the costs for reagents represents another point to consider, as well as a sufficient specificity, which is usually determined by the utilized biomolecular assay.

In order to address these requirements of robustness and cost-effectiveness in the context of miniaturization, sensitivity, and specificity, a certain set of bioanalytical technologies has been proposed based on optical detection in microsystems. This book will introduce this field by surveying promising approaches, presenting the state of the art, and discussing future developments.

Micro- and nanotechnologies are driven by the needs of microelectronics, such as the need for ever smaller functional elements (e.g., transistor) in the context of higher integration at the IC level. This technological development is nowadays also used to miniaturize equipment in other fields such as sensorics, and it is widely

applied for miniaturization of optical elements. A variety of integrated light sources, spectrometer, etc., have been developed and can be adapted into miniaturized and integrated analytical devices. Typical examples—which are also the subject of chapters in this book—represent functional elements for guiding as well as characterizing light. Optical waveguides are a key part of optical systems and enable the miniaturization of such systems. On the other hand, the realization of miniaturized optical approaches for the characterization of light such as interferometers or spectrometers allows the design of compact optical systems with optical waveguides for an on-chip transfer of light as well as the analysis of light using different integrated interferometric approaches.

Optical detection can rely on various properties of light which are used as measurement signal, such as intensity (photometry), the description of light as composed of a set of fundamental primary colors (such as RGB) in colorimetry, the use of molecules exhibiting fluorescence, or the extended spectral decomposition as done in spectroscopic methods. From the point of robustness and costs, this list starts from a rather simple technology and ends rather sophisticated. This is also the justification for colorimetry, which yields less quantitative results as spectroscopy, but is doing this with a simpler technique resulting in a robust and cost-efficient technique. The application and its requirements then decide the choice, because the question is not about the best system possible, but about the sufficient one. On the other hand, more complicate techniques such as fluorescence and spectroscopy allow for a new quality in sensitivity and differentiation, respectively, which could open possibilities for applications not covered by the simpler techniques.

Another important aspect of the described approaches represents the parallelization of analytical assays. Due to miniaturization, the parallel incubation and readout of a number of sensor fields is possible and thereby enables answering not just one but a number of analytical questions in one experiment. This ability represents also a motivation for the use of microtechnologies in the context of bioanalytics, because just one assay could (when a certain sensitivity is sufficient) be also realized using the well-known lateral flow assays (test stripes) as established for pregnancy and similar tests. This parallelization at the miniaturized scale requires also new developments in positioning technology, such as spotting or the described electrochemical approach using microelectrodes for localized deposition of the various complementary binding partners.

In order to increase the sensitivity, labels have been introduced. For optical detection, such labels can be dyes with increased absorption or fluorescent abilities. On the other hand, label-free techniques would be preferred when achieving sufficient sensitivity, because they tend to be more robust from the assay process as well as cost efficient when certain incubation and washing steps can be avoided. Typically label-free techniques detect the binding of target molecules by the mass difference, by electrochemical means, or using optical approaches. Although there are established methods for the detection of mass differences such as quartz crystal microbalance, and even integrated developments in this direction based on microstructured cantilever arrays, the detection is connected with movable parts and not easy to integrate higher. Optical detection techniques are often aiming at the

changed refractive index at the sensor surface, which will change when target molecules bind to the complementary capture molecules located there. An established sensoric principle in this field is the use of surface plasmon resonance where conduction band electrons at metal–dielectric interfaces are excited by the electromagnetic field of light. The resulting resonances depend on the refractive index of the dielectric at the interface and are changed upon binding of analyte molecules there. Already in the 1980s converted into a biosensing technology, such surface plasmon resonance (SPR) sensing approaches are widely used in life science for detection and investigation of molecular binding, which is a key process in biological processes. The limitation of the original SPR sensing approach to just one assay is overcome by the imaging SPR approach (SPRi) as described and discussed in a chapter of this book. This approach allows for the monitoring of several assays in parallel and is another example of the general trend to parallelization in bioanalytics.

Raman spectroscopy is another label-free approach. In contrast to SPR detecting only mass changes, Raman represents a powerful method for identification of the respective compound by their fingerprint. This high specificity allows also multicomponent detection. The inherent limitation of low intensities is usually addressed by the use of surface enhancement at metal nanostructures resulting in surface enhanced Raman spectroscopy (SERS) as discussed thoroughly in this book.

Another point of interest in this context refers to the choice of materials. Materials which allow for a rapid and cost-effective replication of microsystem reactors are needed when a mass application is envisioned. Usually plastic materials fulfill these requirements, as discussed in the respective chapter, and allow for the required number of copies. But as several contributions to this book show, already in the demonstrator setups, one can often find materials like PDMS which are not only easily machined but also highly compatible with the biomolecule solution of interest.

Finally, the integration of the several components such as microfluidics, actuators, and optical detection devices is a key issue for the field. Supported by technical developments in the field of miniaturization and optics, approaches have been established allowing for the combination of optical approaches with microfluidics and microsystems. Numerous examples for successful integration of such optical functional elements into microsystems have been demonstrated, with representative examples thoroughly discussed in this book.

This book shows that proofs of principle for a wide field of promising directions for optical bioanalytics in microsystems have been demonstrated. It also presents the fast development in the field, which ensures that over the next years we will witness these techniques finding increasing applications in real-world applications.

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Contents

Part I
Photometry, Color Sensors, and Fluorescence Labels

Color Sensors and Their Applications

Poenar Daniel Puiu

Abstract This chapter intends to provide an introduction to and a very brief summary of the principal categories of color sensors and of their applications, both relatively little known to the general public.

First, an introduction describes the background of the topic(s) and the overall context. The main differences between two major but complementary techniques, colorimetry and spectrometry, will be presented here, as well as a quick summary of the scientific, technical, and industrial applications of color sensing. The second section will summarize the basic operational principles and architectures of color sensors realized in silicon. Although stand-alone detectors will also be described and discussed, the main focus will be on solid-state microsensors which can ideally be monolithically integrated together with signal processing circuits onto the same chip as "smart sensors" or intelligent microsystems. First, sensors realized only in monocrystalline silicon are summarized, followed then by those fabricated in other materials, with amorphous silicon and its alloys as the key players in this latter category.

Finally, the chapter ends with the sections devoted to Conclusions and References.

This chapter presents only a few of the most relevant aspects related to color sensors and examples of their practical applications. It is, in fact, an extensively abbreviated version of a much more detailed and exhaustive review dedicated to both color sensing and microspectrometry, and which is presently in preparation for future submission to Springer Verlag.

Keywords Smart sensors • Color filter array (CFA) • Two-junction color sensors • Triple-junction color sensors • Thin film color sensors

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Contents

1 Introduction—The Color Sensor: Necessity or Just a Curiosity?

Color is one of the most important characteristics of light, although we may not always be aware of this fact and of its importance, taking it for granted.

However, even though color plays a unique role of great importance in our daily life and—as we shall soon see—in quite a few industrial and scientific applications, the number of sensors dedicated to color sensing is surprisingly small and—more importantly—the existence or operation principles of such sensors are very little known to the general public. When "microelectronics" is mentioned, most of the people think of mobile phones, computer microprocessors, or other familiar applications such as DVD players, GPS, or game consoles. In general, the media typically focuses its attention on the latest developments in these areas which are very familiar to everybody, such as telecommunications, computers, and new consumer electronic gadgets.

However, another extremely important area deals with sensors and actuators. Sensors can be briefly defined as devices or systems which detect relevant signals from the environment and convert them into an electric signal or data that are further processed into electronic circuits. Without sensors, most of our technological equipment, from complicated machinery to gadgets that we use daily, would be unable to operate. Sensors pick up the signals containing precious information necessary to either correctly operate equipments or control processes by providing vital data for their feedback. Presently, the trend is to no longer realize discrete sensors which can then be combined with various integrated circuits on a printed circuit board in order to perform a certain desired function. Instead, the technological advances in the area of microelectronic fabrication made it possible to integrate a complete microsystem on a single chip both the sensor and its intelligent signal conditioning circuits as well as digital processing blocks which manipulate the

obtained data. This high degree of integration is possible due to the progresses in two areas. The first key factor is the miniaturization of integrated circuits which has been going on aggressively in the last decades and which has now reached Ultra-Large Scale Integration (ULSI) levels in which the feature size of the transistors is at submicron scale. For instance, Intel's Atom processors presently integrate features as small as 45 nm using a high-k metal gate technology, and this will soon be replaced by a 32-nm silicon process technology [1]. Such advanced fabrication technology allows the realization of extremely complex circuits like microprocessors and Digital Signal Processors (DSPs).

The second factor is the development of Micro-Electro-Mechanical Systems (MEMS) or Micro-Opto-Electro-Mechanical Systems (MOEMS) that can be fabricated in silicon (or other materials) using modified microelectronic processing derived from IC fabrication in order to achieve various structures with different functionalities other than purely electronic ones. This enabled the realization of new and complex structures that can be used as sensors or actuators of various types.

The co-integration of both the sensor(s) and its/their signal processing circuits is demanded by practical considerations such as the reduction of the useful signal magnitude for smaller scale sensors, and the needs to minimize parasitic components and to perform amplification as well as other signal conditioning operations such as scale linearization, filtration, and elimination or minimization of offset and drift. Additional functions can be easily added subsequently using digital circuits of great complexity, and in certain cases, these can process data not only from a single sensor but also from an array of sensors, as is the case in, for example, the "electronic nose," or can control both sensors and actuators embedded in the same microsystem.

Actuators can be defined—in the most general meaning—as devices performing a function complementary to that of sensors; namely, they convert a type of signal (most often electric ones) into another type which is re-introduced back into the environment, either for its feedback control, or by carrying an information/meaning that can be perceived only by the human used. For instance, the computer monitor can be considered as one such actuator since it converts an electrical signal coming from the computer into an optical signal provided into the environment to the human user.

The term "signal" may refer to six different types of measurands: physical/mechanical (e.g., force, acceleration, or displacement), thermal, magnetic, (bio) chemical, radiative, and electric [2].

Yet, despite their importance and ubiquity, sensors and actuators are much less known to the general public, given that, on the one hand, they are usually "hidden" from view, and, on the other hand, the measurement and control field—to which sensors and actuators belong—are not as often and widely popularized as, e.g., consumer electronics.

This introduction may now explain the seeming obscurity of color sensors: they represent just a diminutive group in the family of optical sensors, which is just a small part in the larger category of sensors, which, on its turn, represents only

a niche domain in the measurement and control field, in itself a smaller and less known area than, e.g., consumer electronics or microelectronics.

The same situation is also reflected even in the area of scientific papers. A very quick review of the papers published in reputed scientific journals dedicated to the general topic of sensors (e.g., IEEE Sensors; IEEE Journal of MEMS; Sensors and Actuators; Journal of Micro/Nanolithography, MEMS, and MOEMS; or Sensors & Transducers, to name just the more prominent ones) would quickly confirm to the curious reader that the amount of papers dedicated to color sensors represents only a relatively small percentage of the total number of papers published in those journals. Indeed, many—if not the great majority—of the optical sensors detect only the intensity of light, while other science and technology applications have required the detection of other parameters, such as polarization or phase (whose manipulation, or capture and storage are necessary in certain applications, of which holography is the best known to the public).

There is also confusion or a lack of clear understanding among most people regarding the definition of what exactly colorimetry is and how it differs from spectroscopy—its older, more widely spread, and much better known relative. Without going too much into details which are beyond the limited scope and space of this chapter, we can clarify this by providing quick definitions and highlighting the fundamental principles underlying these two measurement techniques. This is necessary because the difference between these two major but complementary approaches, the spectrometric and the colorimetric approach, is a very significant and fundamental one.

Thus, the colorimetric method relies on the decomposition, analysis, and description of incoming light by means of a set of fundamental primary colors, such as red (R), green (G), and blue (B). In contrast, spectrometry decomposes the incident light into a large multitude of extremely narrow passbands by using a dispersion element, such as a prism or a grating. This spectral decomposition is carried out within a certain wavelength range of the incident light, and this fact highlights another major difference between colorimetry and spectrometry: colorimetry always processes only *visible* light, whereas spectrometric analysis is not limited to the visible range alone and can also be carried out in many other spectral ranges of interest, e.g., ultraviolet (UV) or infrared (IR). Moreover, each of these ranges is typically divided in sub-domains, each of which can also constitute the subject of detailed spectrometric investigations. For instance, the IR domain is considered to comprise three such narrower domains: near infrared (NIR; $\lambda \cong 0.7$ μm–3 μm), medium infrared (MIR; $\lambda \cong 3$ μm–8 μm), and far infrared (FIR; $\lambda \cong 10$ μm–100 μm). The ultraviolet (UV) range is "partitioned" in narrower domains as well. The application of spectrometry in such a large number of ranges, domains, and sub-domains, as well as its older age and great importance for a great deal of scientific and technical applications, can all explain the much wider usage of spectrometry and the fact that it is better known even among non-specialists.

Because it splits the operational wavelength range in a large number of very narrow channels and provides a "point-by-point" approximation of the entire spectral information of interest of the analyzed light, spectrometry can be

considered to be much more accurate in comparison with colorimetry, which would offer only a more limited, and rather "averaged," evaluation. However, the spectrometric approach has its own major drawbacks.

First, it requires a much more complicated setup which is considerably more difficult to miniaturize, particularly in a monolithic solid-state solution and/or when high performance is desired, although such microspectrometric solutions can be realized and have indeed been reported. Unfortunately, due to the very limited space of this chapter, we cannot review here the interesting solutions found for the implementation of such microspectrometers. However, it is worth mentioning that many of them do indeed require complicated fabrication and that the requirement to use (monocrystalline) silicon for their fabrication (in order to enable easy integration with signal processing circuits) is not always easily satisfied (in fact, it is very rarely satisfied), particularly for the visible range. This is due to two main reasons. On the one hand, a microspectrometer is not just a simple single device, but it is truly a microsystem of increased complexity and which must include many additional (micro)optical elements (e.g., lenses and gratings) besides photosensors and signal processing circuits in order to perform its function. On the other hand, the usage of silicon is much more convenient for the realization of devices that would operate instead in the IR range.

Second, the very fact that spectrometry offers a dramatically increased amount of information from a large number of output channels makes much more challenging the simultaneous processing of all these data. This further increases the complexity of a spectrometric smart microsystem since it requires the addition of many signal conditioning circuits for all the channels, as well as a complicated digital circuit(s) for subsequent data processing.

Third, one cannot find a single standardized solution for all spectrometric systems. Instead, custom solutions have to be found, depending on each type of operational wavelength domain or sub-domain, and the desired performance requirements for the intended specific task. Even for the visible range, a multitude of solutions and implementations can be found, and the variety is also multiplied by the fact that the number of channels is not fixed and may vary from one application to another.

In contrast, colorimetry is a solution which is not only simpler to implement but is also easier to standardize, making it much cheaper. More importantly, the standard RGB output is directly compatible with the human vision response and thus is immediately applicable for imaging applications. All these factors made colorimetry to become a very valuable analytical tool in its own right. If, for the moment, our attention focuses not on the color sensors but on the real-life applications of color detection and quantification, it will quickly become evident that colorimetry is actually quite a well-established technique with many important applications in a large range of fields or industries. The main areas of such applications can be loosely divided into the following categories: Chemistry, life sciences, food & beverages industry, cosmetics, wood & paper processing as well as the printing and textile industries, and electronics & optoelectronics. Given the fact that the importance of colorimetry for practical applications is relatively little

known to the general public, the following subsections will provide several examples of real-life applications of color sensor and colorimetry in some of these fields. The description of color sensors and their operation principles will be subsequently presented in a separate section after this short review.

1.1 Chemistry

Chemistry, and by extension, biochemistry and the Life Sciences, as will be presented in the next sub-section, heavily rely on color detection for many practical purposes. Indeed, chemistry and medicine were most probably the very first fields in which colorimetry has been extensively applied even from their early stages of development. The Beer–Lambert law and the direct connection between the concentration of a substance and its color in a solution are the underlying principles on which are based the technical applications in organic and inorganic chemistry as well as biochemistry.

One of the first—and probably one of the best known—applications of colorimetry was for the measurement of pH, and its subsequent extension to the measurement of pK and even to complex tasks such as the analysis of multicomponent mixtures and precision measurements for various routine industrial chemical processes, had already been achieved before World War II [3–8].

Presently, colorimetry still plays an important role in chemistry, but it is applied in practice using new and different methods, which will be briefly presented here. Thus, one technique relies on the usage of sol–gel films that can be used for measuring pH as well as the concentration of metal ions. These sensors proved to be inexpensive, accurate, unaffected by temperature up to 40°C, fast, and reusable.

Another is the usage of polymers, which have become an attractive alternative due to their availability, low cost, and ease of usage. One such sensor employed a polyester–cellulose acetate double layer onto which a pH indicator dye was covalently immobilized and which could be employed in an optoelectronic setup to determine the pH value in the range 6–10 [9]. Another example is that of an autonomous sensor, i.e., which could be deployed and used repeatedly in varied conditions with minimal or no human input. This was achieved by moving the sensor in either a measurement zone or in regenerative ones (one acidic and one basic). The movement was obtained using a compact trilayer membrane comprising polypyrrole (PPy) outer films as actuating elements and a central polyvinylindene fluoride (PVDF) membrane as the central backing material. The pH sensor itself consisted of a polyethylene film coated with bromocresol green dye [10].

Much more recent trends in this area are the application of "smart textiles," or of nanotechnology. An example of the former was obtained by the immobilization of a dye—to which various concentrations of dimethylamine (DMA) have been added—in a viscose textile matrix. Both color changes and fluorescence quenching resulted as a function of the pH, while reactions with ammonia and methylamine were also observed and they were more efficient than those with the dye solution

alone. This realization has thus proven that "smart textiles" may become in a near future a very convenient, low-cost alternative of high sensibility and reproducibility that is also highly suitable for mass production for easy, fast, and accurate analysis for medical or environmental applications [11].

An example of the latter is the family of chemical sensors which employ color change induced by the swelling of a detecting matrix or another type of modification brought onto a material with a periodic structure. A key advantage offered by such sensing materials that are structurally colored is that they eliminate the need for dyes or tags, hence they do not suffer from any photobleaching effects, while at the same time also simplifying the sample pre-processing. Two types of such chemical sensors can be briefly mentioned here.

An example of the first type is a hologram-based humidity sensor which used the holographic pattern recorded in an acrylamide-based photopolymer as a diffraction grating. Since the material swells or shrinks when exposed to various relative humidity values, the fringe spacing is modified, resulting in a change in the observed color [12]. A related realization employed molecules attached to the gratings surface so that the reflected wavelength (color) shifted correspondingly when target molecules linked to the grating surface's receptors due to the change in the optical path of light coupled into the grating. This technique was experimentally demonstrated to detect the binding of biotin to avidin, of a five amino acid peptide, and the cleavage of a portion of the bound molecule, respectively [13].

An example of the second type of chemical sensors employs a three-dimensional (3D) colloidal crystal realized by embedding mono-disperse polystyrene nanoparticles in PDMS. Because PDMS swells in nonpolar organic solvents, when the resultant colloidal composite was subjected to such substances, its lattice constant and thus the wavelength of the Bragg diffracted light increased, resulting in a colorimetric detection of such volatile organic compounds (VOCs) [14]. Another interesting example of a chemical sensor that also made creative usage of nanotechnology is that of colloidal crystal films formed from composite core/shell nanospheres for selective sensing of multiple vapors at very low concentrations The nanospheres consisted of a core made of a material preferentially responsive to one class of chemicals (e.g., polystyrene), which was then coated with a shell made of a second material which was preferentially responsive to another class of chemicals (sol–gel). The chemical compositions of the core and the shell could be varied depending on the analytes of interest, thus enabling the designer to tune the sensor response. A colloidal crystal array was then self-assembled into a 3D ordered film. Sorption of polar or nonpolar vapors induced a change in the optical lattice parameters of the colloidal crystal array as a function of analyte concentration, determining on its turn a variation in the Bragg diffraction wavelength, i.e., of the film's reflected color [15].

Similarly, a photonic crystal with an inverse polymer-gel opal could be swelled or shrunk by electrochemical oxidation/reduction, resulting in modification of the wavelength of the light diffracted by it in a large range, from UV through the visible to NIR [16]. Another analogous structure comprised 1D multilayer stacks (also known as Bragg stacks) of mesoporous TiO_2 and SiO_2. The color exhibited by

this mesoporous Bragg stack (MBS) is dictated by its high reflectivity at a certain wavelength of light λ_B which is caused by the periodic variation in the effective refractive index (RI). The overall response of this MBS was observed to depend not only on the RI of an analyte but also on other physical properties, such as hydrophilicity. Various such MBS structures were successfully used to detect a large range of volatile compounds, such as alcohols, and exhibited superior sensitivity in comparison with a conventional Bragg reflector with just a single porous layer as the chemical sensing element. The MBS provided different responses even when it was exposed to two substances with very similar RIs [17].

Finally, dyes and chromophores form a vast section of considerable importance in chemistry and biochemistry and with a great many applications. For brevity, only two examples of reported applications in this area will be mentioned here.

In the first one, a copolymer bearing a covalently attached solvatochromic dye (the so-called Reichardt's dye) was used as an optical sensor to detect interactions with β-cyclodextrin [18]. The second one employed chromoionophores (i.e., molecules changing their color on complexation with the compound that is to be measured) in order to detect a metal ion in solution (e.g., Ca^{2+}) [19].

Fluorescence is another area of major relevance within the same section related to dyes and chromophores. Since it was one of the first bio/chemical investigational methods developed more than a century ago, fluorescence has been gradually perfected to a high level of performance and finesse and is presently one of the most convenient detection techniques widely used in numerous variants, including multi-dye (i.e., multi-color) applications and single-photon detection.

A great wealth of work has been carried out and an overwhelming number of papers dealing with the application of fluorescence, particularly in life sciences, have been generated. From this tremendous volume of information, we shall highlight here just a few, related to lesser known applications in Chemistry. For instance, fluorescence was employed for optical pH sensing with aminofluorescein as indicator [20], for detecting Li ions using two-color fluorescence [21], or for monitoring of commercial gasolines by quantifying the intensity of the Stokes-shifted fluorescence from some of the heavier polycyclic aromatic hydrocarbons C_xH_y, $(x,y) \geq (14,10)$, that are present in gasolines as minor constituents [22]. A fluorescence smart detector for capillary analysis was realized and employed for concentration and molecular discrimination from the average wavelength of fluorescence spectrum. The microsystem comprised a buried double *pn* junction color sensor integrated onto the same chip with CMOS signal processing circuits and has been tested using FITC and Rhodamine B in different concentrations with a capillary having an illuminated volume of about 5 nl. The best results have been obtained with FITC, which was detected in concentrations as low as 10^{-10} M [23].

Other applications focused on the challenging task of detecting numerous analytes that are neither intrinsically fluorescent nor can be rendered fluorescent by means of labeling, e.g., pH, O_2, CO_2, ammonia, and glucose. In such cases, a preferred solution is the usage of a "paint" that can be applied (by painting or spraying, or by direct manufacturing as a thin film) on a suitable substrate and

which would modify its optical properties when exposed to the (bio)chemical analyte of interest if/when placed in the system to be studied [24].

More applications dedicated exclusively to life sciences are detailed in the next sub-section.

1.2 Life Sciences

In medicine, the alteration in color of a tissue is typically a clear indication of inflammation or of any other deviation from the normal healthy standard outlook, e.g., edema, burns, or necrosis. Moreover, color interpretation has recently become a key ingredient necessary in interpreting the images captured inside the human body using endoscopic probes. Presently, this endoscopic exploration is no longer limited only to analyzing simple reflected light, but also uses fluorescent light, or light scattered or re-emitted due to some physical process (e.g., two-photon excitation, second harmonic generation, or other nonlinear processes) [25]. However, in this case, the color reading and interpretation are not done by a specialized detector, but by examination and analysis of the image captured by the imaging camera chip of the endoscopic probe. This analysis is increasingly relying on specialized image-processing software which implement various algorithms on the captured digital image in order to extract from it valuable markers that could indicate the onset of a certain disease, or determine its stage of advancement.

Nevertheless, purely colorimetric investigation methods have been applied for a long time in medicine and played quite an important role in various applications. Blood oxygenation and urine analysis are just two of the best known applications of colorimetry in medicine. Blood has a particularly well-established history in this respect, with a distinctive place devoted to hemoglobin measurements that are carried out almost exclusively using such colorimetric methods. Very early work had measured the absorption spectrum of anhydrohemoglobin (Hb), and determined the transition points of the system $Hb \rightleftarrows Hb \bullet H_2O$ as well as the thermodynamic constants for the combination of one molecule of water with the iron atom contained within the Hb molecule [26]. Subsequent studies deduced the oxygen equilibrium in a wide range of concentrations for both deoxygenated and oxygenated hemoglobin using colorimetric methods. This enabled to calculate the oxygen equilibrium constants and the association–dissociation constants for deoxyhemoglobin and oxyhemoglobin. Later, both *transmission* and *reflectance* methods were established (alone or in combination) to determine the blood oxygen content in a quick and reliable manner, and both of them relied on the differences between the absorption coefficients of oxy- and deoxyhemoglobin [27]. Other essential blood-related parameters which were measured colorimetrically are the glucose content [28] and the detection of saccharides [29].

The need to carry out faster bio/chemical analyses in a shorter time, at a lower cost, and using samples of significantly reduced volume demanded the realization of miniaturized microsystems which were implemented using dedicated microfluidic

platforms. An ingenious realization of this type employed rapid prototyping of a CD fluidic platform in PDMS, being successfully used in enzymatic assays [30]. A much more recent development used super audio compact disks (SACDs) and computer optical disk drives for multi-wavelength chemical measurements, and it exploited the availability of optical laser pick up heads that produce the wavelengths of 405, 650, and 780 nm (normally used for reading Blu-ray disks, DVDs and CDs, respectively) for determining the chlorine concentration in water [31].

Other efforts focused on the integration of a color sensor within a monolithic chip that also included microfluidics as well as signal processing circuits. An initial simple version consisted of a photodiode formed on a glass substrate and covered by a recrystallized polysilicon thin film which, due to its extremely high absorbance at very short wavelengths, could detect fluoresced light (450 nm) in the presence of the UV excitation light (340 nm). The device was aimed at monitoring enzymatic reactions that convert nicotinamide adenine dinucleotide (NAD) to its fluorescent product NADH (nicotinamide adenine dinucleotide-reduced form) in any of the subnanoliter cuvettes within a large array [32]. This initial realization was further perfected into a fully monolithic microsystem using SU8 microfluidics on top of a Si substrate that integrated the filtered photodiode array together with the readout electronics [33].

Another example of a miniaturized realization is a DNA microarray employing CMOS Buried Double *pn* Junction (BDJ) detectors and a fiber-optic bundle-based illumination and fluorescence collection system [34].

In the area of medical diagnosis, the identification of different types of bacteria after staining with one (or more) suitable indicator(s) is a very well-known and established practice. However, the standard staining procedures are complicated and time consuming, and the development of new, simpler (or more accurate) solutions could be more convenient and attractive. Thus, a zinc-based chromogenic complex was reported to bind preferentially to adenosine triphosphate (ATP) in aqueous solution at physiological pH, causing a visual change in color. This complex was used as a staining agent for different biological cells which could be viewed subsequently with normal light microscopy. Very importantly, this non-lipophilic zinc-based reagent could even be used for distinguishing both Gram-positive and Gram-negative bacteria (prokaryotes) while at the same time being nontoxic to living microbes (both eukaryotes and prokaryotes). The viability of the stained microbes even after staining was confirmed by their subsequent growth in their respective media, in stark contrast with many other dyes available commercially [35].

A similar and very relevant application in the same area is the fluorescent-based detection of the human immunodeficiency virus (HIV). A fluorescence resonance energy transfer (FRET)-based technique was developed to detect changes in fluorescence caused by viral protein receptor binding [36].

Just as for chemistry and many other fields, nanotechnology-based applications become increasingly common in medicine as well. Thus, a colorimetric method employing fluorescent or immunogold assays was developed using a protein

microarray with the ability of serodiagnosis of IgM antibodies, directed against pathogens such as *Toxoplasma gondii*, rubella virus, cytomegalovirus, and herpes simplex virus [37]. Another example is a simple and rapid colorimetric bioassay for the detection of cholera based on a specifically synthesized lactose derivative that has been self-assembled onto gold nanoparticles 16 nm in diameter [38].

Similar realizations were reported for DNA and protein detection, respectively, and were all based on the usage of Au nanoparticles and of their nanocluster-enhanced absorption [39]. The basic operation principle was a strong color shift (from intense red to deep purple) induced by the aggregation of the nanoparticles in the presence of the antigen to be detected. A rather different approach was employed for DNA detection using the variation in opacity of self-assembled nanometallic particles before and after DNA strands pairing which was monitored with a CMOS image sensor [40].

Other important examples of nanotechnology application in medicine can also be cited. One of them is the usage of aptamer-conjugated nanoparticles for sensitive detection of cancer cells. Just as in the previous case, the aggregation of gold nanoparticles in clusters of different sizes induced by their adhesion to cancerous cells results in chromatic changes [41]. In another realization, unmodified silver nanoparticles were used to implement a sensitive, selective, simple, and label-free colorimetric assay to detect enzymatic reactions of ATP dephosphorylation by alkaline phosphatase (CIAP) and of peptide phosphorylation by protein kinase (PKA). In the absence of the enzymes, unreacted ATP protected the silver nanoparticles from salt-induced aggregation, whereas in the presence of the enzymes, the reaction product of ATP (i.e., adenosine for CIAP and ADP for PKA) did not, resulting in a color change of the initial yellow colloidal silver solution with a strong absorption peak at ~400 nm [42].

Although the applications for medicine and biochemistry are by far the most numerous, plant and animal biology are also part of life sciences, and applications like chromatography, electrophoresis, tissue health estimation, fluorescence reading, etc., heavily depend on colorimetric measurements, either in transmission or in reflection. For instance, colorimetric analysis of spectral reflectance was used for a selective herbicide spraying system that could distinguish between crops and weeds [43].

Additionally, oceanography and pedology also include colorimetry in their metrological arsenal. The former uses color measurement and analysis from absorption and scattering of phytoplankton from which chlorophyll concentration, and consequently the phytoplankton population and its general health and biomass, can be deduced. Such measurements enable a more accurate assessment of the state of the observed ocean area and prevent any undesired large-scale event like algae bloom, or monitor a large variety of other important parameters, such as overall photosynthetic potential and amount of yellow substance and suspended matter. Similar judgments related to photosynthesis and biomass could also be done by monitoring the land vegetation's spectrum [44, 45]. A realization employing a 4-channel color sensor was used for a similar purpose, namely, for measuring phytoplankton pigments. The results revealed a strong dependence between the

concentrations of chlorophyll *a* and carotenoid pigments in the phytoplankton cells [46].

Finally, a color sensor was successfully employed in pedology to assess the content of six nutrients in farmland soil [47].

1.3 Food and Beverages Industry

In the food & beverages industry, color is a very important measurand which can be used either for quality estimation, or for automatic selection of products in different categories. Reflectivity measurements can be easily employed for solid foods, whereas for liquid samples, it is much more appropriate to measure the sample in transmission. The previously mentioned dependence of blood color on the concentration of deoxygenated and oxygenated hemoglobin can also be applied to estimate quantitatively the freshness of fresh meat. Thus, by analyzing the reflectance spectrum of beef meat and its variation in time during storage, it was deduced that the typical spectrum changes in time due to exposure to oxygen, which causes the oxymyoglobin to be oxidized into methemyoglobin, resulting in a quality degradation-induced color change that can be automatically sensed so that packaged meat that exceeded its shelf-life could be promptly removed and replaced with fresh one. Similar color-based quality analysis has also been successfully performed on other foods (for diagnosis of storage conservation or for estimation of their alimentary properties), e.g., milk, orange or strawberry juice, or peach nectar [48].

Another example is the reported realization of an accurate real-time color classification microsystem that integrated the photosensors (in this case, not an array of dedicated color sensors but simple CMOS photosensors), together with pre-processing circuitry and a subsequent neural network processor, onto a single IC. The usage of neural networks offers distinct advantages, such as extreme ease of usage by the user of the final product, flexibility by self-adaptation to new circumstances, and reduced cost and extreme suitability of monolithic integration with standard CMOS fabrication processes. This one-chip smart sensing microsystem had low cost, was robust, was mass-producible using standard commercial CMOS processes, and exhibited a significantly higher performance. The chip was successfully applied practically in freshness tests for several fruits (apples, tangerines, and lemons) [49].

Colorimetry has also been a useful tool in wine characterization for quite some time. An earlier method had been developed by Folin and Ciocâlteu [50] and perfected later by Lowry et al. [51] to determine the total phenolic content of the red wines [52]. The phenolic content is important because phenolic compounds are responsible for the characteristic color, flavor, and aroma in wines and also act as antioxidants, with alleged beneficial effects on human health (reduced incidence of coronary heart disease and certain forms of cancer). More recent research modified the initial spectrophotometric method and improved its performance. Thus, the more modern realization could be used to analyze totally opaque (i.e., highly optically absorbing) samples that otherwise would be difficult to analyze

classically, and did not require any dilution (as was necessary with the previous Folin– Ciocâlteu method). Moreover, the method was equally well suitable for other types of samples, such as pastes, suspensions, and semifluids [52].

Color sensing has been employed not only for fresh produce but also to characterize the cooking of foods. This is important in order to avoid under- or overcooking, and also to ensure objectivity of assessment since human evaluation is highly subjective, may also depend on other variables, and does not guarantee accurate reproducibility. One reported realization for this purpose comprised an optical fiber sensor in conjunction with a small portable Ocean Optics spectrometer in order to monitor the color of the food while it was being cooked by examining the light reflected from both the sample's surface and core [53].

Finally, a system was reported for colorimetric nondestructive online inspection of white foods (e.g., flour, but obviously the method could be equally well applied for milk, cheese, etc.). For the specific purpose of characterizing four flour samples, the selection of just two different wavelengths was proposed: one out of the {440 nm, 480 nm} set, and another out of the {520 nm, 540 nm, 600 nm} set, respectively. Based on this operational principle, a fiber-optic-based system was built and successfully tested in industrial conditions [54].

1.4 Scientific, Technical, and Industrial Applications of Color Sensing

Many modern manufacturing processes require the detection of different colors and hues of visible light, i.e., wavelengths in the range 400–700 nm. Color detection can be used to sort objects, verify position of objects, recognize color sequence, control color in dying and coating applications, and detect changes in color of liquid during titration. Hence, color sensors are becoming an integral part in many industries, e.g., cosmetics, textile, food, publishing, optoelectronics, and image processing, including digital cameras. This sub-section will present some of the most important applications of color sensing in industry. It will be seen, however, that the sensors that can be used in various applications are not necessarily only solid state or monolithic.

For instance, in the textile industry, it is useful to measure color in order to access quantitatively the color variations in printed textile material during the online manufacturing process. Moreover, color monitoring should not be limited only to checking small areas but also large ones. In this latter case, however, employing a colorimeter may no longer be a viable option for real-time monitoring and other solutions had to be found. Such a solution used machine vision and image processing for large-area continuous textile process monitoring [55].

One of the first usages of color sensing was to determine the color of light emitted by various illumination sources by using colorimeters [56–59]. However, one can extend the meaning of "light source" to include other, less obvious,

physical phenomena that either emit light, or which can be characterized using colorimetric and optical methods. A first category of such phenomena is the characterization of plasmas [60], including the online monitoring of thin semiconductor films—initially deposited on a substrate—during their processing with an RF plasma [61].

The second category is related to the characterization of a combustion source either directly (by monitoring the flame color, when this is applicable) or indirectly (by monitoring the properties of the smoke's particulates). Color sensors can, therefore, play important roles in flame monitoring, either for fire prevention or for characterization of the combustion by-products. An illustrative example of this latter category is a system that monitored smoke in order to characterize combustion material and which was capable to identify uniquely a combustion material by variations in lightness and saturation over a range of hue values [62]. This system employed "chromatic modulation" (sampling white/polychromatic light after it was affected by the sample via three separate optical detectors with different but partly overlapping spectral responses). The same principle was also employed to monitor particles of sizes between 2 and 10 μm in a system employed for air pollution monitoring [63]. A relatively similar approach was employed for *in situ* monitoring of chromatic changes in particulate mass fraction and variations in the mean particle size of diesel exhaust particulates. The extracted mass fraction of these particulates in the exhaust gas is an indicator of fuel consumption, while variation in mean particle size is a primary indication of incomplete combustion [64]. Two similar systems were demonstrated practically for liquids. One system successfully discriminated between single-phase liquids (fuel and water) and distinguished between single-phase aviation fuel types and a range of mixtures of water in fuel [65], whereas the other was used for online monitoring of oil in industrial vacuum pumps detecting the quality of these oils as well as the presence of any air leaks [66]. Another system was also reported for liquid fuel quality monitoring, but based on a distinct fiber-optic-based architecture and operation. One version employed neural networks, solution which proved to be more advantageous as it could easily relearn to adapt to various tasks and examined samples, and was also faster and significantly cheaper [67].

A different approach for particulate characterization was based on a three-wavelength scattering technique. A first example of this technique was also employed for smoke characterization in order to determine the average particle size and mass concentration of smoke from transmission data using Mie theory [68]. The second example is an optical fiber-based system that was used to characterize monodisperse particulates in liquid suspensions in terms of their size, refractive index, and density per unit volume. The system was used to characterize both absorbing and non-absorbing particulates, with specific focus on the investigation of suspensions of solid particles in liquids, and excellent agreement between theory and experimental results was obtained [69].

Another example of color sensing application in industry is white balance testing. This is an instrument that adjusts the purity of the white emitted by color TVs, monitors, and PC displays. It employed simple photodiodes in conjunction

with colored filters, and the entire system was controlled by a 16-bit single-chip microcomputer that automatically controlled the emitted luminance level [70].

The wood-processing and paper industries as well as any printing-related application also need colorimetric measurements. One first example is the application of color sensing to assess the effects of thermal treatment of birch with respect to color and mechanical strength. However, it was concluded that color is not a useful parameter for prediction of mechanical strength, and the color homogeneity of the treated boards was not good [71].

A second and much more successful example is the real-time measurement of lignin content in moving paper sheets which enabled high-speed automated sorting of papers according to lignin content in a mixed-waste stream for efficient recycling. However, it was not the actual paper color *per se* that was monitored, but rather the fluoresced one. It was found that the magnitude of the fluorescence peak at 650 nm was directly proportional to the lignin content of the paper, while the dependency on paper weight was nonlinear, saturating at higher paper basis weights. The effect of other important variables was also investigated [72].

A third and different reported application in the same industry investigated the optical surface quality of two different commercial laser print papers before and after printing of R, G, and B color inks. It was observed that the R, G, and B color print inks had influence on the reflectance, transmittance, and optical anisotropy of the print paper [73].

The colorimetric characterization of printers using more than three colorants is another very important practical problem in the printing industry, since typically there is no unique combination of colorants that would exactly reproduce a particular color, not only in standard CMYK printers but also in high fidelity ones which employ RGB colorants in addition to the CMYK ones. Two methods were developed and implemented using software in order to solve this problem by determining the best combinations of colorant amounts which can provide the most suitable match: the variable reduction method and the division method. The variable reduction method used connecting functions to reduce the number of variables controlling the colorant amounts. It was a simple method but did not always utilize the entire color gamut. The division method employed sub-gamuts composed of appropriate sets of three or four colorants that were combined to form the desired final hue. This latter method enabled access to the entire color gamut, but had the drawback that its boundaries tended to cause pseudo-contours due to abrupt changes in one or more colorants [74].

2 Monolithic Color Sensors

As mentioned above, in the *colorimetric approach*, the visible light range (400–700 nm) is analyzed based on its tri-stimulus decomposition based on the usage of “primary” colors, typically red (R), green (G), and blue (B). Although one can envisage many different types of realizations that could perform such a colorimetric analysis, for brevity we shall focus our attention only on monolithic

sensors fabricated in semiconductors. This type of sensors may be implemented in silicon (Si) using either monocrystalline substrates, or polycrystalline, or—more often—amorphous thin films. The next sub-section will briefly introduce the fundamental concepts on which such Si-based color sensors are based. The following sub-sections will then succinctly review some of the most relevant examples of Si-based color sensors of each type, i.e., realized either in monocrystalline substrates or in amorphous thin films.

2.1 Basics of Solid-State Color Sensing

As is well known, a photon with energy greater than the bandgap energy of silicon, $h\nu > E_g$, can excite an electron from the valence band to the conduction band. This causes the generation of an electron–hole (e–h) pair, i.e., free carriers which, under right circumstances, can be separated and give rise to a current in an external circuit. After transferring energy to the electron, the photon disappears and is "absorbed." Conversely, photons with less sufficient energy will not be absorbed and are, therefore, transmitted. This explains why short wavelengths (i.e., with photon energy above the threshold energy equal to that of the material's band gap) will be absorbed, but larger ones will be transmitted. Since silicon has a bandgap energy of 1.12 eV, it can absorb light with wavelengths equal to or below 1,100 nm [75].

However, the absorption process for photons with energy around the bandgap value is strongest only in direct bandgap semiconductors. When a semiconductor does not have a direct bandgap, which is also the case of silicon, the absorption of a photon does not take place unless a phonon (or lattice vibration) also participates in the process. The absorption coefficient of photons in crystalline Si, an indirect semiconductor, is lower than that of a direct material and rises only slowly at energy levels near the bandgap (1.12 eV). Once the photon energy is sufficient to cross the direct gap region (3.4 eV), the absorption coefficient increases rapidly since direct transitions are possible [76].

The absorption of photons in silicon is of particular interest for understanding how one can fabricate color sensors in this material. More specifically, the optical properties of any materials are dictated by the so-called characteristic (optical) admittance of a medium, $\boldsymbol{N} = \boldsymbol{n}\text{–}\mathbf{i}\,\boldsymbol{k}$, which is typically referred to as the "complex refractive index." Its two components are $\boldsymbol{n}$, the refractive index, and $\boldsymbol{k}$, the extinction coefficient. We are particularly interested in the latter coefficient, which is directly related to losses, i.e., the attenuation of light propagating through the respective material. Most importantly, the value of the coefficient $\boldsymbol{k}$ decreases significantly (quasi-exponentially) with the wavelength [77]. The direct consequence of this fact is that the violet-blue hues (short wavelengths) of the visible range will be extremely quickly and efficiently absorbed in an extremely thin layer situated at the top-most of the silicon substrate. As light advances deeper in silicon, the longer wavelengths (green and yellow) begin to be absorbed, though not as well

as the shorter ones, so that these colors are absorbed only mildly at a greater depth (1–3 μm from the surface). Finally, k has the smallest values for the longest wavelengths so that the red hues are only slightly absorbed in Si and at great depths (>5 μm). This means that the orange-red colors can be detected only in a very wide detecting region located deep inside the Si structure within which this radiation can be absorbed and optoelectrically converted into an electrical signal. If a normal Si substrate is used (650 μm thick for a wafer with a 6-inch diameter; even thicker for larger diameters of 8 or 12 in.), then the visible light will be totally absorbed and no transmitted component will appear.

Hence, the silicon's dependence of k on λ is the most fundamental principle on which are based all the color sensors to be subsequently presented in the next subsections. However, this dependence will also prevent practical Si-based solutions to exhibit spectral responses very similar to ideal ones, i.e., which would equally split the visible range in three domains (R, G, and B) and characterized by peaks at 450, 550, and 650 nm, each with FWHM of 100 nm. Additional mathematical transformations would need to be applied to the photogenerated currents collected at the sensor's output terminals in order to address this issue.

2.2 Color Sensing Using Standard Solutions

A pragmatic way to conceive a color sensor (especially for practical real-life imaging applications) is to fabricate it as an array of cells comprising three identical independent photosensors, each of them detecting only one primary color. Therefore, the easiest implementation of such color sensors used colored filters obtained by depositing different polymer dyes on top of neighboring photosensors [78, 79]. In the simplest case, a *pn* junction, i.e., a photodiode, can be used as a photodetector. Figure 1 shows the classical configurations used in typical photodetectors. Various other detectors may also be used depending on the desired application and the available technology used for the fabrication of the sensors and of the adjacent signal processing circuits. The same conceptual solution shown in Fig. 1b is applied, e.g., in photo cameras and camcorders that use either Charge-Coupled Devices (CCDs) or CMOS detectors.

Knowing the transmittance characteristics of the polymer dyes and the photocurrent output of each of the three photodiode elements, it is possible to calculate the unique position of the color of the incident light. However, this *classical approach* of realizing a color sensor has two important *disadvantages*:

1. *It employs polymer dyes as color filters*. This can lead to inherent variations in the color selectivity of the filters (mainly due to aging), and/or incompatibility with other technological steps, especially when smart sensors are desired to be realized.
2. *A complete color sensing cell must employ more than one single photosensing element, such as a photodiode*. A minimal number of at least three photodiodes are necessary for a complete pixel in order to provide the full RGB output, with each photodiode covered by a different filter.

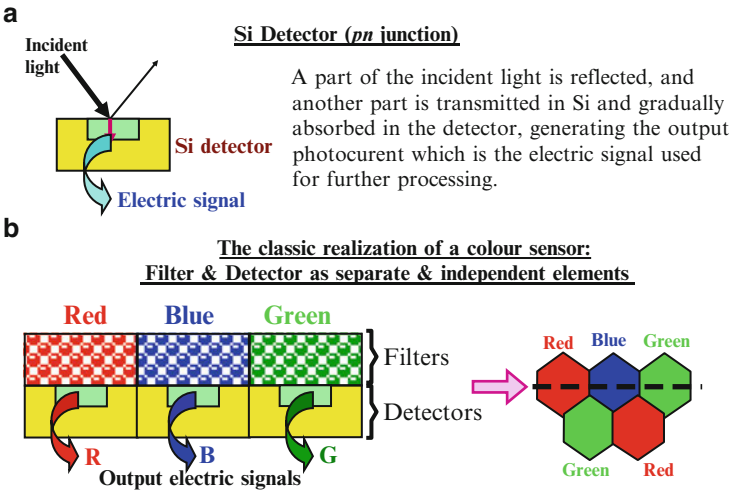

Fig. 1 Classical implementation of optical sensors: (**a**) A simple photodiode; (**b**) the principle used for typical color detection in standard imagers, e.g., video cameras. The detector represented here is a photodiode, but any optical sensor such as a CCD element or a MOS sensor can also be employed, depending on the exact architecture used to fabricate the chip

The 2D arrangement of the photodiodes and of their corresponding overlying colored (polymer) filters forms a *color filter array* (CFA) and is essential for the correct reconstruction of the original illuminated color. Numerous types of CFAs have been developed for different applications, but the most popular one is the *Bayer pattern* developed by Kodak in the 1970s, based on work in spatial multiplexing. Using a checkerboard pattern with alternating rows of filters, the Bayer pattern has twice as many green pixels as red or blue. This is justified by the greater acuity of the human eye toward detail contained in the green part of the spectrum. Hence, the green channel has more weight in generating the luminance (or brightness) data for the image [80–82]. However, special RGB-type CFAs with various alterations in the pattern and employing a different sample reconstruction algorithm had to be designed in order to better capture the properties of the human visual system [83]. Moreover, at least in this specific case of a Bayer pattern with a double number of G filters, a total of four photodiodes would be required to form a complete pixel. In any case, the increased number of photodiodes necessary to provide all RGB signals leads to a large area occupied by one entire pixel, and thus to a larger cost of the final chip, since the latter is directly proportional to the silicon "real-estate" surface. Another important drawback of the CFAs is that severe *color aliasing* can appear, due to the fact that the pixel array is a two-dimensional sampled data system. In practice, this aliasing appears as *Moiré effects* and *color artifacts* [80–82]. Technologically, this means that one can also obtain undesired Moiré effects if errors appear in the alignment/positioning of one (or more) filter(s) with respect to the underlying photodetector. Therefore, special care must be taken

in the design, as well as in the fabrication, of these structures in order to prevent such misalignments taking place.

2.3 The Simplest Color Sensing Approach: The Single Junction

The above-mentioned drawbacks of the classical CFA-based color sensing solutions required the finding of novel alternative solutions which would exhibit improved performance, without their disadvantages. This sub-section and the following ones will briefly explain how a monolithic Si-based color sensor can be realized using *pn* junctions instead of the more conventional solutions, e.g., CCDs. The essential underlying idea is to attempt to stack more junctions vertically one on top of each other. This approach should thus provide a much more compact pixel element due to two reasons: first, more detectors would be integrated in the same physical space of a single pixel, and second, the functions of filtration and detection are now merged together in a single structure. The latter feature appears because silicon's light absorption capabilities which carry out light filtering (due to the spectral dispersion of the absorption coefficient k) are now intrinsic part of the sensor together with the photo-detecting junctions.

This sub-section will present the case when the simplest detector—a single *pn* junction—is used for a color sensor, while the following sub-sections will introduce the more efficient color sensors with two or three vertically stacked junctions.

In the simplest case, just one light-detecting region, namely, a single *pn* junction, is employed. If the *p*-type top layer has a high doping level, the space-charge region will extend almost entirely into the underlying *n*-type region. Since photo-generation must take place only in the depleted region of a junction in order to separate effectively the photogenerated electron–hole pair in order to lead to electric output signals, it logically follows that one can perform color sensing with such a single junction only if the width of the junction's depleted region is modulated. This can be realized practically by fabricating the junction such that the optical absorption peaks around a wavelength in the middle of the desired spectral range. Its spectral response can be electronically tuned by varying the reverse voltage applied across the photodiode, enabling an electrical output that is thus the cumulative or successive contribution of different wavelengths. This principle can be applied in two versions: static or dynamic (at different moments in time). The information regarding the various incident wavelengths can then be extracted using subsequent signal processing.

The static version reported in the literature employed two neighboring junctions of identical depths, both of which were illuminated simultaneously by the incident light. However, different reverse biases were applied: one photodiode was kept at a constant reverse bias (hence it was denominated as reference photodiode), while the bias of other one was varied so as to find the appropriate reverse voltage value required for detecting a fixed ratio of the two photocurrents. In practice, the reference photodiode may have a much smaller exposed area than that of

the sensing one, in order to maximize the overall optoelectronic efficiency of the sensor. The imbalance in photocurrents between the two junctions caused by the unequal sensitive areas (assuming an initial identical reverse bias voltage applied on both of them) can be compensated by decreasing the bias unto the sensing photodiode (or alternatively, increasing that unto the reference photodiode), until current balance (at the desired ratio) is achieved. Practically, however, the two junctions should be identical in size in order to present identical electrical characteristics, the most important being the dark current. The difference in sensing areas (or, rather the equivalent resultant difference in photocurrents) can then be implemented either by covering a part of the reference photodiode's exposed area with an opaque material (e.g., metal), or (more conveniently) by simple subsequent signal conditioning of its photocurrent.

Therefore, the basic operation of this color sensor using two separate single junctions can be summarized as follows. The reverse voltage across the reference photodiode was held constant, while that applied unto the sensing photodiode was first adjusted using a feedback loop until the photocurrents were in balance. After obtaining stationary conditions, the variation in the reverse voltage across the sensing photodiode (as dictated by the feedback) could be monitored continuously. An almost linear relation between the reverse voltage ratio and the "average color" of the incident light was then obtained. Further elements were added in the subsequent version to boost the performance of the sensor [84–87].

A first modification of the static concept employed just a single junction as sensing photodiode, and the bandpass central wavelength of it spectral response was electronically tunable by varying the applied reverse voltage, while readout circuits integrated in the same chip enabled easy decoding of the incident light by providing an output proportional to its "average" color [88–90].

Starting from the latter type of realization, we can easily propose a fully dynamic approach in which the reverse bias of the photodiode would be scanned periodically using a sawtooth voltage. The colorimetric information could then be extracted using complex subsequent signal processing of the photocurrents detected at different moments in time. The concept is illustrated in Fig. 2.

However, one can also easily realize that the single-junction-based color sensors are characterized by some severe drawbacks:

1. The static approach (i.e., with a depleted region of constant width in time) can provide an output dependent on the "average" color, not a truly RGB output. Moreover, the presence of an additional reference junction next to the color sensing one defeats the purpose of minimizing the area occupied by a single pixel necessary to provide the desired colorimetric output.
2. When the depleted region's width is varied in the proposed dynamic approach, since a single absorption curve is used (although dynamically modified by the periodically varying reverse bias modulation), one cannot achieve a very good selectivity between very different wavelengths.