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## Optical sensors (optodes) for multiparameter chemical imaging: classification, challenges, and prospects

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Chemical gradients and uneven distribution of analytes are common in natural and artificial systems. As a result, the ability to visualize chemical distributions in two or more dimensions has gained significant importance in recent years. This has led to the integration of chemical imaging techniques into all domains of analytical chemistry. In this review, we focus on the use of optical sensors, so-called optodes, to obtain real-time and multidimensional images of two or more parameters simultaneously. It is important to emphasize that multiparameter imaging in this context is not confined solely to multiple chemical parameters (analytes) but also encompasses physical (e.g., temperature or flow) or biological (e.g., metabolic activity) parameters. First, we discuss the technological milestones that have paved the way for chemical imaging using optodes. Later, we delve into various strategies that can be taken to enable multiparameter imaging. The latter spans from developing novel receptors that enable the recognition of multiple parameters to chemometrics and machine learning-based techniques for data analysis. We also explore ongoing trends, challenges, and prospects for future developments in this field. Optode-based multiparameter imaging is a rapidly expanding field that is being fueled by cutting-edge technologies. Chemical imaging possesses the potential to provide novel insights into complex samples, bridging not only across various scientific disciplines but also between research and society.

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### Introduction

“A picture is worth a thousand words.” It is sayings like this that demonstrate how important visual representations are for us as humans and for that matter scientists. It is much easier to understand if we can see something with our own eyes. Maybe, therefore, chemical imaging has become so popular. Using as diverse methods as mass spectrometry,<sup>1,2</sup> Raman spectroscopy,<sup>3,4</sup> or X-ray spectroscopy,<sup>5,6</sup> scientists have developed approaches to visualize chemical elements or molecules in 2 or even 3 dimensions. While vastly different in terms of the underlying analytical methodology, all those methods can be referred to in one way or the other as chemical imaging. It is beyond the scope of this review to address all these methods, rather this review focuses on chemical imaging using optical sensors, so-called optodes.

Contrary to several other chemical imaging methods, optodes often enable the real-time visualization of small molecules (gasses and ions) and often use rather cheap instrumen-

tation. Furthermore, optode-based chemical imaging can also be used to image multiple analytes and parameters at a time. This multiplexing possibility will be at the center of this review. The ambition is to identify and describe different approaches to obtain multiparametric images and to discuss the respective advantages and challenges.

In the field of chemical imaging, it is crucial to establish a clear definition of sensors and their application. There exist various interpretations of the term “chemical sensors”; however, we will focus on two definitions that are relevant to our discussion. The first one is the “Cambridge” definition: “Chemical sensors are miniaturized devices that can deliver real-time and online information on the presence of specific compounds or ions in even complex samples”.<sup>7</sup> The second one implies that sensors aim to “obtain information about the substantial matter, especially about the occurrence and amount of constituents including information about their spatial distribution and their temporal changes”.<sup>8</sup> Thus, chemical sensors should be designed and utilized in a manner that provides information on the spatial distribution of the analyzed parameter(s), *i.e.*, provides the possibilities for chemical imaging.

Chemical sensors are categorized based on their operating principles, and one of those is based on utilizing optical

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signals. Optodes convert changes in analyzed parameters (e.g., analyte concentration) into changes in optical phenomena. “Compared to several other imaging techniques, optodes can often be applied in a non-destructive way and respond almost in real-time with typical response times in the range of seconds to minutes and in a reversible fashion. Furthermore, optodes are flexible in their design, allowing chemical imaging over a wide range of scales from single cells (sub- $\mu\text{m}$ ) to larger organisms or small ecosystems ( $\text{dm} \times \text{dm}$ )”.<sup>9</sup>

One of the main advantages of optodes is the possibility of multiparameter imaging at the exact same spot and time. In this review, we use the term “multiparameter” as the synonym for “multianalyte”, “multiple”, and “multiplexed”. Conducting chemical imaging with optodes for multiparameter sensing requires addressing various challenges. Those challenges arise as chemical imaging is a highly interdisciplinary field building on developments in fields such as electronic noses/tongues,<sup>10</sup> organic synthesis,<sup>11</sup> chemometric data processing,<sup>12</sup> colorimetric sensor arrays,<sup>13</sup> device development,<sup>14</sup> etc. In this regard, chemical imaging requires bringing all those aspects together and even further utilizing them to address biological or medical questions.

In 2007, Stefan Nagl and Otto S. Wolfbeis claimed the features of true multiparameter sensors:<sup>15</sup>

- (a) Two or more parameters can be sensed at the very same site and simultaneously;
- (b) They enable chemical sensors to be compensated for the effects of temperature if combined with a temperature sensor chemistry;
- (c) They enable unspecific sensors to be made more specific;

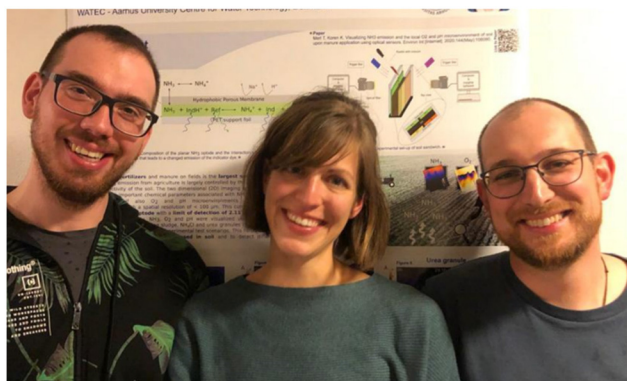
(d) They enable smaller sample volumes to be analyzed for more parameters than with monosensors;

(e) They enable the sensing of several parameters in cases where the sample volume is limited (such as in the case of blood), or in the case of microscopy or microfluidic devices.

For the last 15 years, several comprehensive reviews of imaging with optical sensors and multiparameter analysis have been published (Table 1). This review will focus on the systemization of the present and generally possible approaches to multiparameter chemical imaging with optodes rather than duplicate previously published information.

## Historical overview

To provide robust two-dimensional (2D) imaging, planar optical sensors have been utilized for almost three decades. The first “conventional” imaging setup, which was devoted to  $\text{O}_2$  determination in benthic communities, was introduced by Glud *et al.* in 1996.<sup>22</sup> However, only one analyte could be measured in 2D at that time. Since then, a lot of progress in this field has been made including approaches to provide simultaneous determination for two or more analytes. Nevertheless, before delving deeper into multiparameter imaging, let us see how it became possible to develop the first imaging setup based on optodes. To do that, we would like to indicate three main sections, the advancement of which made Glud’s work possible eventually: *Fluorescence and optode development*, *Sensitive paint utilization and dual sensing*, and *CCD and CMOS implementation in chemistry* (Fig. 1).



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(from left to right)

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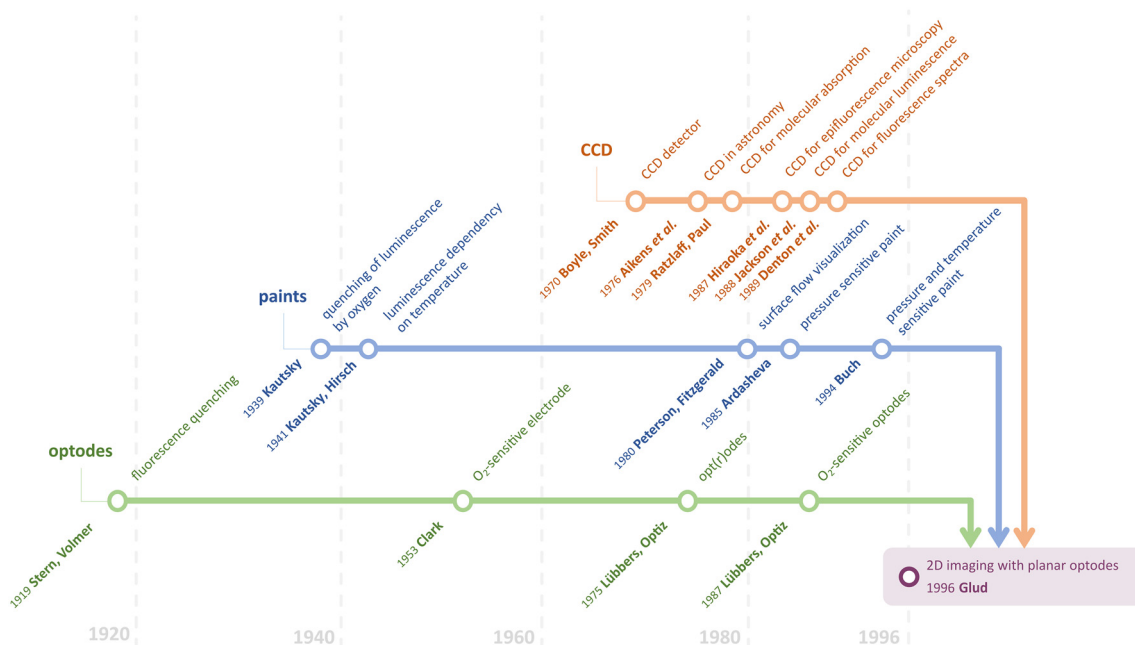
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Klaus Koren obtained his PhD from Graz University of Technology, Austria, under the supervision of Prof. Ingo Klimant in 2012. After working as a postdoc in the laboratory of Prof. Michael Köhl (Copenhagen University, Denmark) he started his independent research group in 2018 at Aarhus University. His research focuses on the development and application of chemical sensors to better understand complex biological systems. Klaus has published more than 60 peer-reviewed scientific articles and received the Fritz-Feigl prize from the Austrian Society of Analytical Chemistry in 2021.

**Table 1** The most important reviews of imaging with optical sensors published since 2007

Title	Authors	Year	Summary	Ref.
Optical multiple chemical sensing: status and current challenges	Stefan Nagl, Otto S. Wolfbeis	2007	The first systematic review of true dual optode used for imaging. The authors envisaged introducing triple sensors and sensing schemes for important analytes such as O <sub>2</sub> , pH, CO <sub>2</sub> , and temperature	15
Multiple fluorescent chemical sensing and imaging	Matthias I. J. Stich, Lorenz H. Fischer, Otto S. Wolfbeis	2009	A critical review of the state of the art in terms of spectroscopic principles and materials (mainly indicator probes and polymers), and gives selected examples for dual and triple sensors	16
The art of fluorescence imaging with chemical sensors	Michael Schäferling	2012	A review of the basic functional principles of fluorescent probes, the design of sensor materials, and approaches of multiple sensors development and methods for their signal readout	17
Optical methods for sensing and imaging oxygen: materials, spectroscopies and applications	Xu-dong Wang, Otto S. Wolfbeis	2014	A comprehensive review of optical methods for sensing oxygen including spectroscopies and readout schemes in luminescent sensors and multiple sensing techniques	18
Planar optode: a two-dimensional imaging technique for studying spatial-temporal dynamics of solutes in sediment and soil	Cai Li, Shiming Ding, Liyuan Yang, Qingzhi Zhu, Musong Chen, Daniel C.W. Tsang, Gen Cai, Chang Feng, Yan Wang, Chaosheng Zhang,	2019	A comprehensive overview of the research progress on 2D imaging with planar optodes in sediment and soil including the progress in multiparameter imaging	19
Optical sensing and imaging of pH values: spectroscopies, materials, and applications	Andreas Steinegger, Otto S. Wolfbeis, Sergey M. Borisov	2020	The first comprehensive review of methods and materials for use in optical sensing of pH values and on applications of such sensors including commonly used optical sensing schemes, luminescent sensors design, imaging of pH values, and selected applications of these sensors	20
Optode based chemical imaging—possibilities, challenges, and new avenues in multidimensional optical sensing	Klaus Koren, Silvia E. Zieger	2021	A perspective article on novel state-of-the-art technologies for optode-based chemical imaging including the discussion of possibilities for multiparameter imaging	9
Fiber optic sensor designs and luminescence-based methods for the detection of oxygen and pH measurement	Jan Werner, Mathias Belz, Karl-Friedrich Klein, Tong Sun, K.T.V. Grattan	2021	A comprehensive review of luminescence-based fiber optical sensors, sensing methodology, optical sensor platforms, and multiple luminescence sensing concepts	21

**Fig. 1** Timeline of milestones in the development of the first imaging setups based on optodes.

## Fluorescence and optode development

The journey to optode development might have begun in 1919 when Stern and Volmer published a theory and equation relating the change in fluorescence quantum yield and fluorescence lifetime as a function of added quencher.<sup>23</sup> Twenty years later, Kautsky provided reports on the possibility of observing the luminescence quenching effect using oxygen.<sup>24</sup> Nowadays, it is generally accepted that almost all the phosphorescence and fluorescence dyes are quenched by molecular oxygen.<sup>18,25</sup> Therefore, it is no coincidence that the first implementation of imaging with optodes was devoted to oxygen determination.

The term “optode” was introduced by Lübbers and Opitz in 1975.<sup>26</sup> By analogy to electrodes, they called such an optical sensor with an indicator film separated by a membrane from the sample an optode. The latter is a reason optodes might still be called opt(r)odes since it is a combination of two words “optical” and “electrode”. They began the investigation of optical measurement of gas pressures (especially oxygen) using fluorescent dyes. It appeared to be a bifurcation point in the era of dissolved and gaseous oxygen measurements predominantly done using Clark electrodes<sup>27</sup> since the O<sub>2</sub> optode did not consume oxygen and a reference electrode was not required anymore. In 1987, Opitz and Lübbers published a comprehensive overview of the theory and development of fluorescence-based optochemical oxygen sensors.<sup>28</sup> In this article, they demonstrated the superiority and perspective of using optodes in general.

## Sensitive paint utilization and dual sensing

The first sensor most similar to 2D optodes was sensitive paint.<sup>29</sup> Pressure-sensitive paints (PSPs) have been developed for measuring air pressure on surfaces. The response mechanism of PSPs is also based on luminescence quenching by molecular oxygen, which was established by Kautsky. Peterson and Fitzgerald demonstrated a preliminary approach for surface flow visualization using spray-coating on shapes of interest in 1980.<sup>30</sup> This approach led to the development of PSPs in 1985 by Ardasheva *et al.*<sup>31</sup> Since that time, it has become possible to determine at least one of the quantitative parameters in 2D. However, this emerging field of chemical imaging unintentionally unlocked the possibility of performing simultaneous dual measurements.

In 1931, Kautsky and Hirsch obtained luminescence dependency on temperature.<sup>32</sup> Although the basis of using luminescence probes for dual sensing had been known for many years, one of the first reports was published only in the 1990s. Buck suggested using perylene dye sorbed on silica to perform simultaneous luminescence pressure and temperature measurements using paints.<sup>33</sup> Despite the possibility of dual sensing with optical sensors, the temperature cross-sensitivity was a significant interfering factor for numerous real applications. Thus, researchers tried not to utilize this feature of the luminescent dyes but to eliminate it. Coyle and Gouterman proposed a method of correcting lifetime measure-

ments for temperature by adding a non-oxygen quenched, temperature-dependent phosphor.<sup>34</sup>

## CCD and CMOS implementation in chemistry

Unarguably, modern chemical imaging is not possible without digital imaging devices. Widely used devices such as monochrome, color, hyperspectral cameras, or scanners are based on the three main sensor types and device architectures, namely, CCDs (charge-coupled devices), CMOS (complementary metal-oxide-semiconductor) and CIS (contact image sensor). To trace the path that led Glud *et al.* to utilize the CCD camera, let us turn to the beginning.

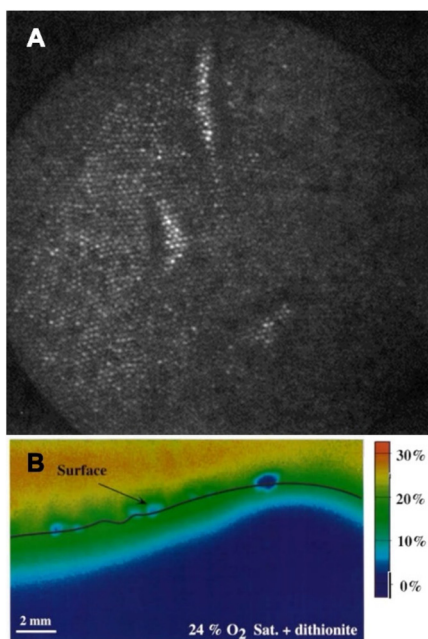
Boyle and Smith developed the concept of a charge-coupled detector in 1969.<sup>35,36</sup> The scientific applications of CCDs were first demonstrated in astronomy research in 1976.<sup>37</sup> However, a chemical application was first demonstrated by the CCDs' utility in a spectroscopic measurement; Ratzlaff and Paul employed a linear CCD for molecular absorption in 1979.<sup>38</sup> They showed that the CCD-produced signals are linear with both intensity and integration time in the visible region. For the proof-the-concept, the absorbance spectrum of the calibration tool, the didymium oxide filter, was recorded within 250–750 nm. Subsequently, the high sensitivity of CCDs made these devices well-suited to molecular luminescence measurements. The properties of CCDs and their application to the high-resolution analysis of biological structures by optical microscopy were shown for three-dimensional imaging using an epifluorescence microscope in 1987.<sup>39</sup> In 1988, the first 2D visualization of fluorescently labeled objects was reported by Jackson *et al.*<sup>40</sup> Furthermore, Denton *et al.* demonstrated the acquisition of fluorescence spectra for the first time in 1989.<sup>41</sup>

Although it is Glud's optode design (Fig. 2B) that one could consider as “conventional” now, the combination of CCD camera and optical sensor fibers was reported by Bronk *et al.* a year earlier, in 1995.<sup>42</sup> The authors used approx. 6000 optical fiber-sensors in a bundle with a 4 μm spatial resolution (Fig. 2A).

Nowadays, although CCD image sensors are still used in some high-end cameras and scientific applications, CMOS sensors, invented in 1963 by Wanlass and Sah,<sup>43</sup> have emerged as the predominant technology in consumer electronics. This transition has primarily occurred due to the cost-effectiveness, energy efficiency, and rapid processing capabilities offered by CMOS sensors.

## The road to multiparameter sensors

Thus, both improving knowledge of physicochemical processes and technological advancements played pivotal roles in devising strategies for chemical imaging. Nearly three decades have passed since 1996. Consequently, what are the most significant changes that have occurred during this period? It is challenging to precisely identify these changes; however, we would like to highlight some we consider essential, namely: (1) advancing chemical materials and utilizing different modes of



**Fig. 2** Different designs of multiparameter optodes. (A) Fiber-bundle sensor design: polyHema/fluorescein-modified imaging fiber with mouse fibroblast cells.<sup>42</sup> (B) Planar 2D sensor design ("Glud's design"): calibrated images of the O<sub>2</sub> distribution at the sediment-water interface with sodium dithionite grains added.<sup>22</sup> Reprinted with permission from ref. 42. Copyright 1995 American Chemical Society.

signal formation/readout, (2) using different approaches to create optodes including nanoparticle formation, (3) utilizing technologies beyond monochrome CCDs including color and hyperspectral devices. Eventually, the combination of these factors has led to a push towards multiparameter imaging.

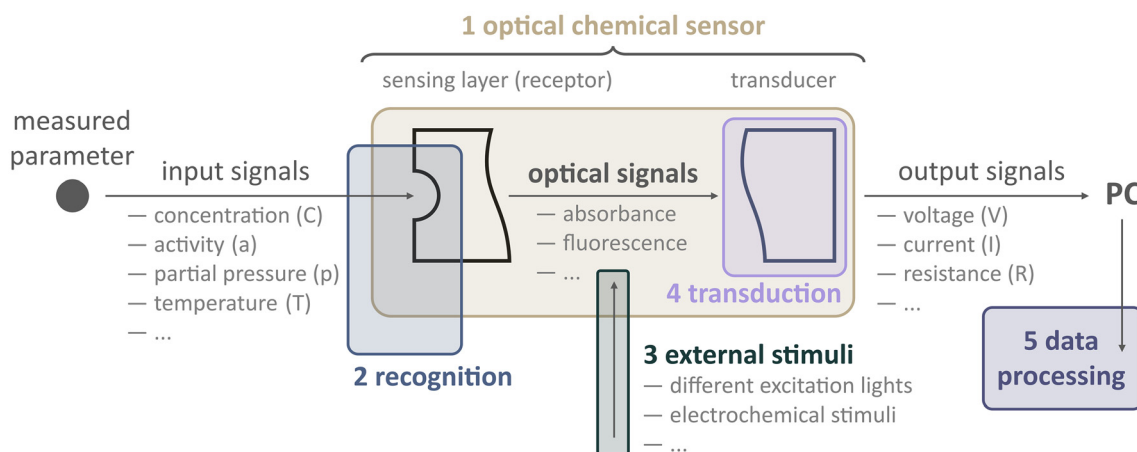
Undoubtedly, the latter is paramount to gaining a more comprehensive understanding of the analyzed system, especially where the distribution of the analytes measured

simultaneously might reveal any intimate interplays between them. For instance, the dual determination of oxygen and carbon dioxide at the same time and in the same spot could shed light on oxidative respiration and photosynthesis process differentiation. This problem was in fact tackled by the first created dual imaging setup based on optodes.<sup>44</sup>

Before diving into examples, we must ask, how can one create a multiparameter optical sensor in general? To address this question, let us look closer at what an optode is as well as what components and steps are involved to obtain physico-chemical information from samples (Fig. 3).

Optodes are optical sensors that translate information about samples into optical analytical signals. The type of information could be different: from physical (temperature, partial pressure) to chemical (analyte concentration or activity). We would like to underline that an optode should be considered not as a probe or sensing film *per se* but rather as a compound device. Conventionally, an optode includes two parts: a sensing element (receptor) and a transduction element (transducer). The receptor provides the recognition of a parameter being analyzed, *e.g.*, the concentration of a measuring analyte, temperature, pH, gas partial pressure, *etc.* Providing that the optode changes at least one of the optical properties (absorption, fluorescence, reflectance, refractive index, optical activity, surface plasmon resonance, *etc.*) depending on the magnitude of the parameter. The transducer converts the optical signal generated by the receptor (the actual concentration value, a non-electric quantity) into a measurable signal that is appropriate for processing, *i.e.*, an electric quantity: voltage, current, or resistance.<sup>8</sup>

Consider, for instance, the first 2D imaging setup described by Glud *et al.*<sup>22</sup> In this case, the sample was a marine sediment, and the authors were interested in measuring O<sub>2</sub> concentration over both space and time. To achieve this, they prepared an O<sub>2</sub>-sensitive luminescent optode. The receptor of this optode kind consists in its most primitive form of an oxygen-



**Fig. 3** Schematic depiction of measurements conducted using optical chemical sensors. The color blocks and numerical annotations indicate the specific sites enabling the simultaneous analysis of multiple parameters, presented in the order listed (see text for more details). PC denotes the personal computer.

sensitive dye (indicator) dispersed within a host polymer, which is applied onto a transparent surface, such as a glass plate or polymer foil.

In addition to those basic components, others can be added, like enhancers (scatters like titanium dioxide or diamond particles) and additional dyes (for referencing). Furthermore, this optode layer might be protected by an optical isolation layer, which serves to minimize the influence of ambient light on the sensor while not interfering with the chemical receptor.

When this optode comes into contact with the sample, excitation light is needed to excite the luminescence of the dye, and a detector (*e.g.*, camera) to record the emission of the receptor. Filters are employed to ensure that only the analytically relevant light reaches the camera. In summary, a basic chemical imaging starter kit needs some sensor chemistry, including indicators and a host polymer, a light source, filters, and a camera. Remarkably, this is the very same configuration that Glud and co-workers used in their pioneering work, and it continues to be employed today in similar research.

In addition to this conventional setup, in recent years, researchers have explored the use of external stimuli to enhance data collection from sensors. In this context, an external stimulus refers to any external changes or influences that can impact the response of a sensor. For instance, the luminescence of a sensor can be affected by temperature changes. Therefore, altering the temperature during analysis can lead to variations in the sensor response. Additionally, when using a luminescence-based optode, it is necessary to use excitation light to trigger the sensor. Let us assume that an optical sensor consists of several indicators. Changing the excitation wavelength, in this case, results in altering the emission spectra of the optical sensor. Consequently, applying external stimuli causes the measurement signal to depend not only on the parameters being analyzed but also on the nature of the stimulus itself. By considering these factors, the use of external stimuli opens possibilities for distinguishing between different analytes or their compositions. This, in turn, facilitates multiparameter analysis.

Thus, to enable simultaneous analysis of multiple parameters, various approaches can be classified based on the processes or designs involved, as shown in Fig. 4. These approaches include (1) sensor arrays, (2) dual/multiple recognition, (3) changing external stimulus, (4) multiple transduction, and (5) utilizing chemometric techniques.

### Multispot sensors and sensor arrays

Multispot sensors and arrays are composed of single sensor systems for given analytes, merged and miniaturized to a compact optical sensing device.<sup>16</sup> Nowadays, the most common approaches to making sensor arrays are using optical fibers (fiber bundles)<sup>21</sup> and the microspheres of optical sensors in microwells.<sup>45</sup> Recently, a similar technique to the last one has been widely utilized in tissue profiling approaches.<sup>46</sup> The recent comprehensive review on the devel-

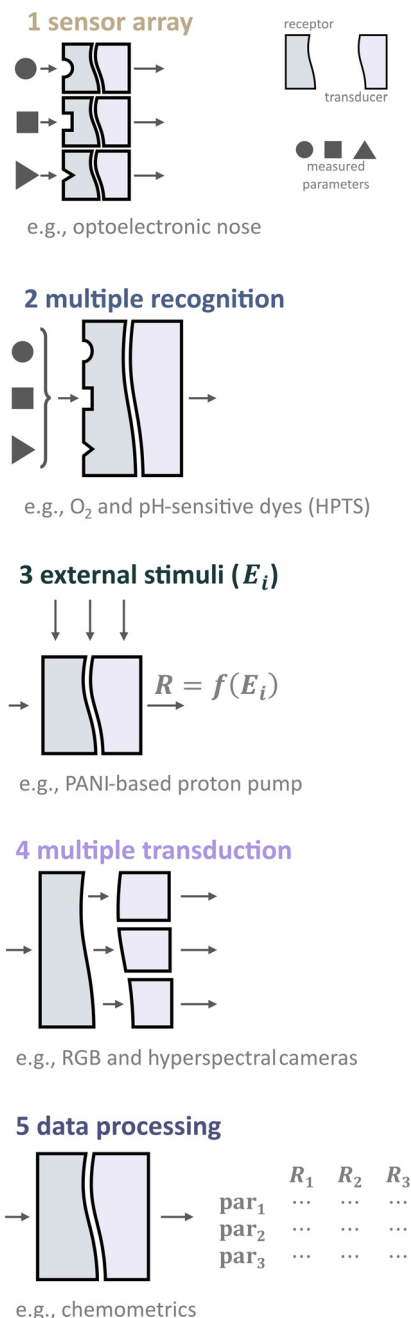


Fig. 4 Designs of optical sensors and involved processes provide multiparameter analysis.

opment and state-of-the-art of colorimetric and fluorometric sensor arrays has been published in 2019.<sup>13</sup>

Although this approach for simultaneous measuring several parameters is cost-efficient and relatively easy, sensor arrays do not fulfill the requirement of determination of analyzed parameters in the same compartment. Usually, the distance between the adjacent elements is 5–20  $\mu\text{m}$ ; however, depending on the array geometry, measurements can be up to several 100  $\mu\text{m}$  apart. This can be considered a large distance, in particular in systems where steep chemical gradients form like within biofilms.<sup>47</sup>

## Recognition

As mentioned, recognition is the first step to obtaining sensor response. Typically for planar optodes, the receptor function is fulfilled by a thin layer that can interact with analyte molecules or participate in a chemical equilibrium together with the analyte.<sup>8</sup> For example, the most important processes underlying the response of ion-selective optodes are ion exchange and co-extraction in the case of cation- and anion-selective optodes, respectively.<sup>48</sup>

One of the key properties of the recognition phase of optode response is selectivity. Selectivity is the ability of the sensor to respond preferentially to the analyte but not to an interfering species or interfering parameter. However, there is no one absolutely selective sensor, and some cross-sensitivities are generally always present. Should one acknowledge such cross-sensitivities as a possible source for multiparameter analysis? We think—yes—if any approaches allow the determination of both the analyzed and the interfering parameters separately.

**Optical detection schemes.** There are various approaches to a read-out of analytical signal occurring due to the changes of optical properties: absorption/reflectance, luminescence intensity, luminescence decay time, two-wavelength referencing, dual lifetime referencing (DLR), Förster resonance energy transfer (FRET).<sup>20</sup> While absorption is often considered a straightforward choice for chemical imaging due to its simple instrumentation, techniques that rely on luminescence intensity and decay time are currently more prevalent. In this section, we want to discuss the possibility of utilizing these two techniques individually and their combinations.

**Luminescence intensity.** In luminescent sensors, the excitation of an indicator dye leads to the emission of photons either *via* fluorescence or phosphorescence. Those photons generally have a lower energy (longer wavelength) than the excitation light and the amount of those photons (intensity) can be correlated to an analyte of interest. Take for example the classical optical oxygen sensor. Paramagnetic O<sub>2</sub> is an effective luminescence quencher resulting in reduced indicator emissions with increasing oxygen concentrations.<sup>18</sup> In this way the intensity of the indicator emission can be correlated to the O<sub>2</sub> concentration following *e.g.*, the Stern–Volmer relation.

Despite this being a very straightforward approach relying solely on luminescence intensity can be problematic as also other factors than the analyte concentration can influence this parameter. Those factors include fluctuations in the excitation light, bleaching of the indicator, or simply uneven distribution of the indicator within the sensor.<sup>49</sup> Currently, these limitations are often overcome by adding a second, inert, dye into the sensor matrix.<sup>50,51</sup> In this way the emission of the two dyes can be set into proportion (ratio) enabling a more robust readout as several of the factors mentioned above can be eliminated or reduced. At the same time, the use of an extra reference dye comes with challenges in terms of multiparameter imaging. Separating the reference signal from the indicator signal is one of those.

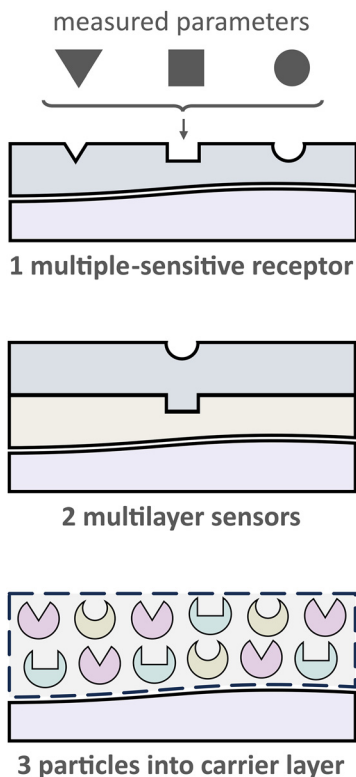
**Luminescence decay time.** Contrary to the above, decay time-based imaging does not use the amount of photons to quantify an analyte, but rather another property of the emitted photons, their decay time. The decay time represents the average duration between photon excitation and emission and can range from nanoseconds to milliseconds, depending on the indicator and the type of luminescence used (*e.g.*, phosphorescence or fluorescence). Measuring decay time offers several advantages over intensity-based methods. Firstly, the decay time is not affected by the concentration of the indicator. No additional referencing (additional dye) is needed as decay times as such is intrinsically referenced. Additionally, fluctuations in the optical setup have less impact on the measurement.<sup>49</sup>

On the flip side, chemical imaging based on decay times requires somewhat more sophisticated equipment. Essentially, cameras used for this approach need to be able to measure (time) when photons are emitted relative to the excitation, either in the time or frequency domain. In a recent article, we evaluated those approaches, and the interested reader is referred to this study.<sup>52</sup> While being technically more challenging, decay time-based imaging enables the separation of multiple signals not only on spectral but also temporal domain. This capability holds significant potential for applications in multiparameter imaging.<sup>53</sup>

**Combination of luminescence and decay time.** It is also possible to combine different detection schemes. For instance, HPTS (8-hydroxypyrene-1,3,6-trisulfonic acid) was used to measure both pH and O<sub>2</sub>.<sup>54</sup> In order to measure pH and the concentration of oxygen, various optical properties were measured. Specifically, pH was determined by analyzing the ratio of intensities between the isobestic point and the emission peak of the HPTS anion. Meanwhile, oxygen concentrations were measured based on fluorescence decay time, which decreased as the oxygen concentration increased.

**Individual/dual/multiple recognition.** The single parameter sensing strategy employs one receptor usually, such as a pH indicator for the pH-optode,<sup>55</sup> oxygen-sensitive luminophores for the O<sub>2</sub>-optode,<sup>51</sup> *etc.* However, to address dual or multiparameter analysis, it is necessary to utilize more than one receptor or recognition process. We differentiate these options into three categories (see Fig. 5): (1) receptors that are sensitive to several parameters, (2) several single-selective receptors in two- or multilayer sensors (known as “sandwich sensor”), and (3) the incorporation of several single-selective receptors into a single-layer sensor (more often (nano)particles into polymer binder).

**2.2.1. Receptors that are sensitive to several parameters.** One evident and desirable solution for multiparameter sensing is using chemicals that are sensitive toward at least two parameters each with a distinct influence on the optical properties, either spectral or *via* decay time (Fig. 5). It is generally challenging to find a suitable dye or pigment to measure two or more parameters simultaneously. However, one can argue that one natural additional parameter for measuring is temperature as it affects every sensor. Nevertheless, the latter enables tempera-



**Fig. 5** Schematic of the three most common designs for creating multiparameter optical sensors *via* different recognition options: (1) multiple-sensitive receptor, (2) multilayer sensors, (3) (nano)particles into carrier layer.

ture compensation which could be essential to reduce measurement errors.

Typically, the fluorescence intensity or fluorescence decay time of a single luminophore is used for multiparameter sensing. In section 2.1.3, HPTS is shown as an example of a probe selective to  $O_2$  and pH simultaneously. Other indicators for the determination of two parameters (pH/ $O_2$ , pH/T,  $O_2$ /T,<sup>56</sup>  $CO_2/O_2$ , pH/ $Cl^-$ ) were proposed. More details can be found in section 10.7 of ref. 20.

Nevertheless, other types of luminescence are also used to achieve multiparameter sensing. Schwendt and Borisov showed that the simultaneous monitoring of  $O_2$  and temperature can be achieved with a single metalloporphyrin-based indicator through thermally activated delayed fluorescence (TADF) and phosphorescence.<sup>57</sup>  $O_2$ /T determination was demonstrated for a naphthoquinone-extended Pt(II) benzoporphyrin embedded in polystyrene, which operated within temperature region from 5 to 55 °C and oxygen partial pressure from 0 to 202 hPa.

Recently, diazobenzocrowns with pyrrole residue as a part of the macrocycle were utilized as a dual  $Pb^{2+}$ /pH indicator.<sup>58</sup> The authors suggested using absorption followed by digital color analysis. Both pH and lead(II) concentration influence optical signal: increasing lead(II) concentration leads to the color change from pink to blue-gray.

In addition, there are functional synthetic probes for multiparameter sensing.<sup>11</sup> Although it is possible to distinguish three main amino thiols (homocysteine, cysteine, glutathione) due to producing three distinct products and fluorescent emission signals in blue, green, and yellow channels, it was demonstrated for imaging in live cells only.<sup>59</sup> Thus, there is enough room for collaboration with organic synthesists and for implementing such probes for planar 2D imaging of similar analytes.

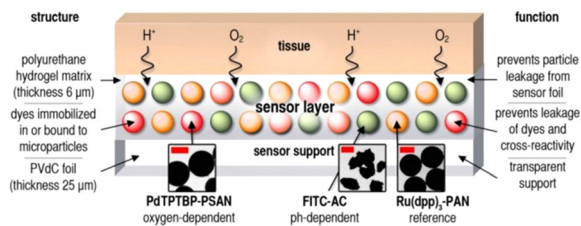
**2.2.2. "Sandwich" sensors.** The scarcity of existing probes for multiparameter sensing *via* a single dye can be overcome by utilizing multilayer sensors (Fig. 5). This kind of sensor is based on the use of two or more layers, each of which contains single-selective receptors. Besides that, different analytical signals from each layer could be utilized to differentiate various parameters. Nevertheless, even though "sandwich" sensors allow to performing true multiparameter sensing at identical sites, there are some drawbacks. Stefan Nagl and Otto S. Wolfbeis revealed these disadvantages as follows:<sup>15</sup> (1) cross-sensitivities concerning the analyte as well as spectral crosstalk including FRET and PET processes, (2) cross-leaching of the components between layers, and (3) increased photo-decomposition and signal drift compared to single sensors.

Despite all the drawbacks, the rather easy fabrication of such multilayer sensors and the increasing popularity of cameras with >3 color channels are the main reasons for the increased use of this sensor type.<sup>60</sup>

**2.2.3. Polymer binder with (nano)particles suspension.** An effective solution to address the challenges described above involves compartmentalizing the sensing chemistry for specific applications. This can be achieved by preparing nano- or micro-particles that are sensitive to a specific analyte.<sup>61,62</sup> In this way, each particle can be designed to provide a favorable microenvironment for the respective sensor chemistry. These multiple particle-based sensors can then be combined into a single layer (see Fig. 6). To hold these particles together, a suitable binding material, or "binder", is needed.

Hydrogels are particularly suitable for this purpose due to their biocompatibility and hydrophilic nature, which helps prevent the often hydrophobic components of the particles from leaching into the surrounding matrix.<sup>15</sup> This approach has enabled the development of multiparameter imaging techniques for various applications. For instance, the collaboration between the research group led by Wolfbeis and medical professionals has demonstrated how extracellular pH gradients and tissue hypoxia affect wound healing in chronic wounds.<sup>63</sup> Those insights have been made possible by using a dual  $O_2$ /pH optode employing three types of particles within the same matrix (Fig. 6).

Importantly, this innovative approach is not limited to medical applications, as shown by Fischer *et al.*<sup>64</sup> In this study, a technique involving spray painting was utilized to color complex structures with a mixture of  $O_2$  and temperature-sensitive particles. This novel pressure-sensitive paint method offers the advantage of easy application through spray painting and straightforward removal by washing the coating off with water. The proposed approach is more environmen-



**Fig. 6** Scheme of multiparameter (pH,  $O_2$ , and T) optical sensor with (nano)particles suspension.<sup>63</sup> Oxygen-dependent palladium(II)-meso-tetraphenyltetraabenzoporphyrin incorporated in oxygen-permeable poly(styrene-co-acrylonitrile) particles (Pd-TPTBP-PSAN), pH-sensitive fluorescein-isothiocyanate bound to aminocellulose particles (FITC-AC), and the pH-independent reference dye ruthenium(II)-tris(4,7-diphenyl-1,10-phenanthroline) in oxygen-impermeable polyacrylonitrile particles ( $Ru(dpp)_3$ -PAN). Microparticles were embedded in a polyurethane hydrogel matrix on poly(vinylidene-chloride) (PVDc) foils. Dye leakage was prevented by binding the dyes to or incorporating them into micro-particles. By embedding the particles in the hydrogel, leakage of particles from the sensor was also prevented. The insets show transmission electron-microscopic (TEM) pictures of the sensor particles (bars: left and right image 0.5  $\mu m$ , middle image 2  $\mu m$ ).

tally friendly while still providing high-resolution images for monitoring of  $O_2$  and T.

### External stimuli

External stimuli could be used to alter the response signal of the sensor. For example, the analytical signal might be dependent on a stimulus such as temperature, pressure, pH, humidity, or light. One could implement this dependency deliberately by incorporating specific materials or components into the optode matrix that are sensitive to these external stimuli. These components can then trigger a change in the optical properties of the sensor, resulting in a measurable change in the response signal. However, conventionally used dyes and other active components could also be sensitive to changes in temperature, pH, humidity, etc.

Moreover, the use of external stimuli can provide a way to control and manipulate the sensing properties of optical sensors, creating more versatile platforms for multiparameter imaging. By tuning the external parameters, the response signal or readout of the sensor can be controlled, providing the ability to discriminate different parameters or to enhance the sensitivity and selectivity of the sensor.

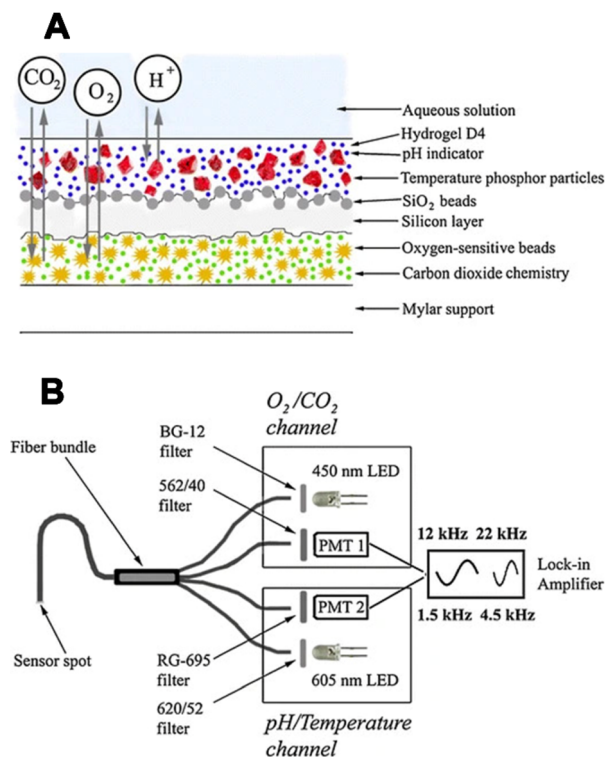
**Using masking agents.** The most obvious external stimulus is using masking agents to eliminate every interfering parameter due to chemical reactions occurring with it. This technique is well established in classical “wet” analytical chemistry and especially in the determination of ionic species. For example, benzothiazole calix[4]arene-based optodes were used for dual  $Ag^+$  and  $Hg^{2+}$  detection.<sup>65</sup> For the determination of  $Ag^+$  in the presence of  $Hg^{2+}$ , the interfering effect of  $Hg^{2+}$  was successfully eliminated by adding EDTA at a pH of 5.0. Otherwise, eliminating the  $Ag^+$  was done by using a NaCl solution that produced  $AgCl$  precipitation.

Unfortunately, this approach is concerned with the destruction of the sample being analyzed. Furthermore, in samples

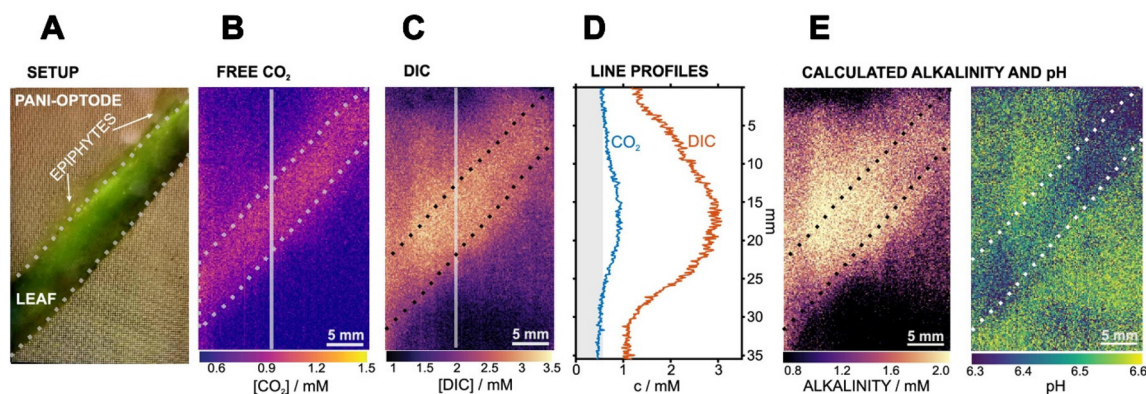
containing intricate matrices, such as blood, the introduced chemicals may easily alter the properties of the sample itself, thus resulting in the infeasibility of analysis or significant inaccuracies in the interpretation of the results.

**Alteration excitation wavelength.** The fluorescent response of most optodes is influenced by the excitation light. For example, using “sandwich” optical sensors is limited due to the overlapping of emission spectra or luminescent lifetimes of dyes being used. Although this issue could be solved for optodes with double sensitive layers, creating optodes for sensing four or more parameters using appropriate chemicals remains a complex task. To address the problem of distinguishing between the emission spectra of different luminophores, the use of dyes with distinct excitation spectra can be employed.

Borisov *et al.* introduced the first quadruple luminescent sensor for measuring  $O_2$ ,  $CO_2$ , pH, and temperature.<sup>66</sup> The multilayer sensor combined two spectrally independent dually sensing systems; the latter was possible due to the use of color filters and two different excitation lights: a blue LED (450 nm) for  $CO_2/O_2$  and a red LED (605 nm) for pH/temperature sensitive layer. The schemes of the sensor and measuring setup are shown in Fig. 7.



**Fig. 7** The first quadruple luminescent sensor for measuring  $O_2$ ,  $CO_2$ , pH, and temperature.<sup>66</sup> (A) Cross-section of the multi-analyte sensor. (B) Schematic representation of the device and the optical arrangement for interrogation of the multi-analyte sensor. The device consists of dual detection channels, each equipped with an LED and a photomultiplier module (PMT). Excitation is achieved with LEDs and appropriate filters, and emission light is filtered accordingly. LED modulation was carried out using a two-phase lock-in amplifier at distinct frequencies for each channel.

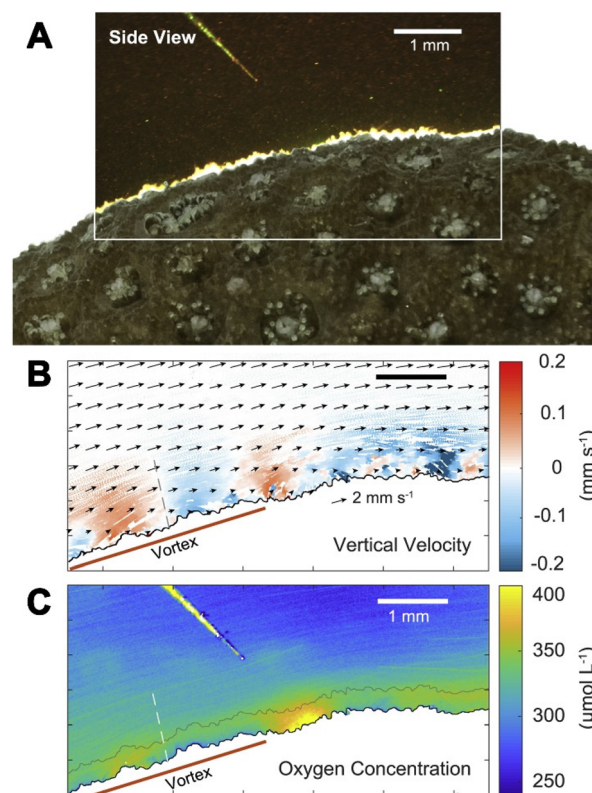


**Fig. 8** Imaging of an epiphyte-covered leaf of the freshwater plant *V. spiralis* during dark respiration with PANI-based  $\text{CO}_2$  and DIC optode.<sup>69</sup> (A) Setup of the leaf on top of the PANI-optode architecture. (B) Image of free  $\text{CO}_2$  after 1 h of incubation in freshwater under dark conditions. (C) Image of DIC right after PANI-based acidification. (D) Line profiles for  $\text{CO}_2$  and DIC are associated with the gray lines traced in (B and C) from the top to the bottom area. The gray area represents values below the  $\text{CO}_2$  optode calibration range. (E) Calculated images for carbonate alkalinity and pH.

**Electrochemically mediated stimuli.** An alternative stimulus could be an electrochemical reaction. Recently, Steininger *et al.* demonstrated that an electroactive polymeric mesh can be placed in front of an optode.<sup>67</sup> This mesh was coated with conductive polymer polyaniline (PANI), known as a proton pump.<sup>68</sup> Protons can be released from the polymer by simply applying a mild potential that alters the pH locally in front of the optode. This change in pH over time can be correlated with the buffer capacity of the sample. Therefore, chemical imaging of pH and buffer capacity at the same time was enabled by combining this 2D proton release with 2D pH imaging, the first-ever attempt at imaging buffer capacity.

In a follow-up study, Wiorek *et al.* combined the same 2D proton pump with a  $\text{CO}_2$  optode, enabling the imaging of dissolved  $\text{CO}_2$  and subsequently total dissolved inorganic carbon (DIC).<sup>69</sup> Based on those two images, total alkalinity, and pH images can also be calculated based on certain assumptions. Thus, this setup represents a four-parameter imaging approach (Fig. 8). However, it should be noted that the acquisition of the two images ( $\text{CO}_2$  and DIC) involves a time delay of approximately 10 minutes, which violates the definition of a sensor should sense both parameters simultaneously at the same point. This limitation should be considered, particularly in fast-changing systems, although it is less problematic for systems in a steady state.

**Particle image velocimetry.** A very different approach toward multiparameter sensing was taken by Ahmerkamp *et al.*<sup>70</sup> This team combined particle image velocimetry (PIV) with  $\text{O}_2$ -sensitive particles (Fig. 9). PIV as such is a method that enables the visualization of flow fields by tracking particle movement in a fluid using high-speed imaging. By incorporating an  $\text{O}_2$  indicator into the seeding particles utilized in PIV, it became possible not only to image the flow field but also to measure local  $\text{O}_2$  concentrations. This rather unique combination of measuring both a physical and a chemical parameter was furthermore used to study the dynamics of oxygen exchange of a living coral with the surrounding water.<sup>71</sup> *Via* this method, it was possible to visualize how the coral trans-



**Fig. 9** The flow and  $\text{O}_2$  visualization around corals with the sensPIV approach.<sup>70</sup> (A) Color image of sensPIV particles around the coral surface (in the top left corner, the  $\text{O}_2$  microsensors used for cross-reference measurements). (B) Combined particle image velocimetry (PIV) and particle tracking velocimetry graph of sensPIV particles. Arrows indicate the flow along the coral's surface. Colored dots represent individual particles, and the color indicates the velocity component perpendicular to the coral. (C) 2D mapping of  $\text{O}_2$  concentration inside the coral boundary layer obtained with sensPIV.

ports  $\text{O}_2$  from areas of  $\text{O}_2$  production to areas of  $\text{O}_2$  consumption by generating surface vortices using cilia located on the coral surface.

This example highlights that multiparameter imaging is not only limited to multiple chemical species but also includes physical parameters. Furthermore, it shows how entwined processes are in nature and how important multiparameter sensing is to be able to untangle these intricate processes.

### Multiple transduction

Generating an optical signal through the recognition stage alone is insufficient for conducting analysis using optical sensors. The next crucial step for quantitative analysis using any analysis tool, in addition to the naked eye, is the transduction stage. As mentioned, conventional devices for chemical imaging coupled with optodes are digital cameras. In this case, metal-oxide semiconductors function as transducers. Here, we want to look at multiple transducers for intensity-based imaging with optodes.

**RGB cameras.** Historically, monochrome CCDs were introduced first. Since such monochrome cameras operate only in one grey mode, only one value could be obtained as an output signal. Thus, even if the sensor consists of several recognition processes, hardly would it be possible to distinguish different optical signals from the different recognition sites, if not filters were exchanged during the imaging process. That was one of the reasons why researchers started using color RGB digital cameras. Typically, color cameras are based on a Bayer filter, which is a color filter array including Red (R), Green (G), and Blue (B) channels. Having had such devices with three different transducers, the information about the analyzed object can be increased by three times. This occurs when the absorbance or luminescence spectrum of the studied object coincides with more than one of the R, G, or B channels.

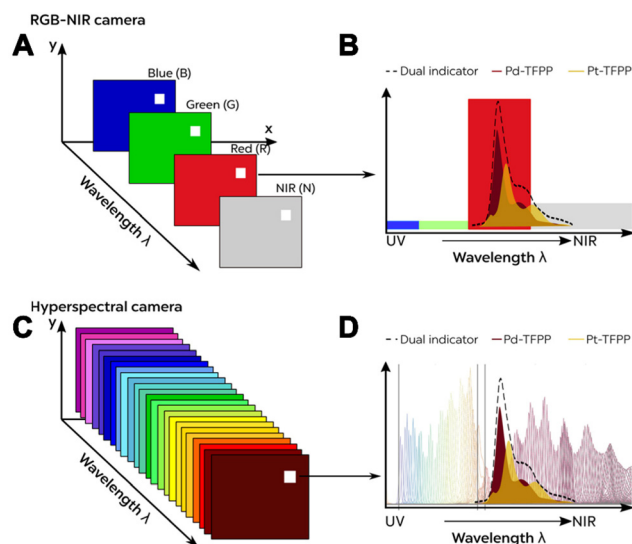
Let us imagine using an RGB camera to measure two parameters independently with color referencing. For that, one needs to use a system based on three dyes: a reference dye, a dye selective to the first parameter, and a dye selective to the second parameter. To minimize the possible errors and uncertainties, each dye must emit light only in R, G, or B channels. It leads to two significant disadvantages: (1) spectral properties of the indicators must match an absorption spectrum of color filters and (2) chosen dyes must have a negligible spectral overlap with each other. Thus, to measure more than two parameters with this approach is almost impossible with RGB digital cameras.

Modern cameras with a dual RGB/NIR (near-infrared) chip might help to overcome the spectral overlapping of several luminophores. Moßhammer *et al.* showed that utilizing RGB/NIR camera provided a dual-analyte for imaging setup optimized to have low cross-sensitivity between the two analytes O<sub>2</sub> and pH.<sup>51</sup> OHButoxy-aza-BODIPY was the chosen pH indicator that had emission in the NIR channel, while Eu (HPhN)<sub>3</sub>dpp was used as an O<sub>2</sub>-sensitive dye with a narrow emission fitting the red channel.

**Hyperspectral imaging systems.** Following the arguments mentioned above, it is only natural that sensor developers and users sought to expand the possibilities of recording even more spectral information in one image, thereby expanding

the possibilities of performing multiparametric analyses. When readout approaches provide more high-quality data and information about the spectral behavior of the sensor, the challenge of assigning different optical signals to different receptors is reduced to a mathematical and chemometric challenge of digital image processing. Devices that have the potential to overcome spectral limitations of common readout systems are hyperspectral imaging systems (HSIS).<sup>72</sup> Hyperspectral imaging systems are camera systems that capture spectral information of an image scene in each pixel with a narrow or nearly continuous spectral bandwidth. It is common for the number of spectral channels in an HSIS to exceed 100 spectral bands at a high spectral resolution of approx. 3 nm (1–5 nm), which preferably matches the spectral characteristics of the sensing layer (Fig. 10). Thus, HSIS has more than 100 transducers, surpassing the capabilities of monochrome CCDs and even that of color digital cameras which are equipped with only 1 and 3–4 transducers, respectively.

For instance, Kühl *et al.* utilized an advanced hyperspectral visible near-infrared (NIR) camera system for imaging O<sub>2</sub> levels and metabolic activity (respiration and photosynthesis) in a single sample.<sup>73</sup> This approach demonstrated that optodes not only enable imaging of physicochemical parameters but also extend to biological measurements. By employing techniques like hyperspectral imaging, microscopy, and O<sub>2</sub> imaging, this study revealed that Chl *f*-containing cyanobacteria in beachrock biofilms exhibit significant NIR-driven oxygenic photosynthesis, particularly crucial for primary production in shaded environments. Such studies provide promising tools for unraveling the ecological importance of intricate interactions within real-world complex samples.



**Fig. 10** Comparison of (A) a 4-channel RGB-NIR system with (C) a 150+ band hyperspectral camera system. (B and D) Respective information obtained for the emission spectrum from individual and combined optical O<sub>2</sub> sensors.<sup>72</sup> Reprinted with permission from ref. 72. Copyright 2021 American Chemical Society.

Hence, from a technical point of view, HSIS approaches pave the way for more promising concepts of intensity-based multianalyte imaging with two or even more analytes. However, the sole use of such HSIS does not grant multiparameter imaging *per se*. To make this ultimately possible, image and signal processing, including chemometric techniques, must be employed. The problem of accessing multiparameter imaging can thus be compared to the task of performing multivariate calibration based on spectral data. In this context, we have already shown that depending on the strength of the optical overlap of the different receptors, signal deconvolution can be achieved either with simpler algorithms such as spectral linear combination and least-square fitting<sup>72</sup> or with more complex machine learning algorithms.<sup>74</sup>

### Chemometric analysis

According to the order-based classification of sensors,<sup>75,76</sup> single-selective planar optical sensors are at least four-order sensors, given that their output signal ( $R$ ) is dependent upon four variables: the magnitude of the analyzed parameter ( $C$ ), chosen wavelength ( $\lambda$ ), and the position in 2D ( $x$  and  $y$ ):

$$R = f(C, \lambda, x, y).$$

In the case of multiparameter imaging, the amount of data in the output signal becomes enormous. Not only that the number of variables change from an individual analyzed parameter ( $C$ ) to a list of  $i$  different parameter values, denoted as  $C = \{C_1, C_2, \dots, C_i\}$ , but the number of wavelengths  $j$  at which the total signal response of the optode is acquired, denoted as  $\lambda = \{\lambda_1, \lambda_2, \dots, \lambda_j\}$ , also increases. In addition, the sensor can be subjected to  $k$  different external stimuli ( $E$ ), denoted as  $E = \{E_1, E_2, \dots, E_k\}$ .

Considering the temporal variation ( $t$ ) being a part of the sensor response as a spatiotemporal variable ( $P$ ), denoted as  $P = \{x, y, t\}$ , the sensor response is a point in the high  $i \times j \times k \times 3$  multidimensional space:

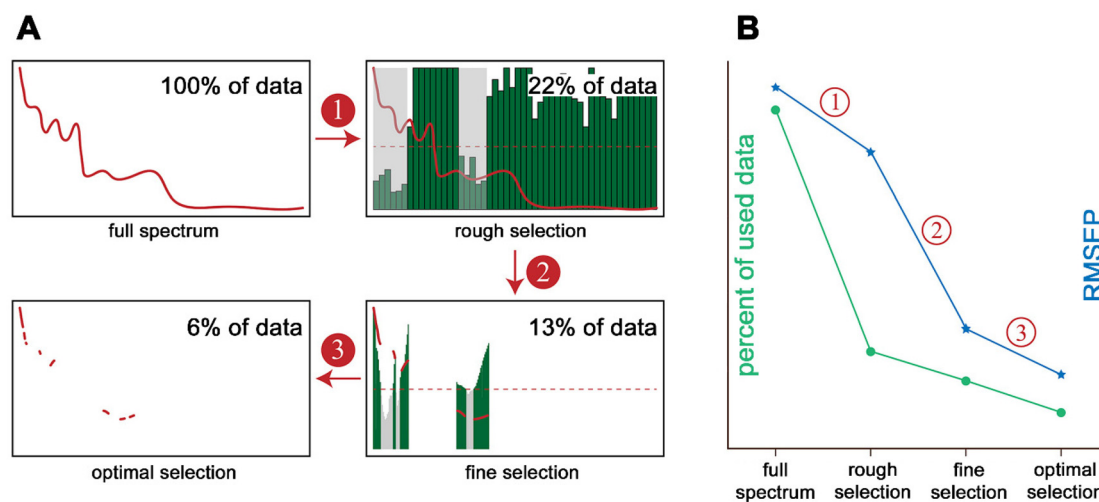
$$R = f(C, \lambda, E, P).$$

To address the challenge of translating this large amount of data into valuable information and to enable multiparameter analysis, diverse chemometric techniques must be applied. Thus, chemometrics as a multivariate approach to assessing overlaid signal responses is an essential tool in imaging with optode sensors and has become increasingly important over the years.<sup>77</sup> It involves extracting chemical information from datasets generated by imaging sensors to identify patterns and relationships between the measured parameters. The most pivotal aim, where chemometrics is employed, is developing multivariate calibration models, which are used to predict the characteristics of unknown samples.<sup>12</sup> However, it should be noted that a significant limitation of chemometrics is the need for extensive experimental characterization and data treatment.

**Chemometrics utilizing for feature extraction.** “Data sets generated by chemical imaging are large, are multivariate, and require significant processing.” Lavine and Workman noted

back already in 2002 in ref. 78 This statement highlights some challenges that chemometrics faces. Since then, computational performance has increased due to advances in computational engineering. However, measured data are still complex; interwoven, and overshadowed by noise, scattering effects, or artifacts coming from the matrix background. To translate the data and extract the true underlying pattern large investment in resources and time as well as extended exploratory and processing algorithms may be required.<sup>12,79</sup> Therefore, to improve the accuracy and robustness of the result and to allow a subsequent analysis model to work more efficiently, it is recommended to implement a signal pre-processing step. Besides smoothing, outlier removal, or baseline correction methods, it might be advisable to focus only on the features in a large data set that contains the most relevant information for the task at hand (so-called discriminative features).<sup>77,79</sup> This step is then referred to as feature extraction.<sup>80</sup> Fig. 11 shows one of the possible approaches for extracting meaningful data related to chemical processing and its separation from “noise”. Utilizing machine learning techniques, it was shown that optimal selection using only 6% of the original dataset leads to drastically reducing the error of prediction.<sup>81</sup> The proposed approach is based on a hybrid strategy involving interval partial least squares, variable importance in projection, modified variable combination population analysis, iteratively retaining informative variables, and genetic algorithm. For completeness, we note that in HSIS, where we obtain a narrow or near-continuous spectrum throughout the image scene and thus obtain high spectral and spatial dimensionality, feature extraction can be performed in either of these dimensions. However, this review focuses on spectral feature extraction methods, which are mainly done *via* multivariate approaches.<sup>82</sup>

Upon selecting the discriminative features, one can either use the specific spectral bands as such or combine these spectral bands to create a new combined feature parameter in a sub-space independent from the original feature space.<sup>83</sup> When selecting certain features from the entire data set, different approaches are available depending on the given data set and the intended analysis (features dependency, quantity of available features, and general data quality). Torrione *et al.*<sup>80</sup> summarized the main approaches for feature extraction relevant to spectroscopic techniques. They classified approaches ranging from feature extraction based on expert knowledge and visible inspection of spectral peculiarities in each data set<sup>84</sup> to automatic and independent feature search, using, among others, discriminant analysis approaches or sequential search techniques, to multivariate statistical dimension reduction, such as principal component analysis (PCA)<sup>83</sup> or partial-least-square (PLS).<sup>80</sup> In addition to these approaches, with the forthcoming of machine learning approaches over the past decade more and more approaches are presented using machine learning models for automated (but sometimes indirect) feature extraction. Two examples that should be mentioned here are the random forest algorithm for feature selection<sup>85</sup> and the autoencoder.<sup>86</sup> While the first algorithm is a



**Fig. 11** Different feature extraction strategies.<sup>81</sup> (A) Flow chart with 3 steps using NIR hyperspectral images of tobacco. Step 1: rough selection, wavelength interval selection method is used to select several intervals with low prediction errors. Step 2: fine selection, make further selection by the methods that can produce a sort of variable based on a criterion and eliminate the variables not satisfying a defined threshold value (red dashed line). Step 3: optimal selection, optimization algorithms are used to search for an optimal variable combination from the variables remained by the steps of rough selection and fine selection. (B) The trend of the percent of data used (number of variables) and root mean square error of prediction (RMSEP) from full spectrum to optimal selection.

machine learning approach combining an ensemble of independent decision trees, the latter one is an approach that aims to reconstruct its input data while performing dimensionality reduction and extracting relevant features.<sup>86,87</sup> However, since the field is constantly expanding and this is beyond the scope of this review, the interested reader is referred to respective reviews.<sup>88</sup>

**Multivariate calibration.** Classical univariate analytical calibration means that a sensor response is solely correlated exclusively with one target compound. While there are several examples in the literature where this is true for calibration of all possible different parameters,<sup>75,76,89–91</sup> reality shows that every fluorescent-based chemical sensor is at least cross-sensitive to temperature<sup>15</sup> or ionic strength in the case of ion-selective optical sensors<sup>92</sup> if not other cross-interfering parameters. In particular, in the field of environmental chemistry and real-world applications, there is a whole series of problems for which univariate calibration is not sufficient. This is obvious, as natural systems consist of complex and intertwined processes that require some kind of complex algorithm to correlate in complex multi-component mixtures.<sup>12</sup> A first attempt to deal with a complex situation could be to extend linear univariate calibrations to multilinear regressions (MLR).<sup>12</sup> A multilinear regression approach will therefore work in most cases in primarily analytical or physical chemistry, where most techniques aim to attain conditions of linearity constraints. However, there are some prerequisites to this approach, such as that the number of variables should not exceed the number of samples in the data, which makes the application of MLR to real-world applications and environmental chemistry challenging.<sup>93,94</sup> While there are some approaches to adjusting the analytical method to maintain linearity, it may be advisa-

ble to use more complex algorithms such as multivariate calibration.

In this context, multivariate calibration means that rather than focusing on and calibrating for a single target analyte, or more broadly parameter, multivariate models aim to calibrate multiple parameters that affect the response of the sensor.<sup>12</sup> Thereby, more information can be obtained about the sample than if each variable were considered separately. This is what Paul Gemperline calls the multivariate advantage.<sup>95</sup> However, this multivariate approach requires more information by collecting more sample parameters, as well as more process steps, including signal separation and classification.<sup>96</sup> Historically, multivariate calibration has been used only for spectroscopic data such as absorbance and fluorescence emission, but it is not limited to this, and one could include other sample parameters if relevant to the problem at hand.<sup>97</sup> In addition, while multivariate calibration was previously omitted by chemometricians, it has become a very popular field in recent years.<sup>93</sup> Early algorithms in multivariate calibration still assume linear contributions of different compounds in complex mixtures, and most approaches use principal component regression (PCR), partial least squares (PLS), or so-called multiway or multimode algorithms, with PLS being the most popular.<sup>95,98,99</sup>

However, with the continuous improvement of mathematical algorithms and signal processing opportunities, chemometricians are striving to incorporate novel algorithms that allow them to analyze increasingly complex situations and to account for non-linear contributions of different compounds in complex mixtures.<sup>79</sup> Sometimes it might already be sufficient to extend the aforementioned approaches into a higher dimension, thus enabling a multi-way analysis for example in parallel factor analysis (so-called PARAFAC).<sup>100</sup>

PARAFAC is a generalization of PCR that requires lightly more complex methodological procedural steps to extract component information from mixed 3D fluorescence data.<sup>101</sup> However, for PARAFAC to be useful, certain requirements must be met, such as variability of analyte contributions (no identical spectra), trilinearity of sample parameters, *e.g.*, emission spectra are invariant across emission wavelengths, and

increase linearly with the concentration and intensity, and that the overall signal is due to the linear superposition of a fixed number of components.<sup>100</sup> Other common expansions to deal with trilinearity besides PARAFAC include the Tucker model and multi-way partial least squares regression.<sup>102</sup> Although these algorithms provide great potential, some complex non-linear systems can only be solved by inherently

**Table 2** Comparison of existing approaches to multiparameter imaging based on optodes

Approach		Advantages	Disadvantages
Sensor arrays		<ul style="list-style-type: none"> <li>• Cost-efficient technique</li> <li>• Simple production</li> </ul>	<ul style="list-style-type: none"> <li>• Not true multiparameter imaging</li> <li>• Need of <math>N</math> sensors for analyzing <math>N</math> parameters</li> </ul>
Dual/multiple recognition	Multisensitive receptors	<ul style="list-style-type: none"> <li>• Unlimited number of analyzed parameters</li> <li>• Possibility of measuring in the same spot and time using only one indicator</li> </ul>	<ul style="list-style-type: none"> <li>• Lack of existing probes</li> </ul>
	“Sandwich” sensors	<ul style="list-style-type: none"> <li>• Possibility of transferring knowledge of single sensing with optodes to multiparameter analyzing</li> <li>• Well-established technique for dual and triple sensing</li> <li>• Feasibility of using distinctly different chemicals and sensing principles in one sensor</li> </ul>	<ul style="list-style-type: none"> <li>• Complex organic synthesis problem</li> <li>• Limited number of layers (max three, typically)</li> <li>• Spectral overlap</li> <li>• FRET and PET processes</li> </ul>
	Polymer binder with (nano)particles suspension	<ul style="list-style-type: none"> <li>• Possible imaging for more than 2 parameters</li> <li>• Possibility of measuring in almost the same spot</li> <li>• Unlimited number of single sensitive sensors</li> <li>• Feasibility of using distinctly different chemicals and sensing principles in one sensor</li> <li>• Simple production</li> </ul>	<ul style="list-style-type: none"> <li>• Increasing response time for several layers</li> <li>• Cross-leaching of chemicals</li> <li>• Increasing photodecomposition and signal drift</li> <li>• Spectral overlap</li> <li>• FRET and PET processes</li> <li>• Possible influence of polymer binder (chemical reactions, sorption, memory effect)</li> <li>• Relatively slow response time</li> </ul>
External stimulus		<ul style="list-style-type: none"> <li>• Increasing chance of discriminating of analyzed parameters</li> <li>• Possibility of using a combination of different stimuli</li> <li>• External control of the system being examined</li> </ul>	<ul style="list-style-type: none"> <li>• Complex instrumentation</li> <li>• Possible influence on the sample (sample decomposition, changing sample composition)</li> <li>• Need for thorough calibration design</li> <li>• Increasing amount of output data</li> <li>• Requirement of sophisticated data analysis including pattern determination</li> </ul>
Multiple transduction in intensity-based imaging	RGB cameras	<ul style="list-style-type: none"> <li>• Cost-efficient technique</li> <li>• Commercially availability</li> </ul>	<ul style="list-style-type: none"> <li>• Limited number of probes used for analysis as they have to fit spectral windows</li> <li>• Typical use for dual- and triple-parameter imaging</li> </ul>
	Hyperspectral cameras	<ul style="list-style-type: none"> <li>• Involvement of spectral techniques in optode-based imaging</li> <li>• Increasing spectral resolution of used probes</li> </ul>	<ul style="list-style-type: none"> <li>• Expensive instrumentation</li> <li>• Vast amount of data</li> </ul>
Chemometric techniques		<ul style="list-style-type: none"> <li>• Revealing hidden patterns</li> <li>• Feature extraction</li> <li>• Possibility of multivariate calibration</li> </ul>	<ul style="list-style-type: none"> <li>• Requirement of sophisticated data analysis</li> <li>• Need for thorough calibration design</li> <li>• Complex and nested dataset</li> <li>• Requirement of knowledge in interdisciplinary subjects (mathematics, statistics, programming, <i>etc.</i>)</li> <li>• High dependency on researchers' interpretation of output results</li> </ul>

non-linear machine learning algorithms, including naïve Bayes regression, *k*-nearest neighbor (KNN), support vector machine (SVM), or artificial neural network (ANN) algorithms.<sup>103</sup> In particular with the increased interest in machine learning, the latter approach became the most popular, and Venturini *et al.*<sup>104</sup> demonstrated how a non-linear statistical model can be used for multivariate calibration for simultaneous sensing of O<sub>2</sub> and T. Due to the great success of machine learning approaches in analytical chemistry, chemists strive to push boundaries in their field and extend the capabilities of joint acquisition even further. The combination of more advanced recording systems, such as multi-sensory systems or hyperspectral cameras, with advanced analytical techniques, allows the assessment of the non-linear effects of multiple components in complex mixtures. Data sets are becoming more extensive, and a wealth of information is available. Machine learning approaches offer thereby a great opportunity to process these data sets and translate them into valuable, multiparametric information.<sup>74,105–107</sup> Moreover, in recent years, more and more deep learning algorithms have been reported not only for image classification but also for multiparameter regression purposes.<sup>108</sup> For more details on recent advances in chemometric calibration methods in modern spectroscopy, the interested reader is referred to the respective review by Wang *et al.*<sup>79</sup>

## Concluding remarks

Since the first optode-based imaging, optical sensors have been constantly developing. Continuous and parallel developments in terms of instrumentation, indicators, as well as data analysis, have shaped the field. Multiparameter imaging with optodes is nowadays possible and finds applications in biology, medicine as well as engineering. The field is nevertheless still in development. Table 2 shows the main trends in multiparameter imaging based on optodes.

At the current state, machine learning and artificial intelligence (AI) are entering all fields of science and are redefining what is possible. While those are for sure promising developments within chemical imaging, it is important to maintain focus on the basics and not to forget that chemical sensors are based on receptors and chemical interactions. AI might be able to squeeze more out of the tools we have now but will reach chemical and physical limits. Focusing on the “clever” indicator and sensor design is still key. In recent years single indicators with dual sensing capabilities have gained attention and certainly are very promising for the field.

Furthermore, multiparameter sensing extends beyond chemical species. Physical or biological parameters are equally interesting and needed to increase our understanding of complex biological systems.

As creatures of the eye, we humans have always been fascinated by images. Looking deeper into this, one would even say that images are pivotal to sharpening our worldview, so we constantly create some of them in our heads. Chemical images are

no exception here. Using the information, we gain from these images to solve complex biological or technological problems is essential. Therefore, it is advised to combine chemical imaging with other methods. For instance, chemical images can be used as “maps” to find hotspots or points of interest in complex biological samples like soils<sup>109,110</sup> or sediments.<sup>111</sup>

Unlike many other analytical methods, chemical imaging can generate data both spatially and temporally. As humans, we often rely on temporal and spatial optical information to understand our surroundings. In this context, chemical images are our window into the chemical environment, revealing the otherwise invisible complex mosaics of chemical processes and interactions. This makes chemical imaging a “bridging technology”, connecting not only various disciplines but also reaching a broader audience. While chemical formulas and reactions may appear complex and difficult to non-experts, the ability to visualize chemistry is perceived as intriguing and fascinating.

Chemical imaging has a bright future ahead. Not only will the coming years witness continuous advancements in this field, but it will also become increasingly integrated into efforts aimed at understanding complex biological systems. Finally, chemical imaging has the potential to spark interest in chemistry itself. As the adage goes, “seeing is believing”, and chemical imaging can make the invisible visible for all to behold.

## Conflicts of interest

There are no conflicts to declare.

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