Biosensors and Bioelectronics 263 (2024) 116632

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Integrating machine learning and biosensors in microfluidic devices: A review

Gianni Antonelli, Joanna Filippi, Michele D'Orazio, Giorgia Curci, Paola Casti, Arianna Mencattini, Eugenio Martinelli *

Department of Electronic Engineering & Interdisciplinary Center for Advanced Studies on Lab-on-Chip and Organ-on-Chip Applications (ICLOC), University of Rome Tor Vergata, Via del Politecnico, 1, 00133, Rome, Italy

ARTICLE INFO

Keywords:
Machine learning
Lab-on-a-Chip
Biosensing system
Intelligent microfluidics
Biosensors integration

ABSTRACT

Microfluidic devices are increasingly widespread in the literature, being applied to numerous exciting applications, from chemical research to Point-of-Care devices, passing through drug development and clinical scenarios. Setting up these microenvironments, however, introduces the necessity of locally controlling the variables involved in the phenomena under investigation. For this reason, the literature has deeply explored the possibility of introducing sensing elements to investigate the physical quantities and the biochemical concentration inside microfluidic devices. Biosensors, particularly, are well known for their high accuracy, selectivity, and responsiveness. However, their signals could be challenging to interpret and must be carefully analysed to carry out the correct information. In addition, proper data analysis has been demonstrated even to increase biosensors' mentioned qualities. To this regard, machine learning algorithms are undoubtedly among the most suitable approaches to undertake this job, automatically learning from data and highlighting biosensor signals' characteristics at best. Interestingly, it was also demonstrated to benefit microfluidic devices themselves, in a new paradigm that the literature is starting to name "intelligent microfluidics", ideally closing this benefic interaction among these disciplines. This review aims to demonstrate the advantages of the triad paradigm microfluidics-biosensors-machine learning, which is still little used but has a great perspective. After briefly describing the single entities, the different sections will demonstrate the benefits of the dual interactions, highlighting the applications where the reviewed triad paradigm was employed.

1. Introduction

The ever-increasing development of microfluidics systems (in particular, Lab-on-a-Chip and Organ-on-a-Chip devices, used to mimic human organ and system functionalities *in vitro*) has made necessary the integration of biosensing elements (Zhu et al., 2021). Physical biosensors (i.e., biosensors probing physical quantities, such as temperature, pressure, or strain) can be used to inspect the environment of the tissues and maintain them more representative of the native conditions (Lu et al., 2019; Sang et al., 2014; Salvo et al., 2017; Haleem et al., 2021; Mohankumar et al., 2021; Cao et al., 2024). On the other hand, biochemical sensors, inspecting chemical analytes and biological compounds, can help in checking cellular functionality and behaviour, keeping them similar to their *in vivo* counterparts (Zhang et al., 2024; Xu et al., 2024; Wang et al., 2024a; Bouquerel et al., 2022). Without such understanding, it is impossible to correlate the outcome of a desired measure to external stimuli and obtain new knowledge about the biological phenomenon under study. However, the unmet need to monitor the status of Lab/Organ-on-a-Chips during experimentation remains to be fully addressed.

Integrating artificial intelligence (and machine learning in particular) was demonstrated to be crucial when dealing with complex biosensor signals (Cui et al., 2020; Maqsood et al., 2022; Teng et al., 2023). Despite the black-box nature of artificial intelligence, the fundamental role of algorithms in processing the massive amount of data coming from biosensing and microfluidics is well-known and has been demonstrated in many applications (Zheng et al., 2021; Kadian et al., 2023; Galan et al., 2020; Raji et al., 2022). One of the significant challenges of such a system is to create an optimal integration among the sensing element, the organ/tissue, and the processing algorithms, as it occurs in the human olfactory and gustatory systems (Covington et al., 2021; Martinelli et al., 2011). Each of the entities alone is not able to

* Corresponding author.
E-mail address: martinelli@ing.uniroma2.it (E. Martinelli).

https://doi.org/10.1016/j.bios.2024.116632
Received 10 June 2024; Received in revised form 1 August 2024; Accepted 2 August 2024
Available online 3 August 2024

completely fulfil the survival requirements in the olfactive/gustative case study and may not reach the ultimate goals of disease healing, drug discovery, and effective therapeutic intervention in Lab/Organ-on-a-Chip. In light of this, in this review, we will investigate the role of each entity in this synergy, demonstrating its indissolubility.

In recent years, numerous papers have been published about integrating biosensors in microfluidic devices. For this reason, many authors successfully reviewed the state-of-art technologies in the field, focusing on different applications and several sensing principles (Zhu et al., 2021; Lakshmy et al., 2024; Lafleur et al., 2016; Estevez et al., 2012; Karthik et al., 2022; Zhou et al., 2024a; Dey et al., 2024). Similarly, other reviews presented machine learning as a tool for analysing biosensors' signals (Cui et al., 2020; Kadian et al., 2023; Raji et al., 2022; Jin et al., 2020).

However, none of the state-of-the-art reviews has presented machine learning algorithms as an integrated component of Lab-on-a-Chip sensing systems, thus introducing a novel triad paradigm (biosensing-microfluidics-machine learning) for real-time monitoring (Fig. 1). The essential role of machine learning in addressing dynamic system modelling and prediction, scenario adaptation, and changes in operating conditions remains undiscovered. In the following, we will provide additional details on each framework, aiming to focus on the critical parts needing the synergic contribution of the other contexts. Ultimately, we will provide future perspectives on the advantages and present criticisms of the combined approach.

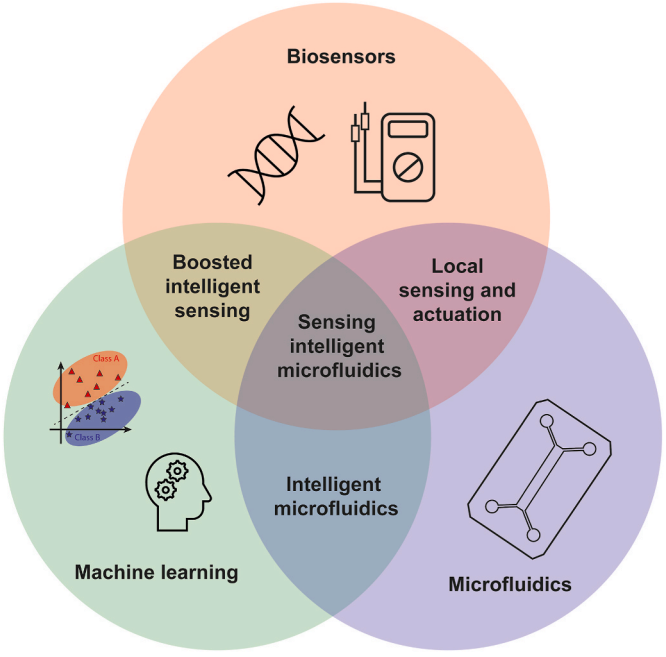

Fig. 1. Euler-Venn diagram showing the interaction between microfluidics, biosensors, and machine learning. Each intersection is demonstrated in this review to solve one of the issues of the entity alone. Microfluidic + Machine Learning intersection generates what the literature is starting to name "intelligent microfluidics", systems able to understand and predict the microfluidic phenomenon of interest. Biosensors + Machine Learning systems can boost biosensors' performance (efficacy, sensibility, reliability), extracting mathematical features from signals that are more insensible to noise or other alterations. Biosensors + Microfluidic guarantees access to the local experimental information and the possibility of actuation depending on the obtained result. The super interaction between the three entities is starting to demonstrate a "sensing intelligent microfluidic", i.e., systems able to detect an analyte by biosensors, make decisions using machine learning, and control the environmental parameters by microfluidic actuation.

2. Microfluidics devices

Microfluidics represents the science and technology of systems that process or manipulate small amounts of fluids using channels with dimensions of tens to hundreds of micrometres (Whitesides, 2006). Characterised by a low Reynolds number, so operating in a regime where viscous forces are dominating concerning the inertial ones, microfluidics is a relatively newly emerged field based on the combined principles of physics, chemistry, biology, fluid dynamics, microelectronics, and material science (Niculescu et al., 2021; Squires and Quake, 2005). Whether used alone or in combination with other devices, microfluidic chips can be exploited for nanoparticle preparation, drug encapsulation, delivery, and targeting, single or multiple cell analysis, diagnosis, and cell co-culture. These miniaturised devices are valuable instruments for carrying out operations on liquids like reactions, separations, or the detection of various compounds (Hamdallah et al., 2020). Depending on the desired application, microfluidic devices can also be exploited as microreactors (Shrimal et al., 2020), Lab-on-a-Chip (Shi et al., 2019; Sengupta et al., 2019; Wongkaew et al., 2019), or Organ-on-a-Chip (Moradi et al., 2020; Sun et al., 2019). Once their destination use is explicit, microfluidic chips can be manufactured using a broad range of materials and implementing diverse fabrication methods (Nielsen et al., 2020). Moreover, due to the increasing research advances, the potential of microfluidics is increasing abruptly, bringing new perspectives to both the academic and industrial sectors.

2.1. *The paradigm of "intelligent microfluidics"*

The integration of machine learning and microfluidics is starting to express in original and inspiring ways, giving birth to a new scientific research branch that the literature is beginning to name "intelligent microfluidics" (Zheng et al., 2021; Galan et al., 2020; Sun et al., 2023; Ma et al., 2023; Song et al., 2021; Mencattini et al., 2023a; Tsai et al., 2023). The association between the two sciences is bringing enormous benefits to both sides (Fig. 2).

Microfluidics is an intrinsic generator of big data, solving micrometrical automated tasks with extremely high efficacy, but to control and extract information from the ongoing process, it is crucial to automatically extrapolate and elaborate the data acquired from the platform (Kheiri et al., 2021; Zhou et al., 2024b; McIntyre et al., 2022; Srikanth et al., 2021; Fukada and Seyama, 2022). One of the more common examples exploiting this interaction is microdroplet generation, one of the most high-throughput studied tasks to which microfluidic is contributing. In this particularly interesting scenario, machine learning algorithms provided the instruments to optimise the microdroplet generation or their recognition by "barcodes", hence a substance (such as DNA, or a dye) or a droplet sequence that can be used to discriminate a droplet content from another one (Srikanth et al., 2021; Ding et al., 2020; Wang et al., 2024b; Zilionis et al., 2016; Nan et al., 2024; Sesen and Whyte, 2020; Molho et al., 2024; Siemenn et al., 2022; Zhang et al., 2023). At the same time, machine learning was also demonstrated to be a new, intriguing way to optimise microfluidic platforms themselves, optimising geometries and shaping flows in the devices (Lee et al., 2018, 2019; Khor et al., 2019; Stoecklein et al., 2017; Desir et al., 2019). Another interesting application in this field was proposed by Rizkin et al., who designed a complex microreactor system to study polymerisation reactions by infrared thermography and deep learning (Rizkin et al., 2020).

Moreover, if we need to study flow distribution in a microfluidic chip from a more theoretical/simulative point of view, the Navier-Stokes equations are known to accurately describe the dynamic of a viscous fluid. However, their generalised solutions are still unknown, being one of the seven Clay Mathematics Institute's Millennium problems (Merrin, 2019; Aref, 2008). Additionally, non-idealities to which microfluidic devices can be subject, e.g., roughness, complex geometries, or multiphysics interaction, can increase more and more the solution of the flow

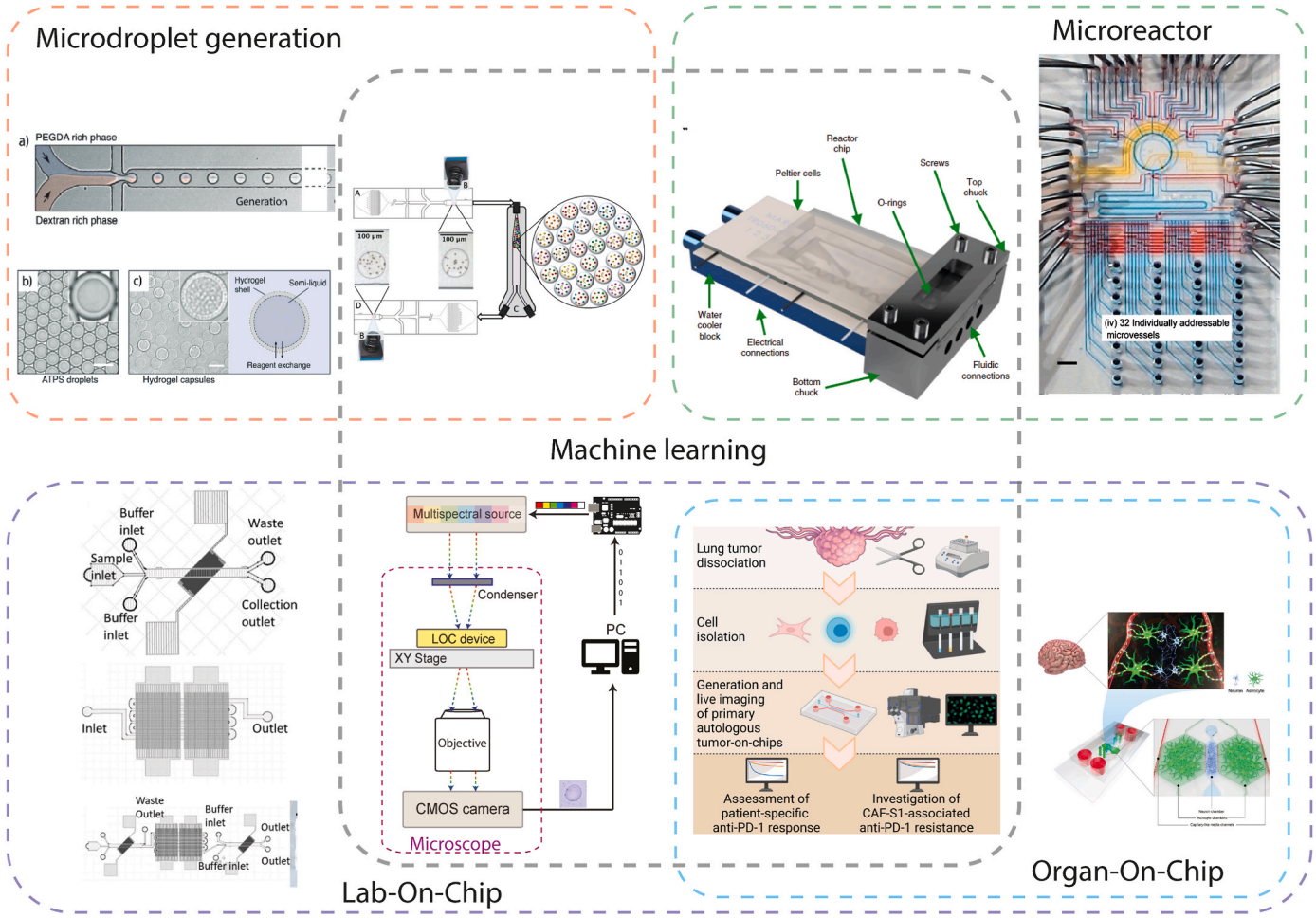

Fig. 2. Microfluidic current trends and their interactions with Machine Learning. Based on the investigation performed in this review, we gave some examples of devices able to generate microdroplets [adapted from (Leonaviciene et al., 2020), with permission from the Royal Society of Chemistry], microreactors [adapted from (Lin et al., 2009), with permission from Elsevier], Lab-On-Chip [adapted from (Ozcelik et al., 2024), with permission of Springer Nature] and Organ-On-Chip [adapted from (Choi et al., 2024)]. Each application is highlighted in their interactions with Machine Learning: microdroplet generation and barcodes [reproduced from (Svensson et al., 2019), with permission from John Wiley & Sons, Inc.]; polymerisation control by thermography and Machine Learning [adapted from (Rizkin et al., 2020) with permission of Springer Nature]; pH inspection in Lab-On-Chip by multispectral imaging and Machine Learning [adapted from (Antonelli et al., 2024), with permission from Elsevier]; tumour-biopsy on chip inspection by fluorescence time-lapse microscopy and Machine Learning [reproduced from (Veith et al., 2024), with permission from Elsevier].

distribution (Zhang et al., 2010; Chen et al., 2009; Lalegani et al., 2018). Machine learning is demonstrating its potential also in this field, approximating the flow dynamics in rapidly variating, real-time scenarios since the usual Finite Element Method (FEM) simulations are extremely time-consuming (McIntyre et al., 2022). For example, Fang et al. demonstrated a deep-learning approach to derive a simulated particle manipulation based on a neural network able to catch the flow physics from the particle movement and fluidic control signals (Fang et al., 2023). The system was then integrated into a reinforcement learning (this approach will be better investigated in the following Sections) controller for an intelligent fluidic micromanipulator.

2.2. Lab-on-a-Chip and Organ-on-a-Chip

The important interaction between these two sciences started to attract other disciplines to participate in this beneficial loop. In particular, from a biological point of view, microfluidic gave its contribution through the development of Lab-on-a-Chips able to outperform complex tasks, such as extra-vesicle isolation (Ozcelik et al., 2024; Contreras-Naranjo et al., 2017), cell sorting (Gao et al., 2024; Shen et al., 2019), encapsulation (Leonaviciene et al., 2020; Aoki et al., 2022), or single cell characterisation (Filippi et al., 2022, 2024; Shukla et al., 2018). The high flexibility of these platforms permitted the extrapolation of crucial information from the biological sample in new and exciting methodologies, which were impossible to implement in a standard culture dish. At the same time, the performed analyses are usually not disruptive and guarantee great cytocompatibility. For example, standard immunoassays such as Enzyme-Linked ImmunoSorbent Assay (ELISA) and Western blotting are known to be tedious, expensive, time-consuming and require the end of the experiment, making troublesome real-time monitoring (Suthar et al., 2020; Laocharoensuk, 2016; Diaconu et al., 2013; Hosseini et al., 2018; Wignarajah et al., 2023).

In this field, Organ-on-a-Chip arose as a new concept of Lab-on-a-Chip devices able to imitate the behavior and/or specific functionalities of an organ. These powerful devices permit the setting up of complex and long-term experiments, guaranteeing at the same time to better mimic and understand the phenomenon under study (Shin et al., 2023; Essaouiba et al., 2020; Chen et al., 2024; Żuchowska et al., 2024; D'Orazio et al., 2024). Numerous organs were implemented, simulated, and studied using this technique in a totally *in vitro* or *ex vivo* environment. For example, Veith et al. (2024) recently presented a work in which they implemented a lung tumour-on-chip to study patients' responses to immunotherapy. Cohen et al. (2021) published an exciting

work assessing the immuno-suppressing and chemotherapic-induced nephrotoxicity in a vascularised kidney-on-chip model. Choi et al. (2024) presented the CANDY chip, an intriguing Organ-on-a-Chip platform able to study the neurotoxic effects of environmental pollutants on neurons mediated by astrocytes.

2.3. Perspective in microfluidics

Even though there are already many available applications, the development of microfluidics is still in its infancy: the existing technology must be further improved, devices can be even more miniaturised, chips can be integrated into various other devices, synthesis processes can be better controlled, new reaction pathways can be investigated, and novel applications may arise.

Moreover, the synergistic convergence of microfluidics and machine learning must be empowered. Nowadays, most microfluidic devices are operated in a non-automatised manner, but it is possible to develop and integrate on-chip multimodal instrumentation. In this way, autonomous and easy-to-use platforms can be fabricated, and the obtained experimental data can be processed by machine learning. Hence, instead of analysing the results of the experiment after it is performed, machine learning allows the device to learn from the data and make accurate predictions to guide and optimise the conducted research. This "intelligent microfluidics" really represents the next generation of platforms for drug discovery, nanomaterials, *in vitro* organ modelling, and developmental biology (Galan et al., 2020; Pulsipher et al., 2018; Ai et al., 2020; Solanki et al., 2020; Burklund et al., 2020; Fallahi et al., 2019; Kulkarni et al., 2019; Jin and Bhujwalla, 2019; Maia et al., 2020; Seleci et al., 2019; Machado et al., 2020; Arduino et al., 2021).

Several other innovative combinations of microfluidics with new domains, such as artificial intelligence, metamaterials, and neuromorphic engineering, may bring about unprecedented technological advancements in the foreseeable future (Kong et al., 2020). By reformulating the paradigm of "intelligent microfluidics", we prefigure a "sensing intelligent microfluidics" able to sense (integrating sensors and, in particular, biosensors) and to process (intelligent) information coming from the experiments.

3. Integrated biosensors in microfluidics

The development of sensitive and reliable sensors (especially biosensors, i.e., sensors combining a bio element to detect a particular analyte) stands as a significant challenge in the advancement of microfluidics. Biosensors are crucial tools in precisely quantifying the physical or chemical properties of substances. Moreover, when compared to standard assays, such as liquid or gas chromatography, immunoassays or Western blotting, these devices can be easily integrated into the Lab-on-a-Chip devices, permitting a non-invasive, real-

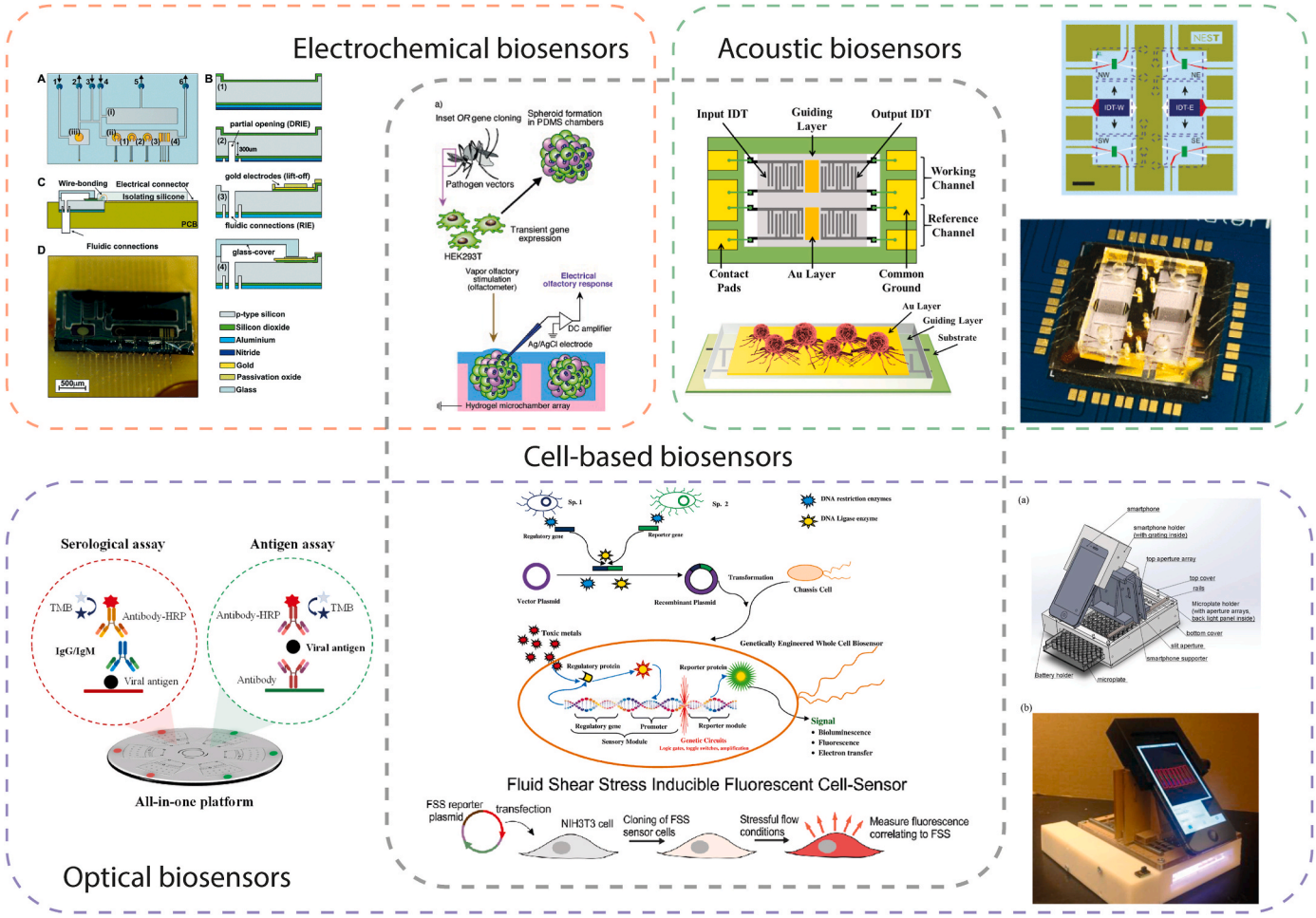

Fig. 3. Different types of biosensors integrable in microfluidic devices. In this review, we focused on the most known types of biosensors: electrochemical [reproduced from (Giménez-Gómez et al., 2020)], acoustic [adapted from (Agostini et al., 2019)] and optical biosensors [adapted from (Wang et al., 2017) and (Teixeira et al., 2022), with permission from Elsevier]. A particular in-depth analysis was given to cell-based biosensors, whose signal behaviour is usually more complex to study and often requires machine learning approaches. Cell-based biosensors can, in turn, be divided into electrochemical [reproduced from (Sato and Takeuchi, 2014), with permission from John Wiley & Sons, Inc.], acoustic [adapted from (Zhang et al., 2016), with permission from Elsevier], and optical [reproduced from (Moraskie et al., 2021) and (Varma and Voldman, 2015), with permissions respectively from Elsevier and the Royal Society of Chemistry].

time, low-cost, easy-to-setup monitoring of analytes of interest. The integration of microfluidics with sensors has propelled technological advancements and yielded innovative solutions applicable across a broad spectrum of research needs. The amalgamation of microfluidic and sensor integration facilitates precise chemical detection and enhanced efficacy in numerous scenarios, such as Point-of-Care devices, clinical diagnostics, drug development and delivery, and pure biological research (Wang et al., 2024c; Gaba et al., 2024; Acharya et al., 2024; Lino et al., 2022; Malhotra and Chaubey, 2003; Lee et al., 2024; Augustine et al., 2024).

For these reasons, the literature investigated how different biosensing strategies can be combined with microfluidics to understand the studied phenomenon in depth. Knowing which kind of biosensor can be integrated into the Lab-on-a-Chip is crucial to understanding the signal of interest, so it can be processed at its best with the correct machine-learning algorithm (Fig. 3).

3.1. Electrochemical biosensors

One of the most straightforward kinds of biosensors is electrochemical, which guarantees a relatively easy and sometimes inexpensive integration, high sensitivity, and low time response. Once immobilised on an electrode, the biological sensing element (enzymes, antibodies, DNA, proteins, etc.) interacts with the analyte, modifying an electric property (resistance, capacitance, voltage, or current), which depends on the type of fabricated sensor. Finally, using proper circuitry, the physical or biochemical information can be encoded in an electronic signal and recorded (Ronkainen et al., 2010; Yang et al., 2024a; Bandodkar et al., 2019; Mahato and Wang, 2021; Mostaccio et al., 2024; Pushparaj et al., 2024). One of the significant drawbacks of this kind of sensor is situated in the signal extraction part, which can sometimes be challenging to integrate directly on-chip, being bulky and cumbersome. Moreover, it was demonstrated that pH and ionic force could negatively influence the responsivity of the biosensor to the analyte (Grieshaber et al., 2008; Wu et al., 2023). Finally, the integration of biosensor arrays in microfluidic systems is still under investigation because the reduction of electrode area, following Cotrell and Randles–Sevcik equations, is correlated with a loss of sensitivity in many types of electrochemical sensors (Grieshaber et al., 2008; Sankar et al., 2024).

3.2. Acoustic biosensors

Another interesting type of biosensor is based on acoustic waves. After 1959, when Sauerbrey proposed the Quartz Crystal Microbalances (QCM) to detect a mass variation by a change in the resonant frequency, acoustic sensors became significantly explored in the literature (Sauerbrey, 1959). A biosensing element is always used as a mediator between the analyte of interest and a physical characteristic of the substrate, which will modify the propagation of the acoustic wave. Its modification will then be translated into an electric signal whose amplitude, frequency or phase will be correlated with the analyte concentration (Zhang et al., 2018, 2021a; Nair et al., 2022; Fu et al., 2017; Huang et al., 2024). Acoustic biosensors are incredibly sensible to minimal mass variation, increasing the detectability of the analyte (Fogel et al., 2016). Moreover, especially for Surface Acoustic Wave (SAW) devices, the acoustic wave is generated far enough away from the sensing area (so from the Lab-on-a-Chip) and can simultaneously act as an actuator (Huang et al., 2021; Länge et al., 2008). A great effort is present in the literature for the miniaturisation of these sensing systems and their integration on-chip (Zhang et al., 2016; Bin Han and Lee, 2024), even though there seems to be still some limitations to a Lab/Organ-on-a-Chip-embedded acoustic biosensing array.

3.3. Optical biosensors

Optical biosensors resulted in being another diffuse and probably the easiest-to-integrate sensing device in Lab-on-a-Chip, mainly because they do not require any external circuitry to be accessed. In this case, the biomolecule is able to change an optical property of the sensor (refractive index, plasmon resonance, colour or fluorescence), making "visible" the sensing information (Panwar et al., 2024; Li et al., 2024; Rasheed et al., 2024; Chen and Wang, 2020). As mentioned, this kind of sensor is relatively easy to fabricate, classically involving standard techniques, such as spin coating, dip coating, drop casting and standard lithography (Fruncillo et al., 2021; Yoshikawa et al., 2015; Crosley and Yip, 2018)–(Fruncillo et al., 2021; Yoshikawa et al., 2015; Crosley and Yip, 2018). However, other interesting and more accessible fabrication techniques, such as 3D printing and stereolithography (Sharafeldin et al., 2018; Muñoz and Pumera, 2020). One of the greatest issues of this type of sensor is in their sensing data extraction, which can sometimes be challenging because of the requirement of complex machinery, such as fluorescence microscopy, spectrophotometers or waveguide couplers. Nevertheless, especially for Lab-On-Chip and Point-of-Care devices, some works are proposing the introduction of custom illumination sources or smartphones to detect easily and reliably the analyte under inspection (Antonelli et al., 2024; Wang et al., 2017; Rasheed et al., 2024).

3.4. Cell-based biosensors

In some applications, whole biological cells were also demonstrated as biosensing elements. Compared to biosensors based on enzymes, aptamers, or other biological molecules, they are cost-effective, simple to set up and fabricate, in addition to being extremely adaptable with different signaling means (Gupta et al., 2019). The analyte of interest interacts with the cell, which is able to produce a signal (usually electrical or optical) detectable by an appropriate instrument (Moraskie et al., 2021; Fang, 2006; Wei et al., 2024). This approach was utilised for numerous applications, spacing from food safety to biological investigations. For example, Varma and Voldman presented an interesting work of a cell-based microsystem able to detect the local fluid shear stress by mechanosensible cell fluorescence (Varma and Voldman, 2015). Larou et al. demonstrated the detection of aflatoxin M1 by modifying the cell membrane of a fibroblast culture with an appropriate monoclonal antibody (Larou et al., 2013). Sato and Takeuchi generated olfactory receptor-modified spheroids in a hydrogel microchamber for the electrophysiological detection of odorants (Sato and Takeuchi, 2014).

Since cells are living entities, some studies demonstrated that their phenotype can be affected by chemicals or external stimuli. For this reason, even if cells' morphological and functional dynamic cannot be strictly defined as biosensors (hence, from IUPAC goldbook, "a device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals" (IUPAC, 2014)), their behavioral modification caused by analytes can be studied by brightfield, fluorescence or confocal microscopy and correlated with the analyte under investigation. For example, some recent works demonstrated that time-lapse microscopy on different cell lines could capture cell phenotype and morphodynamics, discriminating genomic modifications, drug delivery, neurite degeneration, apoptotic events, metastatic properties and motility (Mencattini et al., 2021; D'Orazio et al., 2020, 2022; Pijuan et al., 2019; Solorio et al., 2023; Jia et al., 2020; Aftab et al., 2014). Particularly for these complex phenomena, machine learning, and deep learning have been demonstrated to be crucial to a comprehensive extraction of information from the acquired images.

3.5. Perspective in biosensors integration in microfluidics

As we saw in the previous Section, different types and multiple sensors can be integrated into a Lab-on-a-Chip device to generate a

signal that carries out information about the analyte concentration. The combination of Lab-on-a-Chip and sensors, especially biosensors, ensures access to the local information contained in the biological experiment (Antonelli et al., 2024; Varma and Voldman, 2015; Zhou et al., 2015). At the same time, it is known that the act of measuring slightly modifies the quantity under measurement, inducing the so-called observer effect (Rogala et al., 2016; Lu et al., 2023; Schwickart et al., 2014). This is particularly true for electrochemical measurements, which usually generate redox species on the electrodes that would not usually be present in the environment. Microfluidics can help in this way by helping by recombining measurement-generated redox couples and moving away the reaction products at the electrodes (Sahore et al., 2016; Mo et al., 1979).

Exploiting biosensors' notorious accuracy and reliability, it is possible to continuously and non-invasively inspect the physical and biological information involved in the experiment, as well as the chemical concentration of chemicals and metabolites. As also mentioned in the Introduction section, this is pivotal to a comprehensive awareness of the biological mechanisms.

In this field, microfluidic strongly interacts with biosensors, empowering Lab/Organ-on-a-Chip with fluidic actuation. Microfluidic can generate chemical gradients or allow a drug delivery system setup that sensors can easily detect and monitor. In this scenario, Grant et al. (2022) presented a noteworthy work in which they demonstrated the possibility of establishing oxygen gradients, confirmed by simulations and experimental sensing validations, that they used to study hypoxic epithelial lumen in an Intestine-On-a-Chip. Another interesting work presented by Feng et al. (2019) demonstrated the effect of Vandetanib drug under different fluid shear stress levels and observed cell morphological changes and reactive oxygen species levels, proving that shear stress is a substantial factor for drug toxicity.

As biosensors can capture microfluidic-induced signals, biosensing signals can also trigger a real-time chemical stimulation of the experiment. For example, in a recent paper, Kinet et al. (2024) proposed a system able to "give the cells what they need when they need": after the development of a biosensor able to monitor the nitrogen in an *Escherichia coli* culture, they set up a feedback control system to deliver the correct amount of nitrogen to the culture. Focusing on a more near-to-clinical scenario, Liu et al. (2023) designed a wearable, low-cost control system for insulin. Based on a glucose oxidase electrochemical biosensor, the device is able to deliver insulin in rats with diabetes using an electrochemical micropump. In the optic of building up this kind of biosensors-based control system, we believe that machine learning can be the elaboration step that computes the sensing information, transforming it into an input for the actuation stimulus. Using such a system, the biological experiment's measurables will always be more and more monitored and controlled.

With the progress of this beneficial exchange between biosensing elements and microfluidics, we believe that future technologies will be able to solve current issues in modern devices. For example, in order to be applicable in real clinical, Point-of-Care, or even commercial scenarios, it is crucial to extend biosensors' dynamic range as much as possible, reducing the limit of detection and increasing the saturation level (Prabowo et al., 2021). To achieve this goal is surely fundamental to work on the biosensing molecule. Li et al. (2020) showed that it is possible to work on aptamer distal-site mutation and allosteric inhibition to obtain an extension or a reduction of an electrochemical aptamer-based sensor. Working more on the system setup, Field Effect Transistor (FET)-based biosensors were also demonstrated to be an optimal way to reduce power consumption and noise, while having a broad dynamic range (Sengupta and Hussain, 2024). Furthermore, the cooperation between biosensors, machine learning and microfluidic demonstrated to further enhance the dynamic range also in useful low-cost Point-of-Care devices, able to detect risk for cardiovascular diseases (Ballard et al., 2020).

An additional point to be addressed is that the sensing biological molecule could be subject to degradation, which could limit biosensor applications to single-use. For this reason, in the future, it will be crucial to work on biosensing stability or regeneration, especially for continuous monitoring applications (Jia et al., 2024). Sibug-Torres et al. proposed an interesting Surface-enhanced Raman spectroscopy sensor based on nanogap hotspots, which can be electrochemically regenerated (Sibug-Torres et al., 2024). In this optics, microfluidics can surely lend a hand to microfluidics. For example, focusing on Organ-on-a-Chip-like applications, Aleman et al. draw up a protocol for the continuous electrochemical screening of biomolecules by aptamer or antibodies, suggesting an appealing way to "clean and re-functionalise" the electrodes by microfluidic chemical flow and cyclic voltammetry (Aleman et al., 2021). Of course, these methodologies for restoration are highly desirable for other kinds of sensors, such as acoustic and optical ones.

With the current trend in creating Organ-on-a-Chips with multiple organ types, future efforts will need to be concentrated on the multiplexed detection of biomarkers from several Organ-on-a-Chips, focusing on strategies to perform sensor fusion and multivariate data analysis (Zhu et al., 2021; Liao et al., 2019; Yang et al., 2024b). It would be desirable to have a single multi-sensor platform that can perform both physical and biochemical sensing functions in an automated manner. Furthermore, knowing the various needs and properties of the Organ-on-a-Chip systems, we believe that each multi-sensor platform needs to be customised for the intended Organ-on-a-Chip application, implementing the specificity and the adaptation required to increment the exploitability of the platform. Other issues, such as biofouling (i.e., the aggregation of macromolecules or microorganisms on the biosensor), reliability, and scalability, are yet to be addressed in Organ-on-a-Chip applications (Bhatia et al., 2024).

Lastly, we believe that the development of modular sensing systems that allow plug-and-play will eventually permit the replacement of sensors for sensor degradation or biofouling. In addition, a modular approach would also benefit the Organ-on-a-Chip field to implement additional Organ-on-a-Chip components to the systems when needed. In this field, the literature proposed the development of openable Lab-on-a-Chips (Anwar et al., 2011; Teixeira Carvalho et al., 2023). Possible integration of these relatively new technologies with biosensors in Organ-on-a-Chip could be beneficial for extending its life and eventually customising its final scope (e.g., targeting other analytes by simply changing biosensors) without changing its initial design.

4. Machine learning as integrated tool in sensing systems

As a branch of computer science, artificial intelligence enables computers to simulate the human brain in data analysis and reproduce human decisions with the aim of solving complex problems with minimised human intervention. With the disposal of big data and the increased computing power of computers, artificial intelligence has become a powerful engine driving the development of different disciplines today (Bandodkar et al., 2014, 2019; Mahato and Wang, 2021; Haenlein and Kaplan, 2019; Choi et al., 2020; Di et al., 2015; Lee et al., 2016; Bian et al., 2021; de Castro et al., 2019; Sempionatto et al., 2019; Kim et al., 2017, 2018a, 2018b, 2019; Tiwari et al., 2022; Zhang et al., 2019, 2021b; Nah et al., 2021; Ghaffari et al., 2021; Sonner et al., 2015; Padash et al., 2020; Gao et al., 2016). Machine learning is the real core of artificial intelligence (Bini, 2018). In particular, machine learning algorithms primarily aim to build models from sample data, known as training data, and use them to make predictions or decisions on unknown data. Moreover, after a training session, machine learning algorithms are known to generalise and thus work also on data on which they were not initially trained, automatically acquiring new knowledge (Caro et al., 2022; Doshi-Velez and Kim, 2018) (Fig. 4).

Deep learning is a relatively new concept (Hinton et al., 2006) based on neural network architectures. Taking inspiration from the human brain, it comprises a network of interconnected artificial "neurons", i.e., algorithmic blocks performing simple functions organised in multiple

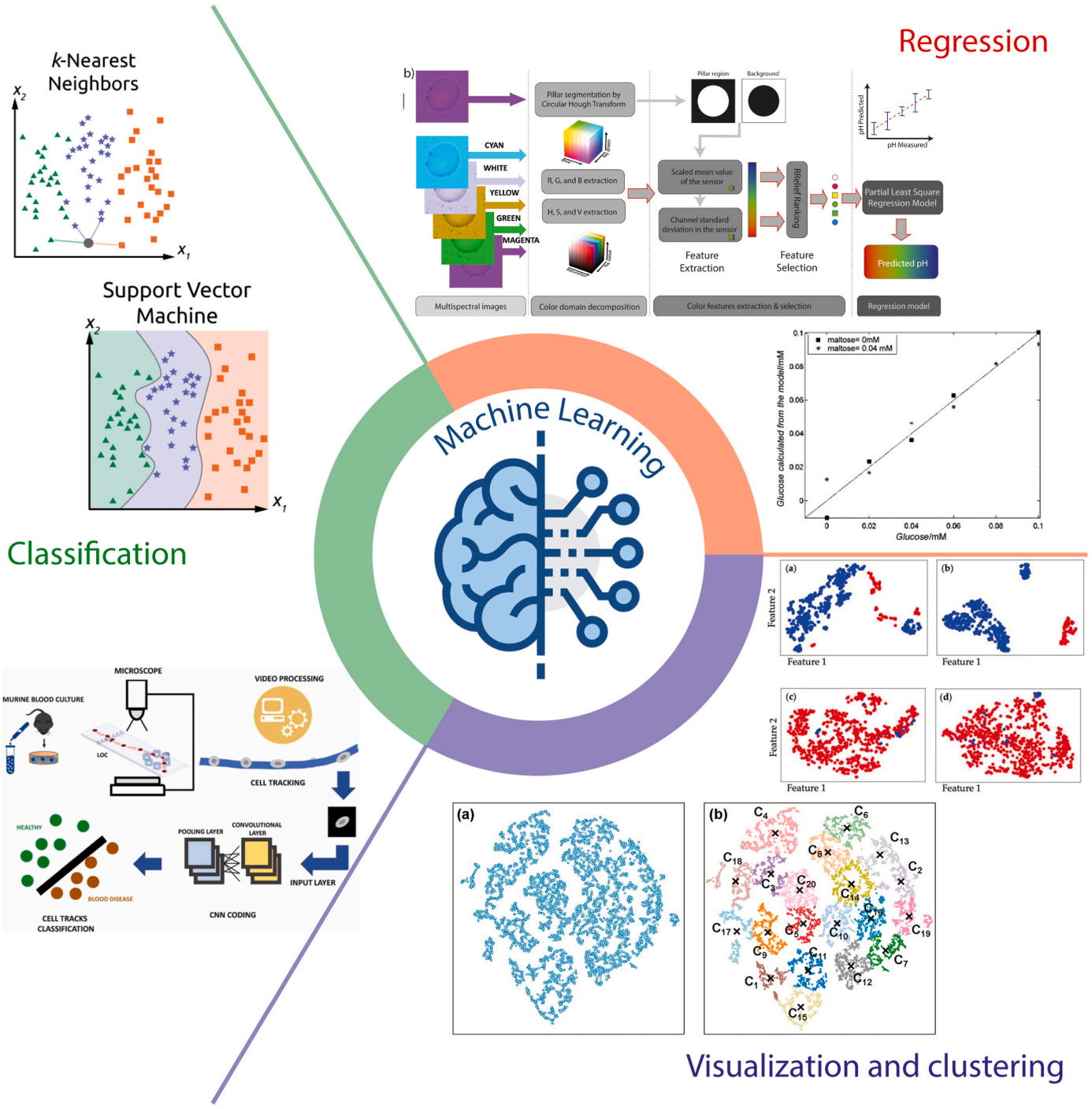

Fig. 4. Some of the machine learning applications discussed in this review. These powerful approaches can help in the reduction of data dimensionality to improve biosensor signals' visualisation [adapted from (Moon et al., 2020), with permission from Elsevier, and (Rong et al., 2023)], their classification depending on the observed phenomenon [adapted from (Mencattini et al., 2023b), with permission from Elsevier, and (Schackart and Yoon, 2021)] or even the extraction of their mathematical relation with analytes [adapted from (Antonelli et al., 2024) and (Lau et al., 2007), with permission from Elsevier].

layers. The extensive interactions among these neurons, paired with the increased depth of processing, enable deep learning to identify hidden correlations and new patterns in raw data, classify them, and even continuously improve. Unlike basic machine learning models, deep learning algorithms make it possible to automatically extract numerous "deep features" by means of their several neural layers, significantly reducing the time and effort required to develop feature extractors from data. This ability is crucial in fields where the mechanism under investigation is partially obscured, as the network can uncover underlying phenomena. This is especially important for biosensors, which, as discussed in the previous section, can integrate well with microfluidic devices but whose signal interpretation can sometimes be not trivial.

With Point-of-Care devices, for example, it is crucial to give the final user the possibility of interpreting the final output without complete knowledge of the device principles. A critical case study in this field during recent years was SARS-CoV-2 virus assays, which aspired to detect the presence of the virus in a few minutes, exploiting the electrochemical, acoustic and optical biosensing principles discussed in the previous Section (Teixeira et al., 2022; Mandal et al., 2022; Asghari et al., 2021; Fabiani et al., 2021). However, while colourimetric sensors

(implying a change of colour in the assay) can be easily interpreted by any final user, other kinds of biosensors still require specific knowledge to be used. For this reason, many researchers proposed to integrate machine learning algorithms in their sensing system to directly integrate human expert knowledge into the biosensing system (Cui et al., 2020; Beisenova et al., 2023; Beduk et al., 2022; Jeong et al., 2020; Un et al., 2021; Arano-Martinez et al., 2022). This case study highlighted the importance of obtaining systems that are more and more intelligent, able to sense the analyte of interest and automatically take action with the help of microfluidic. In this way, while sensors provide information about the investigated analyte and microfluidic give actuation to the platform, machine learning is the "instrument" that permits the processing of the acquired data and, more importantly, the integration and extraction of knowledge inside and from the platform itself.

Depending on how the machine learning algorithm is trained, it can be categorised into three macro-groups: unsupervised, supervised, or reinforcement learning (Morales and Escalante, 2022).

4.1. Unsupervised learning

Unsupervised learning is the branch of machine learning that focuses on analysing unlabelled data, aiming at representing high dimensional data in lower dimensional spaces or at understanding how the data can be clusterised (Xu, 2019; Hiran et al., 2021; Chinnamgari, 2019).

Data visualisation is crucial to understanding their distribution in the space, but this could be quite difficult for higher dimensional data (i.e., big data). Principal Component Analysis (PCA) (Greenacre et al., 2022) and t-distributed Stochastic Neighbour Embedding (t-SNE) (Van Der Maaten and Hinton, 2008) are probably the most known algorithms able to ease big data visualisation. Their use was demonstrated in many biosensors' signals, which can contain complex, highly dimensional content that machine learning algorithms can help extrapolate (Cui et al., 2020; Sosnowski et al., 2020). Malara et al. (2018) presented an interesting Surface-enhanced Organic ElectroChemical Transistor (SeOECT) whose signals are computed by PCA to distinguish between tumor and non-tumor patients using a liquid biopsy.

Since unsupervised machine learning has the intrinsic capability of visualising patterns in unlabelled data, it was also used to assign automatic labels to the data points, i.e., to cluster the input data. K-Means (Sinaga and Yang, 2020), Density-Based Spatial Clustering of Applications with Noise (DBSCAN) (Xie et al., 2019) and Balanced Iterative Reducing and Clustering using Hierarchies (BIRCH) (Zhang et al., 1996) are some examples of algorithms falling in this category and whose application was demonstrated in biosensing microfluidic applications.

4.2. Supervised learning

Focusing on labelled data, supervised learning can find the correlation between complex data as input (features or predictors, but also raw data like signals, images, and videos) and the assigned label as output (responses) (Hastie et al., 2009). It is worth noting that because of the consequences of the No-Free-Lunch theorem, stated and proved by Wolpert and Macready in 1997 (Wolpert and Macready, 1997), especially for supervised learning, there will never be an algorithm that performs better than another for every biosensor, but the algorithm performances will depend mainly on the data characteristics and, eventually, on the preprocessing (Ricci et al., 2016; Tang et al., 2023; Feng et al., 2024). After selecting the most suitable algorithm for the desired problem, the model will be trained on the given data so that it will be able to predict the output, which can be continous (regression) or categorical (classification).

Regression can be particularly useful in biosensor signal processing, helping to find the correct relationship between the signal obtained from the biosensors and the physical, chemical or biological quantity under measurement. The training process typically relies on a calibration step: the biosensor signals are first correlated with a known quantity (e.g., temperature, pH, concentration), and then the model is built up. Many algorithms were proposed in this framework and used to analyse biosensor signals, starting from the most classical Multiple Linear Regression (MLR) (Rodrigues et al., 2020), passing through Principal Component Regression (PCR) (Kim et al., 2008), to more complex algorithms such as Partial Least Square regression (PLS) (Marabel and Alvarez-Taboada, 2013) and Support Vector Regression (SVR) (Brereton and Lloyd, 2010).

Classification problems occur when we need to recognise different groups within the input data (Hastie et al., 2009). This kind of approach can be particularly effective in case studies where biosensors are used to discriminate different analytes in liquid or gaseous environments, e.g., bioelectronic tongues or noses. As with the previous type of algorithms, a calibration must always be performed to teach the model the differences between the several classes. In this category, we can find multiple algorithms like k-Nearest Neighbour (kNN) (Vishalatchi et al., 2024), Partial Least Square Discriminant Analysis (PLS-DA) (Tahir et al., 2024), Linear Discriminant Analysis (LDA) (Subbarao et al., 2024), Support Vector Machine (SVM) (Mao et al., 2024).

4.2.1. Deep learning

A particular mention must be given to deep learning approaches, a series of supervised approaches that, inspired by the neuronal architecture of animals' brains, guarantee multiple layers of processing and a distributed computing architecture to learn patterns that cannot be easily caught from classical machine-learning algorithms (Hinton et al., 2006; Lecun et al., 2015; Schmidgall et al., 2024), especially tailored to work with the high complexity of images and videos. Deep learning networks were demonstrated to be designable to perform both regression and classification, depending on the desired applications. For example, Rho et al. (2022) demonstrated the high efficacy of a deep learning tool named DualWKNet to detect bacteria in arbitrary media using surface-enhanced Raman spectroscopy signals. A Lab-On-Chip for the detection of food pathogens by means of a deep-learning neural network was proposed by Quan et al. (2024), who highlighted an extremely high accuracy and low limit of detection.

The intrinsic complexity of these computing architectures and the high numerosity of trainable hyperparameters make them highly suitable for biosensors based on images and videos, such as cell-based optical biosensors, where the correlation with the analyte under interest can be highly unclear. In these application scenarios, these algorithms can also perform encoding, segmentation, automatic recognition of outliers, or even generation of new phantom data, improving the performances of the algorithm even in noisy environments or with degraded sensors (Casti et al., 2023; Comes et al., 2021; Esmaeili et al., 2023). In the interesting work by McDermott et al. (2024), the authors performed bioimpedance spectroscopy data augmentation using Generative Adversarial Networks (GAN).

One of the biggest drawbacks of training a neural network from scratch is that it requires a big dataset to correctly tune its internal weights. In those issues, the literature suggests as possible solution, the use of transfer learning, thus relying on the use of pre-trained neural networks to extract deep features correlated with the input (Weiss et al., 2016; Bozinovski, 2020). These features can then be used to train another machine/deep-learning algorithm, which will correlate them with the quantity to estimate (Kensert et al., 2019; Qureshi et al., 2023; Yang et al., 2024c). It is crucial to notice that, to enhance the performances of the final model, the extracted features must be selected depending on their significance, the signal-to-noise ratio and correlation with the phenomenon of interest (Mencattini et al., 2023c).

4.3. Reinforcement learning

Differently from supervised and unsupervised learning, reinforcement learning algorithms work in a dynamic environment, and they are trained with their continuous interaction with it (Morales and Escalante,

2022; Milani et al., 2024; Li, 2017). Similar to how newborns learn, an agent (a trainable model based on a machine learning algorithm or a neural network) interacts with the environment under study, being able to observe its current state. Depending on its actions, which will influence the environment, it will get a reward or a punishment. The agent will learn by trying to maximise the rewards for its action in the actual state of the environment (Li, 2017).

Reinforcement learning was successfully implemented in a digital microfluidic biochip by Liang et al. (2024) and used for liquid droplet manipulation. The use of this approach demonstrated the ability to compensate for 2D electrode natural degradation over time, performing profitably an epigenetic bioassay. In another work, Chen et al. (2023) proposed the use of reinforcement learning to find nanostructure designs able to distinguish among different enantiomer chiralities using artificial neural networks and surface plasmon circular dichroism.

Even though many applications display the use of reinforcement learning, we believe that its potential has still not been completely exploited, probably because of the relative novelty of these approaches and the complexity of setting up the control system also in terms of number of continuous measurements.

4.4. Perspective in integrating machine learning in sensing systems

Despite the proven advantages of machine learning and artificial intelligence in microfluidics, several potential challenges and pitfalls still exist. First, expert skills are needed to integrate machine learning into microfluidics, helping reduce the general scepticism about its usefulness. Moreover, the acquisition of labelled data is frequently imperative, albeit it is labour-intensive and subjective. Artificial intelligence, integrating machine learning as a subset of algorithms, results as a black-box model, and, at present, still lacks explainability and easy interpretability of the results. To solve this problem, many researchers are working on models able to explain to the user the reasons leading to a particular outcome (Liu et al., 2022; Fidel et al., 2020; Heuillet et al., 2021).

Although artificial intelligence is a clever approach to increment the "quantity paradigm" allowing massive analysis of sensing signals, it has not totally demonstrated it verifies the "quality paradigm", facing stability and robustness of data (drift, noise, lack of reproducibility over different conditions, etc.). A discussion of such aspects needs to be held next. Artificial intelligence opens new frontiers of biosensing signals analysis, allowing massive analysis and the possibility to extract information from live imaging (optical sensing). This approach allows for the reconstitution of a biologist-like attitude towards the experiment by manipulating spatial multivariate data. This may open the frontiers of correlating spatial information with standard biochemical information, leading to a more exhaustive comprehension of the underlying phenomena.

Indeed, the synergistic combination of high-throughput microfluidic systems and artificial intelligence has created an emerging yet rapidly growing field. Moreover, sharing similar algorithmic architectures may reduce technical barriers to using artificial intelligence in microfluidics, for example, in training high-performance generic image classifiers for a wide range of biomedical image classification tasks. In this way, artificial intelligence could indeed change the underpinning paradigm of microfluidics' use, where massively parallelised efforts could be made

Table 1
A list of the research papers displayed in this review that exhibit at least a dual interaction between microfluidics, biosensors and machine learning.

Description	Reference	Microfluidics	Biosensor	Machine-Learning
Coding microfluidic droplets using coloured beads and machine learning	Svensson et al. (2019)	✓		✓
Polymerisation control by thermography and machine learning	Rizkin et al. (2020)	✓		✓
pH extraction during biological Time-Lapse experiment in Lab-on-a-Chip	Antonelli et al. (2024)	✓	✓	✓
Patients' Lung-tumour-on-Chip to study immunotherapy responses	Veith et al. (2024)	✓		✓
Designing flow shape by reinforcement learning	Lee et al. (2018)	✓		✓
Convolutional autoencoder model for droplet description and stability	Khor et al. (2019)	✓		✓
Deep learning for flow shape definition	Stoecklein et al. (2017)	✓		✓
Flow-flow pattern in a microchannel using machine learning	Desir et al. (2019)	✓		✓
Reinforcement learning for particle manipulation in microfluidics	Fang et al. (2023)	✓		✓
Vascularised Kidney-on-Chip to evaluate nephrotoxicity	Cohen et al. (2021)	✓	✓	✓
Multiplexed, self-calibrating electrochemical sensors for cell cultures	Giménez-Gómez et al. (2020)	✓	✓	✓
Surface-Acoustic Wave biosensors in Lab-on-a-Chip	Agostini et al. (2019)	✓	✓	
Point-of-Care device for detection of respiratory infections	Teixeira et al. (2022)	✓	✓	✓
Modified cell line to make them fluid shear stress sensors in microfluidics	Varma and Voldman (2015)	✓	✓	
Love-wave biosensor to detect marine toxin on-a-Chip	Zhang et al. (2016)	✓	✓	✓
An electrochemical and colorimetric wearable sensor for sweat analysis	Bandodkar et al. (2019)	✓	✓	✓
Detection of microRNA from Cancer Cells using Surface Acoustic Wave	Bin Han and Lee (2024)	✓	✓	✓
A portable Point-of-Care optical glucose biosensor in microfluidic	Panwar et al. (2024)	✓	✓	✓
Dip-coating for kinetically doped biosensors	Crosley and Yip (2018)		✓	✓
A segmentation platform to detect Amyotrophic Lateral Sclerosis and chemotherapeutics	Mencattini et al. (2021)		✓	✓
Single-cell gene expression and drug response estimation by deep learning	D'Orazio et al. (2022)		✓	✓
Screening of apoptosis-inducing drugs by time-lapse microscopy	Aftab et al. (2014)		✓	✓
Monitoring liver injuries on-a-Chip by integrated biosensors	Zhou et al. (2015)	✓	✓	
Effect of fluid shear stress on chemotherapic toxicity in a cell culture on chip	Feng et al. (2019)	✓	✓	
Nitrogen sensing and control to provide the correct amount of nutrients to *Escherichia Coli*	Kinet et al. (2024)	✓	✓	✓
A minimally-invasive wearable system to sense glucose and provide insulin	Liu et al. (2023)	✓	✓	✓
A Point-of-Care device based on deep learning and Vertical-Flow assay	Ballard et al. (2020)	✓	✓	✓
Regeneration of electrochemical biosensors in Organ-on-a-Chip devices	Aleman et al. (2021)	✓	✓	
A Lab-on-a-Chip to estimate Red Blood Cells diseases by deformation	Mencattini et al. (2023b)	✓	✓	✓
Nanoplasmonic biosensor for SARS-CoV-2 immunity profiling	Beisenova et al. (2023)		✓	✓
Detection of all SARS-CoV-2 variants in a platform	Beduk et al. (2022)		✓	✓
Surface-enhanced Organic Electrochemical platform for sporadic tumour risk assessment	Malara et al. (2018)	✓	✓	✓
Photonic biosensors and machine learning for virus detection	Vishalatchi et al. (2024)		✓	✓
PCA and PLS-DA algorithms for Surface-Enhanced Raman Scattering signals	Tahir et al. (2024)		✓	✓
Deep learning for bacterial identification by SERS analysis	Rho et al. (2022)		✓	✓
Identification of pathogens in food using deep learning and Lab-on-a-chip techniques	Quan et al. (2024)	✓	✓	✓
Bioimpedance spectroscopy augmentation by Generative Adversarial Networks	McDermott et al. (2024)		✓	✓
Digital microfluidic chip and reinforcement learning for droplet manipulation	Liang et al. (2024)	✓	✓	✓
Reinforcement learning for chirality detection of biomolecules	Chen et al. (2023)	✓	✓	✓

feasible to solve problems completely impossible with artificial-intelligence-absent efforts. We foresee a sustained upward trend in the utilisation of artificial intelligence within microfluidic systems.

Considering that machine/deep learning's robust analysis of structured data (sequences, images, videos, etc.) can predict complex outputs with unprecedented accuracy, the combination of traditional machine/deep learning and microfluidics should have the potential to address some previously unsolvable problems.

5. Final perspectives

This review explored a new paradigm in which machine learning and artificial intelligence could enhance the performance of integrated biosensors in microfluidic environments, becoming an intrinsic part of the sensing systems, mimicking human sensorial systems (Table 1). Consequently, in the future, it will be crucial to design biosensors and Lab/Organ-on-a-Chip architectures that facilitate machine learning processes. For example, to fully leverage the potential of machine learning in these systems, it will be essential to design experimental setups that can extract the maximum amount of information from the biosensing elements. This will initiate a positive feedback loop, offering numerous advantages to each of the three entities. We have seen in recent applications how biosensors (and, in some applications, microfluidics devices) can be big data generators for machine learning, which can be trained to recognize specific patterns of analytes or flow. On the other hand, machine learning can trigger fluidic actuation in Lab-on-a-Chip devices, closing the feedback loop in the sense/control system. Particularly in Organ-On-Chip applications, biosensing, and microfluidics could help in recreating an environment more similar to the *in vivo* conditions, monitoring key biomolecules (such as Reactive Oxygen Species, proteins, or nucleic acids, etc.) and controlling their distribution in the platform during the experiment (Sabaté del et al., 2024).

This benefit exchange will expand the measurable range of biosensors from electrochemical to optical and acoustic, enabling the collection of comprehensive information involved in biological mechanisms, leading to a complete understanding of biological phenomena or therapeutic intervention effects. Secondly, it will enable the design of microfluidics capable of accommodating a more comprehensive array of biosensors within the cell environment and the selection of existing and new materials to facilitate the sensing process: transparent materials for optical sensing, diverse rigidity materials for acoustics, and conductivity-controlled media for electro-optical investigations, among others. Finally, a platform comprising biosensing and microfluidics will facilitate the acquired and analysed data through an automatic self-regulation process. This would enable complete adaptation to changing biological conditions and greatly extend the duration of experiments, allowing for the study of slowly varying phenomena not observed by current experimental settings.

6. Conclusions

This review proposed the synergic integration of machine learning algorithms, biosensors, and microfluidics devices. Beyond revising the state of the art of the three main domains of application, we focused on how each entity is contributing to the synergy, focusing on the actual limitations of the "dual counterpart" (namely, eliminating one out of the three). The review presented the additional facilities acquired through the implementation of AI in biosignals and microfluidics outcome processing and of using physical and biochemical sensor elements in the monitoring – rather than ad end-point measurements – of microfluidics experiments. We believe this review will concur in promoting the development of new integrated analysis platforms, enabling more high-performance experiments to better understand yet unknown biological mechanisms.

CRediT authorship contribution statement

Gianni Antonelli: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Joanna Filippi:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Michele D'Orazio:** Writing – review & editing, Formal analysis, Data curation. **Giorgia Curci:** Writing – review & editing, Formal analysis, Data curation. **Paola Casti:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Arianna Mencattini:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Eugenio Martinelli:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of Competing interest

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

Data availability

No data was used for the research described in the article.

References

Acharya, B., Behera, A., Behera, S., 2024. Chem. Phys. Impact 8, 100414.
Aftab, O., Nazir, M., Fryknäs, M., Hammerling, U., Larsson, R., Gustafsson, M.G., 2014. Apoptosis 19, 1411–1418.
Agostini, M., Greco, G., Cecchini, M., 2019. IEEE Access 7, 70901–70909.
Ai, Y., Xie, R., Xiong, J., Liang, Q., 2020. Small 16, 1903940.
Aleman, J., Kilic, T., Mille, L.S., Shin, S.R., Zhang, Y.S., 2021. Nat. Protoc. 16 (5 16), 2564–2593, 2021.
Antonelli, G., Mencattini, A., Massimiani, M., Lacconi, V., Filippi, J., Losardo, M., D'Orazio, M., Casti, P., Bragaglia, M., Curci, G., Nanni, F., Campagnolo, L., Martinelli, E., 2024. Sensor. Actuator. B Chem. 401, 135009.
Anwar, K., Han, T., Kim, S.M., 2011. Sensor. Actuator. B Chem. 153, 301–311.
Aoki, H., Masahiro, Y., Shimizu, M., Hongoh, Y., Ohkuma, M., Yamagata, Y., 2022. Sci. Rep. 12 (1 12), 1–13, 2022.
Arano-Martinez, J.A., Martínez-González, C.L., Salazar, M.I., Torres-Torres, C., 2022. Biosensors 12, 710–712 (2022) 710.
Arduino, I., Liu, Z., Rahikkala, A., Figueiredo, P., Correia, A., Cutrignelli, A., Denora, N., Santos, H.A., 2021. Acta Biomater. 121, 566–578.
Aref, H., 2008. Phil. Trans. Math. Phys. Eng. Sci. 366, 2649–2670.
Asghari, A., Wang, C., Yoo, K.M., Rostamian, A., Xu, X., Shin, J.D., Dalir, H., Chen, R.T., 2021. Appl. Phys. Rev. 8, 31313.
Augustine, S., Chinnamani, M.V., Mun, C.W., Shin, J.Y., Trung, T.Q., Hong, S.J., Huyen, L.T.N., Lee, E.H., Lee, S.H., Rha, J.J., Jung, S., Lee, Y., Park, S.G., Lee, N.E., 2024. Biosens. Bioelectron. 248, 115987.
Ballard, Z.S., Joung, H.A., Goncharov, A., Liang, J., Nugroho, K., Di Carlo, D., Garner, O. B., Ozcan, A., 2020. Npj Digit. Med. 3 (1 3), 1–8, 2020.
Bandodkar, A.J., Molinnus, D., Mirza, O., Guinovart, T., Windmiller, J.R., Valdés-Ramírez, G., Andrade, F.J., Schöning, M.J., Wang, J., 2014. Biosens. Bioelectron. 54, 603–609.
Bandodkar, A.J., Gutruf, P., Choi, J., Lee, K., Sekine, Y., Reeder, J.T., Jeang, W.J., Aranyosi, A.J., Lee, S.P., Model, J.B., Ghaffari, R., Su, C.-J., Leshock, J.P., Ray, T., Verrillo, A., Thomas, K., Krishnamurthi, V., Han, S., Kim, J., Krishnan, S., Hang, T., Rogers, J.A., 2019. Sci. Adv. 5.
Beduk, D., Ilton de Oliveira Filho, J., Beduk, T., Harmanci, D., Zihnioglu, F., Cicek, C., Sertoz, R., Arda, B., Goksel, T., Turhan, K., Salama, K.N., Timur, S., 2022. Biosens. Bioelectron. X 10, 100105.
Beisenova, A., Adi, W., Bashar, S.J., Velmurugan, M., Germanson, K.B., Shelef, M.A., Yesilkoy, F., 2023. Sens. Diagn. 2, 1186–1198.
Bhatia, D., Paul, S., Acharjee, T., Ramachairy, S.S., 2024. Sens. Int. 5, 100257.
Bian, S., Ye, S., Yang, S., 2021. Wearable Technology 3, p. 88.
Bin Han, S., Lee, S.S., 2024. Micromachines 15, 249.
Bini, S.A., 2018. J. Arthroplasty 33, 2358–2361.
Bouquerel, C., César, W., Barthod, L., Arrak, S., Battistella, A., Gropplero, G., Mechta-Grigoriou, F., Zalcman, G., Parrini, M.C., Verhulsel, M., Descroix, S., 2022. Lab Chip 22, 4443–4455.

Bozinovski, S., 2020. Informatica 44, 291–302.
Brereton, R.G., Lloyd, G.R., 2010. Analyst 135, 230–267.
Burklund, A., Tadimety, A., Nie, Y., Hao, N., Zhang, J.X.J., 2020. Adv. Clin. Chem. 95, 1–72.
Cao, B., Huang, Y., Chen, L., Jia, W., Li, D., Jiang, Y., 2024. Biosens. Bioelectron., 116378
Caro, M.C., Huang, H.Y., Cerezo, M., Sharma, K., Sornborger, A., Cincio, L., Coles, P.J., 2022. Nat. Commun. 13 (1 13), 1–11, 2022.
Casti, P., Cardarelli, S., Comes, M.C., D'Orazio, M., Filippi, J., Antonelli, G., Mencattini, A., Di Natale, C., Martinelli, E., 2023. Expert Syst. Appl. 232, 120861.
Chen, C., Wang, J., 2020. Analyst 145, 1605–1628.
Chen, Y., Zhang, C., Shi, M., Peterson, G.P., 2009. Phys. Rev. E - Stat. Nonlinear Soft Matter Phys. 80, 026301.
Chen, Y., Zhang, F., Dang, Z., He, X., Luo, C., Liu, Z., Peng, P., Dai, Y., Huang, Y., Li, Y., Fang, Z., 2023. Opto-Electron. Sci. 2, 220019, 220019.
Chen, Y.C., Lee, K.Y., Liao, H.J., Sun, W.L., Huang, W.C., Wang, Y.S., Chang, W.C., Liu, C. H., 2024. Sensor. Actuator. B Chem. 406, 135409.
Chinnamgari, S.K., 2019. R Machine Learning Projects: Implement Supervised, Unsupervised, and Reinforcement Learning Techniques Using R 3.5. Packt Publishing Ltd.
Choi, R.Y., Coyner, A.S., Kalpathy-Cramer, J., Chiang, M.F., Peter Campbell, J., 2020. Transl. Vis. Sci. Technol. 9.
Choi, G., Yang, H.Y., Cho, S., Kwon, D., Kim, D.W., Ko, S., 2024. Adv. Mater. Technol., 2400107
Cohen, A., Ioannidis, K., Ehrlich, A., Regenbaum, S., Cohen, M., Ayyash, M., Tikva, S.S., Nahmias, Y., 2021. Sci. Transl. Med. 13.
Comes, M.C., Filippi, J., Mencattini, A., Casti, P., Cerrato, G., Sauvat, A., Vacchelli, E., De Ninno, A., Di Giuseppe, D., D'Orazio, M., Mattei, F., Schiavoni, G., Businaro, L., Di Natale, C., Kroemer, G., Martinelli, E., 2021. Neural Comput. Appl. 33, 3671–3689.
Contreras-Naranjo, J.C., Wu, H.J., Ugaz, V.M., 2017. Lab Chip 17, 3558–3577.
Covington, J.A., Marco, S., Persaud, K.C., Schiffman, S.S., Nagle, H.T., 2021. IEEE Sensor. J. 21, 12969–12990.
Crosley, M.S., Yip, W.T., 2018. ACS Omega 3, 7971–7978.
Cui, F., Yue, Y., Zhang, Y., Zhang, Z., Zhou, H.S., 2020. ACS Sens. 5, 3346–3364.
de Castro, L.F., de Freitas, S.V., Duarte, L.C., de Souza, J.A.C., Paixão, T.R.L.C., Coltro, W. K.T., 2019. Anal. Bioanal. Chem. 411, 4919–4928.
Desir, P., Chen, T.Y., Bracconi, M., Saha, B., Maestri, M., Vlachos, D.G., 2019. React. Chem. Eng. 5, 39–50.
T. Dey, P. Mitra, B. Chakraborty, A. Sanyal, A. Acharjee, A. Ghosh, D. Mandal, (2024) 263–303.
Di, J., Yao, S., Ye, Y., Cui, Z., Yu, J., Ghosh, T.K., Zhu, Y., Gu, Z., 2015. ACS Nano 9, 9407–9415.
Diaconu, I., Cristea, C., Hârceagă, V., Marrazza, G., Berindan-Neagoe, I., Săndulescu, R., 2013. Clin. Chim. Acta 425, 128–138.
Ding, Y., Howes, P.D., Demello, A.J., 2020. Anal. Chem. 92, 132–149.
F. Doshi-Velez, B. Kim, (2018) 3–17.
D'Orazio, M., Corsi, F., Mencattini, A., Di Giuseppe, D., Colomba Comes, M., Casti, P., Filippi, J., Di Natale, C., Ghibelli, L., Martinelli, E., 2020. Front. Oncol. 10, 580698.
D'Orazio, M., Murdocca, M., Mencattini, A., Casti, P., Filippi, J., Antonelli, G., Di Giuseppe, D., Comes, M.C., Di Natale, C., Sangiuolo, F., Martinelli, E., 2022. Sci. Rep. 12 (1 12), 1–14, 2022.
D'Orazio, M., Filippi, J., Antonelli, G., Curci, G., Casti, P., Mencattini, A., Cidonio, G., Martinelli, E., 2024. Materials 5, 100229.
Esmaeili, F., Cassie, E., Nguyen, H.P.T., Plank, N.O.V., Unsworth, C.P., Wang, A., 2023. Bioengineering 10, 405–410 (2023) 405.
Essaouiba, A., Okitsu, T., Kinoshita, R., Jellali, R., Shinohara, M., Danoy, M., Legallais, C., Sakai, Y., Leclerc, E., 2020. Biochem. Eng. J. 164, 107783.
Estevez, M.C., Alvarez, M., Lechuga, L.M., 2012. Laser Photon. Rev. 6, 463–487.
Fabiani, L., Saroglia, M., Galatà, G., De Santis, R., Fillo, S., Luca, V., Faggioni, G., D'Amore, N., Regalbuto, E., Salvatori, P., Terova, G., Moscone, D., Lista, F., Arduini, F., 2021. Biosens. Bioelectron. 171, 112686.
Fallahi, H., Zhang, J., Phan, H.P., Nguyen, N.T., 2019. Micromachines 10, 830, 830 10 (2019).
Fang, Y., 2006. Assay drug. Dev. Technol. 4, 583–595.
Fang, W.Z., Xiong, T., Pak, O.S., Zhu, L., 2023. Adv. Sci. 10, 2205382.
Feng, S., Mao, S., Zhang, Q., Li, W., Lin, J.M., 2019. ACS Sens. 4, 521–527.
Feng, Z., Liu, D., Gu, J., Zheng, L., 2024. J. Food Compos. Anal. 129, 106090.
Fidel, G., Bitton, R., Shabtai, A., 2020. Proceedings of the International Joint Conference on Neural Networks.
Filippi, J., Di Giuseppe, D., Casti, P., Mencattini, A., Antonelli, G., DOrazio, M., Corsi, F., Della-Morte Canosci, D., Ghibelli, L., Witte, C., Di Natale, C., Neale, S.L., Martinelli, E., 2022. Sensor. Actuator. B Chem. 368.
Filippi, J., Casti, P., Antonelli, G., Murdocca, M., Mencattini, A., Corsi, F., Orazio, M.D., Pecora, A., De Luca, M., Curci, G., Ghibelli, L., Sangiuolo, F., Neale, S.L., Martinelli, E., 2024. Small Methods, 2300923.
Fogel, R., Limson, J., Seshia, A.A., 2016. Essays Biochem. 60, 101–110.
Fruncillo, S., Su, X., Liu, H., Wong, L.S., 2021. ACS Sens. 6.
Fu, Y.Q., Luo, J.K., Nguyen, N.T., Walton, A.J., Flewitt, A.J., Zu, X.T., Li, Y., McHale, G., Matthews, A., Iborra, E., Du, H., Milne, W.I., 2017. Prog. Mater. Sci. 89, 31–91.
Fukada, K., Seyama, M., 2022. Anal. Chem. 94, 7060–7065.
Gaba, S., Chauhan, N., Chandra, R., Jain, U., 2024. Talanta Open 9, 100301.
Galan, E.A., Zhao, H., Wang, X., Dai, Q., Huck, W.T.S., Ma, S., 2020. Matter 3, 1893–1922.
Gao, W., Emaminejad, S., Nyein, H.Y.Y., Challa, S., Chen, K., Peck, A., Fahad, H.M., Ota, H., Shiraki, H., Kiriya, D., Lien, D.H., Brooks, G.A., Davis, R.W., Javey, A., 2016. Nature 529 (7587 529), 509–514, 2016.
Gao, W., Zhang, C., Cai, Y., Su, F., Han, C., Yu, D., Luo, Y., Xing, X., 2024. Sensor. Actuator. B Chem. 410, 135669.
Ghaffari, R., Rogers, J.A., Ray, T.R., 2021. Sensor. Actuator. B Chem. 332, 129447.
Giménez-Gómez, P., Rodríguez-Rodríguez, R., Ríos, J.M., Pérez-Montero, M., González, E., Gutiérrez-Capitán, M., Plaza, J.A., Muñoz-Berbel, X., Jiménez-Jorquera, C., 2020. Lab Chip 20, 823–833.
Grant, J., Lee, E., Almeida, M., Kim, S., LoGrande, N., Goyal, G., Sesay, A.M., Breault, D. T., Prantil-Baun, R., Ingber, D.E., 2022. Lab Chip 22, 1584–1593.
Greenacre, M., Groenen, P.J.F., Hastie, T., D'Enza, A.I., Markos, A., Tuzhilina, E., 2022. Nat. Rev. Methods Prim. 2 (1 2), 1–21, 2022.
Grieshaber, D., MacKenzie, R., Vörös, J., Reimhult, E., 2008. Sensors 8, 1400.
Gupta, N., Renugopalakrishnan, V., Liepmann, D., Paulmurugan, R., Malhotra, B.D., 2019. Biosens. Bioelectron. 141, 111435.
Haenlein, M., Kaplan, A., 2019. Calif. Manag. Rev. 61, 5–14.
Haleem, A., Javaid, M., Singh, R.P., Suman, R., Rab, S., 2021. Sens. Int. 2, 100100.
Hamdallah, S.I., Zoqlam, R., Erfle, P., Blyth, M., Alkilany, A.M., Dietzel, A., Qi, S., 2020. Int. J. Pharm. 584, 119408.
T. Hastie, R. Tibshirani, J. Friedman, (2009) 9–41.
Heuillet, A., Couthouis, F., Díaz-Rodríguez, N., 2021. Knowl Based Syst, vol. 214, 106685.
Hinton, G.E., Osindero, S., Teh, Y.W., 2006. Neural Comput. 18, 1527–1554.
Hiran, K.K., Jain, R.K., Lakhwani, K., Doshi, R., 2021. Machine Learning: Master Supervised and Unsupervised Learning Algorithms with Real Examples, English Edition. BPB Publications.
Hosseini, S., Vázquez-Villegas, P., Rito-Palomares, M., Martinez-Chapa, S.O., 2018. SpringerBriefs in Applied Sciences and Technology, pp. 67–115.
Huang, Y., Das, P.K., Bhethanabotla, V.R., 2021. Sensors and Actuators Reports, vol. 3.
Huang, W., Yang, Q., Liao, J., Ramadan, S., Fan, X., Hu, S., Liu, X., Luo, J., Tao, R., Fu, C., 2024. Biosens. Bioelectron. 247, 115944.
International Union of Pure and Applied Chemistry (IUPAC), 2014. In: The IUPAC Compendium of Chemical Terminology. Research Triangle Park, NC, p. 148.
Jeong, H., Rogers, J.A., Xu, S., 2020. Sci. Adv. 6.
Jia, H., Song, Y., Huang, B., Ge, W., Luo, K.Q., 2020. ACS Sens. 5, 823–830.
Jia, Y., Chen, S., Wang, Q., Li, J., 2024. Nanoscale 16, 2834–2846.
Jin, J., Bhujwalla, Z.M., 2019. Front. Oncol. 9, 1560.
Jin, X., Liu, C., Xu, T., Su, L., Zhang, X., 2020. Biosens. Bioelectron. 165, 112412.
Kadian, S., Kumari, P., Shukla, S., Narayan, R., 2023. Talanta Open 8, 100267.
Karthik, V., Karuna, B., Kumar, P.S., Saravanan, A., Hemavathy, R.V., 2022. Chemosphere 299, 134427.
Kensert, A., Harrison, P.J., Spjuth, O., 2019. SLAS Discovery 24, 466–475.
Kheiri, S., Kumacheva, E., Young, E.W.K., 2021. Front. Bioeng. Biotechnol. 9, 781566.
Khor, J.W., Jean, N., Luxenberg, E.S., Ermon, S., Tang, S.K.Y., 2019. Soft Matter 15, 1361–1372.
Kim, Y.P., Hong, M.Y., Shon, H.K., Chegal, W., Cho, H.M., Moon, D.W., Kim, H.S., Lee, T. G., 2008. Appl. Surf. Sci. 255, 1110–1112.
Kim, J., Kim, M., Lee, M.S., Kim, K., Ji, S., Kim, Y.T., Park, J., Na, K., Bae, K.H., Kim, H. K., Bien, F., Lee, C.Y., Park, J.U., 2017. Nat. Commun. 8 (1 8), 1–8, 2017.
Kim, J., Campbell, A.S., Wang, J., 2018a. Talanta 177, 163–170.
Kim, S.B., Lee, K.H., Raj, M.S., Lee, B., Reeder, J.T., Koo, J., Hourlier-Fargette, A., Bandodkar, A.J., Won, S.M., Sekine, Y., Choi, J., Zhang, Y., Yoon, J., Kim, B.H., Yun, Y., Lee, S., Shin, J., Kim, J., Ghaffari, R., Rogers, J.A., 2018b. Small 14, 1802876.
Kim, J., Campbell, A.S., de Ávila, B.E.F., Wang, J., 2019. Nat. Biotechnol. 37 (4 37), 389–406, 2019.
Kinet, R., Richelle, A., Colle, M., Demaegd, D., von Stosch, M., Sanders, M., Sehrt, H., Delvigne, F., Goffin, P., 2024. Biotechnol. Bioeng. 121, 1270–1282.
Kong, T., Shum, H.C., Weitz, D.A., 2020. Small 16, 2000070.
Kulkarni, N.S., Guererro, Y., Gupta, N., Muth, A., Gupta, V., 2019. J. Drug Deliv. Sci. Technol. 49, 352–364.
Lafleur, J.P., Jönsson, A., Senkbeil, S., Kutter, J.P., 2016. Biosens. Bioelectron. 76, 213–233.
Lakshmy, S., Sanyal, G., Kalarikkal, N., al, Tanjaya, H., Harito, C., 2024. J. Phys. Conf. Ser. 2705, 012018.
Lalegani, F., Saffarian, M.R., Moradi, A., Tavousi, E., 2018. Int. J. Numer. Methods Heat Fluid Flow 28, 1664–1683.
Länge, K., Rapp, B.E., Rapp, M., 2008. Anal. Bioanal. Chem. 391, 1509–1519.
Laocharoensuk, R., 2016. Electroanalysis 28, 1716–1729.
Larou, E., Yiakoumettis, I., Kaltsas, G., Petropoulos, A., Skandamis, P., Kintzios, S., 2013. Food Control 29, 208–212.
Lau, C., Borgmann, S., Maciejewska, M., Ngounou, B., Gründler, P., Schuhmann, W., 2007. Biosens. Bioelectron. 22, 3014–3020.
Lecun, Y., Bengio, Y., Hinton, G., 2015. Nature 521 (7553 521), 436–444, 2015.
Lee, H., Choi, T.K., Lee, Y.B., Cho, H.R., Ghaffari, R., Wang, L., Choi, H.J., Chung, T.D., Lu, N., Hyeon, T., Choi, S.H., Kim, D.H., 2016. Nat. Nanotechnol. 11 (6 11), 566–572, 2016.
Lee, X.Y., Balu, A., Stoecklein, D., Ganapathysubramanian, B., Sarkar, S.. https://doi.org/10.48550/arXiv.1811.12444.
Lee, X.Y., Balu, A., Stoecklein, D., Ganapathysubramanian, B., Sarkar, S., 2019. J. Mech. Des. 141.
Lee, S., Dang, H., Il Moon, J., Kim, K., Joung, Y., Park, S., Yu, Q., Chen, J., Lu, M., Chen, L., Joo, S.W., Choo, J., 2024. Chem. Soc. Rev. 53, 5394–5427.
Leonaviciene, G., Leonavicius, K., Meskys, R., Mazutis, L., 2020. Lab Chip 20, 4052–4062.
Li, Y.. https://doi.org/10.48550/arXiv.1701.07274.
Li, S., Li, C., Wang, Y., Li, H., Xia, F., 2020. Anal. Chem. 92, 13427–13433.
Li, B., Zhai, G., Dong, Y., Wang, L., Ma, P., 2024. Anal. Methods 16, 798–816.

Liang, T.C., Chang, Y.C., Zhong, Z., Bigdeli, Y., Ho, T.Y., Chakrabarty, K., Fair, R., 2024. ACM Transact Des Autom Electron Syst, vol. 29, p. 2020.
Liao, Z., Zhang, Y., Li, Y., Miao, Y., Gao, S., Lin, F., Deng, Y., Geng, L., 2019. Biosens. Bioelectron. 126, 697–706.
Lin, W.Y., Wang, Y., Wang, S., Tseng, H.R., 2009. Nano Today 4, 470–481.
Lino, C., Barrias, S., Chaves, R., Adega, F., Martins-Lopes, P., Fernandes, J.R., 2022. Biochim. Biophys. Acta Rev. Canc 1877, 188726.
Liu, Z., Li, J., Li, J., Yang, T., Zhang, Z., Wu, H., Xu, H., Meng, J., Li, F., 2022. Anal. Chem. 94, 15864–15872.
Liu, Y., Yu, Q., Ye, L., Yang, L., Cui, Y., 2023. Lab Chip 23, 421–436.
Lu, Y., Biswas, M.C., Guo, Z., Jeon, J.W., Wujcik, E.K., 2019. Biosens. Bioelectron. 123, 167–177.
Lu, S.-M., Vannoy, K.J., Dick, J.E., Long, Y.-T., 2023. J. Am. Chem. Soc. 145, 25043–25055.
Ma, X., Guo, G., Wu, X., Wu, Q., Liu, F., Zhang, H., Shi, N., Guan, Y., 2023. Micromachines 14, 972, 972 14 (2023).
Machado, N.D., García-Manrique, P., Fernández, M.A., Blanco-López, M.C., Matos, M., Gutiérrez, G., 2020. Chem. Eng. Res. Des. 162, 162–171.
Mahato, K., Wang, J., 2021. Sensor. Actuator. B Chem. 344, 130178.
Maia, F.R., Reis, R.L., Oliveira, J.M., 2020. Nanomedicine 24, 102139.
Malara, N., Gentile, F., Coppedè, N., Coluccio, M.L., Candeloro, P., Perozziello, G., Ferrara, L., Giannetto, M., Careri, M., Castellini, A., Mignogna, C., Presta, I., Pirrone, C.K., Maisano, D., Donato, A., Donato, G., Greco, M., Scumaci, D., Cuda, G., Casale, F., Ferraro, E., Bonacci, S., Trunzo, V., Mollace, V., Onesto, V., Majewska, R., Amato, F., Renne, M., Innaro, N., Sena, G., Sacco, R., Givigliano, F., Voci, C., Volpentesta, G., Guzzi, G., Lavano, A., Scali, E., Bottoni, U., Di Fabrizio, E., 2018. npj Precis. Oncol. 2 (1 2), 1–13, 2018.
Malhotra, B.D., Chaubey, A., 2003. Sensor. Actuator. B Chem. 91, 117–127.
Mandal, D., Indaleeb, M.M., Younan, A., Banerjee, S., 2022. Sens. Biosens. Res. 37, 100510.
Mao, Y., Cheng, S., Qin, Y., Qin, Z., Tian, S., 2024. Sensor. Actuator. B Chem. 411, 135700.
Maqsood, S., Xu, S., Tran, S., Garg, S., Springer, M., Karunanithi, M., Mohawesh, R., 2022. Expert Syst. Appl. 197, 116788.
Marabel, M., Alvarez-Taboada, F., 2013. Sensors 13, 10027–10051, 10027-10051 13 (2013).
Martinelli, E., Polese, D., Dini, F., Paolesse, R., Filippini, D., Lundström, I., di Natale, C., 2011. Front. Neuroeng. 4, 16333.
McDermott, C., Lovett, S., Rossa, C., 2024. Med. Biol. Eng. Comput. 62, 1177–1189.
McIntyre, D., Lashkaripour, A., Fordyce, P., Densmore, D., 2022. Lab Chip 22, 2925–2937.
Mencattini, A., Spalloni, A., Casti, P., Comes, M.C., Di Giuseppe, D., Antonelli, G., D'Orazio, M., Filippi, J., Corsi, F., Isambert, H., Di Natale, C., Longone, P., Martinelli, E., 2021. Patterns 2.
Mencattini, A., Di Giuseppe, D., Casti, P., D'Orazio, M., Filippi, J., Comes, M.C., Antonelli, G., Di Natale, C., Martinelli, E., 2023a. In: AISEM Annual Conference on Sensors and Microsystems, pp. 45–52.
Mencattini, A., Rizzuto, V., Antonelli, G., Di Giuseppe, D., D'Orazio, M., Filippi, J., Comes, M.C., Casti, P., Vives Corrons, J.L., Garcia-Bravo, M., Segovia, J.C., del Mar Mañú-Pereira, M., Lopez-Martinez, M.J., Samitier, J., Martinelli, E., 2023b. Sens. Actuators A Phys. 351, 114187.
Mencattini, A., D'Orazio, M., Casti, P., Comes, M.C., Di Giuseppe, D., Antonelli, G., Filippi, J., Corsi, F., Ghibelli, L., Veith, I., Di Natale, C., Parrini, M.C., Martinelli, E., 2023c. Commun. Biol. 6 (1 6), 1–17, 2023.
Merrin, J., 2019. Bioengineering 6, 109. Page 109 6 (2019).
Milani, S., Topin, N., Veloso, M., Fang, F., 2024. ACM Comput. Surv. 56, 1–36.
Mo, Y., Lu, Z., Patil, P., Gershenfeld, N., Akinwande, A.I., Buchwald, S.L., Jensen, K.F., 1979. Science 368 (2020), 1352–1357.
Mohankumar, P., Ajayan, J., Mohanraj, T., Yasodharan, R., 2021. Measurement 167, 108293.
Molho, D., Ding, J., Tang, W., Li, Z., Venegas, J., Liu, R., Su, R., Danaher, P., Yang, R., Johnson, J., Brunswick, N., Xie, Y., Tang, J., Molho, D., Ding, J., Tang, W., Wen, H., Venegas, J., Liu, R., Su, R., Xie, Y., Tang, J., Li, Z., Yang, R., Wen, H., Wang, Y., Jin, W., Lei, Y.L., J. 2024 Tang, 2024. ACM Trans. Intell. Syst. Technol. 15, 1–62.
Moon, G., ryul Choi, J., Lee, C., Oh, Y., Kim, K.H., Kim, D., 2020. Biosens. Bioelectron. 164, 112335.
Moradi, E., Jalili-Firoozinezhad, S., Solati-Hashjin, M., 2020. Acta Biomater. 116, 67–83.
Morales, E.F., Escalante, H.J., 2022. Biosignal processing and classification using computational learning and intelligence: principles. Algorithms, and Applications 111–129.
Moraskie, M., Roshid, M.H.O., O'Connor, G., Dikici, E., Zingg, J.M., Deo, S., Daunert, S., 2021. Biosens. Bioelectron. 191, 113359.
Mostaccio, A., Antonelli, G., Capuano, R., Di Natale, C., Martinelli, E., Marrocco, G., 2024. IEEE J. Flex. Electron. 3, 90–99.
Muñoz, J., Pumera, M., 2020. TrAC, Trends Anal. Chem. 128, 115933.
Nah, J.S., Barman, S.C., Zahed, M.A., Sharifuzzaman, M., Yoon, H., Park, C., Yoon, S., Zhang, S., Park, J.Y., 2021. Sensor. Actuator. B Chem. 329, 129206.
Nair, M.P., Teo, A.J.T., Li, K.H.H., 2022. Micromachines 13, 24.
Nan, L., Zhang, H., Weitz, D.A., Shum, H.C., 2024. Lab Chip 24, 1135–1153.
Niculescu, A.G., Chircov, C., Bîrcă, A.C., Grumezescu, A.M., 2021. Int. J. Mol. Sci. 22, 1–26.
Nielsen, J.B., Hanson, R.L., Almughamsi, H.M., Pang, C., Fish, T.R., Woolley, A.T., 2020. Anal. Chem. 92, 150–168.
Ozcelik, A., Demirbolat, G.M., Erdogan, O., Kozan, B., Akkoyun, F., Cevik, E., Gumus, E., Gul, I.B., Trabzon, L., Cevik, O., 2024. Emergent Mater 1–17.
Padash, M., Enz, C., Carrara, S., 2020. Sensors 20, 4236. Page 4236 20 (2020).
Panwar, S., Sarkar, P., Kasim, D.S., Anand, R., Priya, A., Prakash, S., Jha, S.K., 2024. Biosens. Bioelectron. X 17, 100452.
Pijuan, J., Barceló, C., Moreno, D.F., Maiques, O., Sisó, P., Marti, R.M., Macià, A., Panosa, A., 2019. Front. Cell Dev. Biol. 7, 449183.
Prabowo, B.A., Cabral, P.D., Freitas, P., Fernandes, E., 2021. Chemosensors 9, 299. Page 299 9 (2021).
Pulsipher, K.W., Hammer, D.A., Lee, D., Sehgal, C.M., 2018. Ultrasound Med. Biol. 44, 2441–2460.
Pushparaj, K., Catini, A., Capuano, R., Allegra, V., Magna, G., Antonelli, G., Martinelli, E., Agresti, A., Pescetelli, S., Sivalingam, Y., Paolesse, R., Di Natale, C., 2024. ACS Omega 9, 10650–10659.
Quan, H., Wang, S., Xi, X., Zhang, Y., Ding, Y., Li, Y., Lin, J., Liu, Y., 2024. Biosens. Bioelectron. 245, 115837.
Qureshi, S.A., Aman, H., Schirhagl, R., 2023. Magnetochemistry 9, 171–179 (2023) 171.
Raji, H., Tayyab, M., Sui, J., Mahmoodi, S.R., Javanmard, M., 2022. Biomed. Microdevices 24, 1–20, 3 24 (2022).
Rasheed, S., Kanwal, T., Ahmad, N., Fatima, B., Najam-ul-Haq, M., Hussain, D., 2024. TrAC, Trends Anal. Chem. 173, 117640.
Rho, E., Kim, M., Cho, S.H., Choi, B., Park, H., Jang, H., Jung, Y.S., Jo, S., 2022. Biosens. Bioelectron. 202, 113991.
Ricci, F., Vallée-Bélisle, A., Simon, A.J., Porchetta, A., Plaxco, K.W., 2016. Acc. Chem. Res. 49, 1884–1892.
Rizkin, B.A., Shkolnik, A.S., Ferraro, N.J., Hartman, R.L., 2020. Nat. Mach. Intell. 2:4 2, 200–209, 2020.
Rodrigues, D.R., Fragoso, W.D., Lemos, S.G., 2020. J. Chemom. 34, e3271.
Rogala, M., Dabrowski, P., Kowalczyk, P.J., Wlasny, I., Kozlowski, W., Busiakiewicz, A., Karaduman, I., Lipinska, L., Baranowski, J.M., Klusek, Z., 2016. Carbon N Y 103, 235–241.
Rong, G., Xu, Y., Sawan, M., 2023. Biosensors 13, 860, 860 13 (2023).
Ronkainen, N.J., Halsall, H.B., Heineman, W.R., 2010. Chem. Soc. Rev. 39, 1747–1763.
Sabaté del Rio, J., Ro, J., Yoon, H., Park, T.-E., Cho, Y.-K., 2024. Biosens. Bioelectron. 224, 115057.
Sahore, V., Kreidermacher, A., Khan, F.Z., Fritsch, I., 2016. J. Electrochem. Soc. 163, H3135–H3144.
Salvo, P., Dini, V., Kirchhain, A., Janowska, A., Oranges, T., Chiricozzi, A., Lomonaco, T., Di Francesco, F., Romanelli, M., 2017. Sensors 17, 2952. Page 2952 17 (2017).
Sang, S., Zhao, Y., Zhang, W., Li, P., Hu, J., Li, G., 2014. Biosens. Bioelectron. 51, 124–135.
Sankar, K., Kuzmanović, U., Schaus, S.E., Galagan, J.E., Grinstaff, M.W., 2024. ACS Sens. 9 (5), 2254–2274.
Sato, K., Takeuchi, S., 2014. Angew. Chem. Int. Ed. 53, 11798–11802.
Sauerbrey, G., 1959. Z. Phys. 155, 206–222.
Schackart, K.E., Yoon, J.Y., 2021. Sensors 21, 5519. Page 5519 21 (2021).
Schmidgall, S., Ziaei, R., Achterberg, J., Kirsch, L., Hajiseyedrazi, S.P., Eshraghian, J., 2024. APL Mach. Learn. 2, 21501.
Schwickart, M., Vainshtein, I., Lee, R., Schneider, A., Liang, M., 2014. Bioanalysis 6, 1939–1951.
Seleci, D.A., Maurer, V., Stahl, F., Scheper, T., Garnweitner, G., 2019. Int. J. Mol. Sci. 20.
Sempionatto, J.R., Brazaca, L.C., García-Carmona, L., Bolat, G., Campbell, A.S., Martin, A., Tang, G., Shah, R., Mishra, R.K., Kim, J., Zucolotto, V., Escarpa, A., Wang, J., 2019. Biosens. Bioelectron. 137, 161–170.
Sengupta, J., Hussain, C.M., 2024. TrAC, Trends Anal. Chem. 176, 117742.
Sengupta, P., Khanra, K., Chowdhury, A.R., Datta, P., 2019. Bioelectronics and medical devices: from materials to devices - fabrication. Applications and Reliability, pp. 47–95.
Sesen, M., Whyte, G., 2020. Sci. Rep. 10 (1 10), 1–14, 2020.
Sharafeldin, M., Jones, A., Rusling, J.F., 2018. Micromachines 9, 394–399 (2018) 394.
Shen, Y., Yalikun, Y., Tanaka, Y., 2019. Sensor. Actuator. B Chem. 282, 268–281.
Shi, H., Nie, K., Dong, B., Long, M., Xu, H., Liu, Z., 2019. Chem. Eng. J. 361, 635–650.
Shin, Y.C., Than, N., Min, S., Shin, W., Kim, H.J., 2023. Nat. Rev. Bioeng. 2, 175–191, 2 2 (2023).
Shrimal, P., Jadeja, G., Patel, S., 2020. Chem. Eng. Res. Des. 153, 728–756.
Shukla, V.C., rong Kuang, T., Senthilvelan, A., Higuita-Castro, N., Duarte-Sanmiguel, S., Ghadiali, S.N., Gallego-Perez, D., 2018. Trends Biotechnol. 36, 549–561.
Sibug-Torres, S.M., Grys, D.B., Kang, G., Niihori, M., Wyatt, E., Spiesshofer, N., Ruane, A., de Nijs, B., Baumberg, J.J., 2024. Nat. Commun. 15 (1 15), 1–13, 2024.
Siemenn, A.E., Shaulsky, E., Beveridge, M., Buonassisi, T., Hashmi, S.M., Drori, I., 2022. ACS Appl. Mater. Interfaces 14, 4668–4679.
Sinaga, K.P., Yang, M.S., 2020. IEEE Access 8, 80716–80727.
Solanki, S., Pandey, C.M., Gupta, R.K., Malhotra, B.D., 2020. Biotechnol. J. 15.
Solorio, Y.C., Lemaître, F., Jabbour, B., Tastet, O., Arbour, N., Assi, E.B., 2023. PLoS Comput. Biol. 19, e1011449.
Song, H., Liu, C., Lao, J., Wang, J., Du, S., Yu, M., 2021. Geofluids 2021, 1–12.
Sonner, Z., Wilder, E., Heikenfeld, J., Kasting, G., Beyette, F., Swaile, D., Sherman, F., Joyce, J., Hagen, J., Kelley-Loughnane, N., Naik, R., 2015. Biomicrofluidics 9.
Sosnowski, K., Akarapipad, P., Yoon, J.-Y., 2020. Med. Dev. Sens. 3, e10085.
Squires, T.M., Quake, S.R., 2005. Rev. Mod. Phys. 77, 977–1026.
Srikanth, S., Dubey, S.K., Javed, A., Goel, S., 2021. Sens. Actuators A Phys. 332, 113096.
Stoecklein, D., Lore, K.G., Davies, M., Sarkar, S., Ganapathysubramanian, B., 2017. Sci Rep 7, 46368.
Subbarao, M.V., Jaswini, P., Yasaswini, T.N., Ram, G.C., Abudhagir U, S., Kavitha, D.N.S. B., 2024. International Conference on Distributed Computing and Optimization Techniques, ICDCOT 2024.
Sun, W., Luo, Z., Lee, J., Kim, H.J., Lee, K.J., Tebon, P., Feng, Y., Dokmeci, M.R., Sengupta, S., Khademhosseini, A., 2019. Adv. Healthcare Mater. 8, e1801363.

Sun, H., Xie, W., Mo, J., Huang, Y., Dong, H., 2023. Front. Bioeng. Biotechnol. 11, 1208648.
Suthar, J., Parsons, E.S., Hoogenboom, B.W., Williams, G.R., Guldin, S., 2020. Anal. Chem. 92, 4082–4093.
Svensson, C.M., Shvydkiv, O., Dietrich, S., Mahler, L., Weber, T., Choudhary, M., Tovar, M., Figge, M.T., Roth, M., 2019. Small 15, 1802384.
Tahir, F., Kamran, A., Majeed, M.I., Alghamdi, A.A., Javed, M.R., Nawaz, H., Iqbal, M.A., Tahir, M., Tariq, A., Rashid, N., Shahid, U., Hassan, A., Shoukat, U.S., 2024. ACS Omega 9, 6861–6872.
Tang, Y., Xu, Q., Zhu, P., Zhu, R., Wang, J., 2023. Mater. Adv. 4, 5974–5997.
Teixeira Carvalho, D.J., Moroni, L., Giselbrecht, S., 2023. Nat. Rev. Mater. 8 (3 8), 147–164, 2023.
Teixeira, W., Pallás-Tamarit, Y., Juste-Dolz, A., Sena-Torralba, A., Gozalbo-Rovira, R., Rodríguez-Díaz, J., Navarro, D., Carrascosa, J., Gimenez-Romero, D., Maquieira, Á., Morais, S., 2022. Biosens. Bioelectron. 213, 114454.
Teng, Y., Li, W., Gunasekaran, S., 2023. Biosens. Bioelectron. X 15, 100418.
Tiwari, N., Chatterjee, S., Kaswan, K., Chung, J.H., Fan, K.P., Lin, Z.H., 2022. J. Electroanal. Chem. 907, 116064.
Tsai, H.F., Podder, S., Chen, P.Y., 2023. Micromachines 14, 826, 826 14 (2023).
Un, K.C., Wong, C.K., Lau, Y.M., Lee, J.C.Y., Tam, F.C.C., Lai, W.H., Lau, Y.M., Chen, H., Wibowo, S., Zhang, X., Yan, M., Wu, E., Chan, S.C., Lee, S.M., Chow, A., Tong, R.C.F., Majmudar, M.D., Rajput, K.S., Hung, I.F.N., Siu, C.W., 2021. Sci. Rep. 11 (1 11), 1–9, 2021.
Van Der Maaten, L., Hinton, G., 2008. J. Mach. Learn. Res. 9, 2579–2605.
Varma, S., Voldman, J., 2015. Lab Chip 15, 1563–1573.
Veith, I., Nurmik, M., Mencattini, A., Damei, I., Lansche, C., Brosseau, S., Gropplero, G., Corgnac, S., Filippi, J., Poté, N., Guenzi, E., Chassac, A., Mordant, P., Tosello, J., Sedlik, C., Piaggio, E., Girard, N., Camonis, J., Shirvani, H., Mami-Chouaib, F., Mechta-Grigoriou, F., Descroix, S., Martinelli, E., Zalcman, G., Parrini, M.C., 2024. Cell Rep. Med., 101549, 0.
Vishalatchi, S., Murugan, K., Ramrao, N., Sharan, P., 2024. J. Opt. 1, 1–13.
Wang, L.J., Chang, Y.C., Sun, R., Li, L., 2017. Biosens. Bioelectron. 87, 686–692.
Wang, A., Zou, Y., Liu, S., Zhang, X., Li, T., Zhang, L., Wang, R., Xia, Y., Li, X., Zhang, Z., Liu, T., Ju, Z., Wang, R., Loscalzo, J., Yang, Y., Zhao, Y., 2024a. Nat. Protoc. 2024, 1–37.
Wang, J.X., Qian, J., Wang, H., Sun, M., Wu, L., Zhong, M., Chen, Y., Mao, Y., 2024b. Chem. Eng. J. 485, 149467.
Wang, W., Jiang, S., ying Li, Y., Han, Y., Liu, M., ying Meng, Y., yang Zhang, C., 2024c. Anal. Chim. Acta 1300, 342463.
Wei, X., Reddy, V.S., Gao, S., Zhai, X., Li, Z., Shi, J., Niu, L., Zhang, D., Ramakrishna, S., Zou, X., 2024. Biosens. Bioelectron. 248, 115947.
Weiss, K., Khoshgoftaar, T.M., Wang, D.D., 2016. J. Big Data 3, 1–40.
Whitesides, G.M., 2006. Nature 442 (7101), 368–373, 442 (2006).
Wignarajah, S., Chianella, I., Tothill, I.E., 2023. Biosensors 13, 355, 355 13 (2023).
Wolpert, D.H., Macready, W.G., 1997. IEEE Trans. Evol. Comput. 1, 67–82.
Wongkaew, N., Simsek, M., Griesche, C., Baeumner, A.J., 2019. Chem. Rev. 119, 120–194.
Wu, J., Liu, H., Chen, W., Ma, B., Ju, H., 2023. Nat. Rev. Bioeng. 1 (5 1), 346–360, 2023.
Xie, N., Miao, Z., Wang, J., Zhang, Q., 2019. J. Phys. Conf. Ser. 1229, 012056.
Xu, N., 2019. J. Phys. Conf. Ser., 12014
Xu, X., Zuo, Y., Chen, S., Hatami, A., Gu, H., 2024. Biosensors 14, 125, 125 14 (2024).
Yang, Z., Guo, J., Wang, L., Zhang, J., Ding, L., Liu, H., Yu, X., 2024a. Small 20, 2307815.
Yang, W., Li, T., Liao, S., Zhou, J., Huang, L., 2024b. TrAC, Trends Anal. Chem. 172, 117569.
Yang, S.W., Xie, Y., Liu, J.Z., Zhang, D., Huang, J., Liang, P., 2024c. Spectrochim. Acta Mol. Biomol. Spectrosc. 317, 124427.
Yoshikawa, H., Imura, S., Tamiya, E., 2015. RSC Adv. 5, 56423–56429.
Zhang, T., Ramakrishnan, R., Livny, M., 1996. ACM SIGMOD Record, vol. 25, pp. 103–114.
Zhang, C., Chen, Y., Shi, M., 2010. Chem. Eng. Process: Process Intensif. 49, 1188–1192.
Zhang, X., Fang, J., Zou, L., Zou, Y., Lang, L., Gao, F., Hu, N., Wang, P., 2016. Biosens. Bioelectron. 77, 573–579.
Zhang, Y., Luo, J., Flewitt, A.J., Cai, Z., Zhao, X., 2018. Biosens. Bioelectron. 116, 1–15.
Zhang, Y., Guo, H., Kim, S.B., Wu, Y., Ostojich, D., Park, S.H., Wang, X., Weng, Z., Li, R., Bandodkar, A.J., Sekine, Y., Choi, J., Xu, S., Quaggin, S., Ghaffari, R., Rogers, J.A., 2019. Lab Chip 19, 1545–1555.
Zhang, J., Zhang, X., Wei, X., Xue, Y., Wan, H., Wang, P., 2021a. Anal. Chim. Acta 1164, 338321.
Zhang, S., Zahed, M.A., Sharifuzzaman, M., Yoon, S., Hui, X., Chandra Barman, S., Sharma, S., Yoon, H.S., Park, C., Park, J.Y., 2021b. Biosens. Bioelectron. 175, 112844.
Zhang, L., Parvin, R., Chen, M., Hu, D., Fan, Q., Ye, F., 2023. Biosens. Bioelectron. 228, 115213.
Zhang, W., Cai, X., Zhang, X., Zou, S., Zhu, D., Zhang, Q., Chen, J., 2024. Pharm. Res. (N. Y.) 41, 93–104.
Zheng, J., Cole, T., Zhang, Y., Kim, J., Tang, S.Y., 2021. Biosens. Bioelectron. 194, 113666.
Zhou, Q., Patel, D., Kwa, T., Haque, A., Matharu, Z., Stybayeva, G., Gao, Y., Diehl, A.M., Revzin, A., 2015. Lab Chip 15, 4467–4478.
Zhou, J., Dong, J., Hou, H., Huang, L., Li, J., 2024a. Lab Chip 24, 1307–1326.
Zhou, Z., Xu, T., Zhang, X., 2024b. TrAC, Trends Anal. Chem. 173, 117613.
Zhu, Y., Mandal, K., Hernandez, A.L., Kawakita, S., Huang, W., Bandaru, P., Ahadian, S., Kim, H.J., Jucaud, V., Dokmeci, M.R., Khademhosseini, A., 2021. Curr. Opin. Biomed. Eng. 19, 100309.
Zilionis, R., Nainys, J., Veres, A., Savova, V., Zemmour, D., Klein, A.M., Mazutis, L., 2016. Nat. Protoc. 12 (1 12), 44–73, 2016.
Żuchowska, A., Baranowska, P., Flont, M., Brzózka, Z., Jastrzębska, E., 2024. Anal. Chim. Acta 1301, 342413.