Reviews

Pattern-recognition-based Sensor Arrays for Cell Characterization: From Materials and Data Analyses to Biomedical Applications

Hiroka SUGAI,* Shunsuke TOMITA,*,**† and Ryoji KURITA*,**,***,†

* Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan.
** DAILAB, DBT-AIST International Center for Translational and Environmental Research (DAICENTER), National Institute of Advanced Industrial Science & Technology (AIST), Central 5-41, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8565, Japan.
*** Faculty of Pure and Applied Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8573, Japan

† To whom correspondence should be addressed.
E-mail: s.tomita@aist.go.jp (S.T.), r.kurita@aist.go.jp (R.K.)
Abstract

To capture a broader scope of complex biological phenomena, alternatives to conventional sensing based on specificity for cell detection and characterization are needed. Pattern-recognition-based sensing is an analytical method designed to mimic mammalian sensory systems for analyte identification based on pattern recognition of multivariate data, which are generated using an array of multiple probes that cross-reactively interact with analytes. This sensing approach is significantly different from conventional specific cell sensing based on highly specific probes, including antibodies against biomarkers. Encouraged by the advantages of this technique, such as the simplicity, rapidity, and tunability of the systems without requiring *a priori* knowledge of biomarkers, numerous sensor arrays have been developed over the past decade and used in a variety of cell sensing applications; these include disease diagnosis, drug discovery, and fundamental research. This review summarizes recent progress in pattern-recognition-based cell sensing, with a particular focus on guidelines for designing materials and arrays, techniques for analyzing response patterns, and applications of sensor systems that are focused primarily for the biomedical field.

**Keywords:** biosensors, cells, pattern recognition, polymers, nanoparticles, sensor arrays, multivariate analysis, diagnosis
1 Introduction

Characterizing cells is a crucial task for a variety of basic and applied studies, including the diagnosis of diseases,\(^1\text{–}^3\) tracking biological phenomena,\(^4\) controlling cell quality,\(^5\text{–}^6\) and drug discovery from candidate compounds.\(^7\text{–}^9\) Gene expression profiling is a representative strategy for determining cell statuses; however, phenotypes can not necessarily be predicted by mRNA expressions.\(^10\text{–}^12\) Thus, to obtain accurate information, targeting other biomarker molecules (e.g., metabolites and proteins) that are produced in later biological pathways is important.\(^13\text{–}^15\) Most conventional techniques for the characterization of cells based on such biomarkers rely on the use of highly specific probes, such as antibodies, whose binding is evaluated by detection techniques, such as immunohistochemistry\(^16\text{,}^17\) and flow cytometry.\(^18\) In recent years, probes with high specificity for cellular biomarkers, such as aptamers\(^19\) and small organic molecules,\(^20\) have also been developed.

Although this approach is expected to be effective when there is a strong relationship between the biomarker and the cellular characteristic of interest, biomarker expression is often regulated by multiple cellular processes; further, biomarkers that are specific to a given cellular property have been rarely identified.\(^21\text{–}^23\) Therefore, the detection of a particular biomarker by a probe molecule entails substantial uncertainty.\(^24\) In addition, although cell surfaces and inclusions are composed of numerous types of molecules, the determination of cell characteristics based solely on information from several kinds of biomarkers is controversial.

In contrast to analytical methods based on specific cellular biomarkers, analytical methods using “signature-like molecular profiles” that reflect cell characteristics would offer unique opportunities to characterize entire cells based on comprehensive information. As each cell exhibits a unique molecular expression pattern that corresponds to each biological process,\(^25\text{–}^28\)
focusing on differences in this pattern provides an alternative to the so-called lock-and-key approach by using specific probes for cellular biomarkers.

Pattern-recognition-based sensor arrays are a recently emerged analytical technique for evaluating cells. Interest in pattern-recognition-based sensing was triggered by an initial report by Persaud and Dodd in the 1980s, which is now termed as an electronic nose. This concept was applied to chemical sensors by Suslick et al. in 2000, and subsequent advances in statistical methods have led to the generation of numerous sensor arrays targeting a variety of analytes (from ions to biological tissues). It has been ten years since pattern-recognition-based sensors have been applied to cell sensing.

In this review, we describe recent progress in pattern-recognition-based cell sensing. We initially outline the principle of cell sensing based on a combination of array sensors and multivariate analyses. Subsequently, we summarize examples of interesting studies, with a particular focus on materials, mechanisms, and target cellular characteristics. Finally, future perspectives in this field are described.

2 Basic principles of pattern-recognition-based sensor arrays

Pattern-recognition-based sensor arrays are an analytical tool inspired by the sensory functions of biological organisms. Herein, we use the terms “pattern-recognition-based sensor array” and “pattern-recognition-based sensing,” but a standard name has not been established. For example, the tool has been described as an optoelectronic nose/tongue, chemical nose/tongue, chemometrics, cross-reactive sensors, pattern-based sensors, differential sensors, signature-based sensors, fingerprint-based sensors, unbiased sensors, and hypothesis-free sensors. Recent research on pattern-recognition-based cell sensing is summarized in Table 1 with regard to materials, sensing mechanisms, and target cellular
characteristics. Some of these sensing systems are outlined in Sections 3 to 4. In this section, we summarize the general overview of pattern-recognition-based cell sensing focusing on sensing scheme, material design, and data analysis.

2.1 Scheme of pattern-recognition-based sensing

A general scheme for pattern-recognition-based sensing is illustrated in Fig. 1. Unlike conventional specificity-based methods that use, for example, antibodies or aptamers, multiple sensing probes are employed for a single analyte (Fig. 1a). Each probe is designed to interact with each analyte nonspecifically or semi-selectively, rather than specifically (Fig. 1b). The interactions between probe sets and analytes are converted to readable signals, such as light emission (Fig. 1c). The resulting responses are combined and treated as a “pattern” (Fig. 1d). For analytes composed of various molecules, such as cells, the response generated reflects the summation of responses from each component molecule. In other words, the response pattern reflects complex information that describes the entire cell. The obtained response patterns are evaluated by multivariate analyses to determine differences among analytes (Fig. 1e) and to predict unknown analytes (Fig. 1f) based on the principle of pattern recognition.

2.2 Material design to generate response patterns

The materials used to construct arrays determine the effectiveness of pattern-recognition-based sensing. The probes must fulfil two requirements. They must have the ability to interact differently with individual analytes by nonspecific or semi-selective binding (criterion 1) and produce signals that reflect the interactions between sensing probes and analytes (criterion 2), both of which are described in detail below.
2.2.1 The ability to interact differently with analytes

The nature of criterion 1 (i.e., the materials are needed to interact differently with individual analytes) is unique to pattern-recognition-based sensing. Standard design guidelines to meet this criterion are currently not sufficient, but some preliminary guidelines have been suggested as follows. For example, it is essential for probes to cross-reactively interact with analytes via a variety of weak forces (e.g., electrostatic, hydrophobic, $\pi-\pi$, hydrogen bonding, and van der Waals interactions) to obtain comprehensive information on all analytes rather than specific biomarkers in the analytes. In addition, it is chemical-structurally desirable for probes to possess a high density of multiple cross-reactive binding sites to selectively obtain information on large analytes, such as cell themselves and cell-derived samples (primarily, proteins are targeted) while ignoring the effects of small molecules that are not stable in concentration, such as salts and amino acids. The chemical stability of probes is also necessary to obtain a reproducible response pattern. Nanoparticles and synthetic polymers are often used to meet these requirements and to accomplish pattern-recognition-based cell sensing. The details of these materials are described in Section 3.

2.2.2 The ability to output signals

Regarding criterion 2 (i.e., the materials are needed to produce signals that reflect the interactions between sensing probes and analytes), the output signals are roughly divided into electric signals and optical signals. In most cases, the electric signals are based on van der Waals and physical adsorption,\(^\text{32}\) thus posing the challenge of providing relatively simple information. However, it should be noted that an effective example of using electrochemical impedance has been recently reported.\(^\text{45}\) Optical pattern-recognition-based sensors have the advantage of easy access to a variety of analytes because they can provide information on a wider range of chemical interactions, in addition to their high robustness and sensitivity. Therefore, most pattern-recognition-based cell sensors reported to date have used optical signals as output signals.\(^\text{32}\) In
particular, fluorescence is widely used to generate output signals that reflect interactions owing to its sensitivity and established use in biological applications. Various approaches for generating fluorescent signals have been reported, including the release of fluorescent probes from supramolecular complexes with materials capable of quenching or Förster resonance energy transfer as well as changes in the local environment of fluorescent probes introduced into scaffold materials. In pattern-recognition-based cell sensing, alternative optical signals to fluorescence-based detection are also available, including magnetic resonance imaging, thermochemiluminescence, and absorbance.

2.3 Multivariate analysis for pattern interpretation

Multivariate pattern data generated by arrays are too complex for direct interpretations (sometimes including over 10 dimensions), as shown in Fig. 2a (which provides an example of five different analyte patterns from eight-dimensional data). Therefore, the obtained patterns are usually subjected to multivariate analyses with the aim of visualization at a simplified level (2–3 dimensions) as well as subsequent clustering and classification.

Much of the motivation for using multivariate analysis concerns allowing the visual comparison of analytes by displaying complex multivariate data in an interpretable format. Multivariate analysis techniques are divided into those that are unsupervised and those that are supervised, whose main purposes are to understand the sensor principle and to predict the accuracy of the created sensor, respectively. For example, principle component analysis (PCA) (Fig. 2b) and hierarchical clustering analysis (HCA) (Fig. 2c) are commonly used unsupervised methods. These methods, also referred to as model-free, ignore prior information (i.e., analyte labels, such as cell types) during the analysis, and thus are used to obtain information about similarities between analytes or sensor materials. Conversely, a typical supervised method is linear discriminant analysis (LDA) (Fig. 2d), where external information, such as analyte labels,
is input in combination with response patterns. Therefore, LDA provides information about the
classification ability. According to the purpose, the application of an optimal multivariate analysis
method for a sensing system is crucial. Although there is no doubt regarding the significance of
using unsupervised methods together, in the field of pattern-based cell sensing, so far, LDA is the
most commonly used multivariate analysis technique. Therefore, only the LDA results are
discussed in the following examples.

2-4 Advantages and limitations of pattern-recognition-based sensing

Pattern-recognition-based sensing methods exhibit several economic and practical
advantages over traditional specific sensing techniques that used probes specific to target
biomarkers. For instance, (i) it is easy to design and prepare probes due to the structural flexibility
of many available materials and the unnecessity of a priori knowledge of biomarkers; (ii) it is
eyasy to integrate into simple, rapid measurement systems that do not require advanced technical
skills or specialized equipment; and (iii) it provides comprehensive information about entire cells
not only information about a limited number of biomarkers.

However, it should be noted that this approach has limitations, which, it may be more
appropriate to say, are “complementary” rather than representing “drawbacks” to traditional
specific sensing. Namely, it is not optimal for the analysis of individual components (i.e., to obtain
component-by-component information) in complex mixtures. This is because the response from
each component is usually ignored, and a composite response is obtained. This limitation may
render hypothesis testing difficult. However, when the research aim is diagnosis, variation in
precise components need not be characterized in detail. Rather, it is necessary to capture cellular
characteristics and discriminate between highly similar complex mixtures, and pattern-based
sensing is a suitable technique for this objective.
3 Typical materials for identifying cell types

As discussed in Section 2.2, pattern-recognition-based cell sensing requires the provision of a set of probes capable of extensive interactions and the transduction of information. Since this technique was applied to cells in the late 2000s, efforts have been made to develop probe arrays with multiple cross-reactive binding sites to selectively interact with the cells themselves or cell-derived samples in a multipoint manner. Nanoparticles and polymers have received attention as scaffolds with large surface areas for introducing binding sites at high densities. In this section, we focus on a typical application, the identification of cell types, and provide some key examples of materials that have achieved pattern-recognition-based cell sensing.

3.1 Nanoparticles

Pioneering research on cell-targeted sensor arrays was performed by Rotello and co-workers in 2009, who used an array of supramolecular complexes consisting of gold nanoparticles and fluorescent polymers to identify cell types. To improve sensitivity and accuracy, they subsequently developed a system using fluorescence proteins instead of fluorescent polymers. Using supramolecular complexes of cationic gold nanoparticles and anionic fluorescent proteins as a platform, a variety of terminal functional groups were introduced on the surface of the gold nanoparticles to provide the array with diverse interaction capabilities (Fig. 3a). Signal generation is based on the so-called indicator displacement assay (Fig. 3b). Fluorescent proteins are quenched by complexation with gold nanoparticles, but the fluorescence is regenerated when the complexes are disrupted by competitive interactions caused by exposure to cells. These arrays of quenching complexes between structurally diverse gold nanoparticles and indicator fluorescent proteins are capable of producing fluorescence patterns that reflect the properties of analyte cells through interactions with various molecules present on the cell surface (Fig. 3c). In addition, gold nanoparticles/fluorescent protein
supramolecular arrays with similar sensor systems have been exploited as a versatile tool for cell sensing to discriminate tissues,\textsuperscript{58} glycosaminoglycan-mutant cells,\textsuperscript{59} and cancer stem cells.\textsuperscript{60}

Yang et al. have proposed a unique method for obtaining cellular patterns using a sensor array consisting of DNA–gold nanoparticle complexes (Fig. 4a).\textsuperscript{50} Their strategy was based on the adsorption and/or internalization of complexes that varied depending on the cell type. The resulting aggregation of gold nanoparticles was read as a color change by the nanoplasmonic effect. After mixing the cells and complexes, seed-mediated growth of gold nanoparticles with reducing agents was used to amplify the color changes, resulting in the generation of differential response patterns for each cell type (Fig. 4b).

In the examples described above, the interaction and its conversion to light are carried out by different materials. In Rotello’s cases, nanoparticles with diverse peripheries acted to produce diverse interactions with the analyte cells, while the fluorescent materials complexed with the nanoparticles functioned as optical signal generators. Conversely, in the case of Yang et al.\textsuperscript{50}, plasmonic nanoparticles acted as optical signal generators, and the various interactions were generated by further adding DNAs and small molecules. The availability and tunability of nanoparticles encouraged experts on such materials to enter the field of pattern-recognition-based cell sensing.\textsuperscript{61} This has led to analyses of magnetic nanoparticles functionalized with sugars,\textsuperscript{48} quantum dot/dye complexes,\textsuperscript{62} and DNA-modified gold nanoparticle/graphene oxide complexes.\textsuperscript{63}

3.2 Polymers

The use of synthetic polymers that exhibit both of the required functions, i.e. cross-reactive interaction with analytes and generation of optical signals, is effective for simplifying the evaluation process. Recently, we have created block copolymers of polyethylene glycol and poly-L-lysine modified with fluorescein and hydrophobic amino acids as a signal generation unit and
a cross-reactive recognition unit, respectively, to provide the ability to generate fluorescent response patterns unique to different cells (Fig. 5a). A sensor array consisting of three polymers incorporating different hydrophobic amino acids was able to interact with the cell surface in a variety of ways. Because the emission of fluorescein is altered depending on the type of functional group in its vicinity, information on the binding site on the cell surface is transduced as changes in fluorescence. As a result, ten cell types were successfully identified by LDA (Fig. 5b). Similarly, synthetic polymers with other environment-sensitive fluorophores, such as dansyl dye, have been reported as pattern-recognition-based sensor arrays. In addition, conjugated polymers, such as poly(p-phenyleneethynylene), have been also developed.

3.3 Tips for the design of easy-to-prepare sensor arrays

A variety of materials, such as nanoparticles and synthetic polymers, have been designed and synthesized to generate unique signal patterns, resulting in the successful discrimination of cells. However, the synthesis of the large number of probes needed to prepare the arrays requires extensive effort, which can be a problematic constraint in some cases. We recently reported that a sensor array can be constructed using only readily available materials, i.e., commercially available fluorescent dyes, including fluorescein, rhodamine 6G, and acridine orange. Each fluorescent dye exhibits a different affinity against cell surfaces due to their structural differences (e.g., charge and hydrophobicity). Thus, the array of common fluorescent dyes generates unique response patterns to cells. Although this approach has been successful to some extent, commercially available fluorescent dyes are limited. As alternatives, we provide some tips on array design to effectively produce response patterns related to measurement channels, additives, and solvents.

Interactions between chromogenic/fluorogenic dyes and analytes can lead to unexpected peak shifts and/or spectral shape changes. Therefore, signal detection using multiple wavelengths
from a single probe is one of the easiest ways to increase the number of dimensions of sensing.\textsuperscript{50,64--66,68}

For a greater response, additional components can be further added to the reaction vessels or wells of the array. An innovative approach to efficiently double the pattern information using additives has been proposed by Rotello and co-workers.\textsuperscript{69} In this system, supramolecular complexes between benzyl-terminated gold nanoparticles and three fluorescent proteins are used as a platform to produce signals against cells (for details, see Section 3·1). Macrocyclic cucurbit[7]uril can bind to the terminal benzyl groups of gold nanoparticles via a host–guest interaction. Therefore, after a supramolecular complex is mixed with cells and primary responses are detected, macrocyclic cucurbit[7]uril is added to perturb the interaction, generating secondary responses. A multivariate analysis of the secondary response with the primary response improved the accuracy of cell type identification from 63\% to 95\%. Regardless of whether a well-defined host–guest interaction is applied, the further addition of chemicals to arrays may be a general means to extend the dimensionality.

The adjustment of solvent conditions can also effectively increase dimensionality.\textsuperscript{70} For example, the pH of the solution affects the charged state of cellular components, and the addition of salt attenuates the relative contribution of electrostatic interactions by electrostatic shielding effects. Focusing on these properties, we recently reported a one-component sensor array consisting of a positively charged poly-L-lysine partially modified with hydrophobic dansyl dyes (PLL-Dnc; Fig. 6a) dissolved in buffers of different pH values and ionic strengths.\textsuperscript{66} Mixing of the cells with PLL-Dnc under various solvent conditions produced unique turn-on-type fluorescence response patterns depending on cell types (Fig. 6b). A subsequent LDA of the patterns allowed the successful identification of eight types of human cell lines with different tissue origins and states (Fig. 6c).
4 Evaluating cellular characteristics for biomedical applications

In general, the lack of universal biomarkers associated with any perturbation (e.g., diseases, and stimuli) makes it difficult to characterize cells. One of the advantages of pattern-recognition-based sensing is that once training datasets are prepared, it becomes possible to construct an evaluation system for identifying cellular statuses that have even not been fully elucidated in advance. In this final subsection, we present some selected examples that demonstrate this advantage of pattern-recognition-based sensing for biomedical applications.

4.1 Subtyping of cancerous cells

For the diagnosis of human breast tumors, three major biomarkers are commonly employed as targets, i.e., estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2. However, it is difficult to detect triple-negative breast cancer,71 in which all of these biomarkers are not expressed or poorly expressed.72,73 Although triple-negative breast cancer is a troublesome tumor type that exhibits aggressive and malignant features, specific molecular biomarkers have not been determined. Therefore, the early and accurate diagnosis is a major goal in the medical community for the effective treatment of triple-negative breast tumors. Instead of the traditional strategy relying on specific biomarkers, Auguste and co-workers developed an array of supramolecular complexes consisting of fluorescent nanomaterials and graphene oxides for pattern-recognition-based sensing of triple-negative breast cancer cell lines.74 In their system, the fluorescence of nanomaterials was initially quenched by interactions with graphene oxides, and the subsequent addition of cells released these nanomaterials from the complexes, providing a distinct fluorescence response pattern. This sensor array successfully identified cell types, including triple-negative breast cancer cells. Other materials have also been reported for sensor arrays applicable to the subtyping of breast cancer cell lines, such as supramolecular complexes consisting of two-dimensional nanosheets and DNAs75 and dual-ligand cofunctionalized gold.
nanoparticles.  

### 4.2 Identification of cell stimuli

Cell-based assays are increasingly used as an alternative to animal testing, particularly in the area of drug discovery. In conventional cell-based assays, cultured cells are exposed to a drug candidate and the response is assessed by staining or marker molecule extraction. Rotello and co-workers demonstrated that pattern-recognition-based sensing can be used to determine the mechanism of action of drug candidates. They employed an analogous sensing system to that described in Section 3.1, i.e., they used an indicator displacement assay using supramolecular complexes of cationic benzyl-terminated gold nanoparticle and three different fluorescent proteins. This sensor array was developed to detect drug-induced cell surface changes; the addition of drugs to cells causes alterations on cell surfaces, depending on the mechanism of action of the drug. An analysis of fluorescence patterns by LDA allowed the assignment of 15 drugs into seven classes according to the mechanism of drug action. Furthermore, the sensor array enabled the prediction of the mechanism of action for combination therapies, in which multiple drugs mixed at different ratios have synergistic effects. Their strategy rapidly narrows the biological pathways affected by drug candidates and may be promising for high-throughput screening in the early steps of drug discovery.

Recently, Margulies and co-workers reported well-designed multiplexed probes for protein recognition and cell sensing. The branched probe consisted of four fluorophores and three protein recognition sites. These protein recognition sites, containing two small molecule-based binders and a hairpin DNA aptamer, acted as cross-reactive binding sites for different disease-related protein families. Since the fluorophores incorporated into the probes are solvatochromic dyes and Förster resonance energy transfer donor-acceptor pairs, unique emission color and intensity patterns were generated from a single probe according to the binding behavior for a
variety of analytes. The probe successfully identified not only disease-related protein families spiked into complex matrices, such as serum and urine, but also artificially expressed proteins in cultured cells. More importantly, the authors demonstrated that this multifunctional probe was able to discriminate between cells subjected to different stimuli (e.g., heat, oxidative stresses, inflammatory agents, and drugs) that result in different expression profiles of disease-associated proteins. This study demonstrates the potential of pattern-recognition-based sensing to characterize the state of a variety of stimulated cells.

Anslyn and co-workers developed a sensing technique to monitor protein phosphorylation using supramolecular arrays and applied it to the identification of the kinase signaling pathway. This supramolecular sensor array consisted of three-branched structural receptors with Zn(II)-dipicolylamine complexes, a coumarin-based indicator dye, and short peptides with affinity to the kinases (Fig. 7a). The working principle of the sensor was based on an indicator displacement assay. Conjugates of coumarin-based dyes and Zn(II)-dipicolylamine complex releases the dyes via interactions with a variety of phosphorylated proteins. As a result, different fluorescent responses are produced. Diverse interactions were generated by using a combinatorial library of receptors, dyes, and kinase-binding peptides. The resulting fluorescence response patterns could be used to identify types of phosphorylated proteins as well as cells induced with different phosphorylation-associated stimuli. More specifically, different signaling pathways are activated depending on the type of stimulus, and the degree of phosphorylation changes accordingly. For example, cells stimulated with different activators, such as anisomycin and epidermal growth factor, exhibited different fluorescence response patterns that were distinguishable by LDA (Fig. 7b).

4・3 Monitoring of differentiation and senescence

Monitoring biological processes, such as differentiation and senescence of cultured cells,
is crucial for basic biomedical research and cell engineering applications. Most studies of cell sensing in these biological processes have employed the cells themselves and cell lysates as analytes. These techniques, however, required various cell-damaging steps, such as trypsinization, fixation, or cell lysis, to obtain the analyte, making it difficult to use the cells for other purposes or to continue the cell culture. Therefore, noninvasive analytical methods that do not require the analysis of the cells themselves are preferable. The components secreted by cells into the culture medium are possibly key analytes to address this issue. During culture, cells secrete a variety of molecules that reflect their characteristics and states. Of these cell-secreted molecules, the entire set of secreted proteins is called “the secretome” and is regarded as a rich source for identifying various cellular characteristics.\textsuperscript{79–81} Given that, cell culture supernatants are promising candidates for noninvasive cell sensing analytes.

We have recently developed pattern-recognition-based sensing strategy to monitor differentiation of stem cells\textsuperscript{82} and senescence of fibroblasts,\textsuperscript{83} without damage to cells. A polyion complex (PIC) array\textsuperscript{84–87} was used to generate unique signal patterns according to cellular secretory components with different profiles. PICs with diverse cross-reactivity were designed based on both the naturally occurring structural diversity of enzymes and artificial structural diversity of polyethylene glycol-modified (PEGylated) polymers. In this sensor array, PIC formation between anionic enzymes and cationic PEGylated polymers via electrostatic interactions decreased enzyme activity,\textsuperscript{88} whereas incubation with analytes resulted in the release of active enzymes via competitive interactions, thereby producing optical responses (Fig. 8a).

We begin with an example of the application of PIC arrays to cell differentiation.\textsuperscript{89} In the fields of tissue engineering and regenerative medicine, analytical methods for identifying differentiated stem cell lineages are required.\textsuperscript{90} Genetic and phenotypic analyses based on the detection of biomarkers related to the cell state of interest are commonly employed;\textsuperscript{6} however, these invasive techniques are limited for the examination of cell differentiation process because
they damage cells. To demonstrate the applicability of PIC arrays to studies of differentiation, cell culture supernatants of osteogenic-/adipogenic-induced human adipose-derived mesenchymal stem cells (Fig. 8b) were analyzed by a sensor array. An LDA of the optical response patterns supported that use of the PIC array to noninvasively identify osteogenic and adipogenic differentiation in human mesenchymal stem cells (Fig. 8c).

A similar sensing strategy has been applied to cell senescence research. Effective methods to characterize senescent cells are needed owing to the lack of universal biomarkers related to this process and limitations of common biomarkers, such as senescence-associated β-galactosidase, which is detectable upon cell damage. The applicability of the PIC array was investigated using a replicative senescence model constructed by the serial passage of the human fibroblast cell line TIG-1, a representative cell line for in vitro senescence studies. A PIC array was used to obtain patterns of cell culture supernatants at different population-doubling levels (Fig. 8d), followed by an LDA. The PIC array allowed the monitoring of replicative senescence, even at the stage in which the most commonly used senescent biomarker senescence-associated β-galactosidase is undetectable (Fig. 8e).

5 Conclusions and outlook

Over the past decade, extensive research has demonstrated that pattern-recognition-based cell sensing can capture cell characteristics effectively in different ways than the traditional specific sensing approaches. The pattern-recognition-based sensing strategies described in this review are also useful for analyzing other biological samples, such as serum, viruses, fungi, and bacteria. However, there are several important points that must be improved to achieve more versatile and practical applications in cells. In particular, the fabrication of probes that generate differential responses corresponding to analytes and the appropriate selection of statistical techniques are crucial issues in the development of superior sensor arrays. For instance,
a deeper understanding of sensor principles using multivariate analysis will provide useful feedback for probe design, which will assist in the creation of preferred cross-reactive probes. Owing to the diversity of accessible chemical spaces, which is characteristic of materials such as nanoparticles and polymers, this feedback will enable the effective extension of the library. By combining this library with high-throughput and automated measurement systems, the amount of information can be considerably scaled up, potentially reaching the so-called big data level. Advanced analysis of big data using sophisticated machine learning, deep learning, and artificial intelligence technologies will allow pattern-recognition-based sensors to accurately identify challenging analytes with high complexity or only subtle differences, to quantify and predict unknown analytes.

In this review, we described recent advances in pattern-recognition-based cell sensing. In contrast to conventional specific cell sensing strategies, pattern-recognition-based cell sensing techniques have expanded applications that are beyond the examples discussed here; this is because the patterns contain comprehensive information of cellular components. In most cases, pattern-recognition-based cell sensing approaches also have economic and practical advantages, as they are inexpensive, rapid, and require no advanced technical skills or specialized equipment. Statistical methods for data analysis are becoming increasingly accessible owing to the advent of relevant software and packages. Although various improvements are needed, pattern-recognition-based cell analyses are a promising method for disease diagnosis, drug candidate screening, and the monitoring of cellular phenomena.

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References

1. L. A. M. Gravendeel, M. C. M. Kouwenhoven, O. Gevaert, J. J. de Rooi, A. P. Stubbs, J. E. Duijm, A. Daemen, F. E. Bleeker, L. B. C. Bralten, N. K. Kloosterhof, B. De Moor, P. H. C. Eilers, P. J. van der Spek, J. M. Kros, P. A. E. Sillevis Smitt, M. J. van den Bent, and P. J. French, *Cancer Res.*, **2009**, *69*, 9065.

2. K. Theilgaard-Mönch, J. Boulwood, S. Ferrari, K. Giannopoulos, J. M. Hernandez-Rivas, A. Kohlmann, M. Morgan, B. Porse, E. Tagliafico, C. M. Zwaan, J. Wainscoat, M. M. Van den Heuvel-Eibrink, K. Mills, and L. Bullinger, *Leukemia*, **2011**, *25*, 909.

3. M. Kwa, A. Makris, and F. J. Esteva, *Nat. Rev. Clin. Oncol.*, **2017**, *14*, 595.

4. M. L. Whitfield, G. Sherlock, A. J. Saldanha, J. I. Murray, C. A. Ball, K. E. Alexander, J. C. Matese, C. M. Perou, M. M. Hurt, P. O. Brown, and D. Botstein, *Mol. Biol. Cell*, **2002**, *13*, 1977.

5. J. L. Page, M. C. Johnson, K. M. Olsavsky, S. C. Strom, H. Zarbl, and C. J. Omiecinski, *Toxicol. Sci.*, **2007**, *97*, 384.

6. O. Adewumi, B. Aflatoonian, L. Ahrlund-Richter, M. Amit, P. W. Andrews, G. Beighton, P. A. Bello, N. Benvenisty, L. S. Berry, S. Bevan, B. Blum, J. Brooking, K. G. Chen, A. B. H. Choo, G. A. Churchill, M. Corbel, I. Damjanov, J. S. Draper, P. Dvorak, K. Emanuelsson, R. A. Fleck, A. Ford, K. Gertow, M. Gertsenstein, P. J. Gokhale, R. S. Hamilton, A. Hampl, L. E. Healy, O. Hovatta, J. Hyllner, M. P. Imreh, J. Itskovitz-Eldor, J. Jackson, J. L. Johnson, M. Jones, K. Kee, B. L. King, B. B. Knowles, M. Lako, F. Lebrin, B. S. Mallon, D. Manning, Y. Mayshar, R. D. G. Mckay, A. E. Michalska, M. Mikkola, M. Mileikovsky, S. L. Minger, H. D. Moore, C. L. Mummery, A. Nagy, N. Nakatsuji, C. M. O’Brien, S. K. W. Oh, C. Olsson, T. Otonkoski, K.-Y. Park, R. Passier,
H. Patel, M. Patel, R. Pedersen, M. F. Pera, M. S. Piekarczyk, R. A. R. Pera, B. E. Reubinoff, A. J. Robins, J. Rossant, P. Rugg-Gunn, T. C. Schulz, H. Semb, E. S. Sherrer, H. Siemen, G. N. Stacey, M. Stojkovic, H. Suemori, J. Szatkiewicz, T. Tuuri, S. van den Brink, K. Vintersten, S. Vuoristo, D. Ward, T. A. Weaver, L. A. Young, and W. Zhang, *Nat. Biotechnol.*, **2007**, 25, 803.

7. J. Lamb, E. D. Crawford, D. Peck, J. W. Modell, I. C. Blat, M. J. Wrobel, J. Lerner, J.-P. Brunet, A. Subramanian, K. N. Ross, M. Reich, H. Hieronymus, G. Wei, S. A. Armstrong, S. J. Haggarty, P. A. Clemons, R. Wei, S. A. Carr, E. S. Lander, and T. R. Golub, *Science*, **2006**, 313, 1929.

8. A. B. Parsons, A. Lopez, I. E. Givoni, D. E. Williams, C. A. Gray, J. Porter, G. Chua, R. Sopko, R. L. Brost, C.-H. Ho, J. Wang, T. Ketela, C. Brenner, J. A. Brill, G. E. Fernandez, T. C. Lorenz, G. S. Payne, S. Ishihara, Y. Ohya, B. Andrews, T. R. Hughes, B. J. Frey, T. R. Graham, R. J. Andersen, and C. Boone, *Cell*, **2006**, 126, 611.

9. H. Jiang, J. R. Pritchard, R. T. Williams, D. A. Lauffenburger, and M. T. Hemann, *Nat. Chem. Biol.*, **2011**, 7, 92.

10. S. Minucci and P. G. Pelicci, *Nat. Rev. Cancer*, **2006**, 6, 38.

11. C. Vallot, N. Stransky, I. Bernard-Pierrot, A. Hérault, J. Zucman-Rossi, E. Chapeaublanc, D. Vordos, A. Laplanche, S. Benhamou, T. Lebret, J. Southgate, Y. Allory, and F. Radvanyi, *J. Natl. Cancer Inst.*, **2010**, 103, 47.

12. D. J. Hunter, *Nat. Rev. Genet.*, **2005**, 6, 287.

13. J. K. Nicholson, J. Connelly, J. C. Lindon, and E. Holmes, *Nat. Rev. Drug Discov.*, **2002**, 1, 153.

14. L. Wu and X. Qu, *Chem. Soc. Rev.*, **2015**, 44, 2963.

15. G. Várady, J. Cserepes, A. Németh, E. Szabó, and B. Sarkadi, *Biomark. Med.*, **2013**, 7, 803.
16. C. Solier and H. Langen, *Proteomics*, **2014**, *14*, 774.

17. L. L. de Matos, D. C. Trufelli, M. G. L. de Matos, and M. A. da S. Pinhal, *Biomark. Insights*, **2010**, *2010*, 9.

18. A. Adan, G. Alizada, Y. Kiraz, Y. Baran, and A. Nalbant, *Crit. Rev. Biotechnol.*, **2017**, 37, 163.

19. A. Cibiel, D. M. Dupont, and F. Ducongé, *Pharmaceuticals*, **2011**, *4*, 1216.

20. R. Kubota and I. Hamachi, *Chem. Soc. Rev.*, **2015**, *44*, 4454.

21. A. Vidyasagar, N. A. Wilson, and A. Djamali, *Fibrog. Tissue Repair*, **2012**, *5*, 7.

22. G. V Halade, Y.-F. Jin, and M. L. Lindsey, *Pharmacol. Ther.*, **2013**, *139*, 32.

23. M. Pasha, A. H. Eid, A. A. Eid, Y. Gorin, and S. Munusamy, *Oxid. Med. Cell. Longev.*, **2017**, *2017*, 3296294.

24. C. A. K. Borrebaeck, *Nat. Rev. Cancer*, **2017**, *17*, 199.

25. J. S. Draper, C. Pigott, J. A. Thomson, and P. W. Andrews, *J. Anat.*, **2002**, *200*, 249.

26. J. W. Dennis, I. R. Nabi, and M. Demetriou, *Cell*, **2009**, *139*, 1229.

27. M. N. Christiansen, J. Chik, L. Lee, M. Anugraham, J. L. Abrahams, and N. H. Packer, *Proteomics*, **2014**, *14*, 525.

28. D. Ghosh, C. C. Funk, J. Caballero, N. Shah, K. Rouleau, J. C. Earls, L. Soroceanu, G. Foltz, C. S. Cobbs, N. D. Price, and L. Hood, *Cell Syst.*, **2017**, *4*, 516.

29. K. Persaud and G. Dodd, *Nature*, **1982**, *299*, 352.

30. N. A. Rakow and K. S. Suslick, *Nature*, **2000**, *406*, 710.

31. L. Guerrini, E. Garcia-Rico, N. Pazos-Perez, and R. A. Alvarez-Puebla, *ACS Nano*, **2017**, *11*, 5217.

32. J. R. Askim, M. Mahmoudi, and K. S. Suslick, *Chem. Soc. Rev.*, **2013**, *42*, 8649.

33. C. C. You, O. R. Miranda, B. Gider, P. S. Ghosh, I. B. Kim, B. Erdogan, S. A. Krovi, U. H. F. Bunz, and V. M. Rotello, *Nat. Nanotechnol.*, **2007**, *2*, 318.
34. Y. Geng, W. J. Peveler, and V. Rotello, *Angew. Chem. Int. Ed.*, 2019, 58, 5190.
35. P. Anzenbacher, Jr., P. Lubal, P. Bucek, M. A. Palacios, and M. E. Kozelkova, *Chem. Soc. Rev.*, 2010, 39, 3954.
36. Y. Tao, M. Li, and D. T. Auguste, *Biomaterials*, 2017, 116, 21.
37. A. T. Wright and E. V Anslyn, *Chem. Soc. Rev.*, 2006, 35, 14.
38. S. Rana, N. D. B. Le, R. Mout, K. Saha, G. Y. Tonga, R. E. S. Bain, O. R. Miranda, C. M. Rotello, and V. M. Rotello, *Nat. Nanotechnol.*, 2015, 10, 65.
39. S. Tomita, S. Ishihara, and R. Kurita, *Sensors*, 2017, 17, 2194.
40. S. Tomita, H. Nomoto, T. Yoshitomi, K. Iijima, M. Hashizume, and K. Yoshimoto, *Anal. Chem.*, 2018, 90, 6348.
41. H. Sugai, S. Tomita, S. Ishihara, and R. Kurita, *Sens. Mater.*, 2019, 31, 1.
42. S. Elci, D. Moyano, S. Rana, G. Tonga, R. Phillips, U. Bunz, and V. Rotello, *Chem. Sci.*, 2013, 4, 2076.
43. J. Han, C. Ma, B. Wang, M. Bender, M. Bojanowski, M. Hergert, K. Seehafer, A. Herrmann, and U. H. F. Bunz, *Chem*, 2017, 2, 817.
44. W. J. Peveler, M. Yazdani, and V. M. Rotello, *ACS Sensors*, 2016, 1, 1282.
45. L. Wu, H. Ji, Y. Guan, X. Ran, J. Ren, and X. Qu, *NPG Asia Mater.*, 2017, 9, e356.
46. S. Das, A. M. Powe, G. A. Baker, B. Valle, B. El-Zahab, H. O. Sintim, M. Lowry, S. O. Fakayode, M. E. McCarroll, G. Patonay, M. Li, R. M. Strongin, M. L. Geng, and I. M. Warner, *Anal. Chem.*, 2012, 84, 597.
47. S. Rana, S. G. Elci, R. Mout, A. K. Singla, M. Yazdani, M. Bender, A. Bajaj, K. Saha, U. H. F. Bunz, F. R. Jirik, and V. M. Rotello, *J. Am. Chem. Soc.*, 2016, 138, 4522.
48. K. El-Boubbou, D. C. Zhu, C. Vasileiou, B. Borhan, D. Prosperi, W. Li, and X. Huang, *J. Am. Chem. Soc.*, 2010, 132, 4490.
49. H. Kong, D. Liu, S. Zhang, and X. Zhang, *Anal. Chem.*, 2011, 83, 1867.
50. X. Yang, J. Li, H. Pei, Y. Zhao, X. Zuo, C. Fan, and Q. Huang, *Anal. Chem.*, **2014**, 86, 3227.

51. Y. Liu, T. Minami, R. Nishiyabu, Z. Wang, and P. Anzenbacher, *J. Am. Chem. Soc.*, **2013**, 135, 7705.

52. M. Zuffo, X. Xie, and A. Granzhan, *Chem. Eur. J.*, **2019**, 25, 1812.

53. P. Anzenbacher Jr., Y. Liu, M. A. Palacios, T. Minami, Z. Wang, and R. Nishiyabu, *Chem. Eur. J.*, **2013**, 19, 8497.

54. T. Minami, N. A. Esipenko, B. Zhang, L. Isaacs, and P. Anzenbacher, *Chem. Commun.*, **2014**, 50, 61.

55. Z. Li, H. Li, M. K. LaGasse, and K. S. Suslick, *Anal. Chem.*, **2016**, 88, 5615.

56. A. Bajaj, O. R. Miranda, I. Kim, R. L. Phillips, D. J. Jerry, U. H. F. Bunz, and V. M. Rotello, *Proc. Natl. Acad. Sci.*, **2009**, 106, 10912.

57. A. Bajaj, S. Rana, O. R. Miranda, J. C. Yawe, D. J. Jerry, H. F. Bunz, and V. M. Rotello, *Chem. Sci.*, **2010**, 1, 134.

58. S. Rana, A. K. Singla, A. Bajaj, S. G. Elci, O. R. Miranda, R. Mout, B. Yan, F. R. Jirik, and V. M. Rotello, *ACS Nano*, **2012**, 6, 8233.

59. S. Rana, N. D. B. Le, R. Mout, B. Duncan, S. G. Elci, K. Saha, and V. M. Rotello, *ACS Cent. Sci.*, **2015**, 1, 191.

60. Y. Geng, H. L. Goel, N. B. Le, T. Yoshii, R. Mout, J. J. Amante, A. M. Mercurio, and V. M. Rotello, *Nanomedicine*, **2018**, 14, 1931.

61. A. Bigdeli, F. Ghasemi, H. Golmohammadi, S. Abbasi-Moayed, M. A. F. Nejad, N. Fahimi-Kashani, S. Jafarinejad, M. Shahrajabian, and M. R. Hormozi-Nezhad, *Nanoscale*, **2017**, 9, 16546.

62. Q. Xu, Y. Zhang, B. Tang, and C. Zhang, *Anal. Chem.*, **2016**, 88, 2051.

63. C. Li, Y. Yang, L. Wei, X. Wang, Z. Wang, Y. Yin, and G. Li, *Theranostics*, **2015**, 5,
64. S. Tomita, S. Ishihara, and R. Kurita, *ACS Appl. Mater. Interfaces*, 2019, 11, 6751.
65. M. D. Scott, R. Dutta, M. K. Haldar, B. Guo, D. L. Friesner, and S. Mallik, *Anal. Chem.*, 2012, 84, 17.
66. H. Sugai, S. Tomita, S. Ishihara, and R. Kurita, *ACS Sensors*, 2019, 4, 827.
67. A. Bajaj, O. R. Miranda, R. Phillips, I.-B. Kim, D. J. Jerry, U. H. F. Bunz, and V. M. Rotello, *J. Am. Chem. Soc.*, 2010, 132, 1018.
68. M. Mimura, S. Tomita, R. Kurita, and K. Shiraki, *Anal. Sci.*, 2019, 35, 99.
69. N. D. B. Le, T. G. Yesilbag, R. Mout, S. T. Kim, M. E. Wille, S. Rana, K. A. Dunphy, D. J. Jerry, M. Yazdani, R. Ramanathan, C. M. Rotello, and V. M. Rotello, *J. Am. Chem. Soc.*, 2017, 139, 8008.
70. S. Tomita, S. Ishihara, and R. Kurita, *ACS Appl. Mater. Interfaces*, 2017, 9, 22970.
71. W. Foulkes, I. Smith, and J. Reis-Filho, *N. Engl. J. Med.*, 2010, 363, 1938.
72. D. L. Holliday and V. Speirs, *Breast Cancer Res.*, 2011, 13, 215.
73. X. Dai, H. Cheng, Z. Bai, and J. Li, *J. Cancer*, 2017, 8, 3131.
74. Y. Tao and D. T. Auguste, *Biosens. Bioelectron.*, 2016, 81, 431.
75. M. S. Hizir, N. M. Robertson, M. Balcioglu, E. Alp, M. Rana, and M. V Yigit, *Chem. Sci.*, 2017, 8, 5735.
76. Z. Pode, R. Peri-Naor, J. M. Georgeson, T. Ilani, V. Kiss, T. Unger, B. Markus, H. M. Barr, L. Motiei, and D. Margulies, *Nat. Nanotechnol.*, 2017, 12, 1161.
77. D. Zamora-Olivares, T. S. Kaoud, K. N. Dalby, and E. V Anslyn, *J. Am. Chem. Soc.*, 2013, 135, 14814.
78. B. T. Nguyen and E. V Anslyn, *Coord. Chem. Rev.*, 2006, 250, 3118.
79. K. J. Brown, C. A. Formolo, H. Seol, R. L. Marathi, S. Duguez, E. An, D. Pillai, J. Nazarian, B. R. Rood, and Y. Hathout, *Expert Rev. Proteomics*, 2012, 9, 337.
80. S. K. Kapur and A. J. Katz, *Biochimie*, 2013, 95, 2222.
81. Y.-C. Hsiao, L. J. Chu, J.-T. Chen, T.-S. Yeh, and J.-S. Yu, *Expert Rev. Proteomics*, 2017, 14, 737.
82. V. Tabar and L. Studer, *Nat. Rev. Genet.*, 2014, 15, 82.
83. N. Loaiza and M. Demaria, *Biochim. Biophys. Acta, Rev. Cancer*, 2016, 1865, 155.
84. S. Tomita and K. Yoshimoto, *Chem. Commun.*, 2013, 49, 10430.
85. S. Tomita, T. Soejima, K. Shiraki, and K. Yoshimoto, *Analyst*, 2014, 139, 6100.
86. S. Tomita, S. Yokoyama, R. Kurita, O. Niwa, and K. Yoshimoto, *Anal. Sci.*, 2016, 32, 237.
87. S. Tomita, O. Niwa, and R. Kurita, *Anal. Chem.*, 2016, 88, 9079.
88. K. Shiraki, T. Kurinomaru, and S. Tomita, *Curr. Med. Chem.*, 2016, 23, 276.
89. S. Tomita, M. Sakao, R. Kurita, O. Niwa, and K. Yoshimoto, *Chem. Sci.*, 2015, 6, 5831.
90. D. G. Halme and D. A. Kessler, *N. Engl. J. Med.*, 2006, 355, 1730.
91. B. Lozano-Torres, I. Galiana, M. Rovira, E. Garrido, S. Chaib, A. Bernardos, D. Muñoz-Espin, M. Serrano, R. Martinez-Máñez, and F. Sancenón, *J. Am. Chem. Soc.*, 2017, 139, 8808.
92. S. Xu, X. Lu, C. Yao, F. Huang, H. Jiang, W. Hua, N. Na, H. Liu, and J. Ouyang, *Anal. Chem.*, 2014, 86, 11634.
93. N. D. B. Le, A. K. Singla, Y. Geng, J. Han, K. Seehafer, G. Prakash, D. F. Moyano, C. M. Downey, M. J. Monument, D. Itani, U. H. F. Bunz, F. R. Jirik, and V. M. Rotello, *Chem. Commun.*, 2019, 55, 11458.
94. S. C. Hong, D. P. Murale, S.-Y. Jang, M. M. Haque, M. Seo, S. Lee, D. H. Woo, J. Kwon, C.-S. Song, Y. K. Kim, and J.-S. Lee, *Angew. Chem. Int. Ed.*, 2018, 57, 9716.
95. Y. Zhang, J. R. Askim, W. Zhong, P. Orlean, and K. S. Suslick, *Analyst*, 2014, 139, 1922.
96. J. Han, H. Cheng, B. Wang, M. S. Braun, X. Fan, M. Bender, W. Huang, C. Domhan, W. Mier, T. Lindner, K. Seehafer, M. Wink, and U. H. F. Bunz, Angew. Chem. Int. Ed., 2017, 56, 15246.

97. S. Ngernpimai, Y. Geng, J. M. Makabenta, R. F. Landis, P. Keshri, A. Gupta, C.-H. Li, A. Chompoosor, and V. M. Rotello, ACS Appl. Mater. Interfaces, 2019, 11, 11202.

98. C. Sci, K. L. Bicker, J. Sun, M. Harrell, Y. Zhang, M. M. Pena, R. Thompson, and J. J. Lavigne, Chem. Sci., 2012, 3, 1147.

99. H. Pei, J. Li, M. Lv, J. Wang, J. Gao, J. Lu, Y. Li, Q. Huang, J. Hu, and C. Fan, J. Am. Chem. Soc., 2012, 134, 13843.

100. Q. Liu, Y. Yeh, S. Rana, Y. Jiang, L. Guo, and V. M. Rotello, Cancer Lett., 2013, 334, 196.

101. S. Goodwin, A. M. Gade, M. Byrom, B. Herrera, C. Spears, E. V Anslyn, and A. D. Ellington, Angew. Chem. Int. Ed., 2015, 54, 6339.

102. J.-S. Moon, W.-G. Kim, D.-M. Shin, S.-Y. Lee, C. Kim, Y. Lee, J. Han, K. Kim, S. Y. Yoo, and J.-W. Oh, Chem. Sci., 2017, 8, 921.

103. A. M. Gade, M. K. Meadows, Ellington, and E. V Anslyn, Org. Biomol. Chem., 2017, 15, 9866.

104. X. Ran, F. Pu, Z. Wang, J. Ren, and X. Qu, Anal. Chim. Acta, 2019, 1056, 1.

105. N. Das Saha, R. Sasmal, S. K. Meethal, S. Vats, P. V Gopinathan, O. Jash, R. Manjithaya, N. Gagey-Eilstein, and S. S. Agasti, ACS Sensors, 2019, 4, 3124.
Table 1 Examples of pattern-recognition-based cell sensing.

| Materials | Target | Interaction mode | Detection mode | Cell-derived samples | Multivariate analysis | Ref |
|-----------|--------|------------------|----------------|----------------------|----------------------|-----|
| gold nanoparticle/fluorescent polymer complexes | cell type | nonspecific interactions | fluorescence | cell suspension | LDA | 76 |
| gold nanoparticle/fluorescent protein complexes | cell type | nonspecific interactions | fluorescence | cell suspension | LDA | 57 |
| fluorescent polymers | cell type | nonspecific interactions | fluorescence | cell suspension | LDA | 67 |
| magnetic glyconanoparticles | cell type | nonspecific interactions | fluorescence | cell suspension | LDA | 68 |
| catalytic nanomaterials | cell type | nonspecific interactions | fluorescence | cell suspension | LDA | 69 |
| boronic acid functionalized synthetic lectins | cell type | semiselective interactions to glycosaminoglycan | fluorescence | cell suspension | LDA | 90 |
| matrix metalloproteinases inhibitor-modified fluorescent polymers | cell type | nonspecific interactions | fluorescence | cell suspension | LDA | 91 |
| nano/graphene oxide complexes | cell type | nonspecific interactions | fluorescence | cell suspension | LDA | 92 |
| gold nanoparticle/fluorescent protein complexes | tissue type | nonspecific interactions | fluorescence | cell lysate | LDA | 58 |
| gold nanoparticle/quantum dot complexes | cell type | nonspecific interactions | fluorescence | cell suspension | LDA | 100 |
| receptor with Zn(II)-dipicolylamine/oumarin-based dyes and nucleobinding peptide complexes | kinase expression and activity | nonspecific interactions to phosphorylated proteins | fluorescence | cell suspension | LDA | 71 |
| gold nanoparticle/DNA complexes | cell type | nonspecific interactions to glycans on cell surfaces | absorbance | adherent cells | LDA | 50 |
| aptamers | cell type | nonspecific interactions to glycans on cell surfaces | fluorescence | adherent cells | LDA | 99 |
| DNA-modified gold nanoparticles | tissue type | nonspecific interactions to glycans on cell surfaces | absorbance | cell lysate | LDA | 63 |
| graphene oxide complexes | | | | | |
| enzyme/polyelectrolyte complexes | cell differentiation | nonspecific interactions | absorbance | cell culture supernatant | LDA | 69 |
| gold nanoparticle/fluorescent protein complexes | drug mechanism | nonspecific interactions | absorbance | adherent cells | LDA | 38 |
| fluorescent nanomaterial/graphene oxide complexes | cell type (breast cancer cells) | nonspecific interactions | fluorescence | cell suspension | LDA | 74 |
| fluorescent polymer/fluorescent protein complexes | cell type, glycosaminoglycan -mutant cells | nonspecific interactions | fluorescence | cell suspension | LDA | 67 |
| quantum dot complexes | cell type | nonspecific interactions to glycans on cell surfaces | fluorescence | cell suspension | LDA | 67 |
| enzyme/polyelectrolyte complexes | cell type | nonspecific interactions | absorbance | cell suspension | LDA | 67 |
| multiplexed probe possessing fluorophores and protein binders | cell type (disease-related protein families) | semiselective interactions to disease-related protein families | fluorescence | adherent cells | LDA | 76 |
| dual-ligand cofunctionalized gold nanoparticles | cell type (breast cancer cells) | nonspecific interactions | fluorescence | cell suspension | LDA | 36 |
| genetically modified phages | cell type (breast cancer cells) | nonspecific interactions | RGB color | volatile chemicals produced from cells | LDA | 102 |
| transition metal dichalcogenide/thin film/other labeled DNA complexes | cell type (breast cancer cells) | nonspecific interactions | fluorescence | cell suspension | LDA | 75 |
| guest-modified gold nanoparticle/ host molecule/fluorescent protein complexes | cell type | nonspecific interactions | fluorescence | cell lysate | LDA | 40 |
| graphene oxide/variable molecule complexes | cell type | nonspecific interactions | fluorescence | electrochemical impedance | LDA | 45 |
| fluorophore-modified peptide/DNA complexes | cell type | nonspecific interactions | fluorescence | cell suspension | LDA | 103 |
| gold nanoparticle/fluorescent protein complexes | cell type | nonspecific interactions to glycans on cell surfaces | absorbance | adherent cells | LDA | 60 |
| enzyme/polyelectrolyte/immunomagnetic complexes | cell type (cancer stem cells) | nonspecific interactions | absorbance | cell culture supernatant | LDA | 40 |
| MntO nanosheet/thin film/DNA complexes | cell type | nonspecific interactions | absorbance | adherent cells | LDA | 104 |
| small organic fluorophores | | | | | |
| fluorophore-modified polyelectrolytes | cell type | nonspecific interactions | absorbance | cell culture supernatant | LDA | 60 |
| fluorophore-modified nanoparticle complexes | cell type | nonspecific interactions | absorbance | cell culture supernatant | LDA | 60 |
| gold nanoparticle/fluorescent labeled DNA complexes | cell type | nonspecific interactions | absorbance | cell culture supernatant | LDA | 60 |

a. Abbreviations: LDA, linear discriminant analysis; PCA, principal component analysis; HCA, hierarchical clustering analysis; SVM, support vector machine; PLSDA, partial least squares discriminant analysis

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Figure Captions

Fig. 1 Schematic illustration of the pattern-recognition-based sensing approach.

Fig. 2 Typical output of multivariate analyses of patterns. (a) Patterns of five different analytes (1–5) obtained from eight sensing elements (A–H). (b) PCA score plot. (c) HCA dendrogram. (d) LDA score plot. In the PCA and LDA score plots, each point corresponds to a pattern obtained by a single measurement of the analyte. Ellipses represent the confidence intervals ±1 SD for the individual analytes (n = 11). The HCA dendrogram yielded a large cluster including analytes 1, 3, and 5, and the clusters of analytes were located in close proximity in the PCA score plot. Both had the same characteristics, that is, patterns of these three analytes were similar to each other. The distances between the clusters corresponding to analytes 1, 3, and 5 were slightly greater in the LDA score plot than that in the PCA score plot. In addition, the distances between individual points (components) of the cluster were smaller in the LDA score plot than that in the PCA score plot. This difference can be explained by the aims of the PCA and LDA, i.e., maximizing the variance and separation of classes, respectively.

Fig. 3 (a) Chemical structures of gold nanoparticles. (b) Schematic illustration of a supramolecular sensor array consisting of cationic gold nanoparticles and anionic fluorescent protein, and basis for the generation of diverse fluorescence responses in the indicator displacement assay. (c) LDA score plots obtained from the supramolecular sensor array against four cell lines. Reproduced from Ref. 57 with permission from The Royal Society of Chemistry.

Fig. 4 (a) Schematic illustration of the method for obtaining cellular patterns by a sensor array consisting of DNA–gold nanoparticle complexes. (b) LDA score plot obtained from the sensor
array against four cell lines. Reprinted with permission from ref. 50. Copyright 2014 American Chemical Society.

Fig. 5 (a) Chemical structure of three polyethylene glycol/polylysine block copolymers modified with fluorescein and hydrophobic amino acids. (c) LDA score plot obtained from the polymer array against ten cell lines. Reprinted with permission from ref. 64. Copyright 2019 American Chemical Society.

Fig. 6 (a) Chemical structure of dansyl-modified polylysine. (b) Schematic illustration of the dansyl-modified polylysine array, and basis for the generation of unique fluorescence responses depending on buffer conditions. (c) LDA score plot obtained from dansyl-modified polylysine array against eight cell lines. Reprinted with permission from ref. 66. Copyright 2019 American Chemical Society.

Fig. 7 (a) Chemical structure of the components of the sensor array, i.e., there-branched receptors with Zn(II)-dipicolylamine moieties, coumarin-based indicator dye, and kinase-binding short peptides. (b) LDA score plots obtained from the supramolecular arrays against the cells stimulated with anisomycin (Aniso) and epidermal growth factor (EGF) and cells without stimulation (CL). Reprinted with permission from ref. 77. Copyright 2013 American Chemical Society.

Fig. 8 Polyion complex (PIC) array for pattern-recognition-based cell sensing. (a) Schematic illustration of the generation of optical response patterns. (b) Human adipose-derived stem cells stained with Alizarin Red S (osteogenic differentiation) and Oil Red O (adipogenic differentiation). (c) LDA score plot obtained from the PIC array against osteogenic- and adipogenic-induced cells. Reproduced from Ref. 89 with permission from The Royal Society of
Chemistry. (d) Human fibroblast-derived TIG-1 cells stained with senescence-associated β-galactosidase. (e) LDA score plot obtained from the PIC array against TIG-1 cells at four senescent stages. Reprinted with permission from ref. 40. Copyright 2018 American Chemical Society.
Fig. 1 Schematic illustration of the pattern-recognition-based sensing approach.
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Fig. 6 (a) Chemical structure of dansyl-modified polylysine. (b) Schematic illustration of the dansyl-modified polylysine array, and basis for the generation of unique fluorescence responses depending on buffer conditions. (c) LDA score plot obtained from dansyl-modified polylysine array against eight cell lines. Reprinted with permission from ref. 66. Copyright 2019 American Chemical Society.
Fig. 7 (a) Chemical structure of the components of the sensor array, i.e.,there-branched receptors with Zn(II)-dipicolylamine moieties, coumarin-based indicator dye, and kinase-binding short peptides. (b) LDA score plots obtained from the supramolecular arrays against the cells stimulated with anisomycin (Aniso) and epidermal growth factor (EGF) and cells without stimulation (CL).

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Fig. 8 Polyion complex (PIC) array for pattern-recognition-based cell sensing. (a) Schematic illustration of the generation of optical response patterns. (b) Human adipose-derived stem cells stained with Alizarin Red S (osteogenic differentiation) and Oil Red O (adipogenic differentiation). (c) LDA score plot obtained from the PIC array against osteogenic- and adipogenic-induced cells. Reproduced from Ref. 89 with permission from The Royal Society of
Chemistry. (d) Human fibroblast-derived TIG-1 cells stained with senescence-associated β-galactosidase. (e) LDA score plot obtained from the PIC array against TIG-1 cells at four senescent stages. Reprinted with permission from ref. 40. Copyright 2018 American Chemical Society.
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