ACS MEASUREMENT SCIENCE Au
AN OPEN ACCESS JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

pubs.acs.org/measureau
Perspective

Nanosensor Chemical Cytometry: Advances and Opportunities in Cellular Therapy and Precision Medicine

Published as part of ACS Measurement Science Au virtual special issue "2023 Rising Stars".

Youngho Song, Changyu Tian, Yullim Lee, Minyeong Yoon, Sang Eun Yoon, and Soo-Yeon Cho*

Cite This: *ACS Meas. Sci. Au* 2023, 3, 393–403
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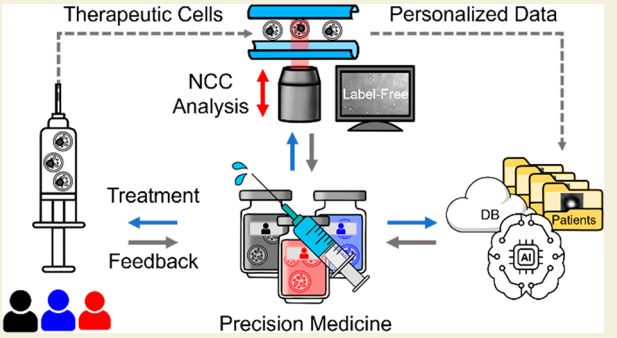
ABSTRACT: With the definition of therapeutics now encompassing transplanted or engineered cells and their molecular products, there is a growing scientific necessity for analytics to understand this new category of drugs. This Perspective highlights the recent development of new measurement science on label-free single cell analysis, nanosensor chemical cytometry (NCC), and their potential for cellular therapeutics and precision medicine. NCC is based on microfluidics integrated with fluorescent nanosensor arrays utilizing the optical lensing effect of a single cell to real-time extract molecular properties and correlate them with physical attributes of single cells. This new class of cytometry can quantify the heterogeneity of the multivariate physicochemical attributes of the cell populations in a completely label-free and nondestructive way and, thus, suggest the vein-to-vein conditions for the safe therapeutic applications. After the introduction of the NCC technology, we suggest the technological development roadmap for the maturation of the new field: from the sensor/chip design perspective to the system/software development level based on hardware automation and deep learning data analytics. The advancement of this new single cell sensing technology is anticipated to aid rich and multivariate single cell data setting and support safe and reliable cellular therapeutics. This new measurement science can lead to data-driven personalized precision medicine.



KEYWORDS: *nanosensor, cytometry, single cell, cell therapy, label-free, precision medicine*

INTRODUCTION

As the definition of therapeutics broadens to include the transplanted or engineered living cells and their molecular products including immune-cellular therapy, hematopoietic stem cell transplant, and regenerative medicine, there is a significant scientific need for fundamentally new analytical approaches to understand this new type of drugs. Cellular therapy is a rapidly and widely growing medicinal innovation with the first U.S. Food and Drug Administration (FDA) approval of YESCARTA (Axicabtagene ciloleucel) and KYMRIAH (Tisagenlecleucel) in 2017, both of which are autologous Chimeric Antigen Receptor (CAR)-T therapies.[1] After this approval of the first CAR-T therapies, monoclonal antibodies such as tafasitamab, antibody drug conjugates (ADCs) such as loncastuximab, and bispecific antibodies such as blinatumomab have recently been FDA approved.

Currently, a wide range of cell types are employed in the clinical trial stage of development including mesenchymal stromal cells (MSC), dendritic cells, and neuronal cells.[2−7] These therapies target diseases such as various malignancies, viral disease, graft rejection, autoimmune disorders, and many degenerative conditions by enabling mechanisms of action and therapeutic approaches that small biochemical compounds and drugs cannot.[8−11] For instance, the differentiation of stem cells into specialized cells, such as hormone-secreting endocrine cells, cytotoxic lymphocytes, or tissue-regenerating cells, can be exploited for therapeutic properties.[12] In addition, cells can be genetically engineered to perform a wide range of biochemical functions[13,14] and can deliver drug payloads with cell-homing properties.[15] Since these therapies utilize the patient's own cells and their derivatives, the role of precision medicine tailored to the distinct immune environment of each individual patient is being emphasized even more to give them a good clinical outcome.

As promising modes of cellular therapeutics are discovered, attention of the field is turning to the cell manufacturing

Received: August 17, 2023
Revised: October 13, 2023
Accepted: October 16, 2023
Published: November 1, 2023

https://doi.org/10.1021/acsmeasuresciau.3c00038
ACS Meas. Sci. Au 2023, 3, 393–403

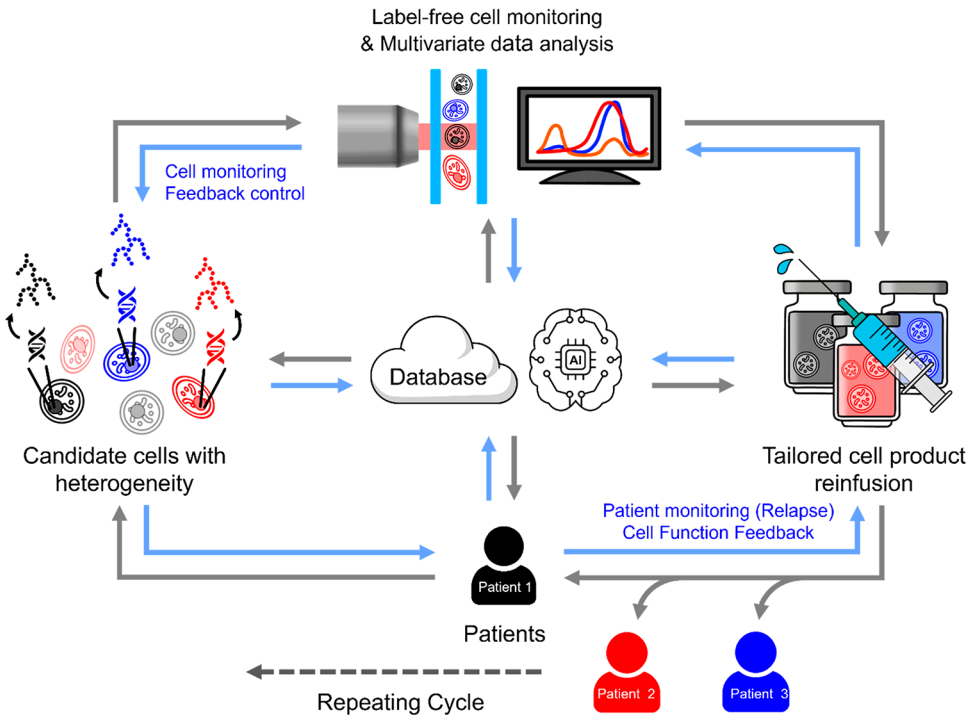


Figure 1. Overview of a data-driven personalized treatment scenario using label-free single cell sensing technology.

process, in particular the challenge of gaining higher levels of engineering control over cell function, population variability, and product consistency at the single cell and subcellular levels during vein-to-vein. The current cell therapeutic treatment involves several major steps.[16] First, extraction and characterization of the source of the therapeutic cells from patients and ensure the identity and safety. Second, isolation and engineering of the required therapeutic cells and determination of the cellular contents and functionalities with quality assurance/quality control (QA/QC) criteria. Third, infusion or implantation of the engineered cell products to patients with follow-up doses of cells as required.[17,18] However, based on the complexity and long manufacturing time of cellular engineering, there should be fundamental gaps between extracted cells from the patients, engineered/reinfused cells, and the desired cell populations in terms of genomic and proteomic properties.[19] This outcome includes molecular heterogeneities, that can complicate therapeutic outcomes combined with patient variability.[20,21]

Therefore, precise monitoring of the molecular heterogeneity such as nucleic acids, metabolites, small molecules, protein contents, and characterization of the activation, persistence, and exhaustion status of target cells in a label-free way is a vital part of the personalized treatment scenario (Figure 1).[22,23] Here, single-cell analysis is of critical importance in revealing molecular heterogeneity of the population in precision, identifying minority subpopulations of interest, as well as discovering unique characteristics of individual cells. Differences between individual cells are invariably observed in any cell population, and the collective behaviors of the population may not reflect the actions of a single cell.[24] Thus, an integrated database containing the versatile status of individual cells, ranging from a patient's cellular extraction to final product is imperative for precision medicine. By establishing this, we can derive an optimal therapeutic efficacy model based on a single cell resolution database. Moreover, this will facilitate the completion of a patient-centered treatment cycle leveraging a feedback model that inherently considers the unique characteristics of each individual patient. The label-free extraction of multivariate cellular characteristic data including both biophysical and molecular attributes at the individual cell level can lead to the most fundamental technological innovation for next-generation cell therapy and, furthermore, the realization of precision medicine. This Perspective describes the trend and limitation of current single cell analysis technology and introduces a new field of measurement science that enables label-free and high-throughput single cell physicochemical sensing and discusses how they can bridge the gap between the potential of cell therapy and the engineering process. We also provide an outlook for the integration of these tools with existing treatment processes and propose what kind of technological developments need to be done to increase the utility and accessibility of these tools to the end-users and finally precision medicine.

CURRENT ANALYTICS FOR SINGLE CELL PROFILING

Recent advances in optics and nanotechnology have led to the development of single cell analytic tools and the rich data collection of multivariate cell traits for therapeutic applications. Representatively, flow cytometry, phase imaging based microscopy, and nano-micro sensor devices have been mainly used for the single cell analytics (Figure 2). Flow cytometry, as a gold standard technique for cellular heterogeneity profiling, allows the user to detect and classify the cells as they pass through a microfluidic channel in front of a laser alignment (Figure 2a). During the flow, injected cells which are prelabeled with fluorescent dyes are stimulated by the excitation laser to emit visible fluorescence. The cell fluid is surrounded by detectors, one aligned with the laser beam for measuring forward scatter (FSC), and the other positioned at a

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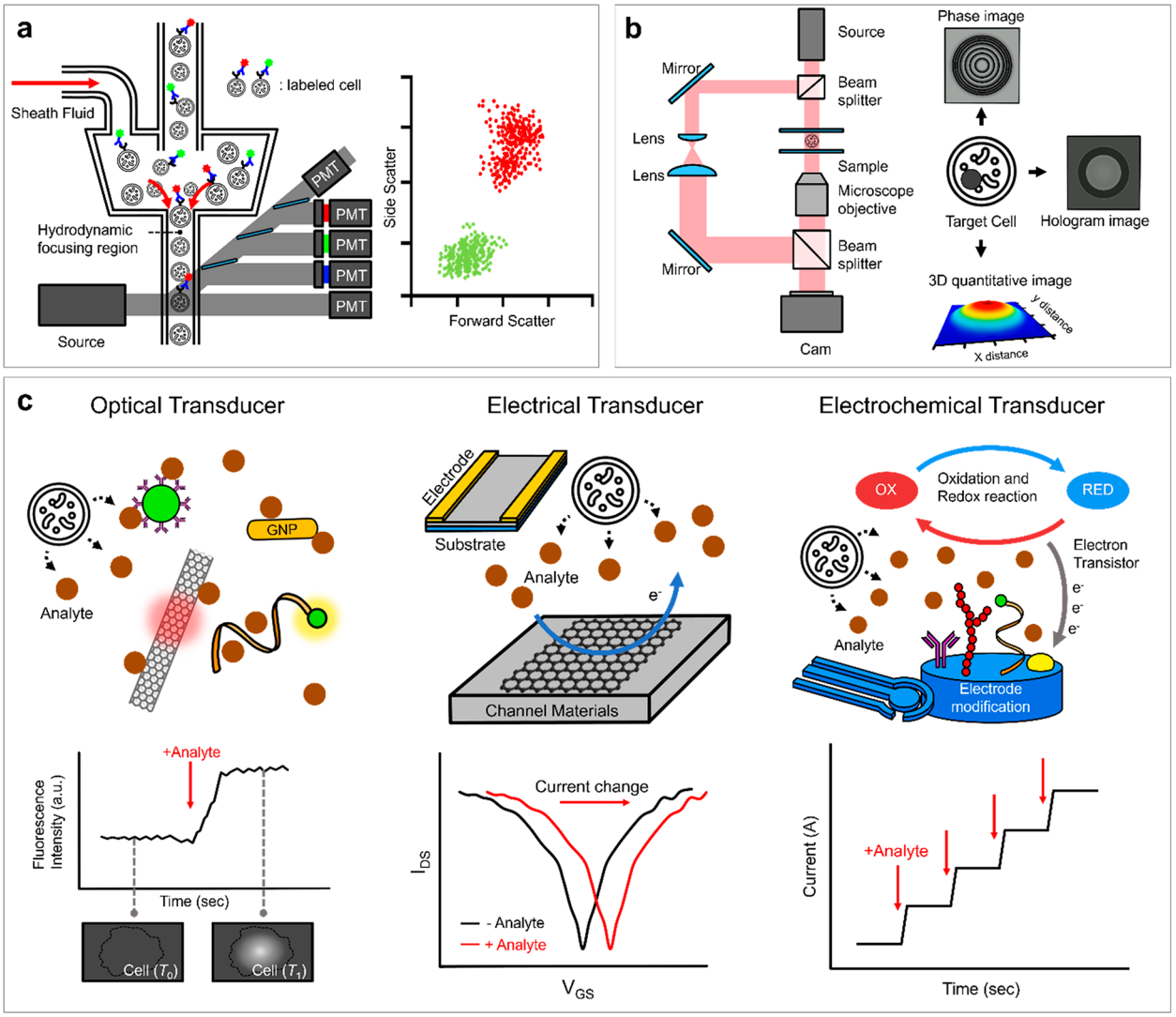

Figure 2. Current analytical approaches on single cell profiling for clinical applications. (a) Flow cytometry system based on labeling and fluorescence scattering. (b) Phase contrast microscopy utilizing 3D structure of living cells for label-free biophysical profiling of single cells. (c) Optical, electrical, and electrochemical nano-micro transducer technology for the real-time molecular profiling of cells.

right angle to the stream for measuring side scatter (SSC). FSC primarily reflects the size or volume of the cells, while SSC provides insight into the internal structure of the cells including granularity.[25,26] Many researchers have used flow cytometry to analyze the therapeutic characteristics of various cell types such as leukocytes and MSCs, based on fluorescence associated with specific labels.[27−29] These tools allow for the discrimination of different cell populations as well as measurement of cell viability and activation status by assessing the fluorescence intensity of single cells. With its ability to provide detailed cellular analysis, flow cytometry plays a crucial role in advancing the study of cell therapy. A significant advantage of flow cytometry is its ability to provide high-throughput data collection of around 10^6−10^7 cells in just a few minutes. Furthermore, it can sort target cells by applying electrical charge along the channel, where the cells labeled with fluorescent dyes are deflected and sorted into the collector. However, a key limitation of the analytics lies in the requirement for cell labeling. A large number number of cell types do not yet have well-defined specific markers for each target trait for therapy. More importantly, labeling reagents may alter the behavior of bound cells through their inadvertent activation or inhibition prior to collection and introduce xenobiotic compounds.[30,31] This label-based method inevitably poses safety and reliability issues when monitoring cells in further downstream analysis or direct patient infusion. Thus, for cell therapy applications, it necessarily requires non-destructive and label-free techniques that can characterize and quantitatively measure cell heterogeneity and engineering control of a directly infused product.

Various label-free cell imaging techniques, including quantitative phase imaging (QPI), are alternatively leveraged for the safe profiling of living cells without the need for labeling (Figure 2b). Phase contrast microscopy constructs the three-dimensional (3D) structure of living cells from multiple 2D optical field images captured at varying laser angles for single cell analysis. The reconstructed cell provides quantitative information on cell morphology, such as refractive index (RI) distribution, along with dry cell volume, mass, and dry cell mass concentration.[32−34] A prominent example of these techniques is digital holographic microscopy (DHM), which creates and digitally records holograms of single cells utilizing a

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ACS Meas. Sci. Au 2023, 3, 393−403

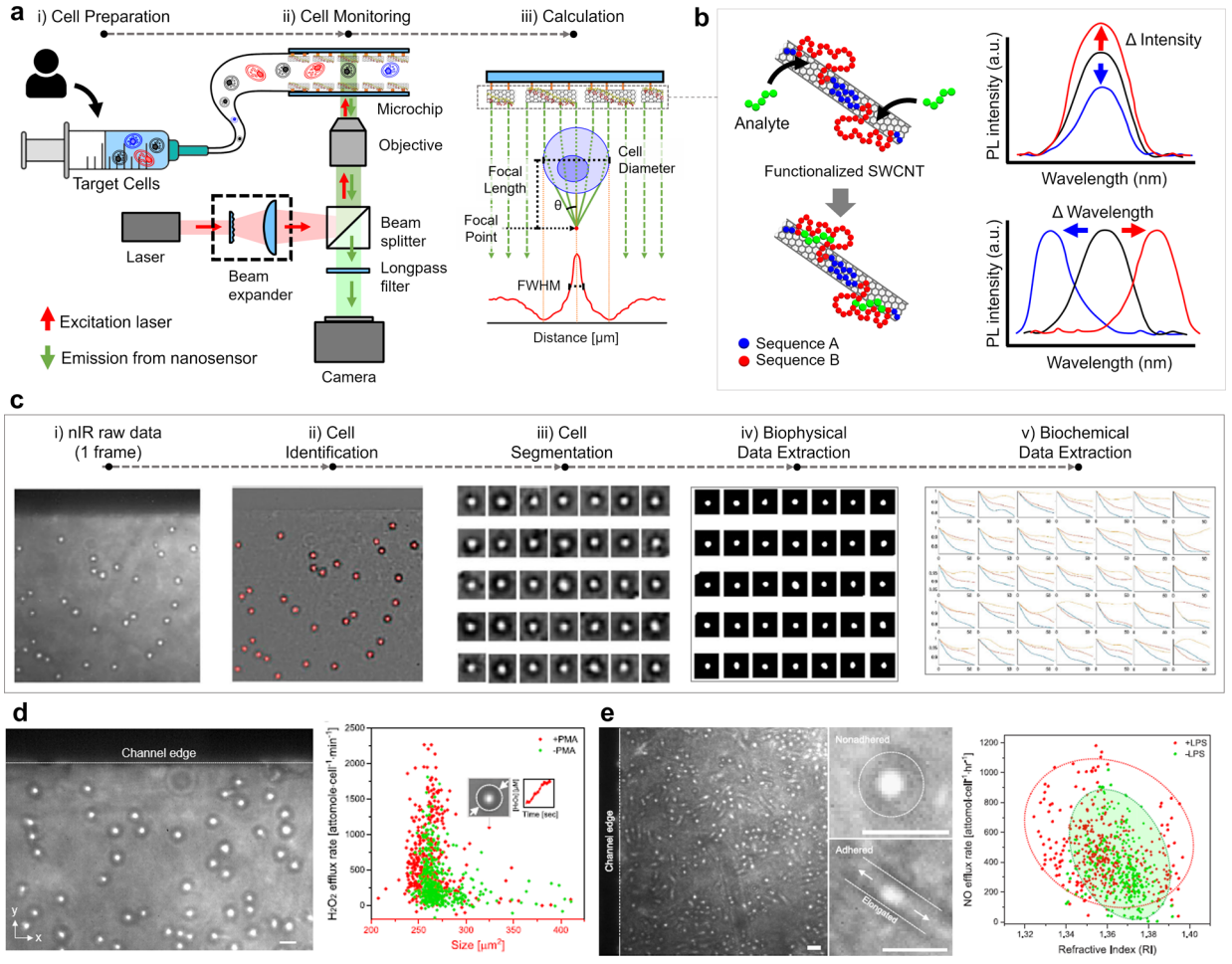

Figure 3. First-generation examples of NCC for label-free physicochemical heterogeneity profiling of single cells. (a) Typical operation process and hardware structure of the NCC technology. (b) Nanosensor design mechanism of NCC for the selective molecular profiling of single cells. (c) Single cell data extraction flow from nIR sensor images using computational code. Reprinted with permission under Creative Commons CC-BY 4.0 from ref 68. Copyright 2021 SPRINGER NATURE. Recent applications of NCC on (d) human monocyte ROS immune heterogeneity monitoring. Reprinted with permission under a Creative Commons CC-BY 4.0 from ref 68. Copyright 2021 SPRINGER NATURE. (e) iNOS heterogeneity monitoring of murine macrophage. Reprinted from ref 69. Copyright 2021 AMERICAN CHEMICAL SOCIETY.

monochromatic light source laser. The sample wavefronts and reference beam combine to create an interference pattern that records a sample imprint on the hologram. The computer then uses a numerical reconstruction algorithm to convert this generated hologram to an image. This method is implemented in various forms, each employing the reference wavefront to obtain intensity and phase information. Such measurements do not influence cells, making them suitable for long-term research and consecutive downstream analysis. QPI facilitates the heterogeneity analysis of diverse cell types, encompassing red blood cells (RBCs) and peripheral blood mononuclear cells (PBMCs), through the quantification of their physical properties, such as cell volume, RI, and dry mass. Through the application of advanced techniques, the biophysical characteristics of postoperative patient blood samples can be analyzed facilitating a straightforward assessment of the progression of inflammation.[35] Additionally, in samples predominantly composed of RBCs, PBMCs, and tumor cells, intentionally introduced tumor cells can be discerned with precision, suggesting significant potential for disease diagnostics.[36] QPI offers the advantage of enabling analysis without the need for prior treatments such as cell fixation or staining with dyes such as flow cytometry, thereby minimizing side effects on therapeutic samples. However, this information is mostly limited to the biophysical properties of single cell such as size, shape, volume, mass, or RI. The changes in the physical properties of cells can occur due to various reasons, such as changes in pH of the extracellular environment or fluctuations in the molecular expression levels within the single cell.[37−39] Therefore, for an accurate assessment of the live single cells, additional single cell information including proteomic or molecular attributes is critically required in addition to the biophysical attributes. To quantify the molecular information from individual therapeutic cells, it is essential to conduct chemical analyses tailored to the specific target analytes including substances secreted by cells, both internally and externally, such as reactive oxygen species (ROS), reactive nitrogen species (RNS), cytokines, or amino acids (AA) playing a critical role in cellular communications and metabolism. Achieving a comprehensive understanding of cellular heterogeneity necessitates a thorough chemical analysis of the cells.

In pursuit of analyzing the substances secreted from single cells, the devices with versatile nano-micro sensing devices have been widely used to real-time monitor the intracellular and extracellular molecular information. Transducer materials

https://doi.org/10.1021/acsmeasuresciau.3c00038
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having optical, electrical, and electrochemical properties in the nano-micro dimensions exhibit high sensitivity and selectivity to a wide range of biochemical molecules of the cells (Figure 2c). Transducers utilize specific recognition elements, such as antibody aptamers or receptors, contingent upon the substance under examination. These elements can be integrated onto materials of nano-micro scale, including 2D materials such as graphene and MoS_2, 1D materials including carbon nanotubes (CNTs) and Si nanowire, and 0D materials including Au nanoparticles (AuNPs).[40−43] Optical signal detection through transducers is characterized by a unique interaction between the active site of the transducer and light signals, which arises from distinct physical, chemical, or structural properties, leading to high sensitivity.[44] Optical transducers utilize a range of spectroscopic techniques, including absorption, fluorescence, and Raman scattering.[45−47] This approach has been utilized to monitor the differentiation state of stem cells including human neural stem cells (hNSCs) and MSCs by analyzing the presence and quantity of specific molecules in various environments.[48−51] Electrical sensors are using conductance or resistance variation between electrodes by adsorption of biochemical molecules on conducting channel materials.[52−55] Electrical transducers can detect and quantify a wide range of cellular environmental changes, from pH shifts to the presence of large molecules such as antibodies and proteins. This allows for distinguishing cancer cells, observing stem cell differentiation, and monitoring immune cell activation.[56] For instance, using impedance responses, these transducers have differentiated between breast cancer cells (MDA-MB-231) and normal epithelial cells (MCF-10A), observed MSC-differentiated osteoblasts, and quantified cytokines expressed from monocytes.[57−59] This allows for the assessment of physiological health within the body and the monitoring of a patient's therapeutic progress. Electrochemical sensor is based on chemical reactions between working and reference electrodes where electronic potential is changed with analytes adsorption.[60,61] Representative electrochemical detection approaches include cyclic voltammetry, potentiometry, and amperometry.[62−64] In peripheral blood mixed with breast cancer cell (MCF-7), successful detection of MCF-7 demonstrated the capability to identify circulating tumor cells (CTCs) directly from whole blood.[65] Additionally, they enhance the effectiveness of cell therapies by monitoring stem cell differentiation, as illustrated by the observation of MSCs differentiation into osteoblasts.[66]

These methods provide the advantage of enabling real-time and label-free observation without any complex pretreatment and optical instruments, thus users can conveniently track cellular changes during therapeutic applications.[67] However, these methods are significantly constrained by a low throughput sensing rate ($1-10^2$ cells/min), and this is also even only for optical transducers that can provide real-time imaging, and electrical and electrochemical transducers usually hard to provide single cell data but just provide an average value of the cell populations. The single cell analytic tools for cellular therapy applications must provide a reliable heterogeneity statistic from multivariate and rich data collections since the miss-calculated data will directly affect the healthiness of the patients. Thus, the nanosensor fields itself is hard to find the answer for this throughput problems. Other challenges of the fields remain in the integration with imaging constructs, which allows user to correlate the measured single singles with their other physical profiles.

NEW CLASS OF SINGLE CELL ANALYTIC TECHNOLOGY: NANOSENSOR CHEMICAL CYTOMETRY (NCC)

To tackle these challenges from the current single analytics fields, there is recent advances called nanosensor chemical cytometry (NCC), which integrates a label-free fluorescent nanosensor array and microfluidic system (Figure 3).[68,69] NCC utilizes the inherent optical lensing effect of live cells in a cell guiding channel to extract single cell information in a completely label-free way and associate biomolecular information with respective biophysical properties. For the first generation of the NCC, a single-walled carbon nanotube (SWCNT), which provides a near-infrared (nIR) fluorescence with visible light excitation, is incorporated as a nanosensor array in the microchips. When a SWCNT is functionalized with versatile surface chemistry including smalls molecules, polymers, or DNA, SWCNT forms specific 3D corona interfaces that enable selective molecular recognition of target analytes (Figure 3b).[70,71] This molecular adsorption instantly changes the bandgap structure of SWCNTs, subsequently inducing variations in wavelength or intensity of fluorescence signal. Versatile constructs of this SWCNT based nanosensor have been designed and synthesized to detect a wide range of biochemical molecules of cells, ranging from small molecules such as ROS to large proteins such as interleukin-6 (IL-6) family cytokines and amyloid-beta, even extending to the plant hormone gibberellin.[72−75] The nanosensors for the cellular target analytes are integrated within microchannels in a uniform array using the evaporation induced self-assembly (EISA) based on silane chemistry.[68,76,77] In the initial stages of NCC operation, excitation laser sources having visible light wavelength for the SWCNT fluorescence are exposed to the nanosensor array from the bottom side of the channel (Figure 3a). Each single cell flowing just below the nanosensor array is strongly interacting with nIR fluorescence from the array, where a highly focused propagating beam from the shadow-side of the cell is generated due to constructive interference of the light field, called a photonic nanojet (PNJ).[78,79] This PNJ phenomenon focuses light in a manner that treats each single cell as a specialized optical lens reflecting the physical characteristics of each single cell. This process is termed the cellular lensing effect. Since this unique optical effect is the main mechanism for single cell data extraction, other types of nanosensors such as electrical, electrochemical, or mechanical transducers are limited to be integrated with NCC. The distinct lensing effect of each single cell can be numerically expressed in terms of focal point, focal length, maximum intensity, and full width at half-maximum (fwhm). This effect is not merely indicative of the cellular RI but also provides insights into intracellular activities including expression and translocation of proteins and RNA transcriptions.[80,81] The nIR image being measured in the microchannel directly includes the biophysical information on each singles cell including size, eccentricity, and RI, obtained through cellular lensing. This accumulated information proves to be instrumental in assessing biophysical heterogeneities. More importantly, the utilization of nanosensors facilitates the exact quantification of molecular substances emitted by each single cell, which is hard to achieve with conventional flow cytometry or a phase imaging technique. By modeling of reaction and diffusion kinetics between cell efflux and nanosensor surfaces, we can translate the fluorescence intensity variations within the

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ACS Meas. Sci. Au 2023, 3, 393−403

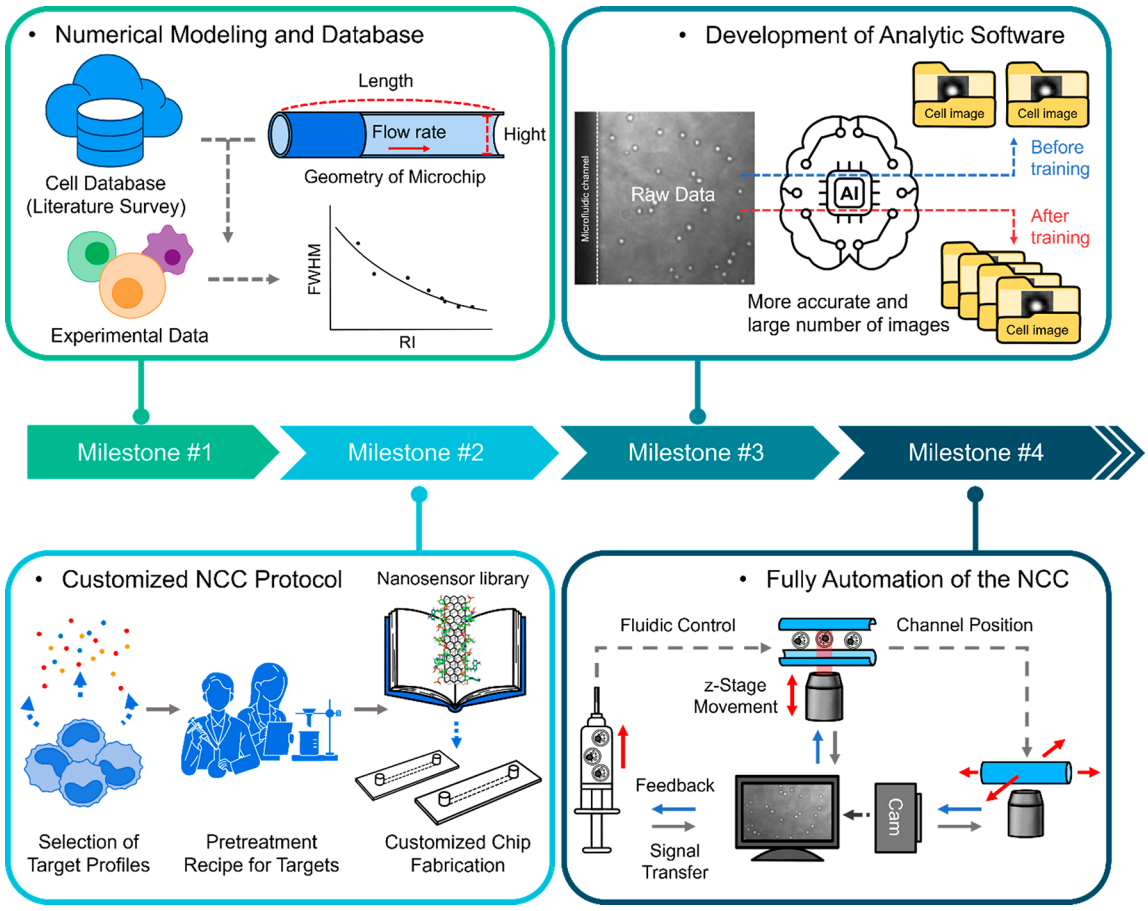

Figure 4. Four stages of the technological roadmap of NCC.

cellular lensing area to real-time biochemical concentration releasing from individual cells even in attomole level (10^{-18} mol).[68] Image processing software was conducted together with NCC to collect a rich multivariate data set from individual cells within a population (Figure 3c). Initially, target cells are identified from raw data to distinguish the background from the cells, and cropped single cell images are modified in contrast and brightness to calculate the biophysical attributes. Then, real-time biochemical efflux is calculated by calculating fluorescence intensity changes of most central parts of each segmented cell image based on the pre-established model.

As the first demonstration of technology applications, NCC has been utilized to quantify the heterogeneities of immune cells on dynamic immune environmental changes by correlating biophysical and biomolecular properties. The cellular lensing effect was successfully demonstrated in different types of cells, including immune cells, epithelial cells such as human embryonic kidney cells (HEK), and endothelial cells such as human umbilical vein endothelial cells (HUVEC).[68,69] Finite-difference time-domain (FDTD) numerical modeling has been exploited to estimate a distinct cellular lensing parameters including focal length, lensing intensity, and fwhm. Based on this computation, biomolecular and biophysical properties of human monocytes are dynamically correlated by employing phorbol 12-myristate 13-acetate (PMA), an agonist of the protein kinase C (PKC) signaling pathway inducing immune activations (Figure 3d).[68] This approach, employing the H_2O_2 nanosensor array, was utilized to investigate the heterogeneity of the physicochemical properties, specifically focusing on variations in ROS that result from real-time immune activation. The results demonstrated that, compared to inactive monocytes, activated monocytes exhibited an 88.9% increase in H_2O_2 efflux, accompanied by a 2.92% reduction in size, a 10.31% decrease in eccentricity, and a 0.3% decline in RI. Furthermore, the standard deviation (STDEV) of H_2O_2 efflux in activated monocytes increased from 344 to 497. There was also a change in the STDEV for size, decreasing from 29 to 24, for eccentricity, reducing from 0.14 to 0.13, and for RI, increasing from 0.05 to 0.06. These findings highlight the shifts in cellular heterogeneity in response to real-time immune activation. Upon monocyte activation, changes in PKC and ROS result in the cell becoming more rounded and smaller, with a subsequent decrease in intracellular RI.[82,83] These observations demonstrate the ability to track such intricate functional changes at the single-cell level. Similarly, the NO nanosensor array was used with NCC to observe RNS heterogeneity generated by inducible nitric oxide synthase (iNOS) in murine macrophages (Figure 3e).[69] Lipopolysaccharide (LPS) was used as a stimulant. Compared to inactive macrophages, activated macrophages exhibited a 35.7% increase in NO efflux and a 0.51% reduction in the RI. Furthermore, the standard deviation (STDEV) of NO efflux in activated macrophages increased from 199 to 206, and the STDEV for the RI rose from 0.009 to 0.015. These applications highlight the capability of NCC to analyze diverse physicochemical heterogeneity kinetics with high-throughput and multivariate analysis using

https://doi.org/10.1021/acsmeasuresciau.3c00038
ACS Meas. Sci. Au 2023, 3, 393−403

specifically designed nanosensor arrays to detect target molecules within cells.

These analyses are based on a completely nondestructive and label-free monitoring highlighting its potential as a label-free and real-time checking of candidate cells for the treatment. For example, this new system can be used to analyze the immune heterogeneity of PBMCs in patients having CAR-T cell therapy to predict clinical outcome. Clinicians can track physicochemical attributes of a patient's immune cells before, during, and after treatment and thereby can systematically predict the therapeutic outcomes by correlating with cohort data. As another possible application, NCC can be used to quantify the maturation or differentiation status of the various stem cells such as neural stem cells (NSC), mesenchymal stromal cells (MSCs) or hematopoietic stem cells (HSCs). By quantifying the molecular indicators of the differentiation process such as neurotransmitters, clinicians can transplant stem cells with the exact level of differentiation desired to the patient. This new approach paints a more comprehensive and detailed portrait of the cell, thereby enhancing our understanding and ability to manipulate cellular functions for specific therapy.

CHALLENGES AND TECHNOLOGICAL ROADMAP OF NCC FOR PRACTICAL APPLCIATIONS

The recent rise of this technology suggests its significant potential use as an effective single-cell analysis tool for future cellular therapy applications. However, as it is still in the early stages, a tailored and systematic technology maturation roadmap is thought to be necessary. We suggest four stages of the engineering components to be developed and integrated into NCC technology for powerful practical applications (Figure 4). In the initial stages, accurate mathematical modeling of nanophotonics within microfluidics should be established. This involves calculating the PNJ of a wide range of cell types under various cellular conditions and microfluidic dimensions using computational tools such as FDTD. Computer simulated data of single cells should be continuously databased and upgraded with actual measurements of the samples for feedback. This process should consider diverse cell conditions, including cell extraction days, patient pretreatment, cell expansion status, activation status, and infusing plan based on the analysis scenario, as well as various channel environment scenarios, such as flowing media, 3D dimensions, fluid rate, or number of channels of the microchip. More importantly, the SWCNT is not the only nanosensor candidate for NCC. Other types of fluorescent nanosensors including quantum dots (QDs), two-dimensional materials (e.g., MoS_2 or black phosphorus), or organic nanoparticles can also be utilized as a nanosensor array for NCC, theoretically. Thus, we need to calculate whether the fluorescence and excitation conditions of each nanosensor material can effectively induce label-free imaging based on PNJ in various cellular environments. If a suitable nanosensor candidate is found, it can provide new sensing functions that SWCNTs cannot provide, expanding the chemical sensing library of NCC. Given these multiple contexts, simulation programs such as Lumerical FDTD, a 3D electromagnetic simulator, or the 3D Finite Element Method (FEM) program in COMSOL can be employed for numerical analyses, thereby enhancing the results of NCC analysis.

As a second technological milestone, user-defined NCC protocol should be established for versatile application based on the patients or clinical environments. This might include target cell attributes selection, cell pretreatment before sensing, microchip customization, or construction of the nanosensor library. For example, human cells vary in size from 6−8 μm such as typical immune cells to 25−40 μm such as skin cells. Depending on the average size of the target cells, the height and width of the microchannel should be precisely controlled, allowing the user to induce the strongest and reliable cellular lensing in the channel. In addition to instrumental aspects, the use of adequate pretreatment reagents such as phytohemagglutinin (PHA), concanavlin A (Con A) or PMA, which are cytokine signaling proteins should be prepared to observe the therapeutic behavior of target immune cells since they enhance cellular molecular efflux analysis enabling more accurate biochemical sensing in NCC.[84−87] Surface functionalization of the nanosensor array is also another pretreatment protocol required for reliable NCC. Target cells can be in contact with the nanosensor array during analysis within channels, necessitating the use of nontoxic nanosensor materials. Fortunately, recent two NCC research demonstrated that cells remained almost 100% viable after subsequent analysis.[68] However, considering the potential use of toxic nanosensor materials and other harsh measurement conditions, adequate surface modifications that can protect the cells while selectively transporting the target molecules to the nanosensor array are required. Moreover, adherent cells, such as macrophages, inherently adhere completely to the nanosensors during analysis. Thus, a pretreatment protocol including collagen treatments that enhance cell adhesion while providing cellular protection should be developed.

In addition to a practical experimental protocol, the development of specific statistical methods and AI-based software is also required to handle a large amount of individual cellular lensing data. Users must be able to accurately identify each single cell and rapidly extract multivariate data in high-throughput such as 10^6 cells/min using solid statistical analysis software. Current research on deep learning based nIR image analysis for single cell quantification is ongoing under various conditions.[88] The deep learning model was built by modifying the structure of YOLOv5, which is an image detection model. Developed model should detect each individual cell in high spatiotemporal resolution and precisely calculate the fluorescence signal changes for each pixel behind the single cell area, even complexly intertwined among impurities and signal noise in various samples status. In addition to the cell image detection model, optical simulation can support the model to define the focal length of the cellular lens and aid in effectively determining the optimal focusing length. Furthermore, a clear understanding of fluidic control in channels of varying heights is crucial, calling for software development that facilitates its efficient application in cellular analysis.

The NCC system should ultimately be completed through a solid combination of automated hardware and software when the previous three stages of technological milestones are achieved. This process must encompass solutions to preempt potential errors that could arise during the standardization and automation of the entire analytical procedure. Microfluidic control, sample stage, sensor camera, and objectives are all integrated with a single visualized user platform such as LabVIEW to automatically measure sensor images from specific channel positions at consistent time intervals. Moreover, under steady fluidic control, data extraction should solely focus on the characteristics of the cell without influencing the

https://doi.org/10.1021/acsmeasuresciau.3c00038
ACS Meas. Sci. Au 2023, 3, 393−403

target cell or other remaining cells themselves. Combining such automated hardware and software to achieve analysis speeds comparable to the throughput of flow cytometry will be the most significant technological hurdle. In addition, if it is also possible to integrate the cell sorting function after analysis like flow cytometry, it would be the best outcome ideally. However, since the NCC is originally based on a label-free imaging and sensing mechanism, further intensive research is needed on how to apply external forces to collect the analyzed cells. As various analytical chemistry techniques have evolved over history, unique measurement science must be followed with solid engineering design to become a reliable hardware product, which then needs to be commercialized so that it can be easily used by researchers all over the world.

CONCLUSIONS AND OUTLOOK

This Perspective highlights that we now have first-generation examples and promising concepts of how new label-free single cell sensing tools can revolutionize cellular therapy and precision medicine. Traditional cellular analysis techniques have often failed to adequately capture the intricate details and individual characteristics of cells, leading to a limited understanding of the complexity and diversity within cellular populations. The limitations of traditional destructive and label-based cellular analysis methods have become particularly evident in important therapeutic procedures, including immune-chemotherapies and transplantation therapies. However, the label-free nanosensor integrated cytometry technique approaches transcend these limitations offering a more detailed and live insight into the unique information on individual cells and their environments during vein-to-vein process. Especially in crucial medical fields such as cellular therapy, these advances are anticipated as indicative dynamic biomarkers to significantly enhance the efficiency and cost-effectiveness of treatments, paving the way for safer and more reliable therapeutic solutions for patients.

Building upon these foundational advancements, the potential of NCC is set to evolve even further. We predict that advances in this technique will provide new cell monitoring options for predicting cellular therapeutic outcomes in the coming decades. In addition to technological advancements, significant economic benefits are also anticipated. Conventional analytics inevitably require time and labor costs due to cell preprocessing steps, including labeling and treatment. On the contrary, NCC can be conducted without sample preparation, and if hardware and software automation are completed, analyses could be accomplished within seconds, potentially reducing the cost-per-patient sample considerably. A risk, however, lies in the cost of the nanosensor synthesis and integration processes. To address this, it will be essential to establish an optimized large-scale synthesis process for various nanosensor materials, including SWCNTs, ensuring both sensing quality and prices. Furthermore, streamlining the integration process with standardized customized chips will be needed. If the nanosensor materials and hardware fabrication processes are solidified, then it promises to be a highly economical medical device. In addition, as the fields are still in the early stages of technological advancement, the reliability of the system should be more carefully demonstrated in conjunction with traditional analytical biochemistry techniques to prevent the risks of standalone use. If milestones from various scientific and engineering fields accumulate one by one, this new technology can be ultimately used as validated and the gold standard analytics for cellular therapy in the future. The combination of the concept of traditional analytical chemistry such as flow cytometry and new technologies such as nanosensors will pioneer a completely new form of measurement science, assisting in the realization of truly data-driven personalized precision medicine.

AUTHOR INFORMATION

Corresponding Author

Soo-Yeon Cho − *School of Chemical Engineering, Sungkyunkwan University, Suwon 16419, Republic of Korea;* orcid.org/0000-0001-6294-1154; Email: sooyeonc@skku.edu

Authors

Youngho Song − *School of Chemical Engineering, Sungkyunkwan University, Suwon 16419, Republic of Korea;* orcid.org/0009-0004-4718-860X

Changyu Tian − *School of Chemical Engineering, Sungkyunkwan University, Suwon 16419, Republic of Korea*

Yullim Lee − *School of Chemical Engineering, Sungkyunkwan University, Suwon 16419, Republic of Korea*

Minyeong Yoon − *School of Chemical Engineering, Sungkyunkwan University, Suwon 16419, Republic of Korea*

Sang Eun Yoon − *Division of Hematology-Oncology, Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul 06351, Republic of Korea*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsmeasuresciau.3c00038

Author Contributions

Y.S. and S.-Y.C. conceived the idea, designed the project, and organized the perspectives with the assistance of C.T., Y.L., M.Y., and S.E.Y. Y.S. and S.-Y.C. wrote the manuscript with input from all the authors. All authors contributed to discussions informing the research. CRediT: **Youngho Song** conceptualization, data curation, formal analysis, visualization, writing-original draft, writing-review & editing; **Changyu Tian** resources, software, writing-original draft; **Yullim Lee** resources, writing-original draft; **Minyeong Yoon** resources, writing-original draft; **Sang Eun Yoon** investigation, writing-original draft; **Soo-Yeon Cho** conceptualization, data curation, funding acquisition, investigation, project administration, resources, supervision, visualization, writing-original draft.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported by the National Research Foundation (NRF) grant funded by the Korean government (MSIT) (No. RS-2023-00222838, RS-2023-00211580).

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https://doi.org/10.1021/acsmeasuresciau.3c00038
ACS Meas. Sci. Au 2023, 3, 393−403