Development of Ultrafast Machine Vision-Activated Cell Sorters and Its Applications

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A cell sorter that performs image information analysis in real-time to separate a large number of cells were desired. However, there is always a tradeoff between the processible information per cell (quality) and the number of cells per time (quantity). Therefore, it has been a great challenge to enable high throughput imaging cell sorter that holds the advantages of optical microscope (high quality) and flow cytometry (high quantity simultaneously). In order to overcome this challenge, we proposed and realized a new approach named "ghost cytometry", originating from a concept that image reconstruction is not always necessary in image analysis when performed by machines, not by humans. This method utilizes the motion of each cell in microchannels to acquire its compressed image signal by a single pixel detector, and directly applies AI-based analysis to the signal, resulting in the development of the ultrafast and accurate image-free "imaging" cell sorter. This technology is expected to be widely applied in various biotechnologies and cell-based clinical methods.

Introduction

Imaging and analyzing many single cells hold the potential to significantly increase our understanding of heterogeneous systems involved in various complex life systems and diseases. Many key applications in these fields require accurate and high throughput isolation of specific populations of cells according to information contained in the high content images. However, there is always a trade-off between the processible information per cell (quality) and processible number of cells per time (quantity). This trade-off is depicted in Figure 1. For cytometry technologies, this trade-off caused critical challenges that have to be simultaneously solved: (1) ultrafast imaging technology that simultaneously meets the needs of high sensitivity, polychromaticity, high speed and continuous acquisition, and (2) ultrafast information technology that continuously performs both computational image production and analysis which is costly in terms of both time and money. As a result, there has been no high throughput imaging-activated cell sorting technology that holds advantages of both optical microscopy (high quality) and flow cytometry (high

quantity).

In a series of works, we proposed and experimentally demonstrated that directly applying machine learning methods to compressed imaging signals measured using a single pixel detector enables ultrafast, sensitive, and accurate image-free (without image production), imaging-based cell analysis as well as sorting in real time, which we call ghost cytometry[1] (GC, Figure 1). GC has enabled the fast cell sorting based on the cells' image information in both fluorescence and label-free modes, which we name machine-vision based cell sorters (ViCS). Furthermore, using this ViCS, we have developed an ultrafast pooled platform for cell image-based phenotypic screening of genes, which can be scaled up at a large scale.

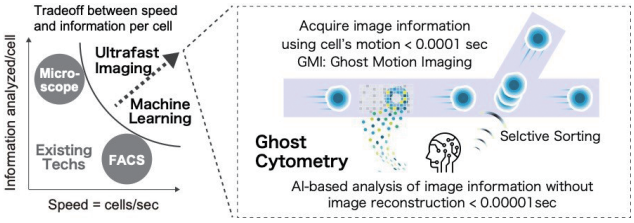

Figure 1 Ghost cytometry simultaneously achieved speed and rich information by "image analysis without image reconstruction"

Moreover, based on the invention, we founded a start-up company ThinkCyte Inc., which develops these ViCS machines through the co-development with renowned companies. Further, we are now striving to realize its practical use in the fields of medical diagnosis, drug discovery, and cell therapy, and a series of joint research with several pharmaceutical companies is underway.

Key feature of our technology 1: ghost cytometry

Our key concept was ghost cytometry (GC), which is an approach of image analysis without image production. In GC, we applied machine learning methods to compressed imaging signals measured using a single pixel detector for enabling ultrafast, image-free, "image"-based cell analysis as well as sorting in real time (Figure 2). Thanks to skipping the time-consuming image reconstruction process, the time required for image inferring was reduced down to 10 microseconds which is orders of magnitudes shorter than other methods relying on image reconstructions. With GC, we developed a series of ultrafast cell sorters which classifies cell based either fluorescence image information or label-free image information. In the case of the fluorescence mode, fluorescent signals are detected by converting image information into temporal waveforms compressively. In the case label-free modes, refractive index distribution of cells is detected; these label-free morphological modalities obtained are similar label free microscopy images including darkfield and bright field images.

Figure 3 shows an example workflow of GC utilizing a machine learning model to classify the cells by directly analyzing the imaging waveforms. It first starts with preparing a training data set: for each cell, we simultaneously measure the GC imaging waveforms and molecular labels that reflect types, states, phenotypes, and other characteristics of cells. Using this training data set, we then develop a model based on machine learning methods. Finaly this model predicts the labels directly from the GC imaging signals of unknown cells.

In the fluorescence modes, GC can classify localization of fluorescence molecules in the cell morphology, even if their total intensities are the same or if the same kind of cell lines are used. This is particularly useful when users know what image pattern to recognize and what kind of specific molecules are their targets which is often the case in drug screening or biological research. With these pre-knowledge, one can chemically or genetically label the molecule of interest to visualize the fluorescent image pattern of interest with a good signal-to-noise-ratio. Using various optical probes, the GC can classify a variety of fluorescent patterns effectively, including nuclear translocation of molecules, organelle morphologies, and protein-protein interactions. On the other hand, that label-free mode can be useful especially when users don't want to stain cells with chemical or biological labels or damage them before the downstream uses. In addition, this approach is also effective when users are not clear about what the target molecules are or what image patterns should be specifically recognized. This lavel-free GC has demonstrated accurate classification of cells based on their kinds, states, and functions without observing molecular labels.

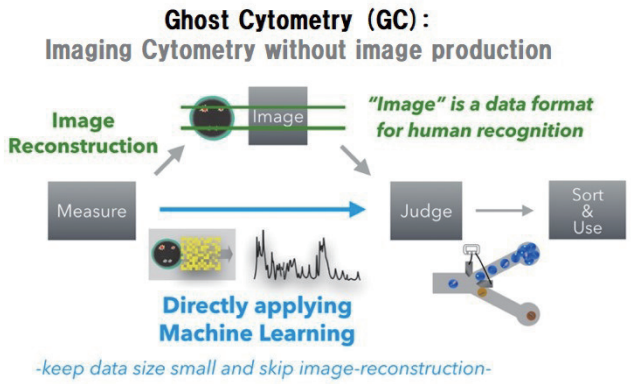

Figure 2 Image-free imaging cytometry driven by machine learning:
In conventional workflows of image-based cytometry, imaging technologies were to reconstruct a clear 2D/3D images for human recognition, followed by analysis based on human recognition. Our radical idea of ghost cytometry (GC) is, "apart from human recognition-based imaging cytometry, reconstructing 2D images is not necessary for machine learningbased analysis (AI)". In GC, its compressive imaging technology works to extract cell's imaging information as a temporal waveform of signals measured by a single pixel detector, followed by analysis without image reconstruction using machine learning methods. Thereby, GC enabled the world's first and fastest image-free "imaging" cell sorter by skipping the most time-consuming and computer-intensive image reconstruction in the data-intensive flow cytometry.

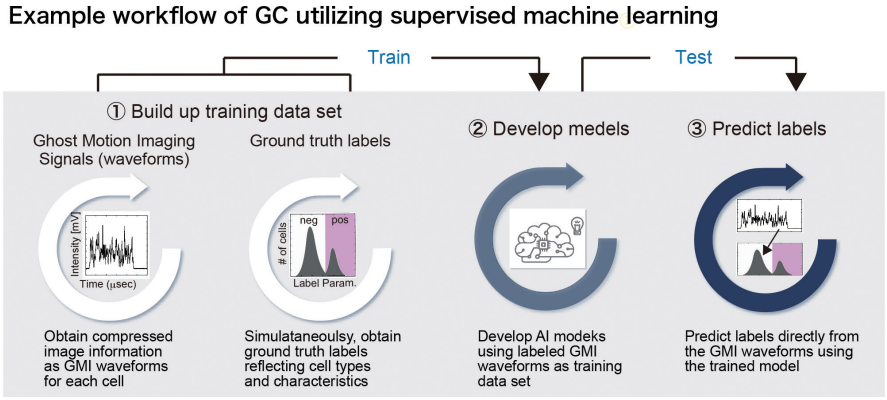

Figure 3 Example workflow of ghost cytometry utilizing supervised machine learning methods

Key feature of our technology 2: machine vision-activated cell sorting

By combining GC's efficient signal processing implemented in a field programmable gate array with a microfluidic device, we realized ultrafast and accurate cell sorting based on real-time analysis of "imaging" data. Figure 4 shows its first prototype and, since then, this machine vision-activated cell sorter (ViCS) has evolved to be more stable and more flexible one through development by ThinkCyte Inc. together with excellent other companies.

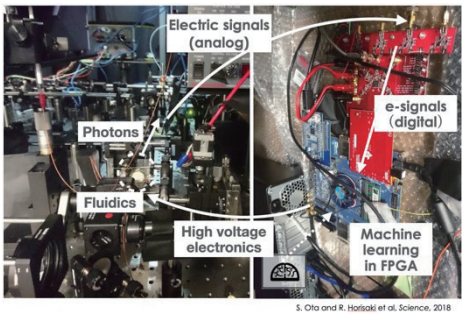

Figure 4 The first prototype of ultrafast machine vision-based cell sorter (ViCS), enabled by combining the optical ghost imaging signal acquisition, the real-time machine learning-based analysis implemented in FPGA, a microfluidic cell sorting technology.

Application

In the field of drug discovery, we have developed a pooled cell phenotypic screening method using ViCS. In this application, the fluorescence mode ViCS is used when the cell phenotype is specifically known (i.e. the localization of a specific molecule or a specific intracellular organelle), and the label-free mode ViCS is used when no specific explicit imaging feature is available. While high-content screening used to take long time (several months) and high cost, our method can significantly accelerate it at lower cost. We believe we can contribute to societ by greatly accelerating drug discovery research and development.

In the field of cell therapy, regenerative medicine, and cell-based production, the label-free GC and ViCS can evaluate cell types and functions without using stains or antibodies, and to enrich cells of interest. This method can be thus used for automatic monitoring of cell production lines and improving cell qualities by removing unwanted cells or contaminating particles. Similarly, it can be used in the evaluation of cells that produces antibodies and drugs. While remarkably effective cell-based drugs currently seem to put pressure on medical costs, such a system may greatly contribute to its reduction.

Conclusion

In conclusion, we proposed and realized "ghost cytometry", an approach based on AI-assisted cell analysis of their "image" information without image production. More concretely, by directly applying AI-based analysis to a compressed "image" signals in temporal domains, we skip a time-consuming image reconstruction process and realized the ultrafast and accurate image-free "imaging" cell sorter. We expect this technology to be widely adoped in biological sciences, and industrial applications including biotechnology, drug discovery, and cell therapy and regenerative medicine.

* Editorial note: This content is based on HORIBA's investigation at the year of issue unless otherwise stated.

References

[1] Ota, S., Horisaki, R., Kawamura, Y., Ugawa, M., Sato, I., Hashimoto, K., Kamesawa, R., Setoyama, K., Yamaguchi, S., Fujiu, K., Waki, K., & Noji, H. (2018). Ghost cytometry. Science, 360(6394), 1246–1251.

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