

# The World as Seen from Cells

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*Thirty years ago, Yokogawa embarked on R&D of high-speed confocal microscopes for real-time imaging of living cells and developed its first product, the CSU10 confocal scanner unit. The company then released the CellVoyager high-throughput screening system, the CQ1 all-in-one image cytometer, the CellPathfinder cell image analysis software, and other advanced products. Yokogawa is a pioneer of live cell imaging technologies and drug discovery support systems. This paper describes the technologies Yokogawa is developing for satisfying expectations in the life science field and for growing its business further.*

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## INTRODUCTION

The smallest unit of an organism is DNA, which carries genetic information. However, various cells are created, differentiated, and developed from the same DNA, and various organs are composed of such cells. Identical twins have the same DNA and look alike but live different lives. Thus, DNAs behave differently depending on the location, time, and environment even though they hold the complete set of information on an organism. The second smallest unit of an organism is proteins of various types, which exhibit different functions depending on the way of folding, interact with each other, and serve as constituents of cells. Still higher-level units are organella and cells.

In the 17th century, Robert Hooke observed cork with a hand-made microscope including a pair of lenses, and discovered a structure of small units, which he called a cell. Microscopes at that time were only capable of showing the shape of individual cells although such microscopes greatly

helped to reveal a world that had remained hidden from humankind. Later, optical microscopes and technologies for clearly observing cells were developed, including phase-contrast microscopes and differential interference microscopes. During such development, the technology of the fluorescence microscope was established, in which target cells are selectively labeled with antibodies and fluorescent dyes. Confocal microscopes were also invented that enable the observation of minute phenomena within cells. In addition, the technology of making cells produce fluorescent proteins has enabled the observation of the behaviors of proteins and micro-organs in living cells.

Optical resolution, which is the minimum distance between two objects distinguishable under visible light, is about 0.2  $\mu\text{m}$ . Since the size of cells is around 10  $\mu\text{m}$  depending on the type, the resolution of optical microscopes is suitable for observing the behaviors of cells. The study of optical systems has continued to develop, and now super-resolution microscopes that can distinguish objects smaller than the optical limit have become available.

## HISTORY OF YOKOGAWA'S TECHNOLOGY FOR OBSERVING CELLS

### Starting with the Confocal Scanner Unit

About thirty years ago, Yokogawa's Central Research Center started to develop a high-speed confocal microscope,

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focusing on a dual-disk confocal optical system with a Nipkow disk and a microlens array disk. By implementing this technology in an attachment product, Yokogawa developed a confocal scanner unit (CSU), which enables existing microscopes to acquire confocal images.



**Figure 1** The CSU10 confocal scanner unit (released in 1996)

Figure 1 shows the first product, the CSU10. In addition to the capability of acquiring confocal images at high speed, this product has an advantage of causing less damage to living cells because it acquires images by repeatedly irradiating a low-power laser. This advantage helped Yokogawa build a strong position in the field of confocal observation of living cells.

For about ten years after its release, major microscope makers tried to develop products that outperform the CSU. However, they finally gave up because the CSU delivered excellent performance with a simple structure. Microscope makers therefore changed their strategies and now actively collaborate with Yokogawa. The successor CSU-X1 (Figure 2) featured a motor-driven mechanism and improved connectivity to various microscopes. The CSU-X1 became a bestseller and the flagship product of the Life Science Center.



**Figure 2** The CSU-X1 confocal scanner unit (released in 2007)

About twenty years ago, the high content screening (HCS) method was developed. The HCS aims to clarify

the conditions of cells by using much information obtained from cell images, such as the shape and size of each cell, the amount of protein expressed in each cell, and the proportion of each cell group. The HCS, which is executed at high speed by automatic microscopes and image analysis technologies, is used in screening candidate compounds for new drugs.

Another term, high content analysis (HCA), also means a method to identify cells by using much information obtained from microscope images. Recently, the two words, HCA and HCS, are used almost interchangeably. Yokogawa prefers “HCA” in order to emphasize that the company’s products extract information from fine images taken through confocal optics.

The major market for the CSU was basic research for biology and medicine. To boost its sales, Yokogawa studied its applicability to other fields in 2003. The drug discovery support system was a promising field, and thus Yokogawa decided to aim at the HCA market.

A microscope is a good tool for observing cells but tells us only what there is and whether the shape has changed or not. A measuring instrument should also provide numerical data such as the amount, surface area, volume, and perimeter of the object, and how and when these values change.

The term, cytometer, is a combination of cyto (cell) and meter (measuring instrument), and means an instrument for measuring cells. In particular, a cytometer that has functions of imaging and image analysis is called an image cytometer. It is also called a high content imaging instrument, meaning an imaging instrument for the HCA.

### Entering the Drug Discovery Support System Market

Yokogawa started market research on the drug discovery field in 2004. This was only about ten years after the concept of the HCS/HCA was established and its applicability was still in the phase of trial and error. However, some leading universities and pharmaceutical companies in the West were developing various applications using image cytometers for the HCA. At that time, Yokogawa had no experience in the market of drug discovery support systems and just started to build a business plan by attending academic conferences and exhibitions both in and outside Japan and asking key persons in this field what features they wanted in image cytometer.

The main technique in the field of drug discovery was the binding assay, in which candidate compounds for a drug are identified by examining their interaction with proteins extracted from cells. Meanwhile, the cell-based assay was gradually attracting attention. In this method, a candidate compound is administered directly to cells and their behaviors and the expression of proteins are examined. However, it was still unclear which item among the obtained data could be used as the index to discriminate the candidate compound. It was also unclear what added value confocal systems could offer.

Yokogawa had an opportunity to develop a screening system in a continued study of a national project “Development of Technologies for the Analysis of Intracellular Network Dynamism,” which was coordinated and funded by the New

Energy and Industrial Technology Development Organization (NEDO). Yokogawa developed a test product called the BTS1000, which was installed in a laboratory of a university and yielded valuable findings. The system was then improved to satisfy screening requirements for the HCA market. By leveraging this chance, Yokogawa started product planning, developed the CellVoyager CV6000 high-throughput cytological discovery system (Figure 3), and entered the market of drug discovery systems.

The CV6000 was an ideal system for live cell screening with a wealth of functions. In addition to the confocal optics, this product featured a cell incubation environment on the observation stage to keep cells alive. It was also equipped with a dispenser to enable cell reactions to be measured immediately after a compound is dispensed to cells to cells.



**Figure 3** The CellVoyager CV6000 high-throughput cytological discovery system (released in 2008)

#### Diverse Specifications

The CSU was an attachment product that enables existing microscope systems to obtain confocal images. To leverage the capability of living cell observation, Yokogawa decided to develop an all-in-one microscope system with an incubation function that can precisely control temperature. This resulted in the CV1000 confocal scanner box, targeting the market of drug discovery as well as the market of basic research for biology and medicine.

As it was named a “high-throughput” system, the CV6000 was intended for high-speed processing. However, users increasingly demanded even faster systems and competitors responded by developing such systems. To outperform them, Yokogawa developed the CellVoyager CV7000, whose processing speed is about four times faster than that of the CV6000. To achieve this high performance, the diameter of the Nipkow disk, which is the core part of the CSU, was doubled. In line with this new specification, the optical design including aberration, vibration insulation, and alignment technologies were improved. The CV7000 successfully gained an advantage over competitors’ products in terms of throughput and became widely popular, particularly in Europe. Following the CV7000, the CSU-W1 confocal scanner unit with a wide field of view (Figure 4) was released to respond to new market needs.



**Figure 4** The CSU-W1 confocal scanner unit with a wide field of view (released in 2012)

Then, Yokogawa aimed at the flow cytometer market which has a longer history than the image cytometer market. In flow cytometers, cells suspended in a solution are aspirated and injected through a narrow flow channel into the sheath fluid. The fluid flow is irradiated by a laser beam, and the scattered light and the fluorescence emitted by the cells are measured. This is the same technique as a method of processing microscope images, which helped Yokogawa enter this market. During observation, a flow cytometer can digitize all data. Therefore, when the measurement of a sample is completed, the system can draw graphs of measured cell data. To achieve a similar usability, Yokogawa developed an on-the-fly method, in which cell images are taken, analyzed, and digitized at the same time. By implementing this method, the CQ1 confocal quantitative image cytometer (Figure 5) comes as a compact, easy-to-use instrument for personal use.



**Figure 5** The CQ1 confocal quantitative image cytometer (released in 2014)

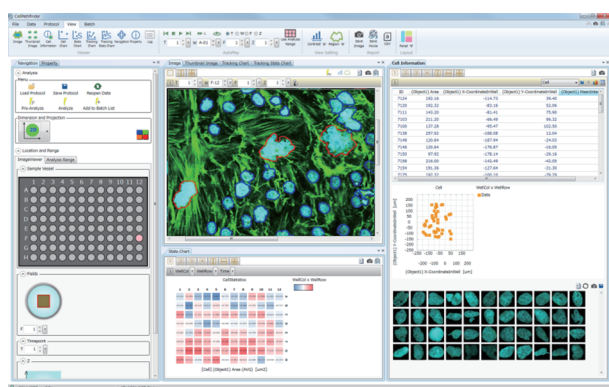
In 2017, Yokogawa released the CellVoyager CV8000 (Figure 6), with the world’s highest performance and improved cell incubation and reagent dispensing functions. This flagship model maximizes the CSU’s feature of causing less damage to living cells.



**Figure 6** The CellVoyager CV8000 high-throughput cytological discovery system (released in 2017)

### Image Analysis Technology

Yokogawa has developed image analysis technology while developing systems to acquire confocal images of cells. In particular, it has focused on the label-free method, which has attracted attention recently, to analyze cell images that are taken under light illumination without using fluorescent dyes. However, Yokogawa struggled to make a breakthrough because the analysis of label-free images was much more difficult than that of fluorescence images. In 2015, Yokogawa acquired label-free image analysis technology from Chip-Man Technologies Oy of Finland, which had a solid track record in this field. By combining this technology with machine learning, Yokogawa released the CellActivision image analysis software, which is dedicated for analyzing phase-contrast images. Around the same time, the Life Science Center independently developed digital phase contrast (DPC) technology for analyzing label-free images. Both technologies were integrated into the CellPathfinder (Figure 7), which is the common platform analysis software for the CV7000, CV8000, and CQ1.



**Figure 7** The CellPathfinder high content analysis software (released in 2017)

Traditionally, animals were used in tests for developing drugs. Recently, however, such tests are avoided in view of animal protection, and are practically prohibited for developing cosmetics in Europe. In addition, recent research results

revealed that animal tests are not so suitable for assessing drug efficacy and toxicity because human beings and other animals have different DNA and show different reactions to a drug, which is another reason for the recent unpopularity of animal testing. In the midst of this trend, technology for obtaining iPS cells was developed. This technology stimulates somatic cells, which were believed to have a lost capability to transform into other types, to differentiate into any cells. This technology has made it possible to assess the effect of toxicity in the early stages of drug evaluation. Yokogawa's instruments are playing important roles in this field.

Figure 8 shows the history of Yokogawa's products described above, as well as its target markets in the future. As seen in the figure, Yokogawa started from components, then developed systems and software products, and established the basis for the life science business. This is also the history of technological evolution. Starting from a simple technology of imaging and visualizing cells, Yokogawa integrated it with its measurement, control, and information technologies and achieved cell-based drug discovery and screening technology. Yokogawa aims to expand its life science business by developing cell handling technology for the areas of diagnostic and regenerative medicine, and manufacturing technology to produce cells.

### PROSPECTS FOR NEW BUSINESSES

This section describes the prospects for Yokogawa's new life science businesses.

#### Expanding Business Areas

##### ■ Cell imaging business

Yokogawa's life science business started with technologies for observing cells and visualizing things invisible to the naked eye. These will remain the core of the company's business. Yokogawa is currently developing the CSU-SR (code name) confocal microscope with a higher resolution than that of the CSU. It is also trying to develop technology for imaging deeper parts than before, such as those of layered tissues.

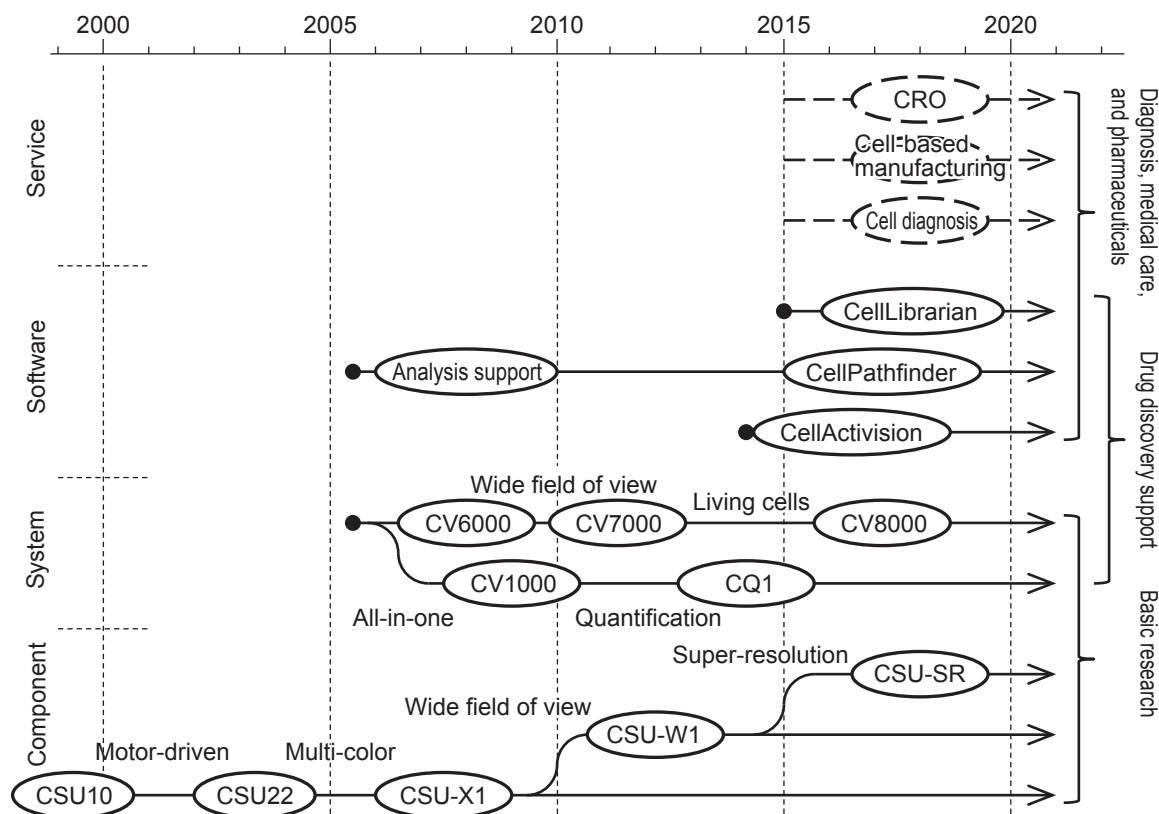
Previously, the ability to image monolayer cells on petri dishes was sufficient. As research for regenerative medicine advances, however, there is an increasing need to image cell aggregations or artificially layered tissues, and new technologies are required for imaging deeper parts of objects that existing confocal microscopes cannot reach. Yokogawa is planning to establish solutions by integrating its original technologies with optical CT, optoacoustics, and other technologies.

Confocal microscopes currently available are expensive and high-end. Thus, the main target of the cell imaging business is academic researchers. Yokogawa is also planning to release mid-range products for a wider range of researchers.

##### ■ Cell measurement and analysis business

The CellVoyagers and the CQ1 acquire, measure, and analyze cell images, and are used in the field of drug discovery. One recent trend in this field is that animal testing





**Figure 8** History of Yokogawa's life science business and future roadmap

is being prohibited. Artificially manipulated human cells such as iPS cells are easily available today and thus will be more widely used for toxicity tests.

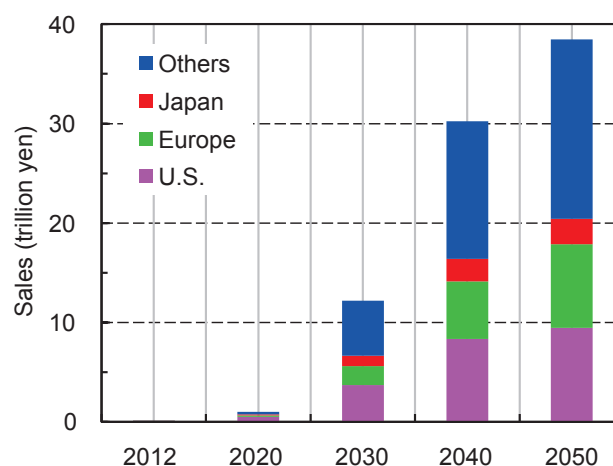
To respond to such trends, Yokogawa joined the corporate sponsored research program, “Human Cell Drug Development Studies,” at the Graduate School of Pharmaceutical Sciences, the University of Tokyo, in order to help the standardization of toxicity testing methods. Once toxicity methods are standardized, Yokogawa will expand its life science business from the sales of products to contract test services.

#### ■ Cell examination and diagnosis business

As data are amassed, the accuracy of cell measurement and analysis will be improved and it will be possible to offer cell examination. The regenerative medicine market is expected to grow substantially and exceed 10 trillion yen globally including peripheral industries by 2030 (Figure 9). Accordingly, the business of examining the quality and differentiation of cells is expected to grow rapidly in this field.

For such examinations, technology for analyzing label-free images using machine learning, which was described above, will be a competitive advantage. In addition, intracellular substance sampling technology will clarify the distribution and metabolism of substances in a specific cell.

Moreover, extracting and analyzing messenger ribonucleic acids (mRNA) will enable the diagnosis of gene expression and signal transmission within cells. This technology is expected to play a crucial role in personalized medicine in the near future.



**Figure 9** Forecast of the regenerative medicine market  
(Source: “Report on the Commercialization and Industrialization of Regenerative Medicine,” February 2013, Ministry of Economy, Trade and Industry)

#### ■ Cell control business

Technology for manufacturing antibody medicines, which is described in another paper in this issue, measures and optimizes the conditions of cell incubation. In other words, this is cell control technology. Although its current target is the manufacturing of antibody medicines, this technology will be applied to the cell-based production of enzymes and biomaterials.

### **Yokogawa's New Business Models**

Yokogawa is preparing to expand its life science business, shifting from a product manufacturer to a contract service provider.

Academic researchers, our major customers, have extensive knowledge on the handling of sophisticated instruments for their research. However, this is not necessarily the case in the field of drug discovery. Customers in this field want test results—quick and precise ones. They do not have enough time to master the manipulation of complex instruments to analyze images. In addition, to optimize personnel arrangements, many pharmaceutical companies are actively outsourcing testing work. Firms doing such contract business are called contract research organizations (CRO).

To enter this contract service business, Yokogawa is currently recruiting and training staff who will become the key personnel who have deep, detailed knowledge on customers' processes.

Instead of a simple test outsourcee, Yokogawa aims to become a service contractor for cell tests that require special skills and create high added value. As described above, once the next-generation toxicity test is standardized by the "Human Cell Drug Development Studies" program, Yokogawa will take the initiative in operating a contract service in this field.

There will be other needs for contract services in personalized medicine and regenerative medicine. By using the intracellular substance sampling technology, which is described in another paper in this issue, a contract business that precisely and accurately examines the characteristics of cells originated from patients will be feasible. Furthermore, a contract test service that uses Yokogawa's cell analysis technology will be promising in the field of regenerative medicine because characterizing a huge amount of cells and assessing their safety will become indispensable.

If the technology for producing antibody medicines is established, a contract manufacturing business will also be possible. Firms doing such contract manufacturing are called

contract manufacturing organizations (CMO). In addition, after establishing a solid manufacturing technology, Yokogawa will be able to make proposals for plants to customers, which will create synergy with the IA business.

### **Improving Competitiveness**

A crucial factor for driving the businesses described above is strengths, which are the source of competitiveness. The CSU is a strength that was built by Yokogawa's engineers in the early days and has driven Yokogawa's life science business. Yokogawa is now trying to develop additional strengths that competitors cannot achieve.

Core technology is essential. A major way to obtain it is in-house development, but forming alliances with other parties is also likely for better efficiency. Fortunately, major customers in the life science business are academic organizations, with which Yokogawa has established good relationships. In addition, its solid track record with confocal microscopes will help Yokogawa form new collaborations with leading academic experts in medical, pharmaceutical, engineering, and other fields. Yokogawa will be able to build an intellectual property network by amassing technological findings based on the core technologies and knowledge of academic people.

### **CONCLUSION**

Life science is a promising business domain; significant growth is expected although paradigm shifts are inevitable. As major manufacturers of electric appliances and machinery are entering this field, competition is becoming increasingly intense. Even so, we believe that Yokogawa will be able to continue growing by reinforcing its existing strengths, monitoring market trends, and driving its business strategically.

\* CSU and CellVoyager are registered trademarks of Yokogawa Electric Corporation.