Providing Solutions for the Life Science Field Using Spectroscopic Analyzers

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We provide solutions using surface plasmon resonance imaging device, particle tracking analysis device, Raman spectroscopic device, etc. for research on drug discovery and formulation of antibody drugs, quality control, and research on medical treatment and drug discovery using exosomes, which have been attracting attention in recent years. I will introduce the outline of these products and measurement examples, I will also introduce leading research and development in the life science field, which we are working on in collaboration with external organizations.

Introduction

The HORIBA Group's scientific analysis device applications are employed in extensive areas of research and manufacturing. Aiming to expand the use of our applications and augment our provision of solutions in bioscience and life science in particular, HORIBA launched our commitment to these fields in 2014. Backed especially by the spectroscopic-technology-based products among all of our scientific instruments, we target the pharmaceutical, food, and cosmetic markets, with a special emphasis on pharmaceutical products. We seek to establish ourselves as a provider of measurement instruments used in all drug-related settings, from drug discovery and formulation studies through manufacturing. By "measurement," HORIBA contributes to not only conventional small-molecule drugs but also diversifying modalities, such as antibody drugs, nucleic acid drugs, cell therapy drugs, and gene therapy drugs. Here I share with you some examples of our business deployment with our spectroscopic technologies in the pharmaceutical market and also our new engagement in the life science field based on collaboration with external organizations.

Technologies and products

HORIBA's measuring technologies are employed in various settings in the life science market as well. Recently drawing much interest in particular is the use of surface plasmon resonance (SPR) imaging, particle tracking analysis (PTA), and Raman spectroscopy for medical research and development and drug quality control purposes.

SPR allows monitoring of label-free molecular interactions. Parameters such as the association constant, dissociation constant, and affinity/binding activity can be assessed based on refractive index changes caused by the interaction between molecules immobilized on the metal film sensor surface and analyte molecules that are injected over the surface. Interactions between not only the same types of biomolecules (e.g., protein-protein, DNA-DNA) but also different types (e.g., DNA-protein) are analyzable, and so are interactions on the cell surface, such as antibody-microorganism interactions. HORIBA's SPR imaging (SPRi) system tracks binding of molecules immobilized in an array format onto the sensor chip; the imaging capability makes simultaneous monitoring of multiple interactions possible.

PTA is a methodology for tracking the scattered light from each laser-illuminated particle. Since the rate of the Brownian motion of particles differs depending on their sizes, the diffusion rate of each particle can be used to determine each particle size and particle count in the sample. PTA thus enables both size measurement and counting of nanoparticles.

Ramon spectroscopy is a spectroscopic technique used to acquire information on the molecular structure and composition of the analyte of interest through detection of Raman scattering from the illuminated analyte. Being a highly molecular specific, non-destructive, and non-contact analysis with no special pretreatment of samples required, this technique is expected to be extensively applied in the bioscience and life science fields.

Here are some examples of antibody drug and exosome analyses to which applications of these technologies were employed.

Figure 1 OpenPlex

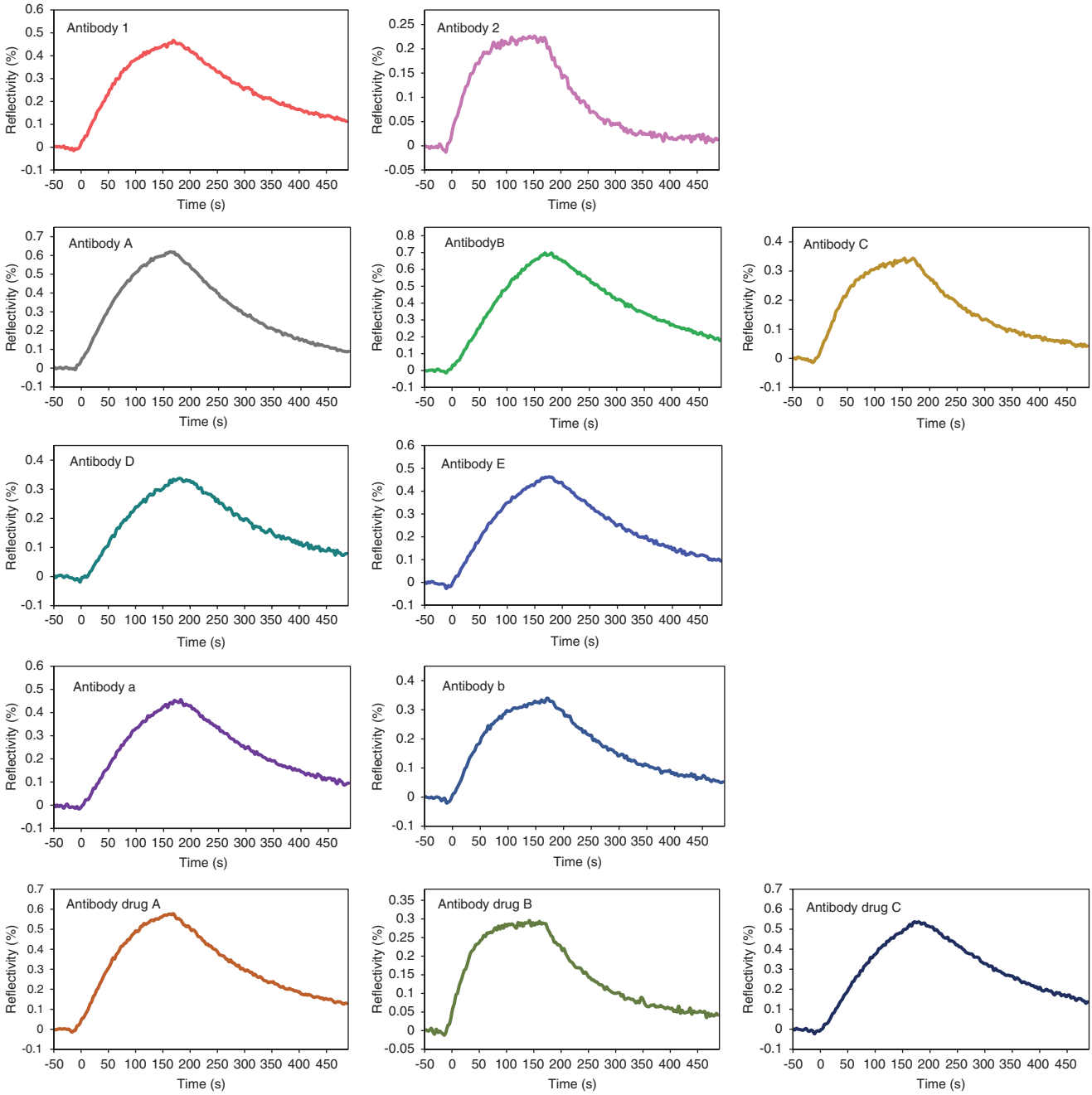


Figure 2 Measurement results with OpenPlex

Applications

Antibody drug analysis

Recent years have witnessed diversification of pharmaceutical forms with the emergence of biopharmaceuticals, taking over conventional small-molecule drugs. Possessing more complicated molecular structures, biopharmaceuticals require different methods for physicochemical property assessment from those for conventional drugs. For antibody drugs, in particular, which have been actively developed, it is essential to analyze and discuss their functional activity, structural stability, and colloidal stability. I would like to present our solutions to such demand using some measurement examples.

(1)SPRi measurement example

The binding potency of antibodies can be analyzed using HORIBA's SPRi system (Figure 1). It enables kinetic analysis for interpretation of interactions between antibodies and target analytes, and its multichannel allows simultaneous measurement of multiple interactions. In a case where interaction analysis was performed with 12 types of antibodies immobilized on the sensor surface over which Fc receptors were injected (Figure 2), slight differences in kinetic parameter values due to antibody glycan structural differences were detected. This result suggests that it may be possible to perform assessment in a way suitable for each different mechanism of antibody-antigen interaction (Figure 2).

(2)PTA measurement example

Antibody aggregation can not only reduce the drug effect but also trigger a side effect(s). Aggregates ranging in size from 100 nm to 10 µm in particular, called sub-visible particles (SVPs), are increasingly recognized as of importance, as reflected in the issuance of SVP guidelines by the U.S. Food and Drug Administration (FDA). PTA is a particle size analysis method with a competitive edge in this regard. Unlike conventional scattering methods, PTA is capable of quantitative colloidal stability analysis based on particle count. With its integrated temperature regulation system, ViewSizer 3000 (Figure 3), our PTA-based instrument, allowed monitoring of increases in particle count over the course of antibody solution heating at 50˚C for a certain time period (Figure 4). This demonstrates that PTA is useful in discussing the colloidal stability of antibody drugs.

Figure 3 ViewSizer 3000

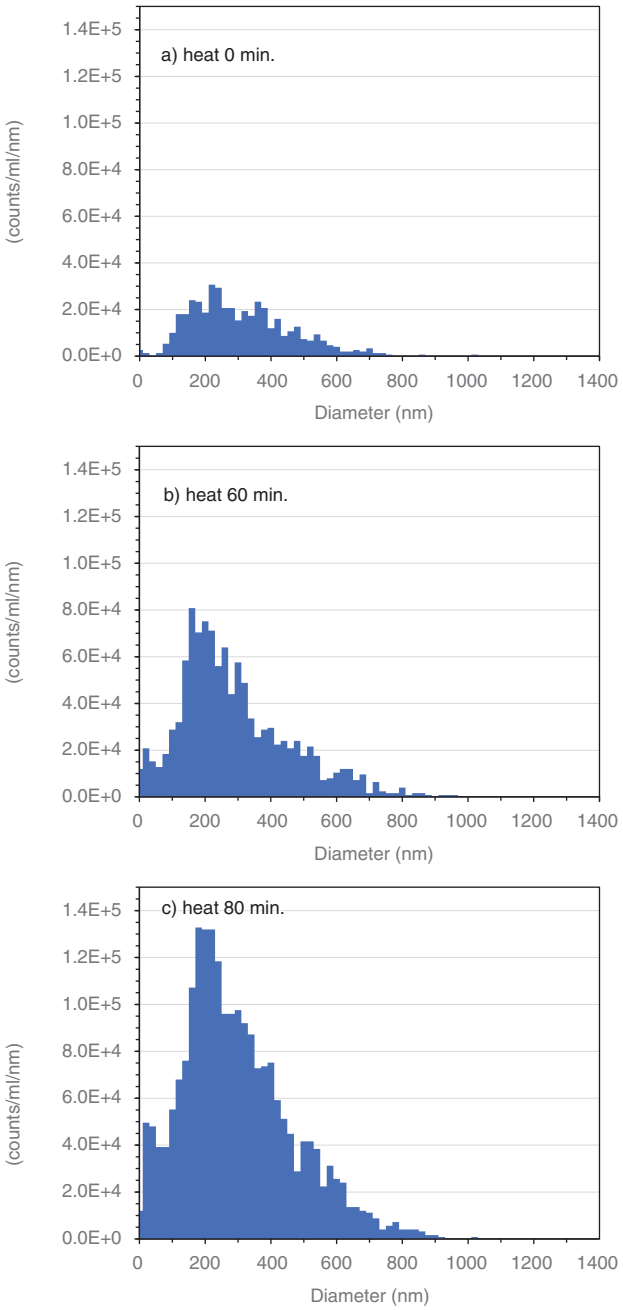


Figure 4 Measurement results with ViewSizer3000

(3)Raman spectroscopy measurement example

In general, antibody drugs are formulated in high concentrations, ranging from several milligrams to several hundred milligrams per milliliter, and thus require search for formulation conditions that inhibit aggregate formation and selection of highly stable antibodies. Aggregates are evaluated using modalities such as liquid chromatography, small angle X-ray scattering (SAXS), static light scattering (SLS), or dynamic light scattering (DLS), all of which solely provide colloidal information. Moreover, it is challenging itself to measure protein solutions in high concentrations (antibody drugs). However, Raman spectroscopy allows direct measurement of high concentrated solutions, providing information that reflects the secondary and tertiary structures of antibodies. This spectroscopic method was expected to be applicable for structural stability assessment of antibodies under formulation conditions; thus, temperature dependence of Raman spectra was evaluated. The results indicate that antibodies' structural stability may possibly be analyzed based on Raman bands of proteins, aromatic amino acids or amides in particular. This may lead to useful findings in the formulation study of antibody drugs.[1]

The use of analysis systems like those describe above is expected to contribute to not only discovery and formulation studies but also quality control of antibody drugs.

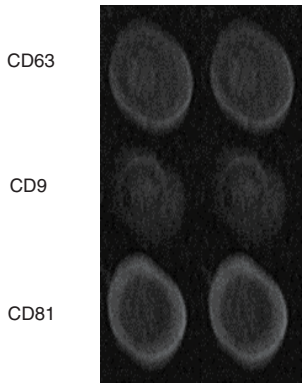
Exosome analysis

Exosomes are extracellular vesicles (Ø50–150 nm) released from cells and are present in our body fluids like serum and urine. They bear surface lipids, proteins, and sugars and contain protein and nucleic acid inside; their constituents differ depending on the cells that release them. This means that exosomes released from disease-related cells exhibit molecular profiles specific to the disease, and such molecules in exosomes can be targeted in liquid biopsies. Exosomes are also studied for possible applications as a drug delivery system, attracting attention from both medical care (clinical testing and diagnosis) and drug discovery (therapeutic drug development) aspects. Here the applicability of SPRi as a method for exosome surface protein identification and that of PTA for exosome particle concentration measurement are discussed using studied examples.

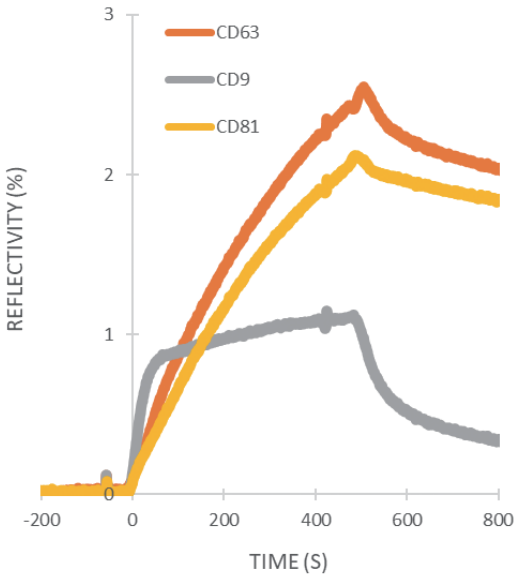
(1)SPRi measurement example

By immobilizing numerous ligands (192 at maximum) at specified spots on the biochip surface, OpenPlex enables simultaneous monitoring of many interactions, and its imaging function allows visualization of the interactions. Further, as another major advantage, analytes relatively in large sizes, such as cells, bacteria, and exosomes, are also assessable on OpenPlex.

Here is an example of SPRi measurement. Exosomes were purified from human serum by ultracentrifugation.



a)



b)

Figure 5 Exosomes detection results with OpenPlex
a) Spot Imaging
b) Reflectance change

The biochip treated for low non-specific binding was used on which antibodies to exosome marker proteins (CD9, CD63, and CD81) were immobilized. An exosome solution 200 µL (≥1010 particles/mL) was injected over the biochip surface for analysis of interactions with the antibodies; interactions with all the antibodies were monitored by way of both imaging and percent changes in reflectivity (Figure 5). This result demonstrated the usefulness of SPRi as a means to identify exosome surface proteins.[2]

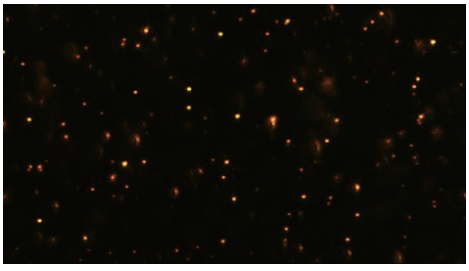
As stated above, OpenPlex is capable of identifying many different exosome surface proteins in a single run thanks to the use of a biochip with various ligands immobilized on its surface. This feature has gained OpenPlex a high reputation and expectations as a tool suitable for exploration and screening of novel markers and for quality control required in drug discovery and application.

(2)PTA measurement example

Equipped with three-wavelength laser light sources, ViewSizer 3000 uses for analysis a red laser for large particles and a blue laser for small ones and is thus capable of measurement of particles of a wide range of sizes. Fluorescently stained particles can also be measured by using fluorescent dyes of matching wavelengths.

Here is an example of exosome measurement by the ViewSizer 3000. Exosomes were purified from human serum using MagCaptureTM Exosome Isolation Kit PS (FUJIFILM Wako Pure Chemical Corporation, Osaka).[3] The resultant exosome solution was diluted 100-fold, and the target exosomes were stained with a fluorescent reagent. The size distribution of fluorescent particles was analyzed on ViewSizer 3000 in the fluorescence mode, and then all particles in the solution were counted. Approximately 40% of all particles were found to be stained ones (Figure 6). The above result indicates that ViewSizer 3000 is a useful tool for determining the proportion of exosomes among all particles. This instrument has thus won a reputation for being effective in detecting target exosomes and is expected to contribute to advancing drug discovery.

As described above, we are committed to disease-specific protein identification research, hoping to make contributions to basic medical science and drug discovery and development in which exosomes are involved. We also seek to contribute more to drug discovery and development for the aforementioned biopharmaceuticals and other various modalities by prompting further extensive use of our analyzers with advantageous features, such as OpenPlex's "multiplexed bioassays" and "analysis of interactions with cells and with bacteria" and ViewSizer 3000's fluorescence mode measurement employing three wavelengths.



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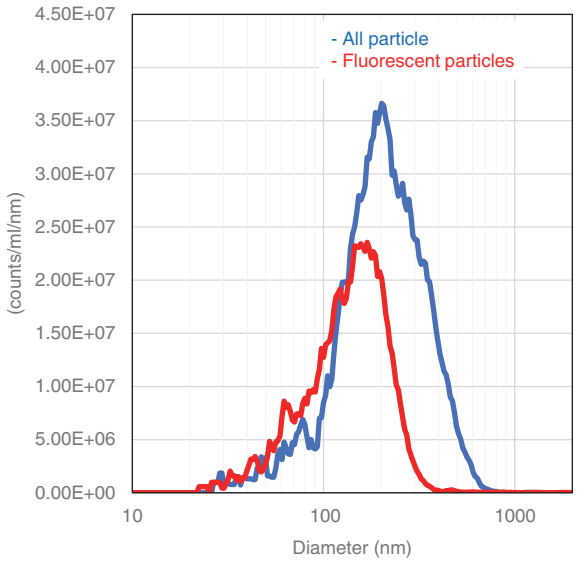

b)

Figure 6 Exosomes detection results with ViewSizer3000
a) Fluorescence imaging
b) Particle size distribution comparison

Collaboration with external organizations

In addition to the above-stated provision of the spectroscopic-analysis-based solutions, we engage in collaborative work with external parties in the life science field.

(1) LC-Raman: a high-performance liquid chromatograph-Raman spectrometer combined system

Shimadzu Corporation and HORIBA Ltd. initiated joint development of the LC-Raman system in 2020 and launched it in the market in June 2021. The high-performance liquid chromatograph separates a mixture sample into components, which are separately loaded into wells of the collection well plate; the Raman spectrum of each component is recorded using a Raman spectrometer. Since each component of a sample is separately analyzed by Raman spectrometry, it is possible to acquire molecular information on each component, which has been hard for mixture samples. This system is anticipated to contribute to the search for unknown natural functional components, biomarkers in biological samples, and more.

(2) Moonshot Agriculture, Forestry and Fisheries Research and Development Project

"Building a platform for sustainable farming by environmental control based on the microbe atlas of the soil"

In line with Moonshot Goal #5 "Creation of the industry that enables sustainable global food supply by exploiting unused biological resources by 2050," established by the Cabinet Office of Japan, this project aims to define the requirements for a soil suitable for crop growing ("what is a healthy soil") and to create a sustainable food supply industry directed at agricultural activation and diet of the future, with eyes on "soybeans" as a future-oriented food. It is intended to establish a "platform for recycle-oriented cooperative agriculture" by applying technologies and software that enable such a form of agriculture on the basis of analysis and control of interactions between soil microorganisms, crops, and the environment. HORIBA Ltd. assumes the task of soil mineral ion measurement over the course of soybean growth, utilizing the electrochemical sensor technology that we have long nurtured. Linking soybeans' mineral ion values and molecular biological information may lead to the development of life-science-field solutions with the aid of not only spectroscopy but also electrochemical sensors as seeds for success.

(3) Cross-ministerial Strategic Innovation Promotion Program (SIP)

"Technologies for smart bioindustry and agriculture"[4]

HORIBA takes part in the research for "technologies for smart bioindustry and agriculture" in SIP. The group for this research aims to establish a smart food chain that allows optimization of food distribution and processing based on data sharing, from production through distribution and consumption. HORIBA is tasked with assessment of residual chemicals in agricultural products. Control of residual agrochemicals is essential for food safety and security assurance. We are working on the development of an immunochromatographic kit suitable for simple on-the-spot measurement of residual chemicals in agricultural products, utilizing a monoclonal antibody for agrochemicals developed in-house.

(4) Other

In addition to the above, we are pursuing various kinds of collaborative work related to pharmaceutical and cosmetic research and development with external partners, such as universities and research institutes. Here I have presented information primarily on biopharmaceuticals, but we are also active in the small molecule drug field, e.g., fluorescence spectroscopic evaluation of drug substances' crystalline nature[5] and Raman spectroscopy-based measurement of skin permeability of active ingredients, which is critical in dermal drug assessment.[6] Our expertise in measurement for the skin is garnering much attention from the cosmetic industry.

Closing remarks

Besides the examples described herein, we are working on measurement instruments and applications suitable not only for drug discovery and development but also for production process and quality control, such as analysis of the active ingredient in a tablet using a transmission Raman spectrometer[7] and Raman spectroscopy- and fluorescence spectroscopy-based analysis of culture media in a mass cell culture bioreactor, which is required for the production of antibody drugs and gene therapy drugs.

We intend to accelerate our contribution to the life science field, including pharmaceuticals, foods, and cosmetics, by further continuing to provide various solutions.

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

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