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Readout

HORIBA Technical Reports

July 2020 No. 54

特集　マイクロプラスチック分析

HORIBA

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HORIBA Technical Reports
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近年，環境リスクの一つとして関心が高まるマイクロプラスチック。海洋や大気汚染，人体への影響も懸念され，世界中でプラスチックの削減対応もおこなわれています。本号では，この世界的な課題に対し長年の環境保全での経験を活かしたHORIBAの取り組みを特集しました。

特集 マイクロプラスチック分析

■表紙写真
撮影：写真家　松井秀雄氏
（二科会写真部　会員）
都会の中の風景として東京湾に掛かるレンボーブリッジとその下を行き交う船の光跡を写してみたいと願っていた。狙いを定めシャッターを切った瞬間その夢が叶った。

■誌名について
誌名 Readout（リードアウト）には，「当社が創造・育成した製品・技術を広く世にお知らせし，多くの皆様に読み取っていただきたい」という願いが込められています。

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Potential impact of Microplastics (MPs) and Nanoplastics (NPs) in the environment has become global concern.
HORIBA products have been applied to environmental monitoring ever since its foundation.
In this issue, we focus on the approach of HORIBA group towards analysis and method development of MPs and NPs.

Rainbow Bridge and light trail of passing vessels has been my old challenge in the genre of "Landscape photograph in big city", and that is realized after getting difficult location and shot timing.
-Photographer MATSUI Hideo-
(Member of Nikakai Association of Photographers)

Name of the book

This book is named "Readout" in the hope that "the products and technology we have created and developed will be read out and so become widely known".

Microplastics and Nanoplastics: Analysis and Method Development

巻頭言

Foreword

世界中のあらゆる水質を守るために

堀場 弾

HORIBA Dan

株式会社 堀場アドバンスドテクノ
代表取締役社長
President
HORIBA Advanced Techno, Co., Ltd.

HORIBAの歴史は，創業者の堀場雅夫が1950年に国産初のガラス電極式pHメーターを完成させた所から始まっており，水の分析はHORIBAの基盤であると言えます。60年代には卓上型pHメーターはコンパクトな形に進化を遂げ，70年代にはガラス電極・比較電極・温度補正電極を一本化した複合電極を開発。80年代～90年代にはカード型，ハンディ型，スティック型と用途に合わせた製品を開発し，オンライン化も実現。2000年以降はそれらの製品ラインナップの拡充と精度・品質・デザイン面においても進化を遂げてきました。電気化学や電導度を用いた分析に加え，分光を用いた技術の発展や計測のオンライン化により，研究開発用途に加えて環境規制や産業プロセスに関わる水質分析機器を幅広く提供してきました。

環境規制に関わる水質分析のニーズは多岐に渡っており，例えば船舶における排出ガス環境規制（一般海域における硫黄酸化物SOxの排出量0.5%以下）の強化を受け，その対応に排ガス浄化装置用水質モニターの需要が高まっています。船舶にはエンジン排ガス中の硫黄酸化物排出抑制のための浄化装置が搭載されており，その浄化に用いられるスクラバー水（浄化水）を船外に排出する前に排水処理が必要となります。国際海事機関（IMO）により定められた規制値を満たした水でなければ船外へ排出が出来ない為，水の採取時点と処理後での計測に弊社の製品（EG-100）が不可欠となっています。

多くの先進国では水利用に不自由する事はなく，水インフラ施設の高水準化の成果を実感する事が出来る一方で，水処理需要の増大による処理施設の増設や効率化追求のための新たな取り組みが日々行われています。また世界の国々では水利用の需要に対する供給の均衡は取れておらず，今この時も浄水，水処理施設やシステム，計測などが必要とされています。加えて，生活排水や産業排水の再利用，環境負荷低減の為の対策も今後取り組みを強化していく国が増加していくと思われます。

Foreword

地域別で見ると，北米では，水不足が深刻化する西部や南部地域では産業排水の再利用化，海水淡水化等の対応が進められ，中南米ではブラジルやアルゼンチンなど上下水処理事業の民営化を推進する国が増加すると見込まれています。また，中国やインドを始め，東南アジア諸国では国の発展と共に環境への負荷軽減の為の規制が強化されており，飲用水・下水・産業排水など様々な水処理プロセスにおいて，効率的で確実な処理とそれらを定量的に表す計測が求められています。グローバルにニーズが拡がる中で，計測データに対する責任を担う我々の役割は重責であると認識しています。

持続可能な社会の実現(SDGs)に関しても，HORIBAは自社の持つ技術や製品とアプリケーションを繋げる事で，多くの社会課題への解決に貢献しています。

これまで述べてきた“水”そのものに対する課題に加え，昨今，マイクロプラスチックのような水に関わる重要な社会的課題も着目されています。マイクロプラスチックは水圏に生息する生物に対する影響だけでなく，我々の飲料水，さらにはボトル飲料の安全性への影響が懸念されており，昨年のG20でも重要な課題として取り上げられました。HORIBAグループでは，これらの課題に対しても，HORIBA Jovin Yvonの主力製品であるラマン分光分析装置を核に，専門家の研究や課題解決に貢献しています。プラスチックは，商業用として多くの物に利用され，我々の日常生活に欠かせないものであるのも事実です。持続可能な社会の実現に対して，“水”のみならず，プラスチックという“固体”に対して，分析・計測のソリューションを提供できるHORIBAグループならではの貢献が求められていると考えられます。

HORIBAは，「世界中のあらゆる水質を守る」事が水計測のプロフェッショナルとしての使命であると考えており，コロナウィルス蔓延による人々の生活が変化した世界でも変わらず，グローバルな環境保全，産業の発達に貢献していける企業であり続けたいと考えています。

Microplastics and Nanoplastics: Analysis and Method Development and the Relationship with HORIBA Instruments Incorporated (HII)

米国HORIBAグループのマイクロプラスチック分析への取り組み

Andrew WHITLEY
アンドリュー ウィトリー

The ecological, human and marine health threat of Microplastics (MP's) and Nanoplastics (NP's) is huge and very real. In order for MP's and NP's to be accurately monitored, understood, legislated and reduced, there remains a significant amount of collaborative work needed between scientists, managers, policy makers and instrument providers such as HORIBA. Harmonized scientific method is required in order to allow legislators and agency managers to determine which issues to prioritize. In North America, HORIBA Instruments Incorporated (HII) is working closely with both scientists and federal and state government agencies. These collaborations are intended to support and develop the science and instrumentation to allow scientists and managers to achieve the directives and advances necessary to apply legislation and reduce the risks caused by MP's and NP's. This review paper explains HII's approach, activities and role in North America to support MP's and NP's analysis and method development towards eventual field monitoring devices and actionable legislation.

近年，マイクロプラスチック（MP's）やナノプラスチック（NP's）の人や海洋生物をはじめとした生体に対する影響が懸念されている。MP'sやNP'sを削減するために正しく測定し，その懸念を理解して必要な規制を制定することが必要である。そのためにはさらに産学官の枠組みでの連携に，HORIBAの様な分析装置メーカーが関与してゆくことがますます重要となる。行政関係者がどの課題を優先的に対処すべきかを決定する上で，このような連携は非常に重要である。我々，HORIBA Instruments Incorporated（HII）は，北米において科学者及び連邦機関や州政府機関と密な連携を図ってきた。我々は，科学技術や測定手法の開発を通じ，科学者や対策を主導する人々の方針立案や法整備に寄与することで，MP'sやNP'sによる環境リスクを低減することを目指している。本総説では，実用的な測定手法の開発と実行可能な規制を検討のために行ってきたMP'sの解析や測定手法の実現に対しての我々のアプローチや具体的な活動をはじめ，北米で果たした役割について報告する。

Introduction

Ever since the very beginning of HORIBA, including the early development work at the end of 1945 and through the very first glass electrode pH meter products in the early 1950's, HORIBA products have been applied to environmental applications to protect our planet. HORIBA found early success at the beginning of the 1960's through the automotive emission analyzer MEXA-1. Since then many of our products have been developed for environmental or related studies and applications. Today our corporate activity towards social responsibility is focused on energy, health, the environment and safety. Some

key examples of these environmentally conscious products include decades of continuing innovation in FT-IR exhaust gas analyzers; our range of XRF analyzers that were applied towards the waste electrical and electronic equipment (WEEE) and the recycling of hazardous substance (RoHS) directives; and our AquaLog fluorescence Absorbance Transmission Excitation Emission Matrices (A-TEEM) spectrometer that was developed for rapid analysis of dissolved organic matter in water to allow environmental and water treatment monitoring. It was only natural therefore, based on the ever increasing concern over microplastics (MP's) and nanoplastics (NP's) in our environment, that HORIBA would be closely involved in leading the development and standardization of analysis methodologies for this pervading pollutant.

The ecological, human and marine health threat of MP's is huge and very real. It is estimated[1] that every year 4.8 to 12.7 million metric tons of plastic waste enter our oceans. One report[2] estimated that up to the year 2014 there were an accumulated number of MP particles, located as a global standing stock of small floating plastic debris, ranging from 15 to 51 trillion particles, weighing between 93 and 236 thousand metric tons, which is only approximately 1% of global plastic waste estimated to enter the ocean in each year. Presumably the remaining 99% of plastic waste ends up in sediment on the ocean floor with some washing up on beaches around the world, and some amount recovered in cleaning exercises.

HORIBA Scientific's North American involvement with MP's began with the development of a close collaboration with Dr. Chelsea Rochman and her research group in 2015 at the Freshwater and Marine Ecology Department at the University of Toronto. At that time Dr. Rochman acquired the XploRA Raman microscope for her groups MP research. In 2019, a second XploRA Raman microscope was delivered to the University and installed in the laboratory of Chelsea's colleague Dr. Robert Andrews in the Institute for Water Innovation. Dr. Rochman is a leading researcher and innovator[3] in the field of MP's, the work of Dr. Rochman's laboratory will be outlined in detail in an article by Dr. Bridget O'Donnell later in this issue of Readout.

In September 2018 the California legislature responded to the increasing threat and public concern towards MP's by enacting two new bills, as outlined below, that require quantification of MP's in various media and development of new management strategies.

Senate Bill 1422: California Safe Drinking Water Act–Microplastics[4]

Senate Bill 1422 (Portantino, Chapter 902, California Statutes of 2018) charges the California State Water Resources Control Board (SWRCB) with developing methodologies and a strategy for monitoring and tracking the concentration of MP's in drinking water. This includes, adopting a standard definition of MP's in drinking water by July, 2020; adopting a standard methodology to test drinking water for MP's by July 2021; adopting requirements for testing and public reporting of MP's in drinking water; and accrediting laboratories to analyze MP's.

Senate Bill 1263: Ocean Protection Council – Statewide Microplastics Strategy[5]

Senate Bill 1263 (Portantino, Chapter 609, California Statutes of 2018) requires the California Ocean protection Council (OPC) to adopt a Statewide MP's ocean and waterways strategy and report to the legislature on implementation by 2025. The bill also requires OPC to develop a prioritized plan to research and support the development of risk assessments in marine habitat by 2021. This includes, development of standardized methodologies for extracting, sampling, counting, and characterizing MP's in the environment; moving forward to characterize ambient concentrations, impacts, sources and pathways of MP's in California waterways; and developing approaches to reduce the introduction of MP's into marine environments, including source control.

With the announcement of these bills the HORIBA Scientific Business Development team, led by Dr. Kentaro Nishikata and Dr. Andrew Whitley proposed a working group meeting to review the analytical instruments and field monitoring required by these bills. We approached Dr. Rochman to discuss planning such a meeting. Dr. Rochman suggested that we collaborate with and hold the meeting at SCCWRP in Costa Mesa, CA, which happens to be just 13 miles from the North American headquarters of HII in Irvine. Chelsea introduced us to Dr. Steve Weisberg, Executive Director of the Southern California Coastal Water Research Project Authority (SCCWRP), and together we proceeded to discuss what was required to create a successful working group meeting. It was agreed that at the meeting it would be necessary to perform a gap analysis between existing methods and summarize the necessary actions to bridge these gaps. From an analytical instrument and environmental monitoring device manufacturer point of view, HORIBA needs

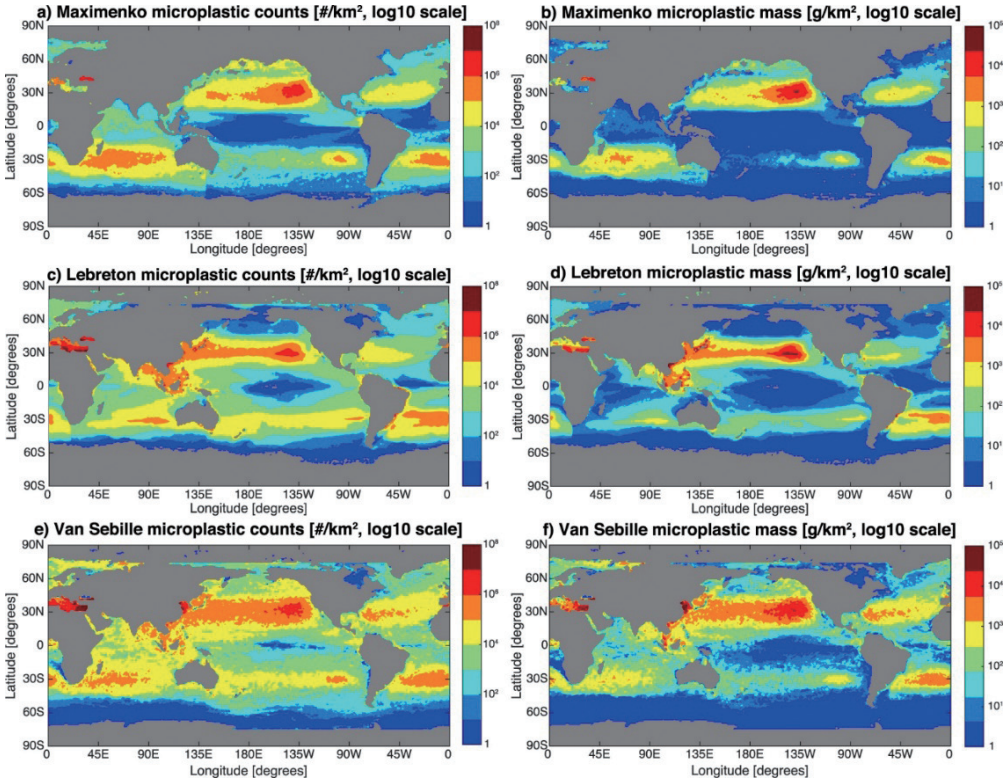

Figure 1 Maps of the solutions of MP's count (left column) and mass (right column) distribution for the three different ocean circulation models. Because fits are done on a per-basin level, there are a few discontinuities visible (e.g. South of Tasmania in the Maximenko solution, panel a)). Figure 3 from "A global inventory of small floating plastic debris". Erik van Sebille et al 2015 Environ. Res. Lett. 10 124006 doi:10.1088/1748-9326/10/12/124006[6]

to work closely with the scientists and managers that are tasked with advising policy makers what instrument and method developments are needed to meet legislation, in this case the two CA Senate bills. HORIBA and other manufacturers require the measurement requirement to be strictly stated so that we can collaborate and advise on possible solutions. Where possible collaborating on method development using existing instruments, but when needed adapting hardware, including sample handling and automation, and software, to meet the measurement requirements. In some cases, where the need is extensive and fully understood, the development of new instrumentation will be done, in the case of MP's, as an example, for in field or treatment plant monitoring.

On April 4-5, 2019 our workshop: "Measuring Microplastics: Building Best Practices for Sampling, Extraction and Analysis", hosted by HORIBA, SCCWRP and the University of Toronto, in coordination with the State of California Water Resources Control Board (SWRCB) and the California Ocean Protection Council (OPC) was held at SCCWRP in Costa Mesa. The main meeting objectives were:

1. Understand policy-maker needs in regards to microplastics methods.
2. Agree on the state of the science and determine the research necessary to reach shared goals.
3. Co-develop a manuscript on best practices for microplastics analyses.
4. Design a study plan to develop harmonized methods, including collection, laboratory and data management, for microplastics analysis.

There were 14 presentations from regulatory and legislation representatives, and scientists and managers from around the world. The meeting presentations were recorded and can be viewed via the link in this reviews references.[7] To begin the day, we heard from Deborah Halberstadt, the Executive Director of the OPC, and Darrin Polhemus, the Deputy Director for Drinking Water at the SWRCP. They shared their perspective and the targets they need to meet which were mandated by SB1263 and SB1422. The scientific presentations

Figure 2 Ann-Marie Cook of the EPA presenting at the "Measuring Microplastics: Building Best Practices for Sampling, Extraction and Analysis" workshop at SCCWRP in Costa Mesa, CA

were separated in to four main topics–extraction, sampling, analytical methods and data analysis. At the end of the first day, we asked everyone to think about what they had learned and what they needed moving forward in relation to their own research, monitoring or management. We asked everyone to answer four questions:

What is your most urgent need at this moment?
What would you like to see in a best practices report?
What types of methods would you like to see developed?
What are some of the key concerns that should be taken into consideration when developing/choosing best methods and practices?

The answers to these questions by all stakeholders were summarized in the final meeting report, they were used to guide day 2 of the meeting and will continue to be used to guide future work. On day 2 of the meeting our goals were to:

1. Create scientific journal review articles (for *Applied Spectroscopy*) to summarize the state of the science towards standardized MP's analysis.
2. Develop a study plan that addresses issues necessary to achieve method standardization.

First we worked on the articles for the special issue of *Applied Spectroscopy*. We spent the morning beginning drafts of each review paper that we were planning to write together and agreeing upon a general outline for the special issue. The special issue will be wrapped up in July, 2020 and come out in early Fall, 2020. Details of this special issue of Applied Spectroscopy can be

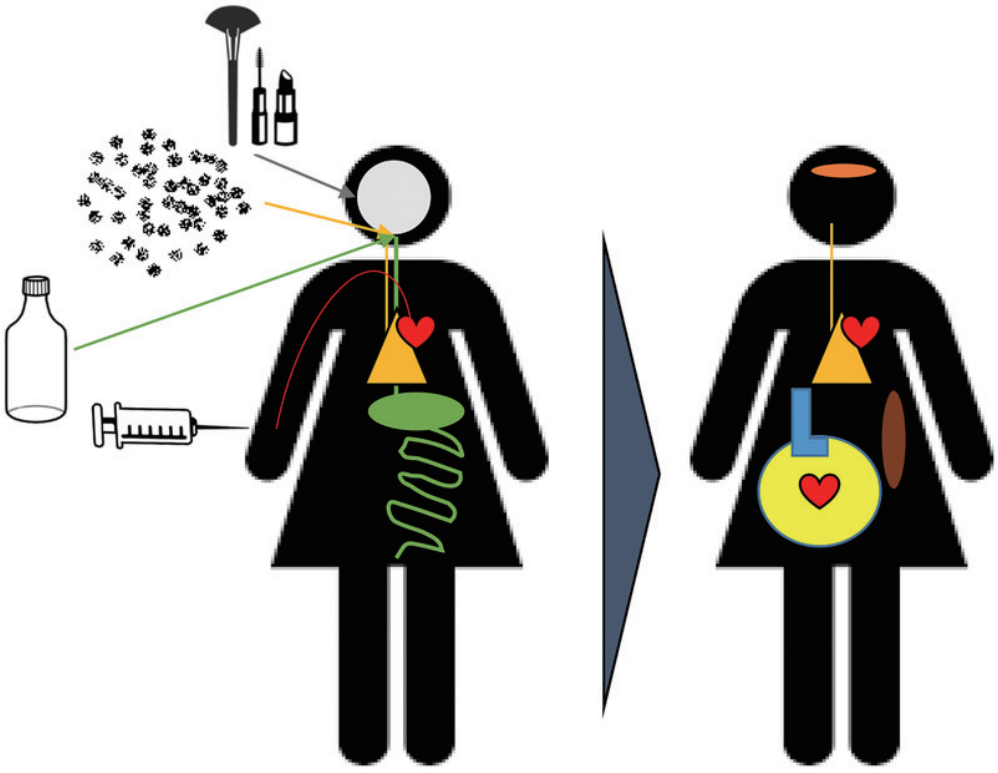

Figure 3 Diagram depicting the routes of NP's exposure (i.e., ingestion, inhalation, dermal, and injection), potential primary systems of impact, and potential secondary toxicity associated with particle deposition. *Reproduced by permission from PA Stapleton, AIMS Environ Sci. 2019; 6(5): 367–378.*[12] *Published online 2019 Oct 22. doi: 10.3934/environsci.2019.5.367*

found later in this review. The afternoon session was spent discussing the format for a multi-lab MP's methods evaluation study plan, this is detailed later in this review paper. The final results and actions of the meeting were then summarized, these were the final report detail to be shared after the meeting with all participants, the collaborative method evaluation study, a special issue in the journal of *Applied Spectroscopy*, and two scientific sessions on MP's analysis (organized by Andrew Whitley, HORIBA and Shelly Moore, SCCWRP) at the October, 2019, SciX conference in Palm Springs. There will also be a follow-up workshop at SCCWRP to share the results of the study plan and discuss how MP's may impact human health.

Toxicological considerations of Microplastics and Nanoplastics

The question of nanotoxicology and which types and sizes of MP's and NP's are most dangerous to human and animal health is still a very underserved research area and significantly more work is required here. It is certainly the case that certain size classifications of MP's down to a few tens of microns, whilst dangerous for wildlife and a pervading concern regarding pollution, do not offer as significant a health risk to humans as the smaller size classifications. Long term this larger size classifications, if allowed to go unchecked, could disrupt and damage marine populations with potentially huge cascading effects further up the marine food chain and on to humans. These larger particles if they are ingested and do not pass through the body can have long term health effects, in particular as they degrade they can leach hazardous chemicals in to the body of marine animals and ultimately humans. These chemicals have been shown to disrupt immune systems and negatively impact growth and reproduction. As a secondary effect MP's can also adsorb chemical contaminants on to their surface, transporting them within the environment or through a biological system. These differential surface absorbents, including biofilms, and particle transformations will impact MP and NP transport and toxicity. This subject is covered in more chemical detail by Dr. Bridget O'Donnell later in this issue of Readout.

The effects of MP's become much more pronounced the smaller the particles are, as they are more likely to pass from the gut and stomach to the bloodstream and other organs. As they become smaller in size these particles also can become airborne. It is known that MP's smaller than 25 microns can enter the human body through the nose or mouth and those less than five microns can end up in lung tissue.[8] Increasing the urgency to understand the impact of airborne MP's and NP's is critical. There is already a great deal of concern, research and attempts at legislating fine particulates in the air formed by burning fossil fuels, including black carbon or soot. These particles have been linked to a number of health impacts including respiratory issues, heart attacks and the impairment of neurological function.[9] Most countries have air pollution standards to limit the volumes of particles less than 10 microns, and especially those below 2.5 microns, respectively known as PM 10 and PM 2.5 standards.[10] However little has yet been done to understand the toxicity or to monitor and legislate the potentially more harmful airborne MP's and NP's pollution.[11] The size of a particle directly relates to the surface area-to-mass ratios. The surface area-to-mass corresponds to the amount of surface area of an object (particle) within a given volume or collection of particles. The fact that NP's have a larger surface area-to-mass than MP's therefore provides a greater surface for biological contact or chemical adsorption. NP's are also

more likely to become surface charged, functionalized and therefore have a further likelihood to have species chemisorbed on their surface. Such surface modifications can aid transport across organ membranes. It is known that surface modification, if cationic, can aid bonding to the brains endothelial cells and therefore become a mechanism to endocytosis and transport across the blood brain barrier. It is established that NP particles can cross biological membranes and influence cellular signaling, however, the cellular and toxic effects of these exposures have yet to be evaluated. Future studies must also identify environmentally and health risk relevant concentrations and take into account the NP physicochemical properties of each NP type analyzed. It is critical that these studies take place rapidly to help guide the necessary development of monitoring and analysis methods to target the most critical size range and MP and NP types that requires the strongest and most urgent legislation.

Analytical Methods for the Analysis of Microplastics

As part of the Applied Spectroscopy special issue on MP's there is an excellent review paper that compares the various analytical techniques used to identify MP's. This review paper[13] "Critical Assessment of Analytical Methods for the Harmonized and Cost-Efficient Analysis of MP's" by Primpke et al includes a contribution by HORIBA's Dr. Bridget O'Donnell. The main focus of this comprehensive review paper is the currently applied identification and quantification tools for MP's. The authors evaluate these techniques and the need to provide a harmonized guideline for future SOPs to cover legislation like the two recent California Senate bills discussed above. The main techniques used for MP's are covered in this paper, these are naked eye detection, general optical microscopy, the application of dye staining–typically Nile Red, flow cytometry, Fourier transform infrared (FT-IR) spectroscopy and microsopy, Raman spectroscopy and microscopy and thermal degradation by pyrolysis–gas chromatography–mass spectrometry (py-GC-MS) as well as thermo-extraction and desorption gas chromatography–mass spectrometry (TED-GC-MS). A guideline to provide the necessary method harmonization in the time frames necessary to support legislation is provided. This includes an analysis of the cost of each method ranging from low cost towards higher analytical demands to measure MP's in an effective way by field laboratories and governmental institutions while maximizing information for risk assessment. It is important to achieve the goals of the California Senate Bills that we create analysis methods that are not only achievable by the most proficient experts, but ones that are transferable and repeatable among a wide array of laboratories, some of which will be introduced to MP's for the first time as a result of new legislation. Methods must also fit in to the requirements of any laboratory accreditation program to ensure the data generated are correct, consistent and traceable. The ability of the current analysis methods to meet all these requirements are being tested through the SCCWRP study plan discussed below. At HORIBA we will use the results of this study plan, our many ongoing MP collaborations and discussions with other government institutions like the EPA, NIST and ASTM to develop rugged, reproducible automated instrumentation and methods.

In Dr. O'Donnell's review of the research work of Dr. Rochman's laboratory she notes that in the characterization of MP's no single technique works for all samples encountered. It is important to use multiple tools to be able to confidently identify all or most collected particulates. This being said it has been

shown and reviewed in the paper by Primpke et al that Raman microscopy does offer a number of significant advantages over other techniques. One of the most important advantages of Raman microscopy is that the spatial resolution is excellent, down to one micron or less. FT-IR microscopy typically has a spatial resolution of between 10-20 microns, and as we discussed above it is the smaller MP's that provide the largest threats to human health. In Dr. Lee's paper, in this issue of Readout, she discusses the optimum instrument and experimental configuration for MP's analysis. Dr. Lee also reviews some of our North American collaborations on MP's. It is clear from these collaborations in North America, and globally, that in order for there to be statistical relevance in studies of the number and distribution of MP's a huge amount of samples need to be analyzed. A single filtered sample of 5 liters of water can capture 1000's of particles. There is clearly a need for automated analysis. Dr. O'Donnell and Dr. Lee show in their papers how HORIBA has worked with researchers to extend development of our ParticleFinder software to start to provide a fully automated analysis of filters containing MP's. Using the XploRA Raman microscope and ParticleFinder software from HORIBA, researchers can automate the location, particle characterization and identification of MP's of their filtered samples.

Now that the measurement hardware and software is starting to be available it is critical to develop standardized measurement methods. MP management strategy requires monitoring to assess the relative contributions of multiple MP sources and assess the progress toward source reduction. Such assessments are of little value if they are confounded by incomparability of measurements among different groups, sample types or over time.[14] Placing results from North America into context of other locations is critical, but only if methods across geographies are consistent enough to warrant such comparisons. It is with this challenge of method harmonization in mind that, at the MP workshop at SCCWRP, see above, it was agreed to undertake an ambitious study plan called "Microplastic Measurment Methods Evaluation Study". The purpose of the study is to assess the consistency of a measurement, characterization and identification of MP's in a number of sample types. The study plan will assess the repeatability of results across a large number of laboratories. The study includes evaluation of five methods (stereomicroscope, staining with Nile Red, FT-IR, Raman and Pyrolysis GC/MS) applied to drinking water, wastewater, sediment and fish tissue matrices. Extraction methods to be assessed include filtration for clean water, peroxide oxidation for wastewater, density separation for sediments and KOH digestion for fish tissue. Each participating laboratory will be given a sample with known blind materials and a standard operating procedure (SOP) for the methods they have agreed and signed up to perform. Most of the expert speakers at the workshop agreed to participate in the study, but other groups have been invited across a range of laboratories, from novice to professional. The proposed timelines for the study plan were pushed back to late 2020 due to initial challenges to prepare the samples and then because of the COVID-19 outbreak in the Spring of 2020. The HORIBA, NJ Lab will participate in this study, along with 35 other laboratories around the world.

Thanks to the high spatial resolution of Raman microscopy it can be used to study MP's and NP's across a wide size range from around 0.5 microns, an order of magnitude less than the lower size definition of MP's, up to, and beyond, the 5 mm upper size limit definition of MP's. Dr. O'Donnell and Dr. Lee recommend, and have developed, a varied menu of Raman applications

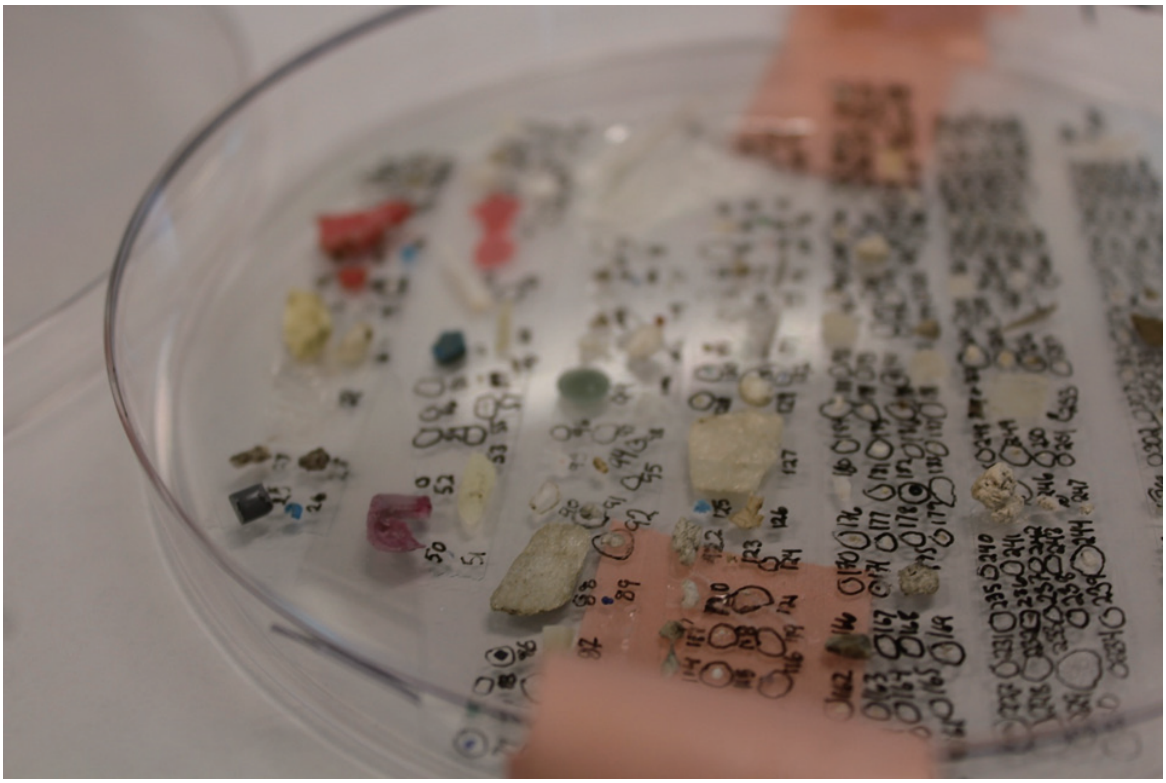

Figure 4 Examples of picked MP particles from a subset of a single experiment showing the large quantity and variation of particles that can be generated in MP analysis studies. Image reproduced by permission from the Rochman group.

methods to be used for the various size classifications of MP's and NP's and for the different morphologies of these particles. It has also been found that in order to maximize the number of particles that can be identified with Raman spectroscopy it is important to have both a 532 nm and 785 nm excitation laser for the analysis. Some particles will also burn under a focused laser, as used in Raman spectroscopy, in these cases it is important to be able to lower the laser power by accessing the laser control through the Raman software or by using neutral density filters to control the laser power reaching the sample.

Future Microplastics business opportunities for HORIBA

There are many institutes and industries that will likely need to monitor MP's and NP's due to legislation in the near future. Apart from the more obvious monitoring requirements for environmental water monitoring, waste water treatment plants and public water plants, there are other industries that will likely be subjected to regulations. These industries include packaged water, beverages and food. Even though there have been reports on MP's in drinking water, there have been no large scale studies on quality control of packaged water and beverages for MP's. It will become, however, most likely mandatory once regulations and reproducible methods are in place. Many companies will have to acquire an analytical instrument or use an accredited analytical service. Proactive companies such as Pepsi and CocaCola have already participated in MP's workshops, and expressed interests in having access to MP counting, characterization and identification capabilities.

The challenge and opportunities long term will be to monitor MP's in flow. One such method that could be adapted and applied to this challenge is flow cytometry, this technique was originally utilized for counting and characterizing cells to monitor growth, degradation, or aggregation processes, for exam-

ple protein aggregation. The sample is typically diluted by a sheath fluid and transported into a flow cell. Once in the flow cell the cells or particles scatter light from a laser beam and are counted based on changes in the optical signal in a forward or side scattering angle. Utilizing different lasers and dye staining methodologies allow for size, quantity, and distribution to be quantified, especially when combined with a strong camera for imaging. Typical size ranges of analyzed objects are between 0.5 and 50 microns.

Another approach for analyzing particles in the range of 2 microns to 1 mm in flowing solution is flow imaging. Here the diluted sample is monitored by a camera system combined with a microscope unit and each particle passing the camera is digitally imaged. The advantage of this technique over flow cytometry are that it visualizes and counts single particles with the options to later validate the counts, removing outliers like bubbles. There are also field deployable units that can be put in the field or treatment plants. In all case of in flow monitoring of MP's pre-filtration and purification will most likely be necessary prior to analysis.

In the case of NP's there is promise that the HORIBA ViewSizer could be used to characterize and count these particles. The ViewSizer tracks scattering from individual particles to determine particle size distribution and concentration. This technique commonly known as nanoparticle tracking analysis (NTA) or particle tracking analysis (PTA). The instrument uses three lasers to simultaneously illuminate the sample and a color video camera for detection, allowing it to analyze the broad size distributions encountered with plastic[15] NP's. Such broad size distributions cannot be analyzed by other single laser systems on the market. Furthermore, there is the potential to discern plastic NP's from other NP's with the use of an appropriate dye such as Nile red. The ViewSizer can be configured to monitor only fluorescent particles and thereby analysis specificity is limited only by the selectivity of the dye. Interest in this technique will grow as concerns about plastic NP's in the environment and NP toxicity converge.

Conclusion

In order for MP's and NP's to be accurately monitored, understood, legislated and reduced, there remains a significant amount of collaborative work needed between scientists, managers, policy makers and instrument providers such as HORIBA. Harmonized scientific method is required in order to allow legislators and agency managers to determine which issues to prioritize. Legislators have great interest in ensuring that there are measurement methods and programs that characterize risk, however it is up to the scientists and managers to determine the specific techniques that are used to achieve the risk assessment and drive policy. HORIBA has an important role to play to develop laboratory instrumentation and methods that allow scientists and managers to achieve the directives of the legislative. Eventually HORIBA's experience and expertise in environmental monitoring systems can help drive and provide for the provision of field deployable monitoring devices for MP's and NP's in liquid and air. Ultimately these tools will be able to support strategies aimed at removal of MP's and NP's at the source, removal in the transport system and ways to remove materials from the ambient environment. Finally it is likely that such tools will be used to monitor imposed limitations on producers that would affect the chemical nature of the source material. HORIBA's intent in North America is to continue collaborations with scientists and management groups

to understand the most urgent laboratory instrument and field monitoring system needs to enable harmonized method development. HORIBA has an important role to play in the environmental understanding, control and reduction of the risks caused by MP's and NP's now and in the future.

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References

[1] JR Jambeck, R Geyer, C Wilcox, TR Siegler, M Perryman, A Andrady, R Naray, Science 13 Feb 2015: Vol. 347, Issue 6223, pp. 768-771, DOI: 10.1126/science.1260352

[2] A global inventory of small floating plastic debris: Erik van Sebille et al, *Environmental Research Letters*, Volume **10**, Number 12, December 2015

[3] https://rochmanlab.files.wordpress.com/2018/09/rochman-et-al-2017-editorial.pdf

[4] https://leginfo.legislature.ca.gov/faces/billTextClient.xhtml?bill_id=201720180SB1422

[5] https://leginfo.legislature.ca.gov/faces/billNavClient.xhtml?bill_id=201520160SB1263

[6] Erik van Sebille et al, A global inventory of small floating plastic debris, Environ. Res. Lett. 10 124006 (2015) https://iopscience.iop.org/article/10.1088/1748-9326/10/12/124006/pdf

[7] https://www.sccwrp.org/about/research-areas/additional-research-areas/trash-pollution/measuring-microplastics-workshop/

[8] PA Stapleton, AIMS Environ Sci. 2019; 6(5): 367–378. Published online 2019 Oct 22. doi: 10.3934/environsci.2019.5.367

[9] Air Quality Guidelines, Global Update 2005, World Health Organization (2006); https://laqm.defra.gov.uk/public-health/pm25.html

[10] Air Quality Guidelines, Global Update 2005, World Health Organization (2006); https://laqm.defra.gov.uk/public-health/pm25.html

[11] Airborne microplastics: a review study on method for analysis, occurrence, movement and risks Christian Ebere Enyoh et al, *Environmental Monitoring and Assessment* volume **191**, Article number: 668 (2019)

[12] PA Stapleton, Toxicological considerations of nano-sized plastics, *AIMS Environmental Science*, Volume **6**, Issue 5, 367–378 (2019) https://www.aimspress.com/fileOther/PDF/environmental/Environ-06-05-367.pdf

[13] S Primpke et al, Applied Spectroscopy Special Issue on Microplatics. "Critical Assessment of Analytical Methods for the Harmonized and Cost-Efficient Analysis of Microplatiscs", express published on line on April 6, 2020. DOI: 10.1177/0003702820921465

[14] C.M. Rochman, F. Regan, R.C. Thompson. "On the harmonization of methods for measuring the occurrence, fate and effects of microplastics." Anal. Methods. 2017. 9:1324-1325.

[15] D. Stramski, J. Tatarkiewicz, R. Reynolds, M. Karr US Patent US9645070B2, "NANOPARTICLE ANALYZER"

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Status of Legislation and Regulatory Drivers for Microplastics in California

カリフォルニア州における マイクロプラスチック法規制と 規制推進体制

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Microplastics are pervasive in the environment, with biological communities exposed to microplastics particles on a continuous basis. Although health risks of microplastics exposure are poorly understood, microplastics have the potential to bioaccumulate through food webs, to serve as an exposure pathway for other contaminants that have stuck to them, and – in the case of smaller microplastics – to translocate into tissues and organs. To comprehensively assess exposure risks, scientists first need to build a foundational understanding of their occurrence and fate in the environment. California is at the forefront of international efforts to vet, standardize and implement measurement techniques that will become part of routine management monitoring. A long legacy of regulatory actions on trash pollution of all kinds has optimally positioned California to serve in this leadership role, including development of the nation's first TMDL (total maximum daily load) regulatory actions to reduce trash in waterways, as well as numerous trash source-control measures. In 2018, the California State Legislature passed a pair of bills that require the State to develop microplastics management strategies for both drinking water and California's coastal ocean. The legislation has become a call to action for the international scientific community to develop clear, actionable recommendations supporting California's microplastics management strategy. Already, a yearlong study has been launched to compare and evaluate various methods and instruments for measuring microplastics levels in water, sediment and tissue matrices. The study will pave the way for California to craft comprehensive, science-informed approaches for effectively managing microplastics in diverse aquatic systems.

マイクロプラスチックは，環境中の至るところに存在し，生物はプラスチック粒子にさらされ続けていると言える。マイクロプラスチックの健康に対するリスクは未だ十分には解明されていないが，食物摂取により生体内に蓄積し，その表面に付着した汚染物質を体内に取り込む媒体として働くことも考えられ，サイズの小さいマイクロプラスチックにおいては，組織や臓器にまで取り込まれる可能性がある。これらの環境リスクを包括的に評価するため，マイクロプラスチックの発生からその末路を的確に把握してゆくことが重要である。カリフォルニア州は，環境モニタリングの軸となる測定技術を精査，標準化し，実装するという国際的な取り組みを牽引している。例えば，水路のごみ削減のため米国内初のTMDL（Total Maximum Daily Load：1日の最大負荷量）規制制定や，多くの発生源対策の実施など，あらゆる種類のごみ汚染に対策を行ってきた。このような歴史に裏付けられるように，カリフォルニア州はマイクロプラスチック問題においてもリーダーシップを発揮している。2018年，カリフォルニア州議会は，マイクロプラスチック管理戦略の開発を行う法案を，「飲料水」と「カリフォルニア沿岸海域」のそれぞれを対象にして可決した。この2件の法律は，国際的な科学コミュニティ

が，カリフォルニア州のマイクロプラスチック管理戦略に沿った，明確で実用的な推奨事項を策定するための行動を促すものになっている。すでに，水，堆積物，および組織マトリックス中のマイクロプラスチックレベルを測定するためのさまざまな手法と機器を比較・評価するために，1年にわたる研究が開始されている。カリフォルニア州は，この研究を進めることで多様な水棲環境でマイクロプラスチックを効果的に管理するための，科学に基づいた包括的な手法で策定を進めようとしている。

Introduction

Numerous studies in recent years have put a spotlight on the pervasiveness of microplastics in the environment. Microplastics have been documented in waterways, in the ocean, in food and drinking water, in the atmosphere, in rain and snow.[1] A wide variety of industrial and consumer goods - from pharmaceuticals to synthetic fabrics - contains microplastics; furthermore, larger plastics break down over time into smaller microplastic particles. Plastic pollution is growing at an exponential rate. Every minute, the equivalent of one garbage truck's worth of plastic escapes into the environment.[2] Although about 14% of all plastic produced worldwide is collected for recycling, plastic pollution is expected to triple by 2060 in the absence of management intervention.[3] The exponential accumulation of microplastics in aquatic environments is a growing management concern. Both wet- and dry-weather runoff are responsible for funneling vast quantities of microplastics into the coastal ocean and other water bodies.[4] Microplastics also can evade wastewater treatment processes and get discharged into the coastal ocean and other water bodies.[5]

Although the health implications of microplastics exposure are poorly understood, both terrestrial and aquatic biological communities are being exposed on a continuous basis. Animals ranging from tiny ocean filter feeders to humans are inadvertently absorbing, breathing and consuming microplastics.[1] Furthermore, many animals cannot distinguish microplastics from food, creating the potential for satiation challenges.[6] Once microplastics enter food webs, they can bioaccumulate and ultimately end up in sportfish consumed by humans and wildlife.[7] Compounding the bioaccumulation challenge is that chemicals and pathogens can stick to microplastics, creating a potential exposure pathway for multiple types of contaminants.[8] Finally, emerging research shows that the smallest microplastics can penetrate cell membranes and translocate into tissue and organs; however, little is known about what health risks these microplastics may pose.[9]

A foundational challenge of assessing health risks from microplastics exposure is that many microplastics are difficult to measure and track in the environment. Although microplastics are typically defined as any plastic particle less than 5 millimeters in diameter, the vast majority of microplastics in the environment are so small that they can only be seen with the aid of a light microscope or even more powerful instrumentation.[10] These smaller microplastics can be difficult to distinguish - visually and/or sometimes spectroscopically - from non-plastic particles with similar physical and chemical characteristics, creating the potential for either under- or over-estimation.[11]

To comprehensively assess the health risks of microplastics exposure, scientists first need to define what constitutes a microplastic particle, so they can focus on developing methods to optimally measure this form of pollution. Although scientists have studied microplastics since the 1960s,[12] international consensus has not yet been reached on a definition.[13] Unlike most-water quality contaminants that are typically dissolved, microplastics are particles with defined solubility, size, shape and chemical composition criteria that are found in various

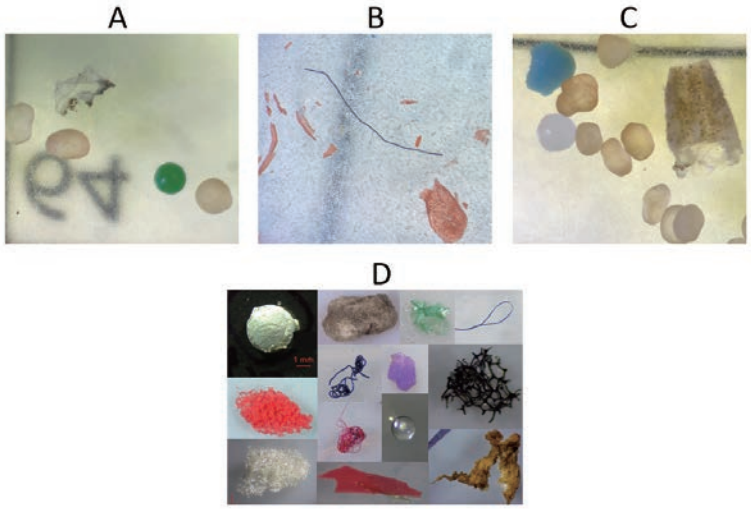


Figure 1 Microplastics are found in various shapes, sizes, colors, and polymer types in the environment. Plastic particles can be difficult to distinguish visually under a light microscope from natural particles (A-C), and may require confirmation of material type using more powerful instrumentation. (A) Microplastic spheres appear similar in shape and size to sand particles; in this case, they are differentiated by color. (B) A dark blue plastic fiber appears next to undigested pieces of fish tissue. (C) Although some microparticles are obviously plastic (blue fragments), other particles could be white sand or gelatin, and may require further spectroscopic identification. (D) Microplastic particles include spheres, fragments, fibers, foams, pellets, film, and fiber bundles. (Photos courtesy of Southern California Coastal Water Research Project Authority and C.M. Rochman, University of Toronto)

possible combinations in the environment.[14] In June 2020, the California State Water Resources Control Board (State Water Board) adopted an official definition of microplastics for its drinking water program: "Solid polymeric materials to which chemical additives or other substances may have been added, that have at least three dimensions that are greater than 1 nanometer and less than 5,000 micrometers." Polymers that are derived in nature that have not been chemically modified (other than by hydrolysis) are excluded under this definition.[15] The adopted definition considers the vast diversity of microplastics found in the environment, and is likely to serve as a foundation - or at least departure point - for additional agencies and organizations that must define microplastics.[16] Finally, the adopted definition may evolve over time with the science.

Scientists will use this agreed-upon definition of microplastics to build a foundational understanding of the occurrence and fate of these pollutants in the environment. By building comprehensive, high-quality data sets, California will gain critical, baseline knowledge of realistic exposure scenarios. However, assembling these data sets will be a challenge, as microplastics monitoring programs are still in their infancy. Even in drinking water systems - where dozens of chemical contaminants are monitored - microplastics are not one of the contaminants that are routinely tracked.[17] Furthermore, monitoring data are not necessarily comparable even among the monitoring programs that do exist, as different programs use different, competing microplastics monitoring and analysis methods. The broader scientific community has not yet vetted any of these experimental laboratory measurement methods or reached consensus on how to standardize them.

California as an international leader on trash management

California is emerging at the forefront of international efforts to vet and standardize microplastics measurement techniques. Not only is California evaluating the performance of the various methods used to identify and quantify microplastics, but the State is working to build capacity to begin monitoring microplastics in water, sediment and tissue. This foundational work will pave the way for scientists to begin reliably measuring and tracking microplastics levels and types in aquatic environments - and ultimately generate the high-quality, comprehensive data sets needed to inform human and ecological health risk assessments.

A long legacy of regulatory actions on trash pollution of all kinds has optimally positioned California to step into an international leadership role in developing capacity to monitor microplastics in aquatic environments. For decades, California has been taking forward-thinking, decisive regulatory actions to curb the entry and spread of trash in the environment, as well as to manage and mitigate the health risks of trash.[18] Much of this work has been borne out of necessity - a consequence of California's population density and the ecological and economic importance of the state's many natural resources. Initially, California's focus was on eliminating macro-sized trash generated by those who frequent beaches and other recreational water bodies. Beachgoers, boaters, anglers and businesses have been targeted with strict anti-littering laws, public education initiatives and outreach campaigns.

Then, in the mid-1990s, the Los Angeles Regional Water Quality Control Board led the state - and the nation - in dramatically rethinking how to curb trash entering waterways.[19] Instead of regulating trash loading one municipality at a time, the L.A. Regional Board placed multiple key waterways in the region on the federal 303(d) list of water bodies with known water-quality impairments. This action enabled the water-quality agency to issue a regulatory target for trash known as a total maximum daily load (TMDL); the TMDL compels the many municipalities and other entities that discharge runoff into these waterways to reduce trash loading. TMDLs for trash have subsequently been issued in other parts of California and beyond.

About 15 years later, seeking to build comparable regulatory infrastructure at a statewide level, the California State Water Resources Control Board amended the master plans that govern management of California's coastal ocean and freshwater systems to include trash as a water-quality impairment. Similar to the L.A.-area trash TMDLs, the State's "Trash Amendments" - which went into effect in 2016 - compel agencies that discharge runoff in areas with high trash-generating rates to either begin installing devices at storm drain inlets to capture all particles larger than 5 mm, or develop an alternate plan for capturing trash at equivalent rates.[20]

As it has become increasingly clear plastic pollution makes up the majority of aquatic trash, California also has targeted plastic pollution specifically. In 2014, California voters approved a statewide ban on carry-out plastic bags at grocery stores and pharmacies.[21] The law went into effect two years later, following an unsuccessful referendum to overturn the ban. In 2018, California passed a law requiring sit-down restaurants to only distribute single-use plastic straws to customers upon request;[22] it went into effect the following year. In enact-

ing these laws, California was not just concerned about entanglement issues as organisms come into contact with these macro-sized plastic particles; California also was cognizant that much of this plastic will break down over time to become microplastics.[23]

Finally, California has taken action to regulate the production of microplastics themselves. In 2008, California enacted strict regulations[24] on facilities that manufacture, handle and transport pre-production plastic pellets, which are particles a few millimeters in diameter that serve as the raw materials for plastic production; these particles can spill and become lost during transport. Subsequently, in 2015, California enacted a ban on the sale of personal care products that contain plastic microbeads.[25] Comparable federal microbeads legislation was passed just months later; California's microplastics bead ban took effect in January 2020.

Developing a comprehensive microplastics management strategy

Even as California has implemented numerous regulatory mechanisms to slow the introduction and spread of microplastics in aquatic environments, the State also is laying a scientific foundation to assess the health risks associated with exposure. In 2018, the California State Legislature passed a pair of bills that require the State to begin building microplastics management strategies for both drinking water and California's coastal ocean and estuaries:

- Senate Bill 1422 requires the California State Water Resources Control Board to develop plans for measuring microplastic particles in drinking water by 2021.[26]
- Senate Bill 1263 requires the California Ocean Protection Council to adopt and implement a statewide strategy for lessening the ecological risks of microplastics to coastal marine ecosystems, especially through research and policy changes.[27]

The State laws are notable for their prescriptiveness and specificity, even in environmentally progressive California. Both laws lay out priority actions, along with deadlines, and explicitly call on two State agencies to take responsibility for executing California's microplastics management priorities. Embedded in each legislative mandate is the need for improved scientific understanding of how microplastics exposure affects both humans and marine organisms, and how much microplastics exposure, if any, is too much.

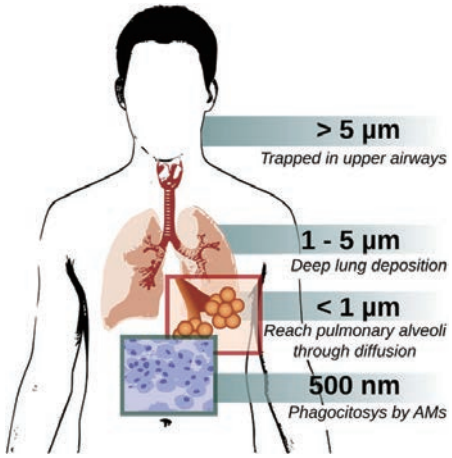
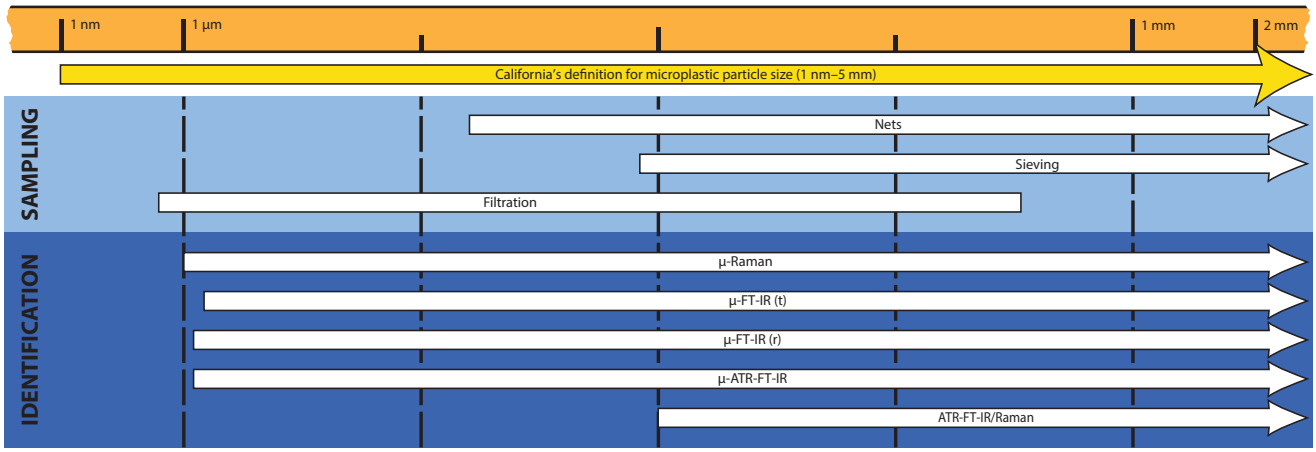


Figure 2 Like other airborne pollutants, microplastic particles can travel deep into the human body.[28] Scientists are just beginning to document the health effects associated with continuous microplastics exposure. (Figure from Costa et al. 2016[29], reprinted with permission)



LEGEND

µ-Raman	Raman microscopy
µ-FT-IR (t)	Fourier-transform infrared spectroscopy microscopy in transmission mode
µ-FT-IR (r)	Fourier-transform infrared spectroscopy microscopy in reflection mode
µ ATR-FT-IR	Micro attenuated total reflection Fourier transformation infrared spectroscopy
ATR-FT-IR/Raman	Attenuated total reflection Fourier-transform infrared spectroscopy

Figure 3 Various methods have been developed for sampling and identifying microplastic particles in the environment; they are designed to measure particles of different sizes.[30-34] California's adopted definition for microplastics encompasses all plastic particles that have at least three dimensions between 1 nm and 5 mm.

As a direct result of the 2018 laws, California has been propelled to the forefront of microplastics research. The pair of laws has made it clear that California intends to immediately adopt, use, and incorporate microplastics science into action and policy. Thus, the 2018 legislation has become a call to action for the international scientific community: Develop clear, actionable recommendations that provide a scientific foundation for California's microplastics management strategy. Meanwhile, scientists recognize that as California goes, so tends to go the rest of the nation. Microplastics measurement laboratories and water-quality managers across the U.S. may follow California's lead - adopting California's regulatory framework for managing microplastics in aquatic systems, and designing routine microplastics monitoring programs based on California's.

Already, California is at the center of an international, year-long study to compare and evaluate various methods and instruments for measuring microplastics levels in water, sediment and tissue matrices. The study's goal is to compare and standardize the many overlapping, experimental approaches that have been developed by microplastics researchers - and variations of these lab methods - to quantify and characterize microplastics levels. The study is being coordinated by the Southern California Coastal Water Research Project Authority on behalf of the State Water Resources Control Board and the California Ocean Protection Council.

More than 35 leading microplastics research labs worldwide have signed onto the study. Each participant will be sent blind samples containing known quantities of microplastics. They will use a variety of methods and instruments to quantify the microplastics in the samples, and compare performance of the various methods and instrumentation; the end goal is to develop recommendations about which methods and which variations of methods produce the most reliable, repeatable, accurate results. HORIBA is among the study's partners, helping to lead training for study participants on the use of Raman spectroscopy, a leading candidate instrument for quantifying microplastic particles so small they can't be distinguished from non-plastics under a light microscope.

California's microplastics measurement methods study is expected to be immediately consequential, resulting in a dramatic consolidation of the nascent microplastics measurement field. The study also will provide clarity to state and federal agencies around the world about how to generate comparable, high-quality data. Finally, the standardized measurement methods are expected to be codified into laboratory accreditation standards. California's Environmental Laboratory Accreditation Program (ELAP), which is charged with overseeing the quality of all environmental data used for decision-making, will create a laboratory inspection process that includes development of performance evaluation samples. Laboratories that collect microplastics data for California will be required to participate in this process.

Ultimately, the foundational R&D work scoped out in the 2018 legislation will help California build capacity to monitor and ascertain the health risks from microplastics exposure. By making it possible for managers to reliably measure microplastics in water, sediment and tissue, and know that data are of high quality and comparable, California stands poised to develop a comprehensive, science-informed strategy for effectively managing microplastics in both drinking water and diverse aquatic ecosystems.

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

References

[1] Hale, Robert C., Meredith E. Seeley, Mark J. La Guardia, Lei Mai, and Eddy Y. Zeng. "A Global Perspective on Microplastics." *Journal of Geophysical Research: Oceans* **125** (1). (2020). https://doi.org/10.1029/2018JC014719

[2] Neufeld, Len, Fabienne Stassen, Ruth Sheppard, and Terry Gilman. "The New Plastics Economy: Rethinking the Future of Plastics." World Economic Forum. Geneva, Switzerland. (2016). http://www3.weforum.org/docs/WEF_The_New_Plastics_Economy.pdf

[3] Lebreton, Laurent, and Anthony Andrady. "Future Scenarios of Global Plastic Waste Generation and Disposal." *Palgrave Communications* **5** (1): 6. (2019). https://doi.org/10.1057/s41599-018-0212-7

[4] Lebreton, Laurent C. M., Joost van der Zwet, Jan-Willem Damsteeg, Boyan Slat, Anthony Andrady, and Julia Reisser. "River Plastic Emissions to the World's Oceans." *Nature Communications* **8** (1): 15611. (2017). https://doi.org/10.1038/ncomms15611.

[5] Murphy, Fionn, Ciaran Ewins, Frederic Carbonnier, and Brian Quinn. "Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment." *Environmental Science & Technology* **50** (11): 5800-5808. (2016). https://doi.org/10.1021/acs.est.5b05416

[6] Ryan, Peter G. "Ingestion of Plastics by Marine Organisms." In Hazardous Chemicals Associated with Plastics in the Marine Environment, edited by Hideshige Takada and Hrissi K. Karapanagioti, 78:235-66. Cham: Springer International Publishing. (2016). https://doi.org/10.1007/698_2016_21

[7] Akoueson, Fleurine, Lisa M. Sheldon, Evangelos Danopoulos, Steve Morris, Jessica Hotten, Emma Chapman, Jiana Li, and Jeanette M. Rotchell. "A Preliminary Analysis of Microplastics in Edible versus Non-Edible Tissues from Seafood Samples." *Environmental Pollution*, March, 114452. (2020). https://doi.org/10.1016/j.envpol.2020.114452

[8] Coffin, Scott, Guo-Yong Huang, Ilkeun Lee, and Daniel Schlenk. "Fish and Seabird Gut Conditions Enhance Desorption of Estrogenic Chemicals from Commonly-Ingested Plastic Items." *Environmental Science & Technology* **53** (8): 4588-99.(2019). https://doi.org/10.1021/acs.est.8b07140

[9] Roch, S., C. Friedrich, and A. Brinker. "Uptake Routes of Microplastics in Fishes: Practical and Theoretical Approaches to Test Existing Theories." *Scientific Reports* **10** (1): 3896. (2020). https://doi.org/10.1038/s41598-020-60630-1

[10] Enders, Kristina, Robin Lenz, Colin A. Stedmon, and Torkel G. Nielsen. "Abundance, Size and Polymer Composition of Marine Microplastics ≥ 10 Mm in the Atlantic Ocean and Their Modelled Vertical Distribution." *Marine Pollution Bulletin* **100** (1): 70-81. (2015). https://doi.org/10.1016/j.marpolbul.2015.09.027

[11] Song, Young Kyoung, Sang Hee Hong, Mi Jang, Gi Myung Han, Manviri Rani, Jongmyoung Lee, and Won Joon Shim. "A Comparison of Microscopic and Spectroscopic Identification Methods for Analysis of Microplastics in Environmental Samples." *Marine Pollution Bulletin* **93** (1): 202-9. (2015). https://doi.org/10.1016/j.marpolbul.2015.01.015

[12] Kenyon, Karl W., and Eugene Kridler. "Laysan Albatrosses Swallow Indigestible Matter." *The Auk* **86** (2): 339-43. (1969). https://doi.org/10.2307/4083505

[13] Hartmann, Nanna B., Thorsten Huffer, Richard C. Thompson, Martin Hassellöv, Anja Verschoor, Anders E. Daugaard, Sinja Rist, et al. "Are We Speaking the Same Language? Recommendations for a Definition and Categorization Framework for Plastic Debris." *Environmental Science & Technology* **53** (3): 1039-47. (2019). https://doi.org/10.1021/acs.est.8b05297

[14] Rochman, Chelsea M., Cole Brookson, Jacqueline Bikker, Natasha Djuric, Arielle Earn, Kennedy Bucci, Samantha Athey, et al. "Rethinking Microplastics as a Diverse Contaminant Suite." *Environmental Toxicology and Chemistry* **38** (4): 703-11. (2019). https://doi.org/10.1002/etc.4371

[15] https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/microplastics.html

[16] Coffin, Scott. 2020. "Staff Report for the Proposed Definition of Microplastics in Drinking Water (June 3, 2020)." Staff Report. Sacramento, CA: State Water Resources Control Board. https://www.waterboards.ca.gov/drinking_water/certlic/drinkingwater/docs/stffrprt_jun3.pdf

[17] US Environmental Protection Agency. "National Primary Drinking Water Regulations; Final Rule. 40 CFR Parts 141, 142, and 143." *Federal Register*, 3526-97. (1991).

[18] https://www.waterboards.ca.gov/water_issues/programs/trash_control/docs/01_final_sed.pdf

[19] http://ftp.sccwrp.org/pub/download/DOCUMENTS/AnnualReports/2016AnnualReport/2016AnnualReport.pdf

[20] https://www.waterboards.ca.gov/water_issues/programs/trash_control/docs/01_final_sed.pdf

[21] https://leginfo.legislature.ca.gov/faces/billNavClient.xhtml?bill_id=201320140SB270

[22] https://leginfo.legislature.ca.gov/faces/billTextClient.xhtml?bill_id=201720180AB1884

[23] https://www.nationalgeographic.com/news/2018/05/plastics-explained/

[24] https://leginfo.legislature.ca.gov/faces/billNavClient.xhtml?bill_id=200720080AB258

[25] https://leginfo.legislature.ca.gov/faces/billNavClient.xhtml?bill_id=201520160AB888

[26] https://leginfo.legislature.ca.gov/faces/billTextClient.xhtml?bill_id=201720180SB1422

[27] https://leginfo.legislature.ca.gov/faces/billNavClient.xhtml?bill_id=201520160SB1263

[28] Lusher, Amy, Peter C. H. Hollman, and Jeremy Mendoza-Hill. Microplastics in Fisheries and Aquaculture: Status of Knowledge on Their Occurrence and Implications for Aquatic Organisms and Food Safety. FAO Fisheries and Aquaculture Technical Paper 615. Rome: Food and Agriculture Organization of the United Nations. (2017).

[29] Costa, Ana, Marina Pinheiro, Joana Magalhães, Ricardo Ribeiro, Vitor Seabra, Salette Reis, and Bruno Sarmento. 2016. "The formulation of nanomedicines for treating tuberculosis." *Adv Drug Deliv Rev* **102**:102-115. https://doi.org/10.1016/j.addr.2016.04.012.

[30] Schwaferts, Christian, Reinhard Niessner, Martin Elsner, and Natalia P. Ivleva. "Methods for the Analysis of Submicrometer- and Nanoplastic Particles in the Environment." *TrAC Trends in Analytical Chemistry* **112** (March): 52-65. (2019). https://doi.org/10.1016/j.trac.2018.12.014

[31] Primpke, Sebastian, Silke H. Christiansen, Win Cowger, Hannah De Frond, Ashok Deshpande, Marten Fischer, Erika Holland, et al. "EXPRESS: Critical Assessment of Analytical Methods for the Harmonized and Cost Efficient Analysis of Microplastics." *Applied Spectroscopy*, April, 000370282092146. (2020). https://doi.org/10.1177/0003702820921465

[32] Cabernard, Livia, Lisa Roscher, Claudia Lorenz, Gunnar Gerdts, and Sebastian Primpke. "Comparison of Raman and Fourier Transform Infrared Spectroscopy for the Quantification of Microplastics in the Aquatic Environment." *Environmental Science & Technology* **52** (22): 13279-88. (2018). https://doi.org/10.1021/acs.est.8b03438

[33] https://www.agilent.com/cs/library/applications/5991-8271EN_microplastics_ftir_application.pdf

[34] https://assets.thermofisher.com/TFS-Assets/MSD/Application-Notes/WP53077-microplastics-identification-ftir-raman-guide.pdf

Context Microscopy and Fingerprinting Spectroscopy of Micro- and Nanoplastics and Their Effects on Human Kidney Cells Using nanoGPS and ParticleFinder

マイクロプラスチックの光学顕微鏡による形態観察と分光法による化学同定ならびにnanoGPSとParticleFinderを用いたマイクロプラスチックのヒト腎臓細胞への影響評価

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Nowadays humans are almost continuously exposed to micro- and nanoplastics (MNPs) through food and air, but very little is known about the exposure level and impact on our health. Here, we focus on bottled mineral water and cultured human podocytes as representative kidney cells prone to accumulation of particles. It is demonstrated that identical MNPs and cells can be precisely relocalized and extensively characterized down to nanoscale in independent instruments using nanoGPS and ParticleFinder technologies developed by HORIBA. Reference particles and particles contained in mineral water were detected, enabling statistical distributions of their mean number, size, and type depending on the bottle and

label materials. The primary effects of MNPs (three standards and tyre wear) on human podocytes were assessed using a cell viability test followed by correlative microscopy and spectroscopy investigations of the same cells. We observed changes in the biological features of MNP treated cells compared to non-treated controls, attributed to cell damage through surface adhesion and uptake of plastic particles. The integration of automatic relocalization and detection of identical objects in a multi-instrument workflow represents a novel analytical approach that can be applied beyond this topic.

Key words
microplastic, nanoplastic, tyre wear, podocytes, kidney, nanoGPS, ParticleFinder, SEM, Raman, correlative workflow, microscopy, spectroscopy

現在，我々は食品や大気を通して，マイクロ～ナノサイズという非常に小さい粒子(MNPs)に日常的に曝されている。しかしながらどの程度曝されているのか，またそれが我々の健康にどの様な影響を及ぼしているかは，ほとんど知られていない。我々は，HORIBAが開発したnanoGPSとParticle Finderの2つの技術を使い，測定対象に対し，正確に位置合わせを行うことで，独立した機器で同一のMNPsをナノスケールまでかつ広範囲にわたり解析を実現した。本項では，容器入り天然水と，粒子の蓄積が起こりやすい代表的な腎臓細胞として培養ヒト有足細胞の2つについて解析を行った結果を示す。まず，リファレンス粒子及び天然水ボトルにふくまれるMNPsを検出し，ボトルやラベルの材質に依存した平均粒子数やサイズ，種類に関する統計解析を行い，その性能を実証した。次いで，ヒト由来ポドサイトに対するMNPs（3種の標準粒子と摩耗タイヤから得た粒子使用）の主な影響は細胞生存率試験で評価され，その同じ細胞を相関顕微鏡法と分光法で解析した（顕微画像とスペクトル測定の同期技術により，対象細胞に関する複数の情報を同一視野角で取得している）。MNPsと共に調製された細胞と無調製の細胞を比較し，MNPsの付着や取り込みによる細胞へのダメージに帰属できる状態の変化を観察し，MNPsの生物学的特徴を評価した。複数の機器を用いた測定により，同一の測定対象の位置合わせだけでなく，自動化できる本技術は，本事例にとどまらず，広く活用が期待される新規分析手法である。

キーワード
マイクロプラスチック，ナノプラスチック，タイヤ摩耗，ポッドサイト，腎臓，nanoGPS，パーティクルファインダー，SEM，ラマン，相関ワークフロー，顕微鏡，分光法

Introduction

Production of plastics has dramatically increased over the last decades and with it the plastic waste in the environment.[1] Plastics are nowadays used almost in all products including packaging, construction, textiles, tires, cosmetics, and so on.[2-4] The major issue is the mismanaged plastic waste that is not collected at all or improperly filtered and recycled, which significantly contaminates the environment on a global scale through the transfer between terrestrial, river, and ocean compartments.[5] Once left in the environment, plastic debris persists and degrades continuously into smaller fragments down to micro- and nanoplastic (MNP) particles, attributed to size classes of < 5 mm and < 1 µm or ≤ 100 nm, respectively.[6,7] With time, these MNPs are assumed to develop into toxic chemical cocktails by increased adsorption of hazardous pollutants and pathogens from the environment given their larger surface areas due to fragmentation, in addition to additives and pigments added during manufacturing of plastics. Moreover, the smaller the plastic particles become (< 1.5 µm), the higher the probability to enter by ingestion and inhalation into human organs and subsequently to accumulate and leach chemicals with still unknown toxicological effects on our health.[8-10]

Microscopy- and spectroscopy-based methods are commonly used to monitor MNPs in environmental samples usually after filtering as well as in various biological matrices and organisms. The employed techniques mainly include optical microscopy with stereozoom, scanning electron microscopy (SEM) with energy dispersive X-ray spectroscopy (EDS), pyrolysis gas chromatography coupled with mass spectrometry (py-GC-MS), Fourier-transform infrared (FT-IR) and Raman microspectroscopies, each method with its benefits and drawbacks.[11-13] Recently, we showed that a correlative approach is needed to avoid overestimation of particles' size and underestimation of particles' number for clustered MNPs as well as to measure Raman without optically visualizing the plastic nanoparticles by overlapping SEM and optical images of high (< 10 nm) and low (~ 1 µm) spatial resolution, respectively. This was achieved by a correlative microscopy and spectroscopy workflow applied to identical MNP particles on large-area filters using an optical zoom microscope and a hyphenated

SEM-Raman instrument (with a bright field optical objective for micro-Raman inside the SEM vacuum chamber).[14] However, such combined systems are limited with respect to the number of measurement techniques available on one instrument compared to stand-alone, method-specific instruments from different manufacturers, in which finding the same micro- and nanosized objects is still a challenge.[15-19]

In this work, the first application of a newly developed relocalization technology for a detailed characterization of MNPs and their effects on human kidney cells in independent instruments is demonstrated. This technology is based on a patented position encoder tag (from HORIBA), called nanoGPS tag, with lithographically defined patterns. These patterns are used to translate the sample coordinates corresponding to the regions of interest (ROIs) into the stage coordinates of different instruments (from HORIBA, Zeiss, Leica in this study), regardless of the sample orientation. Furthermore, the applicability of the ParticleFinder software module (from HORIBA) for automatic detection of microplastic (MP), pigment, and additive particles on large-area filters is shown. Context microscopy and fingerprinting spectroscopy approaches were applied to standard MPs, microparticle contamination of bottled mineral water, and human podocytes that were either untreated or incubated with MNPs. The podocytes exposed to MNPs were under stress and started to die gradually, indicating an overall effect of particle exposure on cell viability.

Experimental

The samples investigated in this study can be divided into three categories: reference micro-sized plastic particles, mineral water from different bottle types bought in Bavarian food stores, and human podocytes cell cultures exposed to MNPs.

Standard MP particles

Commercially available standard plastics (see Table 1) were selected to match the polymer types routinely encountered in the environment.[13,20,21] A mixture of polyethylene (PE), poly(vinyl chloride) (PVC), polyamide-Nylon 6 (PA), polystyrene (PS), and polypropylene (PP) particles were suspended in a solution (ultrapure water and sodium dodecyl sulphate (SDS)) followed by vacuum filtration through polycarbonate (PC) membrane filters (diameter 25 mm, pore size 0.4 µm) previously coated with aluminum (Al thickness 100 nm) as detailed in our previous work.[22] These reference materials were used to evaluate the nanoGPS relocalization technology (hardware and software) and its integration in a correlative microscopy and spectroscopy workflow applied to identical MNP particles (see Figure 1). The nanoGPS tag (4×5 mm^2 silicon piece) is firmly attached next to the filter, which is rigidly stretched and flattened between two metal rings fixed on a SEM holder, to avoid any thermal drift and ensure precise relocalization in different instruments. Along with the corresponding NaviGo software, the instruments' stages involved in the workflow are calibrated and the coordinates of ROIs are recorded.

Mineral water particles

Real mineral water samples packaged in reusable bottles made of poly(ethylene terephthalate) (PET), in single use PET bottles, and in glass bottles (single and reusable) were analyzed for microparticle contamination, taking also into account bottle age as well as label and cap type. Before suspension in SDS solution and vacuum filtration through Al coated PC membranes, calcium and magnesium carbonate particles were dissolved with ethylene diamine tetraacetic acid tetrasodium salt (EDTA) to reduce the number of non-plastic particles.[21] To obtain statistically relevant data given the complexity of bottled mineral water contamination including microplastic, pigment, additive, and mixed particles, we employed an automatic particle detection approach. This is based on the ParticleFinder software that transforms large-area (1 mm^2) dark field optical images obtained by stitching into grey scale images, on which particles are easily detectable using their brightness, counted, classified by size and shape, and their coordinates recorded for further micro-Raman chemical identification. Thus, the mean

Table 1 Details of the plastic particle standards used in the present study to assess the nanoGPS relocalization and the exposure of human podocytes to plastics (PVC, PA, PP). Adapted with permission from Springer Nature.[22]

Material	Type	Manufacturer	Size (µm)
Polyethylene (PE)	Clear microspheres, powder	Cospheric	1-10
			10-106
Poly (vinyl chloride) (PVC)	Powder	Pyropowders.de	< 50
Polyamide - Nylon 6 (PA)	Powder	GoodFellow	15-20 (average particle size)
Polystyrene (PS)	Polybead Micron Microspheres, 2.5% solids in water	Polysciences Inc.	1
Polypropylene (PP)	Chromatographic Grade, powder	Polysciences Inc.	25-85

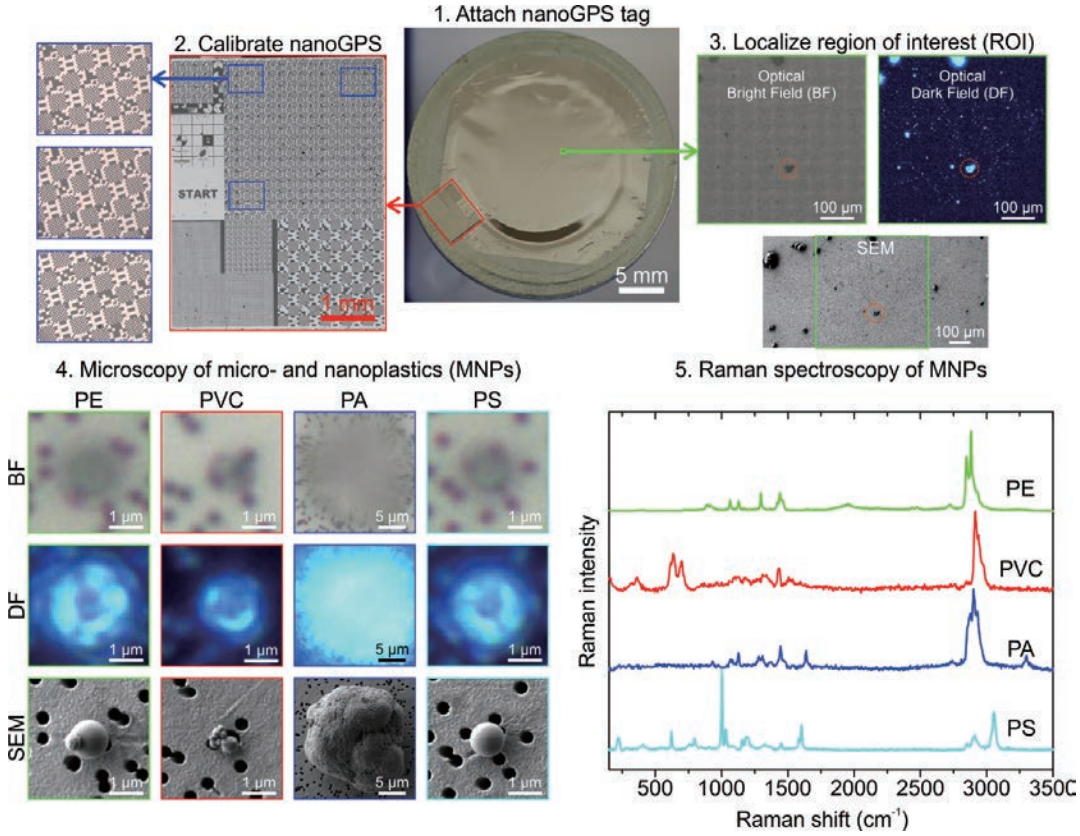

Figure 1 Correlative microscopy and spectroscopy workflow for micro- and nanoplastics on an Al coated PC membrane used to filter MNPs from water. First, a so-called nanoGPS tag is attached directly to the sample. Second, three images are recorded at random positions on a pattern (different patterns correspond to various instrument magnifications) and fed into a software that calibrates the global, stage coordinates into local, tag (sample) coordinates including sample rotation. This procedure is repeated for each instrument to be used in the workflow. Third, identical ROIs are precisely relocalized in independent instruments, regardless of the sample orientation. Fourth, the same single or agglomerated particles are imaged at optical (BF, DF) and SEM spatial resolutions to assess size, shape, number, and surface morphology of MNPs down to nanoscale. DF imaging is used to clearly distinguish MNPs from the porous structure of large-area filters. Fifth, unambiguously chemical identification by micro-Raman spectroscopy is applied. The Raman spectra are taken with permission from the Society for Applied Spectroscopy.[14]

number of microplastic, pigmented, and additive particles (projected to 1 L sample volume), their size, and type distributions were estimated (see Figure 2, additives not included).[21,22]

Human podocytes exposure to MNPs

Conditionally immortalized human podocytes that contain a heat sensitive CV40T antigen were cultured as described previously.[23] Podocytes were proliferated under growth permissive conditions at 33°C and further differentiated through the inactivation of SV40 T-antigen at 37°C. After 7 days of differentiation, cells were treated with different concentrations of diluted standard plastic (PVC: 0.5, 1 mg/ml; PA: 0.5, 1 mg/ml; PP: 2.5, 5 mg/ml) and tyre wear (0.125, 0.5 mg/ml) particles for 7 h to evaluate their possible effects on the cells. In order to decrease the aggregation of particles, they were sonicated before the incubation. Following the particle treatment, cells were washed two to three times with phosphate buffered saline (PBS) and fixed for further biological, imaging, and spectroscopy assays. For this study, the podocytes were grown on the surface of silicon wafers previously coated with platinum (Pt thickness 100 nm) that were attached along with nanoGPS tags to SEM holders to avoid relative sample - tag position shifts when moving between instruments.

Analytical methods

Complementary analytical techniques present on different instruments were used to visualize and detect MNPs on filters and inside cells as well as to determine the changes in cells caused by the contact with MNPs. All measurements have been performed at room temperature. The latter point was first addressed by using a live-dead cell imaging kit based on two-color fluorescence cell viability assay (Thermo Fischer Scientific). Based on this assay, cell-permeable and cell-impermeable dyes were used for staining of live and dead/dying cells, respectively. Following the particle treatment, the live/dead cells were assigned based on the kit instruction. Fluorescent images were collected with the use of an Evos M5000 imaging microscope (Thermo Fischer Scientific) (see Figure 3).

Furthermore, we employed a confocal micro-Raman spectrometer (HORIBA LabRAM HR Evo-Nano or XploRa PLUS), operated by the LabSpec 6 software (with

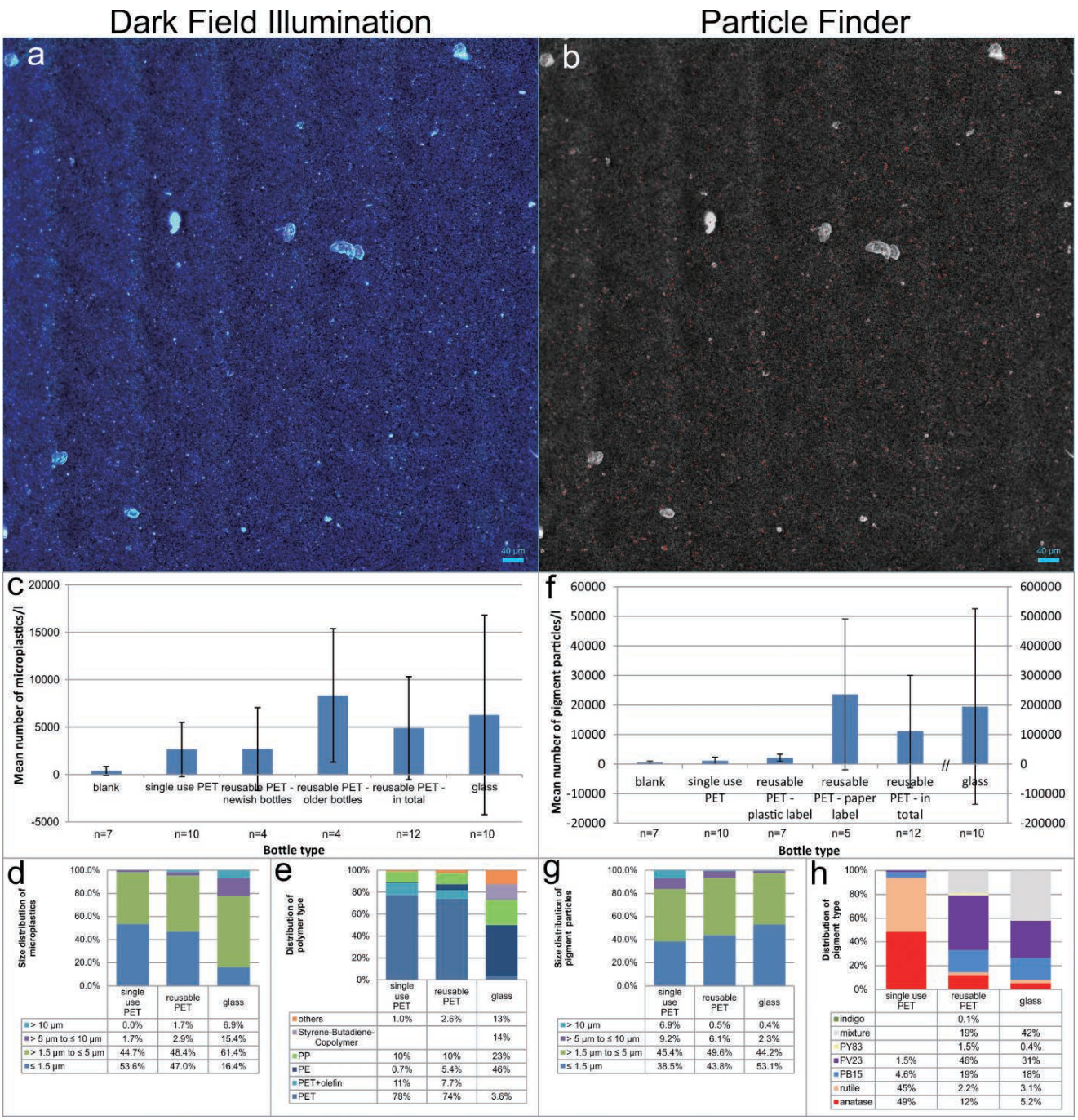

Figure 2 (a) Example of a dark field montage (1 mm^2) obtained by stitching, on which particles from mineral water samples shine brighter than the pores of the Al coated PC membrane filter. (b) ParticleFinder software converts the DF image into a grey scale image used to automatically detect, classify, and measure Raman spectra of individual particles at their center, marked by red points. (c, d, e) Mean number of microplastics ± standard deviation projected to 1 L sample volume, size, and plastic type distributions function of the bottle material. (f, g, h) Mean number of pigments ± standard deviation projected to 1 L sample volume, size, and pigment type distributions function of the bottle material. Adapted with permission from Elsevier.[21]

data analysis and ParticleFinder), equipped with bright and dark field illumination (BF, DF) objectives coupled to a camera to image MNPs and cells (~ 1 µm spatial resolution). Three lasers (532, 633, and 785 nm) focused by 50× (NA 0.75) or 100× (NA 0.9) objectives were used for Raman excitation and collection in a backscattering geometry with laser powers of 1.2 mW or 3.2 mW (532 nm), 11.2 mW (633 nm), and 5.3 mW (785 nm). Two gratings (300 and 600 grooves/mm) and integration times of 1 - 20 s and 2x accumulations were applied. The acquired Raman spectra and maps (step size 1 µm) were analyzed to chemically identify the particles and the structural damage induced by them on the human podocytes. A SEM (Zeiss field emission Auriga, secondary electron detector) was used for a detailed morphological imaging of MNPs and cells (< 10 nm spatial resolution) at a low voltage of 1 kV to avoid modifications caused by electron scanning. The height profiles of the same cells investigated by micro-Raman and SEM were measured by a confocal imaging microscope (Leica DCM 3D), the relocalization of identical cells being realized using the nanoGPS technology (see Figure 4). Moreover, because

of the superposition of Raman bands related to the plastic materials and cells, we applied a classical least squares algorithm (CLS) available in LabSpec 6 to highlight the spatial distribution of MNPs on the mapped cells (see Figure 5).

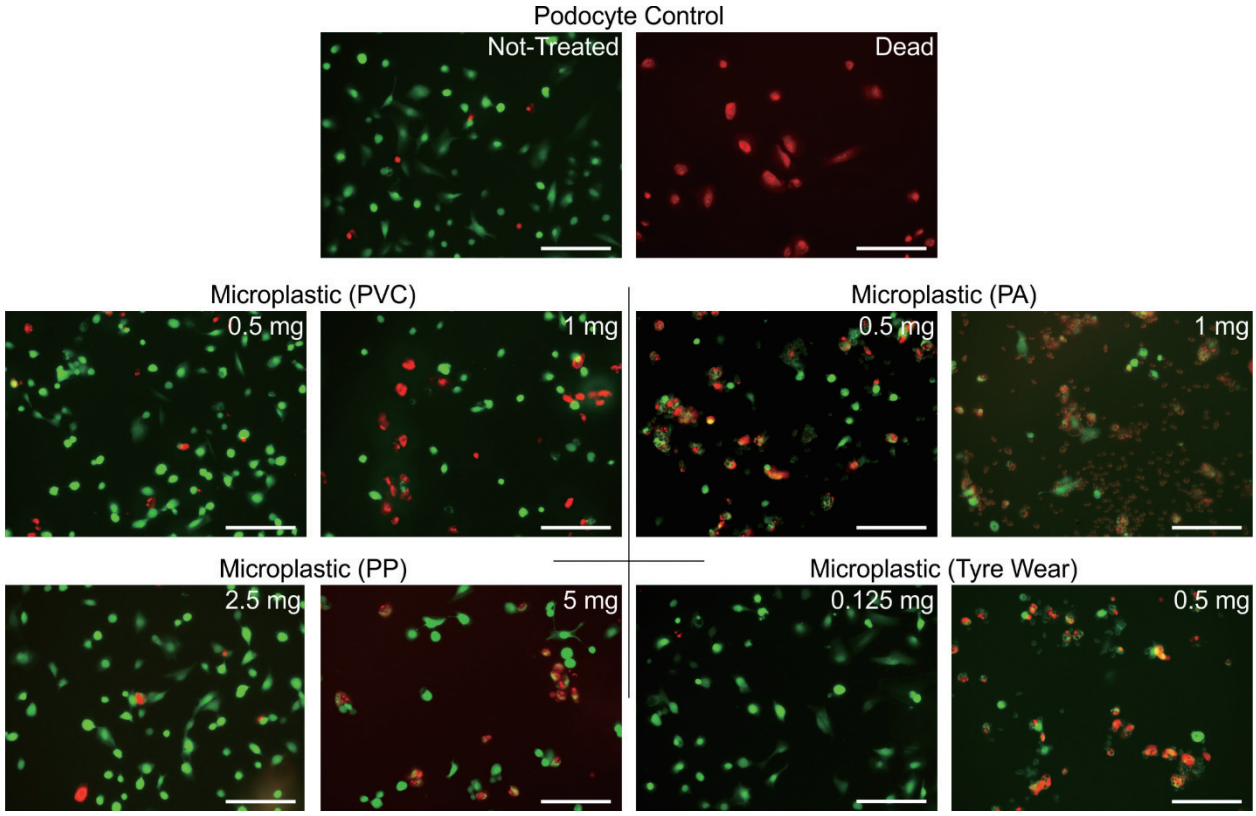

Figure 3 Fluorescence live - dead cell imaging (green - red) to assay the cytotoxicity of microplastic and tyre wear particles on podocytes, following 7 h particle exposure at relevant concentrations (mg/ml) and washing with PBS. The control cells were non-treated or intentionally killed to check the live - dead cell imaging kit. The concentrations to initiate and induce a notable impact on podocytes depends on the polymer type. During particle incubation the cells are under stress and start to die gradually. Consequently, some of the degraded cells are washed away and not assigned with colors. Some attached particles with intrinsic fluorescence are also visible. The preliminary results of this assay are yet mostly qualitative and show an overall effect of particle treatment on the cell viability. Scale bars are 300 µm.

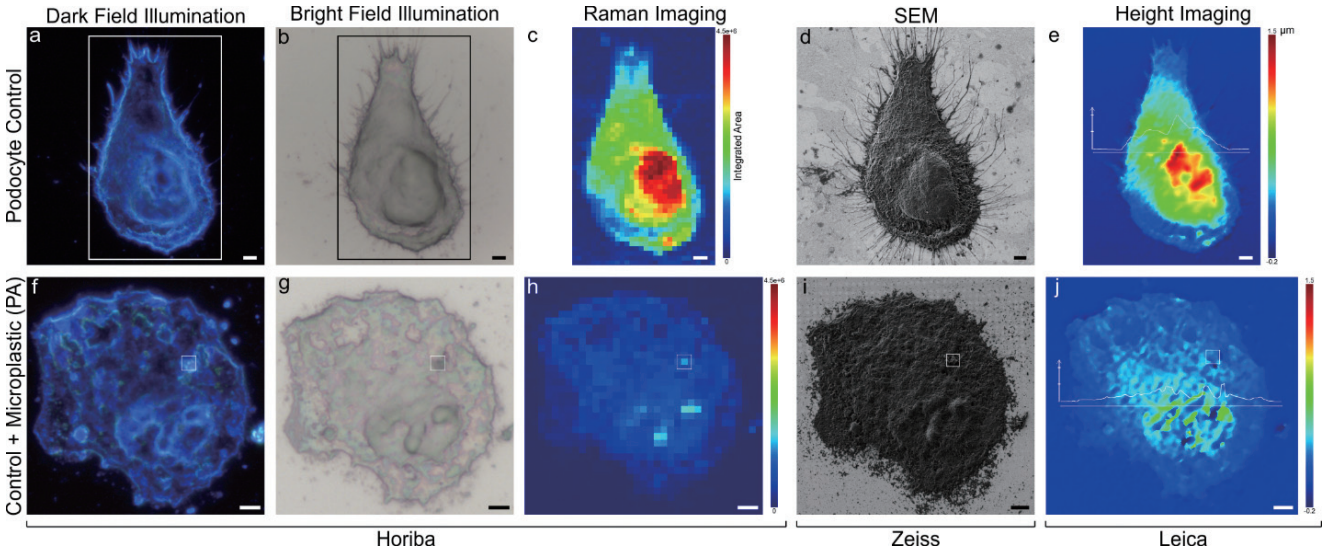

Figure 4 Correlative microscopy and spectroscopy workflow applied to podocytes untreated, control (first row) and particle treated (second row) with 1 mg/ml PA (Table 1 and Figure 3) using the nanoGPS position encoder tag (Figure 1). Two representative cells were easily relocalized and investigated in three independent instruments from different manufactures (Horiba, Zeiss, Leica) with complementary analytical techniques. First, an integrated optical microscope with dark (a, f) and bright (b, g) field illumination and micro-Raman spectrometer are used for a fast visual inspection of cells, followed by Raman imaging (c, h), showing less Raman signal for treated cells (note the same scale) that is an indication of podocytes damage after exposure to PA. Second, SEM imaging (d, i) reveals detailed surface morphology changes at nanoscale induced by the PA treatment and visualizes a PA nanoparticle (~ 30 nm), as confirmed by micro-Raman spectroscopy, delimitated by the square in the second row. Third, an interferometric profilometer is employed to measure the height profile without (e) and with (j) plastic contamination (note the same scale), PA incubated cells being flatter. Two horizontal profiles are also shown (maximum heights of ~ 1.5 µm and ~ 0.8 µm for the control and treated cell, respectively). Scale bars are 3 µm.

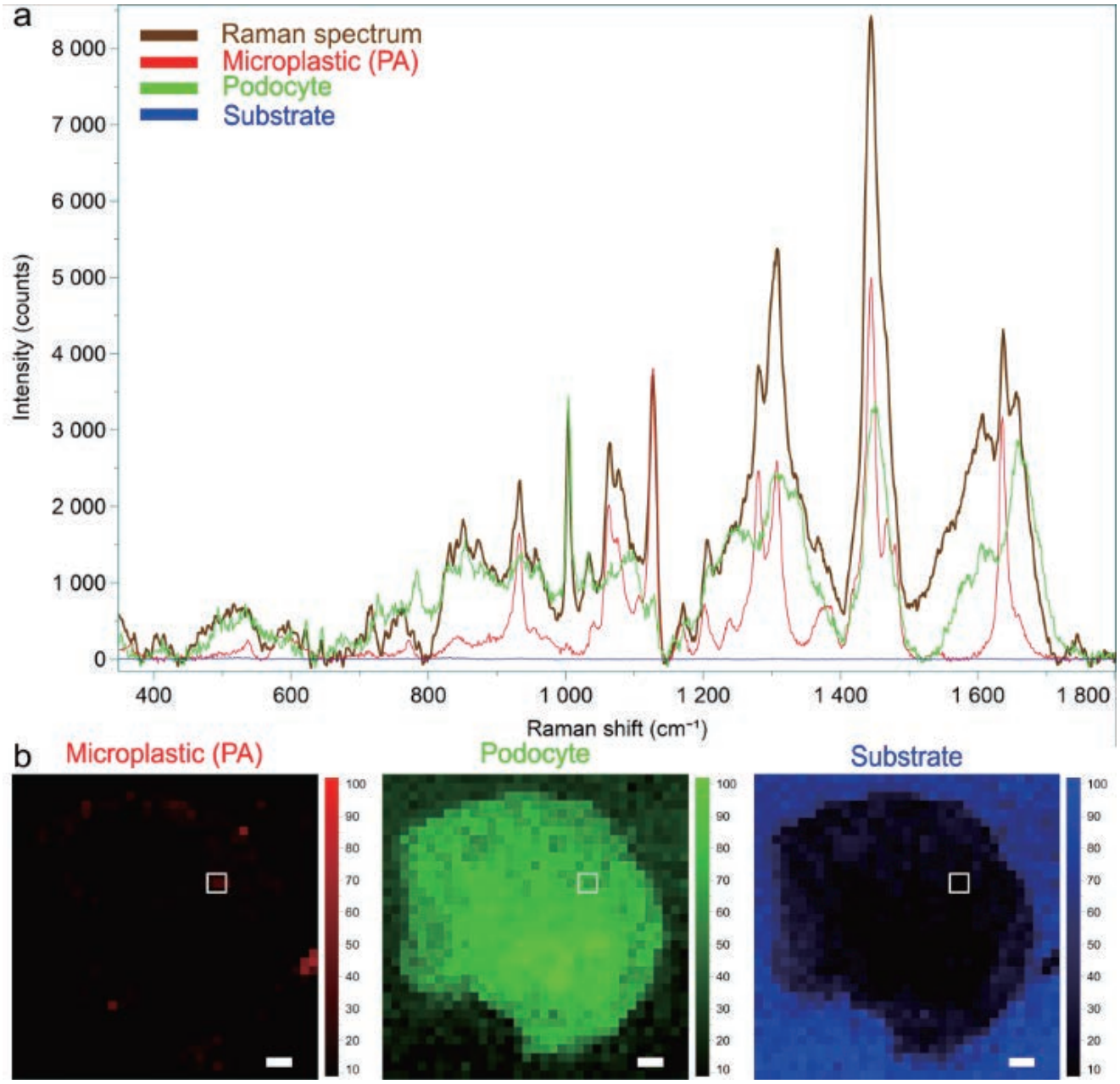

Figure 5 (a) Classical least squares (CLS) fitting is applied to decompose each measured Raman spectrum into its spectral components based on given reference spectra. (b) Separate score maps are generated for each component as illustrated for the podocyte cell treated with PA shown in Figure 4 (second row). The square indicates the position of a PA particle. Thus, despite the superimposed and complex Raman bands of cells and MNP particles, the spatial distribution of MNPs can be clearly localized. Scale bars are 3 µm.

Results and Discussion

nanoGPS relocalization

The nanoGPS relocalization technology for correlative microscopy and spectroscopy investigations is illustrated in Figure 1 for standard micro-sized plastic particles (Table 1), with some particles being by chance < 1 µm. First, a nanoGPS tag is rigidly mounted next to the Al coated PC membrane filter, both on a SEM holder that is moved between instruments, such that the tag and sample keep their positions relative to each other. The smaller the distance between tag and sample, the better the relocalization accuracy that can be further influenced by stage and imaging characteristics. Second, the multiscale and multimodal patterns on the tag are employed to calibrate the stage of each instrument, different feature sizes being used for distinct instrument magnifications (see SEM image of the entire tag). Three images are taken at random positions on a chosen pattern and fed along with the global, stage coordinates into the NaviGo software. In this example, images were recorded with the 10× objective of the optical microscope on the micro-Raman spectrometer. The software automatically determines the local, sample coordinates and rotation with respect to the tag. This calibration procedure is repeated for all instruments in the workflow and can be recalled anytime by recording one single image on the same pattern, independent of stage and sample rotation.

In the third step, one or more ROIs are located on the filter and their sample coordinates are saved in one instrument and retrieved in other instruments by converting sample, local into stage, global coordinates. In our case, large-area optical images acquired by stitching under BF and DF illumination are compared to a large field of view SEM image, with the same particle marked on all overview pictures. Next, MNPs can be directly relocalized and imaged at spatial resolutions of optical and electron microscopies (step four) and their spectral fingerprints determined by micro-Raman spectroscopy (step five) (PP is not shown). While on the BF and DF optical images these particles appear to be single, SEM imaging reveals that PE and PVC are cluster particles. When approaching the filter pore size, particles are barely visible in BF, but clearly noticeable in DF because they shine brighter than the pores, as seen for PVC. Moreover, SEM shows smooth surfaces with spherical and fragment-like shapes for the studied polymer particles. It should be noted that BF, DF, and Raman are usually performed before SEM; however, low-voltage SEM does not damage MNPs, so that Raman after SEM is also possible.[14] All in all, nanoGPS tagging enables sample navigation and observation at different length scales in independent instruments, thus detailed morphological (size, shape, surface, number) and chemical characterization of the same micro- and nanoparticles is achievable.

ParticleFinder

The ParticleFinder software module combined with DF optical microscopy and micro-Raman spectroscopy represents another example of correlative analysis applied here to study contamination by microplastic, pigment, and additive particles in bottled mineral water. 32 samples from 21 different brands of mineral water were investigated to determine the number, size, and type of particles, the results being summarized in Figure 2.[21] DF imaging is used to scan five large-area image montages (1 mm^2) on each sample to warrant significant particle statistics. Such a montage generated by stitching (Figure 2a) is then converted into a grey scale image, on which all particles ≥ 1 µm are automatically detected and individually measured by micro-Raman (Figure 2b).

We identified varying amounts of microplastics in water from all bottle types, partly resulting in large error bars when calculating the mean particle number (Figure 2c); however, some trends are clearly visible. On average, higher number of microplastics were found in water from reusable (PET and glass) compared to single use PET bottles. Interestingly, newish, reusable PET showed less microplastics than older, reusable PET, but similar to single use PET, suggesting that the bottle age can critically affect MP contamination. Regarding the average size distribution, 90.5% of MPs were ≤ 5 µm in all bottles and ~ 50% were ≤ 1.5 µm in PET bottles (Figure 2d), these MP size classes being addressed for the first time in such samples.[21,24] The predominant polymer type detected in PET bottles was PET considered to originate from the bottle material, while some PET particles displayed olefinic or pigment spectral interferences. In glass bottles, we mainly found PE and PS attributed to abrasion of caps on the glass bottleneck as well as PS, styrene-butadiene-copolymer, and PET most likely from the machinery used for the cleaning process (Figure 2e).

In addition to microplastics, pigmented and additive particles were also detected in the analyzed mineral water samples. Large variations in the number of pigmented particles in water from different bottle and label types were observed (Figure 2f). On average, single use PET contained less pigments similar to blank samples, while reusable PET and glass bottles with printed paper labels showed higher amounts of pigments. Alike MPs, older, reusable PET displayed more pigments than newish, reusable PET and most of the pigmented particles belonged to size classes investigated for the first time, 91.5% were ≤ 5 µm and 45.1% were ≤ 1.5 µm (Figure 2g).[21,24] We found that the pigment types mainly correspond to the colors used for printing on the paper labels (Figure 2h). These pigment particles originate from the paper labels and enter into the bottles during the cleaning process. [25] Additive particles were detected in reusable PET bottles and considered to leach from the bottle material (68.6% were ≤ 5 µm and 11.7% were ≤ 1.5 µm). These results demonstrate that ParticleFinder can be used for automatic detection, classification, and Raman measurement of particles < 1.5 µm from real samples, which is very important due to toxicological reasons, since this size class is considered small enough to penetrate deeply into organs.[21,22]

Effects of MNPs on podocytes

The potential risk of plastic particles on human health is addressed in this study using human podocytes as a highly-specialized kidney cell type. Since kidneys are involved in the filtration process and do not regenerate their cells continuously, they are likely to accumulate MNPs over the lifetime.[26] We performed cell viability tests after incubation of podocytes with four different MNP types (standards PVC, PA, PP, and tyre wear) using a live-dead (green - red) cell fluorescent based kit. Representative results for relevant plastic concentrations after 7 h exposure with respect to control cells are summarized in Figure 3. The cytotoxicity response is found to depend on the polymer type, a higher concentration is needed for PP (5 mg/ml) compared to PVC, PA, and tyre wear (0.5 - 1 mg/ml) to achieve a similar cell mortality rate. Two mechanisms are proposed to explain

the damage induced by the plastic particles on podocytes and finally their death. First, particles can attach on the cell surface and limit the nutrient uptake, the degree of attachment depending on particles' adhesion properties and sizes. Some particles still remained attached after three times washing with PBS following incubation and can be visualized based on their intrinsic fluorescence as shown in Figure 3. Second, smaller size particles can be taken up into the cells by phagocytosis as illustrated in Figure 4 for PA particles.

The correlative microscopy and spectroscopy characterization of identical cells using the nanoGPS relocalization technology is demonstrated in Figure 4, exemplary shown for PA treated cells. Two representative podocytes (control and incubated) are localized in three independent instruments and studied with complementary analytical techniques down to nanoscale resolution. Optical imaging (~ 1 µm spatial resolution) under DF (a, f) and BF (b, g) illumination show the degradation and deformation of cells after particle exposure. The structural damage is further confirmed by micro-Raman mapping (c, h), treated cells display Raman spectra with less intensity (note the same scale for the integrated area maps). High spatial resolution SEM imaging (< 10 nm) is used to assay the integrity of cell features at nanoscale, exposed cells do not regularly show normal biological features like heterogeneous surface, nucleus, and foot processes (d, i). Height profile imaging acquired with an interferometric profilometer quantifies the deformation of incubated cells that flatten with respect to control cells (e, j), with height changes from ~ 0.8 µm to ~ 1.5 µm, respectively (note the same scale). Given the complex peak structure of Raman spectra from cells and plastic particles and the large overlap between peaks, we employed a CLS fitting algorithm that decomposes each measured Raman spectrum into its spectral components and provides score distribution maps for each component as displayed in Figure 5. This enables us to spatially resolved MNPs without underlying podocyte and substrate backgrounds, which are shown separately. Taking advantage of the nanoGPS relocalization capability in a correlative workflow, the same PA particle (outlined by the square in Figure 5b and Figure 4 - second row) was imaged by SEM and found to be a nanoparticle (~ 30 nm) most likely taken up into the cell by phagocytosis (Figure 4i). All in all, these preliminary experiments indicate the negative influence of plastic particles on human podocyte cells; however, more assays are needed to account for other relevant polymers present in the environment and their separate and mixed effects on different human organs, tissues, and cells.

Conclusion

The present study introduces an efficient measurement protocol for the assessment of contamination, accumulation, and hazards related to micro- and nanoplastic particles in bottled mineral water and human kidney cells. This protocol combines context microscopy and fingerprinting spectroscopy with automated relocalization (nanoGPS) and detection (ParticleFinder) of the same MNPs and cells in separate instruments from distinct manufactures (HORIBA, Zeiss, Leica). Results on microparticle contamination (average number, size, type) in mineral water and toxicity effects of MNPs (standards PVC, PA, PP, and tyre wear) on podocytes (in-vitro) are reported. It was found that the bottle material (single use, reusable PET and glass), bottle age (older, newish reusable PET), and label print (paper, plastic) affect the distributions of microplastics, pigments, and additives. In contrast to non-treated controls, podocytes incubated with MNPs tend to lack usual cell characteristics such as heterogeneous surface, nucleus, and foot processes, confirming the potential risk of plastic particles on the viability of cells. These findings were revealed by a biological cell test supported by complementary methods involving optical (bright, dark field) and scanning electron microscopy, micro-Raman spectroscopy (with CLS spectra fitting), and height interferometric profilometry. Further work will deal with different plastic types, concentrations, and exposure times.

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References

[1] L. Lebreton, A. Andrady. "Future scenarios of global plastic waste generation and disposal". *Palgrave Commun*. 2019. **5**(1): 6.

[2] R. Geyer, J.R. Jambeck, K.L. Law. "Production, use, and fate of all plastics ever made". *Sci. Adv*. 2017. **3**(7): e1700782.

[3] P.J. Kole, A.J. Löhr, F. Van Belleghem, A. Ragas. "Wear and Tear of Tyres: A Stealthy Source of Microplastics in the Environment". *Int. J. Environ. Res. Public Health*. 2017. **14**(10): 1265.

[4] L.M. Hernandez, N. Yousefi, N. Tufenkji. "Are There Nanoplastics in Your Personal Care Products?" *Environ. Sci. Technol. Lett*. 2017. **4**(7): 280-285.

[5] C.M. Rochman. "Microplastics research—from sink to source". *Science*. 2018. **360**(6384): 28-29.

[6] K. Munno, H. De Frond, B. O'Donnell, C.M. Rochman. "Increasing the Accessibility for Characterizing Microplastics: Introducing New Application-Based and Spectral Libraries of Plastic Particles (SLoPP and SLoPP-E)". *Anal. Chem*. 2020. **92**(3): 2443-2451.

[7] L.M. Hernandez, E.G. Xu, H.C.E. Larsson, R. Tahara, V.B. Maisuria, N. Tufenkji. "Plastic Teabags Release Billions of Microparticles and Nanoparticles into Tea". *Environ. Sci. Technol*. 2019. **53**(21): 12300-12310.

[8] S.L. Wright, F.J. Kelly. "Plastic and Human Health: A Micro Issue?" *Environ. Sci. Technol*. 2017. **51**(12): 6634-6647.

[9] R. Lehner, C. Weder, A. Petri-Fink, B. Rothen-Rutishauser. "Emergence of Nanoplastic in the Environment and Possible Impact on Human Health". *Environ. Sci. Technol*. 2019. **53**(4): 1748-1765.

[10] J.C. Prata, J.P. da Costa, I. Lopes, A.C. Duarte, T. Rocha-Santos. "Environmental exposure to microplastics: An overview on possible human health effects". *Sci. Total Environ*. 2020. **702**: 134455.

[11] W.J. Shim, S.H. Hong, S.E. Eo. "Identification methods in microplastic analysis: a review". *Anal. Methods*. 2017. **9**(9): 1384-1391.

[12] G. Renner, T.C. Schmidt, J. Schram. "Analytical methodologies for monitoring micro(nano)plastics: Which are fit for purpose?" *Curr. Opin. Environ. Sci. Heal*. 2018. **1**: 55-61.

[13] L. Cabernard, L. Roscher, C. Lorenz, G. Gerdts, S. Primpke. "Comparison of Raman and Fourier Transform Infrared Spectroscopy for the Quantification of Microplastics in the Aquatic Environment". *Environ. Sci. Technol*. 2018. **52**(22): 13279-13288.

[14] G. Sarau, L. Kling, B. Ossmann, A.-K. Unger, F. Vogler, S.H. Christiansen. "Correlative Microscopy and Spectroscopy Workflow for Microplastics". *Appl. Spectrosc*. 2020. 000370282091625.

[15] C. Cardell, I. Guerra. "An overview of emerging hyphenated SEM-EDX and Raman spectroscopy systems: Applications in life, environmental and materials sciences". *TrAC Trends Anal. Chem*. 2016. **77**: 156-166.

[16] G. Sarau, M. Heilmann, M. Latzel, C. Tessarek, S. Christiansen. "GaN-Based Nanorods/Graphene Heterostructures for Optoelectronic Applications". *Phys. Status Solidi B*. 2019. **256**(4): 1800454.

[17] G. Sarau, M. Heilmann, M. Latzel, S. Christiansen. "Disentangling the effects of nanoscale structural variations on the light emission wavelength of single nano-emitters: InGaN/GaN multiquantum well nano-LEDs for a case study". *Nanoscale*. 2014. **6**(20): 11953-11962.

[18] G. Sarau, M. Heilmann, M. Bashouti, M. Latzel, C. Tessarek, S. Christiansen. "Efficient Nitrogen Doping of Single-Layer Graphene Accompanied by Negligible Defect Generation for Integration into Hybrid Semiconductor Heterostructures". *ACS Appl. Mater. Interfaces*. 2017. **9**(11): 10003-10011.

[19] G. Sarau, S. Christiansen, M. Holla, W. Seifert. "Correlating internal stresses, electrical activity and defect structure on the micrometer scale in EFG silicon ribbons". *Sol. Energy Mater. Sol. Cells*. 2011. **95**(8): 2264-2271.

[20] S.-A. Strungaru, R. Jijie, M. Nicoara, G. Plavan, C. Faggio. "Micro-(nano) plastics in freshwater ecosystems: Abundance, toxicological impact and quantification methodology". *TrAC Trends Anal. Chem*. 2019. **110**: 116-128.

[21] B.E. Oßmann, G. Sarau, H. Holtmannspötter, M. Pischetsrieder, S.H. Christiansen, W. Dicke. "Small-sized microplastics and pigmented particles in bottled mineral water". *Water Res*. 2018. **141**: 307-316.

[22] B.E. Oßmann, G. Sarau, S.W. Schmitt, H. Holtmannspötter, S.H. Christiansen, W. Dicke. "Development of an optimal filter substrate for the identification of small microplastic particles in food by micro-Raman spectroscopy". *Anal. Bioanal. Chem*. 2017. **409**(16): 4099-4109.

[23] J. Müller-Deile, K. Worthmann, M. Saleem, I. Tossidou, H. Haller, M. Schiffer. "The balance of autocrine VEGF-A and VEGF-C determines podocyte survival". *Am. J. Physiol. Physiol*. 2009. **297**(6): F1656-F1667.

[24] D. Schymanski, C. Goldbeck, H.-U. Humpf, P. Fürst. "Analysis of microplastics in water by micro-Raman spectroscopy: Release of plastic particles from different packaging into mineral water". *Water Res*. 2018. **129**: 154-162.

[25] B.E. Oßmann. "Determination of microparticles, in particular microplastics in beverages". 2020. urn:nbn:de:bvb:29-opus4-132788.

[26] X. Xu, G. Wang, N. Chen, T. Lu, S. Nie, G. Xu, et al. "Long-term exposure to air pollution and increased risk of membranous nephropathy in China". *J. Am. Soc. Nephrol*. 2016. **27**(12): 3739-3746.

Microplastic Analysis in Seawater - Minimum Requirements for Comparative Data Generation

海水中のマイクロプラスチック分析～比較データ生成のために～

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Scientific, regulatory and societal interests in environmental pollution by microplastics has led to the requirement for quality assured and harmonized approaches to assessing samples for microplastics. Many methods for seawater sampling have emerged with varying degrees of comparability. For example, one of the most widely applied field methods - surface net sampling - is limited in comparative data generation for particles < 300 µm. Other developments using different sampling pumps and automated approaches require method validation and harmonization. Furthermore, there are several different analytical approaches with varying detection limits, costs and technical readiness levels for implementation. This lack of inter-comparison complicates a global understanding of microplastics levels in the marine environment. Encouragingly, methods are continuously being improved to further automatize sampling, sample pretreatment and final analysis with a far greater attention to validation. The minimum requirements for comparative data generation in seawater must include careful consideration of sampling parameters, analytical processes and data treatment all conducted with a high level of QA/QC.

マイクロプラスチックによる環境汚染について科学的に検証し，適切な規制の制定や社会の関心に答えるためには，マイクロプラスチック試料を適切に分析・評価することが重要である。適切な分析・評価のためには，分析品質が担保されている統一した手法が重要であるが，特に海洋マイクロプラスチックの試料採取は今まで様々な手法で行われてきたため，そのままデータの比較ができないことも多い。例えば，最も広く利用されている野外での試料採取の手法である表層水のネット採取法(Surface Net Sampling)では，直径300µm未満の粒子のデータ比較を行うことができない。サンプリングポンプ等を用いて自動化を図る場合には，手法(メソッド)の検証と他の手法との整合性(調和)が必要になる。さらに，検出限界，採取作業費用，装備の技術的レベルが異なる様々な分析アプローチが存在する。手法の違いによるデータの相互比較が容易でないという問題は，海洋環境におけるマイクロプラスチックのグローバルレベルでの理解をより難しくしている。しかし，比較検証を意識した上で，より自動化された採取方法，前処理方法，最終分析手法の改善が日々継続的に行われており，今後の成果が期待される。比較検証できるデータを取得するためには，最低でも採取パラメータ，分析プロセス，そしてデータ処理を高レベルの品質保証/品質管理(QA/QC)で細心の注意を払って行う必要がある。

Introduction

Microplastics, or at least particles now considered as microplastics (< 5 mm, GESAMP 2019), were first identified in surface seawater samples dating back to the 1960's during plankton surveys.[1] Investigations which followed generally used similar techniques with nets to sweep surface waters and quantify particles visually[2] and later using a suit of analytical techniques to confirm the presence of plastic polymers, including infrared and Raman spectroscopy and thermal desorption or degradation coupled to mass spectrometry.[3] Surface net sampling has proven valuable in the establishment of long-term data sets.[4-5] Unfortunately, this method is hampered in adverse weather conditions and other limitations related to the determination of accurate sample volumes, lower size limit of particles (mostly > 300 µm) size detection and procedural contamination which can compromise the

results. Researchers began to look at alternative methods of analysis, such as the use of seawater intakes on research vessels, submersible pumps and use advanced sensor systems (e.g. FerryBox) to collect large volume samples of which several have shown promise for their use.[6-9]

Academic, non-profit, government and non-governmental organizations have all become engaged in microplastic research: through the development of methods as well as the inclusion of microplastic sampling into ongoing monitoring programs and strategic projects. As the knowledge surrounding microplastic assessment has increased, researchers have turned their attention to defining methods which allow the collection of robust data with quality an essential consideration for project design.[10-12] There is a strong focus towards the production of quality controlled and quality assured (QA/QC) data, with limited sample manipulation and a general need for automated methods of detection.[13, 14] Currently no sensors or on-line measuring technologies exist due to the large sample volumes needed, pre-concentration of the samples and often large amounts of biological interferences. Therefore, data generation relies on sound sampling methodologies and analytical processing in the laboratory. Limitations to comparative data generation cover themes such as the inclusion and exclusion of certain sizes, sample contamination, inconsistent units of reporting, lack of validation in processing methods, accuracy or representativeness of samples and validation of observed/visual results using analytical techniques.[15] In the following document, the approaches to seawater microplastic assessment are presented with a view towards methods harmonization and minimum requirements for comparative data generation.

Field sampling for microplastics in seawater

Collection of representative samples is of upmost importance. Much literature has assessed the use of different sampling approaches to seawater (Table 1), and the current limiting factor is the collection of large enough volumes to generate a representative sample. Further, when sampling in areas of high biological activity, the samples may be compromised by large amounts of organic matter clogging the collection devices. This is especially true when using small mesh sizes.[12, 16]

Net sampling

This approach is by far the most commonly applied technique for sampling surface water (manta or neuston nets), subsurface waters and the water column (neuston or bongo nets). Nets are towed in surface waters or the water column for a set duration, rinsed on deck and any anthropogenic particles are categorized by morphology (size, shape, color) and sometimes weighed[4, 5]. Many studies using nets sampling focus on the visible identification of the larger fraction of particle 1-5 mm, often these methods do not use spectroscopic confirmation and plastics are only identified with the naked eye. Although net sampling methods enable sampling of large volumes, the disadvantages are discriminating particles smaller than the nominal mesh size, sampling water volumes passing through the net can only be estimated, nets often bounce on the water surface in adverse weather conditions and it can be very difficult to prevent contamination from working on deck of vessels. Further, when clogging occurs the sample may not be representative. Therefore, it is important that samplers can as accurately as possible, estimate the volume of water which has passed through the net, with a flow meter, or through the calculation of distance travelled.[12]

Bulk water samples

There are many approaches to collecting bulk water samples. A volume-reduced water sample consists of pumping water (manually or using a motor) through a filter and out through a flow meter. These samples can be collected from a variety of sampling platforms: large or small vessels, from static platforms and the shoreline. These approaches are generally used when targeting microplastics in the smaller size ranges < 300 µm. QA/QC procedures are fundamental from sample collection into processing, including field and laboratory procedural blanks, which are easier to achieve using bulk water samples. In most cases, samples may be volume reduced in the field where necessary, but the analysis of samples is conducted under controlled laboratory conditions. Researchers began taking bulk water samples using seawater intakes on research vessels in many of the world's oceans[7-9] and have developed to filtering apparatus being incorporated into other seawater monitoring set up, such as the FerryBox system which are deployed on vessels of opportunity.[6] The standard FerryBox system collects continuous data on temperature, salinity, florescence, turbidity, as well as nutrient analysis, continuous plankton recording. The incorporation of microplastics into these systems will allow comparative data generation which can be accurately coupled to the environmental parameters at the time of sampling. Another example of using vessels of opportunity was the inclusion of filtration apparatus on sailing vessels participating in the Volvo Ocean Race, 2017. Samples (n = 68) were collected on board Team AkzoNobel. The analysis was performed in the laboratory used a combination of Raman spectroscopy to identify the particles, and a camera for microplastics particle size.[17] Smaller bulk water samples include the collection of seawater in CTD rosettes.[9]

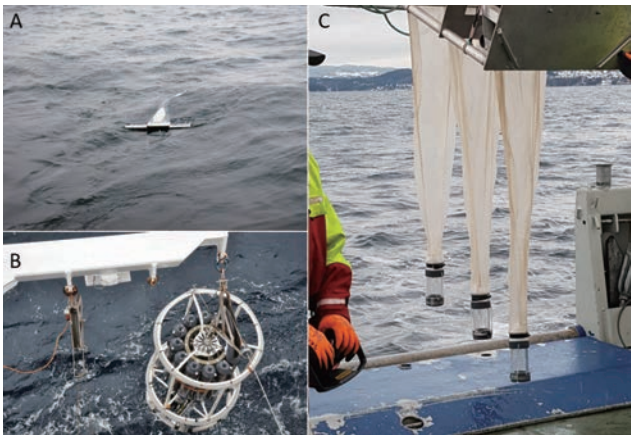

Figure 1 Sampling devices used for microplastic sampling in sea water, A: Manta net; B: CTD rosette bottles, C: multinet

Challenges of continuous measurement

Automated methods, such as continuous measurements, are advantageous as they can collected data without intervention. The representativeness of samples is often complicated by the distance travelled on large vessels or the volume sampled restricted by small water intakes (~in the range of a few cm). As often large volumes of water have to be sampled (> 1000 L), infrequent changing of filters could result in relatively large areas sampled (> 100 km). This is both an advantage, large trajectories can be sampled, but also a disadvantage if a small-scale special resolution is needed. Another challenge includes the presence of organic material which can cause clogging and further compromise collected samples.[12] Coupled with the need for changing filters, it is paramount that QA/QC is strict during sampling. Field blanks can be carried out to monitor the levels of airborne particles in samples, as well as the risk of contamination from the sampler or the equipment. Without these controls it is impossible for researchers to discern the true levels of microplastics in seawater as levels in the marine environment often are low. In summary, the steps made towards automated/semi-automated sample collection are promising, but far from having a readiness for worldwide implementation.

Table 1 Advantages and limitations of sampling approaches to seawater

	Advantages	Limitations
Surface water nets	Long term data sets Visual sorting possible with fraction >1 mm Sample large area	Surface samples only Weather dependent Estimated flow/volume Lower size limit often misses smaller particles (e.g. 300 µm)
Pumping systems - seawater intake e.g. FerryBox	Accurate flow/volume Large volume sampled	Subsurface only
Pumping systems - *In situ pumps*	Can be deployed at variable depths in the water column Accurate flow/volume Large volume sampled	Weather dependent
Bulk water samplers - CTD rosettes	Can be deployed at variable depths in the water column Can collect replicates	Low water volume (~20 litres)

Laboratory processing of seawater samples

Once samples have been collected from seawater, the analytical steps taken are critical to producing robust and comparative data. As with sample collection, a high level of QA/QC is recommended for studies reliant on laboratory and microscopy analysis. This allows the researchers to check the validity of any processing steps introduced before assessment and continue to monitor sources of procedural contamination. Depending on the interfering material collected on the sampling filters and the processing steps needed before the analysis are not discussed herein, the reader is referred to recent reviews on the matter.[18]

A combination of approaches, from visual assessment with the naked eye through to automated spectroscopic methods can be used for the final analysis. A very recent critical assessment of the analytical methods associated to harmonized and coast efficient analysis of microplastics has been published.[3] This review presented the available techniques which includes naked eye detection, optical microscopy, uses of dyes and stains, flow cytometry,

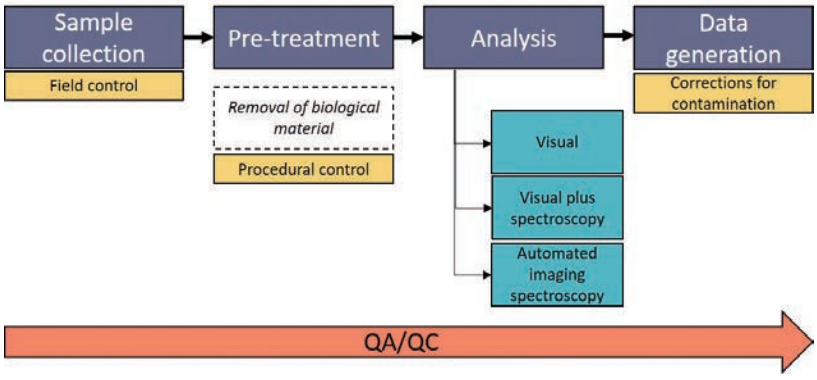

Figure 2 Schematic of steps requires for comparative data generation

Fourier-Transform Infrared (FT-IR) spectroscopy and microscopy, Raman spectroscopy and microscopy and thermal degradation/desorption coupled to gas-chromatography/mass spectrometry. The methods chosen will impede the level of comparison between investigations. Especially when the aim is to quantify the presence of different plastic types. For example, when working with samples contaminating particles from 1-5 mm in size, researchers can use the unaided visual identification with a high level of confidence to identify plastics, but particles < 1 mm require more supported techniques (microscope plus analytical validation) to determine the presence of synthetic polymers as error values can reach up to 70%.[3, 11, 12] Without polymer identification, this may lead to a high level of misidentification, especially when particles size is below 50-100 µm. As such, the use of spectroscopic methods is strongly recommended when working with the identification of microplastics < 1 mm, and fundamental for particles < 100 µm.

FT-IR and Raman spectroscopic methods allow the identification of particle composition by producing a fingerprint spectrum which is unique to different materials. Such that plastic spectra can be differentiated from those produced by natural materials. FT-IR and Raman are both complementary techniques, as molecular vibrations which are inactive with FT-IR, can be active for Raman, and vice versa. Spectroscopic methods can also be coupled to microscope set ups, allowing the application of polymer identification to small particles (µFT-IR ca. 10 µm, µRaman ca. 1 µm).[3] When particles are preselected for FT-IR/Raman using optical light-microscopy by the operator, this can introduce a bias in the analysis, and in some instances transparent or translucent particles as well as very small particles might be overlooked during the preselection process.[3] Hence, a reduced proportion of operator interference is encouraged, and researchers continue to seek advancements in µFT-IR and µRaman approaches. Often, the use of spectroscopic methods is costly and time consuming, as such many studies use subsamples of representative particles.

To counteract this, automated spectral methods are being developed to enable high throughput of samples but are currently still limited to low sample volumes. Furthermore, the automatic samples analysis requires significant sample preparation and clean-up to be effective. Nevertheless, µFT-IR has been seen to be a powerful tool and the atomization reduces time and demand of data generation.[3] Providing the researchers are clear in their approach, choice of methods and use clear reporting guidelines, they can generate comparative data.

Conclusion

In the microplastics field of research there are many different sampling approaches and technologies available to investigate seawater microplastics. Developments are hampered by procedural contamination as microplastics and fibres can be introduced by the sampler, the air or sample equipment. This requires a thorough understanding of potential sources of error and effort to minimize intervention with samples in the field. A high level of QA/QC is required from collection through to data generation. This is especially important when studies reliant are reliant on laboratory/microscopy analysis. Method development is continuously ongoing to further automatize sampling, sample pretreatment and final analysis with a far greater attention to validation.

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References

[1] Carpenter, E.J. and Smith, K.L., 1972. Plastics on the Sargasso Sea surface. *Science*, **175**(4027), pp.1240-1241.

[2] Cole, M., Lindeque, P., Halsband, C. and Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. *Marine Pollution Bulletin*, **62**(12), pp.2588-2597.

[3] Primpke, S., Christiansen, S.H., Cowger, W., De Frond, H., Deshpande, A., Fischer, M., Holland, E., Meyns, M., O'Donnell, B.A., Ossmann, B. and Pittroff, M., 2020. EXPRESS: Critical Assessment of Analytical Methods for the Harmonized and Cost Efficient Analysis of Microplastics. Applied Spectroscopy, p.0003702820921465.

[4] Law, K.L., Morét-Ferguson, S., Maximenko, N.A., Proskurowski, G., Peacock, E.E., Hafner, J. and Reddy, C.M., 2010. Plastic accumulation in the North Atlantic subtropical gyre. *Science*, **329**(5996), pp.1185-1188.

[5] Law, K.L., Morét-Ferguson, S.E., Goodwin, D.S., Zettler, E.R., DeForce, E., Kukulka, T. and Proskurowski, G., 2014. Distribution of surface plastic debris in the eastern Pacific Ocean from an 11-year data set. *Environmental Science & Technology*, **48**(9), pp.4732-4738.

[6] Conchubhair, D.Ó., Fitzhenry, D., Lusher, A., King, A.L., van Emmerik, T., Lebreton, L., Ricaurte-Villota, C., Espinosa, L. and O'Rourke, E., 2019. Joint effort among research infrastructures to quantify the impact of plastic debris in the ocean. *Environmental Research Letters*, **14**(6), p.065001.

[7] Desforges, J.P.W., Galbraith, M., Dangerfield, N. and Ross, P.S., 2014. Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. *Marine Pollution Bulletin*, **79**(1-2), pp.94-99.

[8] Lusher, A.L., Burke, A., O'Connor, I. and Officer, R., 2014. Microplastic pollution in the Northeast Atlantic Ocean: validated and opportunistic sampling. *Marine Pollution Bulletin*, **88**(1-2), pp.325-333.

[9] Kanhai, L.D.K., Gårdfeldt, K., Lyashevska, O., Hassellöv, M., Thompson, R.C. and O'Connor, I., 2018. Microplastics in sub-surface waters of the Arctic Central Basin. *Marine Pollution Bulletin*, **130**, pp.8-18.

[10] Brander, S.M., Renick, V.C., Foley, M., Steele, C.L., Woo, M., Lusher, A., Carr, S., Helm, P., Box, C., Cherniak, S. and Andrews, R.C., 2020, February. Sampling and QA/QC, or how many blanks do I need?: A guide for scientists investigating the occurrence of microplastics across matrices. In Ocean Sciences Meeting 2020. AGU.

[11] Isobe, A., Buenaventura, N.T., Chastain, S., Chavanich, S., Cózar, A., DeLorenzo, M., Hagmann, P., Hinata, H., Kozlovskii, N., Lusher, A.L. and Martí, E., 2019. An interlaboratory comparison exercise for the determination of microplastics in standard sample bottles. *Marine Pollution Bulletin*, **146**, pp.831-837.

[12] Michida, Y., Chavanich, S., Cabañas, A.C., Hagmann, P., Hinata, H., Isobe, A., Kershaw, P., Kozlovskii, N., Li, D., Lusher10, A.L. and Martí, E., 2019. Guidelines for Harmonizing Ocean Surface Microplastic Monitoring Methods.

[13] Hermsen, E., Mintenig, S.M., Besseling, E. and Koelmans, A.A., 2018. Quality criteria for the analysis of microplastic in biota samples: a critical review. *Environmental Science & Technology*, **52**(18), pp.10230-10240.

[14] Koelmans, A.A., Nor, N.H.M., Hermsen, E., Kooi, M., Mintenig, S.M. and De France, J., 2019. Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. Water Research.

[15] Shim, W.J., Hong, S.H. and Eo, S.E., 2017. Identification methods in microplastic analysis: a review. *Analytical Methods*, **9**(9), pp.1384-1391.

[16] Karlsson, T.M., Kärrman, A., Rotander, A. and Hassellöv, M., 2020. Comparison between manta trawl and in situ pump filtration methods, and guidance for visual identification of microplastics in surface waters. *Environmental Science and Pollution Research*, **27**(5), pp.5559-5571.

[17] https://www.geomar.de/index.php?id=4&no_cache=1&tx_ttnews%5btt_news%5d=5977&tx_ttnews%5bbackPid%5d=185&L=1

[18] Lusher, A.L., Munno, K., Hermabessiere, L., Carr, S. Isolation and Extraction of Microplastics from Environmental Samples: An Evaluation of Practical Approaches and Recommendations for Further Harmonisation. Accepted, Applied Spectroscopy (April 20th 2020).

Understanding the Nature of Microplastic Pollution and Identifying Environmental Impacts

マイクロプラスチック汚染の把握と環境影響評価

Bridget O'DONNELL
ブリジェット オードネル

With the large-scale production of consumer plastics, comes the problem of how to deal with the disposal of long-lived single use items. Only a small percentage of plastics are recycled, leaving a significant volume accumulating in landfills or polluting our environment, where they fragment into smaller pieces, termed microplastics. Microplastics are ubiquitous and are present in every part of the environment and in the tissue of organisms, where they have physical and chemical toxic effects. To understand the extent of the problem, it is important to formulate standard methods for the collection, extraction, and identification of microplastics. In addition, laboratory-based research must be performed to understand the capacity of microplastics to effect human and environmental health. This review paper summarizes some of the key research direction in this field, in particular with respect to the research laboratories of Dr. Chelsea Rochman at the University of Toronto, one of the world's leading authorities on microplastic analysis and understanding of environmental and health impacts and risks.

人々が日常的に用いるプラスチックの生産量が増大するとともに，それらをどのように廃棄するかという課題が付きまとうようになってきた。生産されるプラスチックのうちの僅かなものは再利用されるが，残りの大半は埋め立てられるか，マイクロプラスチックとして知られる小さい断片となって我々の環境中に残留することになる。マイクロプラスチックは海洋や大気などあらゆる環境に存在するのみならず，生物の組織にも存在している。そして，生体に存在するマイクロプラスチックの物理的，或いは化学的毒性についての報告例が挙げられる。この問題に関する理解を深める為には，サンプリング方法，抽出方法，そしてプラスチック種類の同定方法の標準化が重要と言える。加えて，実験室レベルでの研究は，人体や環境に影響を与えるマイクロプラスチックの量を理解する上で欠かせない。トロント大学はマイクロプラスチックの環境や生体にあたえる影響や脅威について，最先端の研究を進めている研究機関として世界的に知られている。本項では，この分野における幾つかの報告事例について，主にトロント大学Chelsea Rochman教授による研究成果を中心に紹介する。

Introduction

Starting in the mid-twentieth century, plastics began to replace other materials, since they were easy and inexpensive to manufacture, while still being strong and durable. With the advent of plastics, came the idea of "throwaway living": the idea that consumers could save time through single-use items including tableware and flatware, beverage and food containers, and diapers.[1] With the increase in manufacturing of disposable consumer products, the production of plastics has ballooned since 1950, with an estimated 8,300 million metric tons produced as of 2015. Of that amount, only 30% of manufactured plastics are still in use, while approximately 60% have been discarded and 10% have been incinerated.[2] The same characteristics that make plastics excellent materials for a wide variety of consumer uses, also make managing their disposal difficult. Lifetimes of plastics can range from tens of

years to hundreds of years depending on the nature of the material.[3] Depending on the implementation of waste management standards, plastic waste may be landfilled, incinerated, recycled, or dumped into the environment. Evidence of plastic pollution has been well documented from the Great Pacific Garbage Patch[4] to "pristine" beaches littered with plastic trash.[5]

Plastic litter comprises a wide variety of materials and sizes, including microplastics, particles less than 5 mm in size.[6] Sources of microplastics can be both primary and secondary. Primary sources include pellets from plastic processing plants, microbeads from cosmetic and personal care products, and industrial abrasives. Secondary sources of microplastics arise from the fragmentation and degradation of larger plastics. Examples include microfibers released from textiles and tire wear particles (see Figure 1).[7] Microplastics encompass a wide variety of characteristics. Their morphologies include fibers, films, fragments, pellets, foams, and spheres.[8] Microplastics also incorporate a wide variety of polymers including polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), polyvinyl chloride (PVC), and rubber. Microplastics are also not composed of polymers alone, but may also contain pigments or dyes and additives such as titanium dioxide and calcium carbonate. The transport of microplastics to the environment can occur through a variety of mechanisms, including through air and water, for example from laundering effluent and exhaust.[9] Sinks of microplastics in the environment include sediment, freshwater bodies (lakes, rivers), and saltwater bodies (surface water, arctic ice).[10] They can also be transported between organisms, for example from prey to predator, via trophic transfer.[11] Microplastics have been found in nearly every level of the food chain from invertebrates[12] all the way up to the largest mammals on earth.[13]

There is ample evidence in the literature that microplastics are harmful. Toxicity can take on two forms; physical and chemical. Physical toxicity arises from the accumulation of microplastics in organisms and can have a variety of effects on health, including reduced respiratory function, hepatic stress, and the formation of granulomas through immune response.[14] As microplastics continue to break down into smaller and smaller fragments down to the nanoscale, translocation from the gut can occur leading to harmful effects in other tissues including the heart, lungs, gallbladder, and liver.[15] Chemical toxicity can arise from either additives in the plastics themselves or through the accumulation of toxins like persistent organic pollutants (POPs) or metals on the surface of microplastic particles.[14]

Critical to understanding the source of microplastics and potential toxicity is first understanding the presence and characteristics of microplastics in our environment. This includes elucidating the distribution of polymer types,

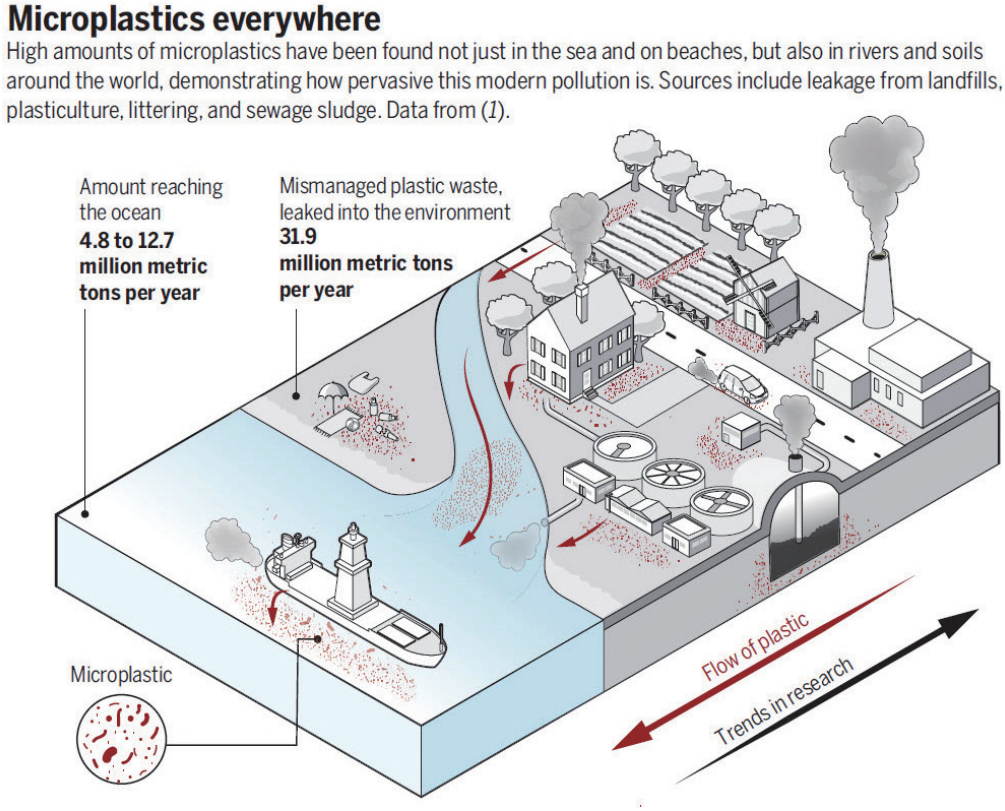

Figure 1 Reprinted with permission from Science Magazine (doi: 10.1126/science.aar7734).

presence of additives, particle morphology, and size distribution. In order to develop a clear picture, methods for collection, extraction, and identification of microplastics must be developed and standardized (or at least harmonized for data synthesis).

Collection Methods

The most common method for the collection of microplastics from marine environments is the neuston net or Manta Trawl. Using this technique, a large volume of surface water can be sampled by towing the net via a boat. Designed for collecting plankton, the net's mesh size is generally in the range of 333-335 μm, so the size of microplastics collected is restricted to those in the larger size range. However, microfibers, which are thought to be one of the most prevalent microplastic morphologies, can slip easily through a net, in addition to any other particulate with ellipsoidal shapes (thin fragments or folded/rolled films). Finally, the material of the net itself may contribute to contamination in collected samples.[16] Another method for collection from marine environments is the grab method. In this method, a 1 L (or larger volume) sample of water is collected, typically in a glass or metal sample container, to avoid contamination. Although the total volume is less than a net, a grab sample can collect plastics down to the sub-micron scale. In addition, because of the simplicity of collection, researchers of any skill level can easily collect samples, including citizen scientists. Samples may also be collected from a variety of environments including shallow tidal pools and wastewater outflow sites.[17]

Sediment samples require alternate collection methods - these include collection from coastal beaches to the deep sea. For sediment samples collected from the seabed, specialized equipment is required. These can include grab samplers which scoop a sample from the top layer of the sea floor (Van Veen, Ekman) and core samplers, which collect columns of sediment, retaining information on the numbers of microplastics in sediment as a function of depth. As in the case of nets, contamination from plastic core samplers is also a concern. Metal is an alternate choice, however the opacity of metal precludes the ability of the researcher to actively monitor the volume of sediment collected.[18]

Other common matrices include biota, which consists of sampling animals from the environment to bring back to the lab for processing. In addition, air samples are also becoming more common and methods for collection continue to be developed to capture both wet and dry deposition.

Extraction Methods

During the collection of microplastic samples, particulate of other types may also be present including minerals, plant matter, biota, and other organic matter. There are a number of methods used to isolate microplastics of interest from other particulate including density separation and chemical digestion. Density separation is used to separate denser particulate (e.g., minerals, silica) from the more buoyant microplastics. In density separation, the mixed sample matrix is immersed in a prepared solution with high density. Those particles with lower density than the solution, including microplastics, float to the surface, while heavier particles sink to the bottom. The solution is allowed to rest for an extended period so that the denser particles have time to settle before carefully extracting the top portion of the sample containing 'floating' particles. This process may then be repeated, through the addition of fresh solution to the settled portion of the sample to ensure that all microplastics are collected. Different solutions with varying density can be prepared including sodium chloride, zinc chloride, calcium chloride, and sodium iodide.[16]

A unique extraction procedure taking advantage of the hydrophobic nature of microplastics was developed and reported by the Rochman lab at the University of Toronto. In this procedure, magnetic iron nanoparticles are functionalized with hydrophobic hydrocarbon tails. These hydrophobic groups preferentially bind to microplastics, which can then be extracted using a neodymium magnet by swirling the magnet in the sample jar and then rinsing it into a clean reservoir. Recovery of microplastics from spiked samples is demonstrated on a variety of size ranges from less than 20 μm to greater than 1 mm. It was concluded that magnetic extraction is beneficial as a secondary extraction technique after density separation or for samples that are relatively clean, as in drinking water. [19] Chemical digestion may be used to remove organic material while leaving microplastic particles behind. These methods include wet peroxide oxidation, alkaline digestion, and acidic digestion. When employing chemical digestion, it is important to ensure that the biological tissue and plant matter are removed without effecting the microplastics being collected. Acidic digestion has been shown to break down certain polymers, including nylon, polyamide, and rubber.[20] It has also been demonstrated in a publication by Munno *et al* that high temperatures generated during digestion (> 60°C) can result in the loss of some microplastics, particularly microbeads used in personal care products.[21] For biological samples, including collection of microplastics from gastrointestinal (GI) tracts, an alternative extraction procedure was developed by the California Department of Public Health, in collabo-

ration with the Rochman lab, to avoid damage to the microplastics under study and to ensure that no biological tissue remains adhered to the surface of the extracted microplastics. In this procedure, the GI tract is dissected, isolated, and sealed in a vial. The vial is then immersed in a water tank and subjected to bursts of ultrasonic waves, termed pulsed ultrasonic extraction (PUE). The sample is then poured through a 1 mm stainless steel sieve and then filtered using a 10 µm core polycarbonate filter. Compared to samples prepared using traditional KOH digestion, samples extracted using PUE showed much cleaner surfaces and resulted in better spectral matches to reference databases.[22] For biota, biota can be extracted whole, or dissected to isolate the GI tract or target organs.[23]

Once an environmental sample has been collected and extracted, it may be sorted into various size fractions. Sieve stacks are used to separate particles into different size fractions down to approximately 300 µm.[24] For smaller particles, vacuum filtration with progressively smaller pore size membranes may be used. Large microplastics can easily clog filter membranes or obscure smaller particles if size fractioning is not employed. For particles greater than approximately 300 µm, samples may be manipulated manually using fine-tipped forceps, while smaller particles are more difficult to manipulate and can be analyzed directly from the filter membrane.[16] The use of size fractioning provides an additional benefit of collecting particles of similar size, which makes manual sorting easier.

Due to the varied nature of techniques and differing laboratory conditions, it is important to follow standard QA/QC techniques to account for any contamination that may be introduced throughout the collection and extraction process. To limit the amount of contamination, it is best to minimize both the number of people handling samples and the amount of time the sample is exposed to air. General QA/QC lab practices include maintaining clean work surfaces, avoiding synthetic clothing, covering samples whenever possible, and installing air filters in the laboratory. In addition, proper QC/QA procedures include the generation of blank samples both in the field at collection and in the laboratory during extraction, which are treated with the exact same procedure used for measured samples. The results of particles found in the blank measurements may then be subtracted from the sample measurements or reported for each study.[8]

Detection and Identification Methods

Visual examination of extracted samples using a stereo zoom microscope is arguably the most prevalent technique for identifying microplastics. Using visual examination, a suspected microplastic can be characterized by color, and morphology. In addition, visual identification can be used to discriminate natural particles from anthropogenic particles. Different modes of imaging can help to improve contrast and aid in identification including reflected/transmitted light, polarized light microscopy, and dark field microscopy. Microscopy images of the particles can be taken and used to record measurements for exact particle dimensions with the implementation of software such as ImageJ.[25] The reliability of visual examination alone to definitively identify microplastics is low: depending on the researcher, false negatives and positives may occur with varying frequency.[24] The addition of fluorescent staining can improve identification using optical microscopy. The most common stain used in the identification of microplastics is Nile Red, which binds to plastics in both exposure experiments in the lab and in environmental samples through hydrophobic interactions. Nile Red fluoresces at a variety of wavelengths and is dependent on the hydrophobicity of the microplastic particle's surface. However, certain types of plastics including polycarbonate, polyurethane, PET, and PVC display weak signals, while microplastic fibers are particularly difficult to stain.[26] In response to these difficulties, alternate stains have been tested in the Rochman lab, including those designed specifically for textiles. For both laboratory tests and environmental samples, different dyes have been identified as promising stain alternatives (see Figure 2).[27-29]

For definitive chemical identification, there are a number of techniques that may be used including pyrolysis gas chromatography-mass spectrometry (GC-MS), Fourier transform infrared spectroscopy (FT-IR), Raman spectroscopy, and scanning electron microscopy (SEM) plus energy dispersive x-ray spectroscopy (SEM/EDS). The application of these techniques has been described in detail elsewhere,[24] therefore a short overview of each technique's use in microplastics research will be given here. Pyrolysis GC-MS works by thermally breaking down the sample under measurement: the masses of the daughter fragments are analyzed in the resulting pyrogram to elucidate the parent molecule. GC-MS is considered a "gold standard" in analytical labs and is a readily available piece of analytical instrumentation. Pyrolysis GC-MS provides simultaneous identification and quantification of microplastics in complex samples. Because of the quantitative nature of this technique (in mass, not number of particles), there is risk of matrix effects from remaining organic matter and materials or chemicals from extraction techniques, so extra care must be taken to accommodate for potential contaminants.[24]

Raman spectroscopy and FT-IR spectroscopy are both

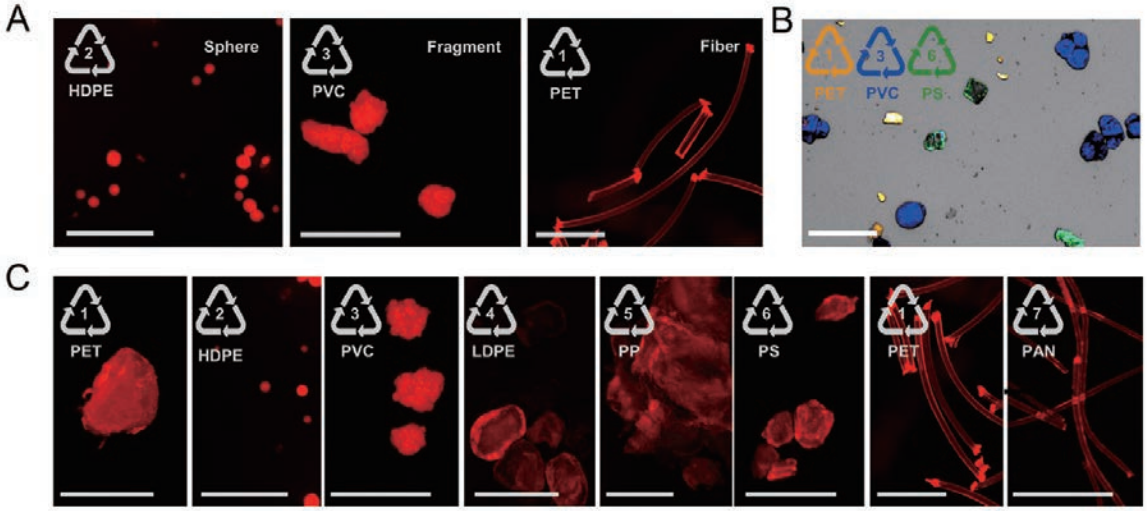

Figure 2 Examples of plastic types and morphologies dyed with different dyes. (A) Different morphologies: spheres (10-90 μm), fragments (50-300 μm), and fibers (30-60 μm/150-5000 μm) dyed with pink dye. (B) Different fluorophores: green (kentucky dye), red (pink dye), and far-red (blue dye). Pseudocolors are applied to different fluorescent channels for the purpose of differentiation. (C) Different polymer types dyed with pink dye: polyethylene terephthalate (PET) fragments (50-500 μm), high-density polyethylene (HDPE) spheres (10-90 μm), polyvinyl chloride (PVC) fragments (50-300 μm), low-density polyethylene (LDPE) fragments (100-500 μm), polypropylene (PP) fragments (500-4000 μm), polystyrene (PS) fragments (100-300 μm), polyester (PET) fiber (30-60 μm/150-5000 μm), and polyacrylonitrile (PAN) fiber (20-50 μm/300-3000 μm). Scale bars are 550 μm. Reprinted with permission from Environmental Science & Technology Letters (doi: 10.1021/acs.estlett.9b00241) Copyright (2019) American Chemical Society.

techniques that probe the vibrational bonds in a molecule. FT-IR uses broadband infrared light to illuminate a sample; when the light is resonant with a vibrational band in the molecule under study, a decrease in the intensity of the infrared light is observed. In Raman spectroscopy, a monochromatic laser source illuminates the sample; most of the light is elastically scattered (Rayleigh scattering), while a small portion of light is inelastically scattered to lower or higher frequency (Stokes and anti-Stokes scattering). The difference in energy between the inelastically scattered photon and the laser corresponds to a vibrational band in the molecule under study. These two vibrational spectroscopy techniques are complementary and provide different structural information on the particle under study. For example, Raman active vibrational modes can provide information on the backbone of a polymer, while infrared active vibrational modes provide information on side chains. In addition, Raman spectroscopy can provide information on additives and pigments or dyes in microplastics, which can help in tracking the source of microplastics. While micro-FT-IR spectroscopy can measure a minimum particle size of approximately 10 μm, Raman spectroscopy can measure particles down to < 1 μm in size.[24] For microplastics, this is critically important because it is generally agreed that, as particle sizes become smaller, the abundance of microplastics increases. Smaller particle sizes also have bigger ramifications when it comes to toxicology, which will be discussed in more detail below.[24,30,31]

SEM/EDS combines scanning electron microscopy and energy dispersive x-ray spectroscopy to provide high resolution imaging at the nanoscale with elemental characterization of heavier elements. SEM focuses an electron beam onto the sample under study and measures the resulting scattered electrons. EDS works in combination with SEM and measures the resulting x-ray radiation from the sample. SEM/EDS provides a means to quickly distinguish plastics from minerals, which in marine environments, are primarily Si (sand) and Ca (shell fragments).[22,24] Each technique described above has advantages, disadvantages, and varying associated costs and measurement times. In the characterization of microplastics, it is important to note that multiple techniques may be required for complete characterization.[24] It is also important to standardize methods across different laboratories to ensure consistency in reporting. This is one of the main goals of the microplastics study plan organized by the Southern California Coastal Water Research Project (SCCWRP) described in a separate article.

As a single microplastics sample can contain hundreds or thousands of particles, a critical part of the process of standardization is automation of sample measurements, in order to reduce the time required for sample analysis. One such method of automation relies on the use of the optical image of a sample, for example microplastic particulate on a filter membrane, to distinguish particles from the background substrate. The optical image provides the spatial contrast needed to identify the particulate, and then the use of a motorized stage together with Raman or FT-IR spectroscopy allows the user to collect spectra at each isolated particle. Using this technique, large areas can be covered without collecting spectra from areas that

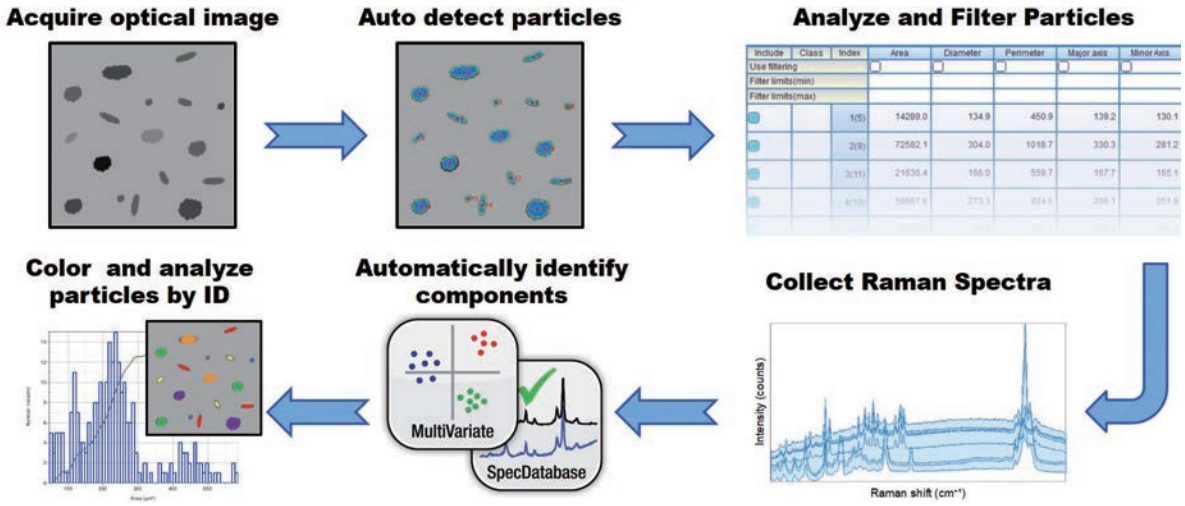

Figure 3 Example workflow for automation of microplastic measurements using Raman spectroscopy and HORIBA's ParticleFinder software module.

are not of interest, for example from the filter membrane itself.[32]

While automated routines like those described above are sufficient for analysis of larger microplastics across an entire filter membrane (for example a 47 mm diameter filter), for analysis of particulate in the lower size range, < 20 µm, measurements across an entire filter becomes prohibitively expensive in both time and data size. As part of standardizing the analysis of microplastics, it is important to formulate sampling and sub-sampling schemes that are representative of the sample under study. There are a number of different ways to define sub-sampling, for example, by percentage of filter area covered in a measurement, or by the percentage of total particles measured. In a paper published by Anger *et al*, it was proposed that sub-sampling by percentage of total particles is most appropriate for two reasons; one, particles may not be evenly distributed across the filter and two, different filter diameters may be used across different laboratories. The proposed working method was to first estimate the total number of particles on a filter using the optical image, and then chemically identify a chosen subset of particles.[33]

Once spectroscopic measurements are complete, it is necessary to determine the number of unique components present. Understanding the complete picture of chemical signatures can provide indication of the source of microplastics and potentially provide information on possible contamination as well (see Toxicology section).[8] Multivariate analysis techniques including principal component analysis (PCA), multivariate curve resolution (MCR), and cluster analysis techniques can be used to determine the number of unique spectral signatures in a sample set.[34] Once a model has been built, spectral matching algorithms may be used to identify the exact species present in a microplastics sample. The development of spectral databases specific to microplastics can improve the quality of spectral matching and produce results that are more relevant to environmental samples, as microplastics encompass a diverse suite of polymers, additives, and dyes/pigments[8]. Existing libraries contain mostly pure polymers, which can only provide limited information. The development of SLoPP and SLoPP-E (Spectral Library of Plastic Particles, Environment) libraries by the Rochman Lab enable better spectral matching and provide much more information on collected samples, as the libraries include reference spectra from particles sourced from everyday products and from the environment.[35] Making these databases freely accessible to the microplastics community helps to ease the cost burden of commercial spectral databases, which frequently come with high annual subscription costs.

Toxicology

While it is important to understand the presence and nature of microplastics in our environment, it is also critically important to understand the impact of microplastics on our ecosystems and potentially on human health as well. There are a number of mechanisms through which microplastics may be harmful including physical and chemical pathways, as described above. Because microplastics can both sorb contaminants[36] and leach harmful additives,[37] bioaccumulation of these toxins may occur in marine organisms.[38] In addition, biomagnification, where toxins consumed by smaller organisms are concentrated in predators that consume them, can also occur, effecting the health of ecosystems across the entire food web. Examples of chemical contaminants in microplastics (sorbed contaminants, chemical ingredients, and chemical byproducts) are shown in Figure 4.[39]

In a laboratory-based study by Rochman *et al*, the effects

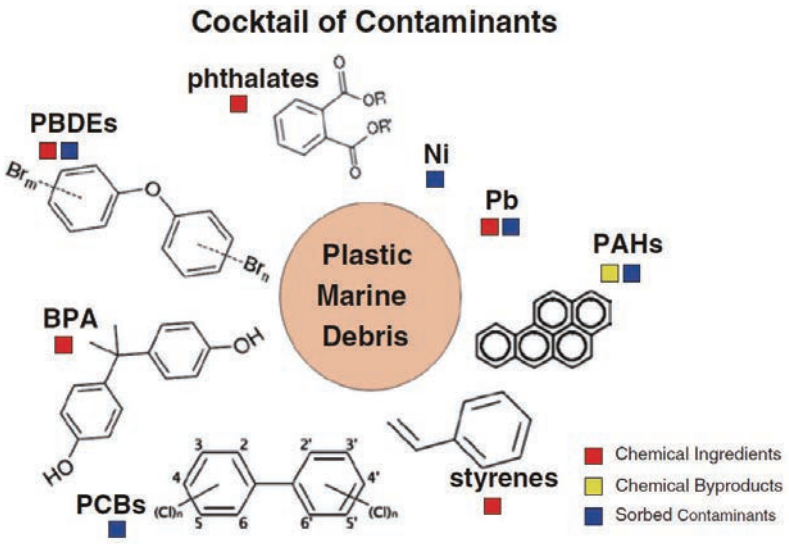

Figure 4 Cocktail of contaminants associated with marine plastic debris. Contaminants associated with marine debris include chemical ingredients (red squares), byproducts of manufacturing (yellow squares) and those that accumulate from surrounding ocean water in the marine environment (blue squares). Reprinted with permission from Marine Anthropogenic Litter (doi: 10.1007/978-3-319-16510-3).

of bioaccumulation were tested using Japanese medaka. In this study, three groups of fish were studied; a control group, a group fed virgin LDPE, and a group fed LDPE that had been deployed in an urban bay. After one and two months of exposure, the amount of polycyclic aromatic hydrocabons (PAH), polychlorinated biphenyls (PCB), and polybrominated diphenyls (PBDE) were tracked in the tissue of the fish. The results showed that not only does bioaccumulation of chemical pollutants occur, but signs of liver toxicity and pathology arise in the groups fed both virgin and deployed LDPE, namely glycogen depletion, fatty vacuolation, and single cell necrosis (in deployed LDPE only). This study demonstrated that (1) bioaccumulation of chemical pollutants through exposure to microplastics occurs in aquatic organisms and (2) exposure to microplastics and associated chemicals may induce hepatic stress.[40]

While evidence for harm from microplastics in marine life has been well documented,[12,39-44] less well understood is the effect that microplastics may have on humans. It is clear that microplastics are found in food consumed, including a variety of species of marine organisms,[45] salt,[46] and canned fish.[47] An example of a potential route of exposure through seafood is shown in Figure 5.[14] What happens once microplastics are consumed is not entirely clear. It is likely that many microplastic fragments are passed as waste, but this does not negate the possibility of physical and chemical toxicity in humans. A well-known example of the effect of chemical toxicity from plastic packaging is bisphenol-A (BPA), a constituent monomer of polycarbonate. It was demonstrated that higher levels of BPA present in urine samples were associated with reported heart disease in American adults.[48] Further research is needed to understand the amount of microplastics consumed, associated chemical exposure, and what health effects arise from such exposure.[14]

Mitigation

To address the global problem of microplastics, governments around the world have begun to enact policies to deal with the staggering problem of plastic pollution. This includes the ban of microbeads from personal care products, the tracking of municipal or commercial waste, and commitments to reduce marine debris.[14] From an industrial perspective, companies have started to implement sustainability practices including manufacturing products from plastics collected from beaches[49], moving towards biodegradable or compostable materials,[50] and transitioning to durable, multi-use packaging.[51] Beach clean-up programs organized by non-governmental organizations (NGO) serve two purposes; to raise awareness of the problem of plastic marine debris and to help remove larger plastics that have the potential to become smaller microplastics.[52] For consumers, there are also strategies that can be used daily to reduce microplastics. While reducing plastic use in general is a good first step (especially single-use plastic items), other sources of microplastics can be addressed in different ways. For example, microfibers from textiles are likely a large contributor to microplastics emissions, where hundreds to thousands of microplastics are generated through the washing of a single garment. [53] Products like the Lint LUV-R filter and CORA ball can help to trap microplastic fibers before they reach wastewater treatment plants, and ultimately are discharged into bodies of water.[9]

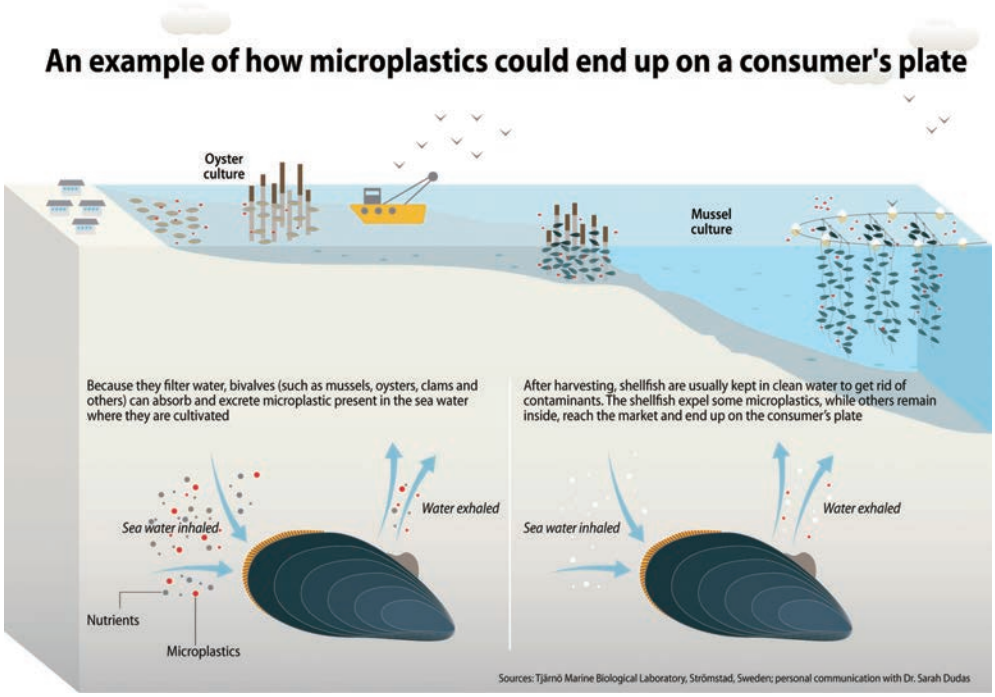

Figure 5 An example of how microplastics could end up on a consumer's plate. Reprinted with permission from Current Environmental Health Reports (doi: 10.1007/s40572-018-0206-z).

Conclusion

Microplastic pollution is a global issue and one of the first steps in addressing the problem is to understand the nature of microplastics. This includes elucidating the major sources and sinks of microplastics in the environment, types of plastics and additives, and the particle morphologies and sizes. Optimization and standardization of laboratory methods for microplastic analysis is critical for reproducibility amongst labs including sample collection, extraction, detection, and identification methods. From this information, researchers can understand more about the sources of microplastics and how best to mitigate the threat to the environment and potentially to human health as well. The work of research laboratories like that of the Rochman group are critical to help drive standardized methodologies and a true understanding of the impact of microplastics and nanoplastics on our environment and health. Only through the development of harmonized reproducible methodologies will government agencies be able to provide the necessary recommendations to state and federal legislative bodies to put in place mandated monitoring and control programs.

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References

[1] Life magazine. Throwaway Living: Disposal Items Cut down Household Chores. *LIFE*. August 1, 1955, p 43.

[2] Geyer, R.; Jambeck, J. R.; Law, K. L. Production, Use, and Fate of All Plastics Ever Made. *Sci. Adv.* 2017, **3** (7), e1700782. https://doi.org/10.1126/sciadv.1700782.

[3] National Oceanic and Atmospheric Administration. Can marine debris degrade on its own in the environment? https://oceanservice.noaa.gov/facts/degrade.html (accessed Apr 15, 2020).

[4] Moore, C.; Phillips, C. *Plastic Ocean: How a Sea Captain's Chance Discovery Launched a Quest to Save the Oceans*, First trade paper-back edition 2012.; Avery a member of Penguin Group (USA): New York, 2012.

[5] Lavers, J. L.; Bond, A. L. Exceptional and Rapid Accumulation of Anthropogenic Debris on One of the World's Most Remote and Pristine Islands. *Proc Natl Acad Sci USA* 2017, **114** (23), 6052-6055. https://doi.org/10.1073/pnas.1619818114.

[6] Arthur, C., J. Baker and H. Bamford. *Proceedings of the International Research Workshop on the Occurrence, Effects and Fate of Microplastic Marine Debris*; Technical Memorandum NOS-OR & R-30; National Oceanic and Atmospheric Administration, 2009.

[7] GESAMP. *Sources, Fate and Effects of Microplastics in the Marine Environment: Part Two of a Global Assessment*; **93**; IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/ UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, 2016; p 220.

[8] Rochman, C. M.; Brookson, C.; Bikker, J.; Djuric, N.; Earn, A.; Bucci, K.; Athey, S.; Huntington, A.; McIlwraith, H.; Munno, K.; De Frond, H.; Kolomijeca, A.; Erdle, L.; Grbic, J.; Bayoumi, M.; Borrelle, S. B.; Wu, T.; Santoro, S.; Werbowski, L. M.; Zhu, X.; Giles, R. K.; Hamilton, B. M.; Thaysen, C.; Kaura, A.; Klasios, N.; Ead, L.; Kim, J.; Sherlock, C.; Ho, A.; Hung, C. Rethinking Microplastics as a Diverse Contaminant Suite. *Environ Toxicol Chem* 2019, **38** (4), 703-711. https://doi.org/10.1002/etc.4371.

[9] McIlwraith, H. K.; Lin, J.; Erdle, L. M.; Mallos, N.; Diamond, M. L.; Rochman, C. M. Capturing Microfibers - Marketed Technologies Reduce Microfiber Emissions from Washing Machines. *Marine Pollution Bulletin* 2019, 139, 40-45. https://doi.org/10.1016/j.marpolbul.2018.12.012.

[10] Rochman, C. M. Microplastics Research—from Sink to Source. *Science* 2018, **360** (6384), 28-29. https://doi.org/10.1126/science.aar7734.

[11] Provencher, J. F.; Ammendolia, J.; Rochman, C. M.; Mallory, M. L. Assessing Plastic Debris in Aquatic Food Webs: What We Know and Don't Know about Uptake and Trophic Transfer. *Environ. Rev.* 2019, **27** (3), 304-317. https://doi.org/10.1139/er-2018-0079.

[12] Thaysen, C.; Stevack, K.; Ruffolo, R.; Poirier, D.; De Frond, H.; DeVera, J.; Sheng, G.; Rochman, C. M. Leachate From Expanded Polystyrene Cups Is Toxic to Aquatic Invertebrates (Ceriodaphnia Dubia). *Front. Mar. Sci*. 2018, 5, 71. https://doi.org/10.3389/fmars.2018.00071.

[13] Lusher, A. L.; Hernandez-Milian, G.; O'Brien, J.; Berrow, S.; O'Connor, I.; Officer, R. Microplastic and Macroplastic Ingestion by a Deep Diving, Oceanic Cetacean: The True's Beaked Whale Mesoplodon Mirus. *Environmental Pollution* 2015, **199**, 185-191. https://doi.org/10.1016/j.envpol.2015.01.023.

[14] Smith, M.; Love, D. C.; Rochman, C. M.; Neff, R. A. Microplastics in Seafood and the Implications for Human Health. *Curr Envir Health Rpt* 2018, **5** (3), 375-386. https://doi.org/10.1007/s40572-018-0206-z.

[15] Stapleton, P.; 1 Department of Pharmacology and Toxicology, Ernest Mario School of Pharmacy, Rutgers University, 160 Frelinghuysen Rd., Piscataway, NJ 08854, USA; 2 Environmental and Occupational Health Sciences Institute, 170 Frelinghuysen Rd., Piscataway, NJ 08854, USA. Toxicological Considerations of Nano-Sized Plastics. *AIMS Environmental Science* 2019, **6** (5), 367-378. https://doi.org/10.3934/environsci.2019.5.367.

[16] Prata, J. C.; da Costa, J. P.; Duarte, A. C.; Rocha-Santos, T. Methods for Sampling and Detection of Microplastics in Water and Sediment: A Critical Review. *TrAC Trends in Analytical Chemistry* 2019, **110**, 150-159. https://doi.org/10.1016/j.trac.2018.10.029.

[17] Barrows, A. P. W.; Neumann, C. A.; Berger, M. L.; Shaw, S. D. Grab vs. Neuston Tow Net: A Microplastic Sampling Performance Comparison and Possible Advances in the Field. *Anal. Methods* 2017, **9** (9), 1446-1453. https://doi.org/10.1039/C6AY02387H.

[18] Tsuchiya, M.; Nomaki, H.; Kitahashi, T.; Nakajima, R.; Fujikura, K. Sediment Sampling with a Core Sampler Equipped with Aluminum Tubes and an Onboard Processing Protocol to Avoid Plastic Contamination. *MethodsX* 2019, **6**, 2662-2668. https://doi.org/10.1016/j.mex.2019.10.027.

[19] Grbic, J.; Nguyen, B.; Guo, E.; You, J. B.; Sinton, D.; Rochman, C. M. Magnetic Extraction of Microplastics from Environmental Samples. *Environ. Sci. Technol. Lett.* 2019, **6** (2), 68-72. https://doi.org/10.1021/acs.estlett.8b00671.

[20] Enders, K.; Lenz, R.; Beer, S.; Stedmon, C. A. Extraction of Microplastic from Biota: Recommended Acidic Digestion Destroys Common Plastic Polymers. *ICES Journal of Marine Science* 2017, **74** (1), 326-331. https://doi.org/10.1093/icesjms/fsw173.

[21] Munno, K.; Helm, P. A.; Jackson, D. A.; Rochman, C.; Sims, A. Impacts of Temperature and Selected Chemical Digestion Methods on Microplastic Particles: Impacts of Temperature and Digestion Method on Microplastics. *Environ Toxicol Chem* 2018, **37** (1), 91-98. https://doi.org/10.1002/etc.3935.

[22] Wagner, J.; Wang, Z.-M.; Ghosal, S.; Rochman, C.; Gassel, M.; Wall, S. Novel Method for the Extraction and Identification of Microplastics in Ocean Trawl and Fish Gut Matrices. *Anal. Methods* 2017, **9** (9), 1479-1490. https://doi.org/10.1039/C6AY02396G.

[23] Lusher, A. L.; Welden, N. A.; Sobral, P.; Cole, M. Sampling, Isolating and Identifying Microplastics Ingested by Fish and Invertebrates. *Anal. Methods* 2017, **9** (9), 1346-1360. https://doi.org/10.1039/C6AY02415G.

[24] Primpke, S.; Christiansen, S. H.; Cowger, W.; De Frond, H.; Deshpande, A.; Fischer, M.; Holland, E.; Meyns, M.; O'Donnell, B. A.; Ossmann, B.; Pittroff, M.; Sarau, G.; Scholz-Böttcher, B. M.; Wiggin, K. EXPRESS: Critical Assessment of Analytical Methods for the Harmonized and Cost Efficient Analysis of Microplastics. *Appl Spectrosc* 2020, 000370282092146. https://doi.org/10.1177/0003702820921465.

[25] Rueden, C. T.; Schindelin, J.; Hiner, M. C.; DeZonia, B. E.; Walter, A. E.; Arena, E. T.; Eliceiri, K. W. ImageJ2: ImageJ for the next Generation of Scientific Image Data. *BMC Bioinformatics* 2017, **18** (1), 529. https://doi.org/10.1186/s12859-017-1934-z.

[26] Stanton, T.; Johnson, M.; Nathanail, P.; Gomes, R. L.; Needham, T.; Burson, A. Exploring the Efficacy of Nile Red in Microplastic Quantification: A Costaining Approach. *Environ. Sci. Technol. Lett.* 2019, **6** (10), 606-611. https://doi.org/10.1021/acs.estlett.9b00499.

[27] Karakolis, E. G.; Nguyen, B.; You, J. B.; Graham, P. J.; Rochman, C. M.; Sinton, D. Digestible Fluorescent Coatings for Cumulative Quantification of Microplastic Ingestion. *Environ. Sci. Technol. Lett.* 2018, **5** (2), 62-67. https://doi.org/10.1021/acs.estlett.7b00545.

[28] Karakolis, E. G.; Nguyen, B.; You, J. B.; Rochman, C. M.; Sinton, D. Fluorescent Dyes for Visualizing Microplastic Particles and Fibers in Laboratory-Based Studies. *Environ. Sci. Technol. Lett.* 2019, **6** (6), 334-340. https://doi.org/10.1021/acs.estlett.9b00241.

[29] Zhu, X.; Nguyen, B.; You, J. B.; Karakolis, E.; Sinton, D.; Rochman, C. Identification of Microfibers in the Environment Using Multiple Lines of Evidence. *Environ. Sci. Technol*. 2019, **53** (20), 11877-11887.

https://doi.org/10.1021/acs.est.9b05262.

[30] Araujo, C. F.; Nolasco, M. M.; Ribeiro, A. M. P.; Ribeiro-Claro, P. J. A. Identification of Microplastics Using Raman Spectroscopy: Latest Developments and Future Prospects. *Water Research* 2018, **142**, 426-440. https://doi.org/10.1016/j.watres.2018.05.060.

[31] Käppler, A.; Fischer, D.; Oberbeckmann, S.; Schernewski, G.; Labrenz, M.; Eichhorn, K.-J.; Voit, B. Analysis of Environmental Microplastics by Vibrational Microspectroscopy: FTIR, Raman or Both? *Anal Bioanal Chem* 2016, **408** (29), 8377-8391. https://doi.org/10.1007/s00216-016-9956-3.

[32] L., F.; I., P.-P.; J., M.; P., S.; C., L.; A., H.; E., R. A Semi-Automated Raman Micro-Spectroscopy Method for Morphological and Chemical Characterizations of Microplastic Litter. *Marine Pollution Bulletin* 2016, **113** (1-2), 461-468. https://doi.org/10.1016/j.marpolbul.2016.10.051.

[33] Anger, P. M.; von der Esch, E.; Baumann, T.; Elsner, M.; Niessner, R.; Ivleva, N. P. Raman Microspectroscopy as a Tool for Microplastic Particle Analysis. *TrAC Trends in Analytical Chemistry* 2018, **109**, 214-226. https://doi.org/10.1016/j.trac.2018.10.010.

[34] Shafer-Peltier, K. E.; Haka, A. S.; Motz, J. T.; Fitzmaurice, M.; Dasari, R. R.; Feld, M. S. Model-Based Biological Raman Spectral Imaging. *J. Cell. Biochem*. 2002, **87** (S39), 125-137. https://doi.org/10.1002/jcb.10418.

[35] Munno, K.; De Frond, H.; O'Donnell, B.; Rochman, C. M. Increasing the Accessibility for Characterizing Microplastics: Introducing New Application-Based and Spectral Libraries of Plastic Particles (SLoPP and SLoPP-E). *Anal. Chem*. 2020, **92** (3), 2443-2451. https://doi.org/10.1021/acs.analchem.9b03626.

[36] Rochman, C. M.; Hoh, E.; Hentschel, B. T.; Kaye, S. Long-Term Field Measurement of Sorption of Organic Contaminants to Five Types of Plastic Pellets: Implications for Plastic Marine Debris. *Environ. Sci. Technol*. 2013, 130109073312009. https://doi.org/10.1021/es303700s.

[37] Halden, R. U. Plastics and Health Risks. *Annu. Rev. Public Health* 2010, **31** (1), 179-194. https://doi.org/10.1146/annurev.publhealth.012809.103714.

[38] Koelmans, A. A. Modeling the Role of Microplastics in Bioaccumulation of Organic Chemicals to Marine Aquatic Organisms. A Critical Review. In *Marine Anthropogenic Litter*; Bergmann, M., Gutow, L., Klages, M., Eds.; Springer International Publishing: Cham, 2015; pp 309-324. https://doi.org/10.1007/978-3-319-16510-3_11.

[39] *Marine Anthropogenic Litter*; Bergmann, M., Gutow, L., Klages, M., Eds.; Springer International Publishing: Cham, 2015. https://doi.org/10.1007/978-3-319-16510-3.

[40] Rochman, C. M.; Hoh, E.; Kurobe, T.; Teh, S. J. Ingested Plastic Transfers Hazardous Chemicals to Fish and Induces Hepatic Stress. *Sci Rep* 2013, **3** (1), 3263. https://doi.org/10.1038/srep03263.

[41] Rochman, C. M.; Parnis, J. M.; Browne, M. A.; Serrato, S.; Reiner, E. J.; Robson, M.; Young, T.; Diamond, M. L.; Teh, S. J. Direct and Indirect Effects of Different Types of Microplastics on Freshwater Prey (Corbicula Fluminea) and Their Predator (Acipenser Transmontanus). *PLoS ONE* 2017, **12** (11), e0187664. https://doi.org/10.1371/journal.pone.0187664.

[42] Rochman, C. M.; Kurobe, T.; Flores, I.; Teh, S. J. Early Warning Signs of Endocrine Disruption in Adult Fish from the Ingestion of Polyethylene with and without Sorbed Chemical Pollutants from the Marine Environment. *Science of The Total Environment* 2014, **493**, 656-661. https://doi.org/10.1016/j.scitotenv.2014.06.051.

[43] Gassel, M.; Rochman, C. M. The Complex Issue of Chemicals and Microplastic Pollution: A Case Study in North Pacific Lanternfish. *Environmental Pollution* 2019, **248**, 1000-1009. https://doi.org/10.1016/j.envpol.2019.03.002.

[44] Rochman, C. M. The Role of Plastic Debris as Another Source of Hazardous Chemicals in Lower-Trophic Level Organisms. In *Hazardous Chemicals Associated with Plastics in the Marine Environment*; Takada, H., Karapanagioti, H. K., Eds.; Springer International Publishing: Cham, 2016; Vol. **78**, pp 281-295. https://doi.org/10.1007/698_2016_17.

[45] Collard, F.; Gilbert, B.; Compère, P.; Eppe, G.; Das, K.; Jauniaux, T.; Parmentier, E. Microplastics in Livers of European Anchovies (Engraulis Encrasicolus , L.). *Environmental Pollution* 2017, **229**, 1000-1005. https://doi.org/10.1016/j.envpol.2017.07.089.

[46] Karami, A.; Golieskardi, A.; Keong Choo, C.; Larat, V.; Galloway, T. S.; Salamatinia, B. The Presence of Microplastics in Commercial Salts from Different Countries. *Sci Rep* 2017, **7** (1), 46173. https://doi.org/10.1038/srep46173.

[47] Karami, A.; Golieskardi, A.; Choo, C. K.; Larat, V.; Karbalaei, S.; Salamatinia, B. Microplastic and Mesoplastic Contamination in Canned Sardines and Sprats. *Science of The Total Environment* 2018, **612**, 1380-1386. https://doi.org/10.1016/j.scitotenv.2017.09.005.

[48] Melzer, D.; Rice, N. E.; Lewis, C.; Henley, W. E.; Galloway, T. S. Association of Urinary Bisphenol A Concentration with Heart Disease: Evidence from NHANES 2003/06. *PLoS ONE* 2010, **5** (1), e8673. https://doi.org/10.1371/journal.pone.0008673.

[49] Proctor & Gamble. The world's first recyclable shampoo bottle made from beach plastic https://www.headandshoulders.com/en-us/whats-new/new-head-shoulders-bottle-to-be-made-with-recycled-beach-plastic (accessed Apr 16, 2020).

[50] Dijkstra, H.; van Beukering, P.; Brouwer, R. Business Models and Sustainable Plastic Management: A Systematic Review of the Literature. *Journal of Cleaner Production* 2020, 258, 120967. https://doi.org/10.1016/j.jclepro.2020.120967.

[51] Terracycle. Loop https://loopstore.com/ (accessed Apr 16, 2020).

[52] National Oceanic and Atmospheric Administration. Marine Debris Program https://marinedebris.noaa.gov/ (accessed Apr 16, 2020).

[53] Hartline, N. L.; Bruce, N. J.; Karba, S. N.; Ruff, E. O.; Sonar, S. U.; Holden, P. A. Microfiber Masses Recovered from Conventional Machine Washing of New or Aged Garments. *Environ. Sci. Technol*. 2016, **50** (21), 11532-11538. https://doi.org/10.1021/acs.est.6b03045.

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Raman Applications on Microplastics
マイクロプラスチックのラマン分析アプリケーション

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Microplastics (MPs) is an emerging and rapidly growing research field, the potential for which the scientific impact and business opportunities are abundant. HORIBA is working with several key opinion leaders (KOLs) help develop and drive the critical thinking to establish analytical standards and regulation guidelines. The availability of harmonized methods and key instrumental and software capabilities will determine how well we can monitor and control MP pollution and health risks in the future. This paper will summarize ongoing collaboration projects with KOLs being performed by the HORIBA US Raman team, and what we've learned from this so far.

マイクロプラスチック(MPs)の研究は比較的歴史は浅いものの，近年急速に注目されるようになっており，MPsの科学的な影響を理解するだけではなく，これらに関わるビジネス創出も大いに期待できると考えられる。HORIBAは，この領域の専門家(KOLs(Key Opinion Leaders))と連携し，様々な角度から本件に対する考察を行いながら，分析基準や規制のガイドライン策定につなげている。一連の検討で確立をめざす手法や装置，そしてソフトウエアを含む解析手法の完成度は，将来どこまでMPsによる環境汚染や健康被害を監視し制御できるかを左右するといっても過言ではないと自負している。本項では，これらKOLsと米国HORIBAグループ(HORIBA Instruments Incorporated)の Ramanチームが現在進めている共同プロジェクトの概要と，これまでに得た情報について報告する。

Introduction

It has been reported that less than 10% of manufactured plastics are recycled. With approx. 30% in use, this leaves 60% of plastics to be discarded into environment, or being incinerated. Discarded plastics take tens (e.g. plastic bags and foam cups) to hundreds (e.g. plastic bottles and disposable diapers) of years to decompose, accumulating on land, including in landfill sites, coastlines, in Arctic sea ice, and on the sea surface and floor.[1] During these long years, plastics fragment into small particulates, forming microplastics (MPs) and nanoplastics (NPs). They are not only hazardous for wildlife, but also pose health risks for humans[2] through air pollution (e.g. nanotoxicity due to enhaling NPs), water (e.g. MPs in drinking water) and food sources.

Figure 1 HORIBA Raman instruments. (a) XploRA PLUS, (b) MacroRAM and (c) XploRA Nano

Governing bodies around the world have recognized the detrimental impact of MPs, and started implementing new regulations. Scientific guidelines are critical for effective regulation, and key opinion leaders (KOLs) are actively working to develop standard methods for sample collection, preparation, analysis, and evaluation. HORIBA has been working with several KOLs to understand analytical requirements for MPs, including software and hardware optimization and automation. These efforts have been successful so far, resulting in various publications and presentations[3]. There is however still a long way to go.

It has been demonstrated[4] that using multiple technologies, including Raman microscopy, is essential to identifying MPs without ambiguity. Raman microscopy is growing in its importance due to its high spatial resolution (necessary for particles on the order of ~ 10 µm and smaller), high tolerance toward wet samples (advantageous for field analysis), sensitivity to additives (useful for brand and source identification) and polymers (useful for chemical identification), and specificity for polymer type (for positive identification) and minerals (for exclusive identification). HORIBA Scientific's XploRA PLUS confocal Raman microscope is well equipped to handle MP analysis, and has been installed at multiple KOL laboratories, successfully proving its performance and usefulness. As research progresses, the demand for Raman analysis (and instruments) will expand to field analysis instruments (e.g. compact Raman spectrometer such as HORIBA's MacroRAM coupled with optical fiber probes), NPs analysis (e.g. AFM-Raman hybrid system) aided by nano-tags (e.g. nanoGPS), and multi-modal imaging and microscopy (e.g. epi-fluorescence and hyperspectral imaging microscopy). HORIBA is well placed to explore the business potential of MPs with its versatile collection of products.

Importance of collaboration

MP analysis is just emerging and rapidly growing as a research field; exponentially increasing in volume and importance as an application area and at critical timing as a business opportunity. Analytical requirements are not well determined, yet. Standards and regulations are yet to be defined. It is critical to work with KOLs to accumulate and exchange knowledge, learn requirements specific for MP analysis, establish Raman as a standard analysis technology for MPs, and establish and brand HORIBA as a go-to vendor for Raman analysis for MPs.

Collaboration with Prof. Chelsea Rochman, University of Toronto[5]

Professor Rochman is a faculty member in the Department of Ecology & Evolutionary Biology at the University of Toronto. The ultimate goal of this collaboration is to develop an automated technique for handling small-size MPs (< 100 µm).

Automating MP analysis must be proceeded by establishing standard operating procedures (SOPs). SOPs are critical in MP analysis because MPs migrate over a long distance (e.g. from river to ocean to shore), and require collective characterization by multiple research groups. Unless every research group follows the same SOP, it will be impossible to combine/compare data, follow the plastics lifecycle as they migrate through the environment, or establish effective guidelines to minimize MPs in the environment.

Dr. Rochman's group has developed SOPs for sample preparation that include:

0. MPs are collected from various matrices (water, sediment, biota)
1. Acid, base, or enzymatic digestion is carried out to remove organic material
2. MPs are size fractioned using sieves down to 100 µm in size
3. Remaining small MPs are filtered into size groups down to 1 µm

When collected from the environment, MP samples contain inorganic materials (e.g. sand), organic materials (e.g. biofilm), and non-synthetic materials (e.g. natural fibers) along with MPs. SOPs developed by Dr. Rochman's group separate MPs from non-plastic materials (step 1 of SOP), and divide MPs into size groups (steps 2 and 3 of SOP). Step 2 divides MPs into multiple size categories using sieves, the smallest size category being 100 µm in diameter. Particles smaller than 100 µm are divided into multi-

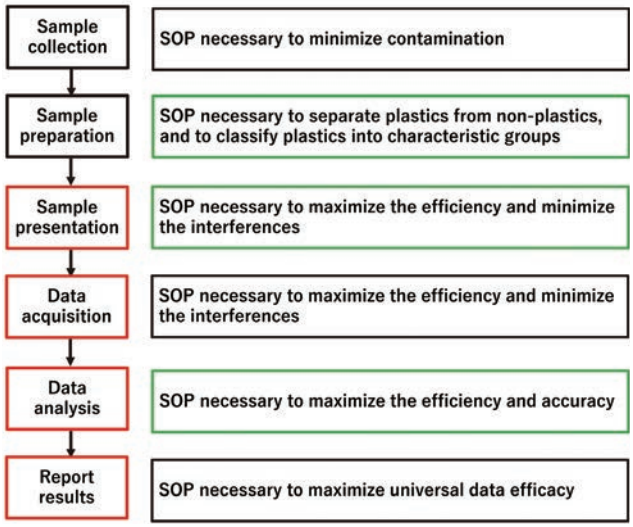

Figure 2 A flowchart of MP analysis. Items highlighted in red mark processes of high demand for automation. Items in green mark Rochman group research focuses at this time.

ple size categories using filters in step 3, the smallest size threshold being 1 µm in diameter. It is not an accident that size thresholds are set at 100 µm and 1 µm. 100 µm represents the smallest size a researcher with a reasonable training can handle manually (e.g. picking up with tweezers). In other words, step 2, with the smallest sieve, separates MPs that can be manually sorted from those too small for that, and designates them for further sample preparation in step 3. 1 µm represents the smallest size an optical microscope can detect, resolve, count, and measure. In other words, step 3, with the finest filter, separates MPs that can be analyzed with an optical microscope i.e. 1 µm or greater, from those too small for that, i.e. < 1 µm, and designates those < 1 µm for nanomaterial analysis by, for example, an atomic force microscope (AFM).

Dr. Rochman's group recognized the advantage of minimizing the number of sample transfers, and utilized filters as a substrate to present the sample to Raman microscopes. This opened the door to further research to optimize the filter material for step 3. Currently multiple filters, commercial and custom developed in the lab, are tested for their efficiency in terms of interference, cost, mass manufacturing, etc.

As the Rochman group's SOPs are being finessed for practicality, the volume of samples they analyze increases, and so do their data accumulation challenges. They have started compiling accumulated data into Raman spectral libraries dedicated for MPs research called SLoPP (spectral library of plastic particles) and SLoPP-E (spectral library of plastic particles aged in environment)[6]. SLoPP is made of pure and pristine MPs spectra, and SLoPP-E spectra of weathered MPs. These libraries are being continuously expanded.

Collaboration with Dr. Ashok Deshpande, NOAA and Dr. Jennifer Lynch, NIST

Raman is a relatively new technology for MP analysis, gaining recognition in recent years. It is important to evaluate a new technology with respect to existing technologies. For MP analysis, the two methods used are mainly pyrolysis gas chromatography-mass spectrometer (pyro-GC-MS) and Fourier transform infrared (FT-IR) spectroscopy. MP samples from Hawaii were analyzed by three technologies for comparison, each at a different laboratory using a different technology. The results proved not the superiority of a single technology but the impor-

Figure 3 Photo of Hawaiian marine debris items, Windward Oahu beach. Kahuku Transect 1[7]

Table 1 Comparison of pyro-GC-MS, FT-IR and Raman

	Raman	ATR-FT-IR	Pyro-GC-MS
Technology	Scattering technique	Absorption technique	Chromatographic technique
Sample	Little to no sample preparation	Sample mounted on diamond crystal and compressed	Sample is completely pyrolyzed (destroyed)
Advantages	Sensitive to molecular vibration based on change in polarizability Sensitive to polymer backbone structure Spatial resolution < 1 µm	Sensitive to molecular vibration based on change in dipole moment Sensitive to polymer side chains Extensive databases available	Sensitive to molecular structure based on breakdown into fragments Quantitative results
Disadvantages	Susceptible to background fluorescence	Susceptible to water absorption	Long measurement time Large sample volume
Analysis by	Bridget O'Donnell, HORIBA	Jennifer Lynch, NIST	Ashok Deshpande, NOAA

tance of multi-technology analysis for accurate identification.

Dr. Jennifer Lynch of the Biochemical and Exposure Science Group of National Institute of Standards and Technology (NIST)[8] is a Research Biologist at and co-director of Center for Marine Debris Research (Hawaii Pacific University). She provided samples in this collaboration, and her student Kayla Brignac performed the analysis using FT-IR.

Dr. Ashok Deshpande of National Oceanic and Atmospheric Administration (NOAA)[9] is a Research Chemist at Northeast Fisheries Science Center (James J. Howard Marine Sciences Laboratory at Sandy Hook). He performed the analysis with pyro-GC-MS.

Dr. Bridget O'Donnell of HORIBA is Manager of Raman Applications at HORIBA Scientific (Piscataway, New Jersey). She coordinated the collaboration, and performed analysis using Raman.

- Identification was 'perfect' for approximately 70% of samples, all three technologies making the same identification, and boosting the confidence in the results by cross-validating each other.
- Identification was 'good' for approximately 10% of samples, two technologies making the same identification. The third technology identifies these as a variety of or similar structure to the accurate identification.
- Identification was 'poor' for approximately 10% of samples, each technology making different identification.
- The remaining 10% of samples were identified by only one technology, the other two technologies failing to yield usable data.

Given the extreme heterogeneity of plastic products with diversity in manufacturing process, this is not surprising. It would be possible to improve these results with further analysis or additional analytical technologies, and calls for adding even more technologies for the MPs' analytical tool box.

Collaboration with Southern California Coastal Water Research Project (SCCWRP)

Southern California Coastal Water Research Project (SCCWRP)[10] is a public Research and Development agency that is leading the project to establish the guideline for MP analysis, which will become the standard method for California State Government regulations. Dr. Steve Weisberg (Executive Director) and Dr. Charles Wong (Department Head, Chemistry Department) initiated and organized a study plan to evaluate MP measurement methods. This is the first step in establishing standard measurement methods that every MP research group will share and follow. In this study plan, they invited researchers around the world, including HORIBA, to participate in drafting measurement methods. SCCWRP has completed drafting a proposal for the method, and is in the process of producing and distributing standard samples in clean water, dirty water, sediment, and biological tissue. Each of the participating laboratories will analyze standard samples with the proposed method, evaluate its practicality, and help finesse improvements. HORIBA contributes expertise on Raman spectroscopy by proposing an augmentation to the study plan, and will evaluate methods for automating MP analysis and for potential field analysis using optical probes.

In preparation for and as a part of the study plan, SCCWRP organized three events in 2019: a workshop and round table discussion in April, sample extraction and preparation training in October, and sample analysis training in November. HORIBA participated in all three events, playing a key role in the sample analysis training.

Many environmental researchers spend limited time in a chemical laboratory, and thus require training operating analytical instruments. The training session at SCCWRP for sample analysis in November 2019 was dedicated to Raman microscopy (provided by HORIBA Scientific) and FT-IR microscopy (provided by Thermo-Fisher). Researchers from key regulatory (e.g. Environmental Protection Agency) and academic (e.g. California State Universities) institutes were present, and trained on Raman using HORIBA Scientific's XploRA PLUS. Researchers will travel back to SCCWRP to perform Raman analysis as part of the study plan, when standard samples are distributed, using the XploRA PLUS.

Instrument requirements of Raman analysis for MP analysis

One of the biggest complexities of MPs (and NPs) analysis is the extremely wide size distribution, ranging from 5 mm to 1 µm (and smaller). As mentioned above, one of the first steps of sample preparation SOPs are classifying MPs into size groups.

MacroRAM with BallProbe[11] (by MarqMetrix) would be suitable to analyze large MPs (in the order of millimeters), especially for field screening. Handheld Raman may be an intuitive candidate for field screening, but is typically designed for bulk analysis. Its measurement spot may be too large for MPs. The focus point of BallProbe is small (in

the order of 200-400 µm), making it a good match for large MPs in size. The MacroRAM is small, light and rugged, and suitable to transport between field basecamps. There are no moving parts, so installation is minimal and alignment is not required: plug it in, and turn it on.

One of the important aspects when selecting a field unit is its compatibility to a lab unit. MPs are sent back to a lab for further analysis if their analysis results are unsatisfactory for various reasons (e.g. too small even for the BallProbe, inconclusive identification, possibility of toxicity, etc.). It simplifies data comparison when the field unit and the lab unit use the same software. HORIBA's benchtop MacroRAM, just like all Raman instruments from HORIBA, uses the LabSpec 6 Spectroscopy Suite (LS6) software, making it easy and simple to compare data to those from a lab unit such as the XploRA PLUS.

XploRA PLUS confocal Raman microscope is well equipped to handle small MP analysis (typically smaller than 1 mm down to less than 1 µm) with a fully featured optical microscope, multiple lasers, confocal spectrometer, motorized stage, and advanced software.

The first step of any analysis is to observe MPs to gauge its color, size, shape and texture. For small MPs (typically smaller than 1 mm), the observation requires an optical microscope. Optical microscopy technologies are dedicated to improve the contrast and the image authenticity. It may sound simple, but it is not trivial to improve contrast or image authenticity. To improve the contrast, one must improve the signal-to-noise ratio (SNR), achieve the best possible focus, and be able to differentiate subtle differences in optical properties (e.g. color, refractive index, birefringence, etc.). To improve image authenticity, one must minimize interference such as 'shadows' from non-uniform illumination, artifacts and distortions from optics themselves, and aberrations from polychromatic light. HORIBA's XploRA PLUS is compatible with all optical microscopy technologies such as darkfield (DF) imaging, polarized light microscopy (PLM), epi-fluorescence imaging, and hyperspectral imaging (HSI) microscopy, allowing the researcher to observe, analyze and classify MPs by their size, color, shape and texture. It is imperative to note that there is absolutely no sample transfer to utilize any or all of these multimodal imaging techniques, which improves the practicality, speed and reliability of correlated analysis immensely.

Chemical composition of synthetic plastics are complex with multiple polymers and additives[12] such as stabilizers, flame retardants, pigments, reinforcements, etc. The complexity increases as MPs undergo weathering in environments, which changes the physical and chemical characteristics. It has been reported[13] that the impact of weathering on MPs is significant, and that the accuracy improves a great deal when spectra from weathered MPs, as well as those from pristine plastics, are used in identification methods. Optimum experiment conditions, naturally, vary depending on MPs' characteristics, the first of which is selecting the 'right' laser to suppress any fluorescence baseline. The XploRA PLUS can house three lasers from blue to near-infrared (NIR), and switch between them easily with a single click, making laser selection a quick and intuitive operation.

One of the most neglected steps in MP analysis is sample presentation. It is necessary to place MPs on some kind of substrate to present it to a Raman microscope. The substrate may be a petri dish, a glass slide or a filter on a filter holder. It is possible to get interference from substrates. For example, the substrate may be Raman active, and its spectral features mix with MP spectra. Confocality of the spectrometer suppresses signals from substrates by suppressing out of focus signal. The XploRA PLUS employs a true confocal optical design for the Raman scattering beam path, making it ideal to acquire 'clean' Raman spectra by minimizing non-sample signals.

As Dr. Andrew Whitley's article in this issue of Readout mentioned, assessing MPs in environments for its quantity, migration and hazard requires statistical analysis of a massive amount MPs. Automated high throughput analysis is absolutely necessary, and a critical research goal. While research groups, with collaboration with HORIBA, are developing SOPs to become the template of automated analysis, a few elements are already identified as necessary.

MPs are often presented for analysis arrayed in a petri dish or retained on a filter. The size of a petri dish or a filter is much bigger than MPs, and navigation requires the stage travel a long distance while stopping at precise positions. A motorized stage with precision control makes navigation easier and more precise, making the experiment more efficient.

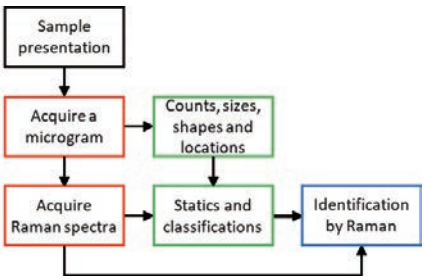

Figure 4 Workflow diagram of PF operation followed by identification by Raman library search. Red boxes highlights data acquisition steps in PF, green results generated in PF, and blue results coupled with library search

Table 2 Summary of HORIBA technologies useful for MPs analysis

Raman spectroscopy	Fluorescence spectroscopy	Particle size analysis	EDXRF† spectroscopy
Chemical identification & differentiation of: • Polymers • Additives • Dyes/Pigments • Natural particles	• Probe effect of MPs on DOM* • Determine concentration of NPs • Quantify MPs for toxicity studies • Measure DOM in control studies	• Measure particle size distribution of possible MPs • Viewsizer - Potential for nanoparticle tracking analysis for NP's	• Analyze metals accumulated on MPs[16]

* DOM stands for dissolved organic matter
† EDXRF stands for energy dispersive x-ray fluorescence

Another advantage of a motorized stage is software control and programmed movements. Coupling this ability to the microscopy functionalities of the XploRA PLUS, HORIBA developed an application module called ParticleFinder (PF)[14] on the LS6 platform. PF acquires a microgram, and analyzes the counts, sizes, shapes, and locations of MPs. PF then moves the stage to each of the MP's location, and acquires a Raman spectrum. The final results include total counts of all MPs, statistics of sizes and shapes, location and Raman spectrum of each MP, and spectral classifications.

As mentioned repeatedly, MPs are highly complex and heterogeneity targets for analysis. Data processing and analysis are proportionally complex[15] requiring sophisticated software. LS6 offers extensive functionalities for data processing (e.g. baseline correction, smoothing, substrate spectrum subtraction, etc.), data analysis (e.g. multivariate analysis, library search, etc.) and visualization (e.g. image rendering) both developed in house and working with business collaborators: Bio-Rad Laboratories, Inc. to incorporate KnowItAll® library search, and Eigenvector Research Inc. for multivariate analysis (MVA).

HORIBA technologies for MPs analysis

The complexity and heterogeneity of MP analysis requires, as demonstrated in the collaboration work with NOAA and NIST above, multimodal imaging and multi-technology approaches. It has, therefore, become a natural focus of interest how to combine, correlate and compare data and results from multiple sources. For now, we are still tackling data from one technology at a time. However, we are preparing for next steps, investigating future development possibilities such as data fusion, correlated microscopy and machine learning.

Conclusion

Microplastic (MP) is a mega trend that is growing even bigger presently. HORIBA is collaborating with KOLs of the field to determine (and influence) the market demand (and trend), and staying relevant to this newly emerging field. We have made an impression as 'the' Raman company for researchers who will provide guidelines to MPs regulations. The scientific and business opportunities are abundant, and we are making good progress.

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

References

[1] Production, use, and fate of all plastics ever made, Roland Geyer, Jenna R. Jambeck, and Kara Lavender Law, Science Advances, 19 Jul 2017, Vol. 3, No. 7, e1700782, DOI: 10.1126/sciadv.1700782

[2] Toxicological considerations of nano-sized plastics, Phoebe A. Stapleton, Environmental Science, 22 October 2019, Vol. 6, No. 5, 367-378. DOI: 10.3934/environsci.2019.5.367

[3] A Comparison Of multiple Techniques, FT-IR, Py-GC-MS, and Raman for Polymer Identification in Plastic Marine Debris; Eunah Lee, Ashok Deshpande, Jennifer Lynch, Kayla Brignac, Melissa Jung, Nigel Lascelles, Dante Freeman, Davielle Drayton, Bridget O'Donnell, 13-18 October 2019, SciX 2019 final program, 58, https://www.scixconference.org/resources/Documents/SciX2019FinalProgramWeb.pdf

[4] Increasing the Accessibility for Characterizing Microplastics: Introducing New Application-based and Spectral Libraries of Plastic Particles (SLoPP & Slopp-e); Bridget O'Donnell, Keenan Munno, Hannah De Frond, Chelsea Rochman, 13-18 October 2019, SciX 2019 final program, 62, https://www.scixconference.org/resources/Documents/SciX2019FinalProgramWeb.pdf

[5] Rochman Lab, Freshwater and Marine Ecology, Ecotoxicology, Environmental Chemistry, Conservation, https://rochmanlab.com/

[6] Microplastic Spectral Libraries: SLoPP and SLoPP-E Raman Spectral Libraries for Microplastics Research, https://rochmanlab.com/slopp-and-slopp-e-raman-spectral-libraries-for-microplastics-research/

[7] Reproduction of Figure S1.C of Supporting Information of Marine Debris Polymers on Main Hawaiian Island Beaches, Sea Surface, and Seafloor, Kayla C. Brignac, Melissa R. Jung, Cheryl King, Sarah-Jeanne Royer, Lauren Blickley, Megan R. Lamson, James T. Potemra, and Jennifer M. Lynch, Environmental Science & Technology, 9 October 2019, Vol. 53, No. 21, 12218-12226, DOI: 10.1021/acs.est.9b03561

[8] The Biochemical and Exposure Science Group of NIST, https://www.nist.gov/mml/csd/biochemical-and-exposure-science-group

[9] Northeast Fisheries Science Center at NOAA, https://nefsc.noaa.gov/

[10] Research interests of Southern California Coastal Water Research Project for Microplastics as a Part of Trash Pollution, https://www.sccwrp.org/about/research-areas/additional-research-areas/trash-pollution/

[11] Details and specifications of BallProbe® by MarqMetrix for touch and immersed Raman measurements, https://www.marqmetrix.com/products/ballprobes/

[12] An overview of chemical additives present in plastics: Migration, release, fate and environmental impact during their use, disposal and recycling, John N. Hahladakis, Costas A. Velis, Roland Weber, Eleni Iacovidou, Phil Purnell, Journal of Hazardous Materials, 15 February 2018, Vol. 344, 179-199, DOI: 10.1016/j.jhazmat.2017.10.014

[13] Application of a Hybrid Fusion Classification Process for Identification of Microplastics Based on FT-IR Spectroscopy, Beauty K. Chabuka, John H. Kalivas, Applied Spectroscopy (in printing)

[14] Details and specifications of ParticleFinder application module on LabSpec 6 Spectroscopy Suite platform, https://www.horiba.com/en_en/products/detail/action/show/Product/ParticleFinder-1657/

[15] Review of Microplastic Data Processing and Interpretation Techniques with Future Outlook, Win Cowger, Andrew Gray, Silke H. Christiansen, Hannah DeFrond, Ashok Deshpande, Ludovic Hemabessiere, Eunah Lee, Leonid Mill, Keenan Munno, Barbara E. Oßmann, Marco Pittroff, Chelsea Rochman, George Sarau, Shannon Tarby, Sebastian Primpke, Applied Spectroscopy (in printing)

[16] Long-Term Sorption of Metals Is Similar among Plastic Types: Implications for Plastic Debris in Aquatic Environments, Chelsea M. Rochman, Brian T. Hentschel, Swee J. The, 15 January 2014, PLoS ONE, Vol. 9, No. 1, e85433, DOI: 10.1371/journal.pone.0085433

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A Focus on HORIBA European Network Activity around Microplastics

マイクロプラスチックの課題に対する欧州でのHORIBAの取り組み

Florian FORMANEK
フローリアン フロマネック

Current research on microplastics, ranging from discovering or confirming their presence in various environments, to quantifying their induced pollution in food matrices, to understanding their impact on wildlife, ecosystems and even human health has become a hot topic worldwide. Europe has always been attentive to environmental issues and prompt to sponsor coordinated programs at the academic level, but also to regulate the different industries whose activities may generate microplastics. This featured article will detail a few initiatives from HORIBA Scientific in Europe to contribute to the harmonization of analytical methods, including sample collection and preparation, as well as to the development and validation of standard reference samples in the field of microplastics research.

近年，環境マイクロプラスチックの研究は世界中で注目を集めている。その研究範囲はさまざまな環境下におけるマイクロプラスチックの存在を確認するところから始まり，食品への混入量，野生生物や生態系，さらには人間の健康に及ぼす影響評価にまで及んでいる。ヨーロッパでは環境問題に対する関心が非常に高く，産学連携という学術レベルでの連携プログラム構築の推進にとどまらず，マイクロプラスチックの発生源になると想定される多くの産業に対し規制をかけてゆく可能性が非常に高いと想定される。本項では，環境マイクロプラスチック研究で必要な標準試料の開発・検証だけでなく，サンプリング，前処理を含む分析方法の標準化に力を入れてきた，欧州HORIBAグループ科学セグメント(HORIBA Scientific in Europe)の取り組みを紹介する。

Introduction

Microplastics (MPs) are present in every environmental compartment, including in the remotest places on earth, and have gained recent interest as a major environmental pollutant. In 2015, the European Union (EU) produced 25 million tons of plastic waste, with 60% still originating from packaging, representing an average of 31 kg per person per year. Worth noting is the fact that the majority of the MPs released in the ocean originate from synthetic textiles, tire dust or city dust.

“Plastic” is not a well-defined term, but rather encompasses a set of synthetic polymeric materials having a wide range of high molecular weight, and whose particle dimensions span 6 orders of magnitude in size, from the nanometer up to 5 mm. MPs present a large variety of chemical compositions: (co)polymers, residual monomers, chemical additives, catalysts or fillers, and can even be contaminated by non-intentionally added substances. While naturally occurring polymers exist, such as rubber or cotton, plastic pollution mostly originates from a few synthetic polymeric families like Polystyrene (PS), polyethylene (PE), polypropylene (PP), polyvinylchloride (PVC) or polyethylene terephthalate (PET).

This diversity gave rise to a search of a variety of methodologies to answer the burning questions in MPs research and to support plastic pollution monitoring and mitigation policies under consideration by state and non-state actors. The existence of various definitions for different regulatory sectors and regions also complicates understanding and implementation of legislation.

Moreover, no validated and harmonized standard methods are currently available for the analysis of MPs and many

analytical protocols and techniques are used. There is still no consensus on the reporting format, in terms of number of particles, mass of size fractions, and an absence of certified reference materials to investigate analytical proficiencies.

Those points were highlighted during the Global Summit on Regulatory Science (GSRS) 2019 Nanotechnology and Nanoplastics which took place in Ispra (Italy) in September 2019,[1] organized by the European Commission Joint Research Center (JRC), whose mission is to provide scientific advice and support to the European Union policy.

All this explains why open interlaboratory studies were recently set up in order to address those shortcomings.

Evidence of microplastics in food

HORIBA Scientific is proud to count world-leading research teams among his customers of Raman spectrometers. Their affiliations reveal the wide range of fields where Raman microscopy is used to study MPs: environment institutes, health and food safety authorities, oceanology and hydrology departments, marine biology agencies, ecotoxicology laboratories, but also water treatment and distribution entities or bottled water companies.

Clearly, the most pressing question on the scientific community agenda is whether or not MPs pose a threat to human health, especially through seafood consumption. To that extend, the European Food Safety Authority (EFSA) Panel for Contaminants in the Food Chain (CONTAM) was asked, following a request from the German Federal Institute for Risk Assessment (BfR), to deliver a statement on the presence of MP (but also nanoplastics) in food, with particular focus on seafood.[2]

This bibliographic review confirmed that MPs can be ingested by many marine invertebrates and have the potential to be transferred between trophic levels, as illustrated in Figure 1. Indeed, the presence of plastic debris, indicated as anthropogenic debris, in the gastrointestinal tract of fished on sale for human consumption was sampled from markets in several countries.

Following on this statement, a team of researchers from IFREMER (French Research Institute for Exploitation of the Sea) and ANSES (French Agency for Food, Environmental and Occupational Health & Safety) has developed a protocol to extract and characterize MPs from seafood tissues, which should be implemented to assure the relevance and comparison of further studies or assess seafood product quality, notably to follow recommendation from the OSPAR Convention for the Protection of the Marine Environment of the North-East Atlantic,

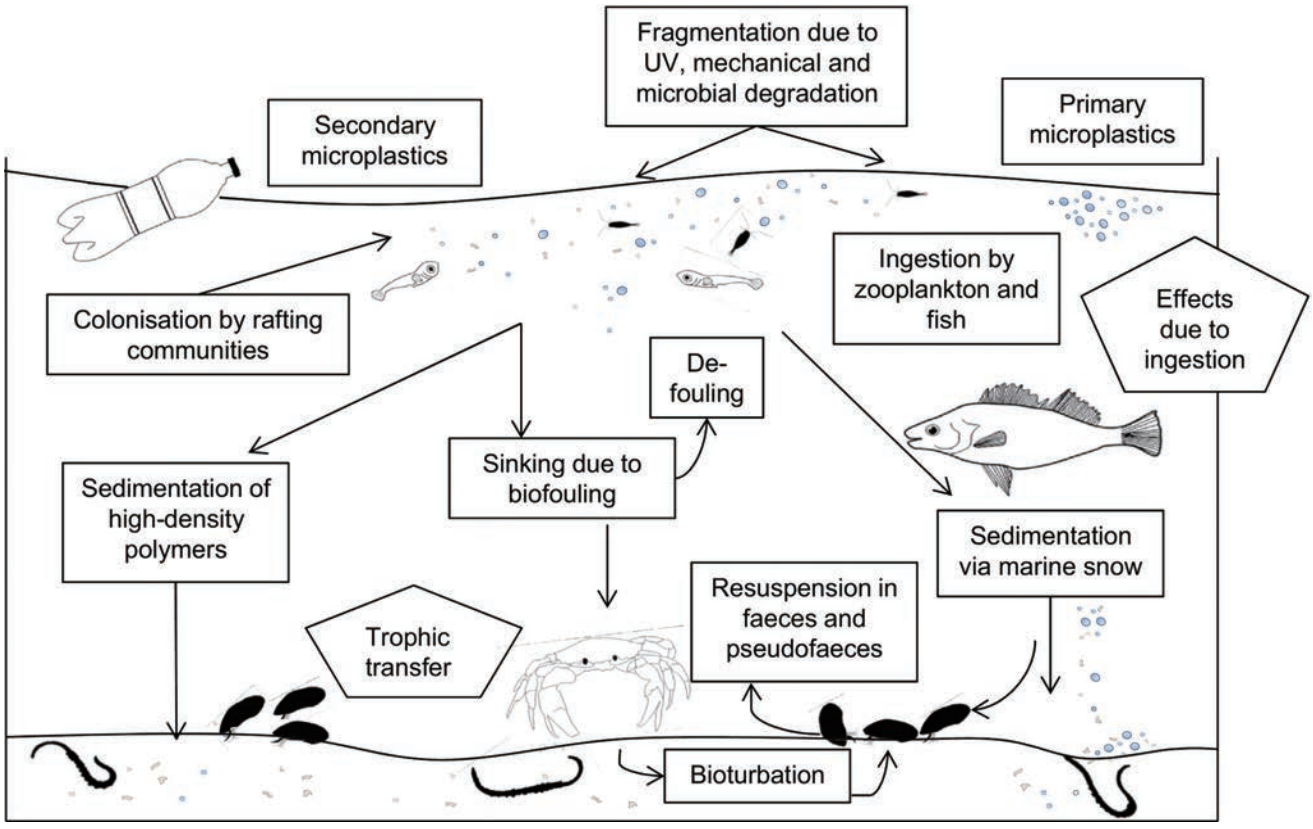

Figure 1 Potential pathways for the transport of microplastics and their biological interactions.[1]

ratified by 15 EU countries in relation with the Marine Strategy Framework Directive.

Plastic integrity and composition was evaluated through microscopic inspection and the use of HORIBA LabRAM Raman spectrometer, before and after digestion by KOH 10% solution with 24 h incubation at 60°C.[3]

Again, related to fish meal pollution, HORIBA Scientific recently participated in a study which demonstrated that cultured (farmed) organisms could be exposed to high levels of MPs via contaminated fish/shellfish used in fish meal production by the aquaculture industry.[4] The most abundant isolated plastic polymer was PE (63.0%) followed by PP (27.8%) and PET (8.8%), while the average size of the particles was found to be 855 µm.

Another publication coauthored by HORIBA Scientific in Scientific Reports[5] made the headlines when it revealed the presence of MPs even in commercial sea and lake salts originating from 8 different countries (Figure 2). This study also raised concerns over the possible transfer of other contaminants associated with MPs into salt, such as pigment fragments, some of them being toxic.

Another milestone article investigated the presence of MPs in mineral waters from different bottle types.[6] Led by scientists from the Bavarian Health and Food Safety Authority (LGL) in Germany, the team focused on small particles (below 5 µm) posing higher toxicological risks as they have the potential to translocate into body tissues and are more likely to penetrate deeply into organs. Using a HORIBA XploRA PLUS system to locate and identify particles down to a size of 1 µm on specially prepared aluminum coated polycarbonatemembrane filters, evidence of higher amounts of MPs in reusable bottles (PET as well as glass) was found compared to single use bottles.

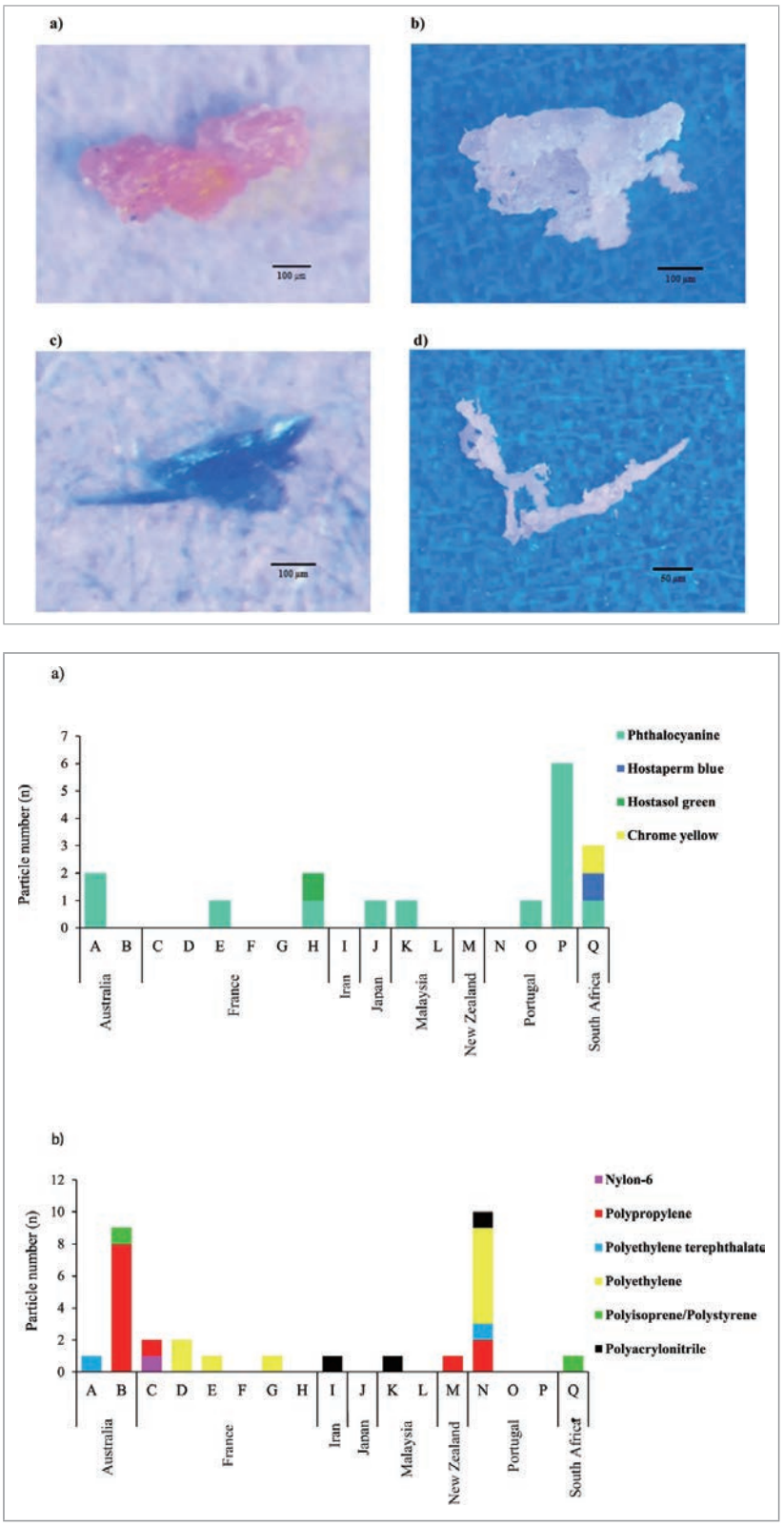

Figure 2 Top: Microscopic images of some of the extracted particles. (a) polyisoprene/polystyrene, (b) polyethylene, and (c) pigment (phthalocyanine) fragment. Image (d) is a nylon-6 filament.
Bottom: Stacked bar chart of the number of (a) plastic polymer and (b) pigment particles isolated from different salt brands.[5]

Open interlaboratory studies

A recent meeting hosted by the Group of Chief Scientific Advisors of the European Commission,[7] supported by the evidence review of the SAPEA Consortium (Science

Advice for Policy by European Academies),[8] concluded on the lack of harmonized methodologies in order to generate standardized data. On this basis, several organizations initiated collaborative programs with the aim of validating internal laboratories quality assessment and competence, supporting environmental data, providing data for national and international stakeholders or supporting accreditation.

One of the initiatives in which HORIBA took part was set up by the Vrije Universiteit Amsterdam (VUA), the Norwegian Institute for Water Research (NIVA) and the WEPAL (Wageningen Evaluating Programmes for Analytical Laboratories) organization based in the Netherland and recognized by the Dutch Accreditation Council (RvA).

This international interlaboratory study on MPs, called QUASIMEME for "Quality Assurance of Information in Marine Environmental monitoring", saw 34 laboratories participating to analyze the test materials between May in August 2019, using several instrumental and quantification methods, with the objective of counting the particles and identifying their chemical family.

Test samples were prepared at NIVA, to enable the analysis by a broad variety of analytical methods and techniques: visual, hyperspectral imaging, Fourier transform Infrared Spectroscopy (FT-IR), Raman and Mass Spectrometry ; and consisted of 6 preproduction pellets, 5 tablets containing microplastics fragments (obtained after filtration of PET, PVC and PS powder) of fibres and 1 blank tablet. The fibres were created by washing polyester blankets in a typical domestic washing machine. While the majority of the participating laboratories used ATR-FT-IR (Attenuated Total Reflection FT-IR) or µ-FT-IR, we employed Raman microscopy (Figure 3), which is favorable for small size particles, typically below 20 µm.

Table 1 shows an example of reported table by the participants for one of the tablet sample. Although some polymer misidentification occurred in some cases, the polymer type was correctly assigned for both larger preproduction pellets (2-4 mm) and particles or fibres added to the tablets (150-300 µm). However, the reported number of particles varied considerably (up to 78% standard deviation), and the standard deviations of the determination of the polymer type in the tablets varied from 29% (for PET) to 99% (for PS).

Overall, the results of this first round indicate that polymer identification and quantification of the number of plastics particles in a sample (especially in smaller size fractions) is not simple of straightforward. Yet, HORIBA's Applications Laboratory was able to demonstrate analytical results on par with recognized European facilities.

This round of the QUASIMEME study will be followed by exercises with increasing complexity and difficulty of samples, including MPs extracted from complex matrices (e.g. sediments and fishes). After several study rounds, the analytical methodologies for MPs are expected to be better comparable and will be included in a routine profi-

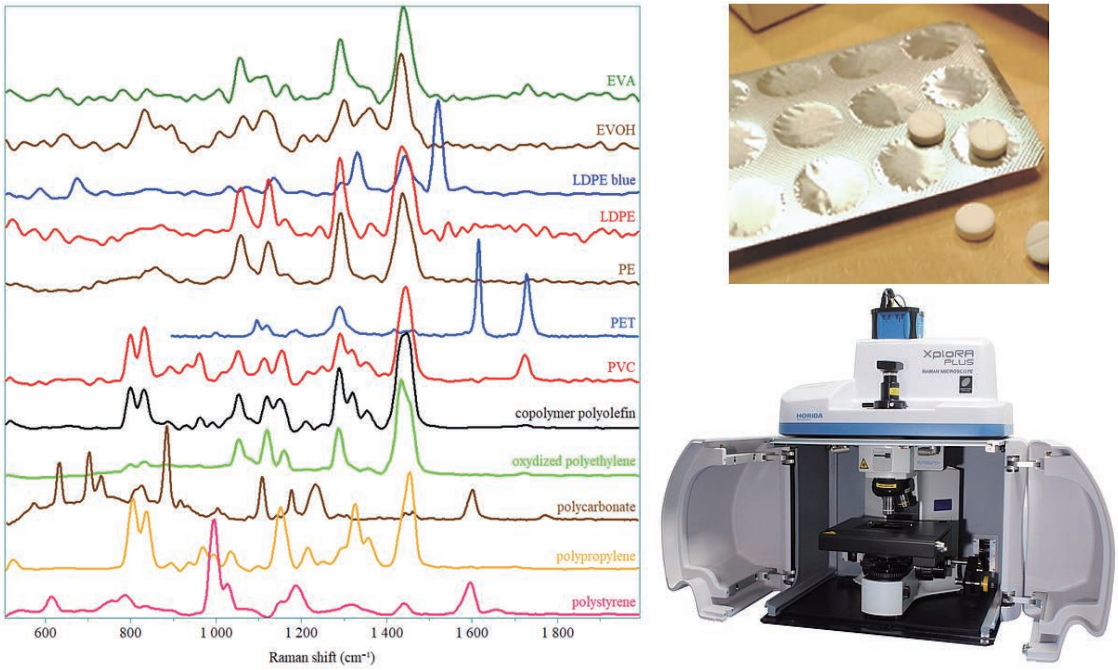

Figure 3 Left: Raman spectra recorded on different polymer families.
Top right: aluminum strip pellet containing 12 tablets sent to participants, that were to be dissolved in analytical grade water to control background contamination.
Bottom right: HORIBA Xplora PLUS Raman microscope with class I laser enclosure used for this study.

Table 1 Type and number of plastic particles reported for table in position no. 10 in the strip pellet shown in Figure 3, by all the participating laboratories of the QUASIMEME study.

Laboratory	acrylonitrile butadiene styrene	Black fiber	Blue fiber	Cellulose	Cellulose fiber black	Cellulose fiber white	Crystaline particles	Grey fiber	Grey piece	High density polyethylene	Low-density polyethylene	polymethylmethacrylate	Polyamide	polybutylmethacrylate	Polycarbonate	Polyester	Polyethylene	polyethylene terephthalate	Polypropylene	Polystyrene	Polytetrafluoroethylene	Polyurethane	Polyvinylchloride	red fiber	TiO2	Unknown	Total particles
H221																44											44
Q101				8														5	< 3	8			14				35
Q104					1	85												9		13			18				126
Q110																		3								4	7
Q114																		2								28	30
Q134											1							7	1	26			26				61
Q152																							34			3	37
Q153																										48	48
Q871																		7	2	37							46
Q968		2						1	1																		4
Q3175			3			6														10				2			21
Q3231																8				13						19	40
Q3239																											
Q3872																	8	11		33			21				73
Q3873	1											1					30		8	39							79
Q3876																				5			10				15
Q3877																											
Q3878																		31		22							53
Q3879																		5	5	6			17				33
Q3882																				11			30				41
Q3883																8				17			19				44
Q3884																				28			27				55
Q3885																		1		6			20				27
Q3887																		7		23			26				56
Q3888	1										8			1				10	6	51	2	1	10				90
Q3889							17			7																	24
Q3890													1					3		29			32				65
Q3891															30			6		12			14	3			65
Q3892																		3		2			11				16
Q3894																											
No. of reporting labs	2	1	1	1	1	2	1	1	1	1	2	1	1	1	1	3	2	15	6	20	1	1	16	1	1	5	27
Average	1	2	3	8	1	46	17	1	1	7	4.5	1	1	1	30	20	19	5.5	4.4	17.1	2	1	20.2	2	3	18.5	42.0
Standard deviation						56					4.9					21	16	4.0	3.9	13.3			9.25			20.9	24.3

ciency testing scheme.

HORIBA Scientific also recently responded to a call to enter an exploratory study organized by the JRC, with support from the German Federal Institute for Materials Research and Testing (BAM).[9] The aim of this proficiency test study on MPs in water in sediments is to help in the identification of possible method candidates for future validation and standardization.

In practice, reference samples employed to benchmark laboratories were developed and qualified beforehand. Those samples were sent to the different laboratories to be prepared on site through a reconstitution protocol, from vials containing a NaCl-carrier with embedded PET particles, a surfactant solution (triton X-100), and deionized water. Participants are to report the number of particles or mass of particles above 30 µm, the particles identified as PET, particles identified as plastic (including PET) and particles of any kind, with a report of the measurement uncertainty.

A workshop will take place during the summer of 2020 to discuss the results and conclusions once the participants report their findings.

Finally, HORIBA France is actively involved in a group of experts within the French Standardization Association (AFNOR) currently working on establishing a regulation on the analysis of MPs in drinking water, through spectroscopic techniques (µFT-IR and Raman). This group, part of the T91M "*Organic micropollutants*" Commission, gathers various governmental, academic and industry organizations, including the Standardization Bureau for Plastics and Plastics Engineering (BNPP), with the objective of drawing up a new norm for the first half of 2021.
This work was presented at the last ISO (International Organization for Standardization) meeting held by the Technical Committee TC 147 on Water Quality in Tokyo, with the purpose of reaching a global consensus in the near future.

Challenge and perspectives

With the improvement of the robustness of analytical techniques, researchers working in the field of MPs will more easily be able to trust their results and compare their studies.

The most pressing question to answer, as little is known at this point, concerns the toxicity of MPs on human health. In particular, an important aspect revolves on the fact that MPs both absorb and give off toxic chemicals and harmful pollutants, which may build up over time and

stay in the environment.

There is also a clear lack of knowledge on nanoplastics (particles smaller than 0.1 µm), which may represent a greater risk to the environment and health. However, their characterization is currently hindered by technical limitations and will require new instrumental developments.

To conclude, it is worth mentioning that the microplastics scientific community will gather at MICRO 2020[10] in Arrecife (Spain), the major biannual international conference focusing on the fate and impacts of microplastics.

HORIBA Scientific will be present !

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

References

[1] https://ec.europa.eu/jrc/en/event/conference/gsrs19-global-summit-regulatory-science-2019-nanotechnology-and-nanoplastics

[2] Presence of microplastics and nanoplastics in food, with particular focus on seafood. *EFSA Journal*, **14**(6), 4501 (2016).

[3] Alexandre Dehaut, Anne-Laure Cassone, Laura Frère, Ludovic Hermabessiere, Charlotte Himber, Emmanuel Rinnert, Gilles Rivière, Christophe Lambert, Philippe Soudant, Arnaud Huvet, Guillaume Duflos, Ika Paul-Pont. Microplastics in seafood: Benchmark protocol for their extraction and characterization. *Environmental Pollution*, **215**, 223-233 (2016).

[4] Samaneh Karbalaei, Abolfazl Golieskardi, Dorothy Uning Watt, Mathieu Boiret, Parichehr Hanachi, Tony R. Walker Ali Karami. Analysis and inorganic composition of microplastics in commercial Malaysian fish meals. *Marine Pollution Bulletin*,**150**, 110687 (2020).

[5] Ali Karami, Abolfazl Golieskardi, Cheng Keong Choo, Vincent Larat, Tamara S. Galloway & Babak Salamatinia. The presence of microplastics in commercial salts from different countries. *Scientific Reports*, **7**, 46173 (2017).

[6] Barbara E. Oßmann, George Sarau, Heinrich Holtmannspötter, Monika Pischetsrieder, Silke H. Christiansen, Wilhelm Dicke. Small-sized microplastics and pigmented particles in bottled mineral water. *Water Research*, **141**, 307-316 (2018).

[7] Environmental and Health Risks of Microplastic Pollution. doi:10.2777/54199

[8] SAPEA, Science Advice for Policy by European Academies. (2019). A Scientific Perspective on Microplastics in Nature and Society. https://doi.org/10.26356/microplastics

[9] https://ec.europa.eu/jrc/en/science-update/call-laboratories-participate-proficiency-tests-microplastics-drinking-water-and-sediments

[10] https://micro2020.sciencesconf.org/

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マイクロプラスチックに関する日本でのHORIBAグループの活動

Microplastics Related Activities in HORIBA Group Japan

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近年，プラスチックが環境中で微細化したマイクロプラスチックによる環境汚染が注目を集めている。HORIBA グループは国内外でマイクロプラスチックの計測技術確立に取り組んできた。本稿では，マイクロプラスチックの分析ニーズに対する日本でのHORIBAグループの取り組みを分析事例も交えて紹介する。

In recent years, plastics pollution has become a widely discussed international problem. Drifting into the ocean from the urban areas, plastics gradually miniaturize into small particles called microplastics, that affect ecosystem in many various ways. HORIBA Group engineers and scientists in Japan and worldwide, are working to establish the measurement techniques for microplastics analysis. In this paper, we will introduce our HORIBA Group's efforts in Japan, in response to the microplastics analysis needs, including application examples.

はじめに

プラスチック材料はその利便性から広く用いられているが，近年，廃棄されたプラスチックによる海洋汚染が国際的な課題となっている。市街地からの流入や海洋への直接の投棄によるプラスチックごみは，物理的な破壊や紫外線などで劣化し微小化する。一般的に5 mm以下のプラスチックの微小片や微粒子がマイクロプラスチック（Microplastics: MPs）と呼ばれている。2019年6月に開催されたG20大阪サミットでは，2050年までに海洋プラスチックごみによる追加的な汚染をゼロにまで削減することを目指す「大阪ブルー・オーシャン・ビジョン」が共有された。日本においても，このビジョンの達成に向け，環境省及び経済産業省が中心となり，(1)リデュース及び代替素材への転換，(2)リサイクル及び資源循環，(3)海洋プラスチックごみ対策，(4)国民運動及び普及啓発など様々な施策が取り組まれている。

MPsの測定と分析機器

MPsの測定は，海洋，河川，湖沼，下水処理水，工場排水処理水，浄水，飲料水など測定対象と目的により，測定範囲や評価項目が異なる。測定対象と分析機器（振動分光）の関係をTable 1に示す。海洋MPsの計測範囲は，粒子サイズが300 μm～5 mmを対象としているが，下水処理水や飲料水では300 μm以下のより小さなMPsを対象とした研究もあり，生態系への影響の研究分野では10 μm以下の更に小さなMPsが対象となる。

MPsの測定方法は，一つに限定するのではなく，目的に応じた測定手法を選択することが有用であると考える。

Table 1　MPsの測定対象と分析機器（振動分光）

調査対象・目的	MPsサイズ	前処理	分析機器
海洋，河川，湖沼	300 μm～5 mm	ピッキング	FT-IR
下水，工場排水	10 μm～300 μm	1次ろ過 ↓ 酸化処理 ↓ 比重分離 ↓ 2次ろ過	顕微FT-IR 顕微ラマン
浄水，飲料水	10 μm以下		顕微ラマン
食品，化粧品			
生態系影響（細胞）			

MPs測定における課題

MPsの測定は，粒子サイズ，粒子形状，組成，質量，表面積，有害化学物質含有量など評価目的により測定項目が異なり，分析方法も異なる。また，対象とする試料により，試料の採取方法，夾雑物の除去や前処理方法が異なり，MPsの粒子サイズが小さくなればなるほど難しくなる。これらは，それぞれの研究者のノウハウとなっているものも多く，試料採取から前処理を含めたMPs測定方法の標準化が重要となる。また，試料採取から前処理は，ほとんど手動で行われるため，多数の試料を測定するには多大な労力を必要と

している。試料の前処理の自動化・半自動化についても今後の課題といえるだろう。

日本での活動事例

前述の背景のもと，日本のアカデミアや産業界では，MPsに関するシンポジウムやセミナーが活発に開催されるようになった。HORIBAは，複数のセミナーにおいて，「MPsの粒子計測とラマン分析」をテーマに，HORIBA製品による測定事例を紹介してきた。

粒子計測においては，静的光散乱法によるレーザ回折／散乱式 粒子径分布測定装置，動的光散乱法によるナノ粒子解析装置，ナノトラッキング法によるナノ粒子径分布・濃度測定装置を紹介し，広い粒子径範囲（mm～μm～nm）における粒子径分布，粒子数濃度，凝集状態の測定を高い再現性で自動化できることを示した。ラマン分析においては，顕微ラマン分光装置と粒子解析機能（ParticleFinder）を組み合わせた測定の自動化への対応事例を紹介し，粒子サイズ，形状，粒子数計測と組成を関連付けて解析できることを示した。**Table 2**にHORIBAが参加したMPs測定に関するシンポジウムとセミナーの一覧を，**Figure 1**にセミナーで紹介した装置の外観を示す。

MPs模擬試料の分析事例

MPsは海洋や河川のような環境水中以外にも，大気，飲料水，食品パッケージから検出の報告[2]がされている。ここでは，環境水中からサンプリングされたMPsを想定した模擬試料の計測例と，大気中から得られたMPsの分析例を紹介する。

試料には，ポリプロピレン（PP），ポリウレタン（PU），ポリメチルメタクリレート（PMMA），ポリエチレンテレフタレート（PET）を粉砕したMPs模擬試料を供し，赤外分光分析により組成分析を，レーザ回折／散乱式粒子径分布測定装置および，装置内蔵オプションのImaging Unit を用いて粒子径分布と画像観察を行った[3]。以下に，それぞれの測定原理を説明する。

赤外分光分析は，試料に赤外線を照射した際の吸収波長と吸収量から得られる赤外吸収スペクトルから組成分析を行う分析手法である。顕微型のフーリエ変換赤外分光光度計（FT-IR）は，赤外線を集光することで約10 μmϕの空間分解能を持ち，かつ電動ステージとの組み合わせによりイメージングが可能な分析装置である。

レーザ回折／散乱式 粒子径分布測定装置は，試料の粒子径分布を計測することができる。試料に一定波長の入射光を照射すると，散乱光強度角度分布は，粒子径の大きさに応

Table 2　HORIBAが参加したMPs測定に関するシンポジウム，セミナー

イベント名	開催日	テーマ	主催
JASISカンファレンス	2019年9月6日	マイクロプラスチックの計測と環境影響	（一社）日本環境化学会 （一社）日本分析機器工業会
JETAセミナー	2019年11月28日	環境水中におけるマイクロプラスチックの測定技術	（公社）日本環境技術協会
AISTシンポジウム	2019年12月2日	マイクロプラスチックの計測・評価を考える	（国立研究開発法人）産業技術総合研究所

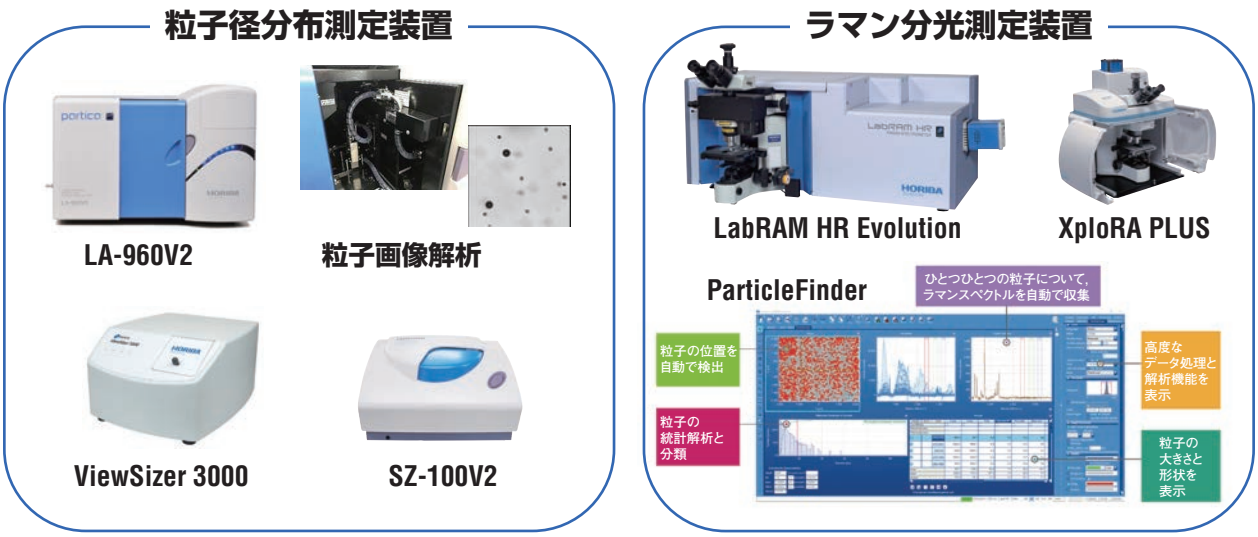

Figure 1　MPsの測定に応用できるHORIBAの分析装置

じて変化する。この回折/散乱光角度分布のパターンを解析することで，試料に含まれる粒子径分布を得ることができる。試料はフローセル内を循環し，数百万個のレベルで粒子を計測することから，顕微鏡などを使った計数方法と比較し，統計的に高い精度と測定再現性を得ることができる。レーザ回折／散乱式 粒子径分布測定装置の光学配置図を**Figure 2**に示す。また，この装置には画像解析に用いるImaging Unitを内蔵することができる。測定装置内のセル背面から白色光を照射し，セル内の粒子をストロボカメラで透過像を撮影する。液中の湿式循環システム内の粒子をリアルタイムに観察し，画像粒子解析結果から粒子径分布のヒストグラムなどを確認することができる。

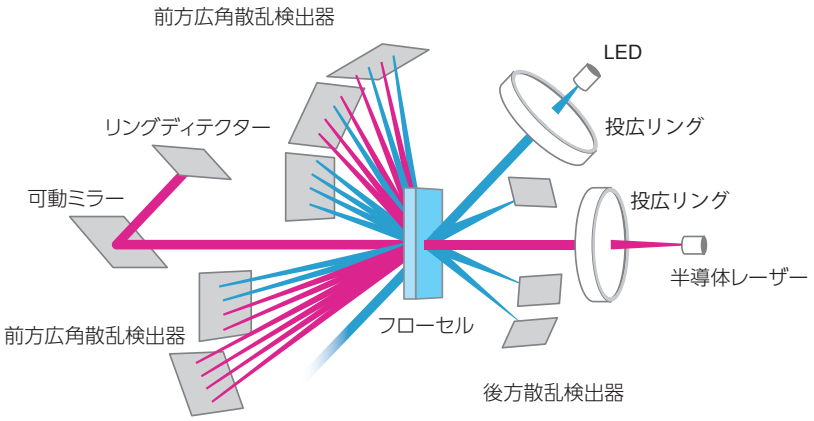

Figure 2　レーザ回折／散乱式 粒子径分布測定装置の光学配置図

MPsの分析には，海洋や河川から採水後，MPsのみを前処理により分離したのち，粒子数の計測とFT-IRによる組成分析を行う手法が広く取り入れられている。そこで，PP，PU，PMMA，PETのバルク材料を粉砕した粉末を作成し模擬試料とした。試料の写真を**Figure 3(a)**に示す。得られた模擬試料を金属基板上に分散し，顕微FT-IRにて赤外反射吸収イメージングを行った。各測定ポイントから得られたスペクトルを主成分解析し，PP，PU，PMMA，PETの分布図を得た。測定結果（**Figure 3(b)**，**(c)**）に示すように，観察像で見られたすべての粒子を同定することができた。

次に，同一の模擬試料をレーザ回折／散乱式粒子径分布測定装置で計測した結果，10 μm～2 mmの範囲で粒子径分布が確認できた。**Figure 3(d)**に示すように，高精度な結果を得られることがわかる。

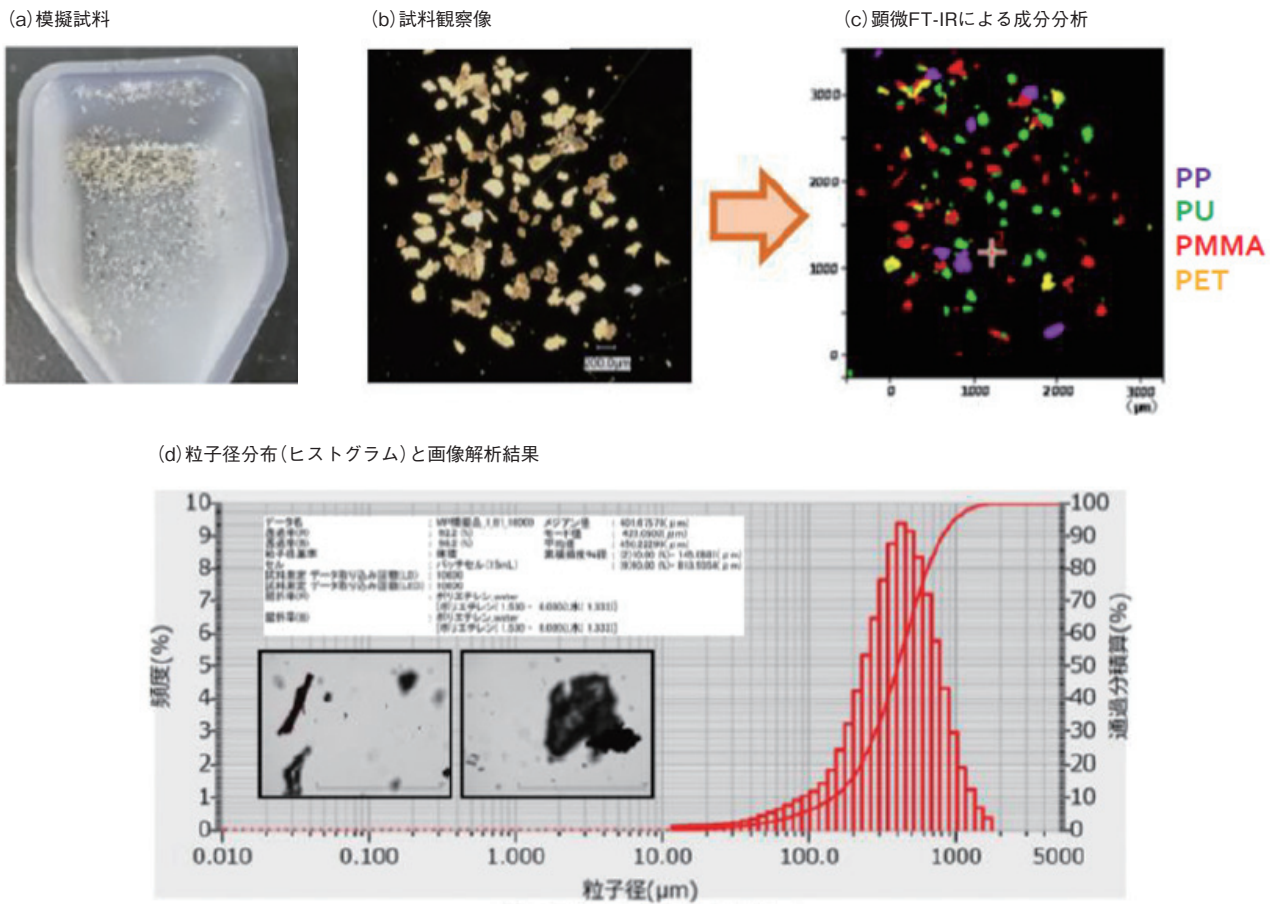

Figure 3　模擬試料の顕微FT-R，粒子計測装置による複合分析結果
＊本データは，株式会社東レリサーチセンター様のご厚意によりご提供いただいた。

また，挿入画像に示すように，同時に画像を確認することで，形状の異なる様々な粒子がある様子を観察することができた。

大気中マイクロプラスチックの分析事例

大気中のMPs（Airborne microplastics: AMPs）は，都市部だけでなく，高山，北極圏からも発見の報告があり，大気を通じてマイクロプラスチック汚染が広がっている可能性が示唆されている[4]。また，大気中に浮遊する直径10 μm以下のMPsは，呼吸を通して体内に取り込まれる可能性があり，環境への影響だけでなく，健康への影響が懸念されている[5]。

自由対流圏におけるAMPsの測定を行った。富士山頂で夜間にサイクロン式PM2.5分級装置付ハイボリウムエアサンプラー（柴田科学）によりテフロンフィルター上にPM2.5を採取し，前処理を行った後，組成分析を行った。AMPsは，想定されるサイズが10 μm以下と小さいため，顕微FT-IRによる測定は困難と考え，空間分解能に優れる顕微ラマン分光装置を組成分析に用いた。

顕微ラマン分光装置は，試料にレーザを照射することで得られるラマン散乱光を測定することで，組成分析や結晶性評価を行うことができる。顕微鏡と電動ステージとの組み合わせにより，サブミクロンオーダーでのイメージングができる。

採取されたAMPsをアルミナフィルター上に移し，4領域（1 mm^2/領域）をマッピング測定し，有機物由来のCH基伸縮振動由来のラマンピーク強度を用いてAMPsを含む有機物粒子の分布図を得た。測定領域からは約30個のAMPsが検出された。顕微ラマン分光装置によってCH基由来のピークが検出された部位をポイント分析し，スペクトル解析したところ，15種類の樹脂に同定された。参考にフィルター上の1粒子の試料観察像を**Figure 4**に，1領域からのCH基のラマンピークの強度分布図を**Figure 5**に示す。また，**Figure 5**の領域から得られたAMPsのサイズと組成を**Table 3**に示す。4領域のマッピングにより検出されたAMPsにはPPの粒子数が37%と最も多く含まれていた。他には，PUに加え，生分解性プラスチックであるポリヒドロキシ酪酸などが同定された。本測定により検出されたAMPsの数を大気中個数濃度に換算すると4.47個/m^3であった。これらの結果から，直径10 μm以下のMPs粒子を含む広い粒子径範囲に対して，組成分析への顕微ラマン分光装置の応用が期待される。

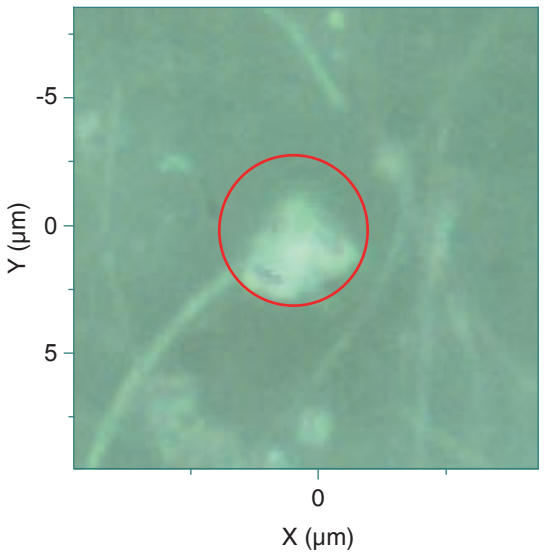

Figure 4　1粒子の観察像

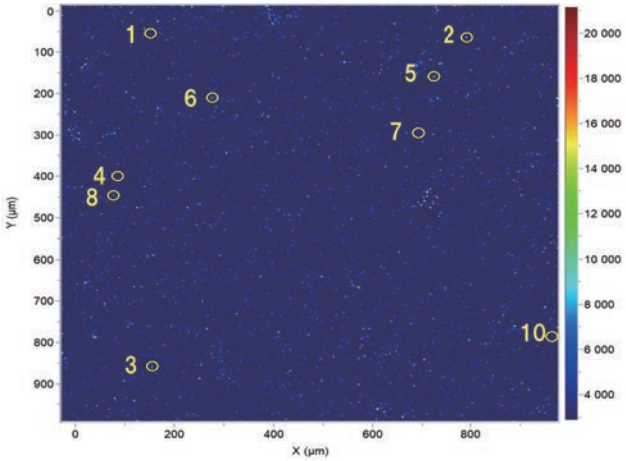

Figure 5　ラマンピーク（CH基由来）強度分布
ポイント1～10から有機物由来のピークを確認。

Table 3　検出したAMPsのサイズと組成

target	Size/μm	identified compound by Library search
1	8（diameter）	Polystyrene（PS）
2	6（Maj axis），4（Min axis）	Unidentified polymer + TiO_2
3	3（diameter）	Polyester
4	4（diameter）	Polypropylene（PP）
5	6（Maj axis），4（Min axis）	Polyurethane（PU）
6	12（Maj axis），3（Min axis）	Polyethylene（PE）
7	1.4（diameter）	Poly-3-Hydroxyl Butyl acid
8	2（diameter）	Polyolefin
9	28（Maj axis），2（Min axis）	Palytetrafluoro ethylene（PTFE）
10	ND	Polyolefin

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＊編集局注：本内容は特段の記載がない限り，本誌発行年時点での自社調査に基づいて記載しています。

参考文献

[1] 亀田豊，公益社団法人　日本環境技術協会　第28回技術交流会講演集(2019)
[2] Laura M. Hernandez, Elvis Genbo Xu, Hans C. E. Larsson, Rui Tahara, Vimal B. Maisuria, Nathalie Tufenkji, Plastic Teabags Release Billions of Microparticles and Nanoparticles into Tea, Environ. Sci. Technol., 53, 21, 12300-12310(2019)
[3] 株式会社東レリサーチセンター，No.0386 マイクロプラスチック分析法の検討と関連技術の研究開発動向調査，https://cs2.toray.co.jp/news/trc/news_rd01.nsf/0/DB586848DFC3BEDF492584B20024AA1B?open,(参照20200424)
[4] 大河内博 他，東京理科大学 研究推進機構 総合研究院 大気科学研究部門 第4回 成果報告会 要旨集,(2020)
[5] 大河内博，認定NPO法人富士山測候所を活用する会　第13回成果報告会　要旨集,(2020)

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蛍光X線硫黄・塩素分析計 MESA-7220V2

X-ray Fluorescence Sulfur/Chlorine-in-Oil-Analyzer MESA-7220V2

上田 英雄
UEDA Hideo
マイク ポール
Michael POHL

自動車用燃料中の硫黄濃度規制値は世界各国で年々厳しくなっており，10 mg/kg以下が要求されている。製油所では，原油の脱硫処理コストを低く抑えるため，原油に含まれる硫黄濃度を正確に把握しておく必要がある。新しく開発したMESA-7220V2は，硫黄濃度が数%を超える原油から，数ppmの石油製品までを簡単に測定でき，ASTM規格D7220に対応し，単色化エネルギー分散型蛍光X線分析法を適用した広い濃度域の硫黄濃度測定が可能な装置である。

キーワード
ASTM D7220
単色化エネルギー分散型蛍光エックス線分析法
単軸湾曲高配向性熱分解グラファイト
バックグランドの低減
検出下限1 mg/kg

The regulations for sulfur concentration in the automotive fuels have now started going down to 10 mg /kg around the world. In the refineries, it is necessary to control the costs for de-sulfurizing the incoming crude oil. In order to do this, it is required to know the incoming sulfur concentration as well as the clean crude oil being distilled in the refinery. The logical technology to employ is Energy Dispersive X-ray Fluorescence (EDXRF) spectrometry as it can easily measure the mass % levels of sulfur in crude oil as well as the ppm (mg/kg) levels of sulfur in the final product. The HORIBA MESA-7220V2 has been developed in order to meet these two concentration ranges and everything in between.

Key words
ASTM D7220
Monochromatic Energy Dispersive X-ray Fluorescence Spectrometry
Singly curved Highly Oriented Pyrolytic Graphite
Lower back ground
Limit of Detection less than 1 mg/kg

はじめに

全ての原油に硫黄は含まれており，精製された燃料の中にも硫黄が含まれている。硫黄が燃焼することで引き起こされる公害や，硫黄による触媒被毒を防ぐため，燃料中の硫黄濃度の規制は強化されている。米国では，ガソリン中の硫黄濃度は，第3次排ガス規制（Tier 3）によって10 mg/kg以下となり，将来的にはさらなる強化が予想されている。

米国での軽油規制に関しては，1990年台中頃に500 mg/kgであったが，2006年には15 mg/kgとなった。これらの規制は当初，自動車燃料だけを対象とするものであったが，建設現場で使用される重機や列車，家庭の暖房燃料にまで範囲を広げ，船舶業界への適用も計画進行中である。MESA-7220V2はこれらの市場の要求に応えるべく開発された。

市場からの要求を原油中の硫黄に限れば，ASTM D4294は非常に有用で，低コストを実現する分析手法である。硫黄

濃度範囲が17 mg/kg～4.6 mass %で，原油分析の要求を十分に満たしている。これらの要求に対応する製品をHORIBAは長年にわたって開発し続けており，現行モデルではSLFA-60，SLFA-6100，およびSLFAS-6800[1]がある。これらの装置は特装車両の中や，船上，試験機関などでの試料分析に使用されている。

近年，硫黄濃度規制値が減少し，D4294では要求される感度を実現できなくなってきた。そのため，分析手法の変更が必要となり，蛍光X線スペクトル上のノイズ成分を減少させ，小さな硫黄ピークでも容易に検出し，定量できる手法が採用された。これは微量硫黄検出専用のX線光学系を用いて，不必要な信号がスペクトルに現れないようにする手法であった。この取り組みによって，MESA-7220とASTM規格D7220が出来上がった。

ASTM規格D7220-12

ASTM規格D7220の改訂版は2012年に作成され，新しい燃料分析の要求に沿った規格となった。その規格は，多くの石油製品を対象としたもので，ガソリンをはじめ，家庭での暖房用やジェット機用の燃料までも含んでいる。濃度範囲は3 mg/kg～942 mg/kgで，エネルギー分散型蛍光X線分析手法を基に，多くの新しいX線光学開発要素を取り込んでいる。例えば，銀またはパラジウムをターゲット材とするエンドウインドウ型のX線管が指定されている。X線管から発せられたX線は，X線回折格子として機能する高配向性熱分解グラファイト（HOPG）の結晶に照射される。この光学系ではブラッグの条件を満たす銀もしくはパラジウムのエネルギーだけに単色化され，分析対象を入れた試料セルに集光される。

試料から発生する硫黄の蛍光X線の検出に用いられるのは，シリコンドリフト検出器（SDD）が一般的で，エネルギー分解能が175 eV（5.9 keVかつ10 kcpsの条件）以下と規定することでスペクトル上の塩素ピークを硫黄ピークから分離することができる。石油製品試料にこれら2つの元素が含まれていても，ピークを分離することができるため，硫黄定量への塩素影響を防止することができる。

また，大気中の微量アルゴンによる影響を避けるために，X線光学系を4.0 kPa以下の真空に保つ必要があり，さらに得られた信号を整え，データを処理する電気回路を備えておく必要がある。これらの技術によって，X線強度計測やスペクトル解析が確かなものとなり，X線スペクトルのバックグランド減算やピークデコンボリューション，そしてピークの重なり補正計算が行われ，最終的にX線強度から硫黄濃度への演算が行われる。

X線光学系とその性能

MESA-7220V2は，銀ターゲットのエンドウインドウX線管と，Ag-L α 線だけを抽出するHOPG結晶を備えた単色化エネルギー分散型蛍光X線分析装置である。単色化されたX線は試料セルに入射し，試料から発生するX線をSDDで検出する。HOPG結晶[2]はポリイミドフィルムを層状に重ね合わせて，高温高圧下で成型されて作製され，得られた黒鉛ブロックは高配向性のカーボン層で構成されている。この材料は，**Figure 1**に示すように，2.98 ± 0.02 keVのAg-L α 線だけを単色化して取り出す回折格子として機能する。この結晶は湾曲しており，その曲率は，X線ビームを収束させるために必要である。曲率半径が2Rに湾曲したHOPG結晶は，半径Rのローランド円[3]と呼ばれる収束光学系を形成する。**Figure 2**に示すようにX線源とHOPG結晶表面の中心，またサンプル表面の焦点が，半径Rの同一ローランド円上に配列されている。この結果，サンプル表面の焦点は図に示す様にライン形状となる。**Figure 3**に，MESA-7220V2の光学系の概要を示す。X線の発生点となる銀ターゲットS，HOPG結晶表面の中央Cと試料セル下面Fはローランド円上に配置され，HOPG結晶は検出されるS-K α 線が最大となるように回転調整できる機構となっている。

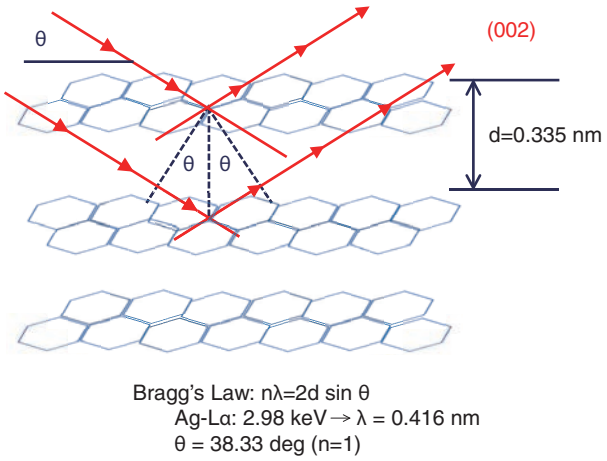

Figure 1　HOPG

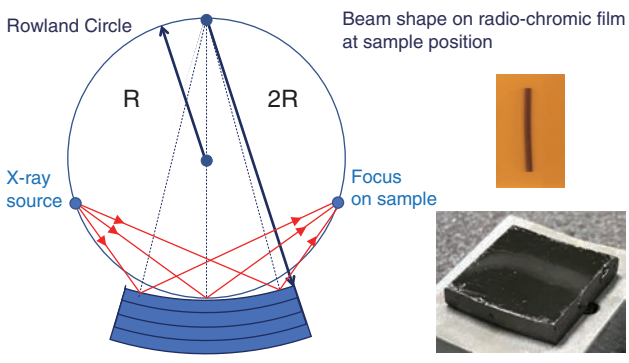

Figure 2　Singly curved HOPG

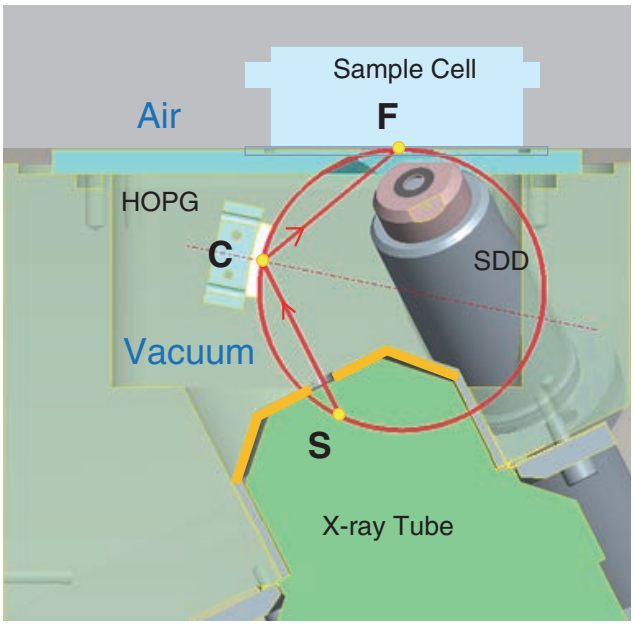

Figure 3　X-ray optics

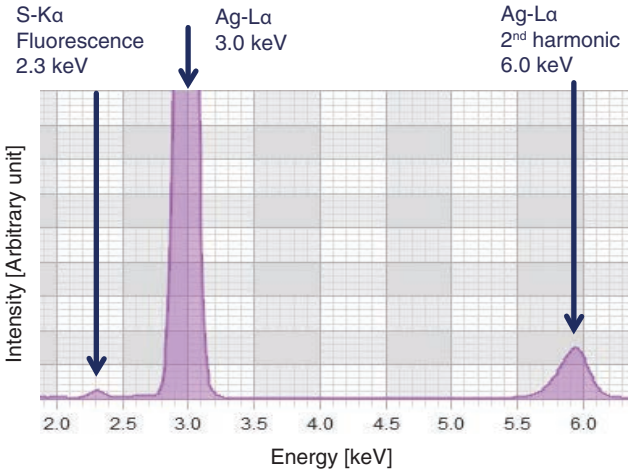

Figure 4　X-ray spectrum

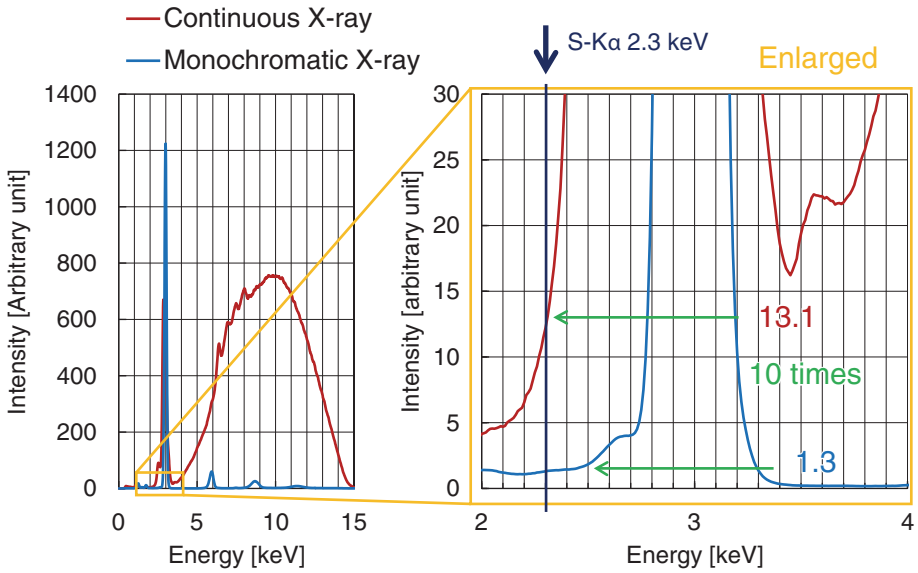

Figure 5　Lower back ground

Figure 4は計測例で，HOPG結晶によって回折されたAg-L α 線とその2倍のエネルギーを持つX線が示されている。前者はX線管のターゲット材である銀の特性X線で，後者はX線管から発せられた連続X線由来のものである。この光学系では蛍光X線と回折後に試料面で散乱したX線の両方が観測されている。

Figure 5にはブランク鉱物油試料(硫黄含有量ゼロ)を連続X線と単色化X線で励起した場合のスペクトル比較例を示す。この比較から，X線の単色化によりS-K α 線計測時のバックグランドが10倍改善したことが判る。検出下限(LOD)は，連続X線励起で3.2 mg/kgであったものが，単色化X線励起で0.5 mg/kgとなり，MESA-7220V2が，優れた感度を有していることが確認された。

MESA-7220V2の特徴と利点

(1)ユーザビリティ(オートサンプラ，真空窓)

新しく開発したターンテーブルは，**Figure 6**に示すような試料交換システムを提供するためのオプションであり，試料セルを8箇所に配置して自動的に測定することができる。単色化されたX線は，ポリイミド膜の真空窓を介して試料セルに入射するが，窓が汚れた場合には，ユーザー自らが簡単に交換することができる。

(2)検量線(測定モード，検量線自動選択)

システムは，硫黄分析，塩素分析，硫黄と塩素の混合分析，および酸素によるX線吸収を補正した硫黄分析の4つの分析モードを提供する。また，さらに拡張された検量線の自

Figure 6 MESA-7220V2 and the optional 8 positions turntable

動選択機能は，S-K α 線の強度に応じて，低，中，および高濃度範囲のどの検量線を適用するかを自動で決定する。これら3つの検量線を組み合わせて，1つの連続した検量線を指定しているかのようなシンプルなオペレーションを実現できる。

アプリケーション

(1)石油製品中の硫黄
多種多様な燃料中の硫黄分析を1 mg/kg以下の検出下限で行うことができる。ASTM規格4294にも準拠しており，原油や残油のような高濃度の硫黄を含む試料にも有用である。

(2)バイオ燃料中の硫黄
環境規制を背景に，E10と呼ばれるエタノールと混合されたガソリンやB85と呼ばれる脂肪酸メチルエステル(FAME)と混合されたディーゼル油等の酸素を含有するバイオ燃料が普及している。酸素補正機能により，これらのエタノール，バイオディーゼルやその混合燃料，および改質ガソリン中の硫黄分析が可能となる。この酸素補正機能は酸素からの散乱X線を分析するもので，専用の検量線を作成することで自動的に補正が行われている。

(3)原油中の残留塩素
Cl-K α 線のエネルギーはS-K α 線よりも，励起X線源であるAg-L α 線に近接しているため，硫黄よりも塩素のほうが高感度で，検出下限は0.6 mg/kgとなっている。しかし，高濃度の硫黄が存在する試料中での微量塩素分析は困難であるとされていた。MESA-7220V2では，適切に硫黄と塩素のピークを分離することにより，ASTM規格D4929に示されるような原油中の数ppmの残留塩素分析にも対応できるようになった。

おわりに

MESA-7220V2はASTM規格D7220とD4294の硫黄濃度分析に対応し，さらにD4929に規定される微量の塩素定量を行うのにも適した装置である。その適用範囲は市場ニーズに合わせてさらなる拡大が期待できる。

＊編集局注：本内容は特段の記載がない限り，本誌発行年時点での自社調査に基づいて記載しています。

参考文献

[1] 大澤澄人：“蛍光X線硫黄分析計 SLFA-60”，p.115，*Readout*（HORIBA Technical Report），**42**(2014)
[2] Cynthia G. Zoski: “5.2.1 Highly Oriented Pyrolytic Graphite”, p.115, Handbook of Electrochemistry(2007).
[3] Jeroen A. Van Bokhoven, Carlo Lamberti: “6.6.2 X-ray emission spectrometers”, p.141, X-Ray Absorption and X-Ray Emission Spectroscopy: Theory and Applications(2016).

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大気監視用二酸化炭素濃度測定装置 APCA-370の開発

Development of the Ambient Carbon Dioxide Monitor APCA-370

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近年，パリ協定で決められた温室効果ガスの削減に向け，温室効果ガスの一つである二酸化炭素（CO_2）の監視が必要になってきており，大気中のCO_2を高精度に測定するニーズが高まっている。APCA-370は大気中の二酸化炭素を測定する装置であり，当社独自の計測技術「クロスフローモデュレーション方式」を活用し，「自己再生型CO_2精製器」を採用したことで，長期的に高精度なCO_2測定を実現した。本稿ではこのAPCA-370の開発経緯とその特徴について紹介する。

In recent years, there has been an increasing need to measure carbon dioxide (CO_2) in our atmosphere with high accuracy. This is due to the necessity of measuring, controlling, and reducing the emissions of the greenhouse gas, CO_2, in each country participating in the Paris Agreement. The HORIBA APCA-370 is a device that measures carbon dioxide in the atmosphere with high accuracy. This has been achieved by adopting our unique measurement technology method, cross flow modulation, and a self-regenerating CO_2 purifier. This paper introduces the development history and features of the APCA-370 in detail.

はじめに

2015年に採択されたパリ協定では温室効果ガスの削減が目標の一つとされており，温室効果ガスの一つと指摘されている二酸化炭素（CO_2）の大気中の濃度の監視が注目されている。特に欧州ではそのニーズが年々高まっている。
1960年代の前半からWMO（世界気象機関）の主導により気象や気候に影響を与える大気成分の地球規模での長期変化を明らかにするために人為排出が少ない地域でCO_2の測定を行っている。日本では気象庁が，綾里村（岩手県），南鳥島（東京都小笠原村），与那国島（沖縄県）で，測定しているデータを公開している。近年では産業革命（1750年頃）以前の平均的な値とされる278 ppmと比べ47％増加し，400 ppmを超える濃度となってきている[1]。
また，石炭火力をはじめとする発電所ではCO_2の排出量を削減することが必要とされており，発電効率の向上と共に排出したCO_2を回収し，地中に貯留する技術が開発されている。この技術においてCO_2の漏えいを精度良く検知する装置が必要とされている。このような市場の背景，ニーズからCO_2を高感度で精度良く，長期的に安定して測定する装置が要求されており，「APCA-370」の開発に至った。

装置の概要

一般に2つ以上の異なる原子からなる分子が赤外線の照射を受けると，その分子の振動及び回転運動のエネルギー準位に基づき，その分子に固有な波長の赤外線を吸収する。
非分散型赤外吸収（NDIR：Non Dispersive Infrared）法はこの吸収量を測定することにより定量を行うものである。赤外線の吸収量は吸収分子（測定成分ガス）の濃度に応じて変化する。この関係はEquation 1のランバート・ベールの法則により表される。

$$I = I_0 \exp(-mcd) \quad \cdots\cdots (1)$$

I_0：入射光強度
I ：透過光強度
c ：吸収分子（測定成分ガス）の濃度
m：吸収係数（分子と波長で決まる定数）
d ：吸収分子層（ガス層）の厚さ

I_0，m，dは測定成分ガス種や装置により決定される定数であるため，透過光強度Iを測定すれば，測定成分ガスの濃度cがわかることになる[2]。
CO_2の測定においては，赤外領域に強い吸収を持つためNDIR法を用いた測定が良く用いられている。

当社のCO_2の測定においてはダブルビーム式NDIR法が用いられてきた。ダブルビーム式NDIR法の基本構造と動作原理を**Figure 1**に示す。光源から出射された赤外光は，測定セルまたは比較セルを通り，検出器へと入射する。この2つの赤外光を回転するチョッパーで断続することによって，測定セルの透過光と比較セルの透過光とを交互に検出器に入射させている。試料セルと比較セルに入射する赤外光は光量調整部でほぼ等しい量に調整されている。

測定セル内にCO_2が存在すると，その濃度に応じた赤外光の吸収が起こり測定セルを透過する赤外光量が減少する。一方，比較セルには赤外光を吸収しないガスが封入されているため，比較セルを透過する赤外光量は減少せず一定である。よってその透過光の差を検出器で検出し，信号として取り出す[2]。

しかし，この方法では大気中の二酸化炭素の測定や漏洩検知などのCO_2を高感度に測定する用途において，精度良く，長期的に安定して測定するには2つの問題点がある。

1つ目はデッドスペース内のCO_2濃度の変動による指示値への影響である。

光学系のチョッパー部や光量調整部には雰囲気空気が入り込むデットスペースがある。人間の呼気などのCO_2を含むガスがそのスペースに入り込み，スペース内のCO_2濃度が変化すると測定値に影響を及ぼす。

この影響はCO_2の高感度の測定においては無視できない誤差になる。そのためデットスペースは常にCO_2濃度が一定になるようにパージを行うか，あるいはデッドスペースに雰囲気空気が入り込まない構造が必要である。

2つ目はドリフトである。

ゼロの値が時間とともに少しずつ変化していくことをゼロドリフトという。

通常，この現象は測定セルにおけるセル窓の表面状態の変化や，セルの内壁の表面の反射率の経時変化などにより起こる。そのため，CO_2の漏えい検知などのゼロ付近の値を測定する際には無視できない誤差となることから，装置の校正する頻度を増やすなど対応が必要となる。

この課題を解決するため，APCA-370はHORIBA独自の手法である流体変調方式NDIR法を採用している。基本構造と動作原理を**Figure 2**に示す。

光源から出た赤外光は測定セルのみを通る。測定ガスと測定成分を含まないガス（比較ガス）を電磁弁などで一定周期で切り替え，交互に測定セルに導入する。この時に検出器に導入される両者の入射赤外光量に差が生じるため，検出器で電気信号として取り出される。

よって流体変調方式NDIR法では，チョッパーや光量調整部などを必要としないため，デットスペースを限りなく小さく設計することができる。また，一つの測定セルに測定ガスと比較ガスを導入するため測定ガスと比較ガスの濃度の差のみを検出することができ，セル窓の表面状態の変化や，セル管内壁の表面の反射率の経時変化が起こったとしても相殺することができる。そのため原理的にゼロドリフトフリーとなる[3,4]APCA-370の装置の外観と主な仕様を**Figure 3**と**Table 1**に示す。

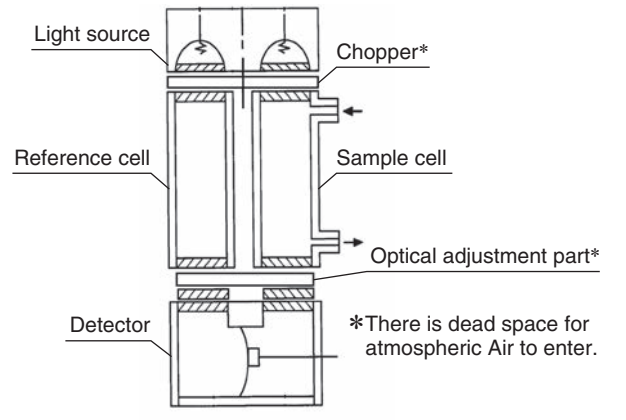

Figure 1　Schematic diagram of a double beam type NDIR

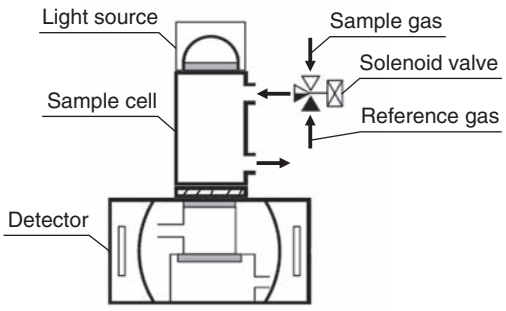

Figure 2　Schematic diagram of a cross-flow modulation type NDIR

Figure 3　External Appearance of the APCA-370

Table 1　Specification of APCA-370

Measurement principle		NDIR
Measurement target		Carbon dioxide (CO_2) in ambient air
Rage		0 to 500/1000 ppm
Lower detectable limit		0.5 ppm (2σ)
Reproducibility (repeating accuracy)		± 1.0% of the full scale
Linearity (readout error)		± 2.0 % of the full scale
Zero drift		± 1.0 ppm/day
Span drift		± 2.0 % of the full scale/day
Response time		60 sec or shoter (T_{90} from the inlet)
Gas flow rate		Approximately 0.7 L/min
External dimensions	(W)	430 mm
	(H)	221 mm
	(D)	550 mm
mass		Approximately 20 kg

自己再生型CO_2精製器

流体変調方式の採用において重要となってくるのは比較ガスに含まれるCO_2濃度である。上記で述べたように流体変調方式は測定ガスと比較ガスのCO_2の濃度差が測定値となる。そのため比較ガスに含まれるCO_2の量が測定の誤差になる

比較ガスとしてはCO_2が含まれないボンベで供給する方法や大気や測定ガスからCO_2を吸着剤によって除去し，比較ガスとして精製する方法がある。

ボンベを使用する場合，常に比較ガスを必要とするためボンベガスを頻繁に交換する必要があり，ユーザの保守経費やボンベ交換の手間など負担を増大させる。

測定ガスからCO_2を吸着剤などにより除去する場合，CO_2を取り除く方法として，化学結合により吸着するソーダライムがある。しかし，再生が難しいことから，定期的にソーダライムを交換する必要があり，ボンベ交換同様の課題がある。

このことから環境負荷も考慮し，APCA-370ではCO_2に対して高い吸着性能を持ち，温度を上げるとCO_2が脱離し，再生するCO_2吸着剤を用いている。

APCA-370では吸着剤を充填した2つの精製器を搭載している。2つの精製器を必要とする理由は，一方が比較ガスの精製をしている間に，他方は精製器の温度を上げてCO_2を脱離させることで精製器を再生させているからである。これを一定時間で交互に精製と脱離を行うことで，常に精製された比較ガスを測定セルに導入することでき，連続測定を可能としている。

精製器の負担を軽減させる工夫

APCA-370では測定セルを通った比較ガスを精製用のガスとして再利用することで，精製器への負担を軽減している。その理由を，**Figure 4**のAPCA-370のガスの流れを現したフローを参照して説明する。試料入り口より導入された測定ガス(Q1)は電磁弁を通り，測定セルに導入される。導入されたガスはそのまま精製用のガス(Q2)として，精製器に導入される。精製器に導入されたガスは比較ガス(Q3)と精製器を再生させる再生ガス(Q4)として使用される。比較ガスは測定セルに導入された後，もう一度精製器に導入される。このため精製器に導入されるガスとしては，CO_2を含んだ測定ガスとCO_2を含まない比較ガスが交互に導入されることになる。よって単純に測定ガスのみを精製するよりも，精製器の負担を低減することができ，精製器に使用する吸着剤の量を40%削減することが出来た。

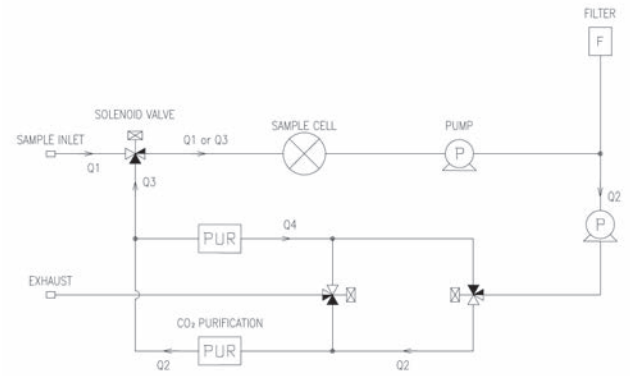

Figure 4　Schematic diagram of APCA-370 gas flow

長期安定性の検証試験

2019年5月24日から12月6日までの約6か月間，当社工場(びわこE-HARBOR)内に設置されている大気汚染監視測定局(**Figure 5**)にAPCA-370を設置し，大気濃度を測定しながら装置の校正は行わず，定期的にゼロガスを導入することで長期安定性の確認を行った。その結果を**Figure 6**に示す。期間中におけるゼロドリフトの値は最大で0.1 ppmであった。これは，最小検出感度以下であることから，ゼロドリフトが少なく，長期的に安定した測定が可能であることを示している。

Figure 5　Air Quality Monitoring Station (AQMS)

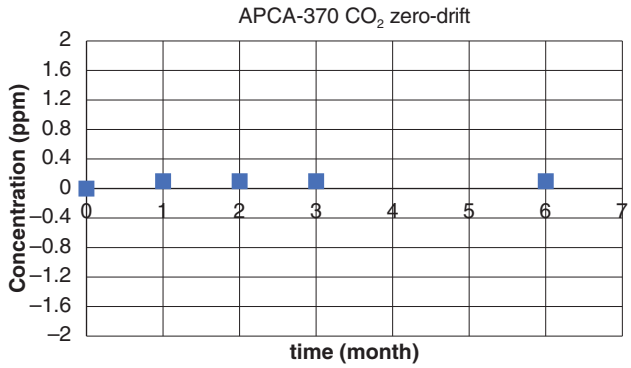

Figure 6　The result of zero-drift

おわりに

現在，環境問題に対する国民の関心が高まっているなかで，高精度で長期的に安定した測定装置が求められている。今回紹介したAPCA-370は，その性能を生かし，人間活動などの影響が少ない地域でのCO_2測定だけではなく，近年，需要が高まっている室内CO_2測定などのさまざまな分野で

の応用が期待できる。今後も引き続き本装置の機能，性能向上を図りグローバル市場を開拓し，顧客のニーズに応え，社会に貢献することが分析機器総合メーカである我々の使命である。

＊編集局注：本内容は特段の記載がない限り，本誌発行年時点での自社調査に基づいて記載しています。

参考文献

[1] “二酸化炭素濃度の経年変化”気象庁. 2020-01-21. https://ds.data.jma.go.jp/ghg/kanshi/ghgp/co2_trend.html, (accessed 2020-02-26)

[2] 青木潤次，ニューマチック赤外検出器，*Readout*，**7**，pp.64-71 (1993).

[3] 水本一徳，マルチガス分析計VA-5000シリーズの開発，*Readout*，**50**，(2018)

[4] 今木隆雄，定点測定用CO2の濃度観測システム，*Readout*，**1**，pp33-39，(1990)

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μTAS技術を用いた遠心方式血液分析装置 Yumizen M100 Banalystの開発

Development of Centrifugal Blood Analyzer Yumizen M100 Banalyst Using μTAS Technology

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糖尿病の指標であるヘモグロビンA1cや炎症マーカーであるCRPを微量の血液で簡便に測定できる検査装置を開発した。μTAS技術を応用して設計された使い捨てのプラスチック製チップを使用しており，検査室にある大型装置で一般的に行われている血液の遠心分離から試薬との反応，光学測定までの一連の分析操作をチップ内で実現している。これにより装置は小型だが高精度な測定が可能となっている。本稿では，チップと装置の構造やユニークな測定原理，μTAS化がもたらす利点を紹介する。

A blood analyzer has been developed that can easily measure hemoglobin A1c, an indicator of diabetes, and CRP, an inflammatory marker, with a small amount of blood. Disposable plastic chip designed based on μTAS technology is used, a series of analysis operations from blood centrifugation to reaction with reagents and optical measurement, which are generally performed in large instruments in a laboratory, are realized in a chip. As a result, the analyzer is small but high precision measurement is possible. This paper introduces the structure of the chip and the analyzer, its unique measurement principle, and the advantages of using μTAS.

キーワード
μTAS, POCT, HbA1c, CRP, CysC

はじめに

検体検査は被験者から採取した血液や尿などに含まれる成分の濃度や活性を測る検査であり，健康状態を把握し病気の発見や治療を行う上で欠かすことができない。近年ベッドサイドや診療所など患者のいるその場所でそのときに検査を行うPOCT（point of care testing）が広く普及してきている。POCT対応機器を用いることで，医師は患者の健康状態を常日頃からモニタリングすることができ，異常が見られた場合には検査結果に基づいた治療方針を即座に決めることができる。このようにPOCT対応機器は医療の質の向上に大きく貢献しているが，一方で検査センター等で使用される大型機器と比較してデータの精度が低いことを指摘されることがあった[1-2]。そのため小型で取り扱いが簡便かつより精度の高いPOCT対応機器が求められており，この要望に応えるべくMicro Total Analysis System（以下μTAS）技術を応用した遠心方式血液分析装置Yumizen M100 Banalyst（以下YM-100）を開発した（**Figure 1**）。

μTASとは

μTASとは，微細加工によって形成されたマイクロメートルオーダーの流路と空間を使って液体や気体の分析を行う分析デバイスである。微小な機能部位がデバイス内に集積されマイクロメートルオーダーの流路で連結されており，それらが相互に連動して動作することで試料の分離・精製

Figure 1　Yumizen M100 Banalyst

から計量，試薬との混合，成分の検出といった分析に必要な一連の操作を実現する。微小空間で操作が行われるため極微量の検体や試薬で検査を行うことが可能であるが，微小空間では我々の日常空間とは異なる物理現象が支配的であり古くから研究が行われてきた。μTASの概念を提唱したのはMantzであり，1990年にMiniaturized Total Analysis Systems(μTAS)のコンセプトを打ち出すとともに[3]，5 ㎜角のシリコン基板上で高速液体クロマトグラフィーを実現している[4]。この時期を境に，それまで研究が進められてきたMEMS(Micro Electro Mechanical System)やマイクロポンプ，マイクロバルブ，ISFET(Ion Sensitive Field Effect Transistor)などの要素技術を集積したデバイスが出現している[5-9]。以降，様々な分析方法が微小空間で検討されるとともに分析対象もDNA，タンパク質，細胞と拡大しており，今日μTASは医療やヘルスケア，創薬，環境分析，食品分析といった様々な分野に応用が期待されている[10]。

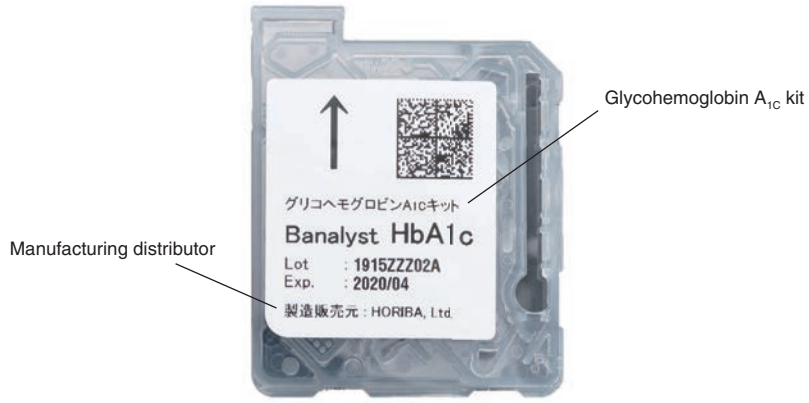

Figure 2 Reagent Chip

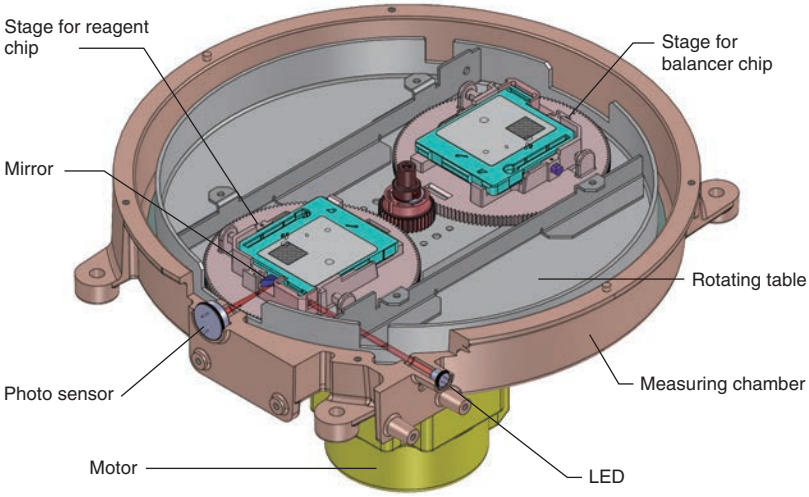

Figure 3 Structure of YM-100

Figure 4 Functional sites of CRP reagent chip

YM-100の測定原理

YM-100は臨床化学分析装置であり，μTAS技術を応用して設計された専用の試薬チップ(40×50×4.5 mm，**Figure 2**)を使用する。**Figure 3**は装置の内部構造を示している。測定室内に回転テーブルが設けられており，回転テーブル上に試薬チップがセットされるステージとバランサー用ステージが設けられている。回転テーブルは連結されたモーターにより最大3000 rpmで回転し，発生する遠心力が試薬チップ内を液体が移動するための駆動力となる。ステージの回転で試薬チップの方向を切り替えて試薬チップ内で液体が流れる方向を制御している。測定室内の温度は37℃に制御されている。また，測定室に取り付けられた光源基板のLEDと受光基板のフォトダイオードにより吸光光度測定のための光学系を形成している。投光用のLEDの波長は630 nmであり，ステージ上にセットされたチップの光学測定部位を透過した光はミラーにより反射され，フォトダイオードで受光される。測定室上面に配置したカメラでチップ表面に貼り付けられた2Dコードの読み取りを行い，項目情報や試薬ロットごとの検量線情報に基づいて濃度演算が行われる。

試薬チップの構造

試薬チップは，糖尿病のマーカーであるヘモグロビンA1c(以下HbA1c)，炎症マーカーであるC反応性蛋白質(以下CRP)，CRPの低濃度域の分解能を高めた高感度CRP(以下hsCRP)，腎症マーカーであるシスタチンC(以下CysC)の4項目を展開している。**Figure 4**はCRP試薬チップ内の各機能部位を示しており，血球分離部，計量部などが，回転テーブ

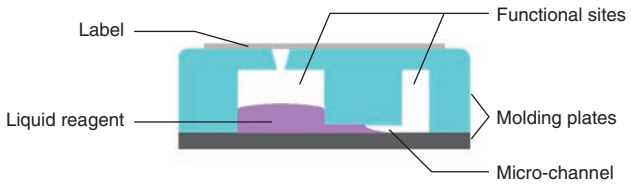

Figure 5 Cross section structure of reagent chip

ルの回転による遠心力とステージの回転による方向切り替えで機能を果たすように形状設計および配置されている。**Figure 5**は試薬チップの断面構造の模式図である。射出成型法で製造した2枚の樹脂基板の一方は表面に凹部を有しており，もう一方は平板である。これらの基板を貼り合わせることで貼り合わせ面に機能部位となる空間とそれを連結するマイクロ流路を形成している。試薬はチップ上面の試薬注入口から注入され，ラベルにより封止されている。

試薬チップ内の試料処理手順

CRP測定用チップを例にチップ内部での試料処理の手順を説明する(**Figure 6**)。検体を吸い上げたキャピラリーを**Figure 4**のキャピラリー挿入部からチップ内に挿入する。遠心力によりキャピラリー内の全血検体が血球／血漿分離部に導入される(**Figure 6-1**)。全血検体は遠心分離により血漿成分と血球成分に分離される(**Figure 6-2**)。上澄みである血漿のみが下流に送液され0.4 μLが計量される(**Figure 6-3**)。血漿計量部は**Figure 6-3**に示すようなV字形状をしており，遠心力により送液された血漿はV字形流路の一端から入り，余剰分が廃液として他端から送液され，V字部分の容積を利用して一定量が計量される。また，同様の計量構造により血漿計量と同時に第一試薬も10 μL計量される。次に，計量された血漿と第一試薬が一次混合部に送液され(**Figure 6-4**)，ステージの方向切り替えと回転テーブルの回転を繰り返すことで混合される(**Figure 6-5**～**Figure 6-6**)。同様に第二試薬も10 μL計量され(**Figure 6-5**)，二次混合部で混合される(**Figure 6-7**～**Figure 6-9**)。最終的に混合液は光学測定部に送液される(**Figure 6-10**)。混合液中では，第二試薬に含まれる抗ヒトCRPマウスモノクロナール抗体感作ラテックスを血漿中のCRPが架橋することで凝集塊が形成され，そのときの濁度増加に伴う吸光度の増加速度からCRP濃度が算出される(ラテックス凝集免疫比濁法)。

このような微量溶液のハンドリングには流路内で発生する毛細管現象を防止する必要があり，マイクロ流路には表面処理が施されている。特に血漿計量のばらつきは測定結果のばらつきに直結するため，血漿計量部は毛細管現象によって血漿が移動しないように構造的な工夫がされており0.4 μLという極微量の計量でもばらつきを抑制している。また，微量溶液の光学測定では，温度変化により溶液中の溶存気体が気泡となり光路にかかることで正確な測定が妨害される。**Figure 6-10**は試薬チップの光学測定部とそこに

Figure 6 Sample handling procedure inside reagent chip
Arrow diagram located at the center of the chip shows the direction of centrifugal force.

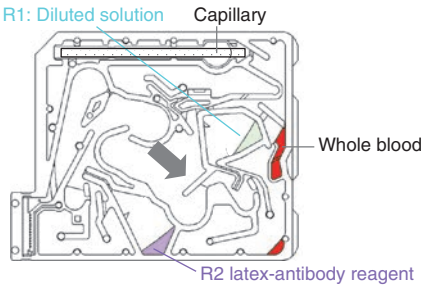

Figure 6-1 : Blood drop (1500 rpm, 5 sec)

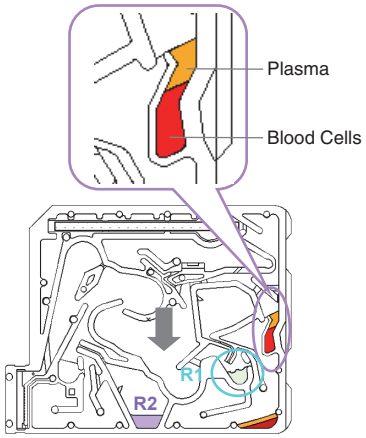

Figure 6-2 : Blood Separation and metering R1 diluted solution (3000 rpm, 120 sec)

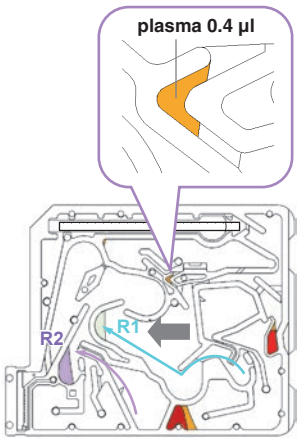

Figure 6-3 : Metering plasma (3000 rpm, 9 sec)

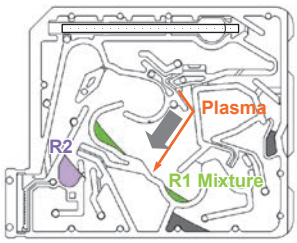

Figure 6-4 : Mixing metered plasma with R1 (3000 rpm, 10 sec)

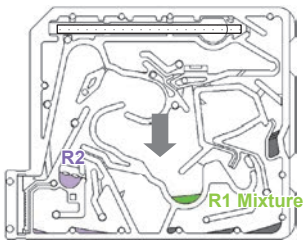

Figure 6-5 : Metering R2 latex reagent (3000 rpm, 10 sec)

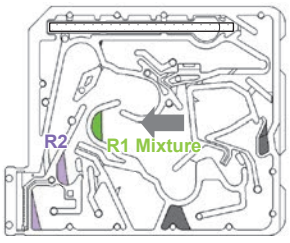

Figure 6-6 : Mixing by changing the direction (1000 rpm, 1 sec)

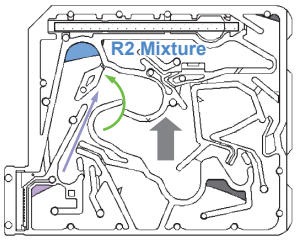

Figure 6-7 : Mixing with R2 latex-antibody reagent
(3000 rpm, 5 sec)

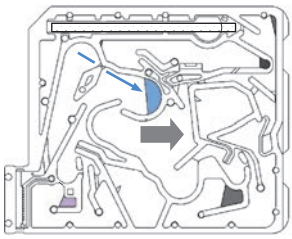

Figure 6-8 : Mixing (3000 rpm, 9 sec)

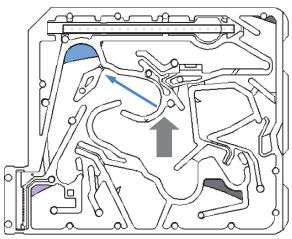

Figure 6-9 : Mixing and change the direction
(3000 rpm, 5 sec)

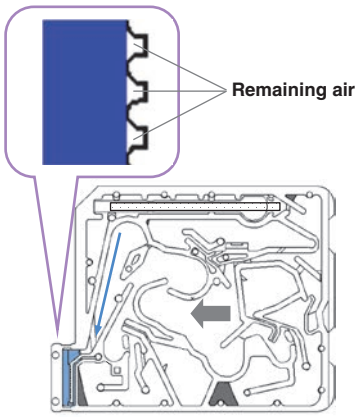

Figure 6-10 : Introduction to the optical sensing site
(2000 rpm, 5 sec)

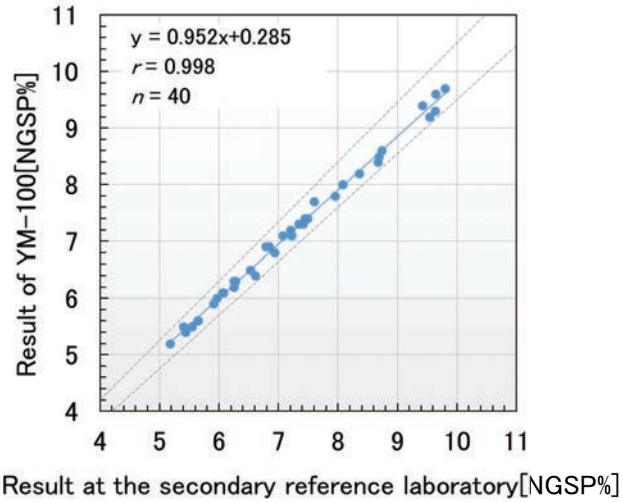

Figure 7 Result of NGSP certification for HbA1c
The dotted line shows the ± 5% of the result at the secondary reference laboratory.

送液された混合液を模式的に示したものである。光学測定部に設けられた複数の凹部は回転の中心側に設けられているため凹部に気泡が閉じ込められる。光学測定中に析出する溶存気体は界面エネルギーを最小化するように閉じ込められた気泡を核として成長する。これにより光軸上に気泡が発生するのを抑止しており，光学測定部の容積は14 μLと微量での光学測定が実現されている[11]。

YM-100の使用方法

実際にユーザーが行う操作は以下のとおりである。付属の専用キャピラリーで全血(CRPの場合4.4 μL，HbA1cの場合4.0 μL，hsCRPの場合9.8 μL，CysCの場合は6.0 μL)を採取し，そのキャピラリーを試薬チップに挿入する。次に試薬チップを装置にセットし，カバーを閉め，スタートボタンを押すことで測定が開始される。CRP，hsCRP，HbA1cの場合1測定当たり約7分40秒で結果が出力される。

性能評価

HbA1cは糖尿病患者の重要な血糖コントロール指標として日常診療で利用されており，開業医での需要が増し，迅速，簡便かつ高精度な測定機器が強く求められている。HbA1cについて，同時再現性，正確性，基準施設との相関性を評価した。

1. 同時再現性，正確性

Table 1は，HbA1c認証実用標準物質JCCRM423-10bの3濃度の試料について各10チップで測定を行った結果である。変動係数(CV値)は，濃度5.59%の試料では1.07%，7.70%の試料では0.92%，10.57%の試料では0.75%であった。HbA1cのPOCT対応機器に要望されるCV値が3.0%以内であることから，同時再現性は低濃度領域から高濃度領域まで良好と言える。また，基準値に対するバイアスは5.59%

Table 1 Reproducibility and accuracy of HbA1c NGSP%

Chip No.	Level M (Certified value 5.59±0.14)	Level H (Certified value 7.70±0.19)	Level HH (Certified value 10.57±0.25)
1	5.6	7.5	10.6
2	5.6	7.6	10.8
3	5.6	7.6	10.6
4	5.5	7.7	10.6
5	5.5	7.6	10.6
6	5.6	7.6	10.6
7	5.6	7.7	10.8
8	5.6	7.7	10.7
9	5.6	7.7	10.6
10	5.7	7.7	10.7
Mean	5.59	7.64	10.66
S.D.	0.06	0.07	0.08
C.V.	1.07	0.92	0.75
Bias	0.00	0.06	–0.09

の試料で0.00%，7.70%の試料で0.06%，10.57%の試料で−0.09%であった。

2. 相関性

HbA1cの国際標準法とのトレースを確認するための試験であるNGSP認証試験に参加し，二次基準測定施設との相関性を確認した。認証機関より送付される認証試験試料40検体を5日間に分けてYM-100で測定した。**Figure 7**は，YM-100での測定値と後日に公表された二次基準測定施設の測定値との相関性である。NGSP認証取得のためには36検体以上で二次基準測定施設での測定値に対し±5%以内であることが求められている。YM-100は全検体で±5%以内の結果が得られ，乖離が小さく高い相関性が確認された。

μTAS化の利点

まず，検査に必要な検体量が少なくて済むことが挙げられる。患者の採血の負担を軽減でき指先からランセットを使って採血しても検査が可能である。次に，検査の迅速性がある。全血を血球・血漿分離する機能が搭載されているため，遠心分離機での検体の前処理が不要でありその場ですぐに検査が可能となる。また，測定とメンテナンスの簡便性も利点である。血球・血漿分離から光学測定までの一連の処理がチップ内で自動的に行われるため，機械操作に習熟していないユーザーでも簡単に使用できる。廃液もチップ内で処理するため，廃液廃棄や洗浄のようなメンテナンスが要らない点でも取り扱いは簡単である。大型の自動分析装置と同じ試薬，同じ測定原理（ラテックス凝集免疫比濁法と吸光光度法）を使用できることから項目の拡張性も高い。

おわりに

YM-100ではμTAS技術を用いることで，微量検体で迅速，簡便かつ高精度なPOCT対応機器を実現できた。診療所や小病院など身近な医療機関での検体検査が一層普及し，病気の早期発見や予後の経過観察に貢献できれば幸いである。本稿では紹介できなかったが，NICU（新生児集中治療室）で注射器のシリンジに残ったわずかな血液でCRPを測定して低体重児の感染症予防を行っている施設があり，低体重児の採血の負荷軽減と検査機会の提供に一役を買っているとの報告がある。このような予想もしていなかったところで有用性を見いだされ使用されていることが嬉しい。今後は，医療現場でPOCT化が望まれている検査項目の開発に取り組みたい。また，試料の分離・精製，計量，試薬との混合といった溶液操作は血液分析に限らずバイオライフサイエンス分野で広く行われる。μTASを使った新たな分析ツールの可能性も探りたい。

＊編集局注：本内容は特段の記載がない限り，本誌発行年時点での自社調査に基づいて記載しています。

参考文献

[1] Lenters-Westra E.; Slingerland, R. J.; Six of Eight Hemoglobin A1c Point-of-Care Instruments Do Not Meet the General Accepted Analytical Performance Criteria. *Clinical Chemistry*, **56**(1), 44-52(2010).

[2] Lenters-Westra E.; Slingerland, R. J.; Three of 7 Hemoglobin A1c Point-of-Care Instruments Do Not Meet Generally Accepted Analytical Performance Criteria. *Clinical Chemistry*, **60**(8), 1062-1072(2014).

[3] Manz, A.; Graber, N. Widner H.M.; Widmer, H. M.; Miniaturized total chemical analysis systems: A novel concept for chemical sensing. *Sensors and Actuators B*, **1**(1-6), 244-248(1990).

[4] Manz, A.; Miyahara, Y.; Miura, J.; Watanabe, Y.; Miyagi, H.; Sato, K.; Design of an open-tubular column liquid chromatograph using silicon chip technology. *Sensors and Actuators B*, **1**(1-6), 249-255(1990).

[5] U.S. Pat. No. 4,883,763, issued November 28, 1989 to Holen, et al. Sample processor card for centrifuge.

[6] Schembari, C. T.; Ostoich, V.; Lingane, P. J.; Burd, T. L.; Buhl, S. N.; Portable Simultaneous Multiple Analyte Whole-Blood Analyzer for Point-of-Care Testing. *Clinical Chemistry*, **38**(9), 1665-1670(1992).

[7] Schembari, C. T.; Burd, T. L.; Kopf-Sill, A. R.; Shea L. R.; Braynin, B.; Centrifugation and capillarity integrated into a multiple analyte whole blood analyser. *Journal of Automatic Chemistry*, **17**(3), 99-104(1995).

[8] David C. D.; Heather L. G.; Joe L.; Norman F. S. Jr.; Gregory J. K.; Microfabricated Centrifugal Microfluidic Systems: Characterization and Multiple Enzymatic Assays. *Analytical Chemistry*, **71**, 4669-4678(1999)

[9] U.S. Pat. No. 5,919,711, issued July 6, 1999 to Boyd, et al. Analytical cartridge.

[10] 金子修一，堀池靖浩. バイオチップ実用化ハンドブック. NTS, 147-610p(2010). ISBN978-4-86043-270-6.

[11] 百瀬俊. 特許公開公報，特開2006-239538特許第4637610(2005年出願)[JP4637610]

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マルチデジタル水質計 WQ-300シリーズの開発

Development of the WQ-300 Series Multi-digital Water Quality Meter

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pH，溶存酸素（DO: Dissolved Oxygen），電気伝導率，酸化還元電位(ORP)およびイオン測定項目から3項目を自由に選択し，同時測定可能なマルチデジタル水質計 WQ-300シリーズを開発した。WQ-300シリーズは，内部液の補充が不要な無補充型比較電極を備えたpHセンサと，幅広いレンジを測定可能とする4極式電気伝導率センサおよび流量影響を受けにくい光学式DOセンサを有している。前機種のポータブル水質計と比較して，カラーグラフィック液晶による全項目同時表示，センサの脱着を簡易にするプッシュプルロック式コネクタ，電池容量を気にせず測定することができるUSB給電，地面に設置しても画面が見やすく，操作性を支援するメータスタンド，保存したデータを無線転送可能なワイヤレス通信機能とユーザビリティを大幅に向上している。従って，WQ-300シリーズはこれまでのポータブル水質計の活用範囲を超えて，環境水測定等に貢献できると期待される。

We developed the WQ-300 series of multi-digital water quality analyzers that can simultaneously select three items from pH, dissolved oxygen (DO), electrical conductivity, oxidation-reduction potential (ORP), and ion measurement items. The WQ-300 series includes a pH sensor with a non-refillable reference electrode that does not require replenishment of the internal solution, a 4-pole electrical conductivity sensor that can measure a wide range, and an optical DO sensor that is less affected by flow rate. Compared to the previous model of portable water quality meter, simultaneous display of all items with color graphic LCD, push-pull lock type connector for easy attachment/detachment of sensor, USB power supply for measuring without worrying about battery capacity, installation on the ground even though the screen is easy to see, the meter stand that supports operability, the wireless communication function that can wirelessly transfer stored data, and usability have been greatly improved. Therefore, the WQ-300 series is expected to be able to contribute to environmental water measurement beyond the range of conventional portable water quality meters.

はじめに

環境水モニタリング，上・下水道の維持管理，更には建設現場や工場廃水の定期検査などを行うにあたり，水温，pH，電気伝導率，溶存酸素など，水の基本特性を測定することは必須となっている。しかしながら，各々の測定項目を個別の水質計で測定することは手間がかかるため，多項目を一度に測定できる水質計がしばしば用いられている。これらの用途に対し，すでにU-50という多項目水質計を販売している。多項目水質計は，持ち運びが可能な大きさの装置内にpH，電気伝導率，溶存酸素，酸化還元電位（ORP），濁度，水深及び水温等のセンサが内蔵されている。しかしながら，深さ方向の計測を必要としない表面水の水質測定や，現場にて採取したサンプルの屋内測定において，多項目水質計は価格も高く，メンテナンスも大変となる。そこで我々は，これらの欠点や市場要求を解決できるWQ-300シリーズを新規開発することにした。

装置概要

Figure 1に，マルチデジタル水質計を示す。メータ及び各センサの重量は，約0.4 kg及び約0.2 kgと非常に軽い。セン

Figure 1　Product outline

サは最大3項目までメータに接続可能であり，センサを試料に浸すことで測定可能である。表示画面には，液晶内に外光を効率よく採光する画面を搭載し，測定項目が一括表示されるだけでなく，暗所はもちろんのこと，直射日光下でも見やすい画面を実現した。従来の水質計はセンサが検出したアナログデータをメータ側でデジタル変換処理していた。しかしながら，この欠点として，メータに内蔵された電子基板に依存した測定項目のみの測定となり，拡張性が無い。またセンサを別のメータに取り換えた場合に，校正データおよび測定設定が引き継がれないなどメータとセンサが対でないと機能しないものであった。WQ-300シリーズは**Figure 1**に示すように，従来，測定設定や校正データなどメータ側に内蔵した電子基板に保存していたが，この電子基板をセンサ側に移行することにより，これらの欠点を解決している。センサヘッドにはデジタル回路を内蔵し測定に関する情報を保持することができる。またセンサはカートリッジ式とし消耗品となるセンサ部分のみ交換可能である。環境にやさしく，現場測定における運営コストとメンテナンス性を向上させている。さらに従来の水質計では外界からのノイズにより測定値にふらつきを与えることがしばしばあったが，センサ直近にデジタル回路を移行したことにより，アナログデータの移動距離が短縮されノイズ影響が少ない測定を可能としている。メータへのデータ転送はデジタル信号となるため，ノイズ影響やケーブル長による内部抵抗増加などの影響を取り除くことができ，今後の水質製品群への拡張性を持つ機能を備えている。

測定に関わる一連の動作に合わせた設計

製品には現場での測定に必要となる備品を全て持ち運べるようキャリングケースを用意した。収納スペースには，メータおよび各種センサに併せ，交換用電池，各種校正用標準液，取扱説明書を収納することができる。これにより現場に向かった後，測定の直前に校正することが可能であり，また現場での測定時に異常を確認した際，標準液測定による評価も可能である。ケースの内部構造は製品の形状に合わせた収納場所および収納時に煩雑になりやすいケーブルの収納場所を設けた。さらに現場では実験室のようにセンサスタンドの準備が困難であるため，校正用標準液ボトルを固定して設置でき，そのままセンサを浸しても自立する構造を備えている。準備後は装置を取り出し測定するだけとなるよう，校正，測定，かたづけの一連の動作がスムーズに対応できる設計とした。したがって，このキャリングケースを持ち運ぶことで全ての測定前準備が完了できる。

Figure 2　Carry Case outline

同時測定に特化した設計

装置をキャリーケースから取り出し，現場にて試料を測定する際にも持ち運びしやすくするために複数のセンサの取り回しに考慮した設計とした（**Figure 3　左**）。装置の移動および測定時にメータをしっかり持てるようメータはゴム材質で全面コーティングし，グリップ力および万が一製品が落下した場合でも衝撃を吸収しやすい構造を備えた。装置の持ち運びの際にはセンサをメータにある3つのホルダーに装着することができ（**Figure 3　中**），かつ現場での測定時にはセンサに備えたフックとホルダーを用いて複数のセンサ同士を接続可能である。この構造により複数のセンサの検出部を揃えて試料の同時測定を可能とした（**Figure 3　右**）。

Figure 3　Sensor holders and hooks

測定環境に左右されない高精度測定

WQ-300シリーズ用に開発したセンサはフィールド測定での高精度測定と操作性に考慮した設計である。新規開発したpHセンサは，日々の測定前準備や現場測定をスムーズに行えるようにするため，バイオポリカーボネート材質の電極筐体内に比較電極内部液の補充が不要なゲル状KClを採用した。ただし**Figure 4**に示すように酸側，アルカリ側においても理論値に対して相関係数0.9997と比較電極内部液補充型のpHセンサとも遜色ない精度で測定できる。

電気伝導率測定に関しては，**Figure 5**に示すように幅広いレンジを高精度に測定できる4極式電気伝導率測定方式を採用した。センサ材質はカーボンを使用しているため，汚濁サンプルによる物理的な汚れの付着に対してブラシ洗浄を行うことが可能でありメンテナンス性が良い。

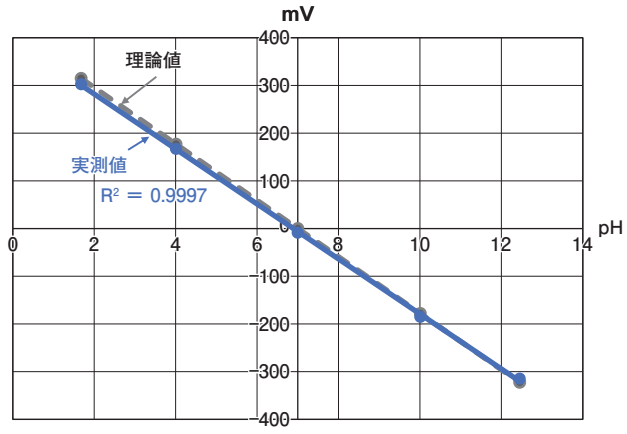

Figure 4　Linearity of non-filled type pH sensor

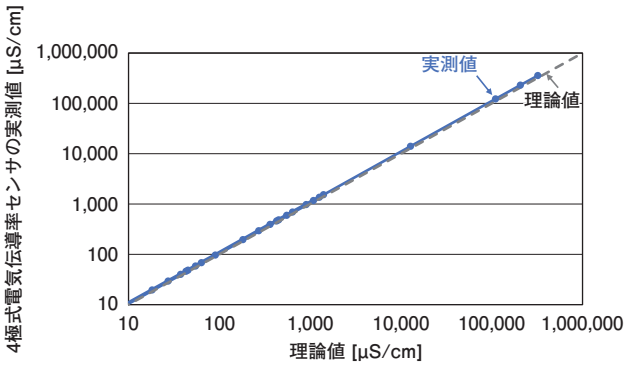

Figure 5　Linearity of 4-cell conductivity sensor

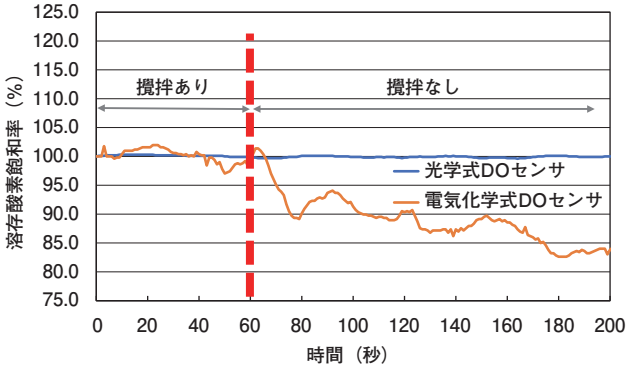

Figure 6　Comparison of flow rate effect of common electrochemical and fluorescent DO sensor

DO測定に関しては，**Figure 6**に示すように流量影響を受けにくい光学式DO方式を採用した。また，メータには，大気圧計を装備しており，測定環境の大気圧で変化するDO値を校正時に自動的に大気圧補正する。さらに電気伝導率センサと組み合わせて測定することにより，測定された電気電率値より塩分濃度を換算し，測定されたDO値を自動的に塩分補正する。このように測定に関わる環境変化を装置内に備えたセンサを用いて補正することにより，測定環境に左右されない高精度測定を可能とした。

おわりに

今回紹介したマルチデジタル水質計 WQ-300シリーズは現場または実験室での測定をスムーズに行える装置設計に併せ，環境変化による測定誤差を自動で補正し高精度測定および幅広いレンジを測定可能とした。従って，環境水の測定を従来よりも簡便かつ高精度に測定できることはもちろんのこと，これまでの水質計では同時測定または環境影響に応じた測定ができなかったような用途にも使用できる。

紺碧の海，美しく澄みきった川，透明度の高い湖沼など，水はあらゆる生命の源であり生きていく上でなくてはならない資源である。しかしその大切な水資源が，生活排水，工場排水，農業排水など，さまざまな人間活動から生じる排水によって，富栄養化，化学物質汚染による環境破壊が広範囲に及んでいる。世界中で同様の問題が起こっており，いかに正確に水質をモニタリングし，管理するツールを提供できるのか，我々分析機器メーカーは大変重要な役割を担っているといえる。今後も水質測定装置の開発を通して，環境問題の解決，実験室での水質評価，上水道の水源管理や下水道の処理工程管理等に貢献していきたい。

＊編集局注：本内容は特段の記載がない限り，本誌発行年時点での自社調査に基づいて記載しています。

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工業用無補充型 pH電極 6155

Industrial Gel-Filled pH Electrode 6155

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MUROGA Tatsuoki

我々は工業用無補充型 pH電極 6155を開発した。本製品は特に日本国内で一般的な内部液補充型pH電極と異なり，比較電極内部に塩化カリウム（KCl）水溶液ではなくKCl水溶液を含有したポリマーゲルを充填している。これにより，既存の当社製pH電極と比較してメンテナンス時のユーザビリティー，および耐圧性能が改善された。また，液絡部を二重構造とすることにより，流量のある低電気伝導率サンプル中の測定誤差を当社製既存電極の十分の一以下に軽減した。本製品の開発により，これまでよりさらに幅広いお客様に満足していただけるpH測定を提供できるようになったと自負している。本稿では，6155電極の特徴を詳細に解説するとともに，フィールドにおける測定事例について紹介する。

We have developed “Industrial Gel-filled pH Electrode 6155”. One of the features of this product is that polymer gel including potassium chloride (KCl) solution, instead of KCl solution itself, is filled inside of its reference electrode. This improves usability during maintenance because there is no need to replenish the internal solution. Besides, water pressure resistance is also improved comparing with general liquid-filled electrodes. Another feature is double structure of liquid junction, which reduced measurement errors in fast-flowing low conductive samples to less than one tenth of our existing electrodes. We are proud to provide satisfying pH measurement to further a wider range of customers by the development of this product. In this article, we explain the feature of the 6155 electrode in detail, and introduce examples of measurements in some fields.

はじめに

1950年に創業者である堀場雅夫が国産初のガラス式pH電極を開発して以降，HORIBAグループは60年以上にわたってpH電極を開発し，世に送り出してきた。現在，HORIBAグループのpH電極はラボ分析だけでなく，各種工場排水や浄水場，下水処理場の各プロセスの水質管理において広く利用されている。このような現場において，pH電極はしばしば過酷な環境にさらされ，その中で連続的かつ長期間に渡る精確な測定を求められる。そのため，我々は金属めっき液などの強アルカリ性サンプルの測定に適した耐アルカリpH電極，ガラス腐食性を持つフッ酸水溶液中で長期使用できる耐フッ酸pH電極など，様々なpH電極をラインナップしている[1]。

一方，お客様の要求は多岐に渡っており，pH電極をより簡易的に，かつ多種多様な現場で使用するためにはまだ改善の余地が多い。例えば，従来の内部液補充型pH電極は洗浄や校正などのメンテナンス時に煩雑な操作を必要とする。また，一般的にpH電極は高圧の掛かる現場や流速のある現場において，寿命の短期化や測定誤差などの問題を生じる。そこで，我々は次に示す三点において優れた性能を持つ工

Figure 1　工業用無補充型pH電極6155の外観図

業用無補充型 pH電極 6155を開発した(**Figure 1**)。

・ユーザビリティー(メンテンナンスの簡易性)
・耐圧性
・低電気伝導率サンプル測定の精確性

本稿では，従来型のpH電極と比較した同電極のアドバンテージについて解説し，フィールドにおける測定事例について紹介する。

pH電極の構造と測定原理

6155電極によるpH測定は一般的なガラス電極法を採用している。初めに，その原理について解説する[2]。

ガラスpH電極はガラス電極と比較電極によって構成されている(**Figure 2**)。ガラス電極をサンプルに浸漬すると，応答膜内外の接液部分に水和層が形成され，内部液とサンプルのpH差に比例した電位が生じる。一方，比較電極は内部の塩化カリウム(KCl)水溶液が液絡を通じてサンプルと繋がっており，サンプルのpHが変化しても一定の電位を示す。比較電極を基準としたガラス電極の電位差と液温により，**Equation 1**に従ってpHが求められる。

$$pH(X) = (Ex - Es)/(2.3026RT/F) + pH(S) \quad \cdots\cdots \quad (1)$$

pH(X)：サンプルのpH値
pH(S)：校正用標準液のpH値
Ex：サンプル中で計測した電位差
Es：校正用標準液中で計測した電位差
R：気体定数
T：絶対温度
F：ファラデー定数

多くのpH電極はガラス電極と比較電極，温度センサを一体化した複合型の構造を有しており(**Figure 3**)，6155電極も複合型pH電極である。

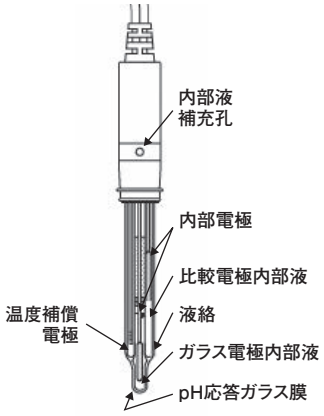

Figure 3　複合型pH電極の構造

6155電極の特徴と既存製品と比較したアドバンテージ

本章では，6155電極の構造的な特徴と，それに基づく既存の当社製品と比較したアドバンテージについて詳細に解説する。

ユーザビリティー

6155電極の一番の特徴は，比較電極内部に飽和KCl水溶液を含有したポリマーゲルが充填されていることである(**Figure 4**)。このポリマーゲルは結合性が強く非水溶性であることから，以下の利点を有する。

① 薬品に分解されにくい
② 微生物の栄養源を含まない
　(微生物由来の汚れが発生しにくい)
③ サンプル中に流出しない(長寿命)

また，ポリマーゲルにはさらにKCl顆粒を添加しているため，長期にわたってKCl飽和状態が保たれる。これらの特長から，6155電極は幅広い現場において内部液補充を行うことなく長期間使用することが可能である。

無補充型であることは電極の設置，校正や洗浄などの定期

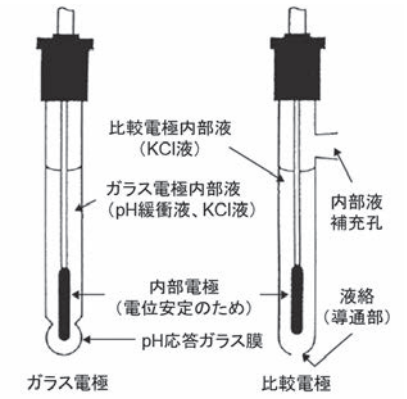

Figure 2　pH電極の原理的な構成図

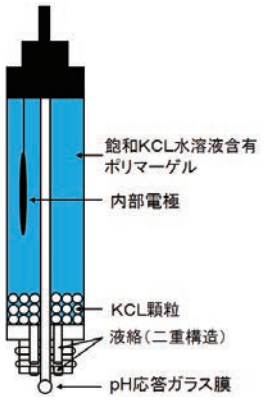

Figure 4　6155電極の構造

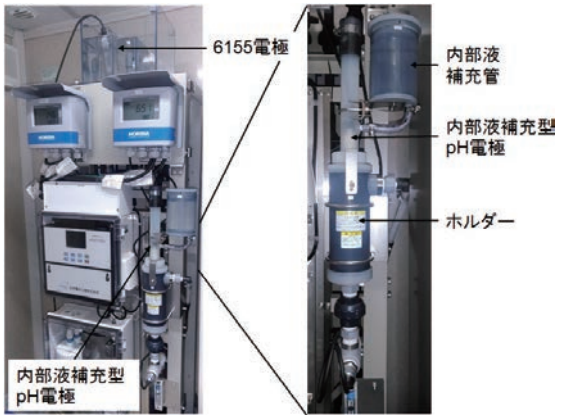

Figure 5　当社製内部液補充型pH電極と6155電極の取り付けの一例

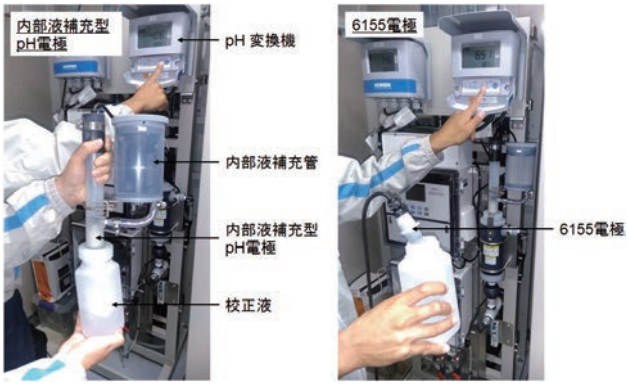

Figure 6　当社製内部液補充型pH電極と6155電極の校正作業の比較

メンテナンスを簡易に行える点において有利である。内部液補充型pH電極はメンテナンス周期を延ばすために内部液容量を確保する必要があり，一般的に電極ホルダに内部液補充管を取り付けて使用される（**Figure 5**）。そのため，ホルダは高重量となるが，垂直を保っていなければ補充管から内部液が零れ出てしまう。ゆえに，メンテナンスを一人で行うことは容易ではなく，充分な作業空間が確保されていない現場では危険を伴うことも考えられる（**Figure 6左**）。一方，無補充型である6155電極は内部液補充管を取り付ける必要がない。したがって，部品点数が少なくなり，設置時の作業が簡略化されるだけでなく，電極ホルダが軽量であるため一人での容易なメンテナンスが可能となる（**Figure 6右**）。

耐圧性の向上

前述したように，比較電極の内部は液絡を通じてサンプルと繋がっている。内部液補充型の電極は，サンプル側から圧力がかかると電極内部へのサンプルの逆流が問題となる。サンプルの逆流は内部液の薄まりや組成の乱れを引き起こすため，精確なpH測定を困難にする。これまで当社では，内部液をサンプル側に向けて加圧することでこの問題に対処していたが，サンプル圧が規定の加圧値を上回った際には逆流を防ぎきれなかった。また，サンプル圧が加圧値を下回ると，流出量増大により内部液の枯渇が早くなり，メンテナンス周期が短くなるという問題があった。

一方，6155電極の比較電極には外部への溶け出しがほとんどない非水溶性のポリマーゲルが隙間なく充填されている。そのため，加圧環境下においてもサンプルの逆流が起こりにくい。したがって，流通配管内などの圧力のかかる場所においても，内部液に関するメンテナンスを行うことなく，安定したpH測定を長期間継続することが可能である。実際，6155電極は0.7 MPa（大気圧の約7倍）のサンプル圧まで適応している。

低電気伝導率サンプル測定の精確性

一般的なpH電極は流れの速い現場において測定値に誤差を生じる（流量影響）。これは毛細管内部を液体が移動する際に発生する流動電位に起因するものと想定される（**Figure 7左**）。流動電位は流れる液体の電気伝導率が低いほど顕著となるため，上水や水道水，イオン交換水などの溶解イオンの少ないサンプルを測定する際には留意が必要である。

そこで，6155電極の液絡部には一般的に用いられる多孔質セラミックに加え，接液部に多孔質ポリエチレン（PE）を組み合わせた二重構造を採用した。こうすることで，PE内部においてサンプルの水流が緩和されるため（**Figure 7中央**），低電気伝導率サンプルにおいても流動電位を低減することが可能であった。実際に，水道水に相当する電気伝導率100 μS/cmのサンプルを用いて，水流が有る場合と無い場合の測定値の変動幅を既存の3種類の電極と比較したところ，6155電極の変動幅は10分の1以下であることが明らかとなった（**Figure 7右**）。また，液絡汚れに対して適切なメンテナンスを行っていれば，6155電極は常圧条件下に

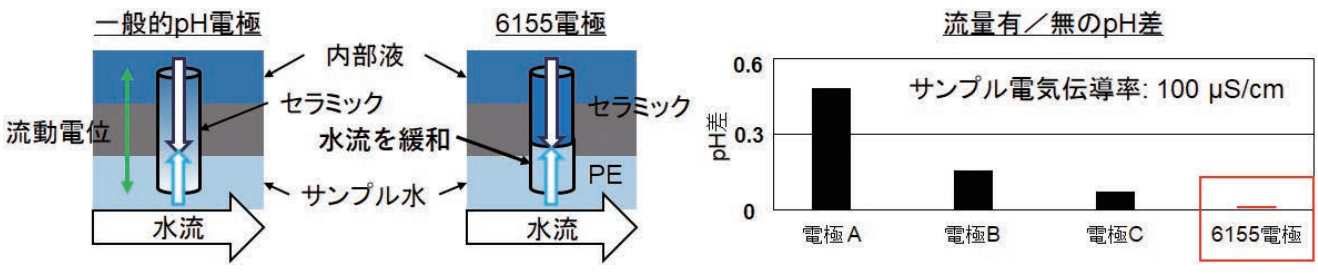

Figure 7　一般的なpH電極と6155電極の比較（左）液絡部の構造（右）流量が有る場合と無い場合のpH差

おいて電気伝導率10 µS/cmのサンプルを誤差0.1 pH以内で測定することができた。

フィールド測定事例

前述したアドバンテージから，6155電極は様々な現場，サンプルにおいて安定したpH測定を継続できることがフィールド評価によって明らかになった。また，無機，有機，微生物など種々の汚れが問題となる現場においても，我々がラインナップしている超音波洗浄機や水ジェット洗浄機などの自動洗浄器と組み合わせることで少ないメンテナンス頻度で使用可能であった。フィールド評価を行った現場の例を以下に示す。

・サンプル電気伝導率約100 µS/cmの浄水場取水口
・サンプル圧0.3 MPaの逆浸透圧膜処理工程
・サンプル圧0.5 MPa，電気伝導率約40 µS/cmの地下水
・排水処理設備活性汚泥層（**Figure 8**）
・排水処理設備嫌気処理層（超音波洗浄器と組み合わせ）
・工場排水放流層（水ジェット洗浄器と組み合わせ）

Figure 8は排水処理設備活性汚泥層において，6155電極と他社製の無補充型pH電極（電極X）の比較電極標準電位を3年間記録したものである。標準電位は内部液の濃度に依存し，個体差もあるが多くの場合は0 mV付近を示す。6155電極の標準電位（ピンク線）は3年間0±10 mVを示しており，これは比較電極に起因するpH推移が±0.15以内であることを意味している。また，3年間電位のドリフト傾向は確認されなかった。一方，電極Xは約1年間6155電極と同等の電位を示したが，その後正方向へのドリフトが確認され，1年半後には30 mV（0.5 pH）以上標準電位が推移した。これはサンプルが内部に混入したことでポリマーゲルに含有されているKCl水溶液の濃度が薄まったためと考えられる。

6155電極のポリマーゲルは耐薬品性に優れた非水溶性ゲルであるため，サンプル混入による含有KCl水溶液の組成変化や薄まりが起こりにくい。さらに，KCl顆粒の添加により多少のサンプルが混入しても飽和状態が保たれる。顆粒はポリマーゲルがサンプルに接触すると徐々に溶解するが，その量は常温，常圧において2年以上の連続浸漬に耐えられるように設定されている。測定環境やサンプルにも依存すると思われるが，本試験により6155電極は実際に2年以上の寿命を持つことが確認された。

また，ある工場の排水処理設備嫌気処理層において6155電極と当社製の超音波洗浄器の組み合わせ評価を行った。同現場では電極の応答膜や液絡に汚れが付着するため，お客様によると既設の他社製電極は精確に測定するために毎日手洗浄を行う必要があるとのことであった。そこで，同現場に設置した6155電極と既設電極の連続測定値を比較したところ，既設電極ではシフトが見られなかった時間帯で6155電極の測定値は顕著にシフトしていた。お客様によると，6155電極は同現場サンプルのpH変化を精確に追随しているとのことであった。さらに，超音波洗浄器を組み合わせて使用することにより，6155電極は同現場において精確な測定を2週間以上継続できることが分かった。これによりお客様の手洗浄の頻度が大幅に削減された。同電極は現在半年以上実装しているが，お客様からは「見事に良好に測定できている」との評価をいただいている。

おわりに

世界では1935年にベックマンにより，日本では1950年に当社創業者である堀場雅夫により初めて製品化され，長きに渡って様々な用途に用いられてきたpH電極であるが，各種現場における多様なニーズに応えるためにはまだまだ改善の余地を有する。我々はメンテナンス時のユーザビリティー，耐圧性，低電気伝導率サンプル測定の精確性にアドバンテージを持つ工業用無補充型pH電極6155を開発した。これまでも多彩な種類のpH電極をラインナップしてきた我々であるが，同電極の開発によりさらに多くのお客様に満足いただけるpH測定を提供できるものと自負している。今後もお客様のニーズに寄り添った水質測定機器を提供し続けられるよう，より一層の努力を行う所存である。

＊編集局注：本内容は特段の記載がない限り，本誌発行年時点での自社調査に基づいて記載しています。

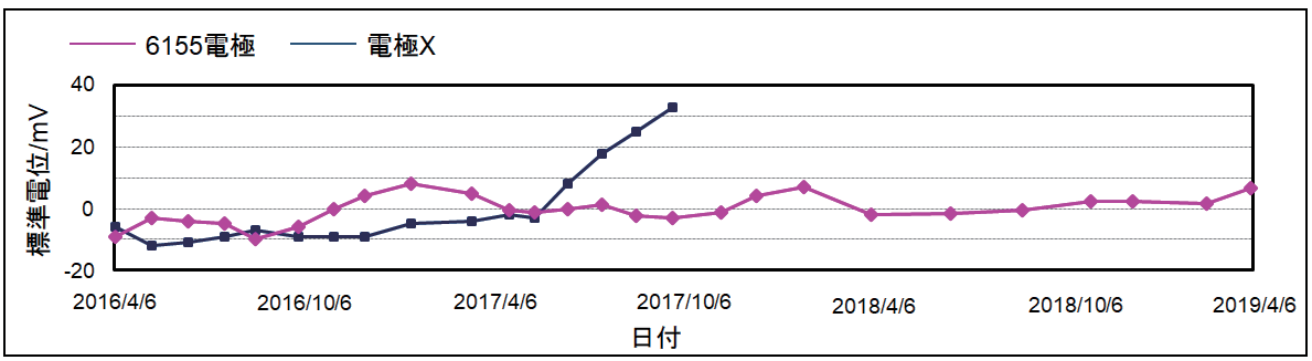

Figure 8　排水処理設備活性汚泥層に連続浸漬した際の6155電極と他社製無補充型pH電極の比較電極標準電位の比較

参考文献

[1] 山内　進，“工業用水質計H-1シリーズの特長”，*Readout*，**37**，88（2010）
[2] 大川浩美，西尾友志，“堀場製作所の基礎技術2　pH電極”，*Readout*，**41**，60（2013）

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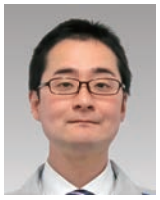

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放射温度計における測定波長の最適化による雰囲気ガス吸収影響低減の評価

Evaluation of Infrared Thermometer for Reduction of Influence of Gas Absorption by Optimization of Spectral Response

藤野 翔
FUJINO Sho

近年，半導体やフラットパネルディスプレーの製造プロセスにおいて，より高精度に温度を測定するニーズが増えており，非接触温度計である放射温度計の要求が高まっている。しかし，半導体の製造工程で高濃度の反応性ガス雰囲気での処理が行われるため，汎用的な放射温度計では反応性ガスによって赤外線が吸収され，正確に測定対象物の温度を測定できない。そこで，反応性ガスの吸収の少ない波長のみを透過させる光学フィルタを用いた放射温度計を開発した。これによって，反応性ガスの吸収によるガス濃度影響を大幅に低減することができ，測定対象物の温度をより正確に測定することを可能とした。

Recently high-accuracy temperature measurement becomes more important in semiconductor and flat panel display (FPD) manufacturing processes. Especially the demand for non-contact thermometer is increasing. However, semiconductor manufacturing equipment uses high concentration reactive gases. These gases make it difficult to measure the temperature with high accuracy and repeatability, because the reactive gases absorb the infrared rays. We have developed the infrared thermometer using an infrared filter through which infrared rays of the wavelength with low reactive gas absorption pass. The influence of the NH_3 gas absorption of the infrared thermometer can be significantly reduced. Furthermore, the temperature of the measuring object in the reactive gas can be measured more accurately.

はじめに

各種工業において，温度計測のニーズはますます高まっている。最近では半導体やフラットパネルディスプレー（FPD）の製造プロセスでも，より高精度に温度を測定するニーズが増えており，特に非接触温度計の要求が高まっている。

非接触式温度計である放射温度計は，測定対象物から放射される赤外線を検出して温度を求めている。そのため，測定対象物から放射温度計までの距離の間の大気の影響を最小限にする必要から水分（水蒸気：H_2O）やオゾン（O_3），二酸化炭素（CO_2）の吸収の少ない“大気の窓”と呼ばれる測定波長（8～14 μm）を採用して，湿度変化や測定距離の変化による測定誤差を最小限にしている。

しかしながら，半導体の製造工程で用いられる熱処理装置等は高濃度の反応性ガス雰囲気での処理を行う必要から，これら反応性ガスの赤外線の吸収により，高精度の温度測定が困難になってきている。そこで，この問題を解決するために，反応性ガスの吸収の少ない波長帯のみ透過する光学フィルタを用いたサーモパイルセンサを搭載した放射温度計を開発した。

本稿では，最初に放射温度計の測定原理を説明し，次に反応性ガスの赤外線吸収スペクトルから，測定波長の選定過程を説明する。最後に，既に販売している半導体製造装置向け放射温度計IT-470F-Hをベースに新規開発した放射温度計を用いて，実際のガス濃度の影響を評価したので紹介する。

原理

放射温度計は非接触で測定対象物の温度を測定できる。

その原理は測定対象物から放射される赤外線を検出し，その赤外線のエネルギー量をプランクの放射則に基づき温度換算している。プランクの放射則とは，物体の温度と物体が放射する放射エネルギーの関係を示したものである（**Figure 1**）。身近な例では，熱せられた800℃程度の鉄板は赤い光を放ち，さらに温度が上がるにしたがって白いまぶしい光を放つ。一方，常温の物体の場合，可視光は放って

おらず，赤外線を放出しているため，目視ではわからないが，手を近づけると，その物体に触れる前に暖かさを感じる。放射温度計は目視では見えない赤外線のエネルギー量を検出し，その物体の温度を測定することができる。

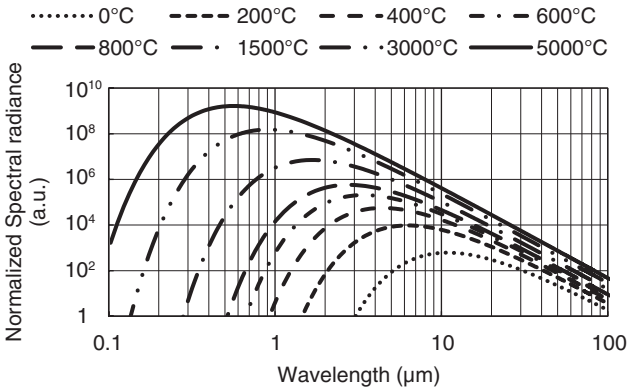

Figure 1 Planck's law
(the relationship between the temperature of an object and the radiation energy emitted by the object)

$$I'(\lambda, T) = \frac{2hc^2}{\lambda^5}\frac{1}{e^{hc/\lambda kT} - 1}$$

$I'(\lambda, T)$: spectral radiation		(J/(s · m² · sr · m))
T	: absolute temperature	(K)
	t(°C)= 273+t	(K)
h	: Planck constant	(J · s)
c	: speed of light	(m/s)
λ	: wavelength	(m)
k	: Boltzmann constant	(J/K) · m

測定波長

放射温度計を用いる際に重要な要素に測定波長がある。一般的に光学フィルタを組み込むことで放射温度計の測定波長を限定している。

大気中に含まれる二酸化炭素(CO_2)や水蒸気(H_2O)等によって赤外線は吸収される。吸収される赤外線の波長はガス種ごとで異なり，そのガスの分子振動のモードでも吸収される波長は異なる。ガスによる赤外線の吸収率は，ランベルト・ベールの法則を用いて以下で表すことができる。

$$A = \frac{E}{E_0} = \exp\left(\frac{-K_A l P}{kT}\right) \quad \cdots\cdots (1)$$

A：吸収率，E：ガス透過後とのエネルギー，E_0：ガス透過前のエネルギー，K_A：吸収係数(cm^2/molecule)，l：光路長(cm)，P：圧力(Pa)，k：ボルツマン定数(m^2*kg/(s^2*K))，T：ガスの温度(K)

この式からもわかるとおり，ガスの吸収率はその光路長，温度，圧力によって変わる。放射温度計の場合，光路長は放射温度計と測定対象物の間の距離となる。この距離はアプリケーションによって様々である。測定波長を誤ると，ガスの吸収によって，測定対象物の温度を正しく測定することはできない。具体的には，季節によって絶対湿度が変わった場合，指示値が変わってしまう。そのため，比較的ガスによる吸収が少ない波長帯で“大気の窓”と呼ばれる8～14 μmを測定波長とする放射温度計が一般的であることが多い。

反応性ガス雰囲気中の測定対象物の温度測定

半導体の製造工程で用いられる熱処理装置等は高濃度の反応性ガス雰囲気での処理を行う必要から，これら反応性ガ

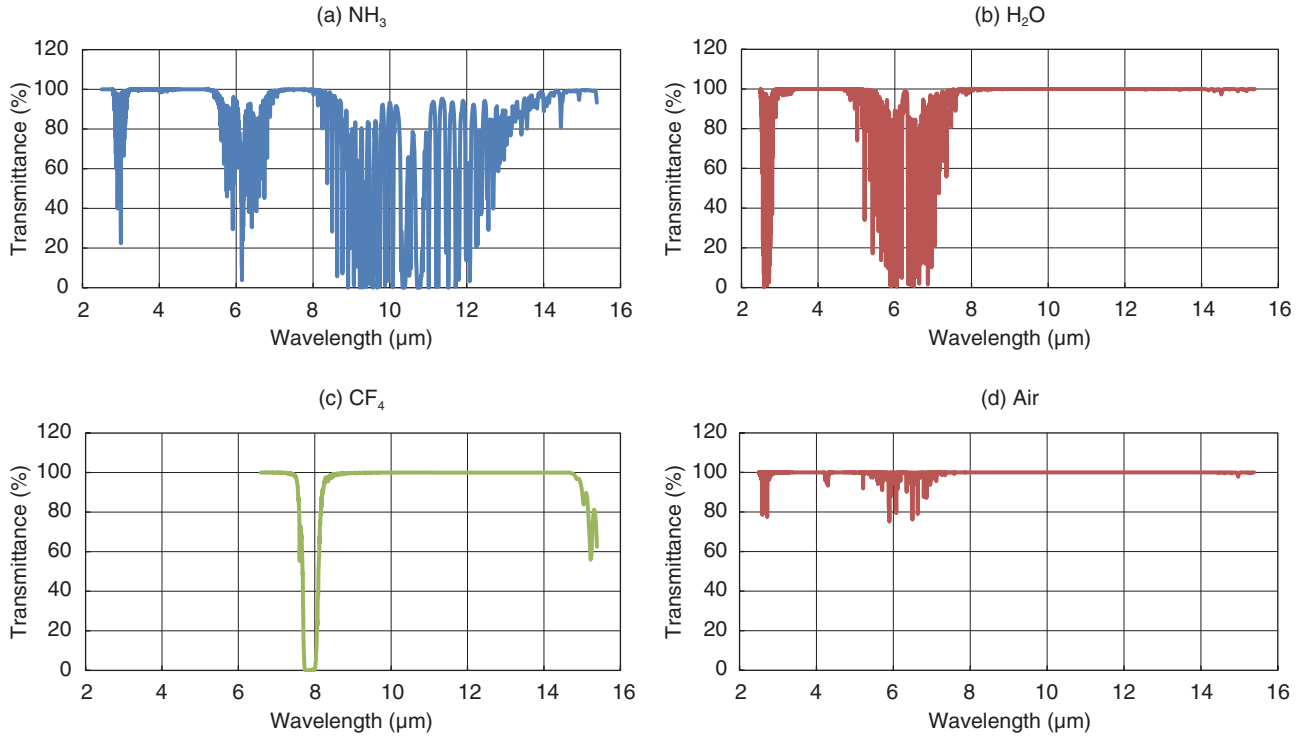

Figure 2 Transmission spectrums of representative reactive gas (1 atm, gas temperature 25°C, Optical path length 10 mm)

スの赤外線の吸収により，高精度の温度測定が困難になってきている。代表的な反応性ガスの透過スペクトルを**Figure 2**に示す。計算条件は，圧力1気圧，ガス温度25℃，光路長10 mm，各ガス濃度100%である。

これらの透過スペクトルから吸収ピークが比較的に少ない波長帯を選択する必要がある。アンモニア(NH_3)ガスの場合，3.5〜5 μm，7〜8 μmに大きな吸収がなく，ガス影響を受けにくいと考える。また，水蒸気(H_2O)の場合は，3.5〜4.5 μm，8〜14 μmが，四フッ化炭素(CF_4)の場合は，9〜14 μmがガス影響を受けにくいと考える。

プランクの放射則に基づく測定波長の選定

NH_3ガスは，半導体の製造工程の熱処理装置等でよく使用されているが，**Figure 2(a)**に示す通り，8〜14 μmに吸収ピークが多数存在するため，一般的な大気の窓を使用した放射温度計での測定は困難である。NH_3ガスの吸収ピークが比較的少ない波長帯は3.5〜5 μmと7〜8 μmの2つ存在する。どちらの波長帯を選択するかは，ガスの吸収だけではなく，測定温度範囲や測定精度を考慮する必要がある。**Figure 3**に測定対象温度と波長の赤外線エネルギー量の関係を示す。波長帯は3.5〜5 μm，7〜8 μmの2つで比較した。8〜14 μmは参考のため載せている。測定温度範囲や測定精度を考えると，赤外線エネルギー量を十分に確保した方が有利である。測定対象温度が0〜200℃の範囲ではエネルギー量が大きな7〜8 μmが有利であるが，200℃〜1000℃の範囲では，3.5〜5 μmの方が有利となる。しかしながら，3.5〜5 μmは200℃以下では急激にエネルギー量が減っており，十分にエネルギー量を確保できない懸念がある。NH_3ガス雰囲気中の測定対象物の温度を測定する場合，0℃〜1000℃の範囲で総合的にエネルギー量を確保するには，7〜8 μmの方が有利であると考える。

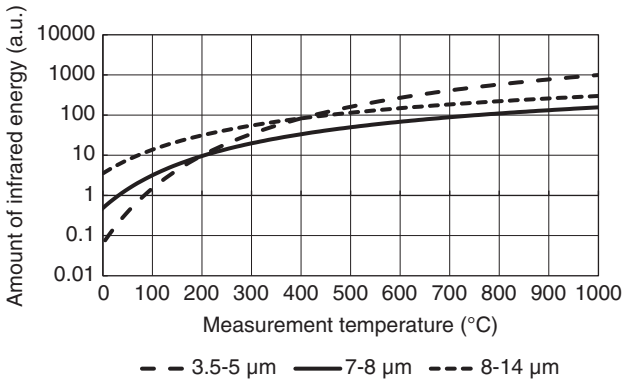

Figure 3 The relationship between the measurement temperature and the amount of infrared energy of each wavelength (Emissivity: 1.000)

アンモニアガス吸収影響を低減させた放射温度計

そこで，実際にNH_3ガスの吸収影響を低減させた放射温度計を設計する。比較的吸収ピークが存在しない波長帯は，3.5〜5 μmと7〜8 μmがあるが，前述したとおり，7〜8 μmを使用する設計とし，既に販売している放射温度計IT-470F-H(**Figure 4**)をベースにて新規開発した。

次に，開発した放射温度計の評価を**Figure 5**に示す実験配置で行った。

反応性ガスによる放射温度計の指示値の変化を評価するため，ガス濃度の影響を調べた。濃度の異なるNH_3ガスを充填したガスセルを用意し，ガスセルを変えた際の放射温度計の指示値を測定した。ガスセルは1気圧，濃度0，15，50，100%の4種類を用意した。ガス濃度の希釈には不活性ガスである窒素(N_2)を用いた。NH_3ガスを充填した部分の厚みは10 mmであり，光路長に相当する。ガスセルに用いた赤外窓は厚さ3 mmのフッ化カルシウムである。ガスセル，放射温度計の温度は常温で安定させている。ガス濃度0%(N_2ガス100%)のガスセルの指示値を基準とするため，ガス濃度0%において指示値が165℃となるように面熱源の温度を調整した。放射温度計は一般的な8〜14 μmの光学フィルタを使用したものと7〜8 μmの光学フィルタを用いたものを準備し，比較することでNH_3ガスの濃度の影響を調べた。

Figure 4 Infrared thermometer for semiconductor production equipment IT-470F-H

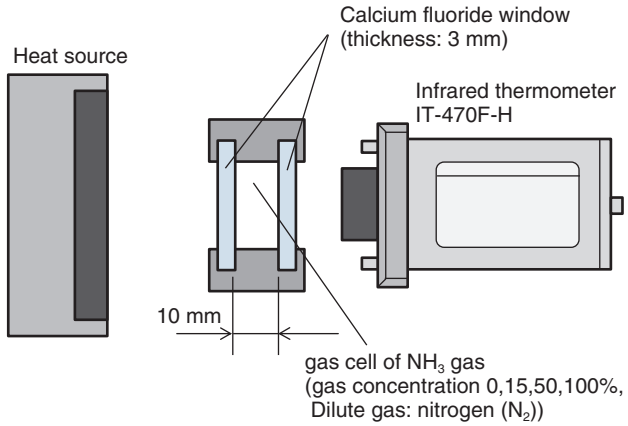

Figure 5 Experimental setup

アンモニアガス吸収影響を低減させた実験結果

Figure 6に実験結果とガススペクトルから放射温度計の指示値を算出した結果を示す。8～14 μmはNH_3ガス濃度が高くなるにしたがって，指示値が低下し，ガス濃度100％で指示値が20℃程度低下しているのに対して，7～8 μmは1℃以内の変動でほぼ一定であることがわかる。そして，計算結果とよく一致した。実験としては，光路長10 mmで測定を行ったため，7～8 μmでは指示値の低下がほとんど見られなかった。一方，光路長が200 mmの場合，NH_3ガス濃度100％で8～14 μmでは約65℃も低下するのに対して，7～8 μmでは約5℃しか低下しないことが予想される。これは光路長が長くなることで7～8 μm帯にも僅かながら吸収ピークが現れるからである。

以上のことから，反応性ガスであるNH_3の吸収ピークを避けて測定波長を選択することで，放射温度計のガス濃度影響を大幅に低減することができた。且つ，計算結果から光路長が長い場合の指示値を見積もることができた。

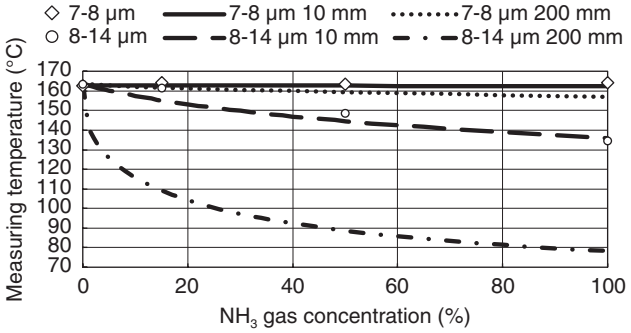

Figure 6 Influence of NH_3 gas absorption about difference of spectrum response
(plot: Experimental result, Dotted line: calculation result)

おわりに

反応性ガスの吸収の少ない波長のみを透過させる光学フィルタを用いた放射温度計を開発した。これによって，反応性ガスの吸収によるガス濃度影響を大幅に低減することができ，反応性ガス雰囲気中の測定対象物の温度をより正確に測定することを可能とした。半導体の製造工程に限らず，今後，多種多様なガス雰囲気中の温度測定のニーズも高まるであろう。我々は，光学フィルタやサーモパイルセンサを自社内で，開発・生産を行っている。そのため，新たなニーズに応えられる解決策を提案できると考えている。

＊編集局注：本内容は特段の記載がない限り，本誌発行年時点での自社調査に基づいて記載しています。

参考文献

[1] 計測技術，602, Vo.**46**, No.2, p.114,（2018）
[2] 大須賀直博，古川泰生，*Readout*, No.**43**, p.70,（2014）

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固定発生源排ガス分析計のサンプリング技術について

Gas Sampling Technology for HORIBA Stationary Source Emission Analyzer

秋山 重之
AKIYAMA Shigeyuki

堀場製作所(HORIBA)は，1968年の大気汚染防止法の制定に前後して工業用赤外線分析計を産業分野へ供給していた。そして，環境規制が具体的に動き出した頃から事前調査として，この分析計を元に煙道排ガス分析計を開発した。規制が始まると同時に自動車排ガス用一酸化炭素(CO)計で培った量産化技術を活かして煙道排ガスSO_2分析計を上市し，煙道排ガス分野へ進出した。初期の分析計は，コンパクトで設置が容易だったこと，さらに量産効果が功を奏し低価格が実現できたことで，より多くの客先に導入され，その結果として多岐にわたる稼働現場から多くを学ぶことができた。本稿では，HORIBAの固定発生源排ガス分析計(以下，煙道排ガス分析計という)の歴史をたどりながらサンプリング技術を中心に解説する。

HORIBA had supplied industrial infrared analyzers to the industrial field before and after the enactment of the Air Pollution Control Low in 1968. The stack gas analyzer was developed based on this infrared analyzer as a preliminary survey when the environmental regulations began to take effect. At the same time as the regulation started, HORIBA launched the sulfur dioxide SO_2 analyzer for stack gas, utilizing the mass production technology cultivated with the carbon monoxide (CO) analyzer for automobile exhaust gas, and entered the stack gas analysis field. The early analyzers were compact, easy to install, and inexpensive by our mass production effect, they were introduced to more customers, and as a result, more I was able to learn on customers' site. In this article, I will focus on sampling technology while following the history of HORIBA's stack analyzers for stationary emission.

はじめに

堀場製作所(HORIBA)は，1968年の大気汚染防止法の制定に前後して工業用赤外線分析計(型式EIA-1A)を産業分野へ供給していた。そして，環境規制が具体的に動き出した頃から事前調査として，この分析計を元に煙道排ガス分析計(型式ESDA-1)を開発し，ユーザとともに排ガス中の二酸化硫黄(SO_2)濃度測定についての調査を行うとともに，社内ではSO_2ガスの溶解度損失の試験を行ってきた。規制が始まると同時に自動車排ガス用一酸化炭素(CO)計で培った量産化技術を活かして煙道排ガスSO_2分析計(型式ESDA-200)を上市し，煙道排ガス分野へ進出した。初期の分析計は，コンパクトで設置が容易だったこと，さらに量産効果が功を奏し低価格が実現できたことで，より多くの客先に導入され，その結果として多岐にわたる稼働現場から多くを学ぶことができた。例えば，環境規制に定められた煙道排ガス中の硫黄酸化物濃度(以下，SO_X)の測定は，JIS K 0103による化学分析方法が基準になっていたが，SO_2計の測定値が化学分析法と比較して低い場合には，分析計本体というよりもサンプリング側に起

因するところが多くあった（化学分析法の測定対象はSO_Xであるが，分析計ではSO_2と異なる）。その後の環境規制の強化や拡大に加え，燃料，燃焼設備及び排ガス処理装置などの多様化に伴い排ガス性状も複雑になった。さらに計測対象もSO_2以外に複数成分の同時計測や高感度化計測が要求されるようになってきた。

本稿では，HORIBA煙道排ガス分析計の歴史をたどりながらサンプリング技術を中心に解説する。特に，排ガス測定のサンプリング技術であるSO_2の溶解度損失の実態調査とその対策，分析計の安定計測のための妨害成分除去用スクラバ*1や窒素酸化物（NO_X，NO及びNO_2の合量）測定用触媒，流量の安定化を図るサンプリング部品の開発について紹介する。

*1：スクラバ：有害ガスを吸着，水洗，薬液中和処理して大気に放出する装置

排ガス計測ニーズの始まり

1960年代，日本経済は本格的な高度成長期に入り，特に重化学工業の発展で，SO_Xによる大気汚染が進行し，都市周辺やコンビナート地域を対象とした大気汚染防止法による「硫黄酸化物に係る総量規制基準」が導入されたことで，ばい煙発生施設からの排ガスを計測するための分析計のニーズが高まった。

ばい煙発生施設としては，火力発電設備，工場ボイラー，廃棄物焼却炉，ガラス溶融炉，鉄鋼プロセス，硫酸プラントなどがあり，規模の大小含めるとその種類は多岐にわたる。計測対象として，硫黄酸化物規制に続いて窒素酸化物規制も始まりNO_X/O_2計が，さらにはダイオキシン類対策特別措置法によりCO計が追加された。以来，HORIBAの煙道排ガス分析計はENDAシリーズとして，環境規制に対応すべく技術の開発により改良を重ねてきた。**Table 1**に環境規制とHORIBAの分析計技術の歩みを示す。

Table 1　環境規制（主要燃料含む）と分析計技術の歩み

年代	1960～	1970～	1980～	1990～	2000～
環境規制と燃料	ばい煙規制法制定（硫黄酸化物総量規制），重油・石炭	窒素酸化物NO_X総量規制，脱硫・脱硝装置増設	ダイオキシン類対策特別措置法，LNG燃料，廃棄物焼却炉	環境基本法 燃料の多様化	自動車NO_X・PM法 大気微小粒子基準
排ガス分析計	ESDA-1 ESDA-200	ENDA-800 ENDA-100	ENDA-900 ENDA-1000	ENDA-2000 ENDA-600	ENDA-5000 ENDA-9000
分析計技術改良点・ポイント，（　）は，下線で示した分析計流量を示す	SO_2計，干渉フィルタ，検出器SO_2ガス封入，ミストキャッチャ開発，電磁式ポンプを開発 （0.5 L/min）	$SO_2/NO_X/O_2$計 干渉補正検出器，NO_Xコンバータ，NH_3スクラバ開発，定圧バブラ定流量化方式 （1.5 L/min）	$SO_2/NO_X/O_2$/CO計 クロスフロー方式開発 高感度化時代，定圧バブラ定流量化方式 （1.5～2.0 L/min）	←，シングルセル複数検出器開発 自社製電子冷却器開発 （0.8 L/min）	←，Push/Pull方式全機種採用，3系列電子冷却器ハロゲンスクラバを開発 （0.6 L/min）

初代SO_2分析計から学んだこと

排ガス中硫酸ミスト（以下SO_3ミスト）の発見

初代SO_2分析計を全国の市場に出荷したが，納入後1～2ケ月経過するとゼロ点が安定しない，測定セルが汚れるなど各地から同様のクレームが入り始めた。中には複数台の点検で毎日のようにメンテナンス作業が欠かせないというユーザからは現地調査の要請が来た。原因がわからないまま，まずは現状把握が大切と決心し装置をご使用の工場を訪問した。

（SO_3ミストによるサンプルセルの腐食を発見）
顧客から分析計の稼働状況の報告を受け，分析計を点検したところ，いずれも流量計のテーパ管が濡れている，2次フィルタの円筒ろ紙が破損している，サンプルセルが汚れていることがわかった。特にドリフトが大きい分析計の2次フィルタケースには粘性のある液体が付着しており，調べると強い酸性物質であることが分かった。このことから，排ガス中のSO_3ミストが分析計内に流入したためサンプルセルが腐食していると判断した。

（実装現場でのSO_3ミスト除去材の開発）
ここで発見したSO_3ミストは，サブμmサイズの硫酸の液滴で試料ガス（気体）中に浮遊し，電子冷却器および微細なろ紙を通過してしまう。このような性質を持つSO_3ミストを捕捉し除去する方法として慣性力を利用してガスの流れる方向に急速に方向転換が可能な多孔質物質の応用を検討した。具体的にはSO_2ガス吸着の少ない無機多孔質材または繊維状部材にターゲットを絞った。

最初の検討素材として，①グラスウール，②セラミックウール，③化学繊維を，容量250 mlカラムに充填したものをドレインセパレータ（サンプルガス中の水分を分離するもの）の後段に設置し，順次ドリフトの低減効果を調べた。設置後2～4日間は性能が安定しゼロ点を示したが，その後は再びドリフトが発生してしまった。各素材を観察すると，いずれも表面に水分が付着し濡れ現象が認められた。

（疎水性素材がSO_3ミスト除去に効果がある）
次に初回の結果を参考にして，疎水性を持つ多孔質素材（パーライト粒子）を選び同様の試験を行った。その結果，1ケ月間継続してドリフトが減り，安定した測定状態（カタログ仕様であるゼロドリフト±2%FS/week）をほぼ実現できることを確認し，表面へ水分が付着しにくい疎水性素材がSO_3ミスト除去に効果があることが分かった。

アンモニア（以下NH_3）共存時のSO_2分析計の指示誤差が大きい

煙道排ガスSO_2分析計ESDA-200が納入先で安定稼働に入った頃，重油ボイラー排ガスの煙突入口SO_2分析計の指示値が化学分析法に比べて低すぎる（化学分析法よりも約20～30%低い）との指摘があった。現地調査の結果，ボイラー排ガスへのNH_3注入と同期してSO_2計の指示値が大きく変動してしまうことが判明した。

多くの重油ボイラーは高温燃焼のためSO_3が発生し，エアヒータ（GGH）や電気集じん機（EP）の腐食や性能劣化の原因になるため，**Figure 1**に示すように，乾式EPおよびGGHの前段にNH_3を注入してSO_3濃度を減少させていた[1]。

原因を調べると，NH_3が加熱配管出口でSO_2と中和反応を起こしていることがわかった（**Table 2**，式（20））。これが契機となりNH_3スクラバを早期に開発することが必要になった。

燃焼排ガスとサンプリングの化学

SO_2分析計の出荷先での排ガス中にSO_3ミストの発見，排ガスプラントへの人為的NH_3注入によるSO_2計の指示値の異常等，過去に知りえなかった排ガス性状に関する情報が多く得られた。これは各設備からの排ガス性状が一様ではなく，燃料やボイラー燃焼方式及び排ガス処理装置の設置で複雑化

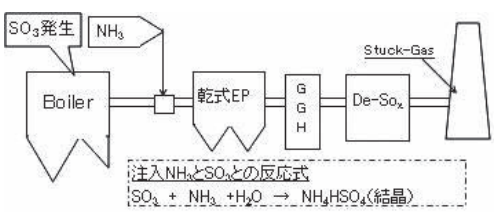

Figure 1　NH_3注入と排ガスフロー

するためで，その対策として，排ガス測定の現場情報，例えばボイラー出口から煙突入口までの排ガス成分の物理化学的性質（特にTable 3に示す溶解度特性）や化学反応についてさらに理解を深める必要があると考えた。Table 2にまとめた排ガス成分の燃焼およびサンプリング時の化学反応例を参照いただきたい。

Table 2　排ガス成分の燃焼時及びサンプリング時の化学反応

燃料と成分元素	燃焼時の排ガス成分の反応性（温度150℃～350℃，水分10～15%，CO_2約10%，残N_2）		試料採取時，凝縮水との反応性（　）内は水溶液のpHを示す			備考：ガス分析計の影響と課題事項
燃料： 重油，石炭，LNG（液化天然ガス），ごみ焼却，バイオマス 成分元素： C，H，O，S，N，Cl	$C+O_2 \rightarrow CO_2$，CO	(1)	$CO_2+H_2O \rightarrow H_2CO_3$	(13)	(5.4)	分析計の干渉影響
	$H+O_2 \rightarrow H_2O$	(2)	H_2O（気体）$\rightarrow H_2O$（液体）	(14)	(7.0)	NDIR法干渉影響
	$N(N_2)+O_2 \rightarrow NO$，$NO_2$	(3)	$NO_2+H_2O \rightarrow HNO_3+HNO_2$	(15)	(<1)	NO_xコンバータ
	$S+O_2 \rightarrow SO_2$	(4)	$SO_2+H_2O \rightarrow H_2SO_3$	(16)	(1.5)	溶解損失
	$2SO_2+O_2 \rightarrow 2SO_3$	(5)	$SO_3+H_2O \rightarrow H_2SO_4$	(17)	(<1)	腐蝕性ガス
	$Cl+O_2 \rightarrow Cl_2$，Hcl	(6)	$Cl_2+H_2O \rightarrow HCl+HClO$	(18)	(<1)	腐蝕性ガス
	$NH_3+SO_3+H_2O \rightarrow NH_4HSO_4$	(7)	←	(19)	(4.5)	結晶化配管系閉塞
	$NH_3+SO_2+H_2O \rightarrow NH_4HSO_3$	(8)	←	(20)	(4.2)	SO_2測定誤差

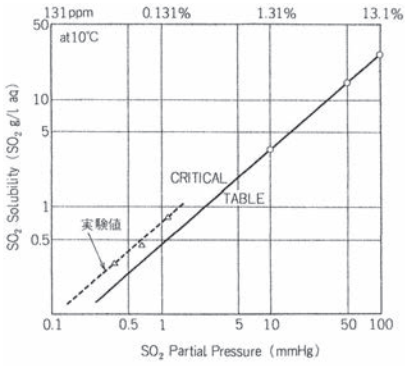

Figure 2　低濃度SO_2溶解度

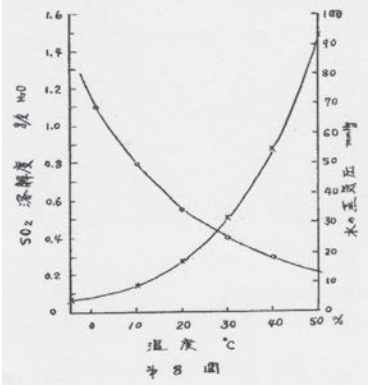

Figure 3　SO_2溶解度と水蒸気圧の温度影響

Table 3　SO_2他排ガス成分の溶解度とその特性

排ガスの主な化学成分		純ガスの各温度における溶解度と特性[kg/100 kgH_2O]		
		5℃	40℃	5℃/40℃比率
二酸化硫黄	SO_2	19.3	5.11	3.78
炭酸ガス	CO_2	0.277	0.097	2.86
一酸化炭素	NO	0.009	0.006	1.39
二酸化炭素	NO_2	—	—	—
アンモニア	NH_3	77.5	30.7	2.52

SO_2計測における溶解度損失とその対策

排ガス中SO_2計測において，サンプリング系のSO_2溶解度損失は計測精度上無視できないとの疑念を持たれたことがあった。Table 3に示すようにSO_2溶解度の温度依存性が大きいこともその理由の一つである。1960年代に入り，煙道排ガス測定におけるSO_2の水への溶解度損失について実態調査を行った。当時，SO_2低濃度域の溶解度に関する技術資料は殆ど見当たらず，SO_2標準ガスを用いて基礎実験を行った。

実験データ（Figure 2の点線部分）において，CRITICAL TABLE値を外挿したデータとの関連性を調べると，約1.5倍となりSO_2低濃度域の溶解度が増大する傾向にあることが分かった[2]。

次にSO_2溶解損失発生のメカニズムを理解するため，SO_2溶解度の温度の影響を調べた。Figure 3に示すようにSO_2溶解度の温度依存性が大きく，また水の飽和水蒸気圧曲線とは互いに温度に対して非対称であることが分かる[2]。この関係を実際のサンプリングの水分除去過程に適用して考えると，急激にサンプルガスの温度を下げて冷却するのではなく，段階的に温度を下げることによりSO_2の溶解度損失を低減することができることが分かる。

従来技術と3段階冷却除湿法について，実際の製品における水分除去フロー図，および，SO_2溶解度損失データの比較をFigure 4に示す[3]。従来法に比べ3段階冷却除湿法の方が，より溶解度損失が低減している様子が分かる。

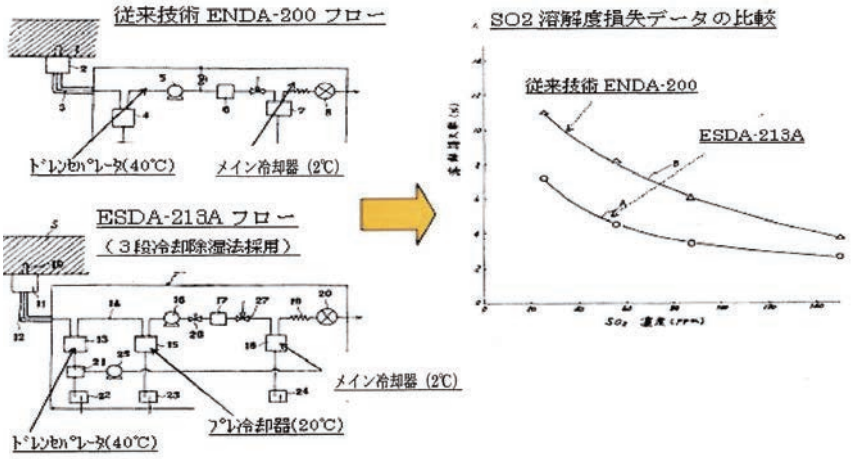

Figure 4　ESDA-213AとENDA-200のSO_2溶解度損失

分析計を支えるサンプリング技術の開発経緯

ガスサンプリング技術の役割

分析計技術の目的は，顧客の要求(購買)仕様を満足する測定装置を提供することである。しかしながら，現実には排ガス性状は，顧客毎で燃料や排ガス設備および採取点位置により異なるため最適なサンプリング技術を構成する必要がある。

環境規制強化とガス分析計技術の進歩

現在販売しているENDAシリーズは，初号機から数えて第5世代目になる。環境規制強化に伴いガス分析計は測定項目を1成分から5成分に増加し，分析計に必要な流量は成分数に比例して，0.5 L/min (1成分)～1.5 L/min (3成分)～2.0 L/min (4成分)まで増加した(**Table 1**)。一方で，NDIR法ガス分析計の光学系の改良(一つの光源と測定セルで複数成分が測定可能な光学系構成を考案)を行うことで，測定項目を5成分(酸素計含む)に増やしても流量を0.6 L/minにまで低減することができた。この技術によりサンプリング系のコンパクト化が実現できた。ENDA-5000のサンプリングシステムを**Figure 5**に示す。

自社開発と運用管理

製品の主要部品については，自社開発の大切さを多くの先輩たちから学んだ。特に，分析部品やサンプリング系部品は，排ガス測定特有の要求性能があるため，改良や独自工夫を加えることで自社の製品技術の強みになることが多々あった。排ガス測定のサンプリング系部品，特に駆動部品としてのポンプおよび電子冷却器は，重要部品としての性能仕様や耐久性を実現するため自社開発を余儀なくされた。

駆動部品

サンプリングポンプは，煙道排ガス計において腐蝕性ガスや水分を含むサンプルガスを吸引するポンプで，流量の安定性および耐久性が要求される。従来のサンプリングポンプには，モータ駆動ダイヤフラム式ポンプが多く用いられてきたが大型で重いことから，小型で軽い2ヘッド電磁式ポンプを開発した。このポンプは，**Figure 6**に示すようにENDA-5000の構成部品として採用されている。

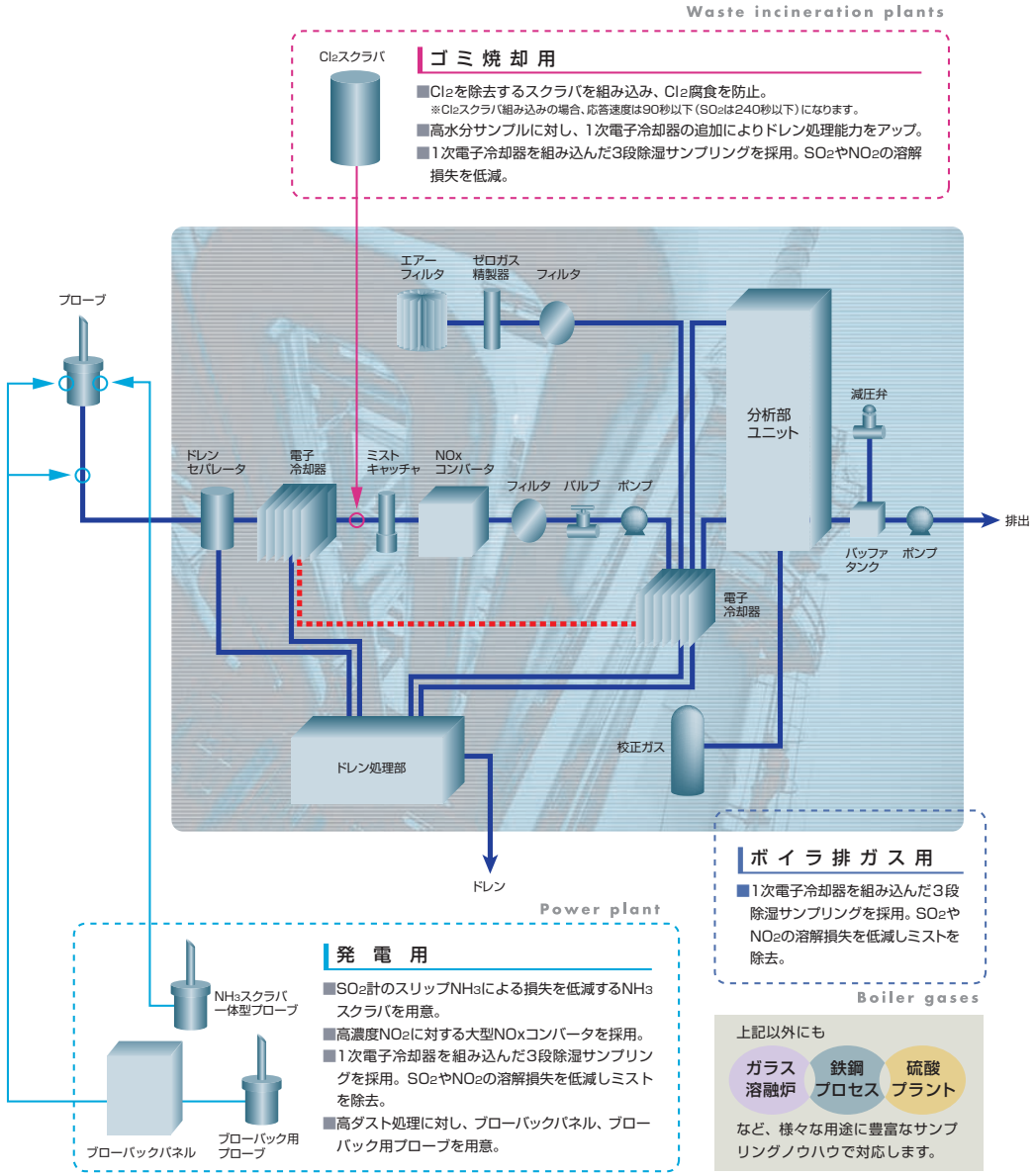

Figure 5　ENDA-5000サンプリングシステム

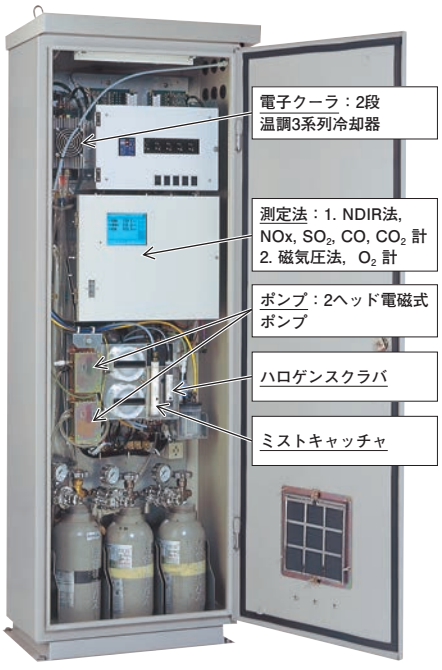

Figure 6　ENDA-5000 構成

電子冷却器

一方，電子冷却器の課題は，HORIBAオリジナル技術であるクロスフロー方式（サンプルガスと基準ガスを一定周期で交互に切り替えてサンプルセルに導入する方式で，長期安定性に優れる）に要求されるサンプルガスと基準ガスの2系列ガスの同時冷却除湿（5℃設定）を行うことであった。さらにSO2溶解度損失を軽減した3段冷却除湿法を適用するため，2段階の温度調節機能（前処理として15～20℃設定）が要求された。

従来技術では設定温度が異なる2台の電子冷却器を使用していたが，ENDA-5000のサンプリングユニットでは，軽量化と省スペース化を目指して**Figure 5**中の破線で結ばれた2台の電子冷却器を1台に集約した。また，1枚のサーモモジュールで異なる温度設定が可能な構造設計を行うことで，省エネ，省資源，コンパクト化を実現し，環境用分析計の構成部品にふさわしいユニット部品となった。

NO_2をNOに還元するNO_xコンバータ触媒

1970年初期，NO_x計に用いるNO_xコンバータには，カーボン系触媒（高純度グラッシーカーボンやグラファイト系）が350℃以上で用いられていたが，共存するNH_3の一部が酸化され窒素酸化物になるため測定誤差の原因になっていた。筆者らは，1970年代に脱硝装置が増えつつある状況下でNO_x計の測定精度を確保するため，いち早く低温型NO_xコンバータの開発に着手した。触媒基材には活性炭を用いて，モリブデン（Mo）塩を担持させた後に不燃処理を行うことで高効率化と長寿命化を実現した。**Figure 7**にNO_xコンバータ本体とNO_xコンバータ効率を示す。

本触媒は，低温動作でかつコンバータ効率に優れていたため，本用途であるNDIR法によるNO_x計の他に，化学発光法（CLD法）を原理とする大気汚染監視用NO_x計（型式APNA-300シリーズ），ポータブルガス分析計（型式PG-200シリーズ）および自動車排ガス分析計（型式MEXAシリーズ）等多くの分析計に用いられている。

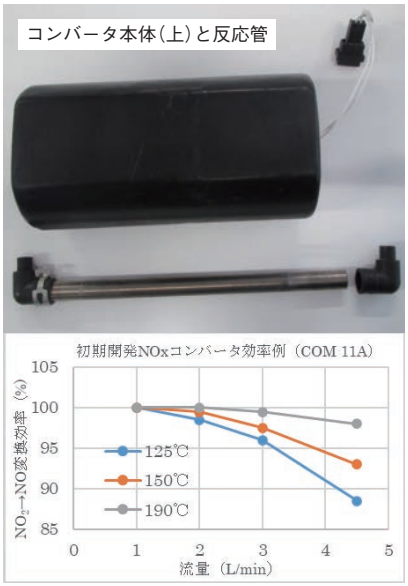

Figure 7　初期開発NO_xコンバータ本体とその効率例

スクラバ類とその運用管理

一般の排ガス施設は，燃料や燃焼設備の種類，環境保全設備などにより，排ガス性状が異なるため，特定の有害物質を除去する専用スクラバを設置する必要がある。以下に示す3種類のスクラバは，排ガス分析計の用途毎に選定して用いられる（**Table 4**参照）。カラムの型式選定は，SO_3濃度，流量，寿命期間を基準に使い分けしている。開発当初は，いずれのスクラバも容量250 mlの大型カラムのみであったが，製品担当者らの発案でモデルチェンジや使用条件の違い，ユーザ要求仕様に合わせて種々のサイズのカラムが自然発生的に製品化されてきた。

Table 4　スクラバ類の型式一覧

スクラバ名称，使用目的，型式の種類等	動作原理について
1. ミストキャッチャ 目的：重油・石炭燃焼，廃棄物焼却炉他全用途 3型式 「MC-025」（常温） 「MC-050」（常温） 「MC-3030」（常温）	ミスト除去原理；素材のSEM写真（上）を示す。無機多孔質物質の微細網目構造にSO_3ミストの衝突現象を利用して，除去・捕集する。この素材は，水分に対して撥水性である。（写真下）
2. NH_3スクラバ 目的：脱硝装置出口高硫黄重油排ガス 3型式 NSU-025」（室温） NSU-050」（室温） ASU-200」（90℃）	NH_3除去原理：不揮発性酸とNH_3の中和反応 100 SO2 濃度（ppm） （ASU-200 有り） SO2 計指示 （ASU-200 無し） 0 100 NH3 濃度（ppm）
3. ハロゲンスクラバ 目的：廃棄物焼却炉，バイオマス，2型式：HS-050（室温），HS-3030（室温）， （上）「実装中のHS-050及びHS-3030」（垂直使用）	ハロゲンガス除去原理：銀塩及びウール状金属線材とハロゲンガスの腐食性反応（写真HS-050） 1. 銀塩コーテイングゾーンが黒色化，2. ウール状金属線変色

ミストキャッチャ：排ガス中SO_3ミストを除去しゼロドリフトやセル腐蝕を防止するミストキャッチャ（MCシリーズ）を開発した。
HN_3スクラバ　：排ガスへのNH_3注入により，重油ボイラー排ガスおよび脱硝設備のいずれの場合もSO_2測定精度に誤差を生じるので，完全にNH_3を除去できるNH_3スクラバ（ASUシリーズ）の開発を行った。
ハロゲンスクラバ：廃棄物焼却炉およびガラス溶融炉排ガス用途にハロゲンスクラバ（HSシリーズ）を開発した。現状では廃棄物焼却炉用途の全出荷製品に採用することで，納入後のハロゲン化合物の検知および除去剤として用いられている。

おわりに

本稿では，煙道排ガス分析計の歴史について，初期のSO_2分析計の開発から製品立上げ，さらに次期後継機種の改良に伴うサンプリング技術とその構成部品について述べてきた。これら分析計技術やサンプリング系主要部品は，排ガス測定分野であるがゆえに独自のものづくりの考えが活かされている。特に，多成分計測でありながら検出原理に全て赤外線分析，クロスフロー方式に集約できたことで，サンプリング系が簡略化でき，ユーザの保守点検の容易さにつながったと言える。

高い山に登る時，登山道は幾通りもあるように，排ガス分析計やプロセスガス分析計の開発も顧客仕様や分析計技術がその機会ごとに異なり常に正解は一つではないと思う。筆者のコラムが，今後のガス分析計開発に携わる若い技術者やガスを扱う研究者の考えのヒントや道しるべとなれば幸いである。

＊編集局注：本内容は特段の記載がない限り，本誌発行年時点での自社調査に基づいて記載しています。

参考文献

[1] 三菱重工業技報，Vol.49，No.4(2012)
[2] 特公昭52-4957(1968年出願)
[3] 特公昭56-25971(1976年出願)

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令和元年度近畿地方発明表彰
Kinki Region Invention Awards 2019

京都発明協会会長賞

液体計量装置及び水質分析装置（特許第6223041号）

表彰の概要

本発明表彰は，近畿地方における発明の奨励・育成を図り，科学技術の向上と地域産業の振興に寄与することを目的としており，近畿地方において優秀な発明，考案，又は意匠（以下「発明等」という。）を完成された方々，発明等の実施化に尽力された方々，発明等の指導，奨励，育成に貢献された方々の功績を称え顕彰するものである。HORIBAからは特許第6223041号(液体計量装置及び水質分析装置)が京都発明協会会長賞を受賞した。

Figure 1　近畿地方発明表彰　表彰式の様子

表彰案件の概要

本発明は，高精度で複雑な制御が不要であり，誤検知を起こしにくい簡単な構成により正確に計量を行うことができる液体計量装置及び水質分析装置に関する発明考案である。本発明は自動全窒素・全りん測定装置（TPNA-500）（**Figure 2**，**Figure 3**）に採用されている。

Figure 2　自動全窒素・全りん測定装置（TPNA-500）

Figure 3　自動全窒素・全りん測定装置（TPNA-500）の内部構造

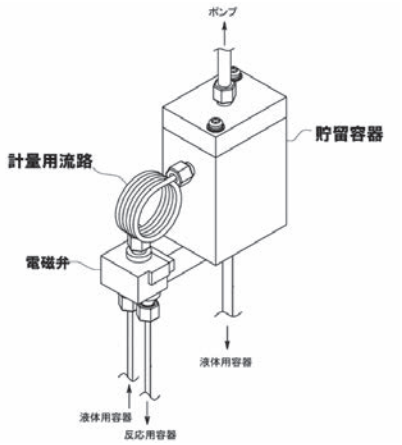

Figure 4　液体計量装置を示す斜視図

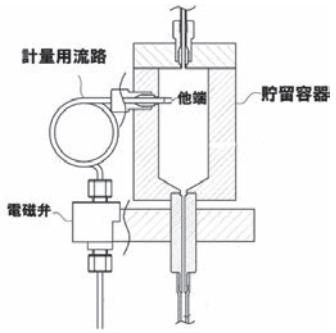

Figure 5　液体計量装置を示す断面図

従来発明等の課題

排水等の水質検査等において，検査結果の精度を向上させるためには，測定試料又は測定試料を処理する試薬等の液体を正確に計量する必要がある。そして，これらの液体を計量するものとしては，シリンジ型の計量ポンプ（シリンジポンプ）を用いて計量する計量装置や，光学式センサ等の液体検知センサを用いて計量する計量装置が考えられている。

しかしながら，両計量装置には欠点がある。シリンジポンプを用いた計量装置では，正確に計量するためにピストンの移動量を高精度に制御する必要がある。加えて，シリンジポンプは液切れが悪く，正確に計量することが難しいという問題がある。また，液体検知センサを用いる計量装置では，計量室に液体を注入する注入手段を液体検知センサの検知信号で制御するため液滴や気泡に反応して誤検知を起こす場合もあり，正確に計量を行うことができないという問題がある。

本発明等の特徴

計量用流路の一端から液体を導入するとともに，計量用流路の他端から液体を漏れ出させる（オーバーフロー）だけで液体を計量することができるため，ポンプ機構による駆動時間（吸引時間）を設定するだけでよく，簡単な装置構成で正確に計量することができる。また，シリンジポンプや液体検知センサが不要となるので，複雑な制御を行う必要がなく，液切れの悪さ，応答遅れ，誤作動等による計量誤差を防ぐことができる。

さらに，本発明の構成では，計量用流路の他端の開口方向が水平方向を向くので，計量用流路の他端の開口方向が垂直方向を向く場合と比べて，計量用流路で計量される液体量の誤差が減り，計量誤差を少なくしてより一層正確に計量することができる（**Figure 4**，**Figure 5**）。計量用流路の他端開口が垂直方向となる場合，計量用流路の他端から表面張力によりはみ出た液体量が液体の粘性などによって変化しやすく，垂直方向上向きの場合，設計によっては計量用流路の他端から漏れ出た液体が貯留容器の上面に付着し，この付着した液体が再度計量用流路の他端に滴下されて，計量用流路で計量される液体量が変化する恐れがあるが，水平方向であればこのような恐れは排除できるからである。

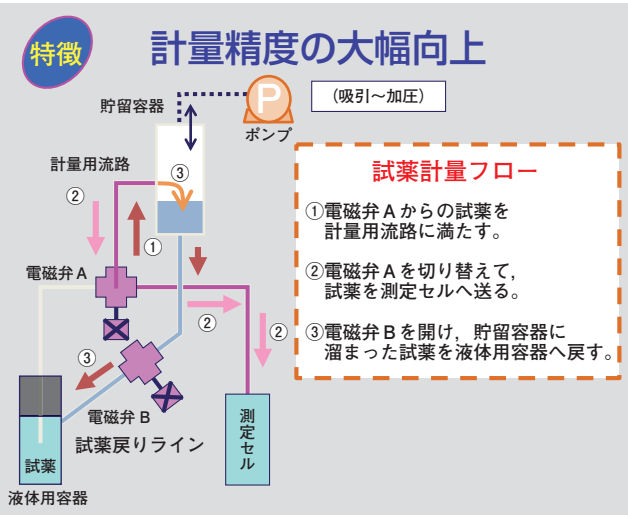

Figure 6　試薬の計量フロー

本発明に関する液体計量装置は，一端が貯留容器に接続されるとともに他端が液体用容器に接続されて，貯留容器に貯留した液体を排出する排出用流路をさらに備え，ポンプ機構が，貯留容器に貯留された液体を，排出用流路を介して液体用容器に送液することが望ましい（**Figure 6**）。このような構成であれば，貯留容器内に貯留された液体を液体用容器に戻すことができるので，貯留容器に液体が溜まりすぎて，計量に不具合が生じることを防ぐとともに，その液体を無駄なく使用することができる。

【登録番号】
特許第6223041号
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その他受賞案件

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発明奨励賞	特許第4519832号	欠陥検査装置	大嘉達夫，池田輝彦，神崎豊樹
発明奨励賞	特許第5715969号	流体抵抗デバイス	矢田秀貴，岸田創太郎，畑板剛久

Figure 7　近畿地方発明表彰　HORIBA受賞者

＊編集局注：本内容は特段の記載がない限り，本誌発行年時点での自社調査に基づいて記載しています。

第9回HORIBA Group IP World Cup Gold Award受賞案件の紹介

Award Winners of HORIBA Group IP World Cup 2019

Figure 1　HORIBA Group IP World Cup

Figure 2　当社適用製品（マスフローモジュール CRITERION D500）

HORIBAグループで生まれた数々の独創的な技術や知的財産（以下，Intellectual Propertyの略語として「IP」ともいう）が事業の推進力となってきた。技術開発とその成果たる知的財産がHORIBAブランドの本質的な要素であり，HORIBA Group IP World Cup（**Figure 1**）は，HORIBA Group is One Companyの精神のもと，事業を牽引する技術・知的財産をグループ全体で賞賛し，次なる成長の起爆剤となる技術・知的財産の創出をさらに奨励していくことを趣旨として創設された。

第9回HORIBA Group IP World Cup*では，海外を含むHORIBAグループの開発拠点から23件の応募があり，株式会社 堀場エステックの「Valve element and fluid control valve（弁要素及び流体制御弁）」がGold Awardを受賞した。この知的財産は，ガスの流量を制御するマスフローコントローラ（以下MFC）等に用いられる流体制御弁に関し，閉塞時におけるシール性を向上させるとともに，長期間に亘って耐久性を維持して安定性を向上させることができる発明考案であり，HORIBAグループを代表する技術として以下に紹介する。

＊第9回では，2018年6月1日から2019年5月31日の間に創作，出願，論文発表，特許登録，または外部表彰を受賞したなどの知的財産を対象としている。

弁要素及び流体制御弁

IP：特願2012-196980

受賞者：林繁之，安田忠弘，太佐和也（株式会社堀場エステック／日本）

Gold Award受賞案件の概要

MFC（**Figure 2**：当社製品）に用いられる流量制御弁において，流体の内部リークを防止するために，弁座面を研磨した弁体を用いて内部リーク防止を行っていたが，弁座面には精度の高い平面度が要求されるため，平面ラッピングを行う必要がある。しかし，樹脂を使用するにはコーティングの膜厚が薄く硬度も低いことから，平面ラッピング時に厚みを制御できず，コーティングが剥がれてしまうことが多かった。このため生産性も悪く，長期耐久性も劣っていた。

本受賞発明は，下記のように3つのステップを経て製造された弁体部材を使用することにより，上記問題点を一挙に解決するためになされたものである。

1. 弁体部材の上面に凹部を形成する（**Figure 3（B）**）。この形成方法としては，切削加工等の機械加工である。
2. 次に，凹部を含む弁体部材の上面全体に凹部の深さ以上となるように樹脂層を形成する（**Figure 3（C）**）。
3. その後，上面に形成された樹脂層を平面ラップング等の研磨処理によって研磨する。つまり，弁体部材の上面に形成された樹脂層を研磨するだけでなく，

弁体部材の上面に形成された凹部の内周部(駆動力作用面に対応する部分)及び外周部(研磨制限ガイド部)も併せて研磨する(**Figure 3(D)**)。このような研磨によって，凹部内にのみ樹脂が残る構成となり，凹部内に残った樹脂が樹脂コーティング膜となる。このように凹部の内周部及び外周部も併せて研磨することによって，凹部内の樹脂を研磨しすぎることを防止するとともに，凹部内の樹脂を均一の膜厚に研磨することができる。また，研磨制限ガイド部が周方向全体に形成されており，この研磨制限ガイド部も併せて研磨されることから，凹部内の樹脂を片削りする心配も無い。

このように構成したMFCによれば，弁体部材の対向面に凹部を形成し，凹部に樹脂コーティング膜を形成しているので，樹脂コーティング膜が弁座部材の弁座面に接触することで，閉塞時におけるシール性を向上させて，内部漏れ量を低減することができる。

また，凹部内に樹脂コーティング膜を形成しているので，樹脂コーティング膜の表面を研磨するときに，凹部以外の対向面部分(特に研磨制限ガイド部)が表面研磨を行うためのガイドとなり，樹脂コーティング膜表面の平面度を確保しつつ，樹脂コーティング膜の膜厚を均一にすることができ，着座面を精度の良い平面度とすることができる。

さらに，弁体部材全体に対して樹脂コーティング膜の膜厚が小さいので，弁体部材の熱膨張量に対して樹脂コーティング膜の熱膨張量を小さくすることができ，樹脂コーティング膜を形成することによる温度影響を小さくすることができる。

加えて，例えば50～150 μmといった薄膜の樹脂コーティング膜によりシール性を確保しているので，樹脂コーティング膜に弁座部材の弁座面が接触したときに樹脂コーティング膜の弾性変形量を小さくすることができ，樹脂コーティング膜に形がつきにくく，長期間に亘ってシール性を確保することができる。

Figure 5　Gold Award受賞者(左から林繁之，安田忠弘，太佐和也)

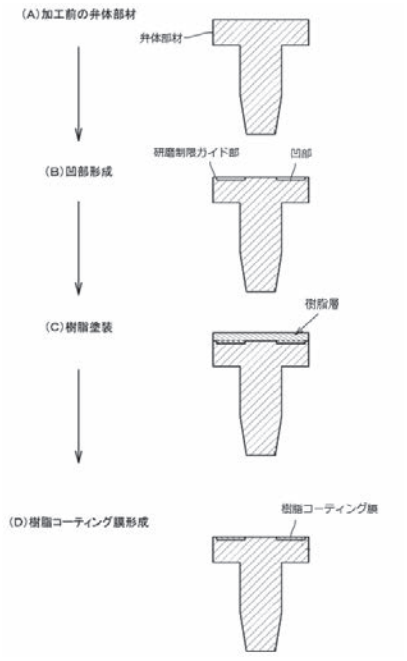

Figure 3　弁体部材の製造方法を示す図

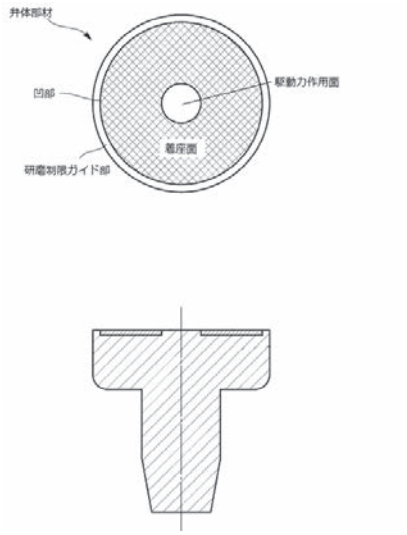

Figure 4　弁体部材の上面図及び断面図

その他受賞IP

【Silver Award】

バッテリモデリングと制御

IP：(社外発表)"Development and Verification of a Distributed Electro-Thermal Li-Ion Cell Model," in 44th Annual Conference of the IEEE Industrial Electronics Society (IECON), 2018, vol. 1, pp. 2044–2049.

受賞者：Richard Stocker, Puneet Mathur, Paramjeet, Michele Braglia
（ホリバMIRA社／イギリス）

概　要：バッテリ挙動を再現するパラメータを任意に設計可能であり，顧客要求に応じた最適なバッテリモデルの作成を実現した技術。

【Bronze Award】

作動距離可変なX線分析装置のための均一照明

IP：特願2018-556538
受賞者：秋山久，上野楠夫，赤松武
（株式会社堀場製作所／日本）
概　要：当社製品XGTの特徴である「部分真空」を維持しながら，照明光を散乱させる拡散板を用いることで，凸凹サンプルの測定時に鮮明な光学像を取得可能とした技術。

【Bronze Award】

トルクマッチングの手法を用いたラボ上でのリアルワールド車両テストの実現

**IP：PCT/US2018/067636,
PCT/US2018/067652**

受賞者：Leo Breton
（ホリバ・インスツルメンツ社／アメリカ），
Thomas Hoehr
（ホリバ・ヨーロッパ社／ドイツ），
Alex Mason（ホリバMIRA社／イギリス）

概　要：実路車速とアクセルを台上で再現し，実路でのトルクを取得し，さまざまな環境条件を変化させた実路を台上で再現可能とした技術。

＊編集局注：本内容は特段の記載がない限り，本誌発行年時点での自社調査に基づいて記載しています。

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