Publishable Summary for 20NRM02 MFMET
Establishing metrology standards in microfluidic devices

Overview

Microfluidics, concerned with fluid-handling in the nano-to-millilitre scale, has major applications in biomedical and chemical analysis however global standards are lacking. ISO/TC48/WG3 has been established to address the standardisation of microfluidic components, interfaces, protocols for associated testing and protocols for microflow control to be applied in the development and the fabrication processes (manufacturing, testing and assembly) of microfluidic devices. This project aims to contribute to the development of globally accepted standards for microfluidics and disseminate them to end users in industry (health and pharmaceutical sectors) and academia.

Need

The increased technical capability required to miniaturise devices along with the growing need for faster, more accessible, and cost-effective solutions for precision analytical tools has led to the rapid and continuous growth of microfluidics in diverse sectors (e.g. pharmaceutical and biomedical industries). According to a recent study, the global microfluidics market size is expected to reach 44.0 billion Euros by 2025 from an estimated value of 15.7 billion Euros in 2020. However, microfluidics and specifically the control of fluids in microfluidic devices still lacks universal solutions and standards. Stakeholders from industry, academia and government have recognised the need for globally accepted metrology standards for microfluidic devices and as a result ISO/TC48/WG3 was established to address this underpinning requirement. Measurement accuracy and traceability of microfluidic devices is critical to improve healthcare, including medical diagnostics and drug development sectors. For example, enabling rapid prototyping of low-cost high-volume point-of-care tests that can be shipped to individuals for rapid in-situ detection of viruses is a critical step in tackling future healthcare crisis, as highlighted by COVID-19. Current on the spot diagnosis that involves clinical input is cumbersome and expensive – microfluidic devices for on the spot diagnosis (such as pregnancy, glucose and PH tests) can provide cheaper, simpler and faster results.

Standardisation of performance characteristics is needed for the different classes of microfluidic components, including test conditions, measurement protocols and guidelines. The increasing demand for passive flow devices has already led National Metrology Institutes (NMIs) to establish protocols and calibrations services for very low flow rates. Traceability to National Standards has been available since 2012 down to 0.1 µL/min through facilities developed under EMRP JRP HLT07 MeDD. Recently EMPIR JRP 18HLT08 MeDDII tackled microflow measurements down to 5 nL/min and introduced new facilities which are now under implementation. These new technologies can now be used to develop microfluidic measurement protocols, and the new microflow pump devised in MeDDII can be used as a traceable flow generator.

In 2016, a first step towards microfluidic standardisation was made through ISO IWA23. The document was created to facilitate the uptake of microfluidic devices by making them easier to use, reducing the cost for assembling and enabling plug-and-play functionality. Recently a new standard, ISO/CD 22916, is being established based on the information from ISO IWA 23 which it will replace; however, this new standard still lacks the metrological specifications required for accurate and reproducible manufacturing.

Objectives

The overall objective of this project is to contribute to the development of globally accepted standards for microfluidic devices used particularly in the health and pharmaceutical industry.

The specific objectives are:

1. To investigate, evaluate and formulate consensus-based flow control specifications, guidelines and protocols to enhance the manufacturing capability of the microfluidics industry supply chain through voluntary compliance.
2. To develop measurement protocols for different flow quantities and liquid properties, in different microfluidics devices to be used in pharmaceuticals, biomedical and mechanobiology applications. A EURAMET guide and a technical report on these measurement protocols will be developed.
3. To define consensus-based standards and guidelines for interfaces and connectivity between fluidic passages and optical/electrical connections of microfluidics components and corresponding measurement standards, from micro to macro size scales.
4. To define guidelines for the standardisation of dimensions and accuracy for modularity (either module-to-module or module-to-world) and sensor integration (combination of sensing elements/materials with microfluidic modules), in accordance with good practices in microfluidic component design and manufacturing.
5. To collaborate with ISO/TC48/WG3 and end users of the standards (e.g. health and pharmaceutical industry) to ensure that the outputs of the project are aligned with their needs and in a form that can be incorporated into standards (e.g. new technical guides, ISO 10991 and ISO/CD 22916) at the earliest opportunity.

Progress beyond the state of the art

In 2012, EMRP JRP HLT07 MeDD established metrological standards in liquid micro-flow in the scope of medical devices performance assessment and calibration.

In 2016, a first step towards microfluidic standardisation was made with the preparation of ISO IWA23. The document was created to facilitate the uptake of microfluidic devices by making them easier to use, reducing the cost for assembling and enabling plug-and-play functionality. This document was now been replaced by the new ISO/CD 22916, still under development by ISO/TC48/WG3.

In 2019, EMPIR JRP 18HLT08 MeDDII followed on from the EMRP JRP HLT07 MeDD to extend traceability to nano-flow rates, from 5 nL/min to 100 nL/min for steady and transient flow rates with a target uncertainty of 1 to 2 % (k=2). A first step toward microfluidics traceability was achieved within MeDDII with the development of a microfluidic pump acting as a transfer standard for micro-flow rate calibration.

By developing a consensus-based harmonisation of the metrological criteria for the design, qualification, and use of flow control devices such as pumps and valves, this project will provide guidelines and standardised protocols and methodologies beyond the state of the art. These will be applicable throughout the entire microfluidics industry supply chain, from the manufacturer to the end user with the guarantee of traceability to the SI.

A EURAMET guide based on the measurement protocol for different flow-related quantities will be developed. Test protocols for flow and liquid properties including documented examples will be produced, as well as a technical report for the manufacturing of transfer standards for microfluidic components, representative of the diversity of the applications, to be used to calibrate testing equipment of end users and industrials. Two microfluidic transfer standards will be manufactured in order to test the protocols developed through WP1 to WP4.

This project will develop harmonised metrological specifications (such as a measurement protocols, guidelines) for the dimensions, positions, physical and material compatibility of the connections in microfluidics components and operational functionality, (such as dimensioning tolerances, leakage and burst pressure) from micro to macro size scales, focusing on fluidic passages and electrical/optical connections of components.

This project will develop a landscape document on component design and manufacturing for interoperability and heterogeneous integration, and measurement protocols for dimensional characterisation, ensuring integrity, functionality and metrological compliance of related devices.

Results

Consensus-based flow control specifications for microfluidics (objective 1)

The partners have performed a literature review and market research to compile and classify flow control components as reservoirs, valves, chambers, external connectors, tubing, etc. Within this research, the partners additionally investigated the definitions, characteristics (e.g. flow range), specifications (e.g. accuracy), application and function (e.g. droplet generation) of the components.

Basic and general concepts and associated terms related to flow control whose definitions were lacking harmonisation were identified and reported together with definitions, symbols and vocabulary of flow control. The information collected by the partners, ISO/TC48/WG3 and The MFA is being inputted in a database/inventory for flow control components, whose software was developed by a partner within the project. A manual to access and use the database was also prepared.

The partners have created a generic specifications list with key parameters for the comparison of flow control components. A future draft will include tentative classification of classes from flow rate intervals. This will be further discussed with the End-user Advisory Board.

The project is heavily engaged in communication with the microfluidics community. And because of the urgent need of the community for guidelines and, in particular, test protocols for flow control focusing leakage and burst pressure, a group of partners together with microfluidics stakeholders have started the work on this topic earlier than foreseen. One white paper (Protocols for Leakage Testing) and one scientific paper (Overcoming Technological Barriers in Microfluidics: Leakage Testing) are now published. Furthermore, guidelines for testing leakage and burst pressure of microfluidic devices have been written.

All the documentation produced can be found on the project's website https://mfmet.eu and Zenodo repository at https://zenodo.org/communities/mfmet.

Measurement protocols for different flow quantities and liquid properties (objective 2)

A generic methodology of accurate measurement of a particular quantity in a microfluidic device has been defined, based on partners' experience, a literature review and results from previous projects (EMRP JRP HLT07 MeDD and EMPIR JRP 18HTL08 MeDDII, and reports published by the MFA).

EnablingMNT performed a survey on microfluidics leakage (completed in September 2021), and also made publicly available three previous surveys carried out in the scope of the MFA. These surveys covered Microfluidic Interconnections and Chips (September 2014), Reliability of Microfluidics based Devices and Components (March 2015), and Microfluidics Flow Control (November 2015). The partners conducted interviews with 11 experts from academia and industry on measurement priorities during fabrication of microfluidic devices and components.

Three flow quantities (flow, flow resistivity, volume) and four liquid properties (viscosity, density, contact angle and refractive index) have been identified and the report presenting the inventory of the above quantities and properties was produced. The partners have carried out a literature review of existing metrology and normative standards related to the flow properties and microfluidic devices and a literature review of existing metrology and normative standards related to the liquid properties and microfluidic devices. The project has produced a document on the development of test protocols for microfluidic devices. Test protocol for liquid properties measurement and several flow quantities have been completed and reports are ongoing. Calibration guide for the evaluation of flow-related quantities in microfluidic devices including an example of three industrial applications is under preparation.

The development of the transfer standards for microfluidics-related quantities such as flow, volume, material, dimensional, and/or optical started earlier than planned in order to facilitate the testing in upcoming tasks. Eight designs have been developed for microfluidic devices made of glass. The polymeric devices are also concluded and will be sent to CETIAT. The glass transfer standards have been delivered by IMTAG in spring 2023.

The transfer standards will be used as reference objects to ensure traceability, comparability, and validity of the microfluidics manufacturers and users' measurement and testing capabilities. They will be characterized at National Metrology Institutes with best available accuracies for flow, flow resistivity, volume, channel dimensions, roughness and surface energy. The design process is driven in accordance with existing ISO standards (such as ISO 22916 for port pitch and dimensions) and MFMET consortium technical recommendations.

General standards and guidelines for interfaces and connectivity (objective 3)

The partners were able to identify the most important and commonly used materials for microfluidics fabrication, the key performance parameter, the type of connectors and the type of fabrication methods, based on survey results and investigation of suppliers of microfluidic components.

A test protocol for hydrophobicity, hydrophilicity and wettability is concluded. The documented example of this test protocol was performed in the liquid flow laboratory at CETIAT and describes in detail the measurand (contact angle) and the property assessed (wettability) quantified by the surface energy of a given material. The materials used in the documented example were glass slides (D263® bio) provided by IMTAG.

The compatibility of at least three microfluidic components was tested and documented. Further tests will be carried out on the golden standards (glass and polymer) and results are expected at a later stage in the project. Guidelines for optical interfaces of microfluidic devices have been developed and published as a White Paper. Further guidelines for the implementation of standardises methods of microfluidic components focusing on port connection from microscale fluidic channels to the macroscale world and associated changes in flow and pressure are still under development.

A database for the surface roughness of materials used in microfluidic was established (COC/COP, glass, PMMA). A test protocol for AFM, confocal microscopy and stylus profilometry has been established, followed by a documented example on glass specimens (D263® bio), which included the bonding and channel area. The roughness results are representative and give a good estimate of the roughness of all surfaces (bonding and channel area) in a glass microfluidic device. Consequently, a list of guidelines for the measurement of key performance parameters of microfluidic connections, which are based on the list of key properties of microfluidic interfaces, has been developed.

Guidelines for the standardisation of dimensions and accuracy for modularity and sensor integration (objective 4)

Interviews lead by EnablingMNT, with strong and instructive feedback from manufacturers and microfluidics customers have been completed. Working meetings have been held between the project partners and industrial representatives. Literature reviews have been completed regarding solutions for assembling modular microfluidic systems and components, and heterogeneous integration of sensors into microfluidic systems and components.

A report on requirements and constrains in modular microfluidic components is already completed. In this context, the preparation of the landscape document to identify standardisation requirements for microfluidic component design and manufacturing with respect to modularity and heterogenous sensor integration is also ongoing and in the final phase.

A list of physical parameters relevant for microfluidic components as well as a list of measurement methods for these parameters is already completed. This will be the foundation for detailed measurement protocols for the dimensional characterisation of microfluidic components, for which the discussion and preparation have started.

Impact

The project webpage (https://mfmet.eu) is regularly updated with news and information such as project reports, and details of project meetings. Since the project's start, the website has been viewed over 34000 times from 68 countries and more than 500 documents download. In collaboration with the Microfluidic Association several surveys have been developed and four whitepapers have been published namely on leakage testing, on Optical Interfaces of Microfluidic Devices, on Flow resistivity and another on hydrophobicity, hydrophilicity, and wettability. Four MFMET Newsletters are now available on the project's webpage. The project's reports and publications are/will be also available on the Zenodo repository https://zenodo.org/communities/mfmet. The project is also advertised at the EURAMET page. A news stories were published by EURAMET in July 2023 regarding the MFMET project: EMPIR project on microfluidic devices presents at major international conference. Two articles were published in the regular press, 14 technical reports/protocols/guidelines have been produced by the consortium and are available in the webpage, so far more than 500 downloads of these documents have been done. The project was presented at EURAMET TC FLOW and at BIPM WGFF. The partners have given four poster presentations to the scientific community e.g., CIM2021, at EUROoCS 2022 and MPS2023. Twelve oral presentations have been given so far in metrology and microfluidic conferences. Three open access publication have been published in international journals. All data and publications are also available on the Zenodo repository under the MFMET community.

Impact on industrial and other user communities

This project is crucial to bridge this gap by providing guidelines as future standards in the areas of design, materials and test. This will enable more reliable products, which is critical in healthcare (e.g. point-of care solutions), enabling the manufacturer to reduce the number of references, cost and ultimately increase its sales. Overall, the outcomes of this project will potentiate testing and improvement or development of new microfluidic devices with increased accuracy and quality, and their joint dissemination with The Microfluidic Association (MFA) will further intensify the early adoption of the practices developed within this project.

Contacts have been made with experts from the American Food and Drug Administration (FDA) and the American Institute of Standards and Technology (NIST) who are much interested in the outcome of this project. The partners are also working together with other experts from outside the MFMET consortium ensuring meeting the expectations of the community and easier acceptance of the project outcomes, e.g., Elveflow that is now a collaborator of the project.

Impact on the metrology and scientific communities

The importance of quantitative measurements with a suitable degree of precision constitutes a basic underpinning framework for the scientific research and technological development. This project will create an early impact as it will allow NMIs to upgrade and adapt their existing facilities for the calibration of microfluidic devices and instruments. By developing transfer standards dedicated to microfluidics applications, the project will allow NMIs to disseminate the traceability chain towards both the manufacturers and end users.

It is generally acknowledged that there is still a lack of understanding of the importance of precision and standards, more so if standards and calibration methods are not available. New calibration methods and microfluidic transfer standards will be developed in the scope of this project, and impact will be created as these methods will be disseminated to the scientific community in relevant publications and EURAMET guidelines.

Impact on relevant standards

In this project, procedures and methods for the calibration of microfluidics devices and microfluidics-related instruments that are already on the market will be developed. The consortium will create impact by supplying this information to the relevant ISO technical committees (TC) and will make efforts to ensure that these results are incorporated in any updates to standards. Thus, this project will adapt existing measurement procedures and define new measurement procedures for different types of devices and instruments used by the microfluidics industry.

The consortium has engaged in several standardisation activities. Within ISO/TC 48/WG 3 Microfluidic Devices contributions were given to ISO 22916 – Microfluidic devices – Interoperability requirements for dimensions, connections and initial device classification, this document was published during 2022. Comments were sent by the consortium on ISO 10991 Microfluidics – Vocabulary, a new version of the document will be published in 2023. A new ISO TS: ISO/CD TS 6417 Microfluidic pumps — Symbols and performance communication is under development (committee draft has been circulated). Further, in ISO/TC 48/WG 5 Liquid Handling Devices- Automatic the development of ISO/DIS 23783- 1, 2 and 3 are followed by IPQ and HSG-IMIT; these documents were published in 2022. IPQ and HSG-IMIT are also involved in the development of ISO/TR 6037 - Automated liquid handling systems – Uncertainty of the measurement procedures. Several partners are now engaged on the work of the new CEN/CENELEC Focus group on Organ-on-Chip that started in 2022, mainly in WG1 – Terminology and WG3 - Engineering.

Longer-term economic, social and environmental impacts

This project will directly benefit society because it will accelerate innovation, by allowing academia, end users in industry (health, pharmaceutical) and microfluidics devices manufacturers to develop and/or use standardised products with clear, traceable and controlled specifications. The rapid production of low-cost high-volume point-of-care tests that can be distributed to patients for swift detection of viruses is a good example of the importance of microfluidics in tackling future healthcare crisis.

Improvements in the accuracy of instruments and devices will reduce manufacturing costs while improving quality and usability. This will be achieved through the wider uptake of traceable calibrations & test protocols and by improved knowledge of how to calibrate instruments involved in the whole manufacturing process of microfluidic devices, from the early stages of chips designs to end-user tests in the laboratory.

List of publications

Metrology challenges for microfluidics. CMM magazine, April 2022, ISSN 2634-9167. http://www.cmmmagazine.com/cmm-articles/metrology-challenges-for-microfluidics

Overcoming technological barriers in microfluidics: Leakage testing. Frontiers in Bioengineering and Biotechnology, September 2022, 10: 958582, https://doi.org/10.3389/fbioe.2022.958582

Cancer Models on Chip: Paving the Way to Large-Scale Trial Applications. Advanced Materials, April 2023, 2300692, 26 pp. https://doi.org/10.1002/adma.202300692

This list is also available here: https://www.euramet.org/repository/research-publications-repository-link/

<table>
<tr><td colspan="2">Project start date and duration:</td><td>01 June 2021, 36 months</td></tr>
<tr><td colspan="3">Coordinator: Elsa Batista, IPQ Tel: +351 212948167 E-mail: ebatista@ipq.pt
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<tr><td colspan="2">Chief Stakeholder Organisation: Microfluidics Association</td><td>Chief Stakeholder Contact: Darwin Reyes</td></tr>
<tr><td>Internal Funded Partners:
1. IPQ, Portugal
2. CETIAT, France
3. CMI, Czechia
4. DTI, Denmark (joined from 1 January 2022)
5. LNE, France
6. NEL, United Kingdom (withdrawn from 19 November 2021)
7. NQIS, Greece (withdrawn from 24 February 2022)
8. RISE, Sweden (joined from 1 September 2022)
9. TUBITAK, Türkiye</td><td>External Funded Partners:
10. CEA, France
11. EnablingMNT, Netherlands
12. HSG-IMIT, Germany
13. IMTAG, Switzerland
14. INESC MN, Portugal
15. microfluidic, Germany</td><td>Unfunded Partners:
16. BHT, Netherlands
17. UofG, United Kingdom</td></tr>
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