European Metrology
Research Programme
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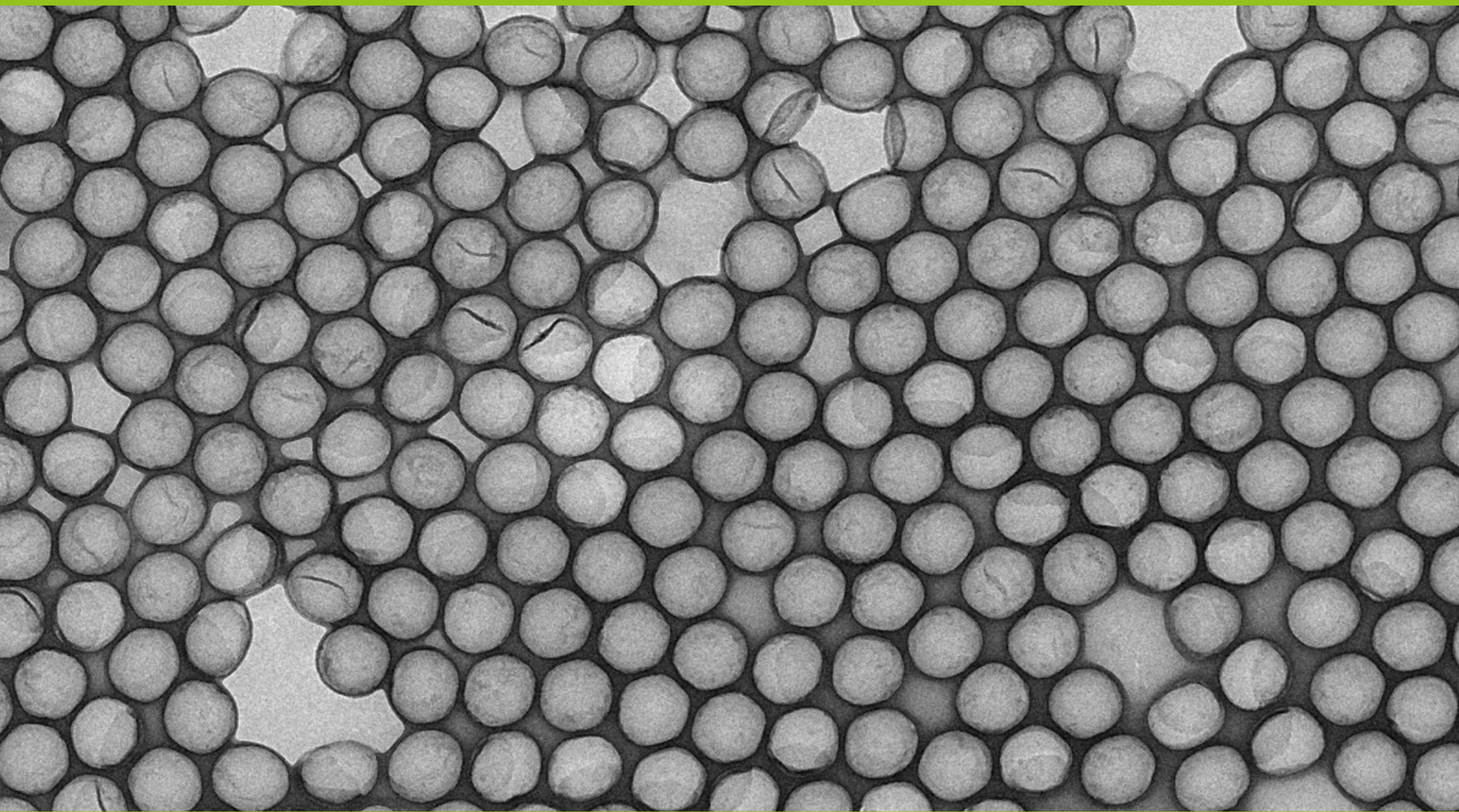


New reference material for light scatter-based sizing of extracellular vesicles

In the 21st century healthcare costs are expected to rise faster than Gross Domestic Product in most European countries. Early diagnosis of diseases such as cancer can help to reduce this economic burden and improve the quality of life and the prognosis of patients. Sub-microscopic particles secreted by human cells have the potential to revolutionise this process but lacked the reference materials needed to standardise measurements.

Europe's National Measurement Institutes working together

The European Metrology Programme for Innovation and Research (EMPIR) has been developed as part of Horizon 2020, the EU Framework Programme for Research and Innovation. EMPIR funding is drawn from 28 participating EURAMET member states to support collaborative research between Measurement Institutes, academia and industry both within and outside Europe to address key metrology challenges and ensure that measurement science meets the future.

Challenge

Extracellular vesicles (EVs) are biological nanoparticles actively released by cells, comprising of an outer lipid membrane, and containing markers indicative of the cell of origin, and housing an inner aqueous core. Most EVs are 100 nm in diameter or less but can range in size from 30 nm to a few micrometres. EVs are found in all body fluids. Moreover, cells affected by disease release altered EV profiles compared to normal cells. Therefore, there is a great clinical interest in the use of EVs as biomarkers to diagnose a wide range of medical conditions.

A common technique used in hospitals to characterise EVs is flow cytometry (FCM). FCM can analyse thousands of individual EVs per second by measuring the light scattered as EVs pass through a laser beam, providing data on size, concentration, granularity, and fluorescent intensity.

However, an EV sample characterised on one cytometer is unlikely to return the same results on a different cytometer. As well as different optical configurations, flow cytometers are commonly calibrated for EV measurements using polystyrene beads with a solid core and a high refractive index (RI, ~1.63) compared with EVs (≤ 1.40). As a result, polystyrene beads scatter light 1000-fold more than similar-sized EVs, leading to misinterpretation of light scattering signals of EVs and making comparisons of clinical results difficult.

Solution

During the *METVES* project, a new reference material for EVs was synthesised by combining a basic amino acid catalysis route with a hard template approach. Silica beads of 200 nm and 400 nm were coated with 1,2-Bis(triethoxysilyl)ethane to form an organosilica 'shell' over the particles. The inner silica core was then 'etched away' to generate Hollow Organosilica Beads (HOBs).

Flow cytometry two-angle light-scattering measurements then confirmed HOBs had light-scattering properties and an effective RI similar to platelet-derived EVs (≤ 1.40). However, during the project, HOB preparations were small scale and problems were encountered in reproducibility of the organosilica shell thickness (5-10 nm).

The *METVES II* project developed procedures to scale up HOB production ten-fold. 'Small-angle X-ray Scattering', a nano-scale measurement technique, was used to obtain the diameter and shell thickness of HOBs. This demonstrated the reproducibility of the core size and the shell thickness (~ 10 nm). Nanoparticle tracking analysis and 'single particle inductively coupled plasma mass spectrometry' confirmed the HOBs concentrations. A protocol was also established to label the HOBs with the fluorescent compound fluorescein isothiocyanate, a commonly used fluorochrome to label EVs.

Impact

Founded in 2015, the goal of Exometry BV, based in the Netherlands, is to promote the use of EVs as biomarkers of disease by applying physics to both understand and push the technological limits involved in EV detection.

The company was a project partner in *METVES II*, which allowed them access to the technology and metrological knowledge to generate traceable data on the HOBs they were developing. After the end of the project in 2022, the company commercialised this new reference material as "Verity Shells", which are applicable for use on all flow cytometers with a scatter detector capable of detecting at least 100 nm polystyrene beads.

The development of more realistic calibration material, such as the Verity Shells, will allow the validation and reproducibility of EV measurements from clinical samples. In turn, this approach will allow cheap and rapid diagnostic tests to replace the more expensive and invasive tests such as solid biopsies and also allow earlier identification of pathological conditions in patients.

Standardising extracellular vesicle measurements for medical diagnosis

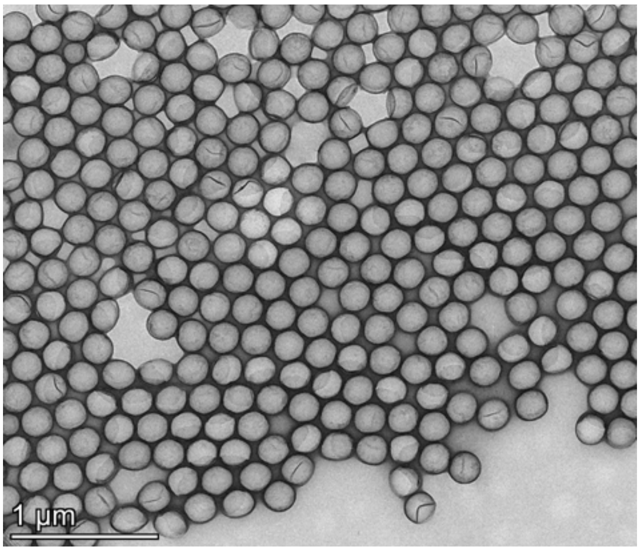
The *METVES II* project developed unique instrumentation for extracellular vesicle (EV) measurements including the world's first precision goniometer and a metrological flow cytometer, along with software to support its use.

Three distinct materials were developed to allow traceable measurements of different EV properties with a low measurement uncertainty:

- Hollow Organosilica beads (HOBs), with a refractive index similar to EVs which were developed into a commercial product.
- Liposomes for traceable measurements of fluorescent properties.
- PEVTES, a unique test sample prepared by isolation of EVs from human blood plasma, which expresses the complexity and heterogeneity of a real human sample.

A global interlaboratory comparison study was performed on 39 different flow cytometers by 24 laboratories from 12 different countries.

The metrological basis developed in the project will become a prerequisite for clinical acceptance and routine application of EV-based diagnostics.



Transmission electron microscope image of "Verity Shells"

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Rienk Nieuwland

VSL, The Netherlands
r.nieuwland@amsterdamumc.nl

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