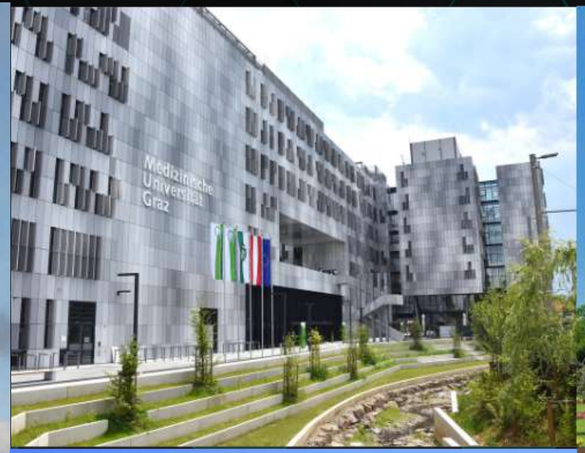
BRAVE®
ANALYTICS

# Seamless monitoring of (nano)particles for optimized quality control of continuous production processes.

Sensor platform.
Continuous 24/7.
In real-time with single-particle accuracy.

# Where we come from ...


Med Uni Graz

BRAVE®
ANALYTICS

**BASIC RESEARCH**
VLPs, liposomes, proteins, EVs

**PHARMA & BIOTECH**
Emulsions, vaccines, anesthetics, infusions

**BEAUTY & COSMETICS**
Skin lotions, sun creams

**COATINGS**
Surface coatings, surface pigments & lacquers

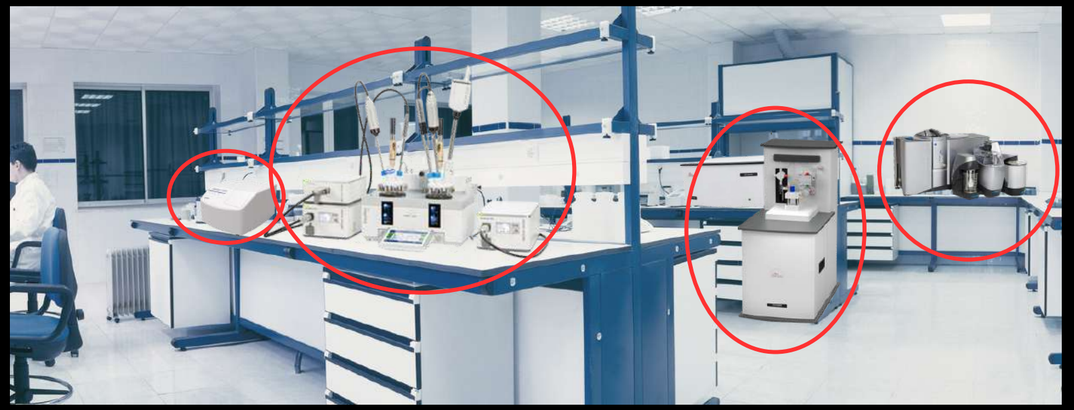
**ENVIRONMENT**
Nanoplastics, wastewater analysis

**MEDICINE**
Ceramics & surfaces in dentistry

# Nanoparticle characterization

(range 5 µm - 100 nm)

| | CURRENT SOLUTIONS | |
|---|---|---|
| | **SNAPSHOTS** | NO representative results<br>NO modification possibilities |
| | **DELAYS** | Cumbersome workflows &<br>complex quality control |
| | **HUMAN ERROR** | MANUAL processes for cleaning & sample preparation |
| | **DURATION** | 8 HOURS for a TOTAL measurement series of ONE batch |



# Nanoparticle characterization

(range 5 µm - 100 nm)

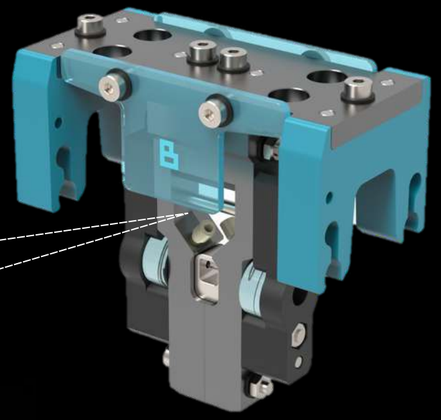
| | THE BRAVE WAY | |
|---|---|---|
| | **DEEP INSIGHTS** | representative results complying with current regulations |
| | **ACCURACY** | continuous measurements for seamless quality control |
| | **RELIEF** | Automated cleaning procedures (within 15 seconds) |
| | **SPEED** | measurement results in seconds (time resolved) |

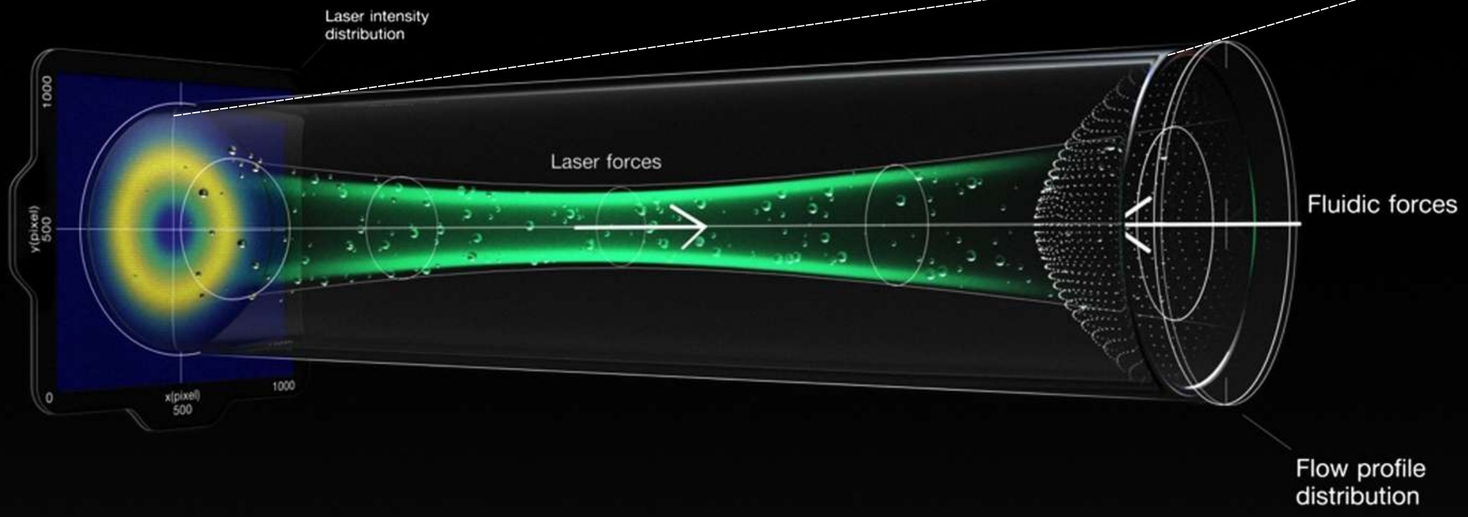
\+ SAVES TIME, WASTE & RESOURCES

# Challenging the state of the art

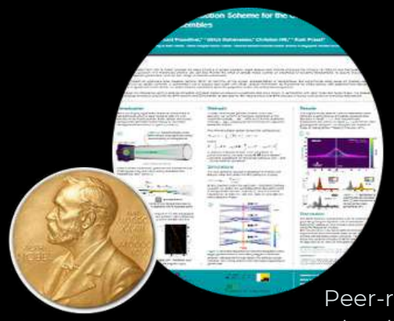
watch video

**OF2i®** actively induced forces of biophotonics and μ-fluidics to break the barrier of Brownian Motion






Thousands of particles are continuously set in motion as they are pumped through the measuring cell. Using a vortex laser beam, the particles are (de)accelerated by optical forces in spiral paths to prevent collision, while an ultramicroscope camera records the change in velocity of each particle, thereby allowing the size of the nanoparticles to be inferred. How? The recorded motion patterns are evaluated using a theoretical model that is parameter-free, making OF2i a calibration-free system. The model is based on Maxwell's equations as well as generalized Mie theory.



Based on Ashkin et al. PHYSICS NOBEL PRIZE 2018

Peer-reviewed technology paper; PhysRevA Simic et al.

PATENTED

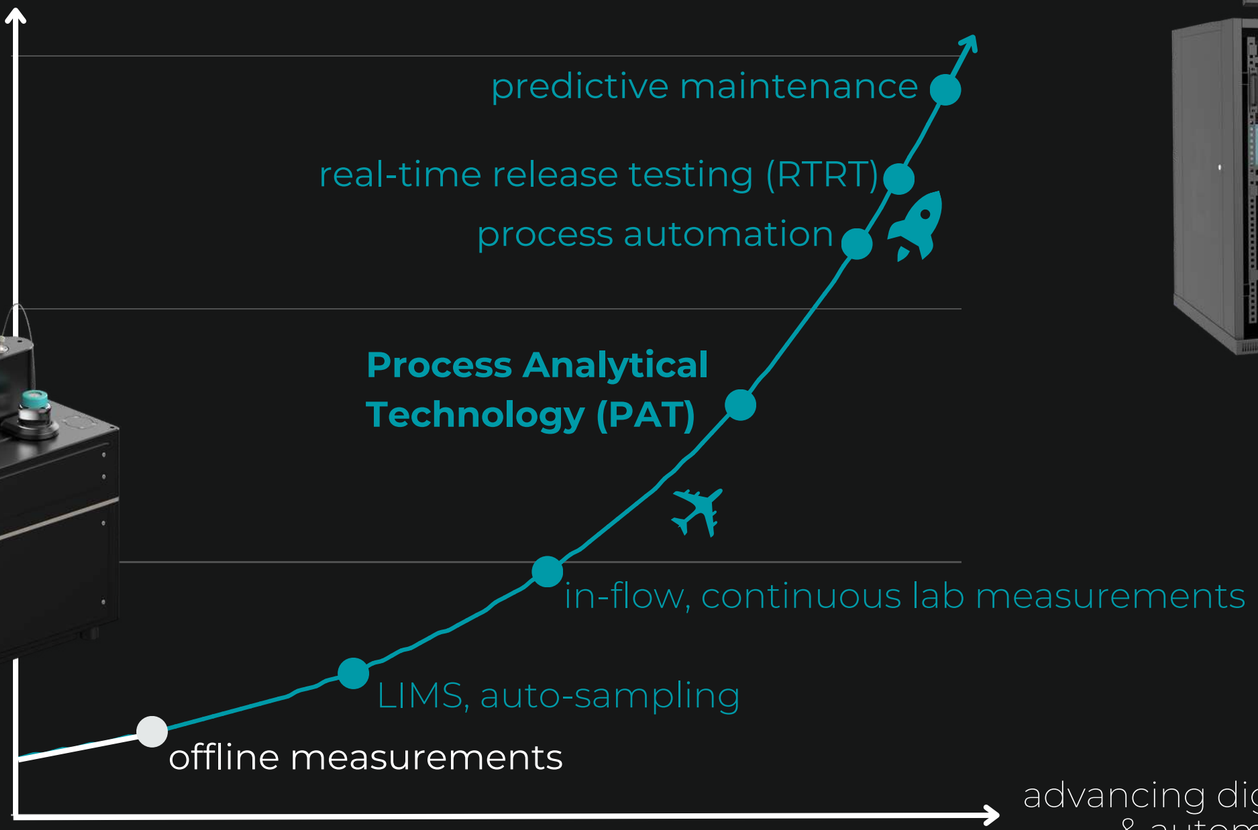
# Ready for scale-up

LAB device with modules for more individual insight

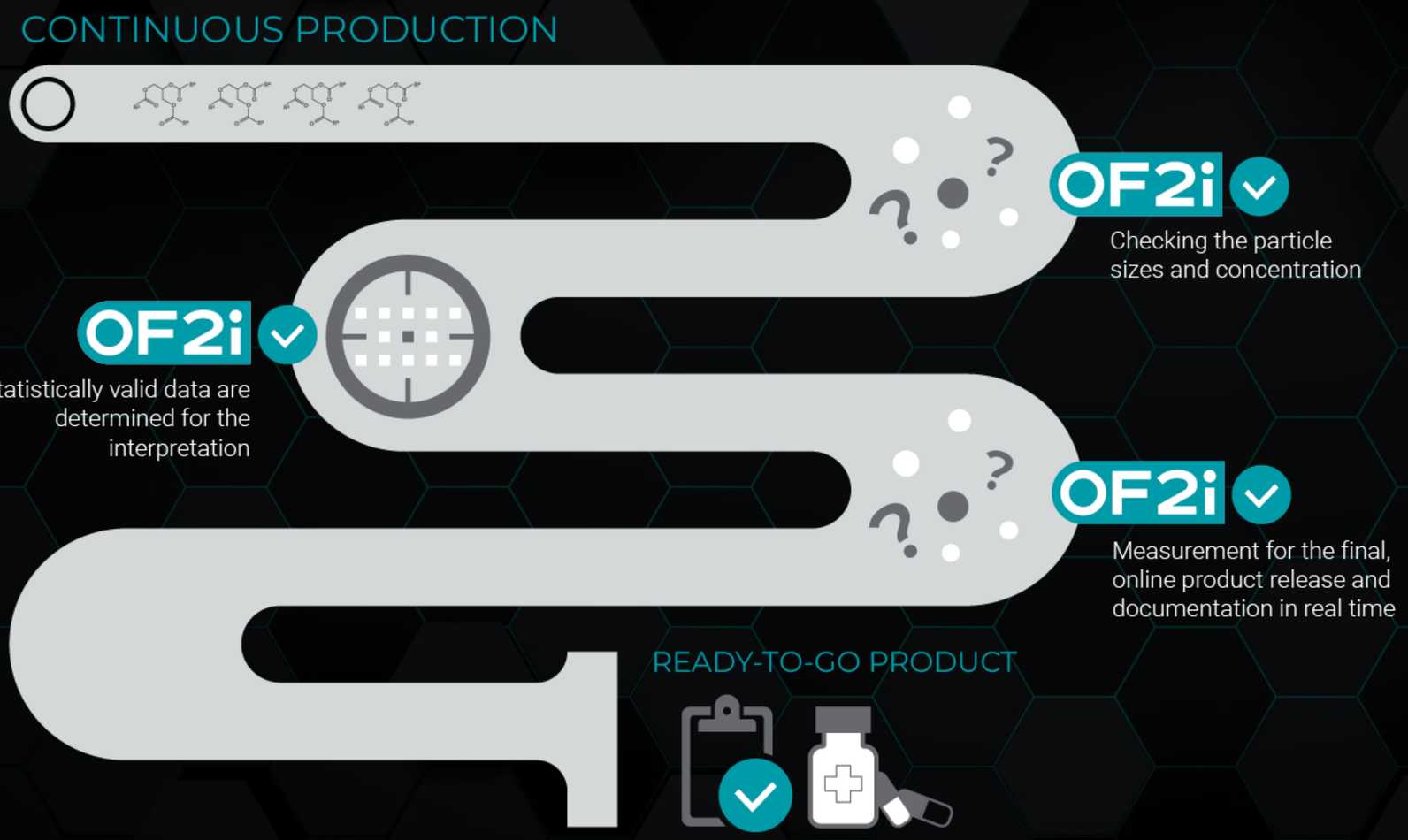
## BRAVE B-Curious

PAT sensor which revolutionizes your production

## BRAVE B-Continuous



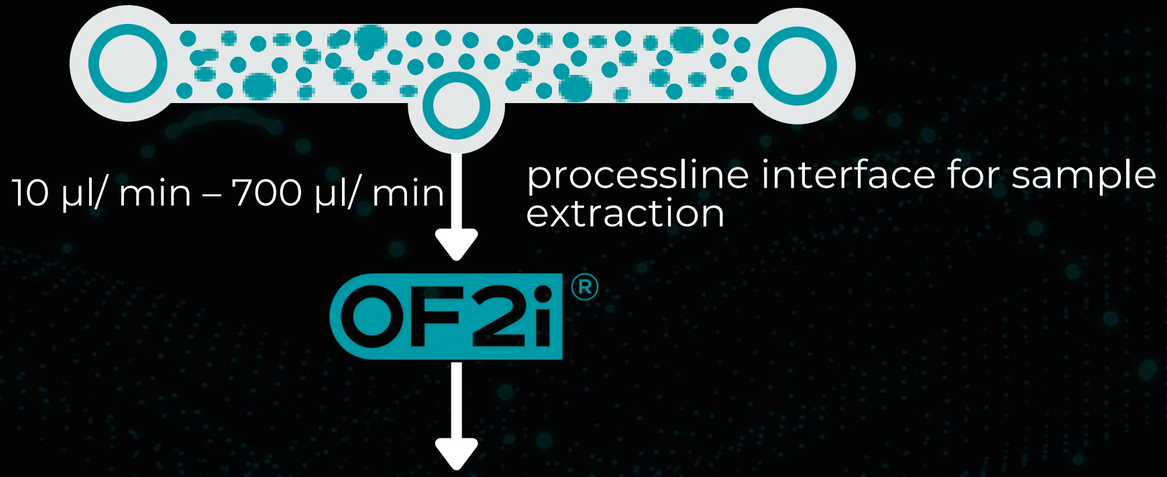
# BRAVE B-Continuous application & extensions



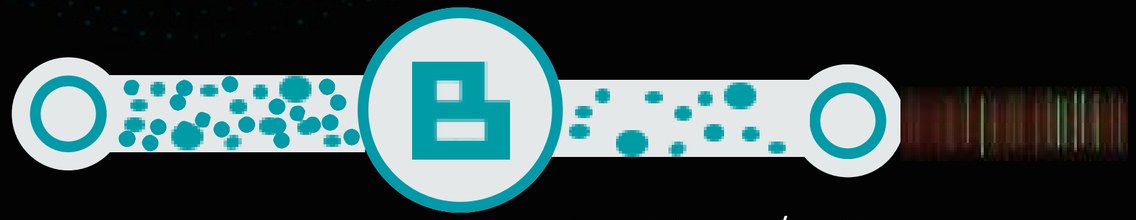

- Identification of Critical Process Parameters (CPP)
- Monitoring of Critical Quality Attributes (CQA)
- Time-resolved Multi-parameter measurements (aggregations, size etc.)
- Coming soon: RAMAN MODULE
- In-Process Quality Control (IPQC)
- Module for LARGE PARTICLE POPULATIONS up to 50 µm
- Module for low volume samples

# Automatic sample preparation unit

## Online measurement




**Counting method** with single-particle accuracy
requires dilution for highly concentrated samples



10 objects/ ml*

## BRAVE B-Prepared

*) sample dependent

# Definition: particle count vs. obejct count

**1** OF2i detected particles

Number-based particle size, PSD and concentration
Sizing range: 100 nm - 3000 nm*
Exampl. value: 3,4 x $10^{13}$ particles/ml
(114 nm - 2750 nm)

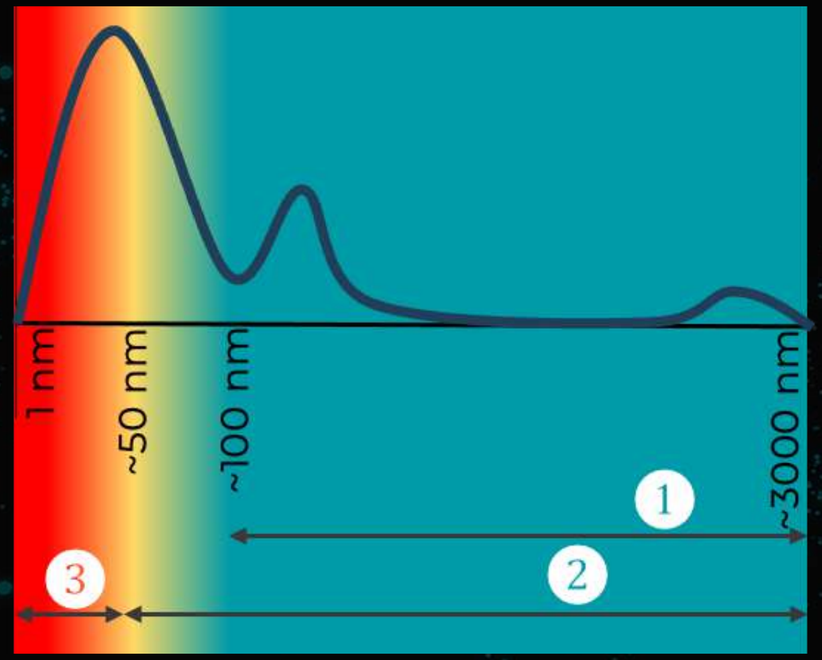
**2** OF2i detected objects

Total of all objects which were detected by OF2i BUT where TOO SMALL to determine their exact size
Exampl. value: 7,6 x $10^{18}$ objects/ml

**3** Full collodial system

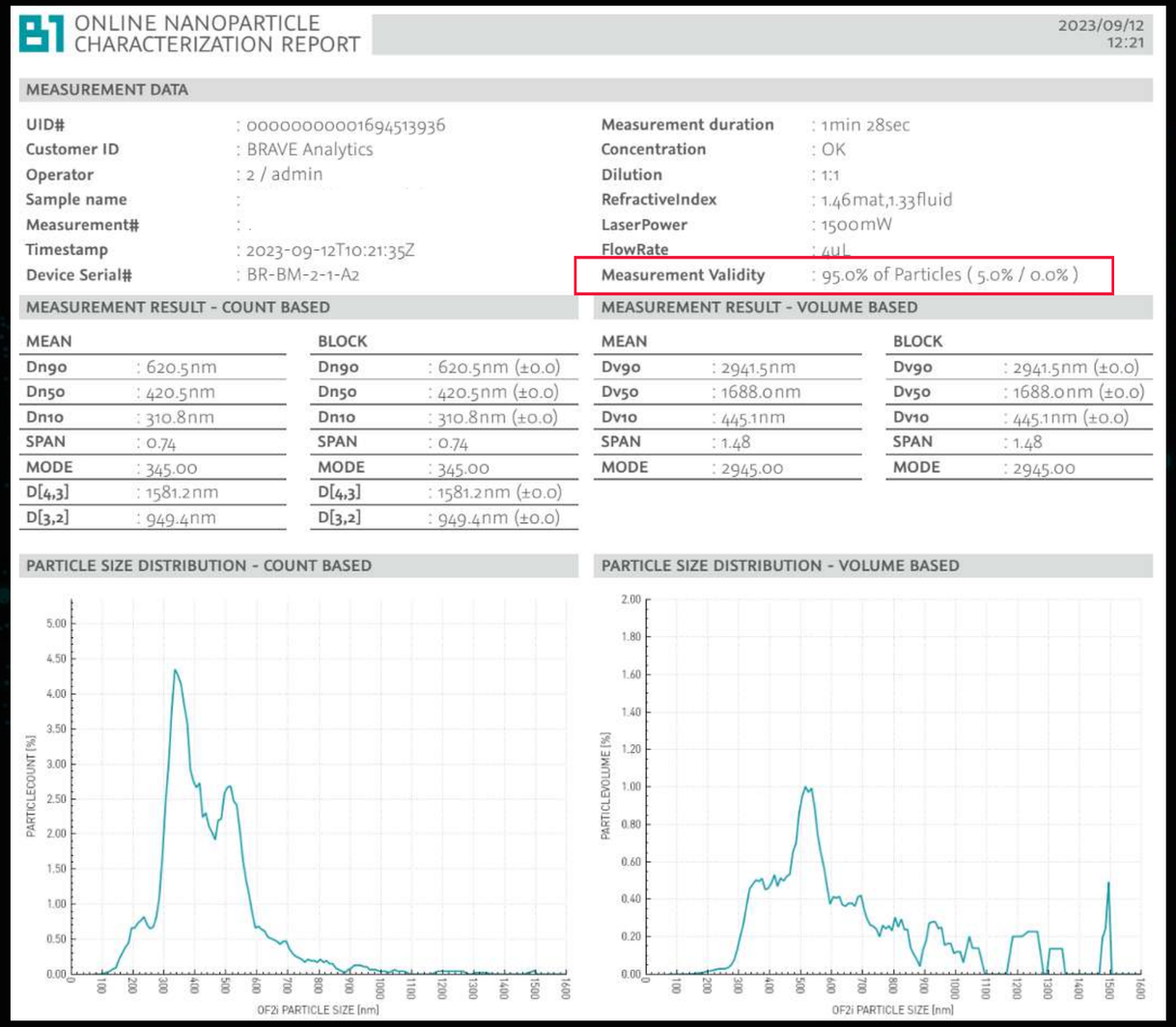
Objects are present but are out of detection range: cannot be detected with OF2i yet.
=> NOT INCLUDED in detected objects value

**OF2i sizing range***




*) sample dependent

# OF2i Report: measurement validity



ONLINE NANOPARTICLE CHARACTERIZATION REPORT

2023/09/12 12:21

**MEASUREMENT DATA**

| | | | |
|---|---|---|---|
| UID# | : 00000000001694513936 | Measurement duration | : 1min 28sec |
| Customer ID | : BRAVE Analytics | Concentration | : OK |
| Operator | : 2 / admin | Dilution | : 1:1 |
| Sample name | : | RefractiveIndex | : 1.46mat,1.33fluid |
| Measurement# | : . | LaserPower | : 1500mW |
| Timestamp | : 2023-09-12T10:21:35Z | FlowRate | : 4uL |
| Device Serial# | : BR-BM-2-1-A2 | Measurement Validity | : 95.0% of Particles ( 5.0% / 0.0% ) |

**MEASUREMENT RESULT - COUNT BASED**

| MEAN | | BLOCK | |
|---|---|---|---|
| Dn90 | : 620.5nm | Dn90 | : 620.5nm (±0.0) |
| Dn50 | : 420.5nm | Dn50 | : 420.5nm (±0.0) |
| Dn10 | : 310.8nm | Dn10 | : 310.8nm (±0.0) |
| SPAN | : 0.74 | SPAN | : 0.74 |
| MODE | : 345.00 | MODE | : 345.00 |
| D[4,3] | : 1581.2nm | D[4,3] | : 1581.2nm (±0.0) |
| D[3,2] | : 949.4nm | D[3,2] | : 949.4nm (±0.0) |

**MEASUREMENT RESULT - VOLUME BASED**

| MEAN | | BLOCK | |
|---|---|---|---|
| Dv90 | : 2941.5nm | Dv90 | : 2941.5nm (±0.0) |
| Dv50 | : 1688.0nm | Dv50 | : 1688.0nm (±0.0) |
| Dv10 | : 445.1nm | Dv10 | : 445.1nm (±0.0) |
| SPAN | : 1.48 | SPAN | : 1.48 |
| MODE | : 2945.00 | MODE | : 2945.00 |

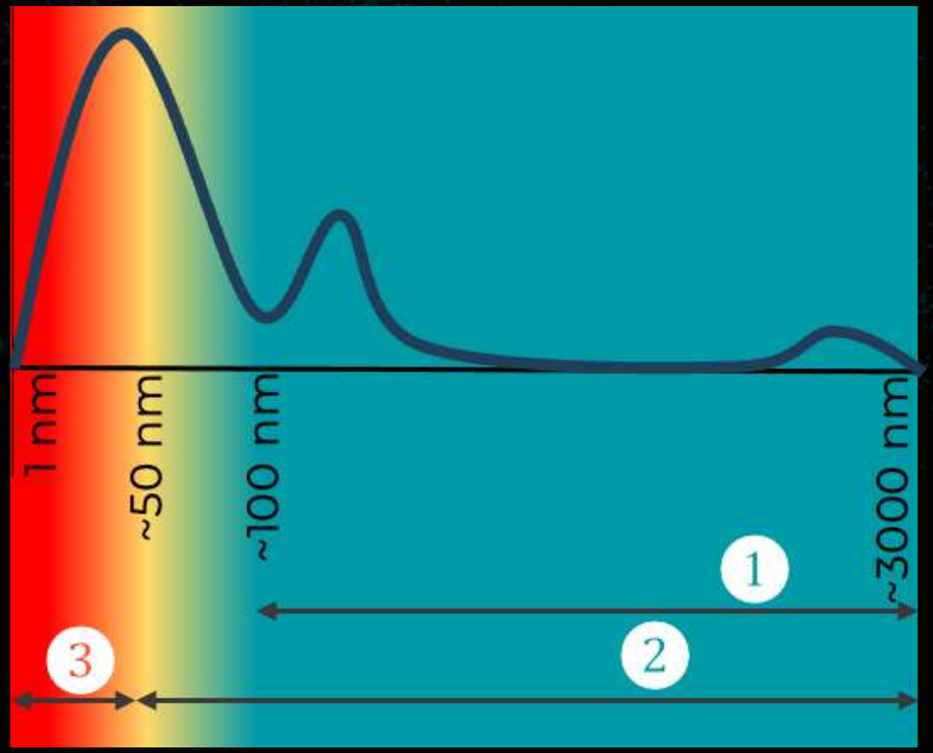
**PARTICLE SIZE DISTRIBUTION - COUNT BASED**




**PARTICLE SIZE DISTRIBUTION - VOLUME BASED**



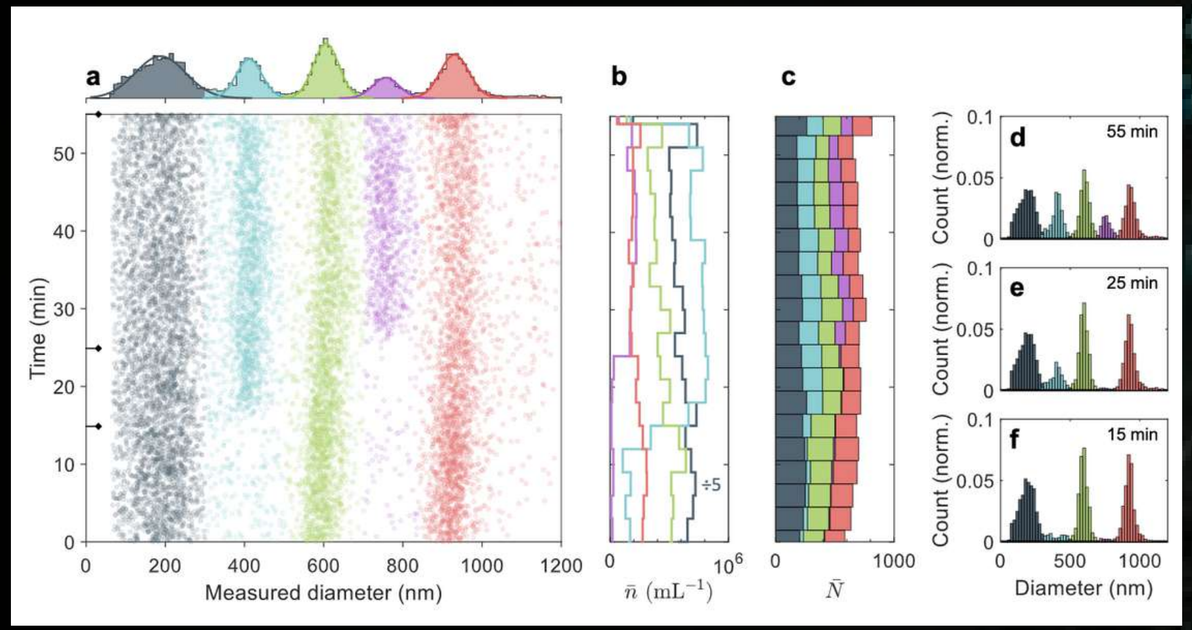

- for 95.0 % of the particles size, PSD and concentration could be measured.
- 5.0 % of the particles were below smallest measurable size (e.g. 100 nm) and could be detected but not sized.
- 0.0 % of the scanned particles were above maximum measureble size (e.g. 3000 nm ).



# OF2i features in a nutshell

| | | |
|---|---|---|
| 100 nm* to 5 µm DETECTION RANGE (*sample-dependent) | REDUCES REJECTS through continuous monitoring | NO WAITING for lab test results. |
| SEAMLESS MEASUREMENTS proven throughput up to 2500 particles/minute. | RELIABLE & REPRESENTATIVE measurement results | PROCESS OPTIMIZATION through real-time data. |
| TIME RESOLVED RESULTS for understanding kinetic processes | BASIS for Real-time Release Testing (RTRT) | MINIMIZES HUMAN ERROR and delays |
| ULTRA-LOW PARTICLE CONCENTRATIONS at high THROUGHPUT | READY TO SCALE-UP from lab to PAT | Enables PREDICTIVE MAINTENANCE |

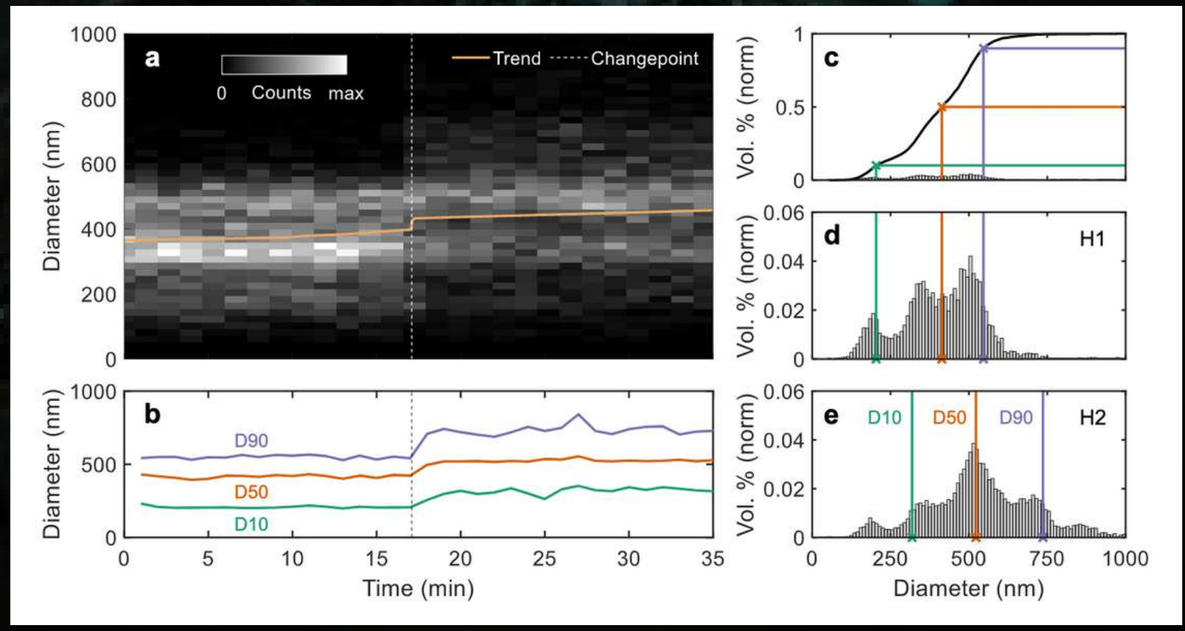
# OF2i® Continuous monitoring: High-pressure homogenization for more efficient quality control




© BRAVE Analytics & Marko Šimić: OF2i® time resolved online characterization as number based size distributions

## Monitoring of production processes:

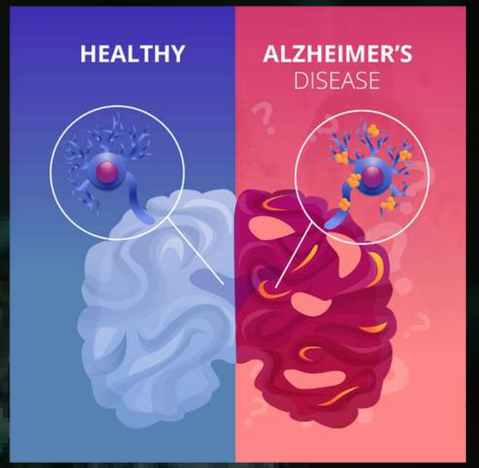
- Object size range: 180 -3000 nm
- Detected substances: biological agents in emulsion
- Measuring mode: online
- Timestep @min 17: detected change homogenization process

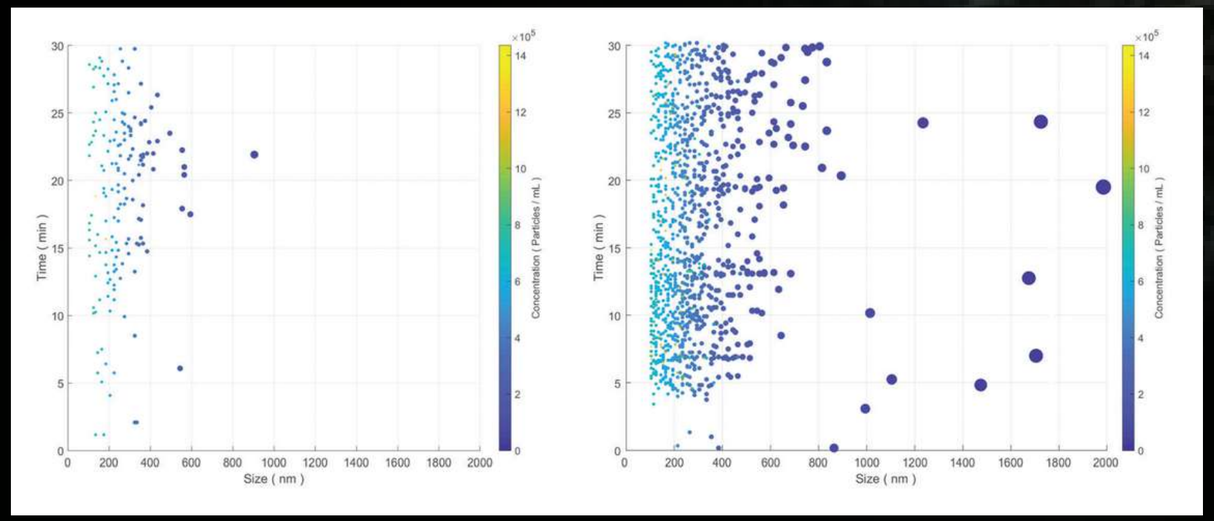


# Biomolecular research: Understanding Alzheimer's Disease

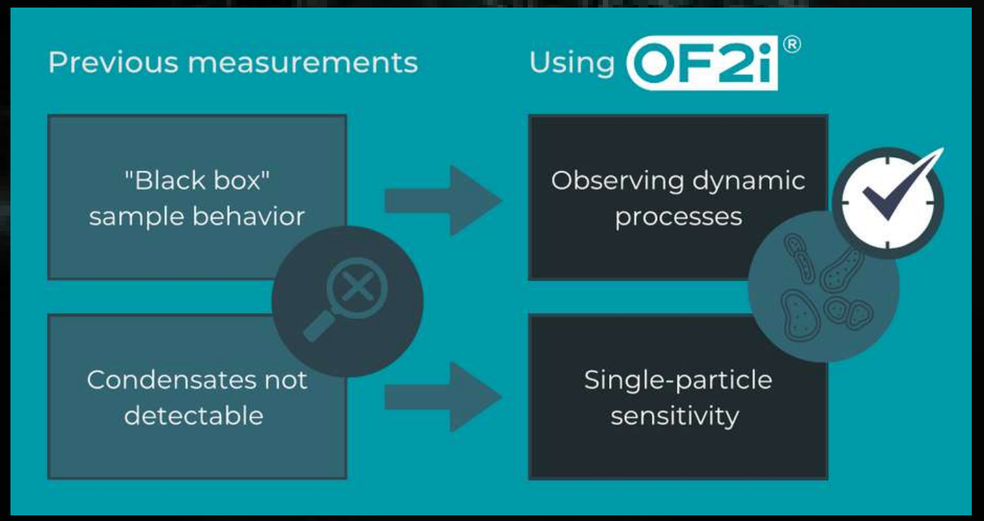
Prof. Tobias Madl

"The OF2i method closes a huge gap and enables in-depth and seamless observation of the proteins as they change over time, in this case time-resolved information over seconds to hours."

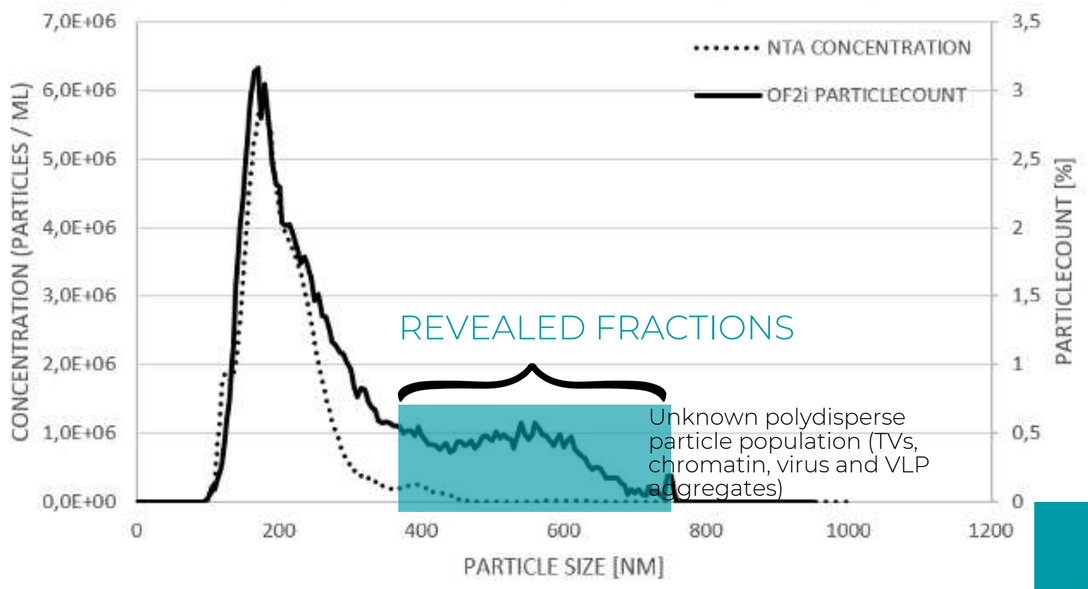



© BRAVE Analytics & Marko Šimić: Time-resolved PSD: Particle formation processes during liquid-liquid phase separation (LLPS) with low (left) and high (right) RNA concentrations over 30 minutes.



# Reliable, statistically relevant results for up- and downstream processes for viruses and VLP polydisperse colloid systems



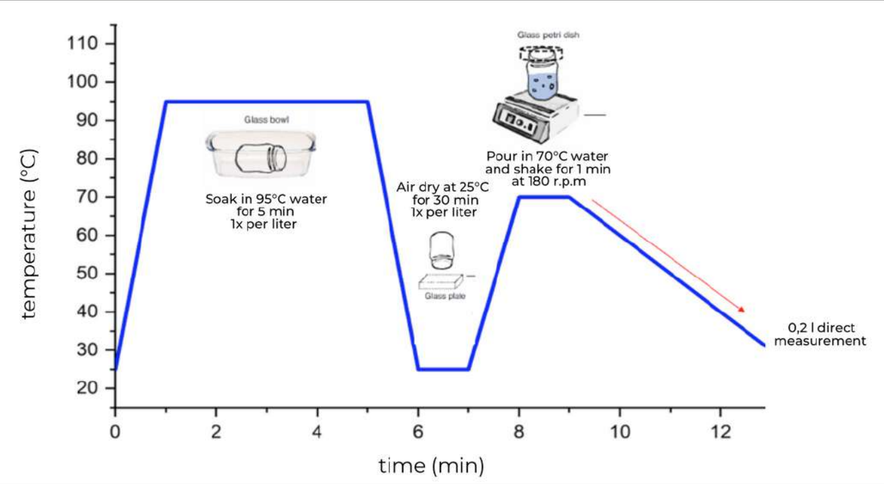

© BRAVE Analytics &. ACIB: OF2i reveals additional larger particle fractions, enabling better process understanding.

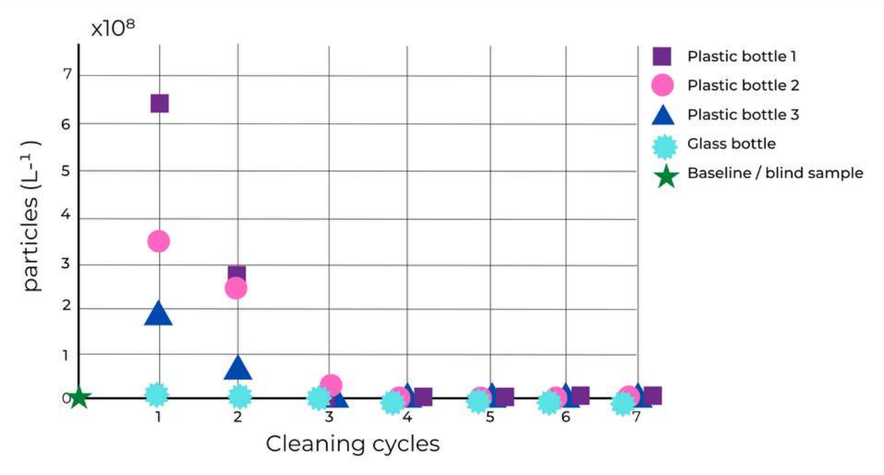
## Monitoring of efficiency of downstreaming operations

- Object size range: 50 - 1000 nm
- Detected substances: biologic virus cultures
- Measuring mode: offline

| | NTA | OF2i® |
|---|---|---|
| Total measurement time | approx. 45 minutes | approx. 8 minutes ✓ |
| Particle throughput | 100/ minute | approx. 360/ minute ✓ |
| Cleaning time after every measurement | approx. 5-8 minutes | approx. 30 seconds ✓ |

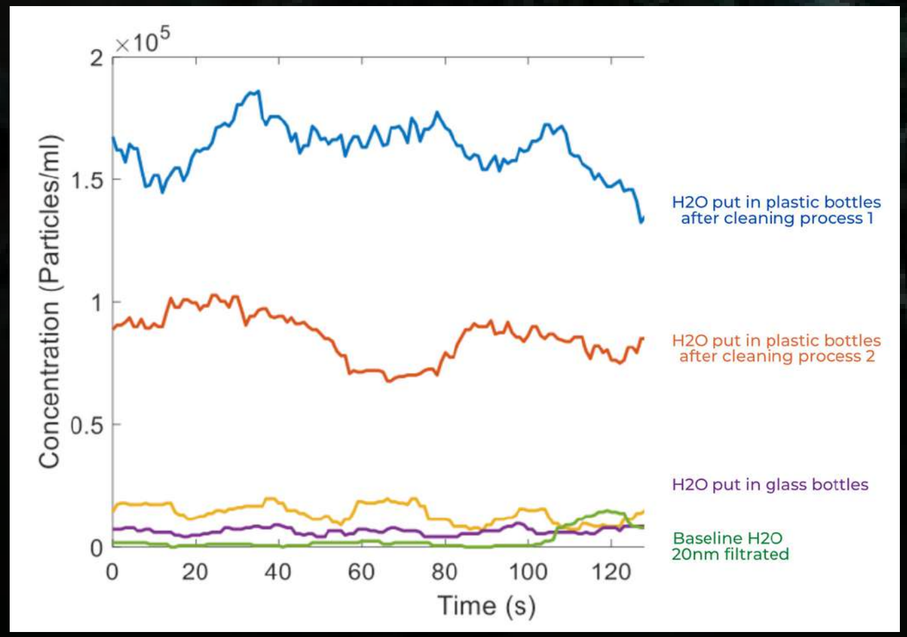
# Online monitoring of the leaching of micro- and nanoplastics into water at ultra-low concentrations




## Understanding of impact of cleaning cycles:

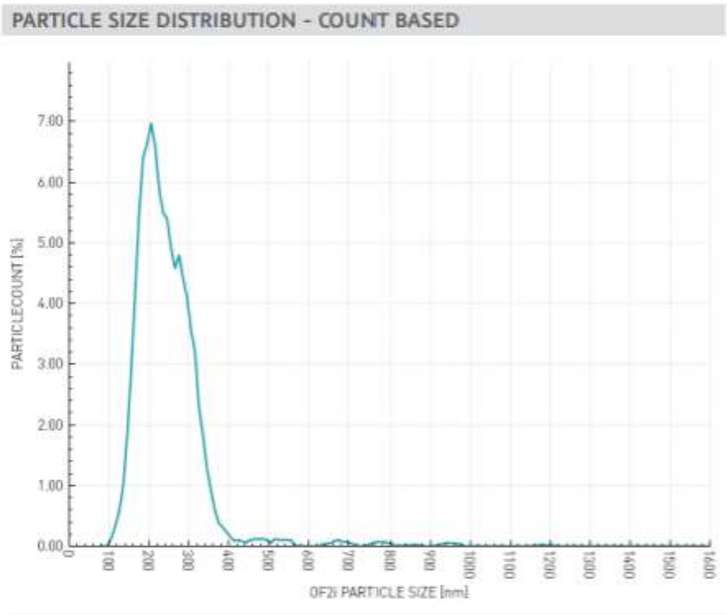
- 7 cycles performed with 20nm filtrated ultra-pure H2O
- Object size range: 50-3000 nm (estimated)
- Detected substances: Si02, polystyrene, PP, PFAS
- Measuring mode: online



# Verification of low-budget screen-printed sensors detecting nano pollutants in surface water

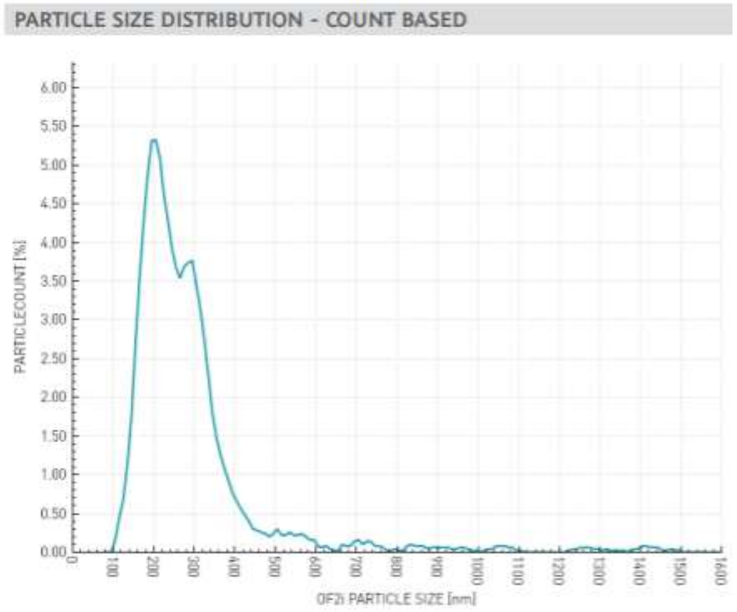
Nanopollutants (from tires) in water suspension

## Sample filtered with 0.22 µm filter




Mean concentration ~ 8E5 objects
D90 - 321.2nm (90% of the population is smaller than 321 nm)

## Sample filtered with 0.45 µm filter




Mean concentration ~ E6 objects
D90 - 406.7nm (90 % of the population is smaller than 406.7 nm)

## Measuring nanoplastic concentration and size changes over time at ultra-low concentrations

- Object size range: 180 -3000 nm
- Filtration: 0.22 µm filter compared with 0.22 µm filter
- Detected substances: car tire abrasion in water
- Measuring mode: online
- Timestep 10: fault detection within homogenization process

# BRAVE market segments

Exosome diagnostics and therapeutics

VLPs and Viruses

Drug delivery

mRNA therapeutics

Ceramics & surfaces in dentistry

Emulsions and infusions

## (nano) Particles in Life Science

### Nanobiotechnology
$ 104.8 BN in 2022
$ 160.3 BN by 2030
CAGR 5.5 %

### Nano medicine
$ 159.53 BN in 2021
$ 427.18 BN by 2030
CAGR 11.7 %

Food
Coatings
Beauty & Cosmetics

### Environmental Analysis
$ 295 BN in 2022
$ 572.2 BN by 2032
CAGR 6.9 %

## BRAVE B-Continuous
**Deep insights into your production**

### Process Analytcial Technology
((pharmaceutical industry)
$ 2.07 BN in 2021
$ 4.91 BN by 2030
CAGR 10.2 %

### High-throughput screening
$ 18.93 BN in 2021
$ 36.84 BN by 2030
CAGR 7.82 %

## BRAVE B-Curious
**The solution for R&D and quality control**

### Global particle size analyzer market
$ 395.37 MN in 2022
$ 695.45 MN in 2032
CAGR 14.4 %

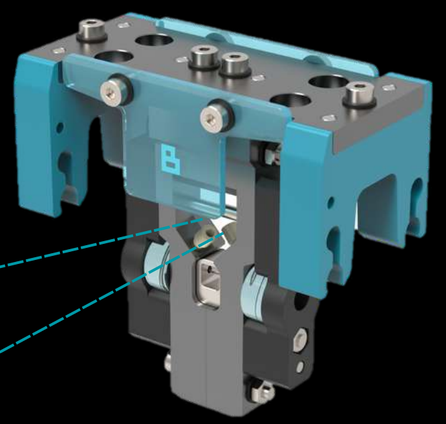
### Wet particle size analyzer
$ 125.33 MN in 2022
CAGR 8.17 %

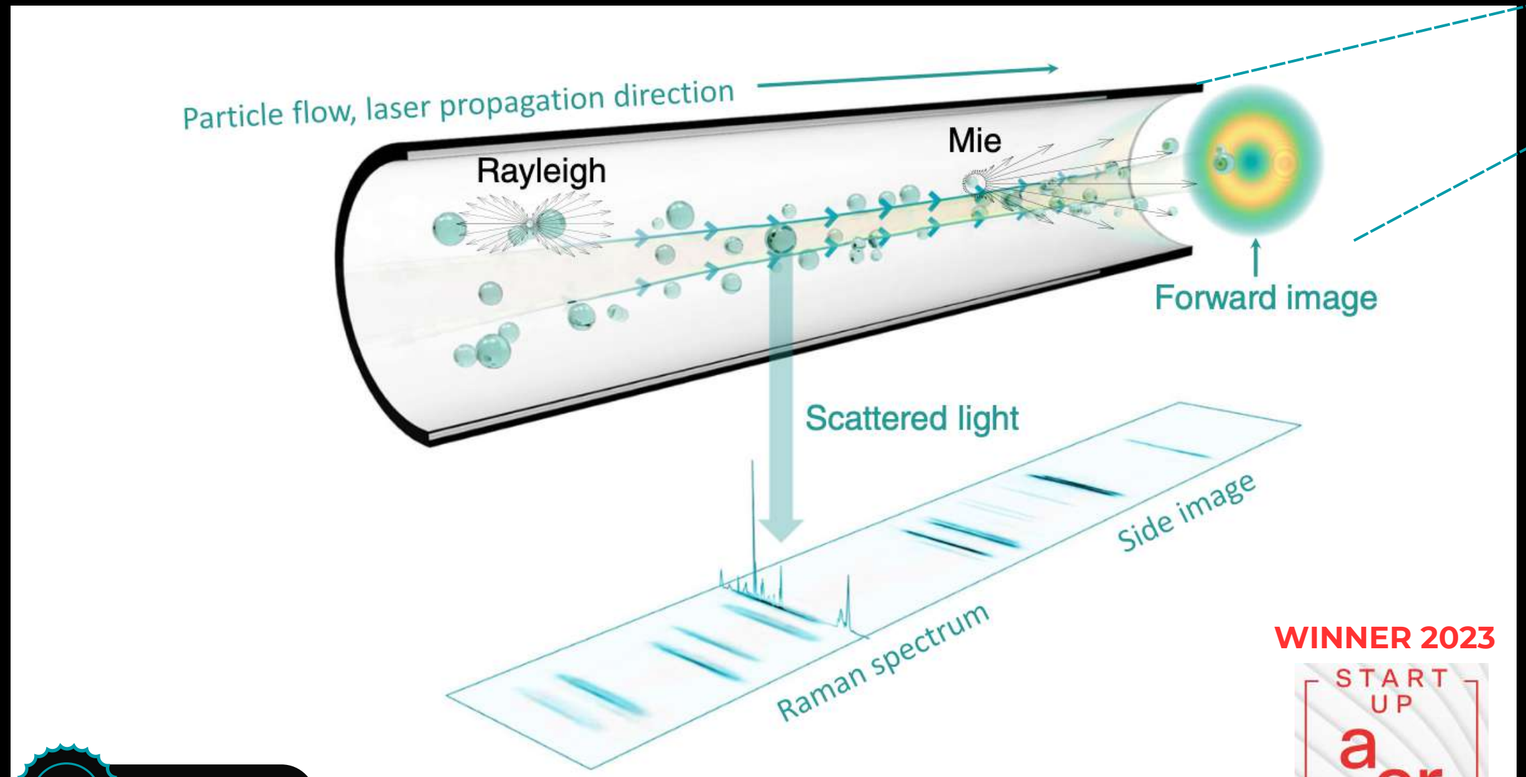
Market share 35 %

### Pharmaceutical manufacturing
$ 425.9 BN in 2021
$ 1,599.9 BN by 2030
CAGR 15.9 %

# BRAVE COLLABORATIONS

# A BRAVE future with OF2i–RAMAN coupling






PATENTED
filed

WINNER 2023
START UP acr PREIS powered by aws

As an addition to the OF2i® technology, which uses elastically scattered radiation, the correlative OF2i®-Raman method employs inelastically scattered radiation for chemical analysis. This approach detects particles smaller than those typically analyzed by regular Raman microscopy (< 500 nm). (publication: Neuper et al.)

# OF2i-Raman for size, size distribution and chemical analysis of nanoparticles in liquids

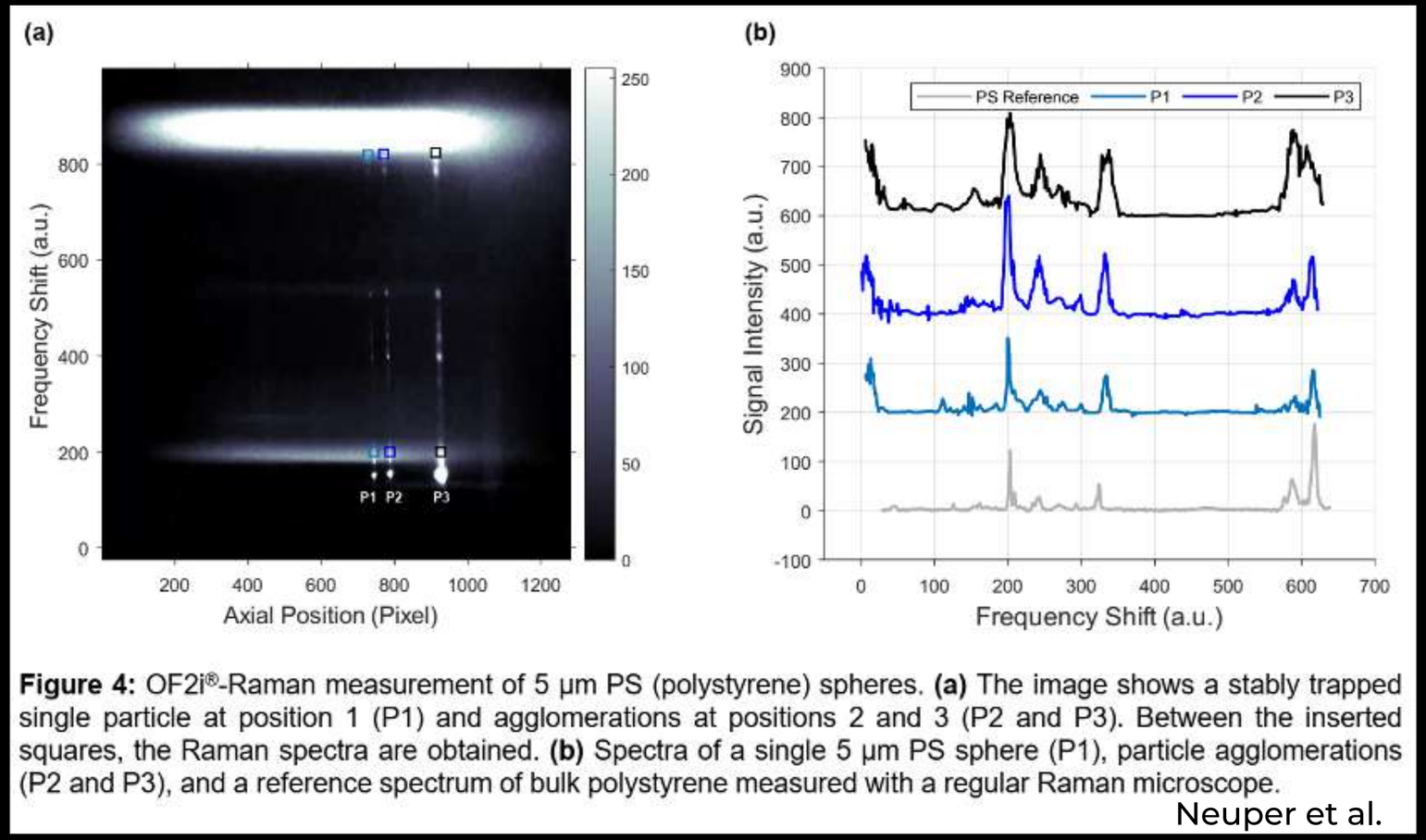
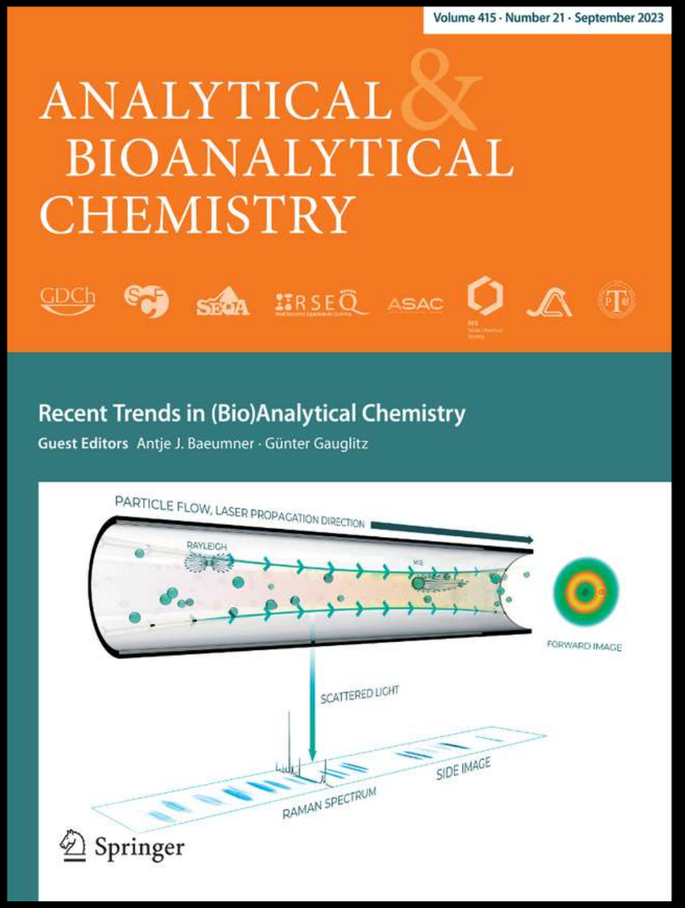
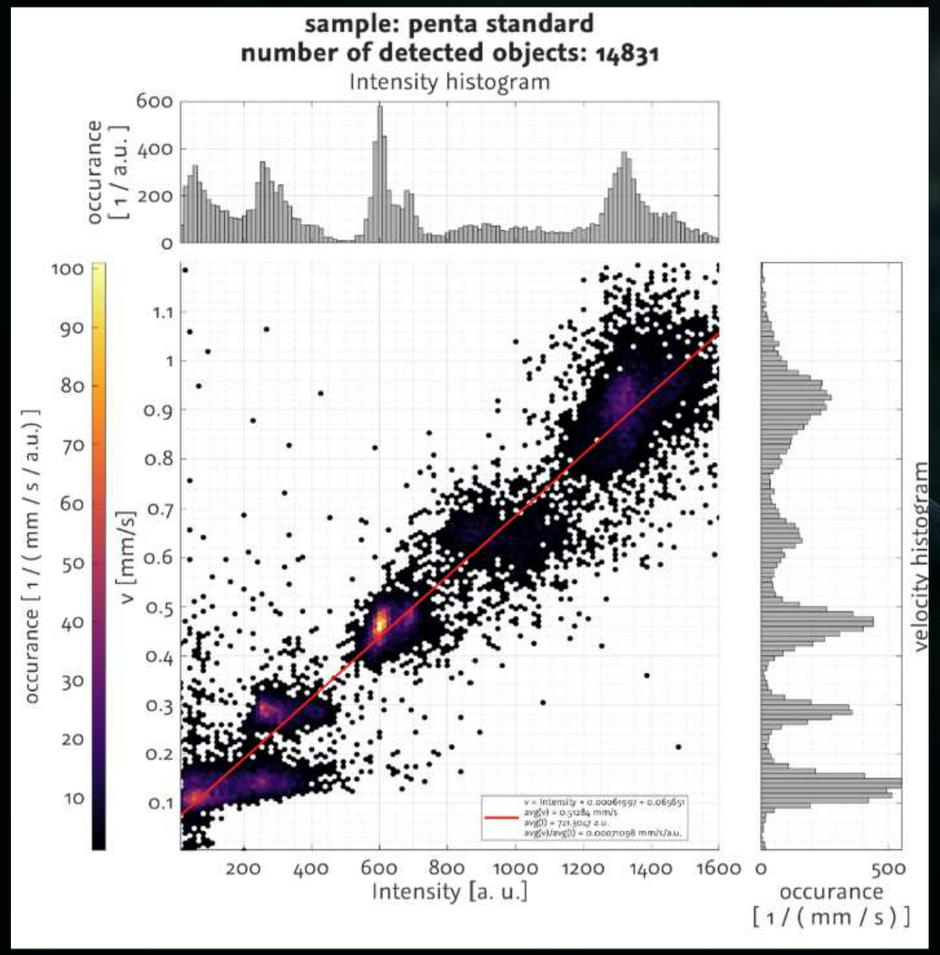



**Figure 4:** OF2i®-Raman measurement of 5 µm PS (polystyrene) spheres. **(a)** The image shows a stably trapped single particle at position 1 (P1) and agglomerations at positions 2 and 3 (P2 and P3). Between the inserted squares, the Raman spectra are obtained. **(b)** Spectra of a single 5 µm PS sphere (P1), particle agglomerations (P2 and P3), and a reference spectrum of bulk polystyrene measured with a regular Raman microscope.

Neuper et al.



# OF2i vs. SingleParticleLightScattering for size, size distribution and chemical analysis of nanoparticles in liquids



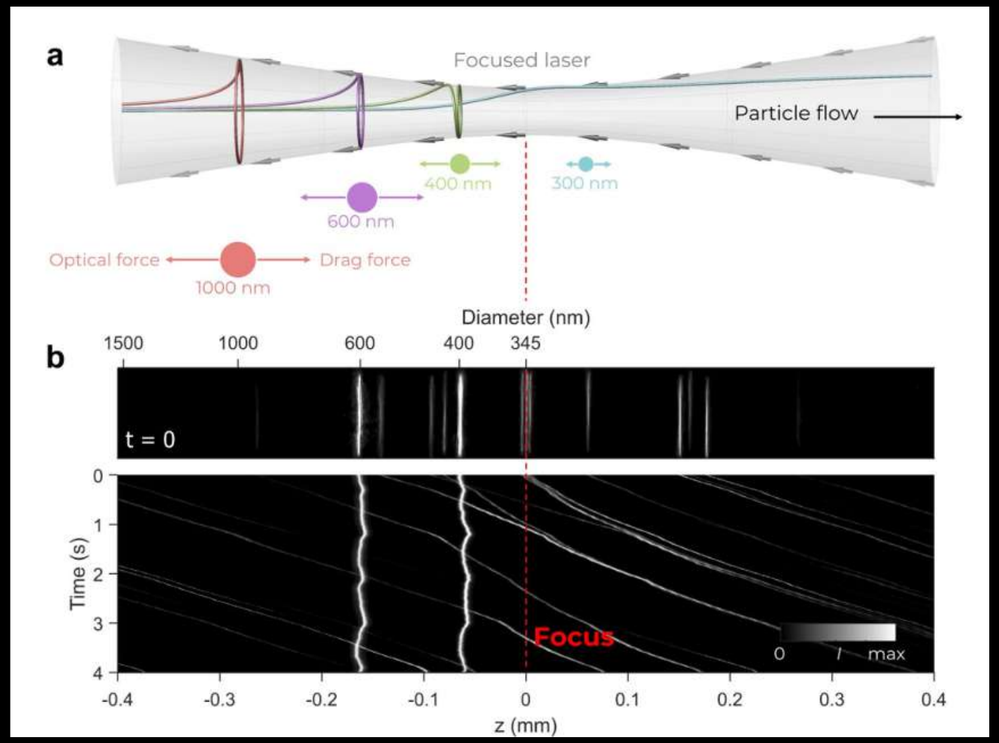

## In house analysis of penta standards

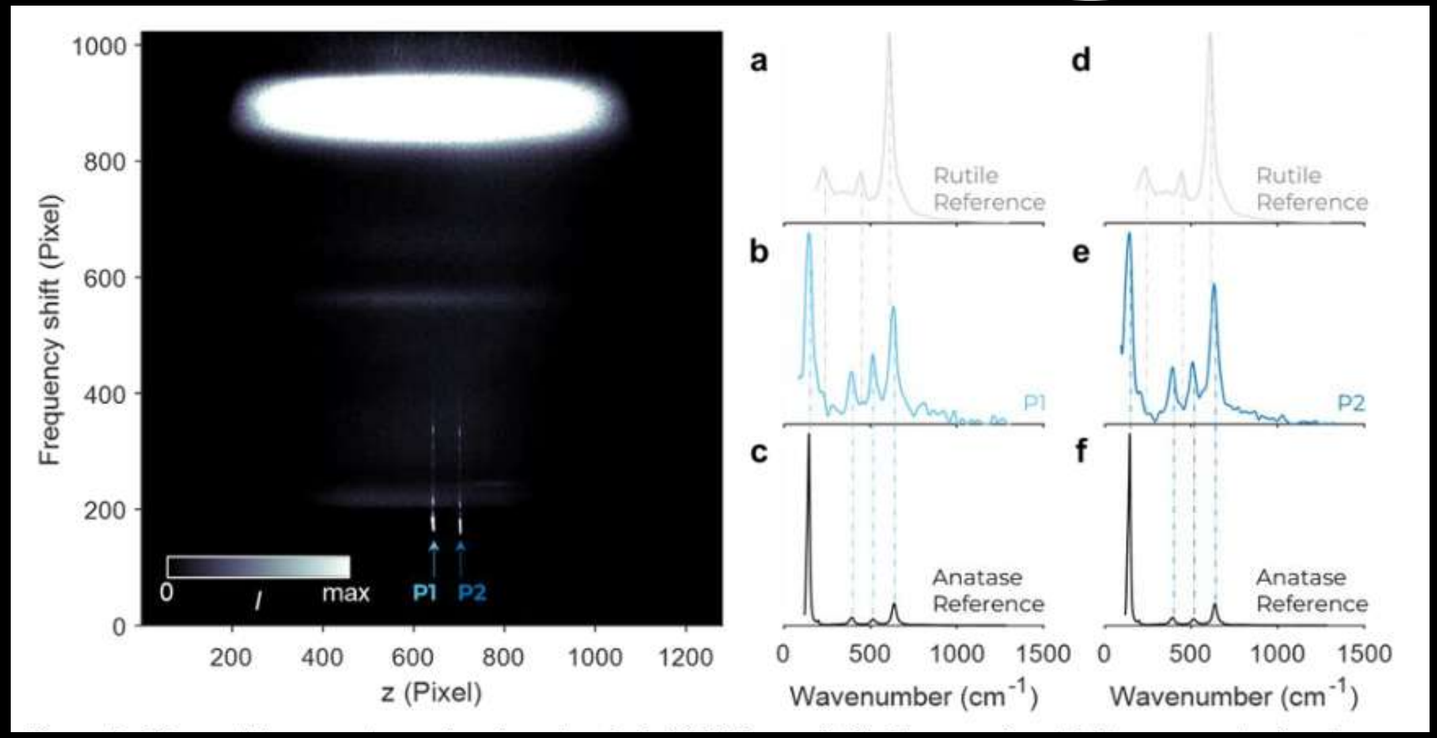
- Trajectory vs. intensity of NIST traceable standards
- Intensity histogram illustrating particle size, size distribution and occurance
- Number of detected objects: 14831

- Scattering of particles changes depending on their morphology => e.g. solid vs. core-shell particles

# OF2i coupled with Raman Spectroscopy and Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

**TiO2 particles were trapped and analyzed via OF2i-Raman. The experimental Raman spectra (b, e) were compared against those of anatase (c, f) as common TiO2 phases enabling a clear identification of the former.**




Optofluidic Force Induction meets Raman Spectroscopy and Inductively Coupled Plasma - Mass Spectrometry: A new hyphenated technique for comprehensive and complementary characterization of single particles. Christian Neuper, Marko Simic, Thomas E. Lockwood, Raquel Gonzalez de Vega, Ulrich Hohenester, Harald Fitzek, Lukas Schlatt, Christian Hill, David Classes. 2023.

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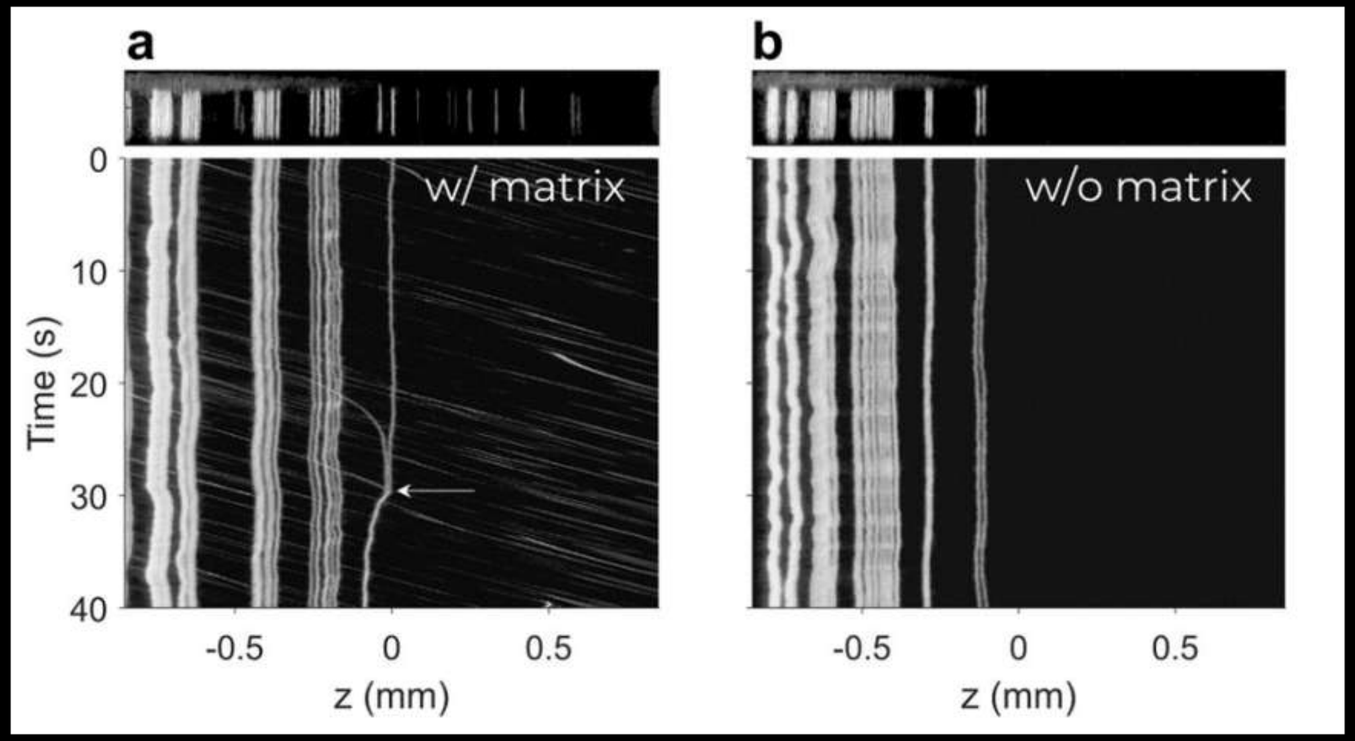


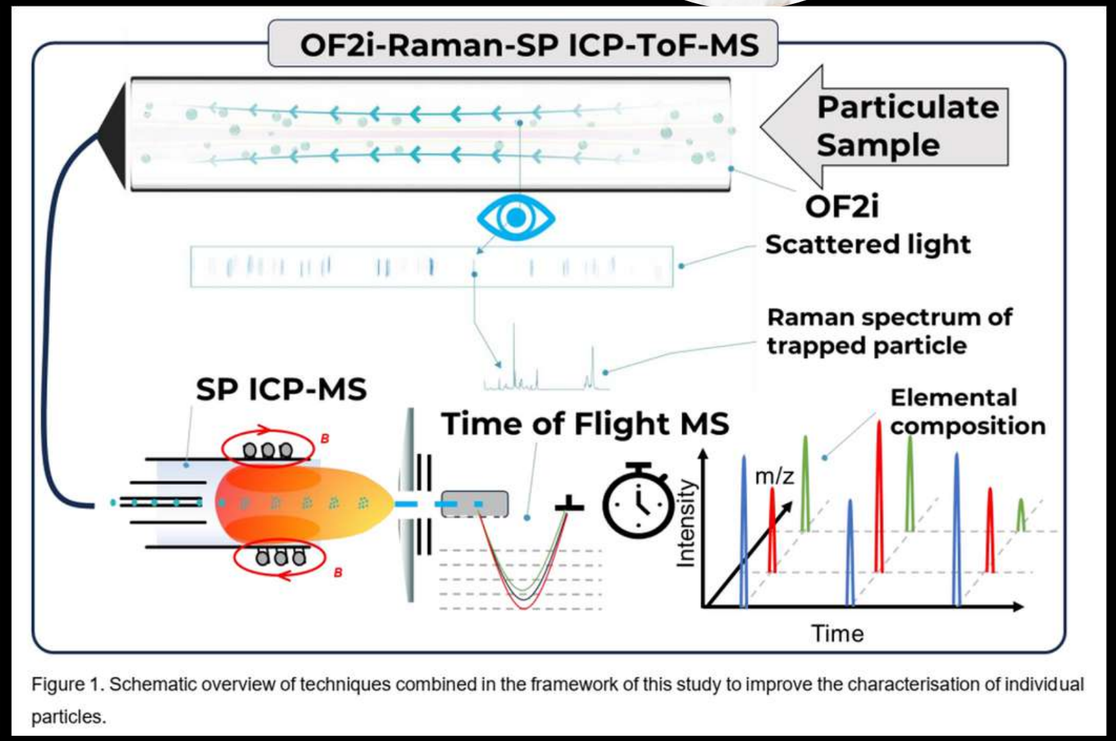


Figure 1. Schematic overview of techniques combined in the framework of this study to improve the characterisation of individual particles.

Optofluidic Force Induction meets Raman Spectroscopy and Inductively Coupled Plasma - Mass Spectrometry: A new hyphenated technique for comprehensive and complementary characterization of single particles. Christian Neuper, Marko Simic, Thomas E. Lockwood, Raquel Gonzalez de Vega, Ulrich Hohenester, Harald Fitzek, Lukas Schlatt, Christian Hill, David Classes. 2023.

# BRAVE Intellectual Property

**STRATEGY**

CONTINUOUS PATENT & COMPETITOR RESEARCH
NETWORK & COMMUNITY BUILDING
while keeping
MATHEMATICAL & PHYSICAL MODELLING CONFIDENTIAL

## TRADEMARKS

BRAVE® ANALYTICS

OF2i®

## PATENTS

PATENT ISSUED:
EP 3422364 B1 (HILL, C.)

OPTICAL PARALLELIZATION
FOR INDUSTRIAL THROUGHPUT
(UK, FR, IT, DE, ES, AT, CZ, NL, JPN, USA, CAN, AUS)

PATENT FILED:
A 50549/2022
CALIBRATION PROCEDURE

PATENT FILED:
A 50829/2023
- OF2i RAMAN SPECTROSCOPY

## IP LICENCE

SIGNED AGREEMENT:
- exclusive exploitation
- iP take-over option
- 4 % IP revenue - EUR 300k cap

Med Uni Graz & BRAVE® ANALYTICS

BRAVE® ANALYTICS

# … an experienced BRAVE TEAM

40 years
lab & application
experience

30 years
programming
experience

45 years
marketing & sales
experience

MARKO ŠIMIĆ
Physics & Modelling

MAGDALENA SCHNEIDHOFER
Business Development & Events

THOMAS GRUBER
Testing & Mechatronics

DORIS AUER
Lab & Application

CHRISTIAN NEUPER
Physics & Optical Engineering

NIKOLA ŠIMIĆ
Physics & Algorithms

RAPHAEL HAUER
Physics & Modeling

MICHAEL SCHNUR
Fluidics, Mechanics & QM

GERHARD PROSSLINER
Founder | COO | CFO

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Product & Sales

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Team Assistant

MICHAEL LONGHINO
Software & IT

SARAH KNIGHTS
Marketing & Communications

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ALEXANDER LELJAK
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# BRAVE MINDS

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- PhD in physical chemistry @ Medical University Graz
- Inventor of OF2i
- Extensive experience in particle technologies, basic research, and analytics
- Researcher/ project manager @ Medical University Graz (2013 – current)
- Tech. project manager @ Anton Paar (2002-2010)

**Ing. Gerhard Prossliner**
**Founder | CFO | COO**
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- Master in Biomedical Engineering @ Technical University Graz
- Experienced prototype development of med-tech devices and industrial smart camera systems and laser applications
- Project Leader for Health tech projects @ Joysys GmbH (2014 – 2016)
- Prototyping, manufacturing, testing of med-tech devices @ CNSystems Medizintechnik (2007 – 2013)

# BRAVE SUPPORT

Med Uni Graz · UNI GRAZ · TU Graz · FELMI-ZFE

FFG Promoting Innovation. · Federal Ministry Republic of Austria Education, Science and Research · austria wirtschafts service aws · Federal Ministry Republic of Austria Digital and Economic Affairs

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