Istituto Nazionale di Fisica Nucleare

ChAMBRe:

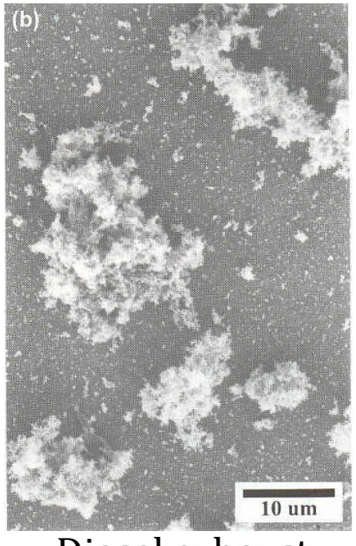
a new atmospheric simulation Chamber for Aerosol Modelling e Bio-aerosol Research

Particulate matter (Aerosol)

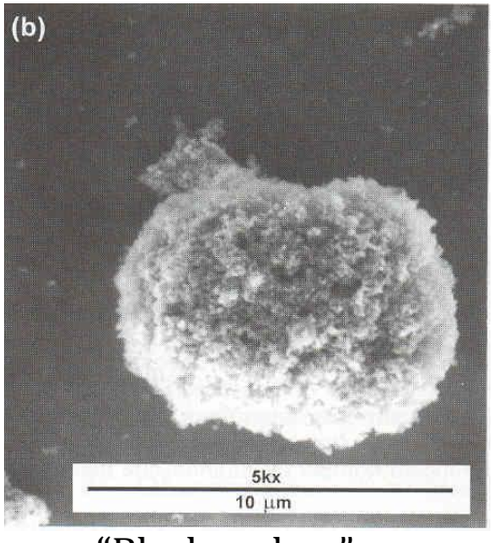
Molecules aggregates, solid or liquid, in atmosphere

with size in the range 10^{-3} – 10^{2} μm

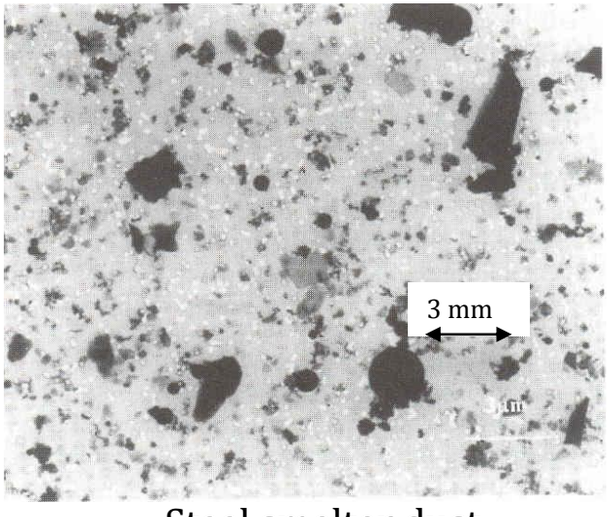
PM EPA's definition: the generic term for a broad class of chemically and physically diverse substances that exist as discrete particle in liquid droplets or solids forms in the air



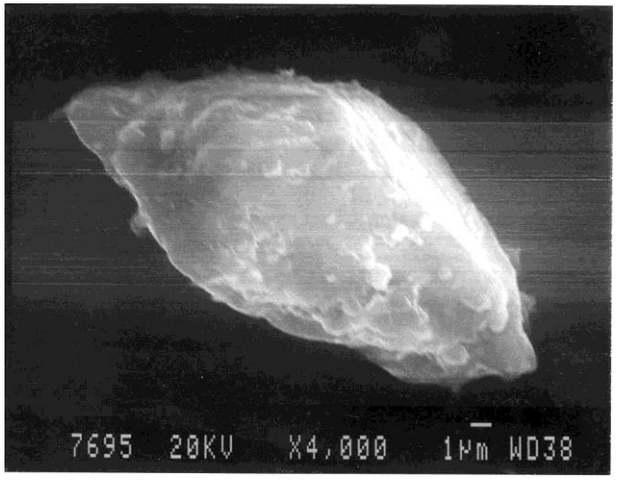
Diesel exhaust



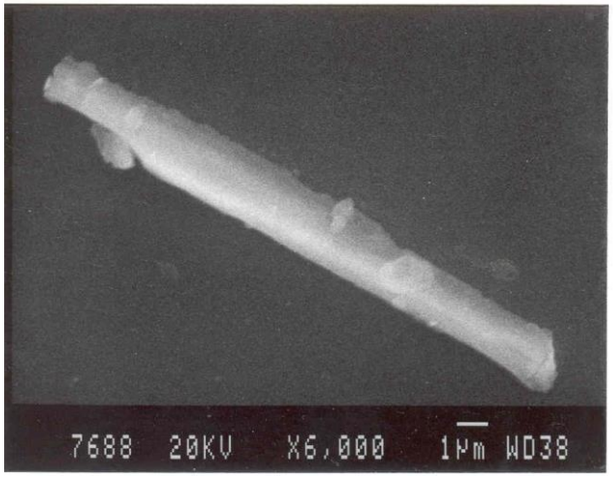
"Black carbon"



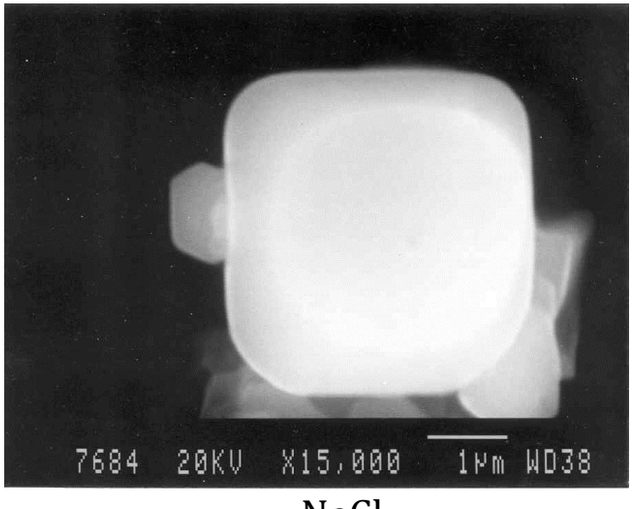
Steel smelter dust



"Rock" fragment



Vegetal fiber



NaCl

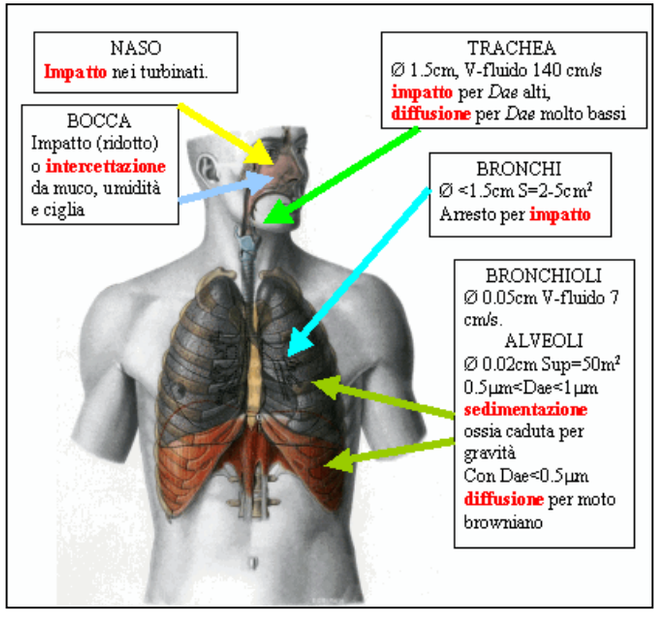
1) Natural sources, like:

2) Anthropogenic sources, such as:

Traffic
Industrial Activities
Domestic Heating
Thermal power plants
Incinerators...

...and many other...

Effects of PM:



- **Effects on Human Health**

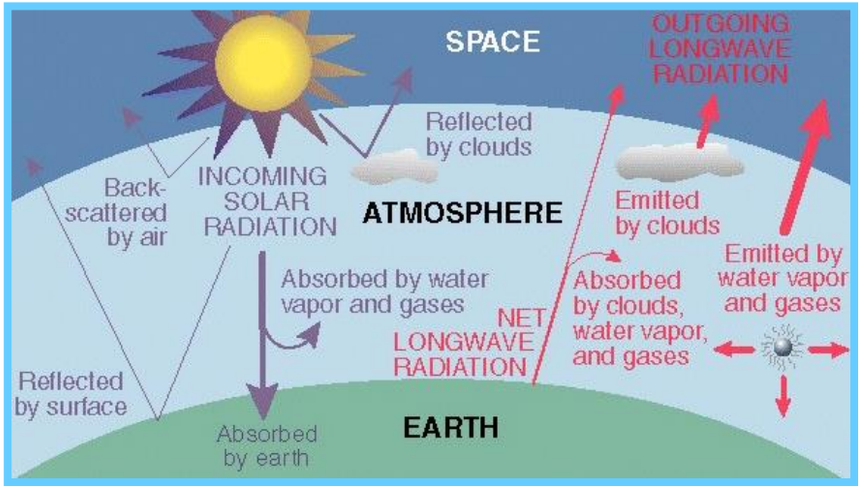
Depending on size, PM can penetrate in the respiratory system, causing serious damage to human health.

Epidemiologic studies highlight the close relationship between PM and respiratory, cardiovascular and allergic diseases.

- **Effects on the Environment:**

In the atmosphere

- Formation and transformation of clouds
- Light absorption and scattering properties
- Act as catalyzer of chemical reactions

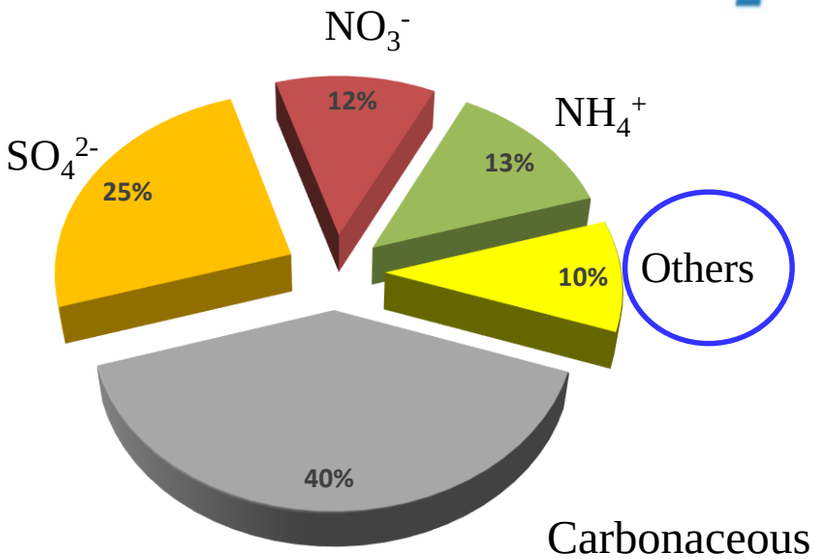


Climate Forcing

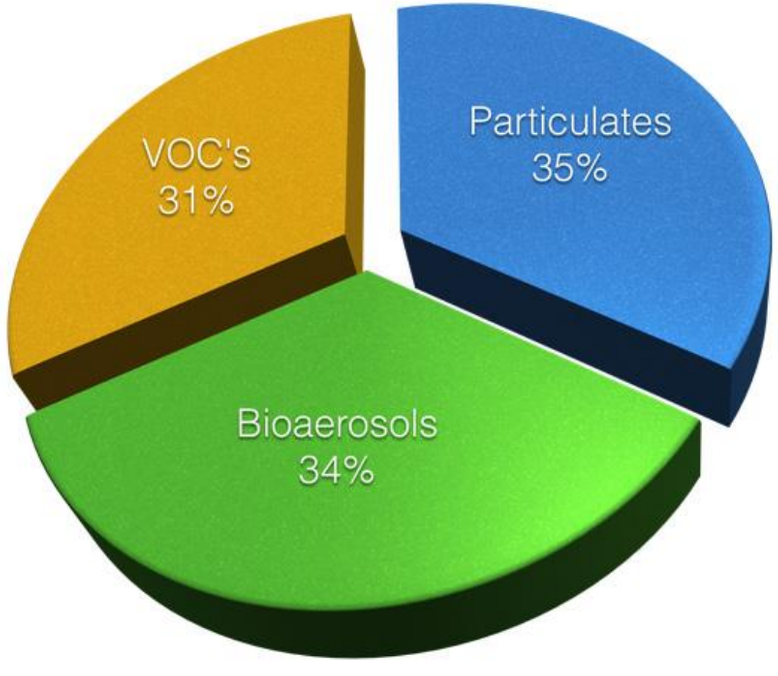
- **Erosion and blackening of materials → cultural heritage**
- **Water and land contamination due to dry and wet deposition**

PM composition:

- **Typical urban PM composition**



- **Typical indoor PM composition**



Particulates
dirt, dust, pollen, spores, pet dander, soot

Bioaerosols
Viruses, mold, bacteria, living micro organisms

VOC's
Volatile organic compounds: chemicals, gases, solvents

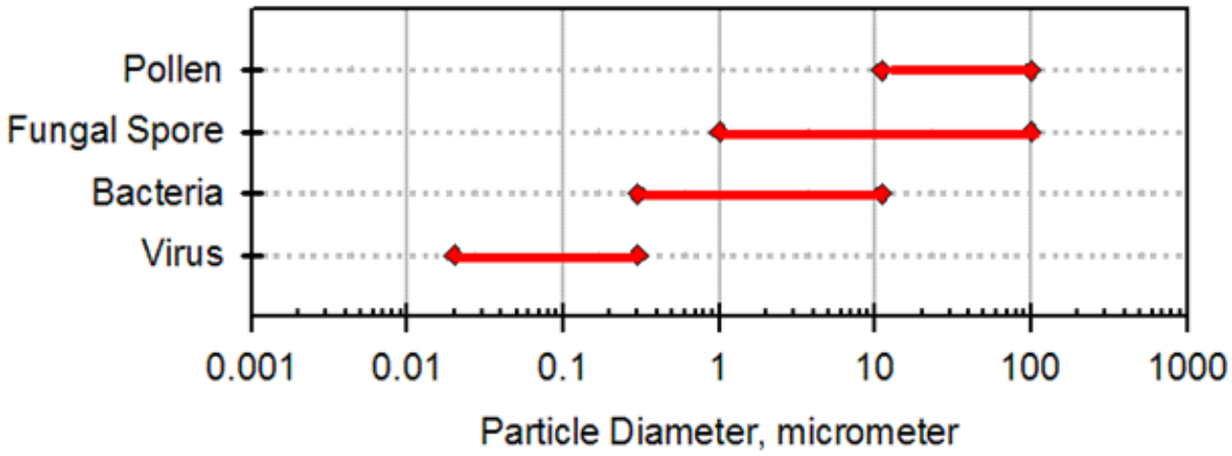
Bioaerosol: an aerosol of biological origin

Two groups:

- Viable (living organisms)
- Nonviable (dead organisms, pollen, animal dander, etc.)

Any respiratory pathogen able to survive aerosolization and air transport is considered a potential cause of airborne disease

Bioaerosol: particle size



- Bioaerosol particles often occur
  - as agglomerates
  - as cluster of organisms in droplets
  - or attached to other airborne debris

Particle size and natural background concentration of bioaerosols:

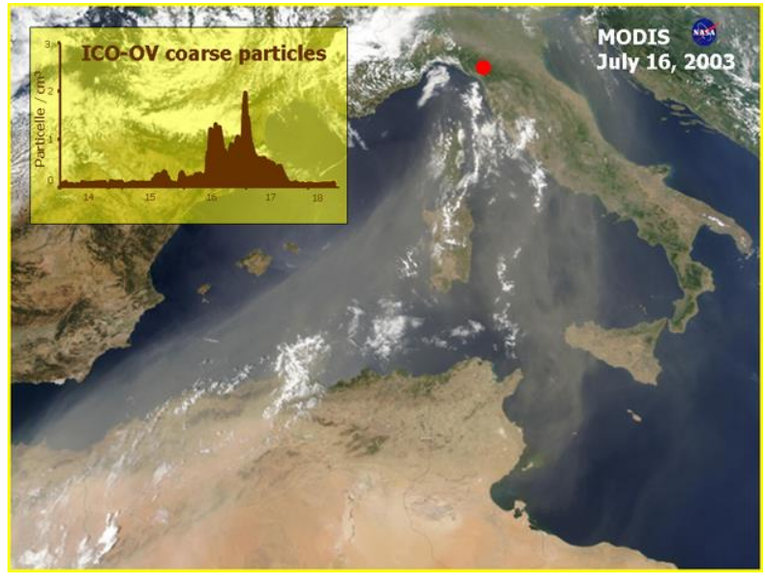
Type of Bioaerosol	Size (μm)	Concentration (#/m^3)
Viruses	0.02-0.3	-----
Bacteria	0.3-10	0.5-1,000
Fungal Spores	0.5-30	0-10,000
Pollen	10-100	0-1,000

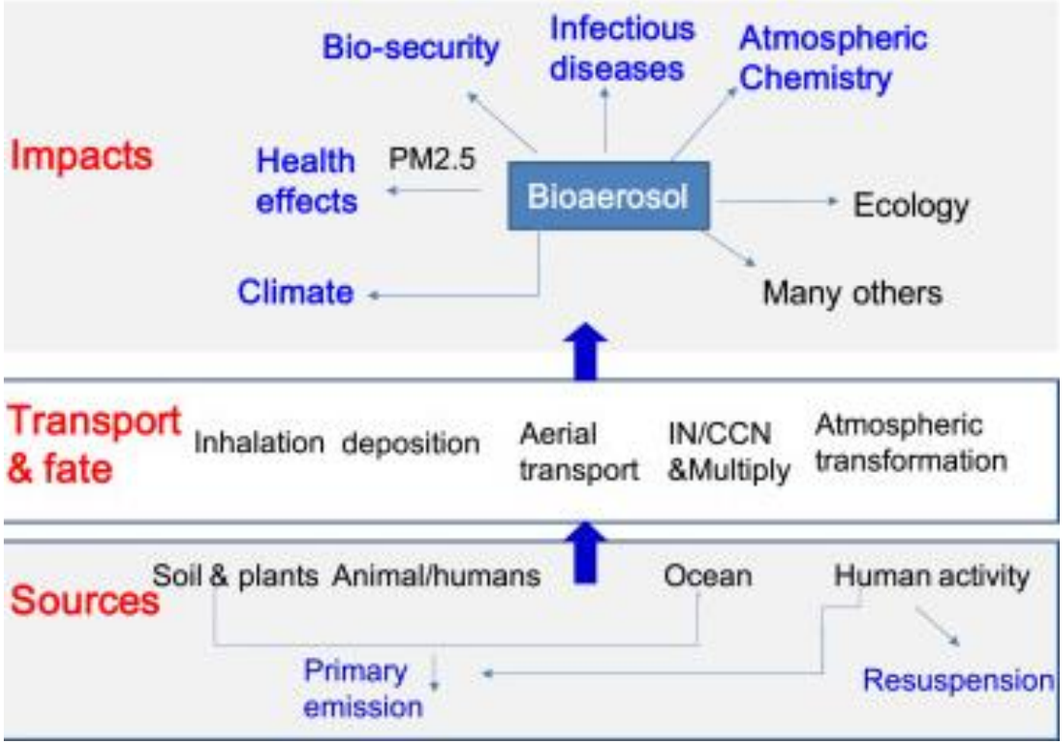
Source: Hinds, W.C. *Aerosol Technology: Properties, Behavior, and Measurement of Airborne Particles, 2nd edition.*

The problem of bio-aerosol and bacterial strains

Four interconnected topics:

- Health issues: exposure to bio-aerosols has been linked to various health effects
- Climate and CCN/IN impact (where viability and proliferation of airborne bacteria are the significant investigation subjects)
- Long-range transport of bacteria linked to dust events
- The role of atmosphere not only as a transport medium but also as a reprocessing system



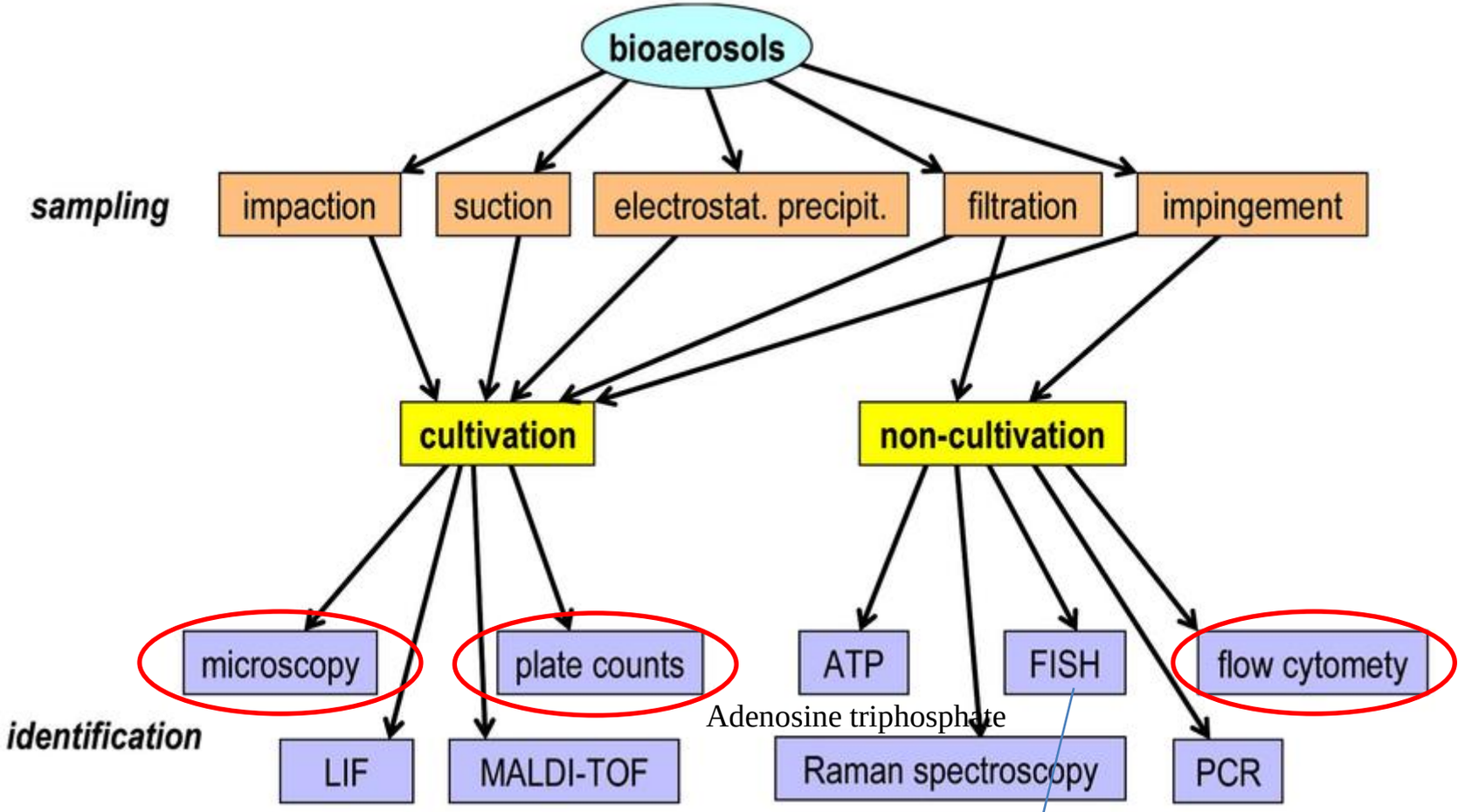


Bioaerosol: stability

It depends on Physical, Biological and Environmental parameters:

- Sunlight (e.g. UV)
- Air movement
- Temperature
- Relative Humidity
- pH
- Oxygen
- Pollutants (e.g. ozone, carbon monoxide)
- Suspended media (e.g. salts, proteins, sugars)
- Others…

Bioaerosol: sampling and analysis techniques



Experimental approaches to aerosol studies

- Atmospheric simulation chambers are exploratory platforms used to study various atmospheric processes in realistic but controlled conditions:

- Atmospheric conditions can be maintained and monitored in real time for periods long enough to mimic the realistic environments and to study interactions among their constituents.

Field (including airborne and satellites)	**Simulation Chamber experiments**	**"Benchtop" laboratory exp^{ts}**
• Very realistic	(somewhere in between ?)	• Very innovatives •Very efficient for small pieces of mechanism
• Complex and hence often difficult to interpret down to mechanisms level		• Not so realistic and hence sometimes difficult to scale to atmosphere

Atmospheric simulation chambers

- **Atmospheric simulation chambers (ASCs)** are the most advanced tools for elucidating processes that occur in the atmosphere.

- ASCs have been used mainly to study chemical and photochemical atmospheric processes:
  e.g. **ozone formation and cloud chemistry**

- The high versatility of these facilities allows for a wider application covering all fields of atmospheric aerosol science.

- Between all the European ASCs, none of them is mainly studying bio-aerosols

- A strong improvement in the understanding of microorganisms behavior in the atmosphere can be provided by atmospheric chamber experiments, that allow for a scientific intermediate approach between “in vitro” and “in vivo” analysis.

Why an ASC to study bio-aerosol?

Thanks to ASC we can:

- Systematic analysis of bio-aerosols behavior in **different atmospheric conditions**, aimed at evaluating the bacteria **viability, survival mechanisms** and endotoxin production

- **Parameterization of microrganisms interaction mechanisms** varying the environmental conditions (meteo, chemical composition, other aerosols concentrations) and development of a **modelling tool** for the bioaerosol description

ChAMBRe will host experiments to study the bacterial viability versus the air quality level, i.e. the atmospheric concentration of gaseous and aerosol pollutants.

ChAMBRe
(Chamber for Aerosol Modeling e Bio-aerosol Research)

ChAMBRe is installed at the ground floor of the Department of Physics of the University of Genoa, where it is jointly managed by the **Italian National Institute of Nuclear Physics** (**INFN**) and the **Physics Department.**

(www.labfisa.ge.infn.it)

- Maximum height: 2.9 m
- Diameter 1 m
- Total volume of 2.23 m^3

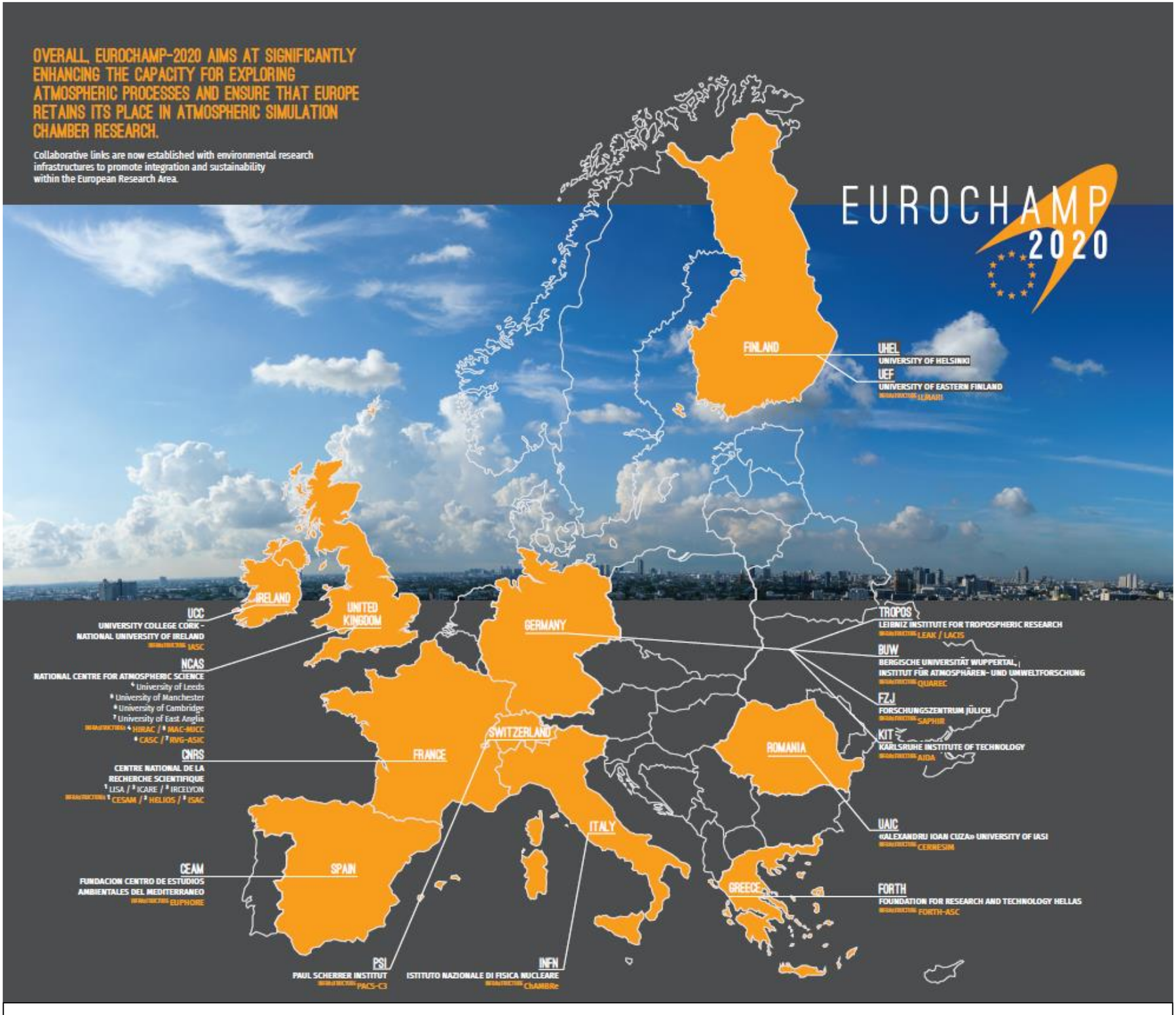
Projects and Funding

- Since the beginning of 2017, ChAMBRe is one of the nodes of the **EUROCHAMP-2020 network:**
  - a world-class infrastructure for research and innovation that include the most advanced European atmospheric simulation chambers.

- Since the beginning of 2018, ChAMBRe (INFN) has joined the **Research Infrastructure ACTRIS** – Aerosols, Clouds and Trace Gases – European initiative related to the research in aerosols, clouds, and trace gases to provide high-quality research infrastructure services to a wide user community

- ChAMBRe will participate, together with the INFN-LABEC (Florence), to the next **PON-IR** (progetto operativo nazionale – Infrastrutture di Ricerca, total budget 326 M€)

- Also a PRIN and a POR projects have been submitted



ChAMBRe is the first chamber mainly dedicated to bio-aerosol studies

TNA

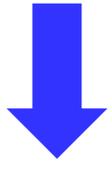
Main Steps:

Chamber characterization (e.g. lifetime vs. particle dimensions)

Definition of a protocol for the injection in the Chamber (linked protocol for the growth and handling of the bacterial strains)

Definition of a protocol for the extraction from the Chamber (linked protocol for the counting of the collected bacteria)

Different sampling techniques can be used depending on what information to be achieved

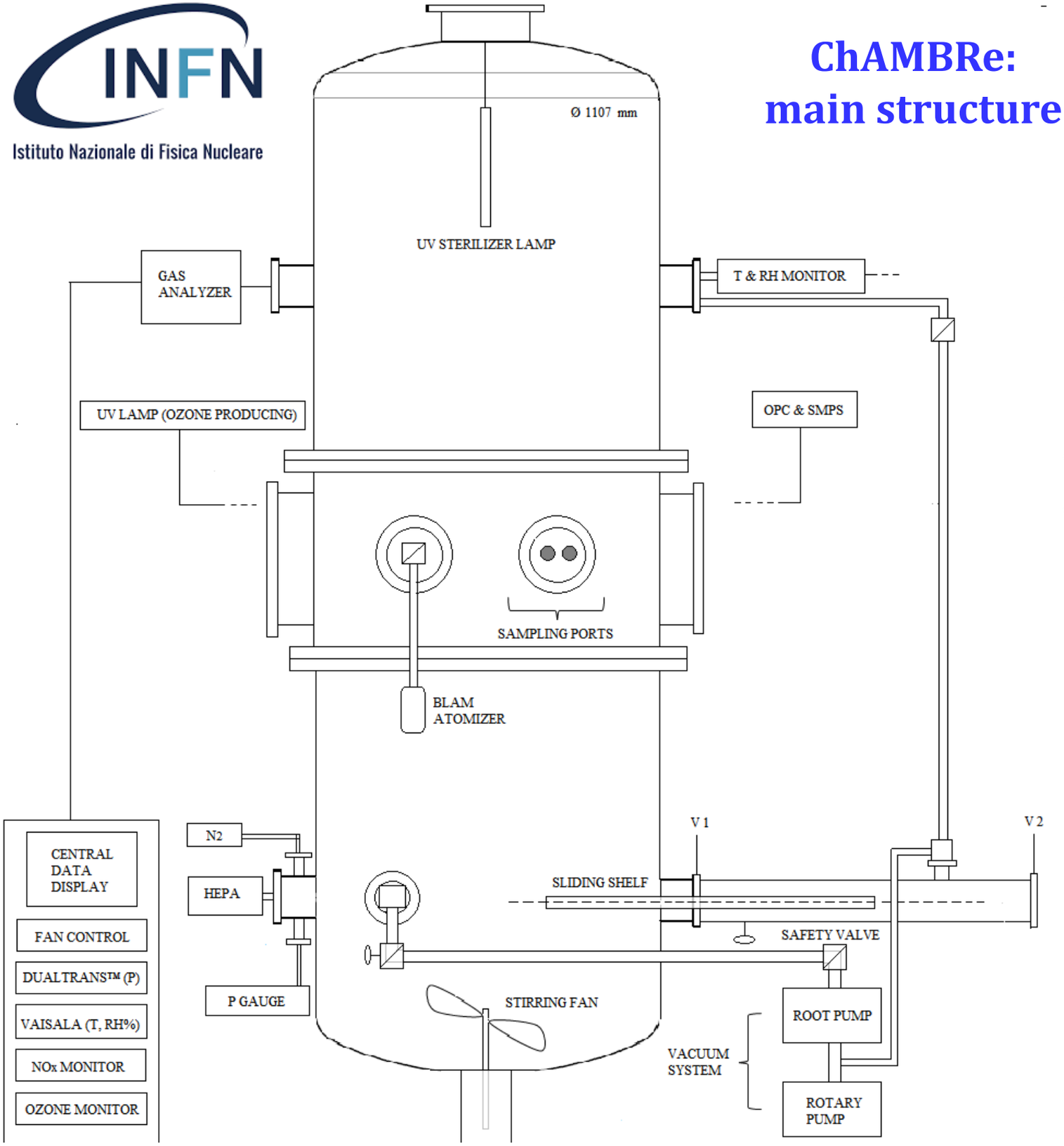


Goal: study of the response of bacteria to different air quality levels, i.e. the atmospheric concentration of gaseous and aerosol pollutants.

ChAMBRe:

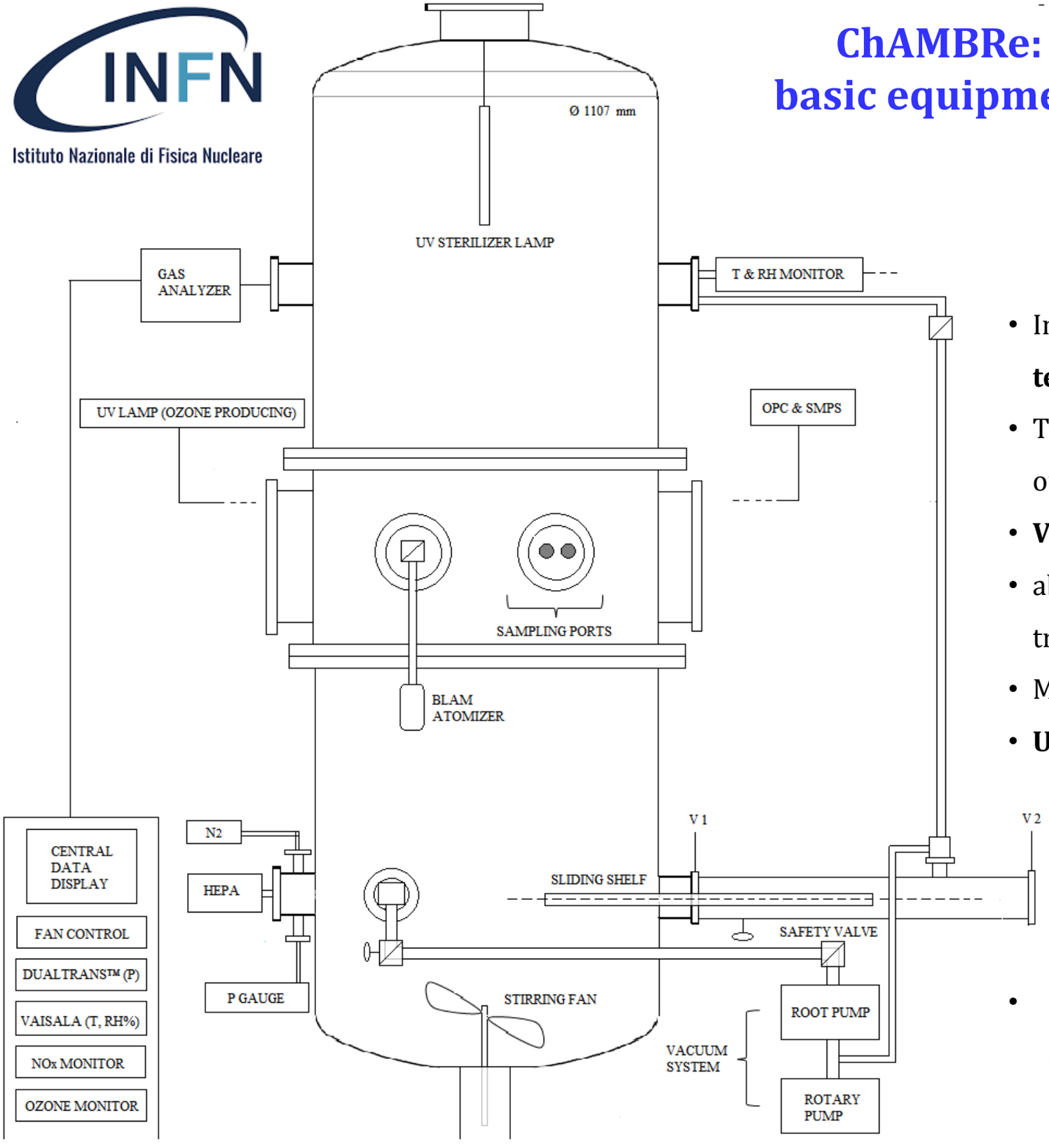
- **Description of the facility**
- **Characterization**
- **Bacterial strains**
- **Protocols to prepare, inject, expose and collect bacteria**
- **Results**

ChAMBRe: main structure



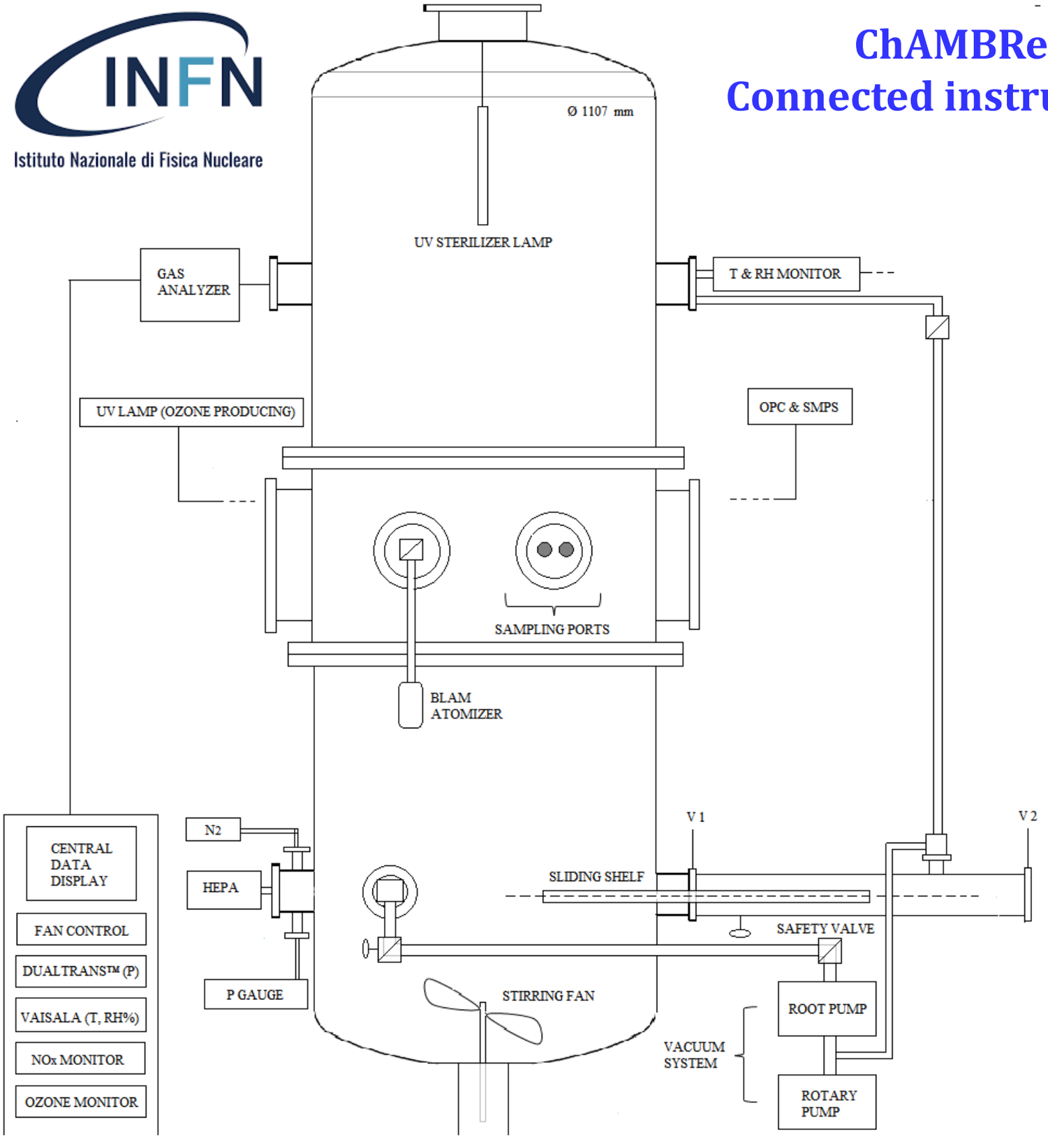
- maximum height and diameter of **2.9 m and 1 m** respectively
- total volume of **2.23 m^3**
- horizontal **cylinder** (length = 1 m) which hosts a movable shelf designed to introduce inside the chamber specific samplers

ChAMBRe: basic equipment



- Internal relative **humidity** and **temperature** gauge
- Two **pressure** gauges (inside and outside the chamber)
- **Vacuum system (root + rotary)**
- absolute **HEPA** filter and a **zeolite** trap
- Mixing fan (speed: 0 to 50 Hz)
- **UV Light sources**:
  - UV **sterilizer** lamp (λ = 253.7 nm)
  - UV lamp **ozone production** (λ <240 nm)
- Ports for gas/aerosol **injection** and **extraction**

ChAMBRe: Connected instruments



- **Real-time** aerosol monitoring:
  - OPC
  - SMPS
  - Aethalometer
- **Aerosol** samplers:
  - Nano-Moudi II cascade impactor
- **Bio-aerosol**-specific samplers:
  - Liquid Impingers
  - Andersen-type cascade impactor
- **O_3, NOx** on-line monitors
- **CO-CO_2** on-line monitor (in progress)

Instrumentation for studying Bacteria

Specific tools for the analysis of aerosol biological parameters and morphological properties:

- Culture methods in vitro (isolation, identification, growth)
- Spectrophotometer (UV/Vis)
- Epifluorescence microscopy
- SEM

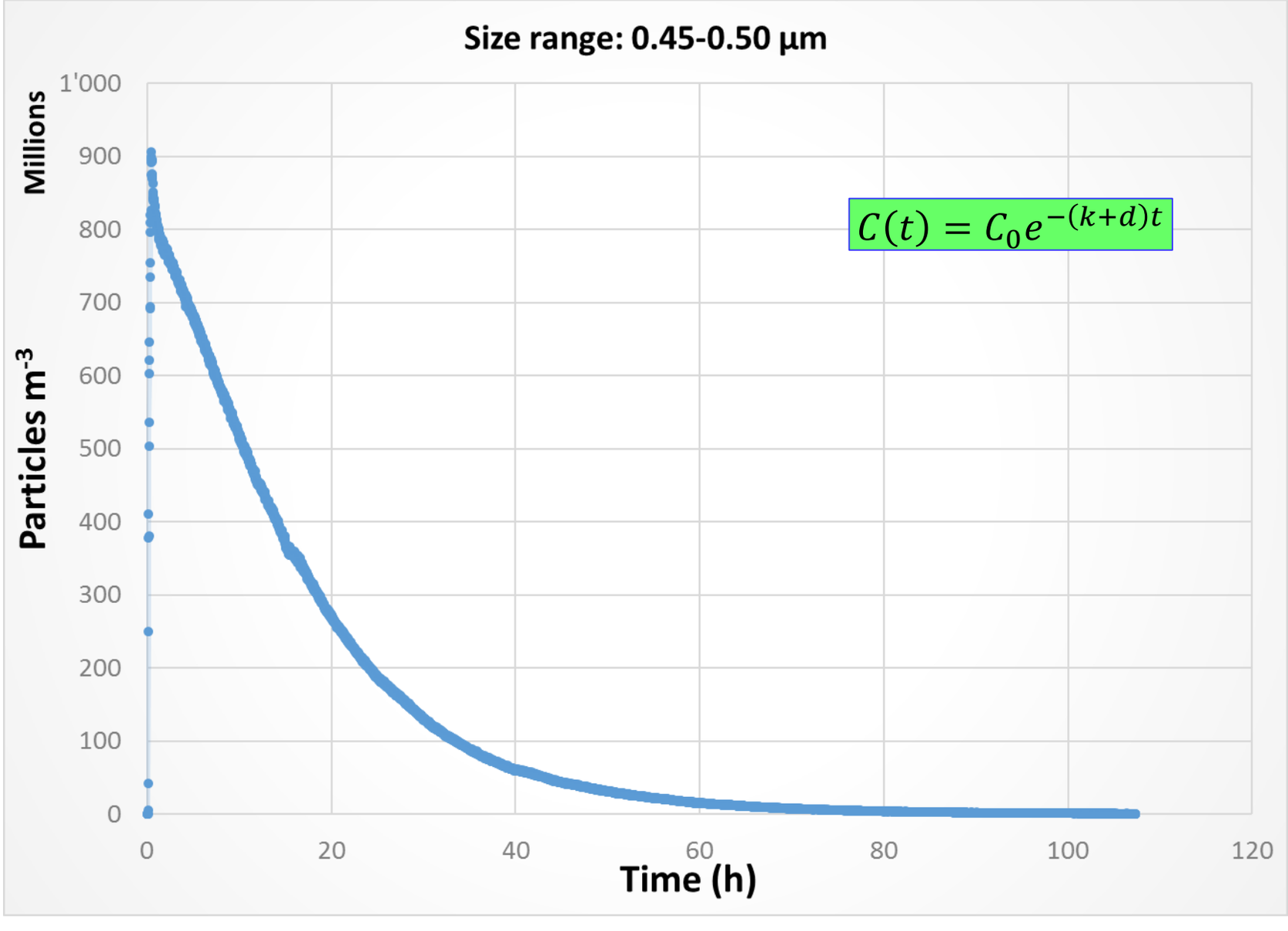
Plus the «standard» equipment for aerosol characterization :

- ED-XRF
- IC, EC/OC by TOT
- MWAA

ChAMBRe:

- **Description of the facility**
- **<u>Characterization</u>**
- **Bacterial strains**
- **Protocols to prepare, inject, expose and collect bacteria**
- **Results**

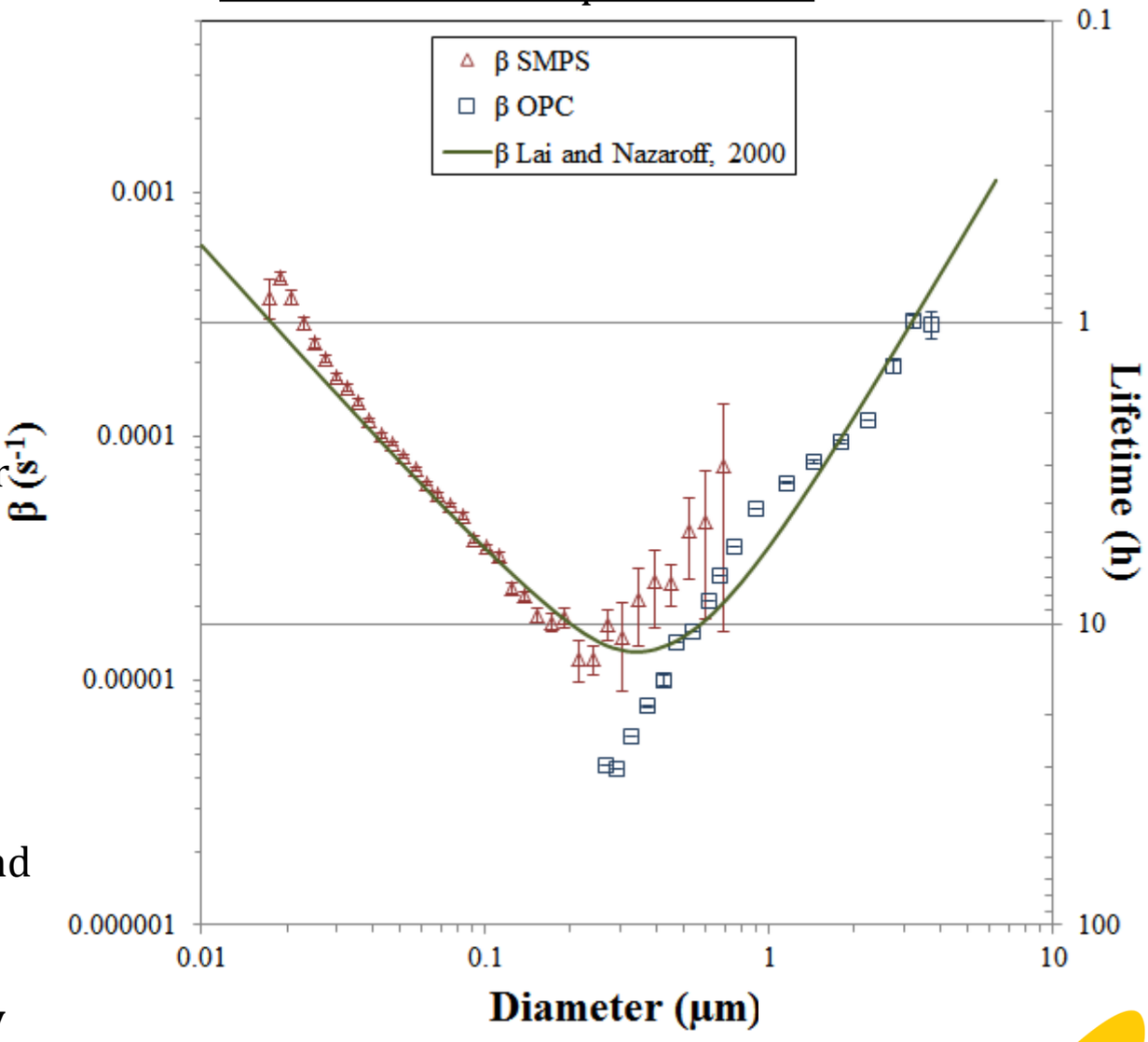
ChAMBRe characterization: aerosol life-time



ChAMBRe characterization: aerosol life-time

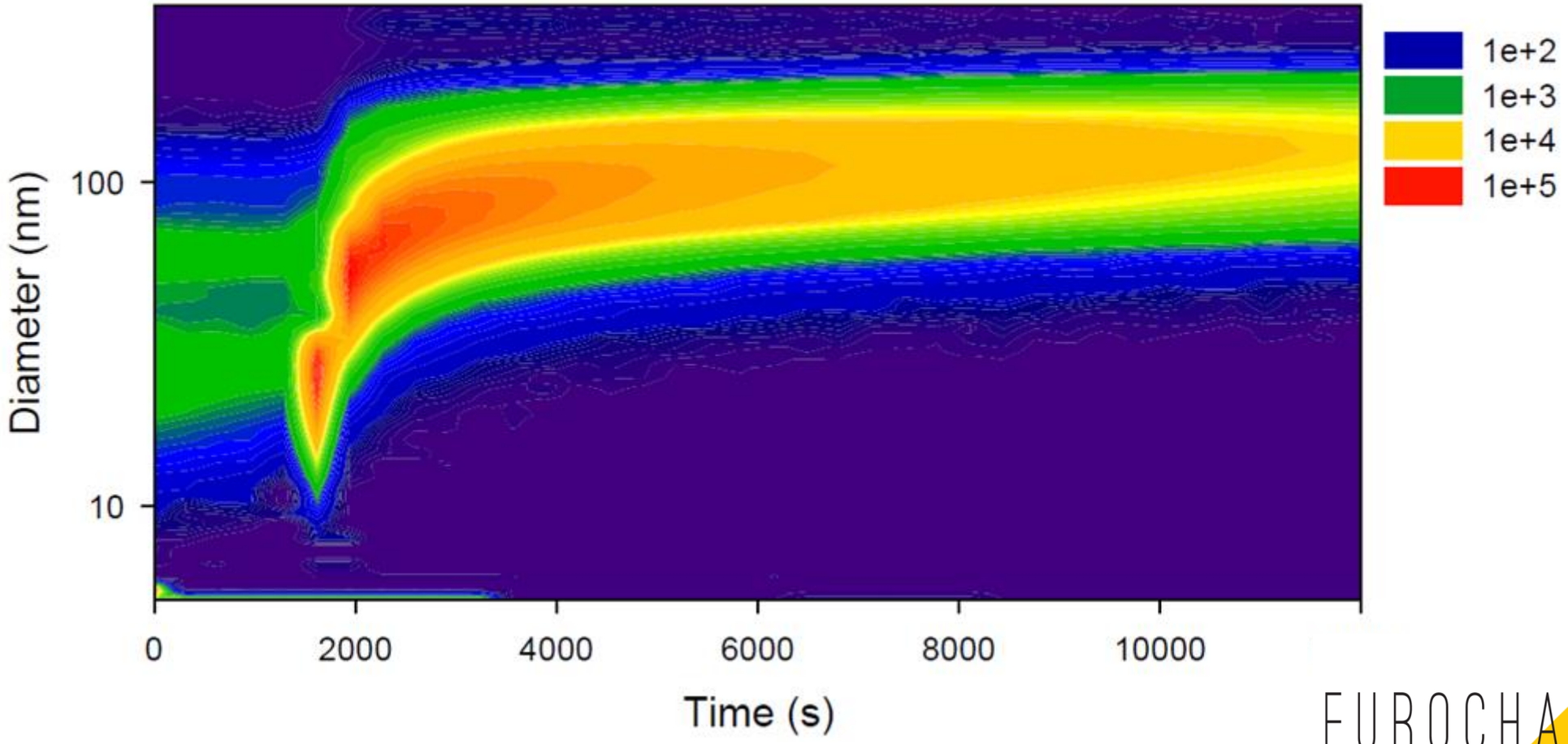
- NaCl solution with different concentration
- polydispersed particles with continuous size distributions from few nm up to about 5 µm
- mixing fan: 5 Hz
- particle lifetime has been determined by fitting the mass decay curve with a first order exponential
- Aerosol dilution was taken into account and properly corrected
- experimental data are nicely reproduced by the wall deposition model described in Lai and Nazaroff, (2000)
- Aerosol lifetime in ChAMBRe varies from **few hours** to about **4 days** depending on particle size

Aerosol lifetime vs. particle size:



ChAMBRe characterization: aerosol life-time

Aerosol production by injection of α-pinene
(130 ppb with 300 ppb O_3)

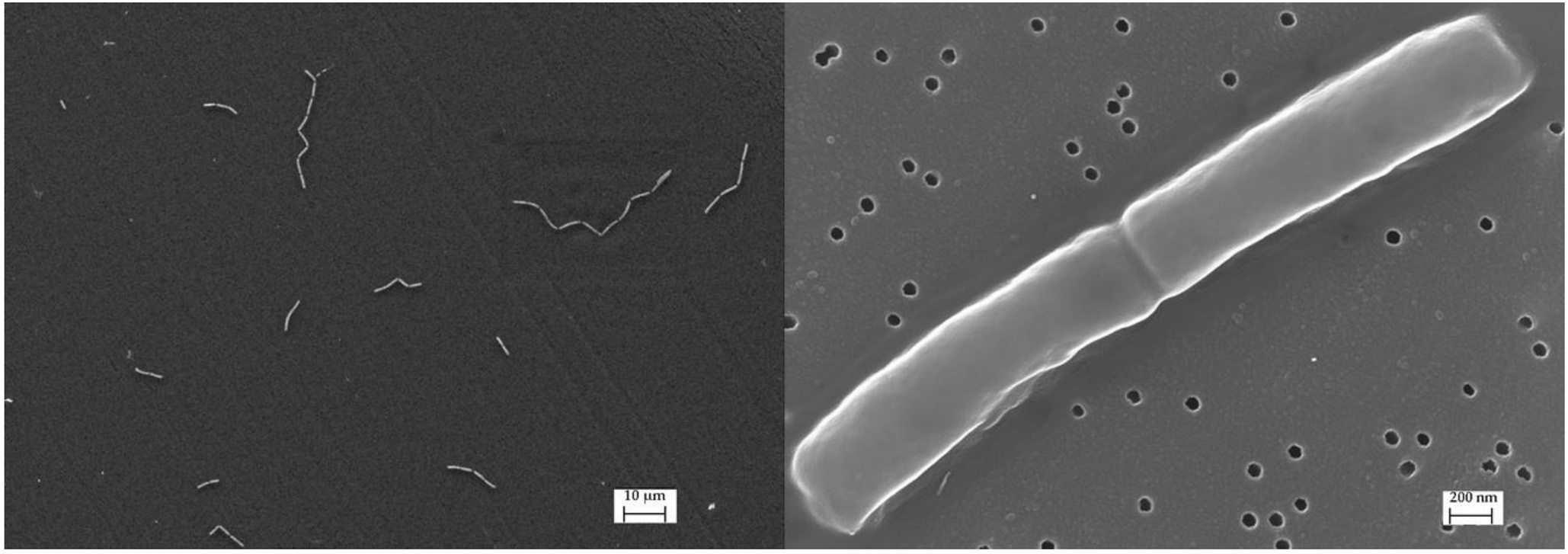


ChAMBRe:

- **Description of the facility**
- **Characterization**
- **<u>Bacterial strains</u>** → We select two tipical model organisms:
  - *Bacillus subtilis*
  - *Escherichia coli*
- **Protocols to prepare, inject, expose and collect bacteria**
- **Results**

Bacillus subtilis

- Gram-positive, rod-shaped bacterium
- length ranging between 2.5 and 6.5 μm
- commonly found in soils
- also observed in other environmental matrices such as water and air

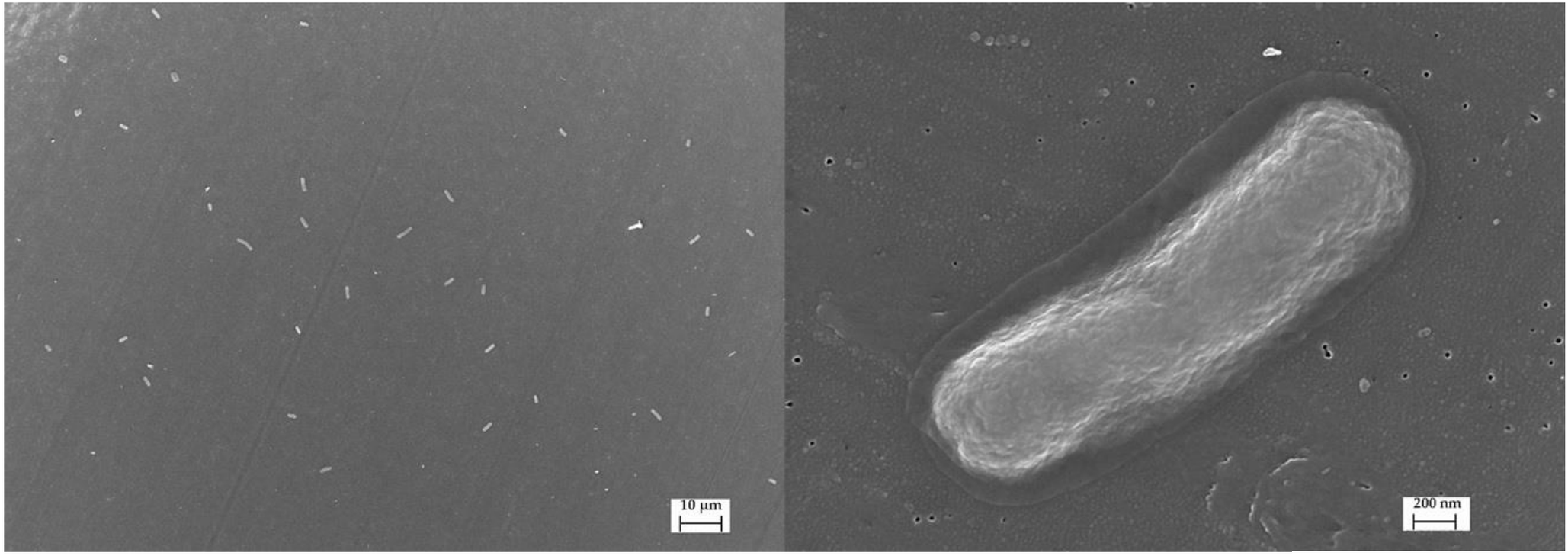


B. subtilis culture preparation:

- inoculation in liquide nutrient broth (TSB) at 37°C, 170 rpm

Escherichia coli

- Gram-negative, rod-shaped, enterobacter
- 1–2 µm long and about 0.25 µm in diameter.
- It is a common inhabitant of the gastrointestinal tract of warm-blooded animals
- specific strains of *E. coli* can also survive in extra-intestinal environments.



E. Coli culture preparation:

- inoculation in liquide nutrient broth (TSB) at 37°C, 170 rpm

ChAMBRe:

- **Description of the facility**
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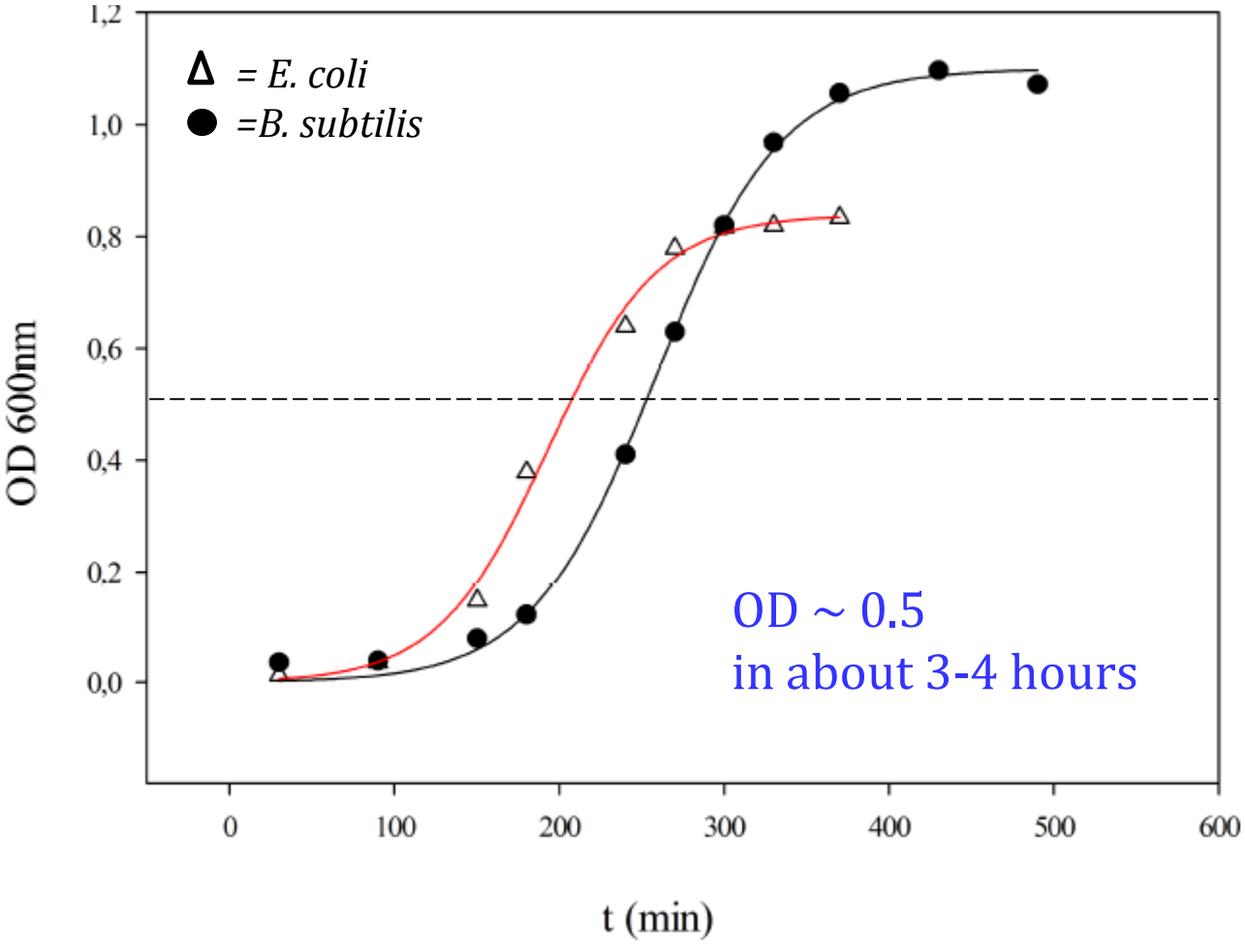
Preparation of bacterial suspension for the injection in ChAMBRe

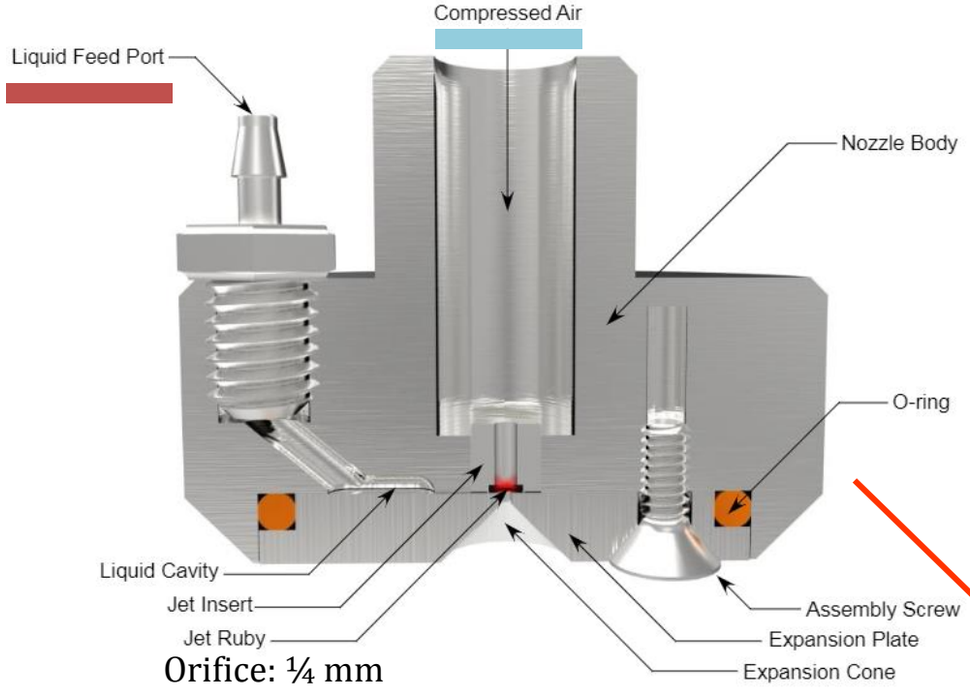


- Growth curve obtained by measuring the Optical Density at 600nm in UV-vis spectrophotometer
- After centrifugation, re-suspension in NaCl 0.9%
- Injectable suspension:
  - ≈ 10^7 CFU mL^{-1} for *B. subtilis* (OD_{600} around 0.5)
  - ≈ 10^6 CFU mL^{-1} for *E. coli* *

* the initial cells suspension with an OD_{600} around 0.6 was diluted (1:10, 1:15, 1:20, 1:40) before the injection, to avoid an excessive bacterial concentration on the Petri dishes

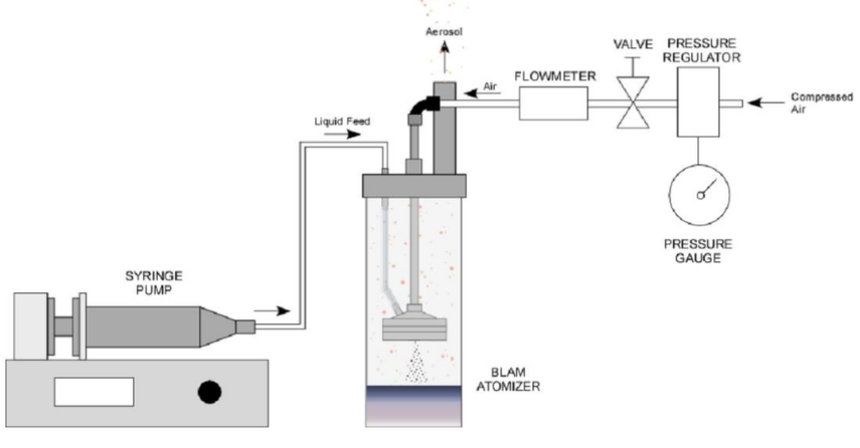
Injection in ChAMBRe

Injection by Blaustein atomizer (**BLAM**):



Orifice: ¼ mm

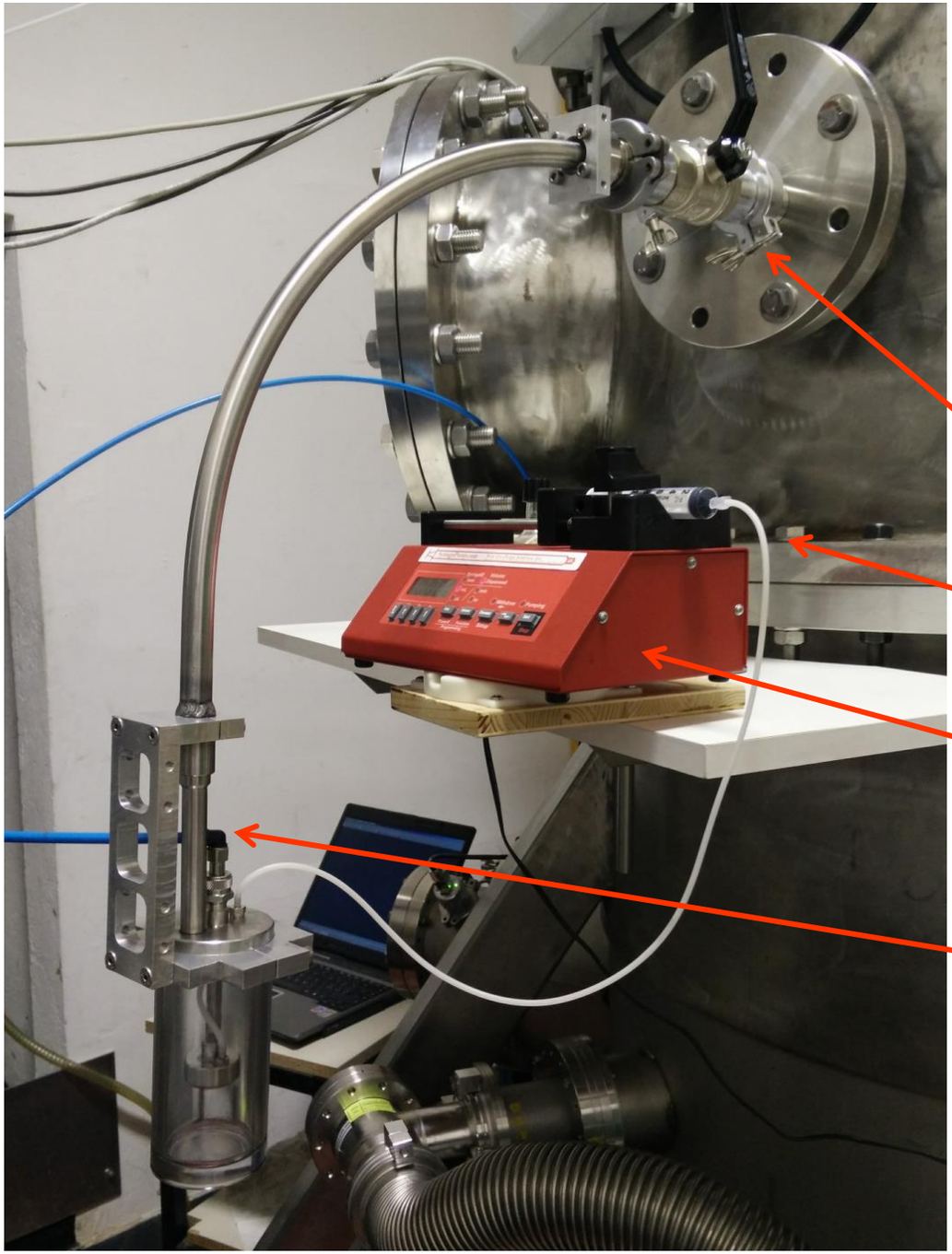
The atomization occurs when the pressurized air coming from the stem pushes at sonic velocity through a precisely laser cut ruby crystal pressed into the Nozzle Body.



To the chamber

Aerosolized Liquid

Injection in ChAMBRe

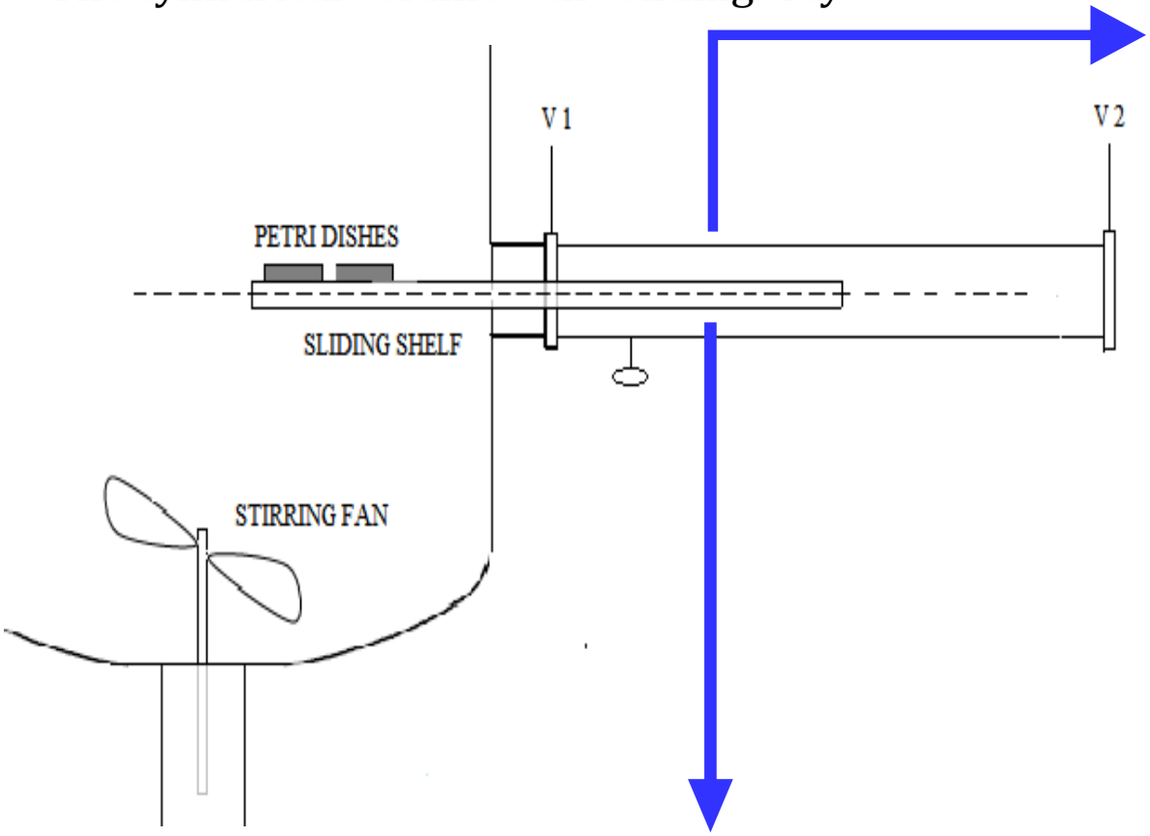


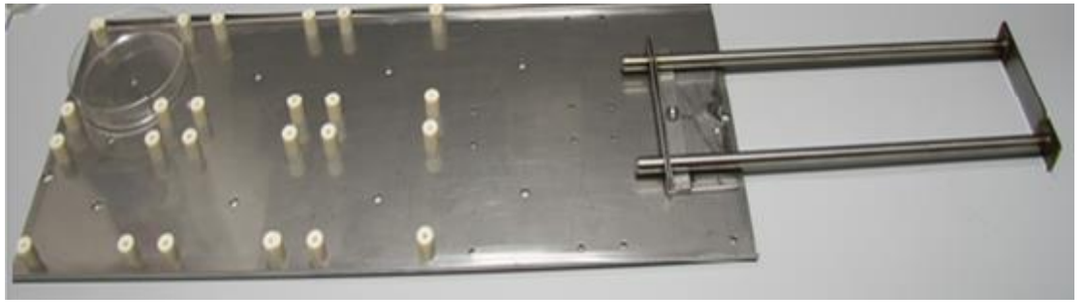
Injection by Blaustein atomizer (**BLAM**):

- Volume: 2 or 3 mL of the cells suspension
- Nebulization efficiency: 1% – 8%
- Cut-off: 2-3 µm

Collection of Bacteria on Petri dishes

The cylindrical volume + the sliding tray

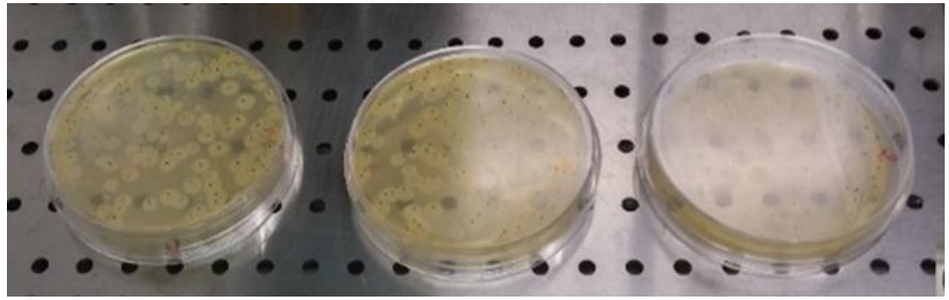




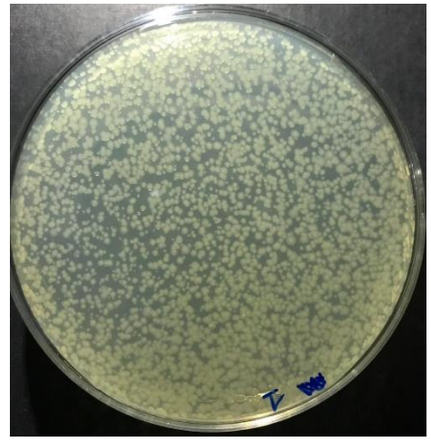
The vessel can host up to 6 Petri

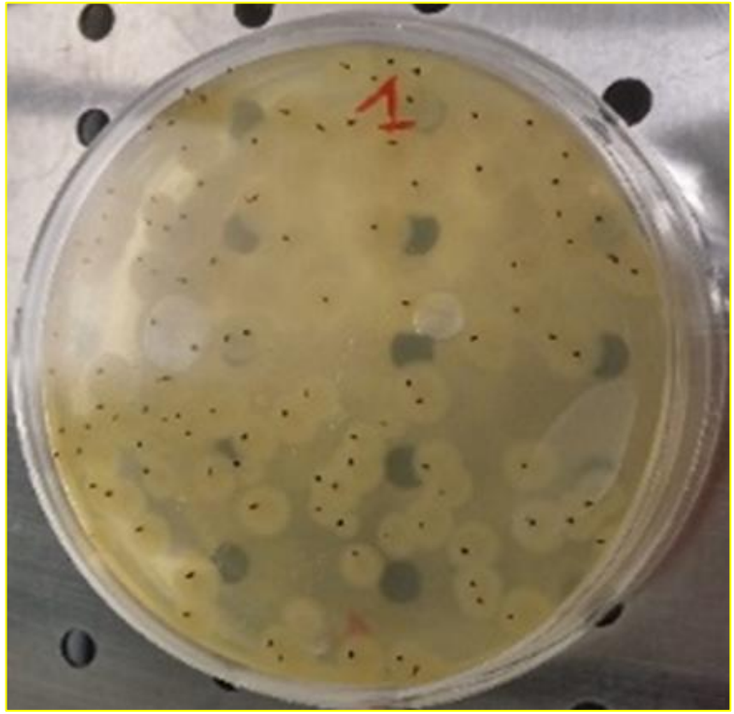
Exposure time inside the chamber: **~5 h**

Incubation

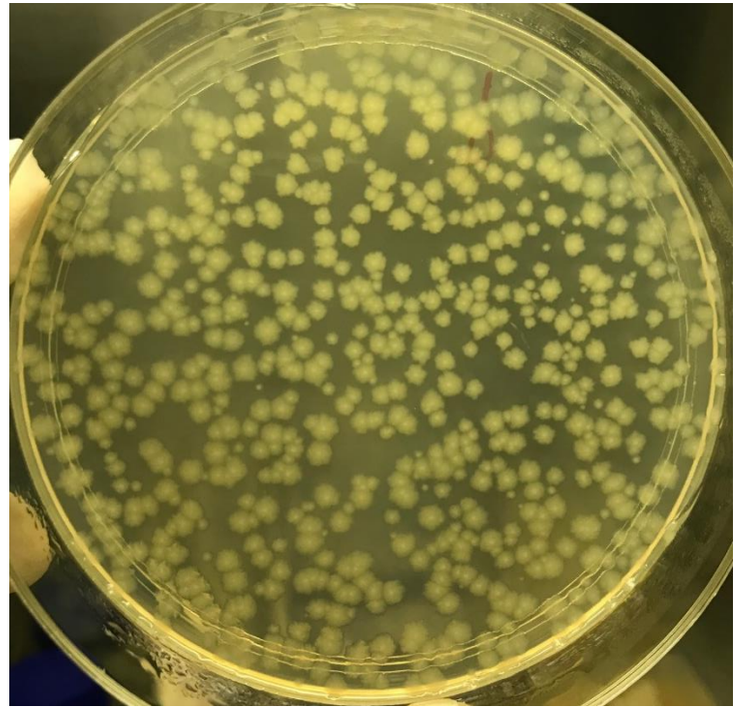


After exposure to chamber atmosphere, Petri dishes are incubated for 24 h at 37° C, after which the number of formed colonies can be counted.





B. Subtilis



E. Coli

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- **<u>Results</u>**

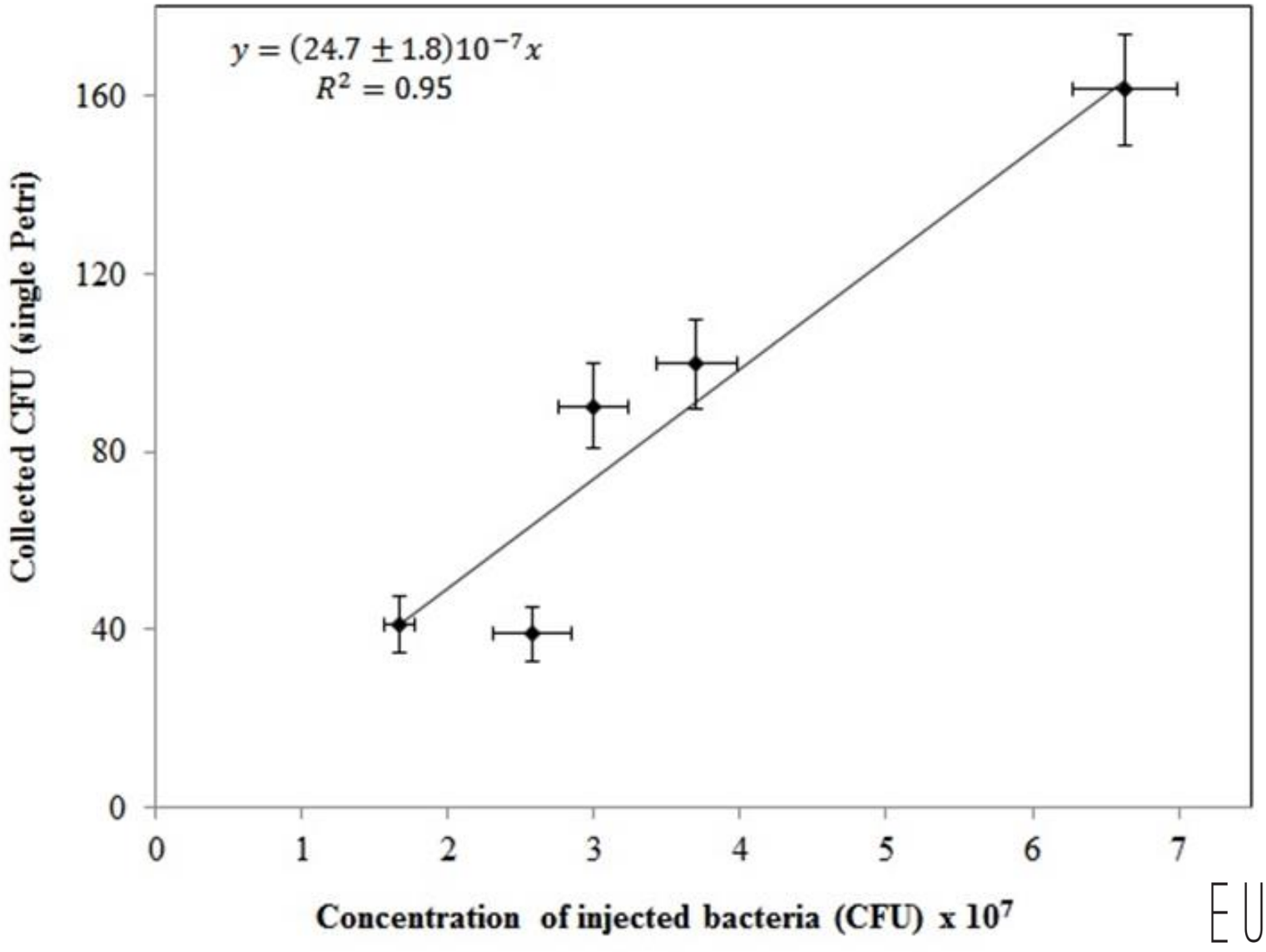
Results and reproducibility: experiments with *B. subtilis*

	Relative humidity range (%)	Temperature range (° C)	Pressure range (mbar)	Petri dishes Exposure time (hh:mm)
Exp. 1	55-85	22.0-21.1	1015-1012	05:00
Exp. 2	44-71	23.7-24.5	1010	05:20
Exp. 3	50-43	23.2-21.3	1014-1015	05:15
Exp. 4	44-70	22.0-22.5	1016	05:05
Exp. 5	75-79	20.1-20.8	1005-1007	05:00

	OD_{600}	Suspension concentration (CFU mL^{-1}) x 10^7	Bacteria injected CFU x 10^7	Average CFU collected
Exp. 1	0.57	1.85 ± 0.14	3.70 ± 0.28	100 ± 10
Exp. 2	0.58	3.32 ± 0.18	6.63 ± 0.36	161 ± 13
Exp. 3	0.58	1.50 ± 0.12	3.00 ± 0.24	90 ± 10
Exp. 4	0.50	0.86 ± 0.09	2.58 ± 0.27	39 ± 6
Exp. 5	0.40	0.83 ± 0.05	1.67 ± 0.10	41± 6

Results and reproducibility: experiments with *B. subtilis*

Collected CFU vs injected bacteria



Uncertainty on the slope of the correlation curve:

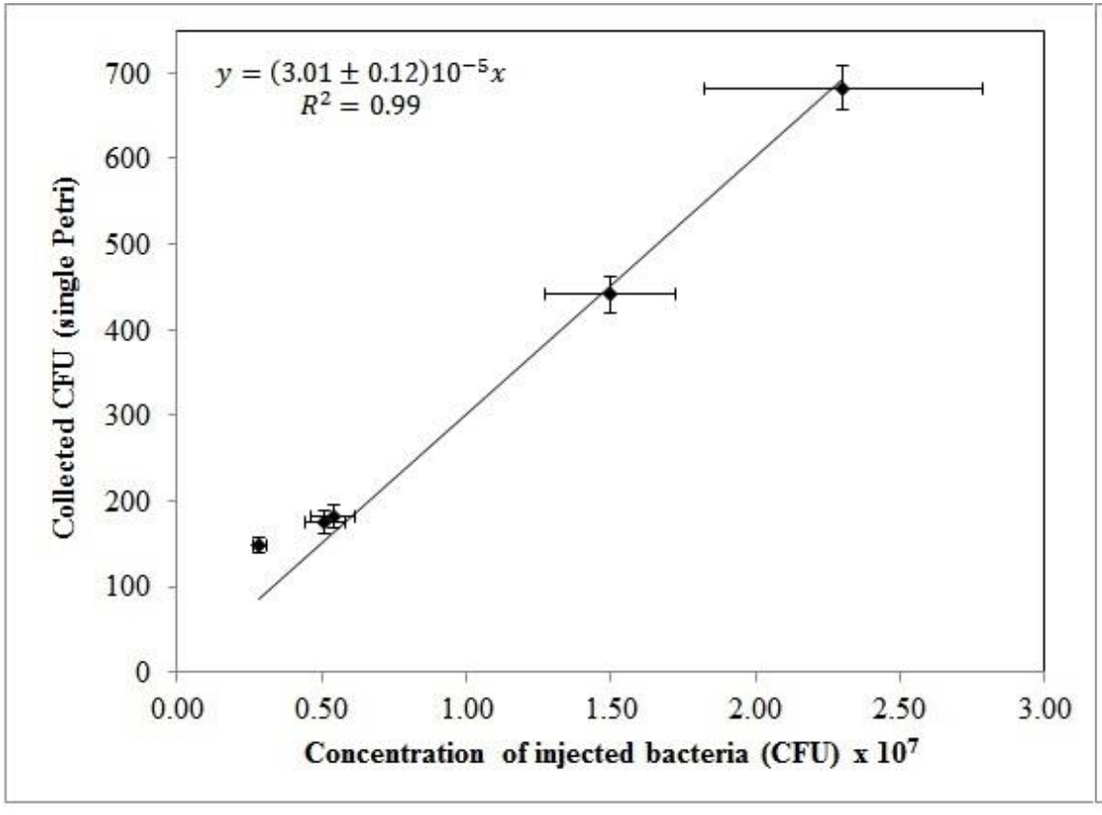
- lower than 10 %

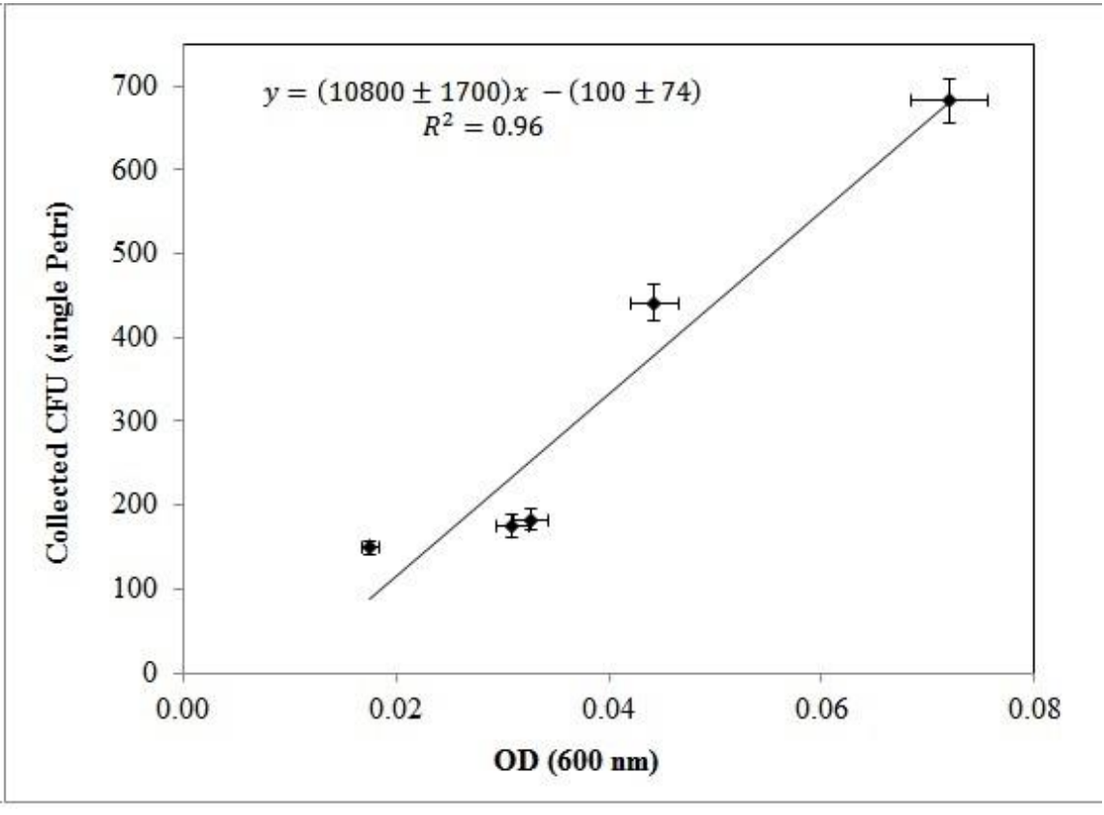
Results and reproducibility: experiments with *E. coli*

	Relative humidity range (%)	Temperature range (° C)	Pressure range (mbar)	Petri dishes Exposure time (hh:mm)
Exp. 1	75-77	15.8-18.7	994	05:00
Exp. 2	73-77	23.1-23.6	992-999	05:00
Exp. 3	78-80	19.0-19.3	1010	05:05
Exp. 4	76-83	18.6-19.0	1007-1009	05:00
Exp. 5	72-80	19.8-20.0	1002-1003	06:05

	OD_{600} (before dilution)	Dilution factor	OD_{600} (after dilution)	Suspension concentration (CFU mL^{-1}) x 10^6	Bacteria injected CFU x 10^6	Average CFU collected
Exp. 1	0.5712	1:20	0.0309	2.55 ± 0.36	5.10 ± 0.71	175 ± 13
Exp. 2	0.6440	1:10	0.0721	11.5 ± 2.40	23.0 ± 4.8	682 ± 26
Exp. 3	0.6037	1:20	0.0327	2.70 ± 0.38	5.39 ± 0.76	183 ± 14
Exp. 4	0.6447	1:15	0.0443	7.49 ± 1.12	15.0 ± 2.25	442 ± 21
Exp. 5	0.6566	1:40	0.0176	1.02 ± 0.07	2.85 ± 0.20	149 ± 9

Results and reproducibility: experiments with *E. coli*





Left panel: uncertainty on the slope of the correlation curve:

- around 5 %
- even better than the same uncertainty related to *B. subtilis* (about 10 %)

Right panel: the correlation between the relative optical density of the cell suspensions and the collected CFU

- For *E. coli* suspension, after an adequate calibration of the procedure, the evaluation of the microbial concentration through the fast and simpler control of the optical density seems sufficiently accurate to perform well controlled experiments.

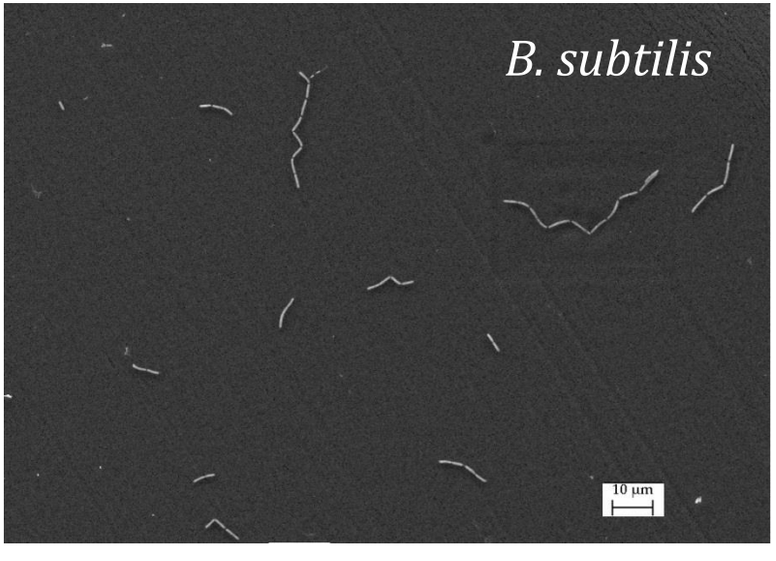
Results and reproducibility: Comparison between *B. subtilis* & *E. coli*

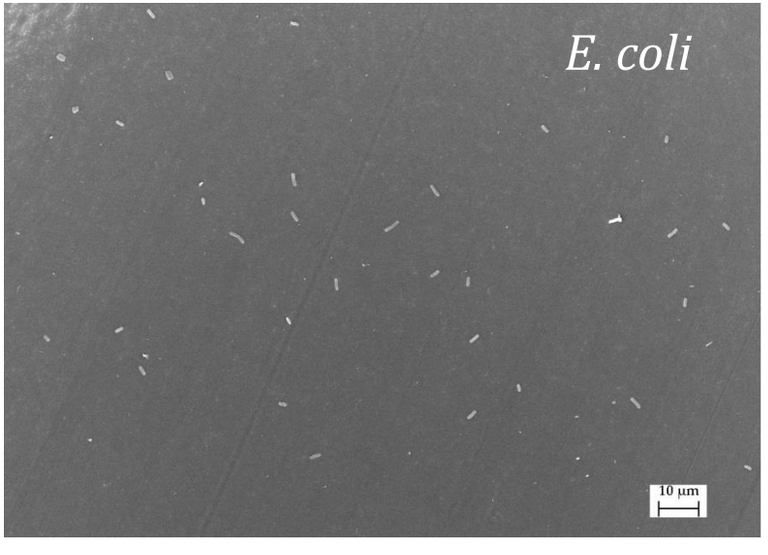
E. coli:

- Injected solution less concentrated but more CFUs were collected
  - humidity in the chamber was generally greater in the second set of experiments, ensuring to Gram negative microorganisms a more comfortable environment,
  - also different behavior of the two bacteria strains.

The FESEM micrographs of the bacteria show that the cells of *B. subtilis* tend to aggregate, forming long chains, while the cells of *E. coli* are mainly present as single individuals:

- in the first case it is quite possible that the colonies counted on the Petri dishes originated from a group of cells, while in the second case each colony results presumably from a single viable microorganism.





Conclusions

- The performance of the new chamber, which may impact on the future experiments on bio-aerosol (i.e. wall reactivity, aerosol lifetime, background levels), has been quantitatively assessed.
- A protocol to handle the injection and extraction phases has been thoroughly tested both with Gram positive and Gram negative bacterial strains.
- With a clean atmosphere maintained inside ChAMBRe, the ratio between injected and extracted viable bacteria turned out to be reproducible at a 10 % level.
- Such result is the first methodologic step in view of a forthcoming systematic study of the correlation between bacterial viability and pollution levels.

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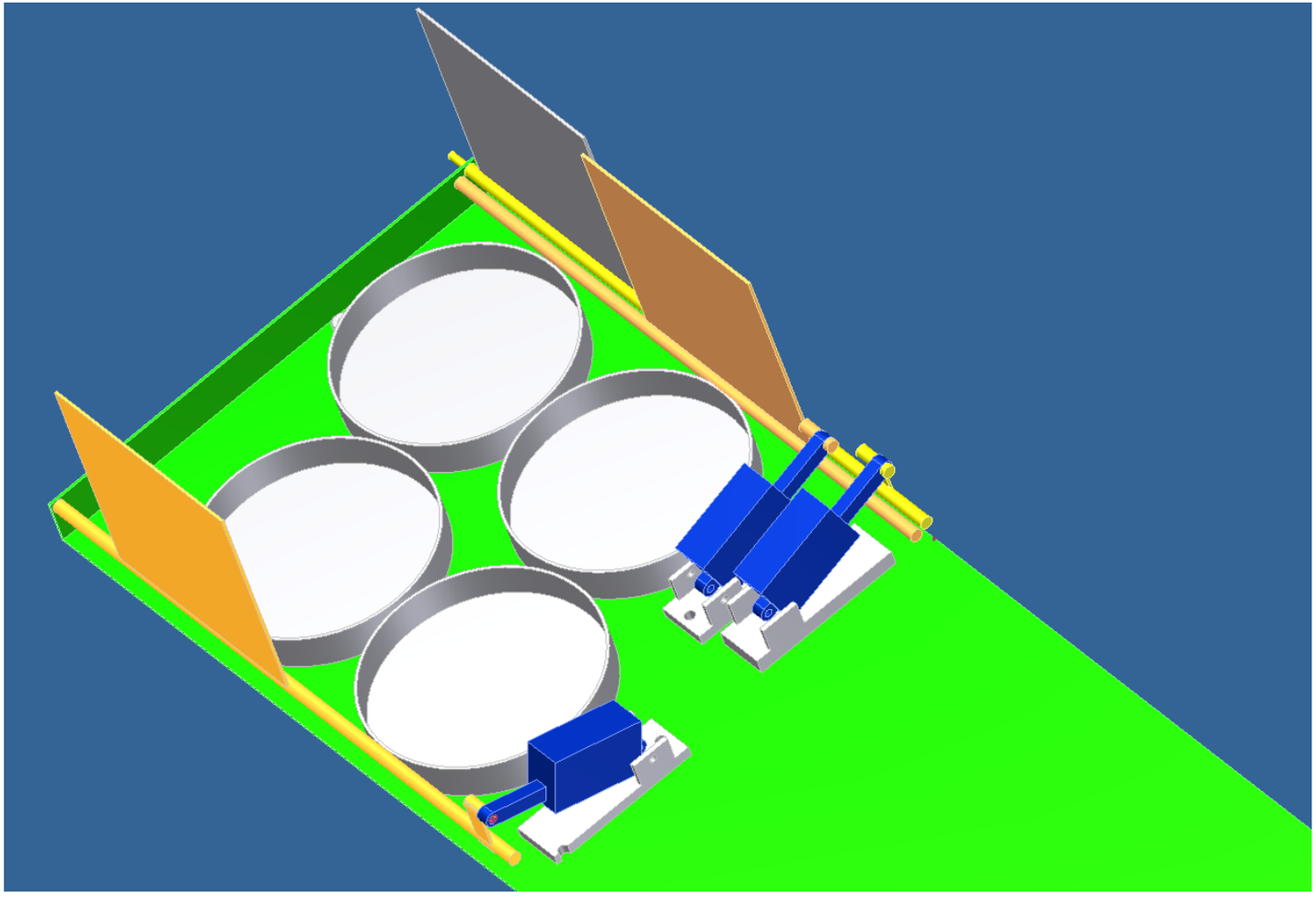
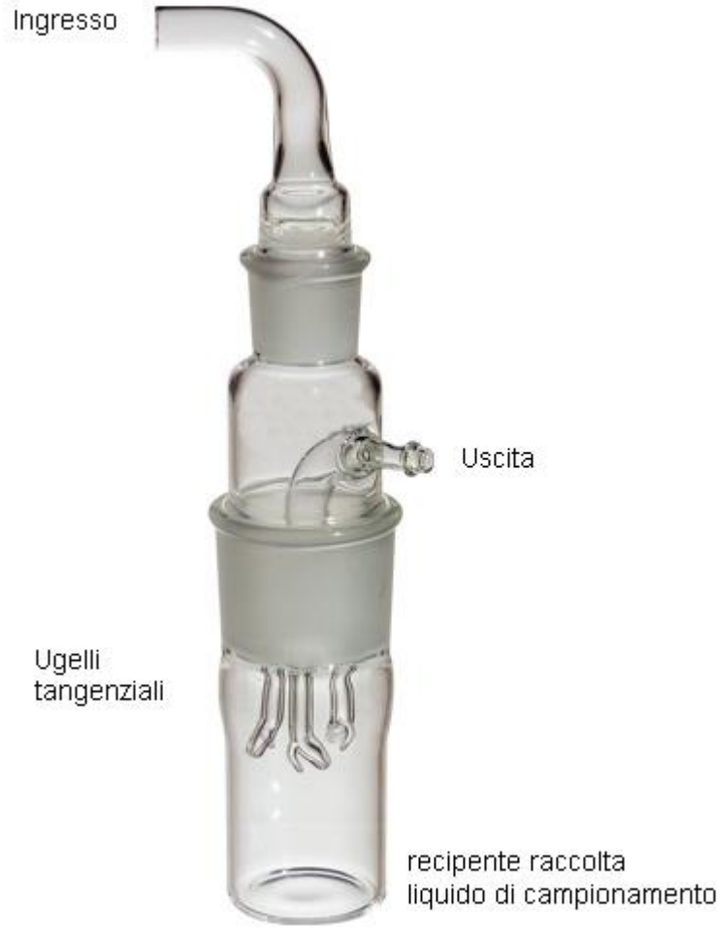
Next Steps

Time-segregated collection



Next Steps

Collection by liquid impinger



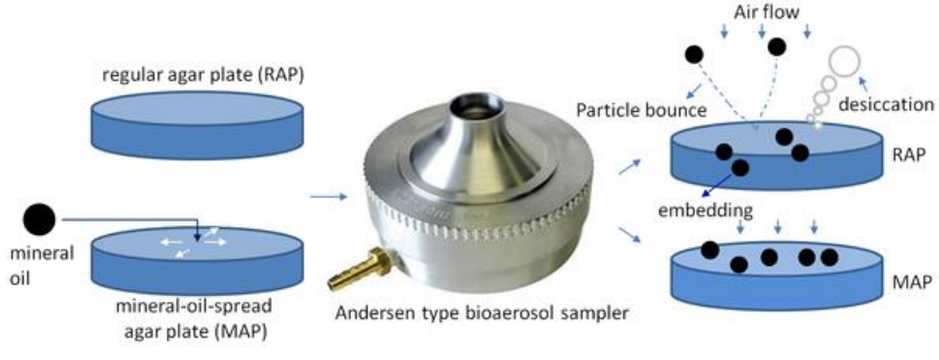
- Flow: 12.5 lpm

Possibility to evaluate **<u>total concentration</u>** and size of bacteria agglomerations by flow citometry

Next Steps

Size-segregated collection

Andersen type multi-stage impactor



Single stage impactor

Istituto Nazionale di Fisica Nucleare

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