ITALIAN NATIONAL AGENCY FOR NEW TECHNOLOGIES, ENERGY AND SUSTAINABLE ECONOMIC DEVELOPMENT

THE FIRST INTERNATIONAL WORKSHOP ON EXOTIC FLOW CYTOMETRY

Checking Small Things... Better!

COMPENDIUM OF ABSTRACTS

EXOFLOWMETRY 2019

13th - 15th NOVEMBER 2019 Rome - Italy

Italian Society of Cytometry

ISTITUTO SUPERIORE DI SANITÀ

Scientific Board

AMALFITANO STEFANO - CNR IRSA
ARIOLI STEFANIA - UniMI
BARCACCIA GIANNI - UniPD
BEVIVINO ANNAMARIA - ENEA
BOON NICO - Gent Univ. BE
CASOTTI RAFFAELLA - SZN
DOLEZEL JAROSLAV - IEB Ceck Rep.
FERRINI ANNA MARIA - ISS
FLAJSHANS MARTIN S. Bohemia - Univ. Ceck Rep.
GALBRAITH DAVID Arizona - Univ. AZ
GIORGI DEBORA - ENEA
GUZZON RAFFAELE - FEM Trento
LETER GIORGIO
LUCRETTI SERGIO - ENEA
MORA DIEGO - UniMI
MULLER SUSANN - UFZ Leipzig GER
O'CONNOR JOSE ENRIQUE - Valencia Univ. SPAIN
RIONDATO FULVIO - UniTO
SANCHEZ MASSIMO - ISS
TIRELLI VALENTINA - ISS
ZOANI CLAUDIA - ENEA

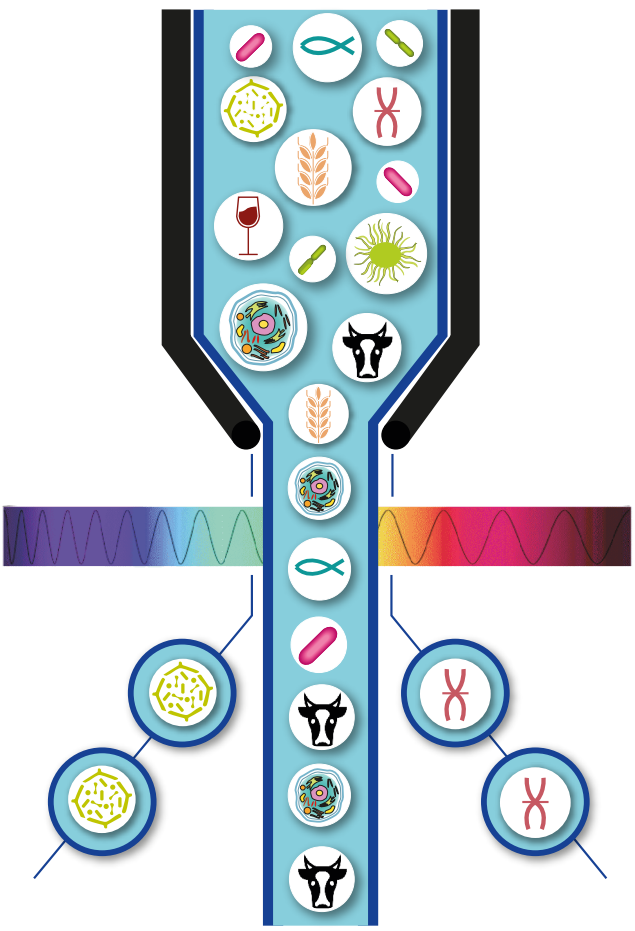
ITALIAN NATIONAL AGENCY FOR NEW TECHNOLOGIES, ENERGY AND SUSTAINABLE ECONOMIC DEVELOPMENT

Italian Society of Cytometry

ISTITUTO SUPERIORE DI SANITÀ

EXOFLOWMETRY 2019

13th - 15th NOVEMBER 2019 Rome - Italy



Supporter

METRO FOOD
METROLOGY IN FOOD AND NUTRITION INFRASTRUCTURE FOR PROMOTING

ISAC
INTERNATIONAL SOCIETY FOR ADVANCEMENT OF CYTOMETRY

SIMTREAA

Golden sponsor

Beckman Coulter italia
BECKMAN COULTER Life Sciences

Silver Sponsors

S.I.A.L.

SONY
Sony Biotechnology Inc.

unisensor
DIAGNOSTIC ENGINEERING

rqmicro
rapid quantitative microbiology

The potential of flow cytometry (FC) is still largely unexpressed in areas different than the clinical and biomedical applications, for which is widely applied since the ’60s of the last century. High processivity, multiparametric and analytical precision are typical features for FC, all well suited to different and unconventional (exotic) applications.
During last years, there has been a significant technological progress in FC and a number of new instruments have been, and are going to be, released to the market or developed as prototypes. New fields of application such as winemaking, diary production, brewery, fish and animal farming, plant breeding and genomics, inner and outer water quality control, are now available to FC characterization and manipulation, and more and more are fast developing.

The First International Workshop on Exotic Flow Cytometry will focus on FC applications other than bio-medical, with the aim to highlight the different fields where flow cytometry is giving a significant contribution, in terms of new components revealed and/or development of new methods of analysis.

A wide overview of the use of FC in food and environment microbiology, plant and animal biotech, green biotechnology and genomics will be offered, as also related to the complex quality control systems of the agri-food supply chain, in the framework of the European METROFOOD - Research Infrastructure.

A demonstrative and practical hands-on day for about 30 persons will conclude the Workshop, during which a selected number of participants will also get a chance to analyze their samples.

FLOW CYTOMETRY AND WATER QUALITY: NEW INSIGHTS FROM THE SPACE RESEARCH

AMALFITANO S., LEVANTESI C., ROSSETTI S.

Istituto di Ricerca Sulle Acque IRSA-CNR, Via Salaria Km 29,300, Monterotondo, Rome, Italy
International Space Station, Water Technologies, Microbial Monitoring
amalfitano@irsa.cnr.it

Space exploration by self-sufficient spacecraft is demanding the development of culture-independent microbiological methods for in-flight water monitoring to counteract possible contamination risks. During longer-lasting future space missions, water renewal by ground-loaded supplies will become increasingly expensive and unmanageable for months. In this study, we aimed at evaluating microbial load data assessed by selected techniques with promising perspectives in space applications (i.e., HPC, ATP-metry, qPCR, flow cytometry), through the analysis of chlorinated and unchlorinated tap waters, groundwaters, river waters, and wastewaters. We identified and presented new alternative standards of water quality based on the assessment of the total microbial load. Our approach is suitable to provide an immediate alert of microbial load peaks, thus enhancing the crew responsiveness in case of unexpected events due to water contamination and treatment failure.

HIGH-RESOLUTION MICROBIOLOGICAL PROCESS MONITORING IN REAL-TIME WITH FULLY AUTOMATED ON-LINE FLOW CYTOMETRY

BESMER M.D.*, SCHIESSL K.*, HAMMES F.**

**) onCyt Microbiology AG, Zürich, Switzerland **) Drinking Water Microbiology Group, Eawag, Dübendorf, Switzerland*
michael.besmer@oncyt.com

Bacteria and other microorganisms are abundant in almost every production system featuring processes involving water and other liquids and in all natural ecosystems of course. They can represent potential operational or even hygienic risks in drinking water, ultrapure water, food and beverage production. At the same time, they are paramount for biotechnological production processes and natural ecosystem services. For all those examples it is crucial to closely monitor the number, composition, and viability of the microorganisms. This allows for better understanding and management of these organisms and their intended or undesired effects.

As most production/treatment systems of are typically subject to operational and/or accidental perturbations, it is absolutely critical to track microbial dynamics at short timescales (seconds to days) and not only with infrequent grab samples. This requires sampling and analysis at very short intervals and ideally in real-time allowing for immediate interpretation/reaction. This can only be achieved through full automation of in-situ sampling, sample processing, and detection. For decades this was impossible with conventional cultivation-based methods but also with advanced molecular methods, as they are still too labour intensive, time consuming, and costly for such applications.

One promising approach in recent years is the automation of flow cytometry. This detection method is now established and has specific advantages including rapidness, sensitivity, reproducibility, accuracy in quantification, differentiation of total and intact cells. By fully automating online/in-situ sampling and sample preparation, the advantages of flow cytometry can be fully exploited. The resulting high-resolution time series of cell numbers, distributions, and viability can be linked with operational data and other online sensors for comprehensive understanding and targeted management.

Given the ever-increasing role of microbiological processes and quality concerns, this technology is rapidly becoming a standard tool in fundamental and applied research but also various industries. Hence, here we present the scientific bases of the method that were established in the last six years.

RAPID & RELIABLE QUANTIFICATION OF PATHOGENS BY FLOW CYTOMETRY

BIEDERMAIER BJORN

Food- and waterborne diseases are of increasing concern for human health. Frequently these go unrecognised and the impact on human health and economy is underestimated. For example, the current incidence rate for Legionnaires' disease is understood to be underreported globally, by as much as eight- to ten-fold. Therefore, rqmicro developed a technology for the rapid and reliable detection of pathogens, such as *Legionella*. Currently, the ISO 11731 culture-dependent method is standard for the isolation and enumeration of *Legionella* from water samples. This approach is inaccurate, time-consuming and cannot detect viable but non-culturable (VBNC) bacteria, thus largely underestimating the number of *Legionella* present in water. We offer an automated, culture-independent immunomagnetic separation (IMS) method in a standalone device. Magnetic nanoparticles are conjugated to highly specific monoclonal antibodies, allowing for the separation of L. pneumophila from diverse aqueous matrices. A second set of highly specific antibodies is used for fluorescent labelling of *L. pneumophila*. Combining the automated IMS with flow cytometry, we are able to enumerate viable L. pneumophila cells, including VBNC bacteria. We successfully detect and quantify *L. pneumophila* in different water matrices and we will present data for the monitoring of disinfection processes. Furthermore, we want to give an outlook on additional assays to quantify *Salmonella* and *Escherichia coli*.

FLOW CYTOMETRY FINGERPRINTING AS MICROBIAL COMMUNITY INDICATORS IN AQUATIC ECOSYSTEMS

BOON NICO

A follow up of water quality is important in different areas, ranging from food and beverage production to waste water treatment. Conventional methods for the determination of water quality, like heterotrophic plate count and molecular techniques, are time consuming and prevent an immediate intervention in case of problems. Flow cytometry is explored as a fast methodology for investigating the microbial community of water, but the data interpretation is until now subjective. During the presentation, the development of a rapid objective method for the assessment of water quality will be discussed. This approach is using the physiological status of single cells within a microbial community using flow cytometry with tailored statistical tools. The method consists of two main parts, first the generation of fingerprint data by flow cytometric analysis and second the analysis of the data by the new statistical tool. The combined method was proven useful for the discrimination and classification of drinking water, detecting . Next to differentiation of microbial communities of different origin, the method was tested to detect changes within a community due to different environmental stress factors. This would allow us to use the method to detect quickly subtle differences in microbial community composition, which would be a reflection of the water quality. Generally, the method can be used as a fast fingerprinting method of microbial communities in aquatic samples. The system can give an indication of the microbial quality within one hour and can as such be used as an early warning system.

HIGH FREQUENCY FLOW CYTOMETRY FOR THE MONITORING OF MARINE MICROBES

CASOTTI RAFFAELLA

Stazione Zoologica Anton Dohrn, Naples, Italy

Marine microbes are highly dynamic and react very fast to variations in ecosystem properties so as to acute pollution events. Most classical methods require the collection of discrete samples at fixed stations and suffer from low reproducibility, long times for responses and high demand for labor. Flow cytometry offers a solution to these constraints, allowing fast, accurate and reproducible counts, and in fact, it has been widely used in marine ecology studies. Flow cytometry is an ataxonomic, single-cell-based method using scatter and fluorescence to discriminate and

count cell types. While photosynthetic microbes can be analyzed directly due to autofluorescence of photosynthetic pigments, non-autofluorescing microbes (such as heterotrophic bacteria) need to be stained before analysis. The staining step requires sample manipulation and incubation times that are needed for a proper assessment of cell concentrations.
Despite its advantages, conventional flow cytometry still requires discrete samples to be collected and, if instruments cannot be transported on board, these need to be fixed and analyzed later on.
Best suited for the analysis of picoplankton, sized 0.2 to 2 microns in size, developments of conventional flow cytometers are available to enlarge the dynamical range of particles analyzed and also to assess their complex morphology. In addition, automated staining modules allow the analysis of non-fluorescent bacteria. Such innovative instruments also allow the on-line connection to water inlet, so to allow a fast, automated and programmable sampling also while the vessel is sailing. Despite the limitation to one sampling depth, this feature allows a much higher spatial resolution and can also be applied to a fixed mooring (for submersible models) for high time resolution.
I will present some past and ongoing works using these conventional and new approaches to achieve a better definition of microbial communities at sea and to define their distribution as a function of water mass properties, also in the context of water quality.
Microbial communities, in turn, appear to be useful markers of changed environmental conditions, that need to be considered and included in water quality assessments such as those sought by the European Directive 2008/56/EC, which aims at reaching a Good Environmental Status (GES) for all European waters within 2020.

CHROMOSOME SORTING HELPS TO CLONE GENES, SEQUENCE GENOMES AND UNDERSTAND THEIR SPATIAL ORGANIZATION

DOLEŽEL JAROSLAV

Institute of Experimental Botany, Centre of the Region Haná for Biotechnological and Agricultural Research, Šlechtitelů 31, CZ-77900 Olomouc, Czech Republic;

dolezel@ueb.cas.cz

Sorting of mitotic chromosomes by flow cytometry is a powerful approach to dissect the full nuclear genome to its natural subunits to achieve a lossless complexity reduction and permit chromosome-centric approaches in genome analysis. Flow cytometry requires suspensions of intact mitotic metaphase chromosomes, which are most conveniently prepared from synchronized meristem root tip cells. To date, the method has been developed for more than thirty plant species. The DNA of flow-sorted chromosomes is intact and is suitable for a wide range of molecular techniques and sequencing technologies. Chromosome genomics facilitated rapid production of draft genome assemblies in important crops with complex genomes such as wheat, barley and rye, study molecular organization of specialized chromosomes such a B chromosomes and sex chromosomes, and was found invaluable to validate whole genome shotgun assemblies in a variety of species. Other important applications include the identification of chromosomes with integrated transgenes, characterization of alien chromatin in introgression lines and development of molecular markers from particular genome regions. This allows analyzing a chromosome of interest isolated from several different genotypes and brings a significant reduction of sequencing costs. Gene cloning has become one of the most important applications of chromosome genomics, recently. The targeted approach greatly streamlines the projects and reduces costs. To date, two chromosome-based gene cloning approaches, namely MutChromSeq and TACCA (targeted chromosome-based cloning via long-range assembly) have been developed and successfully used to clone important genes. A capacity to purify mitotic chromosomes and the recent progress in chromosome conformation capture techniques makes it possible to study spatial organization of DNA in condensed chromosomes. Together with the ability to sequence chromosome proteome this provides powerful approaches to understand the architecture of plant mitotic chromosomes.

APPLICATION OF FLOW CYTOMETRY IN THE ROUTINE CONTROL OF MILK FOR THE TOTAL BACTERIAL COUNT

FERRINI A.M.* , BOLZONI G.**

**LNR latte e prodotti a base di latte, ISS Roma*
*** Centro di referenza nazionale per la qualità del latte bovino, IZS LER, Brescia*

Regulation (EC) 1664 establishes that the reference method for determining the total bacterial count at 30°C in raw milk is EN ISO 4833, however it states that the use of alternative methods is acceptable when they are validated against the reference method in accordance with the protocol set out in EN/ISO 16140 or other similar internationally accepted protocols. In the case of milk, ISO 21187 and ISO 16297 are examples of these protocols.

Acccording to EN ISO 4833 colonies grown in defined conditions must be counted after 72 h of incubation at 30°C whereas flow cytometry instruments count directly free cells independently from their physiological status or their capability to develop into a colony, thus giving results in about 10 min. The counts obtained in impulses must be converted into CFU/mL equivalents, as this is the regulatory unit of measure. This conversion (when calculated by a single laboratory) is the main reason for the low reproducibility of the alternative method in spite of its otherwise better repeatability, rapidity and cost effectiveness compared to the reference method. The necessity to establish national conversion lines is discussed. Currently the flow-cell automatic instruments for total bacterial count are indispensable to the centralized and specialized laboratories in charge of large numbers of milk samples per day. The work performed to establish the conversion line in Italy and its implications are presented.

THE USE OF FLOW CYTOMETRY FOR STUDY OF POLYPLOIDY IN FRESHWATER FISH

FLAJŠHANS, M., LEBEDA, I., HUBÁLEK, M.

University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25 Vodňany, Czech Republic

Polyploidy in fish represents an important evolutionary mechanism which has contributed to the vast biodiversity in present fishes. This biological phenomenon, associated with the evolutionary plasticity of fish genomes, becomes even more evident from the simplicity of experimental induction of higher ploidy levels in fish by various methods, and from its successful, large-scale applications to breeding practice mostly for functional or complete sterility of triploids, their faster growth, balanced quality of market product, or for the desirable fertility of tetraploids. Flow cytometry and determination of relative- or absolute DNA content upon nuclear DNA staining mostly with 4',6-diamidino-2-phenylindole (DAPI) or propidium iodide (PI), represents quick and accurate method of ploidy determination, used globally since the 1980´s to evaluate the success of induced triploidy or tetraploidy in farmed teleosts (salmonids, cyprinids, catfishes, percids and cichlids) and in the study of spontaneous polyploids and diploid-polyploid complexes in various freshwater fish genera, e.g. *Cobitis, Misgurnus, Squalius* or *Carassius*. Sturgeons (Acipenseridae) which have evolved via several polyploidization and hybridization events, display tetraploid - octaploid – dodecaploid evolutionary relationships.They are still prone to interspecific or intergeneric hybridization either between species of the same ploidy level, or of differing ploidy levels and many of such resulting hybrids are fertile. Sturgeons are also prone to spontaneous polyploidization in each generation and similarly, many of such resulting polyploids are fertile. Both cases represent danger for biodiversity conservation efforts. For their rapid identification, flow cytometry can be a first choice tool, followed by microsatellite analysis or comparative genomic hybridization.

In general, flow cytometry allows determination of ploidy level in embryos, prelarvae and larvae, and in older individuals from somatic cells or spermatozoa. Best histograms with coefficient of variation (CV) 1.2 to 1.5% are usually obtained from native samples taken using minimally invasive- (blood cells by caudal vein punction; sperm by artificial reproduction) or non-invasive methods (epitelial cells and fibroblasts by fin clipping). Published methods of fish sample fixation are still rather scarce, sometimes hardly repeatable, often increasing CV over 5%, and sorely lacking standardization.

Results presented in this review were financially supported by the Ministry of Education, Youth and Sports of the Czech Republic - projects CENAKVA (LM2018099), Biodiversity (CZ.02.1.01/0.0/0.0/16_025/0007370) and by the Czech Science Foundation (project No. 18-09323S).

FUTURE FLOW: WHERE WILL CYTOMETRY TAKE US?

GALBRAITH D.W.*

**) School of Plant Sciences, Bio5 Institute, Arizona Cancer Center, Department of Biomedical Engineering, & Institute for the Environment, University of Arizona, Tucson AZ 85721 USA, and School of Biological Sciences, Henan University, Kaifeng, China.*

Flow cytometry and sorting is a mature field of instrumentation and associated methods that has emerged over the last 50 years. It has been commercialized with great success, and instruments permitting the highly multiplexed analysis of the optical properties of cells, and the isolation of subsets of cells having desired characteristics are found in innumerable research and clinical settings. Flow cytometry and sorting has been invaluable in characterizing and treating many notable human diseases, and has provided unique insights into the cell biological, physiological, and developmental properties of living organisms. In my presentation, I will outline some notable achievements in the area of cytometry, highlighting the aspects of the technologies that made these achievements possible. I will then go on to consider how cytometry is currently evolving, how it might be expected to advance in the future, and what future challenges we may face.

FISHIS IN FLOW: RECENT ADVANCES IN FLOW MOLECULAR CYTOGENTICS APPLICATIONS TO CEREAL SPECIES

GIORGI D.*, FARINA A.*, KUZMANOVIĆ L.**, SESTILI F.**, CEOLONI C.** LAFIANDRA D.**,LUCRETTI S.*

**) Department of Sustainability, ENEA Italian National Agency for New Technologies, Energy and Sustainable Economic Development. Via Anguillarese 301, 00123. Rome (Italy)*
***) DAFNE Department of Agriculture and Forest Sciences – University of Tuscia, via S. Camillo de Lellis snc.01100 Viterbo, (Italy)*

Debora.giorgi@enea.it

Polyploidy in fish represents an important evolutionary mechanism which has contributed to the vast biodiversity The combination of FISHIS, a method developed in our lab to fluorescently label nuclei and chromosomes in suspension, with flow cytometry, marks the birth of "Flow molecular cytogenetics". Standard Flow Cytogenetics relies on chromosome analysis and discrimination based on fluorescence emitted by DNA stained with a specific fluorocrome, directly correlated to DNA content and chromosome size. In most plant species analyzed so far, differences in the relative DNA content between chromosomes are too small to allow their discrimination. As a consequence, aneuploid lines, particularly telocentric lines, are to be used to identify and sort specific chromosome types. By contrast, FISHIS in flow, using repetitive sequences with chromosome-specific patterns as probes, allows an effective bivariate flow karyotype analysis, thus enhancing chromosome discrimination and sorting. Being independent of the use of special cytogenetic stocks, FISHIS in flow has widened the application of flow cytometry and chromosome sorting (flow cytogenetics) to any species for which chromosome suspensions and suitable probes can be obtained, including wild species, varieties or breeding lines of agronomic interest. We first applied FISHIS in flow to separate the A and B component genomes of durum wheat (Triticum durum), as well as the majority of individual chromosomes. Through the same approach, the isolation of all chromosomes of the wild grass Dasypyrum villosum was achieved. Recently, flow molecular cytogenetics has been used to characterize particular wheat-alien genetic stocks carrying useful agronomic and quality traits. These include a set of three durum wheat-Thinopyrum ponticum recombinant lines obtained through chromosome engineering and having 23-40% of their 7AL arm replaced by the alien 7el1L chromatin. For them, FISHIS with the repetitive GAA probe was used to isolate the recombinant chromosomes, in view of their sequencing, and to perform comparative genetic analysis of genes putatively involved in tillering and spike fertility. An additional line, a derivative of a secondary triticale crossed with bread wheat, was found to include a rearranged 1R rye chromosome determining a peculiar gluten composition. Such chromosome has been sorted and characterized by post-sorting GISH and by FISH with various probes.

SPERM FLOW CYTOMETRY - IMPACT OF ENVIRONMENTAL TOXICANTS ON SPERM HEALTH

LETER GIORGIO

Laboratory "Health & Environment", Division of Health Protection Technologies
ENEA-Italian National Agency for New Technologies, Energy and Sustainable Economic Development
Via Anguillarese, 301; 00123 Rome, Italy

Gioegio.leter@enea.it

Flow cytometry (FCM) has been extensively used to study mammalian sperm in the areas of reproductive toxicology (to monitor effects from environmental, occupational and therapeutic exposures), veterinary science (to preselect the gender of offspring by sorting X- and Y-chromosome-bearing sperm) and clinical andrology (to assess individual fertility potential). Using FCM, a variety of sperm features can now be rapidly measured on a cell-by-cell basis such as sperm count, viability, acrosomal integrity, mitochondrial function and DNA integrity; the last one is involved in postfertilization failure and embryo toxicity. It is foreseen that only a multiplex approach, which includes FCM assays together with the new genomics/proteomics methods, could increase the assessment of reproductive status and help to identify new biomarkers of susceptibility.

However, continuous follow-up of the methods is a necessity owing to technical developments and the complexity of mapping spermatozoa.

EARLY IN VITRO SCREENING OF PLANT MUTANTS BY FLOW CYTOMETRY: LOOKING AT PLANTS LIKE MICROORGANISMS

LUCRETTI S.* GIORGI D.*, FARINA A*, GROSSO V.*

**) SSPT BIOAG BIOTEC - Department of Sustainability, ENEA Italian National Agency for New Technologies, Energy and Sustainable Economic Development. Via Anguillarese 301, 00123. Rome (Italy)*

Totipotency in plants, or the demonstrated capability each plant cell has to generate a whole organism identical to the mother plant, is a well-known fact that makes them very suitable for cellular and molecular manipulations. And plant tissue can be handled in such a way to obtain protoplasts, or single plant cells in suspension removed of their cellulose cell walls, which very much resemble a "liquid tissue". Then, the definition of "liquid tissue" is the one used to define blood, which in turn is the main substrate for flow cytometry and cell sorting. So, in spite of their solid appearance, trees, vegetables and herbs can be analysed by Flow Cytometry, the main technology for single cell characterization, and their cells can be flow sorted as singles, just like blood cell. This analytical features goes well with the handling capabilities of standard flow cytometers, the ones made for analysing blood samples to exemplify easy, but flow sorters are best suitable to characterize small things also, such as bacteria or microalgae, since their analytical speed and accuracy allow to "see" and counting particle which can't be reliable analysed by microscope observations. Other manipulation techniques, such as chromosomes isolation in suspension, are now highly effective in providing particles in suspension "bacteria size like" which can be flow sorted with high precision and in appropriate numbers for any molecular manipulations, and FISHIS helps in it. From all of this, it appears clear Flow CytoMetry can be applied to discriminate different or "mutant" particles from the normal population, enabling the unique feature of true single cell, or particle, manipulation. But, as a matter of facts, the most diffuse and useful application of FCM still remains DNA content and ploidy evaluation, which discriminates well genomic mutants such as polyploids, partial endoploids and aneuploids, even using just chopped tissue for nuclear isolation in suspension. From 0.1 to 100 micron in size, standard FCM can discriminate mutants and provide an early screening method for identifying particle of interest, helping in setting up protocols and monitoring chemicals effects, and this is good to know.

FLOW CYTOMETRY TO MONITOR AND IMPROVE INDUSTRIAL PRODUCTION OF STARTER CULTURES AND PROBIOTICS

MORO M., PHD.

SACCO System s.r.l., Via A. Manzoni 29 Cadorago (CO)

Flow cytometry application to food microbiology, together with traditional approaches, can give complementary answers and a more accurate picture. By flow cytometry it is possible to assess plasma membrane integrity of the cells, antibiotic resistance, metabolic and enzymatic activity faster than with traditional method. However, the latter cannot be discarded as they provide relevant information about cell viability, morphology and eventual contaminations that are below the flow cytometer's detection limit. In addition, enumeration of bacteria by plate counting remains the gold standard method for bacterial cell viability.
In recent years, the application of flow cytometry in microbiology has transitioned to the industry. At SACCO we are setting up an integrated facility with the goals to i) develop methods apt for quality control, specifically for cell enumeration, and ii) in the future be able to employ this technology to assess assist production by monitoring cell viability, oxidative stress and metabolic activity to improve our production process.
The presentation will focus on some of the data collected by monitoring the stability of starter cultures and probiotics as well as results regarding the biology of these cells when subjected to different conditions.

APPLYING FLOW CYTOMETRY TO EVALUATE OXIDATIVE STRESS: NEW PERSPECTIVES

O'CONNOR J.E.*, HERRERA G.**, MARTINEZ-ROMERO A.*, JAVEGA B*, FELIPO-BENAVENT, M.*

**) Cytomics Laboratory, Joint Research Unit, Valencia University and Principe Felipe Research Center, Valencia, Spain. **) Cytometry Service, Joint Research Unit, Incliva Foundation and University of Valencia, Valencia, Spain*

Reactive oxygen- (ROS) and nitrogen- (RNS) species has been implicated in cellular senescence and aging, as well as in the onset and progression of genetic and acquired diseases and conditions including, inflammatory conditions, cardiovascular diseases and thrombosis, cancer and anticancer chemotherapy, HIV-progression, neurodegenerative diseases and metabolic disorders. However, ROS and RNS serve also important regulatory roles, mediated by intercellular and intracellular signaling and cell-function modifying processes involved both in the destruction of invading pathogens and in the fine tuning of cellular adaptation to endogenous and exogenous stress. Phagocytes use ROS and NOS as a powerful antimicrobial weapon and, in low concentrations, ROS and NOS serve also as second messengers of signal transduction.

Fluorescence-based analysis of oxidative stress and related processes using fluorogenic substrates (probes) is an important aplication of Flow Cytometry (FCM) and more than 4,000 papers have been published since 1989.
To ascertain the specific role of ROS and RNS in oxidative stress studies by cytomic methodologies, it is essential to detect and characterize these species accurately. Unfortunately, the detection and quantitation of individual intracellular ROS and RNS remains a challenge, but recent methodological advances improve the identification, intracellular localization, and mechanistic study of ROS and NOS, as well as their involvement in oxidative- and nitrosative stress. The new strategies that may be applied in the flow cytometric analysis have been made possible by developments leading to:

1. Probes with better specificity towards ROS and NOS.
2. Probes targetted to specific subcellular compartments.
3. Probes detecting oxidized or nitrosylated cellular components.
4. Probes allowing to asses the antioxidant capacity of cells.
5. Genetically-modified organisms with enhanced sensitivity to ROS and NOS (Biosensors)
6. Real-Time FCM kinetic assays to analyze ROS and NOS generation and interplay.
7. Molecular imaging of intracellular ROS and NOS based on Image-in-Flow Cytometry.

In this presentation we will describe and discuss briefly the advantages and limitations of such new approaches.

FLOW CYTOMETRY IN THE DAIRY SECTOR; THE ROLE OF ISO/IDF STANDARDIZATION

ORLANDINI S.*

**) Analytical Equivalence Organization Services (AEOS) Fiumicino (Italy) and International Committee for Animal Recording (ICAR) Rome (Italy)*

Sil.orlandini@outlook.it

Dedicated fluoropto-electronic instruments are used in the dairy sector to determine bacteria cand somatic cells in raw milk. These two parameters are crucial to fulfill the milk hygene law.
Somatic Cell Counting is one of the most analyzed parameters with, more than 500 million of samples analyzed in the world per year.
Somatic cell counting is relevant for milk payment, farm management and breeding programs. The analyses of a single cow milk sample or of bulk tank milk to determine somatic cells is performed using dedicated automatic instruments base on the flow cytometry principle. These instruments are projected to analyse raw milk samples without any sophisticated sample preparation to allow an high throughput of results.
Fluoro-optoelectronic instruments are extremely precise but their accuracy need to be checked with reference materials characterized with microscope method.
The metrological traceability for these parameter is going to built up. This latency is due to the poor precision of microscope method and its rare application.
This presentation will illustrate the standardization processes and efforts of the organizations involved to promote novel projects to fill the gap of metrological traceability.
International Dairy Federation (IDF) and International Committee for Animal recording (ICAR) are coordinating a project Reference System for Somatic Cells Counting (RSSCC). ISO TC 276 Biotecnology is developing new standards for innovative techniques to count somatic cells.
Antoinette project has the aim to validate a method to count somatic cell in raw milk with classical cytometers. Michprüfring Bayern (DE) ed il Qlip (NL) are co-founders of MIAMi (Milk Image Analyses in Milk) project that has the aim to improve the microscope method with modern technologies.

A CYTOFLUORIMETRIC ANALYSIS OF A *S. CEREVISIAE* POPULATION CULTURED IN A FED-BATCH BIOREACTOR

PALOMBA EMANUELA[1], TIRELLI VALENTINA[2], CARTENÌ FABRIZIO[3], DE ALTERIIS ELISABETTA[4], SANCHEZ MASSIMO[2]

[1] RIMAR Dep., Stazione Zoologica "Anton Dohrn", Naples (Italy)

[2] ISS Core Facilities-Cytometry Unit, Rome, Italy

[3] Dep. of Agricultural Sciences, University of Naples "Federico II", Naples, Italy

[4] Dep. of Biology, University of Naples "Federico II", Naples, Italy

The yeast *Saccharomyces cerevisiae* is a reference model for biological system and is one of the widely used microorganisms in biotechnological processes. The importance of S. cerevisiae in food fermentations and other industrial applications led to the improvement of experimental strategies to optimize the biomass yield and the development of mathematical models able to describe and predict its performance. Combined strategies aim to maximize biomass/product yield and avoid by-product accumulation affecting the maximum achieved cell density . Another experimental methodology able to analyze the status of a microbial cell population is the flow cytometry (FCM) now used for diverse applications in food microbiology. In particular, it allows the relative quantification of cells in each cell cycle stage in different points of a microbial population growth curve . Here we propose an FCM application in the analysis of yeast cell cycle using the two currently recommended fluorochromes: SYTOX Green and SYBR Green, with the aim to determine the relative proportion of cells for each cell cycle stage in different times of an of S. cerevisiae fed-batch culture.

References

1. Mazzoleni S, Landi C, Carteni F, de Alteriis E, Giannino F, Paciello L, Parascandola P: **A novel process-based model of microbial growth: selfinhibition in Saccharomyces cerevisiae aerobic fed-batch cultures.** *Microbial Cell Factories* 2015, **14**:109.

2. Delobel P, Tesniere C: **A simple FCM method to avoid misinterpretation in Saccharomyces cerevisiae cell cycle assessment between G0 and sub-G1.** *PloS one* 2014, **9**(1):e84645.

MAN'S BEST FRIENDS AND FLOW CYTOMETRY: DOGS IN THE FCM LAB

RIONDATO F.

Department of Veterinay Science, University of Torino, Largo Braccini 2, 10095 Grugliasco (TO) (Italy)

The main application for diagnostic purposes of flow cytometry in veterinary medicine is in oncohematology of the dog. Most analyzes are performed on lymph node aspirates, peripheral blood and bone marrow in cases of suspected lymphoma or leukemia. Flow cytometric analysis is used to reach a diagnosis of neoplasia, to define the neoplastic cell line and to stage the disease. Flow cytometry provides useful information, together with cytology, for the classification according to the updated Kiel scheme and in specific cases allows the diagnosis of WHO entities (T-zone lymphoma). In more recent years, his role in the prognostic and MRD evaluation is also emerging. In addition to the determination of the immunophenotype, an important role is played by the determination of the proliferative activity (Ki67 and phase S) and of the MFI of some markers (eg MHC-II). A second important use of flow cytometry in the clinical setting is for the diagnosis of immune-mediated anemias. The method in use is highly reliable and represents an important resource for clinicians. T he same method is available for the diagnosis of immune-mediated thrombocytopenia but is affected by poor sensitivity and specificity and it has to be improved. Still in the field of oncology, flow cytometric approaches have recently been described for the diagnosis and staging of mast cell tumour (one of the most frequent neoplasms of the dog) and for the differential diagnosis of effusion (lymphoma, leukemia, carcinoma, mesothelioma, sarcoma).

MULTI-CLASS AND MULTI-RESIDUE SCREENING OF ANTIBIOTICS BY FLOW CYTOMETRY IMMUNOASSAY

SUAREZ-PANTALEON C., GALLO A., WALLEMACQ H., GRANIER B.

UNISENSOR SA

celia.suarez@unisensor.be

Rapid screening analytical methods for the detection of antibiotic residues in the food chain are essential in order to guarantee consumer protection and industrial food transformation processes. Current tendency in food analysis is the implementation of multi-residue technologies which allow simultaneous monitoring of several analytes per sample (multiplexing); thus considerably reducing analysis time and cost. Flow Cytometric Immunoassays (FCIAs) combine the detection of receptor-ligand interactions by immunoanalysis, with the multi-parametric characterization of individually encoded beads provided by Flow Cytometry.

BeadyplexTM is a suspension-based FCIA assay for the simultaneous analysis of more than 80 antibiotic residues from 10 families widely used in the veterinary field, including aminoglycosides, β-lactams, tetracyclines, macrolides, lincosamides, phenicols, fluoroquinolones, sulfonamides, polymyxins and pleuromutilins. Each antibiotic family is associated to a particular population of microspheres, which are classified according to their size and internal fluorescence intensity, thus allowing the specific identification of antibiotics from each family in one single test. This assay can be used for the screening of antibiotics in multiple matrices, including porcine, bovine and poultry muscle, high and low-fat content fish muscle, and milk. Furthermore, this flexible test can be potentially used for other matrices of interest, such as egg, liver, kidney or honey, by adapting the extraction procedure.

The validated assay is particularly appropriate for routine analyses, given the ease of use and the high throughput capability. The performance of the method shows Detection Capabilities (CCβ) for most of the antibiotics within the scope at or below European regulatory limits. This test kit BeadyplexTM allows the rapid detection of antibiotics in different food commodities, and their family identification in one single screening analysis. It is therefore highly recommended for the specific selection of the appropriate confirmation method.

COUNTING CELLS IN WATER FILTERS

VIGNOLA MARTA[a,b], WERNER DAVID[a], HAMMES FREDERIK [c] , and DAVENPORT RUSSELL J.[a]

a) *School of Civil Engineering and Geosciences, Newcastle University, Newcastle upon Tyne, NE1 7RU, United Kingdom.* b) *College of Science and Engineering, Division of Infrastructure and Environment, School of Engineering, University of Glasgow, Glasgow G12 8QQ, U.K* c) *Eawag, Swiss Federal Institute of Aquatic Science and Technology, Überlandstr. 133, CH-8600 Dübendorf, Switzerland*

Sand filtration is a crucial stage in modern drinking water treatment plants. In addition to removing particles from the water stream, sand particles are an excellent support for the development of biofilms; indeed, complex microbial communities have been found populating these systems. However, the quantification of cell abundances in these systems is still an under-appreciated element, essential for assessing filter performance.

In this study we have developed a protocol optimised for the quantification of cells present on the surface grains of sand from water biofilter, using flow cytometry (FCM). Cell detachment from sand particles was optimised testing five different chemical dispersants (NaCl, Triton-X100, CaCl2, sodium pyrophosphate (PP), Tween80 combined with PP); different mechanical pre-treatments (Low and High energy sonication and shaking) and two fixation methods (Glutaraldehyde and Ethanol). The protocol was then validated against other commonly employed methods for biomass quantification in water filter samples (adenosine triphosphate (ATP) quantification, real-time quantitative PCR (qPCR) and volatile solids (VS)). The combination of a surfactant (Tween-80) with an ionic dispersant (sodium pyrophosphate) (TWEEN-PP) produced the best conditions for the detachment of biofilm and their dispersal into single cells. While, four cycles of high-energy sonication in a TWEEN-PP solution was found as the most effective treatment for optimal cell counts. Two common fixation solutions were evaluated for their ability to preserve and protect microbial cells during the protocol proposed (glutaraldehyde and a solution of ethanol:PBS 50%). The results strongly suggested that the fixation methods could affect the extraction performance and the number of both intact and total microbial cells recovered by the treatment. Glutaraldehyde was shown to be the best fixative solution. The protocol developed appeared to be a reliable method for measuring microbial cells in sand samples from water filters. The cell abundances measured with the protocol correlated with the values obtained with other methods for biomass quantification. High correlations were found with counts obtained with ATP and qPCR ($\rho = 0.98$ and $\rho = 0.91$). The VS content was confirmed as an inaccurate method to express biomass in sand samples since it poorly correlated with all the other three methods ($\rho = 0.005$ with FCM, 0.002 with ATP and 0.177 with qPCR). FCM and ATP showed the strongest agreement with a slope of the correlation equal to 0.7, while qPCR seemed to overestimate cell counts. The method proposed could be applied for routine quantification and study of microbial communities in water biofilters and help in assessing their performance unrevealing the ecological mechanisms occurring in such systems.

AMALFITANO STEFANO

PhD in Evolutionary Biology and Ecology and reference person of the Laboratory of Cytometry and Microscopy of the Water Research Institute (IRSA-CNR), I conduct researches in the field of Aquatic Microbial Ecology for the determination of the role of microbial communities in the circulation of carbon and nutrients in aquatic environments. These studies find fundamental applications for the management of water resources. I authored >50 manuscripts in highly ranked international journals (H-index 22; Google Scholar) and currently handle papers as associate editors of the journal Hydrobiologia (Springer-Nature ISSN: 0018-8158).

ARIOLI STEFANIA

Department of Food, Environmental and Nutritional Sciences (DeFENS)
University of Milan
Via Mangiagalli 25, 20133, Milan, Italy
Email address: stefania.arioli@unimi.it

Researcher on fixed-term contract (B) (since 2017)
Work Experience

2016-2017 Post-doc (type B)
University of Milan, DeFENS. Research project: "Study on probiotic properties of Lactobacillus aracasei strains", supervisor Prof Simone Guglielmetti.

2014-2015 Post-doc (type B)
University of Parma. Research project: "Antibiotic resistance transmission mediated by viral populations", supervisor Prof Marco Ventura.

2009-2014 Post-doc (type A)
University of Milan, DeFENS. Research project: "Clinical relevance of antibiotic resistance mediated by bicides in food microrganisms", supervisor Prof Diego Mora.

Dr Arioli is author/co-author of 56 publications (ORCID ID http://orcid.org/0000-0002-5406-8268), focused on i) metabolism and physiology of lactic acid bacteria (LAB), ii) antibiotic resistance in food-associated bacteria, iii) selection and characterization of new probiotic strains, and iv) development of flow cytometry-based applications for the enumeration and the evaluation of the physiology of LAB and probiotics.

BARCACCIA GIANNI

Full Professor of Plant Genetics, School of Agriculture Sciences and Veterinary Medicine, University of Padova, Italy (www.unipd.it) and Adjunct Professor of Plant Breeding, University of Georgia, Athens, USA (www.uga.edu)
Head of the Laboratory of Genomics for Plant Breeding (www.giannibarcaccia.com)
Director of the Department of Agronomy, Food, Natural resources, Animals and Environment – DAFNAE
University of Padova, Italy (www.dafnae.unipd.it)

Education

1991 – M.Sc. in Agricultural Science with Magna cum Laude, Plant Breeding Institute, Faculty of Agriculture, University of Perugia, Italy
1992 – 1995 Ph.D. in Crop Science, Genetics of Plant Reproductive Systems, Faculty of Agriculture, University of Padova, Italy
1996 – 1997 Post Doctoral Fellow in Plant Population Genetics, University of Perugia, Italy

Teaching Activities

2001 – present: Professor of Plant Genetics, also in charge of Applied Genomics, Genetic Traceability of Food Products and Plant Breeding, School of Agriculture Science and Veterinary Medicine, University of Padova (www.agrariamedicinaveterinaria.unipd.it/en)

Scientific Publications
Author of more than 100 peer-reviewed articles (101 and 106 records are available in both the Web of Science and Scopus databases, respectively, and 56 records in the NCBI PubMed database) of which 70% as first or last author or corresponding author (www.researchgate.net/profile/Gianni_Barcaccia/publications).
Books and Book Chapters: 3 academic books (1st and 2nd editions) and 8 book chapters.
Scientific publications in Italian journals: 25 articles on national journals and 2 monographs.

Research Impact Metrics
Citations and Scientific production statistics: about 2.000 citations with H-index=26 in Scopus (35 in Google Scholar and 37 in Research Gate), total IF of publications: 225

Selected Patents
Discovery and analysis of nuclear male sterility in leaf chicory (Cichorium intybus L). International Patent Application PCT/EP2011/058765 and European Patent EP2713705-B1 (https://encrypted.google.com/patents/EP2713705B1)

Last Publications
Several original research articles and reviews were recently published in a Research Topic of Frontiers of Plant Science (www.frontiersin.org/research-topics/8183/genetics-and-genomics-of-plant-reproduction-for-crop-breeding), including Galla et al. (2019) Ovule gene expression analysis in sexual and aposporous apomictic Hypericum perforatum L. (Hypericaceae) accessions (doi: 10.3389/fpls.2019.00654), Alagna et al. (2019) The Paradox of self-fertile varieties in the context of self-incompatible genotypes in olive (doi: 10.3389/fpls.2019.00725), Palumbo et al. (2019) Genomics of flower organ identity in grapevine (Vitis vinifera L.) (doi: org/10.3389/fpls.2019.00316), Palumbo et al. (2019) Construction of the first SNP-based linkage map using genotyping-by-sequencing and mapping of the male-sterility gene in leaf chicory. (doi.org/10.3389/fpls.2019.00276

BESMER MICHAEL

Marchwartstrasse 61
8038 Zürich - Switzerland
Date of Birth: 28 October 1986
Swiss citizen
Phone: +41 43 243 18 50
Mobile: +41 79 718 28 76
E-Mail: michael.besmer@gmail.com

WORK EXPERIENCE
Start-up/Spin-off – onCyt Microbiology from 03.2017
Co-founder and CEO
Engineering Company - CSD Ingenieure Zürich 09.2011 – 12.2011
Internship as team assistant
Electric Utility – Elektrizitätswerk der Stadt Zürich (ewz) 09.2005 – 12.2005
Team assistant in division network services

EDUCATION
PhD in Drinking Water Microbiology 05.2013 – 09.2016
ETH Zürich, Department of Environmental System Sciences
Eawag Dübendorf, Department of Environmental Microbiology
Master of Science ETH in Environmental Sciences 09.2010 – 12.2012
ETH Zürich, Department of Environmental System Sciences
Eawag Dübendorf, Department of Environmental Microbiology
Bachelor of Science ETH in Environmental Sciences 09.2007 – 09.2010
ETH Zürich, Department of Environmental System Sciences

AWARDS
ETH Medal 11.2014
ETH medal for the best master thesis in the department of Environmental System Sciences
Otto Jaag Water Protection Prize 2017 11.2017
Otto Jaag Water Protection Prize for the best PhD in aquatic sciences

BEVIVINO ANNAMARIA

Annamaria Bevivino is the Head of the ENEA Laboratory for AgriFood Sustainability, Quality and Safety, Biotechnologies and Agriculture Division, Department for Sustainability, Rome (ITALY) and Professor of AgroFood Microbiology, Master's Degree in Food Science and Human Nutrition, University Campus Bio-Medico di Roma. Her research activity, performed in the sector of Agro-Food, Environmental and Clinical Microbiology, has been mostly focused on the study of microbial diversity and population structure, microbial population (food, soil and human microbiome), polymicrobial interactions, microbial biofilm, bacterial-host interaction, plant-microbe interactions, plant-growth promoting microbial consortia. She is author of more than 50 peer-reviewed publications and 150 communications at national and international conferences (as invited and/or selected speaker, posters). She is a member of several scientific societies [SIMGBM (Italian Society of General Microbiology and Microbial Biotechnology), Federation of European Microbiological societies (FEMS), SIMTREA (Italian Society of Agro-Food and Environmental Microbiology) and Italian Society of Cystic Fibrosis] and Academic Editor for PlosOne and Frontiers in Microbiology. She actually is WP leader of SIMBA project "Sustainable innovation of microbiome applications in food system", funded by European Union's Horizon 2020 research and innovation programme under grant agreement No. 818431. Scopus: ID: 6602516452 Citations: 1608. h index: 22. Google scholar– Citations 2607; H index= 28; i-10-index: 38.

BIEDERMANN BJÖRN

Björn Biedermann studied biology at the University of Würzburg and the Welcome Trust Biocenter in Dundee (Scotland). He earned his doctorate in the field of stem cell research at the Friedrich Miescher Institute. In 2010, he joined Thermo Fisher Scientific, where he was responsible as a product specialist and later as a sales manager for the cellular analysis product portfolio in Central Europe. In 2017 he joined rqmicro, where he is in charge of sales and marketing.

BOON NICO

Nico Boon is a Full Professor at the Center of Microbial Ecology and Technology (Ghent University) and leading the Microbial Community Engineering group. Since 1998 his research is focused on the applied microbial ecology of managed and engineered ecosystems. The areas of interests have been the development of (molecular) methods for the qualitative and quantitative description of microbial communities. During the last years, the research interests are focussed on the development of new microbial ecological theories to link the microbial community structure to functionality.
Nico Boon is currently project promoter of a major national GOA project "Collaboromics: identifying and engineering core and satellite populations in (synthetic) microbial ecosystems". He is also involved in the ELECTRA project, an EU-China RTD joint initiative (2019-2022). He is promoter or co-promoter of several national projects (FWO, Baeckeland, …) dealing with the microbial ecology of different environments.
Previously, he was coordinator of the most important Belgian research program "Interuniversity Attraction Poles (IAP) Phase VII" of the Belgian Federal Science Policy Office (2012-2017) and coordinator of the FP7 ManureEcoMine. During his career, he participated as partner or WP leader in more than 10 EU projects.
His research has resulted in more than 430 accepted/published international publications in journals with peer review. In 2018 and 2019 he was taken up in the list of ISI Highly Cited Researchers. They have been cited more then 20000 times with a h-index of 73. He has also seven patents. More than 30 international conferences have been attended, and the research results have been presented more then 80 national and international oral presentations. He was awarded the Prometheus Award for Research at Ghent University (2014) and he was laureate of the Royal Flemish Academy of Belgium for Science and the Arts (2013). Nico Boon was supervisor of 51 finished Ph.D.'s and is currently supervising 10 Ph.D.-students in microbial ecology and environmental engineering, and he has supervised more than 130 master students at Ghent University. He had within his group 15 postdocs at a junior or senior level. He coordinates several courses at the Bachelors and Masters level: Microbial Ecological Processes and Environmental Microbiology.

CASOTTI RAFFAELLA

PhD in Environmental Sciences, I am Senior Scientist at Stazione Zoologica Anton Dohrn, Italy. My research interests focus on marine microbial diversity, community structure and interactions using several tools, including High Throughput Sequencing and conventional flow cytometry. I am also working on technological improvements of flow cytometry for the monitoring of marine plankton. I have used flow cytometry on several sampling cruises on board research vessels and I am involved in an augmented observatory initiative in the Gulf of Naples (NEREA) which aims to integrate traditional "omics" approaches and innovative technologies like high frequency scanning flow cytometry to end-to-end investigations from virus to mammals. I have been Visiting Investigator at the Woods Hole Oceanographic Institution (USA) and the Station Biologique de Roscoff (France) and I currently collaborate with several european and oversea institutions. Full CV available at www.szn.it.

DI BERARDINO MARCO

Marco received his PhD in Natural Sciences at the Swiss Federal Institute of Technology Zurich (ETH Zurich) in 1996. After his postdoctoral fellowship at F. Hoffmann-La Roche he worked as project manager and business developer in two start-up companies. During that time he obtained his bachelor degree in business administration at the GSBA Zurich. At Axetris AG, a company of the Leister Group, Marco led the development of the Impedance Flow Cytometer from the feasibility to the prototype and raised the awareness for this technology to the flow cytometry community through numerous publications and presentations. Marco is CTO and Chairman of the Board of Amphasys, a company that he co-founded in 2012 as a spin-off from Axetris AG.

DOLEŽEL JAROSLAV

Jaroslav Doležel is Head of the Centre of Plant Structural and Functional Genomics of the Institute of Experimental Botany in Olomouc (Czech Republic), Research Director of the Centre of the Region Haná for Biotechnological and Agricultural Research in Olomouc, and Professor of Molecular Biology and Genetics at Palacký University in Olomouc. His research focuses on plant genome structure, function and evolution. He has been developing flow cytometric methods to analyze, map and sequence nuclear genomes of economically important crops. In particular, he has pioneered chromosome genomics to facilitate the analysis of complex and polyploid genomes, including hexaploid bread wheat. Doležel published over 350 papers in scientific journals, which have been frequently cited, edited three books and was principal investigator and co-investigator in more than thirty national and international research projects. He is a member of the Learned Society of the Czech Republic and has received several prestigious awards for his scientific achievements including the Award of the Minister of Education Youth and Sports of the Czech Republic for the contribution to science and research, and the National prize of the Czech government „Česká hlava" (Czech Mind) - the highest scientific award in the Czech Republic awarded each year to one scientist.

FERRINI ANNA MARIA

Microbiological food safety and food-borne diseases Unit
Food safety, Nutrition and Veterinary Public Health Department
Istituto Superiore di Sanità- Roma

Education

Degree in Biological Sciences
Post-Degree in Food and Nutrition Science

Position

Senior researcher. Responsible of the Italian National Reference Laboratory for milk and milk product

Activities

Study of the conversion factors for raw milk in Italy; organization of PT on different topics in milk (alkaline phosphatase, residues of veterinary antibiotics); Development of new microbiological methods for the research and identification of antibiotics in milk and meat; Antibiotic resistance: Surveillance of antibiotic resistance in food isolates; Natural antimicrobials: Antimicrobial activity of some essential oils on food pathogens and their possible applications in food preservation; Antibiotic resistance markers: Study of the fate of antibiotic resistance markers in GMO when introduced by diet.

International working groups
WG on harmonization of conversion factors for total flora of raw milk of EUR MMP; FIL/IDF Member of the Standing Committe on Analytical Methods for additives and contaminants ; FIL/IDF Member of the Standing Committe on Analytical Methods for mastitis; Italian NRL group for screening methods for the research of antibiotics in food by screening detection method.
Publications
Author and coauthor of more than 70 publications on national and internation papers and about 70 congress comunications.

FLAJŠHANS MARTIN

Born in 1964 in Prague, Czechoslovakia.

• Master of Science (Dipl.-Ing.) at the University of Agriculture, Faculty of Agronomy, Prague in 1986. Specialization: Animal breeding.

• Doctor of agricultural sciences (Dr. rer. agr.) in 2006 after external doctoral study at the Faculty of Agriculture and Horticulture, Humboldt University in Berlin, Germany. Specialization: Fisheries and freshwater management.

• Associate Professor in 2008 at the University of South Bohemia in České Budějovice, Faculty of Agriculture. Specialization: Special animal breeding.

• Full Professor of Fisheries in 2014 upon proceedings at the University of South Bohemia in České Budějovice.

Employed in Research Institute of Fish Culture and Hydrobiology in Vodňany since 1986 till present. Worked consequently as research fellow, research scientist and since 2005 as head of Department of fish genetics and breeding. In 2009, the institute became an establishing part of newly founded Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice. Since 2009, head of Laboratory of molecular, cellular and quantitative genetics.
Altogether 101 publications on WOS (Core Collection); h-index 21; 1 334 times cited without self-citations from WOS; 15.78 average citations per item from WOS.

Professional interests (1986 – 2019):
Cytogenetics and chromosomal manipulations (induced polyploidy, gynogenesis, androgenesis) in freshwater fish species (cyprinids, silurids, salmonids, sturgeons).
Evolutionary, spontaneous and induced polyploidy in fishes, problems of allopolyploidization and autopolyploidization in sturgeons. Biology and physiology of polyploid freshwater fish species. Flow cytometry and computer-assisted image cytometry of polyploid fish cells.
Interspecific hybridization of sturgeons, their fitness and fitness-related traits. Gynogenesis in sturgeons and development of female stocks for caviar production.
Breeding programme for common carp, tench, European catfish. Conservation of fish genetic resources.
Pilot R & D projects on use of polyploid fish in freshwater aquaculture of SMEs.

FORMARO JORGE

Research Flow Cytometry Applications Support – South Europe

Beckman Coulter LS – December 2012 - presente
Customer Applications Support Sorters MoFlo Astrios, MoFlo XDP, Research Analyzers Cytoflex Platform
Life Sciences Sales Dept.

Instrumentation Laboratory – September 1992 – December 2012
Customer Support analyzers EPICS XL, FC500, Gallios, Navios and cell sorters Altra Hyper Sort, MoFlo Legacy, Cytopeia InFlux, MoFlo XDP, MoFlo Astrios.
Customer trainer.

Coulter Italia - August 1990 – August 1992
Cytometry Application Specialist
Customer support for cell sorters EPiCS C, EPICS 540 series, EPICS 750 series, Elite, Altra and the analysers Profile I, Profile II and XL System 2.
Customer trainer.

Coulter Scientific – November 1985 – July 1992
Trainer of service engineers
Service Engineer Flow Cytometry
Technical Support for the dealer Kontron Instrument with all cytometers : EPICS C, 540 series and 750 series. Experience and service lasers Coherent Innova 90-5 and Innova 60.

GALBRAITH DAVID W.

Dr. Galbraith is a Professor in the School of Plant Sciences, and is a member of the Bio5 Institute, and the Arizona Cancer Center. He has an adjunct membership in the Department of Biomedical Engineering, and is an Associate of the Institute for the Environment.
His research aims at the development of novel bioinstrumentation and methods for the analysis of cells. He is recognized world-wide as a pioneer in the area of plant flow cytometry and cell sorting, and was recently elected Secretary of the International Society for Advancement of Cytometry, the leading scientific society in this discipline. Notable firsts from his laboratory include: (a) methods for analysis of nuclear DNA contents in plants, (b) work defining a gene expression map of the arabidopsis root, (c) identification of endoreduplication in arabidopsis, (d) successful, high-level expression of the Green Fluorescent Protein in plants, and (e) methods for using single nuclei for characterization of gene expression within specific cell types of plants and animals.
He was elected a Fellow of AAAS in 2002. His >180 publications include 3 in Science, 1 each in Nature, Nature Biotechnology, Nature Methods, and Nature Nanotechnology, 3 in PNAS, and 3 in Plant Cell.
Google Scholar lists over 16,000 citations of his publications (h-index = 63).

GIORGI DEBORA

Current position:
Permanent researcher at ENEA, Department of Sustainability, Laboratory of Biotechnologies (BIOTEC).

Main Activities
Her activity deals with molecular genetics and cytogenetics of plant species of agronomic interest belonging mainly to the Poaceae family. She has experience in molecular biology and cytogenetic techniques, such as FISH and GISH, applied to the characterization of wild and cultivated plant species and to interspecific hybrids and recombinant lines mainly between wheat and its wild relatives. In recent years she has became a member of the Italian Society of Cytometry (GIC) and has developed skills in cytometry and flow cytogenetics, developing new tools and resources for plant genomics by genome and chromosome flow sorting. To this aim, she had an important role in developing a new method of FISH labelling of nuclei and chromosome in suspension (named FISHIS), which widen the application of flow cytogenetics in plant, making this approach independent by the use of special cytogenetic stocks. In this frame, Debora Giorgi has been involved in the "International Wheat Genome Sequencing Consortium" efforts, providing pure DNA from 5AL and 5AS wheat chromosome arms. Actually she is involved in an international project named IMPRESA relating to the PRIMA (Partnership for Research and Innovation in the Mediterranean Area) call.
She published several refereed papers in international journals and participated to numerous International Congress with posters and oral presentations. Finally she carried out teaching activities both in the frame of theoretical and practical courses organized by the Italian Society of Cytometry (GIC) and through the preparation of seminars, relating with the applications of Flow Cytometry in plant, in the contest of Genetics and Genomics course of the Faculty of Agriculture, University of Tuscia in Viterbo.

GUZZON RAFFAELE

After graduating in Food Technology at the University of Parma (Italy), he earned the PhD at the University of Trento, with a work about the application of immobilized microbial cultures of lactic bacteria at the oenological fermentation. He works since 2003 at the Edmund Mach Foundation, a research center based in the North of Italy, and currently he coordinates the local food microbiological laboratory, teaching in the local University in the degree course of "Viticulture and Enology". He performs consulting in the fields of microbiology and winemaking in some Italian wineries.
The main fields of scientific activities concern the study of evolution of microbial ecosystems during the winemaking and the impact of oenological practices on them. He carries out experiments to

optimize the winemaking protocols by the use of environmentally-friendly technologies. Winner in 2009 of the Award of the Italian Society of Viticulture and Enology "Italian Research for Development", was a finalist in all subsequent editions. He is the author of numerous publications on scientific and technical journals

LETER GIORGIO

Birthdate & Birthplace: October 1th 1955 - Rome, Italy

Address:
Laboratory "Health & Environment"
Division of Health Protection Technologies
ENEA-Italian National Agency for New Technologies, Energy and Sustainable Economic Development
Via Anguillarese, 301 00123 Rome, Italy
Tel. +39 06 30484201; email: giorgio.leter@enea.it

Dr. Giorgio Leter got his degree in Natural Science at the University of Rome "La Sapienza". In 1979 has been employed by Italian National Committee for Nuclear Energy CNEN. After a previous career in studing nuclear fuel thermo-mechanical behaviors at "Plutonio Laboratory" in Casaccia Research Center he joined in, 1990, the radiation biology group in the Reproductive Toxicology laboratory of the Section of Toxicology and Biomedical Sciences. GL applied the analytical cytology methods, and in particular, flow cytometry and microscopy, on the investigation the physicals and chemicals agent effects on male reproductive system after environmental and occupational pollutants exposure of human populations and on experimental model animals expecially exposed to selected chemical compounds (i.e., endocrine disrupting chemical, EDC) and physical agents (i.e., ionized radiation).
Recently he is involved in sperm epigenetic research field focussed on the development of a flow cytometric methods for assessing global sperm DNA methylation level. New topics in nanotoxicology field is presently studying the in vitro cytotoxicity of nanoparticles. He participated at several national and international funded toxicological research project focussed on reproductive health. He has been author of scientific papers, book chapters and serving as ad-hoc referee for several journals in the field of reproduction science. GL was invited speaker in several congresses and courses in the field of cytometry.

LUCRETTI SERGIO

Sergio Lucretti is permanent staff member at ENEA and research director at the Flownomics group on plant biotech of agronomically important crops. His research is related mainly to cell cycle studies and to analyze, characterize and manipulate genetic variability in plants by means of flow cytometry and flow sorting techniques. Most of his work has been dedicated to protoplast flow sorting and manipulation, and exploring first chromosome flow cytogenetics, developing new molecular cytogenetic tools such as the FISHIS technique. He is author of more than 80 papers on scientific journals and book chapters; holds two patents on new flow cytometry related methods. He is member of Italian and international scientific society and has been involved as investigator and PI in several national and EEC research projects.

MORO MONICA

I am presently Flow Cytometry Manager in the R&D department of SACCO s.r.l.
I am in charge of setting up all the required protocols to support QC, Production and Research departments in order to enumerate, evaluate ad study the starter cultures and probiotics produced in our company. In the last 20 years I developed significant skills in flow cytometry working in different fields such as cancer immunotherapy, regenerative medicine, autoimmune and inflammatory diseases and microvescicles.

E-mail: m.moro@saccosrl.it
mobile: +39 338 3543235

MÜLLER SUSANN

Helmholtzzentrum für Umweltforschung
Department Umweltmikrobiologie
AG Flow Cytometry
D-04318 Leipzig
Phone: +49 341 235 1318
Email: susann.mueller@ufz.de
Present :Senior Scientist (Group leader) for Microbial Flow Cytometry at UFZ, Leipzig

Activities in the scientific community
President of the German Society for Cytometry (DGFZ, 2008-2010)
Associate Editor for Microbiology for Cytometry A (since 2007)
Member Educational Com (2011-13) & ICSP (2013-15) of ISAC
Bord member Local organising Committee (ISME 2018)

Research Fields
- Single Cell Analytics
- Cytomics and other Omics Technologies
- Biotechnology
- Microbial Ecology
- Microbiomes

Summary of scientific publications
ISI cited publications: 79, cites: 1420,
h-index 21, ResearcherID: K-1293-2013
More than 20 other publications (book articles and editorials, Guest Editor of 4 Journal Issues (Advances in Biochemical Engineering and Biotechnology, Cytometry Part A, Current Opinion Biotechnology and Microbiology)

O'CONNOR JOSÉ-ENRIQUE

José-Enrique O'Connor, Ph.D. is Full Professor of Biochemistry and Molecular Biology at the University of Valencia (Spain), and Director of the University Master in Molecular Approaches to Health Sciences. His career in flow cytometry started in 1983, as responsible of the first cytometry unit installed in the Iberian Peninsula. Currently, he is the Director of the Laboratory of Cytomics, a Joint Research Unit of the University of Valencia and Principe Felipe Research Center. His research focuses mostly on in vitro cell-based strategies for predicting acute and chronic drug toxicity to humans. He is Past-President of the Iberian Society of Cytometry and member of the Education and Accreditation Committee of the European Society for Clinical Cell Analysis (ESCCA). He is deeply involved in education and training in cytometry, including e-learning courses and the organization of the ESCCA International Summer School on Cytometry.

ORLANDINI SILVIA

Silvia Orlandini is a Biologist with a long experience in milk analyses and preparation of reference materials for dairy sector.
For 22 years she was coordinator of Italian Breeder Association Laboratorio Standard Latte (AIA-LSL).
Since 2000 she is a very active members of the ISO/IDF committees.
She was chair of the IDF standing committee Statistic and Automation and project leader to revise several ISO standards for the dairy sector with the organization of the associated interlaboratory studies included ISO 13366-1 Microscope determination for somatic cell in milk.. She is still chairing the IDF Action Team Statistic
From 2015 with own company AEOS (Analytical Equivalence) provide consultancy to the dairy sector providing consultancy to ICAR (International Committee for Animal recording) and Milchprüfring Bayern (DE), following the implementation of the ICAR proficiency tests and the improvement of the microscope method to count somatic cell in milk respectively.

PALOMBA EMANUELA

Emanuela Palomba is graduated in Medical Biotechnology at University Federico II, Naples. During her BSc traineeship, she focused on the cellular and molecular aspects of the role of *deiodinase 3*, which inhibits thyroid hormone in skeletal muscle cells and promotes the proliferation of muscle satellite cells, determining muscle regeneration (Tutor: Prof. Domenico Salvatore).
For the MSc traineeship, she had a bioinformatic thesis, acquiring experience in programming in PHP and HTML, and in the use of R packages for data analysis. The final dissertation was on the evaluation of cell duplication and loss in cultured cell populations observed in video time-lapse. The aim of the study was to develop a method to quantitatively assess cell proliferation in relation to other processes such as cell motility (Tutor: Prof. Giovanni Paolella).
From 2017 to 2018, she had a fellowship financed by the project MOD_DEV_CELL (University of Naples "Federico II"). In this period, she worked on experimental and modellistic aspects of microbial cell proliferation, using the yeast S. cerevisiae as a model system. In particular, she studied yeast population dynamics and its dependence on nutrient availability and changes in the growth environment (Tutor: Prof. Elisabetta De Alteriis). Since 2019 until now, she gains a PhD project cofounded by Stazione Zoologica Anton Dohrn (Naples) and NoSelf s.r.l, under the supervision of Prof. Maria Luisa Chiusano. Her research starts from the evidence reported by Mazzoleni et al.,2015 of a significant inhibitory effect of fragmented extracellular DNA (exDNA) on plants' growth. The main aim of her PhD project is the characterization of the molecular response to extracellular self-DNA and the investigation of the sensing in cells of different species in comparison to heterologous DNA.

PIETRELLI LORIS

Is an expert in waste treatment and potabilization processes, his main research activity concern the sustainable waste treatment finalised to the raw material recovery by selective separation techniques. His research concerns also environmental monitoring (heavy metals and microplastics diffusion in particular) and auditing for SME (EMAS and ISO 14000). He developed a good skill in ecology and ornithology and he was involved, as reference person, in some international and national activities. He is working in the frame of the International Cooperation activities (Tambopata- Inambari Basins in PERU', Palestinian Territories, Jordan, Egypt, Ethiopia). He has coordinated several projects at both national and international level.
Expert for EC project evaluation: UE V and VII FP, Expert for EUROSTAR projects. From 2012 contract professor, at La Sapienza University (Rome), of "Sustainable using of polymer materials".

loris.pietrelli@enea.it

RIONDATO FULVIO

Degree in Veterinary Medicine (1999). PhD title in Veterinary and Comparative Oncology (2003). Assistant Professor (2007) and Associate Professor (2012) by the Department of Veterinary Science of the University of Torino. Teaching topics: veterinary clinical pathology and lab diagnostics. Co-head of the Clinical Laboratory of the Veterinary Teaching Hospital. Main diagnostic and research fields: flow cytometry, hematology, oncology, cytology with particular expertise in canine and feline lymphoma/leukemia. Editor of the flow cytometry panel of the European Canine Lymphoma Network (ECLN). Author of more than 40 scientific papers on the topics of interest

SANCHEZ MASSIMO

Expert in flow cytometria and cell sorting; Dr. Sanchez is currently responsible for the cytometry area of core facilities of the health institute (iss).
He has a very strong experience in exosomes and microvescicle characterization, in addition to long studies focused on cartilage cells, rat thyroid cells, differentiation of hematopoietic cells of human origin and murine, studies on the effects of hiv-1 protein nef on the adjustment of immunity functions of macrophagic cells.
He is co-inventor in the canadian patent n. 2462404: "amplification of t cells from human cord blood in serum-deprived culture stimulated with stem cell factor, interleukin-7 and interleukin-2".
Since 2014, He is in the list of experts of the committee for the assessment of the admissibility to the phase I clinical experiment (dm of 22/11/2011, pursuant to dpr 439/2001, of legislative decree 211/2003 and 200/2007 and of the law 08.11.2012 n. 189. Prot 21/02 / 2014-006390); and in the list of AIFA inspectors as observers and as a cell therapy expert. He has received the duty to carry out the analysis of cells taken at the hospital "Spedali Civili di Brescia£ following the inspective visit (in relation to note 25/052012-0020083/bnc.06). Prot. Bcn 06/14/2012-0000542.
He has been a member of the personal board for a technical consultancy required within the penal procedure proc. No. 8903/2015 rgnr.

massimo.sanchez@iss.it

SUÁREZ PANTALEÓN CELIA

Celia Suárez is R&D Manager at Unisensor, a Belgian company developing and producing rapid diagnostic detection methods in the food safety area. Since she joined the company in 2015 she is in charge of the development of Flow Cytometric suspension-based immunoassays for the detection of different food contaminants, including veterinary drugs such as antibiotics or anabolic substances, or food allergens, amongst others.

TIRELLI VALENTINA

Dr. Tirelli, as flow cytometry and sorting expert, will supports the activities of cytometry area of ISS Core Facilities.
Since 2008, she collaborates in different projects that allowed her to acquire an excellent experience and high skills in cytofluorimetric analysis and cell sorting, working specifically with the FACSAria instruments of BD Biosciences and Gallios, Cytoflex LX and MoFlow Astrios cell sorter of Beckman Coulter.
She has a strong experience in haematology and stem cells differentiation. The flow cytometry analysis, combined with molecular biology and cell biology techniques, allowed to characterize and isolate cellular subpopulations of human samples from peripheral blood, cord blood or bone marrow aspirate, both from healthy and pathological samples, with the aim of identifying the in vitro culture conditions that allow the amplification of erythroid cells and their terminal differentiation. She currently collaborates with numerous researchers of the ISS actively contributing to the development of experimental strategies in different areas of scientific research. She is co-inventor of the patent n. WO 2019/122981- PCT/IB2017/058291: "Inhibitor compound of the human GTPase Rac1 for use in the treatment of malaria".

valentina.tirelli@iss.it

VIGNOLA MARTA

Marta obtained her degree in Civil and Environmental Engineer from the University of Bologna; she did her PhD at Newcastle University within a Marie Curie International Training Network with a focus on the microbial ecology of water treatment systems. She then moved to the University of Glasgow to work on the development of tools for the effective design and management of decentralised biofiltration systems. In August 2019 she received a Royal Academy of Engineering research for development fellowship for the development of eco-engineered biofiltration systems for the removal of pesticides from drinking water in collaboration with Brazil. Her research focuses on understanding, quantifying and predicting ecological mechanisms of microbial assembly in engineered environments (particularly in drinking water systems) with the purpose of developing alternative environmental treatment solutions.

ZOANI CLAUDIA

Researcher at the ENEA Biotechnology and Agroindustry Division, graduated in Chemistry with a PhD in Analytical Chemistry, she concluded the courses & research activities of a second PhD in Agriculture, Food and Environment. Specialist on atomic spectroscopic and mass spectrometry techniques and Metrology, she conducts R&D activities on Reference Materials and Methods; measurement uncertainty; food quality, safety and traceability; chemical risk assessment. Member of the Eurachem WG on Reference Materials, IMEKO TC23 Metrology in Food and Nutrition, UNI Committee General Metrology. She is one of the 10 Italian experts on Circular Economy selected by the Italian Ministry for the Foreign Affairs and International Cooperation (MAECI) for the participation to the *Comité des Dix-Italie - Sommet des Deux Rives* initiative. Awarded with the Premio Leonardo UGIS for "research and its communication" on 2014.

She is the Coordinator of the Research Infrastructure METROFOOD-RI – I*nfrastructure for Promoting Metrology in Food and Nutrition*, included in the ESFRI Roadmap 2018 for the Domain Health and Food.

Local Organizing Commitee

Balestra Cecilia - SZN Carella Cintia - ISS Cecchetti Serena - ISS
Farina Anna - ENEA D'andrea Marco - ENEA Del Signore Giuseppina - ENEA
Presenti Ombretta - ENEA Spadaro Francesca - ISS Volpe Silvestro - GIC

Scientific Secretary

De Vita Raffaele - Gic Lucretti Sergio - Enea Tirelli Valentina - Iss*Enquires to*
ENEA Casaccia Research Centre

Enquires to

ENEA Casaccia Research Centre
Department for Sustainability - Division Biotechnologies and Agroindustry
Via Anguillarese, 301 00123 Roma – Italy Phone: 0039 063048-3191/4216/4689

Email for organization

efm2019@italymeeting.it

Email for scientif info

sergio.lucretti@enea.it - debora.giorgi@enea.it - giuseppina.delsignore@enea.it

Websites

www.enea.it - www.italymeeting.it - www.citometriagic.it