



# RESEARCH & TECHNOLOGY SERIES: FLOW CYTOMETRY / qPCR & DIGITAL PCR / LIQUID BIOPSIES

8-9 OCTOBER 2020 – VIRTUAL

## THE RESEARCH & TECHNOLOGY SERIES: Flow Cytometry / qPCR & Digital PCR / Liquid Biopsies

8-9 October 2020

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Global Engage is pleased to welcome you to the Research & Technology Series: Flow Cytometry | qPCR & Digital PCR | Liquid Biopsies, being held virtually on October 8-9.

The focus of these meetings is on advancements in cellular analysis and patient diagnosis. As a well established tool for exploring the surface of cells, flow cytometry offers great opportunities to examine patient biomarkers and support drug development efforts. Liquid biopsies provides a potential to greatly reduce the need for invasive procedures in the diagnosis of cancer, yet difficulties remain in standardising tests and introducing them to clinical practice. As the current pandemic has shown, qPCR and Digital PCR testing is at the forefront of patient diagnosis, but with challenges in accuracy, reproducibility, assay optimization, standardization and translating methods there are still many opportunities to advance this technology.

Bringing together over 60 speakers across three key areas for monitoring patient health and improving disease diagnosis, this meeting provides the opportunity to keep up to date with the latest advancements in technology, research case studies and approaches to clinical implementation. Alongside this diverse program shall be the vibrant virtual exhibition space full of solution providers showcasing their latest technologies and services. With such a breadth of knowledge and experience gathered under one roof, we hope that you will have the chance to develop lasting connections, learn something new, and develop your plans for future research.

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## 2020 Speakers


- Ryan Brinkman, Professor, Distinguished Scientist, British Columbia Cancer Research Center, Canada
- Jochen Guck, Director, Max Planck Institute for the Science of Light & Max-Planck-Institute für Physik und Medizin, Germany
- Nick Jones, Director of Global Flow Cytometry - Pharma Services, NeoGenomics Laboratories
- J. Philip McCoy, Senior Scientist, NHLBI, National Institute of Health, USA
- Romaric Lacroix, Professor in Hematology, Aix-Marseille University, France
- Valerie Coppard, Flow Cytometry Specialist, School of Clinical Medicine, Therapeutic Immunology Group and Microsoft Research, University of Cambridge, UK
- Costa Bachas, Senior post-doctoral researcher, Amsterdam UMC, The Netherlands
- Gareth Howell, Senior Experimental Flow Cytometry Officer, University of Manchester, UK
- Andrea Holme, Director of Facility, University of Aberdeen, UK
- Saskia Santegoets, Senior Research Scientist, Leiden University, The Netherlands
- Ziv Porat, Head of Flow Cytometry Unit, Associate staff scientist, Life Sciences Core Facilities, Weizmann Institute of Science, Israel
- Chris Jones, Associate Professor in Thrombosis and Haemostasis, University of Reading, UK
- Willem van de Veen, Group leader B cell immunology at the Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, Switzerland
- Marij Welters, Assistant Professor, Head of the Immunomonitoring Laboratory, Leiden University, The Netherlands
- Jacqueline Cloos, Professor, Amsterdam UMC, The Netherlands
- Henrik Mei, Scientific Head of Mass Cytometry, DRFZ, Germany
- Sagar Shelake, Scientist, Janssen, USA
- Steve Soper, Foundation Distinguished Professor, University of Kansas, USA
- Dolores Cahill, Professor of Translational Science, University College Dublin, Ireland
- Fred Kramer, Professor of Microbiology, Biochemistry and Molecular Genetics, Rutgers University, USA
- Tony Godfrey, Professor of Surgery and Computational Biomedicine, Boston University, USA
- Ieva Keraite, PhD Student, Heriot-Watt University, UK
- Catherine Alix-Panabières, Director of the Laboratory of Rare Circulating Human Cells (LCCRH), University Medical Center of Montpellier, France
- Bernhard Polzer, Head Cellular and Molecular Diagnostics, Division Personalized Tumor Therapy, Fraunhofer-Institute for Toxicology and Experimental Medicine ITEM-R, Germany
- Patrizia Paterlini-Brechot, Professor of Oncology/Molecular Biology, University of Paris, France
- John A. Martignetti, Professor, Departments of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, USA
- Chamindie Punyadeera, Associate Professor, Queensland University of Technology, Australia
- Geneveive Boland, Assistant Professor of Surgery, Harvard University, USA
- David Guttery, Lecturer in Cancer Early Detection, University of Leicester, UK
- Ellen Heitzer, Associate Professor, Institute of Human Genetics, Medical University Graz, Austria
- Tim Aitman, Chair of Molecular Pathology and Genetics, University of Edinburgh, UK
- Bruno Costa-Silva, Systems Oncology, Group Leader, Champalimaud Foundation, Portugal
- Anders Stahlberg, Associate Professor, PI, University of Gothenburg, Sweden
- Valerie Taly, Group Leader, CNRS Research Director, Université Paris Descartes, France
- Nils Paust, Head of Division Microfluidic Platforms, Hahn-Schickard, Germany
- Wim Trypsteen, Postdoctoral Researcher & Assisting Academic Staff, Faculty of Medicine and Health Sciences, Ghent University, Belgium
- Ed Shuurin, Senior clinical scientist in molecular pathology, Head of the laboratory of Molecular Pathology University Medical Center Groningen, The Netherlands
- Guillaume Gines, Postdoctoral researcher, Gulliver Laboratory – ESPCI Paris / PSL Research University
- Binoy Nadappuram, Research Associate, Imperial College London, UK
- David Zhang, Associate Professor of Bioengineering, Rice University, USA
- Henrik Laurell, Research Scientist, National Institute of Health and Medical Research, France
- Astrid Valles-Sanchez, Associate Director Translational Biology, uniQure, The Netherlands
- Jim Huggett, Principal Scientist, NML at LGC and Senior Lecturer, University of Surrey, UK
- Jörg Tost, Director Laboratory for Epigenetics and Environment (LEE), National Center Research Genomics Humaine, CEA – Francois Jacob Institute of Biology, France
- Ward De Spiegelare, Assistant Professor at the Department of Morphology, Ghent University, Belgium
- Mike Makrigiorgos, Professor of radiation oncology, Dana Farber Cancer Institute and Harvard Medical School, USA
- Philip Day, Reader in Synthetic Biology and Quantitative Genomics, University of Manchester, UK
- Karen Kempbell, Senior Scientist/Project Team Leader, Public Health England, UK
- Mojca Milavec, Research Councillor, Department of Biotechnology and Systems Biology, National Institute of Biology, Slovenia
- Dhavendra Kumar, Visiting Professor, Genomic Policy Unit, Faculty of Life Sciences & Education, University of South Wales
- Vincenzo Dicerbo, Senior Scientist, Cell and Gene Therapy Catapult, UK

# Day One

	Flow Cytometry	Liquid Biopsies	qPCR & Digital PCR
8.50-9.00	Global Engage Welcome Address	Global Engage Welcome Address	Global Engage Welcome Address
	Strategy & Technology	Technologies & Techniques	
09.00-09.40	<p><b>KEYNOTE</b>  <b>Robust, reproducible &amp; interactive QC, cell population identification and biomarker discovery</b>            Manual analysis of flow cytometry data is subjective and time consuming, especially for clinical samples. Automated algorithms have reached a level of maturity that enables them to match and, in many cases, exceed the results produced by human experts. The current state-of-the-art will be reviewed through example applications of these algorithms in automating the entire data analysis pipeline. Examples will include automated quality checking at the event and sample level, rapid, robust and reproducible gating and biomarker discovery algorithms. The utility of these algorithms will be shown through their application to clinical samples for basic research, clinical trials and drug discovery</p> <p><i>Confirmed</i>  <b>Ryan Brinkman, Professor, Distinguished Scientist, British Columbia Cancer Research Center, Canada</b></p>	<p><b>KEYNOTE PRESENTATION</b>  <b>Identification of Different Subpopulations of Circulating Tumor Cells in the Blood of Localized and Metastatic Cancer Patients using Microfluidics</b>            One of the principle biomarkers for oncology-related diseases found in blood is circulating tumor cells (CTCs). The challenge associated with using CTCs as biomarkers for many cancer diseases has been the modest clinical sensitivity demonstrated using the FDA-approved platform. CTCs expressing invasive phenotypes down-regulate epithelial antigens, such as the epithelial cell adhesion molecule – EpCAM, which is typically used for the affinity selection of CTCs. As such, CTCs may have a continuum of phenotypes and thus, a single selection marker may not address all cells comprising the tumor microenvironment. We have developed a CTC selection strategy that employs two microfluidic chips modified with antibodies and connected in series with each selecting a distinct CTC subpopulation from a single blood sample. In addition to the common marker used for CTC positive selection (EpCAM), Fibroblast Activation Protein alpha (FAP<math>\alpha</math>) expressing CTCs were also selected. Using the dual selection strategy, both CTC types were detected from patients with clinical sensitivity that showed significant improvement compared to selection in which only EpCAM was used. Approximately 90% of the selected CTCs were found not to co-express both antigens. Due to the high purity (&gt;80%) and clinical yields (&gt;95% recovery) of the dual selection strategy, molecular analysis of both EpCAM and FAP<math>\alpha</math> CTCs could be carried out including next generation sequencing, and droplet digital PCR. In this presentation, I will discuss the microfluidic chips used for the selection of CTCs, the clinical results secured using these chips, and the molecular profiling of both CTC subpopulations.</p> <p><i>Confirmed</i></p>	<p><b>KEYNOTE PRESENTATION:</b>  <b>Side-by-side use of qPCR and NGS</b></p> <ul style="list-style-type: none"> <li>We are developing molecular techniques based on PCR and NGS that enable ultrasensitive detection of nucleic acids in challenging clinical samples, such as liquid biopsies and single-cells</li> <li>We are combining PCR and NGS to enable the use of both methods in the same experimental workflow.</li> <li>We will demonstrate the clinical use of ultrasensitive analysis in cancer diagnostics including melanoma, sarcomas and pediatric cancers, as well as in other application area, such as immunology and organ rejection.</li> </ul> <p><i>Confirmed</i>  <b>Anders Stahlberg, Associate Professor, PI, University of Gothenburg, Sweden</b></p>

		<b>Steve Soper, Foundation Distinguished Professor, University of Kansas, USA</b>	
<b>9.40-10.20</b>	<p><b>KEYNOTE</b>  <b>Intelligent image-based deformation-assisted cell sorting with molecular specificity</b>  While label-free cell sorting is desirable to provide pristine cells for further analysis or use, current approaches lack molecular specificity and speed. Here we combine real-time fluorescence and deformability cytometry with standing surface acoustic wave-based sorting. We demonstrate basic sorting capabilities of the device using cell mimics and blood not only based on fluorescence but also cell deformability, as a very sensitive inherent functional marker, and other image-derived parameters. In addition, training a deep neural network to identify cells based on images alone, after prior classification using established fluorescence-based markers, transfers molecular specificity to label-free sorting. This approach combines all the advantages of label-free sorting with molecular specificity and opens the door to many novel applications in biology and medicine.  <i>Confirmed</i>  <b>Jochen Guck, Director, Max Planck Institute for the Science of Light &amp; Max-Planck-Institute für Physik und Medizin, Germany</b></p>	<p><b>KEYNOTE PRESENTATION</b>  <b>Promoting Health using Liquid Biopsies: by assessing health, early diagnosis, improved disease diagnosis, adverse events and to compare the cost-effectiveness of treatments.</b>  By combining multiple technologies and Personalised Medicine (P4), Liquid Biopsies have great potential to promote Health by assessing health, early diagnosis, accurate diagnosis, adverse events and to compare the cost-effectiveness of treatments. The importance of Biobanking and accessible sample repositories and the impact of GDPR will be addressed.  <i>Confirmed</i>  <b>Dolores Cahill, Professor of Translational Science, University College Dublin, Ireland</b></p>	<p><b>KEYNOTE PRESENTATION</b>  <b>Potential application of digital PCR in clinics : from sample quality analysis to cancer patient follow-up</b></p> <ul style="list-style-type: none"> <li>• Droplet-based digital PCR allows for unprecedented sensitivity and accuracy for rare sequences detection including genetic and epigenetic cancer-specific alterations.</li> <li>• We will discuss how ddPCR assays could be set-up to highlight presence of ctDNA in several clinical contexts both as a “stand-alone” assay but also in complement to other technologies such as optimized NGS.</li> <li>• We will illustrate their pertinence for overcoming actual clinical oncology challenges by presenting the results of different prospective studies.  <i>Confirmed</i>  <b>Valerie Taly, Group Leader, CNRS Research Director, Universite Paris Descartes, France</b></li> </ul>
<b>10.20-10.50</b>	<p><b>Solution Provider Presentation</b>  <b>Development and Validation of Flow Cytometry Assays for Clinical Trials Requires Specific Harmonization and Optimization Strategies</b></p>  <p>Flow cytometric assessments have become increasingly important for discovering, developing, and validating biomarkers for novel drug-targeted therapies. Specifically designed flow cytometry assays can provide critical information during the clinical phases that can define the effectiveness of therapeutic intervention. Like other technologies, there are many considerations and potential complications that must be addressed to ensure the assay delivers data that is accurate and consistent. Essential to this process is the exchange of specific information between sponsor</p>	<b>No presentation in this session</b>	<p><b>Solution Provider Presentation</b>  Senior representative  <b>Stilla Technologies</b></p> 

	<p>and provider related to the complexity of the assay, regulatory requirements, intended use of the data, and global deployment. Equally important are the critical steps that must be taken to configure the assay components to ensure it performs consistently between different flow cytometry instruments and across all testing sites. One of the most important elements in this process is the selection of appropriate antibodies and fluorochrome combinations, which have a direct effect on assay performance. Furthermore, strict compliance with global SOPs, as well as standardized/harmonized processes for instrument calibration, reagent qualification and data analysis are essential to data consistency.</p> <p>This presentation will illustrate the application of key elements in flow assay design for successful development and implementation.</p> <p><b>Nick Jones, Director of Global Flow Cytometry - Pharma Services, NeoGenomics Laboratories</b></p>		
<b>10.50-11.20</b>	<b>Morning break</b>	<b>Morning break</b>	<b>Morning break</b>
<b>11.20-11.50</b>	<p><b>Deciphering the tumor immune microenvironment of HPV- and HPV+ tumors: linking cytometry data to functional data</b></p> <p>It has been described that patients with HPV-driven oropharyngeal squamous cell cancer (OPSCC) and vulvar squamous cell cancer (VSCC) have a much better overall survival than HPV- OPSCC and VSCC. To study whether the presence of HPV-specific tumor-infiltrating lymphocytes (TIL) in these tumors results in a different immune contexture, we performed extensive in-depth analysis of immune infiltrates in the tumor microenvironment (TME) by using mass cytometry (CyTOF) and flow cytometry (FACS) analyses. To gain more insight into the function of the different immune infiltrates, we link our findings to functional data on antigen-specificity, regulatory and/or stimulatory functionality and cytokine profiles of the immune subsets we identify.</p> <p><i>Confirmed</i></p> <p><b>Saskia Santegoets, Senior Research Scientist, Leiden University, The Netherlands</b></p>	<p><b>Multiplex SuperSelective PCR Assays for the Detection and Quantitation of Rare Somatic Mutations</b></p> <p>SuperSelective primers, by virtue of their unique design, enable the simultaneous identification and quantitation of rare somatic mutations in routine multiplex PCR assays, while virtually eliminating signals from abundant closely related wild-type sequences. These assays are sensitive, specific, rapid, and low-cost, and can be carried out in widely available spectrofluorometric thermal cyclers. The results of these assays will potentially enable the choice, and subsequent modification if necessary, of effective targeted therapies for the treatment of an individual's cancer, utilizing frequent non-invasive liquid biopsies.</p> <p><i>Confirmed</i></p> <p><b>Fred Kramer, Professor of Microbiology, Biochemistry and Molecular Genetics, Rutgers University, USA</b></p>	<p><b>Integration, parallelization and automation of digital assays with centrifugal microfluidics</b></p> <ul style="list-style-type: none"> <li>• A convenient and versatile tool for droplet generation in standard reaction tubes.</li> <li>• Point of care digital testing: Fully integrated sample to digital answer analysis of bacteria and their antibiotic resistances.</li> <li>• Integration and parallelization: To quantify across five orders of magnitudes, two droplet sizes are generated, incubated and analyzed on a single chip. Up to 96 chips are processed in one run</li> </ul> <p><i>Confirmed</i></p> <p><b>Nils Paust, Head of Division Microfluidic Platforms, Hahn-Schickard, Germany</b></p>

11.50-12.20	<p><b>Recent developments of the extracellular vesicle measurements by flow cytometry</b></p> <p>Extracellular vesicles (EV) are small vesicles released by cells and present in all body fluids with a high potential as biomarkers. Among different methodologies, flow cytometry remains the most commonly used technique with the best capability to determine the cellular origin of large extracellular vesicles. Over the past decade, flow cytometry has benefited from significant developments allowing narrowing the gap between research and routine practice in this field.</p> <p>This talk will highlight</p> <ul style="list-style-type: none"> <li>• The potential and limitations of current scatter and fluo-sensitive flow cytometry to measure EV</li> <li>• The quality controls to satisfy regulatory requirements</li> <li>• The standardization strategies available so far to enable multi-center studies</li> <li>• The recent development on EV-dedicated reagents for flow cytometry</li> <li>• A framework for standardized reporting of EV flow cytometry experiments (MIFlowCyt-EV)</li> </ul> <p><i>Confirmed</i>  <b>Romarc Lacroix, Professor in Hematology, Aix-Marseille University, France</b></p>		<p><b>Automated threshold determination methods in dPCR - getting from zero to one</b></p> <ul style="list-style-type: none"> <li>• Overview of threshold determination methods: pro and cons</li> <li>• Challenges for future data analysis: multiplex data</li> <li>• Data analysis reporting guidelines (RDML and dMIQE)</li> <li>• Thresholding in action: case study of HIV-1 clinical trial</li> </ul> <p><i>Confirmed</i>  <b>Wim Trypsteen, Postdoctoral Researcher &amp; Assisting Academic Staff, Faculty of Medicine and Health Sciences, Ghent University, Belgium</b></p>
12.20-12.50	<p><b>Solution Provider Presentation</b>  Senior representative  <b>10x Genomics</b></p> 	No presentation in this session	No presentation in this session
12.50-13.20	Lunch	Lunch	Lunch
13.20-13.50	<p><b>Practical approach to high-dimensional panel design and tissue processing</b></p> <p>This presentation will outline a systematic step-by-step approach to design of a sensitive 20-colour immunophenotyping panel and will present examples of its use to study human tissue-resident T cells. The following topics will be discussed:</p> <ul style="list-style-type: none"> <li>• Generating Spillover Spreading Matrix (SSM)</li> </ul>	<p><b>Single tube SiMSenSeq: So simple Trump could do it (probably)!</b></p> <p>SiMSenSeq is a barcoded NGS library construction approach that facilitates detection of rare variant alleles at frequencies &lt;0.1%. SiMSen-Seq is perfectly suited for applications that require multiplexing beyond the capability of digital PCR but do not require large genome coverage provided by other ultra-sensitive</p>	<p><b>Prediction of tumor response in NSCLC patients treated with targeted or immunotherapy by monitoring of ctDNA levels</b></p> <ul style="list-style-type: none"> <li>• Changes in KRAS mutant levels in plasma cfDNA detected with ddPCR as a potential predictor for tumor response in NSCLC patients treated with immune checkpoint inhibitors</li> </ul>

	<ul style="list-style-type: none"> <li>Choosing appropriate fluorochromes and antibody clones</li> <li>Resolving pitfalls and challenges of tissue processing and staining</li> </ul> <p><i>Confirmed</i>  <b>Valerie Coppard, Flow Cytometry Specialist, School of Clinical Medicine, Therapeutic Immunology Group and Microsoft Research, University of Cambridge, UK</b></p>	<p>NGS approaches. For example, the flexible multiplexing afforded by SiMSen-Seq can be leveraged to generate patient-specific mutation detection panels to monitor treatment response and recurrence in cell-free plasma DNA from cancer patients or to detect clinically actionable evolution of tumors during targeted therapy. This talk will discuss recent applications and reproducibility of SiMSen-Seq, the effect of polymerase fidelity on error correction in barcoded NGS and will introduce a new protocol for single-tube SiMSen-Seq.</p> <p><i>Confirmed</i>  <b>Tony Godfrey, Professor of Surgery and Computational Biomedicine, Boston University, USA</b></p>	<ul style="list-style-type: none"> <li>Comparison of ctDNA assays to detect clinical-relevant mutations using Bio-RAD ddPCR, Roche cobas ctEGFR and Ageno UltraSEEK using NSCLC patients treated with EGFR-TKI</li> <li>Technical evaluation of commercial mutation analysis platforms (ddPCR, UltraSEEK and NGS) and reference materials for liquid biopsy profiling</li> </ul> <p><i>Confirmed</i>  <b>Ed Shuurig, Senior clinical scientist in molecular pathology, Head of the laboratory of Molecular Pathology University Medical Center Groningen, The Netherlands</b></p>
13.50-14.20	<p><b>EARLY CAREER RESEARCHER PRESENTATION</b>  <b>Computational approaches for improved and unbiased residual disease monitoring in acute myeloid leukemia.</b></p> <p>Minimal residual disease (MRD) monitoring in acute myeloid leukemia patients using flow cytometry provides a strong prognostic factor for relapse. Data analysis is however complex and depends on a few experts. In addition, the accuracy of the assay could be improved as 30% of MRD negative patients relapse. We explore approaches to prepare clinical flow cytometry data for computational analyses. FlowSOM, t-SNE and other machine learning approaches are used to assess relevant cell populations features. These features aid expert analyses and are ultimately used to automate flow cytometry based MRD assessment.</p> <p><i>Confirmed</i>  <b>Costa Bachas, Senior post-doctoral researcher, Amsterdam UMC, The Netherlands</b></p>	<p><b>EARLY CAREER RESEARCHER PRESENTATION</b>  <b>PIK3CA mutation enrichment and quantification from blood and tissue</b></p> <p>Four most frequent 'hotspot' PIK3CA mutations (E542K, E545K, H1047R and H1047L) represent predictive biomarkers. Given the current urge for personalized approaches, there is an increasing need for detection methods with advanced less invasive, reliable and low-cost technologies. Therefore, methods to enrich low abundance mutant DNA sequences have a potential for clinical applications including the analysis of cfDNA using blood samples.</p> <p>Thus we aimed to develop PIK3CA mutation specific nuclease based enrichment assay and validate it with clinical blood and tissue samples using crystal dPCR as well as combine it with our developed Sybr Green real-time qPCR detection method. Here we also extended this enrichment method, using reference samples to enable the accurate estimation of the abundance of PIK3CA mutations in samples before enrichment.</p> <p><i>Confirmed</i>  <b>Ieva Keraite, PhD Student, Heriot-Watt University, UK</b></p>	<p><b>EARLY CAREER RESEARCHER PRESENTATION</b>  <b>Isothermal Digital and Multiplex Detection of microRNA biomarkers</b></p> <ul style="list-style-type: none"> <li>Recent development in this field, focusing on the ultrasensitive, quantitative and multiplex detection of microRNA biomarkers</li> <li>Designing a background-free molecular program that can isothermally amplify a fluorescent signal from femtomolar concentrations of microRNA target. Combined with microfluidics, the leakless amplification chemistry makes possible to detect single-molecule compartmentalized in water-in-oil droplets, achieving a digital, absolute quantification.</li> <li>Multiplex and digital detection format relying on the</li> <li>compartmentalization of DNA-grafted particles specific for each target.</li> </ul> <p><i>Confirmed</i>  <b>Guillaume Gines, Postdoctoral researcher, Gulliver Laboratory – ESPCI Paris / PSL Research University</b></p>
14.20-14.50	Speed Dating Networking Session	Speed Dating Networking Session	Speed Dating Networking Session
14.50-15.20	Afternoon break	Afternoon break	Afternoon break



15.20-15.50	<p><b>Topic: considerations for data analysis and management in flow cytometry</b>  <i>Confirmed</i>  <b>Gareth Howell, Senior Experimental Flow Cytometry Officer, University of Manchester, UK</b></p>	<p><b>Viable Single Cell Analysis &amp; Circulating Tumor Cells as Liquid Biopsy in Cancer</b></p> <ul style="list-style-type: none"> <li>• In vitro culture of CTCs and establishment of 9 permanent CTC lines in colon cancer</li> <li>• In-depth characterization of these 9 colon CTC lines</li> <li>• EPIDROP technology: Detection and characterization of functional CTCs</li> <li>• Detection of CTCs expressing PD-L1 as liquid biopsy for guiding immunotherapy in breast cancer</li> </ul> <p><i>Confirmed</i>  <b>Catherine Alix-Panabières, Director of the Laboratory of Rare Circulating Human Cells (LCCRH), University Medical Center of Montpellier, France</b></p>	<p><b>Nano scale tweezers for single cell analysis</b>  Understanding the molecular diversity of seemingly identical cells is crucial to elucidate the genetic heterogeneity of tissues and organs to enable the accurate design of disease models and patient-specific therapies. Traditional single-cell analysis methods require the removal of the target cell from its micro-environment and in most cases, its lysis providing only a transcriptional “snapshot” of individual cells, limiting their ability to perform dynamic studies. To address these challenges, we developed a minimally invasive nanotweezers that can be spatially controlled to trap and extract samples from living cells with single molecule precision. We employed these nanotweezers to extract organelles and nucleic acids for genomic analysis. This work bridges the gap between single-molecule/organelle manipulation and cell biology to enable a better understanding of living cells.</p> <p><i>Confirmed</i>  <b>Binoy Nadappuram, Research Associate, Imperial College London, UK</b></p>
15.50-16.20	<p><b>Advances in quantitative imaging flow cytometry</b>  Developments in image analysis and data processing are rapidly improving our understanding of biological systems, ecosystems and impacting health care. This talk will highlight the advances and new applications as well as known methods in quantitative imaging flow cytometry, and their impact.</p> <p><i>Confirmed</i>  <b>Andrea Holme, Head of the Iain Fraser Cytometry Centre, University of Aberdeen, Scotland</b></p>	<p><b>Exploring the clinical application of liquid biopsy technology</b>  Currently, one in four deaths is caused by cancer, mainly as a result of systemic spread and metastatic disease. Despite new drugs, the currently available therapies are effective only in one in four cancer patients. Monitoring of genetic alterations in the course of systemic cancer disease at close intervals could thus help in recognizing the development of resistance at an early stage and in selecting the most appropriate therapy for each patient. For this, we developed a platform using single cell technologies to follow the genetic evolution of systemic cancer and generate patient-derived models. Moreover, we focus on pushing the liquid biopsy concept beyond blood samples to other body fluids and even tissues to develop tailored strategies for defined clinical questions.</p> <p><i>Confirmed</i>  <b>Bernhard Polzer, Head Cellular and Molecular Diagnostics, Division Personalized Tumor Therapy, Fraunhofer-Institute for Toxicology and Experimental Medicine ITEM-R, Germany</b></p>	<p><b>Massively multiplex quantitative PCR using an integrated label-free microarray</b>  Our group has developed the "Donut PCR" instrument platform, which enables 50- to 1000-plex quantitative PCR from a single sample in less than 60 minutes using a single closed consumable. The platform can reliably detect &lt; 10 copies of human genomic DNA, distinguish single nucleotide changes, and quantitate across 5 logs. We see initial applications in the area of point-of-care infectious disease diagnosis, human genotyping, and gene expression profiling.</p> <p><i>Confirmed</i>  <b>David Zhang, Associate Professor of Bioengineering, Rice University, USA</b></p>

16.20-16.50	No presentation in this session	<p><b>New non-invasive approaches for early diagnosis and follow up of invasive cancers.</b>  Current approaches for early diagnosis of invasive cancers</p> <ul style="list-style-type: none"> <li>• Molecular analysis of cancer cells</li> <li>• Interest of molecular analysis in diagnostics and non invasive theranostics</li> <li>• Advantages and disadvantages of different strategies</li> </ul> <p><i>Confirmed</i>  <b>Patrizia Paterlini-Brechot, Professor of Oncology/Molecular Biology, University of Paris, France</b></p>	<p><b>PCR-based method to measure cell death</b></p> <ul style="list-style-type: none"> <li>• Fragmentation of genomic DNA (gDNA) is a hallmark of cell death. We have developed QSeeD, a qPCRbased method which enables the relative quantification of gDNA fragmentation.</li> <li>• Two different versions of the technology make it possible to measure apoptosis either in a stand-alone context or within an RT-qPCR setup.</li> <li>• The stand-alone version is highly sensitive and allows detection of apoptosis at the unicellular level. The methodology is very simple and generates results comparable to other techniques, such as Caspase-Glo or Annexin V/PI FACS analysis. Unexpectedly, the unprecedented sensitivity makes it possible to detect apoptosis at a very early stage, despite that DNA fragmentation is considered as a late stage event.</li> </ul> <p><i>Confirmed</i>  <b>Henrik Laurell, Research Scientist, National Institute of Health and Medical Research, France</b></p>
16.50-17.20	No presentation in this session	No presentation in this session	<p><b>Brain and biofluid expression of an engineered microRNA for the treatment of Huntington Disease: learnings from preclinical studies</b></p> <ul style="list-style-type: none"> <li>• Use of QPCR evaluate expression of engineered microRNAs delivered by adeno associated viral vectors</li> <li>• Regional expression in brain tissue and in cerebrospinal fluid</li> <li>• Extracellular vesicles as source of microRNAs</li> </ul> <p><i>Confirmed</i>  <b>Astrid Valles-Sanchez, Associate Director Translational Biology, uniQure, The Netherlands</b></p>
17.20	End of day 1	End of day 1	End of day 1

# Day Two

	Flow Cytometry	Liquid Biopsies	qPCR & Digital PCR
9.20-9.30	Opening Remarks	Opening Remarks	Opening Remarks
	Cellular Analysis & Clinical Implementation Case Studies	Clinical Applications of Liquid biopsies	
9.30-10.10	Session starts at 10.10	<p><b>KEYNOTE PRESENTATION</b>  <b>Novel liquid biopsy approaches to detect gynecologic cancers</b>            There is a clear unmet need for a minimally invasive screening test that can detect gynecological cancers prior to symptom onset. Approximately 94,000 women are diagnosed with gynecologic cancer each year in the United States, with ovarian and endometrial cancers comprising almost 90% of cases. Together, ovarian and endometrial cancer will lead to an estimated 26,000 deaths in 2020. At present, there are no screening tests for either of these two female-specific cancers. By contrast, 23 million pap tests are performed yearly the US last to screen for cervical cancer. We describe the background and findings in which we have developed a molecular diagnostic to detect even microscopic ovarian and endometrial cancers.  <i>Confirmed</i>  <b>John A. Martignetti, Professor, Departments of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, USA</b></p>	<p><b>KEYNOTE PRESENTATION</b>  <b>An update on the digital MIQE guidelines (dMIQE2020)</b></p> <ul style="list-style-type: none"> <li>• A review of digital PCR over the last seven years</li> <li>• Example of some of the cutting edge applications</li> <li>• Review of new guidelines</li> </ul> <p><i>Confirmed</i>  <b>Jim Huggett, Principal Scientist, NML at LGC and Senior Lecturer, University of Surrey, UK</b></p>
10.10-10.40	<p><b>FEATURED PRESENTATION</b>  <b>Clinical-oriented applications for Imaging Flow Cytometry</b></p> <ul style="list-style-type: none"> <li>• Imaging Flow Cytometry (IFC) is a powerful technique that combines the high-throughput quantitation of flow cytometry with the information-rich imagery of microscopy.</li> <li>• Malaria is a parasite-induced widely spread disease with grim prognosis.</li> <li>• We used IFC to quantify in an automated, unbiased manner the life stages of the parasite as well as its communication with its host by extracellular vesicles.</li> <li>• Antibody Drug Conjugates (ADCs) are a growing class of targeted therapies, which combine the</li> </ul>	<p><b>Accelerating Personalised Medicine in Head and Neck Cancer through Liquid Biopsy</b>            Personalised medicine is an emerging field in which physicians use diagnostic/prognostic tests to decide which medical treatment will work best for an individual patient. Human saliva is now gaining momentum as a potential diagnostic medium due to its non-invasiveness, ease of sampling and the option of collecting multiple samples by non-healthcare persons. We had the world first discovery of 2mm occult HPV driven throat cancer in a asymptomatic healthy man through serial measurements of salivary HPV DNA. This is the most needed evidence to initiating a targeted screening program. In addition, using a panel of five DNA methylation genes in saliva, gave a sensitivity of 71% and a specificity of 80% discriminating healthy</p>	<p><b>LNA-enhanced qPCR-based analysis of MiRNA profiling in inflammatory diseases and the importance of cell-type selection</b></p> <ul style="list-style-type: none"> <li>• miRNAs can be conveniently profiled using qPCR panels and LNA-enhanced qPCR is the most sensitive and accurate technology for the detection of miRNAs</li> <li>• miRNA expression is strongly deregulated in inflammatory diseases and change with treatment</li> <li>• miRNA profiles differ strongly between disease-relevant sorted blood cell populations</li> <li>• detection of miRNAs in exosomes might be promising biomarker for disease detection and potentially response to anti-inflammatory therapy</li> </ul> <p><i>Confirmed</i>  <b>Jörg Tost, Director Laboratory for Epigenetics and</b></p>

	<p>specific binding of an antibody with the toxic effects of chemotherapy.</p> <ul style="list-style-type: none"> <li>We utilized IFC for development of a screening assay for ADCs, by quantifying both the internalization and co-localization with internal organelles, in a high-throughput manner. This makes IFC an important and useful tool in drug discovery and development.</li> </ul> <p><i>Confirmed</i>  <b>Ziv Porat, Head of Flow Cytometry Unit, Associate staff scientist, Life Sciences Core Facilities, Weizmann Institute of Science, Israel</b></p>	<p>controls (n=122) from head and neck cancer (HNC) patients (n=133). Furthermore, using a multi-marker logistic regression analysis, a panel of nine salivary miRNA demonstrated a sensitivity of 95% and a specificity of 93% (AUC = 0.98) when discriminating HNC patients (n=100) from precancer patients (n=29). We have also demonstrated that circulating tumour cells (CTCs) isolated from HNC patients can predict future metastasis. Identifying HNC patients before clinical will allow us to develop treatment strategies either by intensifying or de-escalating treatments, and could revolutionize the management of HNC patients with concomitant increase in survival rates and significant reduction of healthcare costs.</p> <p><i>Confirmed</i>  <b>Chamindie Punyadeera, Associate Professor, Queensland University of Technology, Australia</b></p>	<p><b>Environment (LEE), National Center Research Genomics Humaine, CEA – Francois Jacob Institute of Biology, France</b></p>
<b>10.40-11.10</b>	<b>Morning Break</b>	<b>Morning Break</b>	<b>Morning Break</b>
<b>11.10-11.40</b>	<p><b>Deep platelet phenotyping by flow cytometry</b></p> <ul style="list-style-type: none"> <li>Summary of well-established flow cytometry assays; utility, limitations and the need for new measures of platelet phenotype</li> <li>Development and utility of real-time assays</li> <li>Development and utility of freeze dried plate based assays</li> <li>Data analysis and novel software for bulk analysis and data handling</li> <li>Phenotypic groupings and clinical implications</li> </ul> <p><i>Confirmed</i>  <b>Chris Jones, Associate Professor in Thrombosis and Haemostasis, University of Reading, UK</b></p>	<p><b>Liquid Biopsy for Cancer - just another Biomarker?</b>  A fraction of the cell-free DNA (cfDNA) in the circulation derives from tumours in patients with cancer. Analysis of cfDNA therefore presents an opportunity for cancer detection, diagnosis, treatment selection and monitoring. However, the field is currently limited by (a) the low concentration of tumour-derived cfDNA in the bloodstream and (b) the lack of prospective clinical trials showing outcome benefits of cfDNA analyses. In this talk, I will review the evidence that cancer outcomes may be predicted and potentially improved by analysis of cfDNA and will present new data in selected cancers that show potential clinical utility alongside other established diagnostic tests.</p> <p><i>Confirmed</i>  <b>Tim Aitman, Professor of Molecular Pathology and Genetics, Director, Centre for Genomic &amp; Experimental Medicine, University of Edinburgh; and Honorary Consultant Physician, NHS Lothian, UK</b></p>	<p><b>Combining and qualitative and quantitative control of DNA and RNA samples using digital PCR</b>  The single molecule resolution of digital PCR enables us to combine qualitative and quantitative control of DNA and RNA preparations. This presentation will discuss the use of dPCR in quality control in different settings. We have used dPCR to evaluate DNA shearing in normal DNA preparations. In addition, a similar method was used to perform quality and quantity control of bisulfite converted DNA for subsequent methylation profiling. Finally we use dPCR for the qualitative and quantitative evaluation of RNA isolated by laser capture microdissection.</p> <p><i>Confirmed</i>  <b>Ward De Spiegelaere, Assistant Professor at the Department of Morphology, Ghent University, Belgium</b></p>
<b>11.40-12.10</b>	<p><b>Detection and characterization of antigen-specific B cells in allergy and cancer.</b></p>	<p><b>The utility of circulating tumour DNA for early detection of monitoring of gynaecological cancers</b></p>	<p><b>New approaches for efficient detection of cancer biomarkers in liquid biopsies using ddPCR and NGS</b></p>

	<p>B cell responses form a key component of the adaptive immune system. In order to better understand the role of B cells in different pathological settings it is critical to identify and characterize antigen-specific B cells. Antigen-specific lymphocytes are present in circulation at very low frequencies, rendering their accurate detection and characterization a challenging undertaking. We used multicolor flow cytometry to identify, characterize and isolate B cells specific for different antigens from allergic individuals that were treated with allergen-specific immunotherapy as well as melanoma patients treated with checkpoint inhibitor therapy.</p> <p><i>Confirmed</i>  <b>Willem van de Veen, Group leader B cell immunology at the Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, Switzerland</b></p>	<p>Circulating tumour DNA (ctDNA), the tumour-derived fraction of cell-free DNA (cfDNA), is quickly becoming recognised as a specific and sensitive biomarker for early detection and monitoring of many cancers, including breast and womb cancer. In patients with metastatic breast cancer, my work has focused on the application of next-generation sequencing of ctDNA towards early detection of endocrine therapy resistance (ETR), complemented by concomitant analysis of circulating tumor cells as a multi-analyte liquid biopsy. My work is now developing similar methods to determine the utility of ctDNA in monitoring womb cancer relapse and progression. Furthermore, I am also developing a programme around combining machine-learning with ctDNA analysis towards improved stratification of cancer patients to more efficacious therapies and early detection of treatment resistance.</p> <p><i>Confirmed</i>  <b>David Guttery, Lecturer in Cancer Early Detection, University of Leicester, UK</b></p>	<p>As the potential of liquid biopsies for prognostic, predictive or early cancer detection applications grows, so does the demand for technical advances to accompany the burgeoning range of applications. We present technical developments that enable rapid assessment of microsatellite instability using ddPCR and NGS, and new protocols that enable targeted re-sequencing for liquid biopsy applications at a fraction of the current cost, while retaining or increasing sensitivity and specificity.</p> <p><i>Confirmed</i>  <b>Mike Makrigiorgos, Professor of radiation oncology, Dana Farber Cancer Institute and Harvard Medical School, USA</b></p>
12.10-12.40	<b>Speed Dating Networking Session</b>	<b>Speed Dating Networking Session</b>	<b>Speed Dating Networking Session</b>
12.40-13.10	<b>Lunch</b>	<b>Lunch</b>	<b>Lunch</b>
13.10-13.40	<p><b>Application of flow cytometry in clinical studies</b>  Immunotherapy of cancer has gained much attention now that it starts to live up to expectations in the clinic. This therapy aims at the restoration of an immune response to tumor antigens to attack the tumor. In clinical studies where patients receive immunotherapy the monitoring of immune responses is important as this also can guide the development of new therapies. Flow cytometry can provide in-depth insight in the cells responding to immunotherapy, both the warranted response (i.e. immunotherapy induced) as well as the unwanted one (for instance the suppressor cells). In my presentation I will show the results obtained by flow cytometry in the multiple clinical trials we have been conducted to demonstrate the value of such type of assay</p> <p><i>Confirmed</i>  <b>Marij Welters, Assistant Professor, Head of the Immunomonitoring Laboratory, Leiden University, The Netherlands</b></p>	<p><b>Extracellular Vesicles: from cell-cell communication to biomarkers discovery</b>  I'll briefly introduce characteristics and biological functions of extracellular vesicles, with special focus in cancer biology and their potential applications for non-invasive liquid biopsy of oncologic patients. I'll also present recent advances of our group in the identification and targeting of pro-metastatic microenvironments and new technologies for the study of populations of extracellular vesicles.</p> <p><i>Confirmed</i>  <b>Bruno Costa-Silva, Systems Oncology, Group Leader, Champalimaud Foundation, Portugal</b></p>	<p><b>Work-flows in Biomarker Validation, Statistical and Empirical Evaluation of Multiplex Data-sets</b>  A plethora of bioinformatics tools are available for interrogating large data sets and for selection of biomarkers for diagnostic and other purposes. Different methods can generate data outputs with performance bias; particularly with regard to sensitivity and specificity. Combining different analytic approaches can increase confidence in selection of appropriate biomarkers. These can also be compared with those from appropriate disease animal model datasets to increase confidence. Empirical evaluation should also be sought to ensure the biomarkers selected are functional and biologically relevant. Validation of select biomarkers using an alternate analytical method is also advised, to minimise selection of biomarkers with poor performance. Here we provide evidence of a multifactorial work-flow to determine optimal selection of biomarkers for diagnostic development,</p>

			with relevance to Tuberculosis and Sepsis. <i>Confirmed</i> <b>Karen Kempsell, Senior Scientist/Project Team Leader, Public Health England, UK</b>
13.40-14.10	<p><b>Flow based MRD assessment for clinical decision making in acute myeloid leukemia</b></p> <ul style="list-style-type: none"> <li>• Methods of flow MRD assessment</li> <li>• Clinical relevance of flow MRD as prognostic factor</li> <li>• Qualification of the MRD assay for clinical decision making</li> <li>• Current research to improve flow MRD to be used as predictive marker for the individual patient</li> </ul> <p><i>Confirmed</i> <b>Jacqueline Cloos, Professor, Amsterdam UMC, The Netherlands</b></p>	<p><b>Circulating biomarkers in melanoma immunotherapy</b></p> <ul style="list-style-type: none"> <li>• Data on the use of extracellular vesicles (EV) in immunotherapy response/resistance</li> <li>• Utilization of plasma proteomics in immunotherapy response/resistance</li> <li>• Applications of liquid biopsy for other applications in immune-oncology (treatment monitoring, drug toxicity, etc)</li> </ul> <p><i>Confirmed</i> <b>Genevieve Boland, Section Head, Melanoma/Sarcoma Surgery, Surgical Director, Termeer Center for Targeted Therapies, Director, Surgical Oncology Research Laboratories , Harvard University, USA</b></p>	<p><b>Digital PCR for support of infectious disease diagnostics: Human cytomegalovirus case study</b></p> <p>Molecular approaches offer the potential to improve management of infectious diseases through increased speed, accuracy, sensitivity and information when compared to conventional microbiological methods. Human cytomegalovirus (hCMV) was used as a model for investigation of dPCR in support of diagnostics. hCMV is a ubiquitous and latent human virus transmitted by body fluids that causes severe morbidity and mortality in immunocompromised and immunosuppressed patients. Treatment of hCMV-infected patients is based on viral kinetics monitoring. Nucleic acid amplification based methods, qPCR and dPCR, have been assessed for their performance characteristics in quantification of hCMV. High resilience to inhibitors and assays with suboptimal efficiency and good reproducibility indicated the potential suitability of dPCR as reference measurement method and as the method for value assignment of reference materials.</p> <p><i>Confirmed</i> <b>Mojca Milavec, Research Councilor, Department of Biotechnology and Systems Biology, National Institute of Biology, Slovenia</b></p>
14.10-14.40	<p><b>Topic: multiparameter immunoprofiling using mass cytometry</b></p> <p><i>Confirmed</i> <b>Henrik Mei, Scientific Head of Mass Cytometry, DRFZ, Germany</b></p>	<p><b>Clinical decision making based on circulating tumor DNA</b></p> <ul style="list-style-type: none"> <li>• Overview of clinical applications of ctDNA</li> <li>• Overview of techniques for molecular profiling and their sensitivities</li> <li>• The use of the Avenio platform in combination with clinical decision support platforms</li> <li>• Examples from clinical studies <ul style="list-style-type: none"> <li>i. Identification of predictive markers from shallow whole genome sequencing and mutations analyses</li> <li>ii. Prediction of response to treatment with ICI in NSCLC</li> </ul> </li> </ul>	<p><b>Single cell analysis of lentiviral transduction to support ex-vivo gene-modified cell therapies</b></p> <p><b>BACKGROUND</b></p> <p>Ex-vivo gene-modified cell therapies are increasingly being developed for the treatment of monogenic diseases and cancers. Product safety and consistency of manufacturing are key to the success of the cell therapy field and require adequate analytics.</p> <p><b>CHALLENGE</b></p> <p>Analytical characterisation is fundamental to monitor product variability and safety, but current methods are often laborious and only partially aid the evaluation of product's attributes.</p>

		<p>iii. Prediction of response to neoadjuvant CTX in localized breast cancer</p> <p><i>Confirmed</i>  <b>Ellen Heitzer, Associate Professor, Institute of Human Genetics, Medical University Graz, Austria</b></p>	<p><b>PROPOSED SOLUTION</b>  We combined single cell technologies and ddPCR to analyse vector copy number and PCR-based transduction efficiency at a single cell level and demonstrated that this is representative of the population of transduced cells. We anticipate that this assay can support the generation of cost-efficient therapies and allow improved monitoring of critical quality attributes required by regulatory authorities.</p> <p><i>Confirmed</i>  <b>Vincenzo Dicerbo, Senior Scientist, Cell and Gene Therapy Catapult, UK</b></p>
14.40-15.10	<p><b>Topic: robust flow cytometry data analysis software to support T Cell therapy development</b></p> <p><i>Confirmed</i>  <b>Sagar Shelake, Scientist, Janssen, USA</b></p>	No presentation in this session	<p><b>HIV: Monitoring a Hidden, Moving Target</b></p> <ul style="list-style-type: none"> <li>Developing nucleic acid-based diagnostics against the rapidly mutating HIV virus requires access to recent and ongoing sequence information, to adequately cover current and emerging strains of the virus.</li> <li>The WHO now recommends that patients and study participants be placed on effective treatment as soon as they are diagnosed as HIV positive. This means that research will continue in an environment where most people's viral levels are undetectable by commercial viral load assays.</li> <li>Cost-effective assays are urgently needed to monitor treatment, particularly in resource-limited settings, otherwise the more wide-spread use of unmonitored treatment will drive the development of drug-resistance.</li> </ul> <p><b>Catherine Kibirige, Research Scientist, Imperial College London, UK</b></p>
15.10	Conference End	Conference End	Conference End



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