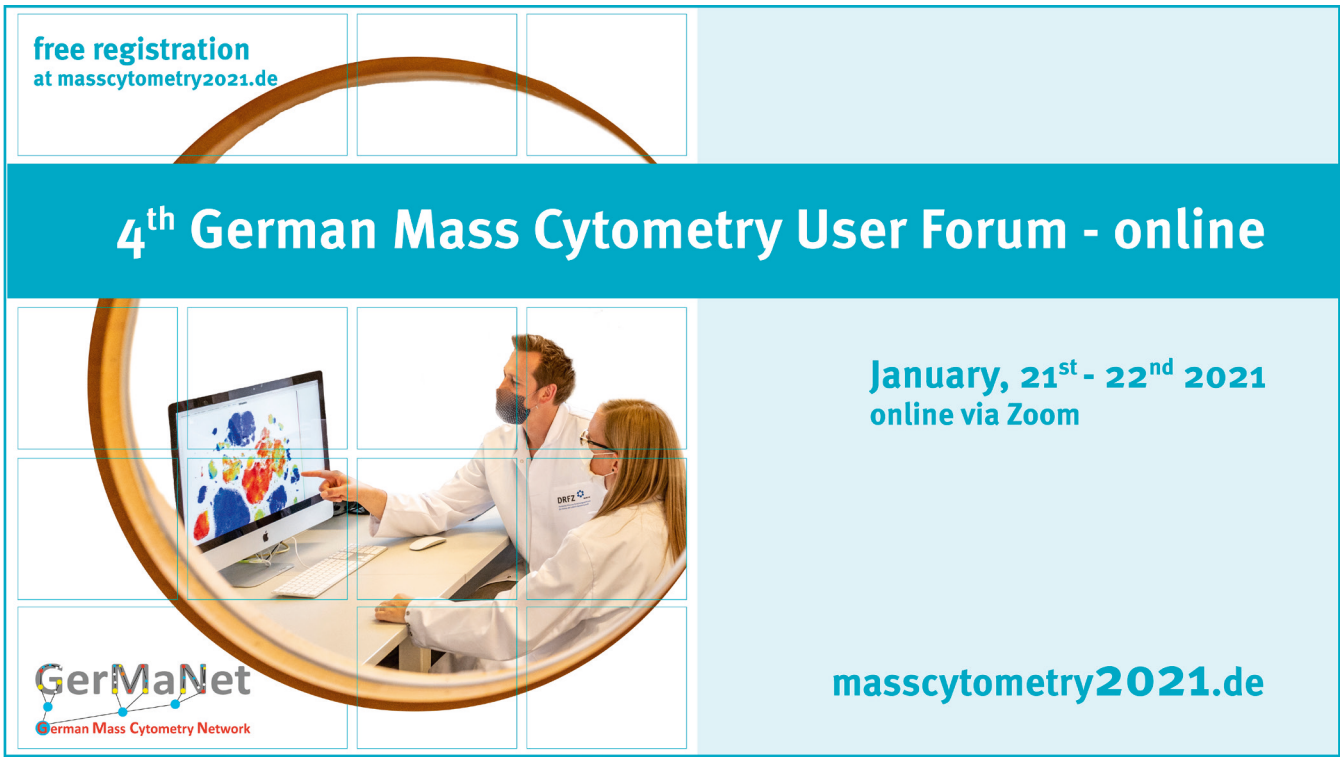


Abstract book

January 21-22, 2021

We are most grateful to our Sponsors and Exhibitors

Welcome to the 4th German Mass Cytometry User Forum - online

Dear friends and colleagues,

It is my pleasure to announce the 4th edition of the German Mass Cytometry User Forum – online.

We have assembled an exciting program with a focus on the application of mass cytometry in the study of SARS-Cov2 and COVID-19, and data analysis techniques.

The meeting will be free of charge for academic participants.

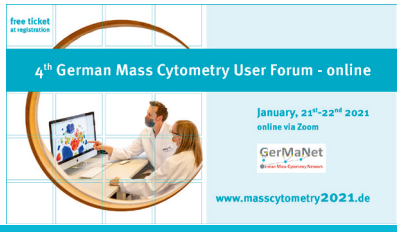
I am looking forward to an inspiring Mass Cytometry User Forum in 2021!

Best wishes, Henrik Mei

Yours,

Henrik Mei

Thursday, January 21st, 2021



10.30 Welcome, Henrik Mei

Getting started... ...with mass cytometry

10:45 Désirée Kunkel, Berlin: Principles of mass cytometry (10)

10:55 Axel Schulz, Berlin: Suspension mass cytometry – basic workflows (20)

11:15 Désirée Kunkel, Berlin: Introduction to Imaging mass cytometry (10)

11:25 Henrike Salié/Bertram Bengsch, Freiburg: Imaging mass cytometry – basic workflows (20)

11:45 Sarah Warth, Ulm, and all presenters: Q&A, tips and tricks (30)
please send questions to: axel.schulz@drfz.de

12:15 Coffee break

Session 1 - Mass Cytometry vs COVID-19

Chairs: Bertram Bengsch & Henrik Mei

13:20 Birgit Sawitzki, Berlin: CyTOF-mediated characterisation of the myeloid cell compartment in severe COVID-19 (15+5)

13:40 Henrike Salié, Freiburg: Spatial single-cell mapping reveals an altered local immune response in COVID-19 brains (15+5)

14:00 Short talk: Malte Lehmann, Berlin: Human small intestinal infection by SARS-CoV2 is characterized by an activation of CD8+ T cells (12+3)

14:15 Short talk: Dena Panovska, Leuven: Mapping the recovery of critically ill COVID19 patients by high-dimensional profiling identifies blood immunotypes following a specific immune trajectory(12+3)

14:30 Coffee break

Session 2 News from … & selected abstracts

Chairs: Marie Burns & Sarah Warth

15:00 … BIH Berlin: Chotima Böttcher – The uses and limitations of single-cell mass cytometry for studying human microglia function (15+5)

15:20 … Ulm: Habib Rahimi: Characterization of acute erythroid leukemia using mass cytometry

15.40 … MPI MG Berlin: Marie-Laure Yaspo, Interpreting bulk RNAseq with single cell technologies

16.00 Short talk: Florian Ingelfinger, Zurich: Single-cell profiling of Myasthenia Gravis identifies a pathogenic T cell signature (12+3)

Webinar by OMIQ

Chair: Axel Schulz

16.20 Chris Ciccolella, Santa Clara, USA: Mastering High-Dimensional Analysis with the OMIQ Platform

16:40 Coffee break

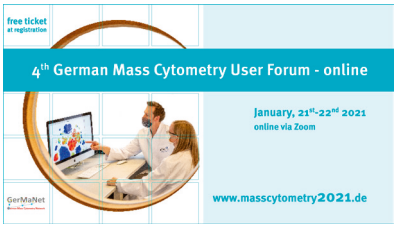
Session 3

Chair: Henrik Mei

17:00 Chris Tape, London, UK
Single-Cell Signalling Analysis of Tumour Microenvironment Organoids

17:40 Good night for today

Friday, January 22nd, 2021



Speed talks

Chair: Axel Schulz, Désirée Kunkel & Sarah Warth

10.00 Speed talks from selected abstracts (3 min each)

11:45 Coffee break

Webinar by Fluidigm

Chair: Henrik Mei

12:00 Speaker: Andrew Quong, Chief Scientific Officer Fluidigm Corporation
Preliminary Title: Mass Cytometry – a glance into the future

12:40 Coffee break

Session 4 News from…

Chairs: Marie Burns

13:00 …DRFZ: Axel Schulz: Severe COVID-19 is characterized by an increased induction of peripheral plasmablasts with an aberrant CD62L+HLA-DRlow phenotype (15+5)

13:20 …Munich: Selina Keppler/Marc Rosenbaum: Establishing CyTOF panels for the analysis of murine immune cells (15+5)

13:40 …Freiburg: Lena Sophie Mayer: Alterations in tissue-resident memory and exhausted-like CD8+ T cells in active UC. (15+5)

14.00 …Granada: Paulina Rybakowska: Experimental and data processing workflow for large-scale immune monitoring studies by mass cytometry (15+5)

14:20 Coffee break

Session 5 Data Analysis

Chairs: Henrik Mei, Axel Schulz

14:40 Lucie Rodriguez, Scilifelab, Stockholm, SE: Multi OMICs data integration (15+5)

15:00 Denis Schapiro, Harvard, USA: „Google Maps" for tissue biology – Mapping the tumor microenvironment with spatial omics technologies (25+5)

15:30 Mark Robinson, Univ. Zurich, CH: Differential discovery for CyTOF experiments (25+5)

Webinar by Beckman Coulter/Cytobank

Chair: Henrik Mei

16:15 Speaker: Dr. Giulia Grazia, Beckman Coulter Life Sciences, Italy
Title: Navigate safely through a sea of data: management and analysis made smart!

16:35 Coffee break

Virtual round table

Chair: Henrik Mei

16:45 Discussion of hot topics, Denis Shapiro, Chris Tape, Chotima Böttcher - Q&A

17:45 Farewell, Poster price ceremony and goodbye

Thursday, January 21th, 2021

Getting started... ... with mass cytometry

Désirée Kunkel

Désirée Kunkel, Flow & Mass Cytometry Core Facility, Charité - Universitätsmedizin Berlin & Berlin Institute of Health (BIH)

Axel Schulz

Mass Cytometry Lab, German Rheumatism Research Center Berlin (DRFZ), a Leibniz Institute

Henrike Salié

University Medical Center Freiburg, Clinic for Internal Medicine II - Gastroenterology, Hepatology, Endocrinology and Infectious Diseases, Freiburg, Germany

Bertram Bengsch

University Medical Center Freiburg, Clinic for Internal Medicine II - Gastroenterology, Hepatology, Endocrinology and Infectious Diseases, Freiburg, Germany

Sarah Warth

Core Facility Cytometry, Ulm University Medical Faculty

Abstract

Our introduction to mass cytometry ensures that everyone is at the same level when talking about this technology. Five experts from the field tell you how mass cytometry works and how it can be used to examine cell suspensions and tissue sections. We will guide you through typical experimental workflows and share our experience with important aspects in the application of mass cytometry, such as metal conjugation, sample barcoding, spillover compensation and batch normalization. You will also learn about the advantages of Imaging Mass Cytometry (IMC) and how to establish a multiplexed antibody panel for it. This is complemented by an introduction to current concepts of data analysis, both for imaging and suspension mass cytometry. Following the introductory talks there will also be time to discuss individual questions concerning mass cytometry and its application.

Biosketches:

Désirée Kunkel is head of Flow & Mass Cytometry Core Facility of the Berlin Institute of Health (BIH) for 11 years. As one of the first CyTOF operators in Germany, she and her core facility provide professional access to suspension mass cytometry since 2014 and IMC since 2018.

Bertram Bengsch is Professor for Translational Hepatogastroenterology and head of the Mass Cytometry Facility at the Clinic for Internal Medicine II, University Medical Center Freiburg. He has worked with mass cytometry starting in 2014 at the University of Pennsylvania.

Sarah Warth coordinates the Core Facility Cytometry in Ulm since 2017. She was formerly working in the core of Désirée Kunkel and where she gained a lot of experience in suspension mass cytometry especially in its application in immunology. The core in Ulm is using a Helios instrument.

Henrike Salié is performing her doctorate studies in the lab of Prof. Bengsch in the Clinic for Internal Medicine II at the University Medical Center Freiburg. Her research focus is on understanding immune responses to malign, autoimmune and viral challenges in tissues, with a focus on the spatial interactions of exhausted T cells, for which she established and applied an imaging mass cytometry approach.

Axel Schulz joined the DRFZ mass cytometry laboratory in 2014 after gaining first experience with the CyTOF technology at the HIMC in Stanford in 2012. As a postdoc, he works on the tech-

nical implementation of the CyTOF technology in various projects and is currently the operator in charge of the institute‘s Helios instrument.

Session 1 - Mass Cytometry vs COVID-19

CyTOF-mediated characterisation of the myeloid cell compartment in severe COVID-19

Birgit Sawitzki

Charité University Medicine, Berlin, Germany

Coronavirus disease 2019 (COVID-19) is a mild to moderate respiratory tract infection. However, a subset of patients progresses to severe disease and respiratory failure. The mechanism of protective immunity in mild forms and the pathogenesis of severe COVID-19 associated with increased neutrophil counts and dysregulated immune responses remain unclear. In a dual-center, two-cohort study, we applied single-cell proteomics of whole-blood and peripheral-blood mononuclear cells using mass cytometry and flow cytometry, respectively, to determine changes in immune cell composition and activation in mild versus severe COVID-19 over time. HLA-DRhiCD11chi inflammatory monocytes were elevated in mild COVID-19. Severe COVID-19 was marked by occurrence of neutrophil precursors, as evidence of emergency myelopoiesis, dysfunctional mature neutrophils, and HLA-DRlo monocytes. Our study provides detailed insights into the systemic immune response to SARS-CoV-2 infection and reveals profound alterations in the myeloid cell compartment associated with severe COVID-19.

Biosketch

Birgit is Professor of “Immune Tolerance” at the Institute of Medical Immunology. She has developed validated immune monitoring tools for application in investigator-driven clinical trials. She is coordinating the immune monitoring of three big multi-center investigator-driven clinical trials, sponsored by the European committee, aiming on e.g. personalized treatment and finally tolerance induction in solid organ transplant patients. In the last month Birgit successfully applied her expertise in deciphering the pathomechanisms of severe COVID-19.

Keywords

Immune monitoring, COVID-19, Immune cell composition and functionality

Publications

1. Schulte-Schrepping J, Reusch N, Paclik D, Baßler K, Schlickeiser S, Zhang B, Krämer B, Krammer T, Brumhard S, Bonaguro L, De Domenico E, Wendisch D, Grasshoff M, Kapellos TS, Beckstette M, Pecht T, Saglam A, Dietrich O, Mei HE, Schulz AR, Conrad C, Kunkel D, Vafadarnejad E, Xu CJ, Horne A, Herbert M, Drews A, Thibeault C, Pfeiffer M, Hippenstiel S, Hocke A, Müller-Redetzky H, Heim KM, Machleidt F, Uhrig A, Bosquillon de Jarcy L, Jürgens L, Stegemann M, Glösenkamp CR, Volk HD, Goffinet C, Landthaler M, Wyler E, Georg P, Schneider M, Dang-Heine C, Neuwinger N, Kappert K, Tauber R, Corman V, Raabe J, Kaiser KM, Vinh MT, Rieke G, Meisel C, Ulas T, Becker M, Geffers R, Witzenrath M, Drosten C, Suttorp N, von Kalle C, Kurth F, Händler K, Schultze JL*, Aschenbrenner AC*, Li Y*, Nattermann J*, Sawitzki B*, Saliba AE*, Sander LE*; Deutsche COVID-19 OMICS Initiative (DeCOI). Severe COVID-19 Is Marked by a Dysregulated Myeloid Cell Compartment. Cell. 2020 Sep 17;182(6):1419-1440.e23. doi: 10.1016/j.cell.2020.08.001. Epub 2020 Aug 5. PMID: 32810438; PMCID: PMC7405822. *shared senior author

2. Sawitzki B, Harden PN, Reinke P, Moreau A, Hutchinson JA, Game DS, Tang Q, Guinan EC, Battaglia M, Burlingham WJ, Roberts ISD, Streitz M, Josien R, Böger CA, Scottà C, Markmann JF, Hester JL, Juerchott K, Braudeau C, James B, Contreras-Ruiz L, van der Net JB, Bergler T, Caldara R, Petchey W, Edinger M, Dupas N, Kapinsky M, Mutzbauer I, Otto NM, Öllinger R, Hernandez-Fu-

entes MP, Issa F, Ahrens N, Meyenberg C, Karitzky S, Kunzendorf U, Knechtle SJ, Grinyó J, Morris PJ, Brent L, Bushell A, Turka LA, Bluestone JA, Lechler RI, Schlitt HJ, Cuturi MC, Schlickeiser S, Friend PJ, Miloud T, Scheffold A, Secchi A, Crisalli K, Kang SM, Hilton R, Banas B, Blancho G, Volk HD, Lombardi G, Wood KJ, Geissler EK. Regulatory cell therapy in kidney transplantation (The ONE Study): a harmonised design and analysis of seven non-randomised, single-arm, phase 1/2A trials. Lancet. 2020 May 23;395(10237):1627-1639. doi: 10.1016/S0140-6736(20)30167-7.

3. Truong KL, Schlickeiser S, Vogt K, Boës D, Stanko K, Appelt C, Streitz M, Grütz G, Stobutzki N, Meisel C, Iwert C, Tomiuk S, Polansky JK, Pascher A, Babel N, Stervbo U, Sauer I, Gerlach U, Sawitzki B. Killer-like receptors and GPR56 progressive expression defines cytokine production of human CD4+ memory T cells. Nat Commun. 2019 May 22;10(1):2263. doi: 10.1038/s41467-019-10018-1. PMID: 31118448; PMCID: PMC6531457.

Spatial single-cell mapping reveals an altered local immune response in COVID-19 brains

Henrike Salié

University Medical Center Freiburg, Clinic for Internal Medicine II - Gastroenterology, Hepatology, Endocrinology and Infectious Diseases, Freiburg, Germany

Abstract

COVID-19 causes neurological symptoms that can be potentially life-threatening in up to 67 % of the patients. To understand the local immune response during SARS-CoV-2 infection at a spatially resolved, high-dimensional single-cell level, we performed a 38-biomarker imaging mass cytometry analysis of the brain stem and olfactory bulb from COVID-19 patients and additional controls. Importantly, utilizing an unbiased image segmentation and cell classification pipeline, we observed a significant immune activation in the central nervous system (CNS) and identified novel context-specific CD8 T cell and microglial clusters. Spatially resolved single-cell analysis identified distinct phenotypes of T cells and microglial clusters, their presence in specific anatomical regions and their cellular interactions. The analysis further highlights microglial nodules and perivascular immune cell clusters as key sites of the local immune response in the brain stem. It also demonstrates that disease-associated neuroinflammation is associated with severe axonal damage as a structural basis for neurologic deficits. Finally, IMC staining for SARS-CoV-2 spike glycoprotein revealed direct evidence of vasculature-associated viral presence in the olfactory bulb and brain stem as well as reactive astrogliosis. Together these analyses identify the immune correlates of a surprisingly high level of neuroinflammation in fatal cases of COVID19.

Biosketch

Henrike Salié is performing her doctorate studies in the lab of Prof. Bengsch in the Clinic for Internal Medicine II at the University Medical Center Freiburg. Her research focus is on understanding immune responses to malign, autoimmune and viral challenges in tissues, with a focus on the spatial interactions of exhausted T cells, for which she established an imaging mass cytometry approach. During the SARS-CoV-2 pandemic she explored the neuroinflammation in brains of COVID-19 patients in a collaborative effort with the Institutes of Neuropathology in Freiburg (AG Prinz), Hamburg (AG Glatzel) and Göttingen (AG Stadelmann-Nessler).

Keywords

COVID-19, Imaging mass cytometry, T cell exhaustion

Short talk:
Human small intestinal infection by SARS-CoV2 is characterized by an activation of CD8+ T cells

Malte Lehmann[1]

Malte Lehmann[1], Kristina Allers[1], Claudia Heldt[1], Franziska Schmidt[2], Yasmina Rodriguez-Silke[2], Désirée Kunkel[2], Michael Schumann[1], Chotima Böttcher[3], Viktor M. Corman[4], Thomas Schneider[1], Christoph Loddenkemper[5], Verena Moos[1], Carl Weidinger[1,3,7], Anja A. Kühl[6,7], Britta Siegmund[1,7*]*

[1]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Campus Benjamin Franklin. Medical Department, Division of Gastroenterology, Infectiology and Rheumatology (including Nutritional Medicine), Hindenburgdamm 30, 12200 Berlin, Germany

[2]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Berlin-Brandenburg Center for

Regenerative Therapies BCRT, Charité | BIH Cytometry Core, Campus Virchow Klinikum, Germany

[3]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, Klinik für Psychiatrie und Psychotherapie, Campus Mitte, Germany Clinician Scientist Program, Berlin Institute of Health

[4]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Berlin Institute of Health, and German Centre for Infection Research, Department of Virology, 10117 Berlin, Germany.

[5]PathoTres, Gemeinschaftspraxis für Pathologie und Neuropathologie, Teltowkanalstr. 2, 12247 Berlin, Germany

[6]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin and Berlin Institute of Health, iPATH.Berlin, Campus Benjamin Franklin, Germany

[7]The Transregio 241 IBDome Consortium

[*]Contributed equally to this work

Symptoms of COVID-19 suggest a multisystemic disease including the gastrointestinal system. Analysing biopsies of the small intestine from COVID-19 patients using imaging mass cytometry we identified histomorphological changes of the epithelium, characterized by infiltrating CD8+ T cells as well as epithelial apoptosis and regeneration. We hypothesize CD8+ T cell activation and migration into the intestinal epithelium upon infection of intestinal cells as a possible cause for gastrointestinal symptoms.

Short talk:
Mapping the recovery of critically ill COVID19 patients by high-dimensional profiling identifies blood immunotypes following a specific immune trajectory

Dena Panovska

Panovska D[1#], Penttilä PA[2#], Van Gassen S[3#], Vanderbeke L[4], Van Herck Y[5], Quintelier K[3], Emmaneel A[3], Claeys A[1], Derweduwe M[1], Verbeke T[1], Chinnaraj R[2], Filtjens J[6], Malengier-Devlies B[6], Ahmadzadeh K[6], Van Mol P[7], Borras DM[8], Antoranz A[3], Bosisio FM[9], the CONTAGIOUS consortium[$], Wauters E[10], Matthys P[6], Saeys Y[2], Garg AD[8], Wauters J[11#], De Smet F[3#].*

[#]equal contribution

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[1]Laboratory for Precision Cancer Medicine, Translational Cell and Tissue Research, Department of Imaging & Pathology, KU Leuven, Belgium

[2]KU Leuven Flow & Mass Cytometry Facility, KU Leuven, Belgium

[3]*Department of Applied Mathematics, Computer Science and Statistics, Ghent University, Gent, Belgium, and Data Mining and Modeling for Biomedicine, VIB Center for Inflammation Research, Gent, Belgium.*

[4]*Laboratory of Clinical Bacteriology and Mycology, Department of Microbiology, Immunology and Transplantation, KU Leuven, Belgium*

[5]*Laboratory of Experimental Oncology, Department of Oncology, KU Leuven, Belgium*

[6]*Laboratory of Immunobiology, Department of Microbiology, Immunology and Transplantation, Rega Institute, KU Leuven, Belgium*

[7]*Laboratory of Translational Genetics, Department of Human Genetics, VIB-KU Leuven, Belgium*

[8]*Laboratory for Cell Stress & Immunity (CSI), Department of Cellular and Molecular Medicine (CMM), KU Leuven, Belgium*

[9]*Translational Cell & Tissue Research, Department of Imaging & Pathology, KU Leuven, Belgium*

[10]*Laboratory of Respiratory Diseases and Thoracic Surgery (BREATHE), Department of Chronic Diseases and Metabolism, KU Leuven, Belgium*

[11]*Laboratory for Clinical Infectious and Inflammatory Disorders, Department of Microbiology, Immunology and Transplantation, KU Leuven, Belgium*

[$]*Additional Contagious consortium members: Michael Casaer, Dieter Dauwe, Jan Gunst, Greet Hermans, Stephanie Humblet-Baron, Diether Lambrechts, Adrian Liston, Natalie Lorent, Kim Martinod, Philippe Meersseman, Johan Neyts, Paul Proost, Jeroen Raes, Stephen Rex, Sabine Tejpar, Karin Thevissen, Thomas Tousseyn, Birgit Weynand, Alexander Wilmer, Carine Wouters.*

Abstract

The COVID-19 pandemic poses a major burden on health-care and economic systems across the globe. Even though a majority of the population only develops minor symptoms upon SARS-CoV2 infection, a significant proportion are hospitalized at intensive care units (ICU) requiring critical care. While insights into the early stages of the disease are gradually expanding, the dynamic immunological processes occurring in critically ill patients throughout their recovery at ICU are far less understood. We have analyzed longitudinally collected, whole blood samples of 40 surviving COVID-19 patients during their recovery at ICU using high-dimensional cytometry by time-of-flight (CyTOF) and cytokine multiplexing. Based on the neutrophil to lymphocyte ratio (NLR), we defined 4 sequential immunotypes during recovery that correlated to various clinical parameters, including the level of respiratory support at concomitant sampling times. We also identified classical monocytes as the first immune cell type to recover by restoring HLA-DR-positivity and by reducing the immunosuppressive CD163+ monocyte population, followed by the recovery of CD8+ and CD4+ T cell, and mDC populations. The determined immunotypes also correlated to aberrant cytokine and acute-phase reactant levels. Finally, integrative analysis of cytokines and immune cell profiles showed a shift from an initially dysregulated immune response to a more coordinated immunogenic interplay, highlighting the importance of longitudinal sampling to understand the pathophysiology underlying recovery from severe COVID-19.

…BIH Berlin:

The uses and limitations of single-cell mass cytometry for studying human microglia function

Chotima Böttcher

Charité - Universitätsmedizin Berlin

Abstract

Microglia, the resident innate immune cells of the central nervous system (CNS), play an important role in brain development and homeostasis. Studies in animal models reveal the origin and development of microglia, and how these cells alter their transcriptional and phenotypic signatures during CNS pathology. However, little is known about their human counterparts. Recent studies in human brain samples have harnessed the power of mass cytometry (CyTOF) to provide a comprehensive molecular view of human microglia in healthy and diseased brains. CyTOF is a powerful tool to study single-cell protein expression of human microglia (huMG), which can be combined with scRNA-seq for comprehensive analysis, as it allows single-cell analysis of post-translational modifications of proteins, which provides insights into cell signalling dynamics in targeted cells. In addition, imaging mass cytometry (IMC) has recently been demonstrated for analysing multiple cell types in human brain sections. IMC leverages mass spectrometry to acquire spatial data of cell-cell interactions on brain sections. Here, the use and limitations of CyTOF in studing huMG are discussed.

Biosketch

Chotima Böttcher is a group leader and principal investigator at the Charité – Universitätsmedizin Berlin, Germany. Dr. Böttcher obtained her PhD at Institute of Pharmacy, at Martin-Luther-University Halle-Wittenberg, Halle/Saale, Germany. Her research focuses on systems immunology in neuroscience, with particular emphasis on myeloid cells including monocytes and brain microglia/macrophages. The main goal is to identify cellular complexity and heterogeneity of the myeloid compartment of the human central nervous system and to further investigate how these signatures alter during neurodegeneration/ neuroinflammation.

Publications

1. Böttcher C*, Schlickeiser S*, Sneeboer MAM*, Kunkel D, Knop A, Paza E, Fidzinski P, Kraus L, Snijders GJL, Kahn RS, Schulz AR, Mei HE, NBB-Psy, Hol EM, Siegmund B, Glauben R, Spruth EJ, de Witte LD, Priller J: Human microglia regional heterogeneity and phenotypes determined by multiplexed single-cell mass cytometry. Nat Neurosci 22, 78–90 (2019). (*equal contribution)

2.Sankowski R*, Böttcher C*, Masuda T, Geirsdottir L, Sagar, Sindram E, Seredenina T, Muhs A, Scheiwe C, Shah MJ, Heiland DH, Schnell O, Gru¨n D*, Priller J*, Prinz M*: Mapping microglia diversity in the human brain through the integration of high-dimensional techniques. Nat Neurosci 22, 2098–2110 (2019). (*equal contribution)

3.Böttcher C*, van der Poel M*, Fernández-Zapata C*, Schlickeiser S, Leman JKH, Hsiao CC, Mizee MR, Adelia, Vincenten MCJ, Kunkel D, Huitinga I*, Hamann J*, Priller J*. Single-cell mass cytometry reveals complex myeloid cell composition in active lesions of progressive multiple sclerosis. Acta Neuropathol Commun 8, 136 (2020). (*equal contribution)

… Ulm

Characterization of acute erythroid leukemia using mass cytometry

Habib Rahimi

Institute of experimental cancer research, University Hospital Ulm

Acute erythroleukemia (AEL) is a rare subtype of acute myeloid leukemia (AML) which accounts for less than 5% of all de novo AML cases. There have been several efforts to characterize AEL at a molecular level, describing recurrent alterations in TP53 and in the NPM1 and FLT3 gene (Iacobucci et al. Nat Genet 2019). A comprehensive genomic analysis of AEL cases confirmed the complexity of this AML subtype. Despite these advances, the underlying biology of AEL is still not precisely defined and the prognosis is dismal with a median survival of only 2-3 months for pure erythroid leukemia. Marker combinations suitable for 1) the identification and characterization of leukemic stem cell (LSC) candidates, 2) monitoring minimal residual disease during chemotherapy treatment and 3) the development of innovative targeted therapies are missing. Along with a comprehensive multiomics approach, flow cytometry and murine bone marrow transplantation experiments, we developed a mass cytometry marker panel for an in-depth characterization of human AEL bone marrow samples in comparison to other AML subtypes and bone marrow (BM) from healthy donors. A total of 8 AELs, 30 AMLs and 5 BM controls were successfully analyzed. Marker combinations, identified and validated by conventional flow cytometric analysis, were able to separate erythroid from myeloid blast populations and might help in identifying MRD.

Biosketch

Habib Rahimi is currently a PhD Student at the Institute of Experimental Cancer Research and the International Graduate School in Molecular Medicine in Ulm, Germany.

His research mainly focuses on acute myeloid leukemia, hematopoietic stem cells and aging. He is a member of the Cellular and Molecular Mechanisms in Aging (CEMMA)-research group (GRK 1789) and is involved in projects within the CRC Experimental Models and Clinical Translation in Leukemia (SFB 1074) and the Aging-related epigenetic remodeling in acute myeloid leukemia consortium (FOR 2674).

Keywords

Acute erythroleukemia (AEL), Minimal residual disease (MRD), Leukemic stem cells (LSC)

… MPI Molecular Genetics Berlin

Interpreting bulk RNAseq with single cell technologiest

Marie-Laure Yaspo

MPI Molecular Genetics, Berlin

Short talk:
Single-cell profiling of Myasthenia Gravis identifies a pathogenic T cell signature

Florian Ingelfinger

Florian Ingelfinger[1,2], Sinduya Krishnarajah[1], Michael Kramer[3], Sebastian G. Utz[1], Edoardo Galli[1], Mirjam Lutz[1], Pascale Zwicky[1], Ayse U. Akarca[4], Nicole Puertas Jurado[1], Corinne C. Widmer[5], Luca Piccoli[3], Federica Sallusto[3,6], Nicolás G. Núñez[1], Teresa Marafioti[4], Didier Schneiter[7], Isabelle Opitz[7], Antonio Lanzavecchia[3], Donatella De Feo[1], Sarah Mundt[1], Hans H. Jung[2], Bettina Schreiner[1,2],†,, Burkhard Becher[1],†,*.*

[1]*Institute of Experimental Immunology, University of Zurich, Zurich, Switzerland.*

[2]*Department of Neurology, University Hospital Zurich, Zurich, Switzerland.*

[3]*Institute for Research in Biomedicine, Università della Svizzera italiana, Bellinzona, Switzerland.*

[4]*Department of Cellular Pathology, University College London Hospital, United Kingdom.*

[5]*Department of Medical Oncology and Hematology, University Hospital Zurich and University of Zurich, Zurich, Switzerland.*

[6]*Institute of Microbiology, ETH Zurich, Zurich, Switzerland.*

[7]*Department of Thoracic Surgery, University Hospital Zurich, Zurich, Switzerland.*

[†]*These authors jointly supervised this work.*

[*]*Correspondence to: becher@immunology.uzh.ch and bettina.schreiner@uzh.ch.*

One Sentence Summary

Single-cell immunophenotyping reveals a pathogenic T cell signature accumulating in the inflamed thymus of Myasthenia gravis patients.

Abstract

Myasthenia Gravis (MG) is an autoimmune disease characterized by impaired neuromuscular signaling due to autoantibodies targeting the acetylcholine receptor. Although its auto-antigens and effector mechanisms are well defined, the cellular and molecular drivers underpinning MG remain elusive. Here we employed high-dimensional single-cell mass and spectral cytometry of blood and thymus samples from MG patients in combination with supervised and unsupervised machine-learning tools to gain insight into the immune dysregulation underlying MG. By creating a comprehensive immune map we identified two dysregulated subsets of inflammatory circulating memory T helper (Th) cells. These signature ThCD103 and ThGM cells populated the diseased thymus, were reduced in the blood of MG patients, and were inversely correlated with disease severity. Both signature Th subsets rebounded in the blood of MG patients after surgical thymus removal, indicative of their role as cellular markers of disease activity. Together, this in-depth analysis of the immune landscape of MG provides valuable insight into disease pathogenesis, suggests novel biomarkers and identifies new therapeutic targets for treatment.

Biosketch

Florian Ingelfinger is a PhD student in the lab of Prof. Dr. Burkhard Becher at the Institute of Experimental Immunology, University of Zurich, Switzerland. His research interest focusses on the integration of single cell technologies in clinical studies to decipher complex cellular interactions leading to inflammation and autoimmunity. Utilizing computational tools he seeks to understand the contribution of genetic and environmental drivers in the development of neuroimmunological disorders like Multiple Sclerosis or Myasthenia Gravis.

Webinar by OMIQ

Mastering High-Dimensional Analysis with the OMIQ Platform

Chris Ciccolella

Co-Founder & CEO, Omiq, Inc., Santa Clara, USA

Abstract

Before biology can be controlled, it must be understood. To be understood, it must be measured. Mass Cytometry gives us incredible power to measure biology. The process of understanding, i.e., data analysis, is a rich and evolving field with a considerable amount of detail to learn, and is often a challenging obstacle for researchers. In this talk, the principles of effective Mass Cytometry data analysis will be presented in context of the OMIQ Data Science Platform (www.omiq.ai), an advanced cloud software for cytometry data analysis

Biosketch

Chris is originally a cell biologist and cytometry expert. In 2013 he moved from the bench to the computer to focus on software engineering and data science. Following a tenure at Cytobank, he co-founded Omiq in 2018 to focus on analytical innovations to accelerate the progress of human health.

Keywords

High-dimensional analysis, algorithms, discovery

Session 3

Single-Cell Signalling Analysis of Tumour Microenvironment Organoids

Chris Tape

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Abstract

Organoids are self-organising stem cell-derived ex vivo cultures widely adopted as biomimetic models of healthy and diseased tissues. As complex heterocellular systems, organoids are especially well-positioned to take advantage of emerging high-dimensional single-cell technologies. Here we present a Cytometry by Time-Of-Flight (CyTOF) method for single-cell analysis of post-translational modification (PTM) signalling in organoids and tumour microenvironment organoid co-cultures. Integrating single-cell PTM analysis with thiol-reactive organoid barcoding in situ (TOBis) enables 35-plex and 126-plex comparison of signalling networks between organoid co-cultures. Cell-type-specific PTM analysis of colorectal cancer organoid co-cultures revealed that oncogenic mutations cell-autonomously mimic signalling states normally induced by stromal fibroblasts and macrophages.

Biosketch

Chris received his Ph.D. from Prof. Gillian Murphy's lab at the CRUK Cambridge Institute (University of Cambridge). He was then awarded a Sir Henry Wellcome Postdoctoral Fellowship between The Institute of Cancer Research (ICR) (with Dr. Claus Jorgensen and Prof. Chris Marshall) and Massachusetts Institute of Technology (MIT) (with Prof. Doug Lauffenburger) to study how oncogenes signal across multiple cell types in cancer. Chris now leads the Cell-Communica-

tion Lab at UCL CI under a CRUK Career Development Fellowship (supported by the CRUK Werth Trust).

Keywords

Organoids, Barcoding, PTM Signalling

Friday, January 22nd, 2021

Speed talks

Speed talks from selected abstracts

Beckman Coulter/Cytobank donates a year's licence of the Cytobank Premium SW for the best Speed Talk at the 4th German Mass Cytometry User Forum!

1. Longitudinal, multi-center study reveals unique immune signatures of severe COVID-19

Stefanie Kreutmair

*Stefanie Kreutmair[1,2], Susanne Unger[1,3], Nicolás Gonzalo Núñez[1,3], Florian Ingelfinger1,3, Chiara Alberti1, Donatella De Feo[1], Sinduya Krishnarajah[1], Manuel Kauffmann[1], Ekaterina Friebel[1], Benjamin Gaborit4, Sepideh Babaei[5], Mirjam Lutz[1], Nicole Puertas Jurado[1], Nisar P. Malek[5], Siri Goepel[5,6], Peter Rosenberger[7], Helene A. Häberle[7], Ikram Ayoub[8], Sally Al-Hajj[8], Manfred Classen[5,10], Roland Liblau[8,10], Guillaume Martin-Blondel[8,9,10], Michael Bitzer[5,10], Antoine Roquilly[4,10], Burkhard Becher[1,10],**

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Abstract

The currently ongoing COVID-19 pandemic is continuing to spread around the world, with rising numbers of deaths. There is clear evidence that severe cases of COVID-19 which require hospitalization due to respiratory distress are mediated by immunopathology. Thus, it is vital to understand how the immune system reacts specifically to SARS-CoV-2 infection.

For this purpose, we analyzed 150 PBMC samples of a multi-center, longitudinal COVID-19 cohort together with non-SARS-CoV-2 critical pneumonia patient samples (n=25) and healthy controls (n=21). High-throughput, high-dimensional sing-

le-cell spectral cytometry and algorithm-based analysis resulted in a complex immune signature network underlying COVID-19 severity. By comparing the immune landscape of severe COVID-19 with non-SARS-CoV-2 critical pneumonia, we were able to extract immune features specific to SARS-CoV-2 infection. While COVID-19 and non-SARS-CoV-2 pneumonia share an emergency monopoiesis and numerous features of adaptive immune dysfunction, pathological immune signatures exclusive to COVID-19 were concentrated in the T and NK cell compartment. Interestingly, predominantly but not only the severe COVID-19 cohort presented several immune alterations which did not recover at the end of our study (14 weeks after hospital admission), pointing to a prolonged immune dysregulation. Bench-to-bedside translation of the identified immune signatures in severe COVID-19 offered potential biomarkers for outcome prediction. On top, we discovered a low but clearly detectable ACE2 expression on a CD4+ T cell subset, which opens the door for so far unknown potential underlying mechanisms in the immunopathological network of COVID-19.

Overall, the comparison of two severe infectious lung diseases driven by different pathogens allowed us to uncover unique immune signatures in SARS-CoV-2 mediated disease, revealing the outlines of a complex immune landscape which can serve as a basis for translational treatment strategies effectively blocking the origin of the immunopathologic cascade in severe COVID-19.

2. SARS-CoV-2 infection is associated with a pro-thrombotic platelet phenotype

Melissa Klug

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Abstract

We compared the activation state and the expression of transmembrane proteins in platelets of 8 hospitalized COVID-19 patients not requiring intensive care support and platelets of healthy controls, both with and without in-vitro stimulation with thrombin receptor-activating peptide (TRAP). We detected a hyper-activated phenotype in platelets during SARS-CoV-2 infection consisting of highly expressed platelet activation markers which might contribute to the hypercoagulopathy observed in COVID-19. Additionally, several transmembrane proteins were higher expressed compared to healthy controls.

3. Deep phenotyping by mass cytometry to define the T cell signature of childhood allergic asthma

Hartmann Raifer

Hartmann Raifer [1,2], Axel R. Schulz[3]*, Johanna Krusche [4]*, Addi Romero [1], Andreas Böck [4,]*

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Abstract

Allergic asthma (AA) in childhood is characterized by Th2-driven immunity and defects in contra-regulation by Th1 cells and/or Tregs. It can be influenced by genetic mechanisms including polymorphisms in interferon regulatory factor 1 (IRF1), which associates with atopy in childhood. We applied single-cell mass cytometry to define the T cell signature and assess a possible interrelationship with IRF1 polymorphisms in childhood AA. Using manual gating and algorithmic analysis we found increased CD4/CD8 T cell ratios in AA which correlated with eosinophilia linked to disease severity. Furthermore, a Th2 cell cluster with high ICOS and TIGIT expression was overrepresented in AA. The ratio between this Th2 population and a specific Th1-clade as well as the abundance of naïve/resting Tregs were increased in AA, suggesting a modulation of Th subset contra-regulation. The abundance of CD8+ T cells was decreased in AA, and they displayed increase in naïve at the expense of memory phenotype. This cellular signature associated in part with IRF1 status, indicating a possible mechanistic contribution of IRF regulatory elements. Our approach demonstrates the utility of high-dimensional mass-cytometry in combination with genetic analysis to interrogate cellular signature in context of a specific genetic parameter, thereby providing basis for further studies to reveal predictive biomarkers in childhood AA.

4. Deep phenotypical characterization of human CD3+CD56+ T cells by mass cytometry

Addi Romero-Olmedo

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[*] These authors contributed equally to this work.

[**] These authors jointly directed this work.

Abstract

CD56+ T cells are a group of pro-inflammatory CD3+ T lymphocytes with characteristics of natural killer cells, and are involved in antimicrobial immune defense. Here, we performed a deep phenotypic profiling of CD56+ T cells of peripheral blood of normal human donors and individuals sensitized to birch-pollen or/and house dust mite by high-dimensional mass cytometry combined with manual and computational data analysis. A co-regulation between major conventional T cell subsets and their respective CD3+CD56+ cell counterparts which we herein demonstrate, appeared restricted to CD8+, MAIT, and TCRγδ+ T cell compartments. Interestingly, we find a co-regulation of several CD3+CD56+ cell subsets in allergic but not in healthy individuals. Moreover, using FlowSOM, we distinguished a variety of CD56+ T cell phenotypes demonstrating a hitherto underestimated heterogeneity among these cells. The novel CD3+CD56+ subset description comprises phenotypes superimposed with naive, memory, type 1, type 2, and type 17 differentiation stages, in part represented by a phenotypical continuum. Frequencies of 2 out of 19 CD3+CD56+ FlowSOM clusters were significantly diminished in allergic individuals, demonstrating less frequent presence of cells with cytolytic, presumably protective, capacity in these donors consistent with defective expansion or their recruitment to the affected tissue. Our results contribute to defining specific cell populations to be targeted during therapy for allergic conditions.

5. Immunity, the beginning: Characterizing immune development in preterm infants using mass cytometry.

Tomer Salame

Tomer M. Salame[1], Shlomit Reich-Zeliger[2], Chava Rosen[2,3], Lisa Buchauer[4], Jacob Rimer2, Tzipora Strauss3 and Nir Friedman[2]

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Mortality of preterm infants due to infections is a major challenge. An immature immune system is a main determinant of susceptibility to pathogens. To better understand the developing immune system, we mapped it in preterm and full-term infants, using mass cytometry, which provides unprecedented information on immune cell types. Using data from longitudinal samples and the clinical course of the infants, we generated signatures of immune state and repertoire composition. Approximately 20 immune cell populations were characterized. We demonstrate that preterm babies born at extreme early week of gestation (23-26 weeks) show all the immune cell populations, but in a different composition compared to more mature preterm babies (32-36 week of gestation) or full-term babies. PCA analysis reveals that trajectories tend to have similar starting and end points for babies born in the same gestation week, and are more similar among twins. Additionally, a large cell population which is negative to CD45 and did not significantly express any other markers was found. It was identified by Single-cell RNA-Seq as nuc-

leated erythrocytes. This population is remarkably detected mainly in extreme preterm babies (or at multiple births) and diminishes over time. Moreover, preliminary analysis links the immune state with clinical data, including antibiotics treatment. We show here a comprehensive study of the neonate immune system components which reveals patterns and similarities throughout its development and clinical state.

6. Cell type-specific dysregulation of CD38 expression in Systemic Lupus Erythematosus

Marie Burns

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by pathogenic autoantibodies secreted by plasma cells (PC). Among novel plasma cell depleting strategies, CD38 has been identified as promising target. The monoclonal anti-CD38 antibody daratumumab is approved for treatment of multiple myeloma and provided a therapeutically relevant depletion of PCs in patients with SLE (Ostendorf et. al, NEJM, 2020). However, CD38 is widely expressed across immune cells, and the cellular targets of daratumumab beyond PC, especially in patients with SLE, are largely unknown. Therefore, we used mass cytometry to systematically characterize the expression of CD38 in peripheral blood leukocytes to identify potential target cells of CD38-directed therapies that may contribute to or limit therapeutic benefits in SLE.

In a cohort of 20 SLE patients, CD38 was highly expressed on plasmablasts, basophils, NK cells, monocytes and plasmacytoid dendritic cells (pDC), the latter being important sources of type I interferons. CD38 expression in B and T lymphocyte subsets was heterogeneous, ranging from absence to very high levels. NK cells, pDC, class-switched memory B cells, marginal zone-like B cells, and CD8 central and effector memory T cells expressed significantly increased levels of CD38 in SLE compared to healthy controls. Naïve CD4 T cells and CD4 TEMRA cells were the only subsets exhibiting significant reduction of CD38 expression in SLE. CD38 expression levels were not associated with clinical activity of SLE. Spearman correlation analysis revealed coordinated CD38 expression in immune cells, with direct associations between most innate cell types and T cell subsets in healthy controls, while associations between innate and lymphoid cell CD38 expression levels were rare and limited to naïve T cells. By contrast, SLE patients displayed a heavily reconfigured correlation landscape, including multiple SLE-specific associations between lymphocyte subsets and innate immune cells.

In conclusion, we provide a comprehensive map of CD38 expression across the peripheral blood immune cells in SLE and controls. The dysregulated, commonly moderately increased expression of CD38 in SLE may establish increased susceptibility of immune cells to anti-CD38 in patients with SLE. In detail, our study identifies plasmacytoid dendritic cells, NK cells, and subsets of T and B cells as additional, potential targets of anti-CD38 treatment. Reconfigured cell-type specific CD38 expression in SLE suggests the presence of a common, likely inflammatory mechanism involved in tuning CD38 expression levels in leukocytes. While mechanistic studies will be required to evaluate the effects of anti-CD38 on the different CD38-expressing cells in SLE, our overall data support the rationale of anti-CD38 treatment in SLE.

7. Single-cell immune profiling of aged mouse tissues reveals ubiquitous contraction of the lymphoid compartment and organ-specific phagocyte adaptation.

Sinduya Krishnarajan

Sinduya Krishnarajah, Florian Ingelfinger, Ekaterina friebel, Dilay Cansever, Ana Amorim, Mirjam Lutz, Sarah Mundt, Frederike Ridder, Sebastian Stifter, Susanne Unger, Melanie Greter, Sonia Tugues, Donatella De Feo, Burkhard Becher.

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Ageing exerts profound and apparently paradoxical effects on the immune system, at once impairing cellular proliferation, cytotoxicity and phagocytosis, and inducing chronic inflammation. Previous studies have focused on individual tissues or cell types, while a comprehensive multi-system study of tissue-resident and circulating immune populations during ageing is lacking. Here we reveal an atlas of age-related changes in the abundance and phenotype of immune cell populations across twelve mouse tissues. Using high-parametric CyTOF-based single-cell mapping of samples from young and aged animals we identified conserved and tissue-type-specific patterns of both immune atrophy and expansion. We uncovered clear phenotypic changes in both lymphoid and myeloid lineages in aged mice, and in particular a contraction in natural killer cells and plasmacytoid dendritic cells. These changes correlated with a skewing towards myelopoiesis at the expense of lymphocyte production in aged mice. Taken together, this atlas represents a systematic and thorough resource of the age-dependent alterations of the mammalian immune system in lymphoid, barrier and solid tissues.

8. Multidimensional spatial profiling of immune contextures in colorectal cancer reveals heterogeneity within Consensus Molecular Subsets

Marieke Ijsselsteijn

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Colorectal cancers (CRCs), the 3th most commonly diagnosed cancer worldwide, can be categorized according to the presence or absence of DNA replication repair defects but also by a cancer's transcriptional signature. Defects in the DNA mismatch repair (MMR) system and, less frequently, in the proof-reading domain of polymerase ▫ (POLE), explain the high tumor mutation burden observed in up to 20% of all colorectal cancers. These cancers are also frequently assigned to the consensus molecular subtype (CMS) 1, which is characterized by a prominent contribution of immune cells towards the transcriptomic signature of this subset. Cancers with DNA replication repair defects are also more likely to respond to state-of-the-art immunotherapies as consequence of their immunogenic profiles. Nevertheless, evidence for the occurrence of anti-tumor immune reactions has also been found in other CMS types: CMS2, CMS3, and CMS4, that are characterized by Wnt signaling activation, metabolic adaptations, and dominance of a TGF-β-related signature, respectively. However, for a full overview of the biological complexity of cancer microenvironments and, specifically, the study of anti-tumor immunity, proteomic data is paramount.

We applied a 40-marker imaging mass cytometry (IMC) panel to characterize the cancer microenvironment in a cohort of colorectal cancers. This analysis reveals additional heterogeneity within CMS subsets and confirms previous observations that suggest the presence of inflammatory responses in the CMS4 subtype that could be exploited from a therapeutic point of view. Of note, while the CMS4 subtype appears to be equipped with T helper cells and (pro-inflammatory) myeloid subsets that could support an immune response, most notably, it lacks the presence of cytotoxic T cells, in particular intraepithelial CD8+

T cells. This profile is similar to some tumors classified in the CMS2 subtype while the CMS3 subtype appears to be the most deprived from anti-tumor immune responses. A strong inflammatory profile was confirmed in the CMS1 subset that was accompanied by a profuse infiltration by granulocytes. Interestingly, the immune suppressive molecule IDO was specifically overexpressed in CMS1 cancers and may constitute an important immune escape mechanism adopted by these tumors. Finally, we discovered an elusive immune cell subset, enriched in CMS4 tumors, displaying overexpression of CD38. Among other proposed functions, CD38 is involved in the production of adenosine, a potent immune suppressive molecule that may play a role in the suppression of immune response in colorectal cancer, most notably in CMS4 tumors.

9. Characterising spatially resolved cell phenotypes in Uveal Melanoma using Hyperion Imaging Mass Cytometry

Anika Novikov

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Uveal melanoma is the most common malignancy of the eye. Recent therapeutic efforts on immunotherapies for metastatic uveal melanoma, for example with IMCgp100, are designed to bring T lymphocytes in the vicinity of tumour cells. It is known that one of the most important variables for both disease progression and therapy sensitivity is the tumour microenvironment (TME), made out of blood vessels, non-malignant cells and immune cells. For the development of cancer therapies, especially immunotherapies, it is therefore crucial to assess the type of cells present in the TME and their interactions with cancer cells. In the T20p project, we have analysed 31 metastatic uveal melanoma samples with Hyperion Imaging Mass Cytometry (IMC), which is especially suited for TME analysis due to the possibility of investigating the spatial distribution of up to 40 antibodies/markers simultaneously.

We have developed a pipeline for identifying the phenotypes of cells observed in the Hyperion IMC images. The first step is the segmentation of the image pixels into single cells using Cellprofiler and Ilastik as proposed in Bodenmiller et al. ("A flexible image segmentation pipeline for heterogeneous multiplexed tissue images based on pixel classification; 2019; available at https://raw.githubusercontent.com/BodenmillerGroup/ImcSegmentationPipeline/development/documentation/imcsegmentationpipeline_documentation.pdf). Signals from single cells are then denoised, their intensity values are transformed and are clustered using flowSOM. Finally, the clusters are classified into phenotypes based on the presence and absence of specific markers. The pipeline will be made available in a user-friendly fashion.

IMC provided a good extension to NGS analysis generated from the same tumors, since it validated whether high expression of a gene came from the tumour or the surrounding cells. With regards to IMCgp100 therapy, we could confirm the infiltration of the tumour by lymphocytes during treatment, which has been described by Carvajal et al. ("Safety, efficacy and biology of the gp100 TCR-based bispecific T cell redirector IMCgp100 in advanced uveal melanoma"; 2018; Investigative Ophthalmology & Visual Science). Furthermore, we found an overall negative correlation between the level of immune infiltration and the number of KI67-positive replicating cells inside the tumour.

10. cyCombine: Robust integration of single cell cytometry data sets

Christina Bligaard Pedersen

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Abstract

Combining single cell cytometry data sets increases the analytical flexibility and the statistical power of analyses. However, technical heterogeneity is commonly observed between data from different experimental batches. We have developed a method to robustly combine data from cytometry experiments, regardless of whether the batch effects are subtle differences caused by the instrument being run at different times or by different operators, or major differences resulting from the use of different antibody clones, different reporters, at different sites, or even with different models of instruments. We demonstrate that regardless of the source of the batch effects, both the biological signals and the inherent structures of the data are retained, while minimizing technical noise. Our method is independent of controls (anchor samples), and computation scales linearly with the number of events. Additionally, we facilitate the merging of datasets with non-overlapping markers, enabling the extension of data depth without compromising breadth. We demonstrate the flexibility, robustness, and scalability of this algorithm on multiple different datasets and demonstrate quantifiable superior accuracy compared with existing methods.

11. Regressing out cell volume and unwanted covariances in CyTOF data

Rosario Astaburuaga

Rosario Astaburuaga1[,2] , Thomas Sell1[,2], Anja Sieber1[,2], Nils Blüthgen[1,2]

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Abstract

In single-cell measurements, the gene or protein expression might be confounded by cell-volume e□ects and other sources of unwanted covariance (eg.: labelling e□ciency), leading to spurious correlations between markers.

We propose a method to regress out unwanted covariances from CyTOF data. The approach consists on building a multivariate model for the expression of each measured protein yi , where the predictors are five surrogates of unwanted covariance: (1) Mean normalized iridiums (DNA), as the closest cell volume surrogate, given that we observed a strong positive correlation between mean normalized iridium and mean normalized ruthenium (Rapsomaniki et al. 2018), (2) Mean normalized palladiums used for barcoding, (3) platinum staining, (4) total ERK, (5) pan AKT.

Fig. 1

$$y_i = \beta_{0,i} + \underbrace{\sum_{j=1}^{5} \beta_{j,i} \cdot X_j}_{\text{surrogates}}$$

Then, the modified expression of each measured protein yi* is determined by the original mean

value of the marker and the residuals of the model:

Fig. 2

$$y_i^* = \beta_{i,0} + \underbrace{(y_i - \hat{y}_i)}_{\text{residuals}}$$

With this approach, we remove dependencies that the marker might have on any of the five surrogates of cell volume and unwanted covariance. After applying this method; (a) the marker distributions are thinner, indicating the removal of unwanted heterogeneity, (b) the spurious correlations are removed and authentic correlations are kept, allowing us to di▫erentiate between activated and non-activated signalling pathways, (c) the relationship between di▫erent samples change, allowing us to better detect di▫erences between conditions, and (d) the heterogeneities arising from di▫erent batches can also be removed by including an additional factor in equation (1).

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12. Computational reconstruction of changes in intracellular signalling networks of colon epithelium cells over the course of differentiation

Matthias Fischer

Matthias M. Fischer[1,2], Thomas Sell1, Mareen Lüthen[1], Markus Morkel[1], Christine Sers[1], Nils Blüthgen[1,2]

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Abstract

Colon epithelium cells are in a constant process of differentiation from stem to terminally differentiated cells. We used diffusion maps to reconstruct the phenotypic differentiation trajectory of human colon organoids to study the changes in intracellular signalling during differentiation. We found considerable effects of differentiation state on intracellular signalling which also depended on organoid culture conditions, stressing the importance of understanding intracellular signalling networks as non-static entities experiencing dynamic alterations during cellular differentiation.

13. A comprehensive unsupervised workflow for mass and flow cytometry

Guillaume Beyrend

Guillaume Beyrend, VisuaLyte, France

Abstract

The development of single-cell platforms offered an unprecedented number of dimensions to comprehensively characterize immunological data. Such a progress regarding immunological phenotyping has been enhanced by the development of flow, mass and spectral cytometry. This exceptional high-resolution exploration should be followed by an appropriate analysis of the data, where manual intervention of the users, like gating strategy, should be limited to guarantee a proper identification of subsets.

Packages or other analysis tools are continuously released, but the lack of a proper training to analyze flow and mass cytometry data needs to be overcome to completely take advantage of those new technologies.

We present a comprehensive training based on

open source software, in eight steps, for beginners, to ensure immunologists get the opportunity to apprehend themselves the data they generated. We showcased our workflow on two different datasets on flow and mass cytometry data and present the most used technologies and visualization tools that most immunologists would need in their research.

14. Deep Profiling of the Naïve Immune Response to P. *vivax*

Florian Bach

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Plasmodium vivax is the causative agent of a neglected tropical disease, vivax malaria, which accounts for more than half of all malaria cases in the Americas and South-East Asia. Globally, ~14 million annual cases present a significant clinical and economic burden, mainly in lower and middle-income countries. P. *vivax* poses a unique challenge for elimination: its dormant liver stage, the hypnozoite, can cause multiple episodes of malaria after just one infectious mosquito bite. The drugs that kill hypnozoites can lead to life-threatening hae-molysis in individuals with G6PD deficiency, a highly prevalent genetic polymorphism in many endemic populations. A vaccine would be a able to overcome this issue, but vaccine development e□orts are hampered by a lack of understanding of how naturally acquired immunity develops in humans, in part due a lack of culture-adapted parasite strains.

To address this, we developed the first P. *vivax* human challenge model in Europe and infected six volunteers with a Thai field isolate. We leveraged CyTOF, multi-analyte plasma profiling and whole blood RNAseq to create detailed time-courses of infection from baseline to convalescence for each volunteer. During infection, volunteers mount a potent systemic inflammatory response concurrent with pronounced lymphopenia. Both the lymphopenia and systemic inflammation largely resolved within six days after treatment. At this time-point, CyTOF revealed high levels of activation of all major T cell subsets, including CD4+, CD8+, MAIT and γδ T cells. Collectively, this activated fraction constituted 10-20 % of the peripheral T cell pool, far more than what is reported for many other human infections. More than half of all activated cells were CD4+ and exhibited diverse phenotypes. The dynamics of this T cell response did not correlate with systemic inflammation, but were associated with elevated levels of alanine aminotransferase in plasma, a marker for liver damage.

Our study resolved the human immune response to P. *vivax* in unprecedented detail. This forms a reference for future reinfection trials that can examine the acquisition of im-munity. The fulminant, possibly polyclonal, T cell activation associated with a first infection exhibited features of immunopathology and correlated with organ damage, emphasising the role of T cells in malaria pathogenesis.

15. Immune responses to controlled malaria infection in malaria naïve protected Europeans using mass cytometry

Yoanne Mouwenda

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Abstract

Malaria is a major health issue. Although drugs are available, it is still killing people worldwide, particularly in endemic regions and therefore much effort is put into developing a vaccine to prevent infection. The only well advanced malaria vaccine so far is RTS'S. However, it has partial protection (53%) against malaria in young children. There are now alternative vaccines being developed. A successful approach has been the development of attenuated parasites as vaccines. Attenuation can be either by irradiation of sporozoites or by chloroquine. In a recent study, protection was achieved by using the attenuation by chloroquine. To this end volunteers are put on chloroquine prophylaxis and then are given either live P. falciparum (vaccinated group) sporozoites or saline (control group). This ensures that the parasites are killed and induce a strong immunity. After 10 weeks of this vaccination period, the volunteers are exposed to malaria parasites and are followed up to see if they develop parasitemia or not and their PBMCs were collected. Here, we investigate the protection in 16 volunteers though controlled human infection. The control group (5 out of 5 participants) all developed parasitemia, while 6/11 from the vaccinated group were not protected, 5 out of 11 showed strong protection. Samples (PBMCs) collected at c-1 (day 1 before CHMI) and d11 (11 days after the CHMI) were immunophenotyped using mass cytometry. Two panels of antibodies, one directed at phenotyping and the other to assess function by measuring cytokines, were applied. Analyses are underway to identify immune subsets involved in protection against malaria and the cytokines that are produced.

Webinar by Fluidigm

Mass Cytometry – a glance into the future

Andrew Quong

Chief Scientific Officer Fluidigm Corporation

Fluidigm is committed to empowering the immunology community with research tools to interrogate immune cell function and tissue microenvironments in high dimensions. Using mass cytometry and genomics workflows, you can obtain unprecedented insight into cellular phenotypes and changes in rare cell populations.

Fluidigm technology can support the response to the global COVID-19 pandemic, enabling applications for SARS-CoV-2 virus detection and the monitoring of the immune system's response to COVID-19 disease. To that end, our technologies are well-positioned for use in providing a robust public research response in support of policies and treatments aligned with local, state, and national recovery.

Fluidigm CyTOF® technology with the Maxpar® Direct Immune Profiling Assay™ provides best-in-class immune monitoring with the cost, flexibility, and consistency needed for standardized COVID-19 disease immune monitoring research. Imaging Mass Cytometry™ adds the capability of spatial visualization of the immune response in tissue samples. This newly developed technology enables the study of clinical outcomes and changes in the inflammatory or immune function directly from whole blood samples or tissues. In addition, labs around the world have leveraged the benefits of Fluidigm microfluidics for research and have implemented high-throughput lab-developed tests (LDT) for SARS-CoV-2 detection.

Whether you seek to target new biomarkers and pathways or optimize the effectiveness of an immunotherapy or vaccine, Fluidigm can help you reach your next research breakthrough. Together we'll transform the future of care. Engage with us at fluidigm.com.

Life is complex. Simplify it. Standardized immune profiling with CyTOF technology.

Session 4 News from…

…DRFZ:

Severe COVID-19 is characterized by an increased induction of peripheral plasmablasts with an aberrant CD62L+HLA-DRlow phenotype

Axel Schulz

Mass Cytometry Core Facility, DRFZ Berlin, a Leibniz Institute

Abstract

The new pandemic coronavirus SARS-CoV-2 emerged in late 2019 and causes acute respiratory syndrome, leading to hospitalization, mechanical ventilation, and death in severe cases. Acutely induced plasmablasts (PB) play a central role in the adaptive immune response against SARS-CoV-2 by producing virus-specific, potentially neutralizing antibodies that help to resolve the infection. Here, we investigated and compared the dynamics and phenotypes of PB in the course of mild and severe SARS-CoV-2 infections.

We used two mass cytometric datasets that included up to three different time points of 9severe (WHO 5-7) and 8 mild (WHO 2-4) COVID-19 cases, as well as 9 healthy donors (HD), previously analyzed for dysregulations in the myeloid compartment (SchulteSchrepping et al., 2020, Cell). CD38hiCD27hi PB were identified using manual gating and opt-SNE and further characterized in OMIQ.ai. The use of SmartTube technology for whole blood preservation ensured the analysis of PB frequencies and phenotypes unaffected by any blood processing method.

We observed elevated frequencies and absolute counts of PB in both severe and mildCOVID-19 courses compared to HD. At early time points (day 4 – 12 post symptom onset) severe cases showed higher PB counts and frequencies among B cells, compared to patients with a mild course of disease (median severe 29%; mild, 19%; p=0.09). We further observed phenotypical differences of PB between both groups. Severe cases showed substantially higher proportions of CD62L+HLA-DRlow PB (p=0.06) and tended to have lower proportions of CD62LHLA-DRhi PB (p=0.2) than mild cases, an observation that became even more pronounced when the severely affected cohort was stratified by fatal outcome (p=0.02).

The strong induction of abberant CD62L+HLA-DRlow PB in peripheral blood, rarely detectable in steady state or induced by systemic vaccination in healthy controls, may indicate an inadequante PB response in severe SARS-CoV-2 infections. Their appearance early after symptom onset could help identify patients at risk of developing a severe or fatal course of COVID-19.

Biosketch

Axel Schulz joined the DRFZ mass cytometry laboratory in 2014 after gaining first experience with the CyTOF technology at the HIMC in Stanford in 2012. As a postdoc, he works on the technical implementation of the CyTOF technology in various projects and is currently the operator in charge of the institute's Helios instrument.

…Munich:

Establishing CyTOF panels for the analysis of murine immune cells

Selina Keppler/Marc Rosenbaum

MRI and TranslaTUM, Technical University Munich

Abstract

Based on the standard mouse phenotyping and intracellular cytokine panels, our work recently focused on establishing CyTOF workflows for the analysis of murine B cells, plasma cells, and regulatory T cells (Tregs). We will give a short update on challenges we experienced and solutions we came up with.

In order to achieve a deep phenotypic profiling of B cells and plasma cells, we used CyTOF analysis to investigate the expression of immunoglobulins, activation markers as well as co-stimulatory molecules in cells of gut-associated lymphoid tissues in our mouse models of inflammatory bowel disease. With the aim to obtain a detailed analysis of Tregs in the tumor microenvironment, we employed a subcutaneous tumor model in the Foxp3eGFP-Cre-ERT2 mouse strain. Tregs were identified with the Treg lineage marker Foxp3, CD25 and the presence of EGFP reporter expression, and suppressive markers expressed on Tregs such as CTLA-4, PD-1, ICOS and Perforin as well as cytokine production were determined.

Biosketch Selina Keppler

Selina currently is a Junior group leader at the Translational Cancer Center (TranslaTUM) at the Klinikum rechts der Isar in Munich. The Keppler lab is especially interested in the crosstalk of B cells with specialized inflammatory niches during autoimmunity, such as the gut or the kidney. In order to understand the complexity of autoimmune processes we combine imaging approaches with high-parametric flow cytometry, mass cytometry (CyTOF) and in vitro culture systems to define drivers of inflammation during homeostatic and inflammatory conditions.In addition to leading her research group, Selina is responsible for the training of users of the Core Facility of Cell Analysis in theory of flow cytometry, handling of the BDCanto and BDFortessa flow cytometers as well as multi-parametric panel design.

Biosketch Marc Rosenbaum

Marc is a senior post-doctoral researcher in the laboratory of Prof. Ruland at the Translational Cancer Center (TranslaTUM) at the Klinikum rechts der Isar in Munich. He has a long-standing interest in immunology, which he developed during studying Molecular Medicine at the University of Freiburg, the University of Western Australia, and the Max Planck Institute of Immunobiology and Epigenetics in Freiburg. Currently, he investigates the role of the CARD11-BCL10-MALT1 signaling complex in regulatory T cells. Besides, he trains users of the Core Facility of Cell Analysis in Amnis Imaging Flow Cytometry. In early 2020, he started working with the CyTOF technology and is interested in a detailed analysis of Tregs in the tumor microenvironment.

Keywords

mouse CyTOF panels, B cells, regulatory T cells

…Freiburg:

Alterations in tissue-resident memory and exhausted-like CD8+ T cells in active UC

Lena Sophie Mayer

Lena Sophie Mayer, Freiburg University Hospital and University of Pennsylvania

Abstract

T cells play a central role in the pathogenesis of inflammatory bowel disease (IBD) and represent a key therapeutic target. We analyzed intestinal and peripheral T cells from 116 IBD patients and 29 healthy controls (HC) using CyTOF panels focused on T cell polarization, memory, effector function, homing, and exhaustion. Relative to HC, IBD biopsies showed decreased abundance of CD8+CD69+CD103+ tissueresident memory T cells (TRM) and a concomitant increase in CD8+ T cells that upregulated PD-1 and other markers of T cell exhaustion (TEX-like). We also observed increased abundance of regulatory and conventional CD4+ T cells in IBD relative to HC. TEX-like cells were particularly prominent in active ulcerative colitis where distinct subsets corresponding to terminally differentiated and progenitor-like TEX were evident. Moreover, TEX-like cells retained expression of CD69, a key marker of tissue residence. Importantly, response to therapy was associated with restoration of conventional CD69+CD103+ TRM and downregulation of TEX signatures. Ongoing analyses will define the lineage relationships between TRM and TEX-like cells in IBD.

Biosketch

Lena Mayer holds a medical degree from the RWTH University, Aachen, Germany. In 2015, she completed her doctorate of Medicine at the Institute for Immunology, RWTH University, Aachen. Following that, she started her training as a doctor of Internal Medicine at the University Hospital of Freiburg in the Department of Medicine II. From 2018-2020, she completed a DFG-funded post doc in the Tomov and Wherry labs at the University of Pennsylvania, Philadelphia, USA, focusing on the role of T cells in Inflammatory Bowel Disease. From January 2021, she is continuing her residency and working as a clinician scientist at the Freiburg University Hospital.

Keywords

Inflammatory bowel disease, Tissue residency, T cell exhaustion

….Granada:

Experimental and data processing workflow for large-scale immune monitoring studies by mass cytometry

Paulina Rybakowska

Department of Medical Genomics, GENYO, Centre for Genomics and Oncological Research, Pfizer/University of Granada/Andalusian Regional Government, PTS, Granada, Spain.

Abstract

Mass cytometry is a powerful large-scale immune monitoring technology. It requires a careful experimental and analytical design to ensure a maximal data quality. Here I present an experimental protocol for whole blood analysis together with an r-based data analysis pipeline, which ensures the minimization of the experimental artifacts and batch effects, while ensuring data

reproducibility. Whole blood samples are fixed and frozen for the phenotyping study just upon drawing or after stimulation. Thus, this protocol is particularly suitable for multiday, multicenter and retrospective studies.

Biosketch

Paulina Rybakowska held her Master's degrees in Molecular Biology from University of Warsaw. She received a strong immunology background during a two-year internship in the laboratory of Umesh Deshmukh at the University of Virginia, Charlottesville and Oklahoma Medical Research foundation, Oklahoma City, as a scholar of the Visiting Research Graduate Traineeship Program (VRGTP). Currently, she is a PhD student at Marta Alarcón-Riquelme´s laboratory at GENYO (Centre for Genomics and Oncological Research). Her main interests are systemic autoimmune diseases and the application of single-cell technologies like flow and mass cytometry to immune monitoring studies. As an EMBO scholar she spent half a year at the Yvan Saeys laboratory in Ghent (Belgium), where she received training in programming and high dimensional cytometry data analysis.

Keywords

Retrospective studies, automated data preprocessing, whole blood immunophenotyping, reference sample

Session 5 Data Analysis

Multi OMICs data integration

Lucie Rodriguez

Karolinksa Institutet/ SciLifeLab, Dept. of Women's and Children's Health, Stockholm, Sweden

Abstract

Systems immunology is a recent research field nested under the field of systems biology that consists of measuring a variety of immunological functions as a way of discovering previously unknown phenomena as well as relationships. It is also a powerful way to get an understanding of the immune system as a whole. Due to the fact that immune cells and proteins do not function in isolation but rather continuously communicate with each other, using a multi-omics approach is helpful. Here I will present different approaches to integrate CyTOF data for multi-omics data analyses with examples stemming from systems-level immunomonitoring COVID-19 studies as well as Myalgic encephalomyelitis (ME/CFS) in order to better understand immune system regulation and function at the systems level.

Biosketch

Lucie is currently a Ph.D. candidate in Petter Brodin's lab at Karolinksa Institutet and Science for Life Laboratory in Stockholm, Sweden. Her research mainly focuses on systems-level analyses as a means to dissect the immune system in patients with poorly-defined inflammatory conditions.

Keywords

Omics integration, Systems Immunology, Phenotypic characterization

Publications

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„Google Maps" for tissue biology – Mapping the tumor microenvironment with spatial omics technologies

Denis Schapiro

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Biosketch

Dr. Denis Schapiro is currently an Independent Fellow at the Laboratory of Systems Pharmacology at Harvard Medical School and the Klarman Cell Observatory at the Broad Institute focusing on spatial transcriptomics and proteomics analysis. He is a Damon Runyon Quantitative Biology Fellow mentored by Prof. Peter Sorger and Prof. Aviv Regev. Previously, he was supported by the SNFS Mobility Fellowship.

Denis obtained his PhD from the University of Zurich and ETH Zurich in the laboratory of Prof. Bernd Bodenmiller where he worked on Imaging Mass Cytometry and corresponding analysis tools focusing on highly multiplexed image analysis. Denis is the lead developer of the histology topography cytometry analysis toolbox (histoCAT) and the multiple choice microscopy pipeline (MCMICRO).

Prior to this, he received his diploma (Dipl. Biol. (t.o)) at the University of Stuttgart and Harvard Medical School working with Prof. Peter Sorger and Prof. Alfred Goldberg. He was also an intern at the Complex Systems Modeling Group at Bayer AG in Leverkusen focusing on PBPK modeling.

Keywords

Spatial-omics, Quantitative Biology, Pathology

Differential discovery for CyTOF experiments

Mark Robinson

Statistical Bioinformatics, University Zurich, Switzerland

Biosketch

My research interests are diverse, but more-or-less encompass the general application of statistical methods and data science to experimental data with biological applications. Often, this is within the context of genomics data types, but we are interested in methodological challenges and robust solutions in data, generally. We also try to be modern scientists, with a focus on reproducibility (repos for code) and open science (preprints).

Webinar by Beckman Coulter/CytobankBy Beckmann Coulter

Navigate safely through a sea of data: management and analysis made smart!

Dr. Giulia Grazia

Beckman Coulter Life Sciences, Italy

With the advent of a global pandemic such as the one we are experiencing with COVID-19, the importance of understanding the immune response to viral infection is become increasingly important to clarify mechanisms at the basis of disease severity. Flow and mass cytometry are excellent techniques for such goals, being able to analyze many parameters simultaneously and several samples at a single-cell level. Every time you run such experiments, though, you end up with large data sets that must be analyzed, archived, shared and rendered traceable and auditable. Learn how to leverage the Cytobank platform, a leading cloud-based solution for high dimensional data management and analysis, to navigate safely in a sea of data: well-organized data sets are huge resources for future discoveries and with the new interface, creating meaningful figure in our platform is even easier. We'll also show you how to use the Application Programming Interface (API) to accelerate your analysis.

Virtual round table

Select speakers – Discussion of hot topics, Q&A

Chair: Henrik Mei

- **Denis Shapiro**
- **Chris Tape**
- **Chotima Böttcher**

Farewell and good bye

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