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Mass Cytometry—A Tool for the Curious: Networking in Berlin

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In the past decade, mass cytometry has been revolutionizing cytomics due to its ability for high-dimensional characterization of single cells using isotopetagged antibodies enabling the simultaneous interrogation of 40+ protein targets. It thereby captures the complexity of cellular systems at unprecedented depth and has been key to advances in human systems biology, in particular in the field of immunology and cancer biology. Cytomics by mass cytometry has also become an integral part of multi-OMICS studies. Recently, imaging mass cytometry and related techniques have been developed. These permit studying high-dimensional cellular features in histological sections using a similar approach, thereby allowing the analysis of cellular phenotypes and their location in solid tissue in outstanding detail (1). Improvements in mass cytometry protocols have overcome many initial shortcomings and now allow reliable and standardized sample processing. Along with that, mass cytometry has transitioned into an established technology. However, pioneering sites initially faced struggles with implementing the highest standards of the technology. As a consequence, in 2016, a group of mass cytometry experts teamed up to connect and found the German Mass Cytometry Network (GerMaNet) to promote the further development, implementation, and applications of mass cytometry primarily in biomedical research. Today, the network spans platforms at the DRFZ Berlin, the Berlin Institute of Health (BIH), the Center for Regenerative Therapies in Dresden (CRTD), the TranslaTUM at the Technical University Munich, the Max-Planck-Institute for Molecular Genetics in Berlin (MPI-MG), the University of Ulm, the Center for Molecular Medicine in Cologne, the Jena University Hospital, and the University Medical Center of Freiburg, with imaging units available at the BIH, MPI-MG, and in

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Freiburg. Close interactions exist with platforms in Prague (Czech Republic) and other platforms in Europe, the United States, and Australia.

Mass cytometry projects are commonly highly multidisciplinary research efforts, and their progress is much quicker when domain experts collaborate to contribute their expertise to achieve highest quality standards for mass cytometry data generation and interpretation. Relevant areas include

- instrumentation and basic mass spectrometry,
- chemistry underlying assays and reagents,
- experimental workflows suitable for large scale trials and minimal batch effects,
- efficient data logistics and data preprocessing, and
- data analysis software tools suitable for handling of high-dimensional single-cell data.

The complexity of mass cytometry projects frequently presents significant risks for individual researchers. To minimize hurdles, the German Mass Cytometry Network introduced several strategies to facilitate the exchange of expertise across different sites and helped to critically advance several projects. These strategies included mutual visits, joint troubleshooting, assay and data acquisition training, and shadowing for specific protocols. GerMaNet also helps the swift distribution of new reagents and data analysis tools among the centers. This approach has proven highly effective, as reflected by several joint publications within the GerMaNet (2–6).

As an important integral part of overall networking efforts, GerMaNet started hosting small but impactful and well received annual meetings primarily for national audience, the *German Mass Cytometry User Forum* (Table 1). Steered by academic mass cytometry labs and core facilities, the Forum provides a hub for exchange in the mass cytometry community in central Europe.

The past three meetings in 2018, 2019, and 2020 have attracted primarily academicians from Germany, but also numerous international guests and participants from 14 European countries, Israel, and the United States. In addition to presentations by leading researchers in the field, young scientists and students were particularly addressed by integrating a poster session and selection of oral presentations from submitted abstracts. The gathering also provided a forum to discuss novel solutions regarding mass cytometry instrumentation, lab devices, reagents, assays, and data analysis, including commercial products.

Table 1. The German Mass Cytometry User Forum in figures

	2018	2019	2020
Participants	83	109	105
from abroad	9	16	31
Industry	7	7	7
Abstracts	23	12	24
Invited speakers	5	7	7
Short talks	10	8	11

As the mass cytometry community grew larger and the technology gained maturity through multiple iterative assay and hardware improvements, the focus of the Forum shifted from technical aspects toward customized workflows to address diverse applications in immunology, immune-oncology, oncology, biology, nanotoxicology, biomarker identification, pathogenesis of immune-mediated diseases such as rheumatoid arthritis, SLE and multiple sclerosis, spanning works with human, mouse and drosophila cells. This development parallels the results from mass cytometry workshops held at CYTO (7,8). Many projects presented at the Forum were later published in peer-reviewed journals (3,4,9–13).

The 3rd German Mass Cytometry User Forum took place from January 23-24th in Berlin, Germany (Fig. 1). Speakers included Michael Leipold from Stanford University, giving this year`s ISAC lecture addressing the challenges of large mass cytometry studies that have been identified as a major hurdle for the implementation of mass cytometry to monitor clinical trials (7). There, unwanted data variation can be minimized by, for example, barcoding, and the careful decision of which samples to combine into a barcoded pool, implementation of instrument and assay controls, and antibody cocktail preservation to minimize reagent variation. Along with that, he stressed the importance of annotating and publicly sharing mass cytometry datasets, for example, via Mendeley, Cytobank, Immport, or FlowRepository.

Burkhard Becher (University of Zurich, Switzerland), Henrik Mei (DRFZ Berlin, Germany), and Marie-Laure Yaspo (MPI for Molecular Genetics, Berlin, Germany) showcased different application areas of mass cytometry for patient immune profiling, exploring the phenotypical setup of a specialized cell type, and precision medicine. For example, immune profiling of Multiple Sclerosis patients revealed an expansion of T helper cells expressing CXCR4 and GM-CSF in the blood, which may serve as a future therapeutic target in MS. Further, it was now discovered that human antibody-secreting plasma cells forming the basis of humoral immunity and memory are composed of a variety of different phenotypes, potentially permitting differential regulation of PC subsets in their bone marrow environment. Finally, the integration of multiplexed pathology data from imaging mass cytometry along with genomic and transcriptional data in a multi-OMICS approach was suggested. This promises direct impact on the care of cancer patients, to increase the benefit of precision medicine.

Mass cytometry hubs often maintain several collaborations, entailing the need for flexible and customizable data analysis solutions. In this regard, two examples for such pipelines were presented by Antonio Cosma (National Cytometry Platform, Luxembourg) and Thomas Höllt (Leiden University, The Netherlands), introducing a Tableau-based workflow and Cytosplore (www.cytosplore.org), respectively. Tyler Burns (Berlin, Germany) then discussed kNN-based preservation of local and global data structure preservation after dimensionality reduction by PCA, t-SNE, and UMAP, with

Figure 1. Impressions from the German Mass Cytometry User Forum. Photographers: Jacqueline Hirscher and Ute Hoffmann.

important implications for the reliability of gating and clustering in dimensionality-reduced data space.

Two workshops were dedicated to news and burning questions in mass cytometry. In the *Basics and Reagents* workshop Michael Leipold, Antonio Cosma, Marjolijn Hameetman (LUMC, Leiden), Henrik Mei, and Axel Schulz (moderator) reviewed and discussed the current needs in the field, that is, to achieve technically consistent data across a large number of samples and measurements, expanding the measurement capacity of mass cytometry, and, to ease the setup of mass cytometry assays. Concerning the latter, benefits of novel pre-made antibody panels suitable to characterize the most common immune cell populations (14) and designed for easy assay handling were discussed. The easy access to such commercially available panels can help speed up standard immune profiling by mass cytometry especially when combined with proprietary software for analyzing this assay: While this strategy may pave the way toward using mass cytometry in clinical practice, its customization is limited.

As doublets may present as artifacts in mass cytometry data, efforts aiming at their selective removal are ongoing. A novel workflow uses doublet discrimination based on Gaussian parameters (15). The panel agreed that this approach helps to improve doublet removal in the absence of barcoding but will need broader verification by the community. Doublet-filtering sample barcoding (16,17) and a moderate cell acquisition speed of approximately 300 cells per second appear as the currently most reliable option to minimize doublets. The discussion also centered around expanding the range of usable mass channels. Recent advances include antibody labeling with cadmium isotopes, adding up to seven channels for antibody-based barcoding or additional analytes. Further, an amine-reactive, column-free metal labeling approach has become available, which suggests itself for very limited probe amounts, or reduction-sensitive probes. The longer term stability of the conjugates and compatibility with a wider range of antibodies and other probes remains to be addressed. The panel highlighted the importance of in-house antibody cocktail stabilization by cryopreservation for the field (10), a method that helps reducing unwanted data variation and pipetting errors. The panel further discussed the implementation of anchor controls for batch normalization and pointed out that the control samples should be as similar to the assay samples as possible. Here, implementing lyophilized PBMC pre-labeled with tantalum were discussed as a possible solution, while important limitations arise from the fact that only those markers preserved in lyophilized cells can later be used for proper batch normalization. Finally, the panel encouraged industry to develop more advanced tools for assay standardization in mass cytometry, and to update standard reagents such as tuning solution and bead preparations to cover the expanding range of additional isotope masses now routinely measured at the far ends of the mass cytometers` detection range, such as yttrium and bismuth. Beads with gradually increasing metal content, similar as "Rainbow" calibration particles in flow cytometry, to calibrate and monitor the sensitivity mass cytometers more precisely were also desired. Recently described osmium-labeled polystyrene beads (9) could serve as a platform for such developments. Finally, the panel agreed that a broader availability

and use of live-cell barcoding options, for example, using antibodies targeting CD45 or beta-2-microglobulin (2,18,19), could significantly help to increase data quality.

The *Data Analysis* workshop moderated by Marie Urbicht, featured Burkhard Becher, Tyler Burns, Thomas Höllt, and Lars Rønn Olsen (Technical University of Denmark). Paralleling the maturation of the mass cytometry field as a whole, this year's lively discussion veered off questions on specific algorithmic tools and more toward the challenges of analyzing larger mass cytometry studies such as batch normalization. A key topic was the challenge to how to distinguish technical from biological variation and how to reliably detect and deal with batch effects. The panel recommended identifying potential batch effects in a study by appropriate visualization, for example, by generating a dimensionality-reduced plot colored by processing day of the samples. Normalization strategies based on anchor samples, which were published in 2019 (12,20) provide promising tools, however, they have yet to be systematically interrogated. Users were encouraged to exclude low-quality samples or entire faulty runs from further analysis rather than risking the accuracy of analysis of the entire dataset.

Again, the panelists emphasized the importance of publishing mass cytometry datasets along with publications. Not only because this should be considered good scientific practice as, for example, defined by the MIFlowCyt guidelines (21), but also because it would allow for a more robust benchmarking of computational tools on more datasets, also comprising non-hematopoietic cell types, such as solid tumors or tissue-resident cells. Furthermore, the development of community-wide standards on data annotation and analysis workflow documentation was identified as another mostly unmet need to ensure quality and reproducibility in dealing with highly multiplexed cytometry data.

The poster tour at the networking evening was organized by Désirée Kunkel (Berlin) and Sarah Warth (Ulm). Tomer Meir Salame (Weizmann Institute, Rehovot, Israel) was awarded this year`s poster prize for his presentation on the role of PD-1/PD-L1 and CCR2 in mouse models of Alzheimer`s Disease. Sufficient time was dedicated to personal communications to aid establishment new collaborations, importantly contributing to the success and serving the aims of the meeting and the network.

In sum, the German Mass Cytometry User Forum meetings have quickly established themselves as a platform for the national and international exchange of mass cytometry related research questions. The meetings have attracted growing numbers of attendees and industry, and increasingly received international attention (Table 1), demonstrating the demand for small and agile conferences that offer excellent opportunities for intimate scientific exchange regarding novel and specialized technologies in a supportive environment.

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