95-plex Deep Phenotyping Assay

Versatile multiparameter single-cell analysis platform enabling the discovery and ultra-deep phenotyping of unknown cellular subpopulations

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Single cell analysis has become increasingly important in basic and translational research. Over the past decade, most biological studies were conducted on cell populations that may be morphologically and genetically identical, but were actually heterogeneous, made up of individual cells with unique protein biomarker signatures.

Single cell analysis enables the study of cell-to-cell variations within a cell population. However, the current flow cytometry protocols are limited to 16-20 protein biomarkers, and the preparation of a high-plex panel is time-consuming. In this brochure, we present an overview of the validation data obtained for ChipCytometry, a groundbreaking and highly versatile imaging cytometry platform enabling several new applications [1,2,3,4,5,6,7]:

- **-easy and fast setup of panels containing 90+ biomarkers for ultra-deep phenotyping**
- **-sample storage during at least 24 months**
- **-sample reanalysis for new biomarkers**
- **-international research collaborations on rare diseases as the samples can be collected at the clinical site and analyzed later elsewhere**

NEXT GENERATION CYTOMETRY

ChipCytometry surpasses the already excellent quantitative phenotyping analysis of flow cytometry, and combines this asset with the unparalleled depth of information gained by microscopy, while preserving the integrity of surface and intracellular markers. Furthermore, ChipCytometry allows the samples to be stored and reanalyzed later, as new biomarkers suddenly become of interest during a study. The sample-to-result workflow is based on sequential cycles of staining, imaging and photobleaching, thus allowing intracellular and surface markers to be assayed on the same cells (Fig. 1).

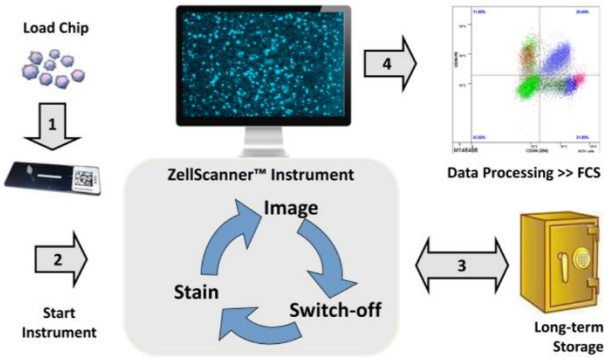

Figure 1: Imaging-based ChipCytometry uses rapid staining-imaging-photobleaching cycles for sequential multiplexing.

Finally, the server converts the raw images into conventional cytometry datasets (e.g. FCS format).

With conventional flow cytometry, the process of sample retrieval/preparation is directly connected to sample analysis. Depending on biomarker stability, the time window between sample preparation and analysis is only 30 minutes to 5 days.

Furthermore, the samples are lost during flow cytometric analysis, and a newly found population cannot be reanalyzed for additional markers using the same samples.

ChipCytometry stores the samples on activated silica surfaces within a microfluidic chip. Once a sample is loaded, the cells are fixed with a fixing buffer, and full biomarker integrity is preserved during at least 24 months. Signal intensities are very consistent over time (not shown) and there is no detectable shift in cytome composition (Fig. 2).

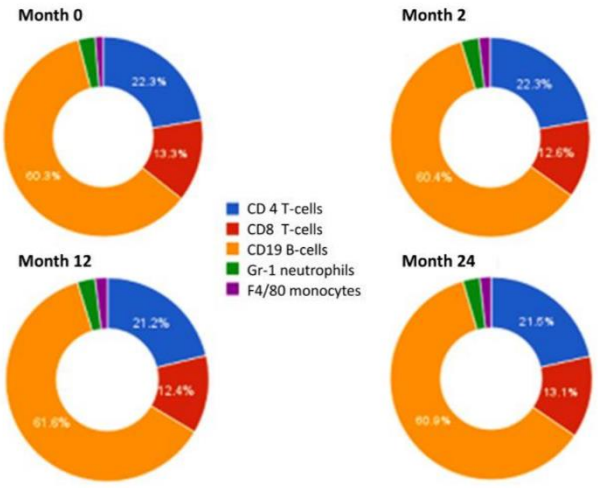

Figure 2: Validation of cytome stability over 24 months

Samples are typically stored at 4°C. Over a time window of 24 months, the same samples can be reanalyzed with additional biomarkers at least 10 times (not shown), similar to clinical samples being analyzed by immunoassays or RT-PCR. Thus, ChipCytometry unlocks the potential of international and even intercontinental collaborations (e.g. studies on rare diseases with only few patient samples per year) because samples can be collected at the clinical site and analyzed in a central laboratory.

95-PLEX BIOMARKER ANALYSIS

ChipCytometry generates multiparameter cytometric analyses even with very low cell numbers. Furthermore, it allows the reanalysis of newly discovered biomarkers on samples that have already been analyzed and characterized. Accordingly, ChipCytometry has become an extremely powerful tool for biomarker discovery and in-depth characterization, as well as biomarker validation and patient stratification. Currently, the human immune-phenotyping panel contains 95 markers (Table 1) and the mouse panel is built upon 39 biomarkers (data not shown).

ChipCytometry is a very versatile tool validated for a broad range of applications including biomarker discovery, ultradeep phenotyping of cell subsets or circulating tumor cells, live cell measurements (e.g. Ca2+ signaling), as well as studies on cell-cell interactions (e.g. immune synapses), receptor occupancy or internalization, and the mechanism of drug action.

Table 1: 95-plex immune cell phenotyping panel

CD3	CD31	CD81	CD195 (CCR5)	IgA
CD4	CD32	CD86	CD196 (CCR6)	IgD
CD5	CD34	CD90	CD197 (CCR7)	IgG
CD8	CD38	CD95	CD206	IgM
CD10	CD39	CD105	CD244	IL1b
CD11b	CD40	CD115	CD257 (BAFF)	IL8
CD11c	CD45	CD123	CD273 (PD-L2)	IL10
CD14	CD45RA	CD127	CD274 (PD-L1)	IL12
CD15	CD45RO	CD138	CD278 (ICOS)	IL17A
CD16	CD54	CD141	CD279 (PD-1)	IL17F
CD19	CD56	CD152 (CTLA-4)	CD319 (CRACC)	Ki-67
CD20	CD57	CD161	CD326 (EpCAM)	LC (κ)
CD21	CD64	CD163	AIOLOS (IKZF3)	LC (λ)
CD24	CD66b	CD172a/b	FoxP3	pan Cytokeratin
CD25	CD68	CD183 (CXCR3)	GM-CSF	RORγ(t)
CD27	CD69	CD184 (CXCR4)	Granzyme B	T-bet
CD28	CD71	CD185 (CXCR5)	Helios	TNFα
CD29	CD73	CD193 (CCR3)	HLA-DR	Vimentin
CD30	CD80	CD194 (CCR4)	IFNγ	Zap-70

INSTRUMENT & CONSUMABLES

Zellkraftwerk offers three types of chips to accommodate various sample types: cell suspensions, solid specimens, and rare cells. The cell suspension chips typically stores 250,000 cells, whereas the chip for rare cells typically stores 1,000,000 cells. Again, biomarker stability was validated for 24 months according to industry guidelines [8,9,10,11,12]. Sample analysis is performed by a fully automated ChipCytometry instrument (Fig. 3).

Figure 3: CYTOBOT™: Automated ChipCytometry instrument

LITERATURE

1. Hennig C. Et al. 2009. A versatile platform for comprehensive chip-based explorative cytometry. Cytometry A 75: 362-370.

2. Happle C. Et al. 2014. Pulmonary transplantation of macrophage progenitors as effective and long-lasting therapy for hereditary pulmonary alveolar proteinosis. Sci. Transl. Med.6: 250ra113.
3. Dijkstra D. Et al.. 2014. Identification and quantification of basophils in the airways of asthmatics following segmental allergen challenge. Cytometry A 95: 580-587.
4. Hennig C. Et al. 2013. High-content cytometry and transcriptomic biomarker profiling of human B-cell activation. J. Allergy Clin Immunol. 133: 172-180.
5. Dijkstra D. Et al. 2012. Basophils from humans with systemic lupus erythematosus do not express MHC-II. Nature Medicine. 18488-489.
6. Woelbeling F. Et al. 2011. Lung function and inflammation during murine Pseudomonas aeruginosa airway infection. Immunobiology 216:901-908.
7. Hennig C. Et al. 2010. Successful treatment of autoimmune and lymphoproliferative complications of patients with intrinsic B-cell immunodeficiencies with Rituximab. Br J Haematol. 148: 445-448.
8. Green C.L. et al. 2011. Recommendations for the validation of flow cytometric testing during drug development: I instrumentation. J. Immunol. Methods 363: 104-119
9. O'Hara et al. 2011. Recommendations for the validation of flow cytometric testing during drug development: II assays. J. Immunol. Methods 363: 120-134.
10. Cunliffe J. Et al. 2009. An approach to the validation of flow cytometry methods. Pharm. Res. 26: 2551-2557.
11. Lee J.W. et al. 2006. Fit-for Purpose Method Development and Validation for successful Biomarker Measurement. Pharm. Res.
12. Bioanalytical Method Validation, FDA Guidance, 2013

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