



Universiteit
Leiden

The Netherlands

Imaging the (un)imaginable of the Barrier Immune system

Guo, N.

Citation

Guo, N. (2023, April 18). *Imaging the (un)imaginable of the Barrier Immune system*.

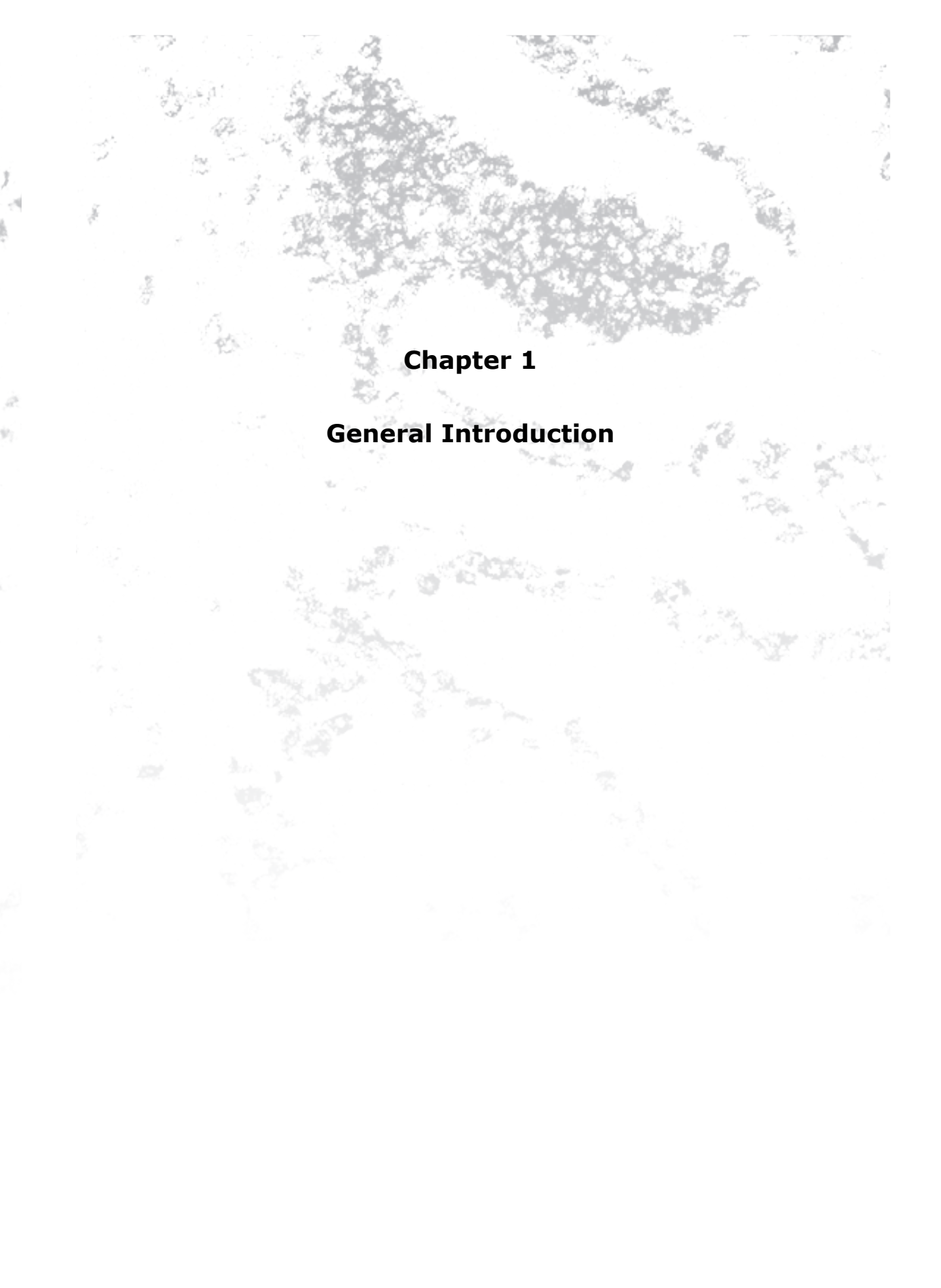
Retrieved from <https://hdl.handle.net/1887/3594146>

Version: Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/3594146>

Note: To cite this publication please use the final published version (if applicable).



Chapter 1

General Introduction

Barrier Organs

Both the skin and intestinal tract have a large surface area that is constantly exposed to a large array of microorganisms. To protect the organism against invading pathogens multiple protective strategies are employed. In the intestine a mucous covered epithelial layer separates the lumen from the intestinal tissue, while in the skin the epidermis forms the first line defense barrier. In addition, the intestinal epithelium and lamina propria are home to numerous immune cells that together form the largest immune compartment in the body. Here, aberrant immune responses to the intestinal microbiota is thought to underly chronic intestinal disease like Crohn's disease and ulcerative colitis. In contrast, under homeostatic circumstances the skin contain relatively few immune cells but is characterized by the presence of Langerhans cells (LCs) in the epidermis and dermal dendritic cells (DCs) in the underlying dermis. Upon infection, however, many immune cells can be recruited to the skin leading to local inflammation and may result in chronic disease condition like psoriasis and atopic dermatitis (AD). Thus, while barrier immunity has developed to maintain local homeostasis by continual crosstalk between environmental signals and the immune system, disturbance of this crosstalk can result in a variety of chronic diseases. Therefore, to characterize the innate and adaptive immune cells that are present in the skin and intestine, the investigation of immune compositions and interactions need to be explored.

Immune system of human intestine

The intestine harbors the largest compartment of the human immune system. The intestinal epithelium and underlying lamina propria are the major effector sites of immune cell accumulation within the intestine. The lamina propria contains B cells, T cells, and numerous innate immune cell populations including innate lymphoid cells (ILCs), DCs, macrophages, whereas the epithelium primarily contains T cells¹. While much is known about the organization and function of the intestinal immune system in adults, little is known about the population and development of the fetal intestinal immune compartment. As such we have a limited understanding of the molecular processes and cellular interactions that establish mucosal homeostasis and prepare the fetus for exposure to the invading microbiota right after birth. Given that disturbance of this homeostasis can lead to chronic inflammatory bowel diseases, more detailed information on how homeostasis is established and maintained will be required to better treat and prevent such diseases.

Immunohistochemistry analysis has shown that mature B cells were found in human fetal intestine at approximately 14 weeks of gestation^{2, 3}, of which around 10% express Ki-67 at gestational week 16 to 20². By 19 weeks, distinct follicles of B cells with variable levels of expression of IgM, IgD, CD5 and CD23 are present and surrounded by T cells³. Mass cytometric analysis revealed that follicular and transitional B cells are enriched in fetal intestines from gestational weeks 16 to 23⁴. Moreover, single-cell RNA sequencing (scRNA-seq) analysis of fetal intestinal B cells from gestational week 8 to 17, identified common lymphoid progenitor, pro-B, pre-B and immature B cells. Also, a subpopulation of pro-B cells were frequently present till gestational week 15, after which there was an increase in the number of immature B cells⁵.

Multiple single-cell cytometric technologies have been applied to investigate fetal intestinal T cells as well^{4, 6, 7}. CD3⁺CD4⁺CD8⁻ T cells were found to be present in both the epithelium and lamina propria of the human fetal intestine at gestational week 13, and their numbers increased with age⁴. Strikingly, tissue resident memory CD4⁺ and CD8⁺ T are abundant in human fetal intestines⁴. Here, multiple subsets of memory-like fetal intestinal CD4⁺ T cells were identified in the second trimester and were found to display distinct TCR repertoire characteristics⁷. Moreover, TNF- α -expressing effector memory T cells have been observed in human the human fetal intestine⁶. Based on the expression patterns of the chemokine receptors CXCR3 and CCR6, the majority of fetal intestinal T cells were found to display a Th1 phenotype⁴.

Innate lymphoid cells (ILCs) are a subset of innate immune cells that promote barrier immunity in the intestine and help to maintain immune homeostasis⁸. Recent analysis of the developmental pathways of the ILC family members indicates that natural killer (NK) cells, and non-cytotoxic helper ILCs, including ILC1s, ILC2s, ILC3s and lymphoid tissue inducer (LTi) cells are separate lineages^{9, 10}. Single-cell mass cytometry data has shown that around 50% of human fetal intestinal ILCs are NK cells^{4, 11}. In addition, ILC3s are also enriched with high expression of CD103¹¹ and CD69⁴ in human fetal intestines, suggesting tissue resident subtypes. Moreover, RORC-expressing LTi, commonly recognized as the initiator of secondary lymphoid organ formation¹², were found in the developing fetal intestines and to express CXCR5 and CCR6^{4, 5} to maintain the congregation of LTi there¹³. In addition, a unique population of the intermediate ILCs (Lin⁻CD7⁺CD127⁻CD45RO⁺CD56⁺) has been identified in the human fetal intestine that can give rise to NK cells and ILC3¹¹.

Antigen presenting cells (APCs) are essential in maintaining homeostasis and are involved in the recruitment of T cells to the intestine by processing and presenting antigens to T cells. Single-cell cytometry analysis has shown that APCs are the most abundant intestinal innate cell in the fetal samples from the 2nd trimester⁴. scRNAseq data of the human fetal intestine have revealed that there is an increased abundance of myeloid cells, including monocytes, DCs, HLA-DR⁺ macrophages, mast cells and megakaryocyte in the 2nd trimester compared to the 1st trimester⁵.

Thus, the fetal intestine already harbors a complex immune system that contains all major immune lineages long before birth. The presence of a sizable proportion of T cells expressing a memory phenotype in the second trimester suggests early exposure to exogenous antigens which may prepare the infant for the bacterial colonization upon birth. In the current thesis, we have explored novel approaches to map and follow the development of the human fetal intestinal immune system throughout the second trimester.

Immune system of skin

Historically, the skin is seen as an organ comprising an outermost layer, the epidermis, and a subjacent connective tissue, the dermis, that together provide a physical and biological barrier against chemical, physical and pathogenic insults. The skin is home to a variety of innate and adaptive immune cell populations that ensure protection against pathogens whilst maintaining tolerance to innocuous antigens, but can also contribute to the pathology of many inflammatory skin diseases¹⁴ (**Figure 1**). Skin barrier dysfunction is critical in inducing cutaneous inflammatory diseases, such as psoriasis as a chronic inflammatory skin disease, but also mycosis fungoides (MF) as the most common type of cutaneous T cell lymphoma.

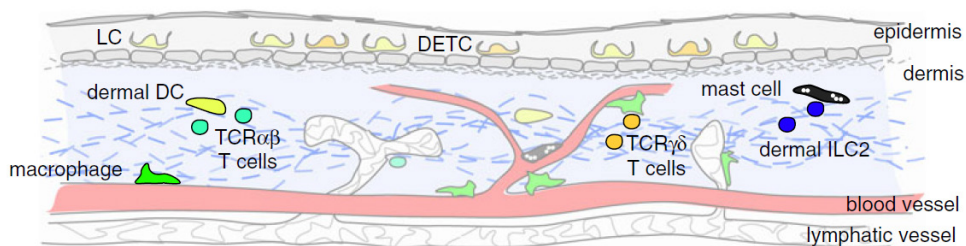


Figure 1. Schematic of skin resident immune cells. Dendritic epidermal T cell (DETC); Dendritic cell (DC); Group 2 innate lymphoid cells (ILC2); Langerhans cell (LC). Figure adapted from Tay et al. (2014)

Psoriasis

Psoriasis is an immune-mediated disorder primarily affecting the skin, in which T cell infiltration into epidermis and dermis is closely linked to disease pathogenesis and maintenance of inflammation¹⁵. Several T cells subsets, including central memory T cells, tissue-resident memory T cells, and exhausted and activated cytotoxic CD8⁺ T cells were identified in psoriasis by single-cell RNA sequencing analysis^{16, 17}. Psoriasis was considered to be a Th1-mediated skin disease for many years, because of the relative increase of circulating and skin-residing IFN- γ -producing T cells and upregulation expression genes involved in the IFN- γ immune response^{18, 19, 20}. However, since IL-17A-producing CD4⁺ T cells (Th17) were observed to be involved in the pathogenesis of psoriasis in mouse models²¹, Th17 cells were described as main instigators of psoriasis^{22, 23}. Moreover, Th17/Tc17 cytokines such as *IL17A*, *IL17F*, *IL26*, *IFNG*, and *CXCL13*²⁴ were predominantly produced by skin-resident memory T cells in psoriasis¹⁶.

1

Compared with uninflamed skin samples many myeloid subsets are increased in psoriasis. These include CD68⁺CD163⁺ macrophages, CD14⁺ classical monocytes and an inflammatory monocyte population with expression of *IL1B* and *IL23A*, HLA-DR⁺CD301⁺ DCs and CD207⁺ Langerhans cells, and mast cells²⁴. Among these innate immune subsets, DCs play a critical role in the development of psoriasis in both of the initiation and maintenance phase²⁵. LCs are a distinct immature DC subset that resides in the epidermis, and constitutes the first innate immune barrier against invading pathogens, but they have also been implicated in tolerance induction^{26, 27}. While the density of LCs is decreased in psoriatic skin due to epidermal hyperplasia, their numbers and morphology are similar to normal skin²⁸. However, mobilization and migration of LCs is profoundly impaired in psoriasis, even in non-lesional skin²⁹. A mouse model of psoriasis has identified resident LCs and monocyte-derived LCs, and it was observed that monocyte-derived LCs produce higher level of IL-23 to induce differentiation/activation of Th17 cells³⁰. For dermal DCs, a double immunofluorescence-based *in situ* study has shown that most CD11c⁺ dermal DCs co-express CD1c^{28, 31}. *In vitro* analysis of single-cell suspensions from psoriasis lesions increased numbers of both CD11c⁺CD1c⁻ resident DCs and CD11c⁺CD1c⁻ inflammatory DCs. Moreover, both DC types induced proliferation of T cells and the production of IL-17 and IFN- γ , while normal skin dermal DCs have no this ability³². In psoriatic lesions mature DCs present as dense cellular clusters, frequently aggregated with infiltrating T cells^{33, 34, 35, 36}. Thus, interfering with DC/T cell interaction has been considered to be an effective therapeutic strategy for psoriasis. In addition, it has been confirmed

that macrophages enable to impact on the pathogenesis of psoriasis especially the initiation phase. Studies in mice have demonstrated that the depletion of macrophages improved psoriasis inflammation^{37, 38}, and reduced the production of Th1 cytokines like IL-23 and TNF- α in the psoriatic lesion^{39, 40}. In human psoriatic plaques dermal macrophages expressed TNF- α ⁴¹. Moreover, the number of CD163⁺ macrophages was increased in psoriatic skin lesion in human, and decreased to non-lesional skin levels after treatment with TNF- α inhibitors⁴². So, next to DCs, it is worthwhile to study the contribution of T cells and macrophages to the development of psoriasis as well.

Mycosis fungoides

Mycosis fungoides (MF) is the most common type of cutaneous T cell lymphoma, which in the early stage presents with appearance of cutaneous patches or plaques covering a limited area of the skin⁴³. In the early stages, MF typically exhibits an indolent clinical behavior and this has been considered to be points in a disease continuum for many years. However, at some point in time the skin lesions can progress from early stages to the advanced stage, while other patients diagnosed never progress to the advanced-stage disease. So many studies aim to understand why some patients progress and other not and to identify markers that can predict progression to the advanced stage.

Histopathological analysis of MF lesions has reported skin infiltration of both malignant CD4⁺ and reactive CD8⁺ T cells, which exacerbates as the disease progresses⁴⁴. Here, MF with low numbers of CD8⁺ tumor infiltrating lymphocytes could represent an early stage of the disease⁴⁵. The analysis of scRNAseq and TCR sequencing for T cells from MF has showed that CD4⁺ T cells with a dominant clonotype expressed CTLA4 and KI67, while CD8⁺ T cells with a dominant clonotype exhibited the upregulation of markers associated with T-cell exhaustion and PD1/PDL1 signaling^{46, 47}. Moreover, loss of the cell surface markers CD7, CD2, CD3, and CD26 have been observed in the advanced stage of MF^{48, 49}. While early stage MF is characterized by a dominant Th1 cytokine pattern, there is a shift from a protective Th1 response to an immunosuppressive Th2 response during disease progression^{50, 51}.

Since 1976, the presence of CD1a⁺ DCs has been well established in MF T cell infiltrates⁵², and subsequent studies have investigated the role of DCs in MF progression^{53, 54}. In early stage MF, immature DCs (LCs) of the epidermis migrate to the dermis to become highly efficient antigen presenting cells in areas of

epidermotropic invasion of tumor T cells⁵⁵. The proportion of CD1a⁺ LCs and dermal DCs with differential expression of CD1c and CD207 are increased through all stages of MF, however, most significantly in the tumor stage of the disease^{47, 56}. Macrophages were previously designated to promote tumor growth by releasing many pro-angiogenic cytokines and growth factors^{57, 58}. The number of CD68⁺ and CD163⁺ macrophages significantly increases as the MF disease progressed^{47, 59}. Moreover, CD163⁺ macrophages were located in the invasive margin of the tumor in MF patients⁶⁰, and the CD163/CD68 ratio was highest at the tumor stage of MF, indicating that alternatively activated macrophages (also called M2 macrophages) are associated with disease progression⁵⁹, which fits with other observations that associate M2 macrophages with tumor cell growth and metastasis through modulation of angiogenesis and tissue remodeling^{57, 61}. Thus, ongoing interactions of T cells with DCs as well as macrophages in lesions of MF could lead to chronic T cell activation and inflammation associated with these lesions.



Although these immune subsets in the inflammation skin conditions has been explored, the investigation of composition and spatial characteristics of the cutaneous lymphocytes and myeloid cells infiltrate simultaneously has been incompletely characterized at single-cell level. Mass cytometry and spectral flow cytometry are novel tools to apply over 40 heavy metal or fluorochrome labelled markers as reporters for dissecting the immune composition in individual patients and diseases. Combined with imaging mass cytometry that enables the simultaneous spatial and phenotypic evaluation of up to 40 biomarkers in the tissue contexture. Therefore, these novel techniques can substantially contribute to a better understanding of the role of immune subsets in disease progression, knowledge that may be used for improved differential diagnosis and therapy of skin diseases.

High-dimensional single-cell cytometric technologies

Fluorescence-activated flow cytometry has been used for decades for the surface/intracellular protein expression single cell level. As such flow cytometry has been a key technology to unravel the complexity of the immune system. However, with conventional flow cytometry only a relatively limited number of antibodies can be applied simultaneously, prohibiting a detailed analysis of the ever expanding number of distinct immune subsets simultaneously. Novel single cell analysis technologies have emerged in the last decade that allow the incorporation of a far greater number of markers. Spectral flow cytometry, for example, can incorporate up to 40 antibodies can be measured at the single cell level in a single tube in a

single experiment^{62, 63, 64, 65}. These antibodies are labelled with spectrally diverse fluorochromes that can be simultaneously detected by the use of multiple lasers in combination with detectors that capture the emitted fluorescence across the full-spectrum. Here, the application of algorithms based on the Least Squares Method enables separation of overlapping fluorescent spectra (**Figure 2**)⁶³. Moreover, spectral flow cytometry can separate distinct autofluorescence signals, and the unmixing algorithm subtracts the autofluorescence signal to allow more accurate true fluorescence detection, thus improves signal-to-noise ratios and allows a more accurate phenotypic characterization of a large number of immune subsets in complex cellular mixtures⁶⁶. Nevertheless, researchers applying spectral flow cytometry are frequently facing challenges when it comes to the distinction of the individual emission patterns of the applied fluorochromes. The development of suitable antibody panels, therefore, is often a laborious process. Nevertheless, spectral flow cytometry allows a more detailed analysis of immune system heterogeneity.

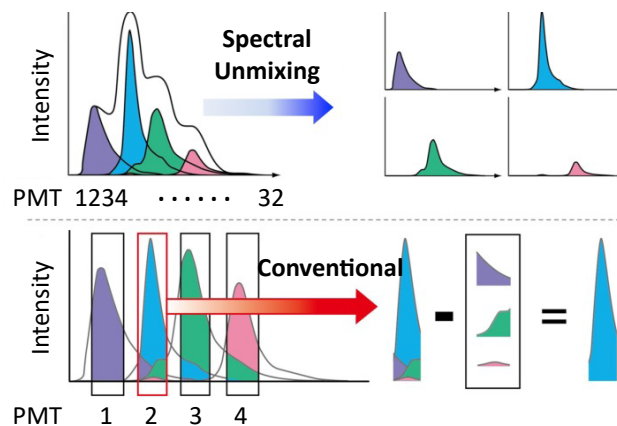


Figure 2. Principle of "Spectral Unmixing" and separation of fluorophores. The spectral flow cytometry unmixing algorithm uses all the emitted fluorescence as basic spectrum patterns to separate different spectrums (upper). In contrast, conventional flow cytometry utilizes only a narrow band of emitted light to remove overlapping fluorescence emissions (lower). Figure adapted from Koji et al. (2015)

Next to spectral flow cytometry, mass cytometry (CyTOF, also known as cytometry by time-of-flight) has been developed to utilize isotopically purified metal-tagged antibodies for the analysis single-cell suspensions. In mass cytometry, the antibody-stained cells are analyzed by breaking the individual antibody labelled cells to the atomic level followed by measurement of the cell bound metals with a time-of-flight mass spectrometer. A schematic overview of the mass cytometry

workflow⁶⁷ is shown in **Figure 4**. Currently, mass cytometry systems typically measure around 40 parameters per cell, but theoretically mass cytometry enables the detection of over 100 parameters simultaneously^{68, 69}. An important advantage of mass cytometry or flow cytometry is that the distinct masses of the applied metal tags allows for baseline separation, minimizing the amount of signal spillover from one parameter to another, thus eliminating the problem of fluorochrome spectral overlap in flow cytometry (**Figure 3**). Moreover, there is no interference from autofluorescence in mass cytometry. However, throughput rates per second by mass cytometry are much lower than modern flow cytometers, and only around 60% stained-cell were acquired. Also, unlike flow cytometry, it is not possible to collect cells after measurement by mass cytometer, as they are nebulized during the analysis procedure. Despite these disadvantages, mass cytometry has in the past decade been widely utilized to study the composition of the immune system in both health and disease, providing novel insights into the potential role of distinct immune subsets in chronic diseases and cancer.

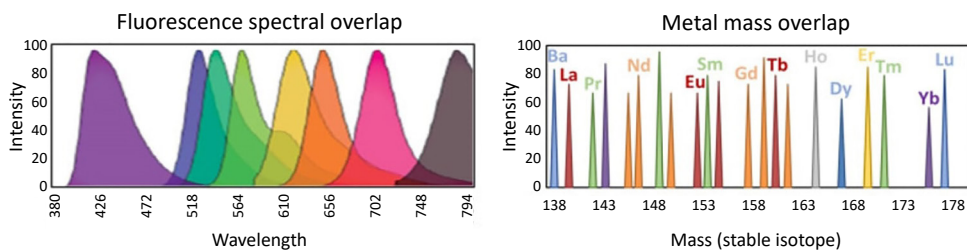


Figure 3. Overcoming spectral overlap via mass cytometry. Histograms demonstrating the emission spectra overlap that exists between different fluorophores by flow cytometry (left), and metal mass overlap that exists for heavy metals by mass cytometry (right). Figure adapted from Blair et al. (2019)

Single cell analysis with a 40-antibody panel simultaneously yields highly complex datasets that are difficult to interpret using the conventional gating strategies commonly used in flow cytometry. Since the inception of mass cytometry, t-distributed Stochastic Neighbor Embedding (t-SNE) has been widely used as a non-linear dimensionality reduction algorithm to obtain an unbiased overview of the immune subsets present in the dataset and visualize with the differential expression patterns of the various markers by which these subsets are defined^{70, 71}. However, tSNE can only analyze a limited number of cells and requires long computing time. To overcome these problems, Hierarchical Stochastic Neighbor Embedding (HSNE)⁷², optimized t-SNE (optSNE)⁷³, and Uniform Manifold Approximation and Projection (UMAP)⁷⁴ and have been developed to allow to analyze large high-

dimensional datasets on millions of cells rapidly. In addition, clustering-based algorithms are employed for the in-depth, including unsupervised Gaussian mean shift (GMS) clustering in Cytosplore⁷⁵, and FlowSOM in Cytobank⁷⁶. Moreover, a data science platform, OMIQ (<https://www.omiq.ai/>) has been built, which contains stepwise operations to accomplish cytometric data analysis in a workflow.

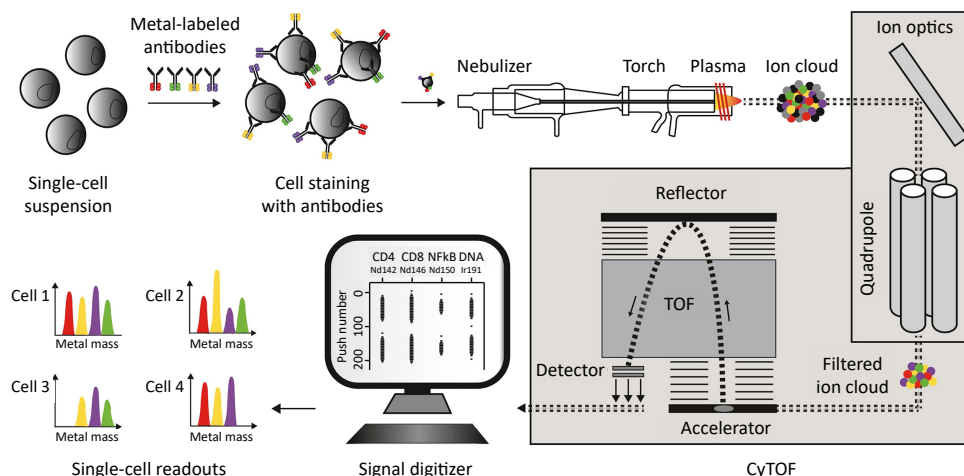


Figure 4. Sample preparation and analysis by mass cytometry. Adapted from Spitzer et al. (2016)

Imaging mass cytometry

Imaging mass cytometry (IMC) is an extension of mass cytometry, using metal-conjugated antibodies to label tissue samples, either formalin-fixed paraffin-embedded (FFPE) or snap-frozen samples that are subsequently ablated by a UV laser spot by spot with the use of the Hyperion⁷⁷. The ablated tissue with a 1- μ m resolution is then analyzed with the CyTOF instrument. Superior to conventional immunohistochemistry (IHC) and immunofluorescence (IF) techniques, IMC allows the simultaneous interrogation of over 40 markers in a single tissue section.

Figure 5 displays the workflow of imaging mass cytometry till the pre-analysis of data⁷⁸. In general, MCD™ Viewer is used to examine the data quality during the optimization of the staining procedure^{79, 80}. After validation of the full IMC antibody panel staining, computational approaches to visualize IMC data at the pixel-level and cell-level have been developed, like Cytosplore Imaging, Imacyte⁸¹ and Histocat⁸², which allow an analysis of the immune cell composition, their spatial organization, and cell-to-cell interactions in *situ*.

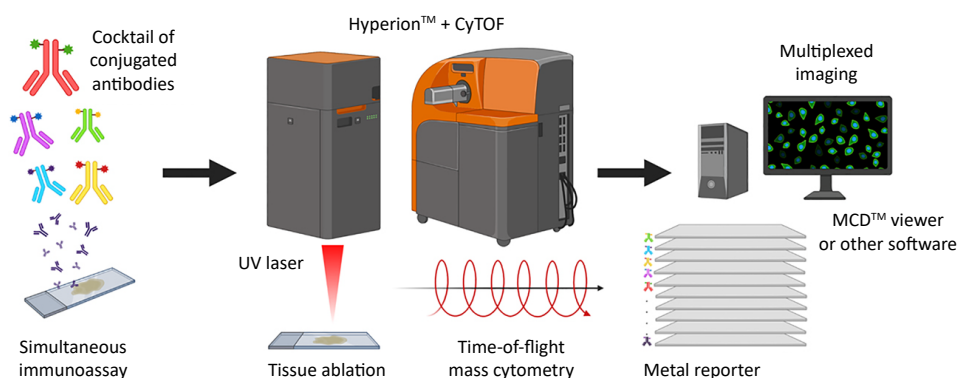


Figure 5. Imaging Mass Cytometry (IMC™) Workflow. Adapted from Cereceda et al. (2021)

Outline of the thesis

The novel high-dimensional cytometric techniques spectral flow cytometry and mass cytometry have provided an opportunities for high resolution comprehensive immune profiling of single-cell suspensions. Due to the dissociation of tissue materials by mechanical and enzymatic methods, however, spatial information on the distribution and interactions of immune cells is lacking. Therefore, imaging mass cytometry (IMC) complements the single cell technologies. In this thesis, I describe my work to characterize the intestinal and skin immune system by integration of single-cell immune profiles with their spatial distribution in the tissue contexture.

In **Chapter 2**, we provide an optimal IMC protocol for snap-frozen human fetal intestinal samples, which were also applied to snap-frozen human adult intestinal samples. Moreover, we present the tissue architecture and spatial distribution of the stromal cells and immune cells for both types of intestinal samples, revealing the colocalization of T cells, ILCs, and various myeloid cell subsets in the lamina propria of the human fetal intestine.

In **Chapter 3**, we further analyze a large number of human fetal intestinal samples from gestational week 14 through 22 by spectral flow cytometry and IMC. The spectral flow cytometry analysis identified stable clusters of Ki-67-expressing cells within all identified immune subsets that remain present in time. Moreover, with the use of the antibody panel developed in **Chapter 2**, IMC analysis revealed

formation of lymphoid follicles just below the epithelium in the developing intestine from week 16 onwards, harboring B, T, ILC and myeloid cells and confirmed the presence of Ki-67⁺ cells in the various immune subsets *in situ*. Finally, we observe that a CD69⁺CD117⁺CD161⁺CCR6⁺CD127⁺ phenotype is shared by subsets of fetal intestinal CD3⁺CD7⁺ ILCs and T cells and that these cells preferentially reside in the lymphoid structures, and harbor Ki-67⁺ cells, indicating a role in the development of these follicles. Overall, these observations indicate the presence of immune subset-committed cells capable of local proliferation, contributing to the development and growth of organized immune structures throughout the 2nd trimester in the human fetal intestine, presumably preparing the infant for the microbial colonization right after birth.

In **Chapter 4**, we use single-cell mass cytometry and IMC to obtain a comprehensive analysis of the immune system in skin biopsies of early stage MF patients, with the goal to improve recognition of early stage MF faster and more accurately. We identified phenotypically distinct subsets in both the CD4⁺ T cell and myeloid cell compartment that are shared by most MF patients. In addition, we find distinct CD4⁺ T cell subsets that are exclusively present in particular patients, potentially representing more advanced stages of disease where phenotypically distinct CD4⁺ T cells expand. Moreover, substantial numbers of CD4⁺ T cells co-localized with both CD1a⁺CD1c⁺ HLA-DR⁺ and CD1a⁺CD1c⁺HLA-DR⁺ DCs in the dermis. Importantly, the identification of prominent cellular aggregates between CD4⁺ T cells and myeloid cells in the dermis with a patient-unique cellular composition may provide a framework for improving mycosis fungoides diagnosis and development of treatment tailored to the characteristic features of these aggregates in individual patients.

In **Chapter 5**, we apply an updated IMC antibody panel to non-lesional and lesional skin from psoriasis patients to explore the complexity and organization of the immune compartment *in situ*. For this, we performed pixel-based analysis in *Cytosplore Imaging* for samples from nine psoriasis patients, by which the pixel-clusters associated with the major immune subset (CD3⁺CD7⁺ ILCs, B cells, CD4⁺ T cells, CD8⁺ T cells, DCs, macrophage and mast cells) were quantified and visualized in non-lesional and lesional skin from psoriasis patients. We demonstrate that clusters of pixels can be identified that have features of both T and myeloid cells and likely represent pixels at the interface between these cell types, allowing an unbiased quantification of cellular interactions in these tissue samples. The analysis of these “double feature” pixels revealed that T cells frequently colocalize with CD207⁺CD1c⁺ DCs in the dermis of lesion skin, and allow a classification of

these psoriasis patients in distinct groups. These data might guide more patient oriented treatment of psoriasis.

In **Chapter 6**, we discuss the main findings of this thesis and discuss potential future directions.

Reference

1. Mowat, A.M. & Agace, W.W. Regional specialization within the intestinal immune system. *Nat Rev Immunol* **14**, 667-685 (2014).
2. Golby, S. *et al.* B cell development and proliferation of mature B cells in human fetal intestine. *J Leukoc Biol* **72**, 279-284 (2002).
3. Spencer, J., MacDonald, T.T., Finn, T. & Isaacson, P.G. The development of gut associated lymphoid tissue in the terminal ileum of fetal human intestine. *Clin Exp Immunol* **64**, 536-543 (1986).
4. Stras, S.F. *et al.* Maturation of the Human Intestinal Immune System Occurs Early in Fetal Development. *Dev Cell* **51**, 357-373.e355 (2019).
5. Elmentaite, R. *et al.* Cells of the human intestinal tract mapped across space and time. *Nature* **597**, 250-255 (2021).
6. Schreurs, R. *et al.* Human Fetal TNF- α -Cytokine-Producing CD4(+) Effector Memory T Cells Promote Intestinal Development and Mediate Inflammation Early in Life. *Immunity* **50**, 462-476.e468 (2019).
7. Li, N. *et al.* Memory CD4(+) T cells are generated in the human fetal intestine. *Nat Immunol* **20**, 301-312 (2019).
8. Artis, D. & Spits, H. The biology of innate lymphoid cells. *Nature* **517**, 293-301 (2015).
9. Spits, H. *et al.* Innate lymphoid cells--a proposal for uniform nomenclature. *Nat Rev Immunol* **13**, 145-149 (2013).
10. Constantinides, M.G., McDonald, B.D., Verhoef, P.A. & Bendelac, A. A committed precursor to innate lymphoid cells. *Nature* **508**, 397-401 (2014).
11. Li, N. *et al.* Mass cytometry reveals innate lymphoid cell differentiation pathways in the human fetal intestine. *J Exp Med* **215**, 1383-1396 (2018).
12. Krishnamurthy, A.T. & Turley, S.J. Lymph node stromal cells: cartographers of the immune system. *Nat Immunol* **21**, 369-380 (2020).
13. Sécca, C. *et al.* Spatial distribution of LTI-like cells in intestinal mucosa regulates type 3 innate immunity. *Proc Natl Acad Sci U S A* **118** (2021).
14. Tay, S.S., Roediger, B., Tong, P.L., Tikoo, S. & Weninger, W. The Skin-Resident Immune Network. *Curr Dermatol Rep* **3**, 13-22 (2014).
15. Conrad, C. *et al.* Alpha1beta1 integrin is crucial for accumulation of epidermal T cells and the development of psoriasis. *Nat Med* **13**, 836-842 (2007).
16. Cook, C.P. *et al.* A single-cell transcriptional gradient in human cutaneous memory T cells restricts Th17/Tc17 identity. *Cell Rep Med* **3**, 100715 (2022).
17. Liu, J. *et al.* Single-cell RNA sequencing of psoriatic skin identifies pathogenic Tc17 cell subsets and reveals distinctions between CD8(+) T cells in autoimmunity and cancer. *J Allergy Clin Immunol* **147**, 2370-2380 (2021).
18. Austin, L.M., Ozawa, M., Kikuchi, T., Walters, I.B. & Krueger, J.G. The majority of epidermal T cells in Psoriasis vulgaris lesions can produce type 1 cytokines, interferon-gamma, interleukin-2, and tumor necrosis factor-alpha, defining TC1 (cytotoxic T lymphocyte) and TH1 effector populations: a type 1 differentiation bias is also measured in circulating blood T cells in psoriatic patients. *J Invest Dermatol* **113**, 752-759 (1999).
19. Szabo, S.K., Hammerberg, C., Yoshida, Y., Bata-Csorgo, Z. & Cooper, K.D. Identification and quantitation of interferon-gamma producing T cells in psoriatic lesions: localization to both CD4+ and CD8+ subsets. *J Invest Dermatol* **111**, 1072-1078 (1998).
20. Zhou, X. *et al.* Novel mechanisms of T-cell and dendritic cell activation revealed by profiling of psoriasis on the 63,100-element oligonucleotide array. *Physiol Genomics* **13**, 69-78 (2003).
21. Langrish, C.L. *et al.* IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* **201**, 233-240 (2005).
22. Lowes, M.A. *et al.* Psoriasis vulgaris lesions contain discrete populations of Th1 and Th17 T cells. *J Invest Dermatol* **128**, 1207-1211 (2008).
23. Cheuk, S. *et al.* Epidermal Th22 and Tc17 cells form a localized disease memory in clinically healed psoriasis. *J Immunol* **192**, 3111-3120 (2014).
24. Liu, Y. *et al.* Defining Patient-Level Molecular Heterogeneity in Psoriasis Vulgaris Based on Single-Cell Transcriptomics. *Front Immunol* **13**, 842651 (2022).

25. Glitzner, E. *et al.* Specific roles for dendritic cell subsets during initiation and progression of psoriasis. *EMBO Mol Med* **6**, 1312-1327 (2014).
26. Kissenpfennig, A. & Malissen, B. Langerhans cells--revisiting the paradigm using genetically engineered mice. *Trends Immunol* **27**, 132-139 (2006).
27. Romani, N., Holzmann, S., Tripp, C.H., Koch, F. & Stoitzner, P. Langerhans cells - dendritic cells of the epidermis. *Apmis* **111**, 725-740 (2003).
28. Zaba, L.C., Krueger, J.G. & Lowes, M.A. Resident and "inflammatory" dendritic cells in human skin. *J Invest Dermatol* **129**, 302-308 (2009).
29. Cumberbatch, M. *et al.* Impaired Langerhans cell migration in psoriasis. *J Exp Med* **203**, 953-960 (2006).
30. Lee, M. *et al.* Resident and monocyte-derived Langerhans cells are required for imiquimod-induced psoriasis-like dermatitis model. *J Dermatol Sci* **91**, 52-59 (2018).
31. Collin, M., McGovern, N. & Haniffa, M. Human dendritic cell subsets. *Immunology* **140**, 22-30 (2013).
32. Zaba, L.C. *et al.* Psoriasis is characterized by accumulation of immunostimulatory and Th1/Th17 cell-polarizing myeloid dendritic cells. *J Invest Dermatol* **129**, 79-88 (2009).
33. Lowes, M.A. *et al.* Increase in TNF-alpha and inducible nitric oxide synthase-expressing dendritic cells in psoriasis and reduction with efalizumab (anti-CD11a). *Proc Natl Acad Sci U S A* **102**, 19057-19062 (2005).
34. Nograles, K.E., Davidovici, B. & Krueger, J.G. New insights in the immunologic basis of psoriasis. *Semin Cutan Med Surg* **29**, 3-9 (2010).
35. Kim, T.G. *et al.* Dermal clusters of mature dendritic cells and T cells are associated with the CCL20/CCR6 chemokine system in chronic psoriasis. *J Invest Dermatol* **134**, 1462-1465 (2014).
36. Kim, T.G., Kim, D.S., Kim, H.P. & Lee, M.G. The pathophysiological role of dendritic cell subsets in psoriasis. *BMB Rep* **47**, 60-68 (2014).
37. Clark, R.A. & Kupper, T.S. Misbehaving macrophages in the pathogenesis of psoriasis. *J Clin Invest* **116**, 2084-2087 (2006).
38. Wang, H. *et al.* Activated macrophages are essential in a murine model for T cell-mediated chronic psoriasiform skin inflammation. *J Clin Invest* **116**, 2105-2114 (2006).
39. Ward, N.L. *et al.* Depletion of antigen-presenting cells by clodronate liposomes reverses the psoriatic skin phenotype in KC-Tie2 mice. *Br J Dermatol* **164**, 750-758 (2011).
40. Wang, Y. *et al.* Monocytes/Macrophages play a pathogenic role in IL-23 mediated psoriasis-like skin inflammation. *Sci Rep* **9**, 5310 (2019).
41. Marble, D.J., Gordon, K.B. & Nickoloff, B.J. Targeting TNFalpha rapidly reduces density of dendritic cells and macrophages in psoriatic plaques with restoration of epidermal keratinocyte differentiation. *J Dermatol Sci* **48**, 87-101 (2007).
42. Zaba, L.C. *et al.* Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. *J Exp Med* **204**, 3183-3194 (2007).
43. Pimpinelli, N. *et al.* Defining early mycosis fungoides. *J Am Acad Dermatol* **53**, 1053-1063 (2005).
44. Wong, H.K., Mishra, A., Hake, T. & Porcu, P. Evolving insights in the pathogenesis and therapy of cutaneous T-cell lymphoma (mycosis fungoides and Sezary syndrome). *Br J Haematol* **155**, 150-166 (2011).
45. Goteri, G. *et al.* Density of neoplastic lymphoid infiltrate, CD8+ T cells, and CD1a+ dendritic cells in mycosis fungoides. *J Clin Pathol* **56**, 453-458 (2003).
46. Gaydosik, A.M., Stonesifer, C.J., Khaleel, A.E., Geskin, L.J. & Fuschiotti, P. Single-Cell RNA Sequencing Unveils the Clonal and Transcriptional Landscape of Cutaneous T-Cell Lymphomas. *Clin Cancer Res* **28**, 2610-2622 (2022).
47. Rindler, K. *et al.* Single-cell RNA sequencing reveals markers of disease progression in primary cutaneous T-cell lymphoma. *Mol Cancer* **20**, 124 (2021).
48. Ormsby, A., Bergfeld, W.F., Tubbs, R.R. & Hsi, E.D. Evaluation of a new paraffin-reactive CD7 T-cell deletion marker and a polymerase chain reaction-based T-cell receptor gene rearrangement assay: implications for diagnosis of mycosis fungoides in community clinical practice. *J Am Acad Dermatol* **45**, 405-413 (2001).
49. Zhang, Y. *et al.* Molecular markers of early-stage mycosis fungoides. *J Invest Dermatol* **132**, 1698-1706 (2012).
50. Campbell, J.J., Clark, R.A., Watanabe, R. & Kupper, T.S. Sezary syndrome and mycosis fungoides arise from distinct T-cell subsets: a biologic rationale for their distinct clinical behaviors. *Blood* **116**, 767-771 (2010).
51. Wilcox, R.A. Cutaneous T-cell lymphoma: 2017 update on diagnosis, risk-stratification, and management. *Am J Hematol* **92**, 1085-1102 (2017).
52. Goos, M., Kaiserling, E. & Lennert, K. Mycosis fungoides: model for T-lymphocyte homing to the skin? *Br J Dermatol* **94**, 221-222 (1976).
53. Nestle, F.O. & Nickoloff, B.J. Role of dendritic cells in benign and malignant lymphocytic infiltrates of the skin. *Dermatol Clin* **12**, 271-282 (1994).
54. Pimpinelli, N., Santucci, M., Romagnoli, P. & Giannotti, B. Dendritic cells in T- and B-cell proliferation in the skin. *Dermatol Clin* **12**, 255-270 (1994).
55. Lüftl, M., Feng, A., Licha, E. & Schuler, G. Dendritic cells and apoptosis in mycosis fungoides. *Br J Dermatol* **147**, 1171-1179 (2002).
56. Cioplea, M. *et al.* Dendritic cell distribution in mycosis fungoides vs. inflammatory dermatosis and

- other T-cell skin lymphoma. *Oncol Lett* **17**, 4055-4059 (2019).
57. Qian, B.Z. & Pollard, J.W. Macrophage diversity enhances tumor progression and metastasis. *Cell* **141**, 39-51 (2010).
 58. Sugaya, M. *et al.* Association of the numbers of CD163(+) cells in lesional skin and serum levels of soluble CD163 with disease progression of cutaneous T cell lymphoma. *J Dermatol Sci* **68**, 45-51 (2012).
 59. El-Guindy, D.M., Elgarhy, L.H., Elkholy, R.A., Ali, D.A. & Helal, D.S. Potential role of tumor-associated macrophages and CD163/CD68 ratio in mycosis fungoides and Sézary syndrome in correlation with serum sCD163 and CCL22. *J Cutan Pathol* **49**, 261-273 (2022).
 60. Günther, C. *et al.* Up-regulation of the chemokine CCL18 by macrophages is a potential immunomodulatory pathway in cutaneous T-cell lymphoma. *Am J Pathol* **179**, 1434-1442 (2011).
 61. Gordon, S. & Martinez, F.O. Alternative activation of macrophages: mechanism and functions. *Immunity* **32**, 593-604 (2010).
 62. Park, L.M., Lannigan, J. & Jaimes, M.C. OMIP-069: Forty-Color Full Spectrum Flow Cytometry Panel for Deep Immunophenotyping of Major Cell Subsets in Human Peripheral Blood. *Cytometry A* **97**, 1044-1051 (2020).
 63. Futamura, K. *et al.* Novel full-spectral flow cytometry with multiple spectrally-adjacent fluorescent proteins and fluorochromes and visualization of in vivo cellular movement. *Cytometry A* **87**, 830-842 (2015).
 64. Ferrer-Font, L. *et al.* Panel Optimization for High-Dimensional Immunophenotyping Assays Using Full-Spectrum Flow Cytometry. *Curr Protoc* **1**, e222 (2021).
 65. Roederer, M. Multiparameter FACS analysis. *Curr Protoc Immunol* **Chapter 5**, Unit 5.8 (2002).
 66. Peixoto, M.M. *et al.* Identification of fetal liver stroma in spectral cytometry using the parameter autofluorescence. *Cytometry A* (2022).
 67. Spitzer, M.H. & Nolan, G.P. Mass Cytometry: Single Cells, Many Features. *Cell* **165**, 780-791 (2016).
 68. Bendall, S.C., Nolan, G.P., Roederer, M. & Chattopadhyay, P.K. A deep profiler's guide to cytometry. *Trends Immunol* **33**, 323-332 (2012).
 69. Chevrier, S. *et al.* Compensation of Signal Spillover in Suspension and Imaging Mass Cytometry. *Cell Syst* **6**, 612-620.e615 (2018).
 70. Van der Maaten, L. & Hinton, G. Visualizing data using t-SNE. *Journal of machine learning research* **9** (2008).
 71. Van Der Maaten, L. Accelerating t-SNE using tree-based algorithms. *The Journal of Machine Learning Research* **15**, 3221-3245 (2014).
 72. van Unen, V. *et al.* Visual analysis of mass cytometry data by hierarchical stochastic neighbour embedding reveals rare cell types. *Nat Commun* **8**, 1740 (2017).
 73. Belkina, A.C. *et al.* Automated optimized parameters for T-distributed stochastic neighbor embedding improve visualization and analysis of large datasets. *Nat Commun* **10**, 5415 (2019).
 74. McInnes, L., Healy, J. & Melville, J. Umap: Uniform manifold approximation and projection for dimension reduction. *arXiv preprint arXiv:1802.03426* (2018).
 75. Comaniciu, D. & Meer, P. Mean shift: a robust approach toward feature space analysis. *IEEE Transactions on Pattern Analysis and Machine Intelligence* **24**, 603-619 (2002).
 76. Van Gassen, S. *et al.* FlowSOM: Using self-organizing maps for visualization and interpretation of cytometry data. *Cytometry A* **87**, 636-645 (2015).
 77. Giesen, C. *et al.* Highly multiplexed imaging of tumor tissues with subcellular resolution by mass cytometry. *Nat Methods* **11**, 417-422 (2014).
 78. Cereceda, K., Jorquera, R. & Villarroel-Espíndola, F. Advances in mass cytometry and its applicability to digital pathology in clinical-translational cancer research. *Advances in Laboratory Medicine / Avances en Medicina de Laboratorio* **3**, 5-16 (2022).
 79. Ijsselstein, M.E., van der Breggen, R., Farina Sarasqueta, A., Koning, F. & de Miranda, N. A 40-Marker Panel for High Dimensional Characterization of Cancer Immune Microenvironments by Imaging Mass Cytometry. *Front Immunol* **10**, 2534 (2019).
 80. Guo, N. *et al.* A 34-Marker Panel for Imaging Mass Cytometric Analysis of Human Snap-Frozen Tissue. *Front Immunol* **11**, 1466 (2020).
 81. Somarakis, A., Unen, V.V., Koning, F., Lelieveldt, B. & Höllt, T. ImaCytE: Visual Exploration of Cellular Micro-Environments for Imaging Mass Cytometry Data. *IEEE Transactions on Visualization and Computer Graphics* **27**, 98-110 (2021).
 82. Schapiro, D. *et al.* histoCAT: analysis of cell phenotypes and interactions in multiplex image cytometry data. *Nat Methods* **14**, 873-876 (2017).

