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"OMIC Skin Histology & AI: The path to a new era of skin complexity analysis"

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1. Introduction

Understanding the structural and functional organization of skin tissue is essential for elucidating the mechanisms of skin homeostasis and its dysregulation in disease. As a dynamic and heterogeneous organ composed of multiple specialized cell types and extracellular components, the skin presents complex spatial relationships that are critical to its barrier function, immune surveillance, and regenerative capacity. A systems-level analysis of these interactions requires technologies capable of preserving and dissecting the native tissue architecture [1,2].

While high-dimensional single-cell approaches such as RNA sequencing and cytometry have advanced our understanding of cellular heterogeneity [3–5], they generally lack spatial context, which is crucial for studying tissue integrity, cell positioning, and intercellular communication in situ [6]. This limitation is particularly relevant in skin, where microanatomical compartmentalization and gradients of signaling molecules influence cell behavior and pathology [1,7].

Imaging Mass Cytometry (IMC) overcomes these challenges by enabling spatially resolved, multiplexed protein analysis within intact tissue sections. IMC combines laser ablation (with a lateral resolution of ~1 µm²) and time-of-flight mass cytometry to simultaneously detect up to 45 protein markers labeled with metal-tagged antibodies [8]. This allows for single-cell resolution analysis while maintaining tissue morphology, thereby facilitating the spatial mapping of cellular phenotypes and their microenvironmental context [9].

In skin tissue, IMC provides a powerful platform for profiling diverse cell types—including keratinocytes, fibroblasts, endothelial cells, and immune cells—while preserving information about epidermal and dermal architecture [1,7]. It enables detailed characterization of skin immune niches, detection of rare cell populations, and identification of spatial signatures associated with inflammation, aging, wound healing, or tumor development [1,5,10].

Compared to fluorescence-based multiplexed imaging, IMC avoids limitations such as spectral overlap and tissue autofluorescence, and does not require serial sectioning or iterative staining [6,9]. These advantages make IMC particularly suited for high-content, spatially resolved studies of skin pathology and response to treatment [10,11].

IMC has already demonstrated its power in oncology by revealing tumor-immune heterogeneity and spatial biomarkers across multiple cancer types [10–12]. However, its application is expanding beyond medical research into the dermo-cosmetic field, where understanding skin architecture and microenvironmental dynamics is increasingly important. Addressing the skin as a heterogeneous complex system allows the analysis of its cellular and acellular component phenotypes, functions and interactions and obtain a complete snapshot of the skin at each investigated state.

2. Materials and Methods

IMC antibody-panel : IMC, a High-Plex single-cell proteomic spatial imaging system was used to simultaneously visualize up to 40 markers and quantify over 200 targets in a unique skin histological section after a one staining step and image acquisition.
A 40-marker antibody panel was designed and optimized for formalin-fixed paraffin-embedded (FFPE) skin samples. The validation of the final antibody panel includes IHC/IMC staining comparison step and IMC staining condition optimization step. The validated panel allows the analysis of cellular and acellular skin components of epidermis and dermis layers with their functional specifications (Figure 1).

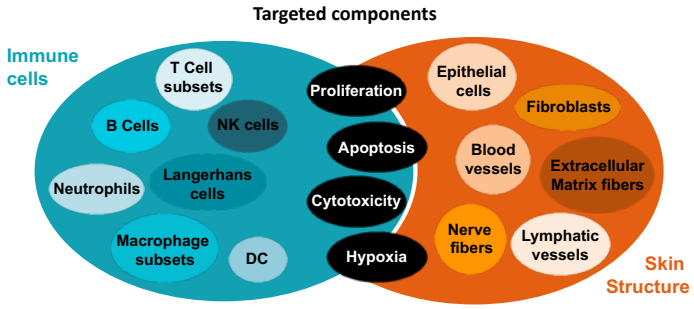

Figure 1. Scheme of the targeted skin compotents using the developed IMC antibody-panel allowing the analysis of key structural skin components, main dermis and epidermis resident cells and several infiltrating immune cells with their functional status, at once.

Image acquisition and computational analysis : Stained samples were imaged using a mass-cytometry-based imaging system (IMC) that is based on metal-conjugated antibody detection, avoiding the biases of fluorescence-based imaging methods. Combined to a tailored AI-based image analysis algorithms, quantitative evaluation of skin components' variations was performed to decipher active' effects.
The standard quantitative analysis process of high-dimensional images begins with the initial step of cell segmentation, followed by the identification of critical cellular characteristics like shape, location, and target expression (Figure 2). The accuracy of the initial single cell segmentation step significantly influences the quality and reliability of the biological outcomes attained through a study, underscoring its pivotal role in the data processing pipeline. We developed an end-to-end cell segmentation tool, and we inserted it into a framework oriented towards the improvement of the performance-to annotation cost ratio.

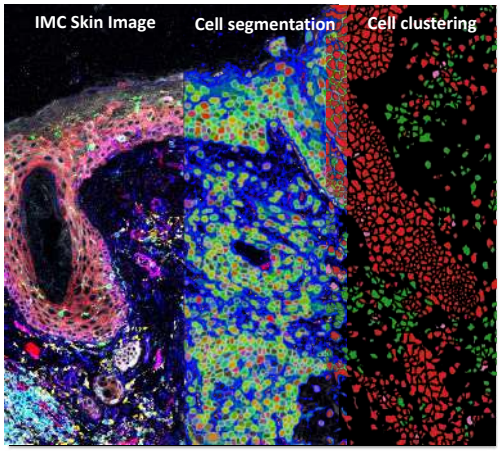

Figure 2. Steps of image analysis from raw IMC image to cell segmentation and cell clustering.

Tissue Material: 4 µm FFPE healthy skin sections from eight healthy donors explants were putchased from France Tissue Bank.

Antibody performance assessed by chromogenic immunohistochemistry (IHC): Sections were deparaffinized and submitted to antigen retrieval then endogenous peroxidase activity and unspecific protein-binding blockad. Each slide was then incubated with the primary antibody for 1 h 30 at room temperature. After washing, the slide was incubated with secondary horseradish peroxidaseconjugated antibody then antibody binding was revealed with diaminobenzidine as chromogenic substrate. The slide was then counterstained and dehydrated before mounting. The detailed protocole was previously described [17].

IMC high-plex staining : the detailed procedure was published in the method paper Elaldi et al. 2021 [17].

3. Results

IHC and IMC antibody clone validation before high-plexing:

Skin tissue IHC staining allows the validation of antibody clone specificity and efficiency (eg CD3 for T lymphocytes in Figure 3A). Antibody clone specificity is also validated with IMC co-staining, after antibody conjugation with metals. IHC staining and IMC staining are compared focusing on cell morphology, staining level and background level (Figure 3B). Colocation (eg CD3 and CD45) of cell specific markers is used to validate clone specificity (Figure 3C).

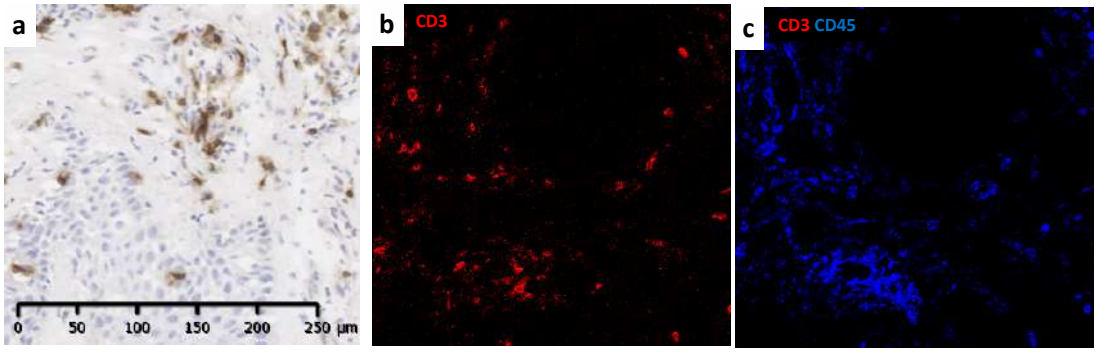

Figure 3. anti-CD3 clone validation using IHC (a) and IMC (b) single staining and high-plex staining for colocation validation with CD45 (c).

IMC staining optimization:

Staining conditions defined with IHC are optimized for IMC staining modulating antibody concentration and incubation time. Data are analyzed on 2 regions of interests (ROI1, ROI2) for each tested concentration for each antibody of the panel. Five cells were randomly picked in each ROI and maximum intensity values were recorded. Background was also evaluated on each ROI on 5 zones. Mean expression values for each concentration were calculated to choose the optimal condition (Figure 4).

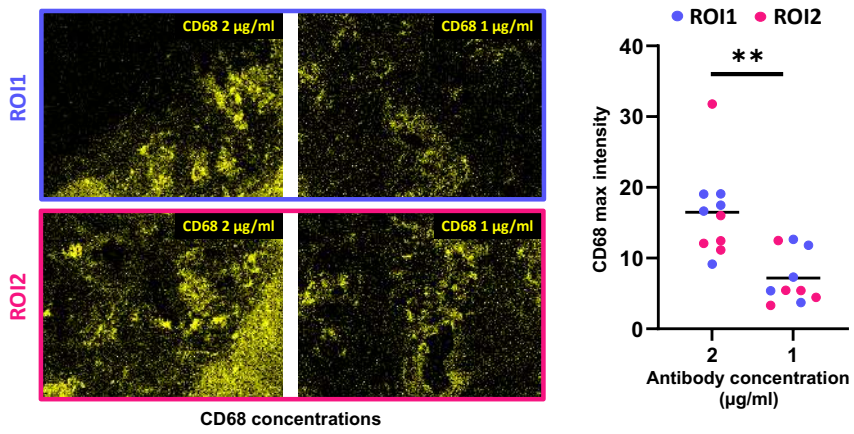

Figure 4. The anti-CD68 clone validated using IHC test for two concentrations (2 and 1 ug/mL).

Skin layers analysis using tissue segmentation:

The specific markers of the skin layers, transglutaminase, fillagrin and loricrin, claudine1, integrinA2 and collagen I and V, as shown in Figure 5A, allow (i) the automatic segmentation of each epidermis/dermis layer, stratum corneum, stratum granulosum, stratum spinosum, stratum basal and the dermis, respectively, and (ii) the quantification of the expression level of each targeted component (i.e.: Collagen, fibrillin, Filagrin…) and the mesurment of layers' thickness (Figure 5B-C).

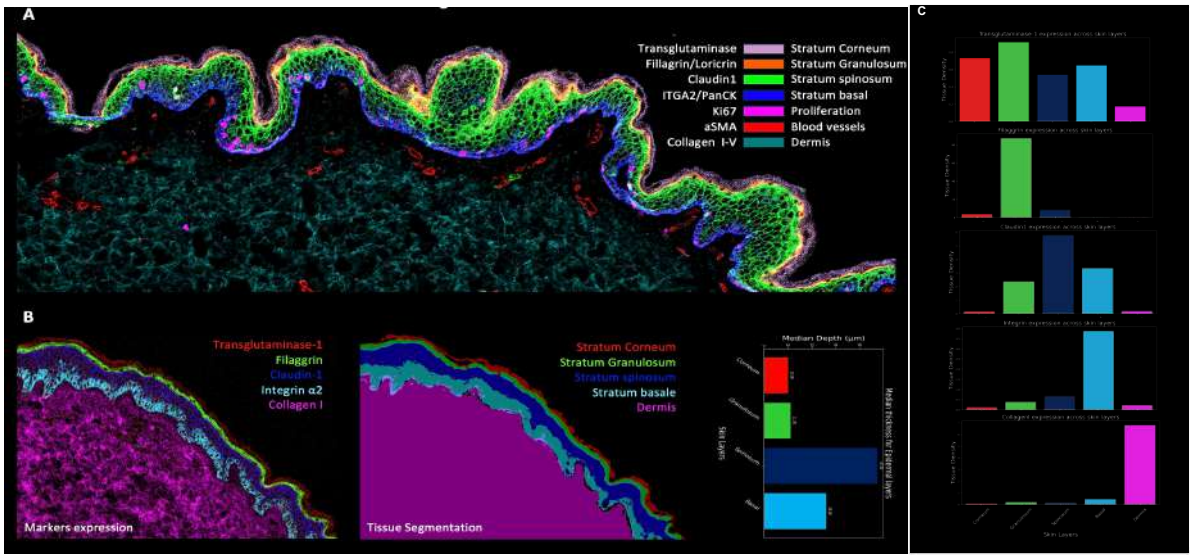

Figure 5. Skin layers analysis using dedicated markers (a) for automatic stratum delimitation, thickness measurement (b) and marker expression level per layer and stratum (c)

Skin inflammation and immune cell infiltrate analysis:

The cell type-specific markers allow the identification of skin resident and infiltrating cells (Figure 1), as shown in Figure 6, and define cell function for each type in each layer.

Immune cells are identified independently from skin structure cells (Figure 6A). Inate (macrophages) and adaptive (T lymphocytes) immune cells are clustered separatly (Figure 6B) and eight different macrophage subsets are defined based on the expression level of the used markers (CD68, CD204, CD206, CD209, CD163, CD14, HLA-DR) and their location in the skin (Figure 6C). A specific subset of macrophages expressing CD206, CD163, CD209 and CD14 markers is identified in the group of inflammed skins (Figure 6D).

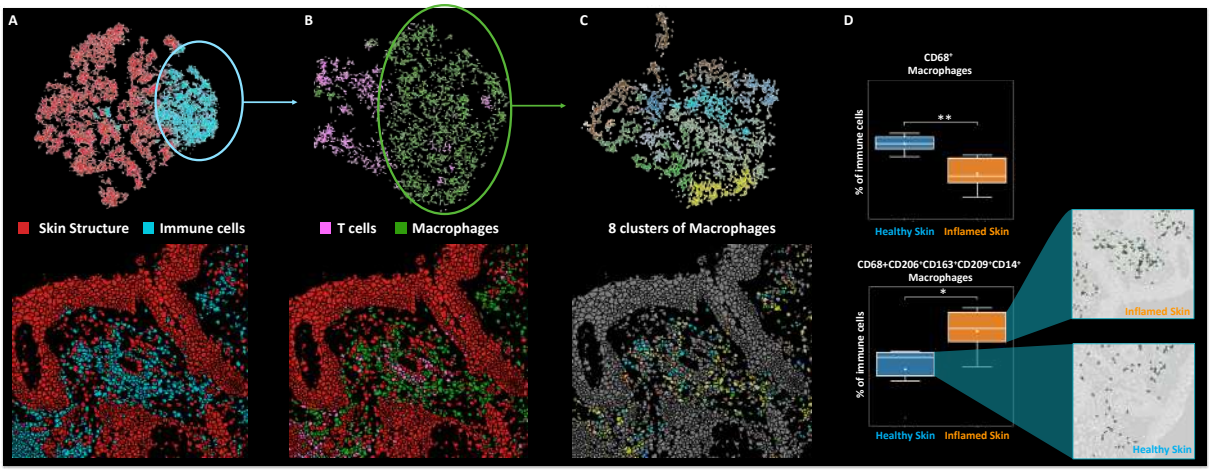

Figure 6. The designed IMC panel is used to characterize and compare the tissue immune infiltrate of inflamed skin (n=5) in comparison with healthy skin (n=6). The immune cells expressing CD45 are separated from Skin structure cells for downstream analysis (A). Within the Immune cell cluster, macrophage subsets and T cells are identified (B). Macrophages deeper characterization shows 8 clusters representing macrophages subsets (C). The statistical comparison of macrophage subsets abundance between the 2 analyzed skin groups, allows the identification of a significantly higher proportion of pro-inflammatory monocyte-derived macrophages expressing DC-SIGN in the inflamed skin while in healthy skin the majority of skin macrophages are resident cells and don't express any of the pro-inflammatory markers (D).

4. Discussion and Conclusion

Imaging Mass Cytometry technology allows to visualize more than 40 markers on one skin section and to analyze different tissue components and cell phenotypes in a quantitative way, avoiding tissue autofluorescence and fluorochrome issues.

The use of a selected skin markers allows the identification and the segmentation of the several skin layers. The combination of the cellular mask and layer definition in skin sample analysis enables a precise quantification of markers' expression across skin layers. The processed analysis drives to a global scheme of skin structure. Looking ahead, this approach can be adapted to explore various aspects of skin biology, such as aging, actives penetration, cell recruitment, tissue regeneration and disease progression (e.g., psoriasis, vitiligo, or skin cancer), and to identify biomarkers for personalized skincare and precision medicine.

In the presented study case analyzing inflamed skin immune infiltrate and deciphering skin microenvironment, several macrophage subsets are highlighted located mainly in the dermis. A deep computational analysis allows the identification of the proinflammatory macrophage phenotype that infiltrate inflamed skin. The computational analysis of the obtained skin high-plex images gives access to skin inflammation mechanisms, skin structure integrity and breakdown and skin regeneration from one image.

Such a comprehensive and integrative analysis that combines the insight of tissue distribution and the granularity of single cell analysis, drives to a global scheme that depict skin balance breakdown and allows deeper investigation of products' effects.

Refrences :

1. Salzer B, Ortner D, et al. Single-cell analysis of human skin identifies tissue-resident memory T cells. Nat Immunol. 2021;22(5):494–503.

2. Doe A, Nguyen T. Skin as a complex system: structure and signaling. Cell Commun Signal. 2020;18:42.
3. Li Y et al. Mapping skin cell phenotypes in health and disease. Nat Rev Dermatol. 2022;18(7):385–400.
4. Patel R, Cohen L. High-dimensional analysis of skin tissue using single-cell technologies. Cell Reports. 2023;34(5):108947.
5. Van Hove H, Martens L, et al. A single-cell atlas of mouse dermis and wound healing. Nature. 2020;582(7812):548–553.
6. Jones D et al. Limitations of single-cell RNA-seq for spatial tissue profiling. Genome Biol. 2022;23:12.
7. Aran D, Looney AP, Liu L, et al. Reference-based analysis of lung single-cell sequencing reveals a transitional profibrotic macrophage. Nat Immunol. 2019;20(2):163–72.
8. Salzer B, Ortner D, et al. Single-cell analysis of human skin identifies tissue-resident memory T cells. Nat Immunol. 2021;22(5):494-503.
9. Doe A, Nguyen T. Skin as a complex system: structure and signaling. Cell Commun Signal. 2020;18:42.
10. Li Y et al. Mapping skin cell phenotypes in health and disease. Nat Rev Dermatol. 2022;18(7):385-400.
11. Patel R, Cohen L. High-dimensional analysis of skin tissue using single-cell technologies. Cell Reports. 2023;34(5):108947.
12. Van Hove H, Martens L, et al. A single-cell atlas of mouse dermis and wound healing. Nature. 2020;582(7812):548-553.
13. Jones D et al. Limitations of single-cell RNA-seq for spatial tissue profiling. Genome Biol. 2022;23:12.
14. Aran D, Looney AP, Liu L, et al. Reference-based analysis of lung single-cell sequencing reveals a transitional profibrotic macrophage. Nat Immunol. 2019;20(2):163-72.
15. Elaldi R, Hemon P, et al. High Dimensional Imaging Mass Cytometry Panel to Visualize the Tumor Immune Microenvironment Contexture. Front. Immunol. 2021;12:666233.