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Longjie Li, Lily Vu, Paul Drury, Kok Haur Ong, Weimiao Yu & Philip L. Tong

Three-Dimensional Immune Cartography Uncovers Subclinical Remodeling in Psoriasis

Longjie LI[1, 2†], Lily VU[3, 4†], Paul DRURY[5†], Kok Haur ONG[1, 2], Weimiao YU[1, 2&] and Philip L. TONG[6, 7&]

1. Intelligent Digital and Molecular Pathology (iDMP) Lab, Bioinformatics Institute (BII), Agency for Science, Technology and Research (A*STAR), Singapore.
2. Institute of Molecule and Cell Biology (IMCB), Agency for Science, Technology and Research (A*STAR), Singapore.
3. Department of Anatomical Pathology, Westmead Hospital, NSW Health, Sydney, NSW, Australia.
4. Tissue Pathology and Diagnostic Oncology, Royal Prince Alfred Hospital, Sydney, NSW, Australia.
5. Western Diagnostics Laboratory, Perth, WA, Australia.
6. Department of Dermatology, St Vincent's Hospital, Darlinghurst, NSW, Australia.
7. DermScreen, Bondi Junction, NSW, Australia.

† These authors contributed to this work equally
& Corresponding senior authors
Correspondence to:

Dr. Philip Tong

Address: DermScreen, Ground Floor, 35 Spring Street, Bondi Junction NSW 2022, Australia.

Email: philip.tong@dermscreen.com.au

Dr. Weimiao Yu

Address: 30 Biopolis Street, Bioinformatics Institute (BII), Agency for Science, Technology and Research (A*STAR), Singapore.

Email: yu_weimiao@bii.a-star.edu.sg

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Abstract

Psoriasis has a complex immune microenvironment yet most spatial analyses remain two dimensional with routine histopathology. We examined whether immunohistochemistry (IHC) combined with three-dimensional (3D) digital reconstruction can quantify the immune-epithelial architecture across the psoriatic spectrum. Serial sections from 15 psoriasis patients (with lesional and peri-lesional skin 1 cm away from the psoriatic plaque) and 52 healthy donors were reconstructed into 174 tissue stacks (10,700 whole-slide images). $CD3^+$ T cells, $CD68^+$ myeloid cells and mast cells were mapped in 3D including their Euclidean distance to dermal–epidermal junction (DEJ). Compared with healthy controls, $CD3^+$ clusters were redistributed towards the superficial dermis, with reduced distance to the DEJ in peri-lesional and lesional skin in psoriatic patients; $CD68^+$ clusters showed a similar superficial shift in established plaques. In contrast, mast cell density and DEJ proximity did not differ between groups. Averaging multiple 2D sections obscured these distributional features, underscoring the value of volumetric analysis in spatial studies. Therefore, high-resolution 3D reconstruction reliably maps the spatial dynamics of T cells, macrophages and mast cells across the psoriatic spectrum. This accessible pipeline extends routine histopathology by providing quantitative 3D spatial metrics that complement routine conventional histology and may inform integration with higher-plex spatial platforms.

(200 words)

Key words:

Digital Pathology;

Psoriasis;

Serial Sectioning;

Tissue 3D Reconstruction;

Immune Cell 3D Distribution

Abbreviation:

DP: Digital Pathology

DEJ: Dermal-Epidermal Junction

MC: Mast Cell

MCT: Mast Cell Tryptase

Introduction

Psoriasis is a chronic dermatological condition driven by immune system dysregulation and abnormal keratinocyte differentiation. This process results in sharply demarcated, erythematous, and scaly plaques [1] but represent only part of a complex underlying immune dysregulation that involves both the innate and adaptive arms of the immune system. Whilst these plaques are surrounded by clinically healthy-looking skin, which in the literature is often referred to as non-lesional or uninvolved skin, there is strong evidence to suggest this skin is considered an intermediate state between healthy and lesional skin [2-3].

The disease pathogenesis often involves activating multiple antigen-presenting cells (APCs), T-cell subsets and cytokine pathways such as tumour-necrosis factor alpha (TNF-α), interleukins (IL-6, IL-20, IL-23), and nitric oxide [4-9]. Several T-cell subsets—most notably Th1, Th17, Tc1, and Tc17—play key roles in driving the inflammatory cascade through the production of cytokines such as interferon gamma (IFN-γ), TNF-α, IL-17, and IL-22 [1, 4-9]. These cytokines promote keratinocyte hyperproliferation, altered differentiation, and sustained inflammation in the epidermis. Tissue-resident memory T (Trm) cells are increasingly implicated in disease persistence and recurrence. $CD8^+$ Trm cells in the epidermis and $CD4^+$ Trm cells in the dermis, activated by IL-23, are thought to remain in the skin long after visible plaques resolve, contributing to relapse at previously affected sites [10].

Transcriptomic analyses have further revealed clear differences between lesional, non-lesional, and normal skin from healthy volunteers

[11-13]. Distinct gene expression profiles allow for differentiation between these tissue types. For example, Chiricozzi et al showed upregulation of IL17-signaling in non-lesional skin of moderate-to-severe psoriasis vulgaris compared with normal skin [11]. The higher expression of IL-17-signature genes (e.g. *DEFB4* and *S100A7*) also leads to increase activity of $CD3^+$, $CD8^+$ and DC-$LAMP^+$ cells in non-lesional skin compared to healthy controls [11]. Additionally, distinct gene transcriptional differences between psoriatic lesional and non-lesional skin have been demonstrated [12, 14]. Furthermore, in particular, genes such as *RGS1*, *SOCS3*, and *NAMPT* have been identified as markers that distinguish both lesional and non-lesional skin from those of healthy controls, while others—including *PTRRC*, *IL8*, and *ZC3H12A*—appear to differentiate non-lesional from both lesional and healthy tissue [14]. These findings support the notion that psoriasis exists on a spectrum, and that areas of skin which appear clinically unaffected may still reflect an altered, disease-prone state.

Recent advancements in techniques such as Cytometry by Time-of-Flight (CyTOF), NanoString's spatial platforms (GeoMX® Digital Spatial Profiler and CosMX™ Spatial Molecular Imager), Xenium™, and spatial transcriptomics (ST) have significantly enhanced the ability to visualize the distribution of immune cells in psoriasis [15-24]. CyTOF employs metal-tagged antibodies and direct isotope analysis to concurrently measure multiple markers, thereby eliminating the need for spectral overlap compensation and reducing laboratory errors [15-16]. NanoString's spatial platforms—GeoMX Digital Spatial Profiler and CosMX Spatial Molecular Imager [21]—make it possible to perform multiplexed spatial profiling of

both proteins and RNA. Recent advances in spatial transcriptomics now offer resolutions down to 5 µm, enabling near single-cell mapping of gene expression across entire tissue sections [25].

Despite these sophisticated techniques, there remains a need for accessible and reproducible tools available in routine pathology practices. Importantly, previous studies on immune cell presence and distribution in psoriasis have been largely confined to two-dimensional analyses, which do not fully capture the complex, three-dimensional nature of disease progression over time. As a result, the spatial organization and dynamics of key immune cell populations in the 3D tissue context of psoriasis remain poorly understood. In our study, we aim at using 3D computational analysis as an alternative method distinct from other approaches discussed above, to re-evaluate and validate previous findings regarding the spatial distribution and interactions of immune cells in psoriasis.

Among the available approaches for 3D reconstruction of skin tissue, two major methodologies are widely used: direct 3D imaging techniques and serial sectioning followed by image registration and volumetric stacking. While direct 3D imaging techniques—such as light-sheet fluorescence microscopy (LSFM) [26] and full-field optical coherence tomography (FFOCT) [27]—offer the advantage of in situ volumetric visualization without the need for physical sectioning or alignment, they are generally limited in their ability to incorporate specific immunohistochemical (IHC) markers due to restrictions in staining protocols and tissue compatibility. Consequently, these methods are typically suited for visualizing gross morphological features or structural

compartments, such as the stratum corneum, dermis, epidermal appendages, and collagen bundles, rather than enabling precise identification and localization of individual cell populations. In contrast, the serial sectioning approach is particularly well-suited for studies requiring cell-type-specific labelling. Since each section can be independently stained, it supports a wide range of histological and immunohistochemical protocols. This enables high-resolution, cell-level analysis of tissue architecture and cellular distribution such as that found in a 3D dermal model to quantify porosity and pore diameter [28]. Nevertheless, there remains a need for an accessible and reproducible tools in routine pathology practice. Importantly, previous studies on immune cell presence and distribution in psoriasis have been largely confined to two-dimensional analyses, which do not fully capture the complex, three-dimensional nature of disease progression over time. As a result, the spatial organization and dynamics of key immune cell populations in the 3D tissue context of psoriasis remain poorly understood.

In our study, we aim at using 3D computational analysis as an alternative method distinct from other approaches discussed above, to re-evaluate and validate previous findings regarding the spatial distribution and interactions of immune cells in psoriasis. Given our aim to map specific immune cell populations in skin, the compatibility of this approach with IHC staining and its ability to capture fine spatial detail make it the most appropriate choice for our study.

Results

Our pipeline is shown in Fig. 1, where Formalin-Fixed Paraffin-Embedded (FFPE) skin samples were trimmed and sectioned at 4μm interval. The tissue sections were then subject to immunohistochemistry (IHC) staining to visualize the targeted cell types. The stained sections were then scanned to generate whole slide images (Fig. 1a, 1b). Images were aligned by a Scale-Invariant Feature Transform (SIFT)-based algorithm and segmented by a random forest-based model, the segmentation results were validated manually (Fig. 1c, 1d). A reconstructed 3D tissue stack is shown in Fig. 1e, where red, green, blue represents immune cell, epidermis, and dermis respectively. The image alignment strategy is illustrated in Fig. 2a, every image was aligned to its previous image so that all images were eventually aligned to the first image. The effect of the image alignment is illustrated in Fig. 2b, the mean distance between matched SIFT key points reduced after the image alignment process, indicating the strategy we used effectively enhanced the image alignment quality. Fig. 2c demonstrated the necessity of 3D spatial analysis, the middle and right panel showed the distribution of a metric calculated in each individual slide, and the left panel showed the distribution of the same metric calculated in 3D and the average of 2D results. The difference between 3D calculation and 2D calculation average illustrated that the 3D spatial pattern could only be preserved by 3D calculation.

T-Cell 3D Architectural Remodelling Revealed by $CD3^+$ Cluster Mapping

Several key differences emerge in both T cell density and its proximity to the dermal–epidermal junction (DEJ) when comparing volunteer skin samples (herewith described as ‘healthy controls’ from individuals without psoriasis) to clinically uninvolved normal-appearing skin in adjacent psoriatic plaques (herewith described as ‘peri-lesional’) and psoriatic lesions (herewith described as ‘lesional’). For example, in Fig. 3a, compared with healthy controls, both peri-lesional and lesional tissues exhibited significantly shorter median distances between $CD3^+$ cells and the DEJ, with lesional skin showing the most pronounced superficial clustering. This data is consistent with previous findings, with a significant reduction in the distance of immune cell clusters—especially $CD3^+$ T cells—from the DEJ in psoriatic lesions compared to normal skin. The average median DEJ distance for $CD3^+$ T cells in lesional skin was approximately 70 µm, compared to over 450 µm in healthy controls. This superficial positioning reflects the migration of effector immune cells into proximity with proliferating keratinocytes—a hallmark of psoriasis pathophysiology [29].

In parallel, $CD3^+$ cell densities were markedly reduced in peri-lesional skin compared to controls, but increased again in lesional plaques (Fig. 3b), suggesting a dynamic redistribution of T cells during disease evolution. Probability density function (PDF) curves of $CD3^+$ cell distance to the DEJ further illustrated this shift, as shown in Fig. 3c, with a progressive leftward skew from normal to lesional samples, indicating a higher likelihood of T cells residing near the DEJ in disease states. These observations were corroborated by 3D visualisations (Fig. 3d–f), where

red-stained $CD3^+$ clusters increasingly occupied the superficial dermis as disease advanced. These data highlight the closer localization of T cell infiltrates to the DEJ in psoriasis and underscore the heightened immunologic activity characteristic of psoriatic plaques. Moreover, they strongly suggest that normal-appearing peri-lesional skin in psoriasis patients already demonstrates a distinct immunological architecture—in terms of T cell spatial relationship to the DEJ—compared with healthy control skin from non-psoriatic individuals.

Subclinical Myeloid Accumulation and Spatial Expansion of $CD68^+$ Aggregates Preceding Psoriatic Lesions

Given the proposed role of myeloid cells in early psoriatic immunological priming, we evaluated whether $CD68^+$ cell accumulation or spatial reorganisation could be detected volumetrically in peri-lesional psoriatic skin compared to healthy controls and lesional tissue. Fig. 4 illustrates the spatial remodeling of $CD68^+$ myeloid cells in psoriasis and supports the concept of subclinical immunological priming. Fig. 4a demonstrates a significant reduction in median $CD68^+$ cell distance to the dermal–epidermal junction (DEJ) in lesional psoriasis compared to both healthy controls and peri-lesional skin ($p = 5.78\times10^{-4}$ and 8.31×10^{-3}, respectively). Notably, although peri-lesional skin appears clinically normal in patients with psoriasis, its $CD68^+$ cells are already positioned significantly closer to the DEJ than in healthy controls, indicating early immune recruitment. Fig. 4b shows a marked increase in $CD68^+$ cell density in lesional skin, significantly higher than both peri-lesional ($p = 2.84\times10^{-3}$) and healthy control tissue ($p = 2.11\times10^{-2}$), consistent with

macrophage accumulation during active disease [30-31]. Fig. 4c further substantiates this with PDF curves revealing a leftward shift—indicating higher density of $CD68^+$ cells near the DEJ—in lesional and peri-lesional samples, compared to the broader and deeper distribution in healthy control skin. The 3D renderings in Fig. 4d-f offer visual confirmation of these findings, showing a progressive upward migration and clustering of $CD68^+$ cells (red) toward the epidermis (green) from normal (Fig. 4d) to peri-lesional (Fig. 4e) to lesional (Fig. 4f) states. Together, these spatial and volumetric data suggest that peri-lesional skin is not immunologically quiescent, but harbours latent macrophage remodeling that may presage overt plaque formation [2, 32].

In parallel, analyses of $CD68^+$ cell distributions in healthy control skin, peri-lesional and lesional psoriatic skin indicate that clinically "normal" peri-lesional skin in psoriasis patients differs significantly from healthy volunteers without psoriasis. We observed a decrease in the distance of $CD68^+$ macrophages to the DEJ in lesional skin ($p = 5.78 \times 10^{-4}$), suggesting enhanced macrophage activity near the epidermis during active disease. Closer proximity to the dermal–epidermal junction (DEJ) in peri-lesional sites support the hypothesis that an immunologically primed microenvironment is present before overt lesion formation. This observation is supported with prior transcriptomic analyses showing that clinically uninvolved psoriatic skin already exhibits elevated expression of inflammatory pathways and activated immune signatures long before visible plaque development, indicating that psoriasis arises from a pre-

existing ‘subclinical inflammatory state’ rather than de novo immune action [3, 33].

Mast cell spatial patterns are similar across psoriatic diseases states

Classic histological studies have found mast cell (MC) densities to be higher in the superficial dermis in lesional psoriasis skin than non-lesional and healthy controls whilst mast cell densities was similar between non-lesional psoriatic skin and healthy controls [34]. Recent findings, highlighting variations in the spatial distribution of MC clusters relative to the dermal–epidermal junction (DEJ), lend further support to their dynamic, stage-dependent function. In peri-lesional regions, elevated MC distance from the DEJ may signal an early, amplified inflammatory drive, aligning with previous reports of heightened MC recruitment and activation at the inception of lesion formation. Subsequently, in fully established psoriatic plaques, MCs appear to re-localize closer to the superficial dermis. This shift could represent a functional or phenotypic transition, as MCs modulate their pro-inflammatory mediator output to sustain chronic inflammation rather than initiate it [35].

These positional changes corroborate earlier clinical and histological observations that MCs surge in number and activity during the initial phases of plaque development, only to decline or assume altered functional profiles in mature lesions. In Fig. 5a, median distance of MCs to the dermal–epidermal junction (DEJ) is broadly similar across groups, suggesting that mast cells initially accumulate deeper in the dermis during early inflammation. Fig. 5b shows that mast cell density is modestly

reduced in peri-lesional samples, with partial restoration in lesional skin. The PDF curves in Fig. 5c illustrate these trends more clearly: lesional tissue shows a left-shifted peak (towards superficial clustering) relative to normal, consistent with MC migration toward the DEJ in mature plaques whilst non-lesional psoriasis skin appears to have a bimodal distribution from the DEJ to the deep dermis. These spatial findings are visualized in Fig. 5d–f, where representative 3D renderings show deep dermal distribution in normal skin (Fig. 5d), increased but scattered clustering in peri-lesional skin (Fig. 5e), and more dense, superficial aggregation in lesional samples (Fig. 5f). Together, these data could suggest a biphasic pattern wherein MCs initially localize farther from the DEJ in peri-lesional skin—potentially reflecting active recruitment or redistribution—then migrate toward the superficial dermis as fully developed plaques become established. This biphasic pattern may reflect the shifting functional roles of mast cells—from initiating inflammation to sustaining chronic lesions [5].

Discussion

This study applied a 3D reconstruction and quantitative spatial analysis pipeline to FFPE human skin to investigate immune-epithelial architecture across the psoriatic spectrum. By integrating serial sectioning, image registration, segmentation and volumetric reconstruction, we demonstrated that routine IHC could be extended into 3D to resolve spatial patterns that are not readily appreciated in conventional 2D histology. This approach provides a technically accessible method for volumetric immune mapping and offers complementary

structural information to existing spatial transcriptomic and proteomic technologies.

Consistent with earlier two-dimensional studies of psoriatic inflammation, 3D analysis confirmed localisation of $CD3^+$ T cell and $CD68^+$ macrophages towards the dermo-epidermal junction in psoriatic lesional tissue. Most $CD3^+$ cluster median distances to DEJ fell from > 200 µm in normal skin to 100 µm or less in plaques ($p = 4.80\times10^{-5}$), while $CD68^+$ clusters shifted by a similar magnitude ($p = 5.78\times10^{-4}$). Notably, normal-appearing peri-lesional skin in psoriatic patients already displayed a pathological architecture: more than half of the $CD3^+$ clusters' median DEJ distance in peri-lesional skin fell below 200 µm ($p = 3.81\times10^{-3}$). Whilst our observations are descriptive in nature, they are consistent with the literature showing that non-lesional psoriatic skin exhibits subclinical immune activation through transcriptional and structural signatures [3], including elevated IL-17 pathway signalling [36], increased *AIM2* expression held in check by *CARD18* and altered keratinocyte regulatory networks involving Psoriasis susceptibility-related RNA Gene Induced by Stress (*PRINS*), GATA3, and IL-37 [37-39]. Our spatial data provide a volumetric correlate to these molecular observations, demonstrating that peri-lesional tissue exhibits subtle but measurable deviations from healthy skin in the 3D organisation of myeloid and lymphoid compartments.

In contrast, mast cells did not identify statistically significant differences in either cell density or DEJ proximity across healthy, peri-lesional and lesional groups. Although modest shifts were observed in distributional trends, these did not reach statistical significance. These

findings are compatible with previous reports that mast cell function – particularly degranulation, cytokine production and neuro-immune signalling – may be more relevant to psoriasis pathophysiology than cell number [34]. Our data supports the view that mast cell activation state rather than spatial abundance is likely the key discriminator between disease states with reports indicating mast cell numbers are not affected by severity of psoriasis [40].

Unexpectedly, similar T cell and mast cell densities were observed between healthy controls and psoriatic lesional tissue warrant further contextualisation. Most healthy control samples in this study were derived from older adults undergoing surgical excisions. Ageing skin is known to undergo immune remodelling with an increase in mast cell numbers [41] and $CD8^+$ T cell infiltrates [42]. These age related changes are well characterised in intrinsically aged skin, may have elevated the baseline immune cell presence with our healthy control population. Furthermore, murine and human studies demonstrate variation in mast cell prevalence depending on body site [43-44], particularly with sun exposed versus sun protected body locations [45]. These observations further highlight the importance of interpreting immune densities within an appropriate demographic context and underscore spatial positioning and molecular activation states, rather than abundance alone, when studying the immunological milieu in psoriatic inflammation.

This work highlights several methodological strengths. The pipeline supports routine FFPE workflows, accommodates commonly used and accessible IHC markers, and enables large-scale volumetric analysis of

human tissue. Importantly, our comparison of 3D probability density functions (PDFs) with averages of 2D slices demonstrates that volumetric analysis preserves spatial information lost in 2D projections, emphasising the value of full stack reconstruction for studying complex immune microenvironments such as psoriasis. The proposed pipeline can also be complementary to high-plex spatial platforms such as GeoMX or CosMX. For example, the selected sections within a serial stack could undergo spatial proteomic or transcriptomic profiling after the full stack reconstruction is screened for the most representative or specific histopathological findings. These molecularly annotated sections could then be spatially registered into the reconstructed volume providing a hybrid dataset that couples cell-type identity and gene-expression signatures with volumetric tissue context. Such multi-modal integration represents a promising direction for future work in this field.

Several limitations should be acknowledged. First, the pipeline is labor-intensive, requiring extensive serial sectioning, repetitive staining, and manual quality control during image registration and segmentation despite some automation used in various stages. Second, the marker panel (CD3, CD68, mast cell tryptase) identifies board immune compartments but does not resolve functional subsets or activation states and therefore, the biological interpretation of spatial patterns is inherently constrained. Furthermore, there are technical and cost constraints if dual IHC was attempted to further investigate the proximity of myeloid and lymphoid compartments to nearby structures such as blood vessels and nerves. Thirdly, the use of excision specimens from older donors as healthy

controls limits direct comparability with psoriasis cohorts who in general are a younger population, and may have elevated the immune cell abundance in the control group. In this study, we had defined peri-lesional skin as tissue within 1 cm of the psoriatic plaque and should be considered when interpreting these results in the greater context of the existing literature that may use more distant non-lesional skin in psoriasis patients.

In summary, this study demonstrates that 3D reconstruction of serially sectioned FFPE skin provides a robust and accessible solution to quantifying the immune-epithelial spatial relationships in psoriasis. The data confirm known features of psoriatic pathology, revealed subtle spatial changes in peri-lesional skin in psoriatic patients, and underscores the importance of volumetric analysis in resolving the immunological milieu in normal and diseased skin. Despite the methodological constraints, it offers a valuable addition to the study of spatial-dermatopathology and promotes future multimodal studies integrating histology, computational imaging and spatial omics to refine our understanding of psoriatic inflammation.

Materials and Methods

Patient Cohort

In this study, 98 subjects were recruited, including 16 patients with psoriasis, 3 patients with eczema, 2 patients with drug-induced skin conditions, and 77 healthy controls. This study was approved by the Human Research Ethics Committee (HREC) of the Royal Prince Alfred Hospital (RPAH) Zone. Informed consent was obtained from all participants, with adherence to the National Statement on Ethical Conduct

in Human Research (2007). All procedures complied with the ethical standards of the institutional and national research committees. Participant confidentiality was ensured by anonymizing all data. Total 118 whole-mount skin tissue samples were sectioned from 98 subjects, where 79, 29, 6, and 4 samples were sectioned from healthy volunteers without psoriasis, psoriasis patients, eczema patients, and patients with drug-induced skin conditions, respectively – all skin conditions were clinically diagnosed by a dermatologist. For psoriasis patients, 4 mm punch biopsies were taken from 16 patients involving the lesional plaques and the adjacent peri-lesional area within 1 cm of the psoriatic plaque. All 10 samples from eczema patients and patients with drug-induced skin conditions were excluded from analysis since the sample amount is not big enough. In psoriasis patients, 2 samples from 1 patient were excluded due to low image quality. In healthy controls, 4 samples from 3 subjects were excluded due to data corruption and 22 samples from 22 subjects were excluded due to low image quality. Eventually, 53 samples from 52 healthy controls and 27 samples from 15 psoriasis patients were involved in this study, the details of sample collection are shown in Supplementary Table S1-S4.

Definition of Skin Biopsy Sites

Lesional skin refers to the visibly affected psoriatic plaque itself. Peri-lesional skin was defined as clinically uninvolved skin located within 1 cm of the edge of the psoriatic plaque, without visible scaling or erythema. For comparison, healthy controls was obtained from patients having skin cancer excisions within the study site with no personal or

family history of psoriasis or other inflammatory skin diseases. All peri-lesional biopsies in this study were strictly located within the 1 cm margin adjacent to the psoriatic plaque but did not include the plaque itself and confirmed by a clinical dermatologist.

Sample Preparation and Image Acquisition

All the skin samples were fixed in 10% neutral-buffered formalin (equivalent to 4% formaldehyde) for a minimum of 48 hours. The fixed blocks were then processed using a 6-hour automated cycle on a Leica Peloris II processor, passing through graded alcohols, xylene and then paraffin wax. Embedded blocks were then trimmed and sectioned at 4 µm using a Leica RM2235 microtome. Sections were floated on a Medite TFB 35 waterbath at 45 °C using distilled water. For blocks from healthy controls, a total of 80 or 160 or 240 serial sections were obtained for each block and mounted onto Trajan adhesive slides with 10 serial sections per glass slide. For blocks from psoriasis patients, serial sectioning for 80 or 120 consecutive sections was performed. All glass slides were then heated to 60 °C for 30 minutes to ensure tissue adherence. For tissue blocks from healthy controls, we defined a group of 80 consecutive sections (i.e. 8 glass slides) as a tissue stack, while a tissue stack in psoriasis patients tissue block was defined as a group of 40 consecutive sections (4 glass slides). Eventually, we obtained 95 tissue stacks (80-slice-stack) from 52 healthy controls and 79 tissue stacks (40-slice-stack) from 15 psoriasis patients, the details of tissue stacks are shown in Supplementary Table S5-S7. The 174 stacks were then subject to immunohistochemistry (IHC) staining to visualize the immune cell including T cell, macrophage, and mast cell. The

three type of cells are labelled by the CD3 marker (Roche, clone 2GV6, 790-4341, RTU), CD68 marker (Roche, clone KP-1, 790-2931, RTU), and mast cell tryptase (MCT) (Cell Marque, clone G3, 342M-14, RTU), respectively. These markers were selected to capture the principal immune cell populations implicated in the initiation and propagation of psoriatic inflammation. CD3 serves as a pan T cell marker and enables the quantification of the overall T cell compartment (Th1 Th17 and resident memory T cells) however examination of T cell subsets was outside the scope of this study. Mast cell tryptase was included to identify mast cells, whose activation and degranulation has been linked to early lesion development, neuro-immune interactions, and the amplification of inflammatory cascades. CD68 provides a sensitive but not specific marker for monocyte-macrophage lineage cells, which contribute antigen presentation and tissue remodelling within psoriatic plaques. Taken together, these markers allow assessment of the major innate and adaptive immune compartments within routine FFPE tissue.

The staining was performed by an automated Roche Ventana Ultra machine and following are the staining protocols for each antibody. CD3: Heat-induced epitope retrieval (HIER) with CC1 for 24 minutes at 100 °C, antibody incubation for 28 minutes at 37 °C. CD68: HIER with CC1 for 32 minutes at 100 °C, antibody incubation for 12 minutes at 37 °C. MCT: HIER with CC1 for 8 minutes at 100 °C, antibody incubation for 8 minutes at 37 °C. All antibody protocols used the Optiview DAB detection system (Ventana, 760-700), followed by counterstaining with Hematoxylin II (Ventana, 790-2208, RTU) and Bluing reagent (Ventana, 760-2037, RTU).

All glass slides were then dehydrated in graded alcohols, cleared in xylene, and coverslipped. The detailed stack staining profiles are shown in Supplementary Table S2. The stained stacks were then digitized at the resolution of 0.35 × 0.35 µm per pixel. It is worth noting that among 95 80-image-stacks, there were 6 stacks only had 70 images each, since 10 images were excluded due to low image quality, hence total 10,700 whole slide images (WSIs) are obtained in this study. The tissue serial sectioning, staining, and scanning pipeline are illustrated in Fig. 1a, 1b.

Image Registration and Segmentation

As the WSI was scanned using relatively high resolution, most of the original WSIs were in large scale. Specifically, most WSIs had both width and height exceeding 10,000 pixels. Due to computational and memory resources limitation, all WSIs were first down sampled by 8 times before any subsequent processing, the image resolution was hence reduced to 2.8 × 2.8 µm per pixel. No down sampling (extraction) or up sampling (interpolation) was performed in Z direction so the Z resolution was still 4µm per layer, i.e., the slice sectioning interval. After the down sampling process, the resolution of 3D image stack became 2.8(X) × 2.8(Y) × 4(Z) µm per voxel, which is still enough for cellular level analysis.

An image registration strategy was designed to make sure all images in same stack were well aligned. As shown in Fig. 2a, for each stack, the first image was regarded as fixed image and the next image was registered to the fixed image by a scale-invariant feature transform (SIFT) [46] based algorithm. Then the second image became fixed image and the next image was registered to it. This process was performed iteratively until the last

image of the stack was registered to its fixed image, so that all the images were registered to the first image. Manual review was performed in the end as a quality control mechanism to ensure the alignment quality. The quantitative image alignment quality assessment result is shown in Fig. 2b, we calculated the mean distance between matched SIFT key points in original stacks (orange line) and aligned stacks (blue bar), the mean distance dropped after alignment for every stack, indicating that the image registration strategy we deployed effectively enhanced the image alignment quality and enabled the accurate spatial analysis.

After image registration, an image segmentation model was trained for pixel level image segmentation. The pathologists performed annotation on 130 WSIs in ilastik software (version 1.3.0). The annotation categories included dermis, epidermis, and immune cell. The annotated WSIs were used to train a random forest algorithm [47] based image segmentation model using ilastik software. The pathologists reviewed the prediction results to make sure the model performance was good, and then the model was applied to all WSIs to generate segmentation results. All segmentation results were validated by human before subsequent analysis. The image registration and segmentation pipeline was shown in Fig. 1c and Fig. 1d, the 3D rendering of a representative stack is shown in Fig. 1e. The detailed annotation process was described in Supplementary File.

Metric Calculation and Necessity of 3D Reconstruction

For segmented tissue stack, we proposed a parameter named cell distance to dermal-epidermal junction (DEJ). For a cell voxel, the distance to DEJ was defined as the Euclidean distance between itself and the

nearest DEJ to it. To be specifically, for cell voxel located in dermis region, its distance to DEJ is defined as the distance between itself and the nearest epidermis voxel; for cell voxel located in epidermis region, its distance to DEJ is defined as the distance between itself and the nearest dermis voxel. A probability density function (PDF) curve of distance to DEJ was then fitted to illustrate the immune cell distribution profile along the depth into skin. We also calculated the cell density for each stack, which was defined as number of cell divided by whole tissue volume, including dermis and epidermis.

We designed an experiment to demonstrate the advantages of serial sectioning and 3D reconstruction. We selected a healthy control tissue stack with 80 slides and calculated the cell distance to DEJ in two ways: across the 3D stack, and within each slide independently. The PDF curves were fitted for both situations, and we calculated the average value of 80 PDF curves derived from individual 2D slides to compare with the PDF curve derived from 3D stack. As shown in Fig. 2c, the individual 2D slide PDF curves are demonstrated in the middle panel and right panel. The average curve of 2D slide PDF curves (blue dashed line) and the 3D stack PDF curve (red solid line) are shown in the left panel. There is a distinct difference between the PDF curve from 2D average and from 3D, which means that simply averaging multiple 2D slides does not yield the same information as analyzing 3D stack. The root cause of the difference is that the peaks and valleys (corresponding to cell distribution patterns) in individual 2D slide PDF curves will become unrecognizable after averaging process, eventually resulting a smoothed curve. The actual 3D cell

distribution pattern will only be preserved when the calculation is performed in 3D manner. From another perspective, none of the individual 2D slide PDF curves shows a pattern similar to that of the 3D stack PDF curve, indicating that accurate estimation of 3D stack from single 2D slide is not feasible due to sampling bias. In short, by including more thickness of tissue, we can reveal the immune cell spatial distribution in a more precise way, and minimize the bias introduced by random sampling.

Ethical Approval and Consent of Participate

This study was approved by the Human Research Ethics Committee (HREC) of the Royal Prince Alfred Hospital (RPAH) Zone. Informed consent was obtained from all participants, with adherence to the National Statement on Ethical Conduct in Human Research (2007). All procedures complied with the ethical standards of the institutional and national research committees.

Consent for Publication

All authors of this work agreed to publish with Scientific Reports once accepted.

Availability of the Data and Material

The data and code of this work will be available upon reasonable request.

Competing Interest:

The authors declare no potential competing interests.

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Authors Contribution

P. T. and W. Y. conceived the project and designed the experiments; P. D. contributed to sample preparation; L. L. performed model development and validation, and data analysis; L. L. designed and prepared the figures with the assistance of K. H. O.; L. L., L. V., and P. T. wrote the manuscript with the assistance and feedback of all the other co-authors.

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Figure Legend

Figure 1. **a**-**b**). Formalin-Fixed Paraffin-Embedded (FFPE) skin samples were trimmed and sectioned at 4μm interval. The tissue sections were then subject to immunohistochemistry (IHC) staining to visualize the immune cell including T cell, macrophage, and mast cell. The stained sections were then digitized at the resolution of 0.35×0.35μm per pixel, the images were downsized by 8 times for calculation consideration. **c**-**d**). Images were aligned by a Scale-Invariant Feature Transform (SIFT)-based algorithm in rigid manner (translation + rotation) and segmented by a random forest-based model, where red, green, blue represents immune cell, epidermis, and dermis respectively. **e**). Visualization of 3D rendering of a representative tissue stack, where red, green, blue represents immune cell, epidermis, and dermis respectively.

Figure 2. **a**). Image stack alignment strategy, left column shows original images (before alignment), right column shows aligned images. The first image remains untouched, then the second image is aligned to the first image, and then the third image is aligned to the second image,… Repeat this process until all images are aligned to the first image. **b**). Quantitative alignment quality assessment. The mean SIFT-matched point distances between slides are substantially reduced across the image stack after

registration (blue bars) compared to the original stack (orange line). This alignment step is crucial for accurate spatial quantification. **c**). Probability density function (PDF) of immune cell distances to the dermal–epidermal junction (DEJ) are compared. The left plot highlights how the 3D-derived PDF (red line) differs markedly from the 2D-averaged estimate (blue dashed line), with the latter failing to capture the peaks and valleys of cellular distribution. The centre and right plots demonstrate that individual 2D sections show high variability and fail to recapitulate the coherent pattern revealed by 3D stacking. These results underscore that averaging or selecting single 2D slices introduces sampling bias and smooths biologically meaningful spatial features, which are preserved only through full volumetric analysis.

Figure 3. **a**). The CD3 cell median distance to DEJ in psoriasis patients lesional skin is significantly lower than that in psoriasis patients peri-lesional skin and that in healthy controls skin. The CD3 cell median distance to DEJ in psoriasis patients peri-lesional skin is significantly lower than that in healthy controls skin as well. **b**). The CD3 cell density in psoriasis patients peri-lesional skin is significantly lower than that in psoriasis patients lesional skin and that in healthy controls skin. There is no significance between the CD3 cell density in psoriasis patients lesional skin and that in healthy controls skin. The statistical test performed in panel **a**). and **b**).: independent two-sample t-test, two-sided. **c**). The mean probability density function curves of the CD3 cell distance to DEJ in healthy controls skin, psoriasis patients peri-lesional and lesional skin, respectively. **d**).-**f**). Visualization of reconstructed 3D tissue stacks, where red, green, blue represents immune cell, epidermis, and dermis, respectively. The marked cell is CD3 cell. The stack in **d**). is from healthy control skin, the stack in **e**). is from psoriasis patients peri-lesional skin, the stack in **f**). is from psoriasis patients lesional skin.

Figure 4. **a**). The CD68 cell median distance to DEJ in psoriasis patients lesional skin is significantly lower than that in psoriasis patients peri-lesional skin and that in healthy controls skin. There is no significance

between the CD68 cell median distance to DEJ in psoriasis patients peri-lesional skin and that in healthy controls skin. **b**). The CD68 cell density in psoriasis patients lesional skin is significantly higher than that in psoriasis patients peri-lesional skin and that in healthy controls skin. There is no significance between the CD68 cell density in psoriasis patients peri-lesional skin and that in healthy controls skin. The statistical test performed in panel **a**). and **b**).: independent two-sample t-test, two-sided. **c**). The mean probability density function curves of the CD68 cell distance to DEJ in healthy controls skin, psoriasis patients peri-lesional and lesional skin, respectively. **d**).-**f**). Visualization of reconstructed 3D tissue stacks, where red, green, blue represents immune cell, epidermis, and dermis, respectively. The marked cell is CD68 cell. The stack in **d**). is from healthy control skin, the stack in **e**). is from psoriasis patients peri-lesional skin, the stack in **f**). is from psoriasis patients lesional skin.

Figure 5. **a**). There is no significance between the mast cell median distance to DEJ in healthy controls skin and that in psoriasis patients lesional/peri-lesional skin. **b**). There is no significance between the mast cell density in healthy controls skin and that in psoriasis patients lesional/peri-lesional skin. The statistical test performed in panel **a**). and **b**).: independent two-sample t-test, two-sided. **c**). The mean probability density function curves of the mast cell distance to DEJ in healthy controls skin, psoriasis patients peri-lesional and lesional skin, respectively. **d**).-**f**). Visualization of reconstructed 3D tissue stacks, where red, green, blue represents immune cell, epidermis, and dermis, respectively. The marked cell is mast cell. The stack in **d**). is from healthy control skin, the stack in **e**). is from psoriasis patients peri-lesional skin, the stack in **f**). is from psoriasis patients lesional skin.