

Spatial Biology Congress

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Spatial atlas of inflammatory skin disease and cutaneous T cell lymphoma

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Unil
UNIL | Université de Lausanne

Introduction

Benign Inflammatory skin disease

AD

Eczema

PS

Psoriasis

LP

*Lichen
Planus*

DAR

*Darier
Disease*

Benign



Malignant



Cutaneous T Cell Lymphoma (CTCL)

MF

*Mycosis
Fungoides*

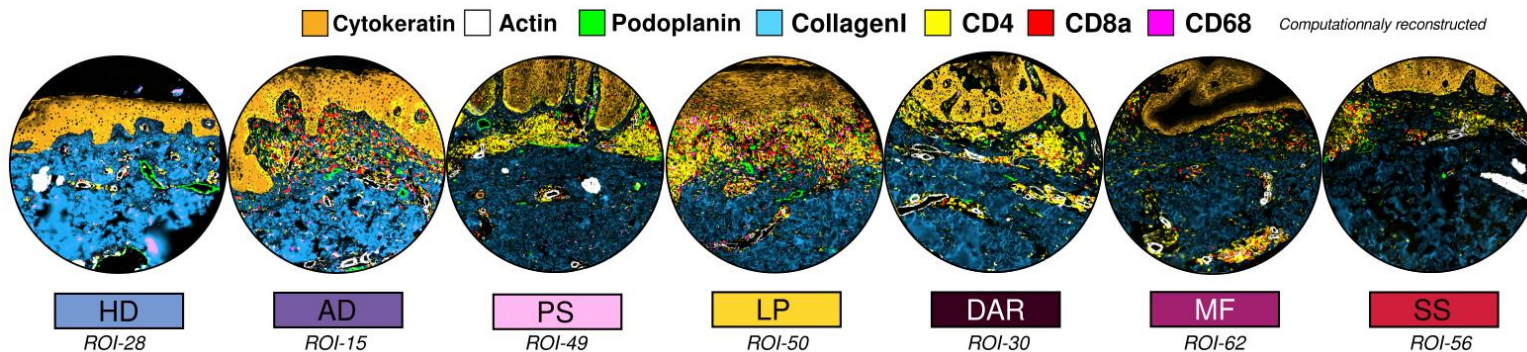
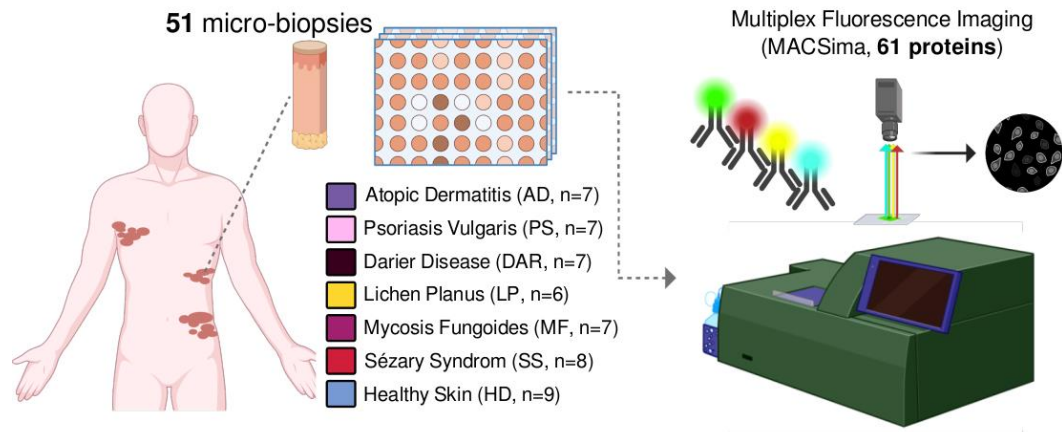
SS

*Sézary
Syndrom*

- Benign inflammatory skin diseases and CTCL are both **deregulation of T cells in the skin**
- To improve cure of the malignant diseases, multiple challenges remain:
 - **Enhance diagnostic**
 - **Predict response in malignant disease**

Roediger et al., T cells in the skin: Lymphoma and inflammatory skin disease (2022)

Multiplexed protein imaging of skin diseases

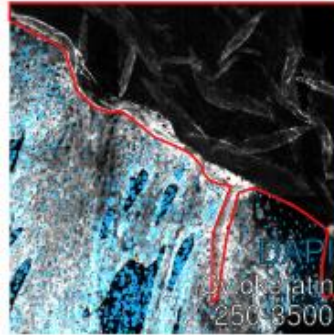


- 1) What characterizes malignant skin disease ?
- 2) Can we predict response to treatment ?

But ... Challenges in analyzing highly multiplex fluorescence images

- Manageable artifacts

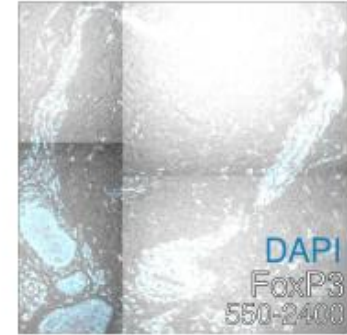
- Folds



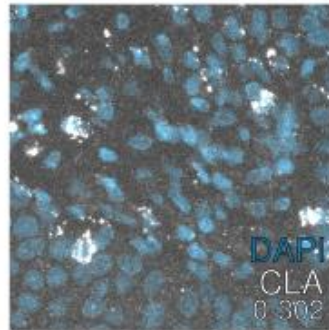
- Stains



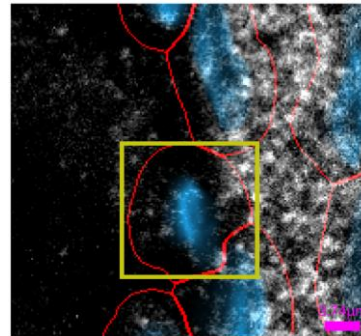
- Whole punch spills



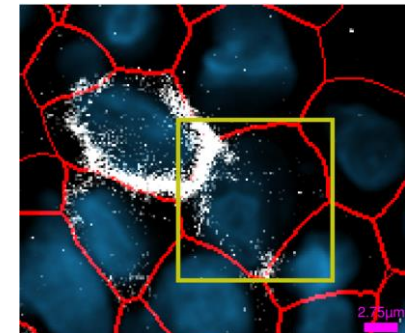
- Varying intensity range



- Low Signal/Noise



- Spillover

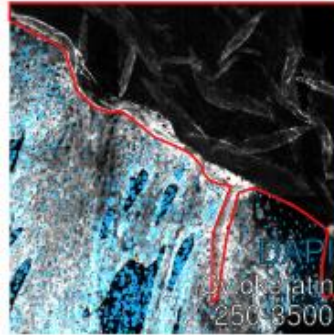


- More challenging artifacts

But ... Challenges in analyzing highly multiplex fluorescence images

- Manageable artifacts

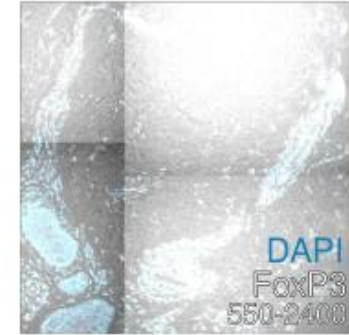
- Folds



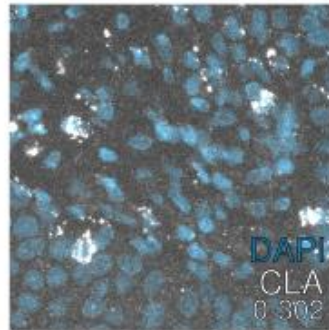
- Stains



- Whole punch spills

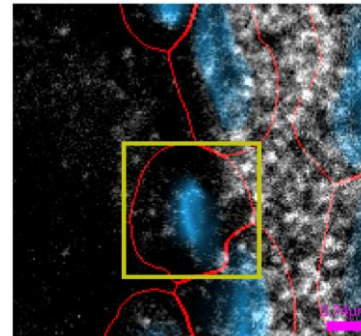


- Varying intensity range



- More challenging artifacts

- Low Signal/Noise



- Spillover

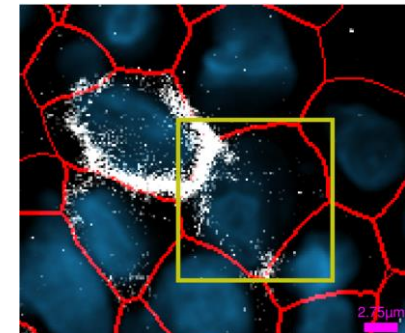
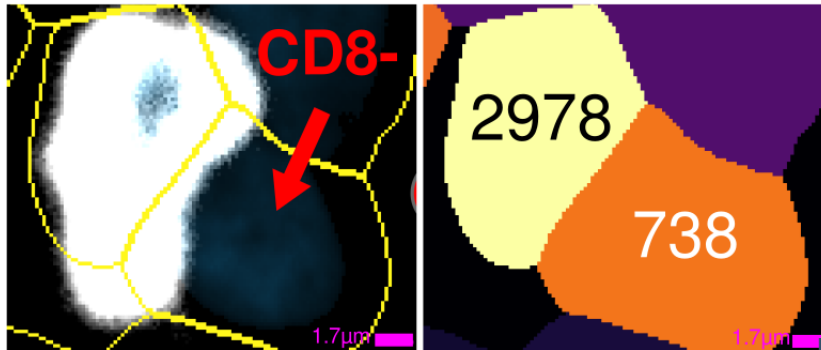


Image vs average intensity

- Image
- VS
- Average intensity

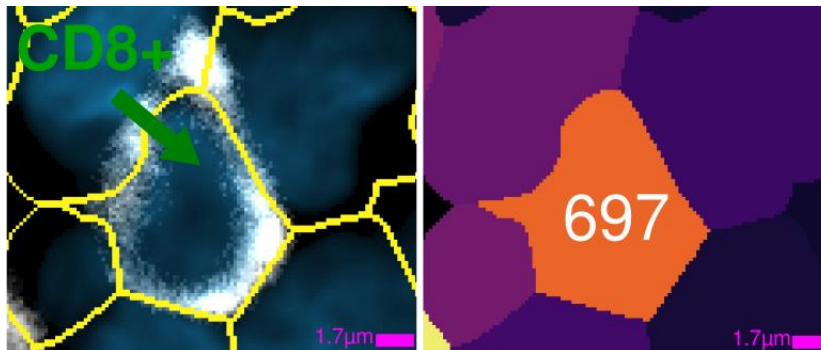
- Spillover



Averaging intensity **hides the pattern.**

“Classical” tools, based on the average intensity suffer from this fact.

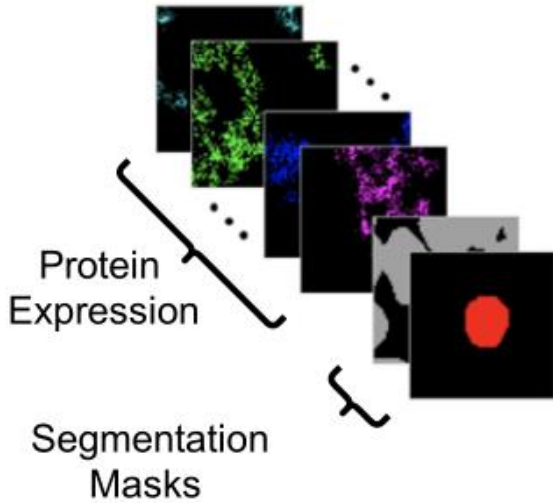
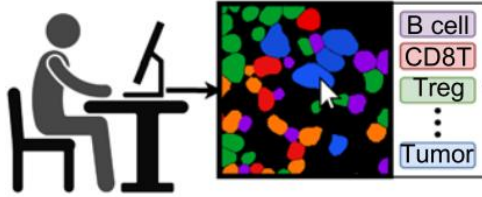
- Low intensity cell



Deep-learning based cell phenotyping reveals heterogeneous cell composition

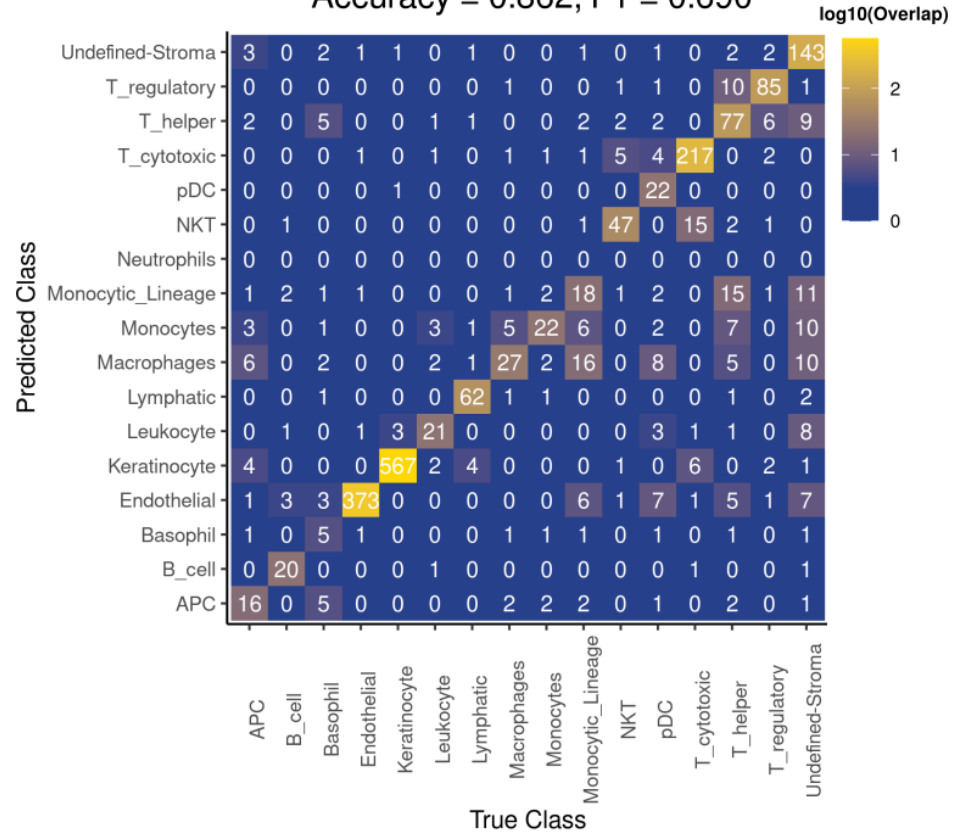
CellSighter¹

Annotated 17 celltypes in 11 images (>8000 cells)



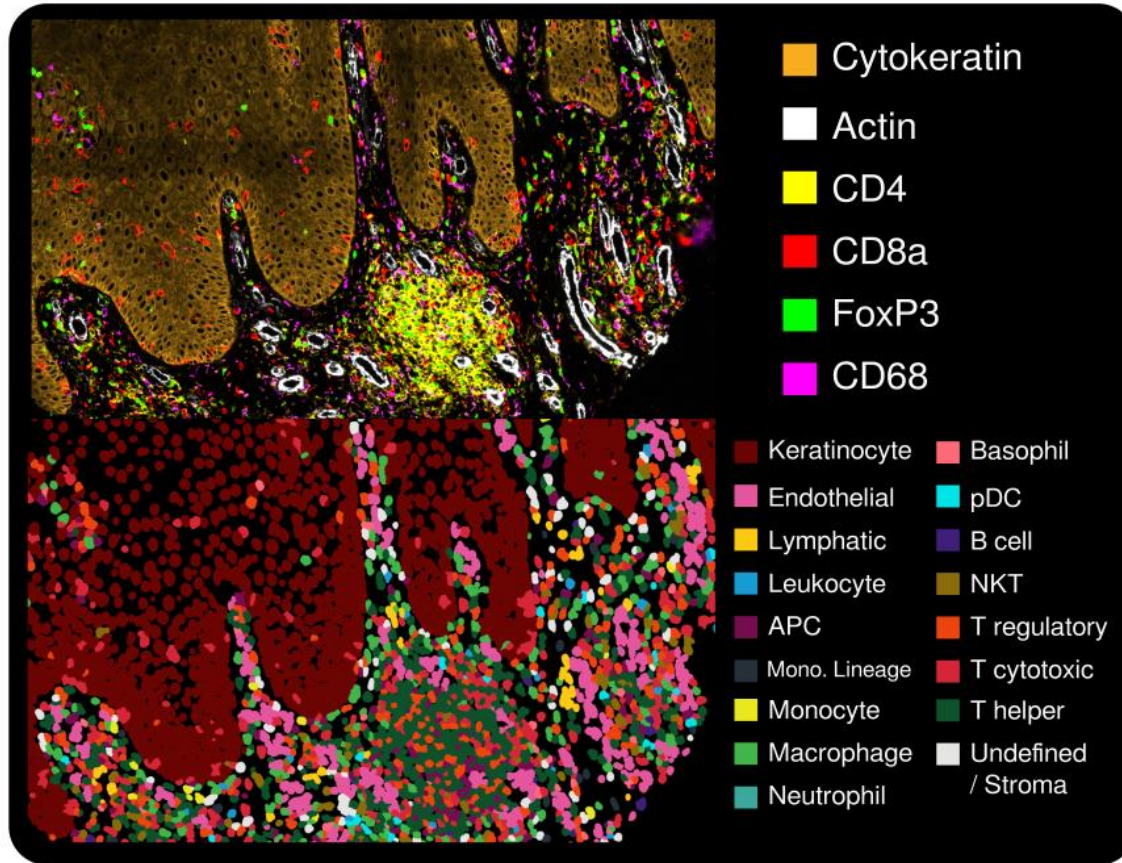
Prediction vs Ground Truth (Validation)

Accuracy = 0.862; F1 = 0.690

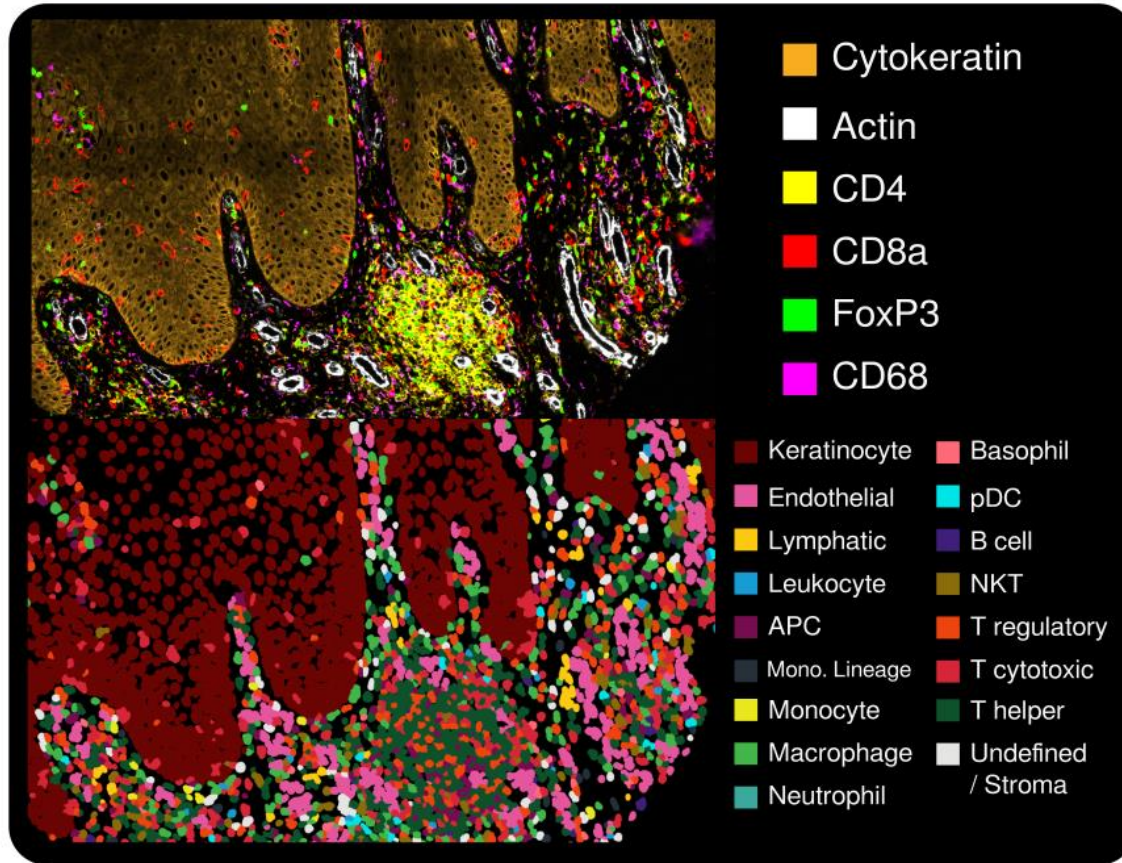


¹CellSighter: a neural network to classify cells in highly multiplexed images, Amitay et al., Nat. Comm. (2022)

Deep-learning based cell phenotyping reveals heterogeneous cell composition



Deep-learning based cell phenotyping reveals heterogeneous cell composition

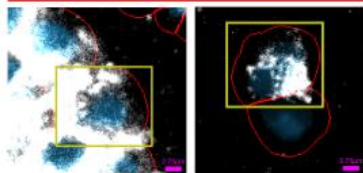


What about the markers ?

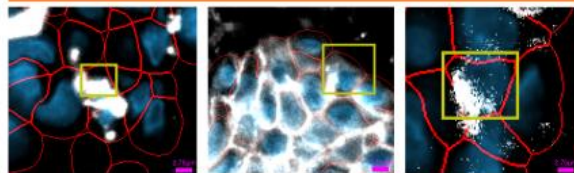
“Hacking” CellSighter for image-based positive marker detection

Positive Cells

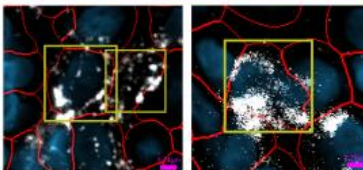
Enriched over Background



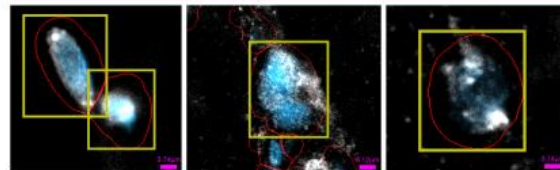
Cytoplasmic staining



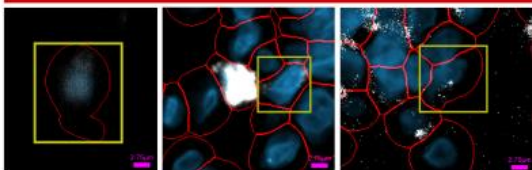
Spotty but enriched in cell



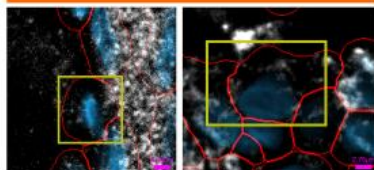
Nuclear staining



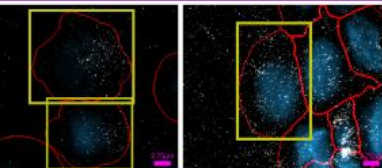
Not different from background



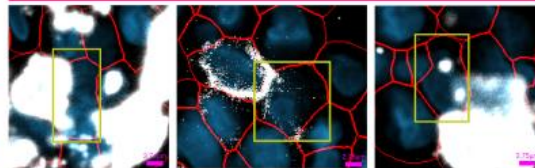
Not cell-centered



Starry night appearance



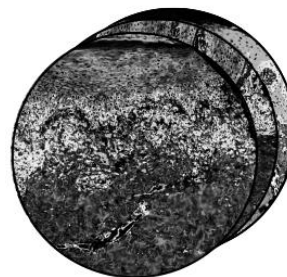
Spillover



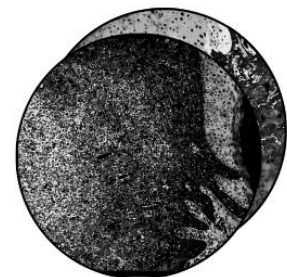
Negative Cells

60 markers

Total annotated cells: 82134



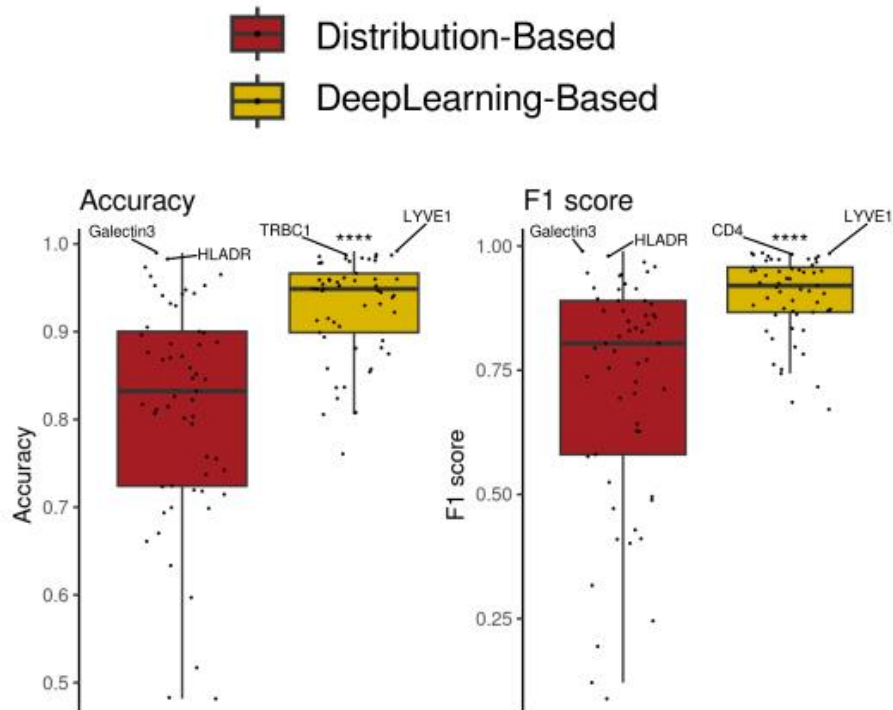
Training
6 punches



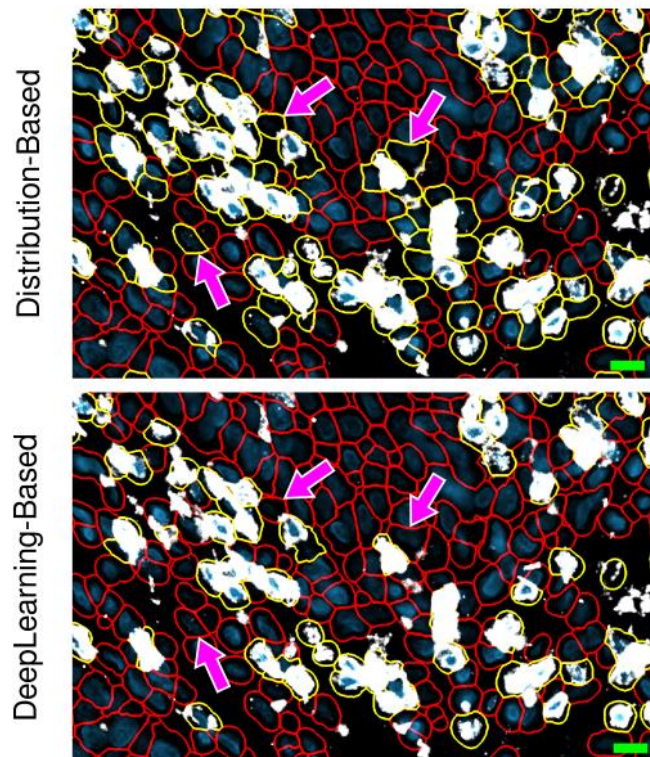
Validation
5 punches

¹CellSighter: a neural network to classify cells in highly multiplexed images, Amitay et al., Nat. Comm. (2022)

Leveraging deep-learning for positive marker detection



ROI-19 ; CD3+ mask 10µm

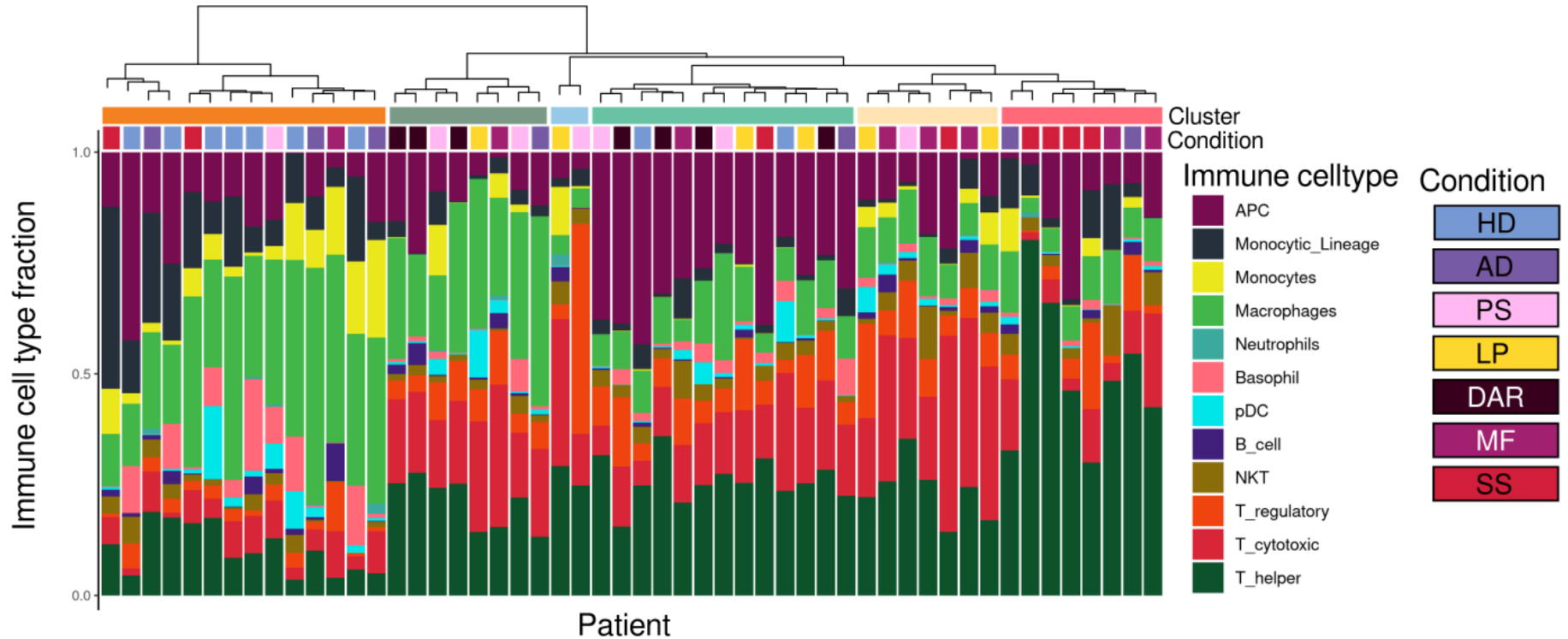


Examples of cells misclassified in distribution-based classifier

Cell classified as CD3- Cell classified as CD3+

Back to the biology

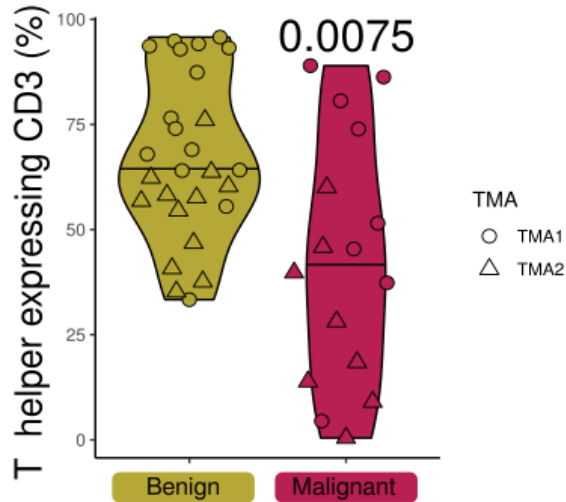
Cell composition reveals strong heterogeneity within diseases



- Strong heterogeneity of cell composition
- Some malignant patients have similar composition to healthy / benign

Increased T cell densities and CD3 loss in malignant skin disease

T helper



Migration / regulation of T cytotoxic & NK cells

▪ T cell activity / TCR signalling

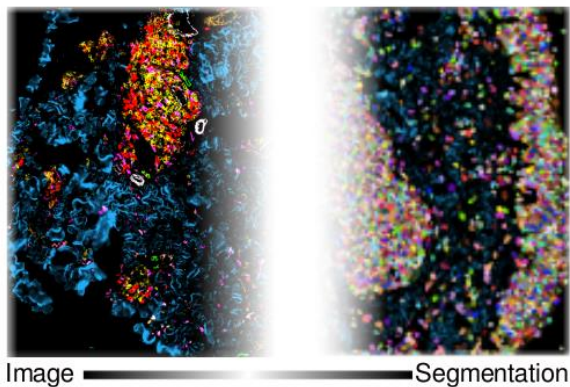
- Strong reduction of the hallmark CD3 marker in T helper in malignant disease

Sano et al., Immunological study on CD3 defective cutaneous T cell lymphoma cells from a patient with Sézary syndrome. Clin Exp Immunol. (1998)

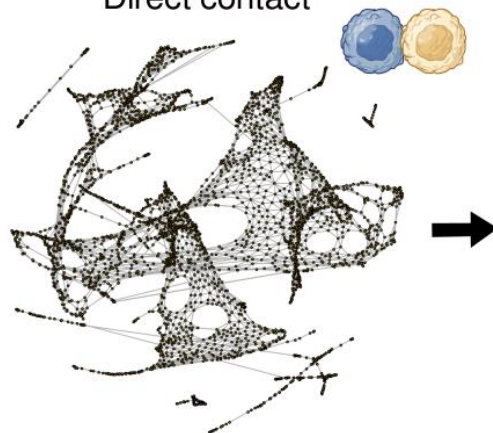
Liu et al., Single-cell transcriptomics links malignant T cells to the tumor immune landscape in cutaneous T cell lymphoma. Nat Commun 13, 1158 (2022)

Spatial configuration : the «immune pathological infiltrate»

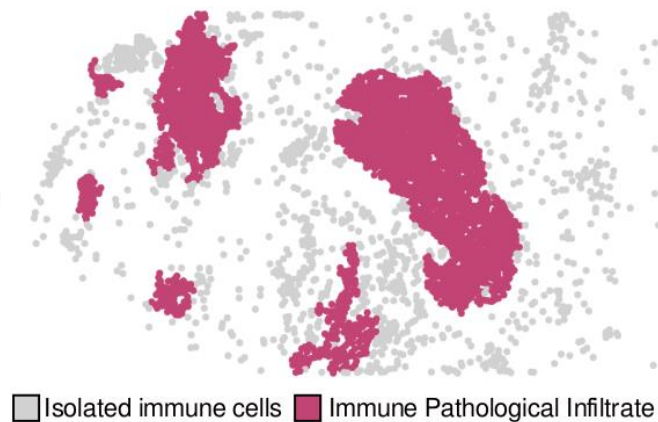
Cell segmentation using DeepCell



Direct contact

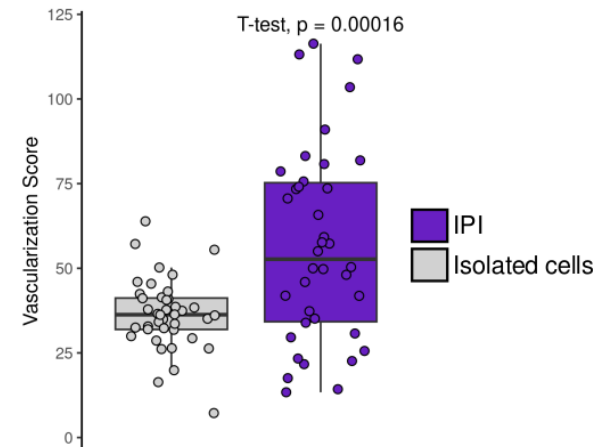
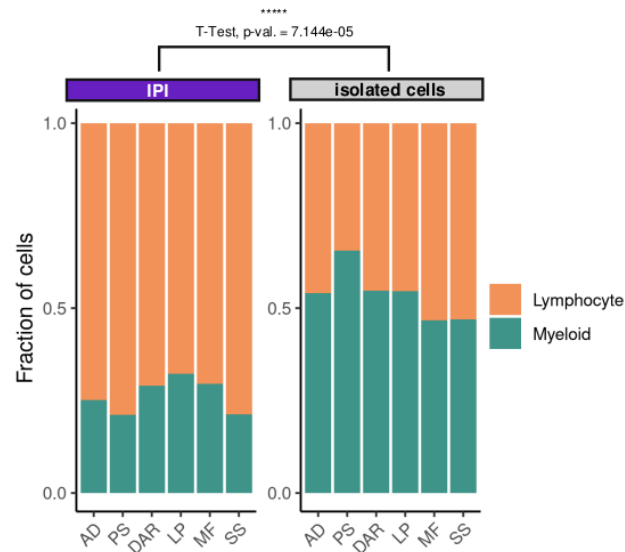
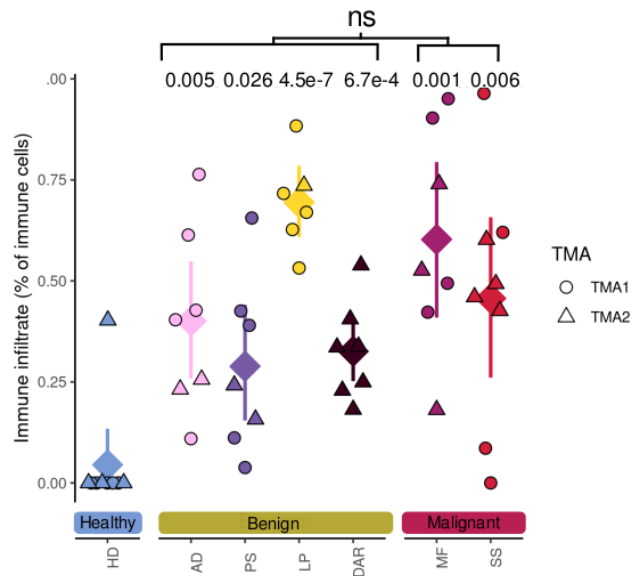


Community detection using Louvain



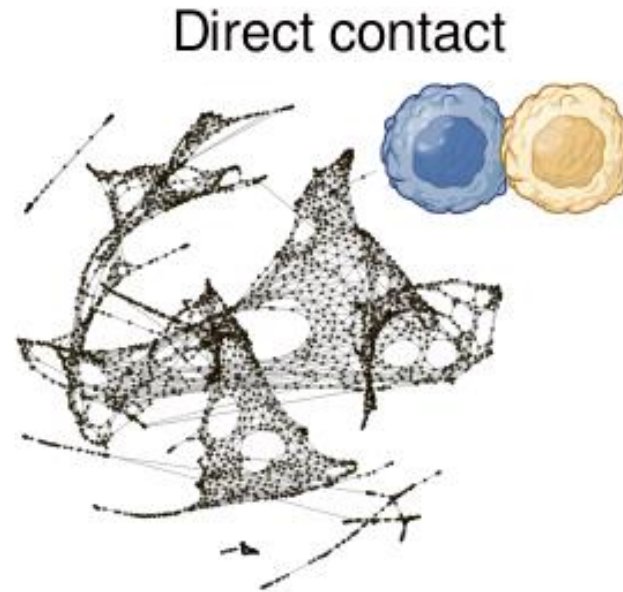
- Building from cell-in-contact network we find communities of immune cells using
- louvain clustering (keeping those > 30 cells).

Spatial configuration : the «immune pathological infiltrate»



- The IPI is increased in malignant compared to most benign skin NCD.
- They are highly lymphocytic compared to the rest and are significantly more vascularized.

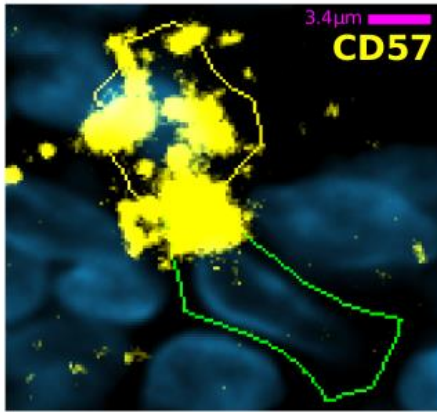
Diving further : finding «immunological synapses»



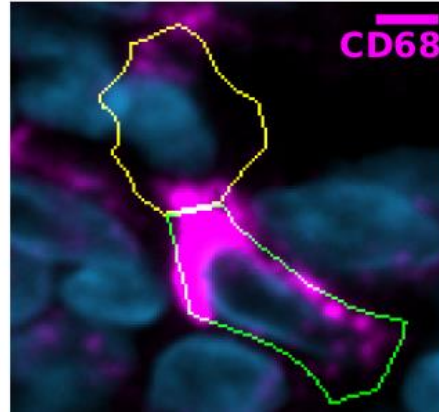
Diving further : finding «immunological synapses»

ROI-57
cells 1454 <--> 1422

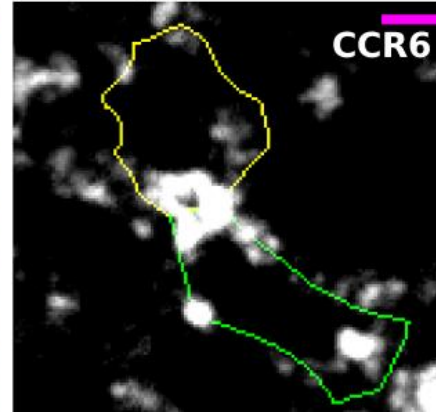
NKT Cell



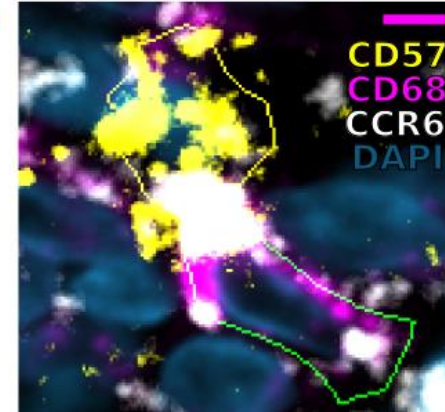
Macrophage



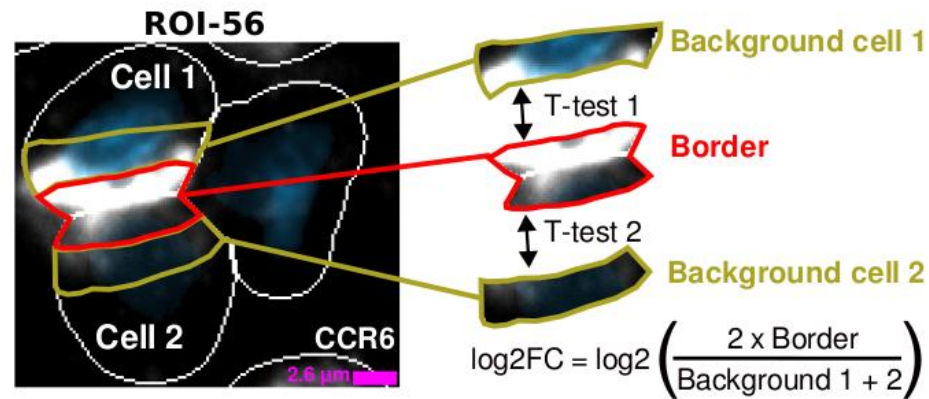
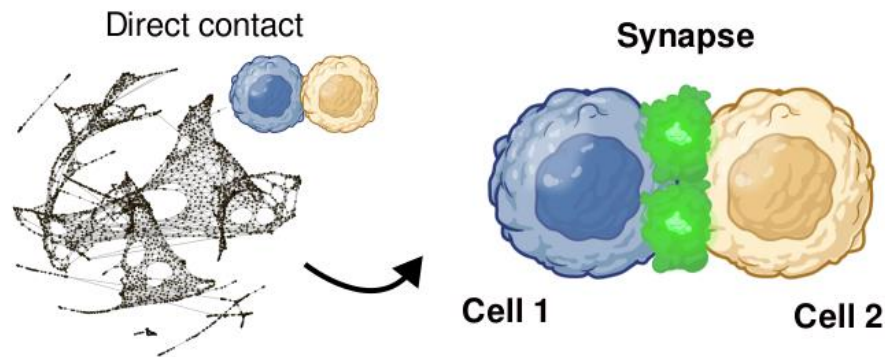
CCR6



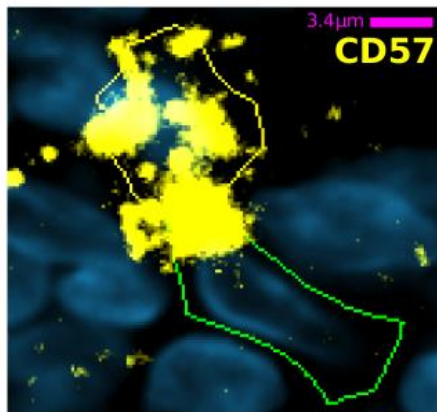
Combined



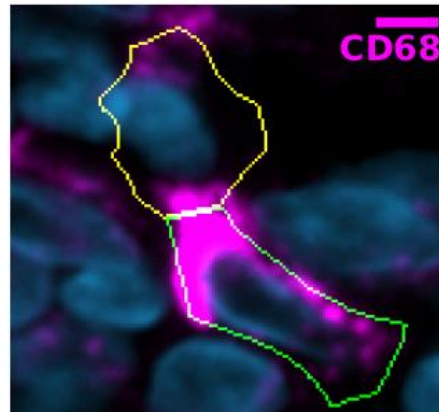
Diving further : finding «immunological synapses»



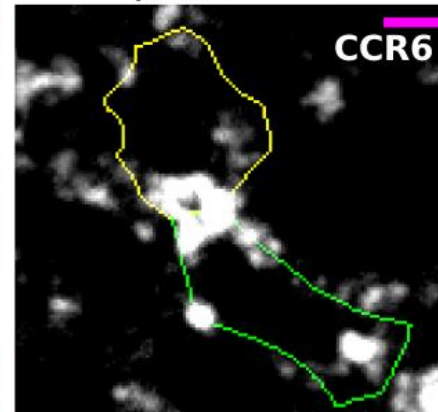
NKT Cell



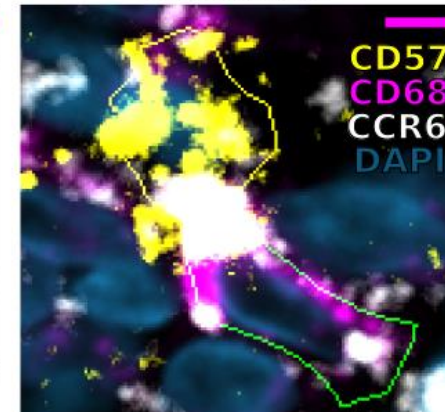
Macrophage



$\log_2FC = 1.70$
 $p.value1 = 1e-66$
 $p.value2 = 1e-23$

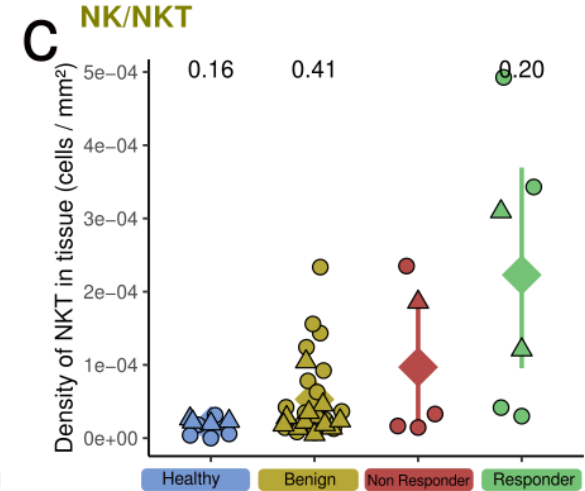
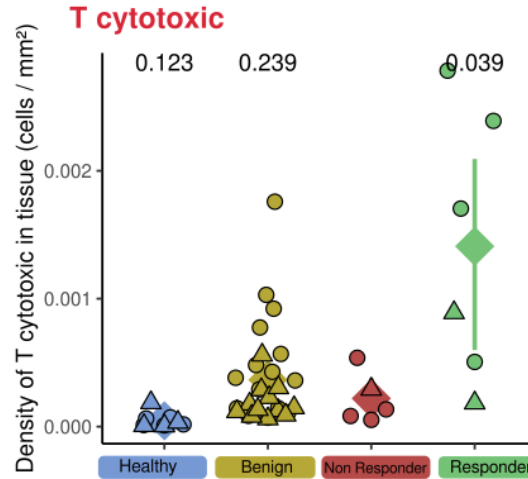
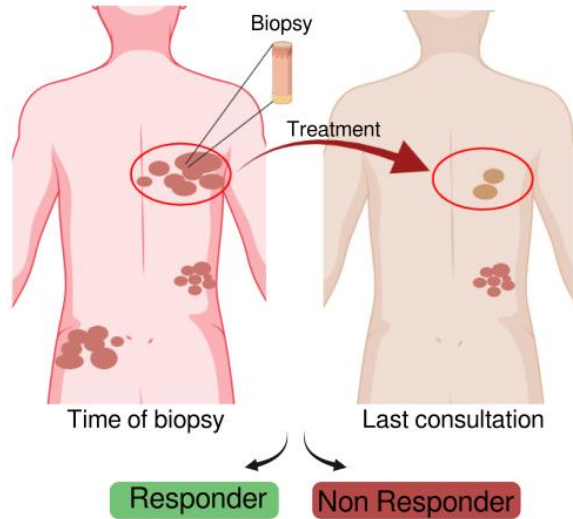


Combined



T cytotoxic fraction improves response to treatment

CAILS score evolution of the biopsied lesion in CTCL



- Classifying patients according to evolution of CAILS score (Responder vs Non Responder)
- Responsive patients have in average **2-3 times greater proportions of cytotoxic T cells at time of biopsy** (validated on survival of additional 34 patients)

Conclusion

- We build a spatial atlas of immune skin diseases with precisely phenotyped cells and functional markers
- T helper cells in CTCL shift their program to evade immune surveillance by cytotoxic T cells
- The best predictor for lesion evolution is the fraction of cytotoxic T cells at time of biopsy

See the story online



Spatial atlas of non-communicable skin diseases

← 1/15 →

Skin is our body's largest organ, serving as the first line of defense against infection. Within the skin are various immune cells that protect us from pathogens. Yet sometime, skin diseases arise from deregulations in T cells which are also strongly influenced by all other immune cells present in the skin.

Multiplex imaging technology provides unprecedented access to the spatial organization and protein expression of single cells within the skin. This powerful tool allows us to better characterize different immune diseases, particularly distinguishing between benign conditions like Psoriasis and Eczema, and malignant conditions such as Cutaneous T-cell Lymphoma (CTCL) which can be life-threatening.

To achieve this, we collected several skin biopsies from patients with various skin diseases and employed multiplex imaging to map their protein landscapes.

Get ready for the tour!

Image adapted from "T cells and the skin: from protective immunity to inflammatory skin disorders", Ho and Kupper, Nat. Rev. 2019, DOI

Table of Contents

1. Introduction
2. Healthy skin under the microscope

Legend:

- DAPI
- Cytokeratin
- all cells
- Monocytic_Lineage
- Keratinocyte
- Leukocyte
- Unknown-Stroma
- Endothelial
- pDC
- Monocytes
- T_cytotoxic
- Lymphatic
- B_cell
- T_helper
- T_regulatory
- Macrophages
- NKT
- APC
- Basophil
- Neutrophils

<https://bit.ly/skin-atlas>

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Fondation Recherche Cancer

Fondation Dind/Cottier