

Comp683: Computational Biology

Lecture 15

March 16, 2025

Today

- Trajectory inference, with SLICER
- Spatial immunophenotyping
- LEAPH for identifying cellular microenvironments.

Project Related Announcements

- Please sign up with your group members and topics here,
- Project proposal writeup is due March 19.
- First round of presentations on Wednesday. Please keep your talk to 5 minutes and check the google doc to know your date and time. You can trade with each other, but please update the google sheet.
https://docs.google.com/spreadsheets/d/1oVms1L0QiRPsuQeLAJhJqgc_mdxBSmKuBW0a-S5gdXU/edit?usp=sharing

Reviewing Diffusion Distance

$$D_t^2(\mathbf{x}, \mathbf{y}) = \sum_{i=1}^{n-1} \lambda_i^{2t} (\psi_i(\mathbf{x}) - \psi_i(\mathbf{y}))^2$$

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- When you find the l such that there is a large difference between l and $l + 1$ eigenvalues (an elbow), you can use the sum up to the l -th term as an approximation for diffusion distance. The first l eigenvectors correspond to the diffusion components.

This diffusion map approach was the beginning of thousands of people starting to think about cellular differentiation.....

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- Use locally linear embedding to capture non-linear relationships between gene expression levels and progression through a process
- Define ‘geodesic entropy’ and use it to define branches
- Capture unique trajectory patterns such as bubbles.

SLICER Overview

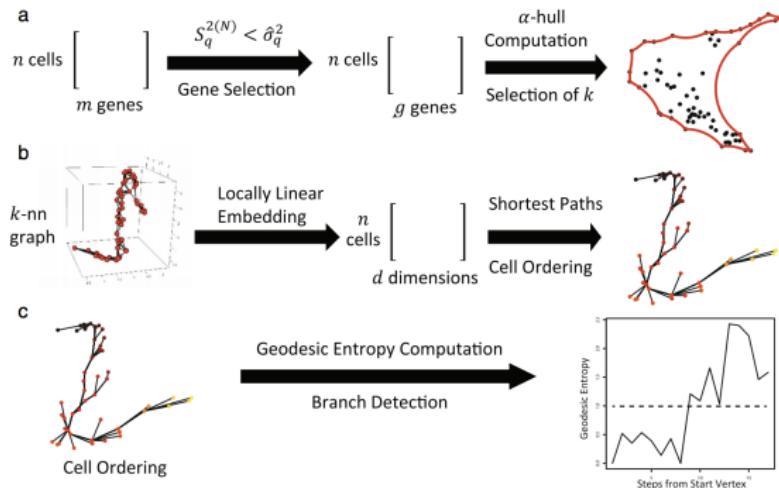


Figure: from Welch *et al.* Genome Biology. 2016

Step 1: Selecting Features to Use (Intuition)

Establishing some intuition about what makes a good ‘trajectory feature’

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- If a feature is involved in progression along a trajectory, expect gradual change in that feature along the trajectory
- A feature not involved should not fluctuate along the trajectory.
- In real life, we have no idea what is happening with this trajectory. Use similarity within neighborhoods to study ‘segments’ of a trajectory.

Neighborhood Variance

Interesting features are those whose variance is greater than some level of neighborhood variance. Specifically, for the g th feature, we can compute its variance within a neighborhood and compare it to the overall variance.

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Interesting features are those whose variance is greater than some level of neighborhood variance. Specifically, for the g th feature, we can compute its variance within a neighborhood and compare it to the overall variance. The neighborhood variance is defined as,

$$S_g^{2(N)} = \frac{1}{nk_c - 1} \sum_{i=1}^n \sum_{j=1}^{k_c} (e_{ig} - e_{N(i,j)g})^2$$

- k_c is the number of nearest neighbors needed for each node for the graph to be connected.
- Each e_{ig} is representing the value of feature g in cell i .
- $e_{N(i,j)g}$ is representing the feature value of the j th nearest neighbor in cell i .

Selecting Genes Most Likely to Be Involved in a Trajectory

Given computed neighborhood variance, $\hat{\sigma}_g$ and neighborhood variance $S_g^{2(N)}$, we seek genes varying more globally than within a neighborhood so that $\hat{\sigma}_g > S_g^{2(N)}$.

Local Linear Embedding ($d = 2$)

Step 1: Find the weights (w_{ij} s) that can best reconstruct the original data (e.g. the E_s cell \times feature) in terms of k nearest neighbors as,

$$W = \operatorname{argmin}_W \sum_{i=1}^n \left| E_i - \sum_{j=1}^k w_{ij} E_j \right|_2^2$$

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Step 2: Find optimal d -dimensional embedding, so in this case, L

$$L = \operatorname{argmin}_L \sum_{i=1}^n \left\| L_i - \sum_{j=1}^k w_{ij} L_j \right\|_2^2$$

k -NN graph and shortest path

- Compute k -nearest neighbor graph between cells in terms of the LLE-determined coordinates.

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- Compute k -nearest neighbor graph between cells in terms of the LLE-determined coordinates.
- Specify a starting point (like a stem cell), and use a shortest path algorithm like Dijkstra to find the shortest path to some cell of interest.

Detecting Branches with Geodesic Entropy Measure

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- $H_k = -\sum_{i=1}^n p_{ik} \log_2 p_{ik} \rightarrow$ look at high entropy

SLICER Applied to Synthetic Data

Studying geodesic entropy over k . Higher entropy in terms of steps corresponds to the 'bubbles' in the data.

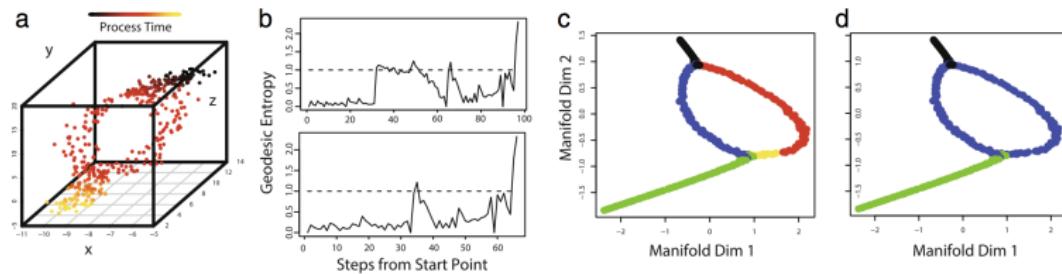


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Neural Stem-Cell Differentiation Data

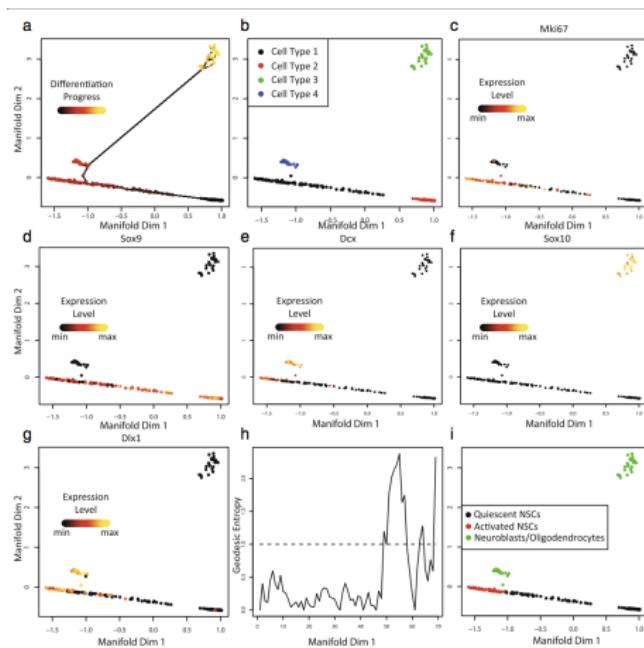


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Switching gears to spatial immunophenotyping with imaging cytometry modalities.....

CyTOF + Spatial Resolution

An upgrade of regular CyTOF to image 32 proteins and their modifications at cellular resolution.

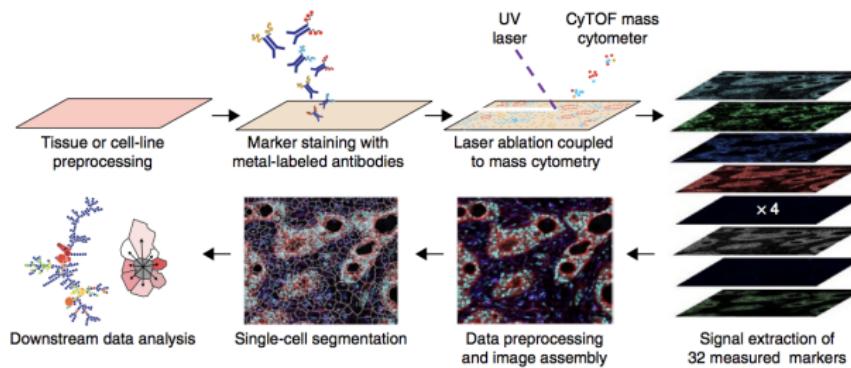


Figure: from Giesen *et al.* Nature Methods. 2016

Why Do We Care?

Understanding the spatial organization of cells (for example, tumor and immune cells) can provide a more mechanistic understanding of the underlying biology. This can further translate to more accurate prediction of prognostic outcomes.

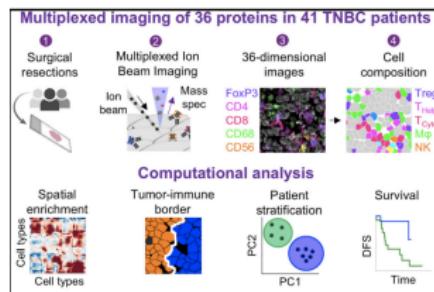


Figure: from Keren et al. Cell. 2018.

Recent Advances in Study The Relationship Between Immune Cells and Tumor

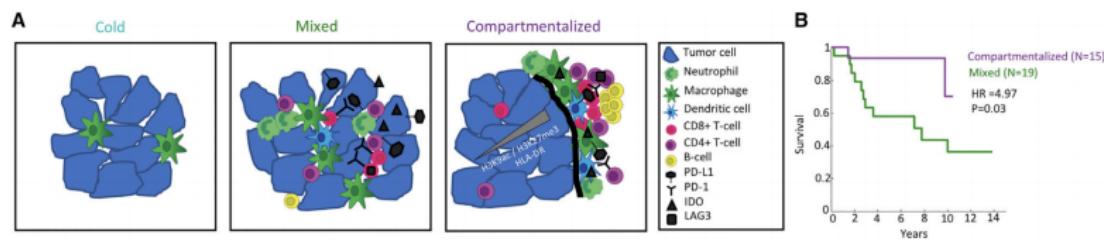


Figure: from Keren *et al.* Cell. 2018.

Studying Aging

Older mice were observed to have infiltrating T-cells in their neurogenic niches (the collection of neuronal progenitor cells)

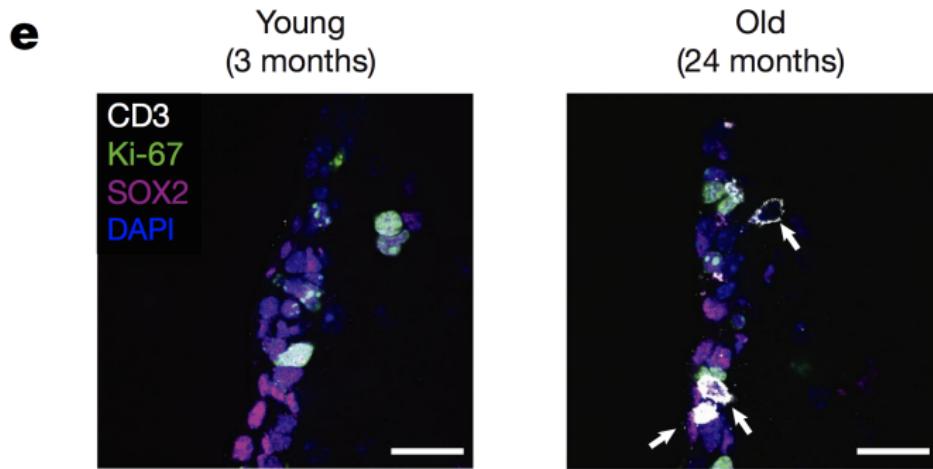
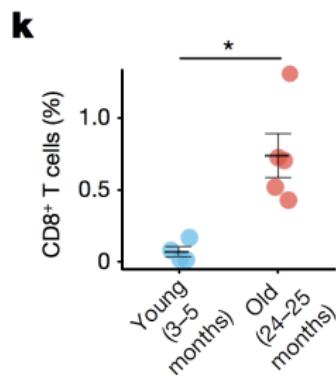


Figure: from Dulken *et al.* Nature 2019

Counting CD8+ T-cells

You can even compare the proportion of CD8+ T-cells there are in neurogenic niches between young and old mice. It's a pretty striking difference.



General Steps in Analyzing These Data

- Segmentation of cells

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- Identify microenvironments or characteristic co-occurrences of particular cell-types within a region.

Example-Cell Phenotype Map

Cells are clustered and phenotyped according to protein expression.

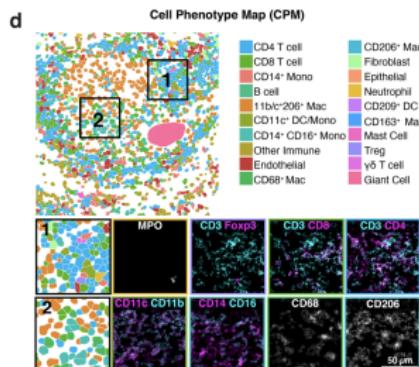


Figure: from <https://www.biorxiv.org/content/10.1101/2020.06.08.140426v1.full.pdf>

End-Goal of Identifying Particular Microenvironments

Ultimately, an objective is to identify ‘micro-environments’ or spatially-localized subsets of cells with characteristic frequency patterns that are predictive of some outcome of interest.

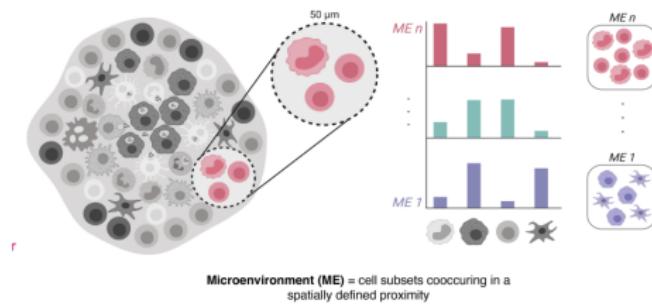


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A New Problem: Identifying Microenvironments

Welcome LEAPH. One of the first methods out there to identify phenotypically distinct microdomains of spatially configured cell phenotypes.

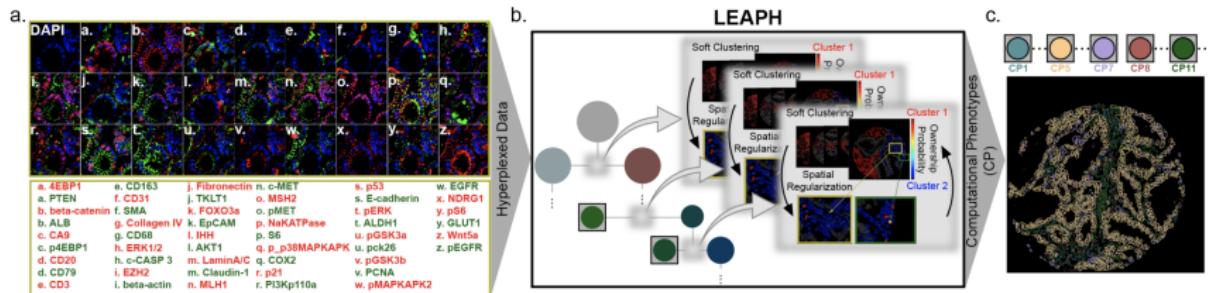


Figure: from Furman et al. Cell Reports Methods. 2021.

LEAPH Overview

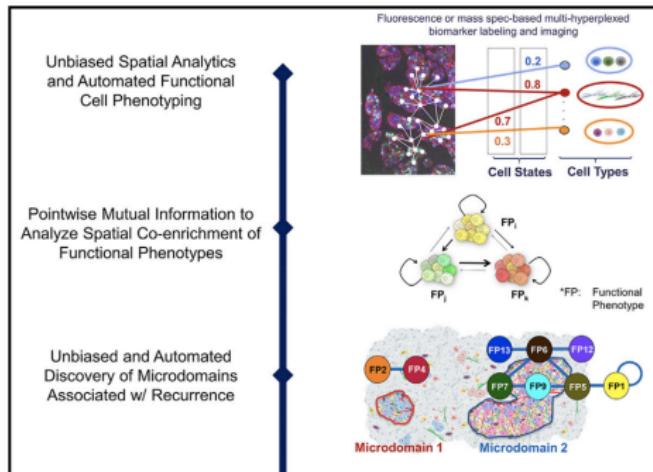


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$$\mathbf{x}_i = \Lambda \mathbf{z} + \boldsymbol{\mu} + \mathbf{v}$$
 - Loadings in $\Lambda \in \mathbb{R}^{p \times k}$
 - Latent variables, $\mathbf{z} \in \mathbb{R}^{k \times 1}$
 - Noise term via, $\mathbf{v} \sim \mathcal{N}(0, \Psi)$
 - Mean vector, $\boldsymbol{\mu} \in \mathbb{R}^{p \times 1}$

Mixture Model

Each $p(\mathbf{x}_i)$ is computed as

$$p(\mathbf{x}_i) = \sum_{j=1}^M \pi_j \mathcal{N}(\mathbf{x}_i | \boldsymbol{\mu}_j, \Lambda_j \Lambda_j^T + \Psi)$$

- π_j is the mixing weight for cluster j .

Practicalities

- Overall, parameters being estimated are $\{\pi_j, \mu_j, \Lambda_j\}_{j=1}^M, \Psi$.

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- This gives a soft clustering interpretation for each cell.

Spatial Regularization Intuition

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A new Ω is optimized that encodes spatial information as follows,

$$\min_{\Omega} - \sum_{i=1}^N \sum_{j=1}^M \Omega_{ij} \log_2 (\Omega_{ij}) + \lambda \sum_{(j,k)} w_{jk} \|\Omega_j - \Omega_k\|_2$$

Unpacking

$$\min_{\Omega} - \sum_{i=1}^N \sum_{j=1}^M \Omega_{ij} \log_2 (\Omega_{ij}) + \lambda \sum_{(j,k)} w_{jk} \|\Omega_j - \Omega_k\|_2$$

- w_{jk} is a weight, calculate as the reciprocal of distance between cells j and k in the image
- The first term is basically an entropy term of ownership confidence
- The second term is promoting spatial coherence.
- λ controls the tradeoff between spatial coherence and membership confidence.

Effect of Spatial Regularization

In particular in the first example, a cell with a highly predicted assignment towards CP1 transitioned towards a phenotype of CP2 after spatial regularization.

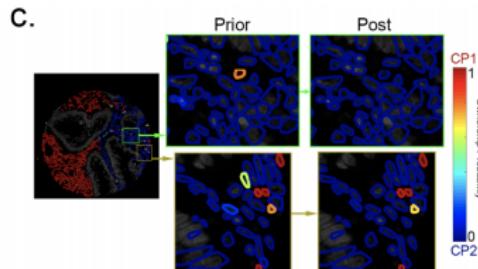


Figure: from Fig. 2 of <https://www.biorxiv.org/content/10.1101/2020.10.02.322529v3.full.pdf>

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- Based on the Ω , assign each cell to one of the M phenotypes based on the j that gives the maximum probability.
- For a particular patient, p , create a feature vector \mathbf{f}_p which gives the proportion of its cells assigned to each of the cell phenotypes.
- At times, the authors refer to specialized cell-types (membership probability $> 95\%$) in contrast to transitional and rare cells.

Recap and Transition

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- Calculate a probability that each cell was from each of these latent factors
- Add penalties that enforce spatial coherence and certainty of assignment
- **Next step:** Identify microdomains with a collection of cells that are predictive of some phenotype of interest.

Predicting Time to Recurrence in Breast Cancer

- Consider cohorts of patients with the following properties.
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The goal is to translate the distributions of cell phenotypes that spatially co-occur to a signal that can be used for prediction.

Constructing a Cell Network For Each Patient

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- For a pair of cells, m , and n , connect them with a weights, $w_{mn} = 1$ if their spatial distance, $d_{mn} < 1$.
- Otherwise, $w_{mn} = 0$ and there are no edge between the cells

Identifying Spatial Co-Occurrence Between Cell Phenotype Pairs

Consider two phenotypes, f_i and f_j for a given set (e.g. a subset of patients, etc). The pairwise mutual information between these two phenotypes is defined as,

$$\text{PMI}_s(f_i, f_j) = \log_2 \left(\frac{p(f_i^s, f_j^s)}{p(f_i^t) p(f_j^t)} \right)$$

- $p(f_i^s)$ is the probability of a particular phenotype, i occurring in a network set, s .
- $p(f_i^t)$ is the background probability of phenotype i .

Calculating Joint Phenotypic Probability for a Single Patient

Letting Ψ encode the set of edges for a particular patient, the joint probability of phenotypes i and j is given as,

$$p(f_i^s, f_j^s) = \frac{1}{z} \left(\sum_{(m,n) \in \Psi} w_{mn} \left(\vec{\Omega}_{mf_i} \vec{\Omega}_{nf_j} + \vec{\Omega}_{mf_j} \vec{\Omega}_{nf_i} \right) \right)$$

*Here z is a normalization over all combinations of i and j according to the computational phenotypes.

Specifying a Background Distribution

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Ultimately, for each cell phenotype pair, (f_i, f_j) compute the PMI for each sample and consider how this relates to the patient re-occurrence outcomes.

Looking at Significant Microdomains Between Groups

There were a few cellular phenotypes that tended to co-occur between the two patient groups.

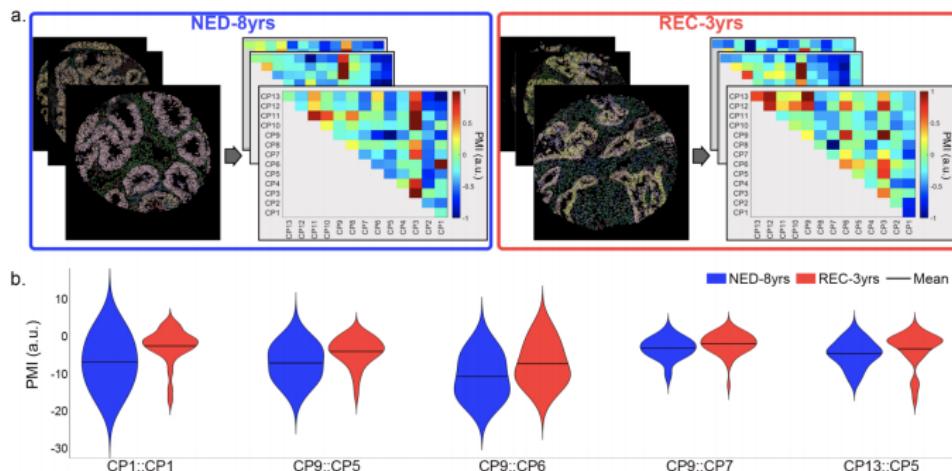


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