Deciphering the identity, composition, and interaction of highly refined cell types within complex tissues

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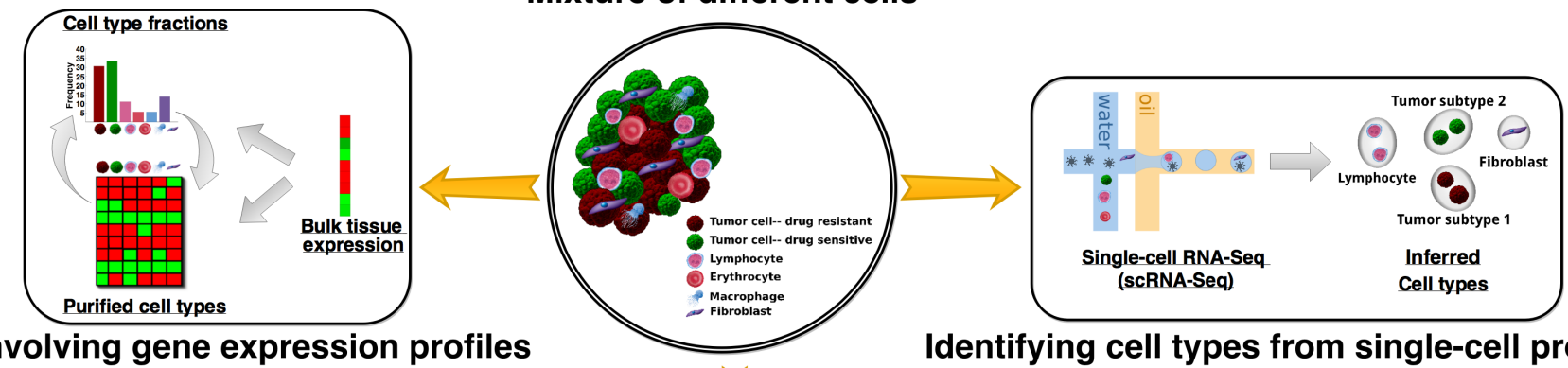
Overview

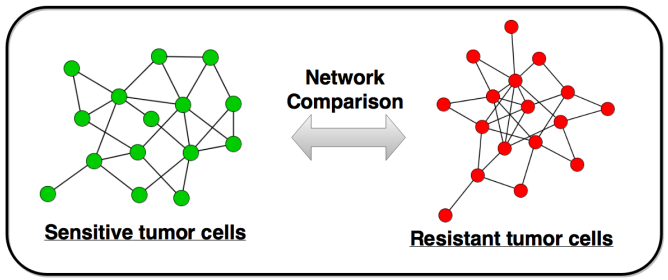


Deconvolving gene expression profiles

A Comprehensive Survey of Deconvolution Methods for Separating Cell types in Complex Tissues, 2016

Identifying cell types from single-cell profiles

Take ACTION to identify high-resolution cell types and associated transcriptional pathways, 2016



Constructing cell type-specific networks

A convex optimization approach for identification of human tissue-specific interactomes, 2016

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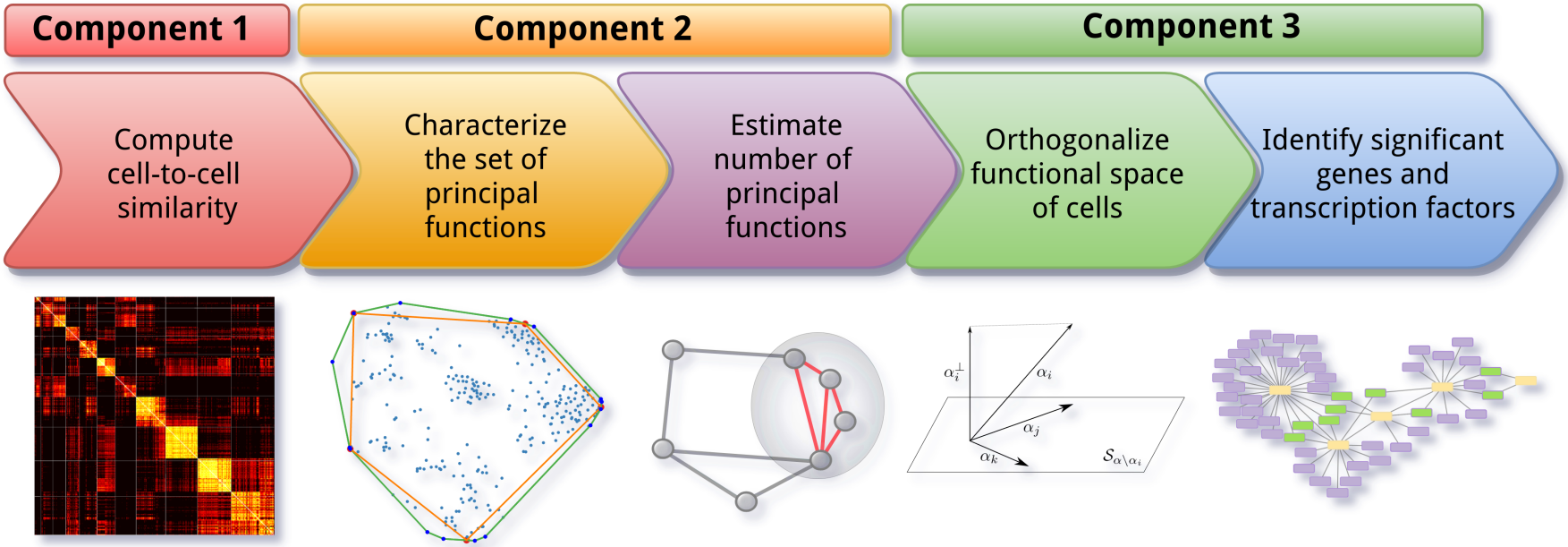
Overall Workflow



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Underlying hypothesis

Transcriptional profile of cells is dominated by housekeeping genes, whereas their functional identity is determined by a combination of weak but preferentially expressed genes.

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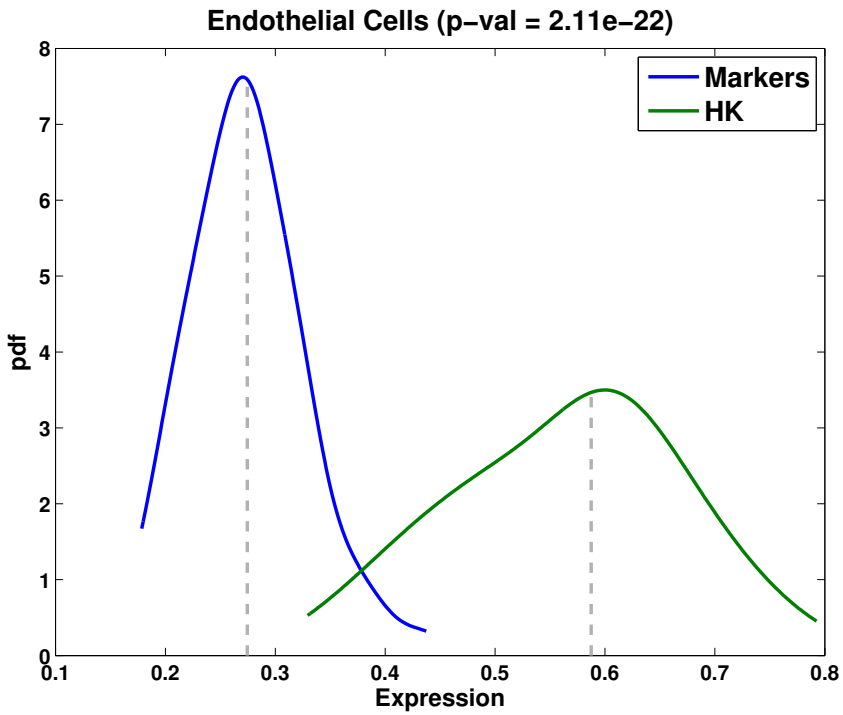

Figure: Endothelial Cells

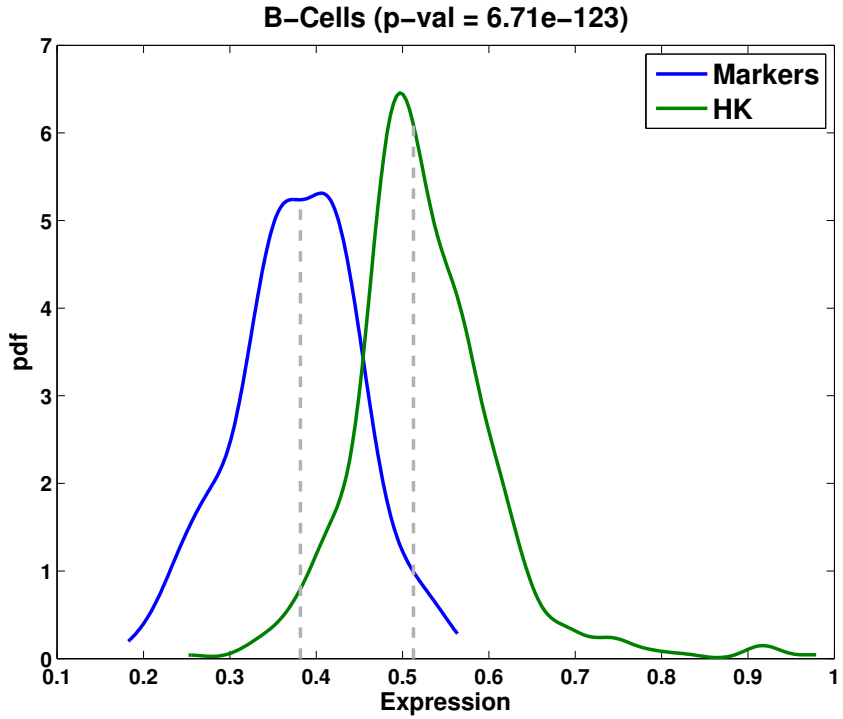

Figure: B-Cells

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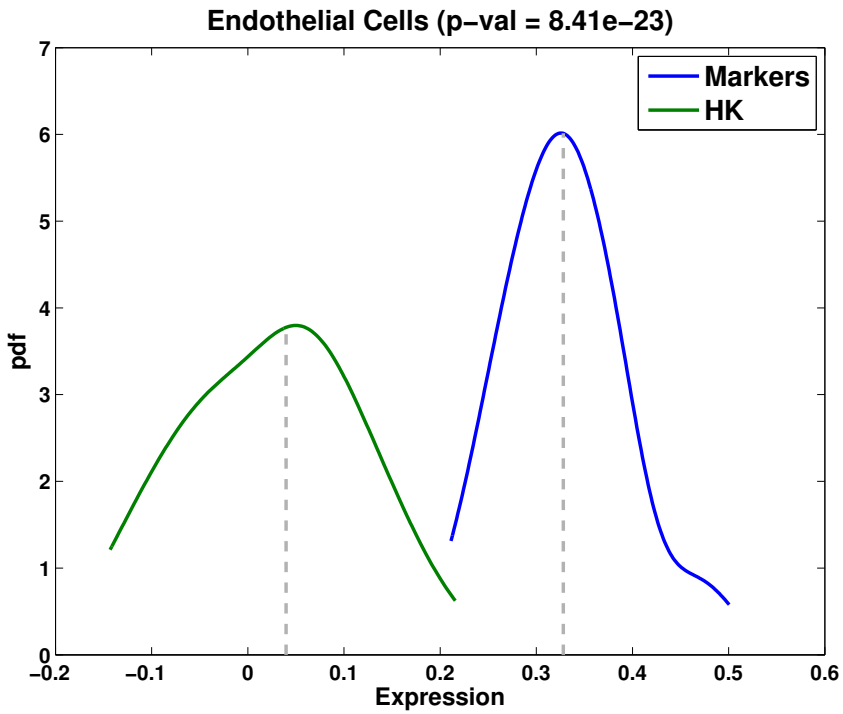

Figure: Endothelial Cells

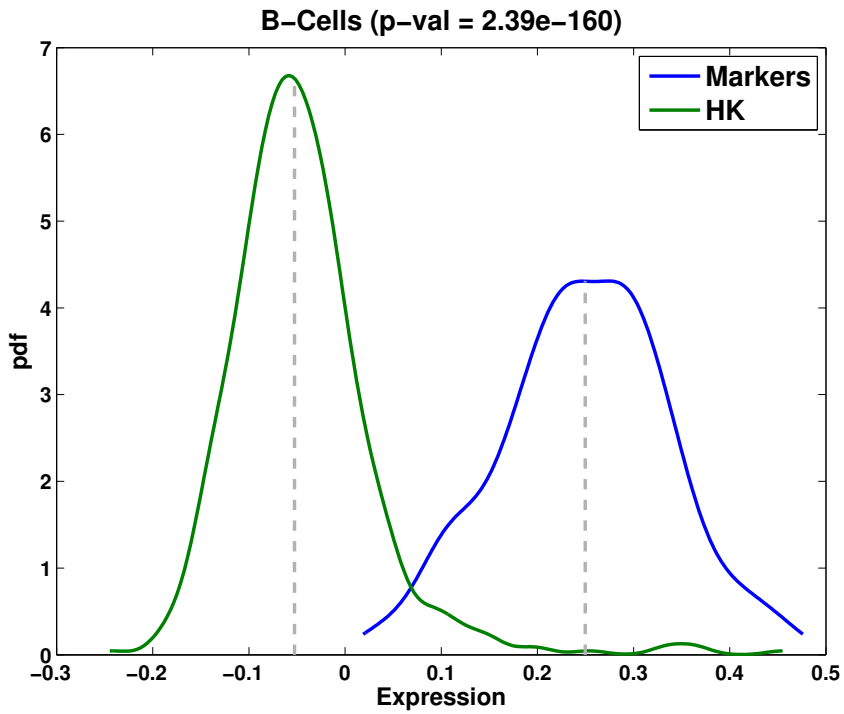

Figure: B-Cells

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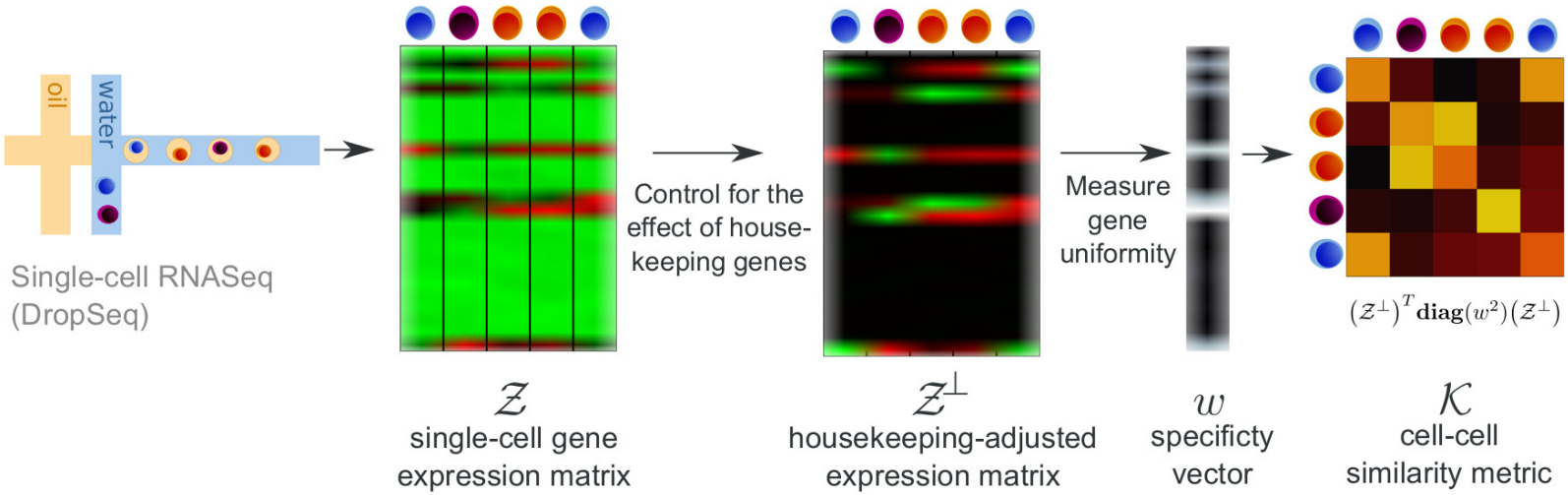
Component 1

Overall flow – cell similarity kernel



- The main steps involved in identifying similarity between cells

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Goal: Identify the shared subspace of genes

Low-rank decomposition

$$A = U_r \Sigma_r V_r = \sum_{i=1}^{r} \sigma_i u_i v_i^T,$$

Example decomposition choices:

- Mean vector
  - *Optimal in a least-square sense when the chance of observing a gene is uniform across all cells.*
- Singular Value Decomposition (SVD)
- Nonnegative Matrix Underapproximation (NMU)
- Sparse NMU

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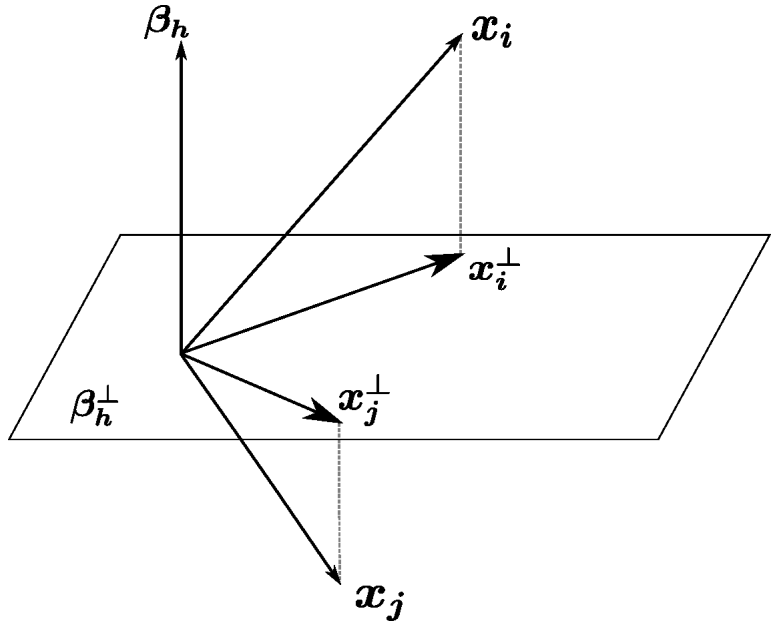
Goal: Remove the effect of common subspace

- $\boldsymbol{x}_i$ and $\boldsymbol{x}_j$: tissues/cell types i and j
- z-score normalize $\boldsymbol{x}_i$ to compute $\boldsymbol{z}_i$
- $\boldsymbol{\beta}_h$: the common signature
- z-score normalize $\boldsymbol{\beta}_h$ to compute $\boldsymbol{z}_h$
- Project to the orthogonal subspace:

$$\boldsymbol{z}_i^{\perp} = \left(\mathbf{I} - \frac{\boldsymbol{z}_h \boldsymbol{z}_h^T}{\|\boldsymbol{z}_h\|_2^2}\right)\boldsymbol{z}_i.$$



Similar in nature to the partial Pearson's correlation

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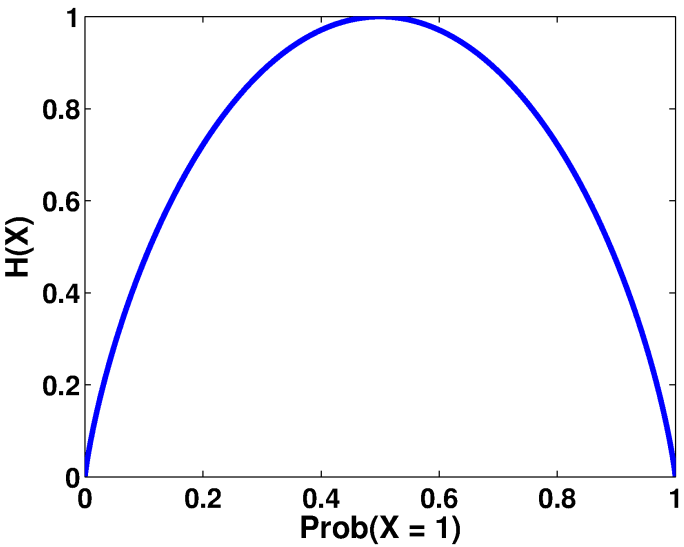
Goal: Estimate expression-specificity of genes across different cells



- Entropy as a measure of expression uniformity: $H(i) = -\sum_j p_{ij} log(p_{ij})$
- How informative observing a gene is with respect to the cell type that it came from
- Maximum entropy when probability of a gene coming from all cell types is equal
- For each gene i, compute a specificity factor w_i.

Similar formulation have been previously used for marker detection.

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ACTION-adjusted cell signatures

$$\mathbf{Y} = \boldsymbol{diag}(\boldsymbol{w})\mathcal{Z}^{\perp}$$

ACTION metric (kernel)

$$\begin{aligned} \mathbf{K}_{ACTION} &= \mathbf{Y}^{T}\mathbf{Y} \\ &= \left(\mathcal{Z}^{\perp}\right)^{T} \boldsymbol{diag}(\boldsymbol{w}^{2})\left(\mathcal{Z}^{\perp}\right) \end{aligned}$$

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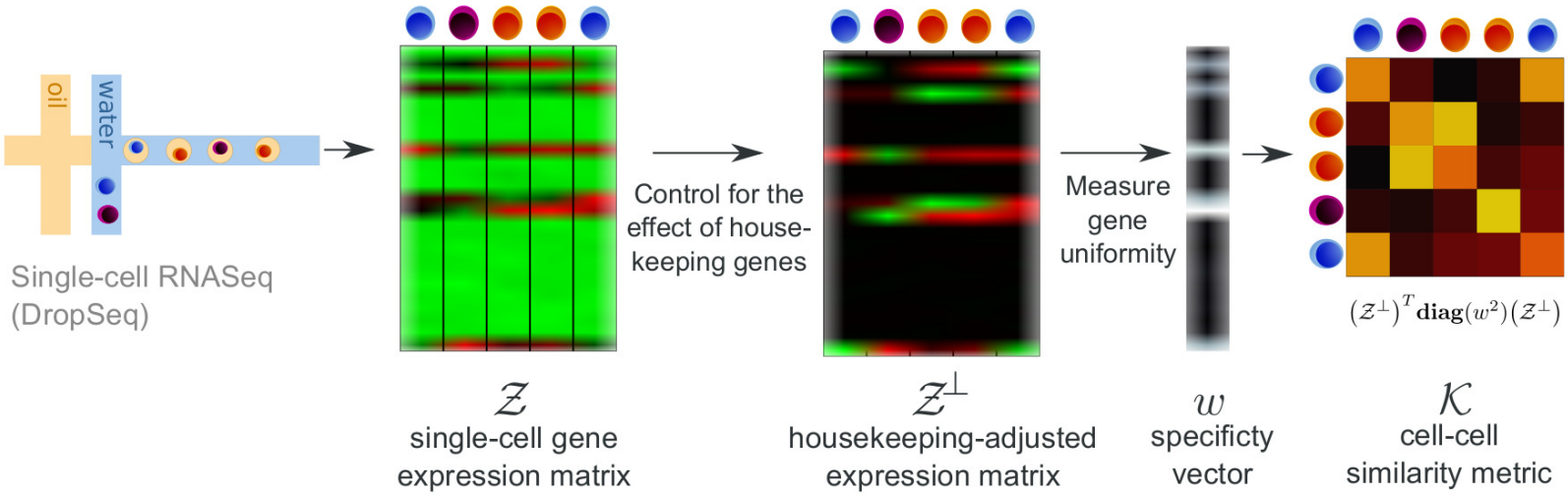
Component 1

Cell similarity kernel – revisited



- Now we have computed the ACTION kernel

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Benchmark datasets

- **Immune:** 1,522 immune cells from mouse hematopoietic system (30 different types of stem, progenitor, and fully differentiated cells)
- **Melanoma:** 4,645 malignant, immune, and stromal cells isolated from 19 freshly procured human melanoma tumors (7 major types, including T, B, NK, CAF, Endo, Macro, and Tumor)
- **MouseBrain:** 3005 cells from the mouse cortex and hippocampus (7 major types, including *astrocytes-ependymal, endothelial-mural, interneurons, microglia, oligodendrocytes, pyramidal CA1*, and *pyramidal SS*).
- **Pollen:** Small set of 301 cells spanning 11 different cell types in developing cerebral cortex

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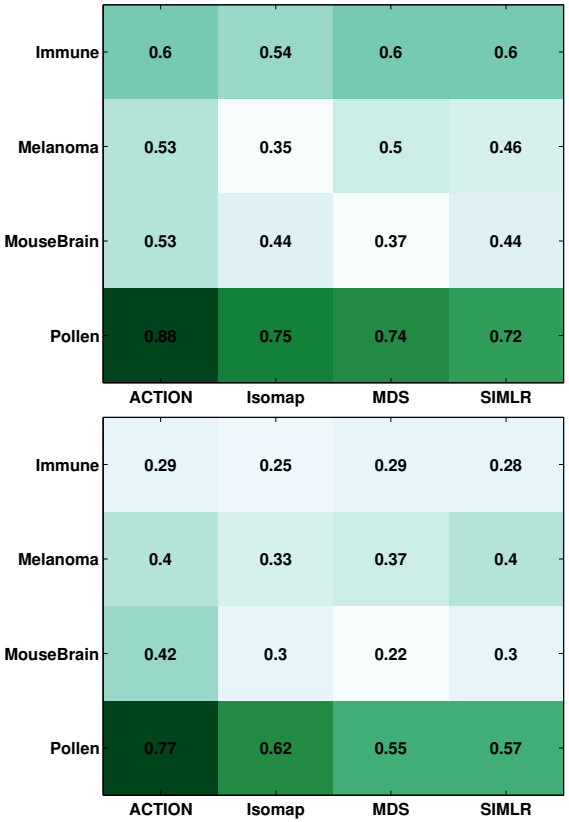
Performance of ACTION Kernel



- Benchmarks:
  - SIMLR: Specifically designed for single-cell data
  - IsoMap,MDS: General purpose dimension reduction
- Tested a range of parameters (5:5:50). Reported best case.
- Ties:
  - *Immune* (NMI: ACTION/MDS/SMLR, ARI: ACTON/MDS)
  - *Melanoma* (ARI: ACTION/SIML)
- In all other cases, *ACTION* metric significantly outperforms all other methods.

- *ACTION* metric performs equally good or better than other methods

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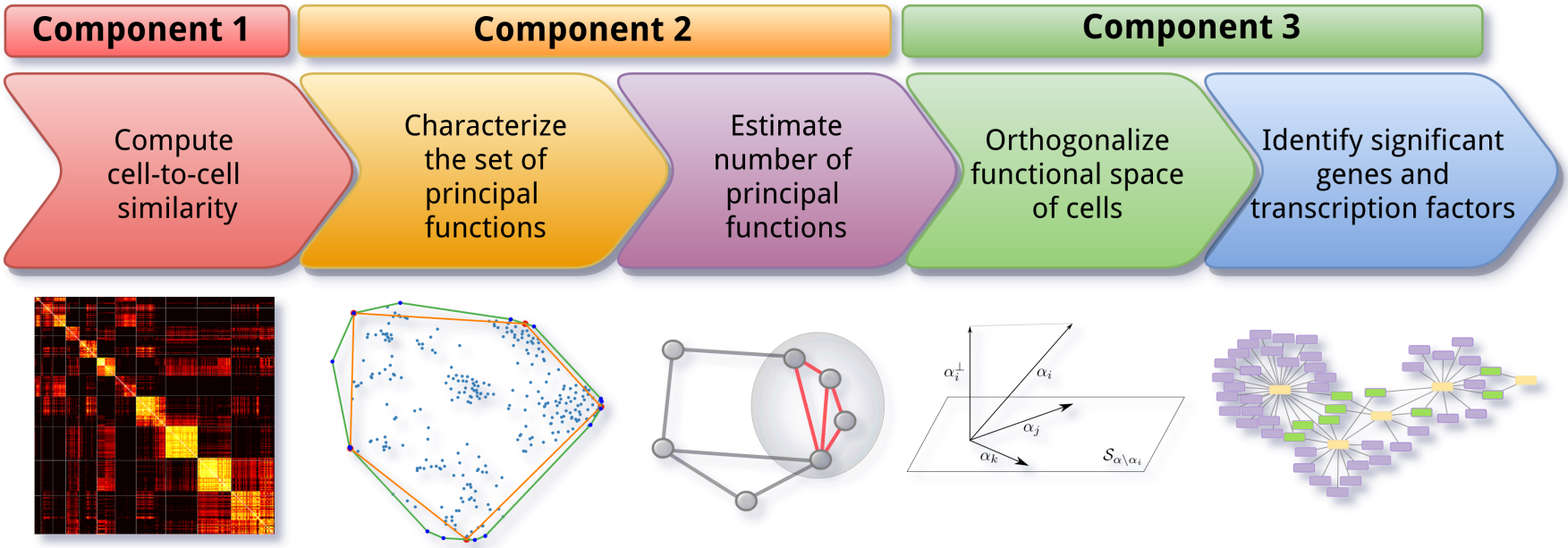
Overall Workflow

Component 2



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General framework

$$\begin{aligned} \underset{\mathbf{C},\mathbf{H}}{\text{argmin}} \quad & \| \mathbf{Y} - \underbrace{\mathbf{YC}}_{\mathbf{W}} \mathbf{H} \| \\ \text{subject to:} \quad & \| \mathbf{C}(:,i) \|_1 = 1. \\ & \| \mathbf{H}(:,i) \|_1 = 1. \\ & 0 \leq \mathbf{C}, 0 \leq \mathbf{H} \end{aligned}$$

Various algorithms can be cast using this formulation

- K-means: $\mathbf{C} \in \mathbb{R}^+, \mathbf{H} \in \{0,1\}$
- K-medoids: $\mathbf{C} \in \{0,1\}, \mathbf{H} \in \{0,1\}$

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There are fundamental problems with K-means/medoids:

- They use hard assignment, whereas many cell types are believed to form a continuum.
- They are sensitive to initialization.
- They are dependent on k.

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Convex NMF

$$\begin{aligned} \underset{\mathcal{K},\mathbf{H}}{\text{argmin}} \quad & \| \mathbf{Y} - \mathbf{Y}(:,\mathcal{S})\mathbf{H} \| \\ \text{subject to:} \quad & \| \mathbf{H}(:,i) \|_1 = 1, \mathbf{H} \in \mathbb{R}^+. \end{aligned}$$

- It uses the same formulation as k-medoid, but relaxes the hard assignment of cells: $\mathbf{C} \in \{0,1\}, \mathbf{H} \in \mathbb{R}^n$
- Unlike k-medoid and k-means, it has an optimal global solution.
  - Under near-separability assumption: there exists for each cell type an ideal example in the population.
- A modification of the *Gram Schmidt* process.

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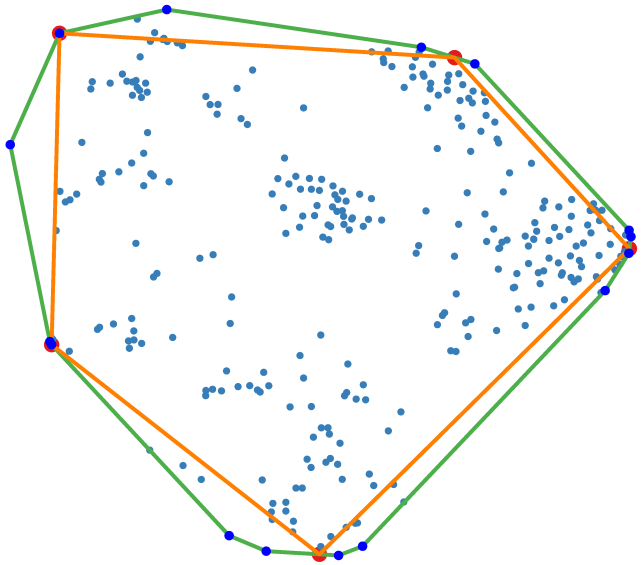

Geometry of functional space: each point is a cell and red points are the "pure cells"

- Picking k corner points/archetypes from the convex hull of the cells, such that they optimally "contain" the rest of cells.
- Each archetype is an ideal example of a cell type with a distinct set of principal functions.

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Goal: Understand the behavior of near-separable NMF

Performance guarantee

$$\max_{1\leq j\leq r}\ \min_{s\in\mathcal{S}} \parallel \mathbf{Y}(:,s) - \mathbf{W}(:,j) \parallel \leq \mathcal{O}\Big(\epsilon\kappa^2(\mathbf{W})\Big)$$

- For any near-separable matrix, multiplying it with any nonsingular matrix $\mathbf{Q}$ preserved separability, where matrix $\mathbf{W}$ is replaced with $\mathbf{QW}$.
- In this case, we have the following modified upper bound: $\mathcal{O}\Big(\epsilon\kappa(\mathbf{W})\kappa^3(\mathbf{QW})\Big)$.

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- It further relaxes matrix **C**: $\mathbf{C}, \mathbf{H} \in \mathbb{R}^{+}$.
- It can handle cases where pure pixel assumption is violated.
- But it no longer has global convergence guarantee $\rightarrow$ it is also dependent on the initialization
  - To address this issue, we use the solution of convex NMF for initializing A.A.
- In essence, it allows local adjustment of the Convex NMF solution.
- A variant of block-coordinate descent for optimization.

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Component 2
Finding the number of archetypes (k)

Goal: To identify when we should stop adding new archetypes.

- Idea is simple: keep adding archetypes till we sense "oversampling."
- Oversampling happens when we start adding archetypes that are "too close" to each other.
- Each archetype is a cell $\rightarrow$ we can compute their similarity of using the ACTION metric.

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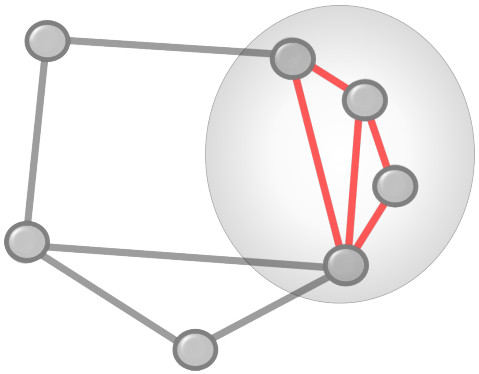
Component 2
Statistical significance of oversampling



- We build and threshold an archetype-archetype similarity graph.
- For each connected component in this graph, we assess its statistical significance using ER model.
- Probability that there exists in $\boldsymbol{G}$ a subgraph of density $\delta(Z)$ and size at least $|Z|$:

$$\Pr[\exists H \subseteq \boldsymbol{G}, |H| \geq |Z| \ : \ \delta(H) = \delta(Z)].$$

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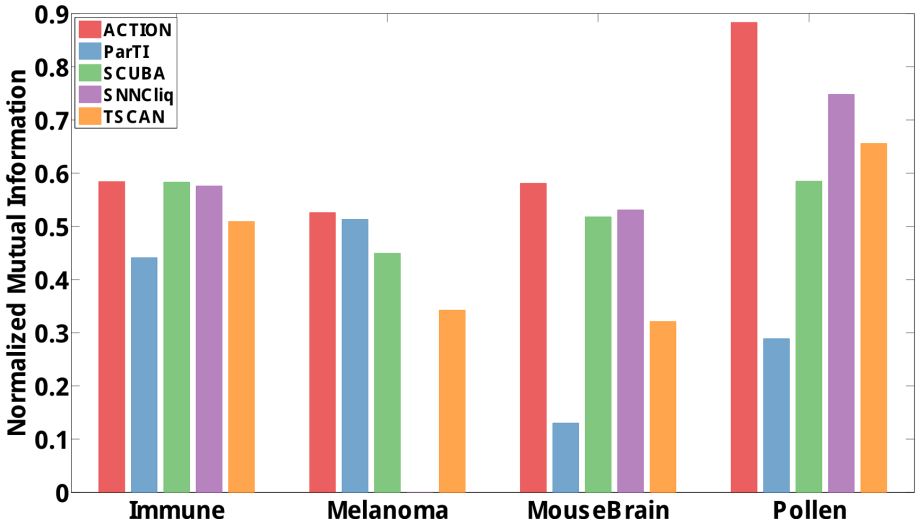
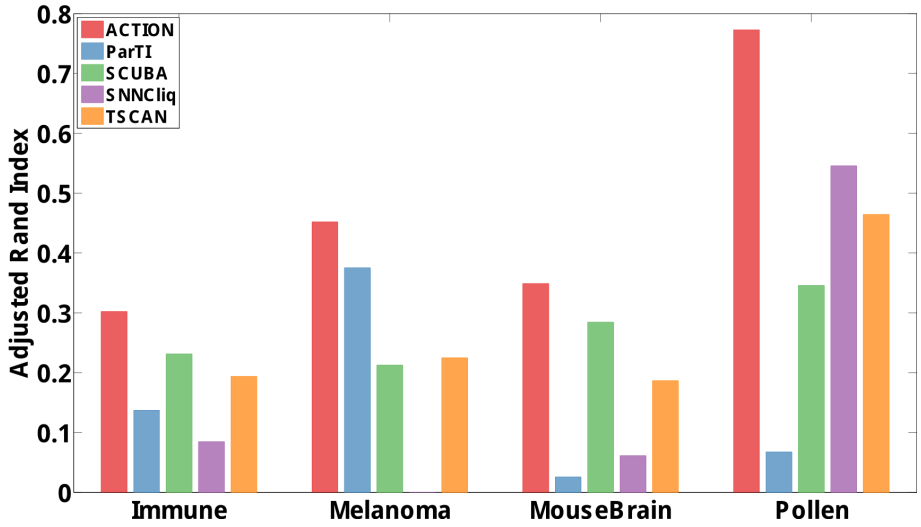
Component 2

Test 1: Identifying cell types using closest archetype

a



b



- ACTION excels in identifying underlying cell types in all cases

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- Use matrix $\mathbf{H}$ instead of $\mathbf{Y}$ in visualization:
  - We are interested in the relationship between cells and their surrounding archetypes.
- Initialize using Fiedler embedding
  - Position according to the dominant eigenvectors of the Laplacian matrix: $\mathbf{L} = \mathbf{diag}(\Delta_{\mathbf{Y}}) - \mathbf{Y}$.
- Update using t-SNE

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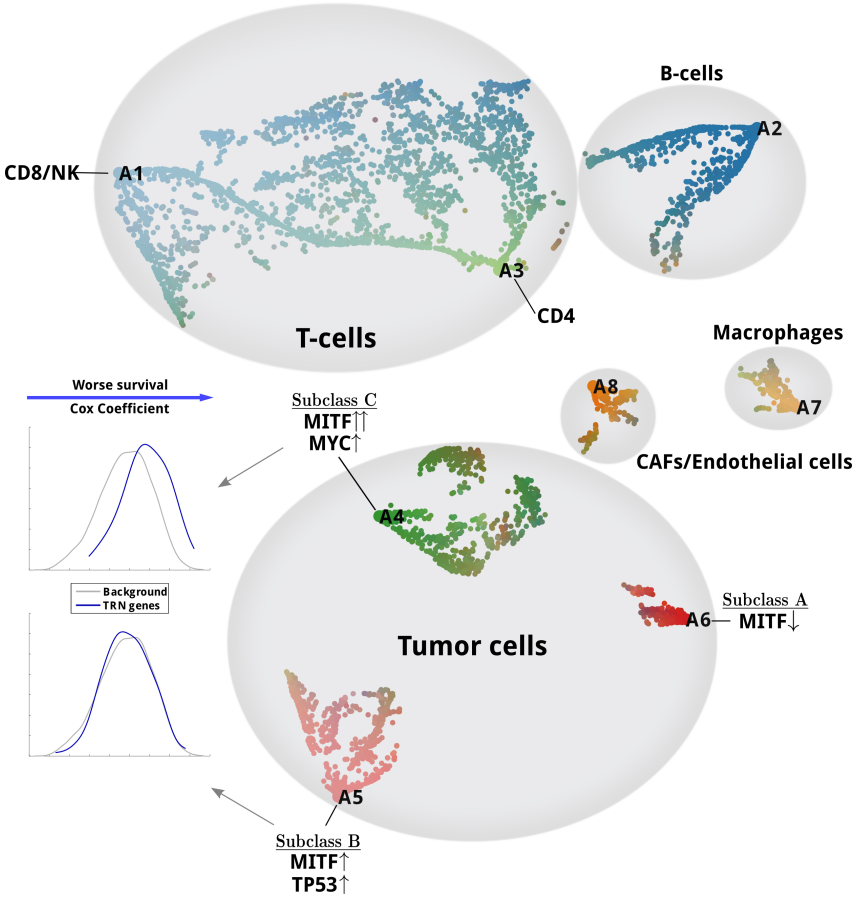
Continuous view

Case study in the Melanoma dataset



- T-cells reside in a continuum of states (Thogerson *et al.*).
- Tumor cells form compact groups.
- Two subclasses of MITF-associated tumors significantly differ in terms of their survival.

- ACTION sheds light on the underlying topology of cell types

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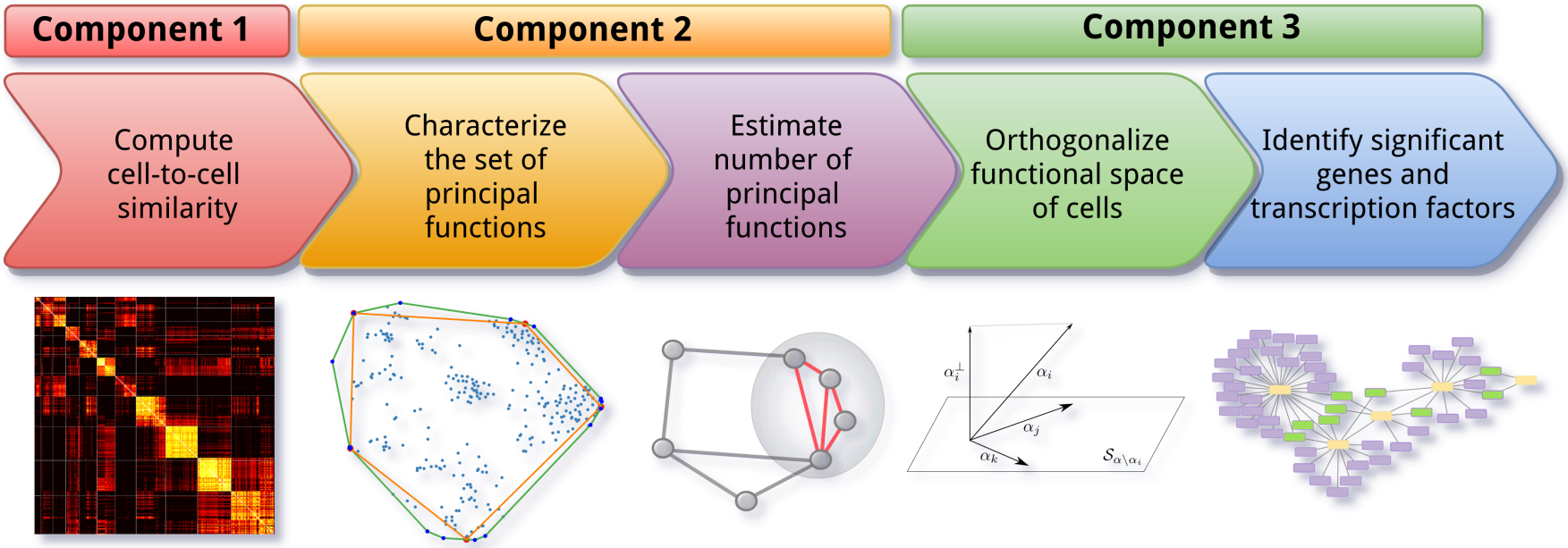
Overall Workflow

Component 3



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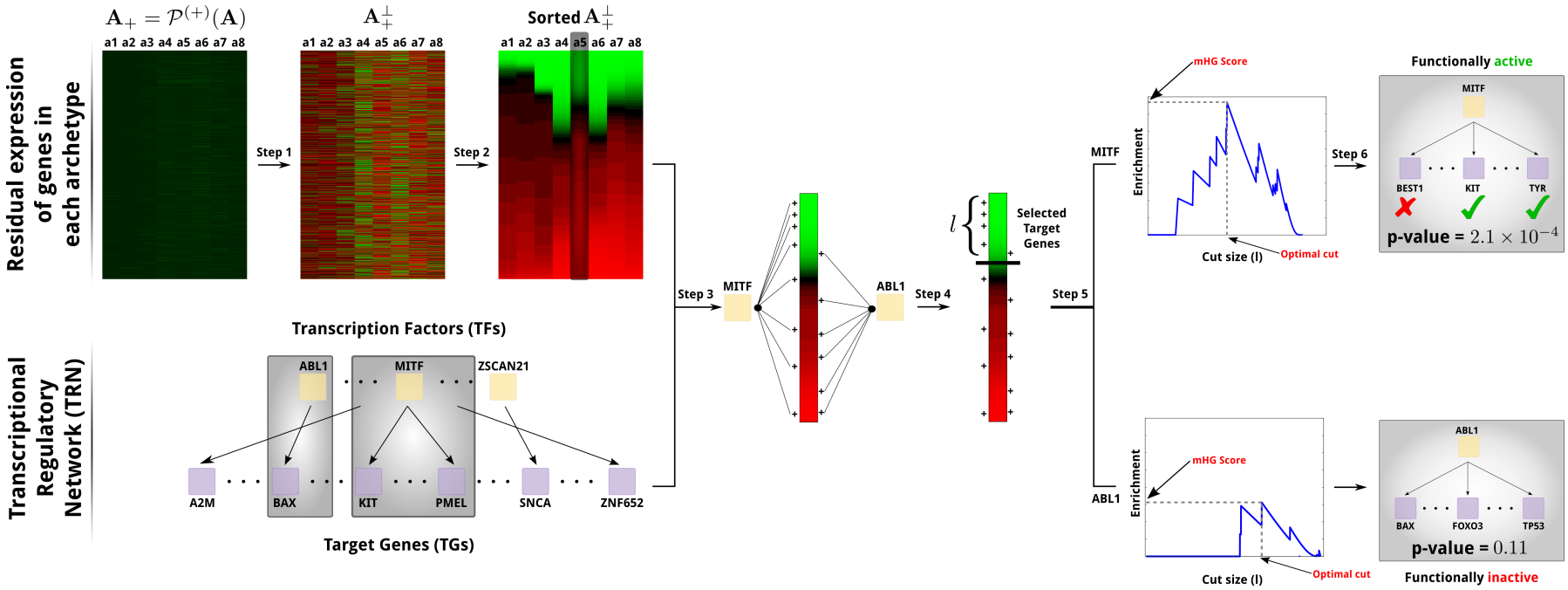
Component 3
Constructing TRN



Continued Goal: Identifying key regulatory elements that drive each cell type

1. Archetype Orthogonalization ($\rightarrow$ Only over positive projection)

$$\boldsymbol{a}_i^{\perp} = \left(\mathbf{I} - \mathbf{A}_{-i}(\mathbf{A}_{-i}^T\mathbf{A}_{-i})^{-1}\mathbf{A}_{-i}^T\right)\boldsymbol{a}_i$$

2. Assessing significance of TFs/TGs

$$\begin{aligned} p\text{-value}(Z = b_l(\lambda)) &= \text{Prob}(b_l(\lambda) \leq Z) \\ &= \sum_{x=b_l(\lambda)}^{min(T,l)} \frac{\binom{T}{x}\binom{m-T}{l-x}}{\binom{m}{l}} \end{aligned}$$

Use Dynamic Programming to compute exact p-value.

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Functional activity of transcription factors (TFs)

Key point!

We identify "functional activity" of transcription factors (TFs) by aggregating transcriptional activity of their downstream targets, not the transcriptional level of TFs themselves. TFs can, and typically do, get regulated through post-translational mechanisms.

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- Both *Subtype A* and *Subtype C* exhibit high activity of *MITF* and *Sox10* transcription factors, which are canonical markers for melanoma cells in the "proliferative" (as opposed to "invasive") state (Verfaiilie *et al.*).
- These two subtypes are significantly enriched for marker genes in the proliferative state:
  - *Subtype A:* 9.3×10^{-14}
  - *Subtype B:* 7.9×10^{-11}
- Subtype A has higher MITF activity (according to its activated targets):
  - *GPNMB*, *M1ANA*, *PMEL*, and *TYR* are shared between two subtypes.
  - *ACP5*, *CDK2*, *CTSK*, *DCT*, *KIT*, and TRPM1/P1 are uniquely upregulated in subtype A.

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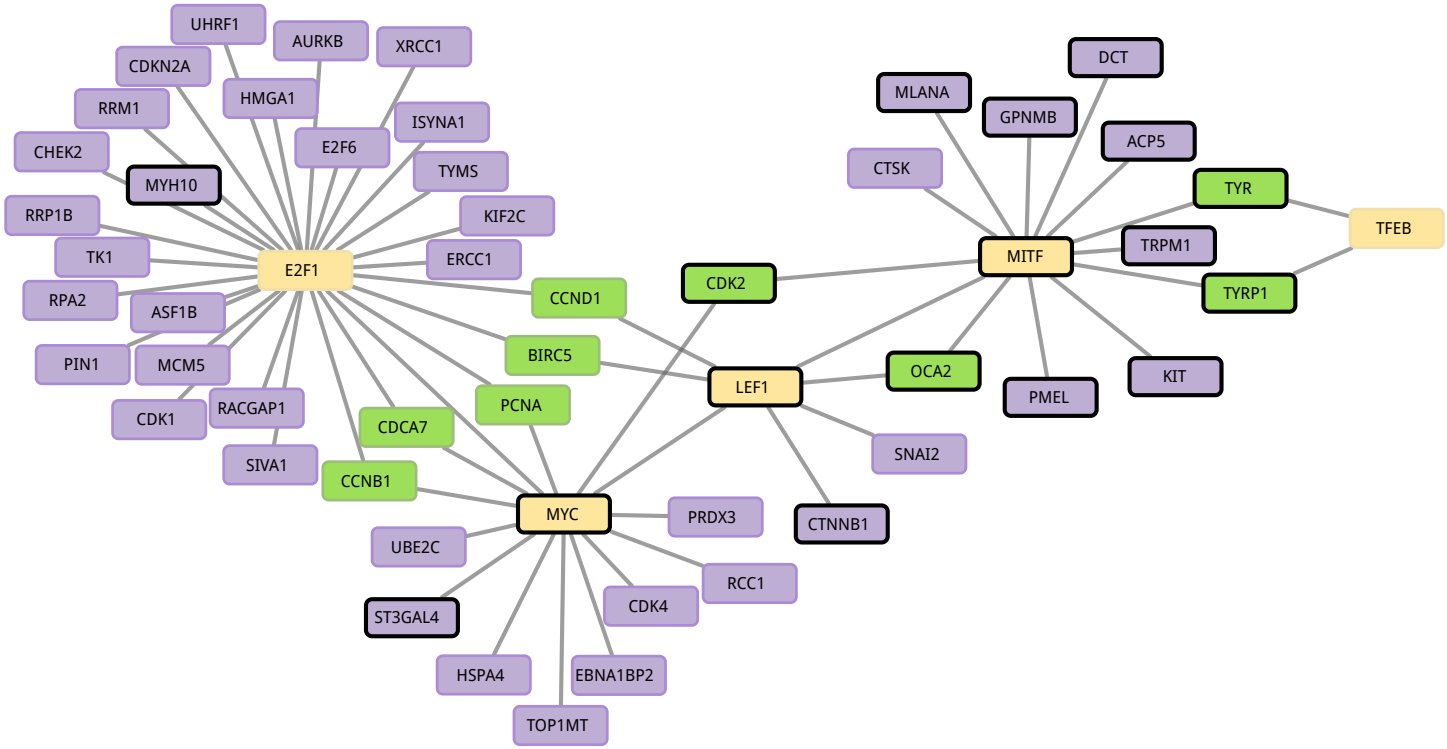
Dissecting transcriptional controls of Melanoma subclasses

Case study in MITF⇑⇑/MYC↑ subtype



- 19 "functionally" active transcription factors in subtype A (p-value ≤ 0.05)
- We focus on the five most significant TFs and their targets (p-value $\leq 10^{-3}$)

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Case study in MITF⇑/MYC↑ subtype

Core transcription factors

- MITF is one of the most well-known markers for classifying melanoma patients (Hartman *et al.*: MITF in melanoma: mechanisms behind its expression and activity).
- Overexpression of the E2F1 is common in high-grade tumors that are associated with poor survival in melanoma patients (Alla *et al.*: E2F1 in melanoma progression and metastasis).
- Melanoma cell phenotype switching, between proliferative an invasive states, is regulated by differential expression of LEF1/TCF4 (Eichhoff *et al.*:Differential LEF1 and TCF4 expression is involved in melanoma cell phenotype switching).
- Amplification and overexpression of the c-myc have been associated with poor outcome (Kraehn *et al.*: Extra c-myc oncogene copies in high risk cutaneous malignant melanoma and melanoma metastases).

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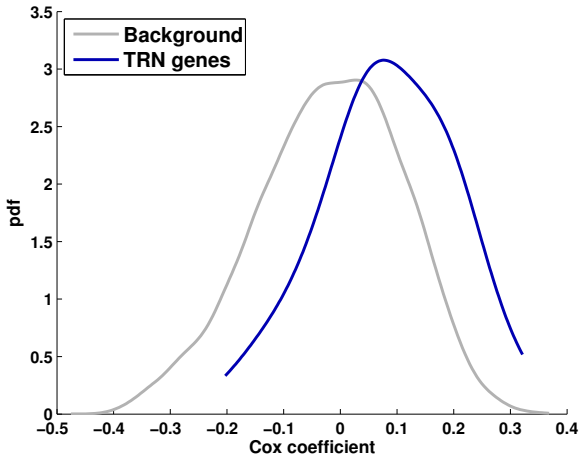
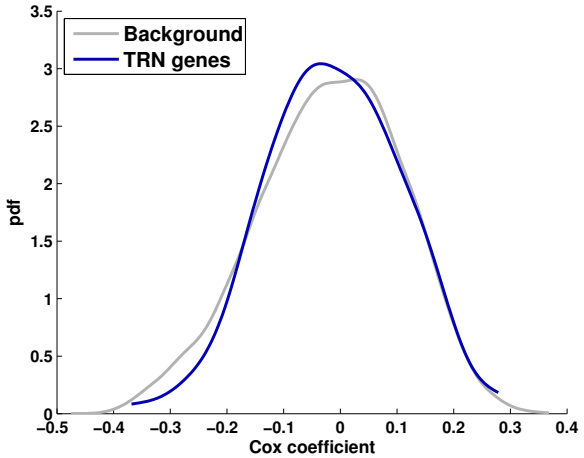
Dissecting transcriptional controls of Melanoma subtypes

Survival analysis



Subtype A: p-value $= 5.4 \times 10^{-10}$



Subtype C: p-value $= 0.31$

- OncoLnc (Jordan Anaya)
- Multivariate Cox regressions
- Gene expression, sex, age, and grade or histology as factors
- Genes associated with Subclass A have significantly worse outcome, compare to the background of all genes

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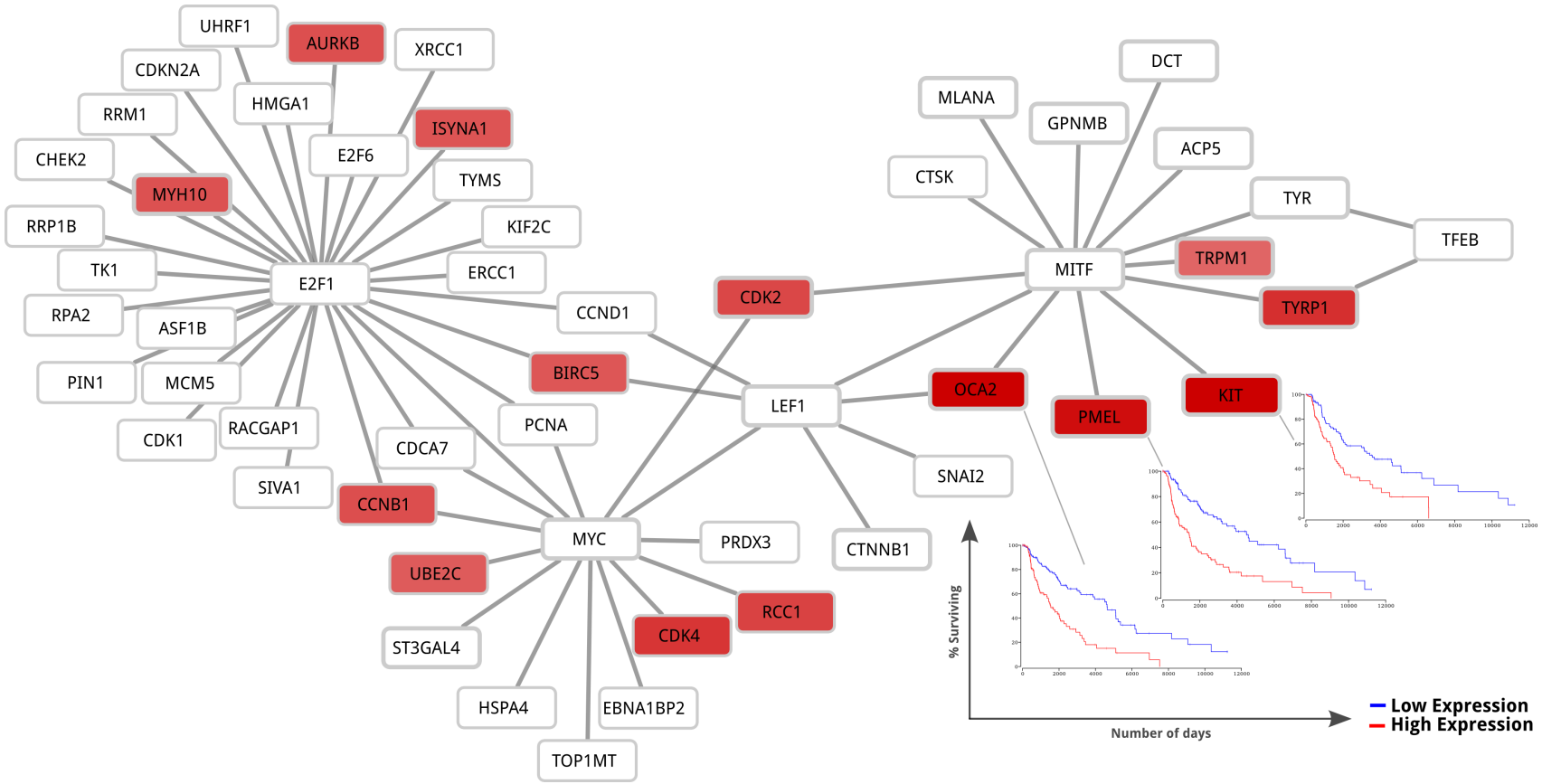
Case study in MITF⇑/MYC↑ subtype

Survival analysis revisited – Kaplan-Meier plots



1. Developed a novel cell similarity metric that is robust to biological noise, while at the same time is sensitive enough to identify weak cell type-specific signals
2. Characterized the functional identity of cells
   - Under the pure cell assumption, this metric induces a convex topology that embeds functional identity of cells
3. Utilized functional identity of cells to identify both discrete cell types and continuous cell states
4. Identified driving transcriptional controls that mediate the functional identity of cells

Clinical significance: Characterization of two MITF-associated subclasses of Melanoma patients, one of which has substantially worse outcomes, along with their underlying regulatory elements.

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Questions?

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