# Comp790-166: Computational Biology

Lecture 11

February 16, 2022

# Good Morning Question

Last time we saw the learned about the data augmentation strategy for single-cell landscapes.

1. Summarize the steps to generate these augmented points, given the initial set of points, $\mathcal{X}$
2. Talk about a concern related to this augmentation approach that is related to changing the biological interpretation of the data.

# Today

- Finish GSP basics
- Filtering - specifically low-pass filtering
- Identifying condition-specific prototypical cells with MELD

# Intermission for Announcements

- Homework 1 is due by 11:59pm eastern time on February 23
- Project proposal template is now online `https://github.com/natalies-teaching/Comp790-166-CompBio-Spring2022/blob/main/Project_Proposal/Project_Proposal.pdf`
- Sign up a time to present your project on March 7 or March 9, `https://docs.google.com/spreadsheets/d/1fX52jKWDWbJO1iB6D7FHv1DNQ53LPs0S8yNCUnUbSwQ/edit?usp=sharing`

# Discussion about Project Proposals

- **Abstract:** Sell your idea in 3-5 sentences. This is really good practice for figuring out what the story is with a project. Convince us why we should care.
- **Formal Problem Statement:** This should be a 1 to 2 sentence summary of what your problem is. Easier said than done.....
- **Contributions:** Think about a list of contributions and then put this into formal writing.
- **Intended Experiments:** Realistically you can aim for 1 to 2 experiments (more if you want!)
- **Implementation:** What is the product that you will give to the scientific community? (e.g. well-documented open source software)

# Graph Signal Processing

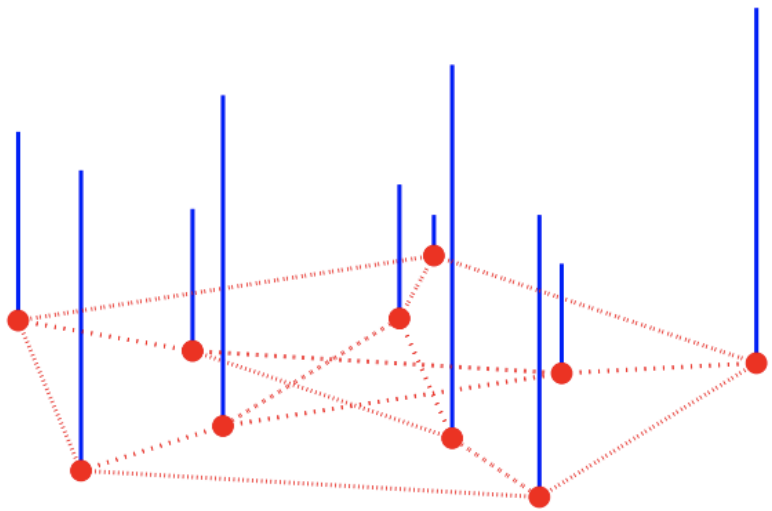


Figure: from Shuman *et al.* ArXiv. The purpose is to study the interplay between some signal and graph connectivity. Often we want to clean up or smooth out a particular signal, given the graph structure.

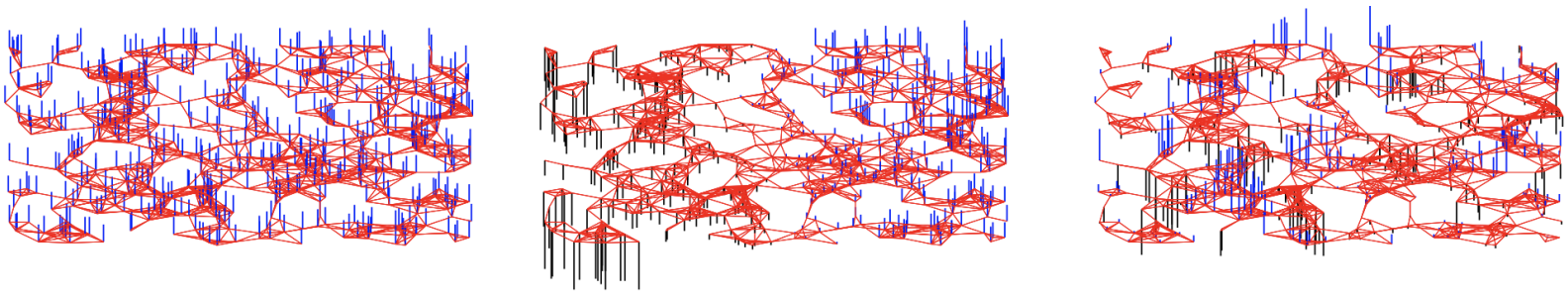
# How Localized is the Signal?

Remember, our friend Graph Laplacian ($\mathbf{L} = \mathbf{D} - \mathbf{A}$),

- Some very nice theory falls out about the eigenvalues of the Laplacian matrix in terms of how 'localized' a graph signal, $\mathbf{f}$, is. For example $\mathbf{f}$ could be an expression of some protein.
  - First re-write $\mathbf{f}$ in terms of eigenvectors of the Laplacian
  - The eigenvectors corresponding to the first smallest eigenvalues of $\mathbf{L}$ are considered **low frequency**, and hence entries of the eigenvector entries corresponding to nodes that are connected should be similar
  - For higher **high frequencies** corresponding to 'later' eigenvalues, the values of the eigenvectors of adjacent nodes will be more different.

# Signal Specificity

Here we visualize eigenvector entries at nodes ($\mathbf{u}_0$,$\mathbf{u}_1$,$\mathbf{u}_{50}$)



$\mathbf{u}_0$ $\mathbf{u}_1$ $\mathbf{u}_{50}$

Figure: from GSP Review https://arxiv.org/abs/1211.0053

# Same Concept Visualized A Bit Differently

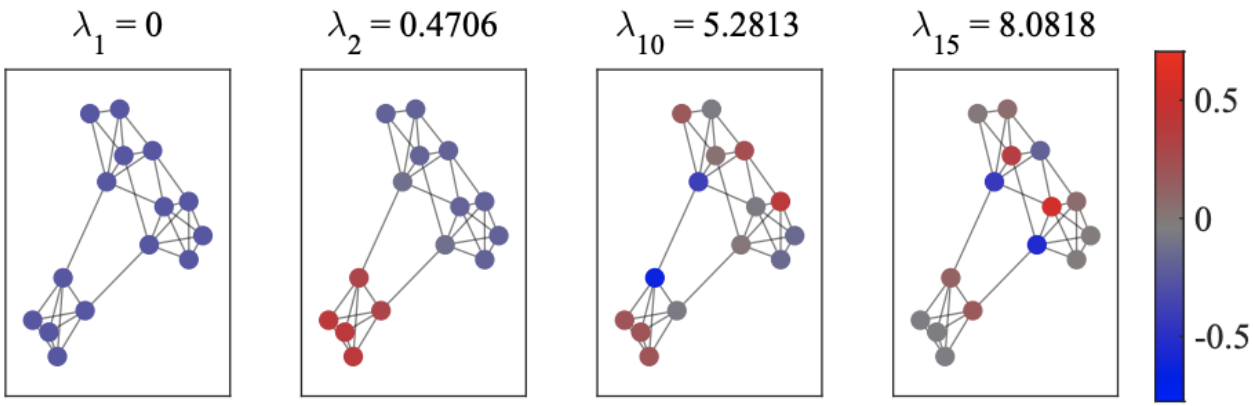



Figure: Notes are colored by their corresponding eigenvector component. From `https://arxiv.org/pdf/2008.01305.pdf`

# Similarly

Zero crossings mean that eigenvector entries are neighboring nodes will be different.

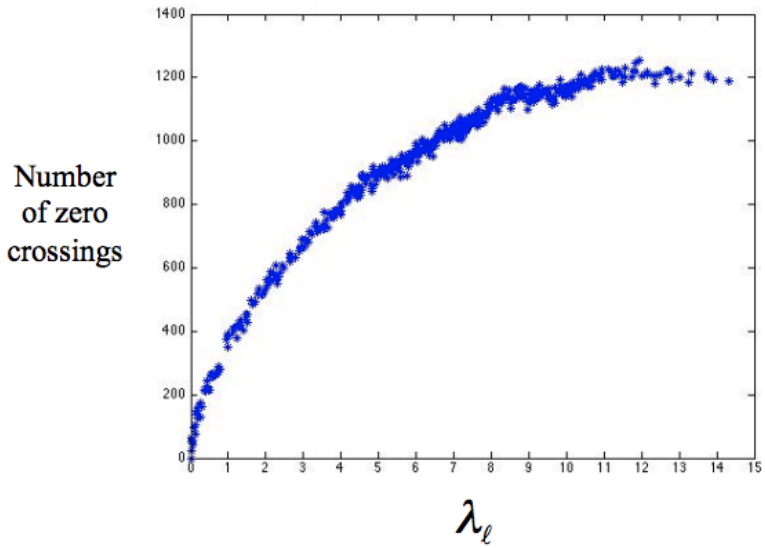



Figure: from GSP Review https://arxiv.org/abs/1211.0053

# What is Graph Fourier Transform (on a high level?)

- Explain frequency content of the graph signal (e.g. experimental measurements/labels/etc) as a weighted sum of the eigenvectors of the Graph Laplacian
- The eigenvectors of the Graph Laplacian comprise the **Graph Fourier Basis and can help to decouple high and low frequency signals**

# Local Variation of a Signal

The local variation of a signal or the sum of differences around a node can be written as,

$$(\mathcal{L}\mathbf{f})(i) = ([\mathbf{D} - \mathbf{A}]\mathbf{f})(i) \tag{1}$$

$$= d(i)\mathbf{f}(i) - \sum_j A_{ij}\mathbf{f}(j) \tag{2}$$

$$= \sum_j A_{ij}(\mathbf{f}(i) - \mathbf{f}(j)) \tag{3}$$

# Local Variation Leads to Total Variation

The total variation of a signal on a graph is defined as follows and is also known as the Laplacian Quadratic Form

$$TV(\mathbf{f}) = \sum_{i,j} A_{ij}(\mathbf{f}(i) - \mathbf{f}(j))^2 \tag{4}$$

$$= \mathbf{f}^T \mathcal{L} \mathbf{f} \tag{5}$$

- Note here I have been assuming that we have an unweighted graph, but you could certainly substitute $A_{ij}$ with a weighted version, $W_{ij}$

# Getting to Graph Fourier Basis

- Start with the eigendecomposition of of $\mathbf{L}$ as $\mathbf{L} = \mathbf{\Psi \Lambda \Psi}^T$
- We can look at eigenvectors, $\mathbf{\Psi} = [\psi_1, \psi_2, \ldots, \psi_N]$ of $\mathcal{L}$
- and eigenvalues, $\mathbf{\Lambda} = [0 = \lambda_1 \leq \cdots \leq \lambda_N]$ of $\mathcal{L}$

# The Graph Fourier Transform of a Signal

The $i$th frequency component of a signal, $\mathbf{f}$ is the inner product between $\boldsymbol{\psi}_i$ and $\mathbf{f}$ and can be written as,

$$\hat{f}_i = \psi_i^T \mathbf{f} \tag{6}$$

The Graph Fourier Transform (GFT) is written as,

$$\hat{\mathbf{f}} = \boldsymbol{\Psi}^T \mathbf{f} \tag{7}$$

# GFT Will Be Used to Filter

- A filter on the graph will take in a signal and attenuate it according to a frequency response function.
- **Low-Pass Filter:** We filter or preserve only frequencies corresponding to eigenvalues below some threshold, $\lambda_k$. So, consider frequencies $\lambda_b$, with $\lambda_b < \lambda_k$
- **High-Pass Filters**: Preserve only frequencies corresponding to eigenvalues above some threshold, $\lambda_k$. So, consider frequencies $\lambda_b$, with $\lambda_b \geq \lambda_{k+1}$

# A Simple Low-Pass Filter

Define some filter $h$ as,

$$h : [0, \max(\mathbf{\Lambda})] \rightarrow [0, 1] \tag{8}$$

Assuming the cutoff is $\lambda_k$,
$h(x) > 0$, for $x < \lambda_k$ and $h(x) = 0$, otherwise

# Defining Notation and Applying Filter to GFT

Define $h(\mathbf{\Lambda})$ as a diagonal matrix of eigenvalues with the filter applied. Based on what we computed with GFT, the filtered signal, $\hat{\mathbf{f}}_{filt}$ can be computed as,

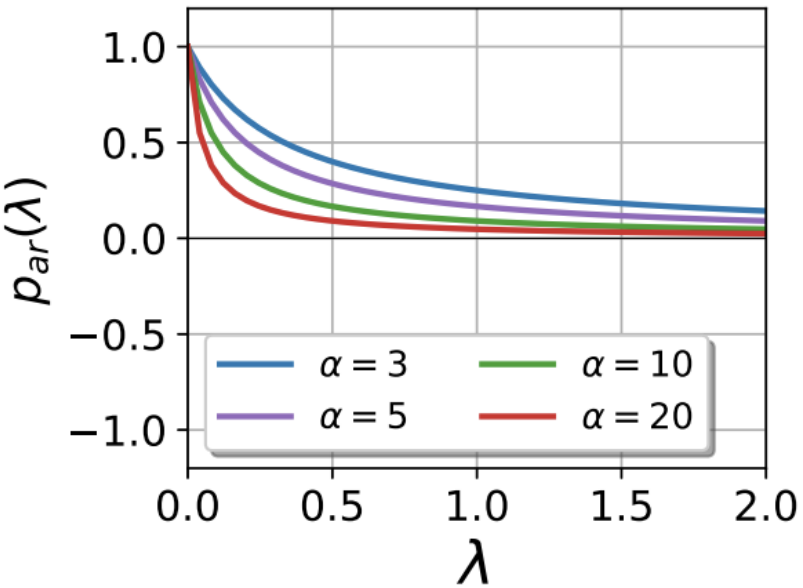
$$\hat{\mathbf{f}}_{filt} = h(\mathbf{\Lambda})\hat{\mathbf{f}} \tag{9}$$

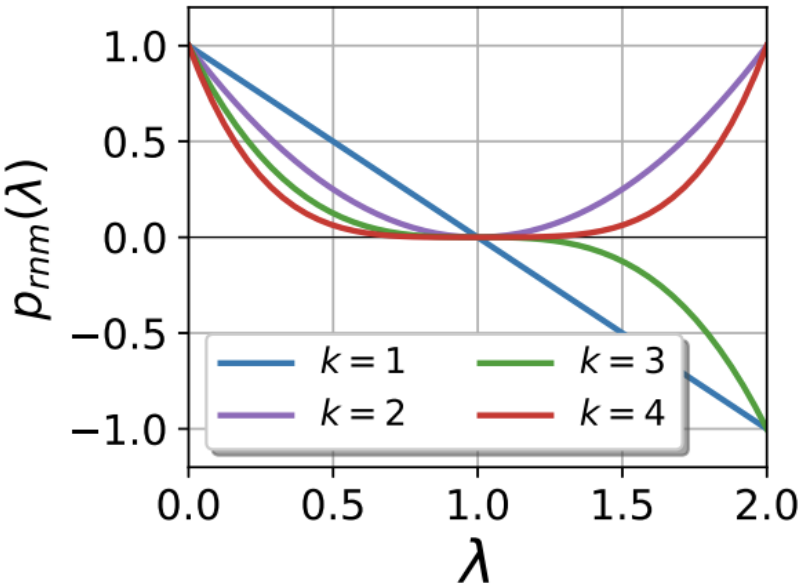
# Applying a Filter in General

In general, you can filter an original signal, $\mathbf{f}$ in general as,

$$\underbrace{\boldsymbol{\Psi}(\mathbf{I} + \alpha\boldsymbol{\Lambda})^{-1}\boldsymbol{\Psi}^T}_{\text{Filtered Graph Laplacian}} \mathbf{f}. \tag{10}$$

# Example Filters




(a) $p_{\text{ar}}(\lambda) = (1 + \alpha\lambda)^{-1}$ (b) $p_{\text{rnm}}(\lambda) = (1 - \lambda)^{k}$

Figure: from `https://openaccess.thecvf.com/content_CVPR_2019/papers/Li_Label_Efficient_Semi-Supervised_Learning_via_Graph_Filtering_CVPR_2019_paper.pdf`

# Example in PyGSP

- Access PyGSP here, `https://pygsp.readthedocs.io/en/stable/tutorials/intro.html`

$$g(x) = \frac{1}{1+\tau x},$$

let's define and plot that low-pass filter:

```
>>> tau = 1
>>> def g(x):
...     return 1. / (1. + tau * x)
>>> g = filters.Filter(G, g)
>>>
>>> fig, ax = plt.subplots()
>>> g.plot(plot_eigenvalues=True, ax=ax)
>>> _ = ax.set_title('Filter frequency response')
```

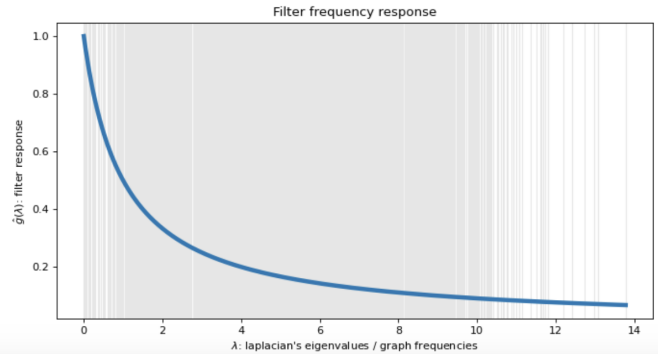
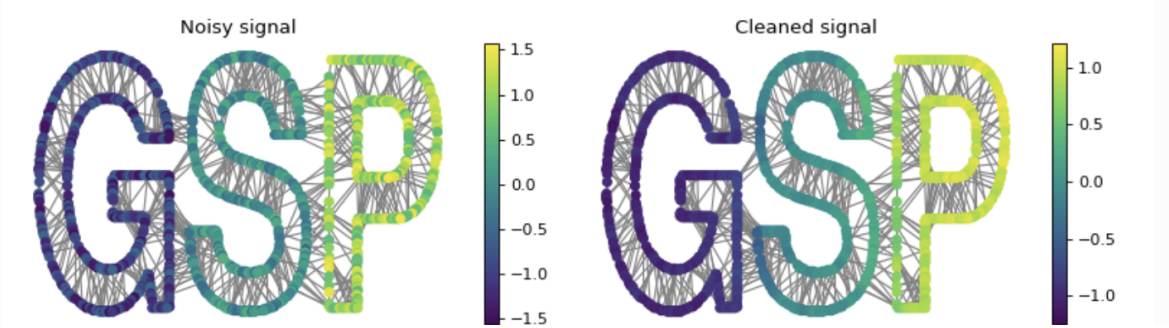



Figure: A simple filter for eigenvalues of **L**

# Low-Pass Filtering a Noisy SIgnal

```
>>> s2 = g.filter(s)
>>>
>>> fig, axes = plt.subplots(1, 2, figsize=(10, 3))
>>> G.plot_signal(s, vertex_size=30, ax=axes[0])
>>> _ = axes[0].set_title('Noisy signal')
>>> axes[0].set_axis_off()
>>> G.plot_signal(s2, vertex_size=30, ax=axes[1])
>>> _ = axes[1].set_title('Cleaned signal')
>>> axes[1].set_axis_off()
>>> fig.tight_layout()
```



# Linking Single Cell Data to External Information

# MELD (an application of GSP!)

The idea of MELD is to model how experimental perturbation alters cell states.

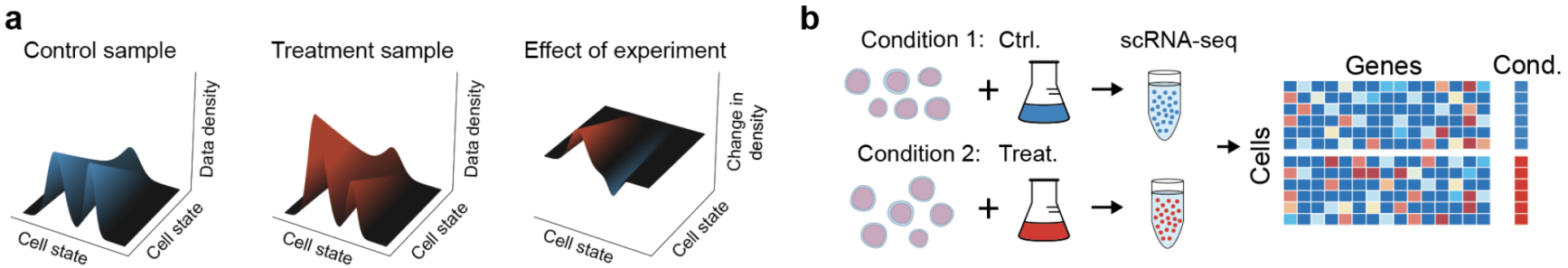



Figure: Burkhardt *et al.*, Nature Biotechnology. 2021. How more or less likely are we to observe a cell in a particular state in a control vs treatment sample?

# A Question for You

If you were just given a bunch of cells and someone told you to find 200 cells that were associated with an experiment or a condition of interest, how would you choose those cells? What would cause you to trust that a particular cell was indeed representative of the experimental label?

# Treatment as a Signal on a Graph

After creating a graph of cells, an indicator of treatment or control can be viewed as the signal on the graph. Interpretations of ‘signal‘ in relation to graph structure should help to inform treatment associated relative likelihood.

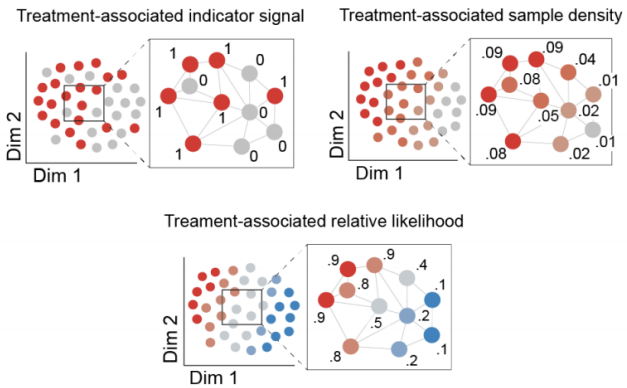



Figure: Burkhardt *et al.*, Nature Biotechnology. 2021.

# What on Earth is a Cell-State

We have seen cell states already!

- Frequency $\rightarrow$ how many of a particular cell-type are there in a sample?
- Function $\rightarrow$ Which proteins are activated in a particular cell-type?

# General Overview of the Steps of MELD

- Build a graph between cells based on gene or protein expression measurements
- **Graph Signals:** Experimental label (a binary indicator) is used to label each cell according to experimental condition
- Using GSP techniques, MELD filters biological and technical noise to look at how much the experimental signal of a cell matches the true experimental label. This quantifies how prototypical each cell is in its condition.
- Relate back to cell-types and features that differ between experimental conditions

# RES vs EES

EES represents the enhanced experimental signal, in comparison to RES, which was the raw, binary signal.

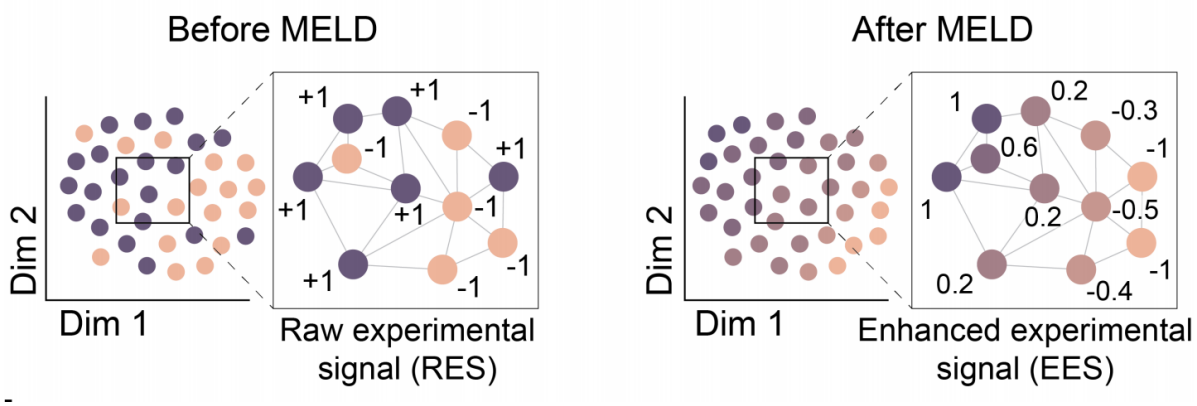



Figure: from Burkhardt *et al.*, Nature Biotechnology. 2021

# Sources of Noise

- Cells with similar feature measurements are said to be in the same state (biologically)
- **High Frequency Noise** : High frequency noise is when the labels of neighboring cells are rapidly fluctuating.
- Graph Fourier Transform is used to study the frequency of a signal over an irregular domain, like a graph.

# Incorporating these ideas into meld

- Low frequency components are thought to be where the true signal comes from (e.g. cell states that can differentiate groups)
- Define a latent variable $\mathbf{z}$ that describes the biological process that differs between the two conditions. This will end up giving us a score for each cell.
- Defining more specific variables
  - $\mathbf{x}$ is the vector of original labels (RES) for each cell
  - $\mathbf{z}$ is the vector of enhanced experimental signals (EES) for each cell.

# Visualizing **x** and **z**

The left is RES (**x**) and the right is EES **z**. **z** is what is being optimized.

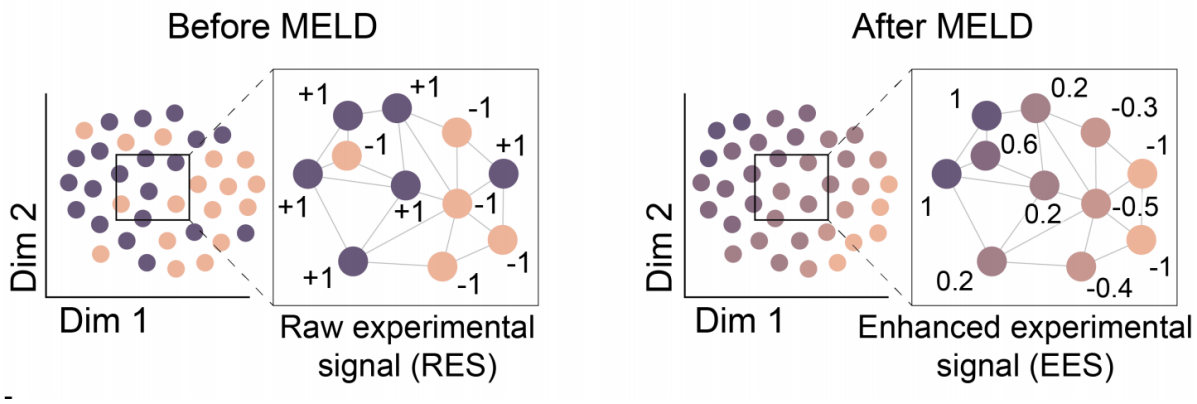



Figure: from Burkhardt *et al.*, Nature Biotechnology. 2021

# MELD Optimization Problem

An optimization problem can be defined for low pass filtering as,

$$\mathbf{y} = \underset{\mathbf{z}}{\operatorname{argmin}} \underbrace{\|\mathbf{x} - \mathbf{z}\|_2^2}_{\mathbf{a}} + \underbrace{\beta \mathbf{z}^T \mathcal{L} \mathbf{z}}_{\mathbf{b}} \tag{11}$$

# Unpacking

**z** is the EES or Enhanced Experimental Signal

$$\mathbf{y} = \underset{\mathbf{z}}{\operatorname{argmin}} \underbrace{\|\mathbf{x} - \mathbf{z}\|_2^2}_{\mathbf{a}} + \underbrace{\beta \mathbf{z}^T \mathcal{L} \mathbf{z}}_{\mathbf{b}} \tag{12}$$

- The Laplacian Regularization (term b) initially encourages smoothness for an input graph signal, **x**
- (**a**) Term a represents reconstruction between **x** and **z**
- (**b**) Term b represents Laplacian regularization or a measure of smoothness on the graph. Recall this looks a lot like total variation.

$$\beta \mathbf{z}^T \mathcal{L} \mathbf{z} = \beta \sum_{i,j} A_{ij} (\mathbf{z}(i) - \mathbf{z}(j))^2 \tag{13}$$

# Introducing the MELD Filter

They adjust the filer a bit as follows. The following allows also for a flexible notion of figure order, $\rho$,

$$\begin{aligned} \mathbf{y} &= \underset{z}{\text{argmin}} \|\mathbf{x} - \mathbf{z}\|_2^2 + \mathbf{z}^T \mathcal{L}_* \mathbf{z} \\ \text{where } \mathcal{L}_* &= [\beta \mathcal{L} - \alpha \mathbf{I}]^\rho \end{aligned} \tag{14}$$

# Takeaway

They show that their Laplacian Regularization is a filter with the following frequency response,

$$h_{\mathrm{MELD}}(\lambda) = \frac{1}{1 + (\beta\lambda - \alpha)^{\rho}} \tag{15}$$

This was a lot to unpack. I recommend staring at the details (if you are interested) in
`https://www.biorxiv.org/content/10.1101/532846v1.full.pdf`

# Filter Variety

Here are some experiments showing what parameters on the MELD filter will do to the frequency response, $h(\lambda)$.

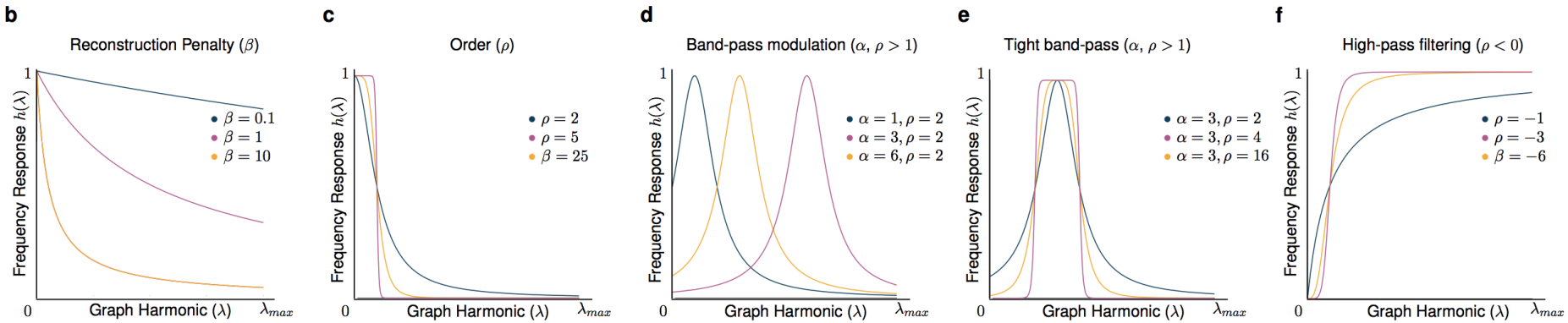



Figure: from Burkhardt *et al.*, Nature Biotechnology. 2021. Negative values of $\rho$, for example, can produce a high-pass filter.

# Meld Results

Computing the EES cleans up some of the noise and helps to better identify prototypical cells in each experimental condition.

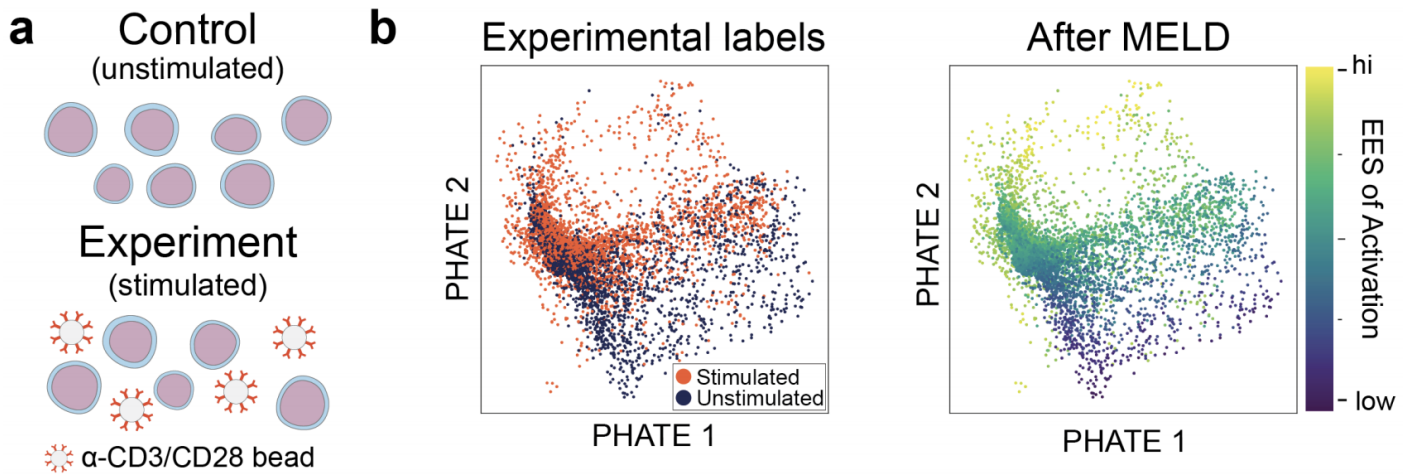



Figure: from Burkhardt *et al.*, Nature Biotechnology. 2021.