## Workflow For Publication Ready Analysis From Raw Data

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## Plugins

**FLOW**JO Exchange

flowjo.com/exchange

#### Apps that extend the functionality of FlowJo<sup>™</sup>

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Stain Index					ViolinBox

#### ViolinBox Bold-Italic → Covered in this webinar

## Analysis Workflow







## Pre-Processing: Stain Index



#### Measure your reagent brightness for good panel design





Identify and gate on high quality events.



FlowJo<sup>™</sup> v10

## Quality Control: FlowAI



#### Identify and gate on high quality events.



Pre-processing



Hierarchical Gating



#### Visualizations

**Quality Control** 

Concatenation

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## Data Annotation: Keywords



#### Annotate your data with descriptive terms

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Concatenation

**Pre-processing** 

**Quality Control** 

Hierarchical

Visualizations

Gating

## **Hierarchical Gating**

### Initial cleanup gates down to population(s) of interest







## Hierarchical Gating Visualizations: Sunburst

Smart visualizations to view your gating strategy and results

### Pre-processing









Concatenation

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To learn more about tree structure visualizations, check out this article by Jürgensmann, et vog informatik uni-rostock.de/~hs162/treeposter/oldposter/treevis_lores.pdf
For any questions or help using this plugin, reach out to the team at BD Life Science - Informatics: flowjo@bd.com

Cancel

Launch







## Hierarchical Gating Visualizations: Mist

#### Smart visualizations to view your gating strategy and results

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## **DownSample and Concatenate**



Merge events from all samples into a single file











### Embed all dimensions within a biparametric plot for ease of

### analysis



FlowJo<sup>™</sup> v10



# Dimensionality Reduction : TriMAP

#### Method to visualize with high fidelity the global structure of nonlinear data\*

TriMap plugin 0.1





How does it work?

- Semi-supervised metric learning
- Initialized with low dimensional PCA embedding, and this embedding is then modified using a set of selected triplets from the highdimensional representation



## **Clustering Algorithms**

# Choosing the correct clustering algorithm is crucial and experiment dependent



For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Adapted from Lie et al, 2019



Subset Name

Count

Sample Name

## **Clustering Algorithm: Phenograph**

PhenoGraph v2.4

#### Group cells based on phenotypic and/or parameter-derived similarities



Dimensionality reduction	PhenoGraph is a clustering algorithm that partitions high-parameter single-cell data into subpopulations. First, it constructs a nearest-neighbor graph of high-dimensional data points and then it applies the Louvain graph partition algorithm to dissect the nearest-neighbor graph into subpopulations. Please select your input parameters: Sample	export_concat_CD1d_1.fcs Priendgraph_AD74_16 export_concat_CD1d_1.fcs Phenograph_AD74_15 export_concat_CD1d_1.fcs Phenograph_AD74_14 export_concat_CD1d_1.fcs Phenograph_AD74_13 export_concat_CD1d_1.fcs Phenograph_AD74_12	441 404 356 793 976
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• Constructs a nearest neighbor graph and partitions the graph into communities

 $\times$ 

Does not require user to input on # of clusters •

Sorting





#### Evaluate identified clusters to ensure they are biologically and not just informatically relevant





# Discovery and Analyze: ClusterExplorer Flow Jot v10

#### Phenotype the different identified clusters lusters Options



Help

TriMap X RC2J

## Discovery and Analyze: Cluster Explorer

#### Identify trajectory of cell maturity or other phenotypes



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iNKT CD1d+ Cells

FlowJo<sup>™</sup> v10

Naïve/Early\* CD4+ CD8-

Effector\* CD4- CD8-

Terminal\* CD4- CD8+

TriMap\_X\_RC2J =

\*As described in Roederer et al.2015. Cell





# Identify the MFI of the different parameters and compare/graph across clusters



## Quantitative Analysis: ViolinBox



# Identify the MFI of the different parameters and compare/graph across clusters



Dimensionality reduction

Clustering



Clustering Quality Control







## Now What?

- You've found one or more interesting populations by high parameter clustering
- You would like to isolate this population for additional study, but don't have a hierarchical gating tree...

Question becomes: How do you sort a population identified with a clustering algorithm?



## **Our Solution: HyperFinder**

#### computes an optimized gating strategy for any population



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## **Our Solution: HyperFinder**



# creates a FACSDiva experiment template —> sort the population of interest



Dimensionality reduction



Clustering



Clustering Quality Control



Discover and Analyze



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## Summary





 Plugins facilitate interaction with bioinformatics programming environments and use of algorithmic tools by bench scientist with limited bioinformatics experience.



• Employing plugins in the proper workflow enables discovery and isolation of complex, potentially novel populations.



 Integration between FlowJo<sup>™</sup> and BD FACSDiva<sup>™</sup> allows transfer of gating information between acquisition and analysis software → sorting of cluster populations using computationally derived gates.

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Stephanie Wildmann

## **Additional Resources**



• Download Plugins:

https://www.flowjo.com/exchange/

Searchable Documentation

https://docs.flowjo.com/flowjo

• FlowJo University

https://www.flowjo.com/learn/flowjo-university/flowjo

Technical Support

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## Thank You!

Questions? Christian R. Aguilera-Sandoval, PhD. Crbech.flowjo@bd.com