

Advancing Cellular Analysis: A Comprehensive Workflow using Label-Free Spectral Flow Cytometry and Imaging

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ISAC SRL Emerging Leader

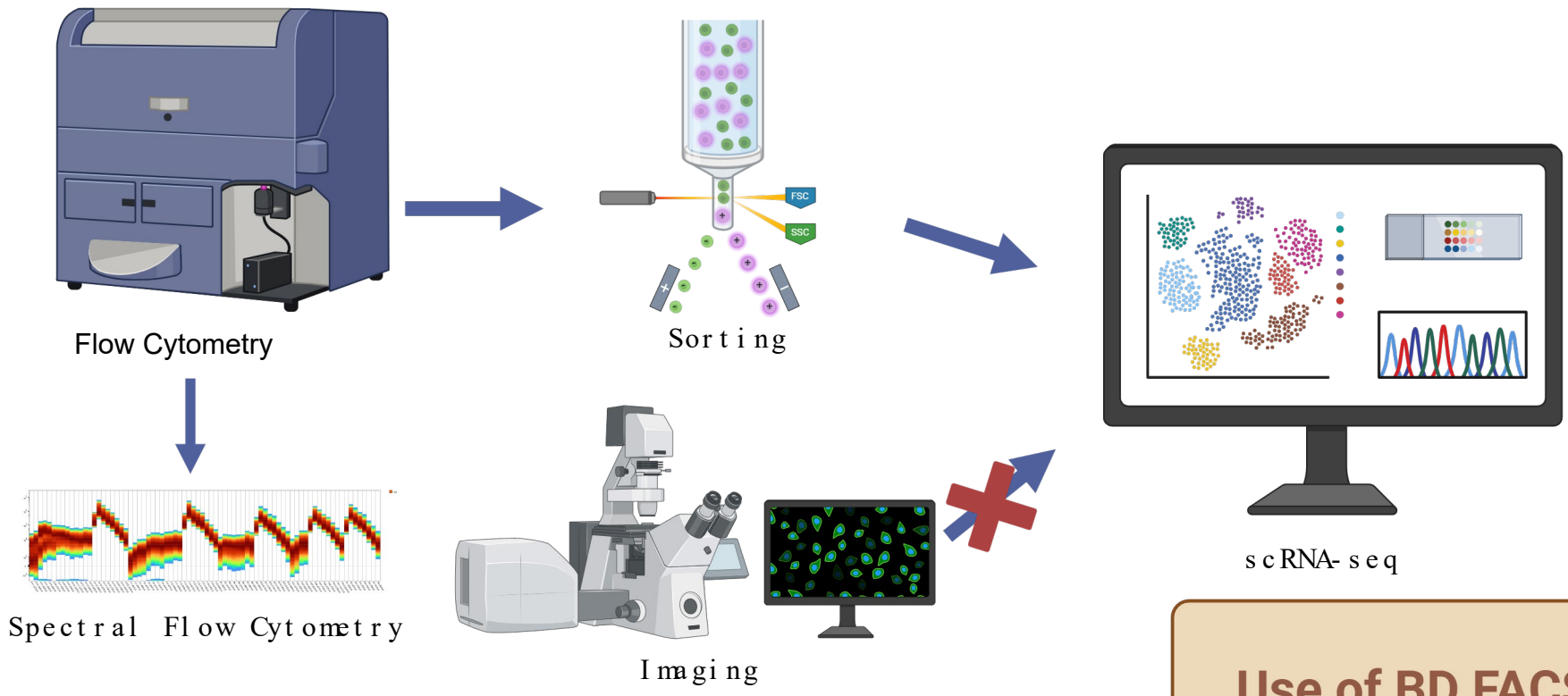


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CYTO 2025
DENVER, CO
MAY 31 - JUNE 4



Introduction – Various advanced methods are used to study cellular and phenotypic heterogeneity



Use of BD FACSDiscover™ S8 Cell Sorter

- Spectral flow cytometer
- Real-time imaging
- Cell Sorter


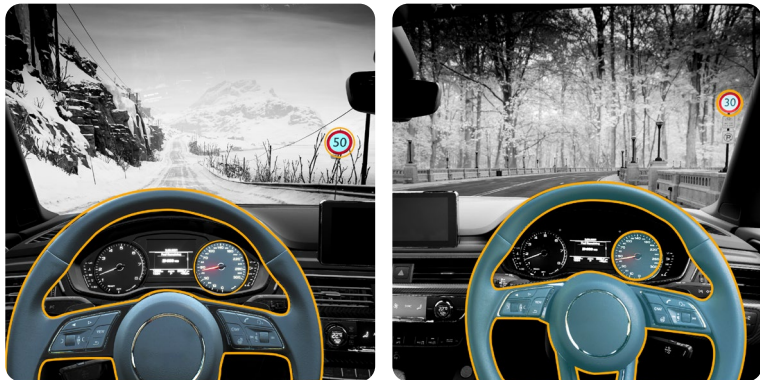
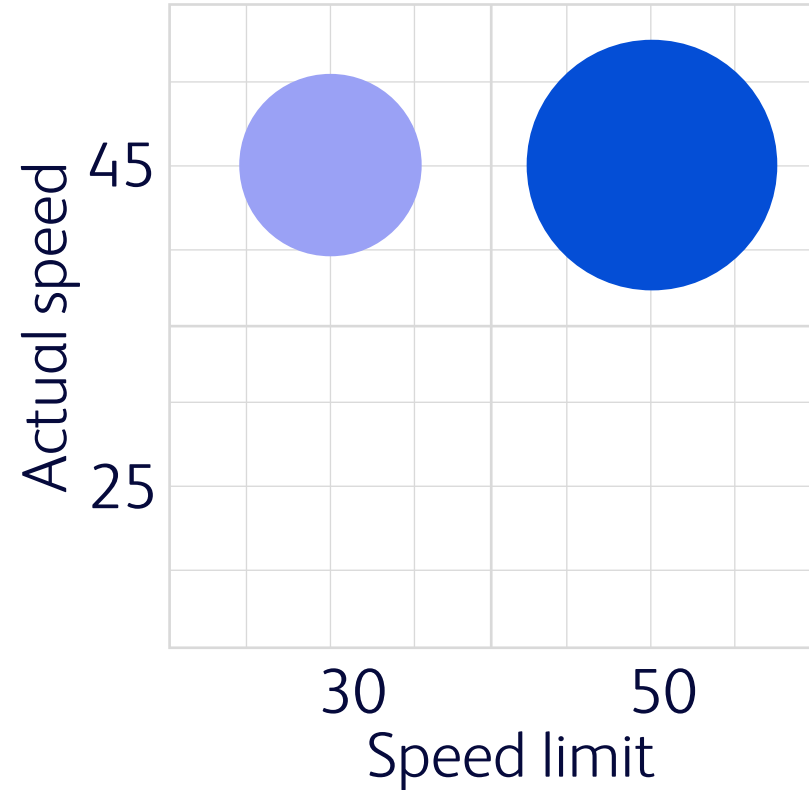
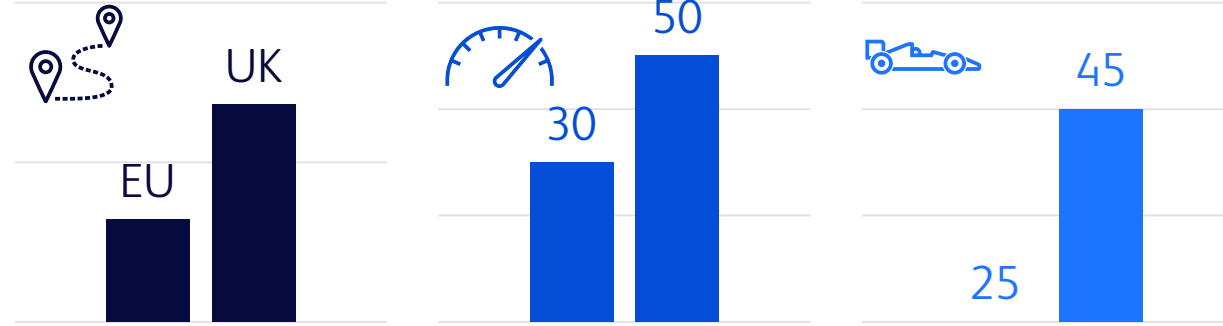


Figure created using biorender.com



BD CellView™ Image Technology: Quantifiable Imaging features



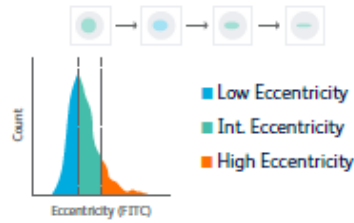
Parameters

- Origin
- Speed limit
- Speed

BD CellView™ Image Technology: Quantifiable Imaging features

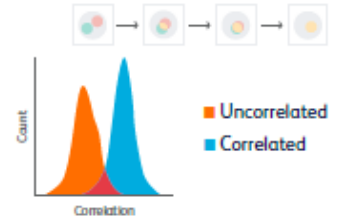
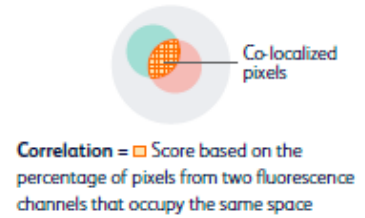
Eccentricity (Scatter and fluorescence)

- » Morphology
- » Cluster identification
- » Doublet discrimination



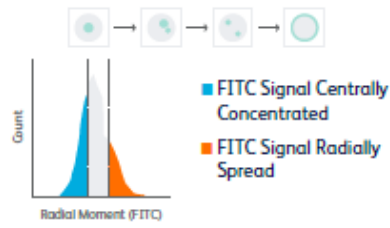
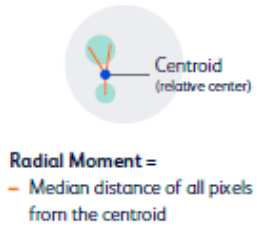
Correlation (Fluorescence: Any two channels)

- » Nuclear translocation
- » Co-localization
- » Intracellular trafficking



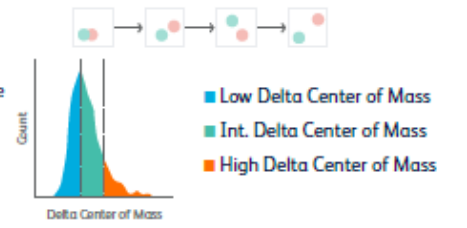
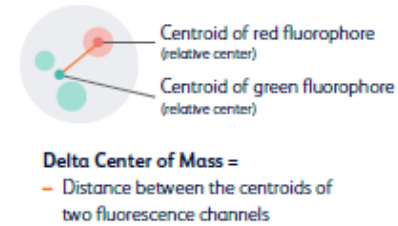
Radial Moment (Scatter and fluorescence)

- » Morphology
- » Cluster identification
- » Doublet discrimination



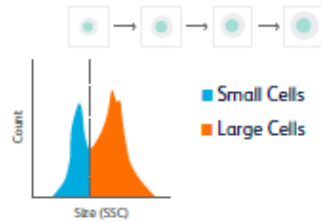
Delta Center of Mass (Fluorescence: Any two channels)

- » Intracellular trafficking
- » Cell-cell interaction



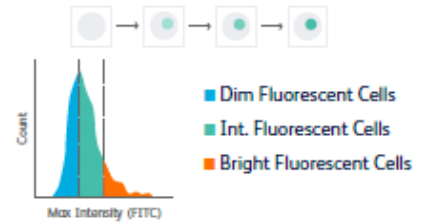
Size (Scatter and fluorescence)

- » Morphology
- » Label-free sorting
- » Cell cycle



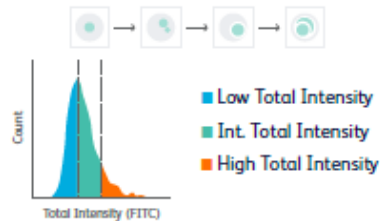
Max Intensity (Scatter and fluorescence)

- » Punctate fluorescence
- » Phagocytosis assay
- » Cell cycle analysis



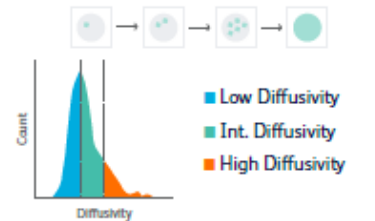
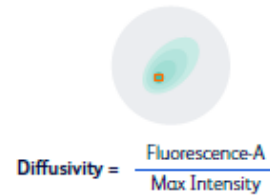
Total Intensity (Scatter and fluorescence)

- » Morphology
- » Imaging FMOs



Diffusivity (Scatter and fluorescence)

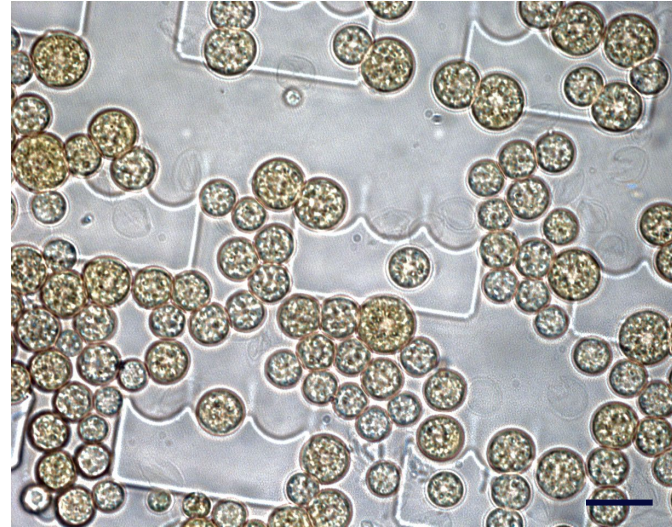
- » Punctate fluorescence
- » Cell morphology
- » Phagocytosis



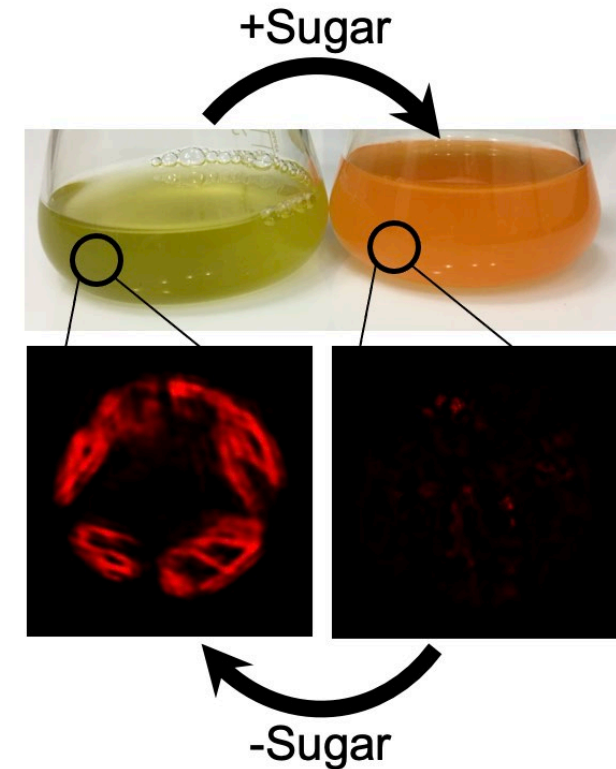
Introduction – biological model system: Green algae *Auxenochlorella protothecoides* (AP) & *Chromochloris zofingiensis* (CZ)

Characteristics:

- Unicellular green algae
- Eukaryotic cells
- Metabolic flexibility
- Photosynthetic switch
- Biotechnology relevant species

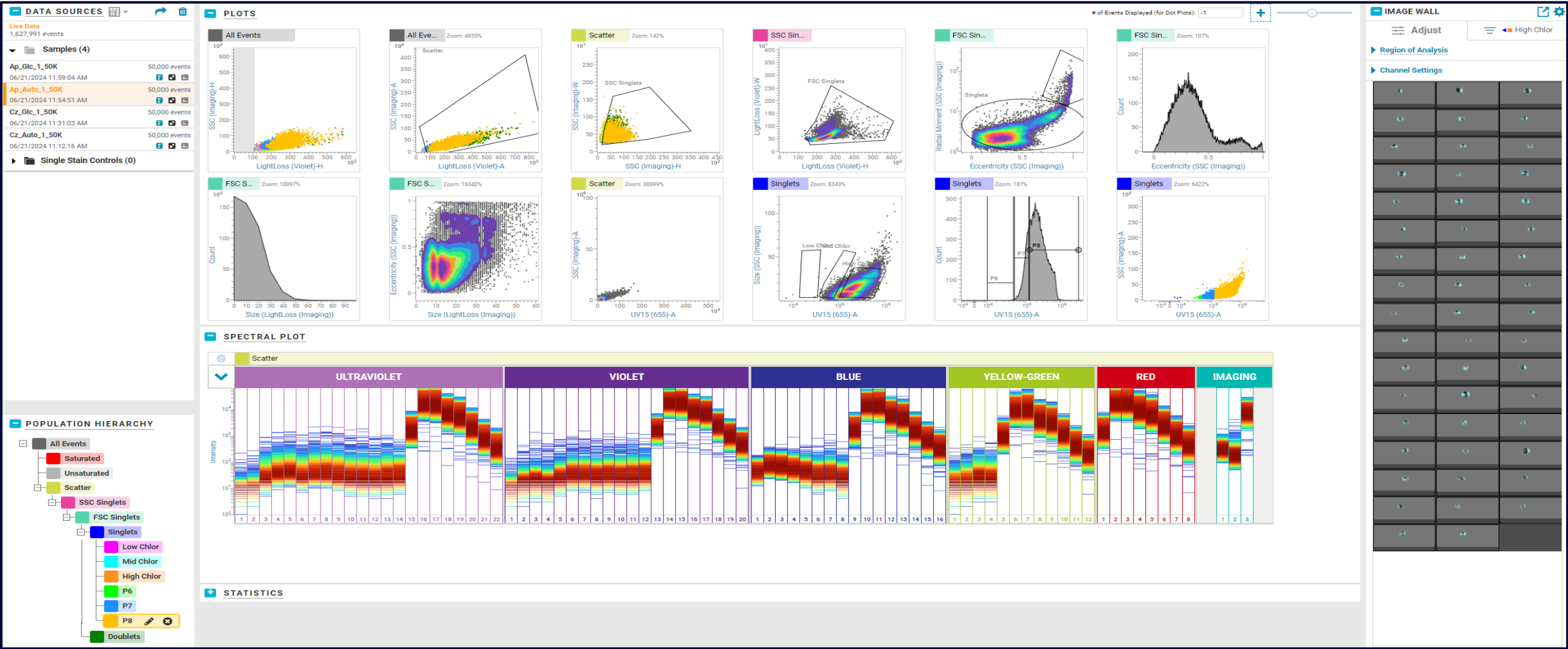
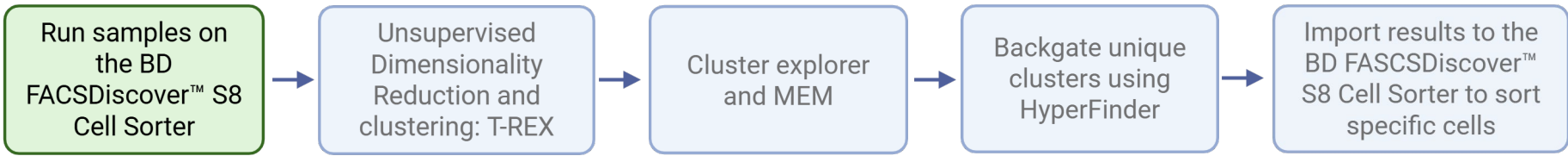


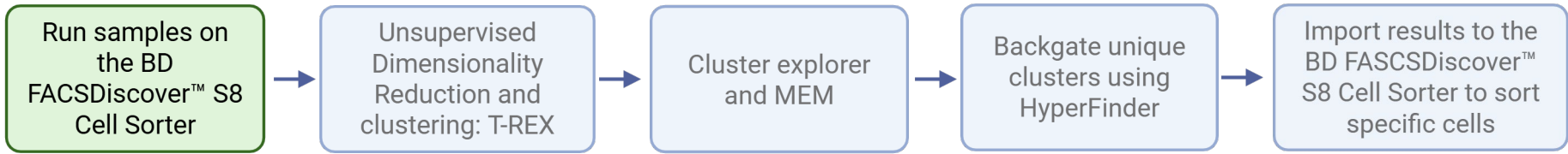
CZ under microscope



Assess species heterogeneity & phenotypic changes that accompany change in metabolism.

Workflow

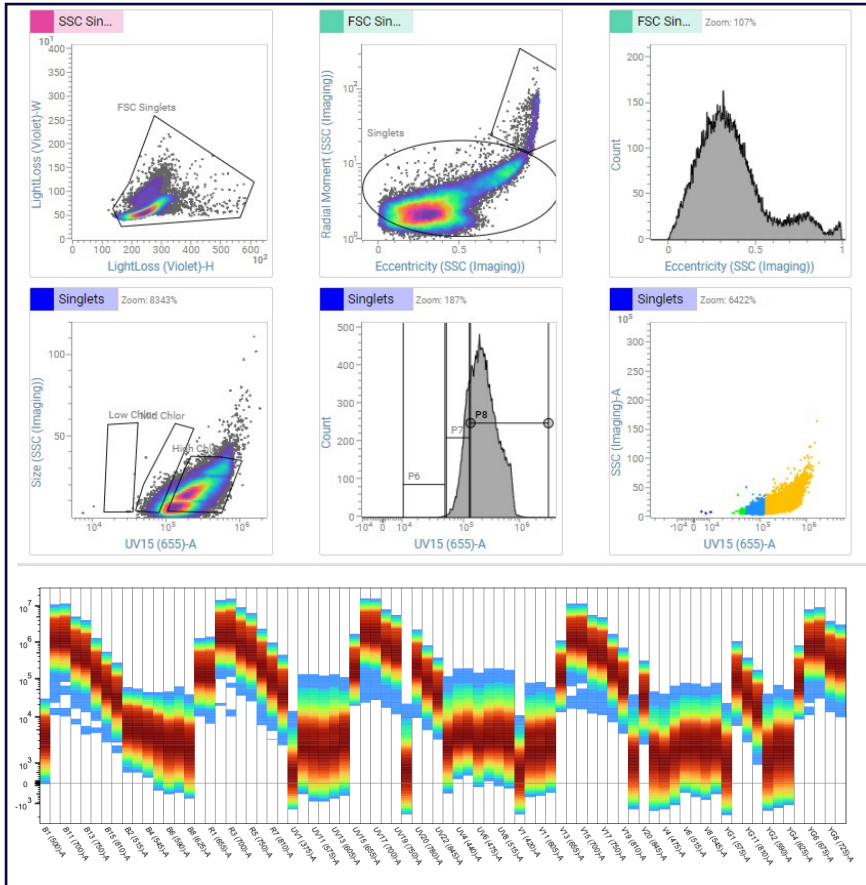




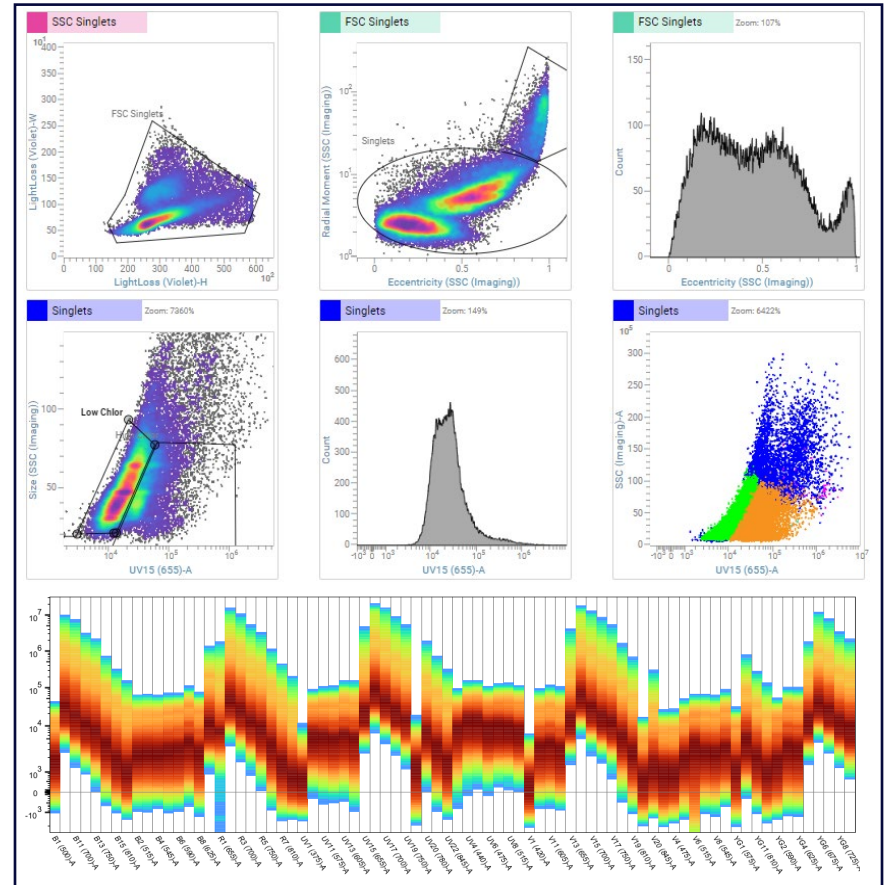
Autotrophic

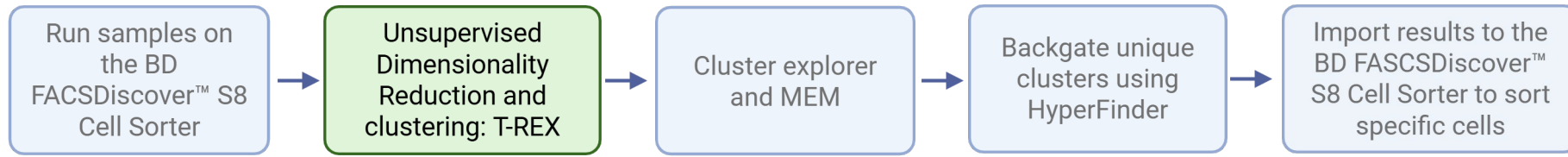
Heterotrophic

Auxenochlorella (AP)



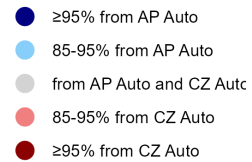
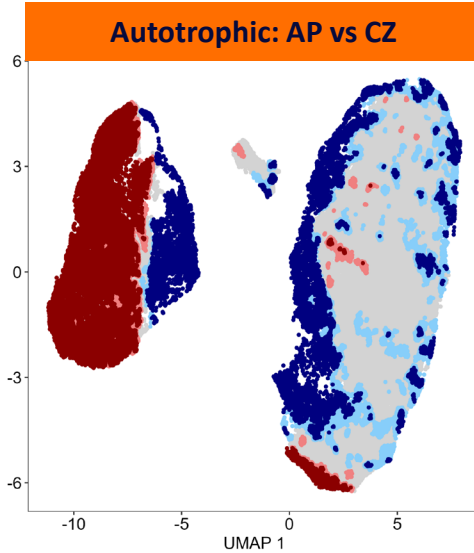
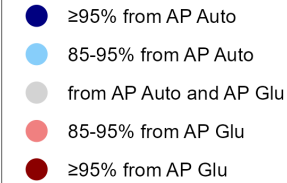
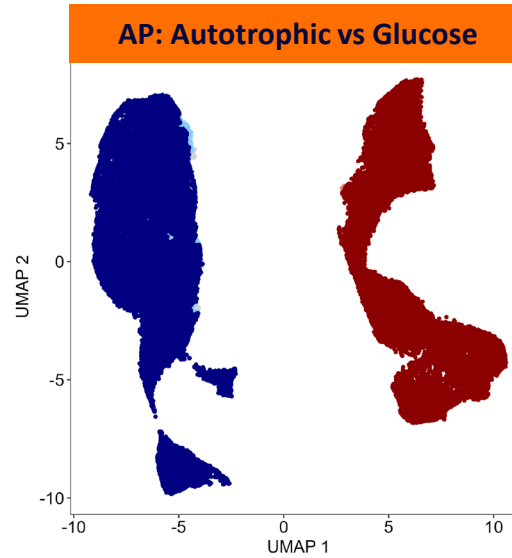
Glucose
→



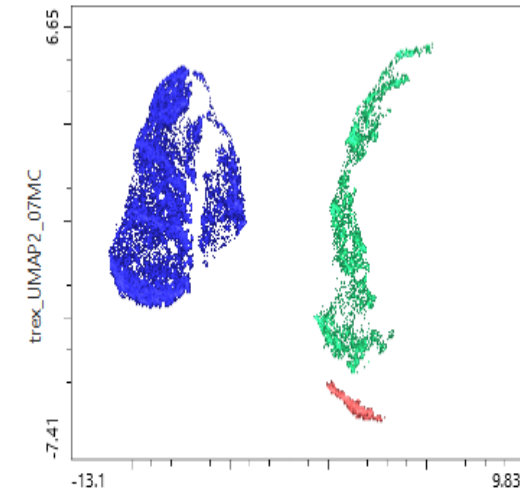
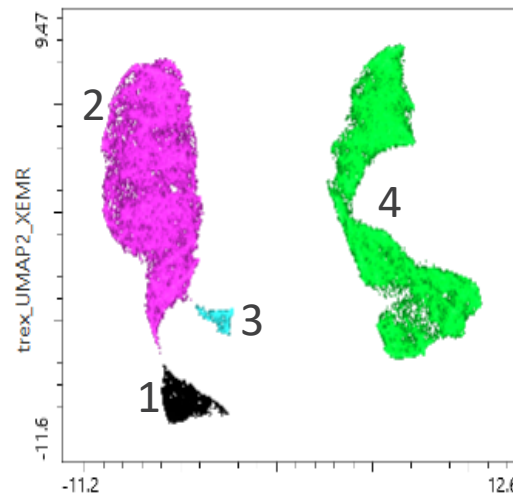


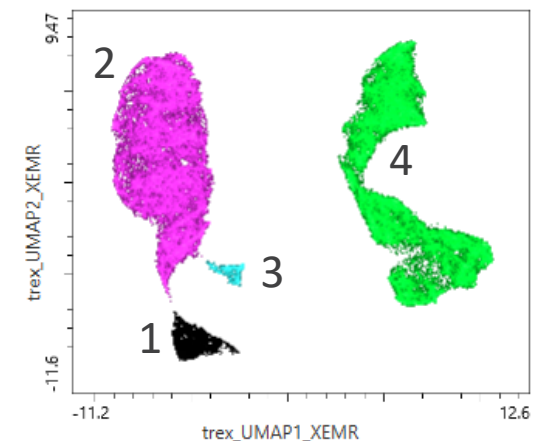
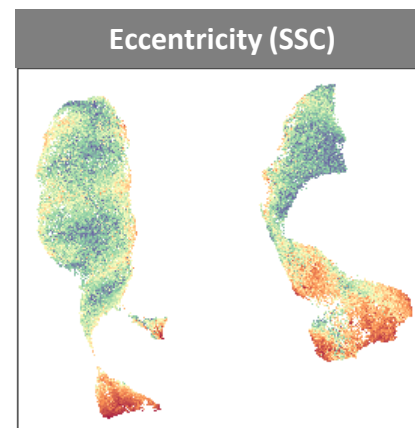
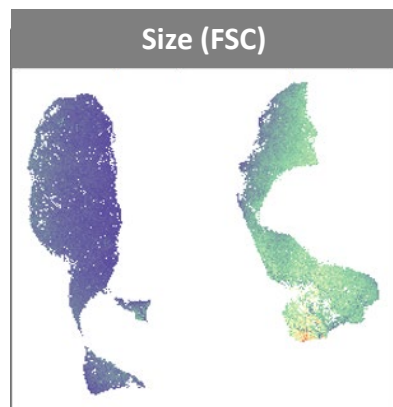
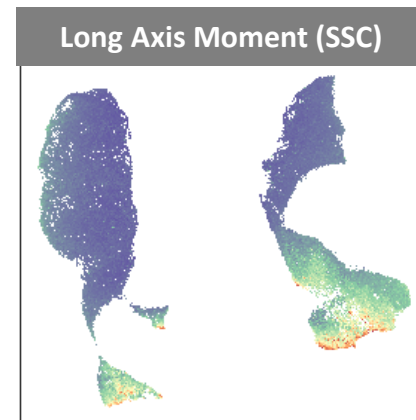
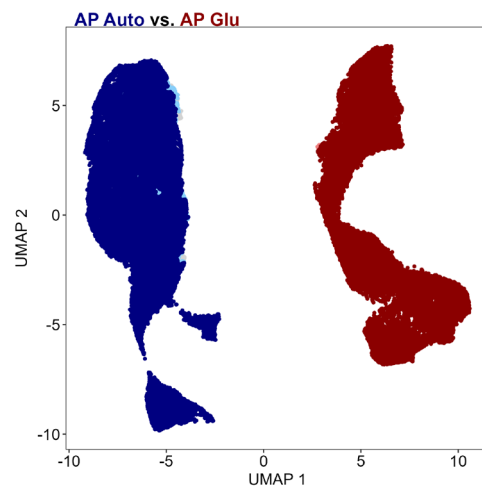
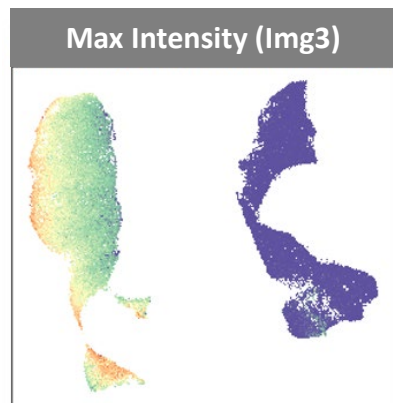
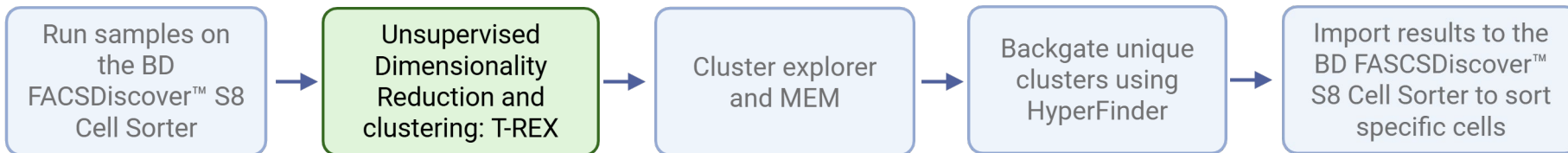
T-REX: Tracking Responders EXpanding tool

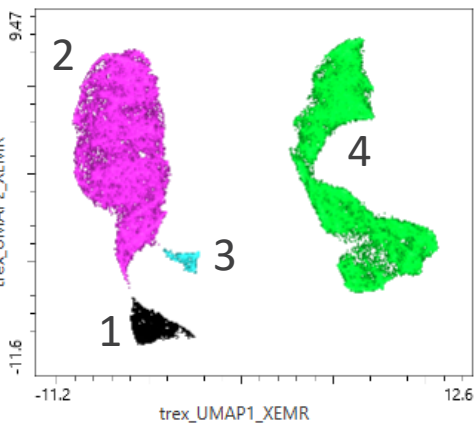
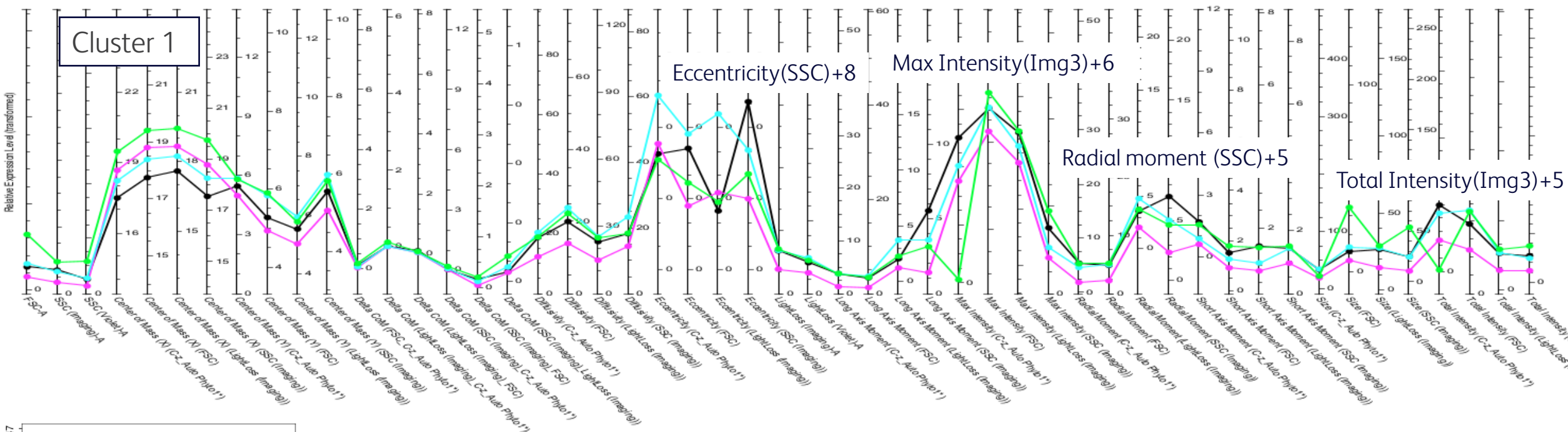
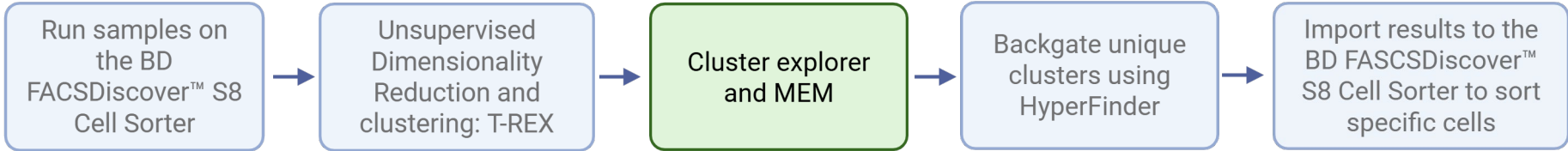
- Input: 2 samples/conditions to compare
- Dimensionality reduction (UMAP or t-SNE)
- K Nearest Neighbors (KNN) - Percent change per cell is then calculated based on the abundance of cells from the two samples in the KNN region.
- For regions expanding or contracting significantly ($\leq 5\%$ and $\geq 95\%$ change), DBSCAN is used to cluster the cells.



↓
Clustering

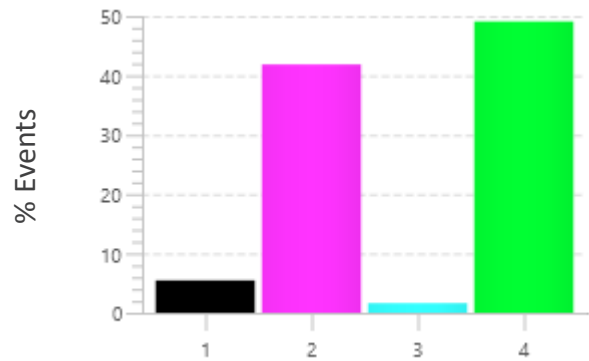


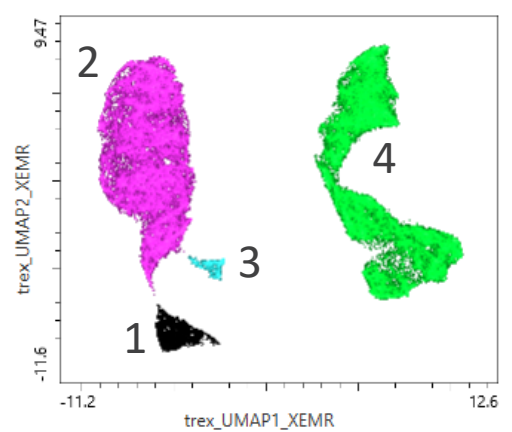
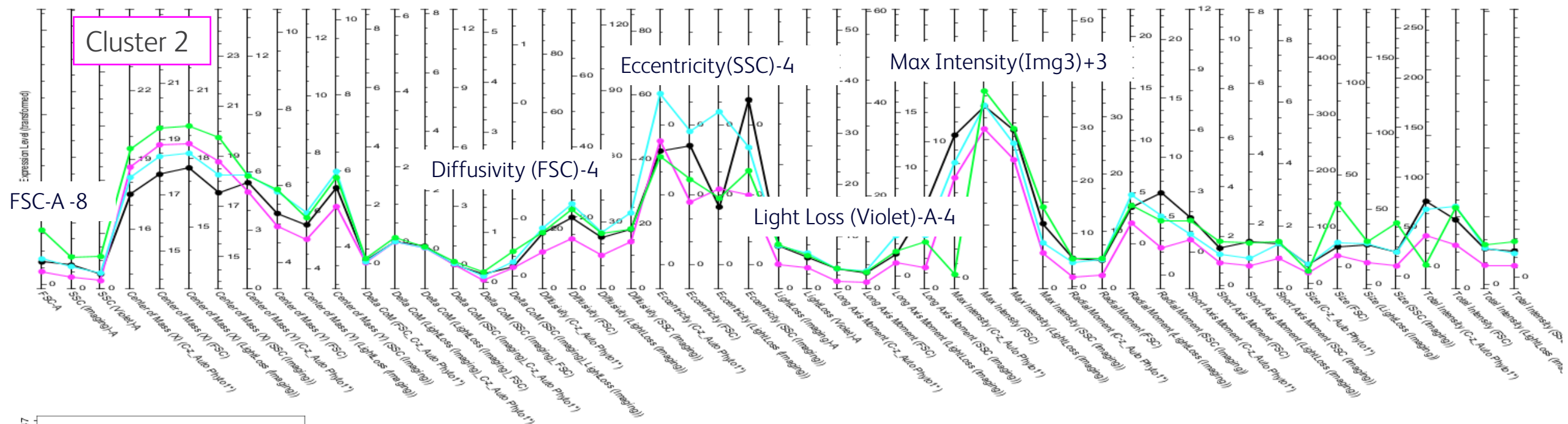
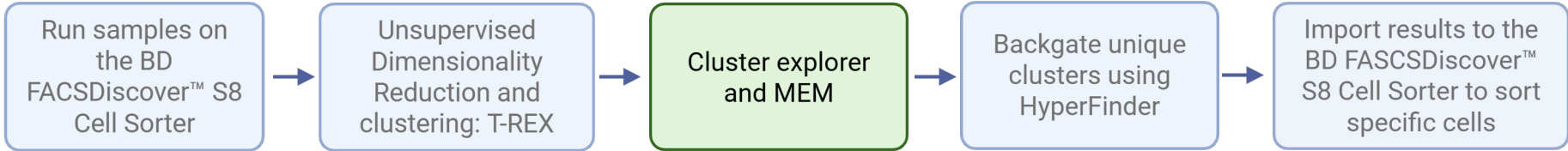




Marker Enrichment Modeling (MEM) – Quantifies relative enrichment by magnitude and interquartile range

Cluster 1: Eccentricity(SSC)+8, Max Intensity(Img3)+6, Radial moment(SSC)+5, Total Intensity(Img3)+5
 Cluster 2: FSC-A-8, Light Loss (Violet)-A-4, Eccentricity(SSC)-4, Diffusivity (FSC)-4, Max Intensity(Img3)+3
 Cluster 3: Eccentricity(Img3)+8, Eccentricity(Light Loss)+6, Max Intensity (FSC)+4
 Cluster 4: FSC-A+9, Size(FSC)+8, Max Intensity(Img3)-8





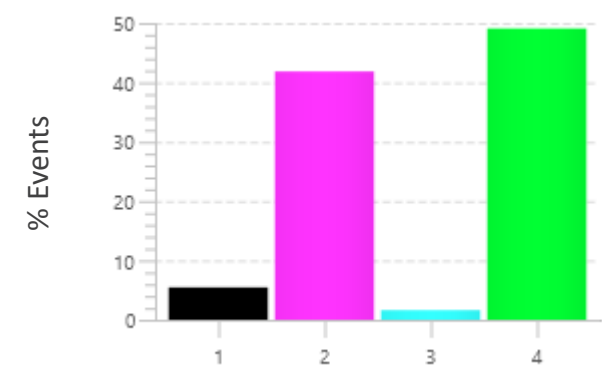
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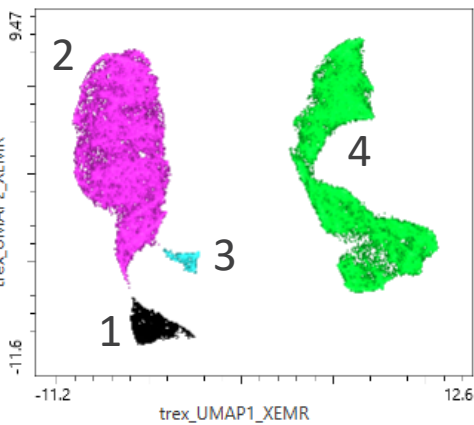
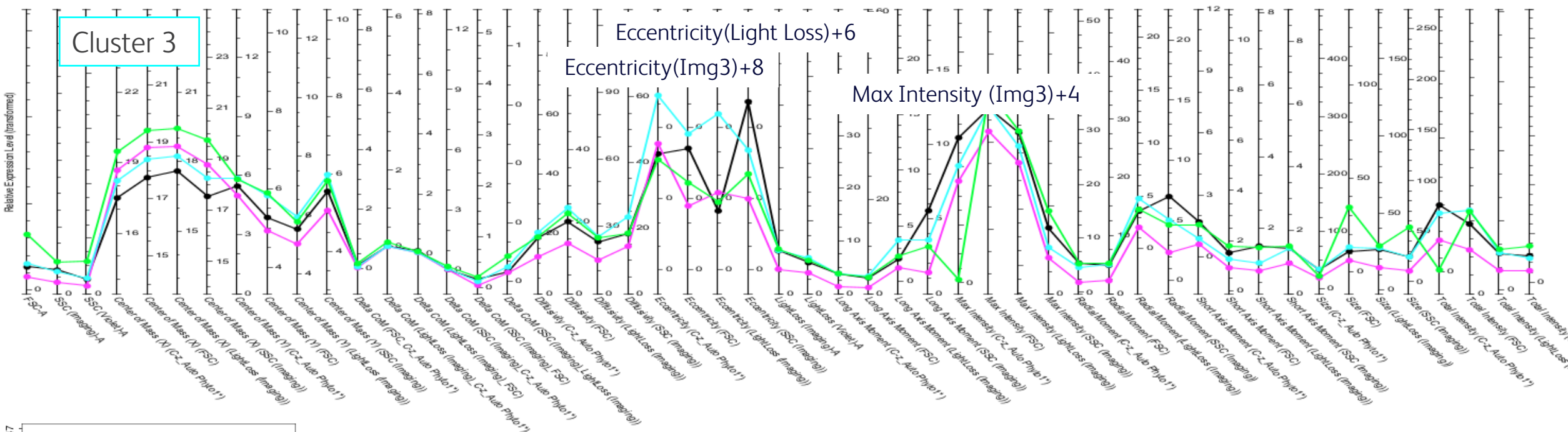
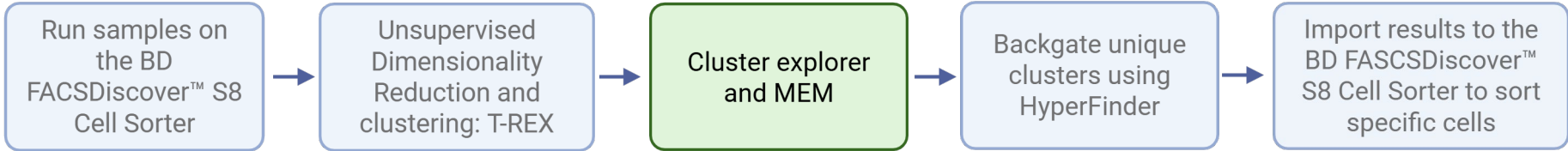
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Cluster 2: FSC-A-8, Light Loss (Violet)-A-4, Eccentricity(SSC)-4, Diffusivity (FSC)-4, Max Intensity(Img3)+3

Cluster 3: Eccentricity(Img3)+8, Eccentricity(Light Loss)+6, Max Intensity (FSC)+4

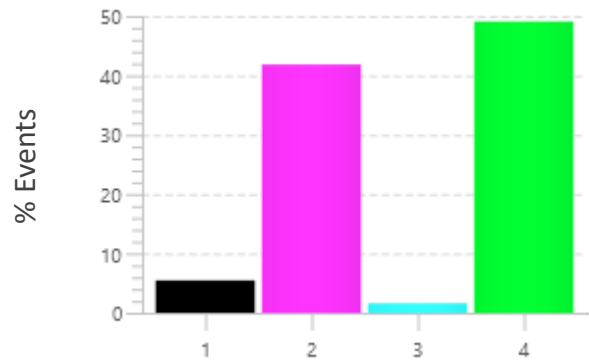
Cluster 4: FSC-A+9, Size(FSC)+8, Max Intensity(Img3)-8

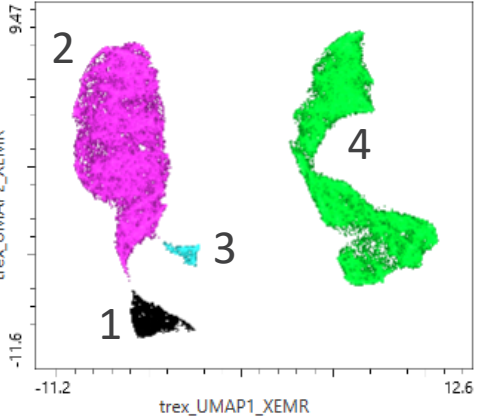
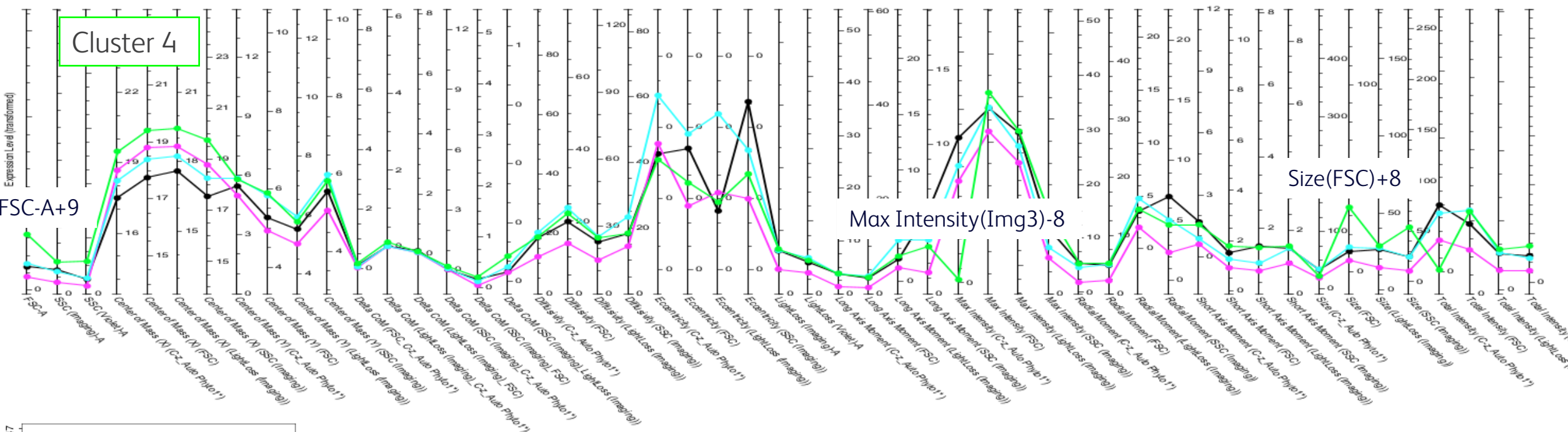
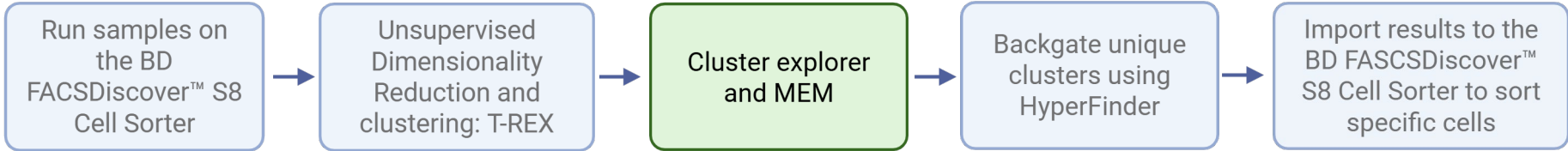




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 Cluster 4: FSC-A+9, Size(FSC)+8, Max Intensity(Img3)-8





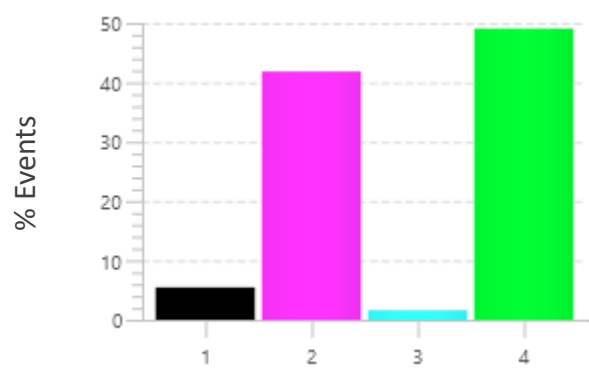
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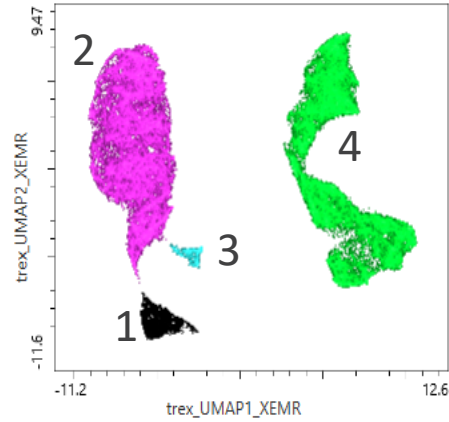
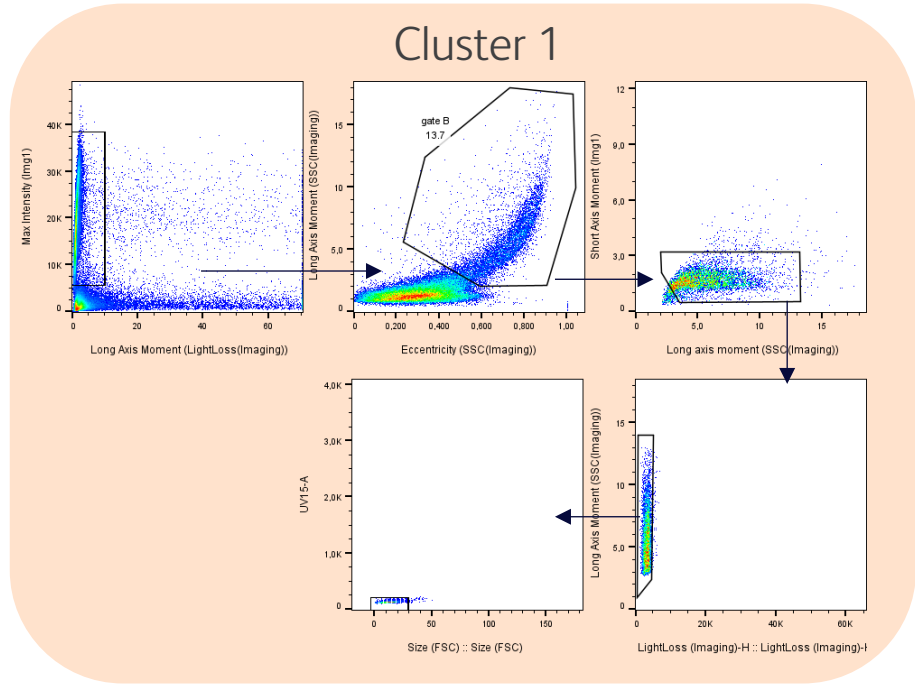
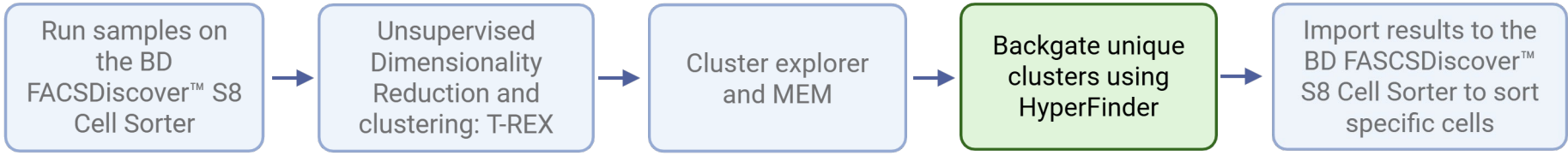
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Cluster 2: FSC-A-8, Light Loss (Violet)-A-4, Eccentricity(SSC)-4, Diffusivity (FSC)-4, Max Intensity(Img3)+3

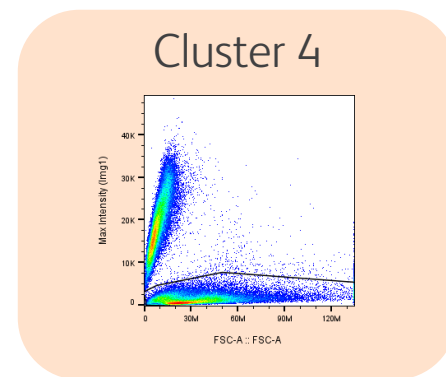
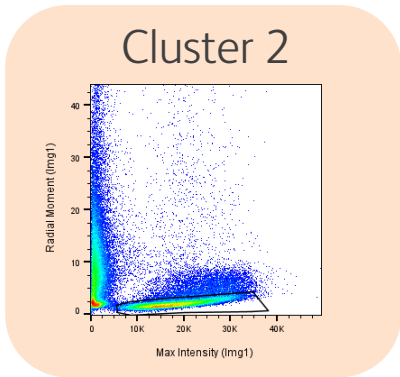
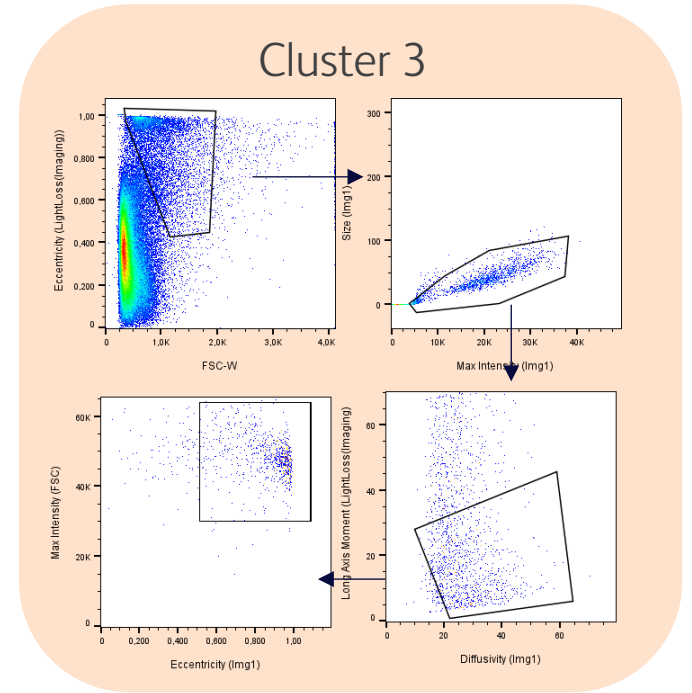
Cluster 3: Eccentricity(Img3)+8, Eccentricity(Light Loss)+6, Max Intensity (FSC)+4

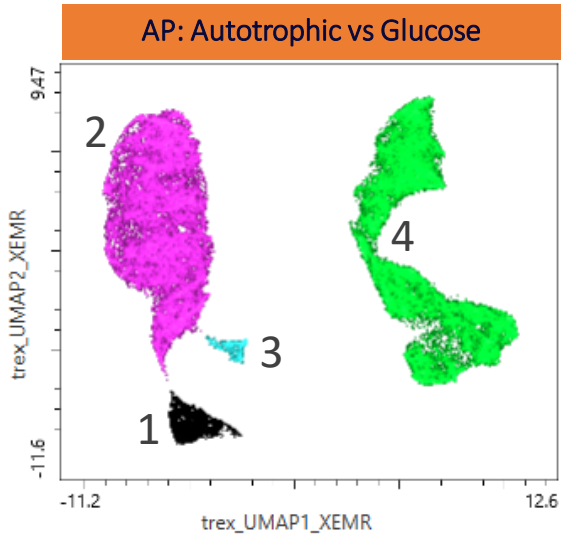
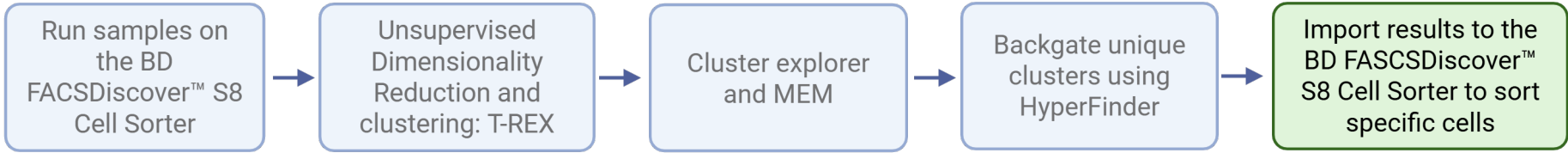
Cluster 4: FSC-A+9, Size(FSC)+8, Max Intensity(Img3)-8



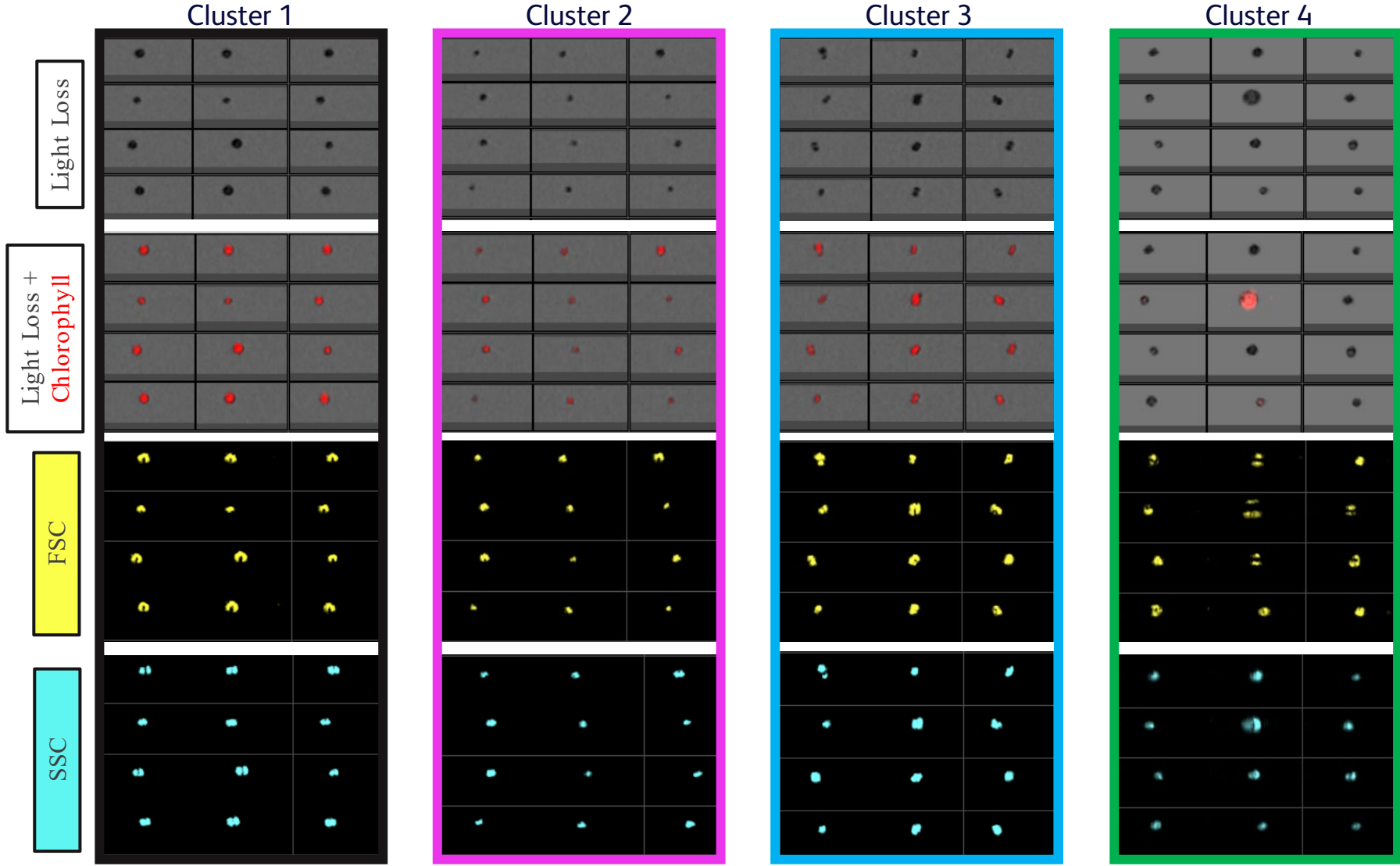


Gating strategy that can be used to sort clusters (or populations) of interest





- ↓ FSC
- ↑ Eccentricity (FSC, Light Loss)
- ↓ Max Intensity Chlorophyll
- ↑ Size (FSC)



Conclusions

- BD FACSDiscover™ S8 Cell Sorter is a system that efficiently combines spectral flow cytometry with real-time imaging and sorting.
- This work demonstrates the utility of Real-Time Imaging and Spectral Flow Cytometry in identifying heterogeneities and generating gating strategies for analysis and sorting of Green Algae by using a Label-Free approach in a biological model with high levels of autofluorescence.
- We were able to assess intra-species heterogeneity & phenotypic changes that accompany change in metabolism of Green Algae by using fluorescent and imaging parameters.
- Quantifiable imaging features generated in real-time provided additional analytical dimensionality to fluorescence parameters and additional information to images alone.

Thank you

Acknowledgements:

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Moen Sen
Aaron Middlebrook
Scott Bornheimer
Shivani Upadhyaya
Melissa Roth



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