Advanced Flow Cytometry Data Analysis Training Course

Professional Development Training Course Outline

Category	Biotechnology and Pharmaceutical Development	**Duration**	10 Days
Base Fee	$4,000 USD	**Delivery Mode**	Online / On-site Hybrid

Course Introduction

The field of life sciences, particularly immunology and oncology, has been revolutionized by the advent of high-dimensional flow cytometry. Modern cytometers, including spectral analyzers, routinely generate complex datasets with 20 or more parameters, which are impossible to fully interrogate with traditional, sequential gating strategies. This technological leap has shifted the primary bottleneck from data acquisition to sophisticated bioinformatics and interpretation. An advanced understanding of computational methods is therefore no longer a specialty, but a core competency. Robust data analysis is essential for extracting meaningful biological insights, ensuring reproducibility, and accelerating translational research.

Advanced Flow Cytometry Data Analysis Training Course moves beyond the fundamentals, focusing on mastery of cutting-edge, computational flow cytometry workflows. Participants will learn to leverage advanced tools, including R/Bioconductor packages, for unbiased data exploration and rigorous statistical validation. From optimizing multi-parameter panel design and navigating the complexities of spectral unmixing to implementing dimensionality reduction and unsupervised clustering algorithms, the program is designed to transform basic users into data analysis experts. The ultimate goal is to equip scientists with the practical skills to handle massive, complex single-cell datasets and publish high-impact research.

Course Duration

Programme Curriculum

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Course Objectives

1. Master High-Dimensional Data Analysis techniques for multi-parameter cytometry.
2. Design and troubleshoot Multi-Color Panel strategies with 15+ fluorescent markers.
3. Implement Spectral Unmixing and manage the Spread Matrix in full-spectrum data.
4. Apply and interpret Dimensionality Reduction algorithms like t-SNE and UMAP.
5. Utilize Unsupervised Clustering for unbiased cell population identification.
6. Develop proficiency in R/Bioconductor for Computational Flow Cytometry workflows.
7. Execute advanced Quality Control (QC), including algorithms like FlowAI and PeacoQC, for data standardization.
8. Conduct statistically Robust Data Comparison and analysis across large experimental cohorts.
9. Identify and characterize Rare Cell Populations and subtle phenotypic shifts.
10. Analyze complex Intracellular Staining assays, including Phospho-Flow Cytometry.
11. Integrate flow cytometry data with other Single-Cell Omics for Multi-Omics Integration.
12. Optimize automated Gating Strategies to enhance data reproducibility and objectivity.
13. Generate publication-quality Data Visualization plots and figure panels.

Target Audience

1. Immunologists studying complex immune cell interactions and subsets.
2. Oncology Researchers focused on tumor microenvironment and cellular profiling.
3. Core Facility Staff managing and supporting high-parameter instrument usage.
4. Pharmaceutical/Biotech Scientist.
5. Senior Graduate Students & Postdocs.
6. Computational Biologists transitioning into single-cell data analysis.
7. Clinical Researchers.
8. QC/Assay Development Scientists needing to standardize multi-color assays.

Course Modules

1. Advanced Panel Design & Optimization

- Using Spread Matrix and Gating Controls.
- Strategies for 20+ colour panel design in Spectral Flow Cytometry.
- Selecting optimal fluorochromes for Antigen Density and instrument configuration.
- Advanced titration protocols for minimal Spillover Spreading.
- Case Study: Designing a 28-color panel for simultaneous innate and adaptive immune profiling.

2. Data Quality Control (QC) and Standardization

- The anatomy of an FCS File and its critical metadata.
- Using FlowAI and PeacoQC to identify and remove bad events.
- Normalization techniques to correct for Batch Effects across multi-site studies.
- Standardizing data acquisition settings and daily instrument QC procedures.
- Case Study: Troubleshooting a multi-site clinical trial dataset with high technical variability due to inconsistent QC.

3. Principles of Spectral Flow & Unmixing

- Autofluorescence and the full-spectrum signature.
- Spectral Unmixing vs. traditional compensation; managing the unmixing process.
- Designing and validating unmixing controls for robust data.
- Fluorochrome Spreading and optimizing detector assignment.
- Case Study: Analyzing a Full Spectrum dataset to resolve two closely-expressing markers with significant spectral overlap.

4. Introduction to Computational Flow Cytometry

- Setting up the computational environment: R, RStudio, and Bioconductor.
- Reading, manipulating, and exporting FCS data in R.
- Basic data transformation, scaling, and quality filtering using R scripts.
- Scripting for high-throughput, reproducible analysis pipelines.
- Case Study: Building an end-to-end R script for pre-processing 100+ FCS files in an automated batch.

5. Dimensionality Reduction

- Theoretical foundation and practical differences between t-SNE and UMAP.
- Optimizing critical hyperparameters for visualization fidelity.
- Interpreting the reduced dimensions and identifying artifacts.
- Best practices for creating reproducible and publication-quality plots.
- Case Study: Using t-SNE and UMAP to visualize the heterogeneity of tumor-infiltrating lymphocytes (TILs) from patient biopsies.

6. Unsupervised Clustering Algorithms

- Introduction to Unbiased Cell Population discovery.
- Implementation of FlowSOM for hierarchical clustering.
- Applying PhenoGraph and SPADE for high-resolution population identification.
- Determining the optimal number of clusters and validating clustering stability.
- Case Study: Discovering a novel, rare immune cell sub-population in a mouse model of chronic inflammation using FlowSOM.

7. Linking Clusters to Phenotype

- Translating computational clusters back to classical biological subsets.
- Automated Gating tools and their validation.
- Using heatmaps and box plots to visualize marker expression across clusters.
- Defining and annotating the final cell populations for downstream analysis.
- Case Study: Systematically annotating 30+ FlowSOM clusters in a peripheral blood mononuclear cell (PBMC) dataset to create a comprehensive immunophenotype map.

8. Data Comparison and Differential Analysis

- Identifying clusters that are statistically different between experimental groups
- Using Differential Abundance Analysis to quantify population shifts.
- Implementing Differential Expression Analysis for marker MFI across clusters.
- Non-parametric statistical tests for flow cytometry data.
- Case Study: Comparing T cell population frequencies and activation marker expression in pre- and post-treatment cancer patient samples.

9. Advanced Compensation and Spread Correction

- Deep dive into the Spillover Matrix and its impact on data interpretation.
- Identifying and correcting residual compensation errors.
- Best practices for handling non-linear compensation and autofluorescence-based compensation.
- Automated vs. manual compensation in high-parameter settings.
- Case Study: Systematically troubleshooting a 15-color panel where residual errors severely compromised the T-cell activation analysis.

10. Rare Event Detection and Analysis

- Strategies for collecting sufficient events for Rare Cell Populations
- High-throughput analysis of large data volumes to enrich for rare events.
- Statistical considerations for analyzing low-frequency populations.
- Using backgating from computationally defined clusters to validate rare event identification.
- Case Study: Identifying and characterizing low-frequency progenitor cells in a bone marrow sample for minimal residual disease (MRD) monitoring.

11. Functional Assays: Phospho-Flow and Cell Cycle

- Panel design and controls specific for Phospho-Flow Cytometry
- Quantitative analysis of signaling pathways using Geometric Mean Fluorescence Intensity
- Modeling and fitting data for cell cycle and proliferation assays
- Troubleshooting fixation and permeabilization protocols for optimal staining.
- Case Study: Quantifying the activation of the MAPK signaling cascade in T cells after a specific drug treatment using phospho-specific antibodies.

12. Multi-Omics Integration

- Foundational concepts of Single-Cell Multi-Omics.
- Matching flow cytometry data with scRNA-seq data to confirm cellular phenotypes.
- Tools and strategies for integrating protein expression with transcriptomics
- The role of flow data in validating and refining single-cell transcriptomic clusters.
- Case Study: Integrating protein-level immunophenotyping from flow cytometry with corresponding gene expression data to refine the identity of a newly discovered macrophage subset.

13. High-Throughput Screening and Automation

- Designing experiments for plate-based, high-throughput flow cytometry.
- Scripting and batch processing for automated analysis of large plate datasets.
- Best practices for data management and archiving of large-scale flow data.
- Implementing machine learning for automated cell classification.
- Case Study: Developing an automated analysis pipeline for a 384-well plate drug screen on immune cells.

14. Data Visualization and Publication

- Generating professional t-SNE and UMAP plots with clear cluster boundaries.
- Creating meaningful heatmaps, dot plots, and statistical summary figures.
- Best practices for data presentation to meet journal submission standards.
- Using ggplot2 for advanced, customizable data plotting.
- Case Study: Revising and optimizing raw analytical plots into a final, high-impact figure panel for a mock journal submission.

15. Advanced Troubleshooting & Experimental Design Clinic

- Systematic approach to diagnosing data acquisition and analysis failures.
- Reviewing common errors in Compensation/Unmixing, Gating, and Cluster interpretation.
- Designing robust controls for complex experiments.
- Developing a complete, documented analysis workflow for a new experiment.
- Case Study: Live review and expert critique of participant-submitted "problem" datasets for collaborative troubleshooting.

Training Methodology

This intensive course employs a mixed-modality approach to ensure comprehensive skill mastery:

- Expert-Led Lectures
- Hands-On Workshops
- Case-Based Learning
- Troubleshooting Clinics

Register as a group from 3 participants for a Discount

Send us an email: info@fineskilltrainingcenter.org or call +254769199797

Certification

Upon successful completion of this training, participants will be issued with a globally- recognized certificate.

Tailor-Made Course

We also offer tailor-made courses based on your needs.

Key Notes

a. The participant must be conversant with English.

b. Upon completion of training the participant will be issued with an Authorized Training Certificate

c. Course duration is flexible and the contents can be modified to fit any number of days.

d. The course fee includes facilitation training materials, 2 coffee breaks, buffet lunch and A Certificate upon successful completion of Training.

e. One-year post-training support Consultation and Coaching provided after the course.

f. Payment should be done at least a week before commence of the training, to Fineskill Training Center account, as indicated in the invoice so as to enable us prepare better for you.

Upcoming Scheduled Sessions

Start Date	End Date	Location	Price
23 Nov 2026	04 Dec 2026	Online	$2,000
23 Nov 2026	04 Dec 2026	Physical	$3,000
23 Nov 2026	04 Dec 2026	Physical	$6,000
23 Nov 2026	04 Dec 2026	Physical	$6,000
23 Nov 2026	04 Dec 2026	Physical	$12,000
23 Nov 2026	04 Dec 2026	Physical	$9,000
23 Nov 2026	04 Dec 2026	Physical	$12,000
23 Nov 2026	04 Dec 2026	Physical	$12,000

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