FLOW CYTOMETRY TROUBLESHOOTING GUIDE

Quickly diagnose and fix common experimental issues

WHAT PROBLEM ARE YOU SEEING?

WEAK OR NO SIGNAL
"Start here first"

CAUSES
- Low antigen expression
- Antibody too dilute
- Low detector sensitivity

FIXES
- Increase antibody concentration
- Use brighter fluorophore
- Adjust PMT voltage

HIGH BACKGROUND

CAUSES
- Non-specific binding
- Excess antibody
- Dead cells present

FIXES
- Add Fc blocking step
- Reduce antibody concentration
- Use viability dye

POOR RESOLUTION
"Most common issue"

CAUSES
- Incorrect compensation
- Spectral overlap
- Suboptimal voltages

FIXES
- Use single-color controls
- Adjust compensation matrix
- Optimize panel design

DEBRIS / CLOGGING

CAUSES
- Cell aggregates
- Unfiltered samples
- Instrument blockage

FIXES
- Filter samples before run
- Wash cells thoroughly
- Perform instrument cleaning

UNEXPECTED POPULATIONS
"Often overlooked"

CAUSES
- Improper gating
- Doublets or aggregates
- Sample contamination

FIXES
- Apply standard gating strategy
- Exclude doublets
- Verify controls and labeling

BEST PRACTICES

Prevent issues before they start

- **TITRATE** all antibodies
- **INCLUDE** proper controls
- **MAINTAIN** instrument QC
- **FILTER** all samples
- **PROTECT** from light

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