BioVaram

EV Analysis Platform

COMPREHENSIVE USER MANUAL

Complete Guide for Optimal Platform Usage

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1. Introduction & Overview

The BioVaram EV Analysis Platform is a comprehensive, research-grade software solution designed for the characterization and analysis of Extracellular Vesicles (EVs). Built specifically for the CRMIT Research Labs, this platform integrates multiple analysis methodologies including Flow Cytometry (FCS), Nanoparticle Tracking Analysis (NTA), and provides powerful cross-comparison capabilities.

Key Features

- **Flow Cytometry Analysis:** Process .FCS files from NanoFACS, ZE5, and other instruments
- **NTA Analysis:** Parse ZetaView data files with size distribution and concentration analysis
- **Cross-Compare:** Compare FCS and NTA results with statistical analysis
- **AI Research Assistant:** Get intelligent insights and guidance on your data
- **PDF Report Generation:** Export professional reports for publications
- **Real-time Alerts:** Quality control notifications and anomaly detection
- **Experimental Conditions:** Track temperature, pH, buffer, and other parameters

■ **TIP:** This platform is optimized for EV characterization following MISEV2023 guidelines. All analysis parameters are calibrated for particles in the 30-1000nm range.

2. Getting Started

2.1 System Requirements

Component	Minimum	Recommended
Browser	Chrome 90+, Firefox 88+	Chrome 120+ (Latest)
Screen Resolution	1366 x 768	1920 x 1080 or higher
Internet Connection	Stable connection	High-speed broadband
Backend Server	localhost:8000	Production server

2.2 Login & Authentication

Follow these steps to access the platform:

→ **Step 1:** Navigate to the platform URL in your browser

→ **Step 2:** Enter your registered email address (e.g., researcher@crmit.com)

→ **Step 3:** Enter your password

→ **Step 4:** Click 'Sign In' to access the dashboard

→ **Step 5:** If you don't have an account, click 'Create Account' to register

■■ **WARNING:** Keep your credentials secure. Do not share your password with others. All activities are logged for security and audit purposes.

2.3 Interface Overview

The platform interface consists of the following main components:

Component	Location	Purpose
Header Bar	Top	Logo, dark mode toggle, notifications, user menu
Sidebar	Left	Previous analyses, settings, filters
Tab Navigation	Below Header	Dashboard, Flow Cytometry, NTA, Cross-Compare, Research Chat
Main Content	Center	Current tab's analysis workspace
Alert Panel	Header (bell icon)	Notifications and quality alerts

3. Dashboard Tab

The Dashboard provides an at-a-glance overview of your analysis workspace, including pinned charts, quick statistics, recent activity, and an AI chat assistant.

3.1 Quick Stats

Quick Stats displays real-time metrics about your analysis session:

- Total Samples Analyzed
- FCS Files Processed
- NTA Files Processed
- Active Alerts Count

3.2 Recent Activity

The Recent Activity panel shows your latest samples with the following actions:

→ **View:** Click to open sample details in a modal

→ **Open in Tab:** Load the sample in the appropriate analysis tab

→ **Delete:** Remove the sample (requires confirmation)

3.3 Quick Upload

Upload files directly from the dashboard without switching tabs:

→ **Step 1:** Drag and drop a file onto the upload zone

→ **Step 2:** Or click to browse and select a file

→ **Step 3:** The file type (.fcs or .txt/.csv) is auto-detected

→ **Step 4:** Analysis begins automatically after upload

3.4 AI Chat Assistant

The Dashboard AI Chat provides intelligent assistance for quick questions:

■■ **NOTE:** The Dashboard AI Chat is a compact version. For full-featured AI assistance with file upload support and detailed analysis, use the Research Chat tab.

4. Flow Cytometry (FCS) Analysis

The Flow Cytometry tab provides comprehensive analysis of .FCS files from nanoFACS, ZE5, and other flow cytometry instruments. This is the primary module for size-resolved EV characterization.

4.1 File Upload

Single File Analysis:

→ **Step 1:** Navigate to the 'Flow Cytometry' tab

→ **Step 2:** Select 'Single File' mode (default)

→ **Step 3:** Drag and drop your .FCS file into the upload zone

→ **Step 4:** Optionally fill in sample metadata:

→ • Treatment (e.g., 'Control', 'Drug A 10µg/mL')

→ • Concentration (µg)

→ • Preparation Method

→ • Operator Name

→ **Step 5:** Click 'Upload and Analyze'

→ **Step 6:** Wait for analysis to complete (typically 5-15 seconds)

Comparison Mode (Dual File):

→ **Step 1:** Click 'Compare Files' button in the upload mode card

→ **Step 2:** Upload the primary file (e.g., Sample A)

→ **Step 3:** Upload the secondary file (e.g., Sample B)

→ **Step 4:** Both files are analyzed and overlaid automatically

■ **TIP:** Use comparison mode to visualize population shifts between conditions (e.g., before/after treatment, control vs. treated, different time points).

4.2 Analysis Settings (Sidebar)

The sidebar contains important analysis parameters. Expand the sidebar to access:

Setting	Description	Default
Wavelength (nm)	Laser wavelength for size calculation	488 nm
Refractive Index	Particle refractive index (n)	1.40
Medium RI	Buffer/medium refractive index (n0)	1.335
Angular Range	SSC collection angle range	15°-135°
Size Threshold (nm)	Minimum particle size to consider	30 nm

Anomaly Detection	Enable outlier detection	On
IQR Multiplier	Statistical threshold for anomalies	1.5

4.3 Interpreting Results

After analysis completes, results are displayed in multiple views:

Statistics Cards:

- **Total Events:** Number of particles detected in the file
- **D10:** 10th percentile size - 10% of particles are smaller than this
- **D50 (Median):** 50th percentile - the 'typical' particle size
- **D90:** 90th percentile size - 90% of particles are smaller than this
- **Mean Size:** Arithmetic mean of all particle diameters
- **Std Dev:** Standard deviation - indicates size distribution spread
- **Polydispersity:** Measure of size heterogeneity (lower = more uniform)

Size Distribution Chart:

The histogram shows particle count vs. size (nm). Look for:

- Peak position = Most common particle size
- Peak width = Size heterogeneity
- Multiple peaks = Different particle populations
- Tail on right = Presence of larger particles/aggregates

Scatter Plot:

The interactive scatter plot shows individual particles. Default axes are VFSC-H (size) vs VSSC1-H (granularity). Use the axis selector to change channels.

4.4 Gating & Selection

Interactive gating allows you to select specific particle populations:

→ **Step 1:** Click and drag on the scatter plot to draw a rectangular gate

→ **Step 2:** Release to apply the gate

→ **Step 3:** The 'Gated Statistics' panel appears with statistics for selected particles only

→ **Step 4:** Click 'Clear Gate' to reset selection

■ **TIP:** Use gating to isolate specific populations (e.g., small EVs only, or exclude debris). Gated statistics update in real-time.

4.5 Export Options

Multiple export formats are available:

- **CSV:** Raw data export for Excel or other tools
- **Excel:** Formatted spreadsheet with multiple sheets

- **PDF:** Professional report with charts and statistics
- **Parquet:** High-performance columnar format for data science
- **Markdown:** Text-based report for documentation

To export, click the 'Export' dropdown button in the results area and select your preferred format.

5. NTA (Nanoparticle Tracking Analysis)

The NTA tab processes data from ZetaView and other NTA instruments. It provides size distribution, concentration measurements, and quality metrics.

5.1 File Upload

Supported file formats:

- **.txt** - ZetaView export files
- **.csv** - Comma-separated data files

→ **Step 1:** Navigate to the 'NTA' tab

→ **Step 2:** Drag and drop your .txt or .csv file into the upload zone

→ **Step 3:** Optionally fill in metadata:

→ • Treatment description

→ • Temperature (°C)

→ • Operator name

→ **Step 4:** Click 'Upload and Analyze'

→ **Step 5:** Review the parsed results

5.2 Temperature Settings

Temperature affects viscosity calculations which impact size accuracy. The platform includes temperature correction:

- Default temperature: 25°C (room temperature)
- Adjust if sample was measured at different temperature
- Higher temperature = lower viscosity = smaller apparent size
- Temperature correction is applied automatically

5.3 Interpreting Results

Key NTA Metrics:

- **Total Particles:** Number of tracked particles across all positions
- **Concentration:** Particles per mL (scientific notation)
- **D10/D50/D90:** Size percentiles (same interpretation as FCS)
- **Mean Size:** Average particle diameter in nm
- **Mode Size:** Most frequently observed size
- **Positions Analyzed:** Number of measurement positions

■■ **NOTE:** NTA concentration is typically expressed as 1.23E+10 particles/mL. This represents 1.23 × 10¹■ particles per milliliter.

5.4 PDF Report Upload

You can also upload ZetaView PDF reports for parsing:

→ **Step 1:** Click 'Upload PDF Report' button

→ **Step 2:** Select the ZetaView-generated PDF

→ **Step 3:** The system extracts tables and charts

→ **Step 4:** Review extracted data alongside raw file data

6. Cross-Compare Tab

The Cross-Compare tab allows you to compare results from different analysis methods (FCS vs NTA) or different samples within the same method.

6.1 Selecting Samples

→ **Step 1:** Navigate to the 'Cross-Compare' tab

→ **Step 2:** Select an FCS sample from the dropdown (or 'Current FCS Analysis' if just analyzed)

→ **Step 3:** Select an NTA sample from the dropdown (or 'Current NTA Analysis' if just analyzed)

→ **Step 4:** Results load automatically when both samples are selected

→ **Step 5:** Click 'Refresh' to reload data if needed

6.2 Statistical Comparison

The comparison table shows side-by-side metrics:

Metric	FCS	NTA	Difference
D10 (nm)	Calculated from scatter	From tracking	Absolute & %
D50 (nm)	Median size	Median size	Absolute & %
D90 (nm)	Calculated from scatter	From tracking	Absolute & %
Mean (nm)	Arithmetic mean	Arithmetic mean	Absolute & %

■ **TIP:** FCS and NTA may show different D50 values due to their different measurement principles. FCS measures optical scatter (sensitive to refractive index), while NTA tracks Brownian motion (sensitive to viscosity). Differences of 10-20% are normal.

6.3 Visualization Options

Available charts in Cross-Compare:

- **Overlay Histogram:** Overlaid size distributions from both methods
- **Discrepancy Chart:** Bar chart showing differences in each metric
- **KDE Comparison:** Kernel density estimation comparison
- **Correlation Scatter:** Scatter plot correlating FCS vs NTA values

7. Research Chat (AI Assistant)

The Research Chat tab provides a full-featured AI assistant specialized in EV research and analysis. It can help you interpret results, suggest experiments, and explain concepts.

Getting Started with AI Chat

→ **Step 1:** Navigate to the 'Research Chat' tab

→ **Step 2:** Type your question or request in the input box

→ **Step 3:** Press Enter or click Send

→ **Step 4:** The AI will respond with relevant information

→ **Step 5:** Continue the conversation as needed

Suggested Questions

- "How do I interpret flow cytometry gating strategies?"
- "What are the key parameters for EV characterization?"
- "Help me analyze my uploaded FCS file"
- "Generate a size distribution graph for my data"
- "Guide me through cross-comparing datasets"
- "What do high polydispersity values indicate?"
- "Explain the difference between D50 and mean size"

File Upload in Chat

You can upload files directly in the chat for analysis:

- Click the upload icon in the chat input area
- Select your .fcs or .txt file
- The AI will analyze and provide insights

Export Chat History

To save your conversation:

- Click 'Export' dropdown
- Choose format: Markdown, JSON, or Text
- File downloads automatically

8. Best Practices

Follow these guidelines for optimal results:

Data Preparation

✓ Ensure samples are well-mixed before analysis

✓ Filter samples appropriately (0.22µm or 0.45µm)

✓ Record dilution factors accurately

✓ Note sample storage conditions and time

File Naming Convention

Use consistent, descriptive file names:

• Format: [Project]_[Sample]_[Treatment]_[Date].fcs

• Example: PC3_EV_Control_20260120.fcs

• Example: HEK_TFF_10kDa_F5_20260120.txt

• Avoid spaces - use underscores instead

Experimental Conditions

Always fill in experimental conditions after upload:

• Temperature at measurement

• Buffer/medium used (PBS, HEPES, etc.)

• Operator name for traceability

• Any relevant notes

■■ **WARNING:** Missing experimental conditions may affect data interpretation and reproducibility. Always document your experimental setup.

Quality Control

✓ Check for alerts in the notification panel

✓ Review anomaly detection results

✓ Verify D50 values are within expected range (30-500nm for EVs)

✓ Compare results with control samples

✓ Document any unusual observations

9. Troubleshooting

Common Issues and Solutions

Issue	Possible Cause	Solution
'Backend Offline' error	FastAPI server not running	Start server with 'python run_api.py'
File upload fails	File format not supported	Ensure .fcs, .txt, or .csv extension
No events detected	Empty file or wrong channels	Verify file contains data
Very high D50 values	Aggregation in sample	Re-filter sample, verify dilution
Analysis timeout	Very large file	Split file or increase timeout
Charts not loading	Browser cache issue	Clear cache and refresh (Ctrl+F5)
Login fails	Invalid credentials	Reset password or contact admin

Getting Help

If issues persist:

- Take a screenshot of the error message
- Note the steps that led to the issue
- Check browser console for errors (F12 → Console)
- Contact the development team with details
- Use the Research Chat to ask for guidance

10. Appendix

A. Keyboard Shortcuts

Shortcut	Action
Ctrl + S	Save current analysis
Ctrl + E	Open export menu
Ctrl + R	Refresh data
Esc	Close modal/dialog
Tab	Navigate between fields

B. Glossary

- **EV (Extracellular Vesicle):** Membrane-bound particles released by cells
- **FCS (Flow Cytometry Standard):** Standard file format for flow cytometry data
- **NTA (Nanoparticle Tracking Analysis):** Method to measure particle size and concentration
- **D10/D50/D90:** Size percentiles (10th, 50th, 90th)
- **FSC (Forward Scatter):** Light scattered in forward direction, related to size
- **SSC (Side Scatter):** Light scattered at 90°, related to granularity
- **Polydispersity:** Measure of size distribution width
- **Gating:** Selecting a subset of particles based on scatter properties

C. Contact Information

Support Type	Contact
Technical Support	tech@crmit.com
Bug Reports	bugs@crmit.com
Feature Requests	features@crmit.com
General Inquiries	info@crmit.com

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