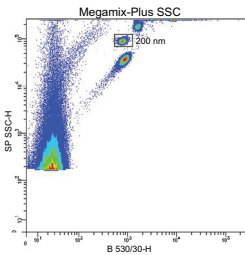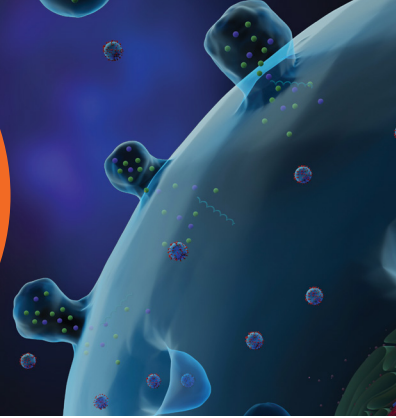# Flow cytometric analysis of extracellular vesicles

with BD FACSymphony™ A1 Cell Analyzer, BD FACSDiva™ Software, FlowJo™ Software and Rosetta Calibration Plugin

Fine tune your small particle detector (SPD)

STEP 1: Prepare your BD FACSymphony™ A1 Cell Analyzer using standard small particle beads

- Prepare SPD bead mixture
- Align SPD using Picomotor Application (if needed)
- Adjust SP SSC voltage to place bead peaks on target
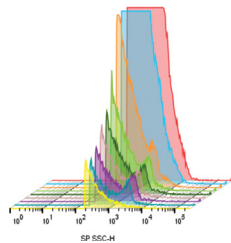
Ensure reproducible low background noise

STEP 2: Perform start-up procedure to reduce background noise

- Run BD® Detergent Solution Concentrate
- Run sample diluent and check background noise

3.1	Buffer only*	3.5	Single-stained controls*
3.2	Buffer with reagents*	3.6	Procedural controls*
3.3	Unstained controls*	3.7	Serial dilution*
3.4	Isotype controls	3.8	Detergent-treated EV samples

Welsh JA, et al. *J of Extracell Vesicles.* 2020;9(1):1713526.

Acquire your EV samples and explore the power of "OR" thresholding

STEP 3: Apply proper controls, calibration and thresholding strategy

- Use "OR" thresholding for SP SSC and relevant fluorescent parameters for maximum information
- Consider running serial dilution series to check for absence of swarming
- Use *MiFlowCyt-EV* framework for publication to guide you on assay controls and SP SSC and fluorescence calibration

Uncalibrated EV data in A.U.

Calibrated EV data in nm
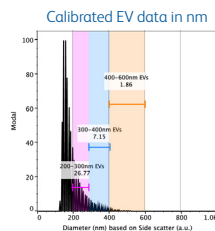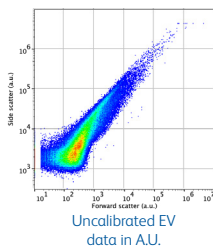
### Measure Rosetta Calibration beads and your EV samples
STEP 4: Optional for EV size calibration

- Run Rosetta Calibration beads and your EV samples at the same scatter detector voltage and gain
- Configure the threshold and voltage of the scatter channel so that you detect the smallest beads in the mixture while retaining maximum dynamic range

BD

Flow cytometric analysis of extracellular vesicles

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and Rosetta Calibration Plugin
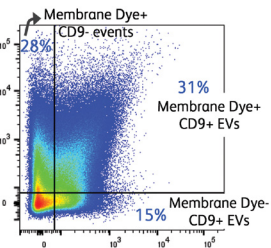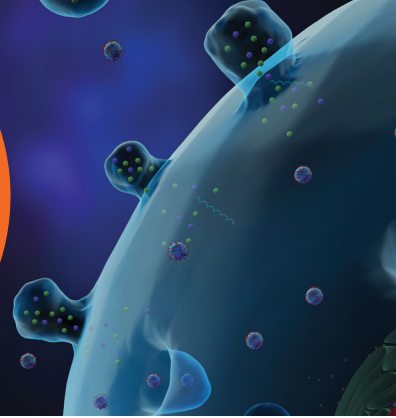
Analyze your EV data

STEP 5: Take advantage of the analysis power of BD FACSDiva™ Software, FlowJo™ Software and the Rosetta Calibration Plugin

- Output files from recommended calibration software compatible with BD FACSDiva™ and FlowJo™ Software for further analysis
- Use the Rosetta Calibration Plugin to derive calibrated size measurements of your EV samples

Still need help?

- Follow troubleshooting guidelines in the BD FACSymphony™ A1 Cell Analyzer User's Guide
- Contact your BD support team for assistance

To learn more about the BD FACSymphony™ A1 Cell Analyzer or the BD® Small Particle Detector, contact your BD sales representative.

Get started with the Rosetta Calibration Plugin today!

Learn more about our free plugins at **https://www.flowjo.com/learn/flowjo-university**

Download Rosetta Calibration Plugin at: **https://www.flowjo.com/exchange/#/plugin/profile?id=61**

Rosetta Calibration webinar: **https://flowjo.wistia.com/medias/92eyr9axmf**

More at: **https://www.exometry.com/products/rosettacalibration**

Plugins are free with a FlowJo™ Software license

BD Life Sciences, San Jose, CA 95131, USA

bdbiosciences.com