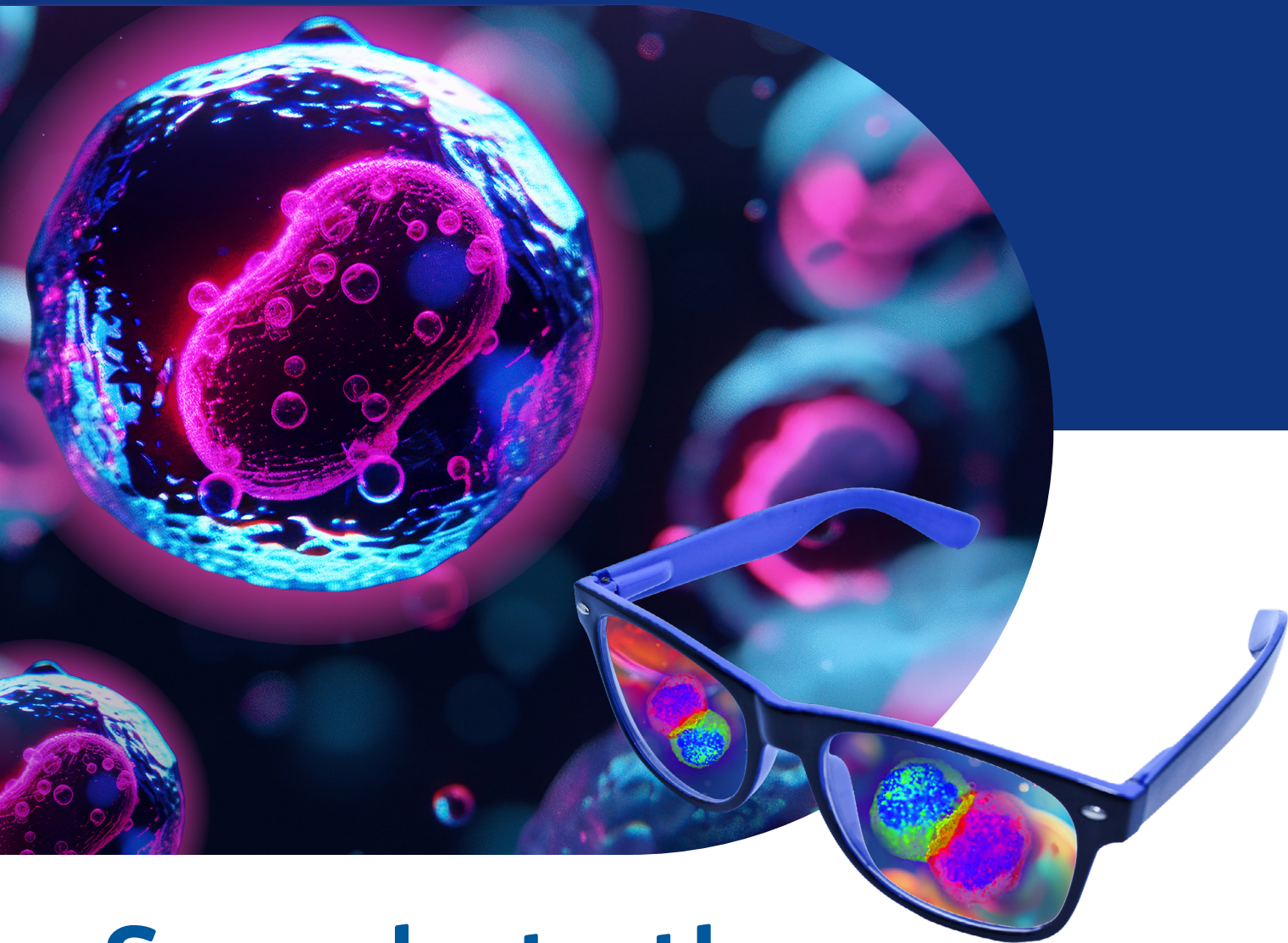




See what others can only imagine!

BD CellView™ Image Technology Applications

- **Application 1:** Truly Distinguish the Cell Cycle
- **Application 2:** Reveal True Phagocytosis
- **Application 3:** Verify True Cells Below 0.01%!
- **Application 4:** Move beyond CAR intensity and discover spatial patterns



Truly Distinguish the Cell Cycle

see G0/G1, S and G2 with confidence

How Accurate is your Cell Cycle Analysis?

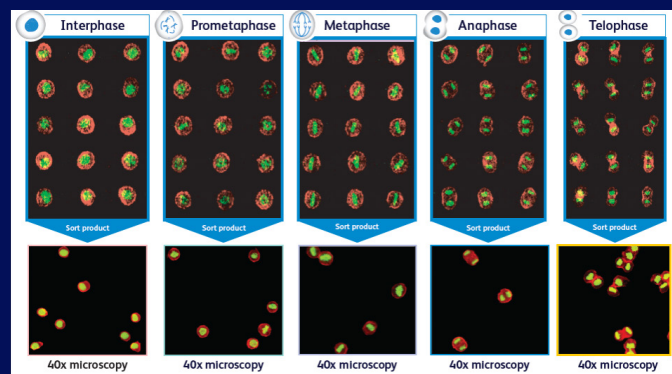
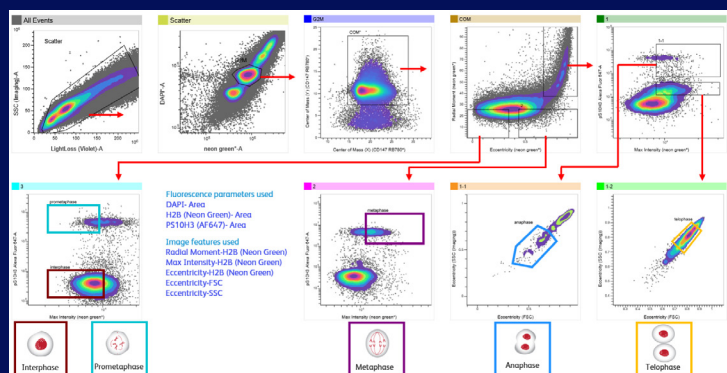
The cell cycle is one of the most fundamental processes in biology and yet one of the most difficult to fully resolve using conventional flow cytometry. While traditional approaches allow separation of cells into G1, S, and G2/M phases, all stages of mitosis are typically grouped into one group.

In this study, the authors demonstrate that all phases of the cell cycle including the five distinct stages of mitosis can be accurately identified and isolated using image-enabled flow cytometry. HeLa cells expressing H2B-NeonGreen were analyzed using classical fluorescent markers (DAPI and phospho-Ser10 histone H3) alongside with quantitative image-derived parameters during acquisition and sorting.

Capabilities with BD CellView™ Image Technology

- **Integrates imaging with fluorescence data** to provide deeper biological insights, including spatial and morphological features.
- **Enables real-time visual verification of gating decisions** during acquisition for greater confidence and accuracy.
- Supports high pure cell sorting using combined imaging - plus - fluorescence (dual-parameter strategy) to enhance sort population purity.

Results at a glance



Microscopy Confirmation of Cell Cycle Sort Products:

HeLa cells expressing H2B-NeonGreen were sorted into five distinct mitotic phases Interphase, Prometaphase, Metaphase, Anaphase, and Telophase using a combination of imaging and fluorescence parameters. Post-sort, cells were fixed with ProLong Diamond Antifade Mountant, cured for 24 hours, and imaged at 40x magnification using a Nikon Eclipse Ti2E inverted microscope. Fluorescence channels for Neon Green (chromatin marker) and PE-CF594 (CD147 surface marker) were captured and overlaid using ImageJ software to visually confirm the accuracy of the sort.

A Paradigm Shift with Real-Time Image Enables Cell Sorting

BD CellView™ Image Technology represents the next generation for flow cytometry capabilities empowering high-throughput, image informed experiments that previously required specialized microscopy all within a single, seamless workflow.

The BD FACSDiscover™ family of instruments with BD CellView™ Image Technology enables direct measurement of molecular localization, structural organization and dynamic morphological changes of cells over time. For laboratories currently relying on traditional flow cytometry, this new approach opens doors to advanced cell cycle experimental possibilities including:

- **Interrogating dynamic biological processes** such as mitosis, nuclear translocation, and organelle remodeling.
- **Defining cell populations using on morphology and localization**, not just fluorescence intensity.
- **Isolating structurally defined phenotypes** for downstream transcriptomic, epigenetic, or functional analysis.

Reveal True Phagocytosis:

Distinguish Internalization from Artifacts - Not Just Fluorescence.

Is your phagocytosis data complete?

Phagocytosis is a fundamental immune process in which monocytes and granulocytes engulf pathogens, debris, or particles. While using Phrodo™ particles enables detection of true engulfment events fluorescence only appears once the particle is internalized.

The challenge with conventional flow extends beyond simple detections. The real difficulty lies in determining where the signal originates and distinguishing true biological events from misleading artifacts.

In this study, the authors demonstrate that previously unresolvable phagocytic subpopulations can be identified and isolated using image-enabled flow cytometry.

Capabilities with BD CellView™ Image Technology

- **Provides spatial and morphological clarity**, distinguishing true phagocytic events from common confounders such as surface-bound particles or debris.
- **Enables detection of subtle structural and shape differences** associated with phagocytic activation, offering deeper insights than fluorescence.
- **Expands analytical resolution**, the combination of 18 fluorescent markers and 20 image-derived features allowed to resolve 27 unique phagocytic clusters, compared with only 13 clusters detected using fluorescence and scatter parameters alone

Results at a glance

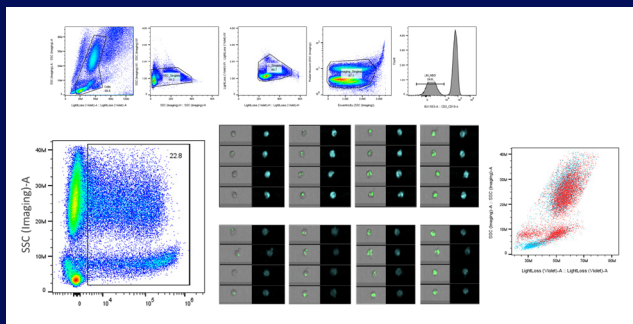


Figure 1. Identification of Phagocytic Cells Using Phrodo Fluorescence and Imaging Features. Representative imaging data from the phagocytosis assay showing side scatter (SSC) and Phrodo fluorescence (FITC) channels. Cells that have internalized Phrodo particles exhibit increased fluorescence, enabling clear discrimination of phagocytic cells. The FACSDiscover S8's imaging capabilities allow for enhanced visualization and classification of these populations based on both fluorescence intensity and morphological features.

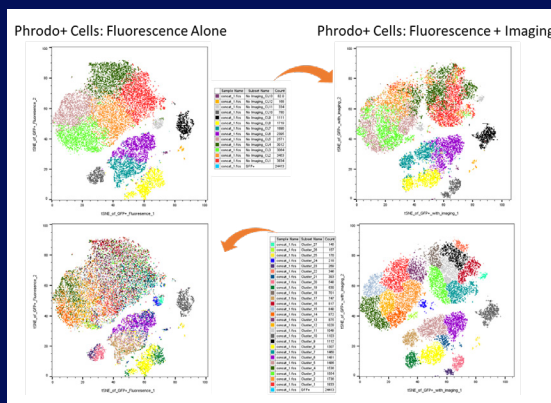


Figure 2. t-SNE algorithm analysis comparing fluorescent parameters with fluorescent imaging parameters. More unique clusters can be identified when combining fluorescent and imaging parameters.

2 Scatter (SSC & LL) +
18 Fluorescent parameters
= **13 Unique Clusters**

↓

2 Scatter (SSC & LL) +
18 Fluorescent parameters +
20 Image derived features
= **27 Unique Clusters**

Discover New Populations through Spatial Localization

Beyond producing imaging data, BD CellView™ Image Technology enhances the precision and interpretability of Phagocytosis assay by intergrading fluorescence-based and image-derived measurements into a unified workflow. This multimodal approach reveals distinct cellular subsets that remain hidden in traditional, intensity-only analyses enabling a new dimension in phagocytosis research.

For laboratories currently relying on conventional flow cytometry, this new workflow enables a range of advanced, mechanistic questions, such as:

- Which populations with similar fluorescence intensities truly represent **different functional pathways**?
- How do **novel phagocytic subsets** emerge under stimulation, drug treatment, or disease conditions?
- Can these **structurally defined populations** be isolated for downstream transcriptomics or functional assays?

Verify True Cells Below 0.01%!

Resolve Rare Events with Confidence.

Are those rare events real?

Rare event detection is one of the most powerful yet challenging applications of flow cytometry. When the target population occurs at frequencies as low as **1 in 10,000**, even minor sources of variability non-specific staining, debris, aggregates, spectral spillover, or thresholding artifacts can produce false “positive” events that closely mimic real cells.

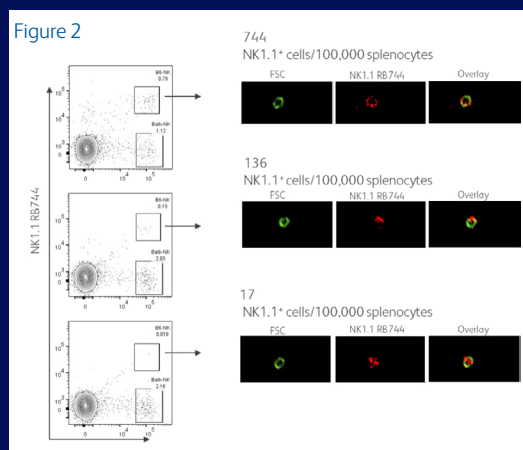
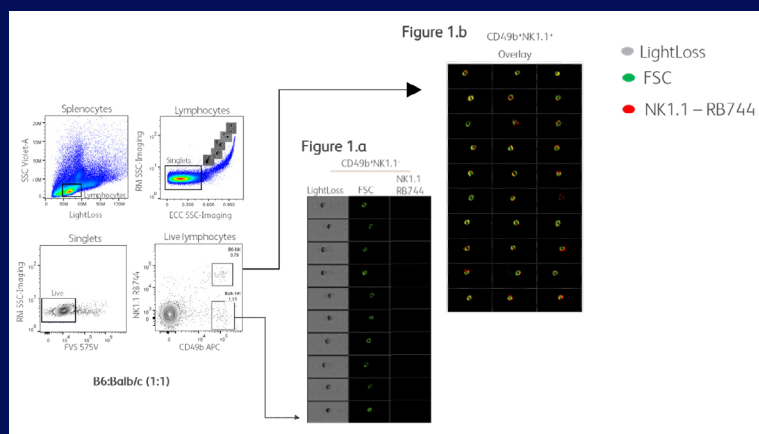
In this application study, the authors evaluate how image-enabled flow cytometry can strengthen confidence in rare-event identification.

A moderately expressed surface antigen (NK1.1) on mouse NK cells within total splenocyte was used to test how morphological information contributes to true-positive verification when diluting NK1.1⁺ events to frequencies below 0.01%.

Capabilities with BD CellView™ Image Technology

- **Combines high-throughput flow analysis with spatial resolution**, leveraging the imaging capabilities of the BD FACSDiscover™ platform to reveal structural details behind each event.
- **Enables visual discrimination between live biological cells and fluorescence-based artifacts**, improving accuracy when analyzing large, sparse populations.
- **Allows cross-checking of rare events for morphological consistency across sample dilutions**, increasing confidence that detected positives represent true biological signals.

Results at a glance



Confirm True Rare Cells Visually

BD CellView™ Image Technology transforms rare-event analysis from a probability driven estimate into a more robust, reproducible measurement. By integrating fluorescence, real-time imaging, and morphological parameters, researchers can:

- Distinguish true rare cells from fluorescence artifacts with higher confidence
- Validate rare-positive events visually in real time
- Strengthen decision-making for assays where every event counts

Why this matters for labs using classical flow cytometry?

Many research areas depend on highly reliable rare-event workflows, such as:

- Minimal residual disease (MRD) analysis
- Circulating tumor cell (CTC) detection.
- CAR T-cell monitoring and persistence studies

Move beyond CAR intensity and discover spatial patterns

Are all CAR-T cells the same?

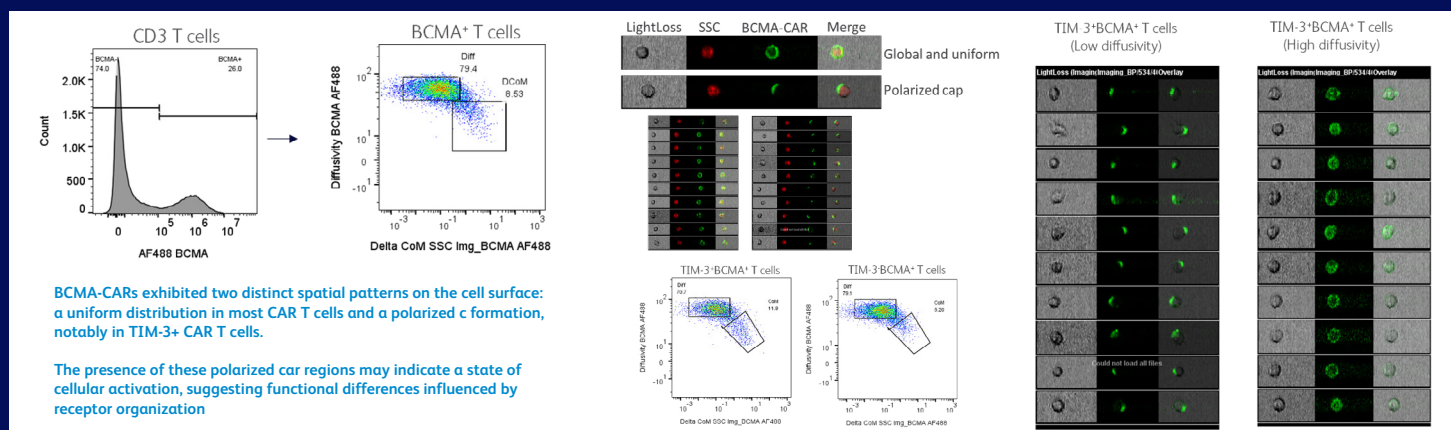
CAR T-cell therapies rely not only on the presence of the CAR receptor but on its expression level and spatial organization on each cell. Yet, with traditional flow cytometry, CAR density and surface distribution patterns such as uniform vs. polarized localization are often difficult thus, the ability to understand construct stability, activation state, or functional heterogeneity across CAR T-cell subpopulations can be challenges.

In this study, the authors demonstrate that single-cell CAR expression patterns can be detected, visualized, and quantified using image-enabled flow cytometry. CAR-BCMA T cells were analyzed on the BD FACSDiscover™ S8 using classical fluorescence intensity measurements alongside quantitative image-derived parameters that capture membrane uniformity, cap formation, and synapse-associated enrichment.

Capabilities with BD CellView™ Image Technology

- **Enables real-time visualization of CAR Distribution pattern**, such as uniform receptor placement or polarized cap formations
- **Integrates imaging with fluorescence data** to reveal spatial and morphological organization of CAR molecules, beyond intensity alone.
- **Provides biological context for subpopulation differences**, including associations between polarized caps and activation markers such as TIM-3.

Results at a glance



BCMA-CAR quantification and analysis of spatial distribution patterns on freshly thawed CAR-T cells using imaging flow cytometry. Using the parameter Diffusivity-AF488 and Delta Center of Mass SSC Imaging _AF488, to measure the CAR-BCMA density and distance from the center of the cell (SSC), we identified two different expression patterns. About 80% of the CAR-T cells presented the CAR uniformly distributed on the surface while 9% of the cells showed a polarized formation

A Paradigm Shift in CAR T-Cell Analysis

BD CellView™ Imaging Technology enables **direct measurements of CAR receptor localization and structural organization**, features traditionally accessible only by microscopy.

For laboratories relying on classical flow cytometry, this approach unlocks new analytical and translational opportunities, including:

- Defining CAR T-cell subsets by morphology and receptor distribution, not just fluorescence intensity.
- Interrogating functional states, such as activation or synapse-associated organization, through spatial receptor patterns.
- Isolating structurally defined CAR T-cell populations for downstream transcriptomic, epigenetic, or functional studies

This approach offers three practical advantages for biological research and translational development:

- Identifies subpopulations that cannot be resolved by intensity-based gating alone.
- Can add a functional dimension that indicate a state of cellular activation .
- BD FACSDiscover™ S8 enables sorting for downstream analysis.

Ready to Expand Your Discovery Power?

Trade in your flow cytometer today to save — and see more.

Step into the future of flow cytometry with the **BD FACSDiscover™ A8 Cell Analyzer**, combining **spectral flow cytometry** and **real-time single-cell imaging** in one seamless workflow. Powered by **BD SpectralFX™ Technology** and **BD CellView™ Imaging Technology**, plus an **integrated autoloader and intuitive guided software**.

The **BD FACSDiscover™ A8 Cell Analyzer** helps you generate richer data, faster, clearer, and with greater confidence.

Why BD SpectralFX™ Technology?

More Colors. More Sensitivity. More Confidence.

- **Full-spectrum detection:** 78 fluorescent detectors across 5 lasers capture complete emission profiles with high sensitivity.
- **Optimized optics:** Modular optical design minimizes signal loss to improve resolution and panel performance.
- **System-aware unmixing:** Real-time detector noise calibration reduces spread and improves population separation, even across different gain settings.
- **Automated setup & QC:** On-board LED for daily detector gain calibration; broad-spectrum setup bead for performance tracking; optimized default gains, no manual voltage adjustments.
- **Guided workflow (BD FACSchorus™ Software):** A simple, step-by-step workflow all in one intuitive software environment.

Design → Set Gains → Single-Stains → Unmix → Analyze → Sort Report

Why BD CellView™ Imaging Technology?

Real time imaging – built into your flow.

- **Richer insights, same run:** Combines flow cytometry parameters with **spatial and morphological** information for faster answers to complex questions.
- **Event-level visibility:** Integrates image and flow data at **every event**, enabling **instant visualization** during acquisition.
- **High-speed imaging without cameras:** Uses **orthogonal frequency domain multiplexing (OFDM)** with existing cytometer electronics/optics for **higher throughput** than camera-based systems.
- **Real-time decisions:** Image data is acquired, quantified, and available immediately for **analysis and sorting** as the sample runs.
- **Flexible by design:** **Imaging mode is optional**, you can **upgrade with BD CellView™ Imaging Technology** to match your workflow and throughput needs.

BD FACSDiscover™ A8 Cell Analyzer

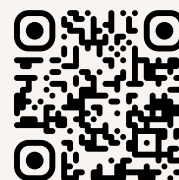
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