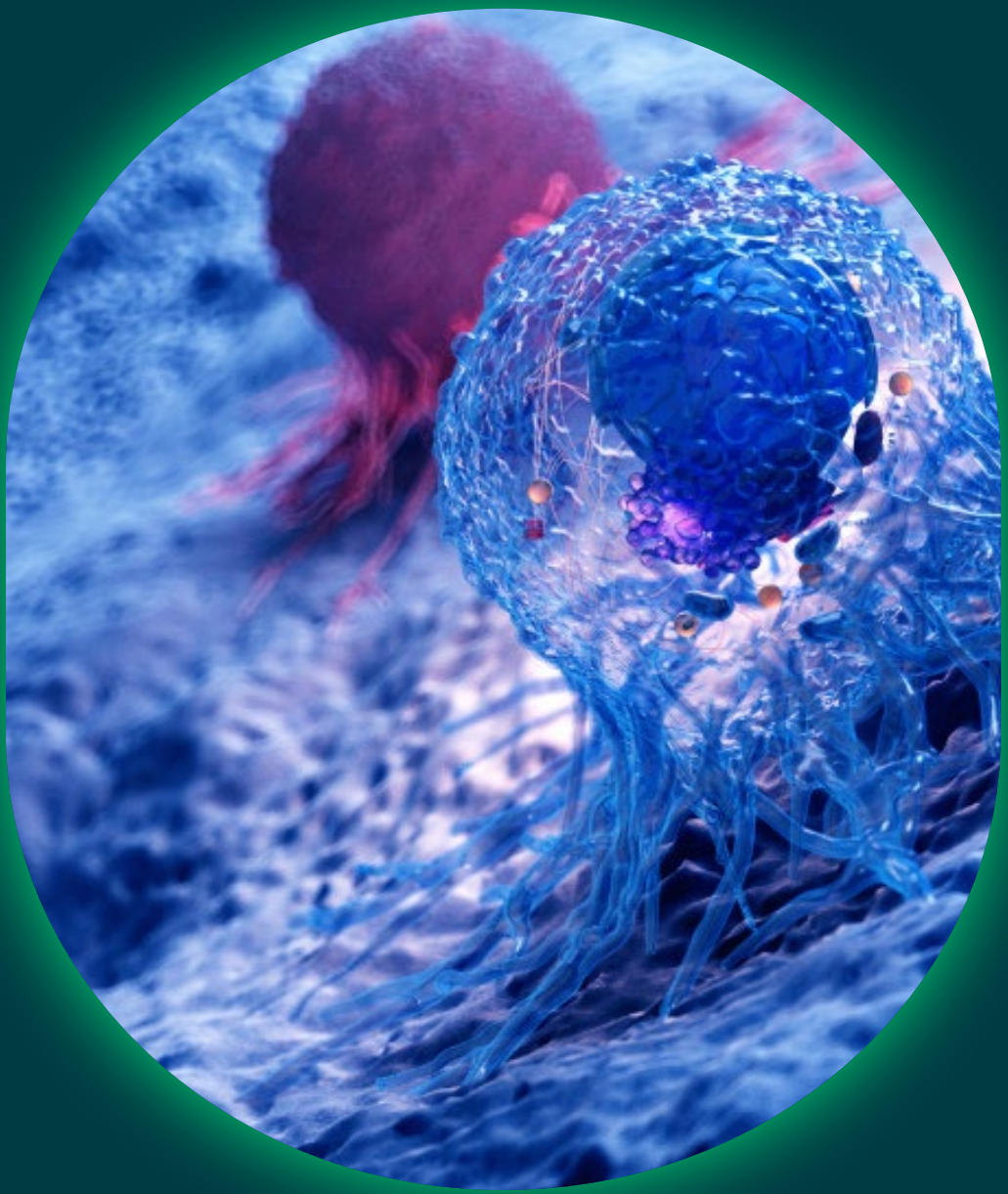
INFOGRAPHIC

From Isolation to Insight:

Fluorescence Imaging of EVs in Liquid Biopsy Workflows



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THE CHALLENGE

1. The Clinical Imperative

Early Cancer Detection Demands Molecular Precision

$23.94B liquid biopsy market by 2034

>90% sensitivity with multi-modal EV analysis

6+ cancers from single blood draw

2. The EV Characterization Bottleneck

Traditional: TEM, NTA, flow cytometry

- Limited throughput
- Size-only characterization
- Limited molecular information

Need:

- Rapid phenotyping
- Multi-marker analysis
- Live dynamics tracking
- 3D spatial context

3. Fluorescence Labeling Strategies for EVs

Lipophilic dyes (DiI, PKH)

Fluorescent proteins (CD63-GFP)

Immunofluorescence (CD81, TSG101)

Covalent labeling (Cy5, Cy7)

Challenge:

Imaging at the diffraction limit while distinguishing labeled EVs from artifacts and free dye.

THE SOLUTION

Fluorescence Microscopy Solutions for EV Analysis

4. A Platform Approach to Multi-Scale EV Imaging

EV research demands imaging flexibility—the ability to rapidly screen samples, resolve 3D tissue context, and visualize nanoscale surface marker distributions. Modern fluorescence microscopy platforms address this need by integrating multiple imaging modalities in adaptable systems that match technique to biological question.

Matching Imaging Approach to Research Need:

EV characterization workflows benefit from three complementary imaging strategies:

1

Widefield fluorescence enables high-throughput screening and rapid particle detection across multi-well formats, ideal for initial EV quantification and concentration determination

2

Confocal microscopy provides optical sectioning for 3D tissue imaging (>500 µm depth), multi-marker colocalization studies, and live-cell tracking of EV uptake dynamics

3

Super-resolution techniques break the diffraction limit (140-180 nm resolution) to resolve EV subpopulations, surface marker clustering, and nanoscale heterogeneity

Oxford Instruments Integrated Solution:

The BC43 and Dragonfly platforms provide researchers with a complete microscopy ecosystem for EV analysis:

BC43 Benchtop Microscope: Compact widefield and confocal system with field-upgradeable super-resolution capabilities (SRRF-Stream technology), designed for ease of use with minimal training requirements (30 minutes to 2 hours)

Dragonfly Confocal Platform: High-speed spinning disk confocal for 3D volumetric imaging, automated tiling/stitching for large-area tissue mapping, and gentle live-cell imaging with efficient light collection

Imaris Analysis Software with Workflows: Imaris 11 introduces Workflows, an intuitive step-by-step protocol system that streamlines EV detection, counting, and tracking in 3D image datasets.
For EV researchers, Workflows enable:

- Automated EV detection and counting across large datasets using reproducible, step-by-step protocols
- Time-lapse tracking to measure EV uptake kinetics, cellular trafficking patterns, and biodistribution dynamics
- Batch processing to analyze multiple experiments with identical protocols, saving time on repetitive tasks
- Shareable analysis protocols that ensure reproducible quantification across users and research teams
- Flexible manual adjustments within automated workflows for precision refinement of detection parameters.

Essential Platform Capabilities:

- Multi-modal imaging flexibility (widefield ➔ confocal ➔ super-resolution)
- Multiple laser lines for multi-channel fluorescence (405-640 nm)
- Sensitive detection (sCMOS cameras) with GPU-accelerated deconvolution
- Motorized stages for automated acquisition and large-area montaging
- Temperature and CO_2 control for live-cell dynamics
- QUAREP-LiMi certification for standardized quality control
- Modular, upgradeable design to adapt to evolving research needs

Integrating Imaging into EV Liquid Biopsy Workflows

5. Six-Step Imaging-Integrated Workflow

Isolate & Label
EV isolation (UC, SEC, immunocapture)
➔ fluorescent labeling
➔ excess dye removal

Characterize (Widefield)
Rapid detection, 4-channel multiplexing, particle quantification, concentration determination

Phenotype (Confocal)
3D imaging, multi-marker colocalization, tetraspanin profiling (CD9/CD63/CD81)

Resolve (Super-Resolution)
Nanoscale imaging, subpopulation discrimination, surface marker spatial distribution

Track Dynamics
Live-cell EV uptake, time-lapse tracking, tissue biodistribution studies

Analyze
Machine learning classification, morphology metrics, spatial statistics, batch processing

6. Advantages of Fluorescence Imaging in EV Research

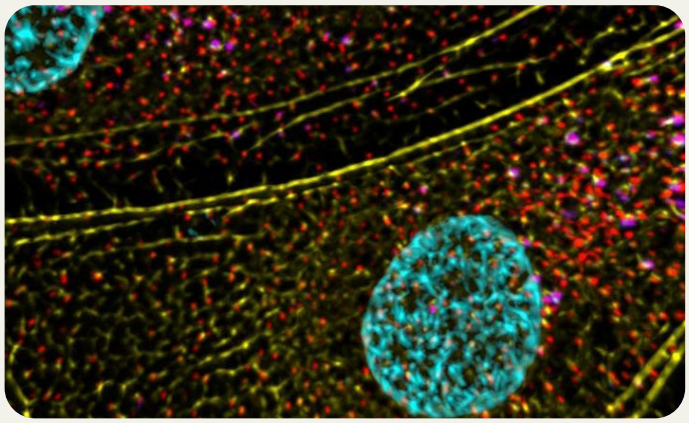
Performance Capabilities

- **Acquisition time:** minutes vs hours (compared to laser scanning confocal)
- **Tissue penetration:** >500 µm depth in fixed or live samples
- **Resolution:** 140-180 nm with super-resolution techniques
- **Multi-parameter analysis:** 4+ channels simultaneously
- **High throughput:** 96-well plate screening compatible

Practical Benefits

- **Moderate** training requirements (hours to days vs weeks)
- **Standardized** protocols and quality control metrics
- **Compatible** with live-cell and tissue imaging
- **Modular** systems adapt to evolving research needs
- **Integration** with automated analysis workflows

7. Applications

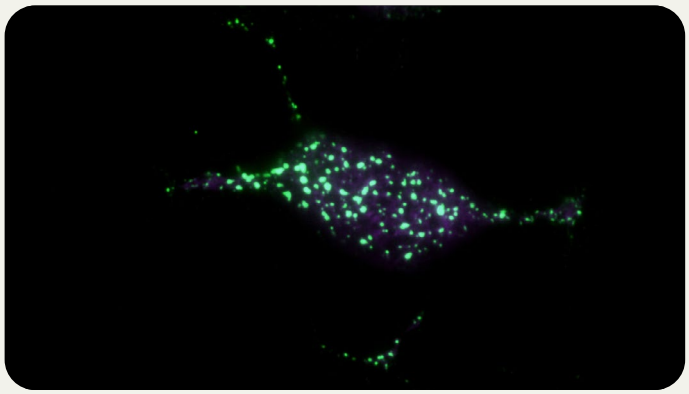


Super-Resolution Protein Localization

Dragonfly in TIRF mode with Sona sCMOS enables high-resolution imaging of vesicular events, while dual-camera setups capture multiple players simultaneously. For live-cell super-resolution, Dragonfly paired with SRRF-Stream delivers speed and clarity, and iXon EMCCD ensures ultra-sensitive, low-light imaging. Imaris completes the workflow with automated 3D vesicle detection and quantification over time.

Technique:

Widefield → Super-resolution (SRRF)

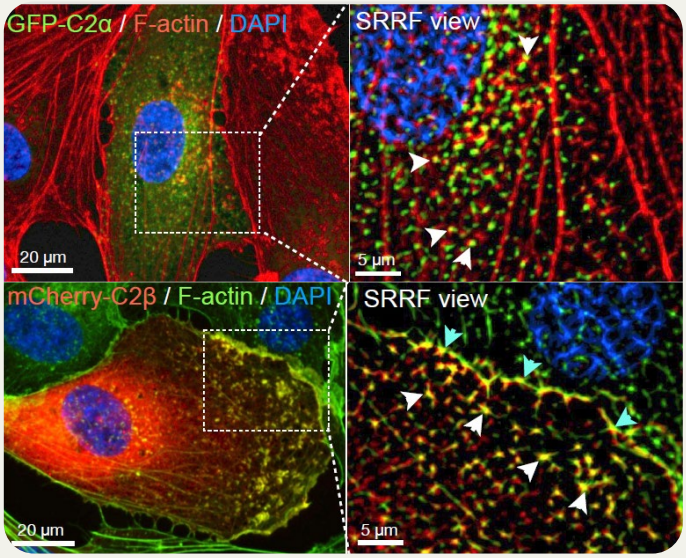


Multi-Parameter Cellular Dynamics

BC43 confocal imaging reveals how VapB (an ER protein) organizes Kv2.1 ion channels into precise clusters at ER-plasma membrane junctions in neurons. This interaction is controlled by specific protein binding motifs and phosphorylation, demonstrating how the BC43 captures the molecular mechanisms that organize cellular architecture. Imaris provides automated 3D analysis of these protein distributions over time.

Technique:

Confocal → Multi-color imaging



Nanoscale Colocalization Analysis

Dragonfly with SRRF-Stream reveals how different C2 domain proteins interact with the cell's structural scaffolding at super-resolution. The system captures mCherry-tagged C2β forming stable associations with F-actin at the cell membrane—a signature of its role in organizing the cortical actin network. In contrast, GFP-tagged C2α shows sporadic F-actin colocalization, suggesting it responds to context-dependent cellular signals rather than maintaining constant membrane contact. This level of resolution distinguishes transient from stable protein-cytoskeleton interactions that would be impossible to resolve with conventional microscopy. Imaris then automates the quantification of these colocalization patterns and spatial distributions.

Technique:

Super-resolution + automated analysis

7. Further Reading & Resources

Key Scientific Literature:

Minimal information for studies of extracellular vesicles (MISEV2023): From basic to advanced approaches

Unveiling Invisible Extracellular Vesicles: Cutting-Edge Technologies for Their in Vivo Visualization

Oxford Instruments Resources:

Dragonfly Confocal Microscope System - Andor - Oxford Instruments

How to Overcome the Challenges of Imaging Vesicle Trafficking

BC43 Widefield and Confocal Benchtop Microscopes

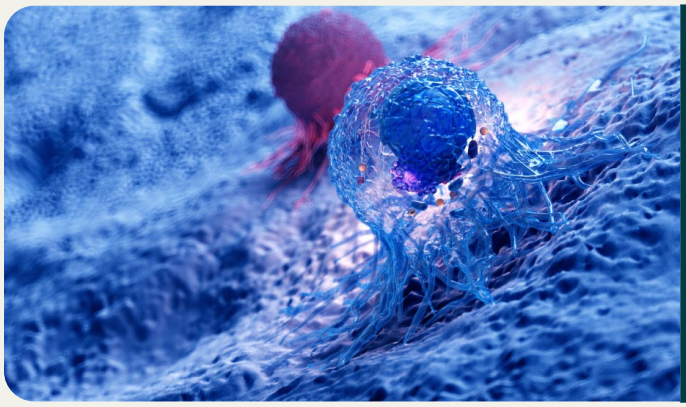
Image Analysis Software for Cancer Research

Imaris AI Microscopy Image Analysis Software

Quantitative Live Cell Imaging of Rab11A and Viral RNA Reveals Differential Manipulation of Transport Pathways

Exploring Microscopy Techniques: Confocal, Widefield, Transmitted Light and Deconvolution

Image Analysis Software for Immunology



Cellular Insights Series:

Unlocking Cancer Insights with Extracellular Vesicles in Liquid Biopsy

Free on-demand virtual event

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