Apoptosis Detection Using the BD Accuri™ C6 Flow Cytometer

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Outline

- What is apoptosis?
- Methods of apoptosis detection
- Detection of apoptosis by flow cytometry

Apoptosis Definition

The process leading to controlled self destruction of a cell. Cells undergo death neatly without damaging their neighbors. Apoptosis is a “programmed event.”

Importance of Apoptosis

- Cell termination
  - Viral infection, cancer
- Homeostasis
  - Tumor, diseases
- Development
  - Organs, appendages, patterning
- Lymphocyte development
  - Thymic selection
- Drug discovery studies

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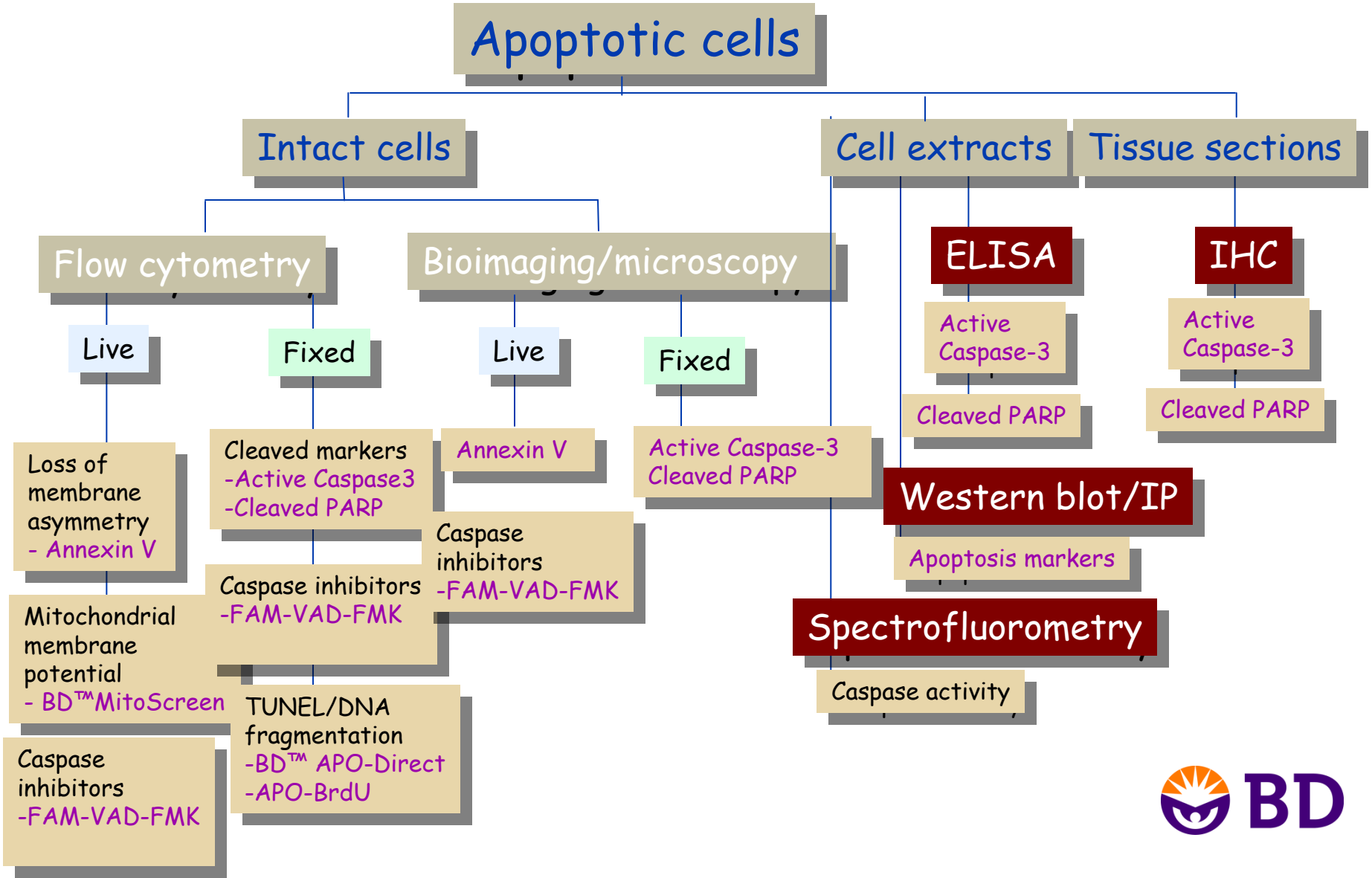
Hallmarks of Apoptosis

- Plasma membrane alterations
- Mitochondrial changes
- Activation of caspases
- DNA fragmentation

Apoptosis Decision Tree



Summary of Apoptosis Assays

Feature Measured	Assay	Key Features
Plasma Membrane Alterations	Annexin V Binding Assay: • Single Conjugates • Annexin V Kits	•Detects early apoptosis •Quick and easy
Mitochondrial Changes	• BD MitoScreen	•Fast and easy
Caspase Activation	• Active Caspase-3 Flow Kit	•Specific antibodies can detect activated versus uncleaved caspase-3 •Can be multiplexed
DNA Fragmentation	• APO-BrdU TUNEL Assay • APO-Direct TUNEL Assay	•Works with adherent cells

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The BD Accuri™ C6 Flow Cytometer System

An affordable, full-featured, easy-to-use flow cytometer
Two lasers and six detectors

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Optics

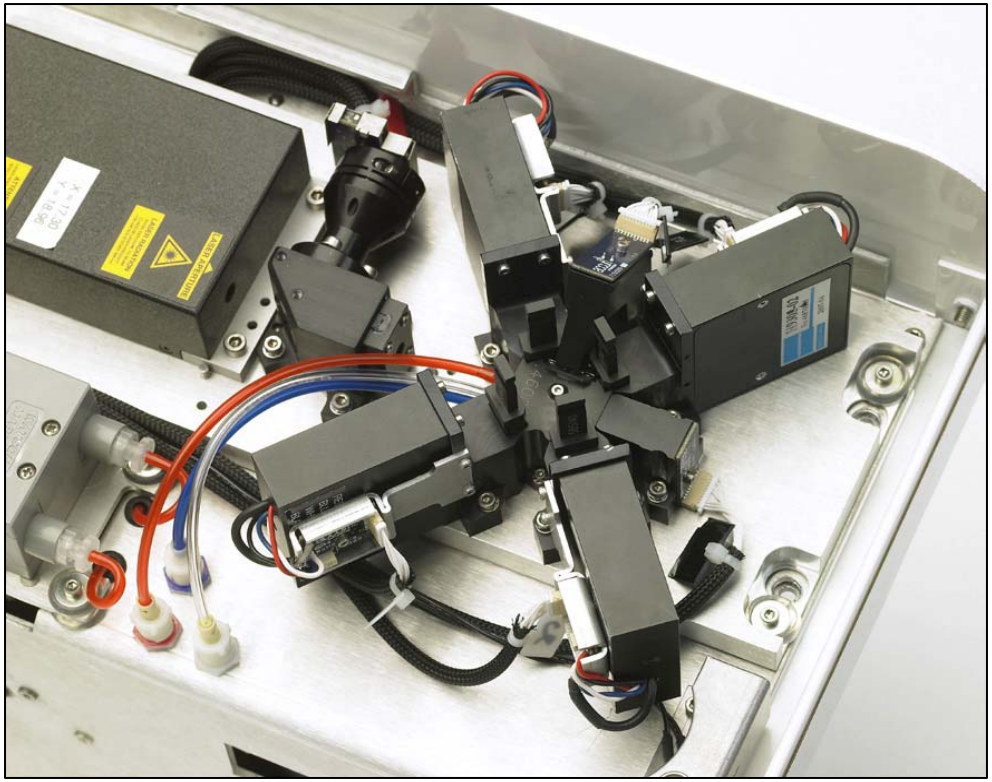
Compact optical system design reduces cost and eliminates alignment issues

488-nm solid state, 21-mW laser (Melles Griot/JDS Uniphase)

640-nm diode, 14.7-mW laser (custom manufacture)

PMTs for fluorescence detection

Diodes for scatter detection



User changeable optical filters

510/15 nm
540/20 nm
565/20 nm
610/20 nm
780/60 nm

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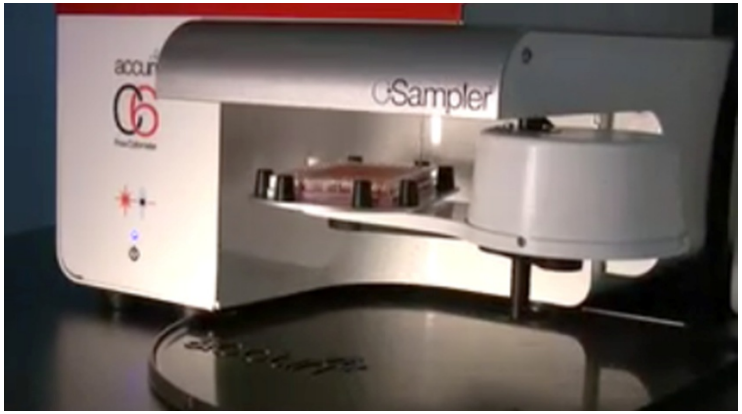
Fluidics

- Microprocessor-controlled peristaltic pumps enable direct volume measurement
- Many types of sample tubes may be used
  - BD Falcon™ fluorescence activated cell sorting tubes
  - Microcentrifuge tubes
  - Ninety-six-well plates with the BD CSampler™ accessory
- Open system conducive for kinetic studies



Detection: Wide Dynamic Range

Intuitive Software

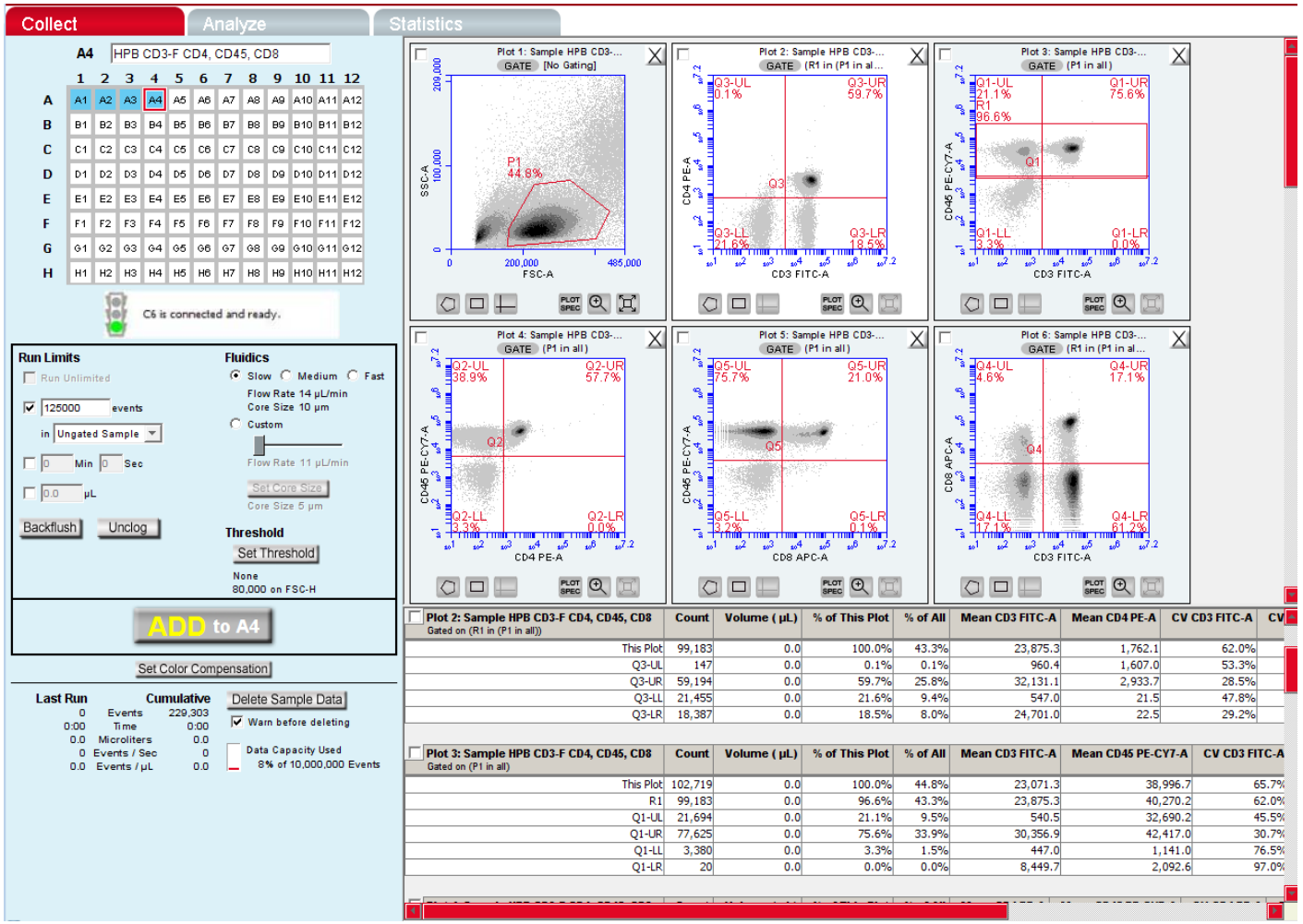
Sample Grid

Cytometer Status

Fluidics Controls

Run Criteria

Real-Time Updates



Histograms

Dot Plots

Density Plots

Analysis and Gating Tools

Plot Statistics

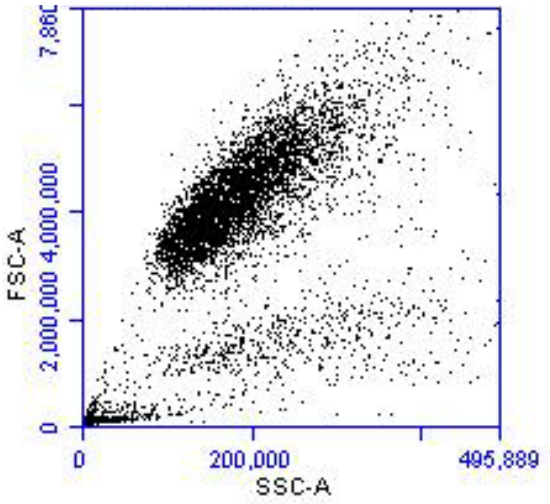
Why Choose the BD Accuri C6 for Apoptosis Measurements?

- Small footprint: fits on a benchtop
- Easy to use
- Locked-down alignment, no voltage or gain adjustments
- Minimal setup and QC
- Affordable
- Intuitive software
- Most apoptosis assays use fewer than four colors

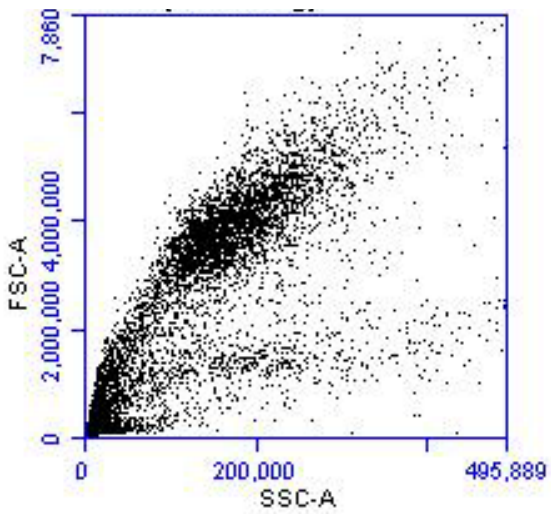
Apoptosis: Scatter Properties

Cell shrinkage during apoptosis is associated with a decrease in forward scatter. Analysis of light scatter is often combined with other assays.

Untreated



Camptothecin treated



Formation of apoptotic vesicles

- **Increases side scatter**

Reduced refractive index of apoptotic cells

- **Decreases forward scatter**

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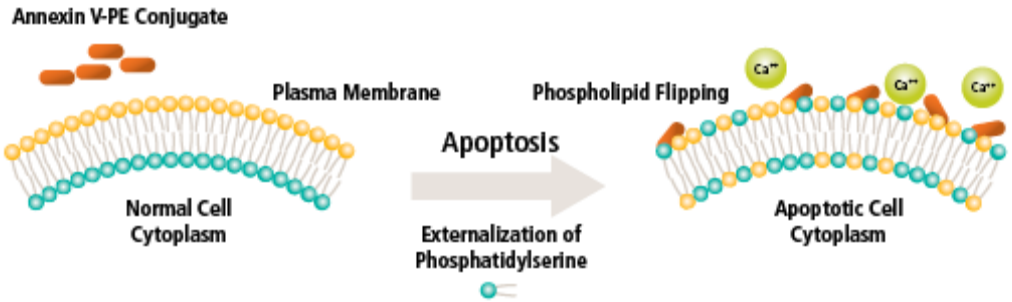
Annexin V

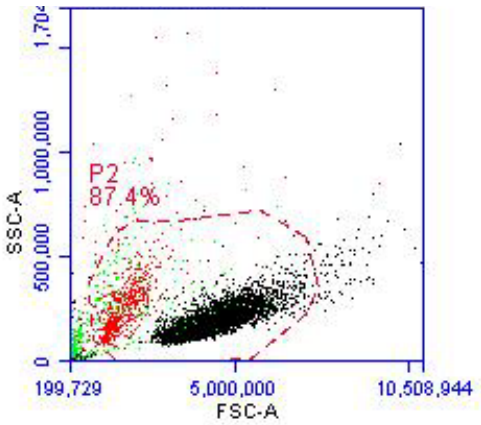


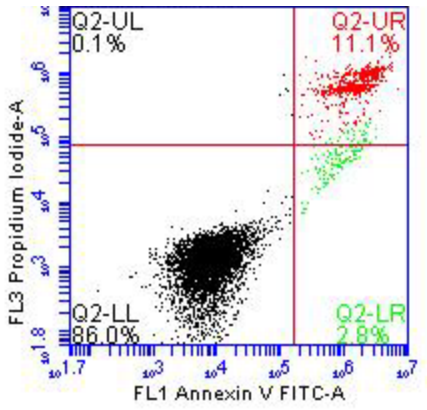
Annexin V is a surface marker and detects early membrane changes associated with apoptosis.
Pros: Rapid confirmation of apoptosis
Uses live, unfixed cells

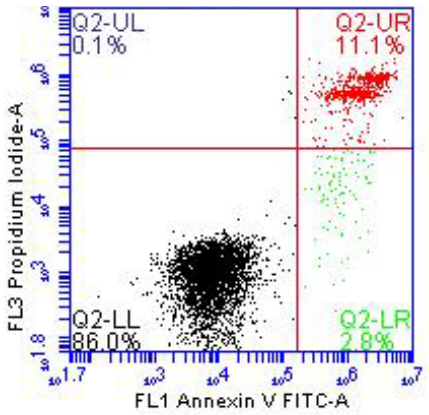
Applications
•Flow cytometry (cells in suspension)
•Fluorescence microscopy (adherent cells)

Annexin V

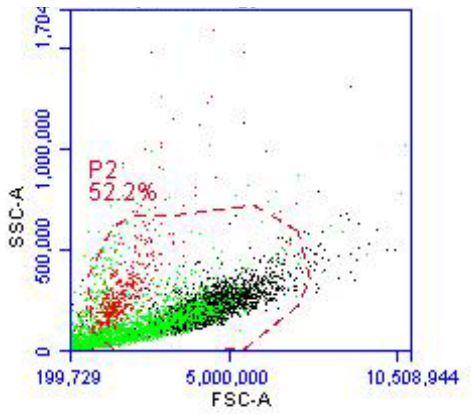
DMSO

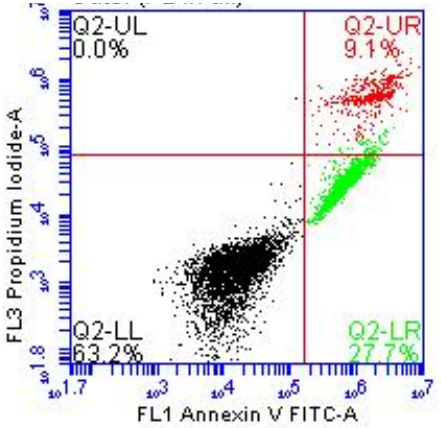


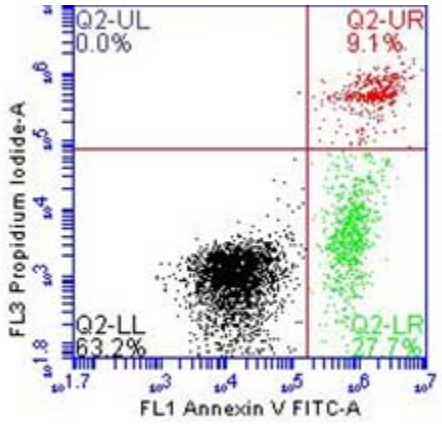




Camptothecin
6 µM







Jurkat T cells were treated with camptothecin for 4 hours and stained with Annexin V FITC + propidium iodide.

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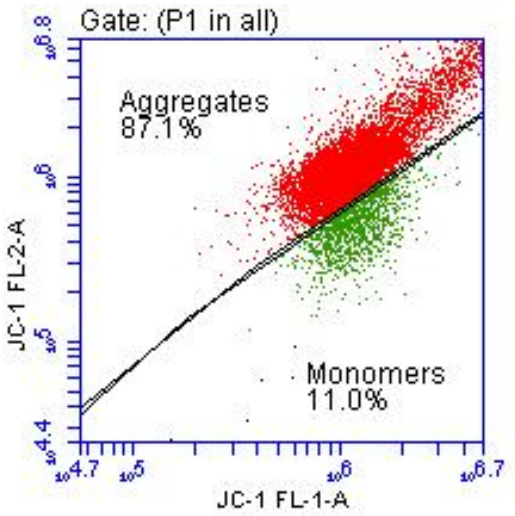
Detection of Apoptosis with JC-1

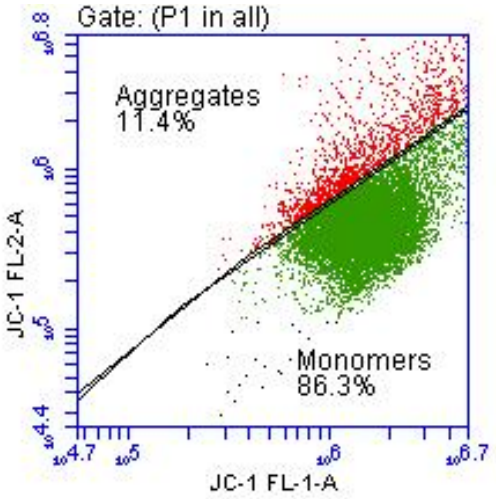
- Changes in mitochondrial potential are another early marker for apoptosis.
- Lypophilic cationic fluorochromes such as JC-1 penetrate cells and form aggregates.
- Monomers and aggregates of JC-1 have different emission spectra.
  - In healthy cells, JC-1 accumulates in the mitochondria and forms aggregates.
  - In apoptotic cells, JC-1 does not accumulate in the mitochondria and remains in the cytoplasm as monomers.
- Changes in membrane potential can be determine by comparing the ratio of fluorescence between the FL1 and FL2 channels.

JC-1 Measurements on the BD Accuri C6

Untreated



Treated



	Median FL1-A	Median FL2-A	Ratio FL1/FL2
Untreated	**1,156,436**	**1,073,827**	**1**
Treated	**1,520,479**	**459,785**	**3**

K562 cells were treated with the compound CCCP (10 µM) for 10 minutes and stained with JC-1.

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Caspase-3

- Caspases are proteases that are activated upon cleavage at aspartate residues at the earliest stages of apoptosis.
- Several caspases are important for apoptosis, including caspases-3, 8, and 9.
- Methods to measure caspase cleavage include fluorogenic substrates and detection with antibodies specific to the cleaved (activated) forms of caspases.

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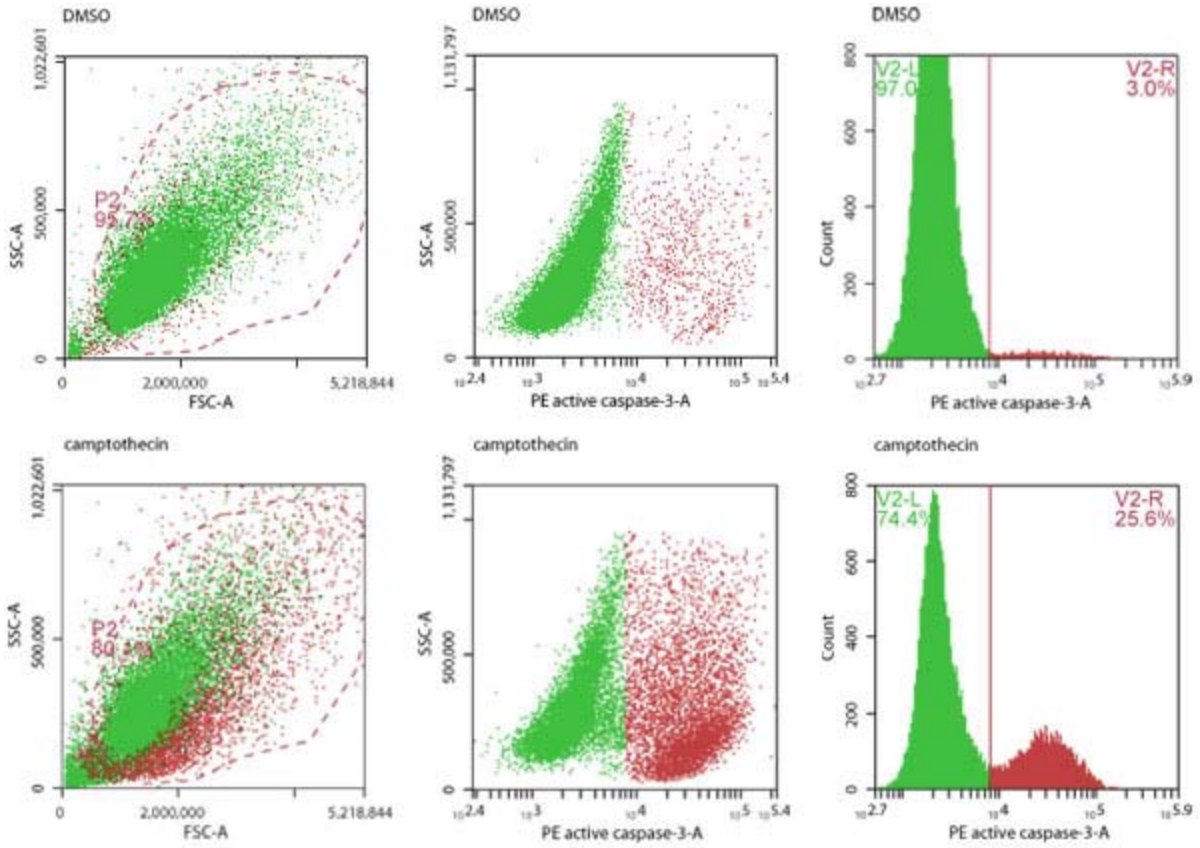
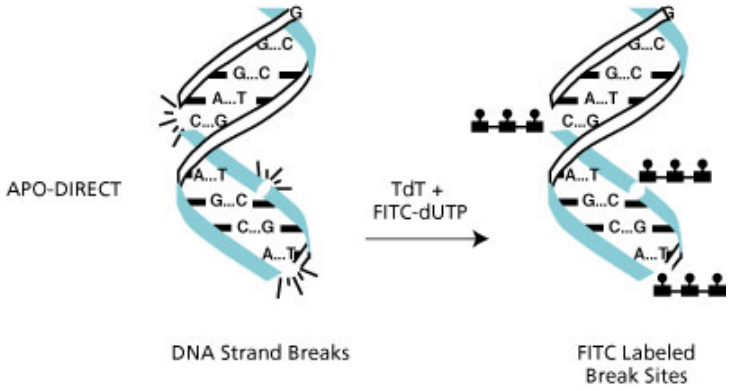
Active Caspase-3



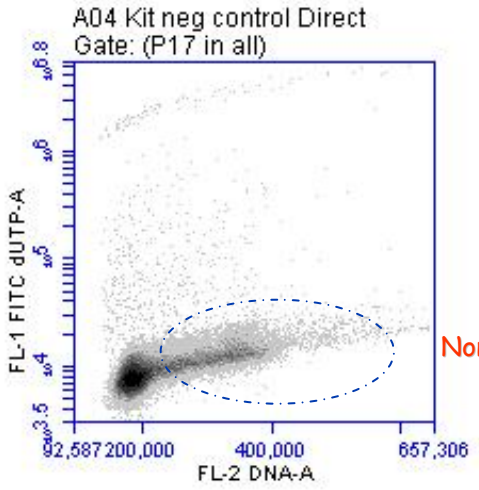
Jurkat T cells were treated with either DMSO or camptothecin (6 µM) for 4 hours, then fixed, permeabilized and stained for active caspase-3.

APO-Direct Assay

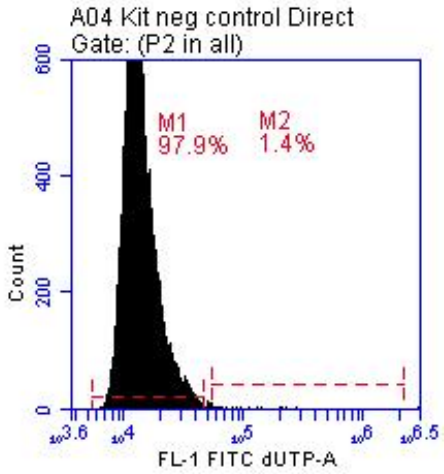


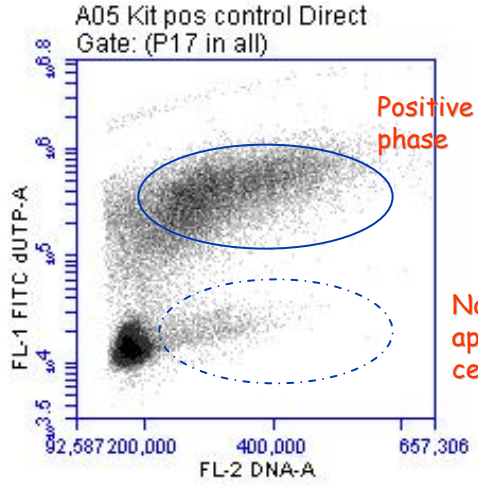
- DNA fragmentation is one of the last steps in apoptosis.
- Fragmented DNA can be detected by the end labeling or TUNEL method.
  - Enzymatic assay catalyzed by terminal deoxynucleotidyltransferase (TdT)

Detection of DNA Fragmentation During Apoptosis by “End Labeling” or “TUNEL” Using the BD APO-Direct Kit



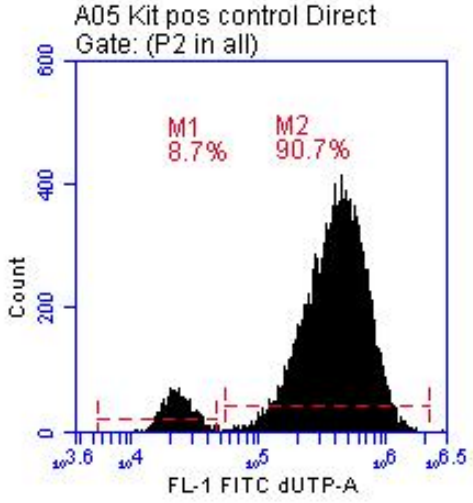
Non-apoptotic cells





Positive apoptotic cells in S-phase

Non-apoptotic cells



Summary of Apoptosis Detection Methods

Method	Advantages	Disadvantages
Annexin V	•Works well on live cells	•Difficult to multiplex •Requires calcium
BD MitoScreen (JC-1)	•Can be performed on live cells •Ratiometric data	•Difficult to multiplex
Caspase-3	•Can be multiplexed with other markers	•Requires fixation and permeabilization of cells
BD APO-Direct	•Useful for detection of late apoptosis	•Difficult to multiplex •Requires fixation and permeabilization of cells

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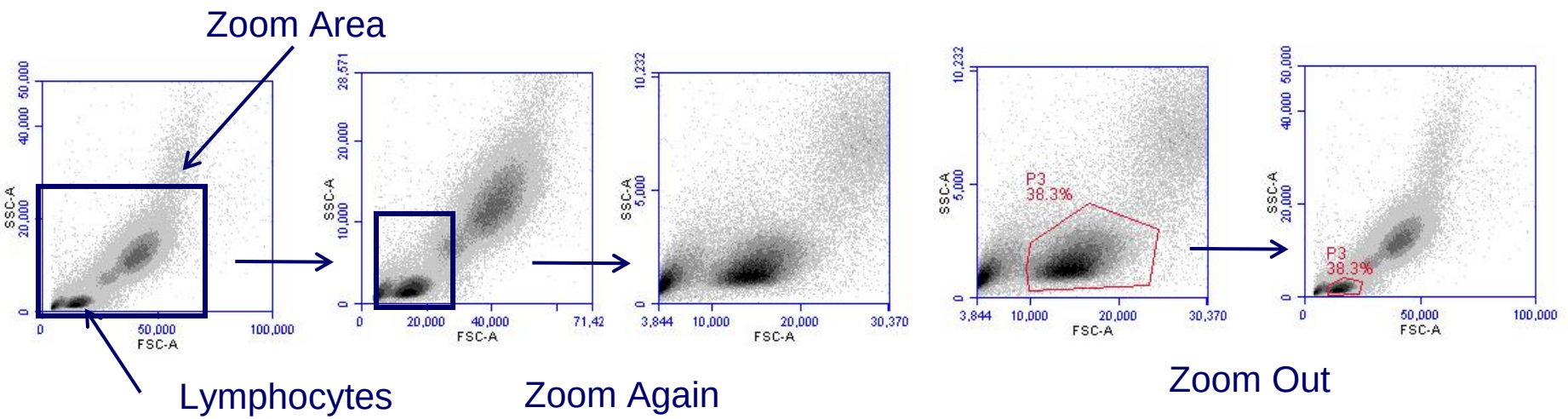
Tips/Tricks

- Keep your system clean.
- Perform *daily* QC with 6- or 8-peak beads.
- Make use of well established positive controls (camptothecin, staurosporine, etc).
- Use light scatter to your advantage.
- Use the Zoom tool to quickly locate populations.
- Start with suggested compensation values.
- Create software templates for common applications.

Suggested Compensation Values

	FITC	PE	PerCP	PerCP-Cy™5.5	PE-Cy™7	APC
FL1 (530 BP)	-	3.50	0.00	0.00	1.00	-
FL2 (585 BP)	7.00	-	0.00	0.00	3.50	0.00
FL3 (670 LP)	1.00	14.50	-	-	-	1.20
FL4 (675 BP)	0.00	0.00	3.00	12.00	0.00	-

Pre-optimized Detectors: Adjust View with Zoom



Conclusions

- There are multiple methods currently available to measure apoptosis.
- Apoptosis detection by flow cytometry can be done easily, using two colors.
- The BD Accuri C6 is a perfect analysis tool for new and experienced users.

Acknowledgments:

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