

# 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.”

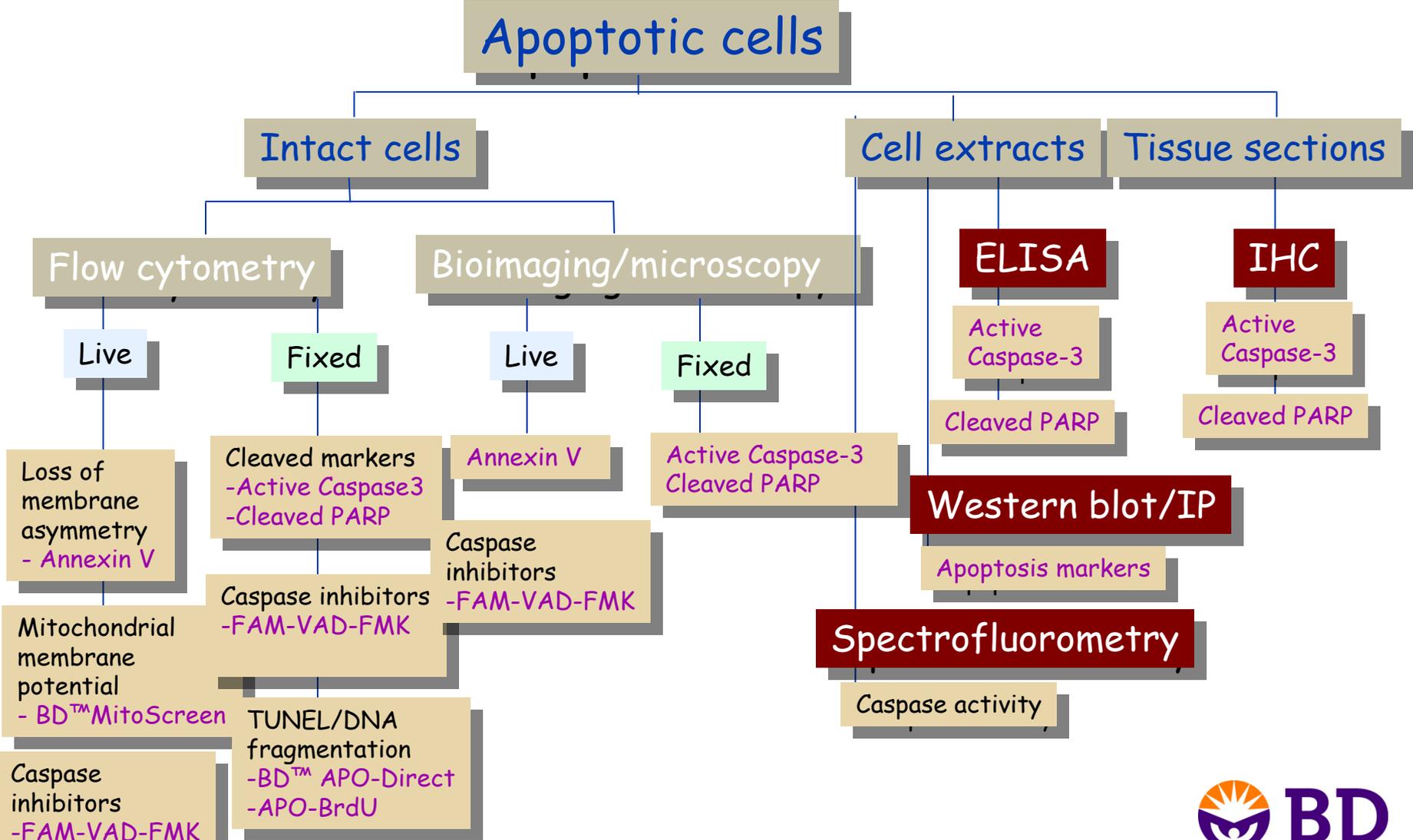
# Importance of Apoptosis

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

# 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: <ul style="list-style-type: none"><li>• Single Conjugates</li><li>• Annexin V Kits</li></ul>	<ul style="list-style-type: none"><li>• Detects early apoptosis</li><li>• Quick and easy</li></ul>
Mitochondrial Changes	<ul style="list-style-type: none"><li>• BD MitoScreen</li></ul>	<ul style="list-style-type: none"><li>• Fast and easy</li></ul>
Caspase Activation	<ul style="list-style-type: none"><li>• Active Caspase-3 Flow Kit</li></ul>	<ul style="list-style-type: none"><li>• Specific antibodies can detect activated versus uncleaved caspase-3</li><li>• Can be multiplexed</li></ul>
DNA Fragmentation	<ul style="list-style-type: none"><li>• APO-BrdU TUNEL Assay</li><li>• APO-Direct TUNEL Assay</li></ul>	<ul style="list-style-type: none"><li>• Works with adherent cells</li></ul>

# The BD Accuri™ C6 Flow Cytometer System



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



# Optics

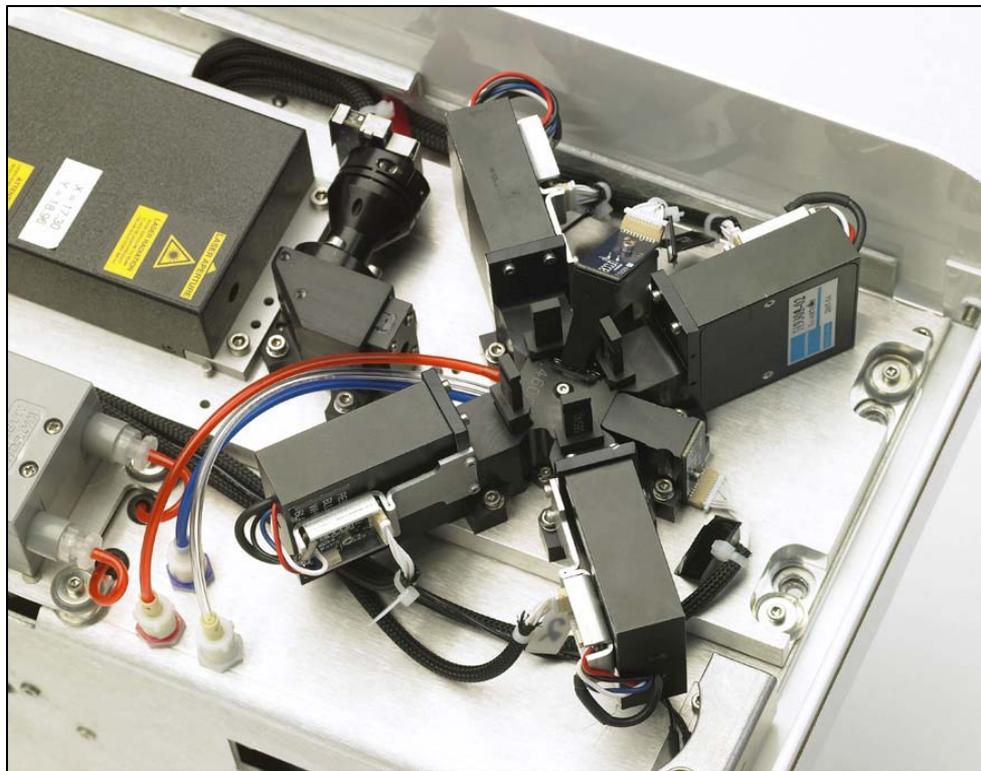
Compact optical system design reduces cost and eliminates alignment issues

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

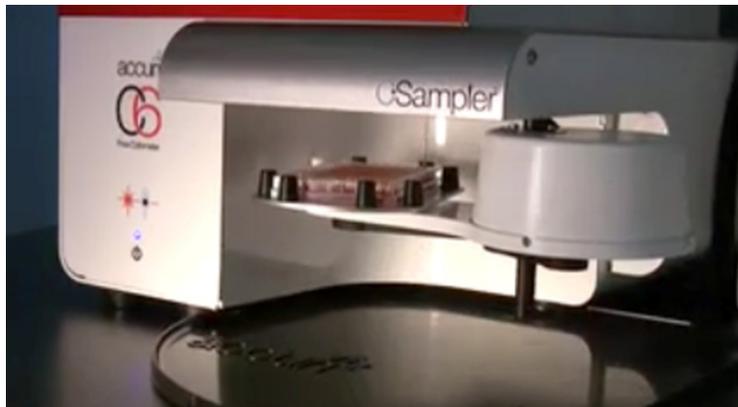


User changeable optical filters

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

# 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

**Collect**   **Analyze**   **Statistics**

**A4** HPB CD3-F CD4, CD45, CD8

1	2	3	4	5	6	7	8	9	10	11	12	
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
D	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12

C5 is connected and ready.

**Run Limits**

Run Unlimited

125000 events

in Ungated Sample

0 Min  0 Sec

0.0  $\mu$ L

**Fluidics**

Slow  Medium  Fast

Flow Rate 14  $\mu$ L/min

Core Size 10  $\mu$ m

Custom

Flow Rate 11  $\mu$ L/min

Core Size 5  $\mu$ m

**Threshold**

Set Threshold

None

80,000 on FSC-H

**ADD to A4**

Set Color Compensation

**Last Run**

0 Events   229,303 Cumulative

0:00 Time   0:00

0.0 Microliters   0.0

0.0 Events / Sec   0

0.0 Events /  $\mu$ L   0.0

Warn before deleting

Data Capacity Used

8% of 10,000,000 Events

**Plot 2: Sample HPB CD3-F CD4, CD45, CD8**

Count	Volume ( $\mu$ L)	% of This Plot	% of All	Mean CD3 FITC-A	Mean CD4 PE-A	CV CD3 FITC-A	CV
This Plot	99,183	0.0	100.0%	43.3%	23,875.3	1,762.1	62.0%
Q3-UL	147	0.0	0.1%	0.1%	960.4	1,607.0	53.3%
Q3-UR	59,194	0.0	59.7%	25.8%	32,131.1	2,933.7	28.5%
Q3-LL	21,455	0.0	21.6%	9.4%	547.0	21.5	47.8%
Q3-LR	18,387	0.0	18.5%	8.0%	24,701.0	22.5	29.2%

**Plot 3: Sample HPB CD3-F CD4, CD45, CD8**

Count	Volume ( $\mu$ L)	% of This Plot	% of All	Mean CD3 FITC-A	Mean CD45 PE-CY7-A	CV CD3 FITC-A	CV
This Plot	102,719	0.0	100.0%	44.8%	23,071.3	38,996.7	65.7%
R1	99,183	0.0	96.6%	43.3%	23,875.3	40,270.2	62.0%
Q1-UL	21,694	0.0	21.1%	9.5%	540.5	32,690.2	45.5%
Q1-UR	77,625	0.0	75.6%	33.9%	30,356.9	42,417.0	30.7%
Q1-LL	3,380	0.0	3.3%	1.5%	447.0	1,141.0	76.5%
Q1-LR	20	0.0	0.0%	0.0%	8,449.7	2,092.6	97.0%

Sample Grid

Cytometer Status

Fluidics Controls

Run Criteria

Real-Time Updates

Histograms

Dot Plots

Density Plots

Analysis and Gating Tools

Plot Statistics



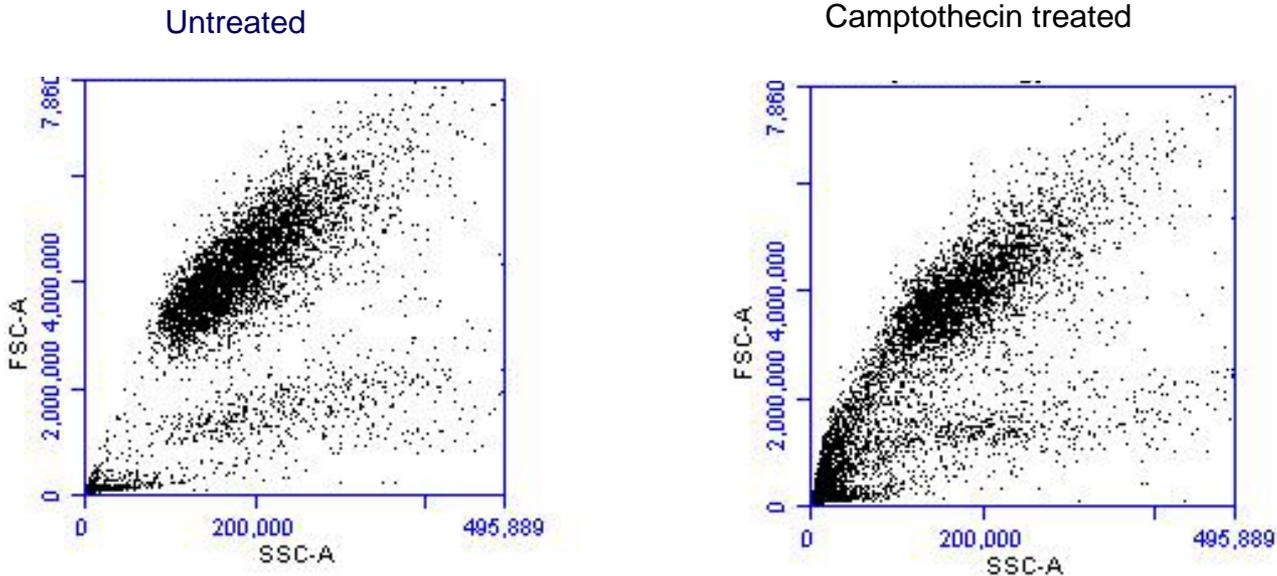
# Why Choose the BD Accuri C6 for Apoptosis Measurements?

- 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.



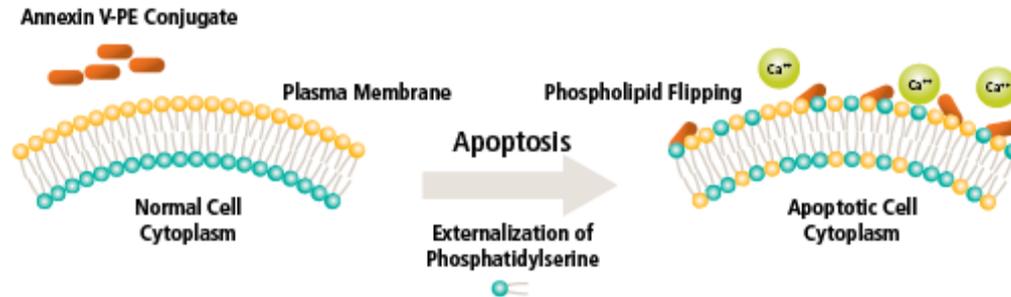
Formation of apoptotic vesicles

- **Increases side scatter**

Reduced refractive index of apoptotic cells

- **Decreases forward scatter**

# Annexin V



Annexin V is a surface marker and detects early membrane changes associated with apoptosis.

Pros: Rapid confirmation of apoptosis

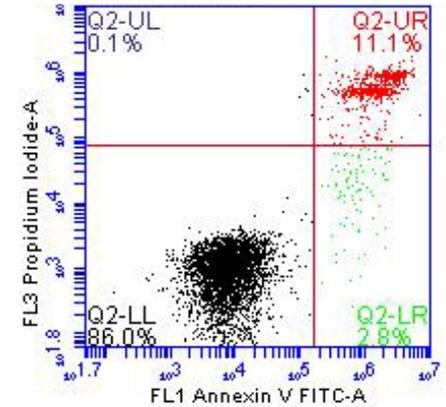
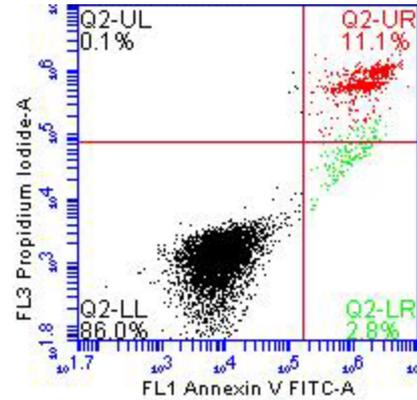
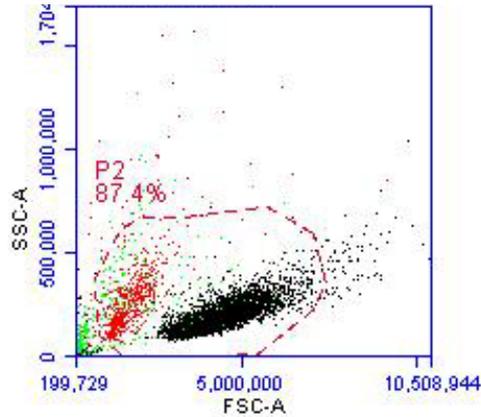
Uses live, unfixed cells

## Applications

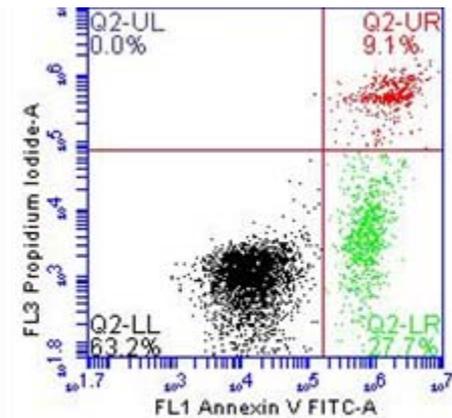
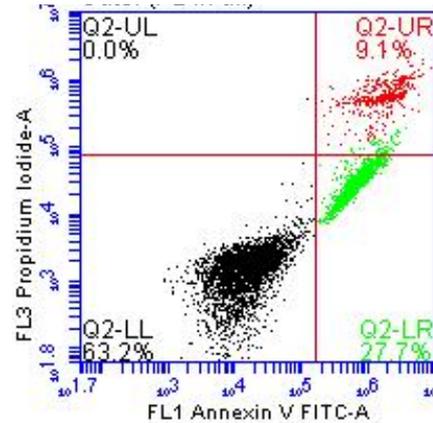
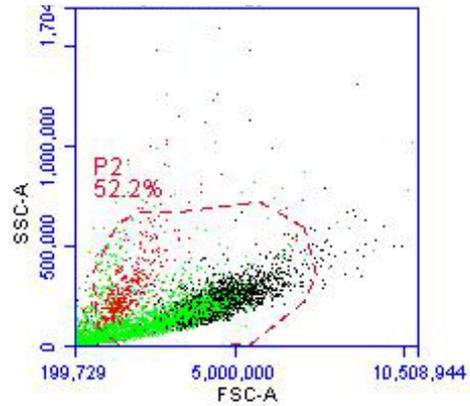
- Flow cytometry (cells in suspension)
- Fluorescence microscopy (adherent cells)

# Annexin V

DMSO



Camptothecin  
6  $\mu$ M



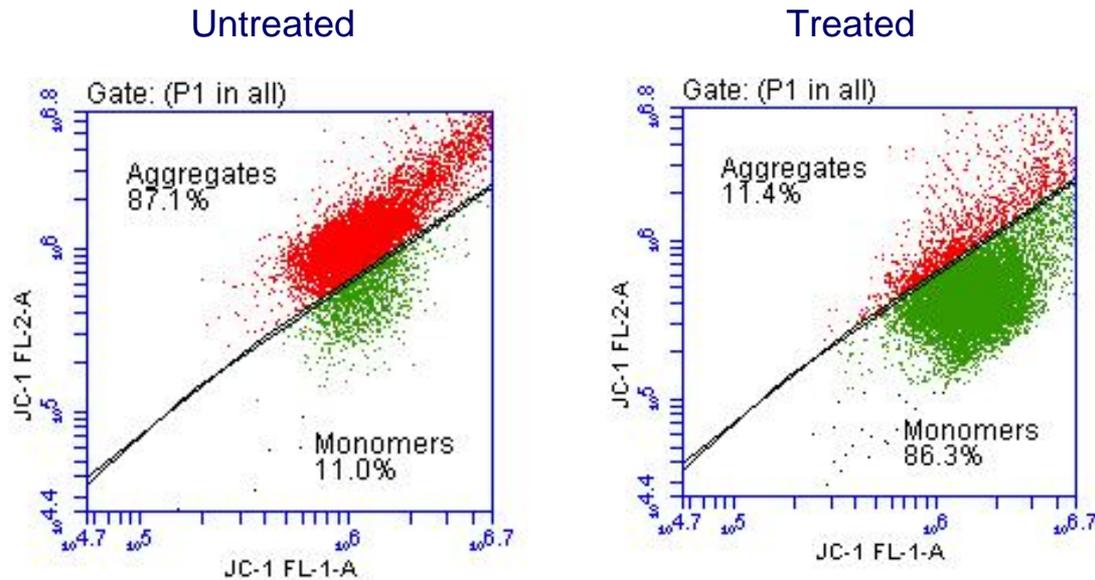
Jurkat T cells were treated with camptothecin for 4 hours and stained with Annexin V FITC + propidium iodide.



# Detection of Apoptosis with JC-1

- Changes in mitochondrial potential are another early marker for apoptosis.
- Lyophilic 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 determined by comparing the ratio of fluorescence between the FL1 and FL2 channels.

# JC-1 Measurements on the BD Accuri C6



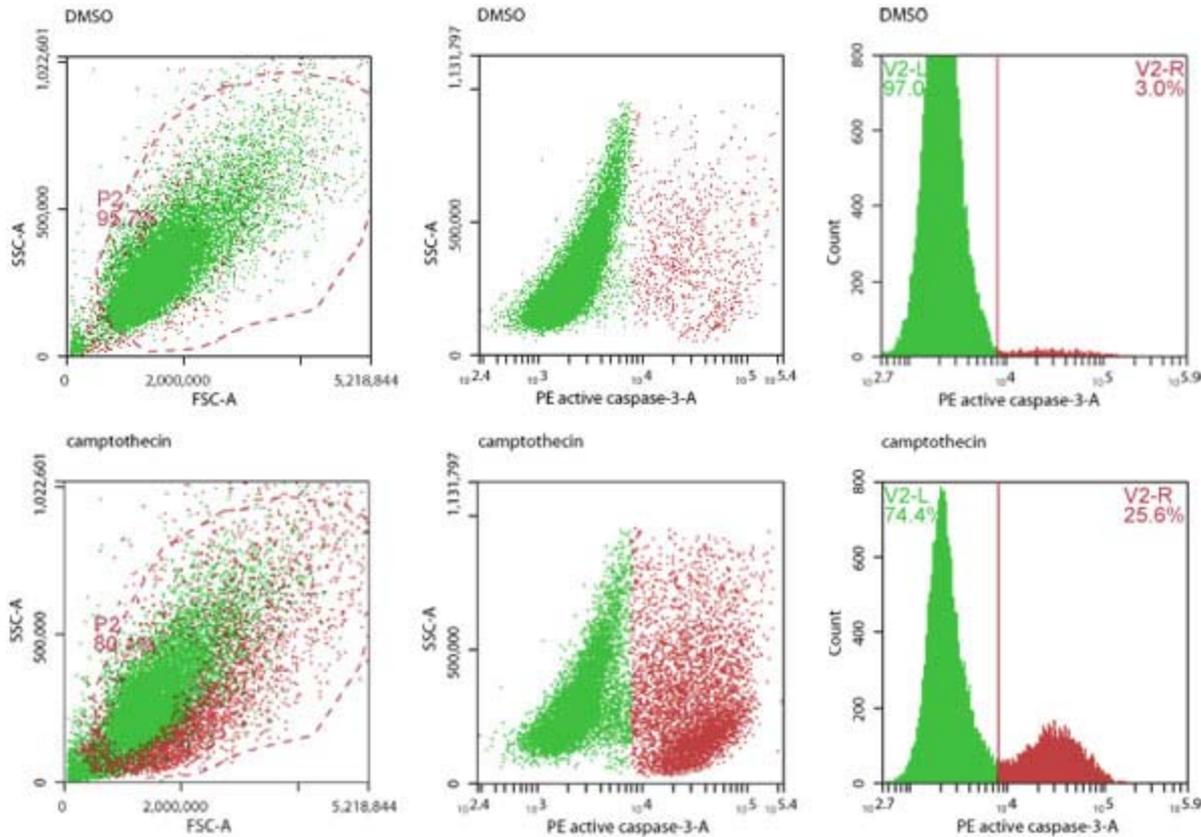
	Median FL1-A	Median FL2-A	Ratio FL1/FL2
Untreated	1,156,436	1,073,827	1
Treated	1,520,479	459,785	3

K562 cells were treated with the compound CCCP (10  $\mu$ M) for 10 minutes and stained with JC-1.

# Caspase-3

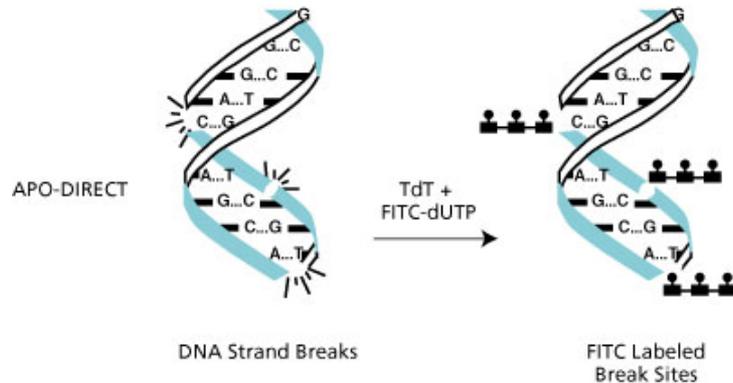
- Caspases are proteases that are activated upon cleavage at aspartate residues at the earliest stages of apoptosis.
- Several caspases are important for apoptosis, including caspases-3, 8, and 9.
- Methods to measure caspase cleavage include fluorogenic substrates and detection with antibodies specific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

# Active Caspase-3



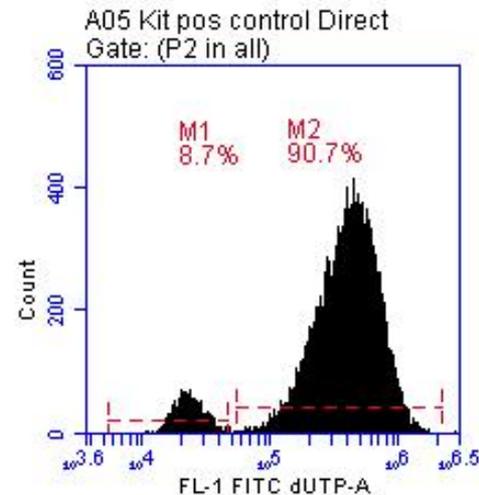
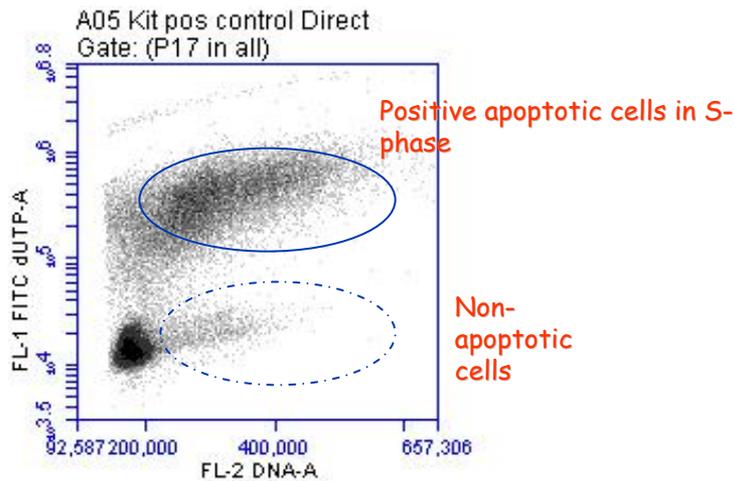
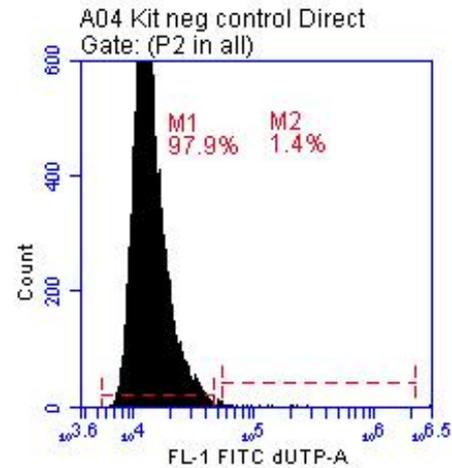
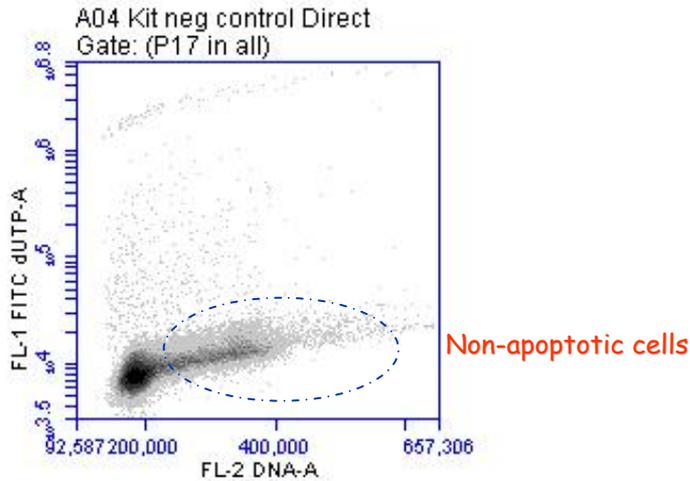
Jurkat T cells were treated with either DMSO or camptothecin (6  $\mu$ M) for 4 hours, then fixed, permeabilized and stained for active caspase-3.

# 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



# Summary of Apoptosis Detection Methods

Method	Advantages	Disadvantages
Annexin V	<ul style="list-style-type: none"><li>• Works well on live cells</li></ul>	<ul style="list-style-type: none"><li>• Difficult to multiplex</li><li>• Requires calcium</li></ul>
BD MitoScreen (JC-1)	<ul style="list-style-type: none"><li>• Can be performed on live cells</li><li>• Ratiometric data</li></ul>	<ul style="list-style-type: none"><li>• Difficult to multiplex</li></ul>
Caspase-3	<ul style="list-style-type: none"><li>• Can be multiplexed with other markers</li></ul>	<ul style="list-style-type: none"><li>• Requires fixation and permeabilization of cells</li></ul>
BD APO-Direct	<ul style="list-style-type: none"><li>• Useful for detection of late apoptosis</li></ul>	<ul style="list-style-type: none"><li>• Difficult to multiplex</li><li>• Requires fixation and permeabilization of cells</li></ul>

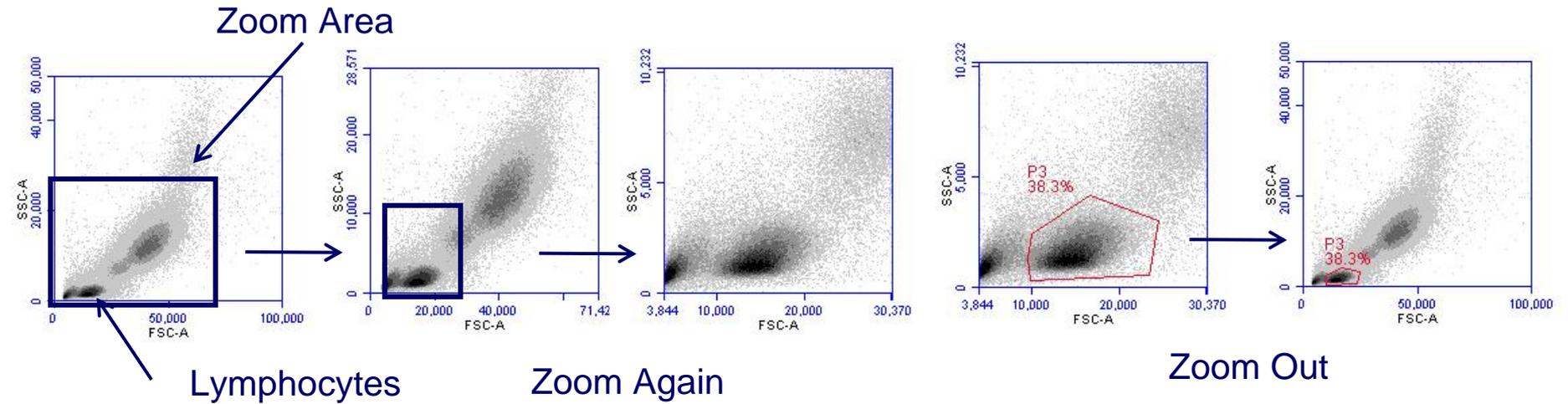
# Tips/Tricks

- 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

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

# 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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