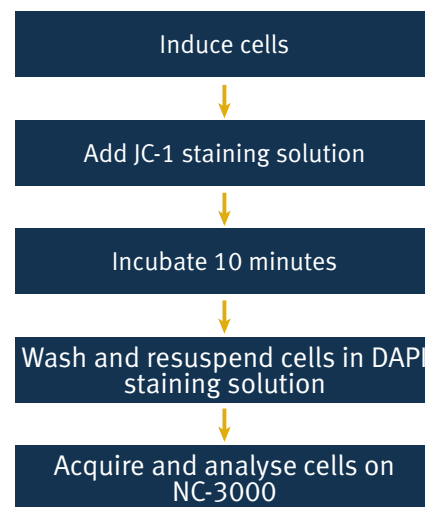


## NC-3000 Mitochondrial Potential Assay

– For easy measurement of changes in the mitochondrial membrane potential

Loss of the mitochondrial membrane potential is known to precede apoptosis and chemical-hypoxia-induced necrosis. The lipophilic cationic dye, **JC-1**, displays potential-dependent accumulation in the mitochondria and provides a simple, fluorescent-based method for distinguishing between healthy and apoptotic cells. In healthy cells, the negative charge established by the intact mitochondrial membrane potential facilitates the accumulation of JC-1 in the mitochondrial matrix. At high concentrations JC-1 forms aggregates and becomes red fluorescent. In apoptotic cells the mitochondrial potential collapses and JC-1 localizes to the cytosol in its monomeric green fluorescent form.

### NC-3000 Mitochondrial Potential Assay

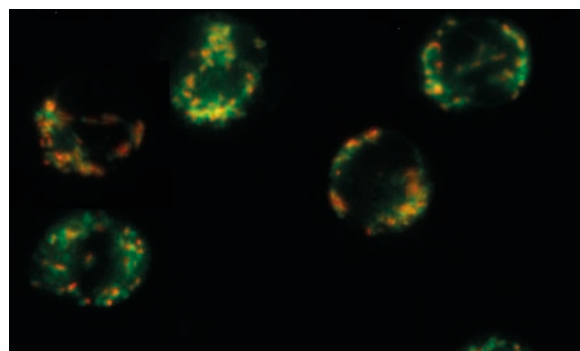


### Key benefits

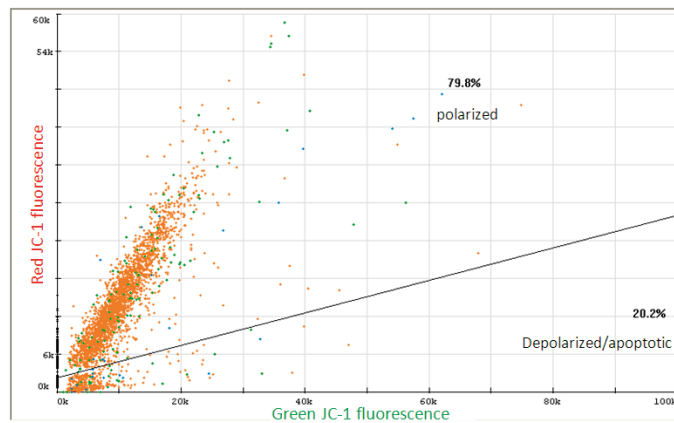
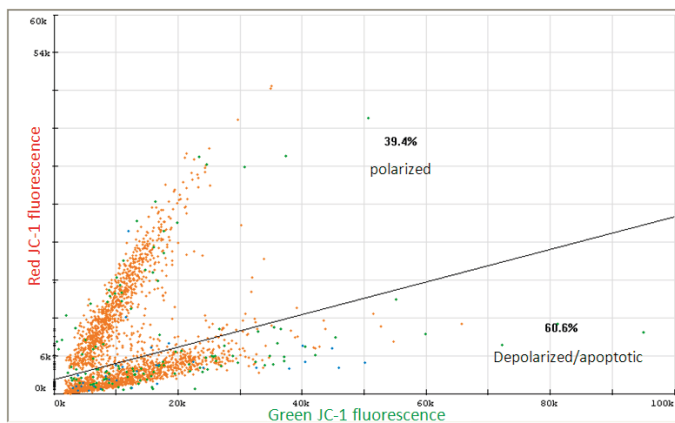
- Easy discrimination between polarized (healthy) cells, depolarized (apoptotic) cells and necrotic/late apoptotic cells
- Fast automated single cell analysis
- Acquisition and analysis in one simple step
- Analysis time less than 20 seconds
- User friendly protocol with predefined settings
- Standardized results – even with different users
- Provides direct cell counts and viability
- No calibration required

## Principle

Using fluorescence microscopy and image analysis, the NC-3000 system automates detection of cells with collapsed mitochondrial membrane potential. Cells are stained with **JC-1** and **DAPI**. Cellular JC-1 monomers and aggregates are detected as green and red fluorescence, respectively. Mitochondrial depolarization is revealed as a decrease in the red/green fluorescence intensity ratio. Necrotic and late apoptotic cells are detected as blue fluorescent (DAPI) cells.



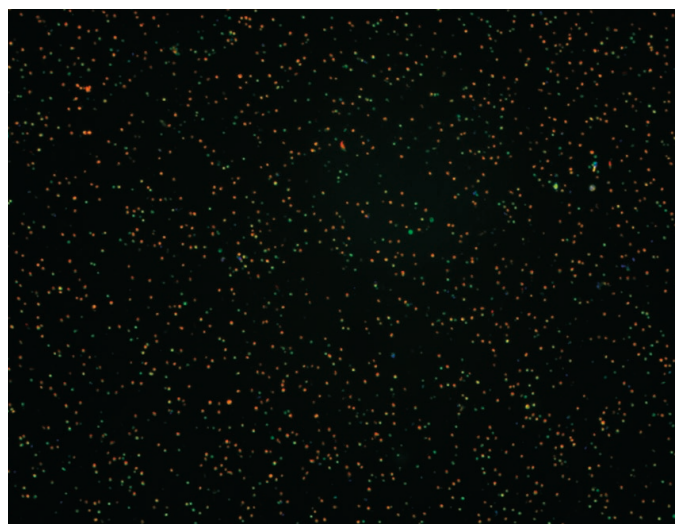
**A**



**B**

Result - Mitochondrial membrane potential			
#	Description	Cell concentration	Image
1	Total cells	4.04E6 cells/mL	Jurkat untreated
	Necrotic/late apoptotic cells	1.48E5 cells/mL	
	Viability	96.3%	
2	Total cells	3.26E6 cells/mL	Jurkat CPT treated
	Necrotic/late apoptotic cells	1.29E5 cells/mL	
	Viability	96.1%	

**C**



Jurkat cells stained with JC-1 and DAPI. Cells were grown in the absence (upper left) or in the presence of 10  $\mu$ M camptothecin (CPT) for 5 hours at 37°C (upper right). Cells were stained for 10 minutes at 37°C, washed with PBS and analysed on the NC-3000 using the *Mitochondrial Potential protocol*. In this example untreated Jurkat cells are 20% depolarized/apoptotic whereas CPT-treated Jurkat cells are 60% depolarized/apoptotic (**A**). The same analysis revealed that the viability of the two samples is similar, being approximately 96% (**B**). (**C**) shows the image acquired from the CPT-treated sample.