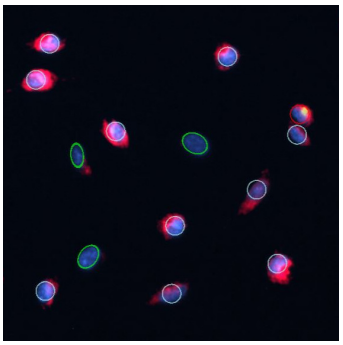
Virtual Cytoplasm (2F)

GENERAL PURPOSE



Virtual Cytoplasm (2F) is a three channel fluorescence application which uses a nuclei staining (e.g. Hoechst or Dapi) to locate an individual cell and two functional fluorescence staining to distinguish between four different populations. These two functional fluorescence markers can be chosen according to your biological demands.

E.g. Apoptosis Test
1) Staining with Hoechst to count the whole cell population
2) JC-1 green to detect dead cells
3) Red J-aggregates to detect viable cells

The major **advantage** of this application is the ability to detect co-localized fluorescence markers regardless of the biological background.

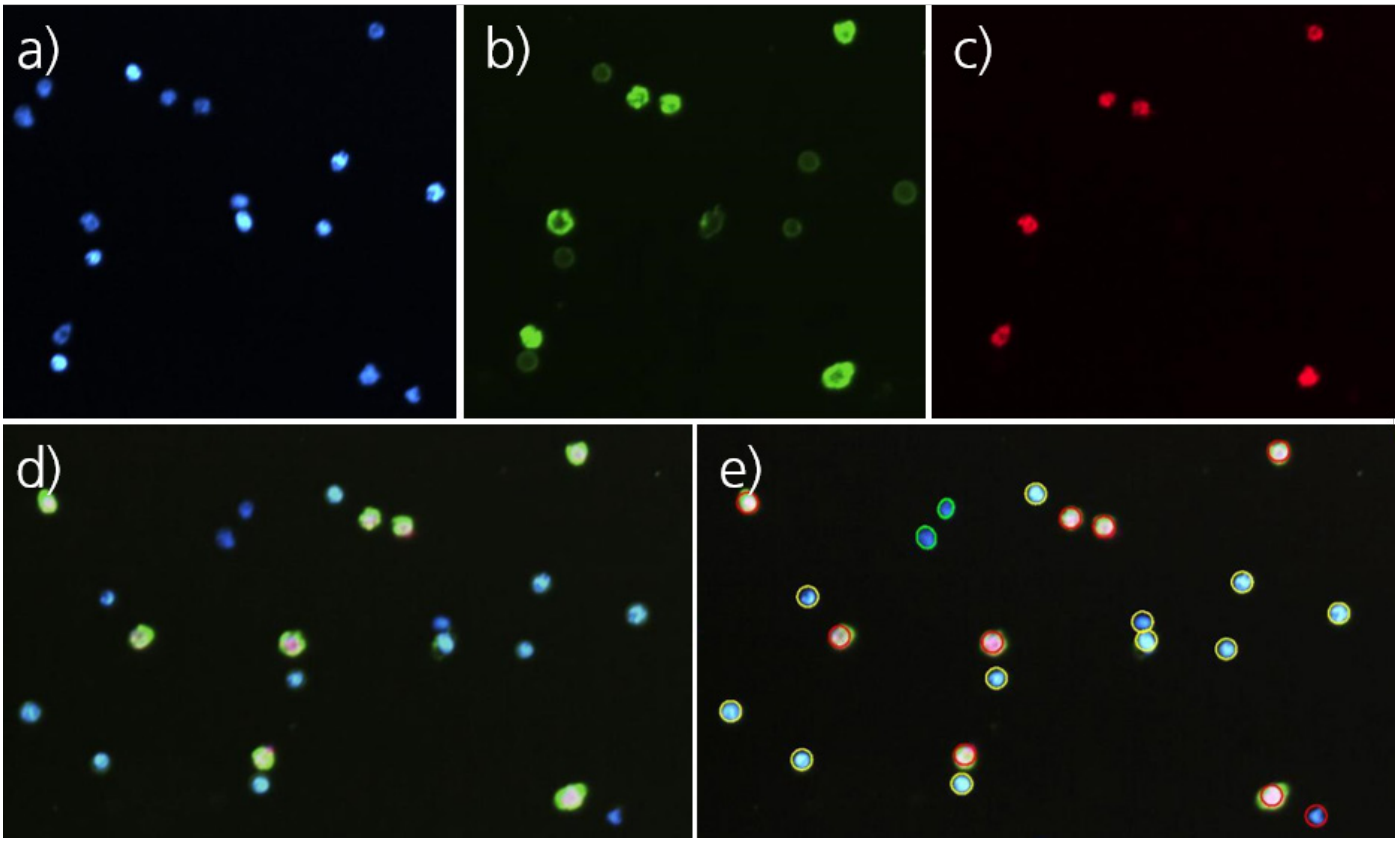
RESULT TABLE

Nuclei Count	Number of Cells
F1 Marker positive	Cells containing fluorescence marker of channel 1
F2 Marker positive	Cells containing fluorescence marker of channel 2
F1 Marker positive percent	Percentage of cells marked with F1
F2 Marker positive percent	Percentage of cells marked with F2
TC-nn	Total cells unstained
TC-pn	Total cells stained with F1 but not stained with F2
TC-np	Total cells with no F1 staining but stained with F2
TC-pp	Total cells containing both stainings
TC-nn percent	Complementary to TC-nn in percentage
TC-pn percent	Complementary to TC-pn in percentage
TC-np percent	Complementary to TC-np in percentage
TC-pp percent	Complementary to TC-pp in percentage
Avg Size of Nuclei	Average size of nuclei
Avg Nuclei Intensity	Average of nuclei brightness
Avg Fluo 1 Intensity	Average fluorescence intensity of all detected cell areas in fluorescence channel 1
Avg Fluo 2 Intensity	Average fluorescence intensity of all detected cell areas in fluorescence channel 2

EXAMPLE

This example shows the Virtual Cytoplasm 2F operator used for an apoptosis test with AnnexinV, which is able to bind to the cell membrane when the phospholipid phosphatidylserine is translocated to the outside when the intrinsic apoptosis pathway starts.

Molt-4 cells were treated with 0.15 µM staurosporine for 2 h to induce apoptosis and stained with Hoechst 33342, AnnexinV-FITC and propidium iodide.



a) Hoechst staining; total cell count. b) Apoptotic and dead cells, stained with AnnexinV-FITC. c) Dead cells stained with PI. d) Overlay image with Hoechst, AnnexinV and PI stainings. e) Overlay image with image processing results, green for viable cells, orange for apoptotic and red for dead cells.

Marked green:	TC-nn –> nuclei staining only
Marked orange:	TC-pn –> nuclei staining AND fluorescence 1
Marked light blue:	TC-np –> nuclei staining AND fluorescence 2
Marked red:	TC-pp –> nuclei staining AND fluorescence 1 AND fluorescence 2

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