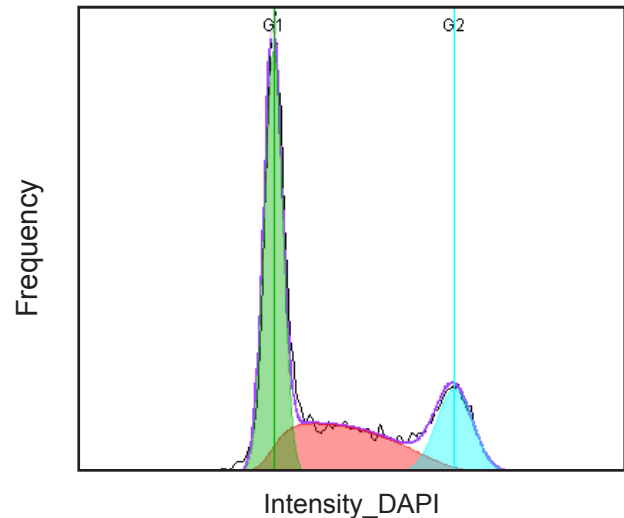


Measuring Phases of the Cell Cycle on the FlowSight

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Abstract

The cell cycle is a series of orchestrated events that leads to cell replication. It is characterized by four distinct stages: three interphase stages (Gap 1, DNA Synthesis or S Phase and Gap 2) and a dividing stage called mitosis. Mitosis is further sub-classified into four primary phases: prophase, metaphase, anaphase and telophase. Progression of cells through the various stages of the cell cycle is fundamental to normal cell function and is regulated through feedback loops that prevent the overpopulation of a particular cell type. It is also a key component of cancer where cancerous cells are able to defeat normal feedback loops and over-replicate. DNA content can be quantified by measuring the per-cell intensity of fluorescent DNA intercalating dyes using flow cytometry. In addition, the change in DNA image texture associated with mitotic events can be measured with image cytometry and used to classify cells in prophase, metaphase, anaphase, and telophase of mitosis.



DNA content histogram of THP-1 cells fluorescently labeled with DAPI and imaged on the FlowSight. The histogram shows G0/G1 (green), S (red) and G2/M (blue) phases of the cell cycle. Using FlowSight imagery and FlowJo FCS analysis software, it's possible to use a cell cycle modeling algorithm to more accurately measure each phase of the cell cycle.

Figure 1.

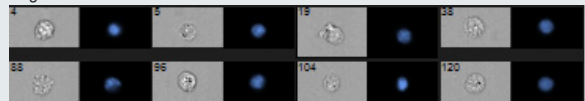
DNA Content Histogram

Monocyte line THP-1 cells were fixed in 2% PFA, permeabilized with 0.1% Tx100 and fluorescently labeled with DAPI. DAPI is a DNA intercalating dye that stoichiometrically binds to T-A rich regions of DNA and allows for quantitative measurements of DNA content. The histogram shows a large G0/G1 peak of cells with 2N DNA content, S phase cells that are synthesizing DNA, and a smaller G2/M peak of cells that are actively dividing.

Images from the Cell Cycle

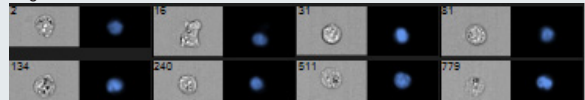
G0/ G1

Brightfield DAPI



S Phase

Brightfield DAPI



G2/M

Brightfield DAPI



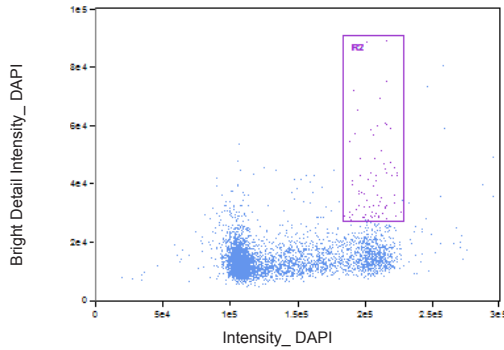
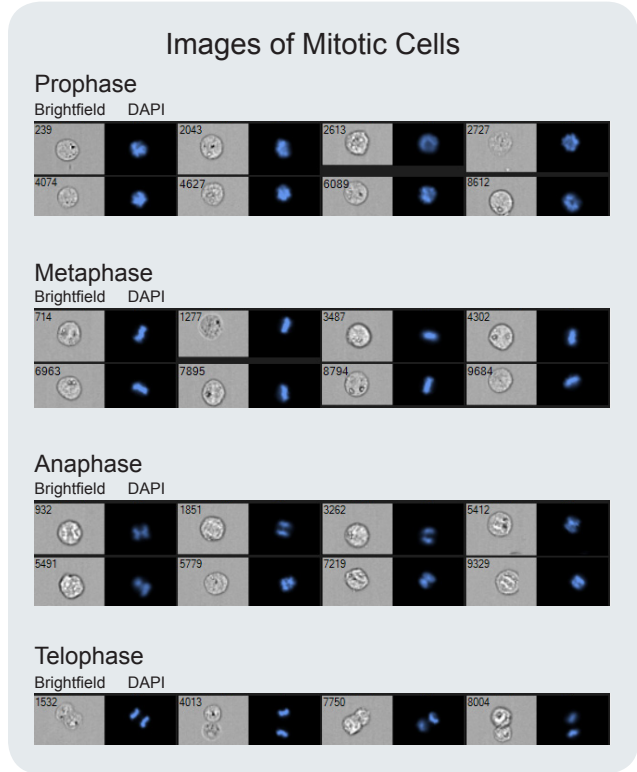


Figure 2.
Measuring Mitotic Index

Mitosis is the process by which cells actively divide, and is regulated by cyclin-dependent kinases. Each phase of mitosis exhibits morphologically distinct DNA staining. In prophase the nuclear envelope is dissolved and DNA condenses into chromosomes. Metaphase is characterized by chromosomal alignment to the metaphase plate, followed by anaphase where sister chromosomes are actively pulled apart to separate sides of the cells. During telophase the cell membrane begins pinching together to form two fully mature daughter cells. The dot plot shows the DNA content as measured by DAPI intensity vs. a morphology based feature that identifies cells with condensed DNA in mitosis. Region R2 identifies cells in mitosis at 2.7% and visual inspection of this region allows for the sub classification of each phase of mitosis.



Conclusions

The FlowSight imaging flow cytometer combines high fluorescence sensitivity with imagery that allows both the quantitative assessment of cell cycle as well as visual verification of mitotic cells.

FlowSight Specifications

Physical Characteristics

17.7 W x 18.3 H x 14.7 D inches (450mm x 465mm x 635mm)

135 lbs. (61kg)

Instrument Capabilities

Images per cell	Up to 12
Event rate	2,000 cells per second
Image modes	Brightfield, SSC, Fluorescence
Automation	Startup, Calibration, Shutdown

Excitation Sources

LASER (NM)	EXAMPLE DYES
405	DAPI, Pacific Blue, eFluor 450, +
488	FITC, PE, PE-TxRed, ECD, PerCP +
561	PE, AlexaFluor 546, Cy3, +
642	AlexaFluor 647, APC, APC-Cy7 +

