The iCys® Research Imaging Cytometer Specifications

The iGeneration LSC technology is a novel platform combining many of the advantages of laser scanning microscopy and flow cytometry, and significantly extending the boundaries of quantitative single-cell analysis. Unlike traditional microscopy's indiscriminate imaging of an entire sample, LSC automatically selects, images and analyzes portions of the sample that meet user-defined criteria (such as fluorescence or laser light loss signal, co-localized signals, size, etc).

Unlike conventional flow cytometry, the technology analyzes samples attached to a horizontal surface, such as cultured cells on slides or in a variety of carriers, tissue sections, and tissue microarray specimens. iGeneration instruments use an automated nanostep X-Y stage to pass the samples by the objective lens, applying up to three different lasers to excite fluorescent emissions or detect laser light absorption and scatter. LSC technology is uniquely able to simultaneously quantitate both fluorescent excitation using an array of four photomultiplier tube (PMT) detectors, and laser light loss and scatter, employing two photodiode detectors. Signal quantification via various segmentation routines is accomplished on cellular or sub-cellular level.

Another distinctive feature of iGeneration technology allows the user to select the level of image resolution, from ultra-high to moderate enabling high degree of flexibility in speeding up assay throughput where image resolution is not of primary concern. Detailed microscopic images are stored and referenced directly to cytometric data.

A highly appealing feature enables the instruments to gather quantitative data from fluorescently and chromatically labeled tissue sections, tissue microarrays, fine needle aspirates, and core biopsies on a microscope slide. The resulting data is stored and displayed in a manner comparable to that traditionally obtained with flow cytometry, complemented by pathologist-like quantitative assessment of the images.

In summary, iGeneration LSC instruments offer a powerful hybrid technology combining imaging and cytometric analysis in a single platform. The result is an ideal, flexible solution for central Imaging Cytometry Core facilities servicing investigators pursuing various diverse basic and translational research projects. The platform provides many researchers with a powerful new way to analyze cells and tissues, enabling in-depth understanding of a broad spectrum of areas of biomedical science, including cancer biology, stem cell research, immunology, autoimmune diseases, and infectious diseases.

- Excitation and emission measurement characteristics
  The system is equipped with 405nm, 488nm, and 633nm monochromatic excitation lasers and blue (438nm-470nm), green (515nm-545nm), orange (565nm-595nm) and long red (650nm-800nm) fluorescent channels; 4th laser option will be available in September 2009.

- Simultaneous fluorescent and light-loss measurement
  iCys measures 2 channels of light-loss simultaneously with up to 4 channels of fluorescence. *This capability allows the mixing of fluorescent and chromatic dyes in the same sample**.

- True quantitative imaging
  Non-confocal by design, the iCys provides true quantitative imaging by virtue of its high depth-of-focus. *This is demonstrated by its ability to provide direct cell cycle measurement comparable to that of a flow cytometer.***

Notes:
**Unique features are shown in italic font.*
*** Not available on camera-based and confocal systems*
**** High resolution scanning is not available on commercially available non-confocal laser scanning systems.*

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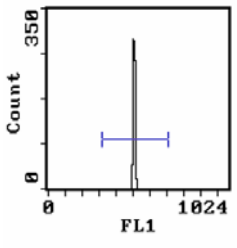
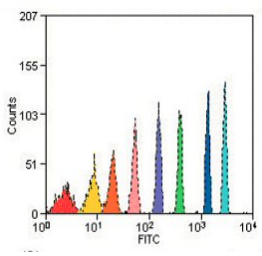
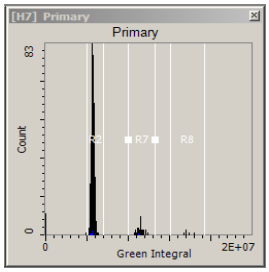
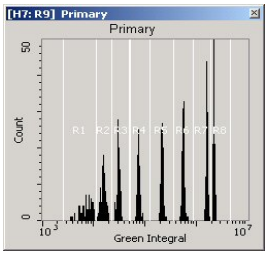
Flow





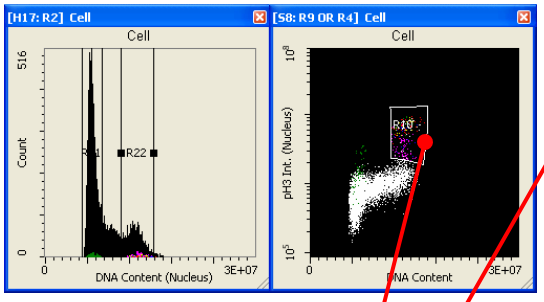
LSC





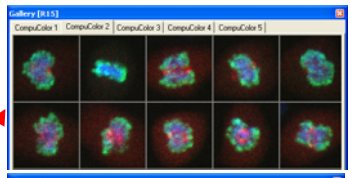
- LSC fluorescence precision - flow check beads –
  - CVs ~ 1.5% - 2.5%
- Sensitivity, Resolution
  - iCys resolves 8-peak “Rainbow” beads
- Dynamic Range
  - iCys – 3 decades of integrated fluorescence

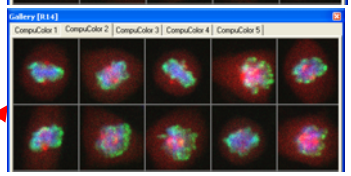
- *Quantitative data is related to image data*
  Each cellular event’s quantitative data (integrated fluorescence signal, maximum pixel level, etc.) can be related to the cell’s image.



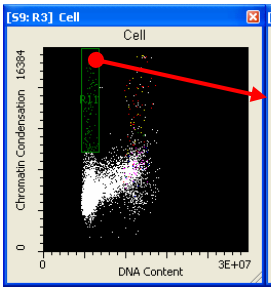
Cell cycle distribution based on DNA content

DNA content vs. phospho-Histone H3 -

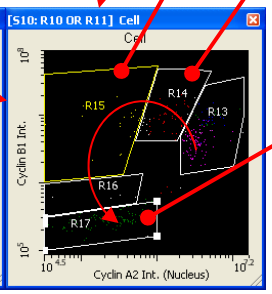




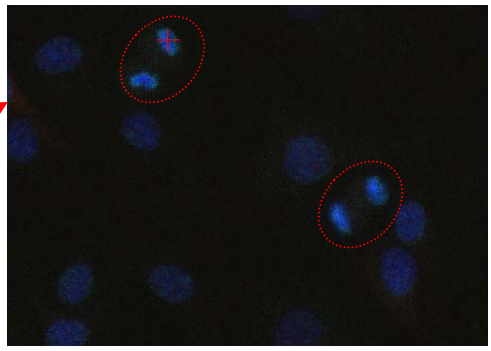
Cell galleries showing cells progressing through Mitosis



DNA content vs. Chromatin Condensation



Cyclin B1 - vs. Cyclin A2 expression



Cells in cytokinesis relocated in Field Scan image

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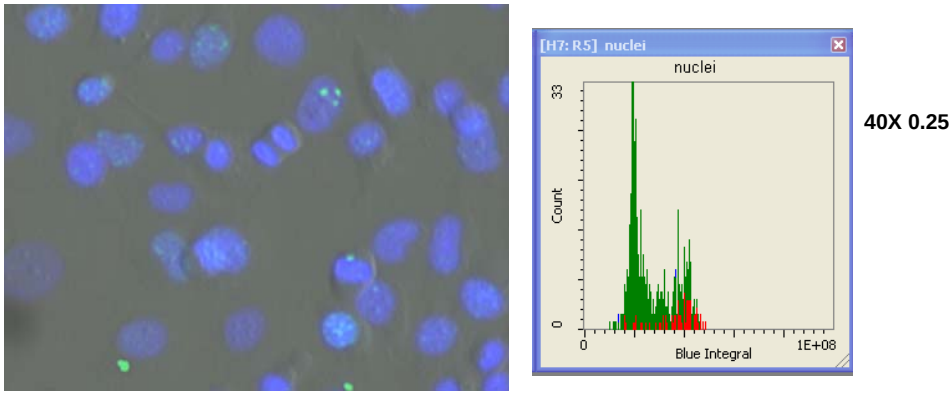
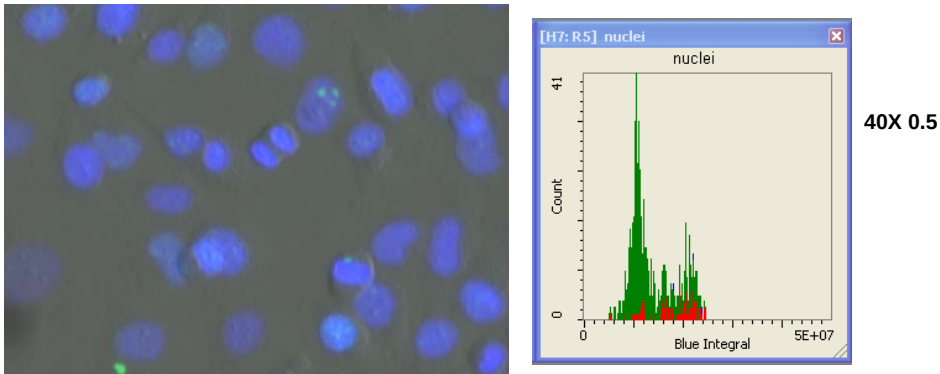
The iCys® Research Imaging Cytometer Specifications

- *Variable resolution scanning*
  Scanning resolution can be varied from high speed - low resolution (20 microns per pixel) for overview scans of specimen to *moderate resolution scans**** (5 to 0 .5 microns per pixel) to *very high resolution**** (0.1 microns per pixel) to suit the needs of each investigation. This scan resolution control is independent of the objective lens magnification thus allowing high sensitivity measurements to be made at lower scan resolution and high throughput.

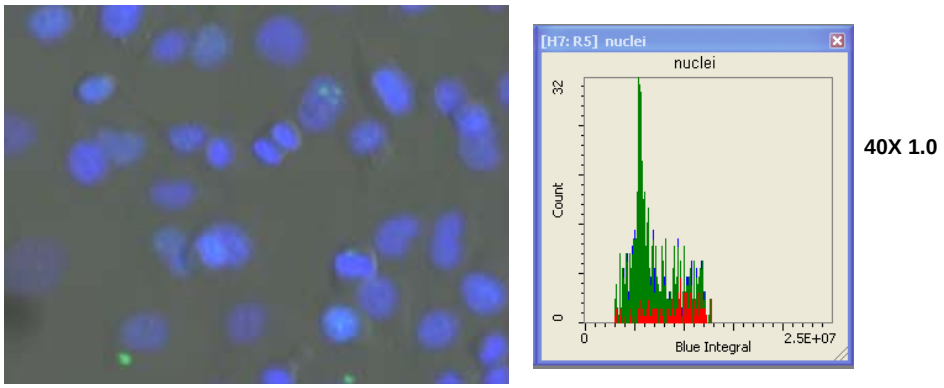
DAPI staining showing DNA content at three different resolutions



40X 0.25



40X 0.5



40X 1.0

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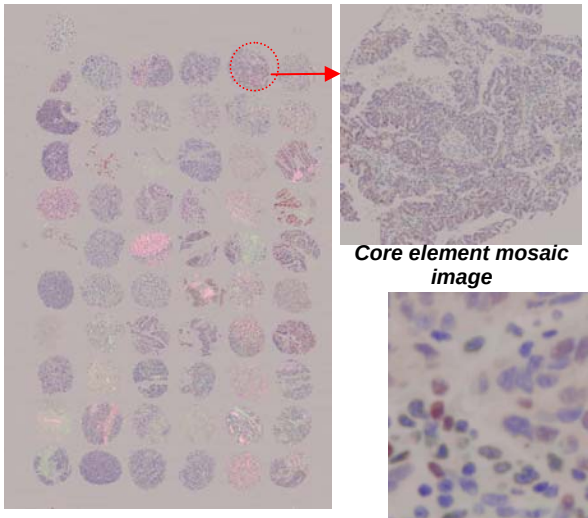
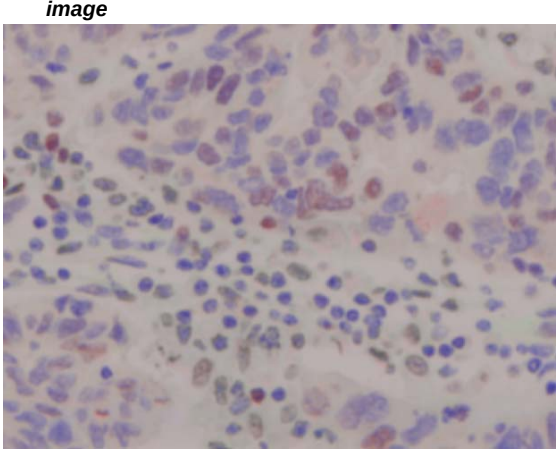
- *Automated "two-scale" scanning*

  Low resolution/ high speed scans are utilized for automated identification of regions of interest within a tissue section or TMA core elements and are automatically followed by high resolution scans of these selected regions. Two-scale scanning can also be applied to cellular specimens.



TMA overview scan

Core element mosaic image



Scan field image

Proliferation marker Ki67 (green, DAB), CD68 macrophages (Permanent Red), countertain Hematoxylin; MaxArray Human Multi-tumor Tissue MicroArray, Invitrogen

- Broad choice of sample types and sample carriers

Sample types	Carriers Types
Adherent cells	Microtiter plates
Live cells	Microscope slides
Fixed cells	Chamber slides
Tissue Sections	Petri dishes
Tissue microarrays	Ability to define "custom
Fine needle aspirates	carriers"

- *Multiple time-point assays*
  iCys can analyze the same cells under different physiological conditions (*i.e.* live and then fixed) and merge the data into a single analysis "run."

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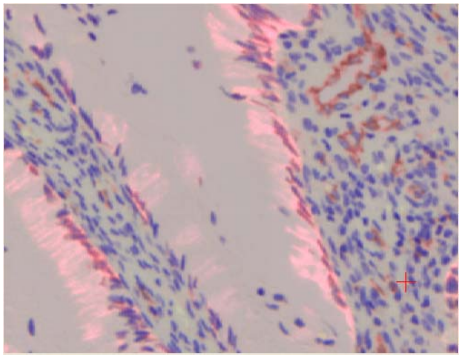
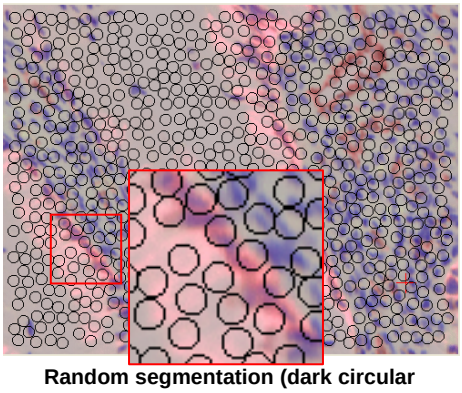
- *Stereological sampling*
  User defined sampling resolution may be effectively utilized to characterize samples where event (cell) - based segmentation is impractical.

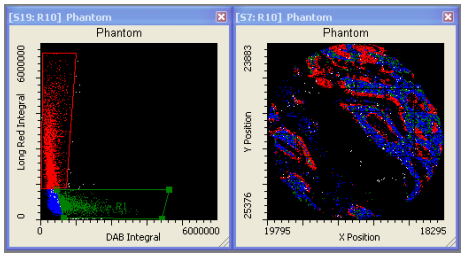
Human Cervical Carcinoma TMA Element



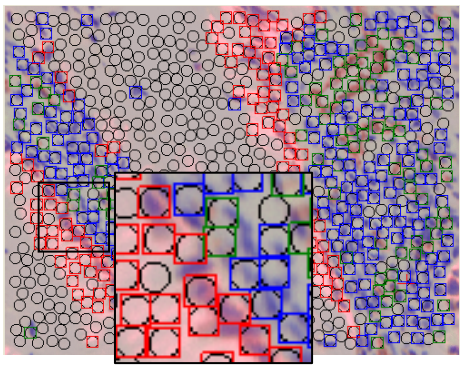
Field Scan image: CD34 endothelium (Permanent Red), Cytokeratins (DAB) and Hematoxylin counterstain



Random segmentation (dark circular regions over image) allow quantification of CD34 expression



High CD34 expression is shown in red in scattergrams and as red rectangles at right.



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