

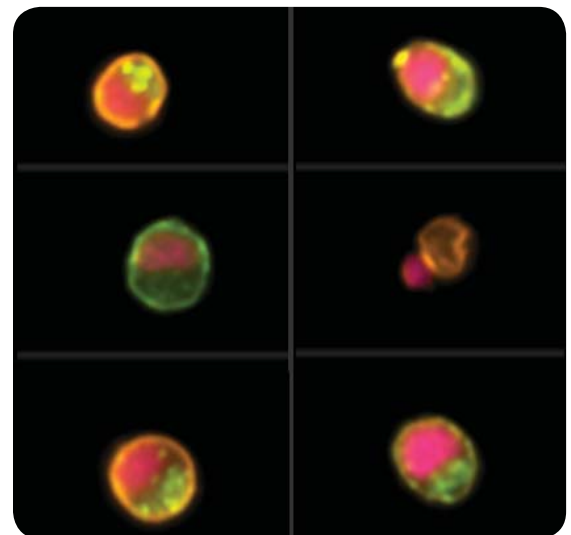
Morphologic and location-based classification of differentiating erythroid lineage cells using the ImageStream

IMAGESTREAM CAPABILITIES FEATURED: internalization / Cell Signaling & Molecular Translocation / Cell-Cell interaction / Cell Cycle & Mitosis / Co-localization / **Morphology** / spot counting / DNA damage & repair / Cell Death & Autophagy / Immunology / Oncology / Biochemistry / Virology / Microbiology / Parasitology / **Hematology** / **Stem Cell Biology** / Oceanography / Toxicology / Drug Discovery /

ABSTRACT

As hematopoietic stem cells differentiate through the erythroid lineage, they undergo a drastic change in size and eventually enucleate while progressing towards becoming RBC. These cells can also be immunophenotypically characterized by staining with anti-CD71 (transferrin receptor) and anti-glycophorinA (glyA) antibodies: especially during the mid-phase of the differentiation program these cells express both markers. Towards the end of the differentiation program erythroblasts continue to gain expression of glyA, but gradually lose expression of CD71. To date, it has been difficult to adequately correlate the morphologic changes with immunophenotyping, especially since CD71+GlyA+ erythroblasts have highly variable morphologies. In this experiment, human CD34+ positive early hematopoietic cells were cultured to promote differentiation into erythroid lineage cells (as described in Kang *et al.*, JBC 283:6997) and during the differentiation program cells were sampled at 10, 13, or 16 days followed by staining with a nuclear dye and for expression of CD71 and glycophorin A, then analyzed immunophenotypically and morphologically on the ImageStream. An initial reduction in both cellular and nuclear area from day 10 to day 13, followed by a loss of CD71 expression from

day 13 to day 16 was observed. Many CD71-/glyA dim cells were either bare nuclei or were in the process of enucleating. This experiment demonstrates the unique ability of the ImageStream to objectively classify thousands of immunophenotypically defined cells using a combination of intensity, morphology, and location-based parameters.

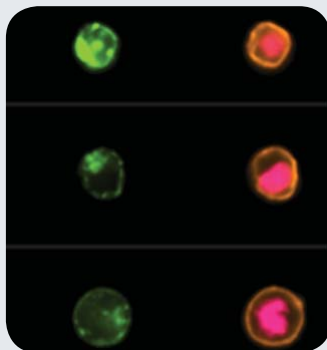


Composite imagery of FITC-CD71, PE-Glycophorin A, and DRAQ5 nuclear dye.

Study Highlights

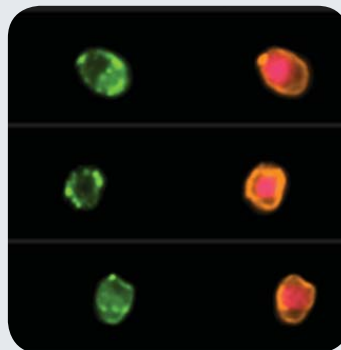
Day 10

High content analysis of non-adherent cells enabled by flow-based imaging.



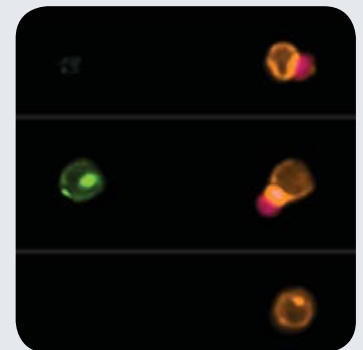
Day 13

Image analysis includes morphology and fluorescence-based measurements.



Day 16

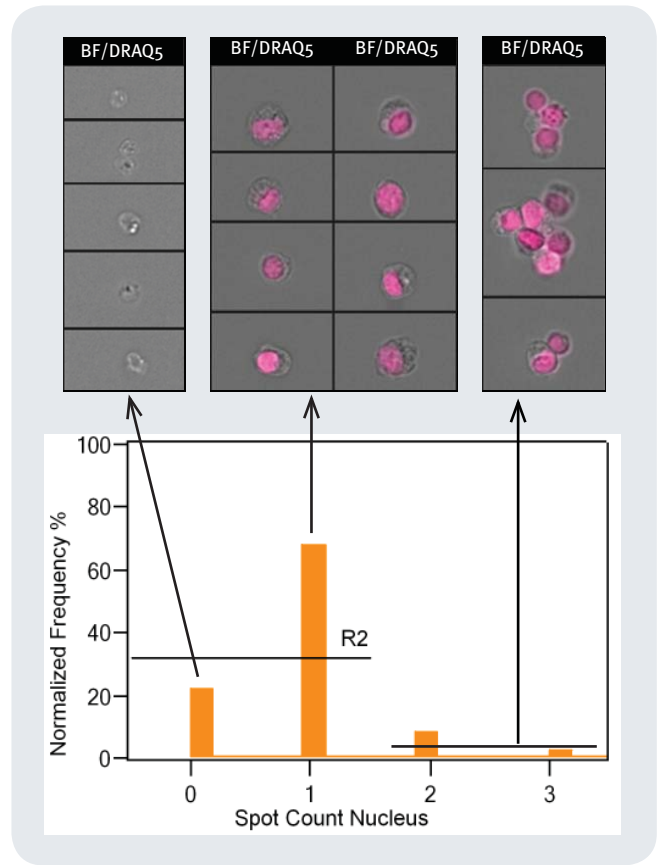
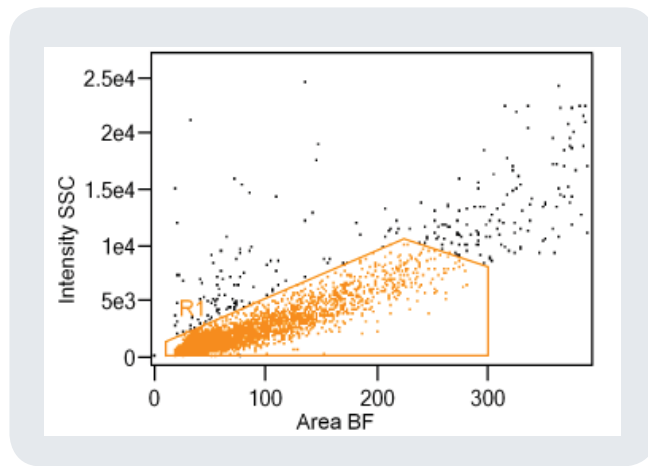
Locate rare sub-populations based on localization of different fluorophores.



IDENTIFICATION OF SINGLE NUCLEATED CELLS

Dr. Amittha Wickrema, University of Chicago

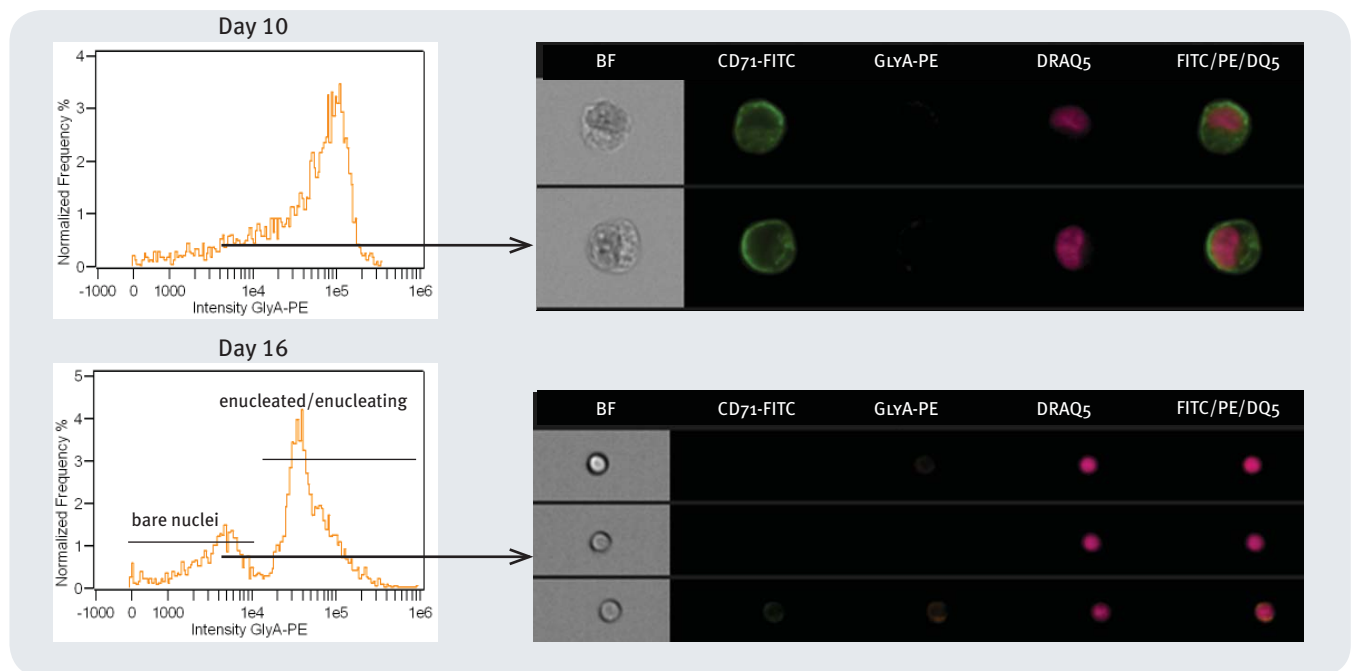
Live cell events with normal size (brightfield area) and scatter intensity are gated (R1) in the first plot shown on the left. Events with zero or one nuclear spot are gated (R2) in the plot on the right to include anucleated reticulocytes and single nucleated events.



EARLY AND LATE POPULATIONS OF INTEREST

While some CD71+GlyA- erythroblasts are still present at day 10 (see images), most cells have progressed to the double positive erythroblast phase. By day 16, many cells have lost expression of CD71 as they progress to

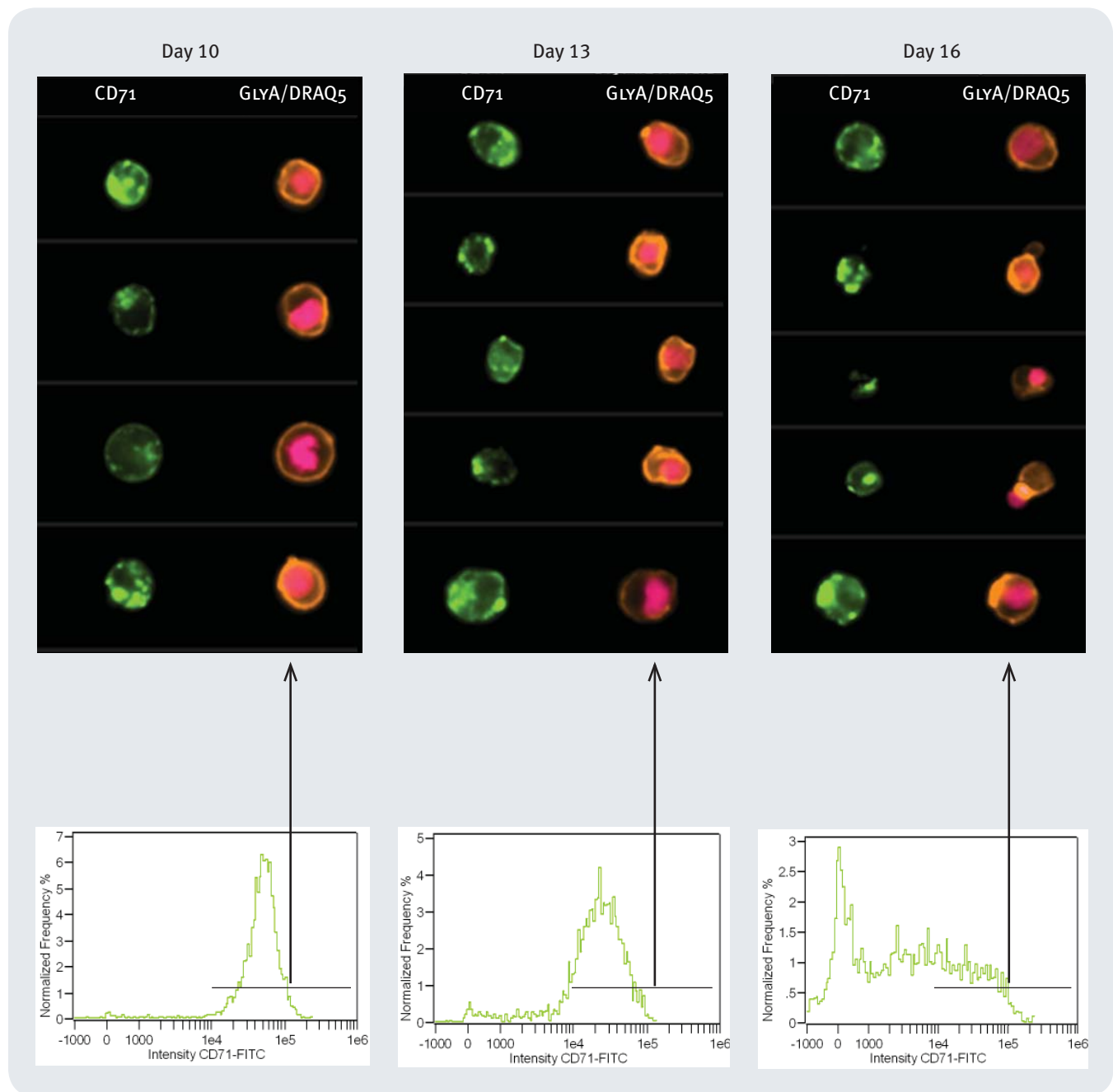
the reticulocyte phase and then to mature RBC. A large population of double negative bare nuclei are seen as well.



CD71/GLYPHORIN A STAINING PROFILES

As hematopoietic stem cells progress to mature RBC, they gain expression of glyphorin A and lose CD71 expression by the end of the differentiation program. Immunophenotypically defined erythroblasts (CD71+GlyA+; see histograms and images below) are a heterogeneous group and include basophilic, polychromatophilic, and orthochromatic subsets,

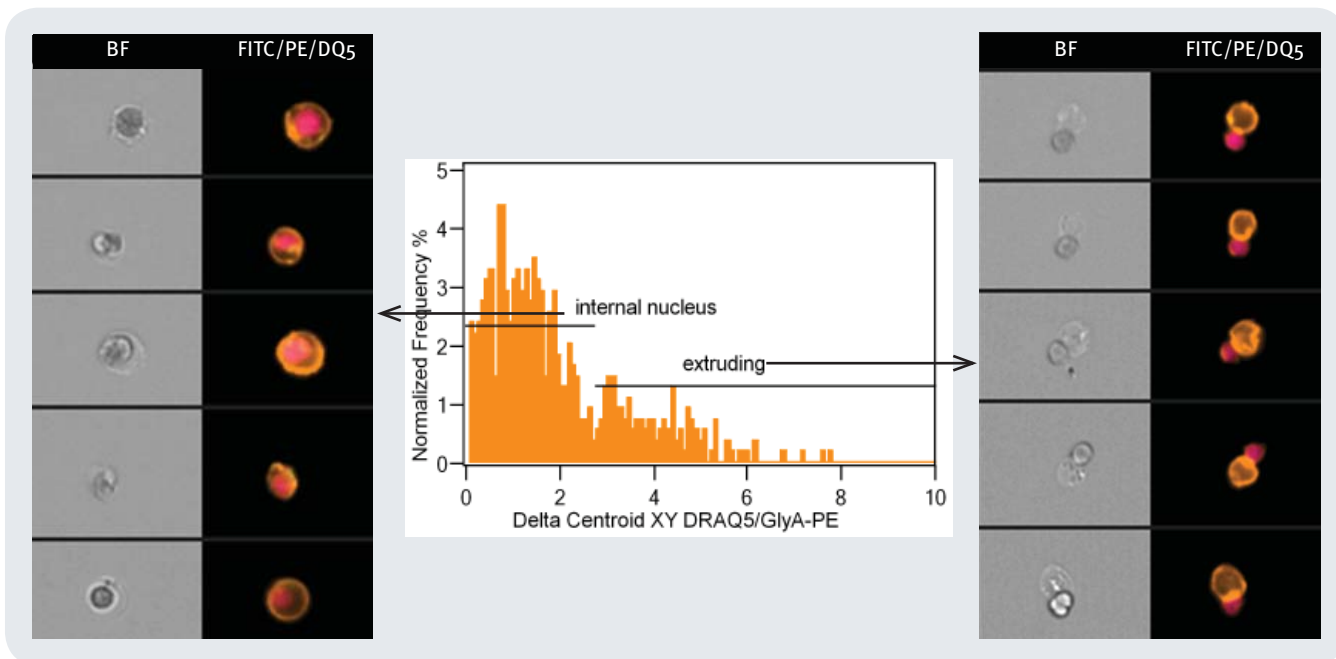
historically identified using histochemical stains (Wickrema *et al.*, Blood 80:1940, McGrath *et al.*, J Immunol Meth 336:91). A variety of morphologically distinct cell types can be distinguished below: as the culture progresses, the cells and nuclei become smaller, and by day 16, some cells have begun to extrude or have already extruded their nuclei.



QUANTITATION OF NUCLEAR SIZE AND ENUCLEATING CELLS

To quantify the percentage of enucleating cells, we used the Delta Centroid XY feature, which measures the distance between the centers of the DRAQ5 and GlyA

images. If the nucleus is in the middle of the cell, the value will be small. If the nucleus is being extruded, the value will be larger than the radius of the cell.



ImageStream^x Specifications



EXCITATION SOURCES

LASER (NM)	EXAMPLE DYES
405	DAPI, Pacific Blue™
488	FITC, PE, ECD, PE-Cy5
560	Alexa Fluor® 546, Cy3
592	Texas Red®, Alexa Fluor® 594
658	Cy5, Alexa Fluor® 647, APC, APC-Cy-7

IMAGING PERFORMANCE

	20X	40X	60X
Magnification	20X	40X	60X
Numeric Aperture	0.5	0.75	0.9
Field of View (µm)	120 x 1024	60 x 512	40 x 340
Imaging Rate (cells/sec)	2,000	1,000	600

INSTRUMENT CAPABILITIES

Images per Cell	Up to 12
Imaging Modes	Brightfield, SSC, and fluorescent
Sample Throughput	1 sample/min nominal
Automated Processes	Startup, shutdown, and self-calibration

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