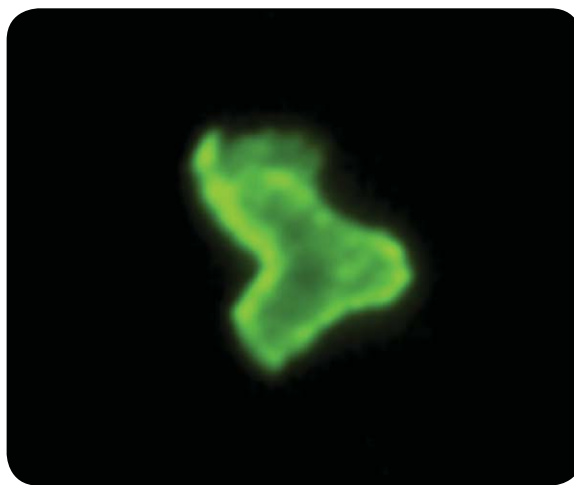


Measuring Shape Change in Monocytes

IMAGESTREAM CAPABILITIES FEATURED: Internalization / Cell Signaling & Molecular Translocation / Cell-Cell interaction / **Morphology** / Cell Cycle & Mitosis / Co-localization / Spot Counting / DNA Damage & Repair / Cell Death & Autophagy / **Immunology** / Biochemistry / Oncology / Virology / Microbiology / Parasitology / Hematology / Stem Cell Biology / Oceanography / Toxicology / **Drug Discovery**

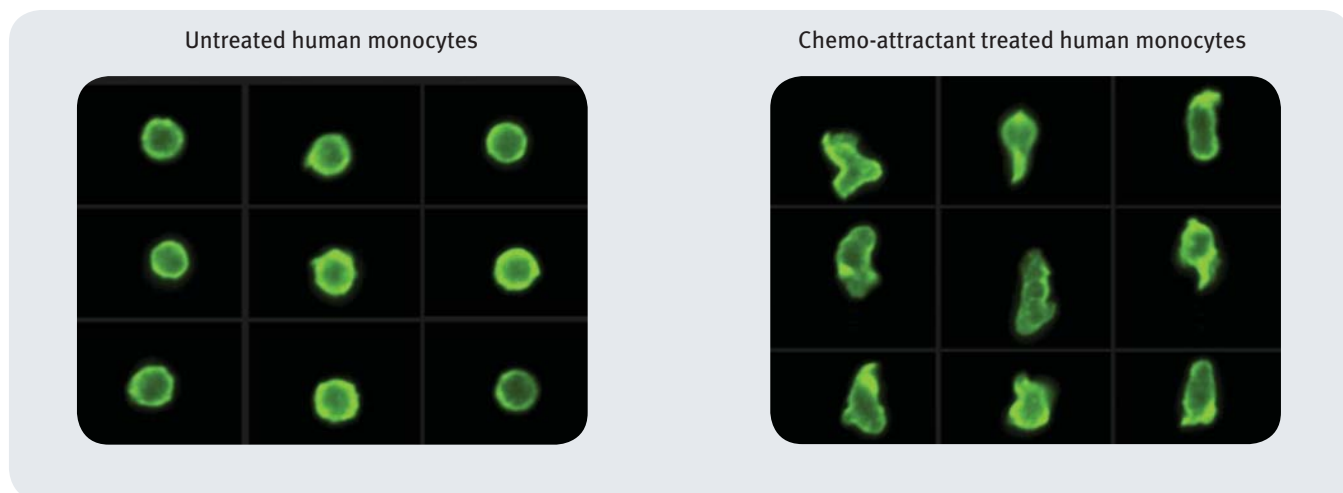
ABSTRACT

Pathogen or autoantigen-induced release of chemo-attractants signal circulating monocytes to leave the bloodstream and migrate to the site of release, where they serve as important early mediators of inflammation. Drugs that target this early response have shown promise for the treatment of autoimmune diseases such as rheumatoid arthritis. Within minutes of binding the MCP1 chemoattractant, the monocyte skeleton reorganizes resulting in a dramatic shape change, followed by migration towards the MCP1 gradient. Migration is traditionally measured *in vitro* using transwell assays, which can be a labor-intensive and time-consuming assay. Here we describe a simple method using ImageStream cytometry for measuring monocyte shape change directly in whole blood samples exposed to chemoattractant.



Human monocyte exposed to MCP-1 showing shape change

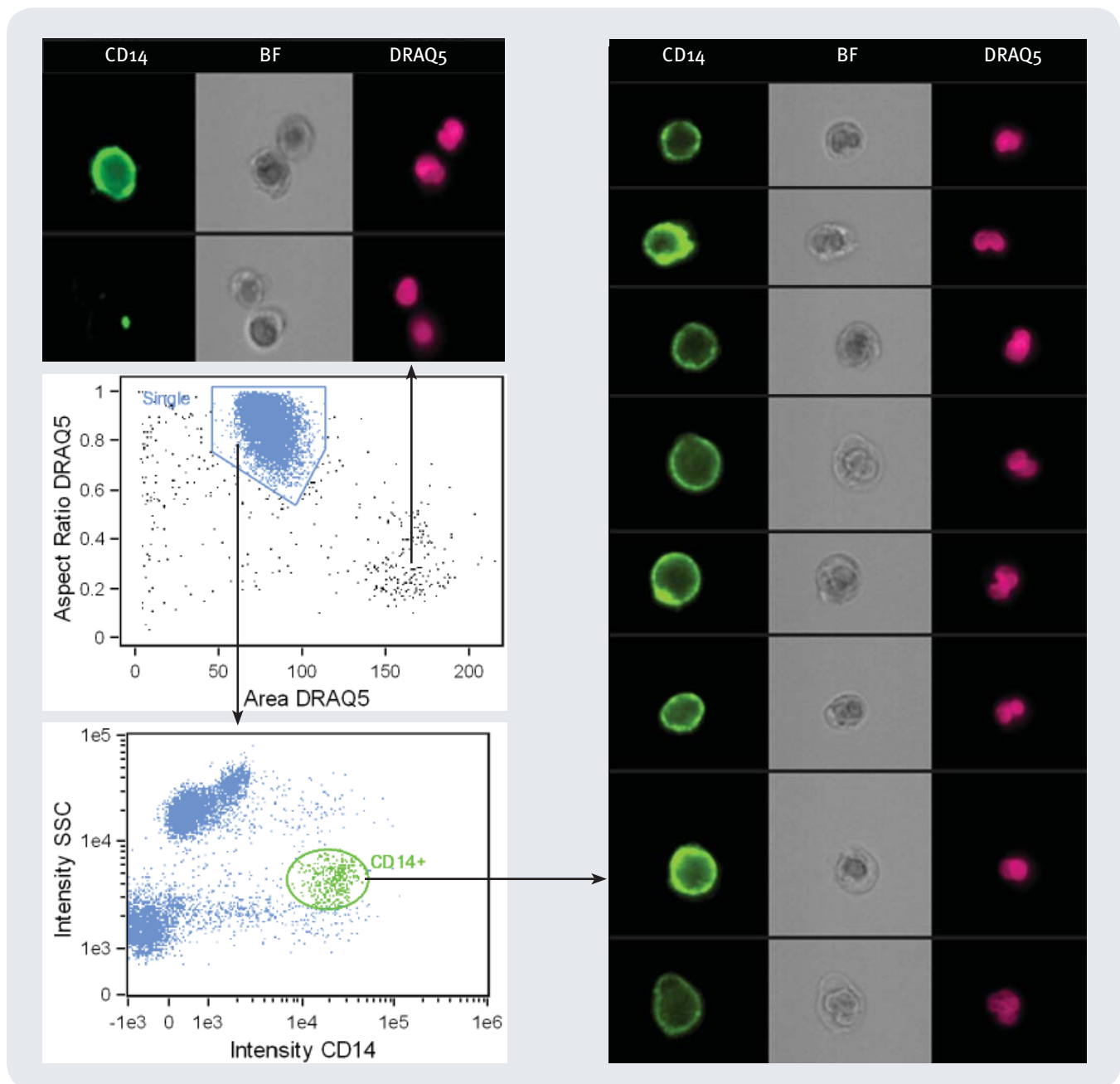
Study Highlights



IDENTIFICATION OF SINGLE CD14+ MONOCYTES

Single cells in peripheral blood were located by plotting the area of the nuclear image vs. the aspect ratio (the ratio of the width and height of the object, *i.e.*, shape) of the nuclear image. Objects with an Aspect Ratio approaching 1.0 are single cells (equal width and height)

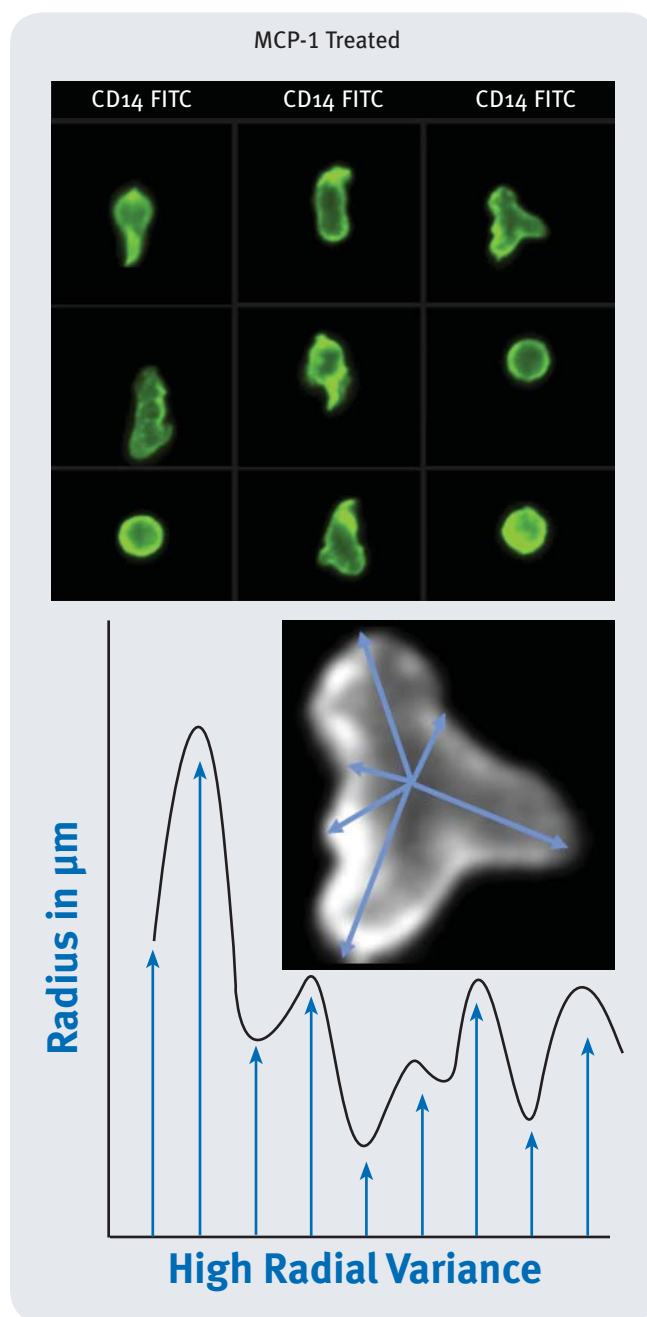
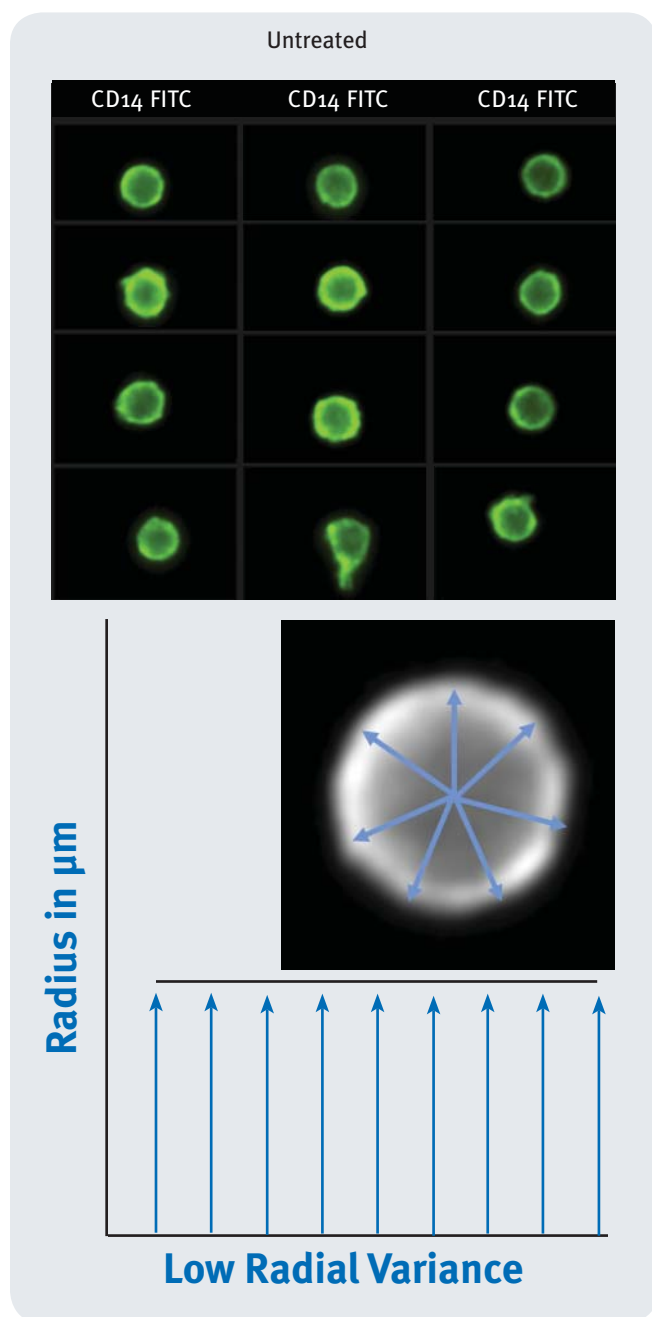
and objects approaching 0.5 are half as wide as they are long (*e.g.*, doublets). CD14 positive monocyte images are then gated for further analysis using the Intensity plot below.



IDENTIFICATION AND QUANTITATION OF CELLS EXHIBITING SHAPE CHANGE USING THE CIRCULARITY FEATURE

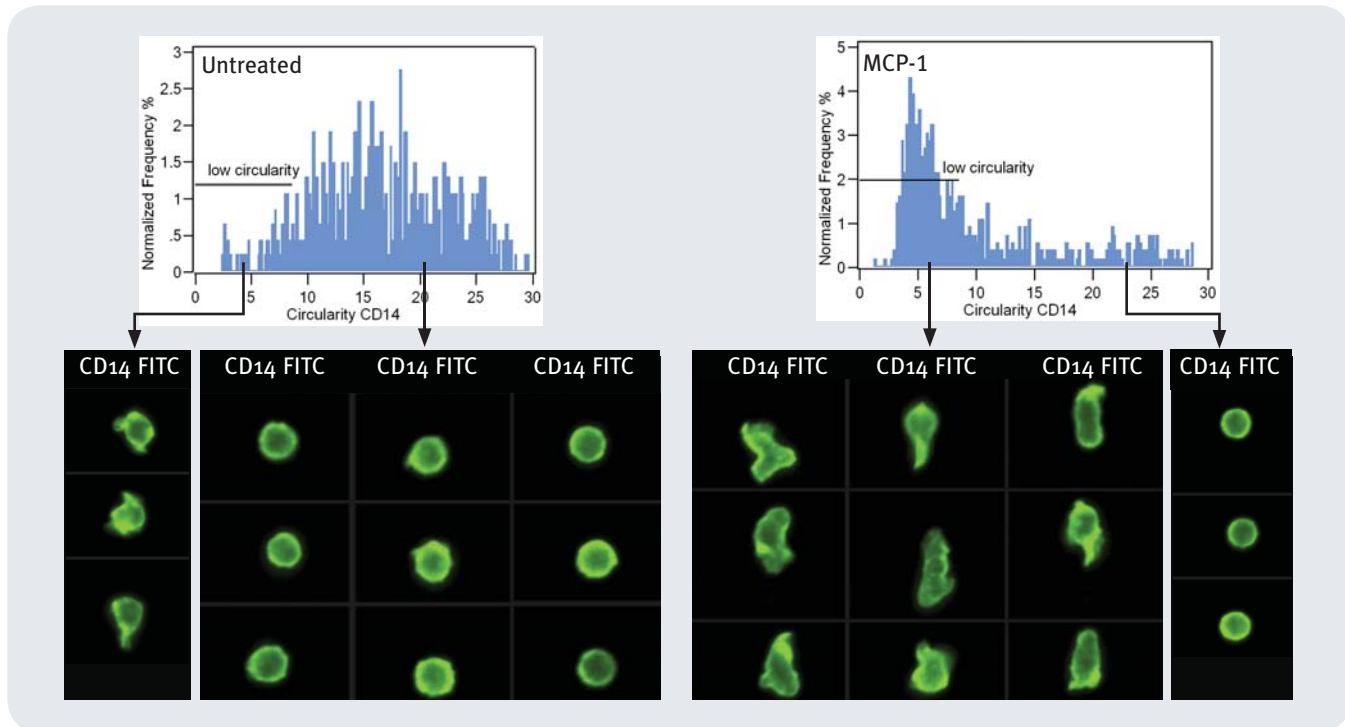
As the images below indicate, MCP-1 induces dramatic monocyte shape change. *In vivo*, these cells would migrate to the site of chemokine release, and many drugs used in the treatment of autoimmune disorders target this migration. To quantify the shape change, we use the circularity feature.

Circularity measures the average radius divided by the variance in the radii. A round cell has low radial variance and thus a high circularity value. A ruffled or elongated cell has high radial variance and thus a low circularity value.



QUANTITATIVE ANALYSIS OF SHAPE CHANGE

Circularity scores for all monocytes in each sample are plotted in a histogram allowing identification of the population exhibiting low circularity (shape change). Representative images corresponding to different areas of the histograms are shown below.



ImageStream^X Specifications



IMAGING PERFORMANCE

	20X	40X	60X
Magnification	20X	40X	60X
Numeric Aperture	0.5	0.75	0.9
Field of View (µm)	120 x 512	60 x 256	40 x 170
Imaging Rate (cells/sec)	2,000	1,000	600

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

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

Pacific Blue™, Alexa Fluor®, and Texas Red® are trademarks of Life Technologies Corporation. Cy® is a trademark of GE Healthcare. ECD® is a trademark of Beckman Coulter, Inc. DRAQ5™ is a trademark of Biostatus, Ltd.