

Identification and Measurement of Bacterial Size using the ImageStream

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


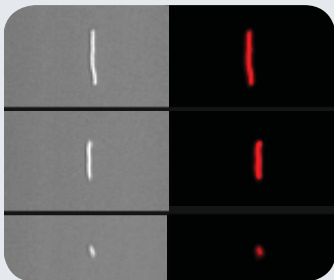
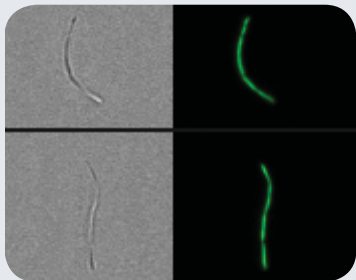
ABSTRACT

In this note, we describe two bacterial studies performed using the ImageStream high speed imaging cytometer. In the first system, pathogenic bacteria are distinguished from microflora and debris normally present in alfalfa seed sprouts. Because the aerobic conditions necessary for sprout growth also foster microbial growth, sprouts harbor a diverse microflora, including potential human pathogens. *Salmonella* spp., probed using a novel FISH method, are detected within complex sprout preparations by combining fluorescence/SSC intensity-based measurements with morphologic confirmation of gated populations. In the second study we measure length of individual bacteria for large populations of *E. Coli* and *Cyanobacteria* to follow bacterial growth in culture. By combining high speed image collection with quantitative image-based measurements, the ImageStream enables accurate identification and classification of bacteria within heterogeneous samples.



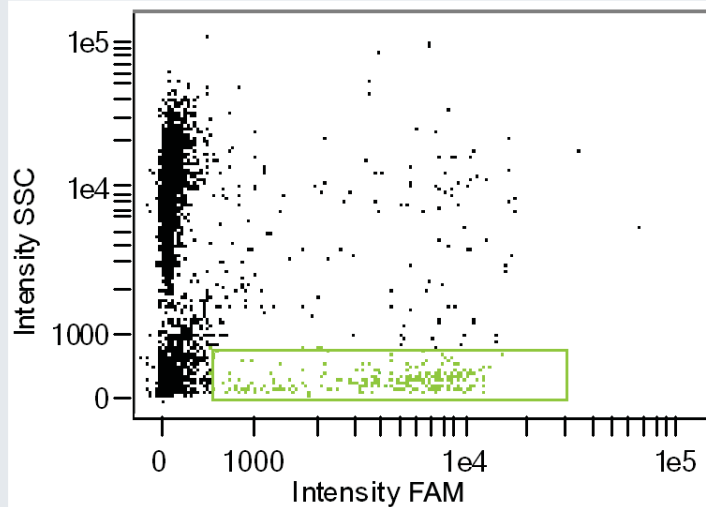
Bacterial identification and bacterial size measurements are enhanced using ImageStream analysis.

Study Highlights

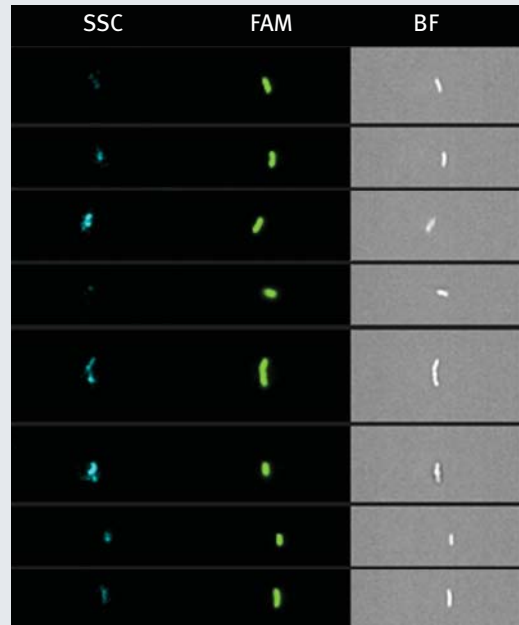
<i>Salmonella</i> Identification	Cyanobacteria Length	<i>E. coli</i> Length
		

IDENTIFICATION OF *SALMONELLA* WITHIN ALFALFA SEED SPROUT PREPARATIONS

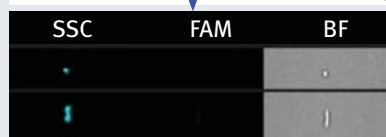
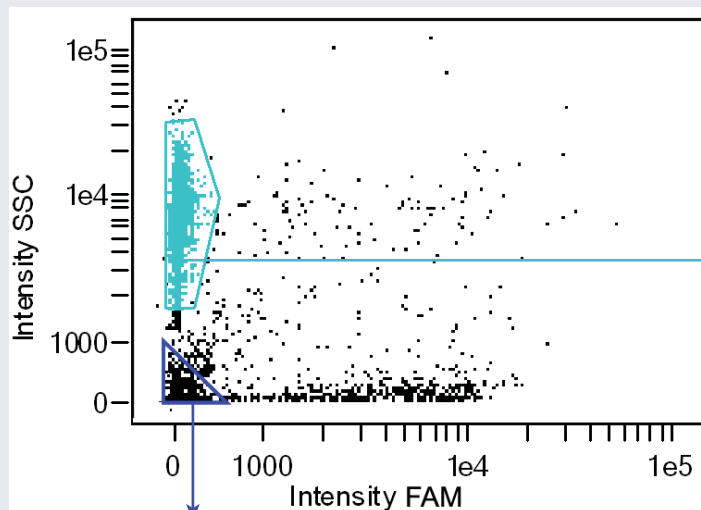
Byron Brehm-Stecher & Bledar Bisha, Iowa State University



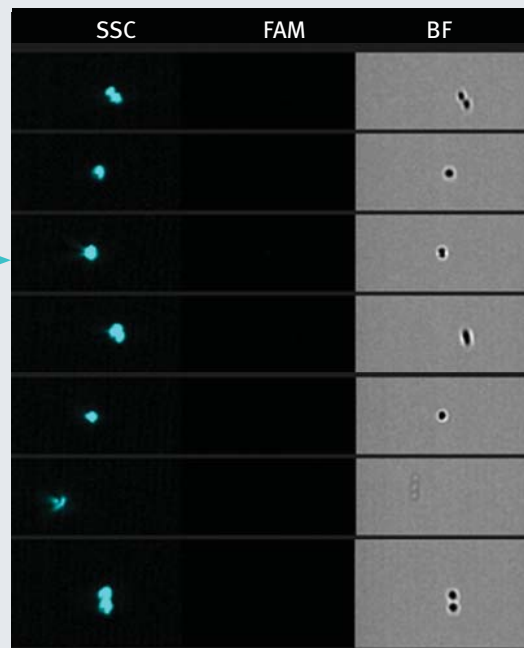
FISH-probed (FAM) *Salmonella* within alfalfa sprout preparations were identified by gating events with high FAM fluorescence and low SSC. Representative gated images shown at right.



NON-*SALMONELLA* OBJECTS WITHIN SPROUT PREPARATION

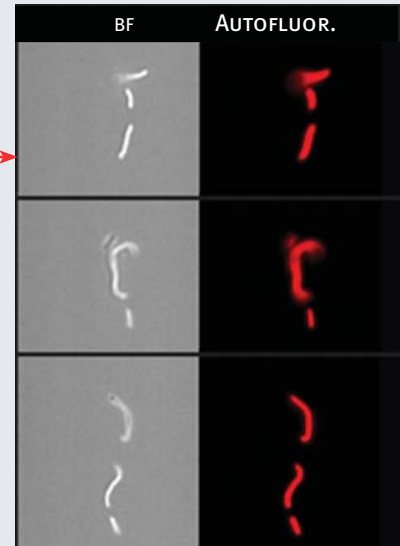
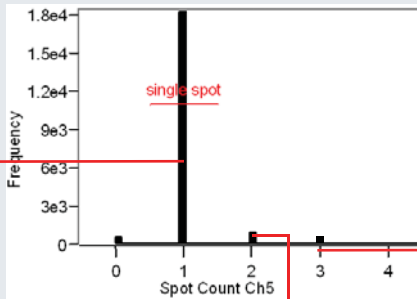


Non *Salmonella* bacteria and debris appear in the FAM negative populations.

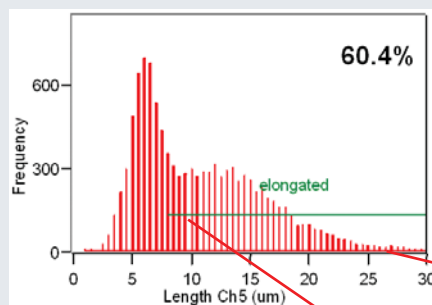
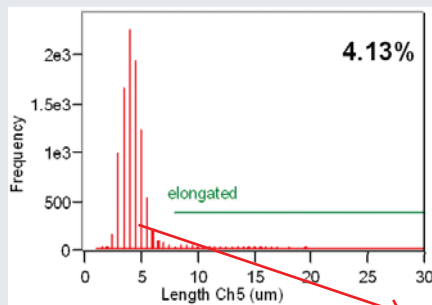


IDENTIFICATION OF SINGLE CYANOBACTERIA

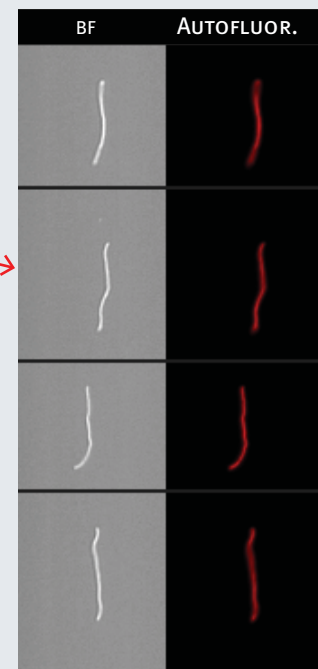
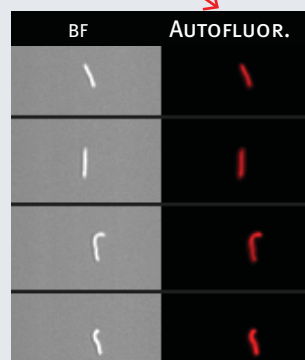
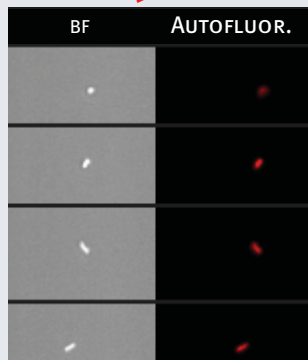
Cultured Cyanobacteria were analyzed using the ImageStream. Single bacteria were identified as those events with one spot in the phycocyanin/ chlorophyll autofluorescence channel.



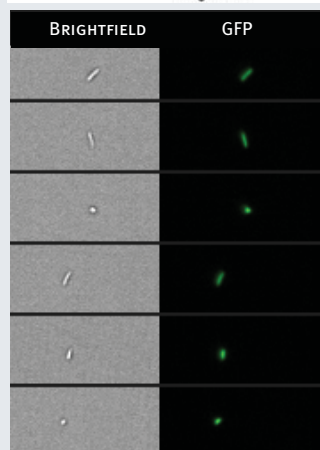
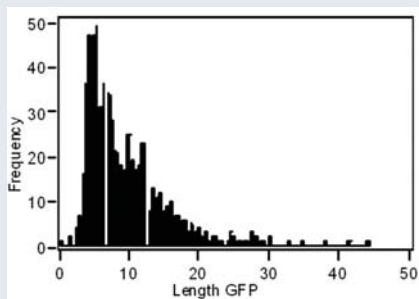
LENGTH DISTRIBUTION OF SINGLE CYANOBACTERIA



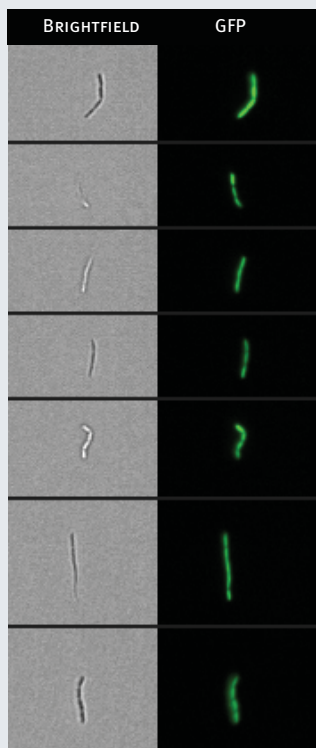
Length (in microns) distribution of single cell populations from resting (left) and growing (right) cyanobacterial cultures, with the percentage of bacteria longer than 8 microns indicated in the upper right of the plots. Representative images from the indicated histogram bins are shown.



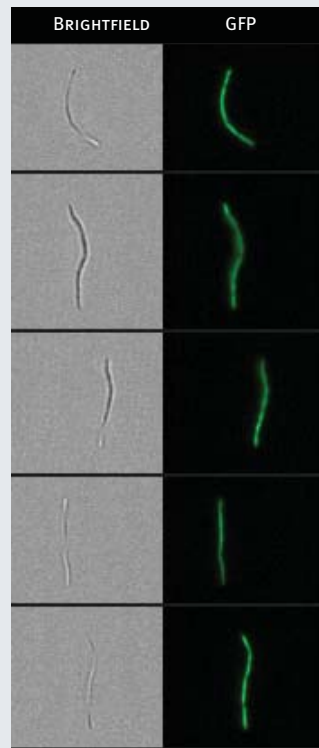
LENGTH DISTRIBUTION OF *E. COLI*



< 5 μm



10-20 μm



20-30 μm

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

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