Microbiology Research using ImageStream Cytometry

The ImageStream system combines high-speed image capture with image quantification to create a statistically robust microscopy platform, enabling robust discrimination of cells based on their appearance. This document highlights applications of ImageStream cytometry to the field of microbiology as described in publications, posters and podium presentations. For more information, check out the website: (https://www.amnis.com/microbiology.html)

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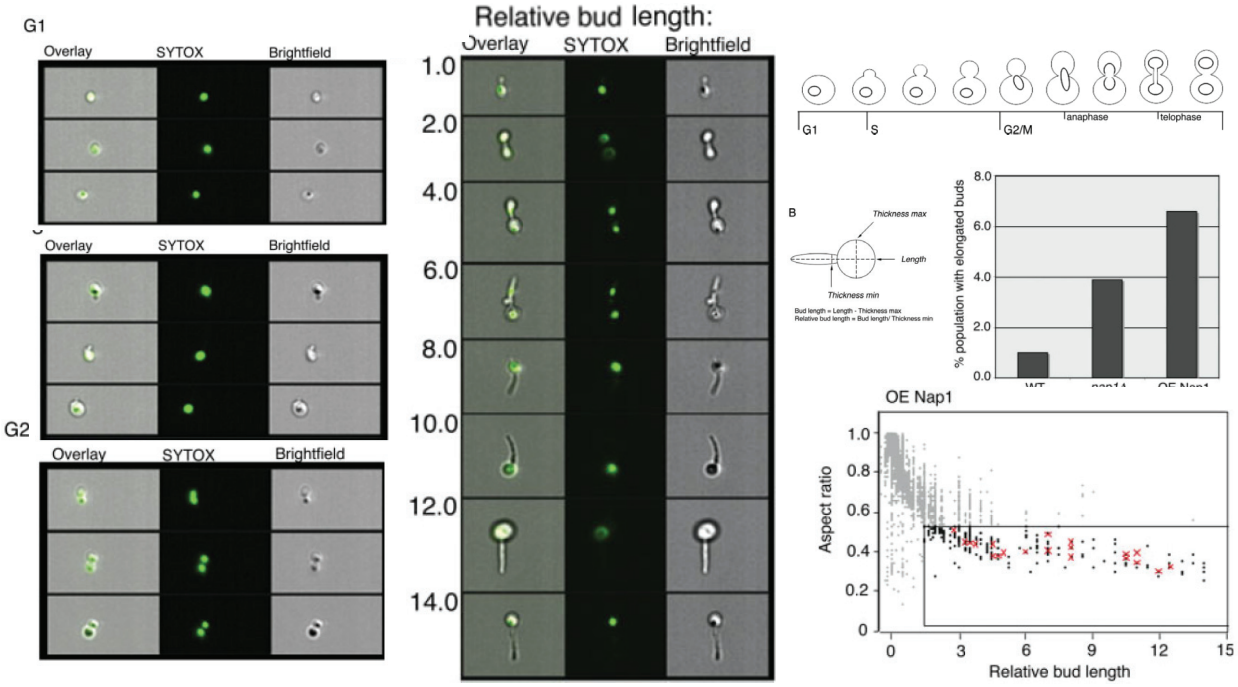
Yeast Cell Cycle Analysis

Summary: This study demonstrates how the ImageStream provides precise quantitation of cell cycle distribution and morphological phenotypes of yeast cells in flow.



"By integrating the analysis of DNA content and budding index, ImageStream allows precise quantitation of the cell cycle by both parameters, and measures S-phase directly rather than predicting the proportions by mathematical modeling. This technique therefore represents a significant improvement over existing methods for performing cell cycle analyses in budding yeast, and simultaneously provides a system for sophisticated quantitative morphological analysis."

Calvert, M. E., Lannigan, J. A., Pemberton, L. F. Optimization of yeast cell cycle analysis and morphological characterization by multispectral imaging flow cytometry. Cytometry A 2008

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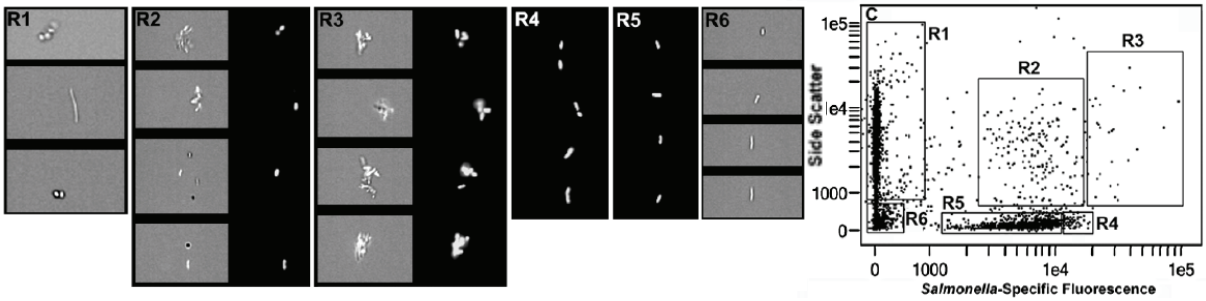
Salmonella Analysis: Protocol Development

Summary: This study demonstrates how the ImageStream can be used as a protocol development tool to further characterize a rapid FISH-based labeling of Salmonella-targeted probe cocktail. The unique ability of the ImageStream of visual verification of "dots" in bivariate plots enabled researchers to distinguish greater detail indiscernible by conventional flow cytometry.



"We have shown here that the ImageStream, combined with "phylogenetic staining" using Salmonella-targeted DNA-FISH probes, enables a more complete and direct visual exploration of this physiologically and phylogenetically complex food system than is possible using either traditional imaging approaches, such as microscopy, or conventional flow cytometry."

Bisha, B., Brehm-Stecher, B. F. Flow-through imaging cytometry for characterization of Salmonella subpopulations in alfalfa sprouts, a complex food system. Biotechnol J 2009

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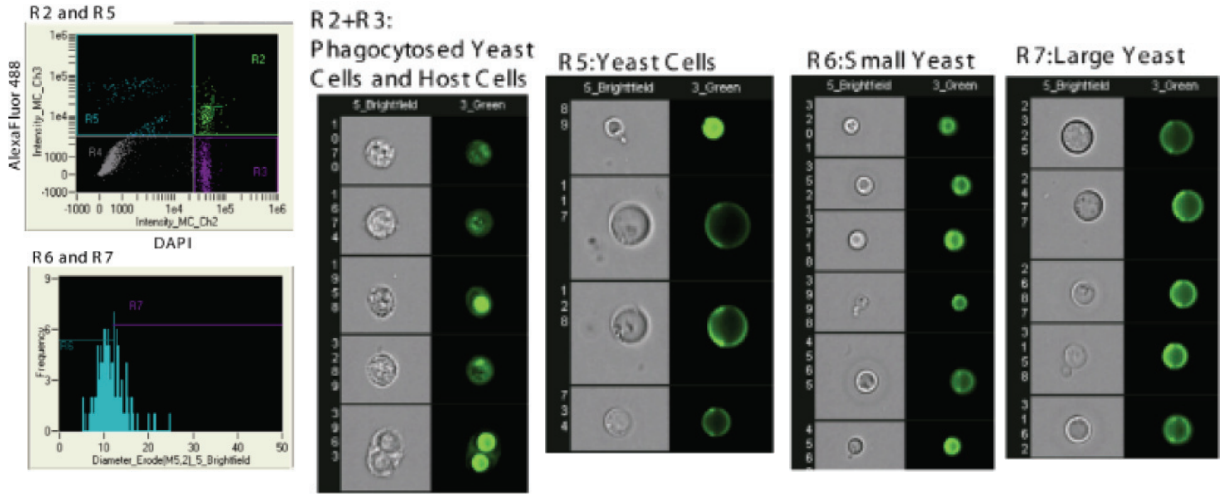
Cryptococcal Cell Morphology Affects Host Cell Interactions and Pathogenicity

Summary: In this experiment the ImageStream was used to differentiate host -phagocytized from non-phagocytized Cryptococcal cells, then to differentiate morphogenesis (size) increases in Titan cell formation.



C. neoformans a + α strains were combined with AlexaFluor 488. Mice were inoculated with an approximate 1:1 ratio of a:α cells. At 3 days post infection, animals were sacrificed and BALs were performed. The resulting cells were fixed; DAPI stained, and analyzed using an ImageStream flow cytometer using IDEAS software (Amnis Corporation). Single, double positive cells with phagocytized. Cryptococcal cells were further differentiated (along with unstained yeast cells). The diameter of the resultant cell populations was measured to separate small yeast cells from large yeast titan cells.

"These results describe a novel mechanism by which C. neoformans evades host phagocytosis to allow survival of a subset of the population at early stages of infection."

Okagaki, L. H., Strain, A. K.,Nielsen, J. N., Charlier, C., Baltes, N. J., Chretien, F., Heitman, J., Dromer, F., Nielsen, K. Cryptococcal cell morphology affects host cell interactions and pathogenicity PLoS Pathog 2010

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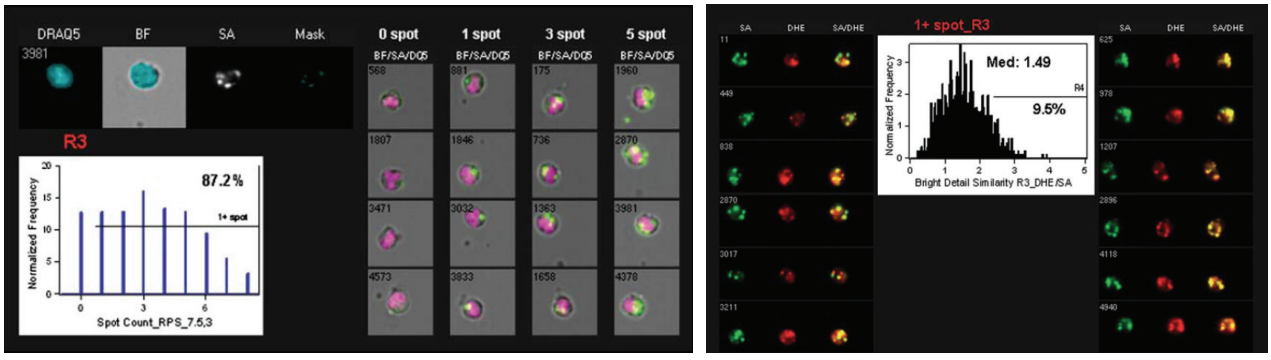
ImageStream Cytometry Extends the Analysis of Phagocytosis and Oxidative Burst

Summary: Phagocytosis and oxidative burst determined by flow cytometry and ImageStream cytometry showed strong correlation. In contrast, ImageStream cytometry easily detected and excluded extracellular adherent bacteria from the measurement of phagocytosis, and enumerated the bacteria within each neutrophil. Using the Bright Detail Similarity score, we identified a subset of neutrophils with intracellular bacteria co-localized to sites of oxidative burst activity.



"ImageStream cytometry provides the spatial resolution to determine the number of bacteria ingested and the sub-cellular localization and trafficking patterns that enables a more complete evaluation of the phagocytic process."

Ploppa, A., George, T. C., Unertl, K. E.,Nohe, B., Durieux, M. E. ImageStream cytometry extends the analysis of phagocytosis and oxidative burst. Scand J Clin Lab Invest 2011

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Pneumocystis Pneumonia Internalization in the study of T-cell mediated clearance and inflammation.

Summary: T cell-mediated immunity that is critical for host defense against respiratory fungal infections also causes PcP-related inflammation and lung injury. Using the Amnis ImageStream, the authors identified alveolar macrophages as the effector cells for T cell-dependent clearance of Pneumocystis from the lung, and demonstrated that macrophage phenotype can be altered to enhance microbe elimination without promoting inflammatory injury. These results suggest that the effector mechanism of T cell-mediated fungal clearance is distinct from the effector mechanism of T cell-mediated lung inflammation and injury.

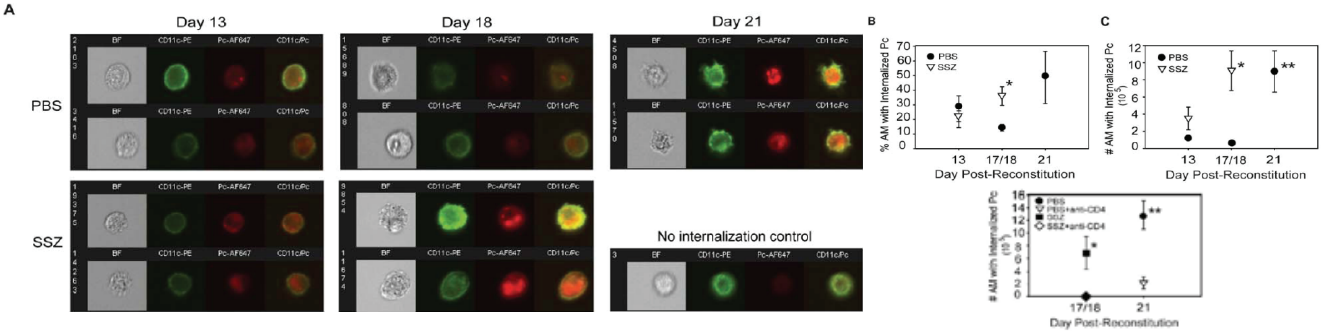

Figure_5. Sulfasalazine (SSZ) enhances CD4+ T cell-dependent alveolar macrophage (AM) phagocytosis of Pneumocystis (Pc). Bronchoalveolar lavage (BAL) cells were collected from PBS- and SSZ- treated mice at day 13,17 or 18 (17/18) and 21 post-reconstitution. Cells were stained with antibodies specific for CD11c (green) and Pc (red). (A) Imaging flow cytometry (Amnis) was used to quantify Pc internalization by AMs. Representative images of brightfield (BF), CD11c, Pc, and merged CD11c/Pc are shown for SSZ or PBS treated mice following immune reconstitution. The no internalization control is a representative CD11c+ cell without internalized Pc. ImageStream IDEAS software was used to quantify (B) percentile and (C) number of AMs with Internalized Pc in immune reconstituted SCID mice treated with SSZ(=) or PBS (N)

"These results suggest that the effector mechanism of T cell-mediated fungal clearance is distinct from the effector mechanism of T cell-mediated lung inflammation and injury."

Wang, J., Gigliotti, F., Bhagwat, S. P., George, T. C., Wright, T. W. Immune modulation with sulfasalazine attenuates immunopathogenesis but enhances macrophage-mediated fungal clearance during Pneumocystis pneumonia. PLoS Pathog. 2010

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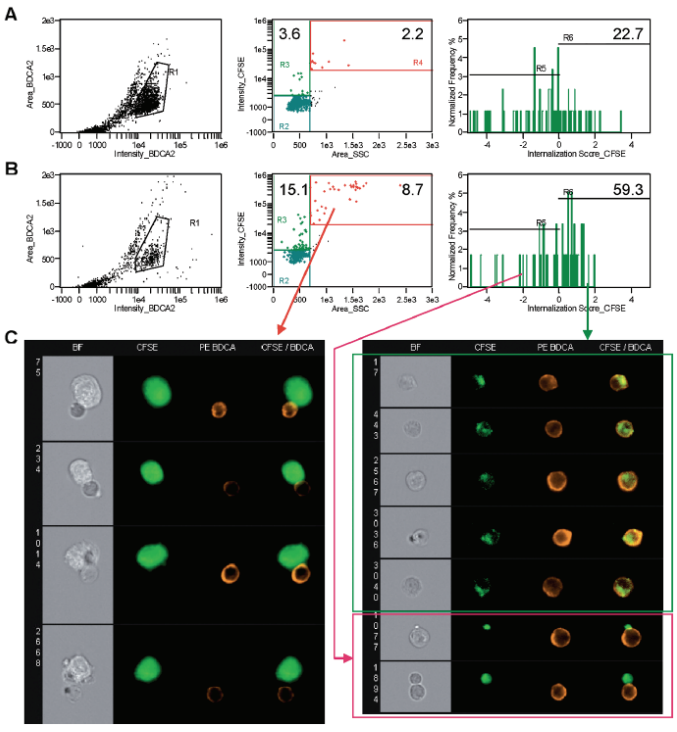
Preferential pDC-MDCC conjugate formation and transfer of cellular components with HSV infection

Summary: Analysis of the interaction of plasmacytoid dendritic cells (pDC) and monocyte-derived dendritic cells (MDDC) by imageStream cytometry combines the statistical robustness of the flow cytometer with the imaging sensitivity of microscopy. We observed that BDCA+ pDC (orange) preferentially formed conjugates (red gate) with the HSV-infected (B) vs. uninfected (A) CFSE+ MDDC (green) and preferentially took-up cellular components from the infected vs. the uninfected cells, as indicated by the increased percentage of single pDC (R3) with internalized CFSE.



"In this study, we have evaluated the interaction of HSV-infected MDDC with pDC using a combination of traditional flow cytometric and imaging flow cytometric techniques. We report here that MDDC infected with HSV are able to stimulate IFN-α and chemokine production by pDC. Additionally, we demonstrate the preferential transfer of cellular components from HSVinfected MDDC vs. uninfected MDDC to pDC. Together, these results indicate that heterogeneous populations of DC interact to generate an effective IFN-α response."

Reference: Megjugorac, N.J., E.S. Jacobs, A.G. Izaguirre, T.C. George, G. Gupta, and P. Fitzgerald-Bocarsly, *Image-based study of interferongenic interactions between plasmacytoid dendritic cells and HSV-infected monocyte-derived dendritic cells.* Immunol Invest, 2007. **36**(5-6): 739-61.

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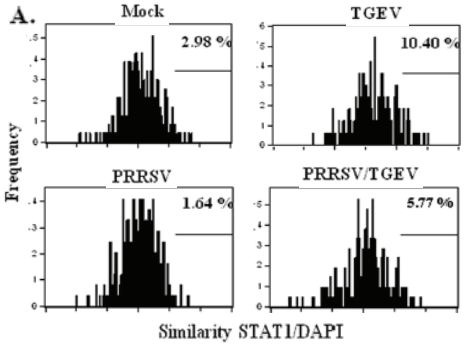
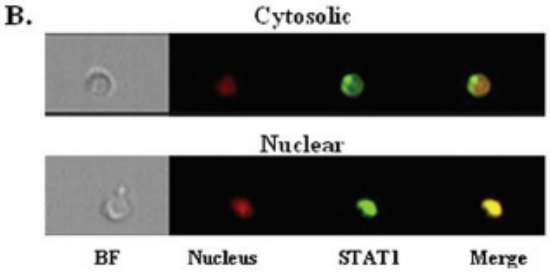
TGEV-induced STAT1 nuclear translocation in porcine pDC

Summary: This ImageStream assay measures STAT1 nuclear localization (by quantifying the similarity between the STAT1 and nuclear images on a per-cell basis) in porcine whole blood pDC cells exposed to transmissible gastroenteritis virus (TGEV) and/or porcine reproductive and respiratory syndrome virus (PRRSV). The ImageStream's unique ability to combine immunophenotyping with objective translocation measurements in large numbers of events allows statistically robust discrimination of the samples, which is of critical importance given their subtle and heterogenous response characteristics.



"Early, IFN-α independent,TLR9 agonist-induced IRF-7 production in human pDC requires the activation and nuclear translocation of STAT1 (66)…. Whereas there was an approximately 3.5-fold increase in the frequency of nuclear STAT1-positive cells during stimulation by TGEV, this augmentation was reduced 62% in the presence of PRRSV. A similar reduction (45%) in this parameter was also observed in regard to non-activated pDC when PRRSV was present. Therefore, PRRSV appeared to partially inhibit the migration of STAT1 into pDC nuclei, even in the absence of external stimulation."



Reference: Calzada-Nova, G., W.M. Schnitzlein, R.J. Husmann, and F.A. Zuckermann, *North American porcine reproductive and respiratory syndrome viruses inhibit type I interferon production by plasmacytoid dendritic cells.* J Virol, 2011.

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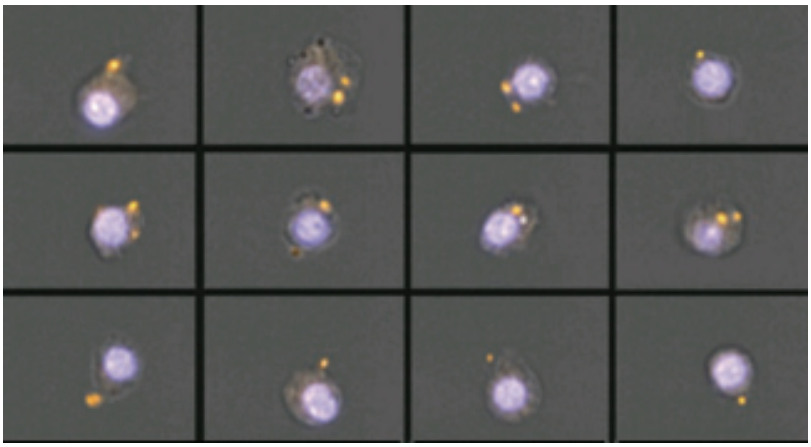
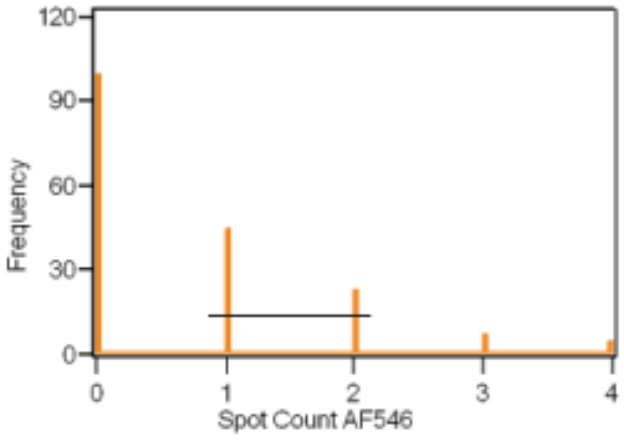
Anthrax Spore Localization

Summary: This ImageStream system allows measurement of spore internalization and spot counting.



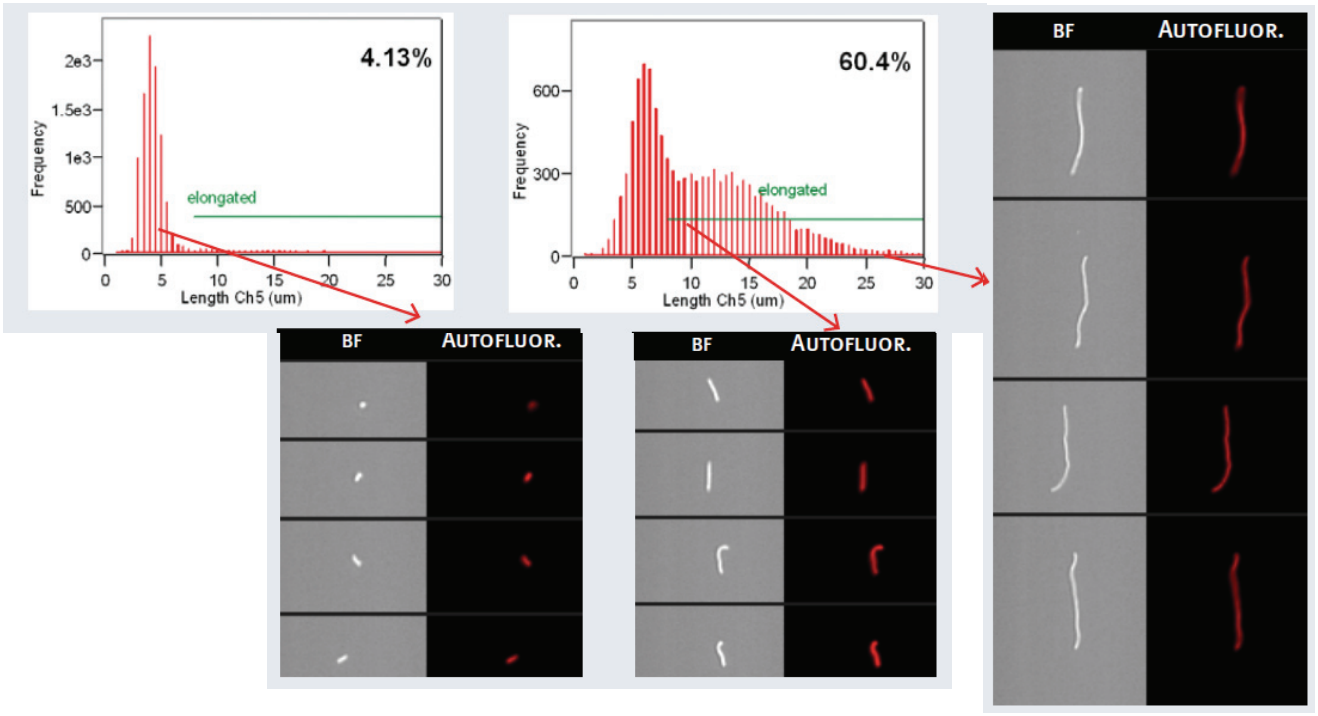


DAPI nuclear image, Brightfield, and Anthrax spore Alexa Fluor 546 composite imagery of macrophages. Macrophages were incubated with Alexa Fluor 546-labeled Anthrax spores, mixed, stained with DAPI nuclear dye, and analyzed on the ImageStream. The plot locates the populations of macrophages which contain zero, one, two, three, or 4 Anthrax spore spots.
Ian Gut, University of Illinois, Urbana Champaign

Amnis. Phagocytosis and Quantitation of Internal Zymosan, L. monocytogenes, M. tuberculosis, and Anthrax Spores (09-003-1-apn). 2009.

Length Distribution of Single Cyanobacteria

Summary: This ImageStream application shows the quantitative capability of counting and of measuring length of Cyanobacteria in culture.



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.

Amnis. Identification and Measurement of Bacterial Size using the ImageStream. (09-002-apn). 2009

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