

GFP Expression and Subcellular Compartmentalization in Trypanosomes: Similarity Score Analysis on the ImageStream Cell Analysis System

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BACKGROUND AND SUMMARY

Green Fluorescent Protein (GFP) has become a commonly used surrogate marker for studies of gene expression in many fields, including pharmaceutical drug discovery. The excitation maximum for GFP is 489nm, a wavelength that is almost perfectly matched to the 488nm laser in the ImageStream system. Analysis of GFP expression with ImageStream technology should, in principle, offer a valuable addition to the repertoire of applications the instrument can support. In this report, we examine expression of GFP in two lines of trypanosomes. These organisms are morphologically irregular single cell protozoa responsible for a variety of dis-

eases in humans, including African sleeping sickness (*T. brucei*) and Chagas' Disease (*T. cruzi*). Trypanosomes are roughly 3 microns by 10 microns and have relatively small nuclei (2 -3 microns). Their irregular morphologies (see Figure 1) and small nuclei present certain challenges to the analysis of GFP localization at a subcellular level. However, the high resolution achieved by the ImageStream system and a powerful similarity analysis algorithm that is part of the companion IDEAS™ software are shown here to be more than sufficient to evaluate the distribution of GFP between the nucleus and cytoplasm of these organisms.

RESULTS

In collaboration with Dr. Marilyn Parsons of the Seattle Biomedical Research Institute, we used the ImageStream system to accurately quantitate the distribution of GFP labeled proteins between the nucleus and cytoplasm in different populations of trypanosomes. Trypanosomes that were stably transfected with either P19-GFP or NP19-GFP were counterstained with DRAQ5 DNA dye. Brightfield, Darkfield, GFP and DRAQ5 images of 10,000 cells from each trypanosome line were collected (Figure 2), and analyzed using the IDEAS® image analysis software.

ANALYSIS

As a first step in the analysis, we identified and gated the subpopulation of cells that expressed GFP (Figure 3). To quantify nuclear GFP translocation we employed a “similarity” algorithm contained in the IDEAS software. This algorithm, which reports a **Similarity score** for each cell, measures the degree of spatial co-localization of signals from the GFP and DRAQ5 spectral channels. Detection of GFP and DRAQ5 (nuclear stain) in the same spatial register results in a higher mean Similarity score than if the GFP is located in the cytoplasm. The mean similarity

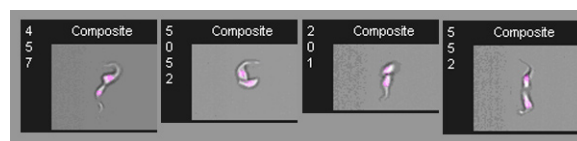


Figure 1: These composite images of bright field and the purple Draq 5 nucleus show Trypanosomes in the late stages of cell division

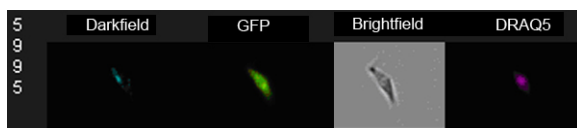
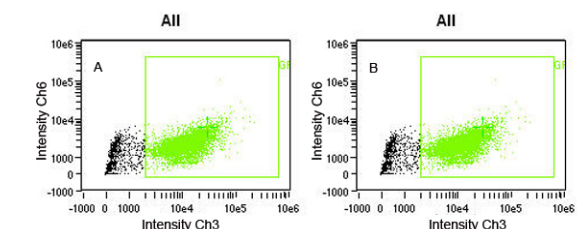


Figure 2: 6 channel imagery of a trypanosome. Channel 1 laser scatter is blue, channel 3 GFP is green, channel 5 bright field imagery is grey and channel 6 nucleus stained with Draq-5 is purple (Note: channels 2 and 4 are not shown in this image).



Population Statistics

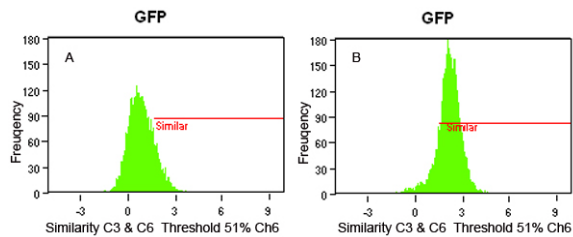
Population	Count	%Total	%Gated
All	10006	100	100
GFP & All	9042	90.3	90.3

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Figure 3 (A) P19 GFP (B) NP19 GFP. Level of GFP expression as measured by the percentage of trypanosomes in a fixed green fluorescence region.

score for the P19-GFP cell line was 0.843, indicating a cytoplasmic distribution for the protein, while the mean similarity score for the NP19-GFP sample was 2.1366, indicating a nuclear distribution for the protein (Figure 4, A and B respectively).

Because data in IDEAS is directly associated with cellular imagery, we can visually inspect the morphology of cells with high and low Similarity scores. The GFP images (Channel 3) of P19-GFP cells with low Similarity scores



Population Statistics

Population	Count	%Total	%Gated	Population	Count	%Total	%Gated
GFP	9289	92.8	100	GFP	9042	90.3	100
Similar&GFP	1368	13.7	14.7	Similar&GFP	7637	76.3	84.5

Mean Similarity Scores

Population	Mean	Std Dev	Population	Mean	Std Dev
GFP	0.843	0.7696	GFP	2.1366	0.7334
Similar&GFP	2.1192	0.3681	Similar&GFP	2.3587	0.5065

Figure 4 (A) P19 GFP (B) NP19 GFP. Similarity scores comparing the channel 3 GFP image to the channel 6 Draq5 nuclear image. In sample A P19GFP only 14.7% of the cells have GFP sequestered in the nucleus as opposed to 84.5% of the cells in the NP19 GFP sample B.

(Figure 5) have dark holes in the position of the nucleus, indicating that these trypanosomes have P19 in the cytoplasm. In contrast, the GFP and DRAQ5 images of NP19-GFP cells with high Similarity scores (Figure 6) are highly similar to one another, reflecting the nuclear localization of the GFP-labeled protein.

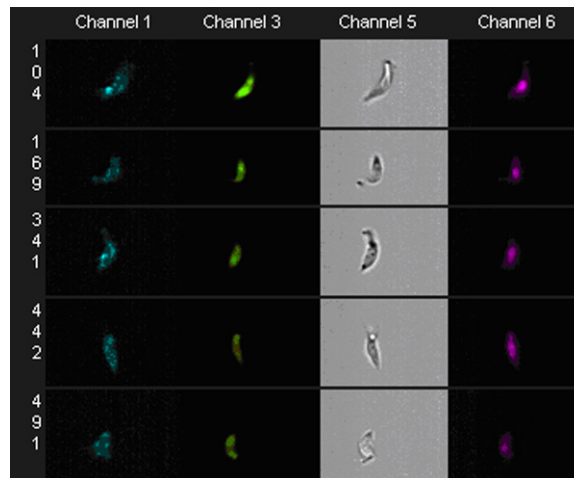


Figure 5: P19 GFP Trypanosomes in the region of low similarity. The channel 3 GFP imagery shows a dark spot in the center of the cells where the nucleus is, indicating that P19 is predominantly cytoplasmic.

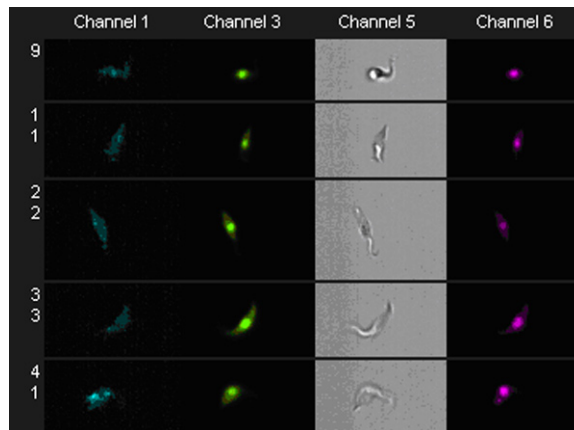


Figure 6: NP19 GFP Trypanosomes in the similar region from figure 4. The channel 3 GFP image shows some cytoplasmic background but for the most part the GFP appears to be sequestered in the nucleus as evident by the bright GFP fluorescence where the channel 6 Draq5 staining is.

CONCLUSIONS

The trypanosomes studied in this report have characteristically small nuclei (3mm) and irregular morphologies. Even so, the Similarity analysis available on the ImageStream 100 was able to distinguish clearly those cells in which GFP was sequestered in the nucleus from cells in which it was found in the cytoplasm. In addition, calculation of mean Similarity scores proved to be a sensitive and reliable metric of GFP localization at the population

level. This experiment demonstrates the functionality of the similarity score approach to track GFP expression, and classify cells based on the staining pattern of the chimeric GFP protein. In a larger sense, the results of this study also support the broader use of GFP as a marker for applications on the ImageStream system.