Fluorescence Image Analysis via Threshold Enhanced Alternative Morphology Guided Image Segmentation

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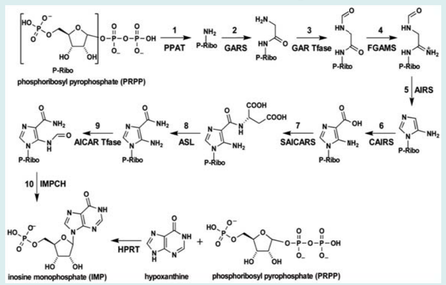
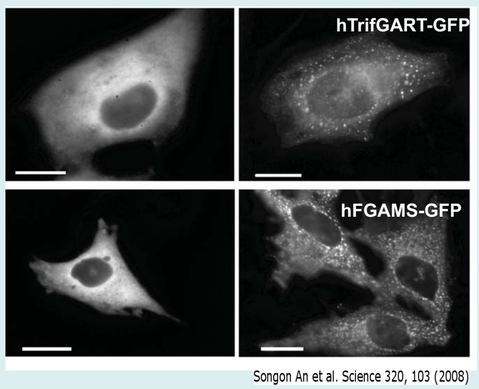
Abstract

The recent push towards high-throughput high-content screening has seen a growing need for fast, accurate, and automated algorithms for cellular content analysis. However, most image segmentation algorithms pose unique challenges for studying cellular components, including fluorescently labeled proteins. Furthermore, no algorithm is able to correctly segment cells or cellular compartments when compared to the visual examination. We focus towards developing such an algorithm which gives any user the ability to perform reliable single cell analysis to sample the number, various sizes, and areas of fluorescently tagged proteins. The designed "semiautonomous" approach is flexible to account for the variance in fluorescent intensity, stochastic cell-to-cell differences, and region-to-region variations introduced during the image acquisition process.
Due to the design of the algorithm, user input is necessary to facilitate verification and correction of each cell after the initial segmentations. We have designed an algorithm to explore 16-bit microscopy images that display fluorescent clusters induced under given stimulated conditions. We have successfully written an executable Matlab function that calculates the cluster sizes of Purinosomes.

De novo Purine Biosynthesis





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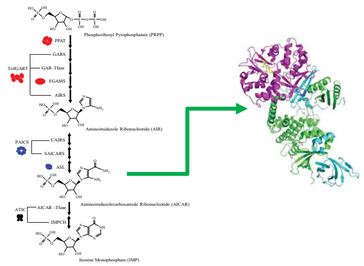
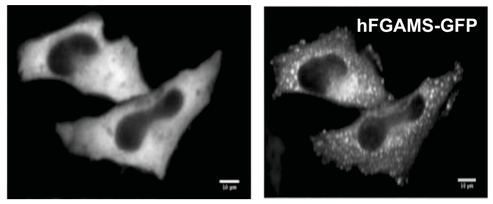
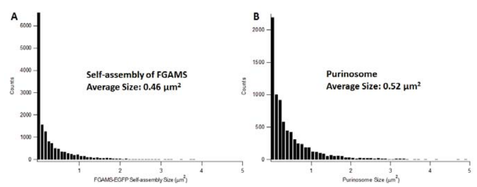
Self-Assembly of FGAMS



Before AMPK Activation **After AMPK Activation**

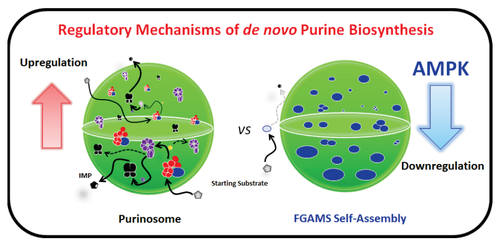


Size Analysis by *ImageJ*-RATS



Human *de novo* purine biosynthesis is a ten step enzymatic reaction. It relies on six enzymes, one trifunctional enzyme (TrifGART), two bifunctional enzymes (PAICS and ATIC), and three mono-functional enzymes (PPAT, FGAMS, and ASL). One of the six enzymes, formylglcinamidine ribonucleotide synthase (FGAMS), forms distinct clusters after the AMP-activated kinase (AMPK) activation.
The graphs above, indicate the average size of FGAMS-self assembly and purinosomes as 0.46 µm^2 and 0.52 µm^2, respectively. Schmitt and coworkers, obtained the average sizes by utilizing ImageJ (NIH) software. They have also proposed that FGAMS-self assembly is independent from purinosome assembly, and that it may provide a mechanism through which cells can downregulate *de novo* purine biosynthesis.

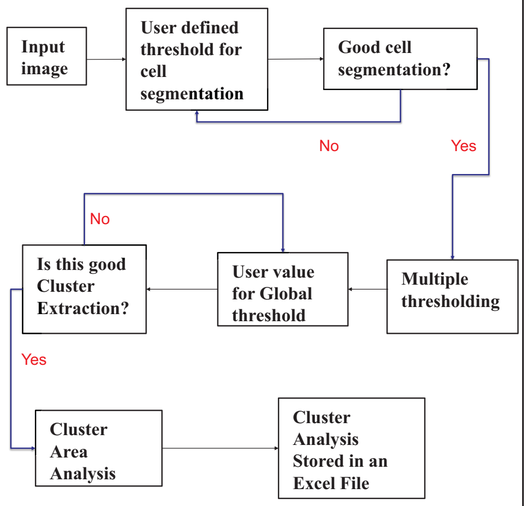
D. Schmitt, *et al*. (2016) , (submitted for publication).



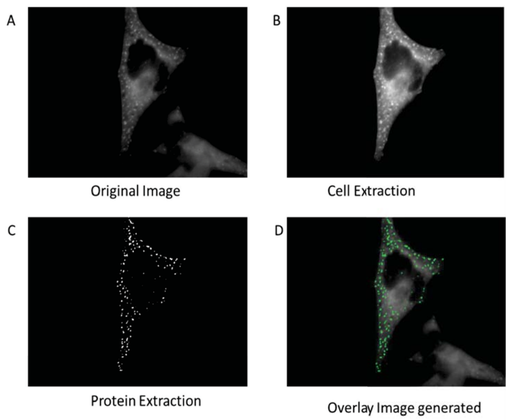
TEAMS Algorithm

The down regulation of *de novo* purine biosynthesis is achieved through requisitioning the FGAMS into FGAMS-self assembly clusters. In-order to confirm that the FGAMS-self assemblies are independent from the purinosomes, we designed and utilized the TEAMS algorithm to obtain average sizes of purinosomes and FGAMS-self assembly clusters.

TEAMS Workflow



Cell Extraction & Analysis



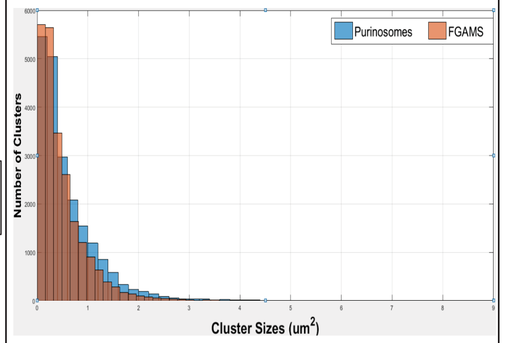
Most image segmentation algorithms pose unique challenges for studying cellular components, including fluorescently labeled proteins. The results indicate that the TEAMS, Threshold Enhanced Alternative Morphology Guided Image Segmentation, is able to handle multiple assays but required some changes in algorithm's thresholding and/or morphology parameters.

Cluster Analysis by TEAMS

Image Set	Protein Marker	Average Cluster Size (µm^2)	Total Cluster	# of Cells
1	Purinosome	0.599 ± 0.004	20975	98
2	FGAMS Self-Assembly	0.492 ± 0.003	23209	156

* The average sizes of FGAMS-self assembly and purinosomes reported in the table (above) are consistent with the averages reported by Schmitt and coworkers. Error reported for TEAMS cluster analysis is a Standard Error, $SE = \frac{\sqrt{\sigma^2}}{Total\ \#\ of\ clusters}$.

Histogram Of Cluster Analysis



Conclusion

The need for fast and accurate algorithms in the field of microscopy is increasing. Especially because high-throughput high-content assays (HT-HCA) have required robust algorithms to perform analysis of hundreds of thousands of fluorescent images per day. Our algorithm is a stepping stone towards understanding the needs for HT-HCA studies. However, TEAMS-algorithm lacks the proper automation and requires greater computational time than what can be afforded for HT-HCA studies.

However, the preliminary trials with the FGAMS-self assemblies have shown that the algorithm successfully handles multiple assays; reveling the average sizes of FGAMS-self assembly and purinosomes to be 0.492 µm^2 and 0.599 µm^2, respectively. These results are comparable to the average sizes reported by Schmitt and coworkers, and confirm that the FGAMS-self assemblies are independent from the purinosome clusters based on the size analysis.

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