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Abstract

Author Summary

Introduction

Single cells measurements allow studying biological processes that cannot be observed at the level of unsynchronized populations. Some examples include intrinsic and extrinsic noise (Elowitz 2002), cycles and . Among the promising techniques for single cell measurements is florescent in situ hybridization (FISH) pioneered by Robert Singer () and Alexander van Oudenaarden().

A major appeal of FISH is the ability of count mRNAs corresponding to different genes without making it's scalability to medium and high-throughput To fully realize those promises, we need robust and reliable algorithms to ... as first developed by Singer at al. In this paper we build upon and those first steps, and extend them by adding new features, including automated cell identification and budding index quantification. All these features are integrated into user friendly software package that can be used both interactively (via GUI) and from scripts for high-throughput image analysis.

Results

Identifying Cell and Cell Buds

Singer at. al. identified cells manually (). We introduce ... A short description who we do it and a fig with 3 panels: 1) Cells, 2) Budded cell, 3) Scatter plot of correspondence between human counting and computer counting

Finding and quantifying spots

algorithm description We developed and tested two algorithms for identifying spots and quantifying their intensities:

Algorithm A: The two most notable features (and differences compared to previously employed approaches) are:

plots of the multi-probe labeled mRNAs

Robust Probabilistic Spot Analysis

Ideally, the separation between the mode corresponding to single probes (M1) and the mode corresponding to multi-labeled mRNAs (M2) should be complete. If the separation is not perfect, using a hard threshold is going to introduce errors (mRNAs will not be counted or single non-hybridized probes will be counted as mRNAs). The bigger the overlap between the two modes (M1 & M2), the bigger the error in mRNA quantification. A second problem with a hard threshold on the intensity of spots is that the position of the threshold can depend very strongly on numerous parameters such as incident light intensity, efficiency of probe labeling, spectral filters, fluorophore quantum efficiency, and even sample preparation. Therefore, establishing a good threshold might require a set of control experiments specific to the equipment and every set of samples, or human decision (and the potential bias) about the threshold position on every single experiment.

TO mitigate those problems, we develop a simple probabilistic approach for determining

Discussion

Materials and Methods

Acknowledgments

References

Figure Legends

Tables