An image based high throughput screen to identify regulators of Imp containing RNP granules
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I. Context

In vivo, RNAs and proteins are frequently packaged into diverse dynamic macromolecular structures named RNP granules. These assemblies form upon phase separation of individual RNA and protein components, a process involving the establishment of multivalent weak interactions and their regulation via post-translational modifications. Deficit in their properties has been associated with several human pathologies. However, our knowledge of these dynamic structures relies essentially on the study of P bodies and stress granules.

We are interested in the highly conserved RNA binding protein Imp whose mammalian counterpart’s overexpression correlates with poor prognosis in several cancers. In vivo, Imp is present in cytoplasmic RNP granules, distinct from P bodies and visible both in neuronal cell bodies and axons. They are also detected in Drosophila S2R+ cultured cells. Taking advantage of this cellular model, we have undertaken a genome-wide RNAi-based visual screen to identify factors that regulate the stability of Imp-containing granules. This approach combining high throughput microscopy with the development of a computational pipeline for automatic image analysis. This pipeline first segments and discriminates healthy from dead marks, storing this information in an interactive SQDB database that enables experimental quality control. Then, GFP-Imp granules are detected using the SPADE algorithm in the cytoplasm of healthy cells. Data from the pilot screen we have performed to validate the experimental design and develop our pipeline for data mining are presented.

II. Pilot screen

Plate map: 240 well

480 X 518 pixels, 10 mc. 12 Mb

In each well 15 views a stack of 17 layers

405 nm : DAPI signal

488 nm : GFP signal

# The pilot screen was performed at the DRSC (Boston, USA)

# Per well 20,000 cells were transiently transfected (L : low, M : medium and H : high level of GFP-Imp expression)

- Two sublibraries were screened:
  - Phosphatase - Kirrasin (controls)
  - 365 genes, 6 plates
  - RNA Binding Proteins: sublibrary 4 plates, 4 plates

# Experiments done in replicates:
  - 24 plates/20 wells/5 views/17 stacks/5 channels ~ 3.10^9 images

II. Pilot screen

A. CellProfiler Object segmentations and Feature extraction

B. CellProfiler Classifier training: - 2 sets of 2505 objects - 4 classes - 25 rules

C. CellProfiler Analytic:

- GFP signal segmentation and analysis

- DAPI signal segmentation and analysis

- Fluorescence

- Statistical analysis

- Genes selected for further studies

III. Workflow

High throughput microscopy-based RNAi pilot screen

Distribution of the stats scores from the pilot second stage: the distribution is robust with a good mean localisation per plates (excluding outliers).

- For each view, DRSC signal MIP

- 93 084 MIP

- GFP-Imp images (A) are first locally-normalised to reduce the effect on a non-uniform illumination. Then, a wavelet-based segmentation produces the corresponding oversegmented (superpixels) image (B). Finally, the interface allows to manually select the superpixels to segment the cells of interest by creating masks (B). In the future, an automatic process for superpixels merging will be developed.

V. Semi-Automatic Cell Segmentation: from GFP MIP images to cell masks

A. GFP MIP images (A) are first locally-normalised to reduce the effect on a non-uniform illumination. Then, a wavelet-based segmentation produces the corresponding oversegmented (superpixels) image (B). Finally, the interface allows to manually select the superpixels to segment the cells of interest by creating masks (B). In the future, an automatic process for superpixels merging will be developed.

VI. SPADE: an algorithm for small particle detection

A. CellProfiler Object segmentations and Feature extraction

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C. CellProfiler Analytic:

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D. The mask generated by the supervised segmentation algorithm (A) is used to extract from the original GFP image presented for enhanced contrast normalisation (B). the GFP positive cytoplasm of healthy cells whose intensity ranges from 100 to 1900 (C). This later is analyzed by SPADE that combines the point process concept of minimizing an energy function with the use of a pre-defined dictionary of shapes, allowing the detection of small objects, ranging from 4 to 25 pixels in a homogeneous context (D) (1). http://spade.ethz.ch (2)

VII. Preliminary data:

A. Plate well

B. RNP

C. % of RNP per spot

D. Supplementary

- Average abs of Imp-RNP per unit area (~SEM)

Images from different control wells (LacZ and water) of one or two independent plates as well as images from 2 possible lines were analyzed as described before (IV to VII). Rosettes show robustness of the controls. As for quality control, we will use a median normalisation per plate (excluding controls). Hits will be identified on the basis of their average number of RNP containing RNP per unit area as well as other criteria to be tested.

VIII. Perspectives

- Automated merging process for image treatment
- Develop the statistical framework to detect hits
- GFP-Imp functional analysis of hits
- Improve the experimental method using additional markers (phalaein, organelle markers)

Collaborators

- DRSC (Harvard, USA) - high throughput RNAi screen

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- Marseille team numbers

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