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 mRNP granules. These assemblies form upon phase separation of individual RNA and protein components, a process involving the establishment of multivalent weak interactions and their regulations via post-translational modifications. Defects in their properties have 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 RNA-binding visual screen to identify factors that regulate the formation of Imp-containing granules. This requires 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 SQL 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 validated the experimental design and developed our pipeline for data mining are presented.

II. Pilot screen

Plate map

<table>
<thead>
<tr>
<th>Well</th>
<th>DAPI signal strength</th>
<th>GFP signal strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Strong</td>
<td>Strong</td>
</tr>
<tr>
<td>2</td>
<td>Weak</td>
<td>Weak</td>
</tr>
</tbody>
</table>

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

For each view:

- DAPI signal MIP
- GFP signal MIP

CellProfiler Object segmentations and Feature extraction

- 90% of the images are correctly segmented

CellProfiler Analysis

- Classifier training:
  - 2 sets of 2593 objects
  - + classes
  - 25 rules

- Gene selected for further studies

V. Semi-Automatic Cell Segmentation:

- GFP MIP images (A) are first locally-normalized to reduce the effect on a non-uniform illumination. Thus, a wavelet-based segmentation produces the corresponding over-segmented (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

The mask generated by the supervised segmentation algorithm (A) is used to extract from the original MIP image perilous for enhanced contrast normalization (B), the GFP positive cytoplasm of healthy cells whose intensity ranges from 100 to 1000 (C). This latter 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). (A) 1 http://spade.igpdb.fr http://www.cellprofiler.org

VII. Preliminary data:

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

VIII. Perspectives

-Automate the merging process for image treatment
- Develop the statistical framework to detect hits
- Improve the experimental protocol using additional marks (phaloids, organelle markers)

Collaborators

- DRSC (Harvard, USA)
- High throughput RNA screen

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