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

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I. Context

In vitro, 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 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 $\Phi$ 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 vitro, Imp is present in cytoplasmic RNP granules, distinct from $\Phi$-bodies and visible both in neuronal cell bodies and axons. They are also detected in Drosophila S2R+ cultured cells.

II. Pilot screen

Plate map

<table>
<thead>
<tr>
<th>Well</th>
<th>Imp granules</th>
<th>GFP MIP images</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>20000 cells</td>
<td>20000 cells</td>
</tr>
</tbody>
</table>

IV. Experimental procedure quality check and selection of healthy cells

- For each well 20,000 cells were transiently transfected (L : low, M : medium, A : high level of GFP-Imp expression).
- Two sublibraries were obtained: Phosphatase - Kirasus sublibrary: 765 genes, 5 plates RNA Binding Proteins sublibrary: 496 genes, 4 plates
- Experiments done in replicates: 24 plates×10 wells×15 views×7 stacks×5 channels = 1,104,912 images

IV. Experimental procedure quality check and selection of healthy cells

- For each view
  - DAPI signal MIP
  - 931 004 MIP

Distribution of the output scores from the pilot screen among living cells is ranked two ways median normalization per plate (excluding controls). In the pilot screen, 812 different RNA targets were used to reduce the expression of one of the 696 genes encoding a RNA binding protein. A 306 different RNA targets were used to reduce the expression of one of the 576 genes encoding a kinase or protein phosphatase (III). Similarly to those of opt, we found that opt and cap69 (RNA degradation factor) cell death and cell survival respectively.

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

GFP MIP images (A) are first locally-normalized to reduce the effect on a non-uniform illumination. Then, a watershed-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 mapping will be developed.

VII. 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 processed for enhanced contrast normalization (B), the GFP positive cytoplasm of healthy cells whose intensity ranges from 100 to 1000 (C).

VIII. Perspectives

- Automate the merging process for image treatment
- Develop the statistical framework to deduct hits

- Improve the experimental readout using additional markers (phaloidin, organelle markers)

VIII. Collaborators

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

VIII. Fundings

- Les Algorithmes
- gforge