Discovering Biosignatures of Cav1 Domains: Computational Methods for Super-resolution Microscopy

LSI Imaging - Super-resolution Microscopy Mini-Symposium

July, 5th. 2016

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Outline

● Nanoscopy: Background and Challenges
● Previous Quantification Methods & Their Limitations
● Proposed Method
  ○ Preprocessing
  ○ Filtering
  ○ Segmentation
  ○ Analysis
  ○ Identification
● Results & Conclusions
Microscopy Becomes Nanoscopy!

Eric et al. won the 2014 Nobel Prize in Chemistry

They are honored for bringing “optical microscopy into the nanodimension”

TheScientist.com October 8, 2014
Nanoscopy Challenges

Provides a plethora of fluorescent signals (events)

Errors in event localization

Big data problem (Computational space and time complexity)

Noisy events unrelated to subcellular structures (e.g. background)
## Related Work

<table>
<thead>
<tr>
<th>Work</th>
<th>Technique</th>
<th>Weakness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Owen et al. 2010</td>
<td>Ripley’s K, L, H-functions for cluster analysis</td>
<td>Global cluster analysis</td>
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<tr>
<td>Pageon et al. 2013</td>
<td></td>
<td>Restricted to analyze homogeneous clusters</td>
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<td>Rossy et al. 2013</td>
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<td>Pereira et al. 2012</td>
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<tr>
<td>Lillemeier et al. 2010</td>
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<td>Lagache et al. 2015</td>
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<tr>
<td>El Beheiry et al. 2013 (ViSP)</td>
<td>Visualization, reconstruction, and density plot of 3D super-resolution data</td>
<td>Limited in quantification capabilities</td>
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<tr>
<td></td>
<td></td>
<td>Assumes the data is clean</td>
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<tr>
<td>Rossy et al. 2014</td>
<td>Univariate/bivariate Getis and Franklin’s local point pattern analysis</td>
<td>Restricted to analyze homogeneous clusters</td>
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## Related Work continued

<table>
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<tr>
<td>Caetano et al. 2015 (MiiSR)</td>
<td>Density-based methods and Ripley’s H-function</td>
<td>Cannot deal with varying clusters densities and hollow clusters</td>
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<td></td>
<td>Sensitive to noisy events</td>
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<tr>
<td>Rubin-Delanchy et al. 2015</td>
<td>Bayesian approach and Ripley’s functions</td>
<td>Sensitive to the prior settings</td>
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<td></td>
<td></td>
<td>Not suitable for small clusters</td>
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<tr>
<td>Levet et al. 2015 (SR-Tesseler)</td>
<td>Voronoi tessellation</td>
<td>Crude segmentation</td>
</tr>
<tr>
<td>Andronov et al. 2016 (ClusterViSu)</td>
<td></td>
<td>Hard to deal with hollow clusters</td>
</tr>
<tr>
<td></td>
<td></td>
<td>It’s multiscale capability are limited</td>
</tr>
</tbody>
</table>
Caveolin-1 Domains

Caveolin-1 is overexpressed and secreted in prostate tumors

Caveolae: Ω-shaped (50-100 nm) invaginations

Scaffolds: Non-Caveolar domains
Caveolin-1 Analysis of PC3 Cell lines

PC3

PC3-PTRF

Widefield (TIRF)

GSD (TIRF)
Proposed Method

Pipeline:

Preprocessing
Filtering
Segmentation
Analysis
Identification

GSD image
3D point cloud
ROIs
Network analysis

Shape
Hollowness
Network
Statistical
Feature Extraction

Identification

Pipeline:

Preprocessing
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Proposed Method

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- Preprocessing
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GSD image → 3D point cloud → ROIs → Network analysis

Shape
Hollowness
Network
Statistical
Feature Extraction

Identification

Segmentation
Filtering
Proposed Method

Pipeline:
- Preprocessing
- Filtering
- Segmentation
- Analysis
- Identification

1. GSD image
2. 3D point cloud
3. ROIs
4. Network analysis

5. Shape
6. Hollowness
7. Network
8. Statistical
9. Feature Extraction
10. Segmentation
11. Filtering
Proposed Method

Pipeline:

Preprocessing
Filtering
Segmentation
Analysis
Identification

GSD image ➔ 3D point cloud ➔ ROIs ➔ Network analysis

Filtering ➔ Segmentation ➔ Analysis ➔ Identification

Feature Extraction

Shape
Hollowness
Network
Statistical
Proposed Method

Pipeline:

1. Preprocessing
2. Filtering
3. Segmentation
4. Analysis
5. Identification
Proposed Method

Pipeline:

1. Preprocessing
2. Filtering
3. Segmentation
4. Analysis
5. Identification

- GSD image
- 3D point cloud
- ROIs
- Network analysis

- Shape
- Hollowness
- Network
- Statistical
- Feature Extraction

Pipeline Diagram:

- Preprocessing
- Filtering
- Segmentation
- Analysis
- Identification
Identification Model

Dataset

PC3 cells
PC3-PTRF cells

Training phase

2 groups

GB
GA
GC
GD

Testing phase

Test image

Filtering & blobs generation

Find the most similar group to each one of the blobs

Blobs identification

Filtering and blobs generation

Learn groups

Match groups / identification

Labeling
Results

Get rid of the multiple blinking of individual fluorophores (artifact) via Iterative merging algorithm.
Results

Filter-out the noisy blinks to get the clusters. Then, segment the clusters (blobs generation)

3D point cloud → Multi-threshold network analysis

Blobs generation ← Filtering
Results

Blobs identification and labeling in both populations
Results

Good Signatures

Hollowness

Sphericity

Avg. distance to centroid (nm)

PC3-PTRF groups
PC3 groups

CS
Results

Good Signatures

Caveolae (hollow structures)

Hollowness

Sphericity
Results

Good Signatures

Caveolae (hollow structures)

G1 is similar to GC & GD in hollowness

Hollowness

Sphericity
**Results**

**Good Signatures**

- **Caveolae (hollow structures)**
- **G1 is similar to GC & GD in hollowness**
- **Scaffolds (less spherical structures)**

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**Hollowness**

![Hollowness Graph](image)

**Sphericity**

![Sphericity Graph](image)
Results

Good Signatures

Caveolae (hollow structures)

G1 is similar to GC & GD in hollowness

Scaffolds (less spherical structures)

Hollowness

G2 is similar to GA in hollowness and sphericity

Sphericity
Results

Good Signatures

Number of blinks / blob

Modularity
Results

Good Signatures

Caveolae have more blinks

Number of blinks / blob

Modularity

- Caveolae have more blinks
- # blinks / blob bar chart
- Modularity bar chart
Results

Good Signatures

Caveolae have more blinks

Number of blinks / blob

Modularity

Caveolae are modular structures
## Good Signatures summary

<table>
<thead>
<tr>
<th>Signature</th>
<th>Caveolae</th>
<th>Scaffolds</th>
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</thead>
<tbody>
<tr>
<td>Sphericity</td>
<td>More spherical</td>
<td>Less spherical</td>
</tr>
<tr>
<td>Hollowness</td>
<td>Hollow</td>
<td>Not hollow</td>
</tr>
<tr>
<td>Avg. clust. coeff.</td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td>Avg. degree</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Modularity</td>
<td>Higher</td>
<td>Lower</td>
</tr>
<tr>
<td>Avg. char. path length</td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td>Density (@ 80 nm)</td>
<td>Lower</td>
<td>Higher</td>
</tr>
<tr>
<td># blinks</td>
<td>Higher</td>
<td>Lower</td>
</tr>
</tbody>
</table>
Results

Caveolae

Blob’s blinks

Blob’s network @ 50 nm

Blob’s network @ 80 nm

Blob’s modules

Scaffold

Z nm

X nm

Y nm

9550 9450 9400 9350 7500 7650

Z nm

X nm

Y nm

9550 9450 9400 9350 7500 7650

Z nm

X nm

Y nm

9550 9450 9400 9350 7500 7650

Z nm

X nm

Y nm

9550 9450 9400 9350 7500 7650

Z nm

X nm

Y nm

9550 9450 9400 9350 7500 7650

Z nm

X nm

Y nm

9550 9450 9400 9350 7500 7650

Z nm

X nm

Y nm

9550 9450 9400 9350 7500 7650
Summary

1. Nanoscopy is a new imaging modality allowing us to study subcellular proteins (e.g. in cardiology and cancer)
2. Large datasets
3. Identification, visualization, quantification of 3D noisy super-resolution data
4. Apply and extend computational tools for new insights
5. Discover biosignatures
Thank You!