# Semi-supervised segmentation of neurons from brainbow images

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# The Brainbow construct



A tool to identify individual neurons by color

## The structural substrate beneath



Livet et al, 2007

# The structural substrate beneath



Livet et al, 2007

## Noise sources



Livet et al, 2007

# 3D image stacks of neuronal tissues



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# Maximum intensity projections





# Noise in the Improved Brainbow



# The task: segment individual neurons

# Partition the set of foreground voxels S = { [x<sub>v</sub> y<sub>v</sub> z<sub>v</sub> r<sub>v</sub> g<sub>v</sub> b<sub>v</sub>] }<sub>v</sub>



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Very large apparent size

# Supervoxels: connected voxels with similar colors

- Identify the foreground
- Obtain an oversegmentation



- Summarize each supervoxel's color with the mean:
- # voxels / # supervoxels ~ 100

#### Collaborative filtering and watershed transformation



#### STD projection of 3d stack to be segmented



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- 764 x 704 x 223 voxels ~ 76µ x 70µ x67µ
- 125K supervoxels



#### A first approach: weighted k-means

- Transform to LUV color space
- Weights: Color SNR  $\alpha \sqrt{voxel count}$



# Utilize both space and color

#### Morphology

"Neurons are connected components."

"Brainbow promises consistent colors."

Color



### 1. Undersegment with weighted k-means



### 2. Retain big connected components



## 3. Cluster-level user manipulation



## Supervoxels are the nodes of a graph



- Edge weights decay with color (and spatial) distance
- Small supervoxels can have local edges only
- Need sparse connectivity due to size
- The task: cluster the nodes of the graph

# Semi-supervised spectral clustering



Kamvar et al, 2003 Kulis et al, 2005

- Calculate affinity matrix (<2% dense)</li>
- Impose edge weights from user input
- Calculate normalized eigenvectors of Laplacian

- Weighted k-means on the <u>feature vectors</u>
- Impose cluster memberships from user input



# 4. Spectral clustering



## Another image stack (BM4d filtered)



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- 1020 x 1020 x 225 voxels ~ 102µ x 102µ x67µ
- 86K supervoxels

# 4. Spectral clustering



# Challenges

- Number of neurons
- Edge weights (spatio-color)
- Warmer starts for more user interaction