# Image segmentation & BPDL in microscopy

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15.03.2018 http://cmp.felk.cvut.cz/~borovji3/ https://www.researchgate.net/publication/323120618

#### Resources

- Publications:
  - Borovec J. (2017). Automatic analysis of gene expressions in Drosophila microscopy images. <u>ftp://cmp.felk.cvut.cz/pub/cmp/articles/borovec/Thesis-TR-2017-07.pdf</u>
  - Borovec J., Svihlik J., Kybic J., Habart D. (2017). Supervised and unsupervised segmentation using superpixels, model estimation, and Graph Cut. SPIE Journal of Electronic Imaging 26(6), 061610, <a href="http://doi.org/10.1117/1.JEI.26.6.061610">http://doi.org/10.1117/1.JEI.26.6.061610</a>
  - Borovec J., Kybic J., Nava R. (2017) Detection and Localization of Drosophila Egg Chambers in Microscopy Images. In: Wang Q., Shi Y., Suk HI., Suzuki K. (eds) Machine Learning in Medical Imaging. MLMI 2017. LNCS, vol 10541. Springer, Cham. <u>http://doi.org/10.1007/978-3-319-67389-9\_3</u>
  - Borovec J., Kybic J., Sugimoto, A. (2017). Region growing using superpixels with learned shape prior. SPIE Journal of Electronic Imaging 26(6), 061611, <u>http://doi.org/10.1117/1.JEI.26.6.061611</u>
  - Borovec J., Kybic J. (2016) Binary Pattern Dictionary Learning for Gene Expression Representation in Drosophila Imaginal Discs. In: Computer Vision – ACCV 2016 Workshops. Lecture Notes in Computer Science, vol 10117, Springer, <u>http://doi.org/10.1007/978-3-319-54427-4\_40</u>

### Overview

- 1. Problem statement
- 2. Used datasets
- 3. Instance segmentation
  - a. Structure (tissue) segmentation on superpixels
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  - c. Region growing on superpixels
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- 5. Conclusion & Future work

### Motivation

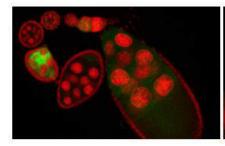
Why Drosophila?

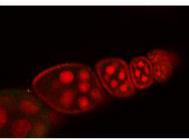
- High gene similarity with mumals (~ 93%)
- Short life cycles -> gene evaluation in generations

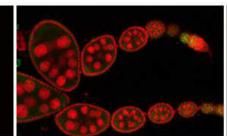
Automatic image processing:



- Analyses require thousands of images to be processed
- Very time consuming for experts

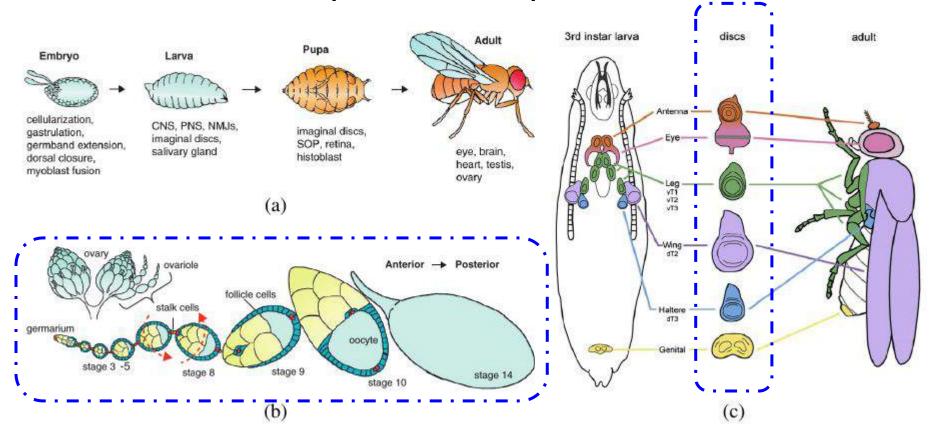








#### Schema of Drosophila development



### Sample images

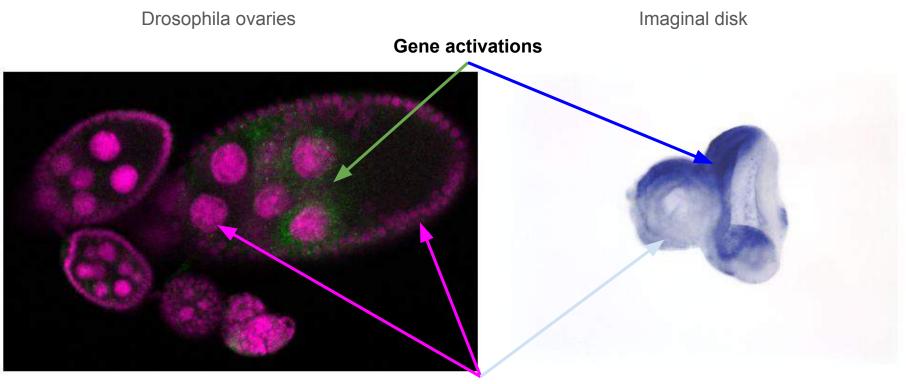
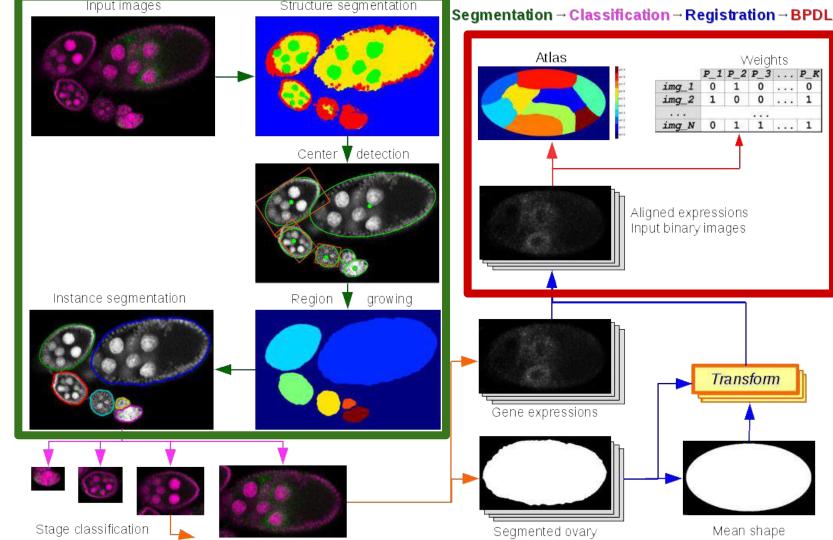


Image analysis pipeline

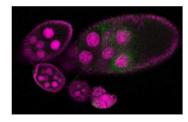


#### **Drosophila Datasets**

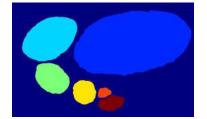
Just a few annotations in biomedical imaging is common issue...

- Noisy images with local deformations
- Low image variance (patterns)

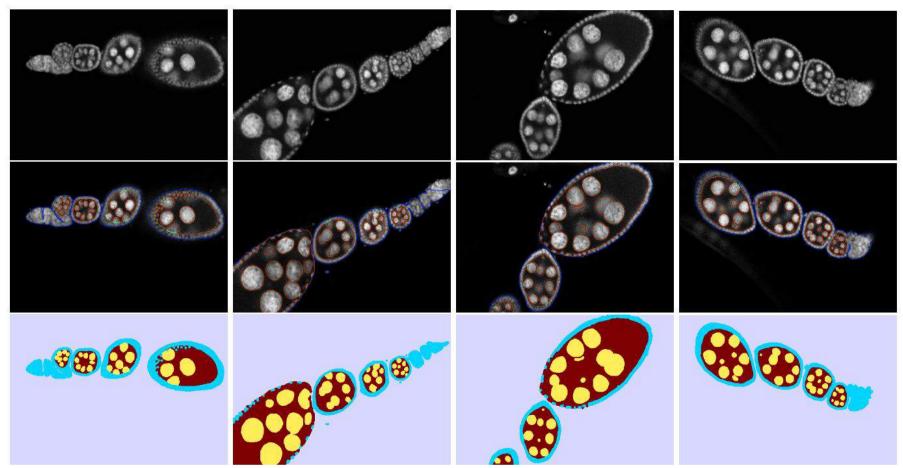
	Domain	Semantic annot.	Instance annot.
Ovaries	2.5D	72	250
Imaginal discs	2D	15	-



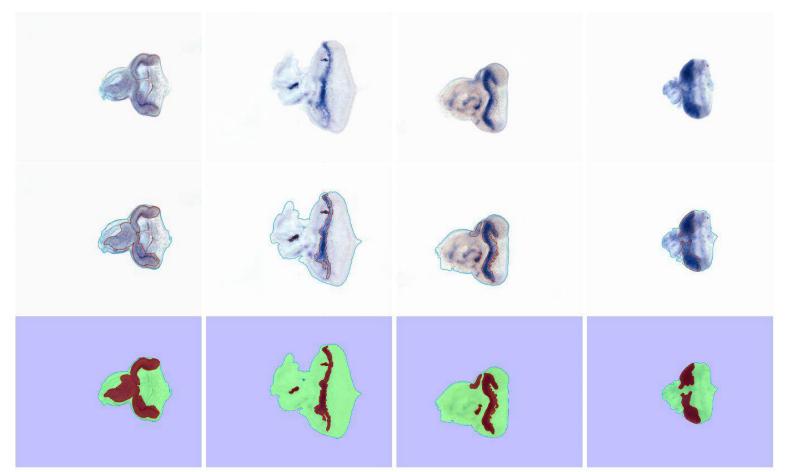




#### Drosophila ovary - semantic segmentation



#### Drosophila imaginal discs - semantic segmentation

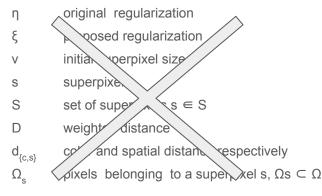


#### Notations

#### Image related

- $\Omega$  set of pixels (image plane)
- I input image function I :  $\Omega \rightarrow Rm$
- L set of labels

#### Superpixels



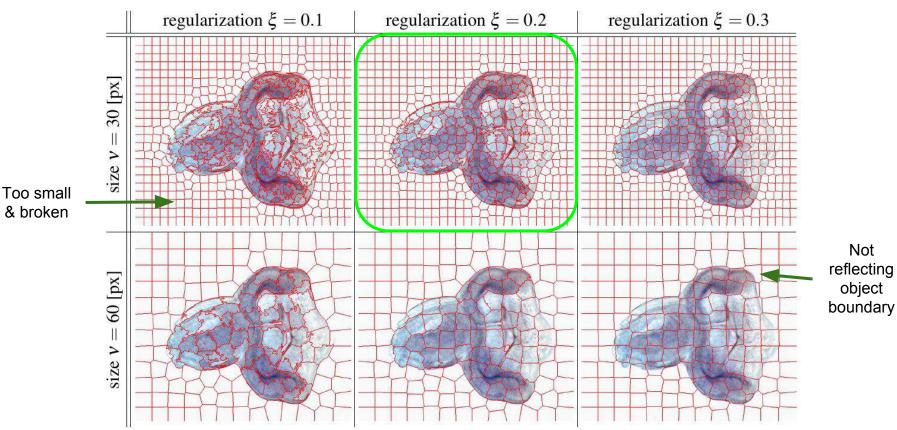
#### Segmentation & Region growing

- $y_{_{\Omega}} \qquad \text{pixel-wise segmentation function } y\Omega:\Omega\to L$
- $Y_{\Omega}$  ordered set of pixel-wise segmentation Y Ω = yΩ (Ω)
- y superpixel segmentation function  $y : S \to L$  with abbrev. for ys = y(s)
- Y ordered set of superpixel segmentation Y = y(S)
- x feature vector
- X set of features  $xs \in X$  for all superpixels
- $d_{_{\!\!\{M,E,T\,\}}}$  Manhattan, Euclidean and Tchebychev distances
- U, B unary (data) and binary (pairwise) term for GC respectively
- g image descriptor
- r vector of ray distances
- c vector of object centers
- m statistical shape model m = [c, r,  $\Theta$ , w]
- M mixture of shape model
- w vector with model weights
- q cumulative probability of spatial prior

#### **Dictionary Learning**

- g image appearance (binary association to a class)
- G set of images  $g \in G$
- $\boldsymbol{y}_{\Omega}$  ,  $\boldsymbol{Y}_{\Omega}^{-}$  atlas (binary patterns, segmentation)
- w vector with binary weights
- W matrix with binary weights  $w \in W$

### Superpixels - Imaginal discs



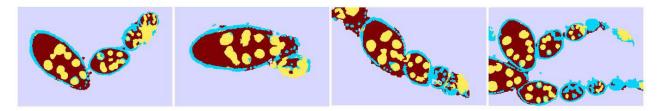
## Superpixels - Drosophila ovary

regularization  $\xi = 0.1$ regularization  $\xi = 0.3$ regularization  $\xi = 0.5$ size  $v = 30 \, [px]$ = 60 [px] 2 size

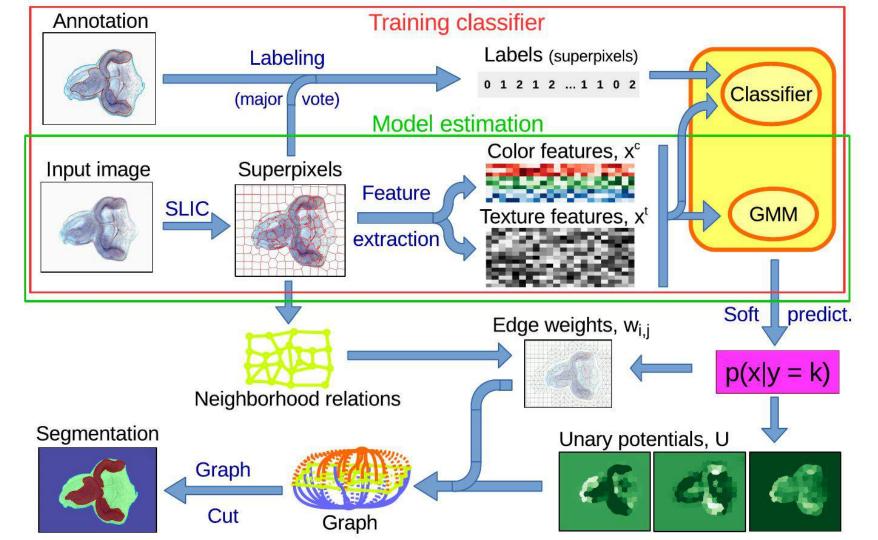
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# Supervised and unsupervised segmentation using superpixels, model estimation, and Graph Cut



Jiří Borovec, Jan Švihlík, Jan Kybic, David Habart, "**Supervised and unsupervised segmentation using superpixels, model estimation, and graph cut,**" Journal Electron. Imaging 26(6), 061610 (2017), <u>DOI:</u> <u>10.1117/1.JEI.26.6.06161</u>0.



### Segmentation method overview

Image segmentation method consisting of the following steps:

- 1. Computation of superpixels SLIC
- 2. Extraction of superpixel-based descriptors:
  - a. Color mean, median, energy, STD
  - b. Texture Leung-Malik filter bank
- 3. Calculating image-based class probabilities:
  - a. Supervised Random Forest, k-NN, Adaboost, ...
  - b. Unsupervised Gaussian Mixture Model
- 4. Spatial regularized superpixel classification using Graph Cut
  - a. Edge weights color, features, model

#### Problem formulation

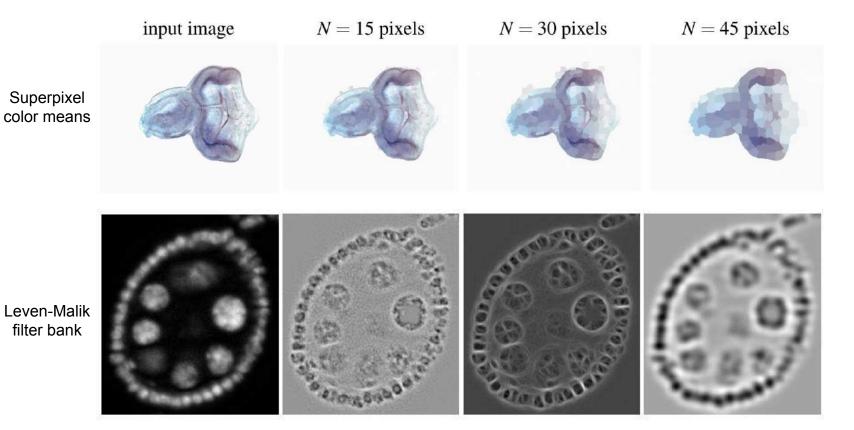
Formulation (standard) as

$$Y^* = \arg \max_{Y} P(Y|X) = \arg \max_{Y} \frac{p(X|Y) \cdot P(Y)}{p(X)}$$
$$P(Y) = \prod_{s \in S} h(y_s) \cdot \prod_{(i,j) \in \mathcal{N} \subseteq S^2} R(y_i, y_j)$$
$$Y^* = \arg \max_{Y} \prod_{i \in S} \left( p(\boldsymbol{x}_i|y_i) \cdot h(y_i) \right) \cdot \prod_{(i,j) \in \mathcal{N}} R(y_i, y_j)$$

Energy minimisation

$$Y^* = \arg\min_{Y} \sum_{s} \underbrace{-\log\left(p(\boldsymbol{x}_s|y_s) \cdot h(y_s)\right)}_{U_s(y_s)} + \underbrace{\sum_{(i,j) \in \mathcal{N}} -\log R(y_i, y_j)}_{\beta w_{i,j} B(y_i, y_j)}$$

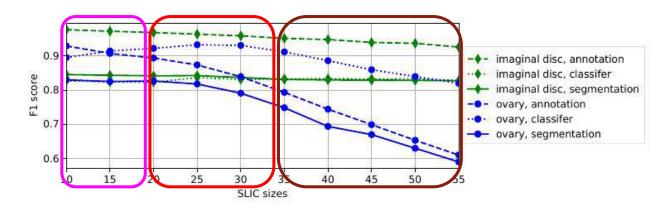
#### Superpixel features



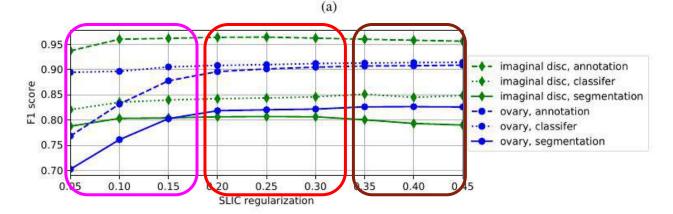
#### Influence of superpixel parameters

Low feature representativeness

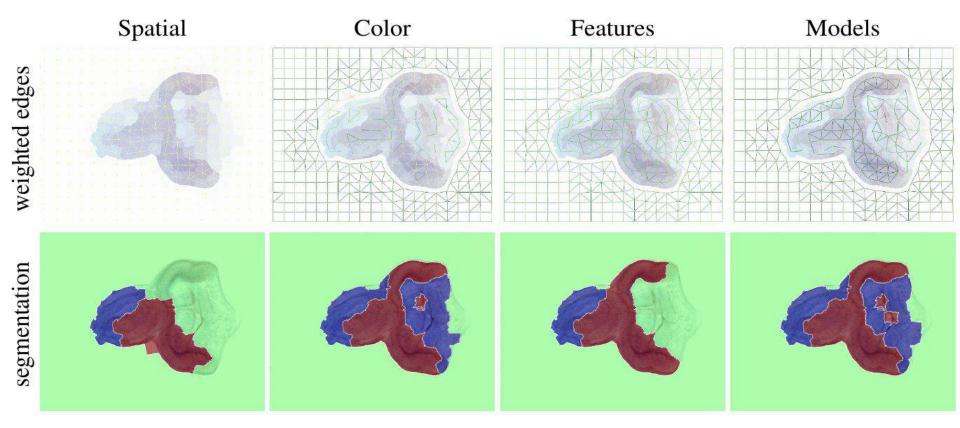
Optimum (~compromise)



Low separability



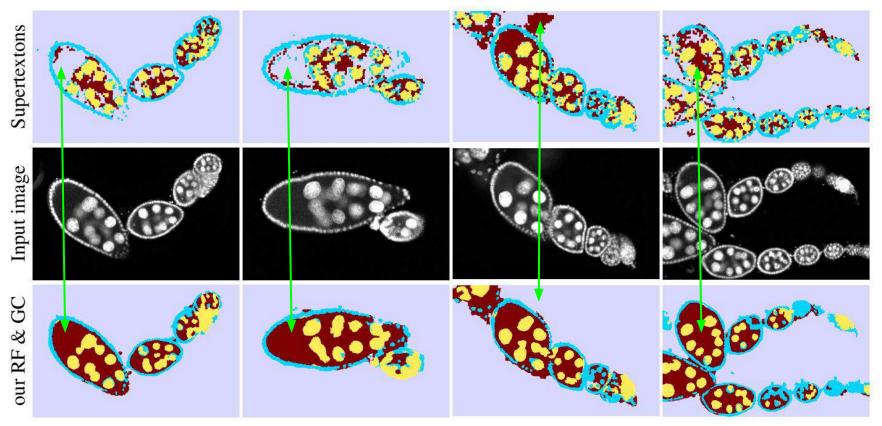
### Graph Cut - Edge weights



#### Segmentation results with SOA (F1-score)

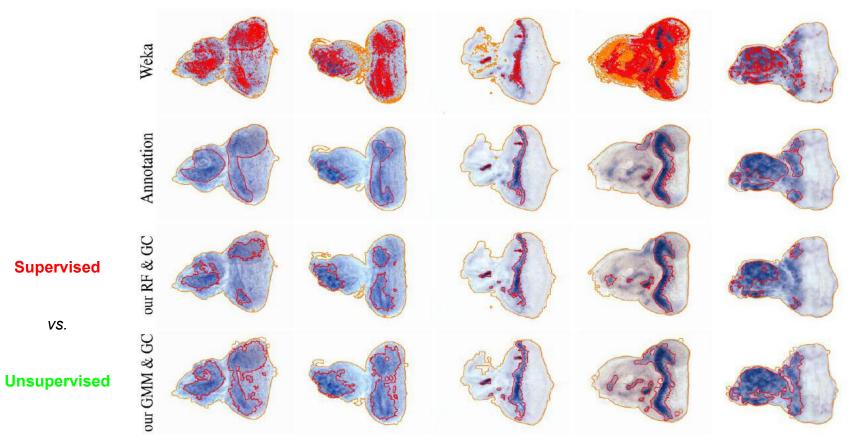
	2	Method	imaginal disc	ovary
Pixel-wise	Supervised	Weka	0.6923	0.5800
		Weka & GC(0, 100)	0.6887	0.5810
		Weka & GC(1, 50)	0.6887	0.5965
		Weka & GC(10, 50)	0.6887	0.1395
		Weka & GC(50, 100)	0.6850	0.6007
	Jup	ideal segm. $Y_A$	0.9696	0.9067
		Supertextons	-	0.7488
tels		our RF	0.8181	0.8201
vid.		our RF & GC	0.8229	0.8600
Superpixels	Unsuper.	our GMM	0.7542	0.5967
		our GMM & GC	0.7644	0.6039
		our GMM [gr]	0.7301	0.6009
		our GMM [gr] & GC	0.7564	0.6083

#### Advantage of using Graph Cut



#### Supervised vs Unsupervised

VS.



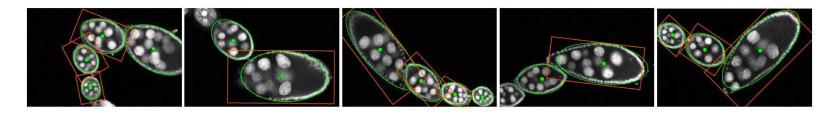
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# Detection and localization of Drosophila egg chambers in microscopy images



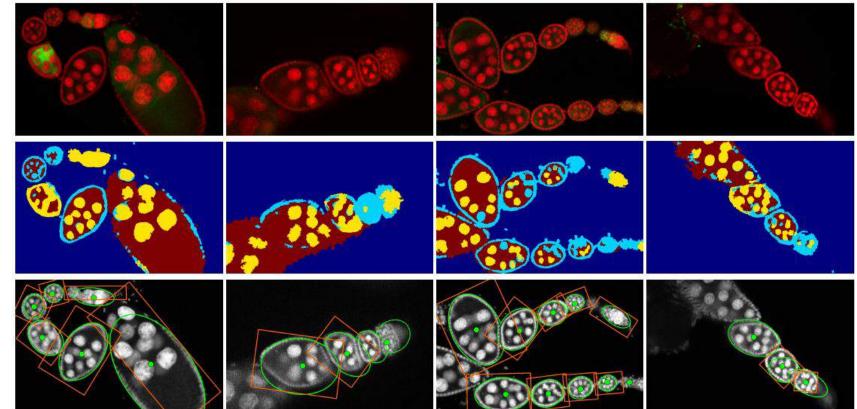
Borovec J., Kybic J., Nava R. (2017) **Detection and Localization of Drosophila Egg Chambers in Microscopy Images**. In: Machine Learning in Medical Imaging. LNCS, vol 10541. Springer, <u>DOI:</u> <u>10.1007/978-3-319-67389-9</u>\_3.

#### **Center detections - illustration**

Image

Input segm.

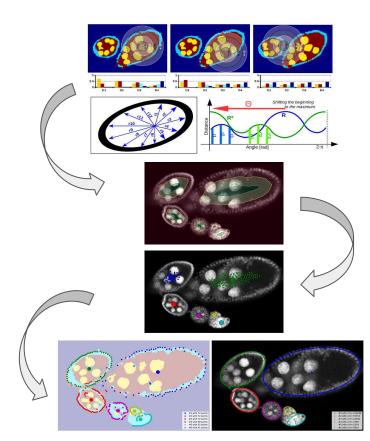
Goal



27

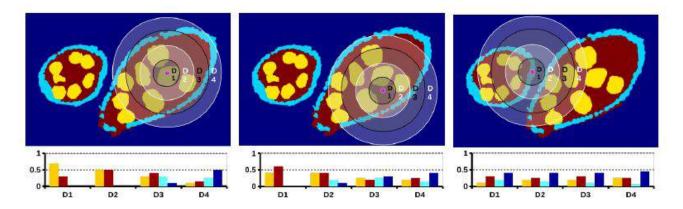
#### Schema

- 1. Extract pixel features
  - a. Label histogram
  - b. Ray features
- 2. Train classifier
- 3. Group center candidates
- 4. Ellipse fitting

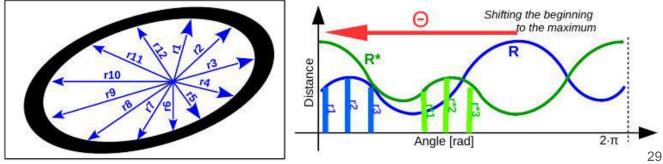


#### Features for center detection

Label histogram 



Ray features 



## Classification & Grouping

Train classifier on 3 zones

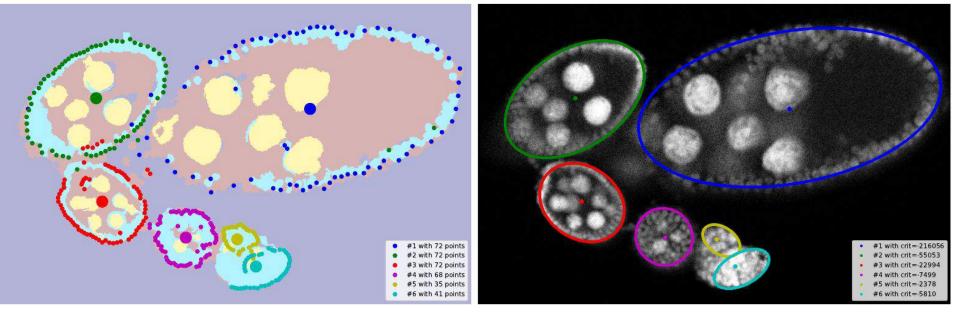
Negative

Clustering of center candidates with DBSCAN

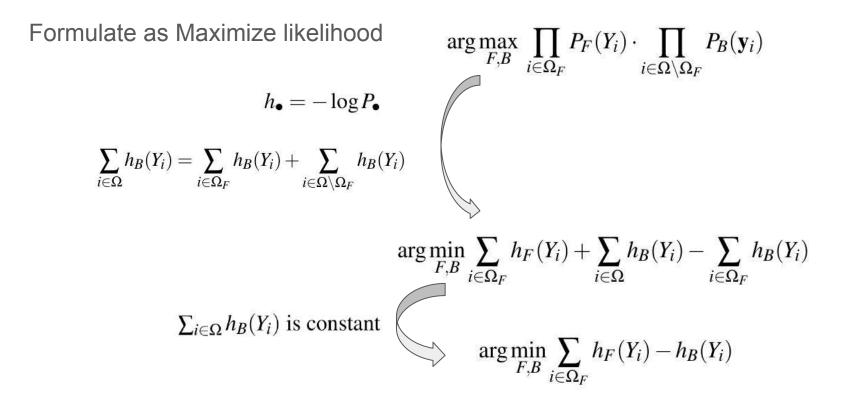
No not case Positive

## Ellipse fitting

Fitting ellipses to boundary points to maximise foreground labels inside ellipse



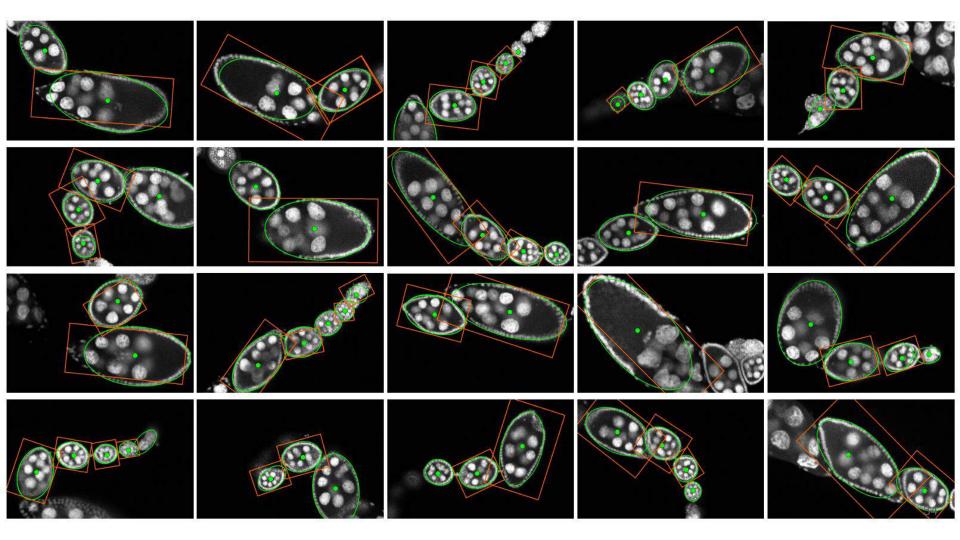
### Ellipse fitting



## Ellipse pruning

Ellipse approximation eliminate multiple center detection in single egg

Egg chambers	Stage					
Egg chambers	1	2	3	4	5	
number	921	1403	865	834	836	
false negatives	306 (33%)	158 (11%)	6 (0.7%)	1 (0.1%)	0 (0.0%)	
multiple detections (MD)	37 (4.0%)	31 (2.2%)	109 (12%)	80 (9.6%)	90 (11%)	
MD after ellipse fitting	18 (2.0%)	13 (0.9%)	27 (3.1%)	20 (2.4%)	30 (3.6%)	
false positives	43 (0.9%)					



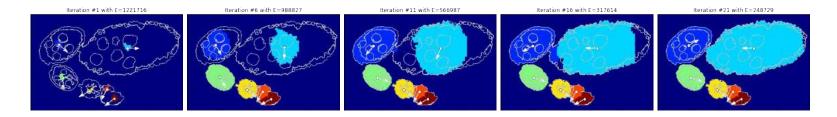
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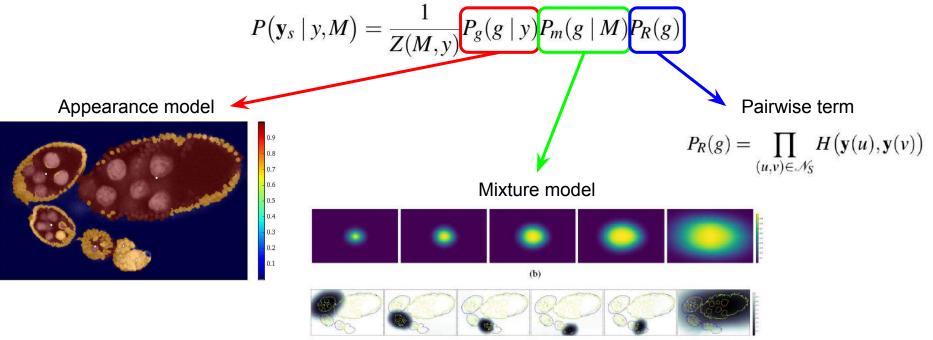
# Region growing using superpixels with learned shape prior



Jiří Borovec, Jan Kybic, Akihiro Sugimoto, "**Region growing using superpixels with learned shape prior,**" Journal Electron. Imaging 26(6), 061610 (2017), <u>DOI: 10.1117/1.JEI.26.6.06161</u>1.

# Region growing - variational framework

Formulated as:



# Region growing - variational framework

Where:

$$P_{g}(g \mid y) = \prod_{i \in \Omega} P_{g}\left(g(s(i)) \mid y(s(i))\right) = \prod_{s \in S} P_{g}\left(\mathbf{y}_{s} \mid y(s)\right)^{|\Omega_{s}|}$$

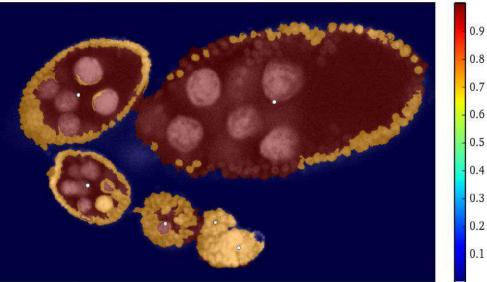
$$P_{m}(g \mid M) = \prod_{i \in \Omega} P_{m}\left(g(s(i)) \mid M\right) = \prod_{s \in S} P_{m}\left(\mathbf{y}_{s} \mid M\right)^{|\Omega_{s}|}$$

$$P_{R}(g) = \prod_{(u,v) \in \mathcal{M}_{S}} H\left(\mathbf{y}(u), \mathbf{y}(v)\right)$$
Resolves in energy minimisation:
$$E'(g) = \sum_{s \in S} \underbrace{|\Omega_{s}| \left[D_{s}(\mathbf{y}_{s}) + \beta V_{s}(\mathbf{y}_{s})\right]}_{U_{s}(y_{s})} + \gamma \sum_{(u,v) \in \mathcal{M}_{S}} B\left(\mathbf{y}(u), \mathbf{y}(v)\right)$$

# Appearance model

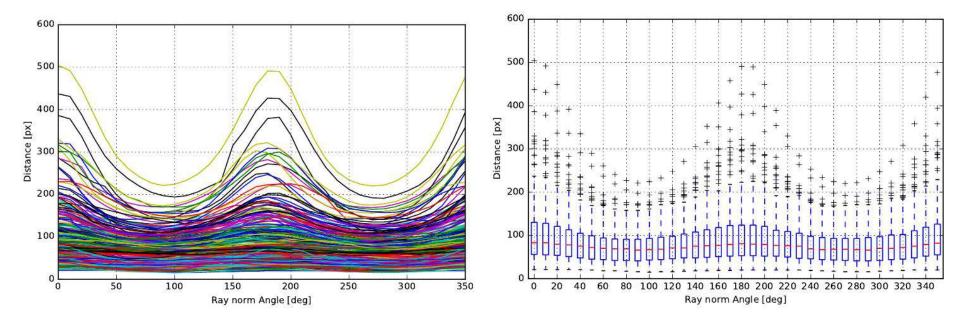
Associating a probability for each pixel (superpixel) whether it belongs to an object or not

$$P_g(\mathbf{y}(s)|g_s) = \begin{cases} P_g(g_s) & \text{for } \mathbf{y}(s) \neq 0\\ 1 - P_g(g_s) & \text{for } \mathbf{y}(s) = 0 \end{cases}$$



#### Measured shapes

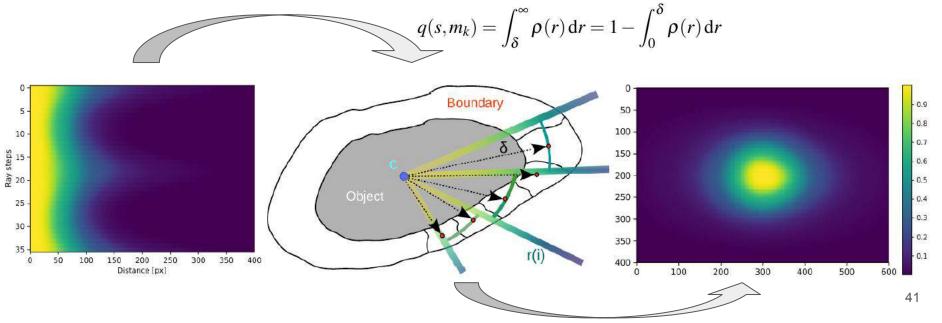
Measured egg shapes as ray features from training examples (~250 eggs)



### Shape model & prior

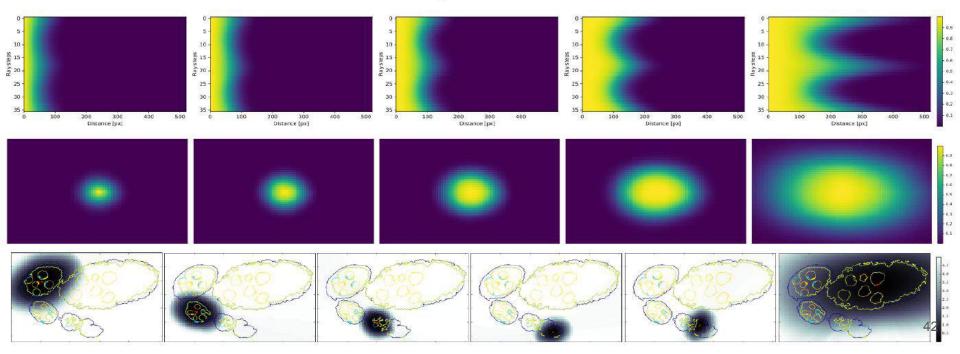
Gaussian model from all measured Ray features

Prior is represented as integral of probabilities



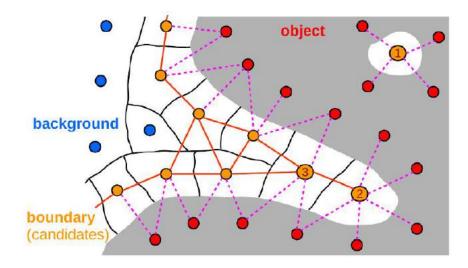
#### Mixture of Gaussian models

$$P_m(\mathbf{y}_s = k \mid M) = \begin{cases} q(s, m_k) & \text{for } k > 0\\ \prod_l (1 - q(s, m_l)) & \text{for } k = 0 \end{cases}$$



# RG - optimisation

- Iterative approach on object boundaries
- Alternating: region growing & update shape prior
- Strategies:
  - Greedy
  - Graph Cut
    - Binary
    - Multi-class
  - Object swapping



Algorithm 1: Region growing.

**Input:** S: superpixels, g: superpixel descriptors,  $c_k$ : initial object centers, M: mixture of statistical shape models

Output: object segmentation y

- 1 compute data cost *D*;
- 2 from object centers  $c_k$  set initial segmentation y and model shape parameters  $m_k$ ;
- 3 compute shape cost V;
- 4 while not converged do
- s update object pose parameters  $c_k$  and  $\Theta_k$ ;
- **if** significant change of center  $c_k$  position, orientation  $\Theta_k$  and object area **then**

update remaining object shape parameters  $m_k$ ;

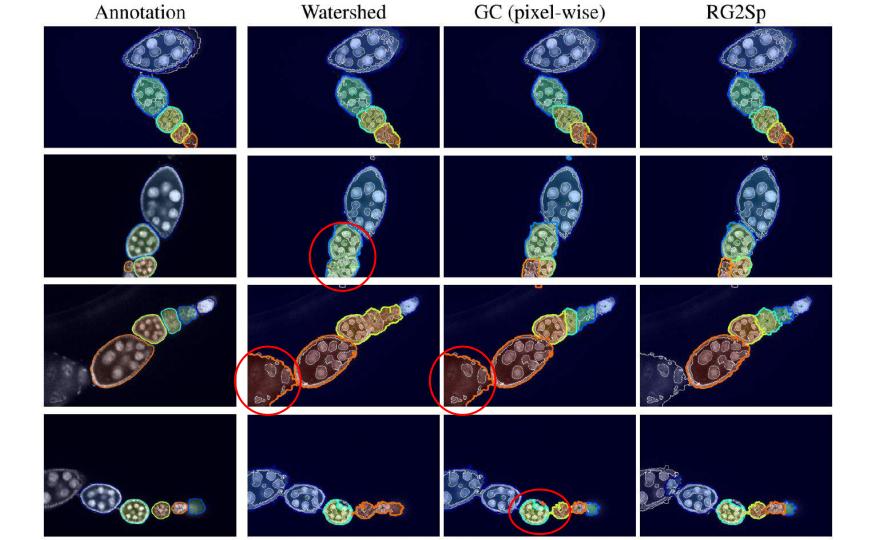
update shape costs V for all s close to k;

- 9 end
- 10 find superpixels  $\partial S_k$  on the external object boundary of k;
- optimize (7.10) wrt **y** by changing  $s \in \partial S_k$  using the greedy or Graph Cut algorithms;

12 end

7

8



# Result compared to SOA

High Jaccard index with reasonable processing time

	Jaccard	accuracy	$F_1$ score	precision	recall	time [s]
Watershed	0.5705	0.9246	0.9246	0.9246	0.9246	5
Watershed (w. morph.)	0.5705	0.9270	0.9198	0.9136	0.9327	7
Morph. snakes (image)	0.4251	0.8769	0.8070	0.9053	0.7987	784
Morph. snakes $(P_y)$	0.6494	0.8812	0.8812	0.8812	0.8812	968
Graph Cut (pixel-level)	0.7143	0.9204	0.9204	0.9204	0.9204	15
Graph Cut (superpixels)	0.3164	0.8643	0.8643	0.8643	0.8643	3
RG2Sp (greedy)	0.7527	0.9577	0.9577	0.9577	0.9577	72
RG2Sp (Graph Cut)	0.7544	0.9568	0.9568	0.9568	0.9568	9

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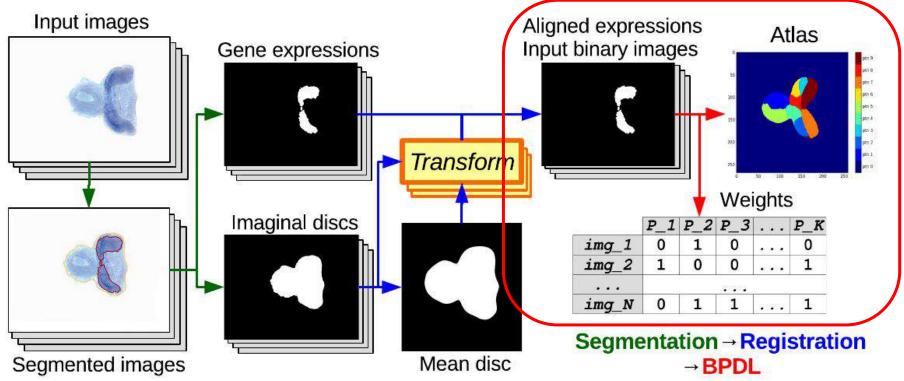
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# Binary Pattern Dictionary Learning of gene expressions



Borovec J., Kybic J. (2016) **Binary Pattern Dictionary Learning for Gene Expression Representation in Drosophila Imaginal Discs.** In: Computer Vision – ACCV 2016 Workshops. Lecture Notes in Computer Science, vol 10117, Springer, <u>DOI: 10.1007/978-3-319-54427-4\_4</u>0.

# Pipeline: segmentation - registration - BPDL



#### **Decomposition methods**

Formulation

$$\min_{Y,W} \|X - Y \cdot W\|^2$$

- Standard approaches
  - Non Negative Matrix Factorisation
  - Fast Independent Component Analysis
  - Sparse Principal Component Analyses
  - Dictionary Learning with Matching pursuit

#### Formulation

• Image representation

• Similarity measure (Hamming distance)

• Regularize neighbouring pixels

Optimization criterion

$$\hat{\mathbf{g}} = \sum_{l \in \mathbb{L}} \mathbf{w}_l \cdot [\![\mathbf{y} = l]\!]$$
  
mmming distance)  

$$F(\mathbf{g}, \mathbf{y}, \mathbf{w}) = \sum_{i \in \Omega} [\![\mathbf{g}_i \neq \hat{\mathbf{g}}_i]\!]$$

$$\text{ng pixels}$$

$$H(\mathbf{y}) = \sum_{\substack{i,j \in \Omega, i \neq j, \\ d(i,j) = 1}} [\![\mathbf{y}_i \neq \mathbf{y}_j]\!]$$

$$\mathbf{y}^*, \mathbf{w}^* = \arg\min_{\mathbf{y}, \mathbf{W}} \frac{1}{N} \sum_n F(\mathbf{g}^n, \mathbf{y}, \mathbf{w}^n) + \beta \cdot H(\mathbf{y})$$

### Alternating minimization

• Update weights - maximise overlap

$$w_{l} = \llbracket P(\mathbf{g}, \mathbf{y}, l) \geq \sigma \rrbracket \quad \text{where } \sigma = 1$$
  
and 
$$P(\mathbf{g}, \mathbf{y}, l) = \frac{\sum_{i \in \Omega, \mathbf{y}_{i} = l} \llbracket \mathbf{g}_{i} = 1 \rrbracket}{\sum_{i \in \Omega, \mathbf{y}_{i} = l} \llbracket \mathbf{g}_{i} \neq 1 \rrbracket} = \frac{\left\| \llbracket \mathbf{y} = l \rrbracket \right\|}{\sum_{i \in \Omega, \mathbf{y}_{i} = l} (1 - \mathbf{g}_{i})} - 1$$
  
Update atlas  
$$\frac{1}{N} \sum_{i \in \Omega} \sum_{\underline{n}} \left| \mathbf{g}_{i}^{s} - \sum_{l \in \mathbb{L}} \mathbf{w}_{l}^{s} \cdot \llbracket \mathbf{y} = l \rrbracket \right| + \sum_{\substack{i, j \in \Omega, i \neq j, \\ d(i, j) = 1}} \llbracket \mathbf{y}_{i} \neq \mathbf{y}_{j} \rrbracket$$

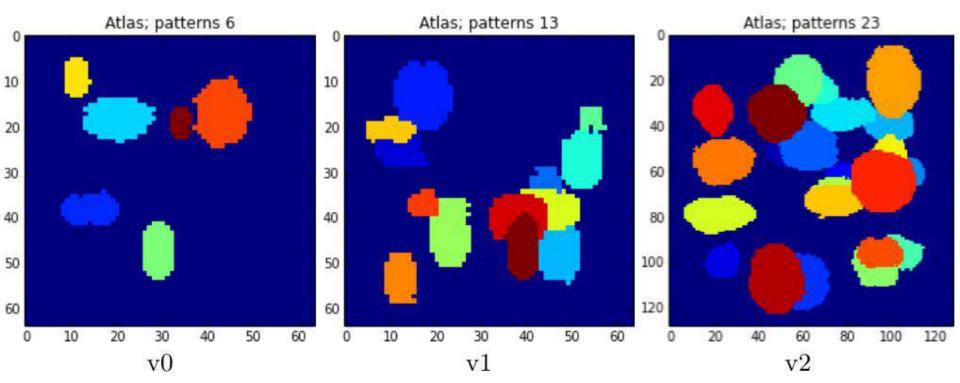
# Algorithm

#### Algorithm 1 General schema of BPDL algorithm.

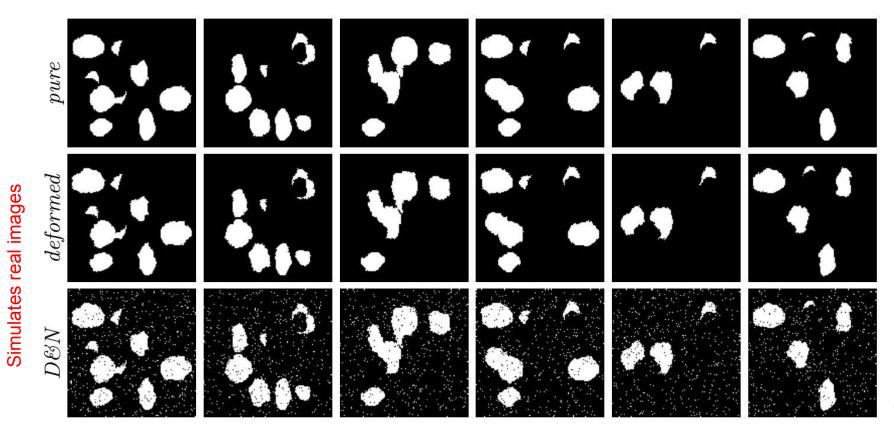
- 1: initialise atlas  $\mathbf{y}$
- 2: while not converged do
- 3: update weights  $\mathbf{w} \in \mathbf{W}$
- 4: reinitialise empty patterns in  $\mathbf{y}^*$
- 5: update atlas  $\mathbf{y}^*$ /via Graph Cut
- 6: end while

It makes the algorithm more robust to initialisation.

#### Synthetic datasets



# Synthetic images



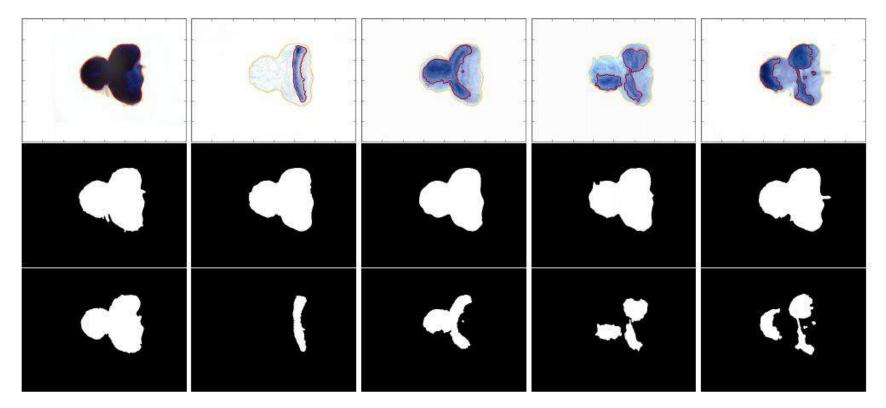
# Comparison on synth. images

data	datasets		FastICA	sPCA	DL	BPDL
v	1	$(size \ 64 \times 64 \ px, \ 13 \ patterns)$				
pure	ARS	1.0	<b>1.0</b> 0.		2 0.995	0.999
	diff.	0.0	0.0	0.029	<b>0.019</b>	0.0
	time	2.333	340.32	18.29	91 737.47	6.029
deform	ARS	0.785	0.948	0.948 0.780		0.992
	diff.	0.017	0.004	0.02	9 0.033	0.005
	time [s]	4.001	312.18	15.00	00 700.03	7.561
	ARS	0.091	0.878	0.00	9 0.0727	0.951
$D \ \mathcal{C} N$	diff.	0.048	0.010	0.06	1 0.0499	0.003
	time [s]	4.490	439.04	11.42	20 697.599	9.562

# Comparison on synth. images

data	datasets		FastICA	sPCA	DL	BPDL	
v	2	$(size \ 128  imes 128 \ px, \ 23 \ patterns)$					
	ARS	1.0	1.0	0.98	9 1.0	0.999	
pure	diff.	0.0	0.0	0.03	7 0.0	0.005	
	time [s]	82.329	5533.4	460.8	32 14786.	88.260	
	ARS	0.818	0.846	0.80	1 0.807	0.970	
deform	diff.	0.019	0.015	0.05	6 0.046	0.004	
	time [s]	144.10	5683.2	477.4	13619.	165.22	
D&N	ARS	0.120	0.612	0.02	4 0.144	0.877	
	diff.	0.036	0.036	0.09	2 0.039	0.013	
	time [s]	77.399	6912.9	485.4	4 13729.	289.51	

#### Input segmented imaginal discs

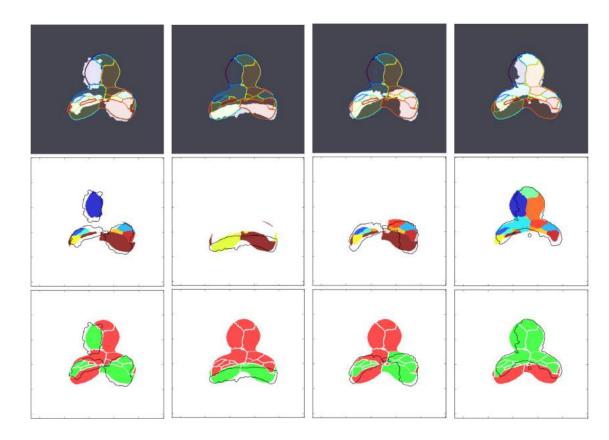


#### Visualise results on imaginal discs

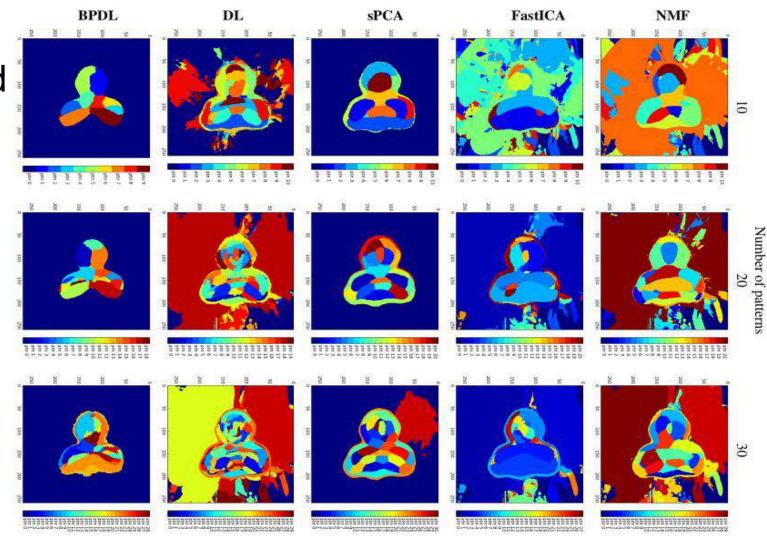
Gene & atlas

Used patterns

Pattern activation



Extracted Atlases for Imaginal discs



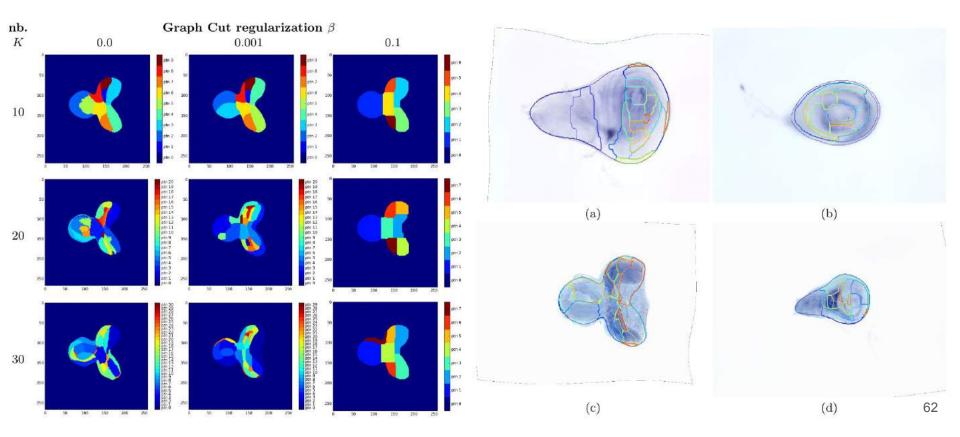
# Results on imaginal discs

No ground truth atlases...

Method	Numb	Time [min]		
	10	20	30	
NMF	0.0939	0.0823	0.0723	10
FastICA	0.1197	0.0779	0.0485	24
sPCA	0.0476	0.0413	0.0352	477
DL	0.0939	0.0648	0.0596	338
BPDL	0.0467	0.0395	0.0361	20

#### Parameter selection

### & More discs



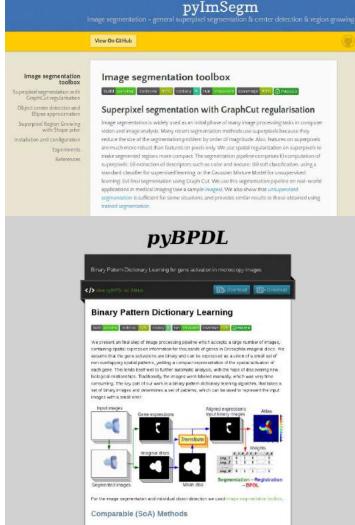
# Overview

- 1. Problem statement
- 2. Used datasets
- 3. Instance segmentation
  - a. Structure (tissue) segmentation on superpixels
  - b. Center detection and ellipse fitting
  - c. Region growing on superpixels
- 4. Binary Pattern Dictionary Learning
- 5. Conclusion & Future work

# Conclusion

- Presented four image processing methods:
  - Image segmentation on superpixels
  - Center detection on segmented images
  - Region growing with shape prior
  - Binary Pattern Dictionary Learning
  - Implementation:
    - <u>http://borda.github.com/pyImSegm</u>
    - <u>http://borda.github.com/pyBPDL</u>
- Future work
  - Complete image analysis pipeline in 2.5D
  - Try instance segmentation with CNNs

0 ...



We have our method BPOL and also as compose it to state of the act, see Extended and

