

branMorph: An image skeletonization based tool for branching cell morphology analysis

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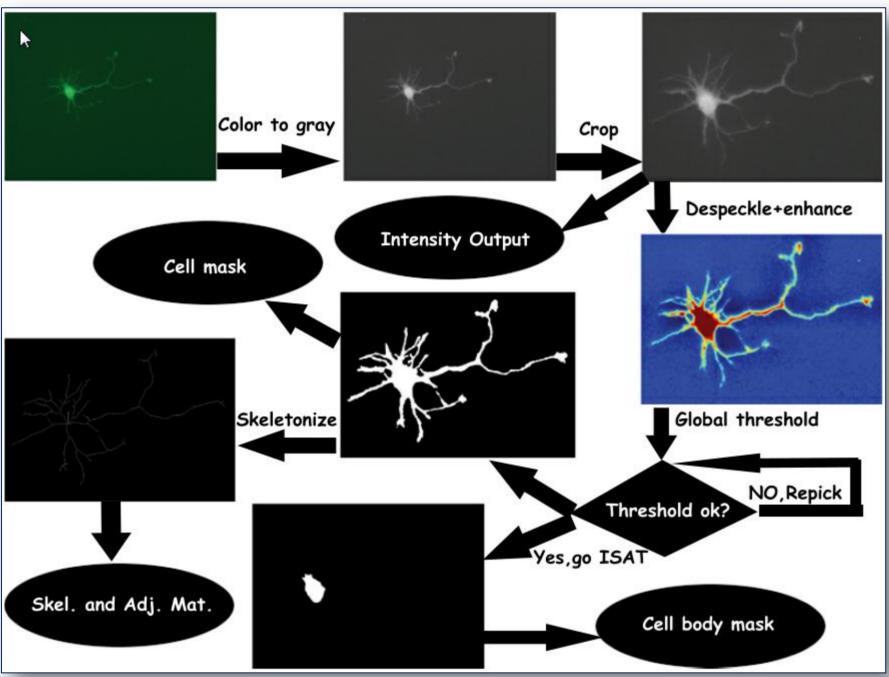
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Introduction

The biological morphology, consisting of shape, color, structure or pattern, has always been an indispensable research object in biological science domain. We devised a computational tool to model and quantify morphological features from sets of cell fluorescence images, which in here, contain pollen tube images and neuron images. A skeletonization based model is proposed to account for the cell morphologies which are shown in Tip-growth cells (TGCs) like pollen tubes and neurons.

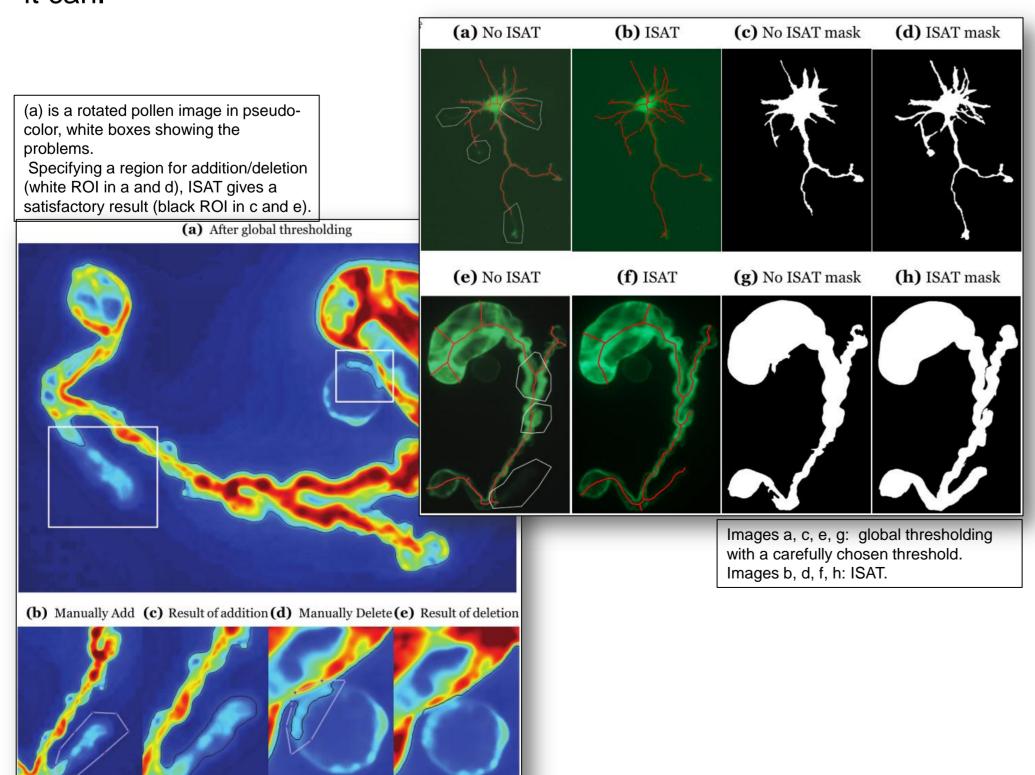
Workflow

The workflow is demonstrated using a neuron image (kindly provided by Dr. Zhi Yang from ION). The four intermediate outputs: intensity image, cell mask, cell body mask and skeleton image are represented in black ellipses. These outputs are used to perform feature extraction later on. Adj. Mat. is short for adjacency matrix.



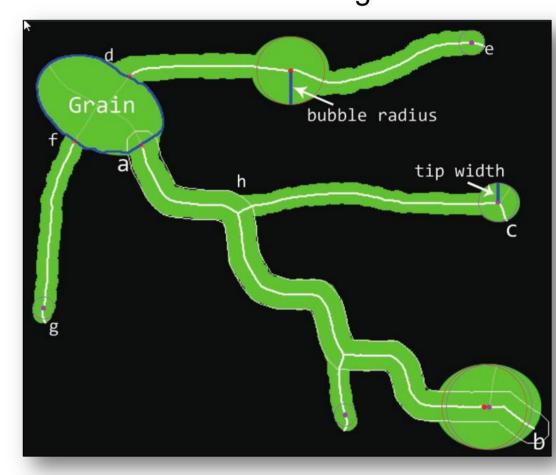
ISAT

Obtaining a proper segmentation is essentially important to feature abstraction. Automatically or manually global thresholding, and image enhancement are usually implemented for such sake. However, from a generality perspective, images having debris or non-interesting cells, complicated by uneven illumination, cause global thresholding to fail. The *Interactive Segmentation Aid Tool* (ISAT) provided by us could easily facilitate the human intervention in an aim of suppressing subjectivity and tediousness in this process to an extent as possbile as it can.



Skeleton based features

After segmentation and skeletonization, the features are extracted along the skeleton. A simulated branching cell is demonstrated below.



Features	Abbreviations	Example values		
Pollen grain area	psArea	9519		
Longest backbone length	bbLen	607		
Longest backbone child number	bbChildNum	2		
Number of branches starting from pollen grain	flBrNum	3		
Relative branching position	sbPos	0.2		
Secondary backbone length	sbLen	304.4		
Longest backbone width	bbWidth	16.4		
Longest backbone tip width	bbTipWidth	44.0		
Secondary backbone width	sbWidth	11.1		
Secondary backbone tip width	sbTipWidth	19.9		
Total bubble number	bubbleNum	2		
Radius of largest bubble	1bRad	43.6		
Ratio of backbone tip width and backbone width	widthRatio	2.7		
The standard deviation of intensities along backbone skeleton	bbIntStd	0		
Ratio of average intensity between tube and grain	avgIntRatio	1.0		
Wavyness coefficient	wavyCoef	4.6		
Number of waves	wavyNum	9		

Validation in classification

We applied the skeleton based features to classification to validates its adequacy to distinguish the different phenotypes observable in our data set based on the first two principal components. To further support this on a more quantitative basis, we examined the cross-validation performance of a random forest classifier. We used 100,000 trees and Leave-One-Out-Cross-Validation (LOOCV) strategy to train and validate the classifier. The *SLIC* features by Murphy et al are used for comparison, and the confusion matrices are shown. Data used in images (a,b) are pollen tubes grouped by morphology by experts, images (c,d) grouped by genes (genes causing different morphs, to be published.)

์(a) Usir	ng skeleton bas	ed feature	es												
True	Predicted	Balloon	Branch	Swollen	Thin	Wavy	Wildtype	_							
E	Balloon	2	0	0	0	0	0	_							
	Branch	0	6	0	0	0	0	_							
5	Swollen	0	0	0	0	0	1	_							
	Thin	0	0	0	1	0	2	_							
	Wavy	0	0	0	0	2	1	_							
W	ildtype	0	0	0	1	1	1	_ □ (c) Using skeleton base	ed fea	tures		(d) Using SLIC featur	res		
/l- \	CLTC Contain							Predicted True	G16	W32	W42	Predicted True	d G16	W32	W42
(b) Usir	ng SLIC feature	!S						G16	10	1	3	G16	6	7	1
	Predicted	Balloon	Branch	Swollen	Thin	Wavy	Wildtype	W32	1	16	0	W32	4	12	1
True								W42	2	0	0	W42	0	0	12
E	Balloon	0	2	0	0	0	0								
	Branch	0	6	0	0	0	0								
5	Swollen	0	1	0	0	0	0								
	Thin	0	0	0	2	0	1	_							
	Wavy	0	0	1	0	0	2	_							
W	ildtype	0	1	0	0	2	0	_							

Conclusion

The skeleton features provide a new way to quantify the morphology of branching cells (or TGCs), and can be used in clustering and classifying high-throughput image morphology data provided that the image quality is consistent.

For lab images bothered by serious noises and pollutants, *ISAT* provides an easy and effective solution to segmentation.