UTILIZING PHASE RETARDATION FEATURES FOR SEGMENTING CELLS IN PHASE CONTRAST MICROSCOPY IMAGES

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BACKGROUND

Label free imaging phase contrast microscopy plays an important role in high-content microscopy, in particular live cell screening. One fundamental task in quantitative biological imaging is cell segmentation. While a number of sophisticated and robust segmentation techniques have been developed for fluorescent microscopy, cell segmentation in phase contrast image sequences remains challenging. Artefacts that are introduced by the specific optics, observed as halo and shade off effects, as well as the lack of clear boundaries among cluttered cells, poor contrast between cells boundaries need to be addressed

OBJECTIVE

We aim to develop a fast, robust and weakly supervised segmentation algorithm that enables the extraction of accurate morphometric measurements at a single cell level.

METHOD

The segmentation method involves 3 main operations (See Fig.1):
1. Feature extraction
2. Generating superpixels
3. Multiclass clustering and region extraction

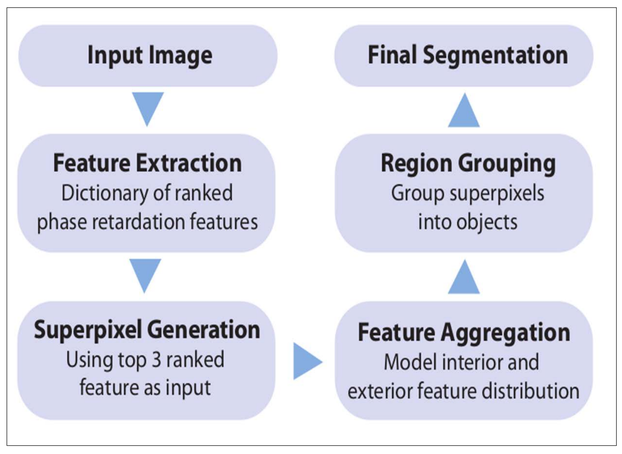


Fig.1: Overview of algorithm showing the main steps in the pipeline

1. Feature extraction

Phase retardation features for each pixel are extracted from a dictionary-based imaging model [1]:

$$g = \sum_{m=0}^{M-1} H_m \Psi_m, \quad s.t.\ \Psi_m \geq 0,$$

where g is the vectorized image intensities expressed as a linear combination of a dictionary of M , H_m bases. Ψ_m is the vectorized coefficients used as pixel feature vector. Fig. 2 shows the 3 principal features mapped onto RGB as a color image.

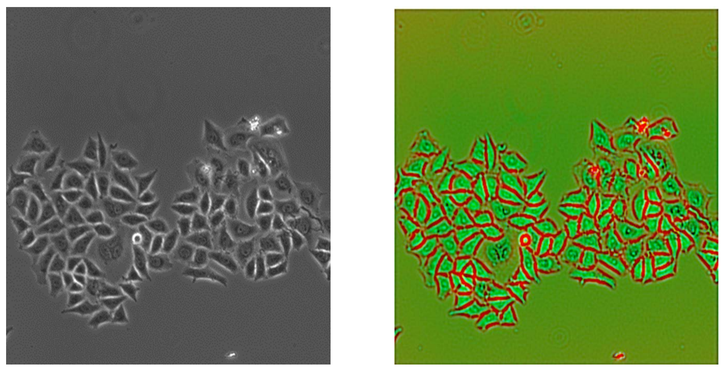


Fig. 2: Sample grayscale image (left) with corresponding top three(3) principal phase features for each pixel mapped onto RGB as a color image (right)

2. Generating superpixels

Similar pixel phase retardation features are then aggregated via SLIC superpixels[2]. See Fig.3. For an *N*-pixel image with phase retardation features $\{\Psi x, \Psi y\}$ for arbitrary pixel locations $\{x,y\}$, the similarity distance measure for aggregating pixels, *ds* is defined as:

$$d_s(x,y) := (\Psi_x - \Psi_y) + \frac{c}{Q}||x-y||_2$$

where ds accounts for both feature variation and distance in pixel location. For K superpixels, $Q=\sqrt{N/K}$ and the compactness, c determines the extent of emphasis on spatial proximity.

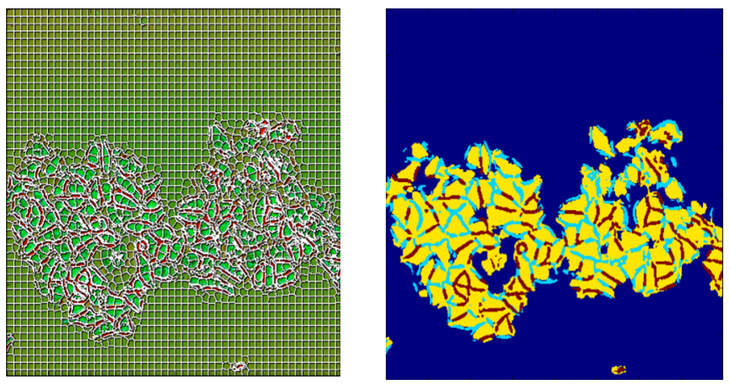
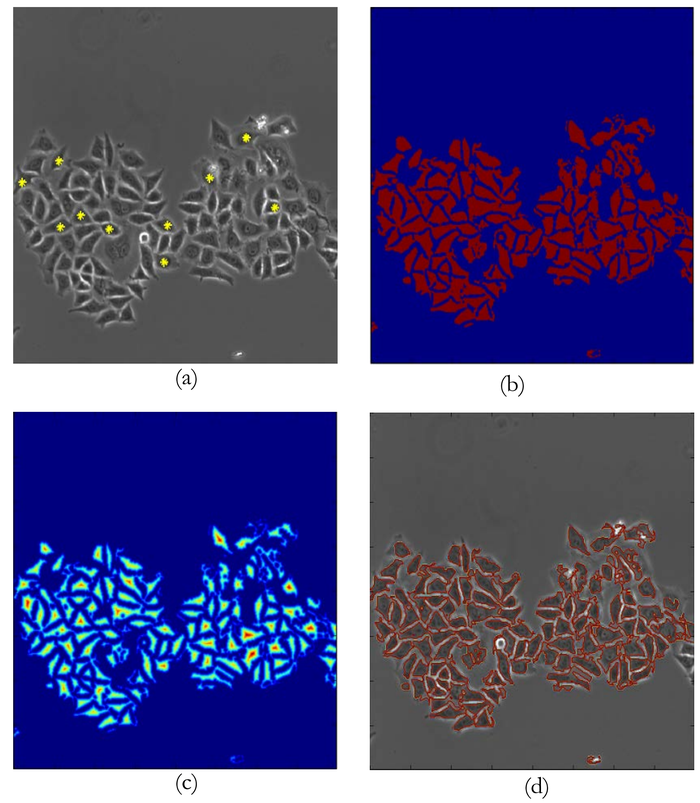


Fig.3: SLIC superpixels over phase retardation features and corresponding Gaussian Mixture Model (GMM) clustering output

3. Multiclass clustering and region grouping

We perform multi-class clustering to group superpixels into clusters corresponding to the main region partitions expected in a typical phase contrast. See Fig.3. We then extract the label associated with cell regions by incorporating prior knowledge of some pixel coordinates indicating cell location via mouse clicks. A marker-controlled watershed routine is then run to obtain the final contours. See Fig. 4



(a) (b) (c) (d)

Fig. 4: Final processing leading to final segmentation output: (a) a priori knowledge of cell location as dots (b) Cell region mask; (c) Euclidean distance transform map; (d) Segmentation contours overlaid on image

RESULTS

Method	Tanimoto Coefficient
Our proposal	0.94 ± 0.03
Su *et al.* [1]	0.94 ± 0.02
Yin *et al.* [3]	0.83 ± 0.06

CONCLUSION AND FURTHER WORK

We have demonstrated cell segmentation method that requires minimal yet weak annotation to achieve results similar to state-of-the-art. We aim to utilize the proposed segmentation for tracking cells in time-lapse sequences.

REFERENCES

[1] Su *et al. (2013)*, Medical Image Analysis,17,746-765
[2] Achanta *et al (2010), EPFL Tech. Report no.149300*
[3] Yin *et al. (2012),* Medical Image Analysis, 16, 1047-1062

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