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Fast recovery of 3D cell morphology from unstained cells using bright-field microscopy

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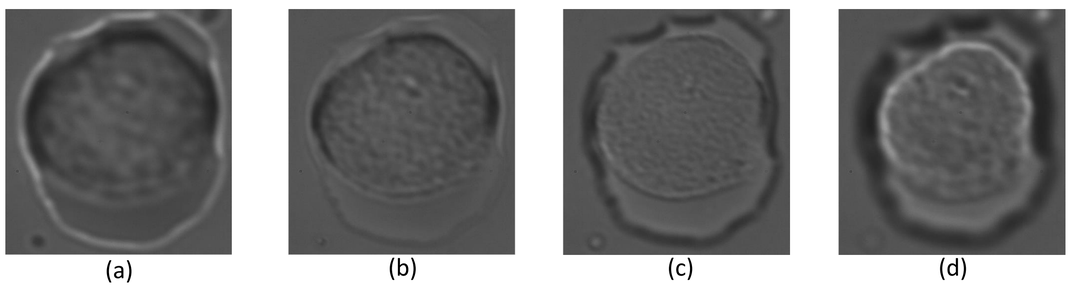
Abstract

We are proposing a fast 3D cell morphology recovery algorithm for unstained cell images recorded by bright-field microscopy. This algorithm requires mechanical motions along the optical axis for incremental scanning of the experimental volume of object under investigation and extract morphology information through block-based focus depths. The core of 3D recovery algorithm is a robust autofocus algorithm we designed for unstained cell images recorded by bright-field microscopy by investigating 15 different autofocus metrics in which tested on unstained cheek, blast and bladder cancer cells. The test results prove the correct focus depth for unstained cell corresponds to the global or local minimum on the focus curve (Fig. 1) instead of global or local maximum for opaque cells. Based on this idea, this autofocus algorithm can be applied in discrete and continuous ways (See Fig. 2). Clearly, continuous way bring us more accuracy and huge computation load at the same time. Thus, GPU implementation is considered to speed up this process.

Autofocus algorithm[1]

15 different autofocus metrics are investigated: 1. Absolute gradient; 2. Square gradient; 3. Netten's filter; 4. Energy laplace; 5. Laplacian; 6. Tenenbaum gradient; 7. Image power; 8. Variance; 9. Normalized variance; 10. Absolute variance; 11. Normalized absolute variance; 12. Vollath's F4; 13. Vollath's F5; 14. Contrast; 15. Histogram entropy. We found that for unstained cells, global minimum indicates the correct focal depth (Fig. 1) rather than global maximum for absorbing objects (Fig. 2).



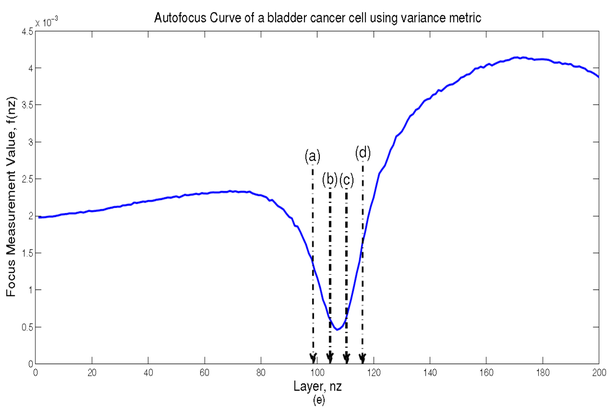


Figure 2: Focus steps: (a) under in-focus plane, (b) cytoplasm in-focus, (c) nucleus in-focus, (d) above in-focus plane; (e) Autofocus curve of the bladder cancer cell using the variance metric where the focus planes that are shown in this figure are clearly indicated using arrows.

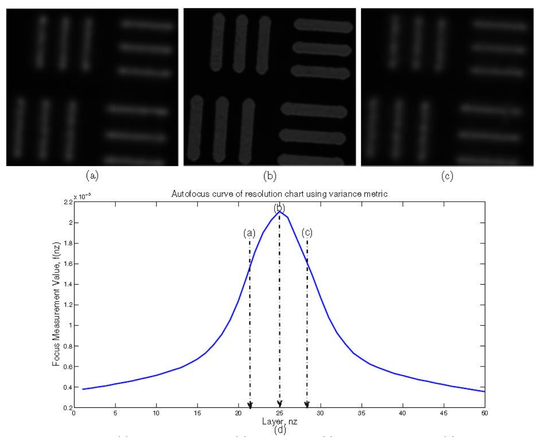
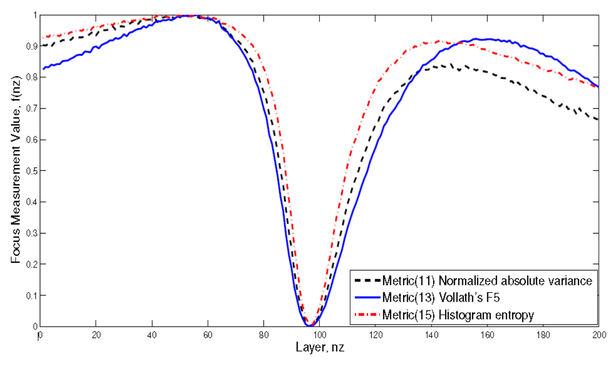


Figure 2: Focus steps: (a) under in-focus plane,(b) in-focus plane,(c) above in-focus plane; (d) autofocus curve of resolution chart using variance metric where we have indicated the position of the planes shown earlier using arrows



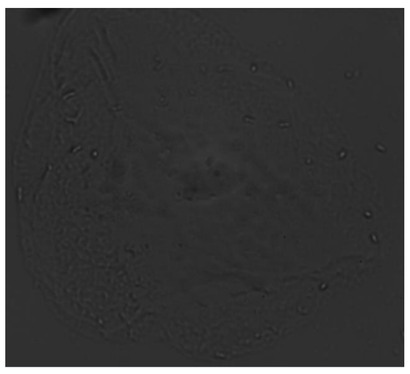


Figure 3: Left: In-focus images of cheek cell (number 97 in the total 200 images in the stack); Right: Comparison of the three selected metrics applied to the cheek cell;

By comparing autofocus curves of all 15 metrics, we believe metric 11, 13 and 15 has most smooth focus curves with distinct global minimum which suits this application. We tested the three optimum metrics on cheek, blast and bladder cancer cells respectively and conformed their robust performance.

3D morphology recovery

Due to cells' three dimension morphology, different parts of the cell like nucleus and cytoplasm come into focus in different depth. Thus, we need to investigate small regions instead of global image.

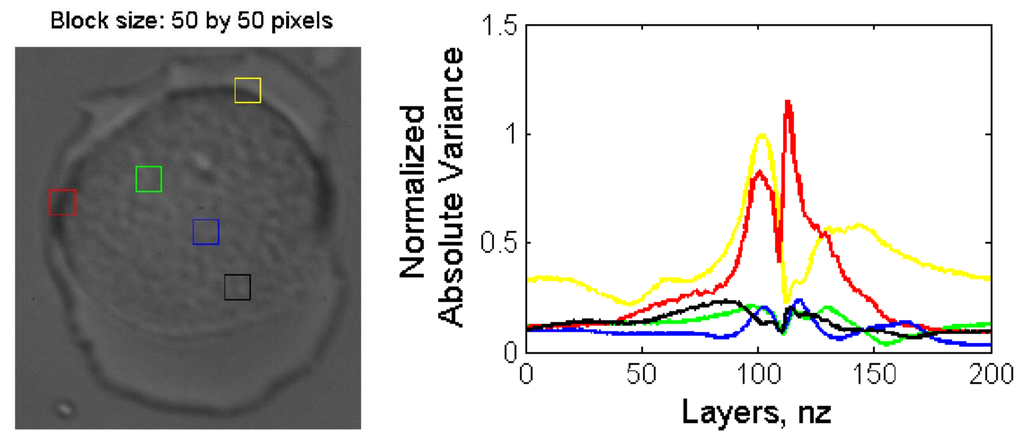


Figure 4: Left: Investigated 50 by 50 pixels blocks represented in different colours; Right: Focus curves corresponds to each coloured block by applying normalized absolute variance metric

Fig. 5 shows the discrete and continuous ways to recover 3D morphology. Discrete way divides each image in the stack into many small equal-sized non-overlapping blocks. By applying autofocus algorithm on each block, the correct focus depth for this particular region will be computed. Continuous way means the block window moves pixel by pixel and the correct focus depth computed for this block returns to the top left pixel in the block shown in red in Fig. 5 (b). At the end of this continuous process, a new image with the same size of the original image will be produced. Every pixel in the new image corresponds to the correct focus depth of each block.

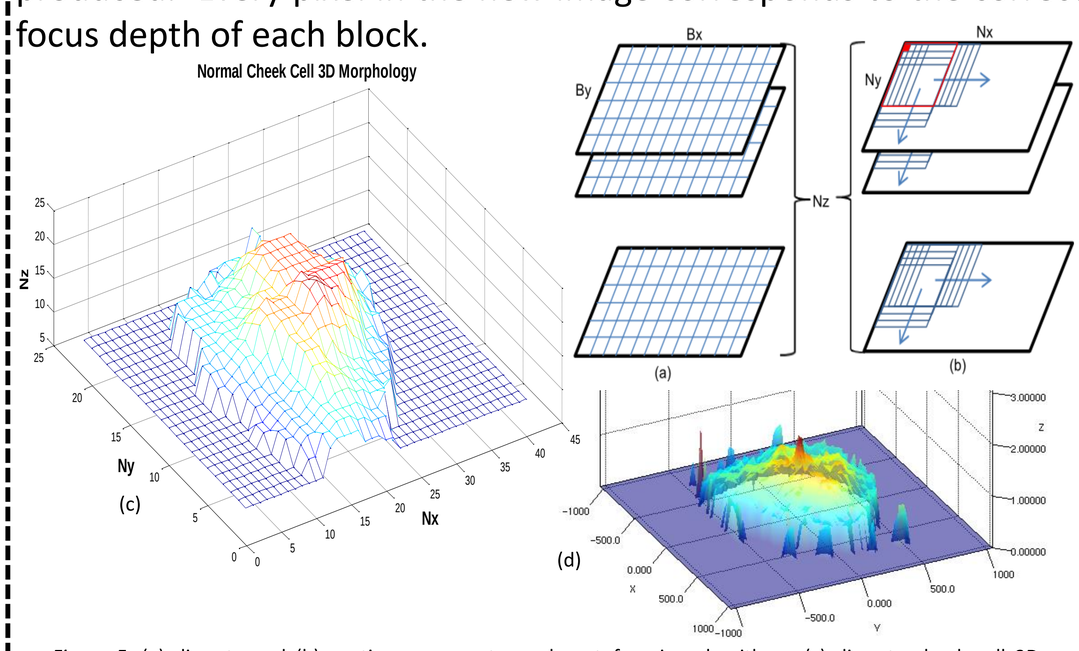


Figure 5: (a) discrete and (b) continuous ways to apply autofocusing algorithms; (c) discrete cheek cell 3D morphology plot; (d) continuous cheek cell 3D morphology plot

Conclusion

By investigating autofocus metrics, we proposed a robust autofocus algorithm for unstained cell images recorded by bright-field microscopy. Optimum metrics: normalized absolute, Vollath's F5, histogram entropy are identified for this application. Making use of this autofocus algorithm, we applied in discrete and continuous way respectively to identify correct focus depth for small region even one pixel to recovery 3D cell morphology. The optimum autofocus metric has been further investigated considering performance on small region and computation speed. At last, normalized absolute variance metric applied on 50 by 50 pixels block size either in discrete or continuous way giving the best result.

Reference

[1] Shu Yu Wu ; Nazim Dugan and Bryan M. Hennelly," Investigation of autofocus algorithms for brightfield microscopy of unstained cells ", *Proc. SPIE* 9131, Optical Modelling and Design III, 2014;doi:10.1117/12.2051944;