Extended Depth of Field Preserving Color Fidelity For Automated Digital Cytology

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In digital cytology the acquired image's depth of field is frequently narrower than the cell clumps thickness [1], resulting in the loss of precious information. To overcome this issue, optical sectioning can be performed and sequences of images (z-stack) are acquired. Extended Depth of Field (EDF) algorithms are thereafter commonly used to ease the analysis of such z-stacks.
Because in cytopathology, specific stains are used to highlight biomarkers, we aim for an EDF algorithm, adapted to digital cytology, with a high color fidelity.

Challenges: The cells are semi-transparent and often superposed. In automated acquisition, a large number of images from the z-stack might not contain useful information.

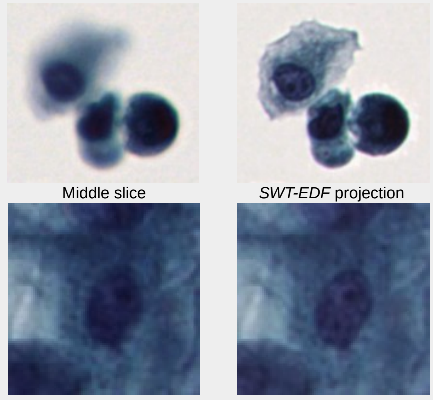

Figure 1: middle slice of the z-stack (left) compared with proposed EDF projection (right)

Proposed approach

- The z-stack is converted to grayscale using Principal Component Analysis (PCA) as proposed in [2].
- Stationnary Wavelet Transform (SWT) [3] applied, allowing great details recovery on superposed semi-transparent cells.
- Level map is used to extract original voxels from the volume.
- In YUV color space, the luminance of the EDF image and the chroma of the original voxels are merged.

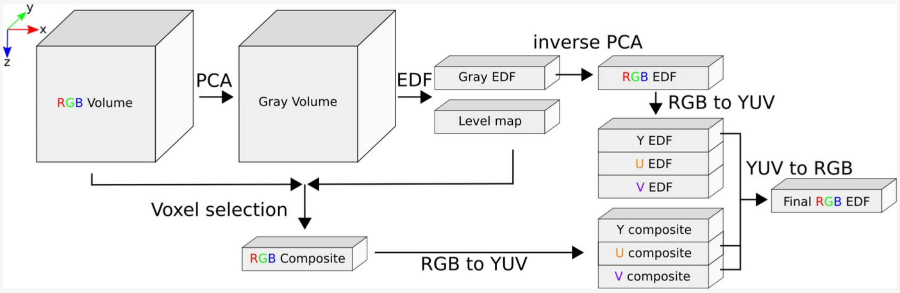

Figure 2: The proposed approach, the output is the concatenation of the EDF image luminance and of in-focus voxels chrominance

Color fidelity experiments

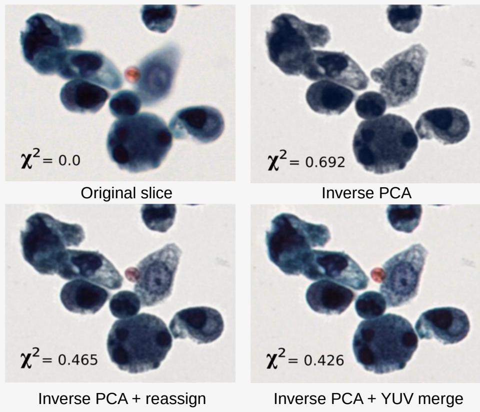

Figure 4: different multi-channel strategies for wavelet transform based EDF algorithm.

Comparison of different multi-channel EDF methods. *Inverse PCA + YUV merge* method recovers colors with a higher fidelity compared to other methods, w.r.t histogram Chi² distance

	*SME**	*CNN-EDF**	SWT-EDF	CWT-EDF
Avg Chi²	*0.173*	*0.331*	**0.586**	1.105

Color comparison between evaluated methods. The *SME* and *CNN-EDF,* selecting in focus pixels, and have perfect color fidelity. Among Wavelet based methods, the proposed *SWT-EDF* achieves highest color fidelity.

Details recovery experiments

Figure 3: Details recovery comparison in presence of overlapping transparent cells

The superposition of transparent cells prevents the methods *SME* and *CNN-EDF*, selecting in focus voxels, to recover as many details as the proposed *SWT-EDF*.

EDF for automated segmentation

Unet trained on best-focus images tested on different projection methods for synthetic cells of increasing thickness. All methods based on volume analysis outperform best-focus selection. Among those, the methods with high color fidelity allow best segmentation.

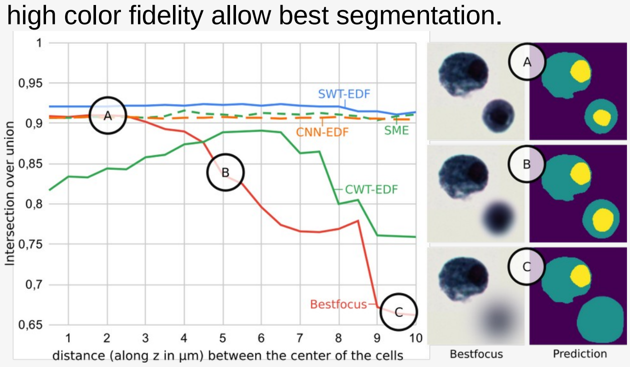

Figure 5: Segmentation IoU, for different projections and cells of increasing thickness

Discussion

The proposed multi-channel EDF is adapted to transparent cells and achieves high color fidelity.
Besides, such color accurate EDF processing is well adapted to automated segmentation of cells of varying thickness.

References

[1] Y. Fan and A. P. Bradley, "A method for quantitative analysis of clump thickness in cervical cytology slides," Micron, vol. 80, Jan. 2016.
[2] B. Forster, et al, "Extended depth-of-focus for multi-channel microscopy images: A complex wavelet approach" IEEE ISBI 2004.
[3] A. P. Bradley and P.C. Bamford, "A One-pass Extended Depth of Field Algorithm Based on the Over-complete Discrete Wavelet Transform," in Image and Vision Computing'04 New Zealand 2004.
[4] A. Shihavuddin, et al, "Smooth 2d manifold extraction from 3d image stack" Nature Communications, vol. 8, no. 1, Aug. 2017.
[5] Y. Liu, et al, "Multi-focus image fusion with a deep convolutional neural network" Information Fusion, vol. 36, Jul. 2017.

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