Morphological restoration: A fast alternative to deconvolution of cells in 3D images

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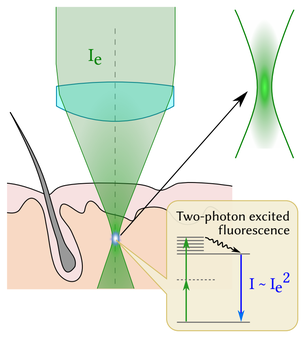
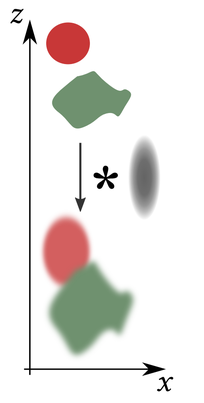
1. Introduction

Multiphoton microscopy (MPM):

✓ a powerful tool for intravital imaging





Point spread function (PSF):

✗ leads to shape elongation along the optical axis

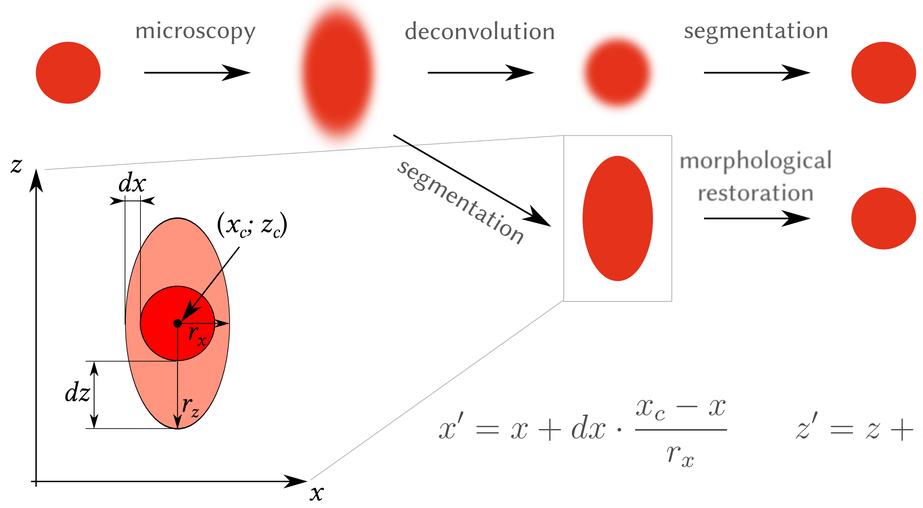
✗ can mislead the analysis of cell interactions

Deconvolution:

✓ crucial to restore objects shape

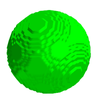
✗ extremely time-consuming

2. Alternative approach: Morphological restoration



$$x' = x + dx \cdot \frac{x_c - x}{r_x} \qquad z' = z + dz \cdot \frac{z_c - z}{r_z}$$

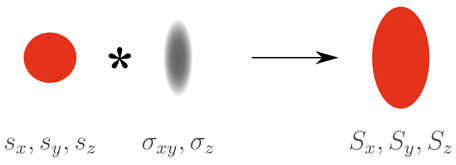
3. Fitting PSF-caused extension from synthetic spheres



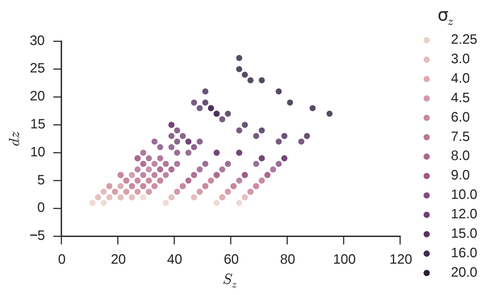
diameter: 10 - 70 pix (3 - 21 µm)

σ_{xy}: 1.5 - 4 pix (0.45 - 1.3 µm) [1]

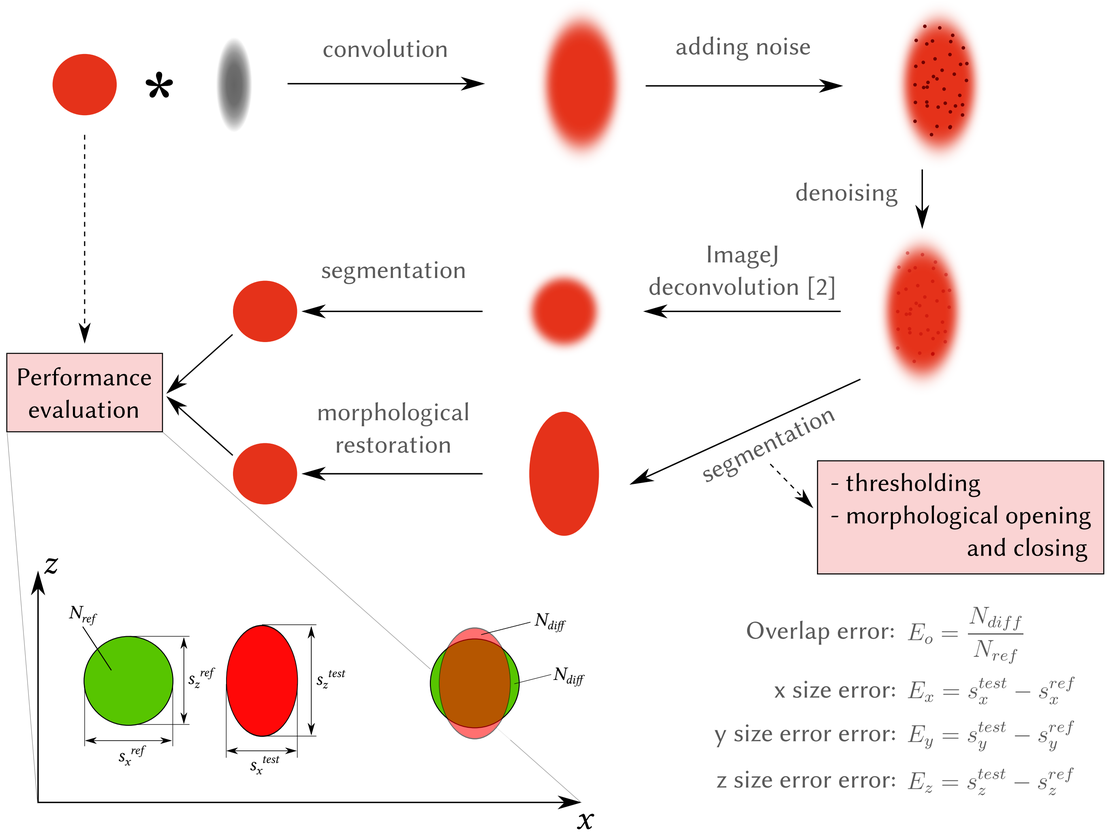
$E_{\mathrm{PSF}} = \frac{\sigma_z}{\sigma_{xy}}$: 1.5 - 5



$$dz = \frac{S_z - s_z}{2} = \sum_{n=0}^{2}\left(S_z^n \cdot \sum_{m=0}^{3} c_{mn} \cdot \sigma_z^m\right)$$



4. Simulation workflow



Overlap error: $E_o = \frac{N_{diff}}{N_{ref}}$

x size error: $E_x = s_x^{test} - s_x^{ref}$

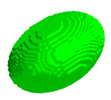
y size error error: $E_y = s_y^{test} - s_y^{ref}$

z size error error: $E_z = s_z^{test} - s_z^{ref}$

5. Estimation of PSF from the "average cell"

1. Measure S_x, S_z
2. $\sigma_{xy} \approx 1$ µm
3. fit dx from σ_{xy}, S_x
4. $s_x = S_x - 2dx$
5. $s_z = s_x$ (spherical cell)
6. $dz = \frac{S_z - s_z}{2}$
7. fit σ_z from dz, S_z

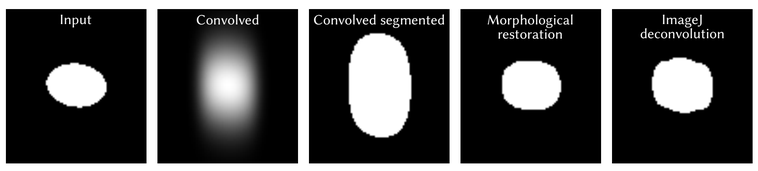
6. Restoration results: Synthetic ellipsoids



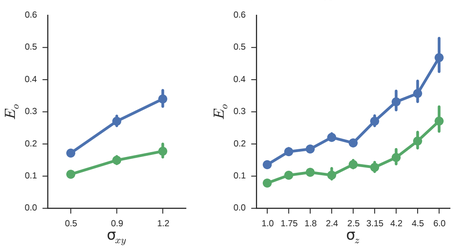
diameter: 10 ± 2 µm
eccentricity: 1 - 2
random orientation
voxel size: 0.3 µm isotropic

σ_{xy}: 0.5, 0.9, 1.2 µm

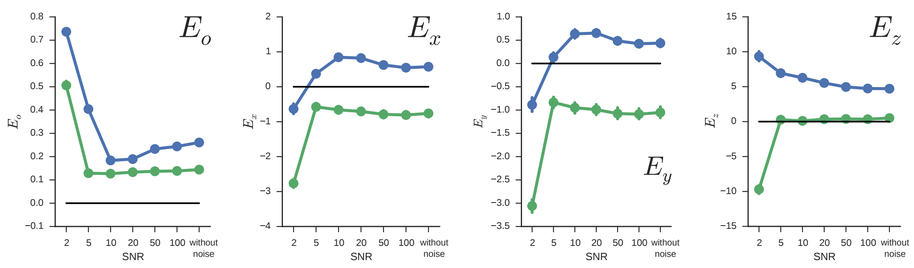
E_{PSF}: 2, 3.5, 5



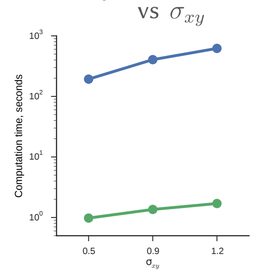
Overlap error depending on PSF



Performance depending on the noise SNR (signal-to-noise ratio)



Computation time vs σ_{xy}

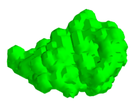


ImageJ
Morphological restoration

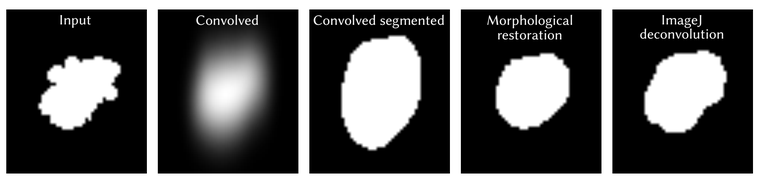
✗ ImageJ: ~ 258 sec (4.3 min) per cell

✓ Morphological: ~ 1.1 sec per cell

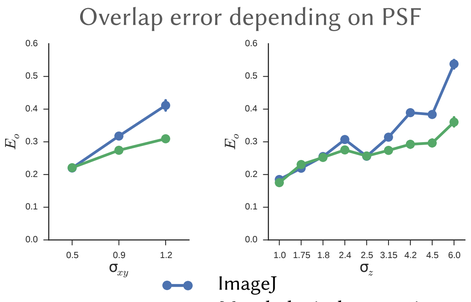
7. Restoration results: Synthetic cells with realistic shapes



diameter: ~ 10 µm
voxel size: 0.4 µm isotropic

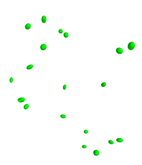


Overlap error depending on PSF



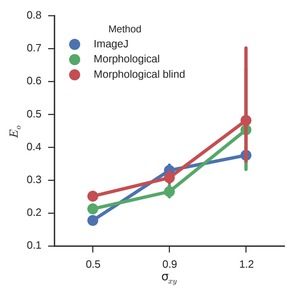
ImageJ
Morphological restoration

8. Restoration results: Synthetic multicellular stacks of ellipsoids

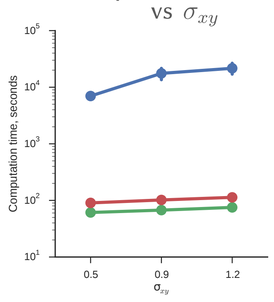


stack size: 307.2 x 307.2 x 60 µm^3
number of cells: 20 per stack
number of stacks: 10
voxel size: 0.3 µm in xy, 1 or 3 µm in z

Overlap error vs σ_{xy}



Computation time vs σ_{xy}



Overlap error vs z-spacing and noise SNR

ImageJ
Morphological restoration

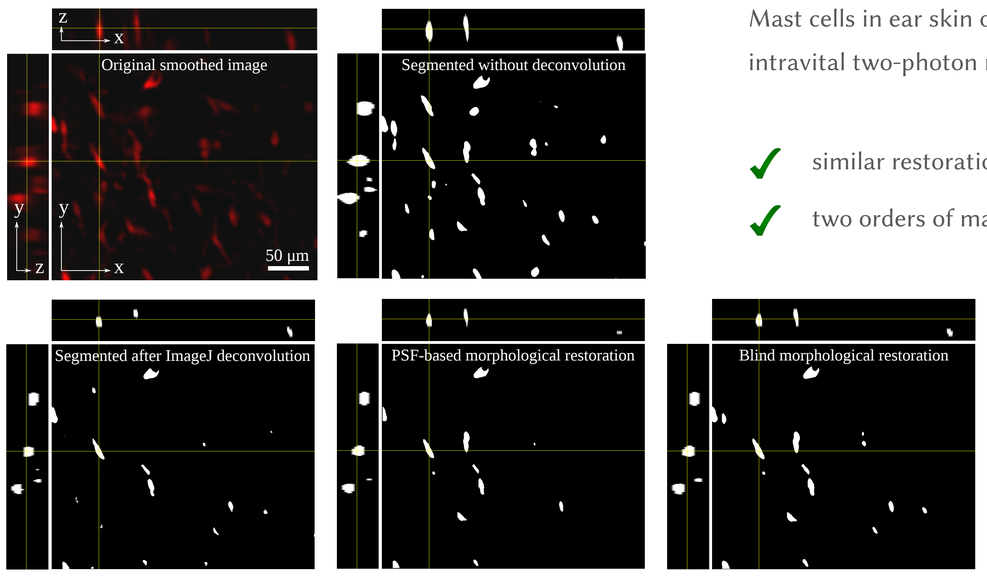
z-spacing
3 µm
1 µm

✗ ImageJ: ~ 152.6 min (2.5 h) per stack

✓ Morphological: ~ 1.1 min per stack

✓ Morphological blind: ~ 1.7 min per stack

9. Restoration results: Experimental data



Mast cells in ear skin of healthy mice, intravital two-photon microscopy

✓ similar restoration results

✓ two orders of magnitude faster

Summary

Morphological restoration vs ImageJ:

✓ considerably faster

✓ comparable accuracy in smaller z-spacings

✗ less accurate in larger z-spacings

Outlook

- optimization for larger z-spacings
- comparison with other deconvolution software
- open source software package

References:

[1] Dong, C.-Y., Koenig, K. and So, P. "Characterizing point spread functions of two-photon fluorescence microscopy in turbid medium," (2003) Journal of Biomedical Optics

[2] Dougherty, R. "Extensions of DAMAS and Benefits and Limitations of Deconvolution in Beamforming," (2005) American Institute of Aeronautics and Astronautics

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