From raw images to abstract surface models: deconvolution, DeConvTest and DynSPHARM

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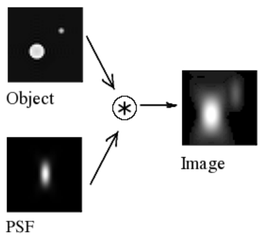
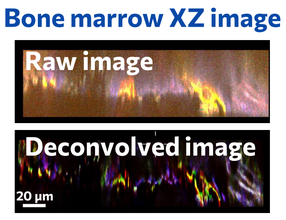
Why do microscopes „ruin" images?



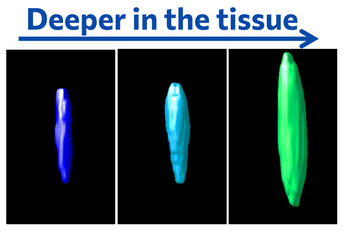
Bone marrow XZ image



Point Spread Functions

Theoretical **Measured**

Deeper in the tissue



Microscopy principle: Object **convolved** with PSF = Image

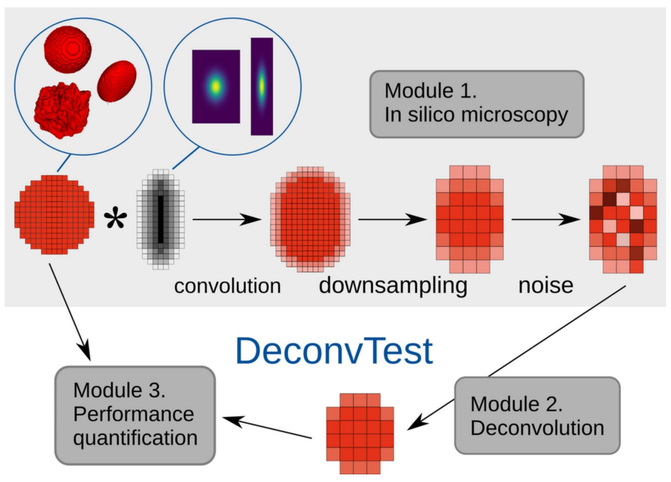
- Raw microscopy images are distorted due to optical imperfections of the microscope-tissue system
- The sum of these effects can be quantified via the Point Spread Function (PSF)
- The PSF can be measured , theoretically calculated or estimated
- By knowing the PSF, we can partially reverse the optical aberrations and approximate the „ideal" image
- We compared several of the existing methods to find the best tool and parameters. [1]

A "perfect image" is thus impossible ⟶ **Deconvolution**

- Using measured images and PSF, the image parameters need to be extracted for the PSF calculations
- Measured PSFs provide higher fidelity deconvolutions and optimized images
- The measured PSF varies with tissue depth due to higher light scattering
- The accuracy of deconvolution can be compared amongst various tools and parameter settings. [1]

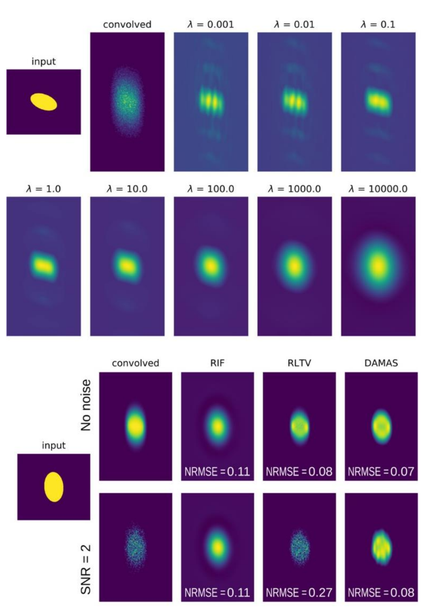
How does deconvolution rescue images? How to find the best deconvolution method and parameters?

Deconvolution parameters for synthetic cells

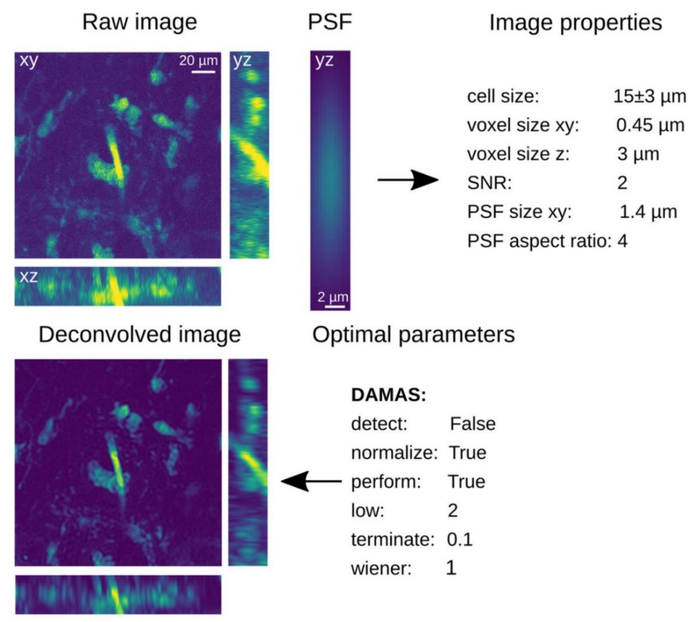


- Choosing the best deconvolution method and parameters must be done objectively
- DeconvTest serves this comparative purpose
- Using measured and simulated images to quantify the precision of deconvolution
- Proprietary, free and open-source systems are readily available for deconvolution
- The image parameters are extracted for the calculations, the deconvolution parameters are varied
- The optimal deconvolution parameter settings are identified via finding the lowest reconstruction errors
- The accuracy of the deconvolution is compared amongst the various methods and parameter settings. [1]

Optimizing the parameters



Deconvolving measured microscopy images



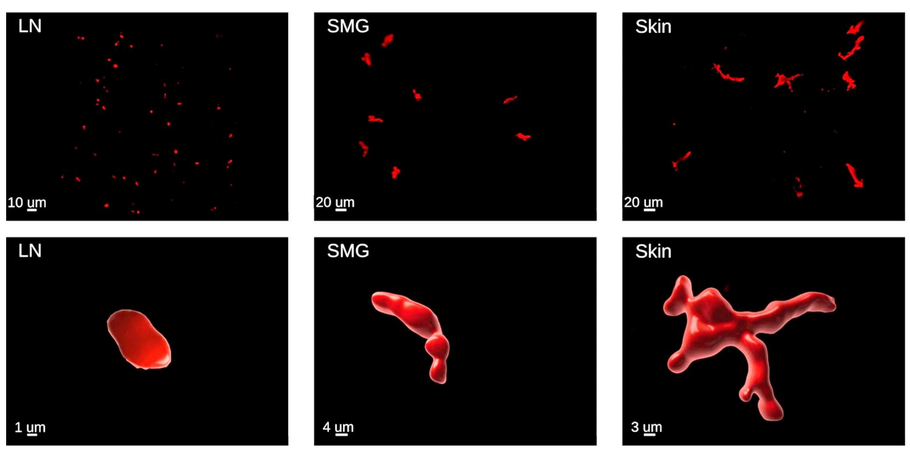
Deconvolution systems

- **Huygens** (proprietary, svi.nl; theoretical and measured PSF)
- **Imaris** (proprietary, bitplane.com, theoretical PSF only)
- **DeconvolutionLab2** (Fiji plugin):
  - Regularized Inverse Filter (RIF)
  - Richardson–Lucy with Total Variance (RLTV)
- **Iterative Deconvolve 3D** (Fiji plugin):
  - Deconvolution Approach for the Mapping of Acoustic Sources (DAMAS).

GitHub https://github.com/applied-systems-biology/DeconvTest

How to classify cells based on their 3D shape changes during migration and immune reactions?

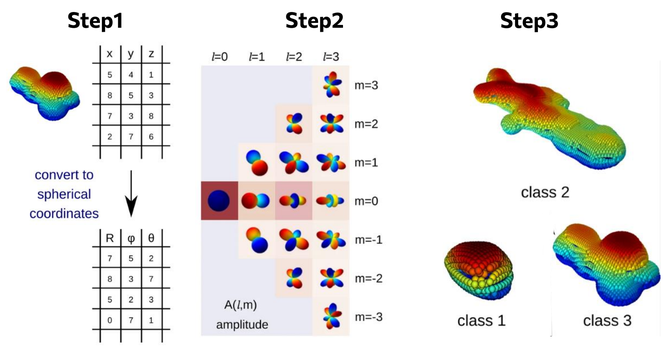
T-cells in lymph nodes, salivary gland, and skin



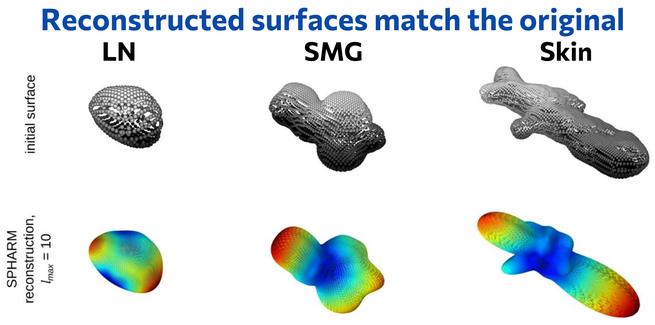
- Cells change their shape during migration, immune reactions, etc.
- The 3D shapes need to be quantified to become comparable
- Decomposing the 3D surfaces into spherical harmonics achieves this
- Using measured and synthetic 3D and 4D surfaces to classify cells
- Adding kinetic information increases cell classification accuracy. [2]

GitHub https://github.com/applied-systems-biology/Dynamic_SPHARM

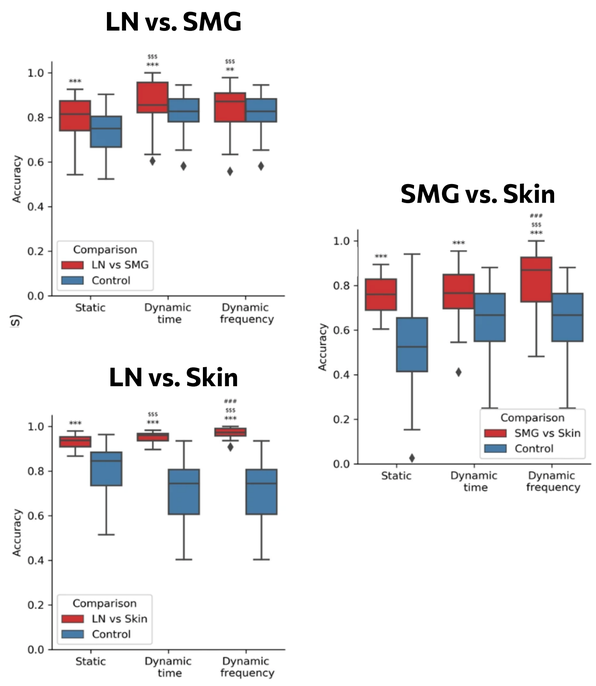
Spherical harmonics as 3D surface components



Reconstructed surfaces match the original



Kinetic information aids cell classification



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References
[1] Medyukhina *et al.*, 2020. *J Biophotonics*. 13(4): e201960079
[2] Medyukhina *et al.*, 2020. *Sci Rep*. 10(1): 1-2

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