

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

A. Medyukhina, Z. Cseresnyes, M.T. Figge

28/10/2021

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

Anna Medyukhina^{1,2}, Zoltan Cseresnyes¹, Marc Thilo Figge^{1,3}

¹Applied Systems Biology, Leibniz Institute for Natural Product Research and Infection Biology – Hans-Knöll-Institute Jena, Germany
²Current address: St. Jude Research Hospital, Memphis TN, USA
³Institute of Microbiology, Faculty of Biological Sciences, Friedrich-Schiller-University Jena, Germany

Why do microscopes „ruin“ images?

Raw microscopy images are distorted due to optical imperfections of the microscope-tissue system
 Point Spread Functions
 Theoretical Measured
 Bone marrow XZ image
 Raw image Deconvolved image
 Deeper in the tissue
 Raw image Deconvolved image
 Microscopy principle: Object convolved with PSF = Image

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
 Deconv/Test serves this comparative purpose
 Using measured and simulated images to quantify the quality of deconvolution
 Proprietary, free and open source systems are currently available for deconvolution
 These methods are extensible. In these 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]

Deconv/Test

Module 1: In-table microscopy

Module 2: Deconvolution

Module 3: Performance optimization

Optimizing the parameters

Deconvolving measured microscopy images

Raw image PSF Image properties

cell size: 150.1 μm
 voxel size x: 0.05 μm
 voxel size z: 3 μm
 PSF size x: 1 μm
 PSF size y: 1.4 μm
 PSF aspect ratio: 4

DAMAS:
 adam: False
 iterative: True
 perfact: True
 tikhonov: 1
 wener: 1

Deconvolution systems

- Huygens (proprietary, svnh; theoretical and measured PSF)
- Imaris (proprietary, bitplane.com; theoretical PSF only)
- DeconvolutionLab2 (Fiji plugin):
 - Richardson-Lucy with Total Variance (RLTV)
 - 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

Spherical harmonics or 3D surface components Kinetic information aids cell classification

Step 1 Step 2 Step 3

LN vs. SMG LN vs. Skin SMG vs. Skin

Reconstructed surfaces match the original LN SMG Skin

Cells change their shape during migration, immune reactions, etc.
 The 3D shapes of different cell types are different
 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/DynSPHARM>

zoltan.cseresnyes@leibniz-hki.de www.leibniz-hki.de

References

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 [2] Medyukhina et al. 2020. *SciRep* 10(1): 1-2

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