deconvolution

- A mathematical image restoration technique capable of:
  - 1. Removing noise / background
  - 2. Increasing contrast
  - 3. Increasing resolution
- by using a priori knowledge of the imaging system: The Point Spread Function (PSF)
deconvolution

Huygens Remote Manager v2.1.2

HTTP://PTBIOPSRv1.EPFL.CH/HRM

Convalaria Sample, MIP top projection
Left: original, right: deconvolved, cmle 40 iterations.
Widefield acquisition: 40X Oil objective xy resolution: 160nm, z resolution: 600nm

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• A microscope is a diffraction-limited system

**DIFFRACTION**: Spreading out of waves past a small opening

• Optical blur is a consequence of light diffraction through the optical system (and particularly through the objective), resulting in the limitation of optical resolution.

*Point Spread Function (PSF)*: System response to a punctual light source
THE PSF IS THE BASIC BRICK OF IMAGING AND THUS DECONVOLUTION

How are the PSF and the optical resolution linked?

- PSF and microscope optical resolution are intrinsically linked.
- The PSF width (FWHM) in the radial and axial directions determine the optical system resolution and vice versa.

Rayleigh criterion

Two Airy disks (points) are resolved if they are farther apart than the distance at which the maximum of one Airy disk coincides with the first minimum of the second Airy disk.

- As deconvolution is performed at the PSF scale, it is necessary that the image is acquired with a correct voxel size, that is, with a voxel size coherent with the optical resolution of the microscope.

According to the Nyquist theorem:  \( \text{sampling \_ step} = 2 \cdot \text{optical \_ resolution} \)

Note that the effective optical resolution of the real microscope is always worse than the theoretical one (lens imperfections, misalignments).
I. The Objective NA
II. Wavelength
III. Pinhole Aperture
IV. Refractive Index
V. Other Factors

What affects the PSF?

Numerical Aperture
NA = 0.8
NA = 1.4

Refractive Index Mismatch

Pinhole Diameter
250nm  500nm  1000nm
Which Elements Influence the PSF?

I. The Objective NA
II. Wavelength
III. Pinhole Aperture
IV. Refractive Index
V. Other Factors

Wavelength

Pinhole Aperture

Left: BPPR = 250 nm, Middle: BPPR = 500, Right: BPPR = 1000 nm.
WHICH ELEMENTS INFLUENCE THE PSF?

- Refractive indexes of the objective medium and the sample mounting medium.
Theoretical vs measured psf

• In practice, the effective PSF can significantly differ from the theoretical one

Theoretical PSF

Measured PSF (λ=488 nm)
Confocal microscope, Zeiss LSM700

Measured PSF (λ=561 nm)
Confocal microscope, Zeiss LSM700
Acquiring Good Images

Good Deconvolution $\iff$ Good Images

Bad Images $\iff$ No Deconvolution
Good quality images

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Good quality images

Noise Reduction
(Pixel Dwell Time, Frame/Line Averaging)

Gain, Offset, Laser Power
(Saturation)

Confocal acquisition using 8-frame average (left) and no averaging (right)

HeLa Cells confocal acquisition showing saturation.

offset saturation
Voxel bit depth

16-bit
pixel value: 0,1... to 65535

12-bit
pixel value: 0,1... to 4095

8-bit
pixel value: 0,1... to 255

1-bit
pixel value: 0 or 1

2-bit
pixel value: 0,1,2 or 3

High Bit-Depth (12 or 16-bits)

8-bit Range Bits

16-bit Range Bits

Image Histogram (Continuous)

Digitalization
Voxel size

Widefield

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Confocal

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Voxel size

A proper voxel size, taken from our deconvolution tables allows you to capture the highest possible amount of information from your sample.
Voxel size

Optimal Voxel Size

Wrong voxel size: Fine detail lost

Voxel Size from Nyquist: Optimal resolution sampling

A proper voxel size, taken from our deconvolution tables allows you to capture the highest possible amount of information from your sample.

Why is the sampling so important?

Resolution Limit

Sample volume (Z direction) acquired at the resolution limit (left) and with deconvolution standards (Right). Notice how we gained more information about the Z localization of the two signals.

SVI Recommendation

Deconvolution

Pixel

Voxel
Voxel Size & Pinhole Aperture

**Slice Thickness Matching**

- **Pinhole Size**
- **Thickness**
- **Match**

The optical thickness is wavelength dependent and thus the pinhole must be a different size for each wavelength.

- Each wavelength is matched to have the same optical thickness.

**Why is the Sampling so Important?**

Sample volume (Z direction) acquired at the resolution limit (left) and with deconvolution standards (Right). Notice how we gained more information about the Z localization of the two signals.

**Resolution Limit**

**SVI Recommendation**

Deconvolution
Deconvolution techniques

- **LINEAR METHODS**
  - Inverse Filtering
  - Wiener Filtering

1. **I. Linear Methods (Filtering)**
2. **II. Iterative Algorithms**
3. **III. (Deblurring Methods)**
4. **IV. Blind Deconvolution**

**Fast** Limited by noise amplification

Original image  Blur (noise free)  Inverse filtering (regularized version)  Deconvolved image

- Blur + additive noise
- Possible ringing

Original image  Ringing Artefact  Deconvolved image
Deconvolution techniques

• LINEAR METHODS
  – Inverse Filtering
  – Wiener Filtering

During division in Fourier space, noise variations are significantly amplified.

Noise amplification can be reduced by making some assumptions about your image. For instance we can assume that the object was relatively smooth and impose some constraint on the estimated solution (deconvolved image).

This approach is called regularization.

\[
F_{\text{Wiener}}(\omega) = \frac{H^*(\omega)}{|H(\omega)|^2 + \left(\frac{\Phi_n(\omega)}{\Phi_g(\omega)}\right)}
\]

with \( \Phi_g(\omega) \) power spectrum of the signal

\( \Phi_n(\omega) \) power spectrum of the noise
**Deconvolution techniques**

- **ITERATIVE ALGORITHMS**
  - **Idea:** An *estimate* of the object is made (typically the raw image). The first estimate of the object is convolved by the PSF to produce a new estimate of the result. The two estimates are compared and an error criterion is established. The error criterion is used to modify the last estimate and produce a new estimate, and so on, iteratively, up to convergence or a defined STOP criterion.

  
  **Mathematical Formulation:** Iterative minimization of a defined cost function.
Deconvolution techniques

- **ITERATIVE ALGORITHMS**
  - Least Squares Estimation
  - Maximum Likelihood

- Minimization of the error between the acquired image and the convolution of the estimated object by the PSF.

\[
\min_{\tilde{f}} \| g - H\tilde{f} \|^2 = \min_{\tilde{f}} \| g - Hf \|^2
\]

**ITERATIVE IMPLEMENTATION**

- **Landweber**
  - (an example among others)
  \[
  f^{(k+1)}_{LS} = f^{(k)}_{LS} + \gamma H^T (g - Hf^{(k)}_{LS})
  \]
  - Previous step solution
  - Correction through the error criterion
Deconvolution techniques

• ITERATIVE ALGORITHMS
  – Least Squares Estimation
  – Maximum Likelihood
• Different formulation of the cost function being minimized:

\[
\min (-\log(p(g | \tilde{f})))
\]

• Iterative formulations are made by making some assumption about the kind of noise affecting the acquired image.

Concluding: ITERATIVE ALGORITHMS

• Give better results than filtering methods
• Noise amplification well controlled (regularization parameter)
• STOP criteria: Minimized Cost Function or Max Iteration number reached
• Computationally expensive
DECONVOLUTION TECHNIQUES

• **DEBLURRING METHODS**
  • **Unsharp masking**: a blurred version of the 2D image is subtracted to the image itself.
  • The blurred image can also be obtained from the upper and lower plane with respect to the considered one (Nearest-Neighbor).
  • Not really a deconvolution: it does not use any information about the acquisition system.
  • Useful in case of thin stacks/single plane images.

**BLIND DECONVOLUTION**

Blind deconvolution is a more recent method.

In this approach, an estimate of the objects is made. This estimate is convolved with a theoretical PSF. The resulting blurred estimate is compared with the raw image, a correction is computed and used to generate a new estimation. This same correction is also applied to the PSF, generating a PSF estimate. The process is iterative.

Good results also on noisy or images with spherical aberrations.
Deconvolution techniques

- COMPARISON

Confocal images of a fixed epithelial cell labeled with Concanavalin A and FITC

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Concerning deconvolution, a possibility is to use an experimental PSF instead of a theoretical PSF.

How to obtain an experimental PSF:

1. Record 3D stacks of fluorescent beads (suggestion: with a diameter of 100-200 nm), with the same microscope parameters, in the same conditions you acquired the images you want to deconvolve.
2. Register and average the beads images (this step is useful to reduce noise)
3. 'Distill' the PSF using Huygens Software
Deconvolution in practice

- **DECONVOLUTION EFFECTS**
  - Improves image resolution, particularly in the Z-direction
  - Improves image contrast
  - Improves image SNR
  - It is a particularly good practice to deconvolve your images when you want to segment objects or do colocalization analysis, besides getting nicer and cleaner images

- **DECONVOLUTION APPLICABILITY**
  - Widefield microscopy
  - Confocal microscopy
  - Spinning disk microscopy
  - Electron microscopy
DECONVOLUTION IS A DELICATE AND VERY SENSITIVE IMAGE PROCESSING TECHNIQUE. IT MUST BE APPLIED ONLY ON CORRECTLY ACQUIRED IMAGES AND THE RESULT MUST ALWAYS BE VERIFIED.

- **TIPS**
  - Acquire your images respecting the Nyquist criterion
  - Do not saturate your images
  - If possible, acquire some black slices up and down the object of interest to avoid border effects.
  - Repeat the deconvolution with different settings of the algorithm parameters (particularly the regularization parameter) and compare your results.
Deconvolution Tlps

• On the BIOP Website

  • LEICA SP5 2P
  • LEICA PS5 WL
  • LEICA SP2 UP
  • LEICA SP2 IN
  • ZEISS LSM 710
  • ZEISS LSM 700 UP
  • ZEISS LSM 700 IN
  • ZEISS LSM 510 Meta

Widefield imaging:
  • LEICA DMI4000
  • LEICA DM5000
  • OLYMPUS AX70

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**LSM 700 710 VOXEL SIZE TABLE**

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<td>63x 0,5 watter</td>
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</tr>
<tr>
<td>40x 1,3 oil</td>
<td>46</td>
<td>82</td>
</tr>
<tr>
<td>63x 1,4 oil</td>
<td>43</td>
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**Theory and Image Processing:**

  • Basics in microscopy
  • Huygens Remote Manager
  • Deconvolution Introduction
  • Colocalization introduction

**SVI Formula**

\[ XY = \frac{\lambda \text{ex}}{(8 n \sin \alpha)} \quad Z = \frac{\lambda \text{ex}}{(4 n (1-\cos \alpha)} \]

**Resel* /2**

\[ XY = \frac{(0.44 \lambda \text{ex}/NA)/2}{2} \quad Z = \frac{(1.5 n \lambda/NA)^2}{2} \]

Nyquist Calculator from SVI = http://support.svi.nl/wiki/NyquistCalculator

Resel* = the resolution can be defined as the radius of the first dark fringe in the the diffraction pattern, or half the diameter of the Airy disc

Preparing for deconvolution

• NOTE

• Deconvolution packages usually implement a series of pre-processing tools which improve deconvolution results:
  • Background correction
  • Bleaching correction
  • Spherical aberration correction (dynamic PSF)
Deconvolution @ biop

• SOFTWARE

• DeconvolutionLab: An ImageJ/Fiji plugin which implements different iterative algorithms and inverse filtering solutions: http://bigwww.epfl.ch/algorithms/deconvolutionlab/

• SVI Huygens and HRM (Huygens Remote Manager). You can ask for an HRM account at http://ptbiopsrv1.epfl.ch/hrm/

• (AutoDeblur, BitPlane)
Use of HRM

Huygens Remote Manager v2.1.2

HTTP://PTBIOPSRV1.EPFL.CH/HRM

Upload Files

SFTP Connection

HTTP://PTBIOPSRV1.EPFL.CH

Build Settings

Launch Deconvolution

Check Quality

Wait

Done

HTTP://PTBIOPSRV1.EPFL.CH/HRM

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Summary

– Deconvolution
  • Powerful technique
  • Very sensitive to imaging system
  • Bad image: Very bad deconvolution
  • Useful for colocalization/segmentation
  • Several methods (Inverse filtering, BD, Iterative...)
  • Softwares
    – Hyugens
    – DeconvolutionLab