Deconvolution in Fluorescence Microscopy-Phase I

Praveen Pankajakshan*, Laure Blanc-Féraud*, Josiane Zerubia*

*ARIANA Project-team, INRIA/CNRS/UNSA Sophia-Antipolis, France

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Problem Statement

1. Images of biological specimens obtained from fluorescence microscopes are corrupted by two primary sources:
   - blurring due to the band-limited nature of the optical system
   - under low illumination conditions, noise due to reduced number of photons reaching the detector.
2. Blurring kernel is unknown (blind deconvolution).
3. Denoising the image can induce artifacts.
4. Restoration of the images is ill-posed.

Poisson Statistic for the Image Formation Model

- The statistics for the image formation is described by a Poisson process as:

$$i(\mathbf{x}) = \mathcal{P}[h * o](\mathbf{x}) \quad (1)$$

where $*$ denotes 3D convolution.

- Likelihood of the observed data i knowing the specimen o is given as,

$$P(i|o) = \prod_{\mathbf{x}\in\Omega} \frac{[h * o](\mathbf{x})^{i(\mathbf{x})} e^{-[h*o](\mathbf{x})}}{i(\mathbf{x})!} \quad (2)$$

Gibbsian distribution with Total Variation (TV)

Gibbsian distribution $P(X = o)$ with TV functional captures the prior knowledge of the object, and is the regularization model.

$$P(o) \propto \frac{1}{Z_\lambda} e^{-\lambda \sum_{\mathbf{x}\in\Omega} |\nabla o(\mathbf{x})|}, \qquad (3)$$

where, $|\nabla o(\mathbf{x})| = \left(\sum_{\mathbf{x}'\in V_{\mathbf{x}}} (o(\mathbf{x}) - o(\mathbf{x}'))^2\right)^{\frac{1}{2}}$; $Z_\lambda = \sum_{o} e^{-\lambda \sum_{\mathbf{x}} |\nabla o(\mathbf{x})|}$

Diffraction-limited Point-Spread Function (PSF) model

- For a fluorescent microscope,

$$h(\mathbf{x}) = |P_{\lambda_{em}}(\mathbf{x})|^2 \cdot |P_{\lambda_{ex}}(\mathbf{x})|^2 \tag{4}$$

where, h is the PSF, $P_\lambda(\mathbf{x})$ is the pupil function for a wavelength λ.

- If the pinhole model A_R is included, then the analytical CLSM PSF model is,

$$h(\mathbf{x}) = |A_R(\mathbf{x}) * P_{\lambda_{em}}(\mathbf{x})|^2 \cdot |P_{\lambda_{ex}}(\mathbf{x})|^2 \tag{5}$$

Parametric approach to PSF modeling

- Assumptions:
  1. Infinitely small pinhole, stationary PSF, ignore aberrations (mirror symmetry about z-axis).
  2. Circular symmetry on xy-plane.
- Diffraction-limited PSF approximation (in the LSQ sense) [*Zhang et al*. 06]:

$$h_{\sigma_r,\sigma_z}(r,z) = \frac{1}{Z_{\sigma_r,\sigma_z}} e^{\left(\frac{-r^2}{2\sigma_r^2} - \frac{z^2}{2\sigma_z^2}\right)}, \quad (6)$$

$$\text{where, } Z_{\sigma_r,\sigma_z} = (2\pi)^{\frac{3}{2}} \sigma_r^2 \sigma_z$$

Bayesian model for the restoration

- From the Bayes theorem, the Posterior probability is,

$$P(X = o|Y = i) \propto P(Y = i|X = o)P(X = o) \quad (7)$$

- Thus, the conditional probability can be written as:

$$P(o|i) \propto \frac{e^{-\lambda \sum_{\mathbf{x}\in\Omega} |\nabla o(\mathbf{x})|}}{\sum_{o} e^{-\lambda \sum_{\mathbf{x}\in\Omega} |\nabla o(\mathbf{x})|}} \prod_{\mathbf{x}\in\Omega} \frac{[h * o](\mathbf{x})^{i(\mathbf{x})} e^{-[h*o](\mathbf{x})}}{i(\mathbf{x})!} \quad (8)$$

Alternate Minimization Algorithm

- The Cost Function to be minimized has the form:

$$\mathcal{L}(o,h) = -\lambda \sum_{\mathbf{x}\in\Omega} |\nabla o(\mathbf{x})| - log[Z_\lambda] + \sum_{\mathbf{x}\in\Omega} (i(\mathbf{x}) log[h * o](\mathbf{x})) - \sum_{\mathbf{x}\in\Omega} [h * o](\mathbf{x}) \quad (9)$$

- Sub-optimal solution alternatively maximizes the joint-likelihood in o and h to find $\widehat{o}$ and $h(\hat{\theta})$ [*Hebert etal*. 1989] satisfying :

$$\mathcal{L}(\hat{o}_{new}, h(\hat{\theta}_{new})) \leq \mathcal{L}(\widehat{o}_{old}, h(\hat{\theta}_{old})) \quad (10)$$

Maximum A Posteriori (MAP) estimate of the specimen

1. Minimizing the cost function (9) w.r.t o,

$$\frac{\partial}{\partial o(\mathbf{x})}\mathcal{L}(o(\mathbf{x})|\lambda,\hat{\theta}) = 0 \tag{11}$$

2. Richardson-Lucy with TV Regularization [*Dey et al.* 2004]

$$o_{n+1}(\mathbf{x}) = [\frac{i(\mathbf{x})}{(o_n * h_{\hat{\sigma}_r,\hat{\sigma}_z})(\mathbf{x})} * h_{\hat{\sigma}_r,\hat{\sigma}_z}(-\mathbf{x})] \cdot \frac{o_n(\mathbf{x})}{1 - \lambda div(\frac{\nabla o_n(\mathbf{x})}{|\nabla o_n(\mathbf{x})|})} \tag{12}$$

PSF parameter estimate

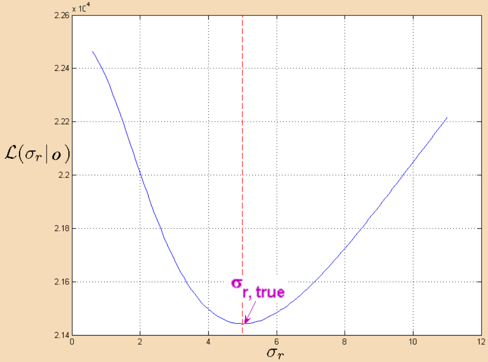
- If we denote $\boldsymbol{\theta} = (\sigma_r, \sigma_z)$ as the unknown parameters, the log-likelihood can be written as:

$$\mathcal{L}(\boldsymbol{\theta}|\widehat{o}) = -\sum_{\mathbf{x}\in\Omega}(i(\mathbf{x})log[h(\boldsymbol{\theta}) * \hat{o}](\mathbf{x})) + \sum_{\mathbf{x}\in\Omega}[h(\boldsymbol{\theta}) * \hat{o}](\mathbf{x}) \qquad (13)$$

- and the gradient w.r.t $\boldsymbol{\theta}$ as,

$$\nabla_{\boldsymbol{\theta}}\mathcal{L}(\boldsymbol{\theta}) = \sum_{\mathbf{x}\in\Omega}((h_{\theta_j} * \hat{o}(\mathbf{x})) - \frac{i(\mathbf{x})}{h * \hat{o}(\mathbf{x})} h_{\theta_j} * \hat{o}(\mathbf{x})) \qquad (14)$$

Analysis of Cost Function



Cost Function as a property of the radial PSF parameter when the initial object and observation are known.

PSF parameter estimate

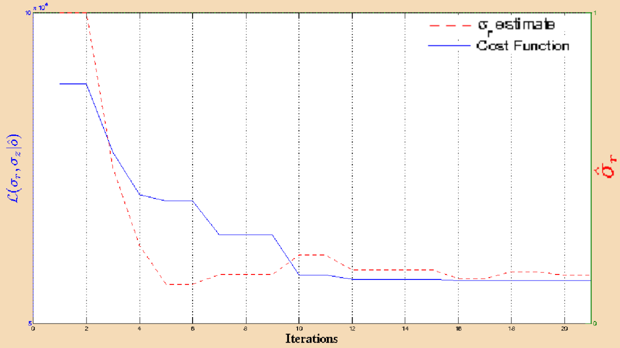
- Conjugate-Gradient algorithm:

$$\hat{\boldsymbol{\theta}}_{k+1} = \hat{\boldsymbol{\theta}}_k - \alpha_k \nabla_{\boldsymbol{\theta}} \mathcal{L}(\hat{\boldsymbol{\theta}}_k | \widehat{o}) \quad (15)$$

- Stopping criteria,

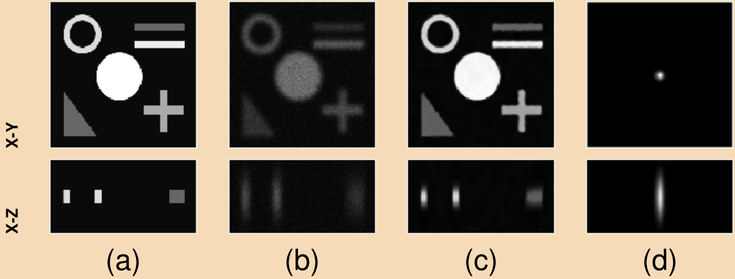
$$\chi_{k+1} = \frac{|\hat{\boldsymbol{\theta}}_{(k+1)} - \hat{\boldsymbol{\theta}}_{(k)}|}{\hat{\boldsymbol{\theta}}_{(k)}} < \epsilon, \ (\epsilon \leq 10^{-3}) \quad (16)$$

Analysis on synthetic data



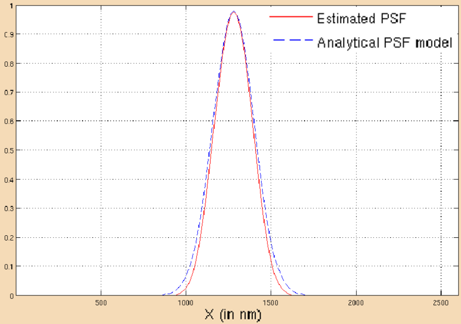
Convergence plot of the radial PSF parameter (σ_r) by the Conjugate-Gradient method.

Results on synthetic data



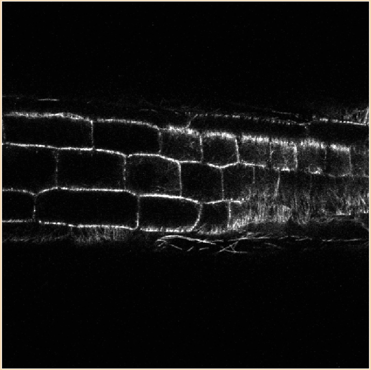
(a) Composite synthetic object, (b) observed image with the analytical blur model and Poisson noise, (c) after RL+TV deconvolution with the estimated PSF, (d) reconstructed diffraction-limited PSF.

Results on Synthetic Data



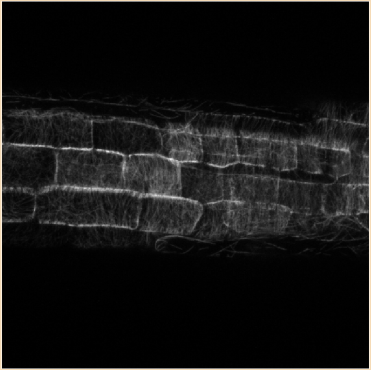
Comparison of the analytically and the estimated parametric diffraction-limited PSF models.

Preliminary results on real data



Root meristem of the plant Arabidopsis thaliana scanned by Zeiss LSM 510, C-Apochromat lens, Δ_{XY}: $0.29\mu m$, Δ_Z: $0.44\mu m$ (depth of about $14.08\mu m$), ©INRA Sophia-Antipolis

Preliminary results on real data



Deconvolved Image after restoration by the RL+TV algorithm and PSF parameter estimation

Conclusions and Future Work

☺ The alternate minimization algorithm jointly estimates a separable 3D Gaussian PSF and the object.

☺ TV regularization preserves borders very well.

☹ Small structures close to noise are not well restored (staircase effect) and some corners are rounded.

☹ Model chosen is for the diffraction-limited PSF and does not include spherical aberrations.

► Additional experimentation on confocal image data of specimens.

► Investigate and extend to the spherically-aberrated PSF [*Gibson & Lanni*, 1991] or [*P. Török et al.*, 1995] and improve the prior representation of the specimen.

References

- [*Hebert*89] T. Hebert and R. Leahy, "*A Generalized EM Algorithm for 3-D Bayesian Reconstruction from Poisson Data using Gibbs Prior,*" IEEE Trans. on Medical Imaging, vol. 8, no. 2, June 1989.
- [*Dey et.al* 04] N. Dey, L. Blanc-Feraud, J. Zerubia, C. Zimmer, J-C. Olivo-Marin and Z. Kam, "*A Deconvolution Method for Confocal Microscopy with Total Variation Regularization,*" Proc. ISBI'2004, pp.1223-1226, Apr. 2004.

References

- [*Gibson & Lanni* 91] S. F. Gibson and F. Lanni, "*Experimental test of an analytical model of aberrations in an oil-immersion objective lens used in three-dimensional light microscopy,*" Journal of Microscopy, vol. 8, no. 10, pp. 1601-1613, Oct. 1991.
- [*Zhang et al* 06] B. Zhang, J. Zerubia and J-C. Olivo-Marin, "*A study of Gaussian approximations of fluorescence microscopy PSF models,*" SPIE Conf. on microbiology, San Jose, Jan. 2006.