Deconvolution in Fluorescence Microscopy - Phase I

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

\[ i(x) = \mathcal{P}[h * o](x) \]  

(1)

where \( * \) denotes 3D convolution.

Likelihood of the observed data \( i \) knowing the specimen \( o \) is given as,

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

(2)
Gibbsian distribution $P(X = o)$ with TV functional captures the prior knowledge of the object, and is the regularization model.

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

where, $|\nabla o(x)| = (\sum_{x' \in V_x} (o(x) - o(x'))^2)^{1/2}$; $Z_\lambda = \sum_o e^{-\lambda \sum_x |\nabla o(x)|}$.
For a fluorescent microscope,

\[ h(x) = |P_{\lambda_{em}}(x)|^2 \cdot |P_{\lambda_{ex}}(x)|^2 \]  \hspace{1cm} (4)

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

If the pinhole model \( A_R \) is included, then the analytical CLSM PSF model is,

\[ h(x) = |A_R(x) \ast P_{\lambda_{em}}(x)|^2 \cdot |P_{\lambda_{ex}}(x)|^2 \]  \hspace{1cm} (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]:

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

where, 
\[
Z_{\sigma_r,\sigma_z} = (2\pi)^{\frac{3}{2}} \sigma_r^2 \sigma_z
\]
From the Bayes theorem, the **Posterior probability** is,

\[
P(X = o | Y = i) \propto P(Y = i | X = o)P(X = o)
\]

(7)

Thus, the **conditional probability** can be written as:

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

(8)
Alternate Minimization Algorithm

- The Cost Function to be minimized has the form:

\[
\mathcal{L}(o, h) = -\lambda \sum_{x \in \Omega} |\nabla o(x)| \log[Z_x] + \sum_{x \in \Omega} (i(x) \log[h \ast o](x)) - \sum_{x \in \Omega} [h \ast o](x)
\]

(9)

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

\[
\mathcal{L}(\hat{o}_{new}, h(\hat{\theta}_{new})) \leq \mathcal{L}(\hat{o}_{old}, h(\hat{\theta}_{old}))
\]

(10)
Minimizing the cost function (9) w.r.t $o$,

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

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

$$o_{n+1}(x) = \left[ \frac{i(x)}{(o_n * h_{\hat{\sigma}_r, \hat{\sigma}_z})(x)} * h_{\hat{\sigma}_r, \hat{\sigma}_z}(-x) \right] * \frac{o_n(x)}{1 - \lambda \text{div} \left( \frac{\nabla o_n(x)}{|\nabla o_n(x)|} \right)}$$ (12)
If we denote $\theta = (\sigma_r, \sigma_z)$ as the unknown parameters, the log-likelihood can be written as:

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

and the gradient w.r.t $\theta$ as,

$$\nabla_\theta \mathcal{L}(\theta) = \sum_{\mathbf{x} \in \Omega} ((h_{\theta_j} * \hat{o})(\mathbf{x})) - \frac{i(\mathbf{x})}{h * \hat{o}(\mathbf{x})} h_{\theta_j} * \hat{o}(\mathbf{x})) \quad (14)$$
Analysis of Cost Function

Cost Function as a property of the radial PSF parameter when the initial object and observation are known.
Conjugate-Gradient algorithm:

\[
\hat{\theta}_{k+1} = \hat{\theta}_k - \alpha_k \nabla_{\theta} \mathcal{L}(\hat{\theta}_k | \hat{o})
\]  

(15)

Stopping criteria,

\[
\chi_{k+1} = \frac{|\hat{\theta}_{(k+1)} - \hat{\theta}_{(k)}|}{\hat{\theta}_{(k)}} < \epsilon, \ (\epsilon \leq 10^{-3})
\]  

(16)
Analysis on synthetic data

Convergence plot of the radial PSF parameter ($\sigma_r$) 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.
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, $\Delta_{XY}: 0.29 \mu m$, $\Delta_Z: 0.44 \mu m$ (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
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

