Restoration of 3D Laser Scanning Confocal Microscopy Images.
Detection of thin strucutres like filaments.

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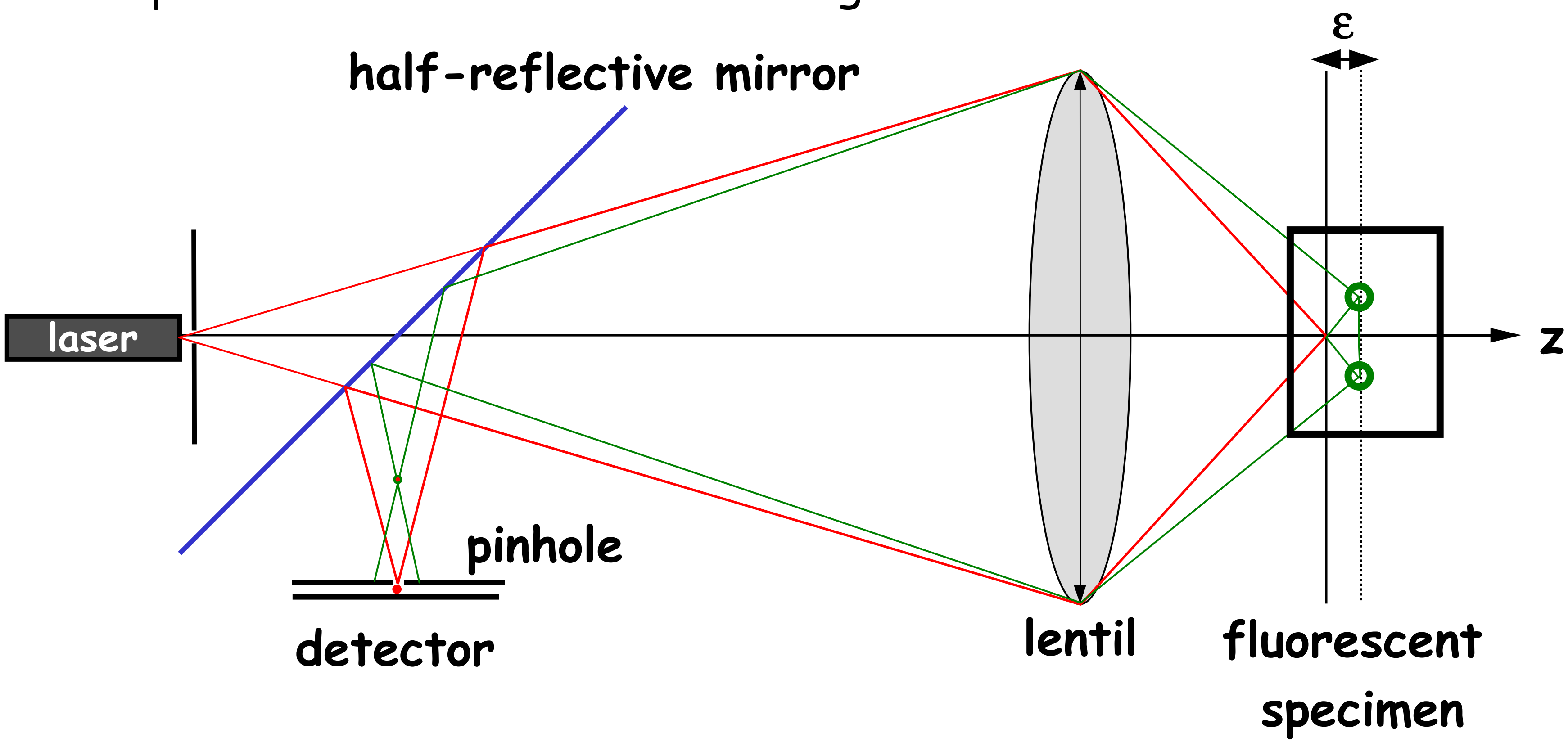
Laser Scanning Confocal Microscopy (LSCM)

- fluorescence: light emission after absorption of photons of high energy.
- Fluorescent specimen will emit photons after been excited by a laser. Detection of these photons give an image of the specimen.
- Extensively used in biology
  - → To see live cells, it can be used GFP (Green Fluorescent Protein) tracer, which makes the cell synthesizes fluorescent proteins. So the cell becomes fluorescent and can be imaged in vivo.
  - → DAPI (Di Amidino-2-Phényl Indole) is a fluorescent molecule which fix DNA.
  - → …

Confocal - 3D object

- Confocal microscope principle
  - pinhole: to limit out-of-focus light



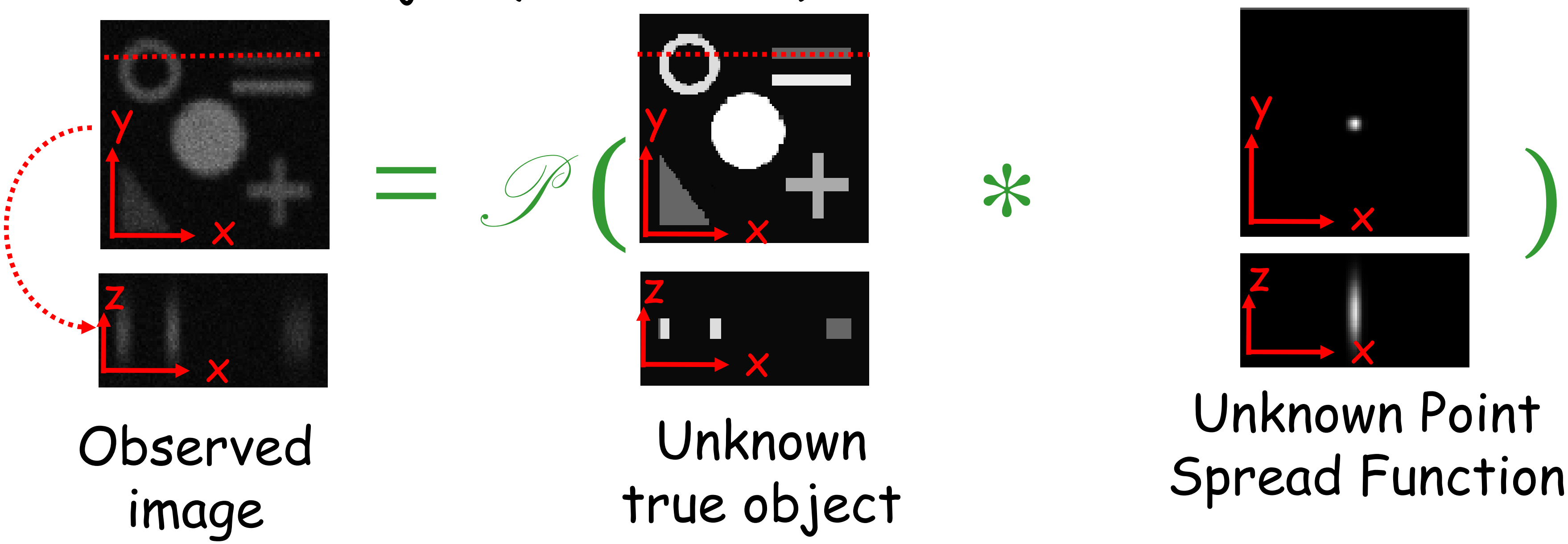
Restoration of confocal microscopy images

- Laser Scanning Confocal Microscopy (LSCM)
  - fluorescence: labels specific structures of the specimen
  - confocal: a pinhole removes out-of-focus light (in XY directions)
  - 3D-scanning: produces 3D images (stacks)

- Confocal microscopy limitations:
  - Diffraction-limited nature of the optical system (finite-size objective aperture): small XY – blur remain. Contribution of non-focussed planes: Z-blur (in depth).
  - Assumption: no aberrations! (spherical, refractive index change…)
  - low-photon imaging: Poisson noise (multiplicative noise)

Image observation model

Simulated 3D object (128x128x64)



$$i = \mathscr{P}(o * h)$$

- Restoration Goal: Given the observation i, recover the object o
  - Degradations are imperfectly known: also recover h
  - Very ill-posed problem

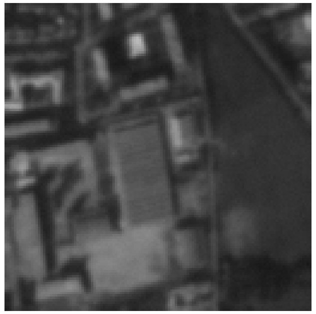
Observation model: Gaussian noise case

Observed images are degraded :

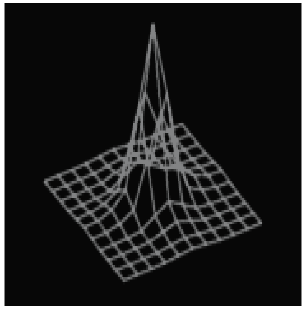
$$i = h * o + n$$

Noise
white Gaussian

Observed Image



Convolution kernel (PSF)



Original Image

- Restoration : retrieve o from i
- Inverse $i = h * o + n$ is an ill posed problem

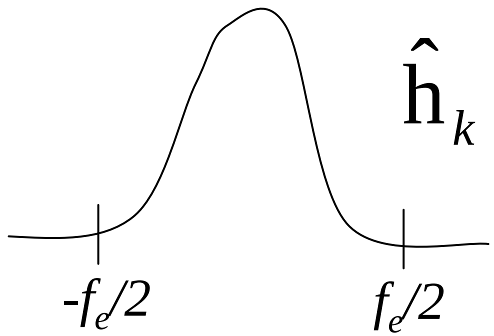
Dans le plan de Fourier

- La convolution discrète circulaire est transformée en un produit simple dans Fourier (conditions de bords périodiques):

$$i = h * o + n \quad \rightarrow \quad \hat{i} = \hat{h}.\hat{o} + \hat{n}$$

- Deconvolution = Inversion dans Fourier si TF PSF non nulle

$$\hat{o}_k = \frac{\hat{i}_k}{\hat{h}_k} + \frac{\hat{n}_k}{\hat{h}_k}$$



Inversion

Image floue

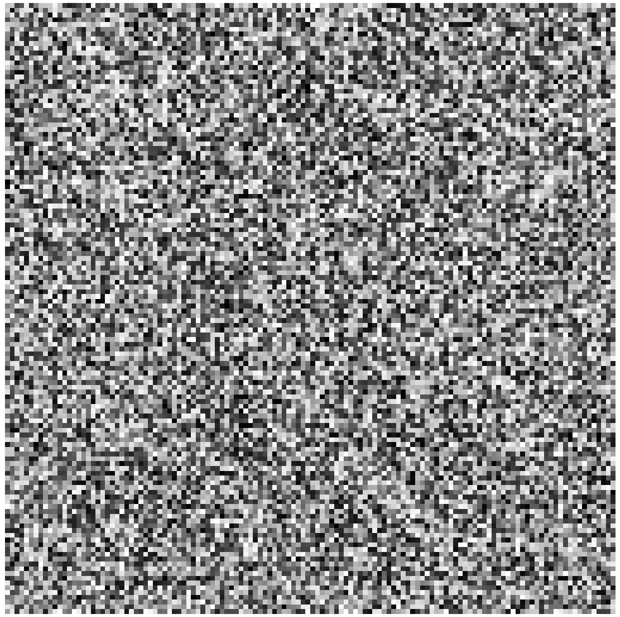
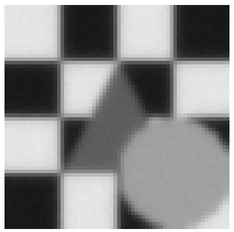
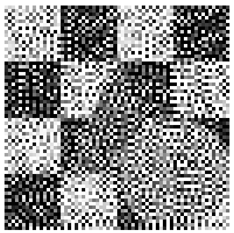
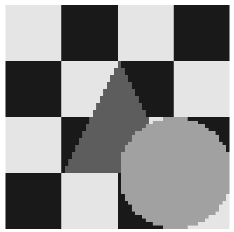


Image déconvoluée
sans régularisation





$\neq$



Probabilistic model

- Image formation:

$$i(x) = \mathscr{P}\big(\big(h * o\big)(x)\big)$$

- Likelihood probability

  - probability of observing i knowing o and h

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

  - one restoration method: maximizing this probability

- Assume first that h is perfectly known
  - Find o that maximizes this probability

Deconvolution algorithm (*h* known)

- Minimizing *-log[p(i/o)]*

  - functional to minimize

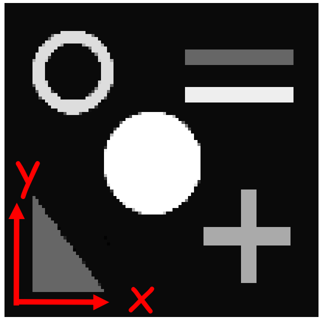
$$J_{data}(i,o) = \sum_{x \in \Omega} [(h*o)(x) - i(x).\log(h*o)(x)]$$

  - Richardson-Lucy: EM algorithm or multiplicative gradient-based method
    → [Richardson74] [Lucy72]

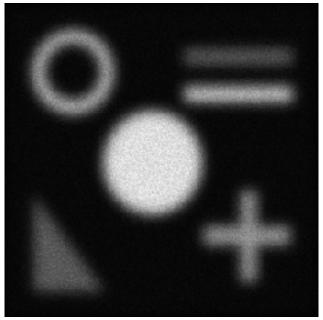
$$o_{n+1}(x) = o_n(x)\left[h(-x) * \frac{o(x)}{(h*o_n)(x)} \right]$$

  - Adapted to Poisson noise distribution, positivity constraint
  - Regularization by stopping the iterations

ML-RL algorithm with no regularization

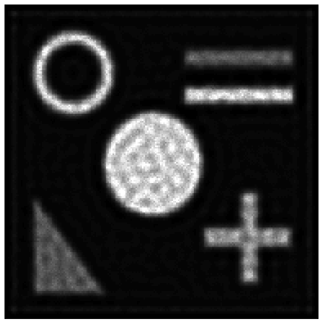


Original image



Degraded image
Blur + Poisson noise

Restored by RL
200 iterations



Restored by RL
500 iterations

➔ Need for Regularization

Regularization

- Incorporate a regularization term:
  - MAP estimation with a priori probability on the object

$$\max_o P(X = o \mid Y = i) \Leftrightarrow \max_o P(Y = i \mid X = o) P(X = o)$$

  - Equivalent to penalized criterion

$$\min_o J_{data}(i, o) + J_{reg}(o)$$

In bayesian formulation:

$$\min_o \sum_{x \in \Omega} (i(x) \log[h * o](x)) - \sum_{x \in \Omega} [h * o](x) - \log[P(o)]$$

Regularization on the gradient

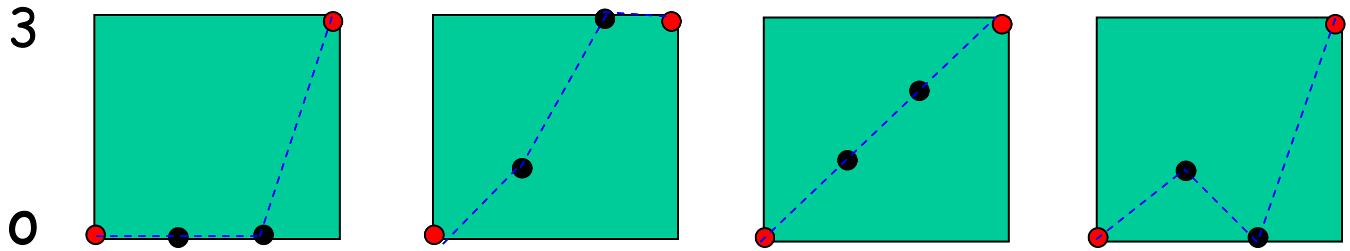
- Incorporate a regularization term into RL (quadratic)
  - → [v.Kempen & v.Vliet97] [v.d.Voort & Strasters95]
  - ➢ Regularizes but smoothes edges

$$J_{reg}(o) = \lambda \sum_{x} |\nabla o(x)|^2$$

- Total variation regularization
  - → [N.Dey & al. 06]
  - ➢ preserves edges in the image
  - ➢ smoothes homogeneous areas

$$J_{reg}(o) = \lambda \sum_{x} |\nabla o(x)|$$

- l^2 / l^1 norms: exemple in 1D.



$\|\|.\|\|_2^2=9$	$\|\|.\|\|_2^2=5$	$\|\|.\|\|_2^2=3$	$\|\|.\|\|_2^2=11$
$\|\|.\|\|_1=3$	$\|\|.\|\|_1=3$	$\|\|.\|\|_1=3$	$\|\|.\|\|_1=5$

$$\|\nabla o\|_2^2 = \sum_i |o_i - o_{i-1}|^2$$

$$\|\nabla o\|_1 = \sum_i |o_i - o_{i-1}|$$

Regularization on the gradient : RL-TV

- Criterion to minimize

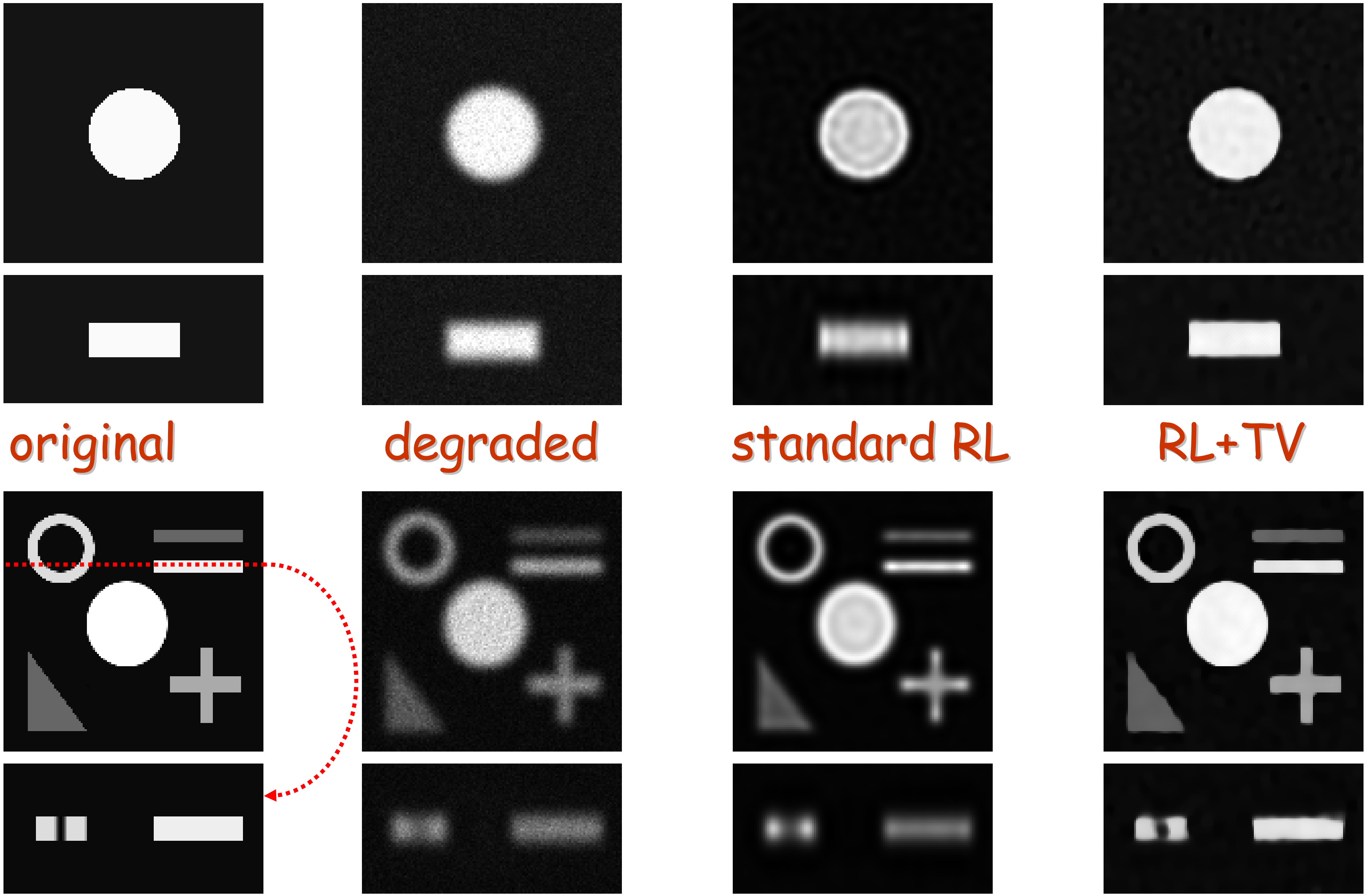
$$J(o) = \sum_x (h*o)(x) - i(x)\log\left((h*o)(x)\right) + \lambda \sum_x \left|\nabla o(x)\right|$$

- Multiplicative gradient descent algorithm : Regularized Richardson-Lucy with Total Variation (RL-TV)

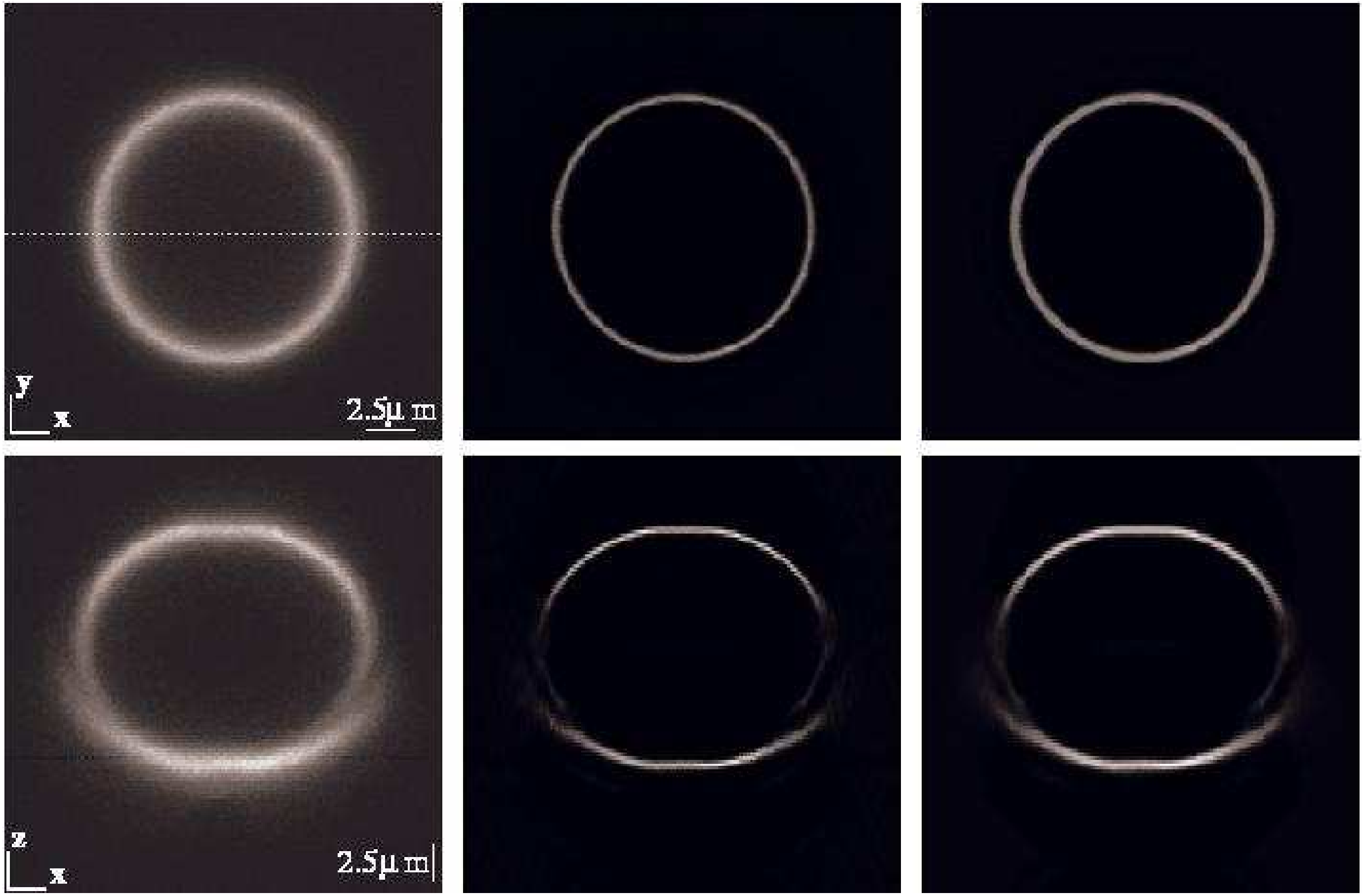
$$o_{n+1}(x) = \frac{o_n(x)}{1 - \lambda \,\mathrm{div}\left(\frac{\nabla o_k}{\left|\nabla o_k\right|}\right)} \left[h(-x) * \frac{o(x)}{(h*o_n)(x)} \right]$$

Applied until convergence

Results comparison



Results on real data



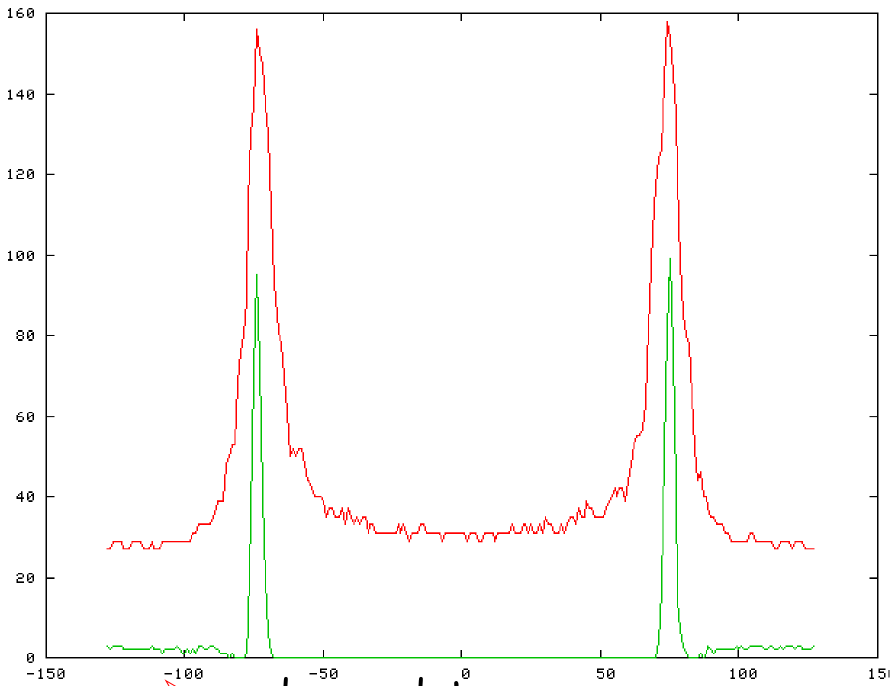
Spherical Shell
@Pasteur

RL algo.

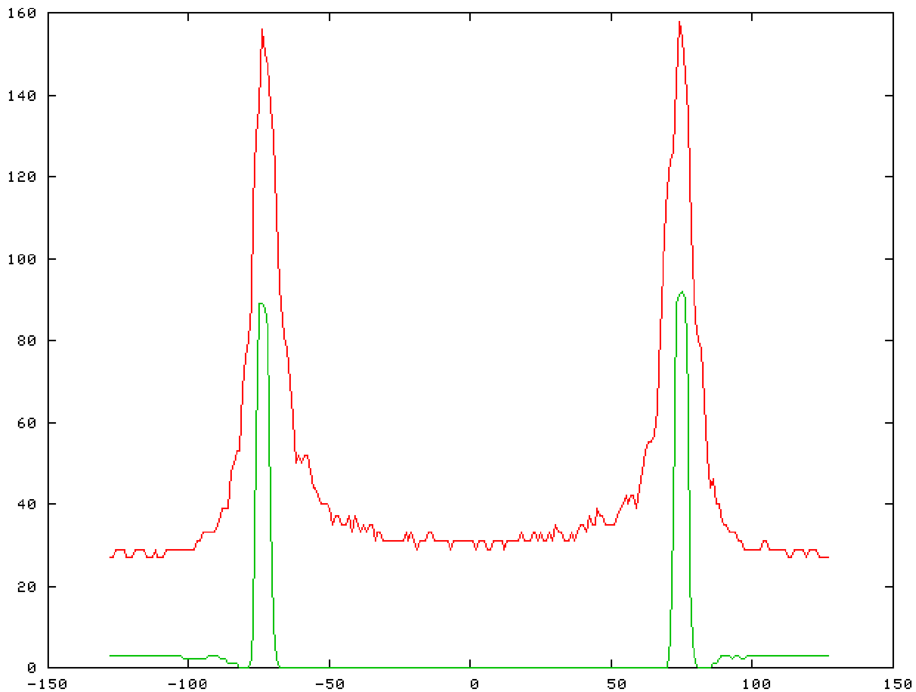
RL + TV algo.

Results on real data

- Profile of one image



- red: raw data
- green: standard RL



- red: raw data
- green: RL+TV

Thickness of the ring: ~500 nm,

Measured on degraded image: ~900 nm

Measured on restored image by RL: ~ 400nm

Measured on restored image by RL+TV: ~500nm

Knowledge on the PSF

- Theoretical physical model for a fluorescent microscope

  - If we exactly know the physical parameters during the acquisition (refractive index, numerical aperture, depth of the specimen under the coverslip) then we theoretically know the PSF, assuming no aberrations.

  - However, this model may differ from the true one
    - → **Physical parameters imperfectly known**
    - → **PSF too much simplified**

- Experimental PSF
  - fluorescent microbeads imaging to approximate the PSF
  - need to average several images of microbeads due to noise
  - Physical acquisition conditions should be maintain during the image acquisition of the specimens.

PSF estimation needed

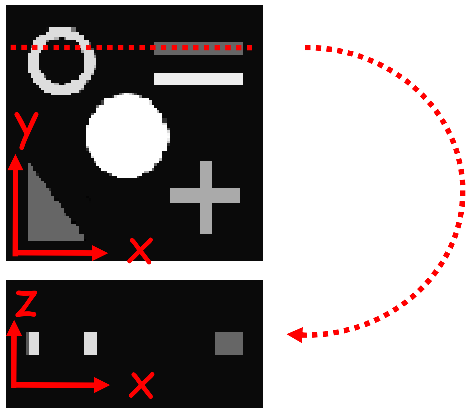
Need for an estimation of the PSF from the observed specimen itself.

- This is a very ill-posed problem: form the observation i recover both o and h form the observation model $i=\mathscr{P}(h*o)$

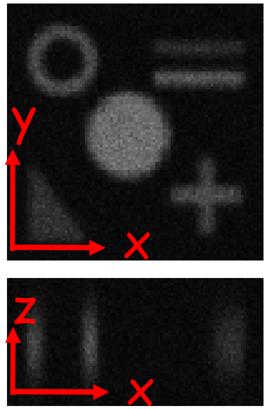
Blind deconvolution problem, no unique solution!

- to reduce the number of unknown, use a parametric PSF

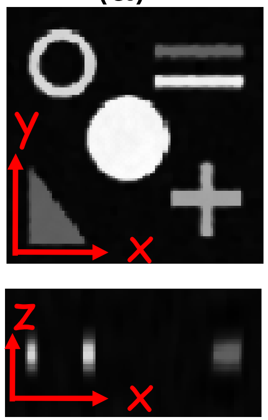
Results on synthetic data



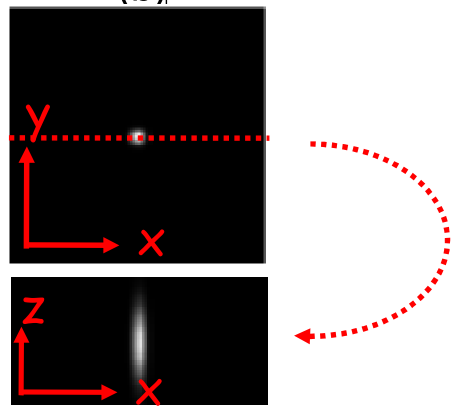
(a)



(b)



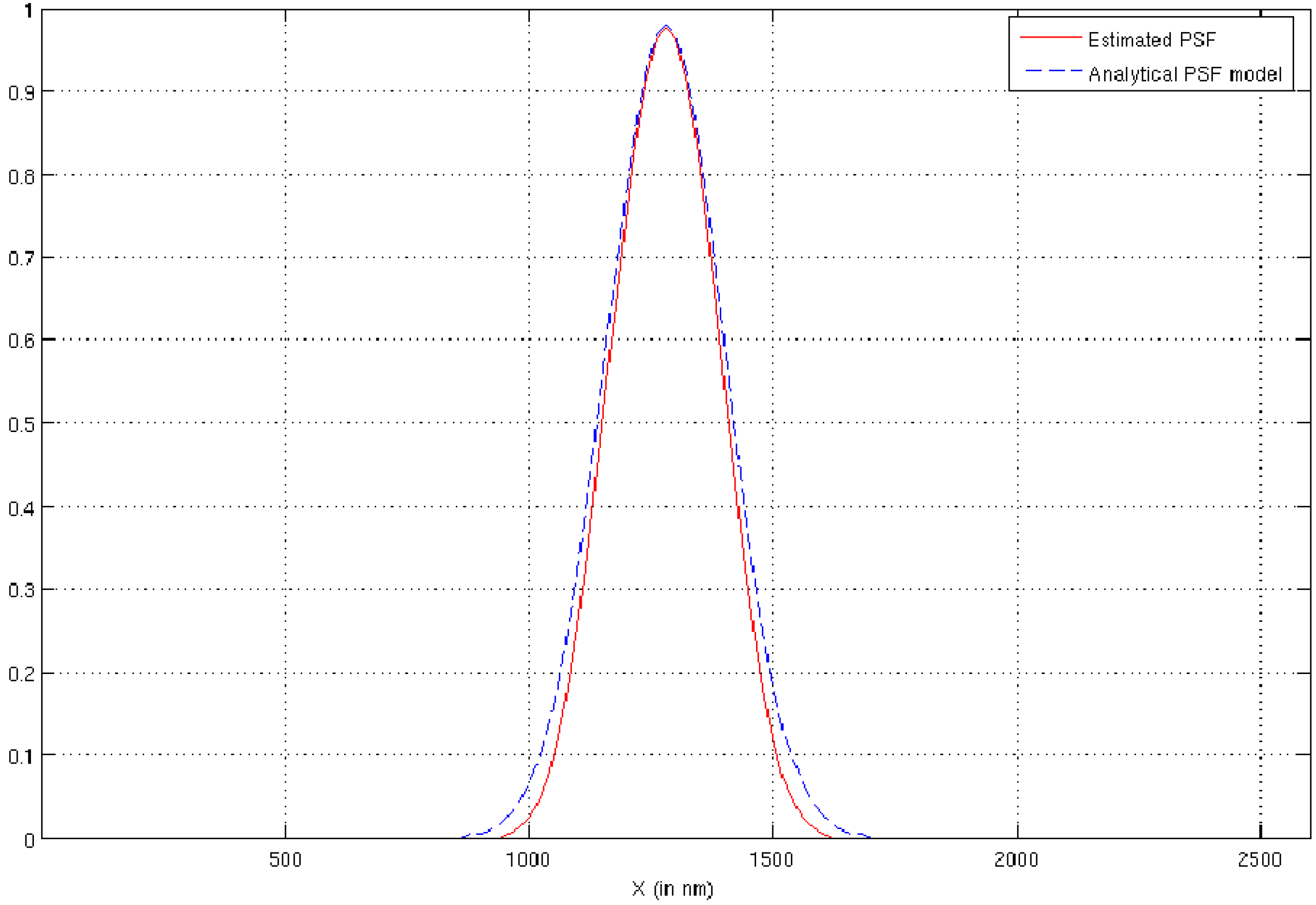
(c)



(d)

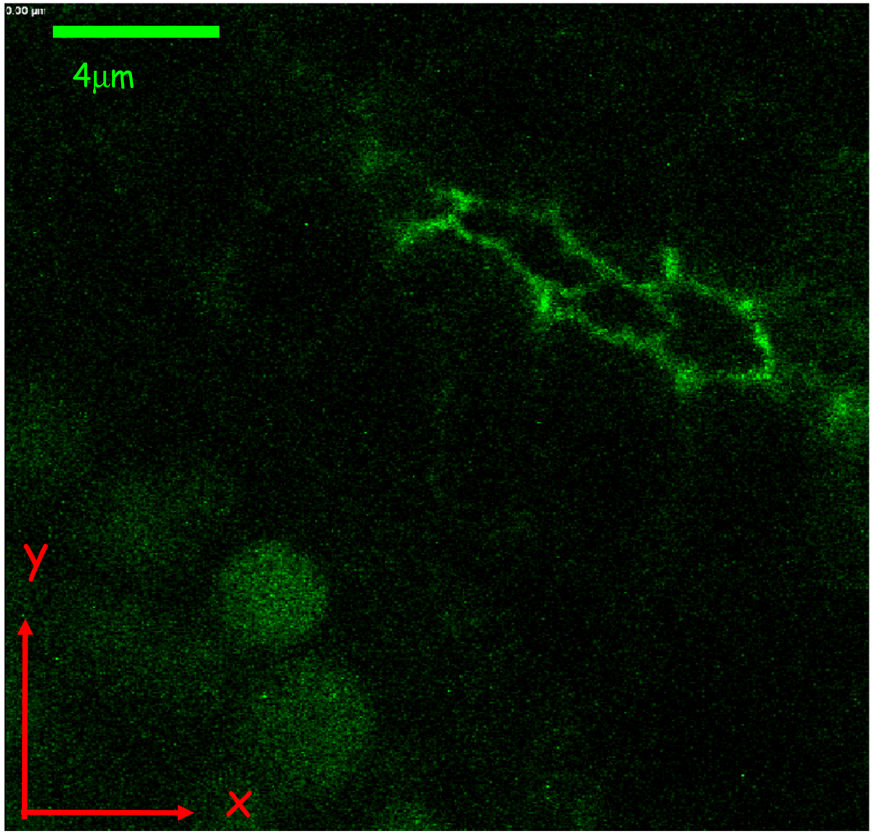
(a) Composite synthetic object, (b) observed image with the analytical blur model and Poisson noise, (c) after RL+TV deconvolution with the estimated PSF, (d) reconstructed diffraction-limited PSF.

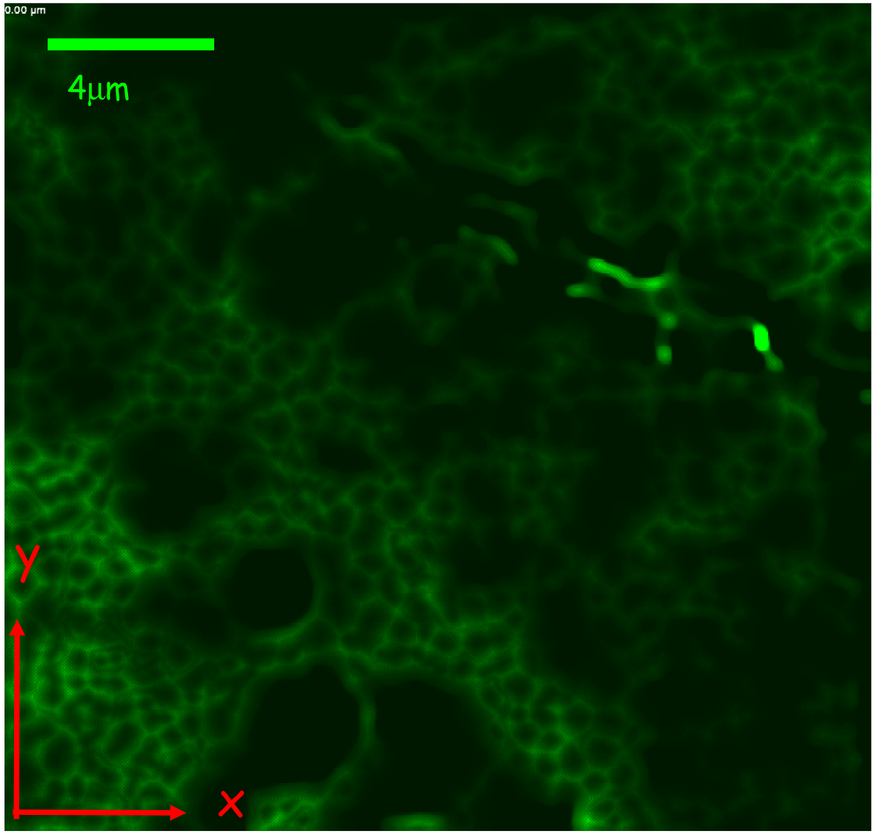
PSF comparison



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

Preliminary results on real data



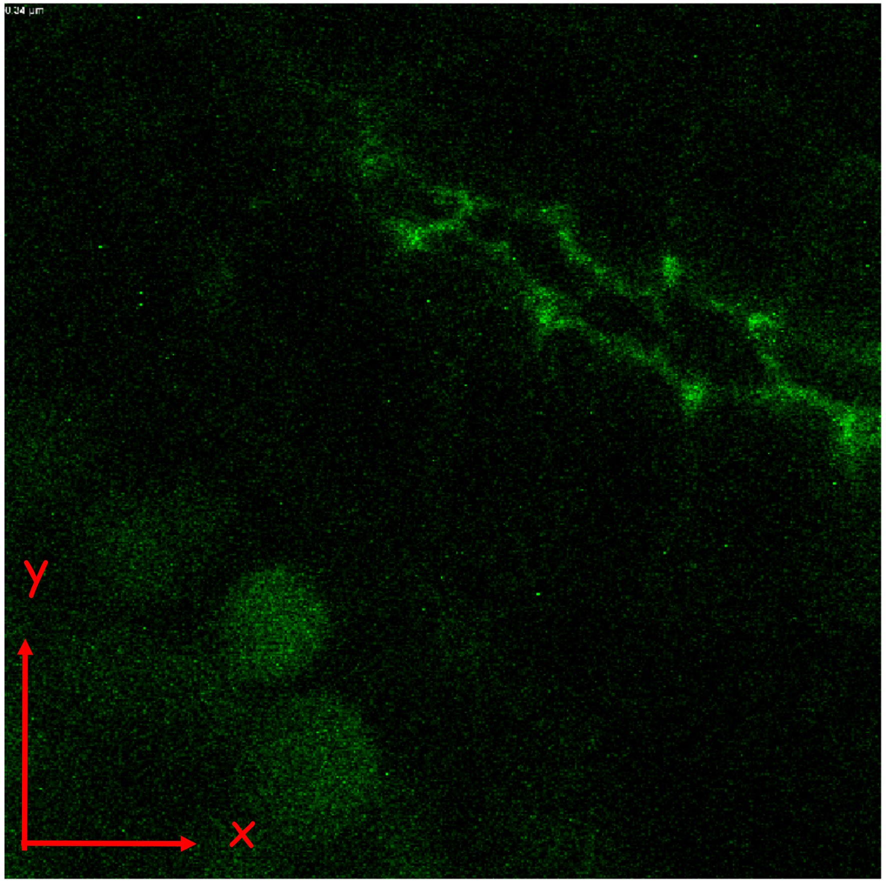


Observed Restored

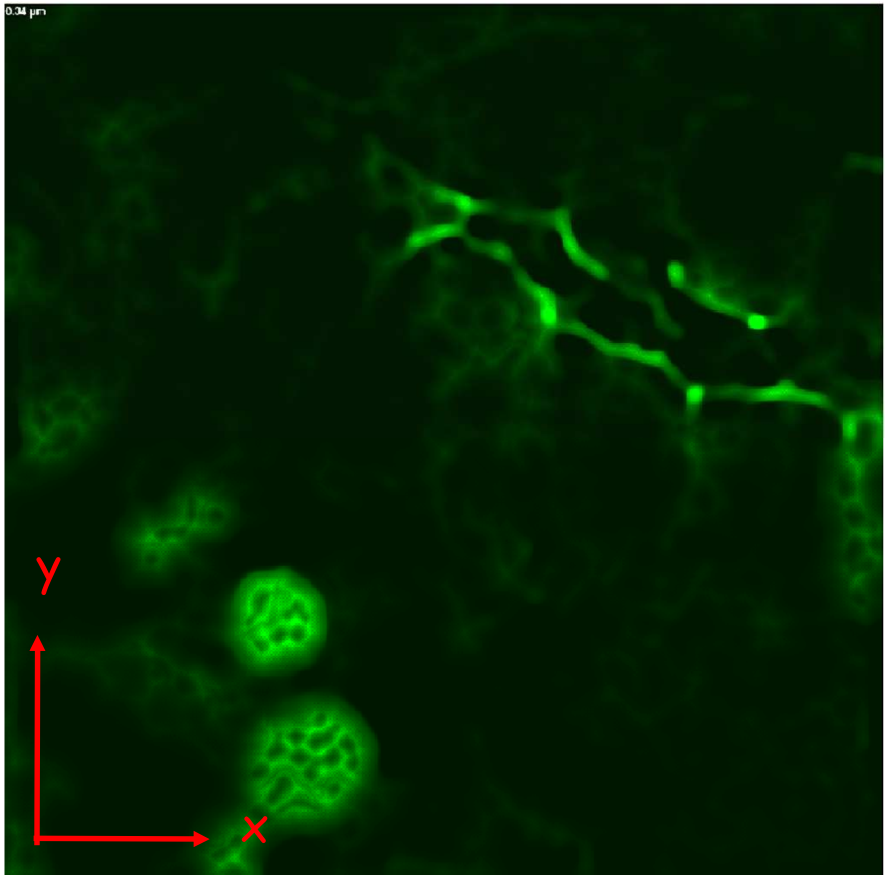
Restoration results at depth of 0µ*m*, ©UNSA, INRIA

Embryonic division of a Drosophilla scanned by Zeiss LSM 510, C-Apochromat lens,
Restoration using the Alternate Minimization algorithm, with λ = 0.05 and ε = 0.002

Preliminary results on real data



Observed



Restored

Restoration results at dept of 0.46µm, ©UNSA, INRIA

Estimated parameters σ_r= 257,9nm, σ_z =477nm

Theoretical from physical parameters : σ_r =76,5nm, σ_z =230,8nm

Filament detection in 3D images

- In order to detect singularities of co-dimension *k* in an ambient space of dimension *n+k* , we need to use a function $u : \mathbb{R}^3 \rightarrow \mathbb{R}^2$
- In fact, the observed image of the filament gives us a noisy and blurred version of the filament :

$$I = G_\sigma * I_0 + n$$

- We will try to
  - first have a pre-detection of the filament by using the Hessian matrix *H* of *I*
  - then affine this detection to have a thin filament and complete the missing part of the filament by using a GL model.

Filament detection

Because the observations are noisy, I is either smoothed or approximated by using an order 2 polynomial. We assume that I is twice differentiable and we denote H its Hessian matrix. Let λ_i, $i=1,2,3$ the eigenvalues such that

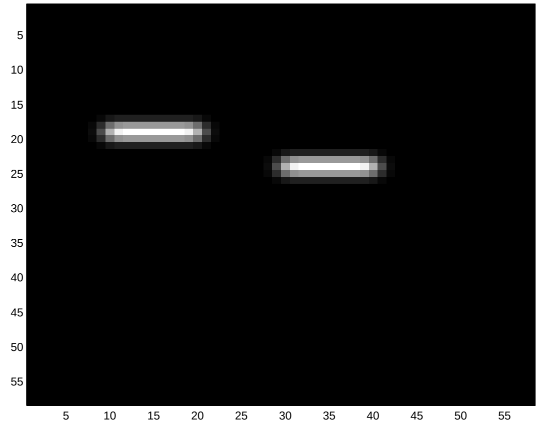
$$|\lambda_1| \le |\lambda_2| \le |\lambda_3|$$

and v_i their associated eigenvectors. Then we know that a filament corresponds to the case where $|\lambda_2|$ and $|\lambda_3| \to \infty$ as $\sigma \to 0$

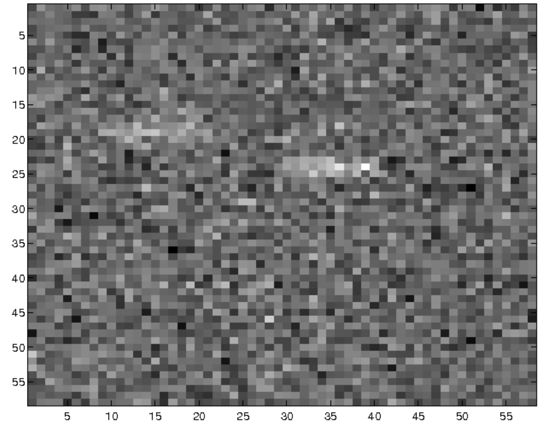
and $|\lambda_1| << |\lambda_2|$

The set $M_\alpha=\{x \in \Omega \;/\; | \lambda_2 \lambda_3 | > \alpha\}$ contains points where I has high variations in almost the two directions v_2 and v_3. So it contains filaments and isolated points.

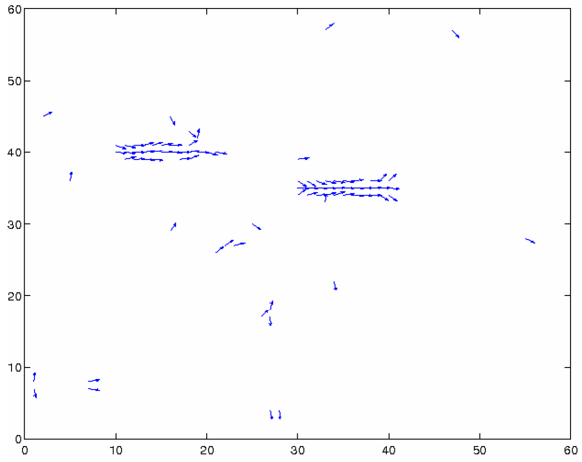
Example on a synthetic image



Blurred filament



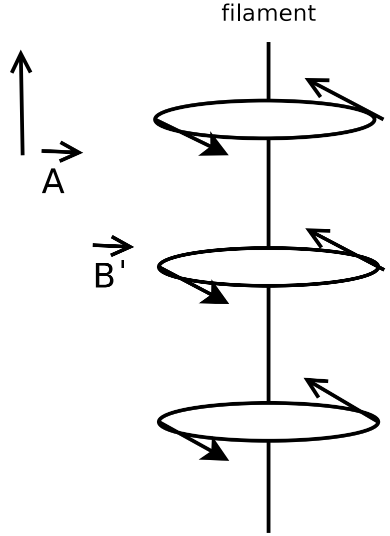
Blurred and noisy filament



v_1 vectors in M_α

Filament representation

- In order to represent a filament we draw our inspiration from magnetostatic. A filament is considered as an electric current line and we construct the associated potential field $\vec{A}$ and magnetic field $\vec{B}$.



$$\vec{A}(x) = \int_{M_\alpha} \frac{v_1(y)}{\|xy\|} dy \quad \forall x \in \Omega$$

$$\vec{B} = rot(\vec{A})$$

- $\vec{A}$ is a regularized version of v_1 since it is the sum over the contributions of all pre-detected points.
- $\vec{B}'$ is the ortho-normalized projection of $\vec{B}$ in the orthogonal plane to $\vec{A}(x)$

Skeleton of a filament

- For a rectilinear filament, $\vec{B}$ is in the orthogonal plane to $\vec{A}$, so $\vec{B}$ and $\vec{B}'$ are equal (up to the normalization). They spin around the filament.
- In this plane, the coordinate of $\vec{B}'$ are (-sin θ,cosθ) where θ is the polar angle. We have

$$\left\|\nabla\vec{B}'\right\|^2 = \left\|\frac{\partial\vec{B}'}{\partial r}\right\|^2 + \frac{1}{r^2}\left\|\frac{\partial\vec{B}'}{\partial\theta}\right\|^2$$

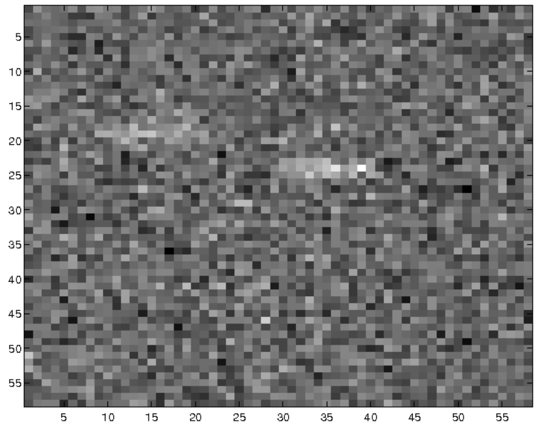
As $\vec{B}'$ does not depend on the radial component *r*, we have

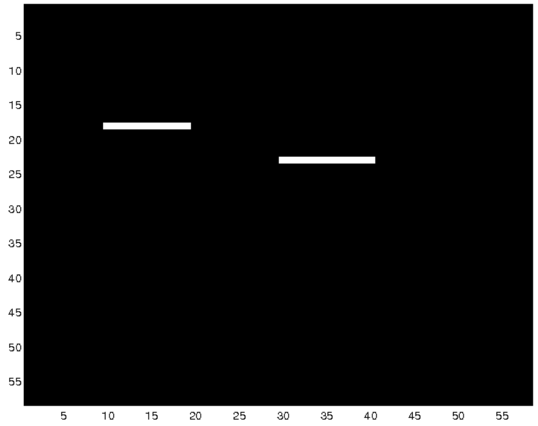
$$\frac{\partial\vec{B}'}{\partial r} = \vec{0} \quad \text{and} \quad \left\|\nabla\vec{B}'\right\|^2 = \frac{1}{r^2}\left\|\frac{\partial\vec{B}'}{\partial\theta}\right\|^2 \quad \text{thus} \quad \left\|\nabla\vec{B}'\right\|^2 = \frac{1}{r^2}\xrightarrow[r\to 0]{}+\infty$$

- We define the skeleton of a filament by

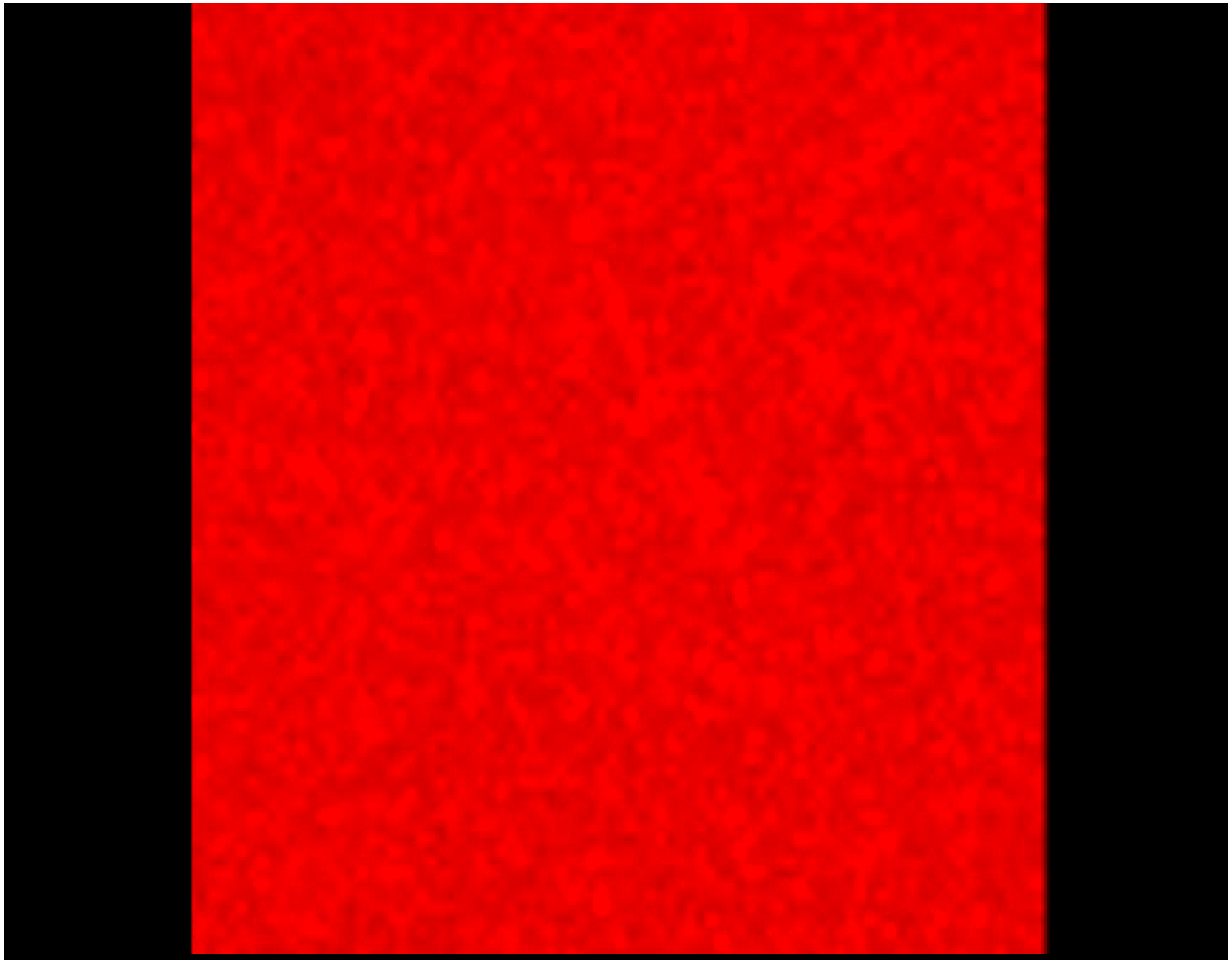
$$S = \left\{x\in\Omega / \lim_{y\to x}\left\|\nabla\vec{B}'\right\| = +\infty\right\}\cap\left\{x\in\Omega / \left\|\vec{A}\right\|\geq\beta\right\}$$

Detection Result

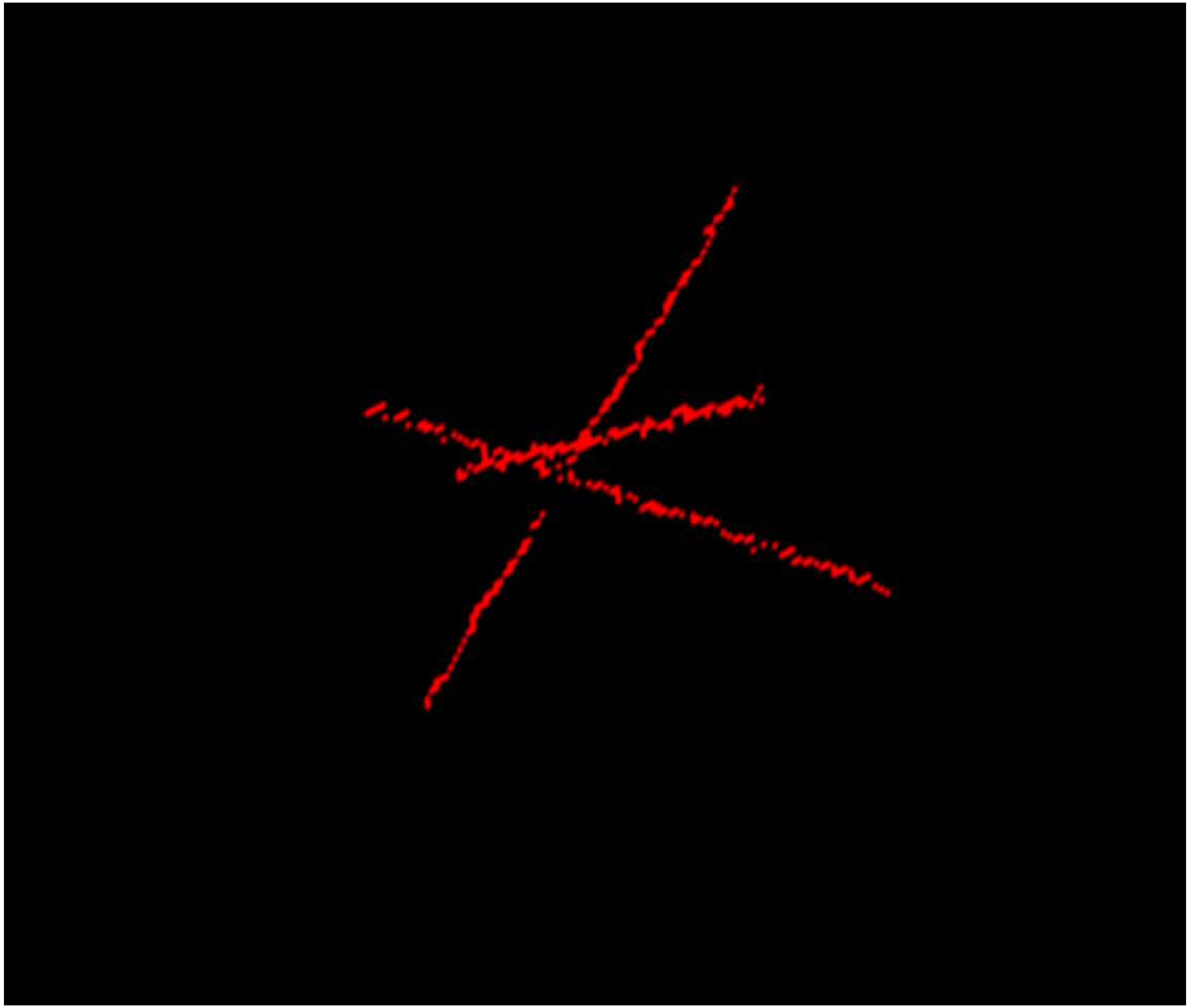




3D detection result



3D detection result



Other works, continuation

- Filament detections: other methods by variational approach (PhD thesis of Alexis Baudour)

- Restoration:

  - Small structures close to noise are not well restored by TV (staircase effect).

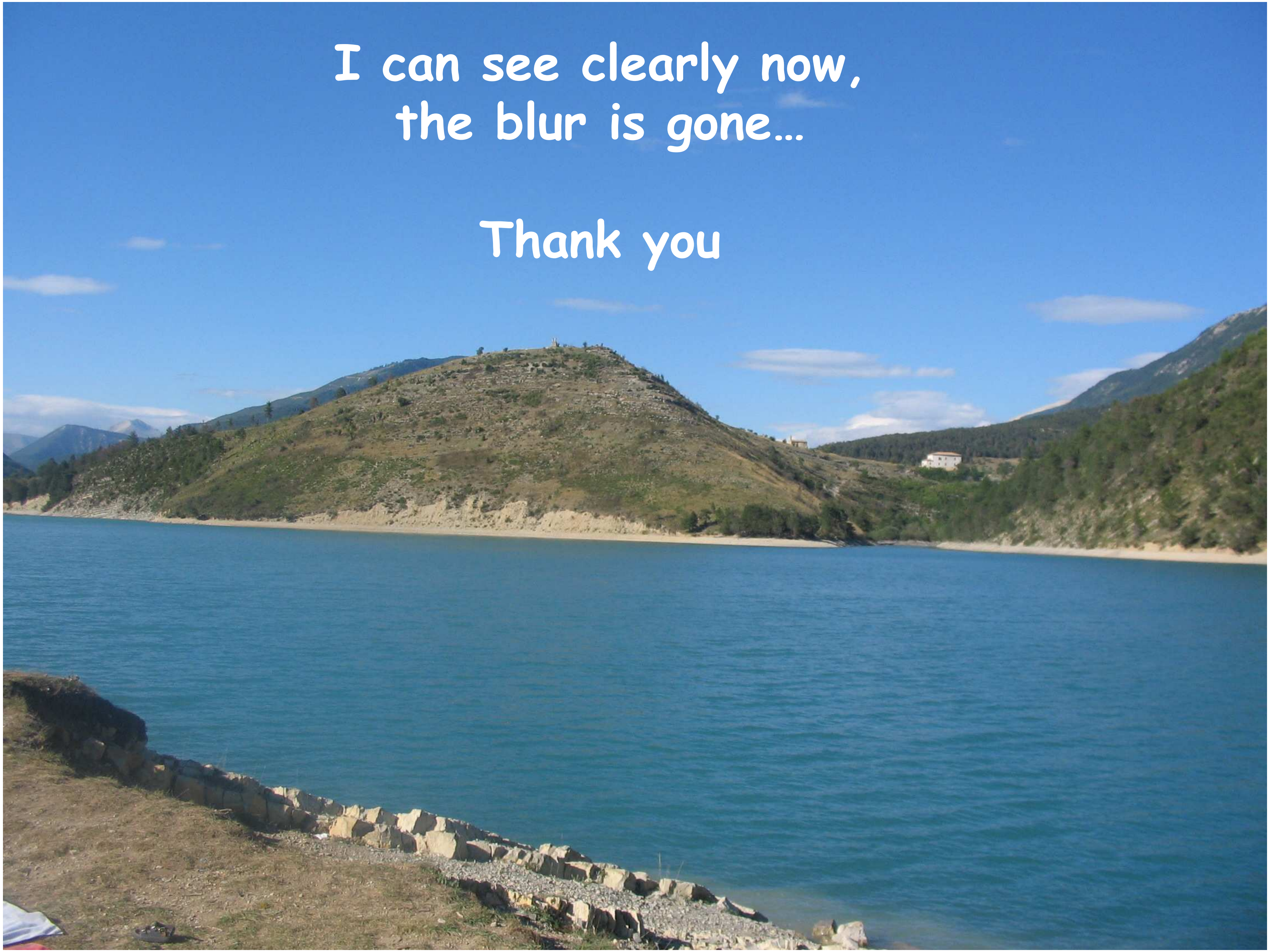
    → l^1 **regularization on wavelet coefficients.**

  - Automatic estimation of regularization parameters

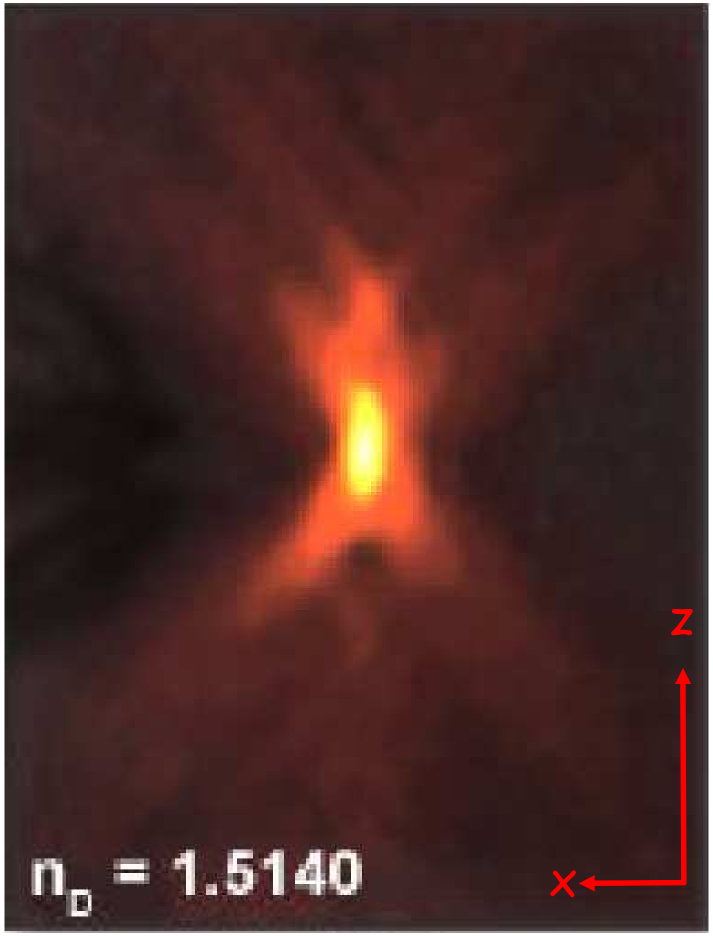
  - Additional experimentation on confocal image data of specimens.

  - Model chosen is for the diffraction-limited PSF and does not include spherical aberrations. Automatic estimation of the PSF from the observation

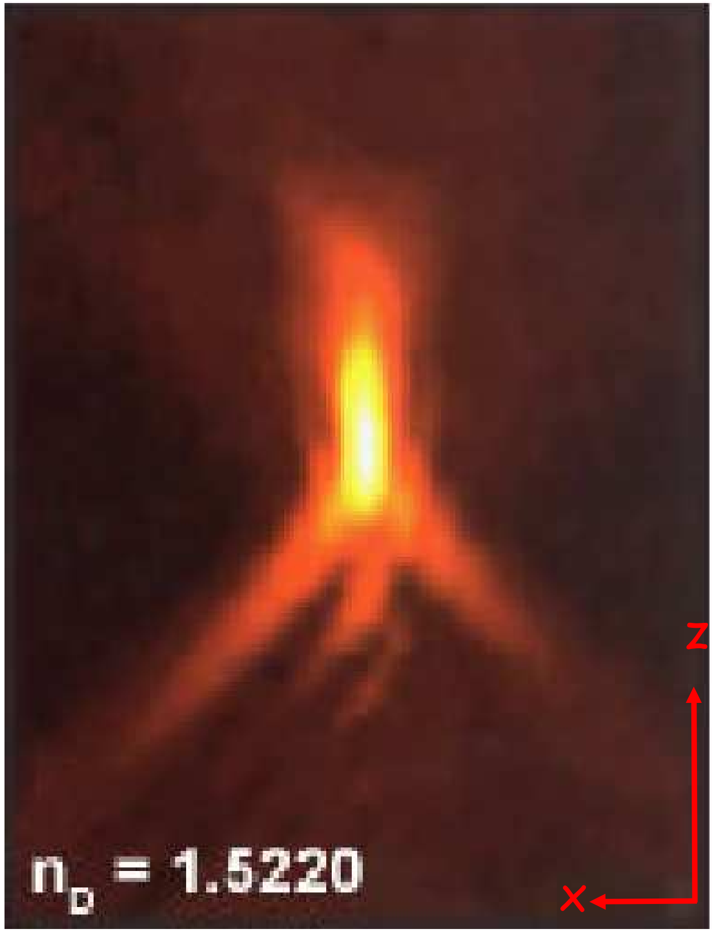
  - Investigate and extend to the aberrated PSF and improve the prior representation of the specimen.


I can see clearly now,
the blur is gone…
Thank you

Effect of Spherical aberrations



Non-aberrated PSF



Spherically aberrated PSF