

# Parameter estimation for fluorescence images deconvolution

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December 12, 2011

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# PHYSICAL ACQUISITION PROCESS

## Fluorescence imaging systems:

- ▶ Diverse noise sources
  - ▶ Some are signal dependent, e.g. photon noise
  - ▶ Some are signal independent, e.g. electric noise, thermal noise etc.
- ▶ Too complex for either Poisson or additive Gaussian noise
- ▶ Next simplest model: sum of Poisson and Gaussian models for signal dependent and signal independent components, respectively.
- ▶ Widely used in the literature [Paul *et al.* 2010]  
[Delibretti *et al.* 2008]

# DEGRADATION MODEL

$$\mathbf{y} = \alpha \mathbf{z}(\mathbf{x}) + \mathbf{w}$$

## Observations

$$\mathbf{y} \in \mathbb{R}^Q$$

→

# DEGRADATION MODEL

## Poisson noise

$$Z_i(\mathbf{x}) \sim \mathcal{P}([\mathbf{H}\mathbf{x}]_i)$$

$\mathbf{z}(\mathbf{x}) = (z_i(\mathbf{x}))_{1 \leq i \leq Q}$  - realization of  $Z_i(\mathbf{x})$

$\mathbf{x} \in [0, +\infty)^N$  - original signal

$\alpha \in \mathbb{R}$  - scaling parameter

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## Gaussian noise

$$W_i \sim \mathcal{N}(c, \sigma^2)$$

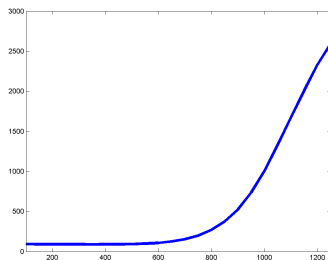
$\mathbf{w} = (w_i)_{1 \leq i \leq Q}$  - realization of  $W_i$

$c \in \mathbb{R}$  - mean  $\sigma^2$  - variance

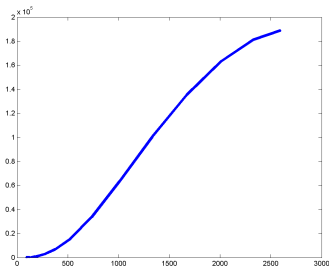
**Observations**

$$\mathbf{y} \in \mathbb{R}^Q$$

# MACROSCOPE GAIN VERSUS NOISE PARAMETERS



(a)

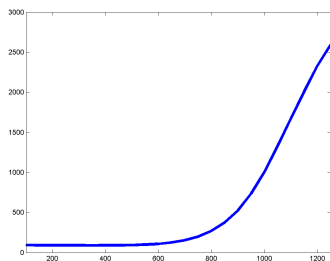


(b)

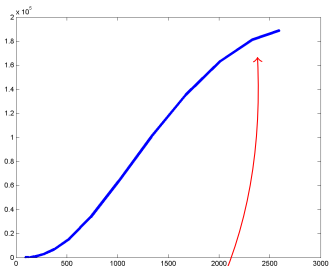
Figure: (a) mean as a function of gain and (b) variance as a function of mean.

**According to the model, measured signal variance should be a linear function of its mean**

# MACROSCOPE GAIN VERSUS NOISE PARAMETERS



(a)



(b)

Figure: (a) mean as a function of gain and (b) variance as a function of mean.

**NOTE: Poisson-Gaussian model is not valid for very low and very high GAIN settings**



# ALGORITHMS FOR RESTORING DATA CORRUPTED BY POISSON-GAUSSIAN NOISE: [BENVENUTO \*et al.\* 2008](#)

- ▶ Authors propose scaled gradient method for restoration
- ▶ Authors propose following approximation of its gradient:

$$\exp \left( - \frac{1 + 2([\mathbf{H}\mathbf{x} + \mathbf{c}]_i) - y_i}{2([\mathbf{H}\mathbf{x} + \mathbf{c}]_i) + \sigma^2} \right)$$

The noise parameters are assumed to be known.

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The noise parameters are **assumed** to be known.

# ALGORITHMS FOR RESTORING DATA CORRUPTED BY POISSON-GAUSSIAN NOISE: GIL-RODRIGO *et al.* 2011

- ▶ Authors propose alternating-minimization method for restoration
- ▶ The mean of Gaussian noise is assumed to be 0
- ▶ Authors propose to approximate the corresponding negative log likelihood with:

$$\frac{1}{2} \left\| \left( D_{\tilde{\sigma}^2} (\mathbf{H}\mathbf{x}) \right)^{-1/2} (\mathbf{y} - \mathbf{H}\mathbf{x}) \right\|_2^2$$

**Covariance matrix depends on noise parameters which are assumed to be known.**

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# ALGORITHMS FOR RESTORING DATA CORRUPTED BY POISSON-GAUSSIAN NOISE: *LUISIER et al. 2011*

- ▶ Authors propose PURE-LET method for denoising
- ▶ Authors derive new unbiased MSE estimate corresponding to Poisson-Gaussian likelihood
- ▶ Authors show that proposed approach is faster and more reliable than Anscombe variance stabilizing transform.

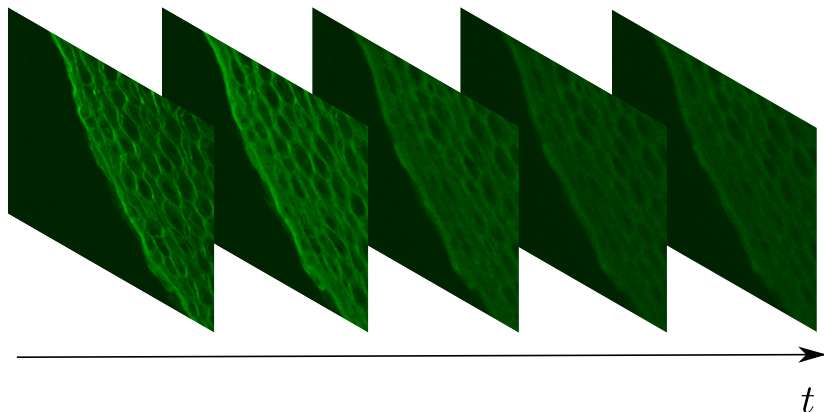
**Unbiased MSE estimate depends on noise parameters which are assumed to be known.**

# METADATA VERSUS NOISE PARAMETERS

**Metadata of fluorescence imaging systems (e.g. PMT Gain, PMT Offset) does not correspond to noise parameters ( $\alpha$ ,  $c$ )**  
**[Paul *et al.* 2010].**

**Noise parameters have to be estimated statistically.**

# TIME SAMPLES - BLEACHING EFFECT



**Bleaching effect** - process of intensity time decay usually modeled with an exponentially decreasing function

# DEPENDENCE BETWEEN NOISE MODEL AND PHOTBLEACHING

**We adopted the same model as the one presented in [Rodrigues *et al.* 2009]**

- ▶ Signal independent noise sources remain the same
- ▶ Intrinsic luminous intensity of fluorophore decays exponentially with time
- ▶ Fluorophore behaviour is not uniform across a sample.

**As a result:**

- ▶ The Gaussian part of the model remains time-independent
- ▶ The exponential decay is put inside the Poisson model.
- ▶ We do not assume a unique decay rate for all pixels



# NOISE IDENTIFICATION - MODEL

$$\forall s \in \{1, \dots, S\}$$

$$\forall t \in \{1, \dots, T\}$$

$$R_{s,t} = \alpha Q_{s,t} + N_{s,t}$$

## Observations

$$r = (r_{s,t})_{1 \leq s \leq S, 1 \leq t \leq T}$$

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# NOISE IDENTIFICATION - MODEL

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## Poisson noise -

$$Q_{s,t} \sim \mathcal{P}(u_s e^{-k_s t})$$

$\alpha \in \mathbb{R}$  - scaling par.

$(u_s)_{1 \leq s \leq S} \geq 0$  - "clean" image

$(k_s)_{1 \leq s \leq S} \geq 0$  - bleaching decay

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$c \in \mathbb{R}$  - mean  $\sigma^2$  - variance

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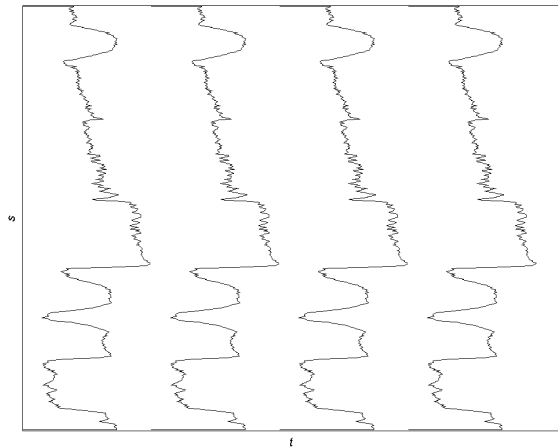
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**Problem**

$$\text{Find } \theta = (u, k, \alpha, c, \sigma^2)$$

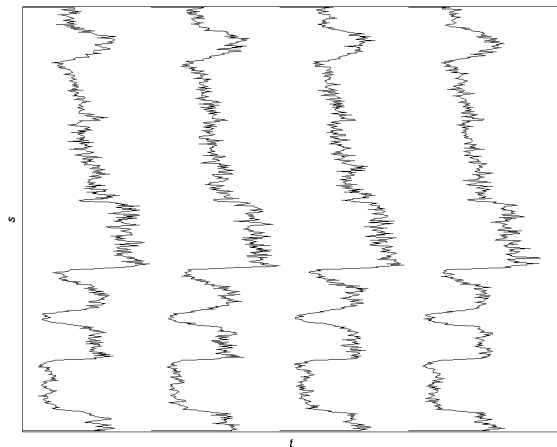


## EXAMPLE

 $U_{s,t}$  $T = 4,$   
 $S = 512$ 

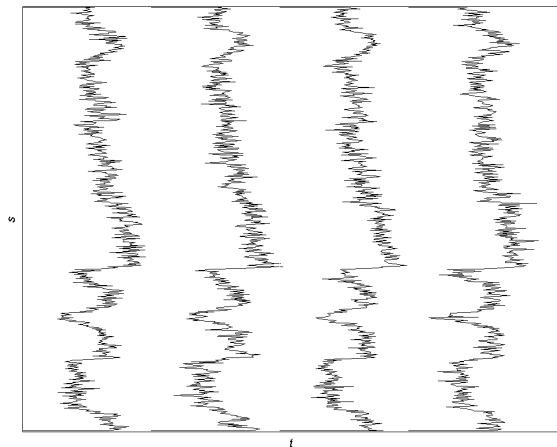
Original signal

## EXAMPLE

 $Q_{s,t}$  $T = 4,$   
 $S = 512$ 

Signal corrupted by Poisson noise

## EXAMPLE

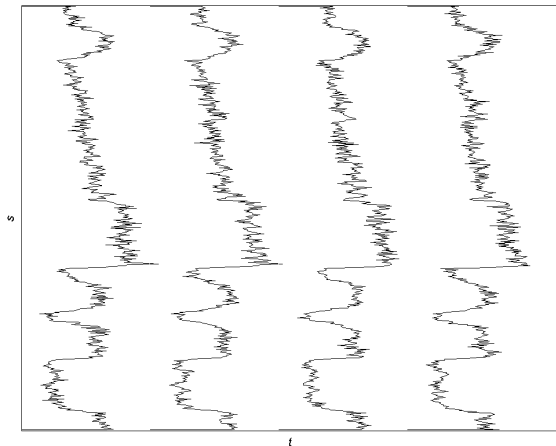


$$U_{s,t} + N_{s,t}$$

$$T = 4,$$
$$S = 512$$

Signal corrupted by Gaussian noise

## EXAMPLE



$$\alpha Q_{s,t} + N_{s,t}$$

$$T = 4,$$

$$S = 512$$

Signal corrupted by Poisson and Gaussian noises



# CUMULANTS

$$\kappa_n[R_{s,t}] = \alpha^n \kappa_n[Q_{s,t}] + \kappa_n[N_{s,t}]$$

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Cumulant of order  $n$



# CUMULANTS

$$\kappa_n[R_{s,t}] = \alpha^n \kappa_n[Q_{s,t}] + \kappa_n[N_{s,t}]$$

Then:

- ▶ mean value

$$\kappa_1[R_{s,t}] = \mathbf{E}[R_{s,t}] = \alpha e^{-k_s t} u_s + c$$

- ▶ variance

$$\kappa_2[R_{s,t}] = \mathbf{Var}[R_{s,t}] = \alpha^2 e^{-k_s t} u_s + \sigma^2$$

- ▶ higher-order cumulants

$$\kappa_n[R_{s,t}] = \alpha^n e^{-k_s t} u_s, \quad n \geq 3$$

# PROBLEM FORMULATION

Using  $E[R_{s,t}] = \alpha e^{-k_{st}} u_s + c$

we have:  $R_{s,t} = a_s e^{-k_{st}} + c + E_{s,t}$

$$(a_s = \alpha u_s)_{1 \leq s \leq S}$$

$(E_{s,t})_{1 \leq s \leq S, 1 \leq t \leq T}$  -  
independent zero-mean  
random variables.

## Optimization criteria

$$(\hat{a}, \hat{k}, \hat{c}) = \underset{a, k, c}{\operatorname{argmin}} \sum_{s=1}^S \sum_{t=1}^T \omega_{s,t} (r_{s,t} - c - a_s e^{-k_{st}})^2$$



# ALGORITHM - STEP 1

## Initialization:

$$c^{(0)} = \min\left\{\frac{1}{T} \sum_{t=1}^T r_{s,t}, 1 \leq s \leq S\right\}$$

## Main Loop:

**for**  $n \leftarrow 1$  to  $N$  **do**

**for**  $s \leftarrow 1$  to  $S$  **do**

$$(a_s^{(n)}, k_s^{(n)}) = \underset{a_s, k_s \geq 0}{\operatorname{argmin}} \sum_{t=1}^T \omega_{s,t} (r_{s,t} - c^{(n-1)} - a_s e^{-k_s t})^2$$

**end for**

$$c^{(n)} = \sum_{s=1}^S \sum_{t=1}^T \omega_{s,t} (r_{s,t} - a_s^{(n)} e^{-k_s^{(n)} t}) / \sum_{s=1}^S \sum_{t=1}^T \omega_{s,t}$$

**end for**

$$\hat{a} = a^{(N)}, \hat{k} = k^{(N)}, \hat{c} = c^{(N)}$$

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end for

$$\hat{a} = a^{(N)}, \hat{k} = k^{(N)}, \hat{c} = c^{(N)}$$



# REMAINING PARAMETERS

## Closed-form expression

$$\hat{\alpha} = \frac{ST \sum_{s=1}^S m_s v_s - \sum_{s=1}^S m_s \sum_{s=1}^S v_s}{ST \sum_{s=1}^S m_s^2 - (\sum_{s=1}^S m_s)^2}$$

$$\forall s \in \{1, \dots, S\} \quad \hat{u}_s = \frac{\hat{a}_s}{\hat{\alpha}}$$

$$\hat{\sigma}^2 = \frac{\sum_{(s,t) \in \mathbb{I}} (v_{s,t} - \hat{\alpha} \hat{a}_s e^{-\hat{k}_s t})}{\text{card}(\mathbb{I})}$$

Where

$$(\forall s \in \{1, \dots, S\})$$

$$m_s = \hat{a}_s e^{-\hat{k}_s} \frac{1 - e^{-\hat{k}_s T}}{1 - e^{-\hat{k}_s}}$$

$$v_s = \sum_{t=1}^T v_{s,t}$$

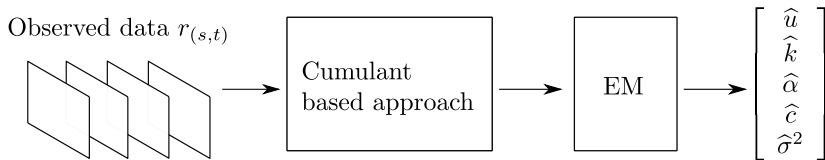
$\mathbb{I}$  - set imposing positivity

# REFINED ESTIMATION

## Cumulant based approach -summary

- ▶ Cumulant approach estimates vector of unknown parameters  $\theta$
- ▶ Unknown parameters are not jointly estimated
- ▶ Possible large error propagation

## Refined estimation



# EM ALGORITHM [JEZIERSKA *et al.* 2011]

Joint density function  $f_R(r | u, k, \alpha, c, \sigma)$  for Poisson + Gaussian:

$$\frac{1}{(\sqrt{2\pi}\sigma)^{ST}} \prod_{s=1}^S e^{-Tu_s} \prod_{t=1}^T \sum_{q_{s,t}=1}^{+\infty} e^{-\frac{(r_{s,t} - \alpha q_{s,t} - c)^2}{2\sigma^2}} \frac{(u_s e^{-k_s t})^{q_{s,t}}}{q_{s,t}!}$$

Expectation step

$$J(\theta | \theta^{(n)}) = \mathbf{E}_{Q|R=r, \theta^{(n)}} [\ln p_{R,Q}(R, Q | \theta)]$$

$R$  - observations,  $Q$  - missing data ;  $\theta$  - vector of parameters

Maximization step

$$(\forall n \in \mathbb{N}) \quad \theta^{(n+1)} = \underset{\theta}{\operatorname{argmin}} - J(\theta | \theta^{(n)})$$

## EM MAXIMIZATION STEP

**Update of**  $(\forall s \in \{1, \dots, S\})$   $k_s^{(n+1)} = -\ln x^{(n+1)}$  **where**  $x^{(n+1)}$  **is the solution in**  $(0, 1)$  **of the polynomial equation:**

$$\begin{aligned} & (1 + Tx^{T+1} - (T+1)x^T) \sum_{t=1}^T \mathbb{E}_{Q|R=r, \theta^{(n)}} [Q_{s,t}] \\ &= (1 - x - x^T + x^{T+1}) \sum_{t=1}^T t \mathbb{E}_{Q|R=r, \theta^{(n)}} [Q_{s,t}] \end{aligned}$$

solved with Halley's algorithm

**Update in a closed form:**  $u_s^{(n+1)}, \alpha^{(n+1)}, c^{(n+1)}, \sigma^{(n+1)}$  assuming known conditional mean  $\mathbb{E}_{Q|R=r, \theta^{(n)}} [Q_{s,t}]$  and  $\mathbb{E}_{Q|R=r, \theta^{(n)}} [Q_{s,t}^2]$  computed in EM Expectation Step.

# EM EXPECTATION STEP

$$\mathbf{E}_{Q|R=r, \theta^{(n)}} [Q_{s,t}] = \sum_{q_{s,t}=1}^{+\infty} q_{s,t} \mathbf{P}(Q_{s,t} = q_{s,t} | R = r, \theta^{(n)})$$

# EM EXPECTATION STEP

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After some calculations, we have:

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After some calculations, we have:

$$\mathbf{E}_{Q|R=r, \theta^{(n)}} [Q_{s,t}] = \frac{\zeta_{s,t}^{(n)}}{\eta_{s,t}^{(n)}}$$

## EM EXPECTATION STEP

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## EM EXPECTATION STEP

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$$\sum_{q_{s,t}=0}^{+\infty} e^{-\frac{(r_{s,t}-\alpha^{(n)}q_{s,t}-c^{(n)})^2}{2(\sigma^2)^{(n)}}} \frac{(u_s^{(n)} e^{-k_s^{(n)}t})^{q_{s,t}}}{q_{s,t}!}$$

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**Problem:** Infinite sums

**Solution:** Adaptive truncation technique

$\mathbb{E}_{Q|R=r,\theta^{(n)}}[Q_{s,t}^2]$  is computed similarly  $\wedge$

# PROPOSED ALGORITHM

## Step 1: Cumulant Method

$$\theta^{(1)} \leftarrow (u^{(1)}, k^{(1)}, \alpha^{(1)}, c^{(1)}, (\sigma^2)^{(1)})$$

## Step 2: EM Algorithm

for  $n \leftarrow 1$  to  $N$  do

    Expectation step

$$E_{Q|R=r, \theta^{(n)}} [Q_{s,t}] \leftarrow \frac{\zeta_{s,t}^{(n)}}{\eta_{s,t}^{(n)}}$$

$$E_{Q|R=r, \theta^{(n)}} [Q_{s,t}^2] \leftarrow \frac{\xi_{s,t}^{(n)}}{\eta_{s,t}^{(n)}}$$

    Maximization step

$$\theta^{(n+1)} \leftarrow (u^{(n+1)}, k^{(n+1)}, \alpha^{(n+1)}, c^{(n+1)}, (\sigma^2)^{(n+1)})$$

end for

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for  $n \leftarrow 1$  to  $N$  do

### Expectation step

$$E_{Q|R=r, \theta^{(n)}} [Q_{s,t}] \leftarrow \frac{\zeta_{s,t}^{(n)}}{\eta_{s,t}^{(n)}}$$

$$E_{Q|R=r, \theta^{(n)}} [Q_{s,t}^2] \leftarrow \frac{\xi_{s,t}^{(n)}}{\eta_{s,t}^{(n)}}$$

### Maximization step

$$\theta^{(n+1)} \leftarrow (u^{(n+1)}, k^{(n+1)}, \alpha^{(n+1)}, c^{(n+1)}, (\sigma^2)^{(n+1)})$$

end for

# PROPOSED ALGORITHM

## Step 1: Cumulant Method

$$\theta^{(1)} \leftarrow (u^{(1)}, k^{(1)}, \alpha^{(1)}, c^{(1)}, (\sigma^2)^{(1)})$$

## Step 2: EM Algorithm

**for**  $n \leftarrow 1$  to  $N$  **do**

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# SYNTHETIC DATA RESULTS

## Evaluation criteria

$$\text{SNR} = 10 \log_{10} \left( \frac{(ST)^{-1} \sum_{(t,s)} (a_s e^{-k_s t})^2}{\sum_{(t,s)} (a_s e^{-k_s t} - \hat{a}_s e^{-\hat{k}_s t})^2} \right)$$

## Results

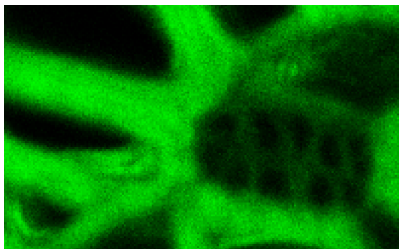
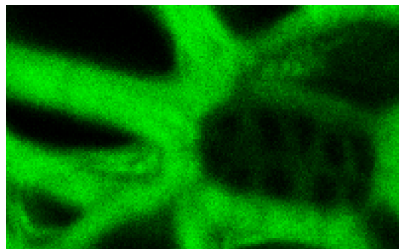
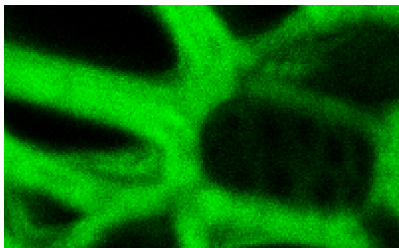
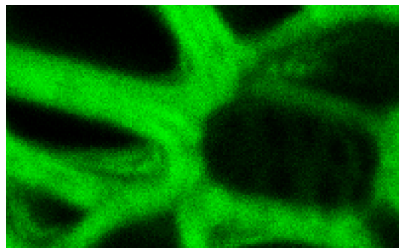
Statistics over 50 noise realizations.

| Method. | $\hat{\sigma}$ |     | $\hat{c}$ |     | $\hat{\alpha}$ |     | $\overline{\text{SNR}}$ |
|---------|----------------|-----|-----------|-----|----------------|-----|-------------------------|
|         | bias           | std | bias      | std | bias           | std |                         |
| Init.   | 357.5          | 3.1 | 1.9       | 1.0 | -0.3           | 0.4 | 39.5                    |
| EM      | 2.9            | 0.9 | 1.4       | 0.8 | -0.3           | 0.4 | 39.7                    |

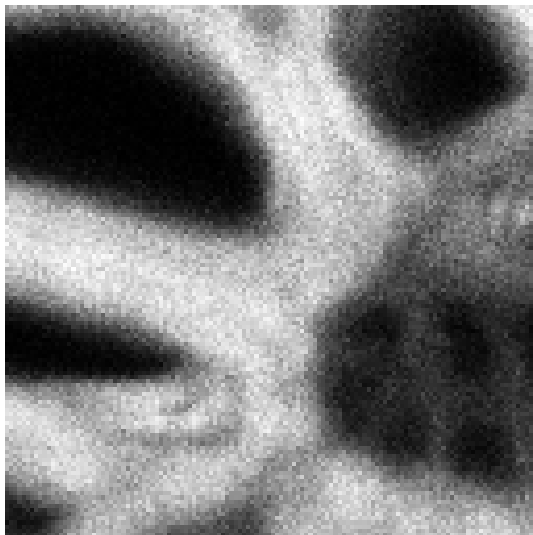
Table: Experiment conditions:  $T = 180, S = 200, \alpha = 30, \sigma^2 = 100, c = 10, u_s \in [0, 150], k_s \in [10^{-4}, 10^{-3}]$





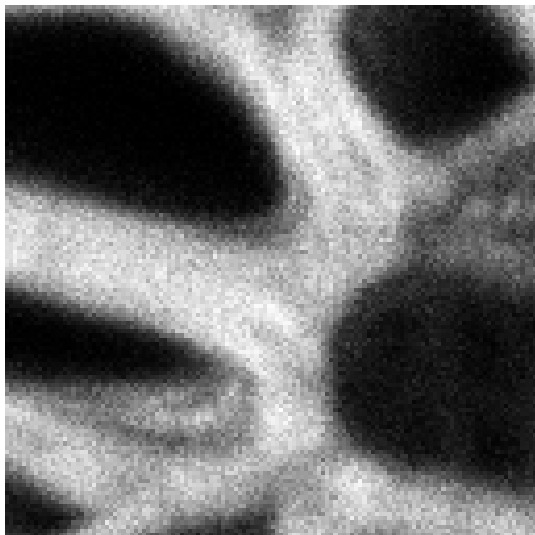
(a)  $R_{s,1}$ (b)  $R_{s,45}$ (c)  $R_{s,90}$ (d)  $R_{s,180}$

# VISUAL RESULTS



Original image  $r_{s,1}$

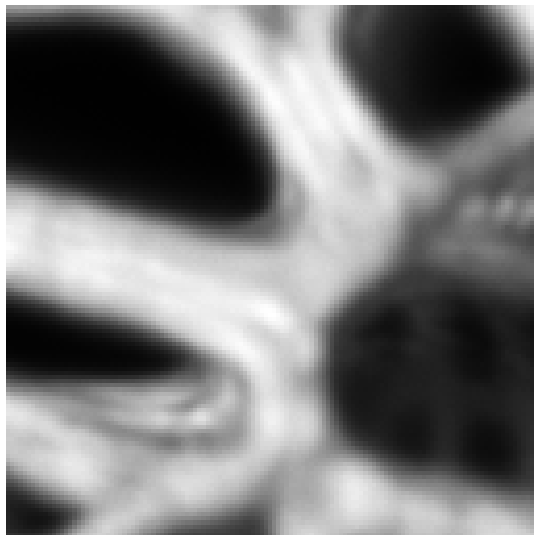
# VISUAL RESULTS



Original image  $r_{s,1}$

Original image  $r_{s,180}$

# VISUAL RESULTS

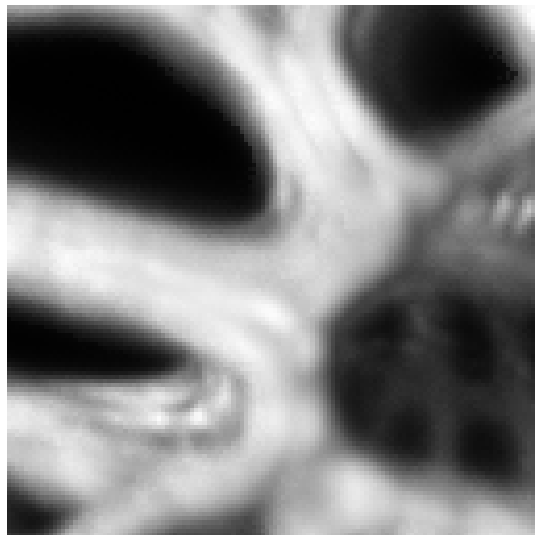


Original image  $r_{s,1}$

Original image  $r_{s,180}$

Mean over  $T = 180$   
realizations

# VISUAL RESULTS



Original image  $r_{s,1}$

Original image  $r_{s,180}$

Mean over  $T = 180$   
realizations

Reconstructed image

Parameters:

$$\hat{\alpha} = 25.8$$

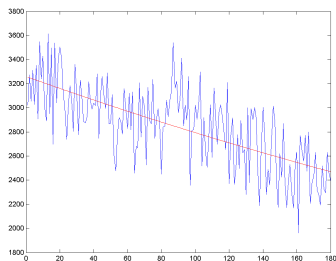
$$\hat{c} = 8$$

$$\hat{\sigma}^2 = 119$$

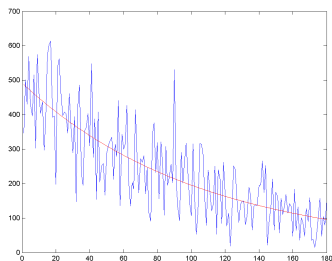
$$\hat{u}_s \in [0, 147]$$

$$\hat{k}_s \in [0, 3.9 \times 10^{-6}]$$

# TIME VARIATIONS



(a)  $25.8 \times 126 e^{-5.7 \times 10^{-7} t} + 8$



(b)  $25.8 \times 19 e^{-3.6 \times 10^{-6} t} + 8$

Figure: (a,b) illustrate time variations for fixed  $s$ . True data  $R_{s,t}$  plotted in blue and estimated time curve (using formula  $\hat{a}\hat{u}_s e^{-\hat{k}st} + \hat{c}$ ) plotted in red.

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- ▶ These algorithms can compute unknown noise parameters for denoising or restoration procedures, which normally are assumed to be known [Benvenuto *et al.* 2008, Luisier *et al.* 2011]
- ▶ Apart from noise parameters, we can also estimate the rate of bleaching.

# FUTURE WORK

- ▶ The study of noise parameters in MACROscope, confocal and widefield case.
- ▶ Image restoration method with proposed noise model and space varying PSF

## SUMMARY

Our work concerning image restoration / denoising, noise identification, and image quantization in the presence of noise

- ▶ A. Jezierska, C. Chaux , H. Talbot and J.-C. Pesquet.  
Image quantization under spatial smoothness constraints,  
Sep. 26-29 Hong Kong, ICIIP 2010
- ▶ C. Chaux , A. Jezierska, H. Talbot and J.-C. Pesquet.  
JMIV, 2010, A spatial regularization approach for vector quantization,
- ▶ A. Jezierska, H. Talbot, O. Veksler, and D. Wesierski.  
A fast solver for truncated-convex priors: quantized-convex split moves  
EMMCVPR 2011, Saint Petersburg, 25-27 July 2011
- ▶ A. Jezierska, C. Chaux , J.-C. Pesquet and H. Talbot .  
An EM approach for Poisson-Gaussian noise modeling  
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- ▶ E. Chouzenoux, J.-C. Pesquet, H. Talbot, and A. Jezierska  
A memory gradient algorithm for  $l_2 - l_0$  regularization with  
applications to image restoration,  
ICIIP 2011, Brussels, 11-14 September 2011.