Prefiltering: Reducing the Noise Sensitivity of Non-linear Image Restoration Algorithms

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Abstract

This paper shows how the performance of image processing algorithms can be improved by reducing the noise influence on the restoration. A procedure, which we call prefiltering, reduces this noisy sensitivity after the image has been acquired. It uses a local smoothing filter to suppress those parts of the image spectrum where the noise dominates the signal. We show, when using a Gaussian filter as smoothing filter that the extra blurring of this filtering can be compensated for by convolving the point spread function with the same Gaussian filter. Experiments, performed on simulated data, show that the performance of non-linear image restoration algorithms can be improved considerable, up to a two-fold reduction of the mean square error.

1. Introduction

This paper presents a method, which we call *prefiltering*, that improves the performance of non-linear image restoration algorithms, by suppressing before the restoration, those parts of the spectrum of the acquired image that hamper the performance of these algorithms.

The performance of the restoration results is strongly dependent on the amount of noise in the image. The Richardson-Lucy algorithm is an unregularized maximum likelihood estimator for Poisson distorted data. It can therefore produce results that are adapted to the noise in the image. In previous experiments (Kempen et al., 1997b) we observed that the performance of the Richardson-Lucy is strongly dependent on the signal-to-noise ratio of the acquired image. Furthermore, results obtained at low signal to noise ratios showed a strong adaptation to the noise realization in the acquired image.

On the other hand, regularized algorithms like the ICTM algorithm or the Carrington algorithm balance their fit to the data with a regularization term making them less sensitive to the noise in the image. The balance between the data and the regularization term is highly dependent on the signal-to-noise ratio in the acquired image. These regularized algorithms may therefore produce solutions that are smoother than desired.

The obvious way to improve the performance of the image restoration algorithms is to increase the signal-to-noise ratio of the image. Given that the noise induced by photon counting is the dominant source of noise in a microscope fluorescence image, this can be

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achieved by collection of more photons per pixel. This however is not always possible. Limits on the total acquisition time, saturation of the fluorescence molecules, and bleaching can limit the signal-to-noise ratio of the acquired image.

We have therefore investigated a method that reduces this noisy sensitivity *after* the image has been acquired. This method, which we call *prefiltering*, suppresses those parts of the image spectrum that do not contain any signal information (or where the noise contribution is much larger than the signal contribution). These frequencies will prevent signal recovery and only amplify noise in the final result.

The proposed method is an alternative method to the method of sieves (Snyder and Miller, 1991), which imposes smoothness to the restoration results to address finite sampling, and to the damped Richardson-Lucy algorithm (White, 1994), which adjusts the functional of the Richardson-Lucy algorithm to make it less sensitive to noise.

In the next section, we formulate a mathematical description of the image formation in a confocal fluorescence microscope and use it to discuss linear and non-linear image restoration techniques. Section three discusses the proposed prefiltering method, and shows how it can be incorporated in the functionals that are optimized by the non-linear restoration algorithms.

Section four presents the results of simulation experiments performed on 3-D spheres and 2-S textured disks. We conclude in section five.

2. Image Restoration

2.1 Classical Image Restoration: The Tikhonov-Miller restoration filter

We assume that the image formation in a confocal fluorescence microscope can be modeled as a linear translation-invariant system distorted by noise

$$m(x,y,z) = N\left(h(x,y,z) \otimes f(x,y,z) + b(x,y,z)\right) \tag{1}$$

In this equation f represents the input signal, h the point spread function, b a (constant) background signal, N a general noise distortion function, and m the recorded fluorescence image. For scientific-grade light detectors N is dominated by Poisson noise.

In classical image restoration, the signal-dependent Poisson noise is approximated by additive Gaussian noise. Using this additive Gaussian noise model for N we rewrite (1) as

$$g(x,y,z) = m(x,y,z) - b(x,y,z) = h(x,y,z) \otimes f(x,y,z) + n(x,y,z) \tag{2}$$

After sampling equation (2) becomes

$$g[x,y,z] = \sum_{i=1}^{M_x} \sum_{j=1}^{M_y} \sum_{k=1}^{M_z} h[x-i, y-j, z-k] f[i,j,k] + n[x,y,z] \tag{3}$$

with M_x, M_y *and* M_z the number of sampling points in respectively the *x*, *y* and *z* dimension. For conveniences we will adopt a matrix notation

$$\mathbf{g} = \mathbf{Hf} + \mathbf{n} \tag{4}$$

where the vectors **f**, **g** and **n** of length M $(M = M_x M_y M_z)$ denote respectively the object, its image and the additive Gaussian noise. The *MxM* matrix **H** is the blurring matrix representing the point spread function of the microscope.

The Tikhonov-Miller filter, a classical image restoration filter, is a convolution filter operating on the measured image. It can be written as

$$\hat{\mathbf{f}} = \mathbf{W}\mathbf{g} \tag{5}$$

with **W** the linear restoration filter and $\hat{\mathbf{f}}$ its result. The Tikhonov-Miller filter is derived from a least squares approach. This approach is based on minimizing the squared difference between the acquired image and a blurred estimate of the original object,

$$\left\| \mathbf{H}\hat{\mathbf{f}} - \mathbf{g} \right\|^2 \tag{6}$$

However a direct minimization of (6) will produce undesired results since it does not take into account the (high) frequency components of $\hat{\mathbf{f}}$ that are set to zero by the convolution with **H**. Finding an estimate $\hat{\mathbf{f}}$ from (6) is known as an ill-posed problem (Tikhonov and Arsenin, 1977). To address this issue Tikhonov defined the regularized solution $\hat{\mathbf{f}}$ of (4) the one that minimizes the well-known Tikhonov functional (Tikhonov and Arsenin, 1977)

$$\Phi\left(\hat{\mathbf{f}}\right) = \left\| \mathbf{H}\hat{\mathbf{f}} - \mathbf{g} \right\|^2 + \lambda \left\| \mathbf{C}\hat{\mathbf{f}} \right\|^2 \tag{7}$$

with $\|\cdot\|^{-}$ the Euclidean norm. In image restoration λ is known as the regularization parameter and **C** as the regularization matrix. The Tikhonov functional consists of a mean square error fitting criterion and a stabilizing energy bound which penalizes solutions of $\hat{\mathbf{f}}$ that oscillate wildly due to spectral components which are dominated by noise. The minimum of Φ can be found by solving

$$\nabla_{\hat{\mathbf{f}}} \Phi\left(\hat{\mathbf{f}}\right) = 2\mathbf{H}^T \left(\mathbf{H}\hat{\mathbf{f}} - \mathbf{g}\right) + 2\lambda \mathbf{C}^T \mathbf{C}\hat{\mathbf{f}} = 0 \tag{8}$$

which yields the well-known Tikhonov-Miller (TM) solution $\mathbf{W}_{TM}$

$$\mathbf{W}_{TM} = \frac{\mathbf{H}^T}{\mathbf{H}^T\mathbf{H} + \lambda \mathbf{C}^T\mathbf{C}} \tag{9}$$

The linear nature of the Tikhonov-Miller makes it incapable of restoring frequencies for which the PSF has a zero response. Furthermore, linear methods cannot restrict the domain in which the solution should be found. This property is a major drawback since the intensity of an imaged object represents light energy, which is non-negative.

The ICTM algorithm, the Carrington algorithm and the Richardson-Lucy algorithm are frequently used in fluorescence microscopy (Voort and Strasters, 1995, Carrington, 1990, Holmes, 1988, Conchello and McNally, 1996, Kempen et al., 1997a, Kempen, 1999, Verveer et al., 1999). These iterative, non-linear algorithms tackle the above mentioned problems in exchange for a considerable increase in the computational complexity.

2.2 Constrained Tikhonov Restoration

The iterative constrained Tikhonov-Miller algorithm

The iterative constrained Tikhonov-Miller (ICTM) (Lagendijk and Biemond, 1991, Voort and Strasters, 1995) finds the minimum of (7) using the method of conjugate gradients (Press et al., 1992). The non-negativity constraint is incorporated by setting the negative intensities after each iteration to zero.

The Carrington algorithm

Like the ICTM algorithm the Carrington algorithm (Carrington, 1990, Carrington et al., 1995) minimizes the Tikhonov functional under the constraint of non-negativity. However the algorithm is based on a more solid mathematical foundation. Carrington used the Kuhn-Tucker conditions (Carrington, 1990),

$$\nabla_{\hat{\mathbf{f}}}\Phi_i = 0 \text{ and } \hat{\mathbf{f}}_i > 0 \quad \text{or} \quad \nabla_{\hat{\mathbf{f}}}\Phi_i \geq 0 \text{ and } \hat{\mathbf{f}}_i = 0 \tag{10}$$

to transform the Tikhonov functional with the added non-negativity constraint to the functional Ψ (on the set $\mathbf{H}^T\mathbf{c} > 0$)

$$\Psi(\mathbf{c}) = \tfrac{1}{2}\left\|P\left(\mathbf{H}^T\mathbf{c}\right)\right\|^2 - \mathbf{c}^T\mathbf{g} + \tfrac{1}{2}\lambda\|\mathbf{c}\|^2 \tag{11}$$

Since Ψ is strictly convex and twice continuously differentiable, a conjugate gradient algorithm similar to can be used to minimize Ψ.

2.3 Maximum Likelihood Restoration: The Richardson-Lucy algorithm

In contrast with the two algorithms discussed previously the Richardson-Lucy is not derived from the image formation model (4) which assumes additive Gaussian noise. Instead the general noise distortion function N is assumed to be dominated by Poisson noise.

A fluorescence object can be modeled as a spatially inhomogeneous Poisson process $\mathbf{F}$ with an intensity function $\mathbf{f}$ (Snyder and Miller, 1991),

$$P\left(\mathbf{F}_i \middle| \mathbf{f}_i\right) = \frac{\mathbf{f}_i^{\mathbf{F}_i} e^{-\mathbf{f}_i}}{\mathbf{F}_i!}$$

The image formation of such an object by a fluorescence microscope can be modeled as a translated Poisson process (Snyder and Miller, 1991). This process models the transformation of $\mathbf{F}$ into a Poisson process $\mathbf{m}$ subjected to a conditional probability density function $\mathbf{H}$,

$$E[\mathbf{m}] = \mathbf{Hf} + \mathbf{b} \tag{12}$$

with $\mathbf{b}$ the mean of an independent (background) Poisson process. The log likelihood function of such a Poisson process is given by (Snyder and Miller, 1991)

$$L(\mathbf{f}) = -\sum \mathbf{Hf} + \mathbf{m}^T \ln\left(\mathbf{Hf} + \mathbf{b}\right) \tag{13}$$

where we have dropped all terms that are not dependent on $\mathbf{f}$. The maximum of the likelihood function L can be found iteratively using the EM algorithm as described by (Dempster et al., 1977). This iterative algorithm was first used by (Vardi et al., 1985) in emission tomography. Holmes (Holmes, 1988) introduced the algorithm to microscopy. Applying the EM algorithm to (13) yields (Shepp and Vardi, 1982, Holmes, 1988, Snyder et al., 1993, Kempen et al., 1997b, Kempen, 1999)

$$\hat{\mathbf{f}}^{k+1} = \hat{\mathbf{f}}^{k}\left[\frac{\mathbf{H}^T}{\mathbf{H}\hat{\mathbf{f}}^{k} + \mathbf{b}}\right]\mathbf{m} \tag{14}$$

The EM algorithm ensures a non-negative solution when a non-negative initial guess $\hat{\mathbf{f}}^0$ is used. Furthermore the likelihood of each iteration of the EM algorithm will strictly increase to a global maximum (Snyder and Miller, 1991). The EM algorithm for finding the maximum likelihood estimator of a translated Poisson process (often referred to as EM-MLE) is identical to the Richardson-Lucy algorithm (Richardson, 1972).

3. Prefiltering

We have investigated a method that reduces the noise sensitivity *after* the image has been acquired. This method, which we call *prefiltering*, suppresses those parts of the image spectrum that do not contain any signal information (or where the noise contribution is much larger than the signal contribution). These frequencies will prevent signal recovery and only amplify noise in the final result.

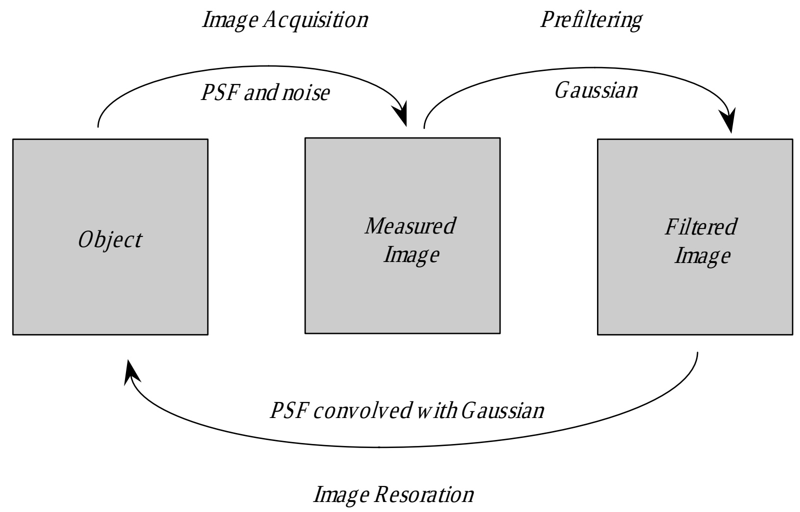

Figure 1 The incorporation of the proposed Gaussian prefiltering in an image restoration procedure.

We have suppressed these (high frequency) parts of the spectrum by convolving the acquired image with a Gaussian filter. Although the Gaussian filter will mainly suppress high frequencies, lower (object) frequencies are also effected. Being a linear filter we can compensate for the extra blurring of the Gaussian filter by convolving the PSF with the same Gaussian filter, as shown in Figure 1.

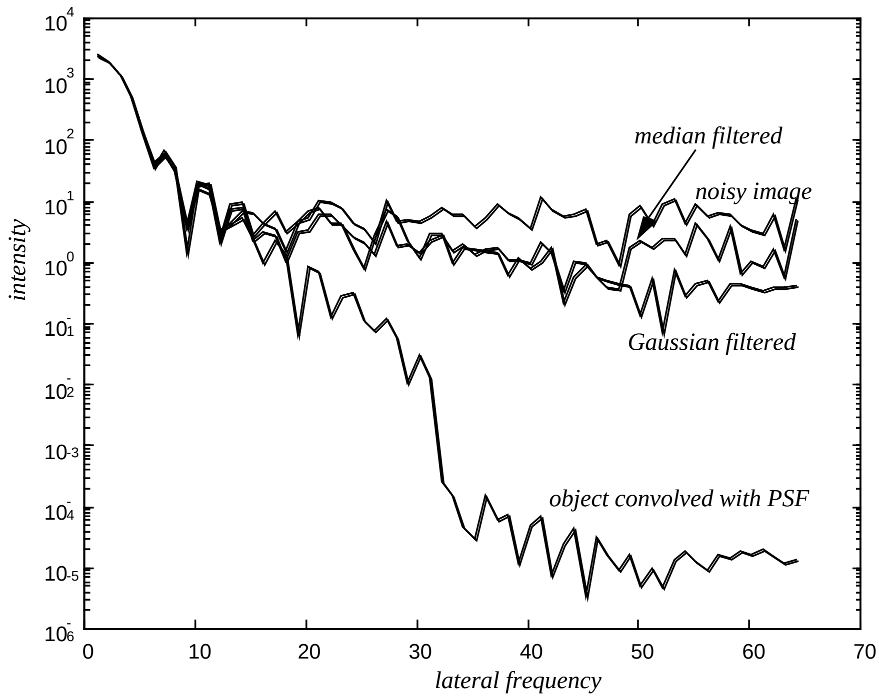

Figure 2 Power spectrum of the original object, the recorded image, and the spectra after smoothing the recorded image by a median and a Gaussian filter.

The above defined Gaussian prefiltering can also be described analytically by incorporating it in the functionals that are optimized by the various restoration algorithms. For the ICTM algorithm and Carrington's algorithm the Tikhonov functional with prefiltering yields

$$\Phi\left(\hat{\mathbf{f}}\right)=\left\|\mathbf{G}\left(\mathbf{H}\hat{\mathbf{f}}-\mathbf{g}\right)\right\|^{2}+\lambda\left\|\mathbf{C}\hat{\mathbf{f}}\right\|^{2} \tag{15}$$

with **G** the Gaussian prefilter matrix. The incorporation of prefiltering in the functional derived from the assumption of Poisson noise distorted data (used by the Richardson-Lucy algorithm) yields

$$L\left(\mathbf{f}\right)=-\sum\mathbf{GHf}+\left(\mathbf{Gm}\right)^{T}\ln\left(\mathbf{GHf}+\mathbf{b}\right) \tag{16}$$

Using these functionals the prefiltering can easily be incorporated in the algorithms based on these functionals.

The Gaussian prefiltering is a linear convolution filter to suppress the noise in the recorded image. Noise suppression by local smoothing however can also be obtained using non-linear filters like the median filter (see Figure 2). It is therefore interesting to compare the performance of Gaussian prefiltering with a median filter.

4. Simulation Experiments

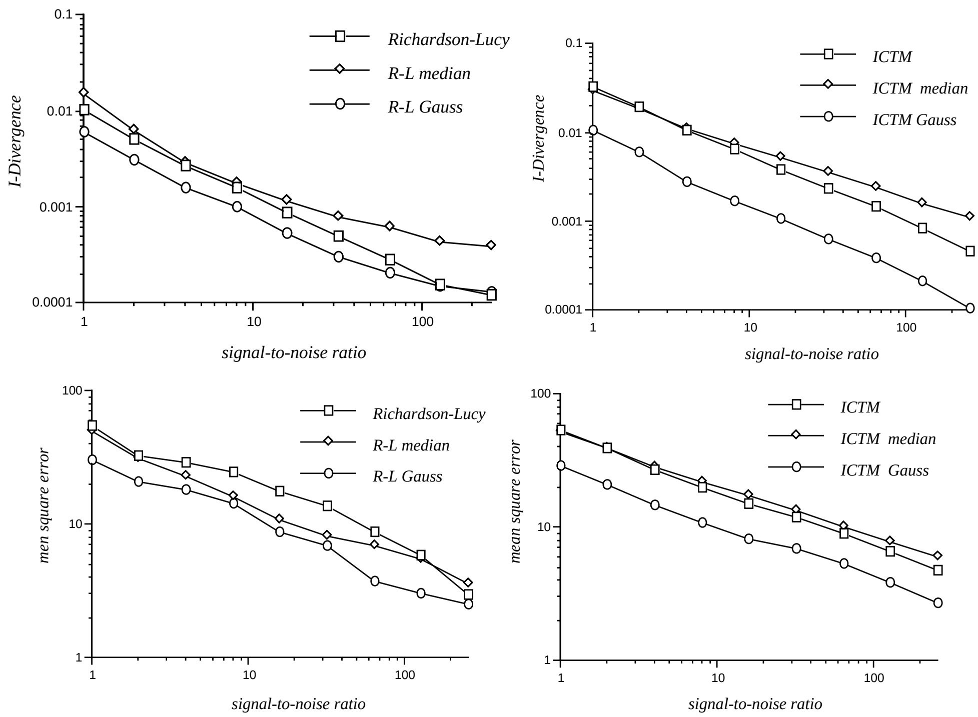

Figure 3 The I-Divergence (*top*) and mean-square-error (*bottom*) performance of the Richardson-Lucy algorithm *(left*) and the ICTM algorithm *(right)* with and without median or Gaussian prefiltering.

We have performed a simulation experiment to test the influence of the proposed prefiltering methods on the performance of the Richardson-Lucy and ICTM algorithm to restore spheres convolved with a confocal PSF and distorted by Poisson noise. The performance of the algorithms is measured with the mean-square-error and I-divergence measure as a function of the signal-to-noise ratio. We have generated spheres with a radius of 1.0 μm, an object intensity of 200.0 ADU and a background of 40.0 ADU. For the prefiltering we have used a Gaussian with a sigma of 1 pixel in all dimensions and a median filter with a size of three pixels in all three dimensions.

Figure 3 shows the I-divergence as well as the mean-square-error performance of the Richardson-Lucy and ICTM algorithms as function of the signal-to-noise ratio. The graphs clearly show the strong dependence of the performance of these algorithms on the signal-to-noise ratio. Furthermore, it shows the improvement of the Gaussian prefiltering on both algorithms. The proposed median prefiltering however does not, in general, improve the results. The center x-y and x-z slices of the restored images of a confocal image with a signal-to-noise ratio of 16.0 are shown in Figure 4.

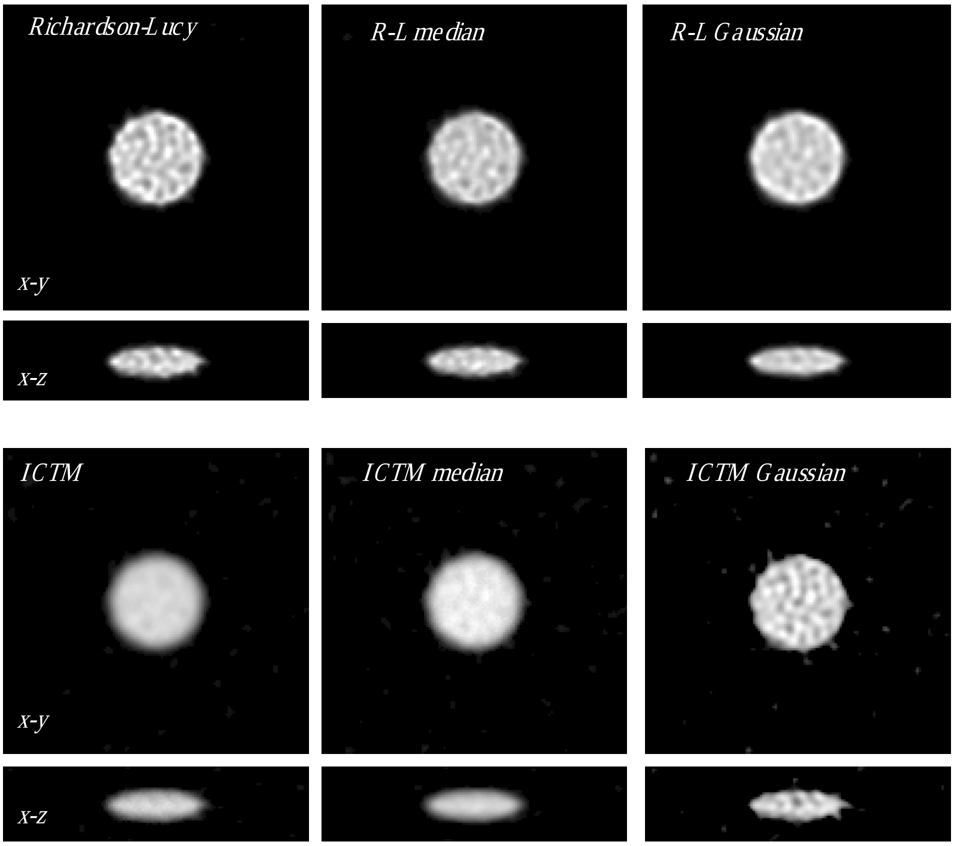

Figure 4 The center *x-y* and *x-z* slices of the results of Richardson-Lucy *(top)* and the ICTM algorithm *(bottom)* are shown without pre-filtering *(left)*, and with median *(middle)* or Gaussian pre-filtering *(right)*. The images are 128 x 128 x 32 pixels in size, the radius of the sphere is 1.0 μm and the SNR 16.0. The images are displayed with eight-bit grey scale resolution, without stretching the intensities.

We performed a second experiment to test the influence of the proposed prefiltering on the performance of the Richardson-Lucy and ICTM algorithm to restore two dimensional textured disks convolved by an in-focus wide-field incoherent PSF and distorted by Poisson noise. The performance of the algorithms is measured with the mean-square-error and I-divergence measures as a function of the signal-to-noise ratio.

We have generated disks with a radius of 1.0 μm, an object intensity of 200.0 and a background of 20 (Figure 5). We have added texture to the disk by modulating the disk in both dimensions with a sine. We have used sine of with a period equal to 1/7th of the image size, and a modulation depth of 20% of the image intensity.
The images are 256x256 pixels in size, the signal-to-noise ratio ranges from 16 to 1024 (12 dB - 30 dB). We have restored the textured disks using the two restoration methods with and without (using three methods of) prefiltering: Gaussian prefiltering (with a sigma of 2.0 pixels), median prefiltering (with a filter size of 5 pixels), and a combined median-Gaussian filtering (median size of three pixels and a sigma of 1.4 for the Gaussian filter. Figure 8 shows the mean-square-error and I-divergence of the restoration results as function of the signal-to-noise ratio.

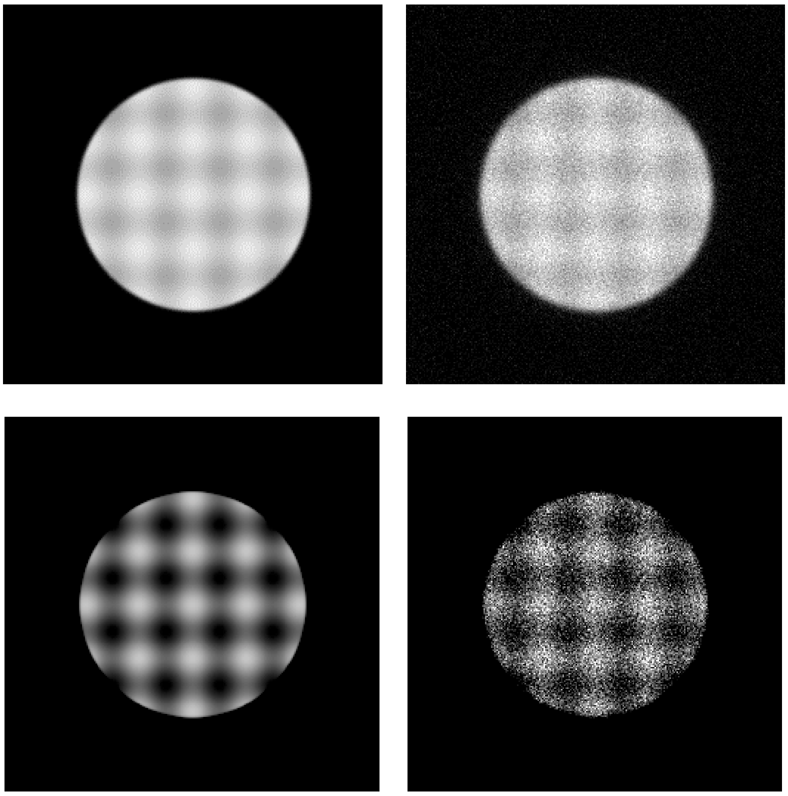

Figure 5 The top row shows the textured disk *(left)* and its image *(right)*, the bottom row shows the same images but with their intensities stretched to make the texture more pronounced.

The restoration results are shown in Figure 6 for an image with a signal-to-noise ratio of 16.0. In this figure we have zoomed in on the texture, by showing only the intensities of the texture. Cross-sections of the centerline of the images shown Figure 6 together with that of the textured disk are shown in Figure 7.

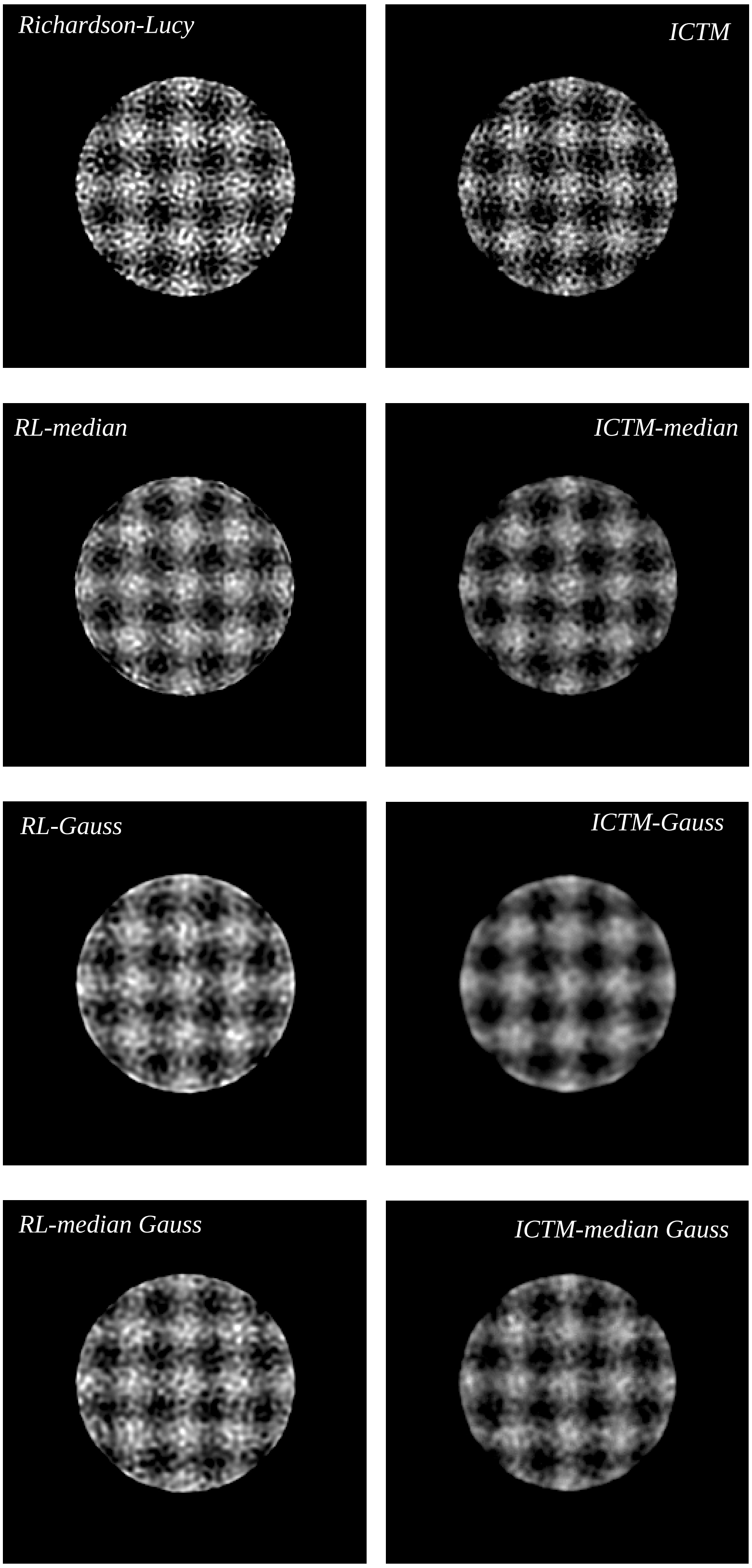

Figure 6 The results of Richardson-Lucy (top) and the ICTM algorithm (bottom) are shown without prefiltering and with median, Gaussian, and combined median-Gaussian prefiltering.

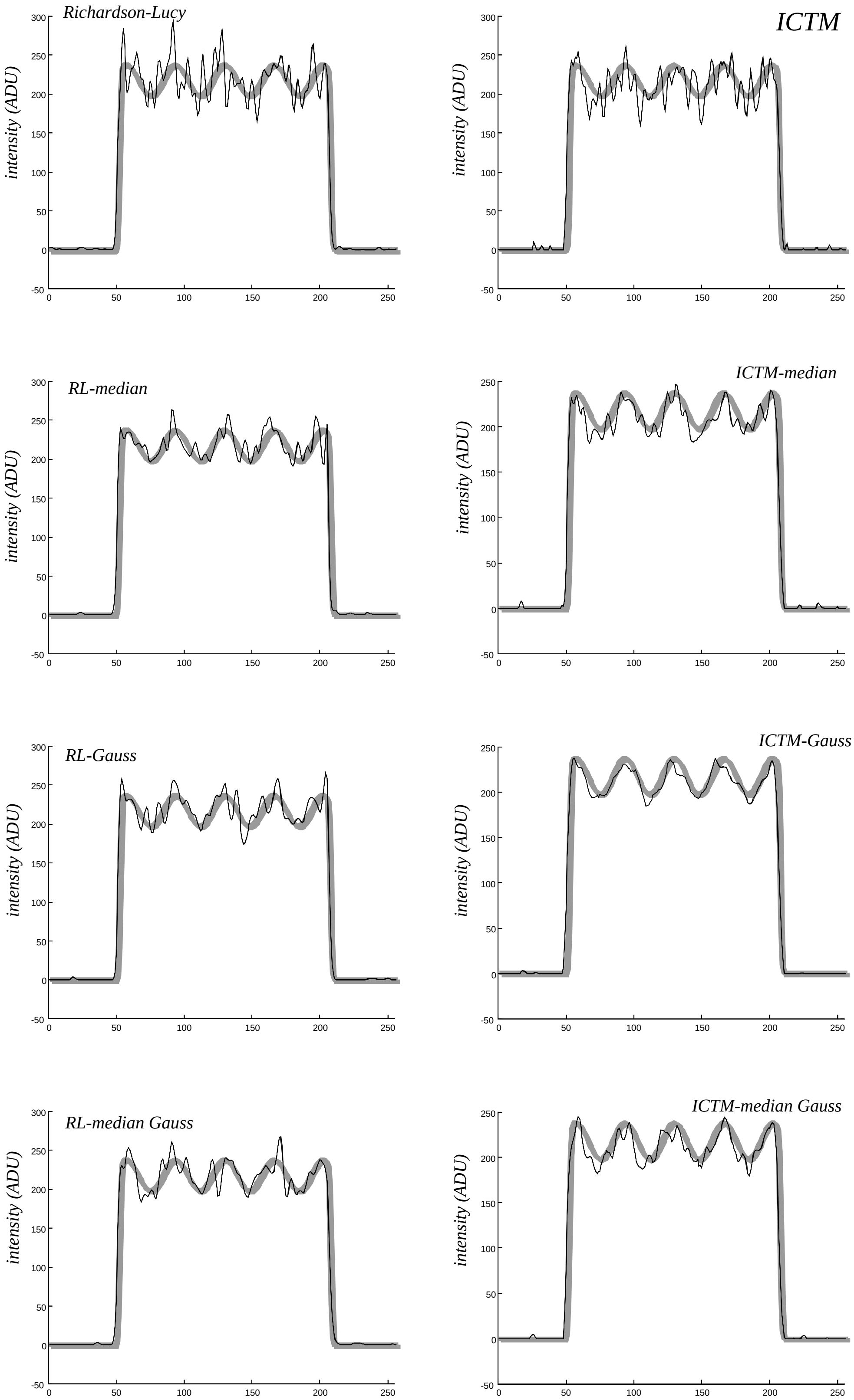

Figure 7 Cross sections of the center line of the textured disk together with the result of the Richardson-Lucy algorithm *(left*) and the ICTM algorithm *(right)* with and without Gaussian, median or combined median-Gaussian prefiltering. The grey line indicates intensities of the original object.

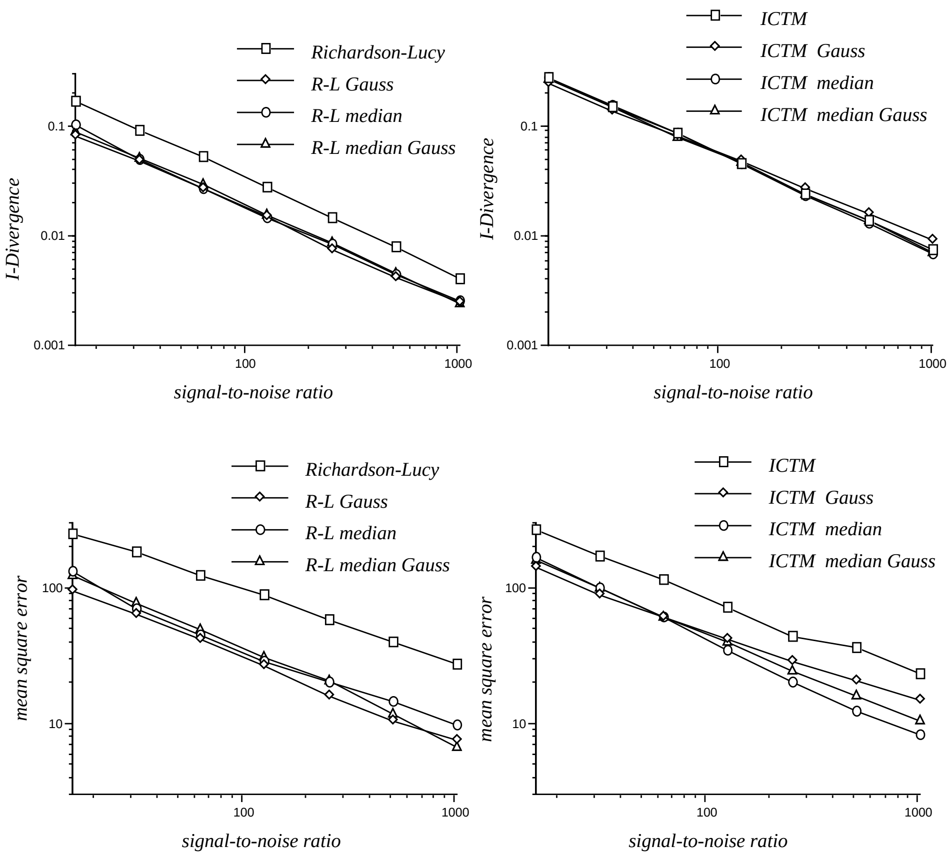

Figure 8 The I-Divergence (*top*) and mean-square-error (*bottom*) performance of the Richardson-Lucy algorithm *(left*) and the ICTM algorithm *(right)* restoring the textured objects with and without median or Gaussian prefiltering

5. Conclusions

We define prefiltering as being a smoothing operation performed on the acquired image *prior* to the image restoration to reduce the influence of the noise on the restoration result. The suppression of noise in the acquired image will make the restoration result produced by the Richardson-Lucy algorithm less sensitive to the noise realization in the acquired image and will relax the influence of regularization in the restoration result for the regularized algorithms. We propose to implement this smoothing with a Gaussian convolution filter. This filtering will add an extra blur to the image but can be compensated for by blurring the point spread function with the same filter. We have also tested the effectiveness of the median filter, a non-linear smoothing filter, as a prefilter. We found however that it does not produce results as good as those obtained with a Gaussian prefilter. Particularly, the mean-square-error performance of the median prefilter is much worse than that of the Gaussian prefilter. This can partially be attributed to the fact that a median filter will shift edges of a curved object inwards (Vliet, 1993) and therefore will not conserve the total intensity in the image. The mean-square-error criterion penalizes the median filter for this, whereas this property of the median filter does not have a large impact on the visual performance of the restoration result.

The filtering of the acquired image will, however, make the remaining Poisson noise correlated, violating one of the assumptions used in the derivation of the various restoration algorithms. In our experiments we did not find any suggestions that this negatively influenced the performance of the algorithms.

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