

Fluorescence Microscopy Deconvolution based on Bregman Iteration and Richardson-Lucy Algorithm with TV Regularization

Steffen Remmele, Madeleine Seeland, Jürgen Hesser

Institute for Computational Medicine, University of Heidelberg
remmele@ti.uni-mannheim.de

Abstract. Fluorescence microscopy has become an important tool in biological and medical sciences for imaging thin specimen, even living ones. Due to Out-of-focus blurring and noise the acquired images are degraded and therefore it can be difficult to analyse them. In the last decade many methods have been proposed to restore these images. One of the most popular methods to restore microscopy images is an iterative Richardson-Lucy Algorithm with Total Variation Regularization. Besides there are some new approaches based on Bregman Iteration to improve the quality of restored images in general. In this paper we formulate a new algorithm for restoring fluorescence microscope images using Bregman Iteration with special attention to the microscopy specific properties. We can prove that the quality of the restored images increases by using the I-divergence and the mean square error criteria.

1 Introduction

1.1 Image Formation Model

Any optical system degrades the acquired images due to the physical properties of the optical aperture and of light itself on the one side and due to the detection process on the other side. These degradations contain two separate parts: the blurring of the image which can be described by a PSF (point spread function) convolution and the additional noise in the image. The type of noise that can be found in the image is dependent on the acquisition process. For fluorescence microscopy it is well-known that there is Poisson noise in the acquired images because it is a low-photon imaginary technique. The Poisson noise will be represented by $\phi(\cdot)$ in this paper. Thus the suitable image formation model where i is the observed image, o the original image, h represents the PSF and \otimes the convolution operator is given by:

$$i = \phi(o \otimes h) \tag{1}$$

In this paper we assume that the PSF is already known. It is possible to use a second image with beads to estimate the PSF in a preceding step or to calculate the PSF due to the physical properties of the used microscope.

1.2 Restoration of Fluorescence Microscope Images

To restore the degradation due to Poisson noise, as mentioned before, it is common to use a Richardson-Lucy (RL) algorithm consisting of an expectation maximization algorithm which calculates the maximum likelihood estimation [1, 2]. Basically the algorithm contains the iterative minimization of the following energy functional H applying the know variables form formula (1):

$$H(o, i) = \int o \otimes h - i \cdot \log(o \otimes h) \quad (2)$$

The basic RL algorithm has a huge disadvantage. It amplifies noise after several iterations while first improving the image. In order to overcome this problem one can denoise the image first and use the RL algorithm to deblurr the image afterwards but it is a better solution to add a separate regularization functional. This additional term is weighted with a regularization parameter. A very popular choice for a regularization term is the Tikonov-Miller (TM) regularization which can be combined with the RL energy functional [3].

$$R_{TM}(o) = \int |\nabla o|^2 \quad (3)$$

∇ is the gradient of the image o and $|\cdot|$ describes the L^2 norm. The TM regularization allows the RL algorithm to converge to a suitable solution but it smooths the edges. This is a well-known problem and it is possible to use another regularization technique named Total Variation (TV) regularization which preserves the edges. A combined RL and TV algorithm for microscopy contains the TV term [4] which is again weighted with a regularization parameter. This method has another drawback because it rounds corners.

$$R_{TV}(o) = \int |\nabla o| \quad (4)$$

Both techniques are very popular and commonly used. The choice for the regularization term should depend on what one wants to analyse in the reconstructed image.

1.3 Restoration Approach using the L^2 Norm and the Bregman Iteration

Another modified approach to reconstruct blurred and noisy images is using the Bregman distance introduced in [5]. This distance was added to a blind deconvolution algorithm [6] and later also used to reconstruct a high resolution image on basis of a set of low resolution images [7]. In both approaches a L^2 norm based deblurring functional with TV is used. The blind deconvolution algorithm [6] consists of two independent parts where alternately the image is deblurred and the PSF is estimated. Here it is assumed that the PSF is already known and so only the deblurring of the image is of interest. To reconstruct the image first a common minimization of the combined energy functional containing the

deblurring and regularization functional is used. Then the observed image i is modified using $i = i + v_m$ for the $(m + 1)$ th Bregman iteration with $v_m = i + v_{m-1} - h \otimes o_m$ being a result of the additional Bregman distance in the reconstruction algorithm.

2 Materials and Methods

2.1 New Algorithm for Restoring Fluorescence Microscope Images

The new algorithm is using the bregman distance D [5] which is defined as shown in formula (5).

$$D_{\vartheta}(x, y) = \vartheta(x) - \vartheta(y) - \langle x - y, \partial\vartheta(y) \rangle \quad (5)$$

In this definition $\langle \cdot, \cdot \rangle$ denotes the inner product and ∂ the sub-gradient of ϑ . In the new algorithm a RL term H is used for deblurring according to the Poisson noise in the observed image as already described in formula (2). Then the TV regularization term R already known from formula (4) is added in order to get a stable and suitable solution. The TV term is weighted with a regularization parameter λ . The general formula to calculate the result of the reconstruction iteratively using the Bregman distance is then:

$$o_m = \operatorname{argmin}_o \{H(o, i) + \lambda \cdot D_R(o, o_{m-1})\} \quad (6)$$

As mentioned before $H(o, i)$ shall be the RL deblurring term containing the PSF and the functional R used in the Bregman distance D is the TV regularization term. It is necessary to start this approach with an empty image and to do the first step without using the Bregman distance and only apply the regularization term R . In order to formulate the algorithm a new term named *Breg* is introduced which contains an additional image with the same size as the observed image and is supposed to be filled with zeros at the beginning.

The algorithm itself consists of two separate steps which have to be executed in every Bregman iteration. The first step is to reconstruct the image iteratively starting with an empty image and then using the result of the previous step as start image afterwards. This reconstruction is done according to the following formula for $m = 1..n$ with n being the requested amount of Bregman iterations.

$$o_m = \operatorname{argmin}_o \{H(o, i) + \lambda R_{TV}(o) - \lambda \langle o, Breg \rangle\} \quad (7)$$

The formula (7) has its origin in formula (6) with the constant parts being removed and the additional *Breg* term. The second step of the algorithm in each iteration step is the modification of the Breg term according to the Bregman distance in formula (5) and formula (6). This modification is given by the fact that the minimization of the considered functional in formula (7) yields the property that the derivative is zero for a minimum of a functional.

$$Breg = Breg - \frac{1}{\lambda} \partial H(o_m, i) \quad (8)$$

In formula (8) $\partial H(o_m, i)$ describes the gradient of the RL functional $H(o, i)$.

Table 1. Results

	I-div	MSE
Original & Degraded	1.304.030	22.459.100
Original & Reconstructed (RL & TV)	67.671	21.536.600
Original & Reconstructed (New algorithm)	56.951	18.058.600

2.2 Measure the Quality of the Results

To quantify the quality of the restored images it is usual to use the I-divergence and the mean square error (MSE) criteria, as you can see in formulas (9,10), measured between two images A and B (both are 3D and of the same size):

$$I_{A,B} = \sum_{ijk} \left\{ A_{ijk} \ln \left(\frac{A_{ijk}}{B_{ijk}} \right) - (A_{ijk} - B_{ijk}) \right\} \quad (9)$$

$$MSE_{A,B} = \sum_{ijk} \{A_{ijk} - B_{ijk}\}^2 \quad (10)$$

It is a problem to use these criteria on real images because the original image is not known exactly. However, for simulated data it is a good measurement of the quality of the restoration comparing the original image and the restored one. The original image is degraded according to the physical assumptions of the fluorescence microscopy serving as basis for the restoration algorithm.

3 Results

The results of simulated images can be seen in table 1. In the first row the I-div and the MSE of the original and the degraded image is shown. In the second row the quality measurement of the well-known RL approach with TV is listed and in the last row the results of the new algorithm can be seen.

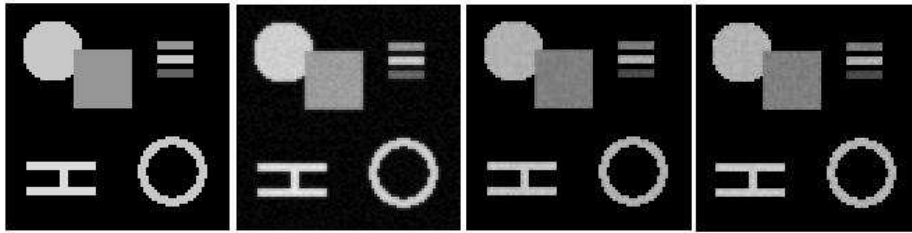
In figure 1 the result of the algorithms can be seen. In 1a) the original image is shown and in 1b) the noisy and blurry image used for the simulation. Figure 1c) shows the result of the RL with TV algorithm and 1d) the result of the new developed algorithm.

4 Discussion

We have proved that the quality of the reconstructed images can be improved by using the new algorithm compared with RL and TV, as shown in table 1. Both the I-div and the MSE show an approximately 15% better value for the reconstructed images. The new algorithm is able to reconstruct finer structures in the image as well.

The result of the new algorithm is highly dependent on the amount of Bregman iterations. With a very high number of iterations the algorithm would reconstruct the degraded image itself which is not the requested result. To start

Fig. 1. a) original image b) blurry and noisy c) RL & TV d) new algorithm



the algorithm an empty image is used which is reconstructed iteratively. In every step finer structures are reconstructed which means that after some iterations the noise is reconstructed as well. During the iterative process before the noise is reconstructed the intermediate result is very close to the original image which is a very good solution for the reconstruction. It is difficult to stop the algorithm in case of real images where the original image is not known. One possibility is to use the discrepancy principle [8] to stop the algorithm and we plan to add that to the new algorithm in future.

References

1. Lucy LB. An iterative technique for rectification of observed distributions. *Astronom J* 1974;79(6):745–65.
2. Dellard F. The expectation maximization algorithm. Technical Report number GIT-GVU-02-20, 2002.
3. Van Kempen GMP, Van Vliet LJ. The influence of the regularization parameter and the first estimate on the performance of Tikhonov regularized non-linear image restoration algorithms. *J Microsc* 2000;198(1):63–75.
4. Dey N, et al. Richardson-Lucy algorithm with total Variation regularization for 3D microscope deconvolution. *Microsc Res Tech* 2006;69:260–6.
5. Bregman L: The relaxation model of finding the common points of convex sets and its application to the solution of problems in convex programming. *Comput Math Math Phys* 1967;7:200–17.
6. He L, Marquina A, Osher SJ. Blind deconvolution using TV regularization and Bregman iteration. *Int J Imaging Syst Tech* 2005;15(1):74–83.
7. Marquina A, Osher SJ. Image Super-Resolution by TV-Regularization. 2007.
8. Lie J, Nordbotton J. Inverse scale space for nonlinear regularization. *J Math Imaging Vis Arch* 2007;27(1):41–50.