Reconstruction of High-resolution Fluorescence Microscopy Images based on Axial Tomography

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ABSTRACT

For a reliable understanding of cellular processes, high resolution 3D images of the investigated cells are necessary. Unfortunately, the ability of fluorescence microscopes to image a cell in 3D is limited since the resolution along the optical axis is by a factor of two to three worse than the transversal resolution. Standard microscopy image deblurring algorithms like the Total Variation regularized Richardson Lucy algorithm are able to improve the resolution but the problem of a lower resolution in direction along the optical axis remains. However, it is possible to overcome this problem using Axial Tomography providing tilted views of the object by rotating it under the microscope. The rotated images contain additional information about the objects and an advanced method to reconstruct a 3D image with an isotropic resolution is presented here. First, bleaching has to be corrected in order to allow a valid registration correcting translational and rotational shifts. Hereby, a multi-resolution rigid registration method is used in our method. A single high-resolution image can be reconstructed on basis of all aligned images using an extended Richardson Lucy method. In addition, a Total Variation regularization is applied in order to guarantee a stable reconstruction result. The results for both simulated and real data show a considerable improvement of the resolution in direction of the optical axis.

Keywords: High-resolution microscopy, Axial Tomography, image reconstruction, Total Variation regularization

1. INTRODUCTION

In biological sciences, fluorescence microscopy is an important tool for imaging specimen like human cells. However, fluorescence microscopes degrade the acquired images like any other optical system. Out-of-focus light blurs the image and can be described by a point spread function (PSF) convolution. In addition, Poisson noise further degrades the microscopy images since it is a low-photon imaging technique. A suitable image formation model where $i$ represents the observed image and $o$ the original image is given by formula 1. Hereby, the Poisson noise is represented by $\phi(\cdot)$ and $\otimes$ denotes the convolution operator.

\begin{equation}
  i = \phi(o \otimes PSF)
\end{equation}

It is not trivial to deblur microscopy images and since this problem belongs to the class of ill-posed inverse problems.\textsuperscript{1} A major problem for ill-posed inverse problems is to cope with noise which is amplified if it is not handled properly. A Richardson Lucy (RL) algorithm\textsuperscript{2,3} considering the Poisson noise in the microscope image allows an improvement of the image quality.\textsuperscript{4} This iterative approach maximizes the likelihood distribution $P(i|o)$ of the underlying noise distribution which leads to an expectation maximization (EM) algorithm. However, due to the ill-posed nature of the reconstruction problem, it is still not guaranteed to get a stable result and noise is amplified after several iterations. In order to further improve the result of the deblurring, it is necessary to add a priori knowledge $P(o)$ to the algorithm. Thus the maximum a posteriori (MAP) distribution $P(o|i) = P(i|o) \cdot P(o)$ is used and the a priori knowledge is realized as additional regularization term. A very popular choice for a regularization term is the Tikhonov-Miller (TM) regularization.\textsuperscript{5} This regularization term suppresses noise amplification successfully and guarantees a stable solution.\textsuperscript{6} Nevertheless, applying TM regularization leads to smoothed edges and thus Total variation (TV) regularization was introduced\textsuperscript{7} which overcomes this problem. TV regularization was combined with the RL algorithm and applied to microscopy deblurring problems.\textsuperscript{8}
Unfortunately, the resolution of the acquired volumes in direction along the optical axis is considerably smaller than in transversal direction resulting in an anisotropic PSF and thus an anisotropic optical transfer functions (OTF). The OTF is the Fourier transform of the PSF and shows the areas in the frequency domain which are imaged by the microscope. There have been various approaches to overcome this problem like theta fluorescence microscopy, 4Pi-confocal microscopy or multiphoton excitation. With an Axial Tomography approach, an object is imaged separately from different angles resulting in rotated OTFs which provide additional information about the object. Using the additional information, it is possible to reconstruct a three-dimensional volume with a high resolution in all three dimensions. The specimen can be imaged from different angles since they are located on a round glass fibre which can be rotated. The set up for this device is shown schematically in figure 1 with some beads on the round glass fibre. The basic idea of the Axial-Tomograph is then to rotate the glass fibre and thus to image the specimen from different viewing angles.

It is possible to image a specimen in a full 360° circle as it has been done in a project investigating mouse embryos. Hereby, 20 rotation steps with a stepsize of 18° are used. By the way, it is possible to image the interior of such relatively thick specimen since the density of the material allows the fluorescence photons to exit the specimen and therefore their detection. In order to reasonably enhance the resolution of the microscopy images, at least three different angles are recommendable. In another research project, beads and human cells have been imaged separately from three different angles (−36°, 0° and +36°) and we use image data sets from this project in this paper. The information included in the three different images is combined in order to reconstruct a single image with an enhanced resolution. In this way, a larger area of the frequency domain can be covered and all of this information is used to reconstruct a high resolution image. An example of a combined OTF based on three different images is displayed in figure 2 and shows the available information. In addition, the PSF and OTF of one single base image is shown.

In order to reconstruct a high resolution image, the images from the different angles and corresponding PSFs are used in according reconstruction algorithms. Note that in axial tomography, the different images have to be aligned before the actual reconstruction can be done. Hereby, possible translational and rotational shifts between the tilted views have to be corrected. The tilted view reconstruction problem was first addressed by Shaw et al. The relative rotation and translation between a pair of tilted views is estimated from the images themselves by a modified phase cross correlation function. Thereafter, the reoriented and deconvoluted images are merged treating the amplitude and phase data separately. In the work of Satzler and Els, the image is reconstructed by preserving the frequencies with the highest amplitudes from the different tilted views in each single object point. This method provides a gain in axial resolution without a significant decrease in lateral resolution. However, simulations demonstrated that this method is sensitive to noise and to even small misalignments of the datasets. An Axial Tomography reconstruction method using a RL approach has been published by Heintzmann. This method includes an automatic alignment determining the relative angles of rotation between the different image data sets. The alignment method uses the measured image data and is based on the computation of a modified (high frequency enhanced) cross-correlation function. For each unknown
rotation angle, an iterative technique is used comparing one image with another at a time. In the next step, the three aligned data sets are simultaneously used to iteratively reconstruct a single high resolution data set using an extended RL algorithm. This method combines the quality improvements gained by RL deconvolution with the refinement allowed by the use of additional information acquired from different viewing angles. The whole micro axial tomography process starting with cell preparation, image acquisition and finally the reconstruction was then investigated and automated as far as possible.\textsuperscript{21} For that purpose, a special software module has been developed. The software module then drives all hardware components required for automated Axial Tomography and performs the image acquisition. Finally, the already discussed reconstruction algorithm\textsuperscript{19} using the point-wise maximum in Fourier space is used to obtain a high-resolution image. This automated method was then used to image fluorescence in situ hybridisation-stained (FISH) cell nuclei fixed on a glass fibre.\textsuperscript{15} In addition, a method for the preparation of cell nuclei attached to glass fibres has been developed and the advantages of a glass fibre compared to a glass capillary are discussed.

In this paper, we present an advanced method to reconstruct an axial tomography image based on a data set with three different viewing angles. Our method contains a multi-resolution registration with a rigid versor transform correcting rotations and translations between the three images in one step. In addition, the aligned images are resampled on an isotropic grid structure suitable for an enhanced resolution. The reconstruction is performed by an extended RL method including a TV regularization in order to cope with the ill-posed nature of the reconstruction problem. First, we test our methods using simulated image data sets and compare the obtained results to previously proposed methods. Finally, real images containing beads and human cells\textsuperscript{17} are processed.

2. METHODS

2.1 Bleaching and background correction

It is well known that fluorescence proteins tend to bleach and thus the signal strength is decreasing after a certain excitation time.\textsuperscript{22} The Axial Tomography images are acquired with the same microscope one after the other and thus the second and the third image suffer from bleaching. Furthermore, due to a different background activity and possible differences in the exposure times, the background signal can vary from image to image. Both, the background signal and bleaching have to be corrected before the images can be processed. In order to estimate the background signal, the cells and their surrounding areas which are illuminated by the cell fluorescence and therefore influenced by bleaching are removed. A bleaching factor is then determined by evaluating a certain amount of the brightest voxels after removing the background signal and used to correct the bleaching. Like that, all images are of the same appearance and can be registered and later reconstructed.

2.2 Registration

The Axial Tomography images are rotated and shifted against each other and have to be aligned before the reconstruction can be done. In addition, the grid structure of a microscopy image is usually anisotropic and a high sampled isotropic grid structure is required for the actual reconstruction. It is not possible to reconstruct
a resolution enhanced image on a grid structure which does not allow an improvement of the resolution due to a large sampling distance. In case of the underlying microscopy images, the sampling distance in axial direction is of factor two larger than in lateral direction. An illustration of the given grid structures regarding sampling, rotation and translation is given in figure 3.

There is a well-established open source framework for registration in ITK (www.itk.org) and we use this framework to estimate the rotation and translation in each image data set. In general, each registration algorithm consists of a transform, an optimizer, a metric and an interpolator. The transform has to be able to correct the misalignments in the images and we use a VersorRigid3DTransform containing a rotation and a translation. The optimizer is chosen according to the transform and we use the VersorRigid3DTransformOptimizer which is especially designed for the VersorRigid3DTransform. In addition, we use a B-spline interpolation for the final alignment of the images allowing a higher quality than other methods like e.g. linear interpolation which are used in intermediate steps because they are less computational intensive. The metric has to be adapted to the image types and we use a mean square metric. Such a metric is suitable for microscopy images if bleaching and background are corrected and the intensity values of all images are on the same level.

When using the rigid versor transform, there are six parameters describing the rotation and translation and thus a six dimensional optimization problem has to be solved. Since the Axial Tomography images are relatively large, the registration is very computational intensive especially when the distance from the initial transform to the optimum is quite large. We use the set up rotation parameter of the Axial Tomograph as initial parameter but since the translation is unknown, we use a multi-resolution approach which is also suited in case of an unknown initial guess. Hereby, the basic idea is that the registration is first performed at a coarse scale where the images have a considerably smaller size and thus the computational effort is reduced. The result from this step is then used as initialization of the next finer scale. This process is repeated until it reaches the finest possible scale. Therefore, with growing computational effort, the initialization is closer to the optimum and less iterations are necessary. In addition, we use a linear interpolator in the first steps because the coarse scale images do not require a high performance interpolation and the registration process can thus be accelerated. A B-spline interpolation is used in the final step in order to allow an accurate final estimation of the misalignment.

Interpolations always cause small errors especially if the images are resampled or relocated. In our case, the images have to be resampled and aligned correcting rotation and translation and it is necessary to resample the images on an isotropic grid structure before the registration can be done. Instead of using the resampled images to create the aligned images used for the reconstruction, the rotation and translation information which are estimated by the registration are used together with the required resampling distances to create the aligned images in one step. Thus, unnecessary errors which are caused by additional interpolations are avoided. In addition, a B-spline interpolation is used to create the aligned images.

2.3 PSF

The PSF of a fluorescence microscopy image can be approximated by a Gaussian function with a certain variance. This model is often used because it is simple and can be well described by few parameters. In the simulations
which we performed to evaluate our Axial Tomography reconstruction method, a Gaussian PSF was also used. As mentioned before, the axial resolution is of factor two to three worse than the lateral resolution and thus the variance $\sigma_z$ in z direction is different from the variances $\sigma_x$ in x direction $\sigma_y$ and y direction which are equal. A function to describe a three dimensional Gaussian PSF is given by formula 2.

$$f(x,y,z) = \frac{1}{\sqrt{2\pi \sigma_x \sigma_y \sigma_z}} e^{-\left(\frac{(x)^2}{2\sigma^2_x} + \frac{(y)^2}{2\sigma^2_y} + \frac{(z)^2}{2\sigma^2_z}\right)}$$

Such a PSF is well suited for simulations but can always cause errors for real images since they represent an approximation. For real images, we estimate the PSFs using beads. The first processed Axial Tomography image data set consists of beads and the images of single beads are used to extract the PSFs which are neccessary for the reconstruction. Hereby, one PSF is required for each rotation angle and thus three different PSFs are extracted. The cell images are aquired with the same microscope and therefore we use the same PSFs as for the bead images. However, the PSFs have to be rotated according to the registration results for the different image data sets.

### 2.4 Reconstruction

The reconstruction algorithm used for the Micro Axial Tomography images is based on existing deblurring techniques. In contrast to normal microscopy deblurring methods, three images and three corresponding PSFs are used. As mentioned before, the RL algorithm is well-suited for microscopy deblurring since it considers the Poisson noise in the image. Hereby, the likelihood distribution of the Poisson noise is maximized by the minimization of the functional $J$ in formula 3 with $i$ being the recorded, $o$ the original image, $h$ the PSF and $\Omega$ the image domain.

$$J(o) = \int (o \otimes h) - i \cdot \log(o \otimes h) + \log(i!)d\Omega$$

After removing the constant parts of the functional, an iterative multiplicative form of the RL algorithm which minimizes $J$ is then given by formula 4. Hereby, a start image $o_0$ is necessary and we always use an empty image for that purpose.

$$o_{k+1} = \left\{ \frac{i}{o_k \otimes h} \right\} \otimes h^*$$

Since noise is amplified after several iterations, it is necessary to add regularization. We use a TV regularization (Formula 5) which guarantees a stable solution without smoothing edges. The TV regularization term is weighted by $\lambda$ in order to maintain the balance between regularization and data fit.

$$R_{TV}(o) = \int |\nabla o|d\Omega$$

An algorithm containing the RL functional with the TV regularization is given by formula 6 in a multiplicative form. This algorithm is well-suited for microscopy image deblurring since it is adapted to Poisson noise and the TV regularization guarantees a stable solution with sharp edges. In the following, this algorithm is called singleRLTV.

$$o_{k+1} = \left\{ \frac{i}{o_k \otimes h} \right\} \otimes h^* - \frac{o_k}{\lambda |\nabla o_k|}$$

In the reconstruction algorithm for the Axial Tomography, one single, isotropic, high resolution image is reconstructed and three recorded images ($i_1$, $i_2$ and $i_3$) with three PSFs ($h_1$, $h_2$ and $h_3$) have to be considered. These images are already registered and resampled on basis of the same isotropic grid structure. A multiplicative
RL method for the axial tomography is described in formula 7 and is denoted as \textit{axialRL} in the following. This method contains no regularization and is based on the original RL algorithm.

\begin{equation}
\begin{aligned}
o_{k+1} &= \left\{ \frac{i_1}{o_k \otimes h_1} \otimes h_1^* + \frac{i_2}{o_k \otimes h_2} \otimes h_2^* + \frac{i_3}{o_k \otimes h_3} \otimes h_3^* \right\} \frac{o_k}{3} 
\end{aligned}
\end{equation}

In order to cope with the ill-posed nature of the reconstruction problem, we propose a RL algorithm is then extended by a TV regularization weighted by \(\lambda\). The combined functional has to be minimized to reconstruct the image \(o\) and an iterative algorithm presented in formula 8 is used for that purpose. Our algorithm is called \textit{axialRLTV} in the following.

\begin{equation}
\begin{aligned}
o_{k+1} &= \left\{ \frac{i_1}{o_k \otimes h_1} \otimes h_1^* + \frac{i_2}{o_k \otimes h_2} \otimes h_2^* + \frac{i_3}{o_k \otimes h_3} \otimes h_3^* \right\} \frac{o_k}{3} \left(1 - \lambda \nabla \frac{\nabla o_k}{|\nabla o_k|}\right)
\end{aligned}
\end{equation}

2.5 Evaluation of results

There are various methods to evaluate the results of reconstruction methods. In case of synthetic data, the original image is known and can be used in the evaluation by comparing it directly with the reconstructed image. Therefore, the mean square error (MSE) criterion shown in formula 9 is used.

\begin{equation}
MSE(o, i) = \sum_{x \in \Omega} (o(x) - i(x))^2
\end{equation}

3. RESULTS

3.1 Simulations with synthetic data

First, we used synthetic 2D images to evaluate our new reconstruction method \textit{axialRLTV} compared to the previously used algorithm \textit{axialRL} and a single view reconstruction with the TV regularized RL algorithm \textit{singleRLTV}. Hereby, we only want to evaluate the reconstruction methods and not the other steps like registration or PSF extraction. Therefore, synthetic rotated PSFs are used for both degrading of the original image and again in the reconstruction. The variances of the PSFs are \(\sigma_x = 1.5\) and \(\sigma_y = 4.5\) and the rotation angles are set to 60\(^\circ\) and 120\(^\circ\). The degraded images used for the reconstruction are created by a convolutions of a given original image with the PSFs and additional Poisson noise. In total, we use two different synthetic test images containing different objects to test the reconstruction methods. The original and the degraded images of the first test image are shown with their Fourier transforms in figure 4. The Fourier transforms displayed in the lower row show the areas of the frequency domain which are covered by the different degraded images.
The results of the different methods can be seen in figure 5 and the axialRLTV reconstruction algorithm produces the best result. In addition, the Fourier transform shows that the information given in all three degraded images is used. The combined information is also given in the result of the axialRL algorithm but the result is degraded by some artifacts due to the lack of a suitable regularization. The Fourier transform of the singleRLTV algorithm using just one degraded image shows the inferiority to the Axial Tomography despite an improved and denoised reconstructed image. Figure 6 shows the images and the results of the second synthetic data set and similar results are obtained in this case.

Since the original images are known for the simulated image data sets, the MSE is used to evaluate the reconstruction results quantitatively and according values are given in table 1. Additionally, the MSE between the original and one degraded image is shown as well. In both cases the axialRLTV reconstruction achieves the best results. The additional information considered in the axialRL algorithm allows an improved result compared to the singleRLTV algorithm although no regularization is used.

### 3.2 Reconstructions based on real data

Two real Axial Tomography image data sets are reconstructed using our own method. First, an image data set containing beads with a diameter of 100\text{nm} is processed and then a second data set which shows a human cell. The used cell is called CAL-51 and belongs to a human breast cancer cell line taken after irradiation and chemotherapy. These cells are a rare example for tumor cells with a normal karyotype. The CAL-51 cells grow adhesive, i.e. its shape is long and thin in contrast to suspension cells and the actinium fibres in the cell are stained with the Alexa Fluor 488 Phalloidin fluorescence agent. Both specimen have been imaged separately.

<table>
<thead>
<tr>
<th></th>
<th>degraded</th>
<th>singleRLTV</th>
<th>axialRL</th>
<th>axialRLTV</th>
</tr>
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<tbody>
<tr>
<td>MSE image 1</td>
<td>1.478.588</td>
<td>710.011</td>
<td>556.935</td>
<td>430.215</td>
</tr>
<tr>
<td>MSE image 2</td>
<td>5.238.654</td>
<td>2.535.474</td>
<td>2.100.555</td>
<td>1.455.254</td>
</tr>
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Table 1. MSE of simulation results and one degraded image
from three different angles ($-36^\circ$, $0^\circ$ and $+36^\circ$) and these angles serve as initial guess for the registration. The cell as well as the beads are located on a glass fibre with a refractive index $n = 1.5168$ and Immersol$^{TM}$ with a refractive index $n = 1.518$ is used as immersion medium in the image acquisition.

The bead image data sets and the results of the axialRLTV reconstruction are shown in figure 7. In the top row, a maximum projection of the aligned and resampled 3D images and the reconstruction result in direction pointing along the glass fibre is displayed. The beads are located on the surface of the glass fibre. In the first step, only the background signal had to be corrected since there is no bleaching in the bead images due to a synthetic and very robust fluorescence agent. After the registration is completed, the aligned images are used to extract the PSFs which are necessary for the reconstruction. Therefore, the background is removed and the bead images are centered and normalized. The reconstruction result is then shown on the right side in the figure. In the middle row, a magnified section of the top row can be seen and the Fourier transform is displayed in the lower row. The aim of the Axial Tomography reconstruction is to obtain an image with an isotropic high resolution using the information included in the three different images. Analysing the Fourier transform of the registered images and the reconstruction result clearly shows that this aim is reached. The Fourier transforms of the aligned images show the limited frequency support regions due to the underlying OTFs and the reconstruction result contains their combined support regions and illustrates the enhanced resolution.

Last but not least, the CAL-51 cell image data set was processed. Projections of the original images together with the aligned images used for the reconstruction are shown in figure 8. The aligned images are bleaching corrected, resampled and relocated using rotation and translation. Hereby, the estimated bleaching factors are 2.5267 for the for left image ($-36^\circ$) and 1.6204 for the right image ($36^\circ$).

Projections of the reconstruction result compared to the original images are displayed in figure 9. Our axialRLTV algorithm was used for the reconstruction based on the three aligned images. The PSFs which are extracted from the bead images are adapted to the rotation estimation of the three cell images and then used for
Figure 8. Maximum projections in two directions of original image data set with \textit{CAL}-51 cell (Left) and aligned image data set (Right) used for the reconstruction.

Figure 9. Projections of original image data set with \textit{CAL}-51 cell (Left and middle) and reconstruction result with \textit{axialR}LT\textit{V} (Right).

the reconstruction. The reconstructed image demonstrates the improved image quality of the Axial Tomography.

4. CONCLUSION

In this paper, we presented a method to reconstruct a resolution enhanced microscopy image on basis of Axial Tomography image data sets. Before the actual reconstruction can be done, bleaching has to be corrected and the images have to be aligned. We therefore estimate a bleaching factor and use a multi-resolution registration method. A high resolution image is then reconstructed on basis of the aligned Axial Tomography images using the information included in all images. Due to the ill-posed nature of the reconstruction problem, we used a TV regularization in order to guarantee a stable result. The synthetic results showed the improvements gained by the new regularized axial reconstruction method compared to previous methods or a regularized reconstruction based on one microscopy image. The Fourier transforms of the registered bead images and the corresponding reconstruction result illustrate the enhanced resolution.

REFERENCES


