RESTORATION OF THREE DIMENSIONAL MICROSCOPIC IMAGES USING THE ROW ACTION PROJECTION METHOD

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ABSTRACT

Three dimensional (3-D) microscopy is widely used in medical applications and biosciences where a high quality magnified image of a specimen is desired. The 3-D object or specimen which is placed under the microscope is sectioned at regular intervals to acquire a sequence of two dimensional (2-D) images. These images are blurred by in-focus and out-of-focus information due to the finite circular aperture of the microscope. We use a 3-D reflecting microscope that has a high numerical aperture (to keep the blurring effect reduced) and at the same time, provides unity magnification and a large field of view. The microscope consists of two large diameter concave mirrors that face each other and share a common optical axis. We mathematically formulate the blurring effect on the 2-D images and use the row action projection (RAP) method to remove the blur. With this set of 2-D clean images, one can use any visualization software to create a 3-D view of the object. Experimental results show that this method yields a very good performance and is computationally feasible.

1. INTRODUCTION

Three dimensional (3-D) microscopy is widely used in medical applications and biosciences where a high quality magnified image of a specimen is desired [1]. For example, details of the human eye structure enables examination and diagnosis by ophthamologists. A sequence of two dimensional (2-D) images of the specimen are taken by moving the plane of best focus of a microscope through the object. Reconstruction of the 3-D image is performed from the 2-D images. The 2-D image formed at each plane of best focus or in-focus plane is commonly blurred by in-focus plane information and out-of-focus plane information (particularly from the adjacent planes above and below the in-focus plane). This is due to the circular aperture of the microscope which scatters the incoming light. Therefore, each 2-D image must be deblurred or restored by removing the out-of-focus information. Image restoration [2] has been conventionally performed by linear inverse filtering techniques [2][3]. In this paper, we investigate the use of the row action projection (RAP) method [4] for restoring the sequence of 2-D images to get the overall 3-D image. For 3-D microscopy, the RAP algorithm iteratively estimates the solution of an underdetermined system of linear equations (fewer equations than unknowns) thereby avoiding Fourier transform calculations that is required for inverse filtering methods.

2. REFLECTING MICROSCOPE

The 3-D reflecting microscope (3DM) [5] we use for collecting the images has a high numerical aperture and consists of two large diameter concave mirrors that face each other and share a common optical axis. Each of the mirrors has an aperture at its vertex, the point where the mirrored surface is cut by the optical axis. The microscope encloses a cylindrical volume that of diameter 9 inches and height 4.7 inches (includes the 1.7 inch gap between the two mirrors). The object is placed in one of the apertures and a charge coupled device (CCD) camera is placed in the other for image acquisition. There is a light-stop at the center of the mirror system. This prevents any light arriving at the CCD directly from the object (at low angles), since these light rays have not utilized the high numerical aperture (NA) of the system.

To utilize the high NA, the object needs to be illuminated by light rays arriving at large angles. If the light rays from the source were to arrive at low angles, the rays would hardly reach the CCD as most of it would be blocked by the light stop. Effective illumination is achieved by using a series of wide-angle light emitting diodes (LED) that are kept along with the CCD array so that the light would appear to arrive from the periphery of the CCD. There are six LEDs that are connected through a variable resistor to vary the brightness. The six LEDs that achieve the proper lighting or illumination are fixed to the mirror's opening and hence cannot be moved. Around this opening, there is a circular space. The CCD camera is on a 3-axis movable stage in which the motion in the circular space can be controlled. The LEDS are placed around the camera but fixed to the mirror's opening. The CCD array is further connected to a serial port of a PC which controls the position of the CCD camera in 1 micron increments. The CCD camera's ouptut is connected to a Dat-

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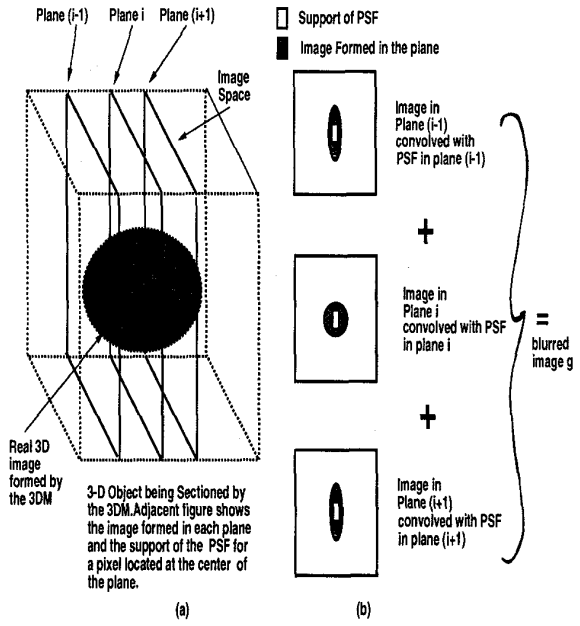

Figure 1: Optical sectioning of an image

acube's real-time image processing system. Image sequences are acquired through the Datacube system (image acquisition device) onto the SUN host (hardware and software control) with the PC used for synchronizing the movement of the CCD array.

From the geometry of the mirror system, light leaving the object from one of the mirrors is captured by the other mirror and reflected back to the first mirror. Since both mirrors are at the focus of each other (confocal), the reflected light rays come to focus at the second mirror. Thus, a 3-D image of the object is formed at the aperture of the second mirror. Now, the image is sectioned as a sequence of 2-D images which are acquired by moving the CCD array. Magnification of the image is introduced by the changing the spacing of the CCD pixel array. The diameter of the mirrors is sufficiently larger compared to the specimen. Practically, all of the light leaving the specimen is captured by the first mirror and focussed upon the camera positioned at the aperture of the other mirror. Because the entire object is illuminated rather than a portion of it, as in a slit lamp scanning microscope, the entire image can be rapidly acquired in a fraction of the time taken by the slit lamp device.

3. MATHEMATICAL FORMULATION

The 3-D object is optically sectioned to get a set of 2-D images. The 2-D images are captured by the CCD camera and are blurred by both in-focus plane information and out-of-focus plane information (particularly from the adjacent planes above and below the in-focus plane). The in-focus and out-of-focus degradation is represented by a set of point spread functions (PSF) which describe how the light is scattered by the circular aperture. We will describe how these PSFs are measured later in the paper. The 2-D images are deblurred by a restoration algorithm that uses the acquired images and the measured PSFs thereby resulting in a set of clean images. With this set of clean images, one can use any visualization software to create a 3-D view of the object. In deriving the mathematical formulation, consider one of the blurred 2-D images (denoted by **g**) without loss of generality. The concept of optical sectioning that leads to the formation of **g** is shown in Fig. 1.

The 2-D blurred image formed at the plane of best focus has pixels arranged as a M by N matrix. The individual pixels are denoted by $g(m,n)$ and **g** is a column vector formed by stacking the rows of the pixels $g(m,n)$. The equation for each pixel is given by

$$g(m,n) = \sum_{i=-p}^{p} f_i(m,n) \star h_i(m,n) \qquad (1)$$

where $\star$ is the convolution operator. The true deblurred image at the plane of best focus is $f_0(m,n)$ and $h_0(m,n)$ is the point spread function (PSF) for the plane of best focus. It is our objective to recover $f_0(m,n)$. The other images at the different out-of-focus planes are given by $f_i(m,n)$ for $i = -p$ to p and excluding $i = 0$. The corresponding PSFs are $h_i(m,n)$. Equation (1) can be expressed in matrix form as

$$\mathbf{g} = \mathbf{Hf} \qquad (2)$$

where **f** is a column vector formed by stacking the rows of the images $f_i(m,n)$ in each plane and **H** is a rectangular block Toeplitz matrix containing all the PSFs $h_i(m,n)$. Since **g** is a function of all the images $f_i(m,n)$, the system of equations is underdetermined in that there are fewer equations than unknowns. The blurred image **g** and a measured estimate of the PSFs are acquired. The row action projection method is used to get **f** and hence, $f_0(m,n)$ which forms part of the vector **f**.

4. MEASUREMENT OF THE POINT SPREAD FUNCTIONS

To measure the actual PSF's, a point source that fills the mirrors was developed. The configuration of the light source is shown in Fig. 2. This was found to satisfactorily fill the 8 inch diameter mirrors. It consists of a long tube with a fibre optic source at one end followed by a diffuser, an iris diaphram, a pinhole (both 10 micron and 25 micron pinholes have been used) and a 0.85 NA microscope objective. The point source of light is mounted in the object space. It is

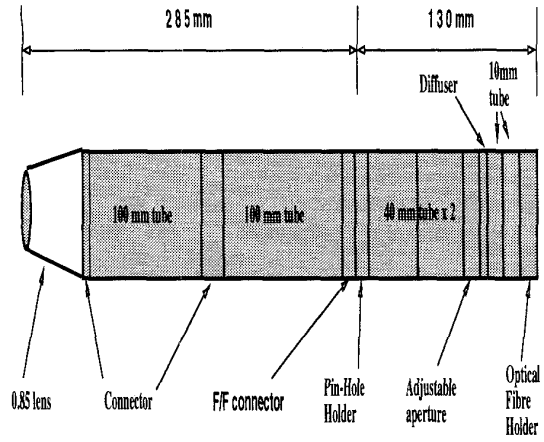

Figure 2: Point source for measuring PSFs

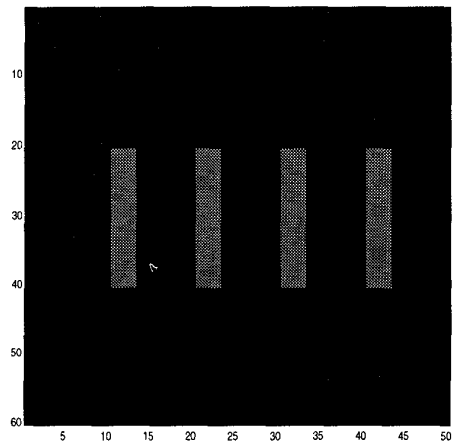

Figure 3: Original image

then beamed into the microscope at the plane of best focus and at various distances from the plane of best focus to get the PSFs. Then, smoothing is performed to get circular symmetry. Therefore, each of the PSFs can be considered to be a circularly symmetric linear phase finite impulse response (FIR) filter [6]. Once the PSF's are measured, the degradation introduced by the system is known. This measurement of the PSFs need only be done once.

5. ROW ACTION PROJECTION (RAP) METHOD

The RAP method has been used for image restoration involving only one convolution operation and one impulse response that represents the image blur [4][7][8]. In this case, the number of equations and unknowns are the same. In this paper, we use the RAP for the first time for 3-D microscopic image restoration in which an undetermined system of equations is involved. The RAP method forms an iterative estimate of $\mathbf{f}$ in which the update is given by

$$\mathbf{f}^{k+1} = \mathbf{f}^{k} + \frac{\lambda e_i H_i^T}{H_i H_i^T} \tag{3}$$

where k is the iteration number, $\mathbf{f}^k$ is the estimate of $\mathbf{f}$ at the kth iteration, λ is a scale factor for the update and H_i is the ith row of the matrix $\mathbf{H}$. The variable e_i is the error for the ith equation as given by

$$e_i = g_i - H_i \mathbf{f}^k \tag{4}$$

where g_i is the ith element of $\mathbf{g}$. The constraint $0 < \lambda < 2$ is required to ensure convergence. The RAP algorithm solves the system of equations by iterative projections onto the hyperplanes specified by each equation. The solution is the intersection of all the hyperplanes.

6. EXPERIMENTAL RESULTS

The practical issues in implementing the RAP method include the initial condition used, number of iterations and the value of λ. Since we have an undetermined system, there are many solutions. Converging to the right solution depends on choosing a good initial condition. We experimented with various initial conditions and found that using $\mathbf{g}$ as the initial condition worked well. We present the results for which the degraded image is formed as a special case of Eq. (1),

$$g(m,n) = \sum_{i=-1}^{1} f_i(m,n) \star h_i(m,n) \tag{5}$$

where the out-of-focus planes are 50 microns away from the plane of best focus. Degradation only takes place due to the adjacent planes which is common in practice.

The signal to noise ratio (SNR) is used to compare the original image with the degraded and restored images. When comparing the original image $f_0(m,n)$ and the degraded image $g(m,n)$, the SNR in decibels (dB) is given by

$$\mathrm{SNR} = 10 \log \frac{\sum_m \sum_n f_0^2(m,n)}{\sum_m \sum_n [f_0(m,n) - g(m,n)]^2} \tag{6}$$

From Eq. (6), the SNR between the original and degraded image is calculated as -16.05 dB. The SNRs when comparing the original and restored images for varying λ and number of iterations are determined by using the formula in Eq. (6). The results are presented in Table 1. From Table 1, it is observed that increasing λ and the number of iterations improves the restored image in that a higher SNR results. The SNR does not improve significantly beyond 1000 iterations. An

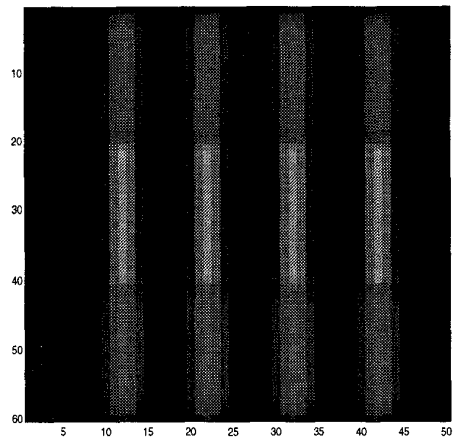

Figure 4: Degraded image

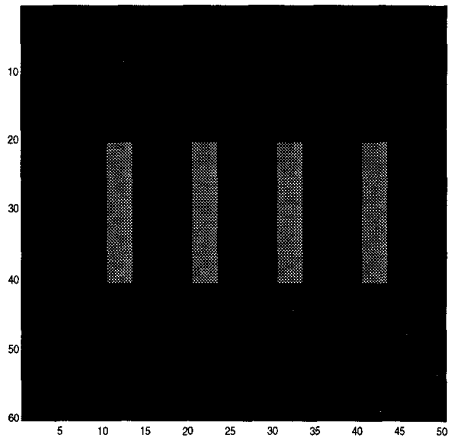

Figure 5: Restored image

Number of iterations	Value of λ		
	0.5	1.0	1.5
20	3.91 dB	5.27 dB	5.76 dB
50	4.45 dB	6.17 dB	6.58 dB
100	4.91 dB	6.84 dB	7.43 dB
200	5.50 dB	7.73 dB	8.70 dB
500	6.71 dB	9.63 dB	11.22 dB
1000	8.21 dB	11.75 dB	13.14 dB

Table 1: The SNRs when comparing the original and restored images for varying λ and number of iterations

improvement in SNR of about 29 dB is achieved by the RAP approach.

Figures 3, 4 and 5 show the original, degraded and restored images for $\lambda = 1.5$ and 1000 iterations. It is demonstrated that for 3-D image restoration, an undetermined set of equations results and the RAP method is highly useful. It is of vital importance to get good PSF estimates and use a proper initial condition. The method is fast in that 1000 iterations results in a good SNR when comparing the original and restored images.

7. SUMMARY AND CONCLUSIONS

The row action projection (RAP) method is highly useful for restoring 2-D images that are blurred due to the scattering of light through the circular aperture of the reflecting microscope. Results show that the SNR between the restored and original image is high when using 1000 iterations of the RAP algorithm. The key points for having a successful method are to use a good initial condition, a high value of λ and getting good estimates of the point spread functions (PSF).

8. REFERENCES

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