Partially Blurred Light Microscopy Image Restoration

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***Abstract*—Blur is a natural issue in imaging systems and may be relevant for several fields which rely on image-based analysis techniques, such as medicine, biology and material science. These fields are frequently related to microscopes, optical instruments capable of generating magnified images of small objects and structures. The main causes of blur are motion and defocus. Dealing with this property is a challenging process.**

I. Introduction

BLUR is a natural issue in imaging systems and may be relevant for several fields which rely on image-based analysis techniques, such as medicine, biology and material sciences. These fields are frequently related to microscopes, optical instruments capable of generating magnified images of small objects and structures. This work attempts to restore light microscopy images and compare the results concerning their quality and relative sharpness.

II. Objectives

This work aims to perform image restoration with four different filters: Inverse Filter, Wiener filter, Iterative Richardson-Lucy Filter and Laplacian Kernel Convolution. The quality of the results will be compared quantitatively and qualitatively.

III. Materials

The image database consists of light microscopy images from a sample of *Callisia repens* specimens (known in Brazil as *Dinheiro-em-penca*, acquired with an ZEISS SteREO Discovery v20 microscope, commonly used in biological analysis applications. The set is a courtesy of the *Scientific Computing Group* from the São Carlos Institute of Physics (IFSC), coordinated by professor Odemir Martinez Bruno. Those images where chosen because the stomata are clearly visible. Fig. 1 represents one image from the database.

The point spread function of the microscope will be extracted with the bead technique. This consists of taking a picture of a small white point-like structure, which will be blurred by the imaging system. This image is assumed to contain information about the degradation process of any image acquired by the system. The small point is usually between 100 and 200 nanometers. In this case, the bead was made of graphite powder, which achieved the bead effect when imaged with a 500x magnification. A denoising process was applied in order to enhance the point spread function quality.

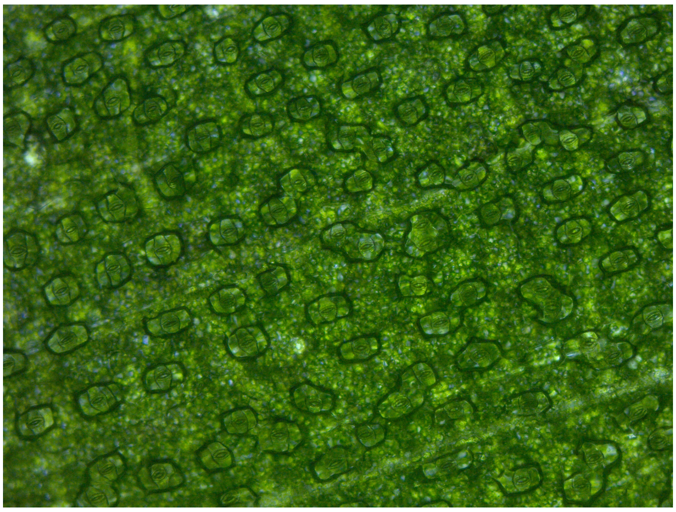

Fig. 1. An image of a *Callisia repens* specimen, magnified 200x.

IV. Methods

The project proposes the use of four different image restoration methods. For all of them, there will be enhanced quality images (this will be done by the ZEISS acquiring software with *Best Fit* and *Exposure* tools or with GNU Image Manipulation Program - GIMP - tools), considered to be the ground truth for the comparison purposes. Those will be compared with each image of the database, before and after the restoration process. The methods are:

- **Inverse Filtering [4]**: considering the effect of blur in images is caused by a linear process, the resultant image may be described by a convolution of the input image with a function $h(x, \alpha, y, \beta)$, called the point spread function of the imaging device. This degradation model is a taken as a constraint for all methods in this work. The process in the continuous domain may be represented by Equation 1

$$g(\alpha, \beta) = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} f(x, y) h(x, \alpha, y, \beta) dx dy \quad (1)$$

  Where $g(\alpha, \beta)$ is the degraded image and $f(x, y)$ is the real image. With the above relation in hands, one can infer that it is possible to obtain $f(x, y)$ performing a convolution of $g(\alpha, \beta)$ with the inverse of $h(x, \alpha, y, \beta)$ matrix.
  The Fourier domain inverse filtering algorithm considering the limitations of zero division may be expressed by Equation 2:

$$\hat{F}(u,v) = \begin{cases} \frac{\hat{G}(u,v)}{\hat{H}(u,v)} & \text{if } u < u_0 \text{ and } v < v_0 \\ \hat{G}(u,v), & \text{if } u \geq u_0 \text{ and } v \geq v_0 \end{cases} \tag{2}$$

- **Wiener Deconvolution [4]**: similar to the inverse filtering approach, the idea is to convolve the PSF by multiplying the matrices in the Fourier Domain. The difference in Wiener filtering is that the matrix $M(u,v)$ is given by Equation 3 in the Fourier Domain:

$$\hat{M}(u,v) = \frac{\hat{H}^*(u,v)}{|\hat{H}(u,v)|^2 + \frac{S_{vv}(u,v)}{S_{ff}(u,v)}} \tag{3}$$

  where $\hat{M}$ is the Wiener filter, $\hat{H}$ is the point spread function and $\hat{H}^*$ denotes its complex conjugate. S_{vv} and S_{ff} are the spectral densities of the noise field and the degraded image. The spectral quotient from the equation above is hard to obtain, due to the possible lack of *a priori* knowledge about what the image should be and how it could be described mathematically. Therefore, it is somehow assumed to be a constant Γ; this creates another problem: the best constant is unique for each image, so there must be several tests in order to find it.

- **Iterative Richardson-Lucy Algorithm [1]**: this method consists in estimating the original image $\hat{f}$ with several iterations. For each of them, $\hat{f}$ is calculated by an inverse filtering operation with the degraded image g, weighted by the point spread function. Initially, the guess is the degraded image itself; later, the results obtained by Richardson in [3] proved that the algorithm can perform a good image restoration with a given PSF and no assumptions of the noise field. The Richardson-Lucy algorithm is denoted by equation 4:

$$f^{n+1} = f^n + H\Big(\frac{g}{Hf^n}\Big) \tag{4}$$

- **Laplacian Kernel Convolution [5]**: a simple spatial domain convolution of the image with some 3x3 Laplacian matrices may be useful for sharpening and deblurring. The Laplacian Kernels have -4, -8 and 9 cores, as showed below:

$$\begin{bmatrix} 0 & 1 & 0 \\ 1 & -4 & 0 \\ 0 & 1 & 0 \end{bmatrix} \quad \begin{bmatrix} 1 & 1 & 1 \\ 1 & -8 & 1 \\ 1 & 1 & 1 \end{bmatrix} \quad \begin{bmatrix} -1 & -1 & -1 \\ -1 & 9 & -1 \\ -1 & -1 & -1 \end{bmatrix}$$

  The image must be converted to floating point values in order to avoid negative values when reconverted to unsigned integers. Kernel cores -4 and -8 require a subtraction of the degraded image by the result of the convolution; the 9 core kernel may be convoluted without that subtraction.

V. Evaluation

For a quantitative evaluation of the results, the chosen metrics are the Root Mean Squared Error (RMSE) and the Peak Signal-to-Noise Ration (PSRN). Those were taken between the ground truth images, the original blurred images and the restored ones. The RMSE is supposed to weight the differences between the two images proportionally, and is defined by Equation 5:

$$RMSE = \sqrt{\frac{1}{MN}\sum_{i=1}^{n}(f(x,y) - \hat{f}(x,y))^2} \tag{5}$$

Where $f(x,y)$ is the ground truth image and $\hat{f}(x,y)$ is the restored image which may be $g(x,y)$ (the degraded image). The result of the RMSE computation is used in PSNR, which is a measure of each value in comparison with the maximum value of an image, 255. Equation 6 denotes the relation between those metrics:

$$PSNR = 20\log_{10}\left(\frac{255}{\sqrt{RMSE}}\right) \tag{6}$$

The equations 5 and 6 shows that the metrics follow an inverse relation; therefore, the restoration quality will be better with lower RMSE values and higher PSNR values.

VI. Results

Tests were performed with all proposed filters and ten *Callisia repens* light microscopy leaf images. The ground truth images for those cases were enhanced and restored GIMP, with the *Unsharp Mask* tool. The parameters were 200 and 0.5 for radius and amount. The results shown in Table II - RMSE and PSNR between the ground truth images and degraded (g) or restored ($\hat{f}$) ones - are the mean values for each method and prove that Inverse and Wiener methods in fact achieve some amount of restoration, but the effects might not be relevant for a very degraded image. The Laplacian Kernel convolution also achieved some relevant results. Table I denotes the environment in which the tests were made (RL stands for Richardson-Lucy).

TABLE I
TEST CONDITIONS

OS	Debian 9.4
Environment	Python 3.5.3
CPU	Intel Core i7
Memory	8GB
Image Size	2560x1920
RL iterations	15

TABLE II
RESULTS

	RMSE	RMSE	PSNR	PSNR	
	$\hat{f}$	g	$\hat{f}$	g	t
Inverse	0.0729	0.2178	70.9622	61.5405	4.6339
Wiener	0.0637	0.2083	72.3017	62.0981	17.0463
RL	0.2144	0.2083	61.5373	62.0981	89.0042
Laplacian	0.1237	0.2083	66.3070	62.0981	0.5066

VII. Conclusions

The Iterative Richardson-Lucy Algorithm did not perform well due to the amount of iterations: it would need 100 and rising. The result table shows that it took too long for the algorithm to give the results, and those 100 iterations were not applicable. Therefore, the dimensions of the image must be considered before using Richardson-Lucy algorithm.

Another issue lies above the point spread function, which was acquired experimentally and may have some limitations due to exposure and noise conditions. In order to achieve better results with techniques that rely on the PSF information, one must estimate it mathematically or experimentally with some sort of reliability. This is a future extension of this work.

There are other techniques which do not require *a priori* information about the image or the degradation process; those are named **blind deconvolution** methods, and will be considered during the research.

References

[1] Shaked, E. and Dolui, S. and Michailovich, O. V. Regularized Richardson-Lucy Algorithm for Reconstruction of Poissonian Medical Images *IEEE International Symposium on Biomedical Imaging: From Nano to Macro* March 30 – April 2, pp. 1754 - 1757; Chicago, IL, USA.

[2] Lang, L. and Xu, Y. Linear and Nonlinear Image Deblurring: A Documented Study *IEEE Signal Processing Letters*, vol. 10, no. 5, (2003), pp. 129-132.

[3] Richardson, W. H. Bayesian-Based Iterative Method of Image Restoration *J. Opt. Soc. Am.* , 62, 55-59 (1972).

[4] Petrou, M. and Petrou, C. *Image Processing: The Fundamentals*. John Wiley & Sons, Singapore, 2nd edition, 2010.

[5] R. C. Gonzalez, R. E. Woods and S. L. Eddin Digital Image Processing Using MATLAB *Upper Saddle River, New Jersey, USA: Pearson Prentice-Hall, (2004), pp. 99 – 103.*