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A restoration technique for defocussed biological images

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The problem of automating quantitative analysis of biological images has been widely discussed by several authors within the context of automatic karyotyping (11), blood cell analysis (2), neuron counting (3), etc. The technical means by which a biologist examines a specimen is the microscope and it seems highly desirable to enhance the power of such an instrument by linking it directly to a computer so that the images may be recalled, processed or displayed. Such a task, although simple in its ultimate goal, is certainly very difficult to achieve (4) since the nature of the biological images is very complex and therefore a very sophisticated software structure is needed if the processing of such pictures must be competitive with the one performed by man.

The biological specimen which is normally under the microscope has a three-dimensional structure but may be only observed by means of an optical system which has in focus one single plane at a time. Therefore the analytical observation of the specimen is generally performed by scanning (manually or automatically if under computer control) the z-axis so that all the components of the specimen may be sequentially focussed. For every position of the object in the z-axis a digitization is performed by the scanning device (FIDAC for instance (5)) and such information is stored in the memory of the associated computer until all possible planes belonging to the specimen have been scanned. At this time all the information contained in the specimen is available in the computer whether the components are in focus or not. For any subsequent processing, which will be decided by the experimenter according to the task, a part of the digitized image will be chosen and processing will begin. The task may be to locate connections between neuron cells, to measure a given area, to find the position of a centromere in a chromosome, etc. In this approach the specimen is considered plane by plane as a set of adjacent sections belonging to a single three-dimensional object. Since the complexity of the tasks is rather high the system often includes an input device (other than the microscope) as a light pen or a Rand Tablet, so that the biologist may interact with the computer. For example, where the location of a specific cell

(i.e. a neuron) is required, and such a cell is embedded in a complex context, the experimenter will point on a display the cell he wishes to process by means of the light pen. From what has been said before, it follows that it is difficult to get all the components (6) of a histological specimen into focus whether one remains on a single focal plane or one scans all focal planes in search of a better one. The purpose of our work is to introduce a local processing algorithm that, when applied to each component out of focus, produces an image which is nearer to the focussed image of the same component.

If we consider a perfectly focussed circular white spot on a dark background and we now defocus the spot we may see on the resulting image a change in the light distribution. The brightness of the centre will be reduced, the contour of the spot will be smeared and the size of the total illuminated area will be larger. The total light intensity will be the same as the one on the focussed spot, isotropically distributed as before but decreasing with the square of the distance from the centre. Such a description considers a slight defocussing within small areas which is what happens with the defocussed components of histological specimens, therefore a nearest-neighbour interaction will be able to compensate for defocussing degradation.

We will now describe a technique, that has recently been presented in a preliminary paper (7), for restoring those components of a picture that are out of focus. Given an array of MN elements on which a digitized image is contained we will assume that such an image is out of focus and therefore its contours will be blurred and the grey values will differ substantially from the original ones as well as having lower maximum grey levels. We will now process the image in order to enhance the grey values which are surrounded by a local average grey level which is high and depress the grey values of elements which are surrounded by a local grey average level which is low. What is high and what is low will be found experimentally by scanning the matrix firstly with a window G. Such a window must have a size of the order of the grey scale areas that represent the digitized images of the histological material to be analyzed. A set of average grey levels will be computed using window G, scanning element by element the whole matrix. The position of window G that gives the maximum average grey scale value corresponds to the histological material that is contained in the image and that must be restored. The assumption implied here is that although theoretically a low grey value element contains the same information as a high grey value one, in practice, at least for defocussed images, it is reasonable to assume as containing more information about the original objects, all those elements which are surrounded by high grey level elements. In the most general case a threshold will be

chosen for every part of the pattern that is processed depending on the local average grey scale (a.g.v.) value. On the other hand, if very different a.g.v. are found and we are not interested in information connected with lower grey values, the maximum a.g.v. found will be chosen as a threshold. If for instance the average grey scale value remains approximately constant then we might consider that value as a threshold. After the threshold has been established the whole MN matrix is scanned with a second window C that behaves as a weighted mask. In the following section the mask will be described. The weights in the mask differ if the average grey value corresponding to the area of the mask is under of above the chosen threshold. An interesting feature of the human visual system is that when a pattern of bars whose grey values vary sinusoidally from white to black is presented to an observer, for any defocussing of such a picture he will always perceive a sinusoidal pattern with the same frequency but different maximum grey values (8). This fact suggested us a test for our method. If our method approximates the process of refocussing the output of a sinusoidal pattern must still remain sinusoidal. For such a test window G is useless and for any window C the test must hold.

Refer to Fig. 1. We have considered a cycle of a sinusoid using 64 grey levels and 72 intervals of 5^{o}each. On such a sinusoid different C windows a common minimum grey of zero and different maximum grey values whilst the frequency remained constant.

Some other tests with simulated patterns have been carried out and finally we have considered a set of 5 different photographs, taken at the optical microscope, of a thin histological sample (Fig.2) which differ from each other since only one of them is in focus and the other four are above and under the first one. The histological material is the diencephalon of rana esculenta. The "out of focus components" of the picture we considered, are the nucleoli of neuron cells. All the photographs (35 mm transparencies) were digitized using a flying-spot scanner with 16 linear grey scale values and 625 by 625 points (Figs. 3, 4, and 5).

Let us divide the image into three equal parts, each one containing a third of all rows. After computing with a three by three window (G window) the a.g.v. of each part, the highest level was chosen as our threshold value. Such a threshold has been used for establishing which weighted mask (C window) to employ on each element of the image to be processed. If the a.g.v. found for a specific elements is above threshold a first mask will be responsible for increasing the grey value of the element after taking into account the contextual grey level weighed by the mask, the second mask will operate in the reverse way so as to decrease the grey value of

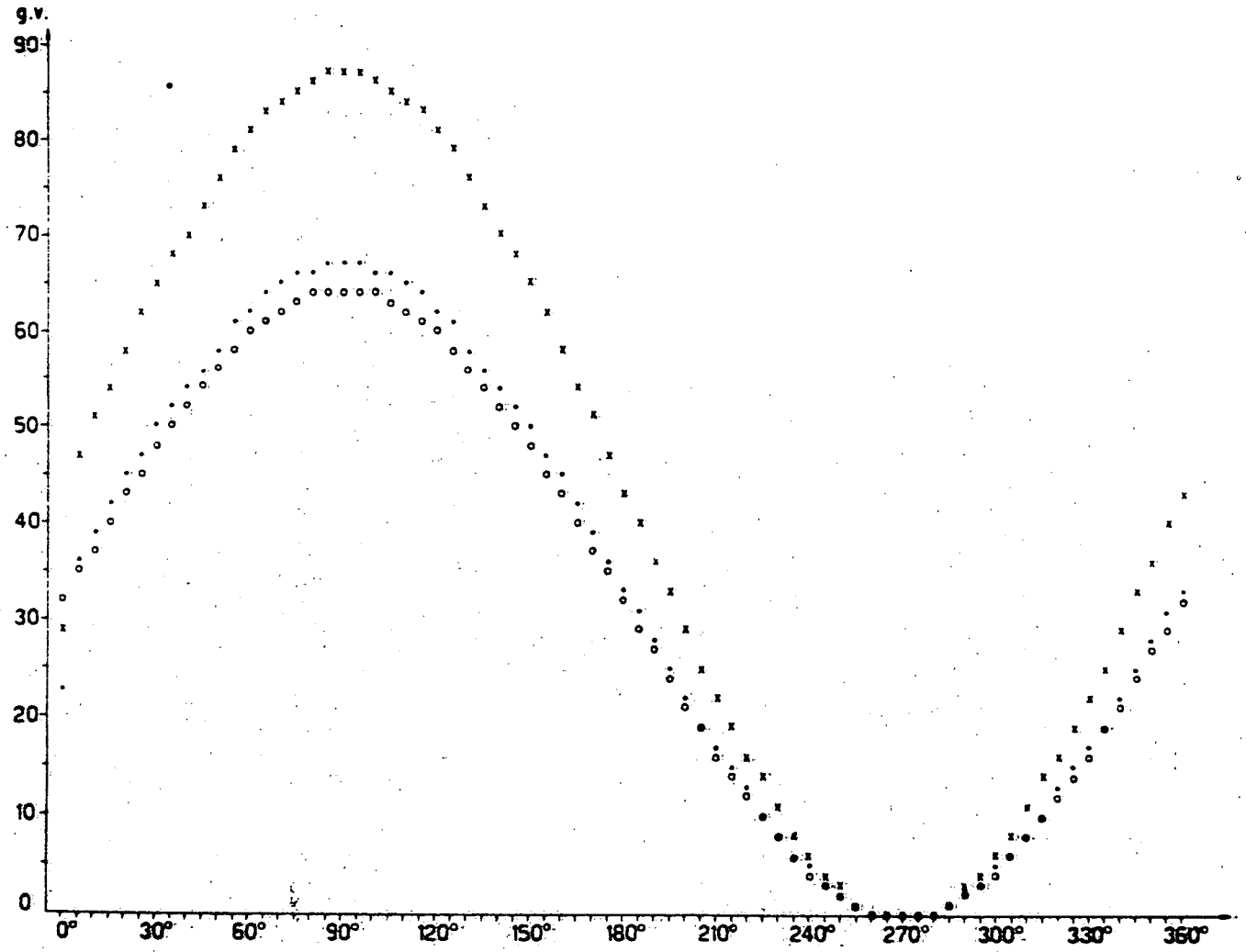
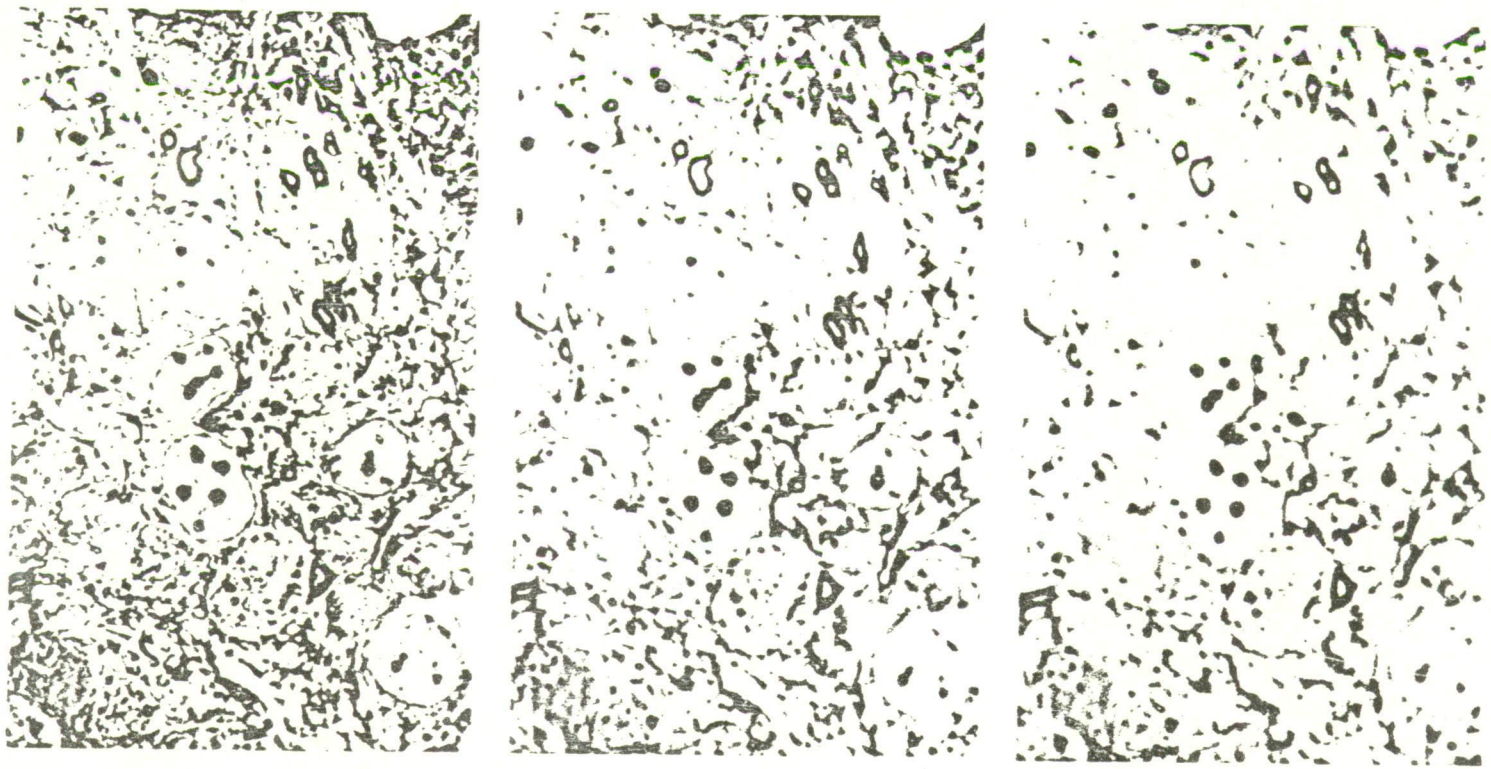


Fig. 1 Test of the sinusoid (o input, x output with C window 1.8, 1, 1.8 / 1, 1, 1 / 1.8, 1, 1.8, output with C window 1.1, 1, 1.1 / 1, 1, 1 / 1.1, 1, 1.1)



20 μ

Fig. 2 Microscope photograph of diencephalon of rana esculenta.
(Kindly given by the Department of Neuroanatomy of C.N.R.,
Arco Felice, Naples.)

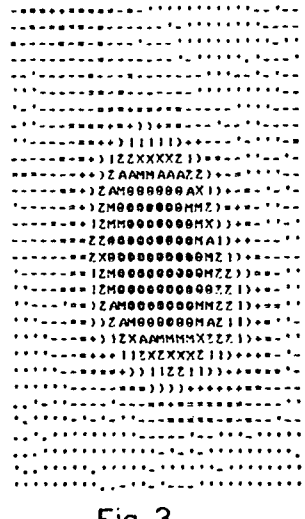


Fig. 3

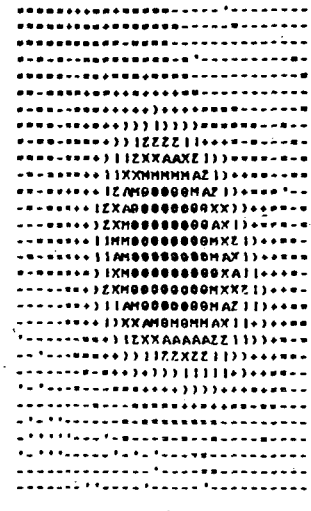


Fig. 4

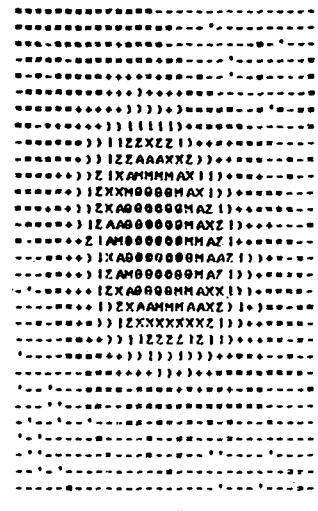


Fig. 5

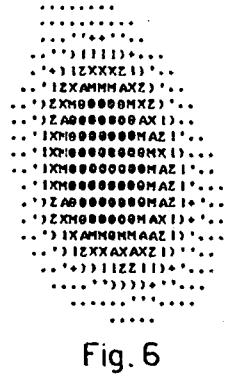


Fig. 6

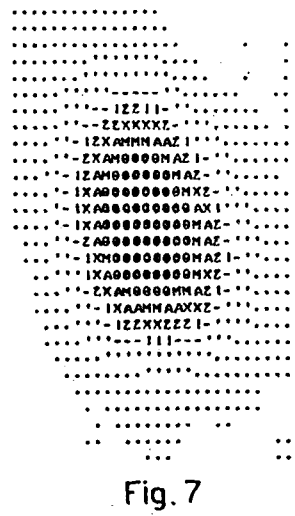


Fig. 7

Fig. 3 Digitized image of in focus nucleolus

Fig. 4 Digitized image of the same nuclolus +0.75 μ out of focus

Fig. 5 Digitized image of the same nucleolus +1 μ out of focus

Fig. 6 Processed nucleolus of Fig. 4 using weights (1.6, 1, 1.6 / 1, 1, 1 / 1.6, 1, 1.6)

Fig. 7 Processed nucleolus of Fig. 5 using weights (1.8, 1, 1.8 / 1, 1, 1 / 1.8, 1, 1.8)

the element. The computations that take place to evaluate each new value of grey level for an element are the following ones: with a three by three mask the grey value of every element is multiplied by the weight of the cell that corresponds to such an element. This is performed for every element covered by the mask, the sum of these nine products is normalized dividing such a number by the area of the mask. The new grey value on the image corresponding to the central element of the mask will be given by the number obtained from the previous operations, and this for all the elements of the input image. More generally we may write:

$$a^{*}_{i,j} = \frac{1}{n^2} \sum_{k=0,\ [n/2]} a_{i\pm k,\ j\pm k} \cdot p_{i\pm k,\ j\pm k}$$

where $a^{*}_{i,j}$ will be the new grey value of the element $a_{i,j}$ after the convolution of the mask, n by n are the dimensions of the mask, $p_{i,j}$ is the weight of the cell i,j belonging to the mask, and square brackets are used to indicate the maximum integer number. For our case, we have used n=3 and therefore the formula becomes

$$a^{*}_{i,j} = \frac{1}{9} \sum_{k=0,1} a_{i\pm k,\ j\pm k} \cdot p_{i\pm k,\ j\pm k}$$

In the general case the scanning of the image with the window n by n will start from the element of the matrix M.N of coordinates (n/2), (n/2). After applying several masks with different weights on the images which were out of focus we have checked that similar masks produced good results on those images that were equidistant in sign and value from the focal plane. It was also seen experimentally that those images which are out of focus because above or under the focal plane (at the same distance in modulus) appear different in size and distribution of grey level as if the defocussing effect would be asymmetrical. To compensate for such a behaviour of defocussing we have used different masks so that when we had to process images that were defocussed above the focal plane the weights were heavier than the corresponding ones for a defocussed equally distant image under the focal plane. It is our intention to automate the process by which, from a given distance to the focal plane, a set of weights for a mask is obtained.

We will present now only those results that appear interesting after having processed more than 25 different biological images with a range of masks that included a variation in size (from 3x3 to 5x5),

1	0	1	0	0	0	0	0	0	0	0	1	1	1	1	2
1	0	0	1	0	1	0	1	1	1	1	1	1	0	2	1
1	0	1	2	2	1	1	0	1	1	0	2	0	0	2	0
0	0	3	2	2	1	1	1	1	2	1	2	0	0	0	1
1	0	2	3	2	1	2	1	1	1	1	2	1	0	2	0
1	2	2	1	2	2	2	2	2	2	1	1	1	1	0	0
1	0	3	3	2	3	3	2	2	2	2	1	1	1	1	1
0	2	2	2	2	2	2	2	2	2	3	1	1	0	0	1
1	1	2	3	2	2	2	2	2	2	2	1	1	2	0	1
1	1	3	3	2	2	2	2	2	2	1	1	2	0	1	1
1	1	2	2	3	1	2	2	2	2	2	1	2	1	1	1
1	1	2	2	2	2	2	1	2	1	2	1	1	1	1	0
1	0	1	1	1	1	1	2	2	2	2	2	1	1	2	1
0	0	1	1	0	1	1	0	1	1	2	0	0	0	0	0
0	0	1	0	0	0	0	1	2	1	1	1	0	1	0	1

a

2	3	2	3	0	1	1	0	0	3	3	3	2	2	2	1
3	3	4	0	0	0	0	0	0	0	0	4	4	2	2	2	2
2	1	0	1	0	0	0	1	0	0	0	1	4	3	1	3
3	0	1	1	1	0	1	1	0	1	0	1	0	3	3	1
1	0	1	1	1	1	0	1	1	1	0	1	0	3	2	3
0	1	1	1	1	0	0	0	0	0	0	0	0	2	3	3
1	1	3	0	0	0	1	0	0	0	0	1	0	2	1	3
1	0	2	0	1	0	0	1	0	0	0	0	0	2	1	3
4	0	1	1	0	0	0	0	0	0	0	1	0	2	0	3
4	1	2	2	0	0	1	0	0	0	0	0	1	2	0	0
4	1	1	1	1	0	0	0	0	1	0	1	0	1	0	4
4	3	0	1	1	1	0	0	1	0	1	0	0	0	0	4
3	4	4	1	0	0	0	0	0	1	1	0	1	0	5	5
3	3	3	4	0	0	1	0	0	1	1	0	0	4	4	3
3	2	2	3	3	3	4	0	1	1	4	4	3	4	3	3

b

Fig. 8a, b Difference matrices (see text)

in weights (1 to 3) and in their distribution along the mask. From all these results we will only examine in this paper those concerning a specific nucleolus, as seen through the microscope, on Fig.2, after digitization on Fig.3 and in Figs. 4 and 5 the digital version of the same biological object only out of focus. Let us now consider Figs.6 and 7, which are processed images with the mask convolution. At this point we may ask ourselves how we can measure the quality of the method on processed images. Such a measurement, if possible, should be a number and it appeared difficult to express the property of local refocussing with such a number. We have therefore chosen to compute the differences of grey values point by point only on that area which contains the nucleolus and to write the absolute value of each difference on a matrix (see Fig. 8a and 8b). 8a is the difference matrix between Fig. 5 and Fig. 3 and 8b is the difference between Fig. 7 and Fig.3. As it may be seen, the most relevant feature that discriminates between 8 a and 8b is given by the set of zeros in the centre for 8b and by low grey values on the border for 8a, which shows that the refocussed image (Fig. 7) is nearer to the original (Fig. 3) in its central part where the nucleolus is contained.

As it has been shown, the restoration process is context sensitive, some of its parameters may be chosen at will by the user, and others will be automatically chosen after the set of preliminary measurements. Furthermore since the masks are chosen according to the a.g.v. of the context it is interesting to note that background noise removal is performed by such a method.

We may conclude by underlining that the restoration properties of this method include the possibility of preserving all the structure of the pattern as contained in the original image (or in the biological material).

This paper briefly reports a technique which we wish to explore on a greater quantity of biological samples. We are presently developing an improvement of such a method which automates the choice of windows G and C for any given biological image.

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References

1. Castleman, K.R., Wall, R.J., Nathan, R., "Automatic Karyotyping with pictorial output", JPL Internal Report, (1971).

2. Preston, K., Ingram, M.L., "Automatic analysis of blood cells", The Scientific American, (1970).

3. Haug, H., "Quantitative data in neuroanatomy", Progress in Brain Research, Vol. 33, Computers and Brains, (1970).

4. Prewitt, J.M.S., Picture processing and psychopictorics, 75,(1970) Academic Press, New York.

5. T.Golab, R.S.Ledley and L.S.Rotolo, "FIDAC - Film Input to Digital Automatic Computer", Patt. Rec. 1971, Vol.3, pp. 123-156.

6. Castleman, R.K., Weinstein, M., "Method and Means for Reconstructing 3-D Specimens from 2-D Sectional Images", JPL Techn. Rep. 30-11864 (1971).

7. L.Cordella, S.Levialdi, S. Vitulano, "Context Sensitive Restoration for Image Processing", Proc. of II Congresso di Cibernetica (1972), Casciana Terme, Italy.

8. F.W.Campbell, "The application of cybernetic principles in investigating the transmission of spatial information through the visual system of cats and man", Proc. of II Congresso di Cibernetica (1972), Casciana Terme, Italy.