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SHORT NOTE

AVERAGING OF LOW EXPOSURE ELECTRON MICROGRAPHS OF NON-PERIODIC OBJECTS

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The investigation concerns the possibility of extending to non-periodic objects the low exposure averaging techniques recently proposed for non-destructive electron microscopy of periodic biological objects. Two methods are discussed which are based on cross-correlation and are in principle suited for solving this problem.

1. Introduction

Recent work on low exposure techniques combined with averaging [1–3] (called 'SNAP shot techniques' in [3]) shows that information can be retrieved from periodic biological objects at higher than conventionally available resolutions [4]. Unwin and Henderson [2] were able to achieve 7 Å image resolution, by reducing the exposure to less than 1 electron/Å^2. Although a number of interesting biological materials exist in the native state as (or can be induced to form) periodic arrays suitable for study by electron microscopy, many others exist only as isolated units or in the form of disordered aggregates. We will investigate how the averaging techniques could be extended to this general case. Of all the possible irregular specimens which are being investigated in biological high resolution microscopy, we are interested in those which form identical particles, sufficiently well separated on the microscope grid so as not to overlap. In order to avoid any three-dimensional complications, we will assume that these particles are flat, with preferential attachment occurring on one side so that all projections in the direction of the axial electron beam are identical.

We note that averaging has been applied to dark field images of molecules labelled with heavy atoms and lying isolated on a carbon film, for removing the noise due to the structure of the supporting film [5, 6]. In these applications, the contrast of the individual marker atom image to be superposed is sufficient for straightforward alignment. However, the requirement of subminimum exposure poses a new problem: the alignment of features that are only faintly visible on a noisy background.

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2. Averaging of a motif repeated on an irregular lattice

2.1. Formulation of the problem

In order to consider this problem, we first assume an arrangement of repeats of a motif (e.g. a virus particle) where each repeat has the same orientation but appears at irregular positions $\boldsymbol{r}_j$. Such an arrangement can be denoted in convolution form:

$$i(\boldsymbol{r}) = m(\boldsymbol{r}) \circ g(\boldsymbol{r}) + n(\boldsymbol{r}) \,, \tag{1}$$

with the irregular point structure $g(\boldsymbol{r}) = \Sigma_{j=1}^{N} \delta(\boldsymbol{r} - \boldsymbol{r}_j)$, and an additive uncorrelated noise term. (For the limits of additive formulation, see ref. [7].)

The idea of SNAP techniques is that a large number of noisy repeats of $m(\boldsymbol{r})$ are averaged to form a noise-free representation of the motif. We immediately see the unique position of the periodic arrangement in that it forces a common orientation on all repeats, and that it makes them appear on a regular lattice, leaving alignment as a trivial problem. So trivial, indeed, that it does not even surface in the usual Fourier treatment [8,9] unless data from different experiments have to be combined, in which case a phase factor must be attached to the Fourier transforms.

In contrast, we have here to consider the position vectors r_j in (1) as unknowns which must be determined from the image.

2.2. Matched filtering

The general problem of accurate alignment of unperiodic patterns can be solved by cross-correlation techniques [7,10]; however, the present problem requires a special treatment.

By applying the idea of matched filtering [11], one could proceed to determine the lattice as follows. From theoretical considerations or some experimental evidence the most probable motif structure $m_0(r)$ ('prototype') may be known. Cross-correlation of $m_0(r)$ with the experimental image (1) gives

$$m_0(r) \otimes i(r) = [m_0(r) \otimes m(r)] \circ \sum_{j=1}^{N} \delta(r - r_j)\,, \qquad (2)$$

i.e. the cross-correlation function of the prototype with the motif $m_0 \otimes m$ appears in each position r_j. Ideally, if the prototype happened to be identical with the motif sought, this cross-correlation function would show a sharp peak, of halfwidth corresponding to the image resolution, indicating the position of the origin of $m(r)$ with respect to the position of each repeat of the motif with precision.

In practice, of course, any degree of knowledge about the motif structure is possible – from complete certainty to total ignorance. Generally, deviations of the motif from the prototype affect the correlation peak in two ways: first, the peak is reduced in height and second, it is blurred out since deviations occur most likely in the high resolution range. For unfortunate choices of the prototype pattern, the correlation peak may in fact be so broad and insignificant that the locations of the repeats cannot be established. To give an extreme example, it will not be of much help to know that the motif is circular with approximate radius R, because the auto-correlation between two circular discs of that radius is a function whose halfwidth is in the order of $2R$.

Once the lattice is established, the noisy image is superposed N-times on itself, shifted by r_j, and the average is computed. The average of the repeats is an enhanced version of the motif structure, whereas the average of the noise portion levels out to a constant background term if N is sufficiently large. To be accurate, the condition for incoherent noise superposition is that the lengths of the lattice vectors r_j and all possible difference vectors $r_j - r_k$ are larger than the auto-correlation radius of the noise function.

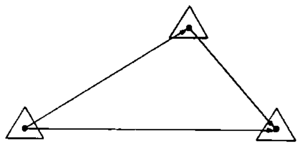

Fig. 1a. Simple structure consisting of three repeats of a triangular motif. Underlying point structure marked by arrows.

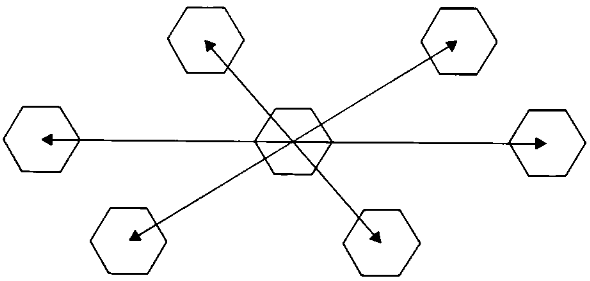

Fig. 1b. Patterson function of the structure in a.

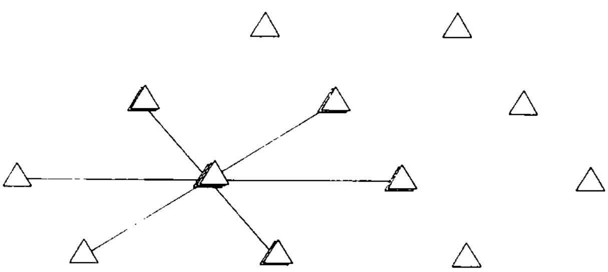

Fig. 1c. The structure a is placed on each point of the Patterson function of its point lattice (endpoints of arrows in b). The number of overlaps in each position is schematically indicated. The "reconstruction" is formed at the centre of the Patterson function, in this case by superposition of three repeats.

2.3. Auto-correlation superposition

The other approach follows from the idea that the repeats present in (1) can act as their own prototypes. In the simple case where the signal-to-noise ratio is sufficiently large, the repeats of the motif can be approximately located in the image by eye. An image area containing a single repeat is then selected and cross-correlated against the whole image. The result is essentially the same as in (2): the autocorrelation function appears superposed on all lattice positions and its peak thus marks the lattice.

We now assume the more complicated case where, due to low signal-to-noise ratio, the repeats cannot be clearly distinguished from the noise background.

By auto-correlating the image one obtains

$$i(\boldsymbol{r}) \otimes i(\boldsymbol{r}) = [m(\boldsymbol{r}) \otimes m(\boldsymbol{r})] \circ [g(\boldsymbol{r}) \otimes g(\boldsymbol{r})] + n(\boldsymbol{r}) \otimes n(\boldsymbol{r}) . \qquad (3)$$

Here the cross-terms between noise and signal are omitted because of the assumption of uncorrelated noise made at the beginning. Apart from the noise auto-correlation $n \otimes n$ appearing in the centre, this is a convolution product of the auto-correlation function of the motif with that of the irregular lattice $g(\boldsymbol{r})$. Following the practice of X-ray structure analysis, we call the auto-correlation function of the point structure 'Patterson function' [12]. We have here the complication that $m \otimes m$ appears repeated on the Patterson function of the lattice rather than on the lattice itself. This function contains points at each possible difference position $\boldsymbol{r}_{jk} = \boldsymbol{r}_j - \boldsymbol{r}_k$ (cf. fig. 1a, b). For an irregular lattice, the number of these points is $N(N-1)+1$ if one counts the multiple zero difference vector $j = k$ only once and observes that each non-zero difference vector appears twice, with opposite directions. The proposal is now that the image should be superposed $N(N-1)+1$ times on itself, shifted by the Patterson vectors found in (3).

The resulting pattern (fig. 1c) will contain single overlaps lying on one half of the Patterson function, and a number of repeats without overlap at the periphery. However, most interesting for our purpose is the fact that exactly N overlaps occur at the origin of the Patterson function, with each position of the irregular lattice contributing only once, so that here the desired average version of the motif is built up. Another way of looking at this result is the following: since the Patterson function of the lattice contains the lattice itself, shifting by all of its vectors assures that all lattice points come to an overlap at one point. Apart from the 'signal' position, our reconstruction contains a noise portion from $N(N-2)+1$ unsuccessful overlaps which tends to be levelled out for large N.

One difficulty has to be mentioned here*: with an increasing number of repeats, the number of difference vectors in the irregular point structure increases, and so does the number of Patterson vectors. As a result, the auto-correlation discs which according to (3) are centred on the end points of these vectors will with increasing probability come to a partial overlap, thus obscuring the Patterson function sought and preventing accurate superposition. One can show by simple geometrical considerations that overlap will be unlikely if the extension of the selected field is large compared to that of a single particle.

This second approach therefore seems to solve the problem of averaging over N noisy occurrences of a motif on an irregular lattice in a general way, although the assumption of equal orientation made at the beginning is a most unsatisfactory restriction. Nevertheless, we may have situations where biological structures appear in irregular intervals in a background matrix with preferred orientation, or where a crystalline supporting film favours a distinct orientation of the support particles.

2.4. The case of arbitrary relative orientation

Following is an outline for a strategy that may be successful in the case of arbitrary relative orientation. The approximate location of the repeats can be found either by inspection or by application of matched filtering with a circularly symmetric prototype function that has the expected radius and radial distribution of the motif. One repeat so located is then rotationally correlated with each of the others. Since the exact position of the origins is unknown at this stage, rotational correlation has to be done by using translation-invariant methods [10,13]. Once the relative orientations of the $N-1$ repeats is found with respect to the reference repeat, these can be rotated so that an image with oriented repeats is produced, which can then be treated by the methods outlined above.

3. Conclusion

As in the case of periodic objects, averaging can be used to build up a high resolution image from noise-

* I would like to thank Prof. W. Hoppe for drawing my attention to this point.

contaminated repeats of a structure lying in random positions but common orientation. Non-destructive electron microscopy which has recently proved successful in the determination of an ordered membrane structure [2] can therefore in principle be extended to biological material in disordered form. The main complication introduced by the random positions is that they are not revealed by Fourier transformation, but have to be determined prior to the averaging procedure. Two methods have been discussed in this context: matched filtering and a new method, called auto-correlation superposition.

While matched filtering requires a certain amount of a-priori knowledge about the structure to be located, the other method makes no such assumption, and entirely relies on the correlation between any pair of repeats.

The question of noise sensitivity which has not been answered here makes a detailed theoretical investigation of the effects of size, shape, resolution, signal-to-noise ratio and nature of the noise on the position-finding algorithms necessary. This will be done in a later study. It is already obvious, however, that the exposure reduction that can be achieved in the case of non-periodic specimens is limited by the requirement of some high-resolution similarity between any pair of repeats which is necessary for accurate positioning, whereas no similar limitation seems to exist for periodic specimens.

As a concluding note to interested biologists, it would be desirable to know at this stage if some interesting biological material occurs in disordered yet oriented form, or can be brought into this form by the use of special supporting films, since this would greatly affect the motivation for extended theoretical studies on these lines.

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