

Automatic Particle Picking From Electron Micrographs

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The present-day interactive manual selection of biological molecules from digitized micrographs for single particle averaging and reconstruction requires substantial effort and time. Thus a computer algorithm capable of recognition of structural content and selection of particles would be desirable. A few approaches¹⁻³ have been proposed in the past. The method by Frank and Wagenknecht¹ is based on the principle of correlation search. Van Heel's method² is based on the computation of the local variance over a small area around each point of the image field. The method by Harauz and Fong-Lochovsky³ is based on edge-detection. The present work was focussed on the detection and classification of particles by exploiting the standard statistical methods of discriminant analysis.

the proposed technique is described in the block diagram (Fig. 1). As illustrated in the figure, the program consists of three distinct segments devoted to, respectively, preparation of the data, training session and automatic selection based on a discriminant function set up in the training. In the data preparation segment, the micrograph is (i) reduced four-fold in size, (ii) low-pass filtered and (iii) run through a peak search algorithm. In the training session, the coordinates of the peak positions are used to window out small images. Manual interaction is mandatory at this point to select comparable numbers of images with particles, noise and "junk" (i.e., particle fragments, stain accumulations, etc.) from the above windowed series. Statistical parameters (i.e., average, variance, skewness, and entropy) evaluated for these selected images serve as input variables to the discriminant function routine which yields the discriminant function and classifies the image set into the three possible categories (viz. particles, noise

and "junk"). In the selection segment of the program, the resulting discriminant function is used to distinguish among the different groups. Only images containing genuine particles as determined by the discriminant analysis are selected from the original unreduced micrograph. Optionally, one can window out the particles from the tilted micrograph simultaneously, as required by the random conical three-dimensional reconstruction of molecules⁴.

This method works very well as long as the particles in the input image are isolated and not clustered together. The program was tested on the 70S *E. coli* ribosome data⁵. When using micrograph pairs with typically about 200-300 particles, it required approximately half an hour for the data preparation, 1.5-2.0 hours for the training and about 1.5 hours for the selection segments of the program, respectively. The precise time depends on the rejection rate. The times quoted here are all wall clock times on a VAX Workstation. For comparison, we mention here the average time taken for manual picking of particles from a similar pair which is about 6 hours. In typical reconstructions, one needs around 1500-2000 particles which would correspond to about ten micrograph pairs. Under such circumstances, this program is very effective since the training needs to be done only once for data of the same type, and the preparation and selection together take approximately a third of the time compared to manual picking⁶.

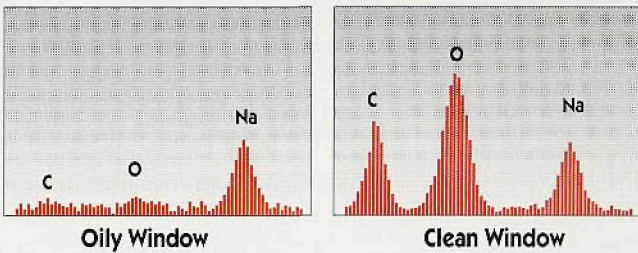
1. J. Frank and T. Wagenknecht, *Ultramicroscopy*. 12(1984)169.
2. M. van Heel, *Ultramicroscopy*. 8(1982)331.
3. G. Harauz and A. Fong-Lochovsky, *Ultramicroscopy*. 31(1989)333.
4. M. Rademacher, *J. Electron Microsc. Tech.* 9(1988)359.
5. P. Penczek, R.A. Grassucci and J. Frank, *Ultramicroscopy*. (1994, in press).
6. Supported, in part, by grant NIH 1R01GM29169 (to J.F.)

Reprinted from *Proceedings of the 52nd Annual Meeting of the Microscopy Society of America*.

https://doi.org/10.1017/S1551929500063203 Published online by Cambridge University Press

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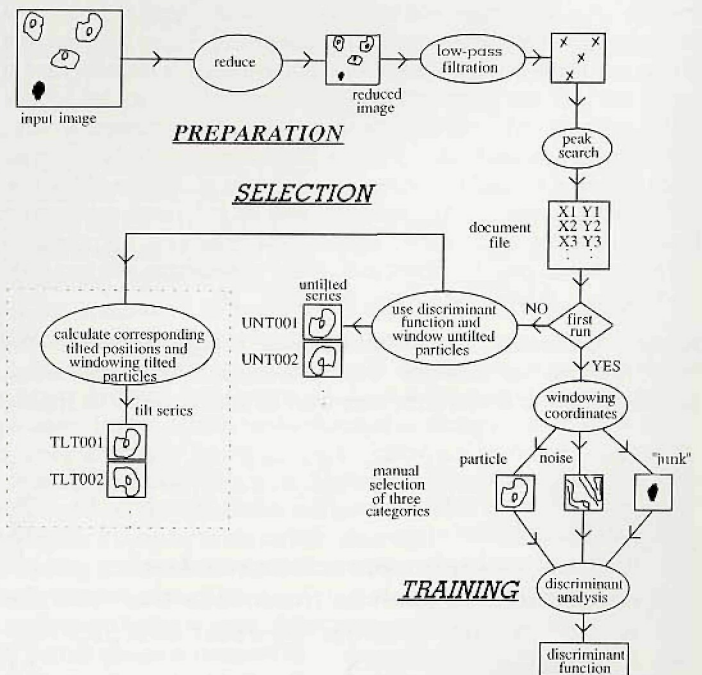
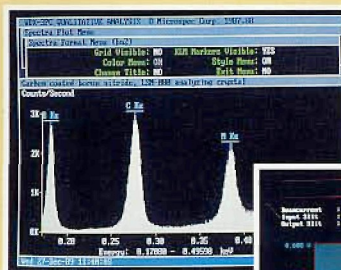


Fig. 1: Block diagram showing the sequence of operations in automatic particle selection. The oval shapes refer to operations performed. The operations within the box drawn within the box drawn in dashed lines are optional

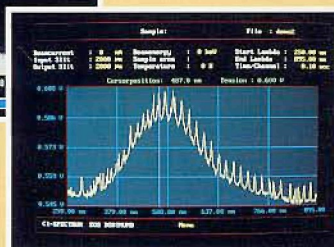
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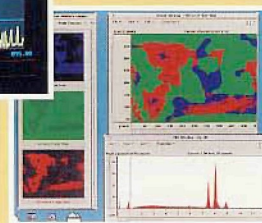
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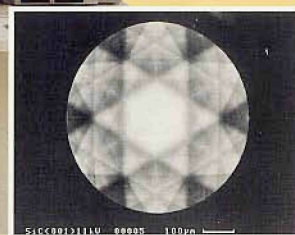
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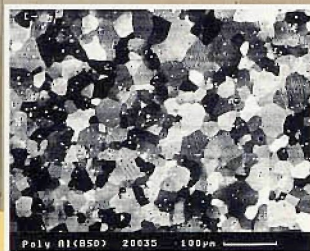
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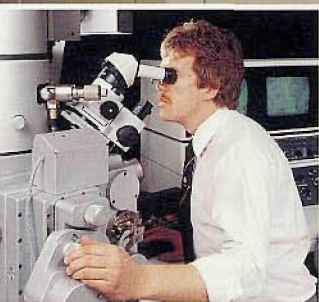
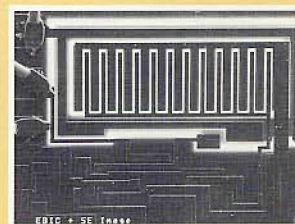
● SAD



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● Absorbed current (AEI)



● Optical

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