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An improved Automatic Virus particle Detection method based on adaptive K-NN classifier

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Abstract: Several automatic and semi-automatic particle detection algorithms have been developed along the years. Here we present a general technique designed to automatically identify the projection images of particles New methods are described that should facilitate high-resolution (5–10 Å) image reconstructions from low-dose, low-contrast electron micrographs and processing of large, digital images produced by new imaging devices and modern electron microscopes. Existing techniques for automatic selection of images of individual biological macromolecules from electron micrographs are inefficient or unreliable. The initial detection of the particles takes place through automatic segmentation of the entropy-proportion image; this image is computed in particular regions of interest defined by two concentric structuring elements contained in a small overlapping window running over the entire image. Morphological features help to select the candidates, as the threshold is kept low enough to avoid false negatives. The candidate points are subject to a credibility test based on features extracted from eight radial intensity profiles in each point from a texture image and Gabor image. For candidate selection use high level Adaptive K-Nearest neighbor Classifier. It's improving the accuracy of candidate selection with the need of low level set features.

Keywords: Adenovirus, Automatic detection, automatic particle selection, electron microscopy images, icosahedral particles, segmentation.

I. INTRODUCTION

The fully automatic selection of biological particles in electron microscopy images is becoming a "holygrail" in image processing, because of the large numbers of particle views that are needed to perform high resolution three dimensional (3D) reconstruction at the ultrastructural level; at, Glaeser points that up to 100.000 particles are needed to achieve a resolution better than 1 nm. This becomes a bottleneck to the procedure, because of the time-expensive human labor involved. Interactive methods can still solve difficult situations, but the need for automatic methods with efficiency better than 75% compared to an expert human observer is the main goal. At a first glance, it's just a classic problem of image segmentation, a challenge that can be trivialor almost impossible, depending on the image and objects of interest, and of its radiometric and geometric characteristics. Three approaches remain relevant: the texture-based method, with an interactive training phase to select data windows representative of three categories — particle, noise and junk — characterized by 8 features plus an estimate of the particle area. These features enable a linear maximum- likelihood discriminant analysis for further classification of other candidate data windows: a supervised classification in a 9-dimensional space of the candidates (located by convolution of the decimated image with a Gaussian of width related to the particle size and a peak search algorithm with specific constraints).

II. LITERATURE SURVEY

1.Model-based particle picking For cryo-electron microscopy
H. Chi Wong ,Jindong (JD) Chen ,Fabrice Mouche ,Isabelle Rouiller

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In this paper, we propose an algorithm for finding particle images in cryo-EM micrographs. The algorithm starts from a crude 3Dmap of the target particle, computed from a relatively small number of manually picked images, and then projects the map in many different directions to give synthetic 2D templates. The templates are clustered and averaged and then cross-correlated with the micrographs.

Disadvantages:
Less specificity.

2.Review: Automatic Particle Detection in Electron Microscopy
William V. Nicholson and Robert M. Glaeser

This paper describes various approaches that have been developed to address the problem of automatic particle selection. The approaches that we cover in this paper include methods that make use of various forms of template matching, local comparison of intensity values, edge detection, quantitative measures of the local image texture/statistics, and neural networks.

Disadvantages:
High Complexity.

3.Three-Dimensional Reconstruction of Icosahedral
Particles—The Uncommon Line
S. D. Fuller and S. J. Butcher

This paper describes the principles behind the most commonly used method of reconstruction of the icosahedral particles. This paper focuses upon a combination of the high-fidelity but low-contrast information contained in these images with efficient algorithms for determining particle orientation and three-dimensional structure. Modifications to the icosahedral reconstruction programs to allow their use for cryoelectron micrographs is also proposed.

Disadvantages:
Lower resolution.

4.Identification of Spherical Virus Particles in Digitized Images of Entire Electron Micrographs
Ioana M. Boier Martin, Dan C. Marinescu, Robert E. Lynch

In this paper we propose the Crosspoint method (CP). Two algorithms for processing large images, one based on image sub sampling, the other on image decomposition, are also proposed. A large image is first compressed and the CP method is applied to the compressed image to produce an initial solution. The information gathered at this stage is used to cut the original image into sub-images and then to refine the particle coordinates in each sub image. An interactive environment for experimenting with particle identification methods is also described.

Disadvantages:
1. Less Specificity.
2. Not efficient.

5.Automatic particle picking using diffusion filtering and random forest classification
Paul Joubert, Stephan Nickel, Florian Beck, Michael Habeck

An automatic particle picking algorithm for processing electron micrographs of a large molecular complex, the 26S proteasome, is described. The algorithm makes use of a coherence enhancing diffusion filter to denoise the data, and a random forest classifier for removing false positives. It uses a training set of manually picked particles. The algorithm consists of the following steps: Segmentation, Normalization, Denoising, Picking, Alignment ,Classification.

Disadvantages:

Not effective.

6. Detecting circular and rectangular particles based on geometric feature detection in electron micrographs
Zeyun Yu and Chandrajit Bajaj

In this paper, we present a method for particle picking based on shape feature detection. Two fundamental concepts of computational geometry, namely, the distance transform and the Voronoi diagram, are used for detection of critical features as well as for location of particles from the images or micrographs. Our approach detects the particles based on their boundary features. The geometric features derived from the boundaries provide a way for locating particles. Our approach is automatic and has been applied to detect particles with approximately circular or rectangular shapes.

Disadvantages:
Less specificity.

7. Identification and classification of human cytomegalovirus capsids in textured electron micrographs using deformed template matching
Martin Ryner, Jan Olov Stromberg and Mohammed Homman Loudiyi

In this project, we have developed a procedure for describing and classifying virus particle forms in electron micrographs, based on determination of the invariant characteristics of the projection of a given virus structure. The template for the virus particle is created on the basis of information obtained from a small training set of electron micrographs and is then employed to classify and quantify similar structures of interest in an unlimited number of electron micrographs by a process of correlation.

Disadvantages:
Less accuracy.

8. An approach to automated particle picking from electron micrographs based on reduced representation templates.

N. Volkmann

Reduced representation templates are used in a real-space pattern matching framework to facilitate automatic particle picking from electron micrographs. Our approach consists of five parts. First, reduced templates are constructed either from models or directly from the data. Second, a real-space pattern matching algorithm is applied using the reduced representations as templates. Third, peaks are selected from the resulting score map using peak-shape characteristics. Fourth, the surviving peaks are tested for distance constraints. Fifth, a correlation-based outlier screening is applied.

Disadvantages:
Not robust and reliable.

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9. A binary segmentation approach for boxing ribosome particles in cryo EM micrographs.

Adiga P.S, Malladi. R, W.Baxter, Glaeser R.M.

This paper describes an approach for automated boxing of ribosome particles in micrographs. Use of a fast, anisotropic non-linear reaction-diffusion method to pre-process micrographs and rank-leveling to enhance the contrast between particles and the background, followed by binary and morphological segmentation constitute the core of this technique.

Disadvantages:
Less accuracy

10. Segmentation of virus particle candidates in transmission electron microscopy images.

G. Kylberg, M. Uppstrom, K.O. Hedlund

In this paper, we present an automatic segmentation method that detects virus particles of various shapes in transmission electron microscopy images. The method is based on a statistical analysis of local neighbourhoods of all the pixels in the image followed by an object width discrimination and finally, for elongated objects, a border refinement step. It requires only one input parameter, the approximate width of the virus particles searched for.

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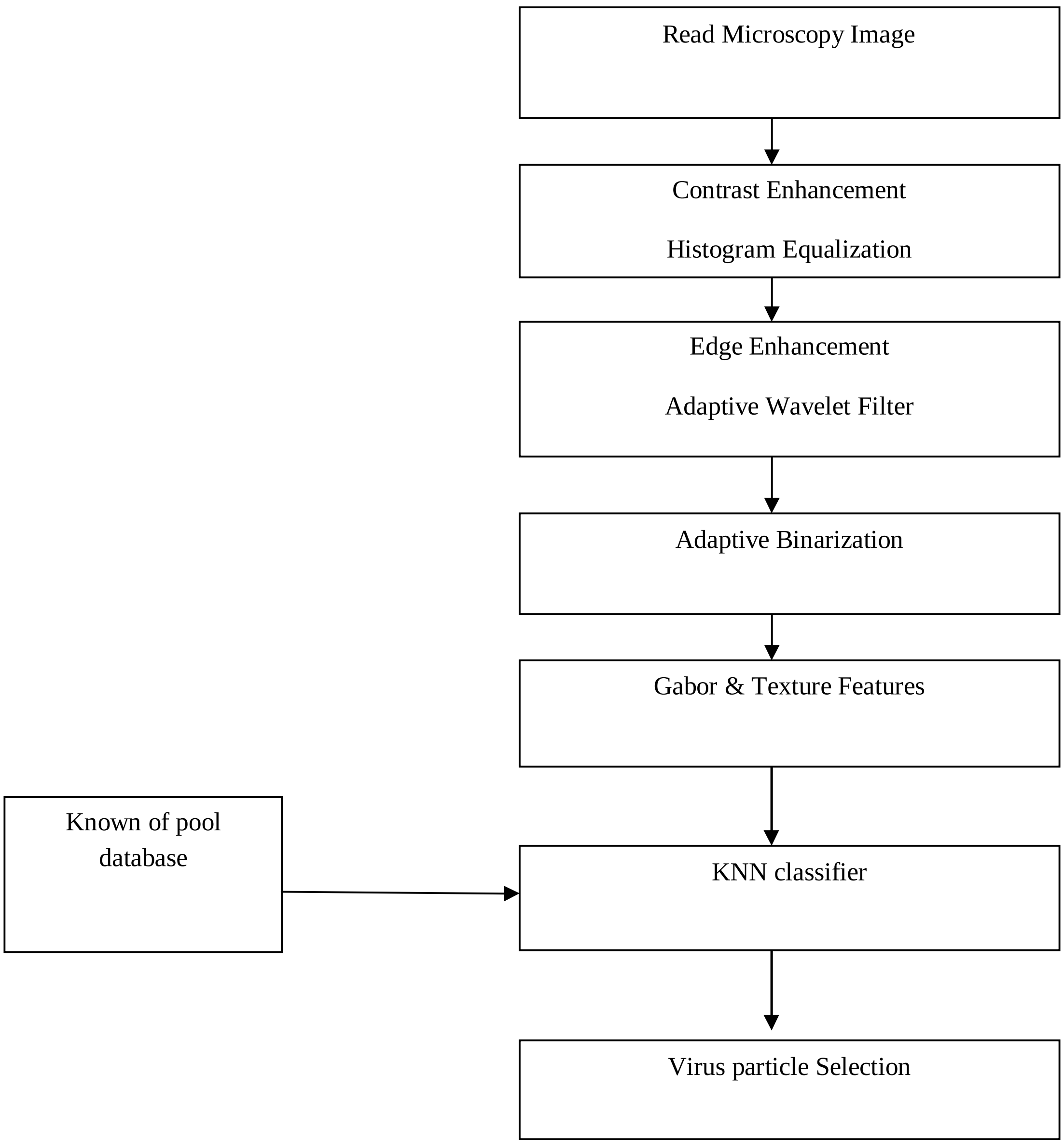
III. BLOCK DIAGRAM



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1. Preprocessing
2. Entropy adaptive threshold
3. Gabor Feature Extraction
4. KNN Classifier

IV. EXISTING SYSTEM & PROPOSED SYSTEM

Existing system:

The texture-based method, with an interactive training phase to select data windows representative of three categories — particle, noise and junk — characterized by 8 features plus an estimate of the particle area. These features enable a linear maximum-likelihood discriminant analysis for further classification of other candidate data windows: a supervised classification in a 9-dimensional space of the candidates (located by convolution of the decimated image with a Gaussian of width related to the particle size and a peak search algorithm with specific constraints).

The cross-point method developed for spherical virus particles, assumes that there is a constant relation between the intensity levels of the pixels inside a particle and the pixels in the background, and explores that relation. Post refinement is achieved by correlation with a model particle built as an average of all the particles detected.

Spherical/icosahedral particles have also been detected by the local average intensity method, an automatic method that locates the initial point candidates comparing the average intensity value in a particle-sized circle with the average in a ring around that circle, and keeping the maxima of that ratio in each square of image with diagonal equivalent to the particle radius; in a second phase, these candidates are evaluated according a set of rules relating the intensities in 8 sectors of the circle and the corresponding sectors in the external ring, reduced to half of the initial thickness. A final pruning limits the possibility of duplicated selection of the same particle in two adjacent squares.

Disadvantage:

Low performance.

Proposed system:

This paper describes a fully automatic approach to locate icosahedral virus particles in transmission electron microscopy images. The initial detection of the particles takes place through automatic segmentation of the entropy-proportion image; this image is computed in particular regions of interest defined by two concentric structuring elements contained in a small overlapping window running over all the image. Morphological features help to select the candidates, as the threshold is kept low enough to avoid false negatives. The candidate points are subject to a credibility test based on features extracted from eight radial intensity profiles in each point from a texture image. A candidate is accepted if these features meet the set of acceptance conditions describing the typical intensity profiles of these kinds of particles.

Advantage:

1. High performance.
2. Presents a low level of initial false positives.

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MODULES

Module description:

1. Preprocessing:

The pre-processing aimed to smooth the radiometric fluctuations inside the particles and to enhance the borders. The background is compensated for irregular brightness with a morphological opening with a disk structuring element of radius 31 pixels, followed by subtraction of the opened image from the original and gray scale adjustment — 1% of the data was saturated at low and high intensities. Pre-processing with a wavelet filter, consisting in decomposition (using a Daubechies wavelet of support of order 11) followed by reconstruction with the details of first level suppressed, provided a smooth filter of local spikes, followed by a contrast-limited adaptive histogram equalization to an exponential function with parameter alpha set to 0.95 in 8 × 8 tiles. A pixel wise adaptive Wiener filtering using neighborhoods of size 11× 11, and final normalization.

2. Entropy adaptive threshold:

A moving data window was displaced over the image with a pre-defined step (5 pixels, in this case) and two entropy values were computed for each window emplacement, giving a local value of the entropy proportion. The local entropy-proportion computation uses two structuring elements, depending on three values, which were set to $R = 45$, $r_{int} = 31$ end $r_{ext} = 37$. The threshold of the minima of the entropy-proportion image is a function of the content of the output image: 7% of the minima are retained and filtered according to morphological criteria — only small areas and high eccentricity ensure the compact spike typical of the coincidence of the set up with a low entropy area.

3. Gabor Feature extraction:

Four intensity profiles — vertical, horizontal and both diagonals — were extracted at each candidate point from the texture image. The features extracted were evaluated against a set of acceptance conditions defined with a set of test images acquired in the same conditions. These include the distributions admissible for the height of the profiles, number of standard widths demanded, and number of intersections admitted, impositions on symmetry and absence of incomplete profiles that must be fulfilled by a candidate point to become an accepted point.

4. KNN Classifier:

The set of candidate points may include false positives and may be losing interesting points, considered as false negatives; the threshold used to locate the regional minima must target a low number of false negatives, at expenses of a high number of false positives, because the erroneous candidates can be dismissed with an evaluation of its neighborhood based in previous knowledge. This evaluation was achieved in a texture image built with a standard deviation filter using a 3D structuring element on a pre-processed input image.

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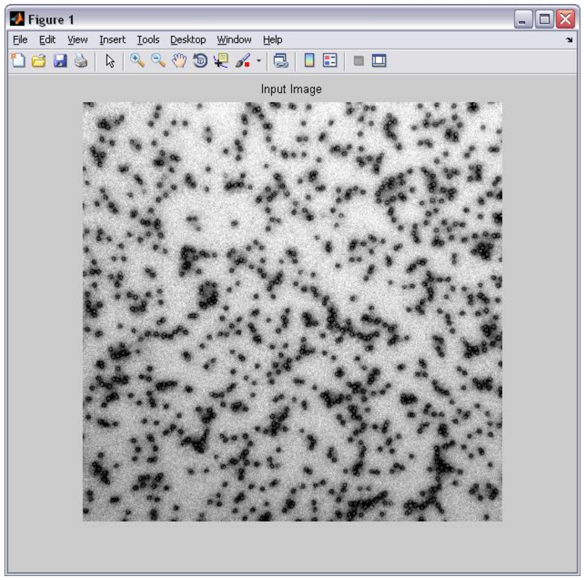
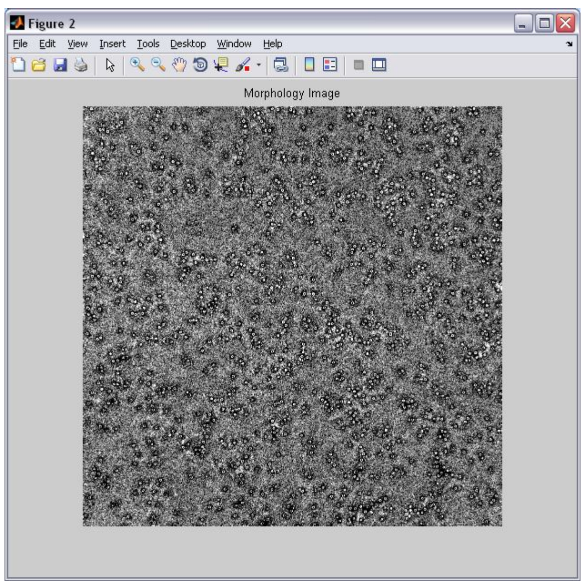
V. RESULT





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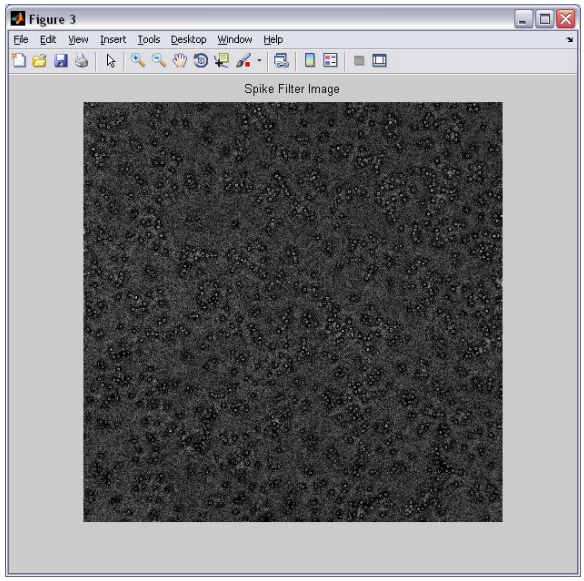

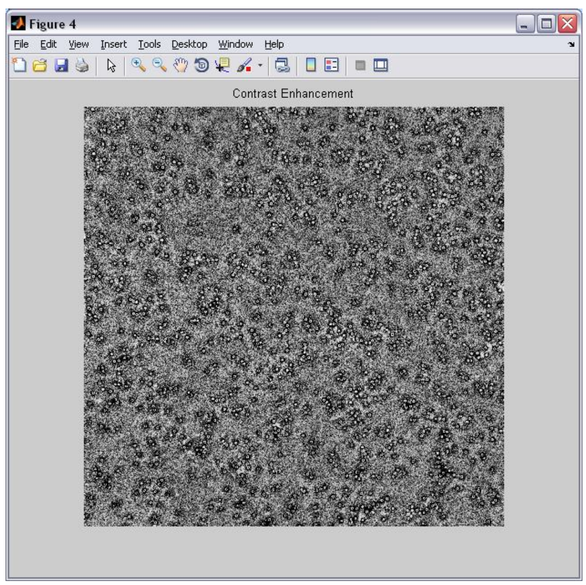

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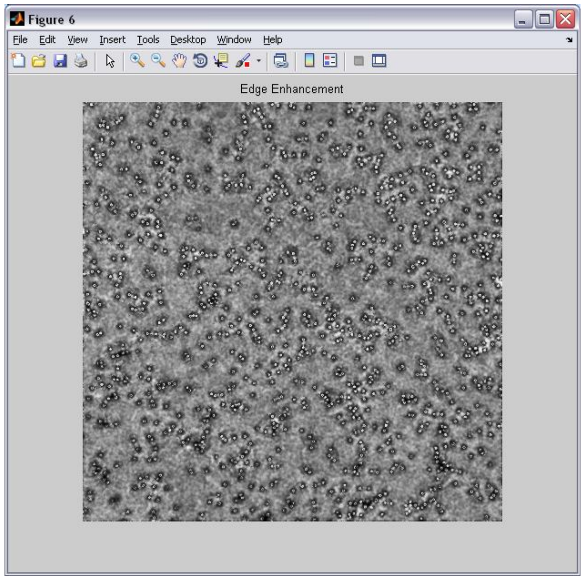

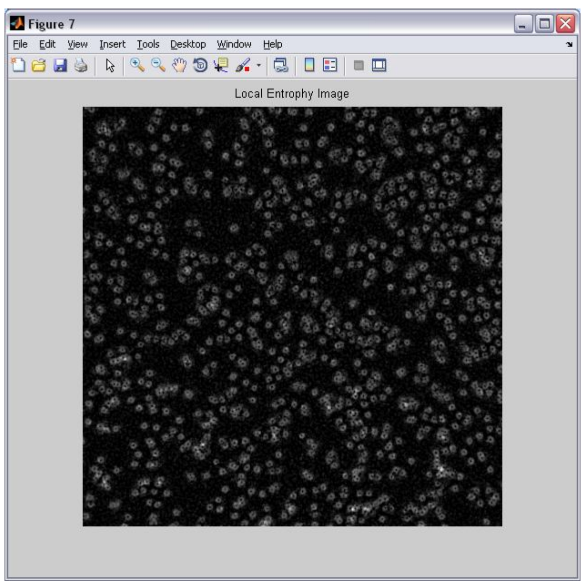

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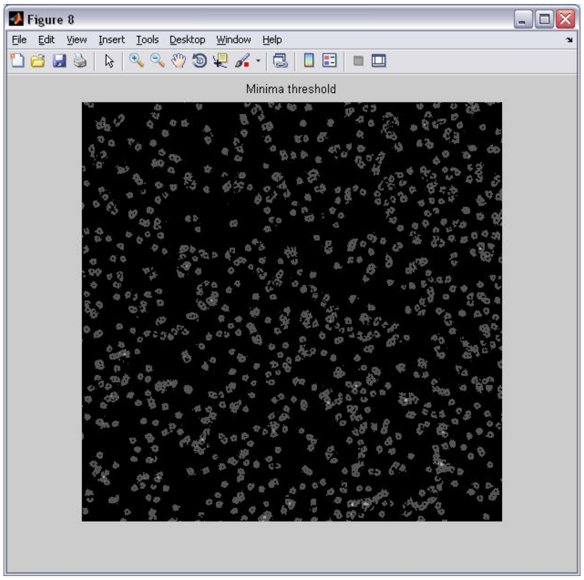

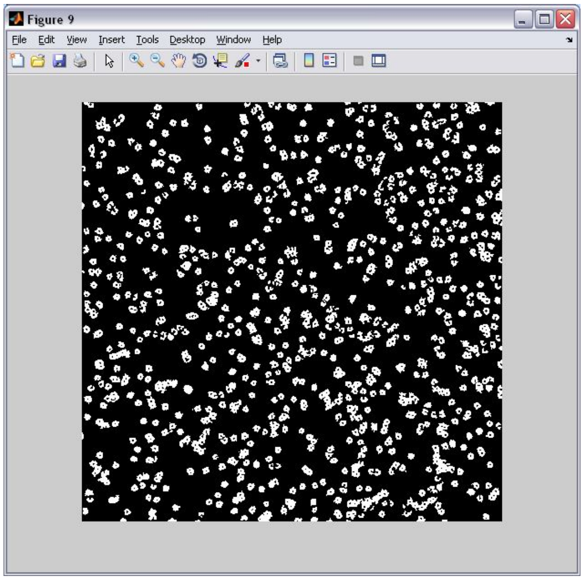

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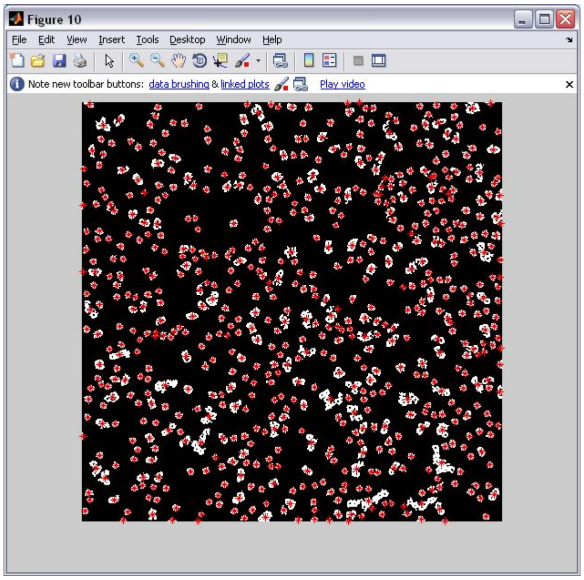

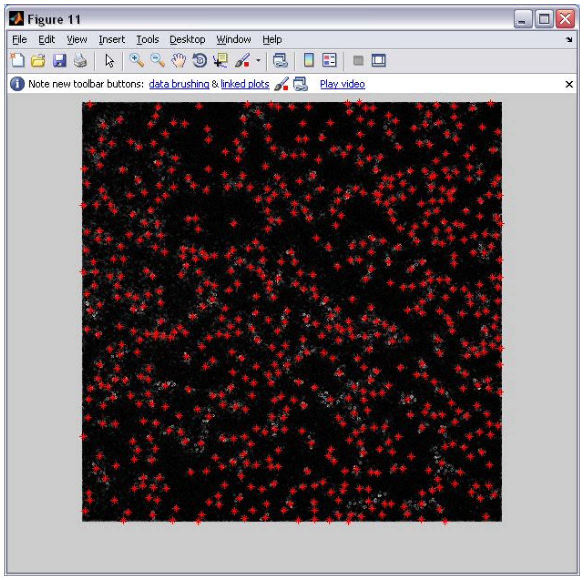

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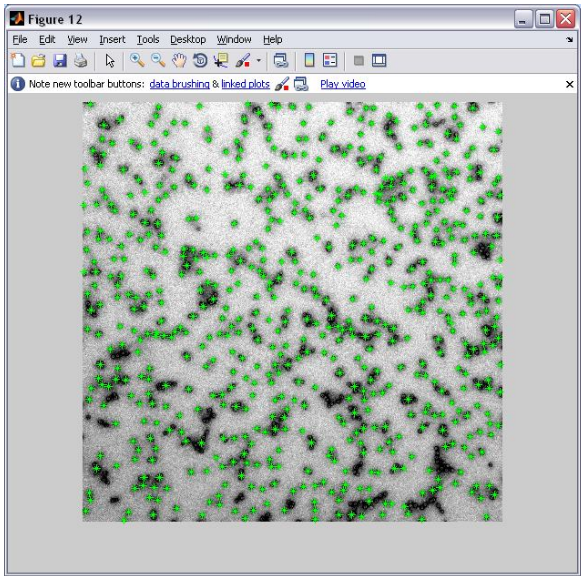

VI. CONCLUSION.

The basics for the entropy approach remains valid as long as an area of low entropy can be associated with the object of interest. The detection of the minima in the entropy proportion image can be achieved by many methods, like a peak search algorithm applied to the complement of the image or any other current method; the use of a threshold dependency of the image characteristics aims to simplify this step of the process, and enables the subsequent selection by morphological char- acteristics of the objects retained.

The evaluation of credibility of the candidates is a function of the particle dimensions, as the profiles range should match the average size of the particle in order to maximize the probability of a correct localization of the borders. The minimum and maximum widths accepted for a profile, and the standard width used as reference in one feature are also a function of the dimensions of the particle; the same kind of dependence is observed in other algorithms. The set of acceptance conditions may be used for any particles with the same radiometric and geometric characteristics.

A comparison with other procedures previously applied to the same kind of images would be possible if the same benchmark datasets and performance metrics were used. Concerning adenovirus, Kylberg et al. report 259 correctly segmented particles in a total of 287 virus particles in 67 images, which represents a sensitivity (defined as the ratio between correctly segmented particles and total number of virus particles) of 0.90. Our results consider the type of particle observed, so a comparison cannot take place without the knowledge of which class of particles were included. In the entropy approach, the balance of false positive and false negative classifications was restricted to very doubtful particles like the possible ones existing in the central zone of Fig. 2; the strongly damaged capsids may or may not be viral particles, depending on the expert's subjectivity. Undamaged unquestionable particles could be clearly identified by this approach, making it a promising tool for massive preliminary screening by unspecialized observers.

In terms of methodology, the entropy approach proposed here presents a low level of initial false positives, inherent to the method; as false positives seems to be a major drawback in many algorithms used for unstained images, we think this approach can be useful as a detection step in a hybrid procedure for cryo-EM images.

As future directions of work, we plan to integrate the entropy approach in a more general procedure to be applied to unstained samples of low contrast, using the publicly available datasets. This should allow for an enlarged comparison of the performance and a measurement of the potential of the proposed approach in one of the most chal- lenging fields of image processing.

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