Automatic Acquisition and Image Analysis of 2D Crystals

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2D-crystallization in combination with transmission electron microscopy (TEM) is one of the few methods for the structural analysis of membrane proteins in their native state. However, the parameters for the generation of large crystalline sheets are typically difficult to identify for a given protein. Many repetitive and time consuming screening steps by TEM are therefore necessary to find the best crystallization and preparation conditions [1, 2]. Although several software packages offer the possibility to control an electron microscope, none is completely adapted for a fully automated and completely integrated acquisition and analysis of 2D crystals. Here we report on the development of a fully automatic screening and on-line analysis software for the fast and automatic survey of large quantities of negatively stained EM samples for 2D crystallography.

The software system designed to achieve our objectives is built of two parts: the computer control of the TEM and the on-line image characterization. The control of the microscope (Tecnai F30 'Polara', FEI Company, Eindhoven, The Netherlands, equipped with a Gatan Imaging Filter and a 2048x2048 CCD camera, 30 µm pixel size) is based on the acquisition of images using the TOM toolbox, a home-made software package utilizing the scientific computing platform Matlab (The MathWorks, Natick, USA) [3]. The image processing tools create an on-line analysis of the images to characterize the sample. An optimized screening process is guided by the analysis outcome simulating the decision of a microscopist. During this process the crystallinity of the sample is evaluated. Figure 1 shows the interplay of the two parts during the three-step process described below. Crystal quality assessment can be done directly using the diffraction mode of the microscope or by calculating power spectra of images at high magnification. However, screening at high magnification is very inefficient since the abundance of suitable 2D crystals can be low and the field of view (FOV) at that magnification (typically less than 1 μm^2) is small compared to the entire grid dimensions (Ø ~3 mm) and the maximal FOV of ~ 2 mm^2. Therefore, two intermediate steps at lower magnifications are required for the complete automation process for the grid quality assessment and the identification of suitable crystals (a segmentation process of images at medium magnification is presented in [4]).

First, at very low magnification (e.g. 175 ×), an area as large as covering 1.96 mm^2 of the grid is screened and the resulting map is processed for an initial quality evaluation (*i.e.* broken carbon film). The position of the suitable mesh squares are extracted by a local histogram analysis method and stored within the program. This step is essential to avoid spending too much time in further acquisitions on regions where there is no information: on the opaque copper grid, and on regions where the carbon film upon which the specimen is placed is damaged. The microscope is then directed to the determined locations for a further analysis at medium magnification (*e.g.* ~ 3,000x). After automatic adjustments (auto-focusing, auto-eucentric height) at this magnification, several images per

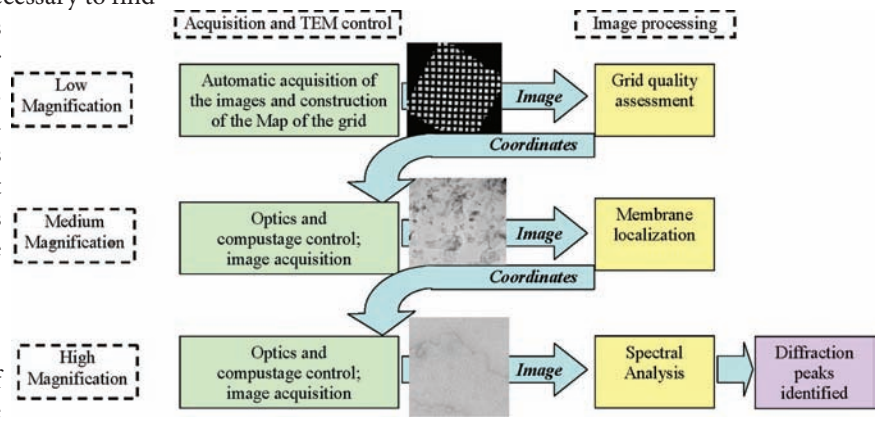

Fig. 1: Automatic control of a TEM for 2D-crystallization evaluation. The diagram describes the interaction between acquisition and TEM control and the image processing tools during the three-step process.

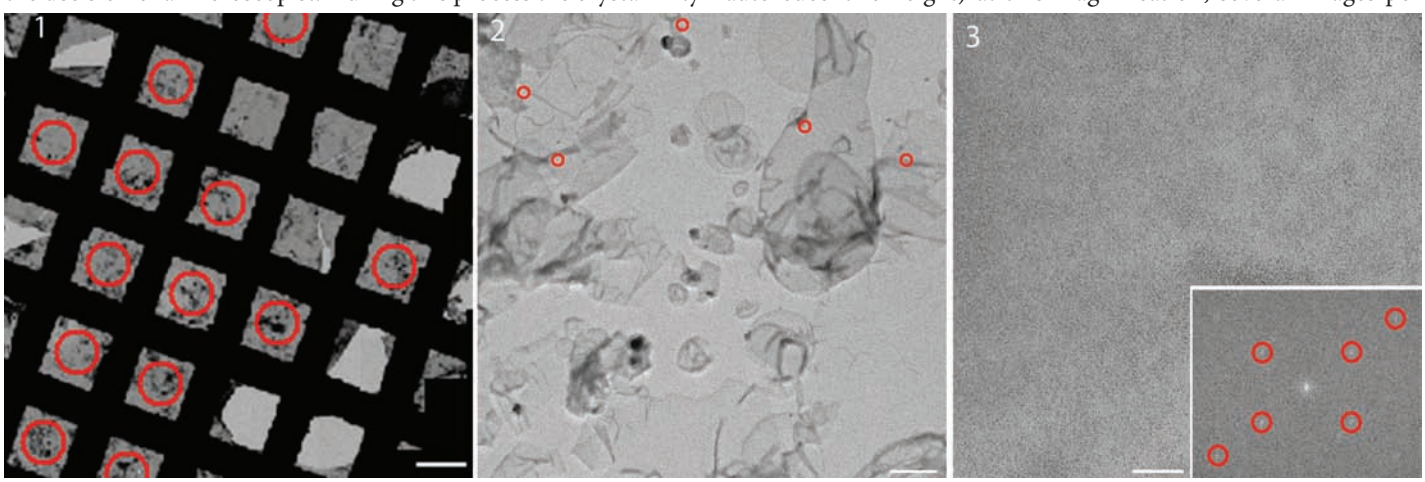

Fig 2: Automated screening micrographs. (1) Map of the grid at low magnification with circles showing the selected squares (scale bar: 20 µm). (2) Image of crystal sheets at medium magnification (scale bar: 1 µm). Circles indicate potential areas for further analysis at higher magnification. (3) High magnified image (scale bar: 10 nm) of such an area and corresponding power spectrum (inset) with the identified diffraction peaks (circles).

grid-square are acquired and analyzed with an edge-based algorithm to identify potentially crystallized areas. Finally, micrographs of these areas are acquired at higher magnification (*e.g.* ~ 50,000x). To unmistakably identify crystals and to validate the success of the crystallization approach, power spectra of the images are computed for a subsequent peak extraction.

Experiments have been conducted on SoPIP2;1, a plant aquaporin recombinantely expressed in *Pichia pastoris* [5], and the results are displayed in Figure 2. Results validate the ability of this method to identify crystal in a fully automatic manner. ■

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