APPARATUS AND METHOD FOR PROVIDING HIGH FIDELITY RECONSTRUCTION OF AN OBSERVED SAMPLE

This invention relates to an apparatus and a method for high fidelity reconstruction of an observed sample and for visual reconstruction of the data.

DEFINITIONS AND ABBREVIATIONS

EM—electron microscopy

ET—electron tomography

CL—common lines method

$F(\overline{X})$—density values at points (x,y,z) in a 3D reconstruction

$m(\overline{X})$—a prior prejudice distribution

C—A chi-squared statistic—a sum of squared differences between a projected 3D reconstruction and an observed projection divided by a measured variance of the observations.

$W(\overline{X})$—an interpolation used for convolution operation given by the fact that a 3D density is built on a 3D grid, said grid values defining a continuous function through a trilinear interpolation and decreasing the resolution somewhat because of decreased bandwidth.

$P^{(i)}$—a projection operation to the ith view

a pupil function based on the deflection of electrons by the iris diaphragm.

CTF—a contrast transfer function

PSF—a point spread function by which each projected image must be subjected to convolution. PSF in Fourier space is the pupil function * CTF.

$T^{(i)}(\overline{X},\overline{x}^{(i)})$—a smearing function, based on the projected PSF

$O^{(i)}(\overline{X},\overline{Y},\overline{x}^{(i)})$—an overlap function

*—multiplication

$\otimes$—convolution

INTRODUCTION

In all data acquisition and signal processing systems resolution is a key factor. The degree of resolution is a direct measure of the size of the smallest detail that can be reproduced. The higher the resolution the better the recording. Resolution, however, is not all. A data acquisition system reacts, not only to the wanted quantity, but also to random processes such as noise and other interferences. When these interferences are of significant magnitude as compared with the resolution of the system, care must be taken in order to ensure that only meaningful information is extracted from the recorded data.

BACKGROUND OF THE INVENTION

Increased resolution and filtering are common practice in data acquisition and processing. The basics of this can be found in any university textbook on the subject, and numerous inventions have been made in this field.

The present invention relates to data acquisition and processing systems of such dimensions that the sheer amount of data poses serious technical problems in implementing conventional techniques. A particular field where these problems occur is the field of three-dimensional (3D) imaging of small objects at high resolution. In this case, the mere size of the calculations needed presents substantial

technical problems. This calls for the use of more refined methods. Once established, such methods could have a broad range of applications. Thus, the teaching from them could also be transferred to other dimensions, for instance 2D and 1D applications. Also, 3D imaging of large sized objects at high resolution is an interesting application.

The fundamentals of for instance 3D reconstruction were investigated in the beginning of this century mathematically by Johan Radon. The idea of 3D imaging of small objects (molecules) was shown and conceived by Aaron Klug and coworkers, but was not implemented for single particles.

Several techniques have since been developed for reconstructing 3D images of different types of objects. 3D imaging of macromolecular complexes lacking symmetry still has technical problems to be solved. In this case, computer power is not enough. The equations to be solved are of such proportions that earlier attempts to solve the technical problem of computing the 3D image reconstruction, from a series of recorded 2D projections from an electron microscope, so that it can be visually displayed as a 3D image, have had limited success due to the limited accuracy and high noise levels.

The technical problems concerned with the reconstruction of 3D images of small objects at high resolution from a number of 2D projections and with a high signal to noise ratio, have not yet been solved in prior art.

One technical problem to overcome is that of the contrast transfer function (CTF) of the electron microscope, or any other input means that is used. CTF is dependent on microscope and focus and most of the parameters are linked to the machine to be used. This makes it difficult to achieve a quantitatively correct reconstruction of the measured quantity.

The noise levels in 3D imaging have been too high for single particle molecular imaging. Using a larger set of tilts increases the risk of radiation damage to the specimen. Averaging has limited effect on artefacts in recorded data. These effects are reduced by averaging but are still present.

Gradient methods have been implemented and used more recently in electron microscopy (EM) 3D reconstructions. However, in EM applications, as well as in some medical radiation therapeutical applications, the use of gradient refinement methods for image restoration seldom results in a substantial improvement in the quality or resolution of the refined 3D reconstruction due to the fact that a large portion of data is missing, as the reconstruction is made from a limited tilt series.

Another technique of 3D reconstruction is to project onto convex sets, which utilises an envelope that engulfs the 3D reconstructed object. Density modulations outside the envelope are regarded as artefactual and are reset to a constant value during the iteration cycles. The iterative refinement cycles proceed until the density modulations become small. The degree of improvement in a 3D reconstruction provided with this method has not been unambiguously established by comparison with an objective model.

In the field of a 3D image reconstruction the resolution which it is desirable to obtain could require such a large number of recorded data sets that calculations could be difficult to accomplish and this thus sets a practical limit for the resolution. The use of symmetry of a crystalline specimen or internal symmetry of a specimen reduces the size of the calculations needed to a technically manageable level. However, not all of the objects in question have internal symmetry, crystalline symmetry, or symmetric arrangement, and it would therefore be advantageous to have a method of