# Chapter 18

# Discrete Tomography in Electron Microscopy

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*ABSTRACT Structural biology is a very fast evolving field that provides key information to understand how biological processes happen in the cell. In essence, its aim is to obtain the three-dimensional structure of biological macromolecules, and then help to establish a link between structure and function. Among the different techniques that provide this three-dimensional information, in this chapter we will concentrate on the one normally referred to as* Three-dimensional Electron Microscopy *(3D EM), which provides information in the resolution range of between 0.5 to about 4 nanometers of protein and of complexes of proteins and nucleic acids by a process of three-dimensional reconstruction from projections. We seek to obtain information at the highest possible resolution level, and to this end we work toward incorporating into the reconstruction process as much experimental as well as* a priori *information as possible. This work is an assessment of the physical considerations that lead us to believe that discrete tomography has a role to play in this field, identifying the main problems to be addressed and the range of possible applications.*

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llowing this lead, and in the context of discrete to-
ss in this chapter the possibility to use this *a priori*
ess of 3D reconstruction. It will be our final con-
ility exists, at least for some key application areas,
r of problems are circumvented. The result of this
ledge should be an increase in resolution in the 3D
uld take us closer to provide definite answers to the
*gical matter around us — function.*

## n microscopy

ee-dimensional electron microscopy is conceptually
logical macromolecule in an aqueous environment.
is complex under the high vacuum conditions of the
icroscope, it is necessary either to cover the sample
*sk* that stabilizes the sample in the microscope (a
he one known as *negative staining*), or to bring the
a special solid phase, known as *amorphous ice*, a
red to as *cryo electron microscopy* of vitrified sam-
f masks normally reduces the achievable resolution,
y has been proved to create a matrix in which the
l preserved up to atomic resolution. (Other prepar-
o possible, although they are not as widespread as
l.)

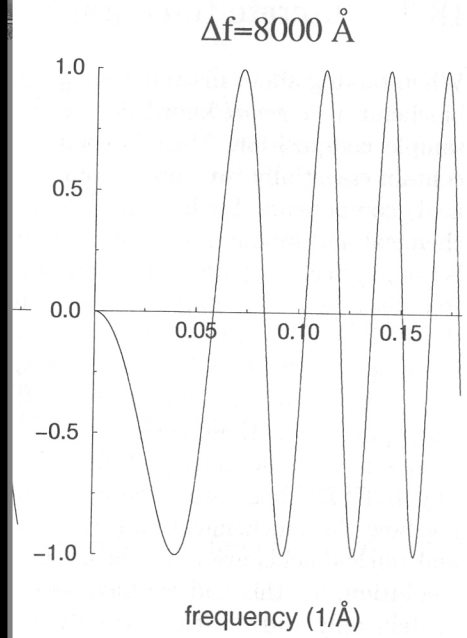
the microscope, a flux of electrons traveling along
microscope goes through the specimen. Under the
teraction of the electrons with the sample is *weak*
ase object — WPO — approximation), the image
can be regarded as a filtered *projection image*. The
the microscope then follows the simple equation:

$$G = Filter \otimes (H \cdot F + N), \tag{18.1}$$

xperimental image, $F$ to the *true* specimen struc-
n operator, $N$ to a recording noise (assumed to be
ilter function and $\otimes$ means convolution. This func-
nation transfer properties of the microscope largely
l parameters of the instrument and the defocus of

sible strategies for data collection as well as 3D re-
that are used in 3D EM (for a review see [6,7]). In
n differentiate between two types of approaches. In
en being imaged is considered *unique*, and then the
ges have to be obtained by tilting the grid inside the

igin in Fourier space (referred to in Fig.
roscope spherical aberration coefficient,
tron wavelength. Plots of this function
resented in Fig. 18.1.




**r function for two defocus values 5.6 mm. and $\lambda = 0.037$ Å.**

lating behavior, and its only free pa-
e electron wavelength $\lambda$ at which the
or a given microscope). The way the
t is first a destruction of the informa-
ros of the CTF) and then a change of
image in between alternate zeros. For
PO approximation is no longer valid,
frequencies), becomes extremely dif-
gh approximate parametric model is

centrate our attention to the case of
t specimens under cryo electron mi-
subnanometer resolution. It is our
the capability to make a significant
has not yet been exploited in prac-
the main concepts that substantiate
ion of some of the practical problems

vercome to solve experimental cases. Finally, we end
ample and the main conclusions.

## tomography in electron microscopy

screte tomography in EM, a key consideration is the
knowledge of the specimen, or, in other words, the
Most biological macromolecules are such that they
(protein and ice) or three (protein, ice, and nucleic
h of these components has known and distinct bio-
cal properties, including the electron density, which
de that is being measured in TEM images. The path
hy application in this area is then to study in detail
ctron density between the different components of
the embedding aqueous media, and then use this
he values in the final 3D reconstruction to those
components.
e performed in this assessment of discrete tomogra-
to design a proof-of-concept test aimed at measur-
al and biophysical differences between ice, protein
eflected in the voxel values of a volume at a given
we have generated 3D volumes made of ice only,
ic acid only. Once these volumes have been gener-
on, we calculate histograms sampling the volume at

g. 18.2) that the situation is as follows: at 0.25 nm,
ms are slightly separated, while protein and nucleic
extensively (although their average densities are
n changes at lower resolution and at 0.75 nm ice,
ls histograms present a negligible overlapping.
proof-of-concept test discussed in this section is
le voxel values corresponding to a biological macro-
hen the biochemical and biophysical *a priori* infor-
constituents is taken into account is indeed limited.
screte tomography approaches is therefore a venue
n experimental cases.

## ng experimental applications:
## ems

basic problems to be addressed when considering
raphy applications in 3D EM: one comes from the



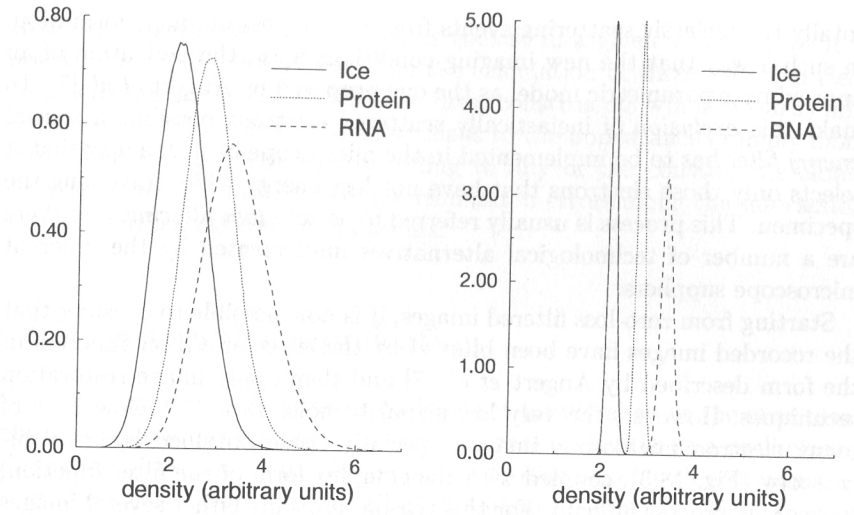



**FIGURE 18.2. Histograms of the voxel values corresponding to volumes sampled using voxels of edge length equal to 0.25 nm (left) and 0.75 nm (right) obtained from volumes composed of either only ice or only protein or only RNA.**

effect of the CTF on the images, and the other one from the discretization of the electron density values.

### *18.4.1 The Contrast Transfer Function (CTF)*

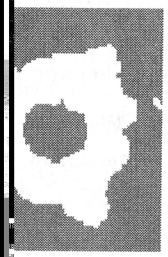
The first problem, the effect of the CTF, has a key influence on the final reconstruction. This is so because the filter function will affect in a very pronounced way not only the "details" of the reconstruction provided by the high frequencies, but also the average values over large areas of the images that are determined by the low frequencies. Since it is clear that in the absence of a proper correction of the CTF the absolute value of each element of the volume is not directly related to the electron density value, it follows that some form of CTF restoration is a must for quantitative 3D electron microscopy and, obviously, for further considerations in a discrete tomography framework.

However, to perform a restoration process it is first very advisable to have an accurate model for the initial degradation process. In this latter respect, while there exits quite good parametric models for the CTF at medium and high frequencies, this is not the case for the low frequency region. This is so because the physical events dominating the behavior at these frequencies are a mixture of elastic and inelastic scattering events that do not lend themselves to be explained as a simple filter function. Indeed, the emerging solution to this low frequency modeling problem is to *exclude*

specimen, as it is
hese averaged elec-
vill necessarily mix
ance of many more
onstituents alone.
ng the interaction

n to the structural
r as a preliminary
with a demonstra-
han, Herman, and
onstructions. (We
ble to use *a priori*

med computer ex-
ata (see Fig. 18.5).
n (Fig. 18.4, left)
e=0.9) (Fig. 18.4,
scribed by Chan,
corrupted by ad-
g. 18.5, left shows
restoration is in
take into account
roposed by Chan,
is application, in
), suggesting that
proved using this



**ored images for**

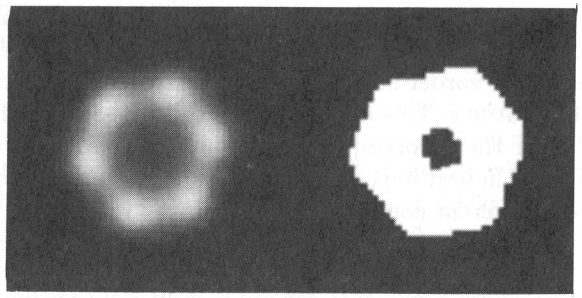


**FIGURE 18.5. Slice of a previously reconstructed volume (namely the protein DnaB helicase of *E. Coli* (unpublished results), which does not contain nucleic acids) and MAP restored image of this slice.**

## 18.6 Conclusions

For the growing field of 3D structural determination by EM at medium and high resolution, it is indeed the case that the specimen can be considered to be composed of discrete and basically homogeneous regions. In order for this biochemical and biophysical fact to became useful for improving the quality of the experimental images, zero-loss filtering, and CTF correction approaches have to be considered. Within the limits just set, the applicability of discrete tomography to this field seems to be justified.

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