III.B. SOURCES OF NOISE IN TEM IMAGES OF BIOLOGICAL SPECIMENS

Noise appears in all micrographs to varying extents and can arise from a variety of sources. **Image processing** methods **identify and separate signal and noise components** in the image. Four major sources of noise include:

III.B.1. Specimen Support Film

Variability in the specimen support film produces the so-called "phase-granularity" (fine, background substructure) superimposed on the specimen image. Variability can arise from unevenness in the film since it is unlikely to be "perfectly" flat and uniformly thick. It is also quite possible that the specimen under observation may be tilted by as many as a few degrees with respect to the electron beam because the underlying support film may itself be tilted. Recall that image contrast, and hence visibility of specimen features, is reduced as the support film thickness increases relative to the specimen.

III.B.2. Specimen

The specimen observed in the microscope environment may not bear a close resemblance to the "native" state for any of several reasons:

a. Impurities in the sample such as the presence of minor contaminants (*e.g.* extra protein) in the specimen sample used for microscopy.

b. Inherent disorder or irregularities at the atomic, molecular, or macromolecular level.

c. Induced disorder caused by the preparation and imaging procedures, for example, from radiation damage, dehydration effects, or stain-induced effects (*i.e.* unfavorable interaction with stains and variability and granularity of stains).

d. Contamination buildup from beam-induced fixation of volatile molecules in the microscope column to the specimen or **etching** due to interaction of the specimen with ions or free radicals produced by the interaction of the electron beam with water molecules in the microscope column (see Sec. II.B.4.b, p. 167).

III.B.3. Microscope

As was outlined in **Section I** (The Microscope), several factors related to microscopy affect the recorded image of the specimen indicating there is a non-simple relationship between the micrograph and specimen. Some of the more significant factors include:

Spherical and **chromatic aberration** in the objective lens

Defocus level of the objective lens (leading to phase contrast effects)

Astigmatism in the objective lens

Thermal drift/Mechanical vibrations

Electrostatic charging (usually in specimens thicker than 50 nm)

Electron statistics (random arrival of electrons)

Random scattering of electrons from the internal parts of the microscope

III.B.4. Photography

Granularity of the **photographic emulsion** limits resolution of the electron image and results in a non-linear contrast transfer function

Non-linear exposure and **development conditions.**

Image processing may be successfully applied whether or not specific sources of noise are identified or quantitated. Nevertheless, processing is more likely to produce meaningful results and is easier to apply if attempts are made to reduce as many sources of noise as possible **during** specimen preparation and microscopy as indicated in Sections I (The Microscope) and II (The Specimen) of the BIO695 notes.