Image Optimization for 3D Electron Microscopy Images

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Compared to ordinary Light Microscopy (LM), Electron Microscopy (EM) offers various advantages. Perhaps the most important one is the high magnification factor. Where LM is able to magnify up to a factor of 2.000, EM allows us to increase this factor up to 2.000.000. This, combined with high resolutions of more than 100 MP, gives opportunities to research areas where the ordinary LM would fail. Research in several fields such as Biotechnology and Healthcare has already proven the vast number of potential applications where 3D EM is useful.
However, images produced by 3D EM are in most cases severely corrupted. These corruptions arise due to a multitude of reasons, e.g. the complex electronics in the system, magnetic lens aberration, heating and motion stability, stray electron scattering, charging, etc. Although the raw, corrupted images are currently used for analysis, their usefulness is limited because the corruptions sometimes make visual distinction and automated analysis very difficult. Another problem arises when one tries to use these images in subsequent processing steps, as the corruptions can influence this in an unpredictable way. In this work, we describe a thorough analysis of noise, as one of the most important corruptions in 3D EM imaging. This analysis is vital for future work on 3D EM image restoration algorithms.

In our experiments, we have analyzed the observed noise extensively, using the Zeiss Merlin 3D EM at VIB Ghent. Several experiments were carried out, aiming to make behavioral prediction of the noise easier. Information such as knowing which noise intensities are most and least common, noise dependencies, etc. is highly important to remove a maximal amount of noise, without destroying the image.
Firstly, we analyzed the noise caused by the receiver electronics, this was investigated by switching off the electron beam in the microscope while imaging. However, it does not take into account the signal-dependency of noise in a real acquired image. Therefore, we have also extracted various patches (from other images with a non-zero signal and the electron beam switched on) where we assume a single constant intensity. The noise contributions to these signals can be obtained by subtracting the mean intensity of the patch from itself, where the noise is assumed to be zero-mean. Even if this assumption turns out to be false, the worst consequence is that restored images have a fixed intensity bias, which we do not consider significant for most applications.
We found that the additive noise, caused by the electronics, is Gaussian distributed, meaning that small noise corruptions are more often to appear than strong fluctuations. This was confirmed using the two types of experiments, described above.
Another noise property we investigated, is stationarity, i.e. the positional dependency of the noise. For example, in CT images noise often shows an isotropic grain structure near the center. Towards the boundaries, however, it becomes more oriented and less varying. This is a property that can be exploited intensively when removing the noise. Therefore, we compared statistical properties of the noise in function of the position within the image: we computed the mean and standard deviation of noise in block shaped regions within a certain environment. The means on the one hand and these

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standard deviations on the other hand should be constant if the noise were to be stationary. We verified this by looking at the variance of the estimated values over all the blocks. Thus, we can conclude that the noise is stationary.

Next, we have investigated the spatial correlation of the noise. This was achieved by computing the Power Spectral Density (PSD) of the noise signal. The PSD expresses the power of specific frequencies in the noise (i.e. hard or soft transitions from noise intensities). For example, MRI images, PAL television video sequences, thermal cameras, etc. suffer from correlated noise. Their PSD indicates that some noise frequencies occur stronger in the corrupted images than others. Note that in our case this frequency is expressed by three components, since we are working with 3D images in a slice by slice fashion. Within each slice, the image is scanned, horizontal line by line. Because of this principle, we expected a horizontal spatial correlation if present. The PSD of our signal-free noise sample confirmed this horizontal spatial noise correlation. Low horizontal frequencies (i.e. nearly flat regions in horizontal direction) appear less strong in the noise signal than middle high horizontal frequencies. The higher frequencies (i.e. stronger horizontal transitions or vertical edges) then appear with less energy. No spatial correlation is found in any of the other directions. This explains the horizontal striping effect we witness in the noise signal. We found that the strength of this horizontal correlation depends on the dwell time parameter used while imaging.

Finally, we determined signal dependencies in the noise. Film grain noise, for example, shows higher amounts of noise in specific intensity regions. To investigate this, we computed standard deviations of noise in patches with increasing intensity, having a constant intensity within that patch. When plotting these standard deviations in function of the signal intensity, signal independency would manifest as constant variance. However, we found a decreasing relationship: low intensity (i.e. dark to black) patches turned out to be corrupted with a higher noise variance than high intensity (i.e. bright to white) patches. This is typically for Poisson noise, which would be consistent with the 'electron counting' principle of the 3D EM.

To summarize, we can state that the noise in 3D EM is stationary and a combination of Gaussian and Poisson noise, with a dwell-time dependent horizontal spatial correlation (this is due to the horizontal scanning of the microscope). The derived noise characteristics will allow us to define an optimal reconstruction algorithm, which will produce noise-free sharp images.