Huygens - what is my SNR

Signal to Noise Ratio questions

How to estimate the signal to noise ratio?

The SNR describes the ratio between signal and noise in the image. The deconvolution algorithms require the SNR of the image as an input parameter. If the deconvolution algorithms are provided with an SNR value that describes the image accurately the restoration will be optimal. However, if the provided SNR value is too high, the image noise will be amplified. If the SNR value is set too low, the noise will be reduced at the cost of the final resolution of the image. Therefore, the SNR can also be thought of as an **artifact limiter** as far as the deconvolution is concerned.

Confocal images

In many confocal images the SNR is due to photon noise. If the background is mostly zero with spikes here and there, then these spikes are probably single photon events. From the height of the average small spike one can roughly estimate how many gray levels correspond with one photon. If, for example, such a single photon event has an intensity value of 5, then the maximum intensity of the image (for example 255) corresponds with 51 photons. The SNR around the maximum intensity area will be: square_root(51) = 7.

With a good confocal image, and when using an 8-bit converter, one can easily get into a situation where one gray level corresponds with more than one photon. In such case, the above procedure fails, but one could still start a run with SNR 20-30 and increase it later on.

Some confocal microscopes are equipped with photon counters like avalanche photo diodes (APD). In this case the SNR is simply the square root of the brightest part of the image.

Widefield images

Images from widefield microscopes equipped with 12-bit CCD cameras usually have an SNR in the range of 40-60. These kinds of images can also be deconvolved with the fast Quick-MLE algorithm for low noise images.
The Huygens Remote Manager is equipped with an automatic SNR estimator free to use here (registration needed). See also Set The Signal To Noise Ratio.

Experimenting with the SNR

In practice, the SNR can be estimated out by calculating the number of photons in the image, or by rule of thumb reasoning. One can then start a first deconvolution with that SNR value and inspect the result for artifacts. If no artefacts are visible, the deconvolution can be continued with a higher SNR setting (say 5-10 higher). If the results are good but there is still a residue background, the deconvolution can be continued with the same SNR value but with a higher background setting.

Are there guide values for the SNR of different image types?

As a very basic approach, the following values can be used as a first estimation of the SNR of different image types:

- Bad quality confocal image: SNR = 10
- Noisy confocal image: SNR = 20
- Good quality confocal image: SNR = 30-60
- Good quality widefield image: SNR > 40

If the image noise is clearly amplified after the restoration then the SNR value provided to the deconvolution algorithm was too high. On the contrary, if the restored image looks too smooth then the SNR value was too low. In both cases, please proceed to restore the raw data again with an adapted SNR value.

See Set The Signal To Noise Ratio.

What is the potential drawback of estimating the signal to noise ratio (SNR) too high?

Too high SNR setting in both MLE and ICTM methods may lead to artifact generation. These artifacts have a different character in the MLE and ICTM method, so they will be treated separately.

- *Artifacts in the ICTM method due to overestimation of the SNR*: There are two sources of artifacts with this class of method: amplification of noise and 'ringing' artifacts, i.e. fringes around sharp edges. Ringing artifacts are greatly suppressed in the ICTM procedure. Moreover, when you switch on 'ringing reduction' the ICTM procedure will lower the SNR setting when it detects the onset of ringing. The drawback of this feature is that it increases the data dependency of the procedure. So, when you are planning to restore and compare a number of images of the same type, we do advise you not to use this switch. More troublesome is noise amplification. The amplification increases with the number of iterations. Most notably it shows up as semi-periodic structures in the background. The ICTM method can be used with the background correction option. This enables you to set as background value the average of the image background. Use Analysis->background from the Operations window to inspect the background. This feature will enable you to remove the background including the ringing phenomena effectively. Conclusion: overestimation of the SNR (say by a factor of 2) won't do much harm. However, since noise amplification artifacts may also occur inside the object, be careful with high SNR values (>100) in combination with large number of iterations (>100).
- *Artifacts in the MLE method due to overestimation of the SNR*: Due to the built-in background correction and its optimal treatment of background (nearly always Poisson type) noise the MLE method is hardly sensitive to background noise amplification. For noisy images, amplification of noise within the object is also less than in the ICTM method. Overestimation of the SNR value in noisy images will result in reduced noise suppression. A potential danger of overestimation of the SNR parameter (>100) in low noise images is 'over-restoration', i.e. objects are estimated too small.

This occurs in combination with high iteration counts, >100. With settings of the SNR parameter below 30 there is not much danger in over-restoring the object. Conclusion: for restoration of high noise images the MLE method is quite safe: there is a straightforward trade-off between noise and resolution gain.

What is the potential drawback of estimating of the signal to noise ratio (SNR) too low?

In both the Maximum Likelihood Estimation (MLE) and Iterative Constrained Tikhonov-Miller (ICTM) methods this will lead to loss of detail in the result. The ICTM method with SNR < 10 and MLE with SNR < 5 will actually lower the lateral resolution. Axial resolution will be reduced in the ICTM method with SNR < 10; in the MLE method with SNR < 3. The figures given here are estimates, they depend on the data. See Set The Signal To Noise Ratio.