Spatial Resolution of Weakly Reflecting Objects in Confocal Optical Microscopy

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INTRODUCTION

Imaging with a confocal scanning optical microscope (CSOM) (Fig. 1) allows for high contrast and high spatial resolution (Wilson & Hewlett, 1991), depth discrimination or optical sectioning (Wilson & Sheppard, 1984) and rejection of scattered light (Hamilton et al., 1981). Such optical systems can be characterized by its point spread function (PSF) (Born & Wolf, 1980) and the resolving power can be related to the full width at half maximum (FWHM) of the effective PSF. For a CSOM, the PSF is obtained from the product of the PSF of the two imaging lenses in a confocal arrangement (Wilson & Sheppard, 1984; Wilson, 1990). These classical resolution criteria can be achieved only for very high signal-to-noise ratio (SNR) images. The image resolution, even for a well-corrected lens, is ultimately limited not by diffraction but by the SNR. A good survey of resolution can be found in den Dekker & van den Bos (1997).

The SNR, and consequently the image resolution, is determined by random and systematic errors in the detected image (Goodman, 1985). In a CSOM, image resolution is further by the loss in sensitivity and photon count due to the size of the pinhole at the detector and source. The pinhole leads to the rejection of out of focus rays in the system and this property result in the unique optical sectioning capability of a CSOM. The enhanced image resolution gained from the confocal configuration of the microscope is offset by the reduced photon count at the detector plane. The quantum efficiency of the photo-detector will also affect the number of photons contributing to the detected image. For our investigation, we shall consider a very fine array of photodetectors with quantum efficiency η at the detector plane. In practice, η is lower than unity and contributes further in lowering of the intensity of the detected light. In addition, a weakly reflecting object, or similarly, a highly absorbing one, will limit the photons that will arrive at the detector pinhole.

In this paper, we show the behavior of spatial resolution of a weakly reflecting object in a confocal setup. In the next section, we discuss the numerical model we used and the instrumentation geometry of the confocal system. The results of the simulation are presented in section 4. A summary and discussion are presented in the final section including some possible applications of non-classical light in photon-limited imaging.

NUMERICAL MODEL AND INSTRUMENTATION GEOMETRY

We use a three-dimensional computer ray-tracing model to simulate the optical behavior of the confocal system. Single photons enter the setup uniformly from a source at a specified position in front of the entrance pupil and

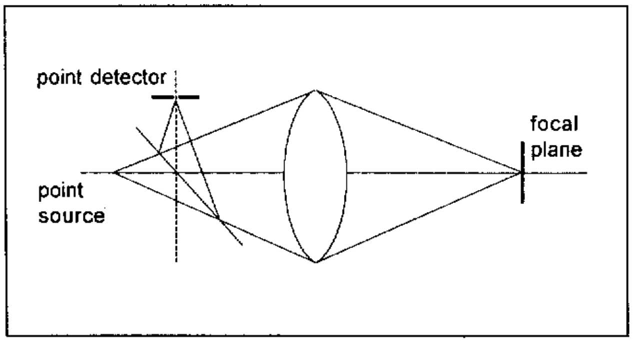

Fig. 1. Schematic diagram of a confocal scanning optical microscope. NA=0.7, f=10 cm for the lens

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is assigned a wave function $\Psi = Ae^{-ikx}$. The rays are then traced through the system on the basis of geometry (Douglas et al., 1995), and diffraction effects are obtained from the phase difference due all the ray paths available for a photon to arrive at a single point on the detector plane. In the limit of infinite rays we obtain the classical point spread function at the detector. A single photon entering the setup will thus fall with a photo-detection distribution probability given by the PSF at the detector plane and its quantum efficiency. We also model the weakly reflecting object with a mirror. We change the probability that a photon is reflected depending on the reflectivity R of the mirror.

The confocal reflecting microscope that we considered has a lens of focal length 10 cm and NA=0.7 (Fig. 1). The PSF profile was built sequentially using the simulation and the FWHM was estimated for different reflectivity R. Furthermore, a 20x20 image was taken at each R to simulate a scan of a reflecting surface such as a plane mirror. We have repeated the simulation for 400 trials with input photon count ranging from 10^6 to 10^8.

RESULTS

In Fig. 2, we show the FWHM approaching a value of 1.12 as one increases N, which is the number of photons detected. The variance of the FWHM is also shown and it becomes smaller with increasing N. The best fit curve for the variance of the FWHM is $0.3014N^{-0.952}$ This decreasing variance suggests that the PSF of the setup becomes more stable as one increases the number of photons you allow into the system. Thus at low N, a point in the object space can have multiple possible PSF or alternatively, a range of possible images. This inability to image precisely a point results in loss of resolution. Increasing N thus results in better resolution because the range at which the PSF varies becomes smaller.

The error of the transverse PSF is shown in Fig. 3 for different reflectivities R. The normalized error was found to decrease inversely with the number of photons detected. In Fig. 4, we vary the quantum efficiency of the system. The behavior with respect to a different η

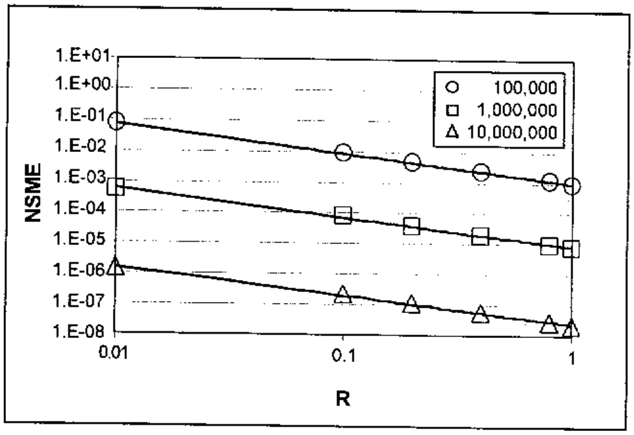

Fig. 3. NMSE vs R for the transverse profile of the PSF. The error was found to decrease approximately inversely with R. NA=0.7, λ=630 nm

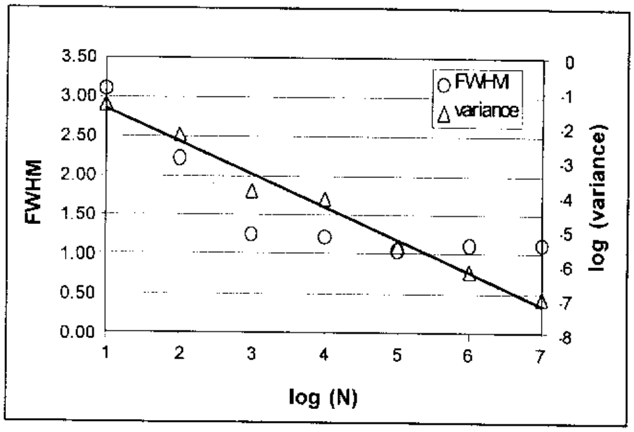

Fig. 2. The FWHM of the transverse PSF vs the number of photons detected (N) and its variance is shown. The best fit for the variance is $0.3014N^{-0.952}$, NA=0.7, λ=630 nm

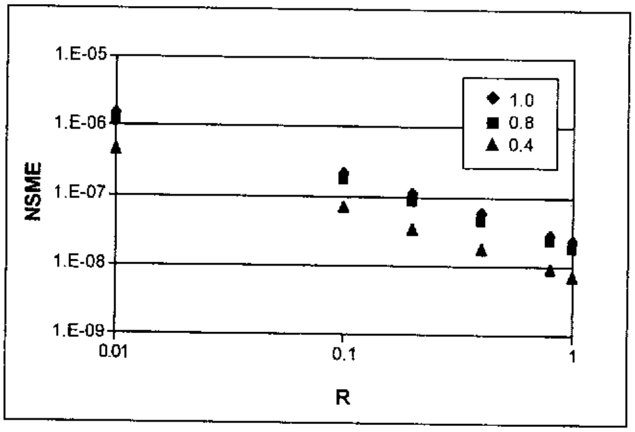

Fig. 4. NMSE vs R at at different h. The error is inversely proportional with R across different η. NA=0.7, λ=630 nm.

of the NMSE was found to lower further the resolution obtained and was still inversely proportional with R across different R. Lower efficiencies may be corrected by compensating for the loss of photons by having a longer observation time.

DISCUSSION

In this paper we have discussed the effect of the reflectivity of the object on the resolution of a confocal imaging setup. At low reflectivities, a reduced photon count is observed at the detector. The resolution of the system is limited by this reduced photon count and by the errors introduced in the imaging process. At low light levels a point source can be estimated by many possible PSF, or equivalently, it can have many possible images. We attribute the loss of resolution to this ambiguity in the PSF. However, as the number of photons detected increase, the range at which the PSF varies becomes smaller and with higher intensity illumination, we can expect that the resolution will improve. Unlimited resolution cannot be achieved because noise will always be present in the imaging process. If noise can be suppressed to yield an increase in the signal-to-noise ratio, better resolution for a given optical setup can be achieved. Non-classical light such as squeezed light can suppress noise (Tapang & Saloma, 2000; Kolobov, 1999) at photodetectors and it would be interesting to explore further its applications to increasing the resolution in imaging systems.

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