An Imaging Modality for Ultrahigh Accuracy Estimation

Extracting Quantities from Image Data with Near-Best Accuracies

The extraction of quantities from image data represents a common yet powerful way of obtaining information about the imaged objects. In the last decade or so, it has come to play an important role in single molecule microscopy data analysis, where attributes of a fluorescent molecule such as its location need to be estimated accurately from an image. Different estimators can be used to determine the quantities of interest with varying levels of accuracy. However, even when an accurate estimator is used, the obtainable accuracies are fundamentally limited by the fact that the estimation is carried out on image data that has been deteriorated by pixelation and detector noise. We describe here an imaging method that produces image data from which quantities of interest can be estimated with accuracies significantly higher than those that could be expected when the quantities are estimated from conventionally acquired image data. Focus is given to a particular implementation that minimizes the deteriorative effects of both pixelation and detector noise.

Estimating Quantities from Image Data and Challenges

The ability to accurately extract quantities of interest from image data is of importance in diverse applications ranging from the determination of interstellar distances in astronomy to the estimation of the positions of focal spots in a Shack-Hartmann wavefront sensor. In single molecule microscopy, the accurate estimation of the locations of fluorescent molecules plays an integral part in the tracking of biomolecules [1, 2] and the super-resolution reconstruction of subcellular structures [3, 4].

Regardless of the particular estimation problem at hand, a fundamental obstacle to the ultrahigh accuracy determination of the quantities of interest is the fact that the acquired image is a deteriorated version of the ideal image, the latter being an image that is captured using a hypothetical image detector that introduces neither pixelation nor noise.
An image produced by a charge-coupled device (CCD) detector or an electron-multiplying CCD (EMCCD) detector, each a commonly used data acquisition device in single molecule microscopy, is pixelated and contains measurement noise that is added during the detector's readout process.

An image produced by an EMCCD detector further contains noise due to the stochasticity of the detector's signal amplification (i.e., electron multiplication) process, the intended purpose of which is to enable low-light imaging by augmenting weak signals to such an extent that the readout noise is rendered insignificant.

Pixelation reduces the image resolution, while detector noise corrupts the signal that is detected in each image pixel. These two major deteriorative effects lower the amount of information that the image contains about the quantities of interest, and therefore decrease the accuracies with which the quantities can be estimated. The lessening of these effects can lead to improved estimation accuracies, and is especially important in low-light imaging where the low numbers of detected photons already mean that the obtainable accuracies will be relatively poor [5].

**Ultrahigh Accuracy Imaging Modality (UAIM)**

We recently introduced an imaging method called the Ultrahigh Accuracy Imaging Modality (UAIM) [6]. To enable estimation with accuracies approaching those that could only be attained if the estimation were carried out on hypothetical image data that contained no detector noise, UAIM specifies the typical use of an EMCCD detector at a high level of signal amplification (i.e., at a high electron multiplication gain setting), but in a highly atypical imaging configuration in which less than 1 photon is on average detected in each image pixel. The specified rule of thumb is the direct result of our theoretical finding that the signal in an EMCCD pixel is least corrupted by detector noise when on average it comprises less than 1 photon. More specifically, by observing the value of a Fisher information-based expression called
the noise coefficient [6, 7] as a function of the average photon count in an EMCCD pixel, it can be seen that the amount of information that a pixel contains about the quantities to be estimated is highest when very small numbers of photons are detected. To increase the information content of an entire image, UAIM extends this idea to every pixel of an image.

Image data acquired using UAIM has an unconventional appearance. Figure 1 shows, for example, that whereas a conventionally acquired EMCCD image of a fluorescent dye molecule is a relatively smooth Gaussian-like profile, a UAIM image of the same type of molecule has a spiky appearance due to the combination of very low pixel photon counts and high electron multiplication gain. Despite the unusual appearance of UAIM data, we demonstrated using maximum likelihood estimation that when fluorescent beads and single molecules are imaged using UAIM, their positional coordinates can be determined with substantially higher accuracies (i.e., lower standard deviations) than when they are imaged using conventional EMCCD imaging [6].

**Approaches to Implementing UAIM**

UAIM may be implemented in many different ways. To reduce the photon count in each pixel of the image data, two general approaches are the temporal distribution and the spatial distribution of the detected photons. As illustrated in figure 2a, the temporal distribution approach could be implemented by splitting what would normally be a single image acquisition into multiple successive and shorter image acquisitions that span the same length of time as would be taken by the single acquisition. Similarly, the spatial distribution approach could be realized, as shown in figure 2b, by dividing a single acquisition into multiple ones. However, the light signal would be physically partitioned and directed to multiple EMCCD detectors, such that the multiple acquisitions would be carried out simultaneously, each by a different detector and over the same length of time as would be taken by the single acquisition. In either scenario, the multiple acquired images would be used together to estimate the quantities of interest with accuracies close to those that could be attained only if the quantities were estimated from hypothetical image data that contained no detector noise. The temporal and spatial distribution approaches are not mutually exclusive, and can be combined to achieve the necessary reduction of photon counts in the pixels.

**High Magnification Implementation of UAIM**

A simple yet powerful way to implement the spatial distribution approach to UAIM is to use an unconventionally high magnification to spread out the detected photons
over many pixels, such that the photon count in each pixel generally averages less than 1 (fig. 3a). For example, instead of imaging with a standard 100x magnification in a fluorescence microscopy experiment, one might use additional magnifiers (fig. 3b) to image at a total magnification of 1000x, as was done to acquire the UAIM image of figure 1.

Compared to the multiple-acquisition implementation (fig. 2b), the high magnification implementation can yield significantly higher estimation accuracies. While both implementations reduce the photon counts detected in the pixels and thus minimize corruption of the signal in each pixel by detector noise, the spreading out of photons via high magnification additionally decreases the effective pixel size of the detector and generates a more finely pixelated, and hence higher resolution, image. The high magnification implementation thus lessens the deteriorative effects of both pixelation and detector noise, and produces an image that more closely approximates an ideal image (i.e. an unpixelated and detector noise-free image). Ultrahigh estimation accuracies approaching those that could only be attained when estimation is carried out on an ideal image are thus possible with the use of high magnification.

Figure 4 provides a quantitative illustration of the high magnification implementation of UAIM by showing that the best possible standard deviation for estimating the positional coordinates of a point source improves (i.e. decreases) with increasing magnification, and approaches the standard deviation of 8.22 nm that could only be achieved in the absence of pixelation and detector noise. For example, whereas a best possible standard deviation of 13.40 nm is attainable at a standard 100x magnification, a best possible standard deviation of 8.82 nm is attainable at a 1100x magnification.

Importantly, demonstrating that the EMCCD detector has traditionally been underutilized in terms of obtaining the highest estimation accuracies possible, figure 4 also shows that the standard deviations attainable at high magnifications with UAIM are significantly lower than those which are commonly believed to be the best possible [8] based on a presumed limitation attributed to the excess noise [9] of the EMCCD signal amplification process.

References

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