Improved Single-molecule Imaging Based On Photon Counting With An EMCCD Camera

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Introduction

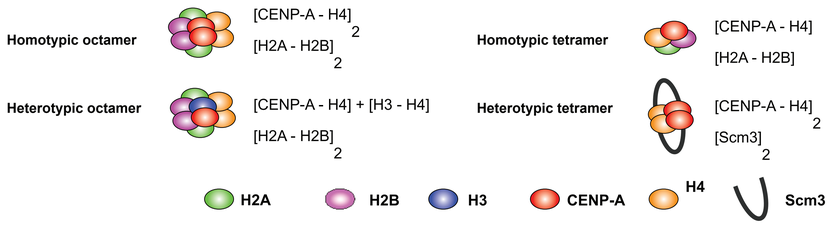
New breakthroughs in digital imaging have historically opened opportunities to scientists in bio-medical research. One important landmark of the last decade has been the development of the electron-multiplying charge coupled device (EMCCD), an imaging detector that uses an avalanche phenomenon to amplify the weak photo-electron signals. EMCCDs are well suited for ultralow light applications in that a single photo-electron can be amplified sufficiently to allow the possibility of photon counting. However, as with all electronic detectors, the noise increases greatly with an increase in gain, diminishing the overall sensitivity and dynamic range and thus compromising images.

Recently, a new camera has been developed by Nüvü Camēras that significantly reduces the noise generated during the read-out process (up to 10 times) and allows more efficient photon counting. This major reduction of the noise threshold represents an opportunity for various low-light imaging applications including single-molecule studies.

We present a demonstration of its applicability in a total internal reflection fluorescence microscopy (TIRFM) assay to visualize single biomolecules. In molecular biology, it is well established that most proteins exert their biological roles in complex with other proteins. Thus, detecting individual components and the structure of these complexes is essential to understand the mechanism of biological events. To this aim, we employ TIRFM imaged in photon counting mode for visualization of single centromeric nucleosomes, containing a histone variant known as CENP-A.

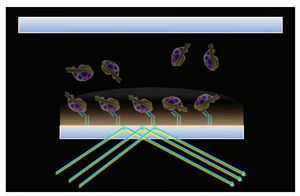
We show that this technique is well suited to TIRFM and that it is capable of detecting single fluorescently labelled CENP-A in the nucleosome with an enhanced signal to noise ratio. The desired and improved properties of the assay such as high sensitivity and photon counting shall pave the way for comprehensive studies of single molecules in this and other biological contexts.

Proposed models for nucleosomal composition at centromeres



Modified from Allshire and Karpen, 2008

Methods



TIRF microscope

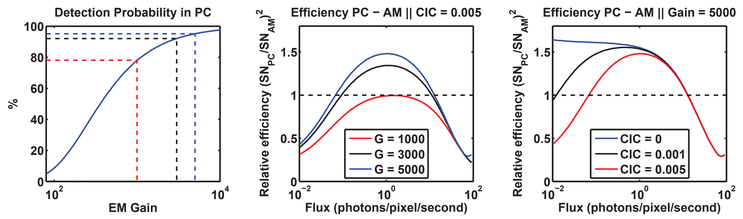
+

Nüvü Camēras EM N2

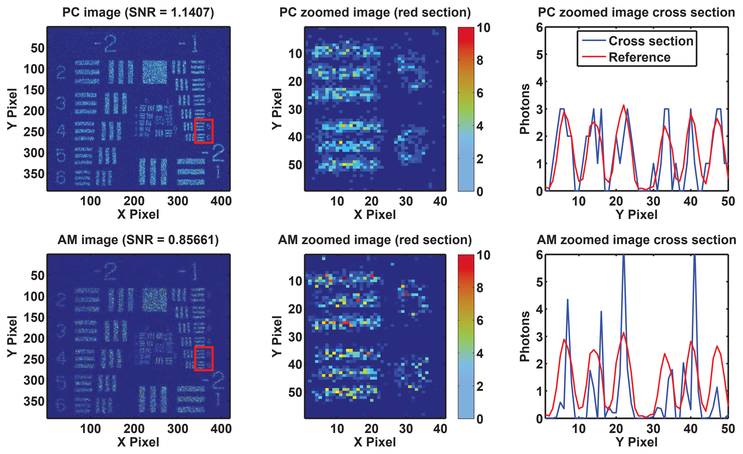
EMCCD camera

Photon counting principles

In EMCCD cameras, the Excess Noise Factor (ENF) usually has the effect of halving the Quantum Efficiency. However, Photon Counting (PC) allows to get rid of it. To attain a plus value over the analog mode (AM), extremely low clock-induced charge (CIC) noise and a high EM gain are necessary.



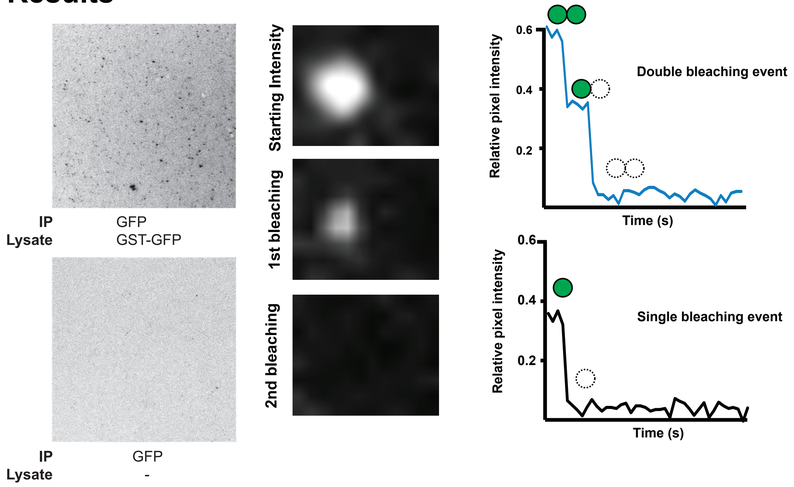
Photon counting demonstration (USAF 1951 target)



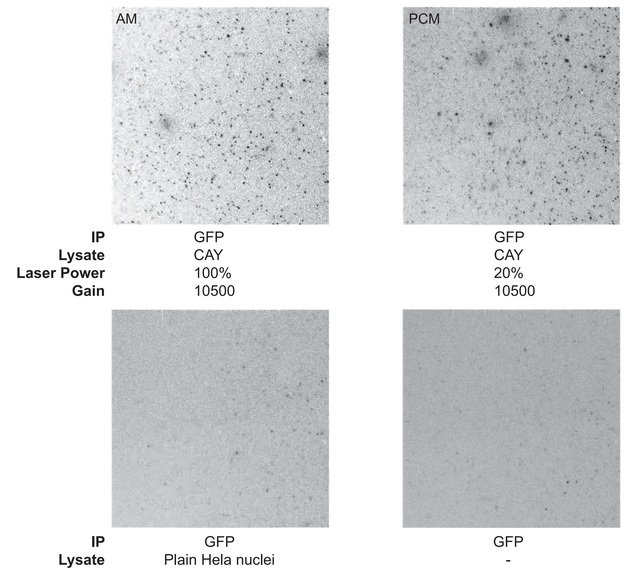
The left and center columns show that with PC, the improved SNR allows for better imaging of the finer details of the target. In the right column, the red curves represent the same cross section obtained from a normalized acquisition with a longer exposure time. It is used as a reference to compare the blue curves.

For ultra low light applications, PC provides better detection accuracy and faster acquisitions.

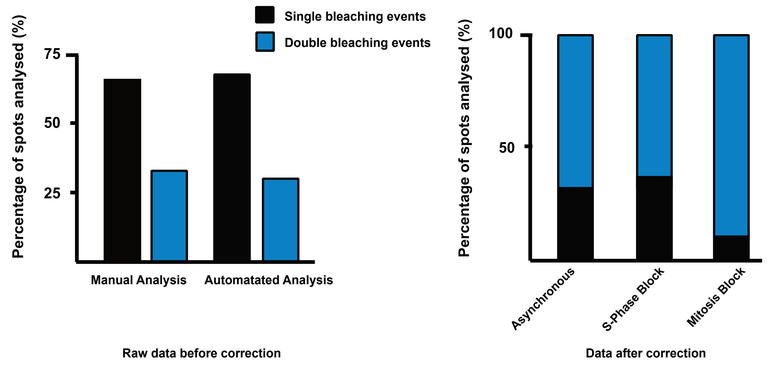
Results



Our TIRFM assay can detect double vs. single bleaching events



PC allows clear detection of individual nucleosomes at lower laser power (20%) versus AM (100%) and thus better time resolution between bleaching events.



Manual automated analysis confirmed the presence of a mixed population of one and two CENP-A containing nucleosomes in Hela cells. Cell cycle analysis also revealed a dynamic change in the proportion of each population.

Conclusions

- Our TIRFM assay is capable of detecting single fluorescently-labeled biomolecules

- Using an EMCCD camera in photon-counting mode enables visualization of single molecules and photobleaching behaviour at a much lower laser power compared with analog mode and thus may provide a better time resolution

- There is a mixture of two different CENP-A nucleosomes in the mammalian system

- Cell-cycle dependent changes appear to occur in the structure of CENP-A nucleosomes

Acknowledgements

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