**Image-Scanning Microscopy and Stochastic Optical Fluctuation Imaging: Making it easy and user-friendly**

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**ABSTRACT**

Recent years have seen a tremendous increase of new and novel methods of high and superresolution fluorescence microscopy. Among them, our group has developed to powerful methods: Confocal Spinning Disc Image-Scanning Microscopy (CSDISM)**Error! Reference source not found.Error! Reference source not found.**, and Superresolution Optical Fluctuation Imaging (SOFI)**Error! Reference source not found.Error! Reference source not found.Error! Reference source not found.**. However, new microscopy techniques that provide not only enhanced image quality and resolution, but they are also simple enough for finding broad application. Here, we present embedding solutions for both CSDISM and SOFI which enable potential users to implement them in an easy and straightforward way into their existing microscopy systems. In the case of CSDISM, we have integrated the method into the environment of the widely used and popular MicroManager Open Source Imaging platform. This allows any researcher who already has a commercial Confocal Spinning Disk microscope to easily implement the image-scanning option and thus to double the spatial resolution. For SOFI, we have developed a dedicated hardware based on a Freely Programmable Gate Array (FPGA) which converts, in real time, image movies taken by high-speed CCD systems into SOFI cumulant images. Thus, all algorithmic complexities and numerical workload of SOFI calculations are taken care of.

1. **INTRODUCTION**

Super High Resolution (SHR) microscopy is a relatively young scientific area, which is framing a diverse group of scientific disciplines. Tremendous insights were gained over the past decade by biochemists, biophysicists and biologists. Nevertheless the subfield of SHR imaging in the field of bioinformatics is relatively inequitably represented, thus we lack a wide range of open source software tools to provide access of those SHR imaging technics to the non-expert group. The investigation of algorithmic complexity for the SHR microscopy would give inference to the general applicability and practicableness of the underlying scientific method. As a consequence of that approach, our aim is to investigate the algorithmic part of our recently developed SHR microscopy methods, termed SOFI and CSDISM. We will present an integrated software approach for the CSDISM which yields every regular spinning disk microscope into a CSDISM microscope with increased resolution. Regarding SOFI microscopy we will present our ongoing work to massively parallelize the algorithm for the image formation within a FPGA processor design.

* 1. **SOFI**

The Superresolution optical fluctuation imaging (SOFI) method was developed recently and might easily be distinguished from most other superresolution imaging technics, by its inherent robustness and simplicity. Instead of switching the molecules sequentially or simultaneously, as it is required for PALM/STORM, the SOFI algorithm derives superresolved information only from stochastic “On” and “Off” states in the temporal domain. Required is the intensity fluctuation of the emission of a molecule over the acquisition time. The root of that intensity fluctuation can technically be anything, from changes in polarization or the alternating bright or dim fluorescence states. The emitters must fluctuate stochastically and independent, thus are not interacting with their neighbor emitters, hence a labeling density greater than 10 nm is essential. In a sample composed of independently fluctuating emitters at location with time dependent molecular brightness, the resulting fluorescence source distribution can be derived with:



Where represents the time dependent fluctuation furthermoreis the constant molecular brightness. The fluorescence signal at position and time is given by the convolution of the Point Spread Function (PSF) with the fluorescence source distribution:



Hence the observable molecules are in stationary equilibrium whilst acquisition, thus fluctuations can expressed as zero-mean fluctuations:



The most primitive case is the second order autocorrelation function:



Hence all cross correlation terms  where  vanish, the second order autocorrelation function reduces to a sum of the squared PSF. Weighted with the squared brightness of each emitter and molecular correlation function. Thus the SOFI image is defined by the value of  with time lag. The intensity of the SOFI image is derived by the brightness and degree of correlation of the fluorescence signal. The PSF is represented by a distribution that is the squared original PSF. It follows from that the width of the new PSF can be reduced by  in all dimensions. Going from the second order correlation function to higher order correlation functions is the next logical step to further increase the resolution, the definition of the  order correlation functions is:



Which can be written as:



The acquired signal fluctuations have to be multiplied for  time lags to generate. Thereafter one can generate higher order SOFI images with transforming higher order correlation functions into higher order cumulant functions. More precise, the  order correlation function represents the  order cumulant function. Hence the cross term contribution of the lower order correlations are eliminated in the  order cumulant function. Thus only terms containing the  power of the PSF are contributing to the order cumulant function. The higher order cumulant function is the following:



The algorithmic complexity yields exponentially for higher order SOFI images where all information from the signal source is used for the final image formation, due to the fact that the  order cumulant is a  dimensional function of.

EVENTUELL DIE NEUEN SACHEN NOCH MIT REINNEHMEN??? FALLS SCHON VEROEFFENTLICHT

* 1. **ISM**

The Image Scanning Microscope (ISM) method utilizes a standard confocal microscope, but the point detector is replaced by an image detector. Thus one is able to record the full illuminated region at each scan position, in particular the full PSF for that particular scan step. Hence the ISM image can be expressed mathematically:



The scan position of the scanner is marked, the position on the CCD is represented with. Whilst integrating  over  the image can be recovered by the confocal microscope. If the data is shifted in  space by the value of  and afterwards integrate over, the image recovering is obtained as in a wide field microscope. The excitation intensity distribution is multiplied with the shifted PSF , since the center of gravity of the product  is shifted by  , which is exactly half the width of a scan step, one must shift the this center of gravity back to the center of the optical axis by the and afterwards integrate over . Thus the image and the PSF can be expressed mathematically in the following form:





The acquired signal on the CCD at the position, with the responding position  in sample space, is added to the final image at the position  at image space.

1. **IMPLEMENTATION**
   1. **SOFI**
      1. **Paralelle correlation etc**
   2. **ISM**
      1. **Kreis encoder problem**
2. **RESULTS**
   1. **SOFI**

**LV FPGA correlation Par vs non par**

Schnell was fpga maessiges zusammenklicken um Geschwindigkeit zu zeigen

Numerische stabilitaet jitter etc

* 1. **CSDISM**

Warum so geil integrierbar durchsatz geschwindigkeit etc

Numerische stabilitaet,jitter etc

NI Karte

* 1. **SOFI**

1. **CONCLUSION**

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