**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**

blab la warum super high.. was gibt es blab la

probleme signal to noise, algorithmisch komplex viel daten etc. parallelisieren etc.

* 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:



* 1. **ISM**

Erklaeren method

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

* 1. **SOFI**

1. **CONCLUSION**

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