SPAD ARRAY CAMERA FOR LOCALIZATION BASED SUPER RESOLUTION MICROSCOPY

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Abstract: Super resolution microscopy by localization is a stochastic based approach, where the resolution is determined by the localization accuracy [1] [2] [3]. The accuracy of localization heavily depends on the statistics of the data obtained with a camera during imaging. Current state of the art EMCCD (electron multiplying charge coupled device) cameras have frame rates up to 200 fps and hence a limited temporal resolution between frames. This can lead to ambiguities in localization. For example, a single fluorescent spot appearing at the same location in two successive frames is not considered for localization, because it is not clear, whether the spot arises from a single fluorophore in ON state for a long time or from two adjacent fluorophores, which switches ON and OFF. In this work, we explore for the first time the use of a single-photon counting SPAD (single photon avalanche diodes) array camera for super resolution microscopy. These cameras can provide high frame rates (up to 375000 fps), with improved temporal resolution between the frames, enabling a more accurate view of events that can be precisely tracked over time. The rich information obtained from such large number of frames leads to more accurate statistical estimations for overcoming the current ambiguities in localization. Also, SPAD array cameras are capable of reading frames having pixels depth of 1-bit. [4]. Such, a fine granularity enables the user to add any number of frames for identifying and localizing individual events with a very high accuracy. SPADs have been successfully used in performing time-resolved imaging measurements like FLIM (fluorescence life time imaging measurements). This allows us to extend the possibility of performing FLIM and super resolution imaging simultaneously. As a result, two different fluorophores can be separated based on their unique life times, enabling multi-channel operations using a single camera. An example of a preliminary image captured using a SPAD array camera is depicted in Figure 1.

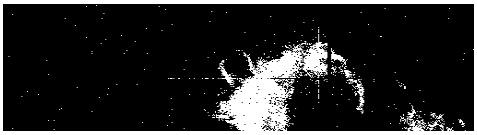

Figure 1. Widefield TIRF image of a Hela cell with rhodamine-labeled actin captured with a SPAD array camera

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