Localization-based super-resolution microscopy with an sCMOS camera part III: camera embedded data processing significantly reduces the challenges of massive data handling

Hongqiang Ma,[1,2] Hiroyuki Kawai,[3] Eiji Toda,[3] Shaoqun Zeng,[1,2] and Zhen-Li Huang[1,2,*]

[1]*Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics-Huazhong University of Science and Technology, Wuhan 430074, China*

[2]*Key Laboratory of Biomedical Photonics of Ministry of Education, Department of Biomedical Engineering, Huazhong University of Science and Technology, Wuhan 430074, China*

[3]*Systems Division, Hamamatsu Photonics K.K., 812 Joko-cho, Higashi-ku, Hamamatsu 431-3196, Japan*

**Corresponding author: leo@mail.hust.edu.cn*

Received April 3, 2013; revised April 24, 2013; accepted April 24, 2013;
posted April 24, 2013 (Doc. ID 188069); published May 20, 2013

We present a camera embedded data processing method for localization microscopy (LM) with faster detectors such as scientific complementary metal-oxide semiconductor (sCMOS) cameras. Based on the natural sparsity of single molecule images, this method utilizes the field programmable gate array chip inside a camera to identify and export only the regions containing active molecules instead of raw data. Through numerical simulation and experimental analysis, we found that this method can greatly reduce data volume (<10%) with negligible loss of useful information (<0.2%) at molecular densities <0.2 molecules/μm^2, thus significantly reducing the challenges of data transfer, storage, and analysis in LM. © 2013 Optical Society of America

OCIS codes: (180.2520) Fluorescence microscopy; (100.6640) Superresolution; (110.2960) Image analysis.
http://dx.doi.org/10.1364/OL.38.001769

Super-resolution localization microscopy (LM) has been recently recognized as one of the most promising tools in biology. The operating principle of LM is relatively simple: sparsely distributed active molecules are localized precisely in each image frame and hundreds or even thousands of image frames are employed to build a final super-resolution image [1]. Therefore, massive amounts of data need to be handled (including data transfer, storage, and analysis) for LM imaging, especially with the advantageous use of scientific complementary metal-oxide semiconductor (sCMOS) cameras [2]. Utilizing better-performing sCMOS cameras will significantly broaden the power of LM imaging in applications where a high imaging speed and large imaging field-of-view are both desired, for example, visualizing the connectivity and dynamics of neural circuits extending over vast volumes [3].

However, using sCMOS cameras in LM imaging faces a huge amount of data flow (up to 1 GB/s), which typically requires the use of several high-speed camera-links and fast frame-grabbers for online data transfer and a complex and expensive redundant array of independent disks system for online data storage. As for further data analysis, it is still not possible even with the fastest analysis software reported in the literatures [4–7]. Therefore, the huge data flow is the major source that hinders the advantageous use of sCMOS cameras in LM imaging. Note that the reported software are capable of achieving online data analysis when using cameras with low data flow, for example, electron multiplying charge-coupled device cameras where data flow is typically <20 MB/s.

It is well known that useful data in LM imaging (that is, the fluorescence spots containing active molecules) are sparsely distributed in raw images. Hence, if we export only the spot regions instead of the raw images to the computer, we can significantly reduce the amount of data flow, data volume, and the computation duty in further data analysis. On the other hand, we realized that sCMOS cameras are typically loaded with field programmable gate array (FPGA) intelligence for offset and gain correction and/or time sequence control. As a general and programmable hardware platform, FPGA provides a unique opportunity for performing embedded data processing (EDP) in cameras.

In this Letter, we take advantage of the power of FPGA programming and demonstrate that data flow in LM imaging can be significantly reduced, if we identify and export only the spot regions through embedded data processed in camera. Through simulation and experimental analysis, we verify that this camera EDP method can perform online data flow reduction with negligible information loss. Although the original images are lost during the EDP process, this method greatly reduces the challenges of massive data transfer, storage, and analysis in LM imaging.

Let us start from a general description of LM imaging with the EDP method [Fig. 1(a)]. First, the emitted photons from active molecules are collected by an objective and delivered to the sensor of a camera. Then, photons are transferred to electrons by the sensor, and the analog signals in each pixel are converted to digital signals with column parallel A/D convertors in the sensor. Next, the pixel values of the sensor are read in FPGA for offset and gain correction. After that, FPGA is used to identify and extract the regions containing active molecules. Finally, the camera exports the spot regions to a computer for further data storage and analysis.

The hardware implementation of the EDP method is shown in Fig. 1(b), which consists of mainly three modules: image denoising, peak finding, and spot extraction.

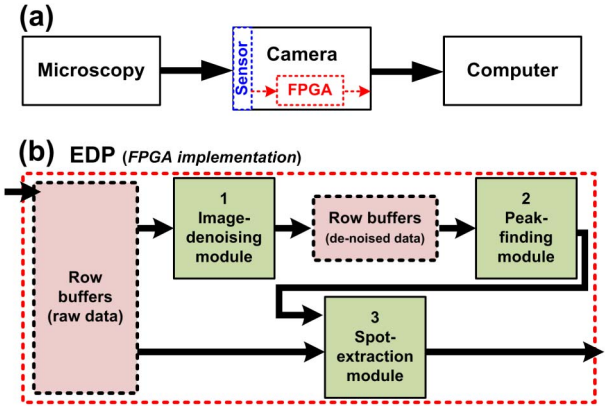

Fig. 1. FPGA-based implementation for EDP. (a) General description of LM imaging using the EDP method. (b) Architecture of the EDP method inside FPGA. Here, the row buffers for pixel caching are built from the internal memory of FPGA, and the logic elements are used for implementing the function modules.

The modules are designed to be consistent with the general processes of spot detection in LM.

First, the **image-denoising module** uses a bandpass filter ([−1/16 −1/16 −1/16 −1/16 −1/16, −1/16 1/9 1/9 1/9 −1/16, −1/16 1/9 1/9 1/9 −1/16, −1/16 1/9 1/9 1/9 −1/16, −1/16 −1/16 −1/16 −1/16 −1/16]) to spatially smooth the raw image and suppress the local background [4]. In addition, a series of row buffers are built for pixel caching, since the output pixels depend not only on the input pixels, but also their adjacent neighborhoods. The de-noised pixels flow out of this module and are cached for further steps.

Next, the **peak-finding module** processes the de-noised data with a nonmaxima suppression filter to find the pixels with local maximum values. Meanwhile, if the peak pixel values are higher than a given threshold (e.g., >3 times the standard deviation of the background noise), these peak pixels are recognized as candidate spot centers.

Finally, the **spot-extraction module** extracts the spot regions if their center pixels are recognized as candidate spot centers. These spot regions are cached and transferred to a computer for further data storage and analysis.

The performance of the EDP method was evaluated both numerically and experimentally with the following parameters: data reduction ratio (DRR), information loss ratio (ILR), and processing speed. Here, the DRR is defined as the total data volume of the extracted spots divided by the data volume of the raw data, and the ILR is calculated and averaged from the photons not preserved in the extracted spots divided by the total photons of the spots. The evaluation was performed on a test environment, which has the following FPGA chip: Altera Cyclone IV EP4CE40. Slightly different from real experimental implementation, the simulated and experimental data were delivered to the FPGA chip from a computer instead of an image sensor. At each clock cycle, four pixels were read in FPGA. Then the EDP method was performed according to the procedures described in Fig. 1(b). Note that sending data from the computer provides a better chance of obtaining statistics.

In the numerical simulation, simulated images with 512×512 pixels were generated according to Ober *et al.* [8], and the centers of the spots were randomly distributed in the center 506×506 pixels of the image. The point spread function (PSF) was modeled with Gaussian function, and the width of Gaussian kernel (σ_{PSF}) was set to be 1 pixel, resulting in a PSF of 6×6 pixels consistent with the experimental LM image dataset used in this study. The total photons of the spots were kept to a log-normal photon number distribution (with a peak at 3000 photons and standard deviation of 1700 photons) to mimic the fluorophore (Alexa 647) used in the experiment [9]. The background was set to be 70 photons per pixel consistent with the experimental dataset. For each molecule density, we generated and analyzed a total number of 1600 images (~420 megapixels), corresponding to the same data volume, which was generated from a Hamamatsu Flash 4.0 sCMOS camera within one second. Considering the photon distribution of the spots in the spot extraction step, we used a box with a radius of 3 σ_{PSF} to extract most (>99.9%) of the photons emitted from active molecules and neglected the photons at the tail of the airy disk. This treatment ensures the DRR, while minimizing information loss.

With the simulated images, we first evaluated the DRR and the ILR of our EDP method. From the simulation results (Fig. 2), surprisingly we found out that the DRR is <10% at molecular densities <500 molecules/frame (corresponding to 0.2 molecules/μm^2 if assuming a pixel size of 100 nm), while the ILR is less than 0.2%. This means that under these molecular densities, the data flow can be reduced to <100 MB/s, a speed that can be easily accomplished by one camera-link and one common hard disk. For higher molecular densities, we found that our EDP method is still effective even with molecular densities up to 8000 molecules/frame, corresponding to ~3 molecules/μm^2 if assuming a pixel size of 100 nm. Note that the molecular densities of >1500 molecules/frame (where the nonoverlapping ratio, NOR < 50%) are already within the realm of high density localization algorithms [9].

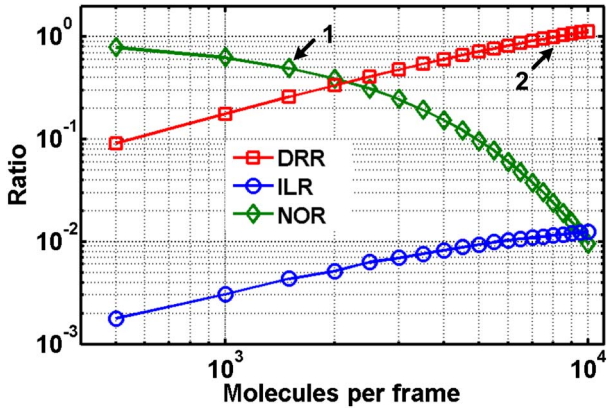

Fig. 2. Performance of the EDP method in simulation data under different molecular densities. Here, the NOR is defined as the number of well separated single molecules divided by the total number of molecules. Arrow 1 shows the molecular density where NOR degrades to 50%, and Arrow 2 points out the molecule density where DRR exceed 100%. Note that the image size is 512×512 pixel.

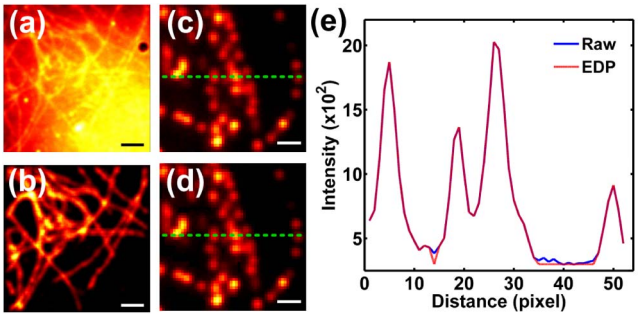

Fig. 3. Evaluating the performance of the EDP method with experimental data. (a) Overlay of a total number of 9990 raw image frames. (b) Overlay of all the extracted spots from the 9990 image frames. (c) Raw high density image frame. (d) Overlay of the extracted spot regions from the raw image shown in (c). (e) Intensity profiles along the dotted lines in (c) and (d). Note that the box size for spot region extraction is 9×9 pixels and the background in (d) was set to be 300 photons per pixel.

Next, we investigated if it is possible to perform online data processing with the EDP method. We found that the EDP method consumed about 0.8 s to process a simulation dataset of 512×512 pixels, corresponding to a speed of 532 *m*egapixels/s. Obviously, this speed is capable of realizing online spot identification and extraction in LM imaging with the Hamamatsu Flash 4.0 sCMOS camera. Furthermore, we combined the EDP method with a fast localization algorithm called MrSE [7] and realized online data analysis in full routine in LM imaging with the sCMOS camera (data not shown), which is not possible by current algorithms [4–6].

It would be of great interest to evaluate the performance of the EDP method in analyzing real experimental data. Therefore, we employed an open LM image dataset where a fixed cell was stained with the widely used fluorescent probe Alexa 647 [10]. Overlay of all the raw images is shown in Fig. 3(a). Overlay of all the spot regions extracted by the EDP method is shown in Fig. 3(b). Surprisingly, we found that the volume of this dataset was reduced ~50 times (from ~300 to ~6 MB), while the sub-cellular structures were well preserved (data not shown). Furthermore, we used a high density experiment dataset [11] to evaluate the performance of the EDP method in high density LM imaging. We compared the raw image [Fig. 3(c)] with an overlaid image from the extracted spot regions [Fig. 3(d)], and found that the structures and information in the raw image are well preserved (with a correlation coefficient up to 0.9995) [Fig. 3(e)]. On the other hand, because the data processing speed is determined by the operating clock of the FPGA chip (133 MHz in this Letter), the processing speed of the EDP method in the experimental data is still the same as that of the simulation data.

The hardware implementation of the EDP method was carried out on a QuartusII (Altera) with the hardware description language named VHDL. We performed the EDP method on a middle-size FPGA chip (Altera Cyclone IV EP4CE40, see http://www.altera.com/literature/lit-cyclone-iv.jsp), and only ~15% of the logic elements (total: 39,600; used: 6000) in this chip were occupied for the EDP method. These findings indicate that many cameras can utilize this EDP method to perform effective data flow reduction with low cost and high performance, which surely brings great benefits for massive data transfer, storage, and analysis. Moreover, we note that (1) the image-denoising and spot-finding strategies used in the current EDP method can be further improved if necessary; (2) although the EDP method is efficient in molecular densities up to 8000 molecules/frame, we recommend performing this method at lower densities (<1500 molecules/frame) to maintain good control on the NOR; and (3) the EDP method is essentially superior to other general data compression algorithms, since data analysis from the extracted spot regions will be much faster than that from the raw image.

In conclusion, we proposed a hardware data processing method to significantly reduce data flow with negligible information loss. The so-called embedded data processing (EDP) method is based on the natural sparsity in LM images and utilizes the existing FPGA chip in a camera. We demonstrated that the EDP method could realize online identification and extraction of fluorescence spots from active molecules, thus significantly reducing the challenges of massive data handling for LM imaging with sCMOS cameras. We believe that this method has great potential to enable the advantageous use of faster detectors in LM imaging.

This work was supported by the National Basic Research Program of China (Grant No. 2011CB910401), the Science Fund for Creative Research Group of China (Grant No. 61121004), and the Program for New Century Excellent Talents in University of China (Grant No. NCET-10-0407). We thank Prof. Suliana Manley from Ecole Polytechnique Federale de Lausanne (Switzerland) for providing the experimental LM images.

References

1. G. Patterson, M. Davidson, S. Manley, and J. Lippincott-Schwartz, Annu. Rev. Phys. Chem. **61**, 345 (2010).
2. M. Baker, Nat. Methods **8**, 1005 (2011).
3. J. W. Lichtman and W. Denk, Science **334**, 618 (2011).
4. T. W. Quan, P. C. Li, F. Long, S. Q. Zeng, Q. M. Luo, P. N. Hedde, G. U. Nienhaus, and Z. L. Huang, Opt. Express **18**, 11867 (2010).
5. R. Henriques, M. Lelek, E. F. Fornasiero, F. Valtorta, C. Zimmer, and M. M. Mhlanga, Nat. Methods **7**, 339 (2010).
6. S. Wolter, A. Loschberger, T. Holm, S. Aufmkolk, M. C. Dabauvalle, S. van de Linde, and M. Sauer, Nat. Methods **9**, 1040 (2012).
7. H. Q. Ma, F. Long, S. Q. Zeng, and Z. L. Huang, Opt. Lett. **37**, 2481 (2012).
8. R. J. Ober, S. Ram, and E. S. Ward, Biophys. J. **86**, 1185 (2004).
9. L. Zhu, W. Zhang, D. Elnatan, and B. Huang, Nat. Methods **9**, 721 (2012).
10. http://bigwww.epfl.ch/palm/?p=tubulin-af647.
11. http://bigwww.epfl.ch/smlm/challenge/index.html?p=datasets-real.