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OpenSTED: Inexpensive and open-source implementation of Dynamic Intensity Minimum (DyMIN) for Stimulated Emission Depletion (STED) microscopy

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Abstract: The DyMIN method reduces photobleaching, a problem in STED microscopy. Labs implementing custom-built STED microscopes would greatly benefit from DyMIN capabilities. We present an inexpensive, open-source version utilizing an FPGA and multiplexer.

1. Introduction

In 2000, Stefan Hell demonstrated the Stimulated Emission Depletion (STED) microscope using an approach called point spread function engineering, which breaks the diffraction limit resulting in as much as a ten-fold improvement in resolution [1]. STED microscopy uses an additional laser beam (shaped like a donut) at the red tail of the emission wavelength, that depletes off-center fluorescence through the process of stimulated emission. To achieve ~40-50 nm resolution, the STED laser must be up to three orders of magnitude higher power than the excitation. One of the most powerful applications of the STED microscope is live cell imaging, but the powers required cause unwanted photobleaching for many samples, limiting the number of frames that can be acquired with sufficient signal-to-noise.

To overcome this problem, the Hell group first demonstrated Dynamic Intensity Minimum (DyMIN), whereby the STED laser is modulated between zero and several incremental powers at a given pixel, depending on the number of fluorescence photons collected [2]. In this way, the maximum STED power is only used when a fluorophore is present within the effective point spread function, and lower (or zero) power is used when there is no fluorophore present. Thus, the total amount of optical energy impinging on the sample is greatly reduced. DyMIN shows dramatic improvement to the achievable signal to noise ratio (SNR) in 3D STED imaging and allows serial acquisition of images over the same FOV without degrading the SNR.

Unfortunately, implementing DyMIN in a custom-made microscope can involve substantial effort and is not commercially available as an add-on. We demonstrated DyMIN using Labview software to control an inexpensive FPGA with an additional multiplexer circuit. Code, materials and design files are available on Github.

2. Results and Discussion

The DyMIN program takes multiple steps at each pixel, with user-selected counts thresholds (T_n), STED power levels (P_n) and times (t_n) for each step. At each DyMIN step, the program counts the fluorescence photons (N_n) collected while the sample is illuminated with STED power P_n for time t_n, with $P_n > P_{n-1}$. The program moves on to the n+1-th step if $N_n > T_n$. If the sample's collected fluorescence fails to reach threshold, the program aborts counting, turns the STED laser off and waits until the pixel clock (PIX CLK) triggers the next pixel to start the DyMIN steps again. At the final DyMIN step the counts N_n are recorded at that pixel to form the STED image.

Our basic experimental schematic is shown in Figure 1. Further details of a custom STED microscope developed by our lab are described in [3]. An AOM and RF driver controls the STED laser power, while the excitation laser (EXC) power remains the same throughout the DyMIN imaging. The fluorescence signal (FLUOR) is detected with an avalanche photodiode (APD) and sent to the FPGA (NI myRIO-1900) programmed with Labview FPGA. Slidebook (3i) is used for image acquisition, synced to a pixel clock sent from the DAQ on the computer (NI PCIe-6259) to the FPGA. The modified/unmodified (DyMIN/Conventional STED) APD signal is sent from the FPGA to the computer DAQ counter input recorded in Slidebook software. Four analog output (AO_n) channels on the FPGA are connected to the analog inputs of a multiplexer circuit (Analog Devices Inc, ADV3221 Evaluation Board). The AO_n values are chosen to achieve STED power P_n when AO_n is sent to the RF driver from the multiplexer. Digital

output channels DO0-3 from the FPGA select which analog output power the multiplexer passes to the RF driver based on the multiplexer's truth table. Our FPGA code and further details on the electronics are publicly available for download at https://github.com/CUNeurophotonics/DyMINSTED4all.

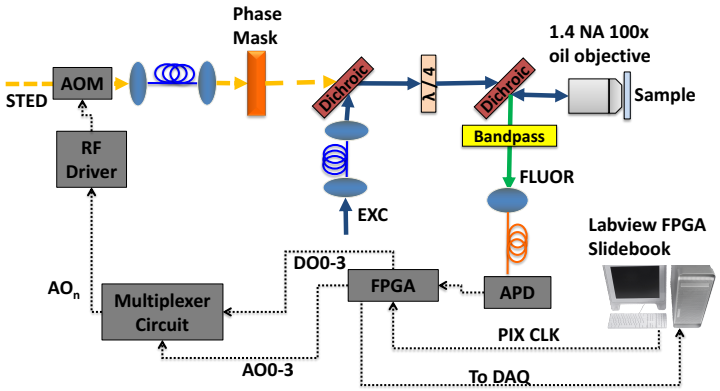

Fig. 1. Schematic of set up for DyMIN implementation

For a first demonstration of our DyMIN implementation, we imaged 100 nm Yellow-Green FluoSpheres™ (ThermoFisher Scientific F8803) with conventional STED and with DyMIN for a time series of 20 images. The DyMIN settings were P_1= 0 mW, P_2=15 mW, P_3=60 mW, T_1=15 counts, T_2=10 counts, t_1=10 µs, t_2=10 µs, t_3=100 µs. A 100 µs dwell time at P=60 mW was used for the conventional STED image. Figure 2A and B show the first DyMIN and the first conventional STED image in the sequence (5 µm x 5 µm), C and D show the line cuts indicated in A and B, and E and F show 20 µm x 20 µm confocal images taken after the DyMIN and conventional STED image sequences, respectively. Figure 2F shows a resulting dark region in the middle due to photobleaching, as conventional STED resulted in near complete photobleaching, while DyMIN reduced this effect. Fig. 2G shows the maximum counts for the image sequence, quantifying the much lower relative bleaching with DyMIN compared to conventional STED imaging over the course of the imaging sequence.

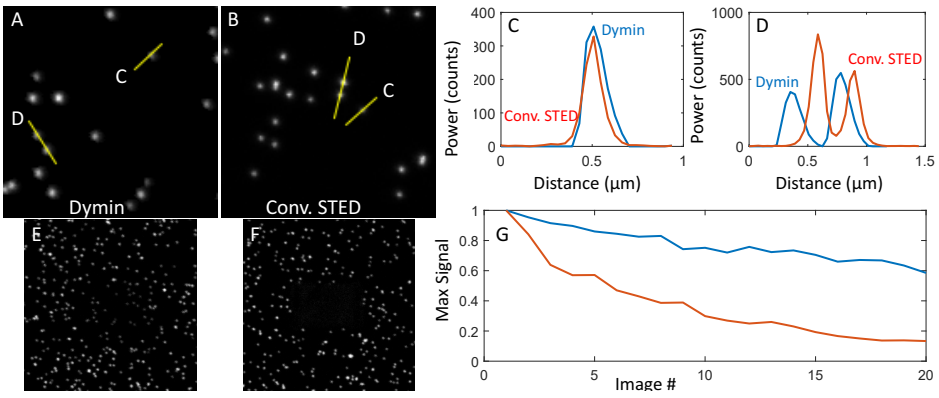

Fig. 2. Images of fluorescent beads comparing DyMIN vs Conventional STED.

In conclusion, we have demonstrated photobleaching suppression during time-lapse STED imaging by implementing the DyMIN technique. Our Labview code and electronics designs are publicly available and can be used by other labs to improve STED imaging, particularly time series imaging of biological samples.

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3. References

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