When higher speeds mean more photons: Using the Kinetix at Kirill Volynski's lab

Dr Louis Keal – Version A1, 3/11/2020

Background

The Volynski lab, led by Kirill Volynski, Professor of Neuroscience at University College London (UCL) are primarily interested in understanding cellular regulation of synaptic release of neurotransmitters which forms the basis of communication among neurons in the brain.

As explained on the lab's website, "Synapses between neurons are critical sites of modulation and plasticity, both in health and in disease. Therefore a detailed knowledge of the cellular mechanisms that regulate synaptic transmission at the level of individual synapses is a prerequisite for understanding the operation of complex neuronal circuits.

"We have recently developed new imaging methods which, for the first time, allow us to study the relationship between Ca2+ entry and vesicular exocytosis, and to probe presynaptic ion channel function in individual small presynaptic terminals. This is based (i) on measuring, with fluorescence microscopy, rapid changes in the concentration of Ca2+ ions, as well as the rate at which small vesicles containing chemical neurotransmitters are discharged, and (ii) on super resolution scanning ion conductance microscopy for patch-clamp recordings from small presynaptic boutons.

"Using these methods we investigate how different channels that mediate Ca2+ influx into the terminal control the release of vesicles, how they influence synaptic plasticity, and how synapses are influenced by other modulatory neurotransmitters acting upon presynaptic terminals."

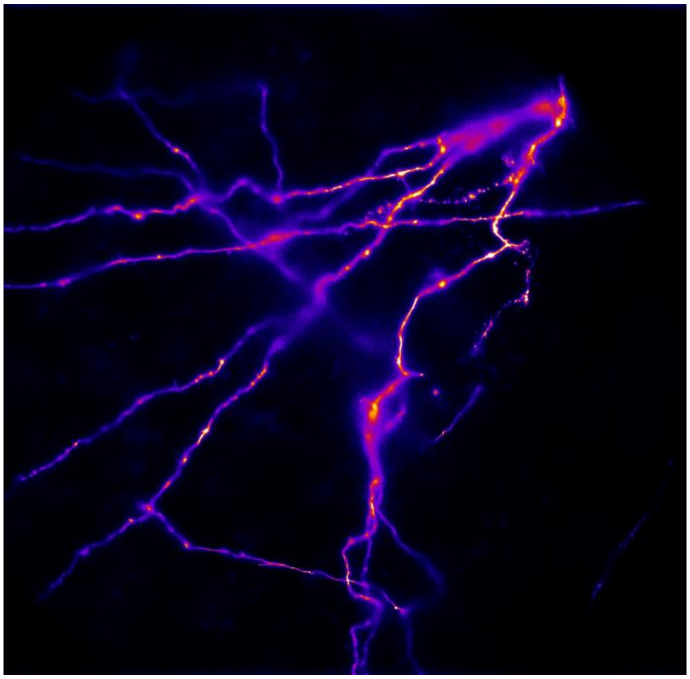

Figure 1: Axonal arbor of a hippocampal neuron in culture expressing glutamate sniffer SF-iGluSnFR probe, acquired with the Kinetix sCMOS.

The Challenge

High speed Ca^{2+} imaging requires both sensitivity and speed. Previously Kirill's lab was using a Prime 95B 25mm to maximise sensitivity and field of view, while achieving high speeds of acquisition. This camera provided a considerable upgrade to the EMCCD they were previously using in speed, field of view, and stability. But the speed of the camera was still a limiting factor both for keeping up with the high speed dynamics in the sample, but also for light acquisition, due to the necessity to use 'pseudo-global shutter' triggering controlling the light source.

Global shutter refers to the simultaneous acquisition of the entire field of view of a camera. This differs from Rolling Shutter, which is the standard sensor design for low-light imaging cameras, in which the acquisition of a frame starts at the top of the sensor, and very quickly sweeps down to the bottom of the sensor. Although the time difference between the top and bottom of the sensor is very small, it can introduce distortions into highly precise high-speed experiments, so 'pseudo-global shutter' must be used.

Sensors that use a rolling shutter such as the Prime 95B are capable of much lower noise and much higher speeds than 'true' global shutter CMOS cameras. We are able to achieve global sensor behavior using triggering of the light source to control when photons actually reach the sensor. This is explained in the diagram below.

In pseudo-global shuttering, the camera begins acquiring a frame at the top of the sensor and the acquisition rolls down with the trigger to the light source **deactivated**, and no photons collected. Only when **all** of the rows of the camera are acquiring does the light source activate, acquiring global information, before then deactivating for the rolling readout process and the start of the next frame.

The 'dead time' required to roll down the sensor and begin acquiring every row, called the 'Frame Time', is directly determined by the camera frame rate. The overall time for a complete frame is this Frame Time plus the 'Trigger On' time, which is sometimes in this context called the 'effective exposure time'.

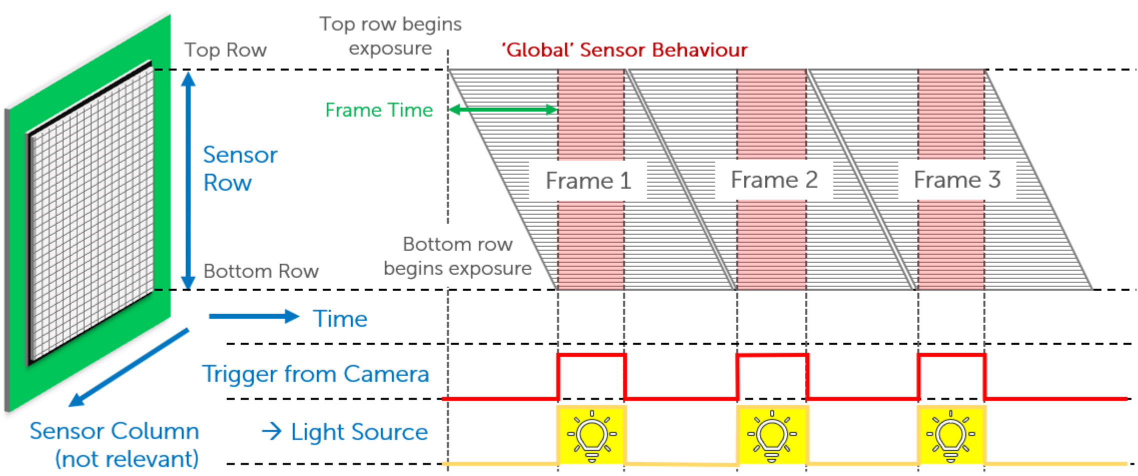

Figure 2: The timing of rolling shutter cameras, and using triggering of the light source to achieve global behavior.

The Kinetix: Faster frame times bring multiple advantages

The Kinetix is a groundbreaking scientific CMOS camera that provides the same near-perfect quantum efficiency as the Prime 95B thanks to its back-illuminated sensor, while measuring signals with more accurately with the low read noise of its 'Sensitivity' mode. But the real improvement comes in both frame rate and sensor size. The Kinetix is a 10 Megapixel camera with an enormous 29.4mm diagonal sensor - in its 'Sensitivity' mode, this entire field of view can be read out at 88 frames per second. But the Kinetix also has a 'Speed' mode, where all 10 MP are read at an astonishing 500 frames per second.

'Sensitivity' mode: Same speed, more than double the illumination time

Thanks to these increases in read out speed, when using regions of interest to achieve high speeds with pseudo-global shuttering either far larger regions of interest can be used to provide more vertical field of view, or as was vital for Kirill's lab, the 'trigger on' time or 'effective exposure time' can be extended as demonstrated in the diagram below.

For an example region of interest of 200 rows (columns does not matter for speed), with an intended acquisition frame rate of 300 frames per second (3.3ms per frame), the Frame Time for the Prime 95B is 2.08ms, leaving 1.25ms for the light source to be on and photons to be collected. The Kinetix on the other hand reads the same number of rows in just 0.71ms, leaving 2.6ms for the light source to be on - more than double that of Prime 95B.

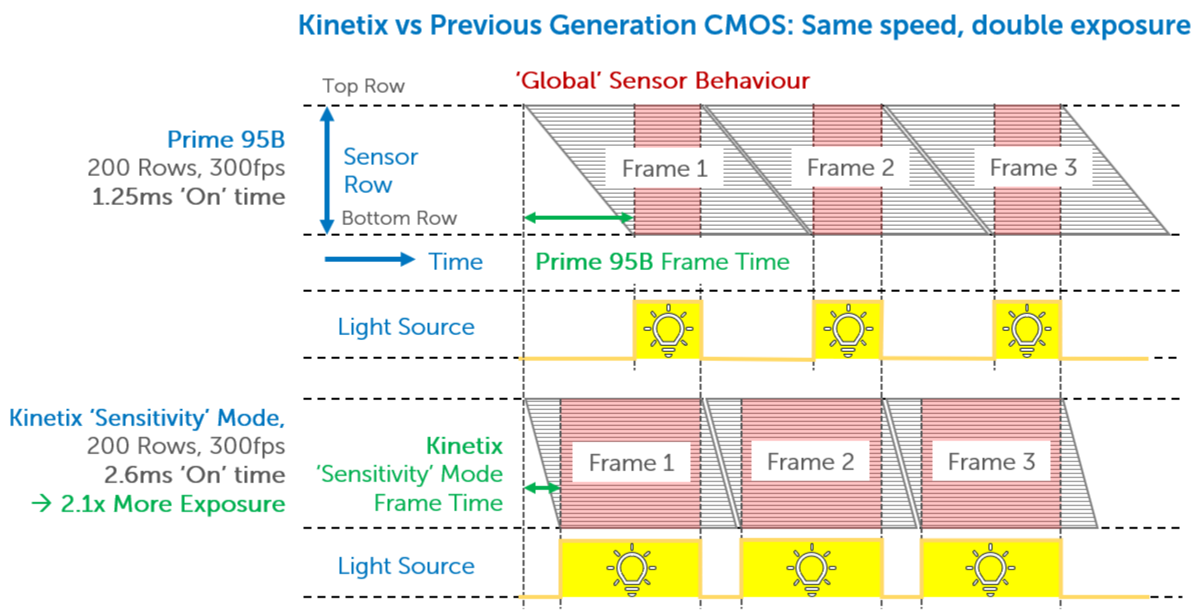

Figure 3: Example comparison of acquisition timing in Pseudo-Global Shutter mode for Prime 95B and Kinetix sCMOS cameras. In this example, a target imaging rate of 300 frames per second is set and a region of interest is used 200 rows in height. Due to the shorter frame time, with the same acquisition speed the Kinetix in its 'Sensitivity' mode is able to achieve a significantly longer 'Trigger On' time during which light can be collected.

Kinetix 'Speed Mode': Increasing speed and illumination time

For Kirill's imaging application, high speeds are also critical. The 'Speed' mode of the Kinetix provides another step change up from previous generation CMOS cameras – this time, the Frame Time is so short, just 0.13ms, that there is time for a 200 row acquisition to run at 600 frames per second. Even despite this doubling of the acquisition speed, the trigger on time is still 20% longer than the Prime 95B at 1.54ms, providing both more speed and more illumination time. This is shown below.

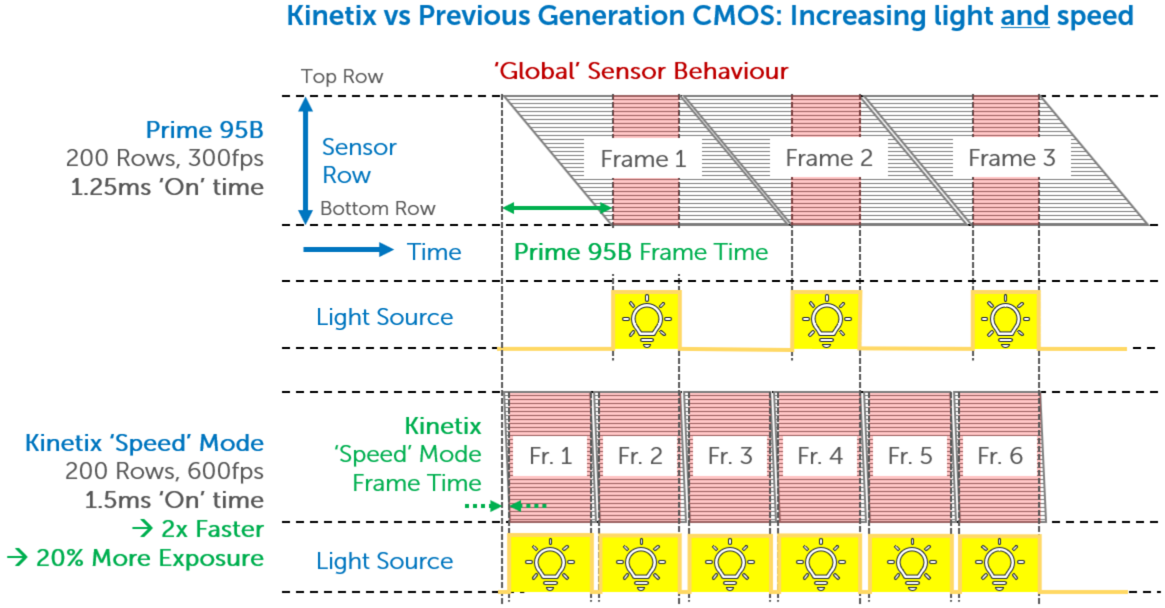

Figure 4: Example comparison of acquisition timing in Pseudo-Global Shutter mode for Prime 95B and Kinetix sCMOS cameras. In this example, a target imaging rate of 300 frames per second is set and a region of interest is used 200 rows in height. In its 'Speed' mode, the frame time of the Kinetix is so much faster than the Prime 95B that twice the number of frames can be collected in the same time period, but also maintaining a 20% longer 'Trigger On' time per frame during which light can be collected.

Conclusion

The speed increase the Kinetix provides over previous generation sCMOS cameras can lead to a significant increase in effective exposure time for increased light collection in pseudo-global shutter applications with its 'Sensitivity' mode. Furthermore, the incredibly fast frame time of the 'Speed' mode can provide speed increase combined with effective exposure time increases.

The Kinetix also does this while delivering an 18% larger horizontal field of view, due to the larger width of the Kinetix sensor (which does not affect speed or frame time).