LightScan PLUS describes a functionality of the Zyla 4.2 PLUS camera that allows the end-user to have more control over the Rolling Shutter scanning mechanism that is inherent to CMOS sensors. This functionality furthermore enables the user to finely synchronize to a range of illumination scan options and to minimize dead time between scans. This scanning mode can be readily adapted to niche optical modalities, such as Scanned Light Sheet microscopy or Line Scan Confocal Microscopy.

Application of LightScan PLUS to Scanning Light Sheet Microscopy

Light Sheet microscopy is utilized to a large extent to investigate the development of living embryos, for which the samples under examination can be relatively large and thick. By virtue of this, it can be quite difficult to acquire highly resolved images due to the scattering of light and low contrast in such samples. In a 2012 paper titled “Scanned light sheet microscopy with confocal detection”,[1] Baumgart and Kubitscheck described an approach whereby a laser beam is swept across the focal plane, resulting in a planar illumination equivalent to a sheet but with improved illumination efficiency and uniform intensity distribution. LightScan PLUS functionality enables the user to readily synchronize the ‘rolling window’ exposure mechanism that is fundamentally inherent to rolling shutter CMOS sensors to the scanning of their illumination beam. Image quality is thus improved since the scan row height can act as a slit detector, rejecting scattered light, improving contrast and SNR and hence providing sharper and more resolved images.

Figure 1 illustrates the Rolling Shutter scanning mechanism, adapted from this paper.[1] As each row starts to expose sequentially down the sCMOS sensor, the sensor essentially acts as a rolling exposure window of pixels that are exposing simultaneously. By synchronizing the scanning light source with the rolling exposure window one can combine the rolling shutter exposure slit with the scanning light source.

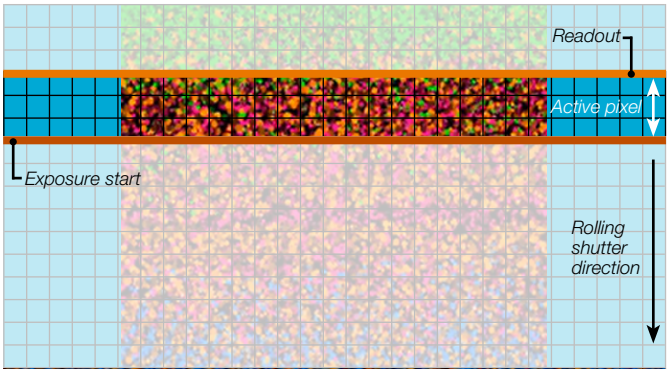

Figure 1: Rolling Shutter mode. The highlighted window of pixel rows is exposed to light. This band sweeps from the top to the bottom of the sensor and can be synchronized to the sweep of an illumination laser.

With LightScan PLUS, the user can scan their light sheet from the top to the bottom of the sensor or vice versa in one continuous sweep. Precision control is available through FlexiScan functionality, allowing independent control over the scan row height (‘slit height’) and line scan speed. Furthermore, LightScan PLUS provides multiple different readout directions allowing the user further flexibility.

Multiple readout directions

The standard mode of operation in sCMOS cameras is to read out from the centre of the sensor out to the edge with two halves of the sensor exposing simultaneously. LightScan PLUS provides the user with a range of different rolling shutter readout options. Firstly, the rolling shutter can now be scanned from the top to the bottom of the sensor or vice versa in one continuous sweep (Figure 2), adapting the sensor to single port readout. It should be acknowledged however that this functionality will halve the rolling shutter frame rate, resulting in a maximum full resolution frame rate of 50 fps.

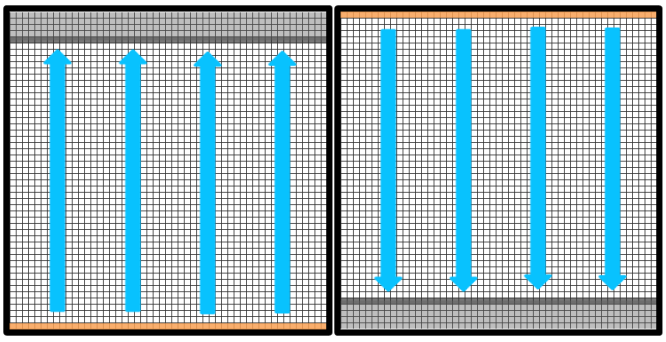

Figure 2: LightScan PLUS enables the rolling shutter to scan the sensor from top to bottom or vice versa in one continuous sweep. This is also known as Single Port Readout.

Furthermore, LightScan PLUS offers the user multiple new scanning directions for the rolling shutter mechanism in which both sides of the sensor can be used simultaneously, synchronized to two scanning lasers, thus providing the option to utilize the full ‘Dual Port Readout’ capability of the sensor and provide 100 fps full resolution frame rate.

These new scanning options for sCMOS are also ideal for dual-wavelength applications where two light sources are scanning across the image sensor

with different wavelengths. The multi-laser readout configurations are illustrated below in Figure 3.

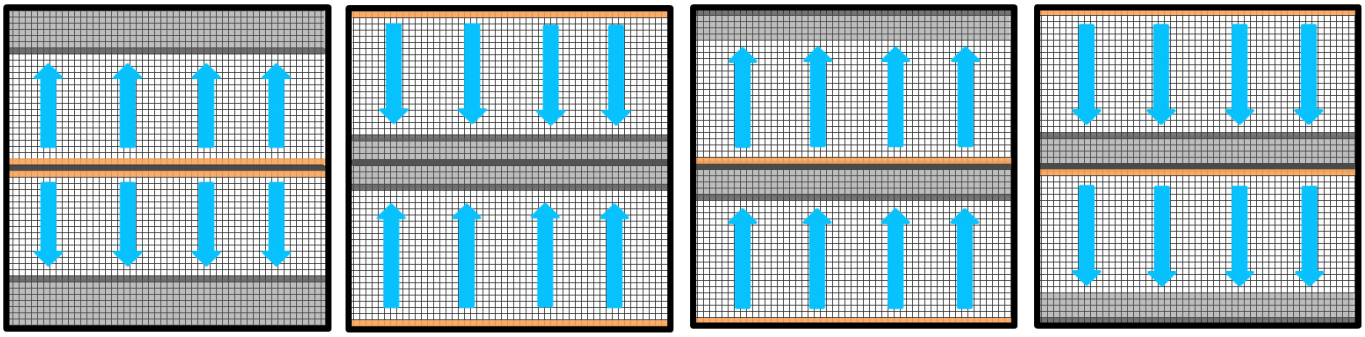

Figure 3: Multiple 'dual laser' scanning options available. The standard rolling shutter scan mode (Centre outwards in both directions simultaneously) is illustrated on the left along with the three additional scanning options.

For each of the various scanning options outlined above, **CycleMax** - a feature of LightScan PLUS - ensures minimum dead time between scans by enabling the laser sweep and corresponding rolling shutter scan direction to alternate from top-bottom to bottom-top, therefore avoiding the need to reset the laser for each subsequent image. Furthermore, a programmable trigger delay has been built into LightScan Plus enabling the user to input a defined delay time between the camera receiving an external trigger and the acquisition start. This is important when using peripheral devices e.g. controlling the illumination source.

FlexiScan

It has been reported previously[1] that since the length of the rolling shutter exposure time is governed by the rolling shutter 'slit width', then as the exposure time is increased the slit width is also increased. This carries the disadvantage that for weakly fluorescent samples for which the exposure time must be extended to increase the signal to noise ratio, then the slit width would also be increased, thereby reducing the contrast and confocality in the final image.

However, it was furthermore stated by the authors that a straightforward solution would be to make the cycle time between activation of rows adjustable by the user. That is to say, in order to have an increased signal and maintain confocality concurrently, it would require that the exposure time and slit width be controlled independently. This is effectively achievable through the FlexiScan feature of LightScan PLUS, by virtue of the user being able to independently adjust the line scan rate, a capability that is inherent to rolling shutter CMOS sensors. Table 1 denotes the scan speed range flexibility of LightScan PLUS in terms of scan speed.

Parameter	*216 MHz	*540 MHz
Line scan speed range (rows/ms)	2.98 - 41.67	7.43 - 104
Resultant scan time for one full image (ms)	49 - 686	19.69 - 275.66
Resultant exposure range for slit width of 10 rows (ms)	0.240 - 3.36	0.096 - 1.344

Table 1: Range of Line Scan Speeds available through FlexiScan for each pixel readout rate. Also shown is the resultant range of scan times for one full image (full top to bottom or bottom to top sweep) and the resultant exposure range, assuming a 'slit width' of 10 rows.

References

1. Optics Express, Vol. 20, Issue 19, pp. 21805 – 21814, 2012.