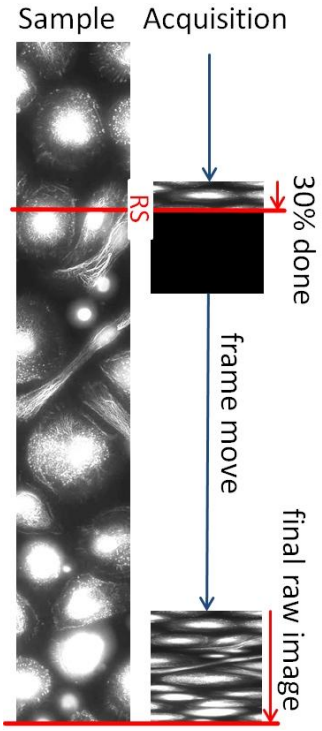
**Virtual objective: Fast continuous slit confocal fluorescence imaging with sCMOS**

**Christian Seebacher, Thomas Naiser, Alexander Hildebrand, Martin Tosstorff, Rainer Uhl**
**TILL I.D. GmbH, Am Klopferspitz 19a, 82152 Martinsried, Germany**

To image large areas with high speed and accuracy it is preferable to use a continuous motion instead of stop and go. Motion blur would be the main problem when imaging these moving samples. With CCD sensors using 'time delayed integration' mode it is possible to image moving samples [1] but modern CMOS cameras cannot support this feature [2].

We present a method that uses a sCMOS camera in combination with continuous sample movement. The rolling shutter of a sCMOS camera is synchronized with a line shaped illumination resulting in a line confocal microscope [3]. We use a PCO Panda that has 2048x2048 pixels and needs a readout time of 12µs for one line. Sample movement perpendicular to the line generates only very small motion blur. A typical 5 pixel width of illumination and rolling shutter has an exposure time of less than 50µs for each pixel. In combination with a simultaneous sample speed, for example 45mm/s, this generates a small motion blur of about 2µm. Choosing the frame movement in the direction of the rolling shutter allows a continuous movement as there is overlap after a single raw image is acquired. This technique allows very fast overview images without changing the objective to lower magnification. With a 60x objective the length of the measured line is about 222µm leading to a area measurement speed of 10mm$^2$/s at 45mm/s sample movement speed. In this example the acquired raw images are compressed in the scan direction by a factor of 6. Excluding acceleration and other overhead a fluorescence overview image of a 25x25mm area with 8 GPixel can be acquired in about 60s. Whereas using a 10x objective with a classical stop and go measurement would need about 40s for the stage movement, even with neglected time for exposure and objective change. Especially when using oil immersion, our method has the great advantage of keeping only one objective in place.




[1] Netten et al. Bioimaging 2 (1994) 184-192
[2] www.pco.de/fileadmin/user_upload/pco-product_sheets/PCO_scmos_ebook.pdf
[3] Schropp et al. Photonics 4 (2017) 33