CMOS descanning and acousto-optic scanning enable faster confocal imaging

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Abstract: Fast pixel random access capabilities of CMOS imagers are exploited to create a virtual pinhole or slit in synchrony with acousto-optic laser beam scanning to significantly speed up confocal microscopy.

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The traditional means of confocal imaging, by tilting a pair of galvanometer-mounted mirrors, reached its speed limit over a decade ago with the invention of resonant galvanometers. This limit cannot be eclipsed substantially through cleverer mirror design as it stems from the fundamental physical limit on tensile strength of materials. For this reason, other physical mechanisms of laser beam deflection have been sought to advance confocal microscopy beyond megahertz pixel rates.

One of the most interesting alternatives is acousto-optic deflection, where an acoustic wave is injected into a transparent material to form a virtual transmissive diffraction grating. The angle of diffraction is controlled by changing the frequency of the acoustic wave, permitting scanning of the diffracted beam with no macroscopically moving parts. The advantages of this scanning method over mechanical deflection are greater speed and lower latency, random access in constant time, insensitivity to environmental perturbations, simple intensity modulation by changing the amplitude of the acoustic signal, and intrinsic lensing ability which enables scanning in three dimensions with the same latency as in two dimensions.

The main impediment to wider adoption of acousto-optic deflection in confocal microscopy has been the diffractive nature of the scanner, which precludes descanning the non-monochromatic fluorescence emitted from the sample. To achieve confocality a separate descanning mechanism must be employed, *i.e.* an image of the scanned plane must be formed on a device that allows the creation of a virtual pinhole or slit, a "synthetic aperture," which is moved in concert with the position of the scanned spot.

The original acousto-optic confocal microscope was implemented in analog using an image dissector vacuum tube [1]. Other early uses of independent confocal descanning were driven by the desire to construct a confocal microscope for transmitted light [2, 3]. Here the virtual pinhole was created by projecting the image plane onto a CCD chip, picking one pixel from the frame and discarding the rest. CCDs are, however, limited to kilohertz pixel rates when descanning due to the necessity of shifting the entire frame even when only a single pixel is of interest. Further, the maximal pixel rate is inversely proportional to the dimension of the imager, making CCDs impractical if high resolution imaging is required. An alternative strategy employed line illumination and an interline transfer CCD to perform slit descanning a little faster [4]. More recently, a digital micromirror device has been used in combination with an acousto-optic deflector to create a virtual pinhole by tilting only one of the mirrors on the array so that it reflects emitted light toward a separate non-imaging detector [5]. This method is also limited to sub-megahertz pixel rates by the mechanical motion of micromirrors.

Scientific CMOS Active Pixel Sensor (APS) imagers made great strides lately [6]. Modern high performance sensors approach the performance of CCDs in every respect; notably, quantum efficiencies in excess of 80% and readout noise levels of a few electrons make the most advanced designs as good as CCDs for many scientific applications [7]. Unlike a CCD, each pixel in a CMOS APS array is an independent circuit and can contain custom logic enabling random pixel access over reset, exposure and readout. For applications requiring independent control and readout of single pixels or small regions, such as the creation of a synthetic aperture, CMOS sensors are vastly superior to CCDs since single pixel access latencies are independent of array size. We exploited this capability in combination with an acousto-optic scanner to descan images faster than possible with other methods.

The diagram of our test bench is shown in Figure 1. Excitation light was provided by a 405 nm laser diode (Sanyo DL-LS5024, pulled from an HD-DVD player), collimated with an f = 35 mm achromatic lens (AC127-030-

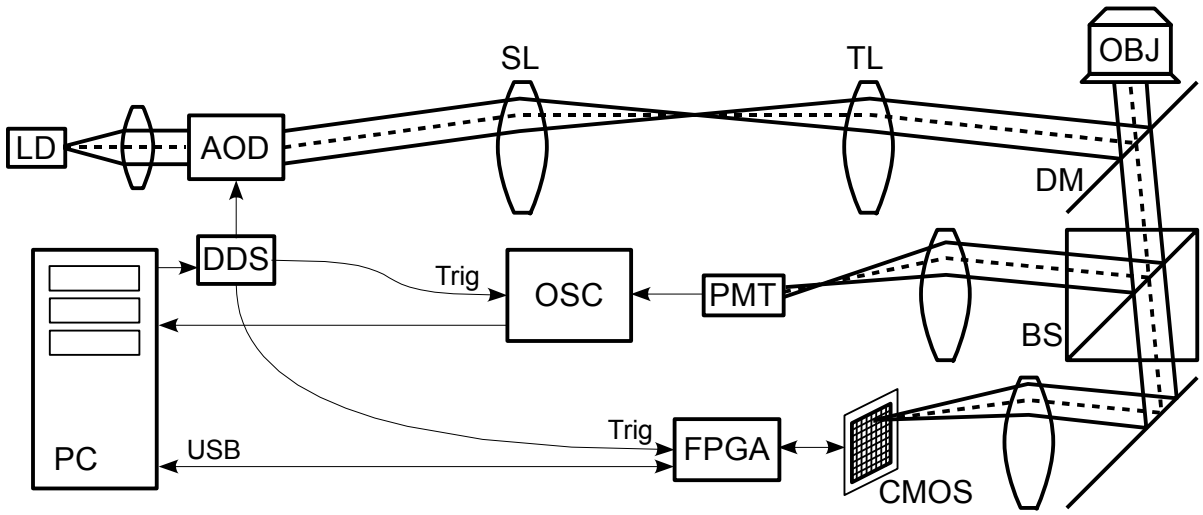

Figure 1 Diagram of our CMOS descanning test bench. LD – laser diode. AOD – acousto-optic deflector. SL – scan lens. TL – tube lens. OBJ – objective. DM – dichroic mirror. BS – beamsplitter. FPGA – field-programmable gate array. DDS – direct digital synthesis of radio-frequency signals for the AOD. PMT – photomultiplier. OSC – oscilloscope. Trig – trigger signal for synchronization of scanning and descanning.

A1, Thorlabs), deflected with an integrated 2D acousto-optic deflector (2DS-100-45-.488, Brimrose), and mapped 1:1 onto the back-aperture of an objective using two f = 100 mm achromatic lenses (AC254-100-A1, Thorlabs). Optical performance of this design was modeled using OSLO Edu software and deemed adequate. Excitation and emission were separated with a dichroic filter (Di01-R405-25x36, Semrock). Emission was further divided using a 50:50 beamsplitter (CM1-BS1, Thorlabs): one arm was collected with a singlet lens (LA1608-A, Thorlabs) and focused ahead of the active area of a photomultiplier module (7422P-40, Hamamatsu) to minimize the visibility of its surface defects; the other arm was projected onto a CMOS imaging chip with a simple two-achromat zoom (AC254-100-A1 followed by ACN254-100-A1, Thorlabs) adjusted to a focal length that maps a CMOS pixel to a size smaller than the diffraction limit of the objective in the nominal focal plane.

For these preliminary experiments we used three different CMOS imagers: an off-the-shelf 1.3 megapixel Micron MT9M413 (used to make Figure 2), and two custom made 128×128 APS chips (both measuring 3×3 mm in 0.5 μm triple-metal, double-poly CMOS fabricated through the MOSIS foundry service), which respectively had lower readout noise and pixel windowing capabilities. The imagers were clocked and read out using either a custom built USB board with a PIC processor and FTDI USB interface, or an FPGA prototyping board (Xylo-L, www.knjn.com) capable of much faster control and higher data transfer rates. Signals for acousto-optic scanning were generated with a Direct Digital Synthesis chip (AD9959/PCB, Analog Devices). The photomultiplier was read out using an oscilloscope (WaveRunner 64Xi, LeCroy). The entire system was controlled using the `neurospy` open source microscopy software. All the software, including Verilog FPGA code, is available under an open source license from the project web site at `http://neurospy.org`.

The fastest of the CMOS chips in our arsenal, the Micron MT9M413, can in principle descan entire lines at a rate of 0.435 gigapixels per second by virtue of its 1280 column-parallel A/D converters. At this speed more than two thirds of the duty cycle is spent in pixel reset, making the light collecting efficiency quite low. This limitation stems from the design of the readout logic built into the chip, which, like most other CMOS chips currently on the market, was optimized for high bandwidth of full-frame readout. The practical limit on the rate of sustained acquisition on our test setup was nearly two orders of magnitude slower than the capability of this chip, limited by the data transfer bandwidth of our USB-based prototype cameras—we were able to reach 26 frames per second with a 512×512 frame. Thanks to inertialess random access the frame rate scaled with the reduced number of scanned lines up to about a kilohertz, limited by USB bus clock. Beyond these easily defeated technical limitations, a further limit was set by the speed of the acousto-optic deflector we had at hand, which was an order of magnitude too slow to take advantage of the full speed of the Micron chip.

Descanning rates in excess of gigapixel per second can easily be achieved in the future using the same chip-making technology by designing a custom imager with logic that synchronously lifts out of reset only the pixels that are about to be illuminated, by using a faster acousto-optic scanner or other scanning technology, and by using a

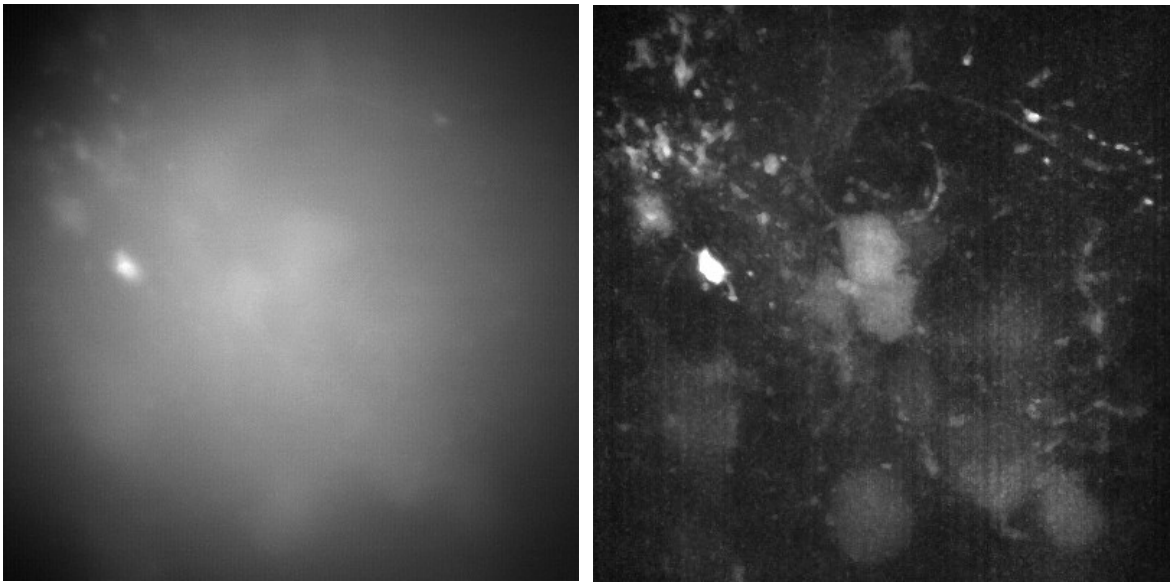

Figure 2 Maximal intensity projections of a 20 μm deep stack taken from the middle of a 40 μm thick slice of mouse brain tissue showing neurons in the dentate gyrus expressing EGFP. Non-descanned widefield detection using a photomultiplier (left) is compared to line-descanned detection using a CMOS imaging detector (right) to illustrate the improvement in contrast and resolution. Field of view is 70×70 μm.

long frame buffer or a data interface with higher bandwidth. Because of the spatio-temporal nature of detection with this descanning method, the pixel rate can actually be pushed beyond the limit set by fluorescence lifetime since photon leakage from one pixel into the adjacent ones is typically small and calculable, and a pixel can be allowed to collect laggard fluorescence photons for some time after the scanning beam has passed its location. Finally, since the optical path to the descanner is independent of the scanner, and multiple descanners are easily synchronized, the presented method is equally well suited to epi-fluorescence, back-scatter, trans-fluorescence and trans-illumination confocal imaging.

In conclusion, we introduce the use of CMOS APS image sensors as descanners for scanning microscopy. This approach can accommodate very high scanning rates and promises great flexibility: only small changes to the design of existing imagers are required to make them suitable for a variety of scanning schemes, from traditional mirror-based devices to exotic multi-spot scanners. The high sensitivities, low read noise, robustness and low cost of mass production of CMOS devices promise to make them a superior replacement for photomultipliers and avalanche photodiodes in scanning microscopy.

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