

Imaging Technology Update

SPRING 2007

VOLUME 3 ISSUE 2



**Exposed!!!
Single Photons**

At Biophysical Society 2007, Booth Number 428:

XR/Turbo-120Z: Single Molecule Imaging at 1000 Frames Per Second

NEW Piper Control™ Acquisition Software Adds Time Lapse and Device Control for Extended Collection of Circadian Rhythm Image Sequences and Extended Support for Turbo™ Speed ICCD Family of Products

NEW: SNF™ Spot Noise Filtering for Dramatic Improvement in Signal-to-Noise at Highest Gains

STANFORD PHOTONICS INC

launched the Mega-10Z™ in 2004, opening up new discovery opportunities by virtue of the product's ability to image single photons at high speeds with virtually no detector background signal. Initial installations were targeted at single molecule fluorescence and in-vivo luciferase imaging in various transfections and transgenics.

Late in 2005, we began working with investigators applying luminescent markers to brain slice and cellular level imaging of long time lapse studies correlated to Circadian Rhythm cycles and processes. In 2006, the use of the Mega-10Z™ continued to expand into new areas where the use of luminescent markers has been challenging, to say the least. The results of some of these studies are spectacular.

Over the past year, we have faced the challenge of showing that the novel technology embodied in the Mega-10Z™ does in fact work in practice as well as it does in our lab and in theory.

One of the best executed impartial benchmarks produced last summer shows clear advantages relative to traditional deep cooled CCD imaging both in signal noise and detection under very demanding conditions.

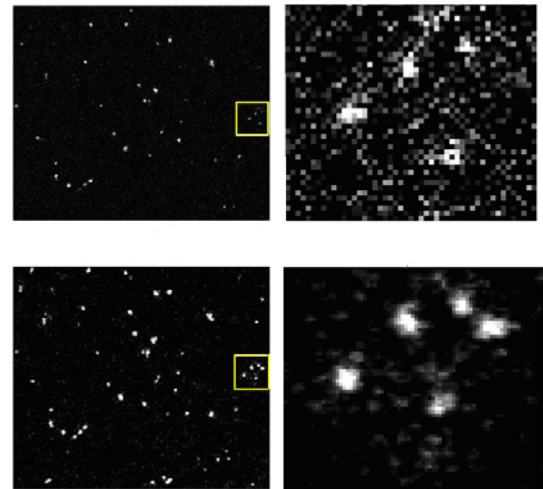
Top left, slow scanned cooled CCD, -90 C. Top right, CCD ROI.

Bottom left, XR-Mega10Z™. Bottom right, XR-Mega10Z™ ROI.

Identical image acquisition set up and conditions. Data collection details and metrics on page 2*.

Images courtesy of Dr. David K. Welsh, Scripps Research Institute

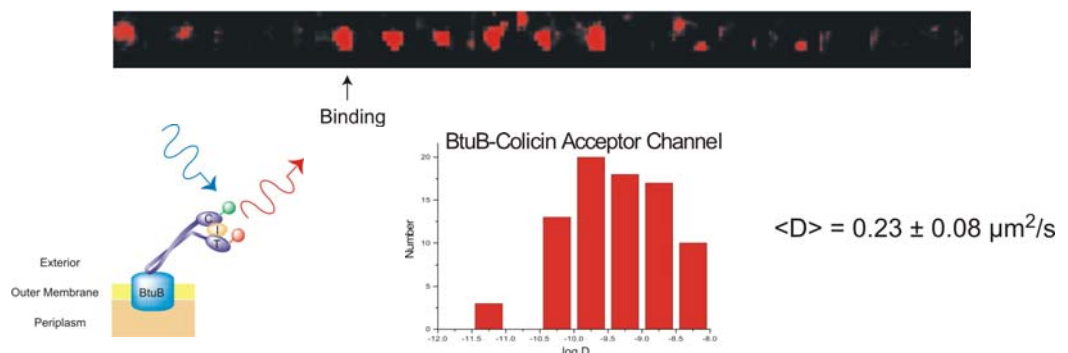
Image Details: SCN cells from Per2-luc knockin mice cultured at postnatal day 3 and imaged after 38 days in culture at 36 C. Images are cropped and scaled for optimal viewing.



XR/Turbo-120Z: Single Molecule Imaging at 1000 FPS

The XR/Turbo-120Z ICCD offers **single photon detection** with virtually zero dark counts at light gains of 2000 to 2M. **Posters 2517 and 2518 (Tuesday, March 6 at 1:45)** will present initial results from Purdue University facilitated by the use of Stanford Photonics' patented technology optimized for imaging at unprecedented speeds and extremely low light levels.

2518-Pos: Single Molecule Tracking of Individual BtuB-Colicin Complexes in the Outer Membrane of *E. coli*
BELOW: Imaging the binding of individual colicin to BtuB in the outer membrane of *E. coli*



We have imaged the binding of Colicin E3 to BtuB and investigated the motion of the complex using a novel FRET-based probe. The probe allows us to determine if the two "heads" of individual molecules of Colicin E3 are together or separated during the observation.

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NEW Piper Control™ Acquisition Software: Turbo Speed Single Photon Imaging

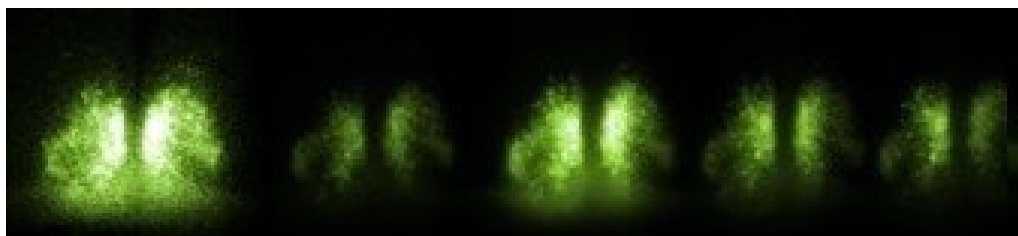
The XR/Turbo™ was first featured in Spring 2005 and was introduced in tandem with a higher speed version of Piper™ software designed to provide full bandwidth, unlimited run length, and TIFF formatted streaming to RAID on consumer priced PC platforms. At Neuroscience 2006 we introduced the **XR/Turbo-120™**. This model has a higher native frame rate of 120 fps at 640 by 480 pixels and supports **unbinned** (10, 15, or 20 micron) pixels with partial scans up to 1000 fps. The unit in our booth has a **dual MCP intensifier** tube which allows the same single photon discrimination and detection as the -Z but at higher speeds and lower cost. Even without the photocathode cooling used in the -Z configuration, dark counts are still below 0.01 per pixel/second when running 640 by 480, 120 fps.



The new Piper Control™ software upgrade facilitates this high speed photon accumulation imaging while providing frame clock synchronous I/O I (TTL, serial and analog) for instrument and mechanism control. In addition to supporting the newest Turbo features, we have also developed, tested, and integrated a proprietary real time pixel-level filtering function into the Piper™ architecture, removing random "scintillation noise" appearing in some images at the highest gain settings. SNF (Spot Noise Filtering) modules allow ICCD imaging at higher gains than any EMCCD products with a dramatic reduction in extraneous noise often associated with image intensifiers. Stop by the Stanford Photonics, Inc. booth to see a live demonstration of SNF.

NEW Piper Control™ Acquisition Software: Time Lapse

While the technical validation of the -Z technology has been a critical step forward for us, the real world results we are seeing from users is most rewarding. The images below are derived from a four day time lapse study performed by Dr. Michael Sellix, UVA: 250 micrometer slice in the coronal plane from the brain of a PER2::luc. transgenic mouse that contains the paired suprachiasmatic nuclei. Collected at 1 minute intervals (integrated 900 frames) and further integrated post-recording at 3 minute intervals. These slides are accompanied by a time chart (below) representing the pixel intensity of the SCN slices. This and other movie sequences acquired via Piper Control™ can be viewed in full at our booth.



Data collection and metrics listed below.**

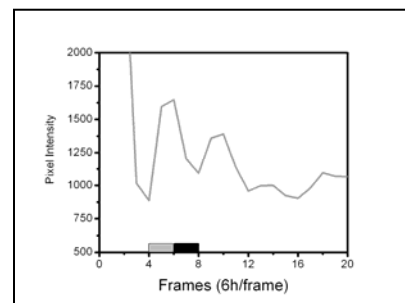


IMAGE DATA AND METRICS FROM Welsh* (Page 1) and Sellix** (see above):

*SCN cells from Per2-luc knockin mice cultured at postnatal day 3 and imaged after 38 d. in culture at 36C. Olympus IX71 microscope with 4x XLFLUOR lens. Spectral Instruments SI800 camera with CCD at -90C, no binning, 50KHz readout, 14.9 min exp. x2. Stanford Photonics camera XR/MEGA-10Z™, ~700 gain, 10 sec integrations per file x30 min, stacked to two 15 min. summed images. For both cameras, cosmic ray artifacts were removed by pixelwise comparison of the two images, as well as using the minimum value for each pixel. The resulting images were cropped to correct for small differences in field of view, and scaled optimally for viewing on an 8-bit display.

**Slides are of a 250 micrometer slice in the coronal plane from the brain of a PER2::luciferase transgenic mouse that contains the paired suprachiasmatic nuclei. The slice was taken with a vibratome and cultured according to the method developed by Shin Yamazaki (Yamazaki and Takahashi 2005). The images were collected at 1 min. intervals (integrated 900 frames) and further integrated post-recording at 3 min. intervals. Each frame represents a 30 min. interval (1/10 frames were included in the movie).

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