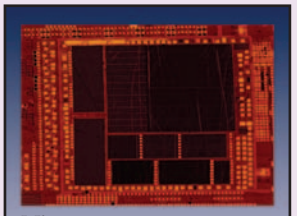
Adaptive Scanning Optical Microscope...Page 1 of 8



Microprocessor

Mosaic Imaging Provides 40mm Diameter Composite FOV Image with a 1.5µm Resolution

Breaking the barrier between large field of view (FOV) and high resolution without moving the sample or microscope. The ASM9600 captures 50,000 x 50,000 pixel mosaic images in just 20 seconds.

ASM9600

ASOM Specifications

- **Composite Field of View:** 40mm (Diameter)
- **Total Observable Field Area:** 1250mm² (3215 Tiles at 0.39mm²/Tile)
- **Resolution:** 1.5µm
- **Static Optical Magnification:** 6.5X (Object to CCD Image Plane)
- **Numerical Aperture:** 0.20
- **Working Distance:** 19mm
- **Operating Wavelength:** 490-530nm
- **Camera Pixel Count:** 1024 x 768
- **Camera Pixel Size:** 4.7µm x 4.7µm
- **Camera Dynamic Range:** 50dB
- **Camera Shutter:** 1/15s to 1/6000s
- **Manual Sample Stage Trasnlation Ranges:** X=1.75", Y=2.25", Z=1.35"

Thorlabs is pleased to offer the world's first optical imaging microscope based upon a new adaptive optic technology that circumvents the trade-off between field of view (FOV) and image resolution. By combining a high-speed steering mirror, a large aperture scan lens, and a deformable mirror, the Thorlabs Adaptive Scanning Optical Microscope (ASOM) is capable of delivering a large field of view (40mm in diameter) while simultaneously providing a uniform image resolution of 1.5µm throughout the entire composite image. Current microscope designs can only generate this type of functionality via moving stages, large microscope arrays, or very expensive lithography-based imaging systems.

ASOM Image Scanning Features

- Moving Object Tracking
- Rare Event Detection
- Spatially Separated Individual Tiles
- Full Contiguous Area Coverage

ASOM System Includes

- Complete Microscope With Transmissive & Reflective Illumination Sources
- All Drive Electronics
- Pentium-Class PC With Windows XP
- Pre-Installed ASOM Software (Described on Page 585)

The composite image of the ASM9600 is formed by using the system's fast steering mirror (FSM) to scan only the portion of the sample that is of interest up to the maximum FOV defined by the large 40mm diameter aperture scan lens. As the imaged area on the sample is changed (by changing the orientation of the FSM) the deformable mirror (DM) is used to correct the wavefront error introduced by the scan lens, thus maintaining the diffraction-limited 1.5µm resolution across the extended composite FOV. The scanning system has a wide range of operating modes that include: object tracking, rare event detection, and localized monitoring of specific locations separated spatially by large distances, all in real time. The ASOM, operating at 200 tile frames per second (optional high-speed camera), can complete one full 40mm diameter image less than 20 seconds.

The sample being imaged by the ASOM system can be illuminated via two separate illumination pathways, each with its own light source. A light source mounted under the sample stage is used for transmissive mode illumination while another light source is used for reflective mode illumination (more details on the illumination system are provided on page 586). Only the portion of the sample being imaged is illuminated, when operating in the reflective illumination mode making optimal use of the available light.

The ASM9600 was designed in collaboration with Ben Potsaid, John Wen, and Yves Bellouard at the Center for Automation Technologies and Systems (CATS) at Rensselear Polytechnic Institute (RPI; Troy, NY).

ITEM#	$	£	€	RMB	DESCRIPTION
ASM9600	CALL	CALL	CALL	CALL	Adaptive Scanning Optical Microscope

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ASOM Specific Components

Scan Lens

The ASOM scan lens is based on a reverse telephoto design and has a 76.5mm clear aperture. It is designed to be used with a 44mm diameter light field to facilitate the 40mm composite FOV. For the 44mm light field diameter, the image space NA is 0.2, resulting in a resolution of 1.5µm across the mosaic image after the wave front is corrected by the DM. The use of the DM and its ability to correct up to 14 waves of wave front distortion at 510nm relaxes the need for a complex scan lens design. The scan lens has an on axis wave front error of 0.539 waves peak to valley, and an off axis error of 3.381 waves (all measured at 510nm).

A large working distance of 19mm is provided to allow sufficient room for samples to be easily loaded and manipulated.

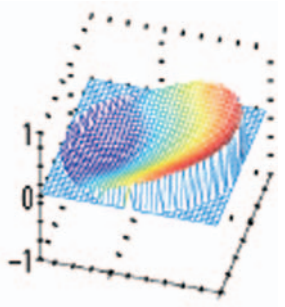
Scan Lens Features
- Large Diameter
- Flat-Field Telecentric Design

ASOM Image Scanning Features
- Moving Object Tracking
- User Defined

Fast Steering Mirror (FSM)

The pitch and yaw fast steering mirror (indicated as FSM in Figure 1) measures 75mm in diameter and is the component that controls the mosaic image formation of the ASOM system. The angular orientation of the fast steering mirror selectively images a particular segment of the sample. Each of these segments is referred to as a tile; each tile measures 0.72mm horizontally by 0.54mm vertically and is comprised of 786,432 pixels (1024 x 768). There are 3215 tiles per composite image when the entire mosaic FOV is imaged. The control system allows both sequential and non-sequential image acquisition, the later provides the ability to track live organisms as they move within the composite FOV.

The total angular range in both pitch and yaw of the FSM is 12°, and the time required to step from one tile to the next is approximately 30ms. As the tile location changes, the system control software automatically updates the shape of the of the DM. To ensure a flat image field, the FSM is located at the back focal plane of the scan lens.



Example Deformable Mirror Profile

Deformable Mirror (DM)

The DM is capable of reconfiguring its surface topography to cancel the wavefront error encountered for each image tile. A factory calibration process ensures that this correction process provides diffraction-limited performance throughout the entire imaged area. See Figure 1 for the location of the DM within the ASOM system.

The DM measures 4.4mm by 4.4mm and is comprised of a 12 x 12 grid of electrostatic actuators on 400µm centers, each with a 3.5µm range. The DM has a broadband enhanced aluminum coating and is capable of operating at approximately 1kHz.

ASOM System Schematic

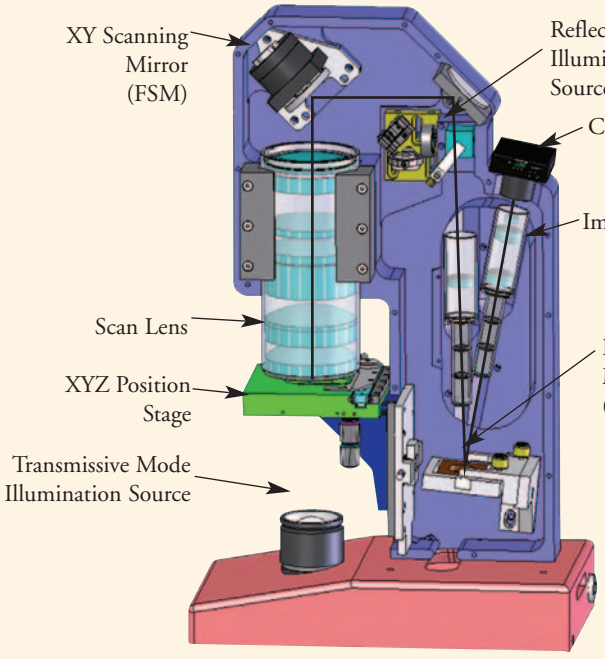
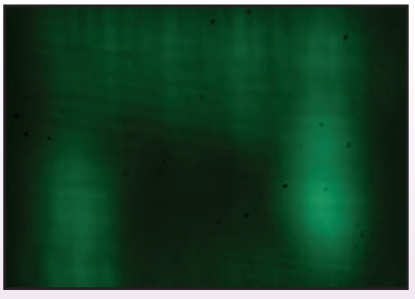
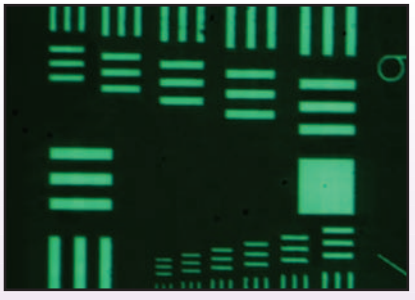


Figure 1.



Air Force target image without the use of the deformable mirror.

Figure 2a.



Air Force target image using the deformable mirror. The smallest lines are separated by 2µm.

Figure 2b.

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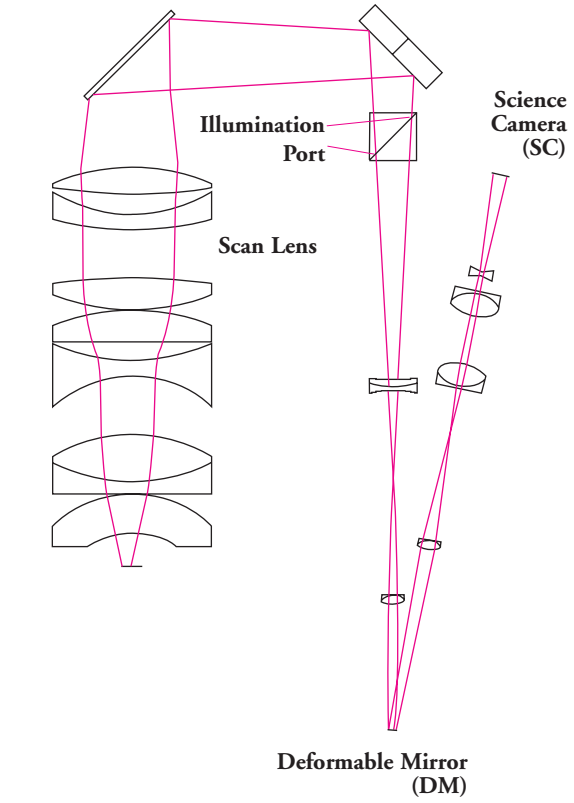
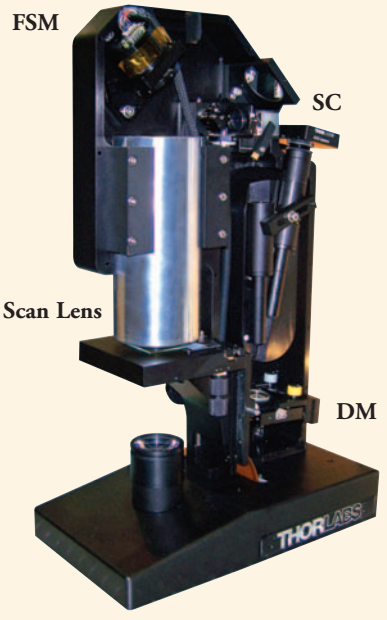


The original ASOM concept was developed in 2005 by Ben Potsaid, John Wen, and Yves Bellouard at the Center for Automation Technologies and Systems (CATS) at Rensselear Polytechnic Institute (RPI; Troy, NY). In November 2006 Thorlabs and RPI researchers initiated a collaborative effort to transform the ASOM design from a research project into a commercial product. The success of the collaboration realized a major milestone in May 2007 when the first fully functional commercial prototype was unveiled as ASOM and was honored with the PhAST/Laser Focus World Innovation Award.

Microscope Conceptual Design

In a traditional microscope, the FOV is small and limited by the objective lens. In order to image large samples at high resolution, the objective must be scanned across the sample (either by moving the microscope or the sample with a moving stage). In contrast, the ASOM uses an FSM to scan across a large diameter objective lens.



First prototype ASOM completed within six months of the initial collaboration with RPI.

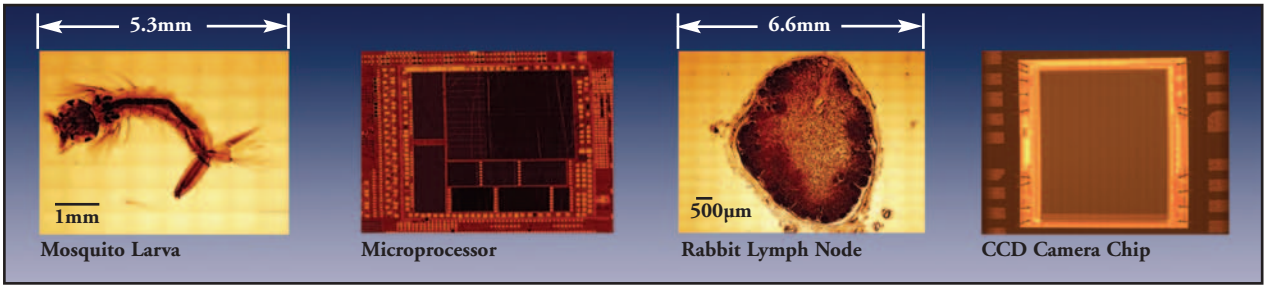
Off-axis light experiences significant wavefront distortions from the objective lens that results in an aberrated image. However, by using a DM with real-time control, the surface of the mirror is optimized to correct these wavefront distortions in order to provide uniform resolution and diffraction-limited imaging over an extended composite FOV.

To image a large sample, the light is scanned across a single square aperture (single tile size is 720 x 540µm). Multi-tile scanning creates an image mosaic providing a high resolution image with a large FOV.

Thorlabs is honored to be recognized by photonics industry professionals who awarded the ASOM First Place in the 2007 PhAST/Laser Focus World Innovation Awards.

ASOM Wins 2007 PhAST/Laser Focus World Innovation Award

Examples of large field images produced by the ASOM.



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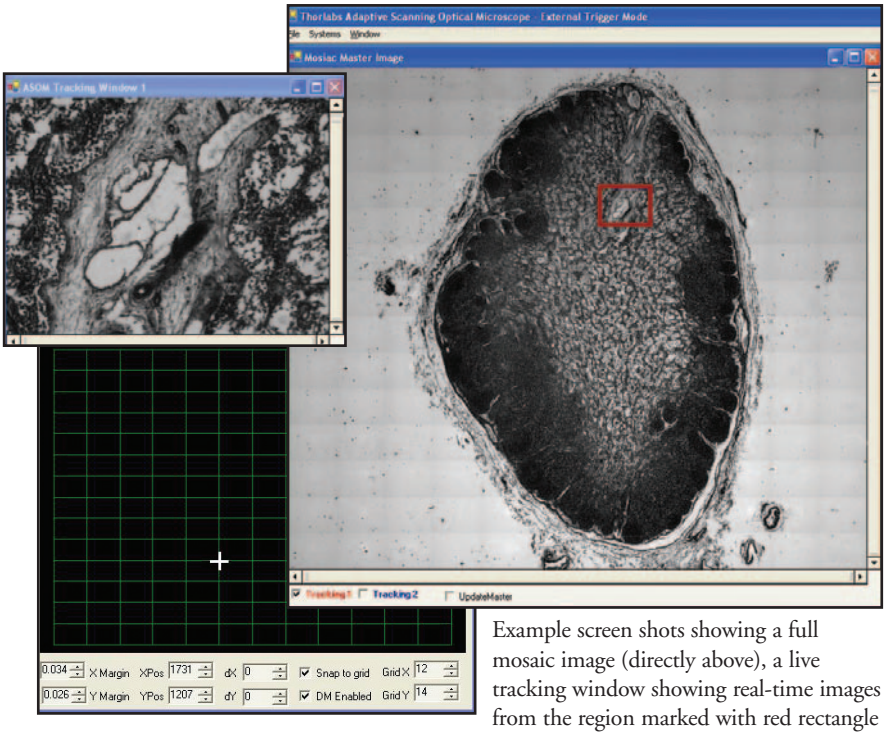
Fully Integrated Software

The ASOM comes as a complete microscopy imaging sytem including scanhead, computer, electronics, and software. The Pentium-class PC running WindowsXP comes with preconfigured software that is loaded and ready for operation.

The software package provides complete system control including self-calibration and self-optimization of the DM and all functions for image scanning, image collection, and data processing. A dynamic display provides detailed information on the FSM position and the DM topography, which provides immediate visual feedback of system functions and performance. An option for manual adjustments of the DM is available for experiment-specific requirements.



Example screen shots showing a full mosaic image (directly above), a live tracking window showing real-time images from the region marked with red rectangle on mosaic scan (upper left), and the FSM interface (lower left).

Mosaic Image Creation

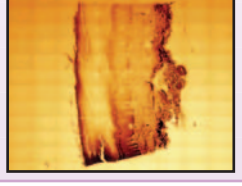
In order to capture the large FOV while maintaining the high resolution, the ASOM software stitches together several sequential camera images (tiles) into a single mosaic image. As a mosaic image is assembled, the ASOM software maintains the full 1024 x 768 pixel CCD data set contained within each tile, preserving the high-resolution data for exploration and analysis. Tile placement within the mosaic image is dictated by the object plane coordinates associated with the angular orientation of the FSM at the time the tile image was captured, the software seamlessly stitches together the composite image.

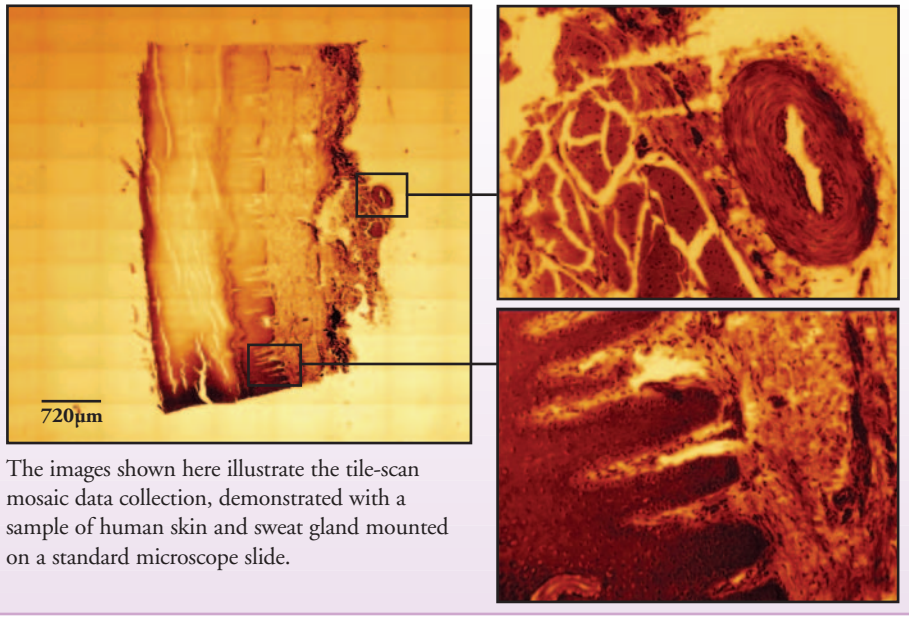
ASOM Mosaic Imaging

Mosaic Image at 1.3 seconds

Mosaic Image at 6.4 seconds

Mosaic Image at 12 seconds*





The images shown here illustrate the tile-scan mosaic data collection, demonstrated with a sample of human skin and sweat gland mounted on a standard microscope slide.

*As the design of the ASOM matures, the acquisition speed is expected to double every 3-6 months.

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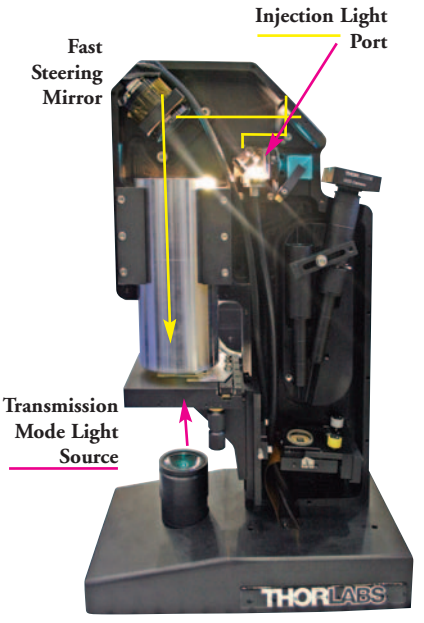
Illumination

The sample being imaged by the ASOM system can be illuminated via two separate illumination pathways, each with a separate light source. The LED light source mounted under the sample stage is used for bright field imaging (transmissive mode), and another internal LED light source is used for epi-illumination (reflective mode). The system comes with standard 528nm illuminators for both illumination modes, but various wavelengths are available (please see our website for details). While the scan lens is designed for operation around 510nm, the use of other wavelengths across the visible spectrum is possible. A substantial change in wavelength (>50nm) will require a small focus adjustment for optimal image quality.

In addition, there is a light injection port that can be used to insert a user supplied light source into the system. The ASM9600 is designed to accept band-pass filters, which can easily be inserted into the illumination pathway to facilitate multi-spectral imaging. Thorlabs is also developing a flexible lighting solution that will be fully compatible with the ASOM system. This light source will feature 6 wavelengths (452nm, 472nm, 498nm, 528nm, 593nm, and 626nm).

When operating in the bright field mode, the entire composite FOV is illuminated from beneath the sample stage. When operating in the epi-illumination mode, the sample illumination is limited to just the active tile. This mode ensures optimal use of the available light, and hence often results in a reduction of the image acquisition time as well as reducing photo-induced damage of live samples.



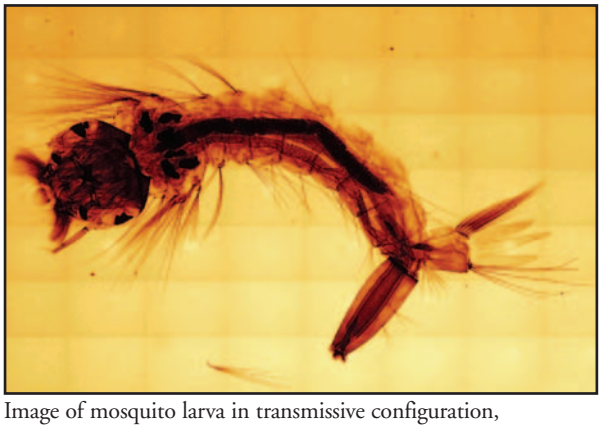


Image of mosquito larva in transmissive configuration, acquired in 8 x 8 tile mosaic scan. Total digital image measures 8,192 x 6,144 pixels.

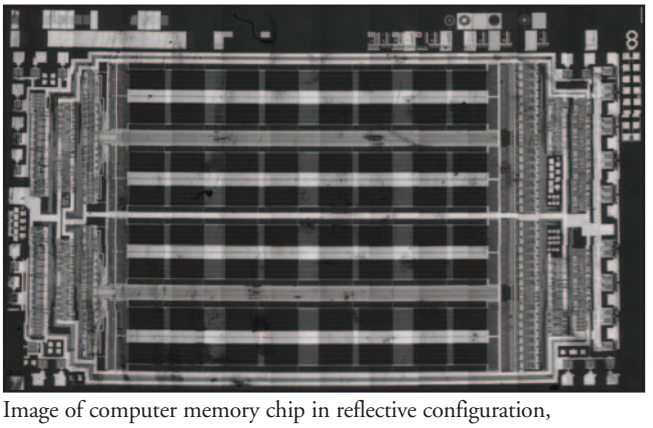
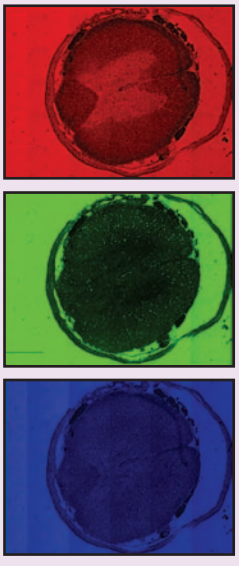
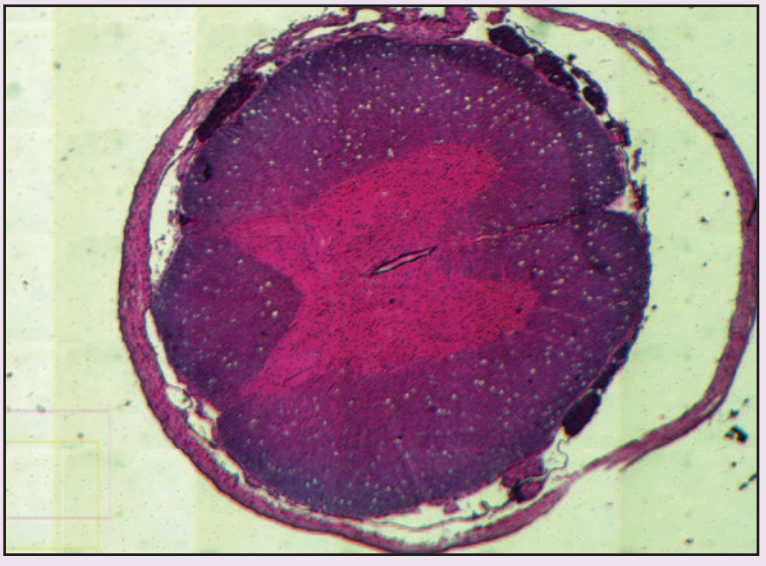


Image of computer memory chip in reflective configuration, acquired in 14 x 8 tile mosaic scan. Total digital image measures 14,336 x 6,144 pixels.

RGB Composite Imaging

This example demonstrates the versatility of the illumination components and software data processing capabilities. Individual images were captured with a black and white CCD camera, each with a discrete dichroic color filter (red, green, and blue). Once the intensity data is captured, the software color adjusts and then combines the RGB data into a single composite image representative of a full-color scan, but without any chromatic effects commonly associated with imaging with a broadband light source.





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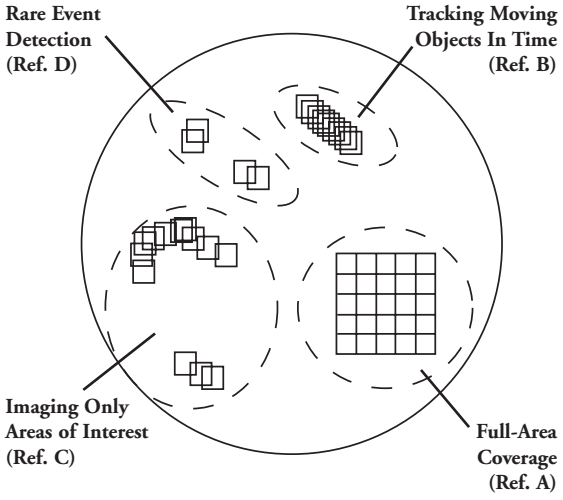
Flexible Scanning Capabilities

The FSM located above the ASOM Scan Lens (see figure on top-right of previous page) is responsible for determining, via its angular orientation, the specific location within the object plane on which the optical system is focused. Since the ASOM software self-optimizes the DM for every unique FSM orientation, diffraction-limited performance is achieved at all times. This dynamic wavefront correction capability allows the ASOM software to generate completely arbitrary scan paths tailored to the specific needs of the individual application. In addition, the software allows the user to adjust the DM topography manually at any location within the FOV so that the wave front may be manually corrected as well.



This dynamically adjustable scan path capability is demonstrated by the schematic to the right, representing four common applications made possible by the FSM. The dashed circles represent possible viewing strategies, while the boxes represent individual scan locations that define the strategy. The standard mosaic image scan mode is ideal for a fast, complete examination of a user-defined area of interest (**Ref. A**). The software may be set for other scan modes such as tracking a moving object (**Ref. B**), viewing multiple spatially separated areas of interest simultaneously (**Ref. C**), and monitoring intermittent processes or rare events over time (**Ref. D**). These scan modes can be used for a wide range of applications, including tracking live samples or micro-robotic grippers in a microelectronic production environment.

Real-Time Live Sample Mobility Tracking

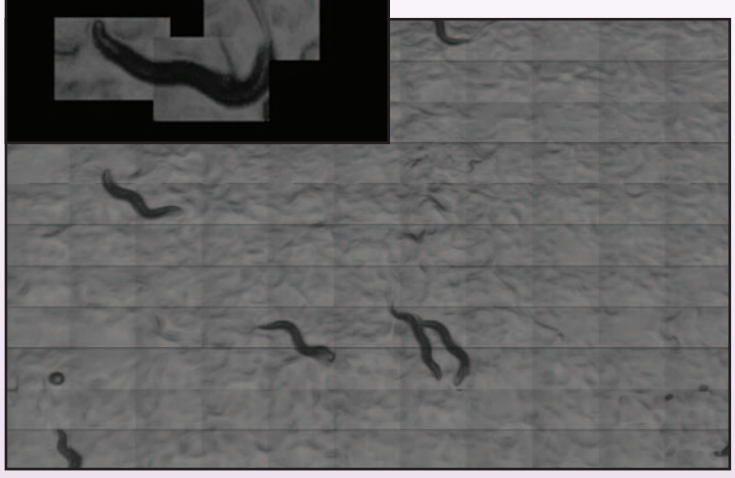
The ASOM design incorporates an FSM for imaging speeds up to 200 tiles per second. A real-time tracking algorithm allows the user to track live organisms without moving the stage, which can blur the image due to vibrations and can disturb the normal mobility tendencies of living samples. The images shown below were obtained by imaging living *C. elegans* on an agar plate. Figure 1 shows the large FOV imaged to identify an organism of interest.

Once this is defined, the software allows the user to zoom in, without changing objectives and track individual organisms that would otherwise move out of the FOV in traditional microscope designs. This application is demonstrated using live *C. elegans*, an organism with a rich body of research literature in molecular biology, developmental biology and neurobiology.

Imaging of Live *C. elegans*

Multiple moving targets can be easily tracked with the ASOM's ability to quickly adjust the focal position with the Fast Steering Mirror. Figure 1 demonstrates three scan "tiles", the first positioned on the head, the second on the midsection, and the third on the tail of a single *C. elegans* worm. These live worm imaging tracking results were obtained by Fern P. Finger, Benjamin Potsaid, and John T. Wen at RPI. Figures 2 and 3 illustrate the tracking of multiple and single worms, respectively.

Figure 1



Back illuminated images of *C. elegans* on agar plate obtained with Thorlabs ASOM-VIS system.

Figure 2

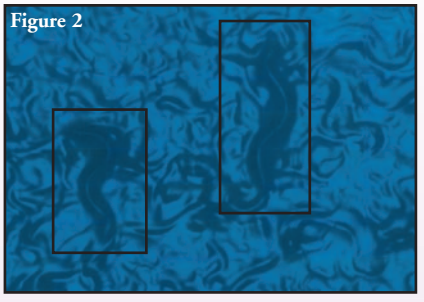
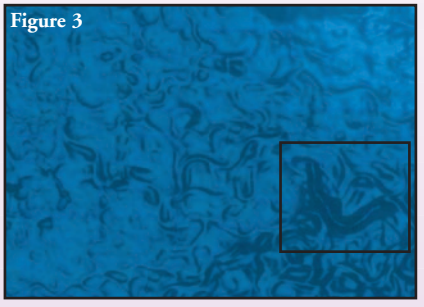


Figure 3



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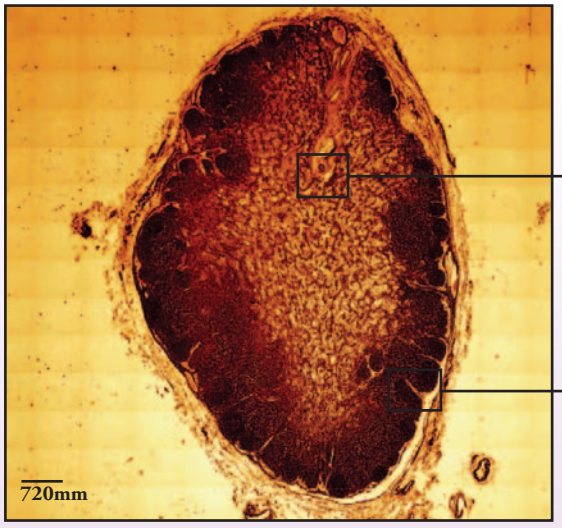


Figure 1

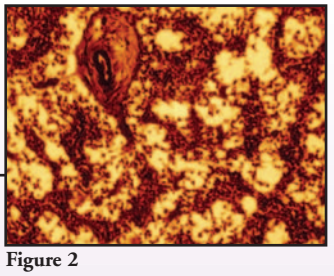


Figure 2

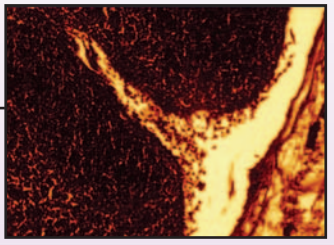
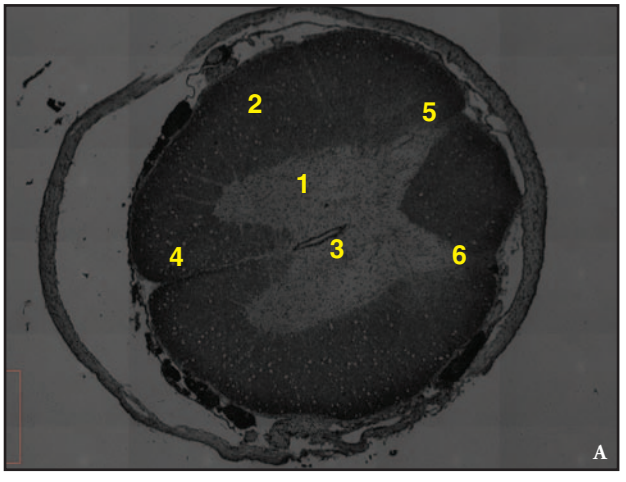
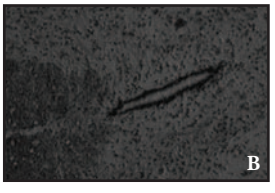


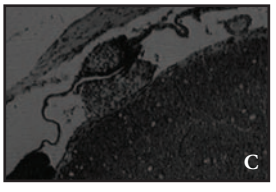
Figure 3

These three images show a slide of rabbit lymph node mounted on a standard microscope slide. The images were taken using the ASOM9600 in transmissive mode. The first image (Fig. 1) shows the entire structure (image size), while the two other images (Figs. 2 and 3) demonstrate cellular-level resolution capabilities of the ASOM, by zooming in on the sinus and a cross-section of a lymphatic vessel.



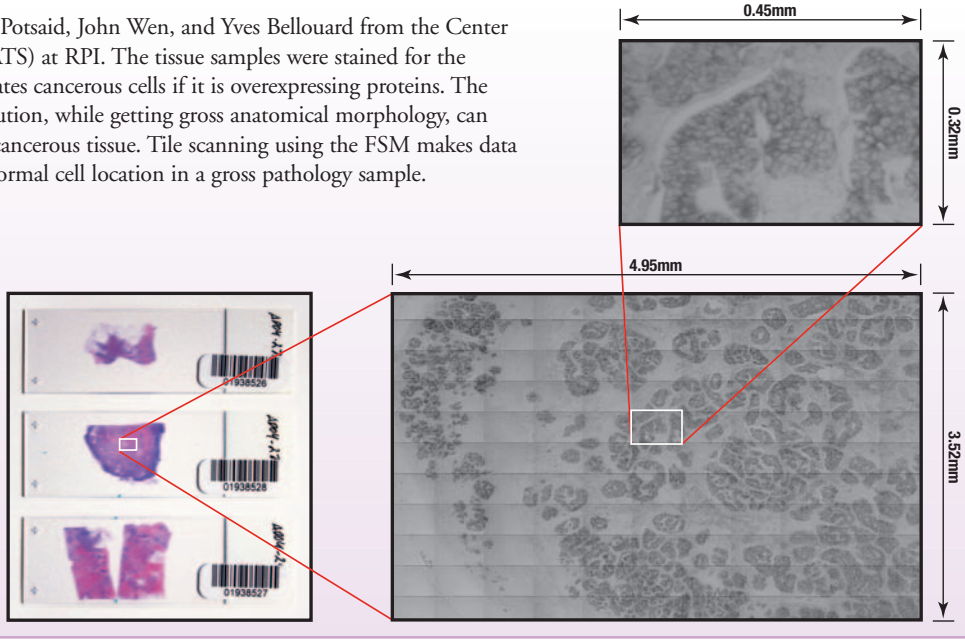
The image shown here is a cross-section of a Rat Anerior Horn (spinal cord) mounted on a standard microscope slide taken with the ASOM9600 in tranmissive mode. Figure A shows the entire sample (4.32mm x 3.24mm), showing the complete structural identification of the gross anatomy and a close-up of the central canal. 1: Gray Matter 2: White Matter, 3: Central Canal, 4: Median Fissure, 5 & 6: Nerve Root. Figures B and C are enlargements of regions 3 and 2 respectively.





Breast tissue slides shown are provided by Ben Potsaid, John Wen, and Yves Bellouard from the Center for Automation Technologies and Systems (CATS) at RPI. The tissue samples were stained for the growth hormone receptor system, which indicates cancerous cells if it is overexpressing proteins. The ability to create images with cellular level resolution, while getting gross anatomical morphology, can greatly aid the detection and identification of cancerous tissue. Tile scanning using the FSM makes data acquisition rapid, while clearly identifying abnormal cell location in a gross pathology sample.

The DM and FSM design incorporated in the new ASOM system makes pathology screening quicker, easier, and more accurate. By providing cellular-level resolution and a large FOV, the full plane of the pathology slide can be rapidly imaged, which eliminates the need for multiple scans with a moving stage, allowing high throughput slide processing. The high resolution (1.5µm) obtained in a single scan enables facile determination of cell abnormalities for accurate clinical diagnoses.



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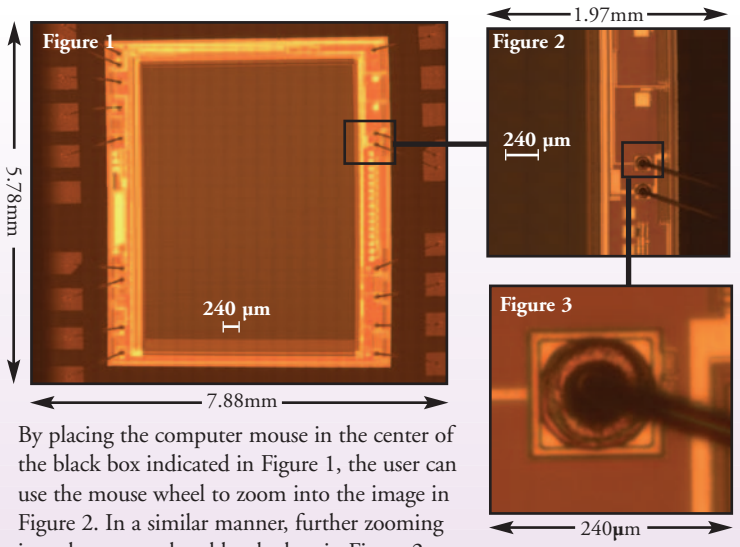
Microelectronics Production and Quality Control

The high-resolution and high FOV provided by the ASOM are ideal for electronic production environments. The image shown is Thorlabs DC310C CCD camera taken with the ASOM in the reflective illumination mode. This technique allows visualization of the entire camera, as well as individual circuit components. The large mosaic scan depicted in Figure 1 contains within it the full data set required to display at a resolution of 1.5µm. By simply zooming into the image with the ASOM software, greater details will be displayed without the need to rescan the sample.



By placing the computer mouse in the center of the black box indicated in Figure 1, the user can use the mouse wheel to zoom into the image in Figure 2. In a similar manner, further zooming into the area enclosed by the box in Figure 2, results in the image shown in Figure 3.

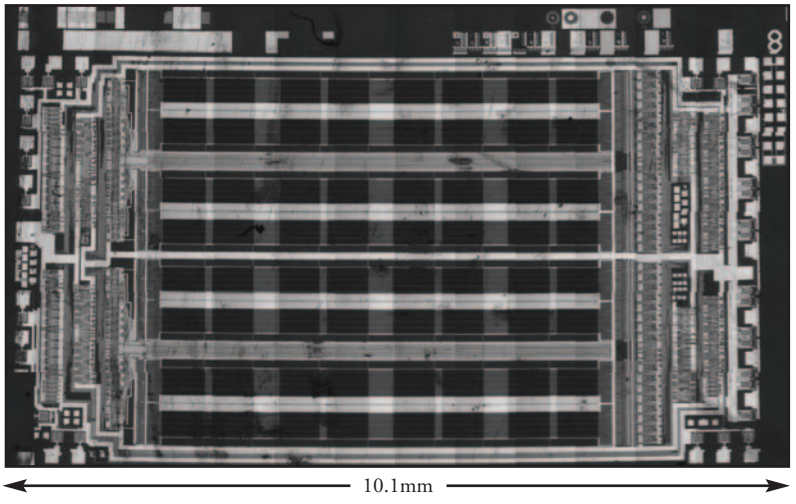
As manufacturing and electronic fabrication advances, larger assemblies can be produced from smaller and smaller components. The ASOM is ideally suited for such applications because of its large FOV and high resolution.

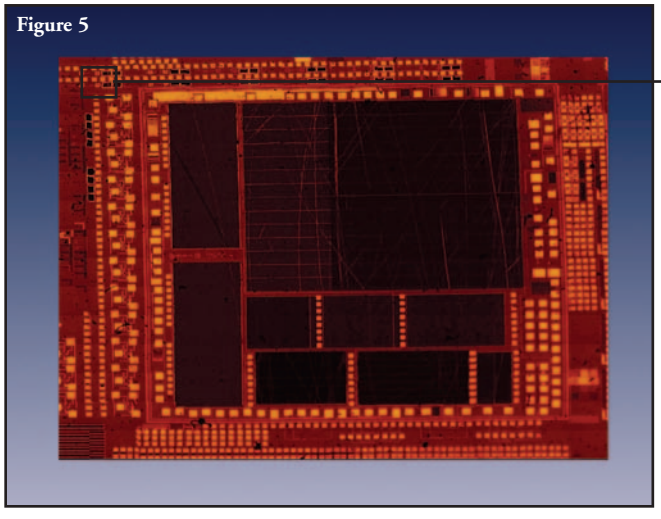
Figure 4 shows an image of an 8-bit memory microchip, showing signs of surface contamination at various locations.

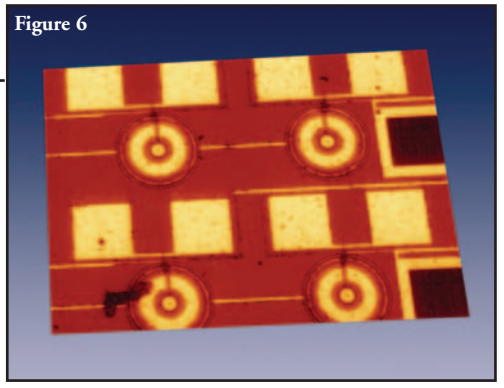
Figure 5 shows an image of an 8-bit microprocessor with signs of surface defects.

Figure 6 shows small surface contaminates on the microchip wafer.

Figure 4







The enlarged image reveals surface defects not readily apparent in the full mosaic scan.