Active Optics Improve Microscope's Field of View

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Introduction

Since the earliest explorations of the microscopic world in the 17th century and continuing to the present day, microscopists have continuously faced a challenge in that there is an inherent tradeoff between the resolution of the microscope and the size of the field of view--as the resolution of the microscope is increased to resolve smaller features, the observable area of the specimen decreases proportionally. While being a long recognized characteristic of the traditional microscope design, this tradeoff is becoming a significant hindrance as optical microscopes are being used in automated systems for advanced biotech research, medical diagnostics, robotic micromanipulation, and industrial inspection. More and more, these applications are requiring high throughput or challenging spatial-temporal observations where the small field size is often the source of a bottleneck in the process or prevents observation of the event of interest altogether. A new microscope design, called the Adaptive Scanning Optical Microscope (ASOM), uses a deformable mirror and a specially designed scanning configuration to effectively enlarge the field of view in optical microscopy. Exhibiting unique performance characteristics, the ASOM is particularly suitable for applications requiring dynamic or low fill factor observations over a wide field of view.

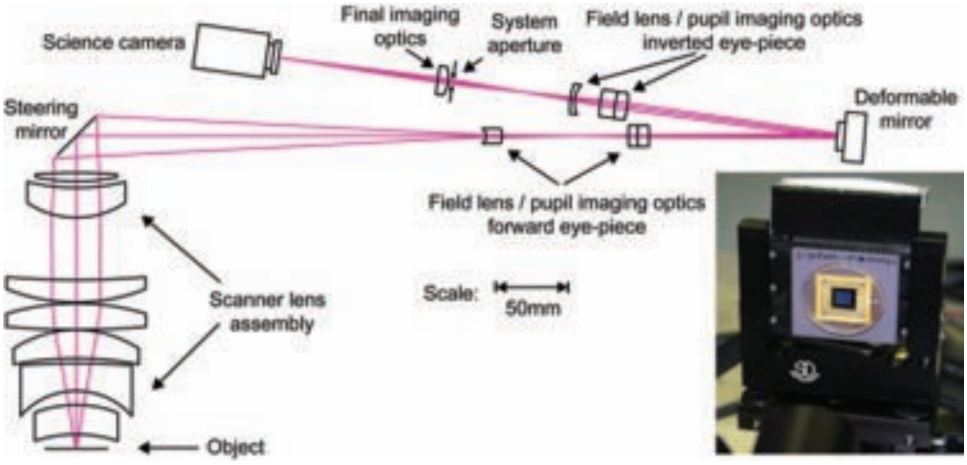


Figure 1: Optical layout of the ASOM with a photograph of the deformable mirror.

In a traditional microscope design, practical considerations related to the optics and signal processing theory are to blame for the small field size. Limits on the angles of incidence of the light on the lens surfaces, off-axis aberrations, and manufacturability of the opto-mechanical assemblies make it extremely difficult to design fixed (static) optical assemblies that simultaneously achieve a wide field of view, high numerical aperture, and a flat image field. But these lens design issues account for only half of the challenge. Signal processing theory requires that the image be sampled at or above the Nyquist frequency to avoid aliasing the image (high spatial frequency object content will manifest itself as false low spatial frequency artifacts in the image). Consequently, the number of pixels in the camera determines an absolute maximum field size for a given resolution, regardless of the quality of the optics. The relatively small field sizes at high resolution that result from these two effects working together can limit the utility of a simple microscope design.

Equipping the microscope with multiple parfocal objects on a rotating turret, motorized moving stages, zoom microscope designs, and line scanning techniques are all examples of approaches to cope with the small field of view and are quite common. However, each method suffers from deficiencies of its own in practice. For example, a wide field of view and high resolution can not be obtained simultaneously when using multiple parfocal objectives. Relatively slow dynamic performance and agitation to the specimen are unavoidable when using a moving stage. Zoom microscope designs can not achieve both a high resolution and wide field of view at the same time. And line scanning techniques require extremely bright illumination, which can be damaging to living biological specimens, or slow scanning speeds when bright illumination is unavailable.

Researchers at the Center for Automation Technologies and Systems (CATS) at Rensselaer Polytechnic Institute were faced with the difficulty of using these traditional solutions for the field of view vs. resolution tradeoff while using microscopes to facilitate robotic micro-manipulation and micro-assembly. Micro-part manipulation often requires high resolution imaging of small features or objects that are separated by distances much greater than a typical field of view. Familiar with the limitations of using multiple microscopes, moving stages, and zoom microscopes, CATS researchers realized that a new microscope design to expand the field of view to cover more of the robotic workspace while preserving resolution and operating at high speeds could be a valuable contribution. Ideally, this new microscope would allow multiple robotic manipulators to cooperatively work together in the workspace. And to facilitate probing, stimulating, and manipulating, the workspace and sample should not move at all. The same advantages that are desirable for micro-assembly would also make it useful for biological and medical imaging. Richard Cole at the Wadsworth Center (New York State Department of Health) and Dr. Jacques Izard (now at the Forsyth Institute) have been involved with the microscope since nearly the start of the program, actively guiding the microscope development towards biological applications.

An Active Optics Based Solution

In designing the new microscope, we approached the imaging challenge from a systems perspective rather than a more traditional static optical design perspective. The recent availability of affordable and high performance active optical components (e.g. dual axis steering mirrors, deformable mirrors, piezo driven elements, etc.) opens the door to novel optical system layouts with performance exceeding even the theoretical capabilities of a purely static optical design. In general, active optical elements and algorithmic components are becoming integral to advanced optical systems as more traditional optical design is expanding to become optomechatronic design. For this reason, we have started the Smart Optics Lab (SOL) at the Center for Automation Technologies and Systems to encourage the use of active and adaptive optics technologies in biomedical and industrial applications. The mission of the Smart Optics Lab is to work with companies and researchers to bridge the gap between research and the real world applications that can benefit from active optics technologies. By having a full range of equipment in our lab, multidisciplinary design tools, and rapid prototyping capabilities, the goal is to transfer the emerging and newly affordable active optics technologies into products and instrumentation. Our ASOM microscope continues to be one of the primary programs exploring the use of adaptive optics at the Smart Optics Lab.

In our new microscope (see Figure 1), two key ideas improve the size of the field of view while both maintaining lens simplicity and using currently available commercial technologies. First, a curved image

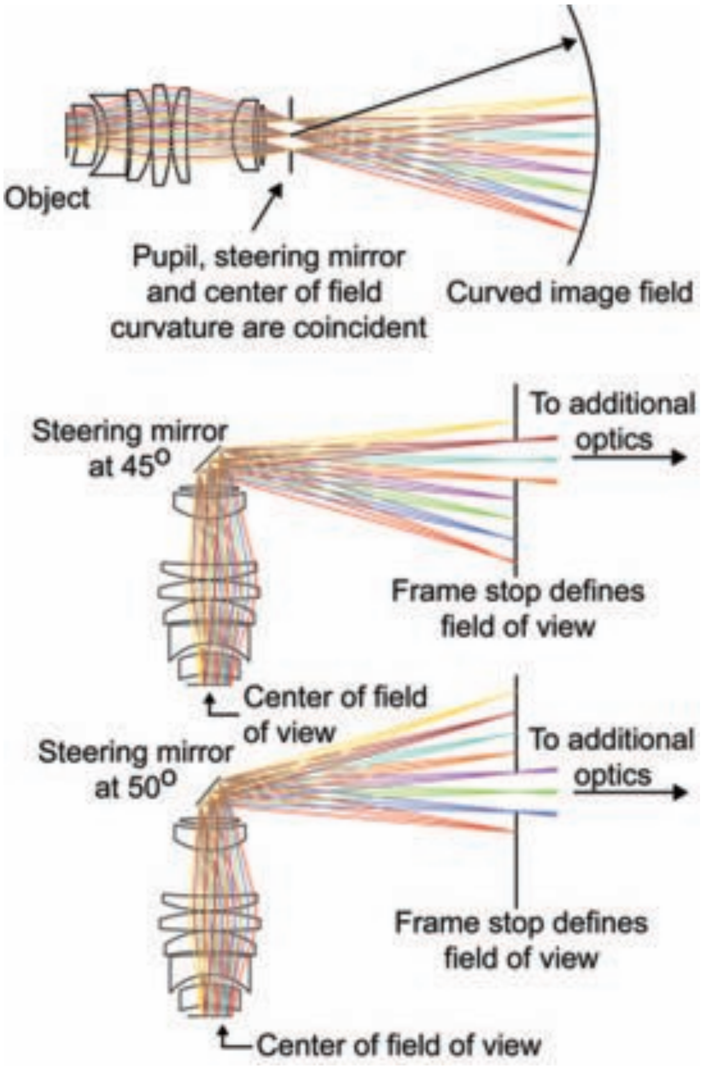


Figure 2: The curved field scanning configuration allows for a simple lens design.

field scanning configuration works with the natural behavior of the positively powered optics. Second, a deformable mirror in the optical path actively corrects for the optical aberrations associated with the simple lens design to produce a diffraction limited and blur-free image over the entire field of view.

One of the greatest challenges for a lens designer is that the natural behavior of a positive lens assembly is to project a curved image field. Considering that available sensor technologies (CCD/CMOS cameras and photographic film) have a flat active area, the projected image must also be flat to avoid blurring of the image towards the edges. Additional glass elements need to be added to the lens design to flatten the field, and the problem gets much worse as the size of the image field increases. With the field size and numerical aperture we required, a flat field imaging system made of a static lens assembly would approach the complexity of a lithography projection lens, which can cost in the millions of dollars to manufacture [1]. To avoid this, the ASOM makes use of a scanning configuration that places the steering mirror precisely at the center of the field curvature as shown in Figure 2. As the angle of the steering mirror changes, the curved image field rotates about its own center. A field stop samples a small portion of the curved image field and additional optics relay and flatten the field to project the final image onto a standard digital camera. Thus, the angle of the scanning mirror determines the location of the center of the field of view on the specimen. At each field position, a complete image is acquired (not a single point as in a confocal microscope). And by taking a sequence of images and assembling a large and possibly disjoint mosaic of the scene, the field of view can effectively be enlarged.

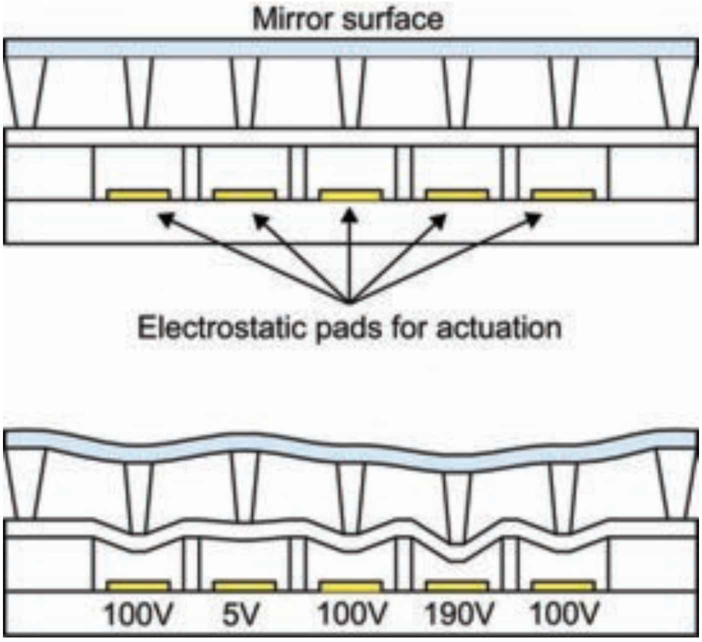


Figure 3: The shape of the surface on the deformable mirror can be controlled by applying different voltages to the electrostatic actuation pads.

The advantages of this configuration are that scanning with a low mass steering mirror can be 10-100 times faster than a moving stage, depending on the stage payload. Furthermore, regardless of the scanning speed, the specimen or robotic workspace are not dynamically disturbed. From an optical and manufacturing standpoint, the scanning mirror and the optics work together to avoid the challenges associated with a high numerical aperture optic that exhibits a large and flat field. It is interesting to note that part of the reason the lens in the human eye can be so simple is that the retina (image sensor) in the eye is curved to match the curved image field projected by the simple positive lens. Similarly, in the ASOM, the wide field scanner lens is relatively simple because the projected image is allowed to be curved.

Managing field curvature is not the only challenge for large field imaging systems, as other off-axis aberrations (spherical aberration, coma, astigmatism, and higher order variants) exist that can cause the image to blur towards the edges. We found a solution by including the latest advances in MEMS deformable mirror technology [2]. The surface of a deformable mirror can be controlled by individual

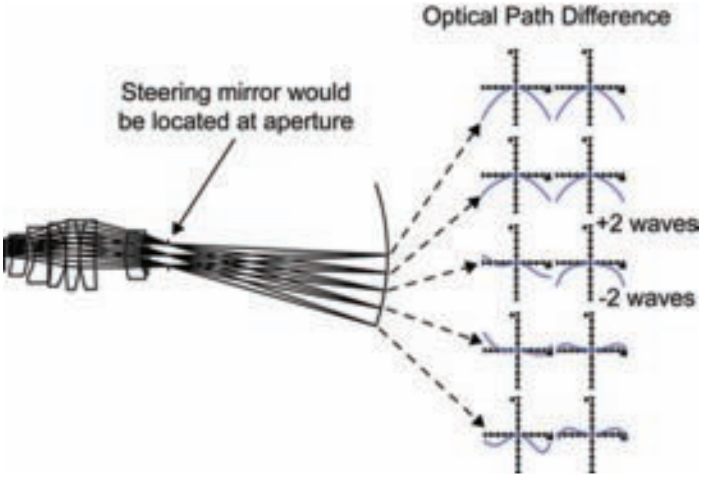
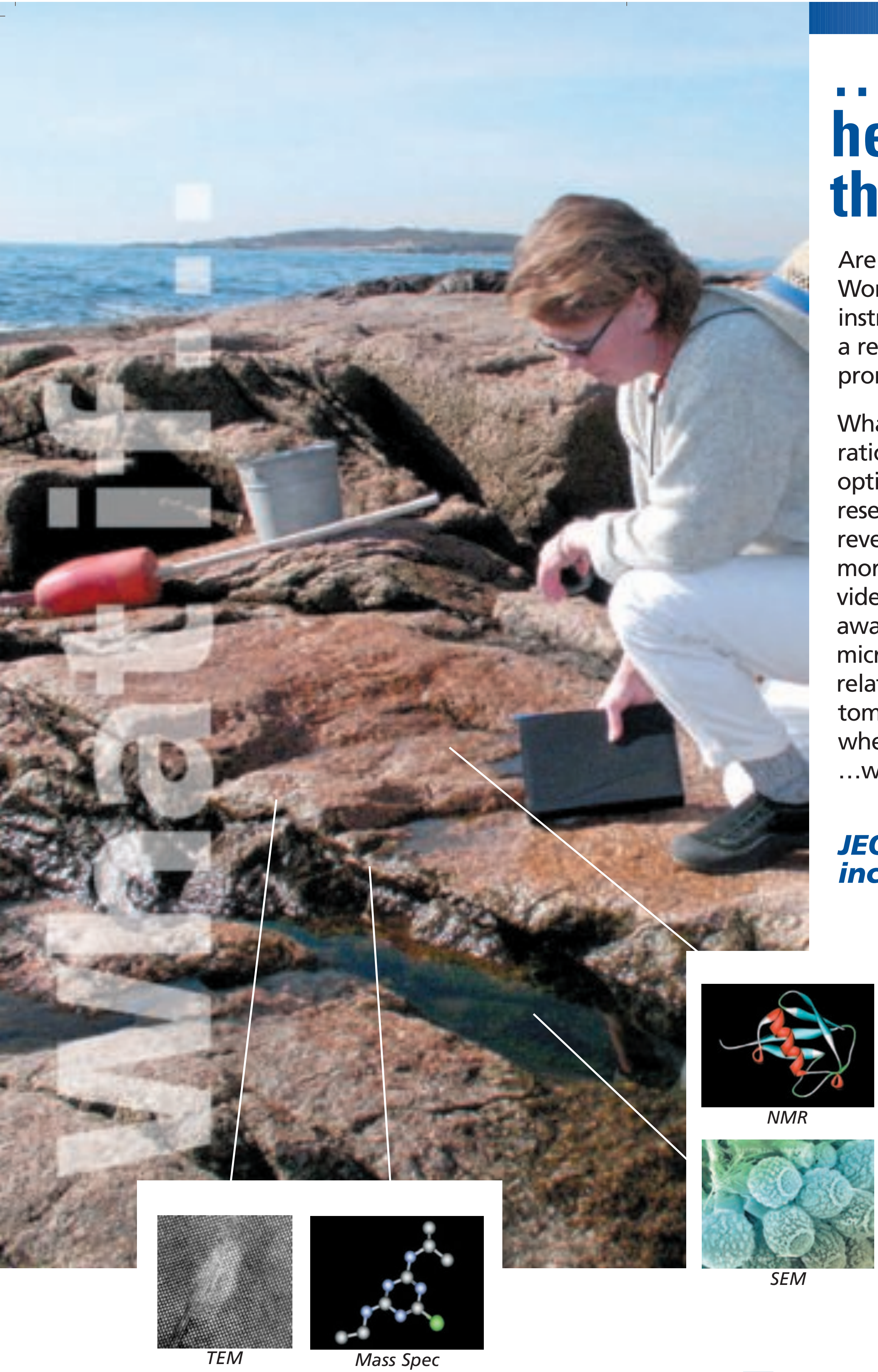


Figure 4: The scanner lens exhibits significant optical aberrations that change with field position. Allowing for this aberration results in a very simple lens design, but the aberrations need to be corrected later in the optical path by the deformable mirror to prevent image blur.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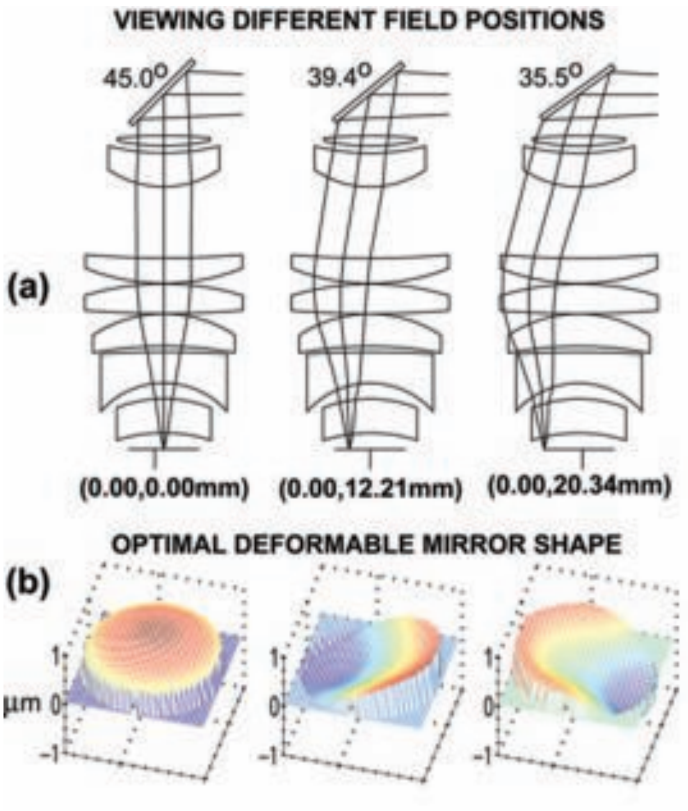

Figure 5: The steering mirror angle defines the location of the center of the field of view. For each field position, a specific deformable mirror shape corrects for the aberrations to beyond the diffraction limit for high image quality over the entire field of view.

actuators as shown in Figure 3. Deformable mirrors and adaptive optics were originally conceived of by the astronomy community with significant advancements made by the military to improve telescope imaging. Here, the problem is that the atmosphere is not homogeneous, but rather composed of turbulent pockets of hot and cold air with different indices of refraction. Consequently, light refracts at the boundary interfaces of the air, causing the stars, enemy satellites, and other astronomical objects to "twinkle". Wavefront sensors can measure the distortions to the light path and by precisely controlling the shape of a mirror surface at a rate of one to several kilohertz, the deformable mirror and adaptive optics control system can effectively remove the twinkle from the object of interest. In our microscope, the optical disturbance is not time varying per say as is the case with telescope imaging, but scan position dependant as shown in Figure 4. Figure 5 shows how a specific deformable mirror shape corrects for the aberrations associated with each field position to above the diffraction limit to achieve imaging nearly indistinguishable from perfect over the entire field of view.

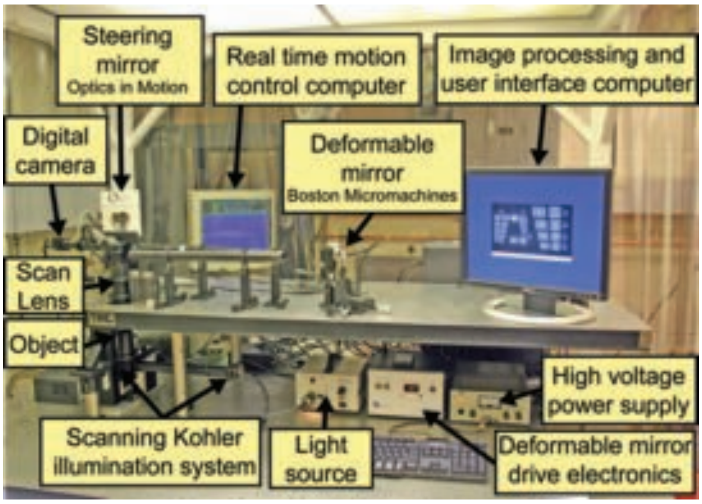

Figure 6: The first ASOM prototype was constructed with off-the-shelf optical elements for low cost and short development time, but demonstrates all of the critical optical characteristics of the ASOM design.

High fidelity simulations using the ZEMAX optical design and simulation software show that the ASOM can achieve a field of view that is approximately 2 orders of magnitude larger in area than a traditional microscope at the same resolution (e.g. one of our ASOM designs achieves a 40 mm diameter field of view at 1.5 micrometer resolution).

Different modes of operation include imaging regions of interest, tracking multiple moving objects, full area coverage, and combining low resolution background monitoring for rare event detection with high resolution imaging of the rare event itself. The array based imaging sensor used in the ASOM is time efficient in low light conditions because the pixels are exposed simultaneously rather than sequentially as is the case in most confocal or line scan imaging approaches. This is especially important for live cell imaging, where light can damage the cells and affect the rate of natural processes. More detailed information about the ASOM design, layout, and theory of operation can be found in [3].

Experimental Implementation

Our simulated results for the ASOM showed very desirable performance, but assumed perfect knowledge of the aberrations to calibrate the shape of the deformable mirror. In practice, perfect knowledge of the aberrations would not be available due to manufacturing and assembly tolerances associated with a real physical system. We also hoped that by performing the deformable mirror shape optimization using an image based method; we could avoid the explicit need for a wavefront sensor in the system. An initial prototype needed to be built quickly and at low cost to verify the idea, especially the experimental calibration and the use of the deformable mirror.

Deformable mirrors have been used since the late 1960's and 1970's, but the high cost associated with the piezoelectric drives, custom design work, and the hand assembly made the technology available only to a select few well-funded researchers and the defense industry. However, in the last 5 years or so, a competing deformable mirror technology based on silicon and using the same semiconductor fabrication techniques as computer chips has emerged, become productized, and readily available from a range of US and international manufactures.

As we were initiating our design work for the first ASOM prototype and evaluating different deformable mirror options, a new 32 actuator deformable mirror became available to us from Boston Micromachines Corp. (Watertown, MA). With only 32 actuators on this deformable mirror instead of 140 actuators as is used on their standard

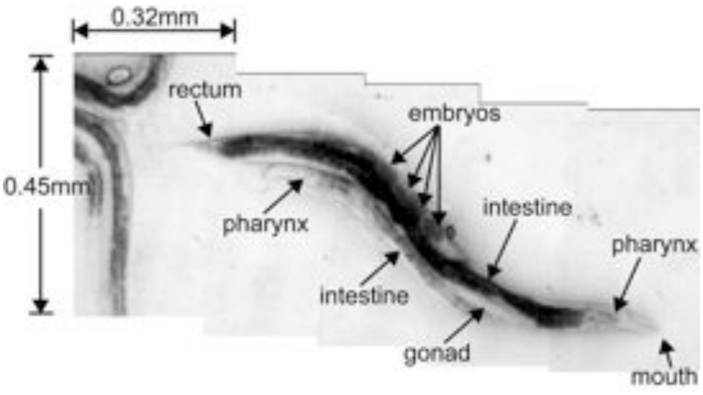

*Figure 7: A five tile mosaic of living and moving nematode worms (*Caenorhabditis elegans*) obtained with the prototype ASOM.*

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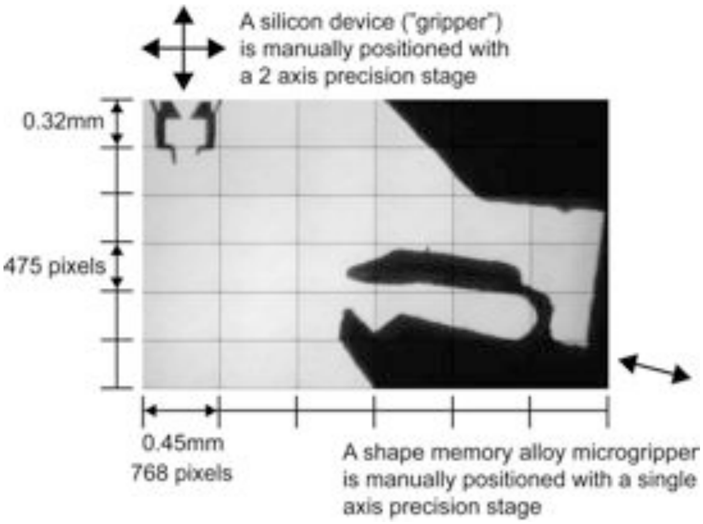

Figure 8: A 6x6 tile image mosaic showing two grippers in a robotic workspace.

mirror, this version was well matched to our prototyping budget. The 3-layer structure of this MEMS deformable mirror allows the mirror to generate relatively large deflections at high spatial frequencies and the small aperture (about 3 mm) helped facilitate a compact size for our microscope.

The rest of the microscope was designed around off-the-shelf lens elements available from a catalog to save cost and take advantage of short delivery times. However, care was taken in the design such that this prototype exhibits all critical optical characteristics of the ASOM. Although the spacing and arrangement of the lens elements has been custom designed and optimized in the optical path, using off-the-shelf optics meant that we had to compromise the performance of the prototype when compared to the simulated results that use custom fabricated lens elements. As such, this low cost prototype (shown in Figure 6) has a 10mm field diameter and operates at 0.1 NA (about 3 micron resolution).

ASOM Imaging Demonstrations

Applications with challenging spatial-temporal observations over a large field can benefit from the wide field and rapid scanning capabilities of the ASOM. Here we describe two demonstrations related to biological imaging and micro-robotic manipulation.

Using the prototype ASOM, we have performed initial imaging demonstrations of living biological cells, medical biopsy samples, and moving multicellular organisms. Collaborating with Dr. Fern P. Finger at the Rensselaer Biotech Center, we are beginning experiments with living nematode worms (*Caenorhabditis elegans*), which are a major model system for neurobiology and developmental biology studies. Current imaging techniques allow observation of either multiple worms at low resolution or high resolution imaging of only an individual worm. Consequently, during mobility characterization studies, the worms are filmed at a low resolution to prevent losing sight of the specimen outside the field of view. Imaged in this way, the movement of several worms can be observed, but the details of the inner organs can not be resolved. The ASOM has the ability to track and follow multiple moving objects and compose image mosaics with high resolution tiles. An example is shown in Figure 7, which shows a five tile mosaic of several worms. Images obtained with the ASOM clearly show both the internal organ structure and movement of the worms as the animals are alive and active on the agar medium. Ultimately, the ASOM imaging system will enable high resolution studies of later developmental events in a large population of freely moving unanesthetized animals. Similarly, the ASOM will allow the high resolution characterization of the behavior of freely moving and interacting animals.

To demonstrate the advantages of the ASOM for microrobotic activities, we automatically track and monitor two moving microgrippers in a workspace much larger than a single field of view. Figure 8 shows a 6 × 6 tile image mosaic of the workspace containing the grippers. Real time image processing routines can identify the location of the gripper and generate the proper steering mirror commands to keep the gripper centered in the field of view. As shown in Figure 9, a first tile (each tile corresponds to a single camera exposure) automatically tracks the smaller gripper and a second tile monitors the region immediately to the right of the gripper. Third and fourth tiles also track the larger microgripper. Notice that in frames (c) and (d) of the video sequence, portions of the shape memory alloy gripper can be seen in the second tile, showing the ability of the system to track multiple moving objects with the possibility of overlapping regions. The observation and tracking of multiple moving objects such as this can not be done with the moving stage technique.

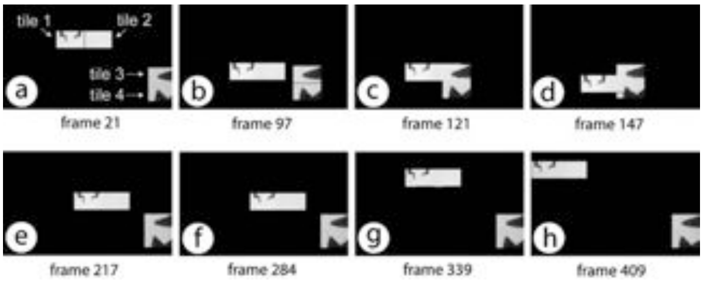

Figure 9: Sequence of video footage showing the capability of the ASOM to track multiple moving grippers in a workspace.

Summary

By replacing optical complexity with active optical components, we have developed a new microscope with unique imaging capabilities called the Adaptive Scanning Optical Microscope. Applications in biology, medicine, industry, and robotics will benefit from the rapid scanning capability that enables observation of challenging spatial-temporal events over a large field. A low-cost proof of concept prototype demonstrates all of the critical optical aspects of the design, but at a compromised resolution and field size. We are now working on developing the follow-on prototype that will make use of custom fabricated optics and a higher performance deformable mirror to fully realize the potential of this new microscope design. We are presently conducting more advanced biological and robotic demonstrations and are working to expand our collaborations with outside partners. As the price on adaptive optics technologies continues to decrease, we hope to bring the ASOM technology to market. ■

Acknowledgements:

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