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Automation of Challenging Spatial-Temporal Biomedical Observations with the Adaptive Scanning Optical Microscope (ASOM)

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***Abstract*— Biological studies, drug discovery, and medical diagnostics benefit greatly from automated microscope platforms that can outperform even the most skilled human operators in certain tasks. However, the common approach of combining a traditional optical microscope design with a moving stage suffers from relatively low dynamic bandwidth and agitation to the specimen. This paper describes an automated microscope station which is based on the novel Adaptive Scanning Optical Microscope (ASOM) that combines a high speed post-objective scanning mirror, a custom design scanner lens, and a MEMS deformable mirror to correct for off-axis aberrations to achieve a greatly expanded field of view. Particularly suitable for observing challenging spatial-temporal biological events, the dynamic performance of the ASOM is 10-100 times faster than a moving stage without any agitation to the specimen. After describing the layout and operating principle of the ASOM imaging subsystem, we present a system architecture for an automated microscope system suitable for the ASOM's unique imaging capabilities. We then describe a low cost experimental prototype of the ASOM that demonstrates all critical optical characteristics of the instrument. Finally, we present initial biological (living nematode worms) and medical (cancer biopsy sample) imaging experiments obtained with the experimental apparatus and discuss the impact of the ASOM on such biomedical activities. The work summarized in this paper is a critical step towards realizing a fully operational and high performance ASOM based imaging platform to perform cutting edge biological research and high throughput medical diagnostics.**

***Index Terms*— Wide field imaging, adaptive optics, microscopy, biology, medicine.**

I. Introduction

The introduction of digital cameras, motorized stages, and image processing to optical microscopy has had a profound effect on fundamental biological research, drug discovery, and medical diagnostics. Combining automation of the imaging hardware with image processing and robotic manipulation allows for monitoring and interacting with biological samples in ways far beyond the capabilities of even the most skilled human operator. For example, such systems can capture and track events that are much too fast for human reaction times (e.g. tracking rapidly moving motile organisms [1]), large samples can be observed over a time frame of days to weeks to glimpse rare events or increase the strength of statistical arguments [2], and large numbers of samples can be automatically processed and scored as required in drug discovery efforts where only a small portion of the prospective treatments turn out to be effective.

In general, the motivation for applying automation to microscope based workstations are related to the following issues or limitations of the optical microscope:

- There is an inherent tradeoff between the field of view and resolution of the imaging system. Thus, large regions of a sample can not be imaged at a high resolution with one camera exposure.
- The depth of field associated with high numerical aperture microscope objectives is quite small, which causes blurring of the sample if it is not at the optimal distance from the objective.
- Automated analysis or processing systems require that the samples themselves be stored, transported, and loaded into the microscope.
- Samples may require precise in vivo manipulation or measurement as a part of the process, experiment, or observation.

Most automated microscopy systems are structured around a traditional optical microscope design. Relatively low cost and standardized microscope objectives make this architecture attractive, but the small field of view at high resolution poses a significant challenge in practice. By far the most common solution to address this field of view vs. resolution tradeoff is to use a moving stage to reposition the sample relative to the microscope optics. Indeed, many automated imaging systems retrofit a moving stage to a commercially available microscope to enlarge the field of view by constructing an image mosaic out of sequence of individual camera exposures [3]. However, as conceptually simple as this idea seems, the governing physics associated

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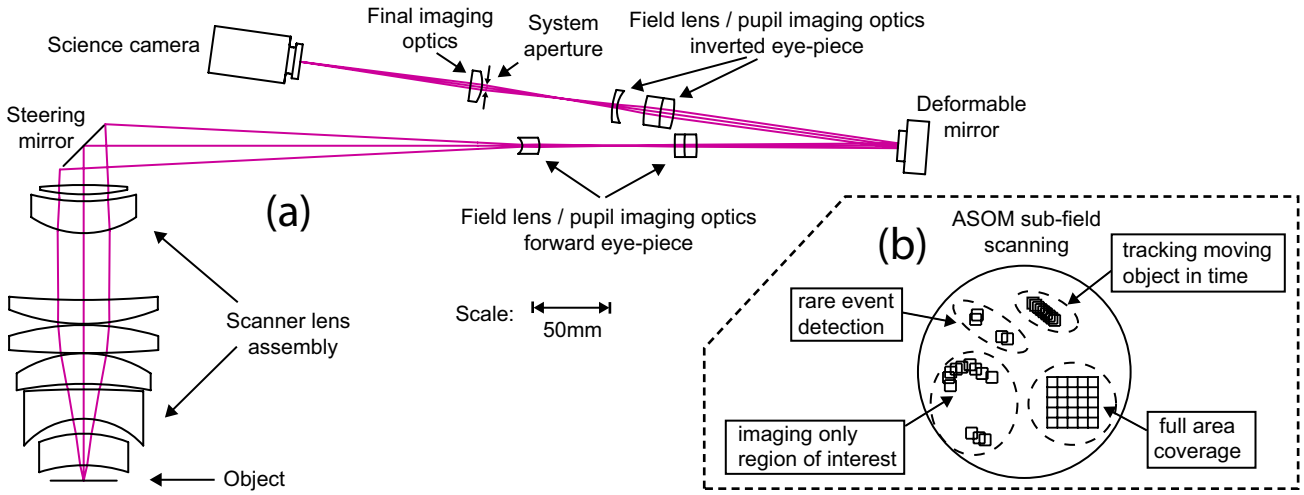

Fig. 1. (a) Preliminary ASOM design (b) ASOM modes of operation (total field size and individual camera exposure regions are not to scale.)

with the relatively large mass of the moving stage result in: a low dynamic bandwidth (stage speed/acceleration) and considerable positioning and repeatability errors. In fact, there is generally a direct tradeoff between the speed of the stage and the amount of positioning error in the stage motion [4]. These dynamic limitations are compounded when the stage is not moving just a glass slide, but a temperature regulated chamber or large well plate. Furthermore, certain sensitive biological observation tasks [1] are adversely influenced by the agitation of the specimen resulting from the moderate accelerations of the moving stage platform. From an automated systems perspective, the moving stage is undesirable because the sample must be loaded and unloaded from the stage platform. The loading and unloading operations do not modify or enhance the sample and it is a critical goal of efficient process implementation to eliminate non-valued added operations from a production line [5]. Because of these dynamic limitations during scanning and the need to load and unload slides, the moving stage is often the source of a bottleneck in biological research or automated medical diagnostics process or prevents the observation of challenging spatial-temporal events altogether.

This paper describes a recently developed wide field microscope concept, called the Adaptive Scanning Optical Microscope (ASOM) and a system architecture that addresses the shortcomings of a traditional microscope/moving stage design. By utilizing fast and dynamic optical components, the ASOM operates in a unique region of the performance domain and exhibits the following desirable characteristics:

- Rapid dynamic scanning to enlarge the field of view while maintaining resolution
- No agitation to the specimen or sample during scanning
- Efficient low light level imaging
- Convenient integration into automated production systems using conveyor transports to facilitate part storage and presentation
- Easy integration with robotic manipulators, sensors, or stimuli that facilitates high speed operation and high positioning accuracy.

The large field of view, relatively long working distance, rapid and flexible scanning capability, and the ability to scan without moving the workspace make the ASOM particularly suitable for challenging biomedical imaging applications. With respect to throughput and dynamic imaging capabilities, the scanning mechanism provides scanning speeds on the order of 10-100 times faster than a moving stage. Furthermore, the area scan camera in the ASOM is more efficient than line scanning cameras in low light imaging applications because the pixels can be exposed simultaneously rather than sequentially. When multiple manipulators, microinjectors, or sensors are required, the moving stage does not offer such an attractive manipulation environment because either (1) the robotic manipulator must be mounted on the moving stage with the consequence of increasing the stage mass and considerably slowing down the stage motion or (2) the robotic manipulator must be fixed to the machine base with the consequence of requiring the motion of the moving stage and the manipulator to be precisely coordinated, compromising speed and accuracy. Active correction of off-axis aberrations with the deformable mirror achieves uniform resolution and diffraction limited imaging performance over the entire field. However, the field of view of the ASOM is not as large as the virtually unlimited field of view associated with a moving stage due to optical considerations in the ASOM design.

Previous work related to this research has demonstrated automatic tracking of moving objects in a robotic workspace and shape optimization of the deformable mirror using an experimental implementation of the ASOM [6]. The ASOM concept, theory of operation, and design methodology are also discussed in greater detail in [7]. This paper discusses an automated imaging platform based on the ASOM and biomedical applications that benefit from the new imaging system and architecture. Section II

describes the ASOM imaging subsystem itself and presents simulated results of an example ASOM design. Section III describes how the ASOM would be integrated into an automated imaging platform and the overall system operation and architecture. Section IV describes an experimental ASOM apparatus, while Section V presents biological applications and images captured with the experimental system. Conclusions and future work are presented in Section VI.

II. ASOM Imaging Subsystem

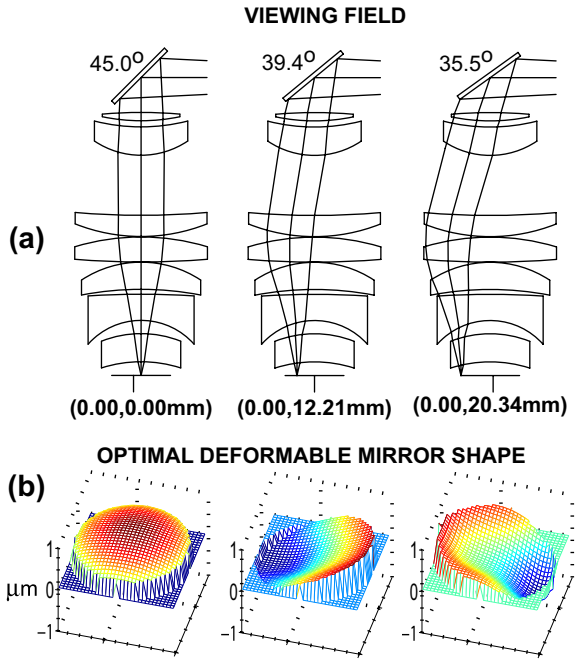

Fig. 2. (a) Scanning (b) Optimal deformable mirror shapes

At the heart of the imaging platform is the imaging subsystem itself, which consists of an Adaptive Scanning Optical Microscope (ASOM). The underlying concept of the ASOM is to use a low mass steering mirror located between the scanning lens and the imaging optics to form a post-objective scanning configuration as shown in Figure 1(a). This allows a small sub-field-of-view to be quickly scanned throughout the workspace so that multiple disjoint or overlapping regions of the workspace can be visited and imaged in rapid succession as illustrated in Figure 1(b). Through image warping and mosaic construction, a large and continuous virtual image of the object or region can then be constructed from the individual image tiles. The advantages of such an arrangement are: a large effective field of view at high resolution, no disturbance to the sample, and high scan rate operation. However, such a system configuration also poses significant design and implementation challenges. Compared to the moving stage or moving microscope designs, there is extensive off-axis imaging (i.e., images are obtained by looking diagonally through the scan lens), which introduces image distortion in addition to contrast degrading and resolution reducing aberrations (e.g. coma, astigmatism, field curvature, etc.) [8] that have typically reduced the effectiveness of such an approach. In the ASOM, we address the off-axis aberrations by:

1) Explicitly incorporating field curvature into the design to greatly reduce the complexity of the scanning lens.
2) Introducing an actuated deformable mirror (DM) into the optical path to correct for the residual aberrations.
3) Image processing to remove image distortion.

Note that by combining dynamic components and algorithmic techniques with traditional static optical elements, the lens count in the scanner lens is greatly reduced compared to a comparable static optics only design. This potentially saves millions of dollars in manufacturing and assembly costs (lithography lenses with similar specifications can cost in the millions of dollars [9]), making the ASOM accessible for research and production activities.

TABLE I
Preliminary ASOM performance specifications

	Specification
Effective field of view diameter	$40mm$
Total observable field area	$1257\ mm^2$
Numerical aperture	0.21
Working distance	$7\ mm$
Operating wavelength	$510\ nm$
Resolution	$1.5\ \mu m$
Magnification	15.2
Camera pixel count	512×512
Camera pixel size	$10\mu m$

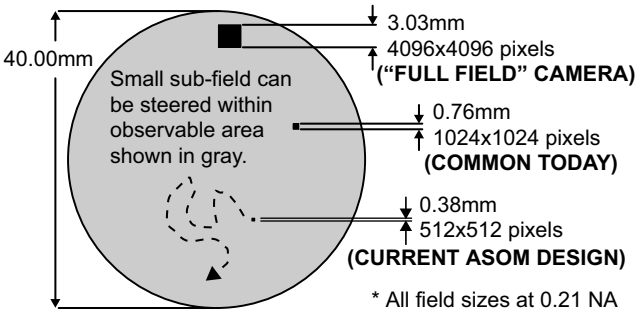

Fig. 3. Field size comparison of ASOM to standard microscopes

Figure 2 shows how the angle of the steering mirror selects the location of the field of view within the workspace. A specific deformable mirror shape corrects for the aberrations unique to each field position to obtain diffraction limited performance. Without the deformable mirror, images obtained at these field positions would be blurred with such a simple scanner lens design. In practice, the deformable mirror is calibrated once during the initial construction. Then during operation, a lookup table with interpolation is used to shape the deformable mirror for each field position. Recent advances in MEMS deformable mirrors have reduced the price of this technology, making adaptive optics more affordable for research and productization [10]. Table I lists performance specifications for the

ASOM shown in Figure 1(a) and the greatly enlarged field of view for the ASOM is compared to existing microscope technologies in Figure 3. See [7] for a more thorough discussion of the ASOM theory of operation.

III. System Architecture

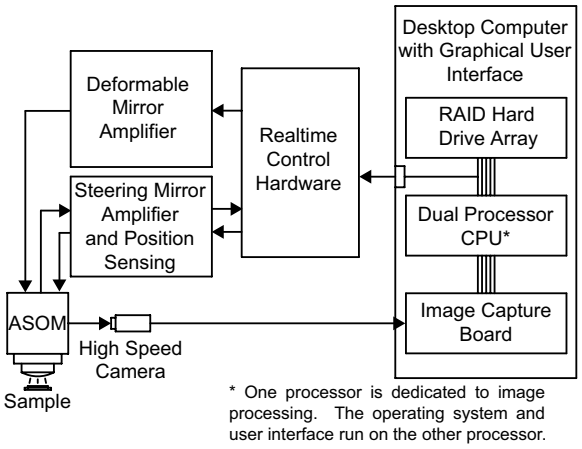

Fig. 4. System Architecture

The overall architecture for the imaging platform we are developing is shown in Figure 4. Light from the specimen is collected by the ASOM imaging subsystem and projected onto the sensor of a high speed digital camera. The high speed camera digitizes the image and transfers the data to a framegrabber residing in a computer with a multi-core processor. One core of the processor is dedicated to image processing while the other core runs the operating system and manages the user interface. The image processing routines locate objects of interest within the image or determine regions of interest. Coordinates for the next scan pattern can then be determined from the processed data and the image data is saved on the computer's hard drive. A Redundant Array of Independent Disks (RAID) is required to maintain the high data transfer rates as continuous data rates for single hard drives are too slow. The coordinates of the regions of interest are then transmitted to the realtime control computer, where the motion path planner generates a new trajectory for the steering mirror. The realtime control computer also controls the steering mirror with a closed loop controller and generates voltage signals to drive the deformable mirror amplifier.

When tracking rapidly moving objects, a moving stage based approach is typically limited to observing only one object [1]. Thus image exposure, data transfer, processing, and motion must be performed *serially* in time as each step depends on the previous step having been completed. The rapid dynamic performance of the ASOM allows multiple rapidly moving objects to be tracked. A significant consequence of tracking more than one object is that image processing can be performed in *parallel* to the data transfer, motion, and image processing events of other objects. This is demonstrated in Figure 5, which shows a possible operational timing diagram of the imaging platform. In this diagram, it is assumed that the system is tracking the motion of three objects and that the frame rate of the camera is constant at 100 frames per second. Events associated with each of the three objects are noted with a circled "1", "2", or "3". First, the deformable mirror is commanded to produce the required shape to compensate for the off-axis aberrations and the steering mirror is commanded to the angle required to steer the field of view into the desired position. After settling of the mirrors, the camera exposure takes place and the data transfer from the camera to the image processing hardware begins. Once transferred, the image is processed and key features are extracted to determine new coordinates for the field of view associated with that object. However, after acquiring images from each object, note that the deformable mirror and steering mirror are free to move into the next position *at the same time* as the data transfer and image processing are taking place associated with the previous object. In fact, the advantage of parallelizing the image processing operations increases with the number of objects being tracked until the data transfer rate or mirror dynamics become the limiting factor.

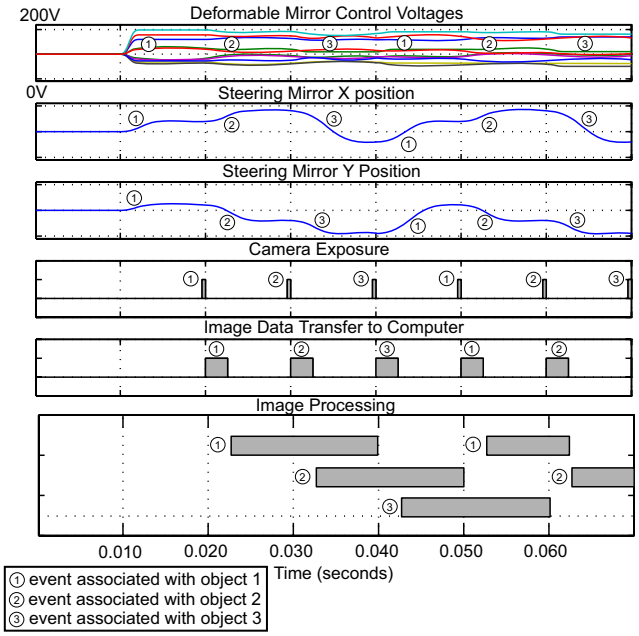

Fig. 5. Timing Diagram

IV. Overview of Experimental Apparatus

The purpose of this experimental ASOM apparatus was to demonstrate all essential optical aspects of the ASOM design, but at low cost and with a short development time. As such, off the shelf optics were used exclusively to avoid the considerable cost of custom fabricated optics and to take advantage of the existing stock of catalog available items that ship within days. However, most stock lenses are designed to be used in a particular manner (e.g. with infinite conjugates) for generic applications and are offered in a coarse range of focal distances, lens diameters, and glass selections. Considering the atypical imaging characteristics of the scanner lens, the experimental ASOM design using

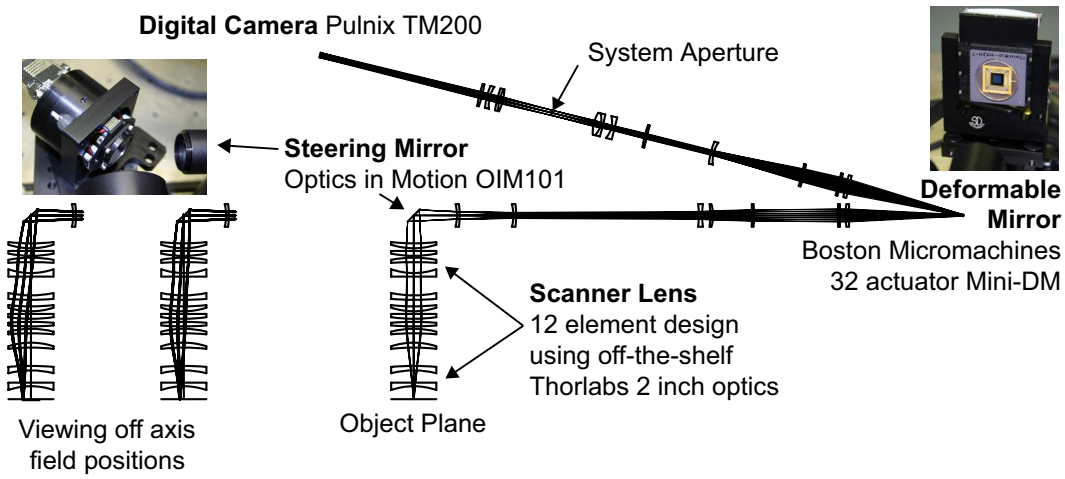

Fig. 6. ASOM Experimental Setup

off-the-self optics only is far from optimal, and as such, exhibits a noticeably high lens count to achieve 0.1 NA over a nominal 20mm field size. However, even with the use of off-the-shelf optics only, this experimental apparatus has been carefully designed to demonstrate all of the *critical optical characteristics* that define the ASOM, including the curved field optical scanning approach and wavefront correcting optics using a deformable mirror. Future experimental work will utilize custom manufactured optics to fully realize the potential of the ASOM concept to achieve higher numerical aperture and a larger workspace.

Figure 6 shows the optical layout of the experimental setup and Figure 7 shows a picture of the prototype ASOM. This prototype utilizes a galvanometer based scanning Kohler illumination system such that only the region being imaged is illuminated by the transmitted light, reducing phototoxity effects on living specimens. Because the current design is very sensitive to chromatic aberration, a $510nm$ wavelength bandpass filter is included in the illumination stage to eliminate much of the light spectra below $500nm$ and above $520nm$. Light transmits through the sample and is then collected by the telecentric 12 element scanner lens assembly. An electromagnetically actuated fast steering mirror (FSM) with a flexure suspension (Optics in Motion OIM101) has two degrees of freedom to steer the sub-field of view within the workspace. Optics project the light onto the MEMS deformable mirror (Boston Micromachines Mini-DM). By precisely controlling the shape of the reflective surface of the mirror to be opposite the shape of the wavefront error (but at half the amplitude), the deformable mirror can correct for the wavefront aberrations to within the diffraction limit. This mirror has 32 electrostatic actuators with $400\mu m$ actuator spacing, a $2.5\mu m$ actuator stroke, and a $2.0mm$ diameter actively controlled area. The $2.5\mu m$ stroke is capable of correcting for up to several waves of aberration, which allows for high image quality even for the off-axis field positions and enables the greatly expanded field of view in the ASOM. Additional optics project the final image onto the CCD camera (Pulnix TM200). A host computer acquires (MATROX Meteor II frame grabber) and processes the images. A second computer is dedicated for real time control running MATLAB xPC Target and implements the digital control/trajectory generation algorithms for the FSM and generates galvanometer position commands for the scanning illumination system. Note that parallel processing has not yet been implemented on this testbed, but is currently under development along with integration of a high speed camera.

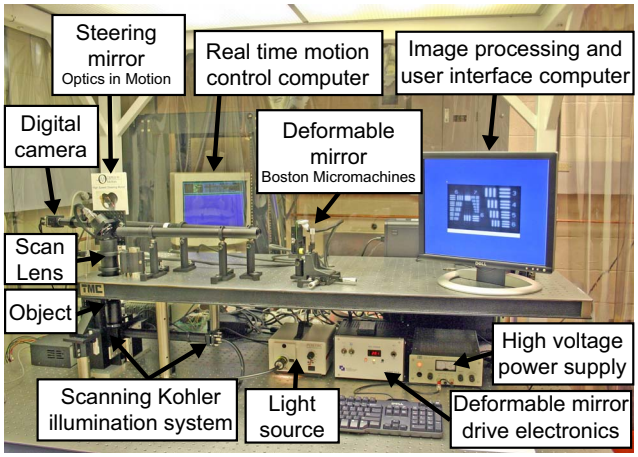

Fig. 7. ASOM prototype built with off-the-shelf optics

V. Biomedical Applications and Demonstrations

Using the experimental apparatus described in Section IV, we have performed initial imaging experiments of living biological specimens and medical samples used for diagnostics. Figure 8 shows a five tile mosaic of several nematode worms (*Caenorhabditis elegans*), a major model system for neurobiology and developmental biology studies. These worms are alive and moving on an agar medium. Ultimately, the ASOM imaging system will enable studies of later developmental events such as gonadogenesis, in freely moving, unanesthetized animals. Additionally, the ASOM will allow the high resolution tracking of the behavior of freely moving and interacting animals.

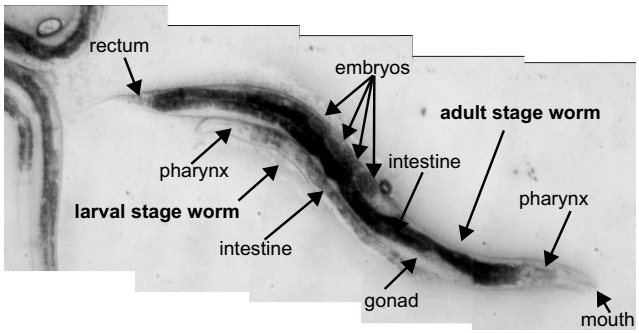

Fig. 8. Five tile mosaic image of *Caenorhabditis elegans* worms

Figure 9 shows an image obtained with the experimental ASOM apparatus of a biopsy sample mounted on a glass slide. The darker stained area indicates the presence of cancer cells while the surrounding tissue is healthy. Increasingly, specialized sample preparation and instrumentation are required in medical diagnostics, requiring that tissue samples be shipped and processed at a centralized facility. Currently, process throughput is a significant bottleneck resulting from slide handling, slide transfer, and slow moving stage dynamics. Furthermore, in the case of biopsies obtained with a needle extraction, the sample is placed haphazardly on the slide and occupies only a small portion of the slide area. The ASOM will be able to perform a very rapid background scan and then a high quality scan of only the biopsy region of interest. The high speed of the rapid background scan will be obtained by imaging without stopping the steering mirror motion. These images will be slightly blurred, but will allow the tissue sample location to be identified. The ASOM will then plan a trajectory to capture the region of interest. High quality images of the regions of interest only will then be acquired by obtaining the images with the steering mirror fully stopped and settled for each exposure to gain a large increase in process throughput.

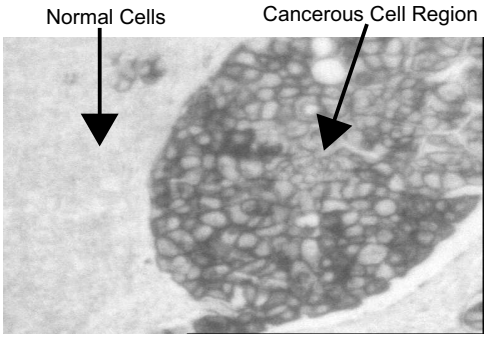

Fig. 9. Biopsy showing normal and cancerous cell tissue

VI. Conclusion

An automated microscope station based on the Adaptive Scanning Optical Microscope (ASOM) would exhibit significant advantages over the existing moving stage approach with respect to dynamic performance, throughout, and by not agitating the specimen. Particularly suitable for challenging spatial-temporal biomedical observations, the novel ASOM configuration achieves its performance advantage by combining a high speed post-objective scanning mirror with a custom design scanner lens and a deformable mirror to correct for off-axis aberrations. During high speed operation, the microscope system will generate considerable amounts of data which requires careful consideration of the system architecture. We propose that high bandwidth data busses, multiple processors, and parallelization of the image processing and motion tasks are thus required. A proof of concept experimental apparatus has been constructed and demonstrates all of the critical optical aspects of the ASOM using off-the-shelf optics, a 32 actuator MEMS deformable mirror, and high speed steering mirror. We have used this experimental hardware to performed initial imaging experiments of living biological specimens and a cancer biopsy sample used for medical diagnostics. We are currently integrating a high speed camera and multiprocessor computer into the ASOM imaging system and will soon begin a series of biological studies with the apparatus. Experiences learned during this first round of studies will help us design the next generation prototype that will include custom manufactured optical elements, an aerospace grade high speed steering mirror, and 100 actuators MEMS deformable mirror to realize the full potential of the instrument.

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