Design of an Adaptive Scanning Optical Microscope for Simultaneous Large Field of View and High Resolution

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Abstract—In microsystems applications from micro-assembly to biological observation and manipulation, the optical microscope remains one of the most important tools. However, it suffers from the well known trade-off between resolution and field of view. Traditional solutions involve moving the sample under the microscope using a moving stage or moving the microscope itself, and switching between low and high magnification objective lenses. In this paper, we present a new optical microscope design that uses a 2-dimensional high speed, high precision steering mirror system to scan the sample. By stitching the images together as a mosaic, we have the potential to achieve both high resolution and large field of view. Working in coordination with a deformable mirror, this arrangement offers certain advantages over the current state of the art. We describe the theory of operation, our design methodology, and present a preliminary simulated design. A reduced functionality experimental prototype has been constructed to demonstrate the basic efficacy of the concept and we demonstrate with both biological and micro-assembly examples.

I. INTRODUCTION

The capabilities of optical imaging systems, which have traditionally consisted of extremely accurate and well calibrated, but primarily static components, are rapidly expanding with the introduction of robotic and motion control technologies applied to the optical elements. These adaptive and dynamic imaging systems allow the pursuit of scientific investigations, military applications, and medical diagnostics that are beyond the theoretical capabilities of a purely static optical design. Along with the recent growth of Biotechnology and Micro-Electro-Mechanical Systems (MEMS) there is a growing need to observe, interact with, and inspect at a scale below the threshold of the naked human eye. The optical microscope has been, and will continue to be a critical tool as these fields advance. While this need has inspired a resurgence of interest in the optical microscope, the essential optical design and operating principle has not changed significantly in the last century and the optical microscope still suffers from a well known inherent tradeoff between the field of view and resolving power of the system. This paper seeks to show that our new Adaptive Scanning Optical Microscope (ASOM) concept can effectively address this tradeoff, and can offer certain advantages over the current state of the art. We do this by integrating active optical elements, motion control, and image processing techniques into the design.

The need for a large field of view at high resolution occurs in biological science, industrial manufacturing, medical diagnostics, etc., and there are several modern approaches to address the situation where a single image can not satisfy both requirements. These solutions include: a moving stage located underneath the microscope, parfocal (equal focus) objective lens switching, zoom lens designs, and moving microscopes. Each method has advantages and disadvantages, which are summarized in Table I. The ASOM design that we present in this paper will excel in applications where critical spatial-temporal observations are demanded, but will not offer the virtually unlimited field of view associated with the moving stage do to optical limitations. Biological applications where the ASOM would be attractive are: observing dynamic cellular events (mitosis, viral attachment, motility) over a large population of living cells. In industry, the ASOM will allow for vision guided micro-assembly and rapid inspection of completed parts, with the potential for higher product throughput.

The motivation for the ASOM came from the micro-assembly and packaging activities at the Center for Automation Technologies at Rensselaer Polytechnic Institute and the conceptual layout was inspired by a machine designed at École Polytechnique Fédéral de Lausanne (EPFL), for laser annealing shape memory alloy [1].

The key idea is a simple one: use a low mass steering mirror [2], [3] positioned in the middle of the optical path to scan the images. The advantages of such an arrangement are: a large effective field of view at high resolution, no disturbance to the sample, and high bandwidth operation. However, such a system also poses significant challenges. In contrast to the moving stage or moving microscope designs, there is extensive off-axis imaging (i.e., images are obtained by looking diagonally through the lens), which introduces distortion, contrast degradation, and loss of resolution [3]. We address the off-axis aberrations by: (1) explicitly incorporating field curvature into the design and (2) introducing an actuated deformable mirror (DM) into the optical path for wavefront
correction.

In this paper, we first discuss wide field of view and high resolution imaging in Section II. Section III-A describes the key features of the ASOM; Section III-B presents our initial design approach; and Section III-C presents simulated performance results. Finally, Section IV describes our current experimental proof-of-concept implementation with demonstrations in micro-assembly and biological observation.

### II. WIDE FIELD OPTICS AND IMAGING

The design of wide field and high resolution microscopic imaging systems are driven by consideration of (1) an image sampling issue and (2) an image quality issue. First, consider an imaging system with optics that are nearly perfect (i.e. the optical aberrations are much below the diffraction limit). Such a system will image two point sources separated by a distance, $d$, as two overlapping Airy patterns in the image field. As the distance between the two points decreases, a critical distance will be reached, $r$, where the two points can no longer be individually distinguished. According to the Rayleigh criteria, this critical distance, called the resolution, is equal to the radius of the central core in the Airy pattern and is related to the numerical aperture, $NA$, of the system and the wavelength of light, $\lambda$, by:

$$
r = \frac{0.61 \lambda}{NA},
$$

where the NA of the system is defined by the index of refraction of the transmitting medium, $n$, and the half angle, $\theta$, of the cone of light collected from the object: $NA = n \sin(\theta)$.

A digital camera must sample with two pixels per Airy core radius to avoid aliasing according to the Nyquist sampling criteria. This observation provides the following maximum theoretical object field width, $W_o$, for a sensor array pixel count per edge, $k$, and resolution, $r$:

$$
W_o = \frac{kr}{2}.
$$

While microscopic imaging systems are often designed with resolutions in the $\frac{1}{4} \mu m$ to several $\mu m$ range, the lower practical limit on CCD camera pixel size is approximately $6 \mu m$ due to noise effects. Therefore, the optics must enlarge the Airy pattern to achieve proper sampling with the required magnification factor, $M$, for a given pixel size, $s$ is given by: $M = 2s/r$. The corresponding image size, $W_i$, is: $W_i = ks$.

Figure 1(a) illustrates the above mentioned equations and represents the imaging optics as a generic black box. The optical design task is to specify the design of the imaging system, i.e., to fill in the details of the black box with specific lens or mirror geometries, glass types, and spacing.

![Fig. 1.](image)

Fig. 1. (a) Imaging with black box optics, (b) microscope objective, (c) lithography lens. The lens prescriptions for the microscope objective and lithography lens were obtained from [4] and are not shown to scale.

The first intuitive approach to designing a large field and high resolution imaging system might be to take an existing microscope layout, such as that shown in Figure 1(b), and simply increase the pixel count of the camera while redesigning the optics to achieve a larger field of view. This approach may indeed be possible, but it is not generally practical as the requirements for field size, flat field, and numerical aperture soon approach those of lithography lenses. The 1998 Nikon
lithography lens (US Patent 5,805,344) shown in Figure 1(c) has a 0.65 NA with field sizes of 93.6mm and 23.4mm. Lithography lenses require near perfect manufacturing and extremely tight assembly tolerances (often requiring an interferometric assembly process), and can cost in the millions of dollars [4]. Also notice the presence of negatively powered elements located at the narrow beam regions in both the microscope and lithography lenses. This design technique is used to achieve a flat imaging field (small Petzval sum) and results in an increase in the lens count. An additional consideration is the size of the image sensor, considering that large commercially available CCD cameras only have approximately 9216x9216 pixels (e.g. Fairchild Imaging CCD595). Smaller CCD arrays can be mosaicked to achieve larger pixel count with the advantage of being able to read data in parallel, but at a cost of additional precision assembly requirements. Consideration of these issues motivated the design of the ASOM.

III. ASOM CONCEPT, DESIGN, AND SIMULATION

A. Theory of operation

The ASOM operates by taking a sequence of small spatially displaced images in rapid succession and then assembling a large virtual image (mosaic) of the scene. The concept of expanding the field of view while preserving resolving power through mosaic construction is well established and has been applied to biological imaging [5] as well as industrial imaging [6]. However, instead of a moving stage as is common, the mechanism and scanning principle in the ASOM consists of a high speed steering mirror working in coordination with a specially designed scanning lens assembly, a deformable mirror, and additional imaging optics. A conceptual layout of the ASOM is shown in Figure 2.

Curved field scanning layout- Different than a microscope objective or lithography lens, the scanning lens on the ASOM is designed to exhibit significant field curvature with a relatively large Petzval sum. This relaxation of the flat field requirement offers the advantage of a greatly simplified optical design with far fewer lens elements (the advantages of curved field designs have been recognized for aerospace applications [7]). Additional characteristics of the ASOM scanning system include:

1) The center of the field curvature, the rotation center for the 2-D steering mirror, the mirror surface, and an optical pupil plane are all mutually coincident.

2) The shape of the projected image surface is nearly spherical instead of the more typical parabolic surface associated with field curvature. This is achieved through higher order aberration control.

Under the above mentioned conditions, as the steering mirror angle changes, the projected curved image surface rotates about its own center as shown in Figure 3. Stationary imaging optics with a matching negatively curved field work with a frame stop to sample a portion of the image surface, providing for an image scanning and selection mechanism as the steering mirror angle changes. This layout exhibits (1) a large positively curved field associated with the scanning lens, and (2) a small negatively curved field associated with the imaging optics, thus avoiding the significant difficulty of designing and manufacturing a large continuous flat field imaging system as discussed in Section II.
Variation in the aberration is allowed between individual field positions throughout the scanner’s range. However, given that the deformable mirror can only achieve one specific shape at a time, the rate of change in the aberration between field positions must be small enough to allow diffraction limited imaging performance over the entire sub-field of view that is selected. This is similar to the concept of the isoplanatic patch in the atmosphere [8] that is widely recognized in the adaptive optics telescope community. By analogy with the ASOM, the isoplanatic patch of the scanning lens must be larger than the selected field of view.

B. Design process

Modern optical design is generally performed using the simulation and optimization capabilities of commercially available lens design software. Local minima searching algorithms can often improve an initial design and are highly dependent on the starting values of the design variables. However, generating a new design form requires a global search. Applied directly to the ASOM design problem, the ZEMAX genetic algorithms (global search) proved ineffective because of the size (number of design variables) and complexity of the system (requires multiple configurations to accommodate the different mirror angles and DM shapes required for each field position). Instead of an all-in-one global optimization approach, we partitioned the system into (1) a scanning lens assembly and (2) the imaging and wavefront correcting optics as shown in Figure 3. The scanning lens assembly was designed as a fully static system (no moving elements) for a curved field with up to several waves of aberration (with the assumption that the deformable mirror would eventually correct for this aberration). The imaging and wavefront correcting optics were then designed separately for diffraction limited performance over the entire field with the deformable mirror held flat. The compatibility of the two systems at the interface was maintained by human intervention with much trial and error. Next, the two subsystems were joined with the steering mirror and deformable mirror added to the system. The local optimization algorithm and the multi-configuration capabilities of ZEMAX allowed for the system wide optimization of the lens geometries and spacing while simultaneously considering multiple field positions, steering mirror angles, and specific deformable mirror shapes. Future work will investigate more formal and automated design approaches and will likely draw heavily from the field of Multidisciplinary Design Optimization (MDO).

C. Specifications and predicted performance

A practical design resulting from the optimization described in Section III-B is shown in Figure 4. The simulated results that follow demonstrate that the ASOM can effectively provide an expanded field of view while preserving resolution when compared to existing microscope technologies. Table II lists performance specifications of the specific ASOM design described in this paper, but with suitable changes to the design, the field area and numerical aperture could be tailored to the observation task at hand. However, in general, as the field area increases, the realizable NA will decrease due to physical and practical limitations.

<table>
<thead>
<tr>
<th>Specification</th>
<th>40mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effective field of view diameter</td>
<td>40mm</td>
</tr>
<tr>
<td>Total Observable Field Area</td>
<td>1257 mm²</td>
</tr>
<tr>
<td>NA</td>
<td>0.21</td>
</tr>
<tr>
<td>Resolution</td>
<td>1.5 µm</td>
</tr>
<tr>
<td>Magnification</td>
<td>15.2</td>
</tr>
<tr>
<td>Camera pixel count</td>
<td>512 X 512</td>
</tr>
<tr>
<td>Camera pixel size</td>
<td>10 µm</td>
</tr>
</tbody>
</table>

Table II
PRELIMINARY ASOM PERFORMANCE SPECIFICATIONS

Figure 5 compares the observable field of view of the ASOM to a fixed microscope with a 4096x4096 camera (considered a full field camera with standard microscope objectives) and with a 1024x1024 camera which is more common. The ASOM offers diffraction limited (Strehl ratio > 0.8) for all field positions based on high fidelity simulation. The field sizes for the fixed microscope designs assume perfect imaging and were calculated using Equations 1 and 2 using a 0.21 numerical aperture with λ = 0.510µm for the wavelength of light (green light is relatively nondestructive and desirable for imaging living biological cells).

We next demonstrate the wavefront correcting capabilities of the deformable mirror. The µDM100 DM from Boston Micromachines with 100 electrostatic actuators, a 3.3mm round aperture, and 2µm stroke was used in this design. Instead of modeling all 100 actuators, we created a user defined surface in ZEMAX consisting of a set of 37 evenly spaced Gaussian basis
functions to represent the mirror surface shape. We assume that the surface generated with the low spatial frequency gaussian shapes can be reproduced by the deformable mirror within the maximum actuator stroke. The amplitude of each basis function becomes a variable in ZEMAX and can be optimized with the other glass geometry and spacing variables as well as the steering mirror angle.

Figure 6 shows how the DM corrects for the specific wavefront aberration associated with each field position. Over the entire field and for all field positions, the Strehl ratio is greater than the diffraction limit of 0.8, resulting in near perfect imaging. Figure 7 shows the worst case Modulus of the Transfer Function (image contrast vs. object spatial frequency) for the ASOM design. For comparative purposes, an optimization was performed with the same initial design, except with no deformable mirror in the system (i.e. the DM shape was fixed to be flat). The system with no deformable mirror shows considerably degraded MTF imaging performance as is illustrated in the simulated images of biological cells.

The results presented are based on idealized simulations ignoring the reality that lenses and optical housings are always subject to manufacturing and assembly tolerances. We will consider these aspects in future work.

IV. EXPERIMENTAL PROTOTYPE

To demonstrate the basic principle of scanning and image mosaic construction, we have built a first generation prototype [9] called the Scanning Optical Mosaic Scope (SOMS), shown in Figure 8. No formal optimization of this design was performed, and the prototype unit was constructed using standard catalog lenses available from ThorLabs, a Sony XC-77BB CCD camera, Matrox Meteor II frame grabber, Cambridge technologies galvanometers and servo drivers, and a TI based DSP board. It differs from the more advanced ASOM design proposed in this research in that: (1) the optical layout is simplified, (2) there is no deformable mirror or adaptive optics, (3) all lenses are available as standard catalog items, (4) the scanning lens is a single standard achromat doublet.

A. Micro-assembly and biological demonstrations

The micro-manipulation demonstration is based on a shape memory alloy microgripper moving between two fixed objects in a workspace. A rudimentary correlation based image matching algorithm and Kalman filter are used to track the motion of the gripper tip. A 3 X 3 tile mosaic images the gripper and the scanning pattern is automatically adjusted to maintain the tip in the center tile. The scan pattern also includes the two stationary objects in the workspace, demonstrating the capability of the SOMS to observe multiple stationary and moving objects in the workspace nearly simultaneously. A sequence of the video footage is shown in Figure 9.

Figure 10 shows a video sequence of living cells. Several events of mitosis (cell division) can be seen occurring throughout the viewing field. The ASOM not only offers the possibility of automatically detecting the onset of mitosis and other events, but can be easily programmed to track and record
multiple events at the same time. While automated quantitative cell analysis using a moving stage has recently been proposed [10], the bandwidth of the overall system is still constrained by the response of the stage and the sensitivity of the cell specimen to motion. The ASOM will address both of these issues.

V. Conclusion

We have presented a new microscope concept that can simultaneously achieve high resolution and large field of view that offers several advantages over the current state of the art for observing certain spatial-temporal events. A proof-of-concept prototype has been constructed to demonstrate the basic efficacy of this approach. The next generation of hardware will include a deformable mirror with custom designed optics and will be based on further systematic design optimization and consideration of manufacturing errors and assembly tolerances. The calibration and online optimization of the active optical elements is a significant challenge and will be a focus of future work.

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References