A FAST SCANNER DESIGN FOR A SCANNING MULTI-PROBE MICROSCOPE

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OVERVIEW
This abstract presents the design, performance evaluation and applications of a fast xy translation stage to create area scans of the optical spot in a metrological confocal microscope system. Scanning is achieved by moving the output of the fiber light source over an area of 60 x 60 µm that corresponds to a scan of around 5.2 µm of the spot size. The fiber scanner has a first mode resonance of 1.4 kHz and scans at rates of up to 80 lines per second are presented.

INTRODUCTION
We have developed a novel three-dimensional metrology stage for biomedical imaging systems that will enable the spatial co-registration of diagnostic information using optical and mechanical probes [1]. This Scanning Multi-Probe Microscope (SMPM) utilizes the mechanical and optical probes which are stationary relative to the instrument frame while the specimen can be navigated in a three-dimensional space in the probing field, translating over an XYZ range of 50 µm by 50 µm by 30 µm in each axis, respectively, at closed loop speeds of greater than 10 line scans a second. FIGURE 1 shows the main components of the system.

Block 1 contains the XYZ scanner and sample holder S, mechanical probe P that can be adjusted in three axes for alignment with the focal spot of the confocal lens C that illuminates the specimen from the underside.

Block 2 is the instrument base that also includes a manually adjustable coarse motion stage to adjust the specimen position and orientation with respect to the confocal probe.

Block 3 is the electrical part of signal conditioner for the mechanical probe and intensity and photon counters for optical measurement.

Block 4 is the hardware control systems for dynamic positioning of the probes relative to the specimen. It comprises several newly integrated hardware controls. Software implementation is achieved using LabVIEW 8.6 Real-Time™ with program code running on 2.6 GHz quad processor computer as a high speed real time controller. The controller connects to a PXI-7852RA through MXI bus. An FPGA based 40 MHz counter is used to count photons to produce the fluorescence image. Digital to analogue convertors (DACs) and ADCs are used to read the signals from the sensors and provide the command voltage to the piezoelectric actuators via a high voltage amplifier. Multi-channel proportional-integral-differential (PID) controllers are used for the closed loop control of the fine stage scanning and probe surface tracking. Three capacitance sensors built into the sample holder measure the displacements of three axes of the fine stage and therefore provide the measured displacement to the controller.

Block 5 is the host computer running under Windows XP. A flexible and user-friendly GUI was designed and built using LabVIEW in the host computer. It realizes the function of sending controls and scanning parameters to the real time controller, receiving, post-processing and displaying the measurement results.

FIGURE 1. schematic diagram of the Scanning Multi-Probe Microscope (SMPM).
Recently, we proposed a novel scanning method for the fluorescence probes microscopy of SMPM in which the fiber is mounted to an XY piezoelectric actuated fast scanner shown in block 6 in FIGURE 1. By scanning the fiber in an XY plane, the focused beam is able to scan the sample object plane. Initial experiment and results show that the scan range of the positioner was around 60 µm in each axis for the corresponding scan of 5 µm range of the spot. Such an attenuation of around 12:1 for this optical system may be of particular benefit for ultra-high resolution, metrological confocal microscopy. The low mass of the optical fiber enables high speed imaging of samples without having to move the more massive sample stage with specimens also sometimes comprising liquid specimen environments.

To achieve smooth, frictionless, fast response a flexure constrained, XY piezoelectrically actuated, scanner has been designed to have a range of 60 µm by 60 µm and more than 1 kHz bandwidth in open loop operation, FIGURE 2. Because of the optical attenuation of approximately 12:1, it is desired that the scanner maintain controller errors within 10 nm over the 60 µm scan range corresponding to a signal to noise target of around 10,000:1.

Because the piezoelectric actuators employed have a maximum displacement of 16 µm, a tilt-tilt design is used to obtain a displacement amplification factor of four, see FIGURE 2. To maximize stiffness (and therefore dynamics) and match dynamic performance in the two axes, it is based on a parallel-kinematic design with two orthogonal pairs of differentially-driven piezoelectric actuators. FIGURE 2c) illustrates the amplification mechanism in one axis. The scanning range can be calculated from

\[ 2\delta = \frac{L}{l} \varepsilon \]  

Equation 1

where \( \varepsilon \) is half of the extension of each PZT and the ratio of lengths is the mechanical lever ratio. Ignoring lost motion, the stroke is calculated to be around 60 µm for the design used in these experiments. Four, orthogonally symmetric, leaf spring flexures on the upper face of the mechanism provide the preload.

Theoretical modeling and finite element analysis was conducted to estimate the system’s static and dynamic performance. The platform with the fiber holder located underneath has a stiffness of about 0.95 N·µm\(^{-1}\) and an angular resonant frequency at around 1.8 kHz predicted using FEA. The fiber holder’s first mode of resonant frequency is about 3.4 kHz. Direct knife-edge displacement sensors for closed loop control feedback sensing[2]. Again scanning and imaging is achieved using the PXI-7852R smart Daq card in LabVIEW Real-time with an FPGA based controller. With the integrated fast scanner and the FPGA-based dual stage controller, the SMPM is able to obtain confocal images of a smaller area of interest within the longer range XYZ fine stage scanning volume. Currently this optical detection is capable of measurement of fluorescent resonance energy transfer, fluorescence anisotropy and single molecule imaging.

**DYNAMIC RESPONSE**

Open loop frequency response of the fast scanner has been measured using an HP 35693A dynamic signal analyzer. The displacement responses of each axis as a function of frequency are measured by the optical displacement sensor. Table 1 lists the measured resonant frequency of each axis for three different damping conditions. Damping has been added by applying Kopr-Kote (a colloidal grease with fine copper particulates) into the gap between the center hole of the base and the tube/cylinder. It decreases the Q by 15-20 while the resonant frequency is maintained at 1445.6Hz. FIGURE 3a) shows the result that the natural frequency of the PZT2 with Kopr-Kote. FIGURE 3b) is the step response of the PZT2 with a settling time of 5 ms. As an alternative damping treatment a 1:40 ratio Sylgard, a type of silica gel, was used to fill the gap. It gives a damping ratio two times larger than the copper oil, as well as adding substantial stiffness which increased the resonant frequency to 6,884 Hz.
FIGURE 2. (a) CAD model of the fast scanner (b) photograph of the fast scanner (c) principle of operation \(L = 46\text{mm}, \ l = 10\text{mm}\)

FIGURE 3. a) Frequency response fiber scanner using copper oil as a damper b) Step response

TABLE 1. Resonant frequencies of each axis with different damping treatments

<table>
<thead>
<tr>
<th>Wn/Hz</th>
<th>AxisX1</th>
<th>AxisY1</th>
<th>AxisX2</th>
<th>AxisY2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>1475.3</td>
<td>1470.4</td>
<td>1475.3</td>
<td>1470.4</td>
</tr>
<tr>
<td>CopperOil</td>
<td>N/A</td>
<td>1445.6</td>
<td>N/A</td>
<td>1445.6</td>
</tr>
<tr>
<td>Sylgard</td>
<td>N/A</td>
<td>6884</td>
<td>N/A</td>
<td>6884</td>
</tr>
</tbody>
</table>

RESULTS

FIGURE 4. a) shows a 23.2 \(\mu\text{m} \times 22.8 \mu\text{m}\) fluorescence images of a calibration grid using the longer range scanner. The grid is fabricated using electron beam lithography. It consists of a thin gold film (30 nm thick) patterned with 400 nm round holes, with a periodicity of 800 nm. The sample is then spin-coated with a PMMA film that contains rhodamine 6G fluorescent molecules. The image is acquired by illuminating in the epi-fluorescence mode from the bottom of the sample with the PMMA film on top of the gold film. The light is then collected also from
the same bottom objective so that bright fluorescence spots are present where the holes of the film exist. FIGURE 4b) is a 5 µm x 5 µm scan range of the grid at a speed of 4 lines per second. FIGURE 4c) shows a zoom section of 5 µm x 5 µm from the image in 4a). The resolution is only a 5.5 pixels per micrometer.

FIGURE 4. Images obtained using large scanner. a) Range: 23.2 µm x 22.8 µm, 128*128 pixels at 4 lines per second; b) Range: 5 µm x 5 µm 128*128 pixels at 4 lines per second; c) section magnifying lower left image in FIGURE 4.a);

FIGURE 5. shows two scanning results using the fiber fast scanner. Both images are obtained at a full scan range. With an optical attenuation of about 12, the laser spot navigates over a range of 5 µm x 5 µm in the sample plane. FIGURE 5a) is captured with a line scanning speed of 4 Hz while in FIGURE 5b) this speed goes up to 78 Hz. The contrast drops in higher speed because the sampling time (therefore photon counting statistics) for each pixel is reduced. The stretch in dimension along the y axis (fast axis) comes from a dynamic amplitude decrease at the scan frequency. Compared with the result in FIGURE 4c), both show a same area of 5 µm x 5 µm on the grating, while FIGURE 5b) has much higher resolution and contrast of 25.6 pixels per micrometer at 1.6 second for a frame. With the fast fiber scanner and original scanner, a five times better pixel resolution than FIGURE 4a).

CONCLUSION

The current fast scanner is working in open loop operation. While the fast dynamic response and high resolution has significantly increased productivity as a biological research tool. There remain many challenges; validation of this methodology for metrological measurement, optimizing damping, filtering for smoothing digitized drive signals, working in resonant mode, a high stiffness mount for the fast scanner, utilizing displacement sensors to remove image distortions, and developing scan algorithms capable of recording and storing large data files. Further data incorporating these enhancements as well as interesting confocal measurement case studies will be presented.

REFERENCES
