Positioning systems for high resolution tissue imaging

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ABSTRACT

Tissue handling systems position *ex-vivo* samples to a required accuracy that depends on the features to be imaged. For example, to resolve cellular structure, micron pixel spacing is needed. 3D tissue scanning at cellular resolution allows for more complete histology to be obtained and more accurate diagnosis to be made. However, accurate positioning of a light beam on the sample is a significant challenge, especially when fine spacing between scan steps is desired or large, inconsistently shaped samples need to be imaged. Optical coherence tomography (OCT) is an application where accurate positioning systems are required to reap the full benefit of the technology. By simultaneously manipulating the light beam position and sample location, a 3D image is reconstructed from a series of depth profiles produced. To automate image acquisition, a fully integrated and synchronised system is necessary. A tissue handling and light delivery system for free-space optical devices is described. Performance characteristics such as resolution, uncertainty, and repeatability are evaluated for novel hardware configurations of OCT. Typical scanning patterns with associated synchronisation requirements are discussed.

Keywords: light delivery, positioning system, optical coherence tomography, galvanometer, synchronisation

1. INTRODUCTION

Light delivery and sample positioning systems add important functionality to imaging devices. By raster scanning a focused beam of light across the surface of a sample, multiple dimensions of data may be obtained and reconstructed to form images. Positioning the light beam on the sample becomes a significant challenge when fine spacing between scan samples is desired. While many medical imaging systems use two galvanometers to achieve two dimensions of positioning flexibility, the system presented here uses one galvanometer and a linear stage. A galvanometer and stage configuration allows significantly larger sample sizes to be scanned than when using a two galvanometer system and does not exhibit distortions from stage movements that are more likely with two galvanometers. The sample size is predominantly limited in length only by the travel of the linear stage and the time allotted for scanning. Using a linear stage, however, does limit the system to scanning excised tissue and does not permit *in-vivo* scanning.

1.1 Application Areas

Light delivery systems have wide application. They are used in laser marking, high-resolution print applications, DNA analysis and drug discovery systems,[1] projection displays, 3D image synthesis,[2] and miniaturized fibre-optic communications switches.[3] In addition, scan systems are used for image stabilization in aerial platforms like the Compact Reconnaissance Imaging Spectrometer for Mars (CRISM) satellite orbiting the planet Mars. CRISM gimbals the sensor to keep the location being imaged in the field of view and results in more stable imagery.[4] Finally, high performance scanning applications are prevalent in medical imaging and enable greater sample coverage and throughput at an attractive cost.

In Section 2 the target medical imaging modality, optical coherence tomography, is introduced. In Section 3, a prototype light delivery and sample handling system is depicted and in Section 4, the system is analysed. Conclusions are presented in Section 5.

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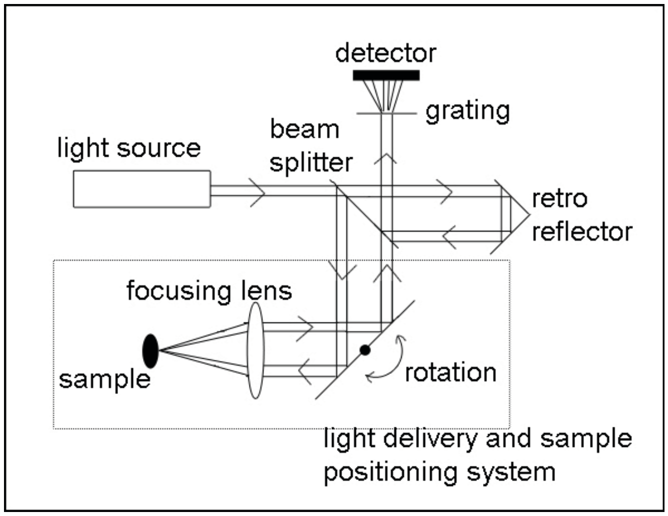

Figure 1. OCT System with the sample handling and light delivery system identified. Beam is shown offset for clarity only to illustrate propagation direction.

2. OPTICAL COHERENCE TOMOGRAPHY

Medical imaging aims to determine a patient's state of health, evaluate anatomy, or to explore spatial relationships between components that yield insight into health. Medical optical coherence tomography (OCT) systems image internal micro-structure in biological tissues by measuring backscattered light.[5] OCT imaging includes many advantageous characteristics: high resolution, non-invasive, non-contact, use in fibre-optics (e.g., in an endoscope), high speed, non-harmful radiation, and inexpensive compared to other imaging systems (e.g., magnetic resonance imaging (MRI)). While OCT, MRI, ultrasound, positron emission tomography, and computed tomography can all be used with live patients *in-vivo*, OCT has better resolution. Confocal microscopy is OCT's closest competitor in terms of resolution, but is often used to scan biopsied tissue and does not have as good depth penetration. The basic configuration of a Fourier domain OCT system is illustrated in Fig.1

OCT splits an incident beam into a reference beam and a sample beam. The interference between the sample arm and the reference arm allows for structural information to be determined from the resulting interferogram. Each wavelength of light interacts with the sample's structures and the deeper the structure, the longer the phase delay. By passing the beam through a diffraction grating, the interfering beam splits to constituent wavelengths and can be read by the sensor in a single exposure. The combined set of broadband phase delays form a sinusoid for each structure. The sinusoids can then be reconstructed to yield a depth profile. By manipulating the location of tissue interaction on the sample, a new depth profile may be obtained.

Scattering is the predominant light interaction between tissue and the OCT sample beam. Light that scatters once within the layers of the sample and is back reflected into the system provides useful information, while light that scatters multiple times adds to the system noise. The multiply scattered light will travel a longer optical path and show up as an inaccurate reflection location. As the ratio of single back reflected light to multiply scattered light decreases, the signal to noise ratio of the resulting OCT image decreases and limits the penetration depth. Scattering generally limits depth penetration of OCT to 1-3 mm.[6]

2.1 Light Handling Requirements

The primary goal of the light delivery system described here is to inject light into a known location within the sample and be able to move the light beam and sample by small enough amounts to image features of interest. To satisfy Nyquist sampling, at least two images must be retrieved for each feature of interest; that is, the imaging beam must be directed to multiple points on a feature in order to differentiate that feature.[7] This is not the only factor, however. Proper design of the system must also take into account the speed of operation, size of tissue samples, and how to synchronise the system's movements with sensor exposures.

2.2 Other Hardware Techniques

There are several configurations of light delivery and sample positioning systems available. Most often, systems use two galvanometers to move the beam across a stationary sample. Galvanometers are specialized motors that have a mirror attached to the output shaft. The range of motion tends to be limited to a maximum of +/-40° optical[1] and the mirror oscillates back and forth according to an input control signal. The movement range is rated in degrees and can be either an optical or mechanical rating. One mechanical degree of movement is equal to two optical degrees since there is a reflection from the mirror. Galvanometers generally use internal PID feedback systems to ensure the mirror head moves to a desired angle and each system must be tuned to accommodate the particular inertia of the mirror attached.[8] The high speed operation of galvanometers allows these systems to change the beam deflection angle quickly, yet maintain accurate positioning of the beam. However, the systems are limited in the size of sample they can accommodate. When galvanometers approach their maximum operation angles, the permissible frequency of oscillation decreases. This effect is due to the inertia of the mirror and the speed at which a galvanometer is able to change direction. The settling time (time until the mirror has stopped moving) is higher for a larger galvanometer movement.[1] Additionally, the further from the optical axis the galvanometer directs the focused beam, the more distortion when using a single lens focusing system. The spot distortion would need to be considered in post-processing and makes the reconstruction of images more difficult.

There are also microelectromechanical system (MEMS) driven mirror devices. The MEMS scanner presented in[6] uses torsion hinges rather than galvanometers to direct the beam. However, at only 2.5 mm x 2 mm, the size of the mirror surface would be a limitation for free space optics. These systems are more appropriate for fibre optic based systems. Their performance does rival larger systems with 45° movements possible. MEMS devices can operate at much higher speeds near resonant frequencies, but would then be fixed to particular frequencies and offer less flexible use.

3. LIGHT DELIVERY SYSTEM

3.1 Hardware Configuration

The following light delivery and sample position system was designed for OCT use. The device is an electro-mechanical system consisting of electronic components, optics, and mechanical hardware. The light is injected into the delivery system from the sample arm of the interferometer in the OCT system. There is a dog-leg intake that allows for acceptance of light at any height level from the interferometer. Two mirrors are used at 45° angles to bring the light beam to the galvanometer. The system is depicted in Fig. 2.

A single axis galvanometer (Nutfield Technologies QuantumScan-30) driven by a class 1 servo amplifier (Nutfield Technologies QuantumDriver-4000) is used. Class 1 servo amplifiers are used when accuracy is more important than speed. The servo amplifier receives an analogue signal from a data acquisition (DAQ) card (National Instruments USB-6251) and based upon the frequency and amplitude of the analogue input, generates a rotational movement in the galvanometer. There is a linear relationship between the input voltage and the output angle of the galvanometer and +/- 10 V input produces +/-20° optical movement. Due to the inertia of the scan mirror, there is a maximum frequency at which the servo amplifier can drive galvanometer oscillations. A sinusoidal input on the order of 50 Hz is within the performance envelope of this system, especially when operating over small angular ranges as is typical in OCT. A linear stage (Nanomotion FB050, HR4 motor) provides sample positioning control. The stage moves perpendicular to the movement generated by the galvanometer. The stage makes use of an encoder (Renishaw RGH25F series) and microcontroller (Galil DMC4010) to provide closed loop feedback control over the position of the stage. The stage is able to translate at a range of microns per second movement to centimetres per second and settle to a specified position with accuracy as high as 50 nm. This model of Nanomotion stage has a stroke of 5 cm translation length.

Broadband light centred at 850 nm, 100 nm bandpass, is used. The light reflects from the galvanometer mirror and is sent through a 50.8 mm diameter focusing lens (Thor Labs, AC508-100-B) which is mounted beneath the galvanometer. The lens has a focal length of 100 mm and the sample is placed upon the linear stage near the focal plane. As the mirror rotates, the galvanometer mirror changes profile from the viewpoint of the incoming beam, but must accommodate the entire beam over the desired angular displacement. As such, the mirror must

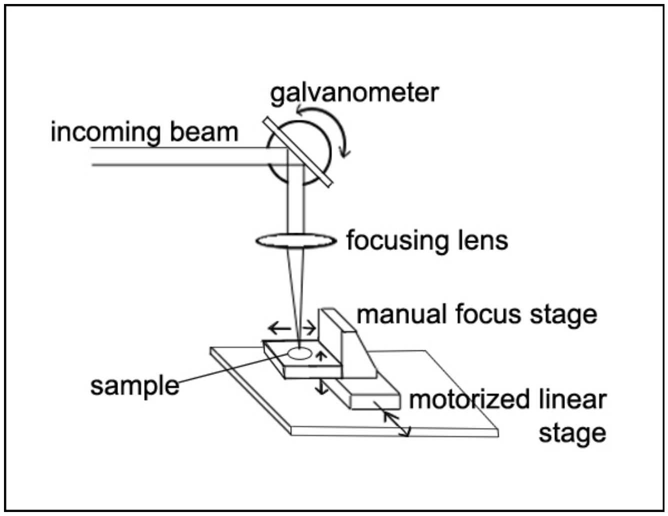

Figure 2. Schematic of an OCT sample handling and light delivery system. A galvanometer and linear stage work together to accommodate larger sample sizes than two galvanometer systems

be oblong shaped. The sample mount is fixed to a Z-axis stage (New Focus, 9064-X) to finely control the vertical position of the sample. To maximize the intensity of the returned light, the surface of the sample is placed at the focus. The backscattered beam moves from the sample delivery system back through the interferometer to the detector (Basler, SPC2048-70k). The sensor is a 2048 pixel, dual line CMOS with a line rate between 1 kHz and 70 kHz. The camera has configurable bit depth from 8-12 bits.

3.2 Scan Synchronisation

Synchronisation in this system is provided by matching all movements to timed trigger pulses. For each trigger the galvanometer moves one position step, the camera takes an exposure, and the linear stage checks if the galvanometer is at the end of its motion range. The DAQ provides the master trigger pulses, as well as the voltage signal controlling the galvanometer. If the galvanometer is at the edge of its range, the stage moves one step forward. This continues until the stage has moved a desired amount. This is best illustrated by the timing diagram in Fig. 3. The number of samples laterally and transversely are customizable and determined before the scan is initiated.

The scan pattern used in the prototype system scans laterally across-track to produce a B-scan and while the galvanometer moves back to the starting position, the stage increments the position forward. Only one B-scan direction is used and stage movements are done in a stepwise fashion. There is evidence that using only one scan direction can increase the overall performance of the system since the currents present within the galvanometer are smaller and time is given for thermal buildup to dissipate.[1] The galvanometer actuation signal resembles a stepped sawtooth pattern. A step-and-hold architecture is used for the galvanometer to ensure the mirror position is held steady during the sensor exposure.

4. SYSTEM ANALYSIS

An important consideration in any light delivery system is the spatial resolution. The spatial resolution determines how much distance may be between sample elements in order to still distinguish the sample locations. If the incoming beam is focused to the smallest spot possible and there are no other limiting aberrations, a diffraction limited system is present. Diffraction limited systems are limited by the inherent characteristics of light and not by the configuration of the electro-mechanics that control beam positioning. The spot size is determined by the Raleigh criterion which states that the smallest distance between two spots is

$$\Delta L = \frac{1.22\lambda}{NA} \tag{1}$$

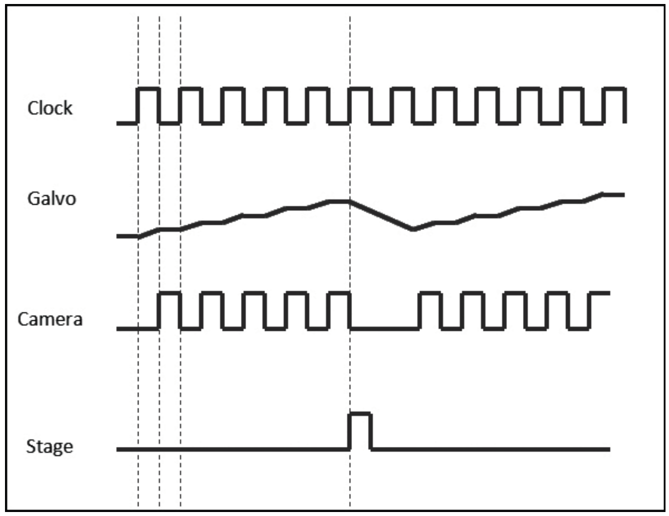

Figure 3. Timing diagram and synchronization scheme for operation of the galvanometer, linear stage, and camera exposures together.

where ΔL is the spatial resolution, λ is the centre wavelength of the broadband source (i.e., 850 nm), and the NA is the numerical aperture (i.e., 0.2 for a 20 mm beam size). For this system, the spatial resolution is

$$\Delta L = \frac{1.22 \times 850}{0.2} = 5185[nm]. \tag{2}$$

Paraxial ray tracing[9] may be performed to find the relationship between the incident angle and lateral displacement of the beam in the focal plane. After simplification, the following equation is derived

$$h = \sigma d \tag{3}$$

where σ is the transmitted beam angle (in radians), d is the distance between the sample and the lens, and h is the distance from the optical axis to the location of the beam. Paraxial ray tracing makes a number of assumptions. The equations are only valid for small angles where the small angle approximation sinθ=θ and tanθ=θ is used. The operational range for OCT is generally less than +/-5° and so the small angle approximation holds. The ray tracing is simple since there is a single lens involved in focusing the beam onto the sample.

To yield diffraction limited operation, the galvanometer angular movements must change the beam location less than the spatial resolution. A general rule of thumb for selecting the sampling frequency is to sample at half the Rayleigh Criterion.[10] If the spatial resolution is approximately 5 microns, 2.5 micron movements are necessary and smaller movements are desirable to give fine control over positioning. With a focal length of 100 mm, the angular change necessary in the galvanometer is 1.43x10^{-3} ° optical to provide a 2 micron lateral movement. Since 1 V translates to a 2° optical change in the galvanometer, 7.15x10^{-4} V steps are necessary to provide a mechanical movement in the galvanometer.

The DAQ provides the analogue signal to the galvanometer and discretises the signal over 16 bits. The galvanometer is driven by +/-10 V, 20 V total, and so the smallest discrete element is $\frac{20V}{16bits} = 3.05\text{x}10^{-4}V$. Since we require 7.15x10^{-4} V increments and the DAQ is able to generate 3.05x10^{-4} V changes, the hardware is suitable. Nyquist rate imaging requires sampling at twice the spatial frequency of the object of interest in order to represent the feature digitally.[7] If there are too few sampled locations (undersampling) for a feature of interest, pixilation occurs and the object cannot be identified. Oversampling occurs if there are more samples than necessary and the efficiency of the scan is decreased.

When designing a light delivery system, there is a general trade-off between lateral resolution and depth of focus. The lateral resolution is governed by the spatial resolution calculations above and for a given wavelength

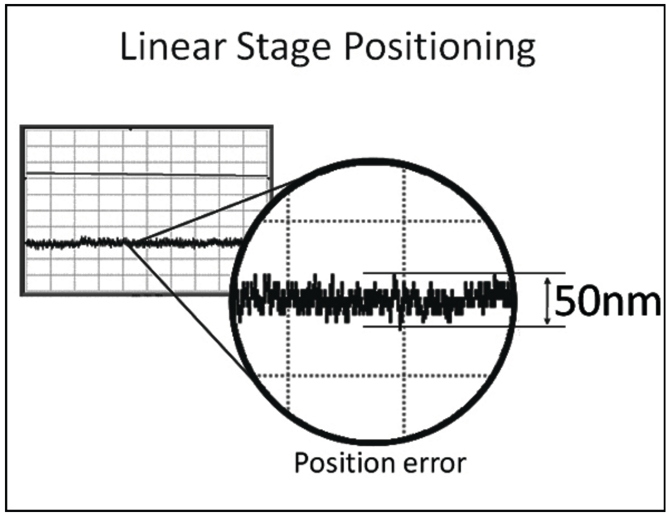

Figure 4. The Nanomotion linear stage with 10 nm encoder can provide operational position stability up to 50 nm during servo holding.

of light and diameter of lens; the focal length is the variable quantity. Choosing a fast lens, with a low f/# will be the main factor in making this a low depth of field system, but will have high lateral resolution. The selection of a slower lens, with a high f/#, will degrade the lateral resolution and increase the depth of field. Both high lateral resolution and wide depth of field are desired and so a lens with a focal length of 100 mm was chosen as a good starting point. The mechanics of the light delivery system allow for alternate lenses to be inserted if an application requires different performance characteristics.

This system is able to generate three dimensional images. The lateral dimension is generated by movements in the galvanometer and the resolution is diffraction limited. The smallest movements do not need to be smaller than the half the diffraction spot size for Nyquist-rate imaging. Using smaller movements allows for the features of interest to be located precisely in the image. The second dimension is provided by the linear stage. The linear stage resolution is determined by the accuracy of the servo motor and feedback loop driving the movements. The Nanomotion linear stage includes a 10 nm encoder that can provide movements as small as 50 nm. The controller driving the linear stage can produce plots of movements, as illustrated in Fig.4.

The final dimension, depth, is inherent to the OCT technology. The resolution of this axial dimension is determined by the spectral resolution of the system and the bandpass of the light.

By using one galvanometer and a linear stage, the size of sample is limited by the angular range of the galvanometer in only one dimension. The other dimension is limited by the stroke of the linear stage. Linear stages are available in many stroke length configurations and this second dimension has potential to accommodate very large samples. Galvanometers operate at high oscillation frequencies, but the linear stage only needs to move once per oscillation and can therefore moves with less stringent parameters.

There are three main characteristics that determine the system operation speed: the movement of the galvanometer, the light returning from the sample, and the integration time of the sensor. Since this system uses free space optics and a large diameter beam, a large mirror must be used with the galvanometer and the overall scan frequency is smaller as a result. Operation is limited to 10's of Hz. In order for an image to be captured, enough light must return through the system to provide information about the internal structures. The integration time of the sensor may be increased to accommodate fewer photons returning and increase the signal to noise ratio. However, increasing the integration time slows the overall performance of the system. For small scan samples, this is of little importance, but when scans achieving thousands of lateral scans (across the galvanometer) and thousands of transverse locations (one scan line per stage location) are desired, the time for scanning is high.

5. CONCLUSIONS

A light delivery and sample handling system for OCT is an important factor in obtaining quality imagery. The device presented can accommodate large sample sizes compared to typical two-galvanometer systems. The linear stage allows for samples that are up to 5 cm in length (the stroke length of the stage) and larger stages are available allowing for even longer samples. Increasing the angular displacement of galvanometers in an effort to increase the sample size yields undesirable effects like increased complexity reconstructing the images, as well as decreased speed due to mirror inertia. The system is designed solely for excised tissue samples and not *in-vivo* samples since the linear stage handles one of the positioning degrees of freedom. Any sample that can be placed upon the linear stage can be imaged. A Z-stage allows for fine focal control of the sample by changing the distance between the focusing lens and the sample surface. The system is designed specifically for free space optics OCT systems and can accommodate a 20 mm beam.

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