Laser Interference Fringe Tomography - A Novel 3D Imaging Technique for Pathology

Farnoud Kazemzadeh[†1], Thomas M. Haylock[1], Lev M. Chifman[1], Arsen R. Hajian[12], Bradford B. Behr[12], Andrew T. Cenko[1], Jeff T. Meade[1] and Jan Hendrikse[2]

[1]Department of Systems Design Engineering, University of Waterloo, 200 University Avenue West, Waterloo, ON, N2L 3G1, Canada

[2]Tornado Medical Systems, 705 - 555 Richmond Street West, Toronto, ON, M5V 3B1, Canada

ABSTRACT

Laser interference fringe tomography (LIFT) is within the class of optical imaging devices designed for *in vivo* and *ex vivo* medical imaging applications. LIFT is a very simple and cost-effective three-dimensional imaging device with performance rivaling some of the leading three-dimensional imaging devices used for histology. Like optical coherence tomography (OCT), it measures the reflectivity as a function of depth within a sample and is capable of producing three-dimensional images from optically scattering media. LIFT has the potential capability to produce high spectral resolution, full-color images. The optical design of LIFT along with the planned iterations for improvements and miniaturization are presented and discussed in addition to the theoretical concepts and preliminary imaging results of the device.

1. INTRODUCTION

Medical imaging devices cover a wide range of imaging resolutions and penetration depths into the tissue being imaged. For most medical imaging modalities, increased resolving power usually comes at the cost of reduced penetration depth. As an example, magnetic resonance imaging has the capability of imaging the entire body at millimeter resolution whereas a confocal microscope can probe on micron or sub-micron levels but can only image to tens of micron depth. In between the extremes of small scale imaging like confocal microscopy and large scale imaging like MRI, we find technologies such as OCT, programmable array microscopes (PAM) and ultrasound imaging. Most of these imaging modalities involve complicated and expensive technology.

Devices such as OCTs illuminate the area of interest and, by deciphering the information encoded in the reflection from the target, are able to produce an intensity map which corresponds to reflection amplitude as a function of depth into the target (depth profile). Assembling numerous depth profiles, a two- or a three-dimensional image of the target can be produced. To accurately measure the depth profile using OCT, an interference pattern is generated from the reflected signal and captured on a detector. Typically, OCT systems use a reference beam and generate an interference pattern by overlapping the reference beam with the scattered or reflected beam returning from the sample. This type of interferometer is referred to as an amplitude-splitting interferometer. Additionally, these devices use a broadband optical light or multi-frequency laser sources, e.g., swept-source OCT,[1] which employ a complex arrangement of optical elements. Devices as such, that could potentially incorporate multiple optical elements in series, tend to fall out of alignment outside of tightly-controlled laboratory settings, requiring verification and adjustment of the alignment prior to any measurement.

The aim of this study is to design and build a simple, portable, and cost-effective OCT replacement with performance akin to commercial systems in use by the medical community. Although the performance of this device will not be able to rival its commercial counter-parts, it seems to be very suitable for less demanding pathological applications. An optical source such as a red or infrared laser can be employed for illumination of the sample. Long wavelength light will result in deeper penetration into the sample and, since we use a monochromatic light source, achromatic optical components can be avoided. This will simplify our optical requirements and provide us with a system that can inexpensively provide excellent imaging in a small and robust package.

Laser interference fringe tomography is an imaging device which produces results similar to a traditional OCT, while being many times simpler and more cost-effective. First, the optical design of LIFT is described, then the mathematical

†fkazemza@uwaterloo.ca

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formalism of the system is briefly summarized. After presenting some imaging results of LIFT and their interpretations along with a brief discussion about some of the limitation of the instrument are presented. Lastly, the future prospects of LIFT are discussed.

2. THE DESIGN

LIFT is designed to be a very simple tomography imaging device working on principle of interferometry. Unlike conventional imaging interferometers, LIFT is a wavefront splitting interferometer. The interference pattern – containing the reflectivity as a function of depth information – is produced by overlapping different parts of the beam of light returning from the sample. This idea is most easily achieved using monochromatic light.

2.1 Optical Design

Below is the optical layout of LIFT, the description following will be referring to this figure.

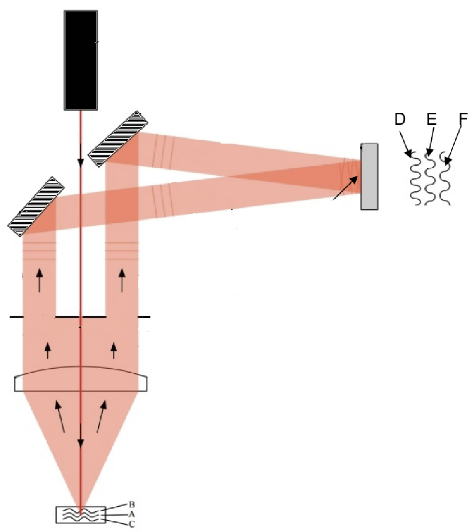

Figure 1. The layout of LIFT.

A laser source can be a very efficient and cost-effective light source for imaging. In our LIFT prototype we use a Helium Neon laser is used for illumination, even though a simple laser light source such as a conventional laser pointer can also be used for illumination. The laser light is focused onto a sample using a lens. The light from the laser falls on the surface of the sample and is scattered/reflected in all directions. It is assumed that the most useful/meaningful light scattered from the sample is concentrated within an angle of fews tens of degrees from the optical axis and all other light is heavily influenced and overpowered by noise. The focusing/collimating lens is chosen to only allow a cone angle of 30 $^\circ$ to go through and be collimated.

The collimated light then encounters a screen which has two small pupils, symmetric about the principle axis of the lens. The pupils allow only two small sections of the collimated light to emerge and carry downstream. The two pupils are then relayed off of two adjustable mirrors and are directed onto a sensor, with the path of one of the two pupils manipulated to overlap the other. If the two beams satisfy the interference condition, the optical path difference (OPD) is an integer multiple of their wavelength, the result of the two overlapping beams tilted by a relative angle between them, is an interference pattern. The frequency of the interference pattern relates to the depth at which the signal is coming back from and the strength (intensity) of the signal portrays the reflectivity at that depth.

2.2 Mathematical Formalism

The two beams will have to be overlapping for any interference to occur. The interference will be visible in the plane of the detector. Maximal constructive interference occurs between the light from the two pupils when the OPD between the two beams is an integer multiple of their wavelength, along with maximal destructive interference from a half wavelength offset. It is important to note that only the light returning from the focal plane of the lens will will be collimated, resulting in the light from the two pupils being parallel. Any light returning from any point off of the focal plane will be under-collimated or over-collimated, originating in front or behind the focal plane respectively. For the purposes of imaging with LIFT the sample is positioned so the laser light is focused on the surface of the sample and any 'depth imaging' is done behind the focal plane (away from the lens). This would result in over-collimation of the light by the lens and by association, the convergence of the light out of the two pupils.

As mentioned before, the frequency of the interference pattern signifies the depth from which the signal is returning from due to interaction with a structure. The following equation predicts the fringe frequency to be expected as the light returns from different depths into the sample.

$$\frac{1}{y_m} = \frac{(d + 2l_{ms}\, tan[tan^{-1}(\frac{2f \pm 2x}{d}) - tan^{-1}(\frac{2f}{d})])}{m\, L\, \lambda} \qquad (\frac{1}{meter})$$

The variable x is the offset from the focal plane. For completeness, both cases of interference due to a response from in front or behind the focal plane are incorporated in the equation. The variables used in the above equation are defined as follows: y_m is the distance between consecutive fringes; d is the separation between the two pupils on the screen; l_{ms} is the distance between the screen and the two manipulating mirrors; f is the focal length of the focusing/collimating lens; L is the distance between the manipulating mirrors and the detector; λ is the wavelength of the light source.

A plot of fringe frequency ($\frac{1}{y_m}$) as a function of distance from the focus (x) is shown below. The fringe frequency has been calculated at steps of 100 micron for a total of 3 mm of distance from the focus. It seems that to the first order, the two are linearly correlated, however there is a slight curvature to the line as it is tending to flatten out at very large x. This could mean that there exists physical limitations in the imaging capabilities of this system as shown here, albeit, this system is not designed to be used to image anything so greatly out of focus.

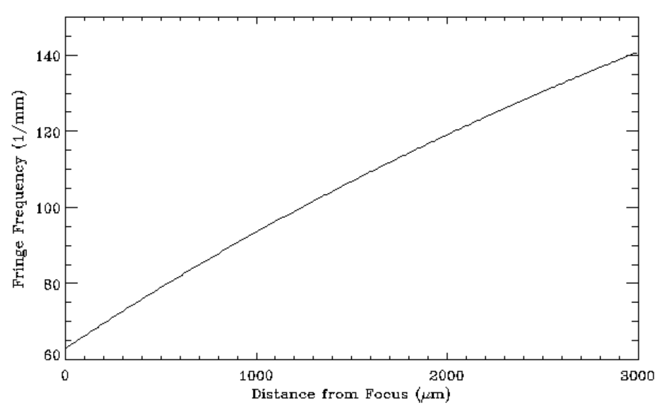

Figure 2. A simulation showing the effect of displacement from the focal point on the fringe frequency.

2.3 Resolution

Imaging systems analogous to LIFT have two resolution elements that classify the system. The two resolution elements are axial and lateral resolution. Lateral resolution is determined by the size of the focused spot onto the sample, which means the incident light is probing a region the size of the area of the spot on the sample. Other than the wavelength of light the lateral resolution depends on the focal length of the lens and the size (diameter) of the beam going through the lens. Decreasing the focal length of the lens will increase the lateral resolution but there is a trade off with the depth of field which is the region around the focal point that stays in focus. With the current configuration of LIFT the lateral resolution of the system is $\sim 65\ \mu$m.

The depth resolution of LIFT determines the smallest discernible length in the depth scan. Another way of thinking of the depth resolution is the smallest detectable amount of movement away from the focal point. If the surface of the sample is placed at the focal plane of the lens, any length departure from the focal point would be into the sample, hence probing the depth of the sample. Depth resolution is strictly the product of the system configuration (all lengths that were used in the fringe frequency equation) and the fringe equation is used to determine the depth resolution. The goal is to determine the deviation from the focal point along the optical axis that would result in one additional fringe, given the physical properties of the system (fixing all other lengths), since the only observable in this system is the number of fringes due to interference or fringe frequency. So, the distance offset from the focal point that would result in one additional fringe is said to be the axial resolution of LIFT.

For the purposes of this study the system configuration is chosen such that the the axial resolution is equal to the lateral resolution, so a cubic voxel is being probed.

2.4 Noise

There are two types of noise inherent in medical imaging devices using diffuse light from a target, specially using lasers. One of which is photon noise, also known as square root noise or Shot noise. This kind of noise exists in any photon counting imaging device. More generally, it is the detectable statistical fluctuations in measurements of any energy carrying particle, photons in this case.[2] The effects of this type of noise are mitigated in LIFT by making numerous observations (i.e., imaging the same interference pattern numerous times, $\sim$ 50-100 times), and averaging the resulting interference pattern, the inherent photon noise can be reduced.

The most dominant source of noise in this systems and other devices that use the imaging principles applied here, is speckle noise. Speckle phenomena is a laser-induces granular intensity distribution resulting from the coherent superposition of random light scattered from a diffuse surface.[3] In essence it is the over- and under-density of photons being reflected by the imperfect surface falling on the detector. In applications that LIFT is aimed for, imaging of tissue, speckle noise will always be present as the most prominent noise source and it will be very unpredictable. There are measures to be taken in order to mitigate this. The most simple thing to do is to physically dislocate the sample from the focal point either away or toward the lens, and image the interference pattern. Align all resulting depth profile curves based on the signal pertaining to the surface of the sample and then average all the curves together. The speckle pattern will change as the result of translation away from the focal point, however the signal from the sample (real signal) will remain constant, although the intensity will change depending if the sample has been moved away or toward the lens. After averaging, the real signal will be amplified over the signal due to the speckle noise.

2.5 Future Iterations

The optical elements comprising LIFT downstream from the screen are designated to be the imaging back-end of the instrument. In later iterations of LIFT, the two manipulating mirrors of the back-end can be replaced by a beam-recombining cube and the interference output as the result of the overlap of the two beams can be imaged. This iteration would decrease the size of LIFT. This would also lower the aligning complication of the instrument. Another change that can be made will result in direct depth imaging by LIFT onto a detector with next to no post-processing. If, instead of a detector, a lens is placed in the imaging plane then the post processing of the interference pattern can be done by the lens and a response as a function of depth can be directly imaged on the detector. The data acquisition to image production of LIFT will be enormously sped up and would allow for responsive medical imaging. Many different color (frequency) lasers can be used in conjunction with dichroic filters and multiple detectors to simultaneously image a sample at many wavelengths to produce a full-color image.

3. RESULTS AND DISCUSSIONS

In this section some of the preliminary depth imaging results of LIFT are shown and briefly discussed. Finding a suitable sample for testing of LIFT was a challenging task. The initial sample chosen for imaging had to be one of no depth so the response of the system based on exact departures from the focal plane could be studied. A possible solution would be to use a mirror as the sample however, a mirror would simply reflect the incident light. LIFT operates on the principle of scattering so in a sense the perfect sample needs to scatter the incident light off of its surface, also known as diffuse reflection.[4,5] The sample that was chosen for these initial tests was a piece of sandblasted Aluminum. Of course not all the scattering from the perfect sample (PS) will be from the surface and some will be coming from small depths into the PS but, it is very minimal when a dense material such as Aluminum is used.

The figure below shows the response of the PS as it is placed at different positions with respect to the lens. The three positions used are: PS at the focal plane of the lens, PS at 250 μm behind the focal plane, and PS at 400 μm behind the focal plane. The purpose of this experiment was to demonstrate that LIFT can accurately measure the position of the PS with respect to the focal plane of the lens. The sample was placed on a linear translation stage and the displacement from the focal plane was measured with a micrometer. The highest peaks in the plots are attributed to the signal coming back from the surface of the PS and it is clear that the surface is displaced, from the zero point, at roughly the expected displacement values. In a way, LIFT is being used as a very low-resolution metrology system.

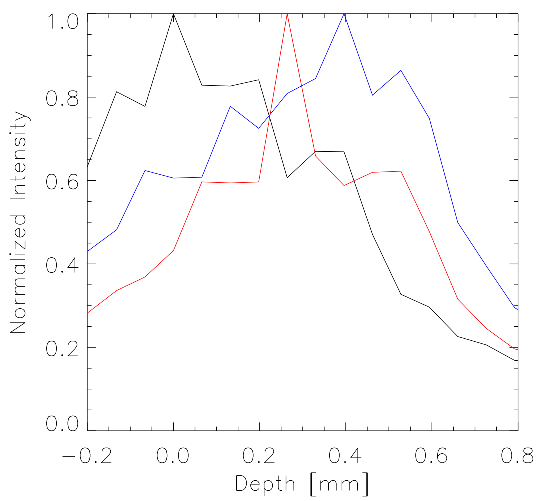

Figure 3. Depth scan of the PS at depths of 0 μm (black), 250 μm (red), and 400 μm (blue).

In the above plot, we clearly see peaks at 0, 250, and 400μm. While significant noise is seen around these peaks, this can be attributed to a combination of the roughness of the sample, the resulting speckle pattern, and the limited spatial resolution of our device. While we are still actively working on reducing this noise, it is clear that our system can accurately measure the positions of our sample.

The next step in imaging using LIFT was to experiment with a sample that would provide response from two layers separated by a small amount, on the order of $\sim$ 200-600 μm, of air or glass. A phantom was created adhering to the mentioned specifications using a microscope slide. The slide of thickness 1 mm was ground down to thickness of 350 μm. The process of grinding the slide to the appropriate thickness also acted to roughen the front and back surface of the slide to produce two surfaces prone to producing diffuse reflection.

The figure below shows the depth scan of this phantom imaged with a conventional OCT system and with LIFT. The most prominent feature in this plot is the signal-to-noise in the two imaging modalities. The OCT scan has enormous response from the front and back surface of the phantom while the noise is very low. The LIFT scan shows the response due to the front and back surface of the phantom at the expected separation, but the signal seems to be blanketed by an overpowering amount of noise, so the peaks are not as pronounced. Nevertheless, it has been demonstrated that LIFT is capable of imaging this particular phantom. The thickness of the glass phantom is observed as expected by the OCT and LIFT.

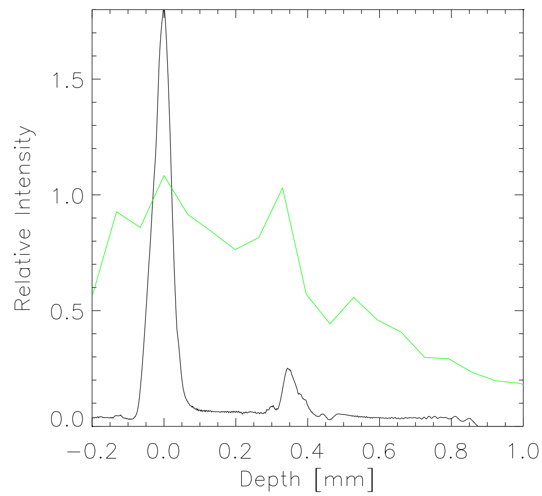

Figure 4. A Depth scan of the microscope slide phantom imaged by an OCT (black) and LIFT (green).

4. SUMMARY

The optical design and mathematical formalism of a new, simple, and cost-effective diffuse light interference imager, LIFT, has been presented. The current system's specifications and imaging capabilities are outlined along with alternate optical designs and miniaturization plans of the system. As seen in the optical design of the system, it uses a few generic (simple and with high tolerance) optical components which makes the system very simple to assemble, set up and use. So far the only imaging with LIFT has been the acquisition of depth profiles, albeit, acquisition of a two- or three-dimensional image is accomplished by simply placing the sample on a two-axis linear translation stage and assembling subsequent depth profiles. LIFT performs very well as a coarse, low resolution, metrology system when imaging a PS. LIFT is able to image a two-layer glass phantom and its measurement was confirmed using an OCT system. Other than competing with OCT in becoming a clinical high-resolution imaging modality, LIFT can be largely useful in applications where the aim is to find margins on the scales of tens of microns. Even though the current imaging capabilities (resolution, penetration depth, and signal-to-noise) of LIFT are far inferior to a conventional, clinical OCT system, future generations of LIFT will be able to bridge the gap in performance. The affordability of LIFT will enable applications yet to be thought of for this instrument.

5. ACKNOWLEDGMENTS

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