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Volume Holographic Reflection Endoscope for In-Vivo Ovarian Cancer Clinical Studies

I. D. Howlett^{*,a,b}, M. Gordon^{a,b}, J. W. Brownlee^{a,b}, J. K. Barton^{b,c}, and R. K. Kostuk^{a,b}

^a Photonic Systems Lab; Department of Electrical and Computer Engineering, University of Arizona, Tucson, AZ 85721

^b College of Optical Science, University of Arizona, Tucson, AZ 85721

^c Biomedical Engineering, University of Arizona, Tucson, AZ 85721

Abstract

We present the design for an endoscopic system capable of imaging tissues of the ovary at two selected imaging depths simultaneously. The method utilizes a multiplexed volume hologram to select wavefronts from different depths within the tissue. It is the first demonstration of an endoscopic volume holographic imaging system. The endoscope uses both gradient index (GRIN) optical components and off the shelf singlet lenses to relay an image from the distal tip to the proximal end. The endoscope has a minimum diameter of 3.75 mm. The system length is 30 cm which is connected to a handle that includes the holographic components and optics that relay the image to a camera. Preliminary evaluation of the endoscope was performed with tissue phantoms and calibrated targets, which shows lateral resolution $\approx 4 \mu\text{m}$ at an operating wavelength of 660 nm. The hologram is recorded in phenanthraquinone doped poly methacrylate and is designed to produce images from two tissue depths. One image is obtained at the tissue surface and the second 70 μm below the surface. This method requires no mechanical scanning and acquires an image at the camera frame rate. The preliminary ex-vivo results show good correlation with histology sections of the same tissue sections.

Keywords

Endoscopy; Ovarian Cancer; GRIN; volume holography

1. INTRODUCTION

Early detection of cancer through medical imaging has a critical impact on patient survival rates. Of particular interest is ovarian cancer which is estimated to have 22,240 new cases diagnosed in 2013 and 14,030 deaths¹. Due to a lack of sufficiently accurate screening tests for early detection of ovarian cancer, many of the cases which are diagnosed are in the later stages of development which decreases the patient survival rate. The current statistic states that only 15% of the new cases get diagnosed at a localized stage¹.

It is essential to know the extent of the disease before beginning any treatment as it is of particular significance for women who want to retain functional ovaries for family planning and/or quality of life. The current treatment options for patients with diagnosed ovarian cancer often include a combination of the following: cytoreductive surgery, chemotherapy and radiation therapy. These procedures require thorough pretreatment imaging that identify and find localized ovarian cancer sites. Additionally, pelvic examination, transvaginal ultrasound, and laparoscopic optical imaging techniques are used to image the surface of the ovaries to attain greater understanding about the extent of the suspected disease.

When assessing the characteristics that make imaging modalities used for cancer detection the following items are expected to be achieved: high lateral and depth resolution; quality information of tissue status with minimized operation times; sensitivity to structure and biochemistry; simple and cost effective design that is minimally- or non-invasive; and can be readily sterilizable for clinical use. The current imaging modalities that exist with these properties are optical coherence tomography (OCT), reflectance confocal microscopy, and fluorescence spectroscopy and fluorescence confocal microscopy. OCT has the ability to resolve spatial structure with high resolutions of 2-20 μm at depths of 1-2 mm using backscattered near infrared light ²⁻⁵. Fluorescence spectroscopy and fluorescence confocal microscopy systems provide information about biochemistry used for early cancer detection with depth weighted average fluorescence ⁶⁻⁹.

While traditional optical microscopy/microlaparoscopy is able to provide high quality images of a single object plane, the multiplexed volumetric holographic imaging allows for simultaneous imaging of multiple depths within a three dimensional object ¹⁰. Images captured on the camera device are displayed side by side on a two dimensional monitor, which can later be reconstructed into a three dimensional image. The group's previously published VHIS design is an ex-vivo system that allows for both epireflectance and epifluorescence imaging at two imaging planes. The system achieved lateral resolution of 2 – 4 μm and an axial resolution of 15 μm , thus providing subcellular resolution at each image plane.

Simultaneous capture of multiple imaging depths within a volume of tissue is possible through the use of multiplexed volume holograms ^{11,12}. The hologram for this study is optically fabricated from a photosensitive polymer, phenanthrenequinone-doped poly(methyl methacrylate) (PQ-PMMA). The multiplexed hologram is created by recording two gratings with laser light into the PQ-PMMA substrate. The finalized hologram spectrally disperses light originating from within the 3D object along a lateral axis. This characteristic allows spatial-spectral encoding of light along the dispersive or X-axis. The Y-axis provides spatial information similar to that of a standard microscopic imaging system. Hologram specifications, such as number of image planes, spatial-spectral range, and field of view are determined during the recording process.

In the design presented here, we investigate for the first time the performance of a volume holographic imaging system (VHIS) intended for in-vivo endoscope imaging. The system is designed to image surface and subsurface morphology in human ovarian tissues. Initial testing has been performed utilizing USAF bar targets and tissue bead phantoms. For this

study we fabricated an endoscopic probe and handle system for ex-vivo imaging, however the system is ready for invivo ovarian imaging studies to begin Early 2014. The clinical use of the endoscope will assist in the process for determining the necessity of prophylactic oophorectomies and possibly reduce unnecessary ovary removal for a large number of patients and improve the quality of life.

2. MATERIALS

The endoscope consists of three 2.7 mm diameter gradient index (GRIN) optical elements. These elements are labeled as followed, in accordance to the Nature Protocol¹³: coupling lens (GoFoton ILW-2.70), relay lens (GoFoton SLR-2.70), and imaging lens (GoFoton ILW-2.70). The relay lens is a 281.4 mm long, 2 pitch design GRIN rod. Affixed to the distal and proximal ends of the rod are the imaging and coupling lens, respectively. Both the imaging and coupling lenses were ordered with a nominal pitch of 0.25, and were later polished down to account for the distal window and rear field flattener. The distal end of the GRIN probe is protected by a 3 mm diameter, N-BK7, window. The window is created from a 3 mm diameter plano-convex lens (Edmund #45-272) which was polished down in-lab to create an optical flat. The window provides necessary separation between the GRIN lens and the patient. The proximal end of the endoscope contains a negative lens (Edmund #84-380) which is used as a field flattener. This element also aided in the reduction of back reflections due to the curvature of the surface and the VIS-NIR anti-reflection coating. The full system is encased in stainless steel 304 hypodermic needle tubing manufactured by MicroGroup. Properties of the optical elements can be found in Table 1.

The LED being used (LED Engin LZ1-00R200) has a peak wavelength of 660 nm. The source is coupled into a 600 μm diameter fiber (ThorLabs M29L05) and connected to the handle assembly through an SMA adapter. The fiber core is imaged onto the rear focal plane of the objective using an 8 mm focal length lens (Thorlabs C240TME-A) producing Köhler like illumination.

The handle of the endoscope utilized a one to one relay set-up as used previously in the ex-vivo set up to relay an image from the objective (Olympus ULWD MSPlan50) to the hologram plane. The relay contains a symmetric configuration of lenses achromatic doublets (AC254-050, AC254-100). After the hologram, an imaging lens (AC254-030) images the two channels onto a CMOS camera (ThorLabs DCC3240N). The final design of the handle will be encased in a 3-D printed shell to protect the optical components from surgical room contaminants, Figure 1.

The holographic optical element (“HOE”) is designed such that the diffracted beams deviate at approximately a 90 degree angle onto the imaging optics and detector. Angular multiplexing is used to separate two images from different depths in a volume of target tissue onto different areas of the same focal plane array. The HOE is recorded in a photosensitized poly-methyl-methacrylate containing 0.5% (wt) (1,9)phenanthrene-quinone and 1.1% (wt) 4-nitro-aniline Using the 514 nm line of an Argon ion laser. The hologram's selectivity is enhanced due to this materials large effective thickness of 2.2 mm yielding an angular bandwidth of each channel smaller than 0.02 degrees at the reconstruction

wavelength. Moreover we avoid the penalty in image performance from detuning by placing the curved grating channel of the multiplex hologram at the surface of the tissue volume under investigation. The second deeper channel (shown in a later section) is in focus at a position 70 μm into the volume of tissue.

3. METHODS

Coupling and imaging lenses were modified in-lab utilizing a polishing procedure, discussed by Kim et al., in order to ensure the system was focused on the surface of the window which will be in flush contact with the tissue. The BK7 window provides a safe working surface for the patient as the GRIN lenses are not approved for contact with human tissue. Once the coupling and imaging lenses were polished to 5.715 mm and 5.780 mm respectively, they were attached to the relay lens using optical adhesive with an index of refraction of 1.625 (Norland 1625). This assembly was then potted into an 11 gauge hypodermic needle tube (MicroGroup 304H11XX) using the same Norland Optical Adhesive.

The negative lens was bonded to the GRIN proximal side using Norland 1625. The window bonded with a medical grade epoxy from Epoxy Technology (EpoTek 301). The full assembly was potted in a 9 gauge hypodermic needle tube (MicroGroup 304H9TW) using the medical grade epoxy.

Upon assembly, the endoscope is clamped onto a threaded retainer which is used to keep the endoscope attached to the handle assembly. Alignment is achieved using the CMOS sensor to view the surface image, then three axis stages and a manual xy-slip plate assist in placing the exit of the endoscope to the focus of the objective.

4. RESULTS

Initial imaging of the endoscope uses the 1951 USAF Resolution Chart (Edmund Optics #38-257) as a target which has a maximum resolution of 228 lp/mm on the Group 7, Element 6 target. The endoscope and handle optics were separately evaluated before assembly. Figure 2 shows the imaging performance of the endoscope evaluated in a bench top set-up¹⁴. The bench top set-up contains an optimized hologram and imaging optics and is used to evaluate any image degradation caused by the endoscope.

The image on the right represents the surface channel, which is in focus. The left channel is a depth channel which is showing a defocused image since the object being viewed is a 2D planar object. The image on the right shows resolution corresponding to 228 lp/mm (4.4 μm).

The handle optics were also tested with and without the hologram in place to check for image quality changes in image quality and scatter. The images shown in Figure 3 are images obtained using the handle's microscope objective without the endoscope probe. Figure 3a shows the image through the endoscope relay and handle optics without the multiplexed hologram. This step was used to align the optics prior to inserting the hologram to realize the depth sectioning capability.

Initial results using the hologram are shown in Figure 3b (left channel in focus) and 3c (right channel in focus), and indicate the ability to resolve features in the Group 7, Element 4 region (181 lp/mm) corresponding to 5.52 μm for each line pair. However contrast is reduced due to scattering in the holographic polymer. Techniques for reducing scatter in the pq-PMMA material are currently being investigated.

Full system assessment was performed with the current hologram set as means of determining image performance and determining image degradation between the two multiplexed channels. This is done by aligning the endoscope such that the target is in flush contact with the window and locking the endoscope in place. The first image obtained in this configuration is the surface channel in focus. To obtain an image of the depth channel, the target is moved away from the endoscope until an in focus image is achieved. This process is shown in Figure 4.

It is seen in above that the surface channel resolution (4a) is read from the Group 7, Element 1 region at 128 lp/mm (8 μm) whereas the depth resolution (4b) is 228 lp/mm (4.4 μm).

Additional imaging was performed on beads imbedded in a polyvinyl alcohol substrate as a volume object. The phantom tissue beads mimics the reflectivity expected from human tissues. The beads which are imbedded into the phantom material are 15 microns in diameter as shown in Figure 5.

In this system configuration, the left channel is the depth channel, while the right channel is the surface channel. Since the beads are not all flush against the surface minor defocus occurs in the image of the beads. Further imaging and handle modifications will allow for phantom bead imaging using the complete handle and endoscope set-up.

5. CONCLUSIONS

We have developed an endoscope that includes volume holographic imaging that is capable of resolving 4.4 μm with optimized bench top optics and 8 μm with non-optimized portable handle optics. We are currently increasing the performance of the portable optics with redesigned components and will use the system in clinical trials for issue imaging.

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Figure 1.

a) Fully assembled schematic showing the assembled endoscope and handle b) Schematic of the endoscope enclosed in the protective case

Figure 2.

Endoscope Imaging Assessment using the endoscope on a bench top set-up and a USAF resolution chart. Left half of the image represents the depth channel 70 μm inside of the target. Right half of the image is the in focus target surface.

Figure 3.

a) Resolution target of handle optics without hologram in transmission configuration b) resolution target image using the hologram, left channel in focus c) resolution target image using the hologram, right channel in focus

Figure 4.
Full System assessment: a) surface channel in focus b) depth channel in focus

Figure 5.
Tissue Phantom Beads a, b, and c are images of different locations and depths

Table 1

Optical Properties of components

Optic	Company	Material	Diameter	Index (n)
SRL-2.7	GoFoton	GRIN	2.7 mm	1.610 on axis
ILW-2.7	GoFoton	GRIN	2.7 mm	1.643 on axis
#84-380	Edmund Optics	N-SF11 (VIS-NIR)	3 mm	1.785
#45-272	Edmund Optics	N-BK7	3 mm	1.517