High Frame-Rate, 3-D Photorefractive Holography Through Turbid Media With Arbitrary Sources, and Photorefractive Structured Illumination

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Invited Paper

Abstract—In this paper, we briefly review our work on low-coherence photorefractive holography and report on the current state of the art. We present what is, to the best of our knowledge, the fastest-ever three-dimensional (3-D) imaging system and present results obtained with imaging at 470 frames/s (fps). We demonstrate the versatility of photorefractive holography using various sources, including LEDs, high-power diode arrays, and a novel, all solid-state broad-band laser. We present preliminary results obtained by combining the technique of structured illumination with photorefractive holography for the first time. We demonstrate that this novel holographic optical sectioning technique may be implemented for both reflection and fluorescence imaging.

Index Terms—Biomedical imaging, broad–band laser source, coherence gating, high frame-rate imaging, low-coherence interferometry, photorefractive holography, 3-D fluorescence and reflection microscopy.

I. INTRODUCTION

FOR IMAGING through biological tissue, the wavelength region between 700 nm and 1100 nm is desirable since this corresponds to a transmission window in the absorption profile. However, the scattering coefficient of tissue is significantly higher than the absorption coefficient in this region and scattered light typically dominates any transmitted image. Photorefractive holography can effectively discriminate against a background of scattered light and select the ballistic (unscattered) component in an image-bearing beam, while optical sectioning may be realized using low-coherence interferometry.

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There are numerous coherent imaging methods to preferentially select the ballistic light from the diffuse, scattered background [1], [2]. These interferometric methods, which include optical coherence tomography [3], heterodyne detection [4], and holography (electronic [5] and photorefractive [6]–[8]), use the principle that only light which retains coherence with a reference beam derived from the same source would interfere with it to produce fringes, whereas any scattered light should have lost coherence with the original beam. Coherent imaging techniques can be applied in reflection using sources with a short coherence length to achieve depth-resolution—and also discriminate further against scattered light. Three-dimensional (3-D) images are built up by axially scanning the interferometer path difference between the object and reference beams. In optical coherence tomography (OCT), coherence gating is combined with confocal imaging to provide further rejection of scattered light. However, OCT requires pixel-by-pixel scanning to build up a two-dimensional (2-D) image and so is not a real-time, whole-field technique, although it can provide images at video rate [9]. A further drawback to OCT systems is the requirement for spatially coherent broad-band radiation, that is typically obtained from expensive, mode-locked Ti : sapphire lasers, or from superluminescent diodes that are limited in power to a few milliwatts.

Whole-field imaging techniques, such as heterodyne imaging or electronic holography, can provide faster imaging rates, since all the pixel information is acquired in parallel, and these techniques can also take advantage of sources with low spatial coherence [10], [11]. Their principal drawback is that much of the scattered light is incident on the whole-field detector, typically a CCD camera, and this can saturate the detector, limiting the dynamic range.

Photorefractive holography provides a whole-field coherence gating technique with the added advantage that the photorefractive effect requires a spatially modulated incident light field [12] and is insensitive to a diffuse background of scattered light. Furthermore, since the signal processing is performed optically in media that have response times of less than 1 ms, it is possible to capture whole-field, depth-resolved images at frame rates approaching thousands per second. As with other whole-field coherent imaging techniques, this technique may be implemented with light sources of almost arbitrary spatial coherence.
In this paper, we report on the state of the art of the photorefractive holography technique, demonstrating a range of broad-band sources with diverse coherence properties, and present what we believe to be the fastest, depth-resolved 3-D imaging system yet demonstrated. We also describe a new optical sectioning technique that combines some of the advantages of photorefractive holography with the technique of structured illumination [13] to provide a sectioning technique that can be applied to reflected light or fluorescence.

II. PHOTOREFRACTIVE ADVANTAGE

Photorefractive materials, such as bulk crystals, e.g., rhodium-doped barium titanate or semi-insulating semiconductor multiple-quantum-well (MQW) devices, have the unique property that when these materials are illuminated by spatially modulated light patterns, their optical properties undergo a change that results from charge migration and trapping by defects [14]. Electrons are liberated in high-brightness regions and drift to the low-brightness regions where they may be trapped by the defects present in these materials. A space–charge field that reproduces the incident intensity pattern is, consequently, set up within the photorefractive medium. The space–charge field then acts either via the Pockels’ linear electrooptic effect [15] (in the case of bulk photorefractive crystals) or the Keldysh [16] and the Quantum confined Stark effects [17] (in case of multiple quantum wells) to induce a refractive index grating within these devices.

As these materials are not sensitive to a uniform light background (since this does not produce a space–charge field), this property can be exploited in imaging through turbid media where scattered light that forms a diffuse background may be rejected by the photorefractive effect. This rejection of diffuse light offers the potential to achieve a very high dynamic range in imaging through turbid media. Using rhodium-doped barium titanate, we have recorded a coherent image against an incoherent background that was $10^8$ times greater than the coherent signal [18].

Photorefractive media are reusable holographic materials with response times varying from picoseconds to tens of hours (for bulk photorefractives), and $\sim$ microseconds to milliseconds for semiconductor MQW devices. For high-speed photorefractives, real-time writing and read-out of the images is possible on a timescale comparable to the response time. This makes these devices attractive for use with in vivo imaging modalities and other applications where the sample may be moving or evolving. Using both bulk crystals and semiconductor MQW devices, we have obtained whole-field, real-time reconstructions of holographic images [19]. The use of multiple quantum wells has also made it possible to acquire images at frame rates approaching 500 frames/s (fps) [20]. We note that this frame rate may be increased to 1000 fps by refining the image acquisition software.

III. DEPTH-RESOLVED PHOTOREFRACTIVE HOLOGRAPHY

Fig. 1 shows 2-D, whole-field, sectioned images of the various cylinders. A 3-D reconstruction of the object was then obtained by using appropriate 3-D rendering software. A depth-resolution of $<100 \mu m$ was measured. These depth-resolved images compare favorably to those obtained earlier by our group using a mode-locked, femtosecond Ti : sapphire laser [8]. To demonstrate depth-resolved, 3-D photorefractive holography, the object used in Fig. 2 was a 3-D object consisting of concentric machined aluminum cylinders with a step-size of 100 $\mu m$. As source, we used a home-developed broad-band laser [21] with a coherence length of $\sim 63 \mu m$.

IV. DEPTH-RESOLVED IMAGING THROUGH TURBID MEDIA USING PHOTOREFRACTIVE HOLOGRAPHY

The criteria for the selection of which photorefractive materials to use for our imaging application is based upon wavelength, sensitivity and response time considerations. We also require the recording medium to have a superior optical quality and a uniform response with spatial frequency. To date, we have demonstrated depth-resolved holography through turbid media using photorefractive crystals such as rhodium-doped barium titanate (Rh:BaTiO$_3$) [18], cadmium telluride (CdTe) [22], and GaAs–AlGaAs MQW devices. This and the following section discuss the selection criteria for these materials in some detail.
Fig. 2. Experimental setup for high frame-rate 3-D photorefractive holography with MQW devices, and using group delay compensating prisms in the interferometer to compensate for “walkoff.”

Barium titanate was found to exhibit a response time of typically 1 s with incident powers of 60 mW/cm² in the signal beam which permitted us to achieve near-real-time imaging. Also, doping this material with rhodium increased its sensitivity in the near infrared, thus rendering it suitable for our biomedical imaging application. For optimum performance, we used rhodium-doped barium titanate with its c axis cut at 45° to take advantage of the large $r_{12}$ electroabsorption coefficient [23]. We have also used the semiconductor cadmium telluride to image through a weak scattering medium. Note that the holograms recorded in photorefractive materials are volume holograms. The depth resolution of the recorded holograms is degraded due to an effect called “walkoff” [23], which occurs when the coherence length is comparable to the diameter of the holographic recording beams. Also, the Bragg matching conditions to read out volume holograms impose constraints on the system design.

V. DEPTH-RESOLVED IMAGING THROUGH TURBID MEDIA USING SEMICONDUCTOR MQW DEVICES

MQWs are photorefractive devices composed of alternating layers of semiconductor materials. The MQW devices that we use are composed of GaAs and AlGaAs layers on a GaAs substrate and are typically about 1 μm thick. An important difference between bulk photorefractives and MQWs is that conversion of the space–charge field into a refractive index grating is achieved either via the Franz–Keldysh [16] or the quantum confined Stark effects [17], depending on whether the field is applied parallel or perpendicular to the wells, respectively. These are nonlinear effects and, unlike the Pockels’ linear electrooptic effect, are dependent on the square of the electric field. The MQW devices that we have employed in our experiments are all Franz–Keldysh devices and require typical electric fields of 8–10 kV/cm.

The advantage of MQW devices over the photorefractive crystals lies in their ease of use (holograms are recorded in the Raman–Nath regime and so Bragg matching is not required), greater sensitivities (due to the higher absorption) and very fast response times—as short as a few microseconds for writing beam intensities of the order of mW/cm². Also, MQW devices have a large optical bandwidth and any writing wavelength may be used to write a hologram as long as it excites above the bandedge [17]. The position of the bandedge can be tuned throughout the infrared region by changing the composition of the wells. These photorefractive MQW devices work best when holographic images are read-out using a laser tuned to their exciton absorption peak. We typically use home-made Cr:LiSAF lasers for reading out the hologram, although we have recently demonstrated that this may readily be achieved by using a grating tuned external cavity semiconductor laser. The exciton peaks of GaAs–AlGaAs MQW devices typically lie in the wavelength region 830 nm–850 nm, which means that it is not possible to record holograms above these wavelengths. This is a problem since, ideally, we would like to use longer wavelengths to take advantage of the reduced scattering coefficient of biological tissue. However, we have recently been
TABLE I

<table>
<thead>
<tr>
<th>Source</th>
<th>Source type</th>
<th>Spatial Coherence</th>
<th>Output Power</th>
<th>Central Wavelength</th>
<th>Spectral bandwidth</th>
<th>Temporal Coherence Length</th>
<th>Experimental Sectioning Strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser diode</td>
<td>McDonald Douglas 160 μm broad stripe multimode diode</td>
<td>Partial (High)</td>
<td>&lt; 300 mW</td>
<td>664 nm</td>
<td>5 nm</td>
<td>88 μm</td>
<td>218 μm</td>
</tr>
<tr>
<td>Broadband c.w. laser</td>
<td>Home-made</td>
<td>Very High (Diffraction-limited)</td>
<td>~100’s mW</td>
<td>870 nm</td>
<td>12 nm</td>
<td>~63 μm</td>
<td>&lt; 100 μm</td>
</tr>
<tr>
<td>Fibre-coupled laser diode</td>
<td>OPC-D007-830FC</td>
<td>Partial (Low)</td>
<td>&lt; 20 W</td>
<td>809 nm</td>
<td>4 nm</td>
<td>173 μm</td>
<td>82 μm</td>
</tr>
<tr>
<td>Mode-locked, femtosecond Ti:Sapphire</td>
<td>Commercially available</td>
<td>Very High</td>
<td>&lt; 1 W</td>
<td>830 nm</td>
<td>7.5 nm</td>
<td>100 μm</td>
<td>&lt;100 μm</td>
</tr>
<tr>
<td>LED</td>
<td>Hitachi HE8404SG</td>
<td>Very low</td>
<td>&lt; 50 mW</td>
<td>807 nm</td>
<td>50 nm</td>
<td>13 μm</td>
<td>8.6 μm</td>
</tr>
</tbody>
</table>

characterizing InGaAs–GaAs MQW devices grown at the University of Tsukuba, Tsukuba, Japan, and have measured output diffraction efficiencies of 0.12% at 940 nm. This would enable us to utilize sources such as high-powered light-emitting diodes (LEDs) and diode lasers emitting above 850 nm. Devices that permit imaging up to 1.7 μm would be ideal since we could then exploit the available high-power fiber laser technology and further reduced scattering coefficient in tissue.

VI. BROAD-BAND SOURCES FOR DEPTH-RESOLVED HOLOGRAPHY

To date, we have demonstrated whole-field photorefractive holography using both bulk photorefractives and MQW devices with light sources ranging from mode-locked femtosecond lasers, LEDs, high-power fiber coupled laser diodes, multimode laser diodes emitting in the red to a novel, all solid-state, broad-band tunable laser source, developed by our group [21]. Table I is a brief summary of photorefractive holography performed with numerous light sources.

VII. IMAGING WITH LASER SOURCES

Depth-resolved images shown in Fig. 1(a)–(d) may be obtained with any sufficiently broad-band source (as long as the photorefractive medium exhibits a response for the particular wavelength used). However, when using spatially coherent sources, such as lasers and laser diodes, the images acquired through solid scattering media are marred by speckle [24], which results from the interpixel crosstalk that occurs when photons that have been scattered out of their coherence cell scatter back into it whilst still retaining coherence (i.e., same path length) with the original reference beam. This problem does not arise for liquid scattering samples since the Brownian motion of the scatterers time-averages the speckle to a uniform background [24]. One way around this problem is to use spatially incoherent sources so as to make the pixels mutually incoherent. We have demonstrated that synthesizing a spatially incoherent source using a rotating diffuser in a laser beam causes a substantial reduction in the amount of speckle [24]. We have also investigated the use of liquid light guides as a low-loss diffusing element for the fiber-coupled laser diode array.

We note that the ability to utilize sources of low spatial coherence make it possible to access such high-power sources, producing tens of watts of average power, for wide-field imaging through thicker turbid media. The higher power should increase the ballistic light signal transmitted through a scattering medium. Of course, photorefractive holography is designed to image very low light levels (<1 μW), and the power incident on the MQW device would need to be limited to ~1 mW to avoid damage due to excessive photocurrents. Thus, a combination of judicious spatial filtering and attenuation would be required to optimize such a high-power photorefractive MQW imaging system. When using bulk photorefractive crystals such as rhodium-doped barium titanate, however, the incident power may be as high as ~1 W with no damage issues. Bulk photorefractive media are generally much slower than MQW devices, but real-time depth-resolved imaging is still possible when higher powers are used [25].

VIII. IMAGING WITH LEDs

An attractive method to combat interpixel crosstalk is to use highly spatially incoherent light sources such as LEDs [26]. These sources have the additional advantage that because of the very short coherence length, the depth sectioning is much better than that obtained with a commercial mode-locked laser source. The fact that these sources are cheap, easily replaceable, and much more robust than lasers makes them useful in the development of portable and inexpensive imaging instrumentation. We note that it has been shown by Leith et al. [10] that imaging with spatially incoherent sources is equivalent to confocal imaging. We have achieved depth-resolved images by using high-power, broad-band LEDs with center wavelengths ranging from 760 to 840 nm. Speckle-free, depth-resolved images with a depth resolution of 8.6 μm have been acquired using this cheap source. However, the images obtained with such a broad-band source as an LED have a limited field-of-view. This is because when beams having a very short coherence length are made to interfere at an angle (1.2° in our case), fringes are only written in the central portion of the interfering
beams leading to a restricted field-of-view. One way around this problem is to use an interference filter in front of the LED thereby decreasing its spectral width at the expense of increased temporal coherence. This increases the field-of-view, but affects the depth resolution adversely [26].

We have shown that walkoff can be overcome whilst preserving the coherence length of the LED by the use of group delay compensating prisms [27]. In doing so, we have made use of the idea that the energy fronts in a beam become displaced with respect to the phase fronts when the beam traverses a dispersive element such as a prism. The visibility of the fringes depends on the overlap of the energy fronts of the interfering beams. If we are able to contrive the tilt between the energy and phase fronts of the holographic recording beams such that, upon reaching the holographic recording device, the energy fronts of the two noncollinear beams are both parallel to the MQW surface, then it should be possible to achieve interference over the full field-of-view. We have accomplished this by inserting appropriate prisms in each arm of the Michelson interferometer, as illustrated in Fig. 2, to achieve the required transverse sweep of the group delay across the beams. Fig. 3 shows holographic images obtained without and with group delay prisms and the corresponding fringe modulations for the two images. These images were obtained with an LED of about 40-nm bandwidth centered around 760 nm. Interference fringes spanning the entire field-of-view were obtained with the LED when used in conjunction with the group delay compensating prisms [27].

IX. NOVEL, ALL SOLID-STATE, TUNABLE BROAD-BAND LASER SOURCE

Although photorefractive holography may be implemented with sources of arbitrary spatial coherence, confocal scanning techniques like optical coherence tomography require spatially coherent broad-band radiation. We have developed a tunable, solid-state, broad-band, diode-pumped continuous-wave (CW) laser source that can provide high average power (hundreds of milliwatts) without the need for mode-locking. Because of its high spatial coherence, this laser source is suitable for OCT and is a cheap alternative to complex mode-locked Ti : sapphire systems. Fig. 4 shows the schematic of this approach, which is generally applicable to almost any laser medium and we have implemented it using a diode-pumped Cr : LiSAF laser [21]. This laser source is tunable from 790 to 900 nm, and provides smooth spectra with widths up to 15 nm and provides discontinuous spectra extending up to 65 nm. It is pumped using a 100-μm broad stripe laser diode emitting at 670 nm. After the pump shaping optics, the beam is focussed through the plane face of the rod into the gain medium which is a plane/Brewster cut Cr : LiSAF rod. The gain narrowing that one expects to observe in a CW laser is reduced by spatially dispersing the frequency spectrum of the radiation within the gain medium using an intracavity prism. Further details of this laser source may be found in [21]. The depth-resolved images shown in Fig. 1 were recorded using this source in our photorefractive holography setup.

X. HIGH FRAME-RATE, WHOLE-FIELD, DEPTH-RESOLVED IMAGING USING PHOTOREFRACTIVE HOLOGRAPHY

We have previously demonstrated video-rate depth-resolved holographic imaging using GaAs–AlGaAs MQW devices [28], and have demonstrated that the photorefractive MQW device response time may be submillisecond, which implies a maximum achievable frame-rate exceeding 1000 fps. To further explore this potential for high-speed depth-resolved imaging, we have replaced our standard video-rate charge-coupled device (CCD) camera with a high frame-rate, low-light-level intensified CCD camera and are aiming to achieve depth-resolved imaging at 1000 fps. So far, we have successfully demonstrated a 3-D imaging system at 476 fps and have resolved the motion of a rotating optical chopper in front of an object. To the best of our knowledge, this is the fastest whole-field depth-resolved imaging system reported to date.

The experimental configuration for this system is as shown in Fig. 2. As source, we used a McDonald Douglas laser diode emitting at 664 nm. The object was a U.S.A.F. test chart that was
mounted on a high-precision $x$, $y$, $z$ translation stage. A chopper was placed in front of the test chart and was driven at 20 Hz, corresponding to a blade velocity of 1.1 m/s. Using this system, we could follow the motion of the chopper blades and images were obtained at a rate of 476 fps. The system was tested with the CCD camera in both binning and nonbinning modes. By plotting the pixel values at a fixed position throughout the whole stack of images, we obtained two rectangular curves illustrating the chopping of the image beam, which are shown in Fig. 5. The current speed of the system is lower than is possible from the CCD camera (DALSA CA-D1), but our current software cannot acquire images at a higher data-rate. We expect to extend the image acquisition rate to $\sim$950 fps in the near future.

XI. STRUCTURED ILLUMINATION WITH PHOTOREFRACTIVE HOLOGRAPHY

Optical sectioning in a conventional microscope has been demonstrated by Wilson et al. [13] by exploiting the observation that it is only the zero spatial frequency component that is not attenuated with defocus. It is possible to realize whole-field sectioning, using either reflected light or fluorescence [29], by illuminating the sample with spatially modulated (structured) light and using suitable post-acquisition processing. This is typically realized by imaging a high spatial frequency grid on to the sample. Although video-rate imaging can be achieved, the requirement for post-processing does limit the maximum achievable frame-rate. Accordingly, it seems attractive to try and combine the simplicity and wide utility of structured illumination with the high-speed processing and potential scattered light rejection of photorefractive holography. We describe here, for the first time, a technique to perform whole-field optical sectioning in real-time using reflected light or fluorescence that realizes this combination.

In our approach, which is illustrated in Fig. 6, a grating with a high spatial frequency is imaged on to the sample using spatially incoherent light. The sample is then imaged on to a photorefractive medium that records the spatially modulated component of the incident light field, according to its photorefractive material properties. After the work of Wilson [13], this is the whole-field, sectioned image that is recorded and which may be read-out in real-time using a simple reconstruction laser beam. By using the photorefractive effect, we have thus been able to perform micrometer-level sectioning in real-time without the need for post-processing. It may be pointed out here that using achromatic gratings technique, Adler et al. have written dynamic white light holograms on an optically addressed multiple quantum well spatial light modulator [30], and that our approach is similar to white light holography.

For our first experiments a (20 lines/mm) grating was imaged on to the sample (a U.S.A.F. test chart) using light from a frequency-doubled neodymium YAG laser at 532 nm (Fig. 7). Incoherent illumination was achieved by placing a rotating diffruser in the laser beam path. The test chart, illuminated with the spatially modulated light, was then imaged on to the MQW device. This image was read-out in real-time by diffracting a CW laser beam at the exciton absorption peak wavelength of the MQW device off the refractive index distribution recorded in the MQW device. The sample was translated axially through the focal plane of the objective and real-time, whole-field sectioning was observed. A sectioning curve of $\sim2.2 \mu m$ was obtained with a times 40 objective. We note that the theoretical sectioning strength for this setup was calculated [13] to be $\sim1.43 \mu m$ and we are currently investigating this discrepancy between theory and experiment. It may be a consequence of spherical aberration in our imaging system. Unwanted spatial modulation may be removed from the read-out image by spatial filtering. This leads to a reduction in the transverse spatial resolution. However, we propose that by translating the grating laterally, using a piezoelectric transducer, at a rate intermediate between the response time of the MQW device and the integration time of the CCD, we could eliminate the unwanted spatial modulation from the sample without compromising the resolution.
We are also planning to develop a low-coherence photorefractive holographic microscope to record and read-out whole-field 2-D images using our high frame-rate camera system. This will be applied to imaging of live biological samples and other dynamic objects.

REFERENCES


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