Full Color Holographic Endoscopy

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Abstract The ability to produce color holograms from the human tissue represents a major medical advance, specifically in the areas of diagnosis and teaching. This has been achieved at Glyndwr University. In corporation with partners at Gooch & Housego, Moor Instruments, Vivid Components and peninsula medical school, Exeter, UK, for the first time, we have produced full color holograms of human cell samples in which the cell boundary and the nuclei inside the cells could be clearly focused at different depths - something impossible with a two-dimensional photographic image. This was the main objective set by the peninsula medical school at Exeter, UK. Achieving this objective means that clinically useful images essentially indistinguishable from the object human cells could be routinely recorded. This could potentially be done at the tip of a holo-endoscopic probe inside the body. Optimised recording exposure and development processes for the holograms were defined for bulk exposures. This included the optimisation of in-house recording emulsions for coating evaluation onto polymer substrates (rather than glass plates), a key step for large volume commercial exploitation.

1. Introduction

In full-color interference based imaging, the produced image is essentially indistinguishable from the object. Three-dimensional holographic images of human tissue may be examined under a microscope in the same way as a tissue sample, without need for biopsy, and can resolve detail of cell nuclei. This project drew several recently developed different strands of technology namely interference based imaging techniques, recording materials and processes, fused fiber couplers, and coupled Red, Green, Blue (RGB) laser sources, for a specific application in medical imaging. Monochrome holograms could be recorded of tissue using cw laser light through a monomode fibre. Additionally, experiments using Red Green Blue (RGB) laser light through a monomode fibre were performed to illuminate recorded colour holograms of tissue samples. These preliminary experiments were aimed at investigating the influence of speckle across the illuminated field. The main focus was completely on the recording of medically useful full color holograms in a fixed rather than fibre-optic holographic setup. The fibre-optic setup will not improve the holographic image quality, but only make it possible to have a flexible recording system suitable for a medical environment. Excellent progress was made and world first full-color holograms of human cells resolving cell nuclei were achieved. The medical assessment of the quality of the holograms was positive, as for the first time, human cell samples in which the cell boundary and the nuclei inside the cells could be focused at different depths was achieved Figure 1. This was the main objective and there are definite routes forward for medical applications, particularly in dermatology. However, for the mass commercialization stage, improvements are needed in both colors recording and rendering techniques as well as recording materials.
2. The coherent source

2.1. Requirement for a ‘white light’ laser source

A fixed laboratory setup with visible CW lasers with long coherence and very stable output at 647nm, 532nm and 476nm were used to record the color holograms. However, flexible, high-quality full-color holographic endoscopy requires both visible combination and polarization-maintaining (PM) transmission integrated within a single device. Gooch and Housego fiber optics laboratories [1] with expertise in the production of laser sources, operating principally in the Red region of the visible spectrum were tasked with the development of a combination device Figure 2(a), with the following characteristics: (i) stable pigtailed sources, operating in the Red, Green and Blue regions, (ii) an output PER of >15dB, (iii) transmission of each visible wavelength between 70% and 90%, and (iv) Red=658nm, Green=532nm and Blue=452nm Figure 2(b). The choice of visible wavelengths was a compromise based on available sources most suited to holographic endoscopy. Realization of the required tasks by Gooch & Housego is achieved by means of a relatively complex adaptation of existing technologies. Globally, this represents pioneering work and to date this has been highly successful.

Figure 2. A flexible combination device with ‘white’ output(a) using RGB lasers (b)
2.2. Improved color rendering

The choice and the number of lasers used affects color rendering. Improved rendering is possible if more than the minimum three primary color laser wavelength (RGB) is used [2]. In house computer simulation Figure 3 based on the Macbeth Color Checker® assuming a perfect recording emulsion confirms this. The choice of the recording material used is also of equal importance and in this case the focus needs to be based on the use of extremely low light-scattering panchromatic materials that are fast in terms of exposure and developed. The main contenders are silver-halide emulsions such as the nano particle size emulsion developed at Glyndwr University’s Centre for Photonics Studies [3] and photopolymer such as the new Bayfol®HX [4-5] from Bayer [6].

![Figure 3](image)

**Figure 3.** Decreasing total average color reproduction error with increased number of lasers

3. Recording Materials

To source the color recording material for the holo-endoscope project a number of commercial suppliers [6] [7] [8] [9] [10] [11] were approached. Despite the long list, the choice is very limited, but some panchromatic (around 450nm – 650nm) recording materials supplied on both Film and Glass substrates could be obtained from Sfera-S, Slavich and Color Holographic (Glass only). However, in order to obtain a recording materials with a diffraction efficiency of higher than 95% (Similar to that from Ultimate and Bayer) it was decided that to reproduce and optimize Glyndwr University’s own ultra-fine grain (8-10 nm) recording emulsion (SilverCross) previously developed as part of an EU framework program [3]. This objective was met successfully. Additionally, for large volume commercial exploitation, optimized recording exposure and development processes for the holograms were defined. The produced emulsion has sensitivity between 4-8 mJ/cm² **Figure 4** and it seems to be one of highest quality color recording materials yet produced. Recording material used: - (i) Sphere-S material with an optimum recording of ~ 1.7 mJ/cm²; (ii) In house coated material ~ 4 mJ/cm² and (iii) Machine coated in house recording material with ~ 6 mJ/ cm².
4. RECORDING FIBRE-DELIVERED ENDOSCOPE HOLOGRAMS

The standard procedure was recording a reflection hologram on a silverhalide material and then chemically processing it in a darkroom.

4.1. Fibre-delivered recording

The following three Continuous Wave (CW) lasers were used to create a coherent ‘white’ beam to record the full color holograms: krypton ion laser (647 nm), frequency-doubled Nd:YAG (532 nm) and argon ion (476 nm). The recording setup is of reflection type using Denisyuk geometry Figure 5. Using multimode fiber, the setup was also used extensively for coherent holographic reconstruction. The reconstructed images were recorded using a digital microscope camera Figure 6.
The original subject matters used for recording, due to their relatively larger size were onion cells. The USAF test chart was used for calibration Figure 7.

4.2. Darkroom processing
The optimum darkroom processing techniques for each recording material were mostly established by trial and error in an empirical manner. Additionally, for large volume commercial exploitation, optimized recording exposure and development processes for the holograms were defined. This included the optimisation of in-house recording emulsions for coating evaluation onto polymer substrates rather than glass plates.

5. Reconstruction
The final recorded reflection holograms were reconstructed using a MEIJI Techno fibre optic (150W) light source Figure 8. A Nikon ECLIPSE ME600L microscope was used to observe the
holograms in detail. The images were then captured using a Nikon DS-Gil 5 Mega Pixel digital camera.

![Image](image_url)

**Figure 8.** Best image reconstruction results were only achieved through non-coherent illumination

5.1. Benchmark tests

These are standard tests looking for bright, noise free and full color holograms. **Figure 9** below, shows the spectral graph of a typical full color hologram made for this project.

![Spectral Radiance Graph](image_url)

**Figure 9.**

The peaks on the graph should match the recording wavelengths of the three lasers used: - i.e. krypton ion (647 nm), frequency-doubled Nd: YAG (532 nm) and argon ion (476 nm). For a good color balance, the size of the peaks should be similar. All color measurements were made using the PR-650 Spectra Colourimeter from Photo Research Inc.

5.2. Image quality and speckle noise

High level of speckle noise was recorded when the single mode fibre was used for exposure. To reduce the noise level the method was abandoned in favour of the multi-mode fibre, but this method did not improve the noise level as the fibre delivery system did not match the hologram recording wavelength used. Eventually, some speckle reduction was successfully achieved by matching the recording wavelengths with the colour sensitivity of the panchromatic recording material (using...
chemical processing). In other words, by matching the recording color with the recording materials’ color sensitivity, the speckle averaging effect is at its optimum, giving us a realistic estimate of the “best possible” colour endoscopic holograms in regard to resolution.

6. **Color holograms of human endothelial**

Early in the holoendoscopic investigations it was demonstrated that monochrome holograms could be recorded of tissue both directly and/or using fibre-optics. Experiments using RGB laser light through a fibre were also performed to illuminate recorded colour holograms of tissue samples. These experiments were aimed at investigating the influence of speckle across the illuminated field. Non-coherent illumination was eventually decided as the hologram reconstruction method of choice. The focus was then completely on recording and displaying of colour holograms of tissue samples without the use of fibre optics. The quality of endoholograms will be the same, independent of if they are recorded in a fixed or fibre-optic holographic setup. The fibre-optic setup will not improve the holographic image quality, but is only required for the next stage of the project to develop a flexible recording system suitable for medical endoscopic applications. The recorded colour holograms of tissue samples were investigated by Peninsula Medical School (PMC). The PMC determined that an RGB colour endohologram of cells recorded has the required quality for medical relevance **Figure 10**.

![Figure 10. Human endothelial cells](image)

7. **Conclusions and the future**

This project developed several different strands of technology and drew them together for a specific application in medical imaging. The main objective was to produce medically approved full color holograms of human cell samples in which the cell boundary and the nuclei inside the cells could be clearly focused at different depths. This objective has been achieved and potentially the recording could be done at the tip of a holo-endoscopic probe inside the body. Additionally, for large volume commercial exploitation, optimized recording exposure and development processes for the holograms were defined. At the Center for Photonics studies laboratories we also developed an optimised version of the Center’s own nano size holographic emulsion with leading-edge characteristics for recording onto polymer rather than glass substrates, a key step for large volume commercial exploitation. However, the mobile holo-endoscope system, designed for flexible use in a medical environment, was not ready for testing in the timeframe of the project, and is not available for immediate commercialisation.
Future Work – With consideration to several advances in applicable technologies during the course of the project, here follows a list of future objectives, grouped by subject case.

A new holo-endoscope design
Analysis of promising recently developed mobile ‘White’ laser holographic recording equipment is of key importance [12], [13], [14].
Consideration for a new device:
• Integrated ‘White’ laser, with a minimum of three lasers, Red, Green, Blue, with all lasers to be fully integrated with PM fibre pigtails.
• Recording requirements - exposure dose ~1mJ/cm², nominal exposure time 1ms requires 1W.
• Full interface from Red, Green, Blue combiner to endoscope with consideration for beam profile for hologram exposure.
• Exposure time and output power balance control for all wavelengths, and shutter mechanism.

Material
• Further development on extremely low light-scattering panchromatic recording materials
• Tests on main recording materials in use today as well as newly developed photopolymer and silver-halide based emulsions.

Illumination
The illumination source to display the recorded holograms is of critical importance and newly reported illuminating system such as [15], [16], should be thoroughly investigated.
Considerations for the development of a new system:
• LEDs - choice of mixing the component LEDs in terms of bandwidth and wavelength, with controller for each LED. This is important as the color temperature of the source influences the color of the holographic image
• Source size partly determines the image resolution. Also, illumination angle and the distance from the source should be considered to avoid aberrations.

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