Ambient humidity control for maximising replay intensity and resolution in aberration-compensated off-axis holograms of underwater objects

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ABSTRACT

In hologrammetry it is usually more desirable to reconstruct the real image than the virtual image, since the latter must be viewed at a distance through the window of the holographic plate itself. In applications where the recorded scene was in water but with replay into air it is necessary to correct for the refractive index difference. This can be done by reconstructing the image with shorter wavelength illumination combined with a change in beam angle to satisfy the grating equation, but these changes mean that the Bragg condition may no longer be satisfied during replay, reducing the diffraction efficiency and making the reconstructed images difficult to see.

Changing the replay beam angle to better satisfy the Bragg condition makes the images brighter, but also renders them unrecognizable by introducing severe optical aberrations. A possible solution is to alter the Bragg properties of the hologram. In particular, the emulsion thickness can be conveniently controlled by altering the humidity of the atmosphere surrounding the hologram without causing any long-term changes or damage to the holographic plate.

The validity of using humidity change to tune the Bragg properties of emulsions during replay has been demonstrated by measuring the brightness and perceived resolution of a reconstructed real image from a hologram over a wide range of humidities. The results have been compared with a simple model based on the Flory-Huggins theory of polymer swelling.

Keywords: holographic emulsions, photographic emulsions, emulsion swelling, emulsion shrinkage, humidity

1. INTRODUCTION

When using holography for dimensional measurement it is often much more convenient to reconstruct the real image, with which it is possible to interact directly, than the virtual image which must be viewed at a distance through the window of the holographic plate itself. As the recorded scene was in water while replay is usually into air, it is necessary to correct for the refractive index difference. This can be done by reconstructing the image with shorter wavelength illumination than used for recording, accompanied by the corresponding change in beam angle to satisfy the grating equation.

The Holomar collaboration is currently developing both *'HoloCam'*, an underwater holocamera for the *in situ* recording of plankton species and distributions, and *'HoloScan'*, an associated hologram reconstruction and analysis instrument. The holocamera [1] uniquely incorporates simultaneous in-line and off-axis holography of overlapping sample volumes (figure 1*a*), allowing the recording of organisms over a wide range of sizes and concentrations. Holograms are recorded on glass plates by a Q-switched, frequency-doubled Nd-YAG laser, operating at a wavelength of 532 nm and a pulse duration of less than 10ns to freeze any motion of the organisms. It is expected to expose up to 25 holograms of each geometry during a dive. Manual analysis of large volumes containing thousands of particles is, however, an enormous and time-consuming task, with operator fatigue an unpredictable source of errors. The overall purpose of the data extraction system is to automatically locate [2] and identify [3] the various organisms within the sample volume, allowing for the first time a quantitative analysis of the spatial relationships between both individuals and species and thus improving our understanding of fundamental biological and chemical processes in the upper layers of the oceans.

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The presence of the thick window (and 120mm air space for the off-axis reference beam), combined with the desire to capture large (metre-scale) volumes makes it impossible to study microscopic objects throughout the sample volume from the virtual image. Thus, although the objects are located in water, image replay is carried out in the laboratory in air using the projected (real) image mode of reconstruction. This change in refractive index can introduce significant aberrations, particularly with off-axis holography, which may be partially corrected by replay at a shorter wavelength [4].

The holocamera's window thickness and the air gap to the plate have been optimised for reconstruction with 442nm illumination from a HeCd laser [4] . A schematic diagram of the *HoloScan* replay machine is shown in figure 1*b*. The holographic plate is illuminated by the collimated beam from 180mW HeCd laser. A movable mirror allows the replay system to be easily switched between in-line and off-axis modes. A CCD-based videocamera is traversed through the projected real image on computer-controlled micro-positioners, which cover a sample volume up to 200mm across and 1000mm deep in 10µm steps, allowing individual organisms to be isolated and located [2, 3]. The video output is captured by a frame-grabber and then processed to clean up the image and identify the true focal plane of each object within the 3-dimensional sample volume.

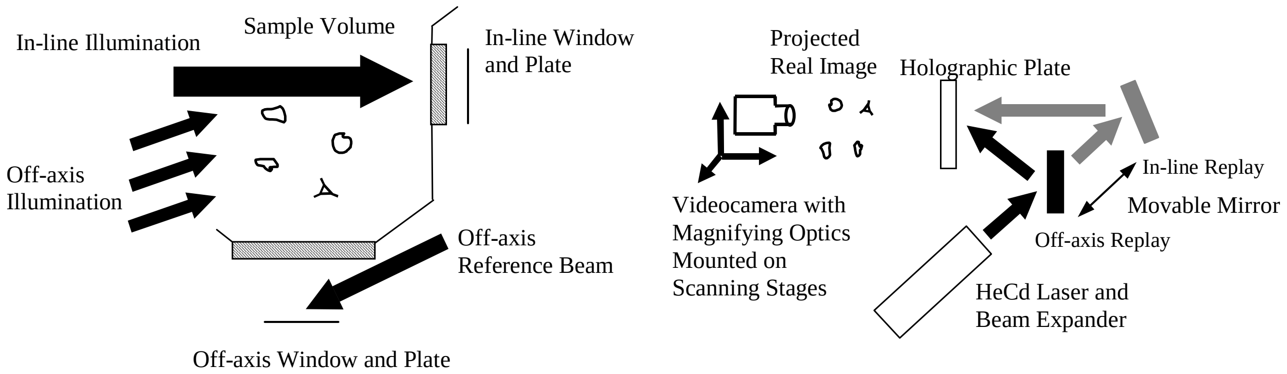


Figure 1: Schematics of (*a*) *HoloCam* sample volume (*left*) and (*b*) *HoloScan* replay system (*right*)

Although the replay laser is among the most powerful commercially available and the videocamera was chosen because of its sensitivity in the blue, many of the off-axis holograms examined during testing produced images too dark to see clearly. This is because the changes in the illumination wavelength and direction mean that the Bragg condition is no longer be satisfied during replay, reducing the diffraction efficiency. Changing the replay beam angle to better satisfy the Bragg condition makes the images brighter, but also renders them unrecognizable by introducing severe optical aberrations. A possible solution is to alter the Bragg properties of the hologram - in particular, the emulsion thickness can be conveniently controlled by altering the humidity of the atmosphere surrounding the hologram without causing any long-term changes or damage to the holographic plate.

2. REPLAY OF UNDERWATER HOLOGRAMS

2.1. Background

Although the scene is recorded in water, it is necessary in the lab to reconstruct the real image in air. The significant aberrations associated with the refractive index change may then be corrected for by replaying with a shorter wavelength [4]. However, the incidence angle of the replay beam must also be adjusted, so that the illuminating beam gives the same phase distribution across the holographic plate, otherwise new aberrations will be introduced. This condition is derived from the grating equation and can be expressed (using the notation of Solymar and Cooke [5]) as:

$$\lambda / \sin \theta_E = \text{constant} \qquad (1)$$

where λ is the wavelength and θ_E the angle of incidence of the replay beam on the holographic plate. Although this condition is sufficient to describe a thin hologram, in many cases Bragg effects may be important even if they do not dominate the diffraction process. For example, off-axis holograms from *HoloCam*, which are recorded with a 532nm reference beam incident at 60°, cannot be treated as thin holograms according to the formalism of Solymar and Cooke [5] (neither do they satisfy the conditions for true volume holograms). The holograms are encoded as a set of fringes within the photographic emulsion which take the form of many parallel planes, similar to a Venetian (louvre) blind. The change of replay beam angle accompanying the wavelength shift leaves the incident illumination unable to pass through the fringe structure.

Off-Bragg replay is possible, but to get useful images one would require a cooled, extra-sensitive (expensive) camera, and the signal-to-noise ratio would remain poor, as effects such as emulsion scatter are much less sensitive to the illumination direction than the Bragg selectivity of the grating, so that off-Bragg images tend to suffer from relatively high levels of background noise.

2.2. Approach

Since most replay parameters of Holomar holograms are pre-defined (the replay wavelength by the recording wavelength and layout, the beam angle by (1)), it is desirable to find a mechanism for tuning the Bragg properties of the holographic plates to improve their diffraction efficiency. As they are a property of the emulsion itself, the spacing and orientation of the fringes can be altered by somehow swelling or shrinking the emulsion layer. Modelling [5] suggests that Holomar holograms would require an emulsion swelling during replay of 10% over that at exposure. The swelling mechanism should not distort the reconstructed image or increase noise, and be either archivally stable or easily reproducible.

Bjelkhagen [6] discusses a number of techniques for the controlled swelling of holographic plates: probably the most well-known being the use of Triethanolamine (TEA) for colour selection in reflection holograms. Unfortunately this tends to cause extra scattering and distortion of the holographic image, and since the TEA is gradually lost from the emulsion the swelling is not fixed over the possible working life of the hologram (possibly decades in applications such as coral reef surveys). Angell [7] suggests adding an organo-silane to the fixer. This binds to the gelatin, cutting down hydrogen bonding between gelatin molecules and thus permanently swells the emulsion slightly; but little is known about the effects on the reconstructed image and the scattering properties of the emulsion, or its long-term stability.

An alternative method due to Young [8] is to swell the developed emulsion with an aqueous solution of *n*-Vinyl 2-Pyrrolidone (NVP) and expose the holographic plate to ultra-violet light causing the NVP to polymerise within the emulsion. While this approach might allow the permanent fixed swelling of holograms without the need for a sealing step, again too little is known about the effect of this process on the replayed image and the scattering properties of the emulsion for its immediate use.

Our chosen approach is to alter the ambient humidity around the emulsion. This gives a very strong swelling and shrinking effect that is easily reversible and repeatable, which shouldn't affect or damage the emulsion (which must of course survive the swelling associated with the development process). Unlike the methods mentioned above, altering the ambient humidity also allows rapid selection of *any* arbitrary degree of swelling.

3. HUMIDITY AND HOLOGRAPHIC EMULSIONS

Very little data appears to have been published that relates the swelling of an emulsion to the relative humidity of the surroundings. Wuest and Lakes [9] have investigated this approach for the colour control of bleached display holograms, but they didn't measure the swelling directly. Their findings can be briefly summarised as follows: the emulsion properties vary approximately linearly at normal humidities (20-70% RH), and then change very rapidly over 80% RH. In order to get a better understanding of the effects of humidity on emulsions we have therefore also looked at data for hardened gelatin.

One complication, as described by Sheppard *et al.* [10] in 1940, is hysteresis: hardened gelatin was found to stay in a more swollen state during the drying process than during wetting (figure 2). The same dimensional state is reached at a 10% RH difference around 50% RH. Sheppard *et al.* also found the swelling of plain gelatin and of hardened types to have a similar variation with humidity.

Due to the lack of published data relating the swelling of photographic emulsions to atmospheric humidity, initially we modelled this process with a simplified form of the Flory-Huggins equation, which describes the interaction between polymers and solvents:

$$\ln (p_S / p_{S,0}) = \ln \phi_S + (1 - \phi_S) + \chi(1 - \phi_S)^2 \qquad (2)$$

where p_S / $p_{S,0}$ is the ratio of the partial pressure of the solvent p_S over the swollen polymer to the saturated vapour pressure of the solvent $p_{S,0}$ (this ratio is commonly known as the relative humidity in the case of water); ϕ_S is the volume fraction of the solvent in the solvent/polymer mixture; and χ is a quantity known as the Flory-Huggins parameter which represents the interaction between a given polymer and solvent, and can be found experimentally. Given that when washing a photographic plate in water the emulsion generally swells to around 5 times its original thickness [6], (2) implies that χ is about

0.6 for holographic emulsions. An important consideration with regard to (2) is that the theoretical basis of the Flory-Huggins equation is the thermodynamics of free (unconstrained) *non-polar* polymers and solvents, and so our expectations should not be too high when using it to model gelatin bonded to glass plates and soaked with water.

Figure 3 shows the swelling of gelatin by ambient humidity, with both experimental data and the predictions of (2) using a Flory-Huggins parameter of 0.62 (a value based on the 92% RH data point of Gehrmann and Kast [11]) . The data of Gehrmann and Kast are for gelatin bonded to a substrate and thus constrained in two dimensions, while those of Sheppard *et al.* and Katz [12] are for lumps of material free to expand in all directions. The general trend of the data is similar to the findings of Wuest and Lakes: a steady swelling from 20 to 70% RH followed by a rapid increase in volume past 80% RH. The Flory-Huggins equation can be seen to provide a reasonable fit to that data at higher humidities (and thus the majority of the swelling range), but noticeably under-estimates the swelling below about 70% RH.

We have used the Flory-Huggins equation to estimate the ambient humidity needed during replay in order to give the required 10% swelling, as discussed in section 2.2. Since *HoloCam* will be sealed and purged with dry nitrogen during deployment to prevent condensation, exposure is assumed to occur at 0% humidity and we expect that optimum replay should require about 30% RH. Thus, replay at normal laboratory humidities should naturally correct for the wavelength shift associated with our final system (indeed, the underestimation of the measured values by the Flory-Huggins equation indicates a risk of over-compensation). Other situations are more complex however; for example some of the laboratory-recorded holograms of collected plankton samples in tanks were recorded at ambient humidities above 70% and thus require playback at humidities in excess of 90%.

The dry atmosphere to be used in the submersible holocamera has other implications: the excessive shrinkage may cause the emulsion to peel from the substrate or micro-cracking leading to increased light scattering within the emulsion. There may also be extra fogging of the plates due to static discharge. On the other hand, the lack of oxygen in a dry N_2 atmosphere should also double the plate sensitivity. These issues will be investigated in more detail on deployment of the holocamera.

4. EXPERIMENTAL VALIDATION OF APPROACH

To demonstrate the validity of using humidity changes to tune emulsion properties during replay, the variation of the brightness and perceived resolution of a reconstructed real image from a laboratory-recorded hologram with replay beam angle have been measured over a range of humidities (figure 4).

The image of a USAF 1951 resolution target has been projected from holograms recorded on Agfa 8E56 glass plates and mounted in a glass-sided enclosure fitted with a humidity sensor (Hycal/Honeywell Humidity Sensor HIH 3602-C, with accuracy of ±2%) mounted on a rotary stage. The air supply was fed from a cylinder of compressed dry, low hydrocarbon air (BOC “laser” air), and passed *en route* through a bubbler filled with distilled water with a fine-bore needle valve connected in parallel with it. The moisture content of the airflow could thus be set to any chosen value by a suitable adjustment of the valve, and held steady for a long enough period of time that the emulsion could reach equilibrium. To mitigate hysteresis effects the humidity was generally increased between successive runs. The hologram was illuminated with a 442nm HeCd laser over a range of replay angles. The real image was projected directly on to the faceplate of a CCD camera (JAI CV-M300, AGC disabled) and the perceived resolution found directly from a live monitor image, while the brightness of the image was measured by capturing the image and finding the average pixel intensity over a fixed region of the bright background of the resolution target. The camera had been checked for linearity, allowing the pixel values to be scaled to compensate for changes in laser power. The air flow was stopped while taking measurements, to avoid any bowing of the holographic plate with pressure.

The two amplitude holograms studied so far were both recorded in the *HoloCam* laboratory mock-up (i.e. with 532nm illumination at 60°) with the resolution target 300mm from the plate, developed with M.A.A. and fixed normally. The first, denoted ‘8E/MAA/Fix’ was recorded at an unknown humidity (estimated 60-70%) and with no water in the sample volume. The change of wavelength on replay will in this case tend to cause rather than cure aberrations. The second plate, denoted ‘GAC1’ was recorded at 66% RH with the sample volume filled with water. Although the latter is a more appropriate choice, a flaw in the plateholder design (the outer edge of emulsion face of the plate was the sealing surface clamped tightly against an O-ring) caused GAC1 to crack at high swelling, so that the majority of the final data is from the less representative case.

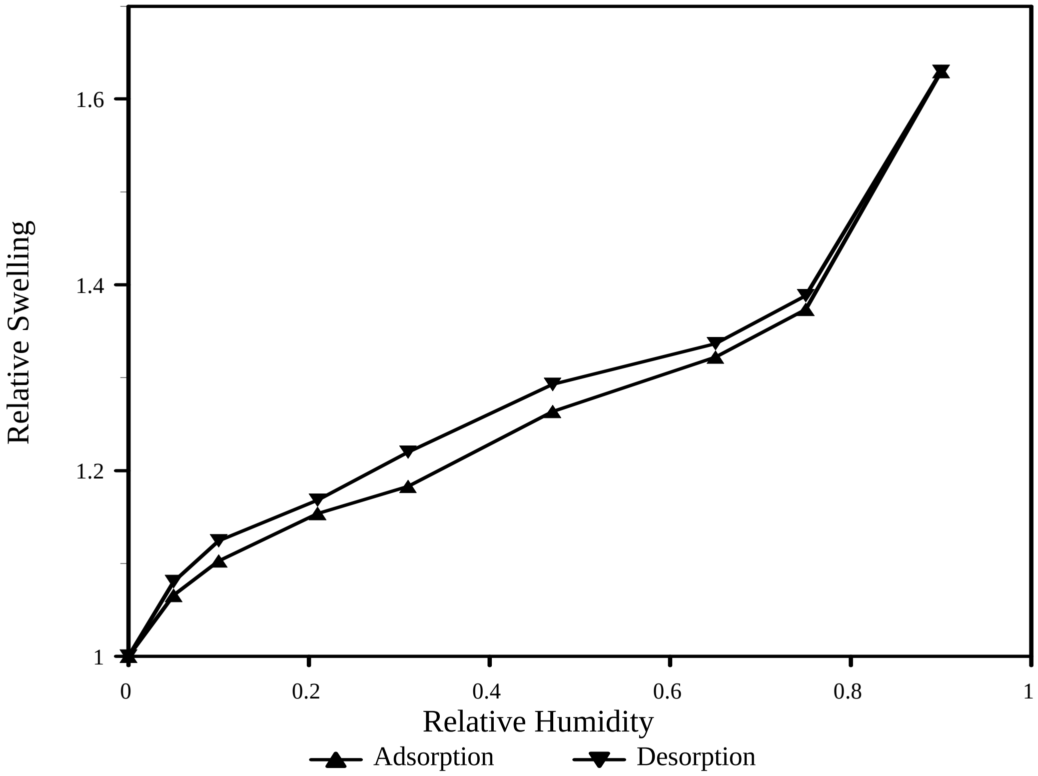
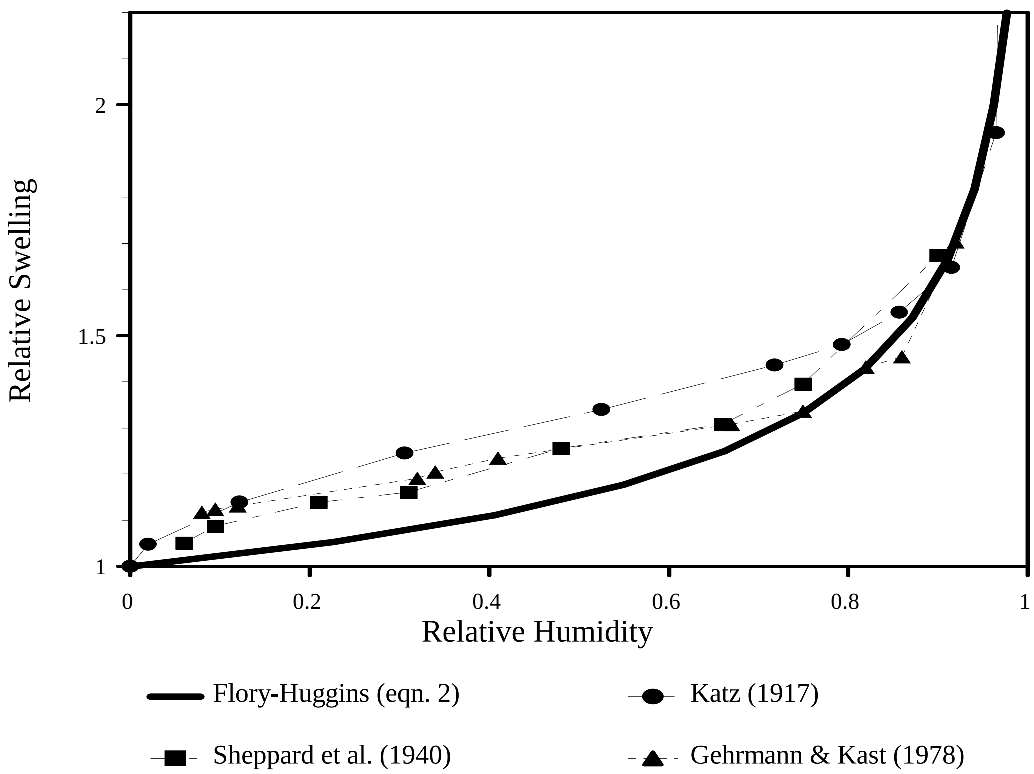


Figure 2: Hysteresis in Gelatin swelling (after Sheppard *et al.*, 1940 [10])

Figure 3: Gelatin swelling by humidity - comparison of Flory Huggins eqn. (with χ =0.62) and experimental data



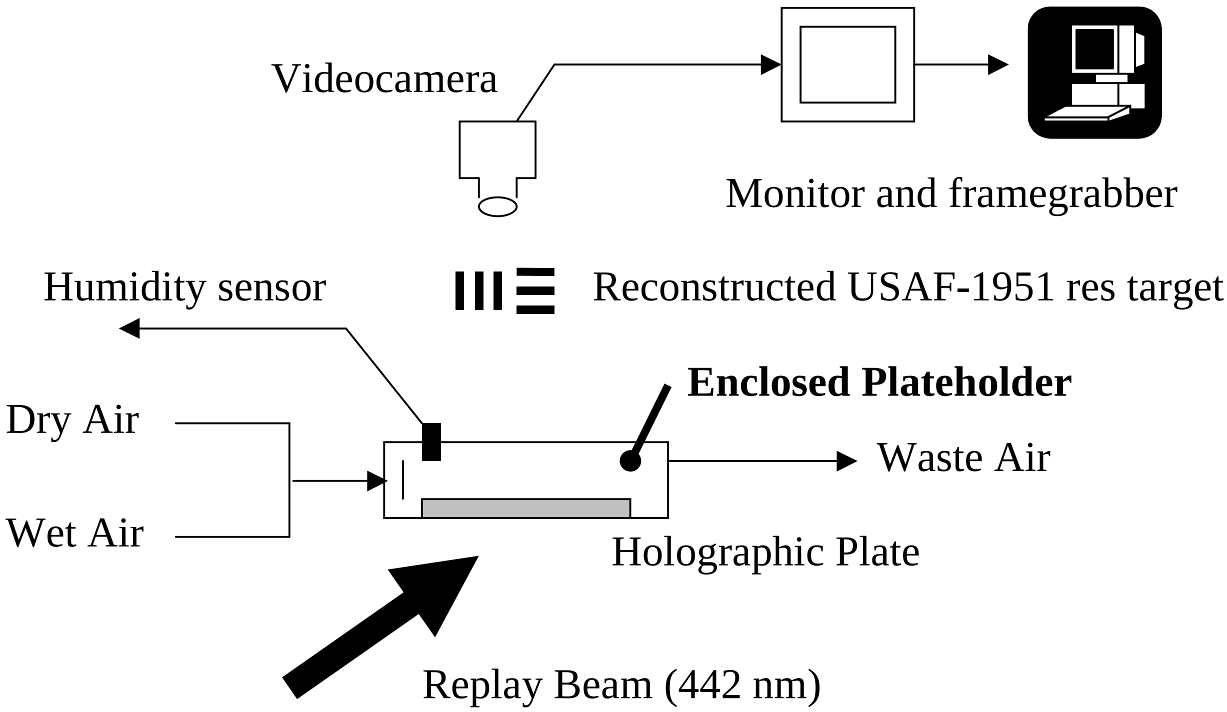


Figure 4: Replay system with a controlled humidity plateholder

For both plates, (1) predicts that aberrations will be minimised at a replay angle of 46°. However, we are interested in maximising the detail that can be studied using our existing video equipment. The effective resolution of of the image recorded by the camera depends on both the intrinsic resolution of the reconstructed image and on its brightness: the most perfectly reproduced image is useless if it is too faint to be seen.

As can be seen from figure 5*a*, at low humidities only mediocre resolution can be obtained from plate 8E/MAA/Fix, and this occurs at a beam angle of 50°. As the humidity is increased, the resolution at higher beam angles remains the same but there is a steady improvement in the best resolution attainable with lower angles of incidence. The reasons for this can be seen in the lower graph: drier emulsions have their peak diffraction efficiency at about 60° beam angles (albeit with excessive astigmatism) while not producing any discernible image when illuminated at 46°. As the humidity increases the emulsion swells, the Bragg selectivity improves and the Bragg angle falls towards the expected optimum replay angle. The improving visibility of the target increases the discernible resolution. When the humidity has reached 90% the brightest replay occurs at the same beam angle as the least aberrated replay, and the camera can therefore pick out the finer detail and thus make use of the intrinsically higher resolution image so that it is possible to see details twice as fine as could be made out with the driest emulsion.

We present two datasets for 49% RH: these represent data obtained at the same ambient humidity but separated by several swelling/drying cycles. It is almost impossible to distinguish these two curves on the lower figure 5, which confirms both the repeatability of the apparatus and that the process does not cause lasting changes to the emulsion. Furthermore, several runs were made with the emulsion being dried (labelled *d*), and it may be noted that the two 49% RH rising and the 43% RH falling curves (and also the 64% RH rising and the 54% RH falling curves) are again almost identical, indicating that the emulsion was swollen to a similar degree at about an 8% RH difference between the wetting and drying cycles, which is consistent with the amount of hysteresis reported by Sheppard *et al.*[10].

The 93% RH data is denoted with an asterisk, as it is not clear that the emulsion had actually reached equilibrium when these readings were taken.

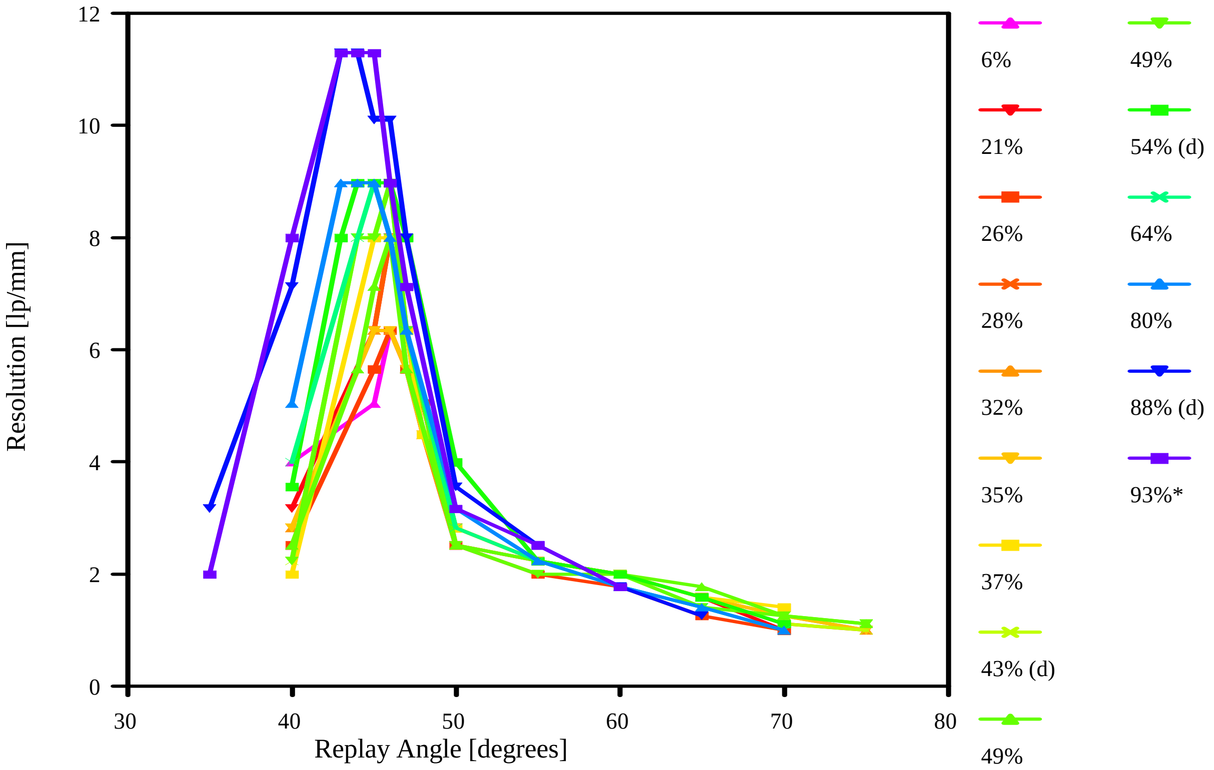
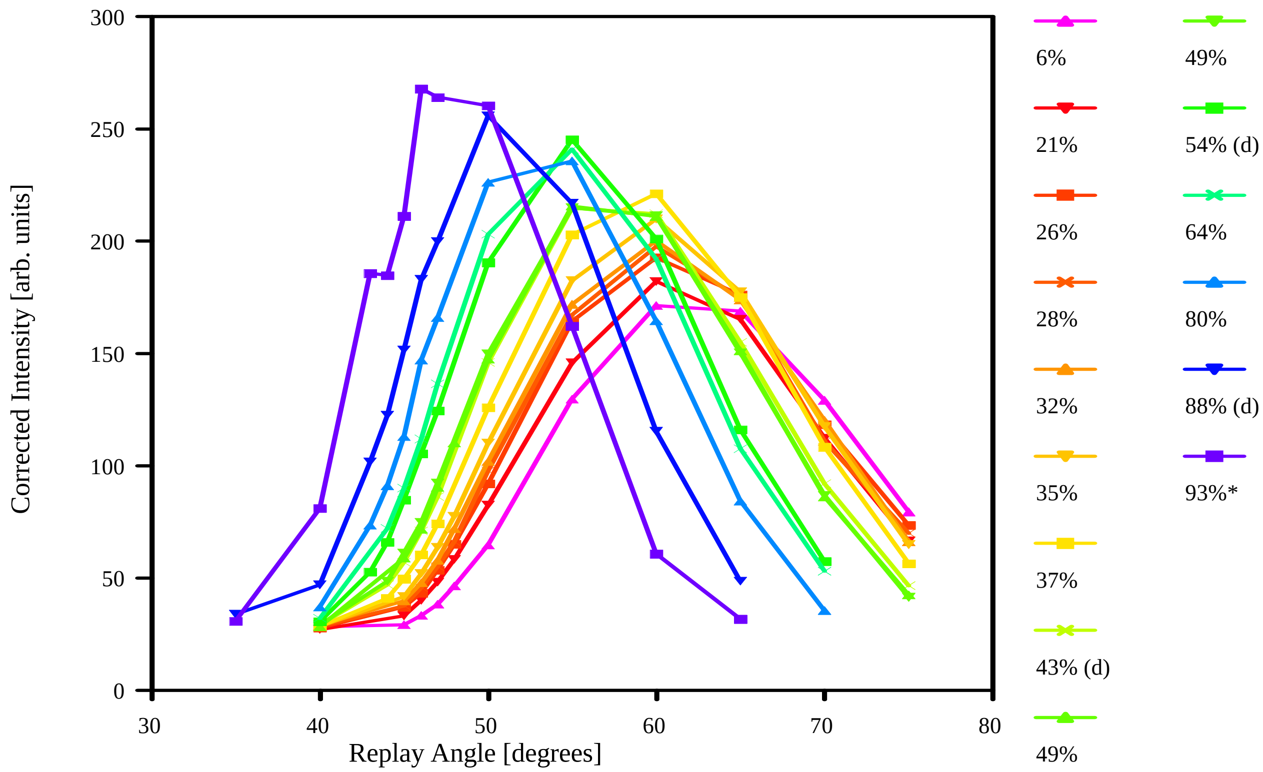


Figure 5: Variation of reconstructed image resolution (*above*) and image brightness (*below*) with replay beam angle, for plate 8E/MAA/Fix at a series of ambient humidities



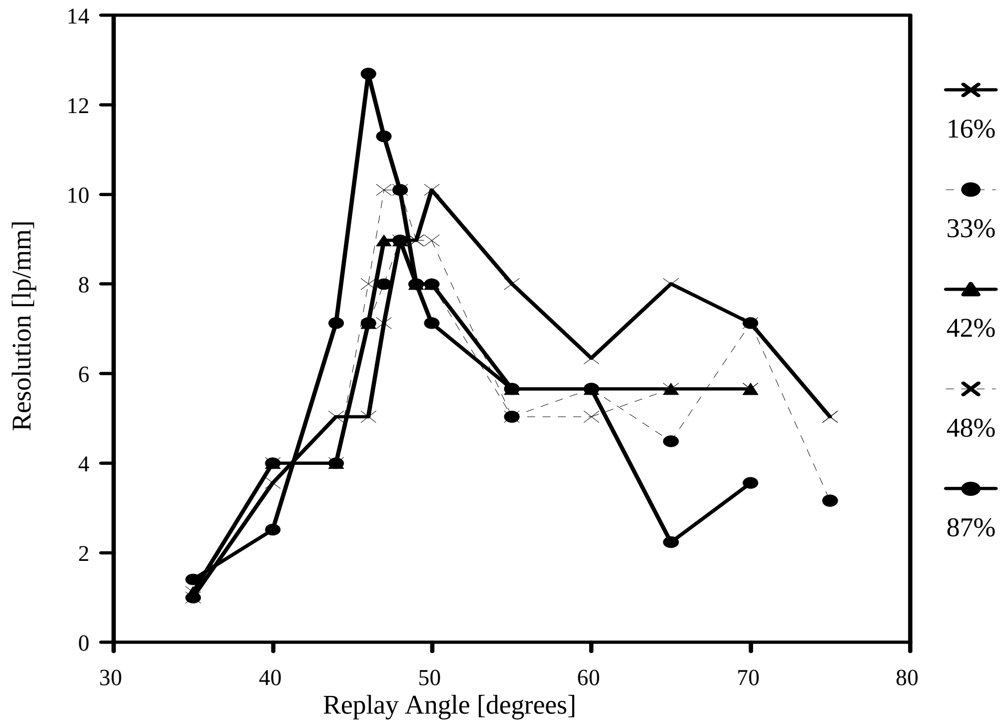
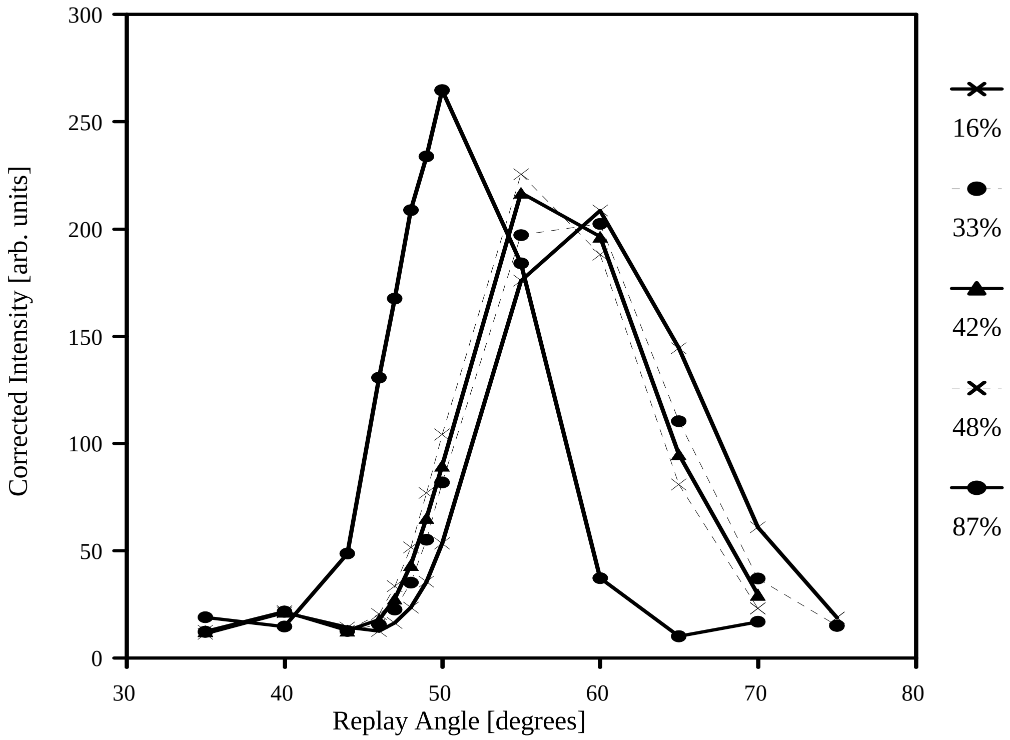


Figure 6: Variation of reconstructed image resolution (*above*) and image brightness (*below*) with replay beam angle, for plate GAC1 at a series of ambient humidities



At this stage there are still some aberrations remaining, mainly due to the wavelength change without any refractive index change. It can be seen from figure 6 that for an object recorded in water the shorter wavelength replay tends to correct the chromatic aberration over all beam angles. Otherwise, the data from plate GAC1 show the same trends as before: as the emulsion swells the higher resolution images become more easily visible. The resolution values show more scatter, possibly due to distortion of the plate by the stresses of the swelling emulsion.

5. DYNAMIC RESPONSE

In order to gain an idea of the speed with which the emulsion can react to changes in environment, the humidity inside the plate enclosure and image brightness were measured while the plateholder was suddenly flushed with dry air. As can be seen from figure 7, the emulsion undergoes significant changes in much less than a minute. In practice the emulsion’s response is limited by the rate at which moisture can be removed from the plateholder, though if this is too rapid further complications may arise in the form of evaporative cooling.

CONCLUSIONS

It has been shown that altering the ambient humidity provides a viable technique for controlling the emulsion thickness of holograms on silver-halide materials. This allows simultaneous optimisation of replay for both minimum aberrations and maximum diffraction efficiency and thus substantially improves the quality of extracted images. The measured responses of the holographic emulsion match the observations of previous workers on both holograms and bulk gelatin, as well as a simple model.

During a significant number of severe swelling/shrinking cycles, no damage or degradation of the emulsion has been observed.

The Flory-Huggins equation has been introduced as a primitive tool for modelling emulsion/humidity interactions using only a single parameter obtained from experimental measurements. It may be possible to improve this model by re-deriving it incorporating the correct (polar solvent and polymer) thermodynamics.

Finally, the *HoloScan* facility is very flexible: although plankton are the immediate subject of this work, the replay system is expected to be equally effective in the automated analysis of other particle fields and underwater applications that require the intrinsic high resolution of holography.

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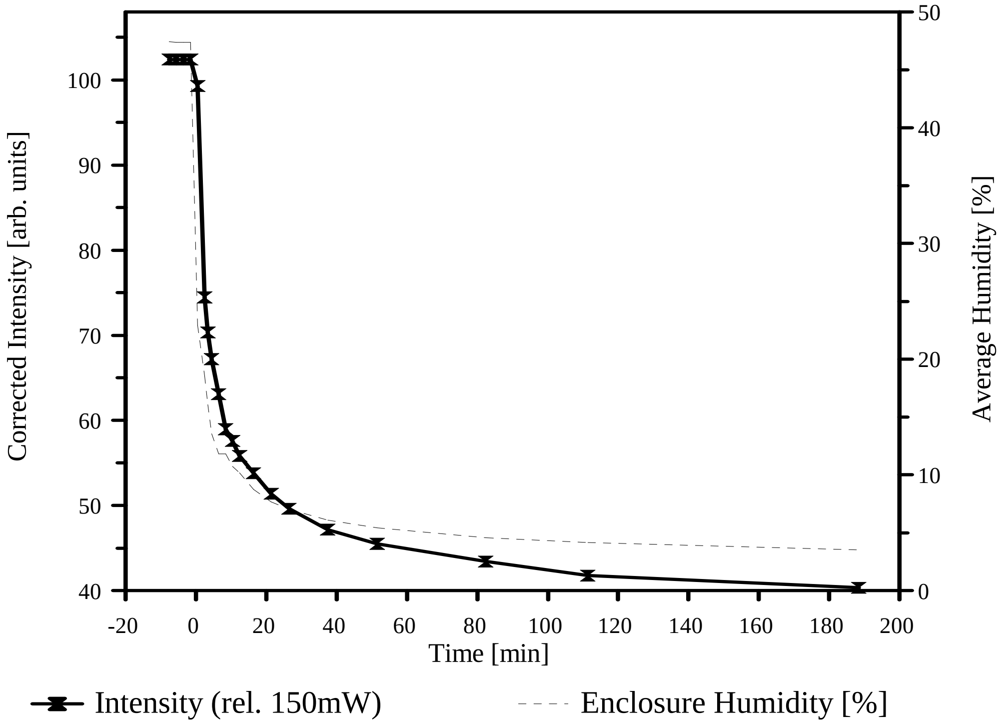
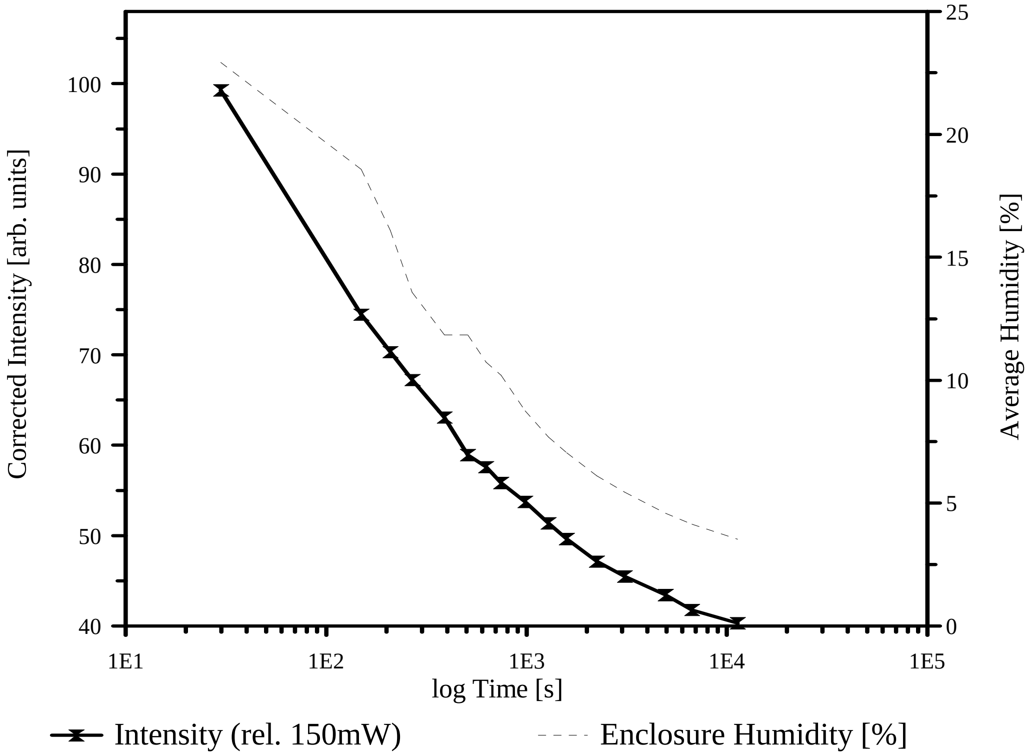


Figure 7: Variation of image intensity (corrected to 150mW of laser power) and ambient humidity inside plateholder enclosure, when subjected to a flow of dry air



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