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Measurements Validating the Confocal Scanning Laser Holography Microscope

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The unique Confocal Scanning Laser Holography (CSLH) microscope is designed to non-intrusively measure the three-dimensional (3D) temperature of a specimen given restricted scanning to a single viewpoint window [1]. The CSLH microscope is principally designed for studying surface tension of a fluid and Marangoni convection in a micro-gravity space environment. The microscope has other applications such as phase-imaging optically transparent specimens with varying composition. A heated fluid specimen was used in experiments to characterize the CSLH microscope and validate the design. The proof-of-concept CSLH microscope uniquely combines holography with a scanning confocal microscope [2]. A focused laser beam is used to probe the specimen instead of a collimated beam to obtain different phase-shift information at each scanning position. A uniquely derived reconstruction algorithm for the microscope reconstructs the 3D refractive index from the scanned holograms and boundary conditions. The probe beam in the specimen is scanned in *xyz*-axis translation with no rotational scanning due to restricted viewing from a single viewpoint. The scan angle is limited to the cone angle of the focused probe beam within the specimen. Typical tomographic 3D reconstruction requires large angle rotational scanning. The CSLH microscope does not allow rotational scanning since vibrations from a rotational mechanism will adversely affect sub-micron fringe measurements in a hologram. This limited viewing angle condition produces a singular reconstruction matrix which can be converted to an invertible matrix with the addition of boundary conditions. The boundary condition temperatures are along the side walls of the fluid-cell. The reconstructed index-of-refraction index is then converted to temperature based on the optical properties of the specimen. The microscope is sensitive to a minute change in refractive index of $7x10^{-6}$. Since the reconstruction algorithm error is negligible the remaining error sources are: 1) phase-shift or fringe translation measurement in a hologram, 2) boundary condition measurement, 3) alignment and positional error, 4) optical aberrations affecting a hologram, and 5) vibrations.

The CSLH microscope optical layout in Fig 1 shows the side-by-side propagation of the object beam that passes through the specimen and the reference beam that bypasses the specimen. The specimen is placed within the focal point of the telecentric lens in the optical loop. The telecentric lens is mounted to a *z*-axis translation stage. The Beam Steering Mirrors (BSMs) move the focal point in the *xy*-axis within the specimen. The beams exit the optical loop and reverse propagate back through the BSM section. The beams are then directed to the confocal optics and the projector lens for overlap onto the camera. The confocal optics consist of stationary pinhole apertures that place a virtual aperture over the focal point in the specimen for any scan position. The focal point at the confocal optics is optically conjugated to the focal point in the specimen. An aerial photograph of the CSLH microscope on a vibration isolated optical table is shown in Fig 2. The lasers are placed in the bottom left, the specimen is placed in the top right, and the camera placed in the bottom right corners of Fig 2. The microscope is configured to measure the phase-shift of weak phase objects where the phase-shift is less than 2π radians between scan positions. The measurement of strong phase objects would require an additional laser to determine phase-shifts that exceed one fringe spacing between scan positions. The 5x5x45mm fluid-cell specimen is shown in Fig 3 with a heater at the bottom to produce thermal gradients. The fluid-cell is mounted to the optics table to minimize disturbance vibrations to the fluid. The reference fluid is matched to BK7 glass with refractive

index to wavelength and temperature specifications. Measurements using the non-intrusive laser probe are compared to a thermocouple as a part of the validation. The needle probe thermocouple is considered invasive as it presents a thermal mass heat sink and obstructs convective fluid flow. The temperature gradients are sufficiently small so that thermal diffusion and conduction dominates convection. Convection is reduced by using a high viscosity fluid. The temperature range for the experiments is 7°C which minimizes convection and provides less than 2π radians phase-shift between the 625μm scan position steps. The measured temperature using the CSLH microscope is shown in Fig 4 as a response to a radially distributed heat source. The temperature profile at a 2.3mm elevation horizontal plane above the heater produces a Gaussian shaped refractive index profile. The CSLH microscope is sensitive to a ±0.02°C average change in temperature change within the fluid. There are 32 reconstructed temperature values along the horizontal plane at 1°C RMS error given 16 boundary condition temperatures and 32 measured phase-shifts from 16 holograms. The CSLH microscope has recently been characterized for operational limits, sensitivity, and optical resolution and considered for applications.

[1] P. Jacquemin, R. McLeod, S. Lai, D. Laurin, R.A. Herring, *Acta Astronautica,* 60 (2007) 723.
[2] R.A. Herring, *Optik* 105 (1997) 65.

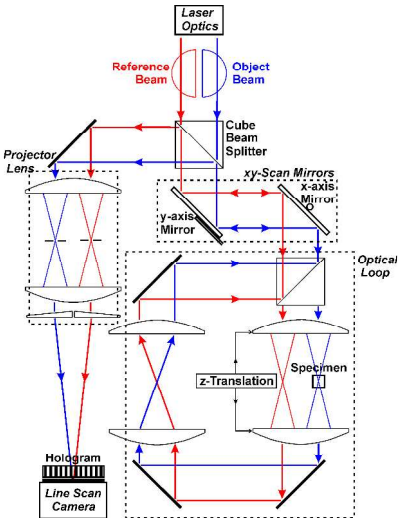

Figure 1. CSLH Microscope Optical Layout

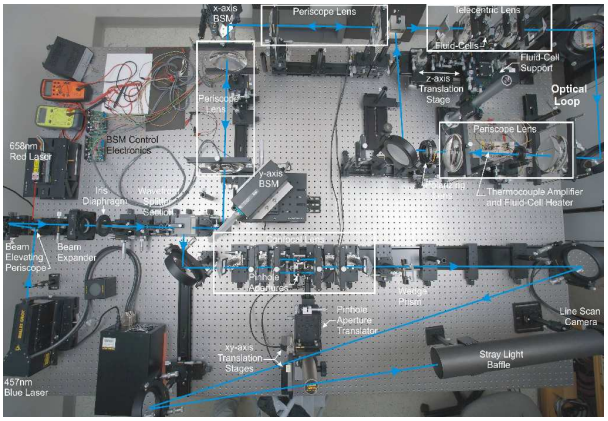

Figure 2. CSLH Microscope Components

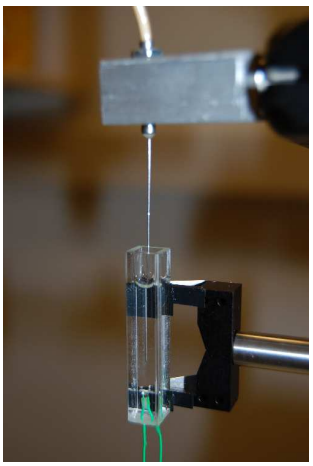

Figure 3. Fluid-Cell with Heater and Thermocouple

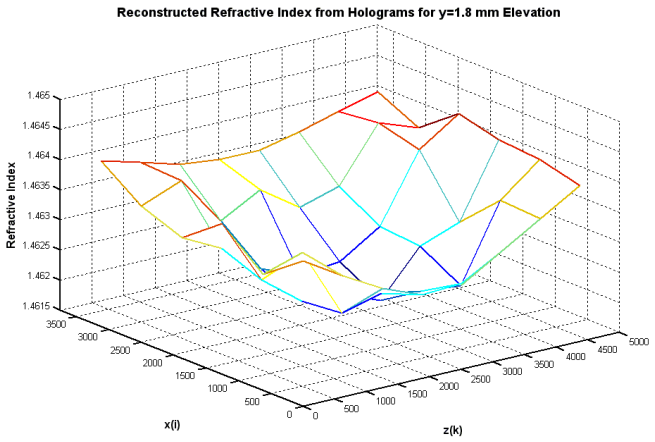

Figure 4. CSLH Microscope Reconstructed Temperature

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