Long working distance DHM for space application

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Microscopy is a powerful tool for Space S & T.
Microscopy usually refers to:

- **Optical microscopy**
  - Only obtain 2D information of the object
  - Resolution and image quality are restricted by object lens

- **Scanning microscopic techniques**
  - Obtain 3D information of the object
  - Complex structure, trivial procedures and rigid working environment

AFM  
LSCM  
STM
Digital holographic microscopy (DHM), which is based on digital holography, aims at investigating small samples, such as life cells, MEMS, micro-optics, etc.

DHM has numerous advantages:

- It is a noninvasive, noncontact and in-situ measurement technique.
- It allows for 3D quantitative analysis.
- Since no scanning is involved, it may operate in real time.
- It is marker-free and no pretreatment is required.
DHM could be applied to:

- 3D imaging & profile analysis
- Deformation & vibration measurement
- Particle field observation
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Introduction to Digital Holography

Holography is a imaging technique that can recover the whole information of the object under inspection from the holographic interferogram.
Digital holography (DH) is based on the optical holography, which is a novel imaging technique that can quantitatively recover both the intensity and the phase information of the object under inspection with the simulated reference wave from the single the hologram recorded digitally by CCD and transferred to a computer.

Introduction to Digital Holography
Introduction to Digital Holography

Mathematical explanation of holography:

Recording: \[ H = |R + O|^2 = (R + O)(R^* + O^*) \]
\[ = R^2 + O^2 + 2|RO|\cos(\Delta \phi) \]
\[ = R^2 + O^2 + OR^* + RO^* \]

- zero-order term
- conjugate terms

\( H \) denotes the intensity of hologram.

\( R \) and \( O \) denote the complex amplitude of reference and object wave, respectively.

Reconstruction: \[ RH = R(R^2 + O^2) + O|R|^2 + R^2O^* \]

If \( |R|^2 = 1 \),

\[ O \]
Once $O$ in hologram plane is obtained, it could be propagated to image plane by simulating a diffraction procedure:

- **Fresnel algorithm: 2D FFT**

\[
b'(n\Delta x', m\Delta y') = e^{i\pi d\lambda} \left( \frac{n^2}{N^2\Delta \xi^2} + \frac{m^2}{M^2\Delta \eta^2} \right) FFT \left[ h(k\Delta \xi, l\Delta \eta) r^*(k\Delta \xi, l\Delta \eta) e^{i\pi (k^2\Delta \xi^2 + l^2\Delta \eta^2)} \right]
\]

- **Convolution algorithm: 2D FFT & IFFT**

\[
b'(n\Delta x', m\Delta y') = FFT^{-1} \left\{ FFT \{ h(k\Delta \xi, l\Delta \eta) r^*(k\Delta \xi, l\Delta \eta) \} \cdot FFT \{ g(k\Delta \xi, l\Delta \eta) \} \right\}
\]

- **Angular spectrum algorithm: 2D FFT & IFFT**

\[
b'(n\Delta x', m\Delta y') = FFT^{-1} \left\{ FFT \{ h(k\Delta \xi, l\Delta \eta) r^*(k\Delta \xi, l\Delta \eta) \} \cdot e^{i2\pi d\lambda} \left[ 1 - \left( \frac{k\Delta \xi}{d} \right)^2 - \left( \frac{l\Delta \eta}{d} \right)^2 \right] \right\}
\]

Then the amplitude and phase image could be calculated.

**Amplitude:** $I(n\Delta x', m\Delta y') = |b'(n\Delta x', m\Delta y')|^2$

**Phase:** $\phi(n\Delta x', m\Delta y') = \arctan \left( \frac{\text{Im} \{ b'(n\Delta x', m\Delta y') \}}{\text{Re} \{ b'(n\Delta x', m\Delta y') \}} \right)$
Nowadays, there are two methods to increase the lateral resolution in DHM:

◆ Pre-magnification approach:

A Microscopic Objective (MO) is used to obtain a magnified image of the sample. The resolution depends on the numerical aperture (NA) of MO.

Only one hologram is needed to retrieve both amplitude and phase image.

The aberrations induced by MO must be compensated.

◆ Synthetic aperture approach:

A series of holograms are captured in different recording conditions. Then these holograms are synthesized to obtain resolution enhanced image.

Since no lenses are involved, the working distance of the system isn’t constrained to can be much longer, and the reconstructed images doesn’t subject to lens aberration.

The record and synthesis of several holograms are time consuming.
Long-working-distance Synthetic Aperture DHM

Research Purpose

• An three-dimensional in-situ microscopy imaging with high resolution based on the digital holography at long working distance for the space in-suit microscopy measurement is proposed. Its superiorities are:

  (1) It is a noncontact, in-situ measurement technique, which can obtain the 3D quantitative information of the object under inspection;

  (2) It is provided of the flexible working distance and high-resolution imaging;

  (3) It is in accordance with the basic design requirements for the miniaturization and the integration of the Load space science exploration.

Technical Specifications

• Capabilities of three-dimensional in-suit microscopy imaging at long work distance, which is varied from the 10cm to the 80cm;

• Lateral resolution could achieve to few tens of micrometers, and axial resolution could be increased to more than a dozen micrometers at the longest work distance.
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Long-working-distance Synthetic Aperture DHM

We developed a synthetic aperture method for DHM in our previous work. Different tilted plane wave illuminations were utilized in this approach.

Distance: $Z_0 = 65 \, \text{cm}$

Schematic diagram of our method
Long-working-distance Synthetic Aperture DHM

USAF-1951 Test Target & 10mm × 10mm × 10mm
Reflective complex field in the object plane:

\[ U_i(x, y) = b(x, y) A_i(\gamma_i, \xi_i) \]

Spatial frequency spectrum of the reconstructed image in the image plane:

\[ \tilde{U}_o(f_x, f_y) = A_0 C\tilde{b}(f_x + \gamma_i, f_y + \xi_i) \text{rect} \left( \frac{\lambda df_x}{L} \right) \text{rect} \left( \frac{\lambda df_y}{H} \right) \]

Spatial frequency spectrum of the synthesized image:

\[ \tilde{U}_o^{\text{sum}}(f_x, f_y) = \sum_i A_0 C\tilde{b}(f_x + \gamma_i, f_y + \xi_i) \text{rect} \left( \frac{\lambda df_x}{L} \right) \text{rect} \left( \frac{\lambda df_y}{H} \right) \]

\[ = A_0 C\tilde{b}(f_x, f_y) \text{SA}(f_x, f_y) \]

Synthetic aperture function:

\[ \text{SA}(f_x, f_y) = \sum_i \text{rect} \left[ \frac{\lambda d(f_x + \gamma_i)}{L} \right] \text{rect} \left[ \frac{\lambda d(f_y + \xi_i)}{H} \right] \]
Multiple holograms are recorded with different titled illuminations.

The complex amplitudes are reconstructed and magnified from these holograms by using digital arithmetic.

The synthesized image is obtained by incoherent superposition.

The resolution is enhanced

The speckle noise is suppressed

365 pixels $\times$ 365 pixels
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These results show that the image quality is effectively improved and the speckle noise is well suppressed with proposed method.
These results were obtained with resolution test target USAF-1951. The spatial resolution is effectively enhanced.

Speckle noise reduction in digital holography by use of multiple polarization holograms

We propose a novel speckle reduction technique for digital holography by recording multiple polarization off-axis holograms. The speckle noise is suppressed by averaging the reconstructed intensity fields, and the resolution is improved.

Coherent noise reduction in digital holographic phase contrast microscopy by slightly shifting object

By slightly shifting the specimen, a series of digital holograms with different coherent noise patterns is recorded. Through averaging these images, the coherent noise is reduced and the quality of phase contrast image is improved.

Complex amplitude of specimen in object plane:

\[ U_b(x, y) = b(x, y) \cdot s(x, y) \]

Complex amplitude of object wave in the recording plane:

\[ U_h(\xi, \eta) = \exp \left( i \frac{2\pi}{\lambda} \cdot \frac{\xi^2 + \eta^2}{2d} \right) \cdot F \left[ U_b(x + \Delta x, y + \Delta y) \exp \left( i \frac{2\pi}{\lambda} \cdot \frac{x^2 + y^2}{2d} \right) \right] \]

Complex amplitude of object wave in the imaging plane:

\[ U_i(x', y') = C \cdot F \left\{ U_h(\xi, \eta) \cdot \text{rect} \left( \frac{\xi}{a}, \frac{\eta}{b} \right) \right\} \cdot \exp \left( -i \frac{2\pi}{\lambda} \cdot \frac{\xi^2 + \eta^2}{2d} \right) \]

\[ = C \cdot b(x + \Delta x, y + \Delta y) \cdot s(x + \Delta x, y + \Delta y) \cdot \exp \left( i \frac{2\pi}{\lambda} \cdot \frac{x^2 + y^2}{2d} \right) \cdot \text{sinc} \left( \frac{ax}{\lambda d}, \frac{by}{\lambda d} \right) \]
Feng Pan, Wen Xiao, et al, “Coherent noise reduction in digital holographic phase contrast microscopy by slightly shifting object”, (accepted by Optics Express)
Gravity strongly influences the growth of living cells.

Microgravity environment was generated by a superconducting magnet system.

Mouse bone cells were cultivated at the 0g point, for 6 or 12 hours. Then we could monitor the growing procedure of bone cells after microgravity stimulation by DHM system.

The benefits of DHM system are as follows:

● It is noninvasive and noncontact.
● It works in real time.
● It permits 3D quantitative analysis.
DHM living cell observation system:

Schematic diagram

Photograph of optical setup
Monitoring of Living Cells Growth under Microgravity

Living bone cell observation results (20x MO):

- Phase image
- Unwrapped phase image
- 3D rendering of phase image
Dynamic analysis of the division procedure of mouse bone cell, after 0g, 12 hours cultivation:

Biological Slices Observation Results

Paramecium

Onion
Profile and Defect Examination of Lens Surface

We have applied DHM to examine profile and defect of lens surface. The in-situ measurement of lens surface could be realized with DHM.

Object under testing

Schematic Diagram
Profile and Defect Examination of Lens Surface

Testing results:

Profile:

Defect:

Phase image

3D rendering of phase image

Fanjing Wang, Wen Xiao, Feng Pan, Shuo Liu, Lin Cong, Rui Li, Lu Rong. “Measurement of optic component by digital holography with least square method”. (accepted by Optic laser Engineering )
Micro-deformation Measurement of the Resistance

Micro-deformation Measurement of the Aluminum
Conclusions

- Digital Holographic Microscopy (DHM) is a useful technique allowing for 3D in-situ measurement. It could be adjusted and applied in space.

- With many advantages, DHM is highly suitable for dynamic observation of biological procedures. It is competitive in the morphological analysis of cells and tissues.

- Although the resolution and image quality are restricted by CCD or CMOS, it would not be such a great problem because the performance of electronic devices will be remarkably improved in the future.
谢谢