REAL-TIME 3D SHAPE MEASUREMENT OF MICRO DROPLET USING DIGITAL HOLOGRAPHIC MICROSCOPY

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Droplet based microfluidic device

Microfluidic device

- Biochemical assay
- Cell manipulation
- Drug discovery
- Diagnosis

Device using droplets

Example

- Use of the droplet for the fluid transport
  - Droplet size pL ~ nL (10 ~ 100 μm)
  - Transportation sample or reagent

- Advantages of using droplet
  - Less dead-volume.
  - Short analysis time
  - Improvement of analysis precision

Importance of understanding the droplet generation for the controlled transportation

➢ Droplet shape and its deformation
## Shape measurement methods

### Current shape measurement methods

<table>
<thead>
<tr>
<th>Commercial product</th>
<th>Measurement method</th>
<th>Measurement time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser displacement sensor</td>
<td>Spot scanning ⇒ 3D data reconstruction</td>
<td>A few minutes</td>
</tr>
<tr>
<td>Confocal laser microscope</td>
<td>2D scanning by Nipkow disk &amp; Z scanning by piezo stage</td>
<td>A few seconds ~ A few minutes</td>
</tr>
<tr>
<td>Interferometric microscope</td>
<td>Single Exposure 2D fringe pattern &amp; 3 Fringe scanning for phase calculation</td>
<td>Sub-second</td>
</tr>
</tbody>
</table>

Only for static object / Invalid for dynamical phenomena

Requirement for 3D & real-time (time-series) measurement
Purposes

1. Development of the real-time and 3D shape measurement technique of microscopic droplet

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2. Demonstration of effectiveness of the developed technique for 3D shape microscopic droplet

Our approach

Use of Digital Holographic Microscopy (DHM) because of its micro-second measurement time (depended on camera’s exposure time)
The principle of the DHM

**Optical Microscopy**
- CCD on imaging plane
  - Recording only image intensity

**DHM (Digital Holographic Microscopy)**
- Incident light
- Objective lens
- Object
- BS
- CCD on arbitrary plane
- Laser
- Mirror

1. **Interferometric recording**
   - ⇒ the digital hologram by added reference light

2. **Diffraction calculation**
   - ⇒ intensity & phase reconstruction on arbitrary plane

**DHM = Optical microscopy + Interferometer**
- ⇒ Intensity & Phase
Developed system

① Table-top DHM microscope
- **Real-time measurement (scan free)**
  by the off-axis formation

② Analysis software
- **Real-time observation (over 30fps)**
  Using the GPGPU

W540 x H640 x D240 mm, 15 kg
Real-time measurement

Off-axis formation
Holographic method that the reference wave plane is slightly tilted relative to the objective wave plane.

Feature
1. Need only a single shot hologram for a single measurement.
2. By the effect of the 0-order beam and the conjugate image rejection, object phase is measurable quantitatively.

—we adopt the off-axis formation which enables the real-time & quantitative phase measurement
Demonstration

① Measurement target

Region of interest

Oil flow
3.0 μL/min

400 μm

Channel depth:
Z 93 μm

Water flow
3.0 μL/min

50 μm

T-shaped microchannel for droplet formation made from PDMS, hydrophobic nature

Working fluid:
water(droplet), silicone oil

② Measurement condition

<table>
<thead>
<tr>
<th>Magnification</th>
<th>10 X</th>
</tr>
</thead>
</table>
| Measurement area| XY: 563 × 563 μm  
Z: 986 μm |
| Resolution      | XY:1.1 μm  
Z: 0.062 μm |
| Frame rate      | 60 fps |
| Exposure time   | 1 ms |
| Light source    | HeNe laser 633 nm |
| Illumination    | Transmission |
Analyzing process for the digital hologram

Hologram of the droplet

Off-axis hologram analysis

Complex amplitude distribution on imaging plane

Reconstructed intensity

Reconstructed phase

Fringe pattern

\( \text{a fringe tone} \sim \text{Amplitude} \)

\( \text{a fringe pitch} \sim \text{Phase} \)

◨ Hologram reconstruction of droplet using only a single shot
Hologram images (raw CCD images)
The movie is obtained by our original software.
Extraction process of 3D shape

Reconstructed images

Intensity

Phase

200 μm

Masking

Phase unwrapping

3D shape

Convert phase to height by the use of the refractive index difference of the each fluids
3D visualization

Reconstructed volume (xyz): 176 μm × 176 μm × 100 μm
Visualized sample: Sequential 4 frames at the moment of droplet formation
Time interval: 16.7 ms (60 fps)
We have succeeded in real-time quantitative measurement of 3D shape of a droplet.
Demonstration with a high-speed camera

Channel:
Cross-shaped microchannel for droplet formation made from PDMS, hydrophobic nature

Working fluid:
water(droplet), silicone oil

② Measurement condition

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Magnification</td>
<td>20 X</td>
</tr>
<tr>
<td>Measurement area</td>
<td>XY:410×410 μm Z: 1078 μm</td>
</tr>
<tr>
<td>Resolution</td>
<td>XY:0.8 μm Z: 0.062 μm</td>
</tr>
<tr>
<td>Frame rate</td>
<td>1000 fps</td>
</tr>
<tr>
<td>Exposure time</td>
<td>0.05 ms</td>
</tr>
<tr>
<td>Light source</td>
<td>HeNe laser 633 nm</td>
</tr>
<tr>
<td>Illumination</td>
<td>Transmission</td>
</tr>
</tbody>
</table>
We have also successfully measured the dynamic change of droplet shape in 3D and in real time at 1 ms time intervals.
Conclusions

1. We have developed the real-time measurement technique of 3D shape microscopic droplet using the DHM

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2. We have demonstrated the effectiveness of the DHM for 3D dynamical microscopic phenomena

【Future tasks】
1. Applications to various microscopic objects
   ⇒ bubble, red blood cell, etc...
2. Product commercialization
Please visit our booth exhibition!  Booth No. 15-16

Photo of booth exhibition

Droplet shape measurement

DHM-PTV
(3D flow measurement)

Tracer particles: φ2 μm
Sequential 20 frames