Dynamic volumetry of flowing cells with doubled field of view by six-pack holography

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Introduction

- Volumetric data of cells can be reconstructed from 3 hologram images acquired at different wavelengths.
  - This technique is limited by camera field-of-view (FOV) and the need for multiple sequential holograms.
- Six-pack holography (6PH) captures 6 unique off-axis holograms simultaneously, without sacrificing FOV or resolution.
- Holographic modules may be placed at the camera port of commercial microscopes to acquire holograms.

Objectives

- Design and implement a holographic module for three-wavelength and two FOV six-pack holography.
- Utilize the module to reconstruct volumetric data of red blood cells across the doubled FOV.

Methods

Six-pack holographic module

- TWL illuminated the sample with a single beam of 692, 532, and 490 nm wavelengths.
- BS1 creates the initial sample and reference beams.

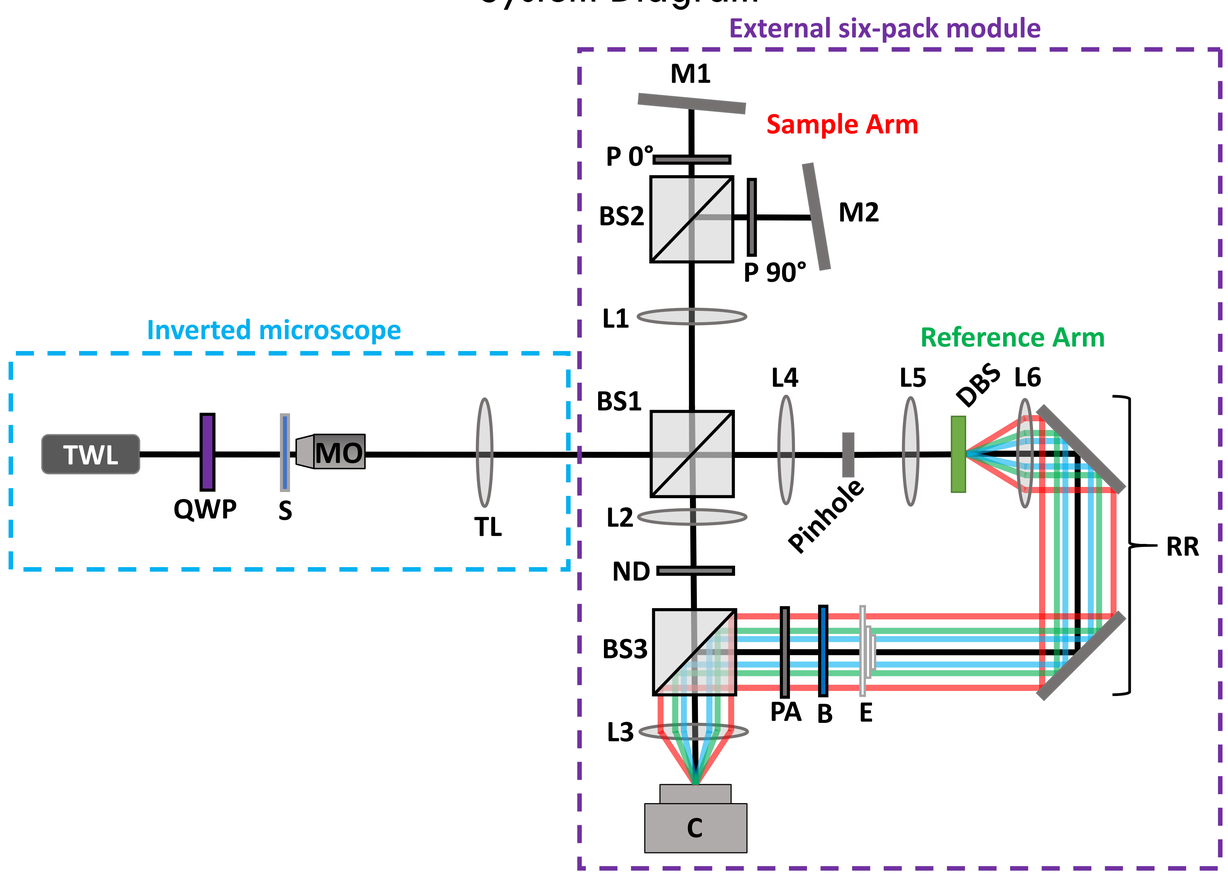
Sample arm

- M1 and M2 are tilted to overlay two FOVs, each with 3 wavelengths, on the camera.
- Polarizers P 0° and P 90° prevent interference between the two sample beams.

Reference arm

- L4 and pinhole clear sample information and create clean reference beam.
- DBS generates the 6 reference beams, 2 of each wavelength, needed for 6PH
- E matches phases of all beams, B blocks unwanted beams, PA polarizes half of the beams to match P 0° and half to match P 90°.

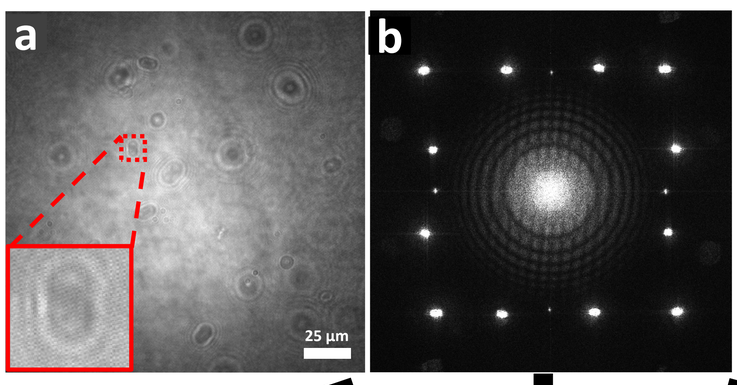
System Diagram

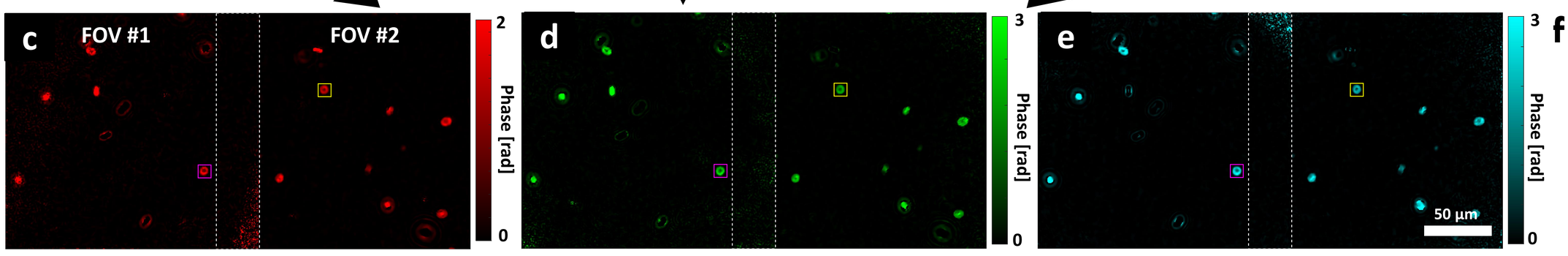


TWL, three-wavelength coherent light source; QWP, quarter waveplate; S, sample; MO, microscope objective lens; TL, tube lens; BS1 – BS3, beam splitters; L1 – L6, lenses; P 0°, polarizer with axis at 0°; P 90°, polarizer with axis at 90° relative to P 0°; M1 – M2, mirrors; ND, neutral density filter; DBS, diffractive beam splitter; RR, retroreflector; B, beam stop; E, echelon; PA, polarizing array; C, camera. Black line indicates the optical axis, colored lines between DBS and C illustrate the reference beam paths per wavelength.

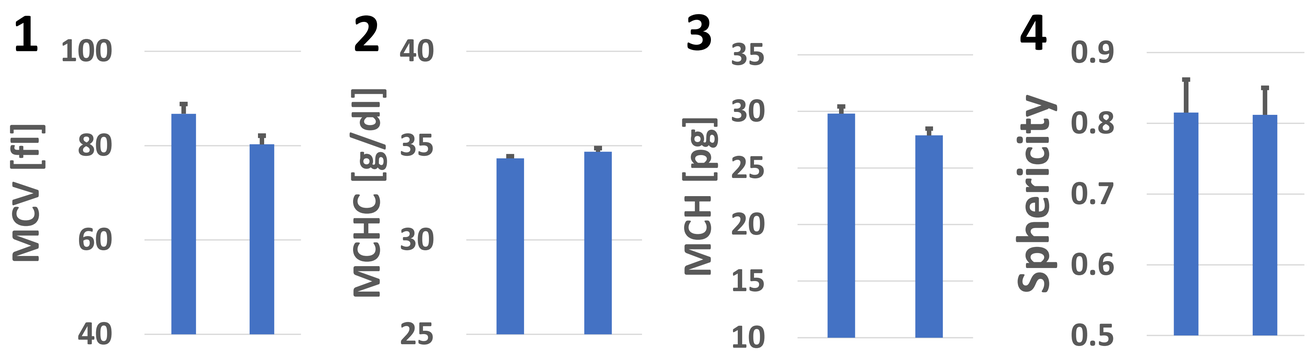
Results

- One six-pack hologram (a) contains six holograms in the Fourier domain (b), phase images shown in (c) – (f).





- Volumetric data extracted, Figs. (1) – (4): mean corpuscular volume (MCV), mean corpuscular hemoglobin (Hb) concentration (MCHC), mean corpuscular Hb (MCH), and sphericity.



Conclusions

- First ever 6PH module and multi-wavelength 6PH achieved.
- The system enables potentially 2× faster acquisition of dynamic volumetric data compared to prior systems.