High Speed Quantitative 3D Blood Flow Imaging by Multi illumination Holographic Microscopy

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Digital and Hardware Solutions, Environmental and Organic Life Modeling

High-Speed Quantitative 3D Blood Flow Imaging by Multi illumination Holographic Microscopy

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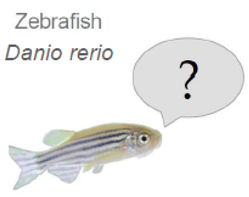
Abstract:

A holographic microscopy method using two illumination beams with a 0.5 aperture objective is proposed to image blood microcirculation in zebrafish larvae. Recent achievements in 3D imaging of blood flow are presented.

OCIS codes: (090.1995) Digital holography; (170.0180) Microscopy; (170.1470) Blood or tissue constituent monitoring; (290.5850) Scattering, particles.

Background

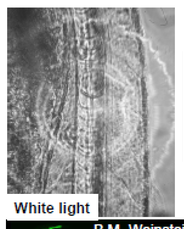


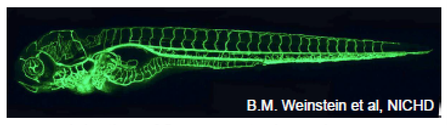
Model organism:
Developmental biology, genetics, neuroscience, biomedicine and behavioral studies.

Blood flow monitoring:
- Assess angiogenesis, tumor vascularization
- Early detection of cardiovascular diseases, age-related macular degeneration

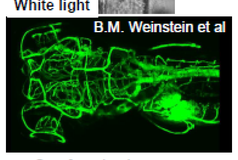
Zebrafish used for:
- A model in drug screening
- Effect of the active substances on the cardiovascular functions
- Zebrafish heart as a model for human heart

Conventional imaging techniques





Injection of fluorescent microspheres



Confocal microscopy

DIC + GFP fluorescence

The techniques commonly used are invasive

Goals

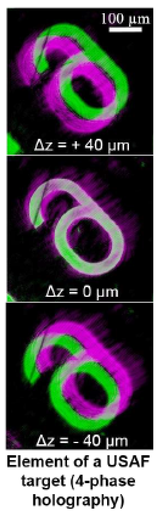
Expected characteristics of the measurement technique

Fast 3D Imaging
- Position of RBCs in volume
- 3D information in one camera frame

Non-invasive operation
- No contrast agents (label-free)
- No genetic engineering

Multimodality, information extraction:
- Integration with conventional microscopes
- Amplitude / phase information
- Quantitative measurement of blood flow

Dual-illumination Holographic Microscopy Setup

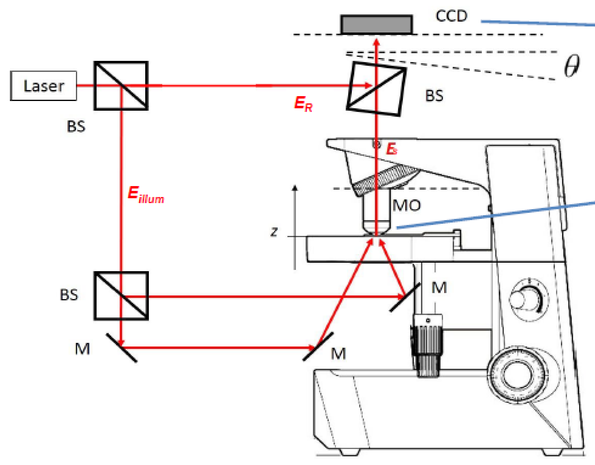


Element of a USAF target (4-phase holography)

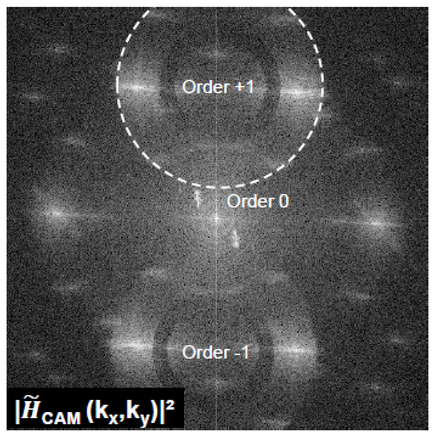
The setup is an upright transmission microscope adapted for digital holography in off-axis configuration. Typical camera frequencies are 100/200 Hz.

A double illumination of the sample is performed to better localize the position of the red blood cells (RBCs) in z.

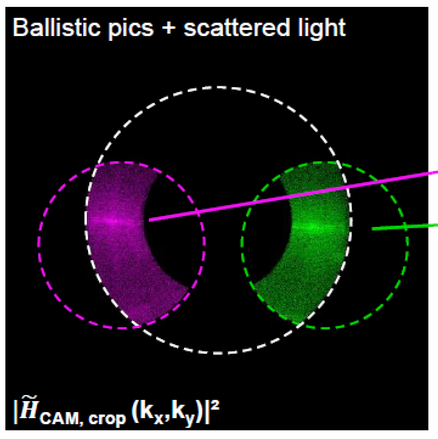
Benefit of double illumination illustrated with an element of a USAF target. Reconstruction in different planes.



2D Holographic Reconstruction of Zebrafish Blood Flow



Fourier Transform of the hologram obtained with a 20x/0.5 microscope objective with a phase ring. The double illumination is visible inside the pupil of the MO.

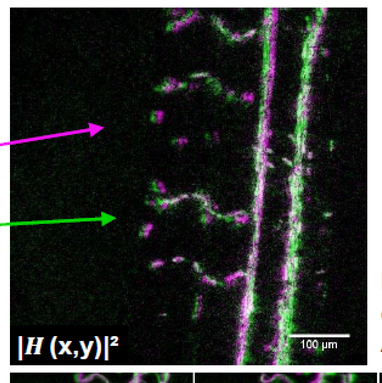


+1 order cropped in the Fourier space. The two directions of illumination are numerically selected and represented in colors.

TF $^{-1}$

TF $^{-1}$

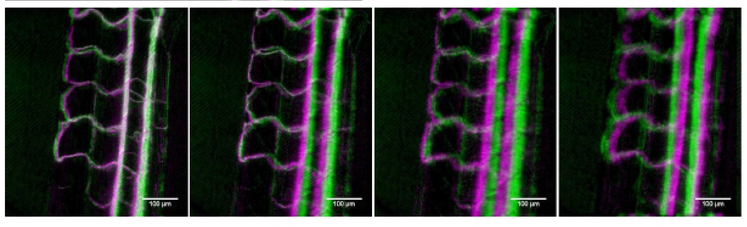
TF : Fourier Transform



Reconstruction of the field in the plane of the sample.
To visualize only the moving red blood cells (RBCs) a difference of images is realized.

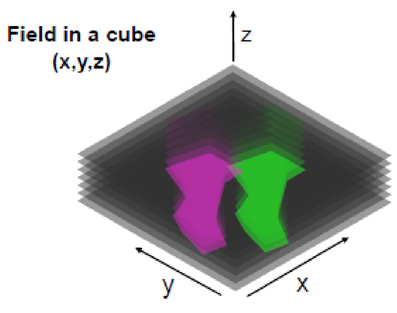
$$H(x,y) = \sum_{k=0}^{N-1} I_k(x,y) e^{i\frac{2\pi k}{N}}$$

Reconstructions in different planes on either sides of the sample.
Averaged over 128 frames

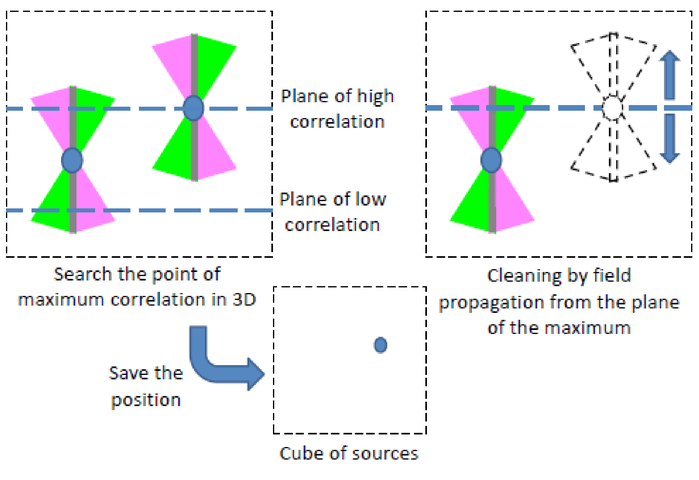


z=0 µm | z=+26.75 µm | z=+48.15 µm | z=-48.15 µm

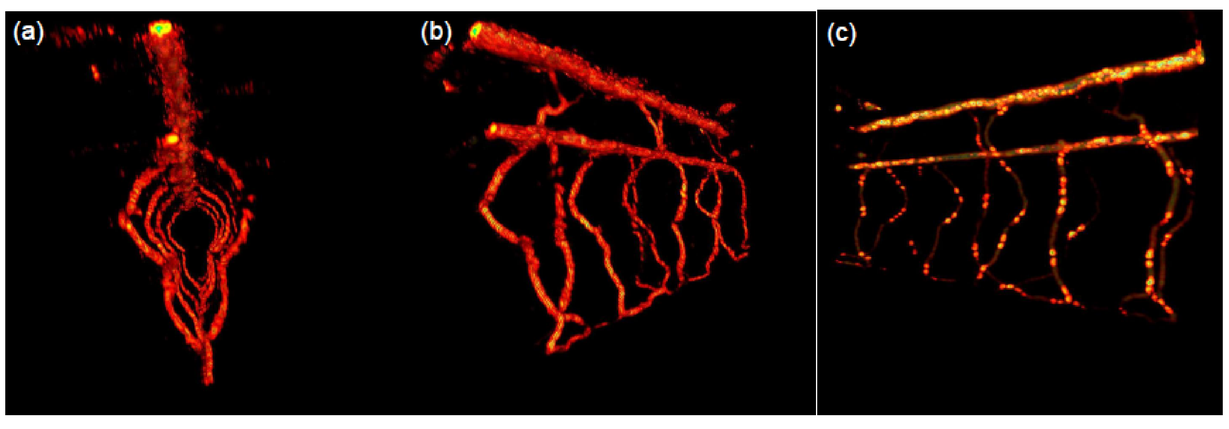
3D Reconstruction with cleaning algorithm



3D cube of data containing the information on the light scattered around the sample.
A cleaning algorithm extracts the positions of the RBCs.



The positions of the RBCs correspond to the points of maximum correlation between the two illuminations.



Reconstructed 3D images of the vascular system of a 5-days zebrafish. (a, b) Averaged in time positions of the RBCs give the shape of the vessels. (c) Positions of the RBCs in one camera frame.

We acknowledge Labex Numev (convention ANR-10-LABX-20) for funding.