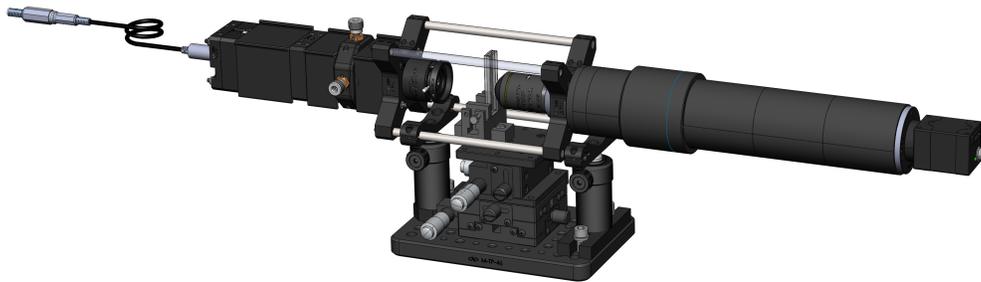


Comprehensive Solution to Imaging and Tracking Particles* in 3D based on Digital Holographic Microscopy (DHM)

MetroDHM - HARDWARE

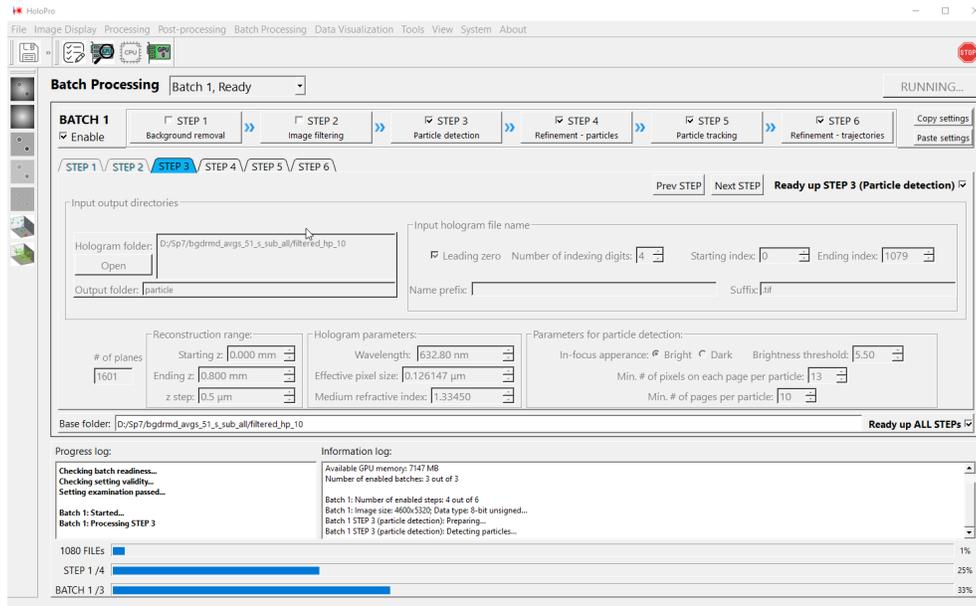
A turnkey inline DHM instrument for microscopic, volumetric, label-free, and 3D holographic imaging



www.metrolaserinc.com/products/metrodhm

HoloPro - SOFTWARE

A hologram processing and analyzing software equipped with GPU acceleration and intuitive GUI for particle detection and tracking

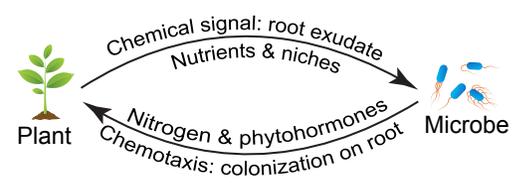


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*biological and non-biological particles

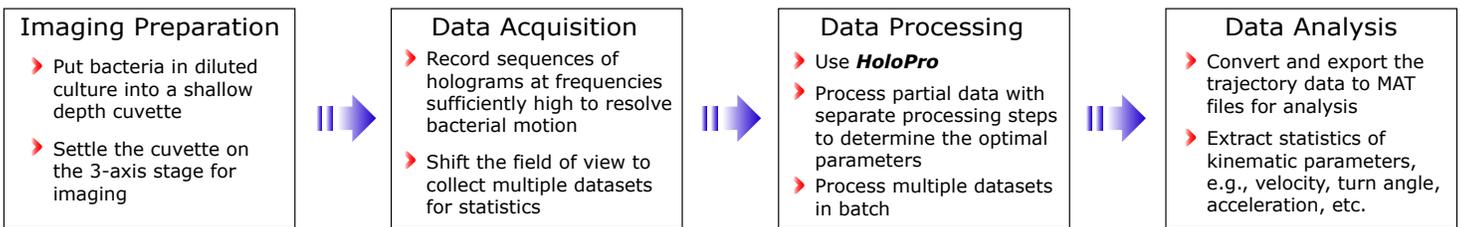
Background

The plant health and crop production are dependent on plant-microbe interactions in the rhizosphere, which is the region around the roots of plants, hosting diverse and dynamic biochemical processes at broad, spatiotemporal scales. Plants send signals through root exudates and bacteria respond via chemotaxis, i.e., the movement of a motile microorganism in a direction corresponding to chemical gradients. Thus, bacterial motility as well as its variability in response to different perturbations (e.g., root exudate) can be used as an indicator to investigate plant-microbe and microbe-microbe interactions.



Application of DHM to Bacterial Motility Characterization

MetroLaser's DHM system is used for volumetric, high-throughput, 3D characterization of bacterial motility. In one realization of the motility measurement, sequential holograms of swimming bacteria in the diluted culture are acquired using MetroDHM, after which HoloPro was used to process the hologram sequence to retrieve the 3D trajectories of the bacteria in the sample volume. Due to the 3D nature of bacterial motion, DHM is intrinsically superior to 2D techniques based on conventional microscopic imaging. The experimentation, data processing, and analysis are performed following the procedures below.



Representative Results

The motility characteristics of the wildtype (Sp7) and a non-chemotaxis mutant strain (CheA4) of *A. brasilense* are revealed using our DHM system. Figure 1 shows 1885 trajectories color-coded with time, determined from a 90-second hologram sequence acquired at 12 Hz. Figure 2 shows an intuitive comparison between the typical swimming patterns of the Sp7 and CheA4 strains, with the former featuring the commonly observed run-reverse motility pattern (manifested by the sequential decelerations correlated with large-angle turns in the temporal profiles) and the latter exhibiting few decelerations/turns.

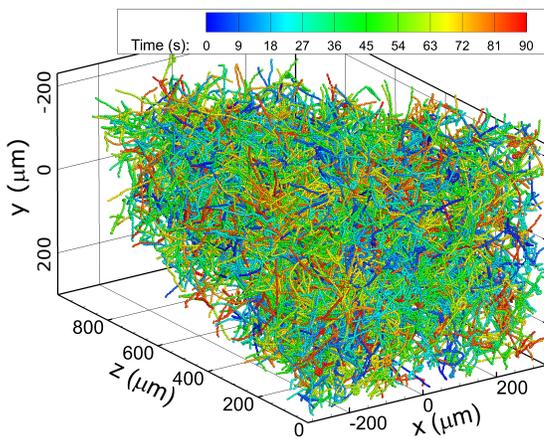


Figure 1. 1885 bacterial trajectories of duration greater than 5 seconds, determined from a sequence of 1080 holograms acquired at 12 fps.

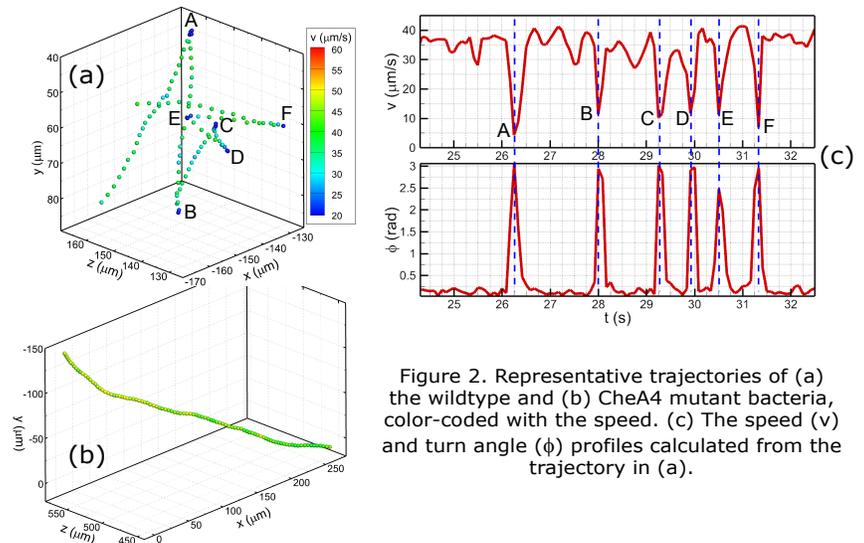


Figure 2. Representative trajectories of (a) the wildtype and (b) CheA4 mutant bacteria, color-coded with the speed. (c) The speed (v) and turn angle (ϕ) profiles calculated from the trajectory in (a).

Reference

Gao, J., Carnahan, J., Alexandre, G., and Trolinger, J., "Digital holographic microscopy for volumetric, three-dimensional characterization of bacterial motility", Proc. SPIE. 12223, Interferometry XXI, 122230E (2022).

Features of ***MetroDHM*** instrument:

- ||➔ Label-free
 - No chemical labeling required
 - Imaging of weak scatters
- ||➔ 3D
 - Resolves 3D motion, which is not accessible using conventional microscopy
- ||➔ Volumetric
 - Eliminates influences of 2D confinement on biological samples and provides sustained observation of samples through the depth
- ||➔ High-throughput
 - Ideal for determining robust statistics
- ||➔ Easy-to-operate
 - Intuitive hardware, software, and the corresponding operating procedures
- ||➔ Compact
 - Small footprint with a total length about half a meter
- ||➔ Affordable
 - A microscopic imaging instrument capable of 4D characterization does not have to be expensive.
- ||➔ Customizable
 - Changeable: field-of-view size, imaging speed, magnification, compatible sample container, imaging wavelength, particle type
 - Additional capabilities: phase retrieval, quantitative phase imaging

Features of *HoloPro* software:

- II ➔ Focused Application but Customizable**
 - Developed and optimized for particle detection and tracking applications
 - Applicable to biological (e.g., bacteria) and non-biological (e.g., bubbles) particles
 - Customizable based on the specialty of customer's application
- II ➔ Comprehensive**
 - Complete processing chain that starts from raw hologram sequences and ends with 3D trajectories
 - Includes: hologram preprocessing, particle detection, particle tracking, refinement
- II ➔ Batch Processing**
 - Automated workflow designed to facilitate processing of multiple datasets
- II ➔ GPU-acceleration***
 - C++ and CUDA based
 - A must-have for processing large datasets
- II ➔ Ease-to-use**
 - Enabled by the intuitive GUI, optimized workflows, and gadgets for setting up and validating the processing
- II ➔ Visualization and Exportation**
 - Interactive data visualization
 - Exports results with formats directly readable to MATLAB, Tecplot, and Excel
- II ➔ Free Lifetime Support**
 - Free upgrades within a major release
 - Upgrades available to incorporate customized functionalities

*Benchmark of GPU-accelerated Processing

Conditions:

GPU: Nvidia Quadro RTX 4000

(2304 CUDA cores, boost clock 1.55 GHz)

Number of holograms in the sequence: **1080**

Included processing: **ALL** (hologram preprocessing, particle detection, tracking)

Size of reconstructed volume (voxel)	Processing time per hologram (second)
1024*1024*1601	1.9
2048*2048*1601	6.5
5320*4600*1601	44.6

HoloPro has versatile functionalities to fulfill the complete hologram processing chain for particle detection and tracking.

Image Display

- Image Display
- Processing
- Post-processing
- Hologram
- Hologram (background-removed)
- Background image
- Reconstruction
- Filtered image

Batch Processing

- Batch Processing
- Create tasks
- Edit tasks
- Add task

System

- System
- About
- Check GPU availability
- Select CPU or GPU
- System parameters

Tools

- Tools
- View
- System
- About
- Convert to MATLAB files
- Particle (.dat)
- Trajectory (.traj)

Data Visualization

- Data Visualization
- To
- Particle distribution
- Particle trajectory

View

- View
- System
- About
- Zoom in (+10%) Ctrl++
- Zoom out (-10%) Ctrl--
- Align image display
- Fit to window

Post-processing

- Post-processing
- Batch Processing
- Refine detection
- Particle
- Trajectory

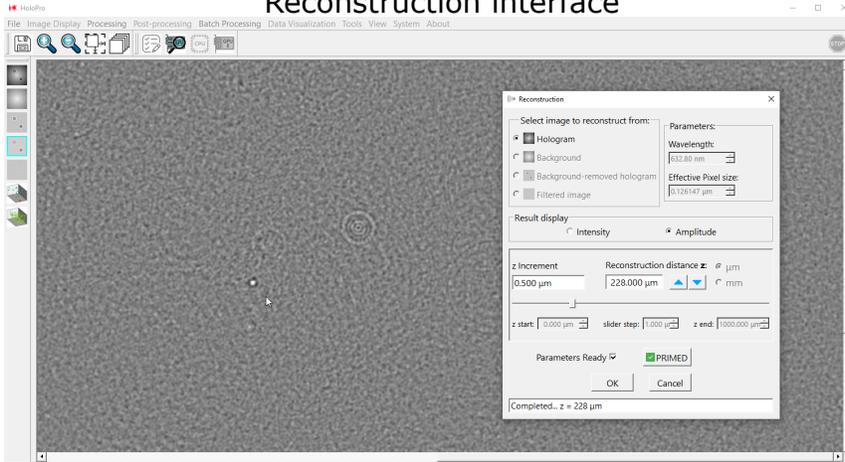
File

- File
- Image Display
- Processing
- Post-processing
- Save image
- Read hologram
- Read background-removed hologram
- Read background image

Processing

- Processing
- Post-processing
- Background Image
- Background removal
- Filtering
- Reconstruction
- Particle detection
- Particle tracking

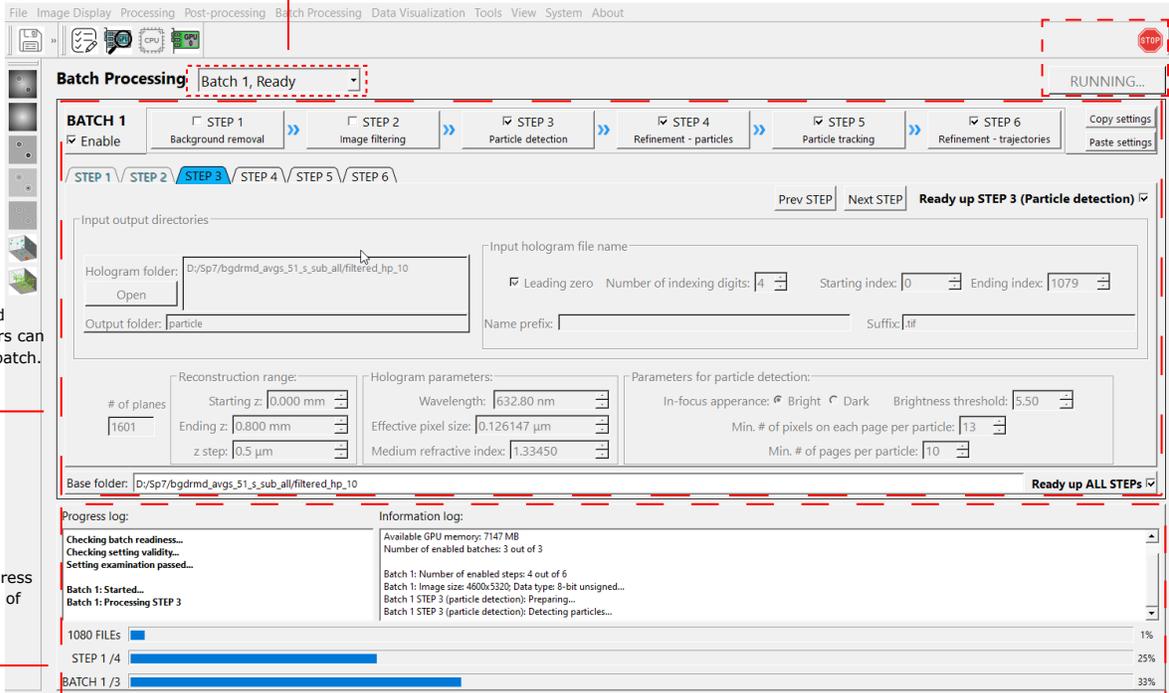
Reconstruction interface



Up to 20 batches of data can be processed in one execution.

The batch processing runs unattended until completion for all datasets but can be easily stopped in the middle of processing.

Batch Processing interface



The processing steps and corresponding parameters can be customized for each batch.

The status logs and progress bars inform the progress of batch processing.

MetroDHM

Wavelength	650 nm
Lateral resolution	0.8 μm (objective dependent)
Magnification	20X (objective dependent)
Field of view	0.69 mm x 0.59 mm x 1.00 mm (depth) (camera and objective dependent)
Imaging rate	12 Hz (camera dependent)
Pixel size	2.74 μm (camera dependent)
Pixel resolution	5320 x 4600 (camera dependent)
Sample stage	Manual XYZ stage, 10 μm precision 13 mm x 13 mm x 13 mm travel range
Dimensions (LxWxH)	550 mm x 100 mm x 150 mm
Weight	5 kg

HoloPro

Development platform	C++
Operating system	Windows 10 (64 bit)
Nvidia GPU	Compute capability 3.5 or higher