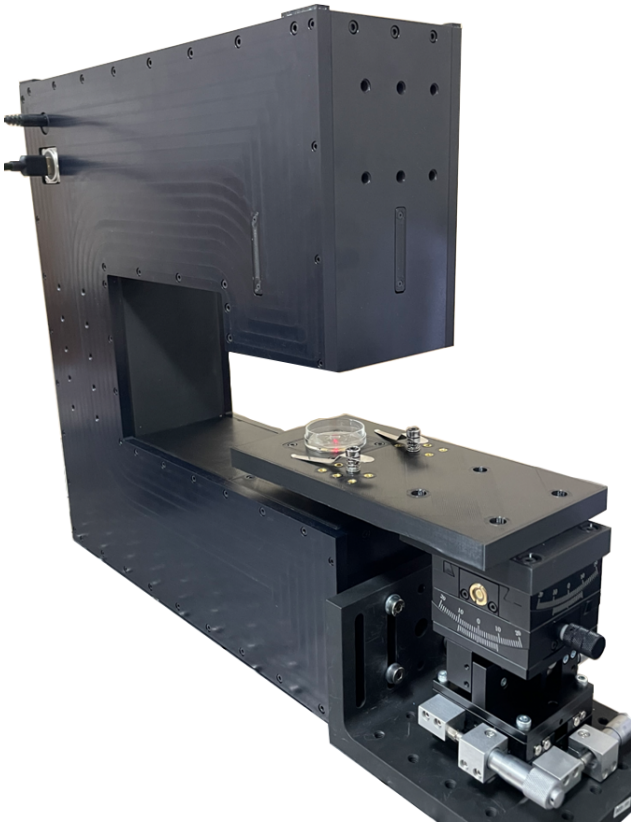
MetroDHM System

MetroLaser, Inc.

Comprehensive Solution to Detection and Tracking of Micro-particles in 3D



MetroDHM-Standard microscope (inverted)

MetroLaser currently offers several MetroDHM microscopes for experimentation and a universal HoloPro software for data acquisition, processing, analysis, and presentation.

Applicable particles include biological particles (e.g., bacteria, archaea, paramecia, algae, and other motile microorganisms) in biological science and abiotic particles (e.g., tracer particles, bubbles, droplets) in engineering subjects.

MetroLaser's microscopes and software can be your ideal tool for analyzing particle dynamics, through scanning-free, label-free, high-throughput, volumetric, and 3D measurements.

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DHM Technique

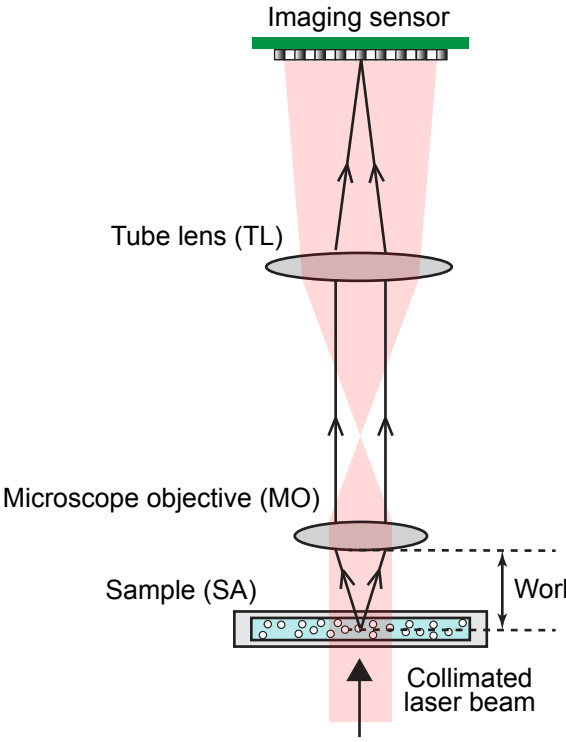


Figure 1. Inline DHM configuration

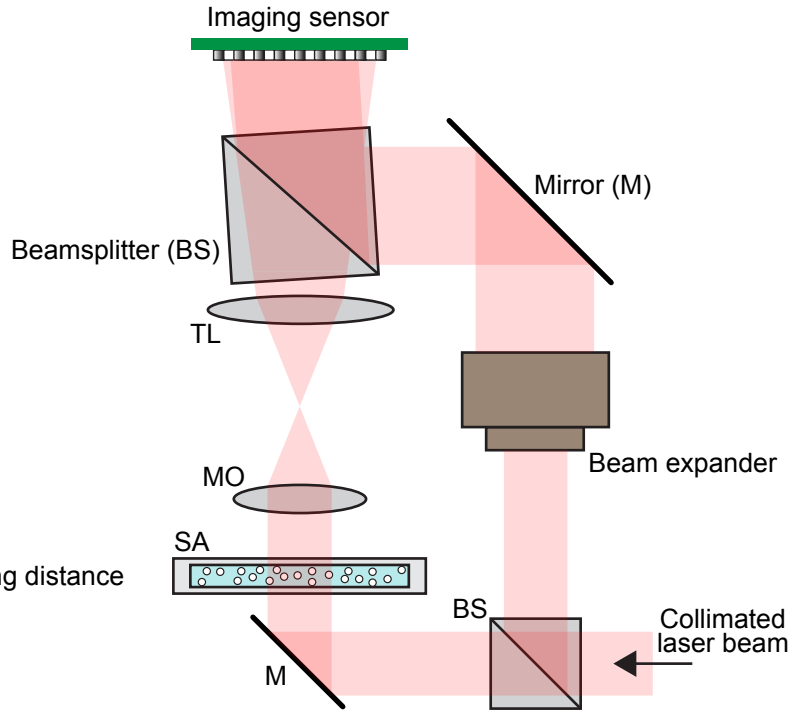
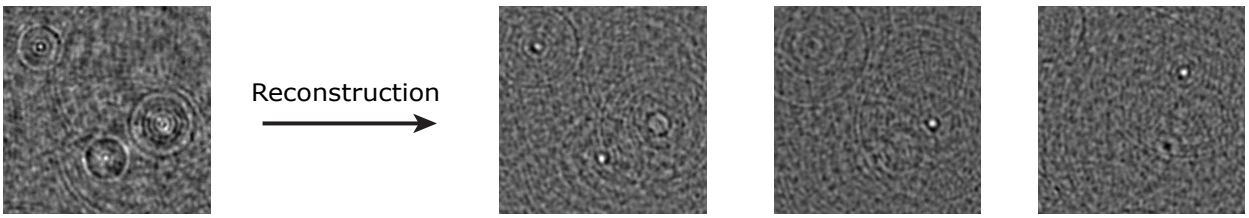


Figure 2. Off-axis DHM configuration (transmission)

Digital holographic microscopy (**DHM**) is a volumetric, label-free imaging technique. The imaging process involves two steps, hologram recording and image reconstruction. In recording, the interference pattern formed by overlapping of the object light and reference light is recorded by a camera as a digital image, i.e., a hologram. The object light is the light scattered by or passes through the objects upon illumination. The reference light comes from the undisturbed part of the same illumination beam in the inline configuration (Figure 1), while in the off-axis configuration (Figure 2), the reference light is split from the initial beam. The interference pattern (fringes) encodes objects' 3D information. In reconstruction, computer algorithms are used to simulate the light propagation from the hologram, back to the planes within the sample volume (specified by the reconstruction distance, z). The reconstruction enables digital refocusing.



Sample inline hologram | Refocused at z=32.7 μm | z=48.0 μm | z=115.4 μm

DHM offers scanning-free volumetric imaging, i.e., the volume acquisition time only depends on the camera exposure time for each frame. The volume information is then retrieved through hologram processing. Thus, DHM is well suited for characterizing dynamic events.

The transmission off-axis DHM is capable of quantitative phase imaging and has been applied to live imaging and monitoring of cell morphology, proliferation, and migration.

Inline DHM is well established for volumetric, 3D particle detection and tracking applications and has been applied to a variety of biological and abiotic particles.

MetroDHM Features

Focus on particle detection and tracking

MetroLaser's DHM system was initially developed for detecting and tracking bacteria. With refinements and augmentations, the latest system is applicable to many more particles of interest and thus can serve as a general tool for high-fidelity particle detection and tracking.

Our MetroDHM system is fast evolving to incorporate more functionalities and features as our R&D progresses. Our customers will enjoy free life-time technical support and software upgradation. The next upgradation of our software will enable machine learning based functions to assist processing and analytics.

For particle detection and tracking applications, our DHM microscopes adopts the inline configuration due to its simplicity.

Quantitative phase imaging option available

For simultaneous particle tracking and quantitative phase imaging applications, we offer a dual-configuration DHM microscope (MetroDHM-Max) that operates in both inline and off-axis modes.

Easy-to-operate microscope

MetroDHM microscopes are designed to be easy-to-use and convenient for experimentation. Users can independently operate the system simply after reading the user's manual and tutorials.

User-friendly and efficient software

The HoloPro software is the hub of our DHM system for data acquisition and processing. Hologram processing is a crucial step in implementing DHM, because it directly determines the quality of measurements. In particle detection, the hologram is reconstructed at a series of planes and object detection is performed on each of those planes to identify the particles in the volume. The particle tracking links the particles detected in sequential frames to generates trajectories, from which parameters describing the micro-particles' dynamics can be calculated. Our processing is GPU-based, ideal for efficient processing of large datasets. The fidelity and accuracy of our processing is verified by both simulations and experiments. HoloPro also interfaces with the hardware, e.g., camera and motorized stage, to automate the experimentation.

Is MetroDHM suitable for your application?

- Are the particles of interest contained in transparent medium for imaging?

  In general, DHM requires the medium to be transparent to achieve the best performance.

- How dense are the particles?

  Higher density could decrease the signal to noise ratio and lower the efficacy of particle detection.

- We'd like to discuss with you on how our DHM system can potentially contribute to your research. Also, we offer demo systems for tryout. Please contact us for more information.

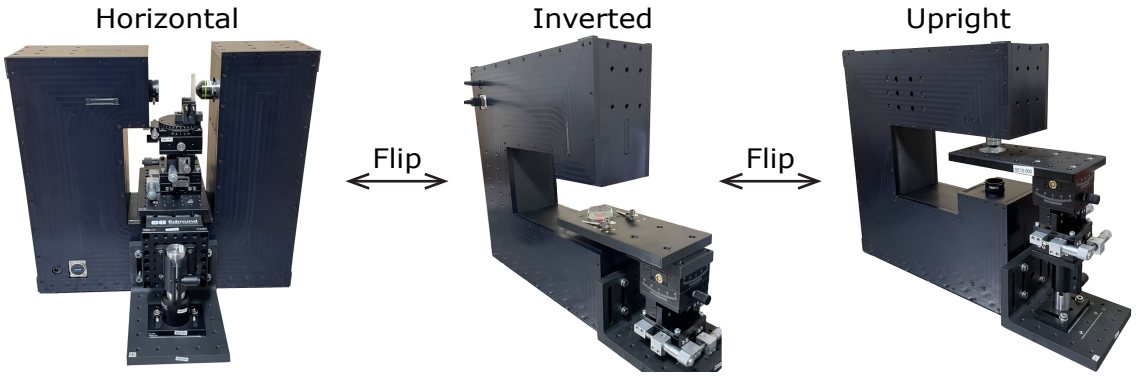
MetroDHM Features

MetroLaser currently offers MetroDHM-Lite, MetroDHM-Standard, MetroDHM-Max microscope models.

Lite: Cost-effective solution to particle tracking, inline configuration, horizontal imaging mode

Standard: Specialized for particle tracking applications, inline configuration, three (horizontal, inverted, and upright) imaging modes to accommodate different sample vessels

Max: Simultaneous particle tracking and quantitative phase imaging, dual (inline and off-axis) configurations, three (horizontal, inverted, and upright) imaging modes



MetroDHM-Standard microscope with three imaging modes

- **Label-free, fast, volumetric, 3D**
  - No chemical labeling, no scanning, no 2D confinement of samples, resolving 3D motion
- **High-throughput**
  - Ideal for efficient determination of robust statistics
- **Easy-to-operate and user-friendly**
  - Intuitive hardware, software, and workflows
- **Automated**
  - Programmable tasks for data acquisition, processing flow with minimal user intervention
- **Comprehensive**
  - From data acquisition to creating publication-quality plots, ALL included
- **Versatile**
  - Applicable to various micro-particles
  - Accommodation of a variety of sample vessels
  - Diverse applications: microorganism motility, cell monitoring, interferometric applications, etc.
- **Customizable**
  - Let us know your unique requirement. We customize the system to fulfill that.
- **Affordable**
  - With MetroDHM, 4D microscopic imaging doesn't have to be expensive.

HoloPro Features

The HoloPro software is an indispensable and powerful tool to work with the MetroDHM microscope for data acquisition and information extraction. During experimentation, it is used to interface with the camera to view and record holograms. During processing, it executes the full processing workflow that starts from loading raw data and ends with producing results (e.g., 3D trajectories). Finally, the results are visualized by 2D and 3D plots on the software GUI with quantitative information, which can be exported as figures for presentation or as Excel or MAT files for further analysis.

Specialized and universal

- Developed and refined for particle detection and tracking applications
- Applicable to biological (e.g., bacteria) and non-biological (e.g., bubbles) particles
- Support for cameras from various manufacturers
- The machine learning upgrade will enable even wider applications.

Comphrehensive

- HoloPro includes every functionality you need to implement DHM.

Batch processing

- Automated workflow designed to facilitate processing of multiple datasets with "one click"

Efficient

- C++ based, GPU-accelerated*

Easy-to-use

- Intuitive and interactive GUI

Powerful data analytics and visualization

- Instantaneous dynamics and statistics can be quantified and displayed on the GUI.
- Trajectories, temporal profiles, and statistics can be visualized and exported as 2D and 3D plots.
- The raw data is readily accessible to users for further analysis.

Free lifetime support

- Free upgrades within a major version
- Custom function can be added upon request.

Unlocked

- HoloPro can process any holograms and work with in-house DHM systems, not limited to MetroDHM.

*Benchmark of GPU-accelerated Processing

Conditions:
GPU: Nvidia Quadro RTX 4000 Mobile
(2304 CUDA cores, boost clock 1.55 GHz)
Number of holograms in the sequence: 1080
Included processing:
Full workflow (hologram preprocessing, particle detection, tracking)

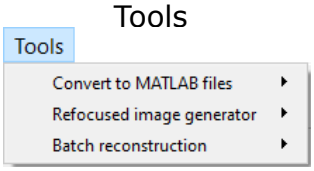
Size of reconstructed volume (voxel)	Processing time per hologram (second)
1024*1024*1601	1.9
2048*2048*1601	6.5
5320*4600*1601	44.6

HoloPro GUI

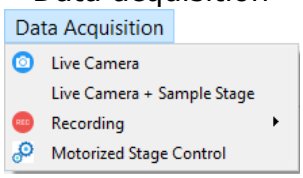
MetroLaser, Inc.

Representative menus and interfaces

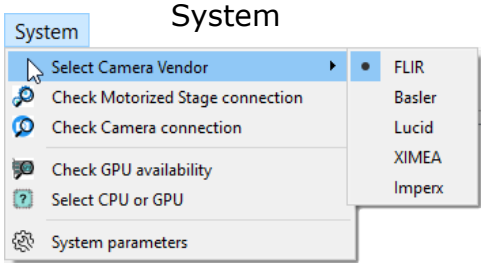
Tools



Data acquisition



System



File

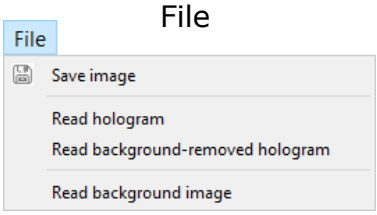
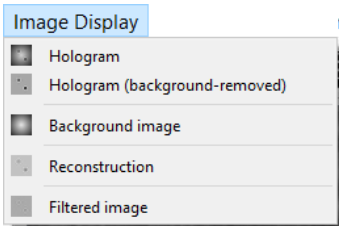
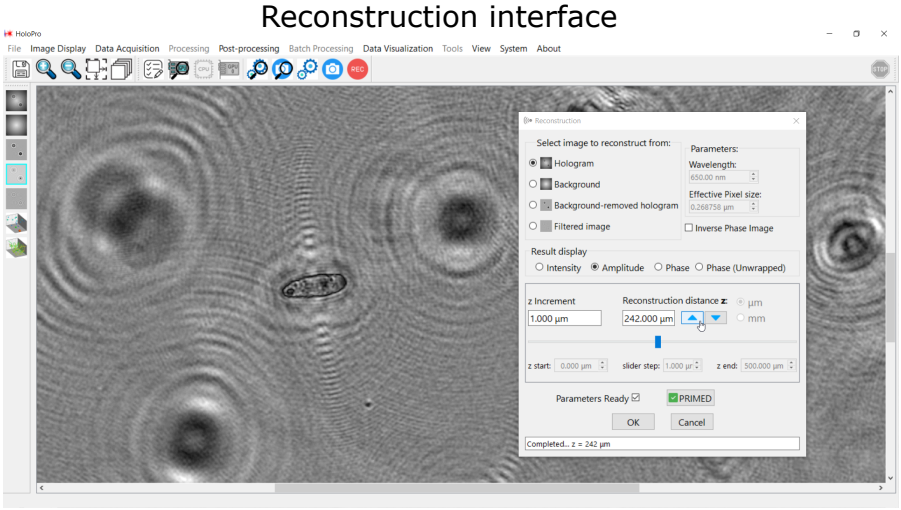


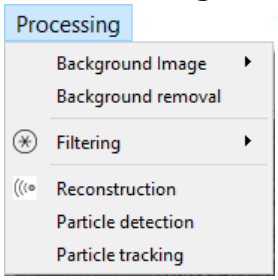
Image display



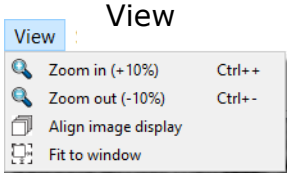
Reconstruction interface



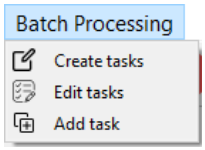
Processing



View



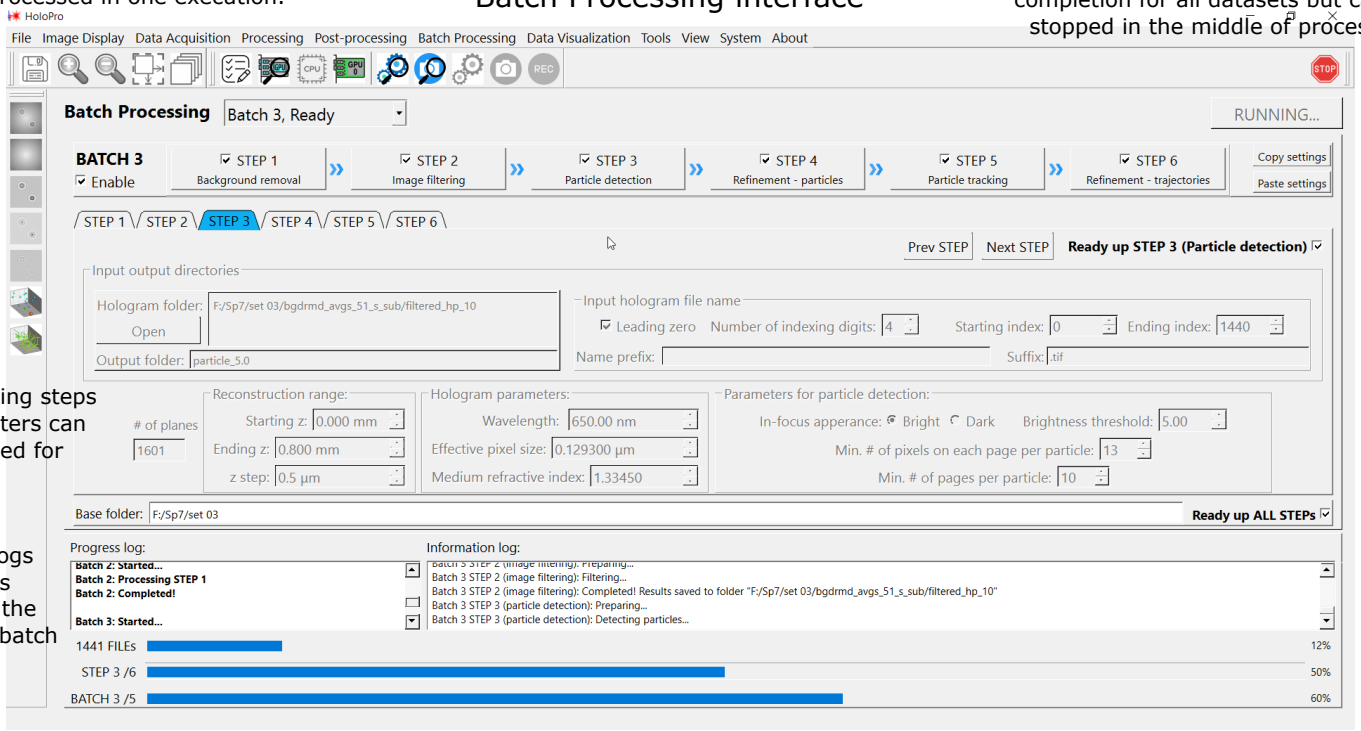
Batch processing



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

Batch Processing interface

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



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

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

Specifications

MetroDHM-Standard

Wavelength*	658 nm
Lateral resolution*	0.8 μm
Magnification*	20X
Field of view*	0.7 mm x 0.6 mm x 1.00 mm (depth)
Imaging rate*	15 Hz
Pixel size*	2.74 μm
Pixel count*	5320 x 4600
Sample stage*	XYZ stage, 10 μm precision 13 mm x 13 mm x 13 mm travel range
Dimensions (LxWxH)	380 mm x 350 mm x 100 mm
Weight	9 kg

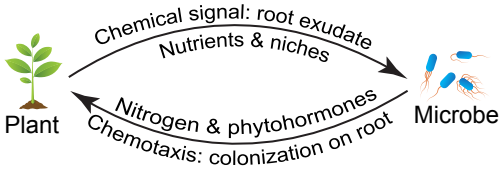
*: Customizable

HoloPro

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

Application Note

Background



Plant health and crop production are dependent on plant-microbe interactions in the rhizosphere, which is the region immediately 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 the direction corresponding to chemical gradients. Thus, bacterial motility response to different perturbations can be used as a direct indicator to investigate plant-microbe interactions.

Application of MetroDHM to Bacterial Motility Characterization

MetroDHM system is used for volumetric, high-throughput, 3D characterization of bacterial motility. Hologram sequences of swimming bacteria in diluted culture are acquired using MetroDHM, then HoloPro is used to process the holograms to retrieve the 3D trajectories of the bacteria in the sample volume. Considering the 3D nature of bacterial motion, DHM is intrinsically superior to conventional microscopic imaging. The experimentation, data processing, and analysis are performed following the procedures below.

Preparation
- Put bacteria in diluted culture into a shallow-depth vessel (e.g., cuvette or petri-dish)
- Settle the vessel on the sample stage

Data Acquisition
- Record hologram video at a proper frame rate to resolve bacterial motion
- Collect multiple datasets for statistics

Data Processing
- Run processing using partial data in separate steps to confirm optimal conditions
- Batch processing of multiple datasets

Data Analysis
- Extract instantaneous and statistical kinematic parameters for velocity, turn angle, etc.
- Visualize and export results using HoloPro

Representative Results

The motility characteristics of the wildtype (Sp7) and mutant strain (CheA4) of *A. brasilense* cells are revealed using MetroDHM. Figure 1 shows 1885 trajectories color-coded with time, determined from a 90-second hologram sequence. Figure 2 shows an intuitive comparison between the "run-reverse" swimming pattern of wildtype cells and the straight path due to defects in making turns of the CheA4 strain, with the former manifested by the sequential pauses correlated with nearly 180-degree turns and the latter exhibiting few pauses or turns.

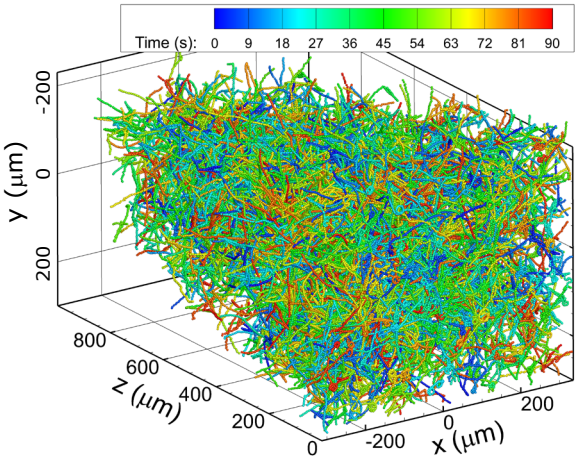


Figure 1. 1885 bacterial trajectories, each longer than 5 seconds, determined from a sequence of 1080 holograms acquired at 12 fps.

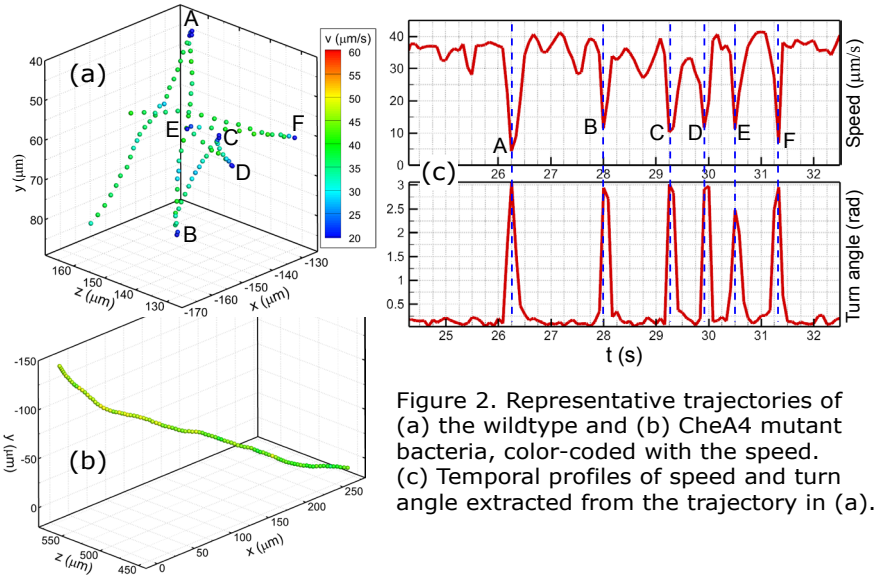


Figure 2. Representative trajectories of (a) the wildtype and (b) CheA4 mutant bacteria, color-coded with the speed. (c) Temporal profiles of speed and turn angle extracted from the trajectory in (a).

Reference

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

J. Gao, H. Parsian, J. Carnahan, G. Alexandre, and J. Trolinger, "Digital holographic microscopy for bacterial species classification and motility characterization," Proc. SPIE 12672, Applied Optical Metrology V, 126720C (2023).