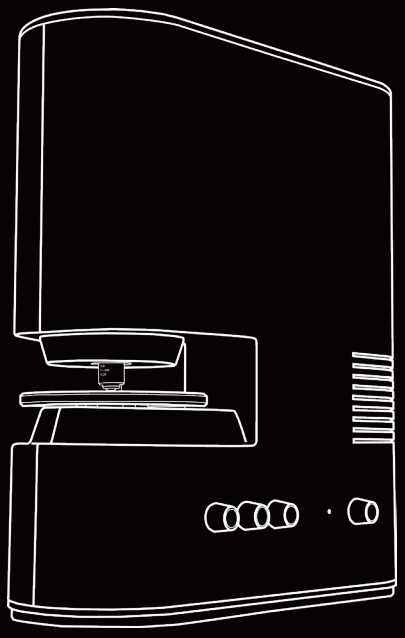
Holotomography

IMAGE DIFFERENT



Tomocube

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www.tomocube.com

info@tomocube.com

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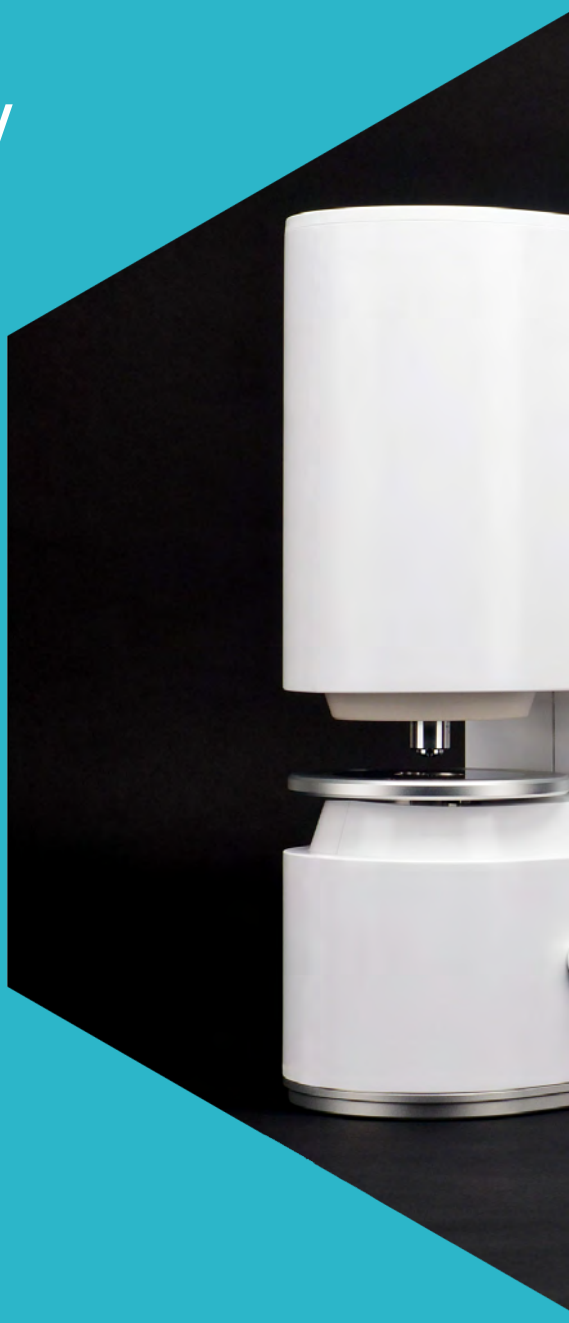
Overview

Revolutionary holotomography (3D holographic microscopy) opens new era for label-free live cell imaging

Cellular analysis plays a crucial role in a wide variety of research fields and diagnostic activities in the life sciences and medicine. However, the information available to researchers and clinicians is limited by the current microscopy techniques. An innovative new tool – *holotomography* – can overcome many of these limitations and open new vistas for researchers and clinicians to understand, diagnose and treat human diseases.



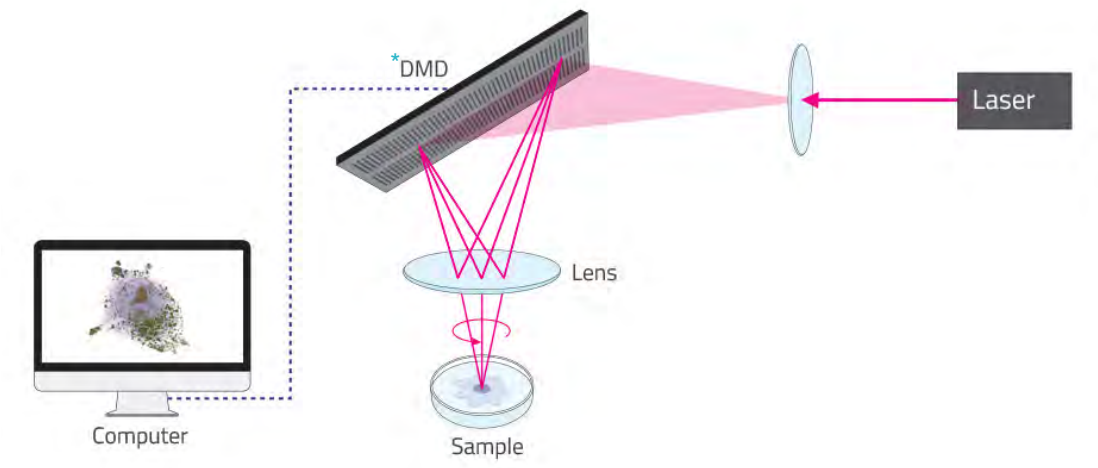
Holotomography (HT) - New era of microscopy

Tomocube's HT technology enable users to quantitatively and noninvasively investigate live biological cells and thin tissues in 3D. Holotomography reconstructs the 3D refractive index (RI) distribution of live cells and provides structural and chemical information about the cell, including dry mass, morphology, and dynamics of the cellular membrane. This can be done very easy and fast, because RI is an instrinsic optical parameter of a material and thus HT does not require any preparation on samples.

Technology

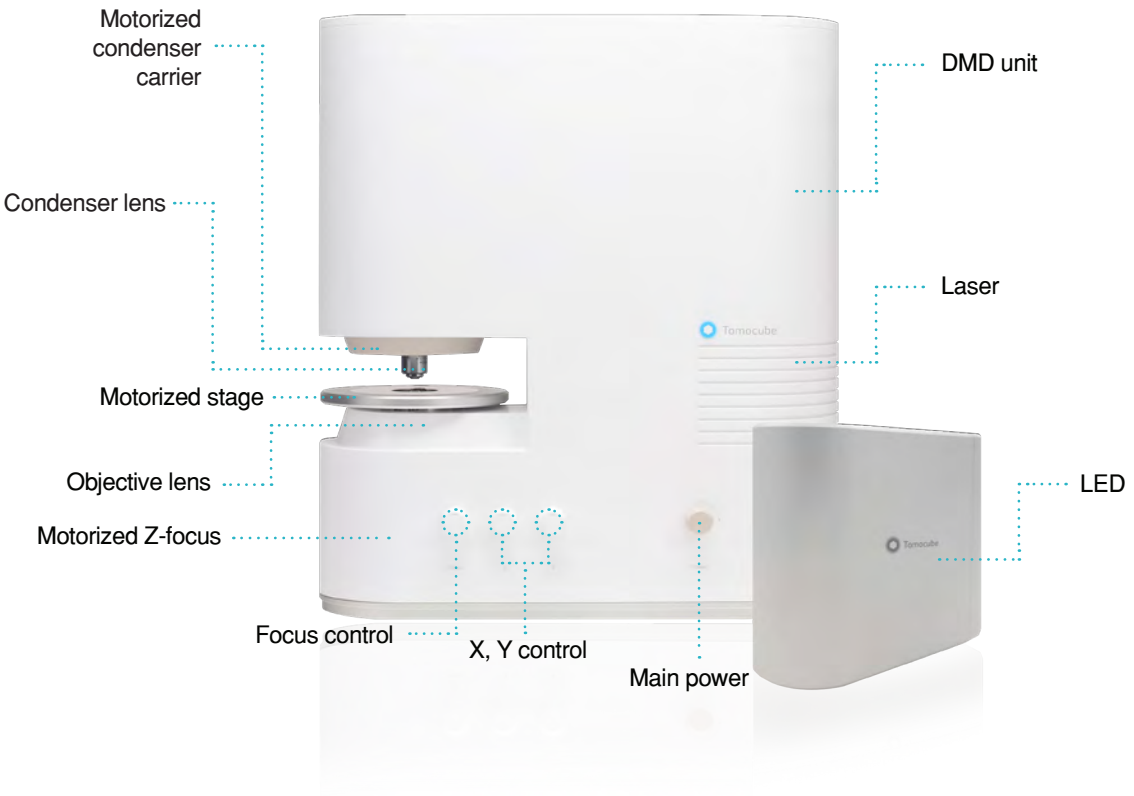
HT is optically analogous to X-ray CT

RI is an intrinsic optical parameter that describes the speed of light passing through a specific material. Light passing through a cell is slower than light passing through the surrounding medium. Analogous to X-ray CT (computed tomography), HT uses a laser beam to measure 3D RI distribution of cells. The system measures multiple 2D holograms of a sample in various illumination angles, from which a 3D RI tomogram is reconstructed via an inverse scattering algorithm. Tomocube presents unprecedentedly precise laser beam control, powered by Texas Instruments™ digital micromirror device (DMD) technology.



* Tomocube's patented technology utilizes a DMD, which allows to obtain multiple 2D images by every angle to reconstuct 3D RI Tomogram without any mechanical movement in the microscope.

HT Series components



HT-1S

60x Lens (dry)

Holotomography

HT-1H

60x Lens (water)

Holotomography (High resolution)

HT-2S

60x Lens (dry)

Holotomography

3D fluorescence microscopy (3 channels)

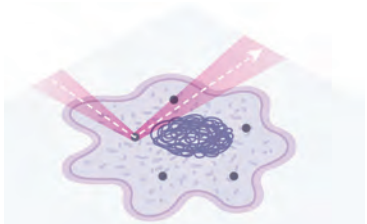
HT-2H

60x Lens (water)

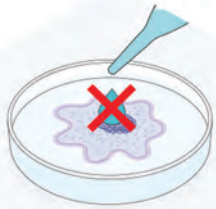
Holotomography (High resolution)

3D fluorescence microscopy (3 channels)

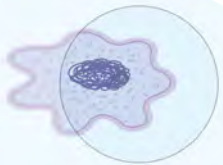
Key features



3D Refractive index
New imaging contrast



No label
Non-invasive method



High resolution
Beyond the diffraction limit

Low laser
532 nm, 0.05 mW

Fast imaging
In 2D, 3D and 4D

Efficient
High impact, Low cost

Benefits

01 Zero stress
Label-free imaging

02 Intact live cell imaging
Long-term imaging with short time interval

03 Time saving
No sample preparation and rapid 3D cell imaging (0.4 sec)

04 High quality 3D image
Optical resolution below 200 nm (Max. 110 nm)

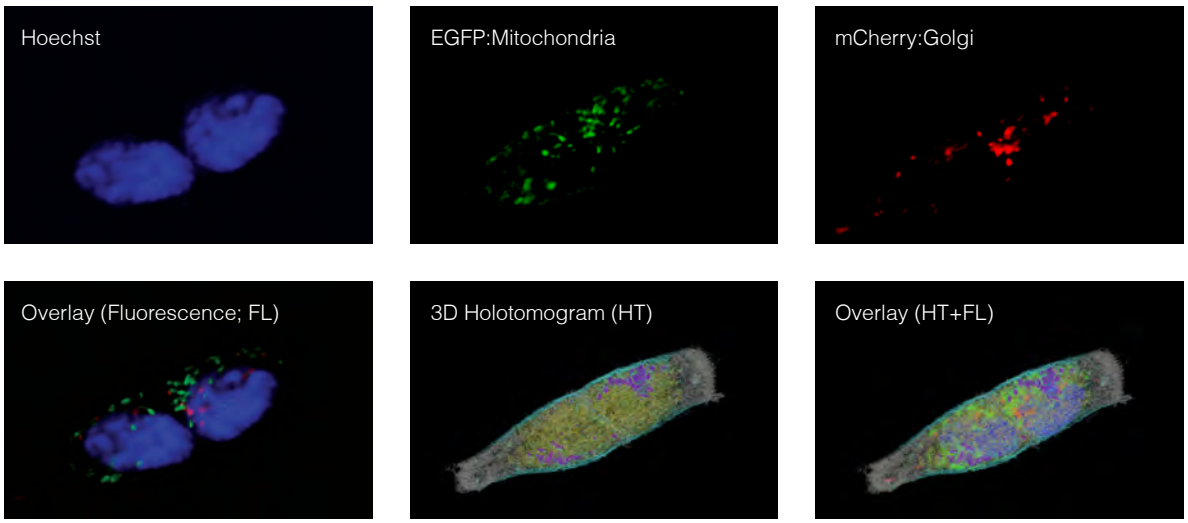
05 Quantitative bioimaging
RI enables quantitative bioimaging
(Volume, local concentration, dry mass, etc.)

HT-2: HT combined with 3D fluorescence imaging

Holotomography powered with 3D fluorescence imaging

HT-2 series opens a new era of 3D correlative imaging, combining the holotomography and fluorescence methods. HT-2 allows the conventional epifluorescence imaging for labeling any specific target (organelle or proteins) in 3D holotomography, minimizing the photodamage of the live cells.

NIH-3T3



* Incubation for 20 hours after Neon electroporation

Advantages

01 Correlative microscopy in one instrument

HT-2 provides high-quality 3D images of both holotomography and 3D fluorescence for each sample.

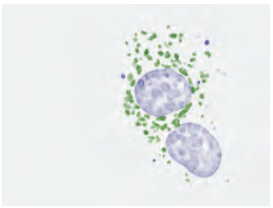
02 Quantitative data marked with fluorescence

HT-2 provides morphological (volume, surface area, projection area and sphericity), chemical (dry mass, concentration) and mechanical (cell deformability) properties of cells with 3D refractive index (RI) tomogram. Moreover, fluorescence image provides information about molecular specificity.

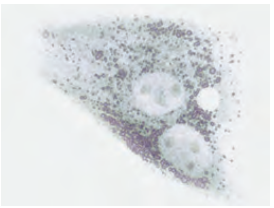
03 Live cell molecular and holographic imaging with minimal stress on cells

Simultaneous measurement capability of time-lapse 3D RI tomography and fluorescence image allows long-time tracking of specific targets in live cells. The fluorescence image provides the position of specific target organelles or structures in live cells, and consecutive measurement of time-lapse 3D RI tomography enables the monitoring the cellular structures with minimal stress.

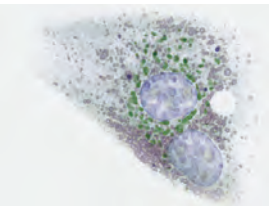
HeLa



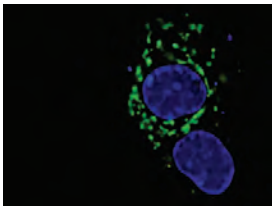
3D fluorescence
(DAPI, GFP-mitochondria)



3D RI tomogram



3D RI tomogram +
3D fluorescence



2D fluorescence
(DAPI, GFP-mitochondria)

Fluorescence capabilities

3-channel LED source (385 nm, 470 nm, 570 nm)

Wavelengths of the LED source can be customized

Z-stack images with a motorized Z-drive (step resolution: 150 nm)

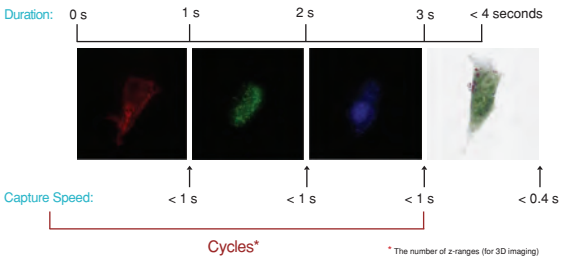
Correlative analysis in 2D, 3D and 4D with HT and fluorescence images

Upgradable: The HT-1 can be upgraded easily to the HT-2 (fluorescence version) in the field

Working scenarios

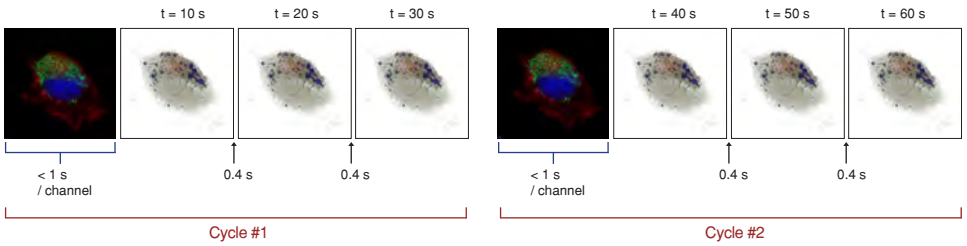
Scenario 1 2D(or 3D) Fluorescence + 3D Holotomogram Snapshot

It is the procedure of “FL+HT” mode (Fluorescence imaging + Holotomography) in TomoStudio™ 2. The capture speed of the fluorescence imaging depends on the number of channels and the range of z-axis (in 3D imaging). Users can merge the fluorescence images with the 3D holotomogram to identify the localization of fluorescence signals.



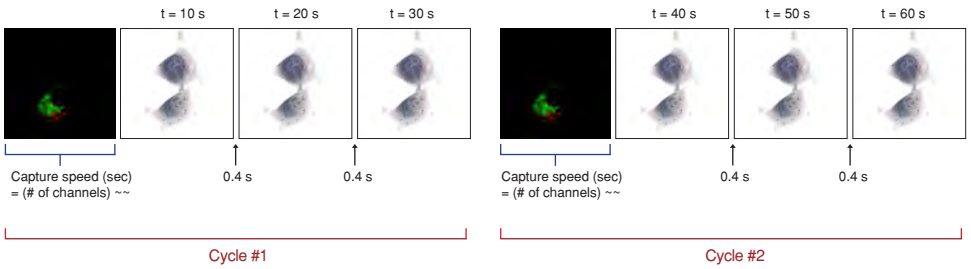
Scenario 2 Time-lapse imaging of 2D Fluorescence + 3D Holotomogram

This scenario minimizes the photodamage of your cells during the fluorescence imaging. Using a single 2D fluorescence image, you can mark your target proteins or organelles in the living cells and follow them by time-dependent manner using 3D holotomograms.



Scenario 3 Time-lapse imaging of 3D Fluorescence + 3D Holotomogram

It is possible to obtain 3D fluorescence images and 3D holotomograms simultaneously, which allows researcher to investigate the 3D morphology of your target in the cells with unprecedented imaging modalities. TomoStudio™ 2 presents sharp and realistic 3D fluorescence images by adapting a deconvolution software.



TomoStudio™

Analysis of 2D/3D/4D holographic images

01

TomoStudio™, the HT series operating software, controls the system and visualizes the captured image in various ways. The flexible user interface provides fast imaging capability and 2D/3D/4D visualization of cellular image based on 3D RI distributions of cells and tissues.

02

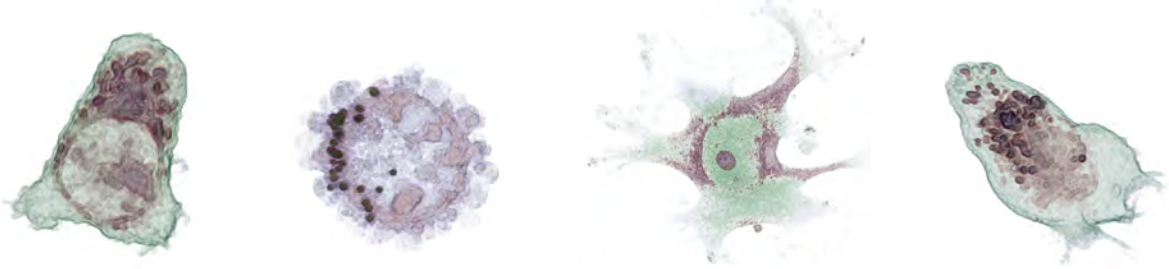
TomoStudio™ provides quantitative information about morphological, chemical and mechanical properties of the sample. Quantitatative and label-free bioimaging capability will open a new avenue for the study of pathophysiology of cells and tissues.

03

Output parameters:

Morphological parameter	Chemical parameter	Mechanical parameter
Volume (μm^3)	Dry mass (pg)	Cell stiffness
Surface area (μm^2)	Concentration (pg/μm^3)	
Projection area (μm^2)	*RBC: Hb contents and concentration	
Sphericity		

* Red Blood Cell



TomoStudio™ provides

01

Work flow interface

User interface allows uninterrupted workflow from using the microscope to analyzing the data.

02

Data backup

Raw data can be stored in the computer for further analysis.

03

Fast image acquisition

HT captures holotomographic images every 0.4 second (2.5 fps) and 2D holographic images every 0.007 second (150 fps).

04

Holographic staining

Digital color coding controller (transfer function) is a graphical user interface that stain the sample digitally based on RI information.

05

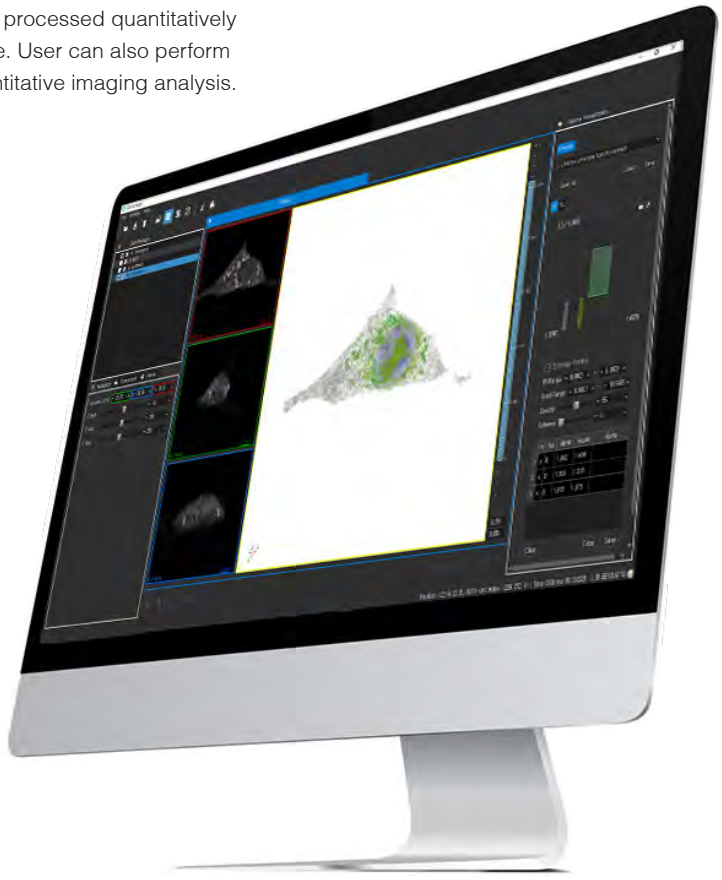
Data analysis

Data can be processed quantitatively and real-time. User can also perform various quantitative imaging analysis.

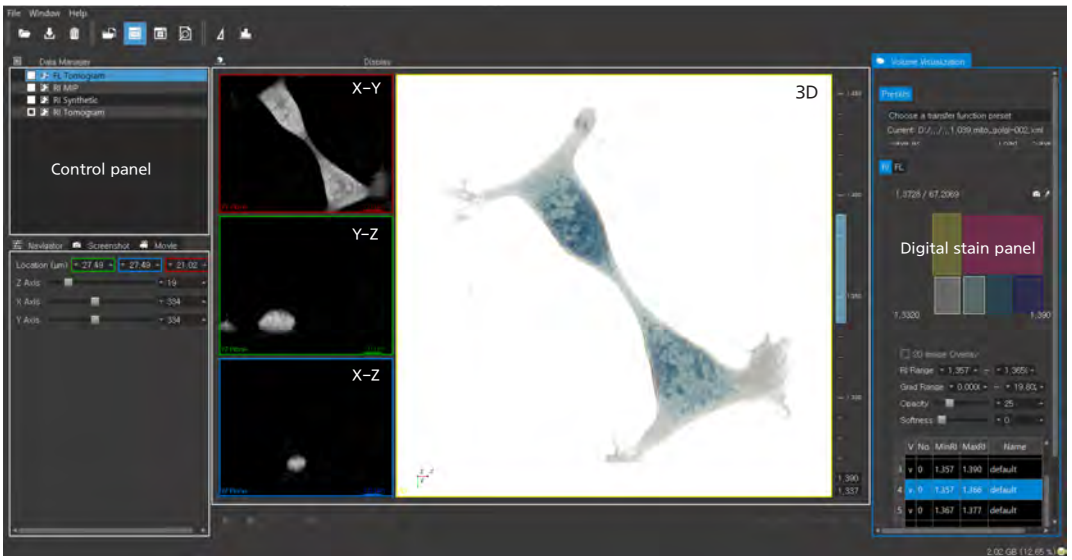
06

Dynamic image processing

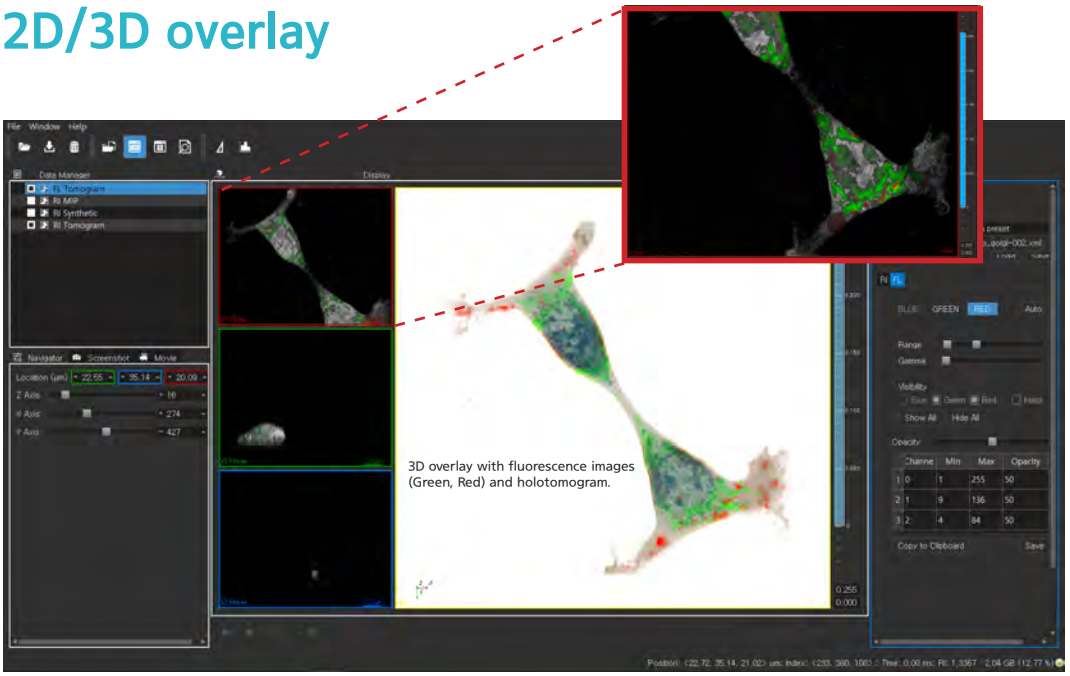
Data processing does not interfere with the image acquisition process. Selective data processing is possible any time.



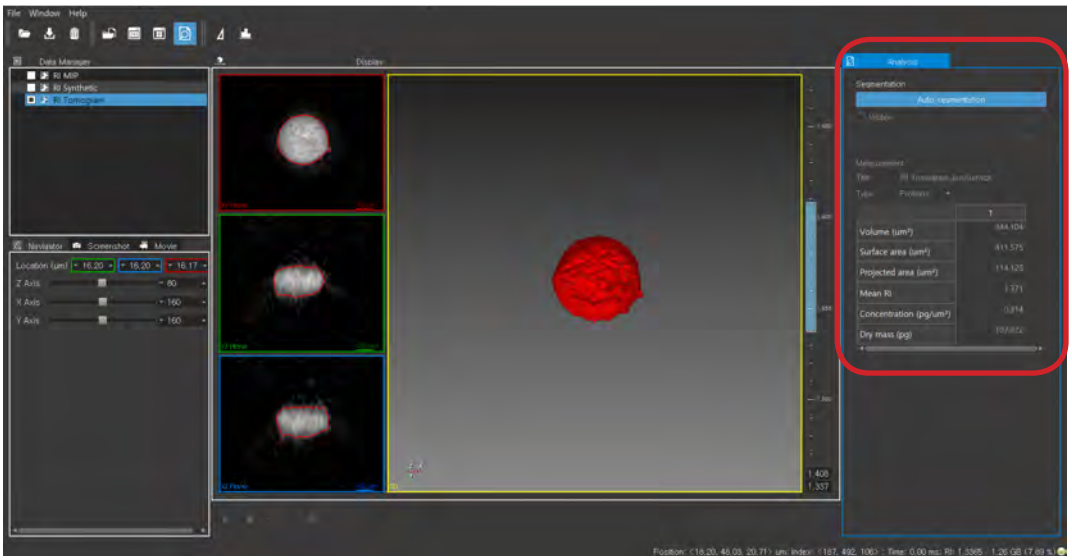
3D digital color coding



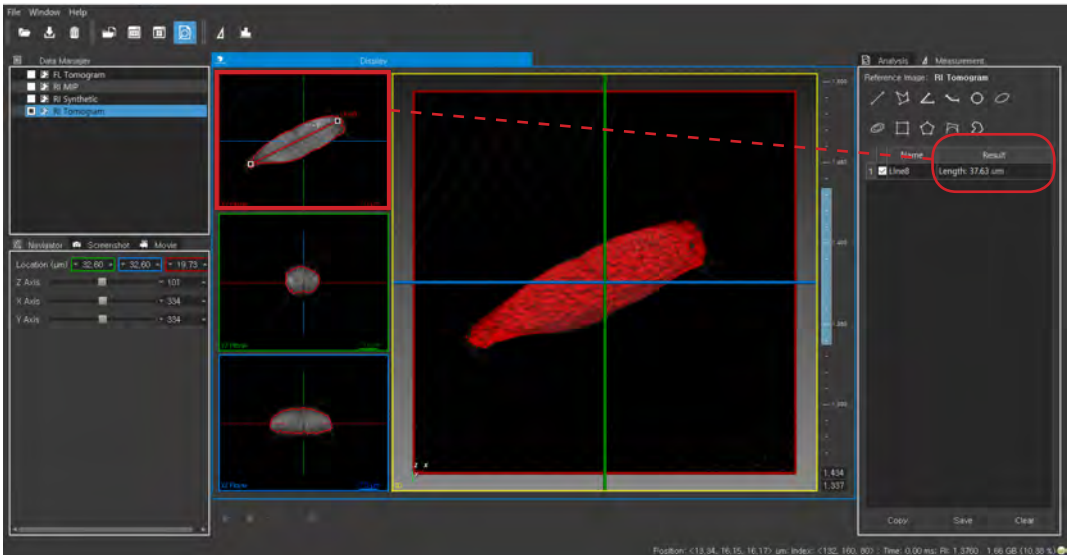
2D/3D overlay



Analysis



Measurement



TomoStudio™ 2 optimized for the HT series

Operating

01
Full control of the motorized stages, condenser and Z-drive

02
Full control of the laser source and camera

03
Data analysis and fluorescence deconvolution

Features

Obtaining 3D RI tomogram 1

'Mark' and 'Find' function for recoding the position of multiple cells 3

2 Obtaining 3D fluorescence images up to 3 colors

4 Control of fluorescence and tomogram with different time series
(Hetero time-lapse: e.g. 1 FL in every 10 tomogram)

Visualization

01

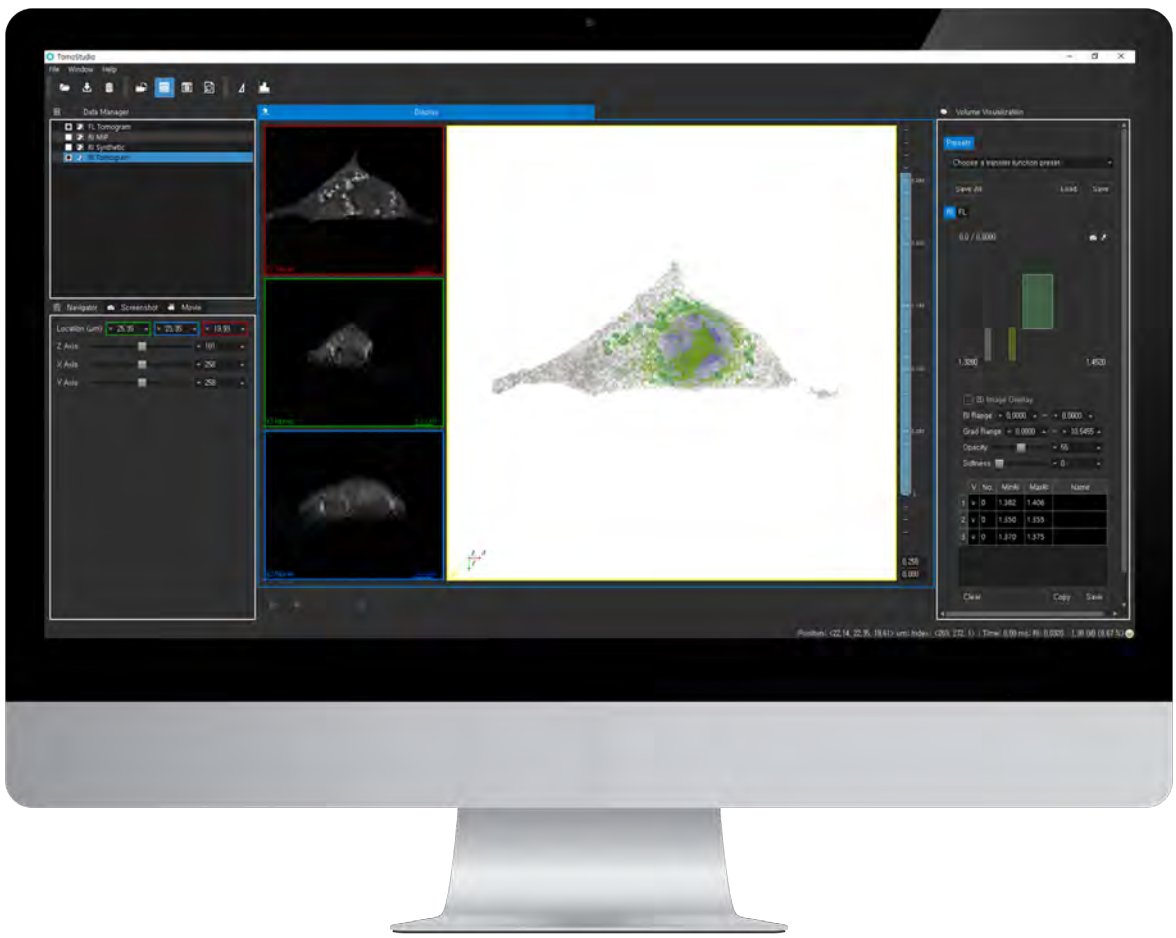
Digital color coding of RI Tomogram with high flexibility

02

Gradient transfer function displays the additional differentiation factor of RIs in the cell

03

Digital 3D overlay of FL and HT images



Accessories

01

TomoChamber

For long-term live cell imaging, it is necessary to secure specific environment to keep cells alive. TomoChamber is an incubation chamber designed to perform time-lapse imaging with HT series. It can be installed in the sample stage, where it maintains the temperature and supplies CO_2.

02

TomoPlate

The laboratory is constantly exposed to mechanical anti-vibration. TomoPlate is a magnetic type compact anti-vibration table specially designed for higher resolution imaging, minimizing the effects of vibration specially designed for getting high-quality images by minimizing the noise signals.

03

TomoDish

Specially designed glass-bottom dish for live cell imaging, allowing easy sample preparation and high-resolution sample imaging. Both adherent and floating cells can be cultured and prepared for imaging very easily.

Applications

Hematology

- Blood analysis
- Malaria infection
- Sickle cell identification

Cell Biology

- Quantitative cell biology (mass, volume, surface area, thickness, etc)
- Cell morphology
- Cell movement
- Intracellular trafficking

Immunology

- Cell-to-cell interaction (e.g. CAR-T)
- White blood cell classifcation
- Cytotoxicity test
- Parasite infection

Microbiology

- Species classification
- 3D structure imaging
- Lipid quantification

Nanotechnology

- Nanoparticle imaging
- Polymer imaging

Capabilities

01 Observe the cellular changes without any labeling or staining

02 Multidimensional acquistion : 2D time (150 fps) / 3D time (2.5 fps)

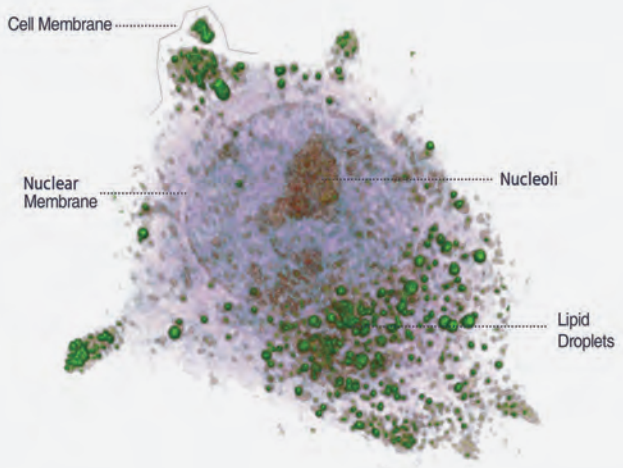
03 Visualize the cellular organelles with 3D RI distribution

04 Identify the changes of the quantitative properties of cells

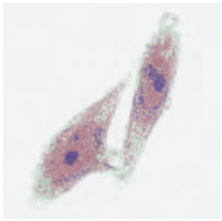
05 Detect the cellular organelles tagged by nanoparticles

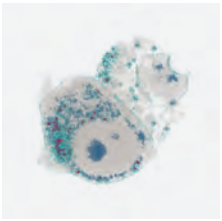
06 Observe the vesicle movement in time-lapse

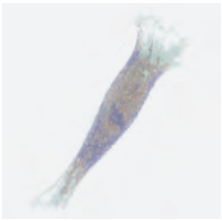
07 2D/3D/4D correlative images with fluorescence

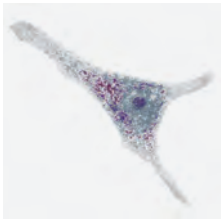


3D HT images of mammalian cells

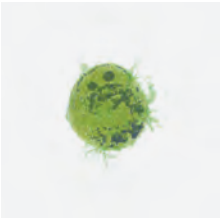

HeLa

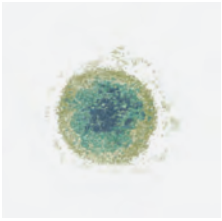

AU565 & NK cell


Schwann


MCF7


NIH3T3

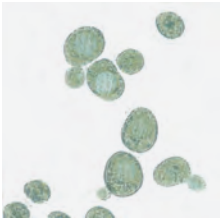

Neurosphere

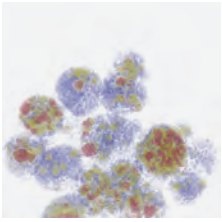

Seal fibroblast

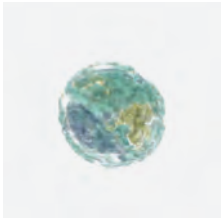

Human sperm

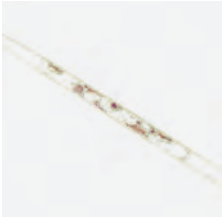
3D HT images of microorganisms


Cyanobacteria

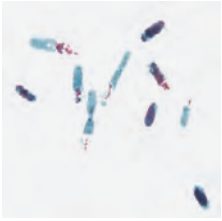

Baker's yeast

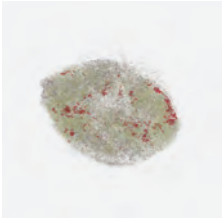

Polychaeta eggs


Chlorella


Diatom


Fission yeast

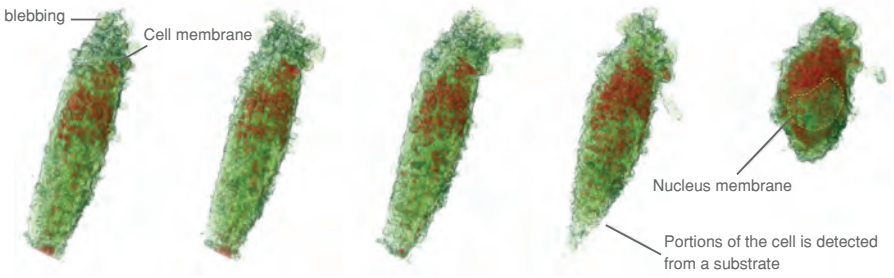

Bacteria (*E.coli*)


Ostreopsis

Time-lapse HT imaging

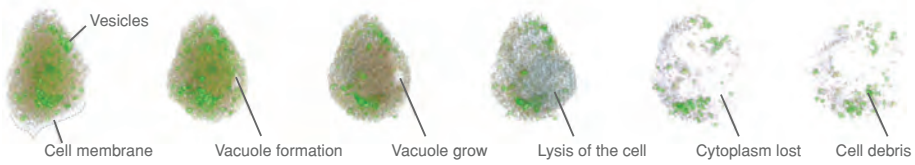
Apoptosis

25 min | 30 min | 35 min | 40 min | 45 min



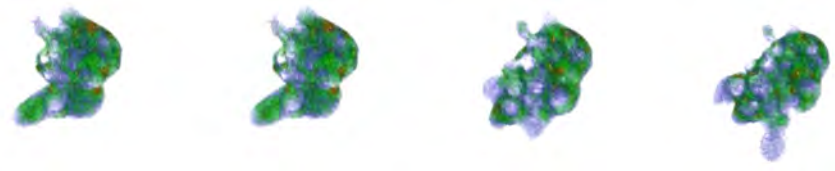
Necrosis

0 min | 3 min | 6 min | 9 min | 12 min | 15 min

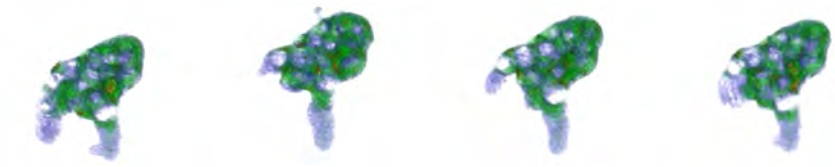


Amoeba

t = 0 sec | t = 1 sec | t = 2 sec | t = 3 sec

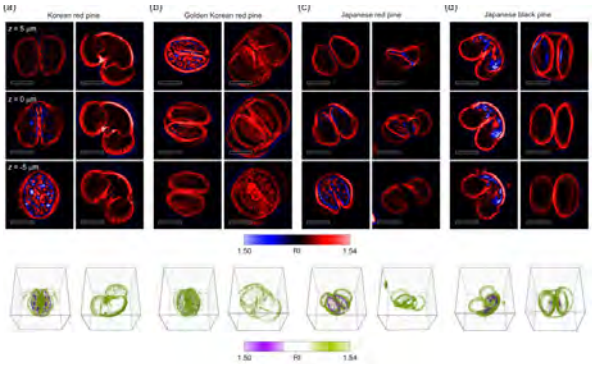


t = 4 sec | t = 5 sec | t = 6 sec | t = 7 sec



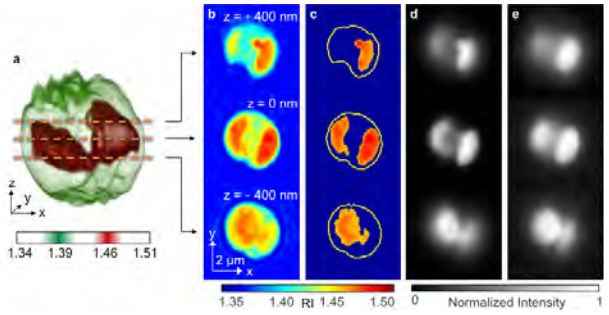
Selected publications

1 SCIENTIFIC REPORTS



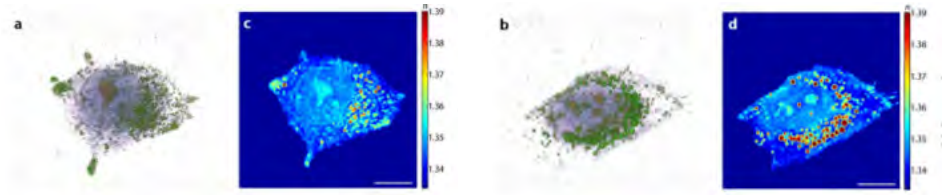
Kim G et al., 3D label-free imaging and analysis of Pinus pollen grains using optical diffraction tomography, ***Scientific Report*** (2018)

2 bioRxiv
THE PREPRINT SERVER FOR BIOLOGY



Jung et al., Label-free non-invasive quantitative measurement of lipid contents in individual microalgal cells using refractive index tomography, ***BioRxiv*** preprint (2017)

3 SCIENTIFIC REPORTS



Kim K et al., Three-dimensional label-free imaging and quantification of lipid droplets in live hepatocytes ***Scientific Report*** (2016)

System

02 TomoStudio™
Software for HT series

01 HT series
Microscope body,
A. Fluorescence LED

03 TomoPlate
Anti-vibration plate for HT series

04 TomoChamber
TomoChamber, the incubator for HT series, consists of **B**. stage top incubator and **C**. controller

PC requirements

Technical specification

HT series technical specification

Model		HT-1S	HT-1H
Objective lens		60x NA 0.8	60x NA 1.2 (Water immersion)
Optical resolution	Lateral resolution	166 nm	110 nm
	Axial resolution	1 ㎛	356 nm
Reconstructed voxel resolution	Lateral resolution	166 nm	110 nm
	Axial resolution	332 nm	220 nm
Field of view		max. 80 ㎛	
Depth of field		max. 40 ㎛	
Imaging speed		150 fps (2D holography)	
		2.5 fps (3D holography)	
Light source (Laser)		532 nm, 0.05 mW, laser class 1	
Max. illumination angle in the sample plane		53˚	63˚
Microscope body		Fully motorized	
Size (W x D x H, mm)		445 x 180 x 500	
Weight		23 kg. / 51 lbs.	
Power requirement		100~240 V, 50 / 60 Hz, 1.5 A, 100 W	

HT-2 fluorescence specification

Model	HT-2S	HT-2H
Light source	Three LEDs for triple channel (λ center = 385 nm, 470 nm, 570 nm)	
Lateral resolution	~ 350 nm	~ 220 nm
Axial resolution	~ 1.6 ㎛	~ 0.7 ㎛
Field of view	80 ㎛ x 80 ㎛	
Imaging	2D/ 3D/ 4D	
Maximum exposure	1 sec	
Life time	> 10,000 hrs	

Environmental requirement

Temperature	15℃ – 30℃, It has to be ensured that the airflow of the air-conditioner is not directed toward the system
Humidity	< 65%

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