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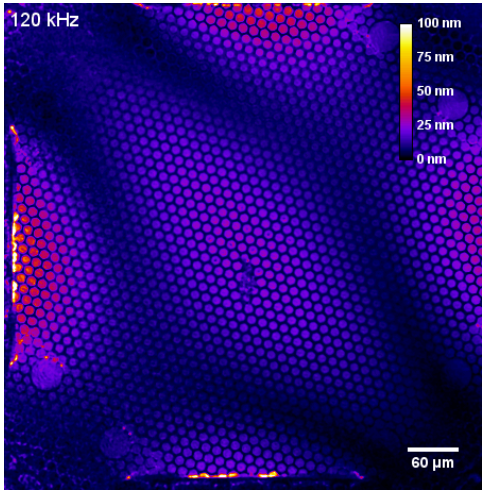
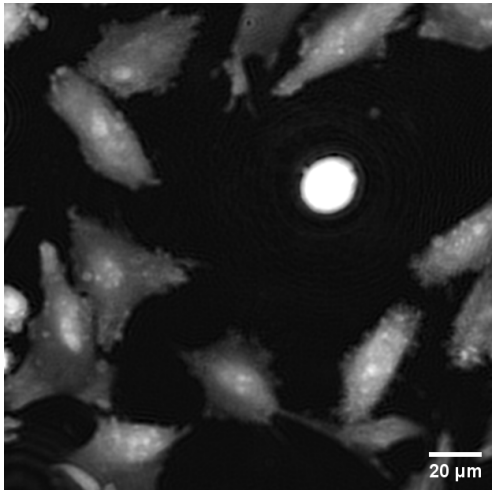
4D profilometry and biomonitoring with our Digital Holographic Microscopes



Vibration amplitude maps of a microphone back-plate



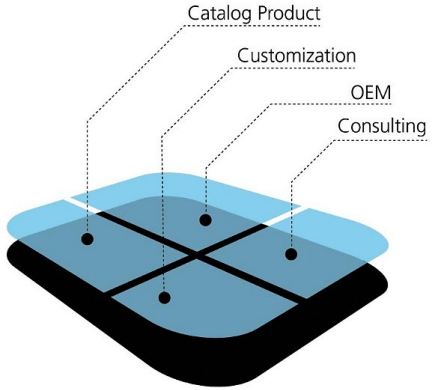
HeLa cells with Colchicine

Lyncée Tec SA

the pioneer and leader in phase imaging

Lyncée Tec supplies directly and through partners universities, industrial laboratories, and manufacturers with a complete range of Digital Holographic Microscopes (DHM®), application software, and accessories.

- Founded in **2003**, in Lausanne, Switzerland
- Presence in **40 countries**
  - A complete range of products (direct and distribution network)
  - OEM partnerships (semiconductor, photovoltaic, industrial metrology, life sciences)
- Lyncée provides **turnkey** solutions **from sample handling to data analysis for**:
  - *Optical Profilometry*
  - *Bio-imaging*



DHM® Camera

Reflection DHM®

Transmission DHM®

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Material sciences applications

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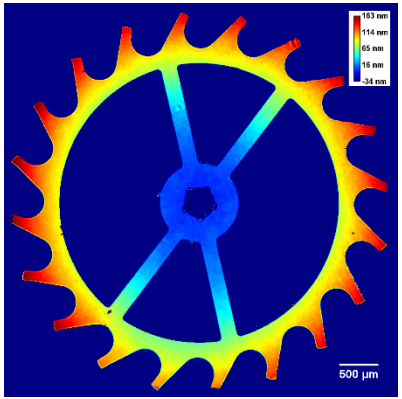
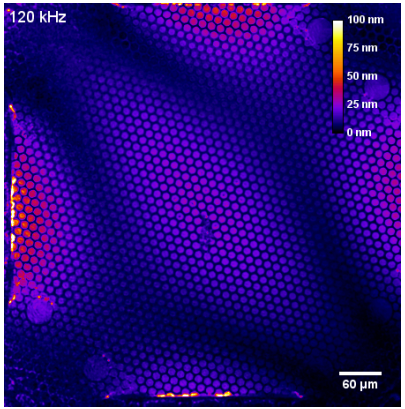
4D profilometry by Digital Holography Microscopy DHM®

Perform measurements that you couldn’t do before



Not only static 3D topography



But time-resolved: 3D + time = 4D

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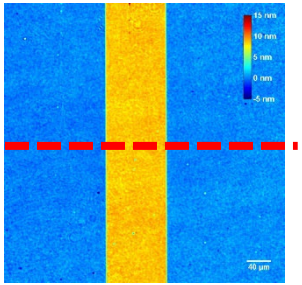
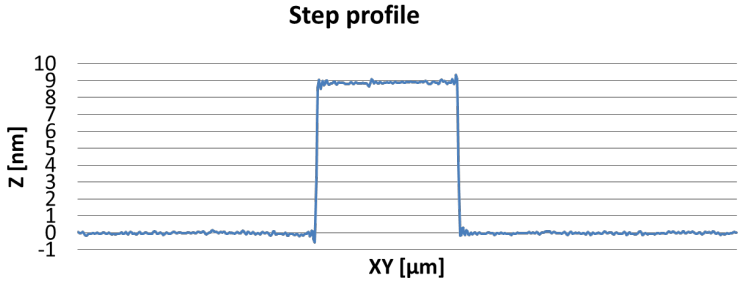
What is a DHM® ?

- **a 3D optical profilometer with interferometric resolution …**

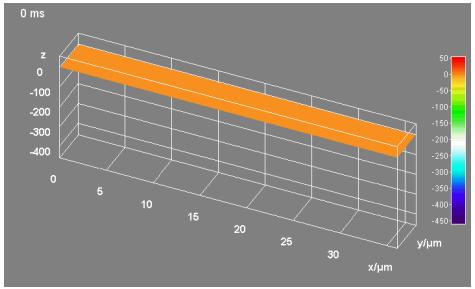


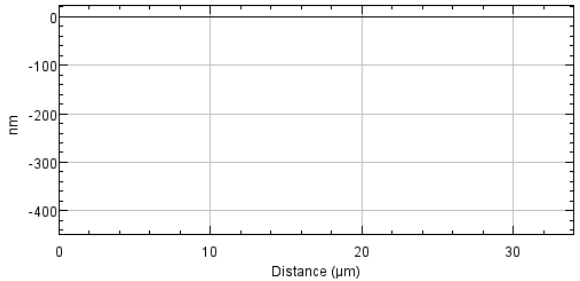


Nanometer height measurements

Certified 9 nm step

- **… enabling time-resolved measurements (4D)**





Milliseconds temporal measurements

Graphene membrane deformation by pressure

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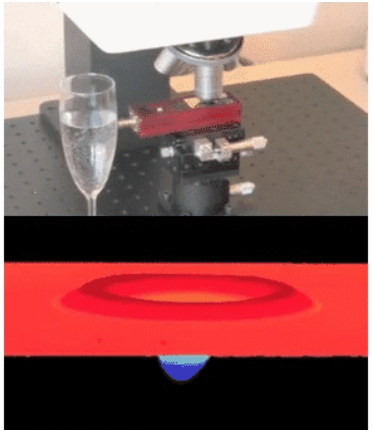
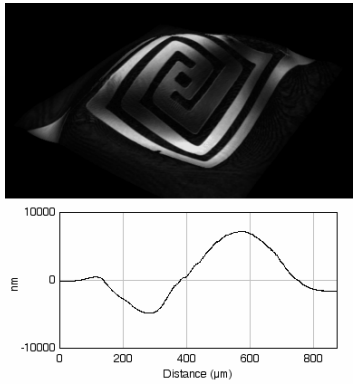
No blur during exposure sensitive measurements

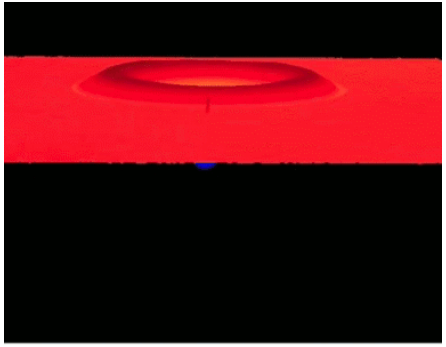
- Acquisition time, down to 10 µs



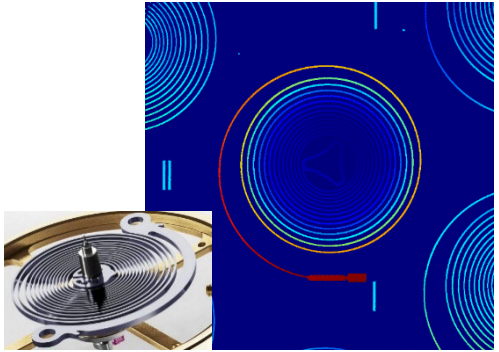
DHM® measurements are not sensitive to surrounding vibrations



Measurements in presence of turbulent air: Micro hot plate varying from 20°C to 800°C DHM® have been used from -196°C to 1500°C



"On flight " measurement of moving samples for fast quality control and screening



Watch spiral spring: a "never static" suspended structures to be caught

Excellent quality control capabilities

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4D: Time-resolved 3D measurements (real-time)

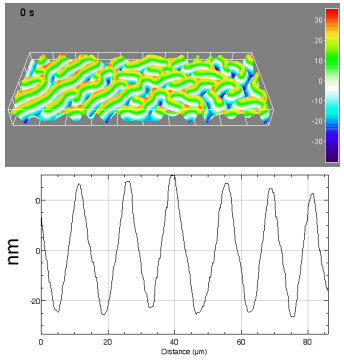
- Acquisition rate standard 194 fps, up to 100’000 fps (full field)

And also

- Evaporation, melting, …
- Dissolution
- Electro - magnetic force
- Measure as you manufacture

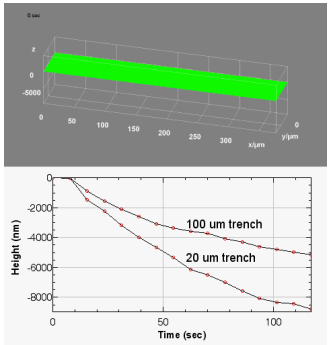
Investigate response of you sample to:

Light irradiance



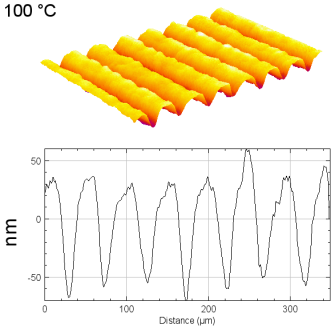
Smart polymer response to UV light excitation

Chemical action



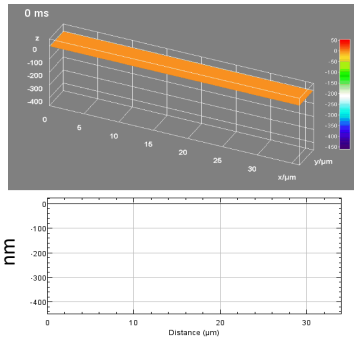
End-point in-situ measurement during Electrochemical etching

Temperature



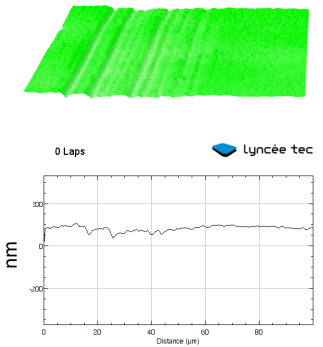
Programmed Liquid Cristal elastomer temperature response

Pressure



Investigation of mechnical properties of graphene membranes

Mechanical wear



Wear track by ball-on-disk vacuum tribometer measured in-situ

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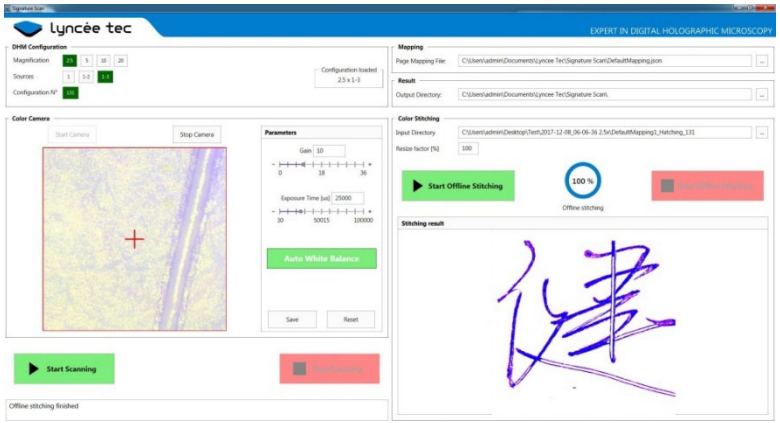
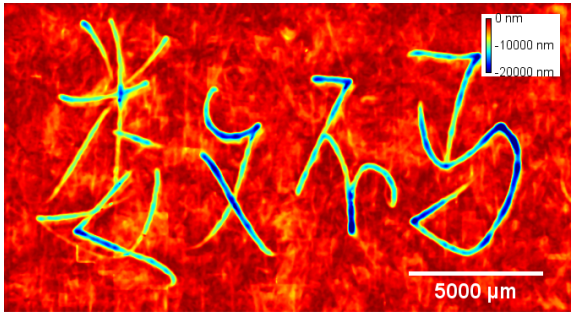
4D: Time-resolved 3D measurements (real-time)

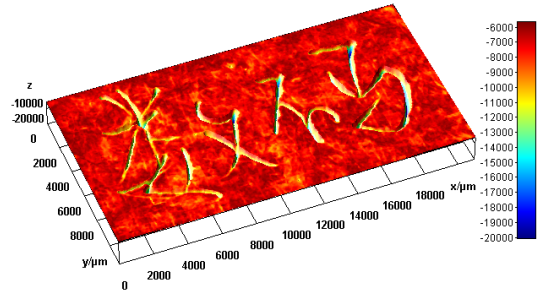
- Acquisition rate standard 194 fps, up to 100’000 fps (full field)

Characterize large surfaces

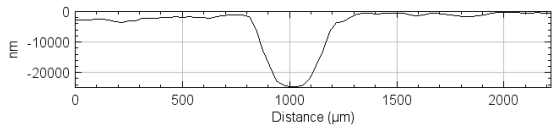
Forensic: automated signature 3D mapping







- Fast scanning of a large area of paper (**2 cm x 5 cm**) (3000 images, <60 s)
- Customized UI for simultaneous white light & DHM image acquisition



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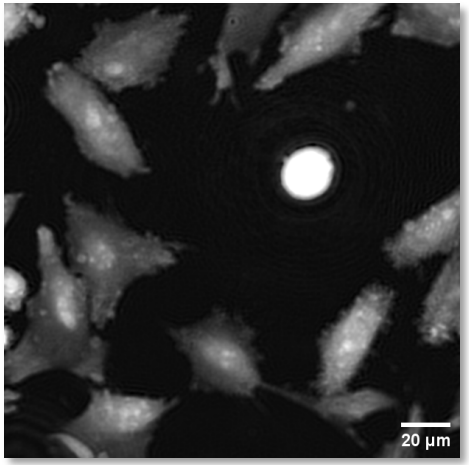
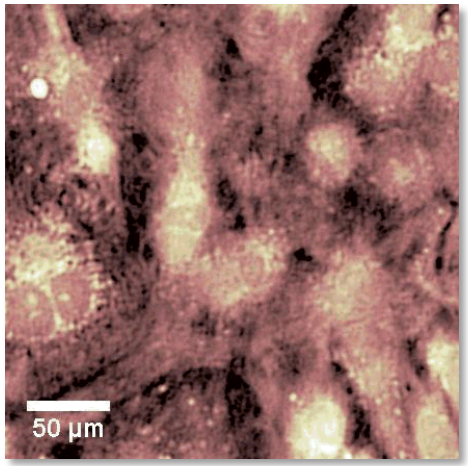
Life sciences applications

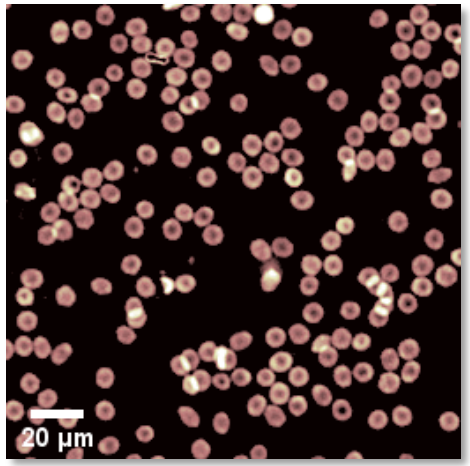
Measure, discover and publish with **Lyncée Tec DHM®**



HeLa cells with Colchicine



Beating cardiomyocytes



Flowing RBC

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Digital Holographic Microscopy (DHM®)

What is it?

A **label- free optical microscope** to observe:

- Cells
- 3D spheroids/organoids
- Swimmers (4D tracking)

What is measured?

Holography measures the phase of light which contains info on:

- **XYZ position:** localization and trajectories
- **thickness**: shape and morphology
- **dry mass**: intracellular content

What can be investigated with these two endogenous markers

- cell health status
- dynamic bioprocesses

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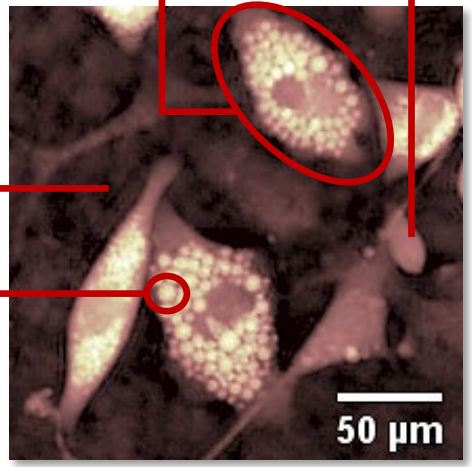
Unique advantages of DHM®

Shape and morphology

Cell health

No marker
no cells washing

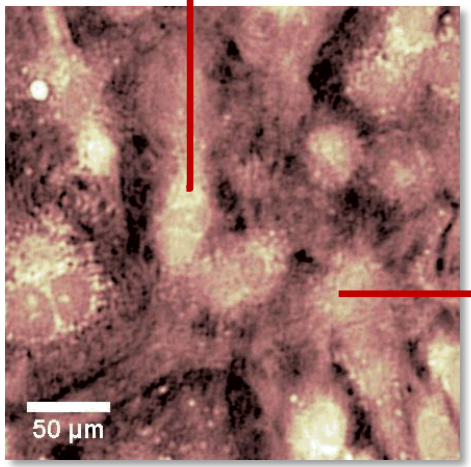
Organelles identification and quantification



Lipid droplets in adipocytes

High-speed (194 fps) dynamic recordings

Non-perturbing measurements



Beating cardiomyocytes

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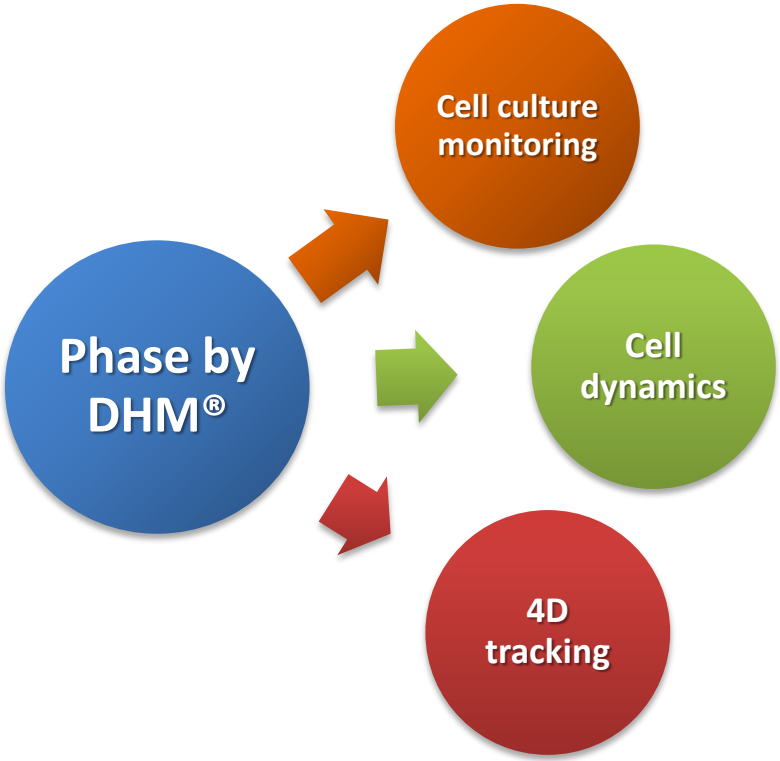
Three key application categories



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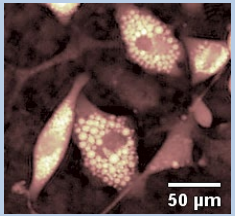
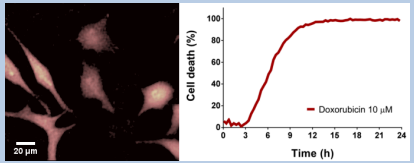
Morphological studies



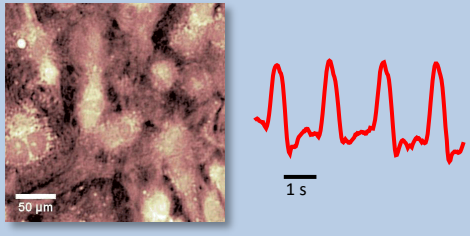
Quantification of lipid production in adipocytes

Cytotoxic assays



HeLa cell death quantification in presence of doxorubicin

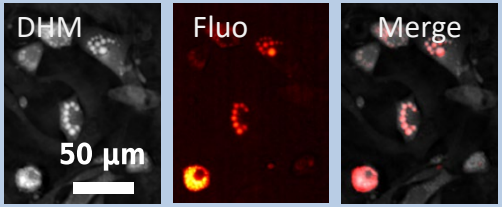
Cell dynamics



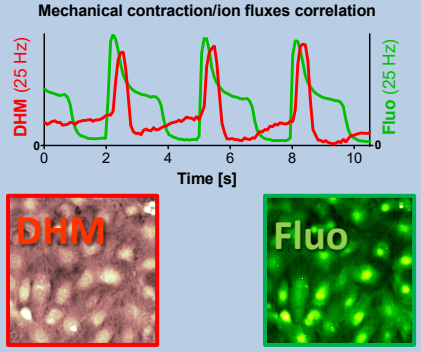
Cardiomyocytes beating cycle quantification

Combine with fluorescence

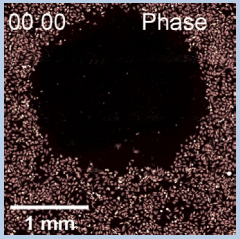
✓ **Colocalize specific structures**



✓ **Monitor dynamic ion concentration changes**

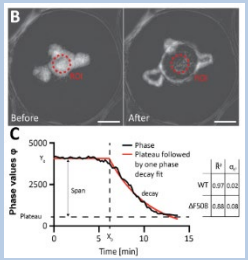


Migration/proliferation



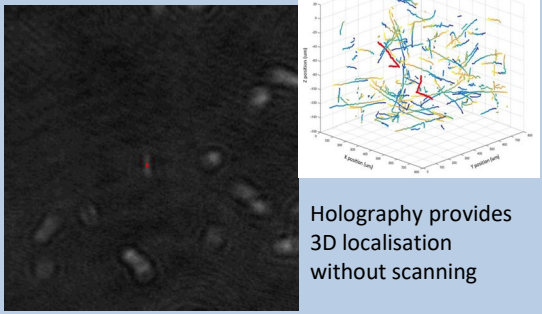
Cell migration inhibition with Cytochalasin D

Spheroids/Organoids



Water influx monitoring upon treatment with fsk.

4D tracking



Holography provides 3D localisation without scanning

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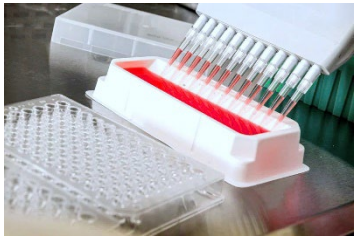
Summary and take-home message

A simple workflow



1. Culture your cells as usual

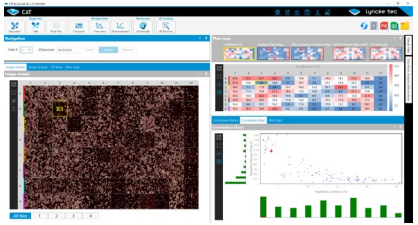
NO NEEDS of:

- specific substrate
- cells staining
- extra pipetting and washing steps

2. Acquisition single or multisite

FROM milliseconds, image captured at camera rate

TO multi-days time lapse



3. Investigate bio processes

CAT software for bioanalysis

provide automatically **data, plots,** and **reports** of your experiment

Morphology and intracellular content (dry mass) measurements without modifying cell behavior

✓ **two key biomarkers** enable monitoring of living cells **health status** and of ongoing **biological processes** that cannot be measured with other methods

Contact information

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DHM® by Lyncée scientific recognition

DHM method and technical developments

- Digital holographic microscopy: a noninvasive contrast imaging technique allowing quantitative visualization of living cells with subwavelength axial accuracy. Marquet P, Rappaz B, Magistretti PJ, Cuche E, Emery Y, Colomb T, Depeursinge C. Opt Lett. 2005 Mar 1;30(5):468-70. doi: 10.1364/ol.30.000468. PMID: 15789705
- Submicrometer tomography of cells by multiple-wavelength digital holographic microscopy in reflection. Kühn J, Montfort F, Colomb T, Rappaz B, Moratal C, Pavillon N, Marquet P, Depeursinge C. Opt Lett. 2009 Mar 1;34(5):653-5. doi: 10.1364/ol.34.000653.
- Living specimen tomography by digital holographic microscopy: morphometry of testate amoeba. Charrière F, Pavillon N, Colomb T, Depeursinge C, Heger TJ, Mitchell EA, Marquet P, Rappaz B. Opt Express. 2006 Aug 7;14(16):7005-13. doi: 10.1364/oe.14.007005.
- Digital holographic microscopy: a noninvasive contrast imaging technique allowing quantitative visualization of living cells with subwavelength axial accuracy. Marquet P, Rappaz B, Magistretti PJ, Cuche E, Emery Y, Colomb T, Depeursinge C. Opt Lett. 2005 Mar 1;30(5):468-70. doi: 10.1364/ol.30.000468.
- Measurement of the integral refractive index and dynamic cell morphometry of living cells with digital holographic microscopy. Rappaz B, Marquet P, Cuche E, Emery Y, Depeursinge C, Magistretti P. Opt Express. 2005 Nov 14;13(23):9361-73. doi: 10.1364/opex.13.009361.

"Optical patch clamp"

- Simultaneous optical recording in multiple cells by digital holographic microscopy of chloride current associated to activation of the ligand-gated chloride channel GABA(A) receptor. Jourdain P, Boss D, Rappaz B, Moratal C, Hernandez MC, Depeursinge C, Magistretti PJ, Marquet P. PLoS One. 2012;7(12):e51041. doi: 10.1371/journal.pone.0051041. Epub 2012 Dec 7.
- Determination of transmembrane water fluxes in neurons elicited by glutamate ionotropic receptors and by the cotransporters KCC2 and NKCC1: a digital holographic microscopy study. Jourdain P, Pavillon N, Moratal C, Boss D, Rappaz B, Depeursinge C, Marquet P, Magistretti PJ. J Neurosci. 2011 Aug 17;31(33):11846-54. doi: 10.1523/JNEUROSCI.0286-11.2011.
- The human CFTR protein expressed in CHO cells activates aquaporin-3 in a cAMP-dependent pathway: study by digital holographic microscopy. Jourdain P, Becq F, Lengacher S, Boinot C, Magistretti PJ, Marquet P. J Cell Sci. 2014 Feb 1;127(Pt 3):546-56. doi: 10.1242/jcs.133629. Epub 2013 Dec 11.

High-content/high-throughput screening

- Digital holographic microscopy: a quantitative label-free microscopy technique for phenotypic screening. Rappaz B, Breton B, Shaffer E, Turcatti G. Comb Chem High Throughput Screen. 2014 Jan;17(1):80-8. doi: 10.2174/1386207311316660062.
- Label-free cytotoxicity screening assay by digital holographic microscopy. Kühn J, Shaffer E, Mena J, Breton B, Parent J, Rappaz B, Chambon M, Emery Y, Magistretti P, Depeursinge C, Marquet P, Turcatti G. Assay Drug Dev Technol. 2013 Mar;11(2):101-7. doi: 10.1089/adt.2012.476. Epub 2012 Oct 12.
- Image-Based Marker-Free Screening of $GABA_A$ Agonists, Antagonists, and Modulators. Rappaz B, Jourdain P, Banfi D, Kuttler F, Marquet P, Turcatti G. SLAS Discov. 2020 Jun;25(5):458-470. doi: 10.1177/2472555219887142. Epub 2019 Nov 28.

Hematology

- Spatial analysis of erythrocyte membrane fluctuations by digital holographic microscopy. Rappaz B, Barbul A, Hoffmann A, Boss D, Korenstein R, Depeursinge C, Magistretti PJ, Marquet P. Blood Cells Mol Dis. 2009 May-Jun;42(3):228-32. doi: 10.1016/j.bcmd.2009.01.018. Epub 2009 Mar 25.
- Comparative study of human erythrocytes by digital holographic microscopy, confocal microscopy, and impedance volume analyzer. Rappaz B, Barbul A, Emery Y, Korenstein R, Depeursinge C, Magistretti PJ, Marquet P. Cytometry A. 2008 Oct;73(10):895-903. doi: 10.1002/cyto.a.20605.
- Quantification of stored red blood cell fluctuations by time-lapse holographic cell imaging. Jaferzadeh K, Moon I, Bardyn M, Prudent M, Tissot JD, Rappaz B, Javidi B, Turcatti G, Marquet P. Biomed Opt Express. 2018 Sep 10;9(10):4714-4729. doi: 10.1364/BOE.9.004714. eCollection 2018 Oct 1.
- Red blood cells ageing markers: a multi-parametric analysis. Bardyn M, Rappaz B, Jaferzadeh K, Crettaz D, Tissot JD, Moon I, Turcatti G, Lion N, Prudent M. Blood Transfus. 2017 May;15(3):239-248. doi: 10.2450/2017.0318-16.

Cytotoxicity

- Early cell death detection with digital holographic microscopy. Pavillon N, Kühn J, Moratal C, Jourdain P, Depeursinge C, Magistretti PJ, Marquet P. PLoS One. 2012;7(1):e30912. doi: 10.1371/journal.pone.0030912. Epub 2012 Jan 31.

Biophysics

- Noninvasive characterization of the fission yeast cell cycle by monitoring dry mass with digital holographic microscopy. Rappaz B, Cano E, Colomb T, Kühn J, Depeursinge C, Simanis V, Magistretti PJ, Marquet P. J Biomed Opt. 2009 May-Jun;14(3):034049. doi: 10.1117/1.3147385.

Cardiomyocytes

- Automated multi-parameter measurement of cardiomyocytes dynamics with digital holographic microscopy. Rappaz B, Moon I, Yi F, Javidi B, Marquet P, Turcatti G. Opt Express. 2015 May 18;23(10):13333-47. doi: 10.1364/OE.23.013333.

Lipid Droplets

- High-throughput, nonperturbing quantification of lipid droplets with digital holographic microscopy, Campos et al. J Lipid Res. 2018 Jul;59(7):1301-1310. doi: 10.1194/jlr.D085217