Past, Present and Future of Data in Biophotonics

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Laserlab-Europe / ELI Workshop
Better data for better science

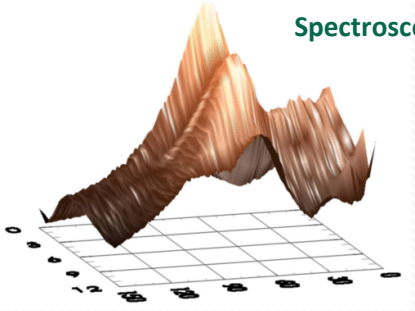
Laserlab Europe

eli

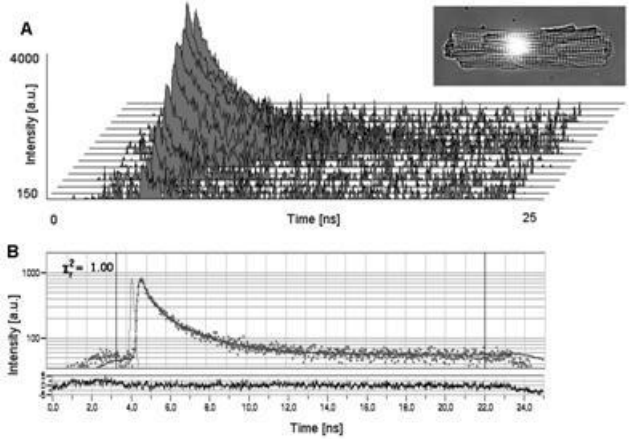
Before 2000

„Classical“ imaging, spectroscopy and time-resolved studies

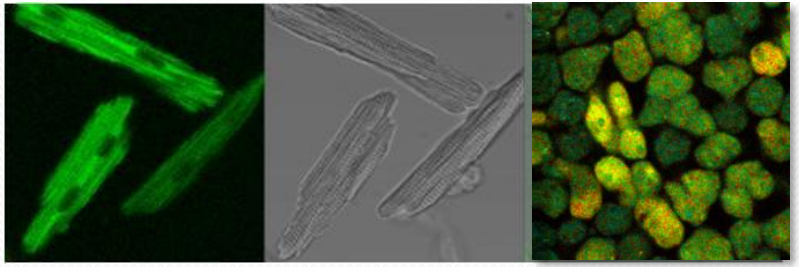
Spectroscopy



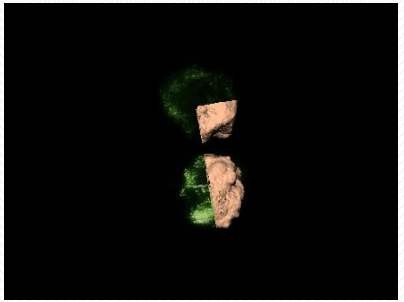
Time- resolved spectroscopy



Level: individual setups and laboratories

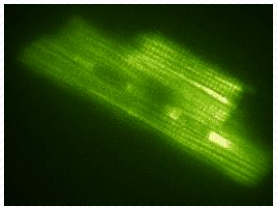


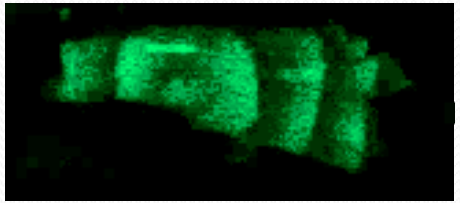
Confocal / fluorescence /DIC / BF ..



3D imaging and vizualization

Imaging dynamics in living cells

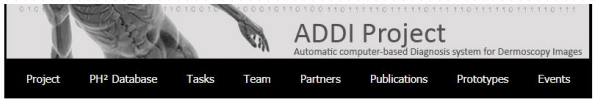




2000-2020

Development from local databases to multi-national global initiatives

PH² database



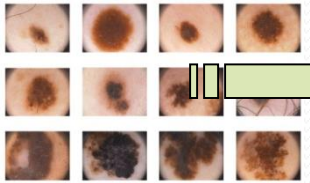
Prototypes

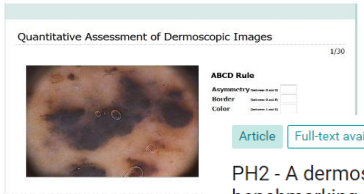
Qadi - Quantitative Assessment of Dermoscopic Images

A web application developed to collect clinical diagnosis of specialists on dermoscopic images of pigmented skin lesions through Abcd Rule and 7 Point Checklist clinical algorithms. Go to Qadi application here.

Next Events

Computer vision and Machine learning events:





Article | Full-text available

PH2 - A dermoscopic image database for research and benchmarking

July 2013 · Conference proceedings: ... Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Conference 2013:5437-5440

DOI: 10.1109/EMBC.2013.6610779

Source · PubMed

Teresa Mendonça · Pedro M Ferreira · Jorge S Marques · Show all 5 authors · Jorge Rozeira

Level: network of labs

Human Brain Project

HBP offers a comprehensive **management and validation** of all data and metadata before it is uploaded and made available in the Knowledge Graph search.

How to share my data

Explore neuroscience datasets shared through HBP's Knowledge Graph **data sharing repository**.

Share Data

Browse through a collection of **HBP supported tools** (reference atlases, elephant, ilastik) to visualise, combine and investigate data.

How to use our data

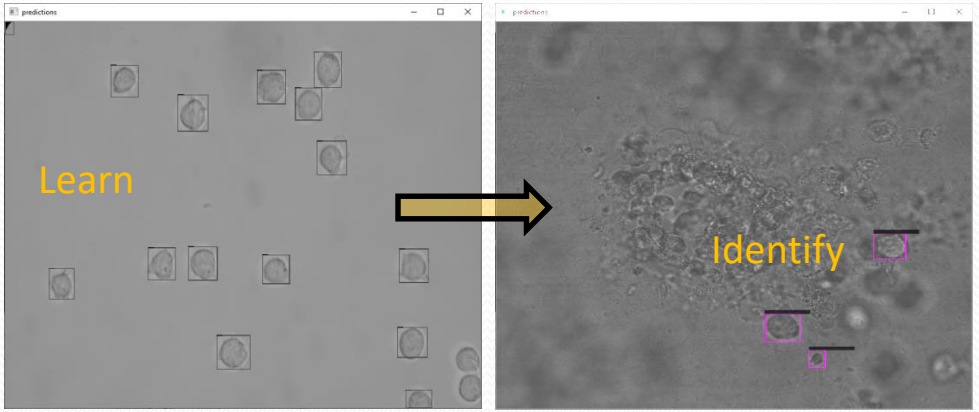
Level: networks of institutes

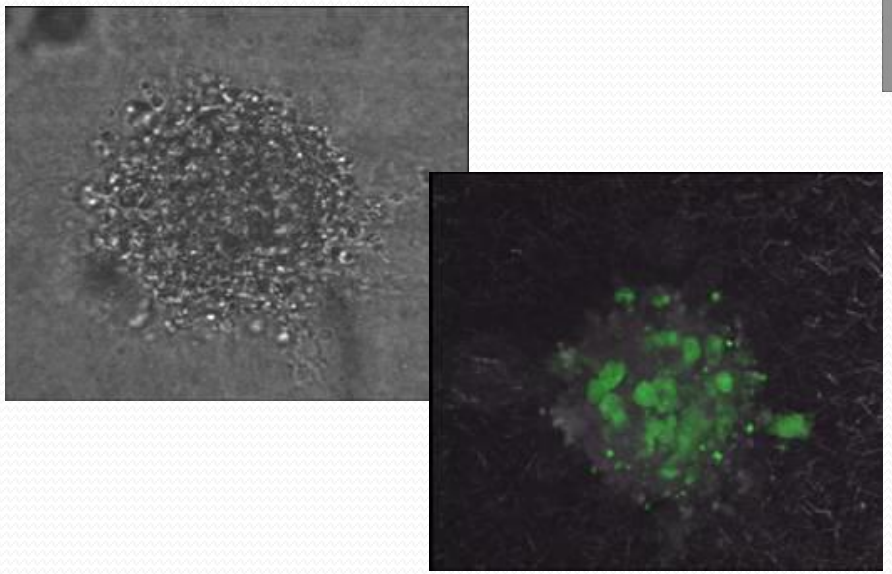
2020+

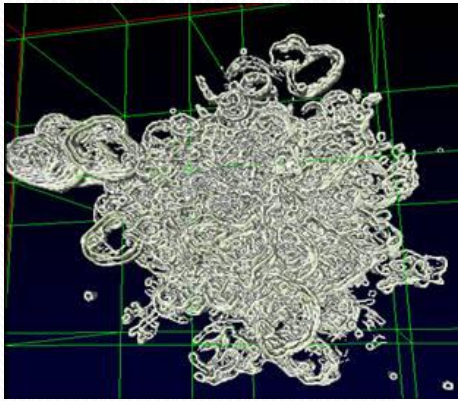
Computational microscopy

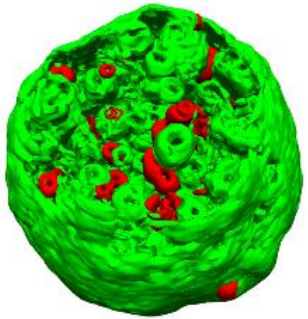
Big multimodal microscopy datasets

- Automatically revealing patterns, shapes, relations
- Bridging the microworld with the macroworld
- Teaching a microscope to understand what it is looking at





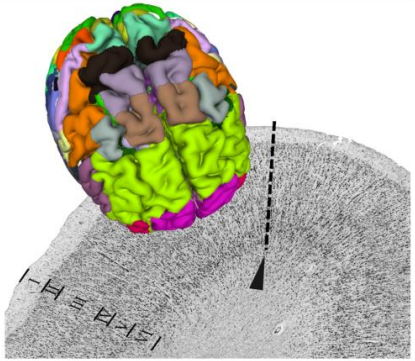




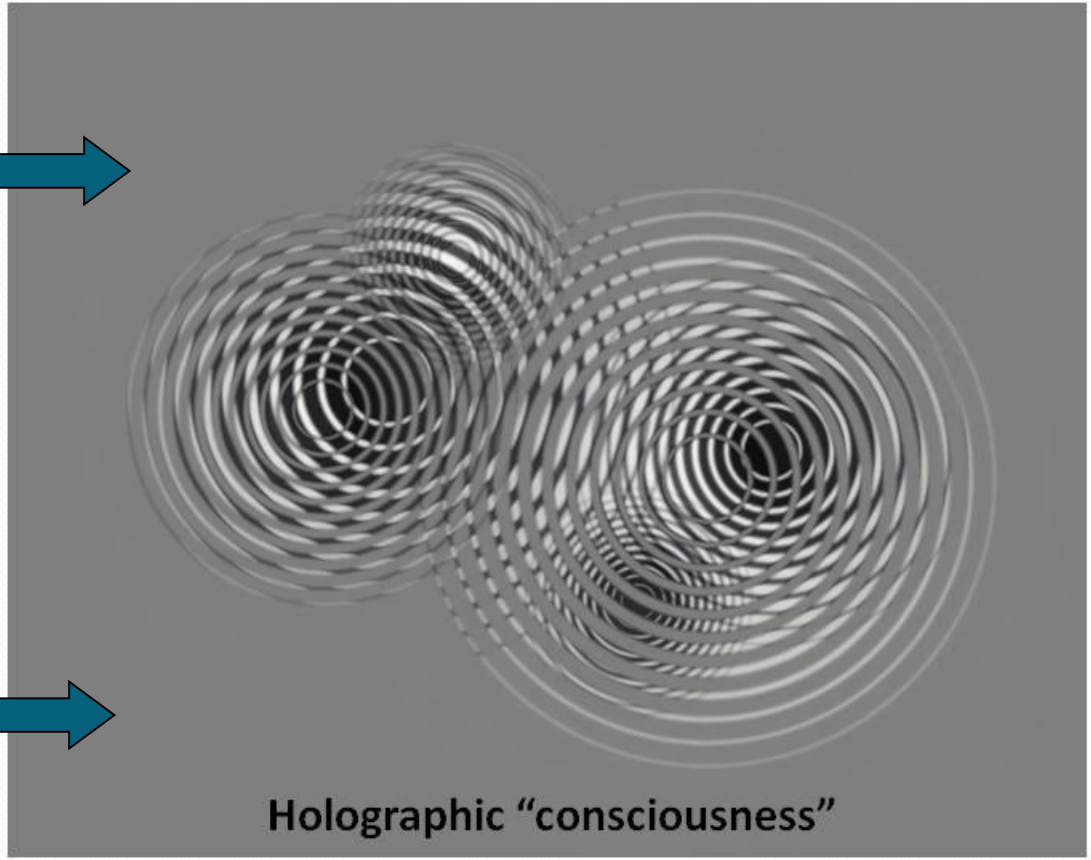
20xx

Pattern recognition using quantum computers

Quantum computers



Understanding of Human Brain

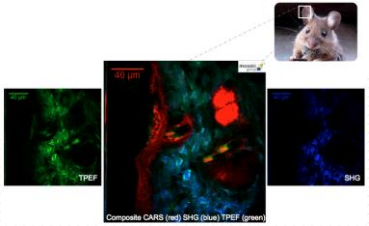


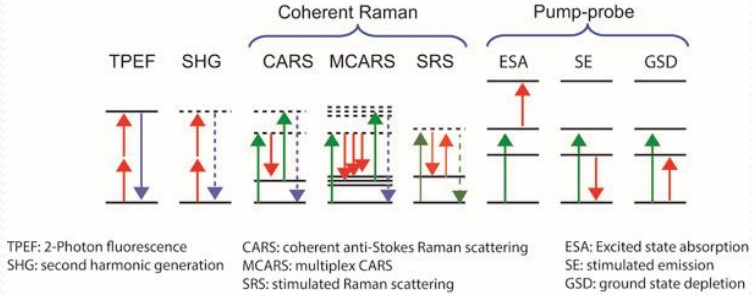
Examples from Laserlab Europe research in Biophotonics

JRA ALTIS, Objective 1

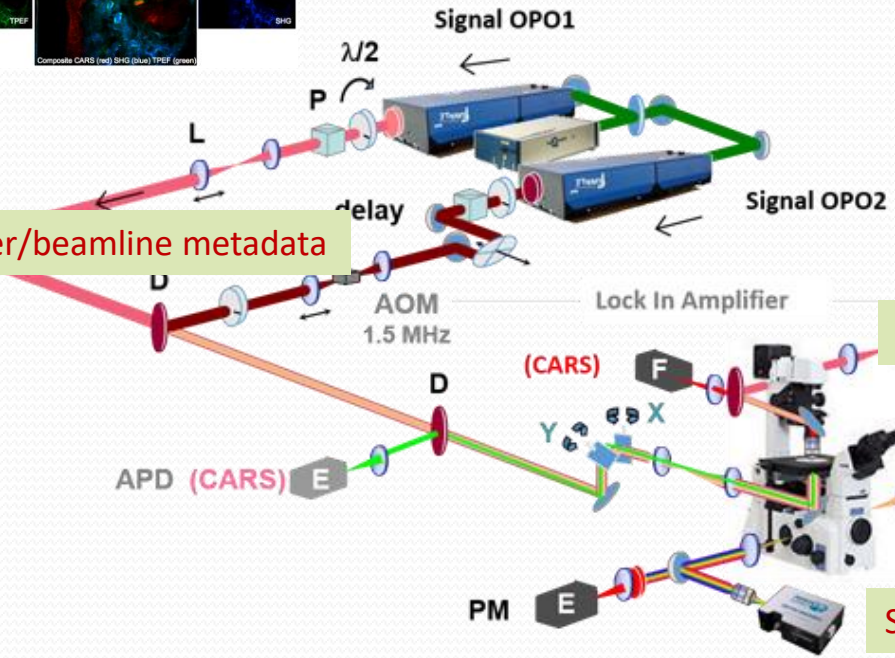
Task 1.3 *Label-free spectroscopy and imaging*

Optical microscopy today





Laser/beamline metadata

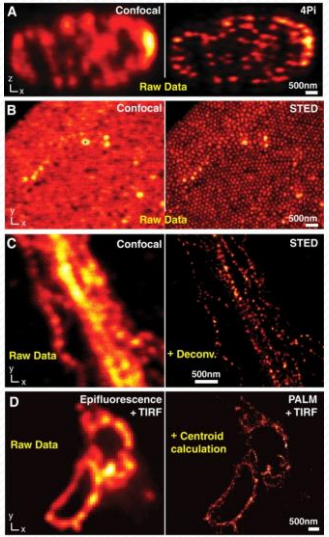


Microscope metadata

Sample/conditions metadata

TPEF, SHG, CARS, SRS multimodal microscope

H. Rigneault et al., www.fresnel.fr/spip/spip.php?article1691



Nobel prize for chemistry 2014
Erik Betzig, Stefan W. Hell, William E. Morner
Nobel Prize for Physics 2018
G.Mourou, D.Strickland, A.Ashkin

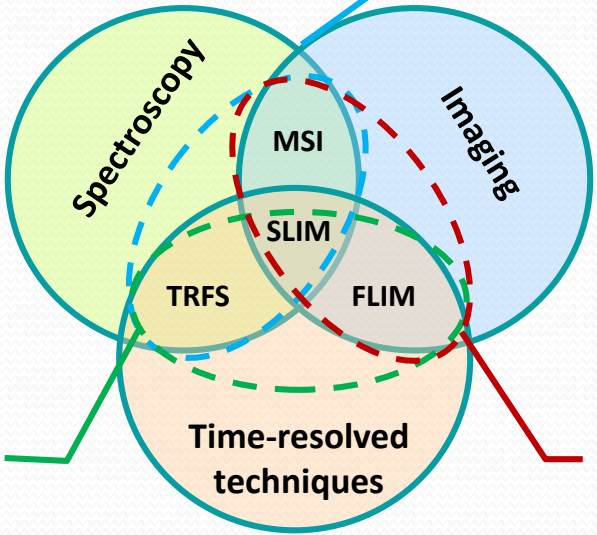
Detection strategies for multi-modal imaging

Spectral decomposition

WHAT

Composition >> Structure

molecular environment classification



Spatial distribution >> Shape

specificity & labeling segmentation

Time-series, modulation lifetime analysis

HOW

Dynamics >> Function

rates, timing, synchrony analysis of behavior

Image analysis / pattern recognition

WHERE

Why the data sharing is important for us

The problem

Different experimental setups often leads to different values / parameters of the same subject.

AIMS

- to create a database of optical fingerprints and features of molecular, cellular and tissue samples obtained by experimental techniques for **label-free imaging and spectroscopy**
- to build a **coherent source for meta-analysis** of **validated data** from different labs
- to reveal **correlations between recorded optical signals and their molecular origins**, and physiological, structural and chemical changes in cells and tissues

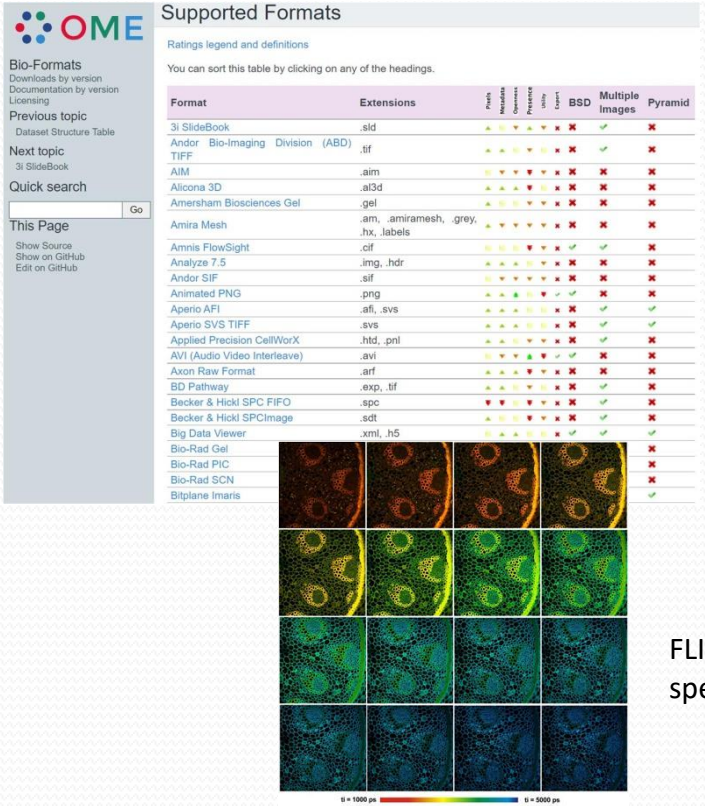
Selected experimental targets>

Properties and orientation of **collagen fibers**, imaged by NLO/SHG microscopy.

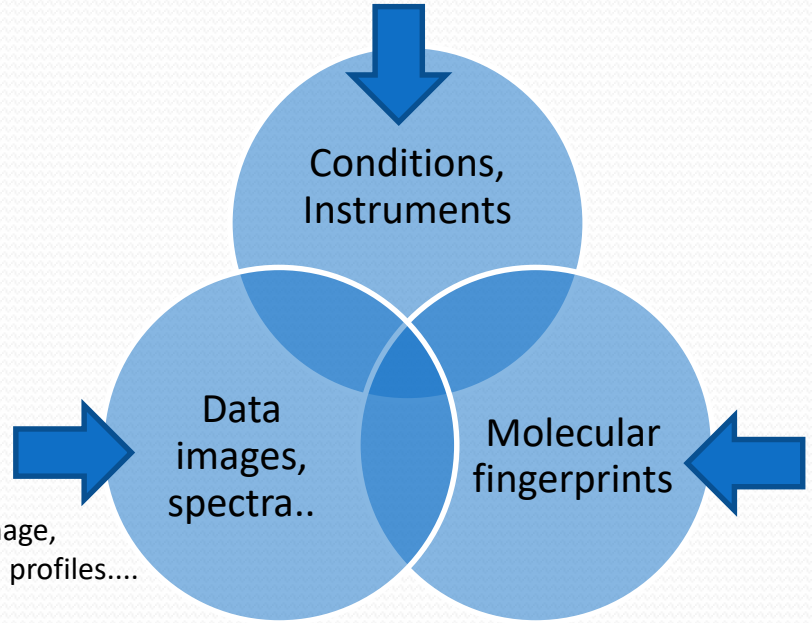
Properties of **NADH** in different physico/chemical environments - model of mechanisms of deexcitation.

Solution: Multi-modal image database

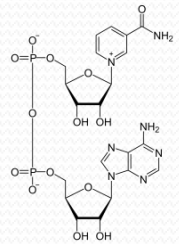
The Open Microscopy Environment



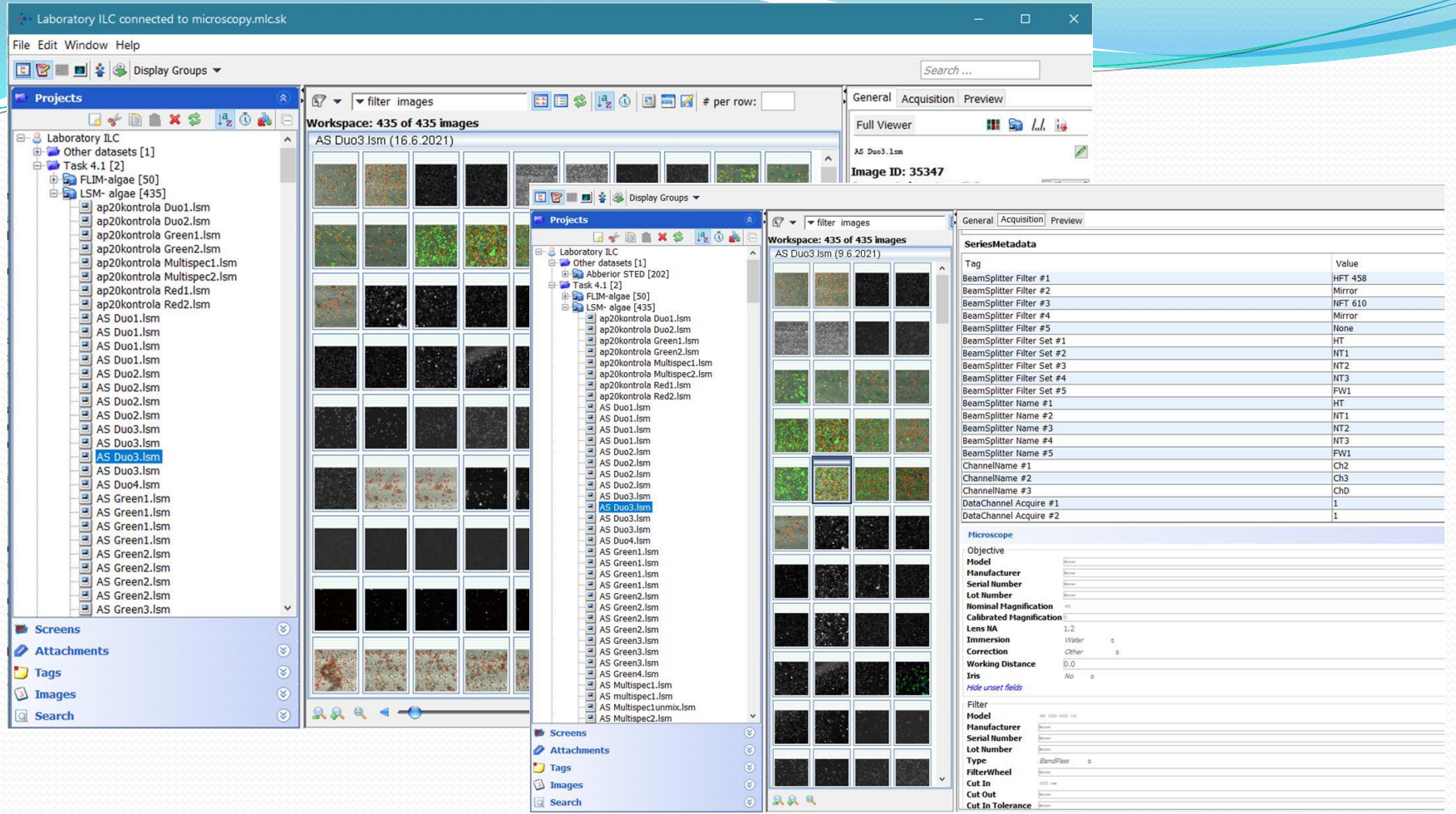
Dedicated database server with open-access database engine OMERO:
http://microscopy.mlc.sk/omero



FLIM image, spectral profiles....



Identified targets (collagen, NADH...)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File Edit Window Help
Display Groups
Search ...
Projects
Laboratory ILC
Other datasets [1]
Task 4.1 [2]
FLIM-algae [50]
LSM- algae [435]
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AS Duo1.lsm
AS Duo2.lsm
AS Duo3.lsm
AS Duo4.lsm
AS Green1.lsm
AS Green2.lsm
AS Green3.lsm
Screens
Attachments
Tags
Images
Search
filter images
per row:
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Acquisition
Preview
Full Viewer
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Value
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HFT 458
BeamSplitter Filter #2
Mirror
BeamSplitter Filter #3
NFT 610
BeamSplitter Filter #4
Mirror
BeamSplitter Filter #5
None
BeamSplitter Filter Set #1
HT
BeamSplitter Filter Set #2
NT1
BeamSplitter Filter Set #3
NT2
BeamSplitter Filter Set #4
NT3
BeamSplitter Filter Set #5
FW1
BeamSplitter Name #1
HT
BeamSplitter Name #2
NT1
BeamSplitter Name #3
NT2
BeamSplitter Name #4
NT3
BeamSplitter Name #5
FW1
ChannelName #1
Ch2
ChannelName #2
Ch3
ChannelName #3
Ch0
DataChannel Acquire #1
1
DataChannel Acquire #2
1
Microscope
Objective
Model
Manufacturer
Serial Number
Lot Number
Nominal Magnification
Calibrated Magnification
Lens NA
1.2
Immersion
Water
Correction
Other
Working Distance
0.0
Iris
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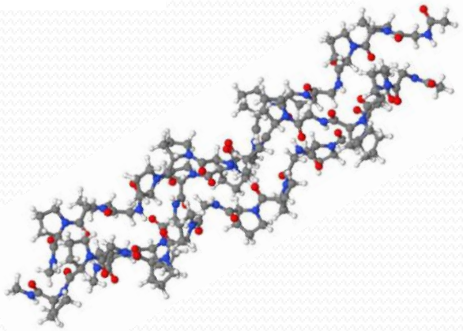
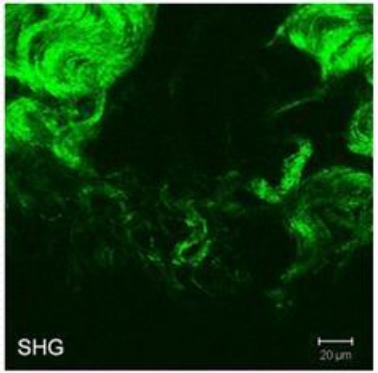
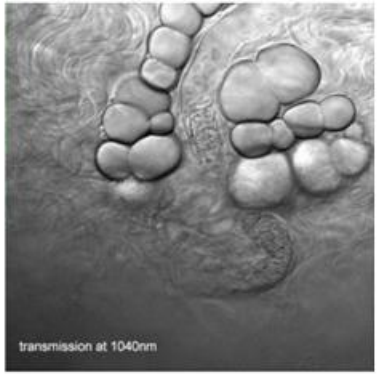
Manufacturer
Serial Number
Lot Number
Type
BandPass
FilterWheel
Cut In
Cut Out
Cut In Tolerance

Analysis of collagen orientation in tissues







SHG is a well-established technique for chracterizing collagen morphology and its organization. However, despite SHG is becoming more and more popular especially among pathologists, a standard method for measuring morphological features of collagen with a quantitative and operator-independent manner **has not been defined yet.**

Problem: it is difficult to compare results obtained in different labs, as the extracted morphological features might be affected by the experimental conditions. Having a standardized method for analysing data is highly desirable and is currently under development.

Methods proposed for analysing SHG images

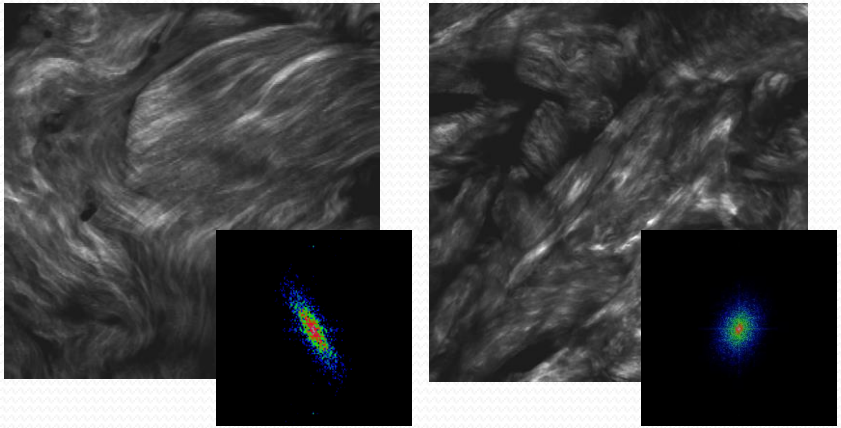
Fast-Fourier Transform (FFT) and Curvelet transform (CT)

FFT approach is useful to perform an analysis of the spatial frequency components of an image. The distribution of spatial frequencies in the FFT can be used to infer information on the pattern of the original image, hence to characterize the geometry of the image texture.

Curvelets represent a generalization of Fourier Transform (FT) that can be used for denoising images and enhancing fiber-edge features. Such property can be exploited by fiber extraction (FIRE) algorithms.

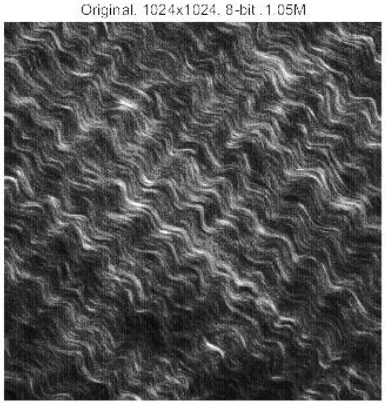
Healthy dermis

Keloid

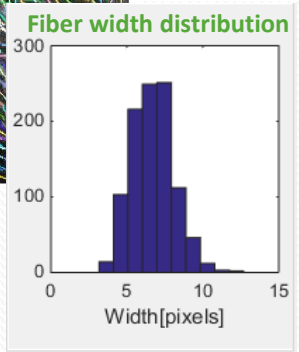


Cicchi et al, J Biophoton (2010)

Equine pericardium



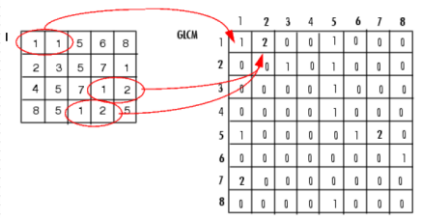
Fiber extraction



monitoring of the fiber anisotropy, length and orientation

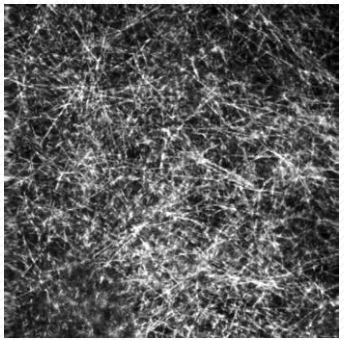
Grey-level Co-occurrence Matrix (GLCM)

The **GLCM** represents the relationships between neighbors of an intensity matrix (e.g. an image). GLCM-derived functions describe image properties such as contrast and correlation between adjacent pixels.



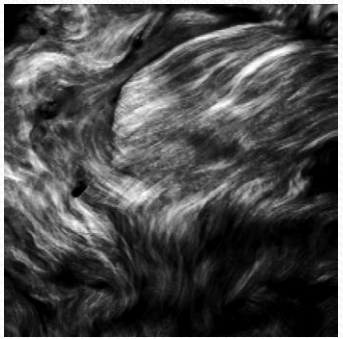
The analysis of the GLCM-correlation function can be used to evaluate the typical size of supra-molecular formations (e.g. the diameter of collagen fibers observed in Second-Harmonic Generation images).

Artificial collagen



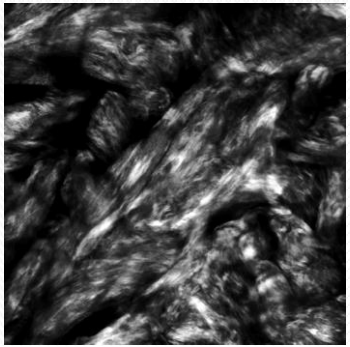
Corr. Length:
1.0 ± 0.1 mm

Healthy dermis

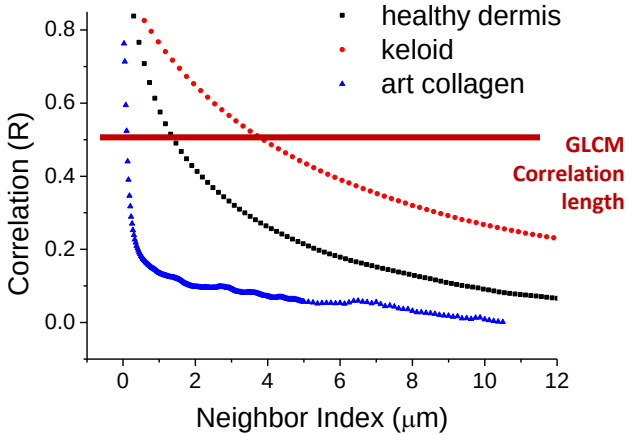


Corr. Length:
3.7 ± 0.1 mm

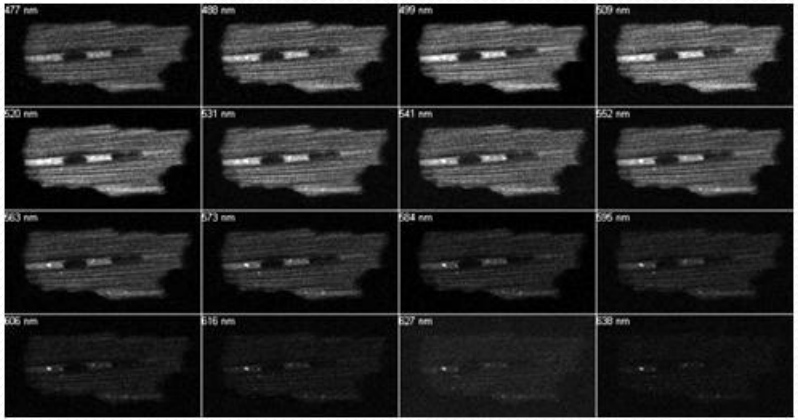
Keloid



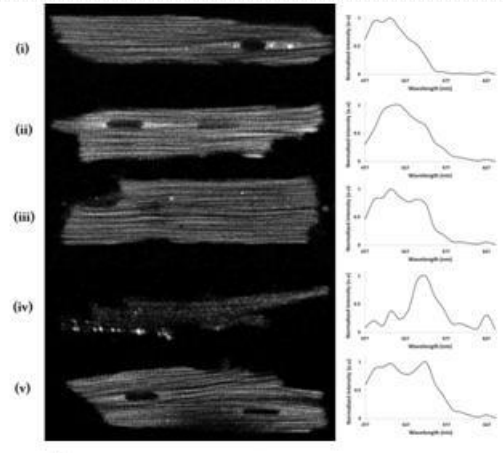
Corr. Length:
6.8 ± 0.1 mm



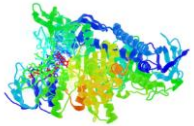
Spectral fingerprinting of cardiac myocytes – metabolic imaging

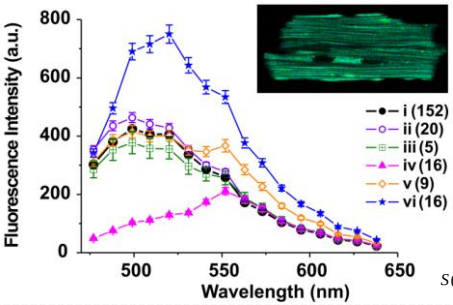


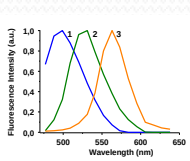
Machine Learning SVM



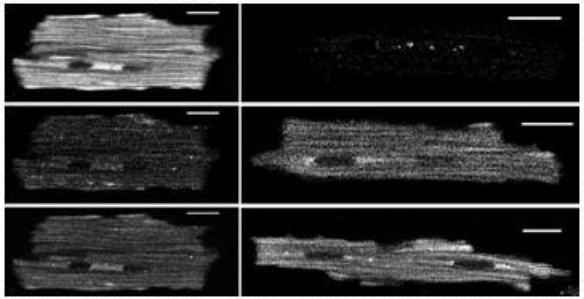
Quest for molecular origins and functional models

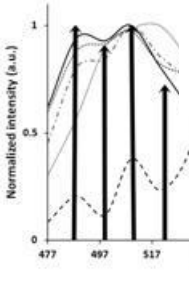






$$S(\lambda)_{sum} = \sum_{i=1}^{3} I_i \times S(\lambda)_i + S(\lambda)_b$$





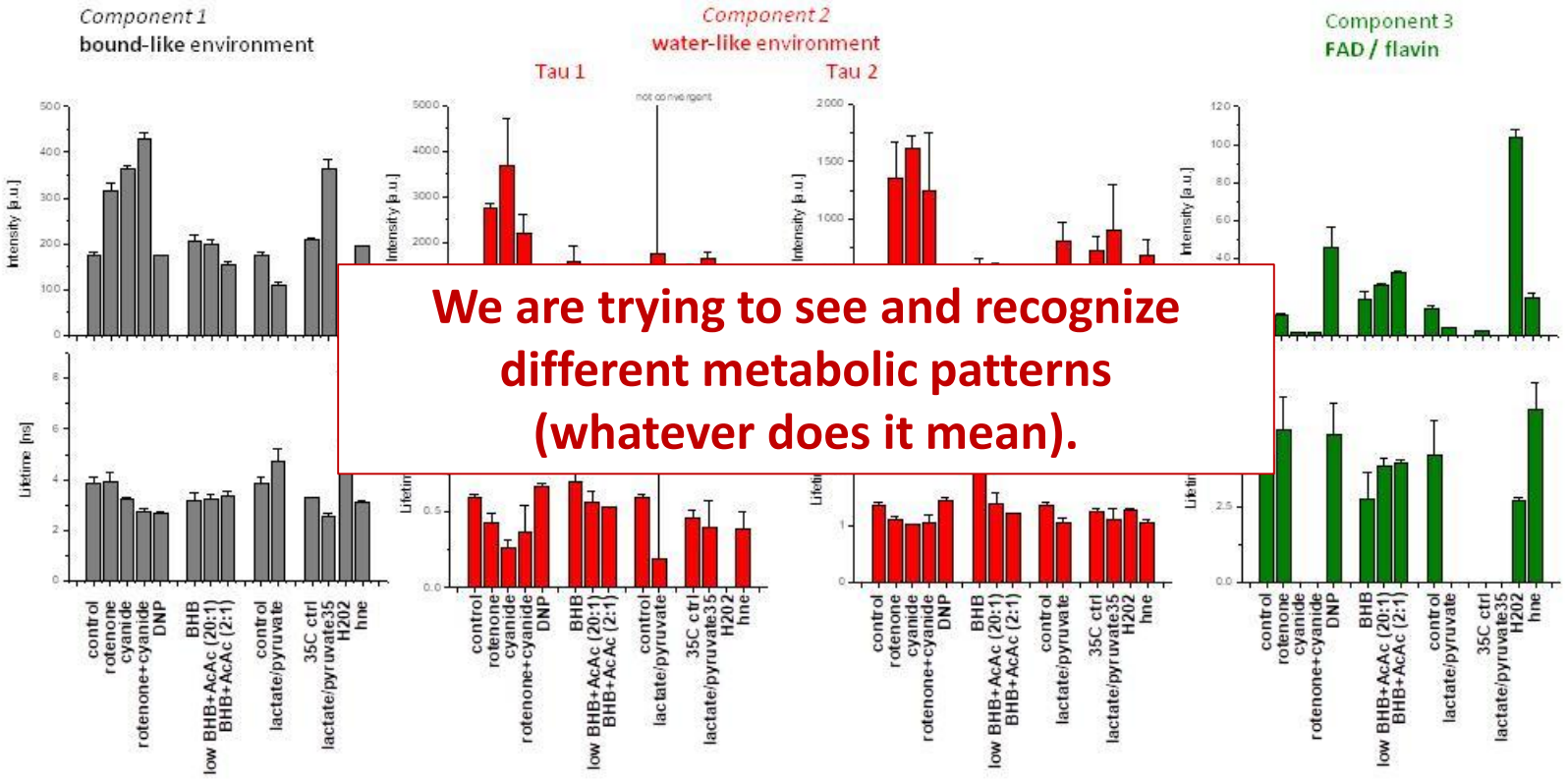
Peculiar studies with models unknown

Metabolic modulation and spectral unmixing „by hand“

Estimated state	SVM method	NMF method
% success	90 - 95	75 - 80

CHORVAT D. JR., KIRCHNEROVA J., CAGALINEC M., SMOLKA J., MATEASIK A., CHORVATOVA A., 2005: *Biophysical Journal* 89 (6): L55-L57.

Patterns of metabolic modulation - NAD(P)H in living cardiac cells



We are trying to see and recognize different metabolic patterns (whatever does it mean).

Effect of metabolic modulation on fluorescence decay components of cardiomyocyte AF.

The problems

Does better data lead to better science?

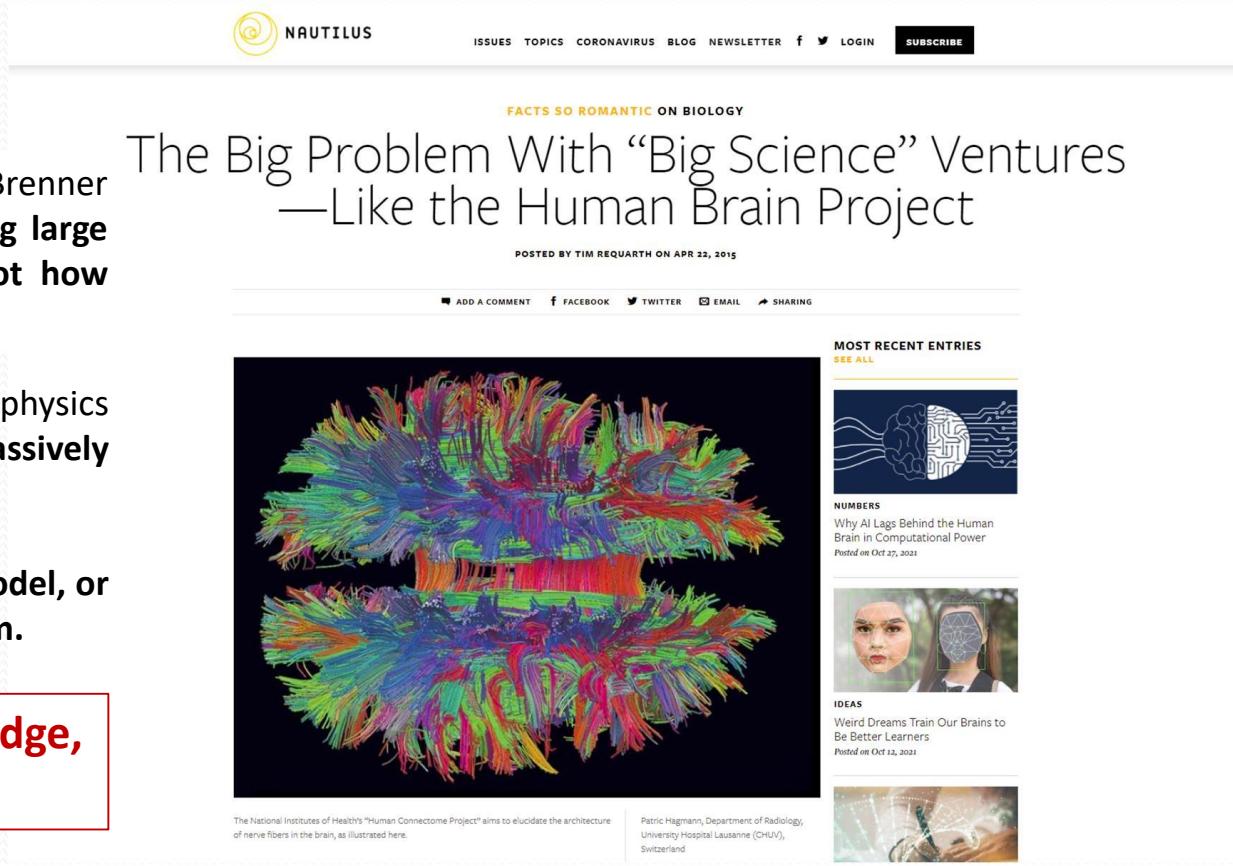
What we can learn from biology

In 2014, Nobel Prize laureate Sydney Brenner warned that just focusing on **generating large amounts of very expensive data is not how progress in science is made.**

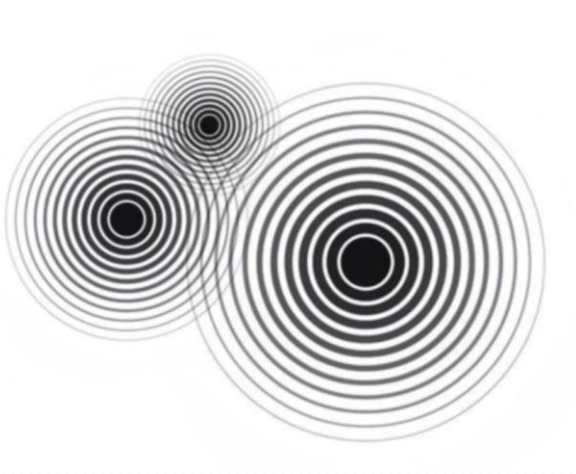
Compared to (even very large) physics experiment, the biological system is **massively more complex.**

Currently we have **no proper tools to model, or even to understand such complex system.**

We miss fundamental knowledge, no (big) data!



What we can learn from physics



The Data

Scientist 1 viewpoint

Publication / hypothesis 1

Does the different results mean these data or hypotheses are wrong?

Scientist 2 viewpoint

Publication / hypothesis 2

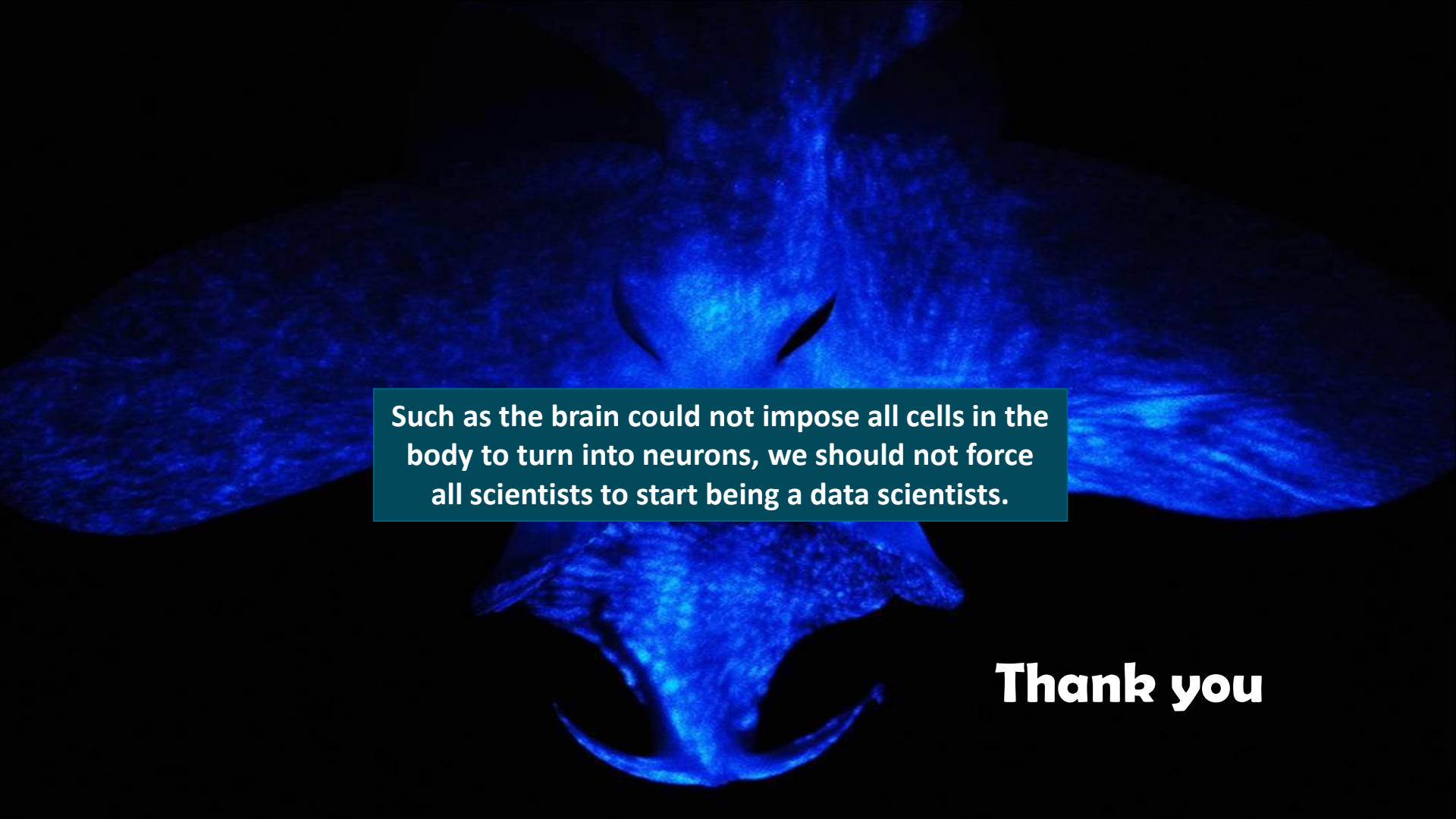
What we can learn from music

Both composing and interpreting are acts of creativity, not a routine!

ORIGINAL DATA, revealed by experiment / simulation

SCIENTIFIC PAPER – interpretation using different instruments

image source: en.wikipedia.org


Such as the brain could not impose all cells in the body to turn into neurons, we should not force all scientists to start being a data scientists.
Thank you