Welcome / Bienvenidos

Microscopía y Procesamiento de Imágenes

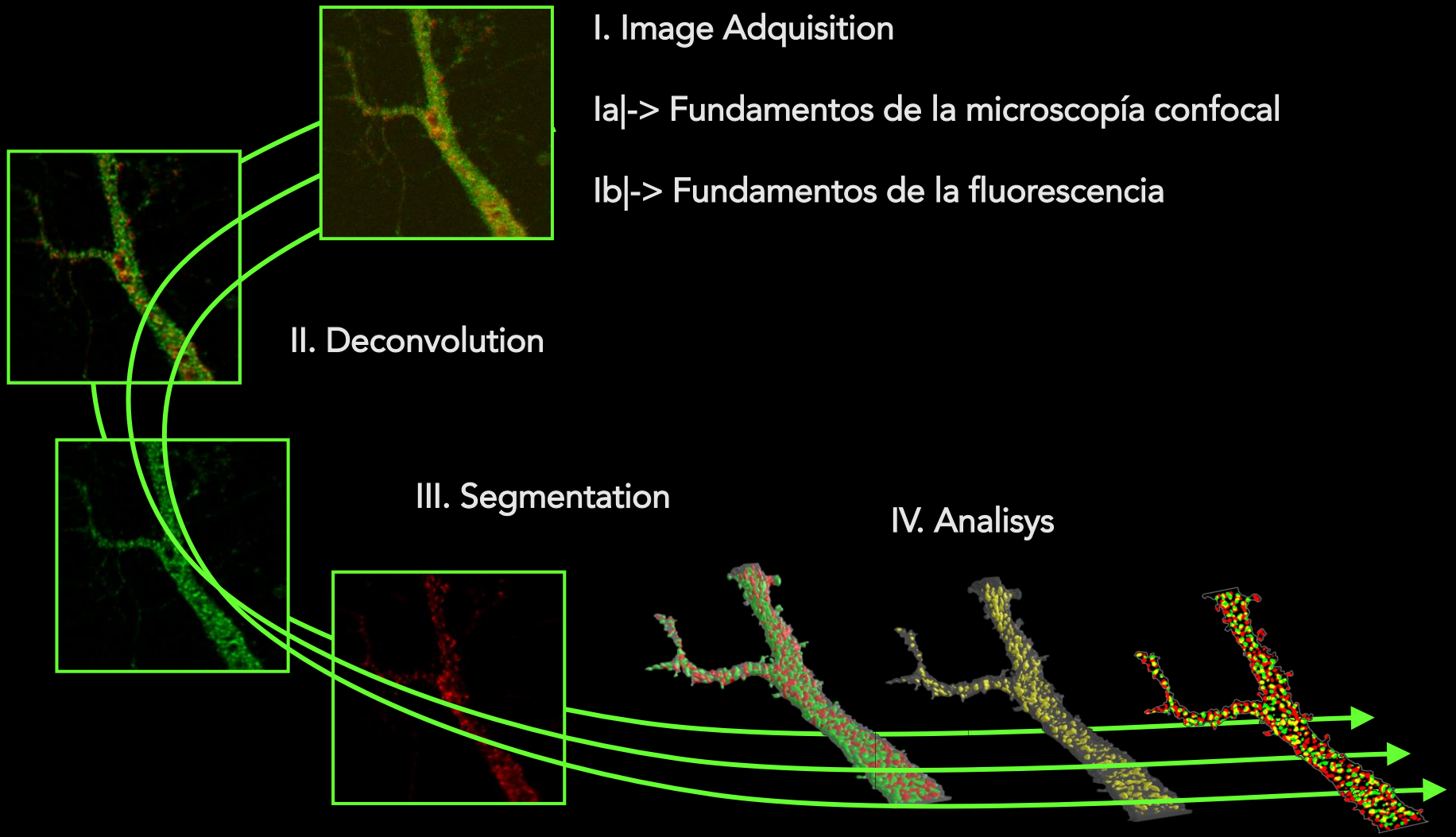
Prof. Dr. Steffen Härtel: www.scian.cl / www.cimt.cl / www.cens.cl

Laboratory for Scientific Image Analysis (SCIAN-Lab)
Centro de Informática Médica y Telemedicina (CIMT)
Centro Nacional en Sistemas de Información en Salud (CENS)
Biomedical Neuroscience Institute (BNI)
Institute of Biomedical Sciences (ICBM)
Anatomy and Developmental Biology Program
Escuela de Postgrado
Facultad de Medicina, Universidad de Chile

Laboratory for SCIAN Scientific Image Analysis

Welcome

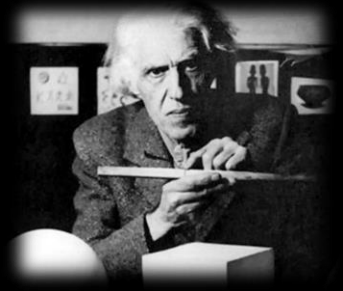
BIOMEDICAL NEUROSCIENCE INSTITUTE



Laboratory for SCIAN Scientific Image Analysis

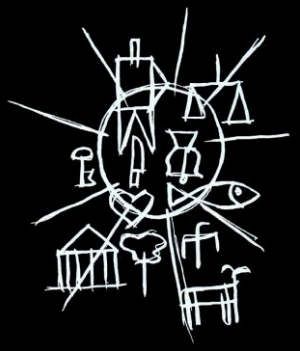
Welcome

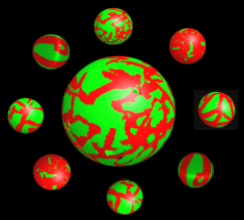
BIOMEDICAL NEUROSCIENCE INSTITUTE



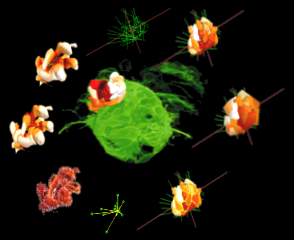
Joaquín Torres García 1874-1949

Richard Feynman (1918-1988)



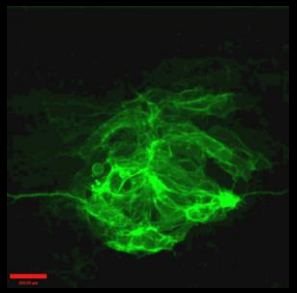


Mats Gustafson 2006 - 2011



Maria Goeppert-Mayer 1906-1972

René Descartes (1596-1650)

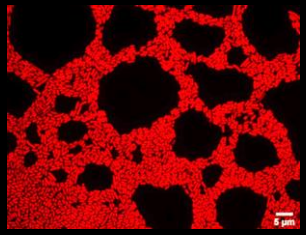


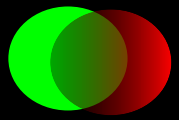
Ernst Abbe 1840- 2005

E Betzig

S Hell

W Moerner





Basic Science	Scientific Platforms	Human Capital Formation & Research in Medical Informatics	Institutes
FONDECYT ANID	FONDEF ANID	DAAD / DFG / STIC-AMSUD / NIH / BID	ICM / ICBM

SCIAN-Lab Members

PIs	●●	Biophysics / Computer Science
PostDocs / Young Academics	●●●●●	Biology / Computer Sc / Electric Engineer / Mathematics
PhD - students	●●●●	Computer Sc / Electric Engineer / Biochemistry
Master - students	●●●●●…	Medical Technology / Electric Engineer / Medical Informatics
Undergraduate	●●●●	Computer Science/Biology
Research – Assistants	●●●●●	Medicine / Computer Sc / Electrical Engineer / Biology
Technicians	●●●	Biotechnology / Labtechnician / Administration

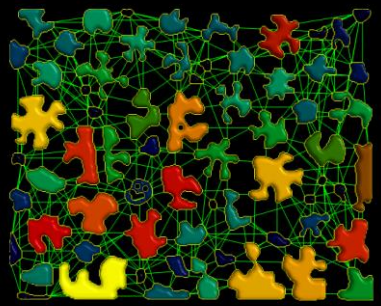
- Image Processing & Microscopy

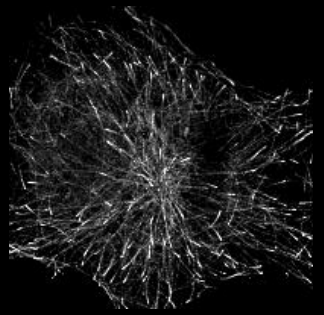
2D/3D · Morpho-topology, Motion estimation, Tracking
2021 Frontiers in Cellular and Infection Microbiology
2020 Journal of Microscopy
2018 Immunobiology
2017 Brain
2017 Nature Communications
2017 Developmental Dynamics
2016 Journal of Physics: Conference Series
2016 Cell Reports
2016 Pathogens and Disease
2015 Acta Tropica
2015 J of Clinical and Experimental Pathology
2015 The American Journal of Tropical Medicine
2014 Current Molecular Medicine
2014 Medical Image Analysis
2013 Development
2012 European Biophysics Journal
2012 PLosOne

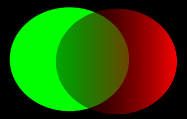
Colocalization
2021 Traffic
2021 Cell Calcium
2017 Frontiers Molecular Neuroscience
2015 Gene
2014 Chromatine Research
2014 Frontiers Molecular Neuroscience
2012 PLosOne

- (Bio)Medical Informatics

2021 Nature Methods
2021 Clinical and Experimental Dermatology
2018 PLOS Medicine
2018 Computer Physics Communications
2017 Computers in Biology and Medicine I y II
2016 Computers in Biology and Medicine I
2015 Science Translational Medicine
2015 IPOL
2013 IEEE Transactions on Pattern Analysis …
2013 Biological Cybernetics







IPOL

Image Processing On Line

ImageJ
Image Processing and Analysis in Java

- Image Processing & Microscopy

2D/3D · Morpho-topology, Motion estimation, Tracking

2021 Frontiers in Cellular and Infection Microbiology
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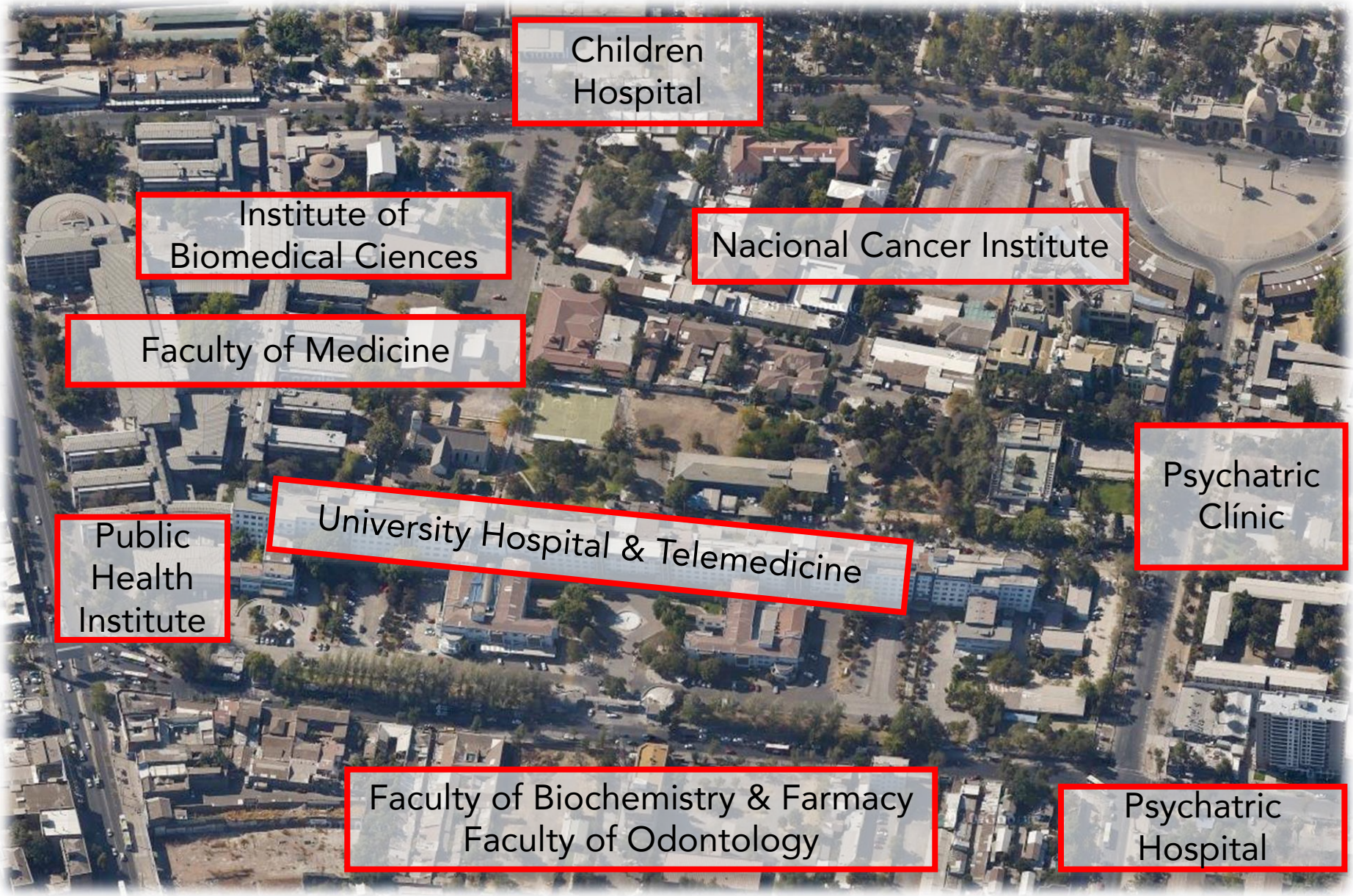
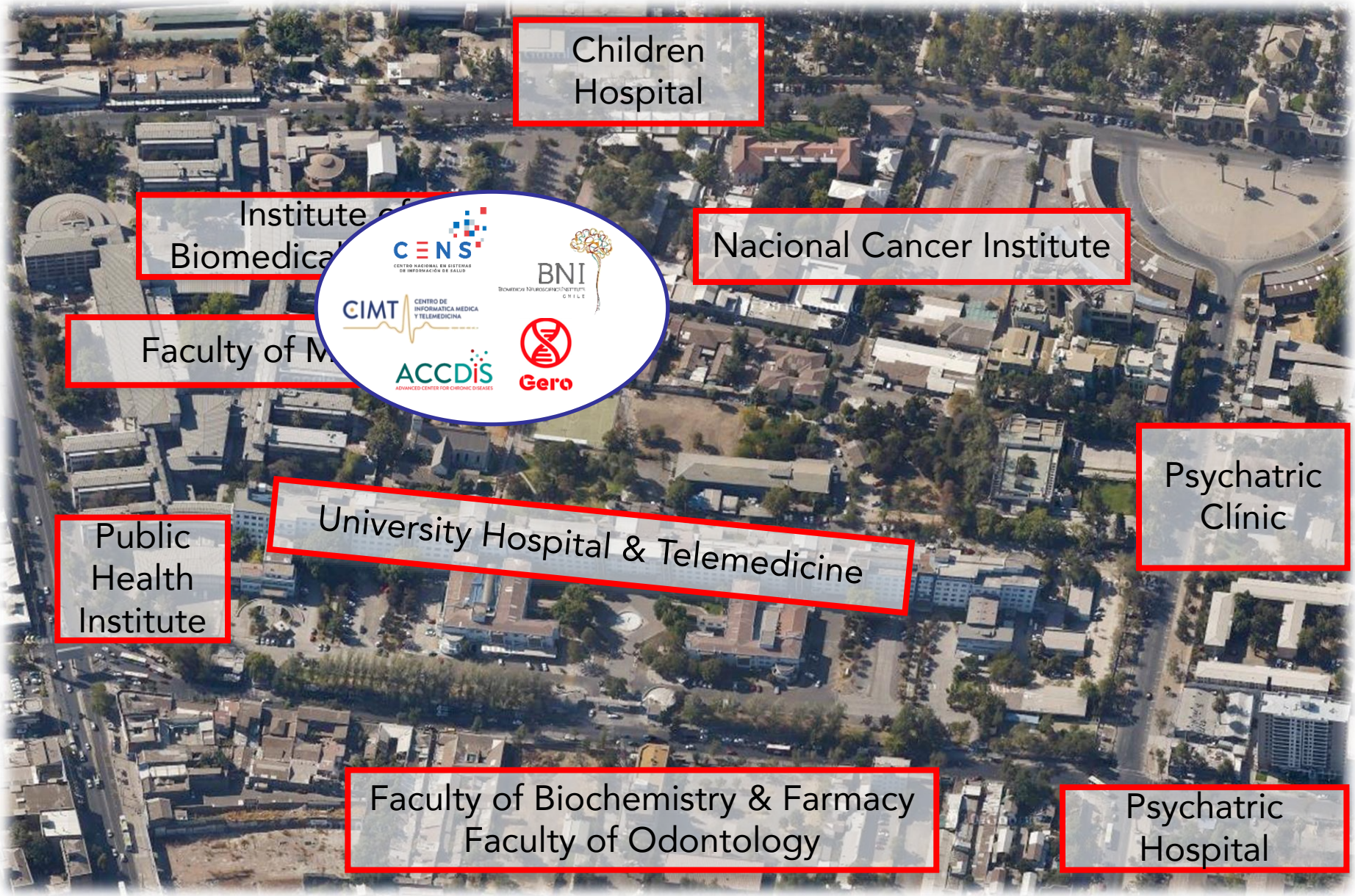

Children Hospital
Institute of Biomedical Ciences
Nacional Cancer Institute
Faculty of Medicine
Psychatric Clínic
Public Health Institute
University Hospital & Telemedicine
Faculty of Biochemistry & Farmacy
Faculty of Odontology
Psychatric Hospital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Nacional Cancer Institute
Faculty of M
CENS
BNI
CIMT
ACCDiS
Gero
Psychatric Clínic
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University Hospital & Telemedicine
Faculty of Biochemistry & Farmacy
Faculty of Odontology
Psychatric Hospital

We are a scientific institution seeking to establish the knowledge production as a fundamental engine for the social, cultural and economic development of the country.

Biomedical Neuroscience Institute
Chile

Sub-cellular functional dynamics | Cellular function & morphology | Supra-cellular development & networks | Plasticity & behaviour | Systems neuroscience | Clinical studies

Biomathematics

Neuropathology

Image and signal processing

Biomedical informatics & telemedicine

Neurodegenerative disease (ALS, Parkinson, Alzheimer)

Psychiatric diseases (Schizophrenia)

Applied Neuromedicine & Technology

Students - Postdocs - Young investigators - Clinicians - Entrepreneurs

Advanced instrumentation - Animal facilities - Communications & events - Scientific & innovation

285 Publications 2011-2017

ISI 5.51 2017

Becas Puente (42/30) y Proyectos Semilla (18)

17 groups
45 magíster
52 PhD
28 postdocs

Latin America Bioimaging

Tecnologías de bioimaging existentes en el país.

Agencia Nacional de Investigación y Desarrollo
Ministerio de Ciencia, Tecnología, Conocimiento e Innovación
Gobierno de Chile

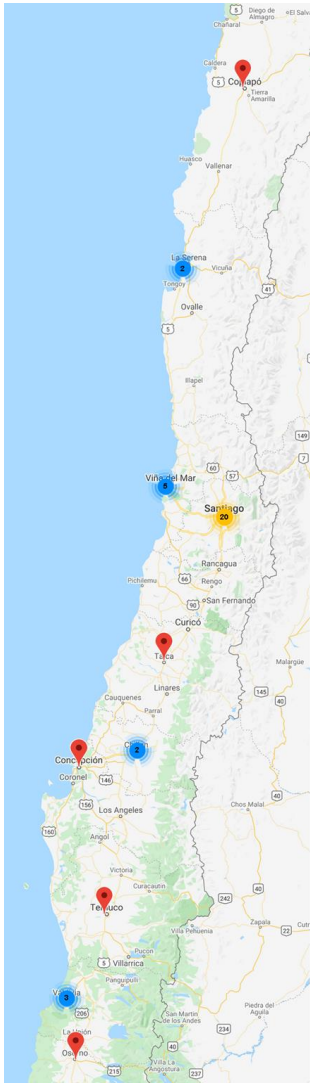
FONDEQUIP ...

~40 microscopios ópticos y electrónicos con financiamiento estatal FONDEQUIP (2011 →)
https://servicios.conicyt.cl/buscadorequipos/#/search_result/microscopio
La Red de Equipamiento Científico Avanzado: https://redeca.med.uchile.cl

Santiago y universidades e institutos públicos/privados concentran la mayor cantidad de equipamiento

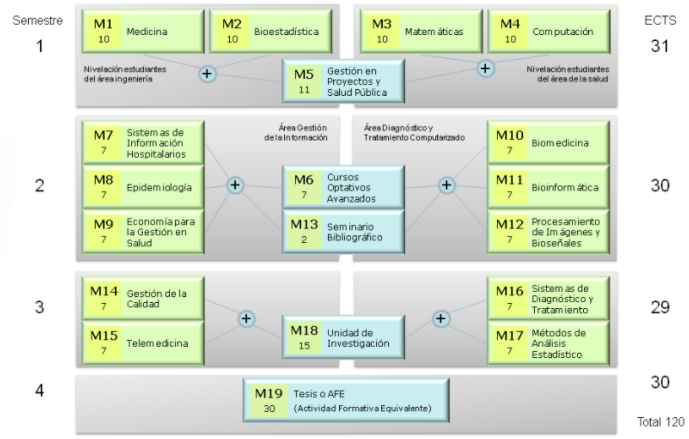
- 10 microscopios @REDECA (F-Med, U. de Chile)
- 7 entre CEMC y otras facultades (F-Med, U. de Chile)

- 6 microscopios ópticos y 2 TEM 2 SEM a @ PUC
- 9 microscopios @ CMA Bio Bio (U. Concepción)
- ...



Organización de la oferta nacional de training/educación/divulgación.

Talleres, Cursos de postgrado,
Summer Schools anuales.

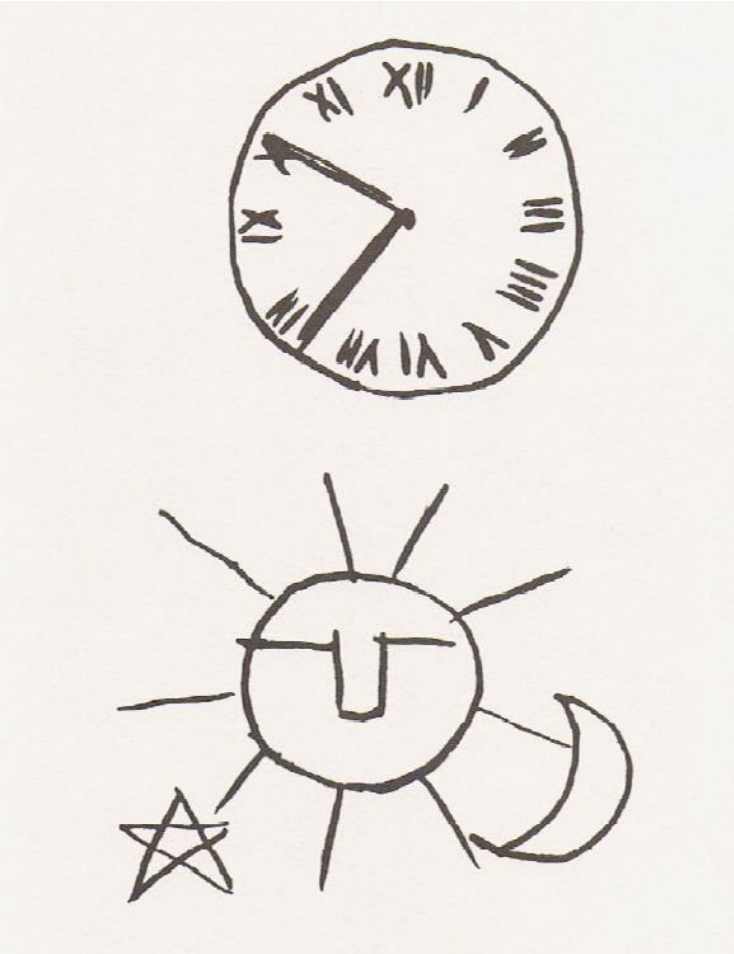


Latin America Bioimaging

https://www.latambioimaging.org

https://www.youtube.com/channel/UCbJbH590dYWqgmtn-0MaIug/videos

https://globalbioimaging.org



Joaquín Torres García

Uruguayo, pintor y pensador,

Montevideo, Barcelona, París, NY …

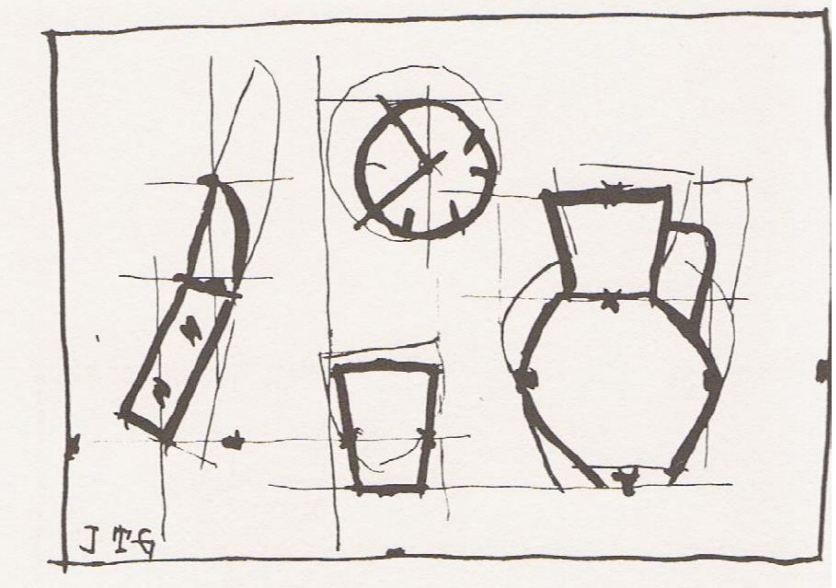
PENSAR ES GEOMETRIZAR

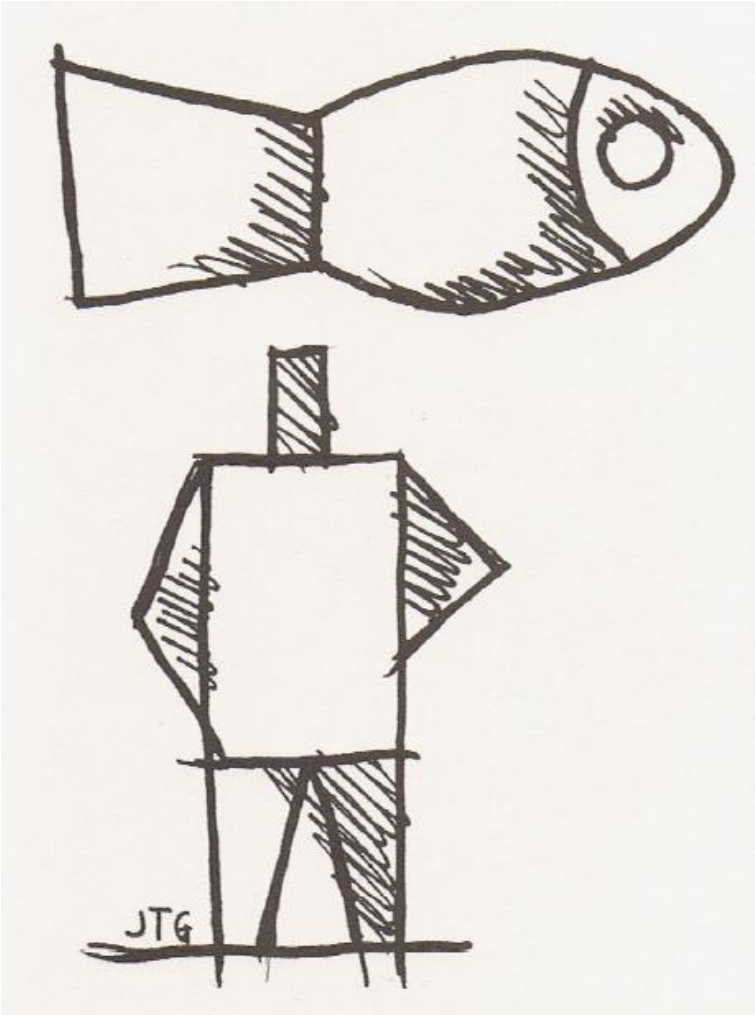
La geometría es como un teclado de lenguajes …

… curvas, regulares o no, rectas, ángulos, circunferencias, arcos …

… son los pocos elementos universales con que se puede expresar todo !!!!

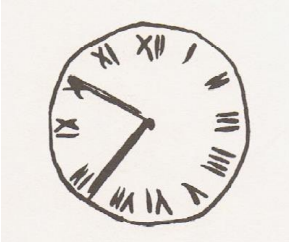
morfología





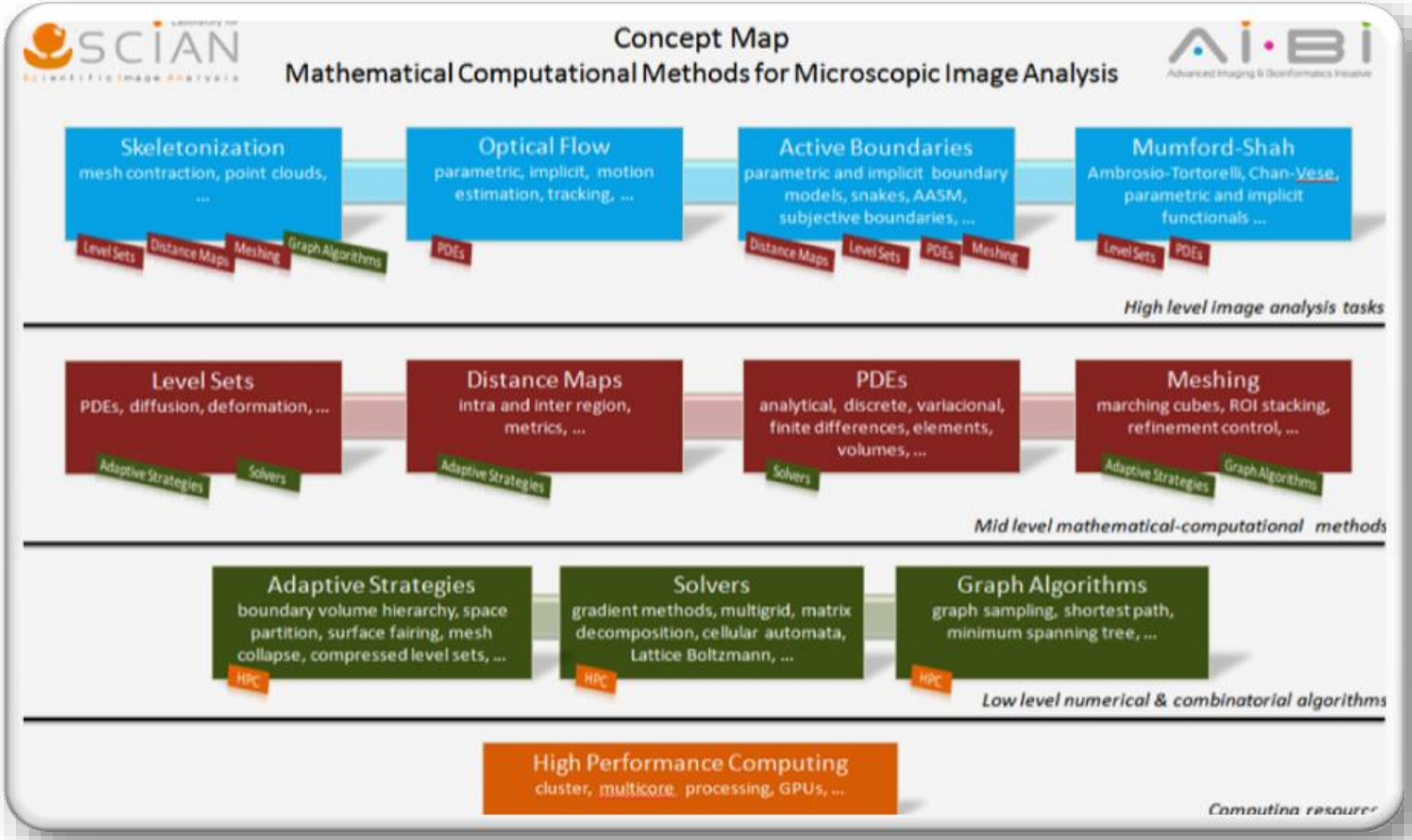
topología

tiempo

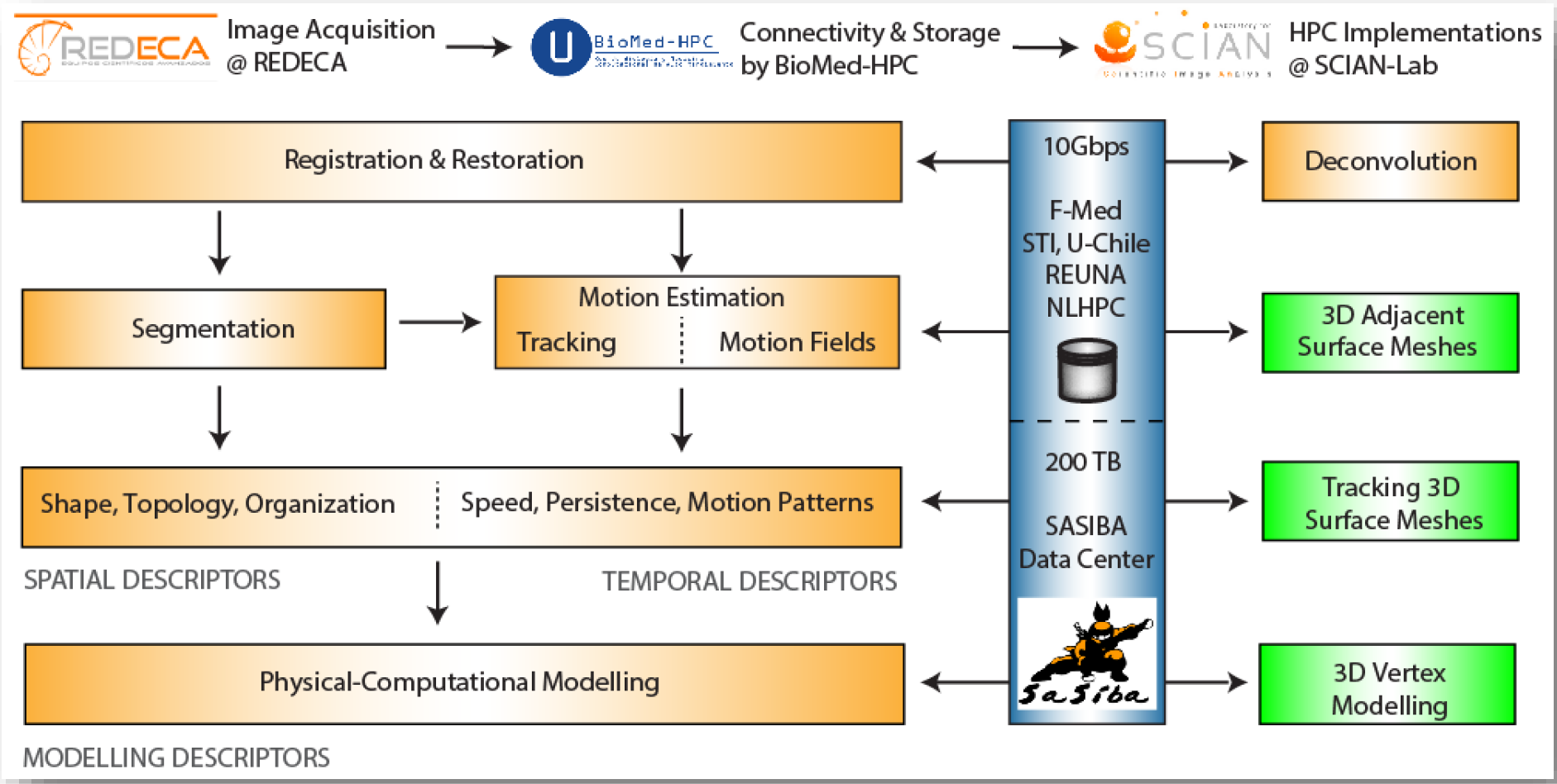


UNIVERSALISMO CONSTRUCTIVO

2014 …

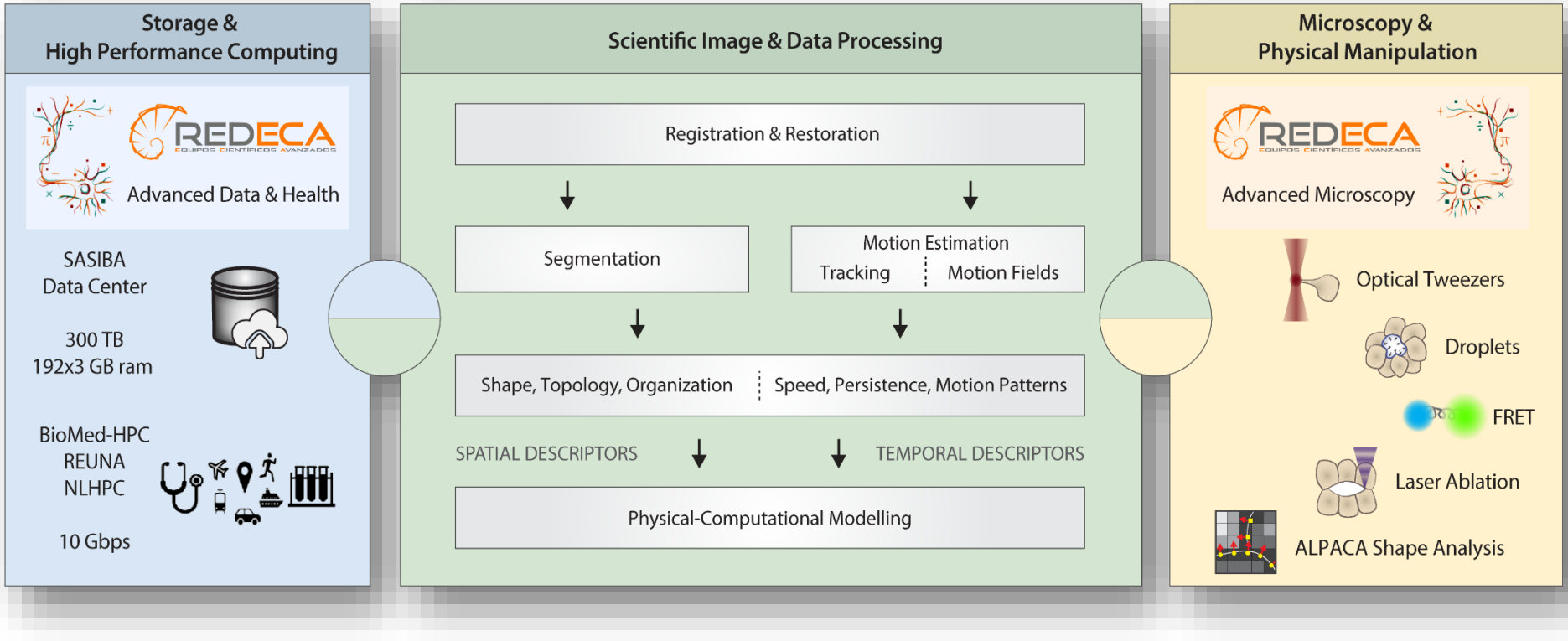

SCIAN
Concept Map
Mathematical Computational Methods for Microscopic Image Analysis
AiBi
Skeletonization
mesh contraction, point clouds, ...
Level Sets
Distance Maps
Meshing
Graph Algorithms
Optical Flow
parametric, implicit, motion estimation, tracking, ...
PDEs
Active Boundaries
parametric and implicit boundary models, snakes, AASM, subjective boundaries, ...
Distance Maps
Level Sets
PDEs
Meshing
Mumford-Shah
Ambrosio-Tortorelli, Chan-Vese, parametric and implicit functionals ...
Level Sets
PDEs
High level image analysis tasks
Level Sets
PDEs, diffusion, deformation, ...
Adaptive Strategies
Solvers
Distance Maps
intra and inter region, metrics, ...
Adaptive Strategies
PDEs
analytical, discrete, variacional, finite differences, elements, volumes, ...
Solvers
Meshing
marching cubes, ROI stacking, refinement control, ...
Adaptive Strategies
Graph Algorithms
Mid level mathematical-computational methods
Adaptive Strategies
boundary volume hierarchy, space partition, surface fairing, mesh collapse, compressed level sets, ...
HPC
Solvers
gradient methods, multigrid, matrix decomposition, cellular automata, Lattice Boltzmann, ...
HPC
Graph Algorithms
graph sampling, shortest path, minimum spanning tree, ...
HPC
Low level numerical & combinatorial algorithms
High Performance Computing
cluster, multicore processing, GPUs, ...
Computing resources

2017 …



V Castañeda, M Cerda, F Santibáñez, J Jara, E Pulgar, K Palma, … Computational methods for analysis of dynamic events in cell migration, Current molecular medicine 14 (2), 291-307

2021 …

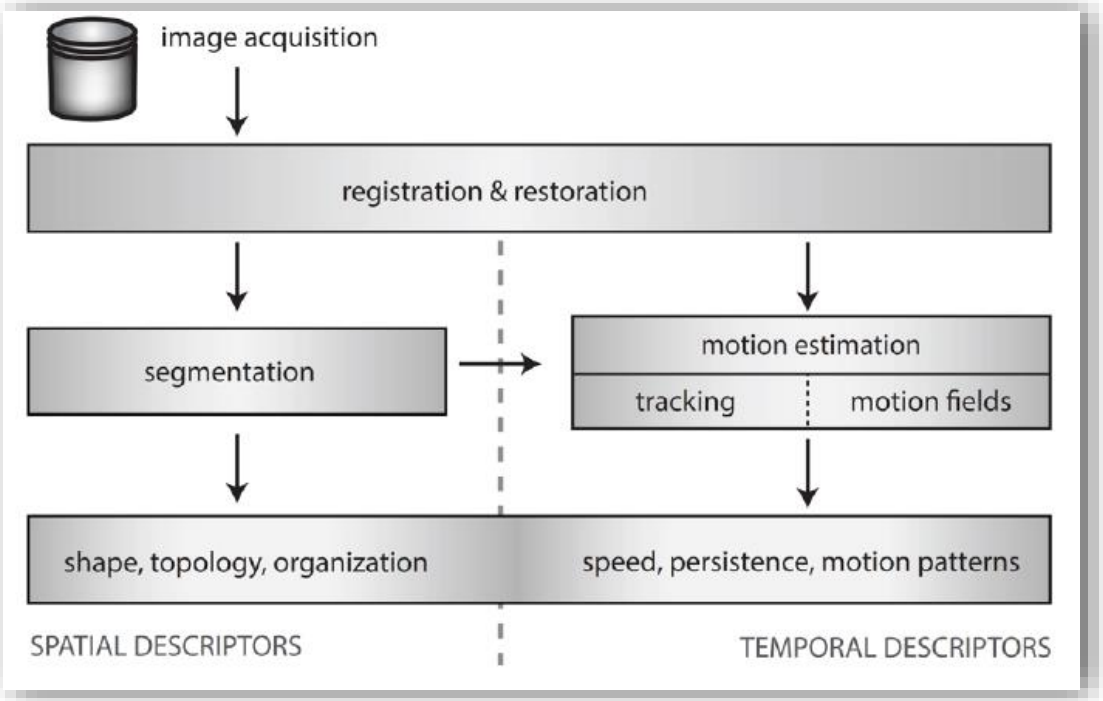


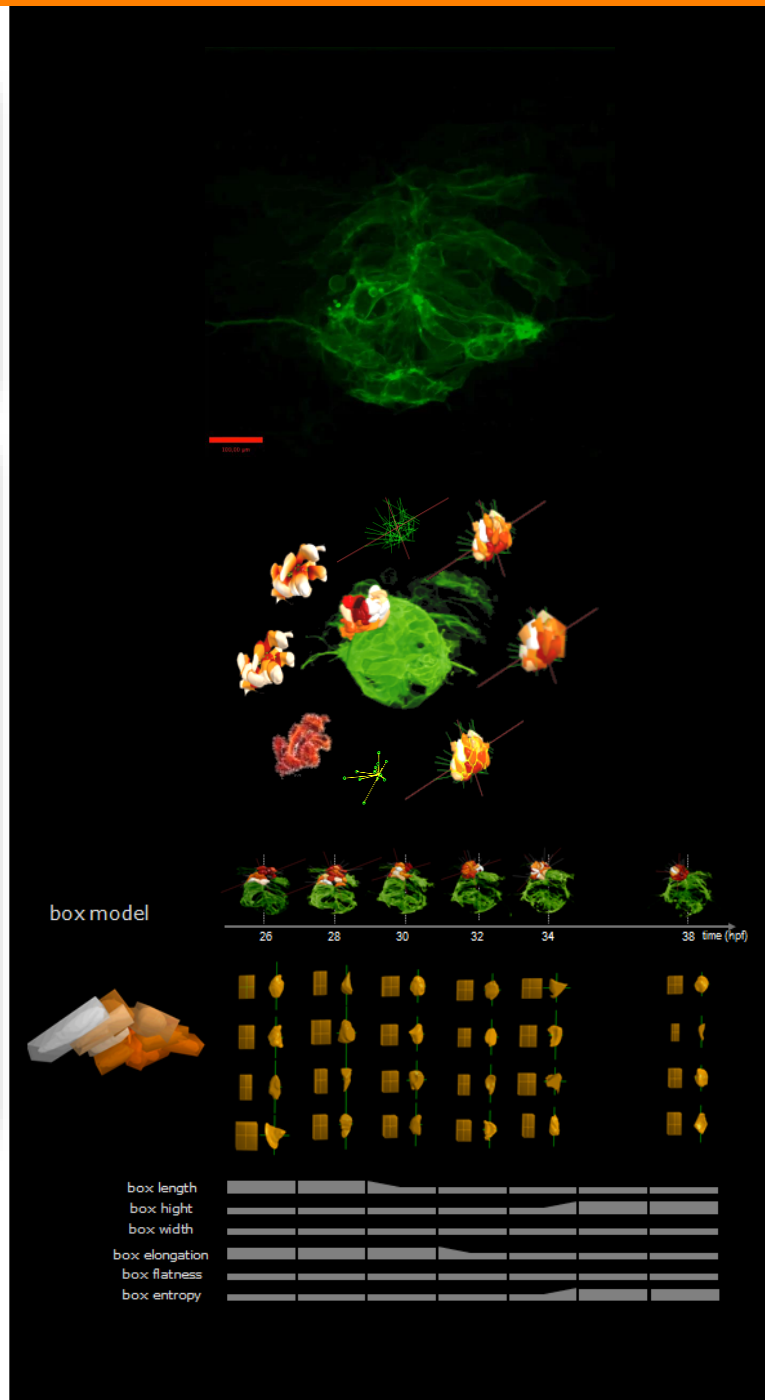
A global view of standards for open image data formats and repositories JR Swedlow, P Kankaanpää, U Sarkans, W Goscinski, G Galloway, ..., Nature Methods, 1-7

Heidelberg Collaboratory for Image Processing

University->Faculty of Mathematics and Computer Science->HCI
University->Faculty of Physics and Astronomy->HCI
Universitv->IWR->HCI

- Computer Vision(Ommer lab)
- Digital Image Processing (Jähne lab)
- Image and Pattern Analysis (Schnörr lab)
- Image Processing and Modelling (Garbe lab)
- Multidimensional Image Processing (Hamprecht lab)





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Computational methods for analysis of dynamic events in cell migration,
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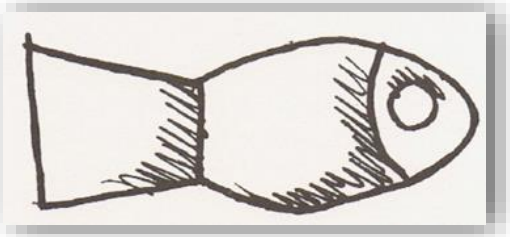
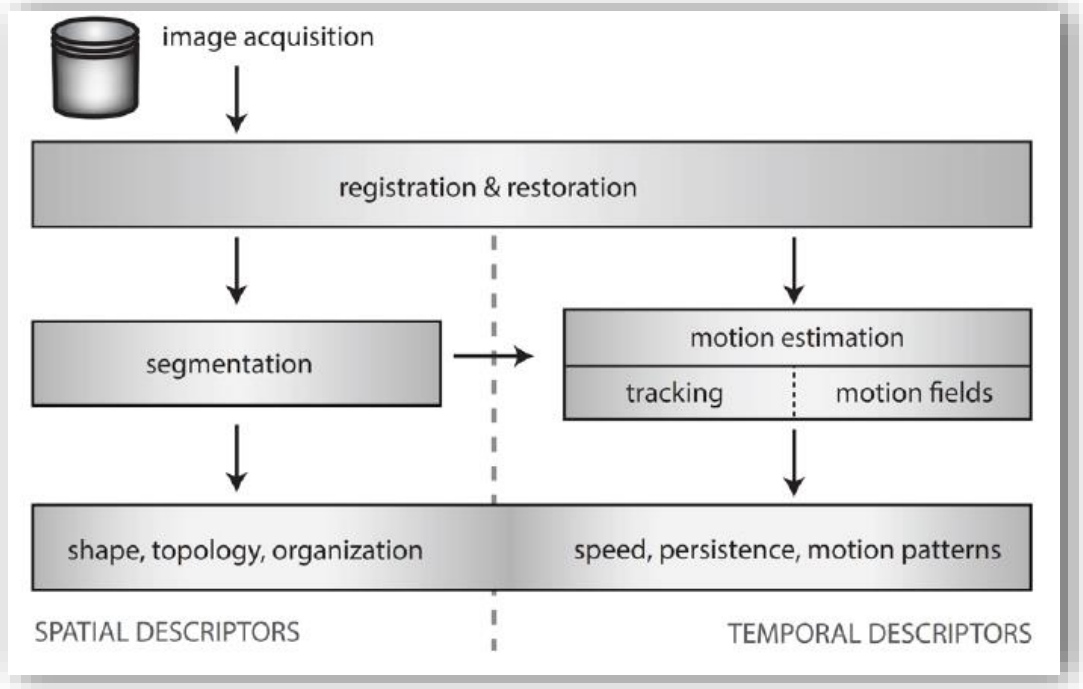
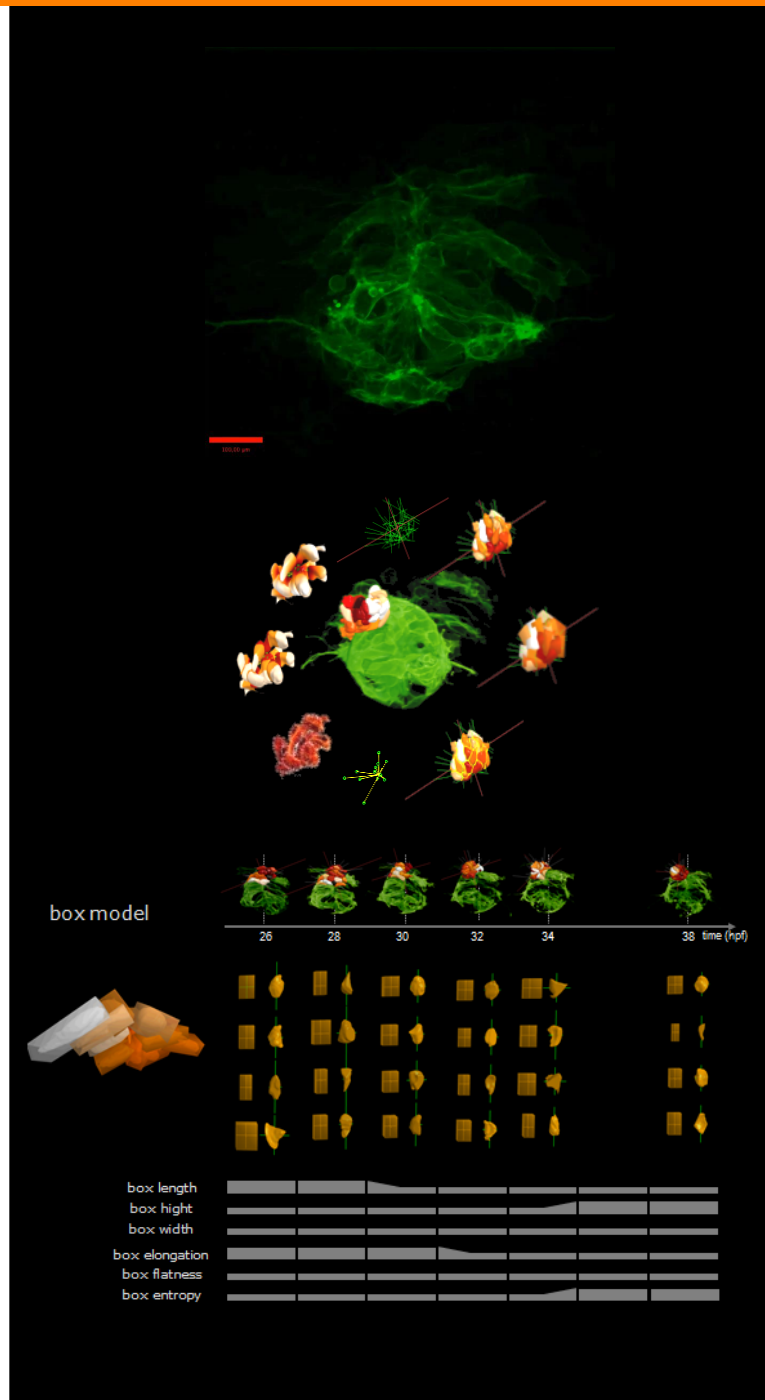
Miguel Concha
Biología del Desarrollo



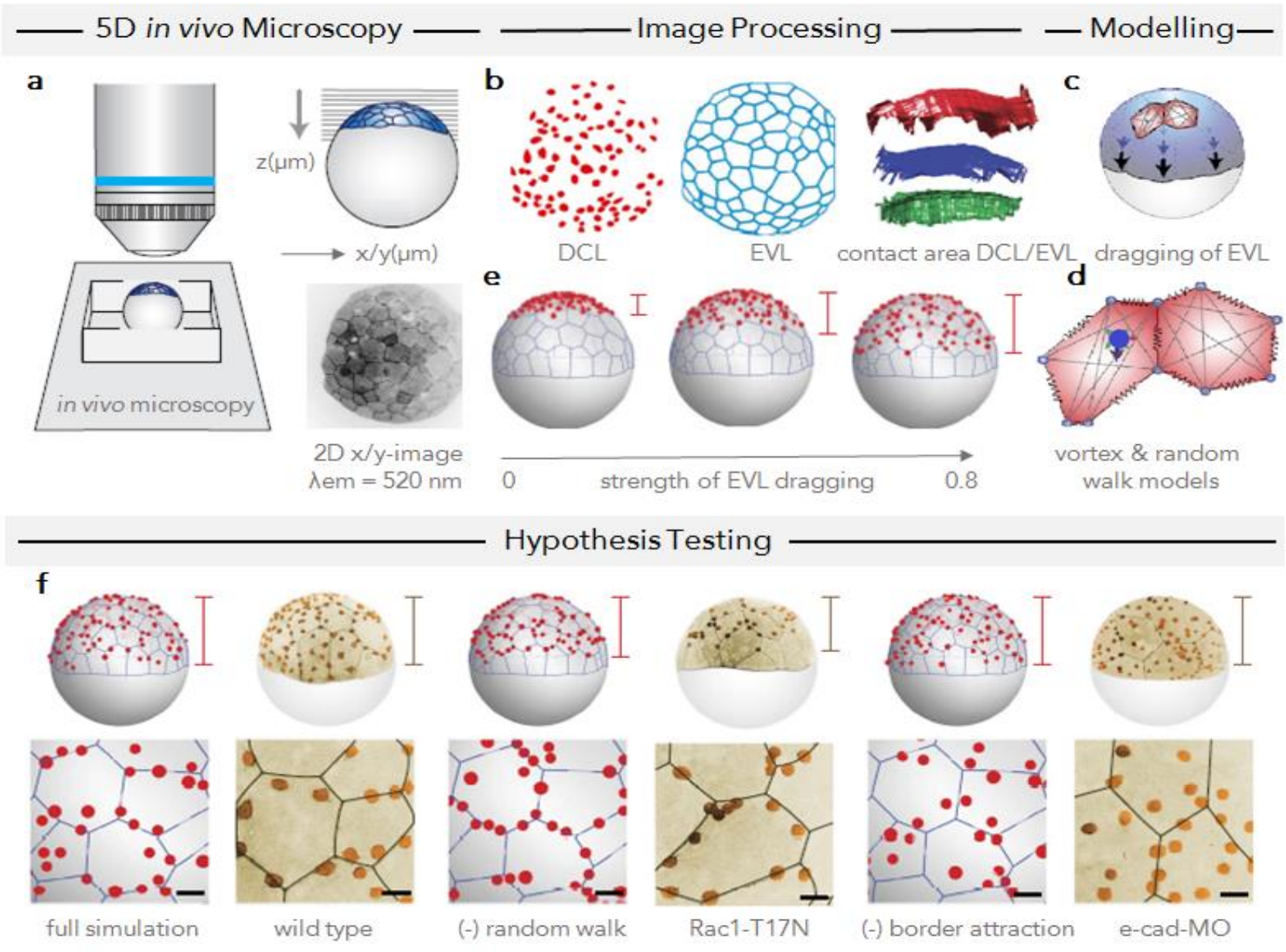




V Castañeda, M Cerda, F Santibáñez, J Jara, E Pulgar, K Palma, ...
Computational methods for analysis of dynamic events in cell migration,
Current molecular medicine 14 (2), 291-307

nature COMMUNICATIONS

2017 Extra-embryonic tissue spreading directs early embryo morphogenesis in killifish. German Reig, Mauricio Cerda, Néstor Sepúlveda, Daniela Flores, Victor Castaneda, Masazumi Tada, Steffen Härtel, Miguel Concha



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Scientific Image Analysis

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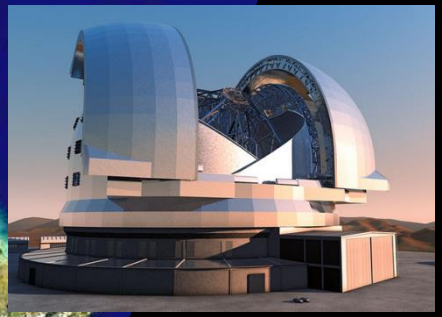
BIOMEDICAL NEUROSCIENCE INSTITUTE

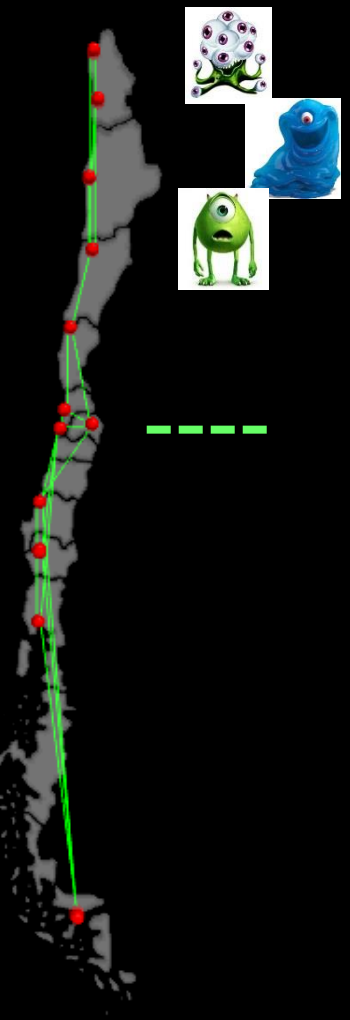
Very Large Telescope (VLT),
4 Telescopes, 8m, 2600 m

Atacama Large Millimeter/submillimeter Array (ALMA), 66 Antenna, 5000 m



E-ELT European Extremely Large Telescope, 39 m, 3000 m

NASA World Wind screenshot



Extraterrestrial Monster Science produces:
TeraB, PetaB, ExaB, ZettaB, YottaB

… Tera is a unit prefix in the metric system denoting multiplication by 10E12 or 1.000.000.000.000 !

… Tera is derived from Greek τέρας (teras), meaning “monster”.

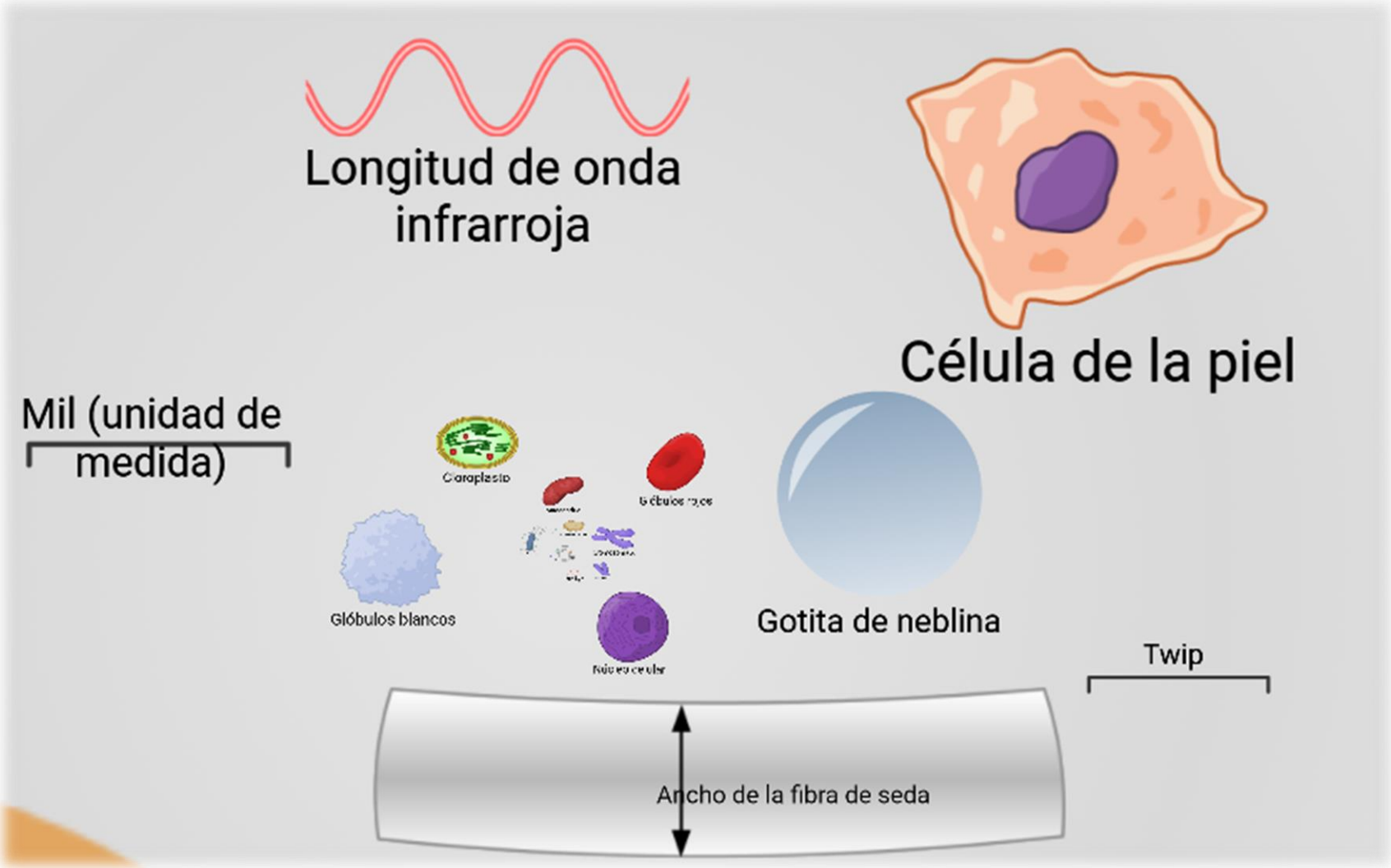
… Tera was confirmed for use in the SI in 1960…. Wiki !

Metric prefixes

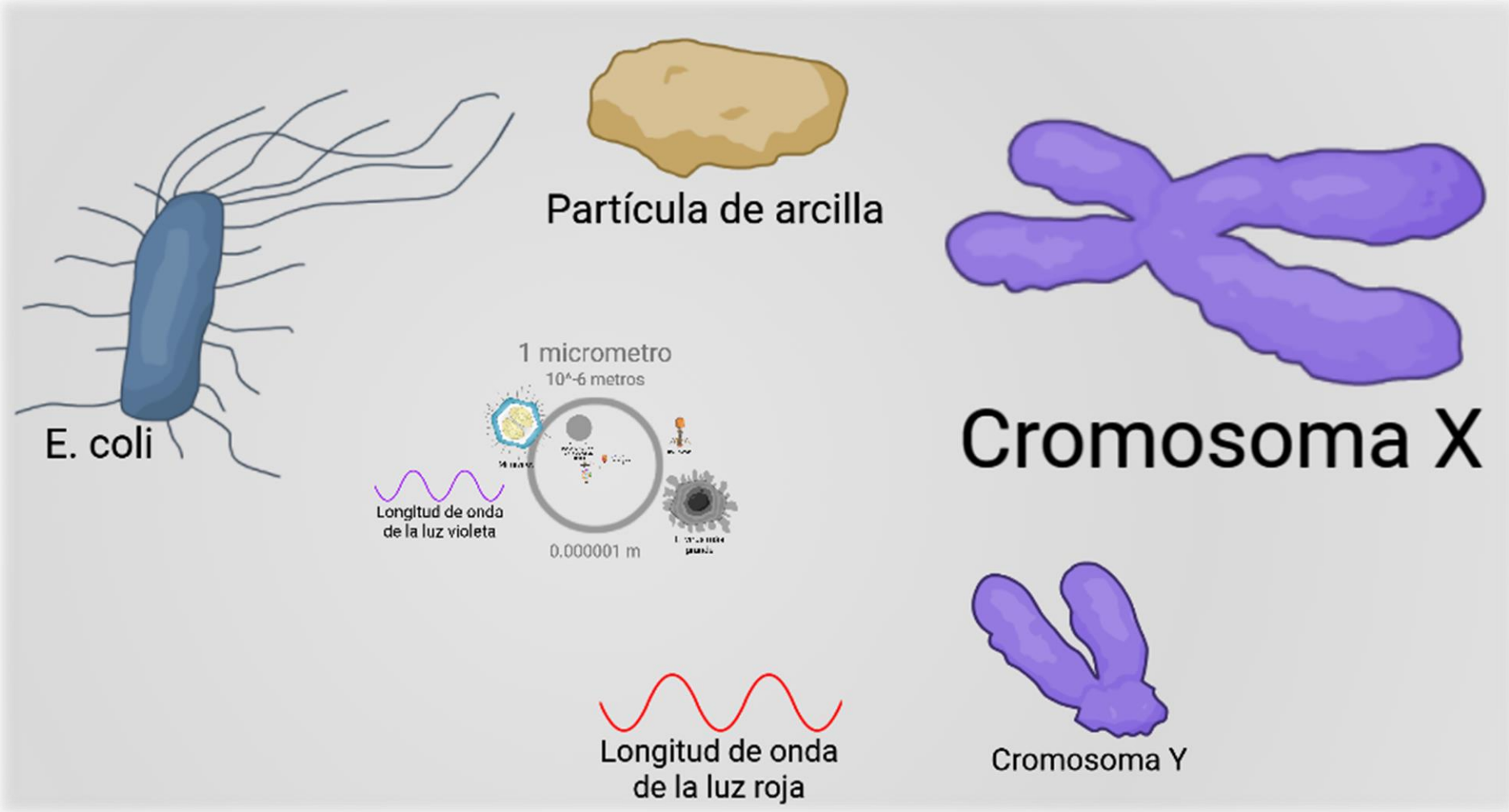
Prefix	Symbol	1000^m	10^n	Decimal	English word[n 1]	Since[n 2]
yotta	Y	1000^8	10^{24}	1 000 000 000 000 000 000 000 000	septillion	1991
zetta	Z	1000^7	10^{21}	1 000 000 000 000 000 000 000	sextillion	1991
exa	E	1000^6	10^{18}	1 000 000 000 000 000 000	quintillion	1975
peta	P	1000^5	10^{15}	1 000 000 000 000 000	quadrillion	1975
tera	T	1000^4	10^{12}	1 000 000 000 000	trillion	1960
giga	G	1000^3	10^9	1 000 000 000	billion	1960
mega	M	1000^2	10^6	1 000 000	million	1960
kilo	k	1000^1	10^3	1 000	thousand	1795
hecto	h	$1000^{2/3}$	10^2	100	hundred	1795
deca	da	$1000^{1/3}$	10^1	10	ten	1795
		1000^0	10^0	1	one	–
deci	d	$1000^{-1/3}$	10^{-1}	0.1	tenth	1795
centi	c	$1000^{-2/3}$	10^{-2}	0.01	hundredth	1795
milli	m	1000^{-1}	10^{-3}	0.001	thousandth	1795
micro	μ	1000^{-2}	10^{-6}	0.000 001	millionth	1960
nano	n	1000^{-3}	10^{-9}	0.000 000 001	billionth	1960
pico	p	1000^{-4}	10^{-12}	0.000 000 000 001	trillionth	1960
femto	f	1000^{-5}	10^{-15}	0.000 000 000 000 001	quadrillionth	1964
atto	a	1000^{-6}	10^{-18}	0.000 000 000 000 000 001	quintillionth	1964
zepto	z	1000^{-7}	10^{-21}	0.000 000 000 000 000 000 001	sextillionth	1991
yocto	y	1000^{-8}	10^{-24}	0.000 000 000 000 000 000 000 001	septillionth	1991

The Scale of the Universe https://htwins.net/scale2

The Scale of the Universe https://htwins.net/scale2



The Scale of the Universe https://htwins.net/scale2



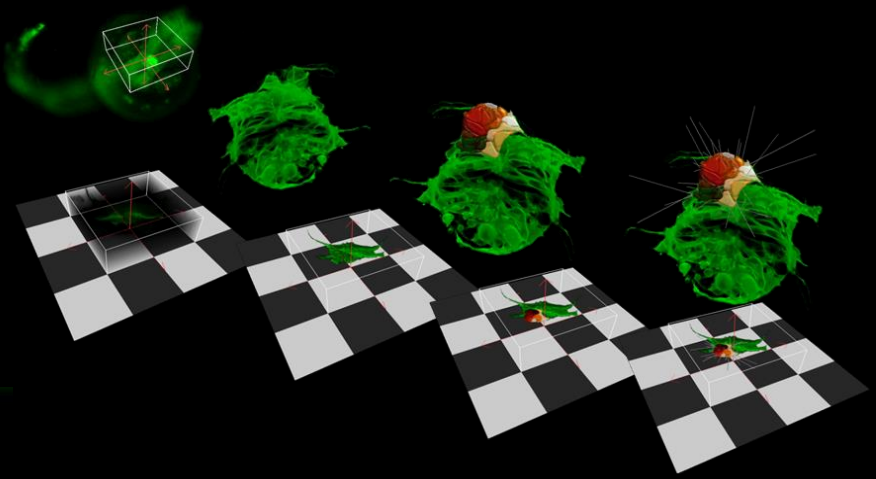
redeca.med.uchile.cl

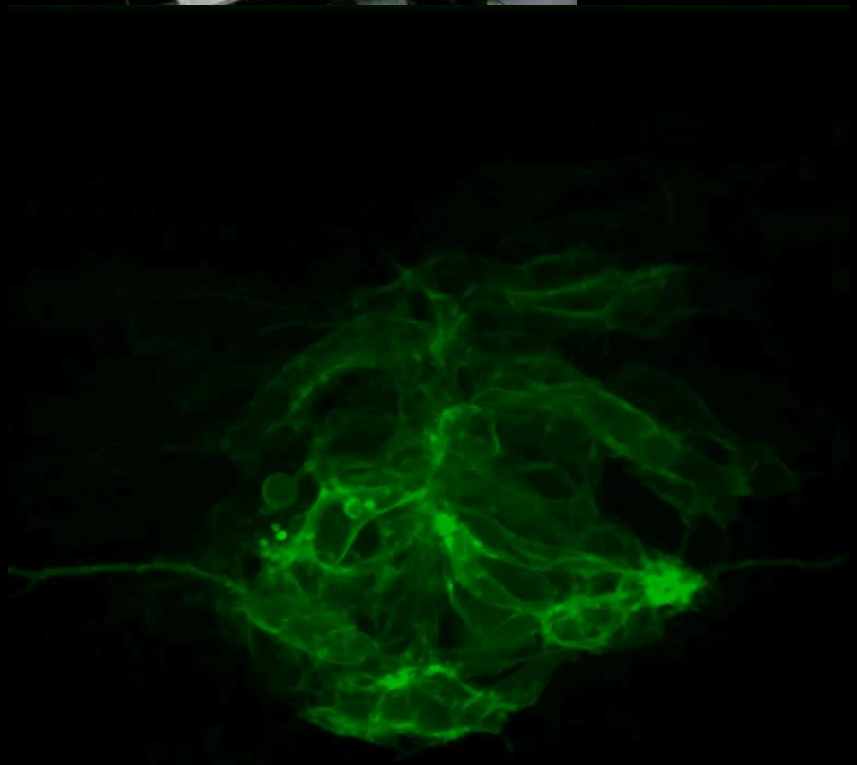
Bienvenid@s a REDECA

REDECA provee apoyo tecnológico, acceso y acompañamiento en el uso de servicios y equipos avanzados. Usuarios de la institución y externos pueden solicitar asistencia para la selección de servicios o equipos adecuados a sus desafíos experimentales, el almacenamiento y el análisis de sus datos.

¡ REDECA… haz ciencia con nosotros !

|-> Perkin Elmer Spinning Disk with laser ablation





|-> Deconvolution

box model

box length

box hight

box width

box elongation

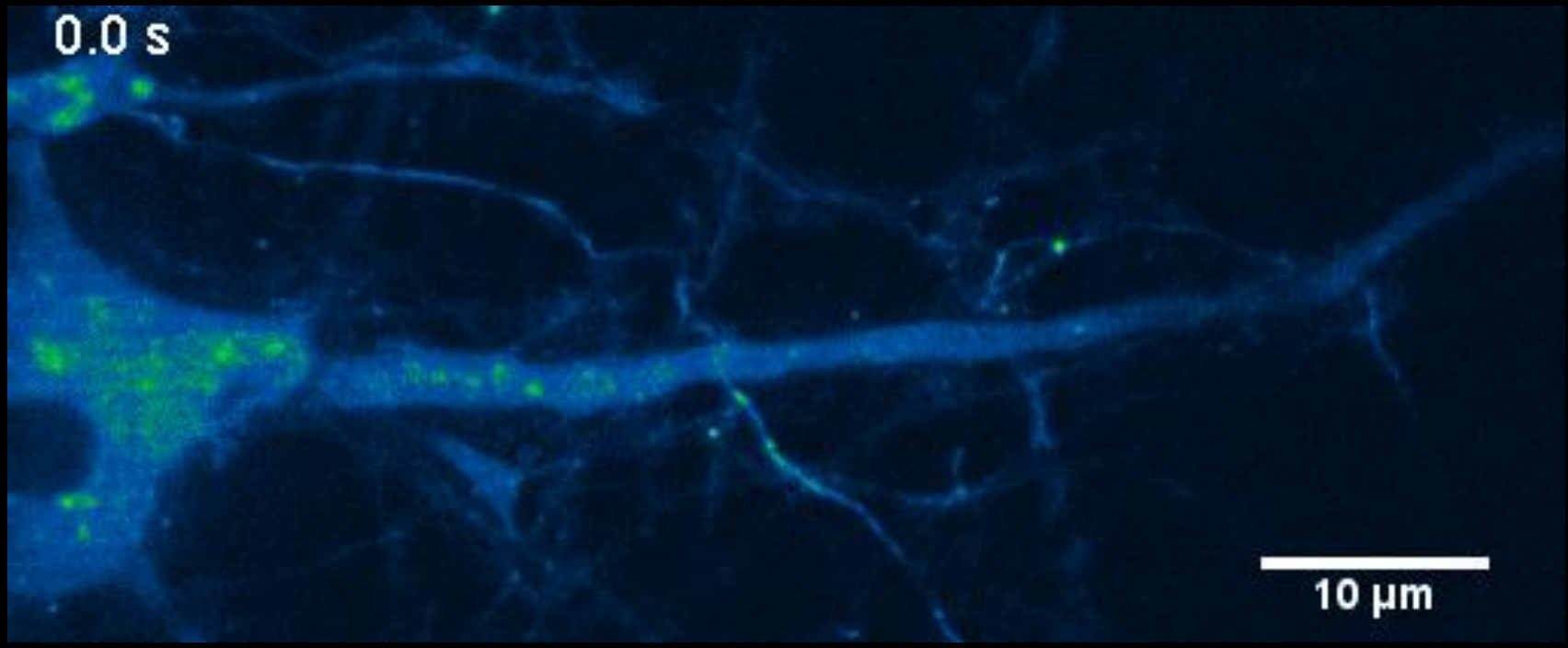
box flatness

box entropy

|-> Perkin Elmer Spinning Disk with Micropoint Laser Ablation.

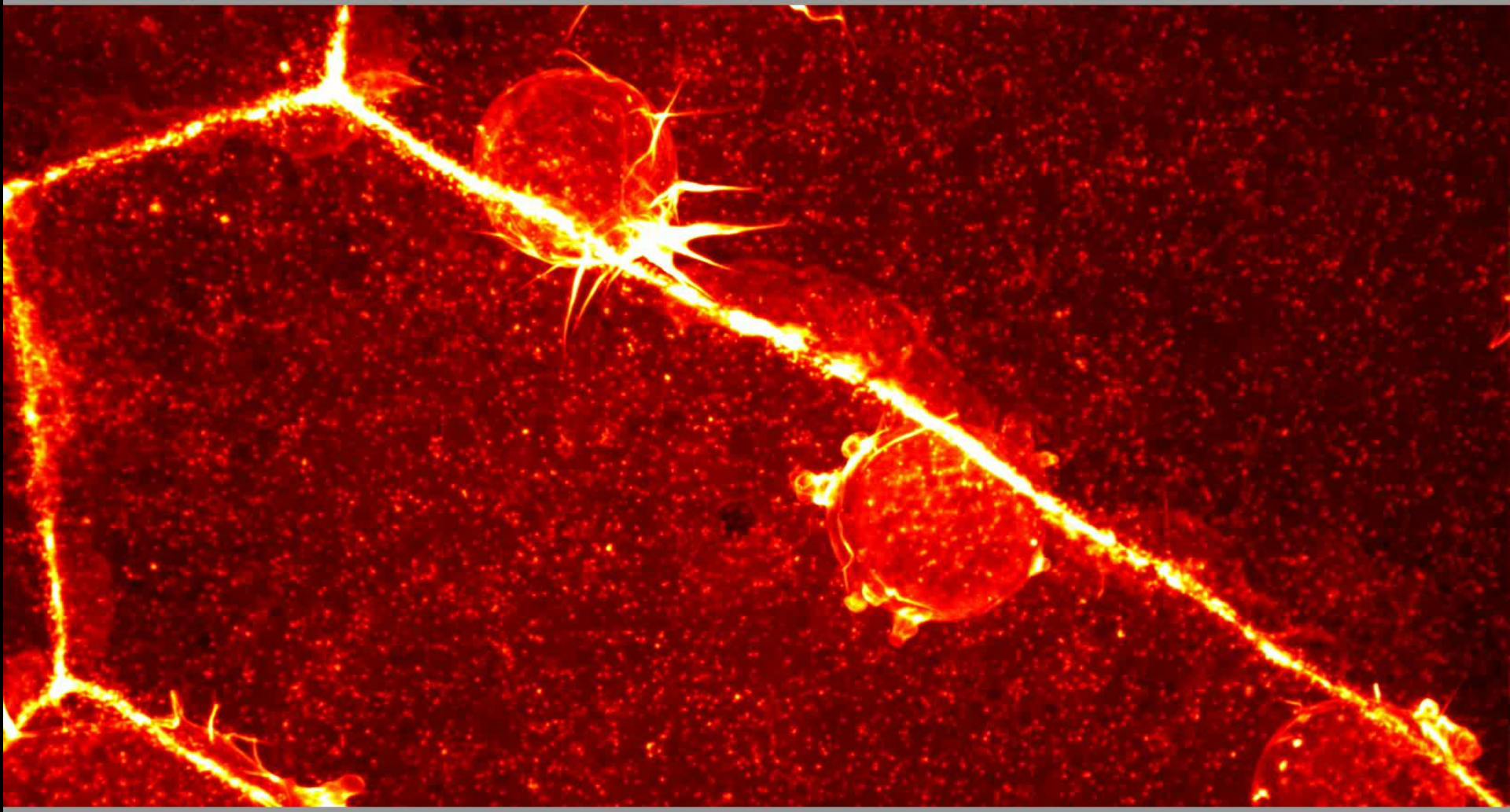
2021 RyR-mediated Ca2+ release elicited by neuronal activity induces nuclear Ca2+ signals, CREB phosphorylation, and Npas4/RyR2 expression P Lobos, A Córdova, I Vega-Vásquez, OA Ramírez, T Adasme, ... Proceedings of the National Academy of Sciences 118 (33)

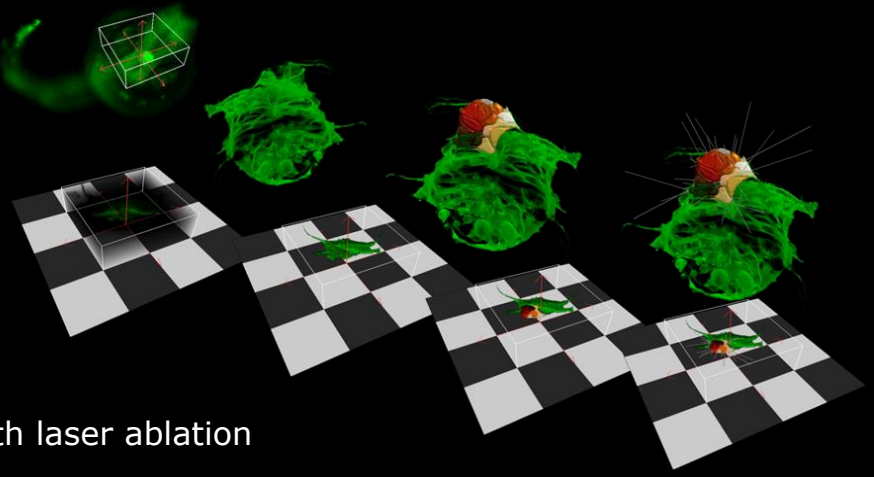
2021 Ryanodine receptor-mediated Ca2+ release and atlastin-2 GTPase activity contribute to IP3-induced dendritic Ca2+ signals in primary hippocampal neurons, OA Ramírez, A Córdova, M Cerda, P Lobos, S Härtel, … Cell Calcium 96, 102399



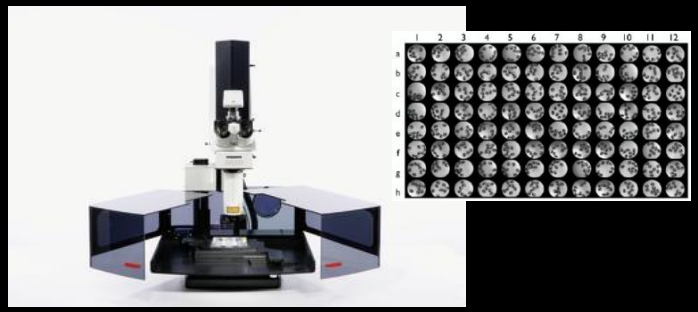
|-> Perkin Elmer Spinning Disk

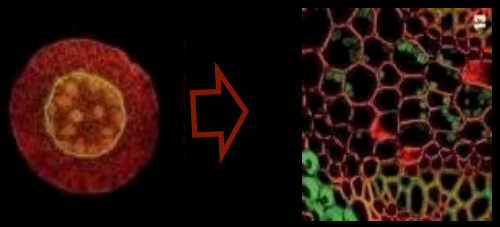
Austrolevias Nigripinnis, Microinyección mRNA para la expresión de LifeAct-GFP (actina). migración de Deep Cells (DC) a través del Enveolpping Layer (EVL). (G.Reig)

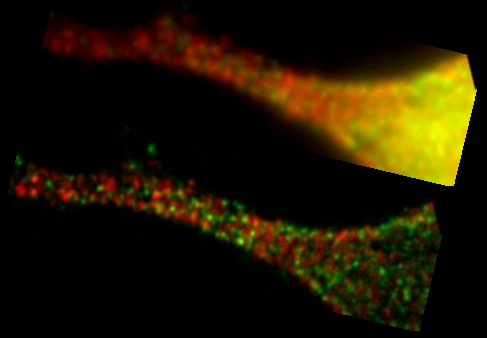




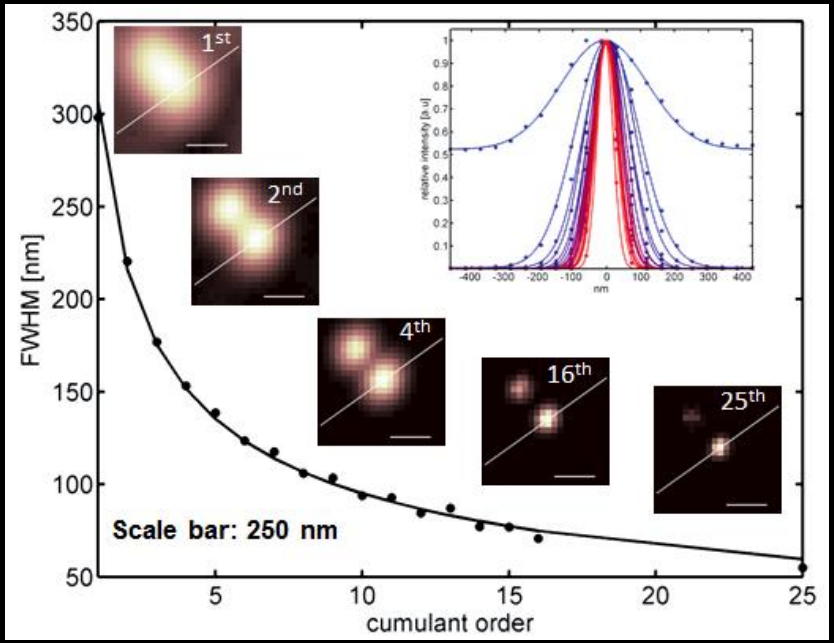
Scian/Leo-Lab: Perkin Elmer Spinning Disk with laser ablation



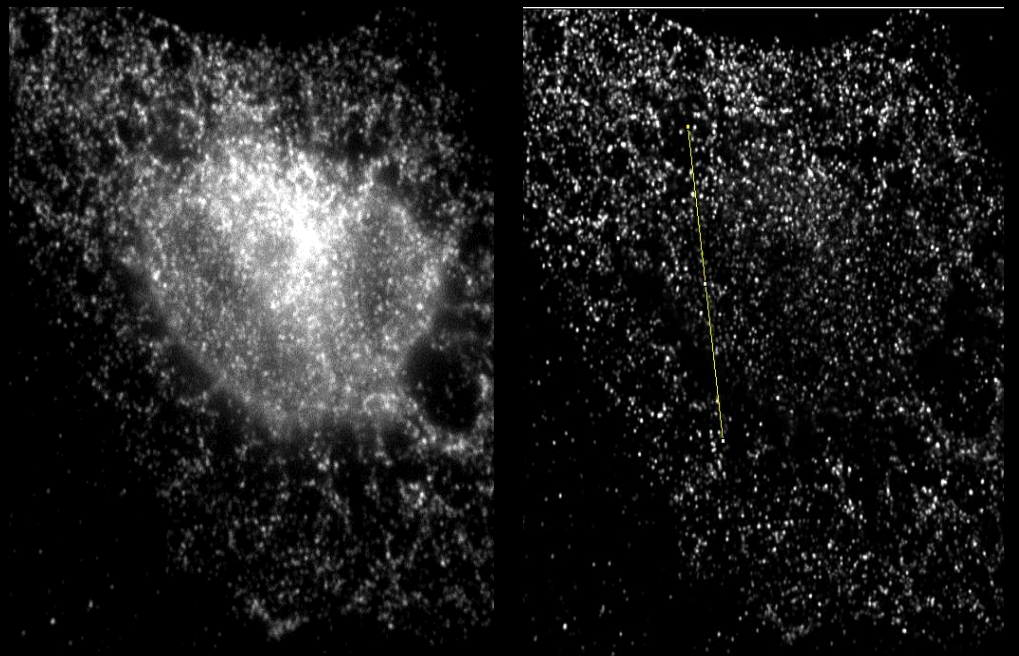




SCIAN-Lab Leica TCS LSI Super Zoom Spectral Confocal + Superresolution Optical Fluctuation Imaging SOFI



Dertinger et al 2009 PNAS

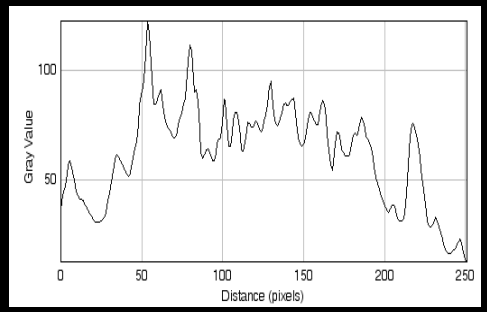


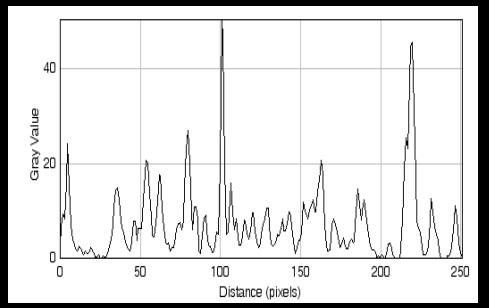
COS7 myc-GABABR1 conjugados con QDots 525 nm

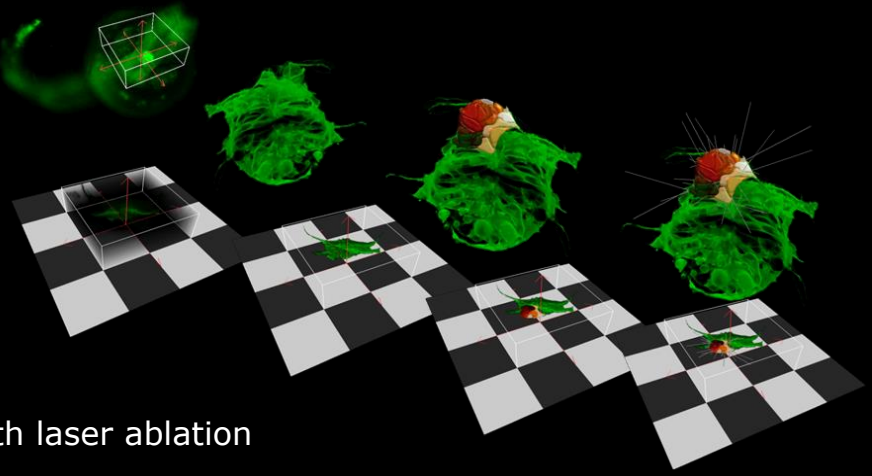
Jörg Enderlein
Gôttingen

Mayor Challenges:

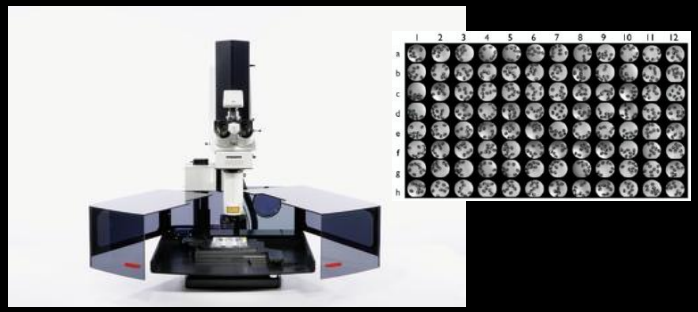
- vibrations
- computational backbone
- sustainability

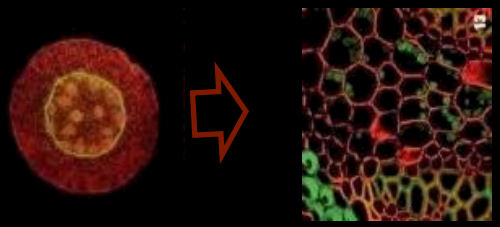


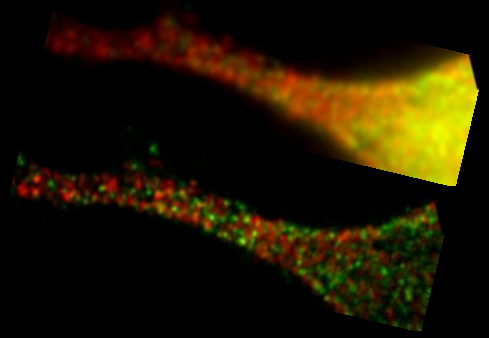




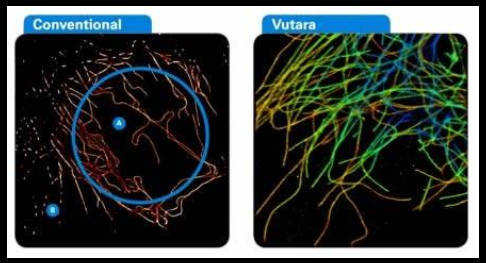
Scian/Leo-Lab: Perkin Elmer Spinning Disk with laser ablation

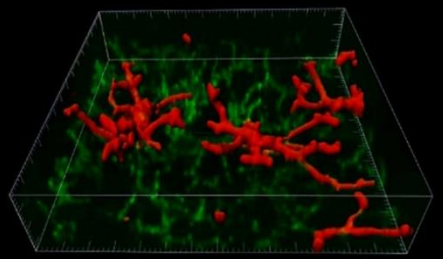






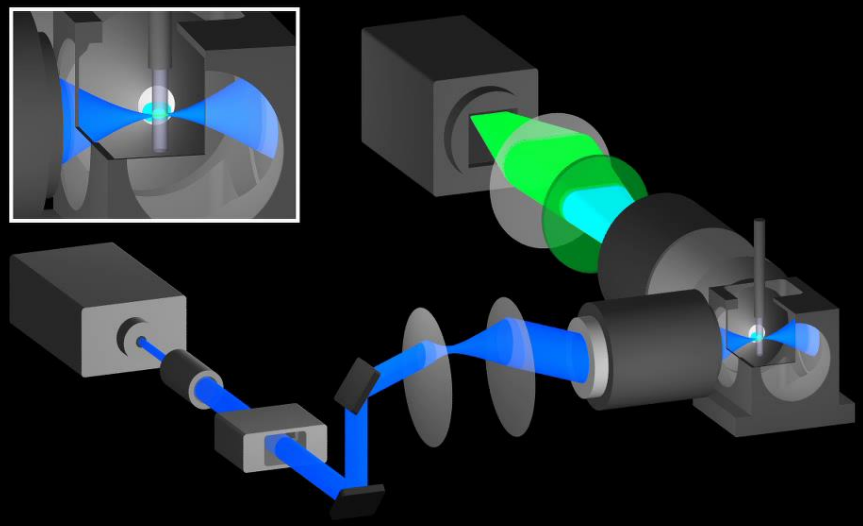
SCIAN-Lab Leica TCS LSI Super Zoom Spectral Confocal + Superresolution Optical Fluctuation Imaging SOFI



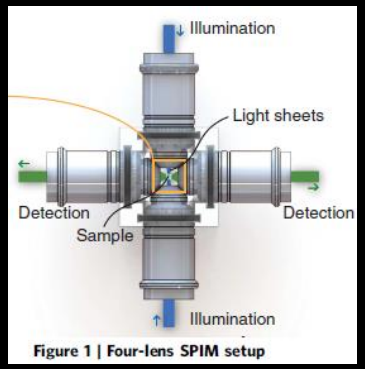


Couve-Lab PALM Vtarar 200, Bliplane 3D

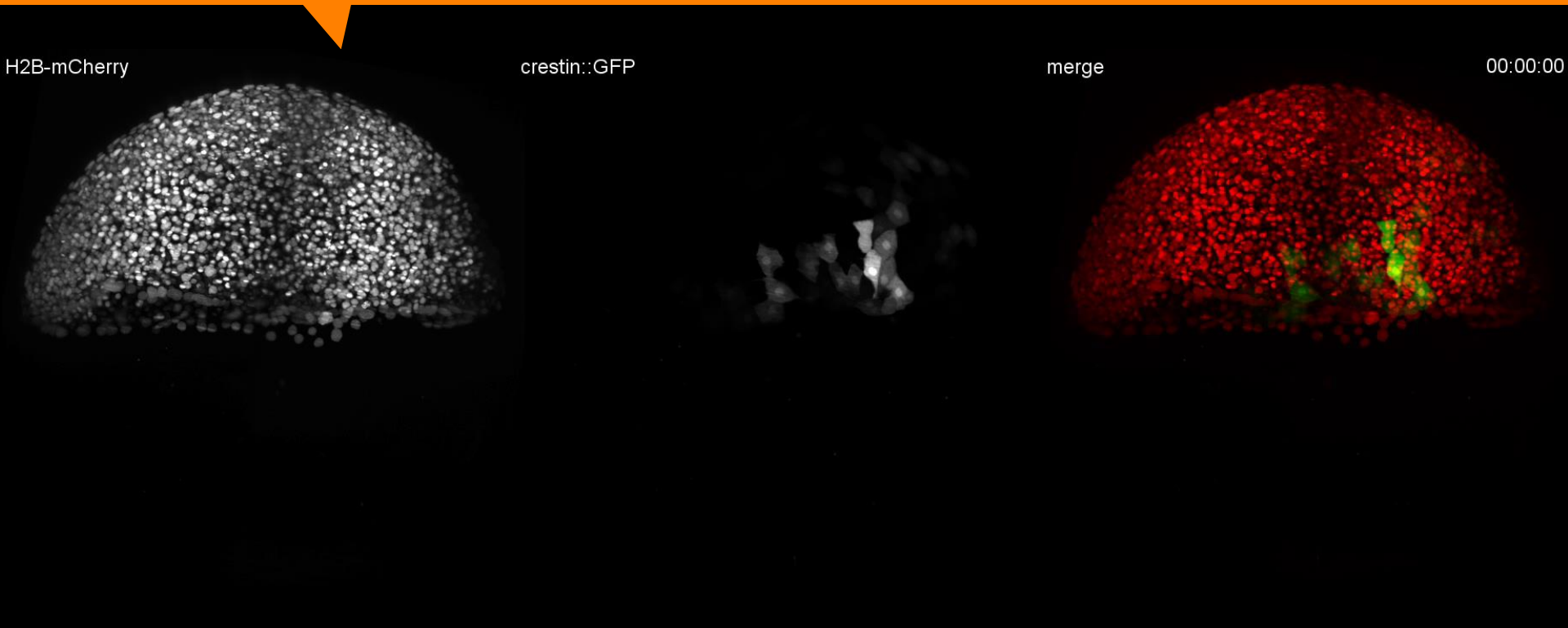
Pulgar, Keller, Concha, unpublished



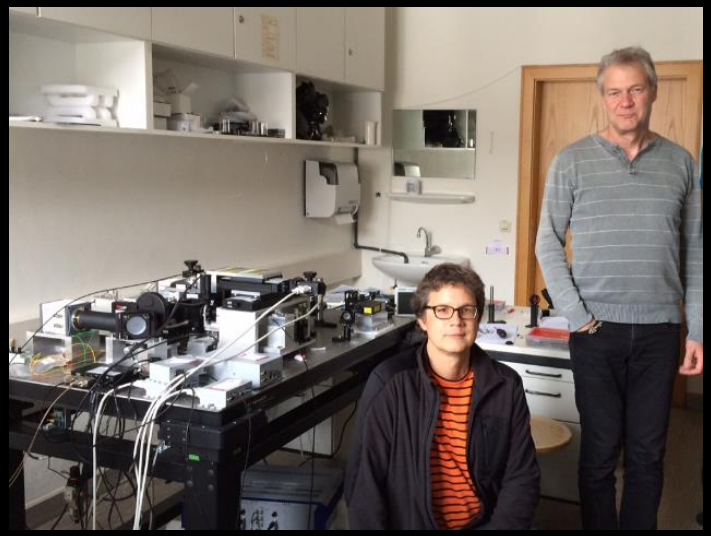
Ulrich Kubitscheck
Bonn



Jan Huisken
Dresden



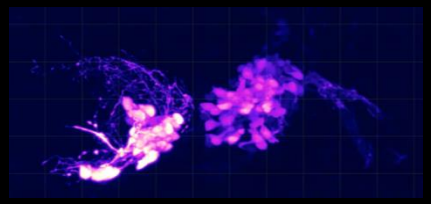
Pulgar, Keller, Concha, unpublished



¿Qué hay de la conectividad a gran escala en alta resolución?

Si no puede mejorar su óptica, amplíe la muestra.

La Microscopía de Expansión puede ampliar el cerebro del pez cebra por factor 4.



Juan Eduardo Rodríguez
Kubitscheck-Lab
Bonn University

Karina Palma
Leo/SCIAN
U-Chile

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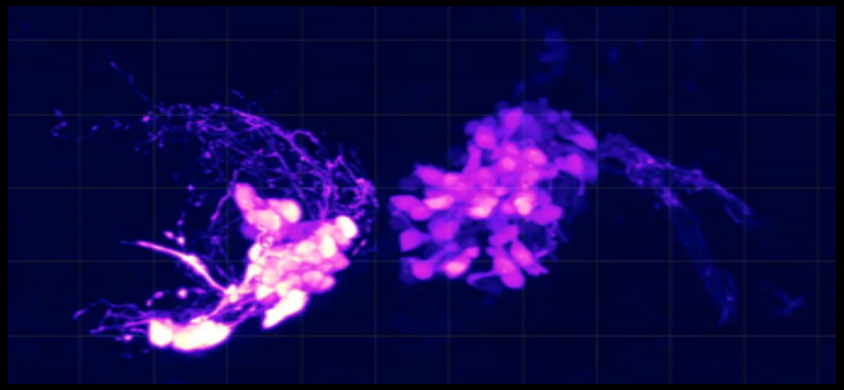

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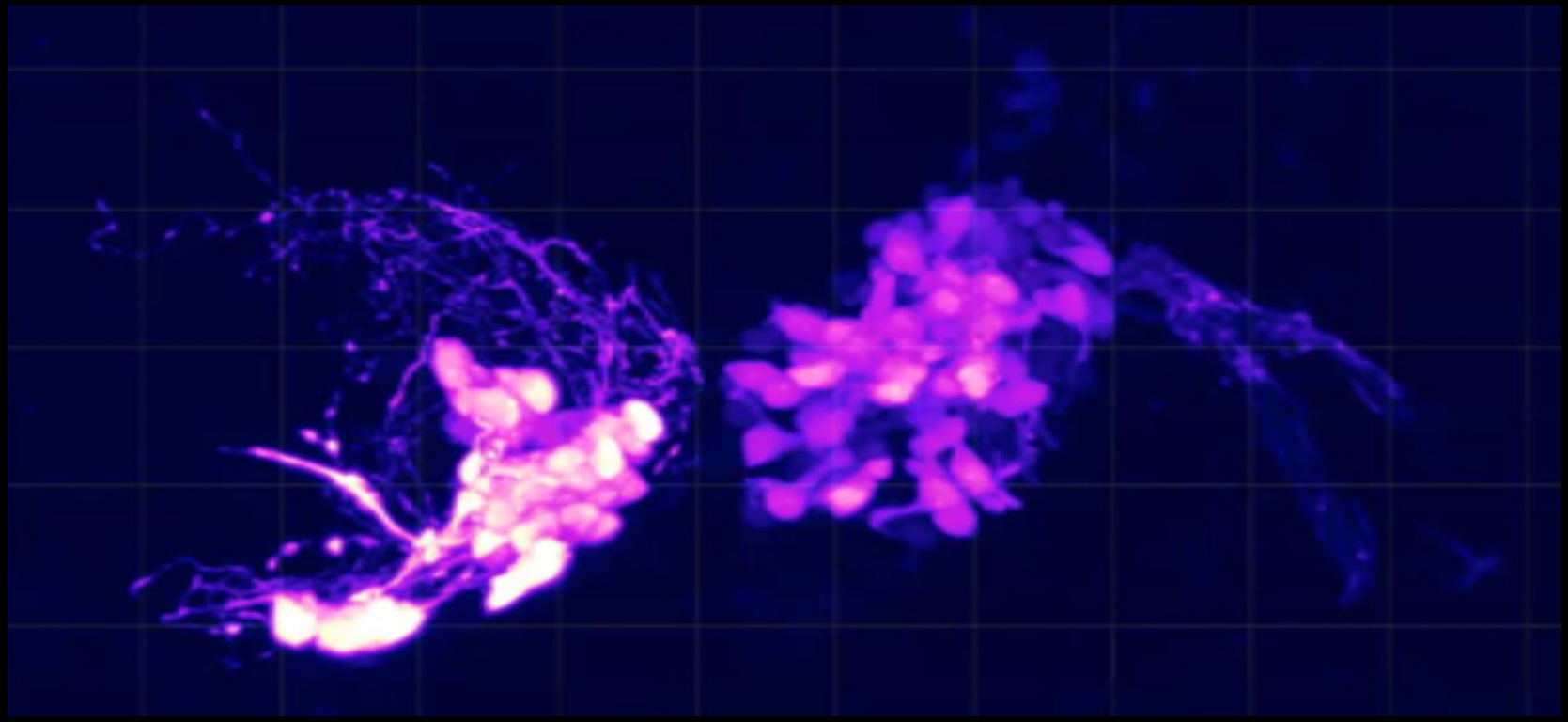
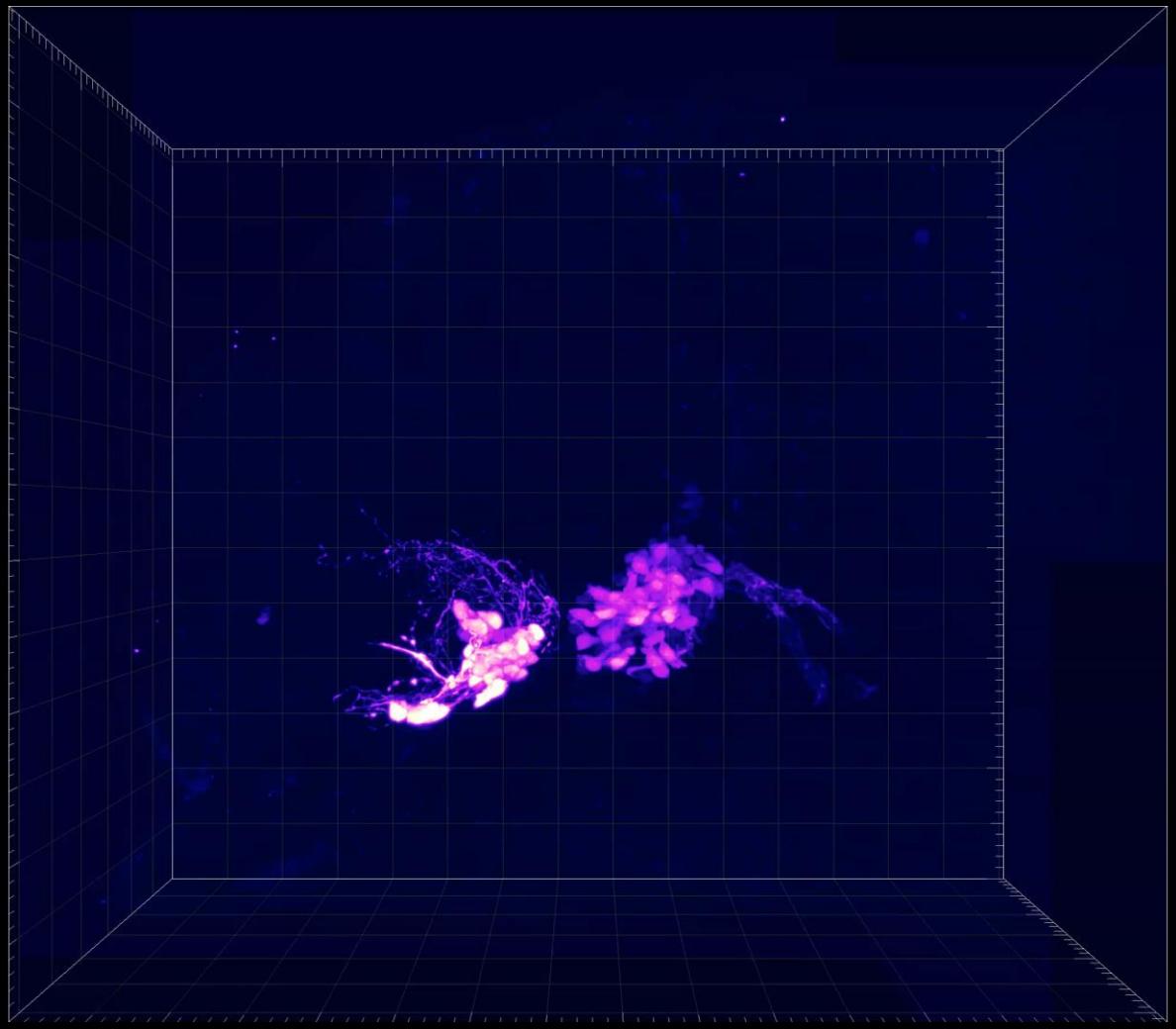


Laboratory for SCIAN Scientific Image Analysis

Zebrafish: Pineal complex

BIOMEDICAL NEUROSCIENCE INSTITUTE

Video of a 3D view comprising 1687 slices. Volume size 1133 x 954 x 954 μm^3 (~113 GB)



Hetz-Lab: Drug Discovery

Natural Chilean compounds

Preclinical models

SCIAN-Lab: Digital Pathology

High Speed Whole Slide Imaging

Digital Patholoy

Quantitative Microscopy

Medical Education & Outreach

BNI
BIOMEDICAL NEUROSCIENCE INSTITUTE
CHILE

R&D: Applied Science

Impact on Health System

Impact on Public Opinion

Análisis Biomédico a Distancia

CED AI SpA

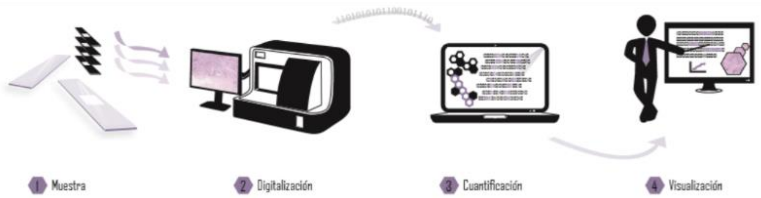
CENTRO DE ESPERMIOGRAMAS DIGITALES ASISTIDOS POR INTERNET

CEDAI Spa, SpinOff creado 2012

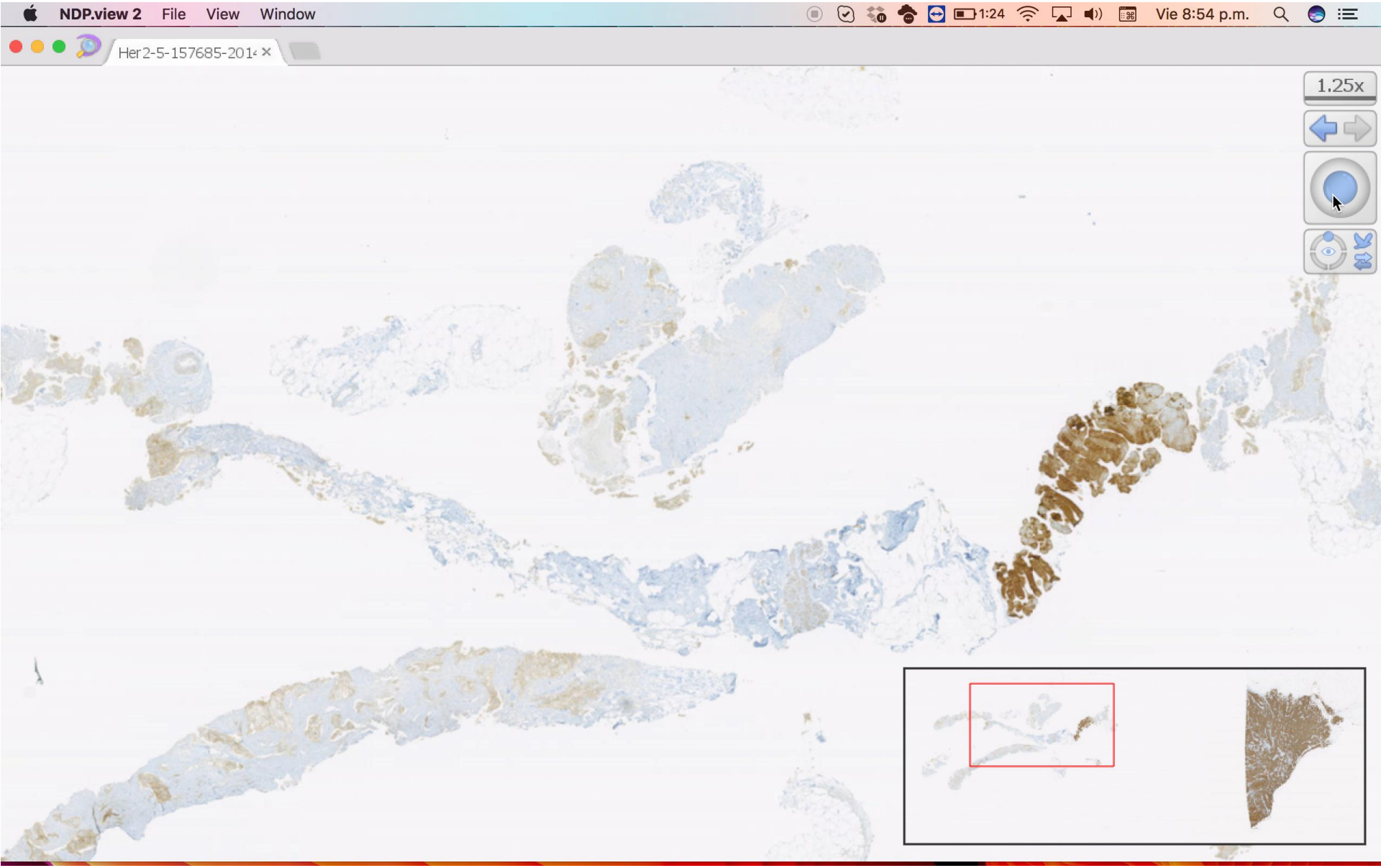
Uno de los dos ganadores de los Premios Iberoamericanos de Innovación y Emprendimiento 2012

CPDAI

CENTRO DE PATOLOGIA DIGITAL ASISTIDO POR INTERNET



CPDAI Digital Pathology as a Service


NDP.view 2
File
View
Window
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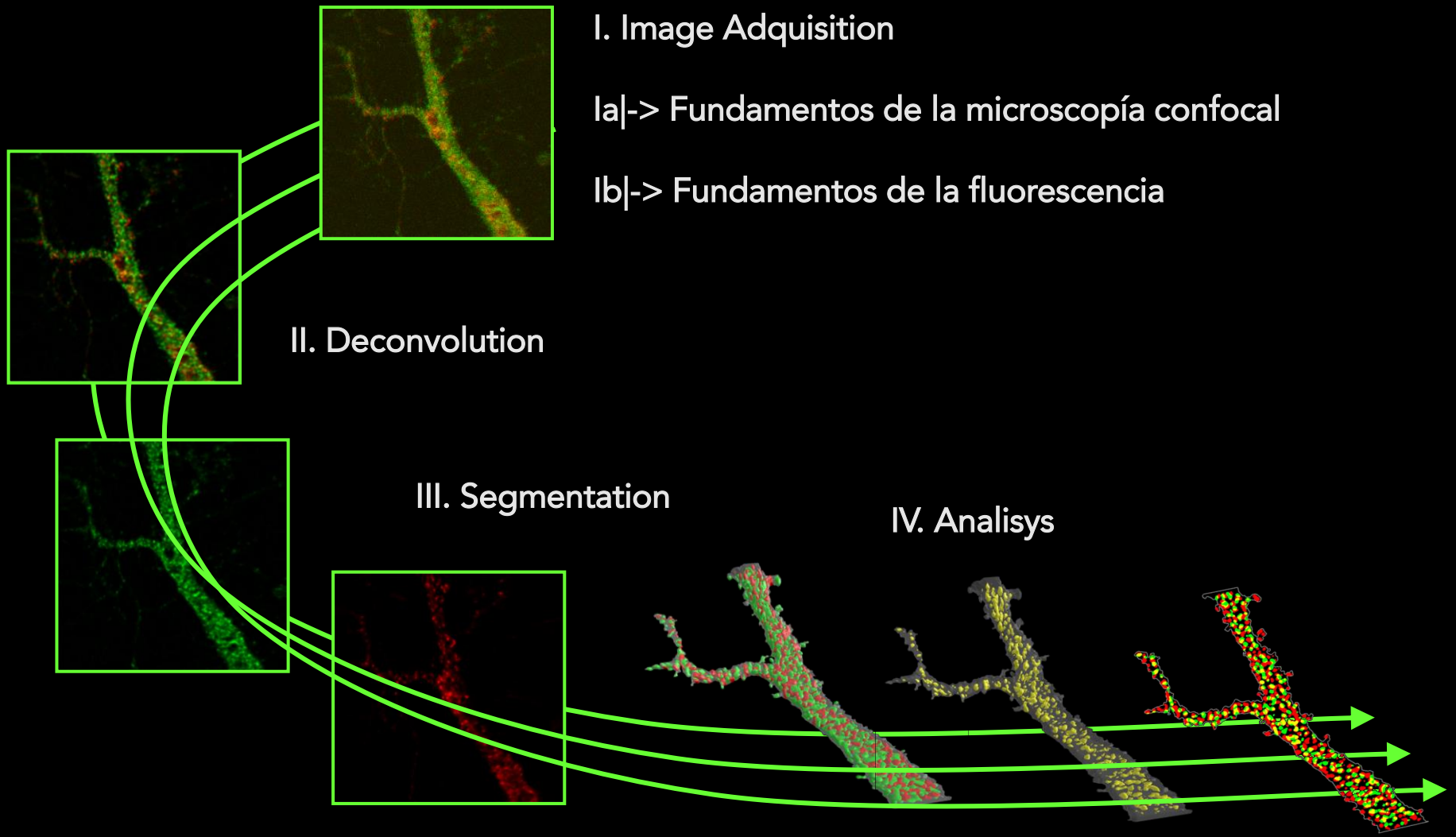
Her2-5-157685-201
1.25x

Laboratory for SCIAN Scientific Image Analysis

Welcome

BIOMEDICAL NEUROSCIENCE INSTITUTE



"It is very easy to answer many of these fundamental biological questions. You just look at the thing !

Make microscopes a hundred times more powerful and many problems of biology would be made very much easier."

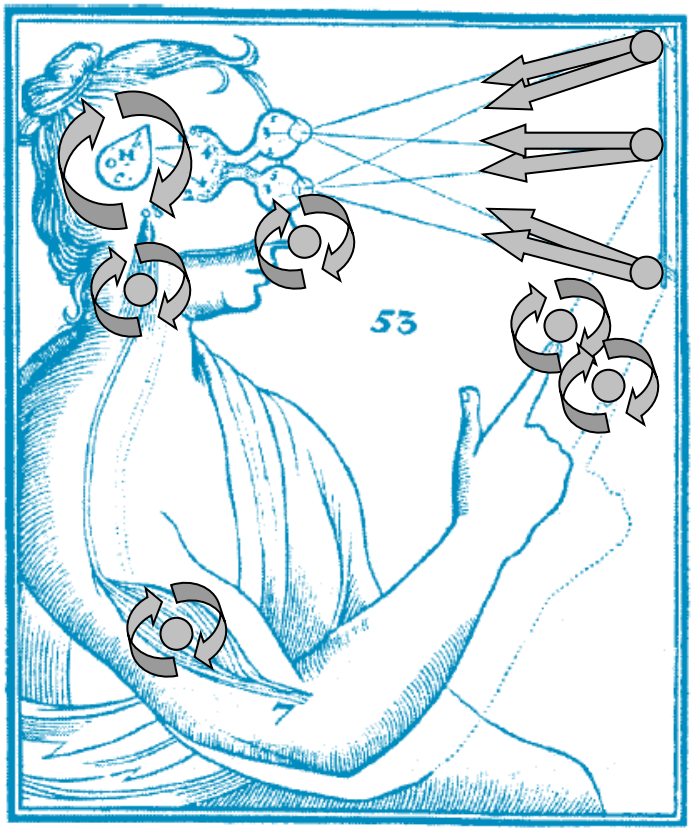
Richard Feynman (1918-1988)

René Descartes (1596-1650)

… just look at the thing …
¿ Human visual perception ?

***Treatise of man* (~ 1637)**

***Passions of the soul* (~ 1649)**



***glandula pinealis* / pineal organ**

A combination of ...

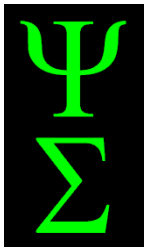
1| direct signals ...

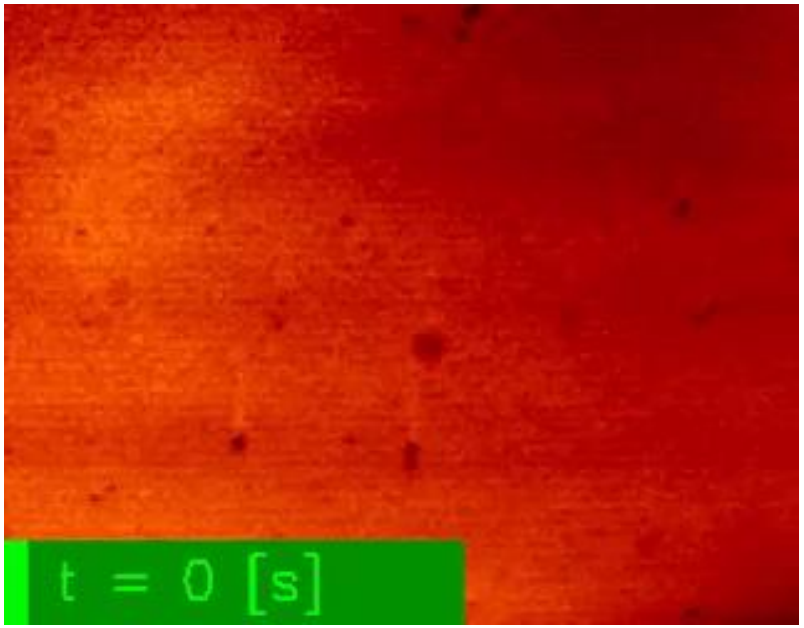
2| signals from other senses ...

3| feedback loops ...

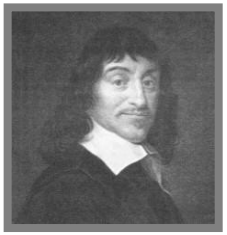
... produce a symbolic representation of an object.

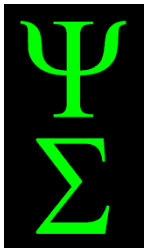
| Best resolution in t & x …

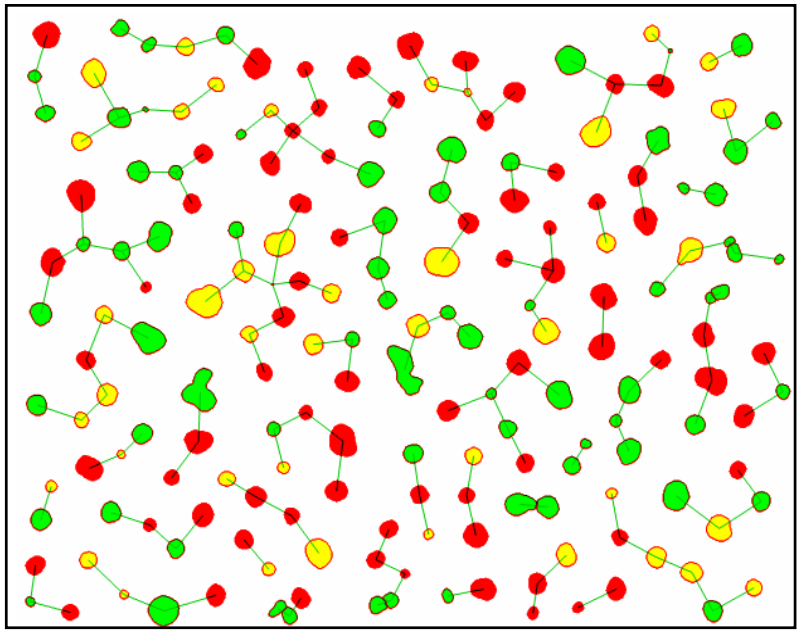


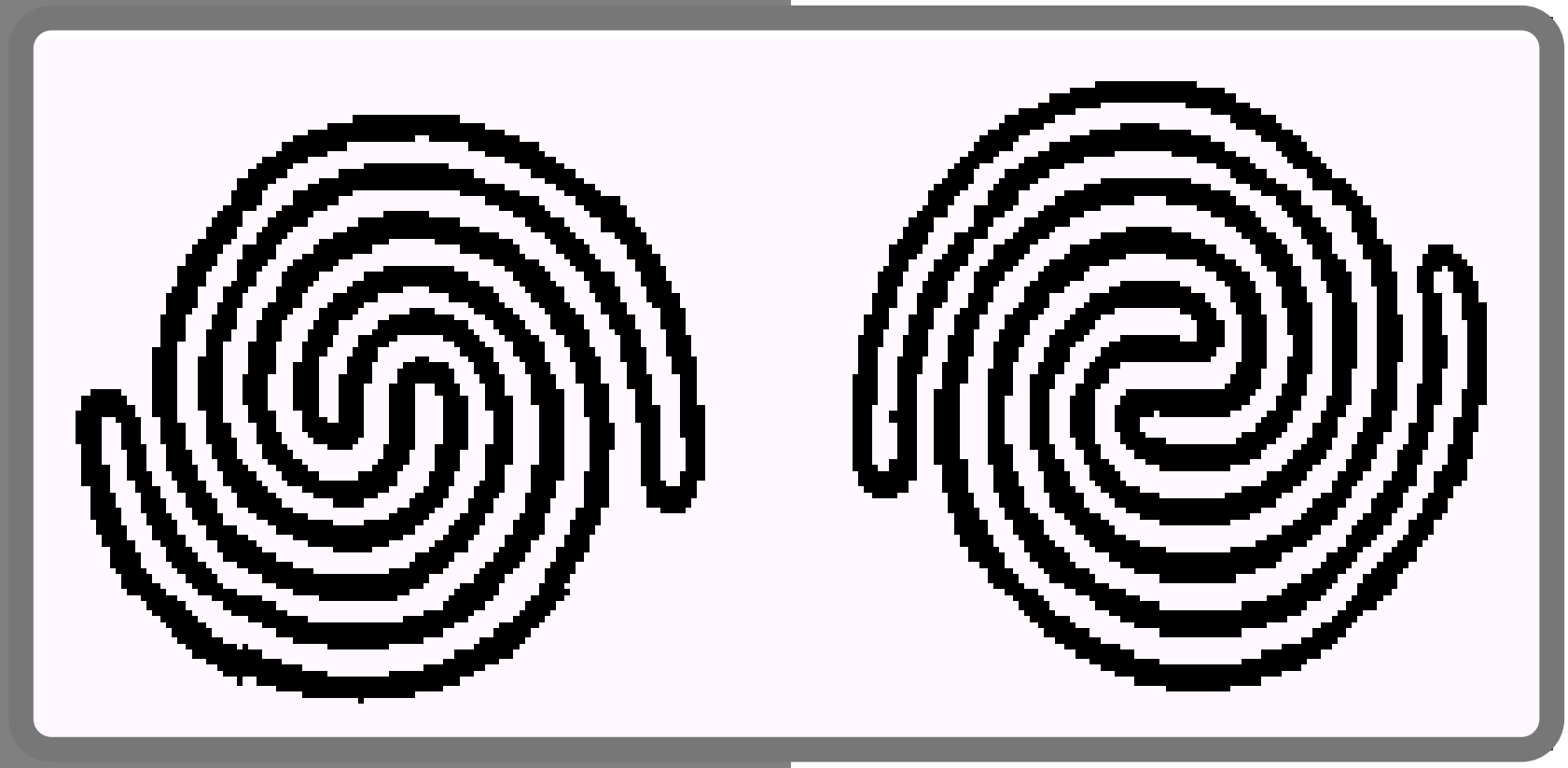


|| Humas vs machine vision





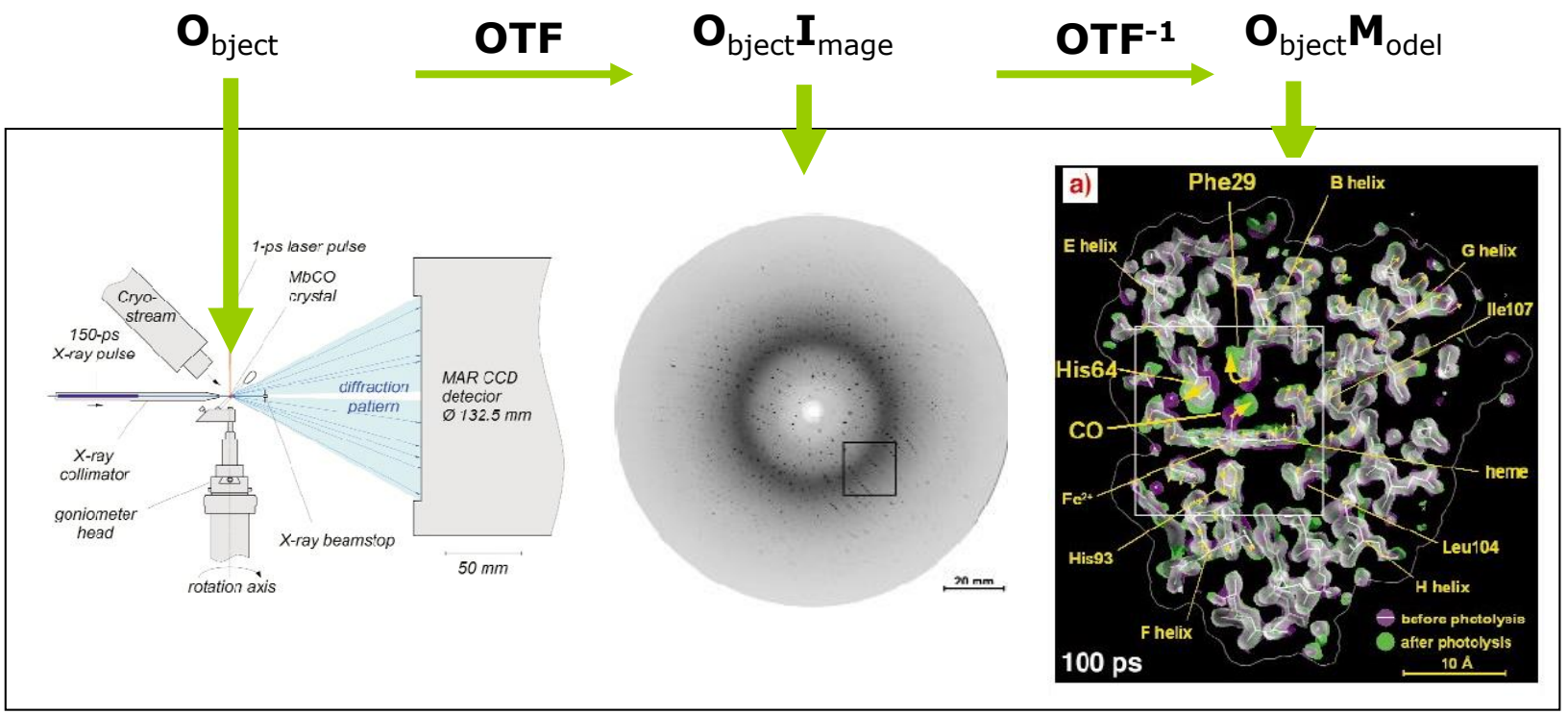




OTF: Object/Optical Transfer Function

Myoglobin in Action |Picosecond Laue Crystallography Diffraction Data

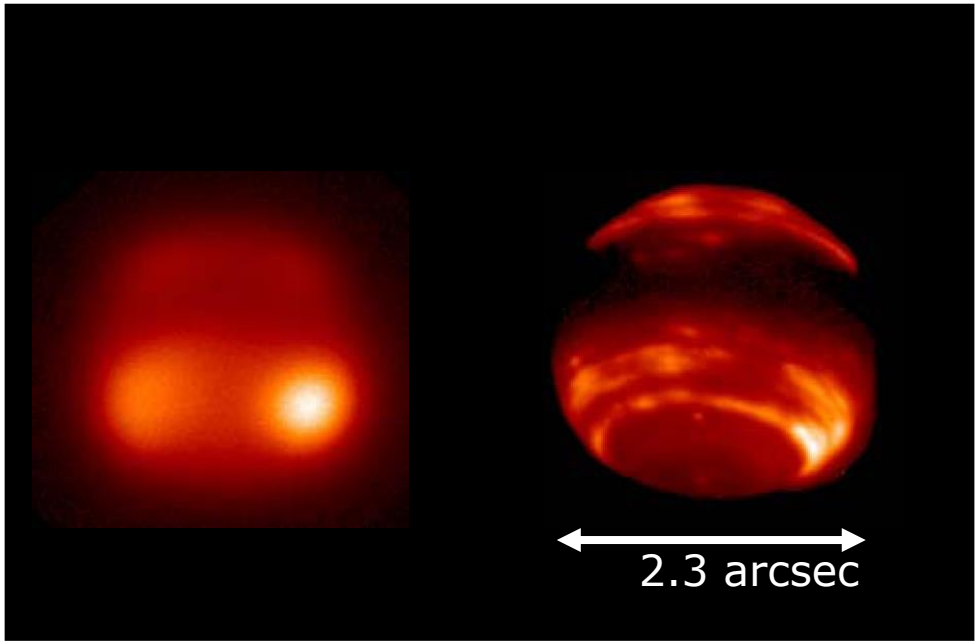
Schotte et al (2003) Science



http://www.youtube.com/watch?v=lnKIBZYarzM

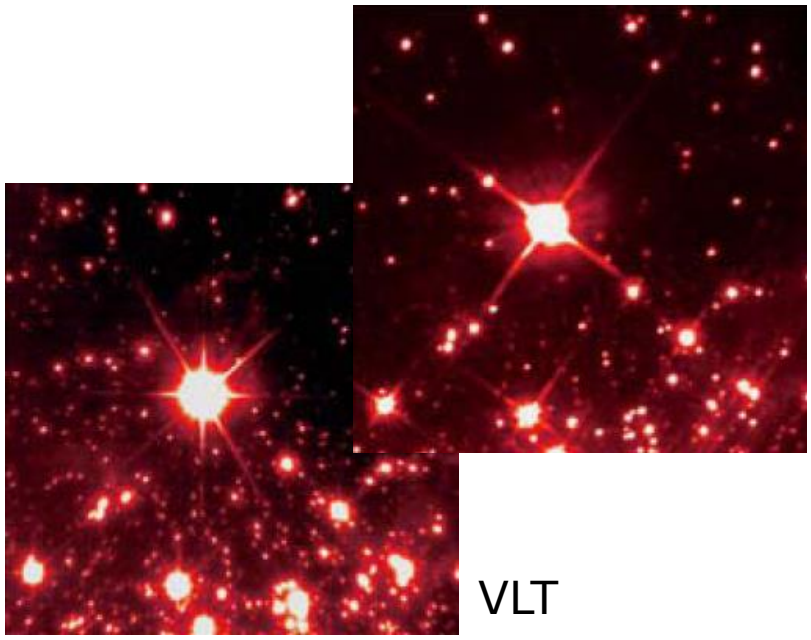
Diffraction Limited Resulution for a 10m telescope ~ λ/D ~ 0.01 arcsec
is limited to ~ 0.5 arcsec by the turbulent atmosphere.
NAOS creates an artificial star at 90 km altitude in the Earth´s mesosphere.
The Laser Guide Star is used to correct atmospheric effects

O_{bject} **OTF** $O_{bject}I_{mage}$ OTF^{-1} $O_{bject}M_{odel}$



Diffraction Limited Resulution for a 10m telescope ~ λ/D ~ 0.01 arcsec is limited to ~ 0.5 arcsec by the turbulent atmosphere.

O_{bject} → **OTF** → $O_{bject}I_{mage}$ → OTF^{-1} → $O_{bject}M_{odel}$



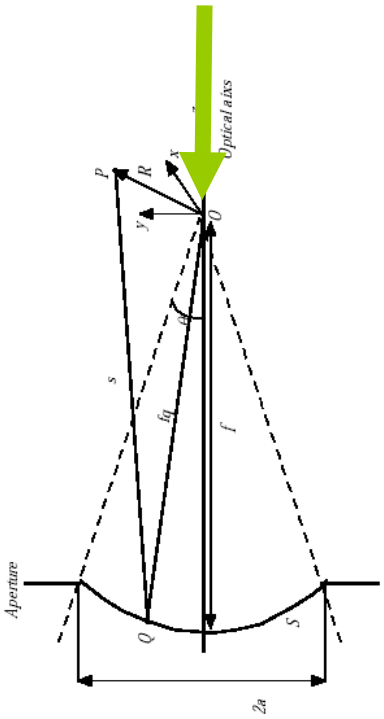
Hubble

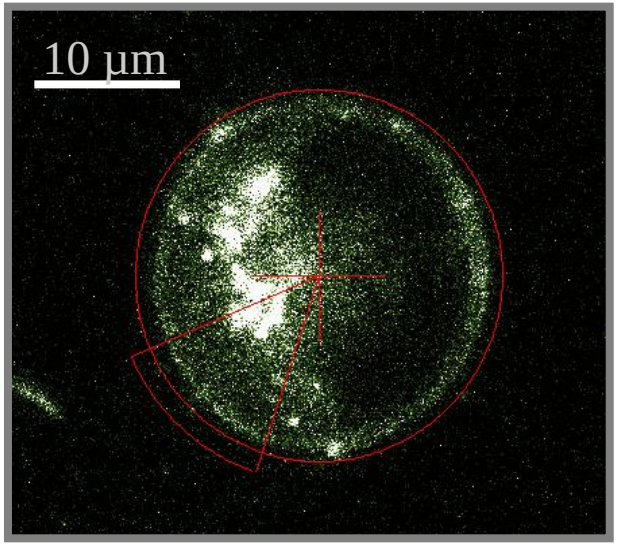
VLT

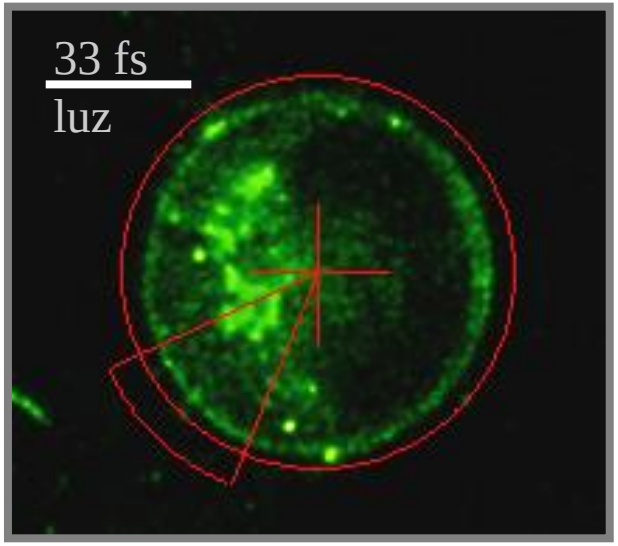
Confocal Microscopy | From Geometric Optics to Diffraction Theory

Diffraction: The deviation of an electromagnetic wavefront from the path predicted by geometric optics when the wavefront interacts with a physical object such as an opening or an edge.

Object → **OTF** → **O**bject**I**mage → **OTF**$^{-1}$ → **O**bject**M**odel



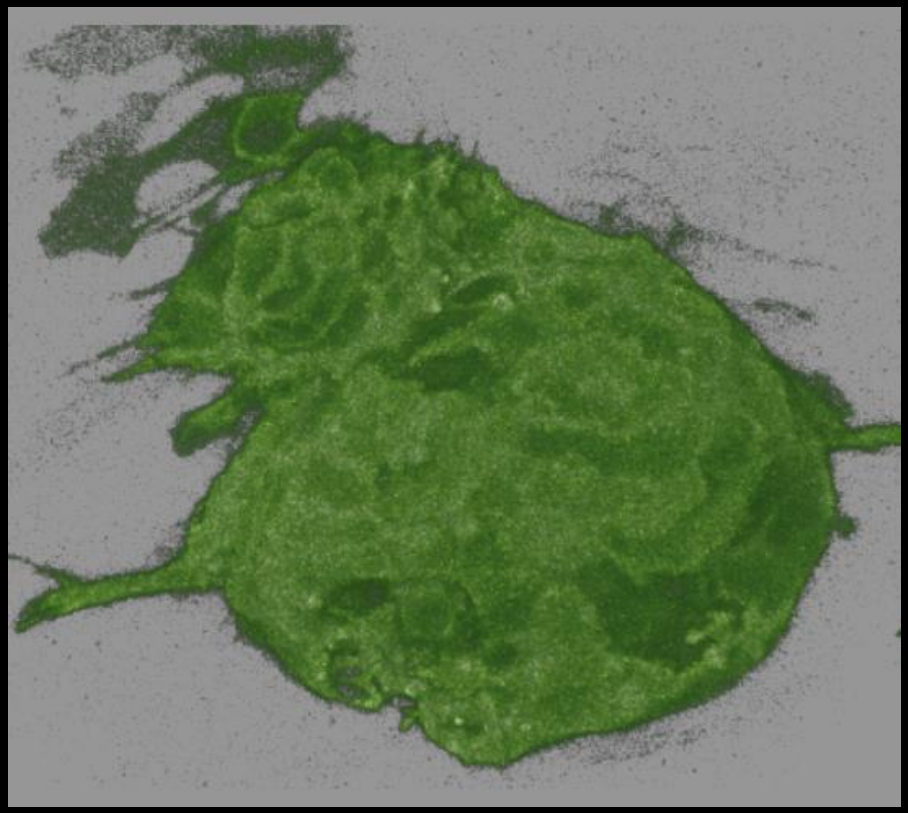


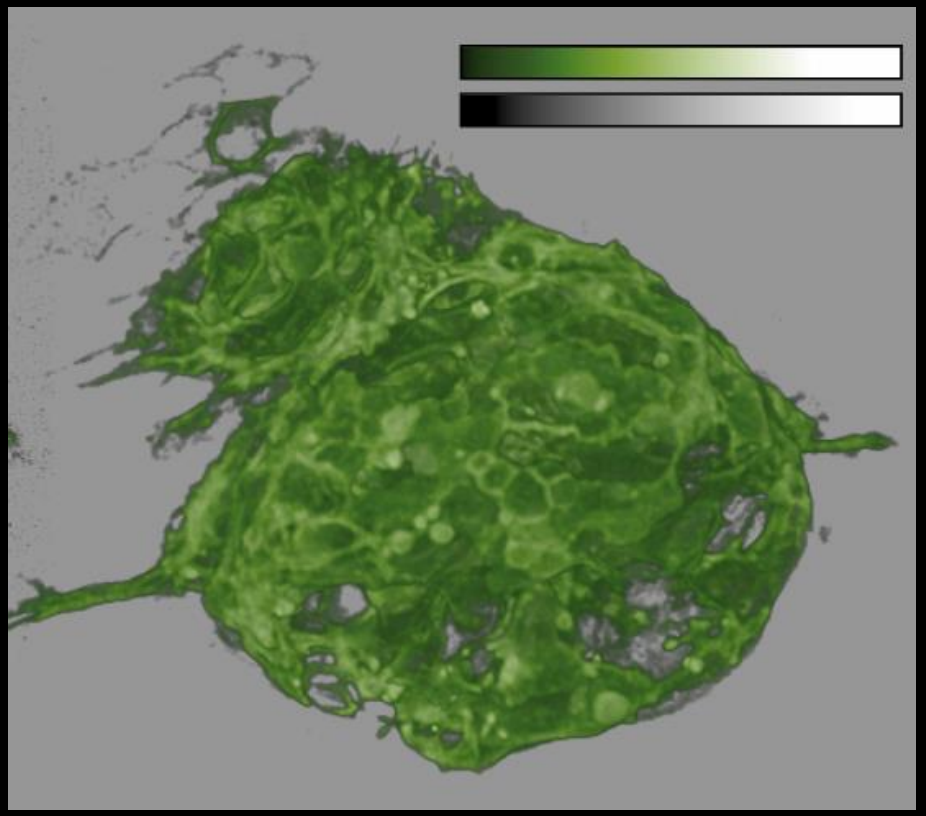


Laboratory for SCIAN Scientific Image Analysis

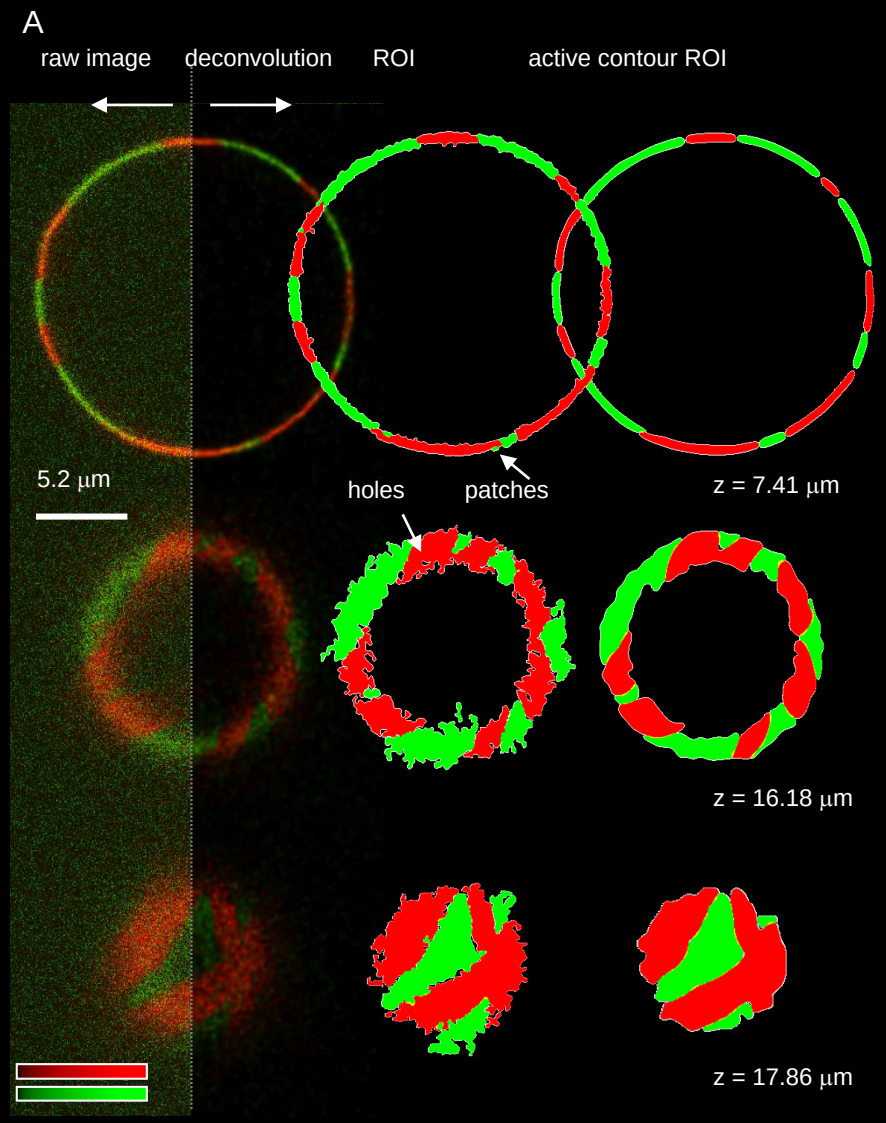
|-> Deconvolution

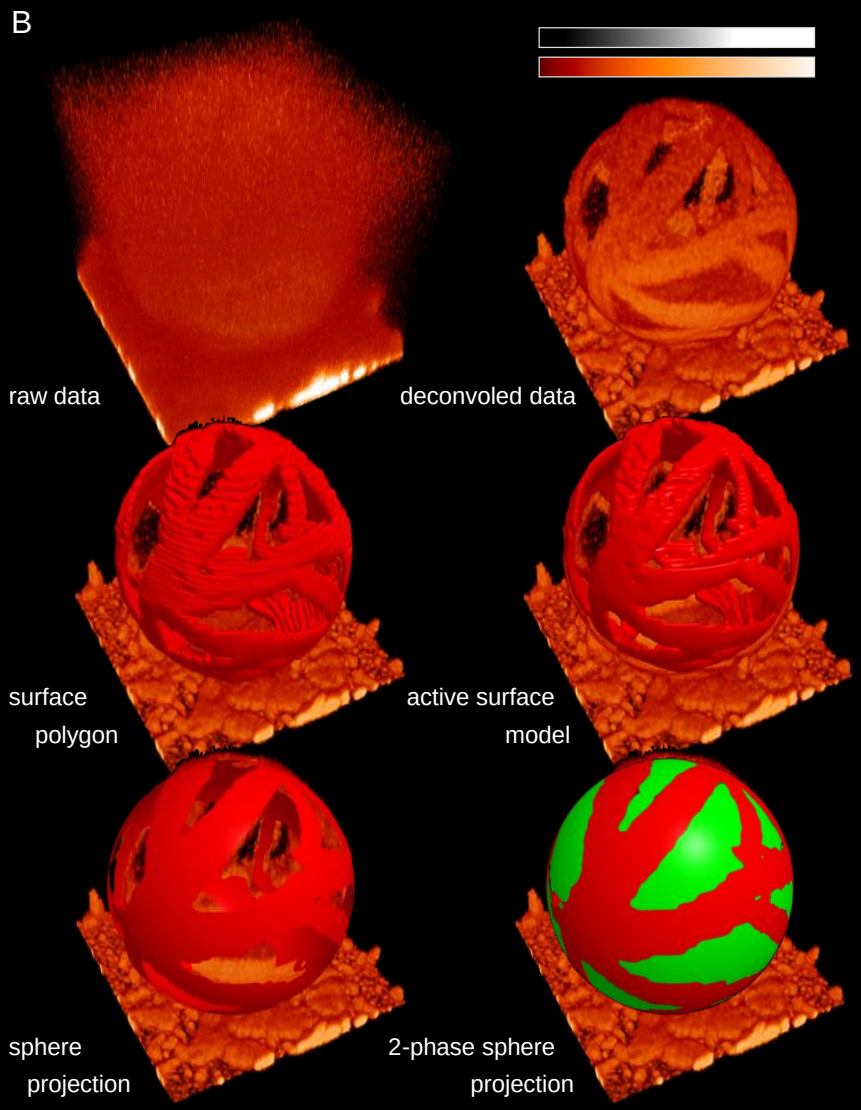
BIOMEDICAL NEUROSCIENCE INSTITUTE





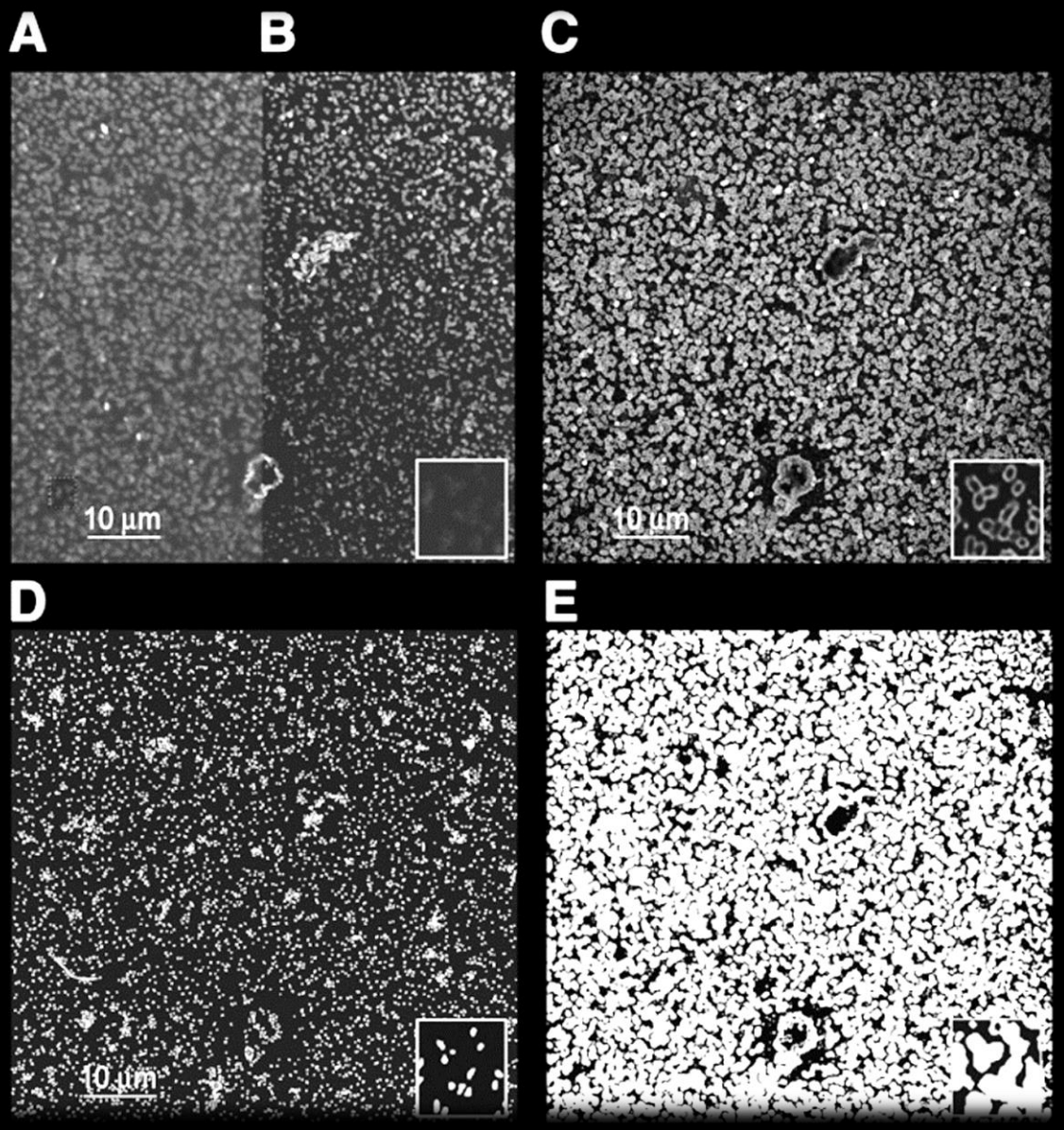
|-> Deconvolution



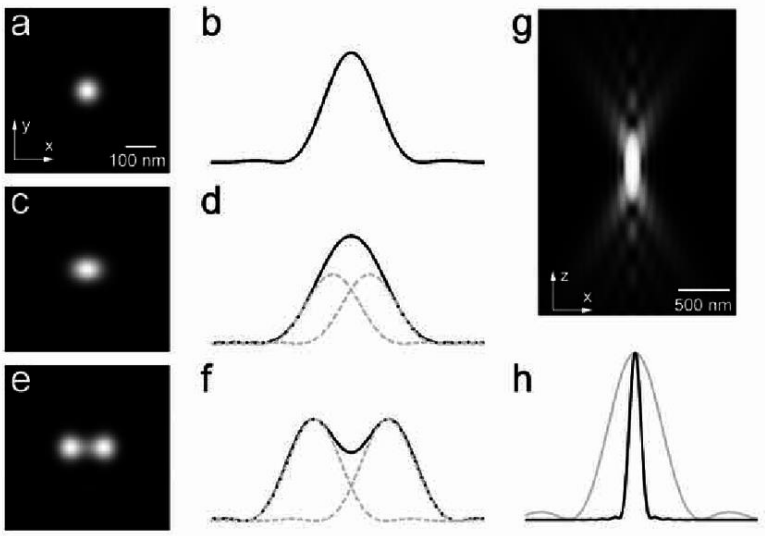


|-> Deconvolution

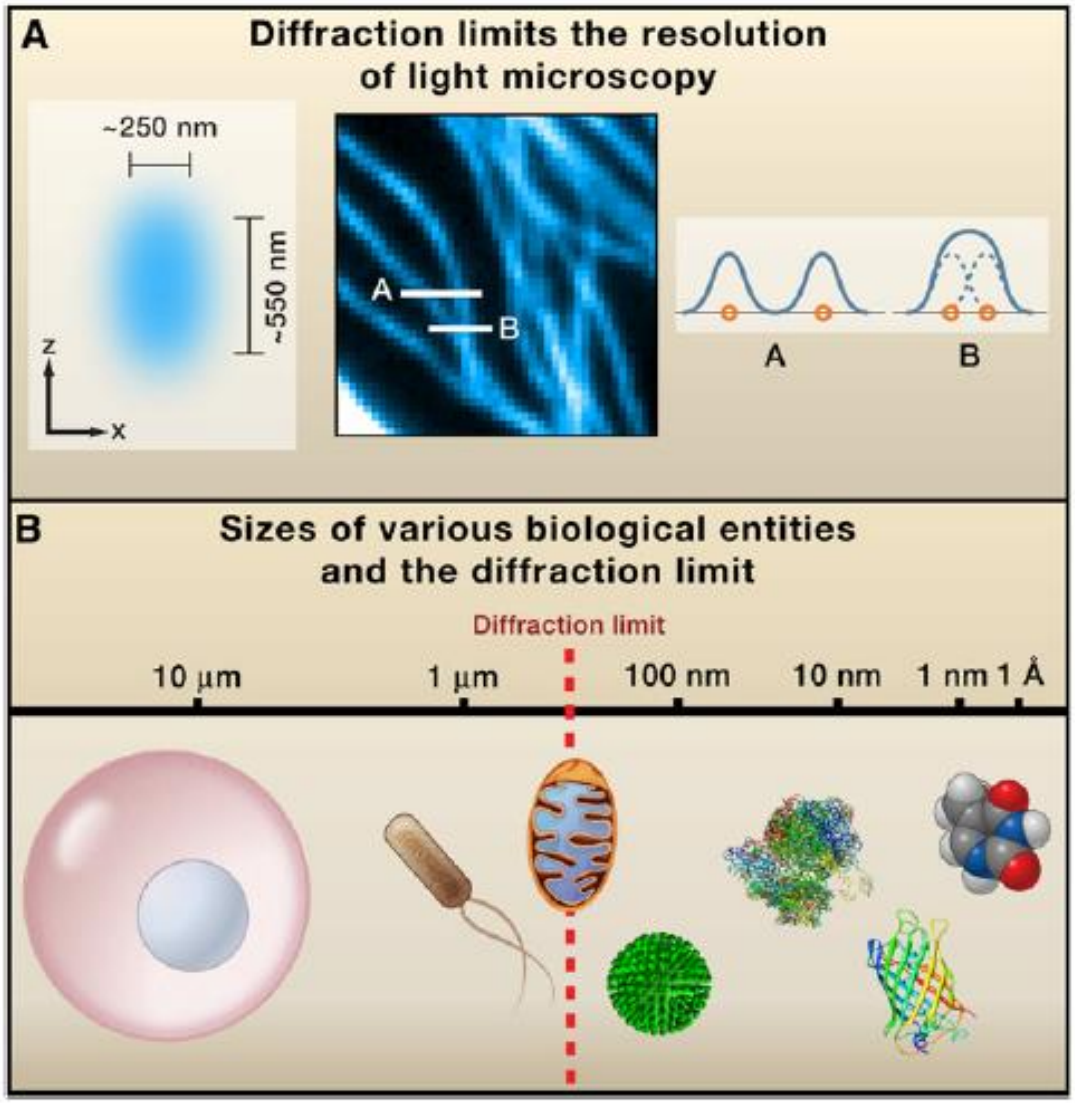
G. Schlapp et al. / Journal of Microbiological Methods 87 (2011)



|-> Diffraction limited Microscopy



Bertocchi, Cristina & Goh, Wah & Zhang, Zhen & Kanchanawong, Pakorn. (2013). Nanoscale Imaging by Superresolution Fluorescence Microscopy and Its Emerging Applications in Biomedical Research. Critical reviews in biomedical engineering. 41. 281-308. 10.1615/CritRevBiomedEng.2014010448.



Bo Huang, Hazen Babcock, Xiaowei Zhuang, Breaking the Diffraction Barrier: Super-Resolution Imaging of Cells, Open Archive Published:December 17, 2010 DOI:https://doi.org/10.1016/j.cell.2010.12.002

|-> Beyond diffraction

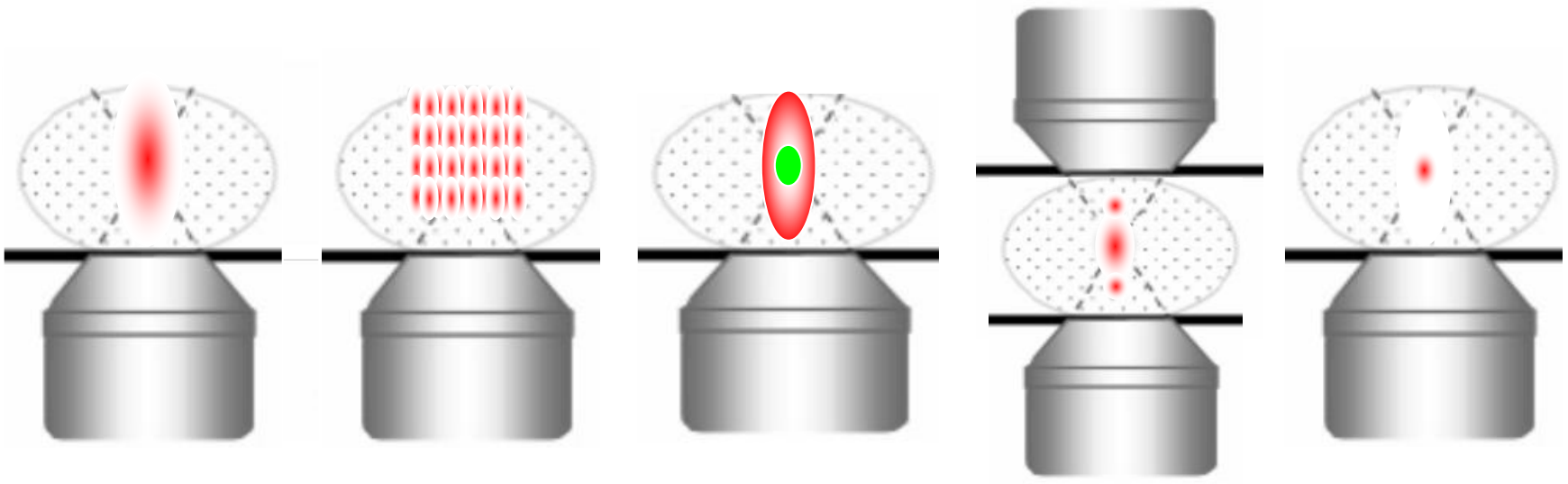
M Goeppert-Mayer
1906-1972

M Gustafson
1960-2011

S Hell
MPI Göttingen
BIOQUANT Hdg

E Betzig
Janelia Farm

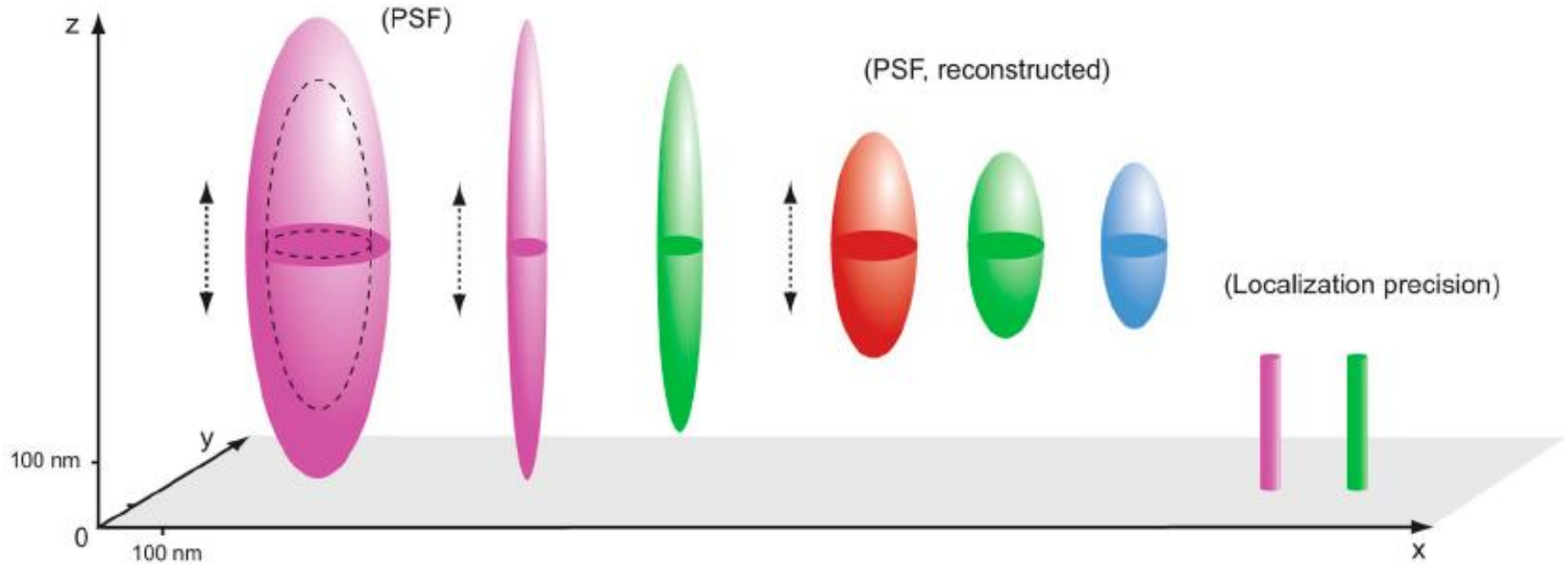
FWHM(xy) ~ λ/2 | ~ λ/4 | ~ λ/∞ | ~ λ/4 | ~ λ/100



2-photon | SIM | STED | 4-π | PALM

|-> PSF overview

	CLSM	STED	CW-STED	3D-SIM			PALM/STORM	
~λ_{em} [nm]	460-670	670	520	620	520	460	670	520



~$D_{x,y}$ [nm]	180-250	60	70	130	110	100	30
~D_z [nm]	500-700	700	560	340	280	250	140 (TIRF-range)
~$V_{x,y,z}$ [*10^{-3} µm^3]	10-23	1.3	1.5	3.0	1.8	1.3	0.1

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Seeing is believing? A beginners' guide to practical pitfalls in image acquisition. Alison J. North. 2006 The Journal of Cell Biology, 172(1):9-18

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JCB: FEATURE

Seeing is believing? A beginners' guide to practical pitfalls in image acquisition

Imaging can be thought of as the most direct of experiments. You see something; you report what you see. If only things were truly this simple. Modern imaging technology has brought about a revolution in the kinds of questions we can approach, but this comes at the price of increasingly complex equipment. Moreover, in an attempt to market competing systems, the microscopes have often been inappropriately described as easy to use and suitable for near-beginners. Insufficient understanding of the experimental manipulations and equipment set-up leads to the introduction of errors during image acquisition. In this feature, I review some of the most common practical pitfalls faced by researchers during image acquisition, and how they can affect the interpretation of the experimental data.

This article is targeted neither to the microscopy gurus who push forward the frontiers of imaging technology nor to my imaging specialist colleagues who may wince at the overly simplistic comments and lack of detail. Instead, this is for beginners who gulp with alarm when they hear the word "confocal pinhole" or sigh as they watch their cells fade and die in front of their very eyes time and time again at the microscope. Take heart, beginners, if microscopes were actually so simple then many people (including myself) would suddenly be out of a job!

All data are subject to interpretation

Deliberate scientific fraud exists, but in modern microscopy a far greater number of errors are introduced in complete innocence. As an example of a common problem, take colocalization. Upstairs in the lab, a researcher collects a predominantly yellow merged image on a basic microscope, naturally interpreted as colocalization of green and red signals. But on the confocal microscope, there is no yellow in the merged images.

"When you employ the microscope, shake off all prejudice, nor harbour any favorite opinions; for, if you do, 'tis not unlikely fancy will betray you into error, and make you see what you wish to see." Henry Baker, chapter 15, "Cautions in viewing objects" of The Microscope Made Easy, 1742.

How can this be? Many factors contribute. Here, I take the reader through the imaging process, from sample preparation to selection of the imaging and image-processing methods. Throughout, we will be on the look-out for problems that can produce misleading results, using colocalization as the most common example. Because one short article cannot be an exhaustive "how to" guide, I have also assembled a bibliography of a few highly recommended textbooks and microscopy web sites, which readers should consult for more extensive treatments of the critical issues introduced here.

Sample preparation

"Garbage in = garbage out" is the universal motto of all microscopists. A worrying tendency today is to assume that deconvolution software or confocal microscopes can somehow override the structural damage or suboptimal immunolabeling induced by poor sample preparation. The importance of appropriate fixation, permeabilization, and labeling methods for preserving cellular morphology or protein localization is well known to electron microscopists (Hayat, 2000), but often underestimated in optical microscopy (Fig. 1).

Many labs use one standardized protocol for labeling with all antibodies, irrespective of whether the targets are membrane- or cytoskeleton-associated, nuclear or cytosolic. However, inappropriate fixation can cause antigen redistribution and/or a reduction in antigenicity. It is therefore important to test each antibody on samples fixed in a variety of ways, ranging from solvents such as methanol to chemical cross-linking agents such as paraformaldehyde and glutaraldehyde (for protocols see Bacallao et al., 1995; Allan, 1999), although glutaraldehyde fixation often reduces antigenicity and increases background autofluorescence. Consult textbooks for notorious pitfalls: phalloidin labeling is incompatible with methanol fixation, while microtubules are inadequately fixed by formaldehyde. Moreover, certain cell types, such as yeast cells, require specialized fixation protocols (Hagan and Ayscough, 1999).

Permeabilization is also critical in achieving a good compromise between antigen accessibility and ultrastructural integrity. Specific detergents will produce different effects (for example, Saponin treatment produces smaller holes in

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The objective lens is the most critical component of a microscope and yet few researchers grasp the differences between specific objective classes.

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Keep the acquisition settings constant between specimens to be compared quantitatively and particularly between sample and control.

"Remember that truth alone is the matter that you are in search after; and if you have been mistaken, let not vanity seduce you to persist in your mistake." Henry Baker, The Microscope Made Easy, 1742.

|-> Microscopy

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