"Biological Physics: what we expect from this frontier at the interface between physics and biology"

Carlos Lenz Cesar – lenz@ifi.unicamp.br
Institute of Physics UNICAMP Campinas Brazil
Midia’s future is always dark: if it bleeds, it leads!

- Planet of the Apes
- Mad Max
- Water World
- NY Subway
But the future can be the best ever seeing

Abundance

THE FUTURE IS BETTER THAN YOU THINK

Health Freedom
Energy Education ICT
Food Water Shelter

PETER H. DIAMANDIS • STEVEN KOTLER

www.gapminder.com

Life expectancy from 1800 to 2010

United States 1800
Japan 1800
Brazil 1800
World population evolution

[Graph showing the evolution of world population over time, with key events marked along the timeline and population milestones marked on the y-axis.]
Mortality in US from 1900 a 2010
Cancer mortality surpasses heart disease

Conclusion: deaths due to external aggressors replaced by internal malfunctioning diseases!
We can make peace with bacterias!
And used them for good
5 Revolutions - ~50-60 years total cycle

1. Industrial Rev. – England – 1771
2. Steam and rail-road – England – 1829  [58 years after the 1st]
3. Steel and electricity – England+USA+Germany – 1875  [46 yrs after 2nd]
4. Oil, cars and mass production – USA – 1908  [33 yrs after 3rd]
5. Information and communications – USA – 1971  [63 yrs]  – [42 yrs after 5th]
Signs of New Era: World is ready to launch the next scientific-technological revolution.

Signs:
- The crisis already appeared
- The Information era evolution is here
Surf wisdom: catch the wave before it breaks

Could Brazil catch this wave earlier?
As you know, scientific discovery … requires the support of a nation. But it holds promise like no other area of human endeavor.
The billion dollars question:

What will be the next [6th] scientific-technological revolution
Our bet:
The 6th scientific-technological revolution will be the control of biology

Watson & Crick 1953
DNA biology software
> 60 years old knowledge
1953: Feynman proposed manipulation of individual atoms

Talk that inspired the nanotechnology!
Information: Britannica on the head of a pin

Nobody implemented this experiment but:
Biological enzymes (ribosomes) function chemically close to Feynman's vision
What I cannot create, I do not understand!
Synthetic biology is the technology of the century. This is going to change how we build things. Biology is fundamentally a manufacturing technology, and we’re on the verge of figuring out how to control that. It's impossible to predict and estimate the impact of that, but it’s going to be massive.

We have very little ability to put atoms exactly where we want them. Semiconductor engineers don't get to put atoms where we want them. Biology puts every atom in the place it wants with precise control. We can use that as a very powerful manufacturing technology.

Be aware that we always overestimate what will happen in five years and always underestimate what will happen in ten.
"Nothing in Biology Makes Sense Except in the Light of Evolution"

1973 quote from Theodosius Dobzhansky

How much synthetic biology needs Darwin?

Distinction between nature and artefact!

Artefacts have no causal connection to historical evolutionary processes.

C. J. Preston, Philosopher, Univ. Montana
Evidences that the next wave is coming faster than we think
Faster than you think:
DNA sequencing cost – similar to microelectronics
Faster than you think: Craig Venter and Synthetic Genomics – San Diego

http://www.syntheticgenomics.com/

First Man-Made Genome; Synthetic Life Comes Next

Custom-design organisms creating biological robots to produce from scratch chemicals humans can use.

Biofuels like ethanol, for example.
Interface physics/biology
Nobel Prize Steven Chu talk at The Annual Meeting of American Society for Cell Biology [ASCB]

Chu’s career: Bell Labs – Stanford – Director of Lawrence Berkeley Labs – Secretary of Energy - Stanford
Science leadership in different centuries:

17th/18th: Mechanics!

19th: which?
Physics [thermodynamics, electricity, statistical mechanics, radio waves]
Chemistry [atomic theory, steel, organic chemistry, periodic table]
Biology [evolution, cell theory, cytology, embryology].

20th: Physics revolution: electron, nuclei, x-rays, quantum physics, atomic theory, nuclear physics, semiconductors, lasers, optical fibers, information network.

Atomic&molecular physics have large impact on chemistry and biology. X-rays opened up cell biology field [DNA double helix].
21st: leaded by biology?
Highest interest of human society!

Reductionism:
Can physics explain biology?
Can physics explain living organisms' processes.
Can molecular physics explain transformation of complex molecules to simplest organisms that can self-regenerate, the step between life and nonlife?
Could physics explain the emergence of conscience?
Simple versus complex!
Validity of reductionism?

A complex system that works is invariably found to have evolved from a simple system that works!
John Gaule [1603?–1687]
2009: Committee on a New Biology for the 21st Century

Ensuring the United States Leads the Coming Biology Revolution; National Research Council

Biology in a revolutionary change integration powerful technologies + new concepts/methods from physics, mathematics, computation and engineering.

The essence of New Biology is integration/re-integration. research community with capacity to tackle difficult scientific and societal problems
Biology at a point of inflection

Detailed information about components of the complex systems: genes, cells, organisms, ecosystems.

Begin to understand how components work together as systems. Powerful tools to probe complex systems – from cellular molecular events to global biogeochemical cycles.

Committee concluded that life sciences reached a point where a new level of inquiry is possible.

Fundamental unity of biology - solutions in life sciences will derive from understanding of core biological processes, common to all living systems.
Why Now?

Life sciences analogous to early 20\textsuperscript{th} century physics. Common sentence: 21st century will be the century of biology! Became increasingly evident over the last decade.

New Biology has already begun to emerge.

Understanding how parts of living systems operate together.

Promises:

- abundant food for everyone
- resilient environment
- good health is the norm
- with sustainable/clean energy
New Biology for the 21st Century:

Recommendations: Inter agency fund support - integration of biology/physics/chemistry/engineering, difficult to capitalize within traditional institutional and funding structures.

The New Biologist is not a scientist who knows a little bit about all disciplines, but a scientist with deep knowledge in one discipline and a “working fluency” in several.

Any knowledge is useful in understanding many others. Biological experimentation shall focus on individual/small numbers of components within a single organizational level. The reductionist approach succeed to reveal many of the basic molecular, cellular, physiological, and ecological processes.

New Biologists as well as physicists or mathematicians
1953 - 1st Bioscience revolution: DNA molecular/cellular biology
2nd Bioscience revolution: genomic revolution
3rd Bioscience revolution revolution: convergence

Combining molecular/cell Biology with physics & engineering.
Economics to support convergence:

Solow showed that economic growth comes from innovation.

Information technology revolution is maturing.

Initial phase of biotech revolution is playing out.

Bets are it will be the next phase of innovation.

USA should capitalize convergence.
Recommendations:

Molecular/cell biology are in the center of next wave.

Funding of integrated research of life sciences with physics and engineering.
Progress can be quick when researchers with different knowledge bases are encouraged to come together.

NIH funds decreased. Conservatism of peer review system: tend to approve only conservative research.

Special multidisciplinary collaborative programs
Suggested 10 years of investments. Reform peer review to open opportunities for such programs.

More multidisciplinary education of next generation
“action most worth watching is not at the center of things but where edges meet, shorelines, weather fronts, international borders”
Anne Fadiman, The Spirit Catches You and You Fall Down
Grand Challenges

1. Synthesizing Lifelike Systems

2. Understanding Brain

3. Predicting Organisms’ Characteristics from DNA Sequence

4. Interactions Earth, Climate, and Biosphere

5. Understanding Biological Diversity
Recommendations to Enable Intersection Research:

Training-Support-Communication Across Disciplines
Connections Between Disciplines
Culture of Separation between the Life and Physical Sciences
Culture and Organization of Academia
Organization of Support for Research
Supporting Transformative Research
Educating Scientists at the Intersection of the Physics/Life Sciences
Enabling Interdisciplinary Research since Undergraduate Level
Integrating Life/Physical Sciences for Graduation/Postdoctor
Trends in Biology: single cell $\rightarrow$ single molecule

Physics + Chemistry: $10^{20}$ identical molecules:
Perfect statistics – strong accumulated signal
Averaging in space and time

Cells are made equal but cells are not identical
Neither in space nor in time

Spatial organization – external /internal signalling

Best thing for us:
CONTROLLABLE
Researchers should apply to universities at which there are already regular collaborations between imaging centres and medical schools or cell-biology departments.

To understand biological heterogeneity, researchers are learning how to profile the molecular contents of individual cells.
Provocative thinking:
Forget about biology – concentrate at the tools

Let’s use reductionism to build complexity from bottom up

Our role as physicists: develop the tools to control biology at single cell/ single molecule level.

What tools?


Observe, measure, manipulate, understand bioprocesses

Write/erase information at DNA level – control of single DNA differentiation
The need of photonics to observe molecular/cell processes

Microscope is not only a visualization tool:

Analytical tool with space, spectral and time resolution.

Single molecule sensitivity

Any optical characterization could be performed in a microscope.
CdTe quantum dot 1 and 2 photons PLE at 40 K
Optics allows multimodality in the same platform

Optical beams do not collide!

Dichroic filters
Why Multimodal?

PROCESS is a sequence of events in time. Time evolution is crucial. Tool needed: capable of real time observations. No more pictures – we need movies!

LABEL FREE

Non destructive – remote – capable to bring biochemical & biomechanical information – spatial resolution sub-cellular level [ideal molecular level] – 3D image reconstruction.

Questions to be answered: where, when and what happened
Resolved in time, space and spectrally
UNICAMP Group’s history:

1988-1990: Pos-Doc Bell Labs - Arthur Ashkin
1991: Optical Tweezers in Brazil
1995: Collaboration with Hemocenter – Blood98
2003: Raman + Optical Tweezers
2005: Multiphoton microscopy + Optical Tweezers
2006: Second Harmonic Generation Microscopy
2007: Fluorescence Lifetime Imaging + Correlation Spectroscopy
2009: Third Harmonic Generation Microscopy
2009: INCT – INFABIC + FAPESP multiuser
2011: CARS microscopy + Multimodal platform
2013: FRET + in Vivo + AFM tip enhancement
INFABIC – Photonics in Cell Biology

Coordinator: Hernandes F. Carvalho Inst. Biology – UNICAMP

Vice-Coordinator: Carlos Lenz Cesar Inst. Physics - UNICAMP

28 sub-projects
40 Principal Investigators
38 PhDs
Biolologists
Physicists
Medical Doctors
Food engineers
Chemists
Veterinarian
Dentist
Electrical engineer

7 Universities
15 unities

Shared Laboratory for cell processes visualization
Super resolution: localization
Present Limit $< 10$ nm: single molecule
Proteínas em tubulina $\sim 30$ nm
The Optics Arsenal

Tools Available
Linear Optical processes

- \( S_1 \) → \( S_{1\text{In}} \) → \( S_0 \)
- Fluorescence

Non Linear Optical processes

- TPE
- Fluorescence
- \( S_1 \) → \( S_{1\text{In}} \) → \( S_0 \)
- Virtual
- \( \omega_{\text{phonon}} \)
- Virtual

Energy transfer:
- donor molecule → acceptor molecule

FRET (Fluorescence Resonance Energy Transfer)
- donnor molecule
- acceptor molecule

SHG (Second Harmonic Generation)
- Virtual
- \( \omega_{\text{phonon}} \)

THG (Third Harmonic Generation)
- Virtual
- \( \omega_{\text{phonon}} \)

Hyper Raman
- Virtual
- \( \omega_{\text{phonon}} \)

CARS (Coherent Anti-Stokes Raman Scattering)
- Virtual
The Platform
Starting with a spectral confocal microscope
Add a confocal Raman Spectroscopy and get: Raman + Hyper Raman + Fluorescence + PLE
Make room to add an Optical Tweezers and laser cutting

- red blood cell
- Trypanosoma cruzi
- Laser cutting

Descanned Array Detectors APDs

Scan horizontal
Scan vertical
Light Sucks"

Biomechanics F~200 pN
Cell rheology
Cell manipulation
12 papers with hemocenter
+ Laser microdissection:
Controlled transfection;
Cell surgery;
Material collection;
Add an AFM/Tip-enhancement system on top

Descanned Array Detectors APDs

Scan horizontal

Scan vertical

Atomic Force Microscopy
Add a femtosecond laser: multiphoton microscopy
2x wavelength – 16 less scattering

In vivo pancreas
Embryos
Mouse  zebra fish

Descanned Array Detectors APDs

fs

Scan horizontal
Scan vertical

Non Descanned Detector

Goat spermatozoid
Second/Third Harmonic Generation comes for free

Descending Array Detectors APDs

Heart fibroma

Mammary duct

muscle

BP filter

NDD

Squid pen

Mouse aorta
Two examples:
**SHG + THG Ovarian Comparison normal vs adenocarcinoma**

- **TACS-2, collagen tangential fibers**
- **TACS-3, radial collagen fibers**

**Cornea Keratoconus: UV + Riboflavin collagen croslinking**
Add a cronometer – get the arrival time of each photon

FLIM – fluorescence lifetime imaging

Descanned Array Detectors APDs

$L^+$

OH$^-$

Hangerham’s cell

After 10 min glucose

Sugar cane lignin

Plant stomata

fs

Scan horizontal

Scan vertical

FLIM Detector
Example 1: In vivo mice pancreas

0.00 min

FLIM after glucose injection

0 min

20 min

Histogram of fluorescence lifetimes after glucose injection
Example 2: Parhyale hawaiensis embryo development

EMBRYO´s Development
Strategical studies to understand stem cell differentiation
Use FLIM to measure distance donor-acceptor by FRET
Förster Resonant Energy Transfer

CFP + YFP
Negative control

CFP - 15 AA - YFP

CFP-Cry-ab + YFP-FAK-CT
Count the fluctuation: Fluorescence Correlation Spectroscopy (FCS)

$$(1 \, \mu m)^3 = 1 \, \text{femtoliter} \quad \text{mass spectrometry?}$$

Hydrodynamic radius extracted from diffusion time

Rodamine

$$\tau_D = \frac{\omega_x^2}{4D}$$

Home made CdSe Quantum dot

R = 1.8 nm

$$R_h = \frac{4k_bT \tau_d}{6\pi\eta\omega_x^2}$$
Coherent Anti-Stokes Raman Scattering (CARS) Chemical specific imaging

The first CARS images of Brazil

- Mouse ear fat gland
- Lung artery
- Heart
Sum Frequency Generation comes for FREE
Myelin sheath of mice sciatic nerve
Make it in vivo:
Mice mammary glands - milk secretion
Integrated techniques into the same platform

3D + time-lapse capabilities

Single/multiphoton fluorescences: intensity spectral

+ FLIM + PLIM + FCS + FRET + F…

SHG + THG

Raman + CARS

Tip-enhancement + conventional AFM

Optical Tweezers + laser cutting

Physiological controlled cell – temperature +
atmosphere
People

Physics UNICAMP:
Wendel Moreira
André Thomaz PosDoc
Diogo Almeida PhD
Vitor Pelegatti PhD
Mariana Baratti Dr
Javier Adur PosDoc
Leverson Faria PosDoc

Max Planck Institute for the Science of Light
Erlangen Germany:
Philip Russel
Tijmen Euser PosDoc
Martin Garbos PhD

NIH Bethesda:
Andrius Masedunskas

Biology UNICAMP:
Hernandes Carvalho
Paulo Mazafera
Paulo Arruda
Ione Salgado
Ana Milena - Colombia

Unif Federal ABC:
Antonio Neves Dr

Univ Federal
Pernambuco:
Adriana Fontes
Beate Saegesser
Patricia Farias

Univ Entre Rios
Argentina:
Javier Adur
Vctor Casco

Medicine UNICAMP:
Sara Saad
Fernando Costa
Kleber Franchini
Konradin Metze
Aline Mara
Gislaine Vieira

CAISM UNICAMP:
Fátima Boetcher
Liliana Andrade
Luciana Pietro

Ophthalmology UNIFESP:
Paulo Schor
Wallace Chamon
Amanda Paz Resident
Thanks for the attention!

Obrigado pela atenção!
Anthony Atala: Printing a human kidney

FILMED MAR 2011 • POSTED MAR 2011 • TED2011

WAKE FOREST INSTITUTE FOR REGENERATIVE MEDICINE
Question for Brazil:

Suppose Venter succeeds!

There is no fundamental law of nature preventing it – if biology does it, it is possible.

How much would our pre-salt oil reserves worth?

What about Russia? Middle east countries?
Only USA, Argentina, Brazil and Australia have enough warm area to grow cattle!

Animal protein is the most expensive food

Anyone could produce in vitro/cultured meat!

How much Money for the meat export?
Would this Picture still mean something?
Irony: we learned how to destroy the world [1945] before understand Biology
DNA – 1953
Photosynthesis – 1945 - 1958

Fukishima’s nuclear reactor
Melvin Calvin: Chemist. Lawrence Berkeley Lab!

Lawrence to Calvin: “do something useful”

1961 Chemistry Nobel Prize for unraveled Photosynthesis:
“The path of carbon in photosynthesis” - work done from 1945 to 1958
"Now is the time to do something useful with radioactive carbon [C-14]"
Winston Churchill in 1932

*We shall escape the absurdity of growing a whole chicken in order to eat the breast or wing, by growing these parts separately under a suitable medium. Synthetic food will, of course, also be used in the future.*


The burger is on the table!
Societal Challenges:

Biological Threats; Early detection/Intervention, Disease susceptibility; Climate/Biology, Renewable Energy, Medicine, Imaging, Treatment Devices, Food, Hydrogenases and Synthetic Photosynthesis, Beyond Combustion, Materials Sciences

Common Themes at Biology/Physics Intersection:
From molecules to organisms and beyond; Dynamics; Multistability; Stochasticity; Self-organization/Self-assembly.

Enabling Technologies/Tools for research:
Physics of Molecular Recognition; Intracell Structures/ Dynamics; Cellular Environment, Cells Interactions, Theory and Simulations, Collective Dynamics, Complex Community Signals.
Researchers concluded true understanding of disease only if they know it at the molecular level inside the cell.

Demonstration of engineered nanoparticle as smart bombs for drug delivery targeting only cancer cells. Brentiximab Vedotin: 70 lethal tumours disappear in 12 weeks. http://www.dailymail.co.uk/
What factors brought biology to this point?

Biology, like physics/chemistry, relies on a small number of core organizational principles:

- DNA is the chemical of inheritance
- Cell is the smallest independent unit of life
- Cells can be organized into complex/multicellular organisms
- All organisms function within interdependent communities
- Photo-systems capture solar energy for all life processes

Any knowledge is useful in understanding many others. Biological experimentation shall focus on individual/small numbers of components within a single organizational level.

The reductionist approach succeed to reveal many of the basic molecular, cellular, physiological, and ecological processes.
FRET for nuclease-DNA interaction

Shimon Weiss, SCIENCE, VOL 283, page 1686.
FRET in molecular rotors: Michael Boersch - Freiburg