INFABiC

National Institute of Science and Technology for Applied Photonics in Cell Biology

inct-infabic.net.br

Share your knowledge, expertise and best practices with others.

Bruna Martinuzzi. The leader as a Mensch, 2009

The continuous body of knowledge, which should be properly named cellular and molecular biology, could be compared to a bridge, which like its equivalents in civil engineering, has two bridgeheads: one in traditional anatomical-morphological sciences and the other in equally traditional biochemistry. The cautious and careful have stayed close to the bridgeheads because the area around them had been consolidated over centuries by the work of their predecessors. The bold and venture-some have ventured on the bridge itself from both directions, because they believed that there was where the action was going to be... As in the old Latin proverb, fortune favored the bold: the bridge proved to be strong enough to support the intense occasionally frantic activity of whole armies of explorers.

George Palade, 1987

***Apud** William Bechtel – Discovering Cell Mechanisms, 2006, p. 190*

2. Coordinator

Hernandes F. Carvalho. Bachelor of Biological Sciences (Unicamp, 1987), Master of Cellular Biology (Unicamp, 1989), Doctor of Biochemistry (UNICAMP, 1993), Postdoctoral Fellow (New Mexico University, Albuquerque), Associate Professor (1997) and Full Professor (2004) at UNICAMP. President of the Brazilian Society for Cell Biology (2006-2008 and 2014-2016). Secretary General of the International Federation for Cell Biology. CNPq research scientist level 1A. INFABiC coordinator (2009-2015).

3. Management Committee

Carlos Lenz César (Vice coordinator)

(Full Professor) (Instituto de Física Gleb Wataghin, UNICAMP)
(CPF 090.585.593-00)

Ruy G. Jaeger

(Full Professor) (USP) (CPF 036.968.758-21)

Sergio Luis Felisbino

(Associate Professor) (UNESP) (CPF 098.097.418-63)

Carmen Verissima Ferreira

(Associate Professor) (UNICAMP) (CPF 775.227.536-00)

Acronyms used in this proposal

Institutions:

CEPID: Centro de Pesquisa, Inovação e Difusão [Research Innovation and Dissemination Center] - FAPESP
CEPOF: Centro de Pesquisas em Óptica e Fotônica [Center for Research in Optics and Photonics]
FM-RP = Faculdade de Medicina de Ribeirão Preto [Ribeirão Preto Medical School] - USP
FMJ = Faculdade de Medicina de Jundiai [Jundiai Medical School]
IB = Instituto de Biologia [Institute of Biology] – UNICAMP
IFES = Instituto Federal do Espírito Santo [Federal Institute of Espirito Santo] – Campus Aracruz
IFGW: Instituto de Física Gleb Wataghin [Gleb Wataghin Physics Institute] – UNICAMP
NIH = National Institutes of Health (USA)
U-o-B = University of Bristol
UFC = Universidade Federal do Ceará [Federal University of Ceará]
UFF = Universidade Federal Fluminense [Fluminense Federal University]
UFG = Universidade Federal de Goiás [Federal University of Goiás]
UFMG = Universidade Federal de Minas Gerais [Federal University of Minas Gerais]
UFPe = Universidade Federal de Pernambuco [Federal University of Pernambuco]
UFPR = Universidade Federal do Paraná [Federal University of Paraná]
UFRJ = Universidade Federal do Rio de Janeiro [Federal University of Rio de Janeiro]
UFSCar = Universidade Federal de São Carlos [Federal University of São Carlos]
UNESP = Universidade Estadual Paulista Júlio de Mesquita Filho [São Paulo State University 'Julio de Mesquita Filho']
UNICAMP = Universidade Estadual de Campinas [Campinas State University]
USP = Universidade de São Paulo [University of São Paulo]

Photonic Techniques and others:
BALM: Bleaching/Blinking Localization Microscopy
CARS: Coherent AntiStokes Raman Scattering
EAD: Ensino à distância [Distance learning]
FCS: Fluorescence Correlation Spectroscopy
FLIM: Fluorescence Lifetime Imaging
FRAP: Fluorescence Recovery After Photo bleaching
FRET: Förster Resonant Energy Transfer
MEC: Matriz extracelular [Extracellular Matrix]
NLO: Non-Linear Optics
OPO: Optical Parametric Oscillator
PALM: Photoactivation Localization Microscopy
PLE: Photoluminescence Excitation Spectroscopy
SFG: Sum Frequency Generation
SHG/THG: Second/Third Harmonic Generation
TPEF: Two-Photon Excited Fluorescence

4. Group of proponents (laboratory chiefs only):

1. Ana Paula Davel (Assistant Professor) (UNICAMP) (CPF 053.909.097-20)
2. André Romero da Silva (Educational Manager) (IFES) (CPF 079.772.818-05)
3. Anibal E. Vercesi (Full Professor) (UNICAMP) (CPF 341.236.608-00)
4. Anita Marsaioli (Full Professor) (UNICAMP) (CPF 110.015.859-68)
5. Carlos Lenz Cesar (Full Professor) (UNICAMP) (CPF 090.585.593-00)
6. Carmen Veríssima Ferreira (Associate Professor) (UNICAMP) (CPF 775.227.536-00)
7. Catarina Segretti Porto (Associate Professor) (UNIFESP) (CPF 878.594.088-72)
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12. Fernanda Ramos Gadelha (Associate Professor) (UNICAMP) (CPF 766.901.057-68)
13. Helena Coutinho Oliveira (Full Professor) (UNICAMP) (CPF 011.788.198-84)
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15. Henrique Souza Marques (Assistant Professor) (UNICAMP) (CPF 004.436.519-57)
16. Hernandes F Carvalho (Full Professor) (UNICAMP) (CPF 552.095.646-49)
17. Jörg Kobarg (Full Professor) (CNPEM) (CPF 217.350.928-43)
18. José Andrés Yunes (Researcher) (BOLDRINI) (CPF 711.727.859-53)
19. José Ferreira Nunes (Full Professor) (UECE) (CPF 031.219.503-63)
20. Konradin Metze (Professor Titular) (UNICAMP) (CPF 137.661.288.76)
21. Leticia Frölich Archangelo (Assistant Professor) (FM-RP/USP) (CPF 252.154.098-62)
22. Lucia Elvira Alves (Assistant Professor) (UNICAMP) (CPF 123.764.208-65)
23. Lucimara Gaziola de la Torre (Assistant Professor) (UNICAMP) (CPF 137.680.368-20)
24. Manoel Biancardi (Assistant Professor) (UFG) (CPF 222.602.608-88)
25. Marco Antonio Ferreira Randi (Assistant Professor) (UFPR) (CPF 068.891.138-50)
26. Marco Aurélio Ramires Vinolo (Assistant Professor) (UNICAMP) (CPF 311.670.698-03)
27. Maria Christina Werneck Avellar (Associate Professor) (UNIFESP) (CPF 082.738.678-89)
28. Maria de Fátima Leite (Associate Professor) (UFMG) (CPF 620.265.936-04)
29. Maria Denise Feder (Associate Professor) (UFF) (CPF 764.426.917.72)
30. Marinilce Fagundes Santos (Full Professor) (USP) (CPF 062.491.238-81)
31. Marisa Masumi Beppu (Associate Professor (UNICAMP) (CPF 137.680.208-27)

32. Mary Ann Foglio (Researcher)(UNICAMP) (CPF 096.776.818.77)
33. Mônica Alonso Cotta (Associate Professor) (UNICAMP) (CPF 083.897.888-61)
34. Patrícia Gama (Associate Professor) (USP) (CPF 126.803.358-80)
35. Paulo Pinto Joazeiro (Associate Professor) (UNICAMP) (CPF 773.096.778-20)
36. Pedro de Campos-Lima (Researcher) (Boldrini) (CPF 432.244.316-87)
37. Roger Frigério Castilho (Assistant Professor) (UNICAMP) (CPF 137.680.638-01)
38. Ruy G Jaeger (Full Professor) (USP) (CPF 036.968.758-21)
39. Sérgio Luis Felisbino (Associate Professor) (UNESP) (CPF 098.097.418-63)
40. Silvana Allodi (Associate Professor) (UFRJ) (CPF 951.356.858-04)
41. Sonia Regina Grötzner (Assistant Professor) (UFPR) (CPF 530.446.149-53)
42. Suzana Côrte-Real Faria (Researcher) (FIOCRUZ) (CPF 352.874.387-53)
43. Suzete Araújo Oliveira Gomes (Assistant Professor) (UFF) (CPF 639.664.257-34)
44. Taize Augusto (Adjunct Professor) (FMJ) (CPF 220.124.188-05)
45. Vanessa Freitas (Assistant Professor) (USP) (CPF 199.948.488-60)
46. Yara Maria Rauh Muller (Assistant Professor) (UFSC) (CPF 247.889.889-68)

5. Functional and Organizational Structure of the Institute

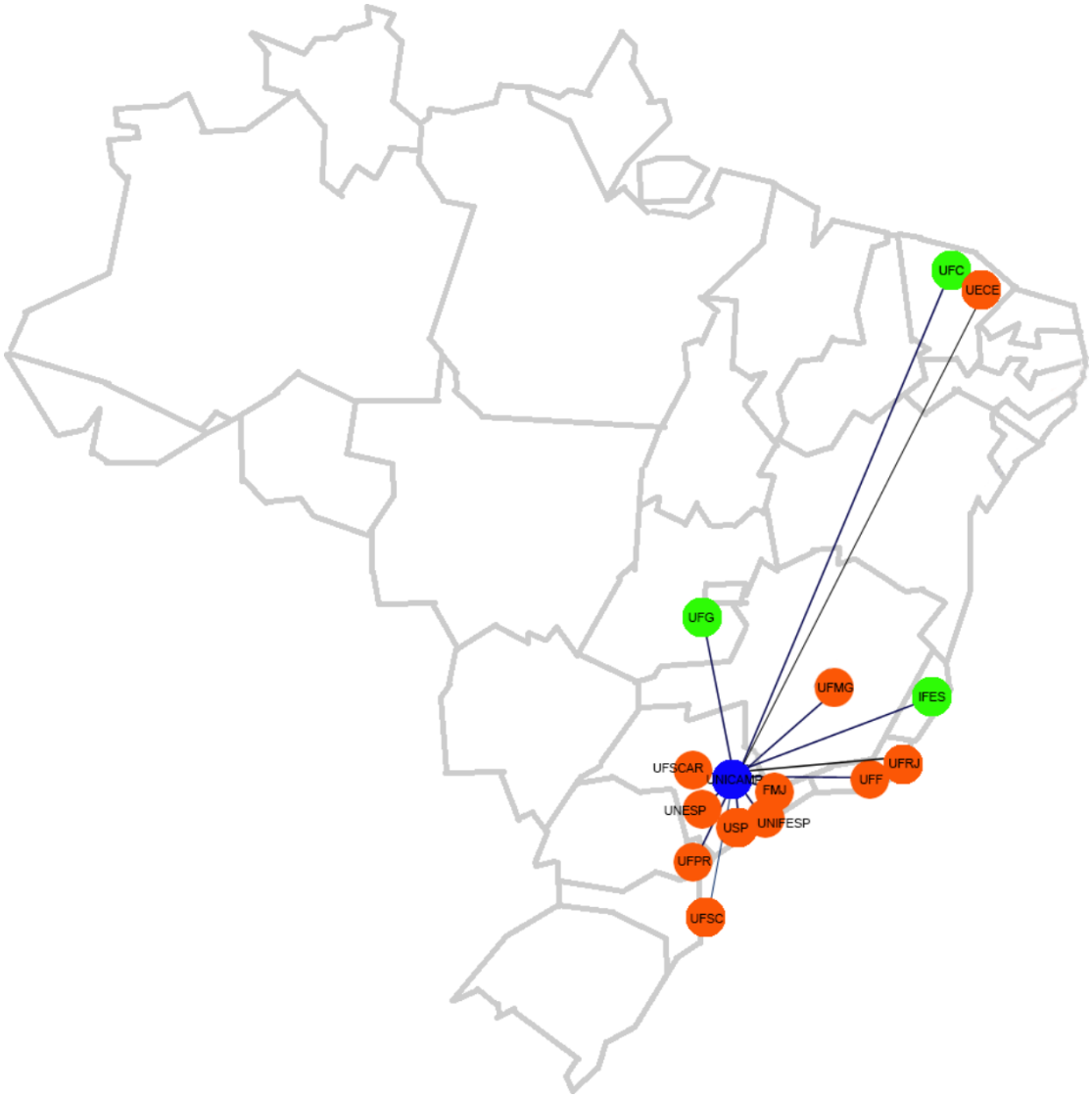


Figure 1. Geographical distribution of INFABIC-affiliated institutions. The headquarters in Campinas is indicated in blue. Type I associated laboratories are indicated in orange, and type II associated laboratories in green. We operate in eight states and four geographical regions of the Federation.

INFABiC Headquarters at UNICAMP (blue in Fig. 1)

INFABiC headquarters is located at UNICAMP, and is officially affiliated with the IB and IFGW institutes. The central laboratory, now in the final stage of construction, is located at IB. Equipment from INFABiC will be installed in a laboratory area of 150 m^2 with air-conditioning and air-filtration systems corresponding to a Class 10,000 cleanroom. This arrangement is designed to protect equipment from variations in temperature and humidity, as well as from the dust and aerosols that are common in Campinas. A contiguous area (300 m^2) is being remodeled to duplicate the capacity for storage and cultivation of cell lines, as well as for processing materials intended for analysis in different types of apparatus. In addition, we plan to build a database of vectors, gene constructs and cells that express specific constructs for analysis of specific cellular organelles and molecules, in a space intended for biofreezers and liquid nitrogen tanks. INFABiC also has a Zebrafish Unit (**Danio Core**), to provide the users with zebrafish strains. This unit is large enough to meet the requirements for fish for experimentation, and it is being fully equipped so that transgenic animals with specific customized features can be created. The INFABiC headquarters also includes the IFGW Biophotonics Laboratory, under the supervision of Professor Carlos Lenz Cesar, which is currently functioning to investigate and transfer technology to the central laboratory. It is expected that both cells and animals (especially Zebrafish) kept at the IB will be utilized in analyses performed at the Biophotonics and Central laboratories. The central laboratory will also concentrate organizational and planning INFABiC activities, while educational and diffusion activities will be conducted at other UNICAMP facilities, depending on the number of participants. Apart from the INFABiC Central Laboratory, Unicamp has multiple associated laboratories also located at the IFGW and IB institutes, as well as at the Chemistry Institute, School of Chemical Engineering, School of Medical Sciences, CPQBA (Multidisciplinary Center for Chemical, Biological and Agricultural Research) and CNPEM (National Center for Energy and Materials Research).

Associated Laboratories (orange and green in Fig. 1)

Associated laboratories (types I and II described below) benefited by INCT must function along the same general lines of dissemination/diffusion, accessibility, training and investigation as INFABiC. They are expected to function as local extension centers of technology and outreach, photonic microscopy, integrated optical microscopy and dynamics, and quantitative and mechanistic aspects of cell biology, focusing on subcellular and molecular aspects.

Associated laboratories are located in established institutions and are connected to INFABiC because they have the same mission, to strive for excellence in research and develop long-term collaborative projects, and benefit from the structure gathered at the Central Laboratory. The associated laboratories broaden the range of topics of interest to INFABiC, disseminate techniques and approaches, conduct research related to the main central topic of INFABiC, and help in offering courses and events. As part of this proposal, INFABiC intends to (i) identify bottlenecks in the conduct of research in the associated laboratories (outside Unicamp), in particular with respect to microscopy, and (ii) decentralize part of the activities conducted in the Central Laboratory, expanding the number of users while allowing the key equipment to remain dedicated to the use of accessories capable of producing more-refined data.

INFABiC plans transformative activities regarding the Type II Associated Laboratories ("Advanced poles"). There are three: Federal University of Goiás (Goiânia), Federal Institute of Espírito Santo (Aracruz) and Federal University of Ceará (Fortaleza). This relationship aims to implement appropriate working conditions, technical and scientific support, and core funding, so they can develop dynamic research in cell biology and start to act as local communicators of INFABiC ideals.

International Partnership

Partners will trade information, exchange students, and function as outposts, communicating useful information of interest to the group. They will also collaborate in research projects conducted by INFABiC, operating in direct contact with their team members. Lectures will be delivered as INFABiC formal activities, as will the submission of proposals for international funding. In special cases, partners will stay for

a given period at the host laboratory or associated laboratories, through specific CNPq and FAPESP programs.

INFABiC`s current International Partners are:

School of Biochemistry (Program on Dynamic Cell Biology)
University of Bristol, UK
Contact:
George Banting – e-mail: dean-fmvs@bristol.ac.uk

Nanoscience and Quantum Information Centre
University of Bristol, UK
Contact:
Mervyn Miles - e-mail: m.j.miles@bristol.ac.uk

Section on Organelle Biology - NIH - Bethesda, USA
Contact:
Jennifer Lippincott-Schwartz - e-mail: jlippin@helix.nih.gov

Yale University, New Haven, USA
Contact:
Michael Nathanson – e-mail: michael.nathanson@yale.edu

Universidad Nacional de Entre Ríos, Argentina
Contact:
Javier Adur- e-mail: jadur@bioingenieria.edu.ar

University of Georgia, Athens, USA
Contact:
Roberto DoCampo - e-mail: rdocampo@uga.edu

Université de Picardie Jules Verne (Amiens), France
Contact:
Pierre Toledano - e-mail: pierre.toledano@u-picardie.fr

6. Assignment definition for each participating institution

Central Laboratory (UNICAMP): Concentrates large pieces of equipment and provides them to users. Offers scientific and technical support. Searches for new technologies, implements them, and disseminates their applications. Organizes INFABiC activities to stimulate collaborative projects with defined goals. Coordinates education and diffusion activities. Organizes events. Collectively defines future strategies. Organizes data and prepares scientific reports. Stimulates interaction among associated laboratories to promote the establishment of sub-networks.

Type I Associated Laboratory (USP, UNIFESP, UNESP, UFSCAR, UFMG, UFPR, UFRJ, UFF, FIOCRUZ, UFPE, Boldrini, FMJ, UFSC): Develops research projects and performs investigations, utilizing facilities offered by INFABiC. Participates in the definition of future objectives and contributes to events. Encourages transit of students and researchers among different laboratories and participating institutions. Conducts activities for local diffusion of INFABiC's research/education/extension/innovation programs, highlighting the importance of approaches at the molecular and cellular levels and assessing their dynamic, quantitative and mechanistic aspects.

Type II Associated Laboratory (Goiânia, Aracruz and Fortaleza): Functions similarly to a Type I Associated Laboratory. These laboratories are led by young researchers, and will have specific support to leverage their activities, whether through new equipment or technical and scientific support from the Central Laboratory and Type I Associated Laboratories.

7. Mechanisms to promote interaction among participating research groups

INFABiC will continue the measures that have ensured high synergy until now, and will implement new activities to further strengthen the interaction among the participants.

Organizational activities:

In addition to the annual meetings of the workshop, and biweekly meetings to advise users, we will include planning meetings of the group leaders to discuss ideas and strategies for generating publications in high-impact journals.

INFABiC will also establish **Monthly Seminars**, advertised as *Webinars,* where different team members and special guests can present the results of their research and suggest collaborative activities; and a **Journal Club,** to be included in INFABiC`s homepage, where members can comment on highlights of the literature, preferably their own publications.

The self-evaluation meeting will include external members of the steering committee, to evaluate our performance and suggest new activities.

We plan to create the Campinas Network for Cell Biology, linking researchers from different fields, who are interested in the cell as an object of study or working tool. This network will cover INFABiC's specific activities in terms of access to equipment, teaching, research, and extension.

Supporting actions

We will increase the support offered to the institutions located in northeastern Brazil, and include researchers from the CW and Espírito Santo in the proponent team. We will also increase the proportion of INFABiC's budget dedicated to travel among associated laboratories, especially for participants living outside Campinas, who will be involved with educational/outreach and training activities. At the present stage of our institute, this action should make a significant difference and should have a more tangible impact on the "asymmetrical" national collaboration with the three centers in Goiânia (UFG,), Aracruz (IFES) and Fortaleza (UFC).

Ongoing activities to foster synergy

The high degree of synergy in INFABiC's activities has resulted from the intersection of the axes (x) instrumentation, (y) technical and conceptual support, and (z) aggregating and interdisciplinary nature of the proponent team. INFABIC efficiently disseminates knowledge, expertise and practices to a large number of researchers. Some examples illustrate the effectiveness of this organization. TPEF, SHG, and FLIM microscopy techniques are in high demand today, but were largely unknown in the country before INFABiC [2009]. We conducted the first study using FRET-FLIM in Brazil, in collaboration with LNBio. This methodology is currently employed by groups in the LNBio, Boldrini Research Center and INCOR.

Our home page provides calendar slots for reserving equipment to any researcher in the country. Moreover, INFABiC has created a committee of users and a set of rules officially approved by IB and IFGW, to ensure continuity of the laboratory operations even if a change in the Managing Committee occurs. Users throughout the country, including members of RENORBIO from Pernambuco and Ceará, and foreign fellows from Argentina, Colombia and Mexico have been using this infrastructure since 2010. The operation of the laboratory involves an expertise that begins with femto/pico second lasers, and extends to linear and nonlinear optics, electronics and software. It also includes sample processing, image analysis, and interpretation of results from cellular and molecular biology and allied research areas.

The following aspects to be continued were critical to the success and the ability to attract a large number of users:

(1) Installing and managing several types of equipment and accessories;

(2) The availability of the coordinators to discuss the possibilities and strategies to answer the users' scientific questions;

(3) The presence of two staff members with PhDs to provide support to the users was critical to encourage them not to be afraid of using and/or damaging complex equipment, so that they can focus on specific aspects of their research, and to preserve any important information that might be shared among users;

(4) Dissemination of knowledge about nonlinear optics and photonic microscopy through the scientific community, and convincing potential users of the usefulness of these methods in solving scientific problems;

(5) Maintenance of close ties with the international community, by attending summer/winter schools and visiting laboratories.

Aspects (4) and (5) above were achieved through several different actions. The simplest was to offer undergraduates and graduates elective courses in (i) Biophotonics, (ii) Cellular and Molecular Biology, (iii) Cancer Biology and (iv) Networks. In addition to theory, these courses included practical lessons about laser alignment, image acquisition, sample processing, identification of artifacts and use of a variety of software. Physics, biology, food engineering, pharmacy, chemical engineering and medical students enrolled in these courses.

The most broad-ranging action, in terms of the number of people involved, was the organization of two-week Theoretical and Practical Workshops at UNICAMP. The three versions of these workshops attracted audiences of over 100 people each. The fourth workshop will take place in late September 2014. The practical classes at these workshops were limited to a smaller number of participants, given the size of the laboratories and equipment available. In addition to lectures and experiments presented by the INFABIC team, the workshops featured Brazilian and foreign speakers, equipment demonstrations by companies, as well as specific training in image processing using Image J (including film editing/animations, Fourier transform, and texture analyses via contrast, energy and entropy, among others). These workshops have created one of the best means of interacting and sharing knowledge inside and outside INFABiC. Professionals and their students, as well as technicians attended these workshops.

On a more global level, we organized the XII International Congress of Cell Biology (in conjunction with the XVI Congress of the Brazilian Society for Cell Biology) (2012) and the Section on Advanced Optical Microscopy in the 17th International Microscopy Congress (2010), both in Rio de Janeiro. In April 2014, we organized the Workshop "At the Interface between Physics and Biology", held at FAPESP headquarters, São Paulo. We also coordinated two versions of the São Paulo State International Cell Biology Day (2012, 2014), both funded by FAPESP.

A significant activity was the publication of several book chapters (see *highlights of production*, item 14). Book chapters are important for two reasons: (i) they contain thorough descriptions and teaching topics that are unsuitable for either a traditional publication or a review; (ii) readers can consult them at any time and without haste.

Interactions with other National Institutes (INCTs). INFABiC maintains a historical and intensive collaboration with other INCTs, such as the INCT-Blood and INCT-Diabetes and Obesity, both part of the Faculty of Medical Sciences/UNICAMP, INOMAT and FOTONICON, also headquartered in Campinas. Outside Campinas there is a strong interaction with the INCT on Photonics and the INCT of Lanthanides, both part of the Federal University of Pernambuco, and INCTTOX, of the Butantan Institute.

8. Personnel Training Program

Secondary education level. Establish partnerships with schools around Unicamp, namely Alberto Freitas, Gabriel Porto and Colégio Múltiplo, for specific programs in teaching/learning Sciences and Cellular and Molecular Biology. Type II Associated Laboratories will liaise similarly with at least one local school.

Higher education level. Release of the 4th edition of the textbook **A Célula** (“The Cell” Carvalho HF, Recco-Pimentel SM eds., Manole) which includes aspects of the interface between cell biology and physics. We will also create a multimedia publication that may eventually replace the printed textbook **A Célula**, for use in teaching **Dynamic Cell Biology.**

Graduate level. Create the Integrated Program for Doctorate Training. This program will expose PhD candidates in their first semester to views, concepts and approaches in other fields. Students will devote 8 hours per week to this activity, which will be linked to a specific course with appropriate allocation of credits. Students will be selected to participate in this program. Two students from different areas will work jointly on their own projects. Before the end of the semester, each pair will be assigned specific problems to solve together. The goal is to extend the understanding through an interdisciplinary study and to stimulate the use of approaches and techniques from other areas in the students' doctoral research. A prototype of this program will be

implemented in early 2015 with students from the Physics (level 7 of CAPES), and Structural and Cellular Biology Programs (Level 6 of CAPES), with subsequent extension to other fields such as Chemical Engineering, Chemistry, Computer Sciences and Physiopathology. Courses on Optics and Photonics, Networks, and Molecular and Cellular Biology will be routinely offered to students of all fields.

Technical Training level. We intend to offer training courses at least once a year for technicians in charge of microscopes and microscopy laboratories throughout the country.

Extension Level. We intend to create Extension Courses (1) Molecular and Cellular Biology, (2) Cancer Biology (available jointly with the Unicamp School of Medical Sciences), (3) Techniques and Practices of Cell Culture *In Vitro* and (4) Image Analysis applying ImageJ.

For this proposal, we incorporate the experience gained at USP in providing updating training for professors in Cellular and Molecular Biology, using distance-learning tools for the state of São Paulo. We intend to link this initiative with professionals from other states and to use the e-Sciences platform from the Institute of Computational Sciences, UNICAMP (Contact: Dr. Siome Klein Goldsmith), to extend this activity to national coverage.

9. Description of the institute activities

In conceptual terms, our proposal is based on the NIH's *Common Fund Programs*[1], as we expect to function in (a) **transformative**; (b) **catalytic;** (c) **synergistic**; (d) **"transverse"** and (e) **unique** ways, working on three levels:

[1] Collins FS, Wilder EL, Zerhouni E. NIH Roadmap/common fund at 10 years. **Science** 345: 274-276

(1) **Instrumentation**. Providing high-cost and complex apparatus with accessories for obtaining images and quantitative parameters, which are difficult for non-specialists to acquire.

(2) **Conceptualization/approach.** The leap required to change to a dynamic, mechanistic and quantitative approach from predominantly descriptive, phenomenological and associative approaches is great, and depends on instrumentation, training and an understanding of the advances afforded by these approaches. The same applies to the different non-linear optics-based methodologies broadly diffused by INFABiC, and for the use of microengineering and microfabrication, which provides efficiency and reproductibility in testing finely controlled variables.

(3) **Organization/ Integration.** The team recruited to develop this proposal and the suggested management system will seek to minimize the difficulties faced in implementing the research. By minimizing bureaucracy and diversion to unrelated issues, we intend to foster the efficient use of the time, individual skills and intellect of researchers to contribute to solving scientific and technical questions prioritized by the team as a whole.

Based on these general principles, INFABiC's functions are:

1. Facilitate and accelerate the work of users, imparting quality to the different stages of research and creating conditions to minimize distraction of researchers from their central focus, encouraging high-risk approaches that could not be implemented otherwise;

2. Encourage interaction among users, promoting collective and cross-linking activities aiming to maintain synergy and increasing the impact on work development;

3. Form partnerships and explore new funding sources to ensure proper functioning of existing equipment and the technological and conceptual work at the forefront of photonic microscopy;

4. Represent the group in multiple instances of their activities and in their best interests;

5. Ensure general principles of conduct for biosafety and ethical animal and human experimentation.

Considering the definition of **program** in the official announcement as a set of steps to achieve goals and objectives, we divide this section into programs relating to research, training of human resources, knowledge transfer, and internationalization. Here we limit ourselves to describing the transformative actions involving methodologies with wider impacts across the whole of INFABiC. The general operation of INFABiC covers several activities, more or less centered on techniques and instrumentation, but also capable of allowing larger steps in the design of mega-projects involving different areas of expertise of participants in the proposing team.

Research Program:

1. **"Far field" Super Resolution**: definition of the super resolution system configuration to be acquired. Importation and equipment installation. Use of the equipment to reproduce standard results in the observation of the trajectory of a single molecule, and the ability to observe super resolution at depths greater than 10 microns in *Drosophila* larvae. Incorporation of this equipment in biochemical studies *in singulo*, including FRET. Dissemination of knowledge in the super resolution field to the members of INFABiC.

2. **"Near field" Super Resolution**: study of fluorescence lifetimes, FRET, generation of second and third harmonics in the vicinity of the metal tip of the AFM system. Anchoring of enzymes in the metallic tip for observations of biochemical reactions *in singulo* via tip-enhancement. FCS measurements in attoliter focal volume and their use in sequencing single-strand DNA. Raman spectroscopy in attoliter volumes.

3. **CARS Microscopy**: availability of CARS/SFG technique. Using CARS together with FLIM and FRET characterization of cellular differentiation, lipid metabolism and mitochondria.

4. **Raman spectroscopy/microscopy**: maintenance in integrated Raman system in the multimodal platform, to study the formation of bacterial biofilms and cell differentiation, with emphasis on DNA methylation.

5. Develop research at the frontier of knowledge in cell biology and Photonics Multimodal microscopy, addressing dynamic, quantitative, subcellular, macromolecular and molecular aspects, including single cells and single molecules. Encourage the use of existing advanced techniques in the group, such as FCS, tip-enhancement, TERS and Raman, together with manipulation and biomechanical measurements. Characterization of stress distributions in cell division using FRET sensors; stress distribution on the cytoskeleton upon the application of external forces; introduction of foreign material through optoporation.

6. Encourage the development of **Megaprojects**. To promote integration, different investigators will submit interdisciplinary projects depending on contributions at various levels (intellectual, experimental, instrumental). A megaproject should include many simultaneous techniques from the INFABiC photonics platform. The very definition of a megaproject includes a high degree of synergy to ensure the most effective dissemination of results through publications in high-impact journals, and will require the expertise of many team members. One example is the investigation of dynamic interactions between organelles acidocalcisomes-mitochondria and endoplasmic reticulum in trypanosomatids, brought to the group by Dr. Anibal Vercesi (FCM-UNICAMP) and his longtime collaborator Dr. Roberto Docampo (University of Georgia in Athens). INFABiC might also be involved in the solution of questions brought to us by other INCTs or CEPIDs.

Program management of the equipment

7. Ensure universal access to instruments and other facilities gathered by the INFABiC team (in accordance with prior commitments to funding agencies and

the host institution). Maintain and preserve public property awarded to INFABiC, ensuring good use and optimizing the organization.

8. Organize an annual self-evaluation meeting with the steering committee, for suggestions on how to improve INFABiC operations.

9. Complete the construction of the Central Laboratory, including the clean room. Acquisition of optical tables for the new laboratory. Planning for transport of current equipment to the new laboratory in order to minimize disruption, possibly dividing the system into two parts and operating one of them while reinstalling the other. Change of equipment for the new laboratory, including cleaning of all optical components. Reinstallation and operation of equipment in the new space.

10. Start the evaluation process of biosafety and bioethics in animal and human experiments in all INFABiC laboratories. Require that associated laboratories follow similar standards in their respective institutions. Physically restructure laboratories that desire to achieve a higher level of biosecurity, redefining their biosafety protocols, including optical ones.

11. Set up a series of time-lapse microscopes to unburden the most sophisticated instrument systems at INFABiC and at the affiliated laboratories. Import and install these new microscopes. Check the quality of their operation via the definition of a standard experiment for all systems.

12. Establish facilities for the production of micro-engineering and microfluidics apparatuses and use them in the integrated devices in a multimodal platform.

Human resources training program:

13. Use INFABiC's infrastructure to develop undergraduate research projects, M.Sc. dissertations, PhD theses and post-doctoral research. Integrate these projects via the Doctoral Training Plan.

14. Teach theoretical and practical courses for multidisciplinary undergraduate and graduate students each semester. Combine courses from different postgraduate programs to optimize the use of human resources while reaching a broad audience.

15. Train technicians responsible for microscopes and/or microscopy laboratories.

10. Main line of research description

Details of the sub-projects are available at

inct-infabic.net.br in "NEW APPLICATION"

Area	Participants	Description
H.1 Extracellular matrix, proteases and its inhibitors	Heloisa H. S. Selistre-Araújo Hernandes F. Carvalho Marinilce F. Santos Paulo P. Joazeiro Ruy G. Jaeger Sergio L. Felisbino	Biosynthesis, organization and degradation of cellular matrix. MMPs and ADAMTs. Heparanase. Protease inhibitors. Substrates for cell invasion and migration assays. Collagen, elastin and proteoglycans. Ultrastructure.
H.2 Cellular Migration	Hernandes F. Carvalho Marinilce F. Santos	Cellular migration on 2D and 3D matrices. Chemotaxis. Collective migration.
H.3 Development Cellular Differentiation	Fernanda C. A. Santos Henrique Marques de Souza Hernandes F. Carvalho Lucia Elvira Álvares Patrícia Gama	Regulation of gene expression during development and cell differentiation. Regulation by TGFβ.
H.4 Reproduction/ Nuclear Receptor Reproductive Immunology	Catarina S, Porto Fátima Lázari José Nunes Manoel Biancardi Maria Christina W. Avellar Sérgio L. Felisbino	Fertility parameters. Spermatogenesis. Prostate induction and development. Prostate cancer. Female prostate.

H.5 Micro-engineering, surfaces, microfluidics	Lucimara G. de la Torre Marisa M. Beppu Mônica A. Cotta	Functionalization of surfaces. Cell "backpacks". Drug targeting. Micro-engineering and microfluidics.
H.6 Organic synthesis	Anita Marsaioli	Enzymes and fluorescent probes for detection.
H.7 *In singulo* biochemistry, lanthanides, quantum dots	Carlos Lenz Cesar André Alexandre de Thomaz Diogo Burigo Almeida Vitor B Pelegati Mariana Ozello Baratti Hernandes F Carvalho	Study of isolated single molecules. Microfabrication and physical-chemical properties.
H.8 Cell cycle, Cancer	Andrés Yunes Assuero Silva Meira Carmen Verissima Ferreira Hernandes F. Carvalho Joerg Kobarg Leticia Frolich Archangelo Marco A. F. Randi Mary Ann Foglio Patricia Gama Sergio L. Felisbino Vanessa Freitas	Cell cycle and its regulation. Cytostatic, antimitotic and death-inducing drugs. Antivirals. Experimental metastasis. Environmental factors.
H.9 Vascular Biology, Angiogenesis and Atherosclerosis	Ana Paula Davel Cláudio C. Werneck Cristina Pontes Vicente Helena C. F. Oliveira Hernandes F. Carvalho Konradin Metze Paulo Pinto Joazeiro	Vascular physiology, thrombosis and endothelial-stem cells. Lipids and atherosclerosis. Extracellular matrix of blood vessels. Smooth muscle cells and vascular contraction.
H.10 Pathology	Konradin Metze	Pathological anatomy, image analysis. Chromatin. Imaging diagnosis.

H.11 Metabolism, Lipids, Mitochondries	Anibal E. Vercesi Helena C. F. Oliveira	Oxygen consumption, oxidative stress. Calcium metabolism. Acidocalcisomes.
H.12 Micro-Immune-Parasitology	Denise Feder Fernanda Ramos Gadelha Marco Aurélio Vinolo Lucimara G. de la Torre Mônica A. Cotta Suzana Corte Real Faria Suzete Oliveira	Biology of trypanosomatids; periodontal disease, *Xyllela fastidiosa*; yeasts.
H.13	Evelise Maria Nazari	Crustaceans and fishes as

Environmental factors	Marco A F Randi Silvana Allodi Sonia Regina Grötzner Yara Maria Rauh Muller	environmental sensors. Environmental neurotoxicity.

11. Qualification problem/justification

Socio-economic and scientific relevance at the convergence of life sciences with physics, chemistry and engineering: The world's fifth scientific-technological revolution – the revolution of information and communication – is reaching the stage of maturity, and a new wave is expected to emerge within the next 10 years. These major waves have a cycle of 50-60 years, with the next one beginning 40 years after the last. Several international analysts suggest that the 6th wave may come from the capability to control biology at the cell and molecular levels, which was unimaginable a hundred years ago. The term bio-economy has been used to refer to this new wave, which may increase our capacity for energy production and for the manufacture of various products with little environmental impact and low costs.

These revolutions are formed through a collective social phenomenon, where the number of people investing in a particular area encourages the entry of more investors, entrepreneurs and researchers.

The large sums of money invested in bio-economic projects by financial and information icons Bill Gates, Warren Buffett, Jeff Bezos, and Sergey Brin is another good indicator of what will become the core of the new wave. Brazilian industrialists are also convinced of the importance of this area, as discussed by the National Confederation of Industry in its 2014 3rd CNI-Harvard Forum on Bio-economy.

The U.S. ***National Research Council*** [NRC] committee on ***Forefronts of Science at the Interface of Physical and Life Sciences*** released the report ***"Research at the Intersection of the Physical and Life Sciences"***[2], evaluating prospects and suggesting strategies to stimulate multidisciplinary research in this field. In parallel, MIT released the white paper ***"The Convergence of the Life Sciences, Physical Sciences, and Engineering"***[3], explicitly stating that "***Information technology***

[2] http://www.nap.edu/catalog/12809
[3] http://dc.mit.edu/sites/dc.mit.edu/files/MIT%20White%20Paper%20on%20Convergence.pdf

revolution is maturing". During the speech "***Remarks by the President at the National Academy of Sciences Annual Meeting***", US President Barack Obama stated "***In biomedicine … we can harness the historic convergence between life sciences and physical sciences that´s underway today…***"[4]. The United States National Science Foundation [NSF] decided to finance 9 interfacing Physics Centers. Two of these centers focus on biological physics, i.e. 22% of the funding in interfacing physics was directed to the interface of physics-biology. One is the University of Illinois[5] ***Center for the Physics of Living Cells***. The other is the UC San Diego and Rice University[6] ***Center for Theoretical Biological Physics***, which is co-directed by the Brazilian Jose Nelson Onuchic.

All the above reports indicate that the concentration of research funds and trial projects in the current classical disciplines is slowing the development of convergence and multidisciplinarity, both of which are necessary for the development of the new technological wave. In addition, the reports are unanimous in concluding that creating multidisciplinary environments is esssential in order to conduct high-impact research and especially to train a new generation in the mastery of interdisciplinary tools. **The multidisciplinary environment is one of the major characteristics of INFABiC.**

Present scientific maturity for study and control of biological processes: From a scientific point of view, one realizes that our own existence, which depends on a multitude of biochemical processes in millions of cells, happening at specific times, and controlled by mechanisms of cell signaling, demonstrates that: (1) biological processes follow laws and standards, and (2) are, fundamentally, controllable. **Control** is the keyword in this context.

Considering the technology of information, the software of biological devices is present in DNA, whereas the hardware is embedded in cell mechanisms, production of proteins, and enzyme activity. Juan Enriquez from the Harvard Business School observed that biological software incorporates manufacturing, as it includes production of the hardware through cell proliferation. Paraphrasing Tom Knight, an MIT engineer who changed fields to biology at 40 years of age and also created the international

[4] http://www.presidentialrhetoric.com/speeches/04.27.09.html
[5] http://www.cplc.illinois.edu/
[6] https://ctbp.ucsd.edu/

competition of synthetic biology called iGEM and Biobricks Foundation: …*biology is fundamentally a manufacture technology and we are at the verge of discovering how to control it. We lack the ability to put atoms exactly where we want them. Semiconductor engineers cannot do it. Biology puts each atom in the place it wants with precise control. We can use that as an extremely powerful manufacturing technology.* Knight's idea fits perfectly with a quotation that Caltech's Nobel laureate physicist Richard Feynman left on his board just before he passed away: *"What I cannot create, I do not understand!"* With the tools available nowadays it is possible to create biological organisms from the beginning, opening a range of possibilities to produce an innumberable series of bio-devices and finding cures for important diseases.

Simple versus Complex: There is a currently established consensus that biology follows rules that are broader than what the immense diversity of beings would suggest, and that the similarity of biological patterns in living beings demonstrates that knowledge obtained in some of them can be extended to the vast majority of living things. This consensus, claiming that any object of study leads to the same set of knowledge, has generated two major strategies for the field's funding and development. The first strategy chooses research in areas with strong socio- economic impacts as objects of study, such as cancer, cardiovascular diseases and the brain. In the second, the object of study is chosen to decrease levels of complexity and facilitate manipulation, therefore speeding acquisition of knowledge in basic life processes. In the second case, the tendency is to focus studies on single-celled microorganisms such as bacteria, which already have a good part of the machinery of higher life forms, are easy to manipulate, and reproduce very quickly, allowing one to monitor processes over short periods. If the study of basic mechanisms allows the understanding of complex processes, as stated by Stephen Wolfram in his book **A Study in Complexity**, the strategy of first studying the simple life forms would be more efficient. On the other hand, whereas the study of complex cases is necessary from the point of view of impacts on health, if the understanding of the simple does not accelerate the understanding of the complex, the first strategy would be more efficient, encompassing understanding of the simple within the process of understanding the complex. In a situation of uncertainty of which strategy might be more efficient, as seems usually to be the case, the best solution would be to invest in a combination of both. Study of the processes of cell differentiation through observation of small organisms and during

embryonic development represents a commitment to analyses somewhere between the simplicity of cellular mechanisms and the complexity of biological systems.

Our proposal consists of a combination of both strategies, between the simple and the complex, unified by the use of the same integrated photonic tools. This proposal involves the formation of three biology mini-consortia: (1) cancer/cell cycle/mitosis; (2) vascular biology/angiogenesis; and (3) microbiology; and three more in physics/engineering: (1) development of studies on isolated molecules; (2) micro-engineering, micro-fabrication/ surfaces, etc.; and (3) quantum dots/lanthanides.

Importance of photonics for research on biological processes

Photonics allows us to remotely and non-destructively monitor cellular processes in real time, with high spectral and spatial resolution. The sensitivity of the present optical detectors, which can detect even a single photon or emit around 1,000 photons per microsecond, allows a dynamic study of single molecules over time. Furthermore, the limit of 200-nm spatial resolution given by diffraction was exceeded in the last decade. A number of photonic microscopy techniques have been attaining resolutions below 10 nm, with some reports of optical microscopy resolution below 1 nm.

This capacity is termed super-resolution. It includes near-field techniques where a device must be located within a certain number of nm of the object under study, and therefore are near-contact techniques. Moreover, we have far-field techniques in which electromagnetic waves focused and emitted by the object of study are observed/manipulated from a distance. Single-molecule studies can be performed through high dilutions or with super-resolution techniques. Photonics has no competition from other techniques of characterization in terms of resolution, sensitivity to individual molecules, ability to obtain real-time information in living cells at room temperature and with chemical discrimination, through linear and non-linear spectroscopy optics. Additionally, photonics includes the ability to perform manipulations and biomechanical measurements using optical tweezers. The convenience of combining and transporting beams of light also allows the development of multimodal systems, combining several photonic techniques with each other or with atomic force and electron microscopy.

INFABiC operation

INFABIC's mission is to generate and disseminate knowledge in the biology/physical interface, ranging from studies on biological phenomena at the molecular scale to observations of small animals *in vivo*. To that end, the Institute has an already established multimodal platform infrastructure, which should be consolidated and expanded in activities termed CONSOLIDATION and EXPANSION. The expansion will focus primarily on assembling a super-resolution system capable of tracking molecular movements.

From the biological point of view, we can classify the possibilities of INFABiC's operation into various categories, meshing at different levels. This classification helps to identify the best techniques, researchers' needs, and basic studies that should be performed in the framework of INFABiC, as well as future directions that are part of the overall strategic aims of our Institute.

INFABIC must function across the following categories

Spatial scale:
A.1 – Single molecules
A.2 – Single cells
A.3 – Cells [tissues and intercellular interactions]
A.4 – In vivo

Observation timescale:
B. 1 – Very fast in small volume [μ s]. Molecular diffusion changes of molecular conformations. Dynamic observation of a fixed point, for example, FCS.
B. 2 – Fast biological processes [μ s-ms] – electrical impulses, action of enzymes, DNA replication, biochemical reactions that depend on molecular diffusion, movement of flagellate parasites, movement of erythrocytes in the bloodstream. Dynamic observation with spinning disk or high-speed cameras spatial resolution.
B. 3- Slow biological processes [min]- living cells/tissues, crawling movement. Dynamic observation with confocal laser scanning microscopy.
B. 4- Very slow biological processes [tens of minutes to days] – development of tumors,

embryonic development, infection processes, movement of leukocytes. Dynamic observation in time-lapse mode.

Functionality of the object of study:
C. 1 – *In vivo* – entire multicellular organisms. Depth difficulties of observation, interest in large volumes and subject to body movements such as breathing and heartbeat. Dynamic observation.
C. 2- *Ex vivo* – functional structures outside organisms. Low movement, small observation volume, dynamic observation.
C. 3- Fixed material. Static observation, frozen in time.

Spatial resolution observation:
D. 0-Atomic force microscope reaches atomic resolution.
D. 1 – Super resolution – between 10-60 nm.
D. 1.1 – Near Field. Tip-enhancement/AFM, SNOM, nano-antennas. Technique of almost contact, slow, usually allows mechanical manipulation/measurements. Can be used in production of structures with nm dimensions and biochemical reactions in the range of 10 nm.
D. 1.2- Far Field. Structured lighting, location techniques [PALM/BALM/STORM], STED. Possible to obtain images with 20/70 nm lateral/ axial resolution, with speeds up to 1000 frames per second, and to track the movement of a molecule with an accuracy of 10 nm.
D. 2 – Normal resolution limited by diffraction [> 250 nm in x-y plane].

Techniques/methodologies:
E. 1 Visualization using fluorescence: single/multi-photon confocal microscopy, FLIM, FRET, FRAP, FCS etc.; confocal laser scanning for platforms or spinning disk.
E. 2 Visualization using nonlinear optics: SHG/THG, SFG/CARS.
E.3 Manipulation and biomechanical measurements: optical tweezers, laser micro-dissection, atomic force.
E. 4 Spectroscopy with spatial resolution: Raman, CARS, PLE of 1 and 2 photons, fluorescence lifetime.
E. 5 Near-field super resolution [AFM/Tip enhancement] – all techniques listed in c. 1; C. 2; C. 3 and C. 4 and can be used in the presence of tip. Validation studies of these techniques will be required, for it is not known how the presence of a metallic tip affects fluorescence lifetimes; interactions of Förster (the FRET); SHG, CARS etc.
E. 6 Far-field super-resolution – structured lighting can be accomplished with non-linear optics. Localization techniques or STED utilize fluorescence. Visualization using super-resolution, with the appearance of commercial equipment, is rapidly coming into wider use. On the other hand, feasibility studies on combining super-resolution with SHG/SFG or FLIM/FRET are in the early stages internationally.

Manipulations and Biomechanical Measurements:
F 1 Multiple Optical Tweezers: movement; capture of microorganisms for time observation; measures of forces between 50 femto-Newtons and 500 pico-Newtons, adhesion studies, elasticity, viscosities of membranes, zeta potential; chemotaxis; cellular deformations, especially combined with FRET stress sensor; characterization of molecular motors, active transport via kinesin or dynein.
F. 2 Laser microdissection: extraction of material for analysis; photoporation;

transfection combined with optical tweezers or AFM.
F. 3 Atomic Force microscopy: measures of forces between 50 pico-Newtons and tens of nano-Newtons; tip-enhancement; anchoring enzyme.
F.4 Micro-engineering, Micro fabrication and Microfluidics in optically transparent cells: fast control of the chemical environment in very small volumes, including the same optical characterization; configuration of gradients; drugs or biological materials.

Materials and sensors:
G. 1 Auto-fluorescence: has the great advantage of being endogenous, not affecting biological processes, considered label-free. NAD and FAD are examples of lignin auto-fluorescence markers. Once considered generators of artifacts, are now considered useful for discrimination of the chemical environment, especially when using FLIM.
G. 2 Exogenous conventional fluorescent markers. Sensors based on fluorescence intensity as well as calcium sensors, pH and O_2 meters, etc. are available commercially.
G. 3 Fluorescent proteins encoded in DNA of organisms: GFP, YFP, CFP, RFP etc. The fact that the body itself expresses the fluorescent protein has led to a revolution in biological markers. Can also be used as a pair of FRET, and CFP-YFP is the most common.
G. 4 Fluorescence sensors through FRET: FRET can be used in the construction of stress, temperature and protein sensors, etc. Any parameter that changes the distance between the donor-acceptor pair can be quantified with FRET. There are commercial FRET sensors and biochemistry laboratories to produce customized sensors.
G. 5 Advanced fluorescent markers. Quantum Dots and Lanthanide ions are examples of modern fluorescent markers. FLIM and FRET allow a large number of applications using this material. Can be discriminated against conventional markers through its lifetime, on the order of 10-40 ns in quantum dots and μs/ms in lanthanides. Lanthanides possess very narrow spectral widths and very long lifetimes, making them important markers for FRET and FLIM. FRET in quantum dots is still at the stage of international study.

Biological issues:

Items H.1 through H.13 in the table on p. 20

Regarding tools and methodologies, the current INFABiC status allows for research under the following items:

A.2; **A.3** and **A.4** (A.1 can be used in high-dilution strategy and tip-enhancement and
D.1.2 of far-field super-resolution requested in this Project)
B.1 (utilizing spinning disk, no integration with other techniques);
B.2; **B.3** and **B.4**
D.1.1 and **D.2** – far-field super-resolution required
E.1; **E.2**; **E.3**; **E.4** and **E.5** available. **E.6** pending on D.1.2
F.1; **F.2** and **F.3** available and engaged for confocal microscopes
F.4 this is possible using groups of researchers trained in micro-engineering, micro-fabrication and microfluidics (Professors Lucimara, Marisa M. Beppu and Mônica A. Cotta). Platform ATM isolated with demonstrated capacity for biological studies. Also links to INFABiC through Professor Mônica A. Cotta.
G.1; **G.2**; **G.3** and **G.4** available in general, with a scientific team well trained in the use

of these markers. Physics group with great experience in Quantum dots. Incorporation of Lanthanides is included in this project through collaboration with the oldest and most experienced group in Brazil in this area, DQF from UFPE.
H.1 to **H.13**; **H.4** and **H.5** represent areas of study already being conducted within the scope of INFABiC. **H.1**, biochemistry study *in singulo*, representing one of the main fields at the border of knowledge, to be developed within the next period, utilizing synergies among researchers who have mastered biochemical aspects and researchers who have mastered observation techniques.

Proposal for consolidation of INFABiC

After the establishment of the laboratory, we realized that most users have requirements for simpler techniques such as fluorescence or confocal 2D, and require more time to use the microscopes, either because of the large number of researchers or the large numbers of samples submitted. On the other hand, two techniques, based on multiphoton microscopy- second harmonic generation (SHG) and microscopy based on fluorescence lifetime (FLIM), were readily incorporated by the simple fact that they can be applied to already set, often-fixed material.

An important, although slowly developing area is the use of living cells and the application of more-advanced techniques that require extensive laboratory time for standardization or that depend on the use of specialized apparatus , and mostly customized for answering questions in different projects such as FRAP, FRET, optical tweezers, intravital microscopy and "tip enhancement".

The strategy developed to increase access under the present conditions, was the establishment of user groups, defined according to their degree of autonomy in the use of technical equipment. Thus, we have **Level 3 users** who have unrestricted access and do not depend on technical support for using equipment, shifted to nights, weekends and holidays. **Level 1 and 2 users** depend on technical support, available only during business hours, for operation of the equipment, and are differentiated according to the expertise/ field of the material examined.

The operation of INFABiC revealed some bottlenecks, which can be addressed with relative ease. Figure 2 shows the relationship between the number of users and the complexity of the technique/methodology employed. As mentioned previously, the majority of users require simpler techniques based on fluorescence, confocal microscopy and obtaining images with SHG. There is a certain inertia inherent in the transition from the use of permanent material, histological sections or fixed cells, to the observation of living cells, including customized apparatus combined with fluorescent

proteins. The use of living animals in experiments, whether mice or zebrafish, is also slow and requires commitment and the creation of appropriate conditions, in addition to enormous effort expended to convince the user to try it. The application of multiple and simultaneous optical tweezers, as well as a more-refined use of spectroscopic techniques that rely on major modifications of equipment settings, standardization and long periods of data acquisition also demand intense application of INFABiC's efforts, since it involves a significant commitment from users.

In terms of instrumentation, our proposal is to acquire new microscopes that are equipped for observation of living cells and that meet the demand for microscopic fluorescence-based images, utilizing confocality supplied by the Apotome system (Zeiss). We hope to extend this measure also for São Paulo (USP, UNIFESP) when installing systems similar to those in their institutions, where there are confocal multiphotons installed. These systems at UFG (Goiânia) and UFSCAR (São Carlos) aim to create centers for experimentation, diffusion, and observation of dynamic aspects of cells.

By doing this we will create a broad base and facilitate work with living cells, recruiting people from different fields for studies at the cellular level, such as physiology and pharmacology. At the same time, we will alleviate the use of confocal that have accessories for more specific and laborious techniques, and encourage the use of more complex techniques, available in the Central Laboratory.

Following the installation and operation of the "**Danio Core**" (zebrafish breeding and maintenance unit), we also want to encourage the use of small animals for *in vitro* studies, as these methods are already in wide use, and represent a promising alternative on several fronts.

In order to add possibilities in microscopy and host-laboratory analysis, we propose the acquisition of a system for super-resolution microscopy, which would be supplied by equipment similar to Nikon N-SIMS or Bruker Vutara 350, depending on particular negotiations with the various companies.

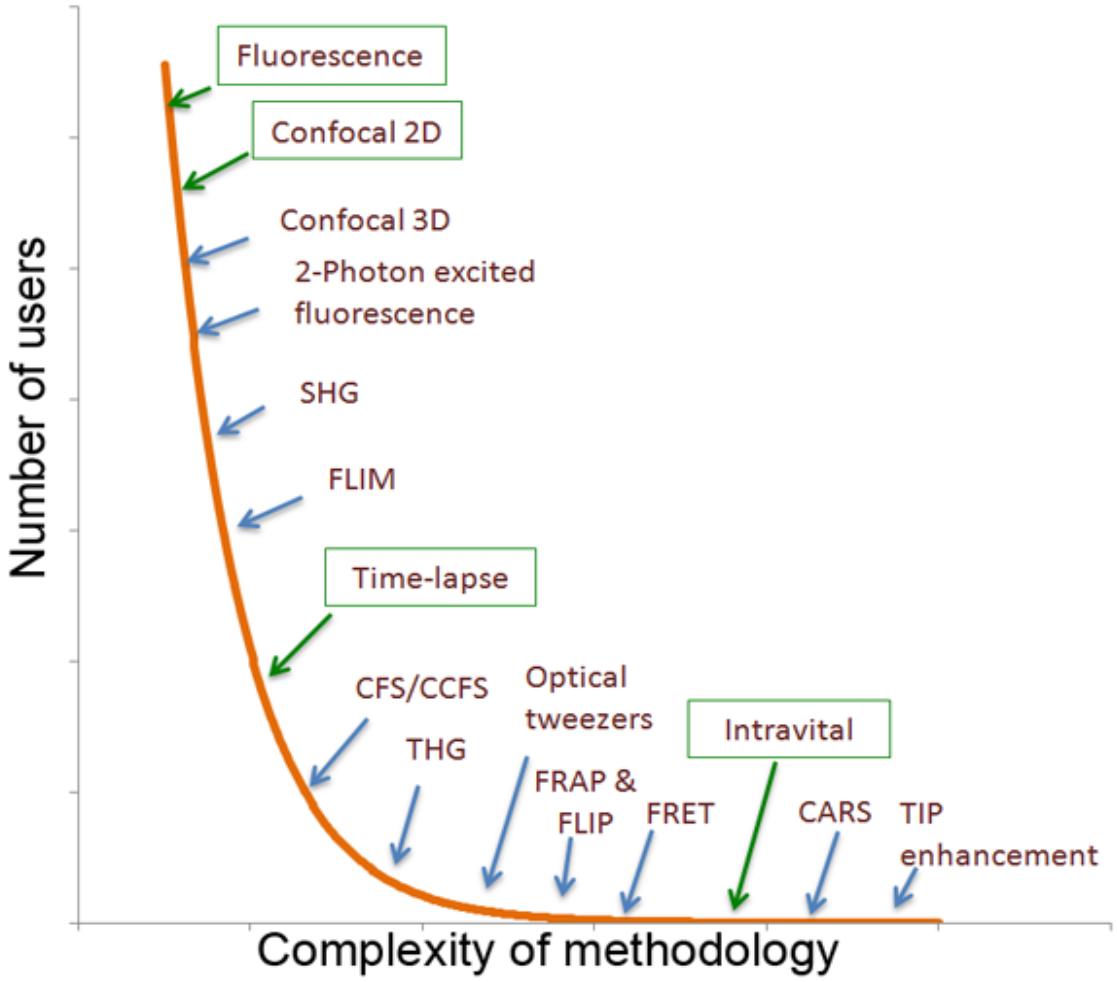


Figure 2. Distribution of the number of users, given the complexity of techniques/ approaches available through INFABiC.

12. Objectives

General objective

The overall objective of the project is to conduct high-impact research on dynamic and mechanistic aspects of cells, organelles and single molecules in various models, providing equipment and methods, prospecting at the forefront of integrated optical microscopy, photonics and microengineering and microfluidics. We will study biological processes from the molecular to the *in vivo* level, using small animals.

Toward this end, we will use and develop tools and methodologies, perform quantitative measurements and manipulate molecules, cells and tissues, and at the same time will integrate a large team of researchers from different institutions around the structural axis of photonics/microscopy and cell and molecular biology. INFABiC will allow the study of biological problems within particular disciplines and will stimulate interactions at the interfaces, thereby expanding the frontiers of cell and molecular biology. The characteristics of INFABiC, in particular its multiuser and multidisciplinary organization, along with continued funding allows us to share knowledge, expertise and best practices to be (a) transformative, (b) catalytic, (c) synergistic, (d) cross-contact and (e) unique, as suggested by the NIH Common Fund Program. The present application proposes strategies for the CONSOLIDATION AND EXPANSION of INFABiC. To consolidate, we need to (1) preserve the unit of the research team, (2) guarantee the proper use and functioning of the installed equipment and accessories, (3) transfer and install all equipment to the new laboratory in the Institute of Biology, (4) continue the organization of teaching/diffusion/extension events, (5) increase/stimulate the use of the integrated and multimodal platform, seeking to explore more deeply the different biological questions, and (6) cross-integrate the team members in new sub-networks and identify new collaborative hubs, particularly using the MEGAPROJECT strategy. To expand INFABiC's influence and approaches, we propose actions with respect tothe composition of the research team, instrumentation, research, education/diffusion/extension and internationalization. (1) **Research team**: New groups were recruited to increase the geographical inclusion and expand the expertise. We seek to foster changes in the associated laboratories, with a predicted strong impact on the asymmetrical collaborations with the three laboratories in the central-western and northeastern regions of the country (including Espírito Santo). We also plan to increase the mobility of the team members among the associated laboratories. (2) **Instrumentation**. (i) acquire, install and support new fluorescence/time lapse microscopes in the associated laboratories, to increase the use of cell culture and dynamic analysis and to (ii) solve bottlenecks in the associated laboratories, (iii) acquire and install a new super-resolution microscope with 20/60 nm lateral resolution and 2O μm deep capacity, with 1,000 frames per second acquisition rate, and capable of 3D single-particle tracking. (3) **Research**. The new research team consists of subgroups working on cell cycle/cancer, vascular biology/angiogenesis, microbiology and microfabrication, among others. We will work in areas such as cell differentiation,

migration, cell cycle; metabolism, lipids, mitochondria; reproduction, nuclear receptors and reproductive immunology; prostate induction and prostate cancer; microbiology-immunology-parasitology of trypanosomatids, periodontal disease, *Xylella fastidiosa*, yeasts; angiogenesis and vascular physiology, extracellular matrix; pathology, microengineering/microfluidics; cell backpacks and drug targeting; organic synthesis and fluorescent probes for measuring enzyme activity, FRET sensors, lanthanids and quantum dots. (4) **Education/extension/diffusion**. New expertise for the use of alternative didactic methods and distance learning has been integrated into the INFABIC team, in addition to new readers and lecturers in cell biology, who will administer classes and lectures. (5) **Internationalization**. In addition to individual collaborations, institutional collaborations will be established with the NIH/USA and the University of Bristol/UK, to share expertise and to promote the bidirectional exchange of researchers and students.

Specific objectives

(1) Maintain INFABiC functionality by keeping the multiuser nature of the installed equipment and its renewal, prospecting at the vanguard of optical microscopy, including super-resolution techniques,

(2) Increase the ability to support users' needs for basic techniques while allowing the use of specific and time-consuming methods with the more complex equipment, while stimulating the adoption of dynamic techniques based on phase-contrast and fluorescence microscopies,

(3) Seek excellence in image acquisition, simultaneous monitoring of diverse parameters, and physical manipulation of cells (using optical tweezers) and molecules (combining expertise on atomic force microscopy and surface functionalization),

(4) Monitor the function of selected enzymes in time and space, particularly for DNA polymerases and carbohydrate synthesis, editing and degradation, such as the bifunctional enzyme N-deacetylase-N-sulfotransferase (NDNST, critical in heparan sulfate and heparin synthesis) and cellulases and chitinases, capable of degrading cellulose and chitin, respectively,

(5) Provide microfabrication techniques for the construction and monitoring of physico-chemical-architectural characteristics of the cell's environment, coupling microengineering/microfabrication and microfluidics with the fabrication of quantum dots and lanthanide complexes, among other customized services, for INFABiC users and outsiders, via the productive sector,

(6) Combine expertise in the construction of vectors, including viruses, and transfection/infection, to obtain customized constructs to achieve molecular specificity in the analysis of subcellular and dynamic aspects of cells,

(7) Stimulate the use of zebrafish as an experimental model, providing the Danio Core Zebrafish Unit and services for the construction and deposit of transgenic strains and non-linear optics for intra-vital imaging,

(8) Coordinate transverse activities to form subgroups interested in cancer, vascular biology/angiogenesis, microbiology and microfabrication,

(9) Integrate INFABiC and two centers of excellence abroad (NIH-USA and University of Bristol-UK), with expertise in dynamic cell biology, stimulating the mutual exchange of researchers and students. Participate in international scientific meetings in the areas of photonics, imaging and cell biology,

(10) Install cell culture and/or time-lapse microscope in the three associated laboratories type II (national collaboration) in Goiânia (GO), Fortaleza (CE) and Aracruz (ES), and

(11) Launch the CampinasVirtual Network of Advanced Cell Biology to coordinate INFABiC's education, diffusion and extension activities.

13. Methodology

The basic function of allowing access to equipment and methodologies is implemented via INFABiC's home page. Once the user's needs are identified, they are instructed how to schedule the use of the equipment and instructed about specific requirements for sample processing. Whenever necessary, the users are referred to the coordinators or other team researchers for (a) a better understanding of the phenomenon involved, (b) refinement of the model or exploitation of additional possibilities, (c)

counseling on sample preparation or protocol adjustments, (d) help with description of methods, and (e) interpretation of results and manuscript writing.

The major means of diffusing photonics and non-linear optics technology is the Spring **INFABiC Annual Workshop**, which offers classes, lectures and courses using the different equipment and accessories, including an Image J training course.

Since 2009, we have been focused on setting up a well-equipped laboratory and on providing infrastructure for its functioning, with minimal commitment of the users in the administration and bureaucracy. In this new application, we will work more effectively in the identification of questions and problems of interest for the team as a whole, prioritizing themes and approaches, defining strategies and gathering expertise that will combine to help us to reach our objectives. We have identified three areas with the critical mass to establish subnetworks: (**i**) cancer (cell cycle, mitosis, invasion, migration, ECM-proteases); (**ii**) vascular biology/angiogenesis (stem cells, ECM-proteases/physiology/atherosclerosis/ultrastructure, both with translational potential; (**iii**) Microbiology (yeast, *Xylella fastidiosa* and other bacteria) with great impact in agriculture, because of their relationships to the host plant or energy production. (**iv**) Microengeneering/microfabrication (surface functionalization, microfluidics, 2D and 3D micropatterns; quantum dots and lanthanides), with great potential for the production of customized products for cell culture.

We also recruited a specialist in vectors and cloning (Boldrini), needed for transfection, transgenesis and recombinant protein production, and two groups who are prospecting for new drugs from the diverse Brazilian fauna and flora (CPQBA and UFC). We also recruited education specialists to expand our activities related to alternative learning strategies (UFPR) and distance learning (USP).

We expect that, by offering access to equipment and other benefits, the team can work in consortia of individuals with a variety of expertise, dedicated to the study of complex problems and questions. To this end, we will explore the idea of MEGAPROJECTS.

14. Scientific contributions and analysis of the current and future situations

INFABiC was created with the initial objective to reinforce the interaction between physics and biology, to prospect and disseminate non-linear optics-based advanced photonic microscopy techniques. This was our major accomplishment. The laboratory exists and provides universal access to researchers from the whole country and from abroad.

We have obtained additional funding that allowed the setup of the laboratory as initially conceived. In particular, the FAPESP's multiuser call (EMU) for large equipment doubled the initial investment from the INCT program. The host university UNICAMP also contributed funds for the construction of the new laboratory.

Highlights of the research conducted in INFABIC

We list below a series of scientific articles, book chapters and one patent, from an average total of 130 articles published per year (reports available at the INFABiC homepage).

(1) Application of FRET-FLIM

Pereira MBM, Santos AM, Gonçalves DC, Cardoso AC, Consonni SR, Gozzo FC, P. Oliveira SL, Figueiredo AR, Cepeda AOT, Ramos CHI, de Thomaz AA, Cesar CL, Franchini KG (2014) "αB-crystallin interacts with and prevents stress-activated proteolysis of focal adhesion kinase by calpain in cardiomyocytes. **Nature Communications** (accepted for publication).

FRET is quite sensitive to the distance between the donor and the acceptor fluorophores. This makes FRET one of the best indicators of interaction among proteins. FRET in live cells is the only way to show that interaction occurs because of a real biological process, not sample processing. FRET measurements must be carefully made, due to some optical artifacts. Merely observing the acceptor emission is not enough to prove that FRET has occurred. It is now accepted that the most robust way to observe FRET is by shortening the donor lifetime observed with FLIM, a technique

known as FRET-FLIM. Our team demonstrated the interaction between Focal Adhesion Kinase (FAK) with αB-crystallin in live cardiomyocyte cells, using FRET. FAK overexpression protects cardiomyocyte depletion of αB-crystallin against the stretch-induced apoptosis. Our studies define a role for αB-crystallin in controlling FAK function and cardiomyocyte survival, through the prevention of calpain-mediated degradation of FAK.

(2) Studies of Pathological Anatomy: INFABiC published a series of seven[7,8,9,10,11,12,13] reports utilizing TPEF+SHG+THG+FLIM in ovary, breast, and colon cancer, and to characterize the genetic bone disorder *Osteogenesis Imperfecta*.

One of the first tasks in this study was related to instrumentation to acquire multimodal images using TPEF+SHG+THG+FLIM, proving that all optical signals obey the expected rules for each NLO method. The THG methodology was the most complicated, as it occurred below 350 nm where microscope optics did not transmit any light. We modified the microscope, designing a special detection scheme to acquire THG images. It was important to assure the reviewers that we had good-quality images without optical artifacts. We then used a multimodal platform to study ovary, breast and colon cancer and a genetic disease called Osteogenesis Imperfecta. We have shown that TPEF+SHG+THG patterns are maintained in H&E-stained tissue sections, and that FLIM signatures are preserved in non-stained paraffin blocks, stored for decades. This means that it is possible to re-evaluate a library of existing pathological case biopsies with these new techniques, opening the possibility to perform retrospective studies that otherwise would require several years of followup. Collagen network of the

[7] Adur J, DSouza-Li L, Pedroni MV, Steiner CE, Pelegati VB, de Thomaz AA, Carvalho HF, Cesar CL. The severity of Osteogenesis imperfecta and type I collagen pattern in human skin as determined by nonlinear microscopy: proof of principle of a diagnostic method. PLoS One. 2013 8(7):e69186.

[8] Adur J, Pelegati VB, de Thomaz AA, D'Souza-Li L, Assunção MC, Bottcher-Luiz F, Andrade LA, Cesar CL. Quantitative changes in human epithelial cancers and osteogenesis imperfecta disease detected using nonlinear multicontrast microscopy. J Biomed Opt. 2012 17(8): 081407-1.

[9] Adur J, Pelegati VB, de Thomaz AA, Baratti MO, Almeida DB, Andrade LA, Bottcher-Luiz F, Carvalho HF, Cesar CL. Optical biomarkers of serous and mucinous human ovarian tumor assessed with nonlinear optics microscopies. PLoS One. 2012 7(10):e47007

[10] Adur J, Pelegati VB, Costa LF, Pietro L, de Thomaz AA, Almeida DB,Bottcher-Luiz F, Andrade LA, Cesar CL. Recognition of serous ovarian tumors in human samples by multimodal nonlinear optical microscopy. J Biomed Opt. 2011 16(9): 096017

[11] Adur J, Carvalho HF, Cesar CL, Casco VH. Nonlinear optical microscopy signal processing strategies in cancer. Cancer Inform. 2014 Apr 2;13:67-76

[12] Pelegati VB, Adur J, De Thomaz AA, Almeida DB, Baratti MO, Andrade LA, Bottcher-Luiz F, Cesar CL. Harmonic optical microscopy and fluorescence lifetime imaging platform for multimodal imaging. Microsc Res Tech. 2012 Oct;75(10):1383-94

[13] Adur J, Pelegati VB, de Thomaz AA, Baratti MO, Andrade LA, Carvalho HF, Bottcher-Luiz F, Cesar CL. Second harmonic generation microscopy as a powerful diagnostic imaging modality for human ovarian cancer. J Biophotonics. 2014 Jan;7(1-2):37-48

extracellular matrix are extensively remodeled in cancer processes, which makes SHG a very valuable diagnostic technique, especially using the SHG/TPEF ratio to obtain the ageing index called SAAID. THG provides images of the nuclei and tissue interface. Numerical automatic processing of acquired images can be used to quantify pattern changes. Fourier transform can provide the collagen fiber orientation, alignment and organization. Moreover, texture analysis using a Grey Level Co-occurrence Matrix provides a set of scoring methods, such as correlation, energy, entropy and anisotropy used to discriminate stromal components of serous, mucinous, endometrioid and mixed ovarian tumors. They can also distinguish normal, benign, borderline, and malignant tumors according to the distribution of collagen fibers and compression levels within the extracellular matrix. The epithelium/ stromal interface, such as the transformation of the epithelial surface, was observed with THG, while the overall fibrillar tissue architecture was observed with SHG. The increased metabolism of cancer cells can be evaluated with FLIM, making the combination of FLIM+TPEF+SHG+THG a very powerful technique to understand cancer processes. We also observed that FLIM images of mucinous cancer are very different from non-mucinous ones. Actually, any abnormal cell proliferation and collagen assembly can be observed with the NLO-FLIM multimodal method. The endothelium family was re-evaluated for colorectal cancer using NLO microscopy, and we found collagen changes in early stages of cancer development, correlated with significant differential gene expression and protein localization, suggesting that ET-2 can be used as an early marker of colon cancer development. Osteogenesis Imperfecta is a genetic disease that affects collagen production, causing brittle bones and other tissue anomalies. The conventional diagnostic technique of bone biopsy is very invasive and time consuming, requiring genetic tests. However, the modification of collagen patterns is easily assessed on the body surface. We showed that a simple skin biopsy using TPEF+SHG could not only distinguish diseased from normal tissue, but also discriminate among OI types and severity.

(3) Assembly of bacterial biofilms

R. Janissen, D. M. Murillo, B. Nizab, P. K. Sahoo, M. M. Nobrega, C.L.Cesar, M. L. A. Temperini, Hernandes F. Carvalho, A. A. de Souza, M. A. Cotta (2014) Phenotypic

changes and spatiotemporal distribution of extracellular polysaccharides mediate *Xylella fastidiosa* adhesion and biofilm architecture. Submitted.

Bacterial biofilms represent a tremendous challenge in medicine and pathology, due to the ability of the colony to protect its individuals. We decided to observe the dynamics of biofilm formation of *Xylella fastidiosa*, a bacterium that causes diseases in important crops, such as citrus and grape. We realized that the blur in the normal confocal microscopy image came from the rotational movement of the bacteria attached to the surface. Using the much faster image acquisition rate of a confocal spinning disk system, we obtained high-quality images that revealed details of biofilm assembly. We observed distinct phases of biofilm formation and altered cell phenotypes, apparently bridging individual colonies. These observations were confirmed by AFM and electron microscopy as well as by confocal Raman spectroscopy.

(4) Caracterização de mobilização de actina nuclear utilizando FRAP e FLIP

[Characterization of nuclear actin mobilization using FRAP and FLIP]

Alexandre Bruni-Cardoso, Viginia A Spencer, Emily Chen, Hidetoshi Mori, Hernandes F Carvalho, Mina J Bissell (2014) Signaling from ECM to nuclear actin leaves to quiescence through exportin-6. Submitted.

In this study, the dynamics of nuclear actin and its relationship to specific stages of tumor progression were investigated in cell-culture models. In particular, from the viewpoint of this application, we have used FRAP and FLIP to determine the predominant mechanism responsible for nuclear actin accumulation, resulting in the finding that actin export is the limiting factor.

(5) Calcium regulation of *Trypanosoma brucei* metabolism

The uniporter calcium transporter was localized in the mitochondria and demonstrated to act decisively in the metabolism and bioenergetics of *T. brucei*, sensitizing this cell to

apoptosis[14]. This study was published in the important journal ***Nature Communications***.

(6) In situ hybridization and confocal microscopy used for the localization of interleukin expression in the hypothalamus

This study, published in ***PLOS Biology***[15], used in situ hybridization and confocal microscopy to locate IL10R and IL6R in the hypothalamus, complementing extensive biochemical and immunocytochemical analyses.

(7) Correlação entre inflamação e progressão do câncer de cólon

[Correlation between inflammation and progression of colon cancer]

We contributed to the identification and counting of intestinal polyps and to the histological characterization of colon cancer progression, in a combined inflammatory and carcinogen administration protocol, resulting in a publication in the prestigious journal ***Gastroenterology***[16].

(8) Structural aspects of the interleukin 7 receptor related to lymphoblastic leukemia

Zenatti PP, Riberio D, LiW, Zuurbier L, Silva MC, Paganin M, Tritapoe J, Hixon J, Silveira AB, Cardoso BA, Srmento LM, Correia N, Toribio ML, Kobarg J, Hosrtmann, Pieters R, Brandalise SR, Ferrando AA, Mijerink JP, Durum SK, Yunes JA,

[14] Huang G, Vercesi AE, Docampo R (2013) Essential regulation of cell bioenergetics in Trypanosoma brucei by the mitochondrial calcium uniporter. **Nat Commun** 4: 2865

[15] Ropelle ER, Flores MB, Cintra DE, Rocha GZ, Pauli JR, Morari J, de Souza CT, Moraes JC, Prada PO, Guadagnini D, Marin RM, Oliveira AG, Augusto TM, Carvalho HF, Velloso LA, Saad MJ, Carvalheira JB (2010) IL-6 and IL-10 anti-inflammatory activity links exercise to hypothalamic insulin and leptin sensitivity through IKKbeta and ER stress inhibition. PLoS Biol. 8(8). pii: e1000465.

16 Flores MB, Rocha GZ, Damas-Souza DM, Osório-Costa F, Dias MM, Ropelle ER, Camargo JA, de Carvalho RB, Carvalho HF, Saad MJ, Carvalheira JB (2012) Obesity-induced increase in tumor necrosis factor-α leads to development of colon cancer in mice. Gastroenterology 143(3):741-753

Barata JT. Oncogenic IL7R gain-of-function mutations in childhood T-cell acute lymphoblastic leukemia. ***Nature Genetics*** 43: 932-939 (2011).

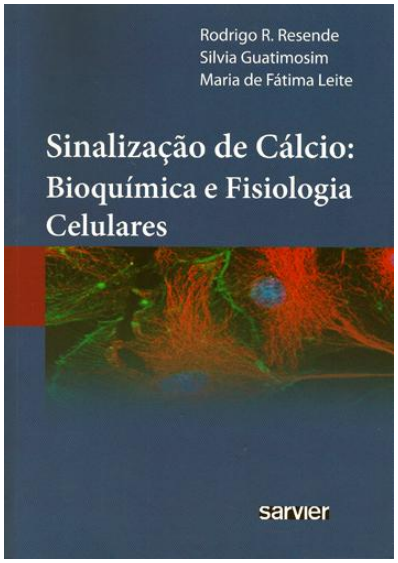
(9) Microfabrication of cell backpacks for use as directed drug carriers

Exploring the possibility of constructing cell backpacks for loading cell surfaces with specific cargoes, we determined that the use of a sacrificial layer based on jacalin-mucin interactions allows the quick release of the backpacks using an inhibitor sugar[17].

(10) Patent application for a probe for the detection and localization of phosphatases.

In collaboration with Dr. Anita Marsaioli, from the Institute of Chemistry – Unicamp, we have submitted a patent application for a fluorescent probe to determine and localize phosphatase in living cells. The patent application number is BR 10 2014 015963 0, and the article "**Fluorogenic probe to detect multi-enzymatic cascade reactions triggered by serine/threonine phosphatases**" was submitted for publication.

(11) Books and book chapters



Sinalização de Cálcio: Bioquímica e Fisiologia Celulares

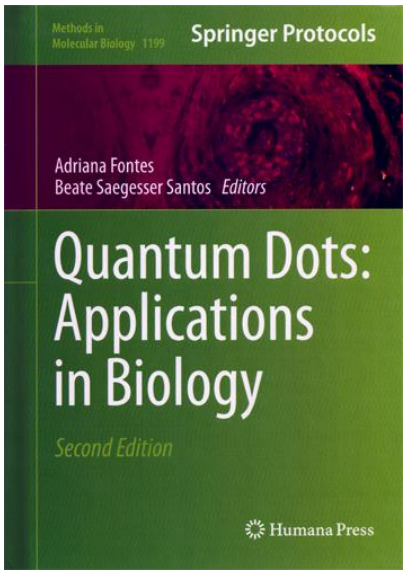
Calcium signaling: cellular biochemistry and physiology

Eds. Rodrigo R. Resende, Silvia Guatimosim and Maria de Fátima Leite

Chapter:

1. Photonics applied to Cell Biology
   Ana Maria de Paula and Carlos Lenz Cesar

[17] Polak R, Crouzier T, Lim RN, Ribbeck K, Beppu M, Pitombo RNM, Cohen RD, Rubner MF (2014) Sugar-mediated disassembly of mucin/lectin multilayers and their use as pH-tolerant, on-demand sacrificial layers. **Biomacromolecules** 15: 3093-3098.



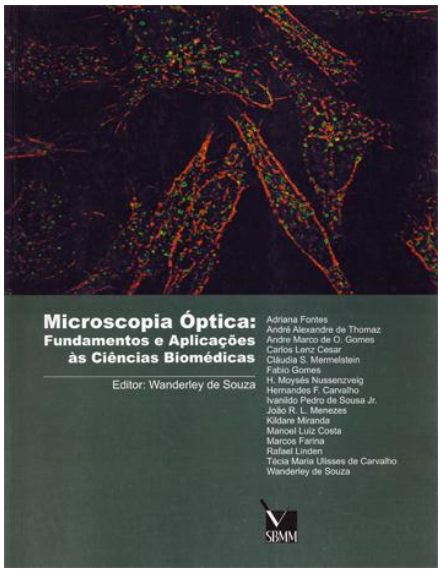
Quantum Dots: Applications in Biology

Eds. Adriana Fontes and Beate Saegesser Santos

Chapters:

1. Quantum Dots as Biophotonics Tools
Carlos Lenz Cesar

6. Mesuring the Hydrodynamic Radius of Quantum Dots by Fluorescence Correlation Spectrocopy
André Alexandre de Thomaz, Diogo B. Almeida and Carlos Lenz Cesar



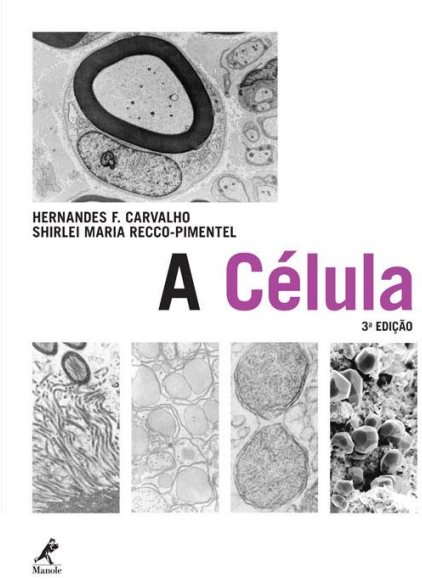
Microscopia Óptica: Fundamentos e Aplicações às Ciências Biomédicas

Optical microscopy: fundamentals and applications in biomedical sciences

Ed. Wanderley de Souza

Chapters:

V. Fluorescência (Fluorescence)
Adriana Fontes, André Alexandre de Thomaz, Carlos Lenz Cesar and Hernandes F. Carvalho
XII. Microscopia de Fluorescência de Alta Resolução (High-Resolution Fluorescence Microscopy
Adriana Fontes, André Alexandre de Thomaz, Carlos Lenz Cesar and Wanderley de Souza



A Célula (The Cell)

Editora Manole; 3rd Edition, 2013

Ed. Hernandes F. Carvalho and Shirlei Maria Recco-Pimentel

16. Technological potential

We believe that the role of INFABiC is broader than the scientific collaboration among its members, and also involves knowledge transfer between the headquarters and the associated laboratories. If the first proposal was strongly based on the physics-biology interaction, the present team added more expertise that will be used to solve specific problems and to develop megaprojects. Moreover, we have identified the potential to make several technologies available to the general community, including certain commercial firms.

In the present configuration, INFABiC can offer services (through FUNCAMP-UNICAMP); agreements with companies or even a Specific Purpose Entity (SPE[18]), to provide:

1. Image analysis, including cell migration parameters (Figure 3a).
2. Stamping molds with adhesive/antiadhesive proteins and micropatterned cell culture dishes (glass or plastic) (Figure 3b and c).
3. Cellular backpacks in several configurations (Figure 3d).
4. Customized fluorescent probes.
5. Functionalized surfaces (including AFM tips, cover slips for cell culture, extracellular matrix degradation assay) (Figure 3e).
6. Microfluidics on demand (Figure 3f).
7. 3D matrix culture.
8. Zymography assays for gelatinase determination.
9. Ecotoxicological assays using Zebrafish as a model.
10. Protein conjugation (antibody, lectin and other proteins) with fluorescent probes, including lanthanides such as Terbium and Europium.
11. Counseling services in multicolor immunocytochemistry and histochemistry.
12. Counseling services in confocal microscopy.
13. Technical training in the INFABiC member expertise areas.
14. Lanthanides probes for environmental conditions, especially in microfluid systems.
15. Quantum dots production on demand.

[18] SPE is a legal entity (usually a limited company of some type or, sometimes, a limited partnership) created to meet narrow, specific or temporary objectives.

We plan to expand our facilities to offer:

(1) Constructs for recombinant protein expression in bacteria, yeasts, mammal cells (CHO, HEK293) or insect cells.

(2) Specific constructs for cell organelle observations.

(3) Systems for lentivirus infections for large reporter-gene constructs or heterologous expression.

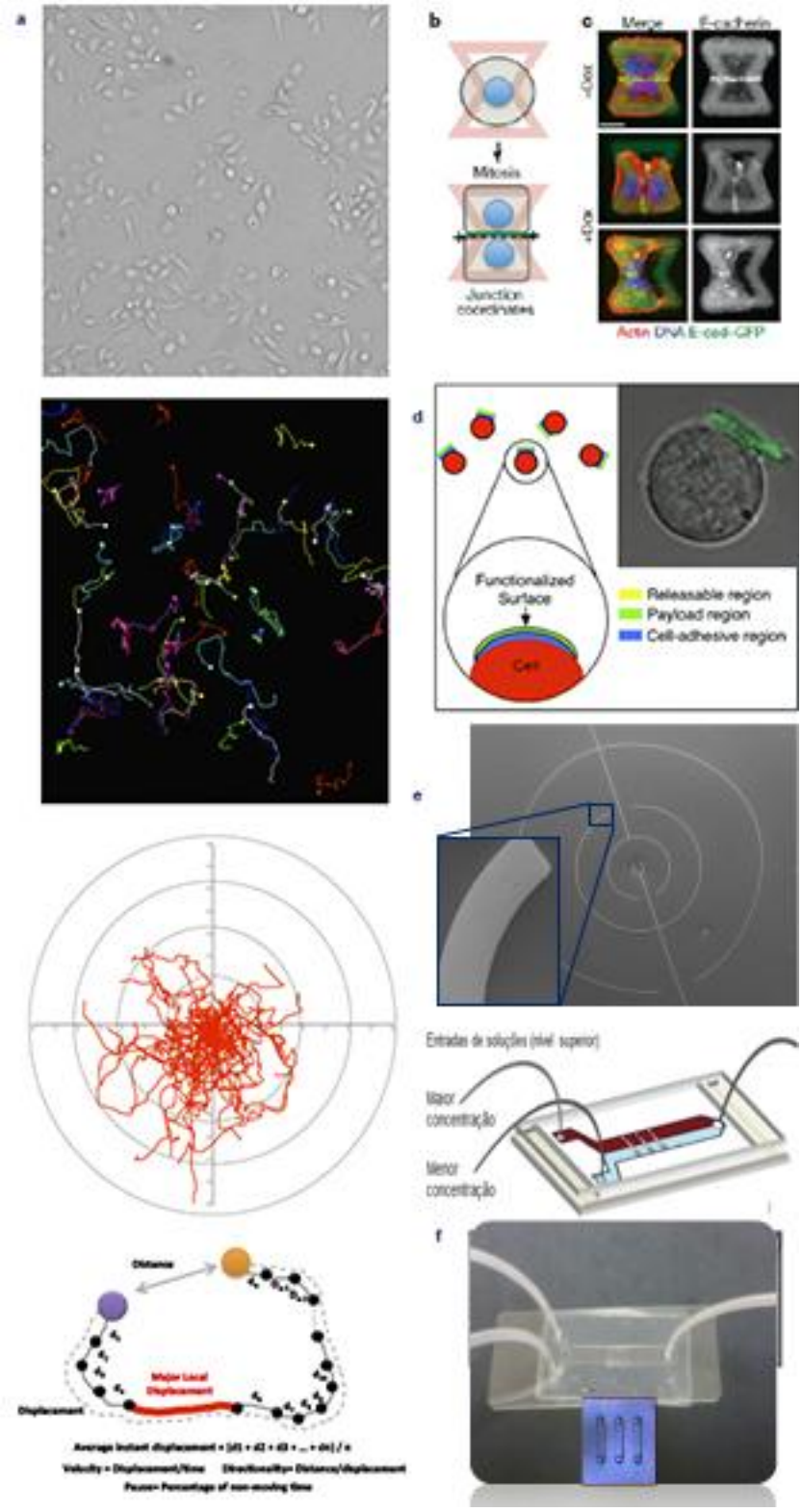


Figure 3. Examples of technology transfer to be explored by the INFABIC. (a) Image analysis parameters including cell migration characterization; (b and c) Constructs of micropatterns to reveal specific cell behavior, such as polarization of mitotic spindles or epithelial cell junctions alignment (Godinho et al. Nature 510: 167-171, 2014) (d) constructs of cellular backpacks with several configurations (Swiston et al. Nano Lett 8: 4446, 2008) (e) constructs of microelectrodes to generate surface electric potential; (f) customized microfluidics.

16. Activities to transfer knowledge to the general society

Diffusion activities

(1) **International training courses in Cell and Molecular Biology** (IFCB/SBBC) simultaneously in three universities (UFRJ, USP-SP and Unicamp), with live transmissions to the whole country, sponsored by the International Federation of Cell Biology (IFCB) and European Molecular Biology Organization (EMBO)

(2) Booklet in the textbook "**The Cell**", with the INFABiC proposals and achievements. Integration of the syllabus of Cellular and Molecular Biology of Brazilian universities, an activity centered on the Brazilian Society of Cell Biology (SBBC)

(3) "Cell Biology for Children" program, to be structured by members of UNICAMP, UFPR, UFSC and the three type II associated laboratories.

(4) Organization of the XVIII Congress of the Brazilian Society of Cell Biology in conjunction with the Annual Meeting of FESBE, with the suggested theme: "**Interfaces of Experimental Biology**"

(5) Organization of the second Workshop "At the Interface between Physics and Biology", preferably as an external activity, with negotiations with UFRJ already initiated.

17. Budget

Budget implementation

	Itemized expenses	
	Operating expenses	**Values in Real (R$) (USD exchange rate = 2.3) (Euro exchange rate = 3.0)**
1	Per diem	150,000.00
2	Consumables - imported	700,000.00
3	Consumables - domestic	360,000.00
4	Tickets	200,000.00
5	**Third-Party Services (various)**	410,000.00
6	Import fees (18%) on item 2	126,000.00
7	Import fees (18%) on items 8-14	866,965.10
	Subtotal 1	**2,812,965.10**
	Permanent Material - Imported	
8	**1** microscope equipped for super-resolution such as the N-SIMS system from Nikon or the Vutara 350 from Brucker	1,604,129.78 USD 697,447.73
9	**3** Axiovert microscopes equipped for time lapse/Videomicroscopy (UNICAMP, UNIFESP, UFSCAR)	873,144.00 EUR 291,048.00
10	**3** Axiovert microscopes equipped for time lapse/Videomicroscopy + Apotome system (UNICAMP; USP, UFG)	1,203,084.00 EUR 401,028.00
11	Permanent license for various softwares	30,000.00
12	**1** femtosecond laser, Spectra Physics Insight or equivalent	733,700.00 USD 319,000.00
13	**4** antivibration optical tables	232,415.00 USD 119,000.00
14	Various optical accessories	140,000.00
	Subtotal 2	**4,816,472.78**
	Permanent Material - Domestic	
15	Furniture for new laboratory	100,000.00
16	Bibliographic material	10,000.00
17	Computers, stabilizers, UPS	50,000.00
	Subtotal 3	**160,000.00**
	Scholarships	**1,973,792.90**
	Grand total	**9,763,230.78**

Justification of the budget

Per diem

This amount is allocated to cover lodging and food during extra-institutional activities; activities of the steering committee, speakers and guests visiting INFABiC's laboratories.

Consumables - imported. This amount is allocated for importation of consumables: plastics, disposables, reagents, culture media, animal diets and animals, among others.

Consumables - domestic. This amount is allocated for the acquisition of consumables in Brazil: plastics, disposables, reagents, culture media, animal diets and animals, among others.

Tickets. These will be used to facilitate travel by researchers and students among the INFABiC laboratories, as well as their participation in scientific events and visits of speakers for the workshops. They will also allow the visit by the steering committee members and for the missions to the international partners. Also includes the participation of the evaluation seminars in Brasília.

Third-Party Services (various)

Allocated for the payment of services not directly related to the researchers' expertise. Design and maintenance of the website, equipment repair, transportation of heavy equipment. Most of this amount will cover import fees.

Superresolution Microscope is the equipment that will promote the greatest advancement in technology over the next six years for the INFABiC, by providing resolution on the order of 20/60 nm lateral/axial and allowing observations on living cells, equipped with an environmental-control system. The system uses either Structured Illumination or Single molecule switching nanoscopy.

The time-lapse/video microscopy systems allow environmental control for monitoring experiments with living cells, using simple contrast and/or fluorescence. Can be coupled to an ApoTome-type system, which allows acquiring equivalent to confocal images, but

is much less costly. As explained in the proposal, these systems will be used to (1) stimulate the transition from fixed-cell research to living cells; (2) to relieve the existing confocal systems (USP, Campinas and UNIFESP), allowing more time to use the accessories intended for more complex experiments; and (3) to widen the basis of skilled users who are interested in more-complex methodologies and the dynamic aspects of cells.

Software licenses

Allocated for the purchase of licenses for software needed for equipment functioning, data and image processing.

Femtosecond laser. This laser provides femtosecond pulses, typically 100 fs, in the range of 680-1300 nm, with average powers above 600 mW, reaching 1600 mW at the peak, in the whole tuning curve. The extended range to 1300 nm facilitates the acquisition of THG and enables observations at large depths, approaching 2 mm. The laser is fully automated and includes mechanisms for group velocity dispersion compensation to maintain the shortest possible pulse in the sample. In the dual version it will be also possible to use it for CARS microscopy and the simultaneous excitation of two fluorophores. It will allow the installation of the microscopes equipped for non-linear optics in the new Central Laboratory, since the present laser belongs to the IFGW and will be needed by the physicists for activities of prospecting for new technologies.

Antivibration Optical Tables are essential to prevent laser misalignment on the microscopes. The AFM technique and tip-enhanced, with resolutions of 10-20 nm, require another platform with an active antivibration platform placed on top of the normal optical table. They will be used for the installation of our microscopes in the new laboratory, given the fact that the present microsc opes belong to the IFGW Biophotonics laboratory.

Various optical accessories. All optical accessories such as filters, mirrors, detectors, photo-multipliers, small monochromators, diffraction gratings, wave plates, etc. Accessories for optical assemblies such as bases, posts, automated x, xy, and xyz translation stages, rotation stages, etc. Electronic components such as controller cards for the translation stages. Computers with hardware control boards for the maintenance of the Institute's website and the storing image bank.

Laboratory furniture. This will allow the proper installation of the new laboratory with drawers, cabinets, shelves, etc.

Bibliographical material. Refers to the acquisition of books and technical manuals.

Computers, stabilizers, UPS. Amount allocated for the purchase of supporting parts for the equipment.

Fellowships. These will fund team members at different stages of their training (Scientific initiation, PhD, Postdoctoral, and the recruitment of young talents from abroad), and will provide support for the research activities (Technical training).

18. Scholarships to be requested from the State Foundations

Technical Training FAPESP TT3 (10 scholarships for 12 months each)

19. Partnerships with companies and public bodies

There are ongoing Collaboration Agreements with the companies TridSkin/Allergisa and Rhea Biotechnologia, both located in Campinas.

20. Availability of infrastructure

Laboratories:

INFABiC´s headquarter laboratory (in the final stages of construction)

IFGW´s Biophotonics Laboratory

Zebrafish Unit (**Danio Core)**

Equipment installed:

Spinning disk system (Andor Technologies)

Upright Zeiss 780 NLO confocal microscope

Inverted Zeiss 780 NLO confocal microscope

Laser microdissection system (PALM-Zeiss)

Module for multichannel FLIM (Becker & Hickel)

Module for optical tweezers (Molecular Machines)

Module for CARS

Human Resources:

High-level technicians

Mariana Baratti (Biologist, PhD in Sciences) hired by Unicamp

Vitor Pellegati (Physicist, Master's in Physics) hired by Unicamp

Administrative Assistant

Ariane Tocci