2 ANNUAL MEETING

SOCIEDAD CHILENA DE IMÁGENES CIENTÍFICAS

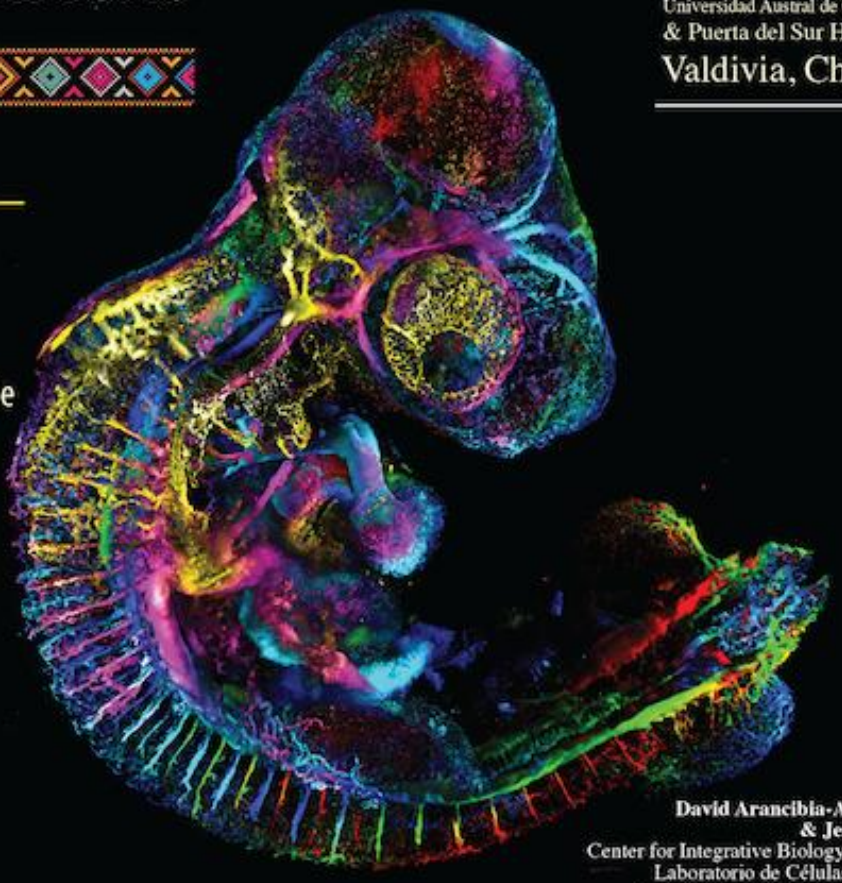
SoChIC

November 6-7, 2026

Universidad Austral de Chile
& Puerta del Sur Hotel
Valdivia, Chile

SPEAKERS

Alessio Accardi · Weill Cornell, USA
Nelson Barrera · PUC, Chile
Cristina Bertocchi · PUC, Chile
Karin Busch · University of Münster, Germany
Juan Pablo Castillo · Universidad de Valparaíso, Chile
Oscar Cerda · Universidad de Chile, Chile
Tomas Falzone · UBA, Argentina
Ginny Farias · Utrecht University Netherlands
Teresa Giraldez · Universidad de La Laguna, Spain
Juan Pablo Henríquez · UACh, Chile
Christian A.M. Wilson · Universidad de Chile
Judit Lisoni · UACh, Chile
Crina Nimigean · Weill Cornell, USA
Valentina Parra · Universidad de Chile, Chile
Andrea Ravasio · PUC, Chile
Simon Scheuring · Weill Cornell, USA
Ivan Sipirán · Universidad de Chile, Chile
Justin Taraska · NIH, USA
Steffen Hartel · Universidad de Chile, Chile



David Arancibia-Altamirano & Jesús Juárez
Center for Integrative Biology, U. Mayor
Laboratorio de Células Troncales y Biología del Desarrollo, U. Chile

Join the Chilean Scientific Imaging community in Valdivia for two days of cutting-edge science, collaboration and innovation



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2nd Annual Meeting SoChIC

Chilean Scientific Imaging Society

06-07 November, 2026

Universidad Austral de Chile - Hotel Puerta del Sur

PROGRAM

Organized by:
Sebastian Brauchi, *U Austral de Chile*
Mauricio Cerda, *U-Chile*
Verónica Eisner, Pontificia Universidad Católica de Chile
Steffen Härtel, U-Chile

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Schedule SoChIC 2026

Friday 06 November, 2026 Auditorio Esteban Rodriguez, FacMed, UACh	
08:30 - 08:45	Accreditation
08:45 - 09:00	Welcome & Introduction
Scientific Session 1: Biology of Organelles / Chair: Sebastian Brauchi	
09:00 - 09:30	**Karin Busch,** University of Münster, DE *Rewiring of ATP Synthase and Cristae After Neuronal Stimulation*
09:30 - 10:00	**Valentina Parra**, Facultad de Ciencias Químicas y Farmacéuticas, Universidad de Chile, CL *The Mitochondria–Lipid Droplet Axis in Chronic Metabolic Diseases*
10:00 - 10:30	**Ginny Farias,** Utrecht University, NL *Shaping the Axonal Proteome*
10:30 - 11:00	**Poster Session & Coffee Break**
Scientific Session 2: Image Analysis in Biomedicine/ Chair: Mauricio Cerda	
11:00 - 11:30	**Steffen Härtel**, ICBM, Faculty of Medicine, University of Chile, CL *Medical Imaging & Image Processing from the Southern Cone*
11:30 - 12:00	**Ivan Sipiran**, DCC, Facultad de CIencias Físicas y Matemáticas, Universidad de Chile, CL *Shape as Phenotype: 3D Geometry Processing for Biomedical Imaging*
12:00 - 13:00	**Student Imaging Competition "My Image in 3 minutes" / Chair: Verónica Eisner**
LUNCH	
Scientific Session 3: Single Molecule Imaging/ Chair: Juan Pablo Castillo	
15:00 - 15:30	**Nelson Barrera,** Pontificia Universidad Católica de Chile, CL *Unraveling the structure and function of membrane channels*
15:30 - 16:00	**Christian Wilson,** Facultad de Cs Químicas y Farmacéuticas, Universidad de Chile, CL *Single molecule studies of chaperone proteins with optical tweezers*
16:00 - 16:30	**Simon Scheuring**, Weill-Cornell Medicine, US *Single-Molecule Kinetics and Single-Molecule Structural Biology by High-Speed Atomic Force Microscopy*
16:30- 19:00	**Poster Session & Coffee Break**
19:00 -20:00	SoChIC Business Meeting - Members only FacMed, UACh

Saturday 07 November, 2026 Auditorio Esteban Rodriguez, FacMed, UACh	
Scientific Session 4: Imaging Cell Physiology / Chair: Ginny Farias	
09:30 - 10:00	**Tomas Falzone,** Universidad de Buenos Aires, AR *Imaging the Neuronal Highway: Visualizing tau mediated effects on polarization and transport*
10:00 - 10:30	**Juan Pablo Henríquez,** Facultad de Medicina, Universidad Austral de Chile, CL *The Shape of Change at the NMJ: a dyna-morph approach*
10:30 - 11:00	**Poster Session & Coffee Break**
Scientific Session 5: Cell Biology / Chair: Cristina Bertocci	
11:00-11:30	**Teresa Giraldez**, Universidad de la Laguna, Tenerife, ES *Rules for nanoscale ion channel coupling revealed by super-resolution microscopy*
11:30-12:00	**Oscar Cerda**, ICBM, Faculty of Medicine, University of Chile, CL *Imaging the Mechanome: Calcium signaling and Voltage Shifts Driving Focal Adhesion Turnover*
12:00-12:30	**Justin Taraska**, National Institutes of Health, US *Imaging the structure of the plasma membrane with light and electron microscopy*
12:30-13:30	*¿Cómo reaccionamos ante los drásticos recortes en la financiación destinada a equipos científicos avanzados y becas internacionales? - How do we react towards severe cutting of funding for advanced scientific equipment and international scholarships ?* *Steffen Härtel, President SoChIC, chair* *Tomas Falzone, Universidad de Buenos Aires, AR* *Ignacio Velasquez, Microx - ZEISS* *Juan Pablo Segal, Galenica - LEICA* *Valentina Parra, U-Chile*
Official Photo Session of all Participants & LUNCH	
Scientific Session 6: Structural Imaging / Chair: Víctor Castro-Fernandez (a confirmar)	
15:00-15:30	**Crina Nimigean**, Weill-Cornell Medicine, US *Structural Mechanism of PIP2 inhibition of rod CNG channels*
15:30-16:00	**Alessio Accardi**, Weill-Cornell Medicine, US *Activation and inhibition of the TMEM16F dual function ion channel and lipid scramblase*
Scientific Session 7: Mechano Biology / Chair: Oscar Cerda	
16:10-16:40	**Andrea Ravasio,** Institute for Biological and Medical Engineering, Pontificia Universidad Católica de Chile, CL *Resolving an elusive connection: the mechanical coupling of cell-matrix and cell-cell adhesions*
16:40-17:10	**Juan Pablo Castillo,** Centro Interdisciplinario de Neurociencia de Valparaiso, CL *Life and death of touch receptor neurons in C. Elegans*

17:10-17:40	**Cristina Bertocci**, Institute for Biological and Medical Engineering, Pontificia Universidad Católica de Chile, CL *Imaging Alternative Routes of Force Transmission in Health and Cancer Progression*
17:40-18:00	**Coffee Break**
Scientific Session 8: New Topics	
18:00-18:30	**Fernan Federici,** Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, CL *Open Source Hardware for Low-Cost Microscopy: Applications in Education and Research*
18:30-19:00	**Judit Lisoni,** Facultad de Ciencias, Universidad Austral de Chile, CL *Correlating imaging with synthesis and functionality in lithium-ion battery electrodes: Fractal nanoarchitectures as a case study.*
19:00-19:15	**Closing Remarks**
20:30 - 22:00	**Social event & Dinner**

- **Registry and oral presentations are located at *Auditorio Esteban Rodriguez*, FacMed, UACh.**
- **Poster suggested dimensions: 80cm x 120cm (vertical layout).**
- **Meal locations will be informed at registration.**
- **Social events for speakers and poster presenters is held at Puerta del Sur Hotel.**

BEST POSTER

Best Poster / Mejor póster
Jennifer Salazar-Tirado
"Characterization of blood-brain barrier repair after penetrating traumatic brain injury in Drosophila melanogaster"
Honorable Mention / Mención honrosa:
Daniel Cárdenas Yunge
"BV2 Microglial Cells and Glucose Metabolism Alterations in the Presence of Alzheimer's Amyloid-Beta Peptide"
Carmen Gloria Lemus
"Quantitative light-sheet microscopy and mathematical modelling reveal spatially patterned deformation and topological remodeling during annual killifish epiboly"

BEST SCIENTIFIC IMAGE

Best Image / Mejor imagen (en 3 minutos):
Gonzalo Barrientos
"Electron tomography Resolution assesment for 3D ultrastructural studies of subcellular nanodomains"
Honorable Mention / Mención honrosa:
Gonzalo Quiñones-Perés
"Cryo-EM Structure of a Giant 24-Subunit Assembly of Phosphoenolpyruvate Synthetase from the Mesophilic Archaeon Methanococcus maripaludis"
Dilan Martínez-Torres
"Three-dimensional single-cell atlas of steatotic liver disease-associated hepatocellular carcinoma links bile acid flux defects to tumorigenesis"

Accepted Poster Contributions (Abstracts)

1. Open-source Raman Spectroscopy-Machine learning workflow for the recognition of FeSO4 in biological relevant media

Authors: Emerson Durán (a,b), Tobias Wenzel (b)

Affiliations:

a) Universidad Santo Tomás

b) Universidad Católica de Chile

Abstract:

Raman spectroscopy is used to identify molecular vibrational fingerprints through inelastic light scattering in organic and inorganic samples. Identifying a sample by visual inspection can be challenging due to weak signal and spectral overlap, which can lead to mistakes, misinterpretations or bias in the sample recognition. This is a problem especially when the sample is diluted in complex media at low concentrations, as when Raman spectroscopy is used to monitor reactions products or interactions between active molecules and bacteria or 2D/3D cell cultures.

Machine learning has been used with Raman spectroscopy to create models trained to recognize specific spectral patterns in different contexts, but generally the Raman workflow (hardware and software) is obtained from proprietary systems that have an optimized better signal, are validated and well calibrated, but expensive and not very flexible. In this study, an open-source Raman-machine learning workflow was tested with ferrous sulphate as an experimental model.

Ferrous sulphate is a widely used oral treatment for iron-deficiency anemia and its interaction with gastric and intestinal fluids is differentiated and plays a crucial role in the gastrointestinal adverse effects of the supplement and its absorption in the organism. Ferrous sulphate alone and pharmaceutical formulations of it (tablets and oral drops) were used in this study to test the capability of the open-source Raman-machine learning workflow to detect it even in the presence of different excipients and at low concentrations in different biologically relevant liquid media (water, PBS, HCl) in the context of iron supplementation studies.

The results of this study can help to promote open-source tools, reducing cost barriers for these complex studies and may support future applications such as in vivo spectral recognition of molecules and machine learning based spatial-spectral identification of molecular signatures in hyperspectral images.

2. Three-dimensional morphological characterization of individual astrocytes from GFAP confocal images using synthetic training data

Authors: Eugenio A. Díaz Urbina (1), Pablo Irarrázaval (1,2)

Affiliations:
(1) Institute for Biological and Medical Engineering (IIBM), Pontificia Universidad Católica de Chile.
(2) Department of Electrical Engineering, Pontificia Universidad Católica de Chile.

Abstract:
Astrocyte morphology changes with injury and disease, but characterizing individual cells in three dimensions from GFAP immunofluorescence remains difficult: GFAP reveals only a sparse cytoskeletal backbone, neighbouring processes overlap at different depths, and the confocal slab truncates many arbors. These ambiguities require prior knowledge of astrocyte form, hard to encode in tracing rules, learnable by a network from examples. Supervised training, however, requires 3D instance annotations of GFAP-positive astrocytes. Tracing them by hand is prohibitively slow and adds annotator bias and inter-rater variability to ground truth used for training and evaluation; none is public.

We therefore addressed two problems. First, we defined a pipeline for morphological characterization from confocal images: three 3D U-Nets detect processes, vote for soma centres and predict soma-directed flow; selection and assignment yield one characterized cell per soma. Second, we implemented a generator of astrocyte structural phantoms with exact cell identity, modelling soma, branching backbone, calibre, territory and slab clipping, with labelled neuropil context and image formation.

Because geometry and ownership are known exactly, image formation can vary with cells fixed. We tested it end to end on 30 unseen synthetic geometries under eight acquisition conditions. At 41 µm spacing, reducing staining contrast to one eighth of calibration lowered true-arbor recall from 70.8% to 57.7%; soma recall stayed 100%, no cells fused, wrong-cell ownership near 1%. At 22 µm spacing, arbor recovery declined first; identity degraded only at lower contrast, when missed somata increased fusion.

In a preliminary sim-to-real test we applied it to ten hippocampal development stacks. Cells characterized per field rose from 7.2 to 14.5, and 33 of 89 boxes from an independent detector lay within 6 µm of a soma. The generator therefore provides training data and a test bed separating failures of cell identity from failures of morphological recovery.

Keywords: astrocytes, GFAP, confocal microscopy, 3D morphological characterization, synthetic training data.

3. Three-dimensional single-cell atlas of steatotic liver disease-associated hepatocellular carcinoma links bile acid flux defects to tumorigenesis

Authors: Cristian Perez-Gallardo 1,2, Dilan Martinez-Torres 1,2, Sebastián Fuller-Vargas 3, Karen Almendras-Durán 1,2, Valeria Candia 1,2, Felipe Aguilera 3, Hernán Morales-Navarrete 4, Fabian Segovia-miranda 1,2.

Affiliations:

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2 Grupo de Procesos en Biología del Desarrollo, Faculty of Biological Sciences, UdC, Chile.

3 Departament of Biochemistry and Molecular Biology, Faculty of Biological Sciences, UdC, Chile.

4 Bio-Cheminformatics research group, Universidad de las Américas, Ecuador.

Abstract:

Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most common liver disease worldwide and progresses through various stages that can culminate in hepatocellular carcinoma (HCC). Previous studies found that bile canaliculi (BC), a bile-carrying microscopic network, are altered in early MASLD, potentially leading to accumulation of bile acids (BA), known carcinogens. However, structural organization of BC in advanced stages (HCC), and its possible contribution to tumour development, remain poorly understood. Could BC alterations promote MASLD-HCC through BA accumulation?

Methods: A MASH-HCC mouse model was generated by neonatal streptozotocin injection and Western diet. Serum metabolism, hepatic gene expression, and BA levels were assessed by biochemical assays. Liver tissue was reconstructed in 3D across MASLD using multiphoton microscopy and deep-learning methods. BA-related transcriptional changes were analysed with public RNA-seq from human HCC. MASH-HCC mice were treated with the drug A4250 for 8 weeks to lower systemic BA prior HCC onset; then tumour number, size and phenotype were analyzed.

Results: The MASH-HCC model exhibited hyperglycemia, inflammation, tumour formation and elevated total BA. 3D atlas revealed altered BC across MASLD and tumour-adjacent tissue disconnection, while perfusion is retained. Also, there was upregulation of BA-related genes and elevated BA in tumours. A4250 treatment reduced tumour number and led tumours to a less aggressive phenotype. The BA-accumulation signature was present in human HCC.

Discussion: BC disconnections isolates tumours, allowing BA influx without outflow, linking BC disruption to local BA accumulation and tumorigenesis. This is backed by the effects of lowering systemic BA, and the conserved BA signature in human HCC. This provides new insights into the pathophysiology of MASLD-HCC and suggests potential therapeutic strategies.

Funding: FONDECYT 1251048, ANID 2024-21241153

4. Hepatocytes divide symmetrically to partition polarized membrane domains and expand the bile canalicular network during liver regeneration

Authors: Dilan Martinez-Torres 1,2, Alejandro Cobo 3, Valeria Candia 1,2, Cristian Perez-Gallardo 1,2, Hernán Morales-Navarrete 3, Fabián Segovia-Miranda 1,2.

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3 Bio-Cheminformatics Research Group, Universidad de Las Américas, Quito, 170504, Ecuador

Abstract:
Introduction: Epithelial cells contain apical, basal, and lateral membrane domains, whose partitioning during division shapes tissue morphogenesis. In simple epithelia, division plane orientation determines whether inheritance is symmetric promoting lumen elongation or widening or asymmetric, restricting a domain to one daughter cell. In the liver this remains unresolved: hepatocytes are multipolar, with multiple apical surfaces forming the bile canaliculi (BC) and multiple basal surfaces facing the sinusoids. Current models come from in vitro studies proposing asymmetric inheritance, contrasting our observations in 3D reconstructions. Here, we dissect how polarized membrane domains are partitioned during hepatocyte division within the liver tissue.

Materials and Methods: We used liver regeneration after 70% partial hepatectomy in mice as a model of tissue morphogenesis. Hepatocytes were 3D reconstructed from immunofluorescence and multiphoton deep-tissue imaging using AI-assisted segmentation. We performed a morphometric characterization of hepatocytes in interphase, metaphase, anaphase/telophase, and cytokinesis. We ran simulations spanning all possible orientations of the division plane and compared them with the real data from 3D reconstructions. FIB-SEM resolved the fate of the BC at cytokinesis.

Results: Simulations predicted symmetric membrane-domain partitioning regardless of division angle, as confirmed by single-cell 3D tissue reconstructions. FIB-SEM further revealed new BC branches forming between sister cells and expanding the BC network.

Discussion: Our findings establish that hepatocyte division is intrinsically symmetric, showing how coordinated cell division builds complex 3D tissue architecture. Yet symmetric division alone cannot explain ordered, directional BC network growth: cell polarity must also be coordinated to orient divisions in space, a question for future studies.

Acknowledgements / Funding: FONDECYT-1251048, ANID-Subdirección de-Capital Humano/Doctorado-Nacional/2023-21230215

5. Mitochondrial nucleoid distribution and dynamics in hiPS-derived neurons carrying disease-causing POLG mutants

Authors: Catalina Muñoz Muñoz 1, Daniel Lagos 2, Nathalie Garrido 1, Constanza Vasquez 3, Matías Berasain 3, Denisa Hathazi 2, Josefa Macuada 1, Sofía Magini 1, Cesar Vásquez-Trincado 1, Paula Amado 1, Mauricio Cerda 3, Rita Horvath 2, Jorge Campusano 1, Verónica Eisner 1

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(3) Universidad de Chile, School of Engineering, Santiago, Chile

Abstract:
Introduction: Mutations in POLG cause mitochondrial DNA (mtDNA) damage, leading to neurological alterations and Alper syndrome. MtDNA is packed in nucleoids and encodes the enzymes involved in oxidative phosphorylation. Nucleoids are distributed in a semi-regular manner within the mitochondrial network, and this organization is important for both the local maintenance of mitochondrial function and the removal of damaged. However, it remains unclear how nucleoids are positioned at specific sites within mitochondria. Although nucleoids have been shown to exhibit intramitochondrial dynamics, these features -particularly in neurons- have not been well characterized as it requires high spatial and temporal resolution techniques, as well as a standardized protocol for the visualization and quantification of intramitochondrial nucleoids and their dynamics. My project aims to characterize the intramitochondrial distribution and dynamics of nucleoids in neurons and to determine whether these processes are altered in models harboring POLG mutations.

Methods: Neuronal cultures derived from induced pluripotent stem cells from patients with POLG mutations and controls were used. Mitochondria and nucleoid were labeled, and time-series images were acquired using an Airyscan confocal microscope. Then, videos were analized with Nuctrack pipeline for nucleoids dynamics quantification.

Results: Our data show that neurons with different POLG mutations exhibit opposing nucleoid dynamics associated with the severity of the model.
Conclusions: Nucleoid dynamics in neurons have not been described before. Our data may contribute to the understanding of factors involved in the disease.

Funding: Scholarship "Doctorado nacional ANID 21222104", "FONDECYT Regular 1231556" and "FONDECYT Regular 1231557"

6. Temperature protects mechanosensory neurons and is associated with changes in calcium and in the distribution of the MEC-4 channel in C. elegans

Authors: Victoria Collio, Juan Pablo Castillo, Andrea Calixto.

Affiliations:
Universidad de Valparaíso, Facultad de ciencias.

Abstract:
The mec-4d mutation is a gain of function variant of the MEC-4 mechanosensitive channel that causes degeneration of C. elegans touch receptor neurons (TRNs). We have observed that increased developmental temperature remarkably prevents degeneration through an unknown mechanism. We evaluated in vivo how temperature affects axonal morphology, intracellular calcium, and the distribution of MEC-4 channel clusters, using epifluorescence, confocal and two-photon microscopy. Control and mec-4d worms were grown between 15 and 25°C. The axonal morphology of the AVM neuron was classified into five categories by epifluorescence. Basal calcium was estimated as the GCaMP6m/TagRFP fluorescence ratio using confocal microscopy, in WT and mec-4d animals. Calcium dynamics after mechanical stimulation were recorded in WT individuals, fitting the fluorescence decay to a single exponential. Recordings in mec-4d mutants were not measured. Fluorescent puncta found in animals expressing MEC-4::mNeonGreen were quantified in WT and mec-4d animals. At 72 h, intact axons in the mec-4d mutant were 0% at 15°C, 60% at 23°C, and 95% at 25°C. In WT animals, basal calcium was higher at 15°C than at 25°C. The time constant (Tau) of the intracellular calcium decay after stimulation decreased modestly as temperature increased. Mechanosentive channel puncta intensity and density at 25°C is higher than at 15°C. In mec-4d mutants, basal calcium did not differ significantly between 20 and 25°C, and at 25°C they showed fewer puncta, lower fluorescence intensity, and greater interpuncta distance than controls. Among protected axonal categories, puncta did not vary. In conclusion, the studied variables cannot explain the high degree of observed protection, which mechanisms is still under investigation.

7. Use of tissue cytometry with nuclear segmentation to quantify sperm-activated NETotic neutrophils in mammals

Authors: Rodrigo Rivera-Concha 1,2,3, Marion León 4, Nikol Ponce-Rojas 5,6, Aurora Prado-Sanhueza 1,6, Pamela Uribe 1,7, Anja Taubert 8, Carlos Hermosilla 8, Raúl Sánchez 1,9 and Fabiola Zambrano 1,9

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9 Departamento de Ciencias Preclínicas, Facultad de Medicina, Universidad de La Frontera.

Abstract:
Upon activation, neutrophils perform three distinct functions: phagocytosis, the degranulation of antimicrobial substances into the extracellular space, and the release of neutrophil extracellular traps. Measuring the expansion of the nuclear area in activated neutrophils to release neutrophil extracellular traps has become essential for demonstrating early neutrophil activation and has become the gold standard procedure. In this study, we present an automated method for measuring nuclear area expansion in two different mammalian species: canines and bovines. In both species, neutrophils were isolated from peripheral blood and co-incubated with fresh spermatozoa for up to 120 minutes in the case of canines, and with recently thawed cryopreserved spermatozoa for up to 240 minutes in the case of bovine neutrophil–sperm co-cultures. We acquired fluorescence images using the TissueFAXS i Plus Cytometry System and subsequently analysed using machine learning algorithms included in the StrataQuest v.7.0 software. A nuclear mask was used for instance segmentation, and at least 2.0×10^4 neutrophil nuclei were analysed. The images show the release of extracellular neutrophil traps following activation by sperm in both species, as evidenced by the colocalisation of neutrophil elastase and DNA staining. Neutrophil nuclei had already expanded by 15 minutes and were detected up to 120 minutes in both species. Analysis using nuclear segmentation showed that the datasets generated for both species were reliable and consistent with previously published methods. The method was developed as an automated alternative for measuring the expansion of neutrophil nuclear area in different species.

8. A virtual parasitology microscopy platform for Chilean parasitology samples

Authors: Martín Saavedra 1,2,3, Alan Oyarce 1, Jorge Jara 2,3, Steffen Härtel 2,3,4,5, Francisca Valdés 2,3,6, Armin Araya 1, Karina Palma 2,3,6, Paulina Ruiz 3, María Isabel Jercic1

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Abstract:
In an increasingly globalized world, migration and the emergence of infectious agents stress the need to strengthen the capacity of healthcare professionals to identify and respond to parasitic diseases. Continuous training and monitoring becomes essential to address these challenges; however, conventional training methods rely on in-person activities and physical slides for microscopy, constraining access in geographically extensive countries such as Chile. To address this limitation, a Virtual Parasitological Microscopy Repository (RPMV) is being developed to both preserve parasitological collections through digitization and provide remote access to microscopy images for assessment and professional training purposes.

We have acquired whole slide image datasets of filariae, chagas, trypanosoma brucei, trypanosoma cruzi, among others; also including their taxonomic information. We have used the repository and samples in 2 training courses in northern and central Chile, and applied basic, preliminary usability tests. So far, these tests yield positive user feedback suggesting that the platform has the potential to support parasitological training and professional development. By preserving valuable specimens and making them accessible regardless of geographical location, we envision a promising tool for expanding training opportunities, reducing geographical barriers, and strengthening diagnostic preparedness for emerging infectious threats.

Funding: ANID-ISP, Concurso Convenio MINCIENCIA 2026.1.3.

9. Electron tomography resolution assessment for 3D ultrastructural studies of subcellular nanodomains

Authors: Gonzalo Barrientos 1, Verónica Eisner 1

Affiliations:
1 School of Biological Sciences, Pontificia Universidad Católica de Chile

Abstract:
Introduction: To sustain cellular function, ATP is synthesized through oxidative phosphorylation, which takes place at mitochondrial cristae. Cristae have diverse morphologies and different nanodomains. Yet, their influence on mitochondrial bioenergetics remains elusive. Electron tomography remains the gold standard for 3D ultrastructure analysis. In this work, we established the framework to study mitochondrial cristae nanodomains with this technique.

Materials and Methods: Mouse embryonic fibroblasts (MEF) WT were processed for electron tomography. Thin sections were obtained with theoretical thicknesses from 150 to 300 nm. Tilt-series (±60° range, 1° step) were acquired at TALOS microscope (200 kV acceleration voltage, 22000X magnification, 2048×2048 detector array). Tomograms were aligned with Inspect 3D and IMOD. Global anisotropic resolution was determined by the Noise-compensated Leave One Out method in Bsoft. Local isotropic resolution was determined by the Even-Odd method in MonoTomo.

Results: Theoretical thickness did not translate into consistent values of experimental thickness. When comparing sections with low (180 nm) and high (350 nm) experimental thickness, the former showed enhanced global isotropic resolution than the latter (7 vs. 12 nm). In contrast, the difference between lateral and axial resolution did not change with increasing sample thickness, maintaining an anisotropy value around 1 nm.

Discussion: This analysis shows that 180 nm sections display enough resolution to detect mitochondrial cristae nanodomains, such as cristae junctions, fenestrations, and interconnections. However, sections over 300 nm may not be suitable for this. Thus, this work establishes the framework for further studies that aim to determine how nanoscopic structures influence mitochondrial bioenergetics.

Acknowledgments / Funding: We thank the Advanced Microscopy Facility (PUC) for TEM sample preparation. This research was supported by Fondecyt 1231557.

10. Gαi2 Functions as a Molecular Compass Coordinating Cytoskeleton Networks During Cranial Neural Crest Cell Migration in Xenopus

Authors: L-Mariana Tovar 1,2, J-Ignacio Leal 1, Jossef Guajardo 1, Teresa Caprile 2, Marcela Torrejón 1.

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2 Axonal Guidance Laboratory, Department of Biochemistry and Molecular Biology, Universidad de Concepción, Concepción, Chile.

Abstract:
Introduction: Cell migration relies on the integration of chemical and mechanical cues that coordinate the actin and microtubules (MT) cytoskeletal networks to drive cell polarization, protrusion extension, adhesion dynamics, and cell body translocation. We previously established that Gαi2, a heterotrimeric G protein subunit, regulates MT dynamics during cranial neural crest (CNC) cell migration in Xenopus. Here, we investigate how Gαi2 regulates cytoskeletal organization and dynamics to support directed CNC cell migration.
Materials and Methods: Live-cell imaging and immunofluorescence (IF) were used to evaluate cytoskeletal dynamics and peripheral localization of tubulin, EB1, CLIP170, actin, Arp2/3 in control and Gαi2 loss-of-function CNC cells. Protein-protein interactions between Gαi2 an the +TIPs EB1 and CLIP170 were assessed by co-immunoprecipitation (Co-IP), proximity ligation assays (PLA), and molecular docking.
Results: Live-cell imaging revealed that Gαi2 loss-of-function disrupts MT dynamics and spatial organization, altering MT polymerization velocity, cortical capture events, and MT orientation. IF further confirmed an abnormal peripheral distribution of EB1, CLIP170, and tubulin. Importantly, Co-IP, PLA, and docking assays demonstrated that Gαi2 interacts with the +TIPs EB1 and CLIP170, suggesting that Gαi2 associates with MT through this +TIPs.
Discussion: These findings suggest that Gαi2 interacts with the +TIPs, EB1 and CLIP170, to regulate MT orientation and dynamics, thereby to the coordination between MT and actin networks. We propose that Gαi2 plays a role in coordinating cytoskeletal networks, and that its loss disrupts this crosstalk, leading to altered cell morphology and impaired directed CNC cell migration.

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11. Gαi2 Acts as a Mechanotransducer Regulating Cellular Tension and Neural Crest Migration

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Abstract:
Neural crest (NC) cells are characterized by their extensive migration during embryonic development. To migrate successfully through complex embryonic environments, NC cells must adapt to changes in mechanical stiffness by engaging mechanosensitive pathways that regulate cellular tension, adhesion, and contractility. While the G-protein subunit Gαi2 is known to control cytoskeletal dynamics and focal adhesion stability, its role in mechanosensing remains unclear. Here, we investigate the function of Gαi2 as a potential mechanotransducer during cranial neural crest (CNC) cell migration in Xenopus.

Using a morpholino-based loss-of-function approach, we analyzed Xenopus CNC explants cultured on fibronectin-coated substrates. Cellular responses to mechanical cues were evaluated using Traction Force Microscopy (TFM) on polyacrylamide gels, stiffness gradient assays to assess durotaxis, and confocal immunofluorescence.

Our findings indicate that Gαi2-depleted CNC cells fail to appropriately adapt their morphology to changes in substrate stiffness and exhibit severely impaired durotaxis. Furthermore, loss of Gαi2 function also results in a significant increase in cellular traction forces, particularly at leading-edge protrusions. This elevated traction is accompanied by increased levels and mislocalization of phosphorylated myosin (p-myosin) within these protruding regions. In addition, Gαi2-depleted cells display a marked reduction in the nuclear YAP localization, suggesting a possible disruption in mechanosensing signaling.

Together, these results support a role for Gαi2 as a mechanotransducer during CNC cell migration contributing to the integration of mechanical cues with cellular adaptability and the regulation of the adhesion and traction machinery.

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12. Relative Movement Plugin: A Fiji Plugin for Quantifying Relative Motion of Subcellular Structures

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Abstract:
In cell biology, organelles like mitochondria have internal substructures such as nucleoids that can actively move within the organelle while the organelle itself also displaces within the cell. Quantifying this internal motion requires separating it from the container's movement, a problem known as relative movement estimation. The SCIAN laboratory at Universidad de Chile developed a MATLAB pipeline that addresses this problem in two scenarios: a 1D case for tubular organelles, based on skeletonization and geodesic distance, and a 2D case for polygonal cell tissues, based on Delaunay triangulation and barycentric coordinates. However, MATLAB requires a commercial license and the pipeline lacks a graphical interface, limiting its adoption by the laboratories and research community.

This work presents the design, implementation, and validation of Relative MovementPlugin, a Fiji plugin written in Java that ports this pipeline to an open-source environment. The plugin integrates two independent graphical interfaces built with Swing: NucTrack for the 1D case and CellTrack for the 2D case. Validation consisted of a quantitative comparison of the plugin outputs against the original MATLAB algorithm using real fluorescence microscopy data as ground truth.

Results show that the plugin correctly replicates the original algorithm in both scenarios. In the 1D case, computed values match MATLAB to six or seven decimal places; residual differences in a fraction of frames arise from non-deterministic tie-breaking in MATLAB's sorting routine, which does not affect biological interpretation. In the 2D case, outputs are numerically identical to the original. Usability evaluation with users of varying computational experience yielded SUS scores in the good-to-excellent range for both interfaces, indicating the tool is accessible to researchers without programming expertise.

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13. Cryo-EM Structure of a Giant 24-Subunit Assembly of Phosphoenolpyruvate Synthetase from the Mesophilic Archaeon Methanococcus maripaludis

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Abstract:
Phosphoenolpyruvate synthetase (PEPS) catalyzes the first step of gluconeogenesis through the coordinated action of three domains: an N-terminal nucleotide-binding domain (NBD), which binds and hydrolyzes ATP; a central phosphohistidine carrier domain (CD); and a C-terminal pyruvate-binding domain (PBD), where phosphoenolpyruvate synthesis takes place. While bacterial PEPS exists mainly in a monomer-dimer equilibrium, hyperthermophilic archaeal PEPS can form higher-order assemblies of up to 24 subunits. However, whether if mesophilic archaeal PEPS exhibits this complex equilibrium remains unexplored. To address this question, we characterized the oligomeric equilibrium and determined the structures of PEPS from the mesophilic archaeon Methanococcus maripaludis (MmPEPS).

SEC-MALS and mass photometry revealed an oligomeric equilibrium comprising monomers, dimers, tetramers, and a 24-subunit assembly, with a KD below 5 nM. Cryo-EM structures of apo and Mg2+-pyruvate-bound MmPEPS were determined at 3.0 and 2.6 Å resolution, respectively. CryoSPARC was used for 2D and 3D classification and map refinement with octahedral symmetry, while atomic models were refined using Phenix and Coot, and atomic interaction interfaces were analyzed using PISA.

Both structures revealed an octahedral 24-subunit assembly mediated exclusively by hydrophobics interactions involving PBD residues in C2 and C4 symmetry axis fold. In contrast, the CD and NBD regions show low or nearly absent local resolution, consistent with their high flexibility. In the Mg2+-pyruvate-bound structure, pyruvate interacts with an octahedrally coordinated Mg2+ ion within the PBD. We report the first structures of MmPEPS, including a Mg2+-pyruvate-bound state, and show that MmPEPS exists in a complex oligomeric equilibrium. These structures provide a framework for understanding the molecular organization of higher-order PEPS assemblies and their potential role in regulating gluconeogenesis in mesophilic Archaea.

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14. Quantifying Nuclear Receptor Oligomerization and Heterocomplex Formation by Number & Brightness and Cross-Correlated Brightness Analysis

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Abstract:
Steroid hormone nuclear receptors (NRs) are ligand-activated transcription factors whose biological activity depends on dynamic changes in their molecular organization and interactions with chromatin. Although traditionally described as dimers, recent imaging and structural studies reveal a more complex, receptor-specific quaternary organization, including higher-order oligomers and receptor–receptor heterocomplexes. Here, I describe fluorescence fluctuation imaging approaches to quantitatively characterize NR oligomerization and heterocomplex formation in living cells. Number & Brightness (N&B) analysis derives molecular brightness from temporal fluorescence fluctuations recorded in raster-scanned image sequences.

Calibration against the brightness of a monomeric receptor allows estimation of the average number of fluorescently labelled receptor subunits contributing to each fluorescent particle, providing a population-average measure of oligomer size with spatial resolution. Using N&B, Presman et al. (PNAS 113:8236, 2016) demonstrated that glucocorticoid receptor (GR) predominantly forms dimeric species in the nucleoplasm, changing to tetrameric assemblies at hormone response elements (HREs). More recently, we showed that mineralocorticoid receptor (MR) forms tetramers in the nucleoplasm and higher-order oligomers upon HRE binding (Fettweis et al., Prot Sci 33, 2024). Structural studies further support the multivalent organization of steroid receptor ligand-binding domains (Alegre-Martí et al., NAR 53, 2025).

In parallel, cross-correlated brightness analysis quantifies correlations between fluorescence fluctuations of two differently labelled receptor populations. Positive cross-brightness (Bcc) indicates coupled fluctuations between the two receptor species, providing a quantitative measure of their molecular association. We have applied this approach to ligand-dependent heterocomplex formation between MR and GR, and between MR and progesterone receptor (PR). Thus, N&B and Bcc provide complementary information: N&B estimates the average oligomeric size of each receptor population, whereas Bcc identifies molecular association between distinct receptor species. Together, these approaches provide a quantitative framework for resolving the molecular organization and heteromeric interactions of NRs in living cells.

15. Spectral resolution of closely spaced fluorescent signals using Spectral Multiplex on the ZEISS LSM 990

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Abstract:
The simultaneous identification of multiple cellular components using immunofluorescence is one of the most versatile tools in research. However, this technique is often limited by the overlap of closely spaced emission spectra and the presence of autofluorescence, which hinder the accurate discrimination of fluorescent signals and limit the number of fluorophores that can be analysed simultaneously. Drawing on the high-end capabilities and tools offered by the ZEISS LSM 990 confocal microscope at the Southern BioImaging Platform (SBIP), the aim of this study was to determine whether a spectral multiplexing approach enables the acquisition and separation of spectrally close fluorescent signals.

To carry out these experiments, three proteins specific to the tracheal submucosal gland cells of the main airways of mice were immunodetected. For these experiments, three secondary antibodies conjugated to peak emission wavelengths of 565, 603 and 617 nm were used. The fluorescent signals were acquired using the spectral detector of the ZEISS LSM 990 microscope. The generation of “spectral fingerprints” and the use of “spectral unmixing” enabled the identification and separation of the fluorescent emission signals from the different fluorophores. In addition, autofluorescence could also be isolated and characterised as an independent spectral component, thereby reducing its contribution to the signals of interest.

In conclusion, the Spectral Multiplex tool on the ZEISS LSM 990 is an effective strategy for resolving spectrally close fluorescent signals obtained from multi-colour immunofluorescence. We believe that its capabilities in spectral acquisition, spectral fingerprint generation and spectral unmixing will facilitate the simultaneous identification of multiple fluorescent markers in complex samples such as tissues, thereby expanding the possibilities for multi-parametric analysis in confocal microscopy.

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16. MoSAIC resolves the epidermal puzzle to reveal that nitrate modulates stem cell development in leaves

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Abstract:
Leaves are essential for plant survival. In the epidermis, stomata regulate gas exchange, while pavement cells provide structural support. A population of diverse stem cells, collectively known as the stomatal lineage, gives rise to stomata and a substantial proportion of pavement cells, making its development crucial for leaf function. Although stomatal lineage development is well studied, how environmental signals regulate this process remains largely unexplored. Nitrate, a major nitrogen source with important agronomical relevance, influences leaf development in the model plant Arabidopsis thaliana. However, its effects on stomatal lineage development remain unknown. Studying these processes relies on live imaging and fluorescent reporter lines, which are technically demanding and time-consuming. Alternatively, cell types can be assigned manually based on morphology and cellular context, but this limits the number of cells analyzed and introduces observer bias. Here, we validate MoSAIC (Morphometric analysis and Shape-based Automated Identification of Cell types), an unsupervised clustering-based tool for the automatic identification of epidermal cell types. Similar approaches have been used in other models, but their use remains largely unexplored in plants. Using MoSAIC, we show that nitrate promotes both division and differentiation within the stomatal lineage in Arabidopsis thaliana.

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17. Open Source Epi-Fluorescence Microscopy and Digital Microfluidics for Decentralized Quantitative Analysis

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Abstract:
Environmental monitoring of pathogens in remote and low-resource areas is an ongoing challenge. The current workflow for sample analysis still depends on transportation from the field to laboratories in urban centers, slowing down the response to existing threats, such as the emergence of new diseases. During the past years, multiple tools have been developed to decentralize these monitoring capabilities. However, quantitative assays are still confined to large laboratories given the need for bulky and expensive instruments like microscopes and microfluidics devices. To address this limitation, in this work we present an open and low-cost epi-fluorescence microscope, built with a 3D printed mobile base and accessible optical and electronic materials. We have combined this microscope with other tools in order to allow portable and quantitative analysis of reactions. First, we have coupled this microscope with an active-matrix digital microfluidics device.

This consists of a chip that uses electrowetting-on-dielectric to manipulate numerous microdroplets in a two dimensional lattice, allowing screening of multiple samples at the same time. As a proof of concept, we have used this to visualize individual droplets filled with fluorescent dyes and cell-free reactions. Also, as an alternative for making digital reactions, we have been exploring the use of particle template emulsification to encapsulate reactions into hydrogel based droplets. So far, we have used this to visualize droplets filled with fluorescent proteins to test the imaging capabilities of the microscope. We intend to combine this technique with nanobody based protein capture for portable digital antigen detection in the near future. As a whole, these systems are projected to allow a more decentralized and accessible monitoring for environmental and human health.

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18. BV2 Microglial Cells and Glucose Metabolism Alterations in the Presence of Alzheimer's Amyloid-Beta Peptide

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Abstract:
Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by amyloid-beta peptide (Aß) accumulation and neurofibrillary tangles, with progression increasingly linked to chronic neuroinflammation and metabolic dysfunction. Microglia, the resident immune cells of the central nervous system, play a dual role by exerting both neuroprotective and neurotoxic effects. Transitions between these states are closely associated with metabolic reprogramming, a process known as immunometabolism. In AD, microglia are recruited to amyloid plaques and attempt Aß clearance; however, inefficient degradation leads to sustained activation and chronic release of pro-inflammatory mediators.

A consistent early feature of AD is a reduced cerebral metabolic rate of glucose (CMRGlc), resulting in neuronal energy deficits. To investigate microglial metabolic responses to Aß, we assessed mitochondrial oxidative capacity in BV2 cells by measuring the intracellular redox state (NADH/NAD ratio) using the genetically encoded sensor SoNar. Responses were compared between BV2 monoculture and a co-culture system with primary neurons and astrocytes. Cell viability was evaluated using Hoechst 33342 nuclear staining.

BV2 cells in monoculture displayed an oxidative metabolic profile consistent with previous reports. In contrast, under co-cultured conditions, exposed to Aβ, Aß exposure induced a marked metabolic shift toward a glycolytic, lactate-producing phenotype, characteristic of classical pro-inflammatory (M1-like) activation and reminiscent of the Warburg effect. Despite these metabolic changes, no significant increase in cell death was observed following Aß treatment. However, nuclear morphology analysis revealed a trend toward alterations similar to those observed in the cell death control.

These findings suggest that Aß does not primarily compromise microglial viability but instead drives a persistent dysfunctional state, characterized by glycolytic reprogramming and a pro-inflammatory phenotype. This metabolic shift may contribute to chronic neuroinflammation and disease progression in AD.

19. MIRO1-dependent regulation of mitochondrial nucleoid dynamics in mouse embryonic fibroblasts

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Abstract:
Mitochondrial nucleoids undergo continuous repositioning within the mitochondrial network, yet the mechanisms governing their intramitochondrial trafficking remain poorly understood, in particular, how mitochondrial positioning and organization influence nucleoid dynamics. Mitochondrial movement within the cytoplasm depends on interactions between the outer mitochondrial membrane and cytoskeletal transport machinery; MIRO1 is an outer mitochondrial membrane GTPase involved in interactions with molecular motor adaptors such as TRAK1/2 and linked to mitochondrial membrane organization through the MICOS complex. Here, we investigated whether MIRO1 contributes to mitochondrial nucleoid trafficking.

Mouse embryonic fibroblasts (MEFs) with genetic deletion of MIRO1 (MIRO1KO) and corresponding wild-type (WT) cells were imaged using lattice structured illumination microscopy (L-SIM) to resolve nucleoid dynamics within the mitochondrial network. Mitochondrial morphology and positioning, nucleoid trajectories, instantaneous velocity, spatial exploration, and mean squared displacement (MSD) were quantitatively analyzed.

MIRO1KO cells exhibited a more perinuclear mitochondrial distribution and a lower proportion of elongated mitochondria compared with WT cells. Nucleoids in MIRO1KO mitochondria displayed lower mean instantaneous velocities and a larger proportion of relatively static trajectories. Consistent with this reduced motility, nucleoids explored a smaller fraction of the mitochondrial landscape in MIRO1KO cells. MSD analysis indicated strongly subdiffusive behavior in both conditions, with a higher scaling exponent (a) in WT cells. Interestingly, the apparent diffusion coefficient (D) showed a higher median value in MIRO1KO despite its lower a.

Together, these observations suggest that MIRO1 loss alters both the magnitude and temporal organization of nucleoid displacement. The combination of relatively larger apparent displacements with lower temporal persistence and reduced spatial exploration could be compatible with a loss of coordinated or directed components of nucleoid motion, resulting in a more temporally uncorrelated trafficking regime. These findings support a role for MIRO1 in organizing mitochondrial nucleoid dynamics beyond its established contribution to mitochondrial positioning.

20. Fluorescence imaging reveals the spatial distribution of Cftr-expressing cells in the murine tracheal epithelium

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Abstract:
Cystic fibrosis is the most common genetic disease in humans and is caused by mutations in the anion channel CFTR, disrupting airway surface liquid homeostasis. Identifying the airway epithelial cells that express Cftr protein has remained challenging due to its low abundance and the limited performance of available antibodies. Single-cell RNA sequencing identified pulmonary ionocytes as a rare epithelial population (<1%) accounting for more than 50% of Cftr transcripts. Ionocytes are mainly identified by the transcription factors Foxi1 and Ascl3; however, their co-expression with Cftr protein and the spatial distribution of Cftr-expressing cells within the airway epithelium have not been investigated.

To overcome the limitations of Cftr immunodetection, our laboratory developed a 2HA-Cftr transgenic mouse model that enables detection of endogenous Cftr through an HA epitope. Here, LightField 4D and Airyscan confocal microscopy were used to investigate the spatial distribution of Cftr-expressing cells and Cftr co-expression with Foxi1 and Ascl3 in the murine tracheal epithelium.

Due to the low abundance of Cftr, HA immunodetection required tyramide signal amplification. Whole-trachea LightField 4D imaging revealed a sparse population of Cftr-positive cells distributed throughout the tracheal epithelium. Selected regions were further analyzed by Airyscan confocal microscopy to resolve Cftr-positive cells at higher spatial resolution and assess their co-expression with Foxi1 and Ascl3.

These studies will define the spatial distribution and cellular identity of Cftr-expressing cells, an important question given that airway Cftr expression has historically been attributed to ciliated and club cells and, more recently, associated with pulmonary ionocytes. Determining where Cftr-positive cells are located and which markers they co-express may provide clues to their functional role in airway physiology.

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21. An in singulo fluorimetric approach to study GPHR stoichiometry

Authors: Luka Robeson, Sebastián E. Brauchi

Abstract:
Ion permeating membrane proteins are some of the most complex structures to study in biophysics. They are embedded in lipid environments, in many cases assemble as multimers, are laborious to purify, and, in many cases, almost impossible to crystalize. State-of-the-Art approaches like Cryo-EM allow us to observe their structure with increasing resolution, but they are still inaccessible for a large portion of scientists over the world. Alternative imaging methods achieving single molecule resolution emerge as relevant for probing these crucial macromolecules of the cell membrane. In this work, we have investigated the stoichiometry of the human Golgi pH regulator, GPR89, at the single molecule level. By designing a dual in vitro approach combining fluorophore binding and bleaching assays on a TIRF microscope we will propose a mutimeric assembly. Data processing and specific analysis workflow for information-theory hypothesis testing is proposed.

22. Elevated MRP4 Expression Correlates with Intratumoral Bile Acid Retention and Altered Cell Architecture in Hepatocellular Carcinoma

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Abstract:
Introduction: Metabolic dysfunction-associated steatotic liver disease (MASLD) represents a major risk factor for hepatocellular carcinoma (HCC). Although MASLD is not traditionally classified as cholestatic, our data show that the bile canalicular (BC) network undergoes progressive disruption during MASLD-HCC progression, becoming particularly pronounced in tumors and leading to localized bile acid (BA) retention. At elevated concentrations, BAs exert potent cytotoxicity that compromises tumor cell viability. Consequently, tumor cells must deploy adaptive mechanisms to evade BA-induced cell death. Basolateral efflux transporters, particularly multidrug resistance-associated protein 4 (MRP4), represent a mechanism for hepatic BA extrusion to counteract this toxic accumulation; however, the role of MRP4 in HCC remains unclear. Here, we investigated whether MRP4 serves as a compensatory survival mechanism against BA accumulation in HCC.

Materials and Methods: In a MASLD-HCC mouse model, total BA concentrations were quantified in tumor and adjacent non-tumoral tissues. MRP4 protein levels across MASLD stages and its spatial correlation with atypical BC structures were evaluated by quantitative immunofluorescence. In HepG2 cells, cell proliferation and death under BA treatment were evaluated. We assessed MRP4 levels in tumor and adjacent tissue in public RNAseq datasets.

Results: In the MASLD-HCC mouse model, BA accumulated predominantly within tumors compared to adjacent tissue. Immunofluorescence revealed marked MRP4 upregulation restricted to tumor lesions, where it strongly correlated with atypical canalicular architecture. HepG2 cells demonstrated that high BA levels induced cytotoxic cell death alongside reduced proliferation. Finally, transcriptomic analyses of human MASLD-HCC datasets showed increased MRP4 levels in tumor tissue compared to adjacent regions.

Discussion: The correlation between intratumoral BA accumulation, selective MRP4 upregulation, atypical BC architecture, and BA-induced cytotoxicity in HepG2 cells suggests that MRP4 may act as a compensatory mechanism enabling tumor cells to survive BA overload through basolateral efflux.

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23. Quantitative light-sheet microscopy and mathematical modelling reveal spatially patterned deformation and topological remodelling during annual killifish epiboly

Authors: Carmen Gloria Lemus 1,2,6, Constanza Vásquez 1,3,6, Javier Cubillos 4,6, Néstor Guerrero 1,2,6, Paulo Casagrande 1,4,6, Carlos Navarro 1, Karia Palma 1,6, Margarita Meynard 1, Germán Reig 5, Steffen Hartel 1,2,6, Ignacio Bordeu 4,6, Rodrigo Soto 4,6, Mauricio Cerda 3,6, Miguel L. Concha 1,2,6

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Abstract:
Early animal development establishes where the embryo forms and how its initial body organisation emerges. Studies in model organisms highlight the fundamental role of extra-embryonic tissues in providing mechanical and geometric constraints that guide these processes. In the annual killifish Argolebias nigripinnis, morphogenesis of the embryonic cell population occurs in close association with the extra-embryonic enveloping layer (EVL), a surface epithelium that spreads from the animal to the vegetal pole, eventually enclosing the yolk. Embryonic cells use the inner surface of the EVL as a migratory substrate and respond to changes in its tension. However, whether the deformations associated with EVL spreading provide mechanical signals that instruct embryo formation remains unclear. As a first step towards addressing this question, we investigated the spatial and temporal dynamics of EVL expansion.

Combining time-lapse multiview light-sheet fluorescence microscopy, quantitative analysis of cell shape and topology, and physical modelling, we show that EVL spreading is spatially patterned, with distinct modes of cellular deformation and topological remodelling across the tissue. Our modelling further suggests that closure of the EVL margin at the end of epiboly reorganises, rather than simply dissipates, mechanical stress, producing a transition from tensile to compressive stresses at the closure site. Notably, this site coincides with the region where embryo formation begins. Together, these findings reveal how regional cellular behaviours coordinate epithelial expansion and stress redistribution, and provide a framework for testing whether the mechanics of EVL spreading contribute to the initial formation and organisation of the embryo.

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24. A Length-Optimized Active Contour Loss Function for Boundary Segmentation in Biomedical Images

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Abstract:
Current image segmentation methods based on deep learning achieve state-of-the-art performance in a myriad of image and object types. In biomedical imaging, however, existing loss formulations used in model training are often challenged when dealing with fine local structures such as cell protrusions or abnormal morphological patterns relevant for disease diagnosis. A promising approach to address this challenge is the design of loss functions that model segmentation with basis on contour features, such as active contour models, that can improve boundary smoothness, by including regularization terms for contour length.

We propose a novel loss function, Length Optimized Active Contour Loss (LOAC), which enhances length optimization through constraints derived from classical image processing principles, including multiple-orientation gradient estimation, magnitude, and orientation penalization strategies, in addition to different normalization schemes. We evaluate LOAC using a U-Net architecture and compare it against conventional loss functions, including Cross Entropy, Dice, and their combination, as well as contour-based approaches such as Normalized Active Contour Loss (NAC) and enhanced classical formulations such as Adaptive Boundary-Enhanced Dice Loss. First experiments have been conducted on the PH2 skin lesion segmentation dataset, showing that both NAC and LOAC achieve the strongest overall performance among the evaluated methods. LOAC attains the highest mean Dice Score, IoU, and Recall, with a statistically significant improvement over NAC in Recall. The results to date suggest enhanced boundary delineation, greater contour consistency, and improved segmentation quality.

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25. Prospero Homeobox 1 transcription factor haploinsufficiency alters the spatial organization of hepatocyte polarity in the adult liver

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Abstract:
Introduction: The liver exhibits an architecture characterized by hepatocyte multipolarity, where the apical domain forms the biliary canaliculi through the sensing of the extracellular matrix in the basal domain. Disruptions in the homeostasis of these domains impair cell polarity, leading to pathological conditions. However, the molecular network regulating and maintaining this balance in adulthood remains poorly understood. We propose Prox1 as a candidate regulator of this process, given its described role in the hepatocyte-cholangiocyte fate decision, which involves a spatial reorganization of the apico-basal polarity, and the fact that its deficiency causes hepatogenesis and extracellular matrix defects in mouse embryos.
Methods: Prox1 haploinsufficiency was studied using Prox1/LacZ knock-in mice. We analyzed liver architecture using multiphoton microscopy and 3D image reconstructions powered by neural network. Extracellular matrix proteins were quantified by immunofluorescence assays.
Results: Prox1 haploinsufficiency alters hepatic architecture at multiple scales. At the cellular level, we observe a reorganization of the apical membrane of the hepatocytes, which impacts the organization of the bile canaliculi at the tissue level, causing atypical branching of the network. These findings correlate with an increase in accumulation of extracellular matrix components in heterozygous tissue.
Discussion: The aberrant phenotype of the heterozygous livers suggests that Prox1 functions could impact on the extracellular matrix turnover. We propose that the accumulation of structural proteins disrupts the sensing cascade, upsetting the balance of the hepatocyte's apical domain.

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26. Subpixel characterization of projected digital patterns for structured illumination microscopy

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Abstract:
Digital micromirror device (DMD)-based structured illumination (SI) microscopy typically uses low-resolution patterns that are well resolved by the camera. While reducing projected feature size increases spatial frequencies and improves the spatial resolution of demodulated images, it introduces a sampling problem. For image reconstruction methods that rely on reference images of the projected patterns, shrinking the projected micromirror size toward the camera pixel pitch decreases the contrast of reference images due to sampling artifacts, degrading optical sectioning demodulation.

Mitigating these artifacts in Hadamard optical sectioning requires precise characterization of the projection system by measuring the delivered pattern scale, position, resolution, and contrast. Here, we measured the projection point-spread function using sub-diffraction fluorescent microspheres translated laterally across single-micromirror-wide DMD lines in 250 nm mechanical steps (0.11 times the camera pixel pitch) to achieve sub-camera-pixel spatial sampling. To explore camera discretization effects in projected patterns approaching that scale, we varied the focal length from 70 to 200 mm of the projection lens, scaling the projected micromirror pitch from 8.59 to 3.12 μm at the sample, corresponding to 3.84x 1.40x of the camera sampling pitch (2.24 μm). Repeating these line scans across known projection defocus offsets while maintaining camera focus on the sample provided estimates of the illumination path effective NA across magnifications. The narrowest line profiles reached 3.6 μm full width at half maximum (FWHM) at the sample, corresponding to 1.61 times the camera pixel pitch and 1.15 times the projected micromirror pitch, and broadened to 11.62 μm FWHM at 73.2 μm axial defocus.

These measurements establish the projected-pattern scale, point spread function width, and effective illumination NA required to model camera-pixel-scale digital SI in large-area fluorescent optical sectioning microscopy.

27. Development of an optical platform for metabolic imaging of the functional ex vivo rat heart

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Abstract:
Imaging cellular metabolism in living, functional cardiac tissue remains challenging due to continuous mechanical motion, complex tissue geometry, and the need to preserve physiological function during image acquisition. Multiphoton microscopy provides an opportunity to monitor cardiac physiology and metabolism at high spatial resolution with reduced phototoxicity and detection of endogenous metabolic signals such as NADH and FAD+. However, imaging the beating heart presents substantial technical challenges due to continuous mechanical motion and the limited accessibility of the tissue which requires specialized optical interfaces and stabilization strategies.

We developed an optical platform for functional and metabolic imaging of the isolated rat heart under controlled perfusion conditions. As an initial step, we first established imaging protocols in cultured cardiomyocytes, characterizing intracellular calcium dynamics and endogenous FAD+ autofluorescence under controlled conditions. Following these experiments, we designed and implemented two custom optical accessories to enable reproducible access to the heart during multiphoton imaging. The first is a perfusion chamber that maintains the isolated heart in a horizontal position while being continuously perfused through a Langendorff system. The second is an optical stabilization device designed to restrict cardiac motion during image acquisition while maintaining access to the myocardial surface and compatibility with the microscope objective.

This platform establishes the technical foundation for subsequent imaging of endogenous NADH and FAD+ fluorescence and quantitative analysis of metabolic and functional signals in the ex vivo heart. Future experiments will incorporate ischemia-reperfusion protocols and combine optical measurements with physiological and biomechanical readouts to investigate the relationship between cardiac metabolic state and mechanical function.

28. Characterization of blood-brain barrier repair after penetrating traumatic brain injury in Drosophila melanogaster.

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Abstract:
The blood-brain barrier (BBB) of Drosophila melanogaster is a specialized structure that surrounds the nervous system and regulates the exchange of molecules between the neural tissue and hemolymph through selective permeability. The BBB consists of two layers of glial cells: subperineurial glia (SPG), which form septate junctions that restrict paracellular diffusion and constitute a secondary epithelium, and perineurial glia (PG), which provide metabolic support and communicate with SPG through gap junctions. Despite the importance of BBB integrity for nervous system protection and homeostasis, little is known about the mechanisms underlying BBB repair following injury in Drosophila. While several models of neural injury have been developed, they have primarily focused on the neural parenchyma. Here, we implemented a penetrating traumatic brain injury (pTBI) model to investigate BBB repair after injury.

Using whole-brain mounts, transgenic lines, immunofluorescence, confocal microscopy, and fluorescence intensity quantification, we performed a structural and functional characterization of the BBB at different time points following pTBI. We observed that both BBB structure and selective permeability were restored as early as 1 day post-pTBI, as assessed by SPG membrane labeling and a dextran-based permeability assay, respectively. Additionally, structural repair was accompanied by increased actin and non-muscle myosin II at the injury site, with actin cytoskeletal reorganization occurring in both SPG and PG.

Using the multicolor FLP-out (MCFO) system, we individually labeled PG cells and characterized their morphological changes after PTBI. PG cells transitioned from a rectangular to a more stellate morphology, extending protrusions oriented toward the injury site.
Together, these findings suggest that the Drosophila BBB can restore its structural integrity and barrier function after injury. Actin and myosin remodeling, together with changes in PG morphology and potentially cell migration, may contribute to the cellular response underlying BBB repair.