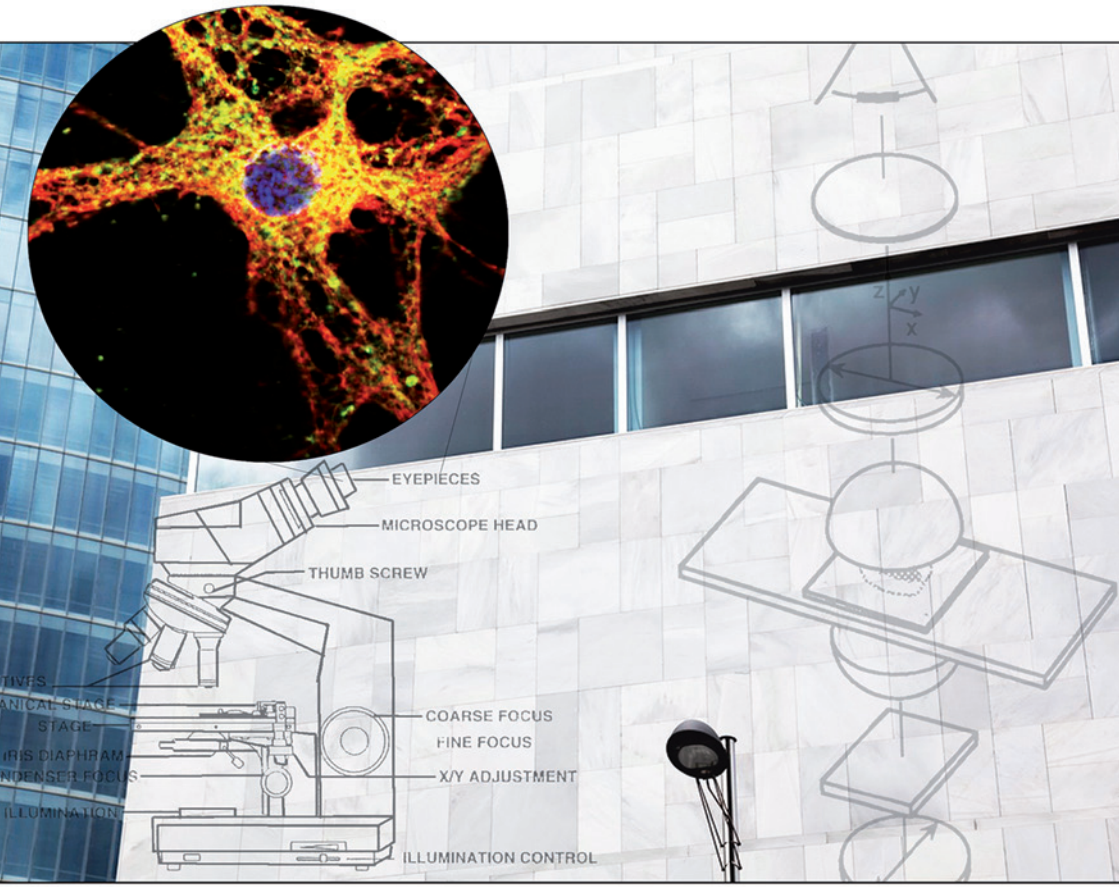
Bilbao (Spain) October 5 - 7 2016

SPAOM2016

Spanish-Portuguese Meeting for Advanced Optical Microscopy

3rd REMOA Conference

REMOA



ORGANISERS:

INSTITUTO BIOFISIKA INSTITUTUA · INSTITUTO GULBENKIAN DE CIÊNCIA · CSIC · Universidad del País Vasco Euskal Herriko Unibertsitatea · Achucarro · IBMC

ORGANISERS

LOCAL ORGANIZING COMMITTEE

- Ricardo Andrade. UPV/EHU. Spain.
- Jose Requejo. Instituto Biofisika, (CSIC, UPV/EHU). Spain.
- Asier Ruiz. Achucarro Basque Center for Neuroscience. Spain.
- Jaime Sagarduy. Achucarro Basque Center for Neuroscience. Spain.

SCIENTIFIC COMMITTEE

- Ana Jesús García. University of Tübingen. Germany.
- Ricardo Henriques. University College London. United Kingdom.
- Dylan Owen. King's College London. United Kingdom.
- Nadine Peyriéras. Institut de Neurobiologie Alfred Fessard. Paris, France.
- JanTonnesen. Institut Interdisciplinaire de Neurosciences. Bordeaux, France.

INVITED TALKS

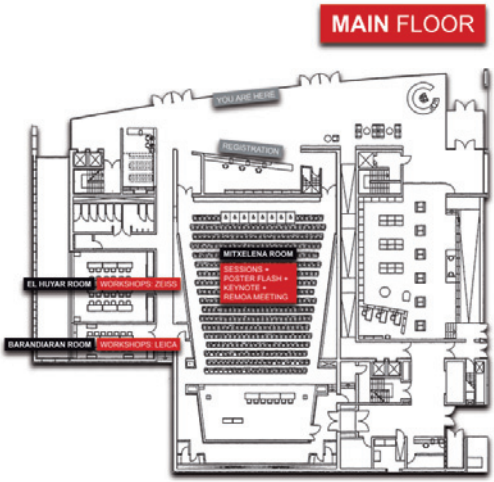
- Julien Colombelli. IRB Barcelona. Spain.
- Katia Consentino. Membrane Biophysics - University of Tübingen. Germany.
- Vadim Frolov. Instituto Biofisika (CSIC, UPV/EHU).
- Rainer Heintzmann. Friedrich-Schiller-Universität Jena. Germany.
- Ricardo Henriques. University College London. United Kingdom.
- Monica Morales. CRG, Barcelona. Spain.
- Valentin Nägerl. Institut Interdisciplinaire de NeuroSciences. Bordeaux, France.
- Dylan Owen. King's College London. United Kingdom.
- Sergi Padilla-Parra. University of Oxford. United Kingdom.
- António José Pereira. Instituto de Investigação e Inovação em Saúde. Universidade Do Porto. Portugal.
- Leopoldo Petreanu. Champalimaud Centre for the Unknown. Portugal.
- Nadine Peyrieras. Institut de Neurobiologie Alfred Fessard, CNRS. France.
- Jorge Ripoll. Universidad Carlos III de Madrid. Spain.
- Jan Tonnesen. Institut Interdisciplinaire de Neurosciences. Bordeaux, France.
- Timo Zimmermann. CRG-Centre for Genomic Regulations. Barcelona, Spain.

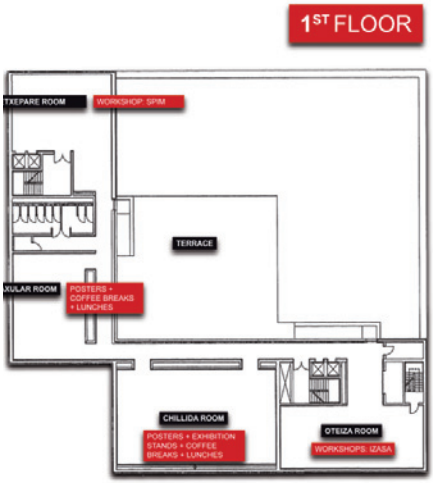
VENUE

Bizkaia Aretoa.

Avenida Abandoibarra 3, 48009, Bilbao.

• **Floor plan**





TECHNICAL ORGANIZATION

Eventokia
Email: info@spaom2016.eu
Tel: +34 946 123 007

During the conference the Technical Organization will be located at the registration desk.

• **Schedules**

Wednesday 5th	**8:30-18:30**
Thursday 6th	**8:30-18:40**
Friday 7th	**8:30-14:00**

SOCIAL ACTS

Wednesday 5th	Welcome Reception	**18:30-20:00**	Bizkaia Aretoa
Thursday 6th	Dinner	**20:30**	Museo Diocesano

INTERNET

Free Wi-Fi access will be available at the venue.

SOCIAL NETWORK

Share and follow everything that is happening at **SPAOM 2016** via twitter: @spaom2016, using the hashtag #SPAOM2016.

ACCREDITATION

Every attendee must pick-up his/her accreditation at the registration desk that it is located at Bizkaia Aretoa's hall. The accreditation must be visible and you will have to keep it with you during all the conference.

• **Speakers**
There will be a special section on the registration desk for the speakers where you will be able to register.

• **Authors**
Authors of contributed talks and poster presentations must register themselves on the regular registration desk. If you need to do any change on your presentation, please go to the speaker's desk corner and contact with the Technical Organization.

CONTRIBUTED TALKS

Every contributed talk will have **10 minutes** to present their oral communication and there will be 5 minutes where the audience will be able to discuss the topic and ask questions. The presentations will take place at Mitxelena room.

Please note that you will have to send your presentation on Power Point format before **September 26th** to info@spaom2016.com.

The order of the contributed talks is listed on the Scientific Programme beside all the information about the sessions.

During all the conferences the printed posters will be exposed at Chillida room and Axular room.

POSTER PRESENTATIONS

Every poster will have **2-3 minutes** to present their poster at the "Poster Flash" session that will take place at Mitxelena room.

Please send to info@spaom2016.com your presentation in Power Point format by **September 26th.**

The order of the "Poster Flash" session presentations is listed before besides all the information about the lecturers.

During all the conferences the printed posters will be exposed at Chillida room and Axular room.

AWARDS

The winners of "Innovation Award" and "Presentation Award" will be announced during the Closing Remarks (Friday 7th, 13:50hrs). We please request you to attend the Awards Ceremony.

Innovation award	**150€**
Presentation award	**150€**

CERTIFICATES

Certificates will be sent by e-mail once the conference has finished.

REGISTRATION FEES

	Early bird fees	Regular fees
	Valid until July 31st 2016	Valid from August 1st 2016
Student	**75€**	**125€**
Academic	**125€**	**175€**
Industry delegate	**200€**	**250€**

The registration includes: access to the conference, documentation, lunch, Welcome Reception and Gala Dinner.

SCIENTIFIC PROGRAMME'S SCHEDULE

WEDNESDAY 5/10/ 16		THURSDAY 6/10/ 16		FRIDAY 7/10/ 16	
8:30 - 9:30	Registration + poster setup	8:30 - 9:00	Registration	8:30 - 9:00	Registration
9:35 -10:55	Session I: Super-resolution. Invited talks	9:00 - 11:05	Session III: Quantitative Microscopy & Biophysics. Invited and contributed talks	9:00 - 11:00	Session VI: SPIM & in-vivo imaging. Invited talks
10:55 - 11:25	Poster Flash				
11:25 - 12:00	Posters + Coffee	11:05 - 11:35	Posters + Coffee	11:00 - 11:30	Coffee Break
12:00 - 12:30	Session I: Super-resolution. Contributed talks	11:35 - 13:00	Session IV: Functional imaging. Invited and contributed talks	11:30 - 12:15	Session VI continued. Contributed talks
				12:15 - 12:55	New and notable
12:30 - 13:00	Poster Flash			12:55 - 13:35	Facility managing
13:00 - 14:00	Industry Workshops	13:00 - 14:00	Industry Workshops	13:35 - 13:50	EuBI / EuBI Spain
				13:50 - 14:00	Closing remarks
14:00 - 15:00	Buffet lunch	14:00 - 15:00	Buffet lunch	14:00 - 15:00	Buffet lunch
15:00 - 15:55	Session II: Advanced Light Microscopy in neurosciences. Invited and contributed talks	15:00 - 16:10	Session V: Functional Imaging: FRET Invited and contributed talks		
15:55 - 16:55	Industry Workshops	16:10 - 16:40	Posters + Coffee		
16:55 - 17:30	Posters + Coffee	16:40 - 17:40	Technical Workshops		
17:30 - 18:30	Keynote Lecture	17:40 - 18:40	REMOA Meeting		
18:30 - 20:00	Posters + Welcome reception	20:30	Gala Dinner		

WEDNESDAY, OCTOBER 5TH

WEDNESDAY 5/10/ 16						
	MAIN FLOOR				1ST FLOOR	
	Hall	MITXELENA ROOM	EL HUYAR ROOM	BARANDIARAN ROOM	OTEIZA ROOM	CHILLIDA ROOM / AXULAR ROOM
8:30 - 9:30	Registration + poster setup					
9:30 - 10:55		Session I: Super-resolution Invited talks				
10:55 - 11:25		Poster Flash I				
11:25 - 12:00						Posters + Stands + Coffee
12:00 - 12:30		Session I: Super-resolution Contributed talks				
12:30 - 13:00		Poster Flash II				
13:00 - 14:00			Workshop ZEISS	Workshop LEICA	Workshop IZASA	
14:00 -15:00						Buffet lunch
15:00 - 15:55		Session II: Advanced Light microscopy in neurosciences. Invited and contributed talks				
15:55 - 16:55			Workshop ZEISS	Workshop LEICA	Workshop IZASA	
16:55 - 17:30						Posters + Coffee
17:30 - 18:30		Keynote Lecture				
18:30 - 20:00						Posters + Welcome reception

THURSDAY, OCTOBER 6TH

THURSDAY 6/10/16									
	MAIN FLOOR				1ST FLOOR				2ND FLOOR
	Hall	MITXELENA ROOM	EL HUYAR ROOM	BARANDIARAN	OTEIZA ROOM	CHILLIDA ROOM	AXULAR ROOM	SALA ETXEPARE	SALA ARRIAGA
8:30 - 9:00	Registration								
9:00 - 11:05		Session III: Quantitative Microscopy & Biophysics. Invited and contributed talks							
11:05 - 11:35						Posters + Stands + Coffee			
11:35 - 13:00		Session IV: Functional imaging Invited and contributed talks							
13:00 - 14:00			Workshop ZEISS	Workshop LEICA	Workshop IZASA				
14:00 - 15:00						Buffet lunch			
15:00 - 16:10		Session V: Functional imaging: FRET. Invited and contributed talks							
16:10 - 16:40						Posters + Coffee			
16:40 - 17:40								Technical Workshop: SPIM	Technical Workshop: DECONVOLUTION
17:40 - 18:40		REMOA Meeting							
20:30	Dinner: Museo Diocesano								

FRIDAY, OCTOBER 7TH

FRIDAY 7/10/ 16							
	MAIN FLOOR				1ST FLOOR		
	Hall	MITXELENA ROOM	EL HUYAR ROOM	BARANDIARAN ROOM	OTEIZA ROOM	CHILLIDA ROOM	AXULAR ROOM
8:30 - 9:00	Registration						
9:00 - 11:00		Session VI: SPIM & in-vivo imaging. Invited talks					
11:00 - 11:30						Coffee Break	
11:30 - 12:15		Session VI: continued Contributed talks					
12:15 - 12:55		New and notable Invited talks					
12:55 - 13:35		Facility managing Invited talks					
13:35 - 13:50		EuBI/EuBI Spain Invited talks					
13:50 - 14:00		Closing remarks					
14:00-15:00	Buffet Lunch						

SCIENTIFIC PROGRAMME

WEDNESDAY, OCTOBER 5TH

08:30-09:30 Registration + Poster setup

09:30-09:35 Opening

09:35-10:55 **SESSION I: SUPER-RESOLUTION. INVITED TALKS.**

Chair: Timmo Zimermann. CRG-UPF, Centre of Genomic Regulation. Barcelona, Spain.

9:35 **Democratising live-cell high-speed low-illumination super-resolution microscopy through Super-Resolution Radial Fluctuations.**

Ricardo Henriques. University College London. United Kingdom.

10:15 **Structured Illumination and the Analysis of Single Molecules in Cells.**

Rainer Heintzmann. Friedrich-Schiller-Universität Jena. Germany.

10:55-11:25 **Poster flash I** (From P1 to P11)

11:25-12:00 Posters + Coffee Break

12:00-12:30 **SESSION I: SUPER-RESOLUTION. CONTRIBUTED TALKS.**

Chair: Jan Tonnesen. Institut Interdisciplinaire de Neurosciences. Bordeaux, France

12:00 **CT1: STED In-vivo imaging in the far red.**

Raquel Garcia[1], Arrate Mallabiabarrena[1], Xavier Sanjuan[2], Timo Zimmermann[1].

1. CRG-UPF Advanced Light Microscopy Unit, Centre for Genomic Regulation. Barcelona, Spain.
2. CRG-UPF Advanced Light Microscopy Unit, Universitat Pompeu Fabra, Barcelona, Spain.

12:15 **CT2: Platinum Shadowing for correlative light and electron microscopy.**

José María Mateos[1], Bruno Guhl[1], Jana Doehner[1], Gery Barmettler[1], Andres Kaech[1], Urs Ziegler[1].

1. Center for Microscopy and Image Analysis, University of Zurich. Switzerland.

12:30-13:00 **Poster flash II** (From P12 to P22)

13:00-14:00 **On-site equipment workshops** (parallel x3)

OTEIZA ROOM	EL HUYAR ROOM	BARANDIARAN ROOM
Nikon superior confocal performance: Enhanced resolution and spectral flexibility.	Zeiss Airyscan goes Fast: an innovative confocal imaging technology allows high resolution, high speed and low noise - simultaneously.	Leica TCS-SP8x: flexible excitation and super resolution.

14:00-15:00 Buffet lunch

15:00-15:55 **SESSION II: ADVANCED LIGHT MICROSCOPY IN NEUROSCIENCES. INVITED AND CONTRIBUTED TALKS.**

Chair: Asier Ruiz. Achucarro Basque Center for Neuroscience. Spain.

15:00 **Applying STED microscopy to understand the electrical properties of dendritic spines.** (Invited talk)

Jan Tonnesen. Institut Interdisciplinaire de Neurosciences. Bordeaux, France.

15:40 **CT3: Automatic analysis of microglial motility with Image.**

Iñaki Paris[1], Laura Escobar[1], Agnes Nadjar[4], Sophie Layé[4], Quentin Leyrolle[4], Julie C. Savage[5,6], Chin-Wai Hui[5,6], Marie-Ève Tremblay[5,6], Amanda Sierra[1,2,3], Jorge Valero[1,2].

1. Achucarro Basque Center for Neuroscience. Zamudio, Bizkaia, Spain.
2. Ikerbasque Basque Foundation for Science. Bilbao, Bizkaia, Spain.
3. University of the Basque Country. Leioa, Bizkaia, Spain.
4. Université Bordeaux Segalen. Bordeaux, France.
5. Centre de recherche du CHU de Québec, Axe Neurosciences. Québec, Canada.
6. Université Laval, Département de médecine moléculaire. Québec, Canada.

15:55-16:55 **On-site equipment workshops** (parallel x3)

OTEIZA ROOM	EL HUYAR ROOM	BARANDIARAN ROOM
Advances in instrumentation and labels for highly multiplexed biological imaging.	Zeiss Airyscan goes Fast: an innovative confocal imaging technology allows high resolution, high speed and low noise - simultaneously.	Leica TCS-SP8x: flexible excitation and super resolution.

16:55-17:30 Posters + Coffee Break

17:30-18:30 KEYNOTE LECTURE

Super-resolution imaging of brain extracellular space in live tissue.

Valentin Nägerl. Institut Interdisciplinaire de NeuroSciences. Bordeaux, France.

18:30-20:00 Posters + Welcome Reception

THURSDAY, OCTOBER 6TH

08:30-9:00 Registration

09:00-11:05 SESSION III: QUANTITATIVE MICROSCOPY & BIOPHYSICS. INVITED AND CONTRIBUTED TALKS.

Chair: Jose Requejo-Isidro. Instituto Biofisika, (CSIC, UPV/EHU). Leioa. Spain.

9:00 **Statistical analysis of super-resolution microscopy data.** (Invited talk)

Dylan Owen. King's College London. United Kingdom.

9:40 **How biomimetic nanotemplates help resolving molecular details of cellular membrane fission.** (Invited talk)

Vadim Frolov. Instituto Biofisika (CSIC, UPV/EHU). Leioa, Spain.

10:20 **Elucidating Bax assembly and pore formation in membranes by advanced microscopy methods.** (Invited talk)

Katia Consentino. Membrane Biophysics - University of Tübingen. Germany.

10:50 **CT4: Analysis of nanoscale compartmentalization of PI(4,5)P2 in the plasma membrane through FRET imaging.**

Maria J. Sarmento[1], Ana Coutinho[1,2], Manuel Prieto[1], Fabio Fernandes[1,3].

1. Centro de Química-Física Molecular and IN-Instituto de Nanociência e Nanotecnologia, Universidade de Lisboa. Lisbon, Portugal.
2. Departamento de Química e Bioquímica, Universidade de Lisboa. Lisbon, Portugal.
3. Research Unit on Applied Molecular Biosciences–Rede de Química e Tecnologia (UCIBIO-REQUIMTE), Universidade Nova de Lisboa. Caparica, Portugal.

11:05-11:35 Posters + Coffee Break

11:35-13:00 **SESSION IV: FUNCTIONAL IMAGING. INVITED AND CONTRIBUTED TALKS.**

Chair: Ricardo Andrade. UPV/EHU. Spain.

11:35 **Shining light on cortico-cortical connections.** (Invited talk)

Leopoldo Petreanu. Champalimaud Centre for the Unknown. Portugal.

12:15 **CT5: Label-free multimodal micro-spectroscopic differentiation of dysplasia stages in mouse tongues as a model of early tumor genesis in head and neck squamous cell carcinoma combined with multivariate data analysis.**

Mona Stefanakis[1], Tobias Krawietz[1], Jörg W. Bartsch[2], Garrit Koller[3], Ines Sequeira[3], Fiona Watts[3], Karsten Rebner[1], Edwin Ostertag[1].

1. Reutlingen University, Process Analysis and Technology. Reutlingen, Germany.
2. Philipps-University Marburg, Neurosurgery. Marburg, Germany.
3. King's College London. United Kingdom.

12:30 **CT6: Enhancement of second-harmonic microscopy images by combining adaptive optics and polarization modulation.**

Francisco J. Ávila[1], Pablo Artal[1], Juan M. Bueno[1].

1. Laboratorio de Óptica (CiOyN), Universidad de Murcia. Spain.

12:45 **CT7: Confocal microscopy and gene expression approaches to analyze auxin dynamics during microspore embryogenesis.**

María C. Risueño[1], Héctor Rodríguez-Sanz[1], Yolanda Pérez-Pérez[1], María-Teresa Solís[1], María-Fernanda López[2], Aurelio Gómez-Cadenas[2], Pilar S. Testillano[1].

1. Biological Research Center, CIB-CSIC. Madrid, Spain.
2. Dep. CC Agrarias y del Medio Natural, Univ.Jaume I. Castellón, Spain.

13:00-14:00 **On-site equipment workshops** (parallel x3)

OTEIZA ROOM	EL HUYAR ROOM	BARANDIARAN ROOM
Andor Dragonfly: Spinning disk based multi-modality platform.	Zeiss Airyscan goes Fast: an innovative confocal imaging technology allows high resolution, high speed and low noise - simultaneously.	Leica TCS-SP8x: flexible excitation and super resolution.

14:00-15:00 Buffet lunch

15:00-16:10 **SESSION V: FUNCTIONAL IMAGING: FRET. INVITED AND CONTRIBUTED TALKS.**

Chair: Jose Requejo-Isidro. Instituto Biofisika, (CSIC, UPV/EHU). Spain.

15:00 **Ascertaining HIV-1 entry and fusion in primary cells with FRET-based biosensors.** (Invited talk)

Sergi Padilla-Parra. University of Oxford. United Kingdom.

15:40 **CT8: Equilibrium parameters derived from FRET experiments in living cells.**

Álvaro Villarroel[1], Araitz Alberdi[1], Carolina Gomis-Perez[1], Pilar Areso[2].

1. Instituto Biofisika, (CSIC, UPV/EHU). Leioa, Spain
2. Dpt. Farmacología, UPV/EHU. Leioa, Spain

15:55 **CT9: Molecular images of plitidepsin-eEF1A complexes in living tumor cells.**

Carolina García[1], Alejandro Losada[2], José Manuel Molina-Guijarro[2], Miguel Ángel Sacristán[1], Juan F. Martinez-Leal[2], Carlos M. Galmarini[2], Mª Pilar Lillo[1].

1. Departamento de Química Física Biológica. Instituto de Química-Física "Rocasolano" (CSIC). Madrid, Spain.
2. Departamento de Biología Celular y Farmacogenómica, Pharma Mar S.A.; Madrid, Spain.

16:10-16:40 Posters + Coffee Break

16:40-17:40 **Technical Workshop** (parallel x2)

- **SPIM (Lightsheet microscopy) and OPT (Optical Projection Tomography) Workshop.**
  Gaby G. Martins. Instituto Gulbenkian de Ciência. Portugal

  Emilio Gualda. ICFO - Institut de Ciències Fotòniques. Barcelona. Spain.

  Jim Swoger, Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology. Barcelona, Spain.

  Julien Colombelli. Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology. Barcelona, Spain.

  Nuno Moreno. Instituto Gulbenkian de Ciência. Portugal

- **Deconvolution for digital microscopy.**
  Sebastien Tosi. Institute for Research in Biomedicine, IRB Barcelona. Spain.

17:40-18:40 **Remoa Meeting**

Chair: Maria Calvo. Universitat de Barcelona. Spain.

20:00-22:00 Dinner

FRIDAY, OCTOBER 7TH

08:30-9:00 Registration

09:00-11:00 **SESSION VI: SPIM & IN-VIVO IMAGING. INVITED TALKS.**

Chair: Gaby G. Martins. Instituto Gulbenkian de Ciência. Portugal.

9:00 **Cellular dynamics in embryonic morphogenesis based on 3D+time imaging data.**
Nadine Peyrieras. Institut de Neurobiologie Alfred Fessard, CNRS. France.

9:40 **Imaging model diseases in transparent organs**.
Julien Colombelli. IRB Barcelona. Spain.

10:20 **3D Live Imaging of Fast Dynamic Processes.**
Jorge Ripoll. Universidad Carlos III de Madrid. Spain.

11:00-11:30 Coffee Break

11:30-12:15 **SESSION VI CONTINUED. CONTRIBUTED TALKS.**

Chair: Julien Colombelli. IRB Barcelona. Spain.

11:30 **CT10: Correction of attenuation artefacts in fluorescence microscopy.**

Jürgen Mayer[1,2], James Sharpe[1,2,3], Jim Swoger[1,2].

1. Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology. Barcelona, Spain.
2. Universitat Pompeu Fabra (UPF). Barcelona, Spain.
3. Institució Catalana de Recerca i Estudis Avançats (ICREA). Barcelona, Spain.

11:45 **CT11: Openspin and opent mesoscopic imaging for cell & developmental biology.**

Gabriel G. Martins[1]; Hugo Pereira[1], Nuno Martins[1], Tiago Vale[1], Emilio Gualda[2], Nuno Moreno[1].

1. IGC - Instituto Gulbenkian de Ciência. Oeiras. Portugal.
2. ICFO - Institut de Ciències Fotòniques. Barcelona. Spain.

12:00 **CT12: High throughput three-dimensional cellular models imaging through flow light-sheet fluorescence microscopy cytometry.**

Emilio J. Gualda[1,2], Pablo Loza-Alvarez[1], Marta F. Estrada[3], Catarina Brito[3].

1. ICFO-Institut de Ciencies Fotóniques, BIST-Barcelona Institute of Science and Technology. Castelldefels, Spain.

2. IGC-Instituto Gulbenkian de Ciência. Oeiras, Portugal.
3. iBET-Instituto de Biologia Experimental e Tecnológica. Oeiras, Portugal.

12:15-12:55 **NEW & NOTABLE. INVITED TALKS.**

Chair: Nuno Moreno. IGC - Instituto Gulbenkian de Ciência, Oeiras. Portugal.

Fluorescent speckle microscopy by speckled photoswitching.

António José Pereira. Instituto de Investigação e Inovação em Saúde. Universidade Do Porto. Portugal.

12:55-13:35 **FACILITY MANAGING. INVITED TALKS.**

Chair: Nuno Moreno. IGC - Instituto Gulbenkian de Ciência. Oeiras, Portugal.

Running a Core Facilities programme: Strategic planning to facilitate research.

Monica Morales. CRG. Barcelona, Spain.

13:35-13:50 **EUBI / EUBI SPAIN. INVITED TALKS.**

EuBI / EuBI Spain.

Timo Zimmermann. CRG-Centre for Genomic Regulations. Barcelona, Spain.

13:50-14:00 Closing remarks

14:00-15:00 Buffet lunch

POSTERS

The **Poster** flash session will take place on Mitxelena's room. They will be presented on the following order:

P1: MULTIPHOTON IMAGE IMPROVEMENT WITH LASER PULSE COMPRESSION.

Juan M. Bueno[1], Martin Skorsetz[1], Pablo Artal[1].

1. Laboratorio de Óptica (CiOyN), Universidad de Murcia. Murcia, Spain.

P2: AUTOMATIC DIATOM IDENTIFICATION FOR WATER QUALITY ASSESSMENT (AQUALITAS).

Gabriel Cristóbal[1], Gloria Bueno[2], Saúl Blanco[3], Carlos Sánchez[1], María Borrego-Ramos[3], Anibal Pedraza[2], Jesús Ruiz-Santaquiteria[2], José Luis Espinosa-Aranda[2].

1. Instituto de Óptica (CSIC). Madrid, Spain.
2. VISILAB, ETS Ing. Industriales, Univ. Castilla la Mancha. Ciudad Real, Spain.
3. Institute of Environment, Univ. León. León, Spain.

*P3: REACTIVE NEURAL STEM CELLS IN THE ADULT BRAIN.

Soraya Martín-Suárez[1], Roberto Valcárcel-Martín[1], Oier Pastor-Alonso[1], José R. Pineda[1], Juan M. Encinas[1,2,3].

1. Achucarro Basque Center for Neuroscience. Zamudio, Spain.
2. Ikerbasque, The Basque Foundation for Science. Bilbao, Spain.
3. University of the Basque Country (UPV/EHU). Leioa, Spain.

P4: OPTIC, GENETIC AND PHYSIC TOOLS TO BETTER CHARACTERIZE TUNNELING NANOTUBE STRUCTURES CREATED BY GLIOMA STEM CELLS.

Jose R. Pineda[1,2], Thierry Kortulewski[1,*], Maya Jeitany[1,*] Laurent Gauthier[1], Marie P. Junier[3,4,5,*], Hervé Chneiweiss[3,4,5,*], Annie Andrieux[6], François D. Boussin[1].

1. CEA DSV iRCM SCSR, INSERM, Laboratoire de Radiopathologie. Fontenay-aux-Roses, France.
2. Achucarro Basque Center for Neuroscience. Zamudio, Vizcaya, Spain.
3. CNRS UMR8246 Neuroscience Paris Seine. Paris, France.
4. INSERM U1130, Neuroscience Paris Seine. Paris, France.
5. University Pierre and Marie Curie UMCR18, Neuroscience Paris Seine. Paris, France.
6. CEA, IRTSV-GPC, INSERM, U836-GIN Site Santé La Tronche. France.

*Contributed equally to this work.

P5: COMPARISON OF FORWARD AND BACKARD EMITTED POLARISATION SENSITIVE SECOND HARMONIC GENERATION FOR THE STUDY OF HUMAN CORNEAL TISSUE.

David Merino[1], Giuseppe Lombardo[2], Rita Mencucci[3], Marco Lombardo[4], Pablo Loza-Alvarez[1].

1. ICFO – The Institute of Photonic Sciences. Castelldefels, Spain.
2. Consiglio Nazionale delle Ricerche Istituto per i Processi Chimico-Fisici, CNR-IPCF. Messina, Italy.
3. Università di Firenze. Firenze, Italy.
4. Fondazione G. B. Bietti. Roma, Italy.

P6: MOLECULAR MONITORING OF BIOMEDICAL SAMPLES BY RAMAN IMAGING AND CHEMOMETRICS.

Monica Marro[1], Pablo Loza-Alvarez[1].

1. ICFO-Institut de Ciencies Fotoniques, The Barcelona Institute of Science and Technology. Castelldefels, Spain.

P7: NEUBIAS: THE NETWORK OF BIOIMAGE ANALYSTS.

Sébastien Tosi[1], Perrine Paul-Gilloteaux[2,3], Kota Miura[1], Julien Colombelli [1].

1. Institute for Research in Biomedicine, IRB Barcelona. Spain.
2. PF MicroPICell IRS-UN 8, Université de Nantes. France.
3. France BioImaging.
4. European Molecular Biology Laboratory, EMBL - Heidelberg, Germany.

P8: REMYELINIZATION STUDIES WITH CONFOCAL FLUORESCENCE MICROSCOPY.

Iñaki Osorio-Querejeta[1,2*], Irantzu Llarena [3*], Maider Muñoz-Culla [1,2], Pedro Ramos-Cabrer [4,5], David Otaegui [1,2].

1. Grupo de esclerosis múltiple, Área de Neurociencias, IIS Biodonostia. San Sebastián, Spain.
2. REEM: Spanish network on Multiple Sclerosis (Red española de esclerosis múltiple -REEM-). Barcelona, Spain.
3. Plataforma de espectroscopía óptica, CICbiomaGUNE. San Sebastián, Spain.
4. Molecular Imaging Unit, CIC biomaGUNE. San Sebastian, Spain.
5. Ikerbasque, Basque Foundation for Science. Bilbao, Spain.

* both authors have contributed equally to the poster.

P9: STEREOLOGICAL ASSESSMENT OF IMMUNE CELLS INFILTRATION IN KIDNEY TRANSPLANT BIOPSIES.

Elena Aguado[1], Konstantin Levitsky[1], Isabel Aguilera[1], Antonio Nuñez[1].

1. Instituto de Biomedicina de Sevilla IBiS/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla. Seville, Spain.

P10: MANAGING IMAGE AND IMMUNOHISTOCHEMISTRY FACILITY CORE IN IDIPAZ, A HEALTH RESEARCH INSTITUTE ENVIRONMENT.

Algarra E[1]. and Vallejo-Cremades MT[1].

1. Image and Immunohistochemistry Facility Core, IdiPaz, La Paz University Hospital. Madrid, Spain.

P11: MULTIMODAL IMAGING OF NANOSTRUCTURED MATERIALS AND BIOLOGICAL SAMPLES IN THE FAR-FIELD AND NEAR-FIELD REGIMES.

Stefan G. Stanciu[1], Denis E. Tranca[1], Catalin Stoichita[1], Radu Hristu[1], Laura Pastorino[2], Juan M. Bueno[3], Carmelina Ruggiero[2], Alexei Antipov[4], George A. Stanciu[1].

1. Center for Microscopy-Microanalysis and Information Processing, University Politehnica of Bucharest. Romania.
2. Department of Informatics, Bioengineering, Robotics and Systems Engineering, University of Genova. Italy.
3. Laboratorio de Óptica, Universidad de Murcia. Murcia, Spain.
4. PlasmaChem GmbH. Berlin, Germany.

P12: DUAL ROLE OF CARDIOLIPIN IN MCL1 MEMBRANE ACTIVITY REVEALED BY MULTIPLE FLUORESCENCE-BASED TECHNIQUES APPLIED TO MINIMALIST SYSTEMS.

Olatz Landeta[1], Hector Flores-Romero[1], Ana Garcia Saez[2], Gorka Basañez[1].

1. Biofisika Institute, Centro Mixto Centro Superior de Investigaciones Científicas (CSIC)-(EHU/UPV). Leioa, Spain.
2. Interfaculty Institute for Biochemistry, University of Tübingen, Germany, Max-Planck Institute for Intelligent Systems. Stuttgart, Germany. And German Cancer Research Center, BioQuant, ImNeuenheimer Feld. Heidelberg, Germany

P13: APPLYING STED MICROSCOPY TO UNDERSTAND THE ELECTRICAL PROPERTIES OF DENDRITIC SPINES.

Jan Tonnesen[1,2], Daniel Cattaert[1,3], U. Valentin Nägerl[1,2].

1. University of Bordeaux. Bordeaux, France.
2. Interdisciplinary Institute for Neuroscience. Bordeaux, France.
3. Institut de Neurosciences Cognitives et Intégratives d'Aquitaine. Bordeaux, France.

P14: LIGHT-SHEET MICROSCOPY IMAGIN OF A WHOLE CLEARED RAT BRAIN WITH THY1-GFP TRANSGENE.

Emilio J. Gualda[1], Jordi Andilla[1], Marzena Stefaniuk[2], Monika Pawlowska[2], Leszek Kaczmarek2, Pablo Loza-Alvarez[1].

1. ICFO-Institut de Ciencies Fotóniques, BIST-Barcelona Institute of Science and Technology. Castelldefels, Spain.
2. Nencki Institute. Warsaw, Poland.

P15: BUILDING A SCANNING FCS SYSTEM.

Unai Lorenzo[1, 2], Gloria de las Heras[2], Jose Requejo-Isidro[2].
1. Fundación Biofísica Bizkaia. Leioa. Spain.
2. Unidad de Biofísica (UPV/EHU, CSIC). Leioa. Spain.

P16: QUANTIFYING EQUILIBRIUM DISSOCIATION CONSTANTS INSIDE THE CELL USING FRET-FLIM.

Gloria de las Heras-Martínez[1], Josu Andrieu1, Banafshé Larijani[1,2], Jose Requejo-Isidro[1*].
1. Unidad de Biofísica (CSIC, UPV/EHU), Leioa, Spain.
2. Research Centre for Experimental Marine Biology and Biotechnology (PiE), University of the Basque Country (UPV/EHU), Leioa, Spain.

P17: CONFOCAL AND VIDEOCONFOCAL IMAGING AS A TOOL TO STUDY LPAR1 ROLE ON THE TRANSITION FROM DEVELOPMENTAL TO ADULT NEURAL STEM CELLS IN THE HIPPOCAMPUS.

Oier Pastor-Alonso[1,3], Juan Manuel Encinas[1,2,3], Jose R. Pineda[1].
1. Achucarro Basque Center for Neuroscience. Zamudio, Spain.
2. Ikerbasque, The Basque Foundation for Science. Bilbao, Spain.
3. University of the Basque Country (UPV/EHU). Leioa, Spain.

P18: SHORT-CHAIN SPHINGOLIPIDS PREFERENTIALLY INSERT INTO TUMOR CELL MEMBRANES AND PROMOTE CHEMOTERAPEUTIC DRUG UPTAKE.

Dalila Ciceri[1], Lilia R. Pedrosa[2], Félix M. Goñi[1], Gerben A. Koning[2], F.-Xabier Contreras[1,3].
1. Instituto Biofísika Institua (CSIC, UPV/EHU) and Departamento de Bioquímica y Biología Molecular, Universidad del País Vasco, Leioa, Spain.
2. Laboratory Experimental Surgical Oncology Section Surgical Oncology, Department of Surgery, Erasmus Medical Centre. Rotterdam, Netherlands.
3. IKERBASQUE Basque Foundation for Science. Bilbao, Spain.

P19: MITOCHONDRIAL DIVISION INHIBITOR 1 MODULATES INTRACELULAR CALCIUM SIGNALING AND EXACERBATES EXCITOTOXIC OLIGODENDROCYTE DEATH.

Asier Ruiz[1,2], Elena Alberdi[1,2], Carlos Matute[1,2].
1. Departamento de Neurociencias, Achucarro Basque Center for Neurosciences, Universidad del País Vasco (UPV/EHU). Leioa, Spain.
2. Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED). Leioa, Spain.

P20: LAURDAN'S TIME-RESOLVED SPECTROSCOPY STUDY OF THE MODULATION OF THE MEMBRANE BIOPHYSICAL PROPERTIES BY AN ETHER-LIPID DERIVATE AND ITS CONSEQUENCES IN METASTASIS DEVELOPMENT.

Ana M Bouchet[1,2], Aurelie Chantôme[1,2], Marie Potier-Cartereau[1,2], Christophe Vandier[1,2], Jose M. Requejo-Isidro[3].

1. INSERM UMR 1069, Université de Tours. France.
2. Ion channel and cancer-Canceropole Grand Ouest network (IC-CGO network).
3. Unidad de Biofisica (CSIC-UPV/EHU. Leioa, Spain.

P21: MICROGLIA EXACERBATES SYNAPTIC DYSFUNCTION IN ALZHEIMER´S DISEASE.

Estibaliz Capetillo-Zarate[1,2,3], Jone Zuazo[1], Carolina Ortiz-Sanz[1], Elena Alberdi[1], Carlos Matute[1].

1. Achucarro Basque Center for Neurosciences, Ciberned and Departamento de Neurociencias, Universidad del País Vasco. Leioa, Spain.
2. IKERBASQUE, Basque Foundation for Science. Bilbao, Spain.
3. Department of Biochemistry, Weill Cornell Medical College. New York, USA.

P22: MEMBRANE INTERACTIONS OF HIV-1 NEUTRALIZING ANTIBODIES ASSESSED BY SCANNING FLUORESCENCE CORRELATION SPECTROSCOPY.

Itziar R. Oar-Arteta[1], Pablo Carravilla[1], Edurne Rujas[1], Nerea Huarte[1], José L. Nieva[1], Jose Requejo-Isidro[1].

1. Biofisika Institute (CSIC, UPV/EHU) and Department of Biochemistry and Molecular Biology, University of the Basque Country. Bilbao, Spain.

*P23: DUAL MODE DIAGNOSTIC TOOL FOR CONFOCAL ENDOMICROSCOPY AND FLUORESCENCE LIFETIME SPECTROSCOPY TO ANALYZE TISSUE BIOCHEMISTRY *IN-VIVO.*

Byungyeon Kim[1], Byungjun Park[1], Seungrag Lee[1], Sucbei Moon[2], Youngjae Won[1].

1. Osong Medical Innovation Foundation, Republic of Korea.
2. Kookmin University, Republic of Korea.

*These posters will not be presented at Poster flash session.

CT1: STED IN VIVO IMAGING IN THE FAR RED RANGE

Raquel Garcia[1], Arrate Mallabiabarrena[1], Xavier Sanjuan[2], Timo Zimmermann[1].

E-mail: timo.zimmermann@crg.eu

1. CRG-UPF Advanced Light Microscopy Unit, Centre for Genomic Regulation, Barcelona, Spain.
2. CRG-UPF Advanced Light Microscopy Unit, Universitat Pompeu Fabra, Barcelona, Spain.

KEY WORDS: Living cells, super-resolution, STED

Using a pulsed depletion laser at 772 nm for Stimulated Emission Depletion (STED) and silicon-rhodamine (SiR) [1] as a cell-permeable fluorescence label we have imaged living cells in super-resolution. Working in the far red range of the spectrum (SiR emission maximum: 674 nm) allows efficient depletion in the near-infrared range (772 nm) minimizes photodamage by the light-intensive imaging method and allows the acquisition of timelapse sequences. Imaging was done with a specially corrected 100x 1.4 NA oil immersion objective and also with a 63x 1.2 NA water immersion objective with a correction collar to minimize spherical aberrations caused by the transition of the light from the coverslip to the watery environment needed for in-vivo imaging.

We have imaged different available forms of SiR conjugates: SiR labelling using SNAP-tags [2], SiR conjugated to cytoskeletal ligands for the labelling of F-actin or microtubules and SiR-Hoechst for the labelling of DNA.

Using these dyes allows relatively easy and flexible in-vivo super-resolution microscopy and is in conjunction with a corrected water immersion objective well matched for imaging at physiologically suitable temperatures.

[1] G. Lukinavičius et al., A near-infrared fluorophore for live-cell super-resolution microscopy of cellular proteins. *Nat. Chem.,* 5, 132–39 (2013).

[2] Keppler A et al., A general method for the covalent labelling of fusion proteins with small molecules in vivo. Nature Biotechnology, 21, 86–89 (2003)

CT2: PLATINUM SHADOWING FOR CORRELATIVE LIGHT AND ELECTRON MPARROSCOPY

José María Mateos*, Bruno Guhl*, Jana Doehner, Gery Barmettler, Andres Kaech, Urs Ziegler.

E-mail: mateos@zmb.uzh.ch

Center for Microscopy and Image Analysis, University of Zurich, Switzerland.

*These authors contributed equally to this work

KEY WORDS: Super-resolution microscopy, Tokuyasu cryo-sections, scanning electron microscopy

Fluorescence microscopy reveals molecular expression at nanometer resolution but lacks ultrastructural context information. Electron microscopy provides this contextual subcellular details but protein identification often requires elaborate protocols. Correlative light and electron microscopy produces complimentary information that expands our knowledge of protein expression in cells and tissue. Even though a number of correlative approaches are currently available, few of these allow subcellular localization in tissue because of the challenges with sample preparation and 3D complexity. Tokuyasu cryo-sections (Tokuyasu, 1980) preserve the sample ultrastructure and antigenicity of most epitopes, however, standard heavy metal exposure generates weak contrast to the samples rendering often interpretation of the data difficult.

We present a quick, simple and reproducible method for protein localization by conventional and super-resolution light microscopy combined with platinum shadowing and scanning electron microscopy to obtain topographic contrast from the surface of ultrathin cryo-sections collected on silicon wafers. Figure 1 shows protein distribution at nuclear pores in the topographical landscape of mouse kidney tissue.

CT3: AUTOMATIC ANALYSIS OF MICROGLIAL MOTILITY WITH IMAGEJ

Iñaki Paris[1], Laura Escobar[1], Agnes Nadjar[4], Sophie Layé[4], Quentin Leyrolle[4], Julie C. Savage[5,6], Chin-Wai Hui[5,6], Marie-Ève Tremblay[5,6], Amanda Sierra[1,2,3], Jorge Valero[1,2].

E-mail: jorge.valero@achucarro.org

1. Achucarro Basque Center for Neuroscience. Zamudio, Spain.
2. Ikerbasque Basque Foundation for Science. Bilbao Spain.
3. University of the Basque Country. Leioa, Spain.
4. Université Bordeaux Segalen. Bordeaux, France.
5. Centre de recherche du CHU de Québec, Axe Neurosciences. Québec, Canada.
6. Université Laval, Département de médecine moléculaire, Québec, Canada.

KEY WORDS: ImageJ Macro, life imaging, microglia, processes motility, two-photon microscopy.

Microglial cells, the immune cells of the central nervous system, continuously survey the brain parenchyma to detect alterations and maintain tissue homeostasis. The motility of their processes is an indicative of the surveying capacity of microglia in normal and pathological conditions. The gold standard technique to study motility involves the use of two-photon laser-scanning microscopy to obtain images from brain slices or the cortex of living animals. This technique generates a large amount of 4 dimensionally-coded images (XYZT) which are manually analysed using tedious and time consuming protocols. In addition, motility analysis is frequently performed using Z-slice projections of image stacks with the consequent loss of three-dimensional (3D) information and accuracy.

To overcome these limitations we have developed ProMoIJ, an ImageJ tool to perform automatic motility analysis of cell processes in 3D. The tool includes several ImageJ macros that allow batch processing for registration, background subtraction, and bleaching correction of the original images. The main core of the tool is formed by two ImageJ macros, by which the microglial process to be analyzed is first manually selected and then its skeleton is automatically 3D reconstructed. Several motility data are extracted from each 3D skeleton: process length at each frame, length variation per minute, retraction (length reduction/min), protraction (length increase/min), tip position, and tip motility. We are currently validating data obtained with ProMoIJ by comparing with data manually obtained.

To the best of our knowledge, ProMoIJ is the first freely available tool for automated analysis of microglial motility. ProMoIJ facilitates the analysis of the motility of cell processes in 3D by reducing the time required to obtain results, and by increasing the accuracy and reproducibility of the data.

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CT4: ANALYSIS OF NANOSCALE COMPARTMENTALIZATION OF PI(4,5)P2 IN THE PLASMA MEMBRANE THROUGH FRET IMAGING

M.J. Sarmento[1], A. Coutinho[1,2], M. Prieto[1], F. Fernandes[1,3].

E-mail: fernandesf@tecnico.ulisboa.pt

1. Centro de Química-Física Molecular and IN-Instituto de Nanociência e Nanotecnologia, Instituto Superior Técnico, Universidade de Lisboa. Lisbon, Portugal.
2. Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa. Lisbon, Portugal.
3. Research Unit on Applied Molecular Biosciences–Rede de Química e Tecnologia (UCIBIO-REQUIMTE), Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa. Caparica, Portugal.

KEY WORDS: Phosphatidylinositol-4,5-bisphosphate; Förster Resonance Energy Transfer; Lipid domains

Phosphatidylinositol-4,5-bisphosphate (PI(4,5)P_2) is a phospholipid concentrated in the inner leaflet of the plasma membrane, to which it recruits proteins involved in several cellular functions, many of which are abrogated in the absence of PI(4,5)P_2, illustrating the importance of this lipid. Protein regulation by PI(4,5)P_2 occurs as a result of spatially and temporally localized fluctuations of its concentration in the plasma membrane. In fact, the distribution of this lipid in the plasma membrane has been proposed to be heterogeneous, and PI(4,5)P_2 clustering is detected on model membranes under specific conditions. Domains highly enriched in PI(4,5)P_2 were also reported at the plasma membrane of specific cell types. However, for most cellular models, scarce evidence has been found for PI(4,5)P_2 segregation/clustering in the plasma membrane. Here, we aimed to characterize the distribution of PI(4,5)P_2 in the plasma membrane of cells where no heterogeneity in PI(4,5)P2 lateral distribution had been previously detected. To this end, FRET microscopy measurements with pleckstrin homology (PH) domains tagged with different fluorescent proteins were carried out. FRET microscopy data is evaluated through comparison with the theoretical expectation for FRET in the case of a homogeneous distribution of PH domains, and evidence for the formation of PI(4,5)P_2 enriched nanodomains is obtained. Results confirm that distinct PI(4,5)P_2 local densities are found in different cellular models, suggesting that PI(4,5)P_2 organization varies significantly between eukaryotic cells. In HeLa cells, cholesterol extraction had no impact on PI(4,5)P_2 compartmentalization. On the other hand, disruption of the cytoskeleton decreased significantly the compartmentalization of PI(4,5)P_2, proving that the organization of a major pool of PI(4,5)P_2 molecules depends on the presence of membrane-cytoskeleton interactions.

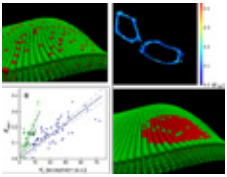

Figure 1. Plasma membrane nanoscale organization of PI(4,5)P_2 is shown to differ signifiacntly for different cellular models.

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CT5: LABEL-FREE MULTIMODAL MICRO-SPECTROSCOPIC DIFFERENTIATION OF DYSPLASIA STAGES IN MOUSE TONGUES AS A MODEL OF EARLY TUMOR GENESIS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA COMBINED WITH MULTIVARIATE DATA ANALYSIS

Mona Stefanakis[1], Tobias Krawietz[1], Jörg W. Bartsch[2], Garrit Koller[3], Ines Sequeira[3], Fiona Watts[3], Karsten Rebner[1], Edwin Ostertag[1].

E-mail: edwin.ostertag@reutlingen-university.de

1. Reutlingen University, Process Analysis and Technology, Alteburgstrasse 150. 72762 Reutlingen, Germany.
2. Philipps-University Marburg, Neurosurgery, Baldingerstrasse, 35033 Marburg, Germany.
3. King's College London, London SE1 9RT, United Kingdom.

KEY WORDS: multimodal optical spectroscopy, micro imaging, multivariate data analysis, head and neck squamous cell carcinoma, mouse model, dysplasia, cancer typing

Head and neck cancer represents the sixth most common type of cancer and encompasses epithelial malignancies that arise in the paranasal sinuses, nasal cavity, oral cavity, pharynx and larynx. Almost all of these epithelial malignancies are squamous cell carcinoma for the head and neck. The early detection of cancer is important for the patient's prognosis.

A mouse model is used to induce early stages of tumor genesis and to investigate the tissues with different optical spectroscopic techniques from the UV and VIS range to the infrared. Figure 1a shows a cross section of a mouse tongue as an example for the investigated organ. The tongues of one mice group are treated with carcinogen 4-Nitroquinoline N-oxide. A control group of healthy mice remains untreated. Several areas of the mice tongues are measured. Our multimodal spectral imaging concept combines optical microscopy with multimodal molecular and elastic light scattering spectroscopy. Laterally resolved spectroscopic information from chemical compounds is recorded together with the elastically scattered light linked to the morphology of the same area of the tissue. The multivariate data analysis, e.g. the principle component analysis (figure 1b), is a tool to extract the valuable information from the large number of measurements. The results are compared to the pathologist's assessment. The combination of the methods can help to get rid of the need for staining and to obtain a higher diagnostic safety for the tumor typing.

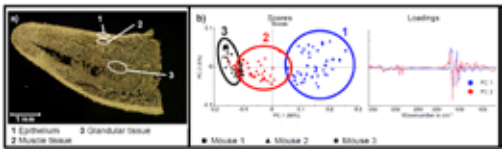

Figure 1. a) Elastic light scattering image of a healthy mouse tongue. Areas of interest are labelled. b) Principal component analysis of infrared spectra from 4000 cm^{-1} to 750 cm^{-1}(spectra are not shown here). Preprocessing: Smoothing Savitzky-Golay (2nd order, 15 points), mean normalization, 1st derivation Savitzky-Golay (2nd order, 15 points). On PC 1 the healthy tissue types can be distinguished.

CT6: ENHANCEMENT OF SECOND-HARMONIC MICROSCOPY IMAGES BY COMBINING ADAPTIVE OPTICS AND POLARIZATION-MODULATION

Francisco J. Ávila, Pablo Artal and Juan M. Bueno.

E-mail: franciscojavier.avila@um.es

Laboratorio de Óptica (CiOyN), Universidad de Murcia. 30100 Murcia, Spain.

KEY WORDS: Multiphoton microscopy, medical imaging, polarization

Second harmonic generation (SHG) microscopy is a non-invasive multiphoton technique to image collagen-based tissues. However, the image quality is limited by the optical aberrations induced by the samples. To compensate this, adaptive optics (AO) procedures have previously been used [1]. On SHG images, different features can be observed depending on both the incident polarization state (IPS) and the spatial distribution of collagen fibers [2]. Here we report a polarization-sensitive multiphoton microscope including AO to improve SHG imaging of tissues containing collagen.

A custom AO backscattered multiphoton microscope [1], has been modified to include a polarization state generator into the illumination pathway. This produces a set of independent IPSs. For each sample, SHG images were recorded without and with AO. Then, with the AO module in operation, the IPS was modulated by the generator. A z-motor coupled to the objective was used to acquire stacks of SHG images of thick samples. Non-stained ocular tissues (cornea and sclera) were used as specimens. The quality of SHG images was evaluated with different metrics as a function of the different experimental conditions. For 3D imaging, the intensity profiles as function of depth were analyzed. Changes in the visualization of ocular structures were also tested.

For a fixed IPS, a significant increase in the SHG signal was obtained and more details were visible with AO. Then, when the IPS was modified, an extra improvement was found and more spatial resolved features were observed. Polarization-control adds extra benefits to the effects produced by AO in terms of image enhancement. Results showed up to 3× improvement in SHG intensity, depending on the sample. This might help for a better detection of changes in the collagen structure, especially at deeper locations within the samples.

[1] J. M. Bueno, E. J. Gualda and P. Artal, “Adaptive optics multiphoton microscopy to study ex vivo ocular tissues,” J. Biom. Opt. 15(6), 066004 (2010).

[2] F. J. Ávila, O. Barco and J. M. Bueno, “Polarization dependence of aligned collagen tissues imaged with second harmonic generation microscopy,” J. Biom. Opt. 20(8), 086001 (2015).

CT7: CONFOCAL MICROSCOPY AND GENE EXPRESSION APPROACHES TO ANALYZE AUXIN DYNAMICS DURING MICROSPORE EMBRYOGENESIS

María C. Risueño[1], Héctor Rodríguez-Sanz[1], Yolanda Pérez-Pérez[1], María-Teresa Solís[1], María-Fernanda López[2], Aurelio Gómez-Cadenas[2], Pilar S. Testillano[1].

E-mail: risueno@cib.csic.es

1. Biological Research Center, CIB-CSIC. Ramiro de Maeztu 9, 28040 Madrid, Spain.
2. Dep. CC Agrarias y del Medio Natural, Univ.Jaume I, Campus Riu Sec, Castellón, Spain.

Auxin is involved in plant development and embryogenesis, but limited information is available on the dynamics of auxin during the two microspore pathways. In the anther, microspores follow the gametophitic pathway to form pollen grains. In contrast, isolated microspores are reprogrammed in vitro by stress, becoming totipotent cells and producing doubled-haploid embryos and plants via microspore embryogenesis. This work involved the analysis of auxin concentration and cellular accumulation; expression of TAA1, NIT2 and PIN1-like auxin biosynthesis and efflux carrier genes during the two microspore developmental pathways in Brassica napus and Hordeum vulgare. Effects of inhibition of auxin transport and action by N-1-naphthylphthalamic acid (NPA) and α-(p-Chlorophenoxy) isobutyric acid (PCIB) in microspore embryogenesis were also analyzed. Results indicated de novo auxin synthesis after microspore reprogramming, accompanying first cell divisions, and its increase during embryogenesis, correlating with expression of TAA1, NIT2 and PIN1. Auxin was evenly distributed in early embryos, whereas in heart-torpedo embryos auxin was accumulated in apical and basal embryo regions. Inhibition of polar auxin transport (PAT) and action, by NPA and PCIB, impaired embryo development. In contrast, auxin levels, TAA1 and PIN1 expression were high at early microspore development, while they progressive decreased during development. Findings indicate different auxin dynamics in the two microspore pathways with different fates.

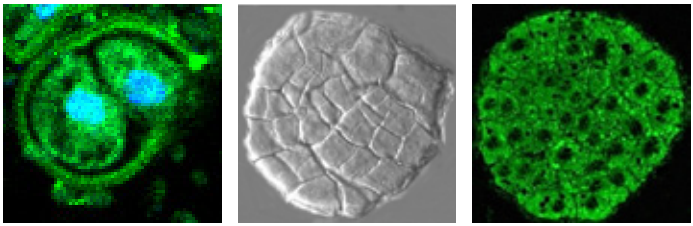

Figure 1. Auxin immunofluorescence on microspore-derived embryos of Brassica napus.

[1] Rodríguez-Sanz et al. (2015) Plant Cell Phys. 56, 1401-1417

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CT8: EQUILIBRIUM PARAMETERS DERIVED FROM FRET EXPERIMENTS IN LIVING CELLS

Alvaro Villarroel[1], Araitz Alberdi[1], Carolina Gomis-Perez[1], Pilar Areso[2].

E-mail: alvaro.villarroel@csic.es

1. Instituto Biofisika, CSIC, UPV/EHU, Barrio Sarriena s/n. Leioa, Spain.
2. Dpt. Farmacología, UPV/EHU, Barrio Sarriena s/n, Leioa, Spain.

KEY WORDS: Living cells, quantitative microscopy, FRET, three-cube FRET, stoichiometry

FRET data contains information about the amount of free and total donor, acceptor and complex. When deconvoluted appropriately, a full description of a ligand-receptor equilibrium could be achieved. This idea has been championed by David T. Yue, and popularized under the label "three-cube FRET" [1; 2]. Although the advantages of obtaining binding parameters in living cells studying sensitized FRET using inexpensive microscopes and simple equipment is very attractive, the error sources coming from cell thickness variability, environmental fluorophore sensitivity, presence of endogenous ligands, etc., are not well understood. In addition, the original description warned that the method is limited to a 1:1 stoichiometry [2].

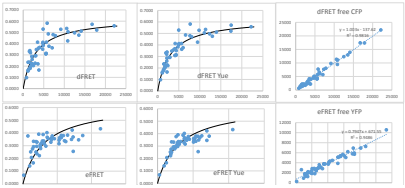

Figure 1. Comparison of data processed with our method (first column) and Yue's method (second column). The third columns contains date from first column plotted against date from second column.

To shed light on this method, we have simulated the impact of cell thickness variations and fluorophore spectral changes. Furthermore, we have developed an alternative method for data processing that does not rely on a 1:1 stoichiometry presumption. Our method complements that of Yue, and reinforces data interpretation when both are used in parallel. Finally, simulations of a 1:2 stoichiometry show that the absolute FRET value taking free acceptor as reference tends to reach half of that obtained when the reference is the free donor. Thus, three-cube FRET could help unveiling the stoichiometry of interactions studied in living cells.

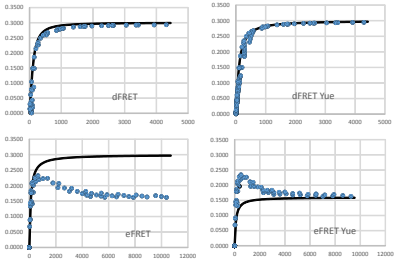

Figure 2. Results of a simulation with a 1:2 stoichiometry analyzed with our method (left), and Yue's method (right) taking free donor (top) or free acceptor (bottom) as reference.

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CT9: MOLECULAR IMAGES OF PLITIDEPSIN-EEF1A COMPLEXES IN LIVING TUMOR CELLS

C. García[1], A. Losada[2], J.M. Molina-Guijarro[2], M.A. Sacristán[1], J. Martinez[2], C.M. Galmarini[2], M.P. Lillo[1].

E-mail: pilar.lillo@iqfr.csic.es

1. Departamento de Química Física Biológica. Instituto de Química-Física "Rocasolano" (CSIC), 28006 Madrid, Spain.
2. Departamento de Biología Celular y Farmacogenómica, Pharma Mar S.A., Colmenar Viejo, 28770 Madrid, Spain.

KEY WORDS: Living cells, quantitative microscopy, functional imaging, fluorescence microspectroscopy, FLIM, FRET, phasor,

Plitidepsin (Aplidin®, APL), a cyclic depsipeptide originally from the marine tunicate *Aplidium albicans*, is currently in a Phase III clinical trial in patients with relapsed or refractory multiple myeloma. It is reported that, in tumor cells, eEF1A2 is the potential target for Aplidin®. Here, we took advantage of two-photon micro-spectroscopy to localize the compound in treated tumor cells and shed light on its *in vivo* interaction with eEF1A2.

HeLa-WT and HeLa-APLR (resistant to Aplidin®) cell lines have been previously described. HeLa AplR present lower eEF1A2 levels than HeLa wt cells. We stably transfected HeLa AplR cells with plasmids encoding GFP-tagged eEF1A1 or eEF1A2, and selected clones expressing physiological levels of the fusion proteins. A fluorescent Aplidin® derivative, APL-DMAC, was used to track the localization and interactions of the compound. 3D-scanning two-photon microscopy was performed with an Olympus IX71 microscope equipped with an UPLSAPO 60× objective, an excitation laser beam (750 nm, MaiTai, SpectraPhysics) and a MicroTime200 (PicoQuant, GmbH).

Using a FLIM-phasor approach we identified different plitidepsin species within cells, depending on their subcellular location and the spectroscopic properties of DMAC in the Aplidin complexes: Plasma membrane, Cell cortex and Cytoplasm. We applied the FLIMphasor approach to quantify species and measure FRET between the fluorescent tags dmac and GFP, thus identifying different eEF1A-APL complexes.

The work was supported in part by Ministerio de Economía y Competitividad grant number FIS2015- 70339-C2-2-R, and by a grant from PharmaMar S.A.

Conflict of interest: Other substantive relationships: AL, JMMG, JFML and CMG are Pharmamar SA employees and shareholders

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CT10: CORRECTION OF ATTENUATION ARTEFACTS IN FLUORESCENCE MICROSCOPY

Jürgen Mayer[1,2], James Sharpe[1,2,3], Jim Swoger[1,2].

E-mail: jim.swoger@crg.eu

1. Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Dr. Aiguader 88., Barcelona 08003, Spain.
2. Universitat Pompeu Fabra (UPF), Barcelona, Spain.
3. Institució Catalana de Recerca i Estudis Avançats (ICREA), Pg. Lluis Companys 23. Barcelona 08010, Spain.

KEY WORDS: SPIM, OPT, OPTiSPIM, light-sheet microscopy, fluorescence microscopy

In recent years, Light-Sheet Fluorescence Microscopy (LSFM) has become an important tool in fields such as developmental biology, neurology, and immunology. Although LSFM techniques can provide high resolution images in 3D mesoscopic samples while causing minimal photo-damage, they often suffer from “stripe” or “shadow” artefacts caused by the attenuation of light by features of the sample. Various methods to reduce these effects have been proposed (e.g. multi-view imaging [1] & mSPIM [2]), but in general the problem remains.

We recently introduced the OPTiSPIM [3], combining Optical Projection Tomography (OPT - an implementation of the idea of x-ray computed tomography at visible wavelengths), and Selective Plane Illumination Microscopy (SPIM, a version of LSFM). While SPIM generates high-resolution 3D fluorescence data sets, OPT can provide a 3D map of the distribution of a sample’s attenuation. This attenuation map can then be used to computationally correct the shadow artefacts in the SPIM data. I will discuss how this is done, and present examples illustrating the usefulness of the method.

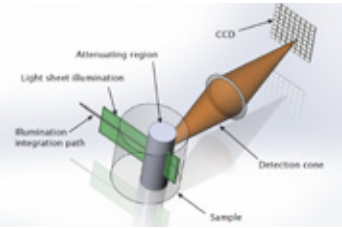

Figure 1. Schematic of light paths subject to attenuation in LSFM imaging.

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CT11: OPENSPIN AND OPENT MESOSCOPIC IMAGING FOR CELL & DEVELOPMENTAL BIOLOGY

Gabriel G. Martins[1]; Hugo Pereira[1], Nuno Martins[1], Tiago Vale[1], Emilio Gualda[2] & Nuno Moreno[1].

E-mail: gaby@igc.gulbenkian.pt

IGC - Instituto Gulbenkian de Ciência. Oeiras. Portugal.

ICFO - Institut de Ciències Fotòniques. Barcelona. Spain.

KEY WORDS: Light-sheet, Optical-tomography, OPT, Mesoscopy, 3D, Development

A standing challenge in developmental biology is the study of morphogenetic processes in vivo and in toto, ie, inside whole embryos. Conventional microscopy relies on thin tissues, typically 1-to-few cell layers, or 3D imaging of larger tissues, often in vivo, using confocal or 2p microscopy. None of these techniques have allowed deep observation in most mid-to-late stage vertebrate embryos. Two recently developed techniques have come to aid, both with fixed and live specimens: optical tomography (aka OPT) and light-sheet microscopy and (1,2). Although there has been an increasing number of commercial implementations of light-sheet systems, currently there is no commercial solution for OPT. DIY is still a viable and valuable access to these technologies. We have developed the OpenSpin microscopy platform (3) which offers a simple solution for implementation and operation of both light-sheet and optical tomography mesoscopy systems. We have made improvements on the original light-sheet setup to allow double-sided illumination and dual camera imaging, and prepared a simpler solution for those interested only in optical tomography, known as OPenT. Here we present examples of high-quality images obtained with these low-cost/high-performance prototypes which can easily be implemented in most developmental biology/microscopy labs without advanced technical capabilities. We also present a simple online anatomical database resource for developmental biologists, being prepared with open-source solutions, known currently as “Haeckaliens” and available online at: www.gabygmartins.info/research/haeckaliens.

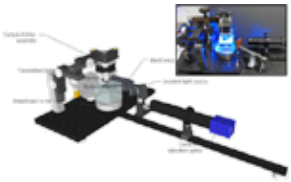

Figure 1. OPenT scanner 3D diagram* (above) and example of a 3D reconstruction of a E14.5 mouse embryo obtained from an OPenT dataset and prepared with the Drishti software.
* THE PROTOTYPE SCANNER WILL BE AVAILABLE AT THE MEETING FOR VISITORS TO SEE AND USE!

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CT12: HIGH-THROUGHPUT THREE-DIMENSIONAL CELLULAR MODELS IMAGING THROUGH FLOW LIGHT-SHEET FLUORESCENCE MICROSCOPY CYTOMETRY

Emilio J. Gualda[1,2], Pablo Loza-Alvarez[1], Marta F. Estrada[3] and Catarina Brito[3].

E-mail: emilio.gualda@gmail.com

1. ICFO-Institut de Ciencies Fotóniques, BIST-Barcelona Institute of Science and Technology, 08860 Castelldefels, Spain.
2. IGC-Instituto Gulbenkian de Ciência, 2780-156 Oeiras, Portugal.
3. iBET-Instituto de Biologia Experimental e Tecnológica, 2780-157 Oeiras, Portugal.

KEY WORDS: light-sheet fluorescence microscopy, flow cytometry, 3D cell culture

Drug screens on complex cell models and organisms are a key factor to understand and treat human diseases. However, fast and effective conclusions have been hindered by the lack of robust and predictable models amenable to high-throughput (HT) analysis. Recently, important advances have been made towards the development of 3D cellular models, using human immortalized cell lines, stem cells and other patient derived cells, which better recapitulate features of tissues. These advances bridge the gap between adherent cell culture and animal models, making 3D cellular aggregates an extremely powerful in vitro model for preclinical research.

A major hurdle, hampering the widespread utilization of complex in vitro models, is the lack of robust analytical tools. Light sheet fluorescence microscopy (LSFM) has been proposed to overcome those limitations. We have created the first flow cytometry system based on LSFM able to handle 3D cell cultures, SPIM-Fluid [1, 2], following an open source philosophy. This novel LSFM configuration, fuses its inherent capabilities with microfluidics, allowing massive live 3D cell cultures studies in real-time and sophisticated cell-based assays in 3D cell cultures.

Using the SPIM-Fluid system, we are able to make HT quantitative analysis of the spatio-temporal organization of the different cell types in spheroids, as well as the response to different environmental conditions with high resolution, high speed and minimal photo-damage. We will show how the use of 3D-cell cultures and full imaging system automation will contribute to measure with statistical relevance a large set of biological parameters to investigate drug response on the central nervous system [3], cancer therapy [4] and cell differentiation.

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P1: MULTIPHOTON IMAGE IMPROVEMENT WITH LASER PULSE COMPRESSION

Juan M. Bueno, Martin Skorsetz and Pablo Artal.

E-mail: bueno@um.es

Laboratorio de Óptica (CiOyN), Universidad de Murcia, 30100 Murcia, Spain.

KEY WORDS: Multiphoton microscopy, pulse compression

Multiphoton (MP) microscopy is based on the quasi-simultaneous absorption of two infrared photons. This is a powerful non-linear tool for biomedical imaging without the need of labelling techniques [1]. MP techniques use ultrafast lasers providing light pulses of some hundreds of femtoseconds. However, due to optical dispersion, these pulses are broadening in time when travelling through the optics of the microscope and the sample. This reduces the peak power and consequently the MP effectiveness. To compensate this, higher laser power is needed, what increases the risk of non-controlled thermal side effects and photodamage. Similar to the pre-shaping operation of an adaptive element to correct for the aberrations [2], it is also possible to minimize the dispersion effects by pre-compensate the broadening of the laser pulse. In this sense, here we propose the use of a variable pulse compressor. This will provide shorter pulses at the microscope entrance and will be used to optimize multiphoton microscopy imaging.

A two-prism pulse compressor was incorporated into the illumination pathway of a custom multiphoton microscope [2, 3]. Thick samples providing different types of non-linear signal were involved in the experiment. Results showed that pulse compression significantly improved the recorded signal and more details were visible. This improvement depended on the type of nonlinear signal and the depth location of the imaged plane. Moreover, the pre-compensated laser beam produces similar images than those acquired with the uncompensated laser beam but with a laser power reduced up to ~45%. The combination of pulse compression effects and MP microscopy has big potential to enhance imaging performance of biological samples.

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P2: AUTOMATIC DIATOM IDENTIFICATION FOR WATER QUALITY ASSESSMENT (AQUALITAS)

G. Cristóbal[1], G. Bueno[2], S. Blanco[3], C. Sánchez[1], M. Borrego-Ramos[3], A. Pedraza[2], J. Ruiz-Santaquiteria[2] and J.L. Espinosa-Aranda[2].

E-mail: gabriel@optica.csic.es

1. Instituto de Óptica (CSIC), Serrano 121, 28006 Madrid, Spain.
2. VISILAB, ETS Ing. Industriales, Univ. Castilla la Mancha, 13005 Ciudad Real, Spain.
3. Institute of Environment, Univ. León, La Serna 58, 24007 León, Spain.

KEY WORDS: water quality, diatom, automatic identification, texture analysis, low cost microscopy

The development of an image analysis system for automatic taxonomic identification of diatoms is presented here, with the aim to facilitate the calculation of biotic indices for network control and monitoring of the environmental status in European rivers. These algae are very useful in monitoring the quality of water, hence the importance of automating the analysis process. The conventional approach has usually consisted of identification and quantification of diatom's valve by optical microscopy [1]. Hence there exists a need for automated recognition techniques for diagnostic tools (networks of environmental monitoring, early warning systems) to facilitate proper management of water resources and decision-making processes. Elsewhere, manual image analysis of such systems is impractical due to the huge diversity of this group of microalgae and its great morphological plasticity. According to a European directive identifying a minimum of 400 valves are needed for determining water quality indices [2,3]. Here, it is intended to realistically cover the entire workflow of a bioindicator system from capture, analysis, identification and determination of quality indices.

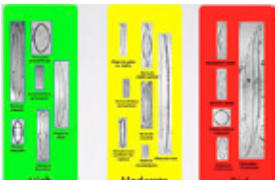

Figure 1. Diatom species under different water quality conditions

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P3: REACTIVE NEURAL STEM CELLS IN THE ADULT BRAIN

Soraya Martín-Suárez[1], Roberto Valcárcel-Martín[1], Oier Pastor-Alonso[1], José R Pineda[1], Juan M Encinas[1,2,3].

E-mail: jm.encinas@ikerbasque.org

1. Achucarro Basque Center for Neuroscience. Zamudio E-48170, Spain.
2. Ikerbasque, The Basque Foundation for Science, Bilbao E-48013, Spain.
3. University of the Basque Country (UPV/EHU). Leioa E-48190, Spain.

KEY WORDS: Neural stem cells, neurogenesis, hippocampus, neuronal hyperexcitation, quantitative confocal microscopy.

In the adult hippocampus of most mammals, including humans, a mostly quiescent population of neural stem cells (NSCs) generates neurons and astrocytes throughout adulthood. New neurons integrate into the hippocampal circuitry and play a role in spatial-related memory and learning, as well as in pattern separation, and responses to fear and stress. Using confocal microscopy-based quantitative imaging in combination with constitutive and inducible transgenic mice, we have recently discovered [1] that after epileptic seizures NSCs become reactive (RNSCs), as they get activated massively and transform into reactive astrocytes (RAs) abandoning their neurogenic potential (Fig.1). This seizure-induced impairment of neurogenesis might explain some of the cognitive deficits and comorbidities associated with mesial temporal lobe epilepsy (MTLE). MTLE specifically affects the hippocampus and related structures and is characterized by poor prognosis and being resistant to drug treatment. We have found that the induction of RNSCs in a rodent model of MTLE is regulated by the epidermal growth factor receptor (EGFR) signalling pathway. Antagonizing EGFR ameliorates the induction of RNSCs and partially preserves neurogenesis. In addition, we have identified the lysophosphatidic acid receptor 1 (LPAR1) as a potential specific marker for RNSC-derived RAs in the epileptic hippocampus using the LPAR1-eGFP transgenic mice, suggesting differences between this cell type and parenchymal astrocytes-derived RAs. Finally, we have found that with aging, NSCs also acquire a reactive-like phenotype that associates with impaired functionality. Together, our results provide new insight into the plasticity and heterogeneity of hippocampal NSCs and highlight their potential as therapeutic targets in epilepsy and aging.

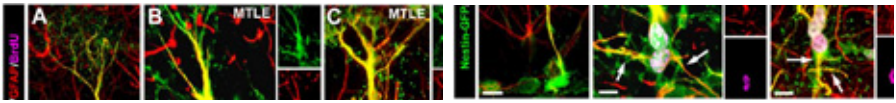

Figure 1. Seizures mimicking mesial temporal lobe epilepsy (MTLE) induce hippocampal NSCs (A) to become reactive. NSCs become massively activated switching to a reactive-like morphology and to a symmetric mode of cell division (B and C) to generate reactive astrocytes. Scale bar: 10 µm.

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P4: OPTIC, GENETIC AND PHYSIC TOOLS TO BETTER CHARACTERIZE TUNNELING NANOTUBE STRUCTURES CREATED BY GLIOMA STEM CELLS

Jose R. Pineda[1,2], Thierry Kortulewski[1,*], Maya Jeitany[1,*] Laurent Gauthier[1], Marie P. Junier[3,4,5,*], Hervé Chneiweiss[3,4,5,*], Annie Andrieux[6], François D. Boussin[1].

E-mail: jr.pineda@achucarro.org

1. CEA DSV iRCM SCSR, INSERM, Laboratoire de Radiopathologie, UMR967, F-92265 Fontenay-aux-Roses, France.
2. Achucarro Basque Center for Neuroscience, Parque Tecnológico de Bizkaia, E-48170 Zamudio, Vizcaya, Spain.
3. CNRS UMR8246 Neuroscience Paris Seine - IBPS; 7 quai Saint-Bernard 75005 Paris, France.
4. INSERM U1130, Neuroscience Paris Seine - IBPS; 7 quai Saint-Bernard 75005 Paris, France.
5. University Pierre and Marie Curie UMCR18, Neuroscience Paris Seine - IBPS; Team GP; 7 quai Saint-Bernard 75005 Paris, France.
6. CEA, IRTSV-GPC, INSERM, U836-GIN Site Santé La Tronche, BP170, 38042 Grenoble. Cedex 9, France.

*Contributed equally to this work.

KEY WORDS: Living cells, electroporation, confocal videomicroscopy, dynamic multiparametric analysis by impedance measurement, Deeper analysis.

Glioblastoma multiforme is the latest stage of malignant tumor of the adult central nervous system. Actual palliative treatments extend the median of survival to less than one year. Surgical resection fails to completely extirpate the tumor given the high infiltrative nature of invading cells with high stemness characteristics identified as glioma stem-like cells (GSC).
One of the key aspects of glioma dispersion is the ability to coordinate cell activities to integrate and synchronize tasks such as migration and invasion. Intercellular bridges, also known as tunneling nanotubes (TNT) were first described in cells of the immune system as an intimate form of long-range communication coordinating cell-division and migration [1]. TNT are de novo formation of a thin membrane channels with F-Actin cytoskeleton that permits direct transfer and share of cytoplasm content recently described in in vivo cancer cells [2].
In this work we use several optic, genetic and physic tools to better characterize TNT structures created by GSC in which Aurora B kinase is strongly involved. Contrary to mid-bodies, TNT structures are devoid of INCENP and accumulation of Aurora B is asymmetrical between cells. Our results show that upon genetical modulation (Aurora B overexpression or silencing by siRNA) or its pharmacological inhibition (using the specific Aurora B inhibitor Hesperadin) TNT alters cell-to-cell detaching and cell adhesion dynamics to substrate, affecting profoundly to the GSC migratory properties, determined by deeper analysis, and promoting a progressive cell clustering determined by both, confocal videomicroscopy tracking and multiparametric analysis by impedance measurement.
The present study demonstrated that Aurora B is a valid target to block GSC dispersion leading to progressive GSC aggregation into a cellular mass where no cell can escape.

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P5: COMPARISON OF FORWARD AND BACKARD EMITTED POLARISATION SENSITIVE SECOND HARMONIC GENERATION FOR THE STUDY OF HUMAN CORNEAL TISSUE

David Merino[1], Giuseppe Lombardo[2], Rita Mencucci[3], Marco Lombardo[5], Pablo Loza-Alvarez[1].

E-mail: pablo.loza@icfo.es

1. ICFO – The Institute of Photonic Sciences, 08860 Castelldefels, Spain.
2. Consiglio Nazionale delle Ricerche Istituto per i Processi Chimico-Fisici, CNR-IPCF, 98158 Messina, Italy.
3. Università di Firenze, 50121 Firenze, Italy.
4. Fondazione G. B. Bietti, 00198 Roma, Italy.

KEY WORDS: nonlinear microscopy, polarization sensitive second harmonic generation, pSHG, cornea, collagen.

Keratoconus is a disease that is believed to affect the structural order and orientation of collagen fibril bundles in the cornea. Although the use of polarisation sensitive second harmonic generation (pSHG) has been used to characterize this structure [1], a non-invasive application of the technique that can be used to do this in-vivo has not been reported to date. We have studied nonlinear signal generated in human corneas affected by keratoconus, from which we acquired simultaneously pSHG images generated in the forward and backward directions, as well as two photon excitation fluorescence (TPEF). Fig 1. shows an example of the results acquired. We have observed correspondence between the direction of the collagen fibers calculated from pSHG signal in the forward and backward direction. This is a very important step toward the implementation of a clinical application of pSHG for the early diagnosis of keratoconus.

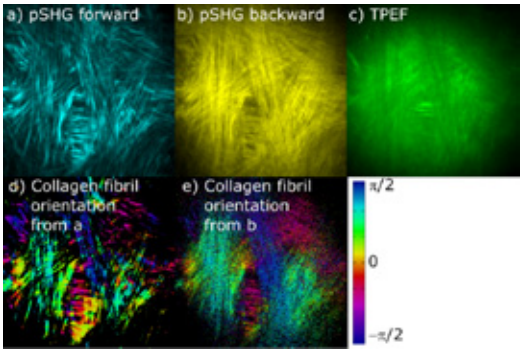

Figure 1. Forward (a) and backward (b) pSHG images and TPEF image (c) of a human cornea affected by keratoconus. Images are taken 125µm deep in the tissue, with a field of view of 250µm. d) Shows the orientation of the collagen fibers calculated from the data in a) and e) is the orientation of the fibers calculated from the data in b).

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P6: MOLECULAR MONITORING OF BIOMEDICAL SAMPLES BY RAMAN IMAGING AND CHEMOMETRICS

Monica Marro[1], Pablo Loza-Alvarez[1].

E-mail: pablo.loza@icfo.es

1. ICFO-Institut de Ciencies Fotoniques, The Barcelona Institute of Science and Technology. Castelldefels, Spain.

KEY WORDS: Raman spectroscopy, chemometrics, cancer, neurodegeneration, food analysis

Raman spectroscopy (RS) is non-invasive, rapid and sensitive. Thus, RS represents a promising technique for studying biomedical samples. However, although RS has the maximum specificity among all optical techniques for detecting molecular changes, the interpretation of Raman spectra is complex. Mathematical methods used in the past, have difficulties to extract meaningful molecular components from the Raman spectra that could be assigned to pure molecules constituting the sample and monitor them during a biochemical process. For this reason, we proposed to apply Multivariate Curve Resolution (MCR) to deconvolve pure molecular components from the Raman spectra and monitor its content in the tissue or cell over the illness or biological process under study. MCR requires minimal a priori knowledge of the system providing objective and chemically meaningful information. We present several successful biomedical applications of this approach in different fields like neuroinflammation, cancer or food analysis. First, retinal tissue is damaged during inflammation in Multiple Sclerosis. We assess molecular changes in murine retinal cultures suffering inflammation by RS [1]. By using MCR analysis, we deconvolved 6 molecular components suffering dynamic changes along inflammatory process. Those include the increase of immune mediators, changes in molecules involved in energy production and decrease of Phosphatidylcholine. Following this work, individual retina cell lines are studied inducing different challenges to investigate which metabolites are synthetized in each cell line. RS combined with MCR allows monitoring the evolution of retina inflammation based in a number of molecular components sensitive to inflammation.

Second, we study the molecular composition of cancerous tissue and healthy tissue by Raman imaging, identifying regions that are compositionally changing during the previous steps of malignancy. This, combined with our previous work on cells studying the Epithelial to Mesenchymal transition (EMT) [2] shows that our approach permitted to deconvolve and track biomarkers for cancer aggressiveness and prognosis.

Finally, applications in food monitoring as the tomato ripening imaging will be shown. Thus, the combination of Raman imaging of biomedical samples and MCR represents a novel methodology that will push forward the applicability of RS for non-invasive monitoring of the biochemical content in vivo.

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P7: NEUBIAS: THE NETWORK OF BIOIMAGE ANALYSTS

Sébastien Tosi[1], Perrine Paul-Gilloteaux[2,3], Kota Miura[1], Julien Colombelli [1].

E-mail: julien.colombelli@irbbarcelona.org

1. Institute for Research in Biomedicine, IRB Barcelona, Spain.
2. PF MicroPICell IRS-UN 8, Université de Nantes, France.
3. France BioImaging.
4. European Molecular Biology Laboratory, EMBL - Heidelberg, Germany.

KEY WORDS: STYLE: **KEYWORDS:** BioImage Analysis, Training, Network, knowledge transfer, repository, Image Analysis tools, workflows, Staff exchange,

Bioimage analysts are new experts that are increasingly wanted, mostly by imaging facilities, to analyze image data in life sciences. We introduce the startup of a network of those experts funded by the COST framework (COST Action CA15124). The network aims at establishing a community to boost the output of bioimaging-based research in Europe. BioImage Analysis (BIAS) is a critical step in imaging-based research and currently is its narrowest bottleneck. A pilot survey showed that only 42% of acquired image data is quantitatively analyzed, that 95% of those surveyed considered BIAS important-to-essential in their research, and that 68% considered more support and training are required. Consequently, the huge investments made by research institutes in imaging systems are not well balanced by the capacity to analyze the data produced. Moreover, size and quantity of image data are constantly increasing and stronger support is inevitably needed. Bioimage analysts are emerging in various research institutions to increase the fraction of analyzed image data and their activity is however mostly local, not well-networked and poorly coordinated.

Our network, NEUBIAS, aims at 1) establishing the identity and career path of bioimage analysts as a bridge between information science and Life/Biomedical science; 2) working jointly to solve bioimage analysis problems with the development of training framework, the organization of analysis tools and their benchmarking, the sharing of workflows, and the establishment of open publication platforms; and 3) becoming a responsible body for keeping the scientific integrity of image data analysis in the Life Science and biomedical fields.

This Action aspires to support the long-term scientific goals of European science and industry by bridging essential fields of scientific excellence. We will explain the objectives and planned activities of NEUBIAS for achieving these aims.

Main Reference: www.neubias.org

P8: REMYELINIZATION STUDIES WITH CONFOCAL FLUORESCENCE MICROSCOPY

Iñaki Osorio-Querejeta[1,2*], Irantzu Llarena[3*], Maider Muñoz-Culla[1,2], Pedro Ramos-Cabrer [4,5], David Otaegui [1,2] .

E-mail: david.otaegui@biodonostia.org

1. Grupo de esclerosis múltiple, Área de Neurociencias, IIS Biodonostia, Donostia-San Sebastian, Spain.
2. REEM: Spanish network on Multiple Sclerosis (Red española de esclerosis múltiple -REEM-), Edificio CELLEX. Barcelona, Spain.
3. Plataforma de espectroscopía óptica, CICbiomaGUNE, 20009, San Sebastián, Spain.
4. Molecular Imaging Unit, CIC biomaGUNE. Donostia-San Sebastian, Spain.
5. Ikerbasque, Basque Foundation for Science. Bilbao, Spain.

* both authors have contributed equally to the poster

KEY WORDS: Multiple sclerosis, remyelination, quantitative microscopy, colocalization analysis.

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) in which myelin is attacked by lymphocytes generating demyelinating plaques. In an early state of the disease, the organism is capable of restoring the myelin sheath and the nerve impulse transmission. Nevertheless, with the disease progression, this ability declines, causing the characteristic neurodegeneration of the chronic state of the disease.

In this work ex-vivo models are used to evaluate the capacity of myelin recovery (remyelination) after an outburst and compare them to control samples. Confocal fluorescence imaging in combination with magnetic resonance imaging (MRI) and western blot (WB) have been used to try to quantify the remyelination progress.

To quantify the remyelination level, colocalization studies of z-stacks at different positions in the sample are performed. These colocalization analyses use to havelarge variabilityand therefore, different analysis strategies will be discussed, suchas the use of the different colocalization plugins (eg: Coloc2 or JaCOP from Image J). This information will be compared with MRI and WB data trying to find the more suitable remyelination quantification method.

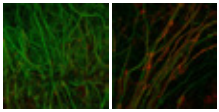

Figure 1. Myelinated axons as control (left) andaxons with depleted myelin (right)

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P9: STEREOLOGICAL ASSESSMENT OF IMMUNE CELLS INFILTRATION IN KIDNEY TRANSPLANT BIOPSIES

Elena Aguado[1], Konstantin Levitsky[1], Isabel Aguilera[1], Antonio Nuñez[1].

E-mail: eaguado-ibis@us.es

1. Instituto de Biomedicina de Sevilla IBiS/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Avda Manuel Siurot s/n, 41013 Seville, Spain.

KEY WORDS: Stereology, Systematic uniform random sampling, Immunohistochemistry, Kidney transplantation, Allograft rejection.

Immune-mediated rejection is the main cause of graft lost in patients who underwent kidney transplantation. Inflammation and interstitial infiltration are diagnosis criteria; however, the immune cells infiltrating the allograft have not been fully categorized. At this point, we propose a methodology to be able to better diagnose patients with graft rejection episodes.

We have designed a protocol that provides an accurate, unbiased, and reproducible tool based on stereology, that will allow the characterization of the cells and the extension of the cellular infiltrates displayed in kidney transplant biopsies with diagnosis of rejection.

This retrospective study includes a cohort of 102 kidney transplant biopsies. According to the histological features, clinical diagnoses have been made by an expert pathologist following Banff criteria. Immune cells such as CD4 and CD8 T lymphocytes, T regulatory lymphocytes, B lymphocytes, plasma cells, NK cells, and macrophages, were identified by immunohistochemistry experiments. Immunostained slides were assessed using our protocol based on Visiopharm® computer-assisted stereology quantification method, which leads to the estimation of number of cells per area.

In this study we have developed a semi-automated cellular quantification protocol, which constitute a novel tool to define cell profiles in an unbiased and accurate manner, in order to contribute to more reliable rejection diagnoses in kidney transplantation.

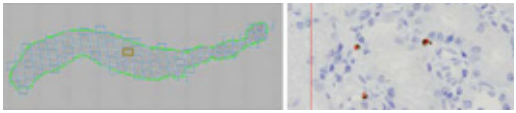

Figure 1. Stereology sampling of a kidney biopsy tissue sample and cell count magnification in a Counting Frame.

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P10: MANAGING IMAGE AND IMMUNOHISTOCHEMISTRY FACILITY CORE IN IDIPAZ, A HEALTH RESEARCH INSTITUTE ENVIRONMENT

Algarra E[1]. and Vallejo-Cremades MT[1].

E-mail: tvallejo.hulp@salud.madrid.org

1. Image and Immunohistochemistry Facility Core, IdiPaz, La Paz University Hospital, 28046 Madrid, Spain.

KEY WORDS: research core managing, Immunohistochemistry, microscopy, services.

The Image Core Unit is an innovative platform supported by Hospital La Paz Research Institute (IdiPAZ) and La Paz University Hospital. At the end of 2014, we moved to new installations at I+D building that offer more quality and security environment for users and staff. The laboratory aims are encompassed in the continuation and improvement of the portfolio of techniques and facilities available to date. The objectives are based on technical quality and innovation criteria to improve the laboratory's facilities and the promotion of the Bioimaging techniques applied to clinical and basic health science research.

The cooperation with other facilities provided by IdiPAZ through its platforms and laboratories enables us to develop and implement new techniques and protocols. The platform offers complete flexibility and adaptability to the specific requirements of each by providing high-end instruments, scientific and technical assistance in the histology and image analysis fields for Biomedical and Health Sciences.

Originally, our research cores were developed by individuals, then, in a few years, it was growing institutional organization and funding systematic investment for infrastructure and staff recruitment. This cooperative research environment allowed facilitating communication between clinical/basic researchers and core scientists. The kind of core facility that we offer could be a centralized *Research*, where everyone gets access with lights and shadows in organization, funding and staff recruitment [1]. This situation allows users minimizing costs; institution absorbs technical oversight and service contracts. The services of the image core facility are available to all scientists of the IdiPaz in collaboration or Do-It-Yourself model and to external research partners in fee model.

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P11: MULTIMODAL IMAGING OF NANOSTRUCTURED MATERIALS AND BIOLOGICAL SAMPLES IN THE FAR-FIELD AND NEAR-FIELD REGIMES

Stefan G. Stanciu[1], Denis E. Tranca[1], Catalin Stoichita[1], Radu Hristu[1], Laura Pastorino[2], Juan M. Bueno[3], Carmelina Ruggiero[2], Alexei Antipov[4] and George A. Stanciu[1].

E-mail: stefan.stanciu@cmmip-upb.org

1. Center for Microscopy-Microanalysis and Information Processing, University Politehnica of Bucharest, Romania.
2. Department of Informatics, Bioengineering, Robotics and Systems Engineering, University of Genova, Italy.
3. Laboratorio de Óptica, Universidad de Murcia, Spain.
4. PlasmaChem GmbH. Berlin, Germany.

KEY WORDS: multimodal imaging, laser scanning microscopy, apertureless scanning near-field optical microscopy, instrumentation development.

We present a multimodal imaging system capable to collect optical data on overlapping field-of-views with multiple imaging techniques operating in the far- and near-field regimes, and a series of applications. The contrast mechanisms of the embedded imaging techniques provide complementary information, which plays an important role in facilitating nanoscale data understanding and interpretation. The architecture of this imaging system is a flexible one, allowing the potential implementation of future upgrades aimed at adding new work modes. Using this multimodal architecture we have imaged nanostructured materials and biological samples using variants of Apertureless Scanning Near-Field Optical Microscopy and Laser Scanning Microscopy, in tandem. The achieved results stand as direct evidence that, although challenging, correlating micro- and nanoscale data by combined imaging approaches holds significant potential for enabling new perspectives in many fields of science such as materials science, biology or medicine.

ACKNOWLEDGMENTS: The contribution of UPB, UG and PlasmaChem was partially supported by the LANIR Project funded by the European Community's Seventh Framework Programme (FP7/2012-2015) under grant agreement n°280804. UPB's contribution was supported as well by the PN-II-RU-TE-2014-4-1803 and PNII-PT-PCCA-2011-3.2-1162 Research Grants, funded by the Romanian Executive Agency for Higher Education, Research, Development and Innovation Funding (UEFISCDI). The contribution of J.M. Bueno was supported by the Spanish SEIDI through the research grants FIS2013-41237-R and FIS2015-71933-REDT.

P12: DUAL ROLE OF CARDIOLIPIN IN MCL1 MEMBRANE ACTIVITY REVEALED BY MULTIPLE FLUORESCENCE-BASED TECHNIQUES APPLIED TO MINIMALIST SYSTEMS

Olatz Landeta[1], Hector Flores-Romero[1], Ana Garcia Saez[2], Gorka Basañez[1].

Email: gorka.basanez@ehu.eus

1. Biofisika Institute, Centro Mixto Centro Superior de Investigaciones Científicas (CSIC)-EuskalHerrikoUnibertsitatea/Universidad del País Vasco (EHU/UPV), Barrio Sarriena s/n, Leioa 48940. Spain.
2. Interfaculty Institute for Biochemistry, University of Tübingen, Germany, Max-Planck Institute for Intelligent Systems, Heisenbergstrasse 3, 70569 Stuttgart, Germany, and German Cancer Research Center, BioQuant, ImNeuenheimer Feld 267, 69120 Heidelberg, Germany.

KEY WORDS: Mitochondrial apoptosis, B-cell lymphoma 2 (BCL2) protein family, Cardiolipin, membrane topology, fluorescence correlation spectroscopy (FCS), Fluorescence resonance energy transfer (FRET).

BCL2 family proteins are key regulators of mitochondrial apoptosis that also contribute significantly to tumorigenesis. Increasing evidence indicates that different mitochondrial membrane properties can modulate the BCL2 interactome and BCL2-related apoptotic pores, but the underlying mechanisms are still poorly understood. Here, we explored the role of membrane lipid composition and curvature in the activity and conformation of MCL1, a prominent antiapoptotic BCL2 family member. To this aim, we used multiple fluorescence-based techniques applied mainly to minimalistic reconstituted systems composed of recombinant proteins and pure lipid vesicles (liposomes). Two-color two-focus Scanning Fluorescence Correlation Spectroscopy (SFCCS) applied to single Giant Unilamellar Vesicles (GUV) and FRET measurements performed on Large and Small Unilamellar Vesicles (LUV and SUV) revealed that MCL1 forms stable heterodimeric complexes with proapoptotic cBID in MOM-like membranes displaying low membrane curvature and cardiolipin (CL) content, whereas high membrane curvature and CL content abolish MCL1:cBID complex formation. Site-directed mutagenesis and fluorescence spectroscopic analyses revealed that in low-CL membranes MCL1 adheres to the membrane surface to inhibit pore formation by proapoptotic BCL2 family members. On the contrary, in high CL membranes MCL1 extensively integrates into the membrane to unleash an intrinsic pore-forming activity. We also identified the minimal MCL1 domain responsible for its pore-forming activity using fluorescence imaging of semi-intact cells, among other techniques. Our results provide novel mechanistic insight into MCL1 function and conformation in the context of a membrane milieu, and add significantly to a growing body of evidence supporting the notion that different mitochondrial membrane components actively participate in BCL2 family protein function.

P13: APPLYING STED MICROSCOPY TO UNDERSTAND THE ELECTRICAL PROPERTIES OF DENDRITIC SPINES

Jan Tønnesen[1,2], Daniel Cattaert[1,3] and U. Valentin Nägerl[1,2].

E-mail: valentin.nagerl@u-bordeaux.fr

1. University of Bordeaux, France.
2. Interdisciplinary Institute for Neuroscience, CNRS UMR 5297, Bordeaux, France.
3. Institut de Neurosciences Cognitives et Intégratives d'Aquitaine, CNRS UMR 5287. Bordeaux, France.

KEY WORDS: STED microscopy, super-resolution imaging, living cells, dendritic spines, two-photon glutamate uncaging, electrophysiology, synaptic plasticity

Most primary neuron types receive synaptic input via sub-micron sized dendritic spines scattered over their dendrites. It has been a long-standing question whether the dendritic spine neck influences the propagation of excitatory postsynaptic potentials from the synapse to the soma. While the importance of the spine neck for biochemical compartmentalization is well established, its role in electrical signaling remains highly controversial. Two main obstacles have hindered progress. 1) The average spine neck diameter is around 150 nm, which is too small to be resolved by conventional light microscopy; 2) The inaccessibility of the spine head to direct electrophysiological recordings.

We have developed a new approach to address this controversy and test the hypothesis that spine necks can provide a sufficient electrical resistance to affect the excitatory postsynaptic potential measured at the soma. It is based on a combination of STED microscopy, two-photon glutamate uncaging and patch-clamp electrophysiology in organotypic hippocampal slice cultures. Through these advanced optical and electrophysiological techniques we can compare structure and function at the single spine level.

We confirm previous studies that relate synaptic strength, in terms of receptors, to spine head size. Furthermore, we disclose a novel and complementary structure-function relation, by identifying a correlation between neck geometry and synaptic efficacy. Specifically, the longer and thinner the neck is, the lower the synaptic efficacy, so that for a given number of activated synaptic receptors the spine will produce a smaller somatic response. In this scenario, the response at the soma will depend not only on the numbers of activated synaptic receptors, but also on the synaptic efficacy. Data-driven numerical simulations enable us to make further predictions about the biophysical effects of spines, predictions that we can test experimentally.

Our results add a layer of complexity to the physiology of dendritic spines, by identifying separate physiological roles of the spine head and neck. The study highlights the usefulness of superresolution microscopy for delineating previously undisclosed physiological properties of synaptic structures.

P14: LIGHT-SHEET MICROSCOPY IMAGIN OF A WHOLE CLEARED RAT BRAIN WITH THY1-GFP TRANSGENE

Emilio J. Gualda[1], Jordi Andilla[1], Marzena Stefaniuk[2], Monika Pawlowska[2], Leszek Kaczmarek[2] and Pablo Loza-Alvarez[1].

E-mail: emilio.gualda@gmail.com

1. ICFO-Institut de Ciencies Fotóniques, BIST-Barcelona Institute of Science and Technology, 08860 Castelldefels, Spain.
2. Nencki Institute, Pasteura 3, 02-093 Warsaw. Poland.

KEY WORDS: light-sheet fluorescence microscopy, optical clearing, brain imaging

Mapping brain circuits that often span distant areas requires imaging of the entire brain at cellular resolution. However, due to light scattering, optical imaging is limited to a depth of several hundreds of micrometers unless chemical clearing is performed. Although cleared brains can be imaged with any microscopy technique, LSFM offers high visualization speeds and a reasonably good 3D spatial resolution, becoming a golden standard for whole brain imaging. Whereas successful attempts to clear and image mouse brain have been reported, a similar result for rats has proven difficult to achieve due to the higher degree of myelination. Herein, we report on creating novel transgenic rat harbouring fluorescent reporter GFP under control of neuronal gene promoter (Thy1-GFP).

We have built a light-sheet fluorescence microscopes dedicated to imaging whole clarified mouse/rat brains. The dual-illumination-dual-view (DIDV) setup includes double-side illumination using cylindrical lenses, as well as double-sided detection with two objectives with different magnification and numerical apertures. This setup uses as a collection lens, a conventional photography macro objective, obtaining an extended field of view of 4.97 x 3.79 mm throughout the whole brain, with a pixel size of 3.7 µm. On the other side of the chamber, an additional microscope objective allows obtaining higher resolution images of 1.05 µm per pixel, over a reduced field of view (1.46 x 1.09 mm). With this setup it is possible to obtain an overall picture of the whole brain using the macro photography objective and then, after rotating the sample, obtain a more relevant picture with increased resolution of the selected area. The system is controlled using the open-source code OpenSpinMicroscopy [1].

We demonstrate efficient imaging of the cleared rat brain using light-sheet fluorescence microscopy [2]. We present data showing that FluoClearBABB was found superior over passive CLARITY and CUBIC methods. FluoClearBABB is a simple, inexpensive clearing protocol yielding robust samples that can be easily imaged and stored in the clearing solution, capable of preserving GFP fluorescence and at the same time providing sufficient tissue transparency for whole-brain light-sheet microscopy imaging.

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P15: BUILDING A SCANNING FCS SYSTEM

Unai Lorenzo[1, 2], Gloria de las Heras[2], Jose Requejo-Isidro[2] (STYLE: AUTHORS).

E-mail: jose.requejo@csic.es

1. Fundación Biofísica Bizkaia. Barrio Sarriena s/n. 48940 Leioa. Bizkaia.
2. Unidad de Biofísica (UPV/EHU, CSIC). Barrio Sarriena s/n. 48940 Leioa. Bizkaia.

KEY WORDS: Scanning FCS, fluorescence correlation spectroscopy

Scanning FCS (Fluorescence Correlation Spectroscopy) allows lipid-mediated protein interactions to be studied quantitatively. To the best of our knowledge, this technique is not commercially available. We have implemented scanning FCS on a Leica SP5 confocal microscope endowed with two APD (Avalanche Photodiode) detectors. The main element of the system setup is a Becker & Hickl TCSPC (Time-Correlated Single Photon Counting) card. We have conditioned the output signals of the detectors and the synchronization signals according to the TCSPC card specifications. We decoded the files with raw data and analyzed it using home-made core-parallelized software written in Matlab.

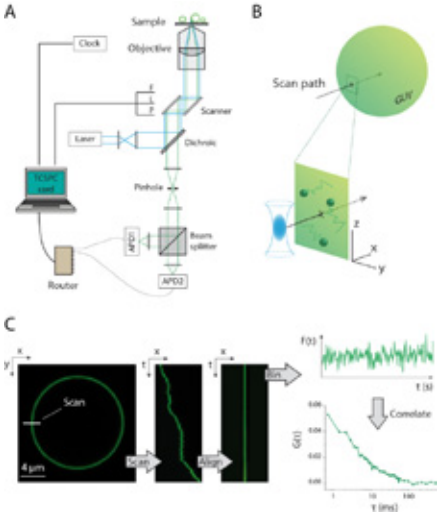

Figure 1. Building a scanning FCS system. A: Scanning FCS setup hardware. B: Scanning FCS in GUVs (Giant Unilamellar Vesicles). C: A single line within the GUV is scanned. Afterwards, the raw data is decoded. Due to the GUV drift its membrane moves during the measurement, therefore the membrane must be aligned before performing the correlation. Finally the data is fit to a 2D diffusing model.

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P16: QUANTIFYING EQUILIBRIUM DISSOCIATION CONSTANTS INSIDE THE CELL USING FRET-FLIM

Gloria de las Heras-Martínez[1], Josu Andrieu[1], Banafshé Larijani[1,2], Jose Requejo-Isidro[1*].

E-mail: jose.requejo@csic.es

1. Unidad de Biofísica (CSIC, UPV/EHU). Barrio de Sarriena s/n, 48490 Leioa. Spain.
2. Research Centre for Experimental Marine Biology and Biotechnology (PiE), University of the Basque Country (UPV/EHU). Leioa 48940, Spain.

KEYWORDS: FRET, FLIM, Equilibrium dissociation constant, protein interaction, GFP, Cherry

FRET is widely used to describe molecular networks assessing interactions between proteins that have been genetically tagged. Intracellular molecular interactions are in most circumstances reliably detected using Fluorescence lifetime imaging (FLIM) of the donor, which readily yields the fraction of donor involved in FRET. Quantification of the interaction in cells using single-channel FLIM, however, is difficult due to the inherent variability of the donor- and acceptor- tagged proteins in every cell. In particular, determination of the equilibrium dissociation constant, *K*d, of an interaction inside the cell requires knowledge of the molecular fractions of both donor and acceptor in complex. Simultaneous time-resolved measurement of the donor decay and the ingrowth of the acceptor emission monitors only those donor and acceptor molecules involved in FRET [1, 2], but they are technically demanding and prone to bleed-through contamination. Besides, spectroscopic heterogeneity and dark states in most fluorescent proteins largely decreases the fraction of interacting molecules that actually undergo FRET [3, 4], resulting in a low apparent interacting population and artefactually high *K*d.

Here, we present a method to determine the intracellular *K*d of the interaction based on imaging the fluorescence lifetime of the donor (single-channel). We exploit the spectroscopic properties of the widely used eGFP–mCherry FRET pair to gain information that is otherwise unavailable, such as the actual fractions of interacting donor and acceptor.

We have validated our method for intracellular *K*d quantification against *in-vitro* data and determined its range of application using Monte Carlo simulations. The uncertainty of the retrieved *K*d using this method is less than 20% for *K*d in the 1 to 100 μM range.

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P17: CONFOCAL AND VIDEOCONFOCAL IMAGING AS A TOOL TO STUDY LPAR1 ROLE ON THE TRANSITION FROM DEVELOPMENTAL TO ADULT NEURAL STEM CELLS IN THE HIPPOCAMPUS

Oier Pastor-Alonso[1,3], Juan Manuel Encinas[1,2,3], Jose R. Pineda[1].

E-mail: jm.encinas@ikerbasque.org / jr.pineda@achucarro.org

1. Achucarro Basque Center for Neuroscience. Zamudio E-48170, Spain.
2. Ikerbasque, The Basque Foundation for Science. Bilbao E-48013, Spain.
3. University of the Basque Country (UPV/EHU). Leioa E-48190, Spain.

KEY WORDS: Living cells, confocal microscopy.

We are interested in the involvement of lysophosphatidic acid (LPA) in the regulation of neural stem and progenitor cells (NSPCs). LPA is a serum-borne phospholipid with hormone and growth factor-like properties. LPA acts as an autocrine and/or paracrine signalling molecule via different G-protein-coupled LPA receptors (LPARs) that trigger a broad range of intracellular signaling cascades, especially the RHOA pathway. This molecule is involved in different cellular processes (cell migration, proliferation and differentiation) of different types of stem cells: on liver stem cells LPA increase is associated to cell proliferation [1] as well as in embryonic murine neural stem cells [2] and P7 postnatal mouse neurospheres [3]. However, in stem/progenitor cells derived from human embryonic stem cells, LPA inhibits neurosphere formation and the differentiation toward neurons [4]. On the other hand, in embryonic rat hippocampus LPA induces morphological changes of neuroblasts and neural progenitor cells through Rho-associated kinases [5].

Given the above mentioned discrepancies, we decided to use confocal images and videoconfocal analysis to further characterize the effect of LPA signaling on the initial steps of murine hippocampal NSPCs, selecting different postnatal time points. For that purpose, we used a transgenic mice line in which the LPA receptor 1 (LPAR1) drives the expression of the enhanced green fluorescent protein (LPAR1-EGFP) [6] and other based on the expression of the NSPC marker nestin, the Nestin-GFP. With the above-mentioned tools we hope to decipher “when” and “how” the population of NSCs gets established in the subgranular zone (SGZ) and unravel the specific role of the LPAR1 in murine NSPCs.

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P18: SHORT-CHAIN SPHINGOLIPIDS PREFERENTIALLY INSERT INTO TUMOR CELL MEMBRANES AND PROMOTE CHEMOTERAPEUTIC DRUG UPTAKE

Dalila Ciceri[1], Lilia R. Pedrosa[2], Félix M. Goñi[1], Gerben A. Koning[2], F.-Xabier Contreras[1,3].

E-mail: xabier.contreras@ehu.es

1. Instituto Biofísika Institua (CSIC, UPV/EHU) and Departamento de Bioquímica y Biología Molecular, Universidad del País Vasco. Leioa, Spain
2. Laboratory Experimental Surgical Oncology Section Surgical Oncology, Department of Surgery, Erasmus Medical Centre. Rotterdam, Netherlands.
3. IKERBASQUE Basque Foundation for Science, 48011 Bilbao, Spain.

KEY WORDS: Short-chain glycosphingolipid, Tumor cell membrane-permeability modulation, Targeting tumor cell membrane, Liposome, Doxorubicin

Insufficient drug delivery into tumor cells limits the therapeutic efficacy of chemotherapy. Co-delivery of liposome-encapsulated drug and synthetic short-chain glycosphingolipids (SC-GSLs) significantly improved drug bioavailability by enhancing intracellular drug uptake. Investigating the mechanisms underlying this SC-GSL-mediated drug uptake enhancement is the aim of this study. Fluorescence microscopy was used to visualize the fate of fluorescently labelled C6-NBD-GalCer incorporated in liposomes in tumor and non-tumor cells via cell membrane lipid transfer. Additionally click chemistry was applied to image and quantify native SC-GSLs in tumor and non-tumor cell membranes. SC-GSL-mediated flip-flop was investigated in model membranes to confirm membrane-incorporation of SC-GSL and its effect on membrane remodelling. SC-GSL enriched liposomes containing doxorubicin (Dox) were incubated at 4 °C and 37 °C and intracellular drug uptake was studied in comparison to standard liposomes and free Dox. SC-GSL transfer to the cell membrane was independent of liposomal uptake and the majority of the transferred lipid remained in the plasma membrane. The transfer of SC-GSL was tumor cell-specific and induced membrane rearrangement, as evidenced by a trans-bilayer flip-flop of pyrene-SM. However, pore formation was measured, as leakage of hydrophilic fluorescent probes was not observed. Moreover, drug uptake appeared to be mediated by SC-GSLs. SC-GSLs enhanced the interaction of doxorubicin (Dox) with the outer leaflet of the plasma membrane of tumor cells at 4 °C. Our results demonstrate that SC-GSLs preferentially insert into tumor cell plasma membranes enhancing cell intrinsic capacity to translocate amphiphilic drugs such as Dox across the membrane via a biophysical process.

L.R. Cordeiro Pedrosa; A. van Cappellen; B. Steurer; D. Ciceri; T.L.M. ten Hagen; A.M.M. Eggermont; M. Verheij; F.M. Goñi; G.A. Koning and F.-X. Contreras, C8-glycosphingolipids preferentially insert into tumor cell membranes and promote chemotherapeutic drug uptake, Biochimica et Biophysica Acta, 1848(8):1656-70 (2015).

P19: MITOCHONDRIAL DIVISION INHIBITOR 1 MODULATES INTRACELULAR CALCIUM SIGNALING AND EXACERBATES EXCITOTOXIC OLIGODENDROCYTE DEATH

Asier Ruiz[1,2], Elena Alberdi[1,2], Carlos Matute[1,2].

E-mail: asier.ruiz@ehu.es

1. Departamento de Neurociencias, Achucarro Basque Center for Neurosciences, Universidad del País Vasco (UPV/EHU). 48940-Leioa, Spain.
2. Centro de Investigación Biomédica en Red en Enfermedades Neurodegenerativas (CIBERNED). 48940-Leioa, Spain.

KEY WORDS: Excitotoxicity, mitochondrial fission, time-lapse microscopy, calcium dynamics, genetically encoded calcium indicator, mitochondrial potential, dynamin-related protein, mdivi-1.

Overactivation of AMPA-type ionotropic glutamate receptors induces a Ca^{2+} overload into the cytoplasm that produces excitotoxic death in oligodendrocytes. Mitochondria play a critical role in intracellular Ca^{2+} signaling but the link between Ca^{2+} homeostasis disruption and mitochondrial dynamics is not fully understood. Mitochondrial fusion and fission events in mammals are controlled by several GTPases of the dynamin family and play a critical role in the regulation of mitochondrial homeostasis. In particular, mitochondrial fission, which is mediated by cytosolic dynamin related protein 1 (Drp1), is essential for removal of damaged mitochondria but may contribute to apoptosis as well. Indeed, Drp1 inhibition or down-regulation provides neuroprotection in models of neurodegeneration and cerebral ischemia. However, the effects of Drp1 inhibition in oligodendrocytes have not been described yet. In the present study, we have analyzed the effects of mitochondrial division inhibitor 1 (mdivi-1), a selective Drp1 inhibitor, on mitochondrial fission and intracellular Ca^{2+} homeostasis during excitotoxicity in optic nerve-derived oligodendrocytes. Using time-lapse confocal imaging in oligodendrocytes expressing Drp1-yfp and mitochondria-targeted fluorescent proteins we found that sublethal activation of AMPA receptors triggered mitochondrial fission, which was inhibited by mdivi-1. Live-cell imaging of the cytosolic Ca^{2+} probe Fluo-4 and a mitochondria-targeted genetically-encoded Ca^{2+} indicator revealed that mdivi-1 reduced both AMPA-induced cytosolic and mitochondrial Ca^{2+} overloads. However, longer exposures to mdivi-1 alone dramatically reduced oligodendrocyte viability. Since Drp1 is important for mitochondrial quality control, we next analyzed whether mdivi-1 induced mitochondrial dysfunction. Rhodamine-123 time-lapse imaging under “dequench” conditions revealed a strong and dose-dependent mitochondrial depolarization after incubation of oligodendrocytes with mdivi-1 for one hour, which was followed by reactive oxygen species (ROS) production and ultimately caspase-3 activation. Mdivi-1 also compromised endoplasmic reticulum (ER) homeostasis, characterized by ER Ca^{2+} depletion and induction of the integrated stress response, and potentiated thapsigargin-induced cell death. On the other hand, toxic insults with AMPA plus cyclothiazide (CTZ) resulted in mitochondrial swelling rather than fission. Mdivi-1 did not prevent excitotoxic mitochondrial swelling whereas cell death was exacerbated. Finally, Drp1 overexpression in oligodendrocytes did not rescue from mdivi-1-, thapsigargin- and AMPA-induced cell death, indicating that mdivi-1 activates a deleterious Drp1-independent mechanism. In summary, our results provide evidence of Drp1-mediated mitochondrial fission during physiological activation of ionotropic glutamate receptors in oligodendrocytes, and uncover a deleterious and Drp1-independent effect of mdivi-1 on mitochondrial and ER function that turns these cells more vulnerable to intracellular Ca^{2+} homeostasis disruption.

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P20: LAURDAN'S TIME-RESOLVED SPECTROSCOPY STUDY OF THE MODULATION OF THE MEMBRANE BIOPHYSICAL PROPERTIES BY AN ETHER-LIPID DERIVATE AND ITS CONSEQUENCES IN METASTASIS DEVELOPMENT

Ana M Bouchet[1,2], Aurelie Chantôme[1,2], Marie Potier-Cartereau[1,2], Christophe Vandier[1,2], Jose M. Requejo-Isidro[3].

E-mail: anamaria.bouchet@univ-tours.fr

1. INSERM UMR 1069, Université de Tours, France.
2. Ion channel and cancer-Canceropole Grand Ouest network (IC-CGO network), www.ic-cgo.fr
3. Unidad de Biofisica (CSIC-UPV/EHU), Barrio de Sarriena, s/n, E-48940 Leioa, Spain.

KEYWORDS: Living cells, quantitative microscopy, phasor plot, Laurdan, Di-4-anedpphq

Many antecedents indicate that during cell migration a change in membrane fluidity occurs in the plasma membrane of cancer cells. This provides evidence of a pivotal role of biophysical adaptation in this biological process that ultimately leads to metastatic development. Specific properties of lipid organisation (fluidity, phase separation) regulate the structure and activity of membrane proteins, including ion channels. Our previous data have shown that the SK3 channel (a calcium-activated potassium channel) is involved in the migration of various cancer cells. Interestingly an alkyl-lipid derivate named Ohmline (1-O-hexadecyl-2-O-methyl-sn-glycero-3-lactose) modulates the activity of this channel [1]. The decrease in channel activity by Ohmline is associated with a reduction of cancer cell migrations [2].

In the present work, we characterise 3 cancer cell lines (colon-HCT-116, breast-MDA-MB-435s and prostate-PC3) with distinct migratory capability using Generalised Polarisation imaging of the polarity probes Di-4-anedpphq and Laurdan and phasor-plot analysis of time-resolved Laurdan emission. Imaging the lipid-packing state of the membrane of living cells before and upon treatment with Ohmline suggests that the modification of the phase sate of the plasma membrane is associated to a decreased migration capability. The implications of this modification of the phase state of the plasma membrane will be discussed in relation to metastatic development.

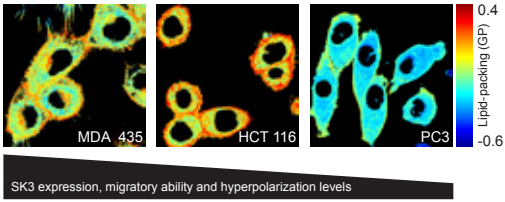


Figure 1. Generalised polarisation of cell lines with decreasing SK3 channel expression measured using Di-4-an-edpphq.

[1] Jaffres et al. (2016) Pharmacol Ther. doi: 10.1016/j.pharmthera.2016.06.003.
[2] Girault A, et al. (2011) Current Cancer Drug Target, 11, 1111

P21: MICROGLIA EXACERBATES SYNAPTIC DYSFUNCTION IN ALZHEIMER´S DISEASE

Estibaliz Capetillo-Zarate[1,2,3], Jone Zuazo[1], Carolina Ortiz-Sanz[1], Elena Alberdi[1] and Carlos Matute[1].

E-mail: estibaliz.capetillo@ehu.eus

1. Achucarro Basque Center for Neurosciences, Ciberned and Departamento de Neurociencias, Universidad del País Vasco. E-48940 Leioa, Spain.
2. IKERBASQUE, Basque Foundation for Science, 48011, Bilbao, Spain.
3. Department of Biochemistry, Weill Cornell Medical College, New York, NY 10065, USA.

KEY WORDS: amyloid, synapse, Alzheimer, microglia, neuron, coculture

Alzheimer's disease (AD) is a degenerative disorder and the most common cause of progressive cognitive decline in the aged population. The progressive and anatomically selective accumulation of β-amyloid (Aβ) peptide and the synaptic dysfunction are the main hallmarks of AD neuropathology. Synaptic dysfunction is the best pathological correlative to cognitive decline, but the cellular mechanism by which Aβ may affect synapses remains unclear.

Microglia are innate immune cells of the brain that mediate responses to pathogens and injury. Recent discoveries pointing to the key role of microglia on synapses in healthy brains opens new research routes in neurodegeration research field. A recent publication also shows that microglia mediates early synapse loss in AD models.

To study the role of microglia in the synapsis in the presence and absence of Aβ we have performed immunofluorescence to measure the levels of synaptophysin in neuronal and microglia-neuron primary cocultures with or without Aβ containing condition media (CM) from Swedish N2A cell line. First, we found that the presence of Aβ in neuronal primary culture reduces 46% the synaptophysin labeling compared to controls. We also found that after adding primary microglía to the primary neuron culture, the synaptophysin labeling increases 60% comparing with the control. However, adding Aβ containing CM to primary cocultures of microglia and neurons, the synaptophysin labeling decreases 69% comparing with the control.

Overall, these results indicate that the presence of microglia in the coculture favors the formation of synapses in normal conditions. Nevertheless, the addition of Aβ to microglia-neuronal cocultures abolishes the synaptogenic potential of microglia and leads to a further synaptic dysfunction. These results indicate that microglia could exacerbate the synaptic dysfunction induced by Aβ in AD.

P22: MEMBRANE INTERACTIONS OF HIV-1 NEUTRALIZING ANTIBODIES ASSESSED BY SCANNING FLUORESCENCE CORRELATION SPECTROSCOPY

Itziar R. Oar-Arteta, Pablo Carravilla, Edurne Rujas, Nerea Huarte, José L. Nieva, and Jose Requejo-Isidro.

E-mail: jose.requejo@csic.es

Biofisika Institute (CSIC, UPV/EHU) and Department of Biochemistry and Molecular Biology, University of the Basque Country, P.O. Box 644, 48080 Bilbao, Spain.

KEY WORDS: quantitative microscopy, protein-lipid interactions, scanning FCS, HIV

Antibodies 4E10 and 10E8 exert broad and potent HIV neutralization after engaging the MPER (Membrane Proximal External Region) helical epitope of gp41, which is stuck into the viral membrane interface. The contribution of direct antibody-membrane interaction to the mechanism of MPER epitope-binding and viral neutralization remains elusive. Here, to obtain further insights into the recognition mechanism, we have produced antigen-binding fragments (Fabs) of 4E10 and 10E8 labeled with Alexa-488, plus non-functional species mutated in the heavy-chain complementarity determining region 3 loop (CDRH3). We have quantitatively studied their interactions with bare and epitope-containing membranes by scanning Fluorescence Correlation spectroscopy (sFCS) and confocal microscopy on Giant Unilamellar Vesicles (GUV) of different compositions. The membrane-binding assays and Fab diffusion coefficients show that 4E10, but not 10E8, interacts nonspecifically with membranes containing anionic phospholipids, and that this process depends on the preservation of the hydrophobic CDRH3 loop and an electropositive paratope surface. Although lipid-4E10 interaction favours MPER epitope binding at the membrane surface, insertion into the membrane is not a prerequisite for recognition.

P23: DUAL MODE DIAGNOSTIC TOOL FOR CONFOCAL ENDOMICROSCOPY AND FLUORESCENCE LIFETIME SPECTROSCOPY TO ANALYZE TISSUE BIOCHEMISTRY IN-VIVO

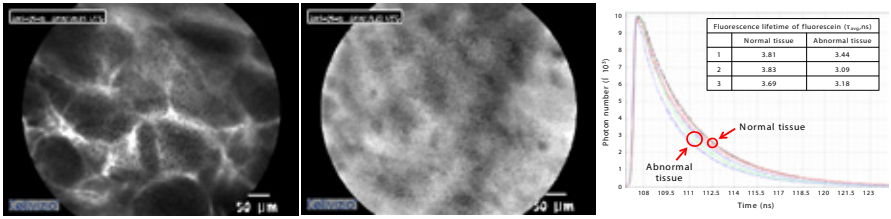
Byungyeon Kim[1], Byungjun Park[1], Seungrag Lee[1], Sucbei Moon[2], and Youngjae Won[1].

E-mail: yjwon000@gmail.com

1. Osong medical innovation foundation, Republic of Korea.
2. Kookmin university, Republic of Korea.

KEY WORDS: Fluorescence lifetime spectroscopy, confocal endomicroscopy, Biochemistry, In-vivo

In this study, we designed a dual-mode experimental setup for confocal endomicroscopic imaging and fluorescence lifetime measurement. A commercially available confocal endomicroscope (Cellvizio, Mauna Kea Technologies, Paris, France) and fluorescence lifetime spectrometer (FluoTime 300, PicoQuant, Berlin, Germany) were linked to the same optical mini-probe. To evaluate the effectiveness of this approach for cancer detection, we subcutaneously injected AsPC-1 activated human pancreatic cancer cells into a living mouse, followed by intravenous injection of sodium fluorescein. The fluorescence lifetime of sodium fluorescein depends on the local pH. Since pH differs between abnormal and normal tissues, they can be differentiated by measuring the fluorescence lifetime of pH-sensitive sodium fluorescein. We morphologically discriminated abnormal and normal tissues by confocal endomicroscopic imaging, and then functionally discriminated them by measuring the fluorescence lifetime of injected sodium fluorescein.



Fluorescence lifetime of fluorescein (τ_{avg},ns)		
	Normal tissue	Abnormal tissue
1	3.81	3.44
2	3.83	3.09
3	3.69	3.18

(a) (b) (c)

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