THE „ECOLE DE PHYSIQUE DES HOUCHES“, FRANCE – MAP

THE „ECOLE DE PHYSIQUE DES HOUCHES“, FRANCE

...is a resort village in the Chamonix valley in the French Alps. Established in 1951, the Physics School is located in a group of chalets surrounded by meadows and woods, at an altitude of 1150 m facing the Mont-Blanc range - a very favourable environment for intellectual activity in ideal surroundings for skiing, hiking or mountaineering. The Mont Blanc is the highest mountain in the Alps, Western Europe and the European Union. It rises 4,810.45 m above sea level and is ranked 11th in the world in topographic prominence.

Ecole de Physique des Houches
La Côte des Chavants
74310 Les Houches, France
+33 (0)4 50 54 40 69

HOW TO GET TO LES HOUCHES

By plane: Geneva Airport is 1 hour drive from Les Houches. The simplest way is to use a shuttle service (approximately 25€ up to the school, book at least three days in advance). Please find here an overview of the different providers:
www.chamonix.net/english/transport/transfers.htm

There is also a regular bus service between Geneva and Les Houches (max. twice a day). You should then take a taxi for the last 5 km from the Les Houches village to the school. You can also travel from Geneva to Les Houches by train (+ taxi from the train station to the School), but it is rather complicated and long (go through Annemasse on the French side or through Martigny on the Swiss side).

By train: arrival at the Les Houches station, with one change at Saint-Gervais (from France), or at Martigny (from Switzerland). There are about 10 trains per day between St Gervais and Les Houches. Then we strongly recommend to take a taxi and to pre-reserve by calling one of the following providers: taxi Garny: +33 6 12 35 30 72, taxi Persault : +33 4 50 54 41 09 and taxi Servoz : +33 6 84 66 86 73 to go up to the school ~5km.

By car: Les Houches are easily accessible from France (A41 highway), from Switzerland (Martigny and Col des Montets) and from Italy through the Mont Blanc Tunnel.

From Geneva and Le Fayet: 8km before Chamonix, 300 m after passing under the tunnel, bear right by the first road out for “Les Houches Bellevue”. When arriving at the cable car station “Bellevue”, turn right and continue upwards (roughly 2 km starting from the teleferic). 500m after the cable car station “Prarion”, turn left and follow small arrows at crossroads. Continue up to the end of Route de la Côte des Chavants. Here you are! From Chamonix: bear right for “Les Houches-Chef-Lieu”, turn right in Les Houches, and go ahead at the cable car station “Bellevue”. Then proceed as above.
Winter equipment for the car is recommended (snow chains). Please park your car at the parking place above the Ecole de Physique.

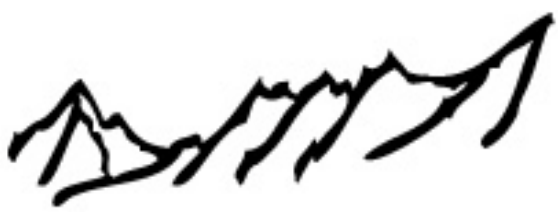
AT THE ECOLE DE PHYSIQUE DES HOUCHES

Arriving is possible on **Sunday January 19, 2014 from 15:00h** onwards. Please note that the school is closed before 15:00h. Within the foyer of the main building you will find a plan indicating the allocation of rooms as well as the instructions how to get in the chalets. Participants are housed in “chalets” (electricity: AC, 50Hz, 220V). The first joint meal will be the dinner at 19:30h.

Breakfast is from 8:00 am to 8:45 am; **Lunch**: at 12:30h; and **Dinner**: 19:30h – sharp!
It is possible to order a lunch packet the evening before. A respective list can be found at the restaurant.

Meals are taken at the school dining room/restaurant. Drinks are not included - aside of coffee and tea.

ÉCOLE DE PHYSIQUE
des HOUCHES



WELCOME TO TOPIM 2014 – THE 8TH "HOT TOPIC IN MOLECULAR IMAGING" CONFERENCE OF THE ESMI

IMAGING THE DEVELOPMENT

by Bertrand Tavitian

There are two main reasons why in vivo imaging is a key to our understanding of development.

The first one is that morphogenesis leading to the formation of a functional adult is a dynamic process during which the forms and shapes of the organism change continuously over time: these changes are best described by images.

The second one is that exploring genetic and molecular positional cues that govern morphogenesis requires long term observations that do not interfere with normal development: the least intrusive method for producing serial and dynamic imaging of an organism is modern in vivo imaging.

Among all domains of biological science, development is one for which the quality and relevance of observations depend drastically on non destructive methods. The secret life of embryos and fetuses remained terra incognita for scientists.

In the last decades the progress of non-invasive imaging has completely changed our view and in situ medical images of developing life are commonplace:

On the research front, immense progress in biophysical and molecular methods to image the development has been obtained recently. The precision of morphogenetic descriptions has improved constantly thanks to progress in ultrasonic and magnetic resonance imaging and further progress are awaited. Cell lineage studies have moved from destructive autoradiographic methods to intravital optical techniques based on genetic molecular engineering, allowing mathematical modeling of the trajectories of individual cells. The role of genetic and epigenetic factors in organogenesis is observed directly in cohorts of laboratory animals, from simple multicellular forms of life to vertebrates and mammals. Multispectral and multimodal imaging techniques such as optoacoustics, paramagnetic resonance imaging, and other, acquire simultaneously several molecular/morphological points of view of the interactions between the development of an organ or an individual and its environment. Progress in Physics for improved imaging methods and in Mathematics for the modeling of the images are moving the research on of development from the statute of a descriptive science to a predictive, holistic knowledge, a topic which is not neutral with respect to the future of interventional approaches.

TOPIM 2014 will cover the state-of-the-art as well as recent progress in the scientific bases for the imaging of development.

Thanks to all of you for your contribution!

TOPIM 2014 PROGRAMME OVERVIEW

time	Monday, 20 January	Tuesday, 21 January	Wednesday, 22 January
08:00 - 08:45			breakfast
08:45 - 08:50	Welcome - Bertrand Tavitian, Paris	Essential Genes for Development: Imaging of Mouse Embryonic Lethals **Mark R. Henkelmann - Toronto**	Adult neurogenesis in physiology and during Parkinson's disease **Jens Schwamborn - Esch-sur-Azette**
08:50 - 08:55			
08:55 - 09:00			
09:00 - 09:05	**Introductory Keynote Lecture** Imaging of Development: an Overview of Dynamic Embryonic Events with a focus on Central and Peripheral Nervous System Development **Lee Niswander - Denver**		
09:05 - 09:10			
09:10 - 09:15			
09:15 - 09:20			
09:20 - 09:25			
09:25 - 09:30			
09:30 - 09:35			
09:35 - 09:40		Introduction to an experimental Hardware Approach of combining MPI and MRI into an integrated Preclinical Hybrid Imaging System. Jochen Franke, Erlangen	Assessing neurodegeneration, neuroinflammation and neurogenesis in an experimental Parkinson's disease model using multimodal molecular imaging. Inga Fricke, Münster
09:40 - 09:45			
09:45 - 09:50			
09:50 - 09:55			
09:55 - 10:00		Rapid 3D light-sheet microscopy with a tunable lens. Florian Fahrbach, Dresden	Intravital Characterization of the inflammatory cells recruitment during the development of Glioblastoma brain tumor. Franck Debarbieux, Marseille
10:00 - 10:05			
10:05 - 10:10			
10:10 - 10:15			
10:15 - 10:20			
10:20 - 10:25			
10:25 - 10:30			coffee break
10:30 - 10:35			
10:35 - 10:40			
10:40 - 10:45			
10:45 - 10:50	**Introductory Keynote Lecture** **Lee Niswander - Denver**	Coffee break & **Poster session 1** **poster # 1-10**	In Vivo Imaging of the Developing Mouse Brain: From Morphology to Molecules **Daniel Turnbull - New York**
10:50 - 10:55			
10:55 - 11:00			
11:00 - 11:05			
11:05 - 11:10			
11:10 - 11:15			
11:15 - 11:20			
11:20 - 11:25			
11:25 - 11:30			
11:30 - 11:35	A Novel Live Imaging System for the Study of Epithelial Dynamics in the Mammalian Embryo. Ray Heather, Denver		Molecular Imaging Studies of the Developmental Emergence of Integrative Brain Function. Evan Balaban - Montreal
11:35 - 11:40			
11:40 - 11:45			
11:45 - 11:50			
11:50 - 11:55	Live-imaging analysis of impacts of apoptosis on the cranial neural tube closure in mouse embryo. Yamaguchi Yoshifumi, Tokyo		Vascular Resistivity Imaging using Ultrafast Doppler : application to the 2D cerebral mapping of preterm infant vascular indexes. Charlie Demene, Paris
11:55 - 12:00			
12:00 - 12:05			
12:05 - 12:10			
12:10 - 12:15			
12:15 - 12:20			
12:20 - 12:25			
12:25 - 12:30			
12:30 - 13:00	lunch		
13:00 - 15:45	break		
15:45 - 15:50			
15:50 - 15:55			
15:55 - 16:00			
16:00 - 16:05			
16:05 - 16:10			
16:10 - 16:15			
16:15 - 16:20			
16:20 - 16:25			
16:25 - 16:30			
16:30 - 16:35	Multiscale Imaging and quantification of tissue morphogenesis: from gene to forces **Yohanns Baillache, Paris**	Targeting endothelial metabolism: principles and strategies **Peter Carmeliet - Leuven**	Ultrafast Ultrasonic Imaging : from Shear Wave Elastography to Functional Imaging of the Brain Activity **Mickael Tanter, Paris**
16:35 - 16:40			
16:40 - 16:45			
16:45 - 16:50			
16:50 - 16:55			
16:55 - 17:00			
17:00 - 17:05			
17:05 - 17:10			
17:10 - 17:15			
17:15 - 17:20	Neuroepithelial slow amplifying progenitors role in the zebrafish midbrain morphogenesis. Gaelle Recher, Gif sur Yvette	Preclinical intravital microscopy of the tumour-stroma interface: invasion, metastasis, and therapy response **Peter Friedl, Nijmegen**	Coffee break & **Poster session 2** **poster # 11-20**
17:20 - 17:25			
17:25 - 17:30			
17:30 - 17:35			
17:35 - 17:40	Computational methods for zebrafish embryogenesis reconstruction. Karol Mikula, Bratislava		
17:40 - 17:45			
17:45 - 17:50			
17:50 - 17:55			
17:55 - 18:00	break		
18:00 - 18:05		break	
18:05 - 18:10			
18:10 - 18:15			
18:15 - 18:20			
18:20 - 18:25			
18:25 - 18:30			
18:30 - 18:35	Pattern formation and tissue morphogenesis during mouse embryogenesis **Magdalena Zernicka-Götz, Cambridge**	Ethical considerations **Pierre-Henri Gouyon, Paris**	Olivier Pourquié cancelled
18:35 - 18:40			
18:40 - 18:45			
18:45 - 18:50			
18:50 - 18:55			
18:55 - 19:00			
19:00 - 19:05			
19:05 - 19:10			
19:10 - 19:15			
19:15 - 19:20	**Pot d'accueil** offered by the Ecole		
19:20 - 19:25			
19:25 - 19:30			
19:30 - 19:45	dinner		
19:35 - 19:40			
19:40 - 19:45			
19:45 - 20:15			
20:15	come together	**open discussion**	come together

TOPIM 2014 PROGRAMME OVERVIEW

time	Thursday, 23 January	Friday, 24 January
08:00 - 08:45		
08:45 - 08:50	Imaging vascular remodeling in reproduction and development **Michal Neeman, Rehovot**	Imaging vessel remodeling at the micro-scale during development: implications for tissue engineering and understanding congenital disease **Mary Dickinson - Houston**
08:50 - 08:55		
08:55 - 09:00		
09:00 - 09:05		
09:05 - 09:10		
09:10 - 09:15		
09:15 - 09:20		
09:20 - 09:25		
09:25 - 09:30		
09:30 - 09:35		
09:35 - 09:40	Human embryonic Posterior fossa venous development based on serial histologic 3D reconstruction. Romain Tonnelet, Nancy	Internalisation movements of the endoderm during gastrulation in the zebrafish embryo. Florence Giger, Paris
09:40 - 09:45		
09:45 - 09:50		
09:50 - 09:55		
09:55 - 10:00	best poster presentation poster session 1	best poster presentation poster session 2
10:00 - 10:05		
10:05 - 10:10		
10:10 - 10:15		
10:15 - 10:20		
10:20 - 10:25		
10:25 - 10:30		
10:30 - 10:35		
10:35 - 10:40		
10:40 - 10:45		
10:45 - 10:50	Non-invasive 3-dimensional imaging of drosophila pupae and fly using raster-scan optoacoustic mesoscopy (R-SOM). Murad Omar, Munich	Reconstructing multi scale in vivo imaging data by multilevel dynamics **Nadine Peyriéras - Gif sur Yvette**
10:50 - 10:55		
10:55 - 11:00		
11:00 - 11:05		
11:05 - 11:10	Live-imaging reveals potential new mechanism of self-renewal. Paula Alexandre, London	
11:10 - 11:15		
11:15 - 11:20		
11:20 - 11:25		
11:25 - 11:30		
11:30 - 11:35	**open discussion**	**open discussion** **conclusion**
11:35 - 11:40		
11:40 - 11:45		
11:45 - 11:50		
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11:55 - 12:00		
12:00 - 12:05		
12:05 - 12:10		
12:10 - 12:15		
12:15 - 12:20		
12:20 - 12:25		lunch and departure
12:25 - 12:30		
12:30 - 13:00		
13:00 - 15:45		
15:45 - 15:50		
15:50 - 15:55		
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16:15 - 16:20		
16:20 - 16:25		
16:25 - 16:30		
16:30 - 16:35	Branch or Expand? New insights into Dll4/Notch dynamics driving vascular patterning **Thomas Mathivet, Leuven**	
16:35 - 16:40		
16:40 - 16:45		
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16:50 - 16:55		
16:55 - 17:00		
17:00 - 17:05		
17:05 - 17:10		
17:10 - 17:15		
17:15 - 17:20	In-vivo imaging of Drosophila melanogaster Development. Jorge Ripoll, Madrid	
17:20 - 17:25		
17:25 - 17:30		
17:30 - 17:35		
17:35 - 17:40	Gap junction control of cell communication and leader cell function in collective cell migration. Antoine Khalil, Würzburg/Nijmegen	
17:40 - 17:45		
17:45 - 17:50		
17:50 - 17:55		
17:55 - 18:00	break	
18:00 - 18:05		
18:05 - 18:10		
18:10 - 18:15		
18:15 - 18:20		
18:20 - 18:25		
18:25 - 18:30		
18:30 - 18:35	Imaging the developing cardiovascular system in mice **Lee Adamson, Toronto**	
18:35 - 18:40		
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19:45 - 20:15		
20:15	come together	

COMMITTEE – TOPIM 2014

Bertrand Tavitian – Paris, France CHAIR

Andreas H. Jacobs – Muenster, Germany

Michal Neeman – Rehovot, Israel

Vasilis Ntziachristos – Munich, Germany

Nadine Peyriéras – Gif-sur-Yvette, France

Like any other organisation, we are the sum of our people - grateful to everyone who is contributing - thanks to all of you for your commitment and dedication.

TOPIM 2014 is co-funded by the EC FP7 project

INMiND - Imaging of Neuroinflammation in Neurodegenerative Diseases.

www.inmind-project.eu

The large-scale integrating project **INMiND** co-funded by the European Commission (GA278850) within the 7th Framework Programme, is a collaboration of 27 research groups from 12 European countries and Ausralia.

The goal of INMiND is to identify novel biological targets of neuroinflammation for both diagnostic and therapeutic purposes and to translate this knowledge into clinical application and patient benefit.

day one: monday january 20, 2014

IMAGING OF DEVELOPMENT: AN OVERVIEW OF DYNAMIC EMBRYONIC EVENTS WITH A FOCUS ON CENTRAL AND PERIPHERAL NERVOUS SYSTEM DEVELOPMENT

Niswander, L.

University of Colorado Pediatrics, Colorado, USA

(Lee.Niswander@ucdenver.edu)

This introductory lecture is aimed at providing an overview of key developmental events that will be touched upon in various animal models during this symposium. During embryonic development, there are numerous highly dynamic processes. Traditionally, however, embryonic development has been studied as static snapshots of time. New imaging modalities are providing novel insights into how cells interact and move relative to one another and the forces that they impart on each other to produce the complex morphogenetic movements that form the embryo. These new insights are beginning to revolutionize the ways in which the field considers cell and tissue interactions during multiple aspects of development.

Insights into the dynamic regulation of development first came from the study of optically transparent embryos, such as C. elegans and zebrafish. Insights were also driven by time-lapse imaging of embryos that are not dependent on the maternal environment (fly, frog, chick). More recently, even the challenges of observing mammalian embryos that develop in utero have been overcome through advancements in embryo culture and in vivo imaging. Technologies and methods discussed by participants in the meeting will highlight these advances and how they are being used to study key aspects of animal development.

This introductory lecture will weave together insights from different animal models to highlight a range of developmental processes - from early cell divisions and establishment of tissue polarity, to the massive cell movements that occur during gastrulation, to neurogenesis and vascular development. These examples will also show how cell polarity, cell division, cell interactions, collective and solitary cell movements, and physical forces help to shape the embryo.

The lecture will be interspersed with research from the Niswander lab. We have used live imaging of fluorescently-labeled mouse embryos to dynamically visualize the formation of the early central and peripheral nervous systems. These imaging methods have been combined with genetic mutants to understand how molecular deficits affect the cell behaviors necessary to drive neural tube closure, neural stem cell proliferation, and neural crest migration.

A NOVEL LIVE IMAGING SYSTEM FOR THE STUDY OF EPITHELIAL DYNAMICS IN THE MAMMALIAN EMBRYO

Ray, H., Niswander, L.

University of Colorado Pediatrics, Colorado, USA

(heather.j.ray@ucdenver.edu)

Introduction: Mouse models can greatly inform the study of human development and developmental disorders. Developmental processes and tissue movements are highly dynamic, yet the study of these events in the mammalian embryo has been hampered due to in utero development. Instead, there has been a heavy reliance on still images of embryos collected at sequential time points. We have now developed a robust and reproducible imaging system to dynamically visualize embryonic morphogenesis in real time and used this system to study epithelial dynamics during neural tube (NT) closure.

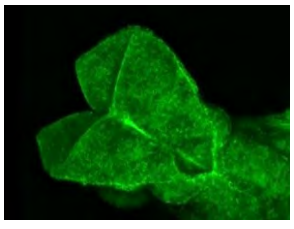
Methods: Mouse embryos are dissected at 4-16 somites, secured on a filter via the embryonic sac and cultured within an environmental chamber mounted to a motorized stage of an inverted Zeiss LSM510 Meta confocal microscope, and imaged for 16 hours. We can image 5 embryos at once at high magnification and high resolution to visualize cellular behaviors. To examine epithelial cell behaviors during the process of NT closure, we mated dual reporter line mT/mG to a non-neural ectoderm (NNE)-specific Cre-recombinase strain (Grhl3-Cre). Grhl2 transcription factor is expressed specifically in the NNE and its loss affects epithelial integrity. Imaging of Grhl2 mutant embryos allows us to study the effect of Grhl2 loss on epithelial dynamics during NT development.

Results: Live imaging of NT closure with focus on the NNE shows three distinct epithelial cellular behaviors. 1) A tight actin-rich cable at the border between the neural ectoderm and NNE. 2) Cellular extensions that span the open gap between opposing neural folds. 3) Collective cell movements to close the gap between opposing neural folds. These various behaviors were observed in discrete regions along the rostral-caudal axis. Our molecular analysis of the NNE transcriptome and NNE mutant for the key transcription factor Grainyhead-like 2 (Grhl2) revealed target genes that a) regulate epithelial characteristics, b) suppress EMT, and c) control EMT induction. Live imaging analysis of mouse embryos mutant for Grhl2 have complemented our molecular data and shown that during NT closure, the actin-rich cable is discontinuous and individual epithelial cells exhibit increased movement within the epithelial layer, leading to a failure of NT closure.

Conclusions: The ability to culture intact post-gastrulation stage mouse embryos in conjunction with time-lapse confocal microscopy has allowed us to image the process of NT closure in real time. Use of the Grhl3-Cre strain and Grhl2 mutants revealed several distinct epithelial cell behaviors that are important for NT closure as disruption of these behaviors causes a failure of this critical developmental process.

Acknowledgement Thank you to Dr. Shaun Coughlin for the Grhl3-cre mice.

References: Pyrgaki, C et al. Dev Biol 353 (2011) 38–49; Pyrgaki, C et al. Dev Biol 344 (2010) 941–947; Massarwa, R et al. Development 140, 226-236 (2013)



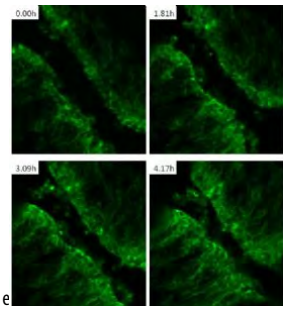


Figure 1 Actin-rich cable at neural ectoderm/non-neural e
Figure 2 Collective cell movement across open neural folds

LIVE-IMAGING ANALYSIS OF IMPACTS OF APOPTOSIS ON THE CRANIAL NEURAL TUBE CLOSURE IN MOUSE EMBRYO

Yamaguchi, Y.[1,2], Shinotsuka, N.[1], Matsumoto, Y.[1], Nakazato, K.[3], Mochizuki, A.[3], Miura, M.[1,4]

[1]Department of Genetics, Graduate School of Pharmaceutical Sciences, University Tokyo, [2]PRESTO, JST, Tokyo, Japan ; [3]RIKEN Advanced Science Institute, Saitama, Wako Japan; [4]CREST, JST, Tokyo, Japan

(bunbun@mol.f.u-tokyo.ac.jp)

Introduction: Many cells die during development, which is called programmed cell death. Dysregulation of apoptosis, a major form of programmed cell death executed by the cystein protease caspases, leads to neural tube closure (NTC) defects including exencephaly and spina bifida. Since both apoptosis and neural tube closure are rapid and dynamic events, observing apoptotic cells in a living context could help elucidate their origin, behavior, and influence on morphogenesis.

Methods: We conducted time-lapse imaging of apoptosis and morphogenesis by using a fast-scanning confocal microscope and a newly developed transgenic mouse that stably expressed a genetically encoded FRET reporter for caspase activation (Figure.1).

Results: We observed that inhibiting caspase activation perturbed and delayed the smooth progression of cranial NTC. In addition, we found that inhibition of caspases affected collective movement of the neural ridge cells surrounding unclosed neuropore after the completion of NTC.

Conclusions: These results suggest that cell removal by caspase-mediated apoptosis facilitates not only NTC completion within a limited developmental window but also the remodeling of the cranial neural tube.

References: Yamaguchi, Y., Shinotsuka, N., Nonomura, K., Takemoto, K., Kuida, K., Yosida, H., Miura, M. (2011) Live imaging of apoptosis in a novel transgenic mouse highlights its role in neural tube closure. J Cell Biol. 195,1047-60.Yamaguchi, Y., Miura, M. (2013) How to form and close the brain: insight into the mechanism of cranial neural tube closure in mammals. Cell Mol Life Sci. 70, 3171-86.

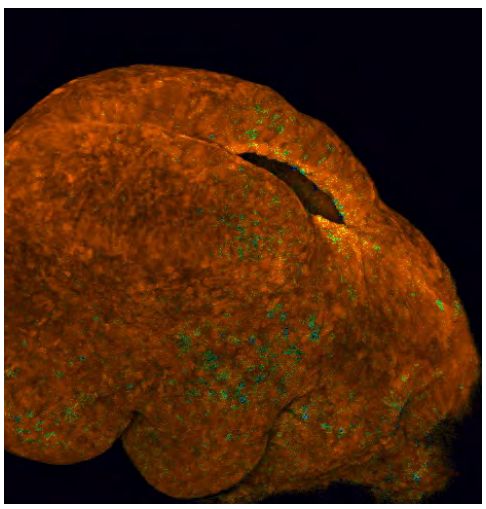


Figure1 A mouse embryo expressing FRET reporter for caspase activation

MULTISCALE IMAGING AND QUANTIFICATION OF TISSUE MORPHOGENESIS: FROM GENE TO FORCES

Bosveld, F., Guirao, B., Bonnet, I., Graner, F., Bellaiche, Y.

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(yohanns.bellaiche@curie.fr)

How proliferative tissues adopt their shape is a central question in developmental biology. Many tissues undergoing extensive proliferation concomitantly adopt their shape during development. Hence, the apparent coordination between growth and morphogenesis underlies the development of organs and tissue of defined size and shape adapted to their physiological function in the organism. The mono-layered epithelium of the Drosophila pupa undergoes extensive proliferation and morphogenesis to shape the Drosophila adult. To study the morphogenesis of the dorsal thorax, we implemented an innovative multi-scale imaging method to follow both cell dynamics (5 minutes time resolution, 200nm resolution) and tissue global morphogenetic (global imaging of the tissue for up to 24 hours) in a living organism. Qualitative observations suggest that the tissue undergoes extensive proliferation (up to three cell cycles) while reshaping by convergence extension movements. Image velocimetry and segmentation based image analysis allow us to describe and quantify tissue morphogenesis from the cell level to the tissue level. Using this novel model system, we have determined the role of the planar cell polarity pathway Fat-Dachsous in epithelial tissue morphogenesis. More generally, our results indicate how global gene expression patterns can trigger local changes in mechanical cell properties to drive tissue morphogenesis.

NEUROEPITHELIAL SLOW AMPLIFYING PROGENITORS ROLE IN THE ZEBRAFISH MIDBRAIN MORPHOGENESIS

Recher, G.[1], Jouralet, J.[2], Brombin, A.[2], Heuzé, A.[2], Mugniery, É.[2], Hermel, J. - M.[1], Desnoulez, S.[1], Savy, T.[1], Herbomel, P.[3], Bourrat, F.[2], Peyriéras, N.[1], Jamen, F.[2], Joly, J. - S.[2]

[1]CNRS N&D-INAF MDAM/BioEmergences, Gif-sur-Yvette, France ; [2]CNRS / INRA, Gif-sur-Yvette; [3]Institut Pasteur Macrophages and Development of Immunity, Paris, France

(gaelle.recher@inaf.cnrs-gif.fr)

Introduction: We aimed at investigating vertebrate neural progenitors behavior *in vivo* by focusing on the peripheral midbrain layer (PML) which is a midbrain sub-territory wrapping the optic tectum (OT) posteriorly, laterally and medially.

Methods: We performed a multilevel morphogenetic description of PML and OT progenitors between 30 and 45 hpf, *e.g.* 1/ lineage and fate, 2/ proliferation modes, and 3/ tissue morphogenesis. This was achieved by long term two-photon *in vivo* imaging of fluorescent proteins in transgenic fish lines and subsequent image processing and analysis with custom software.

Results: We showed that PML cells are neuroepithelial and have large and elongated nuclei suggesting relatively decondensed chromatin, in contrast with OT cells that have a small rounded shape. In addition, 90 % of the analyzed PML cells proliferate by means of symmetric divisions. The PML cells proliferate three times slower (1 mitosis every 1h35) than the OT (1 mitosis every 5h50) [Figure 1]. Furthermore, individual PML cells tracking and global displacement field indicated their contribution to the OT [Figure 2]. This suggests that during the cell population displacement of PML cells toward the OT, cells are undergoing a major change in their gene expression profile. Indeed, the analysis of gene expression patterns showed that PML cells express a specific subset of genes. The latter includes genes involved in nucleotides synthesis and genes encoding nucleolar or ribosomal proteins as reported for *Drosophila* neuroblasts. This idea is also supported by the analysis of a mutant line (*cad*) where PML and OT organization is disrupted with extensive cell death.

Conclusions: This original characterization of PML cells as slow cycling neural progenitors displaced toward their postmitotic location through global cell population movements by 2 dpf serves as a reference for further investigations of midbrain morphogenesis in normal and modified situations (after photoablation, gene expression misexpression…).

Acknowledgement: CNRS, INRA, INSERM, Université Paris Sud, ANR, European Commission [STREP Plurigenes, CISSTEM, FP6 NEST program (Embryomics and BioEmergences EC projects) and FP7 Health program (zf-Health project) to N.P.].

References: Recher G, Jouralet J, Brombin A, Heuzé A, Mugniery E, Hermel J-M, Desnoulez S, Savy T, Herbomel P, Bourrat F, Peyriéras N, Jamen F, and Joly J-S (2013) Zebrafish midbrain slow-amplifying progenitors exhibit high levels of transcripts for nucleotide and ribosome biogenesis. In press - Development.

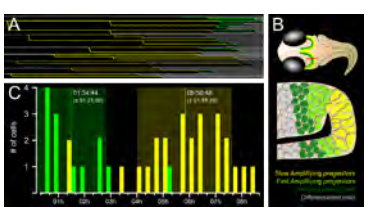

Figure 1 Topology of proliferation rates in the larval zebrafish midbrain

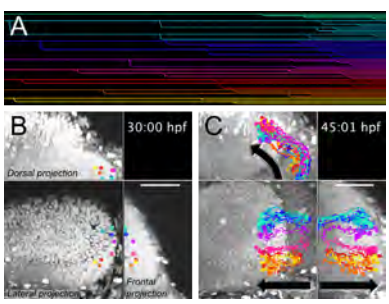

Figure 2 PML slow-amplifying progenitors tracking and lineage analysis

COMPUTATIONAL METHODS FOR ZEBRAFISH EMBRYOGENESIS RECONSTRUCTION

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Introduction: The development of the modern microscopy techniques allows the in vivo imaging of organisms at cell level at very early stages of development without corrupting the cell integrity and normal development of the embryo. The multiphoton laser scanning microscopy is able to deliver 3D+time images of long periods of the zebrafish embryonic development with a relatively short time step. We present an efficient computational strategies for analysis of this type of data.

Methods: Our technique consists of a set of original image processing algorithms based on PDE models. The first step is the image filtering whis is necessary because some noise is always present in any microscope image and its level increases with the speed of image acquisition that is a key issue in the cell lineage tree reconstruction. We perform this step by numerical solution of the so-called geodesic mean curvature flow model [1]. The second step is the level set center detection method used to extract the approximate position and number of cell nuclei centers [2]. Then the 3D image segmentation by the generalized subjective surface method is used to obtain a shapes of cell nuclei and membranes which can be used for various analyses of the embryo [2]. The last step is the detection of cell trajectories and reconstruction of the cell lineage tree by finding an ideal paths in 4D spatio-temporal tubular structures obtained by 4D segmentation [3]. To that goal a constrained distance functions are computed inside the structure by solving the Eikonal equation. Then the suitable potential field is constructed which gives after backtracking in a steepest descent direction the backward cell trajectories which are used for the cell lineage tree construction.

Results: We present and discuss results of image filtering, cell center detection, cell shape segmentation and cell trajectories extraction algorithms on various testing and real 3D+time images.

Conclusions: Our methods show good properties with respect to precision and efficiency of computations in early zebrafish embryogenesis reconstruction from 3D+time images and thus they can be used for large scale image analysis of this type of data.

Acknowledgement: This work is supported by the grant APVV-0184-10.

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PATTERN FORMATION AND TISSUE MORPHOGENESIS DURING MOUSE EMBRYOGENESIS

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The ability to culture and therefore experimentally manipulate and image the development of mouse embryos from the zygote to the blastocyst stage has led to an impressive understanding of the mechanisms behind the first cell fate decisions and the plasticity of pre-implantation development. In contrast, development of the embryo as it implants has been hidden from a direct view and experimental manipulations as it occurs within the body of the mother. Yet these “implantation-stages” are critical: this is the time when the embryo acquires a totally different shape and form, the pluripotent population of cells expands and the anterior-posterior axis becomes established. To gain direct and more precise that thus far possible insight into this developmental transition we have established a new system that enables mouse embryos to develop, be manipulated and imaged throughout implantation stages outside the mother. This has opened a way to provide a new and unexpected insight into how the mouse embryo develops its form and pattern at this previously inaccessible developmental stage. I will discuss these new results at my talk.

day two: tuesday january 21, 2014

ESSENTIAL GENES FOR DEVELOPMENT: IMAGING OF MOUSE EMBRYONIC LETHALS

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One of the greatest challenges to biomedical research in the 21st Century is to understand how the genome gives raise to a normal mammal, and how gene deficits hinder normal development. From a health point of view, we really want to know these genetic effects in the human. However, given the similarity between the human genome and the mouse genome, many of the essential experiments can be carried out in the mouse. There is an International Knockout Mouse Consortium (IKMC) which has knocked out the 23,000+ genes in the mouse one at a time. When these single-gene deficient mice are grown, about a third of them turn out to be embryonic lethal. These are the genes that are absolutely essential for normal development. If they are not expressed, reduced in copy number, or produce a less active product, it is important to know how these effect normal development.

Three-dimensional imaging is the ideal way to study embryonic lethals. The International Mouse Phenotyping Consortium (IMPC) has developed plans to determine at birth whether the knock out embryonic mice are embryonic lethal. A second test for lethality will be conducted at E12.5. If they are lethal after E12.5, they will be imaged at E15.5; and, if they are lethal prior to E12.5, they will be imaged at E9.5.

This talk will describe the imaging methods used for this primary screening. Optical Projection Tomography (OPT) for E9.5 embryos, Iodine-stained Micro-CT imaging for E15.5 embryos, and Diffusion-Sensitized MRI for subviables at post-natal stage P7.

Automated computer analysis techniques for identifying anatomical differences in each of these sets of images will be illustrated. The challenge of comparing mutant embryos with a wild-type reference during the early phases of rapid development will be addressed using a four-dimensional (three spatial dimensions and time) embryonic atlas.

Whole-body imaging of the effects of individual gene molecular products illustrates molecular imaging at its most challenging.

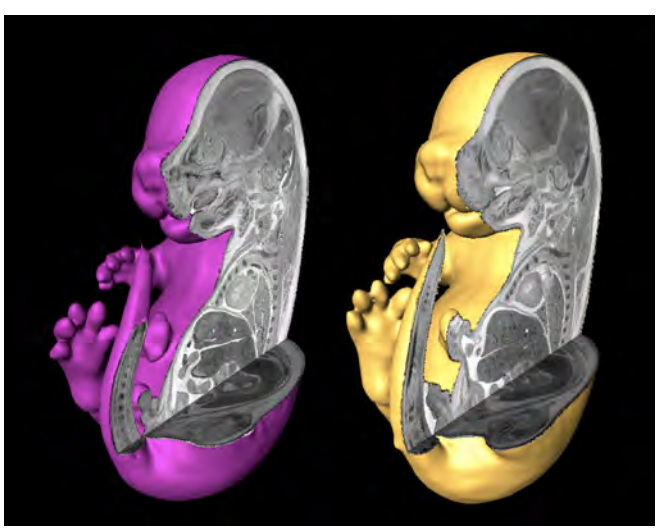

Figure 1 MR images of two embryos: The challenge of phenotyping is to recognize anatomical differences between embryos

INTRODUCTION TO AN EXPERIMENTAL HARDWARE APPROACH OF COMBINING MPI AND MRI INTO AN INTEGRATED PRECLINICAL HYBRID IMAGING SYSTEM

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Introduction: Magnetic Particle Imaging (MPI) is an emerging new tracer-based imaging technology based on the detection of the non-linear response of superparamagnetic iron oxide nanoparticles to an oscillating homogeneous magnetic field [1]. As biological tissue behaves highly linear in respect to an external magnetic field, the MPI signal originates particularly from the administered tracer material which makes an unambiguous assignment to the anatomy difficult. This highlights the need for a reference image dataset which provides the lacking morphological information e.g. data acquired from Magnetic Resonance Imaging (MRI) scanner. In this work, an experimental hardware setup of an integrated hybrid preclinical system, comprising the modality of MPI and MRI, is presented in terms of its specification, realization as well as by preliminary results.

Methods: This experimental setup realizes the generation of two different magnetic field profiles by means of one resistive split solenoid coil magnetic field generator (MFG) which meets the requirements for MPI and MRI, respectively. Dependent on the applied current polarity, the proposed MFG facilitates generation of a homogeneous magnetic field B_z as polarization field (PF) suitable for MRI or a steep magnetic field gradient with zero-crossing as selection field (SF) suitable for MPI (c.f. Fig. 1). Furthermore, to allow for MR imaging, a 2nd order shim system for homogenization of the B_z field, an actively shielded and MPI compatible MRI gradient coil and a Tx/Rx radio frequency coil is included. To allow for MP imaging, a 3D Tx/Rx drive field coil is included, whereas each channel operates at a dedicated resonance frequency around 25 kHz.

Results: This hybrid system is currently under assembly at the research labs of Bruker BioSpin. The magnetic field strength and the magnetic field gradient were measured to be up to B_z=0.495 T and up to G_z=2.2 T/m for the PF and the SF, respectively. It was found that for allowing robust MRI dataset acquisition (c.f. Fig. 2) the PF had to be stabilized to a higher degree in terms of the cooling fluid temperature combined with the usage of self-stabilizing MR sequences using MRI navigators compensating for residual MFG drifts by frequency modulation.

Conclusions: This promising imaging system allows for hybrid dataset acquisition comprising the quantitative MPI tracer distribution as well as morphological MRI information. Due to the concentric topology of all magnetic components, the subject can be scanned sequentially in both modes without the need for subject repositioning and hardware modifications. Using the same software (ParaVision® 6) for both modes simplifies the merging of the complementary data.

Acknowledgement: The authors thankfully acknowledge the financial support by the German Federal Ministry of Education and Research, FKZ 13N11088. References: [1] B. Gleich et al. Nature, vol. 435, no. 7046, pp.1214–1217, 2005. [2] J. Weizenecker et al., Phys. Med. Biol. 54(5):L1-L10. 2009.

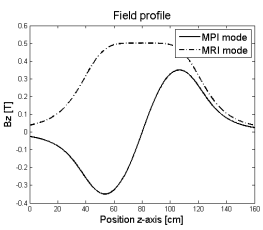

Figure 1: Axial line scan of the magnetic field in the two different modes MPI and MRI, respectively.

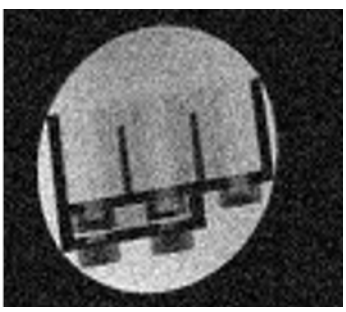

Figure 2: LEGO® phantom MR image using a self-stabilizing Intragate-Flash sequence on the MPI-MRI Hybrid Imaging System.

RAPID 3D LIGHT-SHEET MICROSCOPY WITH A TUNABLE LENS

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Introduction: The analysis of many interesting aspects of developmental biology requires the rapid recording of extended three-dimensional volumes. Fast in-vivo imaging is ideally performed by Selective Plane Illumination Microscopy (SPIM, [1]). To generate a three-dimensional image stack in typical SPIM implementations the sample has to be translated along the detection axis. Due to the fragility of the biological sample there is an upper limit on how fast the sample can be scanned through the light sheet. To keep the sample stationary, both the illumination and detection need to be scanned along the detection axis to acquire image stacks. The light sheet can easily be scanned through the sample using a fast, tiltable mirror. Keeping the image of the illuminated plane on the camera in focus is more challenging. For example, moving the detection lens is not desirable in the case of water immersion lenses.

Methods: We chose a remote focusing approach with an electrically tunable lens (ETL; Optotune AG). ETLs have recently been demonstrated to provide optical focusing for in vivo two-photon microscopy [2]. In our setup (Fig. 1) an ETL is placed in the detection path of a light-sheet microscope, the light sheet is moved along the detection axis by a tiltable mirror and constantly kept in focus of the detection optics by varying the focal length of the ETL [3]. Calibration of the system (mirror angle and focal length of the ETL) in a sample of fluorescent beads before the measurements ensures the perfect overlap of illumination and detection at all times.

Results: In general, the extent of the achievable z-range depends on the effective numerical aperture of the detection objective and its magnification. In combination with a 10x/0.3 water-dipping objective a z-range of 1.1 mm was achieved. Such large volumes can be imaged in the fraction of a second. We recorded the vascular system in the head of a zebrafish (Fig. 2). For faster scanning, due the resonant behavior of the ETL it is best to use sinusoidal driving signals to avoid overshooting of the lens. By the addition of a chromatic splitter in the detection path we are currently able to image two fluorophores in 25 planes over a range of more than 50 microns at a framerate of 60 Hz in each individual plane to study the development of the heart.

Conclusions: The inclusion of a remote focusing unit into a light sheet microscope unleashes the full potential of light-sheet microscopy with modern high-speed cameras and leaves the sample unaffected from any mechanical motion. Our ETL-SPIM is ideally suited for the rapid recording of transient signals, e. g. neuronal activity in the brain, and for the reconstruction of the blood flow in the heart.

Acknowledgement: We thank Michaela Mickoleit & Michael Weber for the preparation of Zebrafish samples. Manuel Aschwanden & Mark Blum (Optotune AG) provided ETLs. We thank Martin Wieckhorst for technical assistance. This work was supported by a grant to F.H. from the Swiss SystemsX.ch initiative (Project Neurochoice) and to J.H. by the Human Frontiers Science Program (HFSP). Furthermore, this work was funded by the Max Planck Society.

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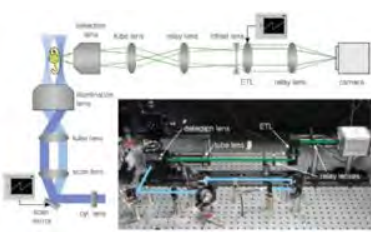

Figure 1: ETL-SPIM setup: The SPIM setup has been modified to include a scan mirror and an ETL lens.

Figure 2: Large volume: Large volume scan with an ETL through the head of a zebrafish (blood cells in red, vasculature in green).

TARGETING ENDOTHELIAL METABOLISM : PRINCIPLES AND STRATEGIES

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Laboratory of Angiogenesis & Neurovascular link, Director Vesalius Research Center (VRC), VIB-KULeuven, Leuven, Belgium

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Angiogenesis, the growth of new blood vessels, plays a crucial role in numerous diseases, including cancer. Anti-angiogenesis therapies have been developed to deprive the tumor of nutrients. Clinically approved anti-angiogenic drugs offered prolonged survival to numerous cancer patients. However, the success of anti- angiogenic VEGF-targeted therapy is limited in certain cases by intrinsic refractoriness and acquired resistance. New strategies are needed to block tumor angiogenesis via alternative mechanisms. Recent studies revealed that targeting endothelial metabolism can be a possible alternative therapeutic strategy for anti-angiogenic therapy. In this perspective, we are also exploring several metabolic pathways.

PRECLINICAL INTRAVITAL MICROSCOPY OF THE TUMOUR-STROMA INTERFACE: INVASION, METASTASIS, AND THERAPY RESPONSE

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Key steps of cancer progression and therapy response depend upon interactions between cancer cells with the reactive tumour microenvironment. Intravital microscopy enables multi-modal and multi-scale monitoring of cancer progression as a dynamic step-wise process within anatomic and functional niches provided by the microenvironment. These niches deliver cell- and matrix-derived signals that enable cell subsets or single cancer cells to survive, migrate, grow, undergo dormancy, and escape immune surveillance. Using intravital infrared multiphoton second and third harmonic excitation, combined with 5-channel fluorescence detection, we here we show how collective cancer invasion follows preformed multi-interface conduits, including perimuscular, vascular and –neural tracks of 1D, 2D and 3D topography. Using molecular interference against beta1 and beta3 integrins, invasion remains unperturbed and metastatic dissemination enhanced, which however in a preclinical therapy regimen was successfully prevented by combining high-dose radiotherapy with anti-integrin targeting. Thus, beyond basic research, intravital microscopy has reached preclinical application to identify niches and mechanisms of cancer dissemination and resistance as well as new therapeutic concepts.

ETHICAL CONSIDERATIONS

Gouyon, P.-H.

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The history of the XXth century showed dramatically how easily the scientific community can promote and even participate in hideous activities on one side and how terrible and disastrous the ideological control over science can be. Today, progress in biological sciences raises fascinating questions at the social level. Indeed, our capacity to investigate profound features of living beings is becoming so powerful that citizens ask for debate and reflection about what we, scientists, are developing.

- Can the social practices resulting from advances in science lead to non-ethical results in terms of medical practices, eugenism, neuro-marketing etc.? Should we, scientists, care about them? If yes, what can we do?

- Experimentation, even when designed to be non-invasive, through imaging for example, can impact organisms, whatever the model, and including human. Can we predict how? Should new regulations be developed?

- All these considerations bring a surprising question: should scientists allow themselves to ask any question or should certain questions be forbidden? We are used to consider that science has no limit. But each human is allowed to have his/her private sphere. How much can we investigate and manipulate the genome, the brain or the body of others?

We propose to explore these questions and promote a debate among the participants. An *open discussion* is scheduled later this evening after dinner.

day three: wednesday january 22, 2014

ADULT NEUROGENESIS IN PHYSIOLOGY AND DURING PARKINSON'S DISEASE

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The adult mammalian brain retains niches for neural stem cells (NSCs), which can generate glial and neuronal components of the brain tissue. However, it is barely established how chronic neuroinflammation, as it occurs in neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, affects adult neurogenesis and, therefore, modulates the brain's potential for self-regeneration.

Here, we demonstrate that differences in the chronicity of TNF-a application to cultured NSCs result in opposed effects on their proliferation. However, chronic TNF-a treatment, mimicking Parkinson's disease associated neuroinflammation, shows detrimental effects on neural progenitor cell activity. Inversely, pharmacological inhibition of neuroinflammation in a 6-hydroxydopamine mouse model led to increased neural progenitor cell proliferation in the subventricular zone and neuroblast migration into the lesioned striatum. Four months after surgery, we measured improved Parkinson's disease-associated behavior, which was correlated with long-term anti-inflammatory treatment. But surprisingly, instead of newly generated striatal neurons, oligodendrogenesis in the striatum of treated mice was enhanced.

We conclude that anti-inflammatory treatment, in a 6-hydroxydopamine mouse model for Parkinson's disease, leads to activation of adult neural stem cells. These adult neural stem cells generate striatal oligodendrocytes. The higher numbers of newborn oligodendrocytes possibly contribute to axonal stability and function in this mouse model of Parkinson's disease and thereby attenuate dysfunctions of basalganglian motorcontrol.

ASSESSING NEURODEGENERATION, NEUROINFLAMMATION AND NEUROGENESIS IN AN EXPERIMENTAL PARKINSON'S DISEASE MODEL USING MULTIMODAL MOLECULAR IMAGING

Fricke, I. B.[1,2,3], Viel, T.[1,4], Collmann, F.[1], Vrachimis, A.[1,5], Worlitzer, M.[2], Faust, A.[1], Wachsmuth, L.[6], Faber, C.[6], Tavitian, B.[4], Dollé, F.[7], Schwamborn, J.[2,3,8], Jacobs, A. H.[1,3,9]

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Introduction: Parkinson's disease (PD) is a slowly progressing neurodegenerative disorder caused by loss of dopaminergic neurons in the *substantia nigra* (SN), leading to severe impairments in motor and non-motor function. Endogenous neural stem cells of the subventricular zone (SVZ) constantly give birth to new cells which might serve as a possible source for regeneration in the adult brain. However, neurodegeneration (ND) is accompanied by neuroinflammation (NI) potentially compromising regeneration. This study aims to non-invasively monitor ND, NI, and progenitor cell migration in a mouse model of PD by multi-modal molecular imaging employing small animal SPECT, PET, MRI, and *in vivo* BLI.

Methods: C57Bl6 mice (10-14 weeks) received unilateral intranigral injections of 6-Hydroxydopamine (6-OHDA) or vehicle. Multimodal imaging was performed at various time points post injection (p. i.), including [^{18}F]DPA-714-PET to assess microglia activation (d7 n=15; d14 n=16; d21 n=13), [^{123}I]Ioflupane-SPECT to determine nigrostriatal degeneration (d3 n=8; d7 n=5; d18 n=16) as well as T2-weighted MRI in order to obtain anatomical information. A lentiviral-based reporter construct carrying the firefly luciferase and mCherry genes under the control of the cytomegalovirus promoter was injected into the SVZ of FVB mice and progenitor cell migration was followed weekly using BLI. After reaching a signal plateau, unilateral 6-OHDA (n=5) and vehicle infusion (n=4) was carried out and signal distribution is being examined over several weeks.

Results: After 3 and 7 days, neurotoxin injection leads to a strong decrease in dopamine transporter ligand accumulation in the left striatum, compared to the normal right striatum. Uptake ratios are further reduced at 18 days post injection, indicating ongoing degeneration after the first week. PET with the TSPO ligand [^{18}F]-DPA-714 reveals an increase in tracer accumulation in the lesioned SN two weeks post injection compared to control SN, which abates 3 weeks post injection. Three weeks after reporter vector injection into the SVZ, first progenitor cell migration towards the olfactory bulb (OB) could be observed using BLI with a stable signal from the OB after 8 weeks.

Conclusions: Induced neurodegeneration of the nigrostriatal system leads to acute neuroinflammation, which both can be assessed by multi-modal molecular imaging. Injection of lentiviral particles encoding firefly luciferase into the SVZ is a suitable tool to follow progenitor cell migration. Ongoing experiments deal with the effect of 6-OHDA lesion on progenitor cells of the SVZ-OB system.

Acknowledgement: The research leading to these results has received funding from the Interdisciplinary Center for Clinical Research (IZKF); Muenster; Germany (project SchwJ3/001/11) and the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 278850 (INMiND).

INTRAVITAL CHARACTERIZATION OF THE INFLAMMATORY CELLS RECRUITMENT DURING THE DEVELOPMENT OF GLIOBLASTOMA BRAIN TUMOR

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Introduction: Our recent data on a preclinical mouse model suggest that drugs transiently reducing Glioblastoma (GBM) tumor growth rate mainly inhibit tumor progression by acting on the stromal environment. A better knowledge of the changes of this microenvironment during tumor progression therefore appears as a pre-requisite to improve the management of GBM patients. Intravital spectral two-photon microscopy on multicolour fluorescent mice models was then used to dynamically characterize the cellular composition of the tumor stroma. Special emphasis was set on the recruited inflammatory cells as they release chemokines and trophic factors likely regulating tumor development.

Methods: Syngenic GL261 DsRed fluorescent GBM spheroids were grafted into the brain of bicolor lysGFP x Cd11cYFP mice where macrophages/monocytes or microglia and dendritic cells are fluorescent. A cranial window was cemented on the opened skull to allow chronic imaging. Following intravenous Cascade blue dextran to label blood vessels, we repeatedly imaged the same tumor at cellular resolution every 3 to 4 days from the second week post-grafting until experimental end-point. From these 4 color images sets, we could characterize and correlate the dynamics of all the cell types on time scales ranging from minutes to hours and weeks.

Results: We found that both LysGFP(+) cells and Cd11cYFP(+) cells were present at high densities in the tumor stroma from the first imaging session (D15). Whereas LysGFP(+) cells were very motile and scrawl around tumor cells, Cd11cYFP(+) cells exhibited stable bodies with highly dynamic processes to sense the environment. Although such phenotypes were expected respectively from the myeloid and microglial origins of these cells, we found that they evolved with tumor stage: LysGFP(+) cells significantly slowed down and accumulated in the tumor while Cd11cYFP(+) cells conversely became more motile and slightly decreased in number.

Conclusions: Our results evidence that the stromal immune signature is dynamic so that it can be used to stage tumor progression. They also support the idea that LysGFP(+) accumulation might be responsible for increased tumor aggressivness.

Acknowledgement We thank the staff of the animal and PicSIL imaging facilities of the IBDM where experiments have been conducted. This work was supported by grants from Agence Nationale de la Recherche (ANR JCJC PathoVisu3Dyn), Fédération de Recherche sur le Cerveau (FRC) to FD and from Cancéropole PACA to CR.

IN VIVO IMAGING OF THE DEVELOPING MOUSE BRAIN: FROM MORPHOLOGY TO MOLECULES

Turnbull, D., Aristizabal, O., Bartelle, B., Berrios-otero, C., Deans, A., Nieman, B., Suero-abreu, G., Szulc, K., Yu, X.

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Extensive genetic information and the expanding number of techniques available to manipulate the genome of the mouse have led to its widespread use in studies of brain development and to model human neurodevelopmental diseases. We are developing a combination of ultrasound and magnetic resonance micro-imaging approaches with sufficient resolution and sensitivity to provide noninvasive structural, functional and molecular data on developmental and disease processes in normal and genetically-engineered mice. Our efforts over the past decade have focused on *in utero* and early postnatal imaging and analysis of the developing brain and cerebral vasculature. The advantages and limitations of both ultrasound and MRI for imaging mouse development will be discussed, and examples provided to illustrate the utility of these approaches for 4D mutant phenotype analysis. Recent advances have also made in the area of molecular imaging, including the generation of novel reporter mice that enable cell-specific imaging with ultrasound and MRI contrast agents. Future directions for molecular imaging of mouse brain development will be discussed.

MOLECULAR IMAGING STUDIES OF THE DEVELOPMENTAL EMERGENCE OF INTEGRATIVE BRAIN FUNCTION

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Introduction: Embryo brains make a developmental transition from a loose network of spontaneously-active cell groups to an organ that functions in a large-scale, highly-integrated and coordinated fashion through mechanisms that remain unknown. We are using molecular imaging to better understand the developmental emergence of organized brain states and the emergence of brain responsiveness to external sensory stimulation.

Methods: Brain metabolic activity from in-ovo chick embryos was measured using sub-millimeter-resolution positron emission tomography (PET) with 2-deoxy-2-[18F]fluoro-D-glucose (18FDG), combined with non-invasive behavioral recording during the final 25% of the fetal period. Structural X-ray computed tomography (CT) of the skeleton was used for fine-scale embryo aging. Embryo PET data was compared to data from 16 awake, sound-exposed 1-2 days post-hatching chickens1.

Results: Non-invasive imaging revealed developmental brain changes that older neurophysiological studies were unable to detect: (a) a previously-undescribed "physiologically-inactive" embryo brain state, which gives rise to a continuum of activated states starting at about 80% of embryonic development; (b) physiological brain activation was inversely related to behavioral activity; (c) exposure to a salient chicken vocalization increased higher-brain activity significantly more than exposure to a ''nonvocal'' noise analog, and (d) patterns of correlated activity between the brainstem and higher-brain areas resembling awake, post-hatching animals were only seen prenatally in chicken-sound-stimulated embryos.

Conclusions: Molecular imaging revealed the developmental emergence of sleep-like behavior and its linkage to metabolic brain states, and that waking-like brain function is present in a latent but inducible state during the final 20% of embryonic life, selectively induced by context-dependent monitoring circuitry. Continuing work combines chronic in-ovo EEG recording and brain temperature measurements in single embryos with PET imaging, and neurophysiological and molecular characterizations of the circuitry controlling the emergence of sleep and waking.

Acknowledgement Support provided by the Fundación BBVA, NSERC (298612), CFI (9908), SISSA, and HFRP (RGP0004/2013).

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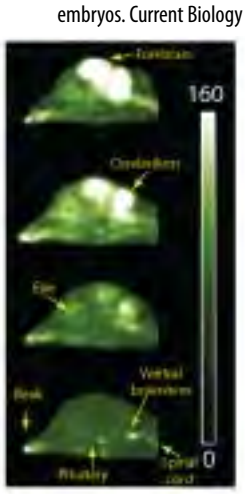

Figure 1: Embryo brain PET images

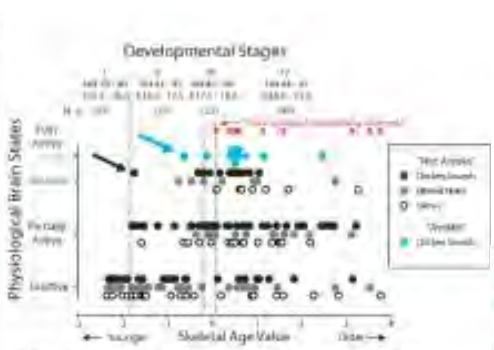

Figure 2: Embryo brain state development

VASCULAR RESISTIVITY IMAGING USING ULTRAFAST DOPPLER : APPLICATION TO THE 2D CEREBRAL MAPPING OF PRETERM INFANT VASCULAR INDEXES

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Introduction: Preterm infant are in a large extent subject to neurological disorders resulting from injuries during brain development. Among the most common lesions, intraventricular hemorrhage and hypoxia-ischemia involve the vascular network 1 and can lead to severe complications such as periventricular leukomalacia, while ultrasound modalities encounter difficulties to give good prognostic parameters. Ultrafast Doppler imaging through the fontanel, used regularly during preterm infant monitoring, could give early insights on changes in Cerebral blood flow, and help to detect those incidents. In this study we investigate the use of this technique to build 2D maps of resistivity/pulsatility index 2 over the entire vascular network in a single cardiac cycle.

Methods: Ultrafast Doppler Imaging uses the recently introduced compound ultrasound plane wave imaging technique enabling both the detection of small vessels (~100 µm) and the acquisition of the complete blood flow speed profile over the entire field of view in a single cardiac cycle, which would not be possible with a conventional focused Doppler sequence. UD Imaging was implemented and validated using an Aixplorer® (Supersonic Imagine, France) and a linear ultrasonic transducer (192 elements, 0.2mm pitch, 6 MHz). Ultrafast Doppler acquisitions were obtained in parasagittal and coronal sections for 10 preterm infants, using a dedicated ultrasound Plane Wave Compound sequence, enabling to acquire 3500 images in 1 sec. For each acquisition, in every pixel an a posteriori pulse wave Doppler like spectral profile is computed 3 and the resistivity index is calculated.

Results: Results showed that the extraction of peak systolic and end diastolic flow speeds in every location of the imaged vascular network was possible with quantitative values in agreement with the litterature (R.I = 0,8 +/- 0.1 in the anterior cerebral artery, RI < 0,2 in veins). We showed that this index mapping was reflecting changes in preterm cerebral hemodynamics in agreement with what was observed on a pulse wave Doppler profile: as a clinical proof of concept we monitored changes of resistivity during mild compression of the fontanel and during rewarming after controlled therapeutic hypothermia 4.

Conclusions: Images of the brain vascular network have been obtained with an unprecedented resolution, all imaging modality taken together, and the feasability of resistivity mapping in a clinical context has been demonstrated. Parametric mapping via Ultrafast Doppler imaging could be a very useful prognostic tool for preterm infant monitoring, but also a ground breaking tool for functional imaging of the infant brain activity.

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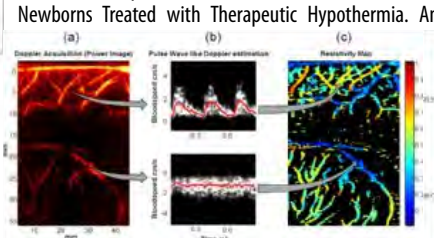

Figure 1: Description of the Resistivity map computing process.

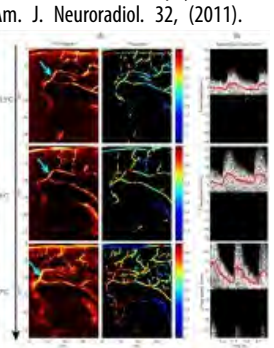

Figure 2: Application of Resistivity mapping to the monitoring of rewarming after controlled hypothermia

ULTRAFAST ULTRASONIC IMAGING : FROM SHEAR WAVE ELASTOGRAPHY TO FUNCTIONAL IMAGING OF THE BRAIN ACTIVITY

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In the last fifteen years, the concept of plane wave transmissions rather than line by line scanning beams breaks the conventional limits of ultrasound imaging. By using such large field of view transmissions, the frame rate reaches the theoretical limit of physics dictated by the ultrasound speed and an ultrasonic map can be provided typically in tens of micro-seconds (giving several 1000 frames per second). Interestingly, this leap in frame rate is not only a technological breakthrough offering completely new ultrasound imaging modes and open new application, but at such frame rates, it becomes possible to track in real time the transient vibrations – known as shear waves – propagating through organs. Such "human body seismology", called **Shear Wave Elastography (SWE)**, provides quantitative maps of local tissue stiffness whose added value for diagnosis has been recently demonstrated in many fields of radiology (breast, prostate and liver cancer, cardiovascular applications,...).

For blood flow imaging, ultrafast Doppler permits high-precision characterization of complex vascular and cardiac flows. It also gives ultrasound the ability to detect very subtle blood flow in very small vessels. In the brain, such ultrasensitive Doppler paves the way for **fUltrasound** (functional ultrasound imaging) of brain activity with unprecedented spatial and temporal resolution compared to fMRI. Examples such as the functional imaging of cerebral blood volume during epileptic seizures will be presented and ill emphasize the potential of this new imaging modality. Finally, it will be shown that SWE and fUS could potentially become great tools to provide new insights in the development of brain during the first steps of life.

My Notes

day four: thursday january 23, 2014

IMAGING VASCULAR REMODELING IN REPRODUCTION AND DEVELOPMENT

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Survival of multicellular organs depends on maintenance of homeostasis via matched delivery of nutrients and oxygen and clearance of waste products. Although diffusion is extremely effective for short distances, beyond 1 mm such delivery cannot be adequately achieved by random Brownian motion, and thus tissue wellbeing and any change in tissue architecture require accompanying remodeling of blood supply. Over the last years we developed MRI tools for monitoring blood flow in the ovary, as it responds to hormonal stimulation during the ovulatory cycle; we followed the vascularization of ovarian grafts; and monitored pregnancy from the early stages of fetal implantation, through the development of the fetal vascular system in organogenesis and the function and perfusion of the placenta. MRI provides multiple functional and molecular readouts, including the use of DCE-MRI to measure blood volume and vessel permeability, BOLD contrast MRI for monitoring oxygen transport, ferritin expression as a reporter gene for MRI, and arterial spin labeling for monitoring perfusion.

Application of these tools on Akt1 deficient mice revealed key effects of Akt1 in mediating vascular remodeling in reproduction. These effects include impact on ovarian graft reception and follicular reserve; effects on fetal bone and heart development, effects on placental function, and remarkably also on wildtype embryos implanted adjacent to Akt1 deficient embryos.

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HUMAN EMBRYONIC POSTERIOR FOSSA VENOUS DEVELOPMENT BASED ON SERIAL HISTOLOGIC 3D RECONSTRUCTION

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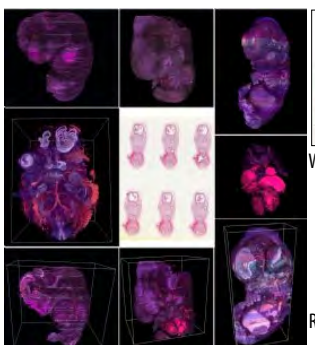
Introduction: Since the mid 1950's, very little work has been done to contribute to a better understanding of brain venous embryogenesis. We present a new analysis method based on histologic sections. This presentation focus on venous evolutive morphology of the brain stem (arachnoido-pial) and petro-cavernous region.

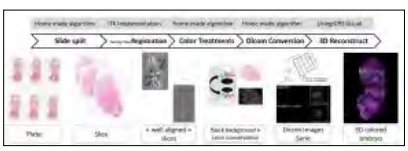
Methods: Thirteen well preserved human embryos were used for our study (Rouvière-Delmas & Tardif universitary collections). The 3D reconstructions were produced using a homemade program (Java and ITK.org based). The reconstruction process included the stages of selecting, registering and filtering histological sections, and volume reconstructions. After having been digitalized (Epson V750 scanner, optical resolution 2400 DPI, i.e. a pixel of 10u²), the microscope slides containing several slices (up to 20 slices by slide) were automatically split using a custom algorithm. Then, each ordered slices were spatially repositioned by pair, using a rigid, intensity-based registration algorithm from ITK. Every slice-registration was then checked and could be manually corrected. Using a specially developed color treatment system we were able to conserve the colorimetric information (histological staining transfert), allowing the use of a black background for 3D volume rendering.The vascular information was retained by inverting the luminance channel of the HSL (Hue/Saturation/Lightness) colorimetric representation. The vascular information available due to the bright red histological staining of the erythrocytes in the embryonic vascular tree was so optimized. The final file was exported as a DICOM file. ORS Visual software (v1.5, Object Research System, Montreal) was used to visualize the DICOM files, providing the display possibilities necessary to obtain the final images (3D VR; MPR).

Results: At first step, we confirmed the embryonic status relatively to the Carnegie classification in assessing the cochlear canal orientation. The evolutive morphology of the brain stem primitive venous network is presented ; at stage XIX, the initial longitudinal veins are separated from the caudal plexus. During the next stages, we demonstrate how they connected to transverse pial anastomoses. The next period (i.e the late embryonic and early post-embryonic period) shows these intrinsic pial veins lying between the arteries and the brain surface leading to a progressive simplication pattern. We also show the embryonic venous pattern of the cavernous and petrosal regions for the same stages. These organisations are compared to the Padget and Streeter works.

Conclusions: We present the preliminary work of posterior fossa venous embryogenesis using 3D and angiographic rendering at the late embryonic stages.

Acknowledgement: The authors are grateful to Pr Delmas, Pr Mercier & Pr Duparc for providing the embryo collection.





Workflow: steps of histological slices treatment

Results: several view of embryos n°151/n°7/n°184

NON-INVASIVE 3-DIMENSIONAL IMAGING OF DROSOPHILA PUPAE AND FLY USING RASTER-SCAN OPTOACOUSTIC MESOSCOPY (R-SOM)

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Introduction: Morphogenesis is a dynamic process on many scales, from fast subcellular rearrangements to structural changes at the whole-organism level. Live imaging approaches based on light microscopy are of key importance to obtaining such information with high spatiotemporal resolution. Although it excels at the cellular and the subcellular levels, it lags in performance at the whole-organism level. To overcome this we developed a raster-scan optoacoustic mesoscopy system (R-SOM) that can image fields of view larger than 4x4mm^2, with resolutions of 30 µm in-plane and 7 µm axially, and at depths reaching up to 5 mm.

Methods: Optoacoustics uses ultrasounds to image absorption contrast, because ultrasounds have less scattering in soft tissue high-resolution images of optical absorption could be generated, at depths beyond the optical diffusion limit. In R-SOM we use a high frequency (25-125 MHz), high numerical aperture (~1), and spherically focused ultrasound sensor for measuring the signals excited with a fast ns laser (pulse width < 2ns, 1.4kHz, 515nm). For acquisition we use a 60 dB amplifier, and a high-speed digitizer. Fast piezo stages allow fast raster scanning of samples, a field of view of 4x4 mm^2 is scanned in ~5-10 min. Besides calibration measurements we have non-invasively imaged a drosophila pupae and a drosophila fly *ex-vivo.*

Results: Both the drosophila pupae and the drosophila fly were genetically modified to express GFP from the wings. Before imaging we embedded the pupae and the fly inside diffusive agar. In the pupae reconstructions, we can see the body of the pupae, and a stronger signal from the middle of the pupae where we suspect the wings grow from. From the fly reconstructions we can clearly see the wings, the body, the head, and the legs of the fly.

Conclusions: The generated images show the potential of R-SOM in imaging morphogenesis, the high spatial resolution coupled with high penetration depths, and large field of view allows the non-invasive imaging of development at the whole-organism level, not only in drosophila but also in other animals such as the zebrafish. Although the first measurements were done *ex-vivo*, *in-vivo* measurements are possible with an estimated time resolution of 2-10 minutes dependent on the FOV. Coupling the mesoscopic method with an optical microscopy method such as SPIM will generate a continuum, where SPIM images at the cellular level and R-SOM images at the whole-organism level, thus image morphogenesis at all levels.

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LIVE-IMAGING REVEALS POTENTIAL NEW MECHANISM OF SELF-RENEWAL

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Introduction: Neural progenitors must be able to self-renew and differentiate during brain development, to promote tissue growth while also producing neurons. We have previously found in a vertebrate central nervous system (CNS) that during asymmetric divisions the daughter cell that inherits the most apical domain becomes a neuron while the basal daughter cell becomes a progenitor. We also observed that the daughter cell that initially loses contact with the apical surface is able to re-attach apically and replenish the apical progenitors pool (Alexandre et al, 2010). We are exploring the cellular and molecular mechanisms that regulate this process in the vertebrate CNS.

Methods: We use the superior optics of the zebrafish brain and live imaging techniques to follow neural progenitors behaviour during and after cell division in an intact embryo. We mosaic labeled neural progenitors with membrane- fluorescently tagged proteins and we monitored apical re-polarisation by following distribution of Par3- and ZO1-GFP.

Results: Time-lapse movies analysis revealed that midbody positions at the leading edge of the basal daughter cell while it regains the apical surface. We also observed that midbody positioning at the apical surface coincides with apical repolarisation. We found that reduction of Msd2 function, a new scaffold protein that localizes to midbody and centrosomes, prevents midbody repositioning to the apical surface; in 10% of these cases it prevented the re-establishment of apical attachment in zebrafish neural progenitors.

Conclusions: Our observations so far suggest that midbody could be critical in allowing neural progenitors to return to the apical surface and reform apical junctions. We are now exploring the potential mechanisms that may reposition midbody and basal daughter cell to the apical and newly reform the junctional belt.

Acknowledgements: Dr Takashi Toda at CRUK and Dr Masa Tada at UCL -collaboration in Msd2 function.

References: Alexandre P et al, Nat. Neuroscience, 2010.

BRANCH OR EXPAND? NEW INSIGHTS INTO DLL4/ NOTCH DYNAMICS DRIVING VASCULAR PATTERNING

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The formation of a hierarchically branched network of blood vessels is critical tissue supply during development and in physiology. What determines whether activated endothelial cells form a new vessel branch, or rather expand the existing vessel has remained unclear. Here we show that the expression of the Notch ligand Dll4 fluctuates in individual endothelial cells within a sprouting vessel in correlation with dynamic cell movement. Studying a novel genetic Dll4 reporter in mouse ES cells, together with the polarity and speed of cell migration, as well as the coordination of individual cell movement and expression levels within the cell population, we find that sprout elongation and branching associates invariably with a highly differential phase pattern of Dll4 between the endothelial cells. Stimulation with pathologically high levels of VEGF, or overexpression of Dll4, leads to Notch dependent synchronization of Dll4 fluctuations within clusters of endothelial cells both in vitro and in vivo, and to corresponding synchronization in collective cell migration. In oxygen-induced retinopathy and in glioblastoma vessels, the loss of vessel diameter control associates with local synchronization of Dll4/Notch signalling. Dynamic imaging in zebrafish tumour vessels identifies cycles of synchronous sprouting and retraction from neighbouring endothelial cells, which leads to vessel expansion instead of branching. Our results demonstrate that the VEGF-Dll4/Notch feedback system normally operates to generate heterogeneity between endothelial cells driving branching, whilst synchronization drives vessel expansion. Based on insights derived from computational simulation, we propose that the sensitivity of this phase transition between differential and synchronized Dll4 dynamics could be a key modulator that is influenced by a variety of environmental factors and signalling pathways, and that therapeutic modulation could be exploited to regulate vessel patterning and therefore vessel network functionality.

IN-VIVO IMAGING OF DROSOPHILA MELANOGASTER DEVELOPMENT

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Introduction: Helical-Optical Projection Tomography (hOPT) [1] is a novel, simple and cost-effective method that allows 3D-imaging of elongated samples. hOPT is based on the vertical translation of the sample while measuring different angular projections, accounting for this movement when performing the 3D reconstructions. Using hOPT it is now possible to obtain 3D-optical images of intact elongated samples without imposing limits on the sample length. Due to the characteristics of this novel approach, hOPT also enables high-throughput tomography of small specimens which we here demonstrate. We show here 3D-reconstructed data of 7 GFP-expressing Drosophila melanogaster pupae which were imaged simultaneously with acquisition times in the order of 20 seconds per fly.

Methods: A hOPT recently developed [1] (see Fig. 1) was used to perform high-throughput time-lapse tomography to follow the development of a total of 7 D. melanogaster pupae for over 12 hours acquiring images every 15 min. Several maximum intensity projection images of 4 individuals are shown in Fig. 2.

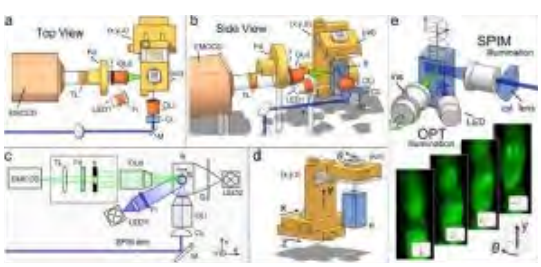
Results: As shown in our results, hOPT successfully allowed the simultaneous observation of several individuals over the time, being possible to follow different developmental steps such as the eversion of the head in different pupae within the same experiment. These results demonstrated the potential of hOPT in developmental studies as a high-throughput tool.

Conclusions: The reduced acquisition time required for hOPT imaging significantly diminishes the risk of phototoxicity facilitating the visualization of in-vivo processes. Thus, hOPT was used to perform high-throughput time-lapse tomography of the development of 7 D. melanogaster pupae for over 12 hours at 15 minutes intervals. Our results demonstrate that hOPT enables the discrimination of GFP-expressing individuals, the observation of multiple structural characteristics in different development phases, and the visualization of in-vivo processes in multiple individuals simultaneously.

Acknowledgement: This research was supported by the E.U. FP7 Collaborative Project "FMT-XCT". A. Arranz acknowledges support from the Marie Curie Intra-European Fellowship program (FP7-PEOPLE-2010-IEF). J. Ripoll Acknowledges the Marie Curie Reintegration Grant CIG12-GA-2012-333632 HIGH-THROUGHPUT TOMO.

References: [1] A. Arranz, D. Dong, S. Zhu, M. Rudin, C. Tsatsanis, J. Tian and J. Ripoll, "Helical Optical Projection Tomography", Opt. Exp. 21, 25912-25925 (2013)

Figure 1: Description of the hOPT set-up



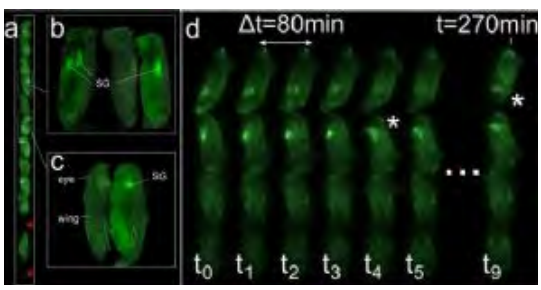


Figure 2: (a)Volume renders of 13 D. melanogaster pupae. Red arrowheads indicate non-GFP individuals. (b, c)Volume render details of two pupae at different developmental stages. SG stands for salivary glands. (d)Time-lapse imaging of a set of D. melanogaster pupae.

GAP JUNCTION CONTROL OF CELL COMMUNICATION AND LEADER CELL FUNCTION IN COLLECTIVE CELL MIGRATION

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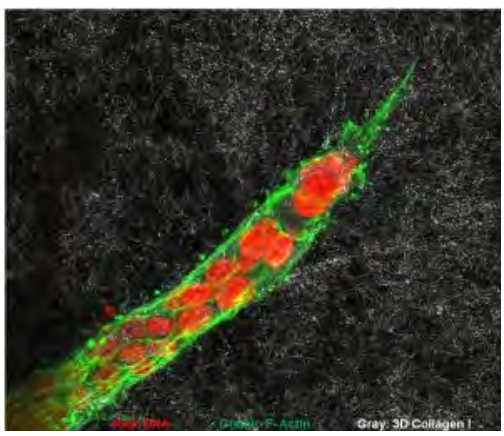
Introduction: During collective invasion cancer cells maintain cell-cell contact and mechanosensory coupling, and undergo coordinated polarization, interaction with tissue and position change. Using 4D live-cell microscopy, the generation of front-rear asymmetry and collective polarization can be identified. Thereby one, or few, leader cells initiate and maintain collective movement by anterior protrusion and elongation while maintaining cell-cell contact to rearward follower cells, however the mechanisms of leader cell selection and communication to follower cells remain unknown.

Methods: 3D collagen invasion assays; 4D live-cell microscopy; Confocal microscopy (Immunocytochemistry of collective cancer cell invasion in 3D lattices and Immunocytochemistry of cancer invasion in ductal carcinoma samples); 3D Gap-FRAP (Fluorescence Recovery After Photobleaching) during collective invasion; QPCR and western blot analysis; Pharmacological inhibition using specific gap junction blockers and purinergic receptor antagonists; RNAi interference

Results: During collective invasion of mammary carcinoma cells in 3D collagen lattices monitored by multiparameter microscopy, leader and follower are connected by gap junctions, as shown by connexin 43 immunolocalization along cell-cell junctions and 3D fluorescence recovery after photobleaching (3D GAP-FRAP). Combined with pharmacological inhibition of connexin function by carbenoxolone (CBX), those data suggest cytosolic continuity among the collectively migrating cells. Interference with connexin-mediated cell-cell connectivity or Cx43 by RNAi, inhibited the initiation of collective cancer cell invasion, suggesting an essential role in leader cell formation. Besides along cell-cell contacts, Cx43 localized at anterior protrusions of leader cells in a focalized pattern, indicative for hemichannel function. We thus addressed whether known hemichannel-released chemoattractants, including ATP and ADP, contribute to leader cell function. Similar to Cx43, several purinergic receptors were polarized towards filopod-like tips of leader cells. Interfering with few purinergic receptors by either pharmacological antagonists or RNAi strongly impaired leader cell development and initiation of collective invasion. Exogenous addition of nucleotides/nucleosides was sufficient to rescue the inhibition imposed by CBX, suggesting hemichannel-mediated nucleotide release as key step in leader cell initiation.

Conclusions: Thus, using cellular and molecular imaging, we suggest a dual role for gap junctions in maintaining cell-cell connectivity and hemichannel function towards autocrine purinergic signaling to initiate leader cell formation and thus collective cancer cell invasion.



Cancer leader cells: A leader cell guiding collective invasion of breast cancer cells in 3D collagen I

IMAGING THE DEVELOPING CARDIOVASCULAR SYSTEM IN MICE

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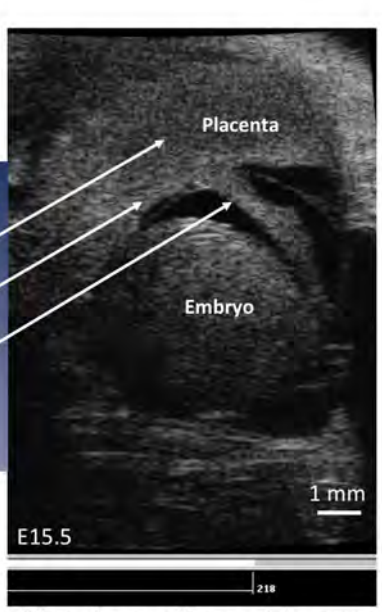
Genetic and environmental factors play important roles in determining the structural and functional development of the cardiovascular system. Working with our collaborators, we use vascular corrosion casting to image the embryonic microcirculation, micro-computed tomography to image and quantify the branching structure of developing arterial trees, and in vivo micro-ultrasound to assess the hemodynamic function of the heart and vasculature in the developing mouse embryo. For studies of development, a strength of these technologies is that the same methods used in tiny mouse embryos can be applied throughout postnatal development into adulthood.

Our lab has used these methods to investigate factors important in the normal growth and development of the maternally-perfused and fetally-perfused circulations of the placenta, the cardiac function of the mother during pregnancy, and of the offspring during embryonic and postnatal development. Maternal exposure to environmental factors including carbon monoxide and chemicals in cigarette smoke, and genetic influences caused by differences in mouse strain or by genetic deficiencies in eNOS, Klf3, Fgl2, and Gcm1 have all been shown to alter cardiovascular development and/or function.

Supported by the Canadian Institutes for Health Research (CIHR), the Heart and Stroke Foundation of Ontario, Canadian Foundation for Innovation, and the Richard Ivey Foundation.

uCT image of placental arterial circulation in the mouse (*Rennie, Whiteley, Adamson & Sled)*



Micro-ultrasound image of the mouse placenta (*Junwu Mu & Adamson)*

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IMAGING VESSEL REMODELING AT THE MICRO-SCALE DURING DEVELOPMENT: IMPLICATIONS FOR TISSUE ENGINEERING AND UNDERSTANDING CONGENITAL DISEASE

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Our group is interested in force-regulated mechanisms that guide morphogenesis and tissue engineering. Understanding the interplay between genetic mechanisms and those regulated by local tissue forces is fundamental to understanding congenital disease. This presentation will focus on imaging studies aimed at defining the role of mechanical forces generated from blood flow in regulating blood vessel remodeling during mouse development. Despite the extensive work showing the importance of blood flow in angiogenesis and vessel remodeling, very little is known about how changes in vessel diameter are orchestrated at the cellular level in response to mechanical forces. To define the cellular changes necessary for remodeling, we performed live confocal imaging of cultured mouse embryos during vessel remodeling. Our data revealed that vessel diameter increase occurs via two distinct processes that were dependent on normal blood flow: vessel fusions and directed endothelial cell migrations. Vessel fusions resulted in a rapid change in vessel diameter and were restricted to regions that experience the highest flow near both the vitelline artery and vein. Directed cell migrations induced by blood flow resulted in the recruitment of endothelial cells to larger vessels from smaller capillaries and were observed in larger artery segments as they expanded. The dynamic and specific endothelial cell behaviors captured in this study reveal how sensitive endothelial cells are to changes in blood flow and how such responses drive vascular remodeling. These events identified in development have profound implications for tissue engineering and strategies to integrate imaging studies in the embryo with tissue engineering approaches will be discussed.

INTERNALISATION MOVEMENTS OF THE ENDODERM DURING GASTRULATION IN THE ZEBRAFISH EMBRYO.

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Introduction: During gastrulation, cells acquire their fate and reorganise within the embryo: ectodermal cells remain outside of the embryo while mesendodermal cells internalise to reach the surface of the yolk. Although this process is crucial to the development of metazoan embryos, the mechanisms underlying germ-layer separation are not yet elucidated. *In vitro* experiments using zebrafish cells demonstrate that germ-layer progenitors can segregate passively by sorting out[1]. In the embryo, the synchronised ingression of cells at the margin makes delicate the analysis of the movements. *In vivo* relevance of cell sorting and whether active processes are involved in germ-layer formation thus remains an open question.

Methods: To assess the question of germ-layer separation *in vivo*, I use a model based on the fact that wherever they are placed in the embryo, endoderm-committed cells internalise and form endoderm[2]. Using single-cell transplants followed by time-lapse microscopy (fig. A) and combined with functional analyses, I provide the first fine description of cell internalisation in Vertebrates.

Results: Endoderm-committed cells are polarised and emit actin-rich cytoplasmic extensions (fig. B). They internalise when, following a contact with the yolk, their cell body rapidly translocates on the surface of the yolk. Loss-of-function analyses demonstrate that the Rac small GTPase is required for the internalisation of endoderm-committed cells, possibly by promoting cytoplasmic extension formation. Rho/Rock/Myosin pathway also seems to be involved in the migration process by playing a role in the retraction of the cell body. These observations strongly suggest an active phenomenon. Strikingly, ectodermal cells are also polarised, with similar actin-rich cytoplasmic extensions. This suggests a two-step model: 1) All cells are polarised and emit cytoplasmic extensions in the direction of the yolk; 2) endoderm-committed cells actively migrate and flatten on the surface of the yolk.

Conclusions: Endoderm internalisation thus results from an active migration rather than a cell sorting. I am currently trying to identify the signals that polarise the cells within the embryo, and those triggering the internalisation of endodermal cells.

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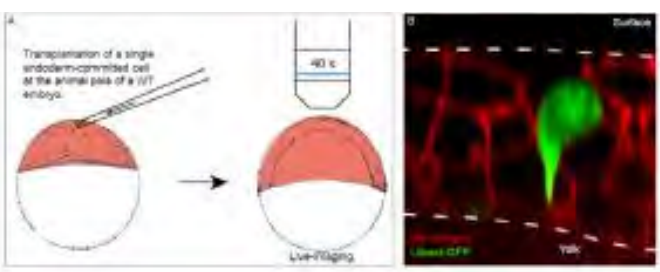

Experimental design: A: Schematics of the experiment. B: Reconstructed section showing an endoderm-committed cell stained with lifeact-GFP, at the animal pole of a WT embryo stained with membrane-bound mCherry.

RECONSTRUCTING MULTI SCALE IN VIVO IMAGING DATA BY MULTILEVEL DYNAMICS

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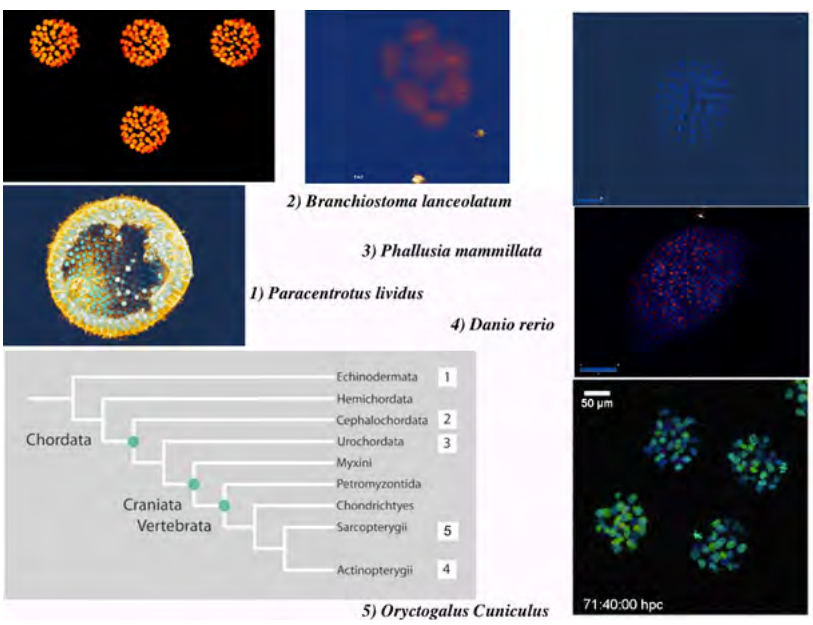
We approach the understanding of model organisms' embryonic morphogenesis, through the quantitative analysis and biomechanical modeling of multiscale in vivo imaging data. The cellular level of organization is taken as resulting from the integration of sub-cellular and supra-cellular processes. Cell dynamics are investigated through 3D+time imaging of developing embryos with fluorescent nuclear and membrane staining (Figure). The automated reconstruction of the cell lineage tree, annotated with nucleus and membrane segmentation, provides measurements for cell behavior: identity, fate, displacement, division, shape and contact changes. This quantitative data is sufficient to find statistical models for cell proliferation and cell descriptors evolution in time and space, and characterize the spatial and temporal length scale of cell displacements and tissue deformations. Confronting numerical simulation derived from a multi-agent based biomechanical model with empirical measurements extracted from the reconstructed digital specimens, is the basis for testing hypotheses for processes underlying early embryogenesis. Further correlating cell behavior, tissue biomechanics and biochemical activities by comparing the patterns revealed by cell fate, velocity, strains or gene expression, is a step toward the integration of multi-level dynamics. This overall framework lays the ground for a transdiciplinary approach of living systems' morphogenesis.



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POSTER

poster #1

DUAL ANALYTE DETECTION FOR BIOLUMINESCENT IMAGING OF TWO BIOLOGICAL EVENTS VIA ENZYMATIC UNCAGING OF D-CYSTEINE AND 2-CYANOBENZOTHIAZOLE

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Introduction: The discovery of biocompatible reactions has had a tremendous impact on chemical biology, allowing the study of numerous biological processes directly in complex systems. However, despite development of multiple biocompatible reactions in the past decade, only few work well in living animals. We have previously reported [1] that the condensation reaction of 2-cyanobenzothiazoles with D-cysteine works in vivo and produce D-luciferin. Oxidation of D-luciferin by firefly luciferase generates light that can be visualized in vivo by bioluminescence imaging, and furthermore allows interrogation of targeted tissues using “caging” approach.

Methods: We therefore applied this reaction for the real-time non-invasive imaging of apoptosis associated with caspase 3/7 through the release of D-cysteine upon cleavage of the sequence (Asp-Glu-Val-Asp-D-Cys) (DEVDc) and quantification of light emission. Similarly, 2-cyanobenzothiazole can be caged by different substrates, therefore making it possible to monitor enzymatic activity. This opens up possibilities for modular construction of bioluminogenic sensors, where both reaction partners could be caged independently, and would be of great interest for monitoring multiple biological events in the same animal. Beta galactosidase is an important reporter enzyme and there is currently a demand for better probes for in vivo non-invasive imaging. We thus developed an assay where it is possible to monitor and quantify apoptosis and ß-galactosidase activity using the dual caging approach.

Results: - In vitro imaging of beta-galactosidase activity by using a ß-galacto-2-cyanobenzothiazole. - In vitro imaging of caspase 3/7 activity by using DEVDc. - In vitro evaluation of dual caging of the split luciferin using purified caspase 3/7 and beta galactosidase enzymes.

Conclusions: By caging both reaction partners D-cysteine and 2-cyanobenzothiazole, we could monitor two different biological processes in the same sample. For example, apoptosis and levels of gene expression are two important concepts in developmental biology. Our already published results using the “split luciferin“ ligation reaction in vivo have important implications for in vivo imaging and open up possibilities for dual caging in order to visualize concurrent biological events by dual analyte detection.

References:[1]Godinat,A.;Park,H.M.;Miller,S.C.;Cheng,K.;Hanahan,D.;Sanman,L.E.;Bogyo, M.; Yu, A.; Nikitin, G.; Stahl, A.; Dubikovskaya, E. A. ACS Chem Biol. 2013, 17;8(5), 987-999.

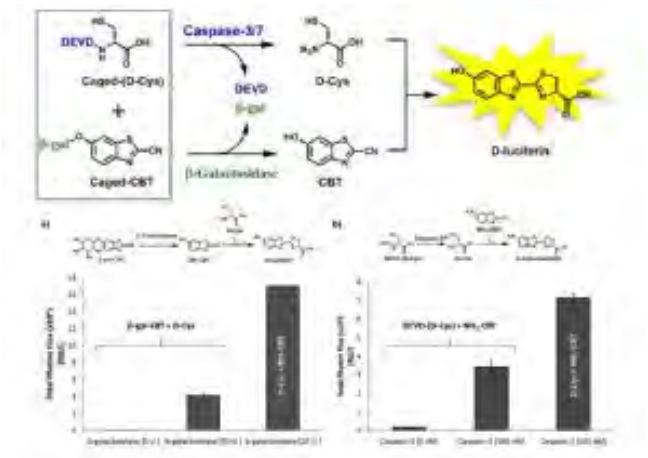

Figure 1: (Top) Selective luciferin formation enabled by dual enzymatic activity; (Bottom) Total photon flux from in vitro enzymatic uncaging by (a)beta-galactosidase and (b)caspase-3/7

poster #2

4D VERTICAL IMAGING OF PLANT GROWTH IN PHYSIOLOGICAL CONDITIONS WITH TWO PHOTONS MICROSCOPY

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Introduction: Root development, growth and architecture are major components of plant nutrient and water use efficiencies [1]. Plants are highly sensitive to gravity and imaging root development under a classical microscope using an horizontal plate does not respect negative root gravitropism thus inducing a “waving” effect on the root growth. Likewise,the small space available between the plate and the objective lens does not make possible the achievement of desirable microenvironmental conditions to provide plant optimal physiological conditions for their growth and development [1] . To overcome these problems, we develop a new vertical imaging set-up allowing plant root growth imaging under controlled environmental conditions and optimal plant physiological conditions.

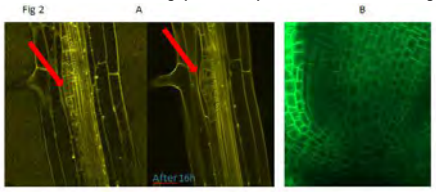
Methods: We use an inverter from LSM tech; this system can be used to convert any inverted microscope into an upright and vice versa. We customised it to be in a horizontal position and the emitted signal was directed into a non-descanned detector that was installed on the other port of the inverter Fig 1. To improve the quality of the acquisition, the sample will be mounted on a rotary stage, this allows to take multiple view of the specimen at different angles then fused to obtain a 4D images with a good resolution [2]. Plants are cultured under the microscope, in a custom made chamber where the temperature can be controlled and the illumination is provided by specific LEDs that are particularly well suited for plant growth. Indeed, in this controlled environmental, the plant is maintained vertically with its roots on agar

Results: Images are taken with the microscope Zeiss LSM 510 META Two-Photon Laser Scanning confocal with Coherent Chameleon Ti:Sa Laser. For acquisition of three-dimensional (3D)-image a piezo stepper for objective positioning (Piezosystem Jena) was installed. Recently following the growth of a lateral root of Arabidopsis thaliana Fig 2.a. for a couple of days in physiological conditions was realized.In addition, we plan to study the plant response to environmental signals (ie osmotic stress, nitrogen and phosphorus availability [3]). Another application that we currently started is to understand the mechanisms that control the cell division in rice root meristems Fig 2.b.

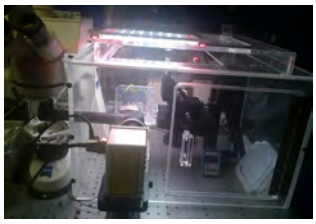
Conclusions: This tool is specially designed and adapted to plant observation and will pave the way to understand molecular and cellular mechanisms that control root meristem formation and development. Besides, it can also be adapted to other plants like corns or with animals.

Acknowledgement: This work is supported by grants from Agropolis Foundation, the INRA and the CIRAD of Montpellier.

References: 1-Assessing the importance of genotype × environment interaction for root traits in rice using a mapping population: II. Conventional QTL analysis. Theor Appl Genet. 2- Imaging plant growth in 4D: robust tissue reconstruction and lineaging at cell resolution. Nature Methods. 2010 Jul. 3-Nitrogen acquisition by roots: physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. Plant Soil 370(1-2).



Plants growth: in panel A, the laterals cells of Arabidopsis thaliana are divided after 16 hours, panel B show a meristem of a rice root



System setup: The modified two photons microscope is shown with the detector in mode NDD, the chamber and the plants in vertical position.

poster #3

LONGITUDINAL MRI OF RETINOPATHY *IN VIVO*

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Introduction: Hypertensive retinopathy predicts the long-term risk of stroke, the major course of neurological disorder. Retinopathy is characterized by abnormal angiogenesis and gliogenesis; some of the keys genes in these processes can be regulated by non-protein coding microRNA (miR). MicroRNAs are a short non-coding RNA with potential to reduce the stability of messenger RNA and thereby regulate protein coding RNA translation. The sequences of miRNAs are usually evolutionarily conserved in vertebrates. Most investigators construct advanced recombinant lentivirus with the capacity to express large quantities of miRNA to regulate (knockdown or knockup) gene expression; there is ample evidence that miRNAs control gene expression. The majority of studies assessing the involvement and effects of miR has been done with postmortem sequencing technology with bio-informatic software. Although angiogenesis in the retina can be examined noninvasively*(1)*, few modalities allow direct imaging and quantitative measurement of specialized cells and miR *in vivo*. Having previously demonstrated seven platforms for imaging microglia activation, regenerative angiogenesis and gliogenesis *in vivo* in living brains using target-guided magnetic resonance imaging (MRI) in a mouse model of cerebral ischemia*(2, 3*), we aim to monitor the progression of retinopathy.

Methods: Based on specific antisense and sense sequence hybridization within cells with unique intracellular RNA expression profiles, we have developed a gene transcript targeting MRI contrast agent by linking phosphorothioate-modified antisense DNA (sODN) and gadolinium (Gd, a T_1 MR contrast agent) or superparamagnetic iron oxide nanoparticles (SPION, a T_2 susceptibility MR contrast agent), which we delivered by intraperitoneal injection or eye drops to live C57B6 mice with blood-brain barrier (BBB) or blood-retina barrier (BRB) disruption, achieving a resolution of 0.005 mm^3 per voxel using high-field 9.4 Tesla MRI*(4*). In human MR scaners, the resolution can be 0.16 mm^3 per voxel using 4.7 Tesla system.

Results: Using a model that shows angiogenesis by pericyte-specific sODN-nestin *(2, 3*), we show Gd-nestin detected angiogenesis in the retina two weeks after cerebral ischemia (Fig 1, left panel). We have detected elevation in neuroglia (Muller cells) using SPION-gfap at 6 months later (Fig 1, upper right panel). The control shows no signal change in the retina (Fig 1). We validated the elevation of neuroglia by postmortem immunohistology (Fig 2).

Conclusions: Our laboratory has developed a platform using longitudinal MRI to monitor various cells involved in gliogenesis, microglia activation and angiogenesis in living retina by MRI.

Acknowledgement: This work is supported by NIH grants: The Boston Area Diabetes Endocrinology Research Center [P30DK057521-14 (J Avruch)], DA029889 and EB013768 (PKL), B Welcome Foundation (HP). The 9.4T MRI system was funded in part by NIH (S10RR025563) to the Athinoula A. Martinos Center for Biomedical Imaging.

References: 1. B. A. Berkowitz et al. Retinal ion regulation in a mouse model of diabetic retinopathy: natural history and the effect of Cu/Zn superoxide dismutase overexpression. Investigative ophthalmology & visual science 50, 2351 (May, 2009). 2. C. H. Liu et al., Noninvasive detection of neural progenitor cells in living brains by MRI. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 26, 1652 (Apr, 2012). 3. C. H. Liu et al., Diffusion-weighted magnetic resonance imaging reversal by gene knockdown of matrix metalloproteinase-9 activities in live animal brains. J Neurosci 29, 3508 (Mar 18, 2009). 4. C. H. Liu et al., Noninvasive delivery of gene targeting probes to live brains for transcription MRI. The FASEB journal 22, 1193 (Apr, 2008).

Fig 1 MRI-PKLiu: Longitudinal MRI of the retina 6 months after cerebral ischemia

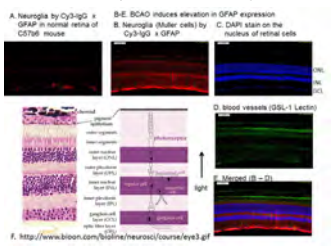

Fig 2 Validation PKLiu: Postmortem validation of retinopathy

poster #4

FUNCTION OF QUAKING RNA BINDING PROTEINS IN MYOFIBRILLOGENESIS

Bonnet, A.[1], Dutrieux, F. X.[1], Lambert, G.[1], Ernest, S.[1], Lobbardi, R.[2], Rosa, F.[1]

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Introduction: Functional muscle cells contain myofibrils, each consisting of numerous sarcomeres, which are the smallest contractile unit of muscle. Sarcomeres are made up of intercalating myosin and actin filaments linked together by many other proteins. One major question concerning muscle formation is to understand how muscle cells mature in order to build such stereotyped and highly organised macromolecular complexes. This question is critical since many muscle pathologies result in defects in myofibrillogenesis. Research has mainly focused on the structural protein content of sarcomere and the interaction between some of these sarcomeric proteins. Different models of myofibrillogenesis have been proposed, but *in vivo* data are lacking to assess their biological relevance. Therefore, we aim to understand how myofibrils are formed *in vivo* and how this process is regulated. We are in particular interested in the function of Quaking (Qki) genes, which encode RNA binding proteins known to regulate various post-transcriptional processes.

Methods: Our project uses the zebrafish model, as it is particularly suited to study muscle development. Indeed muscles derived from somites constitute the most important part of the trunk and they are functional early during development. In order to analyse muscle formation, we use classical fluorescent immunohistochemistry combined to confocal imaging as well as electronic microscopy to reveal muscle fibre ultrastructure during zebrafish development. Moreover, we aim to take advantage of the external development and optical clarity of zebrafish embryos to perform time-lapse analysis of myofibril formation.

Results: Our lab has shown that QkiA is required for the formation of slow and fast muscle fibres during zebrafish development. Searching for Qki homolog genes in zebrafish, we have recently characterized another Qki gene, that we named QkiB. QkiB simple loss of function doesn't seem to affect muscle formation. Interestingly, QkiA and QkiB are both expressed in slow muscle cells suggesting that they could interact. Indeed, Qki double loss of function leads to major defects in muscle formation. We observed accumulation of myosin at the tips of muscle fibres, a phenotype highly reminiscent of the one observed in human myofibrillar myopathies. In order to better characterize these defects in myofibrillogenesis, we are currently setting up time-lapse analysis using different transgenic lines or injection of constructs allowing the expression of sarcomeric proteins coupled to fluorescent proteins.

Conclusions: In conclusion, our results show that Qki RNA binding proteins are important regulators of myofibril formation.

poster #5

IMAGING ACTIVATED ECM REMODELING ENZYMES: FROM BIOPHYSICS TO CLINICAL APPLICATIONS

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Introduction: Extracellular matrix (ECM) remodeling processes are well coordinated, involving versatile dynamic and reciprocal dialogues, between matrix proteins and the cellular components. Importantly, extensive tissue remodeling during developmental processes, in health and disease, beg scientific attention to enzymatic proteolysis of the ECM. Defined enzymes, e.g. matrix metalloproteinases (MMPs), a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), plasmin and cathepsin G (serine proteases), have been shown to degrade various yet selective ECM proteins, while ECM fragments and their receptors were shown in-vivo to be directly involved in tissue remodeling during physiological and pathological processes. This gives credence to the idea that ECM proteolytic reaction mechanisms may well have implications that go beyond modifying the structural environment of cells and tissues, representing master switches in the regulation of critical developmental processes responsible for instructing cell behavior. Subsequently, various ECM molecules and enzymes are currently targeted as key players in many pathological conditions. The hierarchical importance of proteases in a system is influenced by specific activity of the protease, redundancy, expression levels, temporal–spatial distribution, zymogen activation, protease turnover and inhibition properties, all of which are crucial issues in both diagnostic tools and drug development.

Methods: We use an innovative immunization strategy that employs aspects of molecular mimicry to produce inhibitory antibodies (metallobodies) exhibiting hybrid protein:protein interaction mechanisms while binding the catalytic metal scaffold within activated MMPs/ADAMs. We demonstrate the therapeutic and diagnostic potential of these activated protease selective inhibitors using various imaging modalities and a range of mouse models.

Results: Overall we report novel generic approach for generating highly potent, highly selective modulators targeting catalytic metal ions and key elements within native ECM metalloenzyme active sites, and the surrounding protein surface. These nature-like therapeutic and diagnostic molecules targeting activated ECM remodeling enzymes are currently used to image ECM remodeling enzymes in vivo.

Conclusions: Overall we report novel generic approach for generating highly potent, highly selective modulators targeting catalytic metal ions and key elements within native ECM metalloenzyme active sites, and the surrounding protein surface. These nature-like therapeutic and diagnostic molecules targeting activated ECM remodeling enzymes are currently used to image ECM remodeling enzymes in vivo.

poster #6

ANGIOMOTIN PREVENTS PLURIPOTENT LINEAGE DIFFERENTIATION IN MOUSE EMBRYOS VIA HIPPO PATHWAY-DEPENDENT AND -INDEPENDENT MECHANISMS

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Introduction: Cell identity is specified in the early mammalian embryo by the generation of precursors for two cell lineages: the pluripotent inner cell mass (ICM) and differentiating trophectoderm.

Methods: Time-lapse imaging during *in vitro* culture was performed using a confocal spinning-disc microscopy system (Intelligent Imaging Solutions). Time-lapse imaging of pre-implantation embryos was performed on a Leica SP5.

Results: We identify Angiomotin (Amot) as a key regulator of the first cell fate decision. We use *in vivo* imaging, in combination with an *in vitro* culture system to show that loss of Amot leads to differentiation of ICM cells and compromised peri-implantation development. We show that Amot regulates localization of Yap and Yap-binding motifs are required for Amot full activity. Importantly, we also show that Amot function can compensate for the absence of Lats1/2 kinases, indicating ability of Amot to bypass the classical Hippo pathway for Yap regulation. In polarised outside cells, Amot localises apically, pointing to the importance of cell polarity in regulating Yap to promote differentiation.

Conclusions: We propose that both Hippo pathway-dependent and Hippo pathway-independent mechanisms regulate Yap localisation to set apart pluripotent and differentiated lineages in the pre-implantation mouse embryo.

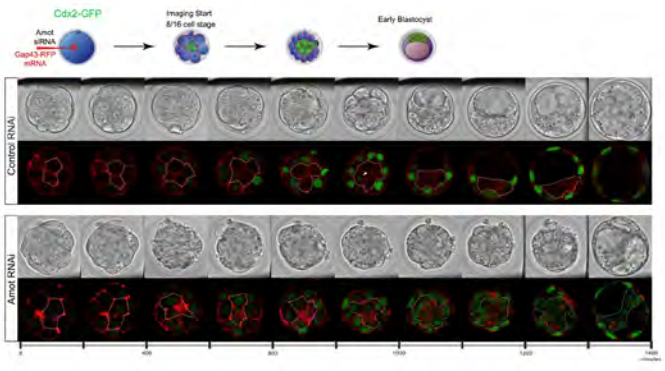

Fig. 1 Live imaging: Live imaging of pre-implantation embryos to elucidate the role of Angiomotin

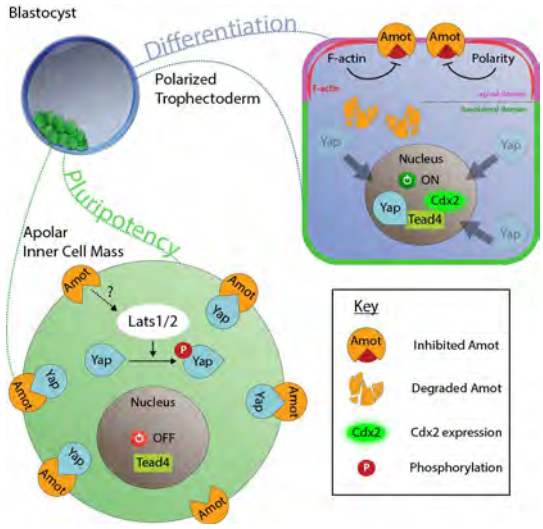

Fig 2 Role of Angiomotin: The role of Angiomotin in the first cell fate decision

poster #7

ROLE OF CDX2 IN EARLY MOUSE DEVELOPMENT

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Introduction: Genesis of the trophectoderm and inner cell mass (ICM) lineages occurs in two stages. It is initiated via asymmetric divisions of 8- and 16-cell blastomeres that allocate cells to inner and outer positions, each with different developmental fates. Outside cells become committed to trophectoderm at the blastocyst stage through Cdx2 activity but here we show that Cdx2 can also act earlier to influence cell allocation.

Methods: In depth analysis of pre-implantation development using non-invasive time-lapse imaging.

Results: Using non-invasive time-lapse imaging approach we show that increasing Cdx2 levels in individual blastomeres promotes symmetric division thereby allocating more cells to the trophectoderm, whereas reducing Cdx2 promotes asymmetric divisions and consequently contribution to ICM. Elimination of zygotic expression of Cdx2 in knockout model leads to defects in the maintenance of the blastocyst cavity, suggesting that it participates only in the late stage of trophectoderm formation. However, we have found that mouse embryos also have maternally provided pool of Cdx2. Importantly, elimination of both maternal and zygotic Cdx2 through RNAi, morpholino or genetic deletion leads to developmental defects before blastocyst formation.

Conclusions: Taken together, our results indicate that Cdx2 participates in two steps leading to trophectoderm specification: appropriate polarisation of blastomeres at the 8 and 16-cell stage and then the maintenance of trophectoderm lineage-specific differentiation.

poster #8

ELASTIC TENSOR IMAGING, TOWARD A NEW MODALITY FOR BRAIN AND CARDIAC IMAGING

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Introduction: Diffusion Tensor Imaging (DTI) is a well known MRI technique for the mapping of cardiac or neuronal fibers. In neuroscience, the mapping of the neuronal fibers network has enabled to better study the brain structures connectivity and their functions. Although it has the potential to bring valuable clinical information about the brain development, DTI cannot easily be applied to fetal or neonatal imaging due to motion. Elastic Tensor Imaging (ETI) is a new imaging technique based on the tracking of shear waves by ultrafast ultrasound imaging that can yield important information on the mechanical structures of tissue and track elastic fibers from the local change of stiffness anisotropy. Even though the technique is in its infancy and not real time yet, ETI is a very promising imaging modality for fetal or transfontanellar brain development monitoring.

Methods: An ultrafast ultrasound scanner (Aixplorer, Supersonic Imagine, Aix-En-Provence, France) was used in Supersonic Shear Imaging mode with a linear probe (SL10-2, 6 MHz). Ultrasonic acoustic radiation force was used to generate a shear wave *in situ* and ultrafast imaging (>5000Hz) to measure its propagation velocity in a specific direction. Using a three-axis mechanical linear translation and rotation device, the shear velocity was measured in several direction locally. From this velocity tensor field, fractional stiffness anisotropy map and elastic fibers were reconstructed. The technique was tested *in vitro* on anisotropic PVA gels and *ex vivo* beef muscle, porcine and ovine myocardial samples and calf brain. Results were compared to DTI (7T MRI with spin-echo-based diffusion sequence, Bruker BioSpin, Germany).

Results: ETI was able to provide anisotropy maps and track elastic fibers in all tested tissues. The resulting images were well correlated with DTI images and gross histology.

Conclusions: ETI is a new ultrasound based imaging modality technique that can track elastic fibers. If the acquisition time is still slow using mechanical translation, implementing ETI on 2D ultrasound arrays will allow near real-time performance well suited for prenatal or transfontanellar imaging. ETI could thus one day be complementary to DTI and provide valuable information for the monitoring of brain development of foetus and neonates in clinical practice.

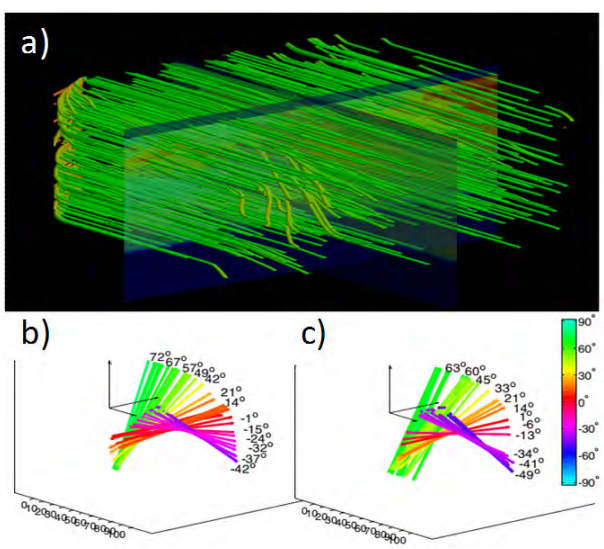

Fig 1: a) Elastic fibers tracking based on ETI in a transversely isotropic PVA phantom. Transmural fiber orientation estimated by ETI (figure b) and DTI (figure c) in the same porcine myocardial sample.

poster #9

THE ANGIOGENIC ROLE OF HYALURONIC ACID DURING EMBRYO IMPLANTATION

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Introduction: Implantation is a critical step in the establishment of pregnancy, during which the embryo attaches to and invades the maternal uterus. One of the characteristics of early pregnancy is a marked increase in the permeability and density of the uterine blood vessels and vascular remodeling that allow the delivery of oxygen and nutrients to the embryo until the placenta becomes structurally and functionally competent. Hyaluronic acid (HA) has been reported to participate in the regulation of vascular development. Specifically, high-molecular-weight HA has been shown to inhibit angiogenesis during development, whereas its enzymatic degradation products are by nature pro-angiogenic [1]. Interestingly, significant changes in HA distribution in the endometrium has been observed during the peri-implantation period, suggesting a potential role of this molecule in endometrial stroma preparation for embryo implantation[2]

Methods: Dynamic contrast-enhanced (DCE) MRI allows non-invasive dynamic analysis of angiogenesis in both normal and pathologic mouse pregnancies. In a study previously conducted in our lab, MRI analysis and subsequently fluorescence microscopy, using biotin-BSA-GdDTPA, allowed detection and quantitative assessment of mouse embryo implantation sites as early as embryonic day 4.5 [3].

Results: Over the course of a study presently carried out in our lab, we have obtained preliminary results, generated by functional MRI inspection of live pregnant mice on embryonic day 6.5. Treatment of theses mice with HA synthesis inhibitor 6-diazo-5-oxo-1-norleucine (DON), revealed a marked increase in decidual blood vessel permeability and accumulation of blood in close proximity to the implanted embryo.

Conclusions: These observations made us raise the hypothesis that HA metabolism participates in the regulation of uterine angiogenesis, vascular development and remodeling during embryo implantation in mice.

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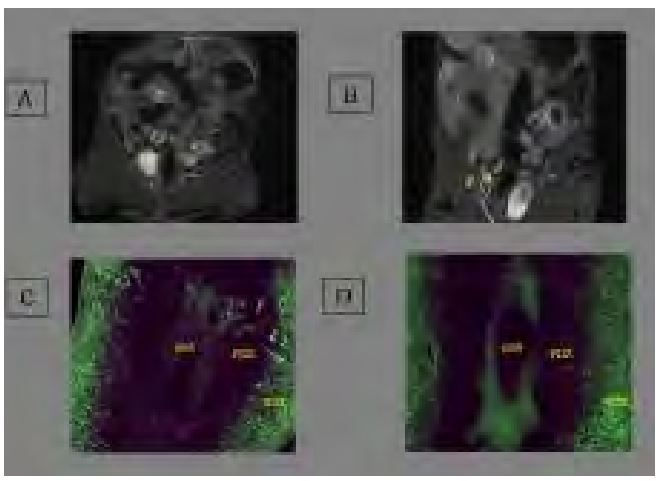

Fig 1: Abnormal morphology of the mouse implantation site in the absence of hyaluronic acid

poster #10

CARACTERISATION OF BRAIN NORMAL TISSUE AND TUMOR ELASTICITY VIA PER OPERATIVE SHEAR WAVE ELASTOGRAPHY: CLINICAL PROOF OF CONCEPT AND IMPLICATIONS FOR BRAIN FOETUS IMAGING DURING DEVELOPMENT

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Introduction: Characterization of brain mechanical properties could be relevant in the follow up of brain development of the human fœtus during pregnancy. Unfortunately current imaging technique like MRI can not evaluate the brain mechanical properties of fœtus in vivo in daily clinical practice. Thus the use of an ultrasound scanner allowing to monitor the elasticity of brain tumors at different stages of the fœtus development could be an efficient tool for the understanding of biomechanical development of brain tissues and potentially diagnosis and prognostic of brain fœtus dysfunctions during pregnancy[1]. As a first step for brain elasticity staging, is presented a clinical study on adult patient for per operative brain normal tissue and tumor characterization. The aim is to make a list of correlation between histology data and *in vivo* tissue mechanical characterization (Young's modulus in kPa) of these brain tumors. A clinical proof of concept of per operative brain elasticity mapping is here demonstrated and discussed.

Methods: An ultrafast ultrasound scanner (Aixplorer, Supersonic Imagine, Aix-en-Provence, France) was used in Shear Wave Elastography (SWE) mode driving a micro convex probe (SMC12-3, 7MHz central frequency). Shear waves are generated by using ultrasonic acoustic radiation force and their propagation is caught in real time by switching in an ultrafast imaging mode (up to 20000 frames/s). Thus shear wave velocity, directly linked to the stiffness of tissues, is calculated in each pixel of the image and color-coded in kilopascals (kPa)[2]. Measures was acquired on 25 adults, on per operating procedure, before resection of brain tumor.

Results: Histology allowed classification of the different types of tumors into four main groups: metastases, meningiomas, low-grade gliomas and high-grade gliomas. SWE was able to characterize each group of tumor by a specific range of elasticity value in kPa. According to *figure 2* gliomas with elasticity inferior to 15 kPa appear to be malignant (high-grade gliomas).

Conclusions: SWE and histology can together characterize brain tumors by quantifying elasticity in kPa. These data give crucial information of the malignance degree of the tumor depending on its elasticity. This study validates the relevance of SWE in the brain. SWE can be used as well in per operating situation. It gives here *in vivo* quantitative elasticity values for normal and tumoral brain tissues on adults. Beyond these initial results, it is envisioned that such elasticity mapping of brain tissues could be performed non-invasively during fœtus development. The extension of SWE to elasticity mapping of the brain tissues of human fœtus during pregnancy will be addressed and discussed.

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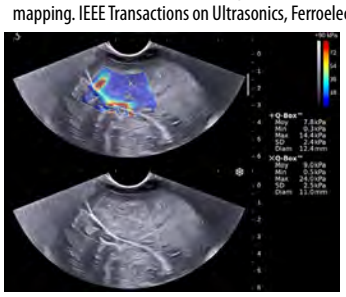

Elasticity map: Elasticity map of a high-grade gliomas superimposed on the classical B mode image. Average tumor elasticity: 8.4kPa

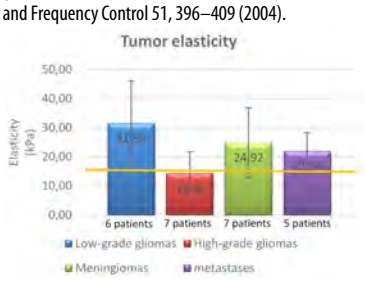

Fig 2: Tumor elasticity: Experimental tumor elasticity depending on the type of the tumor.

My Notes

poster #11

FETAL PROCESSING OF MATERNAL ALBUMIN FOLLOWED BY CONTRAST ENHANCED MRI

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Introduction: The placenta controls the regulated transfer of oxygen and nutrients from the mother to the fetus, while maintaining a tight barrier between the maternal and fetal circulation systems. Dynamic contrast enhanced (DCE) MRI of murine placental perfusion was reported using low and high MW contrast media. Low-molecular weighted gadolinium chelates can cross the placenta and reach the fetal blood pool [1]. In contrast, albumin-based macromolecular contrast agents do not cross the placental barrier, but are actively internalized by trophoblast cells in the labyrinth [2, 3]. The focus of this work was understanding fetal processing of maternal albumin.

Methods: MRI was performed on B6 (C57BL/6J) female mice (n=5) on E14.5 of gestation, using a 9.4 T Bruker scanner. T_1-weighted 3D-GE images were acquired during the 60 min period immediately following contrast-agent (biotin-BSA-Gd-DTPA) administration. The mean signal intensity (SI) was derived for each placenta and for the vena cava. 3D rendering was performed using Amira display software.

Results: Contrast-agent administration produced an initial SI increase during the 3-9 min, which was followed by SI decrease (12-27 min) and then a second SI increase 30-60 min post contrast agent injection [Fig.1 A-D]. The data were analyzed using a three-compartment model, with two main placental compartments -- maternal intravascular compartment and trophoblast cell intracellular compartment -- in addition to the maternal arterial input [Fig.1 A].

Conclusions: The SI reduction between 12-27 min and the delayed SI elevation afterwards is consistent with the hypothesis that the albumin-based contrast agent underwent active uptake into the trophoblast compartment, leading to its accumulation and creating a T_2^* effect (signal reduction). The dynamics of the albumin-based contrast media internalization is superimposed on a gradual accumulation of contrast media in the large maternal placental blood pool [Fig.1 A, C]. However, the model does not explain the perplexing second cycle of signal attenuation. This model study reveals complex and unexplored placental functionality in fetal recycling of maternal albumin.

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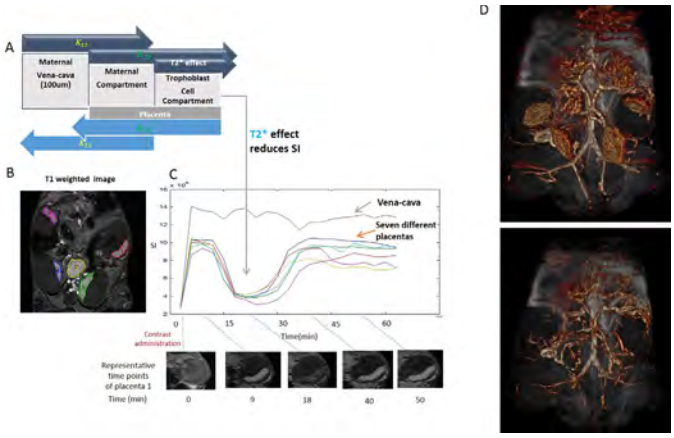

Fig. 1 : Suggested three-compartment model for b-BSA-GdDTPA kinetics during DCE-MRI scanning of the placenta.

poster #12

HIGH FREQUENCY ULTRASOUND FOR IN VIVO PREGNANCY DIAGNOSIS AND STAGING OF PLACENTAL AND FETAL DEVELOPMENT IN MICE.

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Introduction: Ultrasound is a valuable non-invasive tool used in obstetrics to monitor the growth and well being of the human fetus. The laboratory mouse has recently emerged as an appropriate model for fetal and perinatal studies because morphogenetic processes in mice exhibit adequate homology to those in humans, and genetic manipulations are relatively simple to perform in mice. High-frequency ultrasound (HFUS – 40 MHz) has recently become available for small animal preclinical imaging and can be used to study pregnancy and development in the mouse in a longitudinal and non-invasive manner with high spatial and temporal resolution. The objective of the current study was to assess the main applications of HFUS in the evaluation of fetal growth and placental function and to better understand human congenital diseases.

Methods: On each gestational day, at least 5 dams were monitored with HFUS; a total of ~ 200 embryos were examined. 17 morphometric parameters (decidual thickness, gestational sac length and thickness, implantation site length, and thickness, placental length and thickness, crown-rump length, abdominal circumference, antero-posterior abdominal diameter, latero-lateral abdominal diameter, occipital-snout length, biparietal diameter, subretinal space thickness, antero-posterior and latero-lateral lens diameter, stomach diameter) were examined from embryonic day (E) 5.5 to 16.5. Since it is not possible to measure each variable for the entire duration of the pregnancy, the parameters were divided into three groups as a function of the time at which they were measured. Univariate analysis of the relationship between each measurement and E was performed using Spearman's rank correlation (Rs). Continuous linear regression was adopted for multivariate analysis of significant parameters. All statistical tests were two-sided, and a p value of 0.05 was considered statistically significant.

Results: The study describes the application of HFUS to assess changes in phenotypic parameters in the developing CD1 mouse embryo and fetus during pregnancy and to evaluating physiological fetal and placental growth and the development of different organs in the embryonic mouse; representative date are presented in Fig. 1, 2.

Conclusions: HFUS is a valuable phenotyping tool for embryonic mouse research and can be used to answer important questions in developmental biology. A database of normal structural and functional parameters of mouse development will provide a useful tool for the better understanding of morphogenetic anomalies in transgenic and mutant mouse models.

Embryonic Structure	Correlation Coefficient	p
Decidual thickness	-0,0827	0,4257
Gestational Sac Length	0,8983	<0,0001
Gestational Sac Thickness	0,8886	<0,0001
Implantation Site length	0,6234	<0,0001
Implantation Site Thickness	0,7102	<0,0001
Placental Length	0,8386	<0,0001
Placental Thickness	0,7364	<0,0001
Crown-Rump Length	0,9606	<0,0001
Abdominal Circumference	0,7426	<0,0001
Antero-Posterior Abdominal Diameter	0,8602	<0,0001
Latero-Lateral Abdominal Diameter	0,7395	<0,0001
Occipital-snout length	0,6523	<0,0001
Biparietal Diameter	0,9485	<0,0001
Subretinal Space Thickness	0,5559	<0,0001
Antero-Posterior Lens Diameter	0,5787	<0,0001
Latero-Lateral Lens Diameter	0,6255	<0,0001
Stomach Diameter	0,4617	0,0002

Fig. 1: Spearman's correlation coefficient and significance of each embryonic parameter.

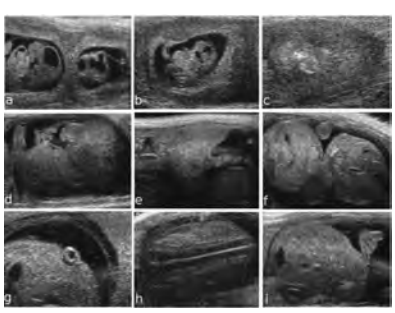

Fig. 2: At E10.5 umbilical cord placenta and eyes (a, b); a dead embryo(c). At E 13.5, crown-rump length(d), placental length and placental thickness(e); fetal thorax and abdomen(f); eyes with lens(g) vertebral colum(h). At E14.5, the occipital-snout length(i)

poster #13

IMAGING DEVELOPMENT OF SEEDS

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Introduction: Plant developmental biology has been strongly impacted by novel imaging techniques that triggered its evolution towards integrative biology. The global analysis of morphological, genetic and biochemical information not only deepens our understanding of developmental processes, it is also the basis for modeling and ultimately plant breeding. Over the past 10 years confocal microscopy has become the tool of choice to obtain 3D and 4D information on the acquisition of organ shape and the establishment of gene expression patterns. However, observations by confocal microscopy necessitate the presence of fluorescent dyes or proteins, the dissection of the structures to be observed and a depth of the structure below 50 µm for 3D reconstruction. Consequently research has focused on the model species *Arabidopsis thaliana* and *Medicago truncatula* due to their small stature and to organs that are easily observable such as leaves, roots or apices. In contrast, seed development has been evasive to imaging because the seed is hidden from direct observation by the fruit case and because the size of the mature seed ranges from 0.7 mm in Arabidopsis to 1.7 mm in *Medicago*. In this communication, we review different imaging techniques that we have tested to gain further insight into the morphogenesis of the seeds of these plant models, and the cellular differentiation between hypocotyl and radicle during the elongation of seedlings.

Methods: We present the monitoring of the development of seeds with magnetic resonance imaging [1], thermography, optical coherent tomography (OCT) and X-rays.

Results: We demonstrate high-throughput capabilities of thermography to differenciate hypocotyle and radicale [2] and OCT to image cellular division [3].

Conclusions: This contributes to identify the good practices for functional and anatomical imaging of the development of seeds.

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poster #14

RESPIRATORY MOTION COMPENSATION IN PRE-CLINICAL PET 3D REGISTRATION

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Introduction: During a PET scan on a small animal, respiratory movements cause artifacts, errors on the apparent size of tumors, quantification of voxel values, and a blur on the apparent kinetic volume observed. Due to the complex tridimensional movement organs, this work proposes to implement a 3D correction of the images based on the reconstructed PET images synchronized to the signal.

Methods: We purpose to correct the image set synchronized with respect to a particular instant of the respiratory. We implemented a 3D optical flow solution to calculate the movement of voxels between each image based on the algorithm of Horn and Shunck (H&S), and its evolution by Brox. This algorithm is based on an assumption of conservation of the intensity of the same voxel in two successive images. The optical flow equation is derived from this. The correct optical flow computation is obtained by an iterative implementation. To do this, they assumed that there is a link between the movement of a pixel and its neighbors. Thus a set of adjacent points in a movement has a direction and a speed equivalent displacement, simulating a smooth and steady movement. The problem is then reduced to minimizing the cumulative error constraints. In 2005, T. Brox added, (i) a resolution Multiscale.This technique speeds up the calculation time and reduce chances of hitting local minima, (ii) the principle of non-linearization of data by changing the shape of the Taylor devlopment of the optical flow equation, (iii) the assumption of constant gradients, introducing the management of any change in intensity in the image and (iv) a function of strength of the non-quadratic shape data and smoothing, in order to compensate for any discontinuity in the vector field. Developments were performed in C + + to optimize the computational time.

Results: Tests were implemented and performed on digital objects, and real heart acquisitions rat and cardiac phantom on the Inveon microPET (Siemens, Knoxville, USA). Rats are under anesthesia with isoflurane (2%), injected with 70 MBq of 18F-FDG. The acquisition time is 20 minutes with a statement of cardiac and respiratory signals. Signals are divided into 16 phases per cycle. Phantoms are filled with 5MBq of 18FDG. Respiratory motion is generated by a platform motion experimental, respiratory signal is recorded by the same sensor type for animals.

Conclusions: With this algorithms, results were in line with expectations products. Indeed, there is a complete recalibration of respiratory movement on a rat. We even got a good reconstruction of a phantom with an amplitude of movement even greater. Note also that the correct results were obtained in processing time quite tolerable, some ten minutes.

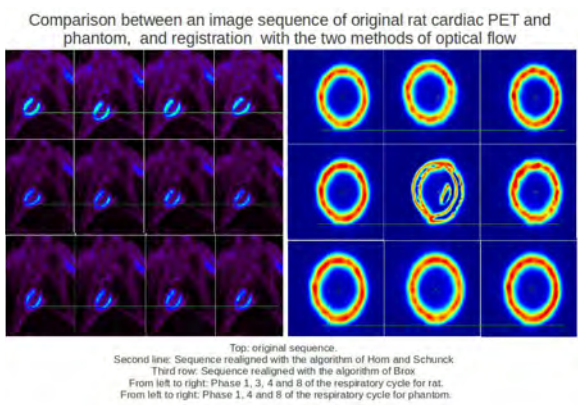

Fig 1: Comparison sequence: Comparision between an image sequence of original rat cardiac PET ant phantom

poster #15

PHOTONIC MICROSCOPY IMAGING OF DEVELOPING EMBRYOS

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Introduction: The possibility to image and record in 3D+time the embryonic development of model organisms is a critical and very challenging issue. This can be achieved with fluorescence microscopy imaging techniques.

Methods: We explored the technical solutions allowing the normal development of different animal species and providing the best compromise in terms of signal to noise ratio, spatial and temporal resolution, imaging depth and overall suitability for the quantification of cell behavior. 2-photon laser scanning and selective plane microscopy (SPIM) are the two options that we explored. Non-linear optics using infrared wavelength is required for deep imaging.

Results: SPIM provides very good signal to noise ratio and temporal resolution. However, the mounting of developing embryos for SPIM imaging is very challenging. Combining non-linear optics and selective plane illumination is still a promising solution. We present the different setups custom designed on the BioEmergences platform.

Conclusions: The setups are suitable for long-term imaging of developing embryos (including mammals) and provide data to build their complete cell lineage tree.

poster #16

COMPLETE AND NON-INVASIVE LIVE IMAGING OF TRIBOLIUM CASTANEUM MORPHOGENESIS

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Introduction: Insect development has contributed significantly to our understanding of metazoan development. However, most information has been obtained by analyzing a single species, the fruit fly *Drosophila melanogaster*. The embryonic development of the red flour beetle *Tribolium castaneum* differs fundamentally from that of *Drosophila* in aspects such as short-germ development, embryonic leg development, extensive extra-embryonic membrane formation and non-involuted head development[1]. Although *Tribolium* has become the second most important insect model organism, previous life imaging attempts have addressed only specific questions and, therefore, no complete life imaging data of *Tribolium* morphogenesis has been available[1-3].

Methods: By combining light sheet-based fluorescence microscopy[4] with the advantages of a novel sample preparation methods, we achieved the complete and non-invasive life imaging of *Tribolium* morphogenesis. The embryos survived the imaging process for up to 50 hours, developed into adults and produced fertile progeny.

Results: This approach allowed us to document all morphogenetic processes from the rearrangement of the uniform blastoderm to the onset of regular muscular movement in the same specimen and along four directions, contributing significantly to the understanding of *Tribolium* development (Figure 1). Furthermore, we created a comprehensive chronologic table of *Tribolium* morphogenesis, aligning our data with previous studies

Conclusions: Based on our observations, we hypothesize that serosa window closure and serosa opening, although deferred by more than one day, are linked (Figure 2). Apart from *Drosophila*, *Tribolium* is only the second insect species whose embryogenesis has been documented extensively with fluorescence life imaging.

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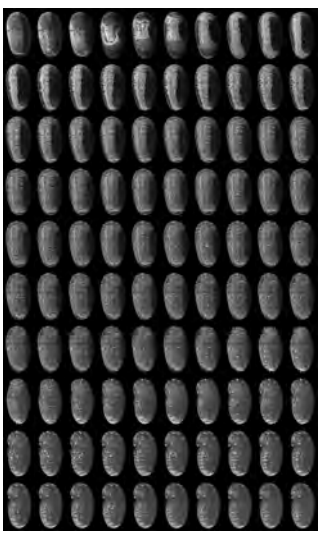

Fig. 1: Ventral view of 50:00 hours Tribolium castaneum morphogenesis from the rearrangement of the uniform blastoderm to the onset of regular muscular movement in steps of 0:30 hours

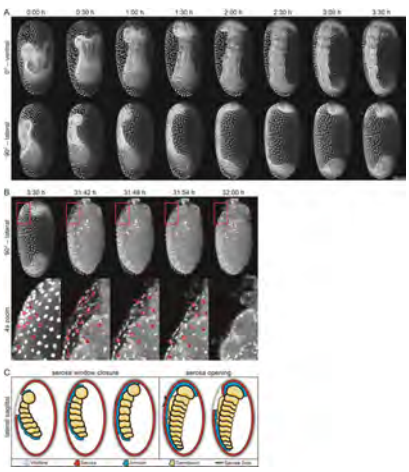

Fig. 2: (A) Serosa window closure and (B) serosa opening, showing that both processes are linked as the opening emerges where the window has closed previously, as shown schematically in (C)

poster #17

MRI VISUALIZATION OF MICRORNA REGULATION BY ALCOHOL IN THE LIVE BRAIN.

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Introduction: MicroRNAs (miRNAs) are essential elements of proper development and function of the nervous system (1). A single miRNA typically targets several protein-coding mRNA transcripts and causes their degradation (2). Alcohol has a detrimental effect on the brain during both, development and adult life. During development, the alcohol insult can cause a range of birth defects known together as Fetal Alcohol Spectrum Disorder (3). During adulthood alcohol affects many brain regions and its consumption can cause intoxication and lead further to dependence and addiction (4). We wanted to determine whether alcohol affects miRNA expression in the live brain, and if so in which brain region, cell type and cellular compartment this regulation is taking place. We focused on miRNA called miR-9 as it is a known alcohol target important for both the developmental teratogenic effects of alcohol as well as for the development of alcohol tolerance, a first step toward addiction.

Methods: We used the following methods: 1/In-house generation of SPION-ASmiR-9 probe for 2/MRI acquisitions performed in a 9.4 Tesla MRI Scanner (5) 3/Alcohol exposure by a single ip injection to reach blood alcohol levels of ~45.2 mM corresponding to intoxication. 4/Immunofluorescence of frozen brain slices as previously (5), stained against NeuN, GFAP, IBA1. 5/TEM of brain slices of nucleus accumbens. 6/Mfold (6) analysis of secondary structure prediction of microRNA precorsors. 7/Murine striatal cultures (7). 8/Northern blots of mature miR-9, mir-9 precursors and miR-9*. 9/ qRT-PCR to determine absolute levels of mature miR-9 (7). 10/Ingenuity Integrated Pathway Analysis for comprehensive pathway and network analysis. 11/Statistical analysis performed on GraphPad Prism IV with $P < 0.05$ used as significant.

Results: We determined that miR-9 targets several key elements of pathways important for neuronal development and function. We observed that alcohol upregulates miR-9 in brain regions belonging to the reward system and relevant to development of addiction. Moreover, we determined that miR-9 upregulation is taking place mainly in neurons, specifically in the rough endoplasmatic reticulum. Finally, we discovered that elevated levels of miR-9 are persistent for several hours despite the absence of alcohol.

Conclusions: We demonstrate that alcohol causes long-lasting upregulation of miR-9 in neurons located in the addiction-relevant brain regions specific to reward. These results broaden our understanding of alcohol effects on the nervous system and can lead to the creation of novel therapeutic strategies for alcoholism and addiction.

Acknowledgement: The work was supported by NIH grants: AA01748 (Career Award) and AA017920 (AZP), AT004974 (JQR), and DA026108, DA029889 and EB013768 (PKL). The 9.4T MRI system was funded in part by NIH (S10RR025563) to the Athinoula A. Martinos Center for Biomedical Imaging; the Microscopy Core of System Biology Center was supported by DK43351 and DK57521.

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poster #18

NUMERICAL MODELING OF THE DYNAMIC OF ULTRASOUND CONTRAST AGENTS IN VASCULAR NETWORK : VALIDATION STUDY

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Introduction: Dynamic Contrast-Enhanced Ultrasonography (DCE-US) is a particularly attractive method to assess the tumor microvasculature from the concentration quantification of ultrasound contrast agents (CA) within lesion. This method does not yet benefit methodological tools imported from physics to characterize the ability of the quantification methods to evaluate the tumor microcirculation. In this context, we developed the first numerical modeling (NM) based on Computational Fluid Dynamics software for studying the quantification methods to describe the tumoral perfusion in a complex vascular network and to apprehend their variations according to the tumor growth, configurations of hemodynamic and CA injections. The aim of this study is to validate this approach in comparison with DCE-US experimentations on simple geometrical configuration composed of 1 bifurcation of fluids.

Methods: NM was performed with Fluent software (ANSYS, France), which modeled blood and CA flows in vascular network with laminar flow described by Poiseuille's law. Geometry of the numerical and experimental phantom was a 31cm length tube and 2mm in diameter with 1 bifurcation with 2 parallel tubes located at 23cm of the input phantom. Volume of injected CA was 0.1mL with 26, 34, 41 and 56mL/min of blood flows. Realistic injection of CA by bolus was implemented in NM. DCE-US experimentations were performed with an Aplio 500 scanner (Toshiba Medical Systems, Japan) and a 12MHz probe with SonoVue® (Bracco, Italy) as CA. Comparison of area under the curve (AUC) from the time-concentration of CA curves was performed between experimental data and NM.

Results: The numerical time-concentration of CA curves were similar to those obtained experimentally and showed the same variations of AUC values according to the different blood flows.

Conclusions: NM with the Fluent software was validated for studying the blood and CA dynamics in a simple geometry.

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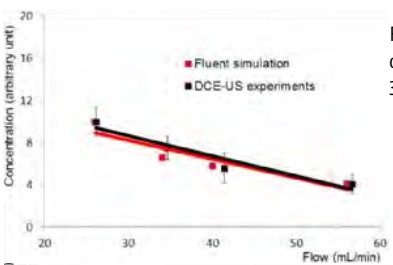

Fig. 1 Concentration flow curves: Curves obtained with differents flows (26mL/min, 34mL/min, 41mL/min and 56mL/min)

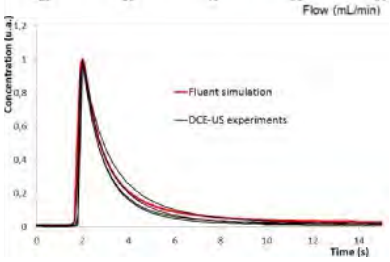

Fig. 2 Concentration time curves: Enhancement curves for the one bifurcation obtained with a 41mL/min liquid flows

poster #19

MOLECULAR MR AND US IMAGING ON KIDNEY TUMOR XENOGRAFTS USING TARGETED CONTRAST AGENTS

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Introduction: The development of targeted contrast agents (CA) is of great interest to increase the specificity of imaging techniques, and to allow early diagnosis, and therapy follow-up of diseases [1]. Molecular imaging (MI) is now extensively used to monitor specific markers of vascularization, and changes in molecular marker expression in oncological and cardiovascular fields. For the MRI imaging, functionalized superparamagnetic iron oxides nano-objects [2] are developed to balance the sensitivity, and the specificity needed to detect molecular markers in a low range concentration.

Methods: Nude mice xenografted with a human kidney tumor (A498) had two imaging sessions, comprising CEUS and MRI, using non-targeted, and αvβ3-targeted CA. CEUS and dynamic susceptibility contrast DSC-MRI were applied. Molecular US acquisitions were performed at 20 MHz. Contrast signal intensity was recorded as a function of time, and the binding of specific CA was evaluated by destructive method 10 minutes after the bolus injection of 50 µL of CA, by taking the difference between the mean pre- and post-destruction signal intensities (dTE). MR acquisitions were then performed at clinical 1.5 T (Philips Achieva, micro23 coil). Apparent diffusion coefficient (ADC) was quantified. Dynamic Susceptibility Contrast (DSC)-MR was performed during one hour pre- and post- injection of 100 µmol Fe/kg USPIO-based nanoemulsion (Guerbet) targeted with RGD binding αvβ3 (P4000), as well as a control nanoemulsion (P3999). MR data were co-localized onto the US 2D acquisition. Tumor size was estimated from US, and from MRI (manual segmentation). ROIs of the whole tumor, hypo - and hyper- vascularized areas were drawn for regional analysis of: 1) ADC, 2) dTE, and 3) relaxation rate R2* (followed during 1 hour to quantify binding to the targeted receptor).

Results: US and MRI tumor sizes correlated well ($R = 0.99$, $p < 0.001$ Pearson test). ADC mean value for all tumors was estimated at $1.65 \times 10^{-3} \pm 0.3 \times 10^{-3}$ mm^2.s^{-1}. ADC and dTE values with targeted CA are inversely correlated on hyper-/hypo- vascularized areas. dTE values evaluated using targeted and non-targeted CA gave significantly different contrast modifications ($p=0.03$, Wilcoxon paired test). For DSC acquisitions, after one hour, targeted CA ΔR_2^* of 16.5 ± 3.9 min^{-1} was significantly different from the one of non-targeted CA, 5.8 ± 1.8 min^{-1}.

Conclusions: The specificity of each targeted CA was shown on kidney tumor xenografts in mice. Histology is needed to further confirm the molecular targeting at level of tumor neovascularization. Multimodal evaluation of targeted CA and various imaged-derived biomarkers may be used to follow-up treatment response in preclinical oncology.

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poster #20

CELL BEHAVIOR IN THE PRE-IMPLANTATION RABBIT EMBRYO

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Introduction: Embryo development is a reproducible process leading to the formation of a normal adult. Although this process seems very similar from one individual to another, it shows an intrinsic multiscale variability necessary to evolve in an ever changing environment. Processes underlying variability are ill-understood and ill-defined. A better understanding of variability requires quantitative measurements and statistical approaches at the cell scale. We aim to characterize sources, range and determinism of variations at different levels.

Methods: Rabbit embryos are harvested 19hpc at the 1-cell stage and immediately micro-injected with H2B-EGFP mRNA (75ng/µL; chromatin). Whole embryos are imaged *in vivo* with two-photon microscopy from the 32-cell stage (70hpc) to hatching (120hpc) with a stack every 15 minutes. Raw data is automatically analyzed with nucleus detection and tracking algorithms. Manual validation and correction with the custom software Mov-IT are necessary to provide an accurate reconstruction of the digital embryo.

Results: Four embryos were successfully imaged from the 35-cell stage to hatching, one of which has been validated to obtain a *gold standard* digital embryo. Single cell behavior was assessed according to cell type (trophectoderm and inner cell mass). Eight apoptotic events were observed between 102hpc and 120hpc, consistent with previous observations in mouse (Brison and Schultz 1997) and indicating no or little effect of imaging on cell survival. The segregation of the trophectoderm and ICM lineage is a major issue. We observed that at 70hpc all inner cells (11 cells) and three outer cells contributed to the formation of the inner cell mass at 100hpc. However this lineage was not irreversible between 100hpc and 110hpc: at least 10% of the inner cells moved to the trophectoderm and conversely at least 4% of the trophectoderm cells moved to the inner cell mass.

Conclusions: Cell scale analysis of rabbit development with high temporal and spatial resolutions and with no harm to the embryo revealed unexpected cell behaviors. We showed that cell position and identity switched from inner cell mass to trophectoderm and from trophectoderm to inner cell mass at later stages. Further analysis will be performed on three other digital embryos to confirm these observations.

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