ZENITH TRAINING WORKSHOP

14-21 NOVEMBER 2021

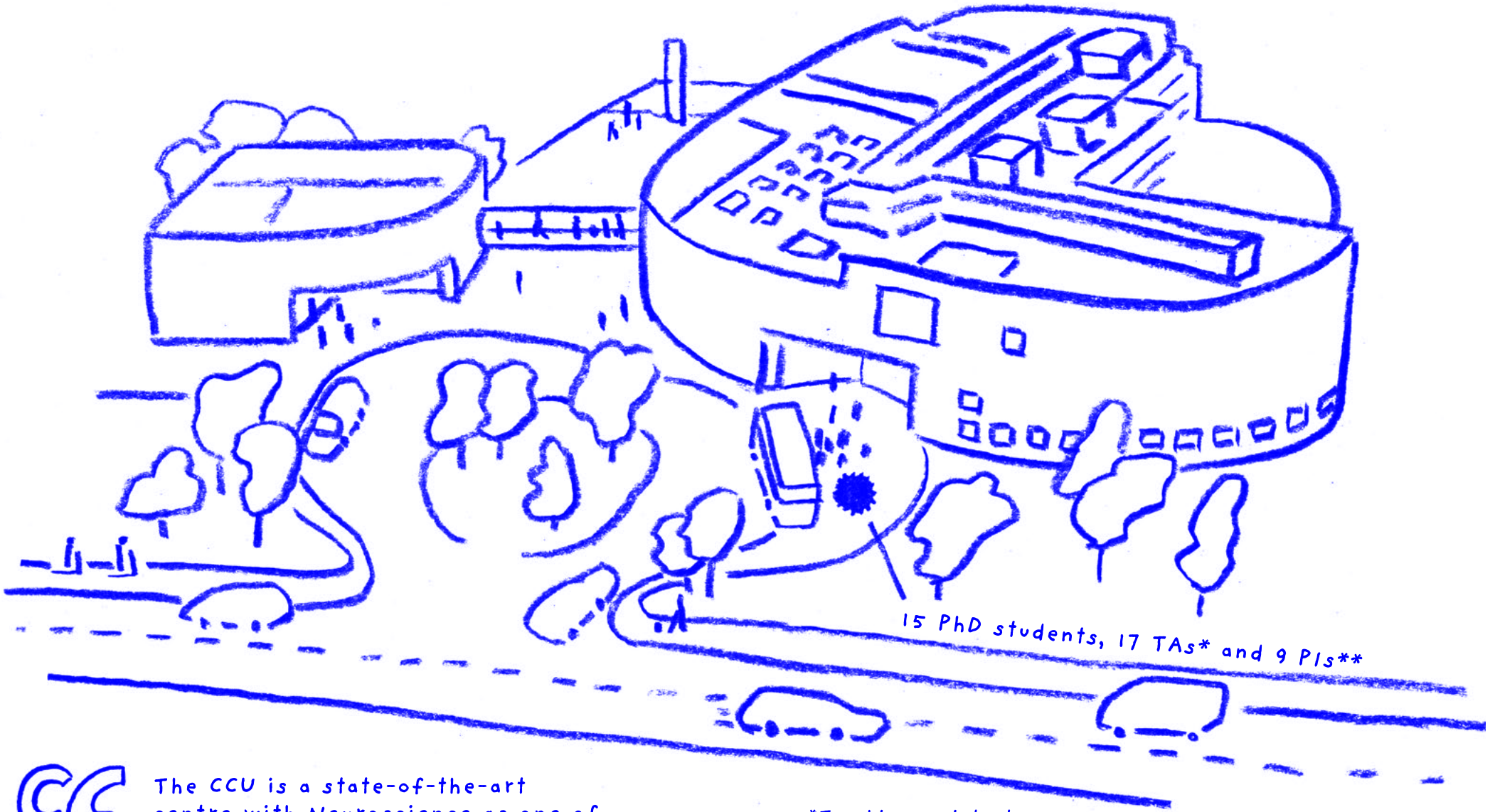
GENETICS, ANATOMY AND BEHAVIOUR

LISBON

ZENITH-ETN.COM

Welcome ZENITH to the champalimaud Center for the Unkwon (CCU)!

Initially focused on a system and circuit approach to brain function and behaviour, the Centre expanded to incorporate molecular and cell biological expertise.



15 PhD students, 17 TAs* and 9 PIs**

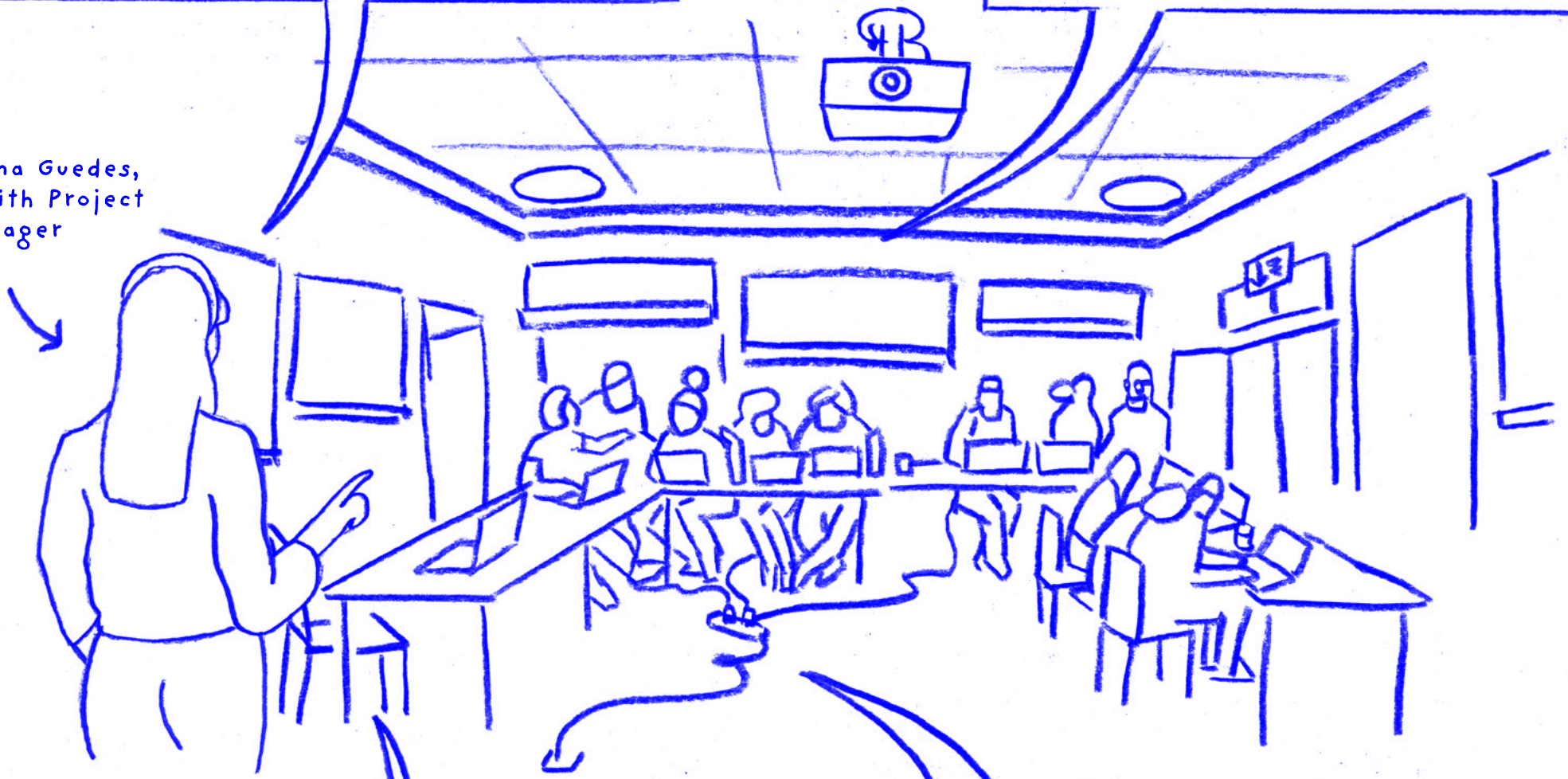
The CCU is a state-of-the-art centre with Neuroscience as one of the core research areas.

*Teaching assistant
** Principal Investigator

Welcome everyone to the ZENITH Genetics, Anatomy & Behaviour Workshop! This training will provide advanced, hands-on training in zebrafish, genetics and anatomy followed by hands-on behavioural experiments and data analysis.

The course will be split into two modules. Module 1 includes an introduction to the zebrafish model, genetics and anatomy. Module 2 focuses on investigating methods for recording specific aspects of behaviour.

Joana Guedes, Zenith Project Manager



The one-week workshop will be taught by the ZENITH faculty and will include theoretical background and practical training supported by several TAs from the different ZENITH labs.

We will start discussing Genetics and Anatomy. But first, an introduction to the Zebrafish model history by David Lyons.

MODULE 1 - GENETICS & ANATOMY - SCHEDULE

DAY 1

Morning

Lecture: Zebrafish neurodevelopment, genetics & behaviour. Historical perspective on origins of the model.
Dave Lyons

Lecture: Transcriptomic analyses
Manuel Irimia

Intro to demo stations: Learning objectives of the demos
Mike Orger/Isaac Bianco

Afternoon

Transgenic and Microscopy Demos:

- Zeiss lightsheet: fli:GFP; gata:RFP
- 2-photon LSM: gad1b:Gal4; UAS:GCAMP6fef05
- 3i spinning disc confocal: fli:GFP; gata:RFP
- SCAPE & Lightfield: vglut2a:Gal4; UAS:GCAMP6fef05
- Lightsheet (homebuild): elavl3:H2B-GCAMP6s

Lecture:Transgenesis & genome editing
Filippo Del Bene

CRISPR design workshop
Victor Ordoñez

DAY 2

Morning

Injections practice (FISH FACILITY)

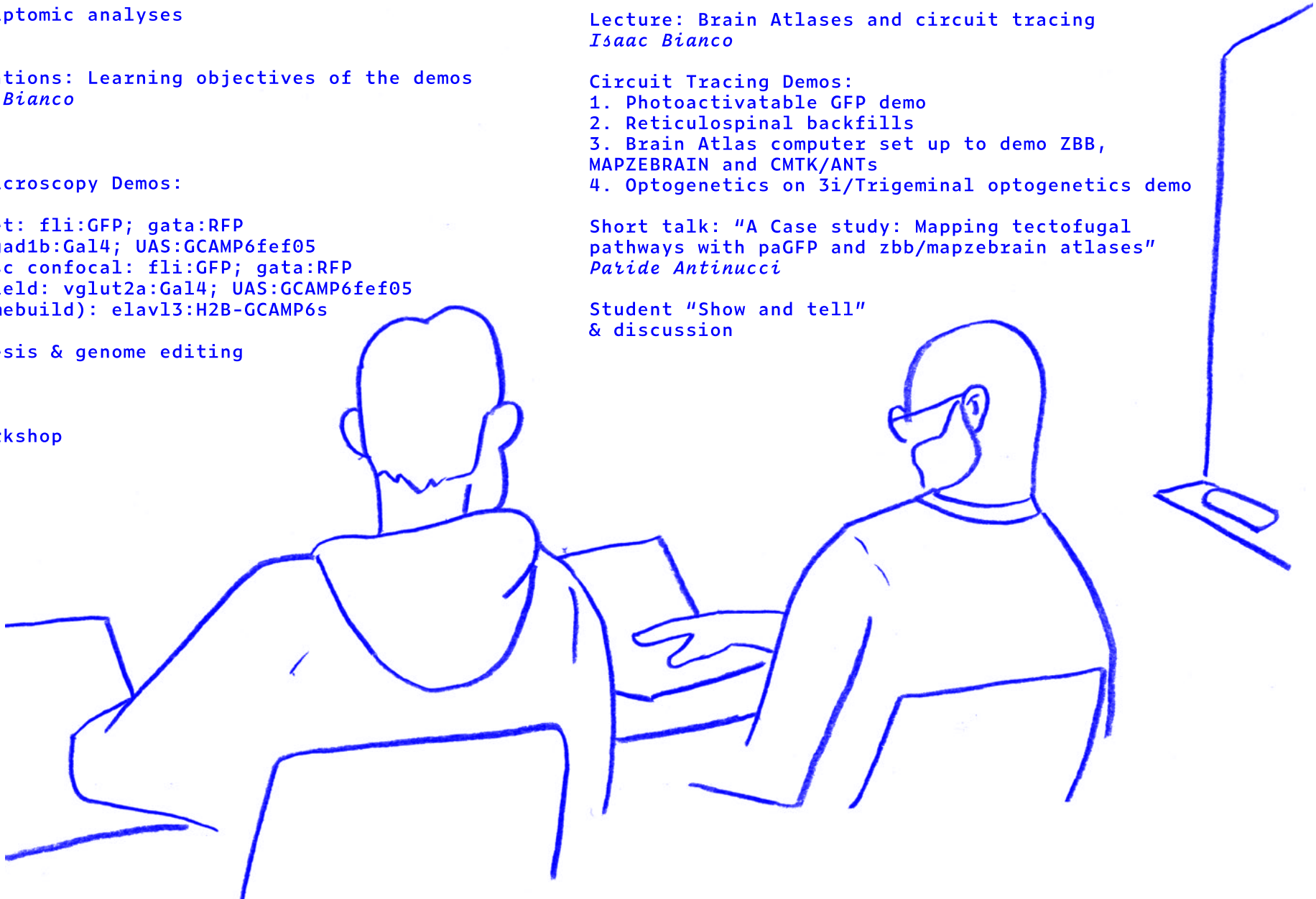
Afternoon

Lecture: Brain Atlases and circuit tracing
Isaac Bianco

Circuit Tracing Demos:
1. Photoactivatable GFP demo
2. Reticulospinal backfills
3. Brain Atlas computer set up to demo ZBB, MAPZEBRAIN and CMTK/ANTs
4. Optogenetics on 3i/Trigeminal optogenetics demo

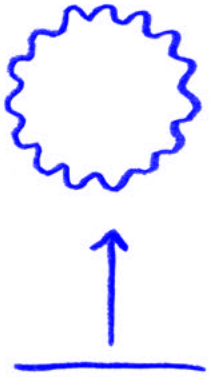
Short talk: "A Case study: Mapping tectofugal pathways with paGFP and zbb/mapzebrain atlases"
Paride Antinucci

Student "Show and tell"
& discussion



live from edimburgh

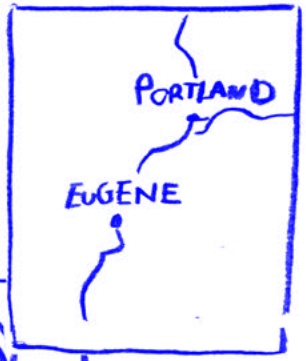
"For a great start, let's hear an «Historical perspective on zebrafish genetics and neuroscience"



Hi everyone, today I would like to share with you how did we reach ZENITH?

A journey through the origins of the Zebrafish model, expansion of the model through screening, right through to "modern" times

"It all started in the far west, back in the late 70's"



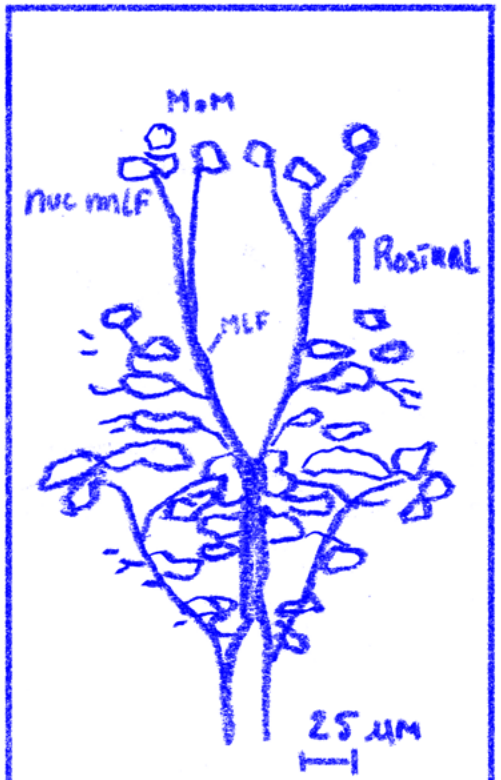
George Streisinger, the father of the field (1927-1984)

David Lyons

University of Edinburgh, UK

Zenith project:
Active Myelination

DAVID LYONS



Dye labelling by backfill

Please take a look at Brain neurons which project to the spinal cord in young larval zebrafish.

KIMMEL ET AL., J Comp Neurol, 1981

You are going to be doing this (40 years later!)

Meanwhile someone in Germany had caught wind of all of this!

"JANNI"

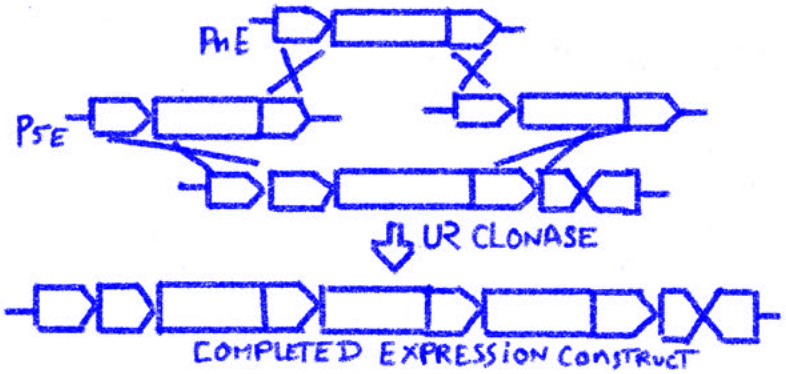
Christiane Nusslein-Volhard (Nobel prize winner Drosophila genetic screens)

WOLFGANG DRIEVER

(PhD student of "Janni") who had "fallen in love" with tech that Janni sent to Eugene to learn about fish and thus postdoc'd to do the same

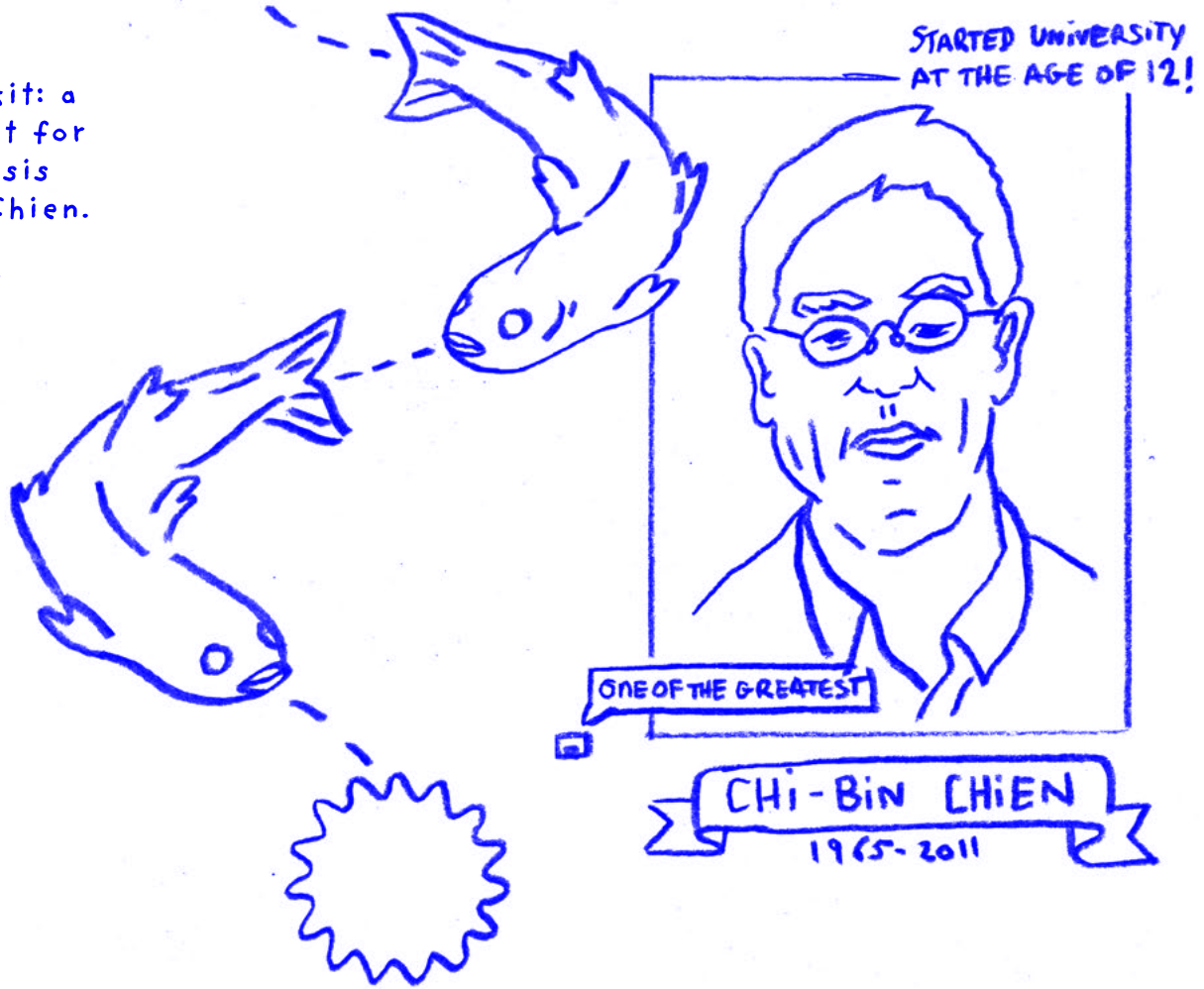
Moving forward, the Tol2kit: a multisite gateway-based kit for Tol2 transposon transgenesis designed by Prof Chi-Bin Chien.

Kwan et al., Dev Dyn, 2007



Now widely used hugely expanded many transgenic lines diverse reporters...

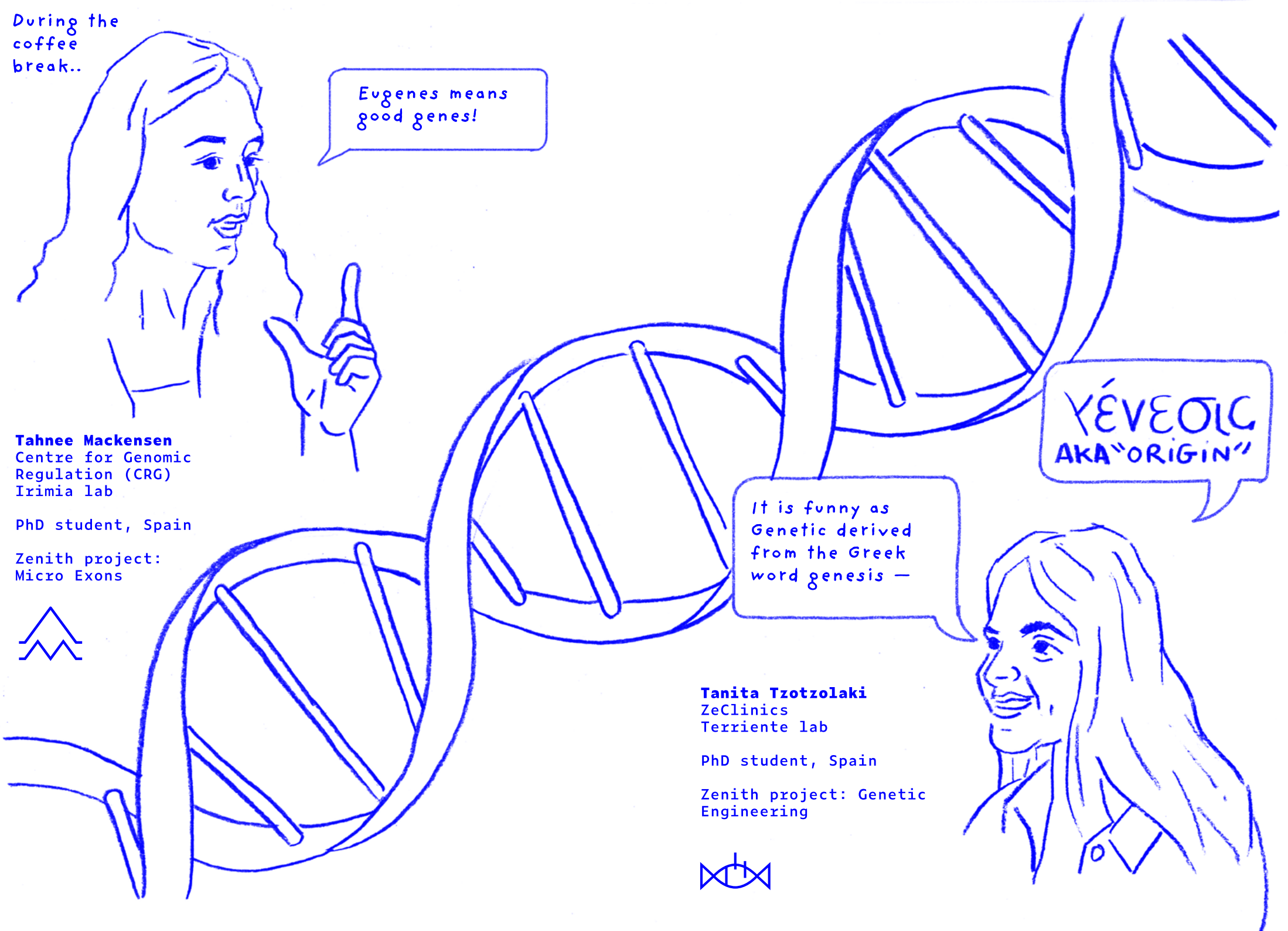
Sadly, Chi-Bin Chien, who was a pioneer in the discipline left us too early.



SO HOW DID WE REACH ZENITH?

- Natural features of zebrafish
- Genetic & cellular tractability
- Functional studies
- Data analysis

And now the ZENITH students need to bring it all together!



Tahnee Mackensen
Centre for Genomic Regulation (CRG)
Irimia lab

PhD student, Spain

Zenith project:
Micro Exons

Tanita Tzotzolaki
ZeClinics
Terriente lab

PhD student, Spain

Zenith project: Genetic Engineering

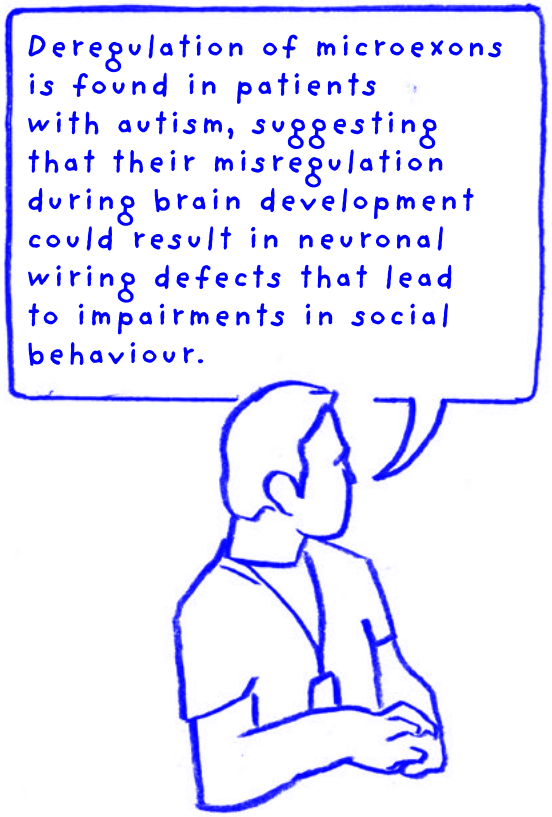
TRANSCRIPTOMIC ANALYSES

"Manu will give a brief intro to transcriptomic analyses and share sources and resources for bulk and single cell RNA-sequencing."

Manuel Irimia

CRG, Barcelona

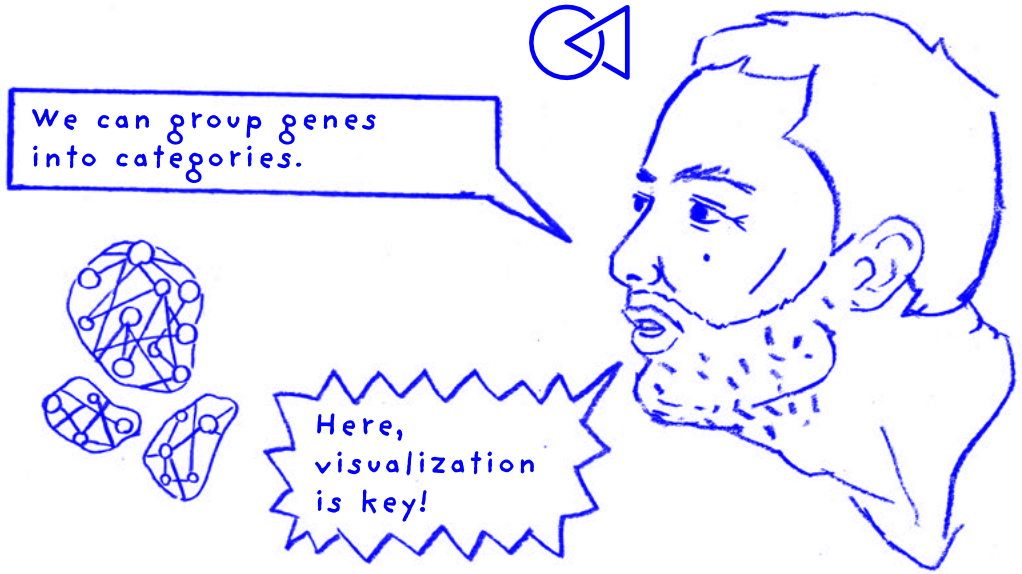
Zenith project:
Micro Exons



Shuhong Huang
Technical University of Munich (TUM) Portugues lab

PhD student, Germany

Zenith project:
Eyes 'n Tail



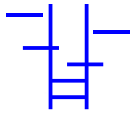
TRANSGENESIS AND GENOME EDITING

"Filo will share general principles on Transgenesis and Genome editing and explain why Zebrafish is such a powerful vertebrate model!"

Filippo Del Bene

Institut de la Vision, Paris

Zenith project: Tectum



Koich Kawakami
National Institute of Genetics
Shizuoka, Japan

In the field, we moved from forward genetic studies with the work of scientists like Kawakami to reverse genetic studies with incredibly powerful tools like CRISPR/Cas9.

CRISPR/Cas9 technology

1. Powerful and efficient Genome editing technology

2. Derived from bacterial immune system against viruses

3. Used to activate and inactivate genes

4.Used to make pathological animal models in research, recently adapted for gene therapy

5. Its optimization is the challenge of genetic engineering

CRISPR design workshop

Victor Ordoñez

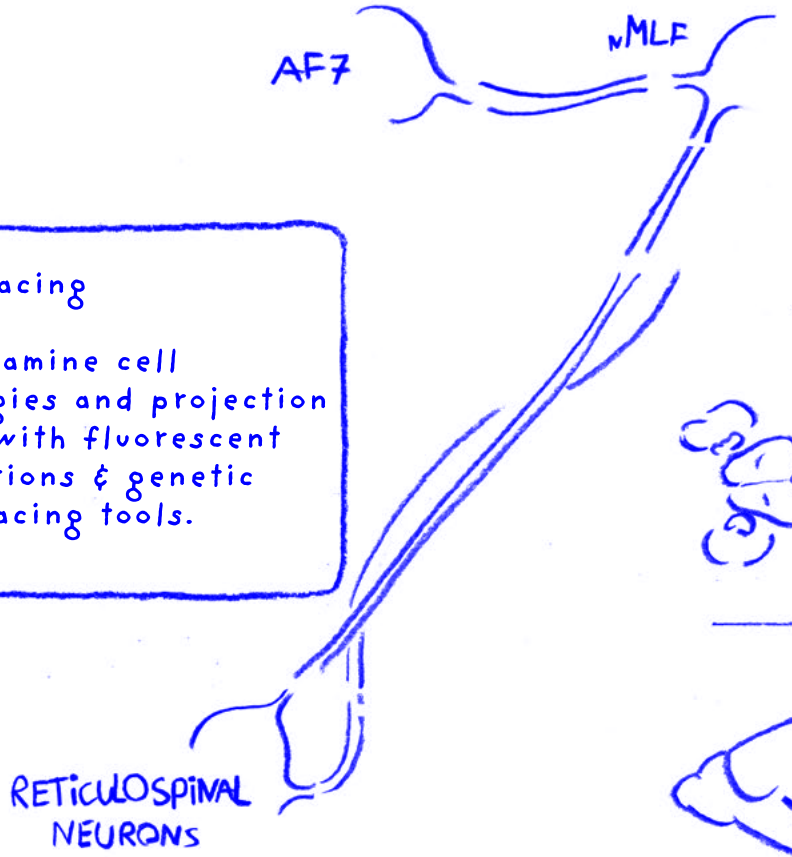
ZeClinics
Teaching Assistant

BRAIN ATLASES AND CIRCUIT TRACING

"Let me introduce you to methods to label, track the morphology and register neurons in the brain atlas"

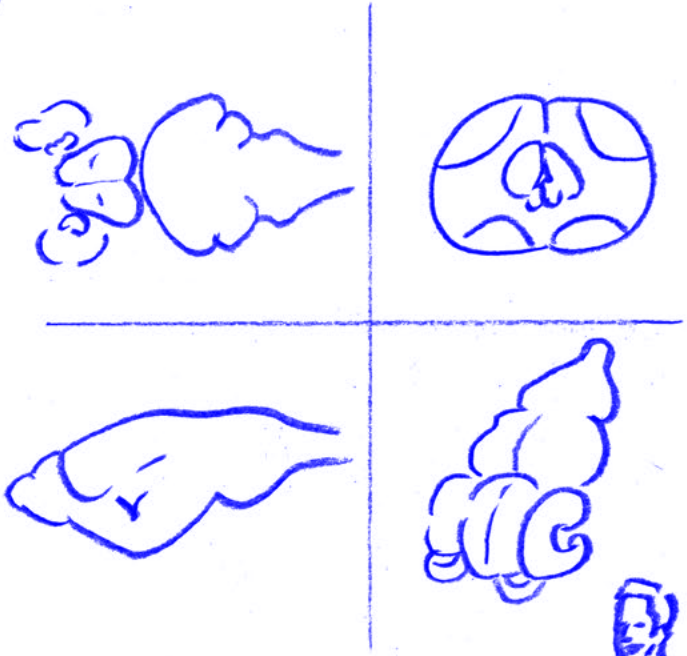
Circuit tracing

we can examine cell morphologies and projection patterns with fluorescent dye injections & genetic circuit tracing tools.



Brain atlases

They are vast repositories of information about the zebrafish brain.



Registering experimental data to an standard brain atlas can help characterize regions of interest, as well as find useful transgenic lines and design experiments.

Isaac Bianco

UCL London

Zenith projects:
Command Action
Visuomotor Control

MAPPING TECTAL-PRETECTAL INPUT-OUTPUT PATHWAYS
WITH PA-GFP AND ZBB/MAPZEBRAIN ATLASES

"The Bianco lab is interested on brain circuits that process visual stimuli to control adaptive behavioural responses. Paride will talk about visually guided control of hunting manoeuvres."

Tectum and AF7-pretectum are required for hunting behaviour. Let me show you an example of PA-GFP photo-activation in AF7-pretectum.

I will also tell you about the Zebrafish Brain Browser Atlas – a.k.a. ZBB and the Max Planck Institute Zebrafish Brain Atlas – a.k.a. Mapzebrain.

Paride Antinucci

UCL, Bianco lab
Teaching Assistant

COFFEE BREAK WITH STUDENTS

Can't wait to start the practicals! Really want to try the injections.

Chung-Yuen (Joe) Chan

Institut de la Vision
Emiliani lab
PhD student, France

Zenith project:
All Optical

And me I'm curious to learn about all the different microscopes!

Elena Putti

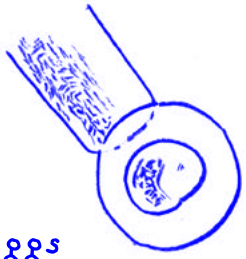
Institut de la Vision
Del Bene lab
PhD student, France

Zenith project:
Tectum


PRACTICALS

INJECTIONS PRACTICE

"Now everyone, let's gather in groups of 4 for a microinjection session in the CCU fish facility!"



1.
Pick eggs using stroke

2.
Drop eggs in plate

"There are different methods to make it work, but I like to align in the eggs in rows"

Thomas Mullen

CCU
Orger lab

Phd Student, Portugal

Zenith project:
Avoidance

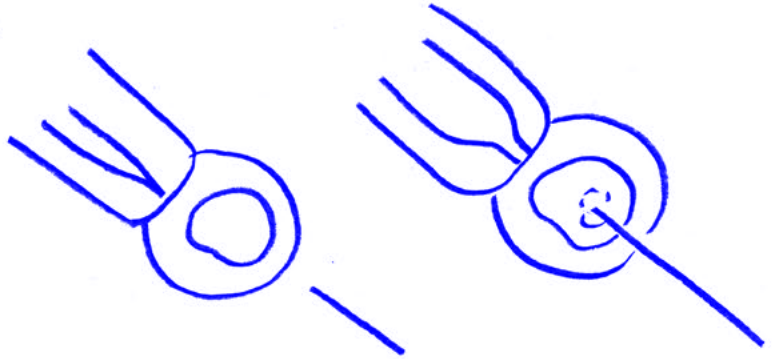
Verity Cook
Charité
Judkewitz lab

PhD student, Germany

Zenith project:
Vocalisation

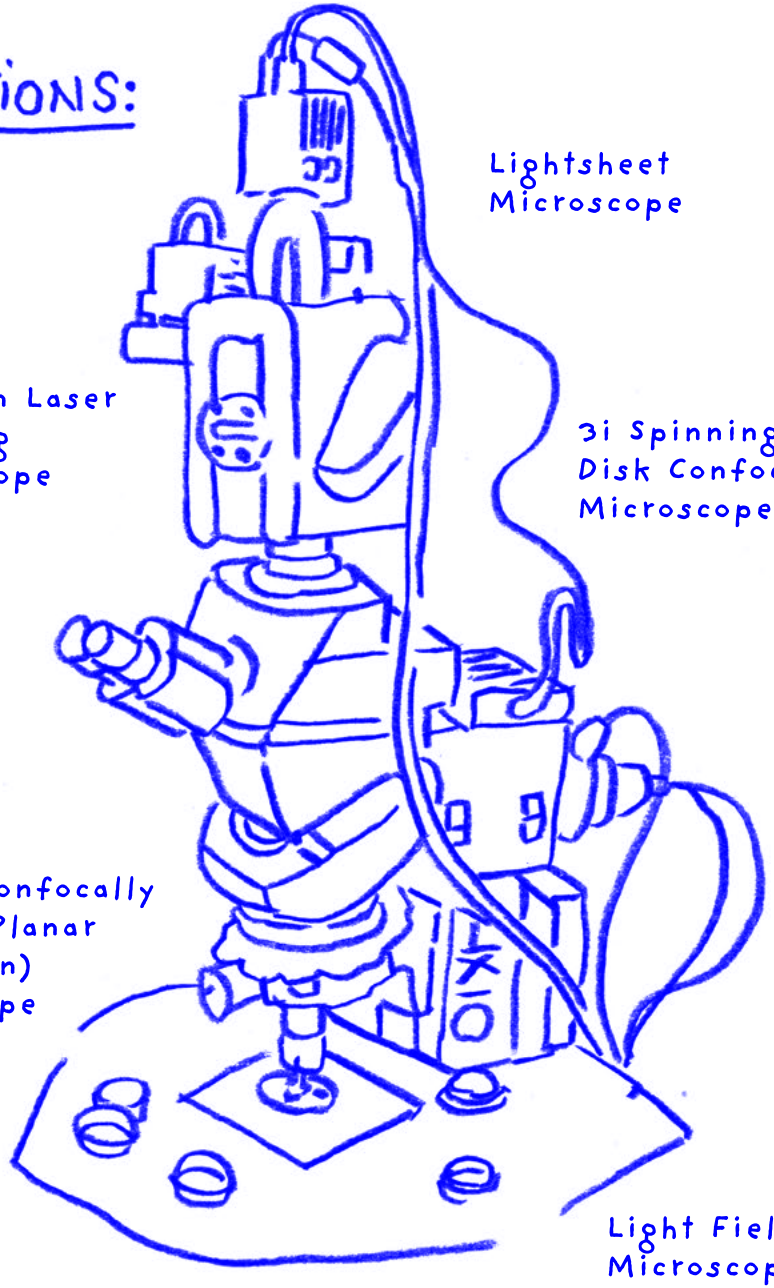
You can see the small sharp needle poking through the center of the egg!

3.
Grab the egg with the stroke, hold your breath and inject!



"In the teaching labs, also in groups, students do rotations in stations with different microscopes and transgenic lines."

STATIONS:



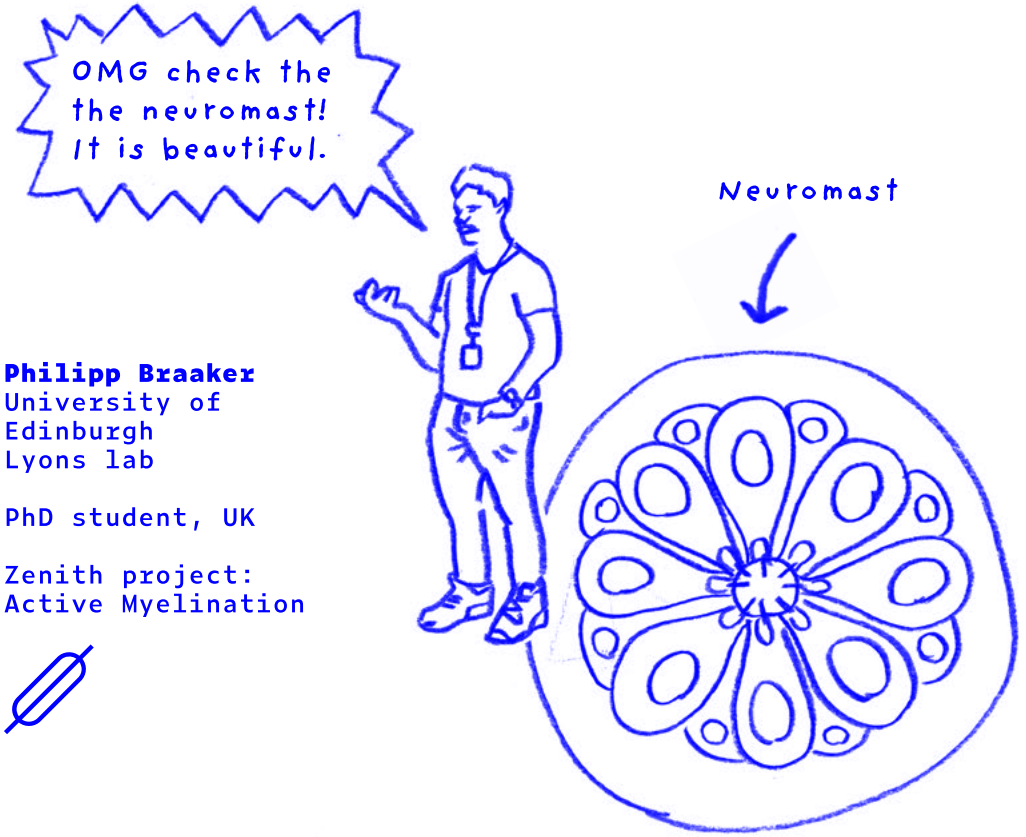
Lightsheet Microscope

2 Photon Laser Scanning Microscope

3i Spinning Disk Confocal Microscope

SCAPE (Swept Confocally Aligned Planar Excitation) Microscope

Light Field Microscope



Philipp Braaker
University of Edinburgh
Lyons lab

PhD student, UK

Zenith project:
Active Myelination

Alizée Kastler
UCL
Dreosti lab

PhD student, UK

Zenith project:
Deep Imaging

PART 2 BEHAVIOUR - SCHEDULE

DAY 3

Morning

Lecture: Tracking and quantification of larval behaviour
Michael Orger

Lecture: Motor learning and computational approaches to behaviour
Ruben Portugues

Afternoon

Stations to learn from:

1.Stytra set-ups - OKR response (restrained) + freely swimming

2.Orger's setups
- Multi fish tracker
- rapid group comparison for closed loop behaviour
- Rigs for free swimming closed loop stimulus presentation
- Top-down projection of UV stimuli

3.Analysis of kinematics and rolling during acoustovestibular escapes and basic exploration

4.Postural control behavioural setup

Visit to Oceanário de Lisboa

DAY 4

Morning

Lecture: Using idTrackerAI to study behaviour of large animal groups
Francisco Romero (Paco)

Intro talks for afternoon projects & Discussion

- Social Behaviour essays
  Tom Ryan
- Analysis of posture and sequence generation
  Claire Wyart
- Studying vocalization/schooling in Danionella
  Benjamin Judkewitz
- Vestibular-driven behaviours
  Volker Bormuth

Afternoon

Demo & Rotating Stations (TEACHING LAB)
DEMO: Record data from juvenile fish and apply ID Tracker

STATIONS:

Analysis of bout sequences using BASS during navigation
Social preference assay for juvenile fish
Danionella vocalization/schooling project
Postural control behavioural setup

Data Analysis group session

DAY 5

Morning

Data collection/analysis, project completion
Breakout sessions for discussion of project progress

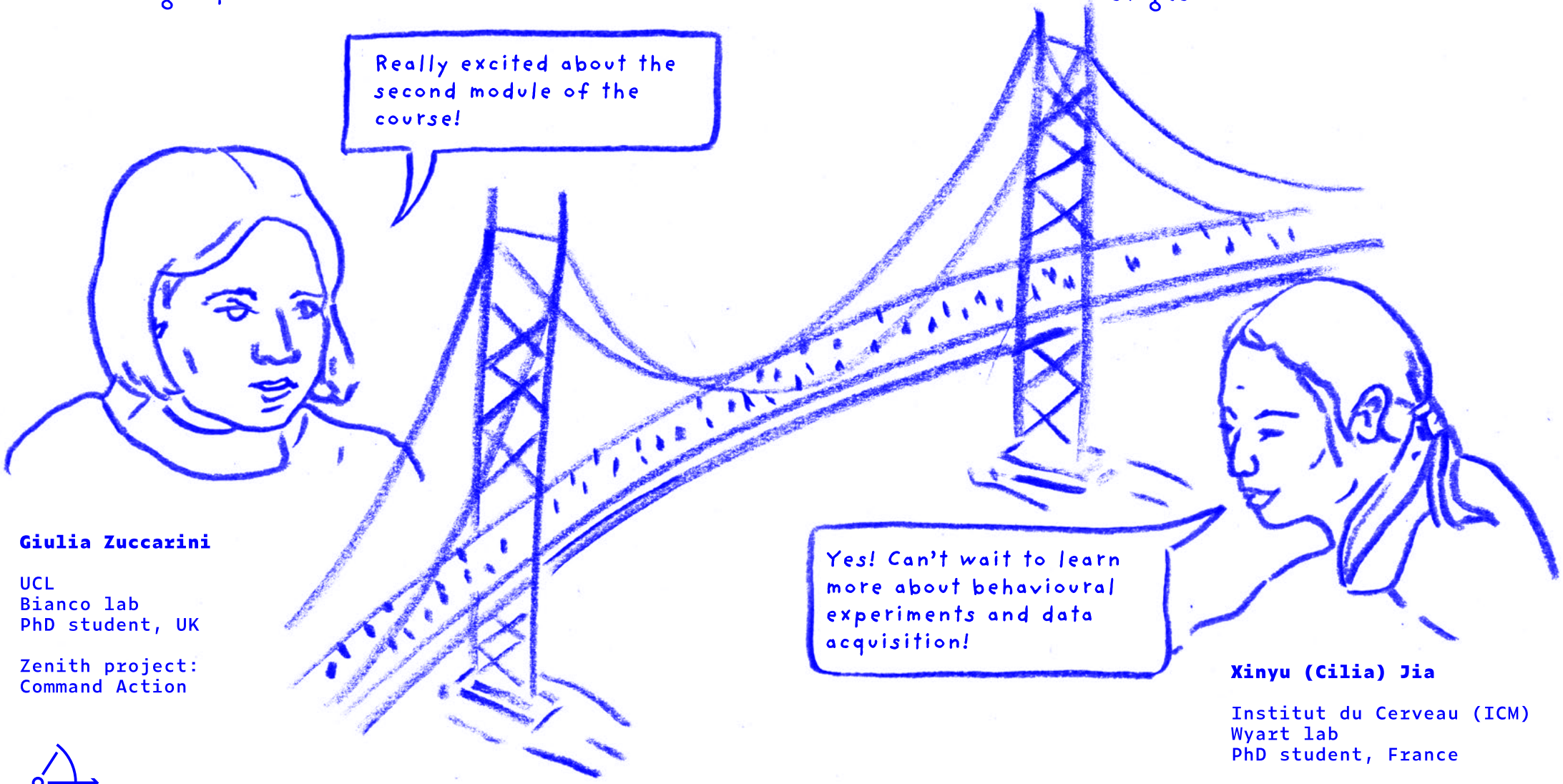
Afternoon

Project completion
Student presentations

PART 2 BEHAVIOUR

"We will now start with the second part of our course: Behaviour! On this module students will acquire simple behaviour data and run the analysis to monitor kinematics of the tail angle for single animals and groups."

Students will also investigate methods for recording specific aspects of behaviour in head-restrained and freely swimming fish at the larval and juvenile stages.



Giulia Zuccarini

UCL
Bianco lab
PhD student, UK

Zenith project:
Command Action

Xinyu (Cilia) Jia

Institut du Cerveau (ICM)
Wyart lab
PhD student, France

Zenith project:
Inner States

INTRODUCTION TO ZEBRAFISH BEHAVIOUR TRACKING

"Mike Orger will introduce us to the module by taking us through different approaches to the quantitative study of behaviour."

Edouard Dumon
UCL
Bianco lab
PhD student, UK

Zenith project:
Visuomotor Control

Sorry for asking a basic question, but what do you define as "behaviour?

When tracking the tail of the zebrafish we are dealing with formula 1 like speeds

Which is why we use high-speed camera that can track the fish at rates 700 frames per a second.

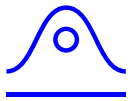
Before tracking a behaviour it's important to think about what the animal perceives in the experiment.

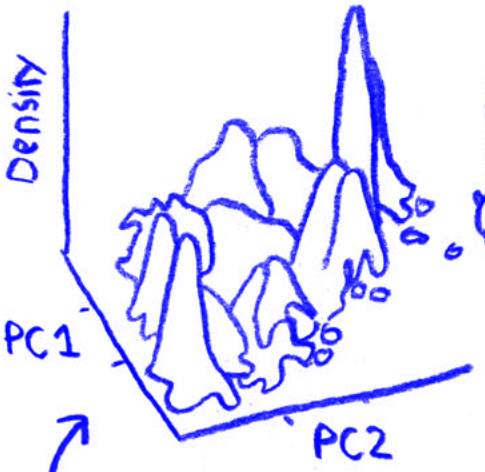
We can distinguish between innate and learnt behaviours.

Michael Orger

CCU, Lisbon

Zenith project:
Avoidance





Marques et al., Current Biology 2018

We are dealing with petabytes of data so we have to be smart in extracting the relevant information.

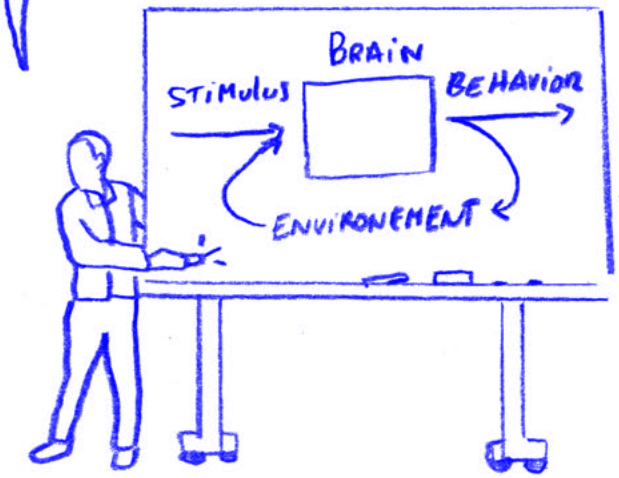
MOTOR LEARNING AND COMPUTATIONAL APPROACHES TO BEHAVIOUR

"The Portugues lab studies zebrafish behaviour and brain function and circuitry using cutting-edge imaging techniques."

"I AM A TOOL"

Illumination is key for behavioural experiments!

Let me tell you about "Stytra" , an open-source, integrated system for stimulation, tracking and closed-loop behavioural experiments



Ruben Portugues

TUM, Munich

Zenith project:
Eyes-n-tail

IDTRACKER AI TO STUDY BEHAVIOUR

"Paco will introduce us to idtracker.ai and other tools to study collective behaviour in juvenile zebrafish."

Collective behaviour:
"... simple repeated interactions between individuals can produce complex adaptive patterns at the level of the group."

Francisco (Paco) Romero

CCU, Collective Behaviour Lab

Teaching Assistant

Fantastic tools to analyse trajectories!

Sharbatanu Chatterjee
Sorbonne University (SU)
Bormuth lab
PhD student, France

Zenith project:
Motor Learning

"The Wyart lab has implemented methods to analyse long term dynamics of zebrafish. Let's hear about it!"

Most of our behaviours are semi-automatic.

Without being conscious about it, we perform recurrent sequences of behaviour.

Claire Wyart

Institut du Cerveau (ICM)

Zenith project:
Inner-states
Navigation

We can observe larval zebrafish to analyze their behaviour and learn how basic sequences are implemented to navigate in space.

1. Complex and naturalistic animal behaviour consists of sequences of discrete actions that cannot be only quantified manually «by eye».

Gautam Sridhar

Institut du Cerveau (ICM)
Wyart lab
PhD Student, France

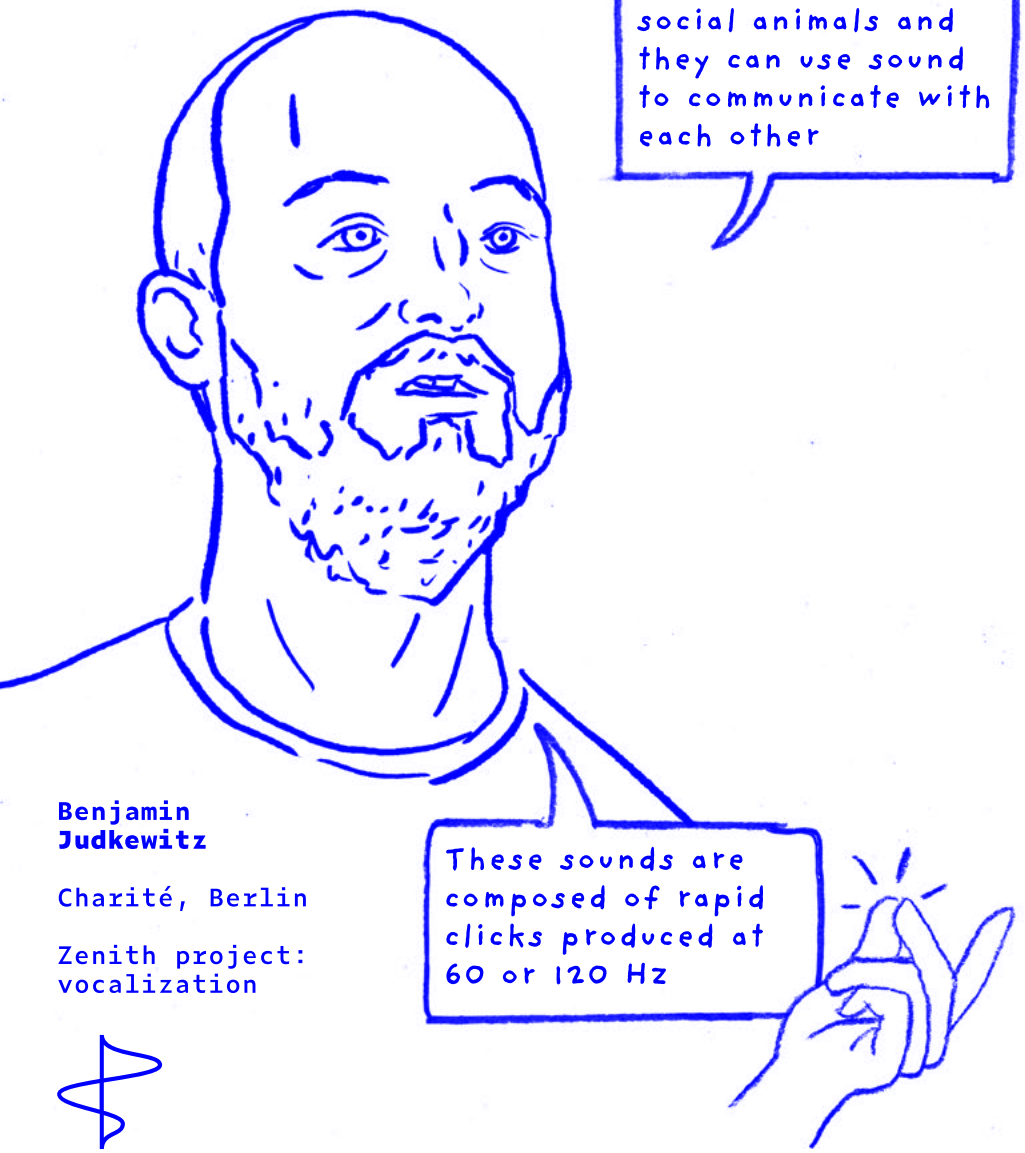
Zenith project:
Navigation

2.
By applying a method that models behaviour like language, we can detect these sequences like finding words from a string of characters.

3.
This can be used to find stereotypical behaviour in larval zebrafish without applying knowledge of the stimulus, like when the fish captures prey.

VOCALIZATION & SCHOOLING IN DANIONELLA

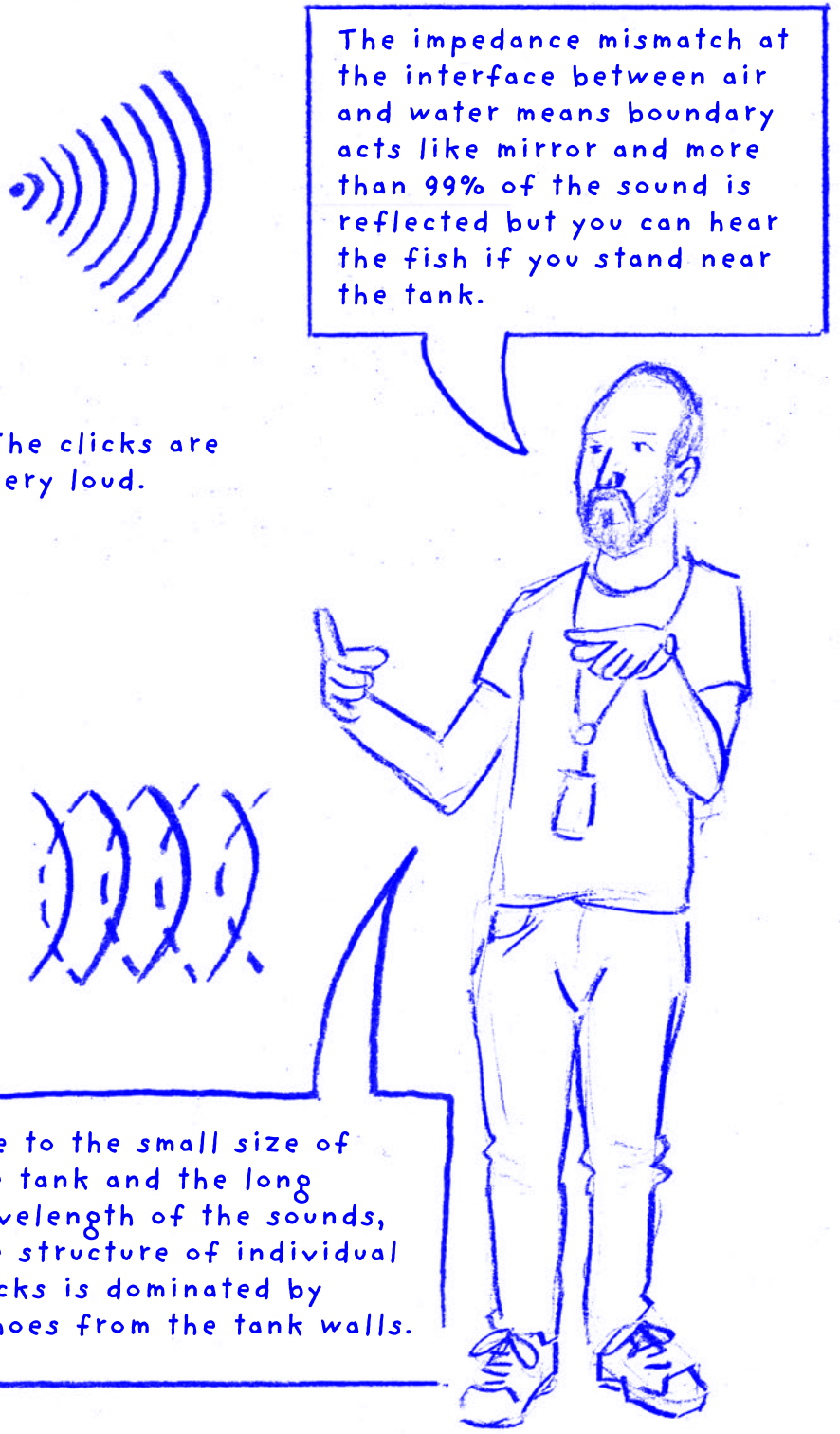
"Zenith is not only about zebrafish, we also explore the power of Danionella! Let's listen to the Judkewitz lab."



Benjamin Judkewitz

Charité, Berlin

Zenith project: vocalization



VESTIBULAR-DRIVEN BEHAVIOURS

"The Bormuth lab combines optical developments, genetics and neuro-computation to obtain insights into the activity of brain-wide neural circuits that process multisensory information."

We look at the response of zebrafish to changing vestibular stimuli - both in their brains & in behaviour

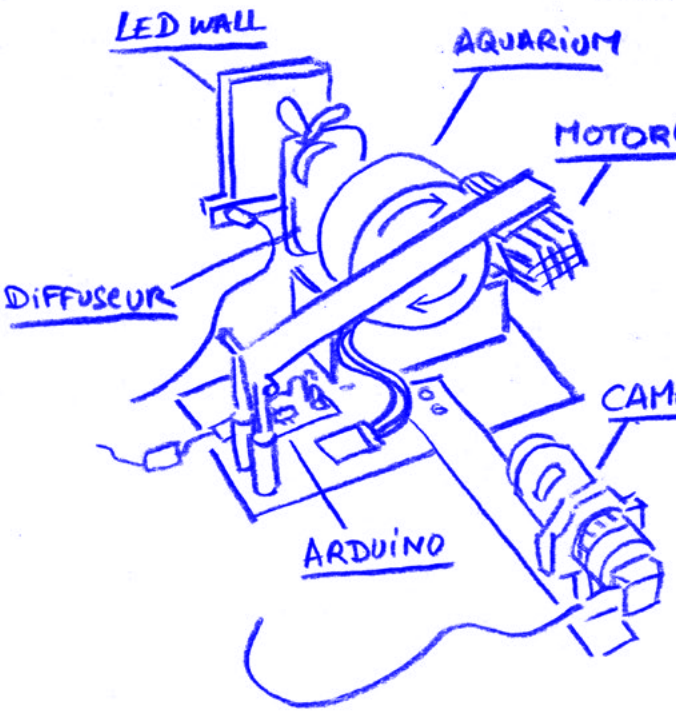
Volker Bormuth

Sorbonne University (SU), Paris

Zenith project: Motor Learning

Behaviours are recorded with high speed cameras

Brains are recorded with powerful microscopes



Very simple DIY experimental setups can reveal behavioural nuances

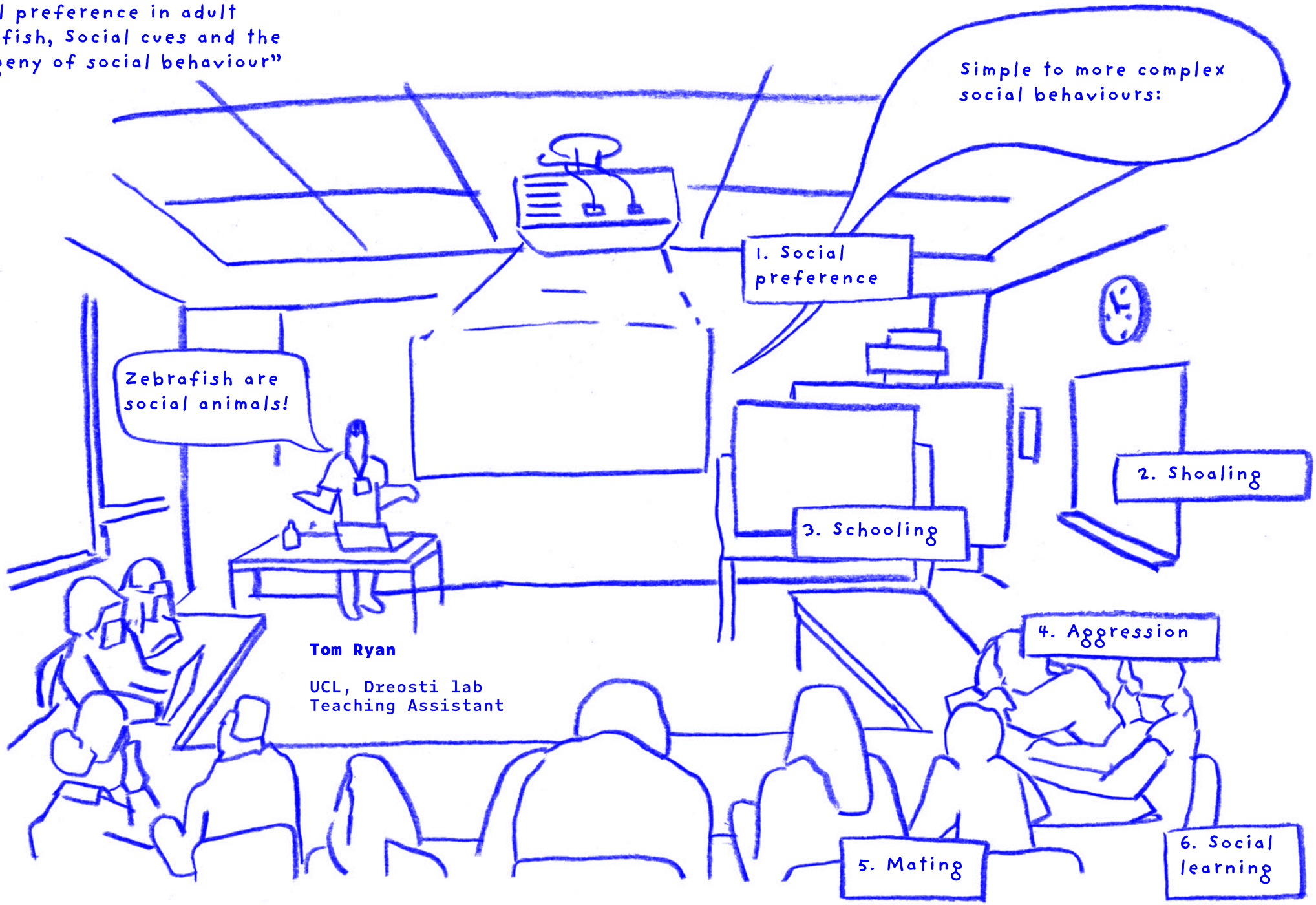
We can, for example find out how drunk fish react to their world rotating fast!

Matteo Dommanget-Kott
SU, Bormuth lab

Teaching Assistant

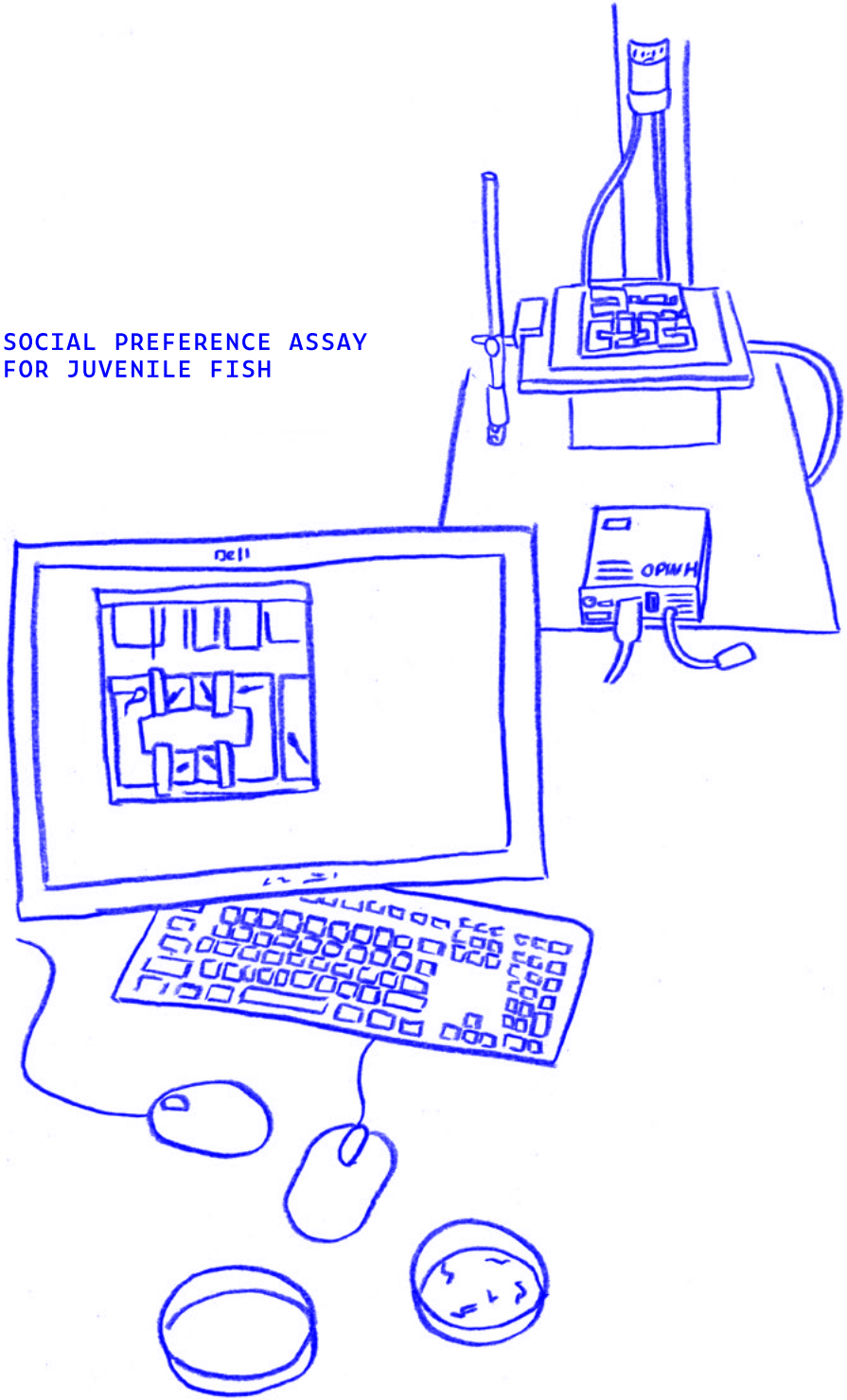
SOCIAL BEHAVIOUR ESSAYS

"Tom Ryan will talk to us about: Social preference in adult zebrafish, Social cues and the Ontogeny of social behaviour"



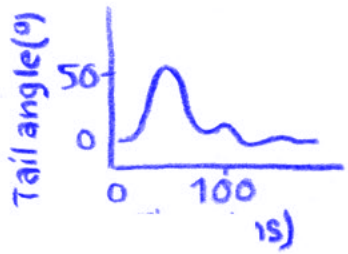
ROTATING STATIONS FOR PROJECTS

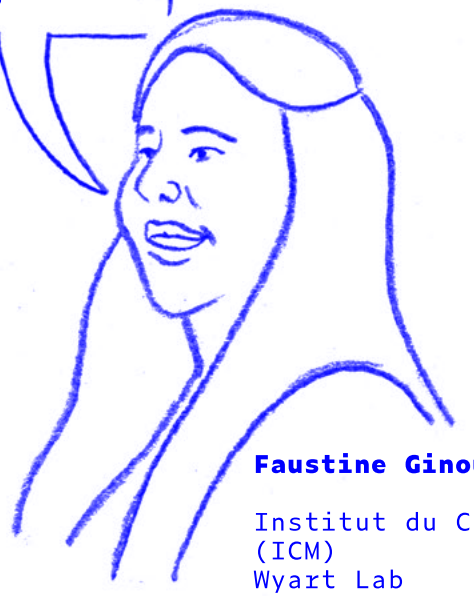
SOCIAL PREFERENCE ASSAY
FOR JUVENILE FISH



ANALYSIS OF BOUT SEQUENCES USING BASS DURING NAVIGATION

Gautam already talked about this, but "BASS" is an unsupervised algorithm to efficiently identify and segment conserved behavioural action sequences transiently occurring in long behavioural recordings.





Faustine Ginoux

Institut du Cerveau (ICM)
Wyart Lab
Teaching Assistant

Sadiq Adedayo

University of Vienna
Grosse-Wentrup lab
PhD student, Austria

Zenith project:
Modelling Behaviour

Faustine and Gautam helped us dive into BASS and detecting sequences of actions in our own datasets!

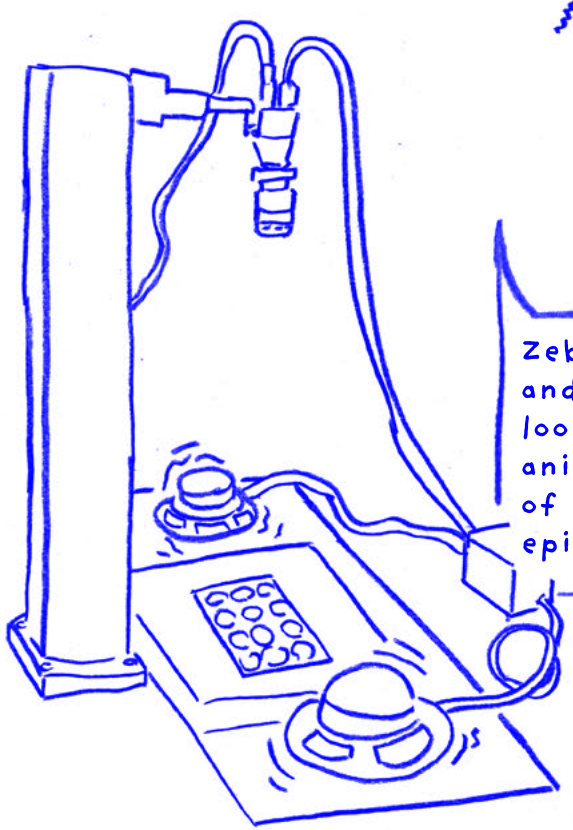
ZEBRAZOOM TRACKING SOFTWARE

ZebraZoom can be used to track the head and tail of freely swimming and of head-embedded larval and adult zebrafish.

We track the head and tail of larval zebrafish in order to analyze the fine kinematics of larval zebrafish.

Olivier Mirat
Institut du Cerveau (ICM)
Wyart lab

Teaching Assistant



Zebrafish larvae are small and we can therefore look at hundreds of animals, doing hundreds of thousands of locomotor episodes.

Larval zebrafish perform extremely fast escape in response to acousto-vestibular stimuli that we trigger with large microphones.

In order to learn the role of genes involved in pathologies in humans such as Parkinson's Disease in humans.

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Teaching Assistant

We analyze the kinematic defects in mutants of important genes whose function is unknown in humans.

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THE ZENITH PHD PROGRAM TRAINS A NEW GENARATION OF NEUROSCIENTISTS IN CUTTING-EDGE APPROACHES THAT BRIDGE BIOLOGY, PHYSICS, AND MATHEMATICS TO UNCOVER THE MYSTERIES OF BRAIN FORMATION AND FUNCTION.

FOR MORE INFORMATION VISIT
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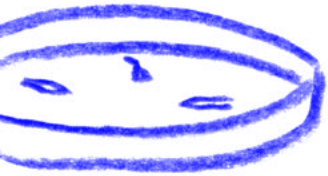

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