Hands-on-School ICTP 2013

Mechanical Oscillations in planarian locomotion

(Olivier Cochet-Escartin and Eva-Maria Schoetz Collins)

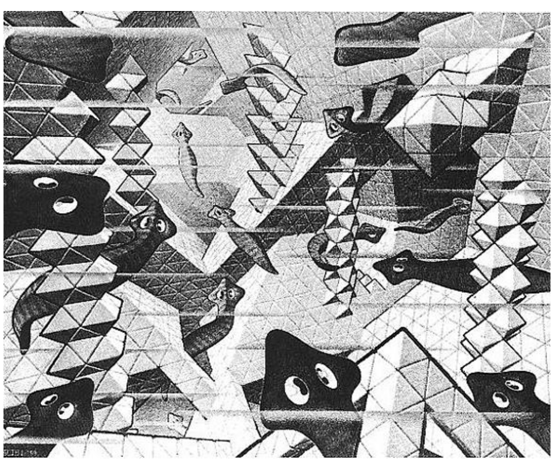
In this lab, you will learn how to cut planarians and study mechanical oscillations in the context of locomotion in these two powerful regenerative organisms. We will employ image analysis in MATLAB and ImageJ (freeware can be downloaded here: http://rsbweb.nih.gov/ij/) to quantitatively analyze the images and the data.

You can often find free-living planarians in a pond when you look underneath rocks; planarians like standing, but clean freshwater. Planarians are best caught using liver in a nylon stocking which is held into the water. Since they really like liver they will come and suck at the stocking.

Some background information before you'll get started:

The name planarian comes from being flat ("flatworms"), and because they are flat they don't need a circulatory system, but can do their gas exchange and nutrient uptake by diffusion. Planarians attracted quite a series of really famous people: There have been reports that Michael Faraday was doing cutting experiments – and Thomas H. Morgan, the father of fruit fly genetics, published a dozen papers on them. It was Morgan who started the myth of 1/279th of a planarian being able to regenerate – now, if you are thinking that he really cut a worm into 279 pieces and kept track of them, you are (unfortunately) mistaken – he just wildly estimated. This is an experiment which would be really worthwhile doing, but takes way too long for this course and it is quite tedious with having to take care of so many worm pieces and making sure that if they die it's not due to contamination with bacteria or fungi, but really because they fail to regenerate. You will, however, get to cut a worm in 5 or so pieces and can then watch it regenerate over the course of a week.

The famous artist M.C. Escher was also fascinated by planarians, and made several drawings of them, including Fig.1 "flatworms" shown here (source: http://bogleech.blogspot.com/), imagining what it's like in "flatland" (the latter, btw, is the title of a highly amusing book by E.A. Abbott (written in 1884) which I strongly recommend if you don't know it).



My absolute favorite though is still James V. McConnell, who did behavioral experiments with planarians in the 60th, and caused a lot of controversy with his studies on "memory transfer through cannibalism". His experiments lead to the creation of an entire planarian journal, "The Worm Runner's Digest".

We won't have time to go into the details about many neat experiments people have done with planarians – after all, we want you to get your hands dirty and not spend all this time listening to us telling stories – but I would like to point out a few highlights and then you can read them and other related papers that interest you in detail later.

A few highlights of planarian experiments:

MEMORY TRANSFER THROUGH CANNIBALISM IN PLANARIANS

JAMES V. McCONNELL, PH.D. *

A Study of Senescence and Rejuvenescence Based on Experiments with Planaria dorotocephala.

By

C. M. Child.

With 14 Curves and 1 Figure.

Eingegangen am 13. Juni 1910.

NOT YOUR FATHER'S PLANARIAN: A CLASSIC MODEL ENTERS THE ERA OF FUNCTIONAL GENOMICS

Phillip A. Newmark and Alejandro Sánchez Alvarado‡*

Clonogenic Neoblasts Are Pluripotent Adult Stem Cells That Underlie Planarian Regeneration (1)

Daniel E. Wagner,[1]* Irving E. Wang,[1]* Peter W. Reddien[1]†

Some basics good to know:

Planarian basics:

We will use a specific planarian strain in this lab – *Dugesia japonica*. Back home, we work with this species and a second species, called *Schmidtea mediterranea*, and since I know much more about the latter, I will tell you some more about that. For the purpose of the lab, however, both species are similar in behavior: when they get stressed out, they start to undergo musculature driven locomotion! (More about that below).

S. mediterranea has a sequenced and assembled genome ($8*10^8$ bp, (7)) allowing for molecular studies, a short regeneration time (~1 week), and is the only known higher organism that can regenerate its entire body from small tissue fragments. The regenerative capability of planarians is based on a population of stem cells (neoblasts), which comprise ~30% of the entire cell population in the adult animal (Fig.2) (8-9) and which have recently been shown to be totipotent (1).

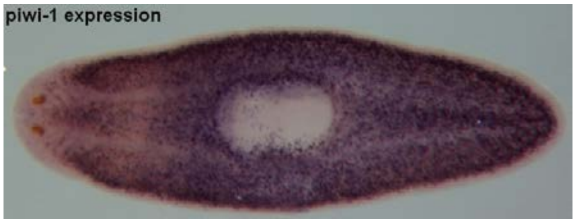


Figure 2: Everything in purple are labeled stem cells.

These stem cells also allow the asexual strain to reproduce by dividing itself (Fig.3a). Isn't that cool? – The worms naturally rip themselves and then regenerate into two new animals, and moreover, the tail piece has to make a brand new head with eyes and a brain possessing a central nervous system (CNS) comprising about 10,000 neurons (Fig.3 left).

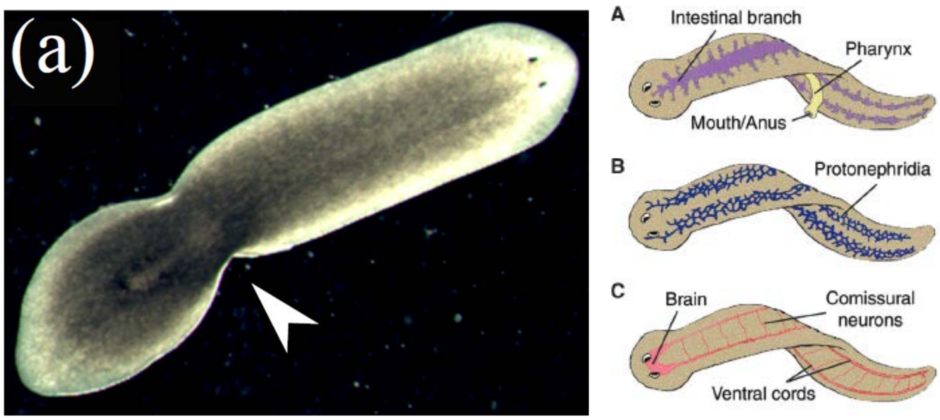


Figure 3: Planarian division zone and planarian anatomy. souces: (10), (6)

Planarians are amenable to drug treatment and displays specific behavioral phenotypes that can be quantified, as we have recently shown (11). They also show specific behavioral responses when stressed out, and this is what we will study in this lab module. Normally, planarians move in a straight gliding motion using cilia on their belly. Cilia are like little hairs (Fig.4) and they beat rhythmically in a layer of mucus that the planarians produce with their excretory cells (see Fig.3, protonephridia). But when a planarian gets stressed out, e.g. by light (they are negatively phototactic) or by us cutting them, they switch to some sort of inchworm motion by which they periodically contract and relax.

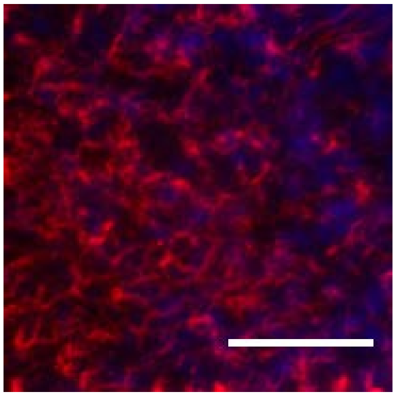


Figure 4: Planarians move using cilia on their belly. The picture shows fluorescently labeled cilia in red using a anti -tubulin antibody and cell nuclei in blue using DAPI. The scale bar is 100um.

Now, let's talk a bit about the experiments you will try in the lab:

I. Planarian anatomy

Look at slides of real samples using the pocket microscope. We have prepared samples showing the stem cell distribution, the brain and the pharynx (eating tube).

II. Stress-behavior of planarians

In these experiments, we want to quantify the frequency with which planarians oscillate using image analysis in MATLAB and determine whether musculature driven locomotion is faster than gliding and whether it actually makes sense as a stress response and allows planarians to escape faster.

Methods:

1) Cut a planarian roughly 1/3 behind the head on the dissecting microscope, observe oscillation locomotion behavior as a stress response.

2) Cut your planarian into a few more pieces (not more than 5). Make sure to try out different cuts (longitudinal, oblique, horizontal). Move the pieces into a separate petri dish with fresh water. Store until next week to observe regeneration. Write your name on the lid of the dish.

3) Learn how to analyze planarian induced oscillator motion data using image analysis in MATLAB. Compare the oscillation frequency with the gliding frequency.

List of materials used for this part:

Flatworms
Plastic pipets
Razor blade
100mm petri dish
Larger plastic arena
Volvic water
You can also make up your own water. E2 medium works well (15.0 mM NaCl; 0.5 mM KCl; 1.0 mM $MgSO_4$; 0.15 mM KH_2PO_4; 0.05 mM Na_2HPO_4; 1.0 mM $CaCl_2$; 0.7 mM $NaHCO_3$)
Ring stand and clamps
Camera
Computer and MATLAB

MATLAB freeware clones:

These two clones are free, but not as well equipped as MATLAB, i.e. they don't have the fancy toolboxes, but you can still do quite a lot with them. And it's free!

http://www.gnu.org/software/octave/

http://www.scilab.org/

Another excellent freeware which allows you to do a lot of the things we showed you how to do in this lab is Python. Maybe you want to attend the Programming in Python session.

http://www.python.org/

Functions we are using in MATLAB for the image analysis:

imread – reads in an image
imshow – displays an image
% invert the image by 255-image
% to find edges/outlines, use edge detection
%to make sure everything is connected, use imdilate
% use imfill to fill in holes
%bwareaopen – filter out noise
%imfilter(bw,hf) – do additional disk filtering; set a filter hf=fspecial('disk', 17)
%imclear border
%cc=bwconncomp(bw); bw is your binary image
% L=labelmatrix(cc); labels all objects it finds after conncomp
%regionpropsl(L,area)

```
%define where to start looking for images
impath = 'C:\ ';
imdir = uigetdir(impath, 'IMAGE folder to read')
savepath = 'C:\ '; cd(savepath);
%where to start looking for a place to save the analyzed data
[savename,savedir] = uiputfile('*.mat','WORKSPACE save-name','tracks_');
cd(imdir);
```

Selected references:

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4. Reddien PW & Alvarado Sanchez (2004) Fundamentals of Planarian Regeneration. *Annu. Rev. Cell Dev. Biol.* 20:725-757.
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10. Dunkel J, Talbot JA, & Schötz E-M (2011) Memory and obesity affect the population dynamics of asexual freshwater planarians. *Physical Biology* 8:026003.
11. Talbot JA & Schötz E-M (2011) Quantitative characterization of planarian wild-type behavior as a platform for screening locomotion phenotypes. *The Journal of Experimental Biology* 214:1063-1067.