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Laminar Flow in Mini-Fluidics Channels Assembly and Its Application in Zebra Fish Embryo Research

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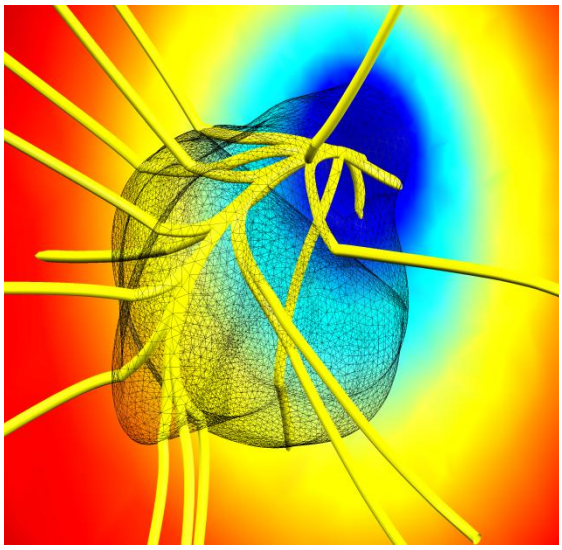

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Instruction Manual

Prepared by

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Laminar Flow in Mini Fluidics Channels Assembly and Its Application in Zebra Fish Embryo Research

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CONTENT

1) Introduction

The mini-fluidic channel system was designed, build and tested as the part of independent research in Biomedical Engineering Minor Program (INT 421) during the fall semester of 2007. The purpose of this system was to produce and sustain a continuous flow of fluid through a series of mini channels. This continuous flow of the fluid generated stresses on the surfaces of small objects that were placed in the channels. A group of Zebra fish embryos was placed into the channels and kept there for a required period of time.

2) Experimental Assembly

While several options were considered during the duration of the project it was established that the final and most practical, economical and manufacturable model would serve its purpose.

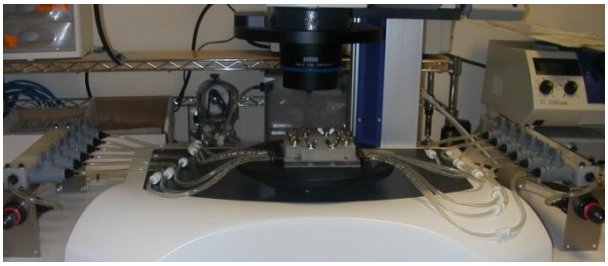

Figure1 The main assembly located under the microscope work station

The main experimental assembly shown in Figure 1 consisted of the following parts: mini-pump, distributive manifold, collecting manifold, storage tank and a mini-channel plate and series of flexible tubes with appropriate fittings.

3) Components of the Assembly

a) Mini Pump RF-100 12/24

The mini pump in this system was a special peril static type of low-flow pump that supplied the system with a variety of flows depending on the DC voltage utilized for the operation.

Figure 2 The mini-pump

The RF Series Peristaltic Pump shown in Figure 2 has a liquid flow rate from 10 ml min^{-1} to 500 ml min^{-1} with three different pumping sizes. The RF series pumps feature a unique dual roller design and a custom stretched tube. They are self priming and can run without fluid. The dual roller combination provides consistent flow capacity with a minimum amount of set in the tubing. This maintains a uniform flow over the life of the peristaltic tube. The RF-100 pump's ball bearing drive features a cogged belt reduction from the motor to the drive sheave. With the quiet belt drive, the motor and pump have an exceptionally long life. The DC drive has a convenient optional power pack that makes it very flexible. The flow depends on the DC voltage that is used. Table 1 shows the performance of the pump.

Table 1 RF-100 Pump Performance[3]

RF-100 Pump Performance Data				
Nonprime Standard	Standard 12V/24V		RF-100-Power Pack 115/60 Converter	
Tube Size OD	Max. Flow ml/min	9 volts ml/min	6 volts ml/min	3 volts ml/min
3/16" ID x 5/16"	500	375	225	100
1/8" ID x 1/4"	220	150	100	45
1/16" ID x 3/16"	45	34	21	10

b) Storage Tank

The storage tank shown in Figure 3 was designed to hold a 20.0 in^3 of fluid. It was made from a ¼ inch thick clear plastic. It has three inlets in the upper portion of the tank and one outlet in the bottom of the tank. Insulated cover prevents fluid evaporation from the tank and keeps the pressure of the system relatively stable with time.

Figure 3 The storage tank

c) Distributive Manifold

The distributive manifold shown in Figure 4a is located on the left side of assembly is made from a plastic block and contains six simple valves for the flow regulation of each individual mini-channel. The block is mounted on a stainless steel supporting frame that also holds one differential valve and one exit valve.

Figure 4a The top view of the distributive manifold

The first valve that the incoming flow encounters is a precision adjustable differential valve shown in Figure 4b. It utilizes color marks on the dial to attain proper position, which regulates the inflow of the fluid to the entire assembly.

Figure 4b The side view of the front differential valve

The blue valve on the exit shown in Figure 4c at the back of the manifold allows for the excess of the fluid to be redirected to the storage tank for further use. The remaining six valves allow for the division of the incoming flow into separate channels with different flow rates each. Depending on the required flow for

each channel these six valves can be adjusted accordingly.

Figure 4c The rear exit valve in the distributive manifold

d) Collective Manifold

The collective manifold shown in Figure 5a is located on the right side of assembly and is made from a plastic block that is serving as a support to eight valves. The block is mounted on top of a stainless steel holding frame. The simple six valves are receiving the separate incoming flows from the mini-channel plate. Depending on the required flow rate for each channel these six valves can be adjusted accordingly to slow down or increase the flow.

Figure 5a Top view of the collecting manifold

The front valve shown in Figure 5b that the out coming flow encounters is a precision adjustable differential valve with a several color marks on the dial, which regulates the outflow of the fluid to the storage tank.

Figure 5b The side view of the front differential valve

The blue exit valve shown in Figure 5c at the back of the manifold allows for the excess of fluid to be redirected from the collective manifold to the storage tank.

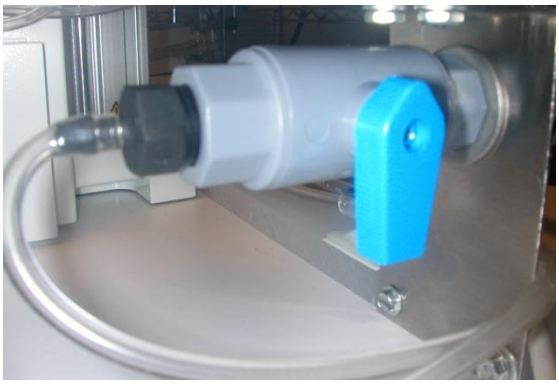

Figure 5c The rear valve in the collective manifold

e) Flow Channels

The mini-channels plate shown in Figures 6a and 6b is designed to provide separate and independent flows. It contains six mini channels that have two different sizes. The larger three channels have width of 3/64 in and should be used with the older embryos. The remaining three smaller channels are 1/64 in and should be used for the younger embryos.

The channels are surrounded separately with a rubber seals that are stopping the individual fluid pockets from mixing. The glass plate is covering the channels and the plastic clamps generate enough pressure for the seal to secure and maintain the flows.

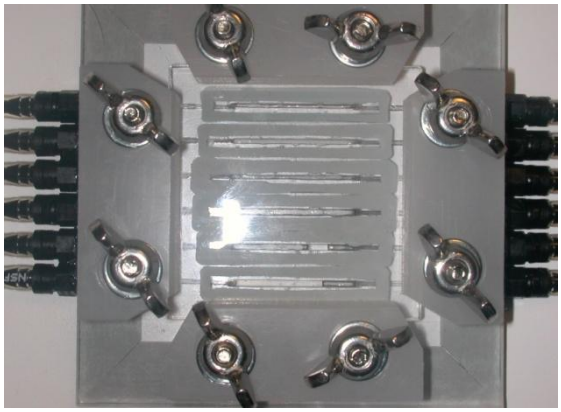

Figure 6a Top view of the mini channels flow plate

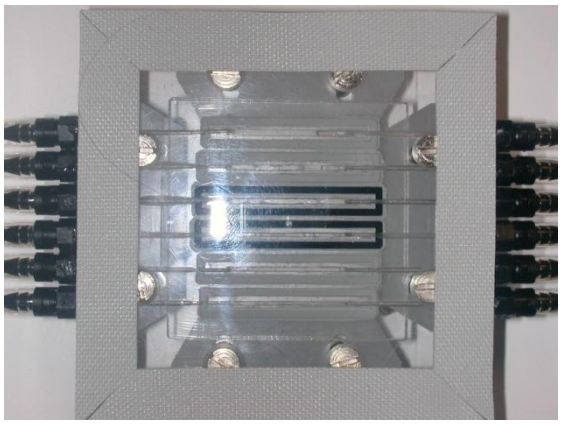

Figure6b Bottom view of the mini channels flow plate

f) Connectors with Caps

The mini piping in the system is distributed continuously and connected with portable female-male connectors shown in Figure 7. This allows for quick and convenient assembly and disassembly of the system. Connectors are located on all inlets and outlets of storage tank, pump and most importantly the mini-channel plate. Once disconnected a corresponding cover caps should be placed on the female and male ends to prevent leakage of the fluid and contamination.

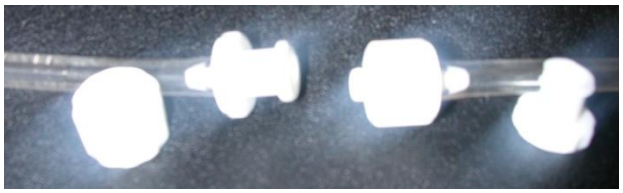

Figure 7 The male-female connectors with their corresponding cover caps

4) Instructions

The following section describes the basic procedures that are applicable during the operation of the flow channels.

a) Start up / Shut Down

To start the system it is necessary to plug in the AC/DC adapter shown in Figure 8 and use the appropriate voltage for the chosen task. Once the adapter is plugged into the wall, a green light should appear. Next, turn on the additional switch at the back of the pump. At this time the pump should turn clockwise. Once finished using, turn of the switch and unplug the adapter from the wall. It is important to unplug the adapter once the operation of the flow channel is completed to avoid overheating of the transformer coil inside the adapter.

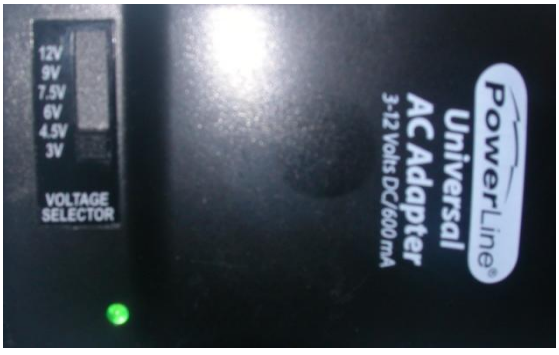

Figure 8 Shows the AC/DC adapter

b) Air Removal from the System

Once the system is turned on it is advisable to remove the air bubbles that have accumulated in the tubes and manifolds. The best way to eliminate air is to use the maximum flow rate (highest voltage-12V) and open all valves to allow unobstructed flow of liquid through the system. Let the pump run for 5-10 minutes. Next, inspect visually that all the air has been removed. The air removal procedure should be performed only after the system has been opened or it has not been used for more than a day.

c) Operation of the Flow Channels

To initiate the flow through the mini channels please proceed in the following way:

1. Open all valves on the collecting manifold (on the right side of the assembly). This includes six grey valves, the front differential valve and the rear (blue) valve. This guarantees that once the flow is generated by the pump the liquid will have a route of exit to the storage tank and the pressure will not build up in the system.
2. In the distributive manifold unblock the differential valve as shown in Figure 9 by pushing the black ring towards the red ring.

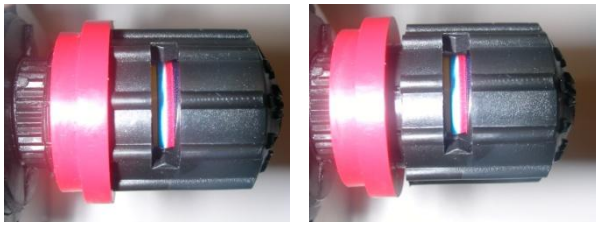

Figure 9 Left-Valve unblocked, Right-Valve blocked

3. Gradually open the differential valve (using the range of colors inside the dial). Next, open completely the three grey valves for the channels where the embryos **are not present** (1, 2, 3 **OR** 4, 5, 6) this will allow for the remainder of the air to be removed from the system.
4. Continuing in the distributive manifold now you can start to open **very slowly** those valves that are leading the flow into the channels into which the embryos were placed in advance. Open one valve at a time and let the flow stabilize and remain constant in each of the active flow channel.
5. Observe the flow under microscope shown in Figure 10 assuring that it is slow and the embryos swim freely in the center of the stream and are not pushed to the end of the channel.

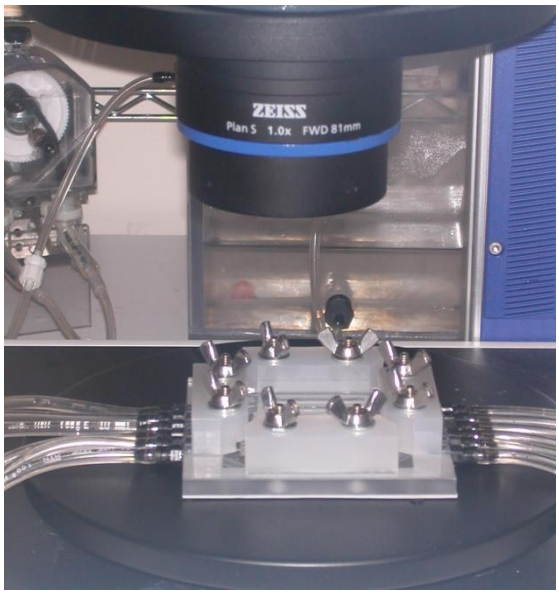

Figure 10 Lens of the microscope above the mini channel plate

6. To increase the flow in the mini channels simultaneously the differential valve can be turned to the next position (using the color

code). Also, to direct some of the flow from the distributive manifold to the collecting tank before it reaches the individual valves, the rear valve (blue) should be opened slowly to obtain desired release of fluid.

7. Now we should have a constant and continuous flow in the mini channels. This can be observed through the microscope. The viewing zone of the microscope allows three channels to be seen. Remembering that the liquid is moving through all channels we should keep all six (grey valves) on both manifolds open to prevent the pressure build up.

8. If it is necessary to adjust the flows in the individual channels, only the grey valves should be used for this purpose. This may be necessary if some of the embryos are lagging behind the others. If only the target channels (with embryos present) are going to be used then the remaining three grey valves can be closed. Caution should be exercised at this point. Closing these three valves will increase the flow in the remaining channels. Thus the main flow should be reduced first gradually with the distributive differential valve.

9. Once the fluid exits from the mini channel plate it enters the collecting manifold individually through the six (or three) grey valves. These valves can be also used to regulate the back flow on the individual basis. The differential exit valve in this manifold gauges the flow into the collecting tank. It allows for the flow to be changed gradually. The rear valve (blue) if opened dumps the excess of exiting fluid into collecting tank.

10. Due to the variety of adjusting options it will take time for the user to work with the valves and produce the desired flow necessary for the embryos to swim continuously in the channels.

11. To stop the flow through the mini channels open the rear valve in the distributive manifold completely and close the individual six(or three) grey valves in the same manifold that deliver fluid to the channels. This redirects the entire flow into collecting tank.

12. Next, to stop the flow completely the pump can be turned off. Close the differential valve in the distributive manifold or leave it open if you want to use the same starting position during the beginning of the next test.

13. Now the fluid is stagnant in the tubes and channels. To remove the mini channel plate, twist the female-male connectors on both sides of the plate. Disconnect them and place the corresponding cups to avoid liquid spilling. This will allow for the embryos to have almost the same amount of liquid inside the channels.

14. To remove the embryos unscrew the eight butterfly nuts and lift the cover plate to have the access to the channels.

d) Placement/Removal of the Embryos into/out of the Test Channels.

Placement of embryos and closing of the mini channel cover plate is probably one of the most difficult and time consuming tasks in the entire

process. The following steps lead to the successful placement of embryos in the channels.

1. Once the mini channel plate is removed from the system and all twelve ends of the tubes are secured with covering caps it becomes a closed and portable system and therefore it can be easily transported.
2. To access the channels the butterfly nuts and four plastic clamps must be removed and the glass plate lifted up. Now you have a clear access to the six channels. The three rear channels are for the smaller (younger) embryos and the three front channels are used for larger (older) embryos.
3. Make sure there is enough fluid in each channel for the embryos to be placed.
4. Pick one embryo at the time with pipette and transport it into a channel. The head of the embryo should be facing left-towards the distributive manifold and against the direction of the flow.
5. Once the embryos are in the channels the cover glass can be placed on top and the clamps tightened with the butterfly nuts.
6. One note of caution. Be very careful when tightening the clamps, because the embryos have a tendency to 'jump' out of the channels and can end up squeezed. Securing of the cover should be done under microscope so you can easily see if the embryos are still located in the center of the channels.
7. The butterfly nuts should be tightened gradually (one turn at a time) and inter changeably (west-east sides first, then north-south sides) until completely tight.
8. Once this is accomplished the mini channel plate can be connected again with the rest of the system using the Female/Male connectors.
9. Removal of the embryos can be done with a pipette and if it is necessary the channel plate can be washed under the sink. Screws and nuts are made from stainless steel therefore possible rusting is eliminated.

5) Applicable Fluid Mechanics Theory

The following basic introductory theoretical background can be used as a starting point in the analysis of forces present in the flow and stresses generated on the embryos.

Fluid mechanics is the study of how fluids move and can be divided fluid statics: the study of fluids at rest, and fluid dynamics: the study of fluids in motion. Fluid dynamics has a wide range of applications. In this project we are dealing with generation of forces and moments on life embryos that are placed inside a laminar flow. Also mass flow rate of the fluid through tubes and channels plays an important role in functioning of the system. Inside the system we have the movement of Newtonian fluid with extremely low laminar flow.

A **Newtonian fluid** is defined to be a fluid where shear stress is linearly proportional to the velocity gradient in the direction perpendicular

to the plane of shear. This definition means that regardless of the forces acting on a fluid, it continues to flow. The constant of proportionality between the shear stress and the velocity gradient is known as the viscosity. Figure 11 shows that the streamlines around an object in the laminar flow are usually symmetrical.

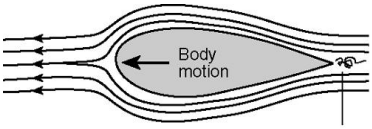

Figure 11 Stream lines of the laminar flow

A body in the laminar flow is experiencing slight stresses. The distribution of shear stresses can be approximated by Equation 1 which describes the case for the Newtonian fluid behavior:

$$\tau = \mu\left(\frac{dv}{dx}\right) \qquad (1)$$

where τ is the shear stress exerted by the fluid, μ is the viscosity and dv/dx is the velocity gradient perpendicular to the direction of shear. A standard visualization of the stresses is shown in Figure 12.

Figure 12 Stress localization in laminar flow

Laminar flow known as streamline flow occurs when a fluid flows in parallel layers, with no disruption between the layers. In fluid dynamics laminar flow is a flow regime characterized by high momentum diffusion, low momentum convection, pressure and velocity independent from time. It is the opposite of turbulent flow.

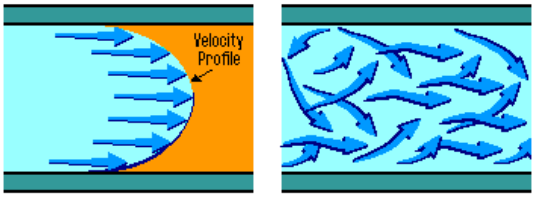

Figure 13 Laminar and turbulent flow velocity profiles

Figure 13 shows the velocity profiles of laminar (left) and turbulent (right) velocity flow profiles. In nonscientific terms laminar flow is "smooth," and is characterized by very low Reynolds number, while turbulent flow is "rough" and much higher Reynolds Numbers can be associated with it.

Reynolds number is the ratio of inertial forces ($v_s\rho$) to viscous forces (μ/L) and consequently it quantifies the relative importance of these two types of forces for given flow conditions. The Reynolds number is defined by Equation 2.

$$Re = \frac{Inertial\ Forces}{Viscous\ Forces} = \frac{\rho v_s^2/L}{\mu v_s/L^2} = \frac{\rho v_s L}{\mu} = \frac{v_s L}{\upsilon} \qquad (2)$$

where v_s is the mean fluid velocity [m s^{-1}], L is the characteristic length [m], μ is the (absolute) dynamic fluid viscosity [Ns m^{-2}] or [Pas], ν is the kinematic fluid viscosity ν = μ/ρ [m^2 s^{-1}], and ρ is the density of the fluid [kg m^{-3}]. If our case can be interpreted as a flow in a pipe/channel then the characteristic length is the pipe diameter (for the tubular section) if the cross section is circular, or the hydraulic diameter, for a non-circular cross section (for the channel portion).

The **hydraulic diameter, D_h,** is a commonly used when handling flow in noncircular tubes and channels. It is defined as D_h = (4A)/U where *A* is the cross sectional area and *U* is the wetted perimeter of the cross-section. The **wetted perimeter** is common in fluid mechanics and heat transfer applications and it is associated with the hydraulic diameter. This term is simply defined using the example of a cross section of channel. The wet perimeter is the perimeter of the cross sectional area that is "wet".

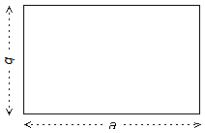

Figure14 Example of wetted perimeter application

In our application the fluid touches all surfaces of the channel and therefore for the rectangular cross-section the wetted perimeter is defined the equation: W_p = 2W +2H or if referring to Figure 14 then W_p = 2a+2b. Consequently the hydraulic diameter is defined by Equation 3.

$$D_h = \frac{4LW}{2(L+W)} = \frac{2LW}{L+W} = \frac{2ab}{a+b} \qquad (5)$$

where L is the depth and W is the width of the channel.

Another important concept in this project is the mass flow rate. **Mass flow rate** is the movement of mass per time with the units of [kg s^{-1}] or [lb s^{-1}]. It can be defined by Equation 4

$$\dot{m} = \rho v A_f \qquad (4)$$

where ρ is density, *v* is velocity and A_f is the flow area. The flow can also be calculated by using the **volumetric flow rate** (Q). It is the volume of fluid which passes through a given surface area per unit time [m^3 s^{-1}] or [ft^3 s^{-1}]. It is usually specified by the pump manufacturer in ml min^{-1} or in ml s^{-1}. In terms of the volumetric flow rate the mass flow rate is expressed by Equation 5.

$$\dot{m} = \rho Q \qquad (5)$$

Accounting for the friction in the tubes and channels, relationships for the flows can be defined by Poiseuille's law. This physical law concerning the volumetric laminar stationary flow Φ of an incompressible uniform viscous liquid through a tube or a channel with constant cross-section is defined by Equation 6.

$$\Phi = \frac{dV}{dt} = v\pi R^2 = \frac{\pi R^4}{8\eta}\left(\frac{-\Delta P}{\Delta x}\right) = \frac{\pi R^4}{8\eta}\frac{|\Delta P|}{L} \qquad (6)$$

where *V* is a volume of the liquid poured in the time *t*, *v* is the mean fluid velocity along the length of the tube, *x* the direction of flow, *R* the internal radius of the tube, ΔP the pressure difference between the two ends, η the dynamic fluid viscosity, and *L* the total length of the tube in the *x* direction This result is also a solution to the Darcy-Weisbach equation in the field of hydraulics, given a relationship for the friction factor in terms of the Reynolds number as expressed in Equation 7.

$$\Lambda = \frac{64}{Re} \qquad \mathrm{Re} = \frac{2\rho v r}{\eta} \qquad (7)$$

where *Re* is the Reynolds number and ρ fluid density. In this form the law approximates the Darcy friction factor-energy loss factor Λ in the laminar flow at very low velocities in cylindrical tube. These relationships are usually presented in the Moody Chart that is included in Appendix.

6) Applicable Examples of Modeling

To visualize how the fluid flow impacts objects that are immersed in it a computational modeling process can be applied. This generates 2D or 3D models that show how fluid behaves while interacting with various stationary or moveable objects. Several studies that are presented here can serve as starting point when considering numerical analysis of various aspects of Zebra fish embryos.

Figure 15 show the results of the results of the laminar flow over circular cylinder. The incoming free stream flow is uniform with a Mach number of 0.2 and a Reynolds number based on diameter of 150. At this Reynolds number, the flow is essentially two dimensional with periodic vortex pairs shed from the downstream side of the cylinder.

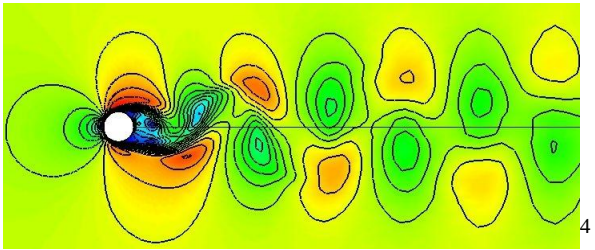

Figure15 Example of laminar flow over a stationary cylinder

Figure 16 shows the results of the simulation of a time-phase resolved combined flow velocity field/structure interaction measurement.

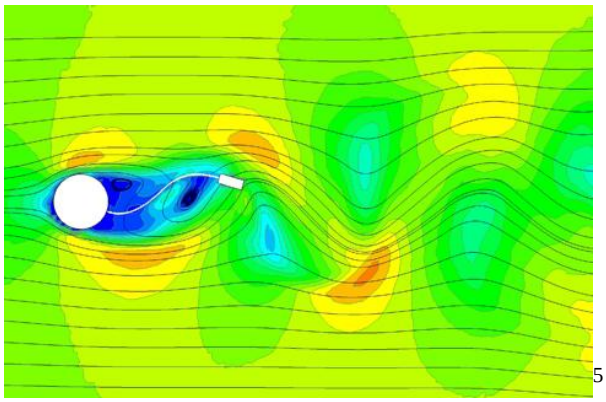

Figure16 Example of flow over a movable cylinder with a flapping effect

These measurements conducted in the context of this project aim the characterization of the resulting structure periodic swiveling motion. Time-phase resolved measurements are typically registered with a time-phase angle resolution of 1^{o} and they comprehend: flow velocity field around the structure, structure attitude and deformation, trajectory of defined points on the structure and also the frequency of the resulting motion.

Figure 17 shows the oxygen partial pressure surrounding a zebra fish embryo in water. The flow lines show the direction of the oxygen flux and here you can see that the yolk does not consume oxygen. Instead, oxygen is consumed in the main body of the fish embryo surrounding the bulky yolk. The model was developed by COMSOL in cooperation with Dr. Sander Kranenbarg of Wageningen University, The Netherlands.

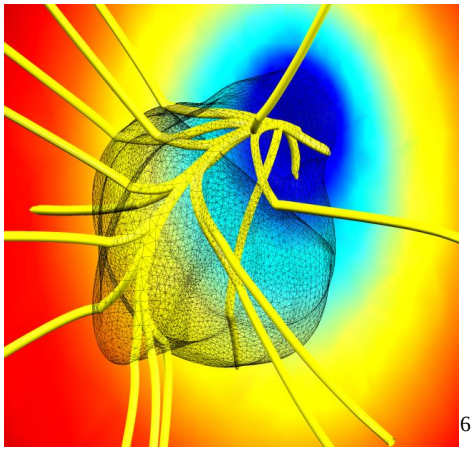

Figure16 Profile of the partial pressure around zebra fish embryo

An interesting research was done regarding the diffusion of oxygen[7] in Zebra fish embryos. Fluid Mechanics and computer modeling was widely engaged as depicted below in Figures 17,18.

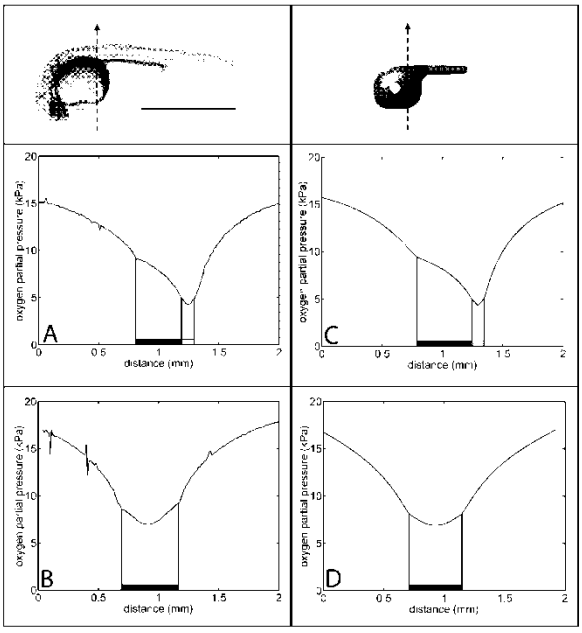

Figure 7.1: Comparison between measured (A and B) and modelled (C and D) oxygen profiles in the zebrafish embryo. (A) and (C), Oxygen partial pressure (in kPa) in ventro-dorsal direction (dashed arrow in the heading figures). B and D, Oxygen partial pressure in lateral direction through the yolk (at the level of the white spot in the heading figures). Note the discontinuity in the slope of the oxygen partial pressure profile at the surrounding medium - yolk boundary. Profile parts inside the yolk are indicated with a light bar, while profile parts inside the tissue of the embryo are indicated with a dark bar. Scale bar is 1 mm.

Figure17 Profile of the oxygen around zebra fish embryo

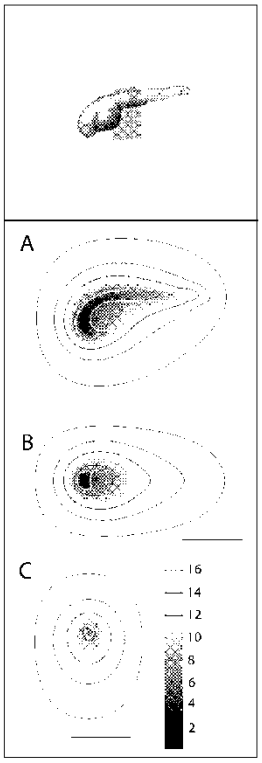

Figure 7.2: Three-dimensional predicted oxygen field surrounding the zebrafish embryo (indicated by the solid white line). A, Sagittal section through the predicted oxygen field. B, Horizontal section through the predicted oxygen field. C, Transverse section through the predicted oxygen field. The figures were obtained from the same simulation as Fig. 7.1. The pressure boundary layer extends both inside the embryo and in the medium surrounding the embryo. Oxygen partial pressure decreases along the long axis of the embryo from posterior to anterior. Minimum oxygen pressures are observed in the center of the tissue of the embryo in the anterior region. Contours of the yolk and animal tissue are indicated in the subfigures. The color bar represents oxygen partial pressure in kPa. The black contour lines represent oxygen partial pressures as indicated in the color bar. Scale bar is 1 mm.

Figure18 Oxygen field around zebra fish embryo

7) Endnotes

[1] http://www.comsol.com/paper/kinetics-of-zebrafish-dorsoventral-patterning-10739

[2] www.greylor.com

[3] Ibid

[4] http://www.grc.nasa.gov/WWW/wind/valid/lamcyl/Study1_files/Study1.html

[5] http://www.lstm.uni-erlangen.de/projekt/flustruc/index_princ_en.htm

[6] https://uk.comsol.com/press/gallery/

[7] http://library.wur.nl/wda/dissertations/dis3241.pdf

8) Appendix - Moody Chart