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NON-NEWTONIAN MICELLAR MICROFLOW VISUALIZATION IN A CONTRACTION GEOMETRY

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KEY WORDS

PIV, blood, pattern, instability~

ABSTRACT

Many biomedical devices work with micellar microflows. The fluids are mainly non Newtonian because of the micelles. These can be red cells, proteins, chemical components, etc… whose flow can interfere in the original organization. This article provides an experimental investigation of an in vitro real and untreated rabbit blood micro-flow. The rabbit blood is chosen for its similitude with human being blood. The channel is made in PDMS and the micrometrical geometry is representing a syringe geometry of the contraction right before the needle. The preparation of such an experiment requires special protocol that's described in details in this paper. Then, after a description of the materials, some trials with a high speed camera are made. We make visualizations and micro PIV with elevated sample rates. The PDMS channel is thin enough in order to get enough contrast of the red cells in the plasma when lighted. The red cells are then tracked as natural enhancement particles usually added for PIV. The use of microscope with several magnification coefficients could enable the catch of high frame rate. The obtained streak lines and velocity fields of the red cells could provide a nice description of the flow qualitatively and even quantitatively. By pushing a bit the velocity and geometry aspects, a swirl could well be identified. So, this report is settling a method on a simple case in order to further increase much more the state of art of biomedical non Newtonian flows.

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1. INTRODUCTION

Two-phases flows are mostly non Newtonian and complex to study. Mixing powders with air or molecules and cells in water are some of the numerous applications. More particularly we know that blood is such a flow and even more complex due to many different proteins present in the plasma.The common unifying thread of research projects currently under way about blood rheology (haematology) is more assumed by medicine body than the fluids mechanics science. Converging, contracting or narrowing configurations in the corporeal body commonly arise as basic constitutive elements in numerous configurations where blood (haematology) is affected: In order to suddenly inject blood in a muscle, veins can contract themselves [1]. By stress, some veins contract as well and the effect is not well: the blood comes back to the heart. Contractions are hydraulic geometries often encountered in nature and even in extracorporeal devices such as syringes when the flow has to pass from the main cylinder volume to the needle and, in the veins as well, due to thrombosis or cholesterol. Particular to these configurations, the velocity before and after the contraction can vary a lot depending on the sections rate and the flow can be subjected to some fluids mechanic instabilities and transitions, shown by [2]. Pressure loss and incongruent structure in the flow can be caused by a certain number of contractions as well which, these ones are not due to mechanical geometries, are due to biological phenomena in the blood: thrombosis amalgamates, etc. [3]. The consequences are important: diseases, pain, stress, and heart degradation. The local and fine fluids mechanics effects are hardly taken into account in the medicine. Most of the time, for example, the viscosity of the blood is calculated macroscopically, it remains the same for the pressure loss and tension. The whole or the resulting tension across the body veins is related to the heart tension with the whole blood when sometimes the problems are very located in different and specified places [4-6].

The structure of the flow itself is not well known because, firstly, the blood is hardly transparent for experimental visualizations and, then, especially for human blood, because the studies of blood are scarce due to hygienic and ethic reasons.

Some alternative fluids [7] to study blood like motion in typical biomedical geometries were tried as blood is presenting difficulties. In water, it is very easy because it is transparent and some adding particles could make it for visualisation. In addition, by adding blood like cells in water such as laponite [8], one can reach a concentration close to human blood but the chemical effects and the elasticity of the cells is impossible to reproduce completely neither finely. Blood is a non Newtonian flow that can as well, but in term of viscosity only, be simulated by solutions [9]. A complete rheology of the blood, as a fluid, is a today's subject that promises lots of interesting discoveries in term of phenomenology.

This is why, in this project, we are developing and evaluating a visualization method for exploring the structure of blood flow in a contraction geometry type flow. We chose this geometry because it is standard and it appears a lot in many fields using blood. This research is exploring not only visual representation techniques, but also environmental compatibilities because of specific blood use requests and in order to take information about the physics of the flow.

This article succeeds to a few recent publications of advances in this field where the investigations were carried upon the motion of the real red cells in the plasma [10] [11]. But the blood hematocrite rate is systematically diluted. And most of the time blood has been treated by anticoagulants. Here we work with rabbit blood because we can use it real and untreated. Moreover, it has the same characteristics as human being blood considering a parameter that has great importance: red blood cells are almost the same, geometrically and in concentration.

The primary objective of this article is to present the experimental set up that enables us to make visualizations in real blood flow, blood with no treatment. As it was not possible to use human blood due to European norms respect in Lisbon University, rabbit blood was found to be the closest choice in term of size and fraction of the red cells. In order to reproduce the converging of a syringe, a design has been chosen and the fabrication protocol of the channel, made of PDMS, will be explained. The mean velocity in the centre of channel at the exit of the convergence was chosen equal 1.5mm/s, which is similar to a small injection rate for insulin. The camera and microscope are technically defined and then, we propose to treat the data in order to use the correlation between frames recorded with the camera to get velocity estimates using PIV technique where red cells are the particles.

2. MATERIAL

In this part we describe the apparatus, and the design of the studied PDMS channel.

2.1 Channel

The channel and pieces of material of the experimentation are prepared knowing that using blood requires biocompatibility of the material in which it is used in order not to deteriorate its properties neither influence the flow. For this, the choice has been made to work with PDMS channels [12-13].

The PDMS technique [14] was followed and realised by ESPCI MMN, Paris. A contraction was drawn with the geometry quotations of Fig.1. The origin for the geometrical locations is taken on the point O. D=50µm is the reference length equal to the height of the exit channel right after the contraction. The *x*-direction is taken as the direction of the main flow. The depth of the channel, in the *z*-direction, is equal to 75µm. This value is small enough to let sufficiently light passing through the blood flow in order to get both contrast and intensity to follow particles in the blood which red colour is due to iron that fixes oxygen. The plasma which the red cells bathe in, is almost transparent.

A thin glass blade dedicated to standard microscope studies, 250µm thick, is supporting the channel in order to proceed to microscope visualizations. The extremities of the channel are connected to medical tubes (BPE-90 from Instechlabs) and the blood is pushed using a screw (Chemix N5000) in order to control the debit of the blood. This screw has shown a precision of 96% for the imposed flow rate.

A whole description of the set up for the experiments is shown in the photograph of Fig.2. The blood flow is pushed by a syringe that is activated by the screw. The syringe is made of glass in order to avoid deformation of the usual plastic syringes.

2.2 Blood

The blood we used was directly taken form a rabbit alive from the Veterinary University, Faculdade de Medicina Veterinária, Universidade Técnica de Lisboa, in the lab of toxicology, Farmacotoxicologica.

The haematocrit was equal to 49% same as human being's. This choice was made following expertise of haematologist department of the University of Farmacology of Lisbon. Directly used for the experiments after its extraction, the blood could keep its integrity until the end of the works which had to be quick. The conditions to use blood and to respect animal chart were totally respected in the lab of the Veterinary Faculty of Lisbon. The choice was decided in a haematologist spirit in order to get results close to the human beings environment. The characteristics are referenced in the Merkvetmanual of the White House Station for the animals. The choice was based on the hematology reference ranges, complete for the following parameters: red blood cells, white blood cells, neutrophils, haemoglobin, hematocrit, mean corpular volume, platletcrit, neutrophils, lymphocytes, moncytes, eosinophils, basophils, in terms of size and fraction.

Each time, from the rabbit, it was possible to take only a small quantity, 3mL, of blood. We let one week for the rabbit to get rest. The volume of blood equal to 3mL was used directly after taking out of the right ear main vein of the rabbit.

After the experiment, in order to reuse the channel, it is the most important thing to clean it with appropriate solvents until the walls of the channel are clean. So a first clean is run with Saur water because it is dislocating the cells and fat acids that stick to the walls of the channel. Then another clean is made with oxygenated water in order to clean the wall from Saur water. In the end a last clean is made with distilled water and the channel is kept with water in order to close the channel from particles and asperities and in order to keep the channel made of PDMS hydrophilic.

Most of the protocols we used were established by the laboratory of the Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL); Molecular and Cell Biology of Eukaryotic Systems Unit of the University of Lisbon. The benchmarking and the preparation of the microscope and visualisation camera were done under their expertise to treat with cells and respect conditions of use.

3. VISUALIZATIONS AND PIV

A high speed camera, a zoom and enlighting lamp were required in order to record frames of the flow.

3.1 Light

A halogen lamp, continuous with varying intensity was used, Olympus KL 1500 LCD. This permits acquiring a high number of frames per second. We could vary the intensity in order to get more contrast and halogen light allowed to capture the red cells with contrast.

A photograph of the blood is presented in the Fig.3 with a zoom in order to check the contrast between the cells and the plasma when lighted by the Halogen lamp.

3.2 High speed camera

The camera (Camera Vision Research Phantom V4.2) could be monitored by computer. The main and classical parameters were the resolution, the number of frames per second and the exposure time. Numerically, the contrast and the windows of caption of the flow were slightly modified depending on the velocity of the flow, the exposure time and the zoom. The software associated to the camera is Phantom software V.9.0.640.

The zooms of 5 and 20 could be used knowing that the camera has a magnification equal to 1.15. When the cells of the blood were the main focus, it was obligatory to use the magnification of at least 40 because the size of a typical cell is standard 8µm diameter and 1µm thick but we will see in the following sections that to process PIV treatment, a zoom of 20 was providing accurate results.

The main results expected are, first, streak lines in order to see the structure of the flow, second, the possibility to make correlations between frames in order to measure velocity of the cells. Streaklines are the locus of points of all the fluid particles that have passed continuously through a particular spatial point in the past. Dye steadily injected into the fluid extends along a so called streakline. Blood cells are considered following the same rule.

3.3 Data treatment

The treatment of the recorded frames was summarily using the classical and elementary technique of PIV. We used the technique developed by [9]. No tracer was added because the red cells are the particles tracked. So we just give the results that are proper to our experiment. The correlation between

windows was reaching a minimum percentage of 95%. Correlating the frames spaced in time of 30 microseconds was not impossible because the high speed camera was acquiring until 30000 frames per seconds. For a velocity *maximum* equal to 1cm/s, a displacement of 1 micrometer was corresponding to a time equal to 100 microseconds. 30µs was chosen as time between frames. The average cross correlation was then processed.

But by going to frames rates equal to 30000 frames per second, with a zoom of 40, we could see some cells slightly deform [10]. This phenomenon can increase errors because a cell can modify in space die to velocity displacement and shape changes. It was the main difference from traditional micro PIV where particles do not deform. Hopefully they did not deform so much in the average. An average of 1000 frames was made. It was measured that cells modify their shape of less than 0.1µm per second, what represent 1% of the displacement due to the flow velocity.

4. CONTRACTION FLOW RESULTS

A first attempt with the whole geometry provided results with low accuracy, the zoom used had a magnification equal to 5. The cells organisation is homogeneous and no piles of plates like or aggregates were found. More accuracy was obtained with a zoom of 20 shown in Fig.4. The streaklines show the structure of the flow. In the non converging areas, the flow is really parallel to the walls. No swirl is encountered and no other instability as well. It seems that even in the convergence, the flow close to the wall is parallel. In order to have more precision on the velocity, the zoom of 20 has been used and PIV processing is made. The velocity profile in the centre of the straight channel at the exit of the contraction (Figs. 5-6) was providing a mean velocity equal to U=1.58mm/s. The mean velocity U is taken as a reference to normalize the velocity profiles.

The velocity profiles of the Figure 6 show that blood flow is established from x=0.5D after the contraction because the velocity profiles are not evolving anymore for x>0.5D. In the section of the exit of the contraction, x=0D, the horizontal velocity profile is resulting from an acceleration across the contraction from x=-1D. The vertical velocity profile is interesting because it shows a relatively constant profile evolution of the vertical component of the velocity inside the contraction but on the exit section, x=0D, this velocity is suddenly diminished because of the section size change. This brutal change may be related to high pressure gradients.

This description of the flow is showing that blood is quite compact and follows very well the lines of the boundaries. It is very important, because of that, to figure out the consequences in term of pressure. Here we could not yet install pressure sensors. But it should be in the next works.

For stronger angles of contraction or higher velocities, swirls appear (Fig. 7). The dynamic description of such a swirl is not yet available because the data need being carefully treated but we show a picture for U=-10mm/s acting as an expansion. This point promises high resonance because blood is known for undergoing aggregates or even amalgamates in high pressure areas and swirls areas.

5. CONCLUSION

This work permitted to achieve a complete study of the dynamics of a blood flow in contraction channel for a very low mean velocity. The micro PIV, the technique of fabrication and treatment of the data could be used with high confidence because blood cells are found being ideal tracers in the flow. Even if the size of these cells, and their shape as well, are quite different from the classical particles used in micro PIV, a high frames number acquisition enabled by the camera revealed much higher quality of the cross correlation than expected. The description of the flow is certainly very simple but the implementation is remaining unsuspected as long as the blood is not assumed being poorly

referenced in today's literature. First, ethic and hygienic committees are seriously controlling human blood use from medical to research uses. The bioethics is nowadays even more binding than the very last years, this is why the research was first dedicated to define a kind of animal blood close to human being's one. Then the problem was to find a place where to run experiments. On the other side, concerning the PIV application, as references are still scarce in literature, we estimate velocities and identify flow structure using this technique carefully. The main results are not as much the description of the flow in the micro contraction than the great confidence into next steps where some more difficult problems will have to be solved in blood flow dynamics area. We could conclude that this study could be the means to the stage being set. The PDMS was tested and different chemical products were tried in order to identify the ones that can clean the elements of the experimental apparatus in which blood systematically sticks, dries, obstructs and amalgamates almost everywhere. In the next plans of research, pressure measurements devices should be implemented in order to relate pressure drops to velocity profiles taking into account the viscosity variations in the blood. Other geometries with different mass rates should be studied in order to see the influence of the geometry on the viscosity of the blood, and, then, if it is possible, the observation of the organisation of the cells (plates, rows, random, ...[15]) should be enabled by use of bigger zooms (magnification equal to 100).

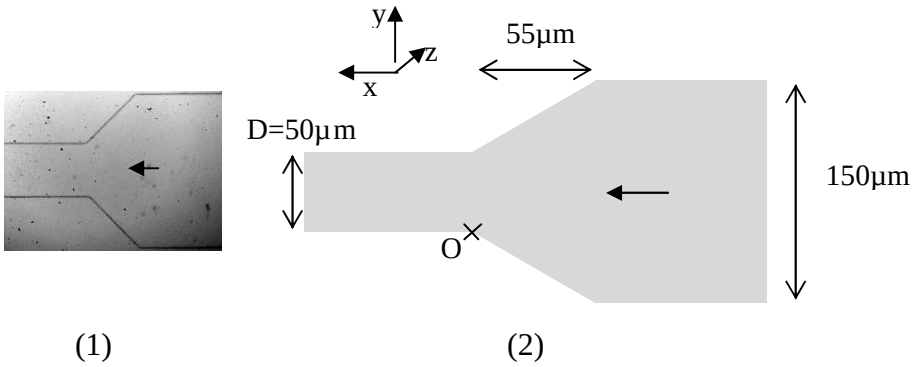


Figure 1: Photograph (1) of the contraction channel and quotations (2).

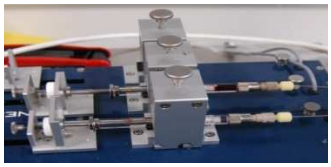


Figure 2: Photograph of the screw pusher. The syringe placed adequately in order to get the blow rate at the imposed conditions.

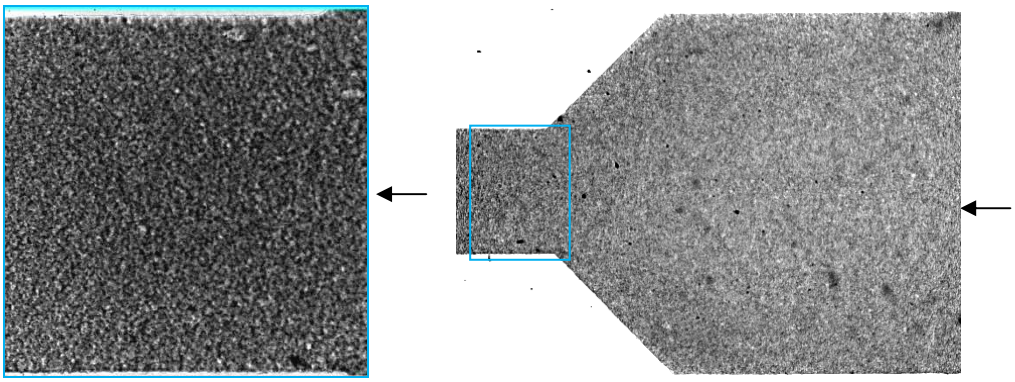


Figure 3: photograph (2) of the blood flow taken with small time exposure equal to 12µs with a zoom magnification equal to 5. A window with a zoom magnification equal to 20 is showing the red blood cells photograph in the photograph (1).

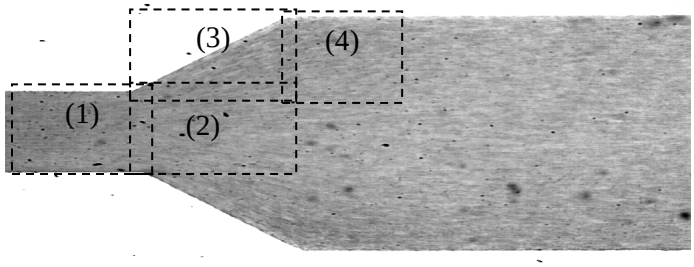
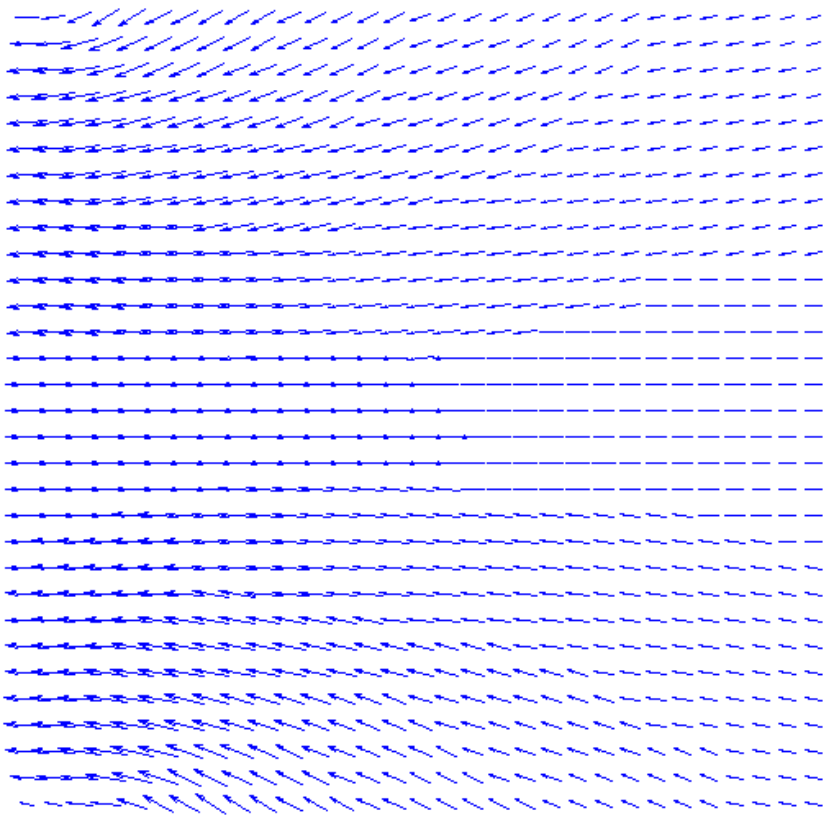
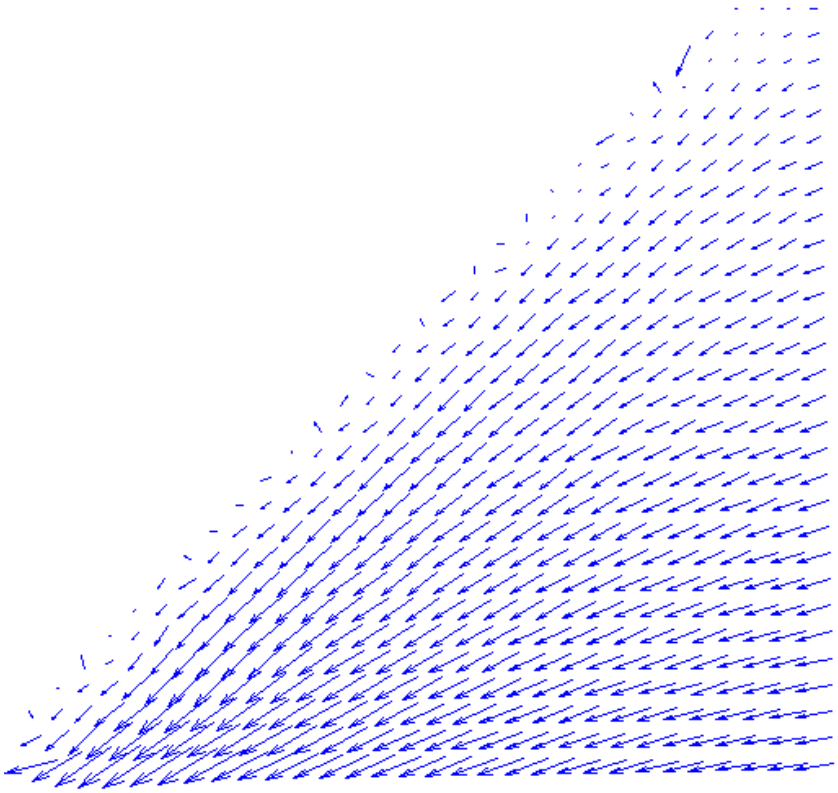


Figure 4: photograph of the streaklines in the contraction, a Zoom of 5 used.

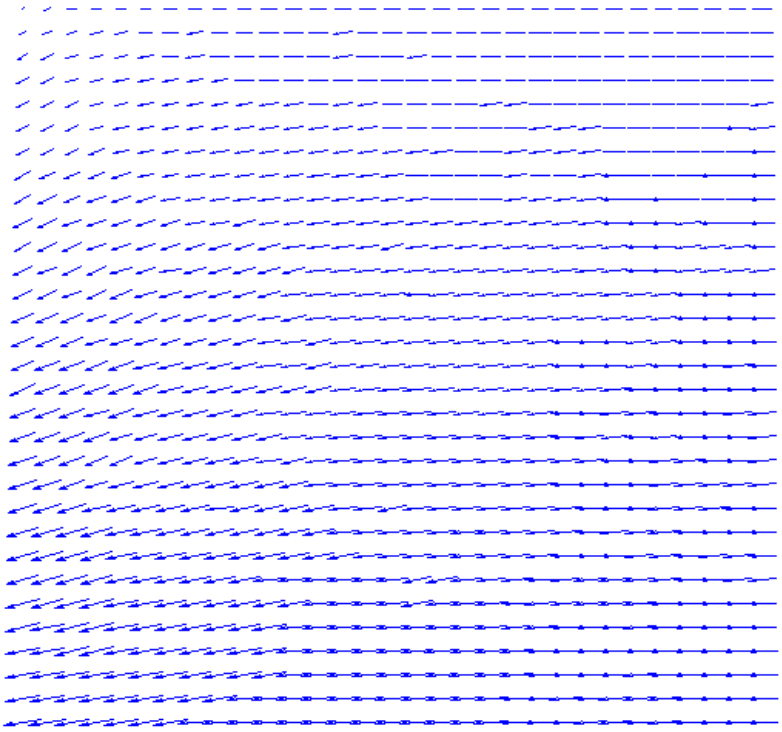
Zoom 20 (1)



Zoom 20 (2)

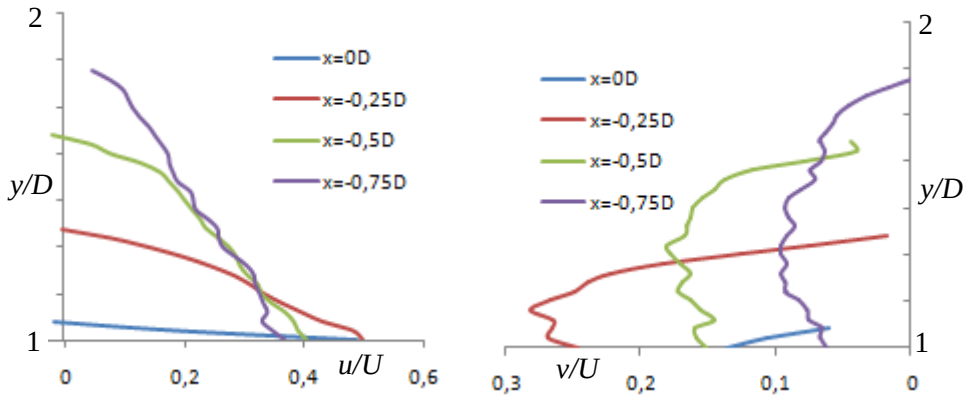


Zoom 20 (3)

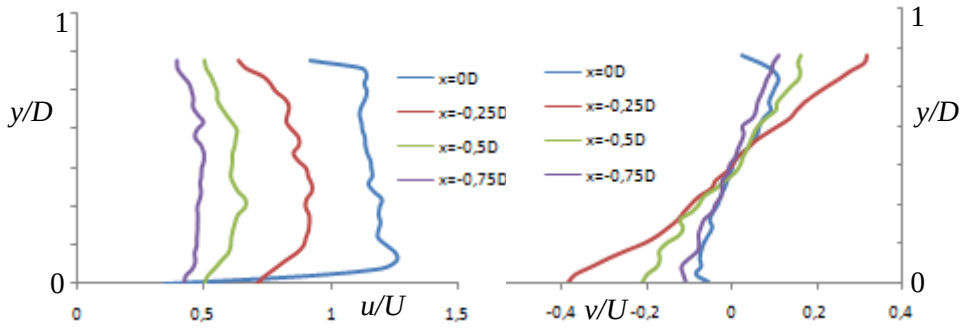


Zoom 20 (4)

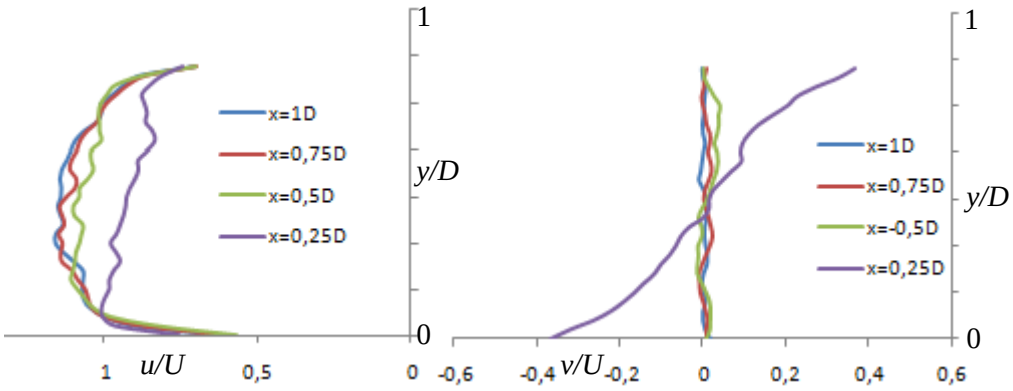
Figure 5: Velocity vectors obtained from PIV results with a zoom of 20 in the different domains of the contraction area (1-4) corresponding to the delimitations of the Fig.3



Evolution of the profiles of the horizontal and vertical velocity *u* and *v* of "zoom 20 (3)" in the Fig. 4.



Evolution of the profiles of the horizontal and vertical velocity *u* and *v* of "zoom 20 (2)" in the Fig. 4.



Evolution of the profiles of the horizontal and vertical velocity *u* and *v* of "zoom 20 (1)" in the Fig. 4.

Figure 6: Different evolutions of the velocity profiles in characteristic locations of the contraction.

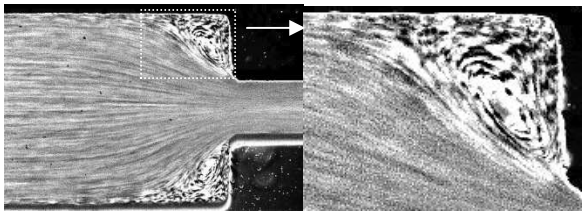


Figure 7: Streak lines for *U*=-10mm/s with a zoom of the swirl in the upper right corner.

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NOMENCLATURE

D	Height of the exit channel	u	mean horizontal velocity
U	Reference velocity	v	mean vertical velocity
		x	horizontal coordinate
		y	vertical coordinate
		z	transversal coordinate

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