ANALYSIS OF VELOCITY PROFILES OF BLOOD FLOW IN MICROCHANNELS USING CONFOCAL MICRO-PIV AND PARTICLE METHOD

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Abstract *The combination of computational and experimental investigations provides an excellent approach to understand complex phenomena involved at a microscopic level. This paper emphasizes a new experimental technique capable to quantify the flow patterns inside microchannels with high spatial and temporal resolution. This technique, known as confocal micro-PIV, consists of a spinning disk confocal microscope, high speed camera and a diode-pumped solid state (DPSS) laser. Velocity profiles of physiological fluids were measured within different microchannels. The measured results agree reasonably well with the predicted analytical values. This new PIV system is a very promising technique to confirm the validity of the data obtained by numerical simulations, such as the MPS particle method.*

1 INTRODUCTION

The main function of the human body circulatory system is to deliver oxygen and materials to the organs and tissues and to carry the waste away from the cells. The exchange of materials between blood and cells occurs mainly at vessels with diameters less than 300 μm which are known as microvessels [1, 2]. The phenomena of blood flow in microcirculation are crucial in maintaining healthy organs and tissues. Although this phenomena have been studied for many years still incompletely understood [2, 3]. It is therefore important to investigate the behaviour of blood flow occurring at microvessels in order to better understand the role of blood cells in the process of delivering oxygen and materials to the organs and tissues.

Over the years, classical numerical techniques, such as finite element method (FEM) or finite volume method (FVM), have been considering blood as a homogeneous fluid in order to model vascular flow [4]. However, to model blood flow in microvessels it is fundamental to

consider several micro-scale effects such as red blood cells (RBCs) deformability, aggregation, vessel geometry and flow shear rate [1, 3, 5]. In this way, recently some computational approaches using discrete particle have been proposed to investigate the dynamic behaviour of blood cells in microvessels [5, 6]. Tsubota and his colleagues [7, 8] have been developing a computer simulation method for microscopic blood flow using a particle method in order to analyse the RBC motion and deformation, plasma flow and their interactions. This new computer simulation method is based on the Moving-Particle Semi-Implicit (MPS) method, proposed by Koshizuka and his colleagues [9]. The particle method developed by Tsubota et al.[7, 8] is an excellent approach to investigate the microscopic mechanical behaviour of blood flow as it considers the human blood as a set of suspended discrete particles. However this proposed computational method needs to be compared and validated with an adequate experimental technique capable to measure and quantitatively evaluate fluid mechanical effects at a microscopic level with high spatial and temporal resolution. According to our knowledge, the best way to achieve those requirements is by using a confocal micro-PIV system.

To date, the experimental studies of blood flow in microcirculation were often obtained by using conventional micro-Particle Image Velocimetry (PIV) systems [10, 11]. However, considerable progress in the development of confocal microscopy and consequent advantages of this microscope over the conventional microscopes have led to a new technique known as confocal micro-PIV [12-15]. This system is a non-invasive optical particle-based flow velocimetry technique where trace particles are seeded to the flow. The confocal micro-PIV system by combining the conventional PIV system with a spinning disk confocal microscope (SDCM) has the ability to obtain in-focus images with optical thickness less than 1 μm (optical sectioning effect), task extremely difficult to be achieved by using a conventional PIV system. As a result, with this system it is possible to achieve not only extremely high spatial resolution but also high temporal resolution. In this way by combining the confocal micro-PIV system with the simulation results from the MPS method we expect not only to validate the computational model but also to complement the numerical investigations in such complex flows.

The aim of the present study is to evaluate the performance of our confocal micro-PIV system in order to validate the numerical simulations performed by the MPS method. Our micro-PIV system is a combination of a SDCM with a conventional PIV technique where due to a new scanning unit (CSU22) enables us to acquire confocal images up to 2000 frames per second.

2 MATERIALS AND METHODS

2.1 Spinning disk confocal microscope

In a conventional microscope the entire depth or volume of the sample is continuously illuminated which leads to the detection of out-of-focus emitted light as well as light from the focal plane of interest. However, with a confocal microscope the sample is scanned with one or more very small spots that illuminate only the plane of focus at one time. In this way, due to the presence of suitable positioned confocal pinholes which act as a spatial filter, out-of-focus emitted light is removed and as a result improves the lateral and axial spatial resolution. This spatial filter is the key to create optical sectioning planes along the depth of the microchannel

[12-15].

The spinning disk confocal microscope employs a spinning disk or Nipkow disk with several thousands of pinholes usually arranged in a helical pattern. Because the disk spins very rapidly (1800 rpm to 5000 rpm) it is possible to capture images in real time which can be directly viewed by eyes or captured by high speed camera. As shown in Figure 1, the scanning method in CSU units developed by Yokogawa Electric Corporation consists of two disks connected together axially, where the upper disk has 20,000 micro-lenses and lower one known as Nipkow disk has also 20,000 matching pinholes with 50 μm in diameter. The excitation light that passes firstly through the micro-lenses is focused onto its corresponding pinhole and then progress down to illuminate the focal plane. Emitted fluorescent light from the focal plane is captured by the objective lens and focused back by the same path onto the Nipkow disk containing the pinhole array. Those pinholes remove the out-of-focus light and by means of a dichromatic mirror, located between the two disks, the treated emitted light from the focal plane is reflected onto a high speed camera to build up the image. As the disks spin very rapidly, thousands of spots of light are scanned simultaneously across the focal plane within a very short time [12-14].

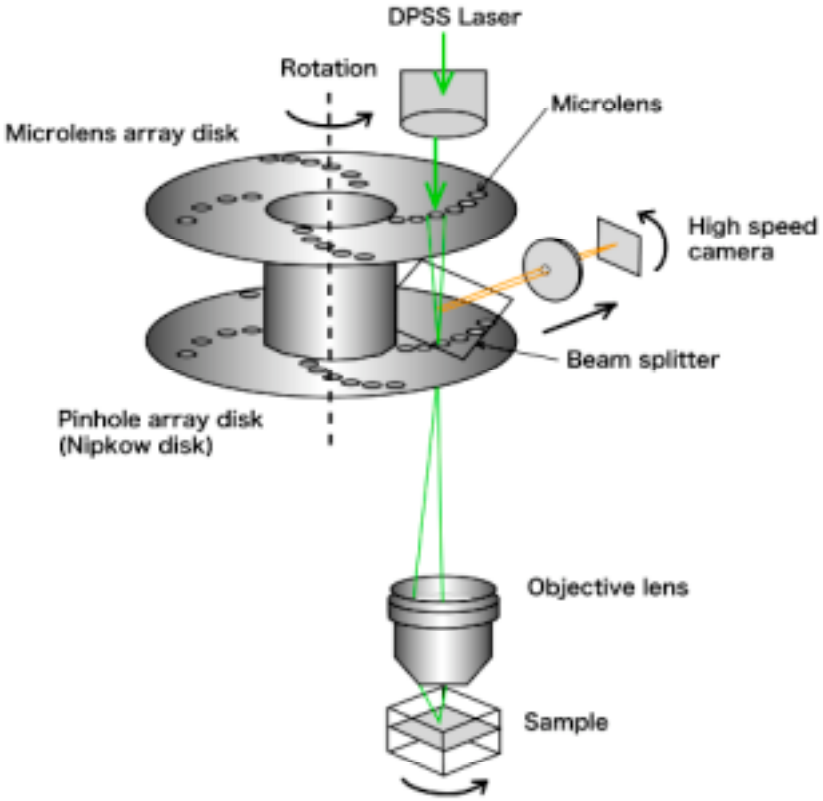

Figure 1: Principle of the spinning disk confocal microscope.

2.2 Rectangular flow microchannels

In microfluidics is common practice to use a steady flow through a long straight channel with constant cross section in order to evaluate the performance of a new measurement flow technique. It is well known that the velocity profile at this kind of channels corresponds to the behaviour predicted by the Poiseuille flow [16]. As shown in Figure 2, the rectangular microchannel consist of: (1) an upper plate of machined and polished acrylic on which the inlet and outlet were drilled, (2) two slide glasses between the upper and lower plates and (3) a glass lower plate. Two rectangular microchannels were used in our experiments. The first microchannel with 55mm long, 140μm deep and 520μm wide was used to evaluate the performance of the confocal microPIV system. The second microchannel with 55mm long, 130μm deep and 120μm wide was used to compare the flow behaviour of two different physiological fluids.

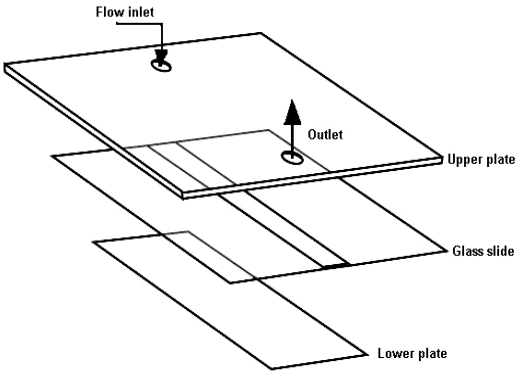

Figure 2: Diagram showing the main components of the rectangular microchannel.

2.3 Working fluids

It is general practice to use a well known Newtonian fluid, such as pure water, to evaluate the performance of a new measurement technique. However, since our main interest is to evaluate the performance of the confocal micro-PIV system to measure physiological flow, we have decided to use physiological saline (PS) as our working reference fluid. This Newtonian fluid is extensively used by biomedical researchers to study several phenomena in hemodynamics. The flow measurements through the microchannel were possible due to the seeding of particles which follow the flow. Two working fluids were used in this study. The first was PS seeded with 1% (by volume) of 1μm diameter red fluorescent solid polymer microspheres (R0100, Duke Scientific). A second fluid was PS seeded with 6% (by volume) of rabbit blood and with 1.3% (by volume) of 1μm diameter red fluorescent solid polymer microspheres (R0100, Duke Scientific). It should be mentioned that the blood was collected from healthy rabbits and heparine was added to the blood in order to prevent coagulation of the red blood cells. The blood was hermetical stored at about 4ºC until the performance of the experiment at room temprature , 20ºC to 24 ºC.

2.4 Confocal micro-PIV system and experimental setup

The confocal micro-PIV system used in our experiment consists of an inverted microscope (IX71, Olympus, Japan) combined with a confocal scanning unit (CSU22, Yokogawa, Japan) and a diode-pumped solid state (DPSS) laser (Laser Quantum Ltd, England) with an excitation wavelength of 532 nm. Moreover, a high-speed camera (Phantom v7.1, U.S.A.) was connected into the outlet port of the CSU22 (see Figure 3). The microchannel was placed on the stage of the inverted microscope where the flow rate of the working fluid was kept constant at 1μl/min (520μm wide microchannel) and 0.32 μl/min (220μm wide microchannel) by means of a syringe pump (KD Scientific Inc. U.S.A.). The Reynolds number used in our experiment was about 0.03, based on the inlet flow velocity and width of the microchannel, whereas the entrance length (Le) was about 6.5μm, considering Le=1.3×(witdh/2) [1]. As the measurements were conducted in the middle of the microchannel we have assured that velocity profiles were fully developed at the recording place. The laser beam was illuminated from below the microscope stage through an air immersion 20× objective lens with a numerical aperture (NA) equal to 0.75. Satisfactory illumination was achieved by seeding fluorescent particles with 1μm diameter, which absorb green light (absorbance peak 542 nm) and emit red light (emission peak 612 nm). The light emitted from the fluorescent flowing particles pass through a color filter into the scanning unit CSU22, where by means of a dichromatic mirror are reflected onto a high speed camera to record the PIV images. Although the camera used in this study can record images at a rate of 2000 frames/s the intensity of the images was too dark to be processed by the PIV data analysis, mainly due to low exposure time of the particles. For this reason and because of the complex fluid (PS with 6% of rabbit blood) used in our study we have decided to capture images with a resolution of 640×480 pixels, 12-bit grayscale, at a rate of 200 frames/s with an exposure time of 4995 ms. After recording the images they were digitized and transferred to the computer in order to be evaluated using Phantom camera control software (PH607). The PIV images of the flowing particles were processed and the flow velocity was determined by using PivView (PivTec) [17]. The images were evaluated by a cross-correlation method, where time between two images was set to 20ms. By using a multiple-pass interrogation algorithm with 32 by 64 pixel interrogation window (step size 15×22 pixel) for the 520μm wide microchannel and 32 by 16 pixel interrogation window (step size 7×9 pixel) for the 220μm wide microchannel, it was possible to obtain the correspondent velocity vector fields.

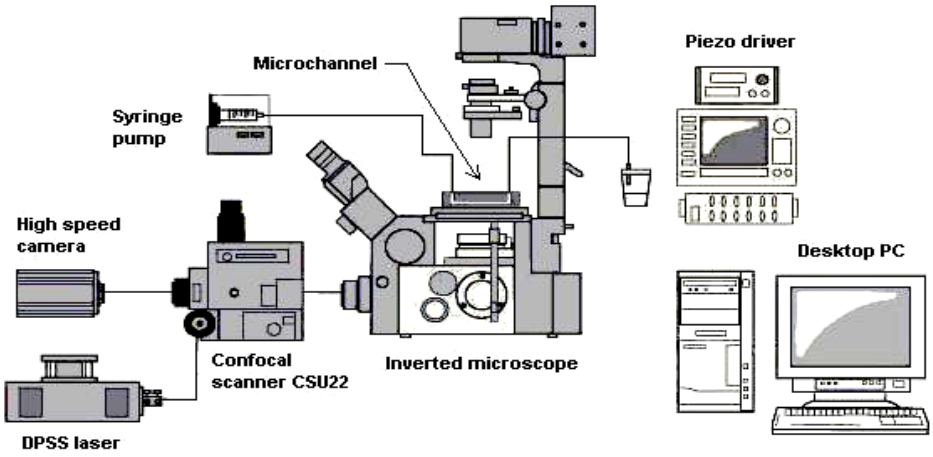

Figure 2: Experimental set-up of the confocal micro-PIV system.

2.5 Spatial resolution of the Confocal micro-PIV System

The spatial filtering done by the pinholes apertures makes optical sectioning possible. This optical sectioning capability enables to obtain a series of optical sections at different focal planes and as a result it is possible to obtain 3D information about the spatial structure of the specimen. It is generally agreed that quality and accuracy of this information depend on the optical section thickness and also on the distance between optical sections (step size). According to Wright [12], optical section thickness should be smaller than the step size [12, 18]. In this way, when using a confocal micro-PIV system it is crucial not only determine the lateral and axial image resolution but also the optical slice thickness.

Resolution can be defined as the ability to distinguish two point objects form each other [12]. In confocal microscopy the pinhole plays an important role in calculating its spatial resolution. Considering that modified pinhole diameter (MPD) can be defined as the pinhole diameter (PD in μm) divided by the magnification (M), and that the airy unit (AU) by the following equation[15, 18]

$$AU = \frac{1.22\lambda_{ex}}{NA} \tag{1}$$

where λ_{ex} is the wavelength of the illuminating laser light and NA is the numerical aperture of the microscopic objective lens, it is possible to determine the spatial resolution of our system by applying the following criterions [18]:

if MPD > 1.0 AU apply the geometric-optical analysis;
if MPD < 0.25 AU apply the wave-optical analysis.

The confocal microscope system used in our experiment follows the geometric-optical analysis, as the MPD = 2.5 μm is bigger than the AU value (AU = 0.865). In this way by considering the geometric-optical analysis it is possible to calculate spatial resolution of our system by applying the following equations [18]

$$R_l = \frac{0.51\lambda_{ex}}{NA} \tag{2}$$

$$Ra = \frac{0.88\lambda_{ex}}{n - \sqrt{n^2 - NA^2}} \tag{3}$$

$$OST = \sqrt{\left(\frac{0.88\lambda_{em}}{n - \sqrt{n^2 - NA^2}}\right)^2 + \left(\frac{\sqrt{2}n \cdot MPD}{NA}\right)^2} \tag{4}$$

where R_l is the lateral resolution, R_a is the axial resolution, OST is the optical slice thickness, λ_{em} is the emission wavelength and n the refractive index of immersion liquid. In the present system, λ_{ex} = 532 nm, NA = 0.75, n = 1, λ_{em} = 612 nm and MPD = 2.5 μm. On substituting the above numbers we get R_l = 0.36 μm, R_a = 1.4 μm and OST = 4.97μm. From these estimated results it is possible to verify that our system can achieve very high spatial resolution.

3. RESULTS AND DISCUSSION

3.1 Performance of the confocal micro-PIV

The confocal micro-PIV system was first evaluated by comparing the experimental results with a well established analytical solution for steady flow in a rectangular microchannel [16]. For this particular case the measurements were performed in rectangular microchannel 55mm long, 140μm deep and 520μm wide. Due to the long width of the microchannel, the field view of our system was not large enough to cover the entire width, therefore two different frame locations (L1 and L2 in the Figure 4) had to be taken and then joined together in order to obtain the complete velocity profile along the entire width of the microchannel. Figure 4 shows the velocity field measurements at the locations L1 and L2 and correspondent velocity vector field for the entire width. Note that for this microchannel the flow rate was 1 μl/min.

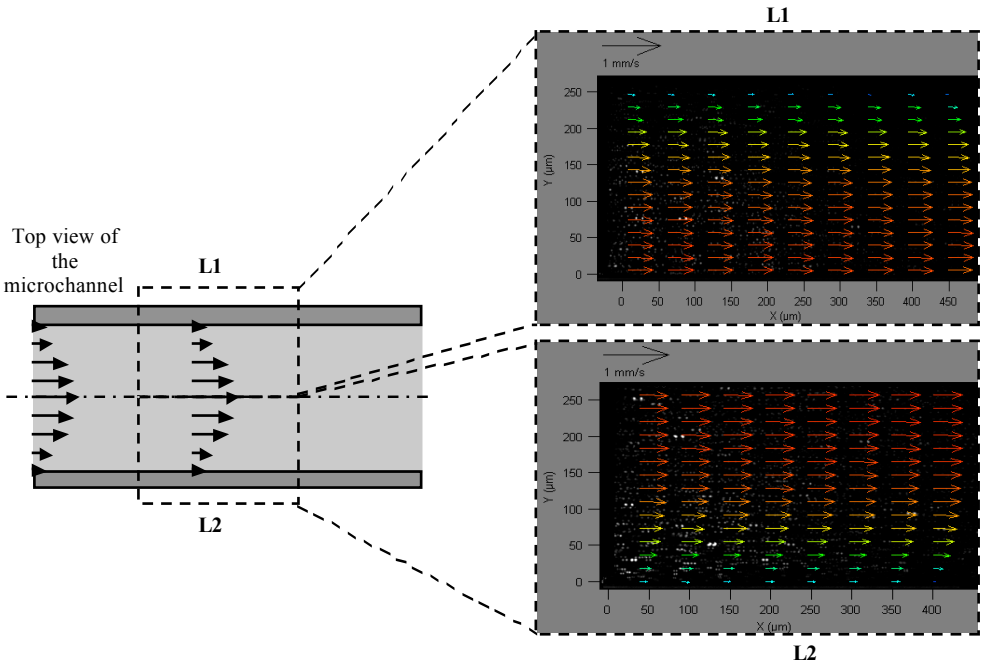

Figure 4: Velocity field measurements at the locations L1 and L2 of the 520μm microchannel.

The determination of experimental results in the center plane (z = hz/2) of the microchannel was performed by using the RT3D system which has sub-micron z direction resolution. It should be noted that, the origin of the z axis (lower plate) was set where the fluorescent particles appeared to be almost static. The average fluid velocities of 12 PIV image pairs are shown in Figure 5. These PIV measurements were obtained with an exposure time of 4995 ms, magnification factor (pixel/μm) 0.865 and time delay of 20ms between two images. According to the results, the averaged velocity data obtained from the confocal micro PIV measurements and the analytical solution for Newtonian flow in a rectangular microchannel[16] show close agreement with errors less than 5%. In addition, Figures 5 and 6 also confirms that a parabolic Poiseuille flow develops along the rectangular microchannel. It should be noted that the marginal deviations are mainly due to some "second-order-effects" such as surface roughness of the wall and some minimal leakage between the slide glasses.

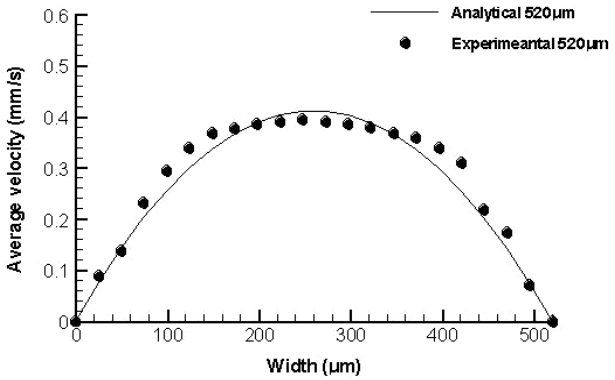

Figure 5: Comparison between experimental and analytical data at the center plane (70μm height).

3.2 Comparison of velocity profiles from different physiological flows

To evaluate the potentialities of our confocal micro-PIV system to investigate phenomena at micro-scale level, we have measured velocity profiles of different physiological flows within a 120μm wide rectangular microchannel and with a flow rate of 0.32μl/min. Figure 5 shows some preliminary results of the velocity profiles of physiological saline and physiological saline with 6% of rabbit blood, at two different xy planes spaced 40μm in depth (z direction).

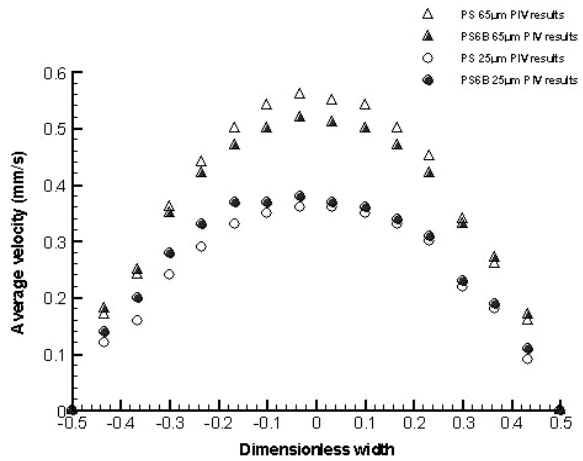

Figure 6: Velocity vector fields at two different z positions of physiological saline (PS) and physiological saline with 6% of rabbit blood (PS6B), as a function of the dimensionless width of the microchannel.

From figure 6 it is possible to observe some deviations between the velocity profiles of the two physiological fluids. These results suggests that our confocal micro-PIV system is capable to investigate several micro-scale phenomena, such as the effect caused by RBC rheological properties in the velocity profiles of the flowing blood. An ongoing work to investigate this phenomenon is under progress.

4. COMPARISON BETWEEN EXPERIMENTAL AND COMPUTATIONAL RESULTS

The MPS particle method is a deterministic method that uses a fully Lagrangian technique to simulate incompressible viscous flow. The method uses a modified kernel function in the particle interaction models and a semi-implicit algorithm is employed for the incompressible model. The algorithm consists in a penalty-like formulation to adjust the pressure whenever density variations occur [9].

Recently the MPS method was applied to study the motion and deformation of red blood cells (RBC) in blood flow. The simulation results have revealed changes in RBC shape and position in flowing blood plasma and as a result it was demonstrated that this proposed particle method is potentially an important and useful approach to investigate the mechanical behaviour of the blood cells under blood flow at a microscopic level [7, 8, 19]. Moreover, the preliminary results from the confocal micro-PIV system also suggest that this experimental technique has the ability to study phenomena of blood flow in the microcirculation. Due to the complex phenomenon of coupling of blood cells motion and blood plasma flow, we believe that by combining both experimental and computational approaches we can obtain at the end a reliable computational model to investigate this phenomenon in the context of multi-scale and multi-physics hemodynamics. In this way, an ongoing work to analyse this phenomenon is under investigation by combining both experimental and computational approaches.

5. CONCLUSIONS

The present study corresponds to a working progress towards improving our understanding of the complicated phenomena of blood flow in microcirculation. An excellent approach to study such phenomena is by combining both computational and experimental methodologies. This paper describes and evaluates a new micro-PIV system capable to measure and quantitatively evaluate fluid mechanical effects at a microscopic level with high spatial and temporal resolution. The confocal micro PIV measurements show that a parabolic velocity profile develops along the rectangular microchannel and that there is a close agreement between the experimental and analytical results. The fact that the micro-PIV results are in satisfactory agreement with the predicted values indicates that the confocal micro-PIV system can be used to evaluate the simulation results from the MPS method. In this way, an ongoing work to analyse the effect caused by RBC rheological properties in the velocity profiles is under investigation by combining both micro-PIV and particle method results. In the near future we expect that this combination will help to understand several physiological phenomena at microscopic and mesoscopic level.

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