Single and two-Phase Flows on Chemical and Biomedical Engineering, Bentham Science, 600-612, 2012.

CHAPTER 23

MICRO-FLOW VISUALIZATION OF MAGNETIC NANOPARTICLES FOR BIOMEDICAL APPLICATIONS

R. Lima[1, 2, *], R. J. Joseyphus [3, ‡], T. Ishikawa[4], Y. Imai[4], and T. Yamaguchi[5]

1. Department of Mechanical Technology, ESTiG, Braganca Polytechnic, C. Sta. Apolonia, 5301-857 Bragança, Portugal
2. CEFT, Engineering Faculty, Porto University, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
3. Department of Physics, National Institute of Technology, Tiruchirappalli 620 015, India. ‡E-mail: rjustinj(at)nitt.edu
4. Department of Bioengineering & Robotics, Grad. Sch. Eng., Tohoku University, 6-6-01 Aoba, 980-8579 Sendai, Japan
5. Department of Biomedical Eng., Grad. Biomedical Sch. Eng., Tohoku University, 6-6-01 Aoba, 980-8579 Sendai, Japan

Abstract: An investigation to measure the flow behavior of magnetic nanoparticles through a 100μm microchannel is conducted. The magnetic field is applied externally by a permanent magnet and by using a micro-PTV system it was possible to measure the flow behavior of magnetic nanoparticles at different flow rates and magnetic fields through a 100μm glass capillary. A strong dependence on both magnetic and hydrodynamic force is observed on the nanoparticles fluidic paths. Based on these in vitro studies, important parameters and issues that require further understanding and investigation are point out.

Keywords: Micro/nano flow, magnetic nanoparticles, superparamagnetism, Fe-oxide layer, hyperthermia, microchannel, micro-PIV/PTV.

INTRODUCTION

Magnetic nanoparticles are used in a variety of biomedical applications such as hyperthermia, drug delivery, bioseparation and MRI contrast agents [1-3]. Drug delivery using magnetic nanoparticles is a more promising application since it is a noninvasive technique where high efficiency could be achieved. The advantage using magnetic nanoparticles in drug delivery is that it could be used for localized drug delivery whereas the conventional drug delivery methods would result in drug associated side effects. The magnetic nanoparticles for biomedical applications are usually coated with a biocompatible surfactant layer which prolongs the circulation time of the nanoparticles in the blood. The size of the magnetic nanoparticles should be less than 100 nm to prevent its capture by mononuclear phagocyte system [4]. Though magnetic nanoparticles of sizes below 50 nm could be easily synthesized and dispersed, the polymeric coating would render the particle size to more than 50 nm [5]. Hence magnetic nanoparticles of smaller sizes are preferred. When the size of the magnetic nanoparticles becomes smaller, thermal relaxation would be dominant and result in superparamagnetism. Superparamagnetism is a phenomenon arising due to the dominance of thermal energy over anisotropy energy (kT>KV). The thermal energy and anisotropy energy are given by kT and KV respectively, where k is the Boltzmann constant, T is the temperature, K is the magnetocrystalline anisotropy constant and V is the volume of the particle. The superparamagnetic particles would exhibit close to zero coercivity and hence could be controlled easily by external magnetic field. However the superparamagnetic

Correspondence Author:** Rui Lima, Department of Mechanical Technology, ESTiG, Braganca Polytechnic, C. Sta. Apolonia, 5301-857 Bragança, Portugal; E-mail: ***ruimec@ipb.pt

nanoparticles are hard to saturate and hence it requires sufficiently larger field. Thus it would be advantageous to use magnetic materials which show larger saturation magnetization even at low magnetic field. Fig.**1 (a)** shows the typical hysteresis loop at room temperature for magnetic materials with high K and **(b)** shows the hysteresis loop for a superparamagnetic material. The difference in magnetic parameters such as coercivity and saturation magnetization is obvious from the fig. **1**.

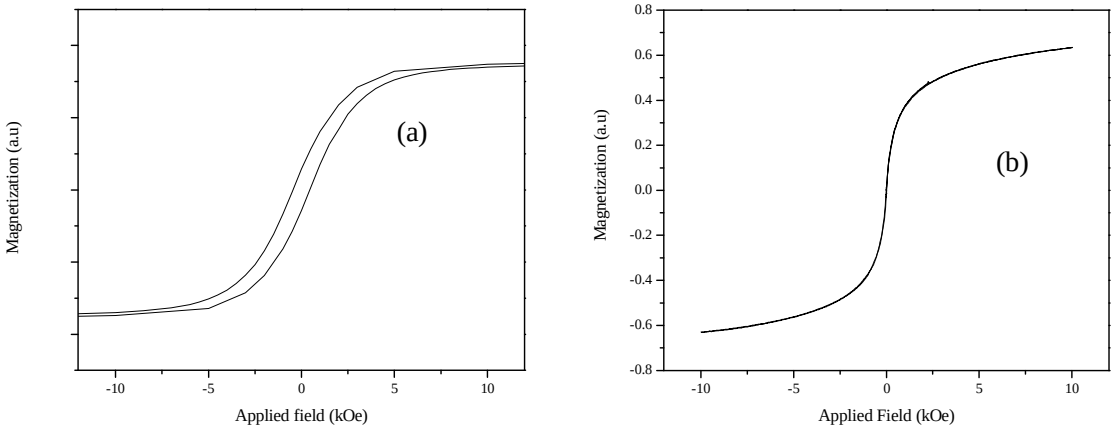

Figure 1: (a) Typical hysteresis loop at room temperature for a high K magnetic material and **(b)** hysteresis loop for a superaparamagnetic material. The superaparamagnetic material shows negligible coercivity.

Although smaller magnetic particles are preferable for biomedical applications, for applications such as drug delivery, the particles should be able to respond to an external magnetic field even at a larger separation. This requires that the magnetic nanoparticles should exhibit very large saturation magnetization. FeCo and Fe are the magnetic materials with very large saturation magnetization of 240 and 218 emu/g respectively [6]. Although FeCo has higher saturation magnetization, the biocompatibility and surface modification are difficult to achieve. On the other hand Fe particles of very high magnetization could be easily synthesized through chemical methods [7]. The biocompatibility could be achieved by a Fe-oxide layer over the Fe particles [8] as the biocompatibility of Fe-oxides is well known. However when the particle size becomes smaller, the Fe particles naturally undergoes oxidation and the large surface area of smaller particles with Fe-oxide layer would reduce the saturation magnetization. This is illustrated in Fig.**2** where larger Fe particles would show higher magnetization.

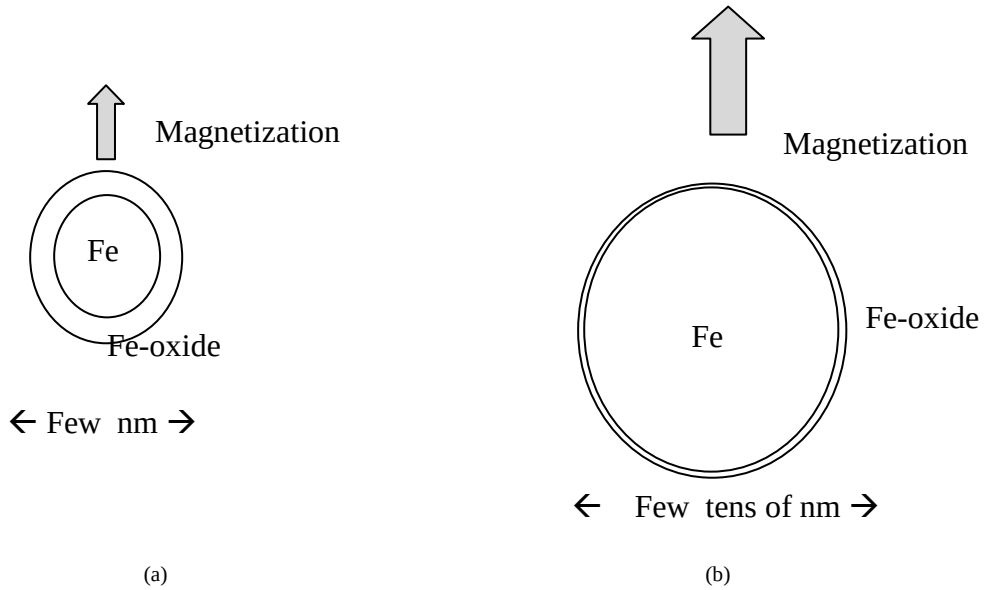

Figure 2: (a) Fe nanoparticles of smaller size have lower magnetization due to the larger fraction of Fe-oxide layer and **(b)** larger Fe particles show higher magnetization due to negligible Fe-oxide layer.

The Fe particles of various sizes could be synthesized using techniques such as inert gas condensation and chemical methods such as borohydride reduction, thermal decomposition and polyol process [9, 10]. The chemical methods such as polyol process is highly advantageous since the Fe particles could be free from contamination and surface modification is also possible. The surface modified nanoparticles could be then dispersed in a biocompatible fluid and injected into the blood stream and targeted to specific sites using an external magnetic field [11]. The drug delivery efficiency for the magnetic nanoparticles to reach specific target is influenced by various factors such as diameter of the circulatory path, force due to blood flow and the magnetic field. Magnetic particles of larger size could be delivered through the venule and arteriole whose radius ranges from 8 um to 0.07 mm whereas particle size should be less than 100 nm to pass through capilaries [12]. Generally magnetic nanoparticles synthesized will have a distribution in sizes and thus the magnetization and flow characteristics in a fluid will vary with size. For a single particle, the forces acting on the particle in a fluid can be obtained from Newton's law

$$m_p \frac{dv_p}{dt} = F_m + F_f + F_g \tag{1}$$

where m_p and v_p are the mass and velocity of the particle. F_m is the magnetic force given by $F_m = (\mu_p.\nabla)\mu_f H$ where μ_p is the effective magnetic dipole moment, μ_f is the permeability of the fluid and H is the external magnetic field. The drag force on the particle, F_f is given by $F_f = -6\pi\eta R_p(v_p - v_f)$ where η is the viscosity of the fluid, R_p and v_p are the radius and velocity of the particle and v_f is the velocity of the fluid. F_g is the sedimentation force due to density difference between the particles and fluid. F_g is given by $F_g = \Delta\rho Vg$, where $\Delta\rho$ is the density difference, V is the volume of a particle, and g is the gravitational acceleration.

Most research in microcirculation has involved experimental studies using optical techniques mainly because they are less invasive for measuring the flow field. However, the fact that measurements of blood flow in microcirculation need to be performed at very small scales, have limited the number of flow measurements techniques suitable for this purpose. For instance, MRI and ultrasonography are not suitable to obtain quantitative flow information in microvessels mainly due to their poor spatial resolution. The majority of the works performed in the past to measure velocity profiles of blood flow *in vivo* and *in vitro* are the double-slit photometry, video microscopy and image analysis, and laser-Doppler anemometry. Recently, the considerable progress in computers, optics and digital image processing techniques made possible to successfully combine the conventional PIV system with an inverted microscope [13]. This combination, known as micro-PIV, has greatly increased the resolution of conventional PIV; as a result, this technique was been recently used to investigate biological flow behavior in microchannels [14-19]. However, to our knowledge micro-PIV/PTV measurements of physiological fluids containing magnetic nanoparticles was not performed in glass capillaries. The present study aims to measure and evaluate the flow behavior of magnetic nanoparticles at different flow rates and magnetic fields through a 100μm glass capillary by means of a micro-PTV system.

MATERIALS AND METHODS

Working fluids, microchannel and magnetic field

The working fluid used in this study was Dextran 40 (Dx40) seeded with magnetic nanoparticles with diameters of 25 nm (Fe25). The microchannel used in this study was a 100-μm circular borosilicate glass microchannel fabricated by Vitrocom (Mountain Lakes, NJ, USA). The microchannel was mounted on a slide glass (~120 μm thick) where it was immersed in a fluid (glycerol) in order to minimize some possible refraction from the walls of the microchannel. (see Figure **3**).

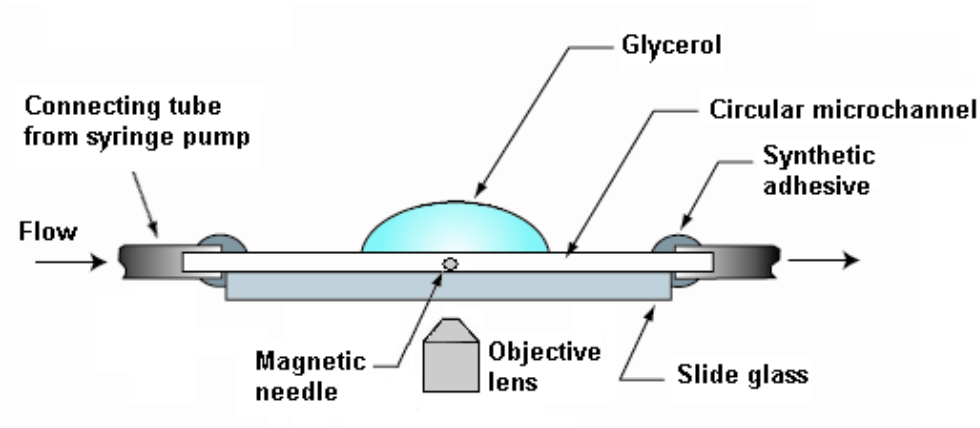

Figure 3: Diagram showing the components of the microchannel device.

Figure **4** shows a schematic illustrating the effect of static magnetic field on the magnetic nanoparticles. Briefly, a permanent magnet was attached into a needle and the strength of the magnetic field was varied by changing the position (L) of the magnet. By placing the needle around the middle plane of the microchannel we were able to create a magnetic field of 0.25mT and 0.4mT. The magnetic field was measured by using a Gauss meter (GM-301, Denshijiki).

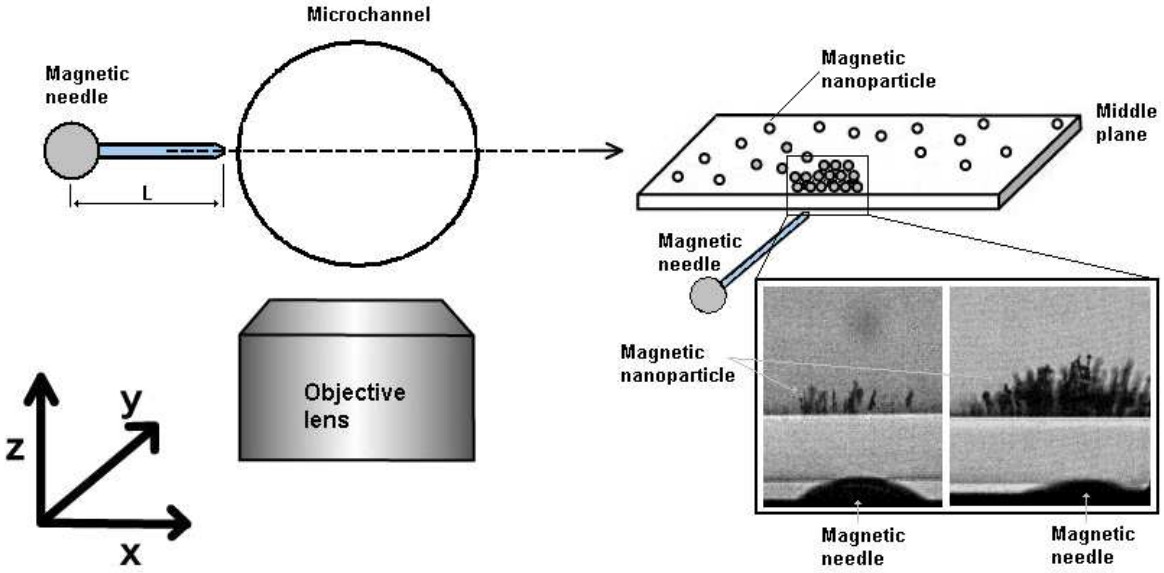

Figure 4: Diagram showing the effect of the magnetic field on the magnetic nanoparticles.

Experimental set-up

The microvisualization system used in this study is shown in Figure **5**. The system consists of an inverted microscope (IX71; Olympus, Japan) combined with a high-speed camera (Phantom v7.1; Vision Research, USA). The circular microchannel was placed on the stage of the inverted microscope and by using a syringe pump (KD Scientific, USA) a pressure-driven flow was kept constant at 0.25 μl/min which corresponds to a Reynolds of ~ 0.01. All the images were captured around the middle of the microchannels with an air immersion 60× objective lens with a numerical aperture (NA) equal to 0.9. The flow images were first recorded with a resolution of 640×480 pixels, at a rate of 200 frames/s (time interval of 5 ms) and then digitized and transferred to a computer to be evaluated by using a Phantom camera control software (PH607),

a Image J (NIH) [20] and by a manual tracking MTrackJ [21] plugin. As a result it was possible to track clusters of nanoparticles flowing within the microchannel.

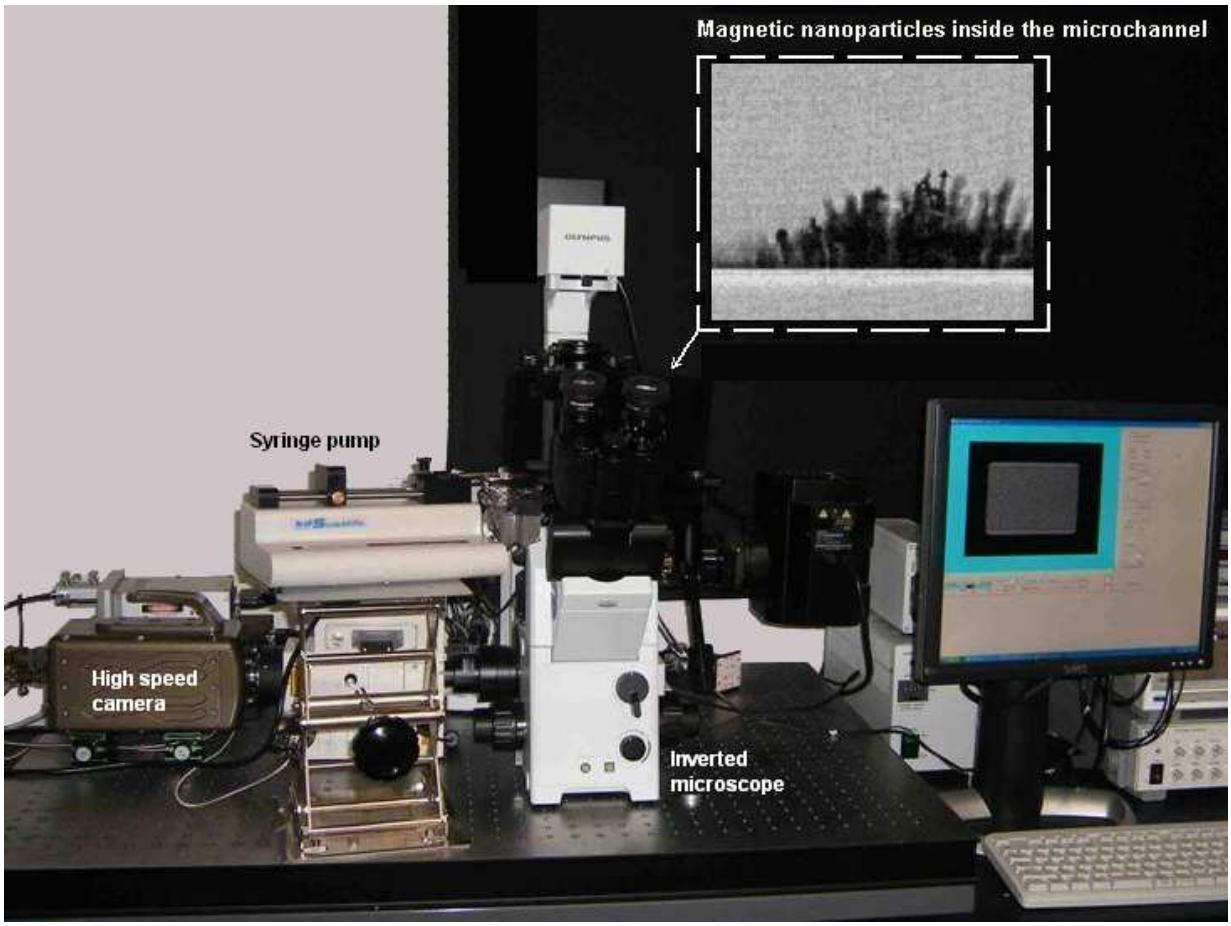

Figure 5: Experimental set-up to investigate the effect of an external magnetic field on the fluidic paths of the magnetic nanoparticles.

RESULTS AND DISCUSSION

Flow visualization

Figures **6**-**10** show the effect of a local electromagnetic force on the flow behavior of magnetic nanoparticles with a diameter of 25nm and a flow rate of 0.25µl/min and 35µl/min. Additionally by changing the position of the permanent magnet we were also able to vary the strength of an external magnetic field. As a result it was possible to investigate the migration behavior of magnetic nanoparticles in an applied magnetic field, i. e., 0.25mT and 0.4mT.

Magnetic field of 0.4mT

First we investigated the effect of a magnetic field of 0.4mT on the nanoparticles flow behavior through a 100 µm circular microchannel. Figure 6 shows clearly that for a flow rate of 0.25 µl/min the magnetic nanoparticles tend to attach to the wall side where the magnetic force is applied. It is also possible to observe that the concentration of the nanoparticles tend to increase as a function of time.

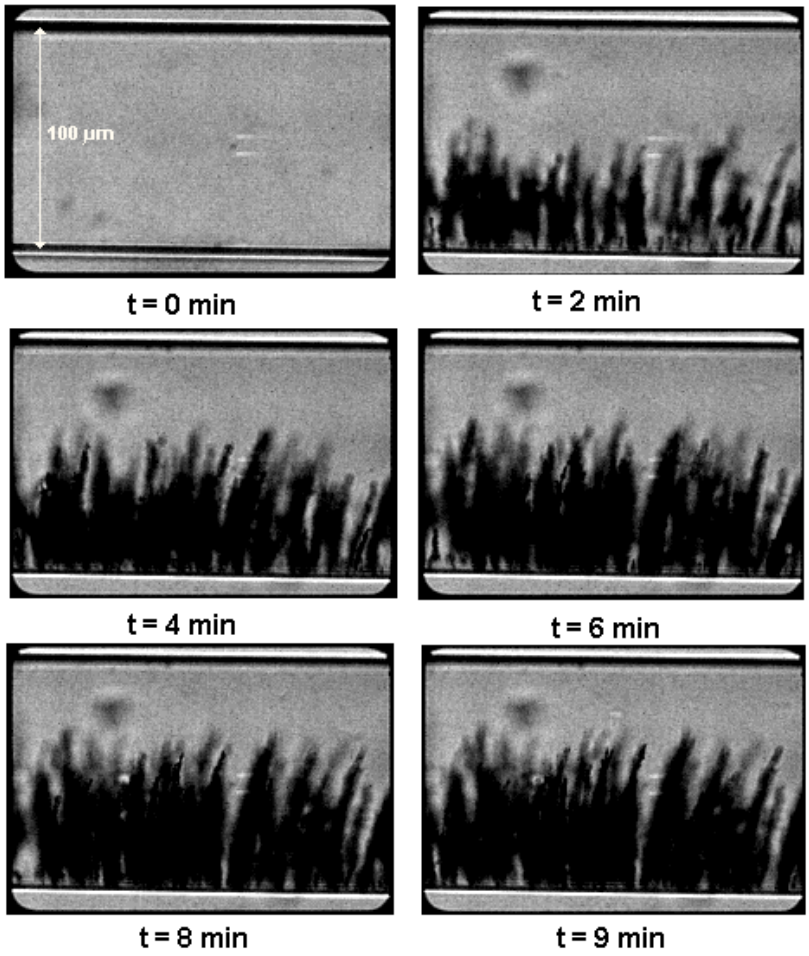

Figure 6: Effect of the local electromagnetic force on the nanoparticles flow behavior in a circular microchannel for a flow rate of 0.25 µl/min. Magnetic field of 0.4mT.

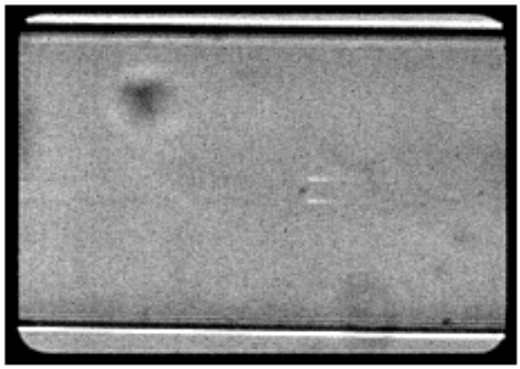

Figure 7: Effect of the local electromagnetic force on the nanoparticles flow behavior in a circular microchannel for a flow rate of 35 µl/min. Magnetic field of 0.4mT.

We also carried out qualitative measurements to investigate the effect of the flow rate for a magnetic field of 0.4mT. Figure **7** shows that for a flow rate of 35 µl/min there is no visible aggregation of nanoparticles on wall of the microchannel. It means that the flow rate strongly influence the binding capacity of the magnetic nanoparticles to attach on the microchannel surface.

Magnetic field of 0.25mT

We also investigated the effect of a weaker magnetic field of 0.25mT on the nanoparticles flow for a flow rate of 0.25 μl/min. The results from Figure 8 show smaller amount of magnetic nanoparticles when compared with Figure **6**. This phenomenon is due to the decrease of external magnetic field.

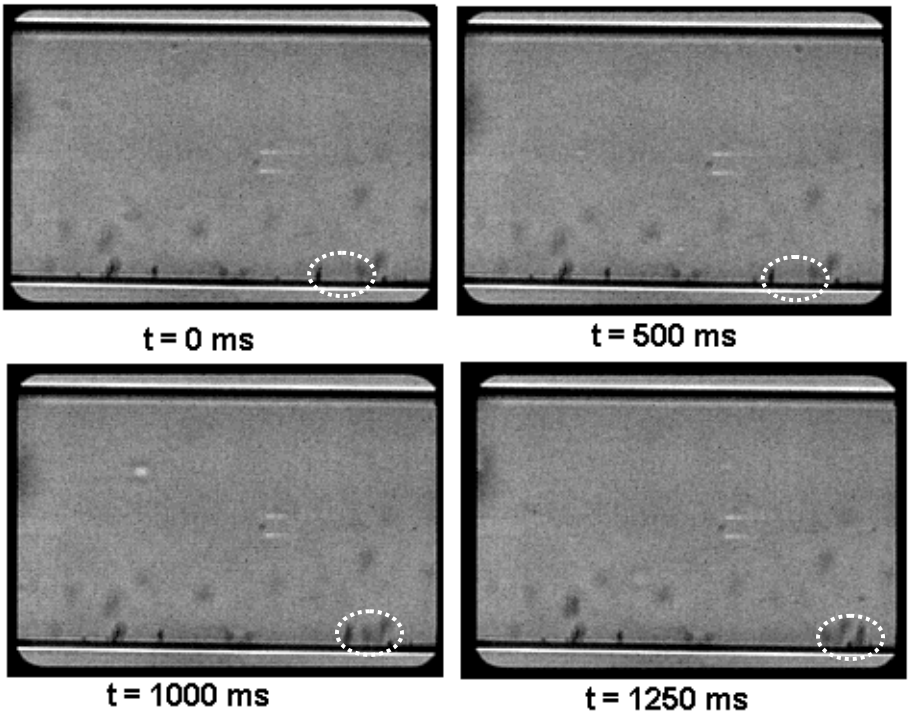

Figure 8: Effect of the local electromagnetic force on the nanoparticles flow behavior in a circular microchannel for a flow rate of 0.25 μl/min. Magnetic field of 0.25mT.

Tracking clusters

By using an high speed camera the flow images were recorded at a rate of 200 frames/s (time interval of 5 ms) and then by using a manual tracking MTrackJ plugin from Image J (NIH) it was possible to track clusters of nanoparticles flowing within the microchannel, as shown in Figures **9** and **10**.

Magnetic field of 0.25mT and flow rate of 0.25 μl/min

Figure **9** shows the trajectories of nanoparticles flowing in middle (Tk2) and near the wall of the microchannel (Tk1). For the case of the middle nanoparticles the applied magnetic field (0.25mT) seems not to interfere in its trajectory. A possible explanation is the higher hydrodynamic force acting on the nanoparticles when compared with the magnetic force generated by the external magnet. In contrast the nanoparticles flowing near the wall the trajectories tend to change their trajectories due the magnetic field strength. Figure **10** clearly shows the effect of the applied magnetic field on the instantaneous velocities on the cluster 1 (Tk1) and 2 (Tk2). The results from the cluster (Tk2) flowing around the middle of the microchannel indicates that the velocity tend be nearly constant. The deviations observed in cluster 2 may be mainly due to the tracking method used in the present study and not to the magnetic field. On the other hand, the cluster (Tk1) flowing near the wall suffer not only an abrupt change on its flow direction but also a significant decrease on its velocity, with values initially around 0.5 mm/s down to nearly 0 mm/s.

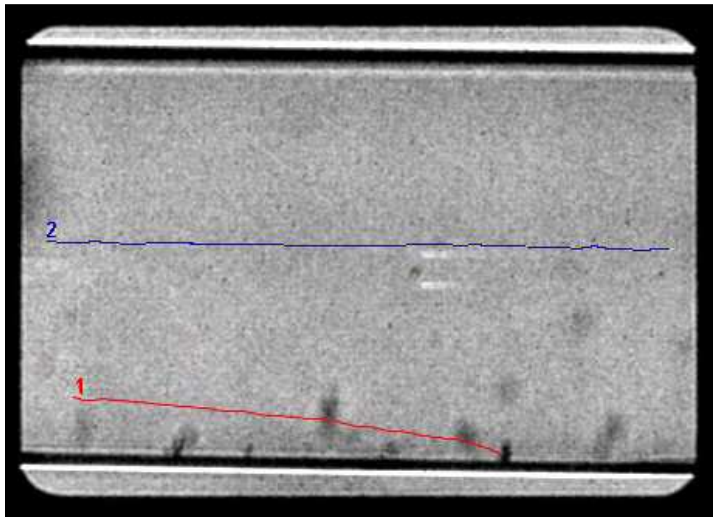

Figure 9: The effect of the local electromagnetic force on the displacement of cluster 1 (Tk 1) and 2 (Tk 2) flowing through a 100 µm glass capillary. Magnetic field of 0.25mT.

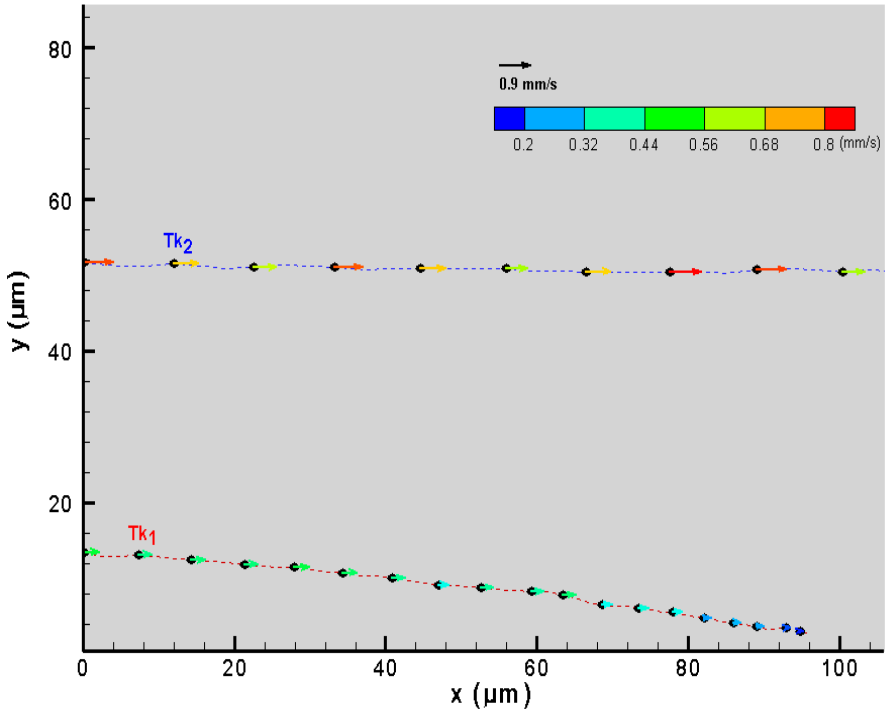

Figure 10: The effect of the local electromagnetic force on the instantaneous velocities of cluster 1 (Tk 1) and 2 (Tk 2) flowing through a 100 µm glass capillary. Magnetic field of 0.25mT.

CONCLUSIONS AND FUTURE DIRECTIONS

The present study corresponds to the first attempt to measure the flow behavior of magnetic nanoparticles through a 100µm microchannel by using a micro-PTV system. Although it was not possible to track individual nanoparticles, the results demonstrated the ability of our system to measure clusters of magnetic nanoparticles generated by an external magnetic field. The preliminary results show that the nanoparticles fluidic paths are strongly dependent not only by the magnetic field strength but also by the hydrodynamic force. For instance, for a magnetic field of 0.4mT and flow rate of 0.25 µl/min the nanoparticles tend to change their fluidic path and attach onto the wall whereas for the same magnetic field and higher flow rate of 35 µl/min the magnetic effects are negligible. Further studies are needed to clarify the effect of the static magnetic field on the flow behavior of magnetic nanoparticles.

In this study, the magnetic field was generated by using an external permanent magnet. In the future we intend to study the flow behavior of magnetic nanoparticles under the influence of an alternating (AC) magnetic field. By using an alternating field the magnetic nanoparticles can generate heat and can be used as novel treatment technique to destroy tumours.

ACKNOWLEDGEMENTS

This study was supported in part by the following grants: Grant-in-Aid for Science and Technology (PTDC/SAU-BEB/108728/2008, PTDC/SAU-BEB/105650/2008 and PTDC/EME-MFE/099109/2008) from the Science and Technology Foundation (FCT) and COMPETE, Portugal and Grant-in-Aid for Scientific Research (S) from the Japan Society for the Promotion of Science (JSPS; No.19100008). We also acknowledge the support from the 2007 Global COE Program "Global Nano-Biomedical Engineering Education and Research Network".

REFERENCES

[1] Pankhurst QA, Connolly J, Jones SK, Dobson J. Applications of magnetic nanoparticles in biomedicine. J Phys. D: Appl Phys 2003; 36: R167-81.
[2] Tartaj P, Morales MP, Verdaguer SV, Carreno TG, Serna CJ. The preparation of magnetic nanoparticles for applications in biomedicine. J Phys D: Appl Phys 2003; 36: R182-97.
[3] Berry CC, Curtis ASG. Functionalisation of magnetic nanoparticles for applications in biomedicine. J Phys D: Appl Phys 2003; 36: R198–206.
[4] Sachdeva MS. Drug targeting systems for cancer therapy. Expert Opin Invest Drugs 1998; 7: 1849–64.
[5] Gupta AK, Gupta M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials 2005; 26: 3995-4021.
[6] Cullity BD, Graham CD. Introduction to magnetic materials. Wiley, NJ, 2009.
[7] Joseyphus RJ, Matsumoto T, Sato Y, Jeyadevan B, Tohji K. Role of Polyol on the formation of Fe particles. J Magn Magn Mater 2007; 310: 2393.
[8] Peng S, Wang C, Xie J, Sun S. Synthesis and stabilization of monodisperse Fe nanoparticles. J Am Chem Soc. 2006; 128: 10676-7.
[9] Hyeon T. Chemical synthesis of magnetic nanoparticles. Chem Comm 2003; 927-34.
[10] Cushing BL, Kolesnichenko VL, O'Conoor CJ. Recent advances in the liquid-phase syntheses of inorganic nanoparticles. Chem Rev 2004; 104: 3893-946.
[11] Jeong U, Teng X, Wang Y, Yang H, Xia Y. Superparamagnetic colloids: controlled synthesis and niche applications. Adv Mater 2007; 19: 33-60.
[12] Arruebo M, Pacheco RF, Ibara MR, Santamaria J. Magnetic nanoparticles for drug delivery. Nanotoday 2007; 2: 22.
[13] Meinhart C, Wereley S, Santiago J. PIV measurements of a microchannel flow. Exp Fluids 1999; 27: 414-9.
[14] Lima R, Wada S, Tsubota K, Yamaguchi T. Confocal micro-PIV measurements of three dimensional profiles of cell suspension flow in a square microchannel. Meas Sci Technol 2006; 17: 797-808.
[15] Lima R. Analysis of the blood flow behavior through microchannels by a confocal micro-PIV/PTV system. PhD(Eng.), Bioengineering and Robotics Department, Tohoku University, Japan, 2007.
[16] Lima R, Ishikawa T, Imai Y, Takeda M, Wada S, Yamaguchi T. Radial dispersion of red blood cells in blood flowing through glass capillaries: role of heamatocrit and geometry. J Biomech 2008; 41: 2188-96.
[17] Lima R, Wada S, Tanaka S, *et al.* In vitro blood flow in a rectangular PDMS microchannel: experimental observations using a confocal micro-PIV system. Biomed Microdevices, 2008; 10, 2: 153-67.
[18] Fujiwara H, Ishikawa T, Lima R, *et al.* Red blood cell motions in a high hematocrit blood flowing through a stenosed micro-channel. J Biomech 2009; 42: 838-43.
[19] Lima R, Ishikawa T, Imai Y, Takeda M, Wada S Yamaguchi T. Measurement of individual red blood cell motions under high hematocrit conditions using a confocal micro-PTV system. Ann Biomed Eng 2009; 37, 8: 1546-59.
[20] Abramoff M, Magelhaes P, Ram S. Image processing with Image J. Biophotonics Int 2004; 11: 36–42.
[21] Meijering E, Smal I, Danuser G. Tracking in molecular bioimaging. IEEE Signal Proc Mag 2006; 23: 46– 5