Multiplexing slanted spiral microchannels for ultra-fast blood plasma separation

Mehdi Rafeie, Jun Zhang, Mohsen Asadnia, Weihua Li and Majid Ebrahimi Warkiani

Supplementary Information

Calculations of the Reynolds number and Dean number

The *Re* number of the spiral microchannel can be obtained as follows.

$$Re = \frac{\rho_{\text{diluted blood}}\, U_{max}\, D_h}{\mu_{\text{diluted blood}}} = \frac{U_{max}\, D_h}{\nu_{\text{diluted blood}}} \tag{S-1}$$

where $\rho_{\text{diluted blood}}$, $\nu_{\text{diluted blood}}$, and $\mu_{\text{diluted blood}}$ are the density, kinematic viscosity and dynamic viscosity of the diluted blood respectively. $U_{\max}$ is the maximum magnitude of the velocity profile, and D_h is the hydraulic diameter of the microchannel. The density of the solution (i.e. diluted blood) can be carried out by considering the following formula.

$$\rho_{\text{solution}} = \frac{m_{\text{solution}}}{V_{\text{solution}}} \tag{S-2}$$

Assume we need to acquire the density of 10x diluted blood.

$$\text{Dilution} = 10x\,, \quad V_{\text{blood}} = \frac{1}{10} V_{\text{solution}}\,, \quad V_{\text{PBS}} = \frac{9}{10} V_{\text{solution}} \tag{S-3}$$

The mass of the solution can then be written as

$$\begin{aligned} m_{\text{solution}} &= m_{\text{blood}} + m_{\text{PBS}} \\ &= (\rho v)_{\text{blood}} + (\rho v)_{\text{PBS}} \\ &= \rho_{\text{blood}} \times \frac{1}{10} v_{\text{solution}} + \rho_{\text{PBS}} \times \frac{9}{10} v_{\text{solution}} \end{aligned} \tag{S-4}$$

Therefore, regarding (S-2), the density of the solution can be obtained as

$$\begin{aligned} \rho_{\text{solution}} &= \frac{\left(1\rho_{\text{blood}} + 9\rho_{\text{PBS}}\right)\frac{v_{\text{solution}}}{10}}{v_{\text{solution}}} \\ &= \frac{1\rho_{\text{blood}} + 9\rho_{\text{PBS}}}{10} \end{aligned} \tag{S-5}$$

The above formula can be rewritten in a general form for diluted blood of λx.

$$\rho_{\text{solution}} = \frac{\rho_{\text{blood}} + (\lambda - 1)\rho_{\text{PBS}}}{\lambda} \tag{S-6}$$

The density of whole blood and phosphate-buffered saline (PBS) are ρ_{blood} = 1060 kg/m^3 and ρ_{PBS} = 1000 kg/m^3 respectively. To evaluate the kinematic viscosity of the diluted blood, we first need to obtain the kinematic viscosity of whole blood and PBS separately.

$$\nu_{\text{blood}} = \frac{\mu_{\text{blood}}}{\rho_{\text{blood}}} = \frac{3\sim4\ centipoise}{1060\frac{kg}{m^3}} = \frac{0.003\sim0.004\ Pa.s}{1060\frac{kg}{m^3}} = 2.830\sim3.774 \times 10^{-6}\frac{m^2}{s} \tag{S-7}$$

$$= 2.830\sim3.774\ cSt$$

$$\nu_{\text{PBS}} = \frac{\mu_{\text{PBS}}}{\rho_{\text{PBS}}} = \frac{0.894\ centipoise}{1000\frac{kg}{m^3}} = \frac{0.000894\ Pa.s}{1000\frac{kg}{m^3}} = 0.894 \times 10^{-6}\frac{m^2}{s} \tag{S-8}$$

$$= 0.894\ cSt$$

The viscosity of a mixture of two or more liquids can be estimated using the Refutas equation [1].

$$\nu = \exp\left(\exp\left(\frac{VBN_{\text{blend}} - 10.975}{14.534}\right)\right) - 0.8 \tag{S-9}$$

where VBN_{blend} is the viscosity blending number of the solution and is obtained by the following equation.

$$VBN_{\text{blend}} = \sum_i^n x_i\, VBN_i \tag{s-10}$$

in which x_i is the mass fraction of each component of the solution, and VBN_i is the viscosity blending number of each fluid blended in the solution defined as below.

$$VBN = 14.534 \ln[\ln(\nu + 0.8)] + 10.975 \tag{S-11}$$

where ν is the kinematic viscosity in centistokes (cSt). The viscosity blending number of whole blood and PBS are calculated as follow.

$$VBN_{\text{blood}} = 14.534 \ln\left[\ln\left(\nu_{\text{blood}} + 0.8\right)\right] + 10.975 = 14.667\sim17.064 \tag{S-12}$$

$$VBN_{\text{PBS}} = 14.534 \ln\left[\ln\left(\nu_{\text{PBS}} + 0.8\right)\right] + 10.975 = 1.668 \tag{S-13}$$

In the general form, the mass fraction of blood and PBS are

$$x_{\text{blood}} = \frac{m_{\text{blood}}}{m_{\text{solution}}} = \frac{(\rho v)_{\text{blood}}}{(\rho v)_{\text{solution}}}$$
$$= \frac{\rho_{\text{blood}} \times \frac{1}{\lambda} v_{\text{solution}}}{\rho_{\text{solution}}\, v_{\text{solution}}} = \frac{1}{\lambda}\left(\frac{\rho_{\text{blood}}}{\rho_{\text{solution}}}\right) \quad \text{(S-14)}$$

$$x_{\text{PBS}} = \frac{m_{\text{PBS}}}{m_{\text{solution}}} = \frac{(\rho v)_{\text{PBS}}}{(\rho v)_{\text{solution}}}$$
$$= \frac{\rho_{\text{PBS}} \times \frac{\lambda - 1}{\lambda} v_{\text{solution}}}{\rho_{\text{solution}}\, v_{\text{solution}}} = \frac{\lambda - 1}{\lambda}\left(\frac{\rho_{\text{PBS}}}{\rho_{\text{solution}}}\right) \quad \text{(S-15)}$$

Thus, the VBN_{blend} is acquired as below.

$$VBN_{\text{blend}} = x_{\text{blood}}\, VBN_{\text{blood}} + x_{\text{PBS}}\, VBN_{\text{PBS}}$$
$$= \frac{14.667 \sim 17.064}{\lambda}\left(\frac{\rho_{blood}}{\rho_{solution}}\right) + 1.668\frac{\lambda - 1}{\lambda}\left(\frac{\rho_{PBS}}{\rho_{solution}}\right) \quad \text{(S-16)}$$

Now, the viscosity of diluted blood samples can be obtained using equation (S-9). The results of calculating the density (ρ) and the kinematic viscosity (v) of the diluted samples used in our experiments are provided in the following table.

Table S1 The density and kinematic viscosity of the blood samples

Dilution (x)	**HCT (%)**	**Density (kg/m^3)**	**Viscosity (cSt)**
90	0.5	1000.67	0.9035
45	1.0	1001.33	0.9131
22.5	2.0	1002.67	0.9328
9	5.0	1006.67	0.9954

The width (w) and heights (h_1 & h_2) of the trapezoidal cross-section of the microchannel are 500, 70, and 40 μm respectively. Therefore, the hydraulic diameter (D_h) of the channel can be obtained as follows.

$$D_h = \frac{4A}{P} = \frac{4\left(\frac{(h_1 + h_2)w}{2}\right)}{w + h_1 + h_2 + \sqrt{(h_2 - h_1)^2 + w^2}} = 99.02\ \mu m \quad \text{(S-17)}$$

where A and w are the area and perimeter of the cross-section of the channel respectively. Finally, the microchannel Re for different blood dilutions can be calculated as below.

$$Re = \frac{U_{max}\, D_h}{\nu_{\text{diluted blood}}} = \frac{\frac{Q}{A} D_h}{\nu_{\text{diluted blood}}} \quad \text{(S-18)}$$

The microchannel Reynolds numbers at the flow rate of 1.5 mL/min are shown in Table S2. In addition, the diagram of the *Re* at all the working flow rates is depicted in Fig. S1. As can be seen, the *Re* slightly decreases for blood samples with higher concentrations denoting that the larger viscosity, the smaller *Re* will be conducted in the channel.

Table S2 The *Re* for different blood dilutions at 1.5 mL/min

HCT (%)	Re
0.5	99.63
1.0	98.58
2.0	96.51
5.0	90.43

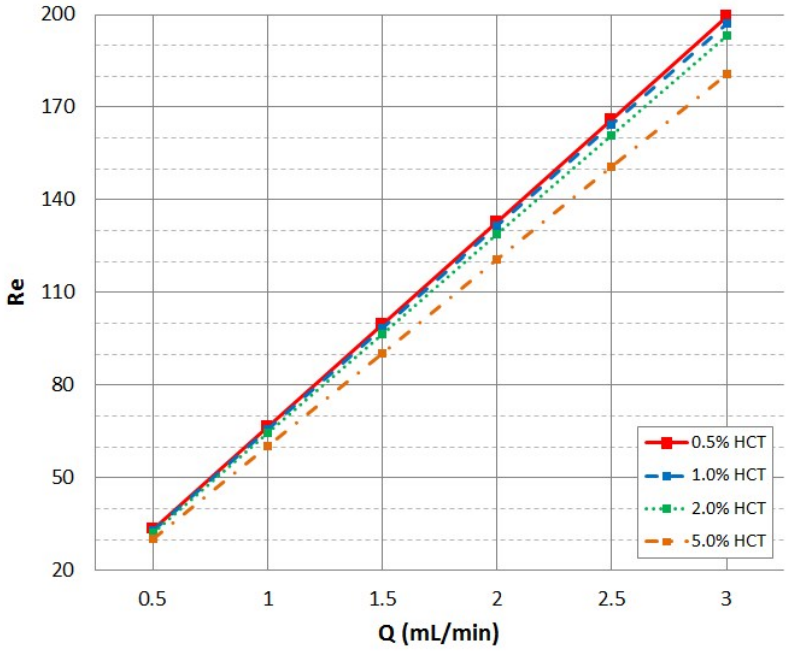

Fig. S1 The microchannel *Re* at all the working flow rates for the different blood dilutions

The Dean number *De* in different loops of the microchannel at a certain section can be calculated using the following formula.

$$De = Re\sqrt{\frac{D_h}{2R}} \tag{s-19}$$

where R is the radius of the channel whose value for all the loops of the microchannel, measured at the same section (Fig. S2), as well as the corresponding *De* are shown in Table S3.

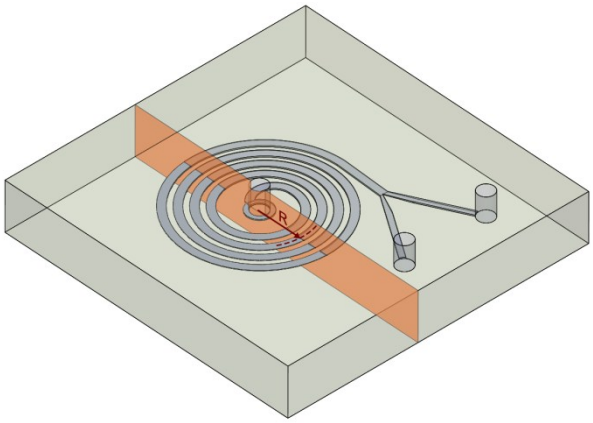

Fig. S2 Measuring the radius of loops based on their centre line

Table S3 The *De* in different loops at 1.5 mL/min for 1% HCT

Loop	R (mm)	De
1	2.75	13.23
2	3.75	11.33
3	4.75	10.06
4	5.75	9.15
5	6.75	8.44

The *De* of the spiral microchannel rises by increasing the flow rate and declines as the flow reaches the outlets. The chart illustrated in Fig. S3 represents the changes of *De* in different loops of the microchannels at all the working flow rates.

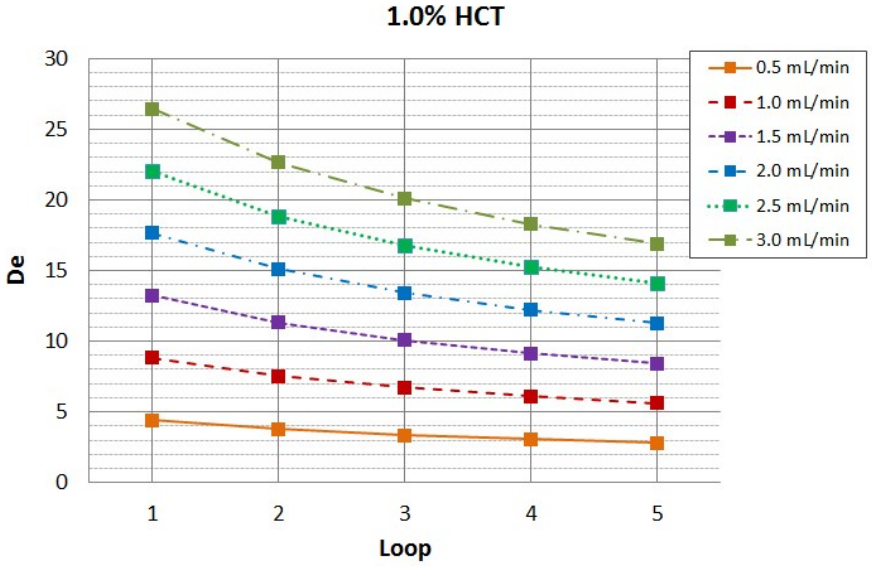

Fig. S3 The *De* in different loops of the microchannel for blood sample of 1% HCT

Performance of the multiplex microdevice

One mL of whole blood can be processed in less than one minute using our multiplexed system. Technically, more spirals can be assembled together to even increase this throughput (if needed). The maximum equivalent flow rate at which the plasma has ever been separated from whole blood is 140 µL/min [2], while the optimum whole-blood equivalent separation flow rate of the microdevice reported in this work varies from 33.3 µL/min (for a single spiral) to 533.33 µL/min (for multiplexed system) with the purity of ~100%.

Table S4 Flow rates of previously reported microfluidic systems for plasma separation

Separation technique		**HCT (%)**	**Dilution**	**Purity (%)**	**Flow rate (Diluted blood)**	**Equivalent Flow rate (Whole blood)**	**Ref.**
Passive extraction	Sedimentation	45	None	N/A	0.1 µL/min	0.1 µL/min	[3]
		9	1:5	99	15 µL/min	3 µL/min	[3]
		9	1:5	100	0.5 µL/min	0.1 µL/min	[3]
		45	None	100	0.83 µL/min	0.83 µL/min	[3]
	Microscale filtration	45	None	~100	0.02 µL/min	0.02 µL/min	[3]
		45	None	~100	50 µL/min	50 µL/min	[3]
		30	2:3	~100	10 µL/min	6.67 µL/min	[3]
	Cell deviation	11.25	1:4	N/A	4 µL/min	1 µL/min	[3]
		45	None	~100	0.16 µL/min	0.16 µL/min	[3]
		45	None	~99	33 µL/min	33 µL/min	[3]
		2.25	1:20	~99	100 µL/min	5 µL/min	[3]
		0.5	1:90	~100	8 mL/min	88.89 µL/min	[3]
	Inertial separation	0.5	1:90	>90	4 ml/min	44.44 µL/min	[4]
		2.25	1:20	~99.75	2.8 ml/min	140 µL/min	[2]
		2.25	1:20	~100	0.7 ml/min	35 µL/min	[5]
	Current work	**1**	**1:45**	**~100**	**24 mL/min**	**533.33 µL/min**	
Active extraction	Acoustic separation	40	8:9	~100	80 µL/min	71.1 µL/min	[3]
	Electrical separation	2.8	1:16	~100	N/A	N/A	[3]

We measured the volume fraction of the separated samples. At each flow rate, the test was repeated three times and an average of the results was taken. The back pressure of the outlets has been designed in a way to let the flow out the inner and outer outlets equivalently. As can be seen in table S5, the recovery ratio of the plasma in the multiplex system is around 50% for 1 cycle of separation which can be increased through recycling the concentrated sample.

Table S5 Volume fractions of separated blood cells and plasma

Flow rate (mL/min)	Volume fraction of blood cells (%)	Volume fraction of blood plasma (%)
0.5	56.5	43.5
1.0	57.2	42.8
1.5	55.6	44.4
2.0	55.7	44.3
2.5	53.8	46.2
Average:	**55.76**	**44.24**

Supplementary videos

Supplementary video 1: Blood plasma separation by the inertial microfluidic device with a single spiral microchannel with trapezoidal cross-section.

Supplementary video 2: Ultra-fast blood plasma separation by the parallelized inertial microfluidic device consisting of eight-coupled spiral channels.

References

[1] R. E. Maples, *Petroleum refinery process economics*: PennWell Books, 2000.

[2] J. Zhang, S. Yan, W. Li, G. Alici, and N.-T. Nguyen, "High throughput extraction of plasma using a secondary flow-aided inertial microfluidic device," *RSC Advances,* vol. 4, p. 33149, 2014.

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