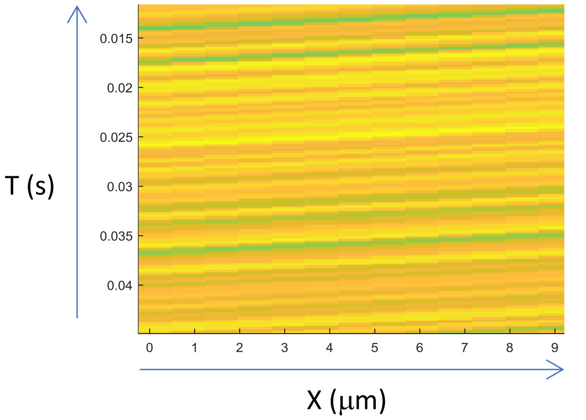
Data File S2. Description of methodology to analyze RBC velocity. Related to Star Methods.

Each RBC flowing through the vessel creates a dark shadow on fluorescent plasma. On XT images (Fig. A1), each of these shadows makes an oblique line which can be used to measure RBCs velocity (Chaigneau et al., 2003; Kleinfeld et al., 1998). Here we used a method adapted from (Autio et al., 2011).

Figure A1



The XT image can be mathematically represented by a field $p_{all}(x,t)$ which is the sum of the lines created by each particle.

$$p_{all}(x,t) = \sum_{j=0}^{n} p_j(x,t) \qquad \text{Eq.1}$$

Where $p_j(x,t)$ is the field created by one particle.

$p_j(x,t)$ can be mathematically described a progressive wave bounded by the edges of the image:

$$p_j(x,t) = s(t + \frac{(x - x_0^j)}{v}) D(x,t) \qquad \text{Eq. 2}$$

With :

x_0^j being the starting position of RBC number j,

v being the speed of the particle,

D(x,t) being a door function equal to 1 if $0 < t < t_{max}$ and $0 < x < x_{max}$, and 0 anywhere else,

s a function which can be approximated to a gaussian function.

$p_{all}(x,t)$ is thus a series of progressive waves :

$$p_{all}(x,t) = \sum_{j=0}^{n} s\left(t + \frac{(x - x_0^j)}{v}\right) D(x,t) \qquad \text{Eq. 3}$$

The two dimensional Fourier transform of this field, can be expressed as:

$$\widetilde{P_{all}}(f_x, f_t) = \sum_{j=0}^{n} \widetilde{P_J}(f_x, f_t) \qquad \text{Eq. 4}$$

where f_x and f_t are respectively the spatial and the temporal frequencies

For each individual component:

$$\widetilde{P_j}(f_x, f_t) = \iint s(t + \frac{(x - x_0^j)}{v}) \, D(x,t) e^{-2\pi i \, (f_t t + f_x x)} dt dx \qquad \text{Eq. 5}$$

As the Fourrier transform of a product is the convolution product of the Fourier transforms of the individual components:

$$\widetilde{P_j}(f_x, f_t) = \iint s(t + \frac{(x - x_0^j)}{v}) \, e^{-2\pi i \, (f_t t + f_x x)} dt dx * \iint D(x,t) e^{-2\pi i \, (f_t t + f_x x)} dt dx \qquad \text{Eq. 6}$$

Where * is the 2 dimensional convolution product.

The first term of the convolution product can be calculated as follows :

$$\iint D(x,t) e^{-2\pi i \, (f_t t + f_x x)} dt dx = \int_0^{t_{max}} \int_0^{x_{max}} e^{-2\pi i \, (f_t t + f_x x)} dt dx \qquad \text{Eq. 7}$$

Thus

$$\iint D(x,t) e^{-2\pi i \, (f_t t + f_x x)} dt dx = \int_0^{t_{max}} e^{-2\pi i f_t t} dt \int_0^{x_{max}} e^{-2\pi i f_x x} dx \qquad \text{Eq.8}$$

As

$$\int_0^{t_{max}} e^{-2\pi i f_t t} dt = t_{max} sinc(\pi f_t t_{max}) e^{-2\pi i f_t t_{max}} \qquad \text{Eq.9}$$

And

$$\int_0^{x_{max}} e^{-2\pi i f_x x} dx = x_{max} sinc(\pi f_x x_{max}) e^{-2\pi i f_x x_{max}} \qquad \text{Eq.10}$$

Where sinc is the sin(x) /x function

Therefore

$$\iint D(x,t) e^{-2\pi i \, (f_t t + f_x x)} dt dx = x_{max} t_{max} \, sinc(\pi f_t t_{max}) sinc(\pi f_x x_{max}) \, e^{-2\pi i f_t t_{max}} \, e^{-2\pi i f_x x_{max}}$$

Eq. 11

The second term of the convolution product can be calculated as follows :

By Change of variable T = t - x_0/v :

$$\iint s(t + \frac{(x - x_0^j)}{v}) \, e^{-2\pi i \, (f_t t + f_x x)} dt dx = \iint s(T + \frac{x}{v}) \, e^{-2\pi i \, (f_t T + f_x x)} e^{-2\pi i \, f_t x_0^j / v} dT dx \qquad \text{Eq. 12}$$

Reformulating :

$$\iint s(t + \frac{(x - x_0^j)}{v}) \, e^{-2\pi i \, (f_t t + f_x x)} dt dx = e^{-2\pi i \, f_t x_0^j / v} \int e^{-2\pi i \, f_x x} (\int s(T + \frac{x}{v}) \, e^{-2\pi i \, f_t T} dT) dx \qquad \text{Eq. 13}$$

But

$$\int s(T + \frac{x}{v}) \, e^{-2\pi i \, f_t T} dT = e^{2\pi i \, f_t x / v} \tilde{S}(f_t) \qquad \text{Eq. 14}$$

With $\tilde{S}(f_t)$ being the Fourier transform of s(t).

Thus

$$\iint s(t+\frac{(x-x_0^j)}{v})\, e^{-2\pi i\,(f_t t+f_x x)} dt dx = e^{-2\pi i\, f_t x_0^j/v} \int e^{-2\pi i\, f_x x}\, e^{2\pi i\, f_t x/v} \tilde{S}(f_t)\, dx$$

Eq. 15

Reformulating :

$$\iint s(t+\frac{(x-x_0^j)}{v})\, e^{-2\pi i\,(f_t t+f_x x)} dt dx = e^{-2\pi i\, f_t x_0^j/v} \tilde{S}(f_t) \int e^{-2\pi i\, f_x x}\, e^{2\pi i\, f_t x/v}\, dx \quad \text{Eq. 16}$$

But

$$\int e^{-2\pi i\, f_x x}\, e^{-2\pi i\, f_t x/v}\, dx = \int e^{-2\pi i\left(f_x - \frac{f_t}{v}\right)x}\, dx = \delta(f_x - \frac{f_t}{v}) \quad \text{Eq. 17}$$

Therefore

$$\iint s(t+\frac{(x-x_0^j)}{v})\, e^{-2\pi i\,(f_t t+f_x x)} dt dx = \tilde{S}(f_t)\delta(f_x - \frac{f_t}{v}) e^{-2\pi i \frac{x_0^j}{v} f_t} \quad \text{Eq. 18}$$

This formula shows a function which is only non-zero for $f_t = \frac{f_x}{v}$.

Putting together the 2 elements of the convolution product :

$$\widetilde{P_j}(f_x, f_t) = \tilde{S}(f_t)\delta(f_x - \frac{f_t}{v}) e^{-2\pi i \frac{x_0^j}{v} f_t} * x_{max} t_{max}\, sinc(\pi f_t t_{max}) sinc(\pi f_x x_{max}) e^{-2\pi i f_t t_{max}} e^{-2\pi i f_x x_{max}}$$

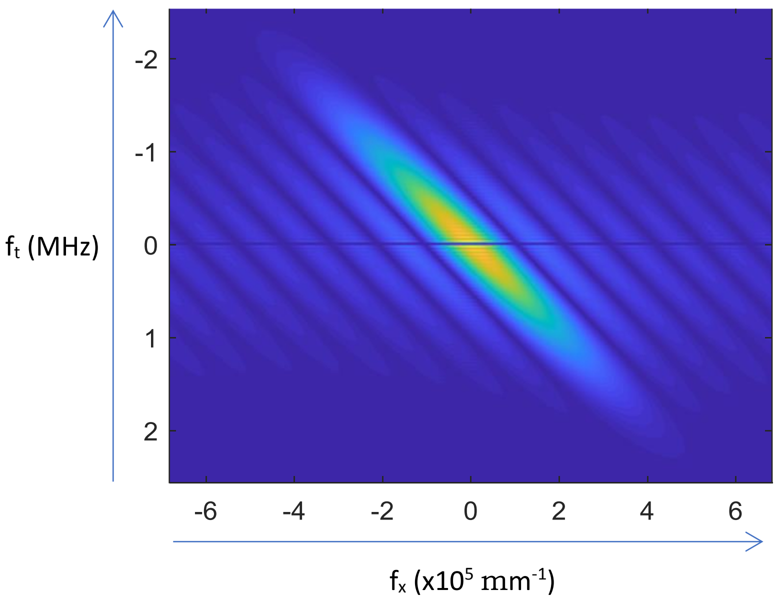
Eq. 19

The modulus of the previous equation has a main lobe centered on zero whose slope gives the speed of the particle, as shown on Figure A2. Therefore, by fitting the maximum for each temporal frequency and computing f_{x0}, the spatial frequency such that $\left|\widetilde{P_j}(f_{x0}(f_t), f_t)\right|$ reaches its maximal, then :

$$f_{x0}(f_t) = \frac{f_t}{v} \quad \text{Eq. 20}$$

Therefore fitting $f_{x0}(f_t)$ as a function of f_t allows calculating v.

<u>Figure A2</u>



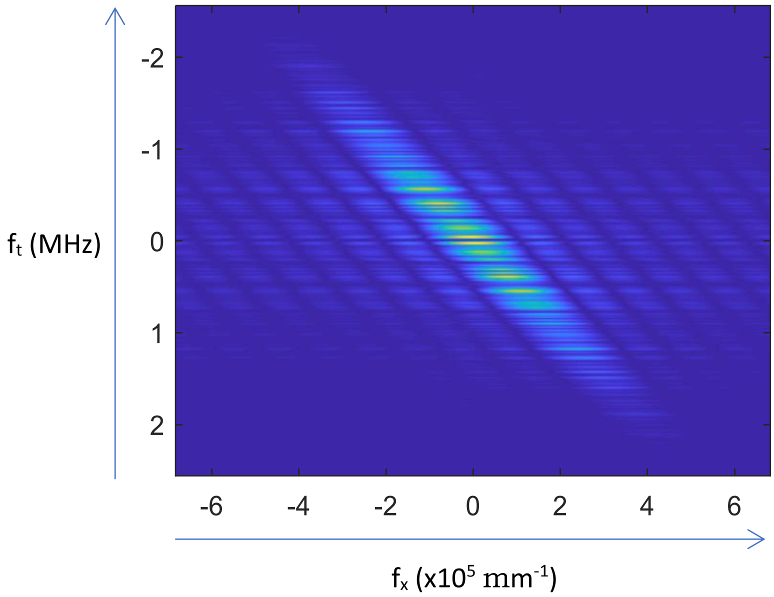
Using Equation 19, the two dimensional Fourier transform of the total field $\widetilde{P_{all}}(f_x, f_t)$ can be expressed as:

$$\widetilde{P_j}(f_x, f_t) = \tilde{S}(f_t)\delta(f_x - \frac{f_t}{v}) \sum_{j=0}^{n} e^{-2\pi i \frac{x_0^j}{v} f_t} * x_{max} t_{max}\, sinc(\pi f_t t_{max}) sinc(\pi f_x x_{max}) e^{-2\pi i f_t t_{max}} e^{-2\pi i f_x x_{max}}$$

Eq. 21

The term $\sum_{j=0}^{n} e^{-2\pi i \frac{x_0^j}{v} f_t}$ will generate random interference within the previous distribution, as shown on the plot of its modulus on Figure A3.

<u>Figure A3</u>



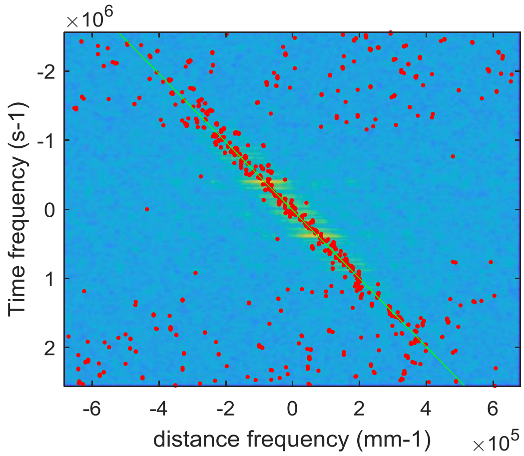
To find v, the strategy of fitting the maximum of $\left|\widetilde{P_j}(f_x, f_t)\right|$ for each temporal frequency and computing f_{x0}, the spatial frequency such that $\left|\widetilde{P_j}(f_{x0}(f_t), f_t)\right|$ reaches its maximal remains valid but needs to be adapted. Indeed, depending on the different starting values of the particles x_0^i (which is random) and the time frequency f_t, the interferences between the different exponential terms in the sum can be destructive. So for some f_t, the term $\sum_{j=0}^{n} e^{-2\pi i \frac{x_0^j}{v} f_t}$ can even drop to zero. For these frequencies it is not possible to measure accurately the maximum of $\left|\widetilde{P_j}(f_x, f_t)\right|$. Moreover, the acquisition noise due to the apparatus, will generate false maxima. Therefore, a recursive algorithm was used to eliminate false maxima and avoid errors in estimating v. This recursive algorithm consists of :

- Fitting f_t versus f_{x0}
- Isolating the point which gives the maximum error to the fit
- Removing this point from the point to be fitted
- Fitting f_t versus f_{x0} without this point.

This algorithm is repeated until half the temporal frequencies to be fit at the beginning are removed.

Figure A4 shows the result of this recursive fitting algorithm, realized with simulated data from Figure 1 on which random noise was added. The red points show the initial distribution of f_t versus f_{x0}. The green line show the final fit found by the recursive algorithm. Movie S1 shows real time analysis on experimental data.

Figure A4


×10^6
-2
-1
0
1
2
Time frequency (s-1)
-6
-4
-2
0
2
4
6
distance frequency (mm-1)
×10^5