Simultaneous Electro-Optical Tracking for Nanoparticle Recognition and Counting

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Supporting Information

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SI.1

Super-resolution imaging

The movies acquired with a fast camera using 1328 fps are analyzed using GDSC SMLM a free ImageJ Plug-In developed by University of Sussex (see http://www.sussex.ac.uk/gdsc/intranet/pdfs/SMLM.pdf). The analysis has been performed considering a pixel dimension of 182 nm, an exposure time of 0.75 ms, an exciting laser wavelength of 488 nm.

The super-resolution images are used to reconstruct the size and shape of the nanochannel analyzing the locations of the nanospheres during the passage from *cis*- to *trans*- microchannel. The software analyses the registered fluorescent spots by means of 2-D Gaussian fits, identifying both position and intensity, so the distribution of particles is mapped on the image describing in details the regions where the particles are localized.

The images reported below in **Fig. S1** and **Fig. S2** are generated from a sequence of 65535 frames. **Fig. S1** allows to appreciate the distribution of nanoparticles in the microchannels, while from the inset it is possible to reconstruct the shape of the nanochannel, in fact the regions where nanospheres accumulate or pass more frequently correspond to larger regions that they can access easily.

In **Fig. S1b** it is possible to observe that there is a region where the fluorescence intensity is very low, integrating the intensity signal of a rectangle (2.7 μm x 0.65 μm, white borders in the inset) placed along the nanochannel and plotting the profile, it is evident that there is a region, nearly in the middle, 500 nm long where the intensity is significantly lower than other regions, this can be considered the effective length of the nanochannel, which correspond to 500 ±50 nm.

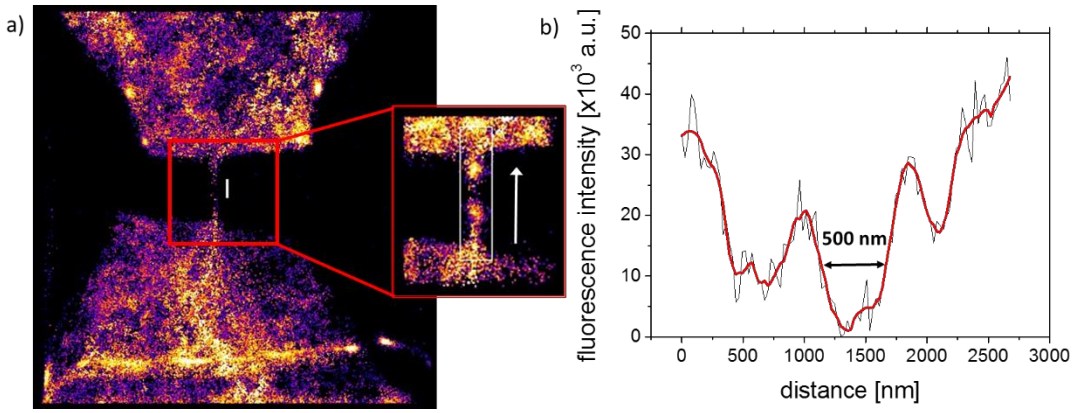

Figure S1. a) Image generated from a sequence of 65535 images of nanospheres moving from cis- to trans- microchannel. Each spot is reported in the position where it has been localized by the 2D Gaussian fit with its signal strength, the image is equalized in order to maximize the contrast. The analysis has been performed separately for the large image and the inset. (scale bar 520 nm) . b) Plot of the fluorescence intensity versus the distance along the white rectangle (2.7 μm x 0.65 μm, following the direction of the arrow).

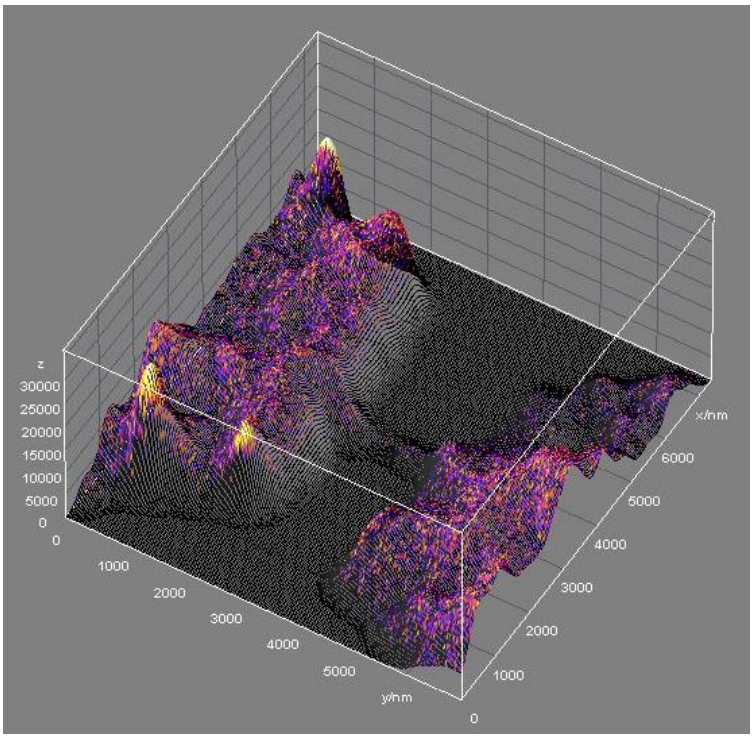

Figure S2. 3D surface plot of the region around the nanochannel.

SI.2

Software for the analysis of the optical and electrical traces.

A home-made MATLAB software has been used for analyzing the electrical traces in order to extract the values of the dwell time t_d and ΔI the maximum variation of the current corresponding to the presence of a nanoparticle inside the nanochannel.

The software works with a very simple principle, it analyzes, one at a time, the current maxima of a trace (above noise), and for each n*th*-maximum, identified by ($t_{n\text{-}peak}$, $I_{n\text{-}peak}$), it determines two local mean current values, one for $t<t_{n\text{-}peak}$ which is set as the threshold $I_{n\_thrshd\_before}$, and the other for $t> t_{nth\text{-}peak}$ corresponding to $I_{n\_thrshd\_after}$. So, for $t<t_{n\text{-}peak}$ each point having $I(t)> I_{n\_thrshd\_before}$ is considered part of the event, and similarly for $t>t_{n\text{-}peak}$ each point verifying $I(t)> I_{n\_thrshd\_after}$. So, the dwell time t_d is the maximum distance on the time scale, between points having $I(t_{n\text{-}min}) > I_{n\_thrshd\_before}$ for $t<t_{n\text{-}peak}$, and $I(t_{n\text{-}max})> I_{n\_thrshd\_after}$ for $t>t_{n\_peak}$, while $\Delta I = |I_{n\_peak}-(I_{n\_thrshd\_before}+ I_{n\_thrshd\_after})/2|$. Examples of traces are reported in **Fig. S3.**

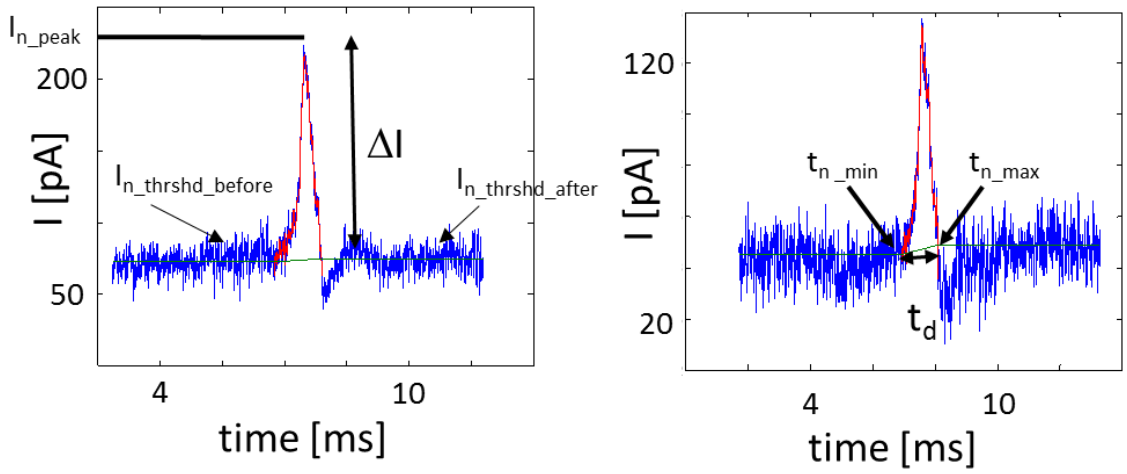

Figure S3.-Examples of current peaks analyzed using a home-made MATLAB software.

SI.3

Determination of the access resistance

The resistance of the microchannel is measured with a solution of KCl 0.1 M, $R_{microchannel}$= 0.76 MΩ

The access resistance can be estimated considering the dimensions of the two access regions reported in **Fig. S4.**

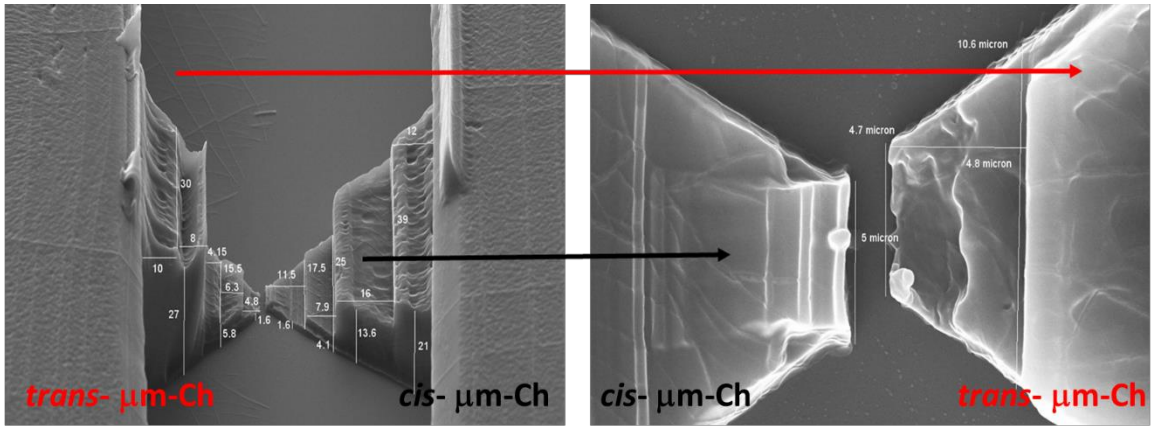

Figure S4. Scanning electron microscopy images of a cast (made of NOA 81 on a silicon substrate) of the region around the PDMS nanochannel.

The access areas consist of 4 major steps (two on the left and two on the right).

The access resistance of the 2 major steps of access regions on each side of the nanochannels can be calculated considering:

Step 1 **Right:** w1=5.0±0.3 µm, h=1.6±0.3 µm, w2=17.5±0.3 µm, L=11.5±0.3 µm

$a=h*w1=8x10^{-12}$ m^2, $b=h*(w2-w1)/L =1.7x10^{-6}$ m^2

$R_{1right}=\rho/b* [\log(a+bL)-\log(a)]$ = 247±64 kΩ

Step 1 **Left**: w1=4.7±0.3 µm, w2=10.6±0.3 µm, h=1.6±0.3 µm, L=4.8±0.3 µm, R_{1left}= 140±39 kΩ

Step 2 **Right**: w1=17.5±0.3 µm, w2=25±0.3 µm, h=4.1±0.3 µm, L=7.9±0.3 µm, $R_{2\ right}$ = 31±7 kΩ

Step 2 **Left**: w1=10.6±0.3 µm, w2=15.5±0.3 µm, h=5.8±0.3 µm, L=6.3±0.3 µm, $R_{2\ left}$= 28±7 kΩ

So $R_{access\ \mathbf{micro}}=R_{1right}+R_{2right}+R_{1left}+R_{2left}$= 446±76 kΩ

Super-resolution images can be used to estimate the real dimensions of nanochannel inlet (in the *cis*-microchannel) and outlet (in the *trans*-microchannel) and make a realistic scheme of the nanochannel structure (reported in **Fig. S5**) in order to estimate the nanochannel access resistance simply applying the Ohm's law. Since inlet and outlet regions do not have a constant cross-section, the relation for calculating the resistance must be modified as follows:

$$R_{cis} = \int_0^{L_{cis}} \frac{\rho \, dl}{S_{cis}}$$

Where $S_{cis} = (w_{1_{cis}} + \frac{w_{2_{cis}} - w_{1_{cis}}}{L_{cis}} l)(h_1 + \frac{h_{2_{cis}} - h_{1_{cis}}}{L_{cis}} l) =$

$$= w_{1_{cis}} h_{1_{cis}} + \left(w_{1_{cis}} \left(\frac{h_{2_{cis}} - h_{1_{cis}}}{L_{cis}} \right) + h_{1_{cis}} \left(\frac{w_{2_{cis}} - w_{1_{cis}}}{L_{cis}} \right) \right) l + \left(\frac{w_{2_{cis}} - w_{1_{cis}}}{L_{cis}} \right) \left(\frac{h_{2_{cis}} - h_{1_{cis}}}{L_{cis}} \right) l^2$$

$= r + sl + t\, l^2$

If $\Delta = s^2 - 4\, t\, r > 0$, R_{cis} can be expressed as

$$R_{cis} = \int_0^{L_{cis}} \frac{\rho \, dl}{r + sl + t\, l^2} = \rho \left[\frac{1}{\sqrt{s^2 - 4tr}} \ln \left| \frac{2tl + s - \sqrt{s^2 - 4tr}}{2tl + s + \sqrt{s^2 - 4tr}} \right| \right]_0^{L_{cis}}$$

The same equations can be applied to calculate R_{trans}.

Considering w_{1_cis}= (250±50) nm, w_{2_cis}= (1200±100) nm, h_{1_cis}= (300±50) nm, h_{2_cis}= (600±50) nm, L_{cis}= (400±50) nm the resistance is R_{cis}=1.3 MΩ

Considering w_{1_trans}= (250±50) nm, w_{2_trans}= (750±100) nm, h_{1_trans}= (300±50) nm, h_{2_trans}= (600±50) nm, L_{trans}= (400±50) nm the resistance is R_{trans}=2.5 MΩ

$R_{access\ \mathbf{total}} = R_{cis} + R_{trans} + R_{access\ \mathbf{micro}} + R_{microchannels}$= 2.5 MΩ +1.3 MΩ + 0.4 MΩ + 0.7 MΩ ≈ 4.9 MΩ

So $R_{nanoopen} = R_{open} - R_{access\ total}$= 20.9 MΩ – 4.9 MΩ = 16 MΩ

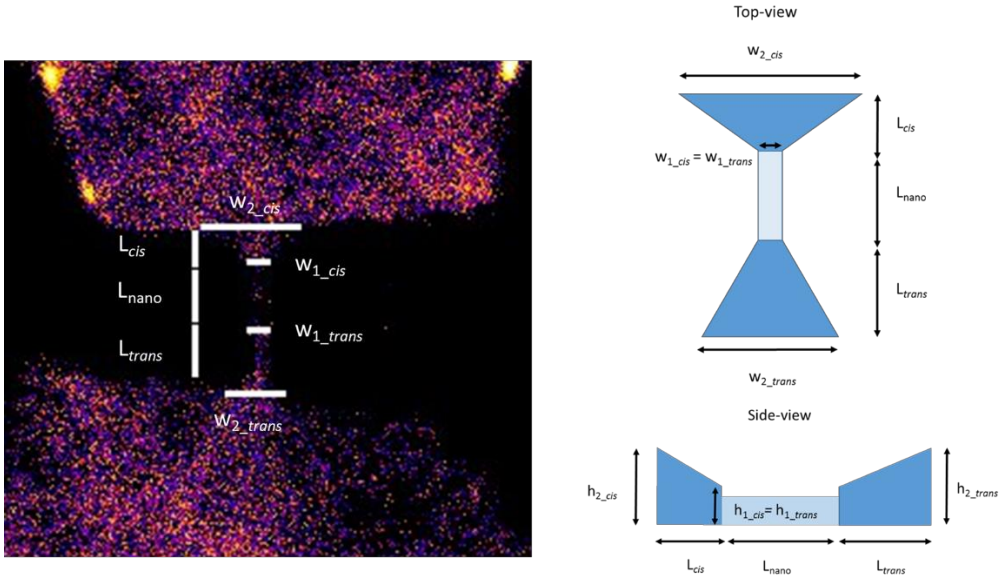

Figure S5. (Left) Super-resolution image of the area around the nanochannel used as reference to reconstruct the scheme of the nanochannel and its inlet and outlet access regions in the cis- and trans- microchannel. (Right) Top view and side-view of the scheme of the region around the nanochannel.

Nanoparticle tracking analysis

To track the nanospheres we acquired fluorescence images at a frame rate of 1328 fps from a ~11x11 μm^2 region surrounding the nanochannel.

Particle positions, as (x, y) coordinates, are registered as time sequence of N images $r_n = r(n\Delta t) = (x_n^2+y_n^2)^{1/2}$, where $[x_n = x(n\Delta t), y_n = y(n\Delta t)|n=1,2\ldots,N]$ and Δt is the data acquisition time interval between consecutive images. The mean square displacement (MSD) of a particle is calculated as the ensemble average of its displacement $\Delta r(\tau)$ from all the positions $r(n\Delta t+\tau)$ and $r(n\Delta t)$ separated by a time lag $\tau = m\Delta t$, i.e.:

$$MSD(\tau) = \langle \Delta r^2(\tau) \rangle = \langle \Delta r^2(m\Delta t) \rangle = \frac{1}{N-m} \sum_{i=1}^{N-m} r_{i+m}^2 - r_i^2 . \quad \text{(eq. 1)}$$

MSD is an informative representation of the mode-of-motion a particle is experiencing. For diffusion[1] in *d* dimensions the MSD scales with a power law according to $MSD(\tau) = 2dD^*\tau^\alpha$ where D* is the generalized diffusion coefficient and the exponent α ($0 < \alpha < 2$) the anomaly parameter. For the simple case of normal diffusion[2] (Brownian motion) $\alpha = 1$ and MSD is given by the expression:

$$MSD(\tau) = 4D^*\tau \quad \text{(eq. 2)}$$

where D* corresponds to Einstein diffusion coefficient. For $\alpha < 1$ the underlying transport mechanism is called sub-diffusive, for $\alpha > 1$ super-diffusive, so analyzing α can reveal the phenomena underlying particle motion. The presence of random errors in the determination of particle position, even in case of simple diffusion, can result in an apparent sub-diffusive behavior for short lag time[3]. In fact, if the mean error on particle position is σ, eq. 2 must be modified to[3-4]:

$$MSD(\tau) = 4D\tau+2\sigma^2 \quad \text{(eq. 3)},$$

to determine the diffusion coefficient, a standard method is to perform a linear fit of eq. 3, the slope D* gives the diffusion coefficient D = D*/4, while the intercept the mean error.

The diffusion coefficient D and mean error σ, of each trajectory has been calculated, fitting the first 7 data points of each MSD *vs* τ (which corresponds to a time lag $\tau = 7\Delta t = 5.73$ ms, $\Delta t = 0.8185$ ms being the frame time for acquiring the movies). For short lag time simple diffusion is dominant, while anomalous diffusion phenomena requires longer time lag to be exhaustively characterized[2].

SI.5

Determination of the nanochannel dimensions by analyzing the electrical traces

In order to calculate nanochannel dimensions based on electrical measurements the value of the resistance variation ΔR caused by the passage of a nanosphere has been considered.

When a voltage V is applied to an open nanochannel, the current measured across it is I_{open}, corresponding to the resistance $R_{open} = V/I_{open}$.

The resistance R_{open} is the series of the access resistance R_{access} and of the nanochannel in open state, $R_{nanopen}$, thus $R_{open} = R_{nanoopen}+R_{access}$

When a nanosphere blocks the nanochannel a current drop $I_{blockage}$ occurs, which corresponds to a resistance $R_{blockage} = V/I_{blockage}$, (which is the series of the access resistance R_{access} and the resistance of the nanochannel when it is partially obstructed by a nanosphere $R_{blockage}$, $R_{blockage} = R_{access}+R_{nanblockage}$) and the current variation is $\Delta I = I_{open}-I_{blockage}$

The resistance variation $\Delta R = R_{blockage}-R_{open}$ can be expressed as

$\Delta R = R_{blockage}-R_{open}= V/I_{blockage}-V/I_{open}$ and $\Delta R = V(\Delta I/I_{open*}(I_{open}-\Delta I))$

Using the first component in the equation derived by DeBlois[5], recently reproposed by Pevarnik et al.[6] to analyze the shape of a nanopore, for a pore of diameter D occupied by a particle of diameter d, and filled with a solution of resistivity ρ:

$$\Delta R = \frac{8\rho d^3}{3\pi D^4}\left[1+\frac{4}{5}\left(\frac{d}{D}\right)^2+\frac{24}{35}\left(\frac{d}{D}\right)^4+\ldots\right] \text{ (eq. 4)}$$

to estimate the cross section S of a nanochannel and considering $D = (4\,S/\pi)^{1/2}$, the expression can be rewritten in the form:

$$\Delta R = \frac{4\pi\,\rho\,r^3}{3\,S^2} \text{ (eq. 5)}$$

Considering ΔI_{ns} as the mode of the distribution of $\Delta I = I_{open}-I_{blockage}$ obtained from the electrical traces recorded for different values of the applied voltage V, $\Delta R = V(\Delta I_{ns}/I_{open}*(I_{open}-\Delta I_{ns})) = 66.3\pm0.7$ kΩ (see **Fig. S6**)

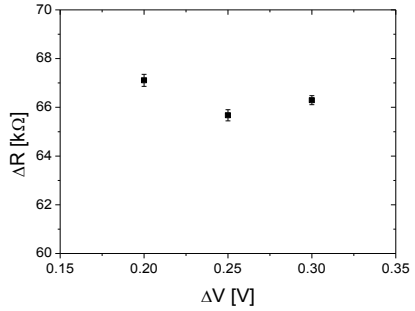

Figure S6. ΔR versus ΔV for three different voltages, $\Delta R=\Delta V(\Delta I_{ns}/I_{open*}(I_{open}-\Delta I_{ns}))$, where ΔI_{ns} is considered as the mode of the distribution of ΔI for all the translocation events analyzed.

So, from the eq. 5 and considering r = (24±3) nm, as reported in Fluorospheres datasheet, it is possible to extract S which is

$S = \left(\frac{4\pi \rho r^3}{3 \Delta R}\right)^{\frac{1}{2}}$ (eq. 6) and results S = (2.6±0.5) x 10^{-14} m^2.

Considering that from the super-resolution images the effective nanochannel length results L_{eff} = (500±50) nm the resistance of the nanochannel is

$R_{nanochannel} = \rho \frac{l}{S} =$ (15±4) MΩ which is compatible with the value of the resistance measured experimentally.

Analysing super-resolution images, nanochannel width results to be nearly (230±50) nm, considering

S = (2.6±0.5) x 10^{-14} m^2, nanochannel height is (220±50) nm.

SI.6

1D diffusion-drift model

The distributions P(t) of the translocation-time t of the particles crossing the nanochannel of length L_{eff} (see **Fig. 5** main text), have been fitted with a 1D diffusion-drift model described by:

$$P(t)=\left(\frac{L_{eff}}{(4\pi Dt^3)^{\frac{1}{2}}}\right)e^{-\frac{(L_{eff}-vt)^2}{4Dt}} \quad \text{(eq. 7)}$$

where the two parameters that are extracted are the particle's mean diffusion coefficient D, and its mean drift velocity *v*. The scatter plot of dwell time t_d *versus* $|\Delta I/I_0|$, obtained for six values of applied voltage $|V_{bias}|$, are reported in **Fig. S7**.

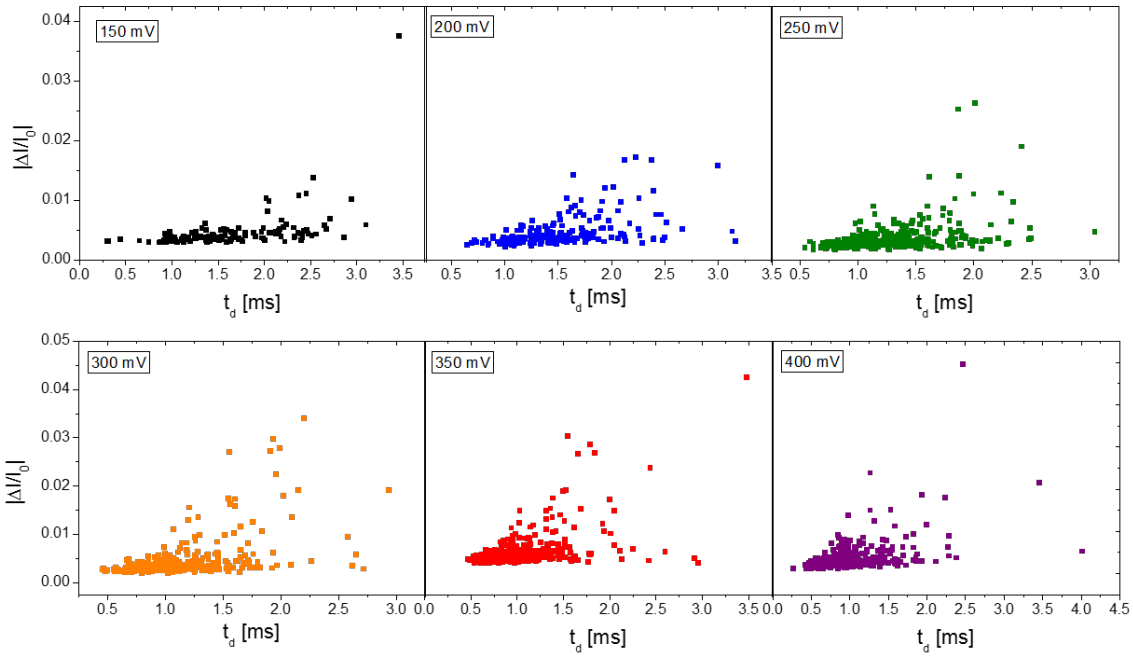

Figure S7. Dwell time t_d versus fractional current $\Delta I/I_0$ scatterplots for 48 nm (nominally 40 nm) fluorescent nanospheres acquired for different values of the voltage $|V_{bias}|$ (150, 200, 250, 300, 350, 400 mV). Each point corresponds to the passage of a sphere through the nanochannel. Translocations become faster and more frequent when voltage (absolute value) is increased.

SI.7

Relation between resistive pulse ΔR and fluorescence intensity signal f

The synchronized electrical and optical signals allow to compare the relative particle size obtained from resistive pulses ΔR and fluorescence intensity peaks measured during translocations, as shown in **Fig. S8 (left)**. A good correspondence between ΔR and fluorescence intensity is confirmed for particles moving inside the microchannels **Fig. S8 (right)**, where the intensity of the spot is determined by 2D-gaussian fits of the peaks. The relation between ΔR and spot fluorescence intensity f, determined as the product of amplitude and spot dimensions on x- and y-axis, is almost linear, which suggests that both parameters are particle volume-dependent properties.

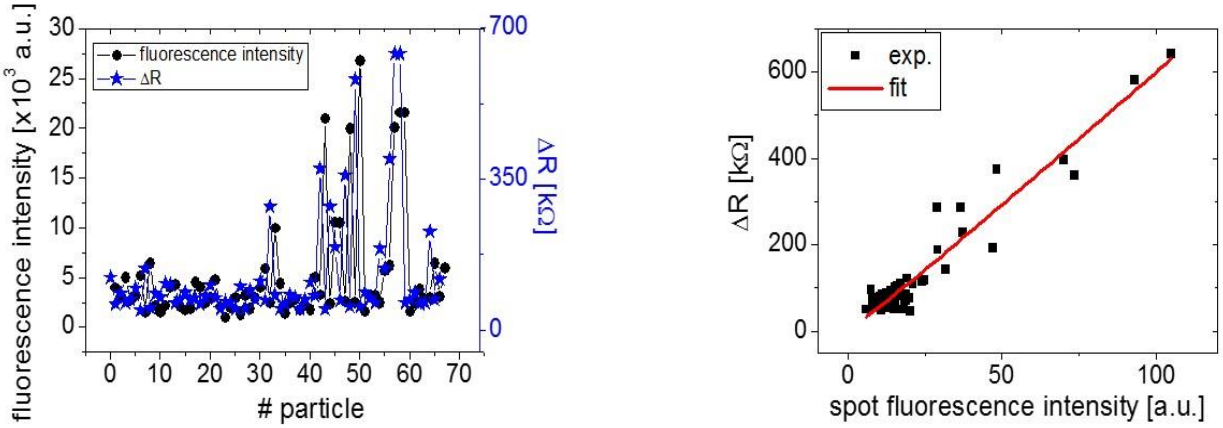

Figure S8. (Left) Fluorescence intensity spikes and resistive pulses ΔR, during the passage of a particle through the nanochannel, show a very good correlation (values for 67 particles). (Right) Resistive pulses during translocation versus fluorescence intensity f of the particle in the microchannels, the fit performed using a power law (f = c+b*ΔR^a) reveals almost linear dependence, in fact a = 1.17±0.09. Fluorescence intensity outside the nanochannel is determined with 2D-gaussian fit of fluorescence spots.

References

[1] Ernst, D.; Köhler, J. *Phys*. *Chem*. *Chem*. *Phys*., 15, 845-849, **2013**, DOI: 10.1039/C2CP43433D

[2] Qian, H.; Sheetz, M. P.; Elson, E. L. *Biophys*. *J*., 60: 910-921, **1991**, DOI: 10.1016/S0006-3495(91)82125-7

[3] Martin, D. S.; Forstner, M. B.; Kas, J. A. *Biophys*. *J*., 83, 2109-2117, **2002**, DOI: 10.1016/S0006-3495(02)73971-4

[4] Dietrich, C.; Yang, B.; Fujiwara, T.; Kusumi, A.; Jacobson, K. *Biophys*. *J*., 82, 274-284, **2002**, DOI: 10.1016/S0006-3495(02)75393-9

[5] DeBlois, R. W.; Bean, C. P. *Rev*. *Sci*. *Instrum*., 41, 909–916, 1970

[6] Pevarnik, M., Healy, K.,* Toimil-Molares, M. E., Morrison, A., Sonia E. Létant, S. and Siwy, Z. S., *ACS Nano* 6 (8), 7295-7302, 2012, DOI: 10.1021/nn302413u