Contribution ID: **189** Type: **poster**

The effects of sub-diffusion in the NTA size measurements of extracellular vesicles

The interest in the extracellular vesicles (EVs) is rapidly growing as they became reliable biomarkers for many diseases. For this reason, fast and accurate techniques of EVs size characterization are the matter of utmost importance. One increasingly popular technique is the Nanoparticle Tracking Analysis (NTA), in which the diameters of EVs are calculated from their diffusion constants. This assumes that the diffusion of EVs follows the Stokes-Einstein relation, i.e. that the Mean Square Displacement is linear in time (MSD$\propto t$). However, we show that this relation is severely violated in biological samples. In fact, at intermediate time periods MSD is strongly sub-diffusive (MSD $\propto t^{\alpha}$, $0 < \alpha < 1$). The sub-diffusive behaviour is well known in statistical physics, but most of its theoretical models do not provide the direct relation between the size of a particle and the generalized diffusion constant D_{α}. We solve this problem by introducing the logarithmic model of sub-diffusion. Applying it into analysis results in the average measured EV diameter reduced by 45% in comparison to the normal diffusion models. To corroborate our analysis, we compare our results with the AFM studies of the same samples. While the average size of EVs in AFM is still twice smaller than the in NTA, this discrepancy is expected due to the systematic differences between these two methods, which we also discuss.

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