

## Testing the spline-method

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The spline method introduced and detailed in Ref.<sup>1</sup> to extract the probability distribution of the speed of swimming bacteria from photon-correlation measurements. Here we propose a test that we have performed to check the robustness of the method. To this aim we focus on one single measurement of the intermediate scattering function.

As a test we check the effect on the  $P(v)$  produced by changing the density of the knots. To do this we change the total number of knots between the first non-zero node at  $1 \mu\text{m/s}$  and  $v_{\text{max}} = 60 \mu\text{m/s}$  (that are kept fixed). This changes substantially the knots' density by a factor going from 0.5 to 2 (number of knots ranging from 6 to 32).

As seen from Figure 1 when the density of knots is too low the  $P(v)$  develops some spurious peak at high speed (see the most blue curve in Fig. 1). Note also that fitting the same data with the standard Schulz distribution (dashed line in Fig. 1) gives a  $P(v)$  whose peak is slightly shifted toward lower speeds. The evolution of this peak determines the significant growing behaviour of  $\sigma$  and a slightly increasing trend of  $\langle v \rangle$  shown in Figure 2. However these parameters (as the  $P(v)$ ) become practically independent on the density of knots when this is greater than  $\approx 0.2 (\mu\text{m/s})^{-1}$ . Note for the analysis presented in the article is performed with a density of knots  $\approx 0.22 (\mu\text{m/s})^{-1}$  as using more dense knots would only slow down the fit while knots very close would bring basically the same information. This corresponds to choosing the knots similarly to Ref.<sup>1</sup>, i.e. 13 knots spaced approximatively by  $5 \mu\text{m/s}$ .

In conclusion the spline-method introduced in Ref.<sup>1</sup> appears as a reliable techniques for the analysis of DDM/ICS data if the knots range and number are chose in a reasonable way.

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<sup>1</sup> G.B. Stock, Biophysical Journal, **16** 535 (1976)

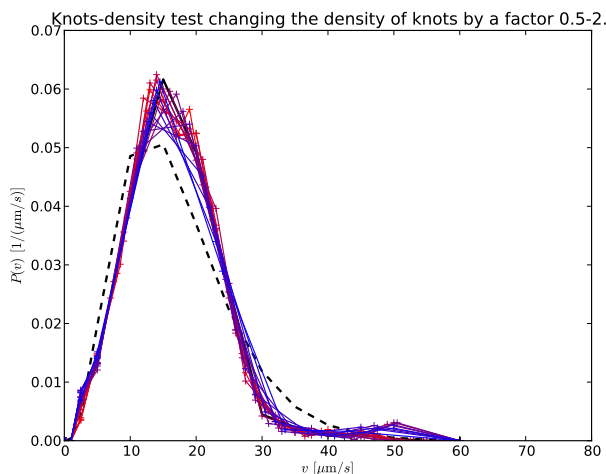


FIG. 1: Fitted spline-probability distribution upon changing the density of knots with original density  $0.22 \text{ knots per } \mu\text{m/s}$  (between  $1 \mu\text{m/s}$  and  $60 \mu\text{m/s}$ ) by a factor ranging from  $1/2$  to  $2$  (from blue to red). The dashed line is the probability obtained from a fit with the Schulz distribution. It is seen that when the knots' density is too low a spurious peak at high-speed develops.

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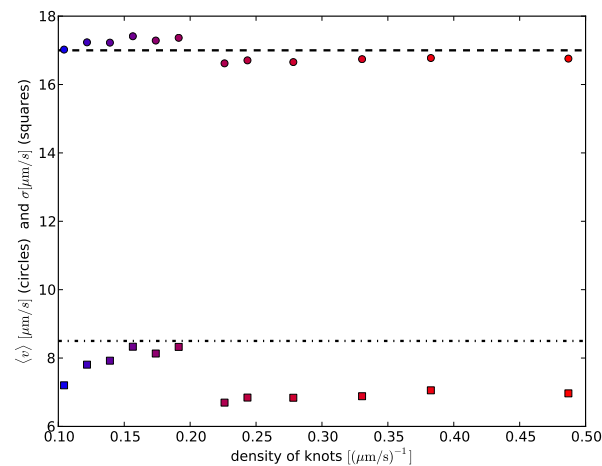


FIG. 2: Average speed and standard-deviation, obtained from the fitted spline-probability distribution (see previous Figure), upon changing the the density of knots. The dashed line and the dash-dotted line are the value of the parameters  $\langle v \rangle$  and  $\sigma$  that are obtained from a fit with the Schulz distribution. On the  $x$ -axis the knots' density is reported (from red to blue). It is seen that the parameters stabilize when we have density  $\geq 0.2 (\mu\text{m/s})^{-1}$  (knots separated by  $4 - 5 \mu\text{m/s}$ ).