Quantification of the number of adsorbed bacteria on an optical waveguide

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A simple method is presented for determining the number of bacteria adsorbed on a planar optical waveguide from measurements of a single effective refractive index. It requires only knowledge of the shape and mean size of the bacteria.

The purpose of this note is to describe a method for determining the number of bacteria adsorbed from solution onto a planar substratum. The technique now widely called optical lightmode waveguide spectroscopy (OWLS) involves measuring the perturbation of the evanescent fields associated with one or more waveguide lightmodes [1]. It is particularly convenient to measure this perturbation with the help of a grating coupler [2], with which light can be coupled in or out of the waveguide. By using a sufficiently shallow grating the perturbation of the waveguide by the grating itself becomes negligible, simplifying analysis.

For adsorbing nanoparticles (including proteins) the adlayer created on the waveguide surface is heterogeneous but Mann et al. have shown that provided the particle diameter $d \ll \lambda$, the wavelength of the light, the particle adlayer can be approximated as a uniform thin film [3]. In the absence of form or other anisotropy this adlayer A can be considered isotropic and characterized by a single thickness d_A and refractive index n_A. It suffices therefore to measure two modes to determine the optical thickness $d_A(n_A - n_C)$, where n_C is the refractive index of the cover medium. Knowing the refractive index increment [4] allows the number of objects or their mass per unit area to be immediately determined. This has been done for proteins [5], nanorods [6], etc.

Bacteria cannot be counted in this fashion because their diameters d_b are typically in the range 0.2–1 μm, i.e. $d_b \sim \lambda$, and the uniform thin film approximation (UTFA) does not hold. Two approaches to this problem are (i) explicit modelling, initially assuming the bacteria to have an idealized uniform shape and size; and (ii) calculating the mass according to the UTFA and then correcting it according to the discrepancies revealed in [3]. (i) is difficult[1] and possibly not worth the effort given the possible severity of approximating the usually microdiverse bacterial population as having a single size and shape; and (ii) seems unreliable unless trial calculations are carried out for objects closely resembling the actual bacteria of interest, which also represents a lot of work. Furthermore it is difficult to determine the refractive index increment of a turbid liquid such as a bacterial suspension.

Here we present a further possibility, based on the fact that the adsorption jamming limit θ_J (i.e., the value of the surface coverage at which no further objects can be randomly sequentially added) is known for the simple geometries (spheres and spherocylinders) corresponding to the shapes of most bacteria [8, 9], also encompassing situations in which the adsorbed layer is able to relax to an equilibrium configuration [10]. Apart from shape and equilibration, θ_J depends only on the projected area of the object.

The typical experimental situation to which the following procedure can advantageously be applied begins with a waveguide equilibrated with the medium used to suspend the bacteria, which are then (at time $t = 0$) flowed in suspension over the waveguide, leading to a gradual increase $b(t)$ in the number of adsorbed bacteria b per unit area until a steady state is reached at which $b(t)$ is constant, with a value of b_{sat}. Usually pure suspending medium is then flowed over the waveguide to verify that the adsorption is irreversible.

The quantity b is, to a good approximation, linearly proportional to an effective refractive index N of the waveguide, say the zeroth transverse electric (TE) one [11]. However, the constant of proportionality depends on the thickness and the refractive index of the waveguide prior to adsorption [2, 1]. To allow for this, we define a normalized effective refractive index $\tilde{N}$ (cf. [12]):

$$\tilde{N} = (N - N_0)/N_0 \qquad (1)$$

where N_0 is the effective refractive index prior to adsorption ($t < 0$). We therefore assume that $b \propto \tilde{N}$. Provided the adsorption is at least quasiirreversible, b will saturate at the jamming limit θ_J. Hence,

$$b_{sat} = \theta_J / a \qquad (2)$$

where a is the area occupied by one bacterium (as

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[1] A simple approximation to this calculation has been presented for eukaryotic cells residing on optical waveguides [7].

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determined from micrographs, along with the shape). This equation (2) provides us with the means to calibrate $\tilde{N}$: the lever rule gives us

$$\frac{\tilde{N}(t)}{\tilde{N}_{\mathrm{sat}}} = \frac{b(t)}{b_{\mathrm{sat}}}, \tag{3}$$

whence elimination of b_{sat} yields

$$b(t) = \frac{\tilde{N}(t)}{\tilde{N}_{\mathrm{sat}}} \cdot \frac{\theta_J}{a}. \tag{4}$$

Although textbook images of bacteria usually show them to be highly uniform in both size and shape, in reality this is far from the case (e.g., [13]). At the very least, a mean area $\bar{a}$ could be used in eqn (4) instead of a, determined from dozens or hundreds of images. This nonuniformity affects θ_J. Size polydispersity (of spherical objects) has been investigated [14–17], but not polydispersity of shape, which is likely to be a fruitful area of further investigation. Given, however, the experimental difficulties inherent in measurements of the adsorption of a living organism, the methodology presented here is likely to be adequate for many purposes, which might include further analysis of the adsorption kinetics.

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