Hi David,

I spent some time reading through everything. Overall, it's a very nice paper for the data coming out of such a condensed span of experiments. I hope I'm not too teutonic for the purpose. However, I'm probably a good test-specimen of a physicist peripherally acquainted with FCS without any real knowledge (the prototype of a reviewer).

Intro

I like the abstract much better now.

The second paragraph is quite difficult to follow.

DL

Theory

I've pondered for ages about [3] before reading 5 lines down that there is a velocity involved. ν has to be mentioned earlier in this section, and it would be nice to have a warning that $g(\tau)$ is for the case with flow.

Signal and noise

It would be nice to know what was simultaneously fitted - as I understand it was diffusion constant and speed?

The sentence surrounding [6] might be somewhat misleading. The factor does not just deviate from unity, it become smaller than unity, and that not just for small diffusion constants, but actually any diffusion constant and any drift velocity.

I understand the thought behind the definition of dg, but it took me some while to figure it out what it is all about. I'm still not 100% sure what the implication is apart from getting a cool plot. As I see it, the strength is that the crossing directly tells you the diffusion constant for known velocity, or vice versa. The path towards that might be

1. Define ΔG (as current)
2. Discuss the crossing (as current)

3. Tell them that the 10% was arbitrary, crossing is the same for any small ΔD.

4. Then linearize and solve (as current)

5. Then say how this can be used

Plot issues:

- You would somehow need to indicate what the varied velocities mentioned in Fig. 3a are.

- The locations of (a) and (b) are non-conventional, and make the scale harder to read than necessary.

- 3 b) is not a scatter plot in my opinion, but is called such in the text.

- Is there anything in particular that can follow from 3b) like that the fits have all similar quality or something like that?

ZMW

Nanofab

Ion beam etching is an age-old technique, and so I would like to use an old citation.
`P.G. Gloersen, J. Vac. Sci. Tech. 12 (1), 28-35 (1975)` or
`R.E. Lee, J. Vac. Sci. Tech. 16 (2), 164-170 (1979)`
would do the trick. It was all the rage in the 70's.

Nearfield optics

I'm not well informed about the literature. What is a in [9]?

I'm also not clear how you used [11] in the context of [4]. The mode volume is most probably smaller than the geometric volume of the "structure". My interpretation of our structure is twofold

1. A deep sub-wavelength aperture that was named ZMW although it does not guide light

2. A region above the ZMW in which there is near-field radiation. This is the more traditional NSOM zone which has been described in detail in the literature.

Samiee et al. have an ideal case 1), we have a mixture. However, since the nearfield intensity decays with R^{-6}, in our active field is not limited by the outer diameter of our crater, but rather a volume similar to the size of the smallest aperture cubed plus the volume of the ZMW region. Hence it is a fortunate coincidence that we get useful fits out of the given formula. I think this is due to the fact that most of the effect and attenuation takes place at the bottom of the crater.

That reduced volume might explain your molarity difference? I am somewhat confused where the factor of 420 comes from. Was the solution that much more concentrated in the ZMW experiments?

If you'd ever have the inclination, I can make better, cylindrical pits with my current technology - it just happened that you managed to take all the data with the first, experimental wafer.

Things:

- I don't understand why A_g has to be made one - do the curves have vastly different correlation amplitudes?
- The top panel is not mentioned in the text?
- The text says that the left center panel contains seven averaged series - Should that be the deviation from the global average - it's never said in prose as far as I can see.
- The long-tail beating looks like Fresnel diffraction at the aperture to me?
- Section 3.3 reads really rough.
- You might want to mention the reason why there is only one curve in 6.a to remind readers like me.

SNR - Figure 7

Looks better now. If you have time and strive to please people who spend hours looking at graphs, you might want to manually shorten the first division of your non-log part to make both fully visible non-log major division lines appear to be of equal width.

Since this figure is the central result of paper, the caption should state explicitly that this is a plot of signal-to-noise ratios, and not just a reference to an equation.

Comments on discussion

Linguistics

"Unimolecular" applies in my opinion to a single input reactant, and not a single product.

Integration constant for free fraction

In your Equation [14] an integration constant would be needed for dimensional consistency and fixing of boundary conditions.

Calculation of time spread

I have some objections to the way that [15] was typeset, i.e.

$$\delta_x = \nu\delta_t = \sqrt{2Dt} \ . \tag{1}$$

One should tell what the δ are, and the last equality is in my opinion not fulfilled. Also, there is already a high-flow assumption at work here. In reality the width of a plug originating at a single time is different on the left than on the right. You could probably kill this with one sentence, but I got carried away in Mathematica - perhaps it is useful. I might have somewhere some missing reasoning, but I think the basic estimate is right. You may already done the whole thing and it is totally useless.

If you assume a concentration of a delta function at $t = 0$ and one has no flow then the normalized spatial distribution is

$$\frac{\partial n}{\partial x} = p_x(x,t) = \frac{1}{\sqrt{4\pi Dt}} e^{-\frac{x^2}{4Dt}} \ . \tag{2}$$

If one adds flow, one gets

$$p_x(x,t) = \frac{1}{\sqrt{4\pi Dt}} e^{-\frac{(x-\nu t)^2}{4Dt}} \ . \tag{3}$$

If one now assumes a constant source, locates oneself at a fixed position x, and asks what the likelyhood is that a molecule entered the flow a time t ago, one should get $p_t(x,t) = \nu p_x(x,t)$. Plugging this into the above, the expected time at a position becomes

$$<t>(x) = \int_0^\infty t p_t(x,t)\mathrm{d}t = \int_0^\infty \left(\frac{\nu\sqrt{t}}{\sqrt{4\pi D}} e^{-\frac{(x-\nu t)^2}{4Dt}}\right)\mathrm{d}t \;, \tag{4}$$

which according to Mathematica is as simple as

$$<t>(x) = \frac{2D+\nu x}{\nu^2} \;. \tag{5}$$

Reassuringly this boils down to your expression for $\nu x \gg D$. The time error could be done similarly as

$$<\delta t>(x) = \sqrt{<(<t> - t)^2>} = \sqrt{\int_0^\infty (<t> - t)^2 p_t(x,t)\mathrm{d}t} \;. \tag{6}$$

The ensuing integral made a bit of trouble for Mathematica, but I strongly believe that the outcome is of the form

$$<(<t> - t)^2> = \frac{2D^2}{\nu^4}\left(4 + \frac{\nu x}{D}\right) \;. \tag{7}$$

Again, the high-flow, long-distance limit works, and your assumption is correct.

After you do all those calculations it may be nice to put in some numbers that show the reader that your assumption of $\delta_{<t>} / <t> \sim 0$ is indeed correct, or for which regime it would be correct. This way it connects to the data that you show in the first part of the paper.

Protected ZMW observation profile - Response to response to Chih-kuan's question

There is an inherent limit to the way that the grazing incidence can be done. Since one is looking at a Gaussian beam waist, the intensity will always fall off with roughly the same speed (at least when you look in the z-direction). I haven't done the math, but I would be surprised if you could get a shallower profile than half the normal z-depth of field. At the same time the effective spot width goes up, and the correlation becomes weaker (... leading to more noise).

One might be able to get that with TIRF, but then the N.A.suffers. Alternatively one could make the channel deep, but than the Taylor dispersion becomes inefficient.

Gold optics

Gold has a $1/e$ distance for *the field amplitude* of around 30 nm, and for *the intensity* around 15 nm, both at 632 nm. Bob may be convinced of different values, so you need solid numbers (which I'm not able to find easily without calculating myself right now). At 488 you go into the resonance and I'm not quite sure which way it all will go, but the length-scale should be similar. Then the Drude skin depth also might not be the right formula.