Comment on Response to “Critical assessment of the evidence for striped nanoparticles”, Ong and Stellacci, PLOS ONE 2015 [DOI: 10.1371/journal.pone.0135594]

We are pleased that Ong and Stellacci have responded to our paper, Critical assessment of the evidence for striped nanoparticles, PLoS ONE 9 e108482 (2014). Each of their rebuttals of our critique has, however, already been addressed quite some time ago either in our original paper, in the extensive PubPeer threads associated with that paper (and its preprint arXiv version), and/or in a variety of blog posts.

Indeed, arguably the strongest evidence against the claim that highly ordered stripes form in the ligand shell of suitably-functionalised nanoparticles comes from Stellacci and co-authors’ own recent work, published shortly after we submitted our PLOS ONE critique. This short and simple document compares the images acquired from ostensibly striped nanoparticles with control particles where, for the latter (and as claimed throughout the work of Stellacci et al.), stripes should not be present. We leave it to the reader to draw their own conclusions.

At this point, we believe that little is to be gained from continuing our debate with Stellacci et al. We remain firmly of the opinion that the experimental data to date show no evidence for formation of the “highly ordered” striped morphology that has been claimed throughout the work of Stellacci and co-workers, and, for the reasons we have detailed at considerable length previously, do not find the counter-claims in Ong and Stellacci in any way compelling.

We have therefore clearly reached an impasse. It is thus now up to the nanoscience community to come to its own judgement regarding the viability of the striped nanoparticle hypothesis. As such, we would very much welcome STM studies from independent groups not associated with any of the research teams involved in the controversy to date.

For completeness, we append below the referee reports which JS submitted on Ong and Stellacci’s manuscript.

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REVIEW 1

I disagree with many of the scientific/technical points raised in this Formal Comment. As it is a reply to our criticism of the authors' work this is, of course, inevitable. For a fair debate, however, the authors should have the chance to make a formal response to critiques of the flaws in their arguments so I will refrain from commenting on these issues at this time. Instead, I am using this review to highlight any instances where this paper clearly misrepresents our paper (or the literature), or makes factually incorrect arguments, in the hope that these issues can be corrected before publication.

Major issues

A recurring theme throughout the Comment is the accusation that we have misrepresented Stellacci et al.'s body of work on striped nanoparticles by suggesting that the stripes were highly ordered. Ong and Stellacci state explicitly that they "do not believe that stripes on nanoparticles are highly ordered and have made this clear in multiple occasions for example in our recent reply to one the authors' paper." This,
however is simply not true. The following are quotes from Stellacci et al's papers describing the nanoparticles:

* "Prefect ordering" in Figure 4 of the original paper [1] and "perfect ripples" is used twice in the main text!
* "domains characterized by highly ordered arrangements." In 2006 [2] when referring to [1].
* "highly-ordered supramolecular structure" In Uzun 2008 [3]
* A title containing "ordered striped nanoparticles" [4]. The same paper has "ordered, striped domains", "phase separation into ordered ripples occurs.", and "ordered phase separation", as well as two more counts of "ordered ripples", and a further count of "ordered striped domains in simulations."
* "ordered alternating phases (ripples)" and "Ordered rings" are in the 2007 Science paper[5]
* "ordered ribbon-like domains" in the abstract of [6]
* "ordered rings of alternating composition" in ref. [7]
* "The third type of NP, 66-34brOT, has identical hydrophobic and hydrophilic content to the 66-34OT, but is deficient of ordered stripes" - implying ordered stripes for 66-34OT [8]
* Finally a rendering of molecules perfectly ordered into stripes is used in refs. [1-12]. This is only an artistic impression, but it is used to suggest highly-ordered stripes.

In contrast, the term "stripe-like" has, according to my literature search, only been used since 2012. As such it is a simply untrue to say we misrepresented the literature when we mention highly-ordered striped patterns. As such, the sections using this argument should be rewritten.

The following three statements are also entirely unfounded:

"The paper dedicates almost half of its length to analyze our first image"; "Now, Levy as one of the authors of reference [19] together with a new team has written a new paper on our work (reference [1]), which re-analyzes for more than half of its length the same images analyzed in 2012"; "the very image they dedicate most of their paper to show how the topography image is similar to the current image."

We spend a little under 2 pages (of 18) discussing the seminal image of the striped particles from ref. [1]. In 2012 [13], this same image and another image from ref. [3] were analysed. If we include our new analysis of these images, this takes this section to a little under 3 pages. The third statement is a reworded repeat of the first. These three statements falsely imply we spent close to 9 pages on a single image from ref. [1].

The paper claims that Lévy et al.'s 2012 paper[13] on striped nanoparticles, and our most recent paper draw different conclusions. This is claimed both in the abstract and in the body of the paper: "There is only one thing that these two papers have in common: they misrepresent our previous work." This, itself, is a clear misrepresentation. We certainly use different analysis techniques as we now have the raw data. Nonetheless, we come to the same primary conclusion as the 2012 paper by Levy et al, i.e. the 2004-2008 STM data appears to simply be feedback ringing. The new paper then goes further to look at newer data and data from other techniques.
The paper also claims "A cursory read of reference [1] would tend to imply that in our literature we had not dealt with scanning probe artifacts". Again, this is simply, and demonstrably, incorrect. We have an entire section entitled "Assessing the statistical analysis used to distinguish artefacts from real structure" which focuses on deconstructing the analysis which claimed to separate real features from feedback instabilities (Figure 3 is part of this section). We also comment on their misconceptions about image artifacts which arise from tip sample convolution under change of scan direction on page 9 and 12. Furthermore, we never claimed (as is suggested in the abstract of Ong and Stellacci's Formal Comment) that STM artifacts were novel. In fact in the conclusion we describe them as "rudimentary flaws".

"It is pointed out that the current range in one of our images is very large (-118.2 nA vs. current setpoint of 0.84 nA). The significance of this observation is unclear." This whole section only mentions the range (with a typo, ranges should be positive), which, while extreme, is not our main point. The main point is that the tunnel current values span positive and negative values, which is clearly unphysical. We then clearly state the significance of this: it suggests that the STM preamp was saturating. To say the significance is unclear sidesteps the argument we have made.

Ong and Stellacci state that "We emphasize that never in our literature have we drawn conclusions on measurements of features below the Nyquist frequency. Indeed the measurements taken through the years have been confirmed in another laboratory by a PSD analysis, and their value is not disputed in reference [1]"

In the first sentence, I assume they mean "above" not "below", as the Nyquist frequency is the highest which can be detected in discrete data. Even with this correction the sentence is a non-sequitur. We stated that their measurements were *near* the Nyquist limit, had uncertainties considerably less than a pixel, and that all measurements sit inside a two pixel range. None of these points were addressed by Ong and Stellacci. The second sentence implies that we do not dispute the values from the PSD analysis. Quite the contrary – we explicitly dispute their methods for extracting spacings from the PSDs.

On page 9, when discussing their Langmuir paper [14], they quote us entirely out of context as saying "We also note that the stripes are clearly visible to the eye before the 1D PSD peak becomes noticeable." These comments are made about *our own simulated striped images*, but are used to imply we agree we can see stripes in their images. The context here needs to be clarified.

Ong and Stellacci: "In reference [1], the main critique to the new images is that PSD might be quantifying random noise and/or scars in our images. Yet there is no explanation for why these random scars should be invariant with imaging speed, with imaging laboratory, but should vary in characteristic length scale upon the change in ligand shell composition. Are there scars that can distinguish between mixed and homoligand nanoparticles?"

First, "scars" are never mentioned in our paper. In addition, in our main criticism of the PSD analysis (Figure 10 and associated text) we do not suggest that this second shoulder arises from noise. We instead compare to randomly positioned speckles, i.e. unordered features with a different characteristic length scale. This entire paragraph misrepresents our argument and should be rewritten.

Ong and Stellacci: "And that the critique that a seven-parameter fit cannot be brought to fit a curve is peculiar to say the least."

This was not our argument at all. Our argument, instead, was that:
a) A seven-parameter non-linear fit is easily biased by initial conditions leading it to converge to a different answer.

b) That during the analysis certain areas were excluded without this being mentioned in the original work.

c) That when we applied these same fits we got poor convergence warnings.

I agree with the authors that they should talk about the fitting approach, however, they should address the arguments we actually made about their fit. Their statement above, re. the seven-parameter fit, is a straw-man argument.

In the section on liquids they write: "If we all agree that in such images there are molecular features". This sentence implies that our paper agreed that these are molecular features. However, in our discussion of the liquid images (Moglianetti et al., Ref 29 in our paper) we discuss tip convolution effects as a possible cause of these features.

**Minor issues**

The terms "proof" and "proved" are used in connection with the evidence presented. As Satoshi Kanazawa said in "Common misconceptions about science I: 'Scientific proof' "[15] :- "Proofs exist only in mathematics and logic, not in science."

The paper refers to our work as that of "Levy and co-workers". Raphaël Lévy indeed played an important role in this paper, but he is neither the first nor the corresponding author, nor did he perform the bulk of the analysis. This should be changed as it could be misinterpreted as referring to the 2012 Small paper from Levy's group[13].

The discussion complains about the "questionable order" of our paper. We presented all STM data chronologically, as it is very hard to understand how evidence develops from any other order. It is worth noting that a referee of our PLOS ONE paper was also firmly of the opinion that by far the clearest and most logical way to present a critique of Stellacci et al.'s work on striped nanoparticles was to do this chronologically. See https://raphazlab.wordpress.com/2014/09/14/stripes-open-access-and-copyright-as-a-form-of-censorship/

**Refs**


**REVIEW 2**

The manuscript has been changed to correct or clarify what I felt was misrepresentation in the original manuscript. Two cases seem to have slipped through. There is still one of the "most of the paper" comments in 'Issue of alignment section', and one reference to our work as that of "Levy and co-workers" at the end of the section 'Biscarini et al. Langmuir'. As the other cases of these issues were removed I assume these were left in accidentally.

However, one new major misrepresentation has been added to the paper. An entirely new paragraph has been added (Not in response to either authors comments) to the section 'Issue of varying gains'. This paragraph incorrectly claims:

"In reference [1] it is claimed that the model we used lacks the correct tip-sample interaction, so that Stirling in reference [1] went on to develop a better model. It is trivial to show that using the model of Stirling one reaches the same conclusion that we reached with our model. Importantly, in reference [1] when Stirling and co-workers wanted to show that feedback loop artifact can produce images that are similar to our striped nanoparticles images (something that in our literature we had shown on multiple occasions but this is presented as new finding) they do not use their own better model but resort to a model de-facto equivalent to the one we used in reference [15]. The nature of this evident contradiction is not clarified in reference [1]."

This statement is factually incorrect and highly misleading on multiple grounds:

1. The different model is not in ref [1], the model was actually published in the Beilstein Journal of Nanotechnology (DOI:10.3762/bjnano.5.38) in response to a model widely used in the SPM community.

2. The new model from the Beilstein paper is transfered from an analytical model to a numerical one for use in [1], but is otherwise not changed.
3. Finally claiming the models simulated in [1] and [15] are de-facto equivalent is simply incorrect. First, the model used in our papers have a second integration step (Equation 3 in Beilstein paper, Line 21 of algorithm provided in file S1 of [1]) when compared to [15]. Secondly, [15] uses the model from the book "Scanning Tunneling Microscopy", this model relies on the dynamics of the peizo-tube for the ringing effect shown in [15] (demonstrating this is the entire basis if the Beilstein paper!). The simulation in [1], however, clearly has no peizo dynamics included.

If Stellacci et al. disagree with the model presented in my Beilstein paper and [1], they are more than welcome to argue specifics on what mistakes they think I have made. However, to argue that [1] doesn't follow the model of the Beilstein paper, or that either of these papers uses the same model as [15] is factually incorrect. This newly added paragraph should either be removed, or should be rewritten to criticise the model in my Beilstein paper.

In conclusion I am happy that (except for two minor omissions) the paper has been amended to answer the misrepresentation in the original manuscript. One new paragraph, however, has been added which fundamentally misrepresents not just the article the authors are responding to, but also another of my papers. I hope that this paragraph can be changed/removed and that this response can quickly get into the literature.