Peer Review File

Manuscript Title: Quantum-enhanced nonlinear microscopy

Redactions – Third Party Material

Parts of this Peer Review File have been redacted as indicated to remove third-party material.

Redactions – Mention of other journals

This document only contains reviewer comments, rebuttal and decision letters for versions considered at *Nature*. Mentions of the other journal have been redacted.

Reviewer Comments & Author Rebuttals

Reviewer Reports on the Initial Version:

Referees' comments:

Referee #1 (Remarks to the Author):

In this work, the authors use a picosecond pulse train that has been squeezed to improve the pair-wise correlations of photons and thus reduce the classical photon shot noise. This light is used as the pump interaction in a stimulated Raman scattering (SRS) microscope. The authors investigate the SRS signals and find that the noise floor is lower, thus improving the signal to noise ratio (SNR). They demonstrate that SRS images can be obtained in this fashion and, according to their analysis, the quantum-enhanced images enable a better assessment of the sample. The main advantage would be that, in principle, less power could be used to acquire the images, and photodamage could be avoided.

The demonstration of SRS with squeezed light is well conceived and the authors show a lowering of the noise floor in the SRS signal. Therefore, this development is interesting. It aligns with the notion that quantum light exhibits unique noise properties because of photon correlation. The lower noise improves SNR and this would reduce the need for higher illumination powers. Overall, this is a concept worth pursuing and it can be expected that the use of quantum light will grow more prominent in the field of nonlinear optical microscopy in the years to come.

Despite the strong concept and the proof-of-principle demonstration, this manuscript overshoots its own aims. My concerns are as follows:
1) The manuscript makes a very strong claim, namely that the use of quantum light (squeezed light) will lower photo damage during SRS imaging. This point is made frequently in the manuscript and appears to be the main motivation for the work. In Figure 3c, there appears to be a small difference in SNR (11%) between quantum enhanced SRS and regular SRS at the onset of photo damage, whereas the actual photo damage appears to occur for both modes of illumination at ~26 mW of pump power. From the Figure it can be seen that the same SRS signal obtained with regular light at 26 mW of pump power can be achieved with ~24 mW of with squeezed light. This is a relatively small difference that is in practice not very meaningful. Since the Figure has no error bars, it can not be concluded that this small difference is actually significant. So, the sweeping statement about photo damage in the abstract, introduction and conclusion is not strongly supported by the actual data.
2) Improving SNR is obtained by lowering the noise, not be increasing the signal. It is not clear how much the sensitivity is improved in practice. A SRS sensitivity measurement usually involves

lowering the concentration of the analyze and observe the signal relative to noise, until the analyze signal becomes indistinguishable from the noise. Such a measurement is not performed. At 26 mW of pump power the authors report that with quantum light the detectable concentration is 20 mM whereas with classical light it is 19 mM. This is not a very meaningful difference.
3) The overall performance here is very underwhelming and is far removed from the state-of-the-art, despite remarks by the authors to the contrary. In regular SRS, pixel dwell times are 1 - 100 us, many orders of magnitude faster than what is reported here. Regular SRS reports sensitivities of ~0.1 mM for small analytes, which seems far lower than the limits discussed here. Despite the much longer integration times used here, the images shown are of inferior quality. The yeast cell image looks distorted and affected by scattering and transmission effects. The small reduction in noise (13%) has certainly not led to better images. In fact, they are worse than what is commonly acceptable.
4) The discussion of Figure 4b is debatable. The authors draw some very ambiguous conclusions. First, the parts of the cell that do not produce signal are probably caused by light scattering at the cell's edges. It is an artifact that has nothing to do with the lack of Raman scatterers in this region. Because it is an artifact, the authors cannot use this signal a Raman null. Second, there is no clear indication of a nucleus or membrane in the image. The image appears so distorted by light scattering that it is hard to assign any features. The only way to ascertain these features is by performing a fluorescence labeling study to prove that the brighter signal belongs to the nucleus. Last, why would the surrounding medium produce signal, but the cytosol of the cell does not? Unless the authors use a special medium, the concentration of organic compounds is much higher inside that outside the cell. Again, the dark regions are most likely artifacts.

Referee #2 (Remarks to the Author):

The authors demonstrate quantum-enhanced imaging based on stimulated Raman scattering (SRS). For 3 μm PS beads, 0.9 dB below shot noise (a 23% increase in SNR) could be reached, and for yeast cells 0.6 dB (a 14% increase in SNR), in images with good resolution giving meaningful detail. This is certainly significant and of importance in view of the fact that damage threshold is always a matter of concern in coherent Raman methods which use intense laser pulses. The threshold for photo-damage was established on PS beads. Also the sensitivity limit was measured using PS beads (not molecules in solution or embedded in a bio-sample such as a cell), assuming an acquisition time of 1s.

The manuscript describing this successful demonstration of true quantum-enhanced imaging is careful and well-written, no specific suggestions or critical points there.

I only have one issue: In my opinion, for this to be of practicality that is worth the effort, the most important question has not been addressed. How does the improvement quantitatively compare with simply increasing the acquisition time, at some suitable reduced intensity setting? The mentioned time of 1 s is not really short (compared to the time-scale of some biological processes). Thus i do not see any reason why one should not be able to increase it by some factor. A quantitative comparison is certainly required here. Of course the signal scales nonlinearly, but if this turns out to be problematic for the detection, then this fact should be included in the discussion.

In conclusion, I think this is a great piece of work, which can be made even stronger by including a comparison between the strategies of exploiting quantum correlations vs. prolonging acquisition time (at lower peak intensity). Without this i am not convinced of the broad impact which would make it a Nature paper. However, i wish to express strong support for publishing this manuscript in another Nature journal, such as **[Redacted]**

Referee #3 (Remarks to the Author):

Dear Editor
This paper presents the first realization of a Raman microscope, where the performances (signal to noise ratio) are enhanced, going under the shot noise limit, by using squeezed light.
On the one hand, squeezed light has already been used for enhancing performances of traditional interferometers (as gravitational wave detectors), of correlation interferometers, etc.; on the other hand sub shot noise has already been beaten in different protocols, as sub shot noise twin beams imaging. Anyway, the number of quantum metrology schemes outperforming classical counterparts is still rather limited and, often, far from practical applications. Thus this work presents significant elements of novelty and interest.
Concerning the methods, they are well described and data clearly demonstrate the quantum advantage. Conclusions summarize well the results and mention possible future developments.
Thus, in my opinion, this work represents a progress worth to be presented to a large audience, that Nature can guarantee.
Nonetheless, a related scheme for Raman spectroscopy appeared recently on ArXiv (with a stronger quantum advantage). The authors briefly mention it, Ref.44. In my opinion before taking a conclusive decision on the acceptance of this paper the authors should discuss more in detail differences and respective advantages/disadvantages of the two set-ups.
Moreover, the authors should consider a few further comments:
- Following a tradition and allowing a clear comparison with other experiments the squeezing should be reported in dB.
- The introduction should be improved, mentioning all previous experiments that exploited squeezed light for beating classical measurements, clarifying where and how shot noise was already beaten in imaging, mentioning also experiments on imaging with undetected photons, quantum illumination etc. when discussing applications of quantum correlations in this area….
- No uncertainty is reported for SNR in Fig.3, may be is too small for being visible.

Referee #4 (Remarks to the Author):

Review report

The manuscript reports the demonstration of a coherent Raman microscope using a squeezed light. The authors claim that they achieved images with the signal to noise ratio beyond the level which is limited by the photodamage caused by the laser illumination.
I have found that the reported experiment is interesting since it may open the door for the use of quantum engineered light in the real world applications in biology. When compared to the related very recent work (ref. 44), although both of the works are done with the similar laser power, this manuscript may attract more attentions since only this manuscript reports the damage threshold for the laser power and the enhancement of the SNR for the image of biological sample.
However, I have also found that many important details on their experimental data and apparatus are lacking in the current manuscript as shown in the comments below, especially for the quantum light source and detectors. In some cases, the uncertainty and reproducibility seems not clear enough. Therefore I am not enough convinced of the validity and reliability of their claims and results. For this reason, I cannot recommend the publication of the current manuscript in Nature.

Comment 1:
The detailed characterization of the squeezed state generated for Stokes light is lacking. For example the pump power dependence of the squeezed/anti-squeezed levels using a homodyne detector normally used for this type of evaluation needs to be provided to understand the quality of the squeezed state the authors generated (without the effect of the detector bandwidth of the resonance detector) .

Comment 2:
Generally, a homodyne detector is used for experiments for shot noise limited experiments to suppress the electric noise of the detectors as is also used in the authors' groups previous work, ref. [3]. In contrast, a single resonant-feedback photodiode (supposed to be optimized for 20 MHz) is used in this experiment. Please give a detailed characteristic of the detector used, for example circuit noise spectrum, sensitivity or gain spectrum and the bandwidth of the detector. With these data, please explain why it becomes possible to perform the shot-noise limited experiment without homodyne detection. Please also add some appropriate references for the resonant-feedback photodiode and related issues.

Comment 3:
Figure 3a just shows the spectrum of squeezed light normalized to the shot noise limit, however, the authors should also show the noise spectra with squeezed light and coherent state (shot-noise) before normalization. Such spectra may be important when the resonant feedback detector is used for the detection. Do the authors observe strong effect at the rep rate of the pulse laser (80MHz)? Why squeezing is limited up to 55 MHz? Please also provide the VBWs in addition to RBWs also for other measurement data.

Comment 4:
Figure 3c is one of the key results for their claim, however, the details how the data was taken for this graph is totally lacking. I assume that the authors might have measured this data using the same single polystyrene sphere with increasing the illumination power and at each power the authors switched the squeezer on and off. In this case, how did the authors switch the squeezer? How fast? How the laser / squeezed light powers are (strictly) controlled? It is also bit strange for me to see that the blue and red data points are changing consistently while "perfectly" maintaining the ratio.
As is explained in detail in Fig. 2b and Fig. 6, there are many different cases for the damages and the average damage threshold is about 60 mW as shown in Fig. 2b. However, currently the authors only show the single experimental result with the damage threshold of 25 mW (the lowest damage threshold in Fig. 2b). It is recommended to discuss some other data should also be discussed to ensure the reproducibility.

Comment 5:
Since it seems that the authors can switch on and off the squeezer, why the authors did not take the same image with the squeezer on and off for Figs. 4 a and 4b? By doing so, one can clearly compare the differences between classical light illumination and quantum light illumination. If the authors provide such figures, it would be definitely better.

Comment 6:
Please provide the pulse widths of the Stokes light and Pump light. How these pulse widths affect the improvement of the signal to noise ratio? I'm wondering the shorter pulse width for Pump light could improve the S/N when we consider the squeezed level of Stokes light changing in time inside the pulse. Are there any experimental data on this issue?

Comment 7:
The authors should more clearly discuss the effect of losses on their experimental results (improvement of SNR, or shot-noise floor). In page 6, the losses are estimated to be around 35%, but in page 9 the authors state the total optical efficiency as approximately 40%. More precise analysis on the effect of losses, which should be more clearly attributed to each of the optical components, and limited detector quantum efficiencies on the degradation of the improvement in SNR or squeezing level at the detector should be given. This is very important to evaluate the validity of the experiments.

Comment 8:
In figure 4, the authors claim 23% increase in SNR, but I wonder the increase in SNR depends on

the signal strength, and the definition is unclear here. (The same is true for 14% increase in SNR claimed for fig. 4b)

Comment 9:
In page 8, the last paragraph before Discussion and outlook section, the authors might infer the membrane cannot be observed without squeezed Stokes light. I think the authors should remove these sentences ("For instance…limited measurement.") since it is bit too complicated and quite misleading.

Comment 10:
In figure 5, the authors show the power scaling of Stokes laser noise variance without squeezing. Please provide this graph for the power up to 4 mW since the most of the key data is taken with the Stokes power at 3.0 mW. I'm afraid there is a slight signature of the saturation of the detector for 2.5 mW and higher powers since those data points are slightly below the fitting line. If so, please discuss the effect of the saturation of the detector for the experiment.

Author Rebuttals to Initial Comments:

Detailed response to Referees

Referee 1

In this work, the authors use a picosecond pulse train that has been squeezed to improve the pair-wise correlations of

photons and thus reduce the classical photon shot noise. This light is used as the pump interaction in a stimulated Raman scattering (SRS) microscope. The authors investigate the SRS signals and find that the noise floor is lower, thus improving the signal to noise ratio (SNR). They demonstrate that SRS images can be obtained in this fashion and, according to their analysis, the quantum-enhanced images enable a better assessment of the sample. The main advantage would be that, in principle, less power could be used to acquire the images, and photodamage could be avoided.

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We thank the Referee for this considered feedback. We agree that a key claim in our work is that quantumlight allows lower photodamage in SRS imaging. We do think, however, that there is a key difference in emphasis between what we aim to show (the proof-of-principle that quantum correlations allow absolute advantage in the presence of photodamage), and the Referees emphasis on the practical level of benefit provided in our particular experiments.

As discussed in the cover letter above, the absolute quantum advantage we demonstrate has not been shown before in microscopy, and is recognised as a significant milestone in the field of quantum technologies (e.g. being one of only two ten-year milestones for quantum-enhanced imaging in the UK quantum technologies roadmap). Indeed, it has not been shown in any previous quantum-enhanced sensing experiment, even with many hundreds of publications demonstrating that relative advantage is possible. As such its impact is broader than microscopy alone (though microscopy is, our minds, a particularly important application area). Our work parallels the dramatic recent demonstration of absolutequantum advantage in computing achieved by Google late last year. Even though the absolute improvement we achieve is relatively modest we believe very much that, as a first proof-of-principle of a longstanding and widely recognised milestone, our work is of sufficient importance to justify publication in*Nature*.

In our original submission we had avoided using the terms “quantum supremacy” or “absolute quantum advantage”, being a bit comfortable with such terms. We had also not clearly put our work in context as amajor milestone in quantum technology, that is identified prominently in technology roadmaps. We recognise now that this may have obscured the significance of our results to the Reviewer. We have updated the abstract, introduction, results and discussion to clearly identify that we achieve absolute quantum advantage for the first time (preferring this term to “quantum supremacy”), as well as to explain the importance of achieving this. We also now clarify that our experiments are the first, in any context, to use quantum techniques to overcome photodamage in both abstract and introduction.

We agree with the Referee that an 11% enhancement in signal-to-noise is not very practically meaningful. The 35% enhancement we now reported for cell imaging is more meaningful, for instance allowing imaging speeds to be reduced by a non-negligible factor (of 35%). Even with this improved performance, though, weaccept that the enhancement remains relatively modest. We see our result as a key proof-of-principle that paves the way for future much higher levels of improvement, and hope to have convinced the Referee that this is an important enough proof-of-principle to warrant publication in *Nature*.

We also agree with the Referee that in the original submission the lack of error bars in Fig 3c made it unclear whether the enhancement we observe is strictly statistically significant. We neglected error bars because they were unresolvable on the figure, though we should have included a discussion of the uncertainties so that the reader could judge the statistical significance of our result. We have now done thisin both the figure caption and main text. To summarise, the statistical one-sigma uncertainty in the signal tonoise values is less than 1%. For polystyrene, the maximum quantum-enhanced signal-to-noise is increased compared to the shot noise limit by 12.5 $\pm$ 0.4% (the quantum enhancement in SNR for cell imaging is 35%). This validates that our experiments do, indeed, achieve absolute quantum advantage given our apparatus and excitation sources, though as the Referee points out this advantage is relatively modest.

2) Improving SNR is obtained by lowering the noise, not be increasing the signal. It is not clear how much the sensitivityis improved in practice. A SRS sensitivity measurement usually involves lowering the concentration of the analyze and observe the signal relative to noise, until the analyze signal becomes indistinguishable from the noise. Such a measurement is not performed. At 26 mW of pump power the authors report that with quantum light the detectable concentration is 20 mM whereas with classical light it is 19 mM. This is not a very meaningful difference.

We agree with the Referee that the improvement in concentration sensitivity that we demonstrate is not very practically meaningful (while, as discussed above, the 35% improvement in imaging speed for fixed signal-to-noise and intensity is somewhat more meaningful). On reflection, the comparison of concentrationsensitivities with and without quantum enhancement, while

informative, was also somewhat distracting fromthe key message and results of the paper. That is, as discussed earlier, we see the main significance of ourwork to be that it uses quantum correlations to overcome photodamage limits for the first time (though we appreciate that this may not have been as clear as we wished in the original manuscript). The (modest) level of improvement in concentration sensitivity is, from this broader perspective, beside the point. We have now shifted the discussion of concentration sensitivity into the new Supplementary Information. We have retained it there because we feel that some readers will find it valuable. We hope that the Referee understands our motivations for this change, and that we can convince them that it is the proof-of-principle of absolute quantum advantage that is important in our work, rather than the absolute level of benefit provided.

3) The overall performance here is very underwhelming and is far removed from the state-of-the-art, despite remarks bythe authors to the contrary. In regular SRS, pixel dwell times are 1 - 100 us, many orders of magnitude faster than whatis reported here. Regular SRS reports sensitivities of ~0.1 mM for small analytes, which seems far lower than the limits discussed here.

We do see the Referees perspective, here. We agree with them to some extent, though not entirely as discussed below. In response we have made efforts to substantially improve our microscope. Our yeast cellimages are competitive with the best Raman images of yeast cells that we have been able to find in the literature (discussed further below and in the cover letter). However, since the key significance in our work is the first demonstration of absolute quantum advantage and not the practical performance of the microscope, we have de-emphasised claims of the microscope operating at the state-of-the-art throughout the manuscript.

We would highlight that our microscope does represent a very significant advance in capabilities for quantum microscopy, bringing such microscopes far closer to the state-of-the-art than has been possiblebefore. Justifying this, we would observe our twelve orders of magnitude higher peak light intensities compared to previous quantum imaging experiments ([20-29] in our manuscript); our eight orders of magnitude higher product of pump and probe intensities compared to previous quantum nonlinear spectroscopy experiments ([46,47] in our manuscript); and the ability to resolve sub-wavelength features.

As far as we can determine, our pixel dwell time is comparable or superior to dwell times reported previously in the Raman microscopy of yeast cells (see comparison below), and provides superior signal-to- noise. We agree with the Referee that our dwell times are well above those of state-of-the-art video-rate Raman microscopes. However, this is the result of technical rather than fundamental constraints – our custom-built microscope does not currently have the galvo-scanning capabilities required to achieve fast scanning. Instead, we are limited to much slower scanning of the sample stage. We hope that the Refereeagrees that this should not significantly impact the broad impact and significance of our work, given its technical, and that it does not compromise our key claims. We would observe that the bandwidth of quantum-enhancement we achieve is sufficient to allow video-rate imaging in future, were we to install appropriate galvo-scanners.

To clarify the above in the manuscript we have added the text:

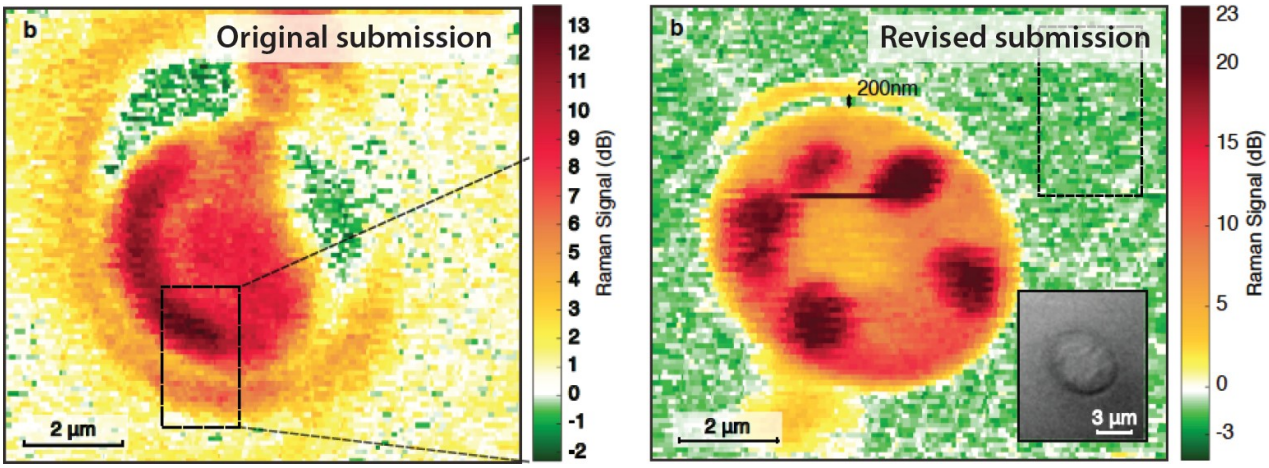
> *A pixel dwell time of 50 ms is used, limited by our stage scanning system. This is comparable with dwell times used in previous Raman imaging of yeast cells [48, 49], butis relatively long compared to state-of-the-art video-rate imaging [35]. The 40 MHz bandwidth of quantum enhancement demonstrated (Fig. 3a) is compatible with the fasterscanning systems necessary for video rate imaging, indicating that quantum-enhanced video-rate imaging should be possible in future.*

Regarding SRS sensitivity, we had compared our sensitivity to those discussed in [Wei *et al. Super- multiplex vibrational imaging* Nature **544** 465 (2017)], which states that without contrast enhancement "*detection*

sensitivity is limited to about 15 mM for typical chemical bonds such as C–H". We now realise that sensitivity beyond these levels is not uncommon, and appreciate the Referee correcting us on this. We have, of course, removed the claim of state-of-the-art sensitivity from the manuscript.

Despite the much longer integration times used here, the images shown are of inferior quality. The yeast cell image looks distorted and affected by scattering and transmission effects. The small reduction in noise (13%) has certainly notled to better images. In fact, they are worse than what is commonly acceptable.

We agree with the Reviewer that the yeast cell image in the original paper did (in retrospect) look distorted.In response to this comment we embarked on a major effort to optimise the squeezed light source used in the microscope, the alignment of the microscope itself, and our sample preparation. This has allowed us toproduce far higher quality yeast cell images, with to our (somewhat untrained quantum physicist) eyes no obvious distortion, scattering or transmission effects, and with 2.5 times higher quantum enhancement (of 35%), and sub-wavelength resolution down to around 200 nm. We thank the Referee for their perceptive assessment of the image in our original manuscript. We found this very helpful in diagnosing issues with our microscope.



For the Referee's convenience we include above a direct comparison between the cell image included in our original submission (left) and that in the revised submission (right). We hope that the Referee agrees that there is a very substantial improvement, and that the revised image is more to their satisfaction. Note,in addition to improved resolution and quantum enhancement, and reduced distortion, the improved microscope alignment has allowed an increase in Raman signal of around 10 dB.

After a careful survey of the literature, we have concluded that our images compare favourably to previously reported Raman images of yeast cells. We show below typical Raman-based yeast cell imagesfrom [Okuno *et al* Angew. Chem. **49** 6773 (2010)], [Svedberg *et al* J. Biomed. Opt. **15** 026026 (2010)], [Furuta *et al* Phys. Chem. Chem. Phys. **19** 13438 (2017)] and [Kochan *et al* Biotech. for Biofuels **11** 106 (2018)], arranged left -to-right in that order. Apart from [Kochan (2018)], each of these images was taken with Coherent Anti-Stokes Raman microscopy (CARS). [Kochan (2018)] was taken with spontaneous Raman microscopy. In our view our new images are significantly crisper, with superior resolution and signal-to-noise.

[Figure Redacted]

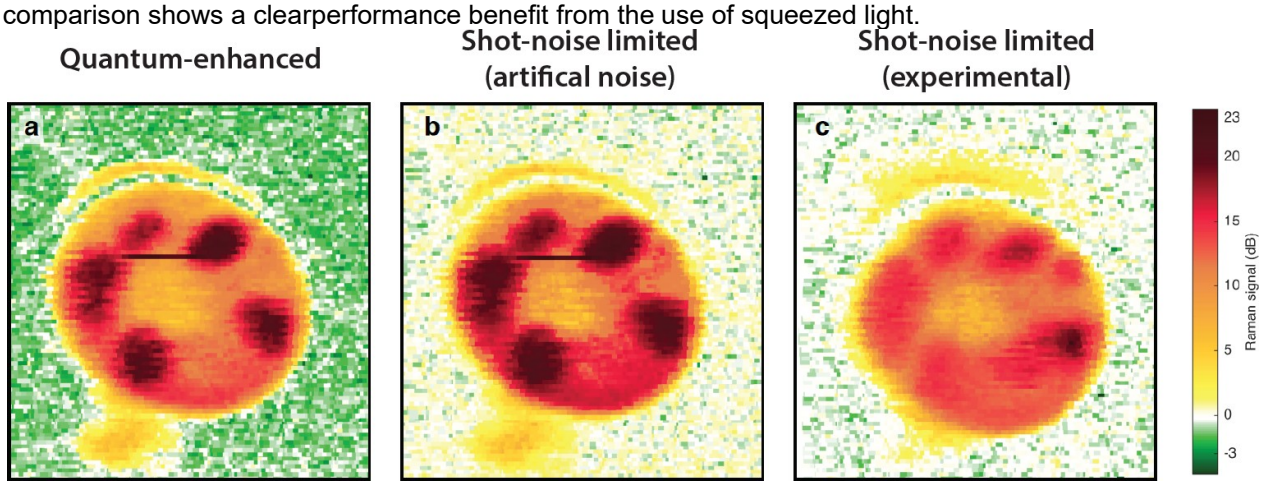
The shortest reported pixel dwell times for these yeast cell images from the literature was 50 ms, equal to ours [Okuno (2010)] (far left image). We note that in that case Raman images were taken simultaneously for fourteen Raman lines, so that the effective dwell time for each pixel could be considered to have been shorter. However, the peak signal-to-noise of around 5 is far inferior to our peak signal-to-noise of 100 (nowstated in the manuscript). For [Svedberg (2010)] (image second

from the left) a dwell time was not reported,but the signal-to-noise was also around 5. For [Furuta (2017)] (image second to the right) the image has a size of 30×30 pixels and was acquired in 8 minutes, corresponding to a 0.5 second pixel dwell time, around ten times longer than ours. [Kochan (2018)] reported a pixel dwell time of 0.1-0.3 seconds, also longer than ours. In both of these later cases it was not possible to quantify the signal-to-noise from the information given in the publications.

We now include the comparison of our images to this prior work explicitly in Section 12.1 of the Supplementary Information. We hope that the Referee agrees that the improved capabilities of ourmicroscope do allow high quality and reliable yeast cell imaging.

To enable a clear visual assessment of the enhancement in imaging due to quantum correlations, we nowinclude the quantum-enhanced image from the main text, along with shot-noise-limited images for comparison in Fig. S10 of the Supplementary Information. We compare to two shot-noise-limited images, one recorded directly after the quantum-enhanced image, and one produced by artificially increasing the noise floor of the quantum-enhanced image to the level of the shot-noise. We do both, (a) because the nonlinear process involved in generating squeezed light changes the spatiotemporal modeshape of the input Stokes beam, and therefore makes a strict comparison between measured quantum-enhanced and shot-noise-limited images invalid (see Supplementary Information Section 8.3), and (b) because the alignment of the microscope can drift over the time between taking images. The image with artificially increased noise floor removes these issues.

We have replicated this comparison between quantum-enhanced and shot-noise limited images below, again for the Referee's convenience. We hope the Referee also agrees that the comparison shows a clearperformance benefit from the use of squeezed light.



We also now include images of cells in both the main text and Supplementary Information that show clear photodamage. These were taken at pump intensities about a factor of two higher than our damage-free cellimages, and demonstrate that the light intensity is indeed tightly constrained by the properties of the specimen. We consider this a nice further addition to the manuscript.

4) The discussion of Figure 4b is debatable. The authors draw some very ambiguous conclusions. First, the parts of thecell that do not produce signal are probably caused by light scattering at the cell's edges. It is an artifact that has nothing to do with the lack of Raman scatterers in this region. Because it is an artifact, the authors cannot use this signal a Raman null. Second, there is no clear indication of a nucleus or membrane in the image. The image appears so distorted by light scattering that it is hard to assign any features. The only way to ascertain these features is by performing a fluorescence labeling study to prove that the brighter signal belongs to the nucleus. Last, why would the surrounding medium produce signal, but the cytosol of the cell does not? Unless the authors use a special medium, theconcentration of organic compounds is much higher inside that outside the cell. Again, the dark regions are most likely artifacts.

We agree. As (relatively speaking) somewhat naïve quantum scientists, we are grateful for the Referee 1's expertise in this area. They have highlighted some significant issues with the yeast cell

image in our originalsubmission. We believe that the improved images in the new submission are far less ambiguous. The Raman signal from the cytosol of the cell is resolvable, and there is no resolvable signal outside the cell.
We observe large Raman signals for five droplet-like features, that are consistent with lipid droplets commonly observed in yeast cells (these can be seen also in two of the yeast cell images from the literatureabove). We also observe a thin semi-circular feature that may be a section of the cell membrane. Since we have no other method to confidently classify these features, we now provide a careful discussion of them in the Supplementary Information (Section 12.1), supported by other results in the literature. We have also endeavoured to be careful in interpreting them both in the manuscript and in the Supplementary Information, describing them as "consistent with" lipid-droplets and cell membrane, rather than attributing them as such with certainty.

We would emphasise that, irrespective of the exact nature of the observed structures, we believe the manuscript does conclusively show the key results of quantum-enhanced imaging and quantum advantage.

Referee 2

The authors demonstrate quantum-enhanced imaging based on stimulated Raman scattering (SRS). For 3 µm PS beads, 0.9 dB below shot noise (a 23% increase in SNR) could be reached, and for yeast cells 0.6 dB (a 14% increasein SNR), in images with good resolution giving meaningful detail. This is certainly significant and of importance in view of the fact that damage threshold is always a matter of concern in coherent Raman methods which use intense laser pulses. The threshold for photo-damage was established on PS beads. Also the sensitivity limit was measured using PS beads (not molecules in solution or embedded in a bio-sample such as a cell), assuming an acquisition time of 1s.

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In conclusion, I think this is a great piece of work, which can be made even stronger by including a comparison between the strategies of exploiting quantum correlations vs. prolonging acquisition time (at lower peak intensity). Without this i am not convinced of the broad impact which would make it a Nature paper. However, i wish to express strong support for publishing this manuscript in another Nature journal, such as ***[Redacted]***

We thank the Referee for their positive assessment of our work. They raise a very important question, of atwhat stage quantum microscopy techniques such as ours will have a true practical benefit. It is, indeed, to this ultimate end that we are putting our efforts. A key challenge is to reach regimes where easy solutions are not available without using quantum techniques. That is to say, where you cannot simply increase illumination intensity to achieve improved performance. As discussed in the cover letter and responses to other Referees, the major contribution our paper makes is to for the first time reach such a regime, and within it to show absolute quantum advantage. Previous experiments were very far away indeed – some twelve orders of magnitude in intensity – so we believe this is a very substantial advance. As discussed in our cover letter and in the revised manuscript, we believe this to be a significant achievement, recognised for instance as a key decadal milestone in the UK Quantum Technologies Roadmap.

The Referee is right that in practical situations one would certainly ask what routes are available, other thanquantum-correlations, to evade photodamage – whether that be to gently measure the specimen at low light intensities for longer periods, or to add contrast agents to improve signal-to-noise, or some other strategy. However, such choices come at a cost. In imaging, the acquisition rate is often a critical parameter. Indeed, in our case we find that the specimen moves and the microscope drifts over timescales not too much longer than the imaging time. Furthermore, some sources of photodamage (such as reactive oxygen species build-up) depend on total irradiation energy, rather than intensity. In this case, performing aslower lower intensity measurement will not protect the specimen from damage, only serving to slow the total measurement acquisition rate. On the other hand, using a contrast agent introduces the risk of perturbing the natural behaviour of the specimen. The key conclusion of our work is showing that quantum correlations provide a pathway to overcome these compromises – offering higher signal-to-noise without longer acquisition, higher intensity or contrast agents. Indeed, quantum correlations offer the only known means to break these compromises.

We would observe that in non-linear microscopes such as coherent Raman microscopes, imaging frame- rate is a crucial concern due to the weakness of the non-linear scattering cross sections (e.g. Ref. [35] in the manuscript). In Raman microscopy this is especially true, since it is in principle possible to perform parallel multispectral imaging (e.g. Ref. [9] in the manuscript) to extract very rich chemical information aboutthe specimens under study, but this requires multiple measurements at different Raman shifts and often requires probing of molecular bonds with far weaker Raman cross sections than C-H bonds. As such, compromises that involve increased measurement duration are not typically preferable.

We have now better clarified in the manuscript these choices and compromises. Specifically, we have included quantitative comparisons between speeds of quantum-enhanced shot-noise limited imaging (as requested by the Referee) in the Quantum-Enhanced Imaging section of the manuscript, a brief discussion of the ability of quantum correlations to break the compromise between signal-to-noise and measurement time in the Discussion and Outlook, and a new section in the Supplementary Information (Section 13) describing some of the compromises in further detail. To perform the quantitative comparison, we recognisethat the signal-to-noise of a shot-noise limited measurement scales linearly with the measurement time (in power units). Therefore, to increase it by some percentage requires the same percentage longer measurement duration. Consequently, to achieve our 35% quantum signal-to-noise enhancement via, instead, a longer measurement would require the measurement to be 35% longer. Following this approach, we have undertaken an analysis of our quantum-enhanced cell image, which allowed us to draw conclusions about the highest quantum and classical imaging speeds possible while still resolving biological Raman signals in our microscope. This is now included in the Quantum-Enhanced Imaging section of the manuscript.

We would note that our pixel dwell time is 50 milliseconds, rather than 1 second, and as we discuss in the new manuscript, could be reduced into the microsecond range before the biological Raman signal becameunresolved. This is more comparable with timescales for biological dynamics, but still slow compared to video rate imaging (as now also discussed explicitly in the manuscript).

We were not 100% sure of the precise nature of additional information the Referee wished to see included in the manuscript, and so have tried to be inclusive. We would emphasise that, in our view, the work reported in our manuscript goes far beyond previous microscopes that use quantum-correlated illumination,both from a fundamental perspective (achieving absolute quantum advantage for the first time), and on a practical level (achieving twelve orders of magnitude higher peak intensities, achieving sub-wavelength resolution, performing imaging directly on living cells, observing photodamage, comprehensively qualifying photodamage…). As discussed in the cover letter and response to other Referees, we feel that the original manuscript perhaps did not emphasise the first proof-of-principle of absolute quantum advantage sufficiently, as the key important result of the paper. We hope that the context provided, as well as the additional text and quantitative comparison in the manuscript and Supplementary Information, cover the issue raised by the Referee to their satisfaction and that they conclude that publication in *Nature* is warranted.

Referee 3

This paper presents the first realization of a Raman microscope, where the performances (signal to noise ratio) are enhanced, going under the shot noise limit, by using squeezed light.

On the one hand, squeezed light has already been used for enhancing performances of traditional interferometers (as gravitational wave detectors), of correlation interferometers, etc.; on the other hand sub shot noise has already been beaten in different protocols, as sub shot noise twin beams imaging. Anyway, the number of quantum metrology schemes outperforming classical counterparts is still rather limited and, often, far from practical applications. Thus this work presents significant elements of novelty and interest.

Concerning the methods, they are well described and data clearly demonstrate the quantum advantage. Conclusions summarize well the results and mention possible future developments.

Thus, in my opinion, this work represents a progress worth to be presented to a large audience, that Nature can guarantee.

Nonetheless, a related scheme for Raman spectroscopy appeared recently on ArXiv (with a stronger quantum advantage). The authors briefly mention it, Ref.44. In my opinion before taking a conclusive decision on the acceptance of this paper the authors should discuss more in detail differences and respective advantages/disadvantages of the two set-ups.

We agree with the Referee that Ref. [46] (Ref. [44] in our original submission) performs quantum-enhancedstimulated Raman spectroscopy in a manner qualitatively similar to our experiments. However, quantitatively, there are very large differences between the works. The most important difference is our ability to observe and evade photodamage – allowing *absolute quantum advantage* to be achieved for the first time. We feel it is very important to clarify this key difference (which we have also emphasised elsewhere in our response). While Ref. [46] demonstrates a relative quantum advantage, it does this with very low light intensity (which is the relevant parameter, rather than power, see below). As such, the advantage demonstrated is not absolute – it could have more easily been achieved simply by increasing optical intensity. Indeed, this argument can be made for all quantum-enhanced imaging experiments prior to ours, but does not hold in our case. Our experiment is fundamentally different in this respect because it operates at intensity levels that cannot be increased without causing photodamage to the sample (and levels relevant to precision microscopy). This, we believe, is the central novelty of our paper.

As well showing absolute quantum advantage for the first time, a second key difference is that our experiments extend the state-of-the-art in quantum-enhanced imaging of biological specimens. We are ableto observe Raman signals from a living biological system, and from nanoscale structures within it, as well as to achieve imaging with sub-wavelength resolution (around 200 nm). We note, also, that Ref. [46] implements only spectroscopy – it does not demonstrate imaging.

We agree with the Referee that the *relative* quantum enhancement that we demonstrate is lower than thanin Ref. [46]. They demonstrate a quantum reduction in the measurement noise floor by 3.6 dB (or 56%) below the shot noise limit, corresponding to a more than 100% improvement in the signal-to-noise ratio. Forcomparison, the best relative quantum advantage we demonstrate is a 35% improvement of signal-to-noiseratio for cell imaging. However, we hope that the above discussion clarifies for the Referee that it is the *absolute* quantum advantage that is the key novelty of our work.

The key scientific differences in our work discussed above are enabled by our use of picosecond pulsed light and specially designed high numerical aperture microscope objectives. The signal from non-linear microscopes depend critically on the peak intensity of the light used. Essentially all nonlinear microscopes use pulsed light and high numerical aperture objectives for this reason. By

contrast, Ref. [46] uses continuous wave light and low numerical aperture lenses. This is far more straightforward to implement, and far more conventional in the quantum optics community. But it is also far less relevant for microscopy. The signal in stimulated Raman spectroscopy scales as the product of the peak pump and Stokes intensities. This product is eight orders-of-magnitude higher for our experiments than in Refs. [46]. This enhanced signal is crucial for achieving meaningful bioimaging results, and not trivial to achieve. The use ofpulsed light and tight focussing also brings the microscope into the regime where photodamage due to, e.g.ablation, can occur. Therefore, it is crucial for both of the main results in our manuscript: the achievement ofperformance beyond photodamage limits and the quantum-enhanced imaging of faint structures in biological media.

We note that, after our submission, Ref. [47] also appeared on the arXiv. That paper reports similar resultsto Ref. [44]. It is differentiated in the same way from our work – eight orders of magnitude lower product ofpeak pump and probe intensities, using low numerical aperture lenses, and not performing imaging.

We wish to avoid being overly dismissive of Refs. [46,47] in our manuscript, since they are a nice papers including nice results. However, we hope that the above discussion clarifies for the Referee that our work isvery much a major advance beyond what they report, as well as beyond other previous quantum-enhancedsensing experiments. To better clarify the differences in the paper, we have replaced the paragraph referring to these works by:

> *"We note two parallel demonstrations of quantum-enhanced nonlinear spectroscopy [44, 45]. Both use peak optical intensities well below the levels used in state-of-the-art nonlinear micro- scopes, and do not observe photodamage. Nor do they perform imaging, though Ref. [44] does report spatially distributed measurements. Our combination of pulsed excitation and high numerical aperture objectives enables orders-of-magnitude higher peak intensities for both pump and Stokes. This brings the microscope into the regime where photodamage is relevant, and increases the nonlinear signal amplitude (proportional to the product of peak intensities) by around a factor of 10^8."*

Moreover, the authors should consider a few further comments:

- Following a tradition and allowing a clear comparison with other experiments the squeezing should be reported in dB.

We agree with the Referee, this is a good suggestion. We had chosen to report percentages since theseare more accessible to a broad audience. We now include both percentages and dB throughout.

- The introduction should be improved, mentioning all previous experiments that exploited squeezed light for beating classical measurements, clarifying where and how shot noise was already beaten in imaging, mentioning also experiments on imaging with undetected photons, quantum illumination etc. when discussing applications of quantumcorrelations in this area….

We thank the Referee for this suggestion. In general, we find referencing to be particularly challenging for manuscripts which cross disciplinary boundaries and have strict length and reference limits. How to appropriately acknowledge all of the very important previous research in both quantum measurement and biological imaging within these constraints is a difficult task. In our approach to writing the introduction, wedecided to address this by focussing discussion of quantum-enhanced imaging on applications rather thantechniques – so phase-contrast imaging or absorption imaging, rather than quantum illumination or ghost imaging. This allowed us to write an introduction that was accessible to the non-specialist, and we believe,covered the required ground in an reasonably appropriate way. Indeed, we note that papers were included both on quantum illumination (Ref. [21]), and derived from the original work on imaging with undetectedphotons (Refs. [28,29]).

Rereading the introduction we do see the Referee's perspective. To try to address this without changing theapproach to the introduction (which we still consider appropriate and more widely accessible than the alternative) we have included the sentence

> *"They have also been shown to improve many other optical measurements in proof-of-principle experiments [5,19]."*

directly after discussing the application of quantum correlations in gravitational wave detection. The references are to a high profile review paper providing an overview of quantum metrology [Giovannetti *et al*,*Nature Photonics* **5** 222 (2011)], and a second review paper focussing on the applications of quantum metrology in biology [Taylor & Bowen *Physics Reports* **615** 1 (2016)]. We have also included two further additional references, one to the original experimental paper on quantum imaging with undetected photons [Lemos *et al, Nature* **512** 409 (2014)] and one to a further recent experimental paper on quantum illumination [Gregory *et al*, *Science Advances* **6** eaay2652 (2020)].

While we feel that, perhaps, these changes are not as substantial as the Referee may have desired, we hope that they do appreciate the balancing act in conveying the science to both communities, appropriatelyacknowledging the work of both communities, and fitting all of this into a few pages!

- No uncertainty is reported for SNR in Fig.3, may be is too small for being visible.

The Referee is correct. The uncertainties were too small to be visible and we had omitted them. Nevertheless, our claim of absolute quantum advantage depends critically on the difference in quantum andshot-noise limited signal-to-noises being statistically resolvable. As such, strictly, we should have included adiscussion of the uncertainties. As also discussed in our response to Referee 1, we have now included this both in the figure caption and in the main text. To summarise here, the uncertainty in each of the signal-to- noise values is less than 1%, and the ratio of the maximum quantum-enhanced to shot-noise limited signal to noise is $12.5 \pm 0.4\%$ before the onset of photodamage.

Referee 4

The manuscript reports the demonstration of a coherent Raman microscope using a squeezed light. The authors claim that they achieved images with the signal to noise ratio beyond the level which is limited by the photodamage caused by the laser illumination.

I have found that the reported experiment is interesting since it may open the door for the use of quantum engineered light in the real world applications in biology. When compared to the related very recent work (ref. 44), although both ofthe works are done with the similar laser power, this manuscript may attract more attentions since only this manuscriptreports the damage threshold for the laser power and the enhancement of the SNR for the image of biological sample.

We thank the Referee for the positive assessment. We would take the opportunity here to highlight very fundamental differences between our work and that of Ref. [44] ([46] in the new manuscript). As discussed also in our response to Referee 2, in nonlinear microscopy it is the product of peak intensities of the pump and probe that matters, rather than the power (both for the strength of signal that is generated, and generally also for the exposure to photodamage). Our work combines picosecond pulsed light (not trivial to integrate with quantum correlations) with high numerical aperture objectives. Ref. [46] on the other hand, uses continuous wave light and low numerical

aperture objectives. While such an approach is more conventional for quantum optics, it very far from relevant for most (if not all) nonlinear microscopy applications. Indeed, the increased peak intensities in our experiments offer an eight order of magnitude larger stimulated Raman signal (as well as, importantly, meaning our microscope reaches the regime wherephotodamage is a critical barrier). We note, also, that imaging was not performed in [46], while the far higher resolution and signal strength in our experiments allow us to perform sub-wavelength imaging of living biological specimens.

We believe that our work makes not only a very important fundamental advance when compared to previous quantum imaging experiments (demonstrating that quantum corrections can provide absolute quantum advantage), but also a very significant technological leap, bringing them into the domain of precision microscopy for the first time. As such, we hope that the Referee understands why we feel it important to clarify these differences (As discussed in our response to Referee 2, we have now updated therelevant paragraph in the manuscript to address the differences more clearly within it).

However, I have also found that many important details on their experimental data and apparatus are lacking in the current manuscript as shown in the comments below, especially for the quantum light source and detectors. In some cases, the uncertainty and reproducibility seems not clear enough. Therefore I am not enough convinced of the validity and reliability of their claims and results. For this reason, I cannot recommend the publication of the current manuscript in Nature.

This is a very good point. We agree entirely with the Referee that improvements in the discussion of how we characterise squeezing and validate our results were required. We have made significant efforts to make these improvements both in the main text and by including twenty four pages of additional technicaldetail in a new Supplementary Information.

Comment 1:

The detailed characterization of the squeezed state generated for Stokes light is lacking. For example the pump power dependence of the squeezed/anti-squeezed levels using a homodyne detector normally used for this type of evaluation needs to be provided to understand the quality of the squeezed state the authors generated (without the effect of the detector bandwidth of the resonance detector).

We agree with the Referee that, given the importance of accurately knowing the squeezing level, these sortof characterisations are vital. We have now included this information in Sections 7–9 of the Supplementary Information. For technical reasons, described below and in the Supplementary Information, it was not possible to perform homodyne detection. Instead, we characterised the level of squeezing and classical deamplification as a function of the optical parametric amplifier pump power using direct detection (shown in Fig. S8). As one would expect, the squeezing improves with increasing pump power. However, perhaps counterintuitively, past a critical pump power the squeezing begins to degrade again. This effect is due to the fact that we do not use a cavity around the nonlinear crystal, and is a key limitation on the amount of squeezing we are able to generate. Any part of the seed for the optical parametric amplifier (which becomes our Stokes beam) that is not spatially overlapped with the pump is neither deamplified or squeezed. When increasing the pump beyond the optimal level, this non-deamplified component of the seed contributes an increasing proportion of the noise measured with the direct detection we use, and for complete de-amplification would result in noise at the shot-noise limit.

We developed a model for this effect which fits our measured deamplification and squeezing very well (Fig. S8), and allows us to extract critical parameters such as the overlap efficiency between the pump and seed,the nonlinearity of the nonlinear crystal, and the detection efficiency. This gives us confidence that we understand the process generating squeezing and the resulting squeezed variance very well.

See below for discussion of the detector design, bandwidth and resonance.

Comment 2:

Generally, a homodyne detector is used for experiments for shot noise limited experiments to suppress the electric noise of the detectors as is also used in the authors' groups previous work, ref. [3]. In contrast, a single resonant-feedback photodiode (supposed to be optimized for 20 MHz) is used in this experiment. Please give a detailed characteristic of the detector used, for example circuit noise spectrum, sensitivity or gain spectrum and the bandwidth of the detector. With these data, please explain why it becomes possible to perform the shot-noise limited experiment without homodyne detection. Please also add some appropriate references for the resonant-feedback photodiode and related issues.

We agree, again, with the Referee. Usually, homodyne detection would be used in measurements of squeezed light, both to suppress electronic noise and also to provide a convenient calibration of the shotnoise floor (by blocking the input signal field).

The primary reason that we chose to use direct detection here is that homodyne detection generally requires a local oscillator power at least an order of magnitude higher than the signal power. Unlike most experiments with squeezed light, in our experiments the signal power must be relatively high to reach parameter regimes relevant to state-of-the-art Raman microscopy and for which photodamage starts to become apparent. The local oscillator powers required for homodyne detection would then introduce significant challenges in detector design. A second reason to choose direct detection is that it avoids the need to mode-match the signal field to another field after it has passed through the microscope, and therefore relaxes requirements on the beam quality of the transmitted signal field. The fact that we work with higher power squeezed fields than most experiments also explains why we are able to perform direct measurements with good clearance from the electronic noise.

We now include in Section 8 of the Supplementary Information a detailed discussion of our design choicesfor squeezed-light detection, and of the designs and performance of the resonant feedback photodiode weuse. This includes the circuit diagram (Fig. S3), as well as the bandwidth (Fig. S4). Fig. S4 shows the electronic noise floor of the detector, with a resonant feature at 20 MHz, as well as the measured noise spectrum for increasing levels of incident shot-noise limited light. The measured noise floor clearly shows the shot-noise limited bandwidth of the detector (see Fig. S6 for a magnified version) as well as the sharp peak at 80 MHz due to the laser repetition rate. The detector design is an unpublished one that we developed in-house. As such, we were unable to find an appropriate publication describing it in detail.
However, we have included reference to the paper [Serikawa & Furusawa *Rev. Sci. Instr.* **89**, 063120 (2018)] which discusses many of the same considerations as well as the noise performance, and presentssimilar designs to ours. We hope that this fully addresses the Referees comment.

We do note that a significant advantage of using homodyne detection would be that the local oscillator onlyamplifies the component of the signal field that is overlapped with its spatiotemporal mode. In this way, it might be possible to avoid the degradation in squeezing we observe at high squeezer pump powers, discussed in our response to the Referee's Comment 1 above. This would be achieved by carefully mode matching the local oscillator field to only the squeezed part of the Stokes field. Here, for simplicity in designand experimentation, we do not do this. We believe that it could be implemented, along with redesigned detectors, to improve performance in future quantum-enhanced nonlinear microscopes. We have added a comment to this effect in Section 9.2 of the Supplementary Information.

Comment 3:

Figure 3a just shows the spectrum of squeezed light normalized to the shot noise limit, however, the authors should also show the noise spectra with squeezed light and coherent state (shot-noise) before normalization. Such spectra may be important when the resonant feedback detector is used for the detection. Do the authors observe strong effect

at the rep rate of the pulse laser (80MHz)? Why squeezing is limited up to 55 MHz? Please also provide the VBWs in addition to RBWs also for other measurement data.

We agree with the Referee also on this point. We have now included the unnormalized spectrum of squeezed light in Fig. S6 of the Supplementary Information, as well as the unnormalized shot-noise limitedspectrum in Fig. S3 (as already mentioned above). It can be seen from Fig. S3, there is indeed a strong peak at 80 MHz due to the laser repetition rate and that the shot noise level sinks into the electronic noise floor at around 55 MHz, limiting the squeezing to lower frequencies. We note that, as now discussed in Section 8.1 of the Supplementary Information, the detector is designed to suppress the signal from the laser repetition rate. However, since it is a very large signal, a strong residual peak remains.

Comment 4:

Figure 3c is one of the key results for their claim, however, the details how the data was taken for this graph is totally lacking. I assume that the authors might have measured this data using the same single polystyrene sphere with increasing the illumination power and at each power the authors switched the squeezer on and off. In this case, how did the authors switch the squeezer? How fast? How the laser / squeezed light powers are (strictly) controlled?

These are fair questions. On re-reading the submission, we realise that some of these important detailed were not sufficiently clear, and thank the Referee for identifying this. Each measurement of the signal-to- noise ratios was performed using data identical to that shown in Fig. 3b. That is, two spectra were recorded, one of the stimulated Raman signal on top of the squeezed noise floor (a quantum-enhanced measurement), and the other of the shot noise floor (measured by blocking both the Raman pump field andthe squeezer pump field, and adjusting the Stokes power on the detector to equal the power used in the quantum-enhanced measurement). Fits of these two measurements provided the Raman signal power, theshot-noise power and the squeezed-noise power, from which the two signal-to-noise ratios could be established. The measurements at each power level took a few minutes to complete. The squeezed light power was controlled by hand to be maintained at 3 mW on the resonant squeezing detector. The Raman pump power was found to be stable to within 1% over the course of the measurements.

We now include a detailed discussion of our approach to characterising the quantum enhancement in Section 8.3 of the Supplementary Information, as well as quantified uncertainties and a more detailed description of how the signal-to-noise ratios are calculated both in the caption of Fig. 3 and in the main text.

The Referee suggests an alternative approach which would in principle be more straight forward – that is make two direct measurements of the signal-to-noise ratio, one using squeezed light and the other using shot-noise limited light. While intuitively attractive, we found this approach to give inaccurate results. The reason for this is that the amplification process in the squeezed light source changes the spatiotemporal modeshape of the Stokes beam (as discussed above, only the complement of the Stokes beam that is well aligned with the optical parametric amplifier pump is deamplified). Since the Raman gain is highly sensitiveto the intensity of the pump, and therefore its spatiotemporal profile, making measurements in this way wasfound to convolve the quantum-enhancement that we wish to show with classical effects on the Raman signal amplitude. We note that other experiments showing quantum-enhancement in nonlinear spectroscopy have encountered similar issues and resolved them in the same was as we do (see e.g. Ref. [47] in the manuscript).

It is also bit strange for me to see that the blue and red data points are changing consistently while "perfectly"maintaining the ratio.

The Referee is correct that the quantum-enhanced and shot-noise limited signal-to-noise ratios

exhibit some common mode fluctuations as a function of pump power. These fluctuations arise from 1%-level driftsof the laser intensity between measurements. They change the Raman signal height, but not the squeezed or shot-noise limited noise floors. Since in our signal-to-noise analysis we use a single characterisation of the Raman signal height for both shot-noise limited and quantum-enhanced signal-to-noises at each power level (as discussed above and Supplementary Information Section 8.3), the fluctuations are common mode.

As is explained in detail in Fig. 2b and Fig. 6, there are many different cases for the damages and the average damage threshold is about 60 mW as shown in Fig. 2b. However, currently the authors only show the single experimental result with the damage threshold of 25 mW (the lowest damage threshold in Fig. 2b). It is recommended to discuss some other data to ensure the reproducibility.

We thank the Referee for this observation. The statistical photodamage data in Fig. 2b&c was derived frommore than one hundred measurements on different polystyrene beads (of traces similar to the one in Fig. 2a). We therefore have high confidence in its accuracy.

In Fig. 3, where we compare the quantum-enhanced and shot-noise-limited signal-to-noise ratios, a series of 36 independent measurements (18 each with and without quantum enhancement) are made as a function of pump power for a single polystyrene bead. Unfortunately, due to major optimisations that we undertook to the microscope, we were not able to provide a comparable series of measurements for a different bead in revision. These optimisations were made to achieve higher quality cell images. While they allowed us to demonstrate both sub-wavelength resolution (as is possible for nonlinear microscopy) and substantially improved quantum enhancement (and therefore, we believe, significantly improve the quality of the manuscript), they also will have most certainly significantly affected the spatial shapes of the pump and Stokes beams at the specimen. As a consequence, further measurements of the photodamage made on polystyrene beads would not be made in the same circumstances as our original measurement, or thosein which we characterised the statistics of photodamage in Fig. 2. These measurements would therefore not be consistent with our existing data.

In order to address the Referees comment given this limitation, we have performed a careful statistical analysis of the uncertainty in our measurements in Fig. 3. From this analysis, we have determined that thestatistical uncertainty in every signal-to-noise data point in the figure is beneath 1%. This level of uncertainty is well below the level of quantum-enhancement observed. Indeed, the ratio of maximum quantum-enhanced to shot-noise limited signal-to-noise-ratios yielded a highly statistically significant absolute quantum enhancement of $12.5 \pm 0.4\%$. Furthermore, the 28 individual measurements in Fig. 3 which are taken at powers beneath photodamage levels closely follow the quadratic trend with pump powerexpected for stimulated Raman scattering, while quantum-enhanced performance is shown consistently forall 18 pairs of measurements. Together, we believe that this allows confident and reliable conclusions to bedrawn from the one series of measurements.

As a minor point, we note that for the polystyrene bead in Fig. 3, significant photodamage begins at a pumppower somewhere in the range of 35-40 mW, rather than 25 mW (at which point the signal starts to diverge from the expected damage-free trend). This higher power, while still somewhat low, is more consistent with expectations given the photodamage statistics in Fig, 2b&c. It is possible that the discrepancy arises from the different approaches used to take the two measurements – in one case, continuously ramping the pumppower up over time, and in the other increasing the pump power in discrete steps and pausing at each step to take both quantum-enhanced and shot-noise limited data. These differences in measurement modality prevent, in our view, a quantitative comparison of the power at which damage typically occurs between the two measurements. We would emphasise that the exact power at which photodamage occurs is not critical to the conclusions of our work, just the photodamage does occur and that quantum light allows a higher signal-to-noise ratio than shot noise limited light within this photodamage constraint.

Comment 5:

Since it seems that the authors can switch on and off the squeezer, why the authors did not take the same image with the squeezer on and off for Figs. 4 a and 4b? By doing so, one can clearly compare the differences between classicallight illumination and quantum light illumination. If the authors provide such figures, it would be definitely better.

This is an excellent question and connects back to the Referees earlier question about how we compare the classical and quantum signal-to-noise ratios (Referee's Comment 4). Because the squeezed light source changes the spatiotemporal profile of the Stokes beam, it is not possible to simply quantitatively compare quantum-enhanced and shot-noise limited images. As discussed also in our response to Comment 4, this issue is not unique to our experiments (see e.g. Ref. [47] which adopts the same solutionas we do to avoid unreliable comparisons). We entirely agree with the Referee that, were it possible to make the direct quantitative comparison, this would absolutely be the natural and appropriate thing to do.

To address the Referees question as best as possible, we have included a new Figure (Fig. S10) in the Supplementary Information (copied also for convenience in our response to Referee 1). This shows a quantum-enhanced image of a cell, a shot-noise limited image of the same cell taken thirty minutes after the quantum-enhanced image, and a shot-noise limited image created by adding an appropriate amount ofartificial noise to the quantum-enhanced image such that its noise floor equals the shot-noise limit. This allows a direct visual assessment of the benefit of using quantum correlated light, along the lines of the Referee's suggestion.

It can be seen from these images that the experimental shot-noise limited image is significant degraded compared to the quantum-enhanced image, both in contrast and resolution. We believe this is caused bymultiple factors: 1) the inferior (non-quantum-enhanced) noise floor, 2) drift in the microscope, and 3) thedifferent spatiotemporal profile of the Stokes beam without squeezing. We therefore believe a direct quantitative comparison of this image with the squeezing-enhanced image is not appropriate. The shot- noise limited image created by adding artificial noise provides a much fairer comparison. Here, it is also possible to see a meaningful increase in contrast using squeezed light, with features within the cell becoming significantly more visible. We hope that this explanation and additional analysis addresses theReferees comment to their satisfaction.

Comment 6:

Please provide the pulse widths of the Stokes light and Pump light. How these pulse widths affect the improvement of the signal to noise ratio? I'm wondering the shorter pulse width for Pump light could improve the S/N when we consider the squeezed level of Stokes light changing in time inside the pulse. Are there any experimental data on this issue?

We have added a Section in the Supplementary Information (Section 2) describing the laser sources andtheir characteristics. How the choice of these pulse widths affects the signal-to-noise ratio is a good (and non-trivial) question. In general, as discussed in the manuscript, the widths should be roughly matched to the decay time of the Raman transition that is being probed. In biology, these widths tend to be in the few picosecond range (as now described in Supplementary Information Sections 2 & 13), matching the pulse widths we use in our SRS system. Indeed, in designing the system we took care to choose appropriate pulse width and repetition rate to give a strong Raman signal, while also aiming to minimise photodamage(i.e. ultimately to maximise the signal-to-noise ratio). We based our designs on previous literature on precision stimulated Raman microscopes (see e.g. Ref. [36]).

Our pump and Stokes lasers were ultimately both sourced from a single Coherent Plecter Duo 1064 nm laser with a fixed 6 ps pulse width. As such we were unable to control the excitation laser pulse widths. It ispossible that tuning of these could improve the signal-to-noise for some Raman transitions. However, based on the literature we believe that our system is close to optimised in this respect. We would note that when claiming absolute quantum advantage we are careful to

specify that this is for the specific spatiotemporal pulse shapes we use in our experiments:

> "*This represents an absolute quantum advantage — with same spatiotemporal modeshapes and apparatus, photodamage prohibits classical techniques from reachingthis level of signal-to-noise*."

One could argue that a wider definition could be used, where the absolute quantum advantage was demonstrated over all possible experimental configurations and excitation laser characteristics. However, absolute quantum advantage of this kind would be exceptionally difficult to prove, and in some sense wouldmiss the point – it is desirable for the quantum advantage to be realised in a microscope close to the sort that are routinely used in precision microscopy the biosciences, such as ours. We hope that the Referee agrees that our choice of definition is appropriate, and that achieving it does represent a significant milestone, leaving the demonstration of advantage over all possible configurations of classical microscope for a future intrepid experimentalist!

We now include discussion in Section 13 of the Supplementary Information of a range of possible strategiesto improve the signal-to-noise of Raman microscopy without using quantum correlations, and the compromises associated with these. This includes discussion of the use of contrast enhancing labels, higher efficiency apparatus, longer measurement durations, and varying the excitation laser pulse widths.
We hope that this sufficiently addresses the Referees comment.

We do agree with the Referee that the dependence of signal-to-noise on the pulse width and shape wouldbe an interesting area for future exploration.

Comment 7:

The authors should more clearly discuss the effect of losses on their experimental results (improvement of SNR, or shot-noise floor). In page 6, the losses are estimated to be around 35%, but in page 9 the authors state the total optical efficiency as approximately 40%. More precise analysis on the effect of losses, which should be more clearly attributedto each of the optical components, and limited detector quantum efficiencies on the degradation of the improvement in SNR or squeezing level at the detector should be given. This is very important to evaluate the validity of the experiments.

This is a reasonable point. We have now included a new section in the Supplementary Information (Section10) detailing the efficiencies of the different components of the apparatus, and the total transmission and detection efficiency for squeezed light. In this Section we also compare our observed quantum- enhancement to predictions based on the total detection efficiency and the known input squeezing, showingthat the predictions and observations are consistent.

The Referee refers to the estimated loss in the manuscript of 35%, and compares this to the total optical efficiency stated as approximately 40%, suggesting there might be an inconsistency. Indeed, were our lossestimates to vary by this magnitude, we would be quite concerned about the accuracy of conclusions that could be drawn. However, these two numbers refer to different optical paths. As stated in the manuscript, the 40% total optical efficiency (corresponding to 60% loss) is the total efficiency of the path from the squeezed light source through the microscope to the detector, and includes the detector efficiency; while the 35% loss refers to the additional loss introduced by the microscope, including pump filters, sample coverslips and polystyrene bead. Both numbers are useful. The first defines what the best possible quantum-enhancement would be, even if the squeezed light source was perfect. The second allows thepredicted degradation in squeezing when the microscope is introduced to be directly determined.

In order to ensure that this is clear in the manuscript we have rephrased the sentence referring to the additional microscope losses so that it reads:

> *"Additional losses of around 35% introduced on transmission through the microscope, pump filters, sample coverslips and polystyrene bead degrade the quantum correlations."*

We do hope that this response reassures the Referee that our results are reliable, and suitably clarifies themanuscript.

Comment 8:

In figure 4, the authors claim 23% increase in SNR, but I wonder the increase in SNR depends on the signal strength, and the definition is unclear here. (The same is true for 14% increase in SNR claimed for fig. 4b)

This is a fair question. The rate of Raman scattering depends on the product of pump and Stokes intensities, which is dominated by the classical coherent amplitudes of the two fields. As such, for fixed intensities, the signal strength is independent of whether the Stokes field is squeezed or shot-noise limited(as now discussed in Section 8.3 of the Supplementary Information). For fixed Stokes and pump spatiotemporal profiles[1] the net effect of introducing squeezing is to reduce the measurement noise floor, and the increase in single-to-noise depends only on this effect.

The signal-to-noise enhancement is defined as the ratio of quantum-enhanced to shot-noise-limited signal- to-noise minus one (e.g. if the quantum-enhanced signal-to-noise was twice the shot-limited signal-to-noise,this would give a signal to noise enhancement of 2-1=1, or 100%). We now define this explicitly where the signal-to-noise enhancement first occurs in the main text. Our method to calculate this ratio is also now clarified in Section 8.3 of the Supplementary Information.

Comment 9:

In page 8, the last paragraph before Discussion and outlook section, the authors might infer the membrane cannot be observed without squeezed Stokes light. I think the authors should remove these sentences ("For instance…limited measurement.") since it is bit too complicated and quite misleading.

We thank the Referee for this feedback. We believe that this point is a nice one to include in the manuscript
– that quantum-enhancement can allow features to be observed that would otherwise be unresolvable. However, we agree that perhaps the phrasing was somewhat unclear in the original manuscript. We havenow made some efforts to improve this. We have modified the relevant section of the main text to read:

> *"A thin semi-circular feature is also resolved that that we tentatively attribute to a section of thecell membrane. Raman signals from the cell membrane are typically faint due to its 10 nm thickness. Here, quantum-correlations allow an approximately 40% longer length of the feature to be resolved than would have been possible with coherent illumination (see Supplementary Information Section 12.3)."*

We have also included a section in the Supplementary Information (Section 12) that outlines (we hopeclearly) how we have estimated the improved ability to resolve this feature that is

[1] As noted earlier in our response to the Referee, and discussed carefully in Section 8.3 of the Supplementary Information, the deamplification of the coherent amplitude of the Stokes field in thesqueezer does change its spatiotemporal mode shape. However, our quantum-enhancement characterisation technique is designed to be immune to these effects.

provided by quantumcorrelations. We hope that, combined, this fully addresses the Referees concern.

Comment 10:

In figure 5, the authors show the power scaling of Stokes laser noise variance without squeezing. Please provide this graph for the power up to 4 mW since the most of the key data is taken with the Stokes power at 3.0 mW. I'm afraid there is a slight signature of the saturation of the detector for 2.5 mW and higher powers since those data points are slightly below the fitting line. If so, please discuss the effect of the saturation of the detector for the experiment.

We thank the Referee for this observation. Clearly, were the detector saturating this would raise significant questions about the validity of our results. We now include measurements of Stokes noise power scaling upto 6 mW in Section 8.2 of the Supplementary Information. As can be seen from these measurements, the scaling is very linear up to 4 mW, after which it begins to saturate. We hope that this additional information clarifies any doubts in the Referees mind about detector saturation.

Reviewer Reports on the First Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

I have read the revised manuscript and the response by the authors. The new images provided are much more convincing than the previous images, and overall the manuscript has substantially improved. The new Figures make a stronger case for the benefits of quantum light for coherent Raman imaging. It is clear that a lower noise floor can improve the imaging quality in a meaningful way. I still believe that a detailed sensitivity analysis would have been worthwhile to report in the main text, because, in practice, the focus on damage threshold is less important than actual sensitivity. What is important is not so much the damage threshold (power can always be lowered if signal remains), but the improvement in signal strength and SNR for a given power and a given concentration of the target.

Overall, I believe the manuscript has merit, but for it to make an absolute impact, it would be wise to focus on parameters that really matter for imaging, which is sensitivity, SNR and signal strength. Clear demonstrations of each (quantum light vs classical light) would convince everyone in the community that the quantum light approach has merit.

Referee #3 (Remarks to the Author):

Dear Editor
The authors made a very significant work for improving their paper.
I my opinion they have answered to most of referees' queries and the paper is substantially suitable to be published in its present form or with a few minor changes.
I only suggest, with the purpose of acknowledging better the previous work on quantum imaging introducing the generic reader to the field, to quote a couple of review papers, e.g Nature Reviews Physics 1, 367 (2019) and Journal of Optics, 18 (2016) 073002.

Referee #4 (Remarks to the Author):

Quantum correlations overcome the photodamage limits of light microscopy
Casacio et. al.

Review report

I have read through the replies from the authors and the revised manuscript carefully. I very much appreciate the significant efforts by the authors for the reply. Although I have found that the most of the issues have been answered properly, and that the manuscript has been improved dramatically, I think there is still one important issue remaining to be clarified.

For my previous comment 4, the authors replied as follows.

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Comment 4:
Figure 3c is one of the key results for their claim, however, the details how the data was taken for this graph is totally lacking. I assume that the authors might have measured this data using the same single polystyrene sphere with increasing the illumination power and at each power the authors switched the squeezer on and off. In this case, how did the authors switch the squeezer? How fast? How the laser / squeezed light powers are (strictly) controlled?

These are fair questions. ….
The Referee suggests an alternative approach which would in principle be more straight forward – that is make two direct measurements of the signal-to-noise ratio, one using squeezed light and the other using shot-noise limited light. While intuitively attractive, we found this approach to give inaccurate results. The reason for this is that the amplification process in the squeezed light source changes the spatiotemporal mode shape of the Stokes beam (as discussed above, only the complement of the Stokes beam that is well aligned with the optical parametric amplifier pump is deamplified). Since the Raman gain is highly sensitive to the intensity of the pump, and therefore its spatiotemporal profile, making measurements in this way was found to convolve the quantum-enhancement that we wish to show with classical effects on the Raman signal amplitude. We note that other experiments showing quantum-enhancement in nonlinear
spectroscopy have encountered similar issues and resolved them in the same was as we do (see e.g. Ref. [47] in the manuscript).
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If my understanding on the above reply is correct, the direct comparison between the cases using coherent light pulses and squeezed light pulses is difficult since the spatio-temporal mode shape of the squeezed Stokes beam is modified during the amplification process.

Does this mean that the Raman signal is stronger for the `classical' Stokes beam without the deformation of the pulse shape due to the squeezer for the same intensity, probably because the spatio-temporal mode of the non-squeezed Stokes light is matching better with that of the pump pulse? In other words, is the Raman signal with the squeezed Stokes beam weakened due to the spatio-temporal mode mismatch? If this is the case, I'm afraid the SNR might be even better for the classical case for the same Stokes/Pump pulse intensities.

Since this is the critical point related to the advantage using squeezed light, I believe this point should be clarified and well explained before the acceptance of this manuscript in Nature.

As a minor issue, I think there may be a small misunderstanding for my previous comment 6. I just wandered short pump pulse could be used as a sort of temporal filter since the squeezed level could be maximum at the center of the 6-ps pulse duration. I completely agree with the authors this should be an interesting open question, and also that the situation would be more complicated when we look at Figure S8 (b).

I have also found some typos in Suppl. Info. P12 line 6 in the second paragraph: "them" may be "then". P22 line 6 from the bottom: "are" is doubled.

Author Rebuttals to First Revision:

Detailed response to Referees

Referee 1

I have read the revised manuscript and the response by the authors. The new images provided are much more

convincing than the previous images, and overall the manuscript has substantially improved. The new Figures make a stronger case for the benefits of quantum light for coherent Raman imaging. It is clear that a lower noise floor can improve the imaging quality in a meaningful way.

We are delighted that, with the revisions we have made to the manuscript, the Referee is convinced that quantum correlations allow the quality of coherent Raman imaging to be meaningfully improved. We thankthem again for their constructive and genuinely insightful feedback. We found this highly motivating, encouraging us to make major efforts to improve our apparatus and results. We were, ourselves, tremendously gratified by how much improvement turned out to be possible.

I still believe that a detailed sensitivity analysis would have been worthwhile to report in the main text, because, in practice, the focus on damage threshold is less important than actual sensitivity. What is important is not so much the damage threshold (power can always be lowered if signal remains), but the improvement in signal strength and SNR fora given power and a given concentration of the target.

Overall, I believe the manuscript has merit, but for it to make an absolute impact, it would be wise to focus on parameters that really matter for imaging, which is sensitivity, SNR and signal strength. Clear demonstrations of each (quantum light vs classical light) would convince everyone in the community that the quantum light approach has merit.

We agree with the Referee that from a practical point of view it is the signal-to-noise, sensitivity and signalstrength that can be achieved that are important for imaging. We have now substantially restructured the manuscript to more strongly emphasize these characteristics, as described in more detail two paragraphsbelow.

Motivated by the Referees comments, we have also made efforts to improve the signal-to-noise and concentration sensitivity of our microscope and to make quantitative comparisons to performance reported in the literature. By optimising the microscope alignment, we have improved the quantum-enhanced signal-to-noise for molecular bonds in polystyrene from 99 to 1730. This provides a concentration sensitivity of 7.8mM over a one second integration period. The Referee was quite right in their previous report that much higher concentration sensitivities have been reported, into the tens of μM range (see e.g. Refs. [11-14] of the Supplementary Information). However, those results are achieved for C≡C bonds, that have extremely high Raman cross-sections. To quantitatively assess the concentration sensitivity we achieve, it is necessary to compare to high quality experiments that probe the same aromatic CH stretch bonds in polystyrene that we do. We have now undertaken a thorough literature review, identifying two appropriate experiments that include sufficient details to estimate their sensitivity (Refs. [8] & [9] of the Supplementary Information). To obtain a fair comparison to those experiments, we normalise all sensitivities to a one second integration period (matching ours) and to 1 mW of Stokes and pump power. Doing this, we find thattheir concentration sensitivity is comparable to ours (within a factor of four). Together with the yeast cell image comparison we made in response to the Referee in our previous submission (Section 12.1 of the Supplementary Information and Response to Referees),

this allows us to conclude that the absolute performance of our microscope is competitive with high performance stimulated Raman microscopes. We now include a detailed comparison in Section 11 of the Supplementary Information, including both a quantitative comparison to Refs. [8] & [9] and a discussion of the superior sensitivity that is possible when probing different molecular bonds (Refs. [11-14]). We also include in the paper proper a brief paragraph quantifying our concentration sensitivity and comparing it to other experiments, as suggested by the Referee.

To refocus the manuscript more towards sensitivity and signal-to-noise we have made the followingchanges:

- We have removed the explicit reference to evasion of photodamage in the title, changing it to"*Quantum-enhanced nonlinear microscopy*".

  When describing our microscope in the summary (abstract) paragraph we now quantify the levels of signal-to-noise and sensitivity enhancement achieved, stating that "*Quantum correlations within[the microscope] allow imaging of molecular bonds in the interior of a cell with 35% improvedsignal-to-noise, corresponding to a 14% improvement in concentration sensitivity.*".

- To better highlight the broad relevance of our work to quantum metrology, we have also rephrasedthe sentence "*This show, for the first time in any context, that quantum techniques can overcome photodamage*." in the summary paragraph to: "*Broadly, this shows that quantum techniques can overcome the optical intrusion that often degrades precision measurements*".
- In paragraph 2 of the Introduction we now refer to the usual trade-off for optical measurements asone between "*signal-to-noise and intensity*" rather than "*signal-to-noise and photodamage*", and rephrased "*The importance of addressing biological damage…*" to "*The importance of improving sensitivity…*". The aim here is to focus on the parameters that ultimately matter, rather than the phenomena that constrains them (photodamage), as suggested by the Referee.
- In the Introduction, to better motivate coherent Raman microscopy and the importance of improvedsensitivity and signal-to-noise we have reordered paragraph 3 and divided it into two. The first newparagraph now motivates the importance of coherent Raman microscopes, rather than our demonstration of the evasion of photodamage. The second paragraph initially focusses on the enhancements in sensitivity, imaging speed and signal-to-noise made possible by quantum correlations. It then give the improvement in signal-to-noise we achieve using quantum correlations, before finally discussing how this allows photodamage limits to be overcome.
- We have changed the title of the section previously titled "*Quantum-correlations allow absolute performance advantage*" to "*Quantum correlations enhance signal-to-noise*" to better emphasisethis key result.
- As discussed above, we now include a paragraph in the paper proper discussing the concentrationsensitivity we achieve for styrene monomers using quantum correlations, and comparing this to both the shot-noise limited concentration sensitivity we achieve and the state-of-the-art. We also include an expanded discussion of this comparison in the Supplementary Information (Section 11).
- We have specified the 14% enhancement in concentration sensitivity we achieve for yeast cell imaging in the relevant paragraph of the "*Quantum-enhanced imaging*" section of the main text.
- We have rewritten the first paragraph of the *Discussion and Outlook*, to first focus on the quantum-enhanced sensitivity we achieve, and the importance of such quantum enhancement for future applications of coherent Raman microscopes. Only after discussing these broader performance advantages, do we discuss our demonstration of performance beyond the photodamage limits of conventional coherent Raman microscopy.

We would note that our quantum correlations do not enhance the Raman signal (only the noise floor), though this may be possible in different configurations (see e.g. Ref. [50]). We now state this explicitly on

page 5, stating that "*The correlations suppress, or "squeeze", the amplitude noise on the Stokes field at the frequency of the stimulated Raman modulation sidebands, while leaving the Raman signal strength unchanged*".

Overall, we hope that the change in focus towards sensitivity and signal-to-noise, together with the inclusion of sensitivity results in the main text and their comparison to the state-of-the-art, put our work in the clearer context that the Referee envisages. We believe that rebalancing the manuscript in this way hasbeen worthwhile, bringing the work into better focus for the broader microscopy community. We thank the Referee for the suggestion to do this. Having said this, we have retained some significant emphasis on photodamage and photodamage evasion for the reasons outlined below.

From the perspective of the quantum physics community, we remain convinced that the ability to overcome hard photodamage constraints is a key novelty of our work, strongly contrasting all previous demonstrationsof quantum-enhanced sensing. We also see the ability to evade photodamage as a key novelty for nonlinear microscopy. Photodamage is quite broadly considered to be a critical concern for nonlinear microscopy (see for e.g. Refs. [1,9] in the manuscript, and extended discussion in the Raman spectroscopyreview of Shipp *et al.* [*Ad. Opt. Phot.* **9** 315 (2017)]). Ultimately, it is photodamage which limits the achievable signal strength, sensitivity and signal-to-noise, by constraining the useable laser intensity.

To take a concrete and directly relevant practical example, it is photodamage which constrains the imaging speed of current high performance coherent Raman microscopes – the laser intensities are chosen to be ashigh as possible, without introducing risk of optical intrusion. Reducing the intensity would prolong the already long acquisition times. For example, Shipp *et al.* states that

> "*As low laser powers are commonly used to avoid damaging biological samples (see below), long acquisition times are usually required to obtain an acceptable signal-to-noiseratio. Acquisition times for a single Raman spectrum vary from around 0.5 s to over 3 min.Even with a relatively "fast" acquisition time of 0.5 s, acquiring a 1024×1024 hyperspectral Raman image would take more than six days. This slow speed is often the largest obstacle for Raman spectroscopy."*

As the Referee rightly points out, so long as the signal strength is sufficient, it is always possible to reduce intensity and therefore avoid photodamage. However, it is not unusual for the targeted signal to lie beneaththe noise floor, and some forms of photodamage depend on the total accumulated energy rather than the intensity, in which case this strategy becomes untenable. Indeed, many interesting signals are so weak thatno conventional microscope could resolve them without photodamage destroying the specimen. Our use ofquantum correlations breaks this compromise, providing the possibility to access biological structures and processes that would otherwise be fundamentally obscured.

We believe that the revised manuscript demonstrates the benefits of quantum light in microscopy in a waythat is well balanced; readily accessible to the light microscopists and the broader audience, and highlighting both the possible practical benefits and the fundamental importance of demonstrating that photodamage can be evaded. We hope that the Referee is now fully convinced of its merits and can support publication in *Nature*.

Referee 3

The authors made a very significant work for improving their paper.

I my opinion they have answered to most of referees' queries and the paper is substantially suitable to be published in its present form or with a few minor changes.

We are pleased to hear that Referee is satisfied that we have addressed most queries, and delighted thatthey now find our manuscript substantially suitable for publication in *Nature*.

I only suggest, with the purpose of acknowledging better the previous work on quantum imaging introducing the generic reader to the field, to quote a couple of review papers, e.g Nature Reviews Physics 1, 367 (2019) and Journal of Optics,18 (2016) 073002.

We thank the Referee for this suggestion to improve the accessibility of our work to the broader audience.As suggested, we have now included the additional reference *Nature Reviews Physics* **1** 367 (2019) in theintroduction of the manuscript. Specifically, in paragraph 2 we now state:

> "*They have also been shown to improve many other optical measurements in proof-of-principle experiments [16]. The importance of improving sensitivity has motivated efforts to apply quantum-correlated illumination into microscopy [5, 17], with recent demonstrations ofquantum-enhanced absorption [18–22] and phase-contrast [23–25] imaging.*"

Here, Ref. [16] is a general review of quantum metrology, Ref. [5] is a review of the applications of quantummetrology in biology, and Ref. [17] is the new reference recommended by the Referee which is a recent review of quantum imaging. Refs. [18-25] are experimental research papers.

We would have liked to also include the Referee's second suggested review paper, *Journal of Optics* **18** 073002 (2016). However, in the end we felt unable to do this due to constraints in the total number of references allowed in a *Nature* paper. These constraints meant that, compared to the previous version of the manuscript, the overall number of references had to be reduced while at the same time including both additional references related to the Raman concentration sensitivity in response to Referee 1, and the additional review paper we have included in response here. Given that *Journal of Optics* **18** 073002 (2016)covers similar subject matter to the material in the more recent review *Nature Reviews Physics* **1** 367 (2019), and in Refs. [5] & [16], we are of the view that omitting it should not significantly diminish the accessibility of the manuscript.

We feel that the referencing in the manuscript is now quite well balanced, given challenges involved in communicating cross-disciplinary research. At a rough count, the revised manuscript includes four quantumimaging & metrology review papers, four nonlinear microscopy review papers, 17 conventional microscopy research articles, and 23 quantum imaging & metrology research articles. We hope that the Referee is comfortable that we have the balance about right, and thank them again for their suggestions of additional references.

Referee 4

I have read through the replies from the authors and the revised manuscript carefully. I very much appreciate the significant efforts by the authors for the reply. Although I have found that the most of the issues have been answered properly, and that the manuscript has been improved dramatically, I think there is still one important issue remaining to be clarified.

We are very pleased that the Referee appreciates the efforts we have made, and sees the revised manuscript as a dramatic improvement. We were happy to receive their thorough review, has been a veryhelpful guide for our revisions. We address the Referee's one important remaining issue, and other minorissue, below.

For my previous comment 4, the authors replied as follows.

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Comment 4:

Figure 3c is one of the key results for their claim, however, the details how the data was taken for this graph is totally lacking. I assume that the authors might have measured this data using the same single polystyrene sphere with increasing the illumination power and at each power the authors switched the squeezer on and off. In this case, how did the authors switch the squeezer? How fast? How the laser / squeezed light powers are (strictly) controlled?

These are fair questions. ….

The Referee suggests an alternative approach which would in principle be more straight forward – that is make two direct measurements of the signal-to-noise ratio, one using squeezed light and the other using shot-noise limited light. While intuitively attractive, we found this approach to give inaccurate results. The reason for this is that the amplificationprocess in the squeezed light source changes the spatiotemporal mode shape of the Stokes beam (as discussed above, only the complement of the Stokes beam that is well aligned with the optical parametric amplifier pump is deamplified). Since the Raman gain is highly sensitive to the intensity of the pump, and therefore its spatiotemporal profile, making measurements in this way was found to convolve the quantum-enhancement that we wish to show with classical effects on the Raman signal amplitude. We note that other experiments showing quantum-enhancement in nonlinear spectroscopy have encountered similar issues and resolved them in the same was as we do (see e.g. Ref.

[47] in the manuscript).

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If my understanding on the above reply is correct, the direct comparison between the cases using coherent light pulses and squeezed light pulses is difficult since the spatio-temporal mode shape of the squeezed Stokes beam is modified during the amplification process.

Yes, the Referee is correct. Direct comparisons using coherent and squeezed light pulses are difficult, since the signal-to-noise is affected both by the quantum-enhancement of the noise floor (the effect we wish to show), and modifications in the Raman signal due to spatiotemporal modeshape changes (a purelyclassical effect). Instead, we use an alternative and previously established method that directly determinesthe enhancement from quantum correlations in a way that is insensitive to spatiotemporal modeshape changes.

Does this mean that the Raman signal is stronger for the `classical' Stokes beam without the deformation of the pulse shape due to the squeezer for the same intensity, probably because the spatio-temporal mode of the non-squeezed Stokes light is matching better with that of the pump pulse? In other words, is the Raman signal with the squeezed Stokes beam weakened due to the spatio-temporal mode mismatch? If this is the case, I'm afraid the SNR might be even better for the classical case for the same Stokes/Pump pulse intensities.

Since this is the critical point related to the advantage using squeezed light, I believe this point should be clarified and well explained before the acceptance of this manuscript in Nature.

We thank the Reviewer for raising this issue, and can understand why they consider it a critical point. Whilewhat we show is that quantum correlations allow improved performance for a Stokes field with fixed spatiotemporal modeshape; from a practical perspective, it could be envisaged that

the modeshape changeassociated with preparing quantum correlations might always degrade the Raman signal, and do this to such an extent that – even with quantum-enhanced noise floor – the raw signal-to-noise of the microscope was reduced. We find that this is not the case in our microscope. Specifically, we find that the quantum- enhanced signal-to-noise exceeds the shot-noise-limited signal-to-noise even when including changes in the Raman signal strength due to modeshape perturbations.

For the molecular vibration in polystyrene that we probe, the modeshape perturbation due to squeezing degrades the Raman signal somewhat (though not sufficiently to remove the quantum-enhancement of the signal-to-noise). In general, however, the Raman signal has complex dependences on the spatiotemporal modeshapes of the pump and Stokes fields, and on the properties of the Raman transition being probed (both the scattering cross-section and the molecular vibrational coherence lifetime). As such, there is no clear rule on whether it will be improved or degraded by a particular change in the Stokes modeshape. For instance, the deamplification used to generate squeezing could, as the Referee suggests, degrade the overlap between the pump and Stokes fields, but at the same time improve the matching of the Stokes pulse duration and molecular coherence lifetime. Combined this could result in a net improvement in Raman signal (rather than the perhaps expected degradation). Indeed, the Raman signal strengths from each molecular bond will be affected differently by a given change in Stokes modeshape – some will be degraded and other enhanced. By comparing quantum and classical techniques for the same spatiotemporal modeshapes we are able to remove this variability, accessing the underlying quantum enhancement of the noise floor that is consistently present for all accessible molecular bonds. This both separates the quantum and classical effects and, we believe, provides a more useful practical metric for theimprovement in microscope performance – one that is general to the microscope, rather than specific to a particular Raman band. It provides an “apples to apples” comparison, quantifying the quantum- enhancement in a way that is independent of classical effects, effects the could be compensated for using standard mode shaping techniques.

We do agree with the Referee that the question they raise is important. To address it within the manuscript,we have significantly extended Section 8.3 of the Supplementary Information, including both new data and new discussion. The new Fig. S6 provides experimental measurements of the power spectrum of the Raman signal when probing the CH aromatic stretch band in polystyrene. These measurements are performed both using squeezed and shot-noise-limited light, allowing a direct comparison. This data evidences our claim that modeshape changes do not remove the quantum-enhancement of the signal-to- noise we measure, and quantifies the level at which they modify the Raman signal. As now discussed in Section 8.3, the Raman signal strength is reduced by 10% due to the deamplification associated with squeezing, while the measurement noise floor is reduced by 14% due to quantum correlations. Overall, thisresults in a 4% improvement in signal-to-noise, even when the spatiotemporal modeshape perturbation is not correct for.

We hope that these changes to the manuscript and discussion convince the Reviewer that our approach isboth accurate and appropriate, and that they are now able to recommend publication in *Nature*.

As a minor issue, I think there may be a small misunderstanding for my previous comment 6. I just wandered short pump pulse could be used as a sort of temporal filter since the squeezed level could be maximum at the center of the 6-ps pulse duration. I completely agree with the authors this should be an interesting open question, and also that the situation would be more complicated when we look at Figure S8 (b).

We thank the Referee for clarifying their previous comment 6, and now understand better the point they were making. We agree that the squeezed Stokes field will exhibit a time-dependent level of squeezing, with the best squeezing occurring at the peak of the pulse. However, we think that controlling the Ramanpump pulse width, by itself, would not allow this higher squeezing to

be accessed.[2] The reason for this is that the detected squeezing level is independent of the properties of the Raman pump pulse. Instead, themain effect of changing the pump pulse duration is to change the Raman signal strength by altering the overlap with both the Stokes field and molecular vibration coherence lifetime, as discussed above.

The Referees observation did, however, lead us to an alternative method to temporally filter the squeezed Stokes field and potentially provide higher levels of measured squeezing. Rather than tuning the pulse width of the Raman pump field, if a homodyne detector was used, the homodyne local oscillator pulse width could be tuned. This could in principle allow detection of only the component of the Stokes field with highestsqueezing or, more generally, allow the signal-to-noise of the detected Raman signal to be maximised. We now include a few additional sentences on page 16 of the Supplementary Information discussing the possibility of improving the observed squeezing by controlling the local oscillator pulse width. We copy themhere for the Referees convenience:

> *Improvements in the observed squeezing could be obtained not only by controlling the transverse spatial modeshape of the local oscillator, but also by engineering its temporal modeshape. The pulsing of the parametric amplifier pump field leads to a time dependent parametric gain, with the highest deamplification occurring at the peak of the pulse. As a consequence, the squeezing of the Stokes field can be expected to be strongest near the peak of the pulse. An appropriately temporally tailored local oscillator could extract out thishighly squeezed component of the Stokes field, lowering the measurement noise floor.*

We think that this is a nice new point, and thank the Referee for bringing the idea to our attention.

I have also found some typos in Suppl. Info. P12 line 6 in the second paragraph: "them" may be "then". P22 line 6 from the bottom: "are" is doubled.

We thank the Referee for bringing these typos to our attention and have corrected them in the revisedSupplementary Information.

Reviewer Reports on the Second Revision:

Referees' comments:

Referee #1 (Remarks to the Author):

I have read the revised manuscript and the response by the authors. The new discussion about SRS sensitivity is very helpful and it has improved the manuscript. I believe that authors have demonstrated that noise levels in SRS can be reduced with using quantum light, which in turn improves the signal to noise ratio. Although the overall effect is rather small, and insufficient to make a real difference in most applications, there is a subset of precision measurements that could truly benefit from the ideas presented in this work.

[2] It is possible that the Referee is referring to the squeezer pump pulse rather than in the Raman pump pulse. If that is the case we apologise for misunderstanding again! Narrowing the squeezer pump pulse would improve the squeezing at the centre of the Stokes pulse, but degrade the squeezing in its wings. Webelieve that this would generally result in poorer overall squeezing. Alternatively, increasing the squeezer pump pulse duration (and indeed transverse waist size) and compensating for the reduction in intensity by increasing the pump power would, we think, result in better overall squeezing, improving the squeezing in the wings.

Referee #4 (Remarks to the Author):

I think the authors have answered to all of my questions appropriately and have revised the manuscript accordingly. I'm happy to recommend the acceptance of the current manuscript for the publication in Nature.

Author Rebuttals to Second Revision:

N/A