2019.10.28

Inspired by our recent discussion with peers from different institutions and research fields, we make the following list. We wish this Q&A list could help further explain our study. The questions here are written (or rewritten) by ourselves, but the answers are mostly what we have used in the discussion. If anything inappropriate is found in this document (or this study), please do not hesitate to contact:

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1. How can an image's details be recovered after its high frequency components are removed by a microscope?

Answer:

On the one hand, we find a "resolvable condition". In this condition, the detail information is included in both high frequency and low frequency components. More generally, it is actually included in any part of the frequency spectrum.

On the other hand, we propose two methods. Each of the methods can recover the detail information from the low frequency components. More generally, they can actually recover it from any part of the Fourier spectrum.

In other words, **the observed image (or its Fourier spectrum) has redundancy in the "resolvable condition"**. Thereby, details can be recovered from part of the observed image, or part of its Fourier spectrum (e.g., low frequency components).

2. Suppose the original image consists purely high frequency where your PSF is zero. How can you recover it through deconvolution?

Answer:

In this study, images usually do not (if not never) have such Fourier spectrum as you mentioned. What we need to figure out is an ROI with limited pixels, and their values are all non-negative. Thereby the image's Fourier spectrum should extend infinitely broad, and includes meaningful low and high frequency parts. The proposed methods have been verified by our repeated tests. Even if such an image does exist, it does not deny the existence of the proposed "resolvable condition".

We have also thought about another relevant question. Assume that two images have the same low frequency part but different high frequency part. Then which one will be recovered from the low frequency part? Presently, we believe such images do not exist (or at least very uncommon) in the "resolvable condition". Actually, high frequency and low frequency components are tightly relevant in such a condition.

3. If there is no noise, it seems this problem has already been solved by existing deconvolution methods. There are a lot of researches on deconvolution, and some can recover images from very blurred images without noise. What is the contribution of your study?

Answer:

For usual deconvolution problem, maybe noise is the most important factor. **But, the problem in our study is another extremely similar but essentially different one.** It is the problem of "the diffraction-limit", even when there is no noise.

I do agree that deconvolution is a very powerful technique. Actually, in some existing literatures, we can find very good results when there is no noise. But in those cases, the PSFs are not ideal low pass filters. That means they do not remove all the high frequency components from images. Usually, those PSFs have limited sizes although they may be very large.

But the problem is essentially different in our study. According to Fourier Optics, a light microscope's PSF extends infinitely broad (with its central area called the "Airy disk"), and more importantly its Fourier Transform (FT) is an ideal low pass filter. From the physical point of view, an image's high frequency components cannot be collected by lens. As a result, there are no high frequency components in the convolved image, i.e., the image acquired by the microscope. In this case, usual deconvolution does not work even without any noise. We believe this is the first reason why the diffraction-limit issue has bothered the world for more than a century.

We do agree that noise is a very important point. But for the diffraction-limit issue, it is still a seemingly "impossible" problem even without any noise. With the help of super-resolution techniques, we are now able to observe structures beyond the diffraction-limit, but "recovering details directly" is still "impossible". That is why we choose to ignore noises and focus on the principle in this study. But after it is solved in principle, we do agree that noise should be treated as the most important issue.

4. Did you confuse mathematically solvable with practically solvable? If there is no noise, it seems we can solve this problem by a simple inversion. For example, divide the observed image's Fourier transform (FT) by the FT of the PSF.

Answer:

This study's task is to extract details from one diffraction-blurred image directly. According to classic theories (about Fourier Optics, the diffraction-limit and Rayleigh criterion), this problem is neither mathematically solvable nor practically solvable. For example, according to Fourier Optics, a conventional light microscope removes the high frequency components of any image. As a result, the FT of the PSF has all zeros in high frequency part. Thereby, the image's high frequency components cannot be recovered by dividing the observed image's Fourier transform (FT) by the FT of the PSF. But our study find an exception, and the image can be recovered without high frequency components. So far, it is still a difficult task in practice.

Actually, the diffraction-limit and Rayleigh criterion apply to various light microscopes, and this paper is based on them. We think this study's conclusion is partly inconsistent with them but do not deny them. Rayleigh criterion is for human vision, while our conclusion is for computer vision (in the "resolvable condition" and using the proposed methods).

5. Why is “isolated lighting” important in your study? Can you provide a counter-example to explain it? Is this study related to Compressed Sensing?

Answer:
Yes, “isolated lighting” is one of the key aspects of the proposed “resolvable condition”. I think the explanation in page 3~5 (especially Fig.1) could be helpful. In short, if “isolated lighting” is not fulfilled, there will be some unknown structures outside the ROI. Their images will overlap the ROI’s image even if they are extremely far away from the ROI. The reason is: the PSF extend infinitely broad, as a result these structures’ images also extends infinitely broad. With these extra unknowns, we cannot solve the unknowns we need, i.e. the ROI pixels. Our methods are first illustrated with two-point situation, and then further generalized to arbitrary ROIs. Yes, we also suspect that there may be some essential linkage between our technique and compressed sensing. We even wish some Mathematician could find out more about this.

6. If there are zeros in the microscope’s OTF (Optical Transfer Function), the ideal image’s high frequency component will be removed. How can the full details be recovered?

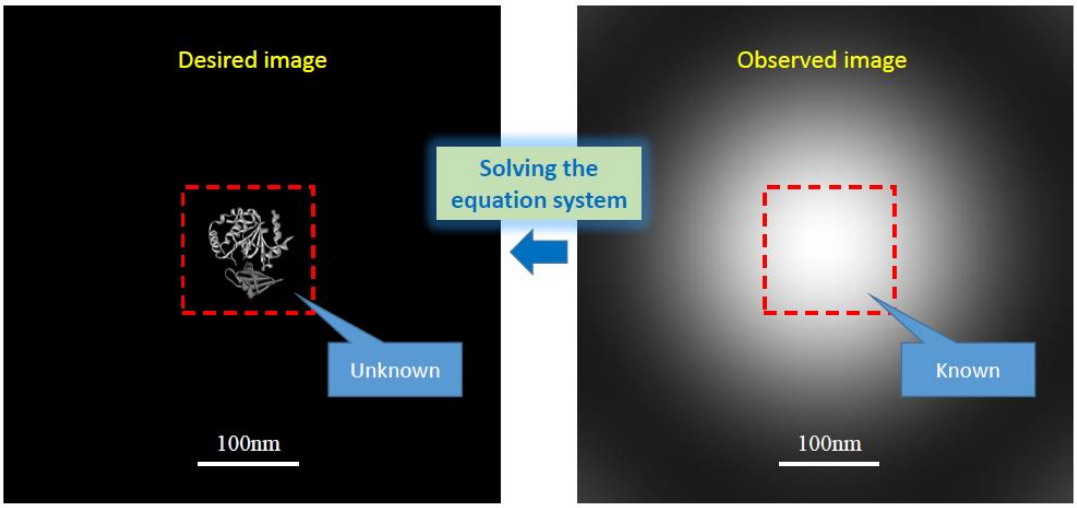
Answer:
In this study, the OTF’s values are actually all zeros except a low frequency part. The simulated microscope removes all the high frequency components of images. But in the proposed “resolvable condition”, full details are recovered merely from the low frequency components.

7. You could add a lot of pixels for each original pixel, but the resolution would not be improved.

Answer:
No, resolution won’t be improved by adding pixels simply. In our study, no pixels are added actually. It only requires to acquire an image in a higher zooming factor. For example, assume that a germ is 10x10 pixels (and sharp) when observed by 1000x microscope. So it may be 20x20 pixels when observed by 2000x. By now, there are more pixels but the resolution is not improved. What we get is a 20x20 but blurred image. Then, our methods will figure out a sharp image from this blurred one. After that, we get a 20x20 and sharp image. By doing so, the resolution is improved now.

8. You can get as many pixels as you want using some techniques such as oversampling or interpolation. But the resolution is not improved. So, are you confusing “the number of pixels” with “resolution”?

Answer:
No. In our methods, "having enough pixels" is of course a pre-condition. But, just like what you say, that does not mean resolution. Then the high-resolution image (with full details) are figured out by solving an equation system:



Please refer to our slides (supplementary material). By now, the recovery of large ROI has only been verified indirectly.

9. Could you discuss the aforementioned pre-condition ("having enough pixels"), in the context of the Nyquist-Shannon Sampling Theorem.

Answer:
Our technique actually has no special requirement on this pre-condition and sampling procedure. I believe the Nyquist-Shannon Sampling Theorem is fulfilled by default in existing microscopes. Our technique is based on these microscopes, and adds no parts that is inconsistent with the Nyquist-Shannon Sampling Theorem. The whole (simplified) procedure is: prepare a suitable sample, observe it (e.g., in 3000x), acquire the blurred image, and then figure out the sharp image using our technique.

10. The proposed methods seem to require zero noise, perfectly known PSF (Point Spread Function), ideal sample, and other ideal conditions. Is it a practical one by now?

Answer:
We do not believe that a very practical technique can be established merely by this single paper. Actually, we would like to treat this paper as just a beginning instead of a perfect ending. This paper mainly focuses on principle. In our previous knowledge, the imaging procedure (convolution) is irreversible even if in ideal situation. But this study finds an exceptional condition, and then proposes methods based on it to further improve the resolution of super-resolution techniques. There are a lot of future work worth doing, especially on the noise. Ideal conditions are the best for this technique to work, while all the imperfections can be modeled as the noise (or distortion) in the

observed data. Therefore, it is an important future work to make the methods less sensitive to noises.

11. This preprint seems relevant to another preprint significantly. Why?

Answer:
In March 2019, we uploaded the preliminary report of this study to preprint websites. It is not formally published in any journal or conference. The preprint here is the extension of that one, and has already cited it (reference [19]).

12. Why does the recovered image in Fig. 10 look almost the same as the ground truth image?

Answer:
In ideal situation, the technique can get very accurate results. The recovery errors are almost unperceivable by human eyes (about 0.45% per pixel averagely in this case). But that is only achieved in simulation experiments by now. It would be more difficult in physical experiments.

13. Why don't you assume that the PSF (Point Spread Function) is a Gaussian function?

Answer:
According to Fourier Optics, a light microscope's PSF is an Airy-disk-shaped function, and its Fourier spectrum is an ideal low pass filter. It removes an image's high frequency components, and make it blurred. According to informatics, details cannot be extracted directly from such a blurred image. For the sake of strictness, we must use an Airy-disk-shaped function when modeling the imaging procedure.
Then we also use an Airy-disk-shaped function when recovering the ideal image. But Gaussian function may also be an option. Actually, it is extensively used in some existing super-resolution techniques, and achieves great success. For example, I believe Gaussian function can also lead to extremely precise results when localizing a blurred point's center. Our study can be treated as the extension of those techniques. Our method tries to further extract its sharp image (*NxN* pixels) from the blurred point after knowing its central position. But it is still sensitive to noise, so we wish the input data can be as precise as possible.

14. Deconvolution is a well-established technique. There are many methods that recover images in non-ideal situations such as unknown PSFs and noise. What is the contribution of this study?

Answer:
We did not propose deconvolution, and of course there are a lot of excellent work on deconvolution. Our key finding is a "resolvable condition". In our previous

knowledge, two or more nearby points cannot be distinguished directly if their distances are shorter than the diffraction-limit. But we find an exception in the "resolvable condition", and full details can be recovered directly from the extremely blurred image. Then we propose two methods based on this condition and existing deconvolution techniques. So we think this study contributes mainly in principle, and still needs future efforts to make it more practical. Solving equation system is not a popular deconvolution technique. But this time we find it works better than our expectation in the "resolvable condition".

15. How does your technique compare to other deconvolution techniques? Can the other techniques also achieve infinitely high resolution?

Answer:
We are afraid not. Our technique combine the "resolvable condition" and deconvolution to further improve the resolution of microscopes, e.g., the resolution of existing super-resolution microscopes. I believe those packages are very valuable. But we find that solving equation system is a deconvolution approach especially suitable for our technique. In usual deconvolution techniques (e.g., wiener filtering), PSFs' high frequency components are used as denominators, and cannot be zeros. By solving equation system, our methods do not have such a problem.

16. How do you implement the PSF? A microscope's PSF has non-zero components going out to infinity. Do you truncate the PSF somewhere? That may cause loss of information.

Answer:
No, we did not truncate the PSF. There are two methods in our paper. For the spatial domain method, only part of the PSF affects the result, in our "resolvable condition" (especial because of its second aspect, please see page 4, or the picture below). The other infinitely many values of the PSF have no effect. For the frequency domain method, all the high frequency components are removed. Thereby, only some low frequency components are used for recovery.

> The second aspect is named *positive effective PSF*, which means that all the values of the effective PSF are positive (i.e., greater than zeros). Where, effective PSF means the part of PSF which affects the convolution results in the ROI. This aspect might be fulfilled in various situations. For example, the effective PSF values are, of course, positive if the PSF is totally positive; this is a little stricter than the situation in usual applications [16]. Or, the PSF may have non-positive values at its "dark rings", but only the central part of the PSF (i.e., the Airy disk) affects the convolution results in the ROI when the ROI is smaller than the diffraction-limit. In this case, the other part of the PSF would only affect the convolution results outside the ROI. Thereby, the effective PSF is the central part, whose values are usually all positive, for normal light microscopes. In practice, approximate solutions might be figured out sometimes even if the conditions are not fulfilled strictly, but the effectiveness would be uncertain.

17. Assume that there are two point objects. Please describe the operation of your simulation, and the deconvolution procedure to resolve the two points.

Answer:

In our paper, we proposed two methods: spatial domain method and frequency domain method. In both methods, we discussed both 1D and 2D situation, respectively. The procedure can be known by reading the 1D situation of the spatial domain method (page 6 and 7). Then the 2D situation further extended it.

In short: when two ideal points are turned on, the observed image is the superposition of two PSFs. Thereby, each pixel of the observed image has two components from the two PSFs, respectively. Picking two of such pixels, we can build two equations whose unknowns are the two ideal points' intensities. Solving this equation system (two equations with two unknowns), we will figure out the two points' intensities. This procedure does not happen in usual imaging conditions. But in the "resolvable condition", not only two points but also multi-point ROIs can be figured out. Please note that pixel coordinates are used in the methods.

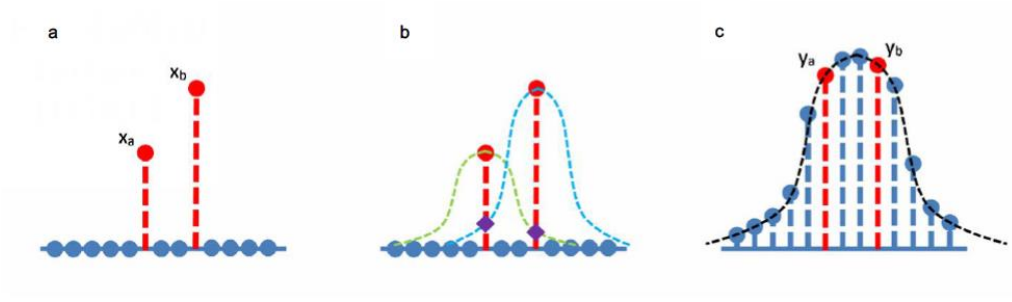


Fig. 2. The 1D situation of the spatial domain method. (a) Before convolution (the ideal signal). (b) During convolution. (c) After convolution (the observed signal).

For example, suppose that the ideal signal is (⋯ 1.2 3.4 ⋯), where the suspension points mean infinitely many zeros; the IRF is (⋯ 0.9981 1.0 0.9981 ⋯), where the suspension points mean arbitrary values (they do not affect the result in this case); therefore, $p = 1$, $q_a = q_b = 0.9981$; then assume that the observed signal is (⋯ 4.5934 4.5977 ⋯), where 4.5934 and 4.5977 are the values of y_a and y_b respectively, and suspension points mean the other values. Substitute these values into formula (2) and we get: $x_a = 1.2$ and $x_b = 3.4$. In other words, the ideal signal is recovered from the observed signal and the IRF.

7 / 23

18. How to practically illuminate a rectangular ROI with such sharp edges? Practically one has to consider excitation and emission PSFs.

Answer:

As discussed in our paper, there can be multiple ROIs, and each ROI can have any

irregular shape. Irregular ROIs are allowed in our methods, but one easy way is to find a bounding box to replace it. Excitation and emission PSFs are handled in the same way as reflection PSFs. Please refer to your paper (page 8).

19. How to differentiate true zeros in the data from noise (if any)? Can your technique do so perfectly?

Answer:
This paper mainly discuss the principle, i.e. how to extract details from seemingly irresolvable images. We think it is just a beginning, and has not solve the noise issue perfectly. We have tested low noises, but noise itself is a difficult research area. There are still a lot of work need to do.

20. Cameras can only detect the intensity of light instead its phase. Thereby, you should use the intensity of the PSF, and the following line should be used:

    psf = abs(psf).^2

    Similarly, the images should not have any negative values.

Answer:
Those negative values are actually not used in our code. The second aspect of the proposed "resolvable condition" requires "positive effective PSF". Accordingly, all the ROIs are set to much smaller than the "Airy disk" (central area of the PSF) in the simulation experiments. Thereby, the negative values have no effect on the results.
The PSF in the code is the Inverse Fourier Transform of a "most typical" low pass filter. But we also wrote another code file "GenPosiAiryPsf.m" in June, which moved the original PSF upward to make it all positive. Similar results and the same conclusion were got. If your code is added into our program, we will also get similar results and the same conclusion. Although our theory is general enough to cover all these cases, it would be better to model more precisely in the future.

21. Is it a right way moving the PSF "upwards" to make it positive? Doesn't that make the simulation even more unrealistic?

Answer:
The main task of this study is to see: if (in what condition) the original image can be recovered directly after its high frequency components are all removed by the microscope. Thereby, the PSF must be modeled as an ideal low pass filter. **This is the key feature that has essential effect on the results and conclusions.**
In the previous answer, our first PSF is the Inverse Fourier Transform of an ideal low pass filter. Thereby, it meets the above requirement. Our second PSF is generated by moving the first one upwards. That only adds a zero frequency component to the original spectrum. As a result, it is also an ideal low pass filter. After an image is convolved by an ideal low pass filter, the original image cannot be recovered in usual condition (even in simulation experiments). That is why we call our "resolvable

condition" an exception. Actually, our method works no matter the PSF is an ideal low pass filter or not. That is because it recovers details merely from low frequency components. Please refer to our paper (especially page 7 and 10) for mathematical proof. It is also supported by our source code, including 19x20 repeated random tests. We have spent some time analyzing the code: psf = abs(psf).^2, and find it also removes high frequency components (just like ours). Since the code works well in our program, it also supports our conclusions.

22. In practice, there are various factors that may limit the accuracy of observed data. But your study ignores those factors. Is it reasonable?

Answer:

Yes, this study assume that there is no or only low noises. We do not believe this paper can solve all the problems perfectly. We treated it as just a beginning instead of a perfect ending. Actually, noise is a hard issue in all kinds of field, and it is a relatively independent research topic (or even area).

The only input data is the observed image and the PSF image, in our algorithms. Any other factors (e.g., shot noise, wavefront distortion, detector limitation, etc.) can be attribute to the noises or distortion of these images. Thereby, our paper treats it as an important future direction. Given the progressive advancement of denoising technology, we believe it can be solved or improved gradually.

Based on the above considerations, input data are assumed to be accurate in this study. Then, our major concern is: if the distance of two points is smaller than the diffraction-limit, and they are imaged simultaneously by a conventional light microscope, are they resolvable in the same image? According to classic theories (about the diffraction-limit and Rayleigh criterion), the answer would be no. Of course, existing super-resolution techniques have already achieved resolutions beyond the diffraction-limit. But to our understanding, adjacent points (or different frequency components) are imaged in different time. However, this study finds an exceptional condition (termed "resolvable condition"), such points can be resolved directly. In such a "resolvable condition", neither profile nor detail information is damaged by diffraction. Thereby, it can be recovered reversibly from a diffraction-blurred image (i.e., an image without high frequency components). This condition is tightly relevant to the imaging condition of existing super-resolution techniques. Then, a method is proposed based on the condition which can achieve unlimited high resolutions in principle.

23. Your paper implies that the inner details of an individual fluorescent molecule can be resolved. But in fluorescence microscopes, the entire protein molecule is not luminescent. Actually, the actual photons are only emitted from the chromophore. How can the "full" details be recovered?

Answer:

Our finding and methods apply to various light microscopes including fluorescence

microscopes. The imaging of fluorescent molecules is one of their possible applications on fluorescence microscopes. Our methods can extract the inner structures of illuminated ROIs. Such ROIs could actually be various objects as long as it fulfills the "resolvable condition". In the future, maybe we can try structures comprised of adjacent molecules, or multiple chromophores? Maybe we can also try to extract the inner structure of chromophores? Or, if a molecule is illuminated by light directly (not in a fluorescent manner), is it possible to extract its inner structure better? These are just guesses, but may worth exploration because they are in accordance with our method's principle.

24. Some existing super-resolution techniques can achieve very high (e.g., 1nm) localization precision. What is the relationship between your technique and them?

Answer:
Our technique can be used based on those techniques. Actually, our methods need to first localize an ROI (e.g., a molecule), and then further extract its inner structures. If an existing super-resolution technique has very high localization precision, it would be helpful for our methods. But rough localization is also acceptable.