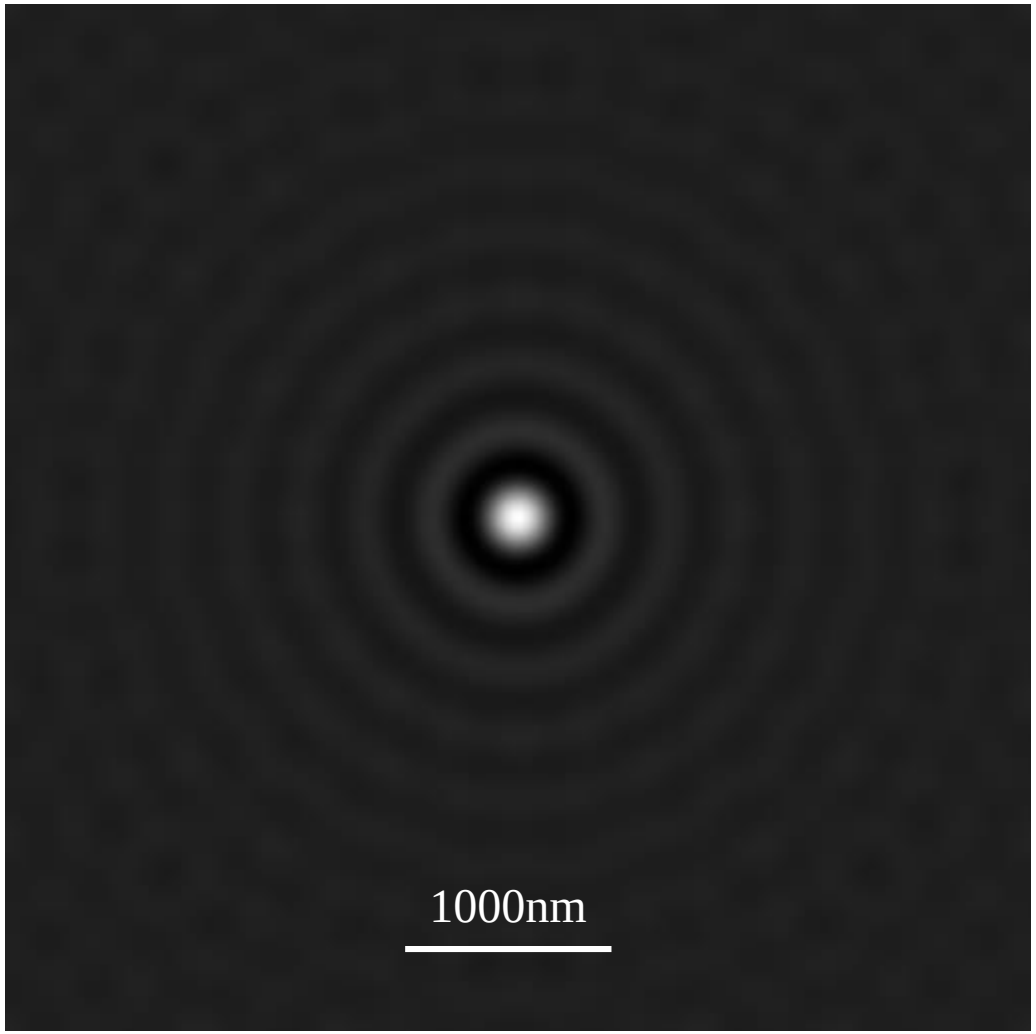
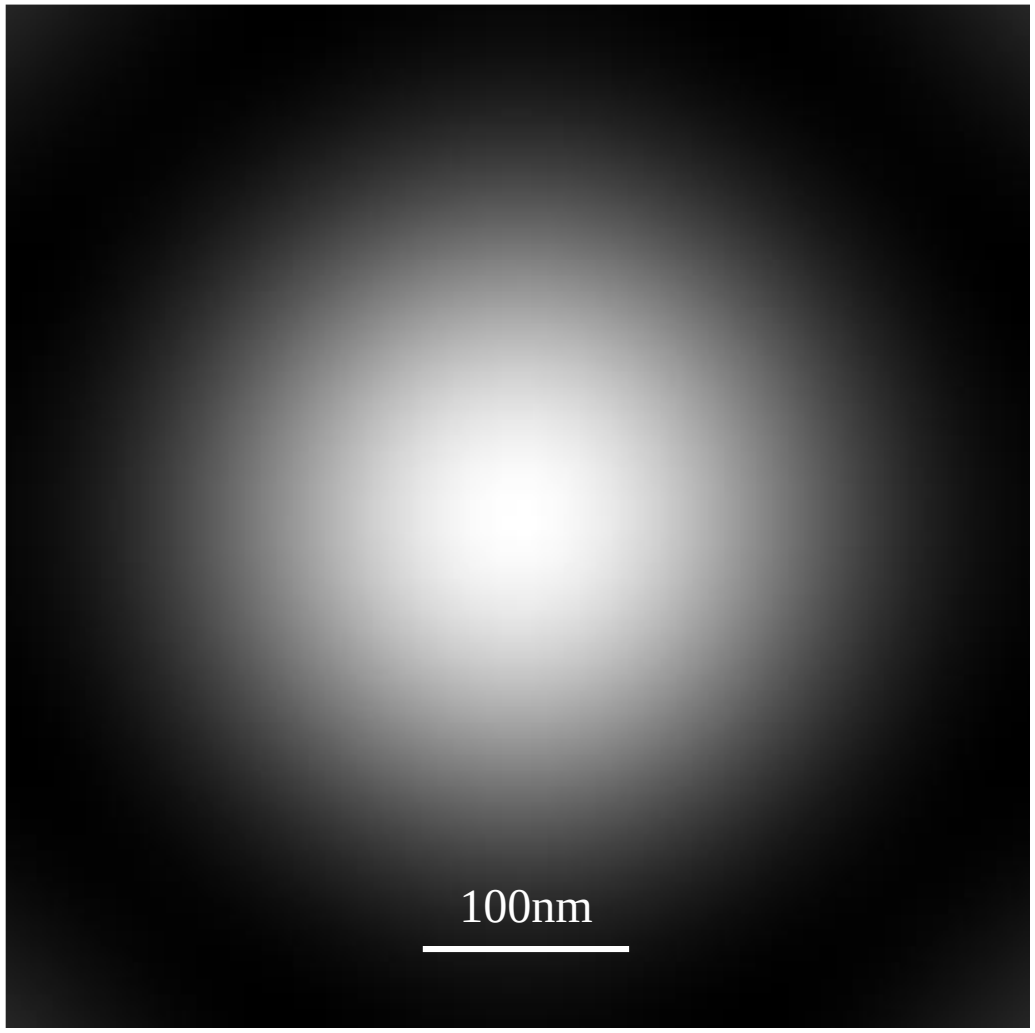
Brief illustration

Extracting super-resolution structures inside a single molecule or overlapped molecules from one blurred image

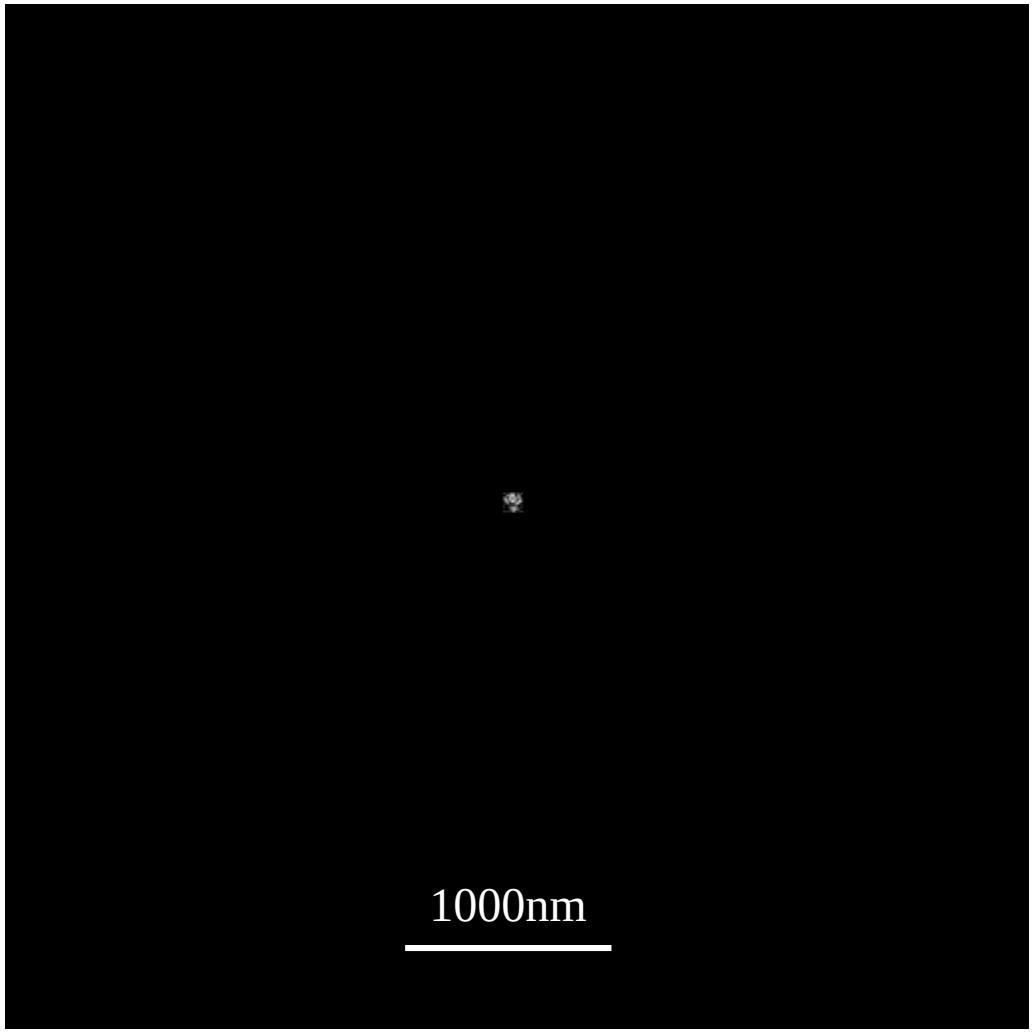
This is a microscope's PSF (Point Spread Function), in different scales.
In principle, it extends infinitely broad (only the central part is shown):

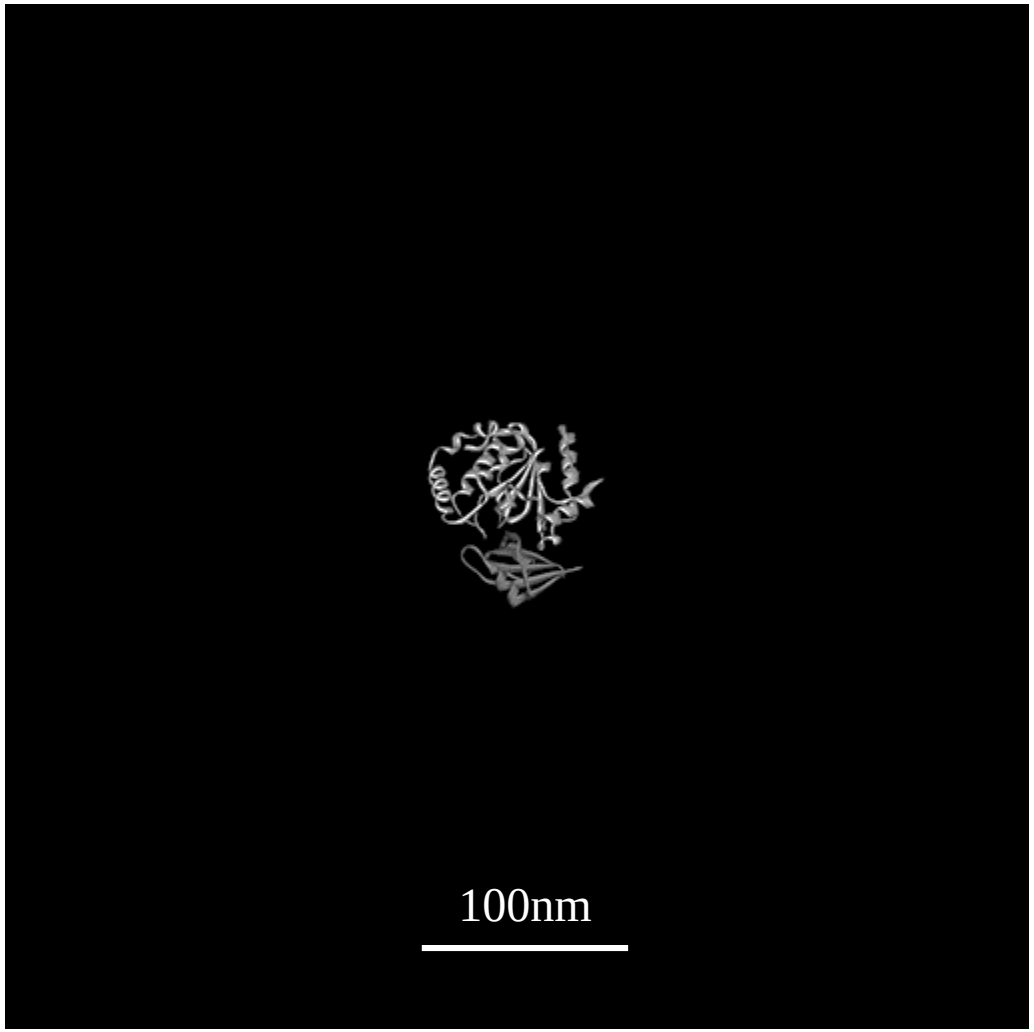




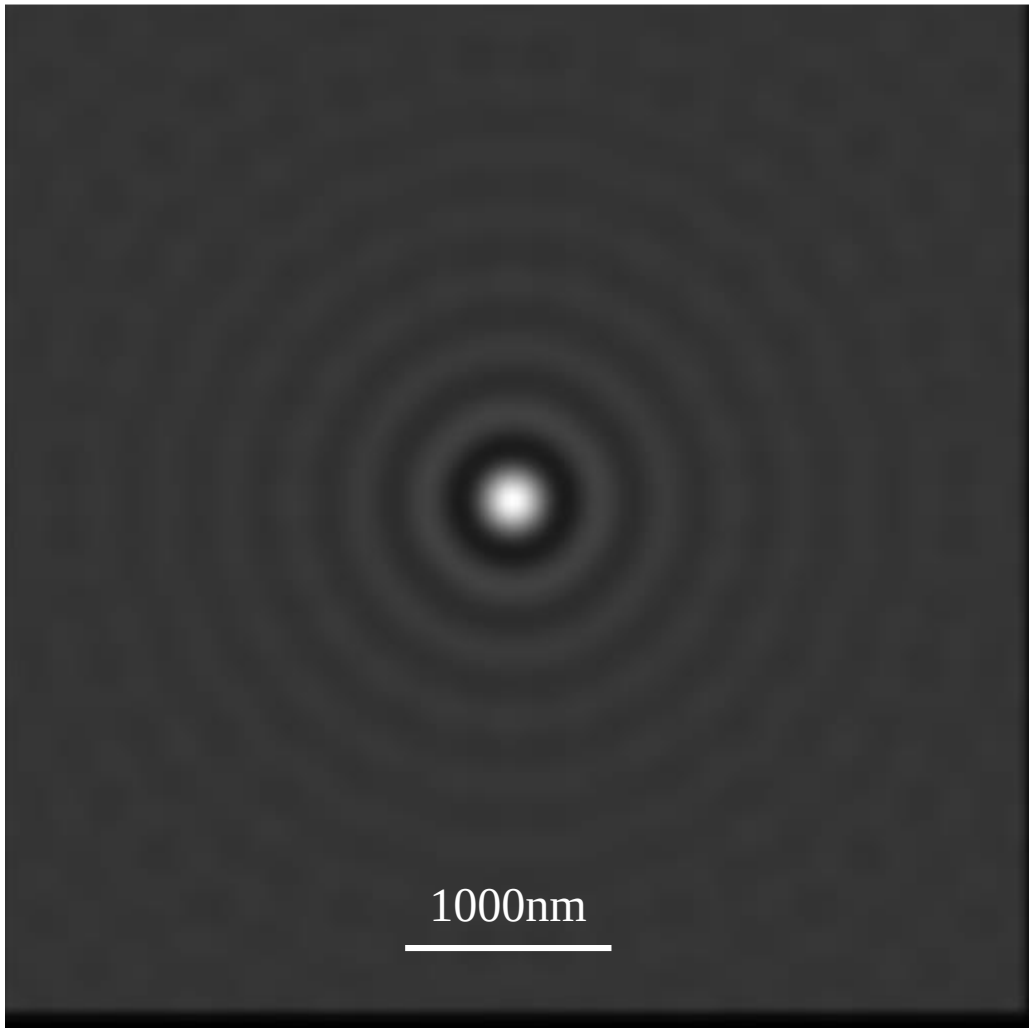
Thereby, points' images overlap and can not be resolved when they are close to one another.
Existing Single-Point-Localization (SPL) techniques turn on adjacent molecules at different times.

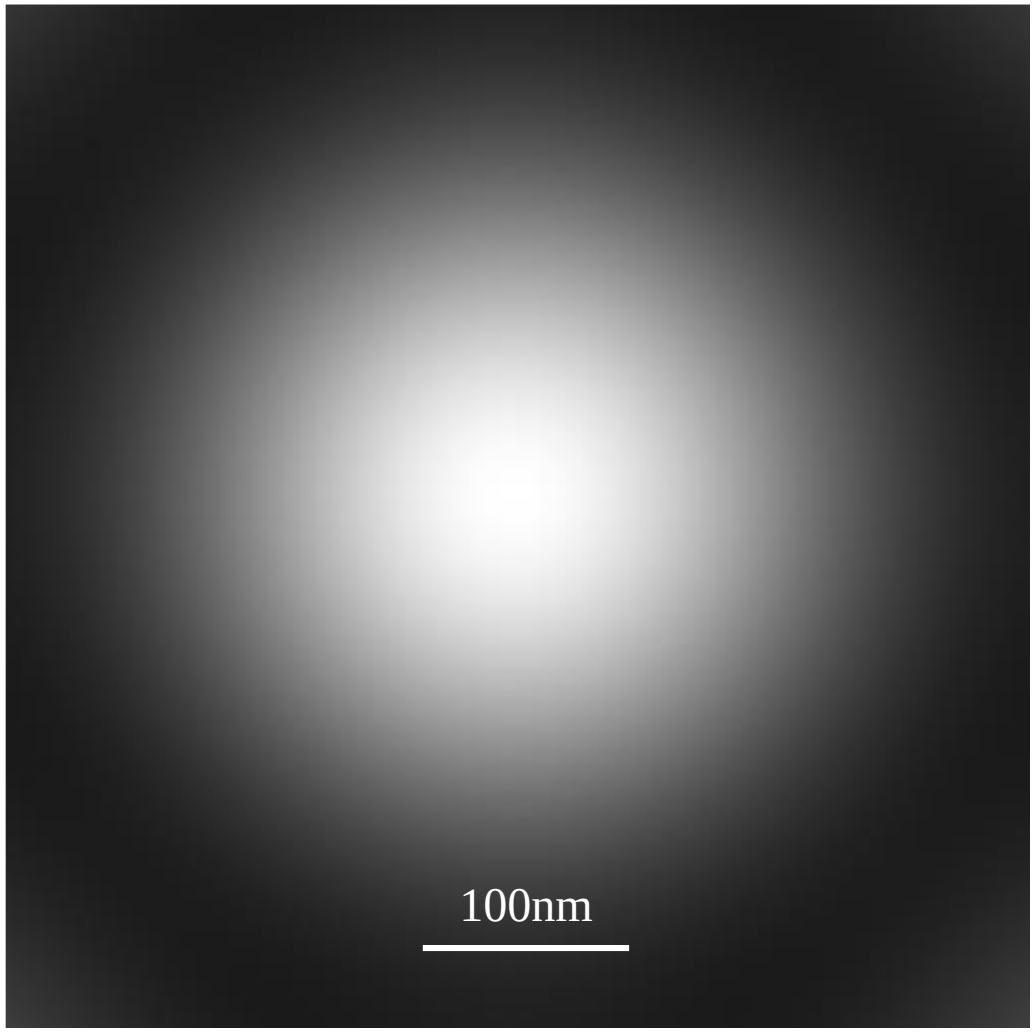
This is the structure of a molecule, in the corresponding scales:





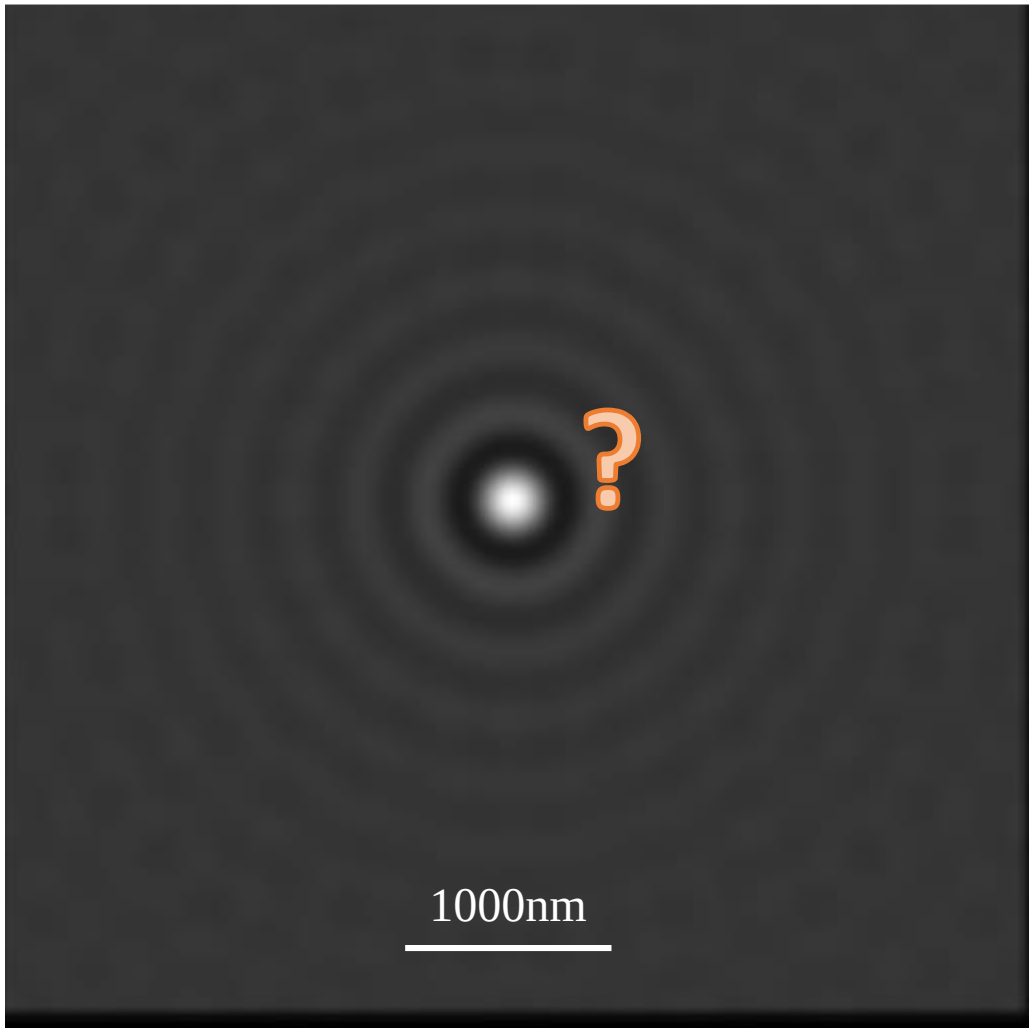
These are images of the molecule observed by the microscope.
In principle, they also extends infinitely broad (only the central parts are shown):

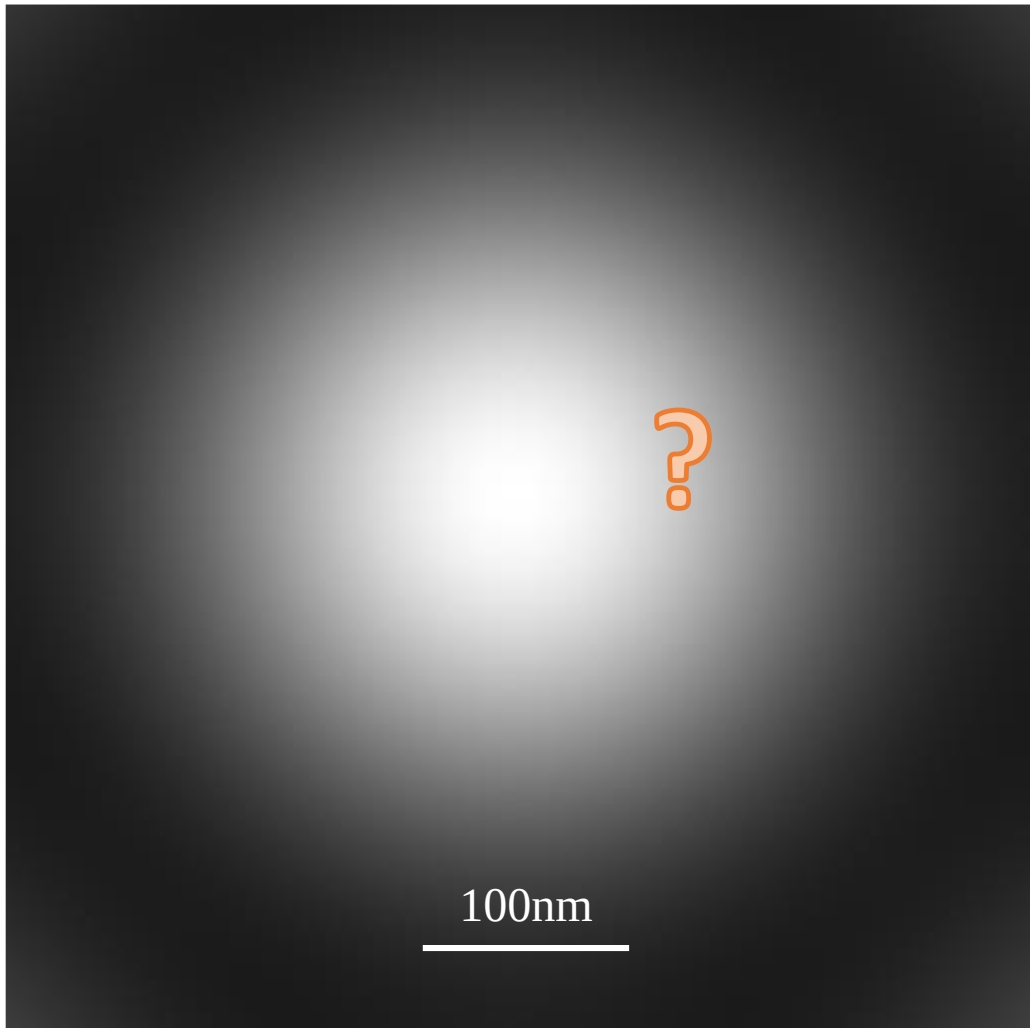




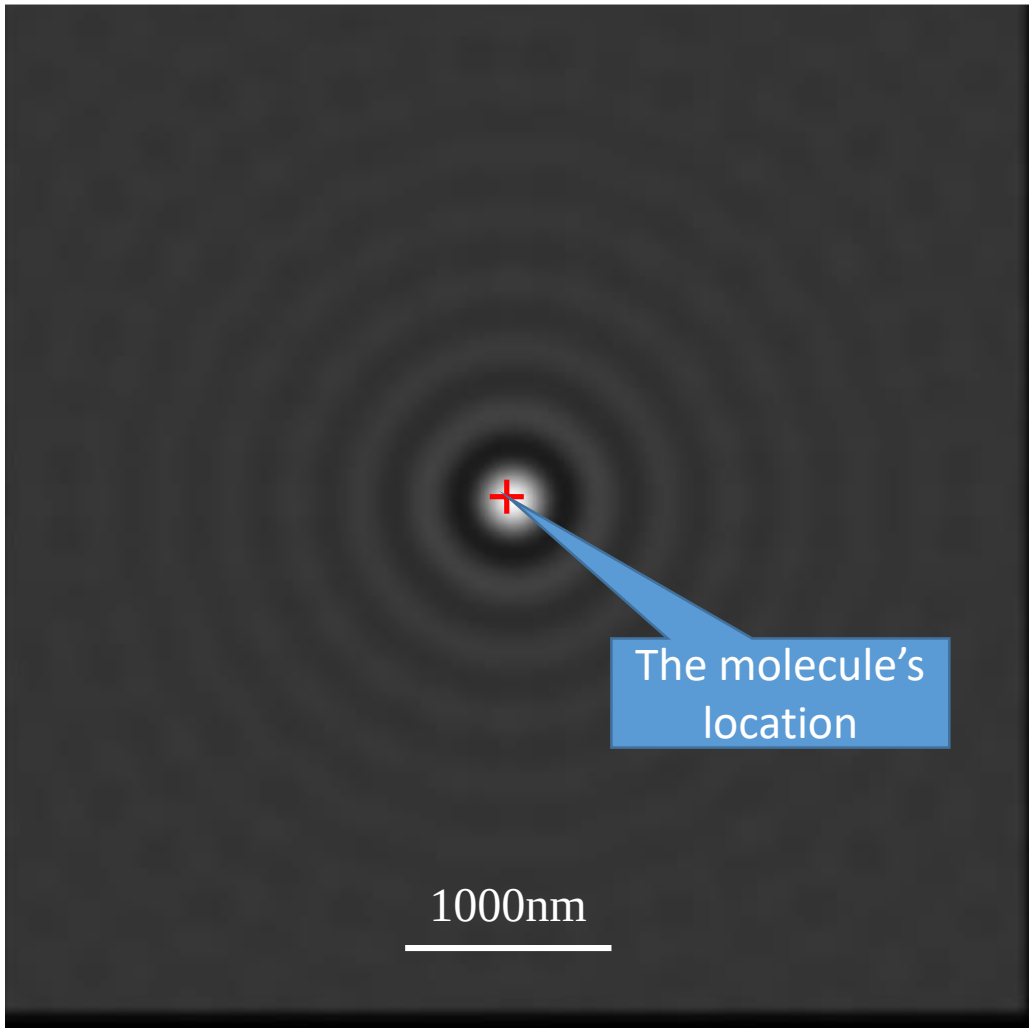
Imaging procedure can be modeled as the convolution of the structure with the PSF.
These results are very blurred, look like the PSF. No structure can be observed directly.

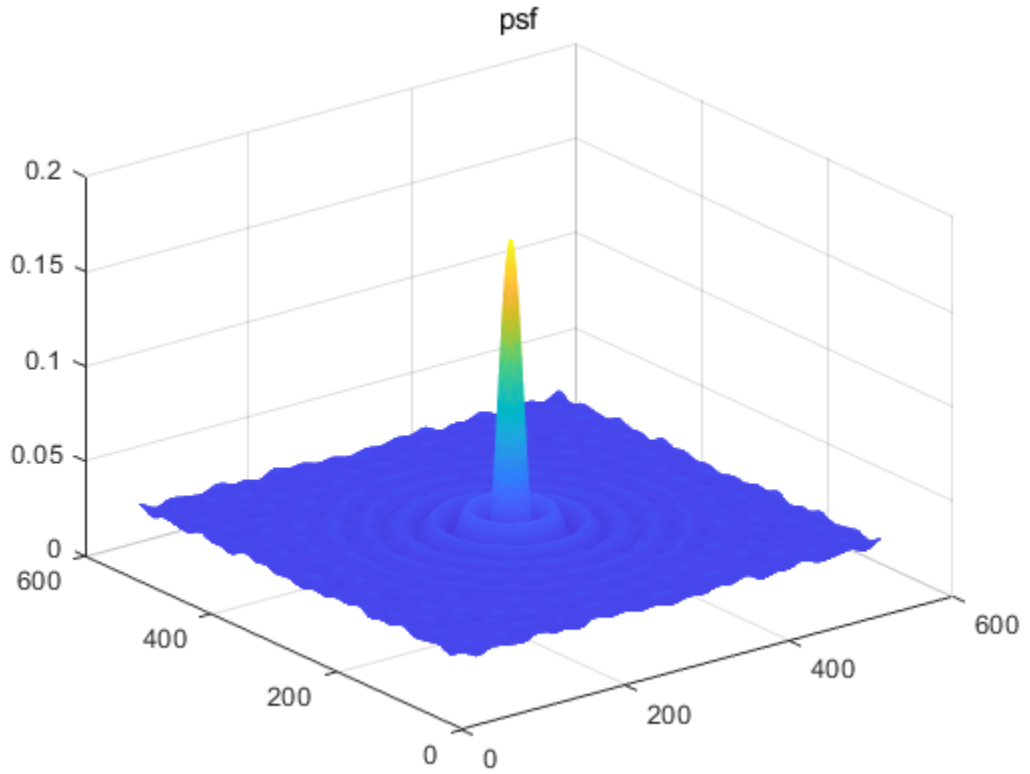
How can we know what's behind such a blur?





Fortunately, the location and light intensity of the molecule can be figured out with **existing Single-Point-Localization techniques**:

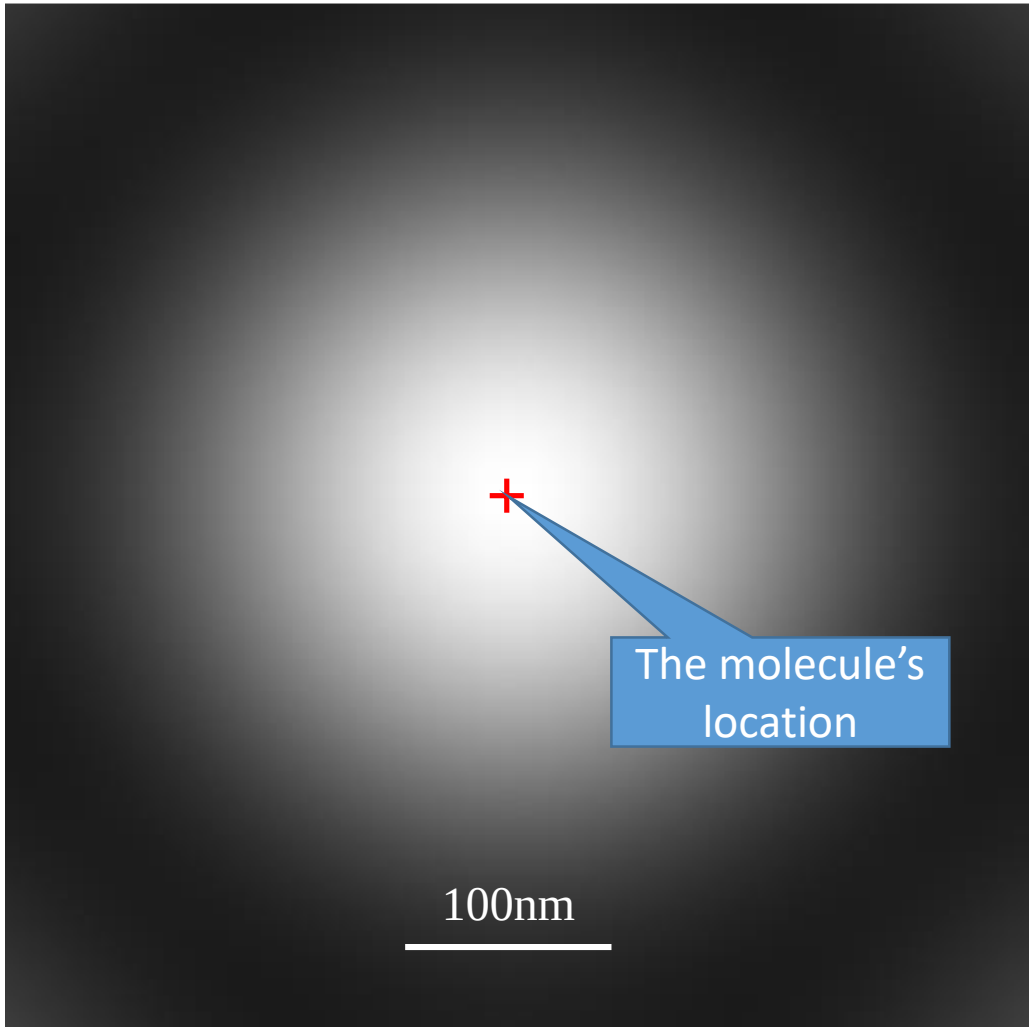


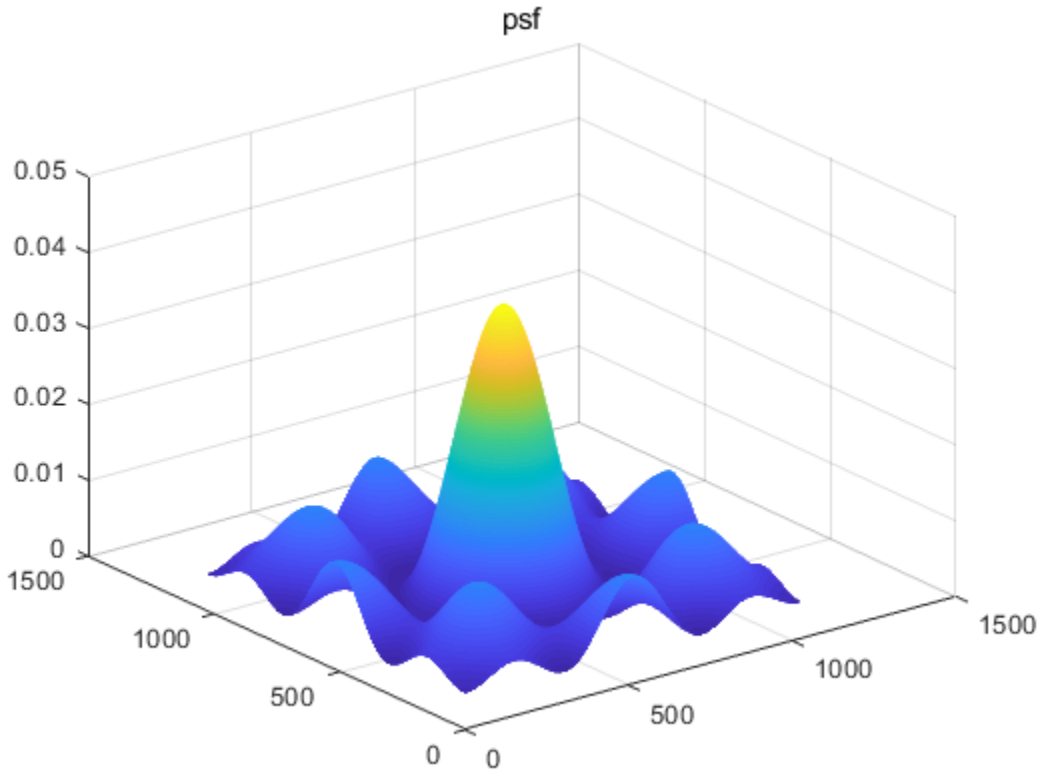


The PSF is known, and sometimes could be treated as a Gaussian Function. Thereby, the location can be figured out by data fitting, etc. (Unit of the coordinates: pixel)

Finally, thousands of such points form a super-resolution image.
The diffraction-limit is overcome, and the resolution is improved significantly.

Similarly, it can also be done in this scale:

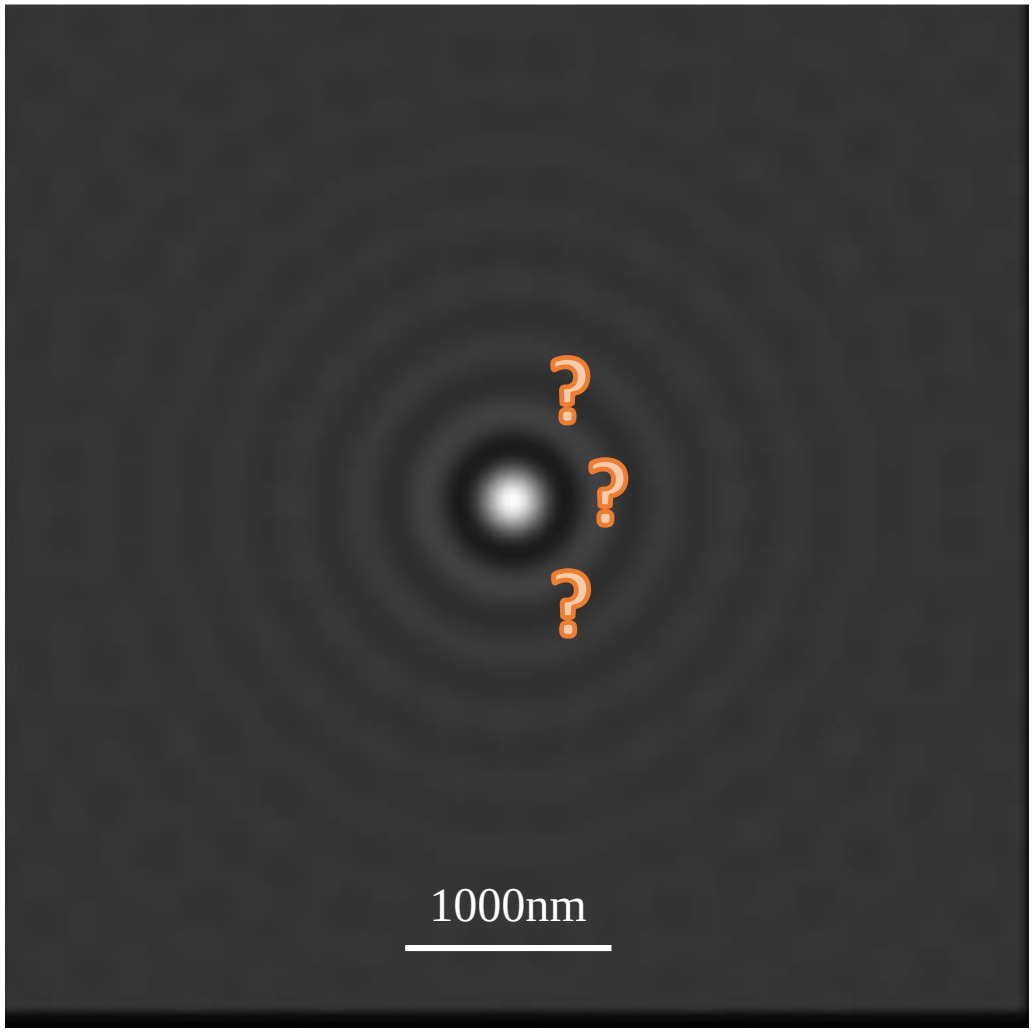


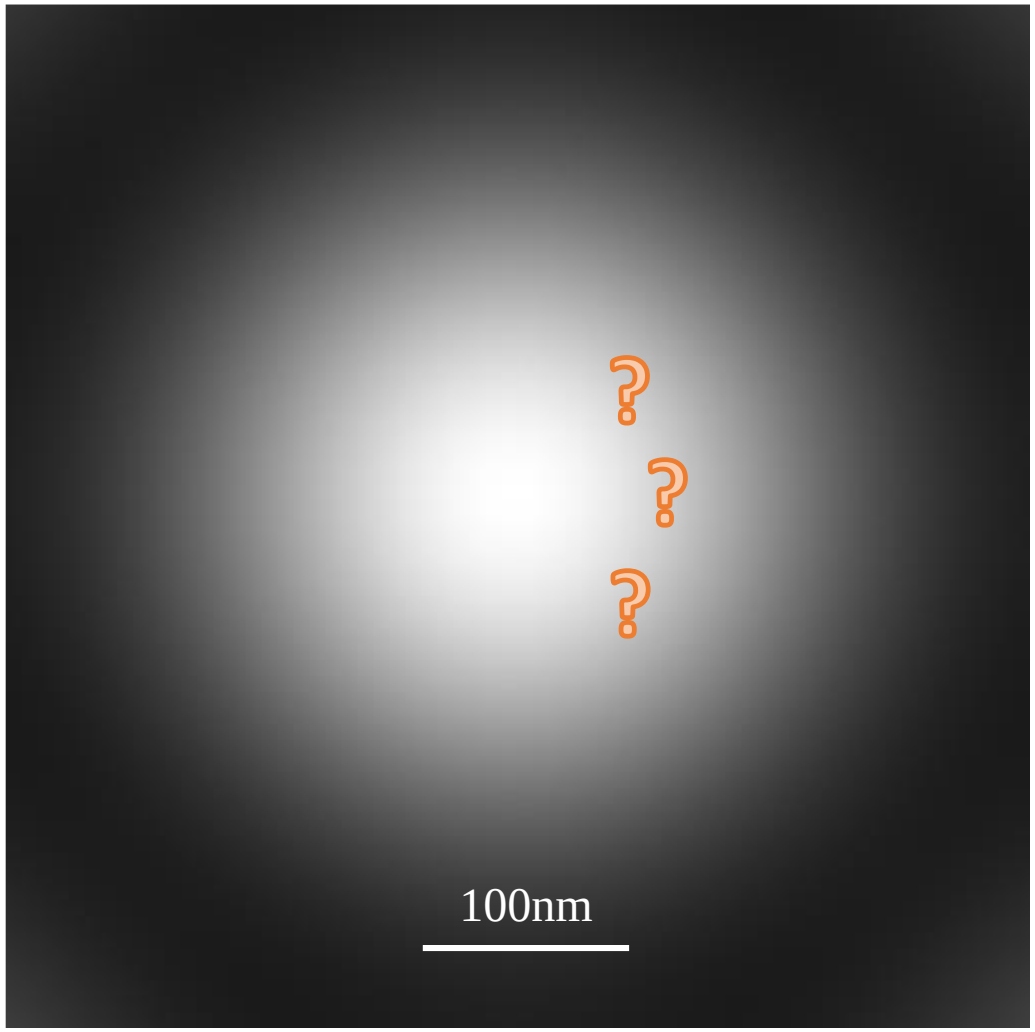


The PSF is known, and sometimes could be treated as a Gaussian Function. Thereby, the location can be figured out by data fitting, etc. (Unit of the coordinates: pixel)

Finally, thousands of such points form a super-resolution image.
The diffraction-limit is overcome, and the resolution is improved significantly.

But, can we know more about the blurred image?





We try to extract more information:

- Existing techniques extract the location and light intensity of a molecule from the extremely blurred image.
- Then, is it possible to extract the **full structure** inside the molecule (including both **profile** and **details**)?
- Or resolve multiple molecules when they overlap and are turned on at the same time?

The proposed technique (DeSu-re):

- According to conventional optics theory, a light microscope filters out high frequency components, and makes the observed image blurred. As a result, detail information **can not be recovered** directly.
- But this study find a "**resolvable condition**".
- In this condition, the structure's full information **can be recovered**, including both profile (low frequency) and details (high frequency).
- Based on the condition, Deconvolution Super-resolution (DeSu-re) is proposed. It works in both spatial domain and frequency domain.

The “resolvable condition” includes two aspects:

- **Aspect 1: “*isolated lighting*” (or “*separated lighting*”)**

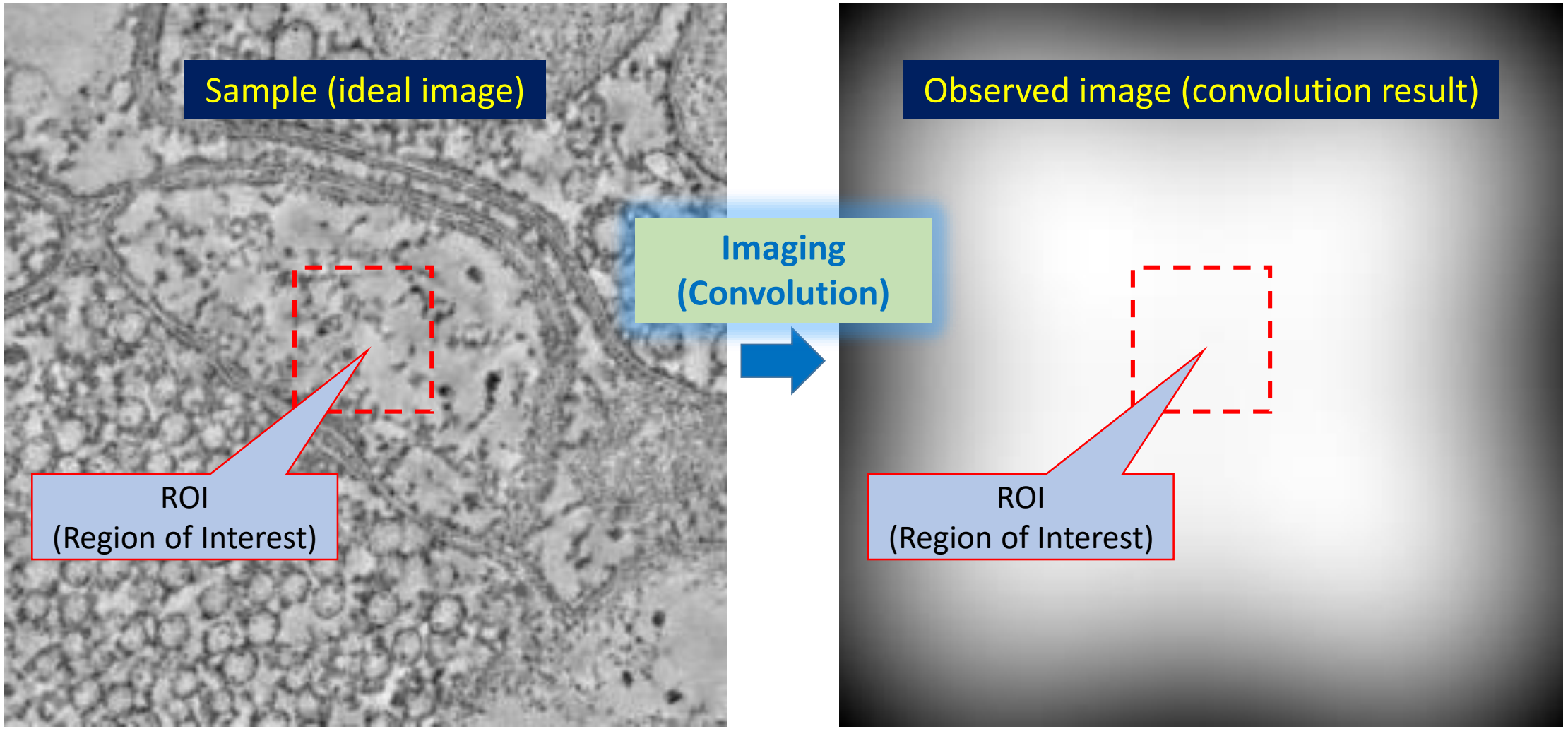
The theoretical extension of the imaging condition of Single-Point-Localization techniques.

- **Aspect 2: “*positive effective PSF*”**

One possible way to fulfill this condition: handling structures which are wholly smaller than the Airy-disk.

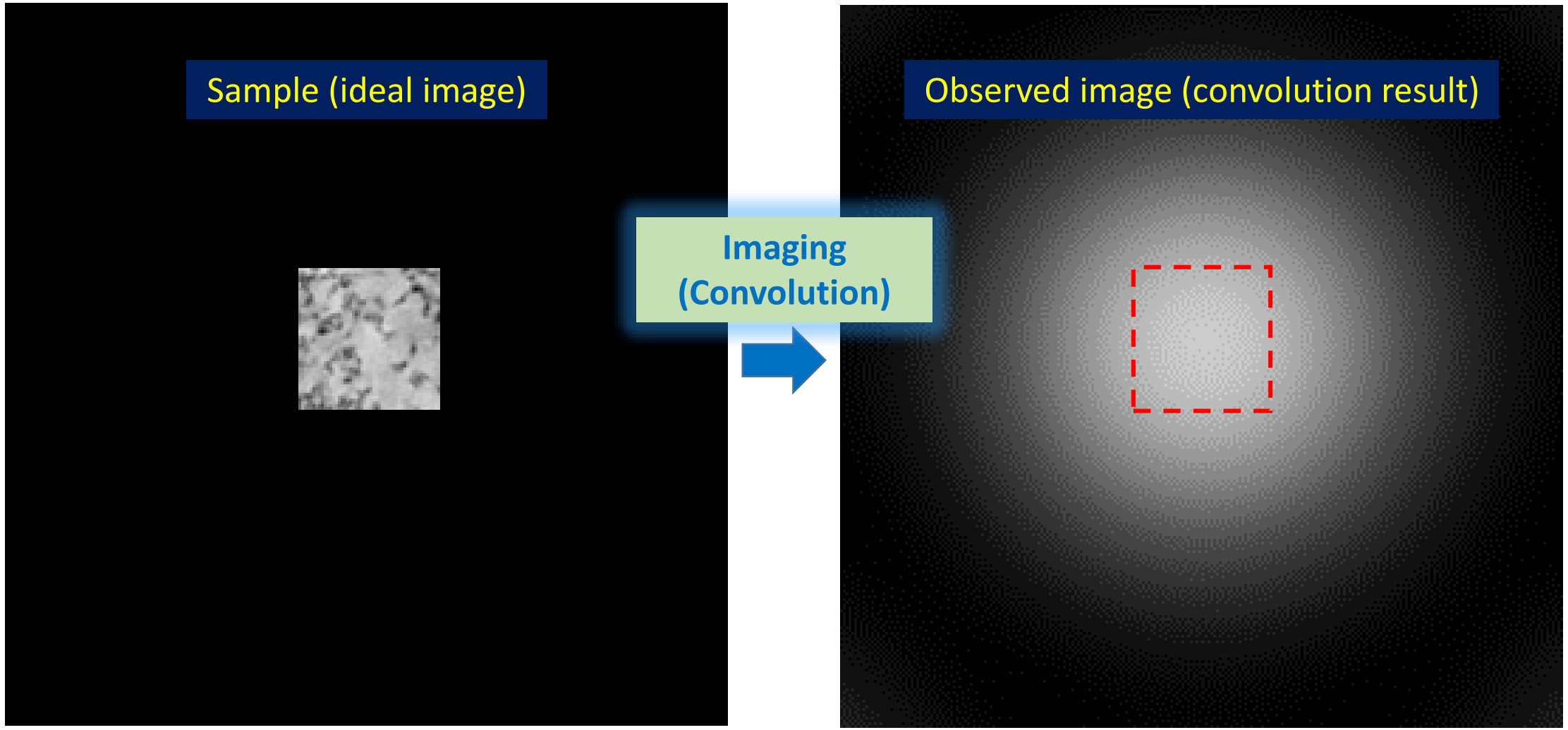
(This is almost opposite the condition of conventional microscope.)

This is an example of normal condition (not *isolated lighting*):



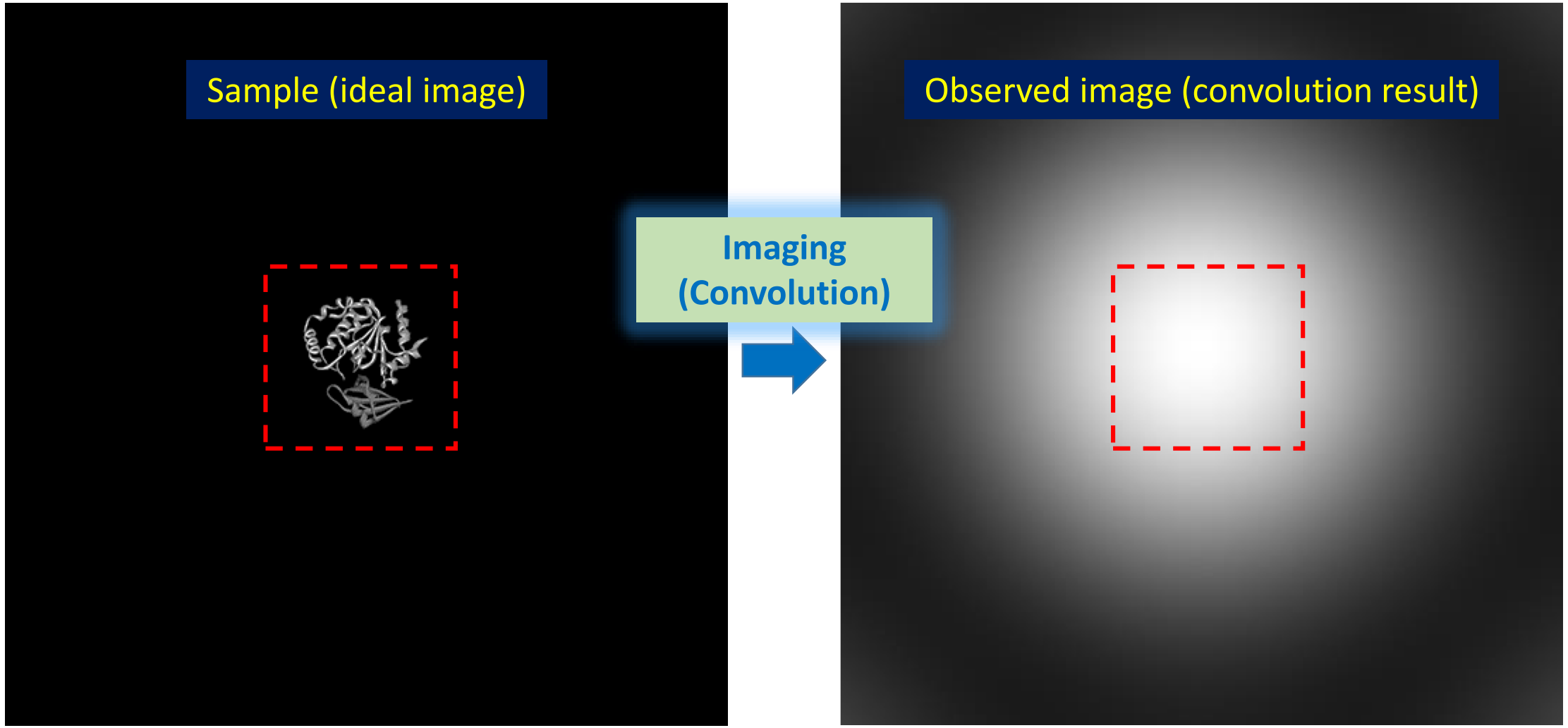
The ROI's light intensities are **submerged by extra lights** (the images of outside structures).
This prevent the ROI from being figured out because the **extra lights are unknown**.

This is an example of *isolated lighting* (or, *separated lighting*) condition:



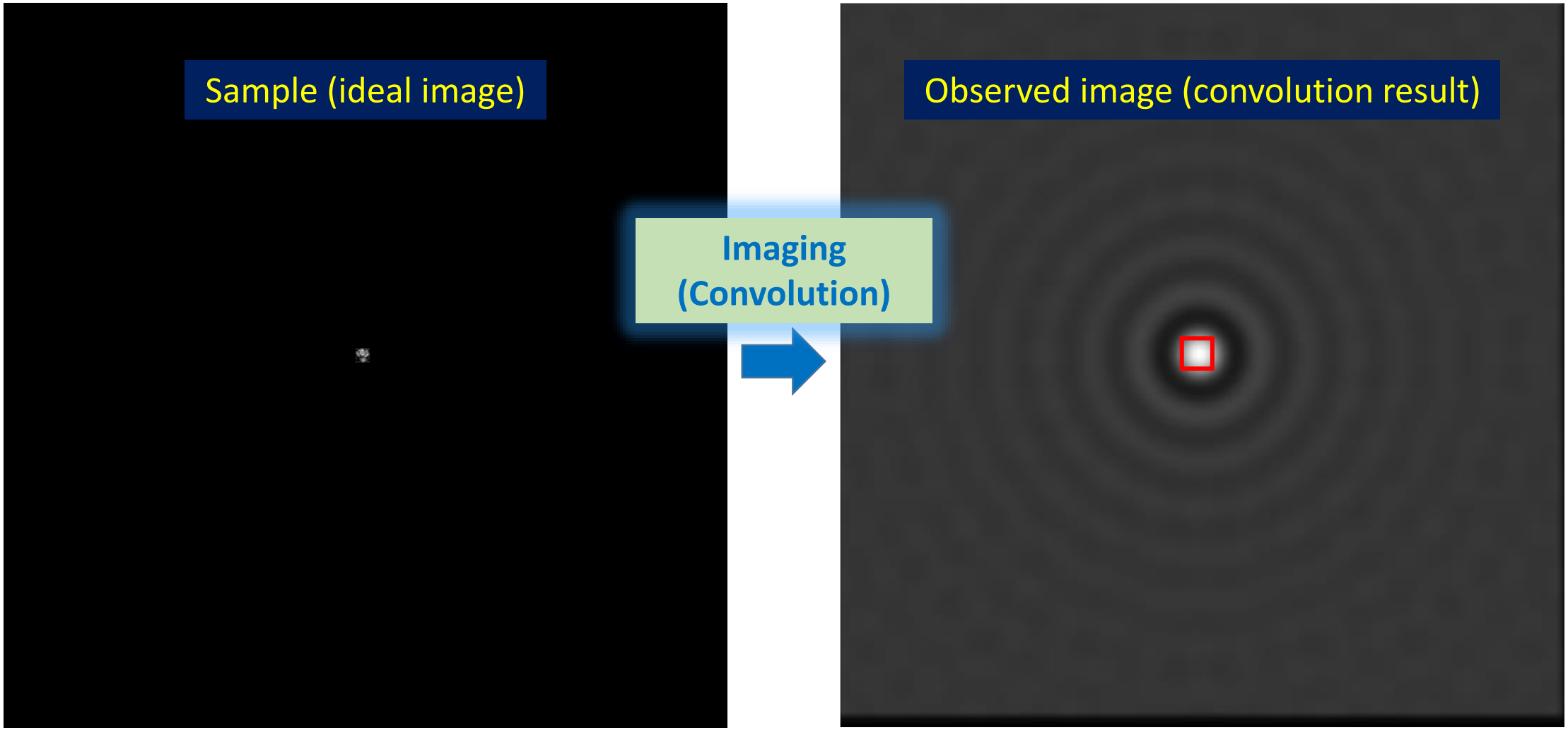
Since there are **no extra lights**, the ROI **can be figured out** using the proposed methods.

Similar situation happens to a single molecule:



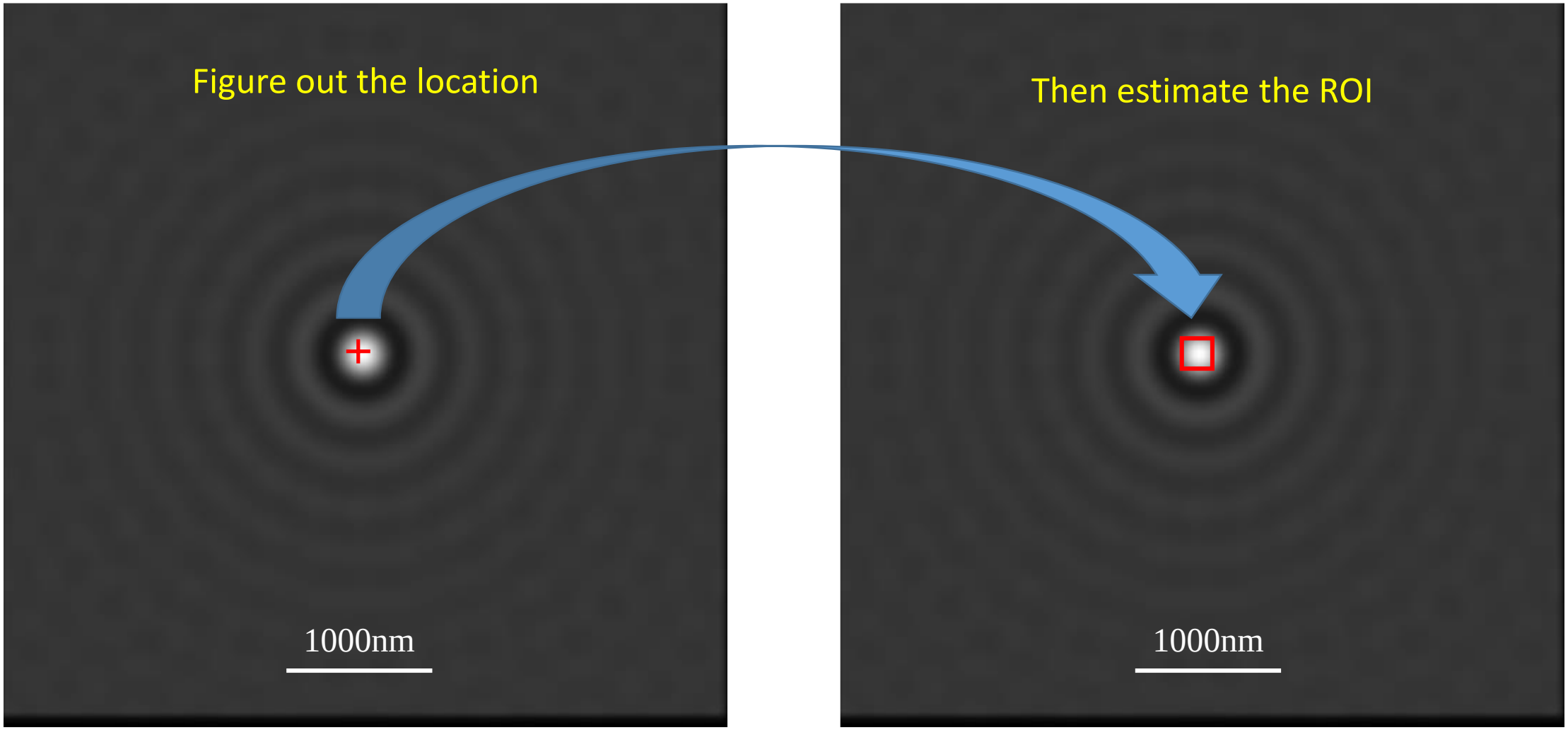
Since there are **no extra lights**, the ROI **can be figured out** using the proposed methods.

And in the other scale also:



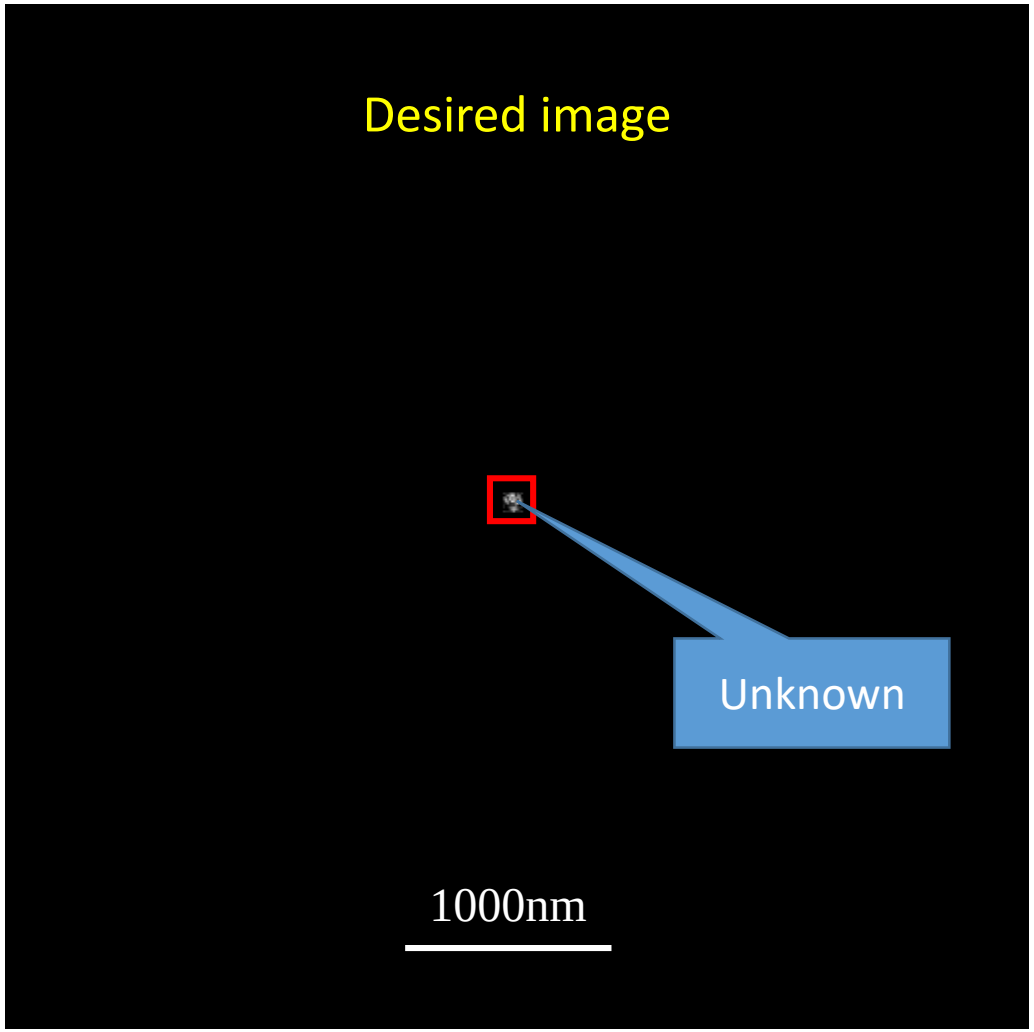
Now we will illustrate the method for spatial domain, taking the single-molecule examples.

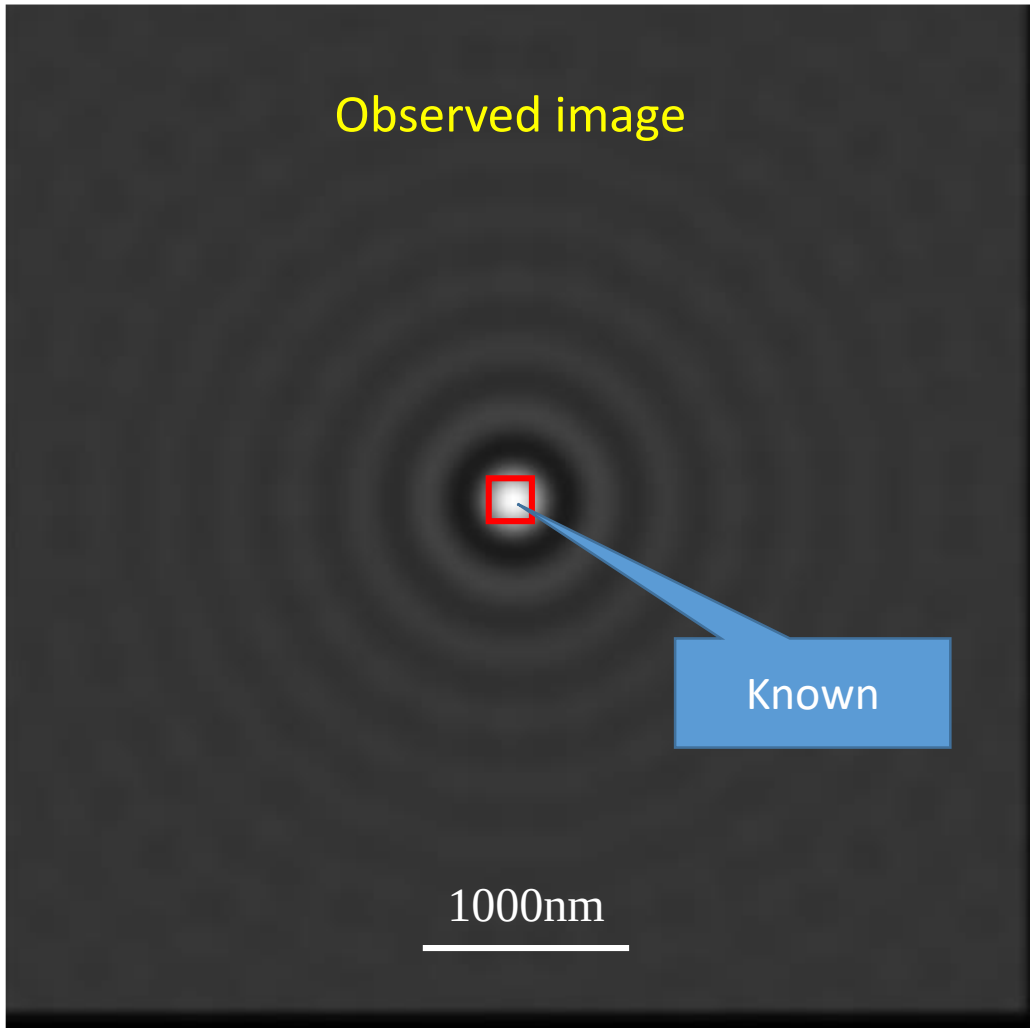
First of all, an ROI, which covers the molecule, needs to be estimated.
This can be done with the help of Single-Point-Localization techniques.



The ROI's size can be determined using prior knowledge, e.g., the molecule's maximum size.

Ideally, there is a mathematical relationship for light microscope:
observed image = desired image * PSF, where * means convolution.



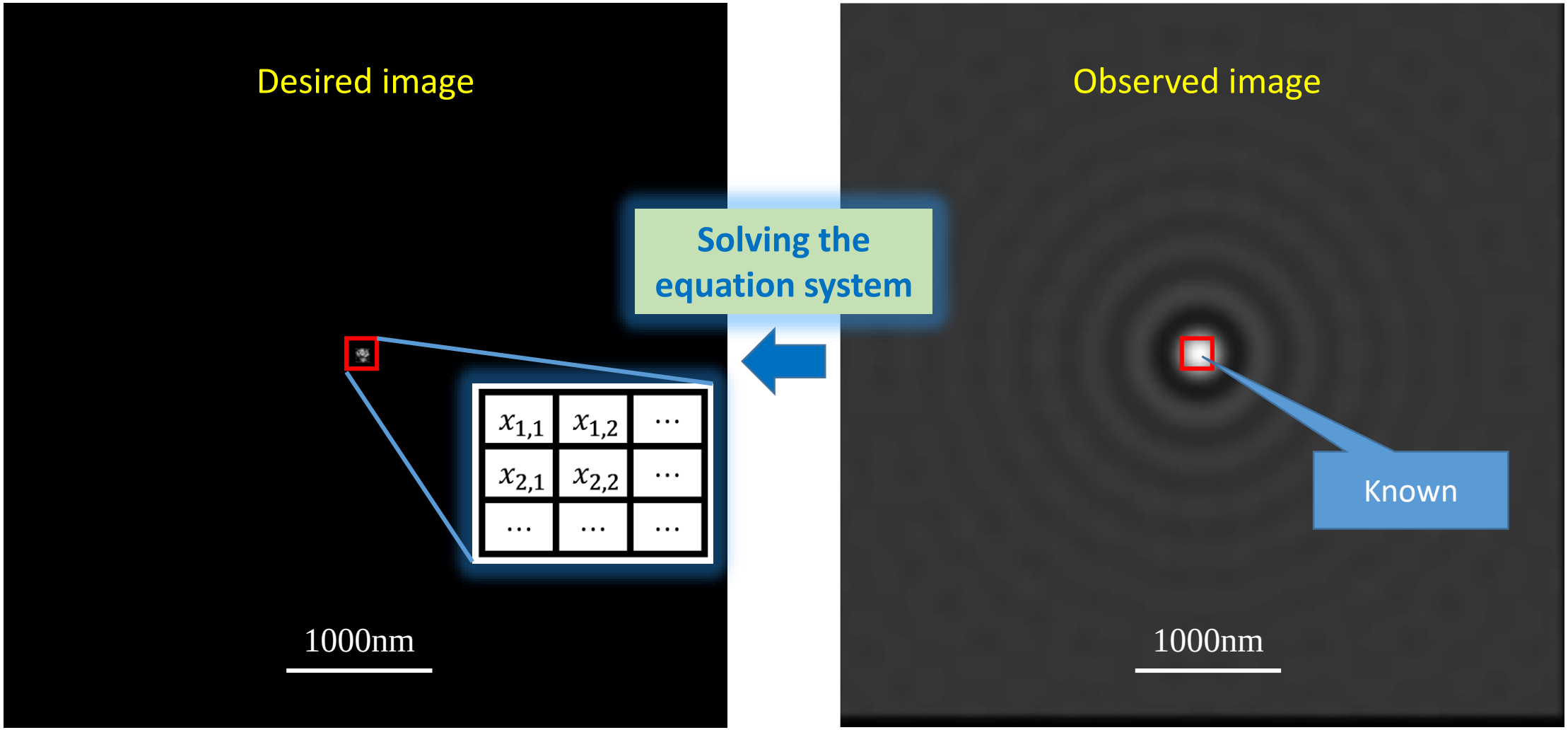


The observed image and PSF is known, and we want to know the **pixels in the desired image's ROI**.

Method for **spatial domain**:

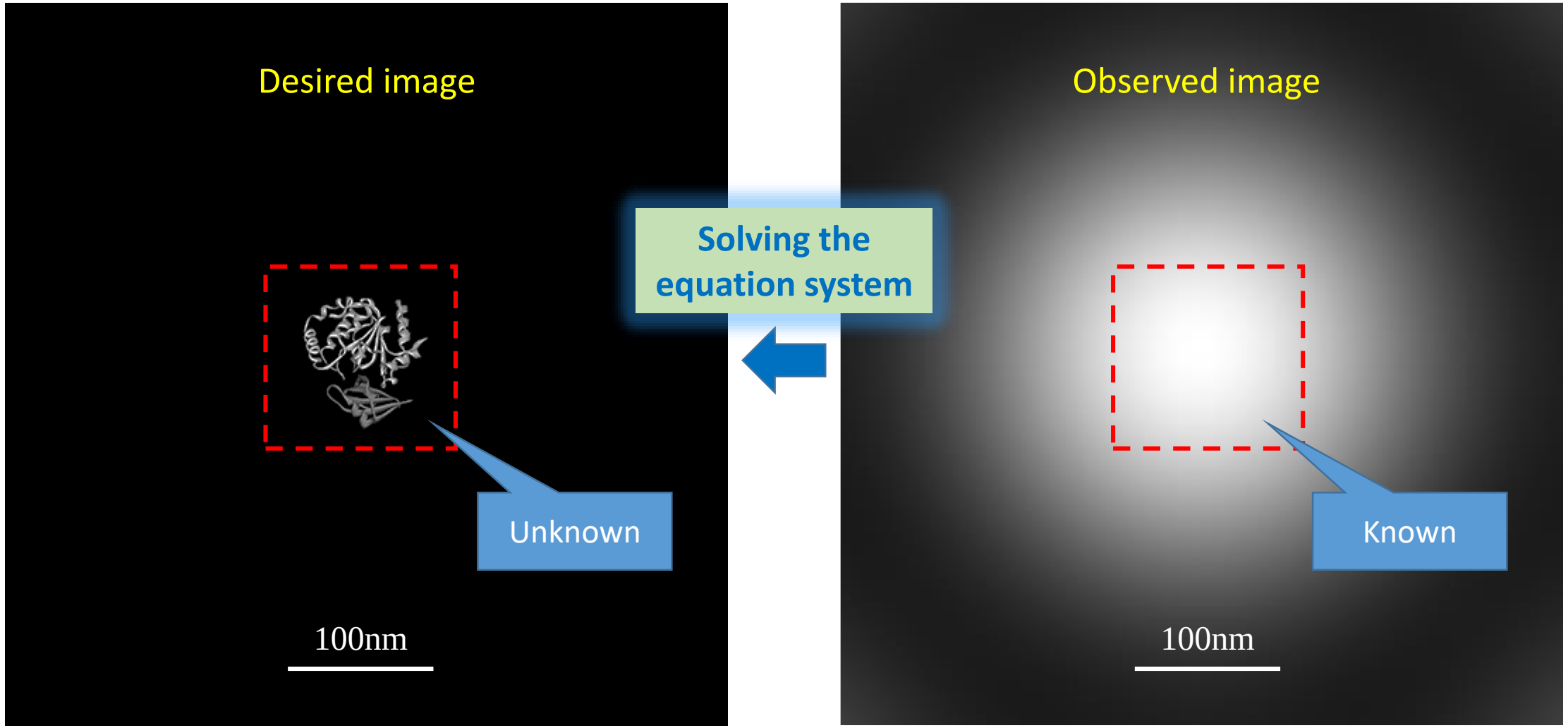
- According to the above relationship, an **equation system** can be built when the condition of "***isolated lighting***" is fulfilled.
- The desired image's pixels in the ROI are the unknowns in the equation system. The other pixels of that image are all zeros.
- In principle, the equation system has an **unique solution** when the condition of "***positive effective PSF***" is fulfilled.
- According to Fourier Optics, both the PSF and observed image extend infinitely broad because the microscope is an ideal low-pass filter.
- But it is enough even if only their central parts are used.

The rectangular ROI is essentially a matrix, and its pixels are the matrix's elements. Thereby, the relationship is actually about these elements.



As a result, the desired image's ROI pixels are recovered by solving the equation system.

If the method is applied to the other scale, higher resolution can be got.
But it requires to solve a larger equation system (more unknowns):



As a result, the desired image's ROI pixels are recovered by solving the equation system.

Method for **frequency domain**:

- Similar procedure can also be used to figure out the desired image in frequency domain.
- Instead of using the observed image and the PSF, the observed frequency spectrum and the OTF (Optical Transfer Function) is adopted to built the equation system.
- Generally speaking, **the details in a signal (image) can not be recovered if its high frequency components are removed**.
- But **this study shows an exception in principle**.

Explanation about the resolution:

- In the result of Single-Point-Localization techniques, each molecule is shown as **a single point**.
- In the result of the proposed technique, it is shown as an ROI with full details. If the ROI has **N×N pixels** (e.g., 10×10), that implies the resolution is improved by **N times** (e.g., 10 times or 1000%).
- This technique can be used to detect the structure inside a single molecule or overlapped molecules, using no high frequency.
- As a theoretical approach, it can achieve **unlimited resolution** in principle. But practical issues may still need further research.