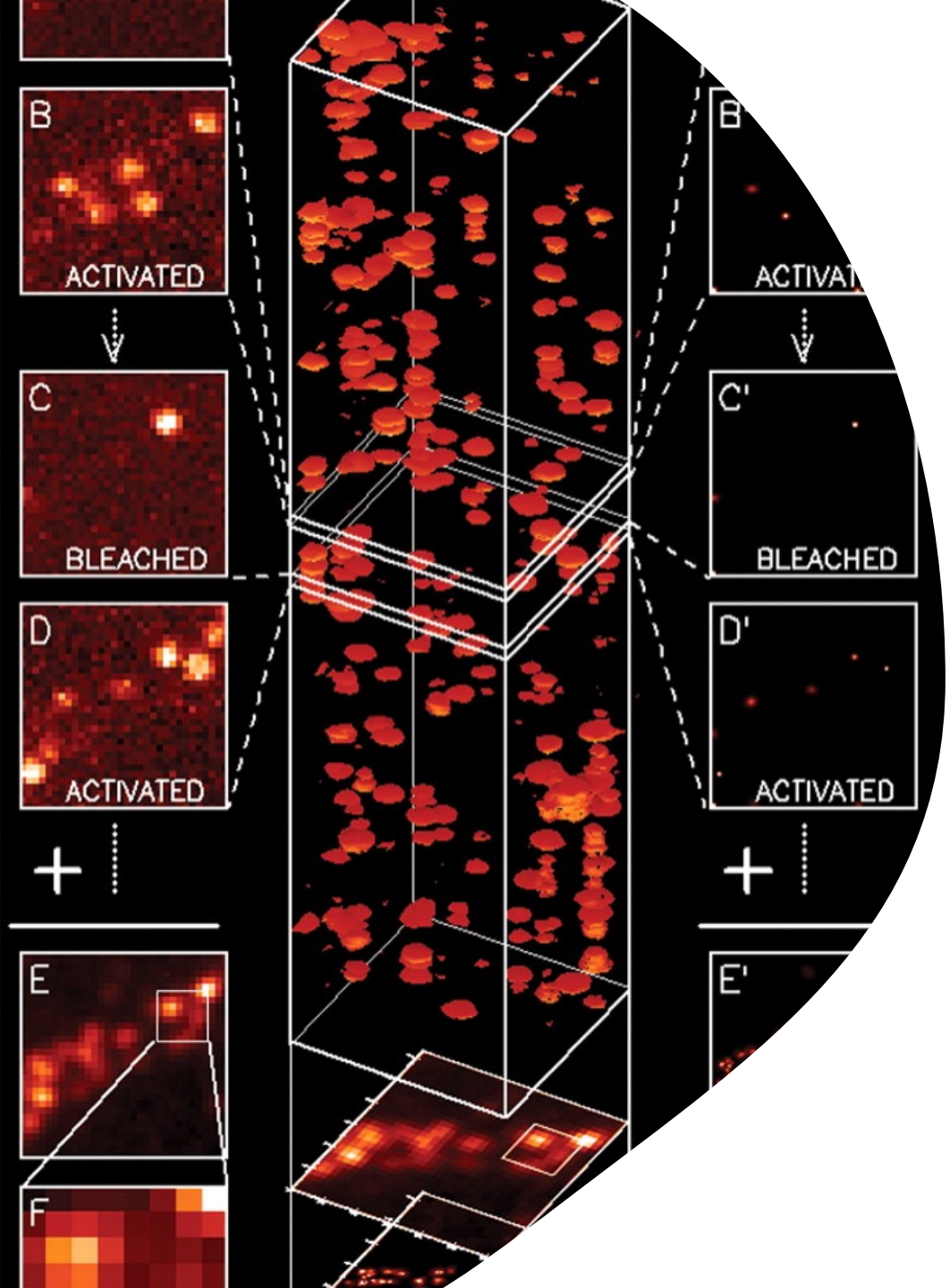
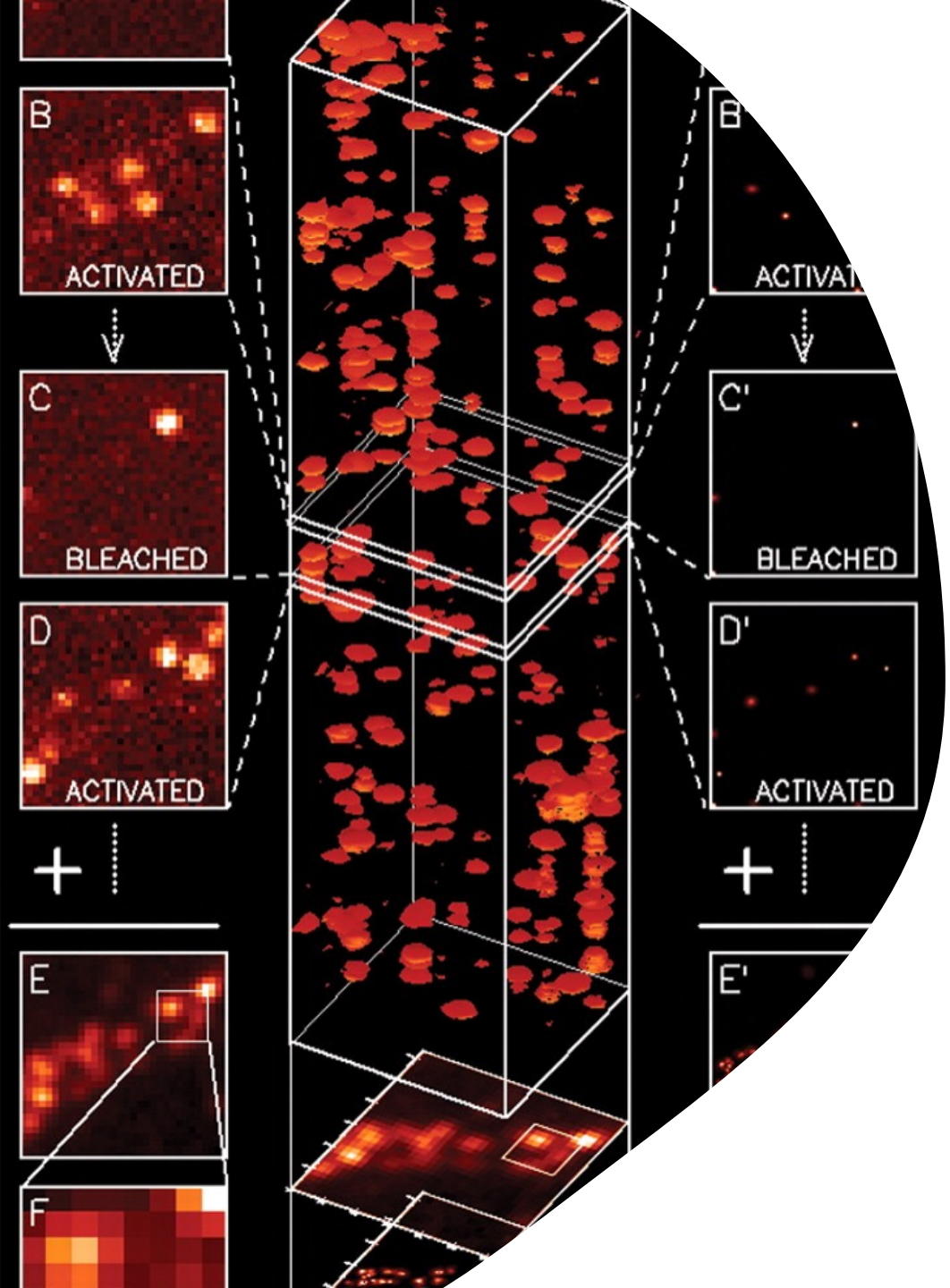


Super-resolution on Photoactivated Localization Microscopy (PALM)

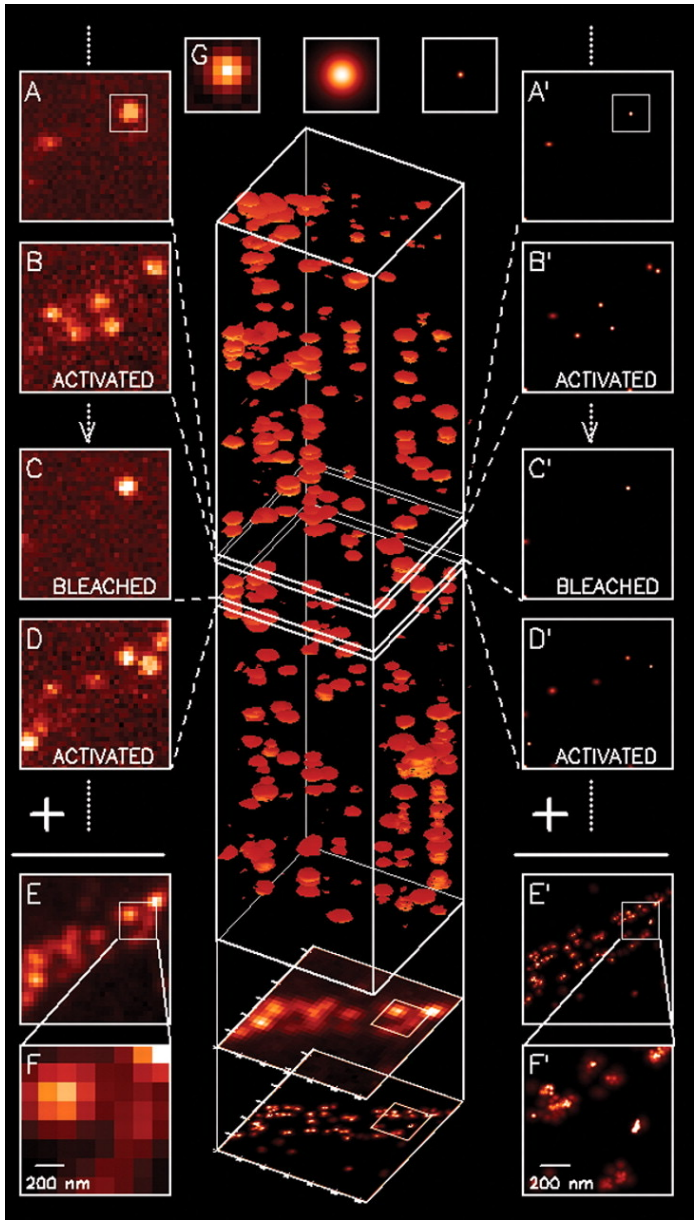
Physics 305 Activity 2
2nd Sem AY 2022-2023

Mark Jeremy G. Narag
2014-64423
PhD Physics



All the codes and files in this activity are available on my Github:

https://github.com/mgnarag/physics305_computational_imaging

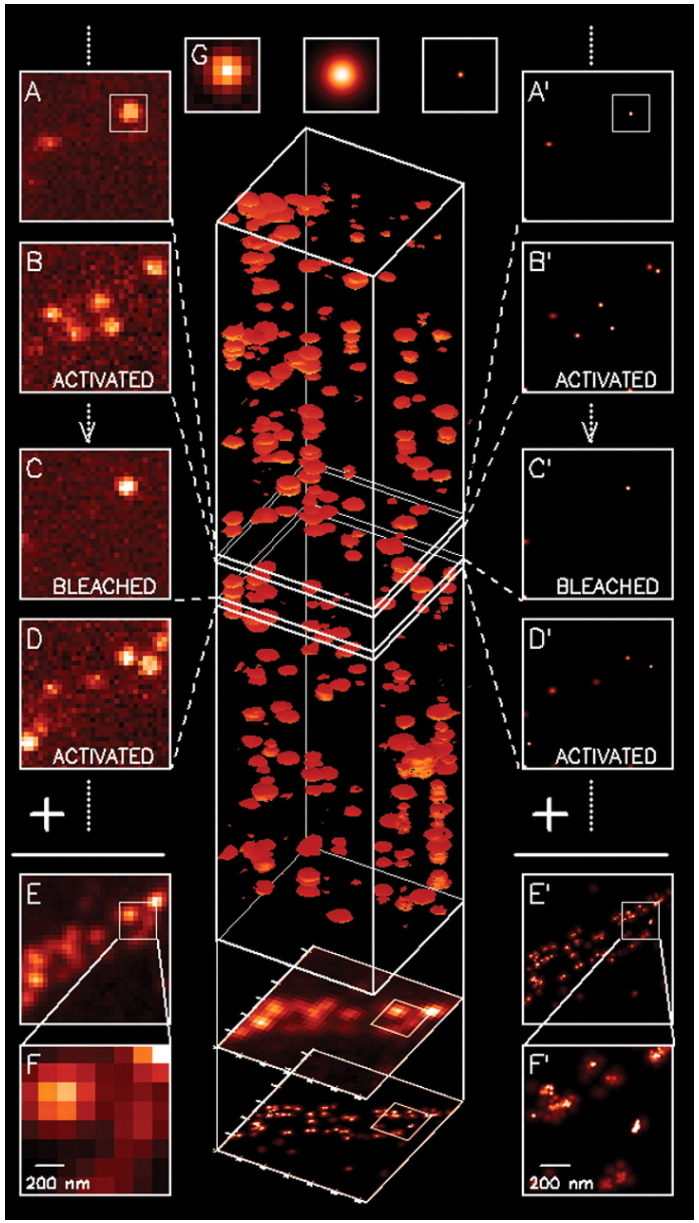


Photoactivated Localization Microscopy (PALM)

- Super-resolution microscopy technique!
- Works by activating and imaging sparse subsets of fluorophores at a time, and then repeating the process multiple times to build up a high-resolution image as shown in Fig 1 (details in the caption)
- featured as Methods of the Year for 2008 by the Nature Methods journal

Fig. 1. The principle behind PALM. A sparse subset of PA-FP molecules that are attached to proteins of interest and then fixed within a cell are activated (**A** and **B**) with a brief laser pulse at λ_{act} = 405 mm and then imaged at λ_{exc} = 561 mm until most are bleached (C). This process is repeated many times (**C** and **D**) until the population of inactivated, unbleached molecules is depleted. Summing the molecular images across all frames results in a diffraction-limited image (**E** and **F**). However, if the location of each molecule is first determined by fitting the expected molecular image given by the PSF of the microscope [(**G**), center] to the actual molecular image [(G), left], the molecule can be plotted [(G), right] as a Gaussian that has a standard deviation equal to the uncertainty $\sigma_{x,y}$ in the fitted position. Repeating with all molecules across all frames (**A**' through **D**') and summing the results yields a superresolution image (**E**' and **F**') in which resolution is dictated by the uncertainties $\sigma_{x,y}$ as well as by the density of localized molecules. Scale: 1 × 1 μm in (F) and (F'), 4 × 4 μm elsewhere. [1]

[1] Betzig, E., Patterson, G. H., Sougrat, R., Lindwasser, O. W., Olenych, S., Bonifacino, J. S., ... & Hess, H. F. (2006). Imaging intracellular fluorescent proteins at nanometer resolution. science, 313(5793), 1642-1645.



Photoactivated Localization Microscopy (PALM)

This technique has both optics and image processing side [2]:

Optics side:

- Photoactivatable fluorescent dyes
- Widefield fluorescent microscope – a Total Internal Reflection Microscope
- A means to switch on and off an activation and readout laser
- A highly sensitive CCD camera

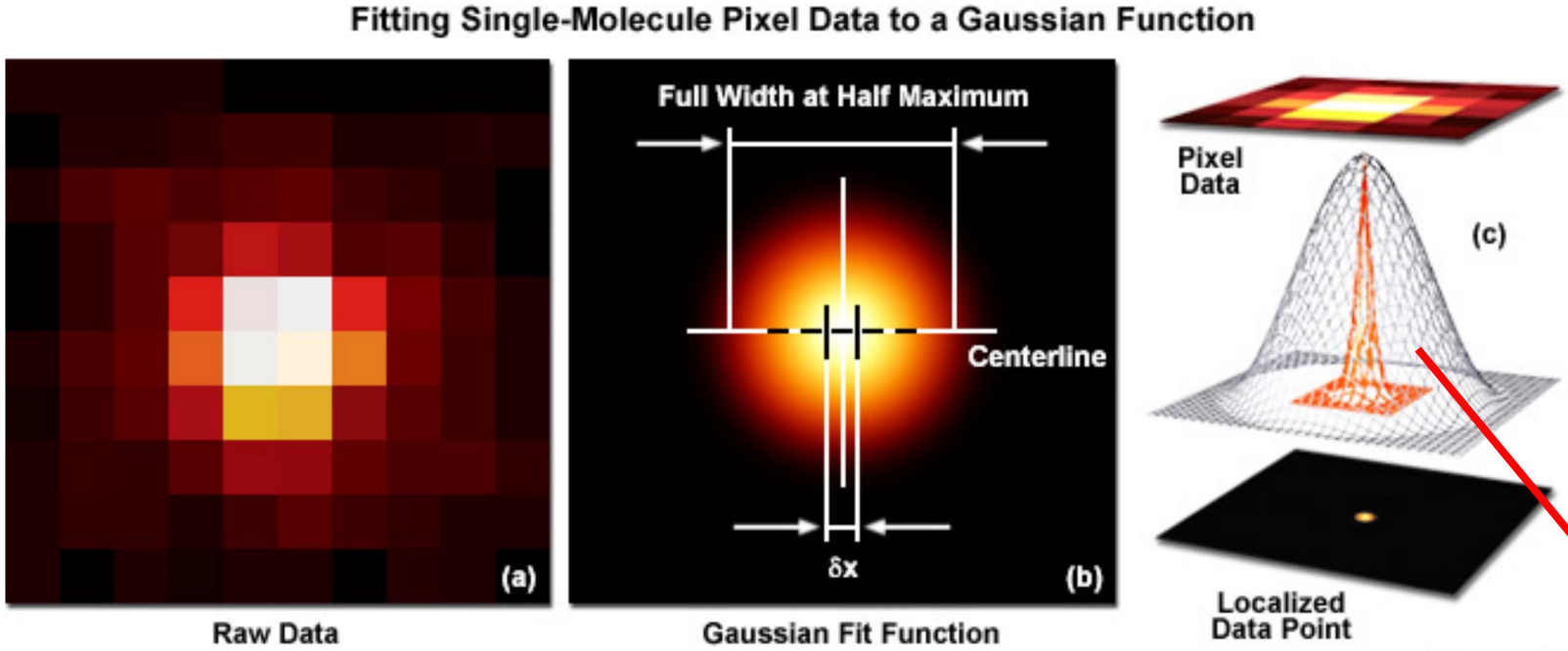
Image processing side:

- Stacking of images
- Gaussian fitting

In this activity, we will do **gaussian fitting** on the stacked images to super-resolve it!

[2] Soriano, M. 2023. Photoactivated Localization Microscopy (PALM). Physics 305 Computational Imaging Manual

Super-resolve it? Gaussian fitting? But why? How?



imaged molecule with **N** detected photons will appear noisy and spread with a width approaching the diffraction limit…

…so we try to apply two-dimensional Gaussian function on the center of the image…

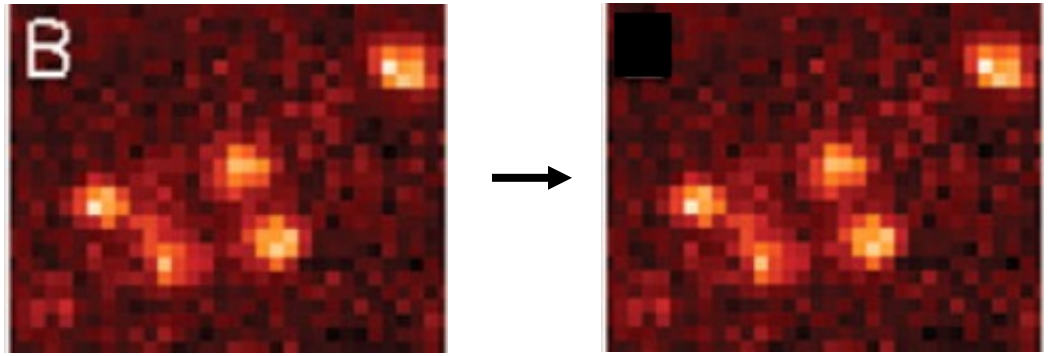
…then this is what we will see as the processed data…

…where this is the Gaussian profile of a point-spread function.

[3] ZEISS Microscopy Online Campus | Practical Aspects of Photoactivated Localization Microscopy (PALM) (fsu.edu)

1. Preprocessing

The sample image has a letter "B" label. This will surely cause an error in the succeeding steps so let's try to remove it by covering it with a black box.

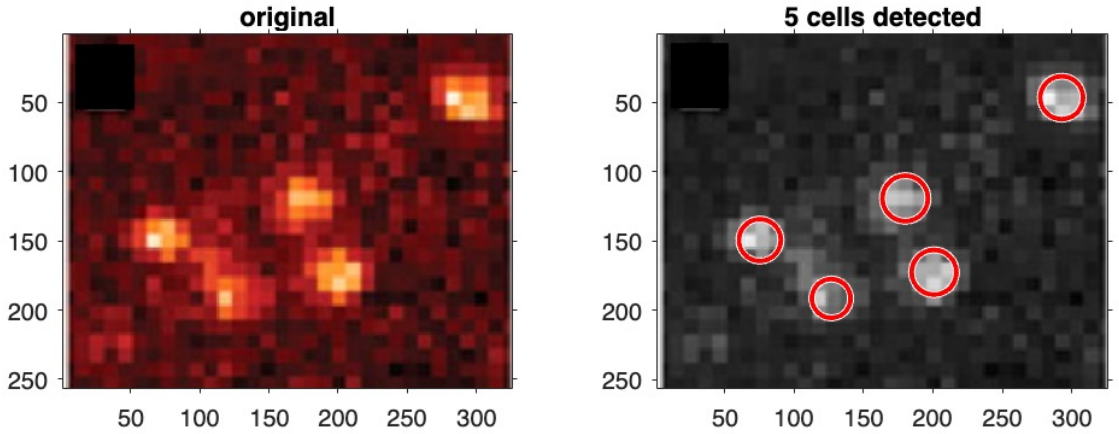


2. Cell detection

Before we apply 2D gaussian, we first need to know where to apply it. In that case, we need to estimate the location of the cells. Matlab has an `imfindcircles(A, RADIUS_RANGE)` where it tries to detect the circles in image **A** given a range of the circle's radius.

It will output the approximated center as well as the radius of the detected circles on the image.

The function takes in one channel only so for simplicity, let's grayscale our image first.



center = *5x2*

292.8177	46.7057
200.7569	172.5755
75.1301	149.3691
179.9637	119.3257
126.6188	191.4335

radius = *5x1*

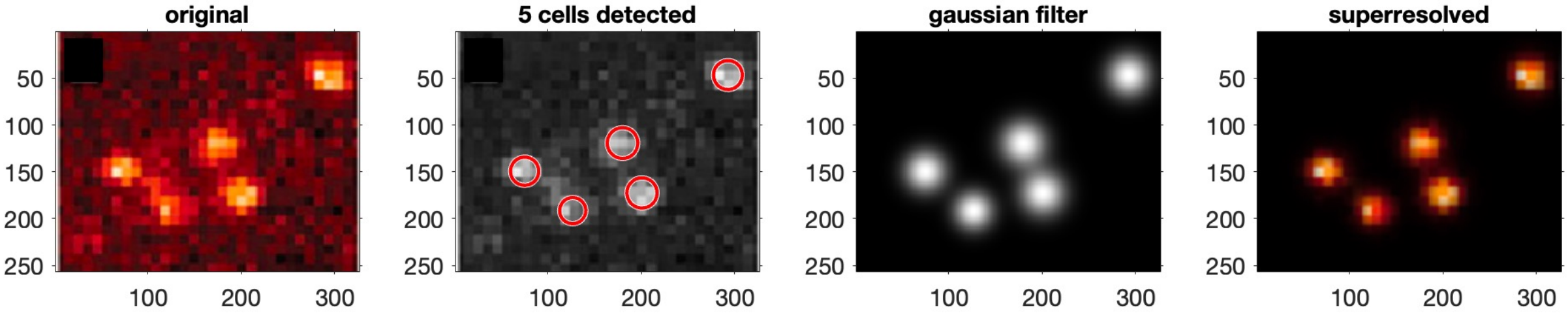
15.4153
16.1052
15.0651
16.3685
13.9501

3. Gaussian filtering

Now that we have an approximation of the center as well as the radius, we can use these information on where we will create multiple 2D gaussian filters:

$$G(x,y) = \frac{1}{\sqrt{2\pi\sigma^2}} e^{-\frac{x^2+y^2}{2\sigma^2}}$$

where I set σ as the radius, x and y as center coordinates. By simply multiplying the two images, we can get the superresolved version of our original



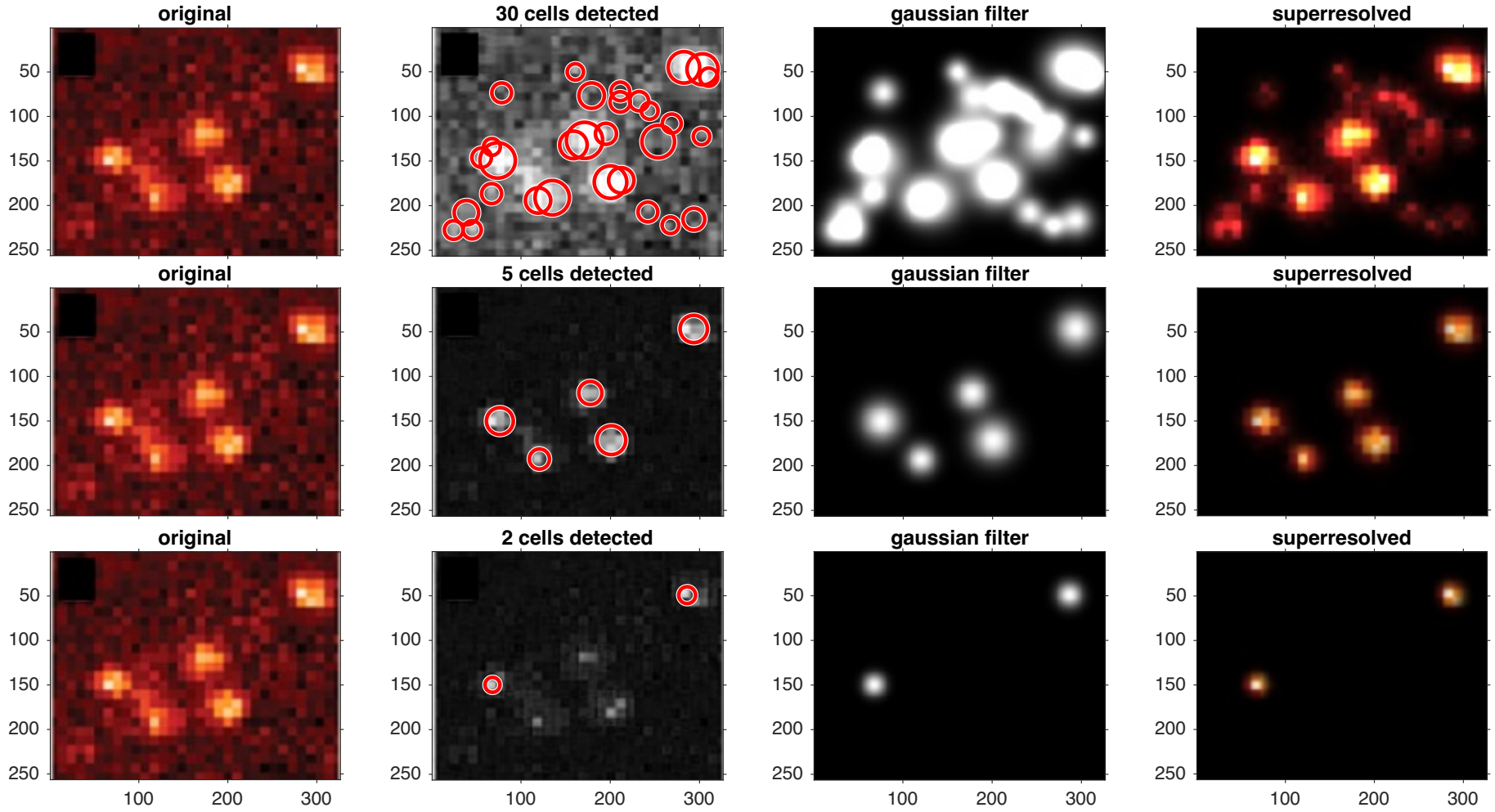
Effect of grayscaling

What if we directly use the original RGB of the image? We can see from results below that G channel closely resembles what we obtained from grayscale. The output in R channel is expected since our image is rich in red colors. The blue channel under-detects the cells.

R channel

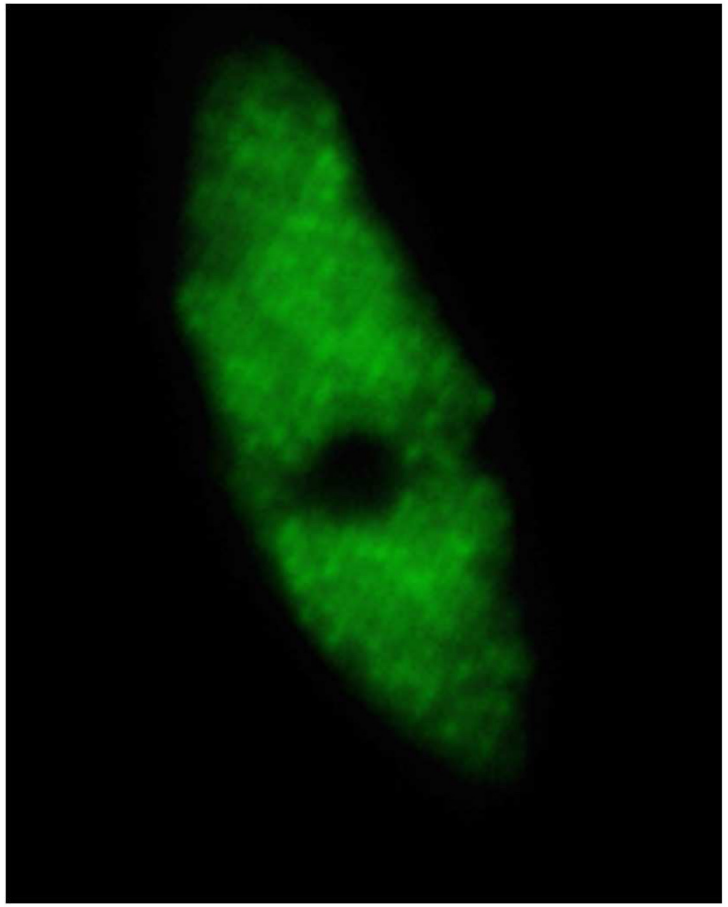
G channel

B channel



Testing on other medical image

I saw this raw picture of RNA polymerase II (RNAPII) on Weisshar et al.'s paper [4] in which they used Structured Illumination Microscopy (SIM) and PALM to capture RNAPII molecues

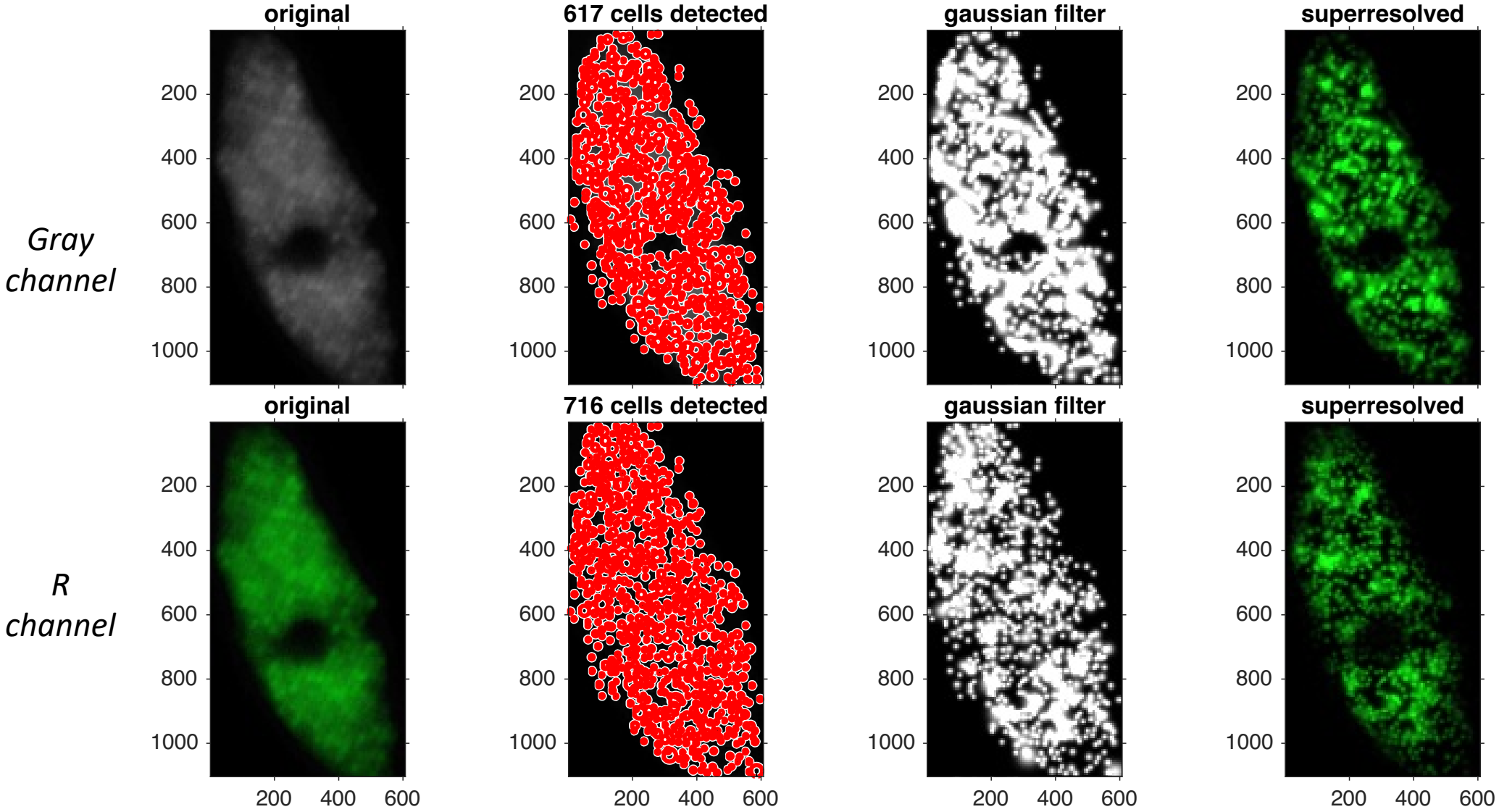


Raw data image. Shown is one of the 21 SIM raw slices (5 phases at 5 rotations) from the z-stack of the RNAPIISer2ph sample [5].

[5] Weisshart, K., Fuchs, J. and Schubert, V. (2016). Structured Illumination Microscopy (SIM) and Photoactivated Localization Microscopy (PALM) to Analyze the Abundance and Distribution of RNA Polymerase II Molecules on Flow-sorted *Arabidopsis* Nuclei. *Bio-protocol* 6(3): e1725. DOI: 10.21769/BioProtoc.1725.

Testing on other medical image

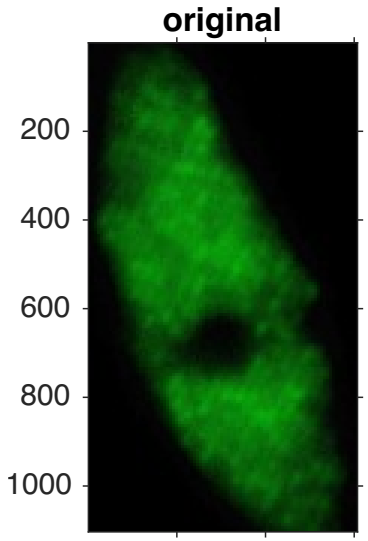
Notice that both the gray and red channel detected cells outside the visible boundary of the image.

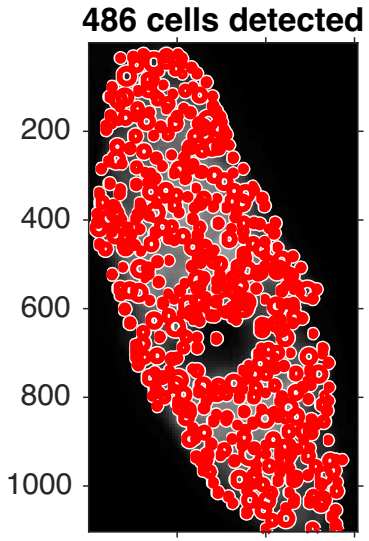


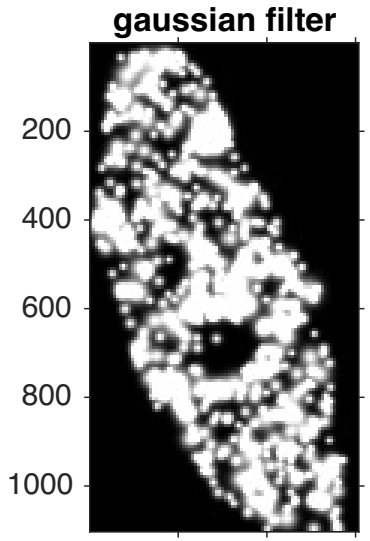
Testing on other medical image

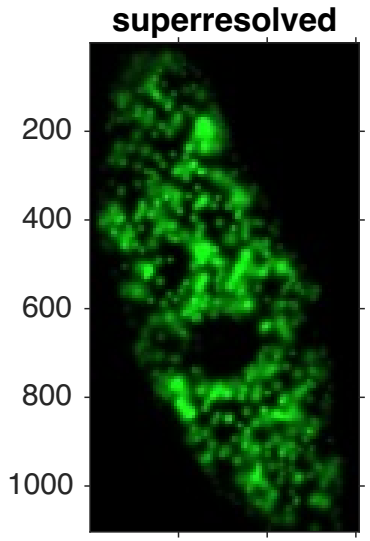
Whereas here, we see that the detected cells are clean in green channel (no cells detected outside visible boundary)

G channel

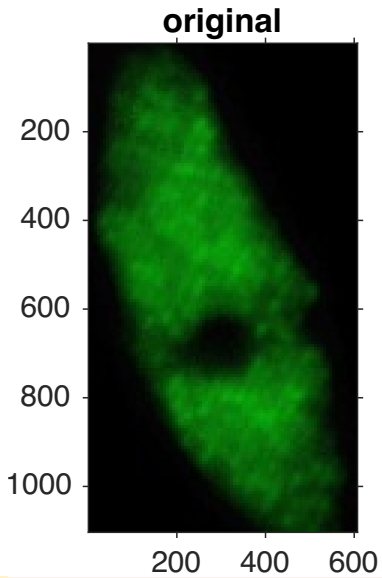


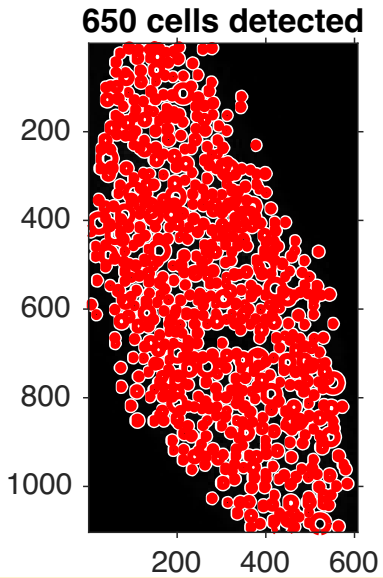


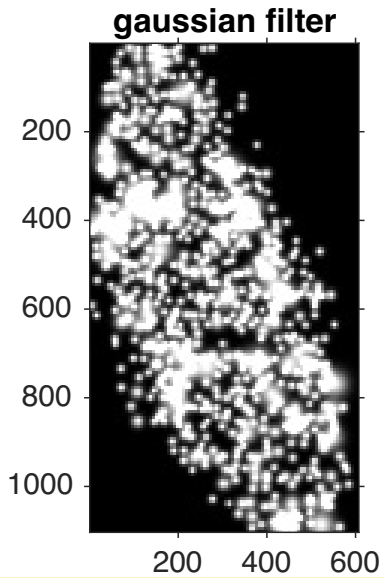


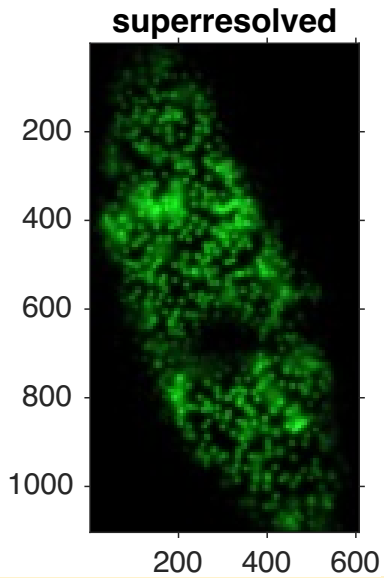


B channel





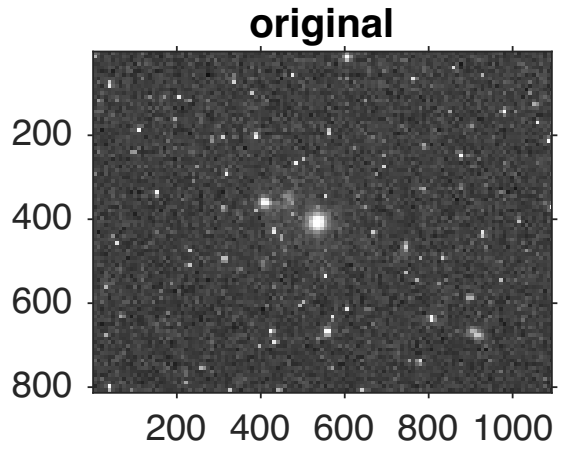


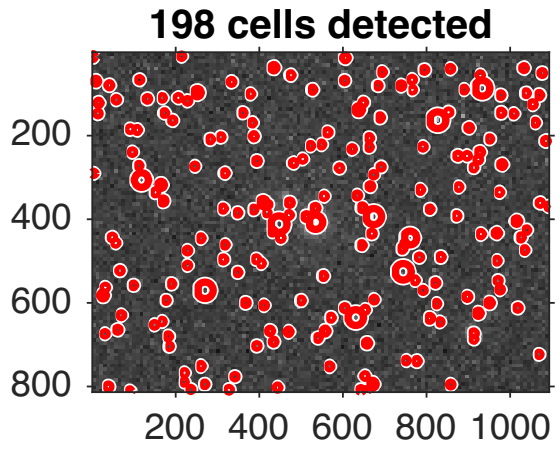


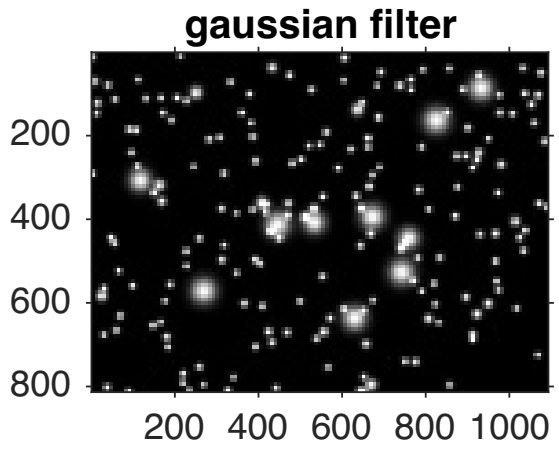
Effectivity on other non-medical image/s

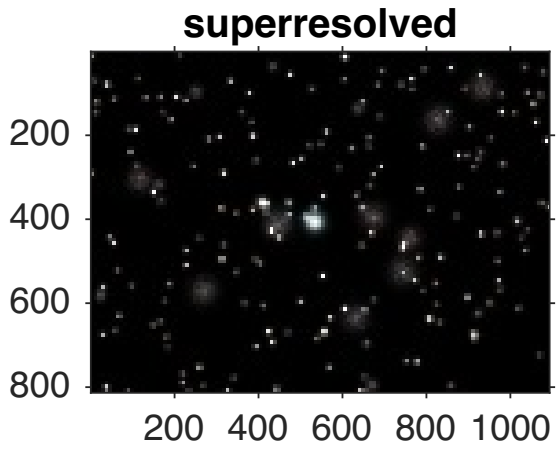
Maybe this can work on noisy outer space images. I tested it on NGC 6543 (Eye Nebula) [4]

Gray channel

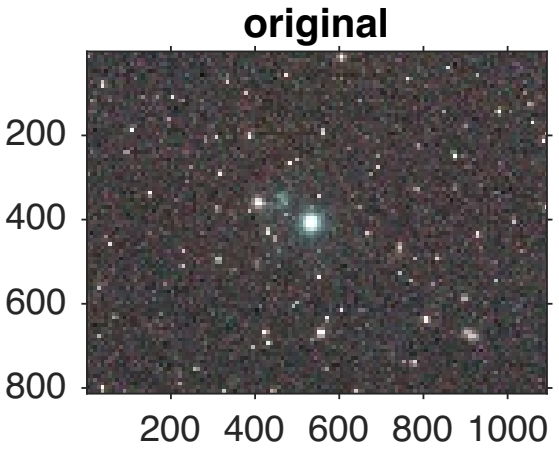


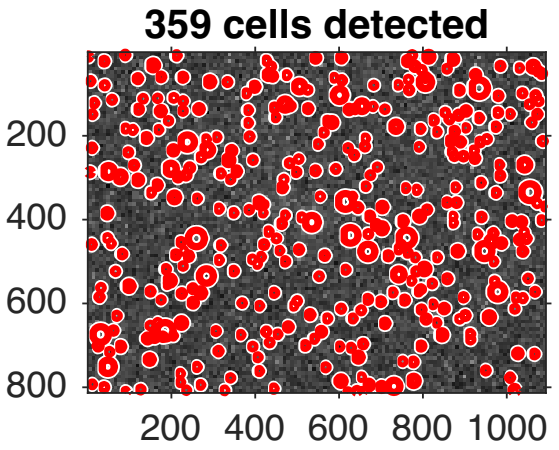


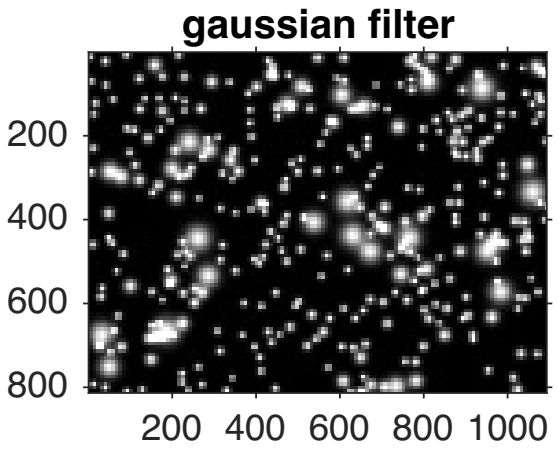


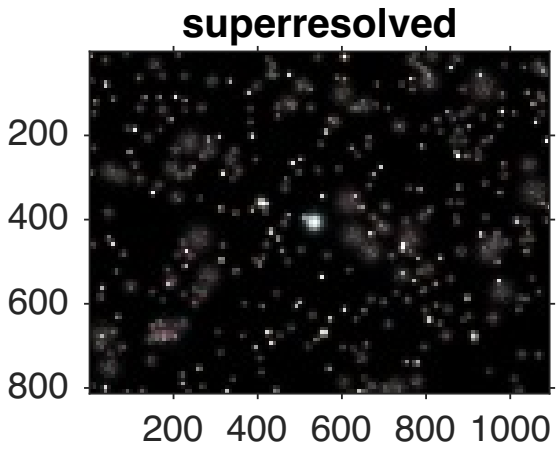


R channel





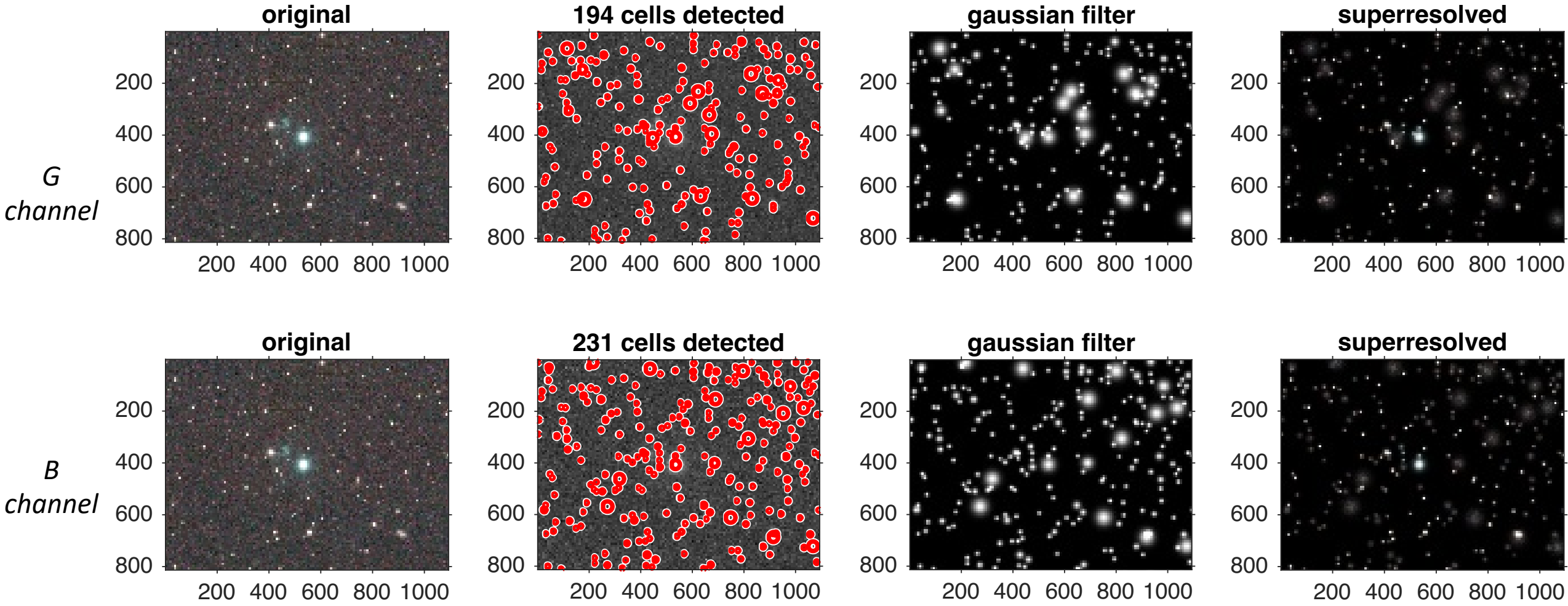




[4] https://www.cloudynights.com/topic/666277-removing-noise-from-an-image/

Effectivity on other non-medical image/s

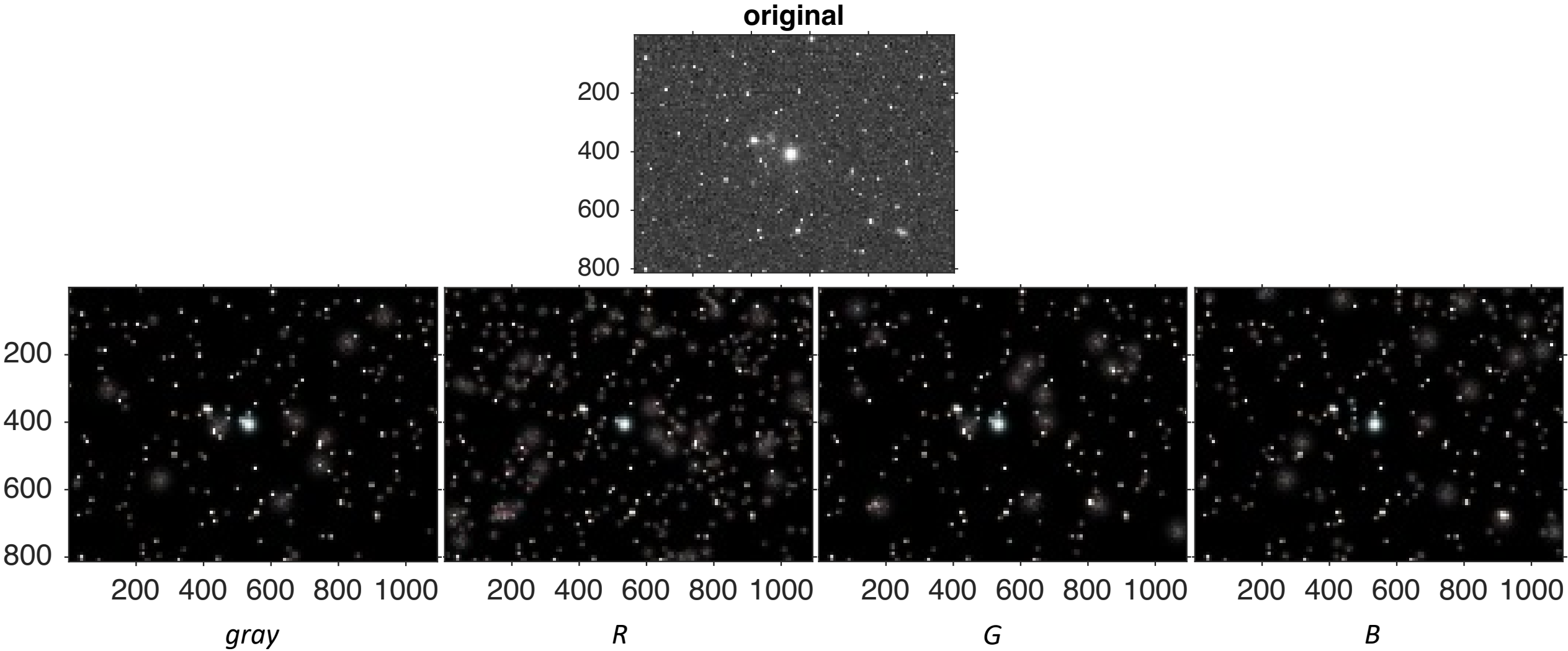
Maybe this can work on noisy outer space images. I tested it on NGC 6543 (Eye Nebula) [4]



[4] https://www.cloudynights.com/topic/666277-removing-noise-from-an-image/

Effectivity in other non-medical image/s

There are still some artifacts like the tails of the gaussian is prominent but nonetheless, it can somehow denoise it! By looking at it, gray and B channel have good reconstruction.



Conclusion

- We are able to super-resolve raw images from PALM, even noisy outer space image! This technique can also be used for denoising several types of images with blobs.

- The reconstruction is better on channel that is dominant on the original raw image (or the color of their illumination):
  - red channel is best in our first image because the cells are illuminated in perhaps a red light
  - green channel is best in RNAPII image because it was illuminated in green
  - blue channel is best in Nebula image because of its bluish appearance

- Higher number of cells/blobs detected does not necessarily mean good detection.
  - We saw this in RNAPII where other color channels, aside from green, detected many cells even outside the visible boundary

- So far, our only judgement is visual.

Reflection

I super enjoyed doing this activity. I was really looking for more images but sadly the papers I saw did now give the raw image huhu could've been fun to superresolve those blurry cells!!

CRITERIA	QUALIFICATIONS	SCORE
Technical correctness	• Met all objectives. • Theory is discussed sufficiently. • Procedures and Results are complete. • Procedures and Results are verifiably correct. • Understood the lesson.	40
Quality of presentation	• All text and images are of good quality. • Code has sufficient comments and guides. • All plots are properly labeled and are visually understandable. • The report is clear.	30
Self-Reflection	• Explained the validity of results. • Discussed what went right or wrong in the activity. • Justified the self-score. • Acknowledged sources (e.g. persons consulted, references, etc.)	30
Initiative	• Experimented beyond what was required. **Yes – applied on other non-medical image** • Made significant improvements to existing code. **Yes – automated gaussian fitting for *N* detected cells** • Analyzed limitations or potential of technique, etc. **Yes**	10
		100