

Balancing Act: Image and Background

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An ensemble of objects in the pool of background

Imaging, a foundational tool in our digital age, permeates nearly every facet of human endeavor. From the microscopic world of scientific research to the macroscopic scale of space exploration, imaging technology serves as a vital lens through which we perceive and interact with our environment.

The philosophy of image processing.

Philosophically, imaging can be seen as a means of bridging the gap between our inner selves (objects) and the external world (background). What we define as object and background largely depends on our perception and knowledge of the object. Imaging algorithms, in essence, allows us to capture and represent the external world in a manner that can be comprehended and interpreted by our minds.

An illustration



Figure 1: We and our Environment, Image and its Background

Ancestry of Digital imaging

- Russell A. Kirsch created a system in 1957 that combined a drum scanner and photo multiplier tube to generate digital data that you could store in a computer.
- Frederick G. Weighart and James F. McNulty (US radio engineer) at Automation Industries, Inc., then in El Segundo, California, co-invented the first apparatus to generate a digital image in real-time in 1969. Their invention played a crucial role in the early development of digital imaging technology, which laid the foundation for advancements in medical imaging and other fields.

From Here to Infinity

Be it bacteria or the black hole - the principles of digital imaging is applicable in the microscopic world as well as in the whole galaxy.



Figure 2: Microscope2telescope

Image of a black hole

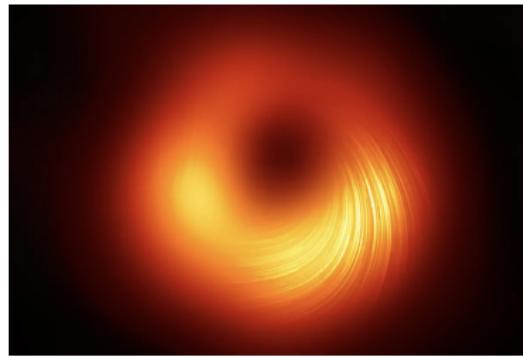


Figure 3: Event horizon 38 billion kilometers

Image of Covid 19

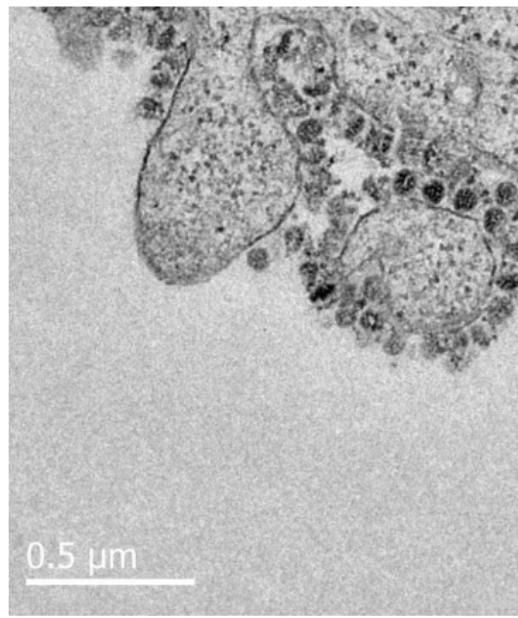


Figure 4: COVID-19 60-140nm

Comparative appraisal of Digital Microscope and Telescope

Applications

While digital microscopy finds application in biology, material science, medical research digital telescopes are used in astronomy, astrophysics, planetary observation.

Imaging

In digital Microscopy one captures detailed images and allows for real-time digital enhancements (e.g., zoom, color processing), on the other hand in telescopes, we may integrate image stacking and long exposures to enhance image clarity.

Illumination

Artificial illumination is used in digital microscope, whereas in digital telescope one relies on natural light (from celestial objects) or faint distant sources.

Basic Attributes of a digital image

- Dynamic range : The dynamic range of a digital image refers to the ratio between the brightest and darkest parts it can capture
- Bit depth :This refers to the number of tonal levels the image can store. An 8-bit image can represent 256 (2^8) brightness levels, while a 16-bit image can represent 65,536 (2^{16}) levels.
- File format -Loss less: tiff, png -Lossy : jpeg, giff -Other:psd,svg,raw

Diffraction limit

The diffraction limit is a fundamental limit on the resolution of an optical system, such as a microscope, telescope, or camera, due to the wave nature of light. It describes the smallest detail that can be resolved by the system.

Explanation

When light passes through an aperture (such as a lens or pinhole), it spreads out or diffracts. This diffraction causes the image of a point source (e.g., a star or a small particle) to appear as a blurry spot instead of a perfect point. The size of this blurry spot sets a limit on how close two points can be to each other and still be distinguished as separate.

Mathematical expression

For a circular aperture, the diffraction-limited resolution is given by the Rayleigh criterion:

$$\theta = 1.22 \frac{\lambda}{D} \quad (1)$$

where θ is the angular resolution in radians (the smallest angular separation that can be resolved and D is the diameter of the aperture (e.g., lens or telescope)).

Microscope based imaging system

In microscopes or other imaging systems, this can also be related to the smallest resolvable distance d , in the focal plane:

$$d = 1.22 \frac{\lambda}{2NA} \quad (2)$$

where NA is the numerical aperture. If n is the refractive index of the medium between the lens and the sample (e.g., air, water, or oil), θ is the half-angle of the maximum cone of light that can enter or exit the lens (also called the acceptance angle):

$$NA = n \cdot \sin(\theta) \quad (3)$$

Does NA change the diffraction limit?

Higher NA would cause d to reduce (practiced in oil immersion microscopy). In light microscope, phase contrast microscope, dark field microscope or DIC, diffraction limit remains typically at the order of 200nm. While the lateral resolution is typically on the order of 200 nm, and the axial resolution is around 500 nm, constrained by the diffraction of light. Changing NA by increasing n from 1 to 1.5 will reduce the diffraction limit by a magnitude $1/1.5 \approx 134\text{nm}$.

Outlines of convolution and deconvolution

Convolution

In microscopy this convolution process mathematically explains the formation of an image that is **degraded by blurring and noise**. The blurring is largely due to diffraction limited imaging by the instrument. The noise is usually photon noise, a term that refers to the inherent natural variation of the incident photon flux.

Deconvolution

Deconvolution is a mathematical operation used in Image Restoration to recover an image that is degraded by a physical process which can be described by the opposite operation, a convolution. This is the case in image formation by optical systems as used in microscopy and astronomy, but also in seismic measurements.

PSF

The degree of spreading (blurring) of a single pointlike (Sub Resolution) object is a measure for the quality of an optical system. The 3D blurry image of such a single point light source is usually called the Point Spread Function (PSF).

Visualizing the PSF

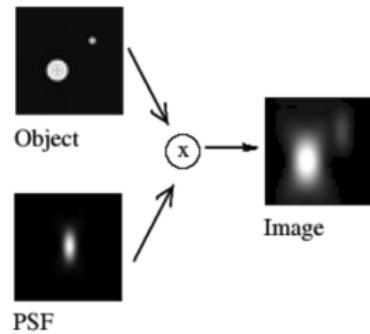


Figure 5: $G=F.H$

Convolution equation

Compute the Fourier transforms F and H of f and h Multiply F times H to obtain G Transform G back to g, the convolved image.

$$g(t) = (f * h)(t) = \int f(\tau)h(t - \tau)d\tau \quad (4)$$

Details of the terms

- G represents the output image. This is the convolved image that results from the interaction between the input image and the point spread function.
- F represents the input image. This is the original image that you want to process or analyze.
- H represents the point spread function (PSF). This is a function that describes how a point source of light is spread out by the imaging system. In other words, it characterizes the blurring or distortion introduced by the system.

Why deconvolution problem is nontrivial

Dividing the Fourier transform of the blurred image by the Fourier transform of the PSF effectively reverses the convolution . However Fourier transform of the PSF has zeros, dividing by these values can lead to numerical instability or artifacts in the deconvolved image. This is known as the “zero division problem”.

Beating the diffraction limit

A typical viva question for a biologist is why we can not see (a) bacteria in naked eye (Eye DL= 100-200 um) (b) nanoparticle in light microscope (DL for ordinary microscope > 100nm) ?

Emerging Microscopic Techniques

- STED uses stimulated emission to deplete the fluorescence of surrounding molecules.
 - Requires high laser intensity
- PALM Photoactivated Localization Microscopy
 - Requires high density of Fluorescent molecules
- SIM Structured Illumination Microscopy, Uses interference
 - Complex image processing algorithm.

Full Width at Half Maximum (FWHM)

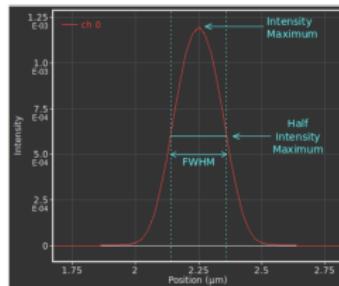


Figure 6: FWHM

$\text{FWHM} = \Delta r$ of the effective fluorescent spot .

STED resolution

$$\Delta r = \frac{\lambda}{2NA} \cdot \frac{1}{(1 + \frac{I_{\max}}{I_{\text{sat}}})} \quad (5)$$

Smaller FWHM will lead to higher resolution. By reducing the width of the PSF, we can better distinguish closely spaced features in the sample. We should also remember:

$$\text{PSF}_{\text{STED}}(r) = \text{PSF}_{\text{exc}}(r) \cdot \exp\left(-\frac{I_{\max}(r)}{I_{\text{sat}}}\right) \quad (6)$$

Principle of STED

STED relies on selectively depleting fluorescence in all areas except a very small region. In STED, after excitation with a regular laser, a second laser beam is applied in a doughnut-shaped pattern to selectively inhibit (or “deplete”) the fluorescence everywhere except in a very tiny region at the center of the doughnut.

How Resolution Enhancement is achieved

By shrinking the region where fluorescence is allowed, the STED technique overcomes the diffraction limit, achieving a much higher resolution compared to traditional fluorescence microscopy. The effective resolution can be as small as **20–30** nanometers.

STED mathematical formulation

The point spread function (PSF) describes the response of an imaging system to a point source. It is a key concept in understanding resolution.

Fluorescence: A molecule absorbs a photon and is excited to a higher energy state, then it emits a photon as it returns to the ground state. The emitted photon has a longer wavelength than the absorbed photon.

Stimulated Emission: A photon of the same energy as the energy difference between the excited state and the ground state can stimulate the excited molecule to emit a photon and return to the ground state.

$$\text{PSF}_{\text{STED}}(r) = \text{PSF}_{\text{exc}}(r) \cdot \exp\left(-\frac{I_{\max}(r)}{I_{\text{sat}}}\right) \quad (7)$$

STED A simple R Code

```
# Load required libraries
library(ggplot2)
library(plotly)

##
## Attaching package: 'plotly'

## The following object is masked from 'package:ggplot2':
##
##     last_plot

## The following object is masked from 'package:stats':
##
##     filter

## The following object is masked from 'package:graphics':
##
##     layout

library(reshape2)
```

Set parameters

```
lambda <- 500 # Wavelength of light in nm
numerical_aperture <- 1.4 # Numerical aperture
I_sat <- 1 # Saturation intensity (arbitrary units)
I_STED <- 10 # Intensity of STED laser (arbitrary units)
sigma <- lambda / (2 * numerical_aperture)
```

Standard deviation of Gaussian PSF

```
# Create a grid of points
x <- seq(-5 * sigma, 5 * sigma, length.out = 100)
y <- seq(-5 * sigma, 5 * sigma, length.out = 100)
grid <- expand.grid(x = x, y = y)
```

Define the excitation PSF (Gaussian function)

```
exc_psf <- function(x, y, sigma) {
  exp(-(x^2 + y^2) / (2 * sigma^2))
}

# Define the STED PSF (doughnut-shaped function)
sted_psf <- function(x, y, sigma, I_STED, I_sat) {
  exp(-I_STED * (x^2 + y^2) / (2 * sigma^2) / I_sat)
}

# Calculate the excitation and STED PSFs
grid$exc_psf <- exc_psf(grid$x, grid$y, sigma)
grid$sted_psf <- sted_psf(grid$x, grid$y, sigma, I_STED, I_sat)

# Calculate the effective PSF
grid$effective_psf <- grid$exc_psf * grid$sted_psf

# Reshape data for plotting
plot_data <- melt(grid, id.vars = c("x", "y"))

# Plot the effective PSF using ggplot2
p <- ggplot(plot_data, aes(x = x, y = y, fill = value)) +
  geom_tile() +
  scale_fill_viridis_c() +
  facet_wrap(~variable, scales = "free", ncol = 1) +
  labs(title = "STED Microscopy Simulation", fill = "Intensity") +
  theme_minimal()
ggsave(filename = "./images/STED_microscopy_simulation.png", plot = p, width = 10, height = 15, dpi = 300)
```

Simulated PSF

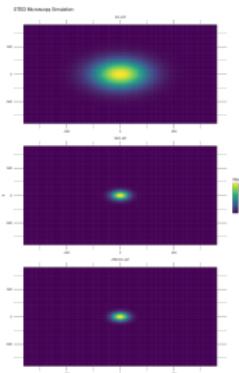


Figure 7: narrowing the PSF

Image Segmentation

- Image segmentation is the process of dividing an image into distinct regions or segments that share similar attributes such as color, intensity, or texture.
- Each segment ideally represents a different object or a part of an object within the image.
- It helps in identifying and locating objects and boundaries (lines, curves, etc.) within an image.

Segmentation Mask

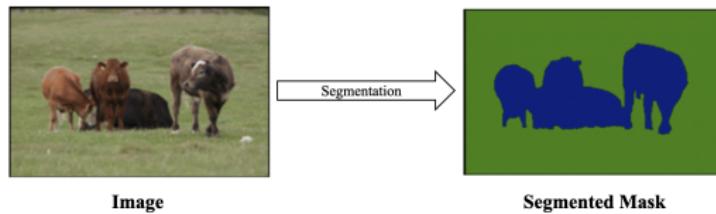


Figure 8: Segmented Mask

Semantic Segmentation

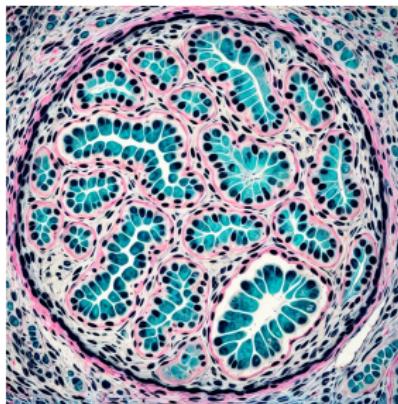


Figure 9: Example of a biological image showing semantic segmentation applied to a histopathology tissue sample. The cell nuclei are highlighted distinctly, separating them from the background tissue.

Instance Segmentation

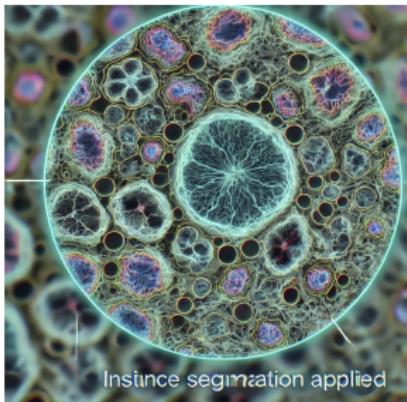


Figure 10: Example of a biological or medical image illustrating instance segmentation. In this image, individual cells or structures are outlined in distinct colors, even when they overlap or cluster together, demonstrating how instance segmentation works in biological contexts

Types of Image Segmentation

- Thresholding-Based Segmentation: Segments an image by converting it into a binary image, distinguishing objects from the background based on intensity values.
- Edge-Based Segmentation: Detects object boundaries by identifying significant changes in intensity, effectively highlighting the edges of objects.
- Region-Based Segmentation: Groups pixels into regions based on predefined criteria of similarity, such as intensity, color, or texture.
- Deep Learning-Based Segmentation: Utilizes neural networks, particularly convolutional neural networks (CNNs), for more advanced and precise segmentation tasks (e.g., U-Net, Mask R-CNN).

Example of deep learning enabled segmentation

REF:>https://www.researchgate.net/publication/350147179_YeastNet_Deep-Learning-Enabled_Accurate_Segmentation_of_Budding_Yeast_Cells_in_Bright-Field_Microscopy/figures?lo=1

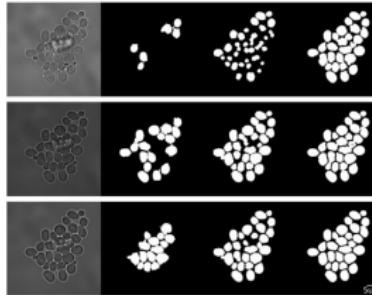


Figure 11: Cell segmentation mask of the same colony at different levels of focus. From top to bottom the input images shown are: in focus, slightly out of focus ($0.6 \mu\text{m}$ above focal plane), more out of focus ($1.2 \mu\text{m}$ above focal plane). From left to right: cropped input image, and segmentations using the non-trainable method, CellStar and YeastNet. Images were obtained using a 60X oil immersion objective. A $5 \mu\text{m}$ scale bar is present to show the scale of yeast cells

YOLO (You Only Look Once) algorithm

YOLO (You Only Look Once) is a state-of-the-art object detection system that excels at real-time performance. Unlike traditional object detection methods that scan images multiple times, YOLO divides an image into a grid and predicts both bounding boxes and class probabilities for each cell. This allows it to process images in a single pass, making it significantly faster than other methods.

Key Features of YOLO

Real-time Performance:

Single-Stage Detection: Unlike two-stage methods like Faster R-CNN, YOLO performs both object classification and localization in a single step.

High Accuracy

Despite its speed, YOLO achieves high accuracy on various object detection benchmarks.

Versatility:

YOLO can be adapted for different tasks, such as person detection, vehicle detection, and custom object detection.

Other applications

Agriculture

- Crop Monitoring: YOLO can help in detecting crop diseases, pests, or growth stages from aerial or ground-level images, improving precision agriculture practices.
- Weed Detection: Identifying and classifying weeds in agricultural fields to optimize herbicide application and reduce crop competition.

Astronomy

- Celestial Object Detection: YOLO can be applied to detect and classify celestial objects like asteroids, comets, and stars in astronomical images or video data.
- Space Debris Monitoring: Detecting and tracking space debris in satellite imagery to prevent collisions.

Lastly

