

Digital Images and Visual Perception

Image Processing & Analysis for Life Scientists

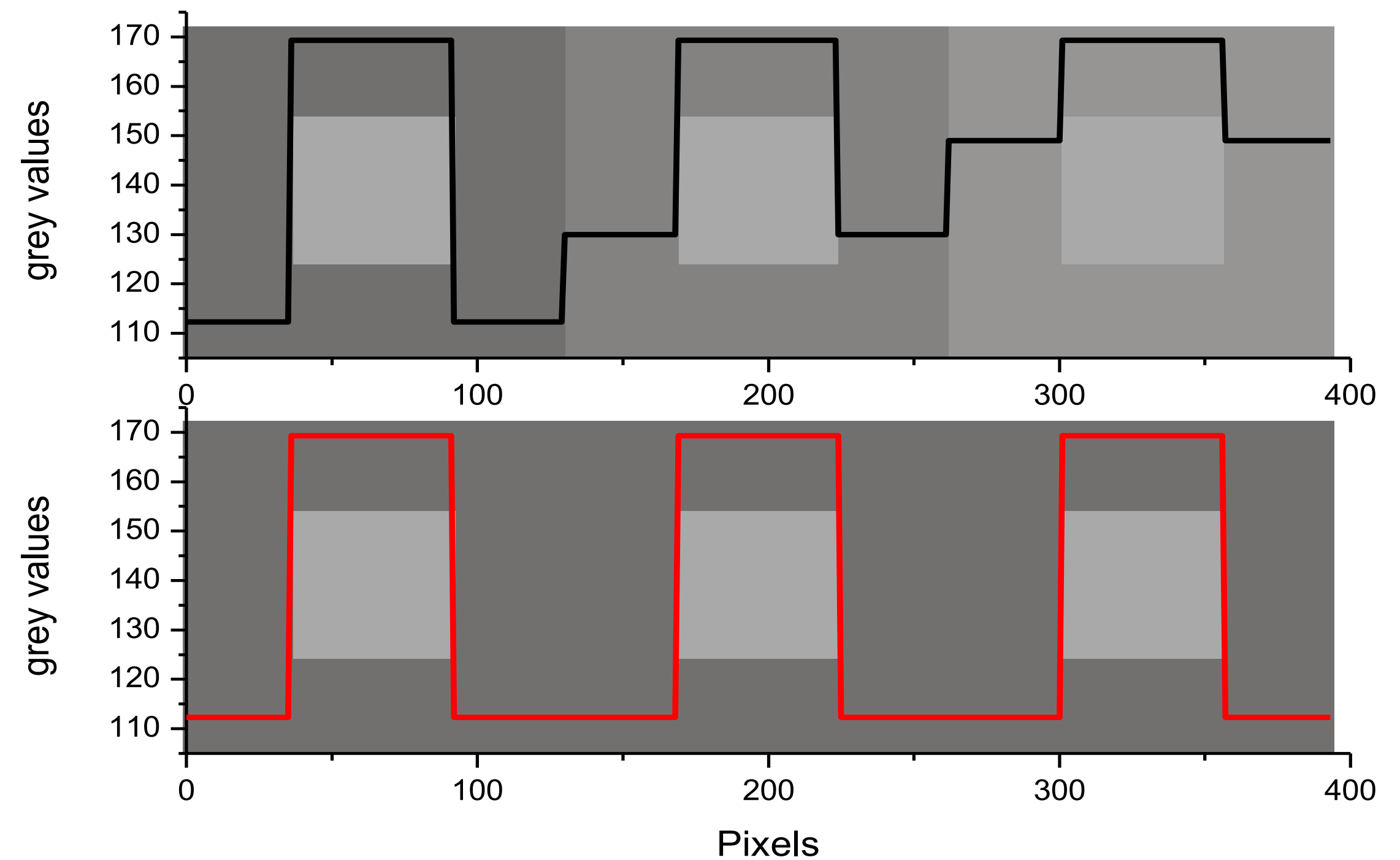
Olivier Burri, Romain Guiet & Arne Seitz

- Visual Perception
- Digital Images
- Resolution/Sampling Frequency
- Acquisition Workflow
- File formats

Visual Perception



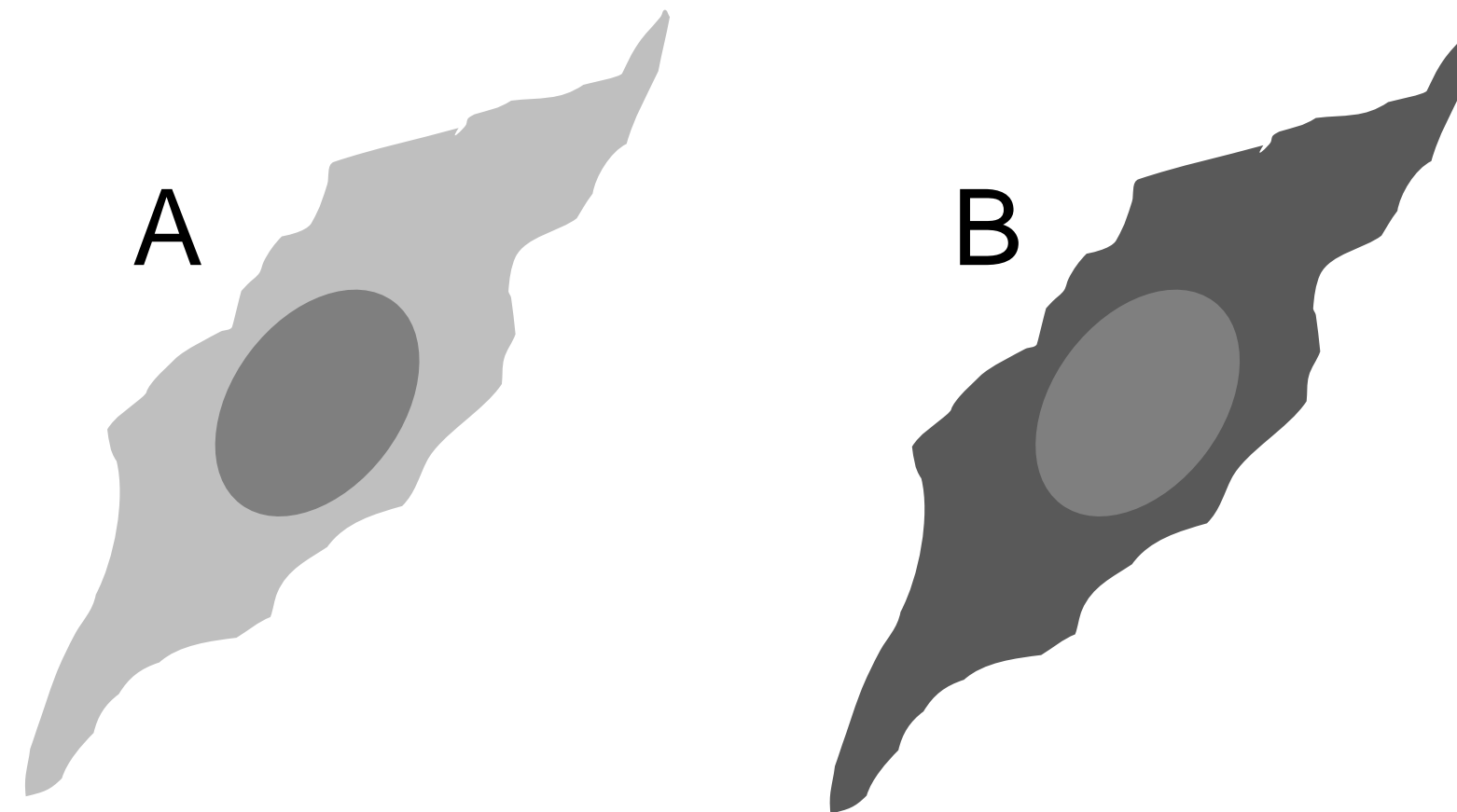
Seeing is believing, measuring is science



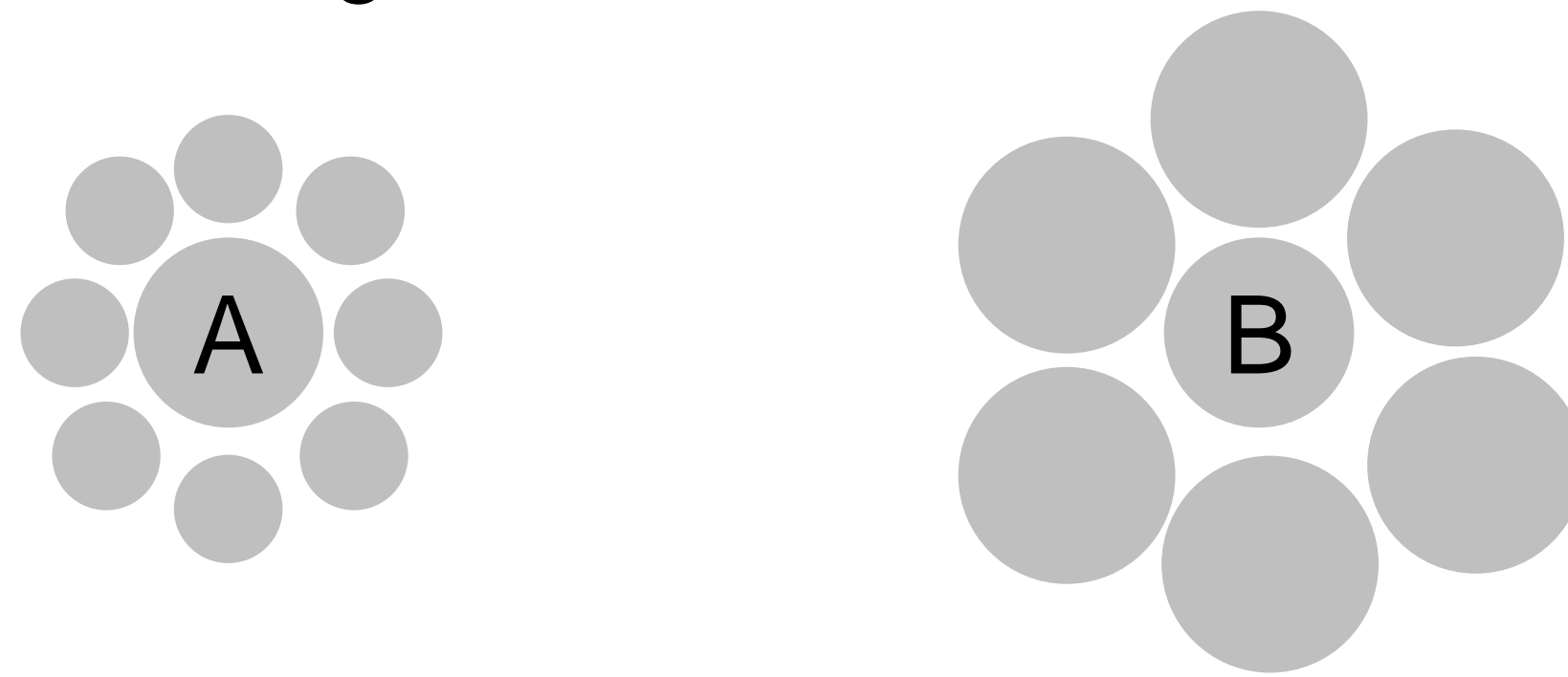
Human image interpretation
is biased due to the context.

Interpretation via Quantification

- Which nucleus is brighter?

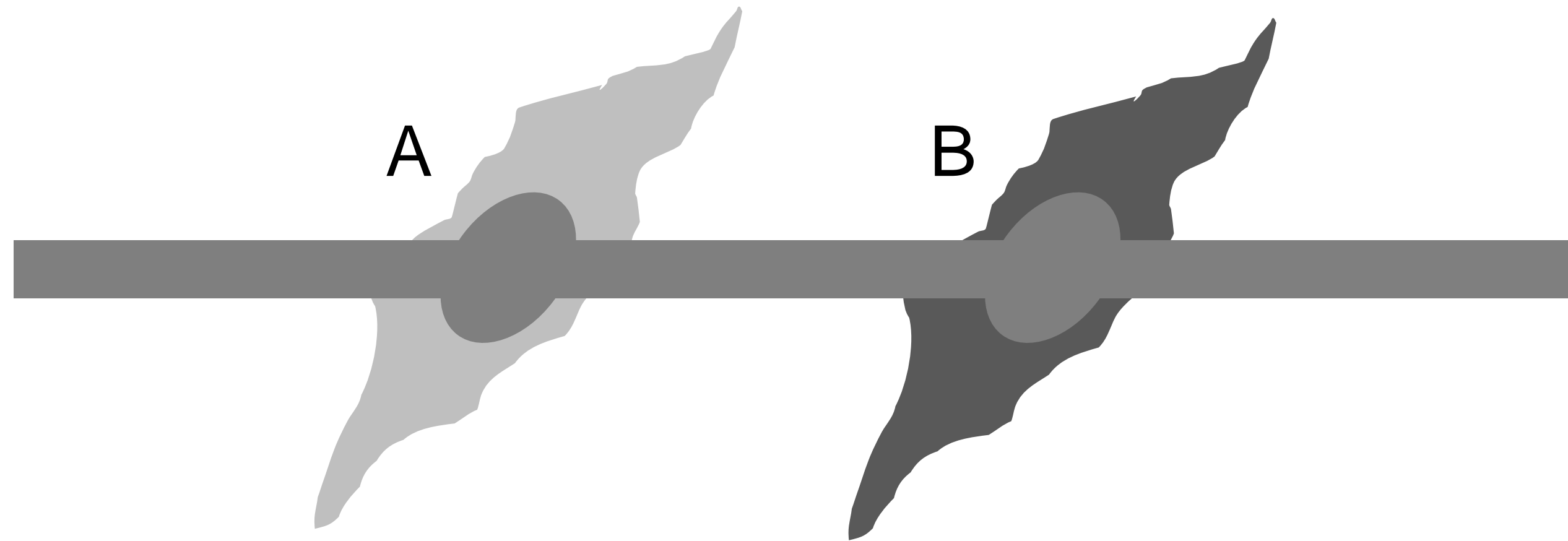


- Which nucleus is larger?



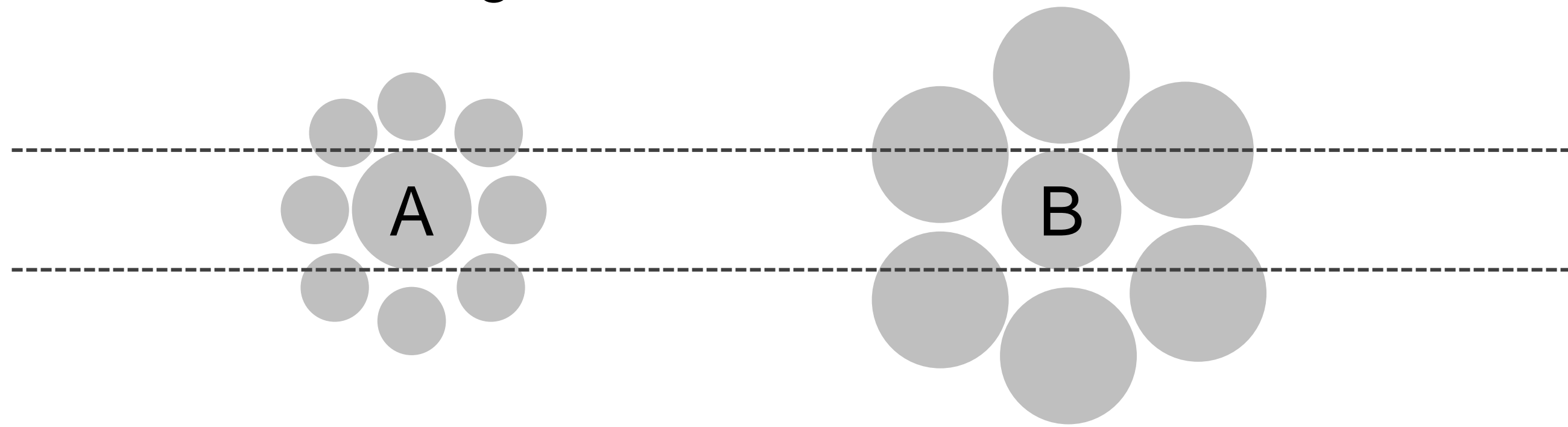
Interpretation via Quantification

- Which nucleus is brighter?



Identical
brightness

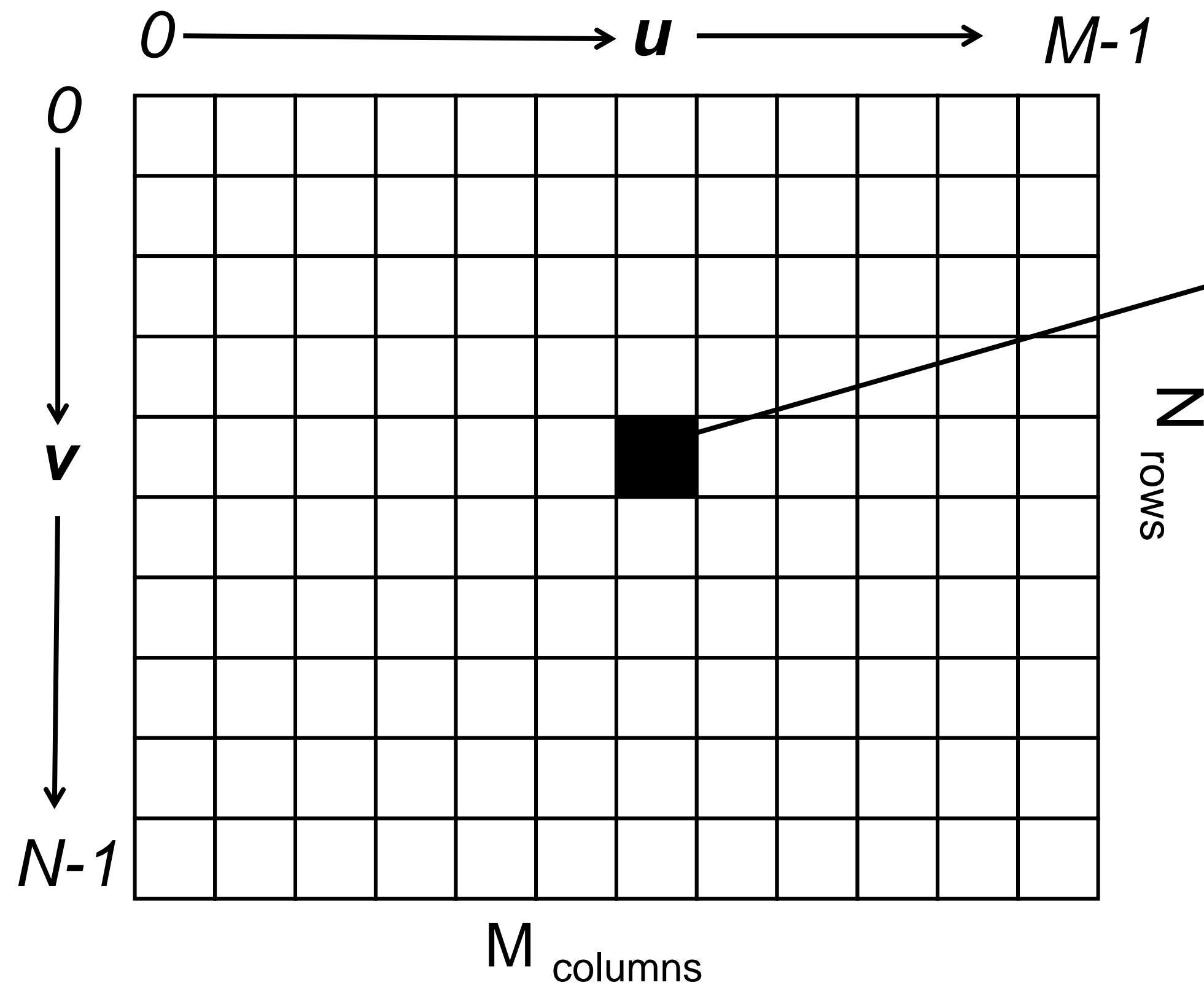
- Which nucleus is larger?



Identical
size

- Reproducible
- High Throughput
- Portable
- Unbiased

Definition Digital Image



$$I(u, v) \in \mathbb{P}$$

and $u, v \in \mathbb{N}$

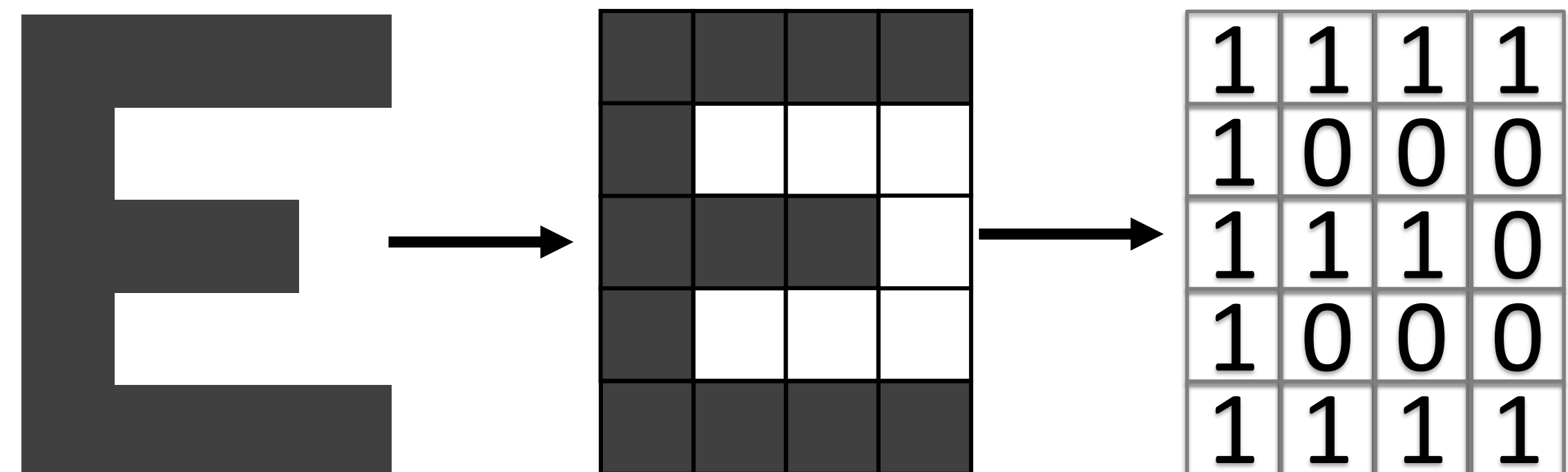
A Pixel Is *Not* A Little Square,
A Pixel Is *Not* A Little Square,
A Pixel Is *Not* A Little Square!
(And a Voxel is *Not* A Little Cube)

Technical Memo 6

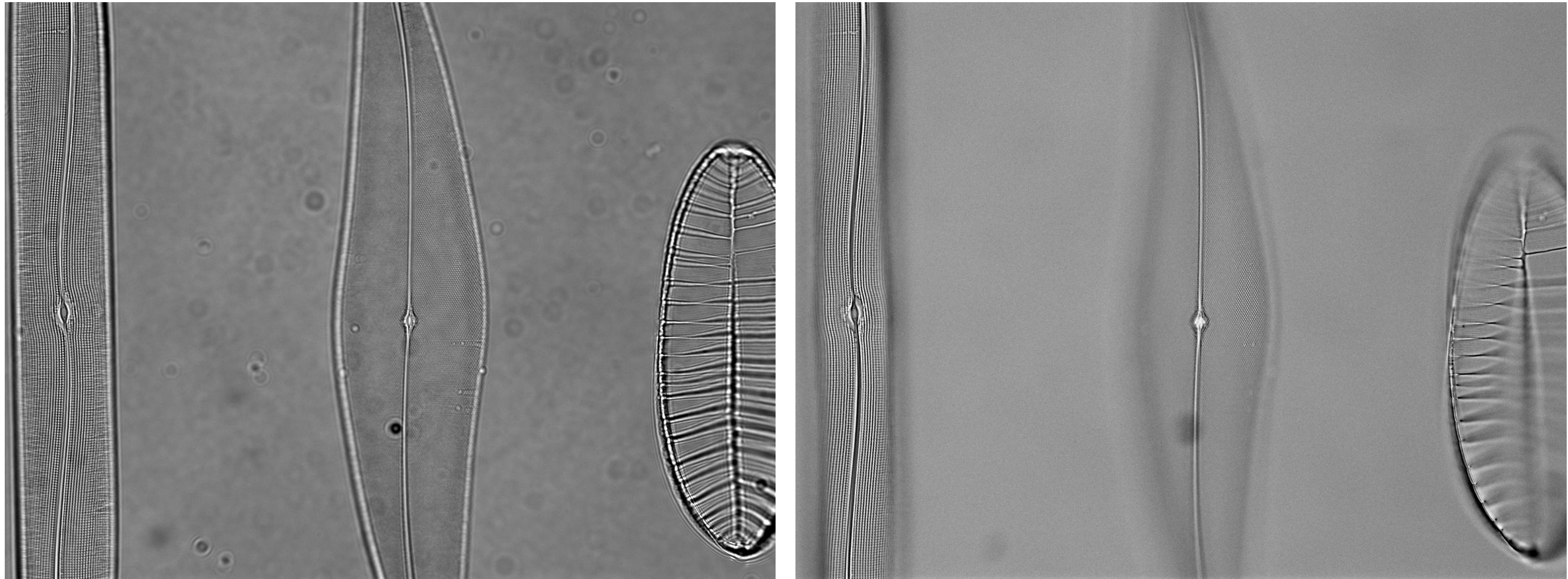
Alvy Ray Smith
July 17, 1995

Definition Digital Image

- Digital Images are raster images. The smallest addressable units are called pixels (or picture elements).
- An intensity value is assigned to each pixel using ones and zeros in the easiest case (=binary image).
- Digital images are classified by their bit depth (8 bit, 12 bit, 16 bit).



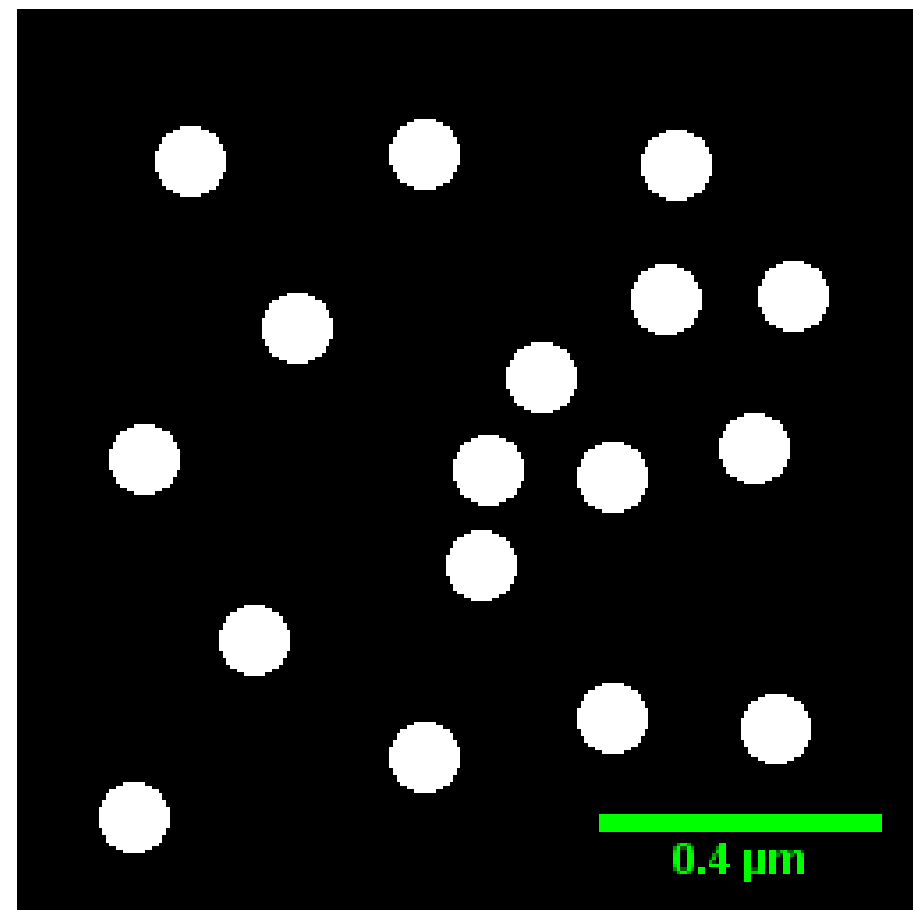
Images are Artefacts



**Two images of same object (sample)
imaged with the same microscope/objective!**

Images are Artefacts

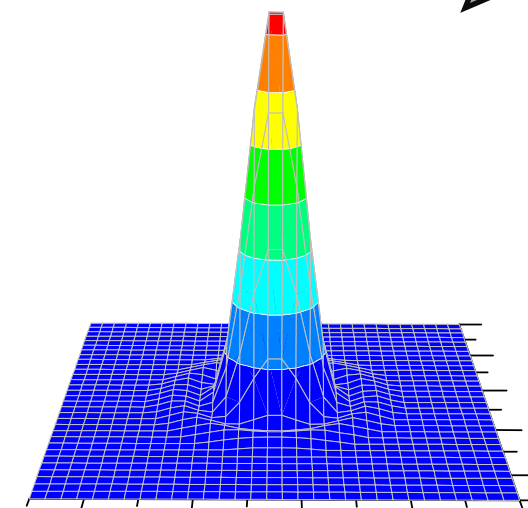
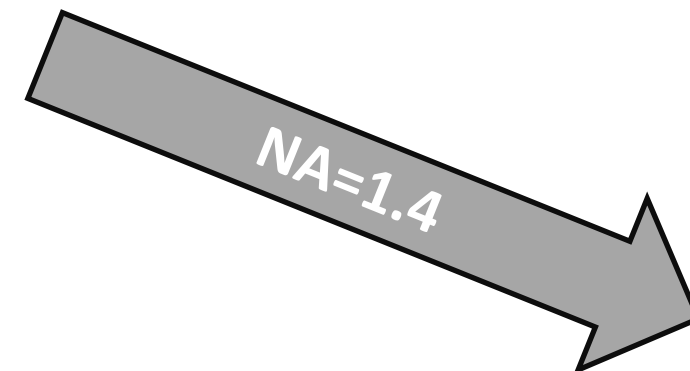
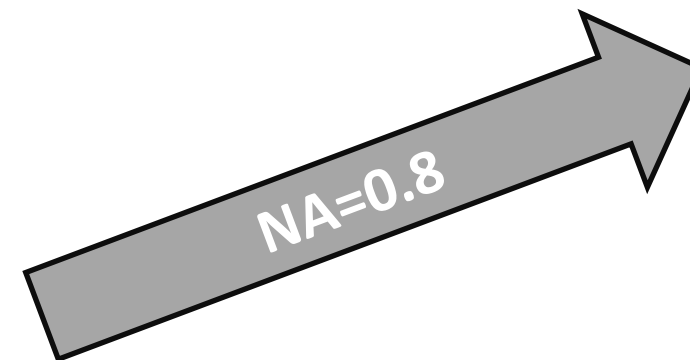
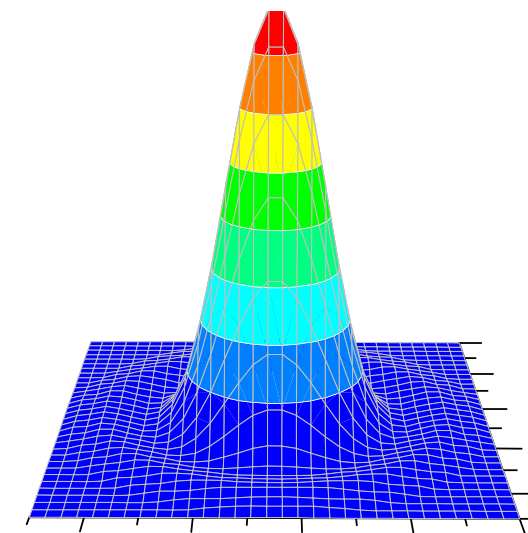
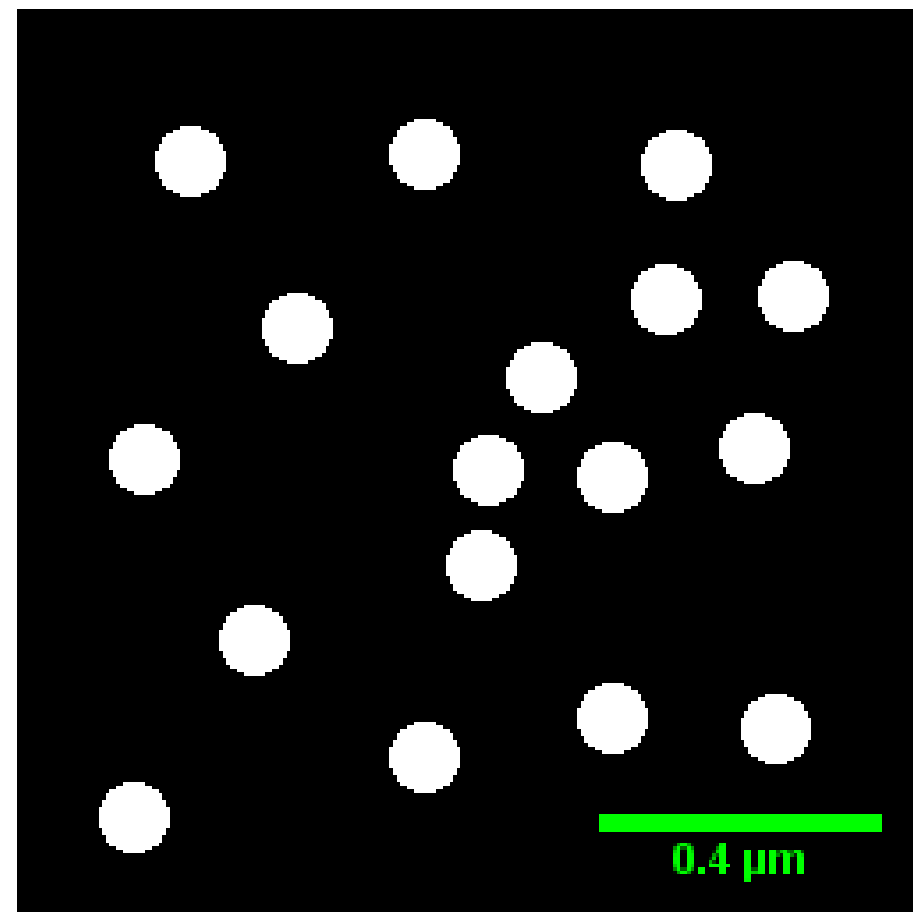
Object



Images are Artefacts

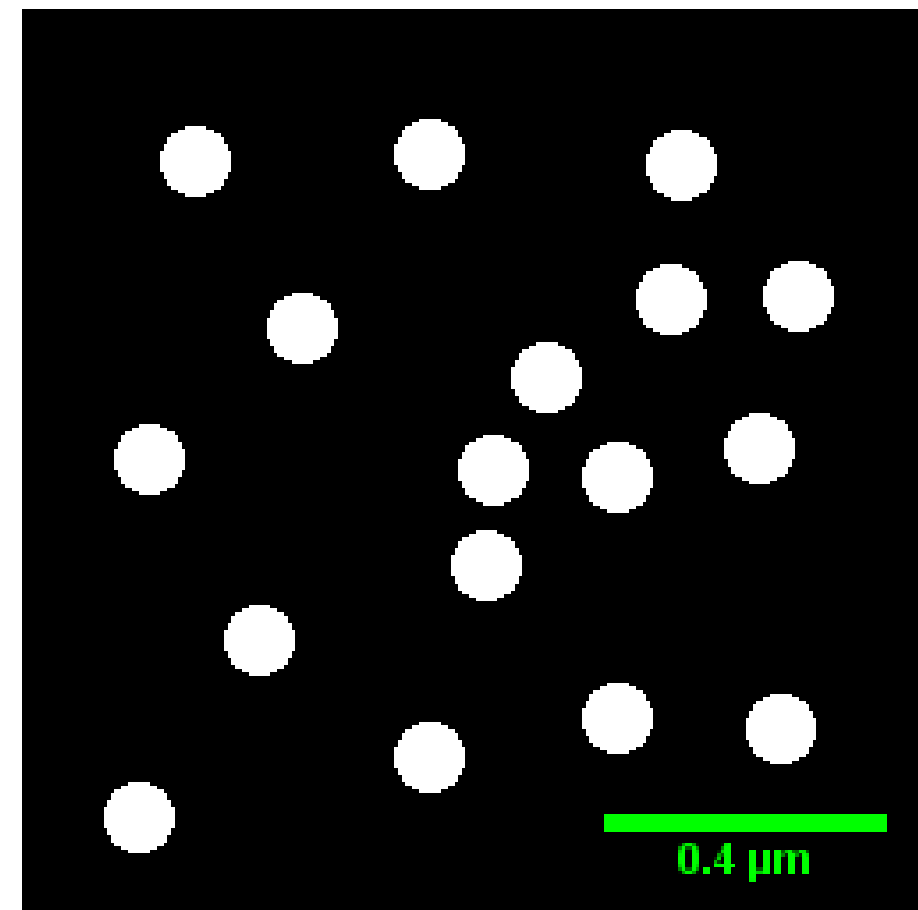
Object

Response

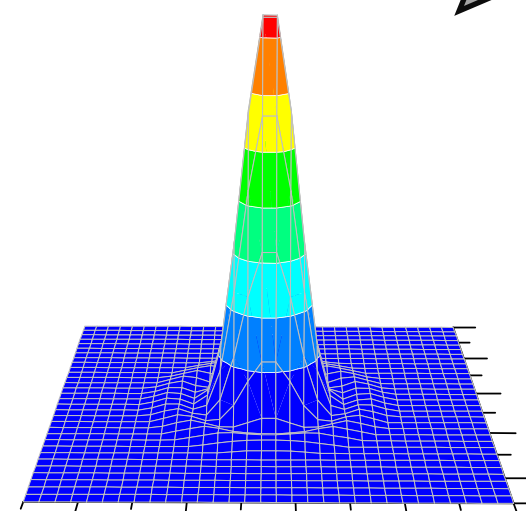
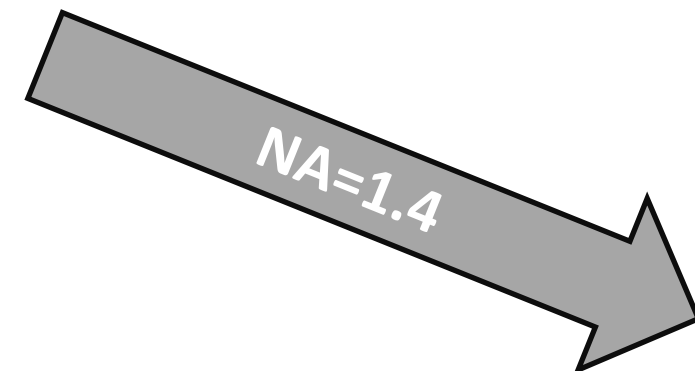
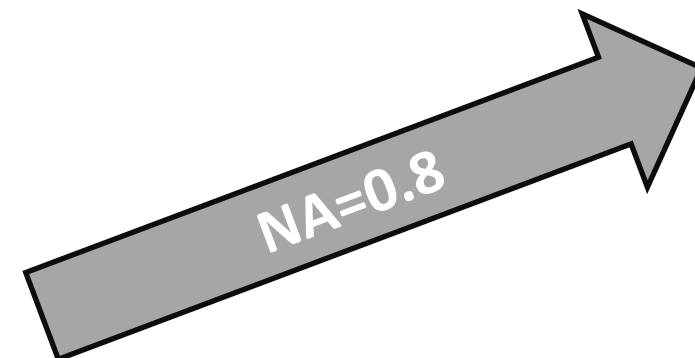
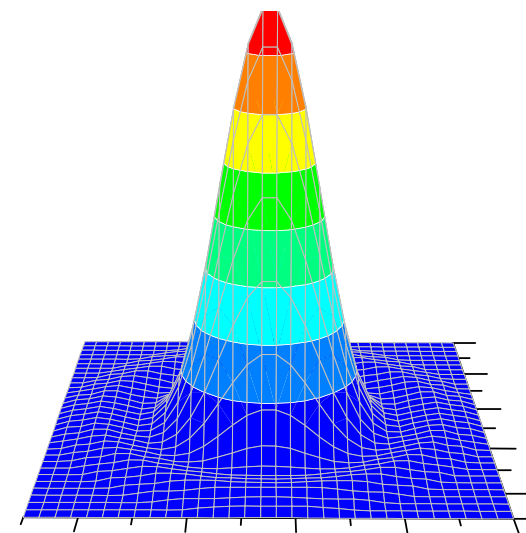


Images are Artefacts

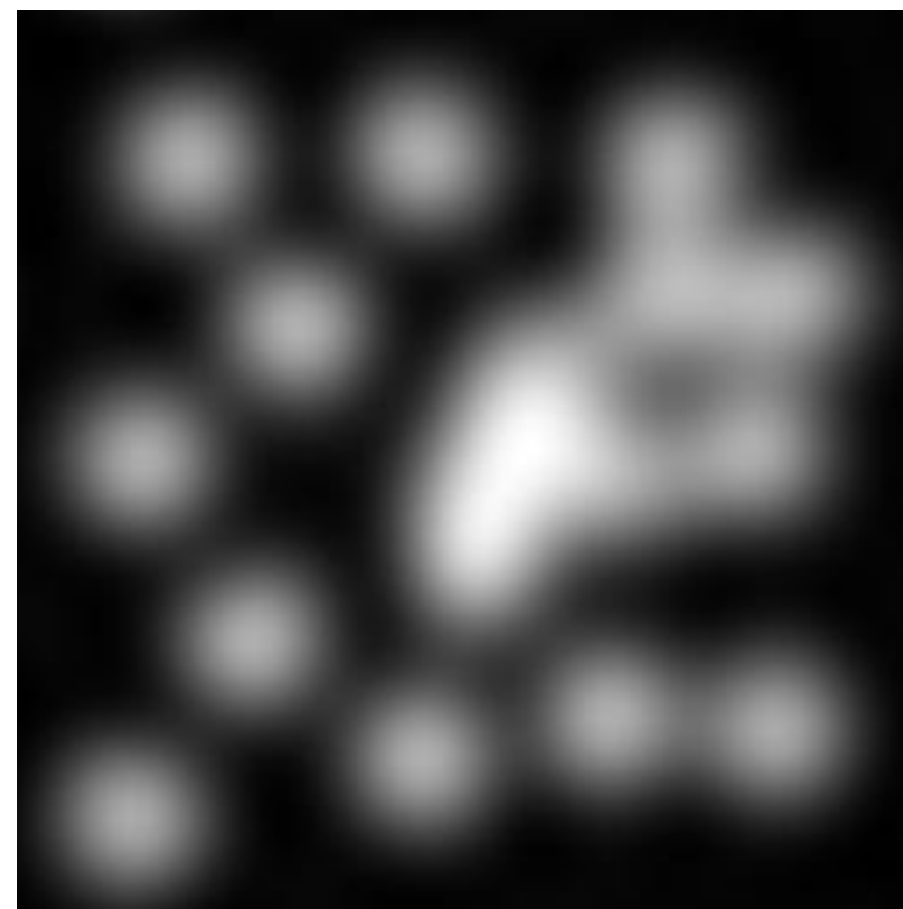
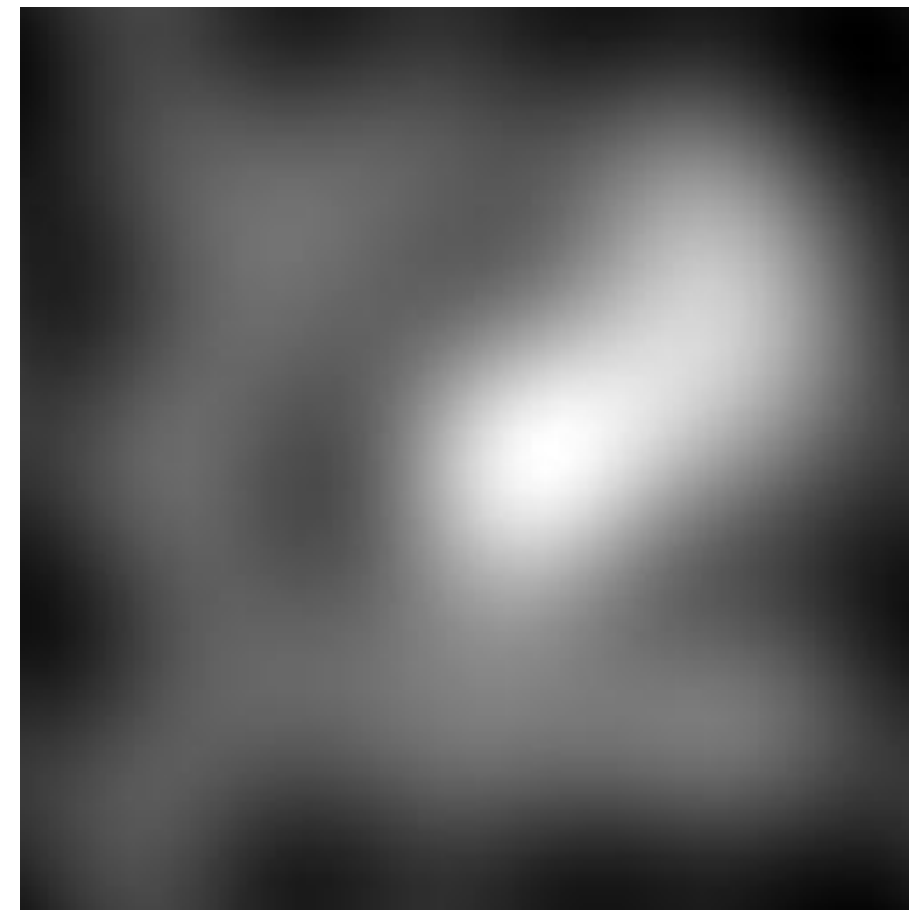
Object



Response

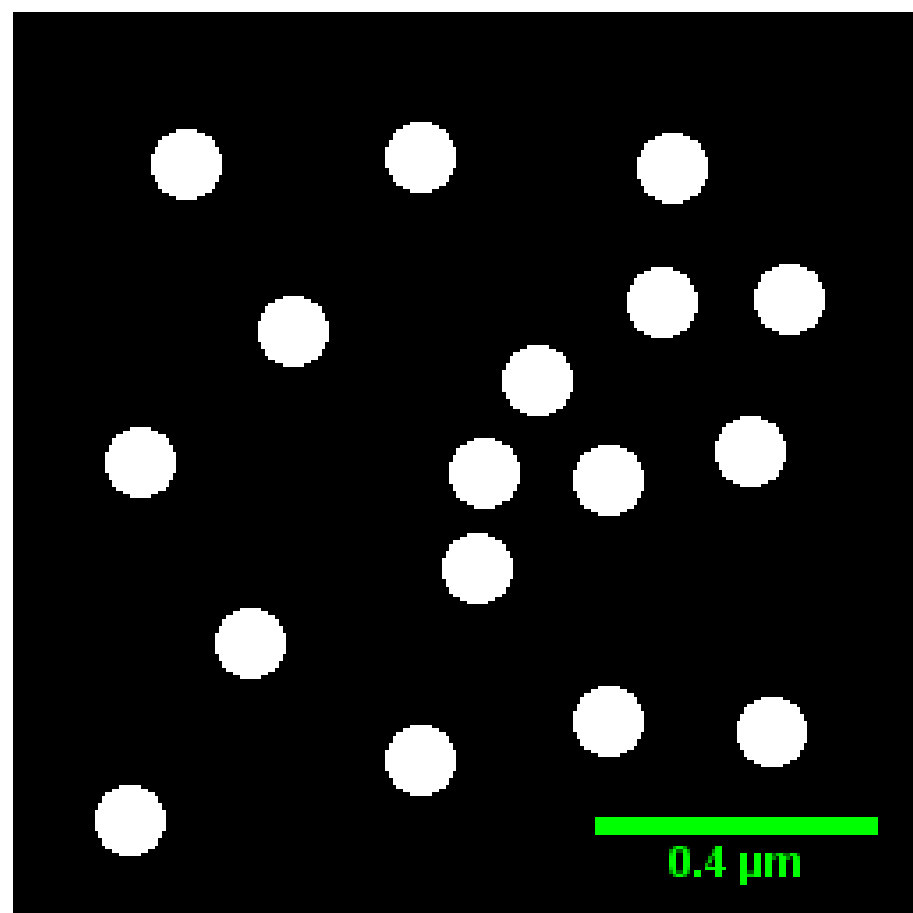


Image

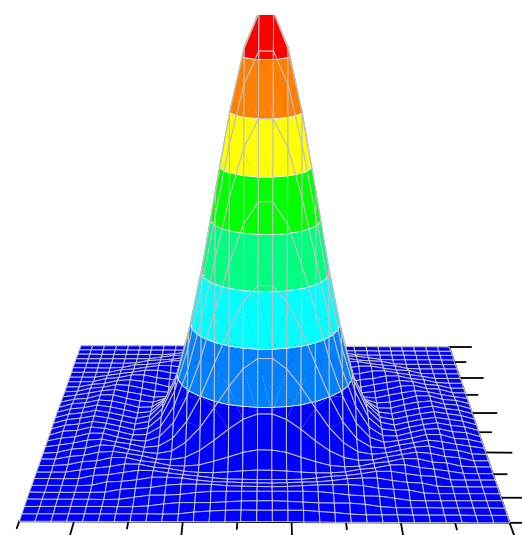


Images are Artefacts

Object

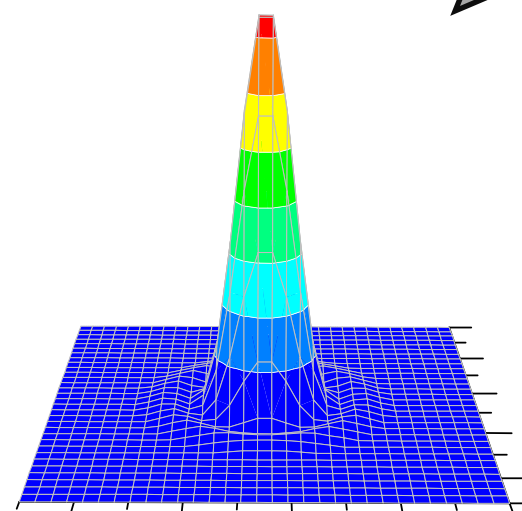


Response

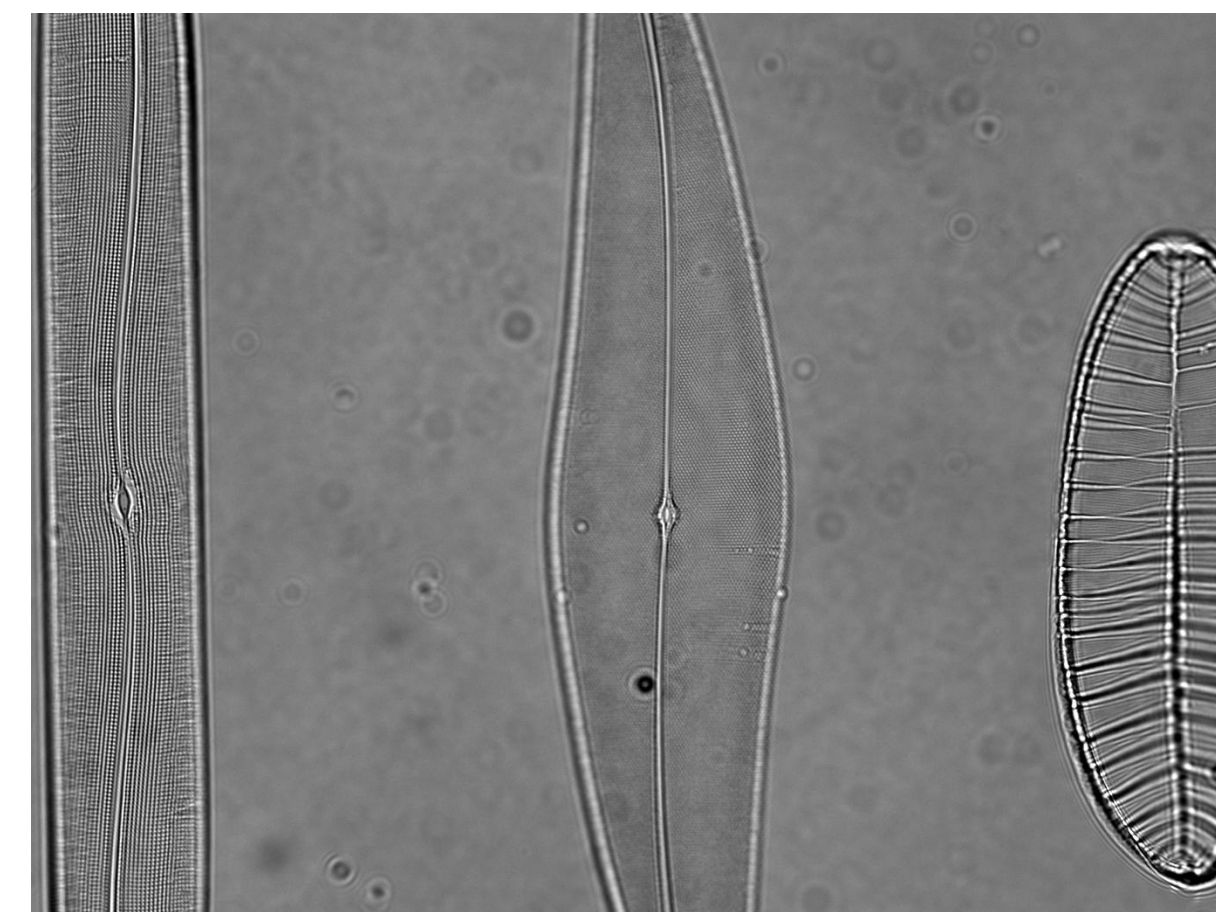
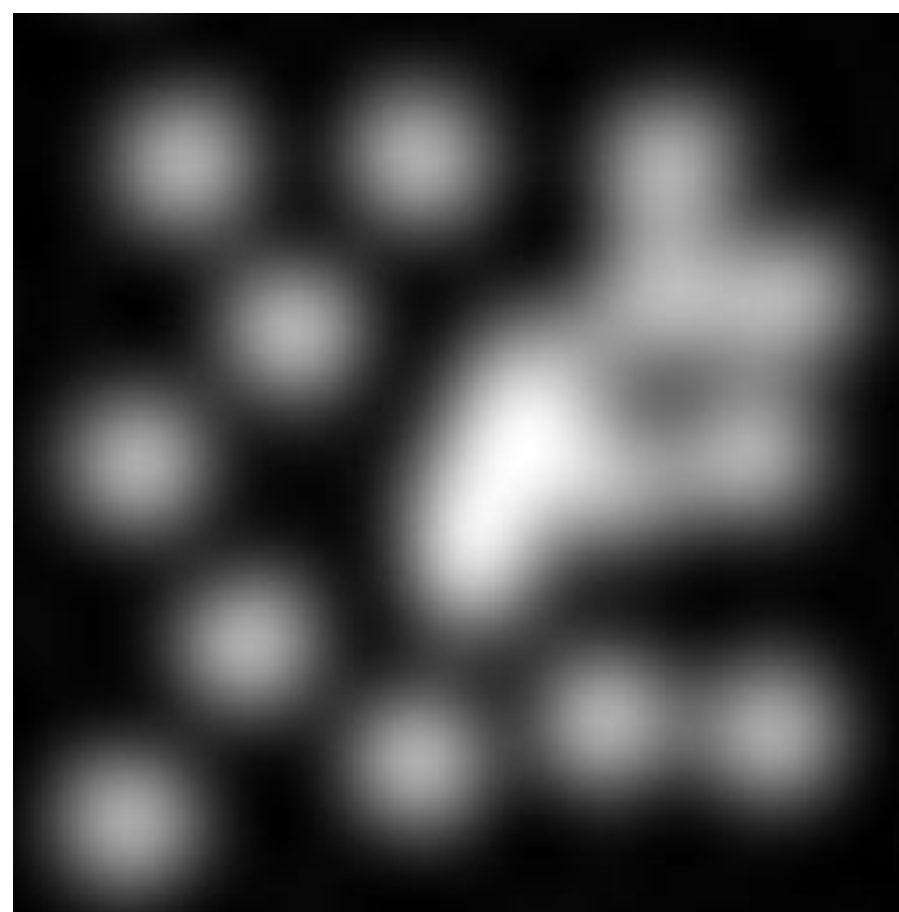
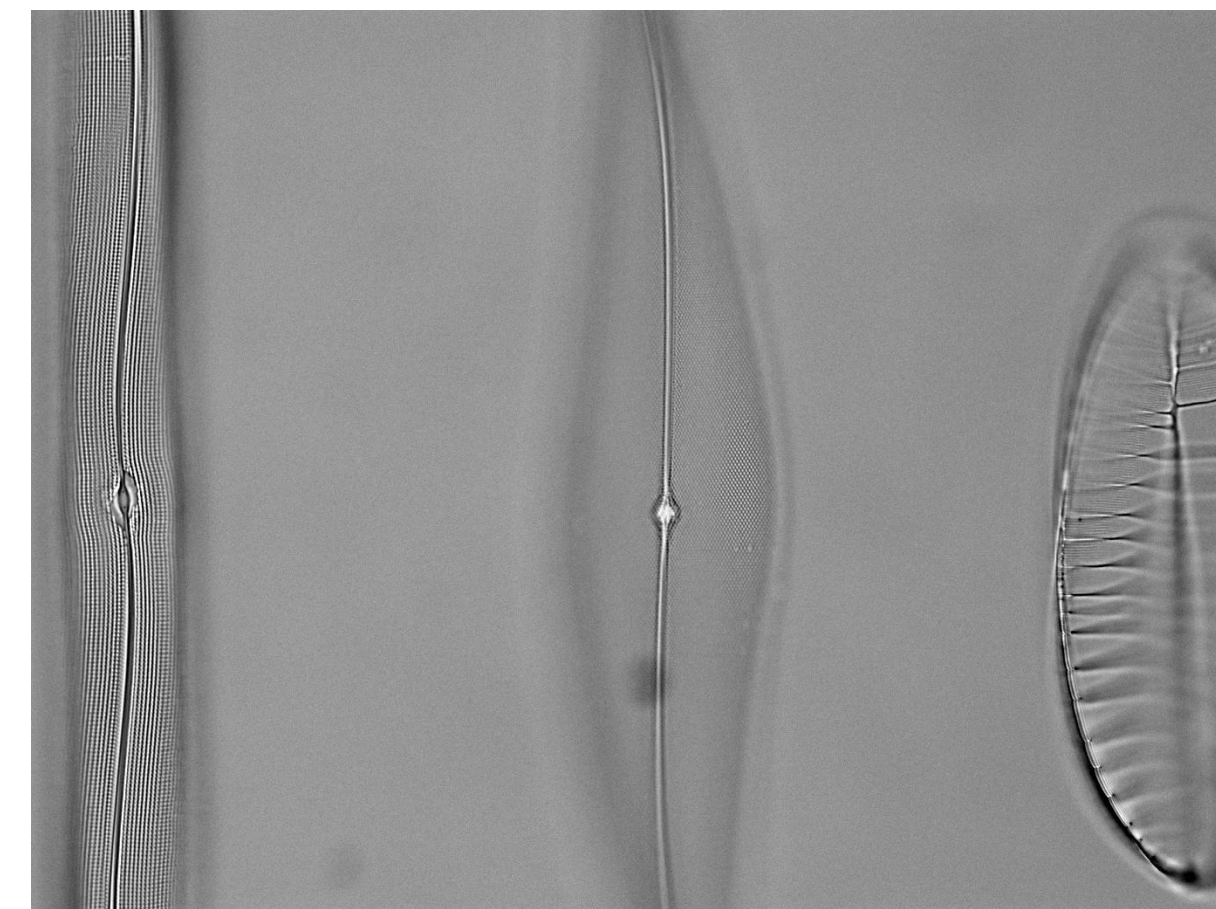
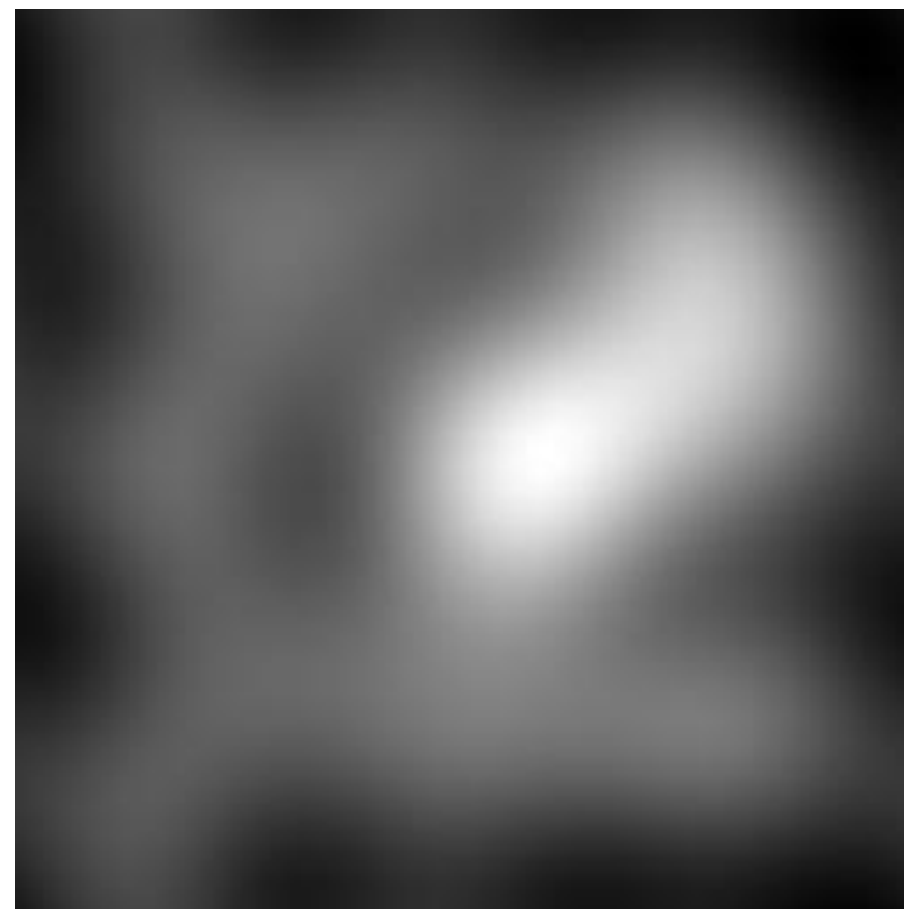


NA=0.8

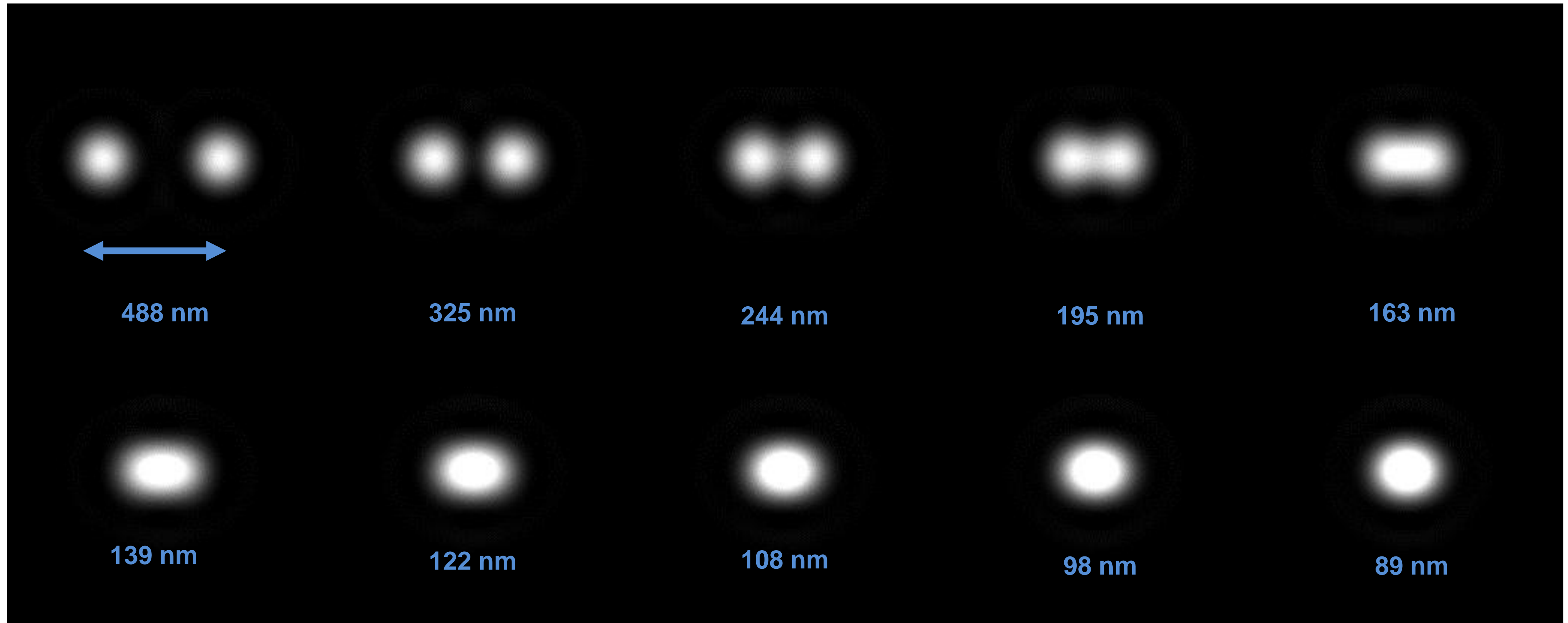
NA=1.4



Image

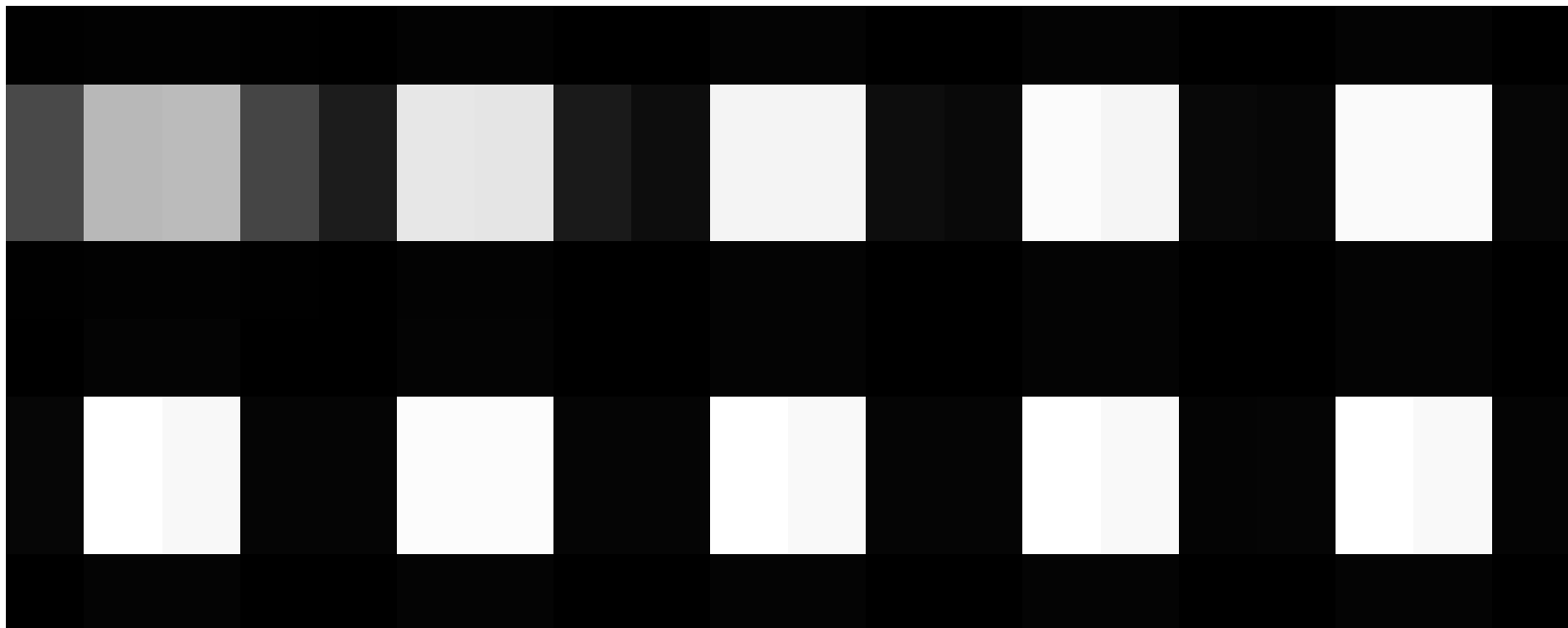
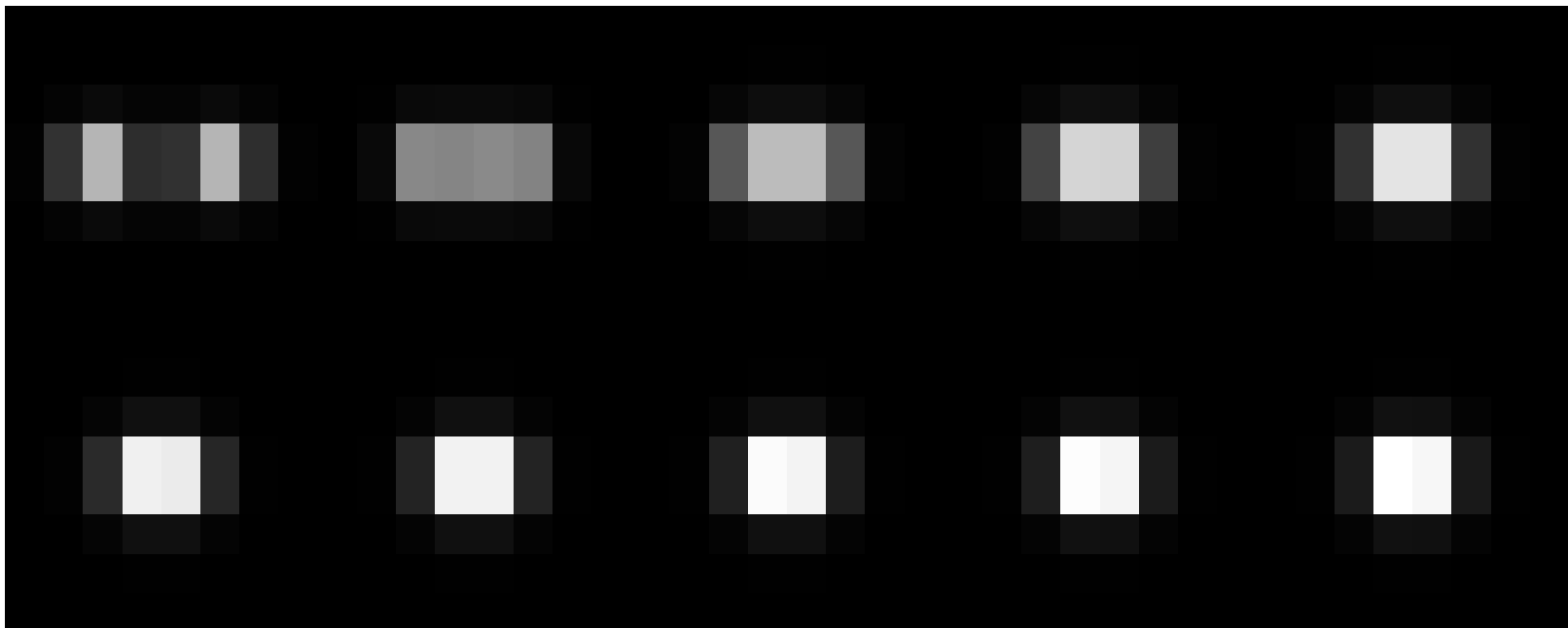
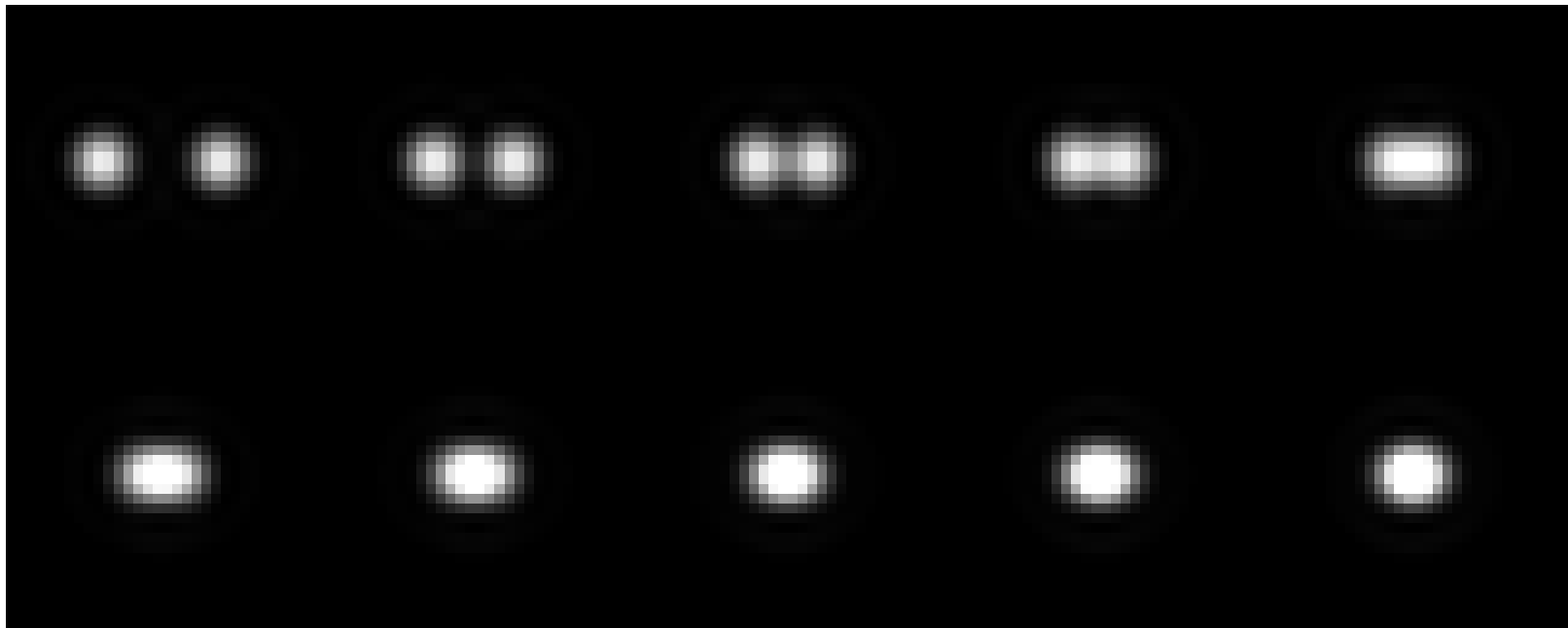
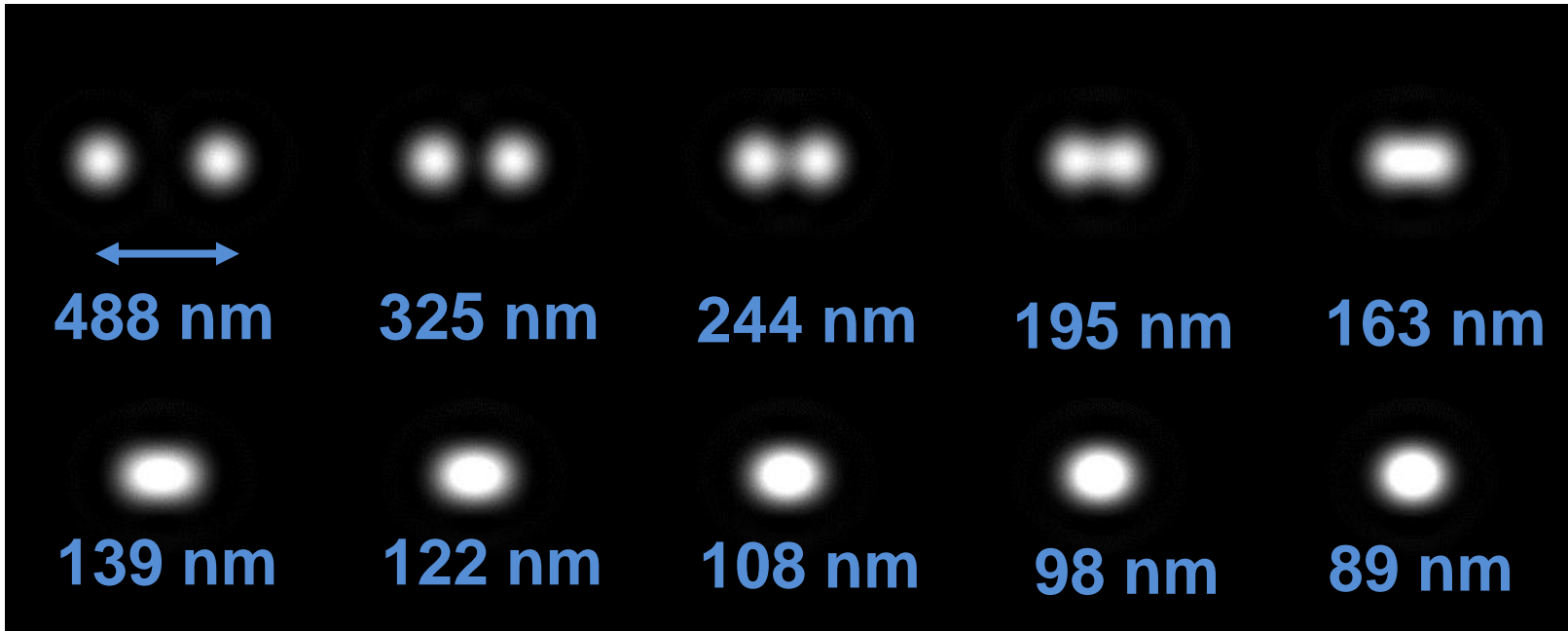


Resolution



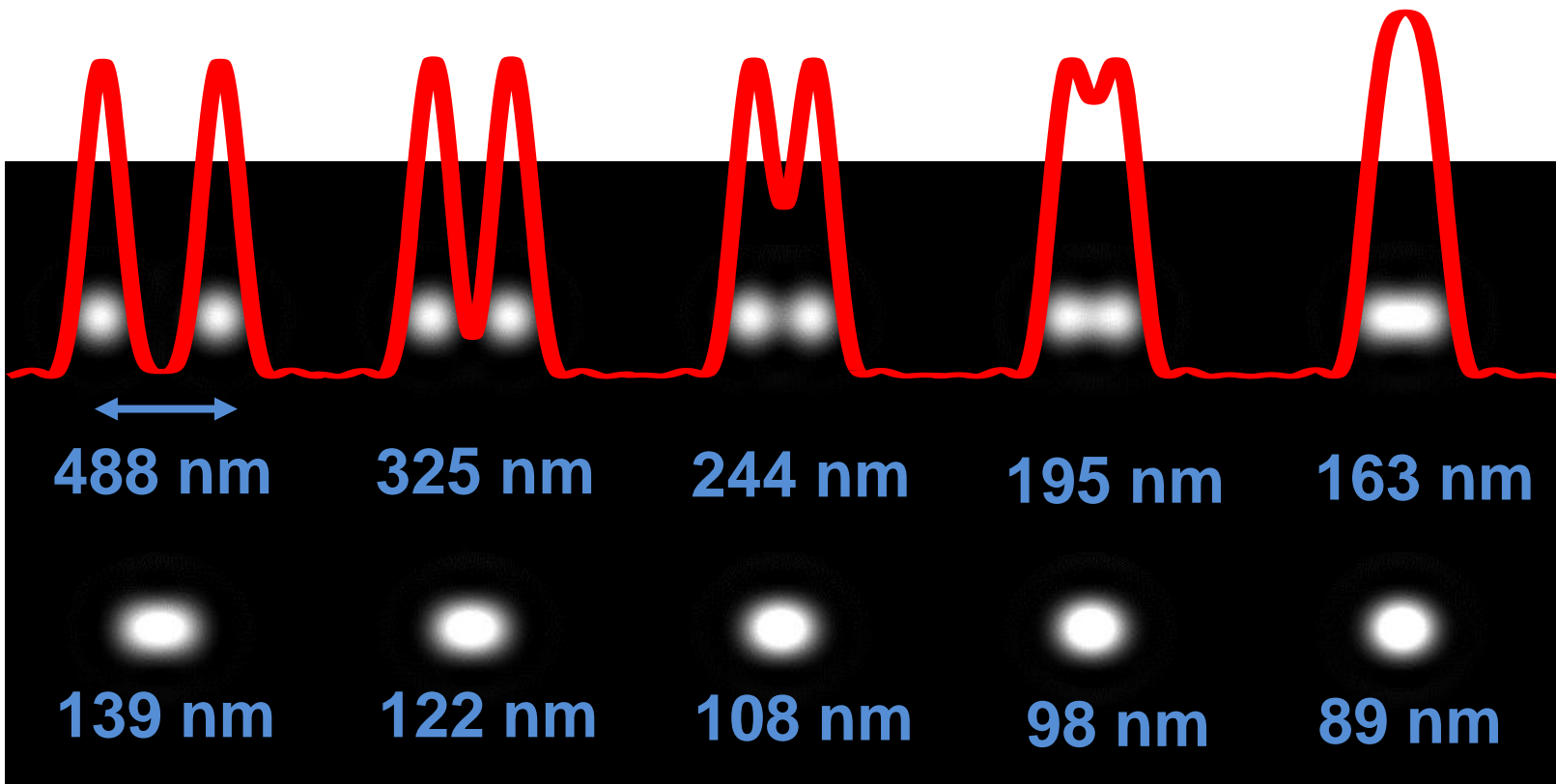
Wavelength: 488nm; NA=1.4 Rayleigh criterion: 212 nm

Digital Sampling

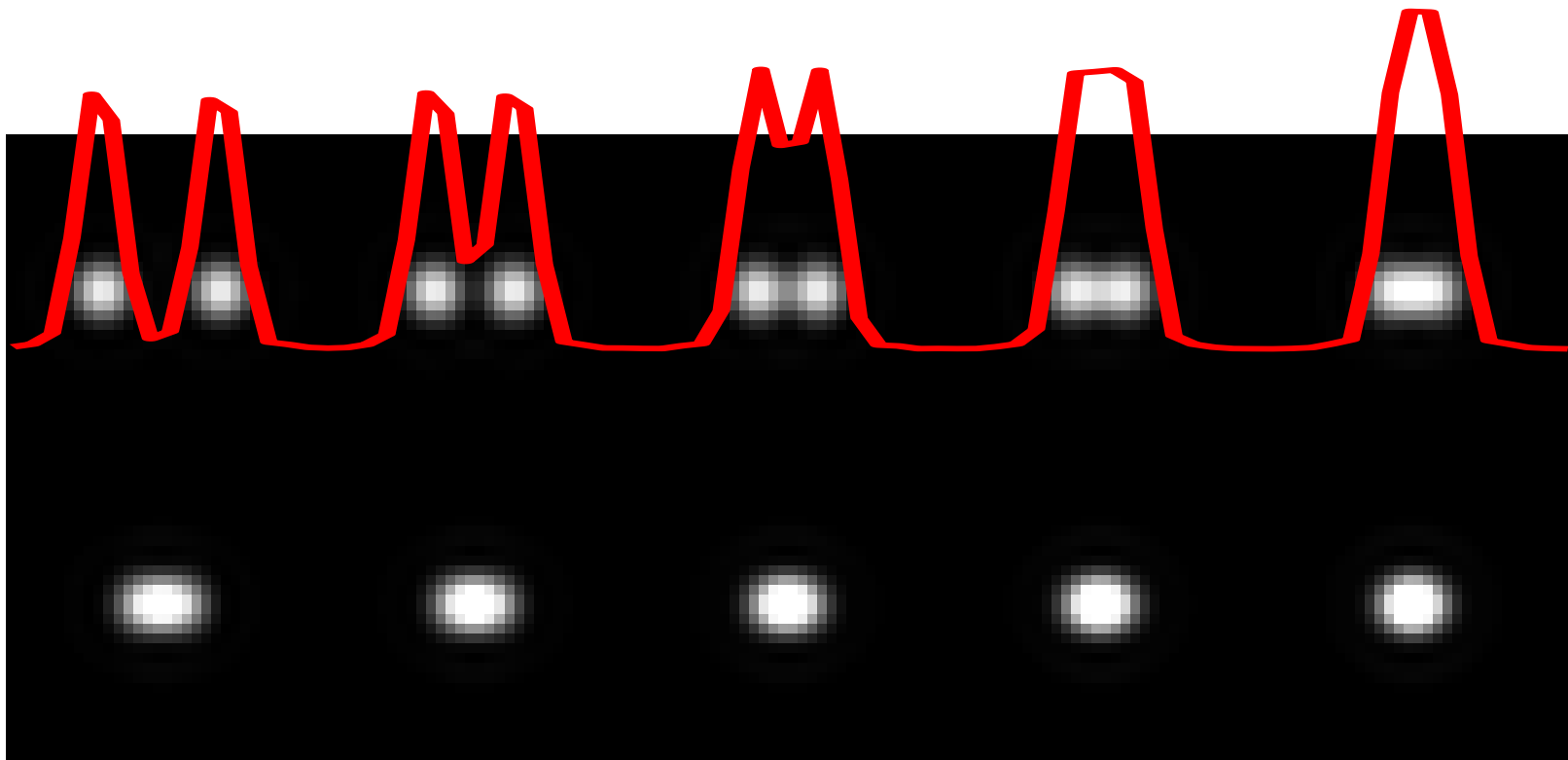


Wavelength: 488nm; NA=1.4 Rayleigh criterion: 212 nm

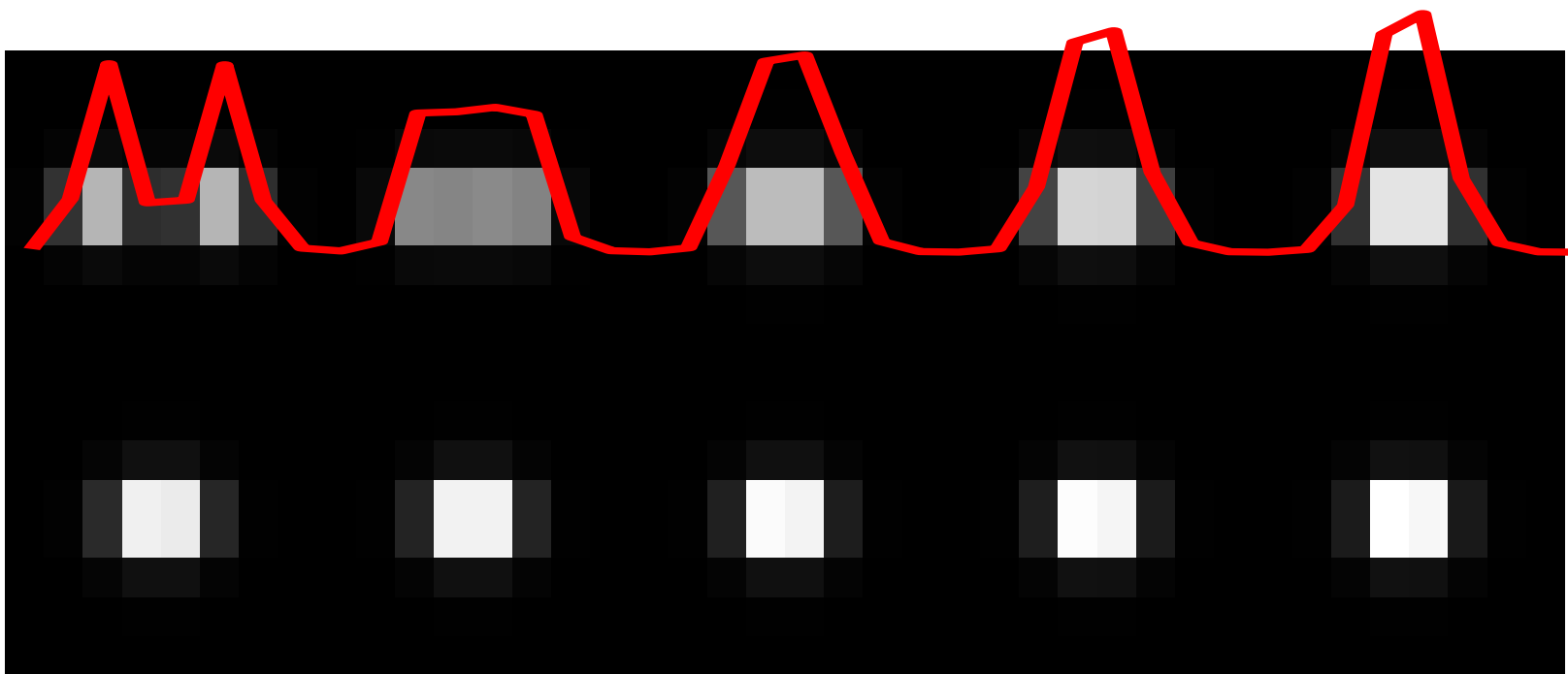
Digital Sampling



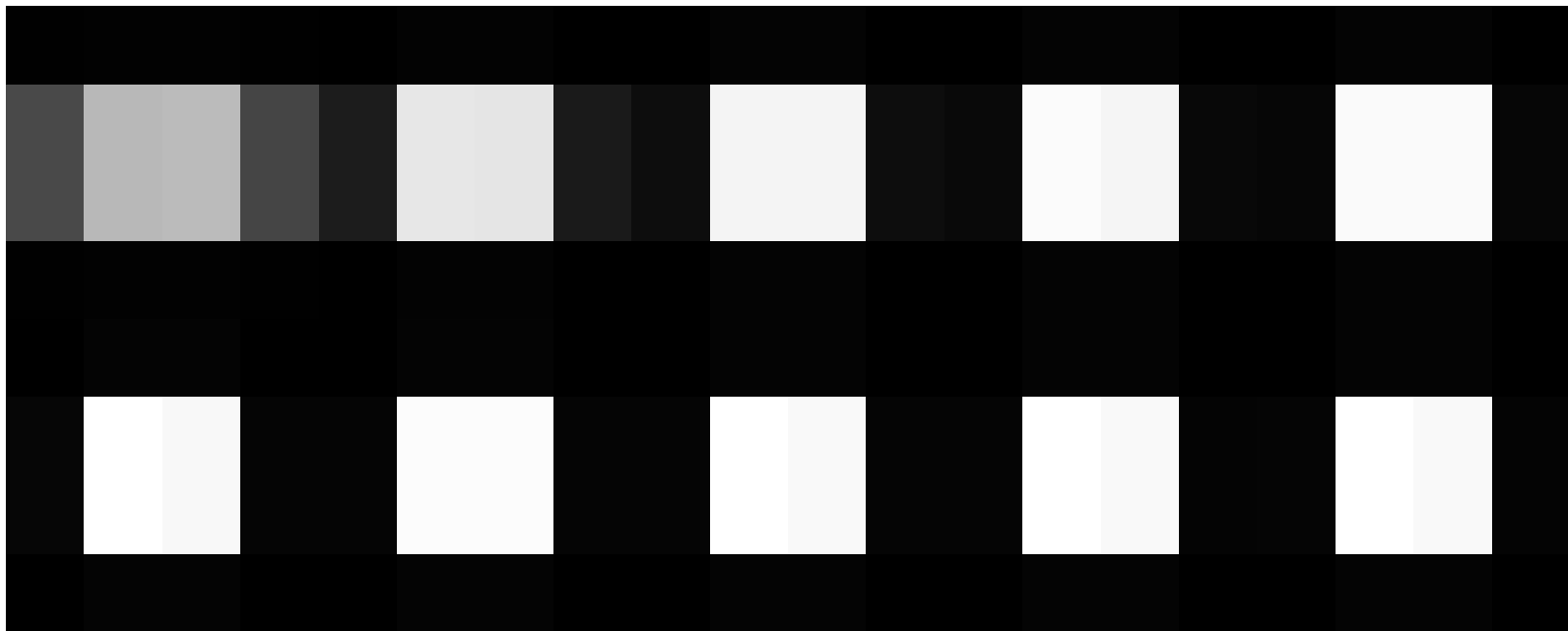
5 nm/pixel



80 nm/pixel



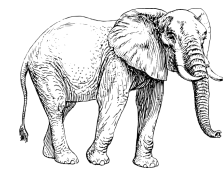
160 nm/pixel



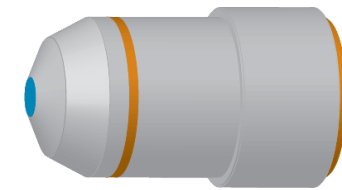
320 nm/pixel

Wavelength: 488nm; NA=1.4 Rayleigh criterion: 212 nm

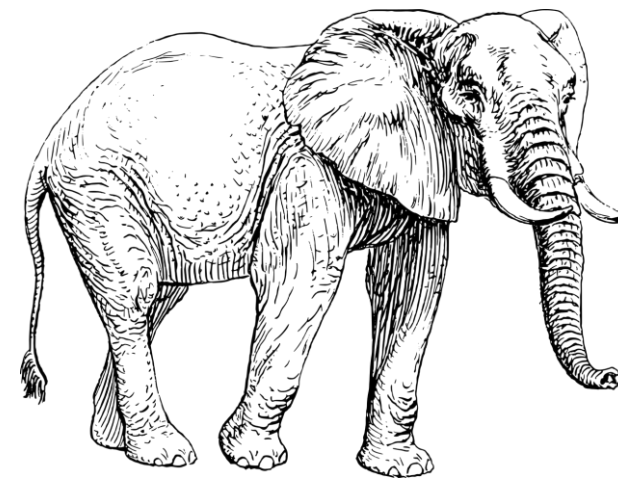
Image formation workflow



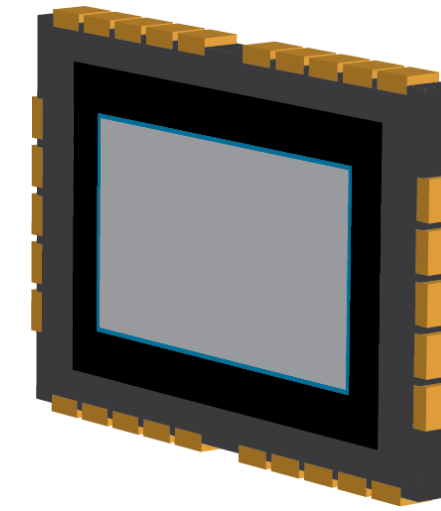
Object



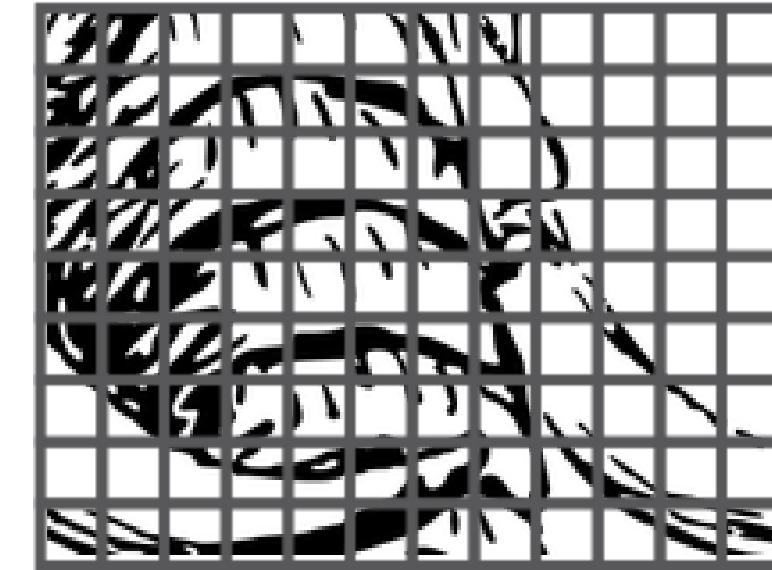
Imaging System



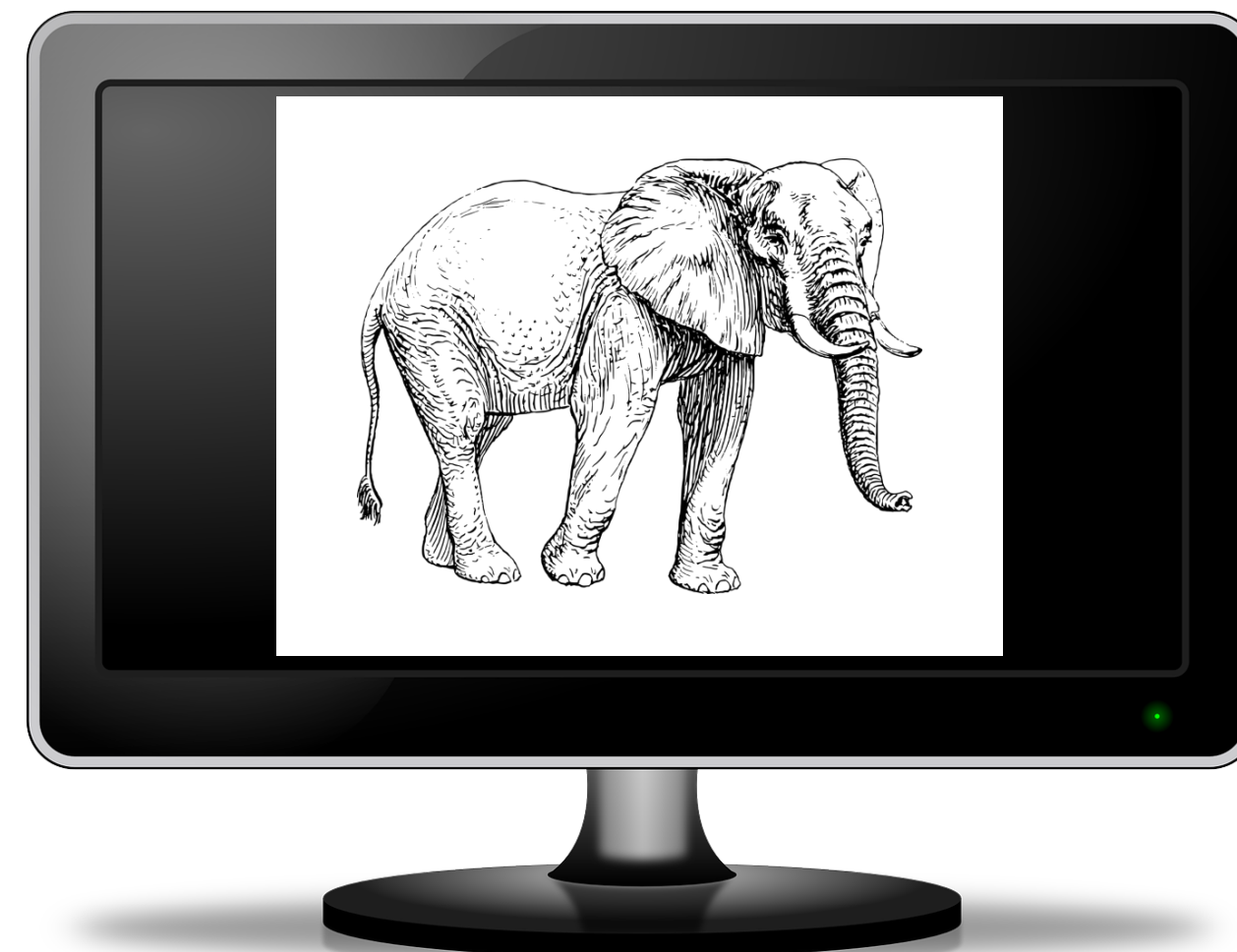
Image



Detection System



Digital Image (Raster)



$$I(u, v) \Rightarrow I(u', v')$$

Pixel Intensity value(s)
e.g. 0...255

Screen luminosity value(s)

Image File Formats

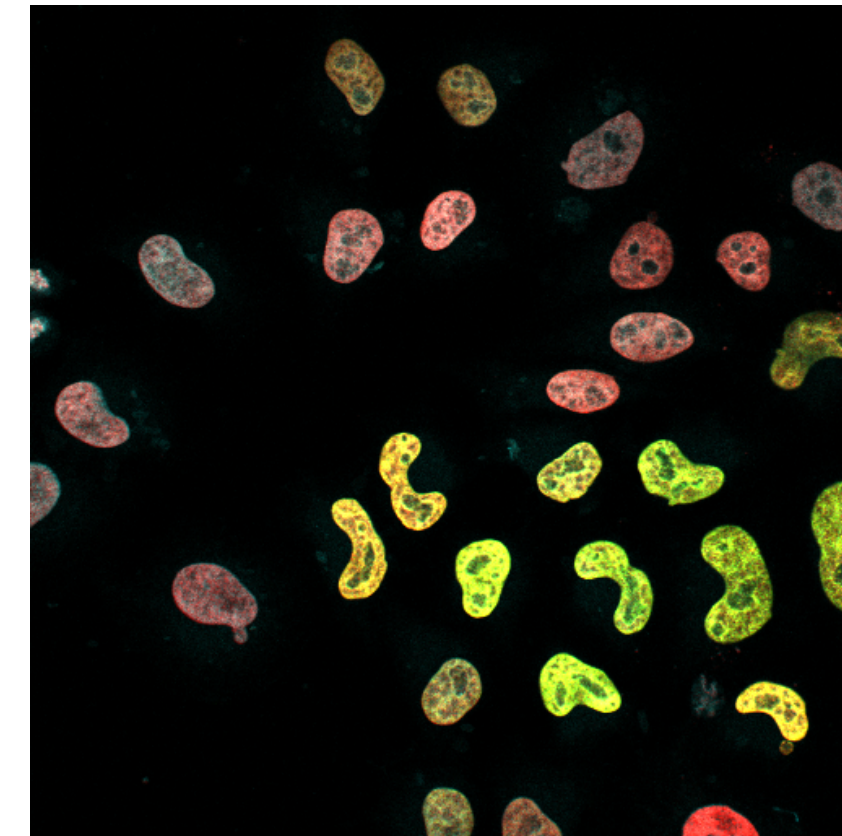
- Lossless compression

- TIFF (Tagged Image File Format)
- GIF (Graphics Interchange Format)
- BMP (Windows bitmap)
- HDF5 (Hierarchical Data Format)

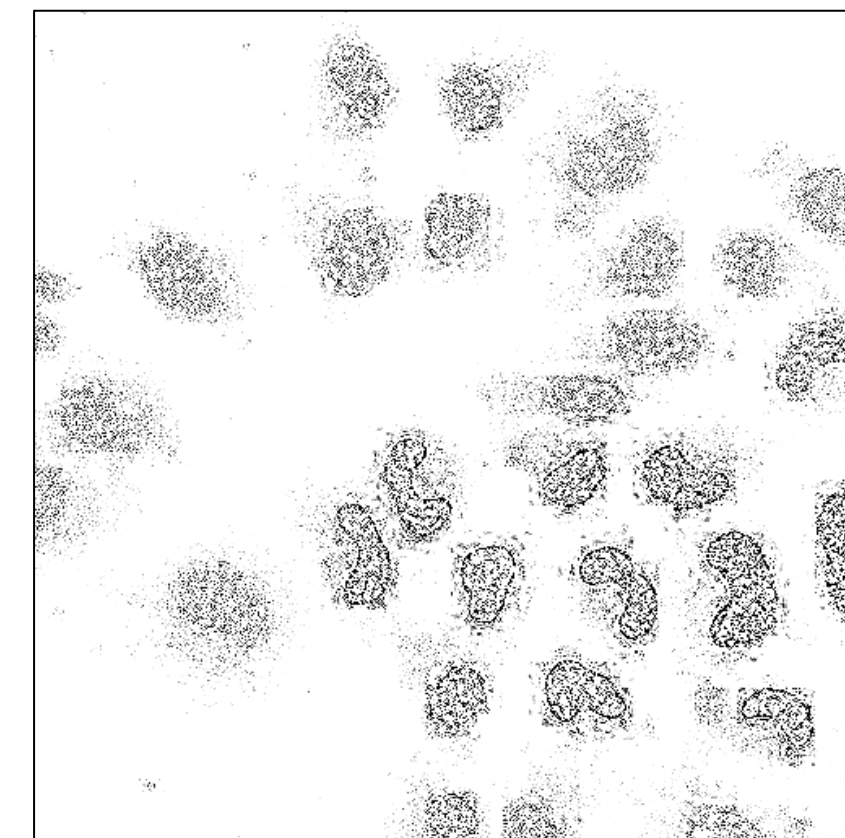
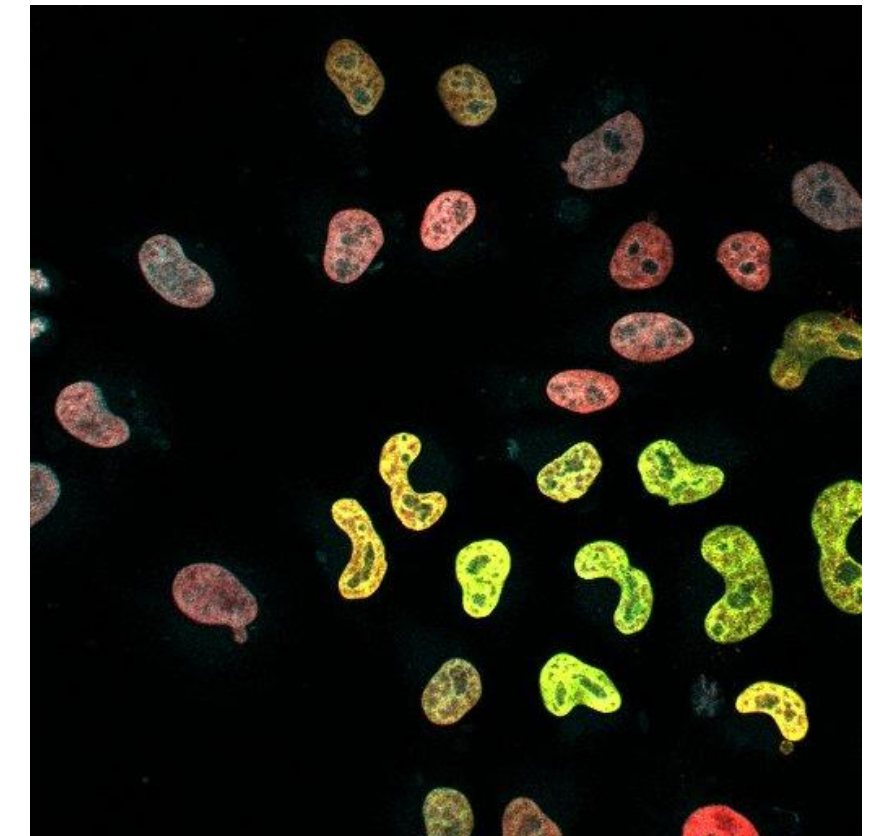
- Lossy Compression

- JPEG (Joint Photographic Experts Group)
- JPEG 2000 (lossless and lossy storage)

Tiff format



jpeg format



Difference

- Image Analysis

- Quantification by avoiding human bias
- High Throughput
- Reproducible
- Portable

- Digital Images

- Carry the response of the acquisition system
- Raster images with discrete intensity values (different bit depth)