

Principles of 2D Image Analysis

BMS 524 - “Introduction to Confocal Microscopy and Image Analysis”

1 Credit course offered by Purdue University Department of Basic Medical Sciences, School of Veterinary Medicine

Part 1

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Morphometry: "The quantitative description of a structure"
(Weibel, ER, 1969. Stereological principles for morphometry
in electron microscopy. Int. Rev. Cytol. 26:235-302.)

Stereology: The extraction/interpretation of 3D data from 2D
data (i.e. sections of objects)

Image processing: Computer enhancement of a digitized
image (i.e., using various filters to remove noise, improve
contrast, pseudocoloring. Enhancement of regular structures
(virus, crystals)

Image analysis: Information extracted from an image (area,
perimeter, length, etc.)

How do humans classify objects?

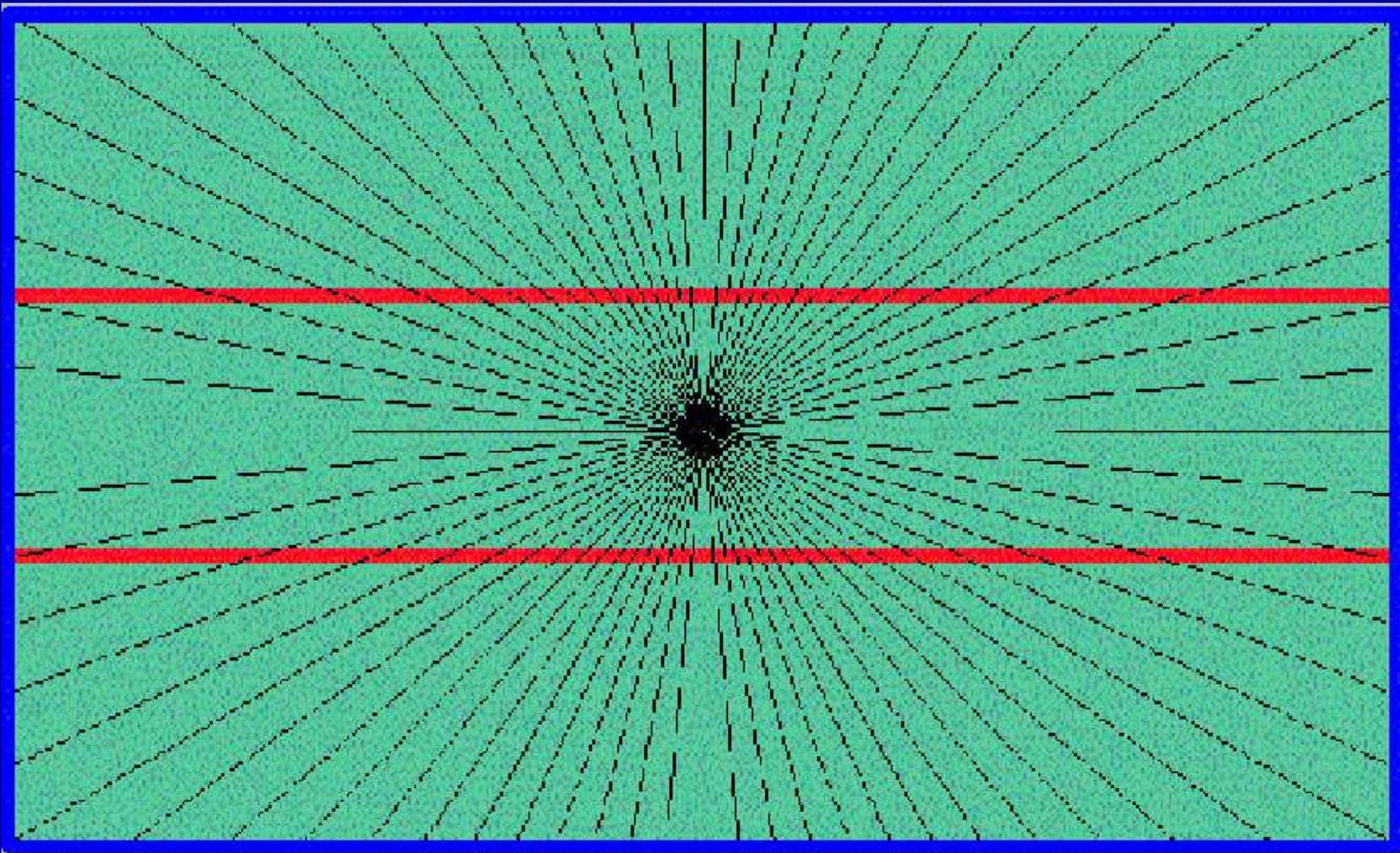
Human method is pattern recognition based upon multiple exposure to known samples. We build up mental templates of objects, this image information coupled with other information about an object allows rapid object classification with some degree of objectivity, but there is always a subjective element.

1. We are sensitive to differences in contrast. We will tend to overestimate the amount or size of an object if there is high contrast vs low contrast.
2. We are sensitive to perspective and depth changes
3. We are sensitive to orientation of lighting. We prefer light to come from above.
4. We fill in what we think should be in the image

Biological structures present a continuous spectrum of change.

Morphometry eliminates subjectivity, it is more reproducible, and has greater limits of detection.

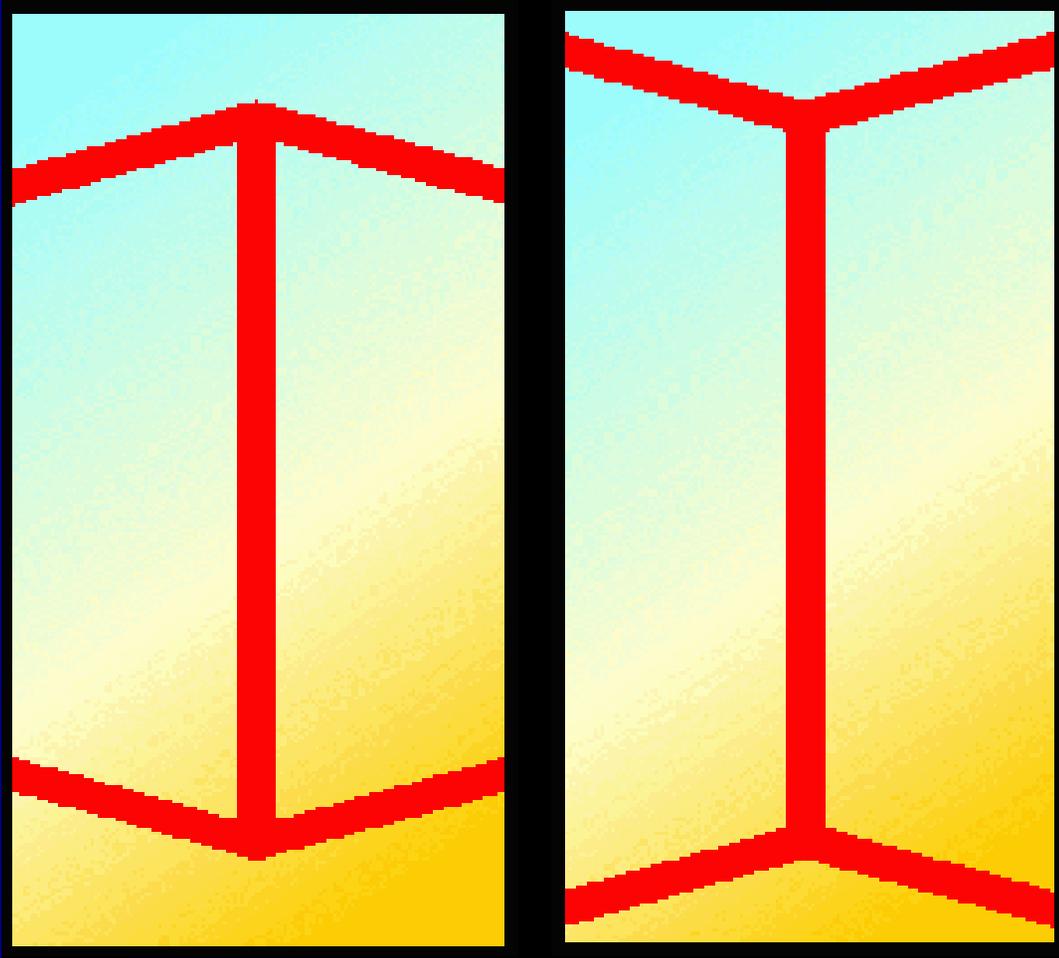




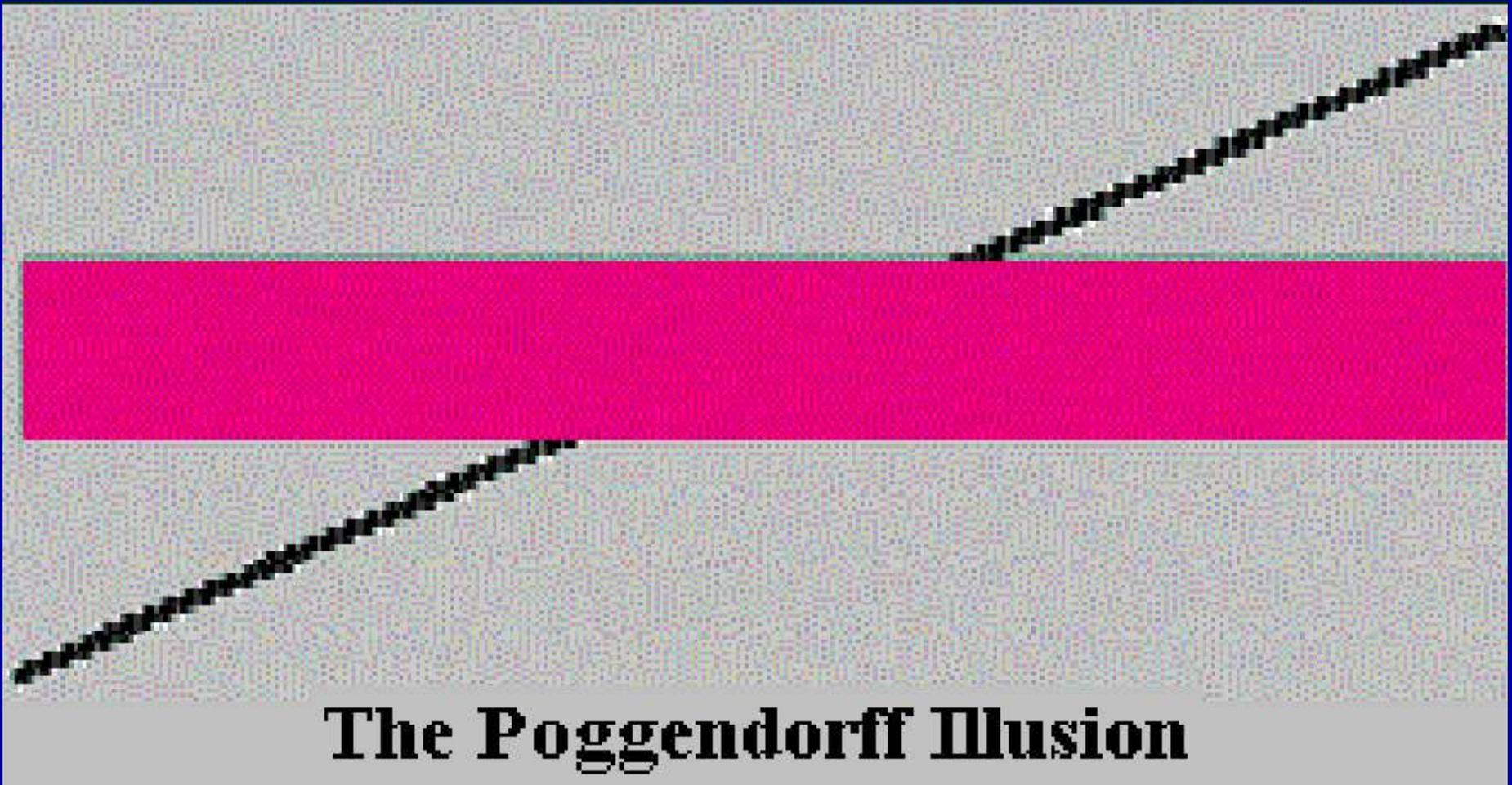
Hering Illusion

This illustration was first published in 1861 by Ewald Hering. Astronomers became very interested in Hering's work because they were worried that visual observations might prove unreliable.

Moller-Franz illusion



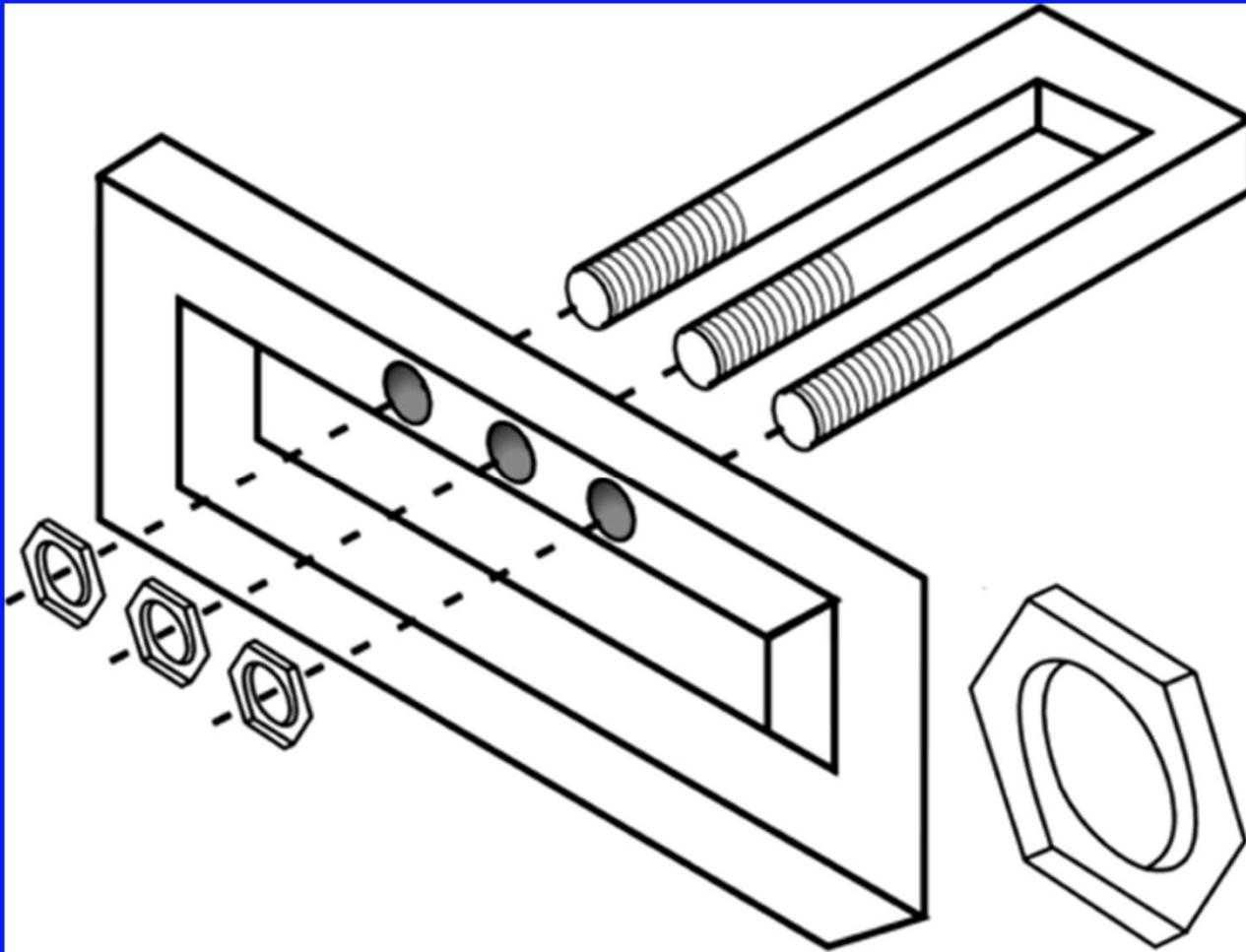
This illusion was created in 1889 by Franz Müller-Lyon. The lengths of the two identical vertical lines are distorted by reversing the arrowheads. Some researchers think the effect may be related to the way the human eye and brain use perspective to determine depth and distance, even though the objects appear flat.



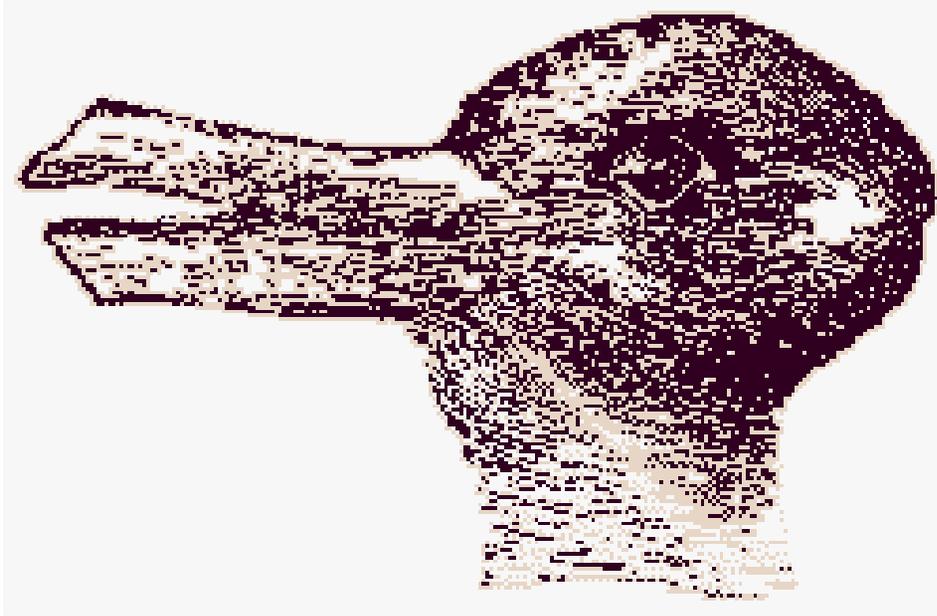
In 1860 Johann Poggendorff created this line distortion illusion. The two segments of the diagonal line appear to be slightly offset in this figure.



This line distortion illusion was first published by Johann Zöllner in 1860. The diagonal lines are parallel, but appear not to be. The illusion was designed to cause errors in optical equipment. It did cause errors, and also great concern among scientists of the time, over the validity of all human observations.



Research scientists who use the trident illusion to test populations, have divided us into two groups: two-dimensional perceivers and three-dimensional perceivers. Presumably, the perspective in this illustration would not be confusing to a two-dimensional perceiver.



This illustration was used at the turn of the century by psychologists to study how our mind learns to cope with conflicting images. The figure is either a duck facing left or a rabbit facing right. When both aspects are finally realized, ambiguity conflict occurs.



New version of the old trick

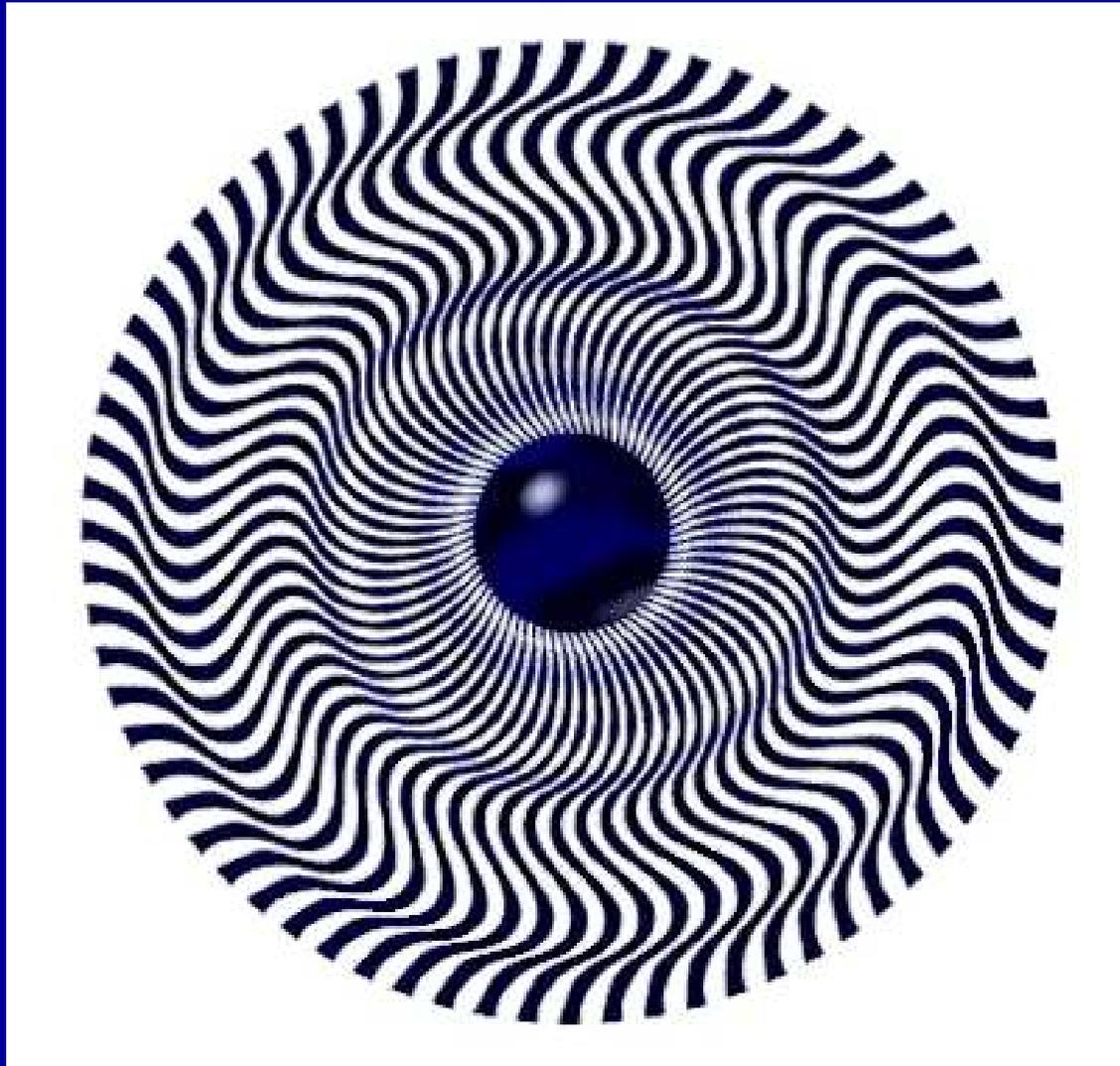


Reversible Goblet: Classic demonstration of figure-background reversal first used by Edgar Rubin in 1915.

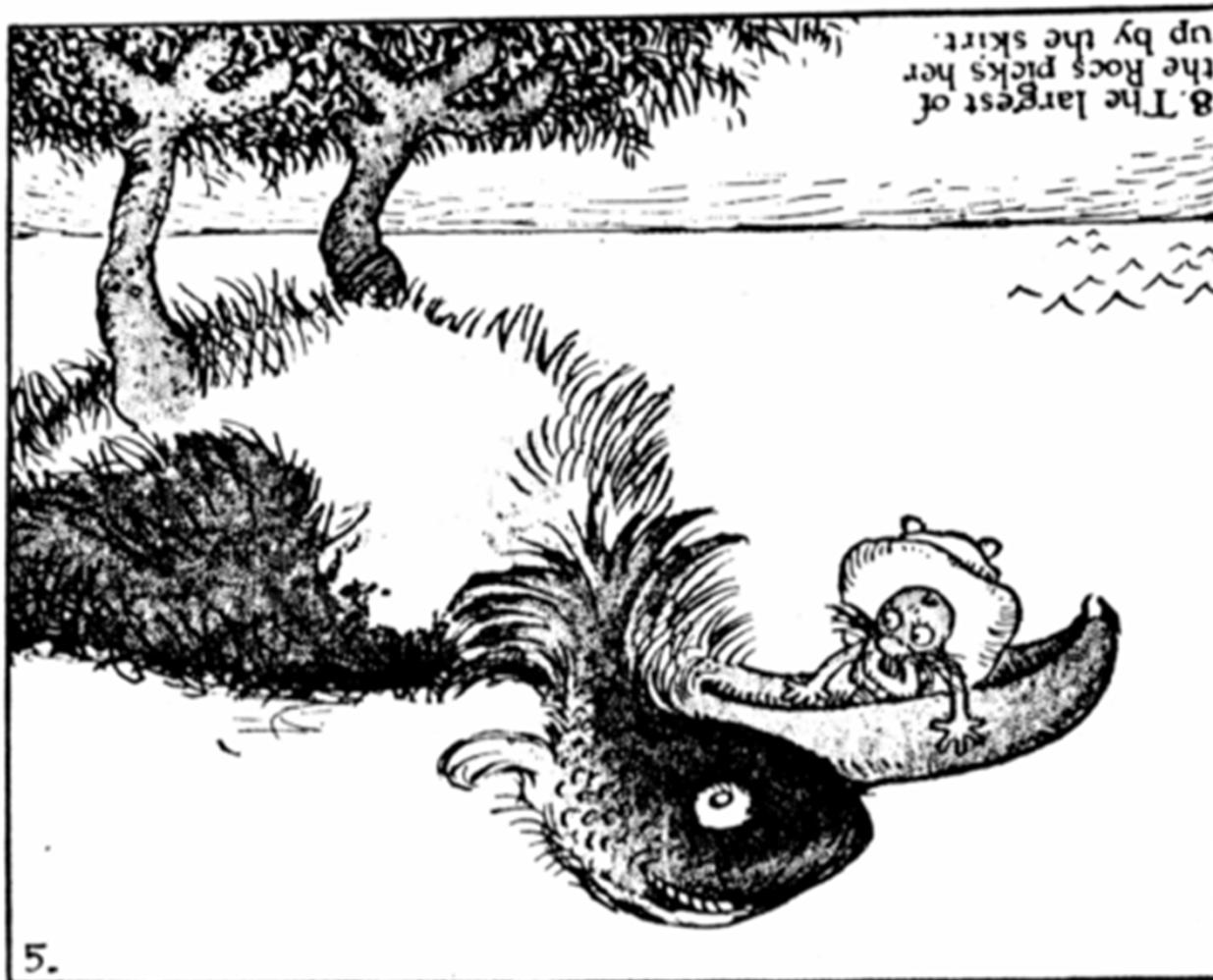


An anonymous German postcard from 1888 (left figure) depicts the image in its earliest known form, and a rendition on an advertisement for the Anchor Buggy Company from 1890 (center figure) provides another early example (IllusionWorks). For many years, the creator of this figure was thought to be British cartoonist W. E. Hill, who published it in 1915 in *Puck* humor magazine, an American magazine inspired by the British magazine *Punch*. (right figure). However, Hill almost certainly adapted the figure from an original concept that was popular throughout the world on trading and puzzle cards.

Mystic Wheel



Several things happen when you stare at the Mystic Wheel. You might see the illusion shimmer or flutter slightly. This is because the lenses of your eyeballs are slightly out-of-round. Parts of the illusion move in and out of focus as your eye scans the image. Looking closer, you get the impression of three dimensions, where some parts look higher or lower than others. Now, follow the ring of curves nearest the outer edge, around the wheel and you will see the geometry go from hump to hollow, an impossible trick in three dimensions.

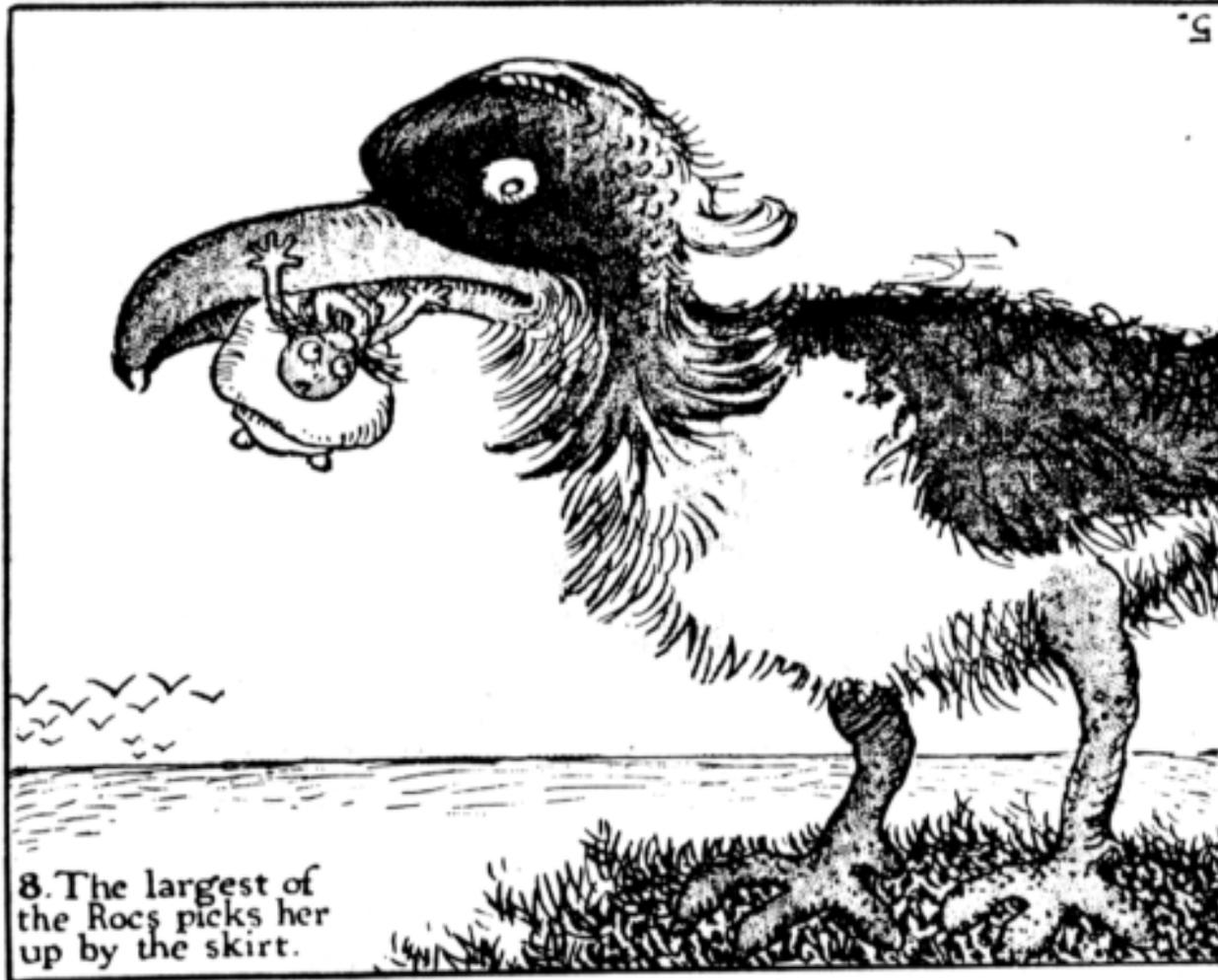


8. The largest of the Roc's picks her up by the skirt.

5.

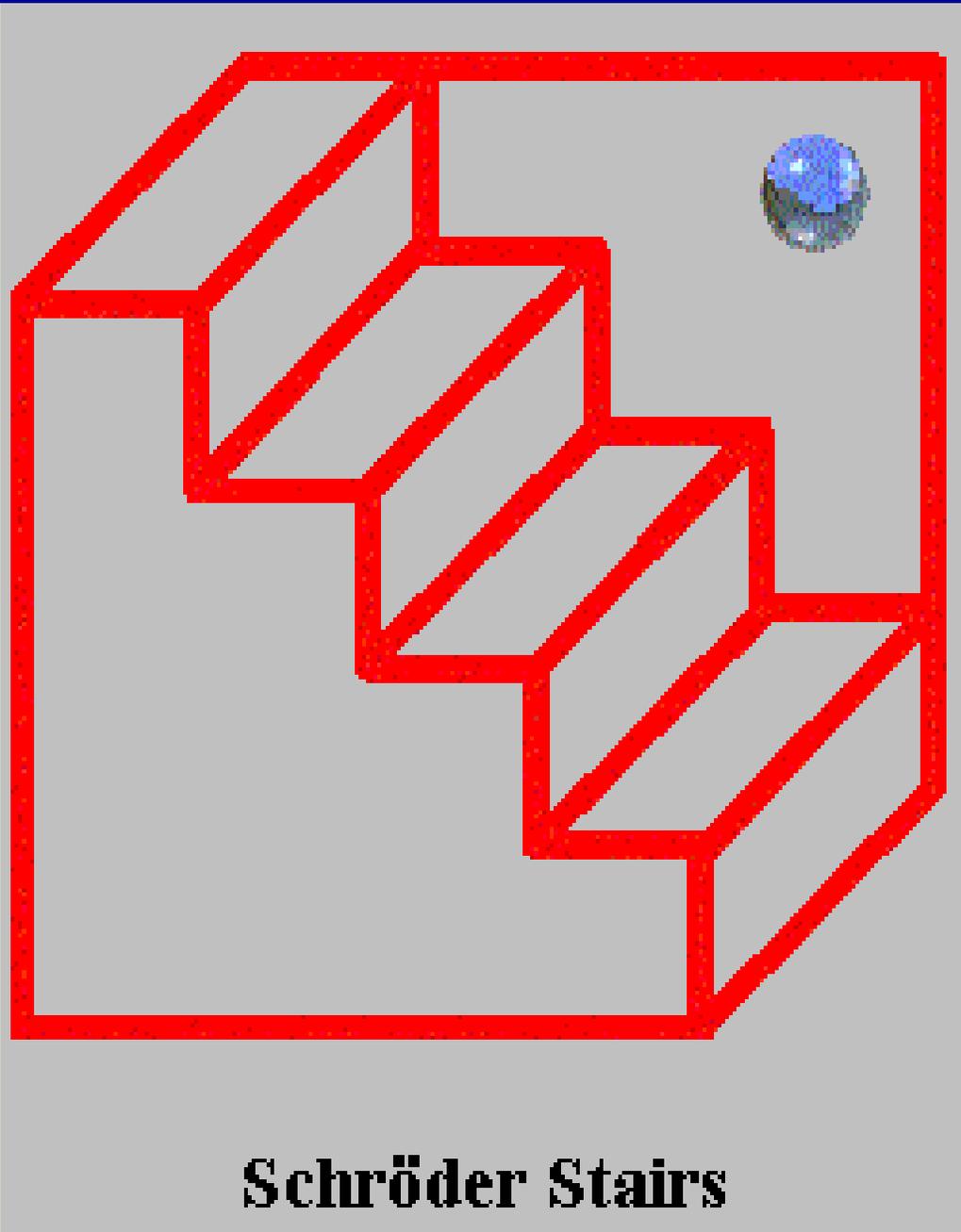
Just as he reaches a small grassy point of land, another fish attacks him, lashing furiously with his tail.

Just as he reaches a small grassy point of land, another fish attacks him, lashing furiously with his tail.



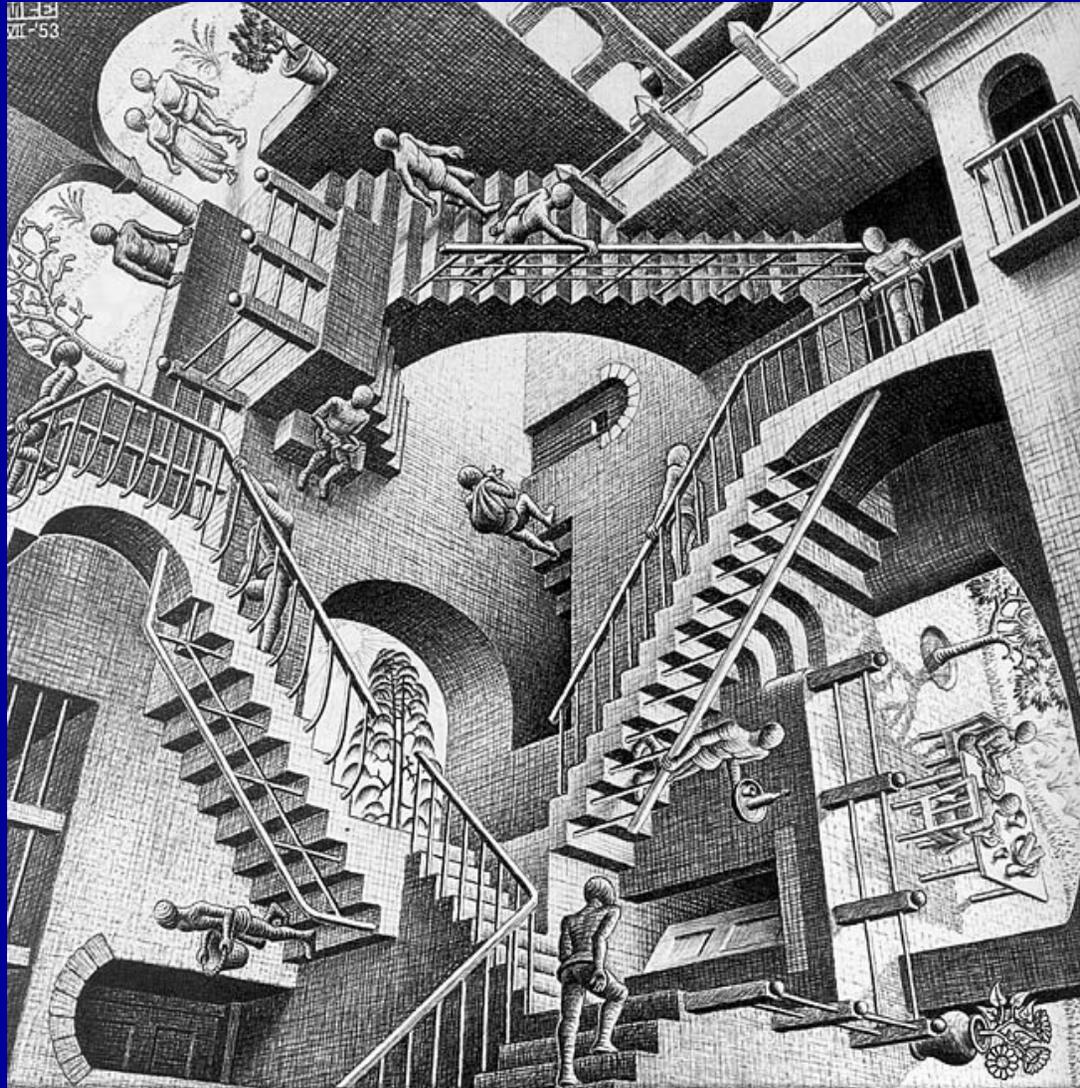
8. The largest of the Rocs picks her up by the skirt.

An ambiguous image by Gustave Verbeek



The Schröder Stairs is a variation on the spontaneous perspective reversal illusion. In this illustration, the stairs will turn upside down during a steady gaze. The wall with the glass bead will shift from the foreground to the background or visa versa. The Schroeder stairs appear in M. C. Escher's works "Relativity" and "Convex and Concave"

Maurits Cornelis Escher "Relativity"

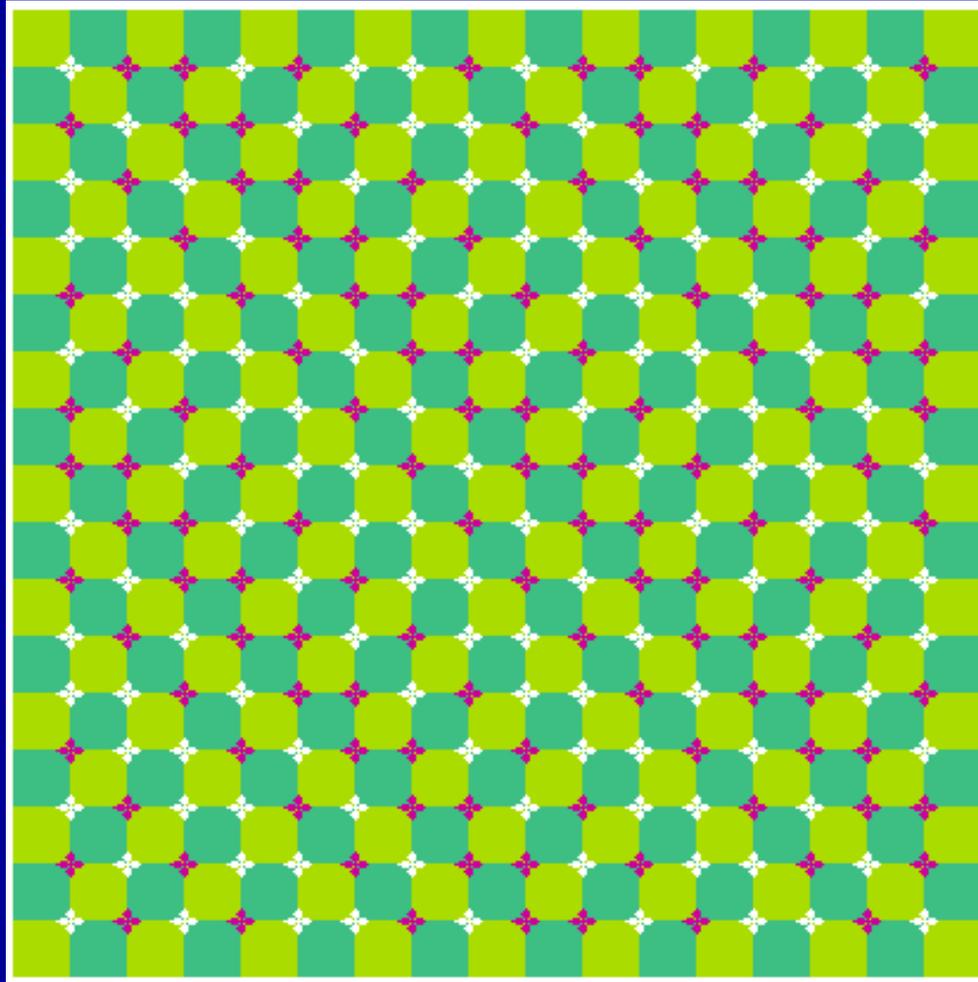


1953, lithograph, 11
1/8 x 11 5/8 inches
(282 x 294 mm),
National Gallery of
Art, Washington D.C.

Context!



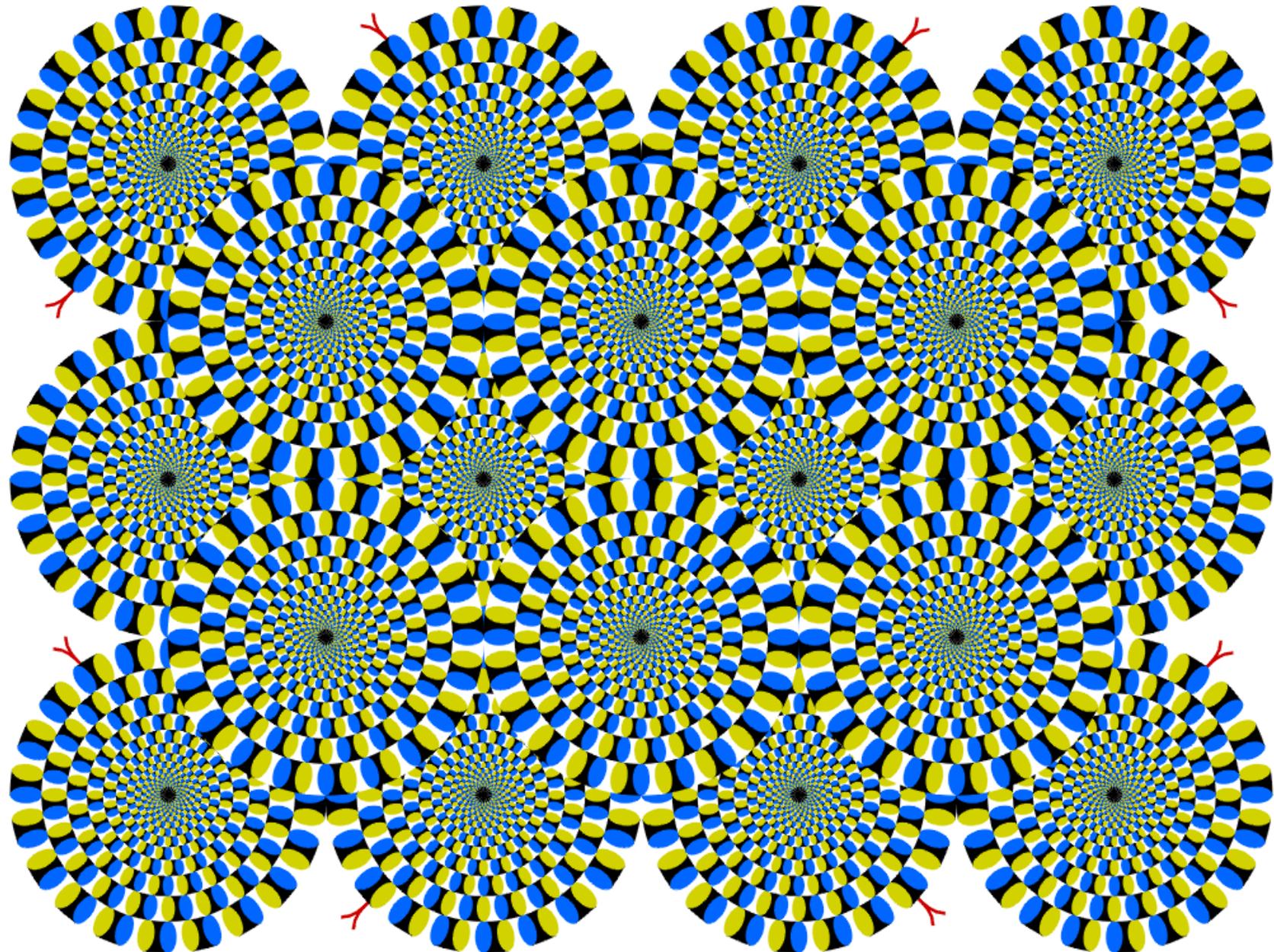
Primrose's field by Akiyoshi Kitaoka



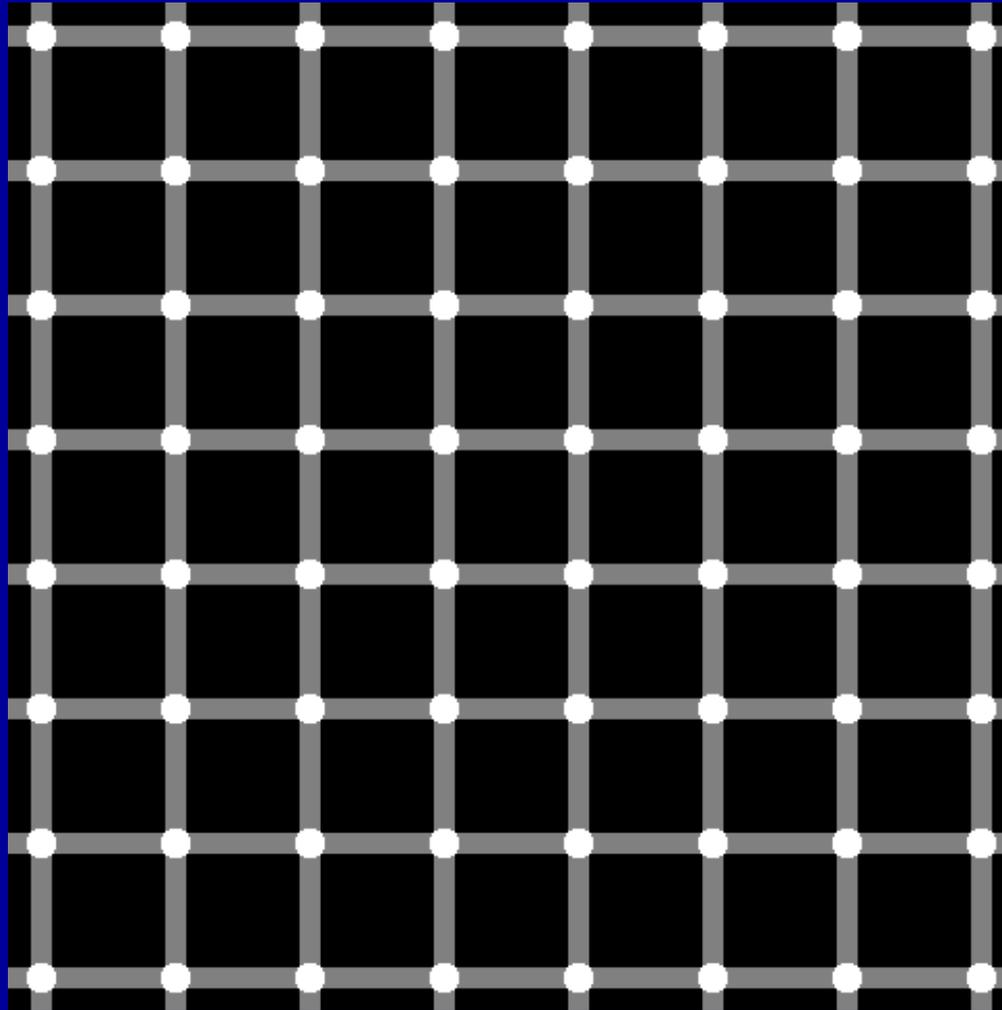
Department of
Psychology,
Ritsumeikan
University, Kyoto,

More can be found at <http://www.ritsumei.ac.jp/~akitaoka/motion-e.html>
and <http://www.ritsumei.ac.jp/~akitaoka/index-e.html>

Rotating snakes by Akiyoshi Kitaoka

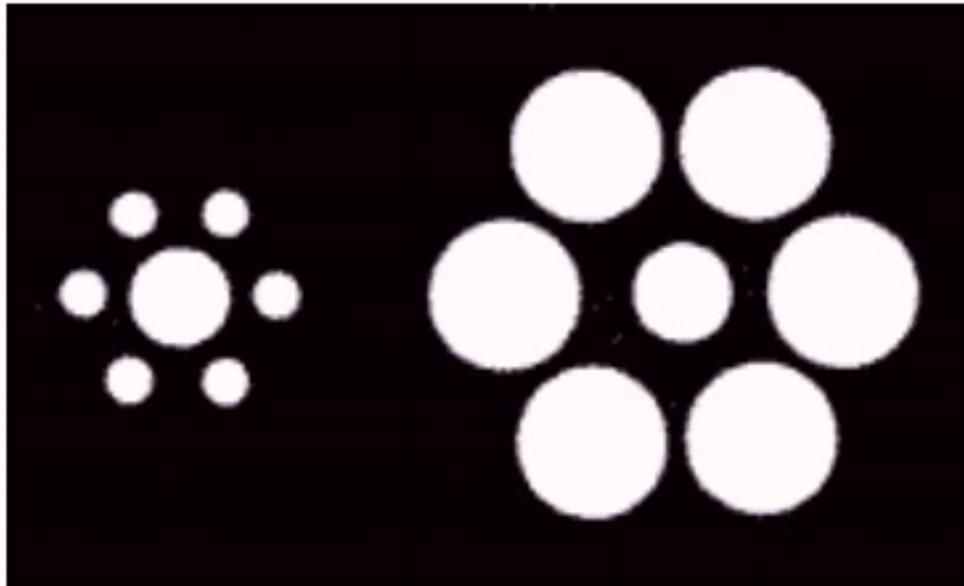


Scintillating grid



Count the black dots!

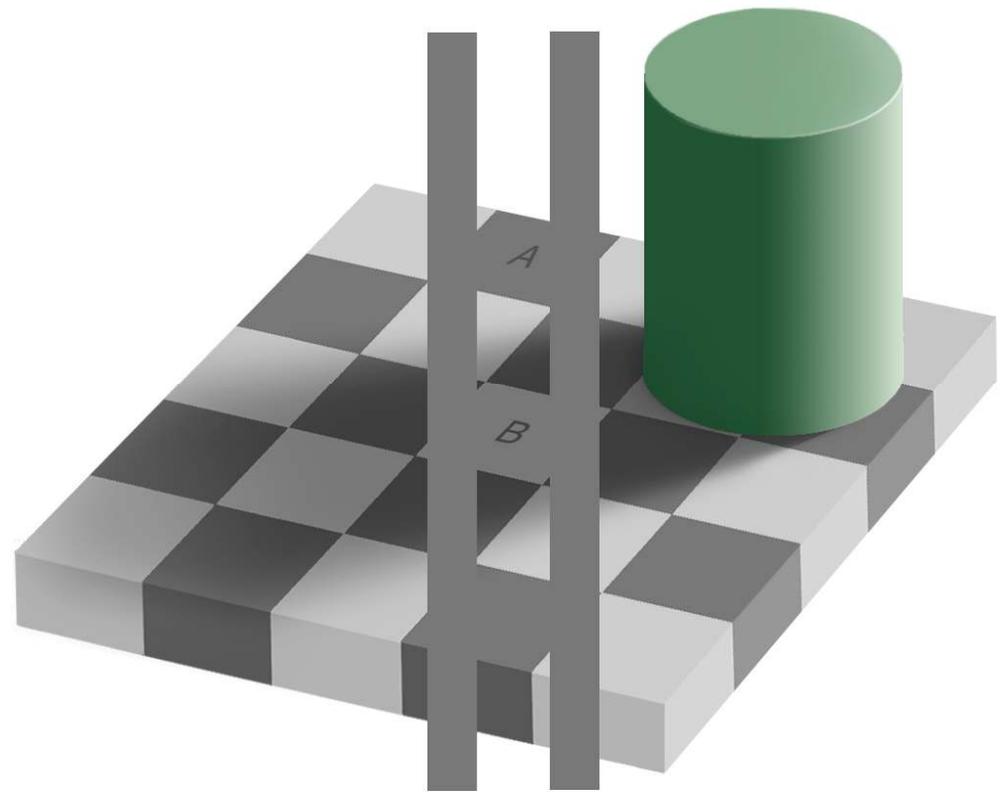
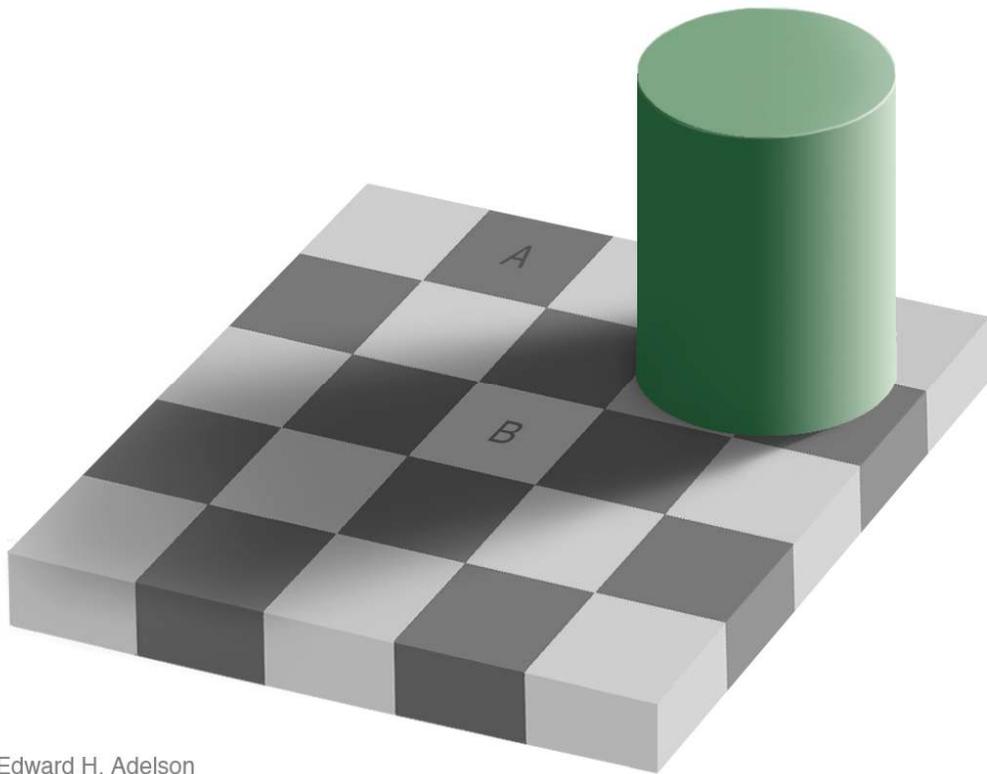
Is the left center circle bigger?



No, they're both the same size



Adelson Illusion

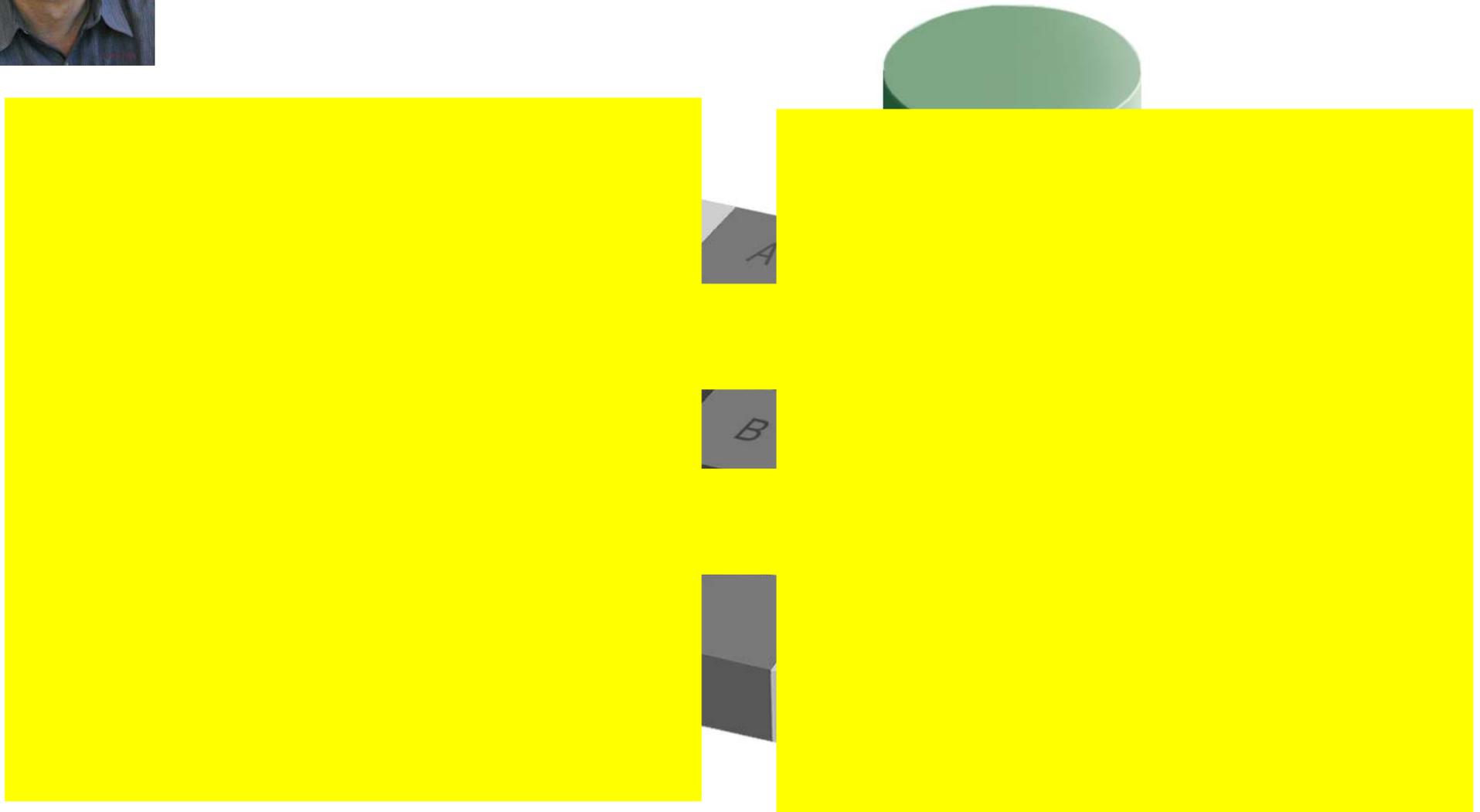


Edward H. Adelson

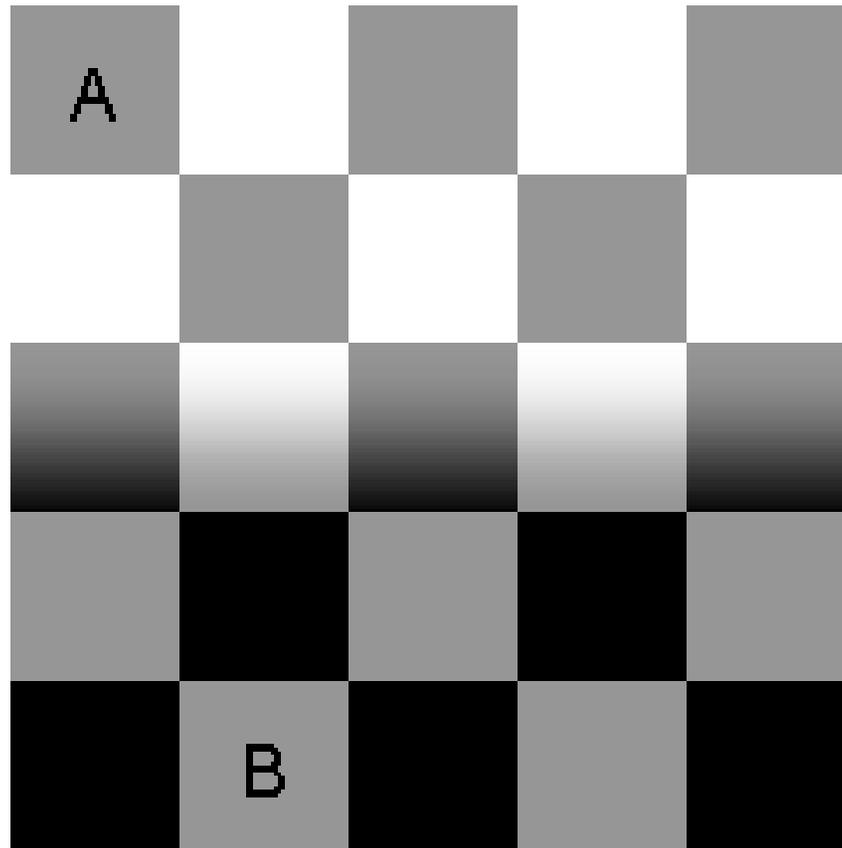
How different are blocks A and B?



Adelson Illusion



Still don't believe?



$$A = B$$

Modified from the checker-shadow illusion
proposed by E. H. Adelson (1995)

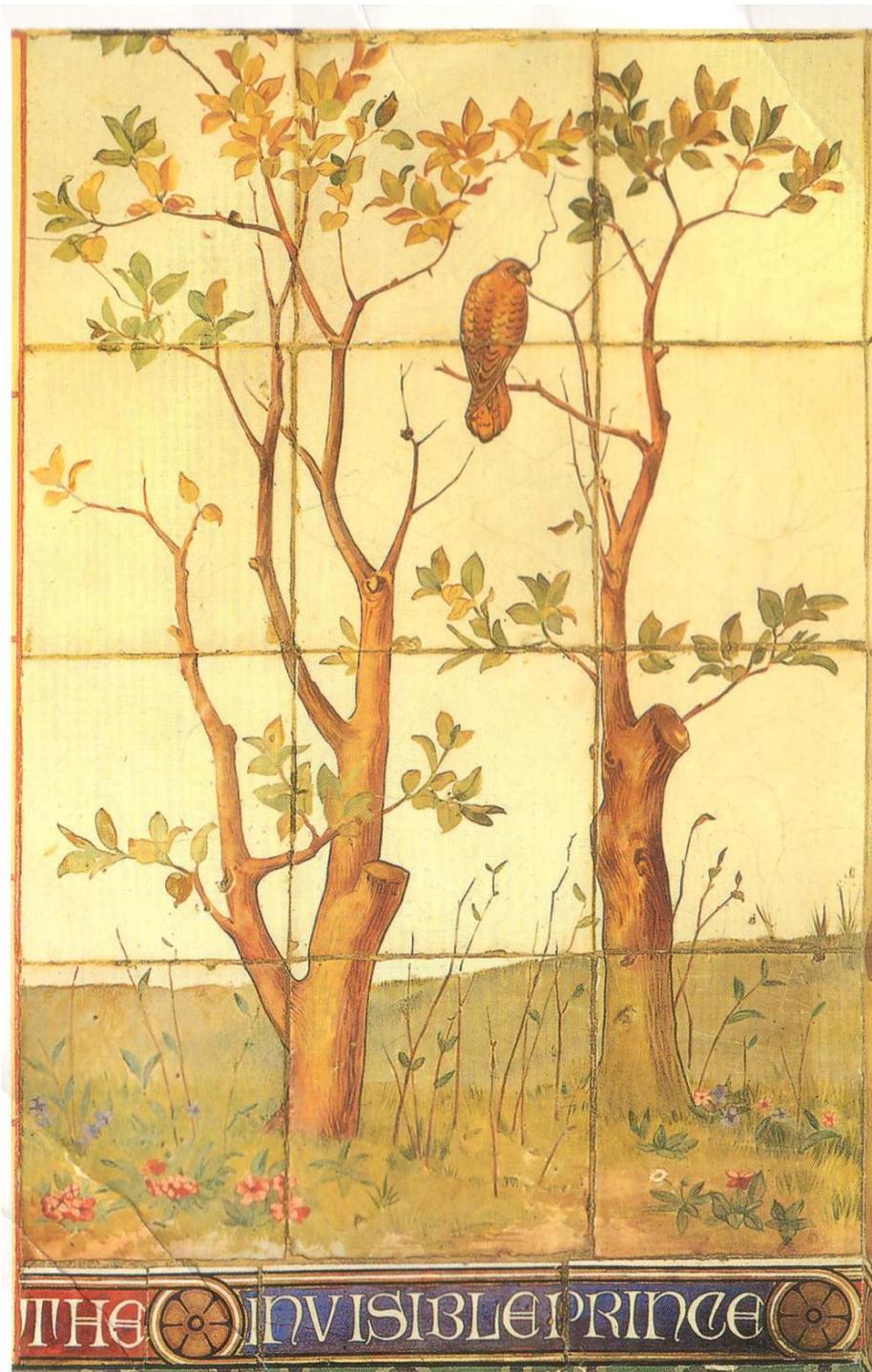
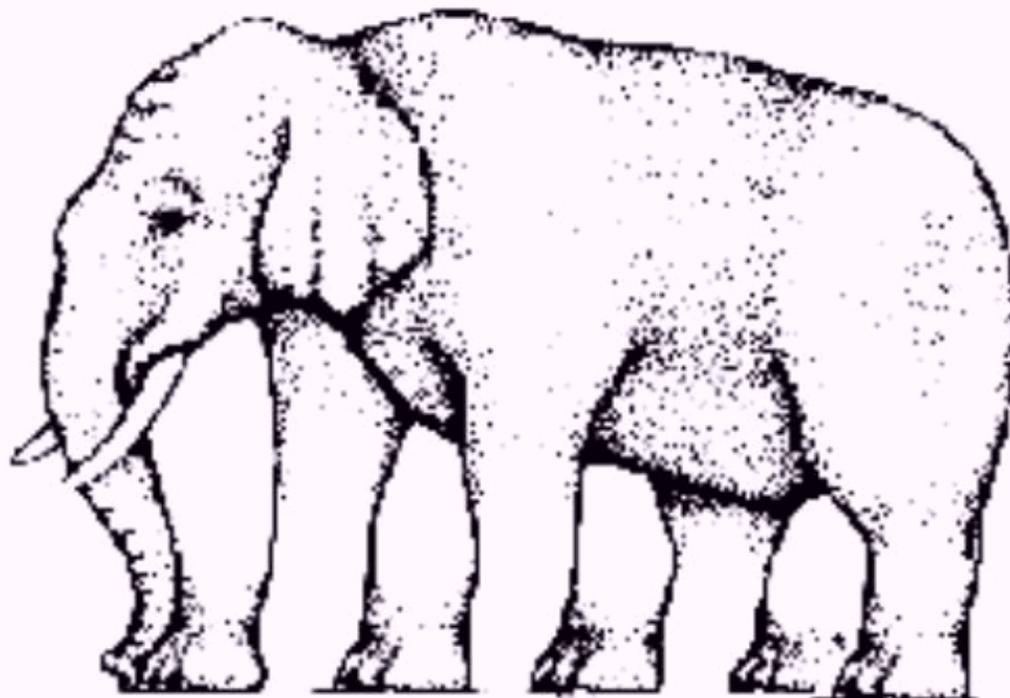


Image from Cardiff
Castle, Cardiff, Wales

Find the “man”



How many legs does this elephant have?



Do you see the face? Or an Eskimo?

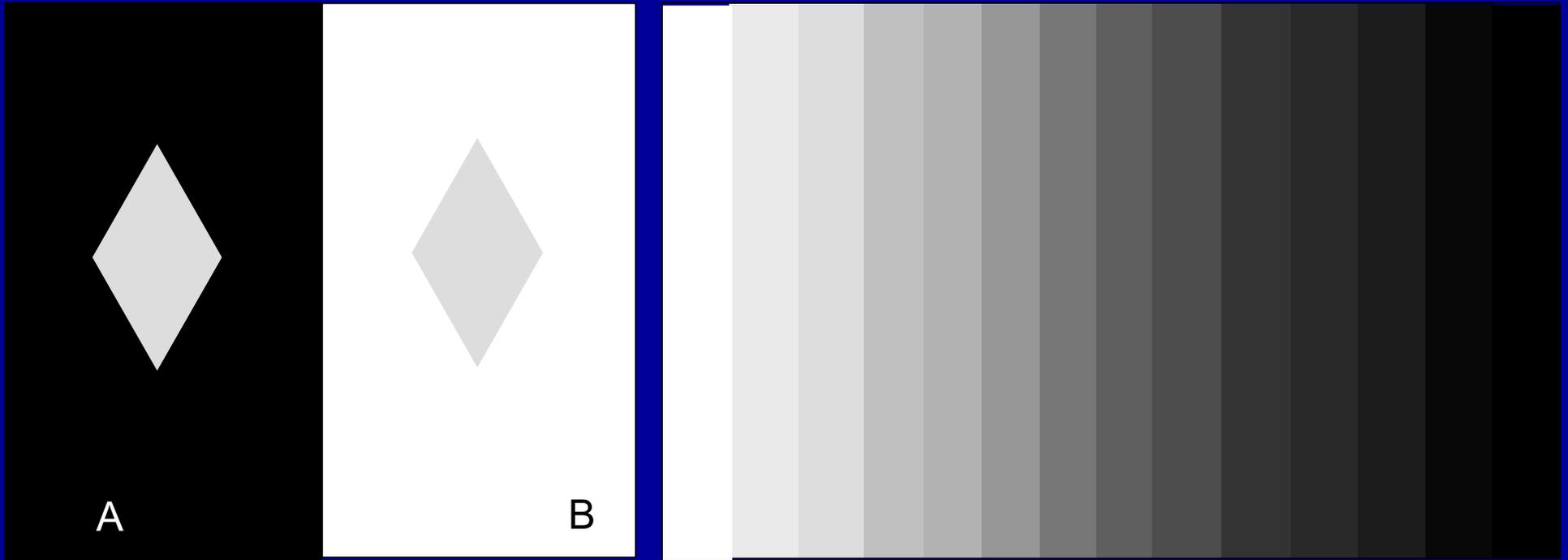
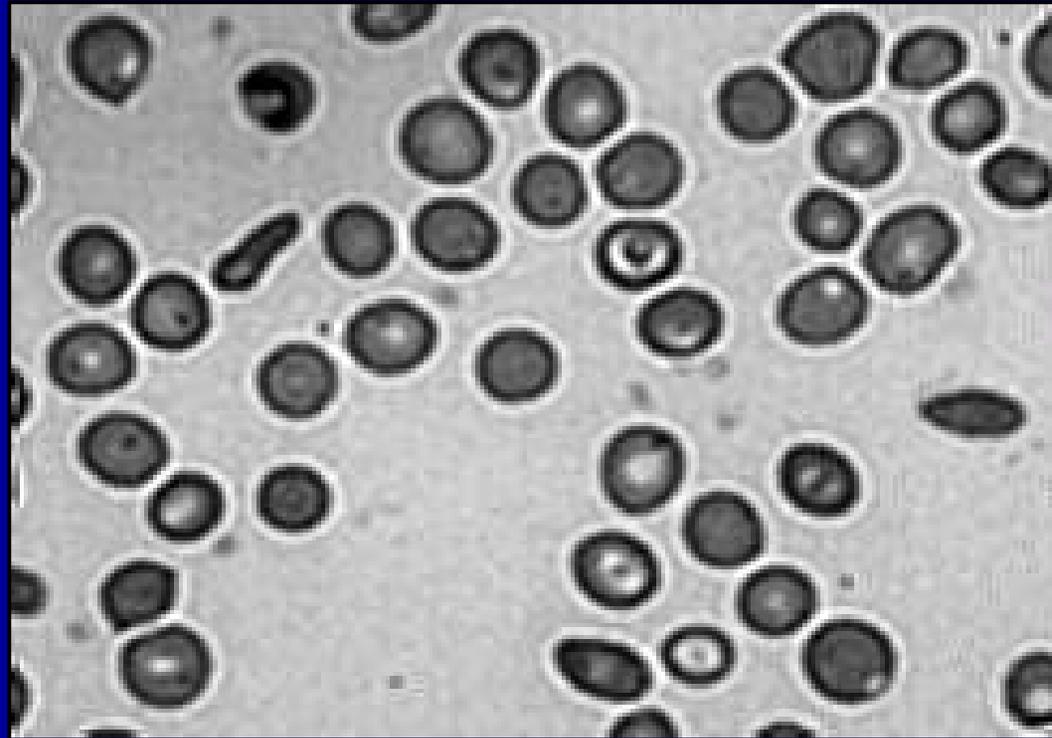


Figure 1. Simultaneous contrast illusion and the Mach band effect

Image Processing and Analysis

Acquire digital image: CCD camera/Scanner



Digital images

- a 2D structure comprised of pixels
- a “matrix” composed of n rows and m columns where each pixel location is denoted by the index (i,j)

for: $0 \leq i < n$ and $0 \leq j < m$

$0,0$	$0,1$	\dots	$0,m$
$1,0$	$1,1$	\dots	$1,m$
\vdots	\vdots	\ddots	\vdots
$n,0$	$n,1$	\dots	n,m

Resolution

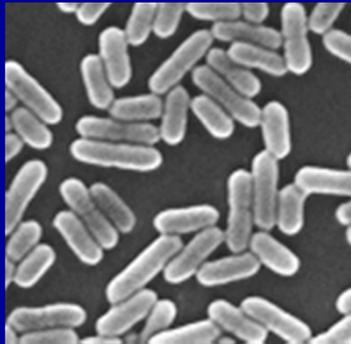
Microscopy

- Rayleigh criterion
- Sparrow criterion
- FWHM

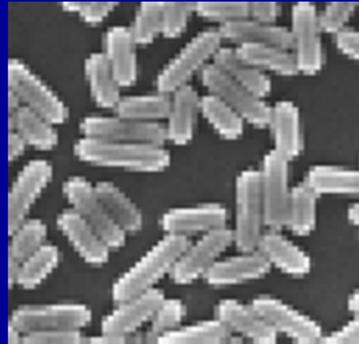
Digital image processing

The spatial resolution of an image is the physical size of a pixel in an image

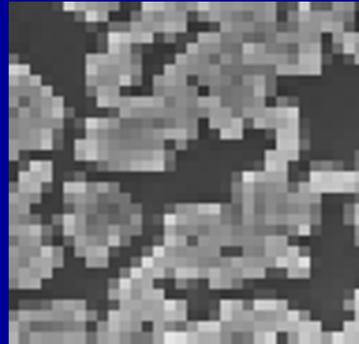
Digital resolution (cont.)



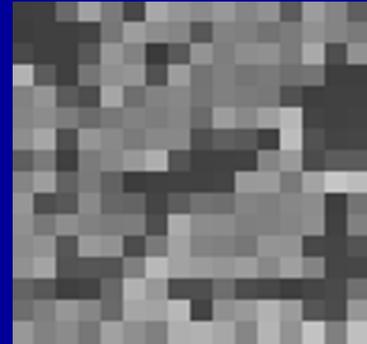
128x128



64x64



32x32



16x16

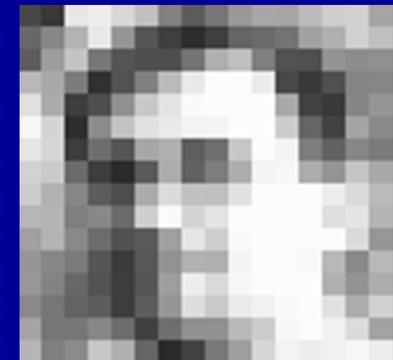
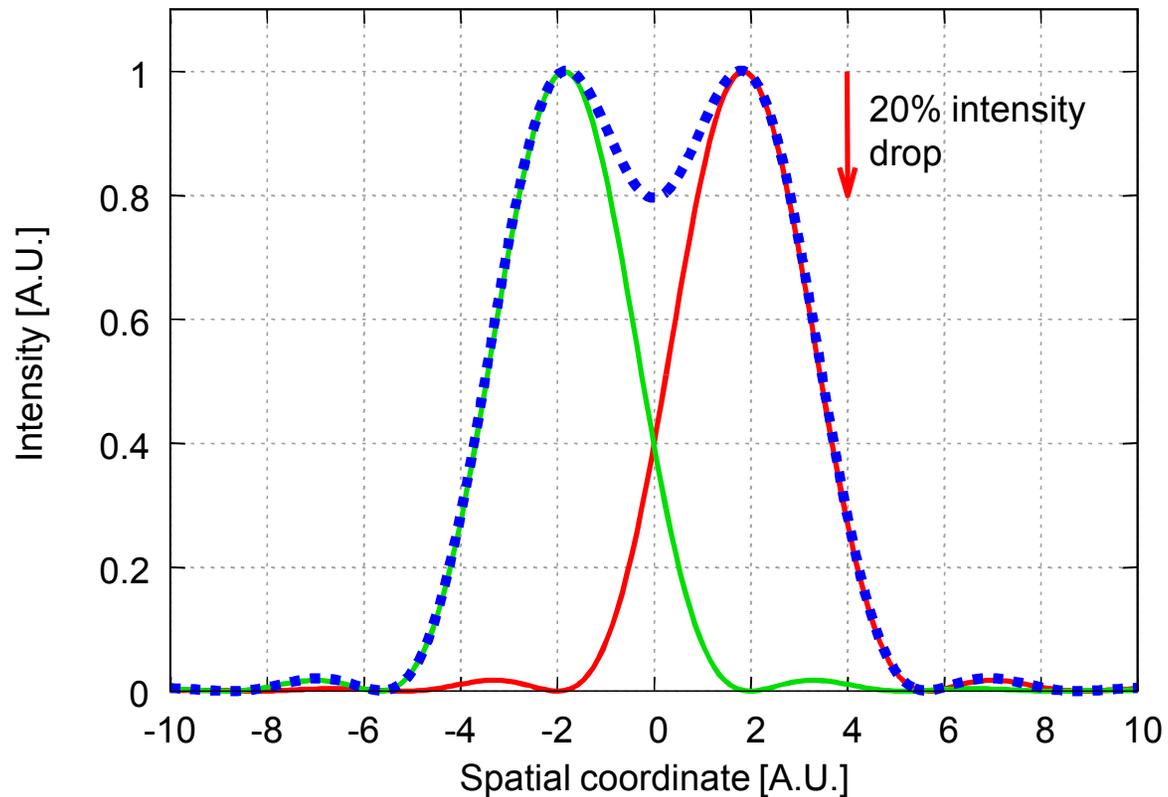


Image resolution in electronic publishing

- **Image resolution** is measured in pixels per inch (ppi).
- An image with a resolution of 100 ppi, contains 10,000 pixels in a square inch (100 pixels wide x 100 pixels high = 10,000).
- The higher the resolution, the more pixels in the image. A 2-by-2-inch image with a resolution of 100 ppi would have 40000 pixels.
- The same image with a resolution of 300 ppi would have 360,000 pixels in the same 2-by-2-inch area.
- To perform image analysis it is necessary that the features of interest are captured so that the measurements are performed on images that are not “**pixelated**”.
- A round object when pixelated will have jagged edges.

Resolution



The **Rayleigh criterion** is the generally accepted, although arbitrary, criterion for the minimum resolvable detail – the imaging process is said to be diffraction-limited when the first diffraction minimum of the image of one source point coincides with the maximum of another.

A little bit of theory: PSF

The point spread function (PSF) describes 3-D light distribution in an image of a point source (for a given lens). An x-y slice through the center of the wide-field point spread function reveals a set of concentric rings: the so-called Airy disk that is commonly referenced in texts on classical optical microscopy.

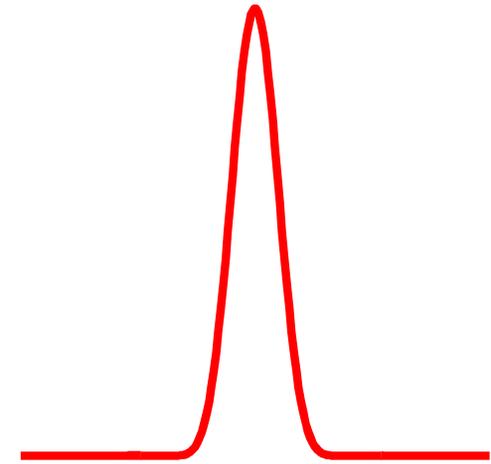
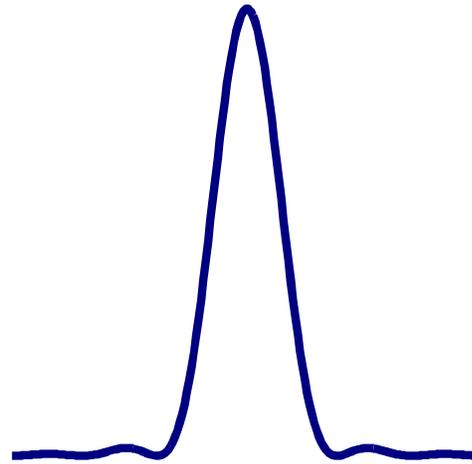
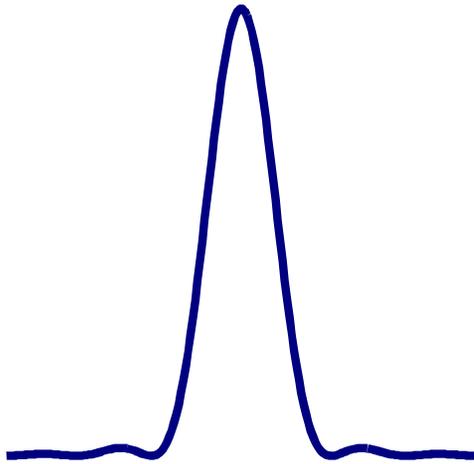
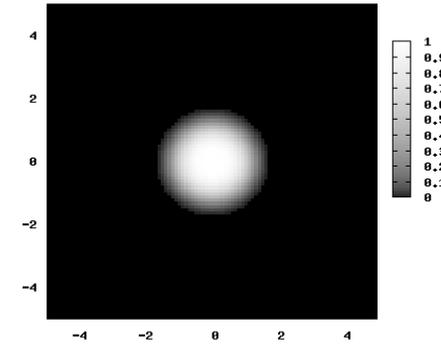
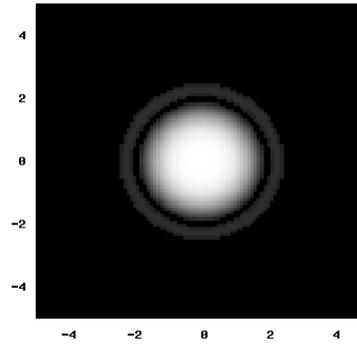
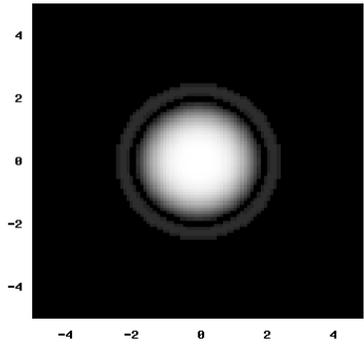
Optical units:

$$v = d \cdot \frac{2\pi}{\lambda} \sin \theta \qquad u = z \cdot \frac{2\pi}{\lambda} \sin^2 \theta$$

$$I(0, v) \propto \left| \frac{2J_1(v)}{v} \right|^2 \qquad I(u, 0) \propto \left(\frac{\sin(u/4)}{u/4} \right)^2$$

Lateral and axial intensity distributions

Confocal PSF



PSF_{exc}

•

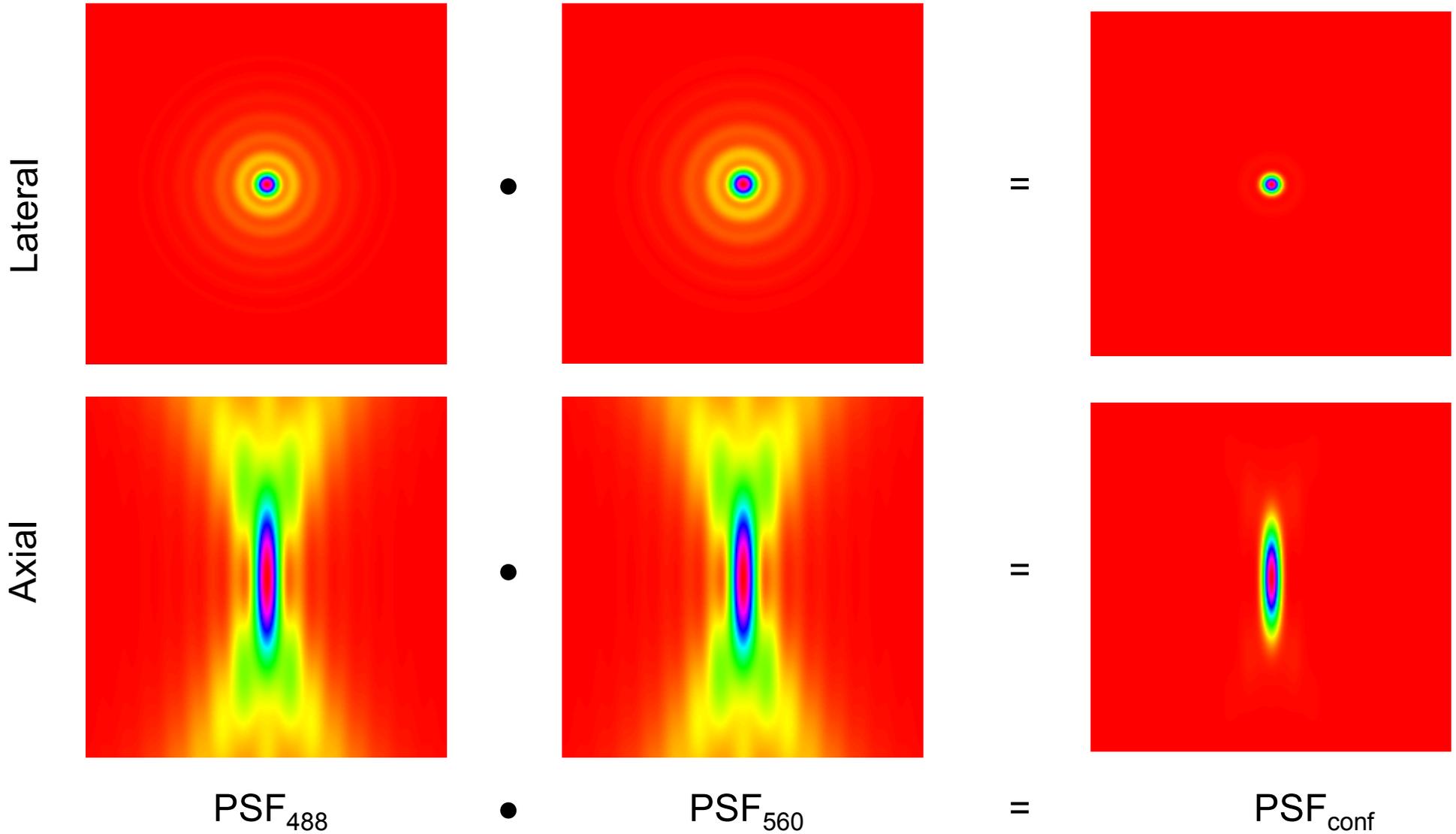
PSF_{det}

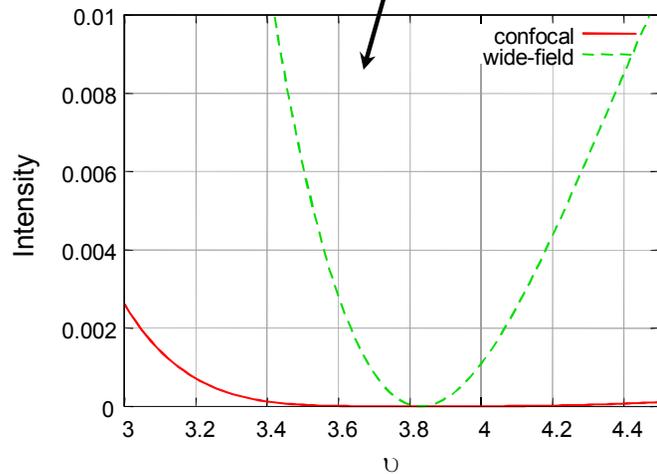
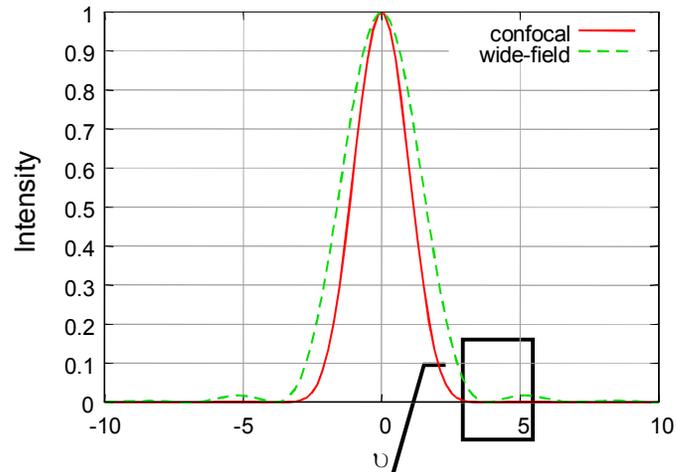
=

PSF_{conf}

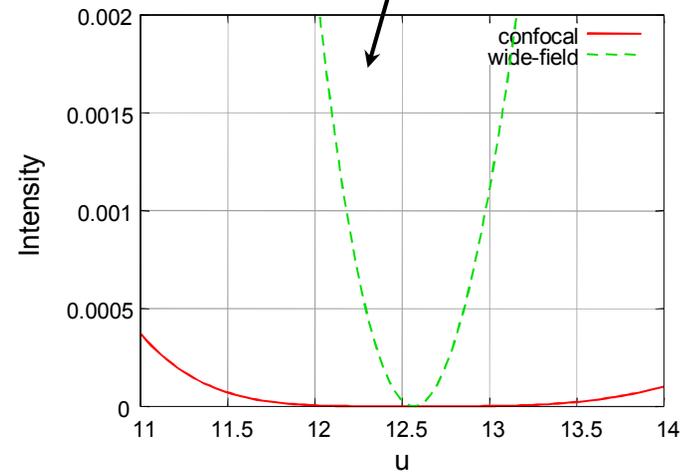
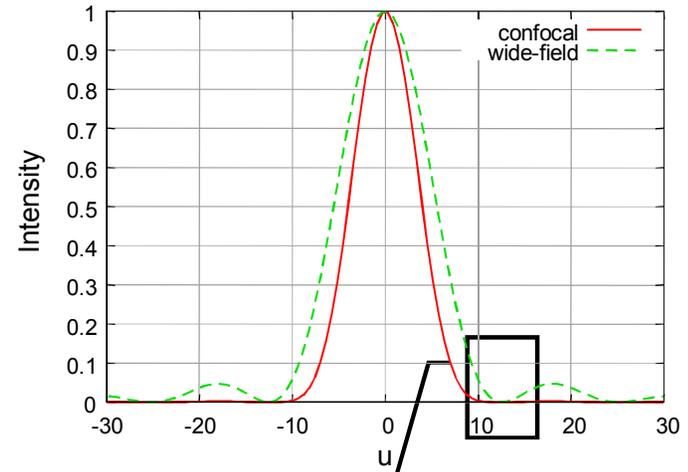
$n = 1$
 $NA = 0.6$

Confocal PSF – example



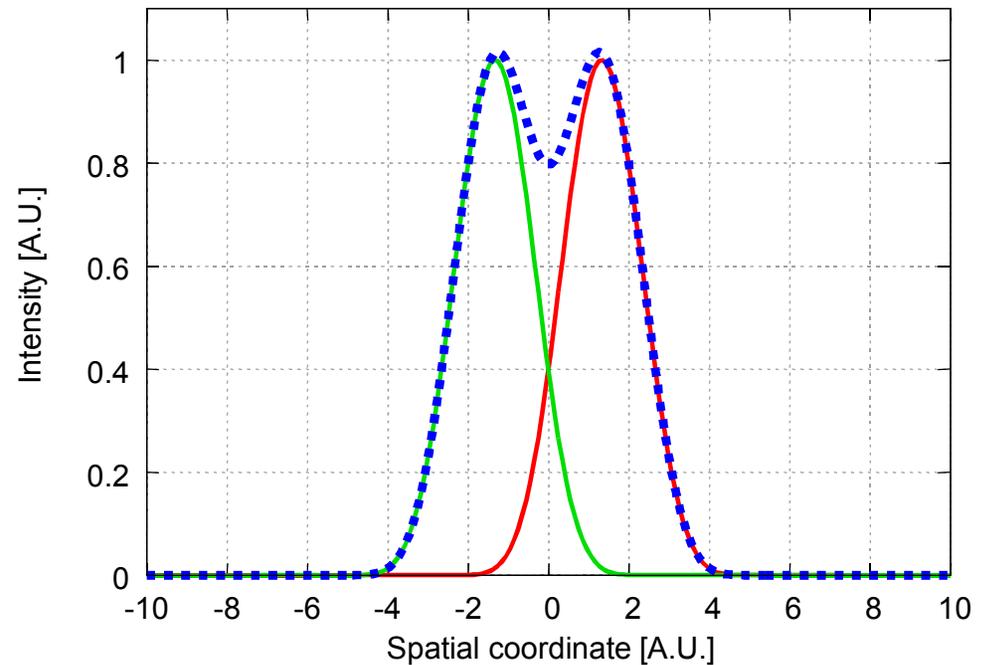
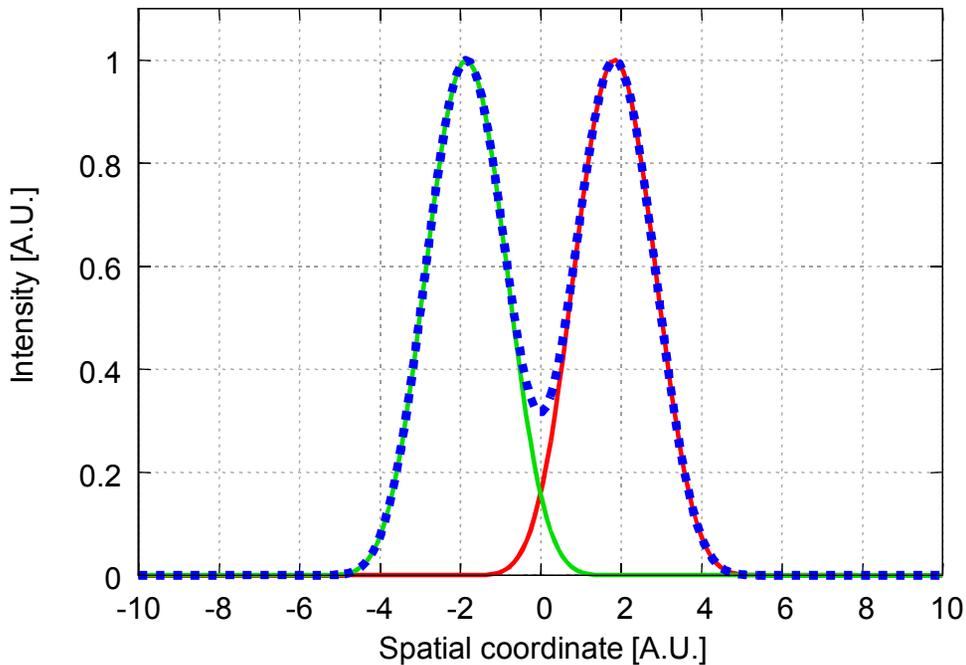


$$r_0 = \frac{0.61 \cdot \lambda}{n \sin \theta} = \frac{0.61 \cdot \lambda}{\text{NA}}$$



$$z_0 = \frac{2 \cdot \lambda n}{(\text{NA})^2}$$

Resolution – confocal microscope



Rayleigh criterion cannot be used directly to define the improvement of resolution in a confocal microscope. The position of the first minimum does not change. The drop in intensity is much larger, though. In fact, we can move two intensity distributions a little bit closer (1.4 times), and still get the required 20% drop in intensity.

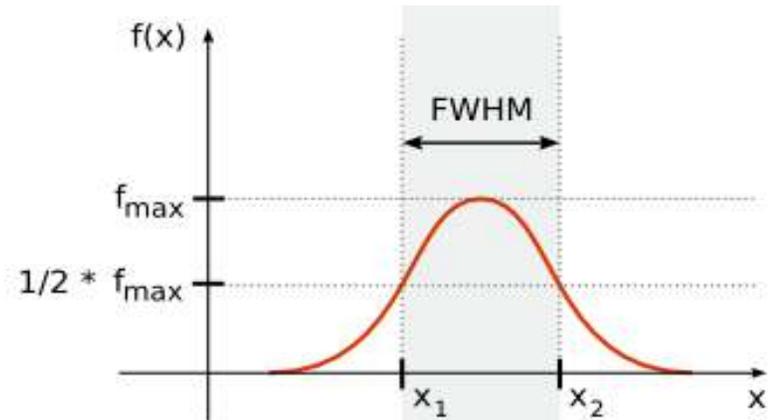
Resolution – FWHM

$$\text{FWHM}_{conf}^r \approx \frac{0.61 \cdot \lambda}{\sqrt{2} \cdot \text{NA}}$$

$$\text{FWHM}_{conf}^r \approx \frac{1}{\sqrt{1 + \left(\frac{\lambda_{exc}}{\lambda_{det}}\right)^2}} \frac{0.61 \cdot \lambda_{exc}}{\text{NA}}$$

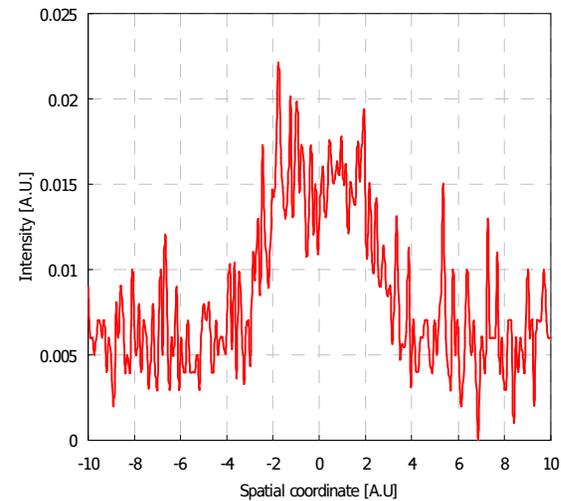
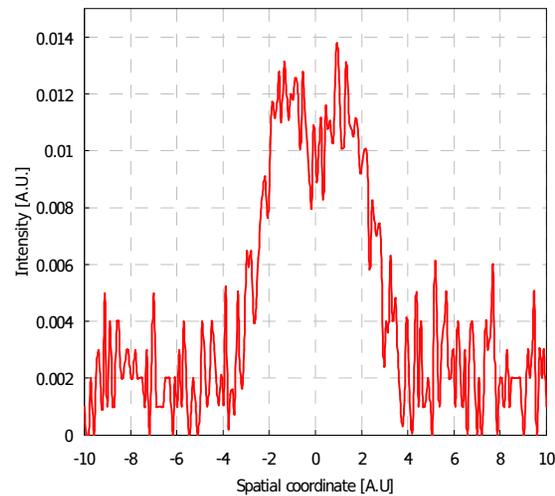
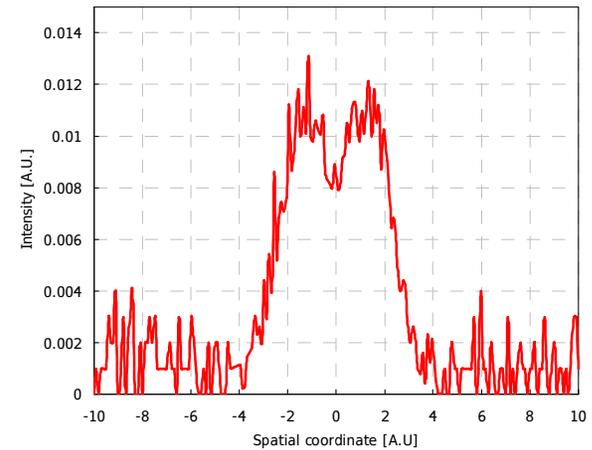
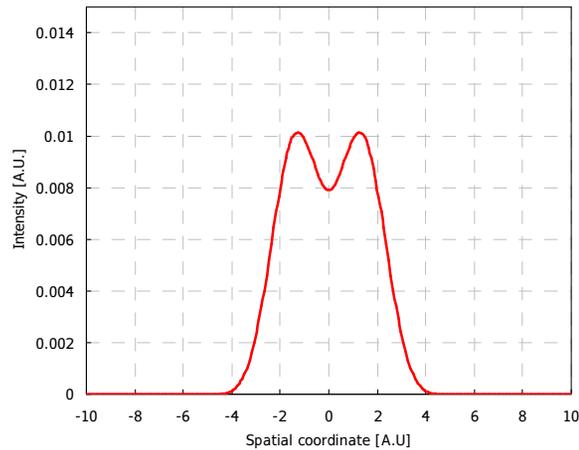
$$\text{FWHM}_{conf}^z = \frac{2 \cdot \lambda n}{\sqrt{2} \cdot (\text{NA})^2}$$

$$\text{FWHM}_{conf}^z = \frac{1}{\sqrt{1 - \left(\frac{\lambda_{exc}}{\lambda_{det}}\right)^2}} \frac{2 \cdot \lambda_{exc} n}{(\text{NA})^2}$$



The full width at half maximum (FWHM) is a parameter commonly used to describe the width of a "bump" on a curve or function. It is given by the distance between points on the curve at which the function reaches half its maximum value.

Signal-to-noise ratio and resolution



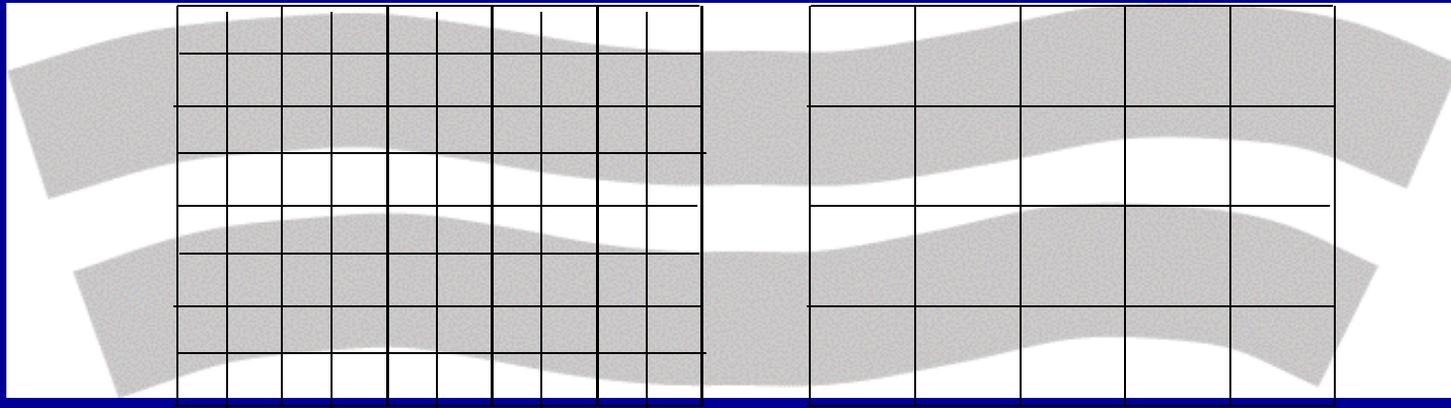
The influence of Poisson noise on two intensity distributions separated spatially according to the Rayleigh criterion.

”What dpi do I need to preserve all of the detail in my image”?

In practice, the dpi necessary to preserve image detail can be quickly determined empirically. The sampling rate (dpi) that is used will depend upon how the image is going to be displayed. If a printed copy of an image is going to be made, there is little value in sampling with a dpi greater than the dpi capability of the printer. An exception to this is if the image is going to be magnified or enlarged in a manner analogous to optical photographic enlargement prior to printing.

cont...

An image 3 x 4 inches that is digitized at a rate of 600 dpi will only be 300 dpi if enlarged 2X and printed. Therefore, a practical sampling guideline for printed images is that the final image should have at least the resolution of the printing device. For light and electron micrographs of biological tissue, prints with a final resolution of 600 dpi will preserve most detail. However, fine granular structures such as autoradiography silver grains or colloidal gold particles used in immunolabeling will not be as sharp as those in a traditional photograph. For very fine granular structures, the final print resolution may need to be 1200 dpi or higher.



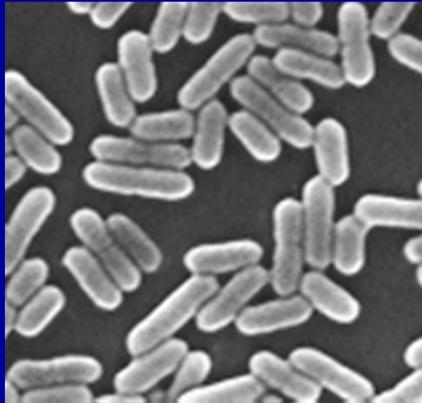
Bit resolution or pixel depth

- This is a measurement of the number of **bits of information** per pixel.
- The pixel depth will determine how much color or gray scale information is available for each pixel.
- Greater pixel depth means more available colors and more accurate color representation.
- Pixels in binary images have a depth of 1 (on or off), and are black and white images
- A pixel with a bit depth of 8 has 2^8 , or 256, possible values; and a pixel with a bit depth of 24 has 2^{24} , or 16 million possible values (Red=8, Green=8, Blue=8).
- Common values for pixel depth range from 1 to 24 bits per pixel.

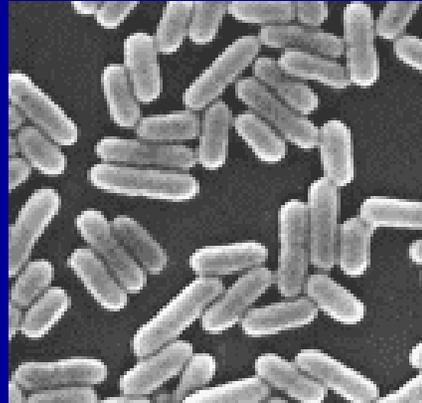
Quantization

- A set of n quantization levels comprises the integers: $0, 1, 2, \dots, n-1$
- 0 and $n-1$ are usually black and white respectively, with intermediate levels rendered in various shades of gray.
- Quantization levels are commonly referred to as **gray levels**.
- n is usually an integral power of two: $n=2^b$, where b is the number of bits used for quantization.
- If $b=1$ then the image is **binary**

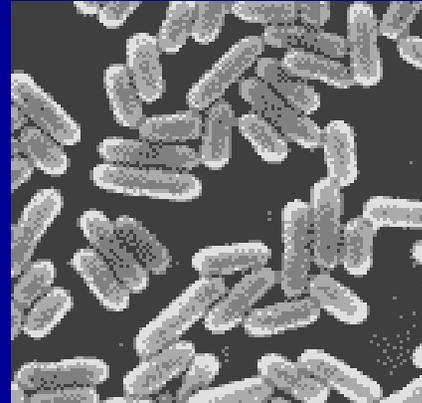
Quantization (cont.)



256



16



4



2



Principles of 2D Image Analysis

- End of Part 1
- Part 2 is the next lecture

