



Confocal Microscopy and Full Field Microscopy

Advanced Fluorescence Microscopy Workshop
August 17th 2015

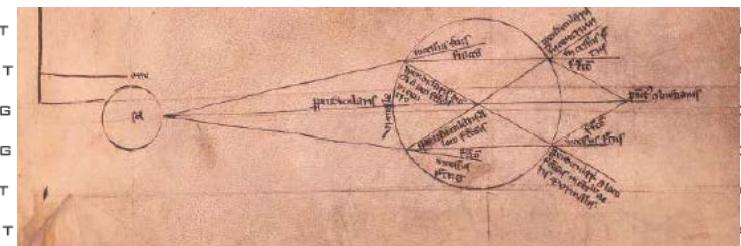
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History

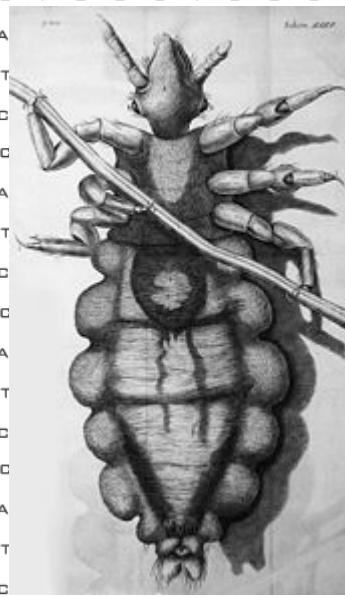
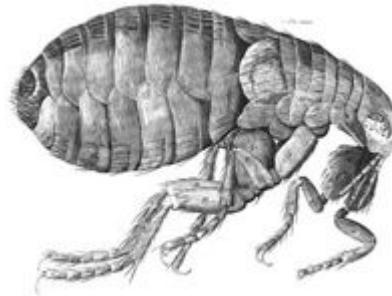
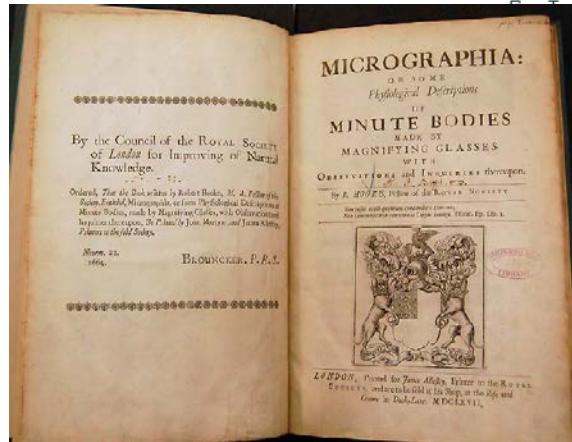
Alhazen Book of Optics 1021



Roger Bacon 1214-1294

Anthony van Leeuwenhoek 1632-1723

Robert Hooke: Micrographia 1665



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History



Galileo 1609



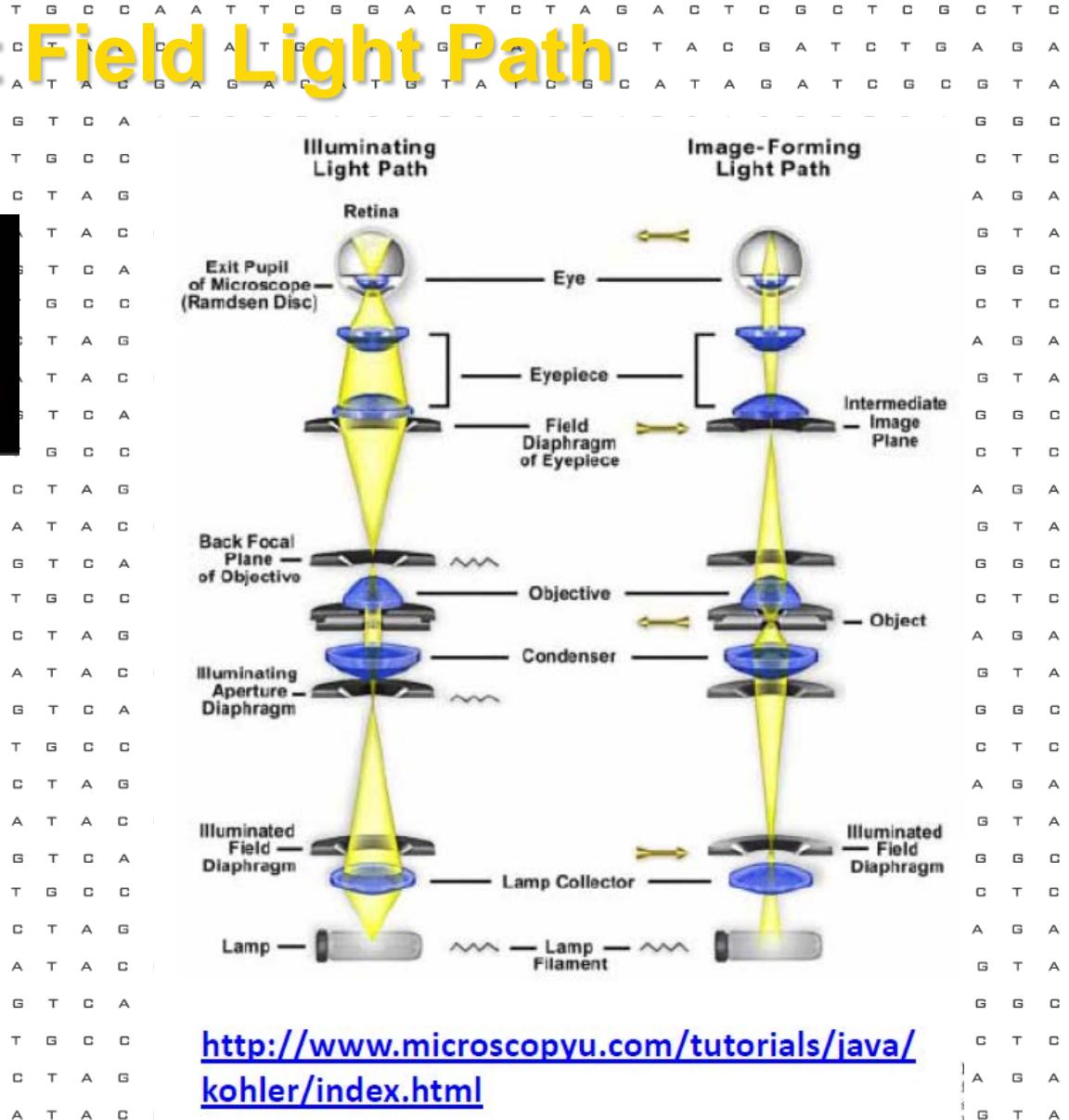
Kepler 1611

The figure consists of two detailed black and white illustrations of microscopes. On the left, labeled 'Fig. 5', is a simple refracting microscope with a single eyepiece lens. On the right, labeled 'Fig. 6', is a more complex compound microscope featuring a eyepiece lens and a objective lens system. Both microscopes are mounted on circular bases with various adjustment knobs and lenses. The background of the entire figure is filled with a grid of DNA sequence data, showing repeating patterns of nucleotide bases (A, T, C, G) across multiple rows.

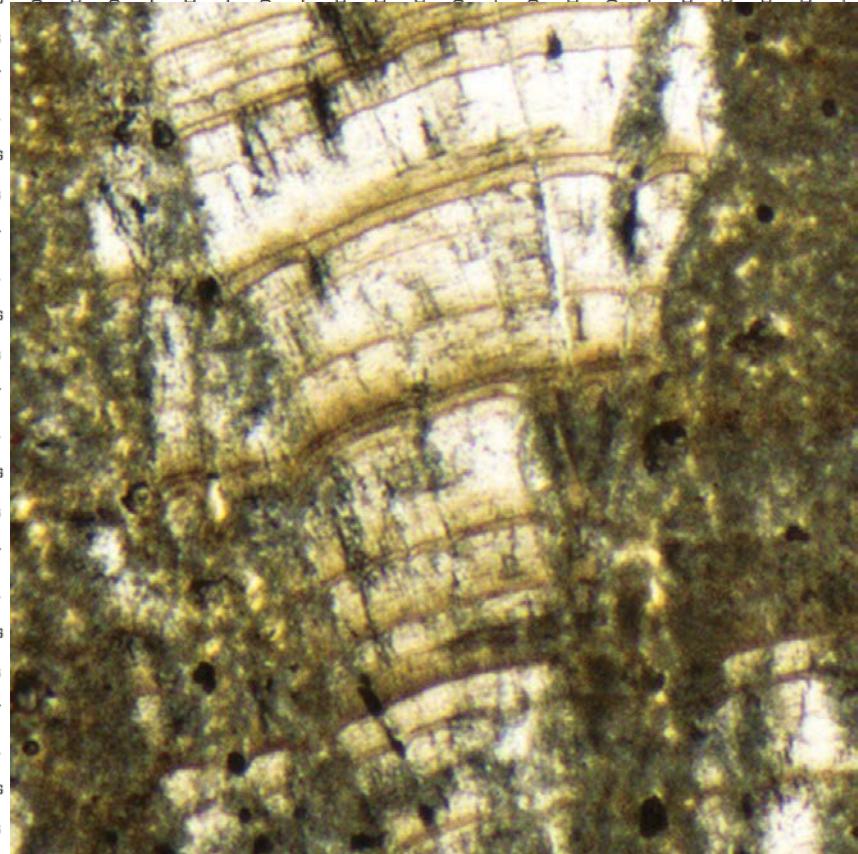


SCIENCEPHOTOLIBRARY

Modern Bright Field Light Path



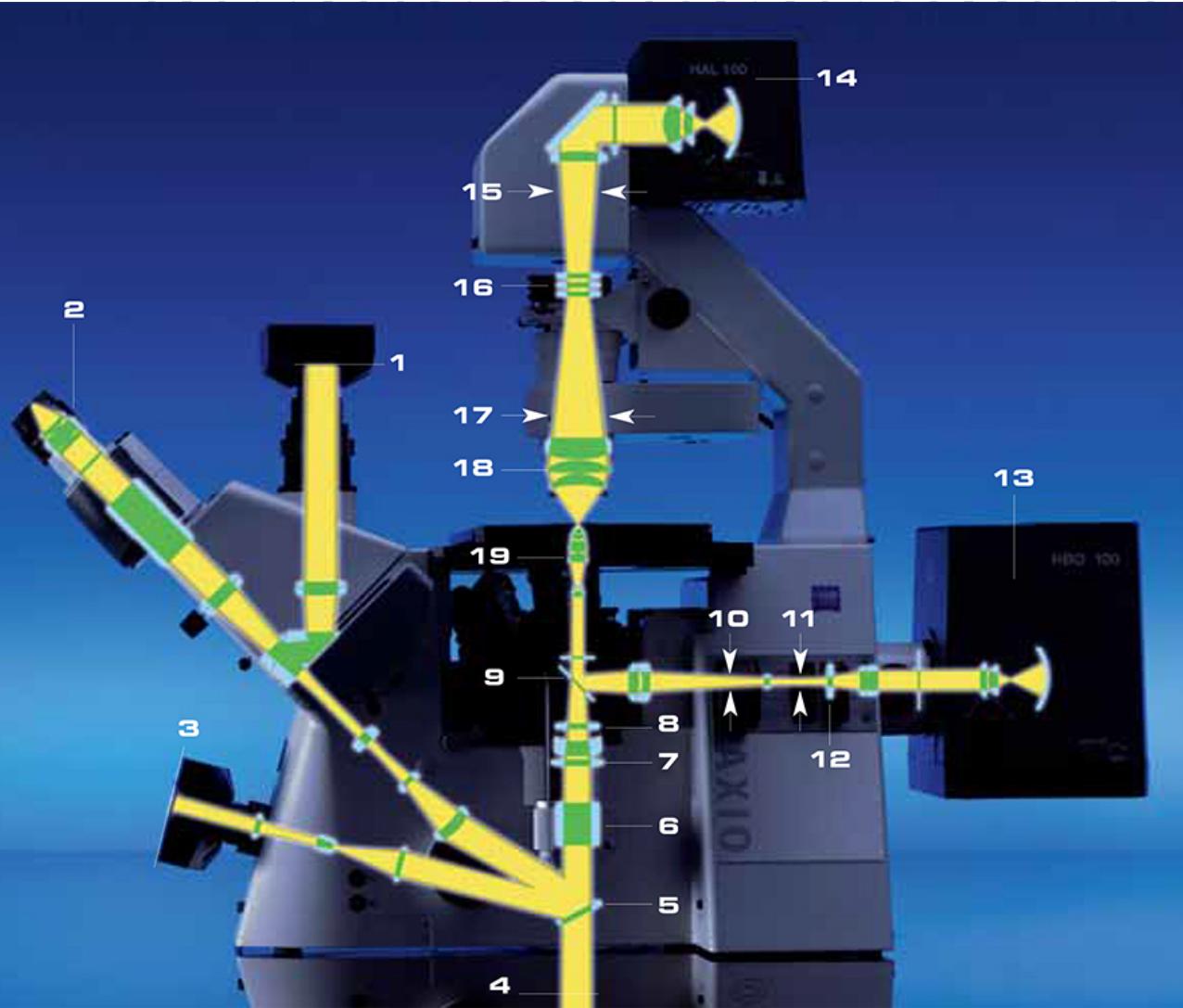
Modern Bright Field Image



The figure consists of two panels. The left panel is a vertical sequence of DNA base pairs, starting with T G C C A A T T C G G A C T C T A G A C T C G C T C G C T C and ending with T G C C A A T T C G G A C T C T A G A C T C G C T C G C T C. The right panel is a square micrograph showing a cross-section of a biological tissue, likely a plant stem or root, exhibiting a distinct radial arrangement of cells and vascular tissue.

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Modern Fluorescence Light Path



Beam path

- 1 Intermediate image plane/phototube
- 2 Eyepiece
- 3 Intermediate image plane/front port
- 4 Intermediate image plane/base port
- 5 Switching beam path between base port/front port/vis. observation
- 6 Side port prisms
- 7 Tube lens
- 8 Analyzer
- 9 Reflector cube
- 10 Field diaphragm
- 11 Aperture stop
- 12 Filter slider
- 13 HBO lamp
- 14 HAL lamp
- 15 Field diaphragm
- 16 Polarizer
- 17 Aperture stop
- 18 Condenser
- 19 Objective

Modern Fluorescence Light Path

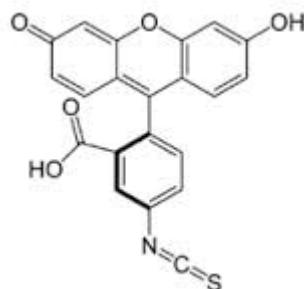
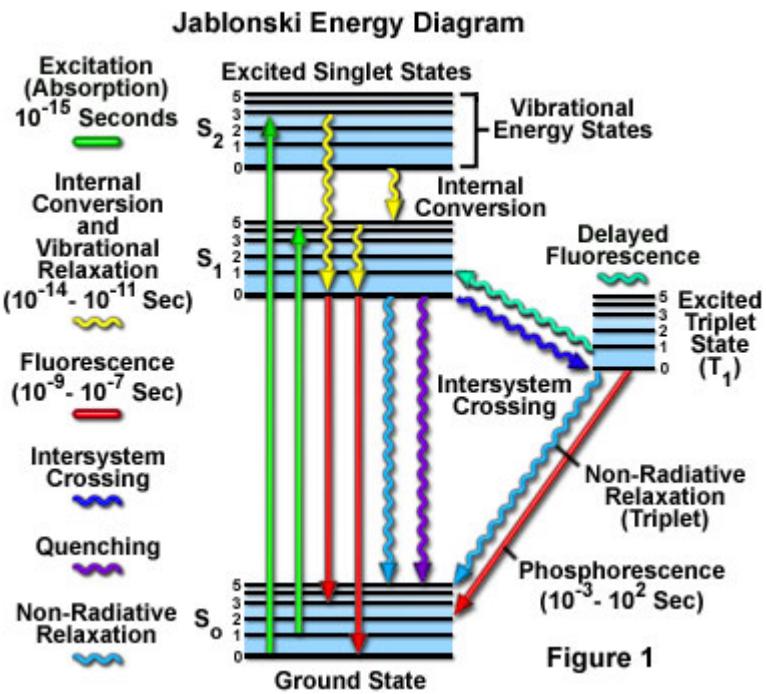


Figure 1



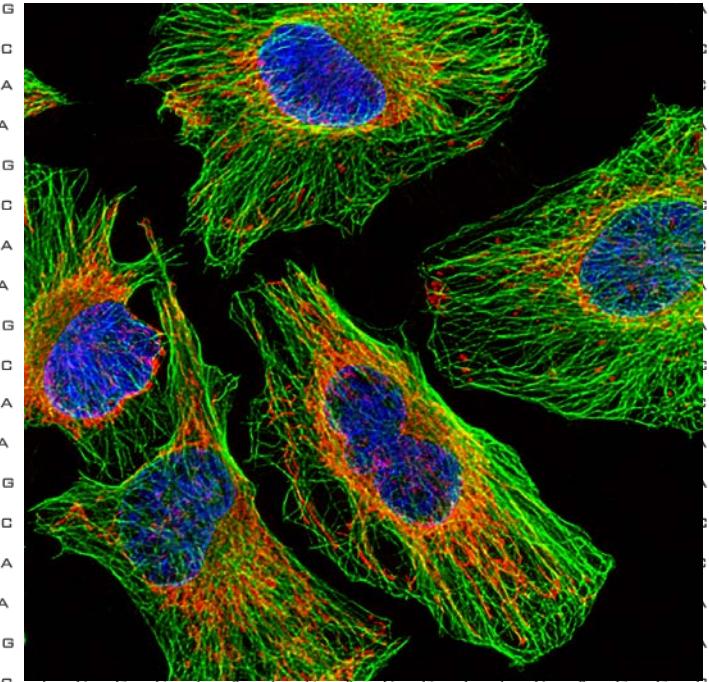
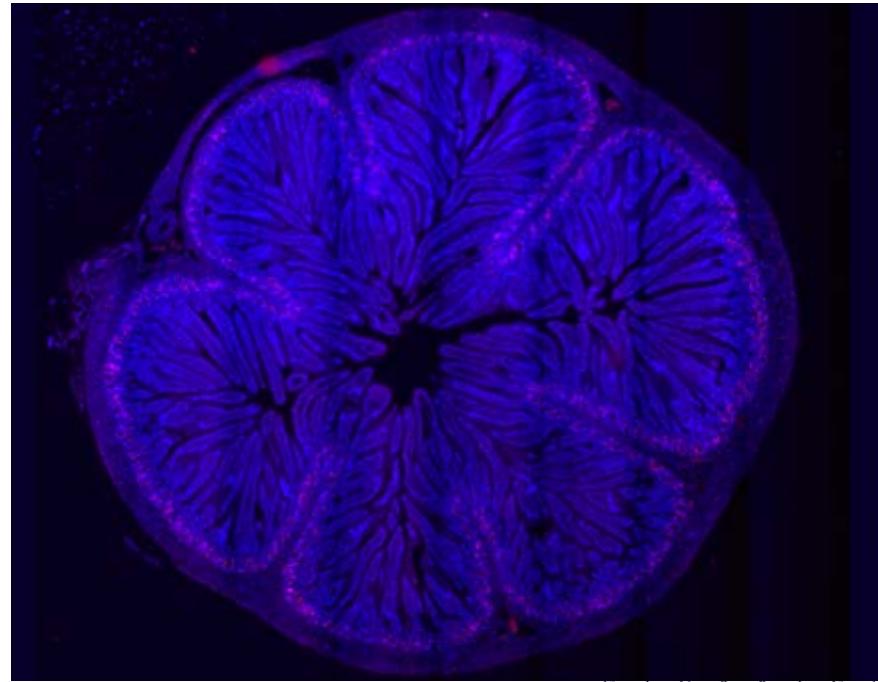
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Modern Fluorescence Image

T G C C A A T T C G G A C T C T A G A C T C G C T C G C T C
T G C C A A T T C G G A C T C T A G A C T C G C T C G C T C
A T A C G A G A C A T G T A T C G C A T A G A T C G C G T A
G T C A A T G C T A G C T C G C T A T C A C G T T T C A G G C
T G C C A A T T C G G A C T C T A G A C T C G C T C G C T C
C T A G C T A T G C T A G G A C T C T A C G A T C T G A G G A



T G C C A A T T C G G A C T C T A G A C T C G C T C G C T C
C T A G C T A T G C T A G G A C T C T A C G A T C T G A G A
A T A C G A G A C A T G T A T C G C A T A G A T C G C G T A
G T C A A T G C T A G G C T C G C T A T C A C G T T T C A G G C
T G C C A A T T C G G A C T C T A G A C T C G C T C G C T C
C T A G C T A T G C T A G G A C T C T A C G A T C T G A G A
A T A C G A G A C A T G T A T C G C A T A G A T C G C G T A

Modern Confocal Light Path

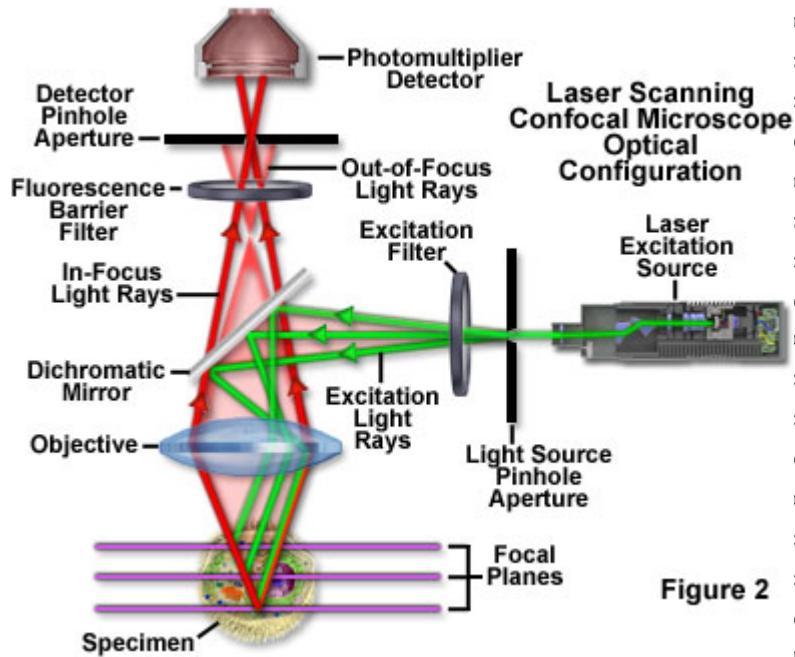
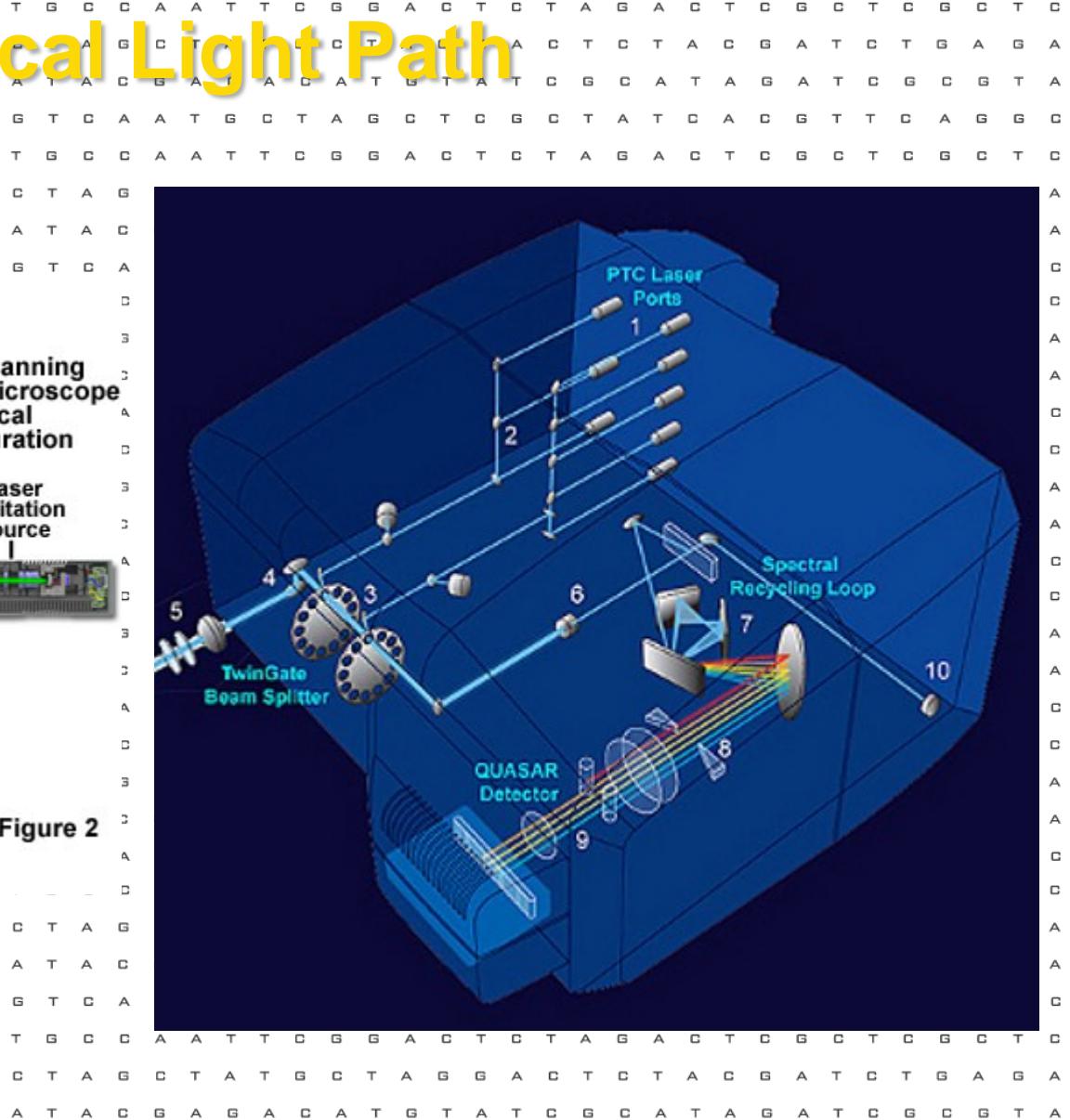
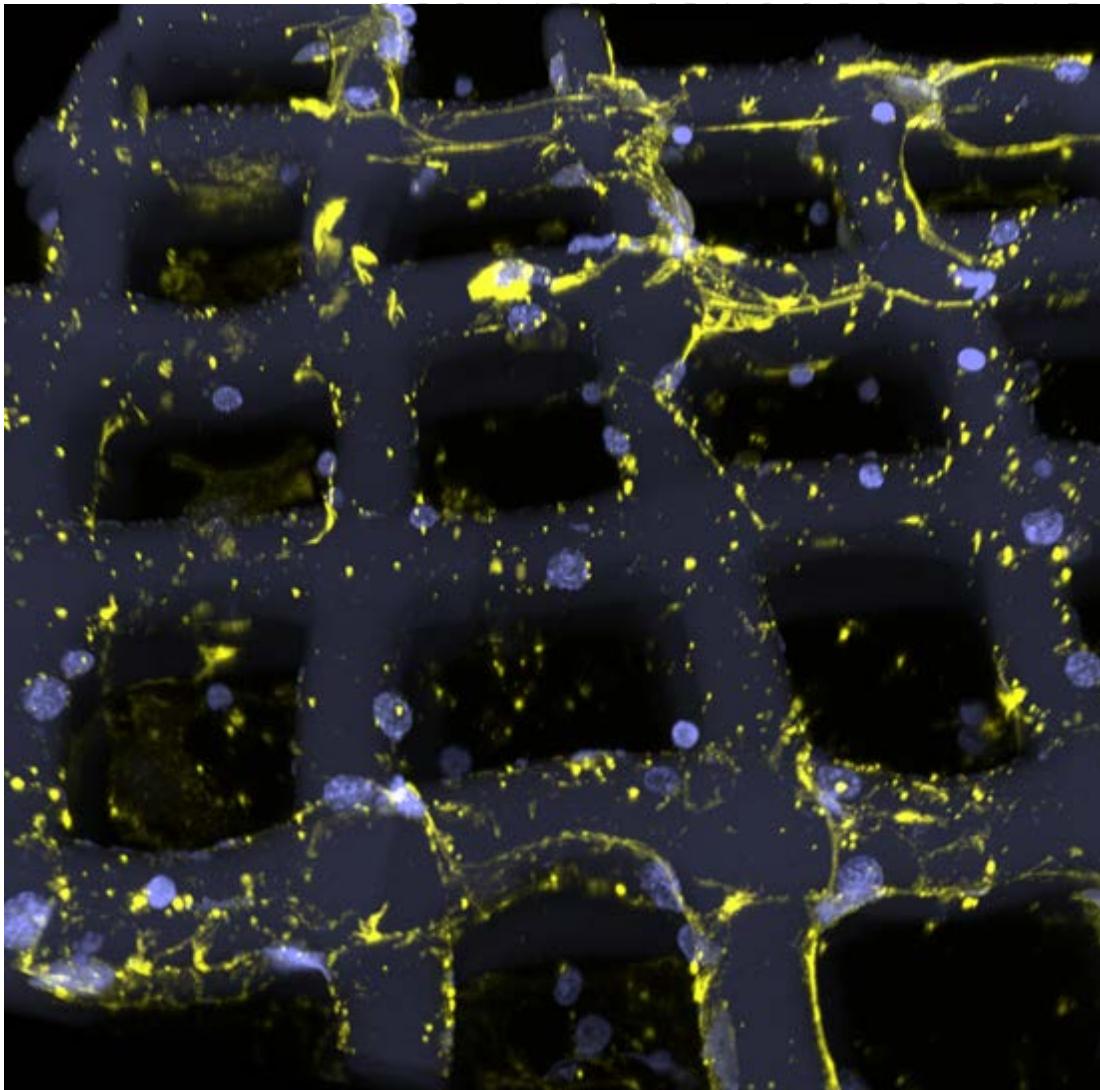


Figure 2



Modern Confocal Image

The image is a composite of two panels. The left panel is a fluorescence micrograph showing a network of neurons stained with yellow and blue. The right panel is a grid of DNA sequence data. The top left corner of the image contains the text "cal Image" in large yellow letters.

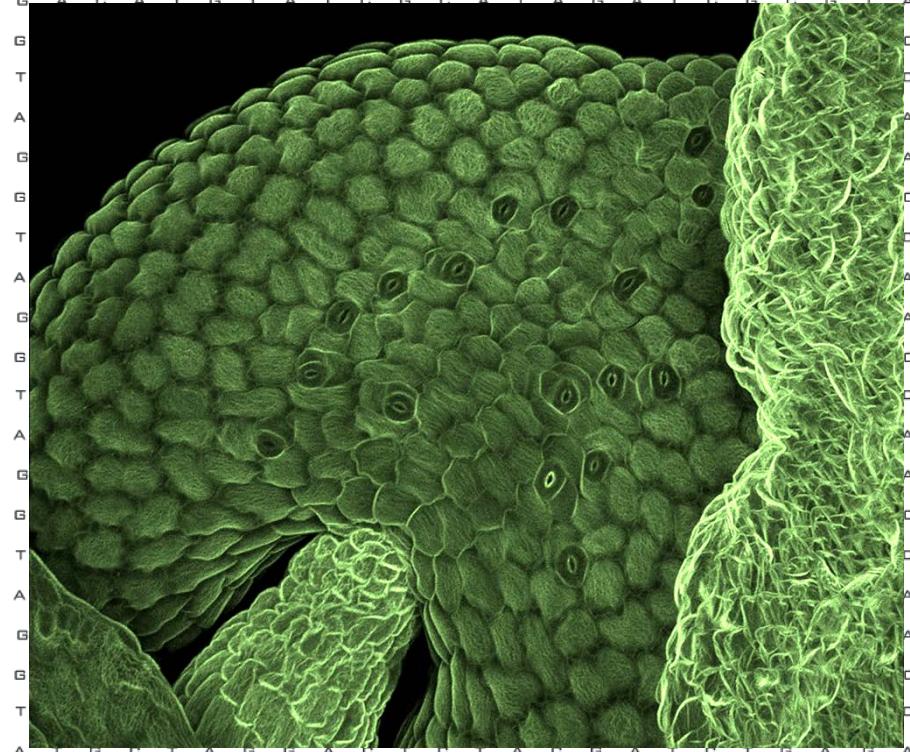
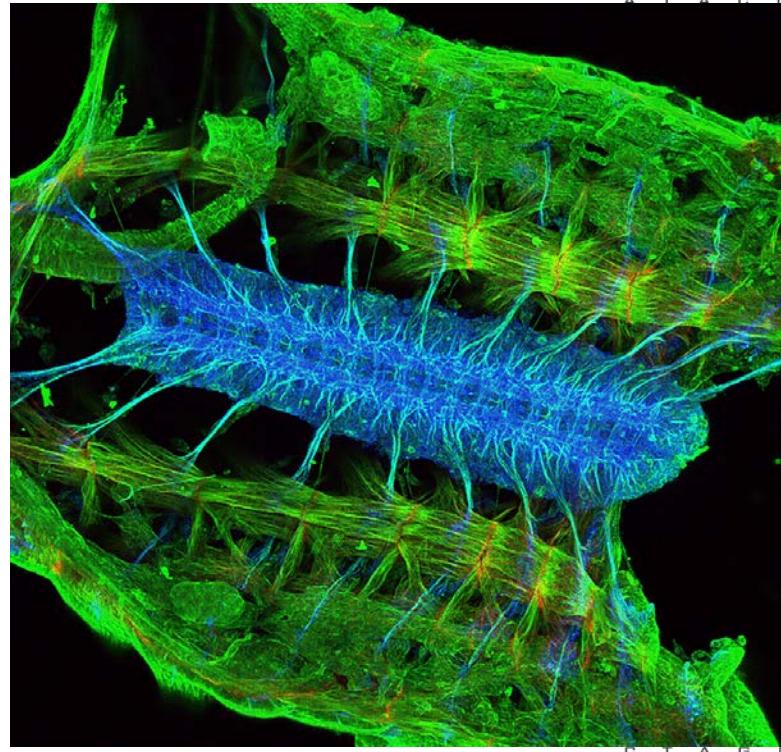


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Modern Confocal Images

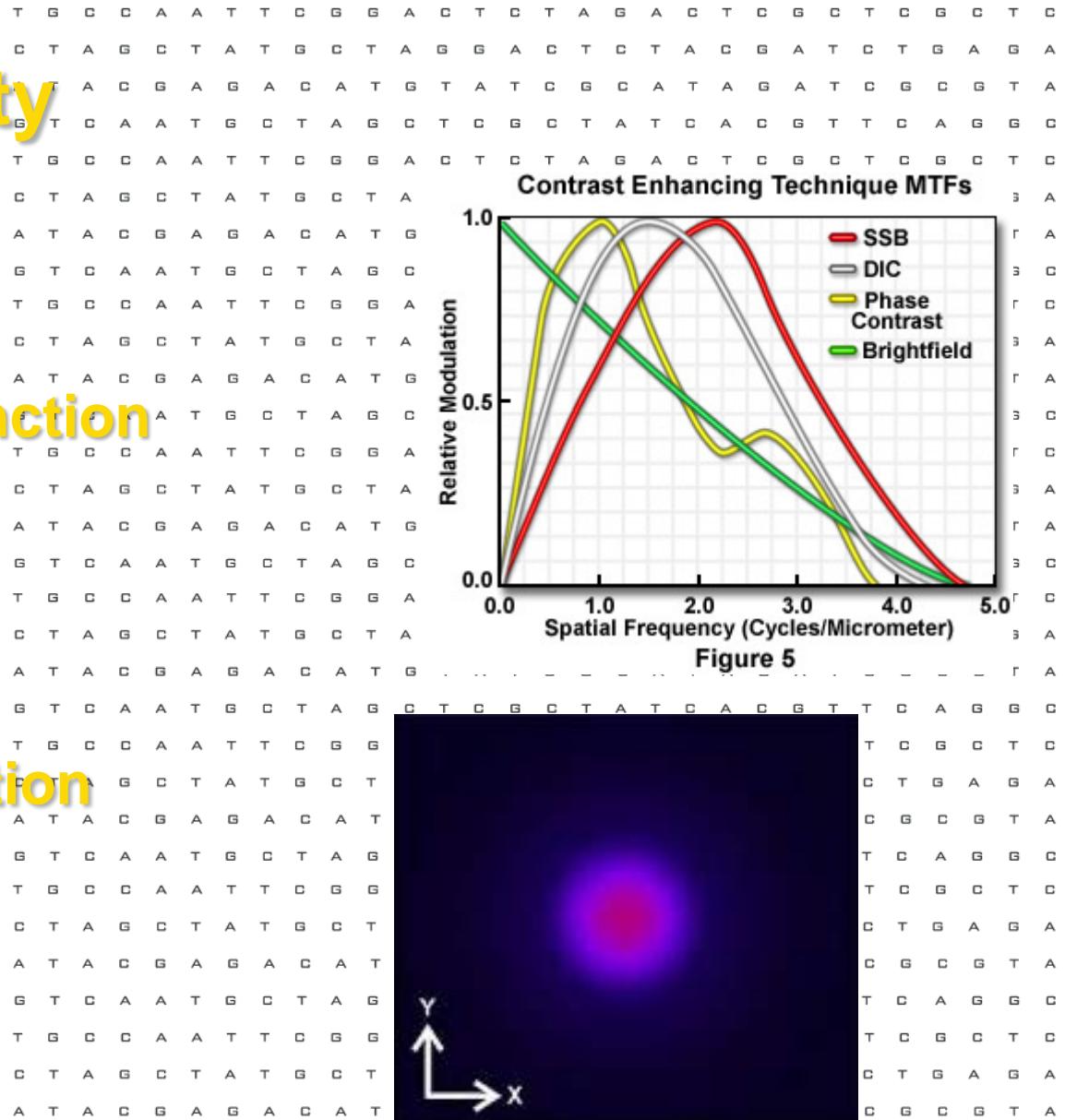


T G C C A A T T C G G A C T C T A G A C T C G C T C G C T C
C A G A G T A G G A C T C T A C G A T C T G A G A
A T A C G A G A C A T G T A T C G C A T A G A T C G C G T A
G T C A A T G C T A G G C T C G C T A T C A C G T T C A G G C
T G C C A A T T C G G A C T C T A G A C T C G C T C G C T C
C T A G C T A T G C T A G G G A C T C T A C G A T C T G A G A
A T A C G A G A C A T G T A T C G C A T A G A T C G C G T A
T G A T A G T G A T G T A T C G C A T A G A T C G C G T A
T A A G T G A T A G T G A T G T A T C G C A T A G A T C G C G T A
T A A G T G A T A G T G A T G T A T C G C A T A G A T C G C G T A
T G A T A G T G A T A G T G A T G T A T C G C A T A G A T C G C G T A
T A A G T G A T A G T G A T G T A T C G C A T A G A T C G C G T A
T G A T A G T G A T A G T G A T G T A T C G C A T A G A T C G C G T A
T A A G T G A T A G T G A T G T A T C G C A T A G A T C G C G T A
T G A T A G T G A T A G T G A T G T A T C G C A T A G A T C G C G T A
T A A G T G A T A G T G A T G T A T C G C A T A G A T C G C G T A
A T A C G A G A C A T G T A T C G C A T A G A T C G C G T A
G T C A A T G C T A G G C T C G C T A T C A C G T T C A G G C
T G C C A A T T C G G A C T C T A G A C T C G C T C G C T C
C T A G C T A T G C T A G G G A C T C T A C G A T C T G A G A
A T A C G A G A C A T G T A T C G C A T A G A T C G C G T A

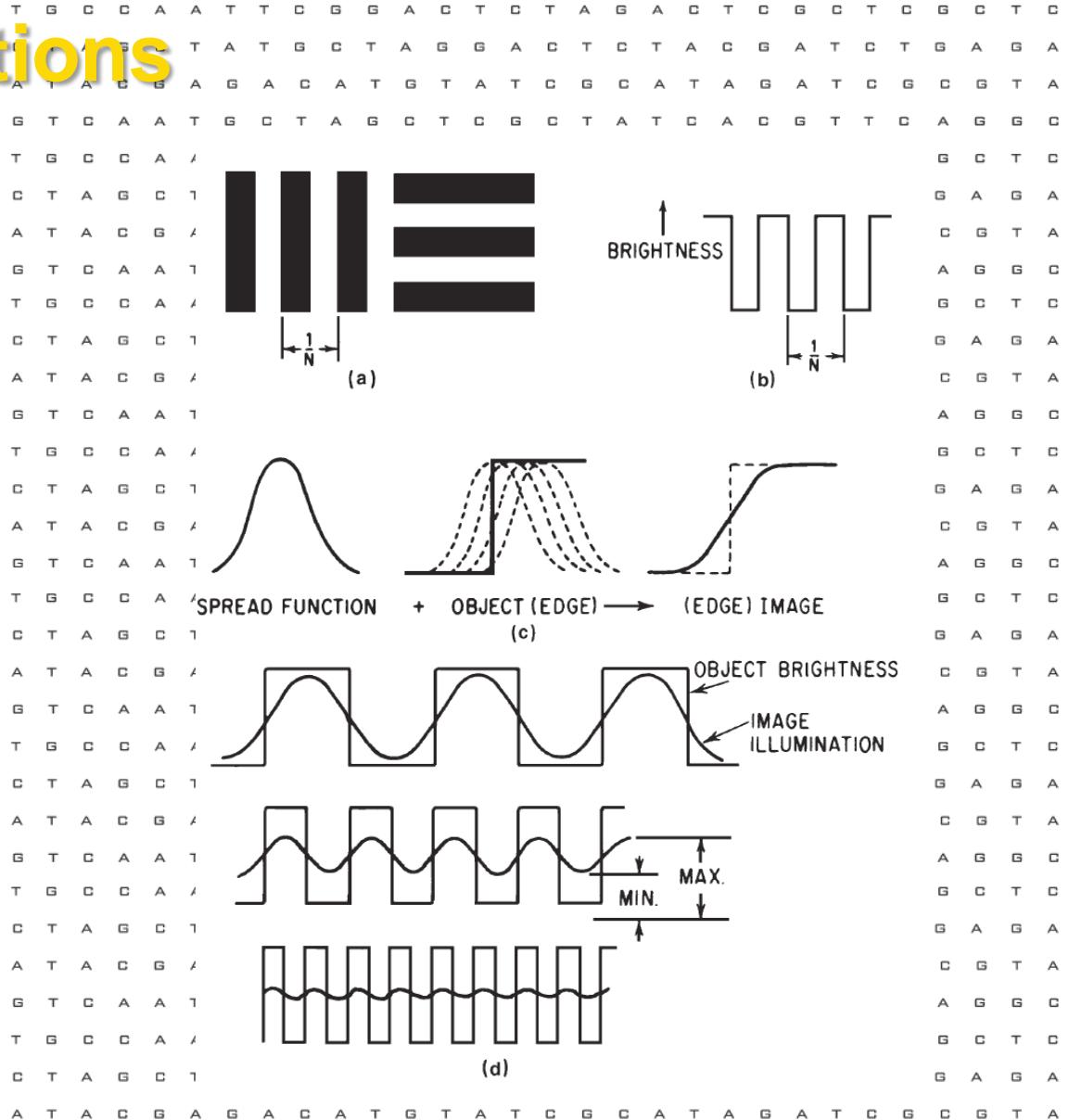
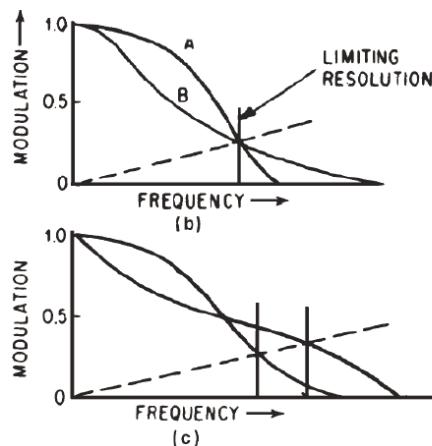
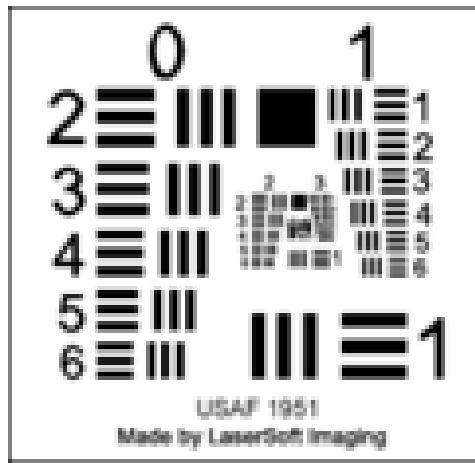
Imaging Quality

Optical transfer function

Point spread function



Transfer Functions



Modulation Transfer Function

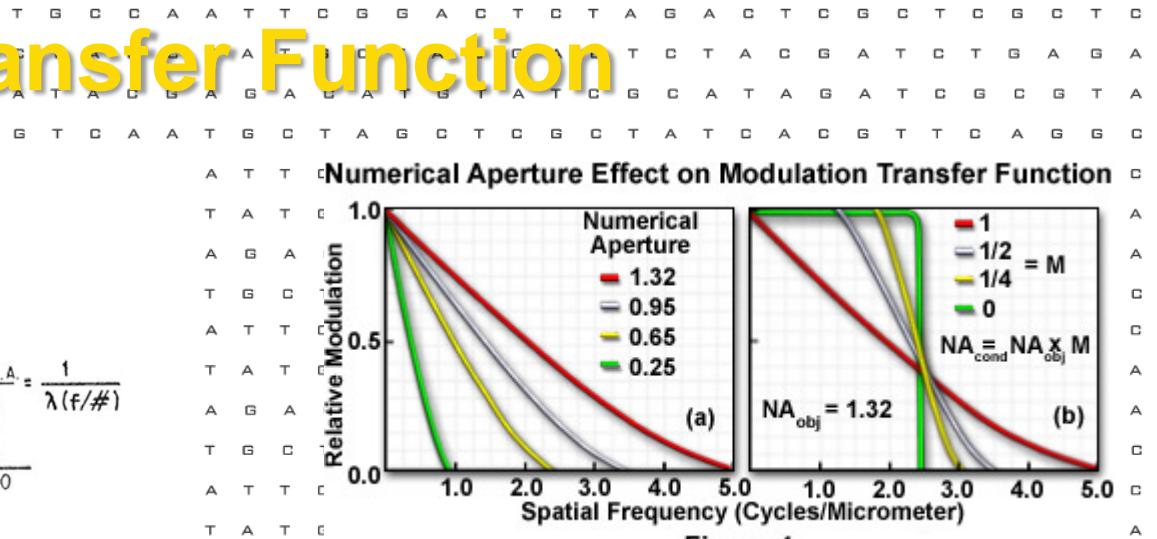
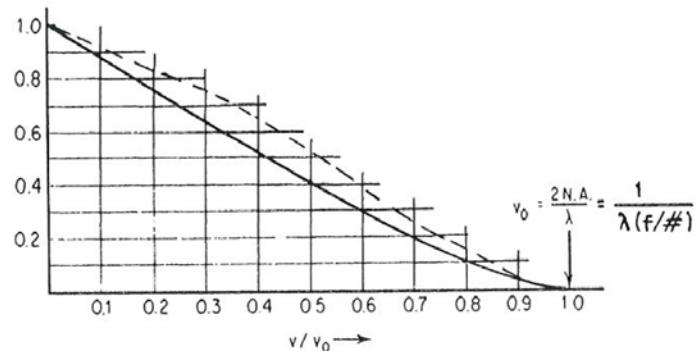
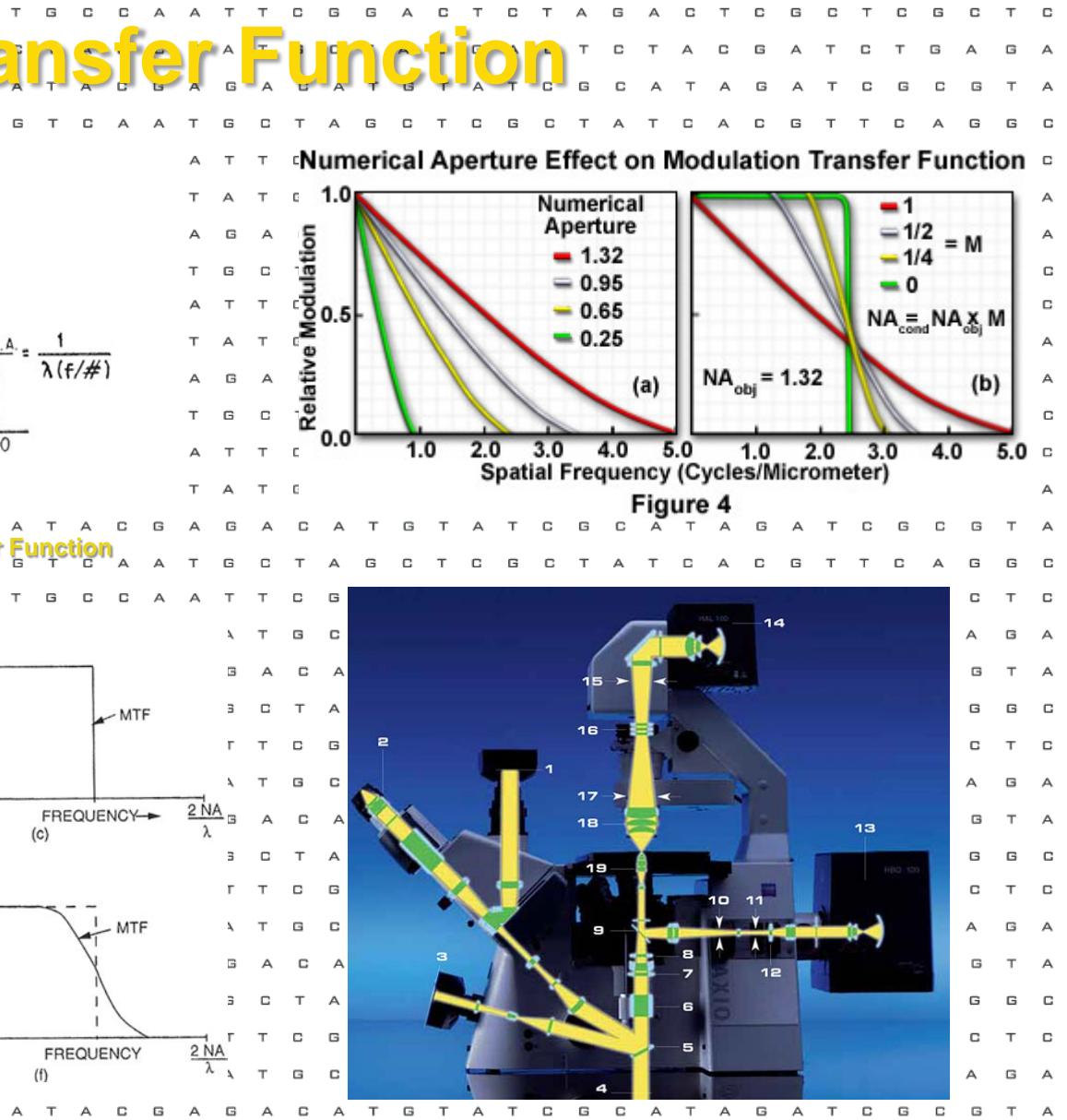
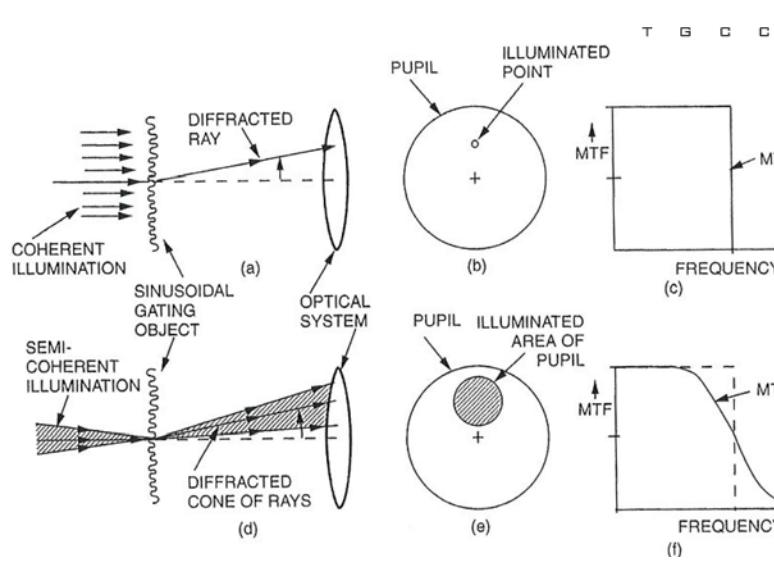


Figure 4

Diffraction Limited Modulation Transfer Function



Modulation Transfer Function

Contrast Enhancing Technique MTFs

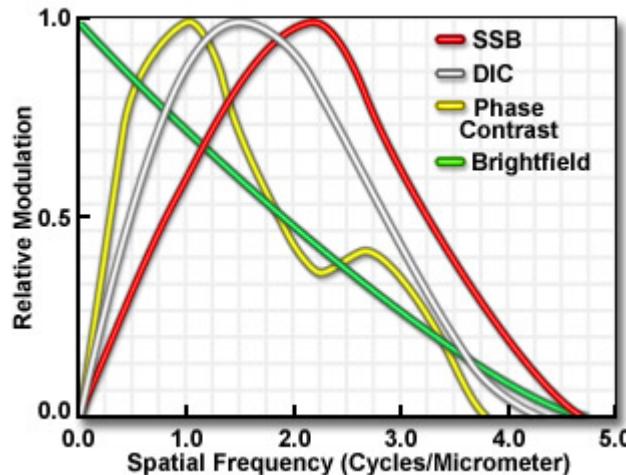


Figure 5

Differential Interference Contrast Schematic

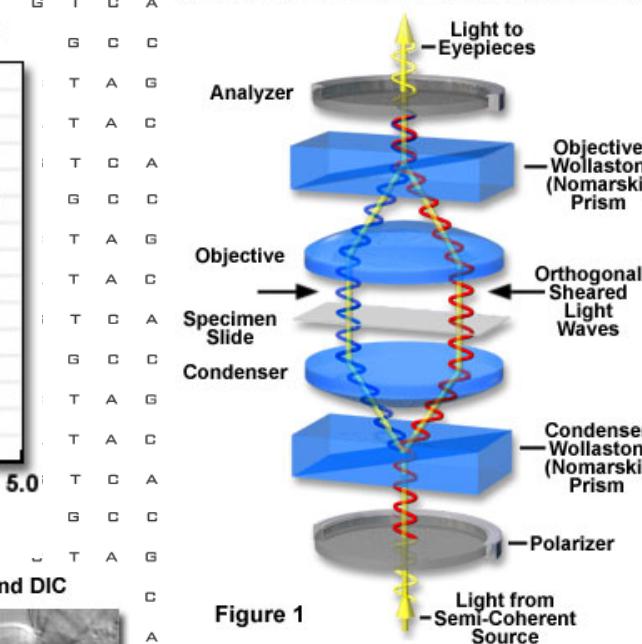


Figure 1

Transparent Specimens in Phase Contrast and DIC

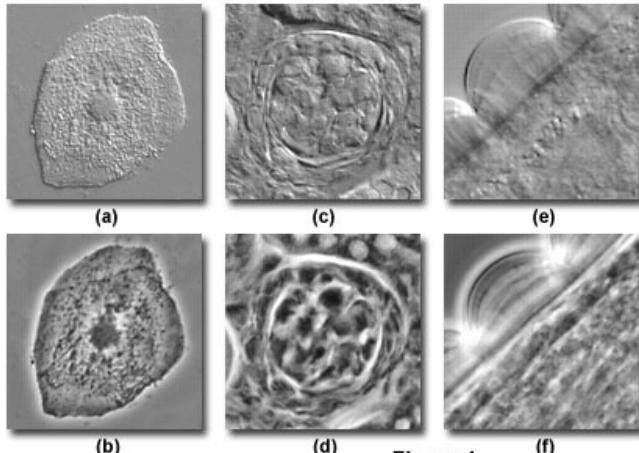


Figure 1

Phase Contrast Light Pathways

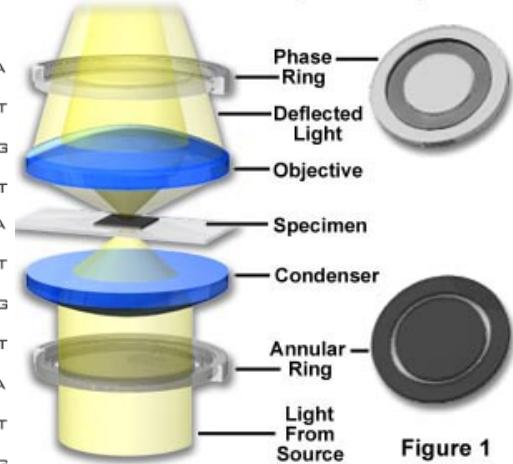


Figure 1

Optical Transfer Function

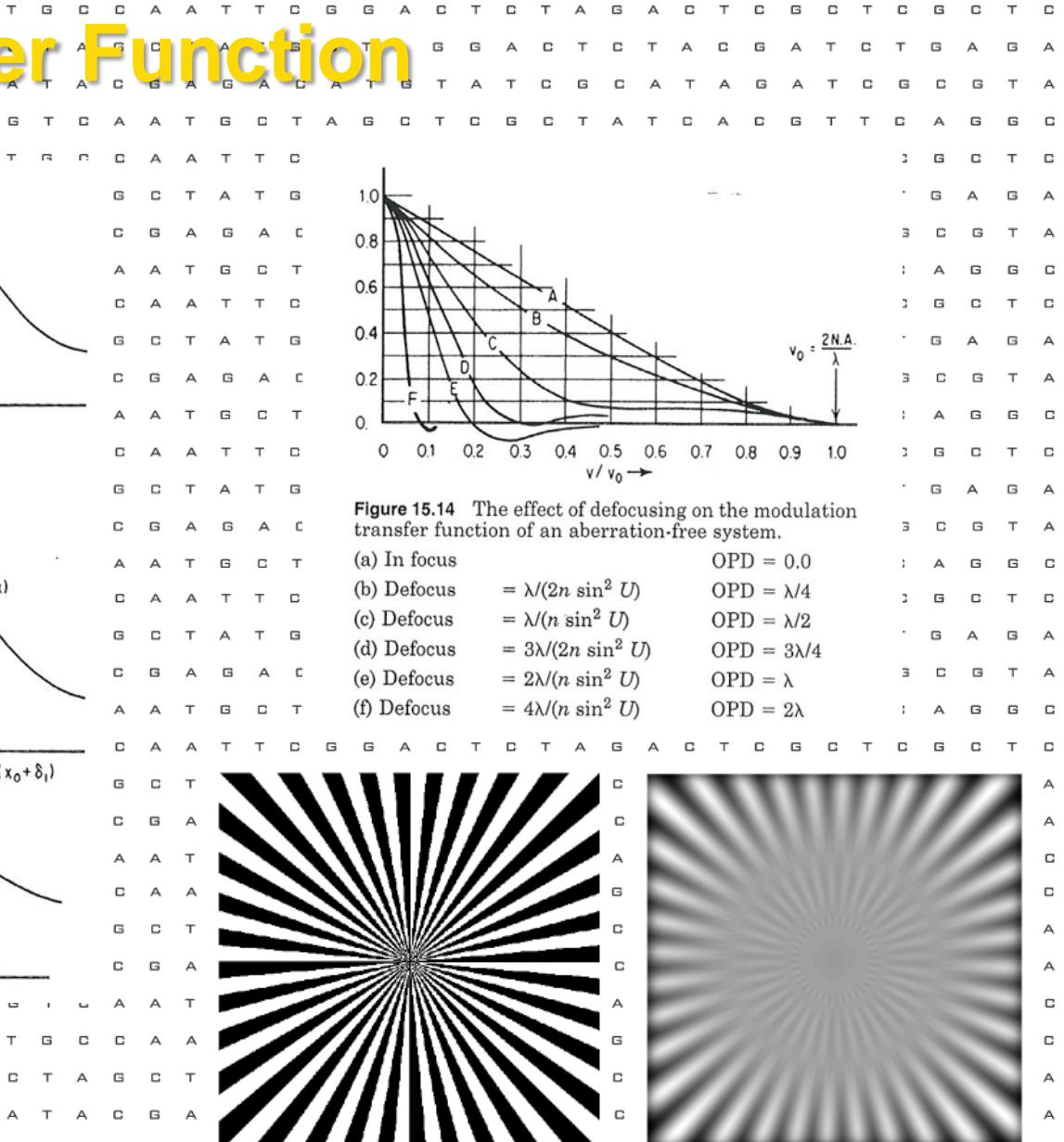
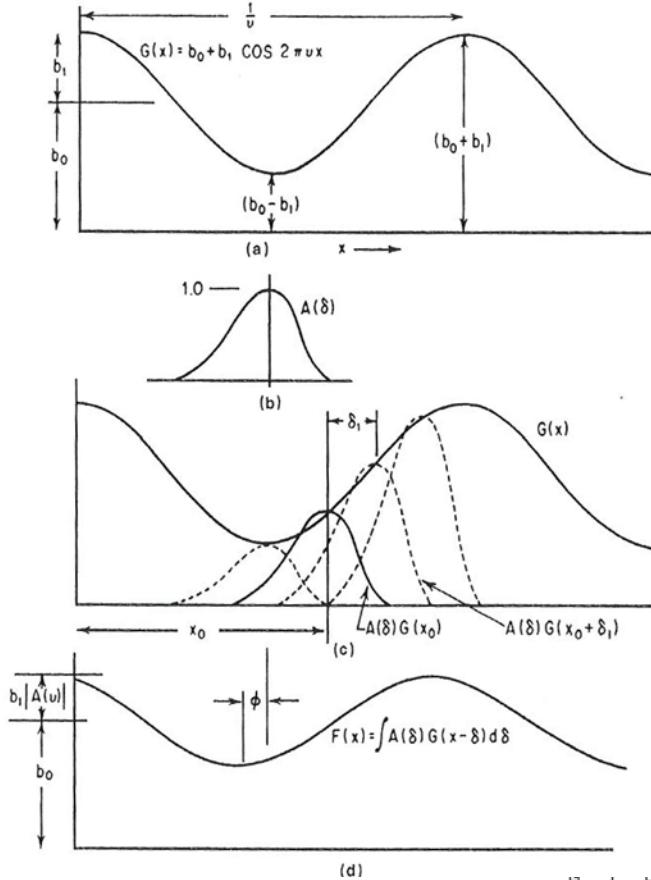
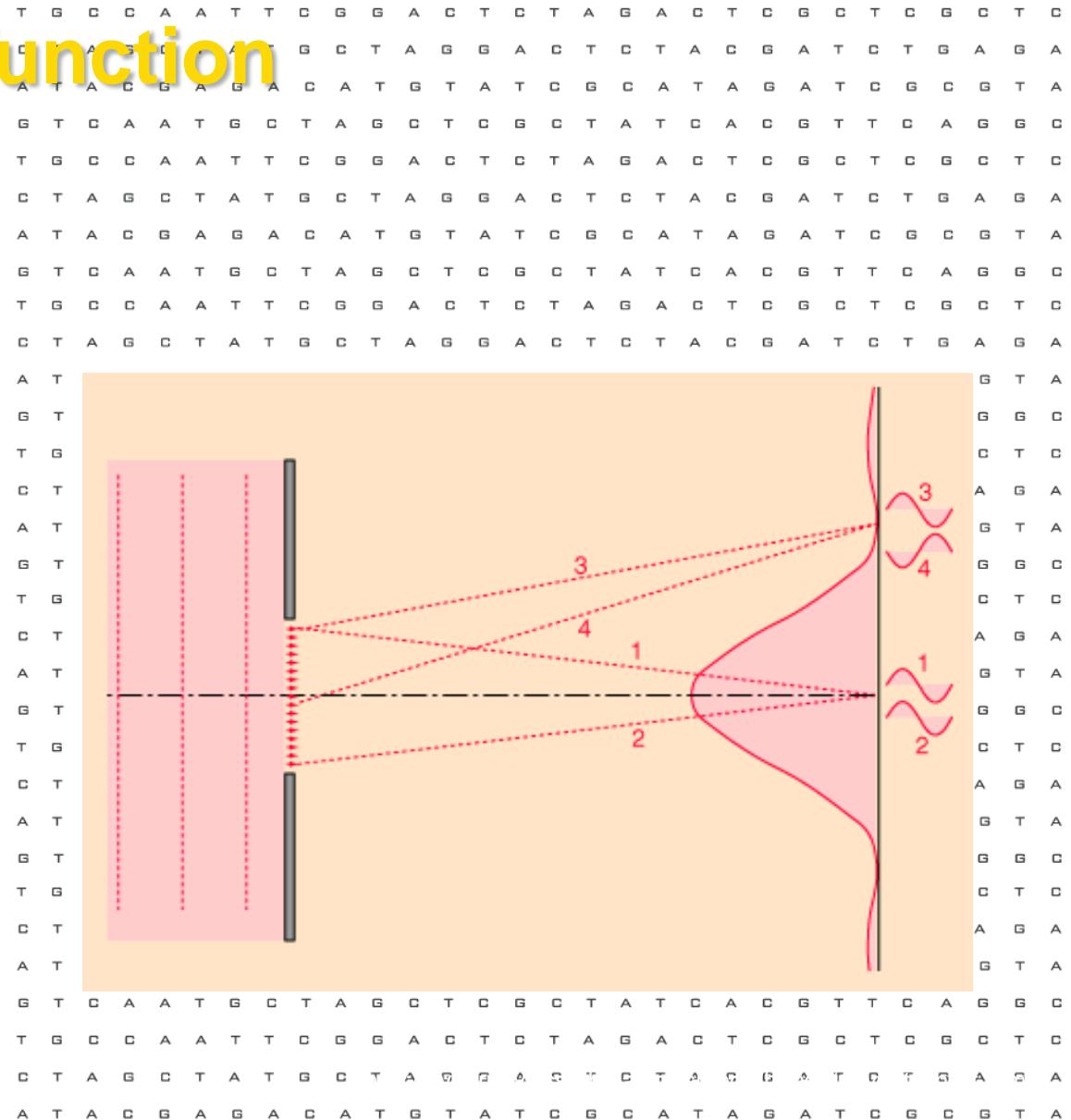
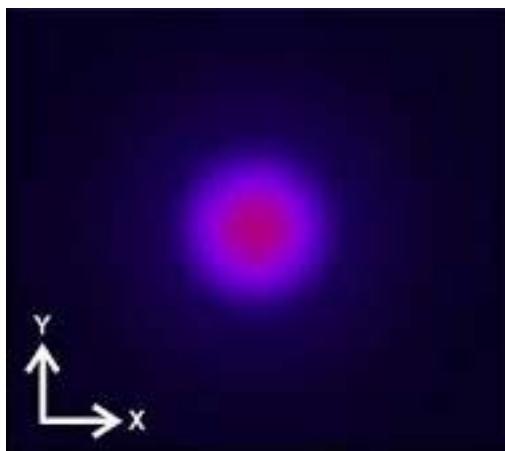


Figure 15.14 The effect of defocusing on the modulation transfer function of an aberration-free system.

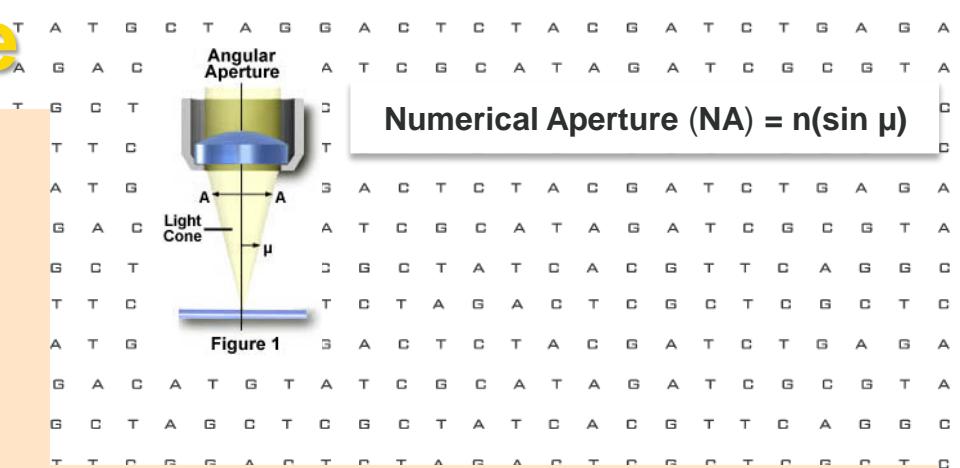
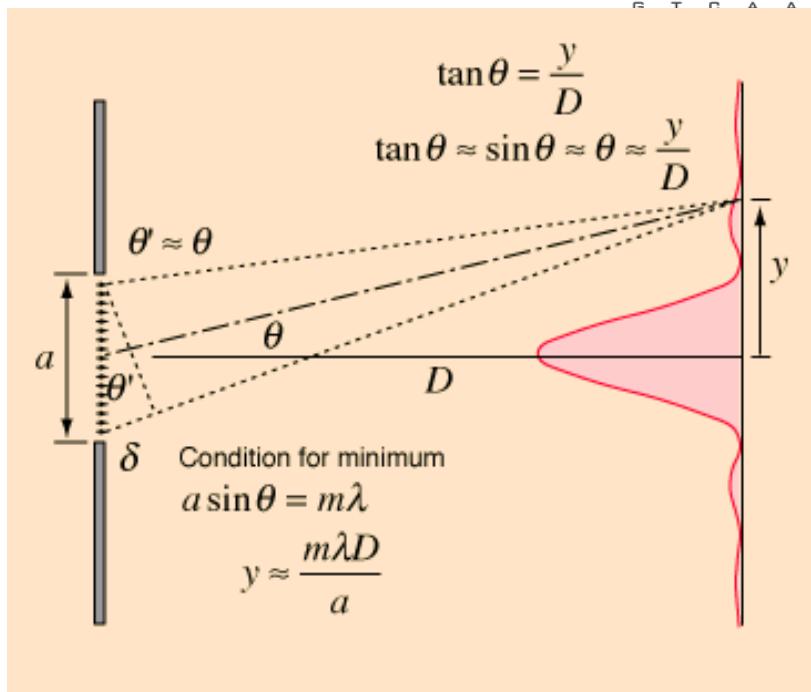
Point spread function



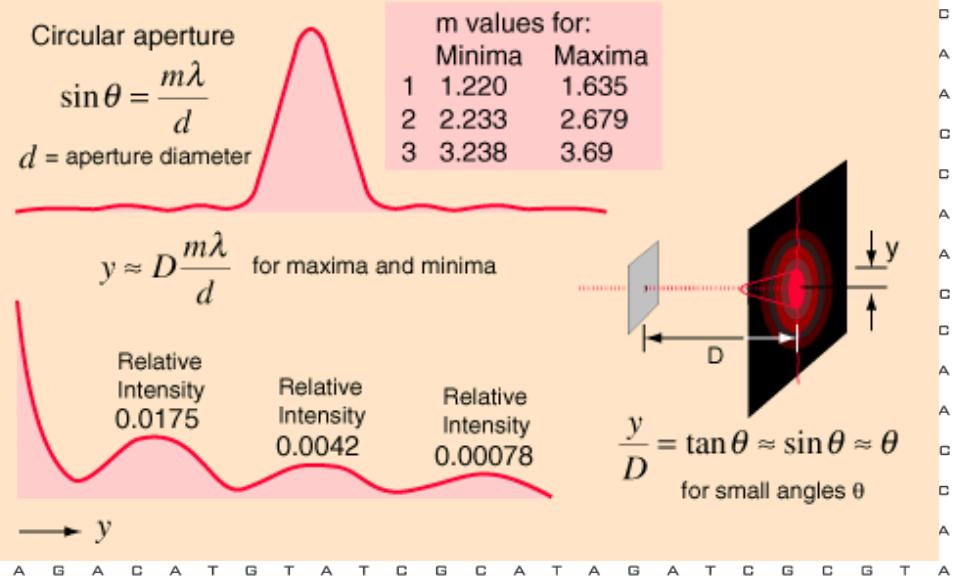
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Numerical Aperture (NA) = $n(\sin \mu)$

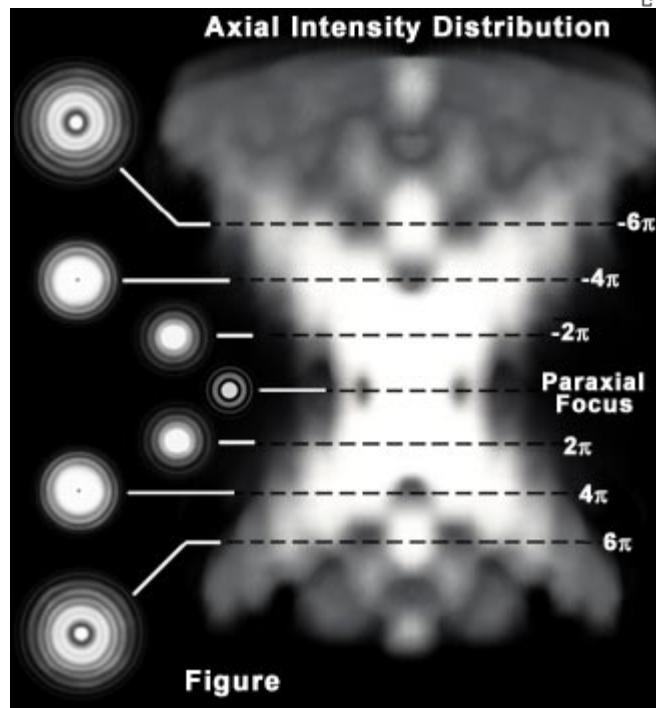
Numerical Aperture



Circular Aperture Diffraction



Point Spread Functions Wide Field



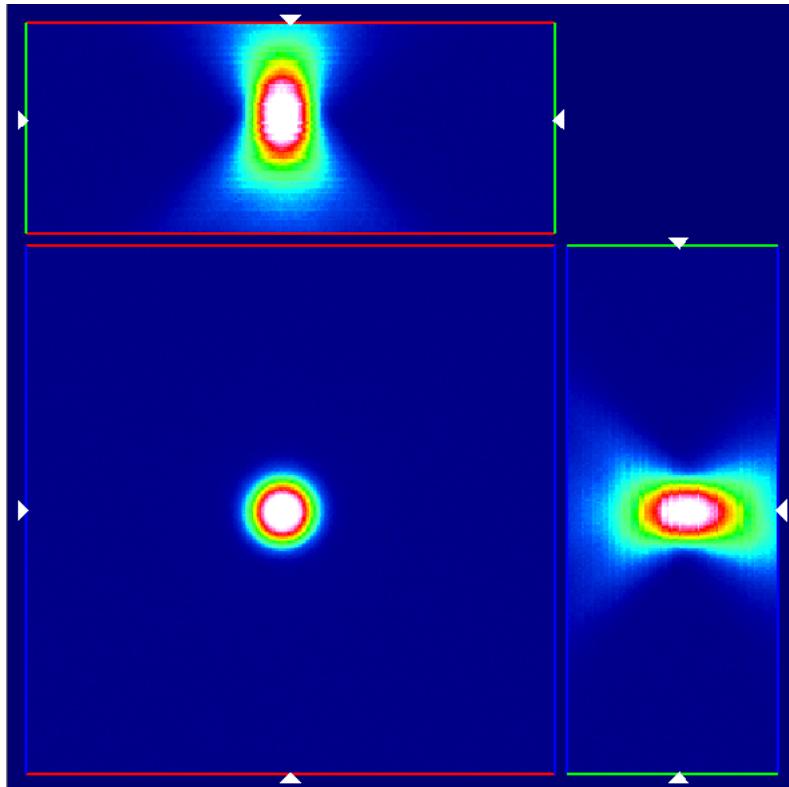
Depth of field

$$d = \frac{\lambda \sqrt{n^2 - (NA)^2}}{(NA)^2}$$

Magnification	Numerical Aperture	Depth of Field (mm)
4x	0.10	15.5
10x	0.25	8.5
20x	0.40	5.8
40x	0.65	1.0
60x	0.85	0.40
100x	0.95	0.19

Shillaber, Charles Patten; *Photomicrography In Theory and Practice.*, John Wiley and Sons, New York, 778 pages(1944).
<http://micro.magnet.fsu.edu/primer/anatomy/focusdepth.html>

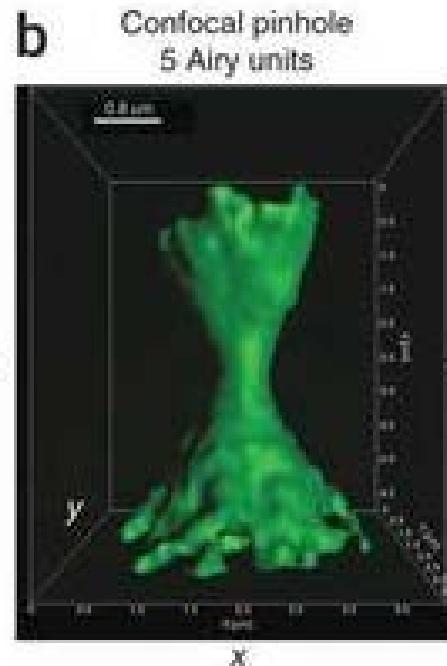
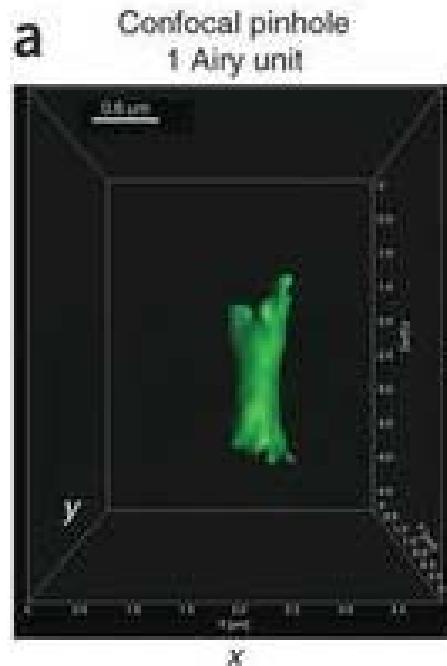
Point Spread Functions Wide Field



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Point Spread Functions Confocal



30% lateral improvement in resolution
compared to wide field

Nature Protocols 6, 1929–1941 (2011)

Resolution

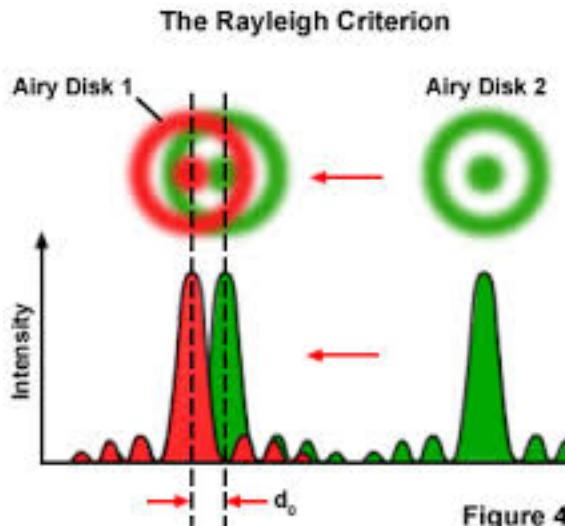


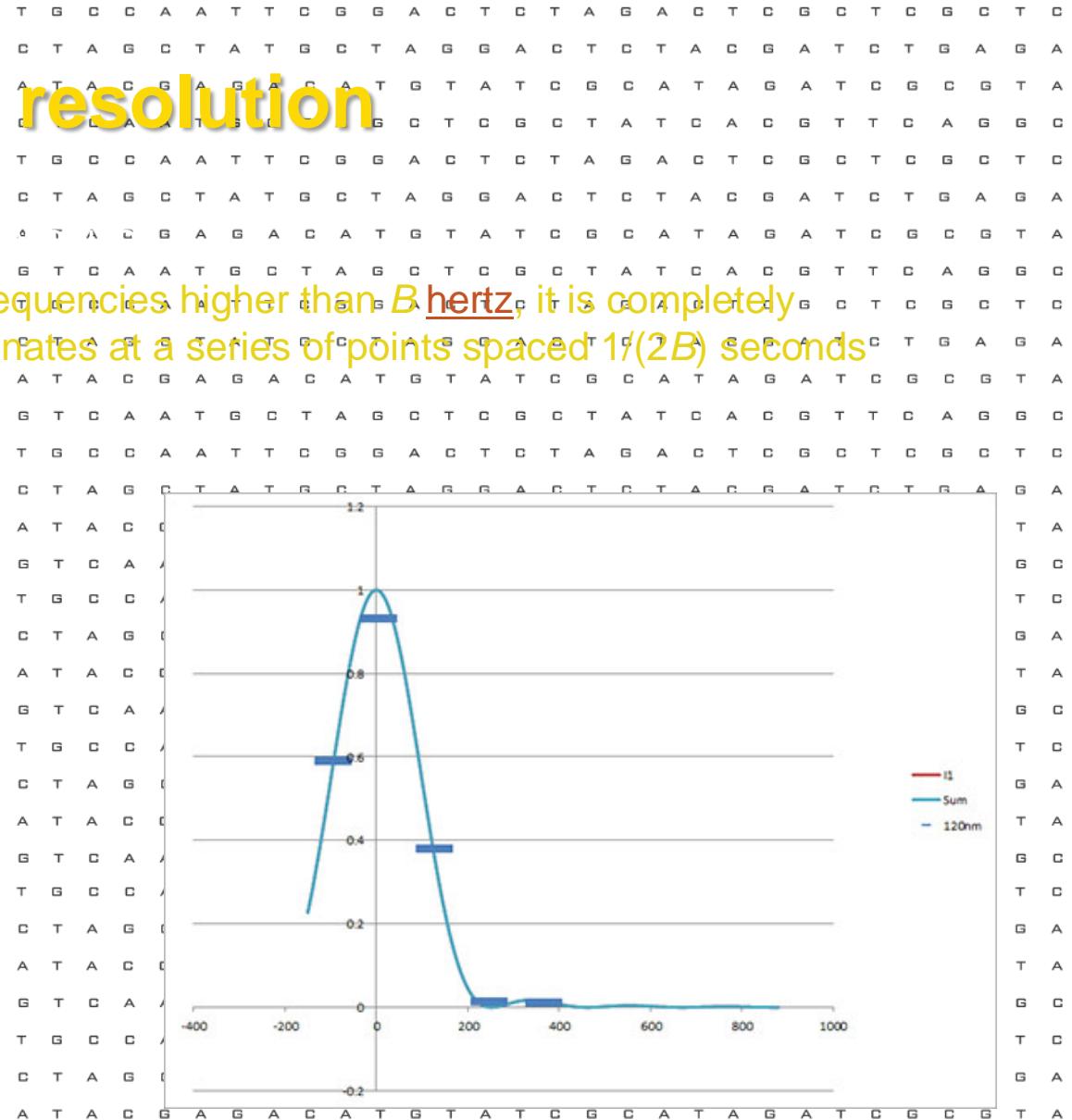
Figure 4

The figure consists of two vertically aligned panels. The top panel is a text-based representation of a DNA sequence, likely from a sequencing gel. It shows multiple rows of bases (A, T, C, G) aligned horizontally. The bottom panel is a photograph of a sequencing gel. The gel has a dark background and features a prominent, bright diagonal band of light that slopes upwards from left to right. This band is surrounded by a color gradient that transitions from orange at the bottom to blue at the top. A white arrow points to the left edge of the gel image.

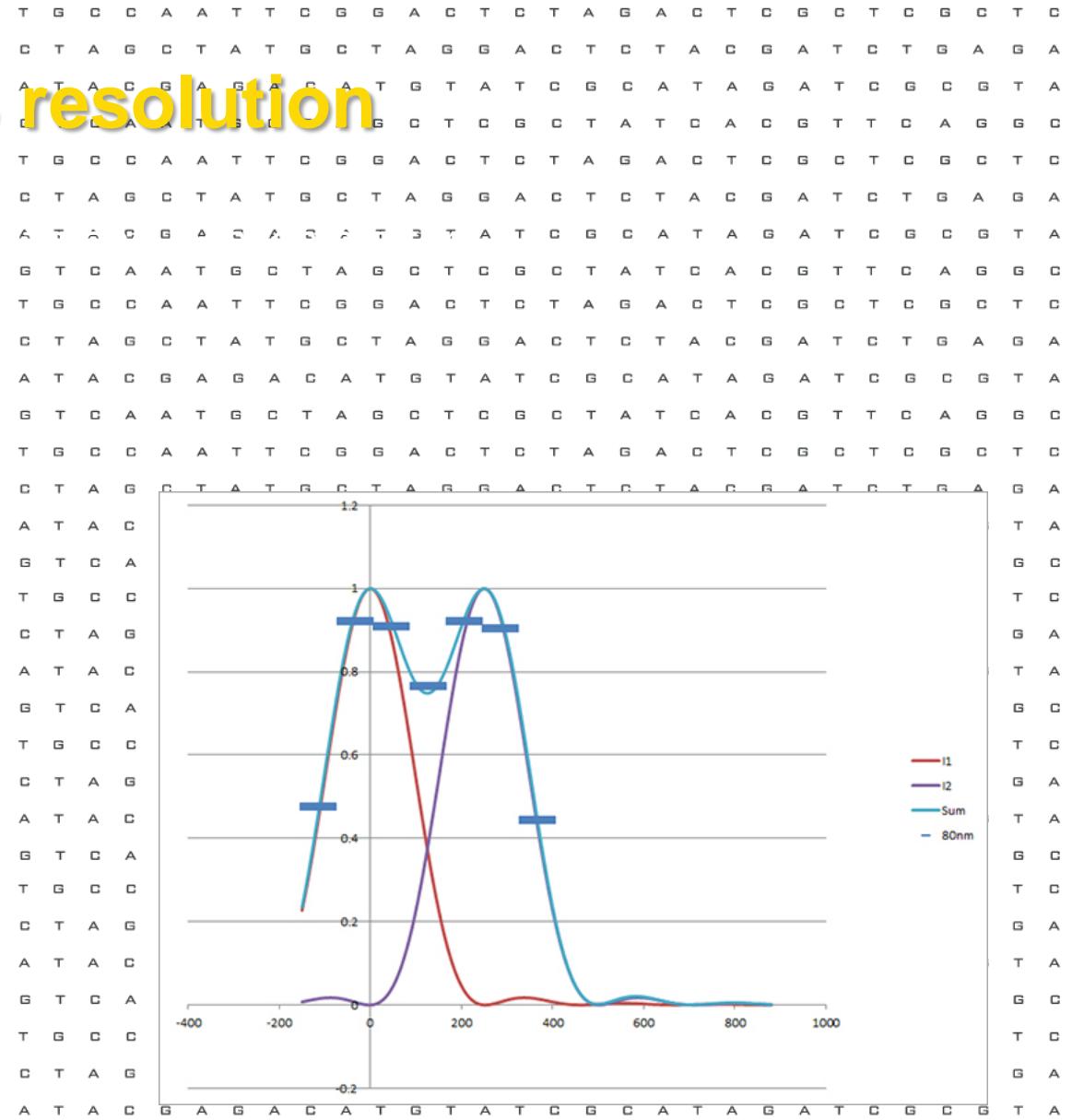


Camera affects resolution

If a function $x(t)$ contains no frequencies higher than B hertz, it is completely determined by giving its coordinates at a series of points spaced $1/(2B)$ seconds apart



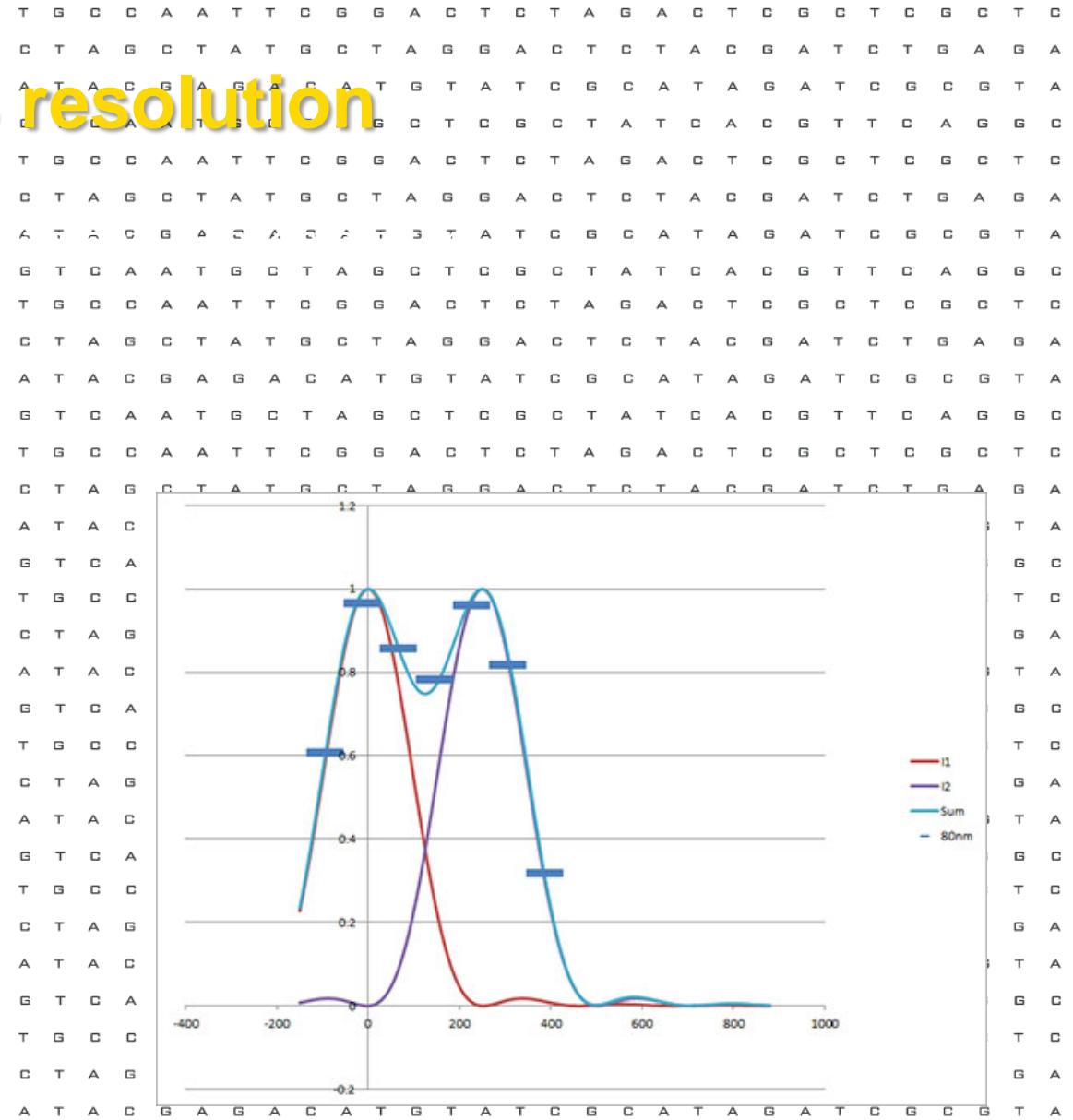
Camera affects resolution



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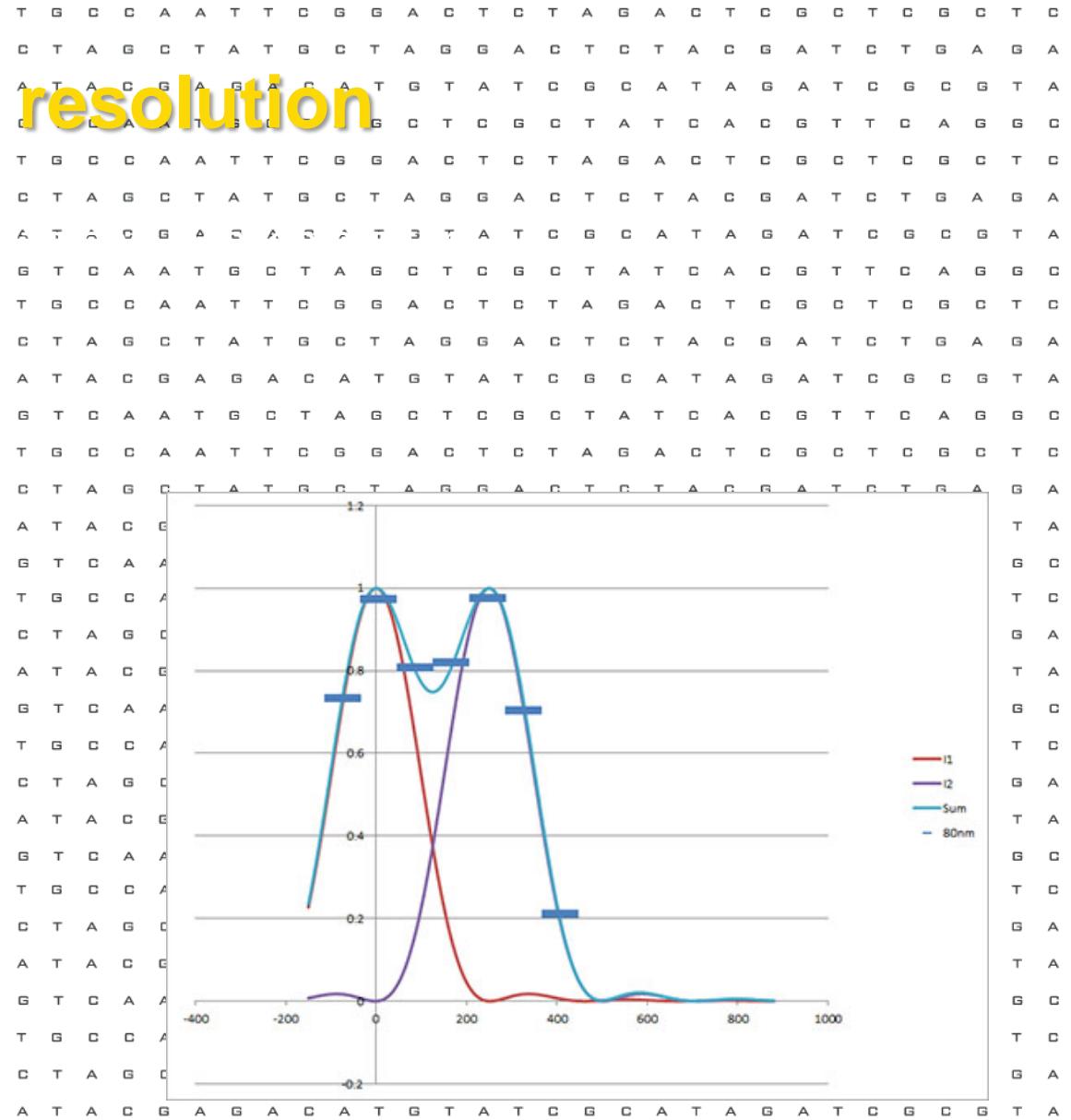
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Camera affects resolution



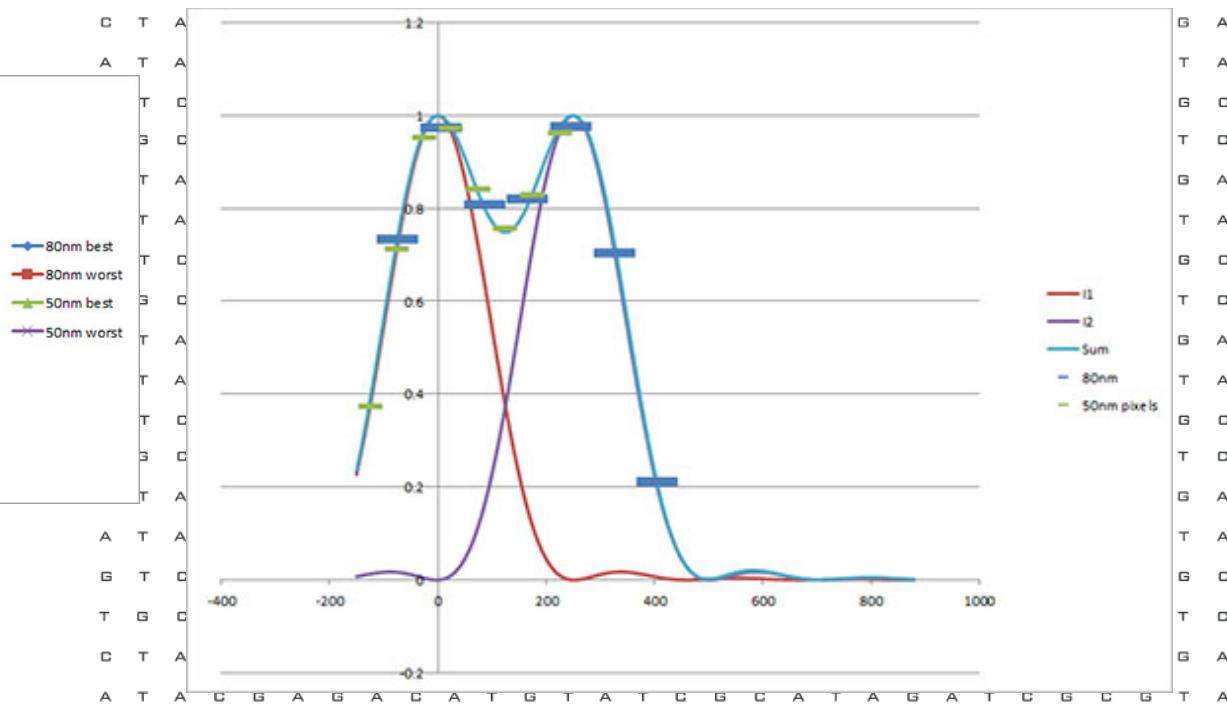
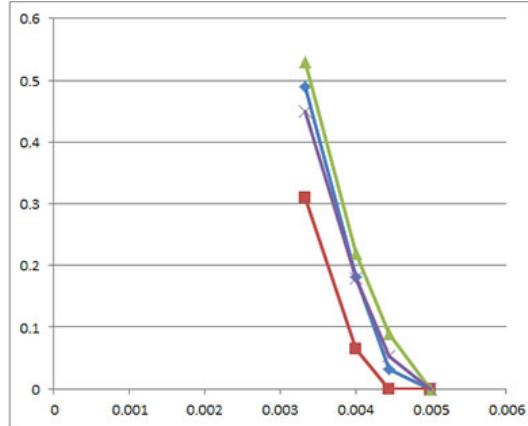
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Camera affects resolution



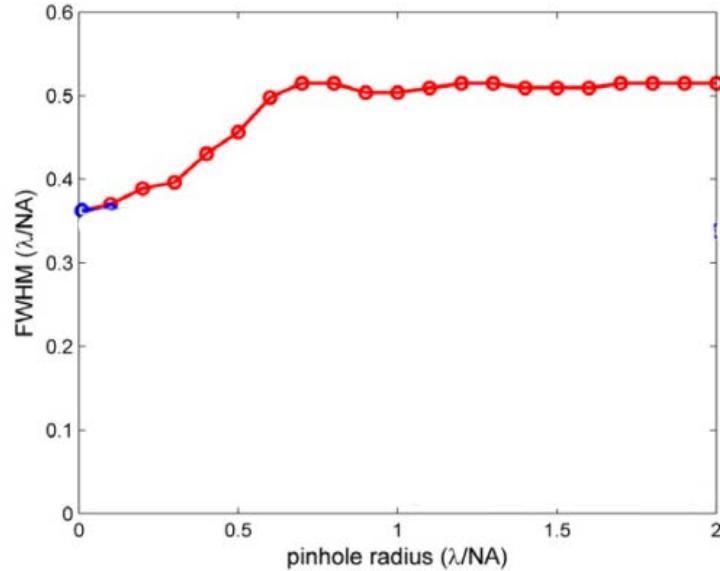
Resolution

spacing	80nm best	80nm worst	50nm best	50nm worst
200	0	0	0	
225	0.032	0	0.089	0.05
250	0.182	0.065	0.22	0.1
300	0.49	0.31	0.53	0.4

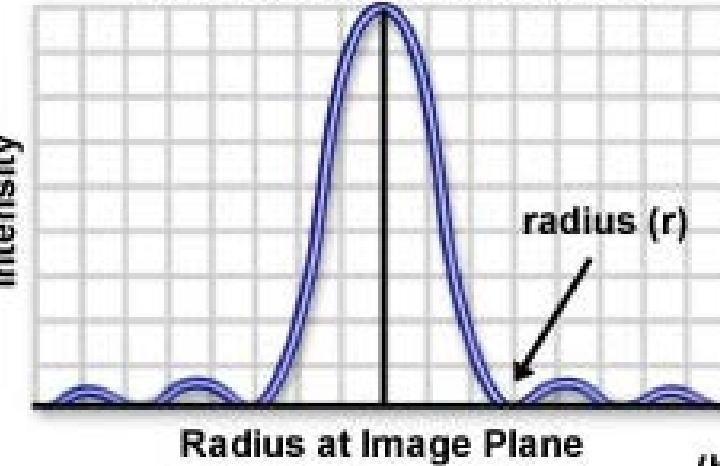


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Pinhole size and resolution



Point Spread Function (PSF)



T G C C A A T T C G G A C T C T A G A C T C G C T C G C T C
A T A C G A G A C A T G T A T C G C A T A G A T C G C G T A
G T C A A T G C T A G C T C G C T A T C A C G T T C A G G C
T G C C A A T T C G G A C T C T A G A C T C G C T C G C T C
C T A G C T A T G C T A G G A C T C T A C G A T C T G A G A
A T A C G A G A C A T G T A T C G C A T A G A T C G C G T A
G T C A A T G C T A G C T C G C T A T C A C G T T C A G G C
T G C C A A T T C G G A C T C T A G A C T C G C T C G C T C
C T A G C T A T G C T A G G A C T C T A C G A T C T G A G A
A T A C G A G A C A T G T A T C G C A T A G A T C G C G T A

Displaced Pinhole and Resolution

Super-resolution in Confocal Imaging

C. J. R. Sheppard,

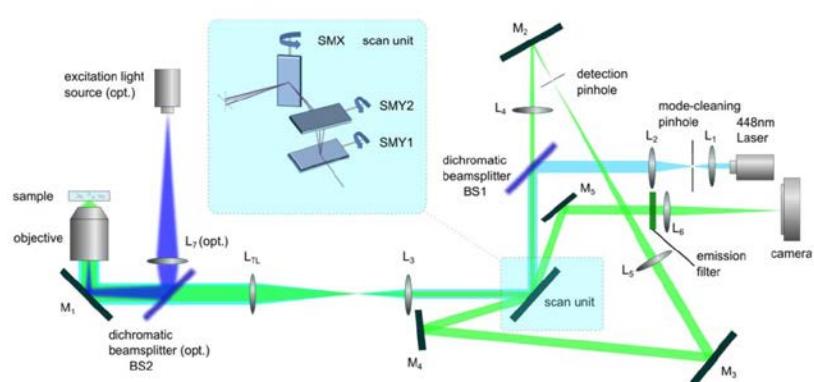
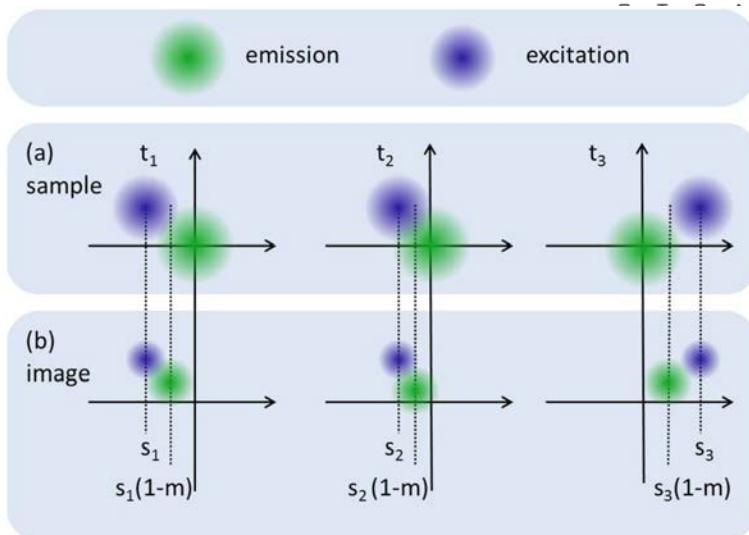
University of Oxford, Department of Engineering Science, Parks Road, Oxford OX1 3PJ U.K.

Received January 5, 1988

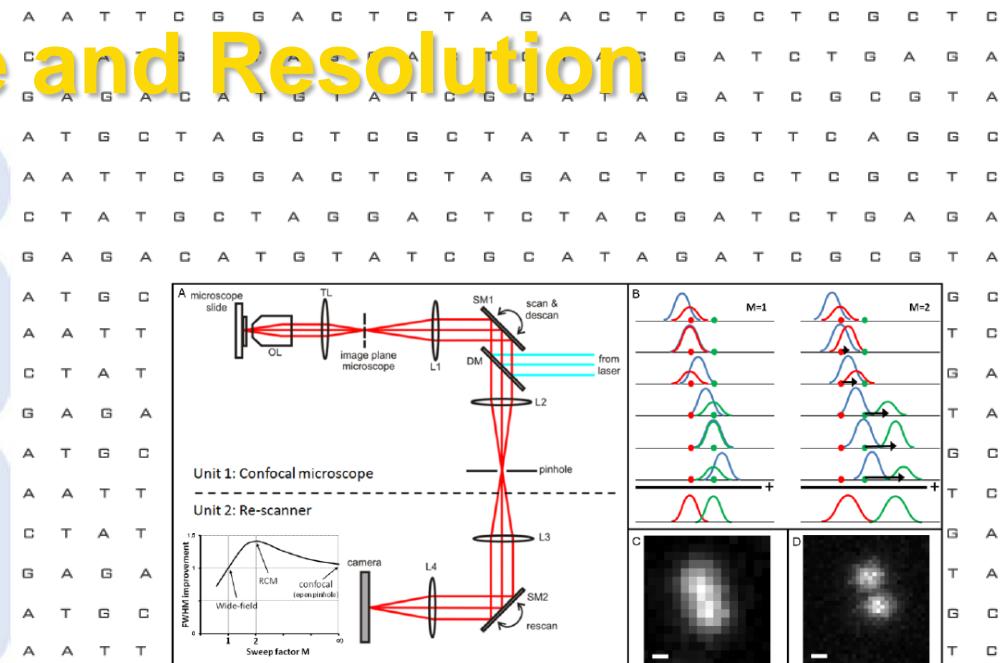
for a displaced point detector (fig. 1), the effective point spread function is given by the product of an Airy disc with a displaced Airy disc. For a system with two equal objectives, this product is symmetrical about a point one half of the demagnified distance from the axis. So a displaced point detector produces an image of improved resolution, but of a point of the object displaced from the optic axis.

this suggests a method of further improving the resolution. The signals from all points of a detector array are measured, but instead of integrating directly as in conventional imaging, each signal is reassigned to its particular image point.

Displaced Pinhole and Resolution



Optical Photon Reassignment Microscopy (OPRA) C T A
Stephan Roth^{1,2}, Colin J. R. Sheppard³, Kai Wicker^{1,2} and
Rainer Heintzmann^{1,2,4} 2013



Re-scan confocal microscopy: scanning twice for better resolution

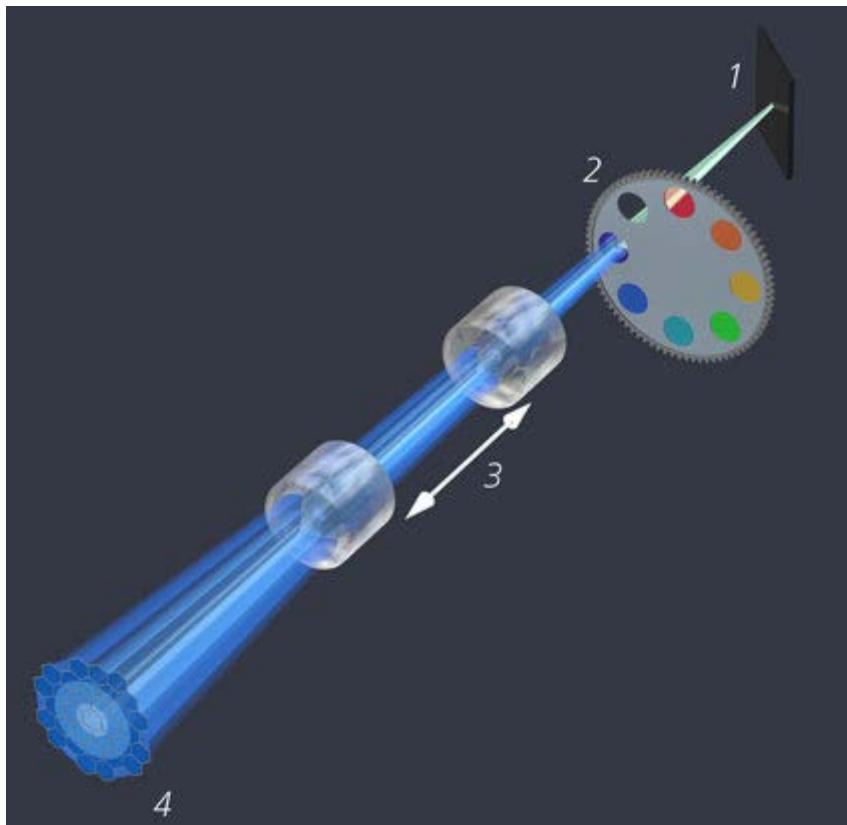
Giulia M.R. De Luca,^{1,7,*} Ronald M.P. Breedijk,^{1,7} Rick A.J. Brandt,¹

Christiaan H.C. Zeelenberg,¹ Babette E. de Jong,¹ Wendy Timmermans⁴

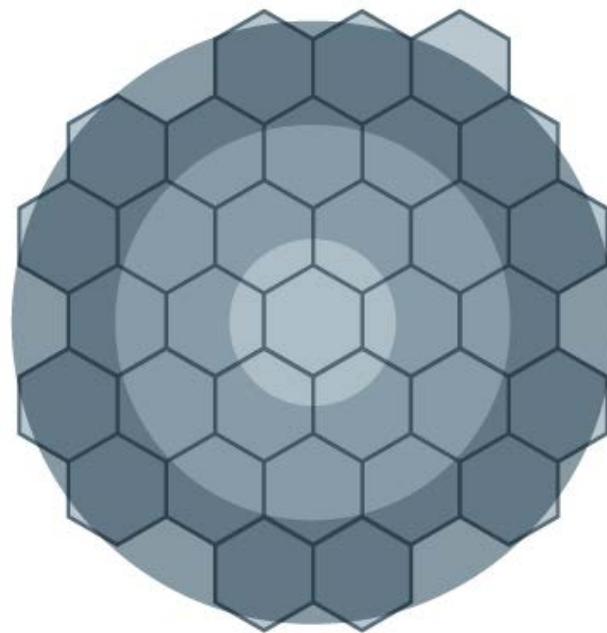
Leila Nahidi Azar,⁵ Ron A. Hoebe,⁶ Sjoerd Stallinga,² and Erik M.M. Manders^{1,3} 2013

C A T G T A T C G C A T A G A T C G C G T A
T A G C T C G C T A T C A C G T T T C A G G C
C G G A C T C T A G A C T C G C T C G C T C
G C T A G G A C T C T A C G A T C T G A G A

AiryScan Resolution

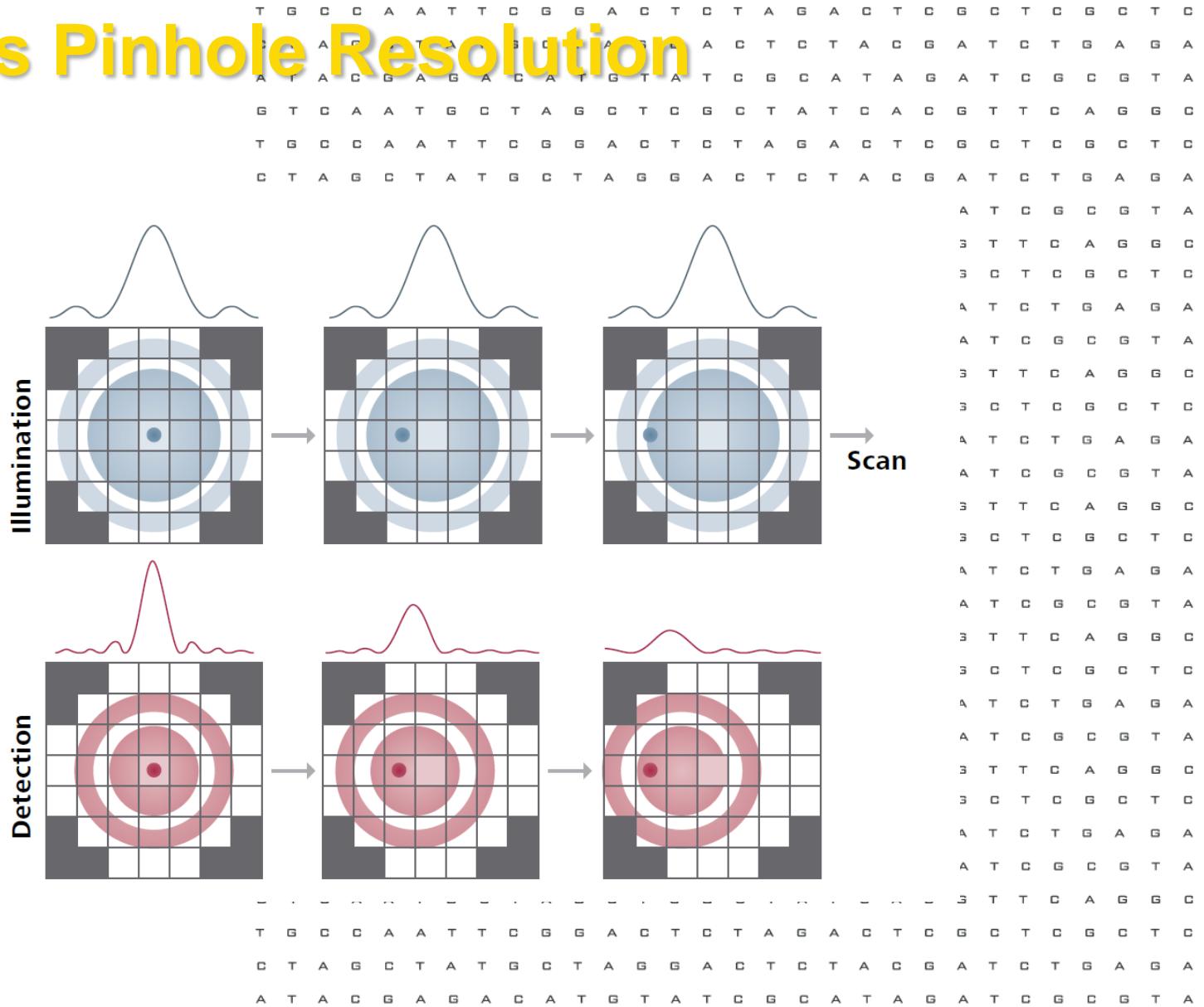


1.25 AU

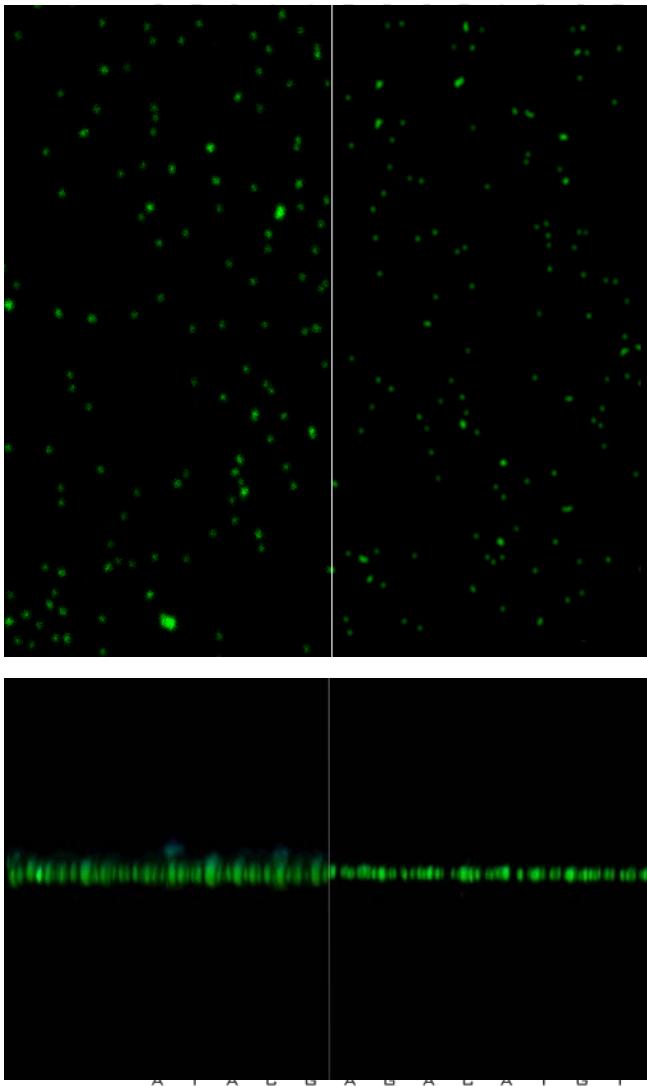


The diagram illustrates the relationship between the length of a DNA sequence and the size of a hexagonal lattice model. On the left, a DNA sequence is shown as a grid of letters. An arrow points from this sequence to a large hexagonal lattice in the center. The distance between the sequence and the lattice is labeled "1.25 AU". To the left of the sequence, there is a small illustration of a robotic arm with colored circular tips, labeled "1".

Off Axis Pinhole Resolution



Confocal vs Airyscan



AiryScan

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T G C C A A T T C G G A C T C T A G A C T C G C T C G C T C
C T A G C T A T G C T A G G A C T C T A C G A T C T G A G A
A T A C G A G A C A T G T A T C G C A T A G A T C G C G T A

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T C G G A C T C T A G A C T C G C T C G C T C
T G C T A G G A C T C T A C G A T C T G A G A
A C A T G T A T C G C A T A G A T C G C G T A
C T A G C T C G C T A T C A C G T T C A G G C
T C G G A C T C T A G A C T C G C T C G C T C
T G C T A G G A C T C T A C G A T C T G A G A
A C A T G T A T C G C A T A G A T C G C G T A
C T A G C T C G C T A T C A C G T T C A G G C
T C G G A C T C T A G A C T C G C T C G C T C
T G C T A G G A C T C T A C G A T C T G A G A
A C A T G T A T C G C A T A G A T C G C G T A
C T A G C T C G C T A T C A C G T T C A G G C
T C G G A C T C T A G A C T C G C T C G C T C
T G C T A G G A C T C T A C G A T C T G A G A
A C A T G T A T C G C A T A G A T C G C G T A
C T A G C T C G C T A T C A C G T T C A G G C
T C G G A C T C T A G A C T C G C T C G C T C
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C T A G C T C G C T A T C A C G T T C A G G C
T C G G A C T C T A G A C T C G C T C G C T C
T G C T A G G A C T C T A C G A T C T G A G A
A C A T G T A T C G C A T A G A T C G C G T A