Confocal Microscopy and Full Field Microscopy

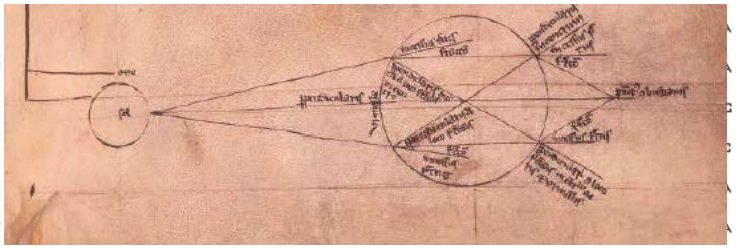
Advanced Fluorescence Microscopy Workshop
August 17th 2015

CARL R. WOESE **INSTITUTE FOR GENOMIC BIOLOGY**
Where Science Meets Society

ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

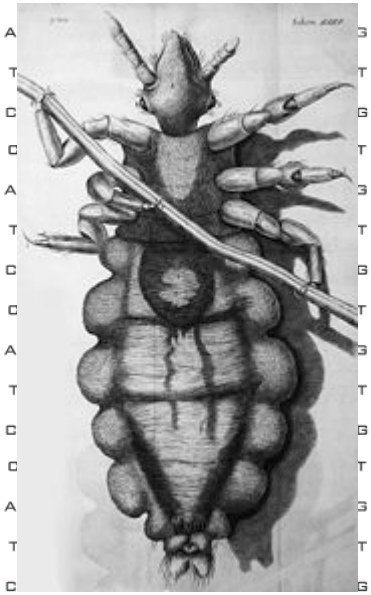
History

Alhazen Book of Optics 1021

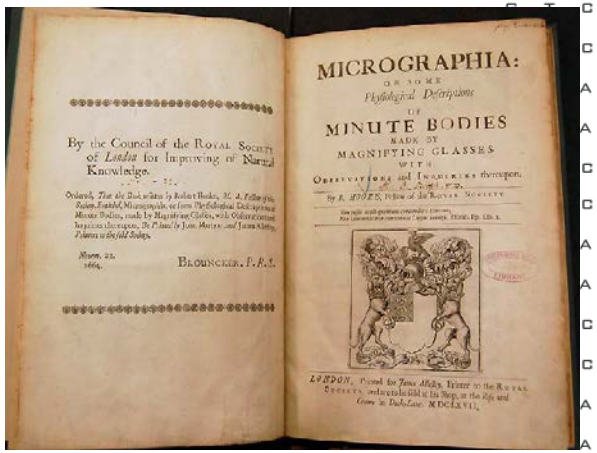


Roger Bacon 1214-1294

Anthony van Leeuwenhoek 1632-1723



Robert Hooke: Micrographia 1665

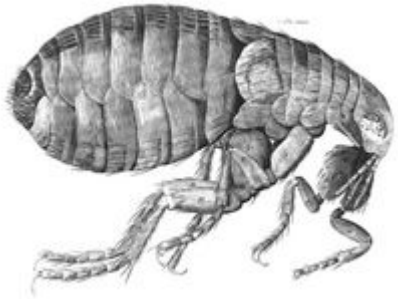


MICROGRAPHIA:
OR SOME
Physiological Descriptions
OF
MINUTE BODIES
MADE BY
MAGNIFYING GLASSES
WITH
OBSERVATIONS and INQUIRIES thereupon.

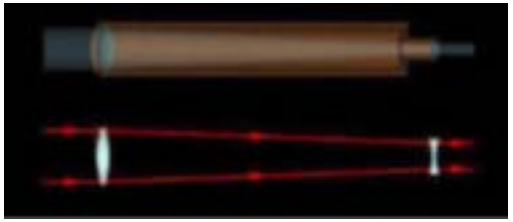
By the Council of the ROYAL SOCIETY of *London* for Improving of Natural Knowledge.

BROUNCKER. P.R.S.

LONDON, Printed for *James Allestry*, Printer to the ROYAL SOCIETY, and are to be sold at his Shop, at the *Rose* and *Crown* in *Duke-Lane*. MDCLXVII.



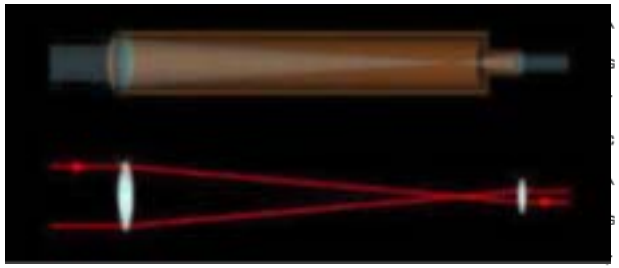
History

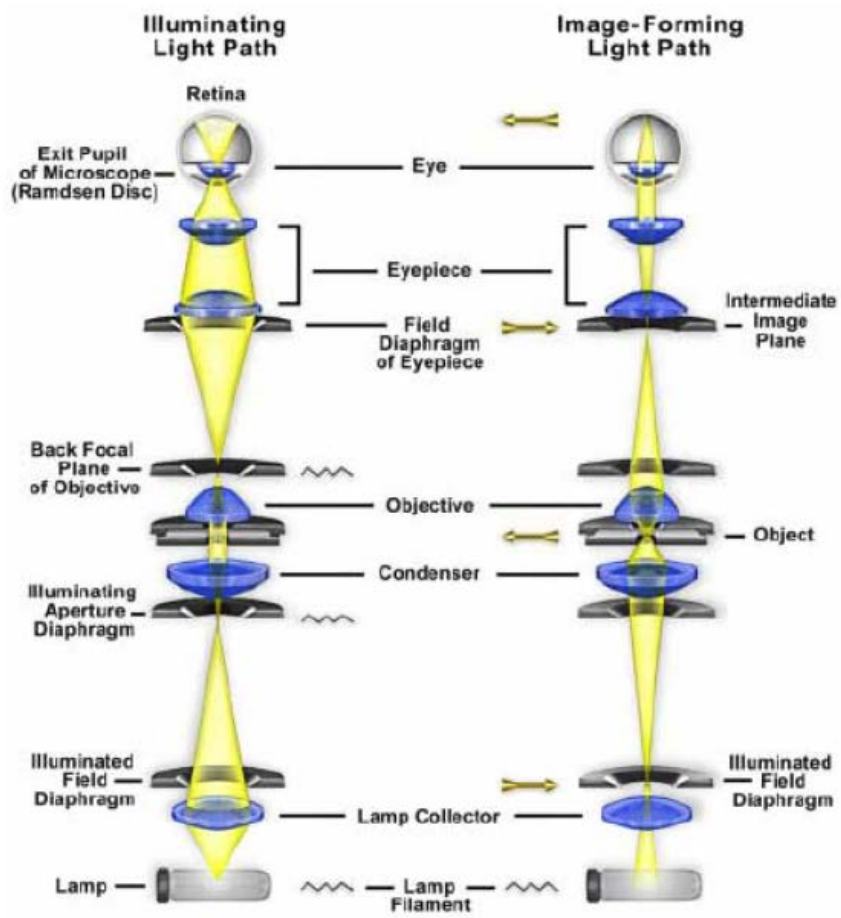


Galileo 1609

Kepler 1611

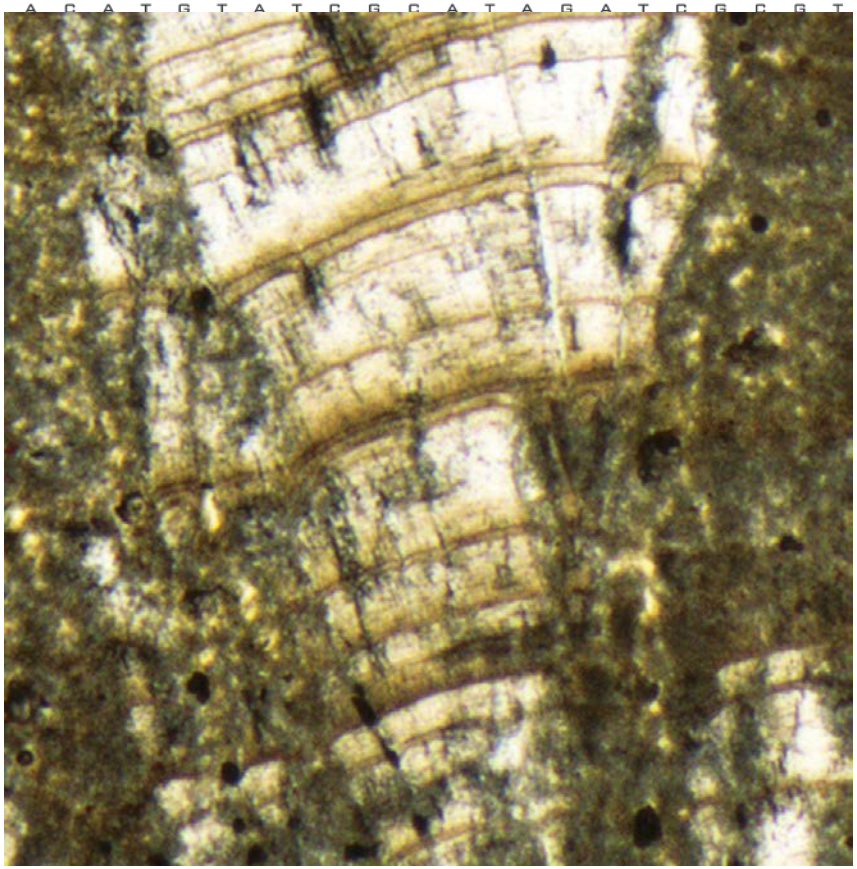
Modern Bright Field Light Path



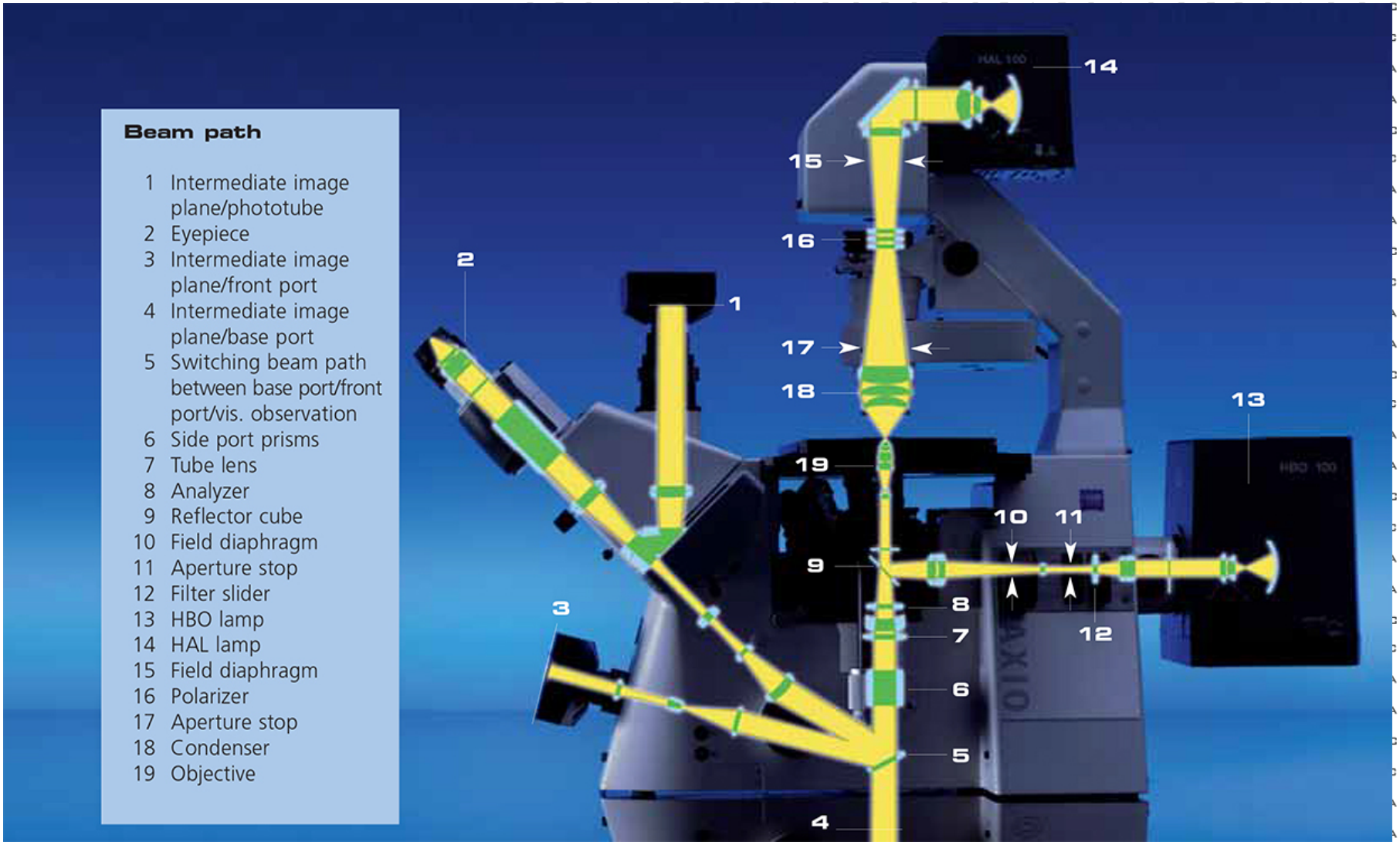


http://www.microscopyu.com/tutorials/java/kohler/index.html

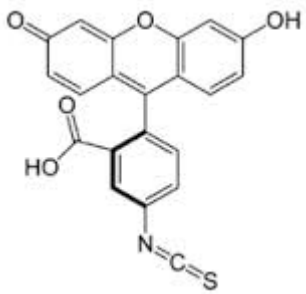
Modern Bright Field Image



Modern Fluorescence Light Path



Modern Fluorescence Light Path



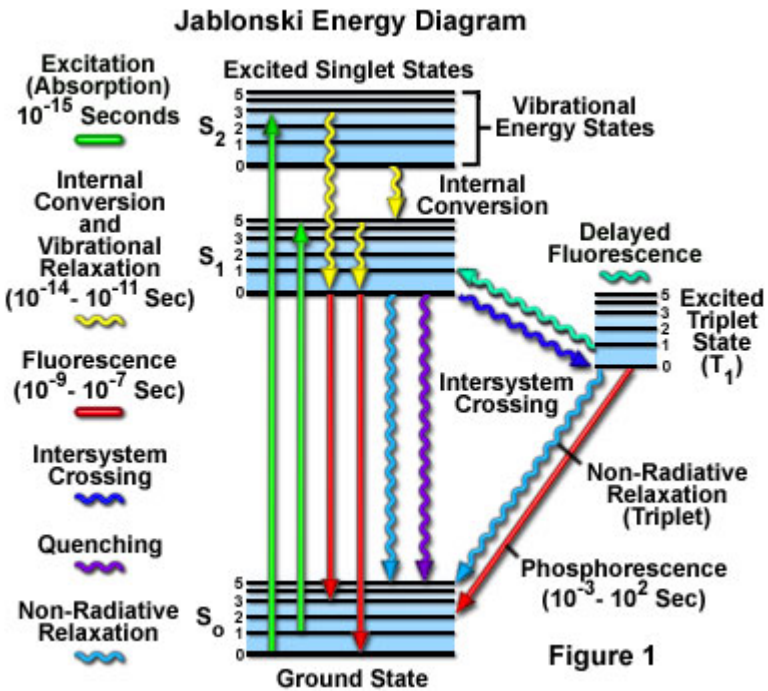
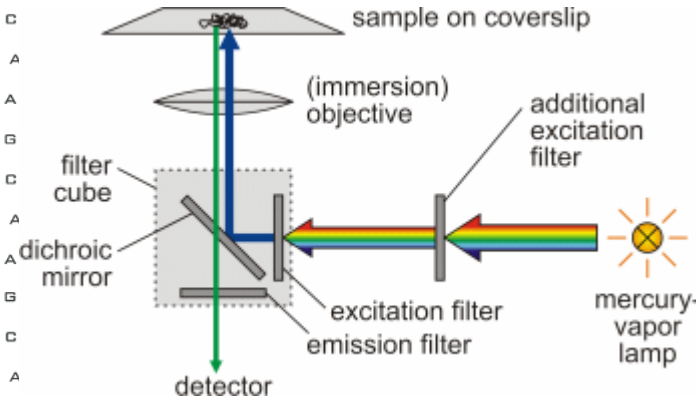
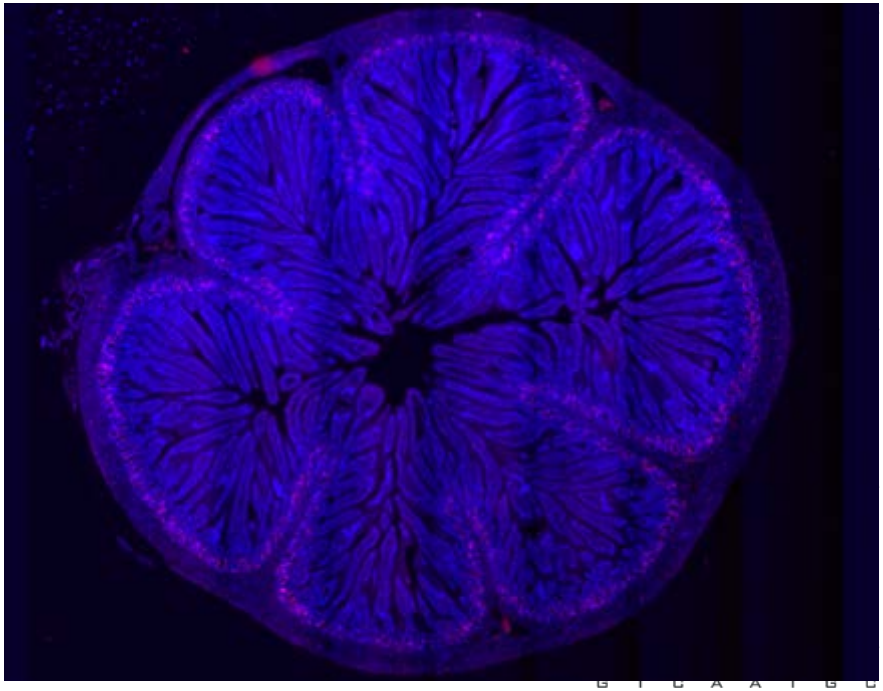
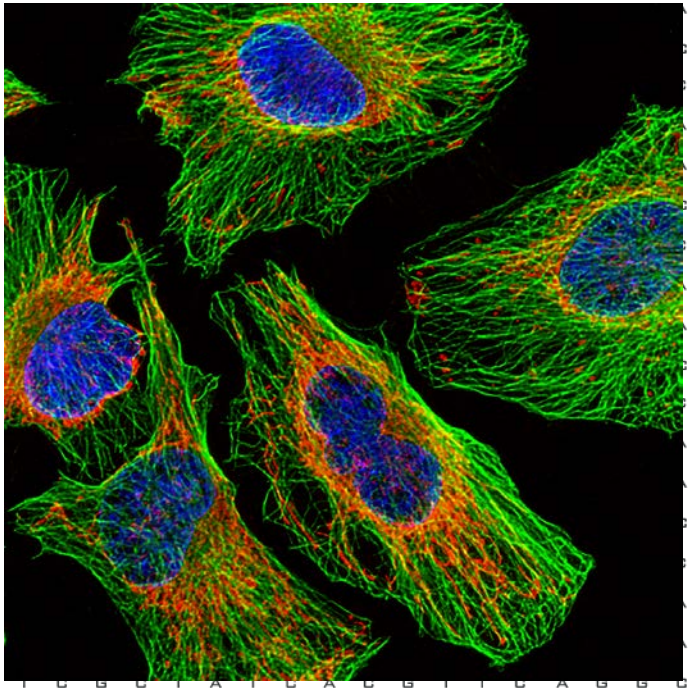


Figure 1



Modern Fluorescence Image





Modern Confocal Light Path

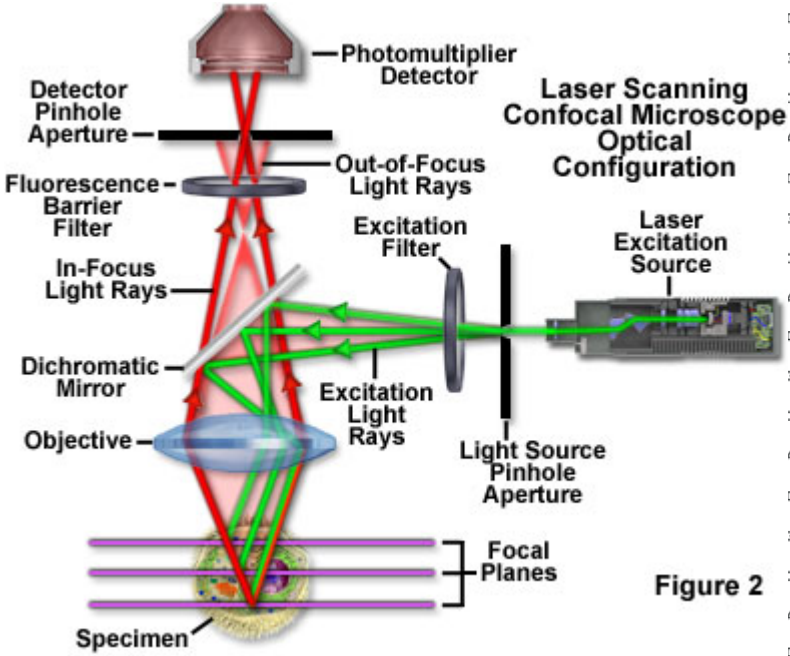
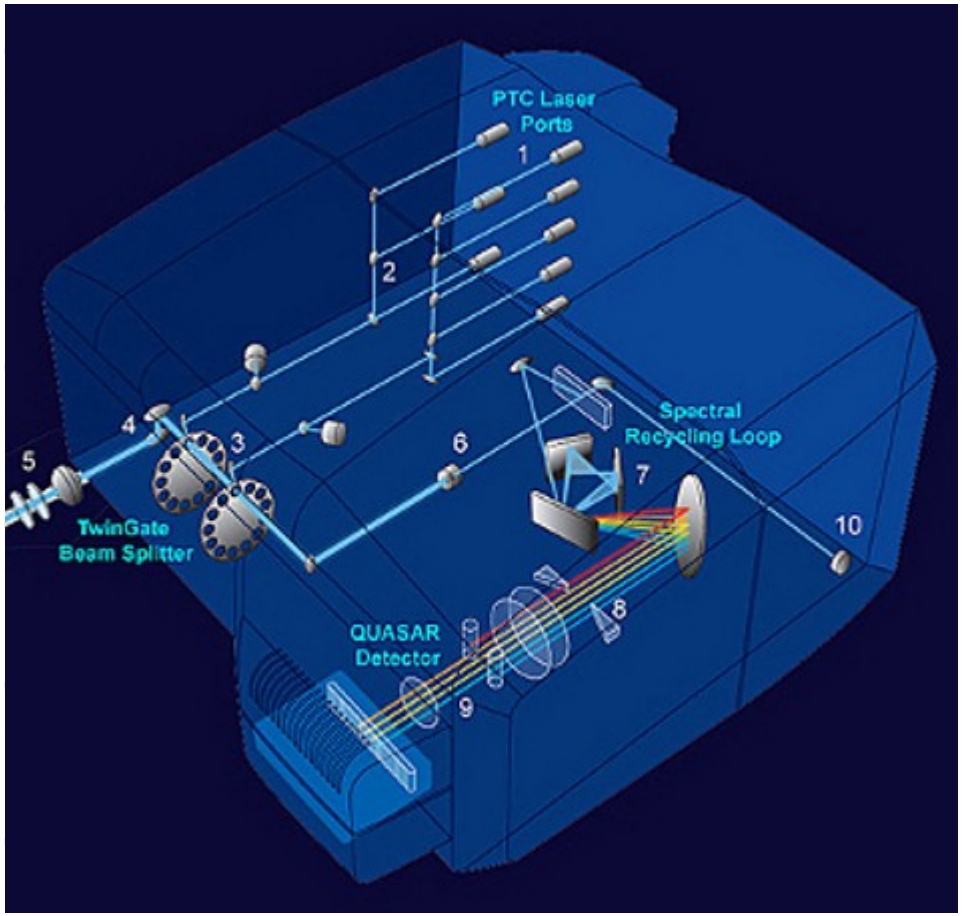
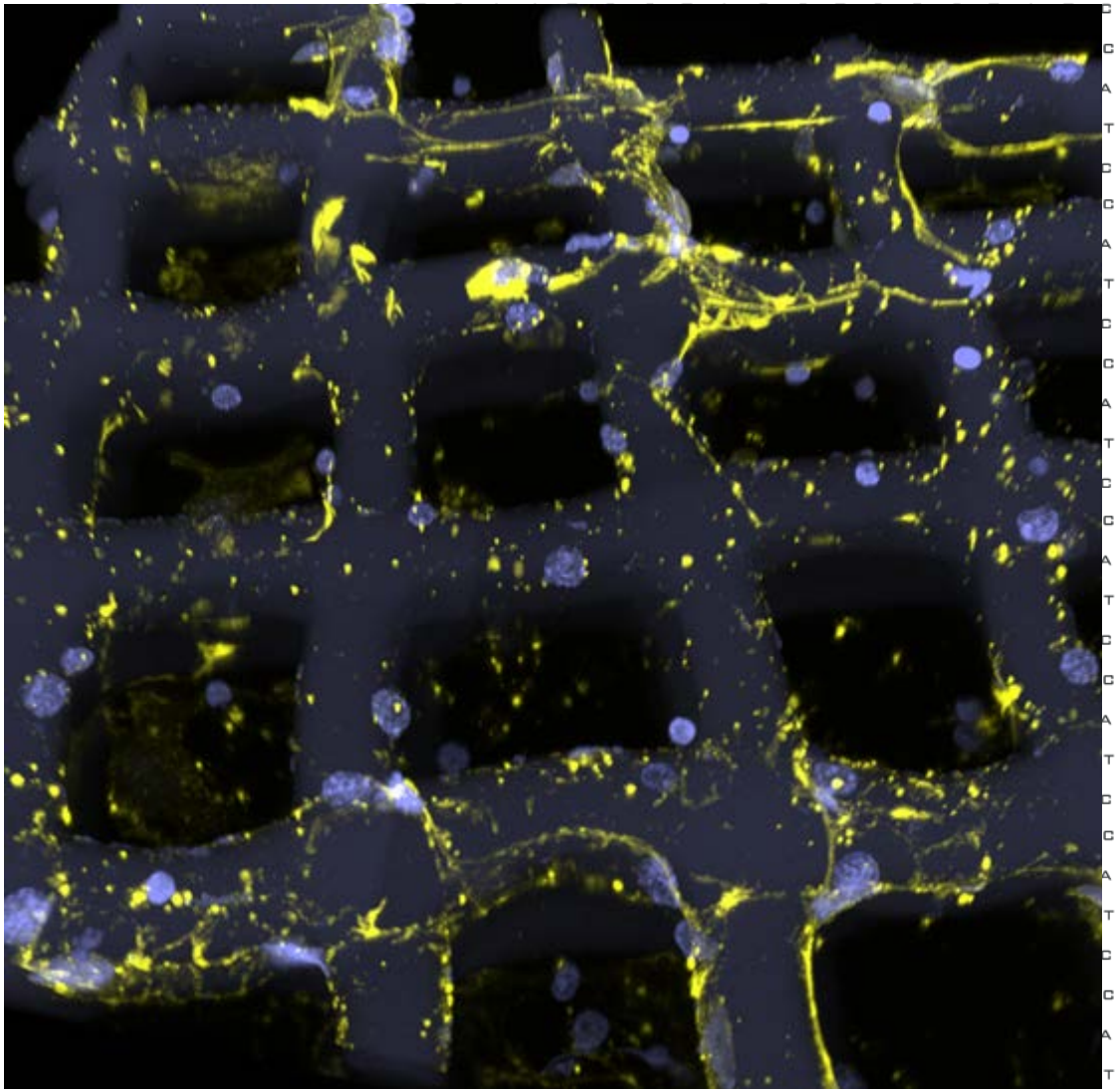


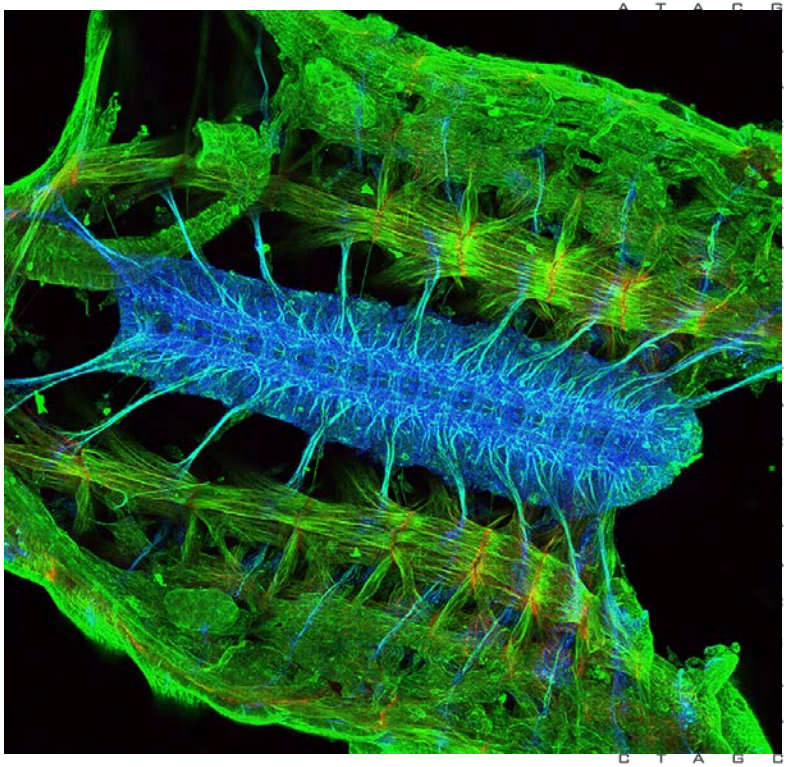
Figure 2

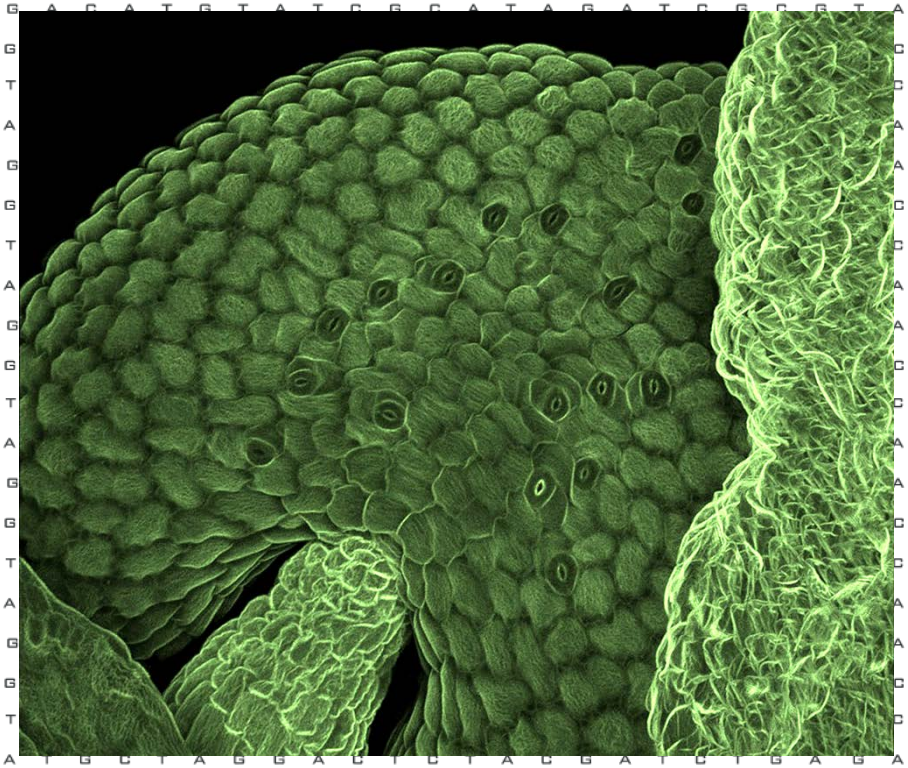


Modern Confocal Image



Modern Confocal Images





Imaging Quality

Optical transfer function

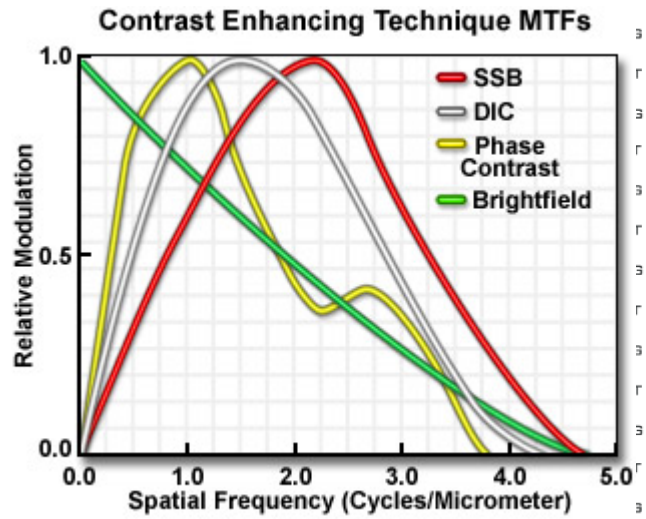
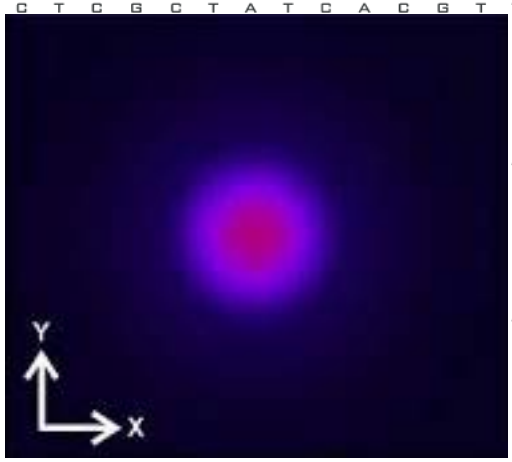
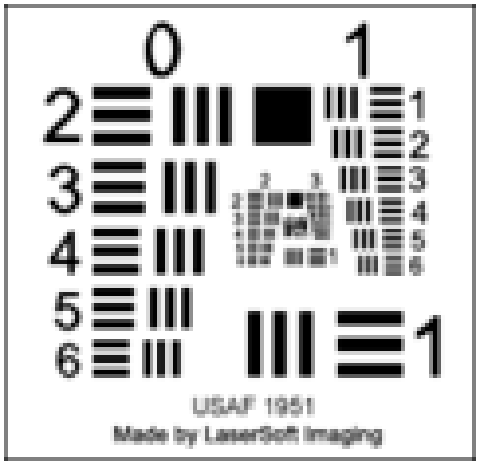


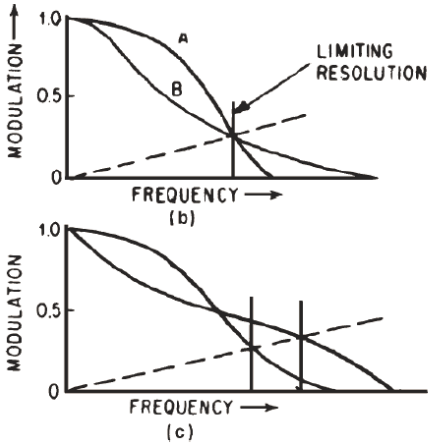
Figure 5

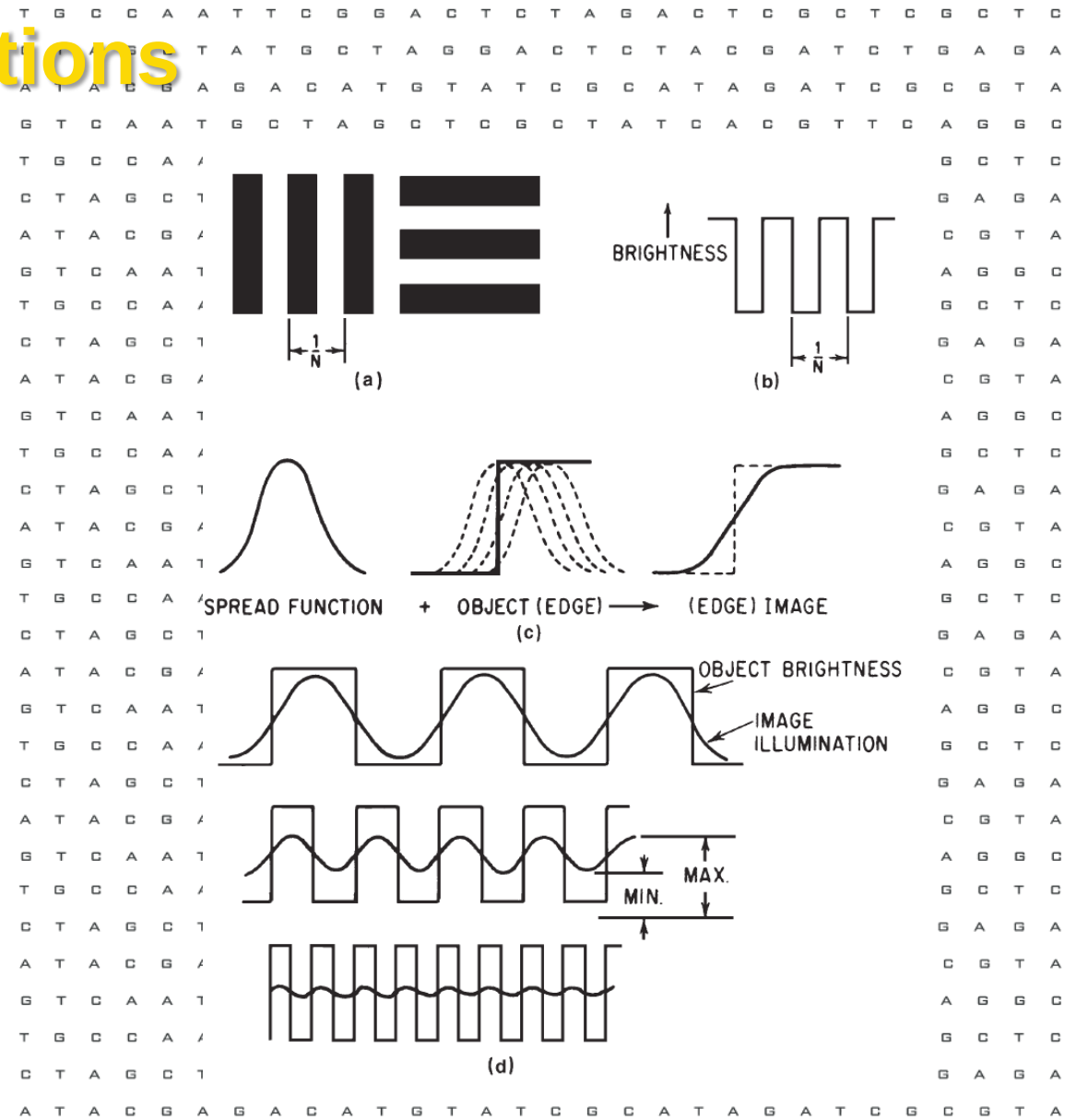
Point spread function



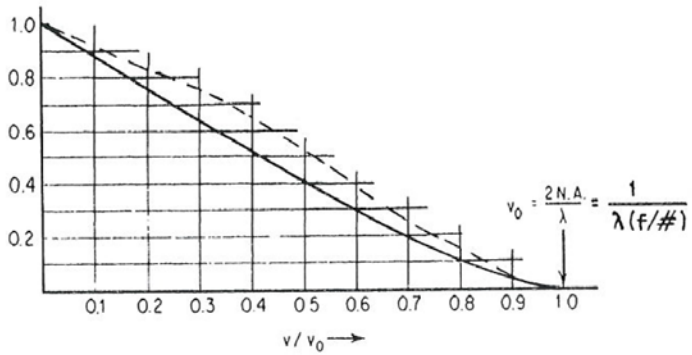
Transfer Functions







Modulation Transfer Function



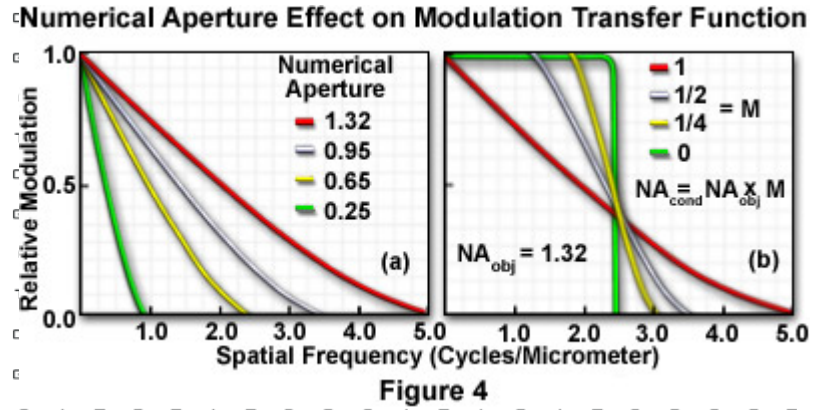
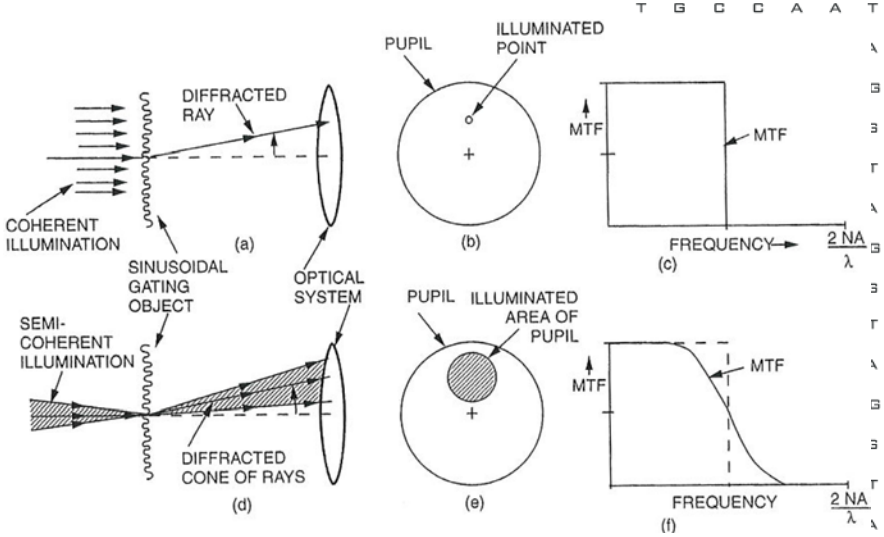
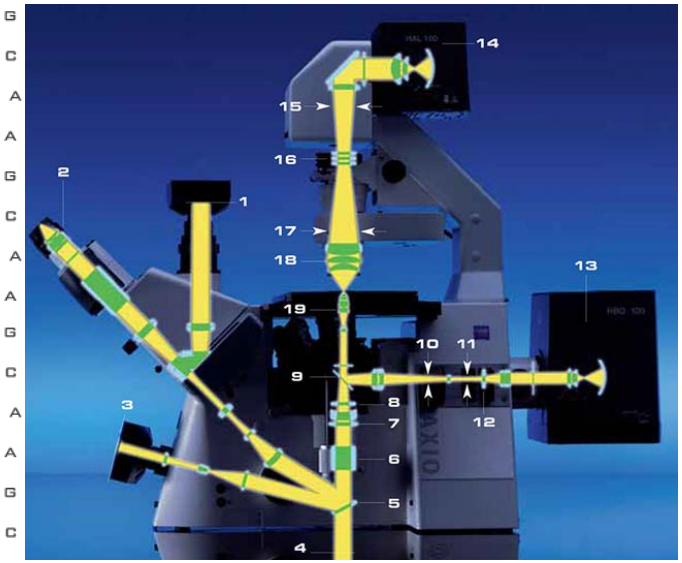


Figure 4

Diffraction Limited Modulation Transfer Function





Modulation Transfer Function

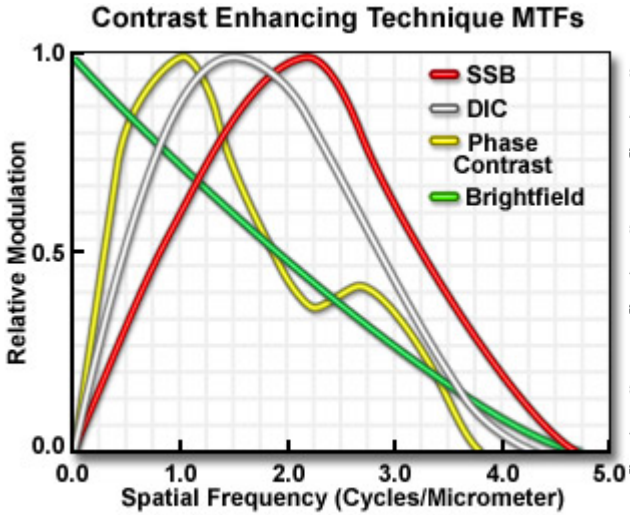


Figure 5

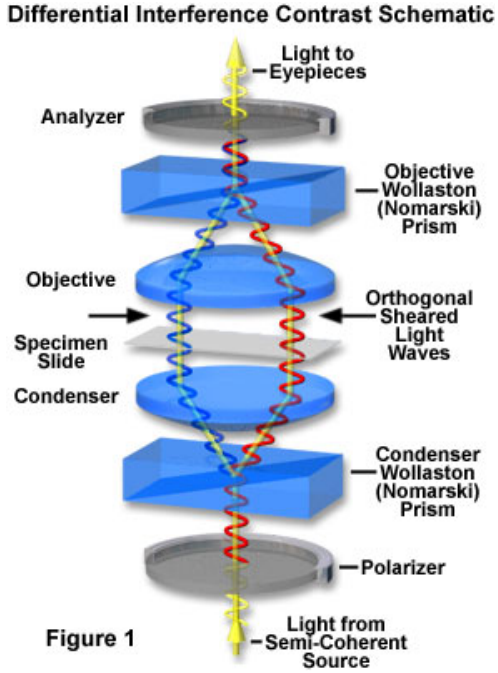


Figure 1

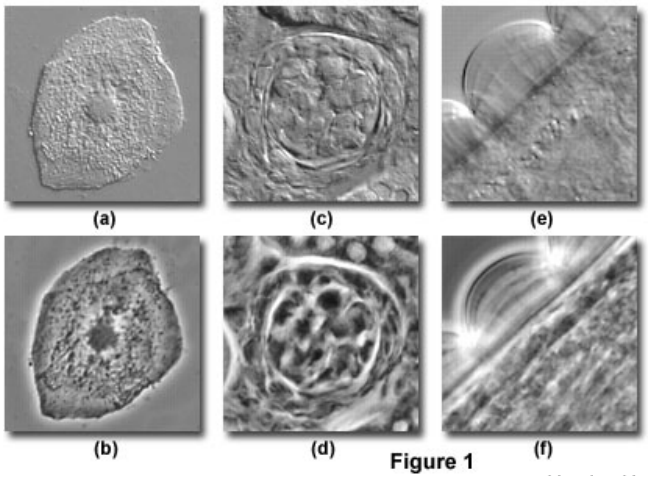


Figure 1

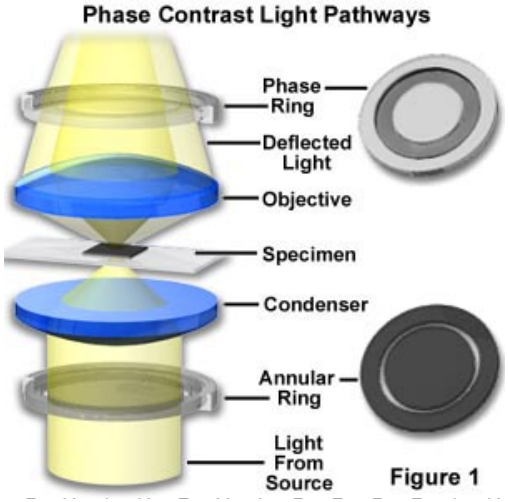
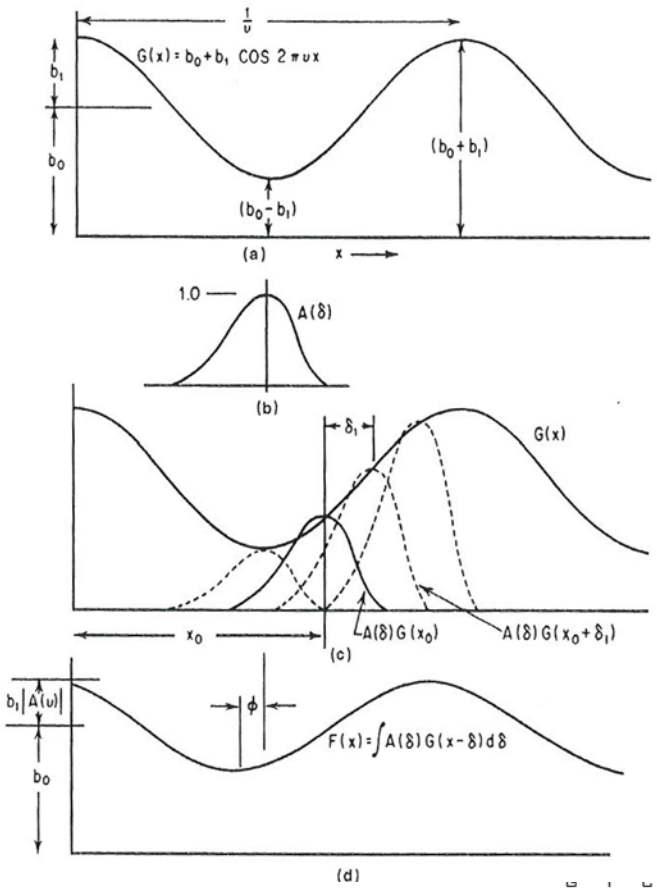


Figure 1

Optical Transfer Function



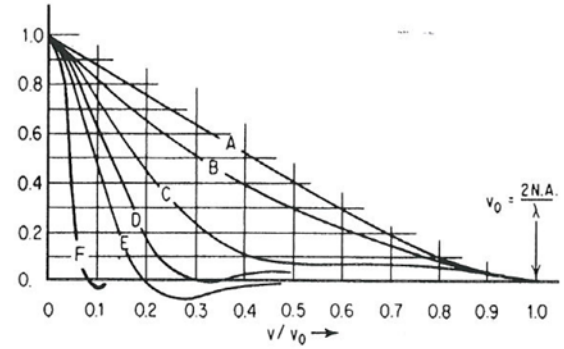
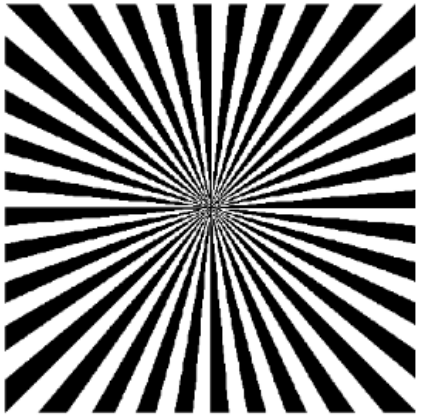
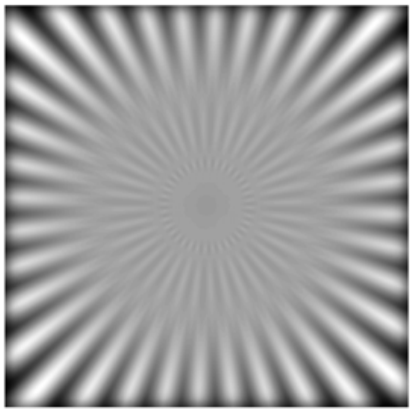


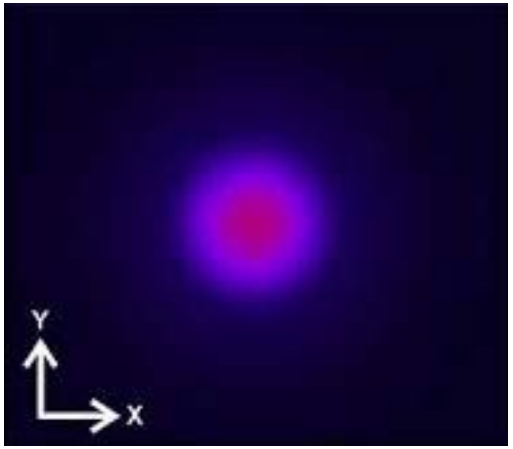
Figure 15.14 The effect of defocusing on the modulation transfer function of an aberration-free system.

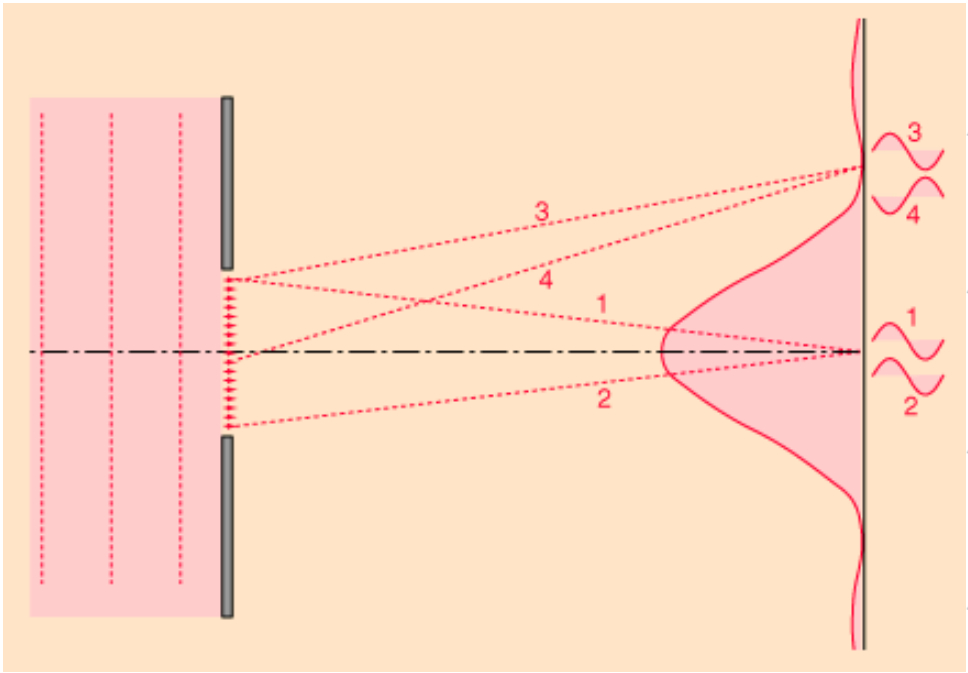
(a) In focus		OPD = 0.0
(b) Defocus	$= \lambda/(2n \sin^2 U)$	OPD = $\lambda/4$
(c) Defocus	$= \lambda/(n \sin^2 U)$	OPD = $\lambda/2$
(d) Defocus	$= 3\lambda/(2n \sin^2 U)$	OPD = $3\lambda/4$
(e) Defocus	$= 2\lambda/(n \sin^2 U)$	OPD = λ
(f) Defocus	$= 4\lambda/(n \sin^2 U)$	OPD = 2λ





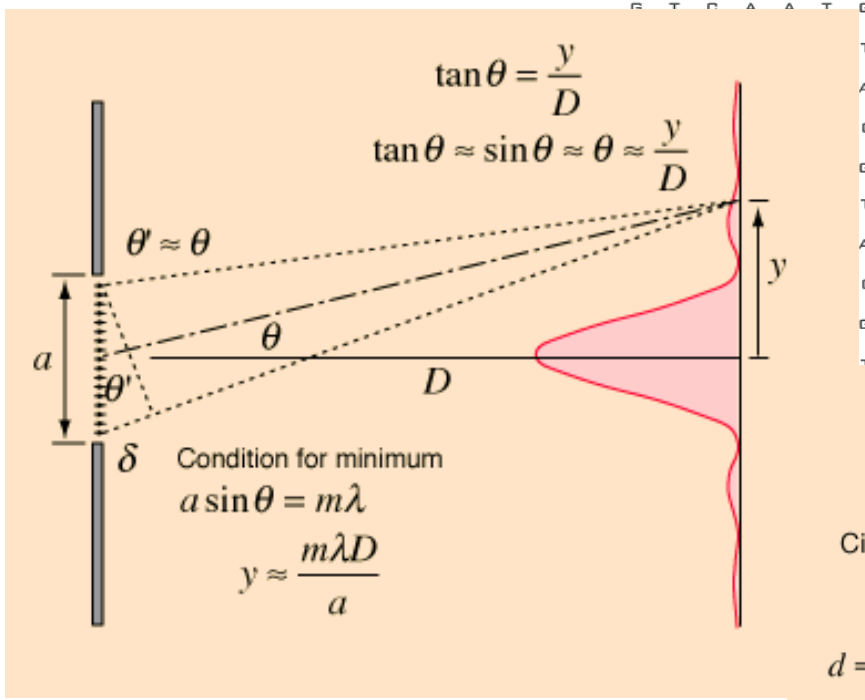
Point spread function

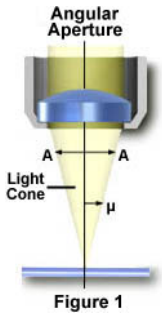




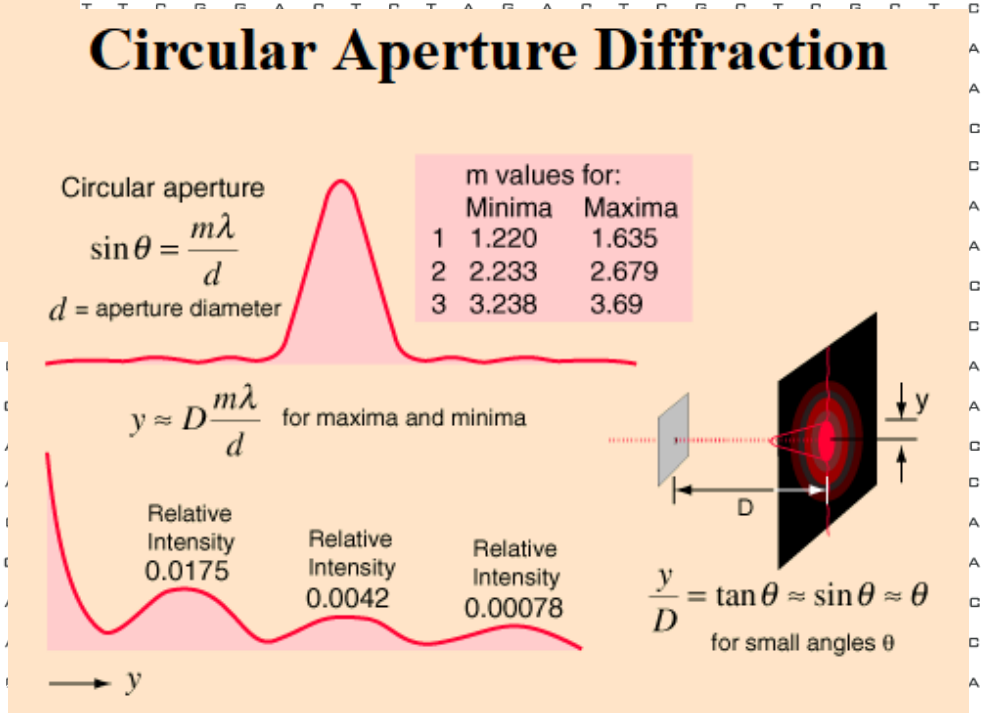
Numerical Aperture (NA) = n(sin μ)

Numerical Aperture

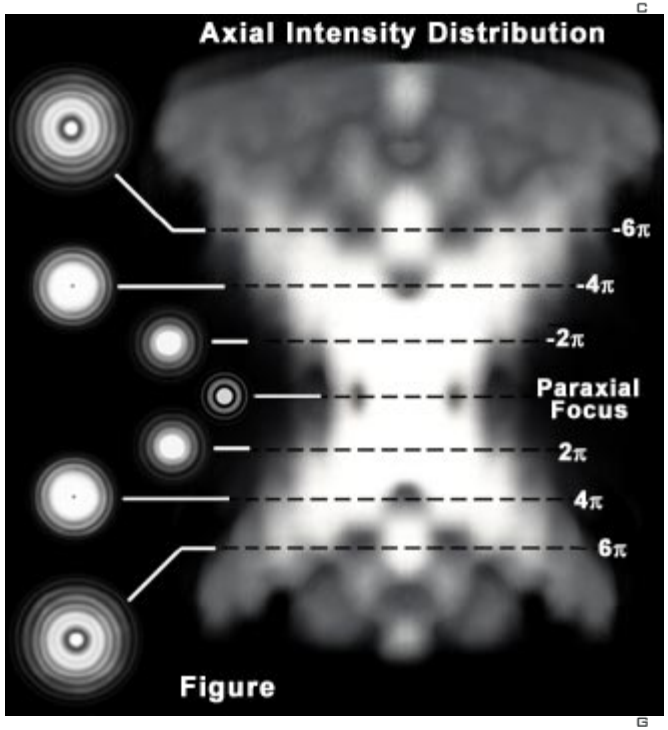




Numerical Aperture (NA) = n(sin μ)



Point Spread Functions Wide Field



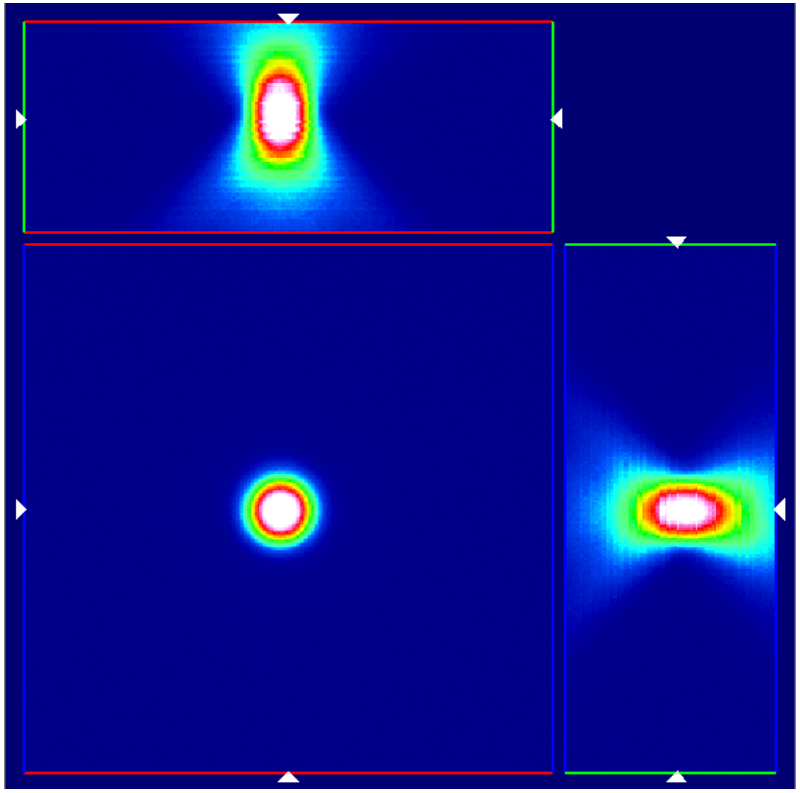
Depth of field

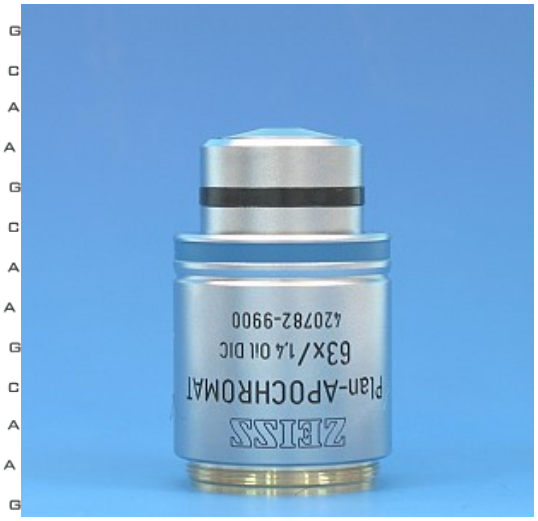
$$d = \frac{\lambda\sqrt{n^2 - (NA)^2}}{(NA)^2}$$

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

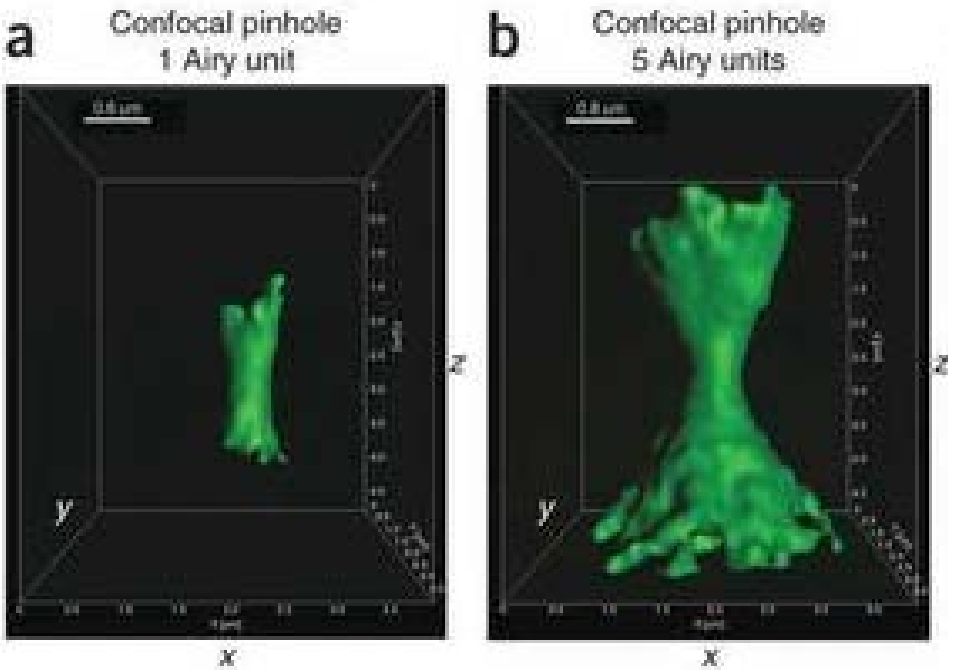
Shillaber, Charles Patten; *Photomicrography In Theory and Practice*., John Wiley and Sons, New York, 778 pages (1944).
http://micro.magnet.fsu.edu/primer/anatomy/focusdepth.html

Point Spread Functions Wide Field





Point Spread Functions Confocal



30% lateral improvement in resolution compared to wide field

Nature Protocols 6, 1929–1941 (2011)

Resolution

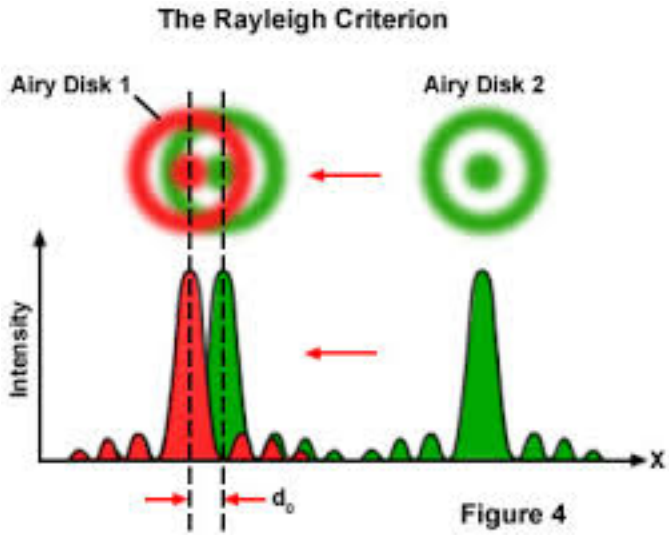
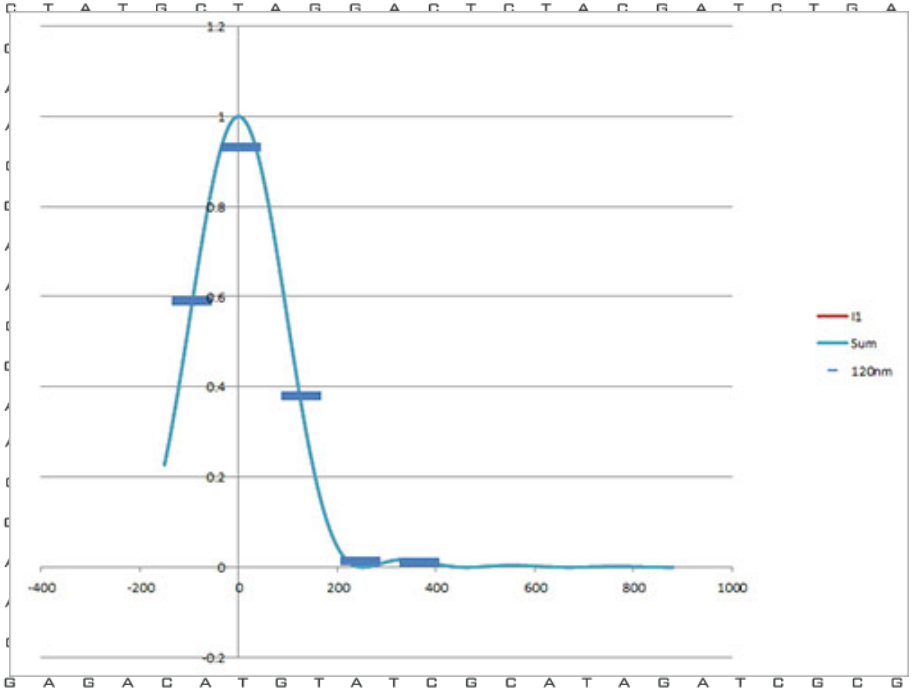


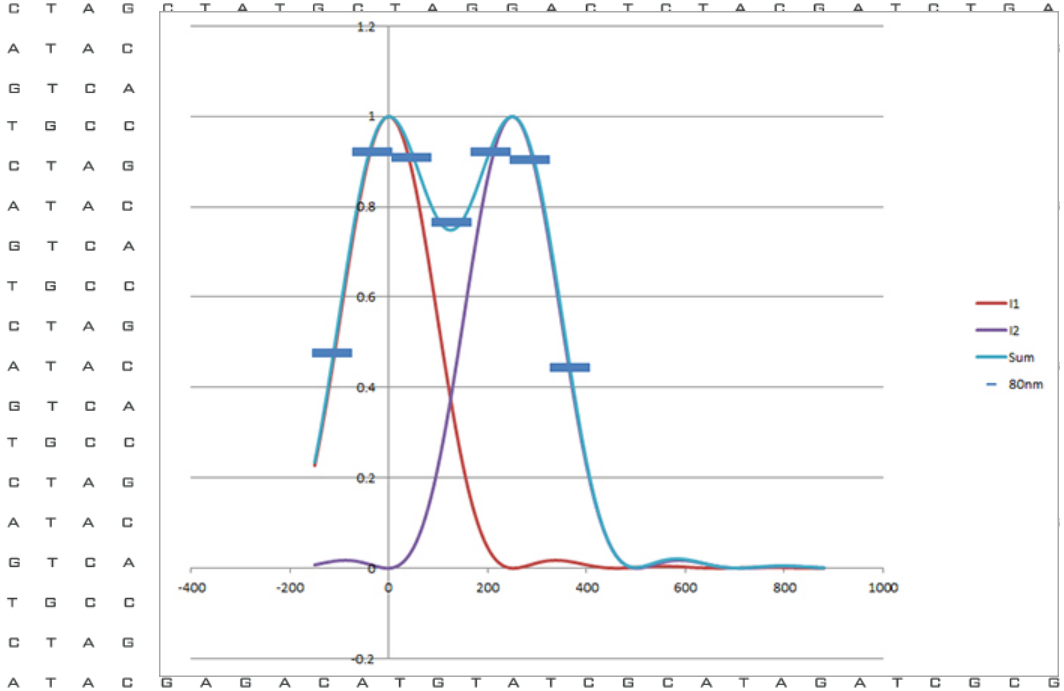
Figure 4

Camera affects resolution

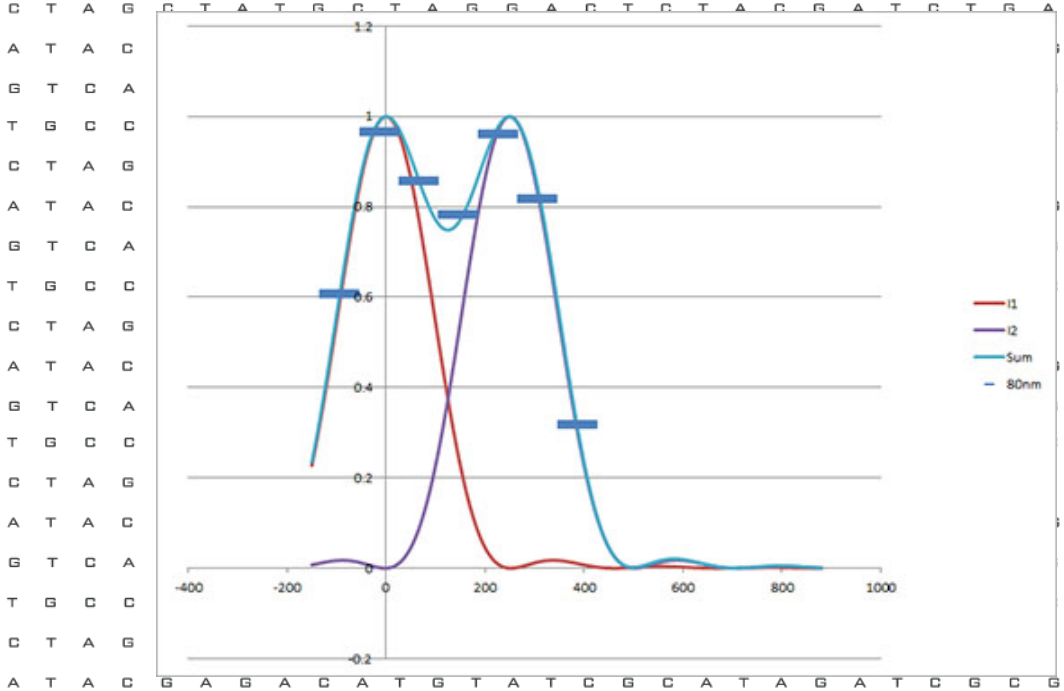
If a function $x(t)$ contains no frequencies higher than B hertz, it is completely determined by giving its coordinates at a series of points spaced $1/(2B)$ seconds apart



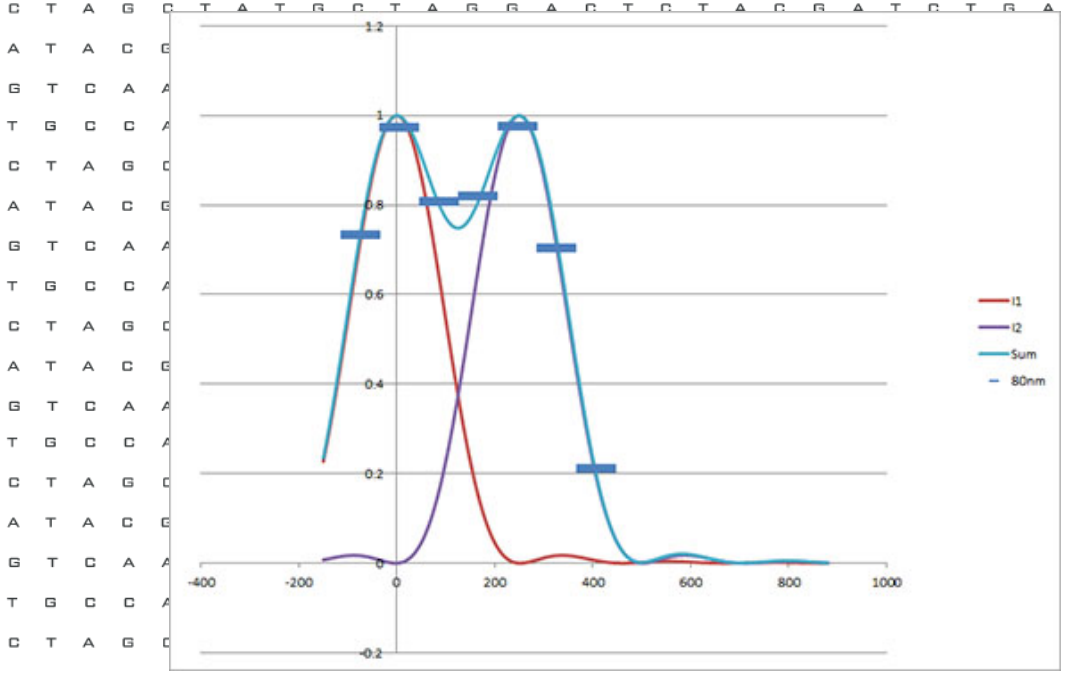
Camera affects resolution



Camera affects resolution

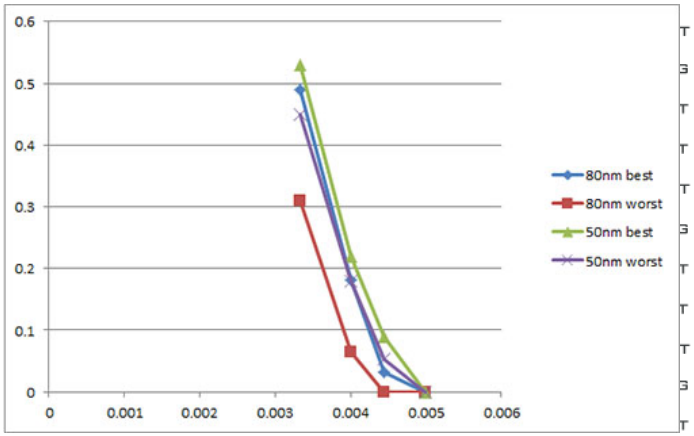


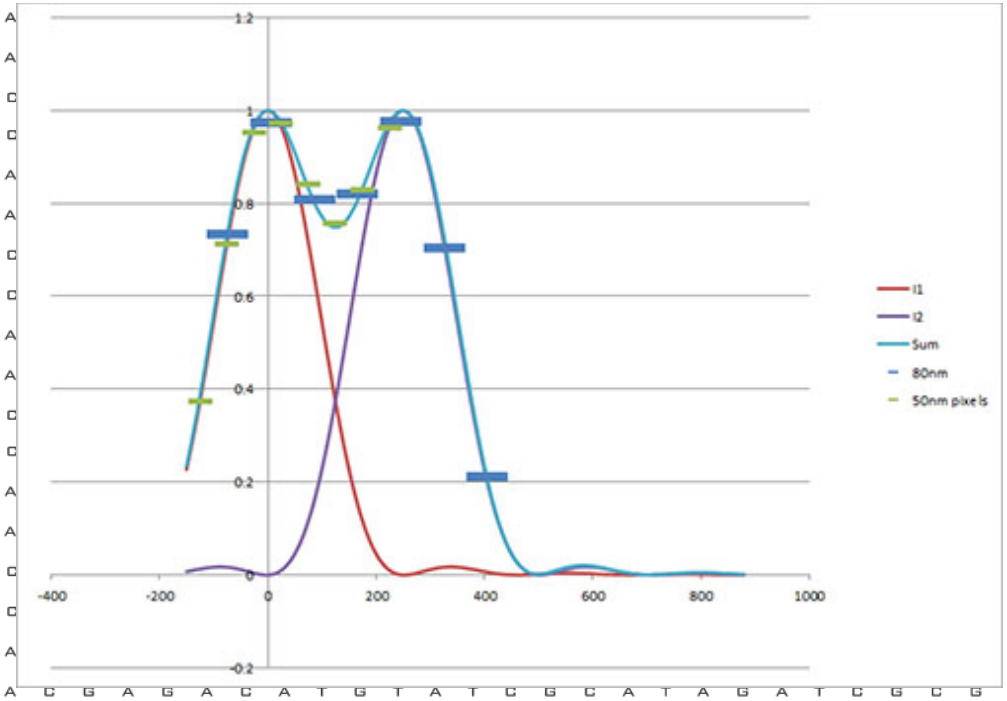
Camera affects resolution



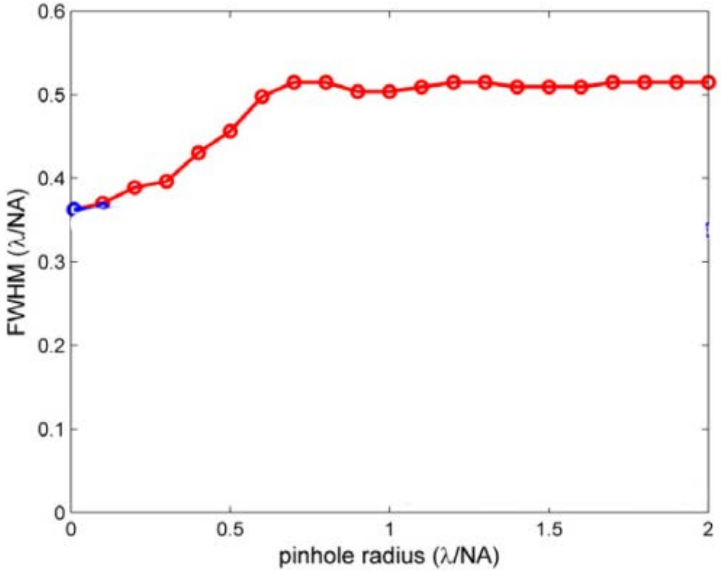
Resolution

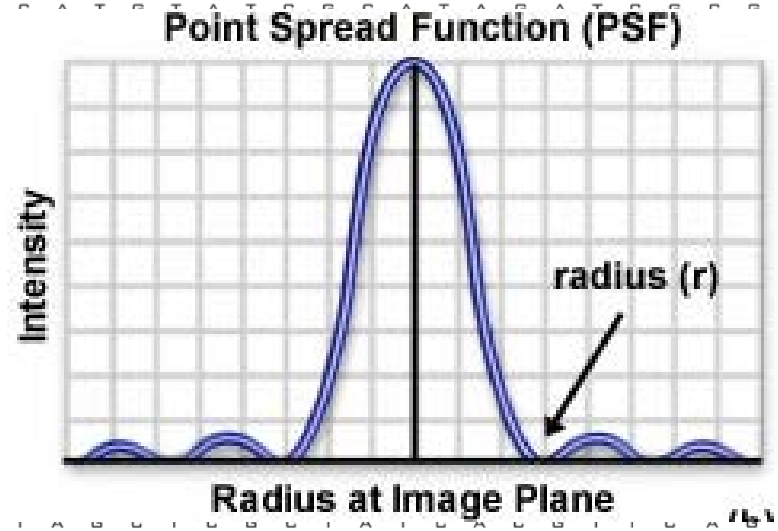
spacing	80nm best	80nm worst	50nm best	50nm worst
200	0	0	0	0
225	0.032	0	0.089	0.053
250	0.182	0.065	0.22	0.18
300	0.49	0.31	0.53	0.45





Pinhole size and resolution





Displaced Pinhole and Resolution

Super-resolution in Confocal Imaging

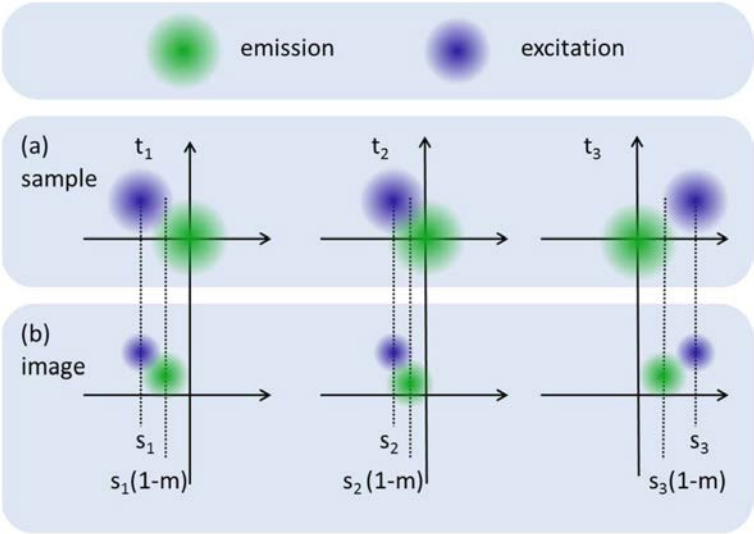
C. J. R. Sheppard,
University of Oxford, Department of Engineering Science, Parks Road, Oxford OX1 3PJ U.K.

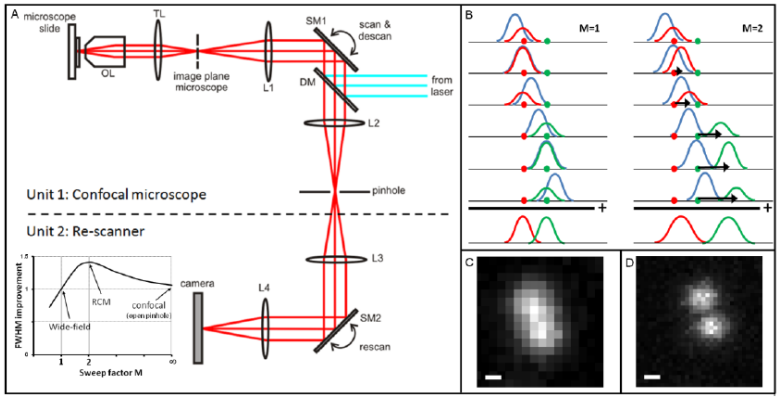
Received January 5, 1988

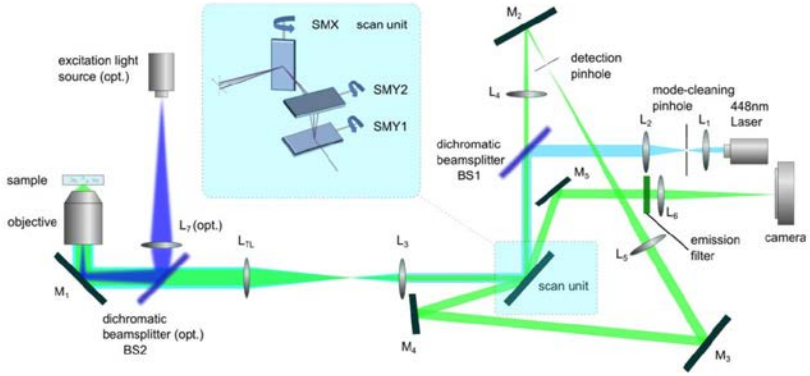
for a displaced point detector (fig. 1), the effective point spread function is given by the product of an Airy disc with a displaced Airy disc. For a system with two equal objectives, this product is symmetrical about a point one half of the demagnified distance from the axis. So a displaced point detector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



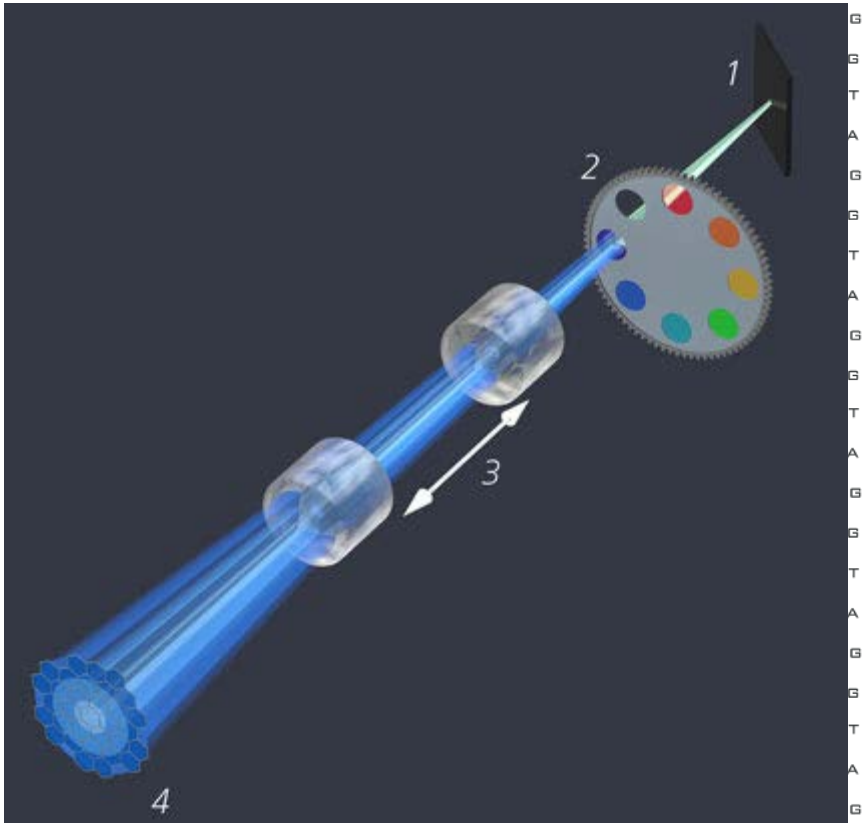


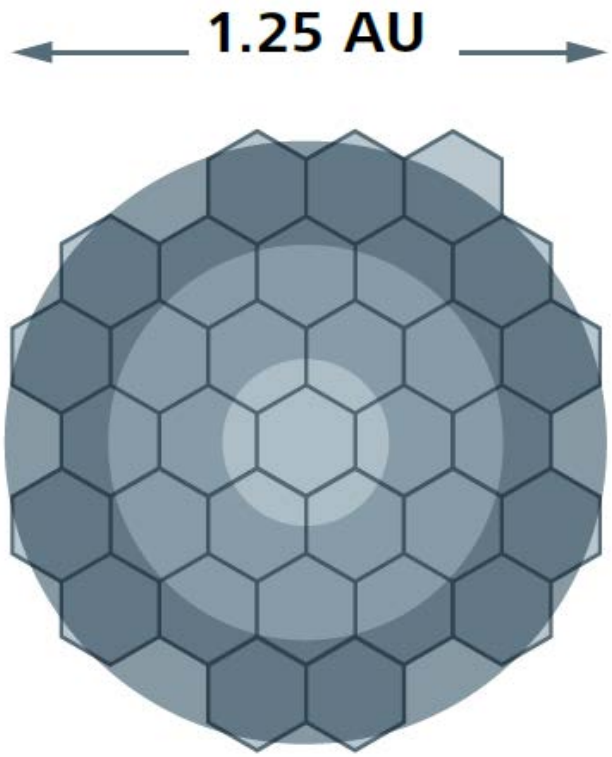


Re-scan confocal microscopy: scanning twice for better resolution
Giulia M.R. De Luca,1,7,* Ronald M.P. Breedijk,1,7 Rick A.J. Brandt,1
Christiaan H.C. Zeelenberg,1 Babette E. de Jong,1 Wendy Timmermans,4
Leila Nahidi Azar,5 Ron A. Hoebe,6 Sjoerd Stallinga,2 and Erik M.M. Manders1,3 2013

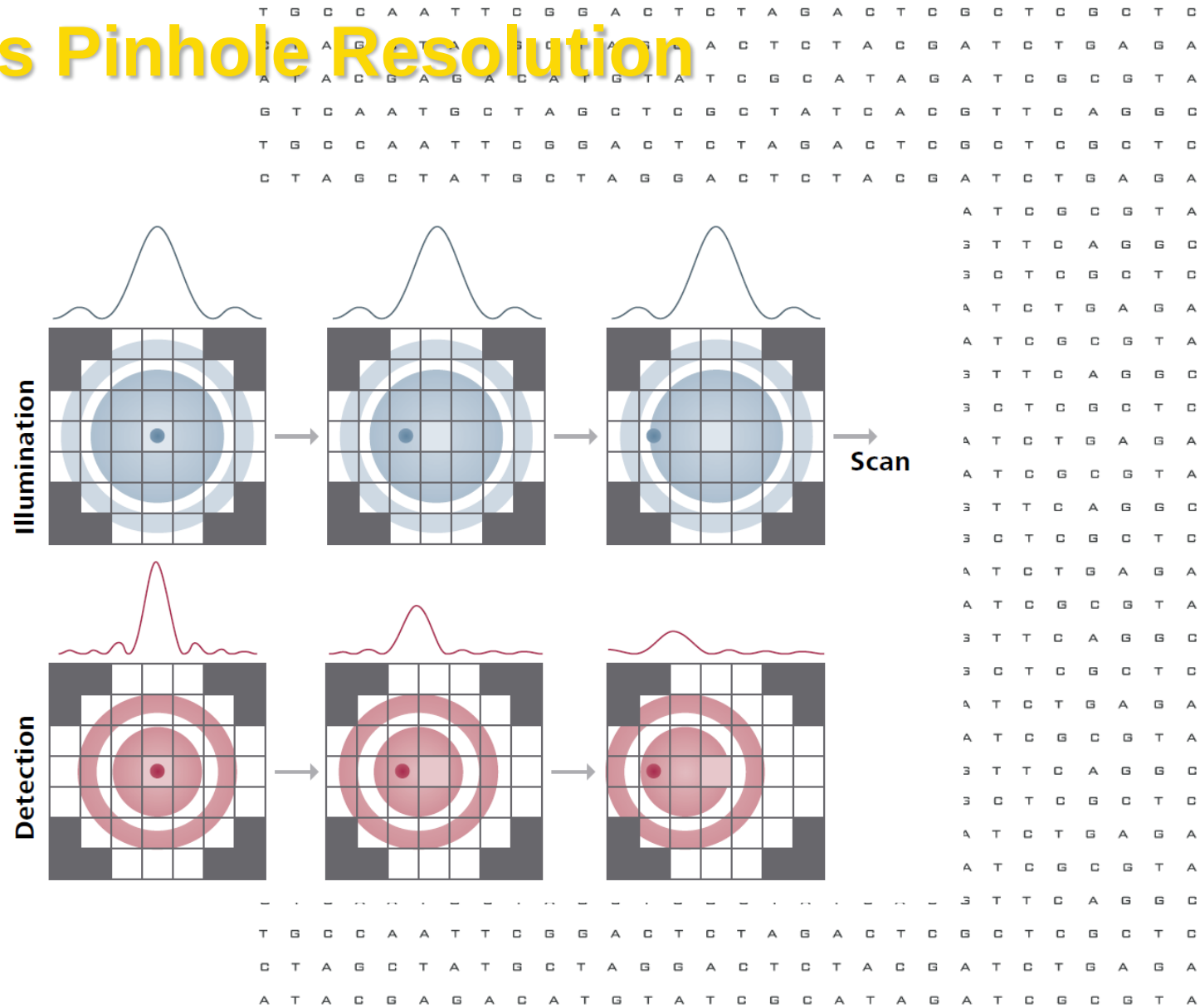
Optical Photon Reassignment Microscopy (OPRA)
Stephan Roth1,2, Colin J. R. Sheppard3, Kai Wicker1,2 and Rainer Heintzmann1,2,4 2013

Airyscan Resolution

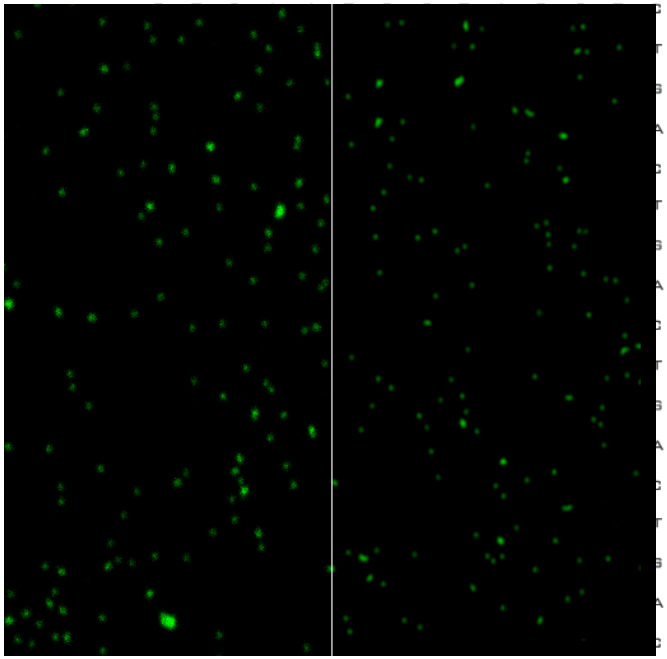


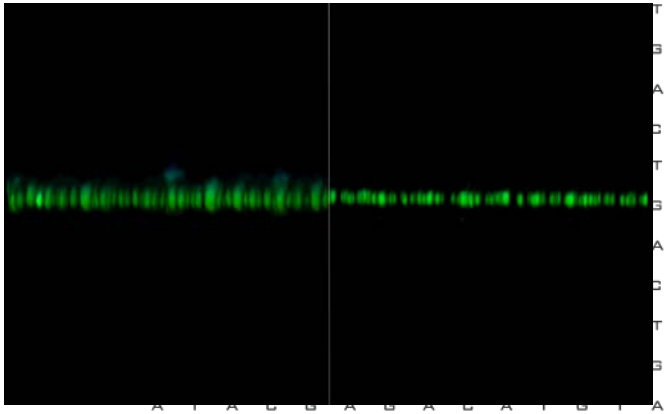


Off Axis Pinhole Resolution

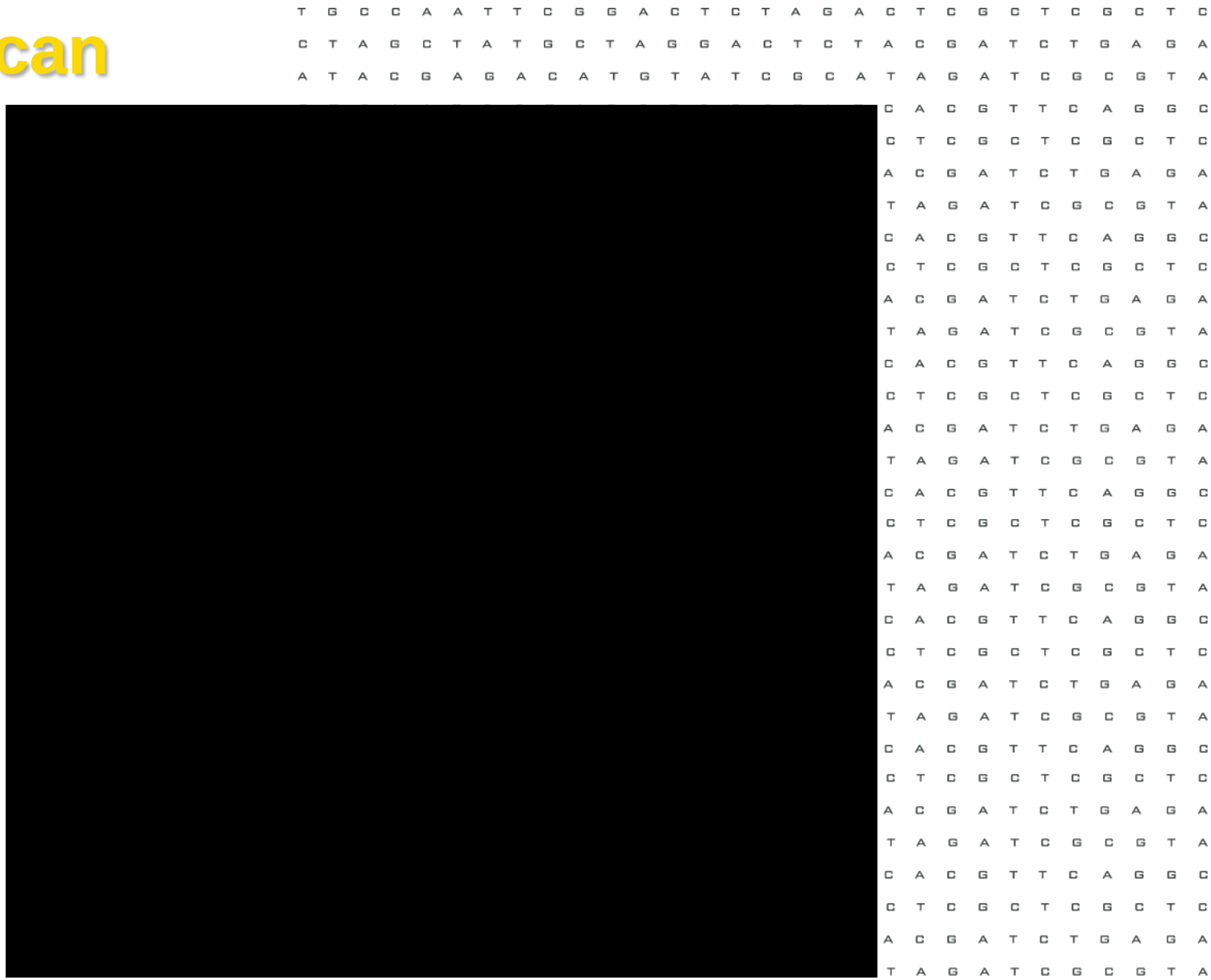


Confocal vs Airyscan





Airyscan



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