


## DIFRACTION









## Transmitted-light. Bright-field



An unusual way of looking at things!


Consider that every ray leaving the object carries some information about fine detail in the object




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# Airy and Rayleigh 



Airy (1801-1892)
Astronomer
The image of a point source formed by a lens of finite diameter was a disk with halos around it (left) whose properties depended entirely on the size of the lens.


Rayleigh (1842-1919) explained how the wave nature of light determined how it was scattered (Rayleigh scattering). In microscopy
he gave the first mathematical analysis of resolution, defining a resolution criterion based on the Airy disk and showing how it was determined by the Numerical Aperture of the objective.

## The Image of a point...is not a point








## Image of a point source: the Airy Pattern

Small aperture
Larger aperture


## The Image of a point....



This is caused by


Image of a point source: The Airy Pattern

Larger aperture and
shorter wavelength
$\square$ smaller disc
better resolution








## What does it mean?

practical example...

$$
\begin{gathered}
\mathrm{r}=\lambda / 2 \mathrm{n} \sin \alpha \\
\mathrm{r}=\lambda / 2 \mathrm{NA}
\end{gathered}
$$

$\lambda=550 \mathrm{NM}$ (GREEN CENTRE OF THE SPECTRUM)

SIN $\alpha=0.65$ (HALF THE ANGLE 40.50; ACCEPTANCE ANGLE 810)
Typical value for 40X objective

$$
\mathrm{r}=\frac{550}{2 \times 0.65}=423 \mathrm{~nm}
$$





## Increasing image contrast



Low contrast


Edge contrast


High contrast


Colour contrast

## Contrast may be altered...

- In the Specimen by Staining
- In the Microscope by optical and illumination techniques techniques

Colour Filters
Dark Field
Phase Contrast
Differential Interference Contrast

- In the Photographic Image by Choice of film

Choice of developer
Choice of printing paper

- In the Video or Digital Image by Electronic adjustments

Computer manipulation

## DARMFED



## Darkfield Microscopy







Fig. 62. Oblique Light with a Condenser.
(From Chamot).
'Digital'

## oblique illumination

- ie using your finger!

A 'no-cost' option for most microscopes

## PHASE

CONTRAST

# The full name of the microscopy technique could be something like 

"phase-strip method for observing phase objects in good contrast, but shortened is phase contrast."


Frits Zernike (1888-1966)

Transforms differences in relative phase of object waves.... to amplitude differences in the image

## Original Phase Contrast Photomicrographs of Human Cells



Brightfield
Phase Contrast




PHASE OBJECT



## Positions where amplitudes are equal



## Positions where amplitudes are equal



In these positions the diffracted ray must have a value of zero

## Positions where amplitudes are equal



In these positions the diffracted ray must have a value of zero

## Positions where amplitude of resultant is less than that of zero order



In these positions the diffracted ray must have a negative value


In these positions the diffracted ray
must have a positive value

## Points for plotting the diffracted ray






## One wavelength



Half a wavelength


Quarter of a wavelength


## Diffracted ray now one half wavelength behind zero order



The diffracted ray is now in a position to interfere destructively with the zero order, but it is of lower amplitude

# Diffracted ray now one half wavelength behind zero order and amplitude of zero order reduced 



New Resultant beam of low amplitude, providing contrast


## Phase Contrast Optical Components



## Phase Contrast Objective



Phase Condenser Annulus Plate Alignment


Phase Contrast Light Pathways


## View of Objective Back Focal Plane for Dark Contrast Phase Objective



## The final result -




## Phase Contrast Gives Contrast to Structural Detail in Transparent Specimens




## View Objective Back Focal Plane

Objective Phase Ring

Miss-Aligned Annular Illumination
 Phase Ring in Objective



Cheek Cell, No
Condenser Annulus





## Polarization of Light Waves



## Light waves



## Crossed polars









## The Michel-Lévy Interference Colour Chart



## Transmission of Polarized Light Through an Analyzer






Nylon Fiber in Polarized Light

(a)

(b)


Figure 11

Chrysotile Asbestos Fibers in Polarized Light

(a)

(b)


Figure 7

$0$

## Constructive Interference



D = Propagation Direction
$\mathrm{A}=$ Amplitude

$A_{w}+A_{w 1}=A_{R}$ (Resultant)
Waves in Phase


Path Differnce $=0$
$\mathrm{C}=$ Vibration
Direction


Young's Double Slit Experiment






## Wollaston Prism Interference Fringes




The final result -


40x



