



# DIFRACTION

































### 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.













# Image of a point source: the Airy Pattern

## Small aperture

### Larger aperture




















#### .....What does it mean?

A practical example...

$$r = \lambda / 2n \sin \alpha$$
$$r = \lambda / 2NA$$

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

SIN  $\alpha$  = 0.65 (HALF THE ANGLE 40.50; ACCEPTANCE ANGLE 810)

**Typical value for 40X objective** 

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



Claude Monet





#### Increasing image contrast



#### Contrast may be altered...

- In the <u>Specimen</u> by Staining
- In the Microscope by optical and illumination techniques techniques Colour Filters Dark Field Phase Contrast Differential Interference Contrast
- In the <u>Photographic</u> Image by Choice of film Choice of developer Choice of printing paper
- In the <u>Video or Digital Image</u> by Electronic adjustments Computer manipulation

## DARK FIELD













#### '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** 











#### Positions where amplitudes are equal



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

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



In these positions the diffracted ray must have a negative value

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



In these positions the diffracted ray must have a positive value

Deter Frees

#### Points for plotting the diffracted ray











O Datas Even





Diffracted beam now approximately half a wavelength behind zero order

### 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





#### PHASE CONTRAST














#### 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



## Brightfield

## Phase Contrast















Cheek Cell, No Condenser Annulus





Cheek Cell, Miss-Aligned Condenser Annulus





Cheek cell, Properly Aligned Condenser Annulus











### Polarization of Light Waves



# Light waves



# **Crossed** polars





Overlapping pieces of Sellotape





Modified from Graham Dunn & Peter Evennett



Crossed polars Rotated 45° Crossed polars + 1 lambda plate

Modified from Graham Duni







Modified from Graham Dunn & Peter Evennett

### The Michel-Lévy Interference Colour Chart



Modified from Graham Dunn & Peter Evennett







#### POLARIZATION









Chrysotile Asbestos Fibers in Polarized Light



**Oolite Thin Section in Polarized Light** 







Young's Double Slit Experiment














## Wollaston Prism Interference Fringes



Wollaston Prism (Crossed Polarizers)



## The final result -







