Manchester Microscopical & Natural History Society

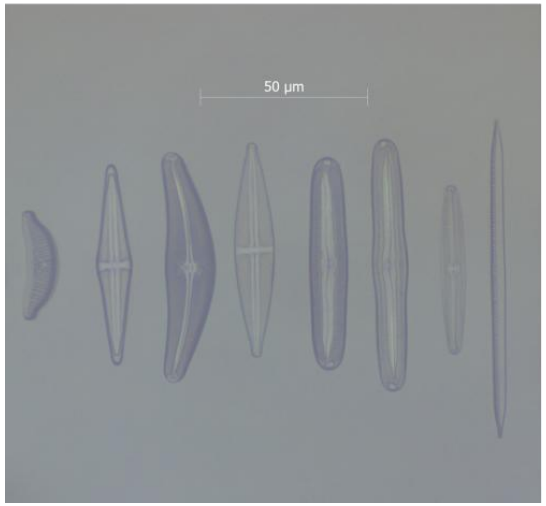
Established 1880

www.manchestermicroscopical.org.uk

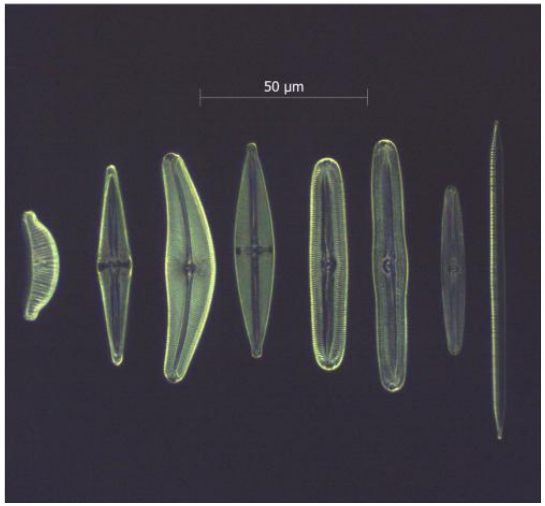
Darkground/Darkfield Microscopy

Another Part of the Microscopist's Armamentarium … for increased visibility

Brightfield



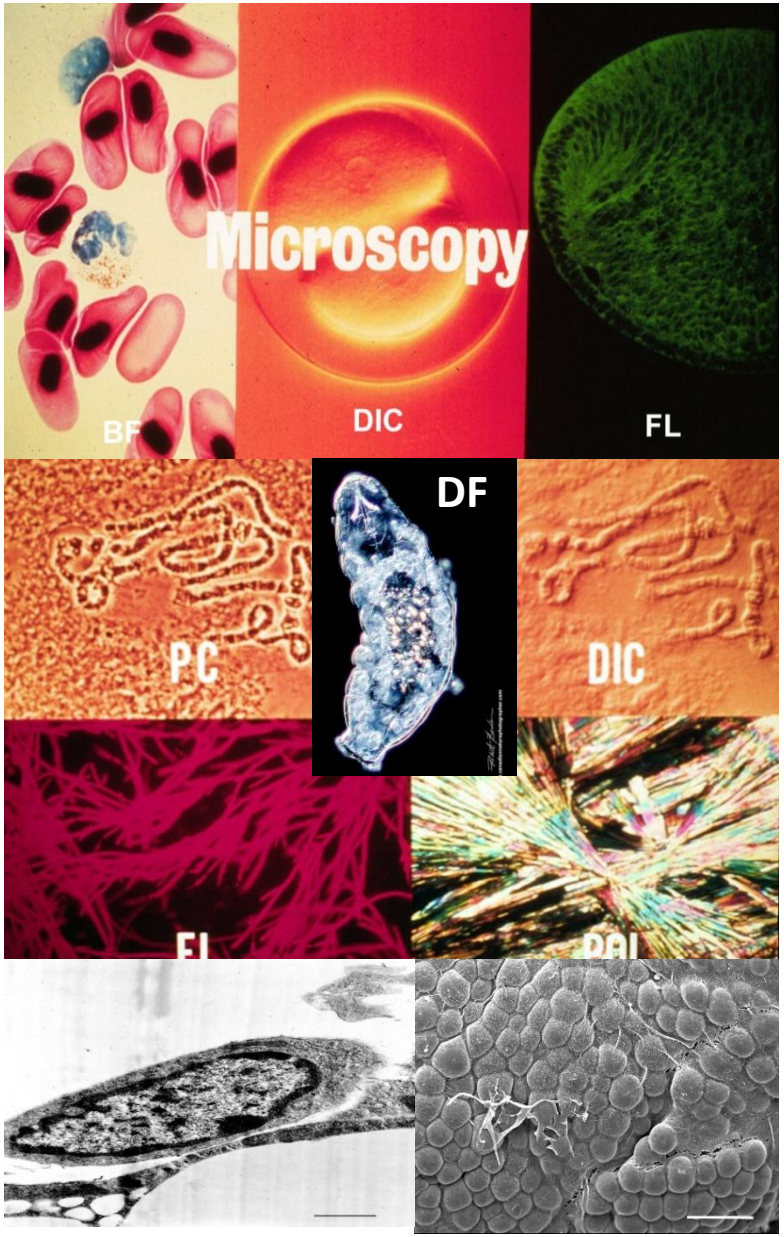
Darkfield



Edinburgh Instruments

Talk &
Demo / Practical

Mike Mahon, *November 21st 2026*



Aim: which technique to use?

- **Light Microscopy** c1600
  - ✓ – bright field + stains
  - – relectance
  - – **dark field** 1800s Add Visibility
  - ✓ – polarising
  - ✓ – phase contrast
  - – interference / DIC
  - – fluorescence
  - ✓ – computerised / confocal
- **Electron Microscopy**
  - ✓ – transmission
  - ✓ – scanning
- **Scanning Probe/Stylus**
- **X-ray, Ion Beam, …**

✓ MMS talks

Darkfield Microscopy Landmarks

From Google AI !!

1. Pre-Formal Observations (17th–18th Century)

- **Robert Hooke & Antoni van Leeuwenhoek:** In the late 1600s, both Hooke and Leeuwenhoek noted that certain highly transparent specimens became much easier to see when lit from extreme side angles (oblique illumination) rather than directly from underneath → **dark background** and **bright specimen**.

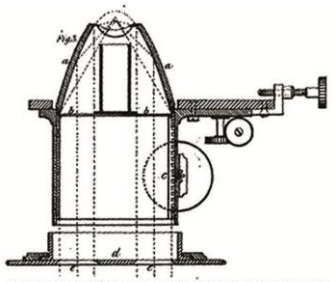
2. Formalization and First Inventions (1830s–1840s)

- **1830:** Darkfield microscopy was formalised as a distinct, intentional technique designed to observe living, unstained microorganisms by **JJ Lister**.
- **The "Spot Lens":** The first dedicated darkground illuminators, known as Spot Lenses, were manufactured during this era. They used a central opaque stop to block direct light, making them perfect for low-power magnification.
- **The Diatom Craze (1840s):** The method gained its first major scientific traction when microscopists used oblique and darkground illumination to resolve the incredibly fine, glass-like structures (striae) of diatoms …
- **1838** Reade, **1850** Wenham, **1851** Shadbolt, **1877** Edmunds, **1879** Stephenson

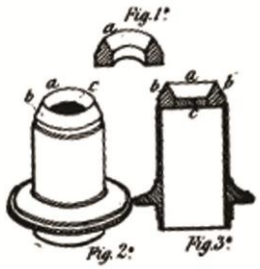
Excellent source article …

[History of Dark Ground (Dark Field) Microscope Illumination](https://microscope-antiques.com/hxdarkfield.html)

https://microscope-antiques.com/hxdarkfield.html



WENHAM'S PARABOLIC REFLECTOR



SHADBOLT'S ANNULAR ILLUMINATOR

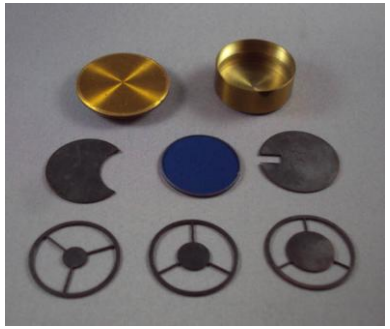


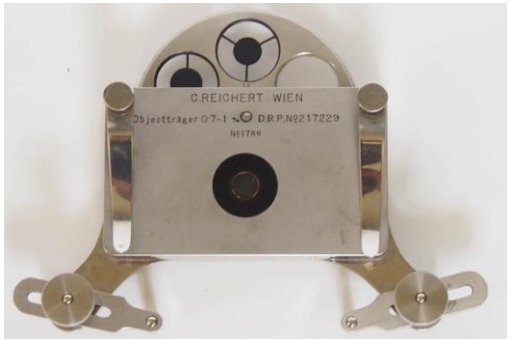
THE WENHAM-SHADBOLT PARABOLOID CONDENSER





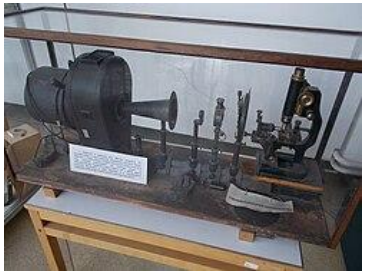






3. The Nanoparticle Breakthrough (1902)

- **The Ultra-Microscope:** In 1902, chemist Richard Zsigmondy and physicist Henry Siedenkopf developed the ultra-microscope, which was the first highly specialized, high-power darkfield microscope.
- This device allowed Zsigmondy to view individual nanoparticles well below the standard limits of light resolution, earning him the Nobel Prize in Chemistry in 1925.



Resolution:	BF	0.25 µm
Visibility	UDF	0.004 µm or 4nm

4. Medical Explosion: The Syphilis Breakthrough (1906)

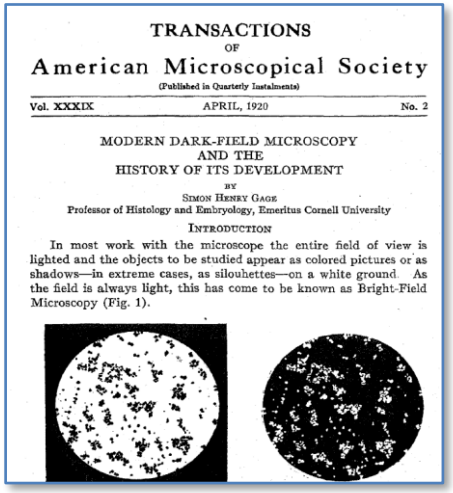
- **Treponema pallidum Detection:** While the technique was centuries old, darkfield microscopy **achieved widespread global popularity in 1906**.
- Scientist Karl Landsteiner & Viktor Mucha introduced darkfield microscopy to isolate and identify Treponema pallidum, the corkscrew-shaped bacterium that causes syphilis. Because the bacterium is completely transparent and incredibly thin, it was invisible under normal microscopes but brilliantly illuminated under darkground conditions. This made darkfield an essential tool in medical clinics worldwide.



More Useful Links …

What Is Darkfield Microscopy? (Olympus)
https://evidentscientific.com/en/insights/what-is-darkfield-microscopy

Modern Dark-Field Microscopy and the History of Its Development (Gage 1920)
https://scispace.com/pdf/modern-dark-field-microscopy-and-the-history-of-its-mmw9x5kt8m.pdf



TRANSACTIONS
OF
American Microscopical Society
(Published in Quarterly Instalments)

Vol. XXXIX APRIL, 1920 No. 2

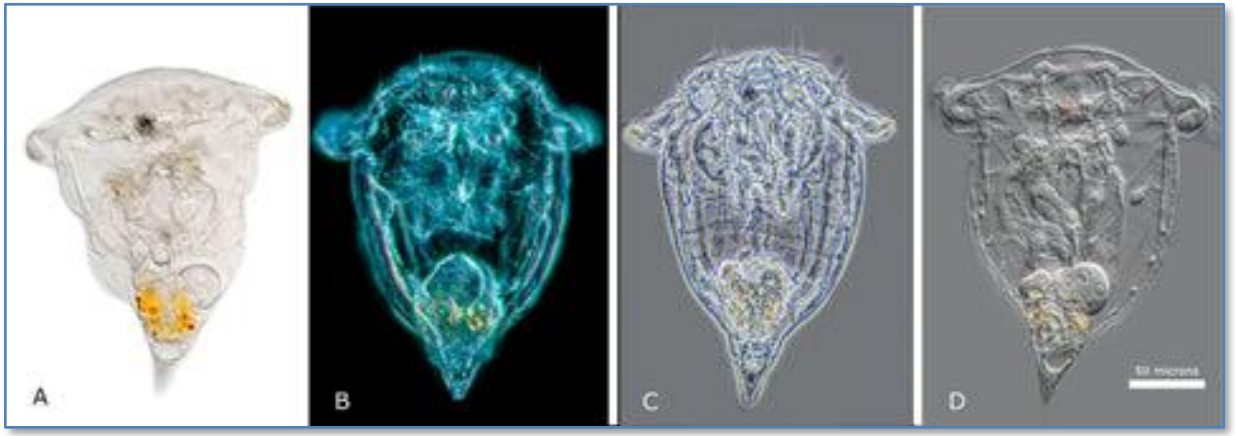
MODERN DARK-FIELD MICROSCOPY
AND THE
HISTORY OF ITS DEVELOPMENT

BY
SIMON HENRY GAGE
Professor of Histology and Embryology, Emeritus Cornell University

INTRODUCTION

In most work with the microscope the entire field of view is lighted and the objects to be studied appear as colored pictures or as shadows—in extreme cases, as silouhettes—on a white ground. As the field is always light, this has come to be known as Bright-Field Microscopy (Fig. 1).

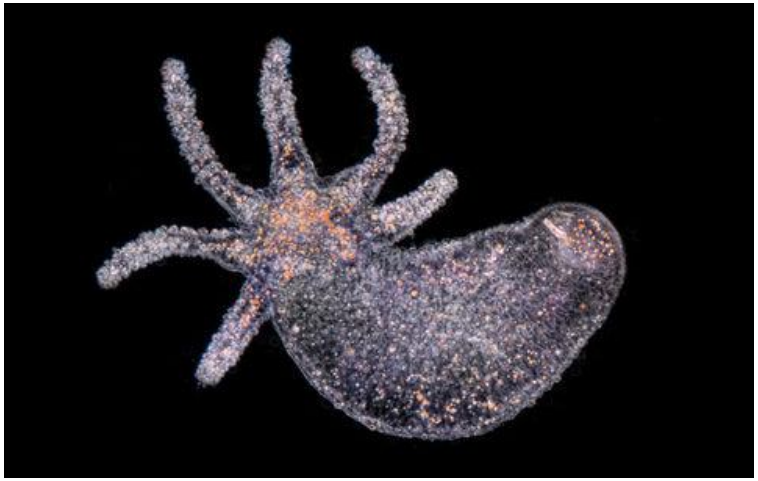
Astronomy ≡ moon & planets at night !

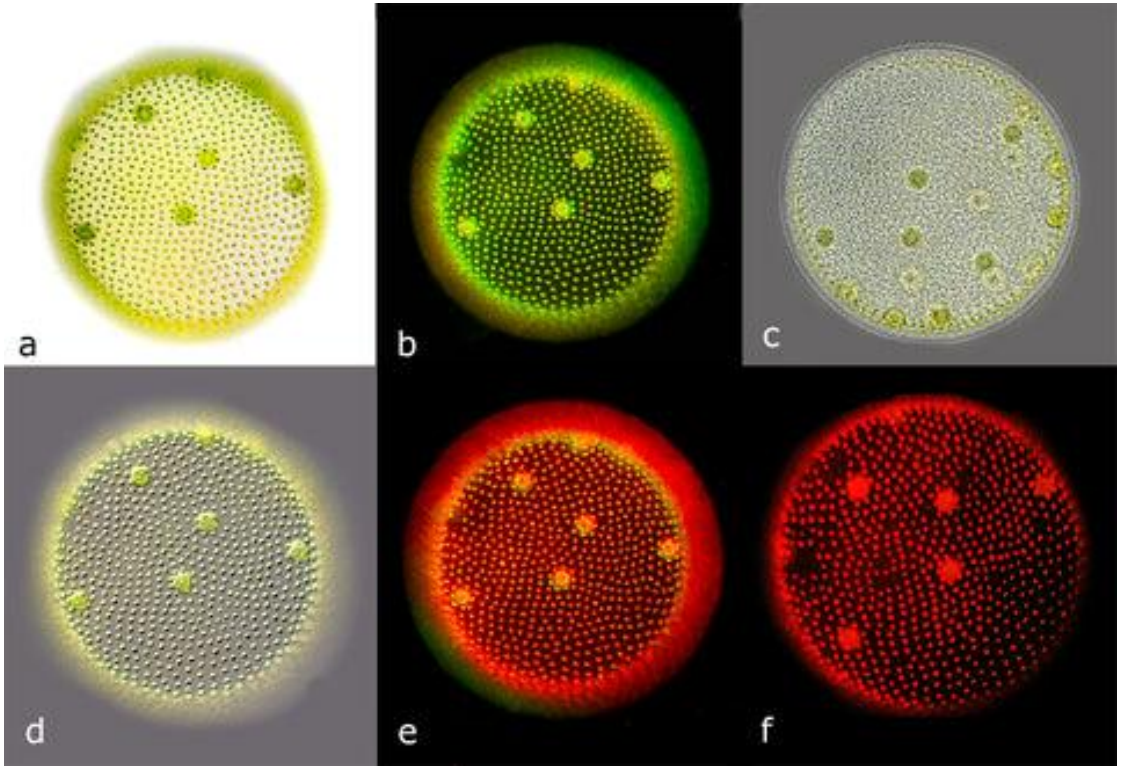


Brightfield Darkfield Phase DIC

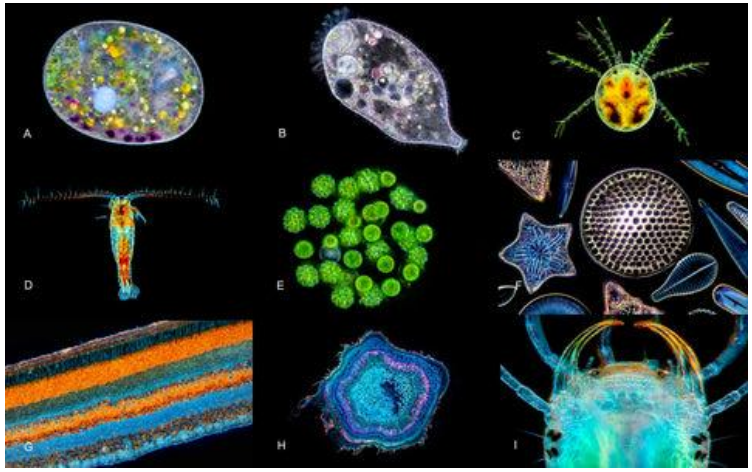
Introduction to Dark-Field Microscopy | Motic Microscopes
https://moticmicroscopes.com/blogs/articles/introduction-to-dark-field-microscopy

Microsoft Word - LM Ch 9 Dark Field.doc (Bagnell et al 2012)
https://www.chem.uci.edu/~dmitryf/manuals/Fundamentals/Dark%20Field%20microscopy.pd

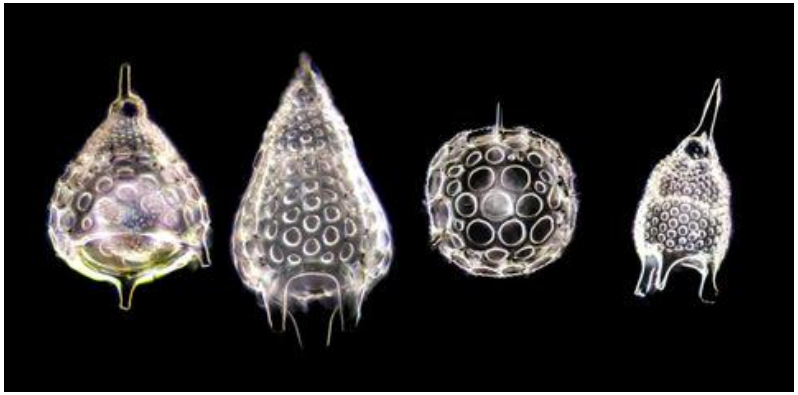




Above is a comparison of different light microscopy techniques on Volvox aureus - a fresh water algae. ***a)*** *bright-field* ***b)*** *dark-field* ***c)*** *phase contrast* ***d)*** *differential interference contrast* ***e)*** *dark-field and a Rheinberg filter* ***f)*** *fluorescence microscopy*



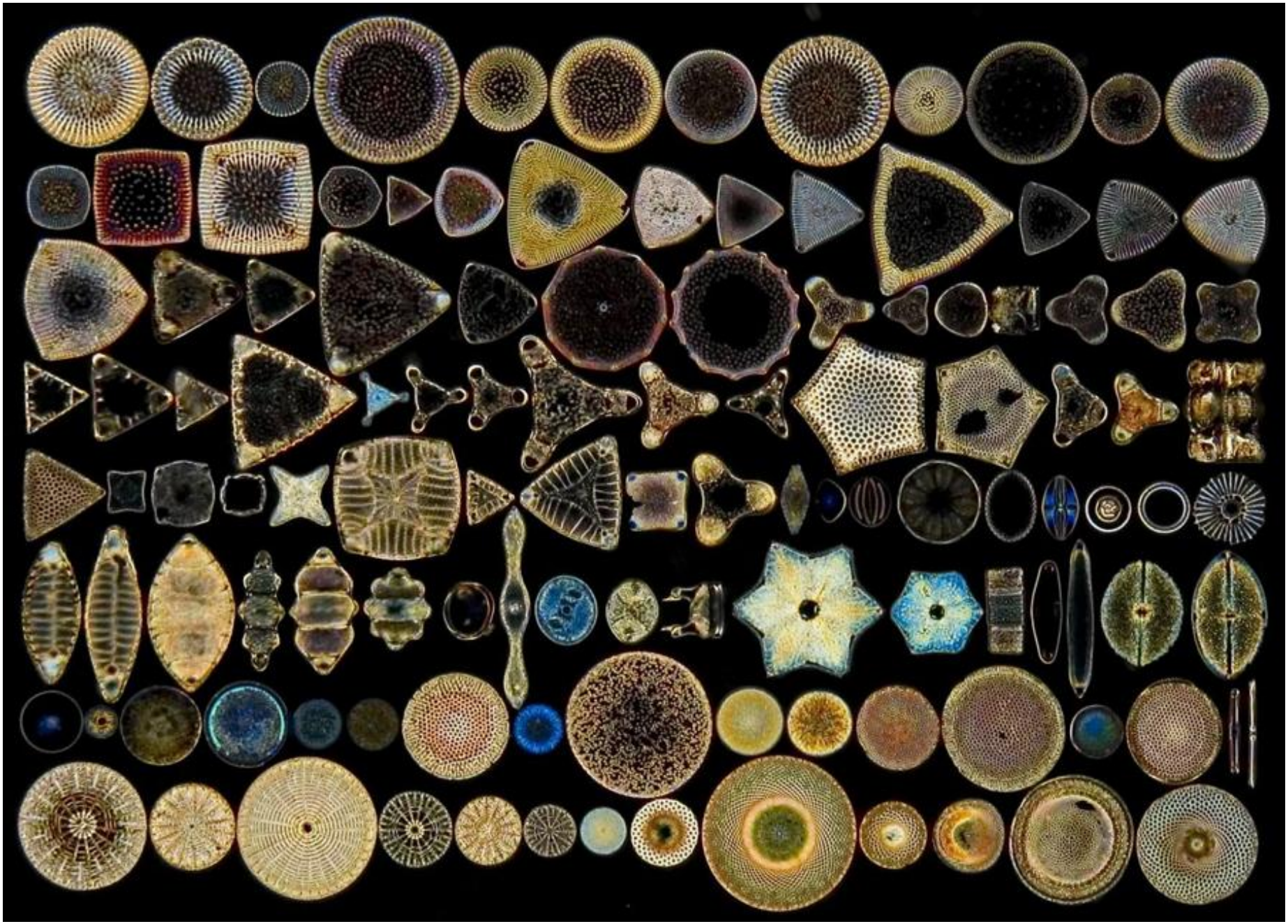




From Motic Website



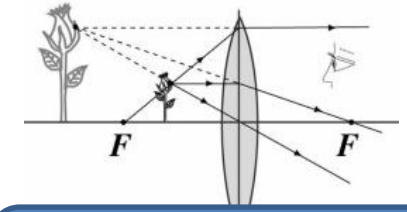
Diatoms – Darkfield Microscopy … from Barone

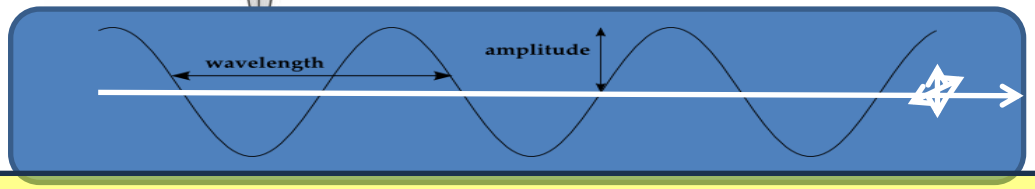


Diatoms – Darkfield Microscopy … from Franchini

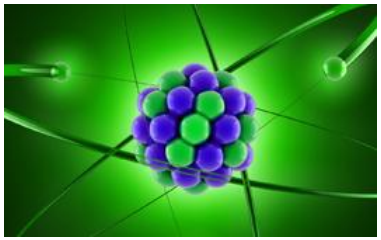
Light Microscope Theory

Properties of Light ⇨ Microscopies



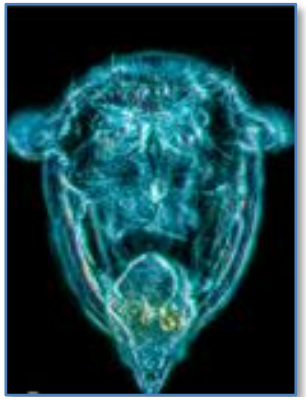


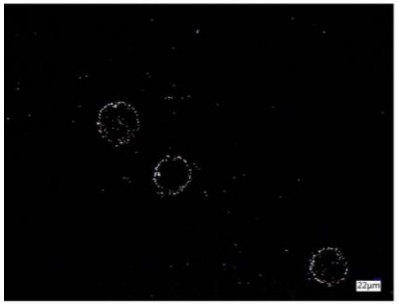
- **Geometric Optics**
  - image location
  - magnification / resolution
- **Wave Optics**
  - direction
  - amplitude
  - wavelength
  - phase
  - **vibrational plane**
  - velocity
  - **reflection**,refraction,**diffraction** / BF / **DF**
  - absorption / BF
  - colour
  - phase contrast, interference
  - polarisation
  - refraction / oil immersion
- **Quantum Optics**
  - formation & destruction
  - fluorescence

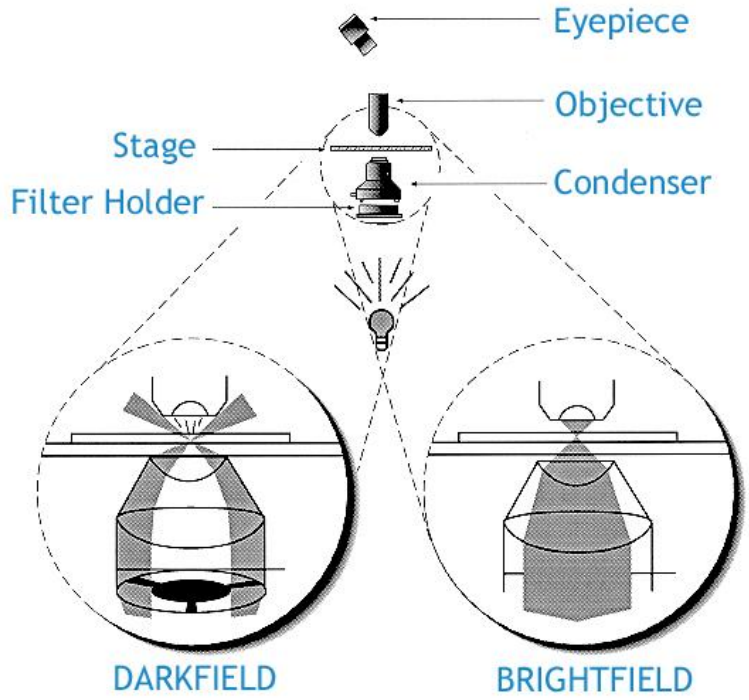


Geometric

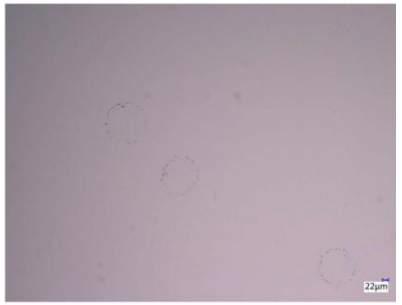
How does it work ? ….











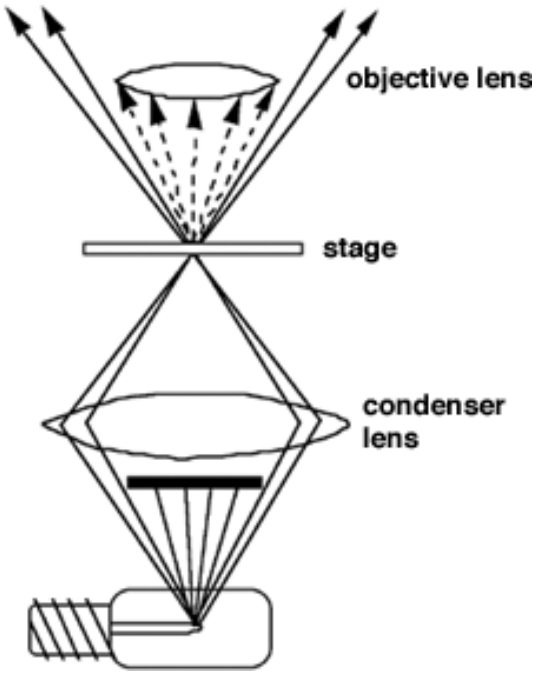
- Central light is blocked by the ‘Field Stop’ leaving a darkfield or dark background
- Light from the outer edges misses the objective unless it hits the specimen …
- then it will reflect or diffract and hit the objective and eyepiece as a bright image
- …so unstained material becomes visible

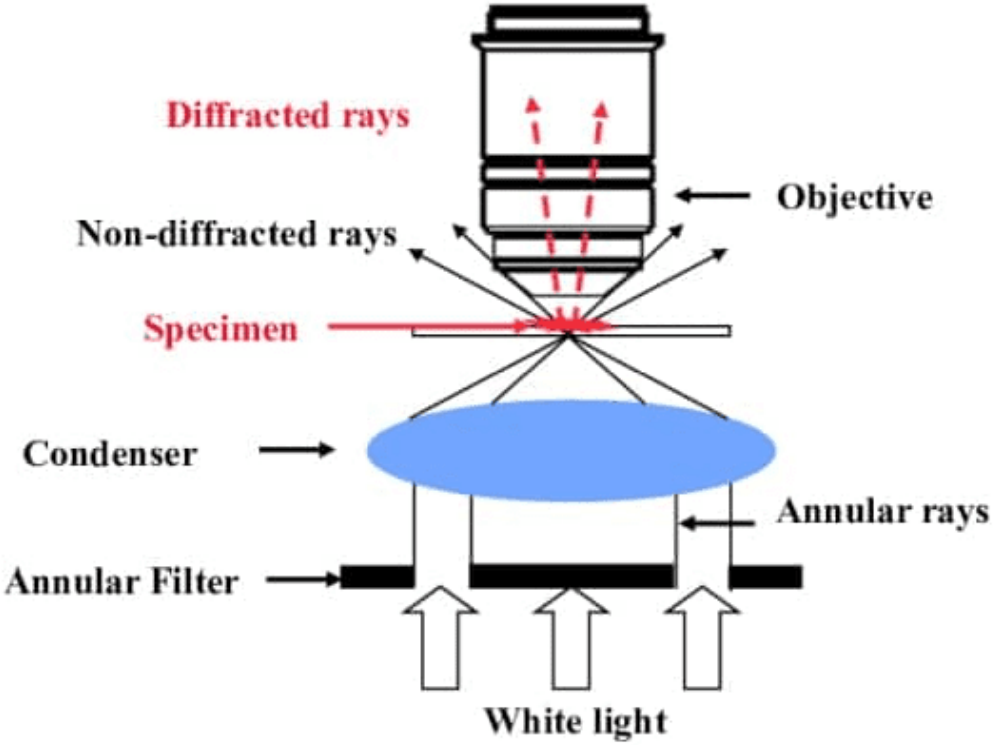
- Central light hits the objective
- If the specimen is dense or coloured it will refract this light and show up in the eyepiece
- If not it will hardly be visible

… purposely misadjusting condenser focus or iris will increase visibility but reduce resolution

How does it work ? ….

Condensor NA needs to be > than Objective NA





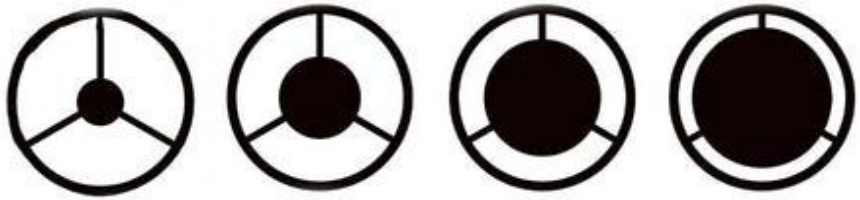
For Low & Medium Power Objectives almost any Condensor and an appropriate sized Central Field Stop will work ….

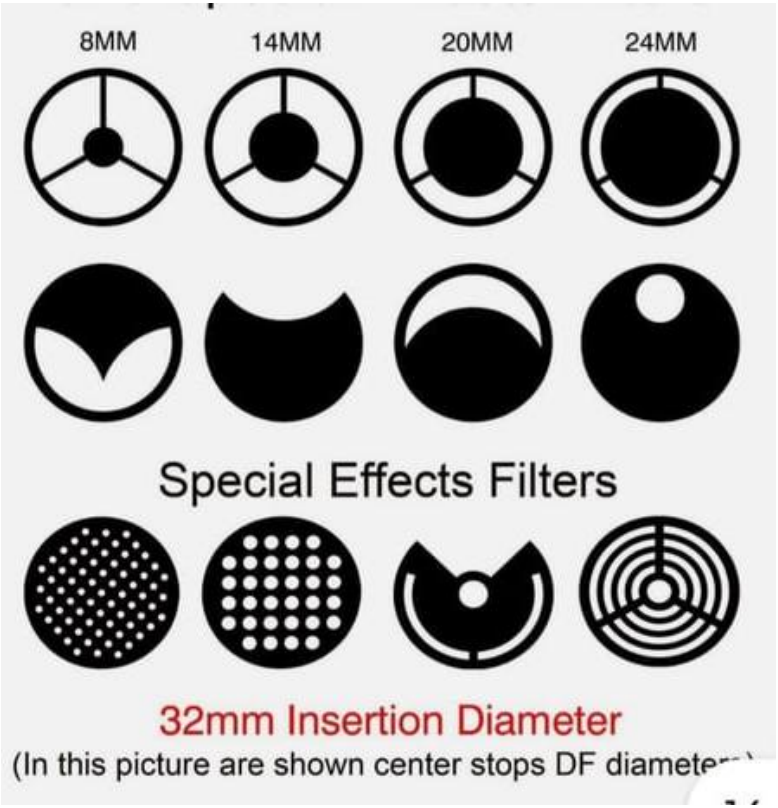
Condensor iris needs to be fully open & centralised

Condensor field stops







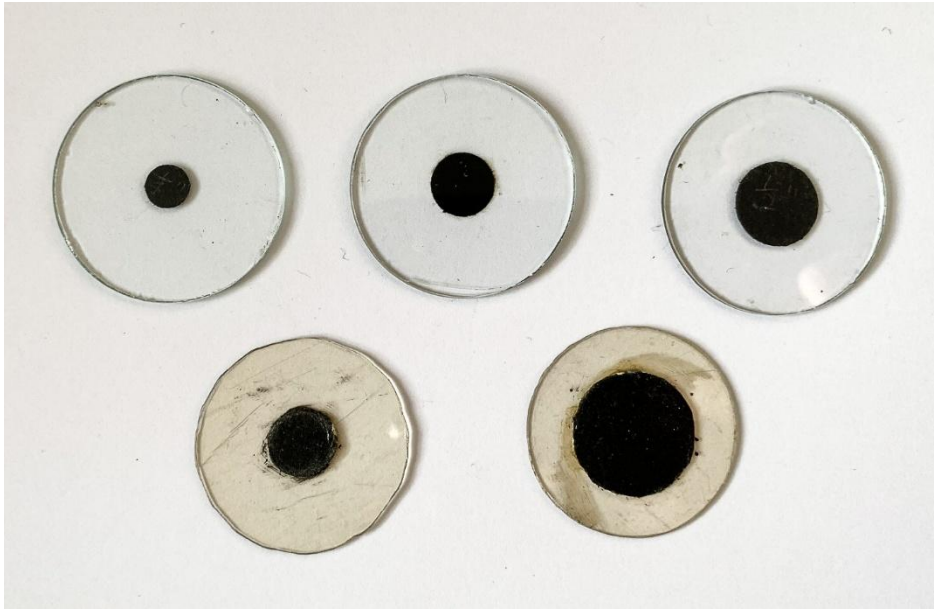




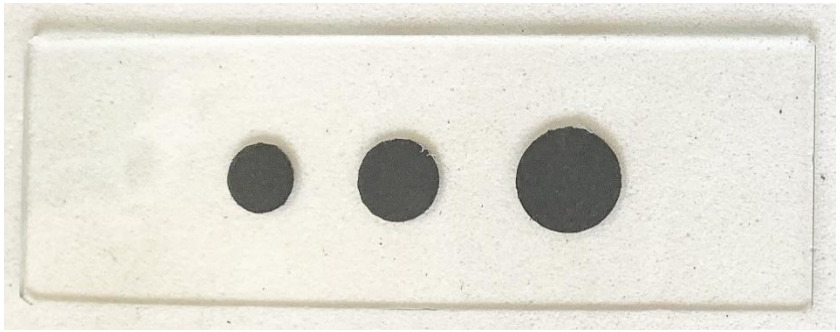
Traviss expandable stop (1906)

Cheap Homemade Solutions …

Black paper or Tin Foil Patch Stops
… on condenser filter tray or Blu-Tak









Coins 18mm 20mm 25mm 26mm

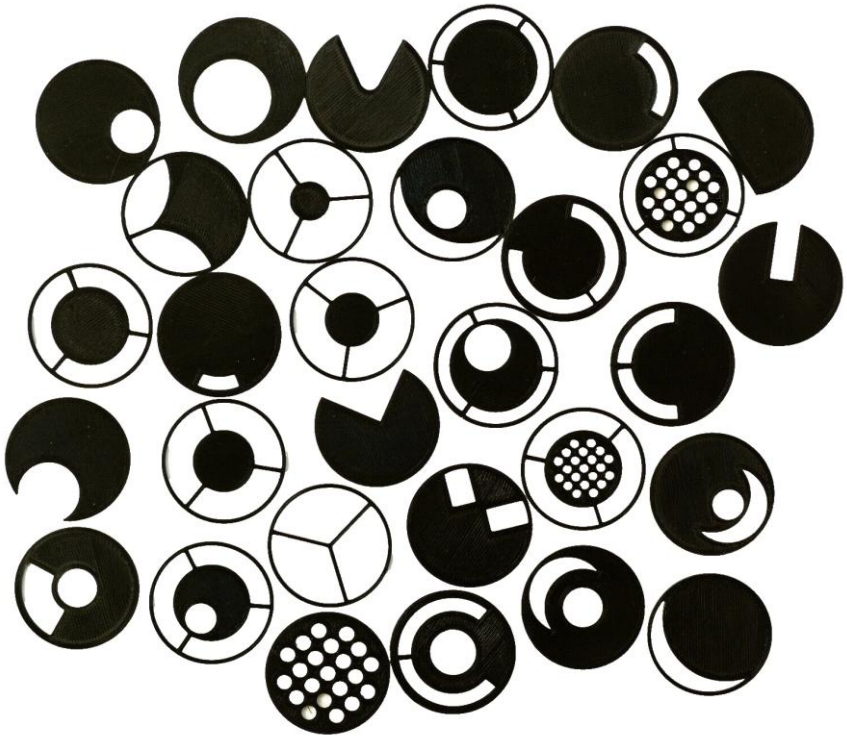


Also use edges of filter tray in the light path …

Online Chinese Versions

£6.00





Full Set
£16 !!

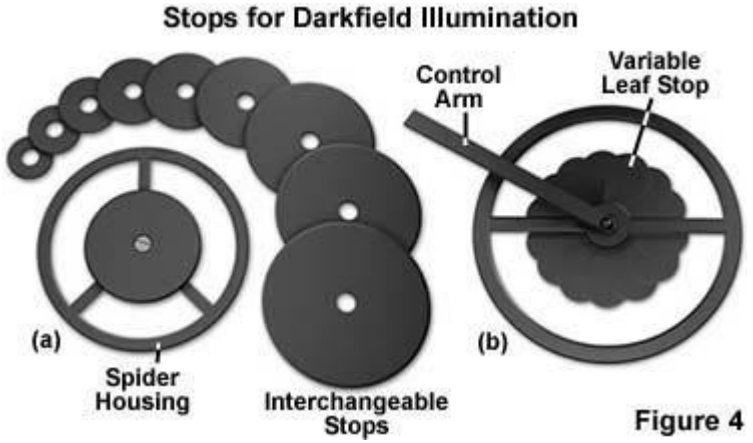
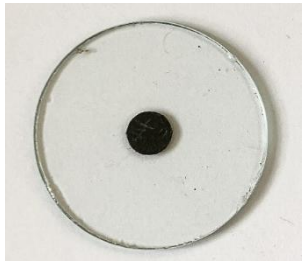


Figure 4

Stop Sizes ….

Mag	NA	Dia mm	inches	≡Coin			
X1	0.03	8	5/16				
X2	0.05	10	⅜				
X4	0.1	12	½				
X10	0.25	17	⅝	5p			
X20	0.4	19	¾ + ↵	1p + ↵			
X40	0.65	21					
x100	1.3	23	1	10p			Poor

Filter holder
sizes 32 mm ..







Sartory – Quekett Microscopical Club

Specialist Darkfield Condensors for High Power Objectives (NA>0.6)

Condensor NA needs to be > than Objective NA

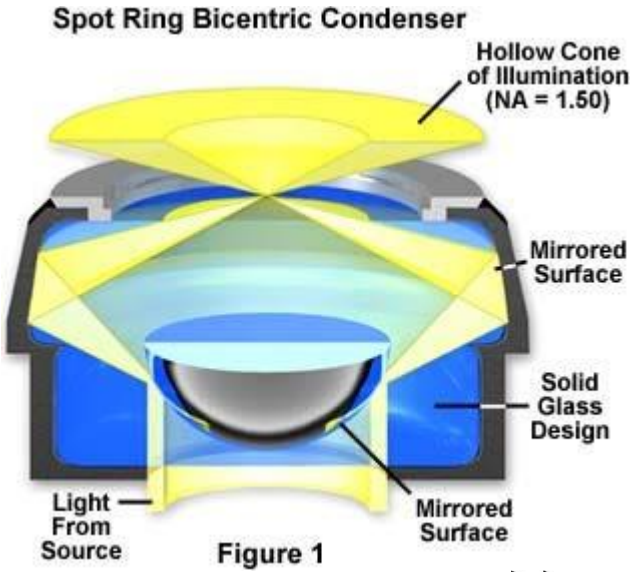


Figure 1



Dry or Wet Condensor

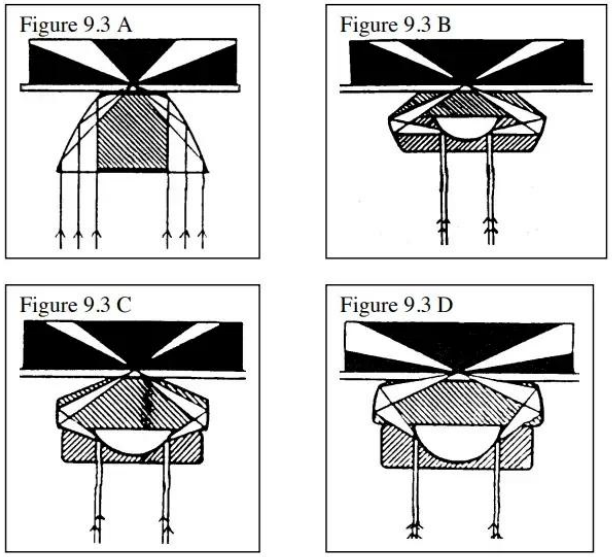
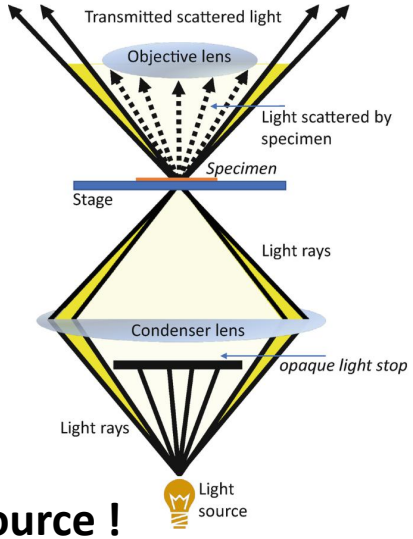


Figure 9.3 Reflecting dark field condensers:
A = Paraboloid, single reflecting type.
B = Bicentric or Bispheric, double reflecting type
C = Cardioid, one spherical and one cardiodial reflecting surface
D = Spot Ring, double reflecting of maximum aperture





+ Need very bright light source !

… or reduce Objective NA via inbuilt Iris

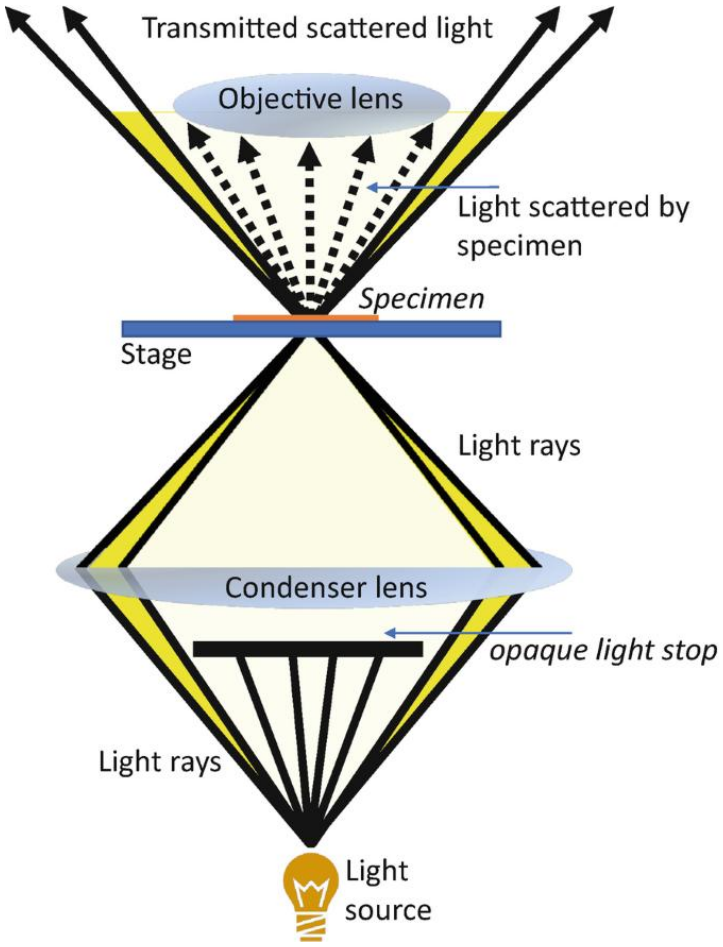


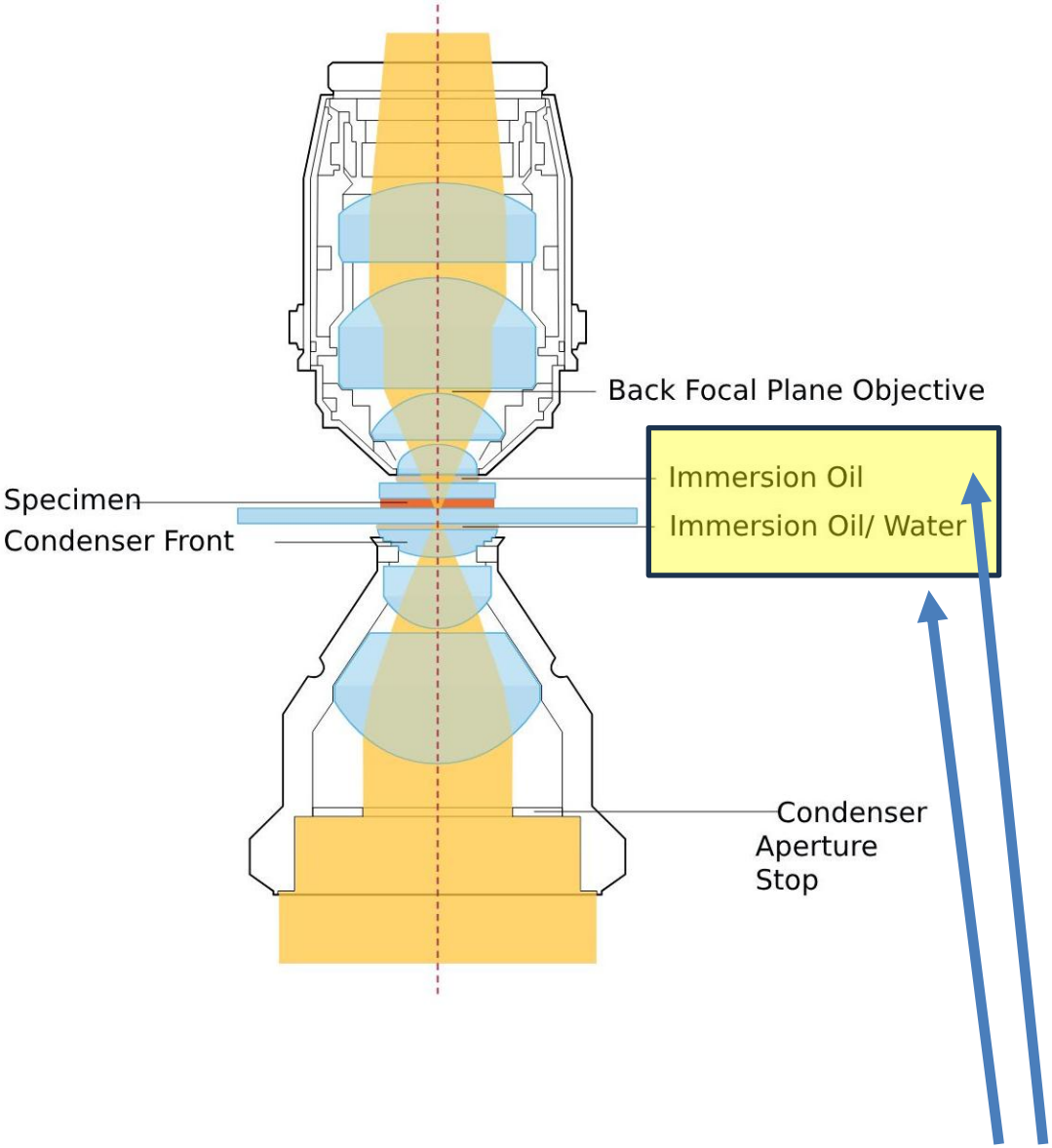
… or insert Objective Funnel Stop



…. Why not combine Traviss stop with Dancer Shutter !!!! (Malies & McCrone)

High Power Darkfield …





Zeiss

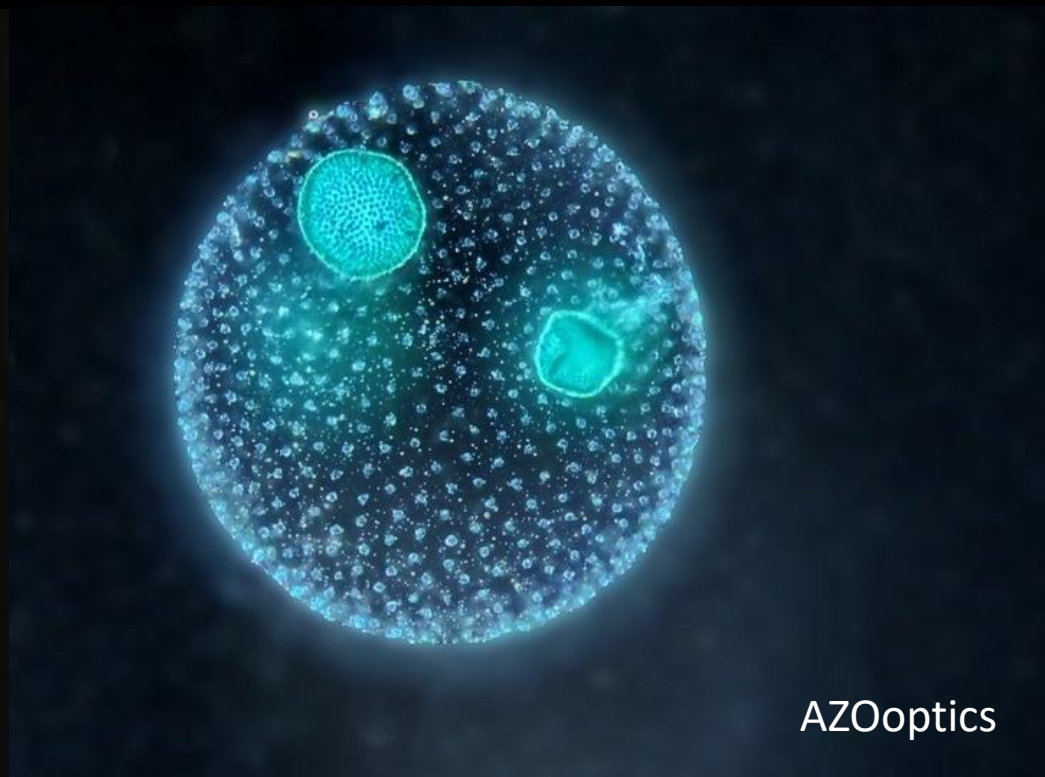
+ need Oil/water on condenser & objective very messy ! …

Examples …



Microscope World

Motic



AZOoptics

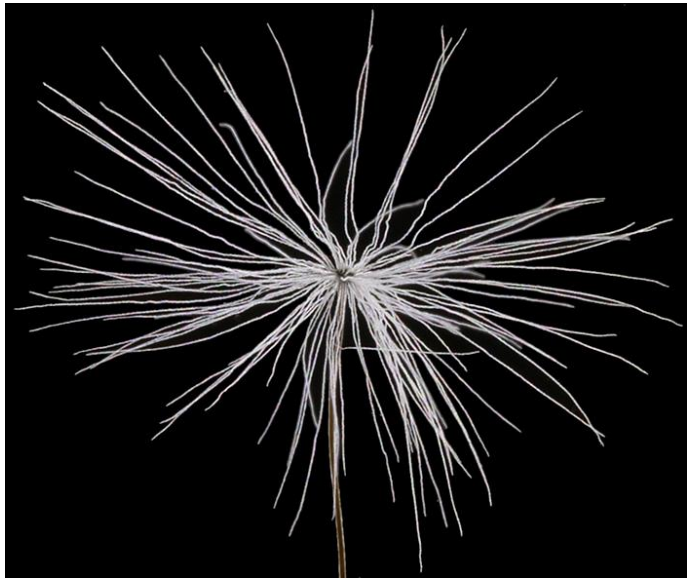
Darkfield for Stereomicroscope



Improvised Darkfield from alanwood.net

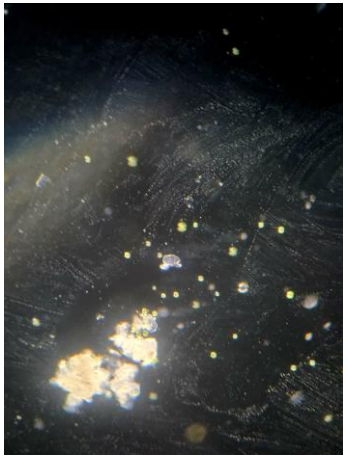
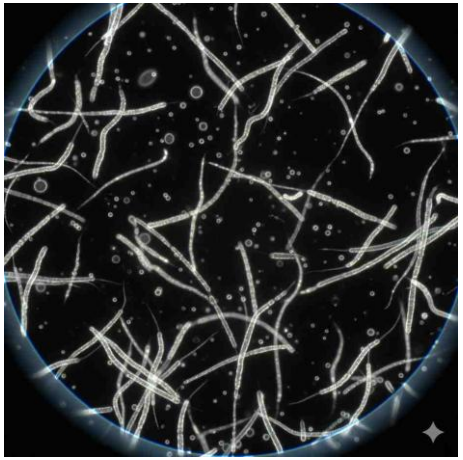








Problems … ideal is BLACK background, no UNWANTED REFLECTIONS

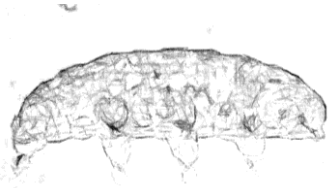




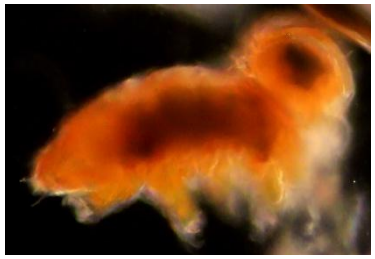

- Condensor needs to be centred, iris open, stop size sufficient ....
- Slide, coverslip, condenser etc need to be very **clean** .. No dust or fingerprints or bubbles !

Otherwise spend ages cleaning up images in Photoshop etc !!

Uses of Darkfield Microscopy

Specimens which are ….. Transparent
Unstained
Low contrast
Thin, Scattered





Common Biological Uses

- **Live Microbes:** Observing live, motile bacteria, spirochetes and protozoa without killing them with chemical stains.
- **Aquatic Organisms:** Inspecting transparent single-cell algae, plankton, and small aquatic creatures.
- **Cellular Structures:** Tracking edges, cell boundaries, and internal refractive details in living tissue cultures, cilia & flagella

Industrial and Material Uses

- **Nanoparticles & Colloids:** Detecting tiny particles and dust counts down to 20–40 nanometres.
- **Surface Imperfections:** Spotting scratches, fractures, or inclusions on thin polymer films, minerals, and crystals.

Darkfield Modifications or Alternatives …

Reflected light df
Inverted df

Oblique illumination
Hoffman Contrast Modulation

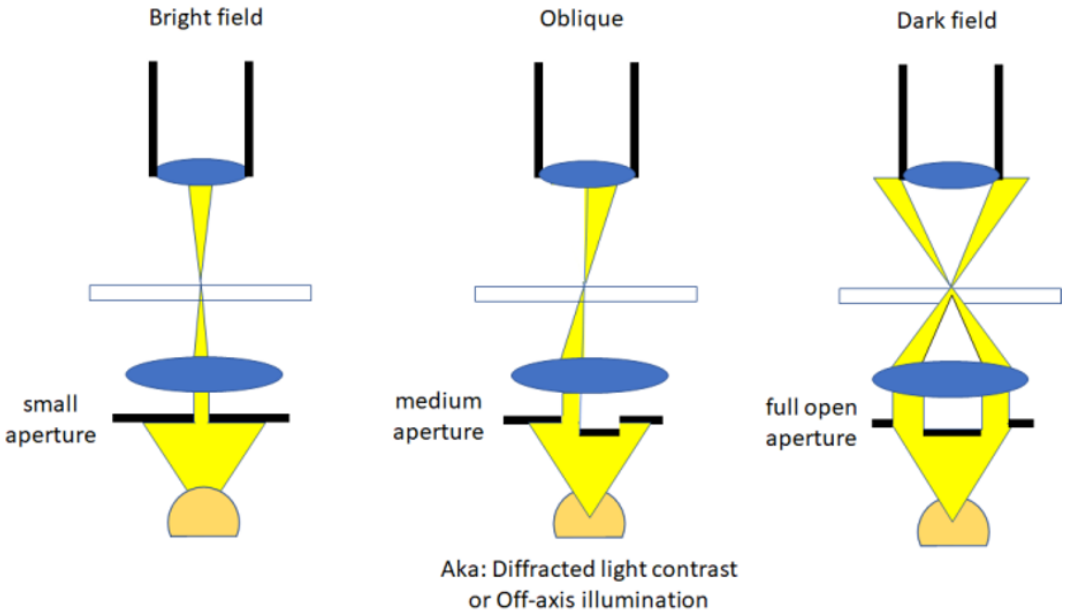
Rheinberg

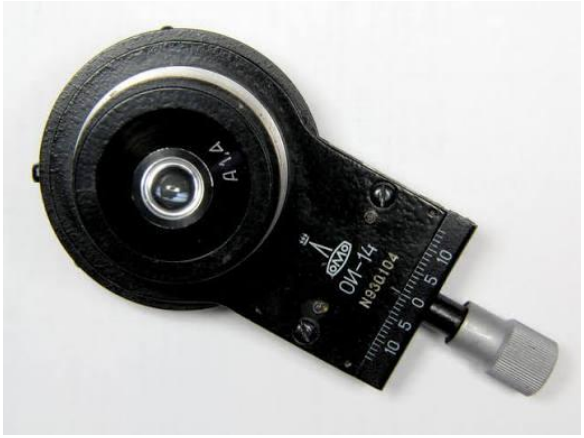
VAC & Spikeberg
Nikon oblique coherent contrast

Darkfield EM
Darkfield Xray microscopy

Defocus condenser … = poor set up but adds contrast and loses resolution

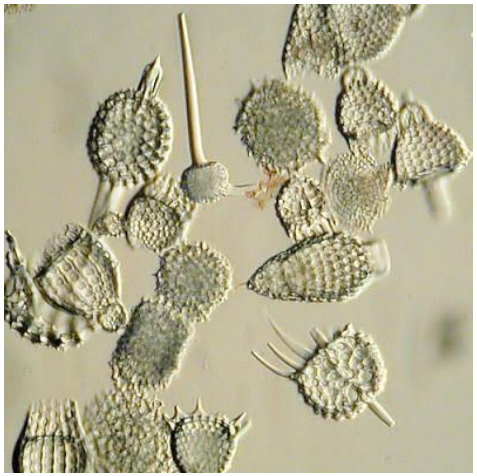
Oblique illumination …



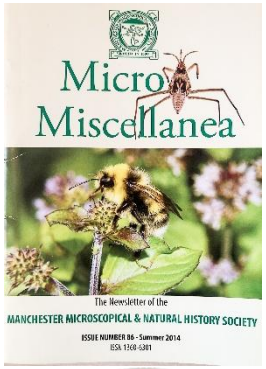


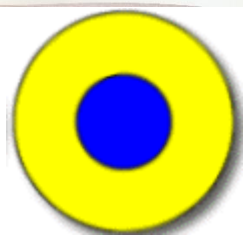






Rheinberg Illumination (Coloured Darkfield)…. 1896

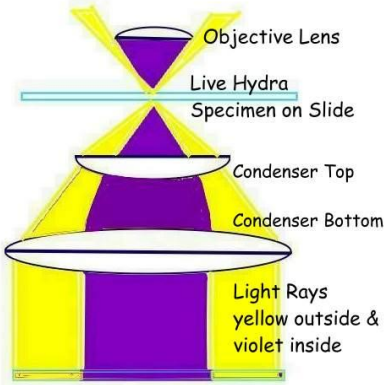




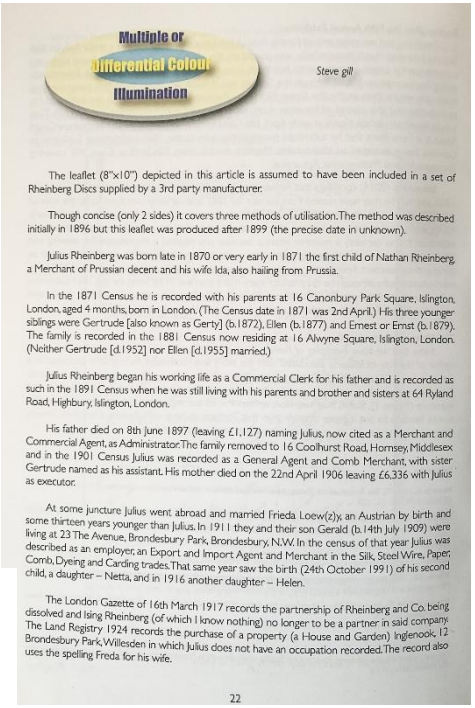
How Rheinberg Filters Work

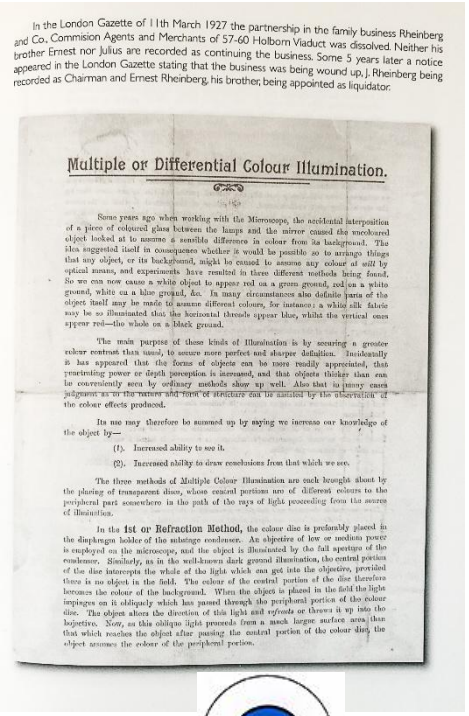


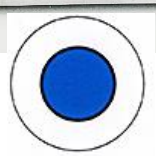
How it looks
in the eyepiece

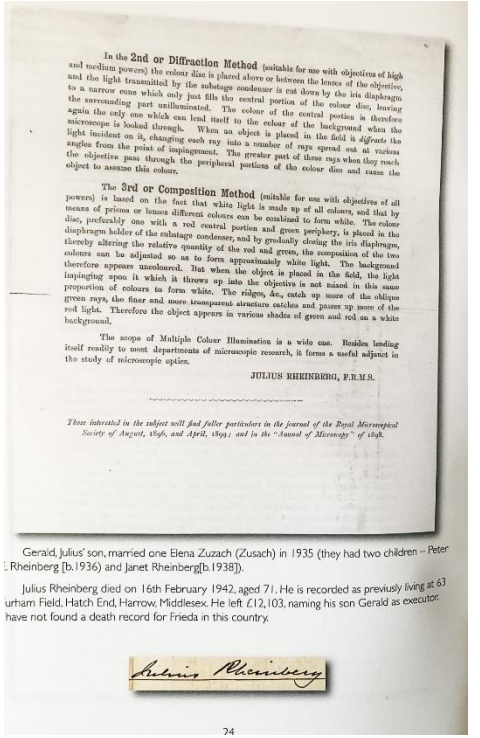


Rheinberg Filter with
yellow annulus outside
and violet center stop inside



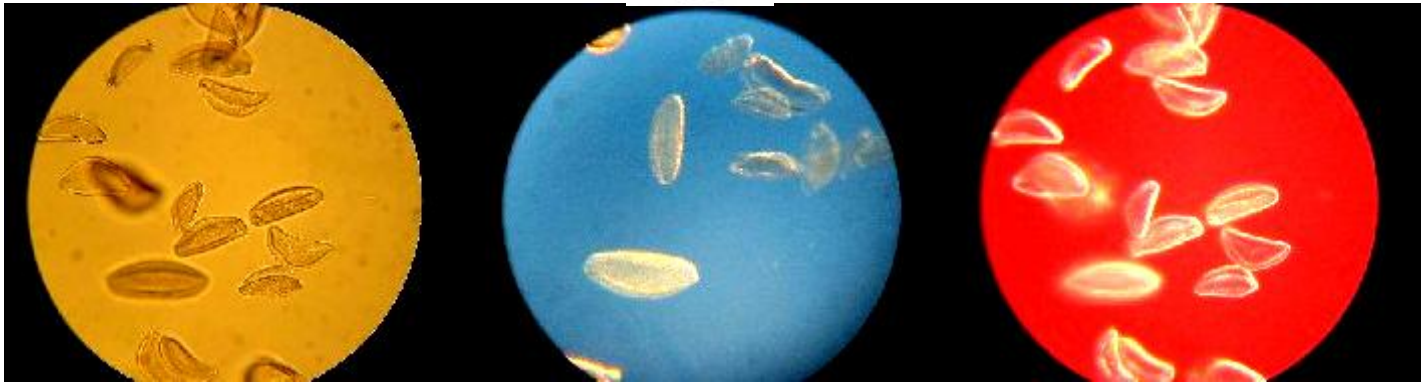




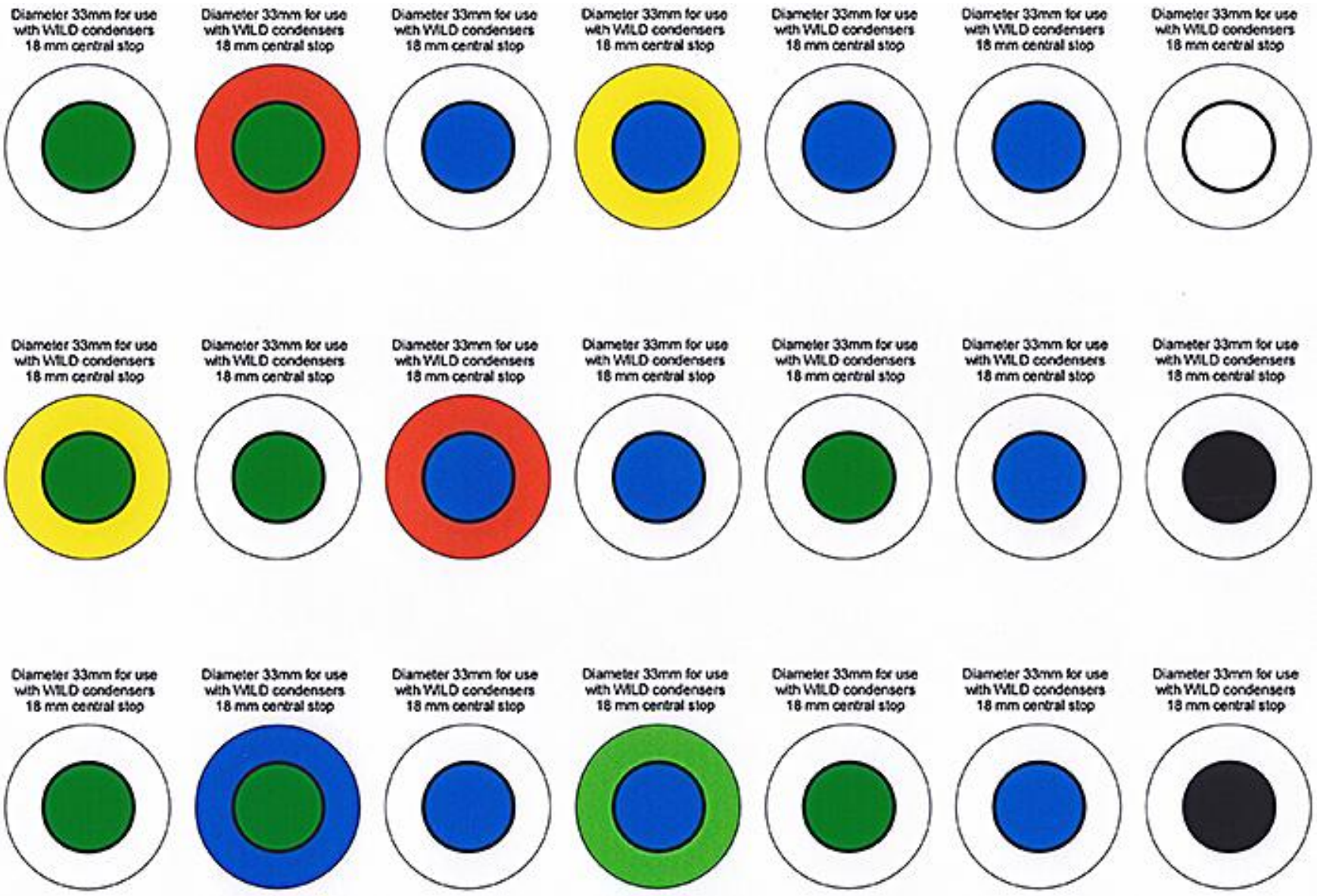


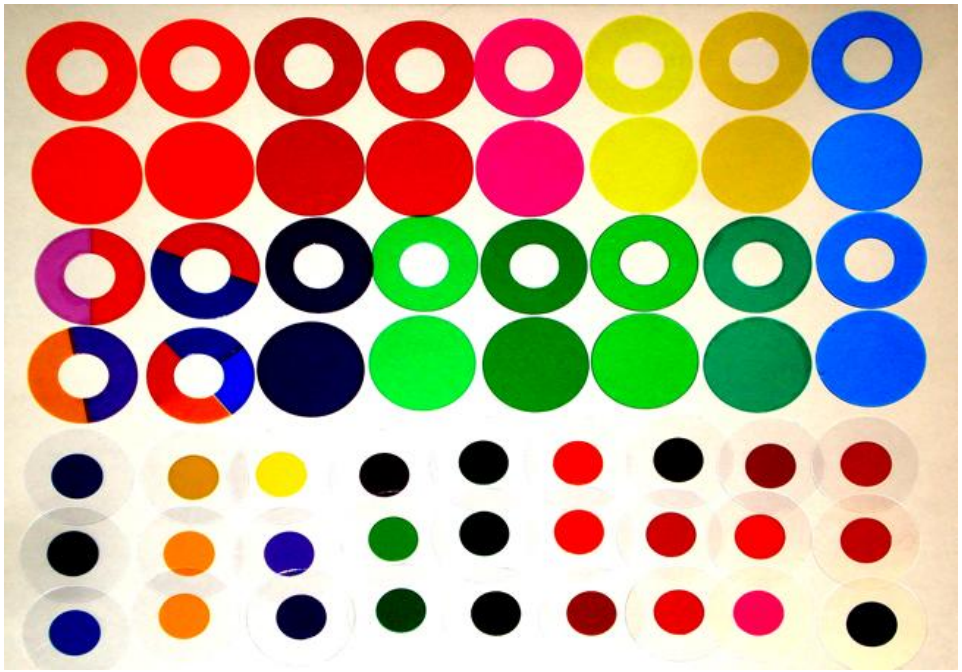
Gerald, Julius' son, married one Elena Zuzach (Zusach) in 1935 (they had two children – Peter Rheinberg [b.1936) and Janet Rheinberg[b.1938]).

Julius Rheinberg died on 16th February 1942, aged 71. He is recorded as previously living at 63 urham Field, Hatch End, Harrow, Middlesex. He left £12,103, naming his son Gerald as executor. have not found a death record for Frieda in this country.



Making Rheinberg illumination discs for a microscope – Quekett Microscopical Club





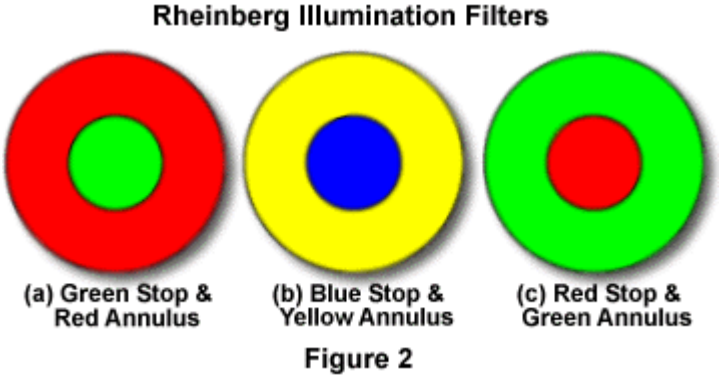
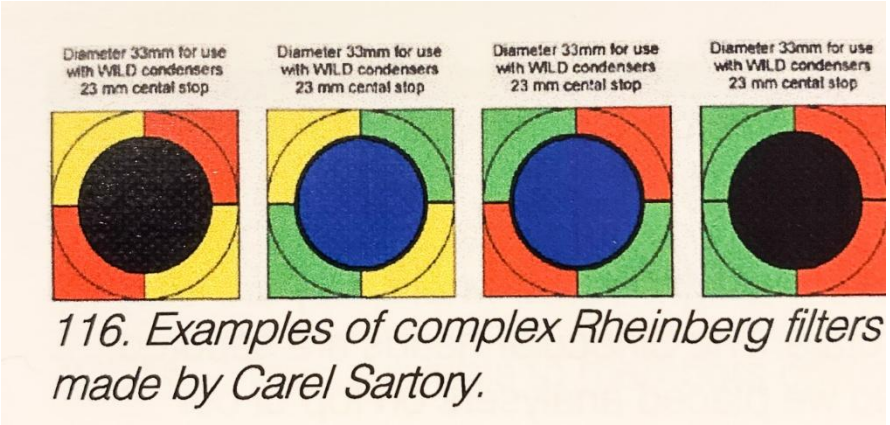
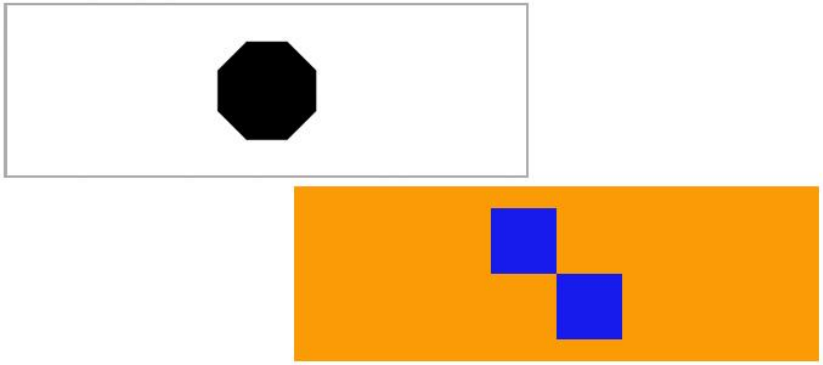


Figure 2



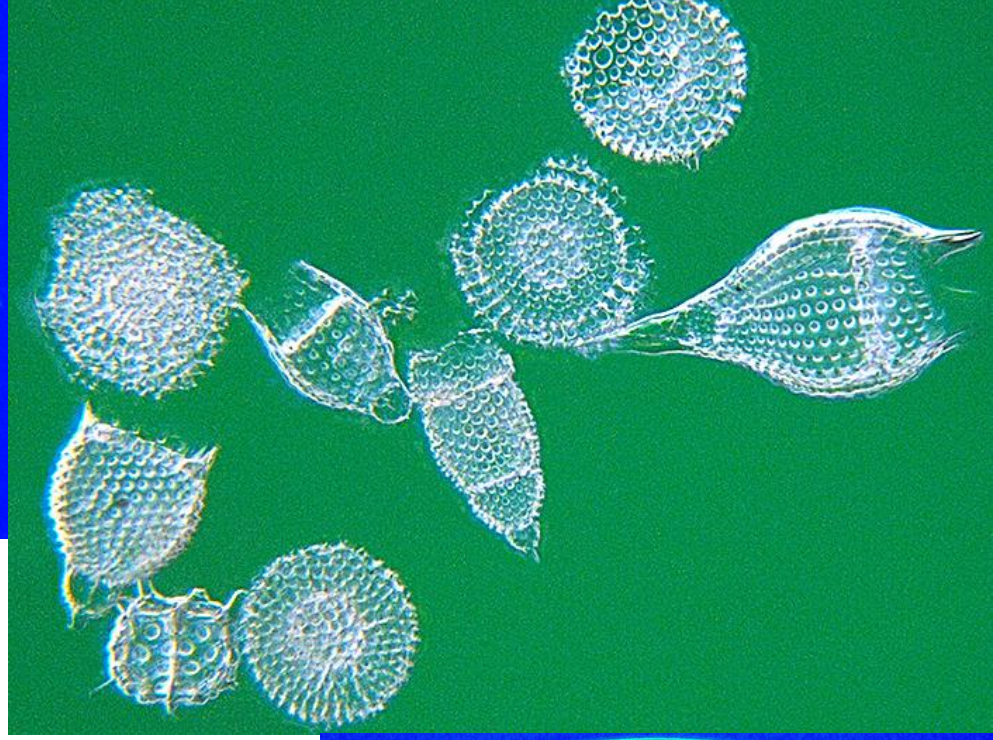
116. Examples of complex Rheinberg filters made by Carel Sartory.





[Making Rheinberg illumination discs for a microscope – Quekett Microscopical Club]

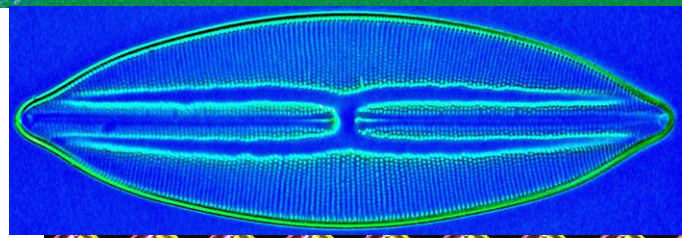


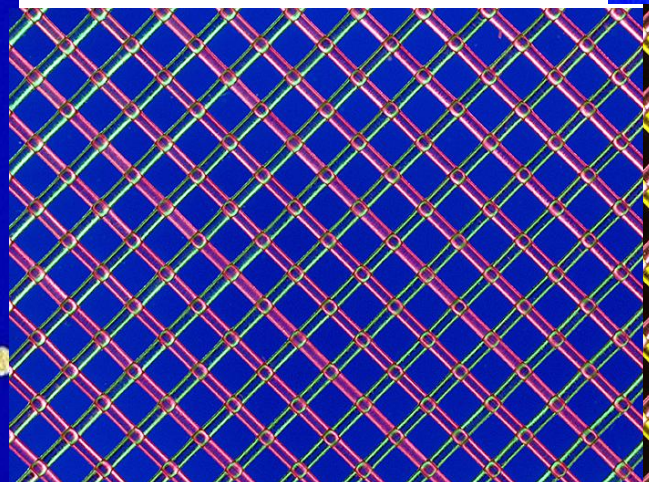


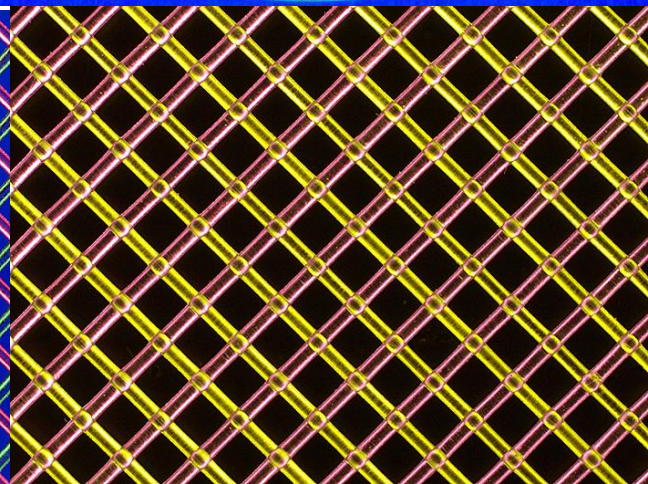


Carel Sartory

[Making Rheinberg illumination discs for a microscope – Quekett Microscopical Club]





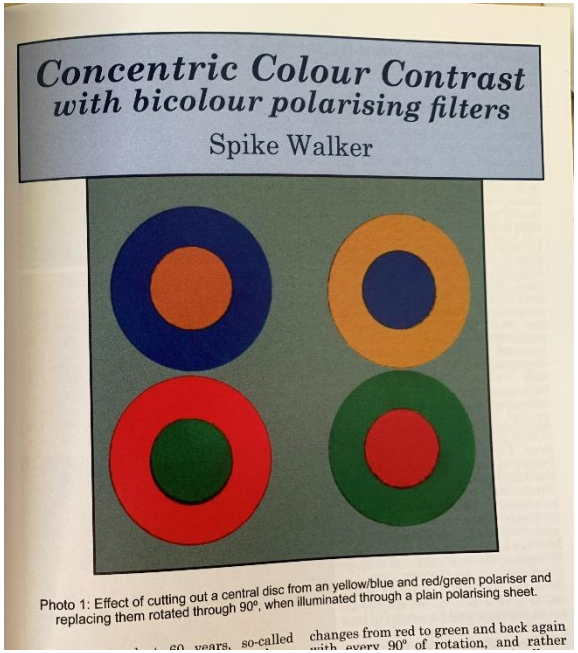


Concentric Colour Contrast – Spikeberg Illumination –

(Phase Contrast or Rheinberg + Polarising)

Spike Walker 2007, 2019, 2023 … Barer 1949

Bicolour Polarising Filters



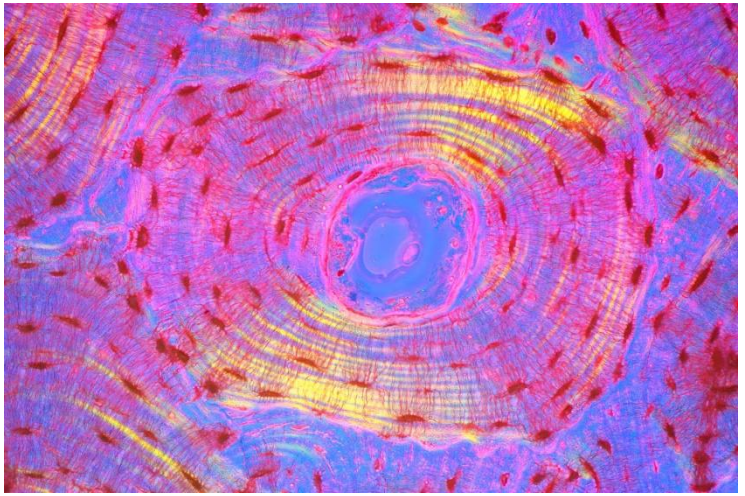
Concentric Colour Contrast with bicolour polarising filters

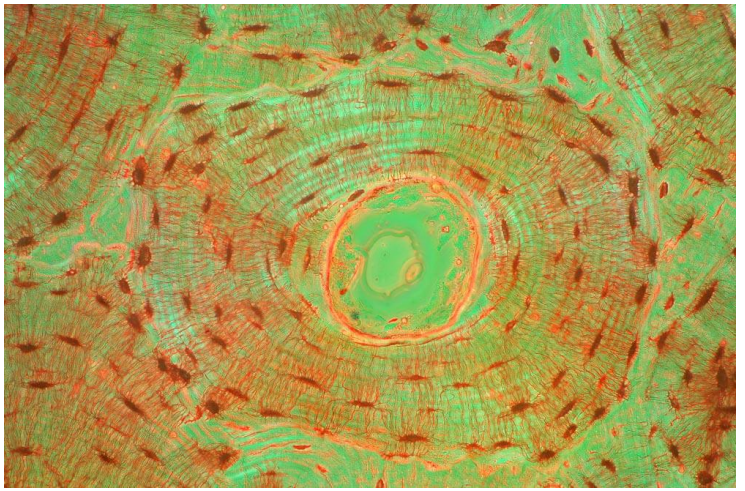
Spike Walker

Photo 1: Effect of cutting out a central disc from an yellow/blue and red/green polariser and replacing them rotated through 90°, when illuminated through a plain polarising sheet.

60 years, so-called changes from red to green and back again with every 90° of rotation, and rather

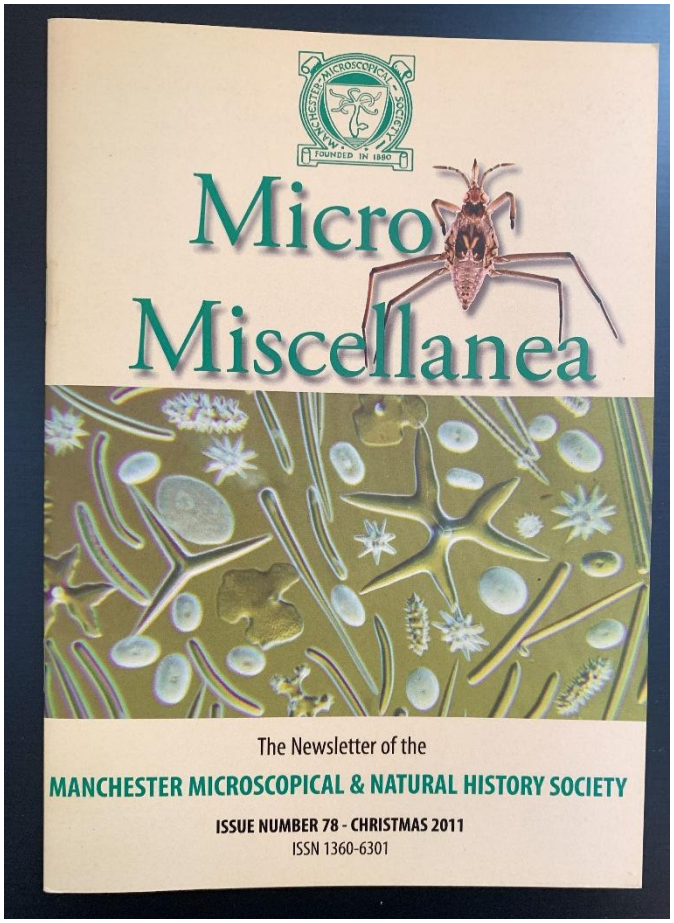


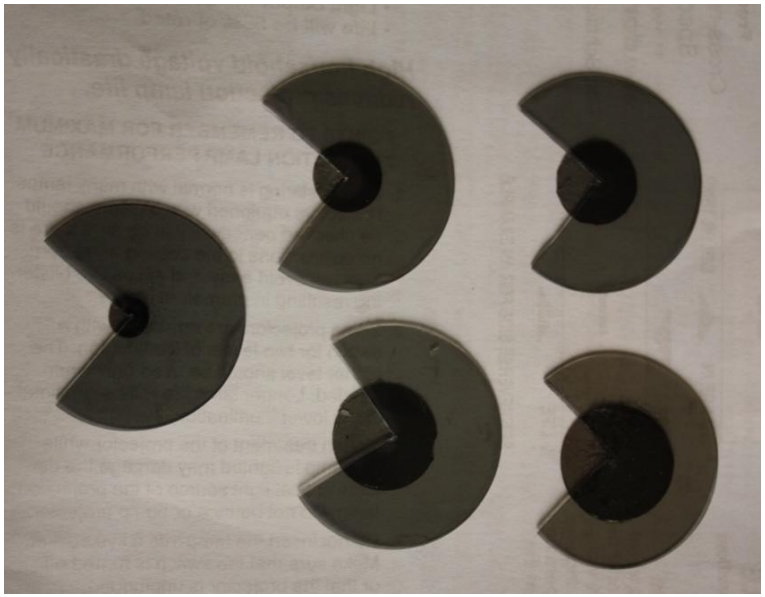




VAC – Variable Amplitude Contrast Microscopy

? DIC on the Cheap





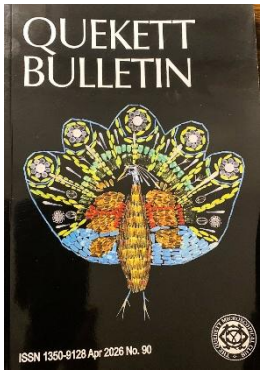


Refs

Arthur Strange (1989) Microscope, Brian Wilkinson (2011) Balsam Post, … also see Barer (1949) Nature

More Reading ….

- Molecular Expressions Website
- Taking a walk on the dark(field) side — San Francisco Microscopical Society
- Quekett Website - Saville Bradbury
- Quekett Bulletin #90 - Alan Wood ++



ECHO all in one

Brightfield
Darkfield
Phase Contrast
Polarised
Fluorescence
Inverted
Auto Stacking



££££

!!!! **END**