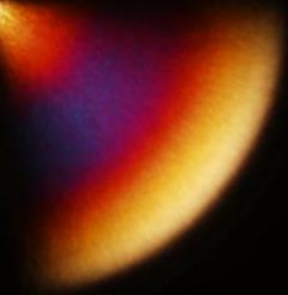

Giant amyloid spherulites reveal their true colours

Michael Smith

School of Physics and Astronomy

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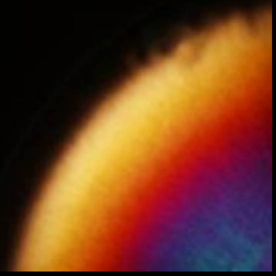


Overview

1) Amyloid aggregates

2) Giant Amyloid Spherulites (GAS)

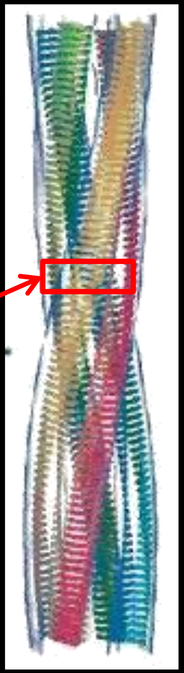
3) Modelling the properties of GAS

Conclusions

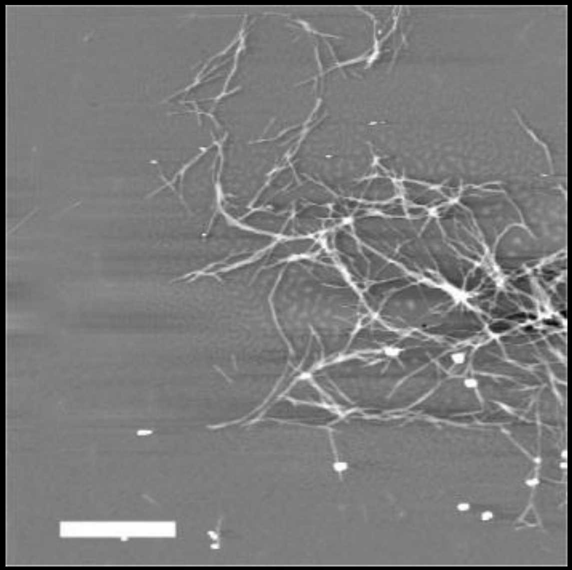
What is an amyloid aggregate?

Amyloid fibrils are protein aggregates formed under certain protein specific conditions (relevant to Alzheimer’s, Parkinson’s)

Change in Protein conformation



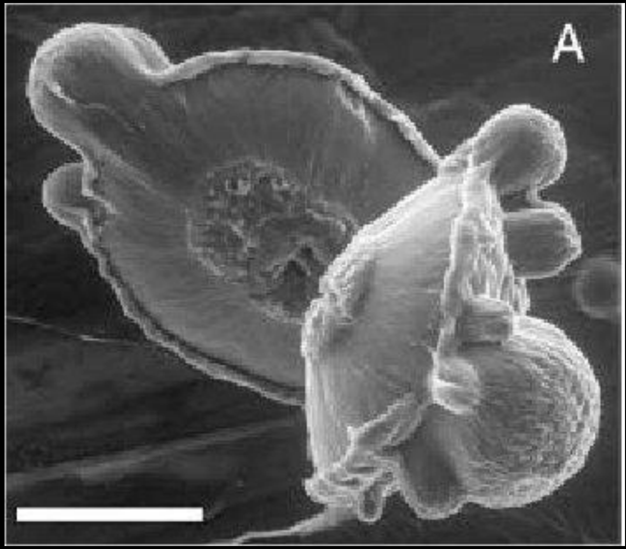
“Sticky” hydrophobic patches cause proteins to form linear chains



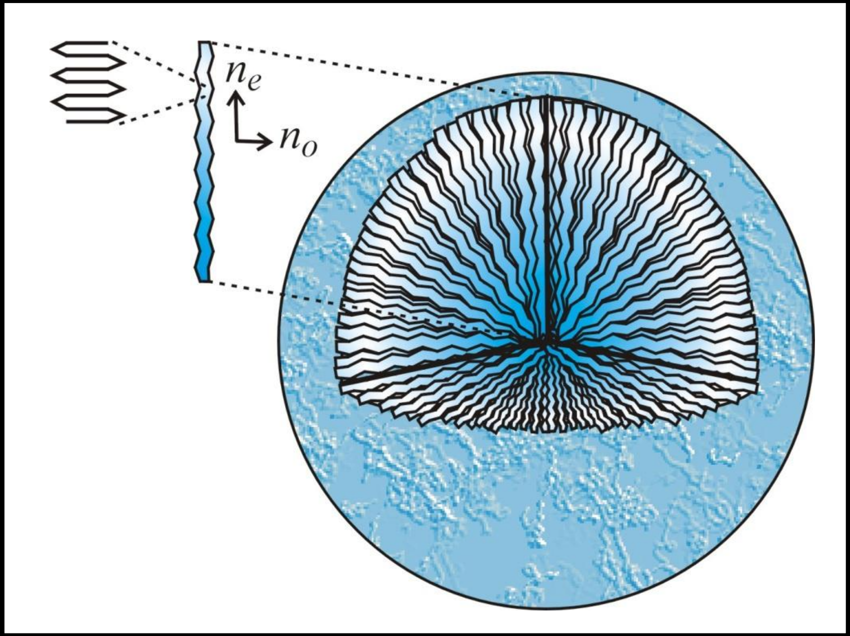
Amyloid fibrils are a few nm wide and many µm in length

Amyloid Spherulites

Fibrils can nucleate from non-specific protein aggregates and foreign particles.



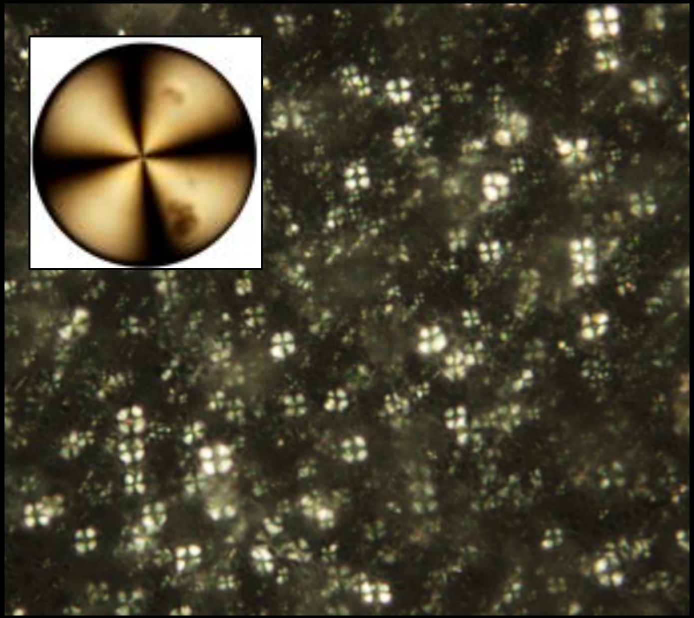
Krebs et al. Biophys. J. 88, 2013 (2005)



The fibrils often grow out from a central core to form a spherulite.

Fibrils are birefringent

Optical microscopy of spherulites



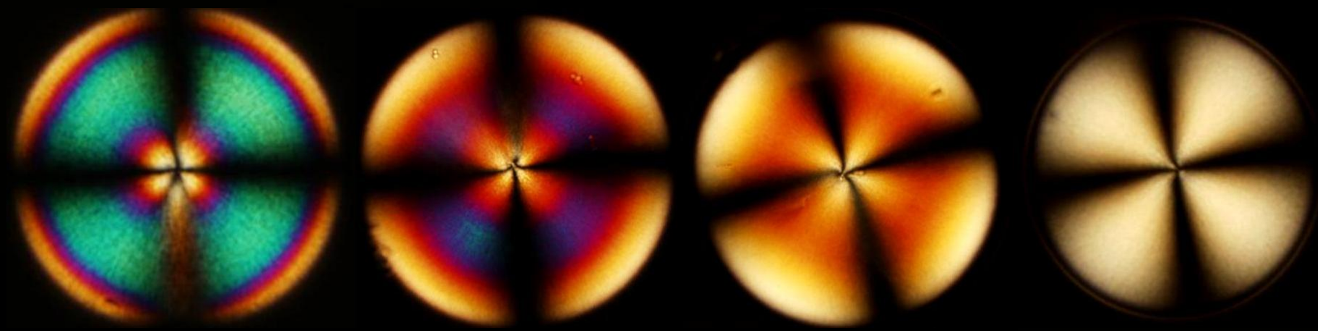
Small spherulites show a distinctive Maltese cross pattern when viewed between cross polarisers (characteristic of birefringence)

Spherulites are typically 5-50 microns in diameter

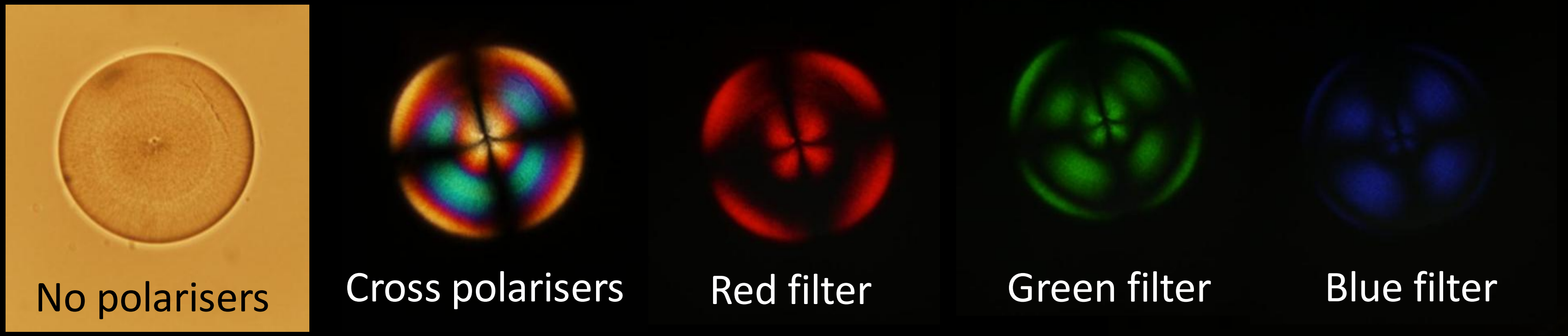
However, under certain growth conditions they can grow up to ~0.8mm in diameter!

[HCl (pH 2.8), 67^{o}C, 25mM NaCl, 10mgml^{-1} bovine insulin, 24 hrs]

Optical studies of giant spherulites



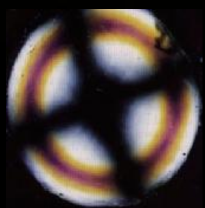
Giant amyloid spherulites can produce a wide variety of coloured patterns (“Isochromes”)



<u>But</u> similar sized spherulites from the same samples can produce very different isochromes

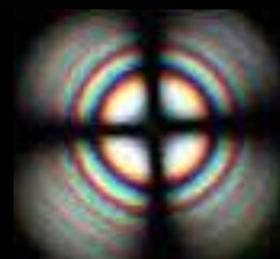
The origin of “isochromes”

Dehydrated fish eyes

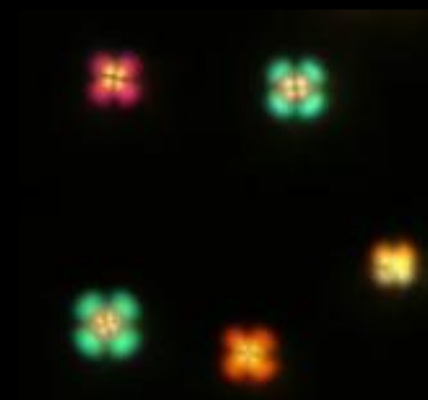


Pierscionek, Exp. Eye Res., **59**, 121, 1994

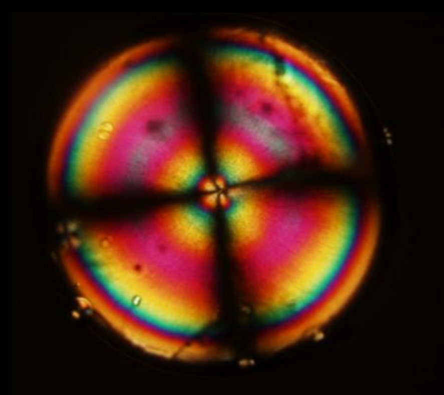
Gemology and conoscopy



Liquid crystal droplets
Nematic drops in an Isotropic LC phase



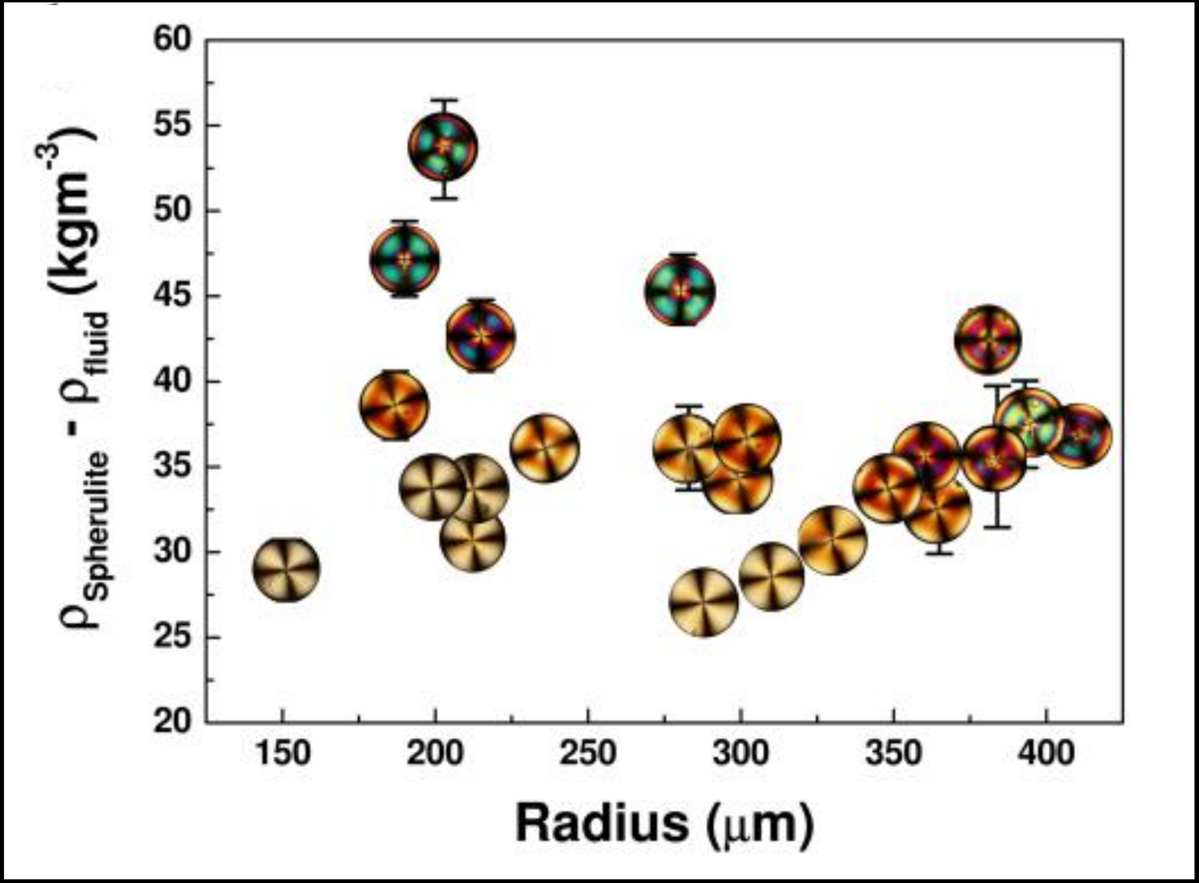
Isochromes in GAS are an interference effect...



Crossed Polarisers

+ QWP

Effects of size and density on optical properties

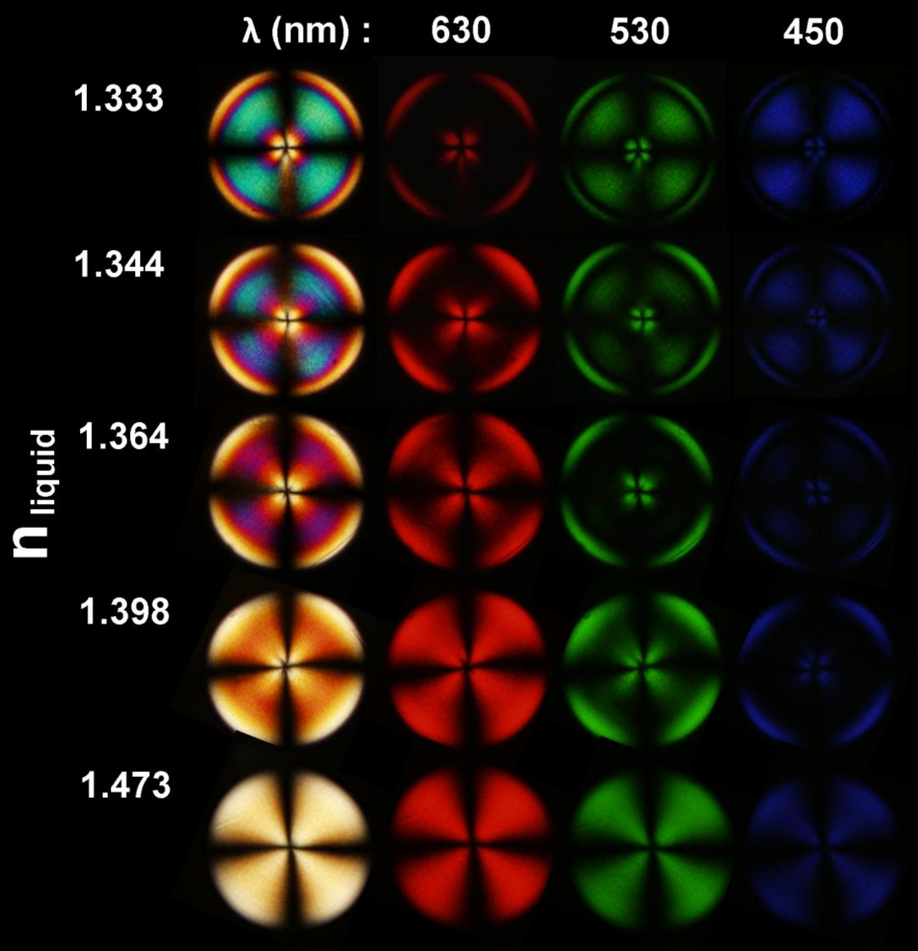


Average density determined from sedimentation velocity of spherulites

Spherulite isochromes depend upon their size and density

Suggests differences in internal structure of spherulites

Effects of changing refractive index of suspending fluid

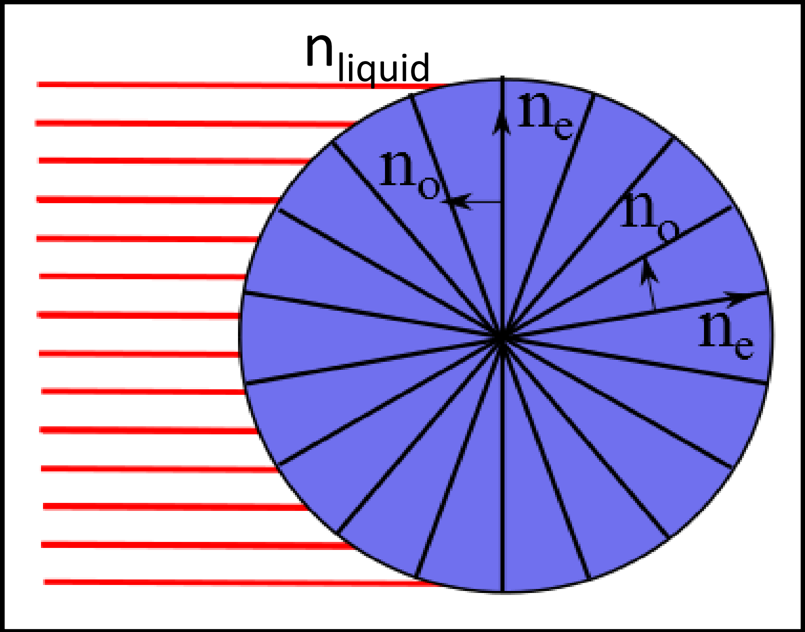


Glycerol-water mixtures used to vary refractive index of surrounding fluid

A single *spherulite* shows different isochromes in different index solvents

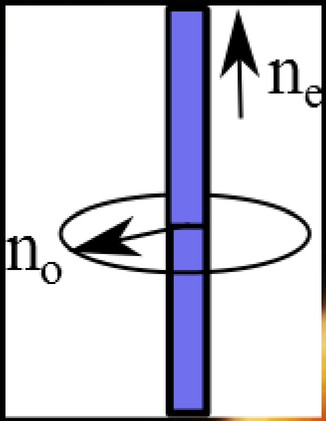
As refractive index mismatch decreases the isochromes disappear

How do we model the optical properties of spherulites?



We consider a 2D slice/disc through the spherulite and split it into ~1000 segments.

Many light rays are passed through the structure and we track their paths and phase changes.

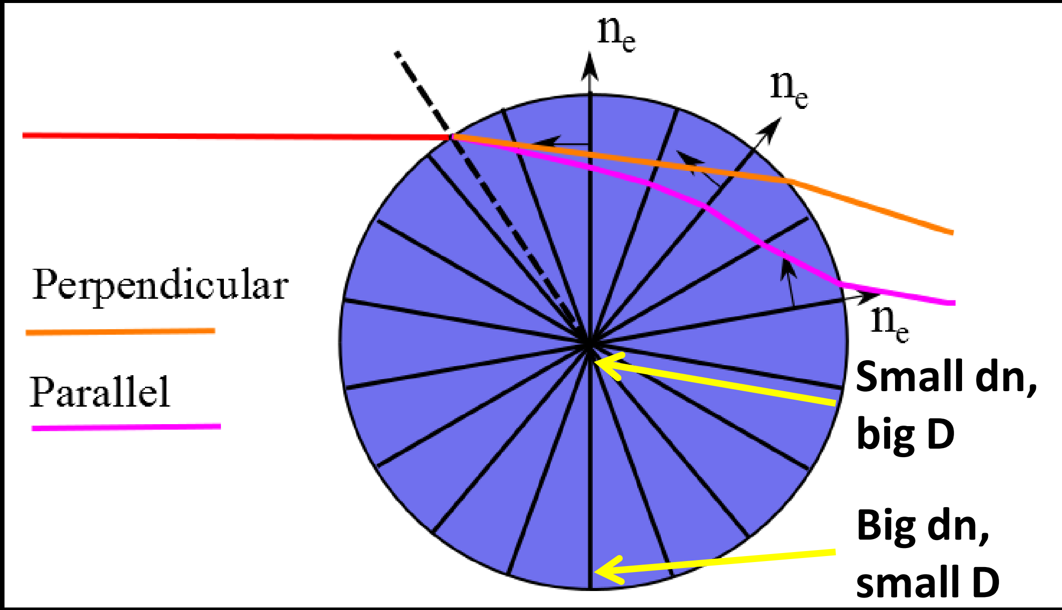


At the interfaces between segments the orientation of fibrils changes - local effective refractive index changes

Polarisation dependence of ray paths

Rays polarised perpendicular to plane of disc 'see ' ***constant*** refractive index n_o

Rays polarised parallel to plane of disc 'see ' **changing** refractive index



Rays with different polarisations follow different paths

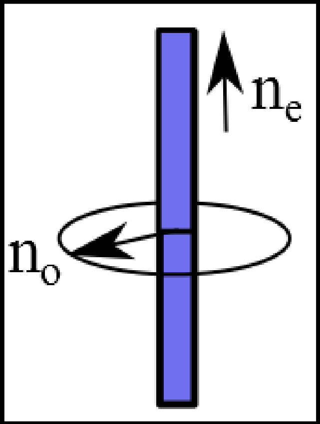
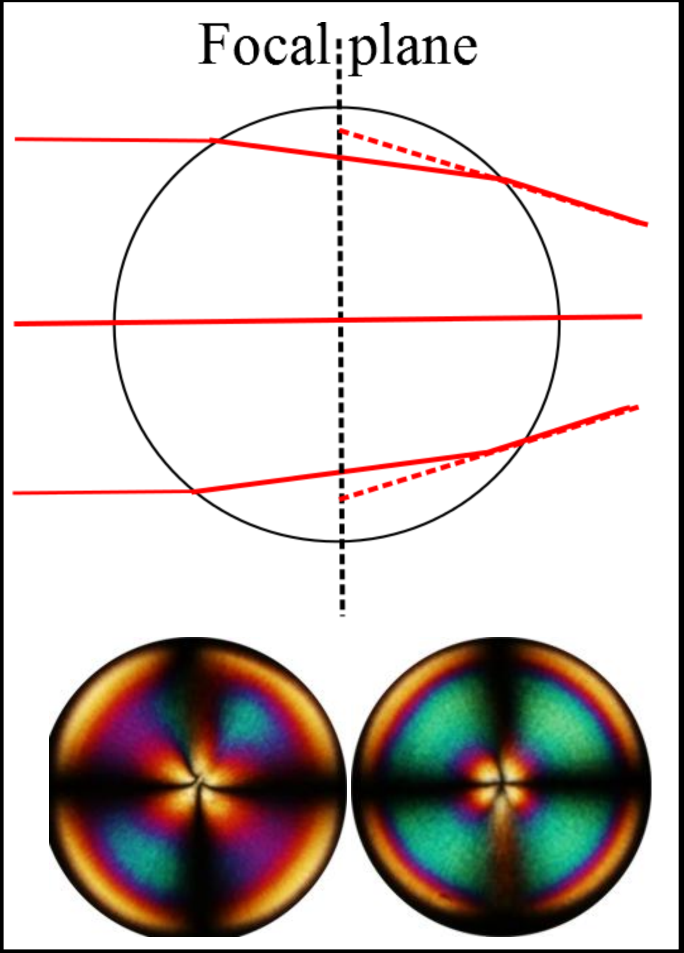


Image Formation

Images are formed by determining apparent source of rays in plane of focus of the microscope.



Local image intensity determined by

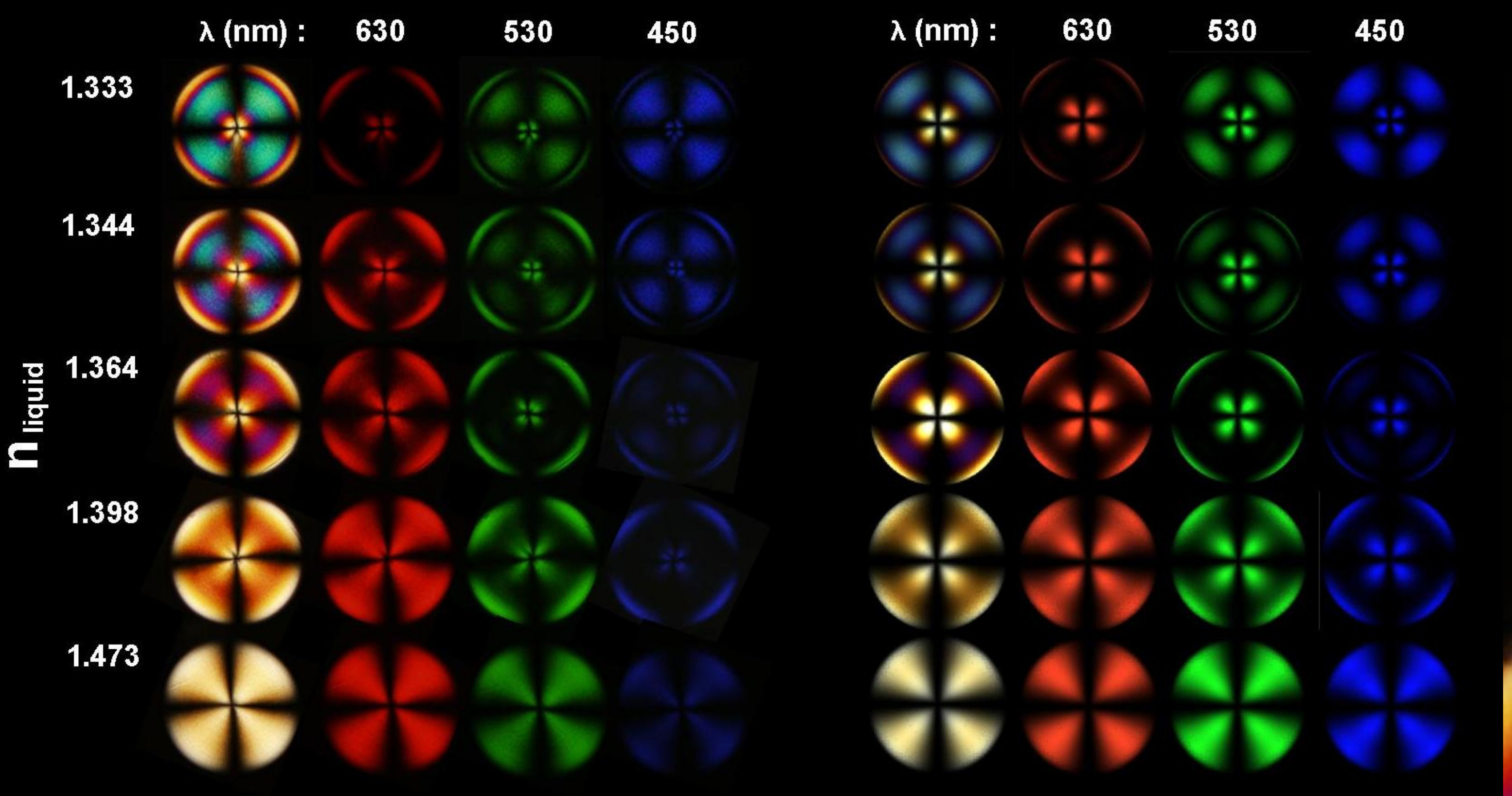
1) number of rays appearing to come from a particular position

2) sum of phase changes of all rays

The 2D slice is then rotated and Jones matrices are used to include the effects of crossed polarisers.

Comparison between data and simulations

190 μm radius Giant Amyloid Spherulite (Average density 1047 kgm^{-3})



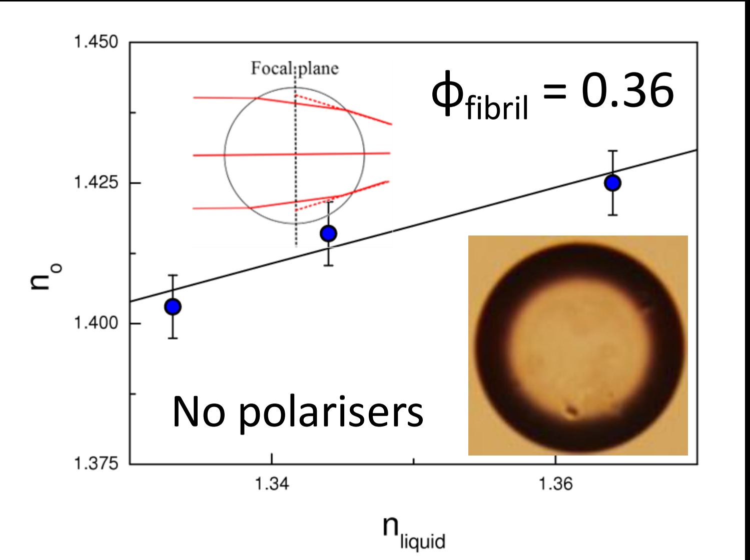
What else can we learn?

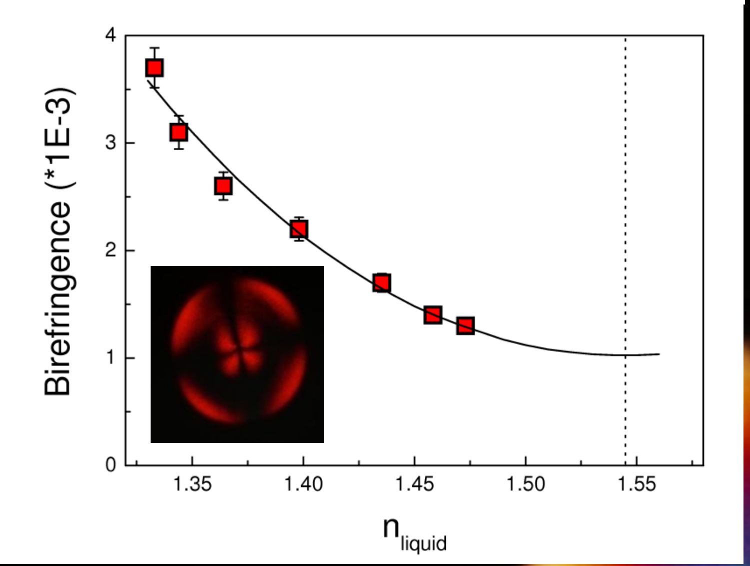
The refractive index and birefringence (dn) of the spherulite can be obtained:

Average refractive index, n_o, can be estimated from the image radius vs. spherulite radius.

dn can then be estimated by matching the measured and simulated radial intensity profiles at each wavelength.

$$dn = S.(dn_{intrinsic} + dn_{form})$$





Intrinsic and Form birefringence

Intrinsic birefringence

Asymmetry in polarisability of molecules .

Form Birefringence

Due to geometry of the fibrils. Shielding of the electric field by the fluid is asymmetric. Depends on n_{liq} and volume fraction of fibrils.

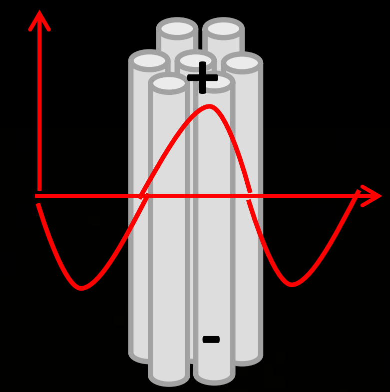
$$dn = S.(dn_{intrinsic} + dn_{form})$$

Calculated value

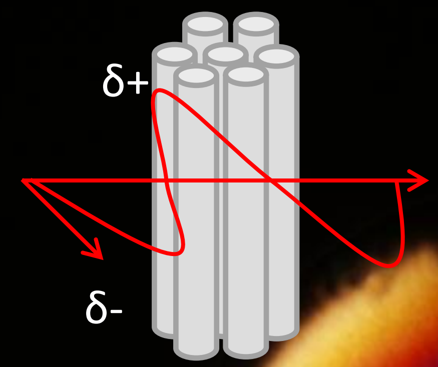
Where S is a local order parameter.

$dn_{intrinsic} = 4x10^{-3}$ $\quad$ $S = 0.27$

No liquid shielding



Liquid shielding



Conclusions

1) Spherulites ~200 – 800µm in radius exhibit colourful patterns when placed between crossed polarisers

2) A ray tracing model captures the essential features of the coloured spherulite patterns.

3) Comparison of model and experiment provides information about the optical properties of fibrils & their arrangement within spherulites

"Giant Amyloids Reveal their true colours"
M.I. Smith, J.S. Sharp, C.J. Roberts
Soft Matter 8 (2012) 3751

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