MICROSCOPICAL EXPLORATION

THIRTY TWO

A COLOURFUL CLOSE-UP OF AMINO ACIDS

Inspired by the excellent work of Dr Robert Berdan in the guise of The Canadian Nature Photographer, in ME32 I will take a close look at a mixture of two of the eleven non-essential amino acids, ß-Alanine and L-Glutamine.

But firstly, a note concerning cleanliness. In my home laboratory I do not consider glass microscope slides to be disposable, and they are, therefore, repeatedly washed and re-used. The washing is, most often, done at 60°C in the kitchen dishwasher, usually in the presence of crockery used during meals, and, due to the presence of food residues, might not be as clean as they appear to be when they come out. So, from the dishwasher the 'clean' slides are transferred to a small plastic box containing purple methylated spirit (shown below) where they are stored until required. Prior to use, each slide is removed from the box and wiped with paper towel until dry and 'squeaky' clean.

Microscope slides stored under purple methylated spirit

Now that the slides are properly clean, let us consider the two non-essential amino acids. Some might say that the term ‘non-essential’ is a bit misleading, insofar as the two amino acids fulfil essential functions in the human body. ß-Alanine has a significant role in buffering lactic acid and improving muscle function, while L-Glutamine is important to the immune and gut systems and is a pre-cursor to the synthesis, in the body, of various proteins. While human body chemistry can, and does, synthesise the ‘non-essential’ amino acids, the amino acids designated ‘essential’ cannot be synthesised and must be provided as part of the human dietary intake.

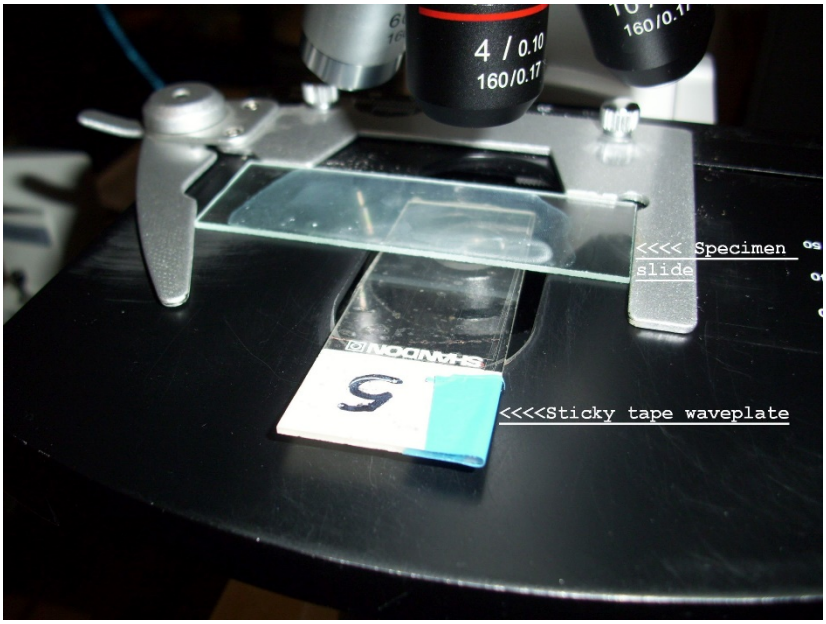
Next, having consulted my copy of the Rubber Bible (CRC Handbook of Chemistry & Physics), in order to look up the solubilities of the two amino acids, I decided to make a solution in 1:1 water: 90% Industrial Methylated Spirit.

I weighed accurately, 0.75gram ß-Alanine and 0.25gram L-Glutamine which I then placed in a borosilicate glass boiling tube and I then added 30mls of the water/IMS mix to the tube. A small PTFE coated magnetic pill was put into the tube and the contents were stirred on a magnetic stirrer for about 20 minutes. The slightly turbid solution thus made was then clarified by filtration through coffee filter paper with a typical pore size of between 10 and 20 µm. 150µL of the resulting clear solution were pipetted onto each of four clean glass microscope slides. Two of these specimen slides were allowed to crystallize slowly at room temperature (slides A & B) and the other two were placed on the fins behind the room central heating radiator to crystallize more rapidly at about 45°C (slides C & D).

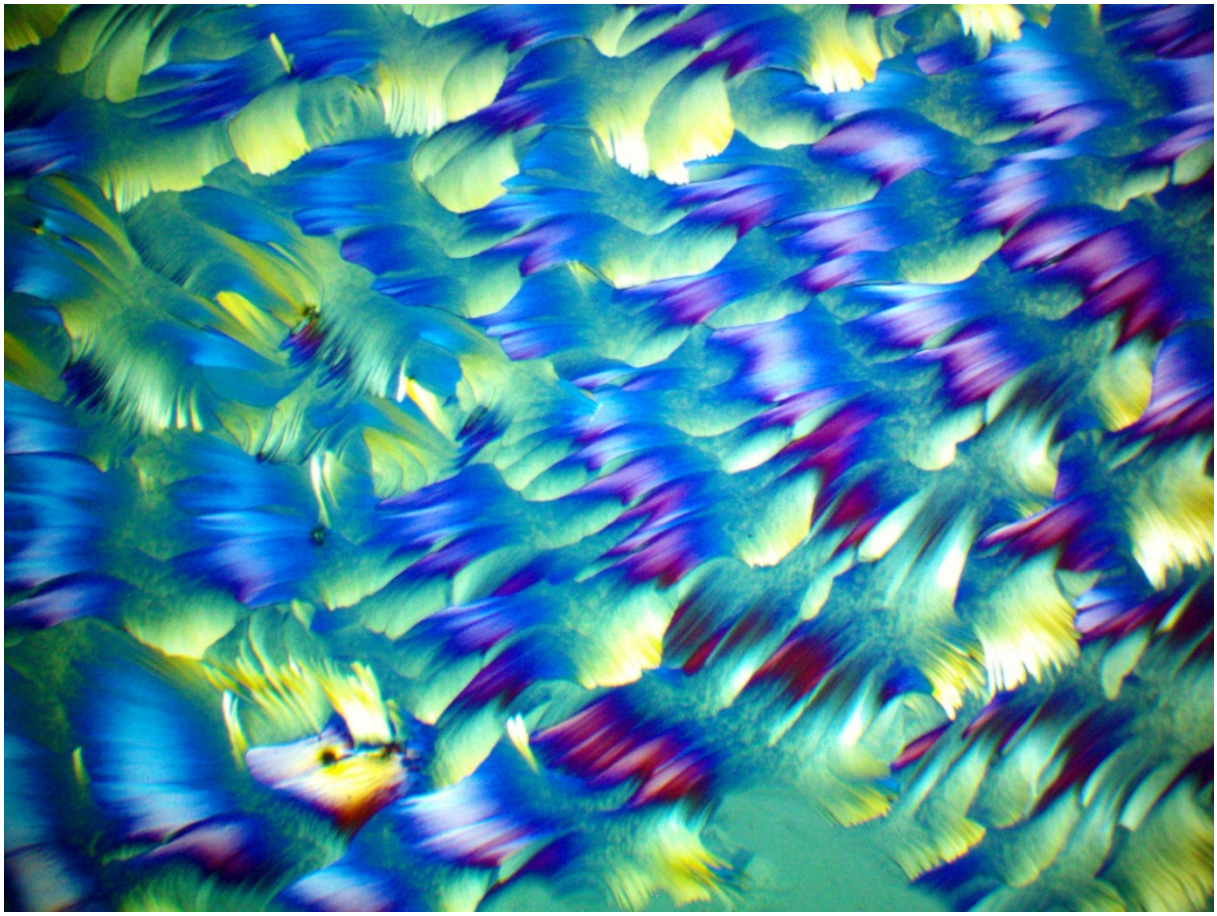
After crystallising for several hours the crystal layer on each of slides A & B appeared, to the naked eye, to be markedly thicker than the layer on either of slides C & D. This can be attributed to the slower evaporation of the solvent at the lower temperature giving rise to larger crystals of the solute on the surface of the slide. On closer microscopical examination, slides A & B were deemed to be unsuitable for the purposes of ME32 and were put aside for cleaning and subsequent re-use.

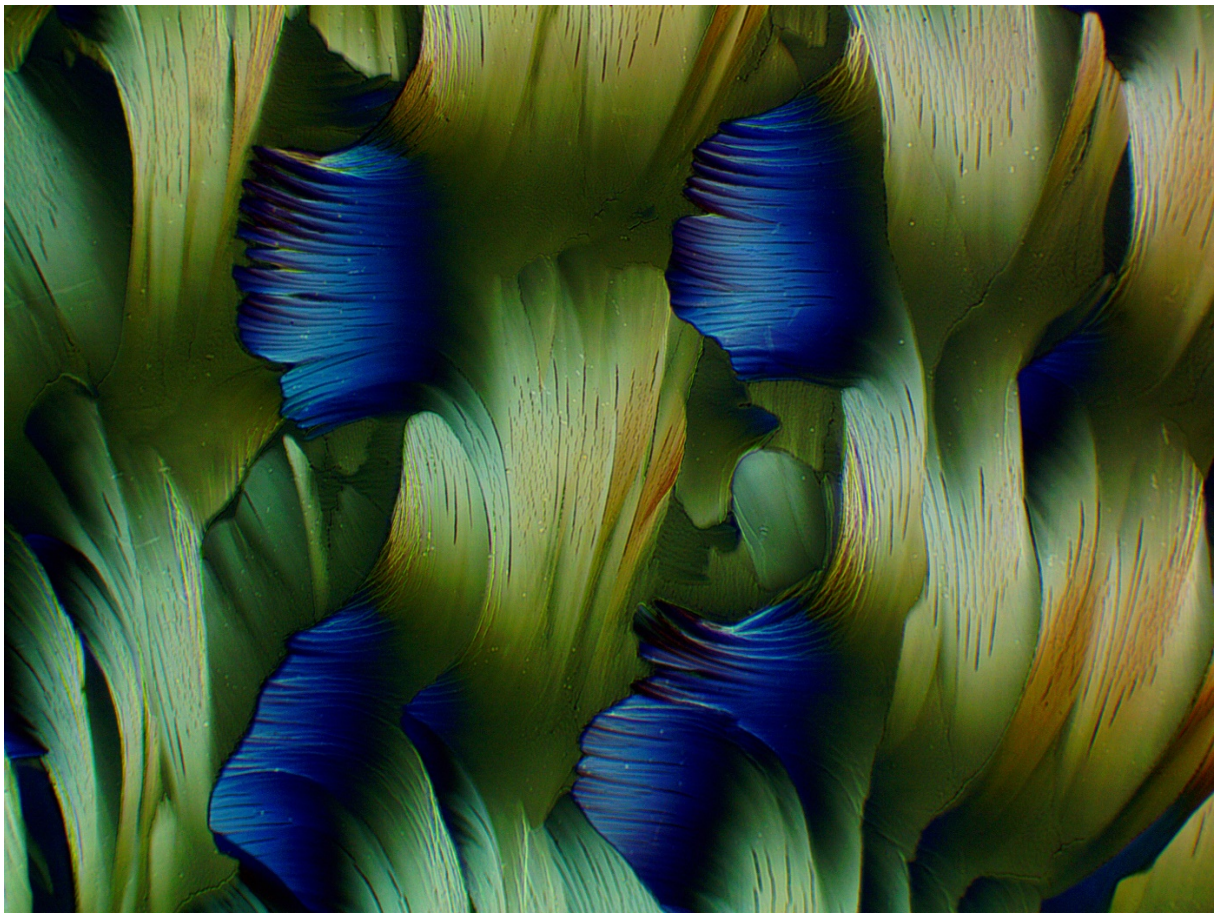
In order to view the specimen slides in polarised light I had to adapt my Swift SW380T trinocular microscope by placing a polarising filter below the condenser on top of the illuminator and placing an analyser in the light path above the objective turret, as shown in this picture.

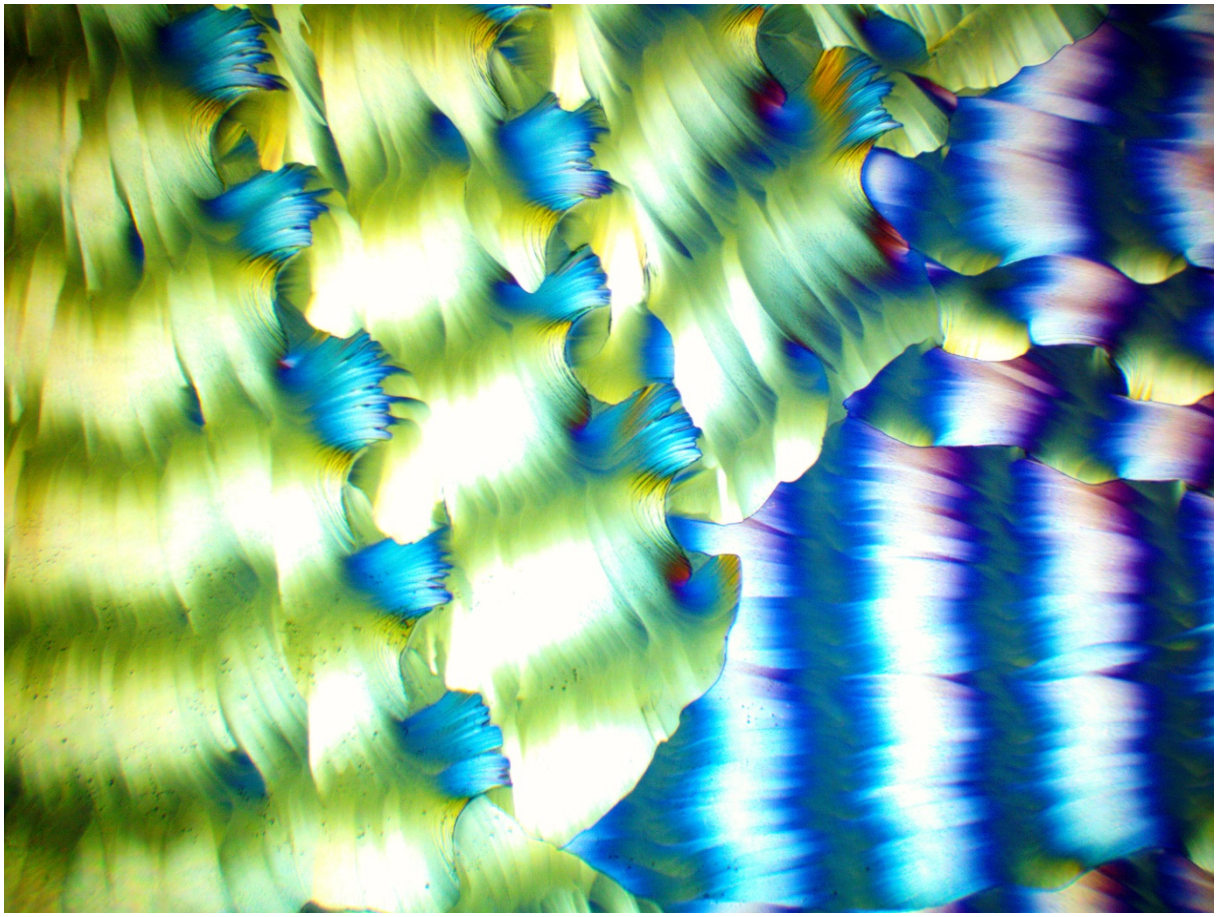
With the illuminator switched on and no specimen slide or waveplate in position on the microscope stage, the polariser was rotated until a dark field was observed through the eyepieces, which indicated that the polariser and analyser filter were in crossed configuration. Next the specimen slides were placed on the microscope stage and observed with various sticky tape waveplates positioned as shown in the next picture.

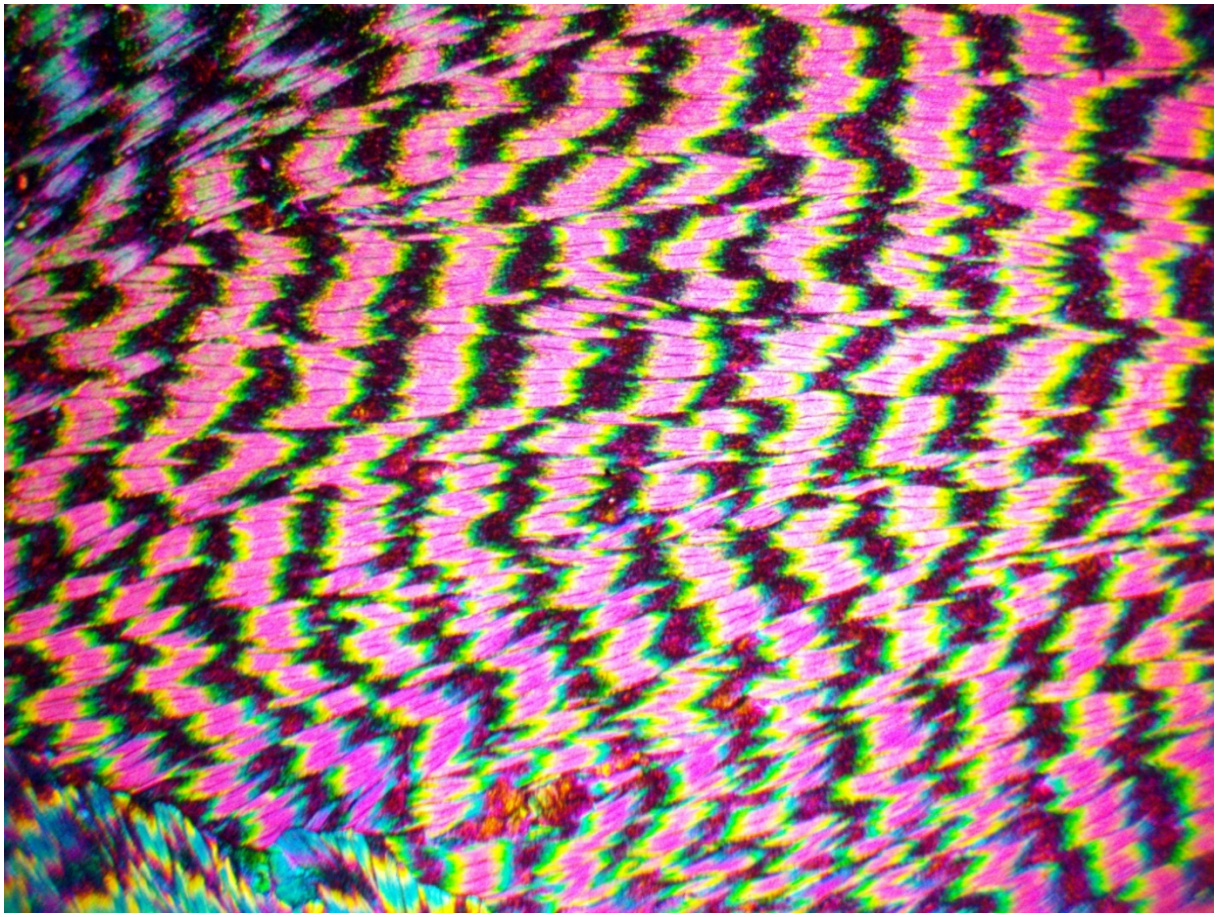


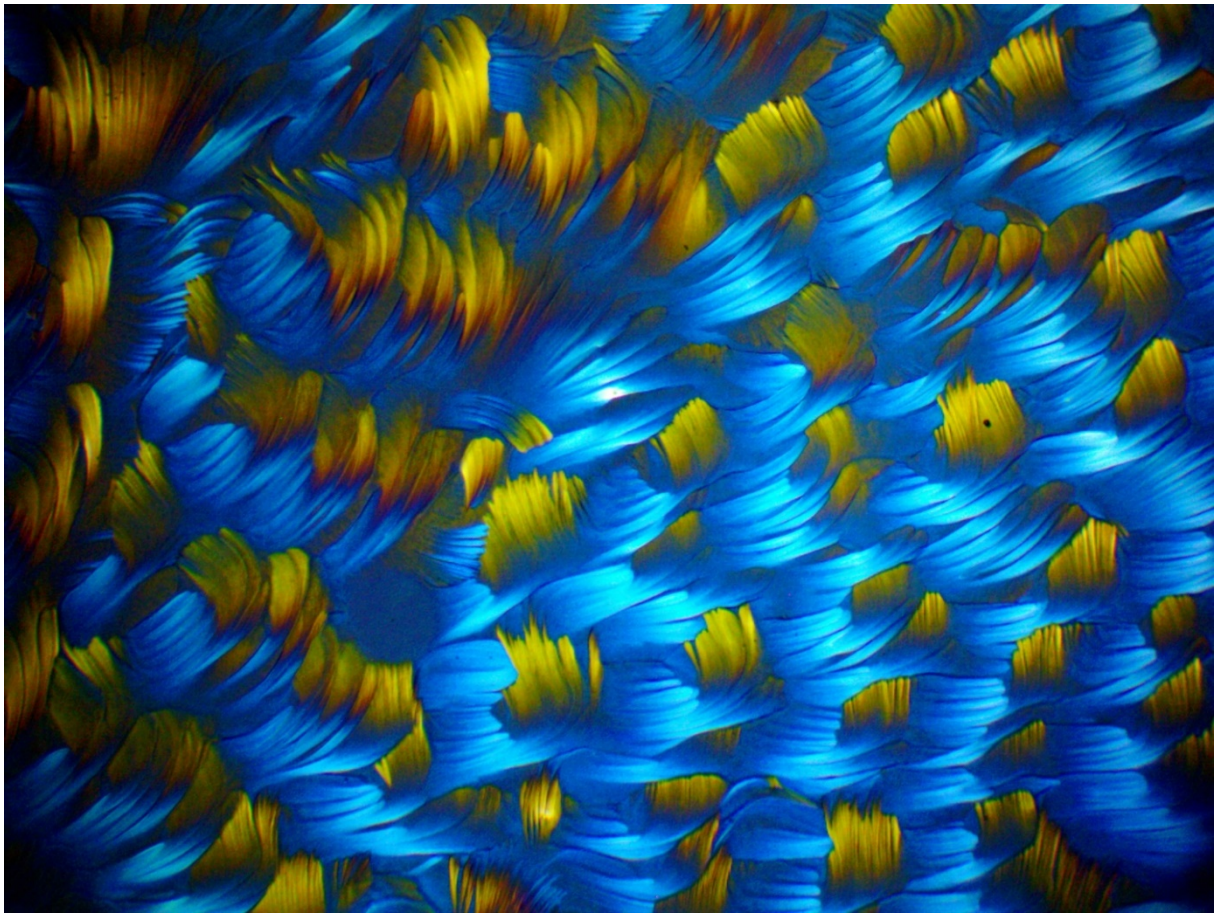
Here are some images of slides C & D observed using the eyepiece camera and recorded with Swift Imaging 3 image capture software.

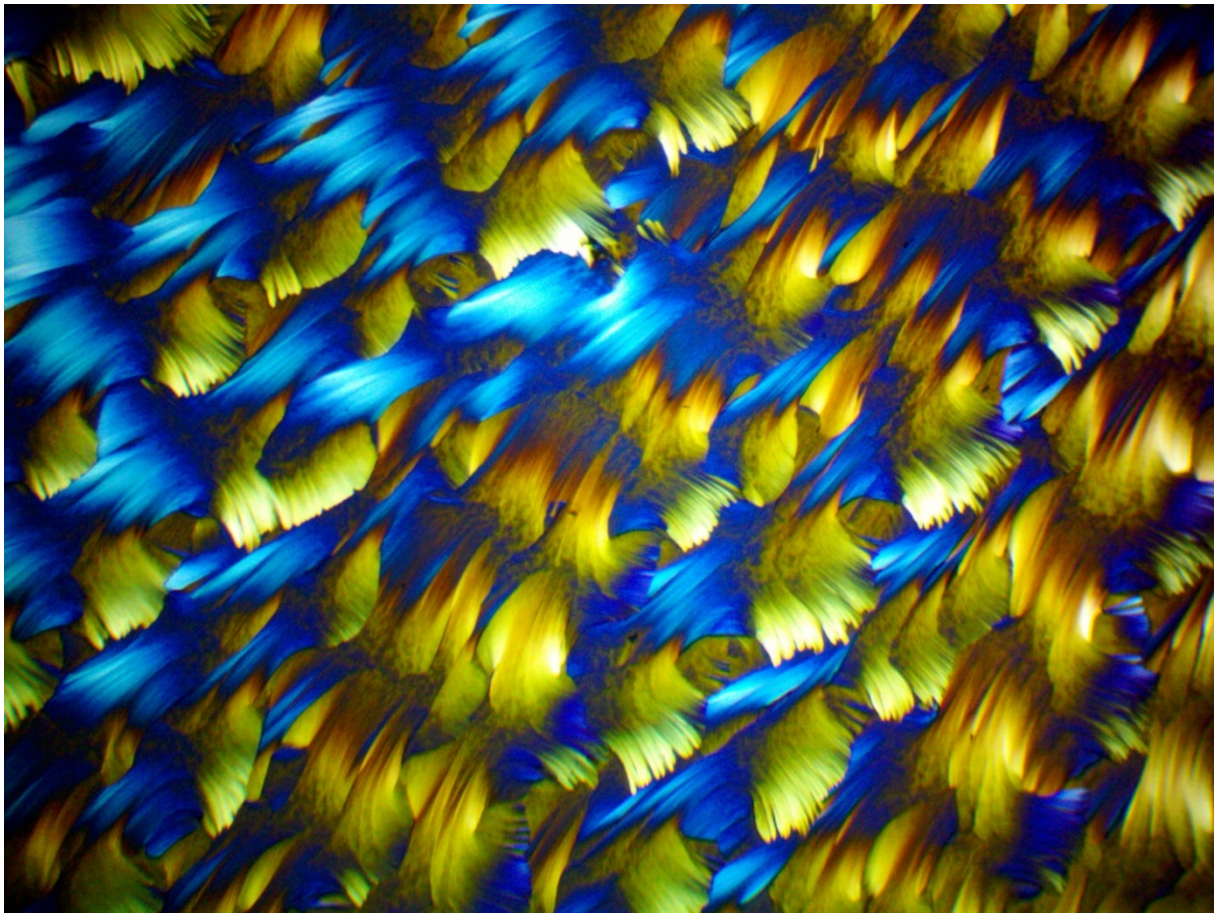


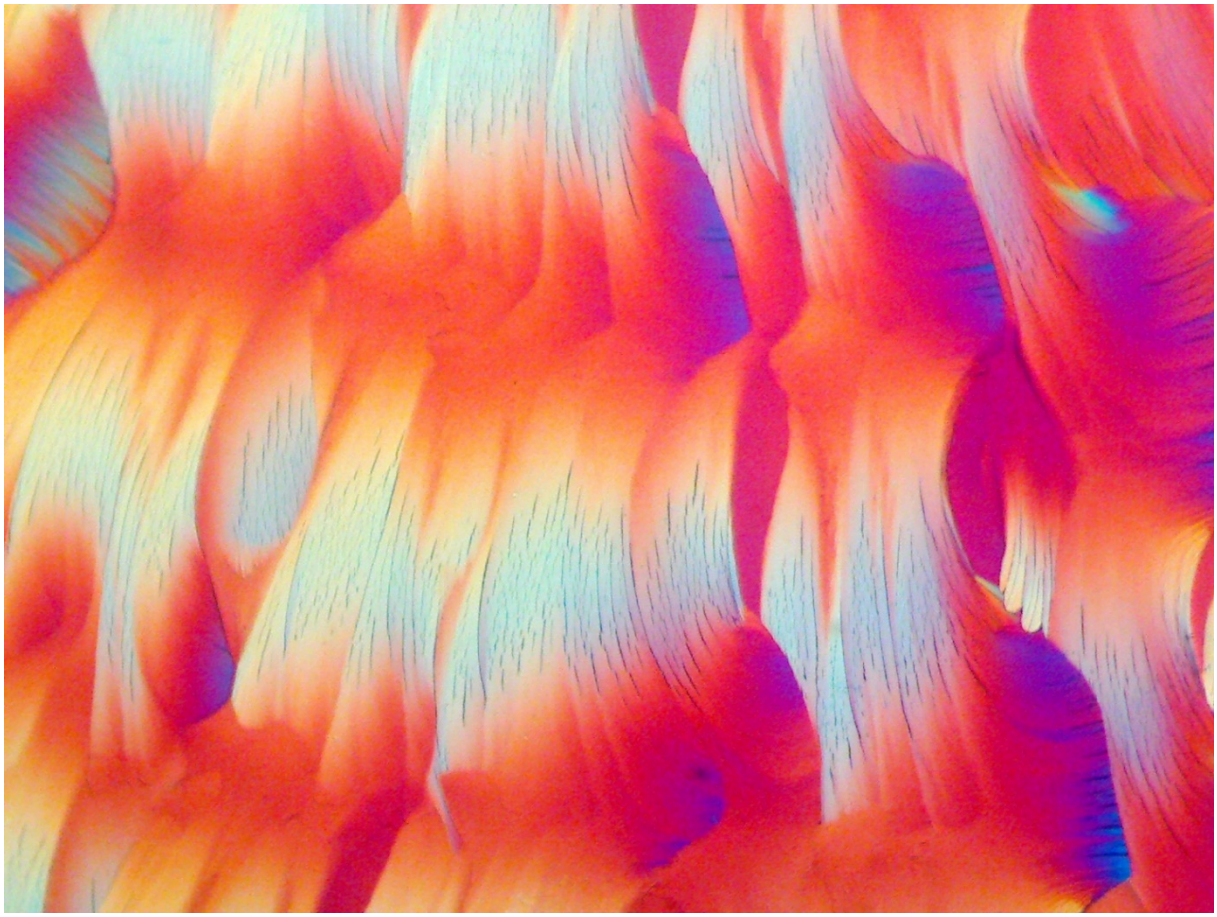


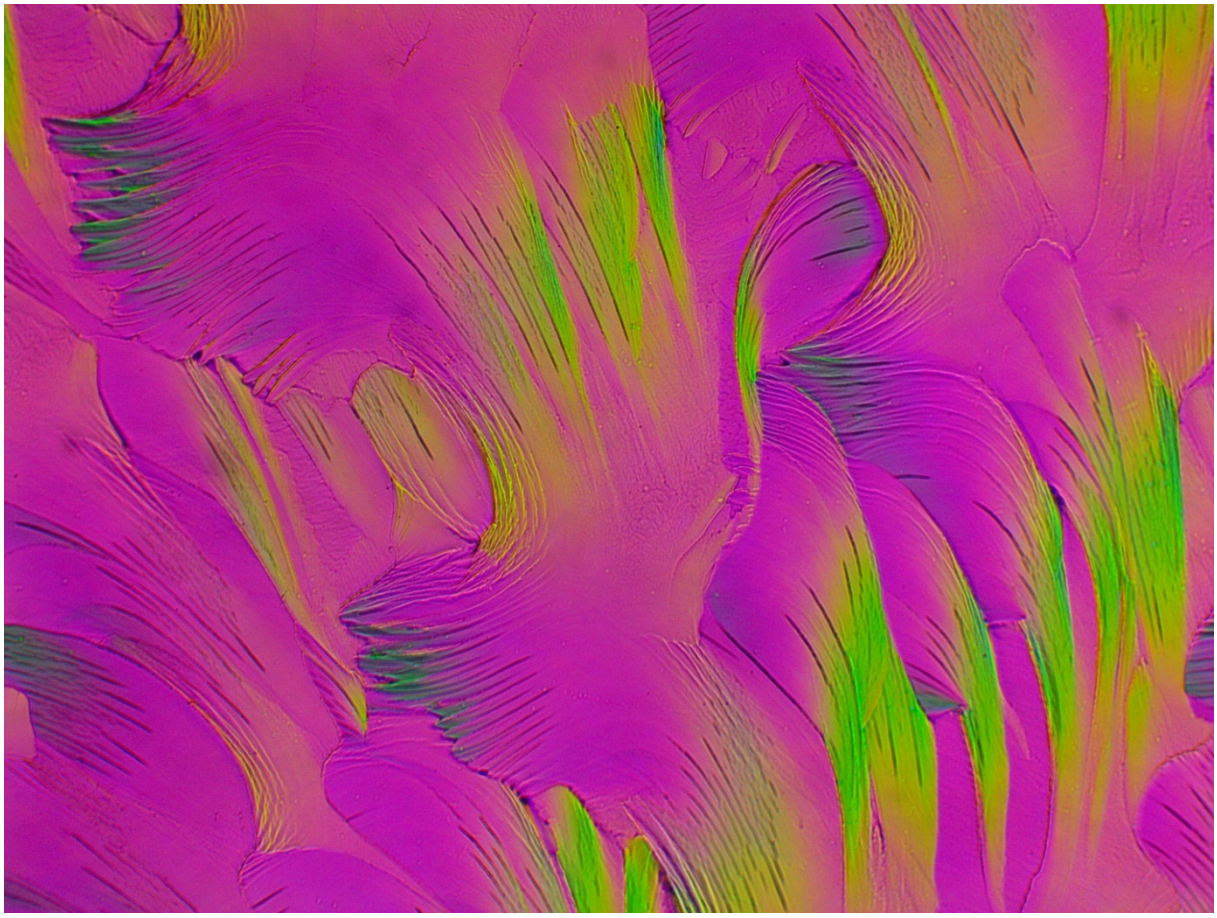


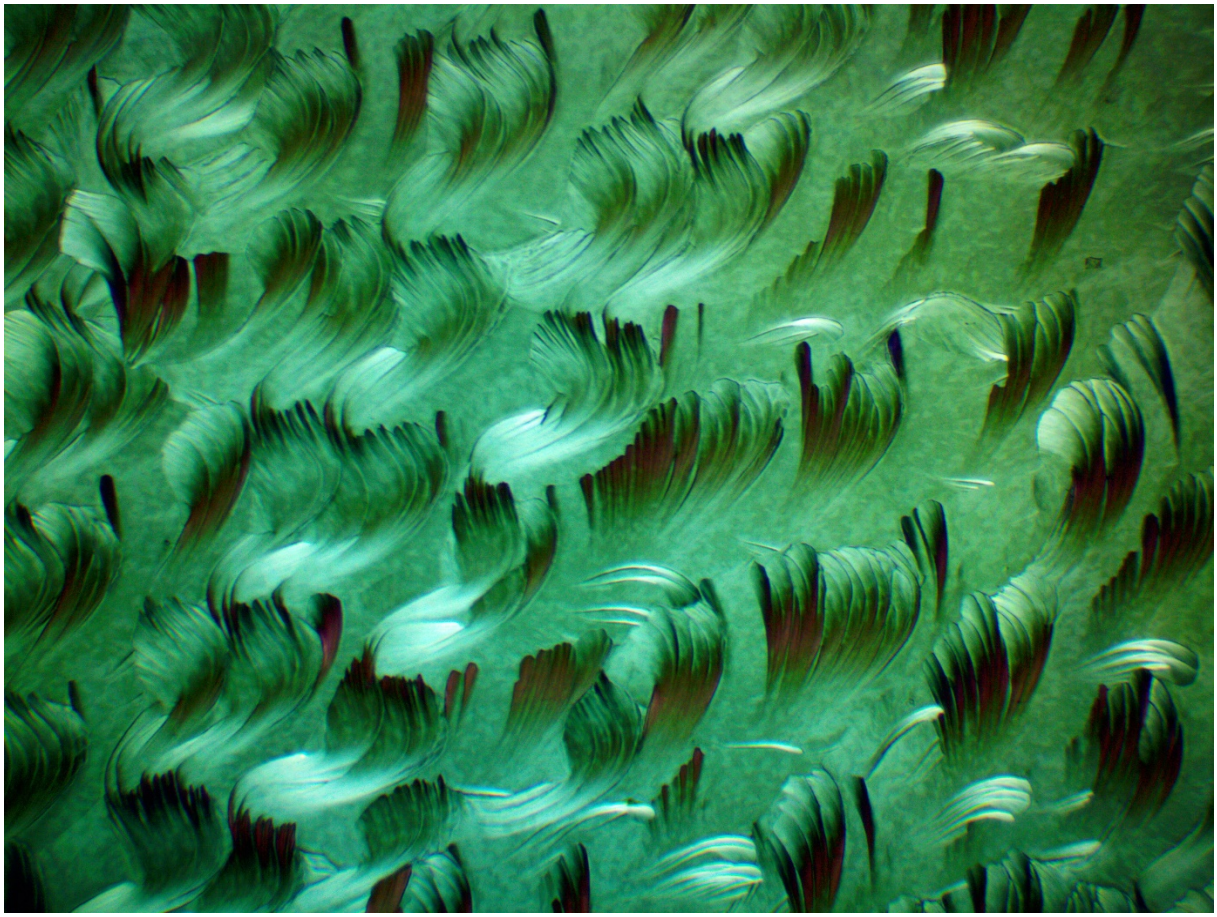


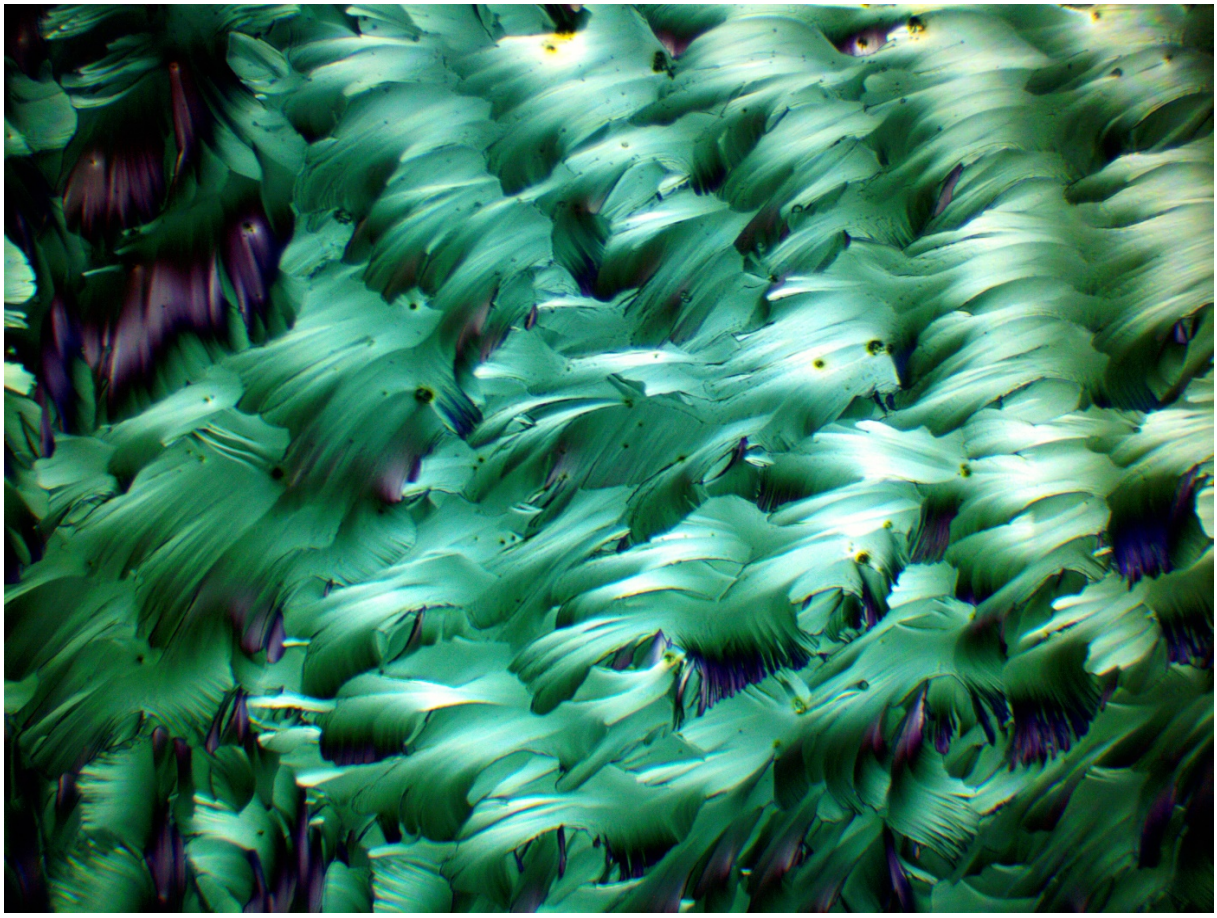












The images above are all single images as they were captured from the microscope, with no image stacking and no colour enhancement.

The Swift SW380T microscope comes fitted with achromatic objectives, which I used to make the observations for this Exploration, and an interesting exercise might be to investigate the difference made by using Plan objectives.

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As we say here in Cumbria:

‘Ave a go yersel’!

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