Nanotech & nature's colours

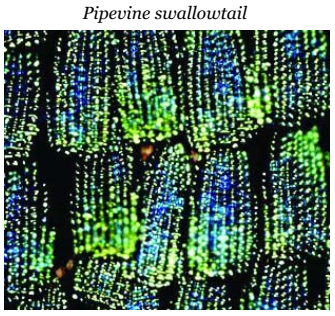
In future we could see structures based on butterflies' wings shining from a currency note or even our passports, says **S Ananthanarayanan**

THE myriad colours that nature is able to display has amazed and challenged scientists through the ages. The *Bible* says, "Consider the lilies of the field, they neither toil nor do they spin. Yet Solomon, in all his glory, was not arrayed as one of these." Dyemasters and chemists have tried their best, but in vain, to create the colours we see in nature.

Even more elusive are the shades that appear in the animal kingdom, in the plumage of butterflies and birds. Here, there are so many shades packed so closely that it is not chemical dyes that are in action but optics and physical structure!

The journal *Nature* carries a report by Mathias Kolle, Ullrich Steiner and Jeremy Baumberg of the University of Cambridge, and Pete Vusisic from the University of Exeter, of advances made in replicating the structures on the wings of *Palilio Blumei*, or the Swallowtail Butterfly, and the technique could have application in industry.

Pipevine swallowtail



The bright green wings of the P. blumei *butterfly result from the mixing of the different colours of light that are reflected from different regions of the scales found on the wings of these butterflies.*

Colours and physics

Colour, of course, is the particular wavelength of light that enters the eye, just as the pitch of sound we hear depends on the wavelength of sound waves that enter the ear. Most things that we see have one or other colours according to the shades of light they reflect or transmit rather than colours they generate themselves. Thus, while we usually see things in sunlight, which is white light, consisting of all the colours that are visible, red objects reflect only the red light and green objects reflect only the green light. This act of reflecting or absorbing light depends on the atomic structure of the surface. There can thus be only as many colours as we have different materials, although a combination of materials can also create new colours, but there is always a limit.

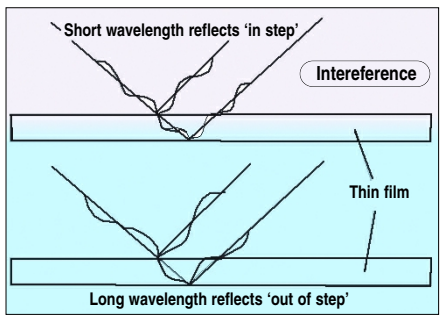
White light, on the other hand, consists of all possible colours. The simple prism is a method of splitting the colours and, in principle, we can isolate nearly every possible colour from white light. There are yet other methods of splitting the colours of white light, and these depend on the wave nature of light.

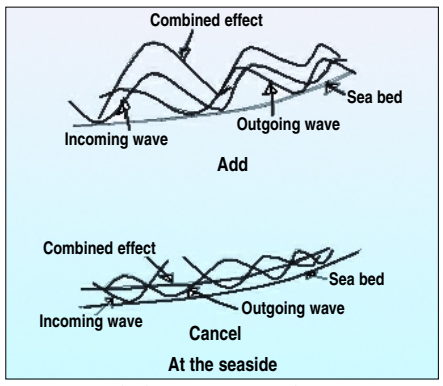
Interference

The rainbow colours we see on oil spilt on water is because of this. As light consists of waves, the waves can be made to add to each other or to cancel each other. It is like the waves that come in at the seashore. There are waves coming in and also waves flowing back. We have seen at the seaside that when the two waves meet "in step", we have a large one. But if they are "out of step", then, the wave fizzles out.

It is the same when light reflects off the two surfaces of a thin film, like oil spilt on water. The waves reflected off the lower surface have travelled a little longer than the waves that bounce off the upper surface. If this extra distance is a whole wavelength, then the two waves are in step and they add. But if the distance is half a wavelength, then the two waves meet out of step and they cancel!

The condition for adding or cancelling is different at any point, for different colours. As the oil film may not be of uniform thickness, the film then shows different colours at different points. The condition also depends on the angle of incidence of the light. The colour pattern can thus be different when viewed from different angles. The whole thing, of course, would also change as the oil film spreads out or the water evaporates, to change the nature of the oil film.





Grating and photonic crystal

Another place where light splits into colours is when it passes through a pair of narrow slits, close together. The beam of light now becomes two beams, originating at each of the slits. There will be points where the distance from both slits is equal or differ by a whole number of wavelengths. At such points, waves will add and show brightly. But where the waves do not come in step, they will interfere and cancel. And the points of brightness or darkness will depend on the wavelength, which is, to say, the colour.

A device to split colours like this is the optical grating, which is a glass plate scored with parallel lines, very close together. The effect is of a series of pairs of slits and there is a clear splitting of colours, like through a prism.

The photonic crystal is an atom scale structure which similarly selects wavelengths to allow free passage or to block. But this is not a manufactured or fabricated device like the crystal. It is the internal structure of a material, depending on its chemical nature and the way it has been prepared.

Butterflies and birds

The plumage of creatures shows colours with the help of intricate, microscopic physical features of their surface. In some cases, there are scorings, like the grating, and in some cases there is a step structure, creating a phase difference between waves reflected from the *cusp* or the *trough*, which also leads to selection of colours. And there are thin, transparent layers that reflect from the upper and lower surface, like the oil spill, and create colours too.

By this combination of physical structures, which can be very sensitively controlled and make for all possible colours and shades, the plumage of butterflies and birds display variety that is not encountered anywhere else. The colour schemes have obvious survival value and the physical features of the plumage are genetically determined. As the colour scheme presented could be different with different viewing apparatus, there are even species of butterfly which appear green and hence difficult to spot among green leaves by to predators, but in different, attractive colours to other butterflies, which would help both for staying together as well as for attracting mates.

Mimicking nature

Mathias Kolle and colleagues used a battery of techniques to deposit layers of optical materials on a silicon surface coated with gold. The process was to first coat the surface with a pattern formed by polystyrene. Gold or platinum is then deposited where there is not polystyrene. The polystyrene is then chemically removed, leaving a nano-scale reflecting pattern.

This is a very simplified account of the methods used, which include deposit by self assembly and atomic layer depositing, depositing materials that not only reflect or absorb by also affect the plane of vibration of light waves, a property known as *polarisation*.

Kolle says, "We have unlocked one of nature's secrets and combined this knowledge with state-of-the-art nanofabrication to mimic the intricate optical designs found in nature… although nature is better at self-assembly than we are, we have the advantage that we can use a wider variety of artificial, custom-made materials to optimise our optical structures."

Understanding how nature manages this astonishing control of colour would have application in commerce and industry. "These artificial structures could be used to encrypt information in optical signatures on banknotes or other valuable items to protect them against forgery. We still need to refine our system but in future we could see structures based on butterflies' wings shining from a ₹ 10 note or even our passports," says Kolle.

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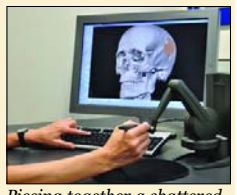
Radical new approach

Virtual cadavers, needle-wielding robots ~ and not a scalpel in sight. **Laura Spinney** meets the research team behind 'virtopsy'

BACKING his four-by-four into the garage one night in 2006, after returning from a drunken party in the Swiss canton of Bern, a man crushed his wife against the garage's rear wall. He called an ambulance but she died before it reached the hospital. The man claimed it was a tragic accident – he had reversed once and hadn't seen what he was doing – and to the police it looked like a case of involuntary manslaughter. But just to be sure, they asked Michael Thali, director of the University of Bern's Institute of Forensic Medicine, for a second opinion.

Professor Thali and his team carried out an autopsy on the woman, but not the kind we're used to seeing in television shows such as CSI and countless police dramas. This was a scalpel-free, virtual autopsy, or "virtopsy" – a radical new approach to forensic investigation in which Professor Thali is one of the pioneers. Using Computerised Tomography and Magnetic Resonance Imaging, the Bern team created a high resolution, 3D virtual double of the woman's crushed corpse. They also scanned the surface of the car so that they could create a virtual model of it, dents and all.



Piecing together a shattered skull could take days. But with virtopsy it can be done with just a flick of a switch.

Combining these with evidence found at the scene, including skid marks on the garage floor, flakes of paint knocked off the wall and fragments from the car's smashed rear lights, they painstakingly reconstructed the events of that night.

When their virtual reconstruction was complete, they were able to tell the police that the man must have reversed twice, because a single backing up could not account for all the damage they had seen. The first time, they concluded, the woman was standing in a doorway and sustained injuries to her arms and legs, before slumping onto a stool. The second impact damaged her internal organs, and it was those injuries that killed her. The man was charged with willful homicide. "Ironically, he died before the trial could take place, of natural causes," says Professor Thali. "We performed the autopsy here."

So how does it work? In its first incarnation, the virtopsy procedure involved taking a 3D surface scan of the body using stereoscopic cameras and a projector that cast a stripe pattern onto the body. The team would then slide the table bearing the body into a CT scanner, which would assemble X-ray slices of it into 3D images of the internal organs. Markers placed strategically on the skin during the surface scan allowed surface and interior to be matched. Later, more high-tech tools were added. While CT is good for revealing bone, it isn't so good for seeing soft tissue, so now they use MRI to image the latter. Taking automation to the next level, they added a robot, the "Virtobot", that can be fitted with a needle and manoeuvred into position by a remote computer, to take a tissue sample should the pathologist need more information.

It hasn't all been plain sailing. Professors Thali and Dirnhofer – who coined the term virtopsy – initially encountered a lot of scepticism from the medical community. But gradually, says Thali, they are bringing people round.

The Independent, London

Blinkers and bottlenecks

There is no known basis for deciding the minimal amount of DNA required by an organism because scientists talk at cross purposes regarding the minimal number of enzymes required for the maintenance of life, writes **Tapan Kumar Maitra**

THE amount and informational content DNA characteristic of an organism or a species must be consistent with its unique requirements for growth, maintenance and reproduction if it is to contribute genetically to the next generation or if the species is to persist. As these requirements increase through the selective acquisition of greater and greater complexity, the amount — and functional diversity — of DNA would be expected to increase correspondingly. On the other hand, an increase in the amount of DNA does not necessarily confer uniqueness of complexity on an individual or a species. The characters that define an individual organism or species can be modified without changing the total amount of DNA and it is equally obvious that a species, for example, the various viruses, can function very effectively with relatively small amounts of DNA.

Considering their mode of life, it is not surprising that the viruses contain minimal amounts of DNA. The satellite necrosis virus contains only enough DNA to code for its own protein coat, borrowing all other necessary enzymatic activities from the host cell it invades. The small fx174 phage contains only 5,000 nucleotide pairs, sufficient perhaps for the coding of 10 to 15 polypeptide chains of average length. Clearly such minimal amounts of DNA would seem to place these viruses in some jeopardy because one might expect that internal deletions of DNA would lead to immediate lethality. Nevertheless, there are many strains of bacteriophage, for example, T4, that can sustain such deletions and still survive. This may be an indication that even organisms at the level of viruses may possess internal duplications of genetic material that can be sacrificed without lethal results. No answer is immediately available, but it is perhaps more reasonable to assume that the deleted portions of the genomes have the effect of restricting the strains of viruses to special environments thus seriously limiting their adaptability.

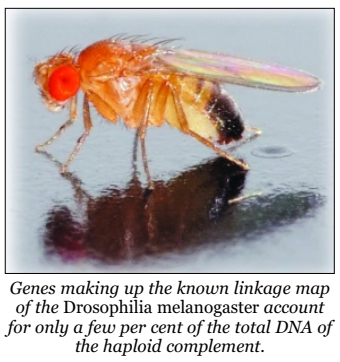
Compared with viruses, E. coli has substantially more DNA, as one might expect from its obviously greater versatility and complexity as an organism, a condition that would demand a greater array of enzymes and, consequently, a greater amount and diversity of DNA to code for these enzymes. However, if one compares the amount of DNA per cell in various plants and animals, a wide discrepancy becomes apparent — Neurospora has 4.5, Drosophila 18 and mall about 1,000 times as much as E. coli.

On a phylogenetic basis, these differences may not seem unreasonable, but then why should Necturus possess 6,000, the lungfish 12,000 and Amphiuma 22,000 times as much as E. coli? It must be obvious that the last three organisms are much less complex than man: they are not polyploid and their chromosomes are no more polytenic than those of other organisms possessing much less DNA. It is thus clear that the amount of DNA per nucleus does not provide a simple index of either structural or functional complexity.

Admittedly there is no known basis for deciding the minimal amount of DNA required by any organism because we do not even know the minimal number of enzymes required for the maintenance of life. Nevertheless, there are reasons for believing that higher organisms contain substantial amounts of repetitive DNA. For example, E. coli is thought to possess the maximum amount of DNA possible without extensive internal repetition of nucleotide sequences. The argument is based on evidence from annealing experiments and, independently, from mathematical considerations. If DNA is melted into single polynucleotide strands and then slowly cooled to permit re-annealing to take place to reform the double helices, the minimal recognition length — the least number of successive bases — for re-annealing is about 12 nucleotides long. The number of non-identical sequences of this length is over eight million (0.5×4^{12}) and this number can be accommodated in an E. coli chromosome. Repetitious sequences of this length are not found among the naked chromosomes of viruses and bacteria; they would be selected against, for they would tend to fold back upon themselves, undergo recombination and thereby create genetic instability.

It can be argued that the complex chromosomes of higher organisms are so constructed as to permit a greater degree of repetition without such danger — a point of view supported by the re-annealing experiments carried out on mouse DNA by Bolton and his colleagues. They have shown that the rate of annealing is very much greater than that expected on the basis of non-repetitive sequences and is, therefore, indicative of substantial repetition brought about through duplication of genes.

A two-dimensional map showing the secondary structure of 16S RNA.



Genes making up the known linkage map of the Drosophilia melanogaster *account for only a few per cent of the total DNA of the haploid complement.*

It has been suggested that the genes making up the known linkage map of D. melanogaster account for only a few per cent of the total DNA of the haploid complement. For example, there are more than 5,000 bands known in the polytene chromosomes of this organism and some 500 known loci. Assuming a 1:1 correspondence of gene to band, this means that about 10 per cent of the DNA has been mapped. Even the X chromosome — the most intensively mapped in any multi-cellular organism — has only one-fifth of its DNA genetically identified (200 genes to 1,000 bands).

What, then, is the function of the remainder of the DNA? Does it consist of genes that control and regulate differentiation? Does it consist of genes governing quantitative characters that are numerous and difficult to map? How much of it is engaged in the manufacture of nucleolar materials, or the several kinds of RNAs needed for cellular synthesis?

An answer to the last question has been provided by FM Ritossa and his colleagues. Following the lead of others who demonstrated that ribosomal RNA is formed in the nucleolar-organiser region and making use of several stocks of D. melanogaster that contain, respectively, one, two, three and four nucleolar-organiser regions, the rate of formation of DNA-rRNA hybrid molecules and the point at which the extracted DNA is saturated with rRNA, they have provided data indicating that there are about 130 copies each of the cistrons that code for the 28S and 18S rRNAs. Presumably, these cistrons exist in a tandem array and have been accumulated through duplication. By the same technique, they have also demonstrated that the bobbed locus, mapped in the nucleolar-organiser region, is a deficiency mutation, resulting in a lowered formation of rRNA.

Comparable studies by this group and others indicate that about 100 rRNA cistrons are present in the genome of the chick, about 1,000 in Bacillus megatherium, and five in E. coli. Continued and often enhanced production of rRNA — particularly at certain stages of development — makes such cistronic linear redundancy explicable in evolutionary terms.

A similar linear redundancy has been found for the tRNAs. There are 60 kinds of tRNA, and in Drosophila each cistron appears to be replicated linearly about 13 times. Ritossa and his colleagues raised the intriguing question whether deficiencies or mutations in tRNA cistrons were responsible for the commonly observed Minute phenotype.

One must assume — in the absence of evidence to the contrary — that all DNA acts as a template for transcription. If this is the case, there is no excess DNA. In fact, the energy requirements for the formation of DNA are so great that nonfunctional or excess DNA would not be tolerated and would be eliminated during the course of evolution. However, there is no completely satisfactory functional explanation that accounts for the acquisition and persistence of such large amounts of DNA as exhibited by the Amphibia, although Keyl has provided a plausible hypothesis to account for its origin through faulty replication. A cytochemical study of hybrids between Chironomus thummi and C. thummi piger shows that certain bands in the polytene chromosomes of C. thummi differ in DNA concentration from those of piger by a factor of 2, 4, 8, or 16. Intermediate values have not been found. This suggests a progressive but localised doubling of DNA, except that the doubling leads to a linear repetition rather than to a lateral redundancy. Experimental evidence, on the other hand, indicates that DNA incorporated into the genome as a duplication might be expected to create genetic imbalance at the time of incorporation: the situation at the Bar locus in D. melanogaster is a representative example of an upset in development caused by such linear replication. The fact that, in the long run, such duplications are not only tolerated but are actually integrated into the genome without imbalance is of great cytogenetic interest.

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