Taking math to heart

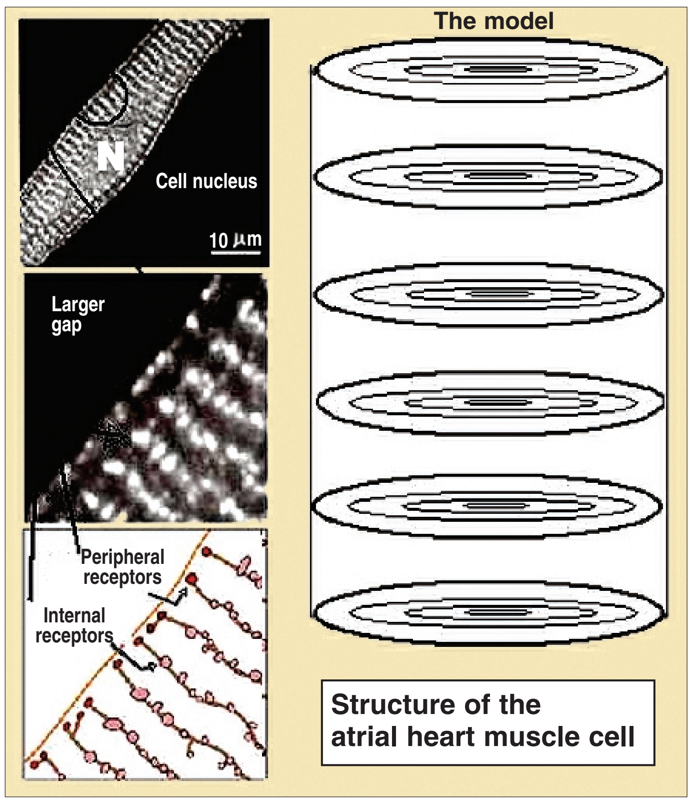
s ananthanarayan reports on what this means for the cardiac specialist

USING mathematics to model physical systems has been routine. For instance, the distance travelled is exactly modelled by "speed multiplied by time", and this model works for the distance travelled by anything that moves. A more complex example is the path of a satellite around an attracting body. The effect of attraction due to gravity can be modelled by the properties of the ellipse and the parabola and mathematics helps exactly chart the path of the heavenly bodies, past or future.

A further abstraction would be when the motion considered is not physical motion but an effect like growth or increase in population. The various components of a system, some that increase growth and some that stop or reverse growth, can be modelled mathematically and the behaviour of the system can, over a period of time, be viewed without leaving the desktop.

The availability of computers has made it possible to build in a great number of components and to view how the system behaves over millions of successive computations. The importance given to each component can be varied to see how the model behaves so that assumptions made in developing the model can be tuned to correspond to experience, and then the model can be used to extrapolate, or make estimates of how things would be under unknown conditions. While these estimates would need to be accepted with caution, modelling can show the way to experiments that help correct assumptions and improve our understanding of the process.

Areas where modelling has been used is for weather forecast, population studies and mechanical performance. The weather is typically dependent on huge numbers of variables and a change in any one can affect all others, including the variable itself. Complex models are developed and thousands of values, which are collected over a large area, are used to make weather predictions, a matter of immediate, economic importance. An application at a different scale is the study of the climate over decades, centuries or millennia.



Structure of the atrial heart muscle cell

Rüdiger Thul

The likely outcome of global warming, in 20, 40 or 60 years, for instance, is being studied with the help of models of the factors, including political and economic, that are involved. Population studies depend not only on birth and death rates but also on migration. Physical, political and economic factors thus need to be built in. In the design of industrial products, performance over long periods needs to be known, but the designer needs the benefit of experience even as he sets out. Modelling and simulating the use of the product over a long period, with the help of a computer, is a way to help the designer.

Muscles of the heart

"A human heart beats more than a billion times during the average lifespan, and is required to do so with great fidelity," say Rüdiger Thul of the School of Mathematical Sciences, Nottingham, and his colleagues, who are from the Laboratory of Signalling and Cell Fate and the Department of Pharmacology, Cambridge, UK. The multidisciplinary group reports in the *Proceedings of the National Academy of Sciences* that they have created a mathematical model of the muscle cells in the human heart, as seeing its working is still beyond the scope of imaging technology.

The main function of the heart, of course, is to pump blood. The pumping action is by the muscle cells that surround the large chambers, the *ventricles*, of the heart. But along with this action of the ventricles is the action of the smaller chambers, to provide a steady flow of blood to the ventricles and also a part of the pressure for pumping. This enabling and helping action of the *auricles*, or the *atrium*, also known as the *atrial kick*, accounts for a substantial portion of the overall output of the heart. But with age or disease, this action of the atrium can be impaired or mistimed and is estimated to be the main reason for irregularity in the beating heart, a factor that accounts for some 15 per cent of heart strokes.

The contraction of the cardiac muscles, finally, is at the level of the muscle cell, which responds to essentially electrical signals delivered through nerve ends. The effect of the signal is to open gates, or channels, in the muscle cell to the entry of calcium ions, which initiate responses that lead to contraction. The actual pathway is elaborate, resulting after a number of steps, in a movement of some 10-12 nanometres of each muscle cell, with the return of calcium to the medium outside the cell once the cell has relaxed. In the case of atrial cells, unlike in ventricle cells, it is also found that passages to the interior of the cell are not present, with the calcium ions arising at the periphery of the cell. The action of the atrial cell thus includes the hormone motivated passage of calcium, with reducing concentration, from the periphery to the centre, a movement known as *centripetal* progress or a centripetal wave.

This orchestration has a number of components, any of which could affect both the function of the cell as well as other components. It is necessary to observe the whole cell in action and manipulation of only a few of the components has not been fruitful in identifying and measuring their affects on the performance of the cell. Hence the interest in developing a model, to try out and test and which could stand in the place of the cell itself.

The model

The structure of the muscle cell is that it contains "tranverse striation" of calcium ion channels, or a stack of calcium receptors, running into the axis of the cell. The calcium ion "spark" that is detected at the periphery then needs to be passed down to the centre, to cause maximum muscle contraction. Based on the known structure of the atrial cell, the Nottingham team modelled the calcium signal path in the muscle cell as a stack of 51 disks, 12 microns in diameter and two microns apart, in the form of a cylinder 100 microns long (a tenth of a millimetre).

These disks correspond to the planes that have the calcium-sensitive receptors in the cell. The receptors on the periphery of the cylinders are the ones that are nearest the signal that arises from the nerve and the inner receptors need to receive the signal from the outer ones. The distance between the inner circles of receptors is one micron, but the distance from the outermost ring to the next, inner ring is two microns, to reflect the observed gap in communication in real cells. Positioning of real, observed calcium ion release sites then allows a study of the passage of the signal, to the centre of the disks and also from disk to disk.

With this geometric structure in mind, modelling provides mathematical entities to represent concentrations and electric potentials at different locations and their effects on tissue, to lead to contraction, and reverse flows of calcium ions. The model can then be worked, with each parameter under independent control, to see how the model muscle cell would work under different conditions. The results of the model have been verified in respect of known results, to give credence to simulations. "...For the first time we can manipulate cellular properties throughout a whole atrial muscle cell in order to deduce which conditions give rise to abnormalities," says Dr Thul.

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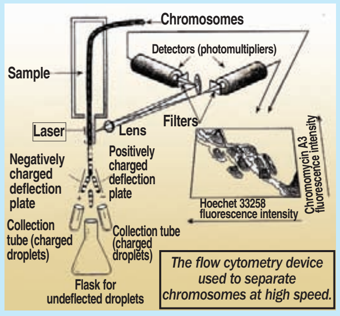
Mapping purposes

tapan kumar maitra discusses a high-speed sorting method based on fluorescent staining and flow cytometry

TO facilitate the creation of recombinant genomic libraries for mapping purposes and for other reasons, it is useful to be able to isolate individual human chromosomes. Towards these ends, several methods have been developed to isolate chromosomes. Here we discuss a high-speed sorting method based on fluorescent staining and flow cytometry.

DNA can be treated with several fluorescent dyes. Chromosomes can then be recognised individually by their relative fluorescent intensities. The dyes Hoechst 33258 and chromomycin A3 are a valuable combination because they respond to different wavelengths of light and they bind DNA differently. Hoechst binds preferentially to DNA rich in adenine and thymine, whereas chromomycin binds preferentially to DNA rich in guanine and cytosine. Thus, since every human chromosome has a unique ratio of bases, the relative intensity of each chromosome is different when fluoresced. Chromomycin fluoresces in the presence of a laser tuned to 458 nm, and Hoechst fluoresces in the presence of a UV laser. The chromosomes can be identified when their relative fluorescence in the two lasers is plotted, producing a flow of karyotype. Modern flow cytometry techniques then allow the isolation of these identified chromosomes.



The flow cytometry device used to separate chromosomes at high speed.

In practice, chromosomes are isolated in large numbers from cells that have been arrested in metaphase by treatment with colcemid, which inhibits spindle formation. These chromosomes are then purified in buffer and treated with the two dyes. The chromosomes are separated at high speed (200 chromosomes per second) in a flow cytometry device. As the chromosome-containing buffer passes through the laser beams, identification is made. The liquid is then forced to form minute droplets (215,000 per second) by passing through a vibrator. Specific droplets carrying the identified chromosomes are then charged, either positively or negatively, and passed between deflection plates.

Positively charged droplets pass one way, and negatively charged droplets pass the other way, thus allowing the simultaneous isolation of two different chromosomes. At a rate of 200 chromosomes per second, it is possible to isolate 0.1 g of DNA in less than an hour. 0.1 g of DNA is adequate for library construction and represents about 5 x 105 average chromosomes.

The technique is not perfect. During isolation, debris and clumps of chromosomes are produced that cause contamination problems. Then, some chromosomes are so similar in their fluorescence that they are hard to separate. This is true, for example, for chromosomes nine to 12. Also, chromosome 21 is hard to separate because its fluorescence tends to fall into the debris area.

Some of these problems, however, can be overcome by using hybrid cell lines of hamsters, for example, containing only one human chromosome. It is much easier to isolate the human chromosome from the hybrid line. Purity values of 90 per cent are not unreasonable, with some in excess of 95 per cent.

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'The applications can be used to meet various challenges'

sanjana majumdar catches up with W Selvamurthy, who heads the nanotechnology mission in India

DISTINGUISHED scientist W Selvamurthy, popular amongst the fraternity for his strong conviction, calm and amiable demeanour, goal-oriented approach and outstanding research contribution, has been serving the Defence Research and Development Organisation since 1973. The recipient of the prestigious Technology Leadership Award (2010), the Lifetime Achievement Award (2006-2007) in "Clinical and Preventive Cardiology", the Rastre Sresht Nidhi Award (2004), Best Academician Award (2004), National Citizens Award (2001), Scientist of the Year Award (1989), among other coveted honours, his research contribution has enabled our soldiers to acclimatise in extreme conditions, especially high altitudes.

Presently chief controller (R&D) for Life Sciences and International Cooperation, DRDO, he also heads the nanotechnology mission in India. Excerpts from an interview:

In 1951, celebrated physicist Richard P Feynman, in a lecture to the American Physical Society titled "There's Plenty Of Room At The Bottom", hinted about the concept of nanotechnology. Now that nanotechnology, in reality, has gained a strong foothold, tell us how it has affected the world?

Nanotechnology is not new. It existed long back; say, for instance, the brilliance of the wings of a butterfly, the shell of a snail, seashells… nature has had all these examples of nanotechnology for ages. It is just that now we are trying to apply the knowledge based on nanoscience. There has been a sudden exponential growth in the study of nanocomposites and nanoparticles. Nanotechnology has helped us interpret traditional systems. In the past 10-15 years, there has been tremendous development in the improvement of nano-based products and processes. On the agriculture side, nanoparticle-encapsulated biosensors can be put into the soil that will monitor the health of the soil.

Low doses of nanofertilisers will be sufficient to meet requirements, thus reducing the negative environmental impacts and promoting green technology. Nowadays, nanoscience and nanomaterials have revolutionised the healthcare sector.

What has the DRDO's role been in advancing and promoting research in nanotechnology?

Nanotechnology-based research activities at the DRDO have been oriented to meet national needs, to ease the life of soldiers deployed in extreme environments and to promote military sciences in order to aid in realising the challenges of the Armed Forces. Nanotechnology has several application areas and research activities in this field have been distributed amongst several DRDO laboratories situated in different parts of the country. For example, research on utilising nanocomposites for structural programmes is being carried out at the Research and Development Establishment, Pune, Defence Materials and Stores Research and Development, the Defence Mettalurgical Research Laboratory, etc. Similarly, studies on nanoelectronics are conducted at the Solid State Physics Laboratory, the Defence Research and Development Establishment and the Defence Laboratory Jodhpur, among other places. Again, a host of laboratories is conducting researching on Nuclear, Biological and Chemical technologies. A separate group is looking after nanomaterials for stealth. Thus, research by the DRDO is customised to meet national needs.

An explosive "Detection Kit" has been developed by the DRDO for the speedy identification of explosives. Nanosensors have also been developed for detecting chemical and biological agents. During the Commonwealth Games, the DRDO was asked to sanitise the stadiums to ensure security. It has also developed novel drug formulations for decorporating inhaled radioactivity, which can be treated now as an essential part of the rescue team kit for nuclear accidents. Silver nanoparticle-impregnated clothing fibres have been developed for protection against NBC agents.

In the healthcare sector, we have developed vitamin-fortified milk in nano-emulsion form which, on consumption, has shown an increased bioavailability or intake of vitamins. Our soldiers have to survive in extreme conditions, like high altitudes or even deserts. The food they carry needs to be preserved properly. We have thus developed nanocomposite food packaging film with increased thermal tolerance that can preserve food from minus to plus-50 degrees Centigrade. At high altitudes, soldiers suffer from various cold-induced injuries and respiratory problems. To address this issue, we have developed nano-salbutamol ointments to tackle the ischemic effects of cold-induced injuries and a nanosal capsule that can be used with inhalers. These capsules optimise the dosage with 80 per cent efficiency in contrast to 20 per cent efficiency in the case of ordinary capsules.

At the DRDO laboratories, we have applied nanotechnology to increase the efficiency of various systems. These include enhancing the mechanical strength of bridges and assault vehicles, developing high performance anti-corrosive coatings, anti-fouling paints, self-healing materials and anti-baffling materials for enhancing the effectiveness of sonar, thus aiding naval surveillance. Nanocomposites used in the cone of the Agni missile has shown a 40 per cent increased tensile strength enabling it to attain a 5,000-km reach capability. Another application has been in developing piezo-electric transducers that, when embedded in shoes, generate current that can be used by a soldier even in remote locations to recharge, say, a mobile phone battery.

How will nanotechnology influence the employment scenario to alleviate poverty in India?

India must invest adequately because the applications of nanotechnology can be used to meet various challenges. In the next 20 years the value of the emerging world market will rise to \$35,000 billion from \$300 billion due to the nanotechnology-related industry. Thus there is a growing market for nano-related products. If we can set up new industries related to nano-products, then our export potential will increase, which will in turn facilitate foreign exchange flow.

Renowned scientist and chairman of the Prime Minister's Scientific Advisory Council, Professor CNR Rao said, "India should make nanotechnology a cottage industry to get the full advantage from this technological revolution." What are your views and suggestions about the initiatives that need to be undertaken?

R&D related to nanoscience and nanotechnology is already moving at a fast pace. Today, about 2,676 nanoparticles from 164 suppliers are available and the product range is increasing by the day. Nano-products have grown fivefold from 212 to 1,015 since 2006. Now we need to further sensitise the Indian market about nano-products.

Nanofoundries should come up from where customised products will be available. Nanotechnology centres should be created and nanotechnology research in institutes and industry should be oriented to meet national needs. Our focus should be on fostering collaborations between institutes and industry in India. "Collaboration" is the mantra we should employ in 2012. We should not work in cocoons because now we need a continent of excellence, which is possible through collaboration as it acts as a force multiplication system. Again, market-active databases should be collected and a website on nanotechnology should be formed to network various stakeholders.

What would be your message to young Indian scientists and students?

Life is full of excitement and mysteries to be understood through science and technology, which is the key to development. Do research. Goal-oriented research. Always keep a mission and, yes, put a milestone or a deadline for achieving your goals.