Counting nanoparticles in real time

They're difficult to detect and even harder to count but a team at the University of California in Santa Barbara has cracked the code, says s ananthanarayan

EVEN before the advent of nanoscience or synthetic nanoparticles, nature made effective and widespread use of the very small as mediators in complex physiological processes. On the other side of the fence are the viruses, with diameters less than 150 nanometers, which take millions of lives every year. But because such particles are too small to detect, any defence against viruses has to be by blanketing measures in affected areas, measures that affect the virus and healthy cells alike. In *Nature*, a multidisciplinary group at the University of California, Santa Barbara, reports on improved equipment that can detect and count nanoparticles as small as 10 nm.

"This device opens up a wide range of potential applications in nanoparticle analysis," says Jean-Luc Fraikin, lead author on the study. "Applications in water analysis, pharmaceutical development and other biomedical areas are likely to be developed…"

The first sally into detecting and measuring very small particles was perhaps in 1676 when Antoni van Leeuwenhoek discovered red blood cells under the microscope. The process has become routine in the pathology lab, where constituents of the blood or body fluids are studied for diagnosis and therapy. Estimates of population of target organisms are typically made by physically counting the numbers of instances in a marked area in the field of view. But this method, apart from being approximate and subjective, is also only good for particles that are visible in the microscope. For the smaller scale, nanometer level particles, the method of choice was *dynamic light scattering*, where the intensity and frequency of light scattered by a sample provides information of the quantity and size distribution of suspended particles.

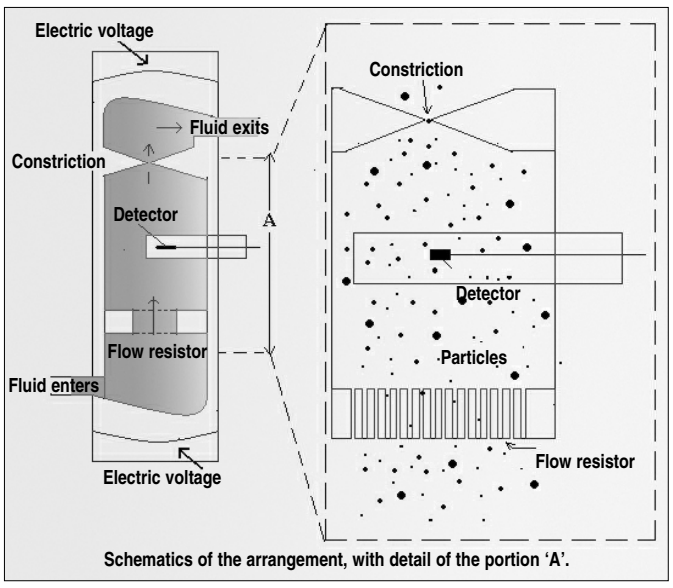
Another method is *disk centrifuging*, where the sample is spun inside a stack of conical disks and components are separated in the space between disks. But these methods are slow, cumbersome and call for sizeable samples of the fluids under study. Individual nanoparticles can be studied under the electron microscope, but this is hardly practical at the routine level and these methods are not successful when the particles to detect are present in small numbers.

An indirect method that has been used for over half a century is the *Coulter counter* method. In this, there is a microscopic aperture in an insulating membrane. An electrical gradient is applied between the two sides of the membrane, which is immersed in a conducting liquid. As the membrane is an insulator, any current can flow only through the aperture, and this current is measured. Now, if there are cells or other particles in the liquid and one of them passes through the aperture, it will effectively block the current path while it is passing through, which would register as a drop in the current. Such changes in current strength are detected and counted to provide data of particle movement.

The Coulter counter has become an important tool in hospital laboratories, primarily for quick and accurate analysis of Complete Blood Counts, which shows the number or proportion of white and red blood cells in the body. The traditional method was to prepare a blood cell stain and manually count each type of cell under a microscope, a process that could take half an hour. Coulter counters are now used in different fields, like paint, ceramics, glass and food manufacture and for quality control.

The same principle is used in the *nanopore*, which can detect much smaller particles like DNA or protein molecules. The insulating membrane could be a double layer of oil-like molecules, with the pore being a local modification in the molecular structure, to work as a protein channel. It could also be a protein channel planted in a synthetic membrane or a physical hole in a solid laminar membrane.

But while these methods have been useful in the fields where they were developed, they are cumbersome and cannot provide rapid count rates, which is required in many nanoparticle characterisation applications.



Schematics of the arrangement, with detail of the portion 'A'.


Jean-Luc Fraikin

Santa Barbara analyser

The arrangement developed by the Santa Barbara group allows a rapid and automated count of up to half a million particles every second, simultaneously measuring the volume of each particle to keep count separately of different types of particles. The arrangement is a version of the Coulter counter and combines a simply fabricated passage for the fluid, under an electric field and provided with a nanoconstriction, and a detector of the electric resistance of the arrangement.

Every time a particle passes through the constriction, the electric path is blocked and the sensor picks up the drop in current. The signal is electronically processed, for count as well as the size of the particle, from the extent of the drop in current. The arrangement can be configured to provide very rapid response, with precise size measurement, for analysis of complex mixtures. The arrangement has been able to detect virus particles suspended both in saline solution as well as in blood plasma.

Measurements on blood plasma have revealed a vast number of smaller sized nanoparticles, probably cell-derived bubbles of material, called vesicles, which are thought to be involved in different physiological and pathological processes. Earlier studies of these particles required extensive sample preparation and purification, for electron microscope study. Use of the Santa Barbara analyser permits speedy and detailed study with minimal preparation, which is a study of the plasma particles in their native state.

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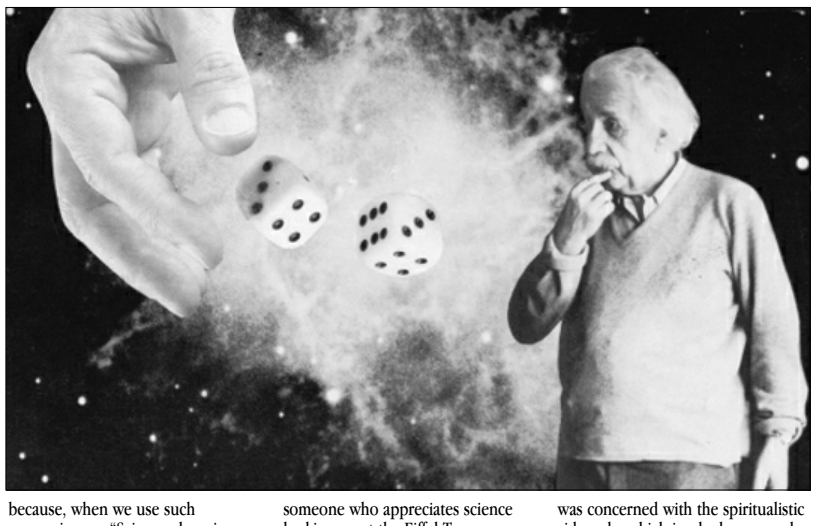
Albert & the relativity of God

Once Einstein took the trouble to judge the excellence of his subject in terms of its contribution to life, he was bound to realise that life was so diverse that no one single philosophy alone was sufficient for its healthy growth, writes durjoy ghosh

HAVING studied Albert Einstein's writings wherein he attempted to reconcile science with religion, one is finally able to catch the invaluable question: What shall we do with "only science"? There is need for caution, though, because the question is not if scientific knowledge is useful, but whether such knowledge alone is sufficient for us to realise our ultimate goal — humanity. Interestingly, Einstein answers in the negative because, as he argues, "science can only ascertain what *is*, but not what *should be*, and outside of its domain value judgments of all kinds remain necessary".

The point is clear. Science can explain, and even also help us discover, the immense energy that rests in an atom. But how we use that knowledge and energy, whether to destroy nations or turn deserts into grasslands, cannot be determined by science, as scientific knowledge is objective, not based on value judgments. The difficulty further complicates itself, as Einstein points out, because while "the knowledge of truth (scientific truth) as such is wonderful, it is so little capable of acting as a guide that it cannot prove even the justification and the value of the aspirations toward that very knowledge of truth". This exposes, in the words of Einstein himself, "the limits of a purely rational conception of life".

To be precise, scientific knowledge may be knowledge of facts, and since facts alone are, in a way, dependable, it may also be treated as the knowledge of truth. But why Galileo made it his life's ambition to pursue such knowledge cannot be clarified in a strictly scientific way because, when we use such expressions as "Science alone is true" or "Science is the best way to analyse the world", we actually make value judgments. Therefore, even science, in order to be valued, needs something other than science, already in existence, to cultivate values. This "something other than science", Einstein tells us, is, or should be, religion.



To discuss what Einstein understood by religion, there is an important question that begs an answer: Does scientific knowledge, which is objective, at all need, in order to be valued – these values subjectively and sometimes even superstitiously — be cultivated? No, because we could say that science can as well be appreciated by its own contribution to humankind. But the difficulty is that the urge to conceptualise, which initiates all sciences, is an epistemological concern that is not restricted to the question whether such concern is materially profitable to us or not.

Had this been the case, astrophysics would have been completely irrelevant, because we certainly do not benefit from knowledge concerning the expansion of the universe. So someone who appreciates science looking up at the Eiffel Tower (contribution of science) may soon lose all enthusiasm once the same tower, God forbids, collapses and kills thousands of people.

What, then, is that which causes the human urge to conceptualise? The answer, according to Einstein, is "amazement at the harmony of natural law, which reveals an intelligence of such superiority that, compared with it, all the systematic thinking and acting of human beings is an utterly insignificant reflection", And, once he traced the inception of scientific inquiry to "amazement at the harmony of natural law", it becomes *roses, roses, all the way*. Einstein was able to connect science to religion. "It is the experience of mystery – even if mixed with fear – that engendered religion. A knowledge of something that we cannot penetrate, our perception of the profoundest reason and the most radiant beauty which only in the most primitive forms are accessible to our minds – it is this knowledge and this emotion that constitute true religiosity."

Here we should remember that religion has two distinct sides, ritualistic and spiritualistic. Einstein was concerned with the spiritualistic side only, which is why he escaped the doubt over whether dogmatism, which is a continuous outgrowth of the ritualistic side of religion, could benefit scientific investigation at all. And the values that he believed could justify "aspirations" towards scientific knowledge are basically human values like "kindness, beauty and truth", without which the cultivation of science, either for civilisation's progress or for its own shake, may indeed become inconsequential.

In an article titled *Religion and Science: Irreconcilable?* —, published in *The Christian Register* in 1948 — Einstein regrets, "The dependence of science on the religious attitude… in our predominantly materialistic age, is only too easily overlooked."

One may, of course, ask, "Are kindness, beauty and truth religious values?" Well, in a way, they are. Every religion, to some extent, does breed these values. Besides, these are values Einstein had always admired in Judaism, his own faith. "… the Jewish tradition also contains something else, something which finds splendid expression in many of the Psalms, namely, a sort of intoxicated joy and amazement at the beauty and grandeur of this world, of which man can form just a faint notion. This joy is the feeling from which true scientific research draws its spiritual sustenance, but which also seems to find expression in the song of birds."

Now, whether Einstein is right or wrong may be a topic for everlasting philosophical debate. There are issues that cannot be addressed properly in the short space we have. What is important is Einstein's stern rejection of "a purely rational conception of life" which, he was apprehensive about, science might encourage and mislead humankind. This is the anxiety that turned him from a mere great physicist to a humanist of the highest order, who, in spite of being sincerely occupied in his specific field of investigation, continued to question the usefulness of his philosophy in human progress.

And it is here and only here that an Einstein, a Bertrand Russell or a Marvin parts ways with any scholar who remains, throughout his life, a university figure satisfied with the appreciation of his subject in scholastic seclusion. For once he took the trouble to judge the excellence of his subject in terms of its contribution to life, he was bound to realise that life was so diverse that no one single philosophy alone was sufficient for its healthy growth, and science, or philosophy, could genuinely contribute to life only when it left its dogmatic professional flow and merged with a number of other equally essential (so-called unscientific) ways of looking at life.

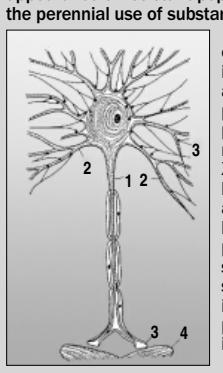
The writer is a freelance contributor

Modern pesticides

The widespread use of organophosphorus compounds is due to their high insecticidal and acaricidal activity, writes tapan kumar maitra

ORGANIC phosphorus compounds are one of the most important groups of modern pesticides. The widespread use of these compounds is due to their high insecticidal and acaricidal activity, the broad spectrum and rapidity (high initial toxicity) of action on pests, their low stability in biological media, to their decomposition with the formation of products non-toxic to humans and animals, their relatively rapid metabolism in the organisms of animals and the absence of an ability to be deposited in them, to the systemic action of a number of toxicants and in this connection to the smaller danger to entomophags, their low rate of use per unit of treated area, their rapid decomposition in the soil and water as well as their moderate toxicity to fish.

Among the negative features of most organophosphorus compounds is their high toxicity to humans and animals and the relatively rapid appearance of resistant populations of pests after the perennial use of substances of this group.

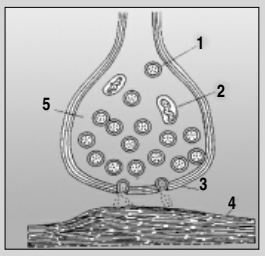


Structure of a neuron – axon (1), dendrites (2), synaptic platelets (3) and muscle fibres (4).

Modern organophosphorus insecticides and acaricides are compounds of pentavalent phosphorus. Its structure reveals that the bond of X with the phosphorus has an anhydride nature and the substance itself has the properties of a phosphorylating agent. A substance having such a structure, when getting into an organism, phosphorylates vitally important substrates.

Indeed, it has been established that the enzyme contained in nerve tissues — acetylcholinesterase, which plays an exceedingly important role in the transmission of a nerve impulse — is such a substrate.

The fundamental structural element of an animal's nervous system is a nerve cell (neuron) whose designation is to receive, interpret and transmit information in the form of nerve signals (impulses). The short and numerous branches (dendrites) of a neuron connected to the axons of other cells gather information, while the single long branch (axon), terminating in a bulb-shaped thickening (a synaptic platelet), transmits the information. Hence, a nerve impulse in the form of an original electric signal travels along a neuron, always from a dendrite to the axon and further from the latter to a dendrite of another cell or to a muscular fibre.



Nerve-muscle synapse: synaptic vesicles (1), mitoch-ondria (2), presynaptic membrane (3), postsynaptic membrane (4) and synaptic platelet (5).

The ending of a nerve and the membrane of another cell or muscle are separated by a synaptic cleft from 30 to 50 nm wide. The cleft is filled with a gel-like substance and has a tremendous electrical capacitance; therefore, an electrical signal cannot pass through it. A nerve impulse is transmitted through the synaptic cleft with the aid of chemical substances (mediators or transmitters) secreted through the presynaptic membrane. Acetylcholine and noradrenaline are the most widespread transmitters. Synapses where a nerve impulse is transmitted with the aid of acetylcholine are called cholinergic and those where noradrenaline is the mediator are called adrenergic.

The free acetylcholine in the inactive form bound to proteins accumulates in the ending of a nerve in vesicles. The consumed acetylcholine is constantly replenished by its synthesis: by the acetylation of choline. Hence, the process of synaptic transmission is an involved biochemical cycle of acetylcholine exchange. Acetylcholinesterase has an exceedingly important significance in this cycle because the inhibition of activity leads to the accumulation of free acetylcholine in the synaptic cleft. As a result, the normal passing of nerve impulses is disrupted, convulsive activity of the muscles sets in that transforms into paralysis and other features of self-poisoning of the organism by surplus acetylcholine appear.

An active centre of acetylcholinesterase consists of two sections — an anionic one (A) containing an ionised carboxyl of asparaginic and glutamic acids and an esterase one (E) containing a hydroxyl of serine. The acetylcholine decomposes in three steps: a) sorption of the acetylcholine on the anionic section that fixes a molecule of the transmitter on the surface of the enzyme and sharply increases the probability of a reaction; b) acetylation of the enzyme with the formation of choline; and c) desorption of the choline and hydrolysis of the acetylated enzyme; here the enzyme is regenerated and acetic acid is evolved.

The entire complicated process occurs exceedingly rapidly (in several milliseconds). An unfailing condition for the reaction to proceed is the strict polar and steric correspondence of the transmitter and the enzyme. All organophosphorus insecticides imitate the ester part of acetylcholine and when they enter an organism usually react with the esterase section of the acetylcholinesterase.

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