Measuring cells with light

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Figure 1 Red blood cells.

Physics focus

Ideas about **momentum** explain how refraction of **photons** gives rise to a **force**, and how a bead in an optical trap experiences a force like that of a **spring**. The forces can be applied to cells, yielding information about their **mechanical properties**.

This article is about current research that might change the way you think about physics. There are two surprises, and I give them away right now:

1 Visible light, from an 'everyday' source such as a bright laser pointer, is enough to deform some materials

2 There are more and more people with training in physics who are now working on topics that look like biology, possibly changing the latter — and not for the first time.

First, I will describe an experiment studying red blood cells (Figure 1) that is possible with new technology. Then I will argue about why this is interesting for people who like physics and why biology needs the help of physicists.

Radiation pressure

The most difficult bit of physics that I need to tell you about is radiation pressure and, in particular, the fact that light carries momentum. Here it is convenient to think of light as being made of particles, called photons, which have a momentum. They are like snooker balls in that a force needs to act on them to change their direction. A photon does not have a mass but it has momentum and travels at the speed of light; it could not have momentum otherwise. It would travel more slowly if it had mass, so these properties are connected. The details are beyond the scope of this article but Box 1 gives some detail (see also 'Faster than light?', Physics Review Vol. 19, No. 3, February 2010, pages 10–13).

Photons will travel straight unless a force acts on them. When does the path of light bend? Familiar examples are reflection (mirrors) and refraction (in glass or water). It follows from this that a mirror hanging on the wall is pushed towards the wall by every photon that it reflects. How big is this pressure? It depends on the light intensity and wavelength (Box 1). In everyday situations this force is tiny, but on a small enough object a tiny force can go a long way.

Optical trap

A colloid consists of small particles, typically ranging from 1 nm to a few micrometres, dispersed in a fluid. It is possible to make (or buy) perfectly spherical particles with any size in this range. In the work discussed here, 3-μm-diameter spheres are used. These colloidal beads can be suspended in water and settle out

Box 1 The force of light

The momentum p of a photon is related to its energy E by

$$p = \frac{E}{c}$$

where c is the speed of light. Pressure is force/area and force is rate of change of momentum. The rate of change of momentum per unit area is the radiation pressure.

If photons are absorbed by a surface, the pressure is the power per unit area divided by the speed of light. If the radiation is totally reflected, the radiation pressure is doubled because the change of momentum is doubled. The radiation of the Sun at the Earth has a power per unit area of $1422\,\mathrm{W\,m^{-2}}$, so the radiation pressure is $4.8\,\mu\mathrm{Pa}$ if it is absorbed.

For electromagnetic radiation, the momentum is related to the wavelength by the de Broglie equation:

$$p = \frac{h}{\lambda}$$

where h is the Planck constant and λ is the wavelength. Momentum is a vector, which means it has magnitude and direction. Changing direction is a change of momentum and so even a change of direction for a photon requires a force.

only very slowly. They are strongly buffeted by the thermal motion of the fluid, causing Brownian motion.

In an optical trap, only light 'touches' the bead. A laser beam is focused so that its intensity is maximum on the axis of propagation. The refractive index of the beads is higher than that of water, so beads bend light by refraction (Figure 2a). Many photons are refracted and so change their momentum. The particles therefore experience a force, and the addition of all the forces that result from deflecting light is the average net force. It is not so easy to show — but it is true — that all the deflections of photons passing through the spherical bead in all possible places add up so that the optical force tends to keep the bead pinned to where the light is strongest. This is an optical trap.

If the bead is displaced from the focus of the laser light by Δx, it feels a force pulling it back. This force grows linearly with the displacement in exactly the same way as if it were exerted by a perfect spring attached to the bead (Figure 2b). If we have calibrated this trap stiffness k, and we also know the position of the laser beam and the position of the bead, then we know the force acting on the bead which is:

$$F = k\Delta x$$

The typical stiffness or spring constant k of optical traps is between 1 and 100 pN/μm (compare this to the springs you have in your lab or in a set of kitchen scales). The typical range of Δx is between 1 nm and 1 μm, so the forces that can be measured are small, up to a maximum 100 pN.

This all works, because the laser is carefully steered through the optical setup, and the bead position is measured (automatically by a computer) by looking at the image taken through a video camera (Figures 3 and 4).

What has been described up to here is a laser trap, capable of holding colloidal beads and of measuring and applying forces. Almost every university physics department in the UK has at least one optical trap, because they are useful. A lot of science is done to study single proteins and other individual molecules and 100 pN is in fact a huge force when applied to single molecules. In this case, scientists work on the lower end of the force range, around 1 fN which is 1×10^{-15} N (see Box 2).

Box 2 Small forces

On Earth's surface, 1 N is roughly the gravity force acting on a 0.1 kg mass. That's something tangible: the weight of a slice of bread. Think of cutting that slice into two, then again, and do this 20 times. Those tiny specks now each have a mass 1×10^{-7} kg and are barely visible. Each has a weight of 1×10^{-6} N, and a very good balance is required to measure that. It is a small force, but in this article the forces are 1×10^{-12} N, and that's another 'world' below. Thermal forces acting on micron-sized objects suspended in liquids are of this magnitude. The forces arise from the fast random motion of the liquid molecules and they cause a slower random motion of the larger object. This can often be observed in a microscope and is called Brownian motion.

Remember that 10^{-3} is milli, 10^{-6} is micro, 10^{-9} is nano, 10^{-12} is pico, 10^{-15} is femto.

The initial letter of the prefix is used in labelling units, e.g. 1 fN is 1×10^{-15} N.

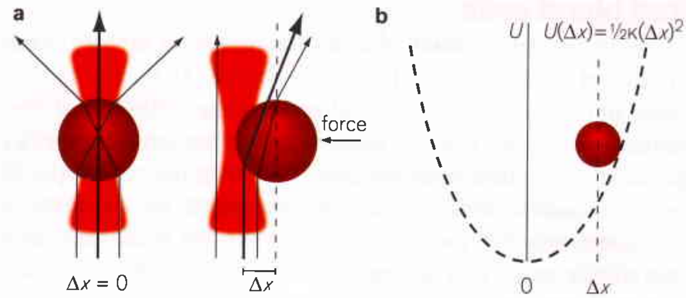

Figure 2a The arrows show how photons would be refracted on entering the bead. When the bead is on-axis the forces are in equilibrium horizontally but when it is off-axis there is a net force pushing the bead back towards the axis.
b The optical trap acts like an ideal spring.

Figure 3 The layout of the optical trap before it was boxed in for laser safety.

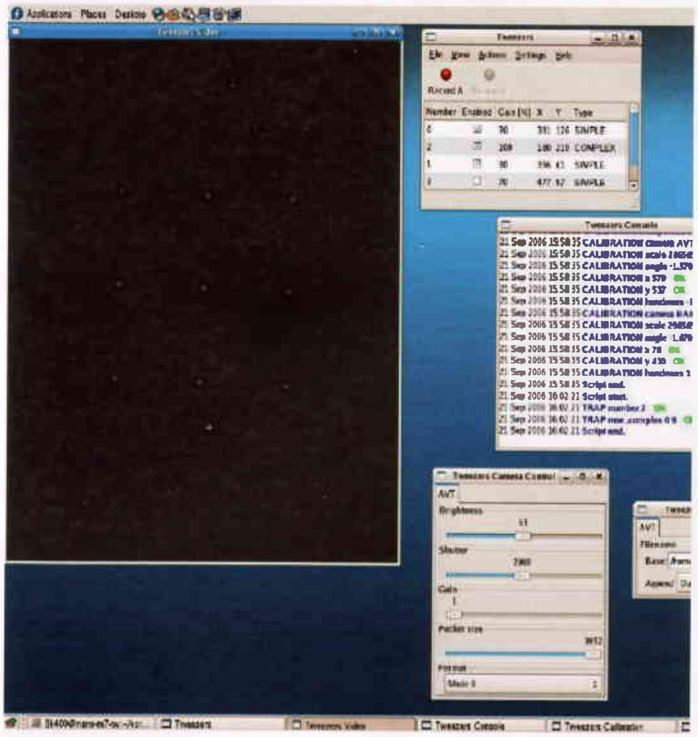

Figure 4 Screenshot of the control panel on the computer. The window on the left of the screen is showing live video from the sample; in this case nine beads are trapped in a 3 × 3 array and another two beads are trapped higher up.

Red blood cells

Many of the applications of optical traps are in biology. Cells are one of the fundamental structures in life. All life on Earth is made of cells — or, rather, we often limit the definition of 'life' to those organisms that are made of cells. This settles the tricky problem of whether a virus is alive. There are over 200 types of cells in humans, which is not a lot compared, for example, to the vast number of proteins. Of these, the red blood cell has a special role and a special structure. Its main purpose is to carry oxygen from the lungs to tissues and carbon dioxide back. Red blood cells are formed in the bone marrow (around 2×10^{11} new cells are made every day), and each one circulates, on average, for 120 days before it gets damaged and is destroyed. During this time cells circulate through vessels of varying size, the smallest of which are capillaries as thin as 5 µm in diameter (Figure 5). They can be smaller than the cell's diameter, which is around 8 µm. Cells are therefore constantly being deformed.

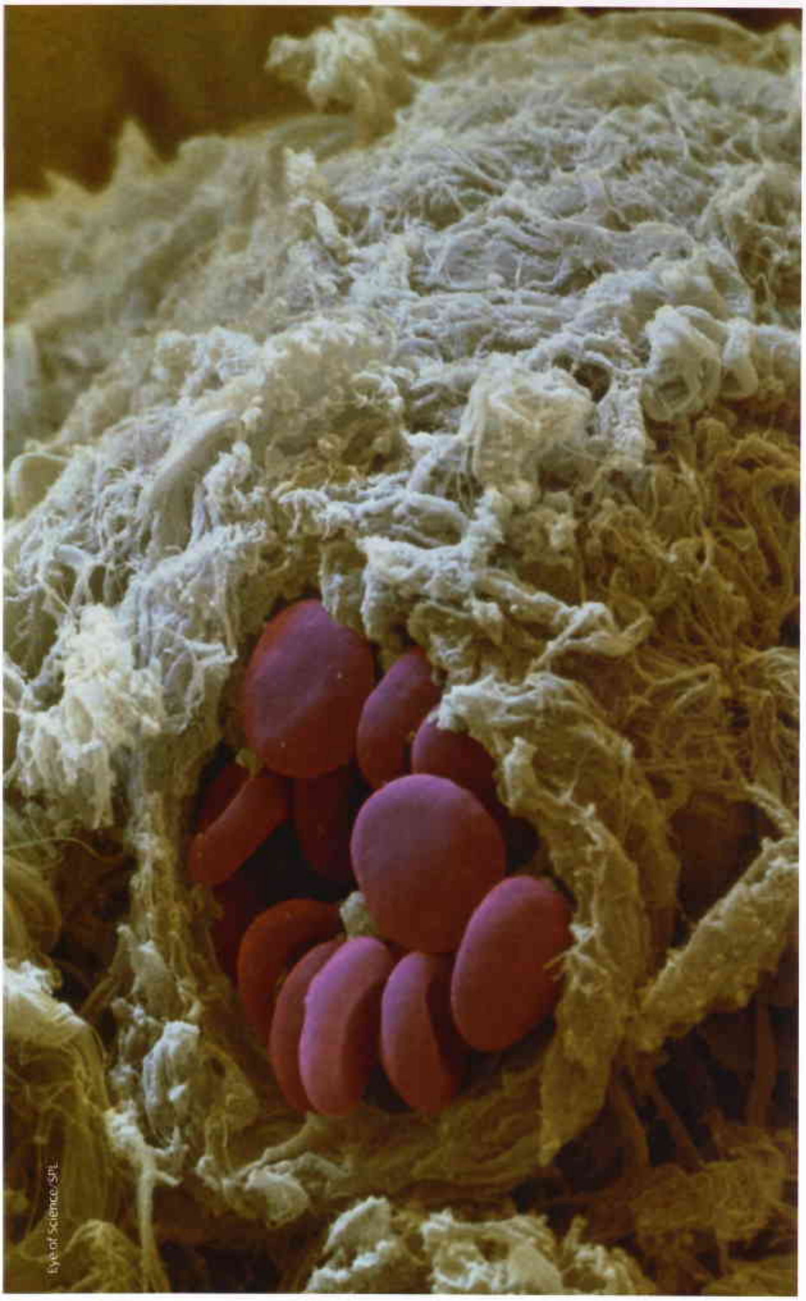

Figure 5 Red blood cells in a capillary.

Healthy red blood cells have a very characteristic bi-concave ('discocyte') shape, which helps them to be deformable. These cells make up around 35% of the volume in blood and their presence is the reason for the high viscosity of blood, much higher than water, and more generally its complex flow properties. The red cells are subjected to deformation due to the gradients in blood velocity and each cell is capable of both storing and dissipating elastic energy. The cell's ability to store elastic energy comes primarily from a network of protein (spectrin) filaments that is anchored on the inside of the surface membrane. You could think of the cell as having a very thin external shell made of rubber which gives it resistance to a shape change. On the outside is a double layer of lipids which acts as a barrier, preventing (or regulating) the exchange of chemicals between inside and outside. Together, the spectrin and lipids form the 'external membrane'. Inside, confined in a sac, is a solution of haemoglobin, which has the properties of a viscous liquid and is not elastic. Of all 200 human cell types, this is the simplest; there is no nucleus and none of the interior structures that are found in skin, bone, immune system cells, etc.

Studying red blood cells

Despite the red blood cell having one of the simplest cell architectures, its mechanical properties have puzzled scientists for decades and continue to do so. These properties are important to understanding the flow of blood in healthy people, but there are also many diseases, for example diabetes and malaria, in which the red blood cells lose the ability to deform easily and recover their shape, causing potentially severe circulatory problems. Of all the puzzles that exist, this is one worth working on.

The typical experiment in our lab consists of stretching one red blood cell at a time, using a pair of colloidal beads as 'handles', and using the optical trap to hold the colloidal beads, as shown in Figure 6. What we found, when we stretched the cell at various speeds and by various amounts, was a rich set of results that we are still in the process of interpreting. One result, however, is very clear; Figure 7 shows the force that the cell exerts on the trapped

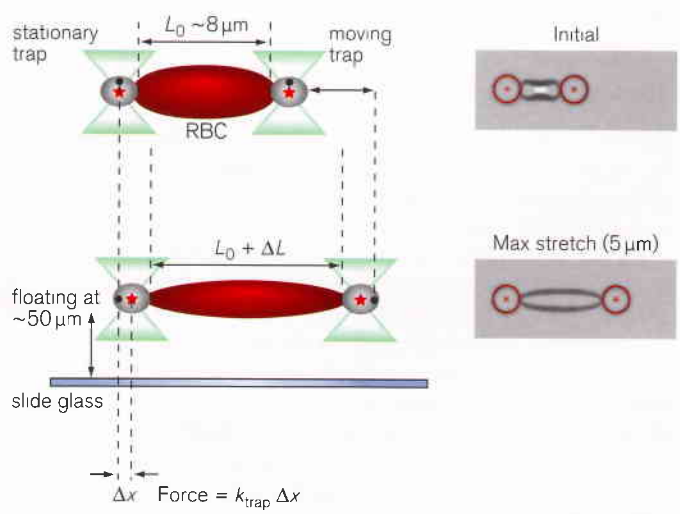

Figure 6 Using a pair of beads to lift a red cell (RBC) off the bottom of the sample and then to stretch it. The images show snapshots as seen by the video camera through a microscope. The bead position is calculated automatically by the computer and is shown with red circles and asterisks.

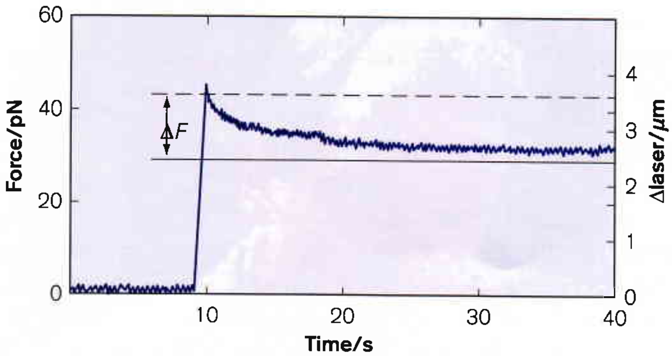

Figure 7 At time just before 10 s, one of the laser traps holding the red blood cell is displaced by 4 μm (dashed line, scale on the right). The trapped bead follows the laser trap, thereby stretching the cell. The stretched cell exerts a force on the trapped bead, shown by the blue line.

beads, as a function of time. We stretch the cell at time $t = 10$ s, when the force first increases sharply, and then over around 30 seconds relaxes to a lower value. A perfect spring or a good rubber band would not give the same result: the force would be steady. Our work has been to understand why this decay takes place and how it is related to the structure of the red blood cell. The relaxation in force is an indication that the molecules in the cell itself are rearranging themselves in response to the change in shape. It may seem surprising in this age, where complex things like space travel and mobile phones work so well, that the apparently 'simple' situation of a material that rearranges when subject to a deformation is not really understood.

Conclusion

I have written about the red blood cell experiment because this is a clear example of physics helping biology by providing a new tool, the optical trap, and new theory, how a soft object can respond to deformation. In doing so, new physics is also discovered. It is often said that 'physics is what physicists do'. This is not so obvious in the school curriculum but it is true in the science that physicists aim to discover in universities and research laboratories worldwide. In the last decade, biology has highlighted new challenges and opportunities that require the skills of people trained in physics. The relation between cell mechanics and cell structure and function, the topic of this article, is one of these developing areas, but there are other challenges too. On a previous occasion similar challenges from the life sciences were taken up by physicists and resulted in a cultural revolution and the discovery of DNA structure and function in 1953. This has developed into the vast and crucially important task of understanding how the genome works. New branches of physics and biology were born then, with the dawn of molecular biology. Now a new revolution is in the air. Physics is providing an understanding of the detailed properties and behaviour of cells. ■

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