In Search of Lost Contrast

Optical coherence tomography (OCT) – a kind of optical counterpart of ultrasound imaging – is continually being improved as image contrast boosting techniques are developed.

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Most people are familiar with ultrasound as a medical imaging method. Although it is most commonly associated with pregnancy monitoring, its applications are far broader. Generally, the technique involves emitting ultrasound – very high-frequency sound waves – at the object under investigation; these waves are reflected and registered by a detector.

There is also an optical alternative to ultrasound, involving illuminating the sample with light (usually in the near-infrared range) and registering the echo of the light wave. Since light propogates far more quickly that ultrasound waves (up to 200,000 times faster!), it is not possible to measure the echo directly. This is solved by using interferometry. Light reflected from the surface of the object is "mixed" with light reflected from a reference mirror; the resulting interference signal is used to recreate the structure of the object under investigation. This method, known as optical coherence tomography (OCT), most commonly uses a Michelson interferometer. The inventor of the technique, Albert Abraham Michelson, was born in 1852 in the town of Strelno (now Strzelno in present-day Poland). When he was four years old, his family emigrated to the United States; some fifty years later, in 1907, he became the first American to win the Nobel Prize in physics.

OCT is used for imagining morphology of tissues with a resolution of 10 micrometers (for comparison, a human hair is around 60–80 micrometers thick). For the majority of biological tissues, measurements are limited to depths of 1–2 mm under the tissue surface, since light cannot penetrate much deeper. The eye is an exception in this respect: since it is partially translucent, it was one of the first tissues to be regularly examined by OCT. OCT systems are now routinely used by ophthalmologists.

Imaging based just on registering light scattered by layers or structures of the given tissue, however, does not always make it possible to distinguish between healthy and diseased cells. This is due to insufficient contrast. Luckily, the technique can be improved by "retrieving" the lost contrast. So far, researchers have devised a number of ways this can be done.

Seeking movement

One way of improving contrast involves imaging the differences between static regions of tissues and those where movement occurs naturally. An example of such dynamics can be found in blood flow. As blood flows through vessels, it alters the signal registered by

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Three photos of a translucent comb placed on a glass plate and illuminated with light from an LCD source. Coating the comb with glycerin, which has a similar refractive index to the plastic the comb is made of, results in a significant reduction of contrast (center). Contrast can be returned by imaging in cross-polarized light (right).

OCT. If the measurement is taken at least twice and only those places where the OCT signal changes are marked, it is possible to create a map of the circulatory system.

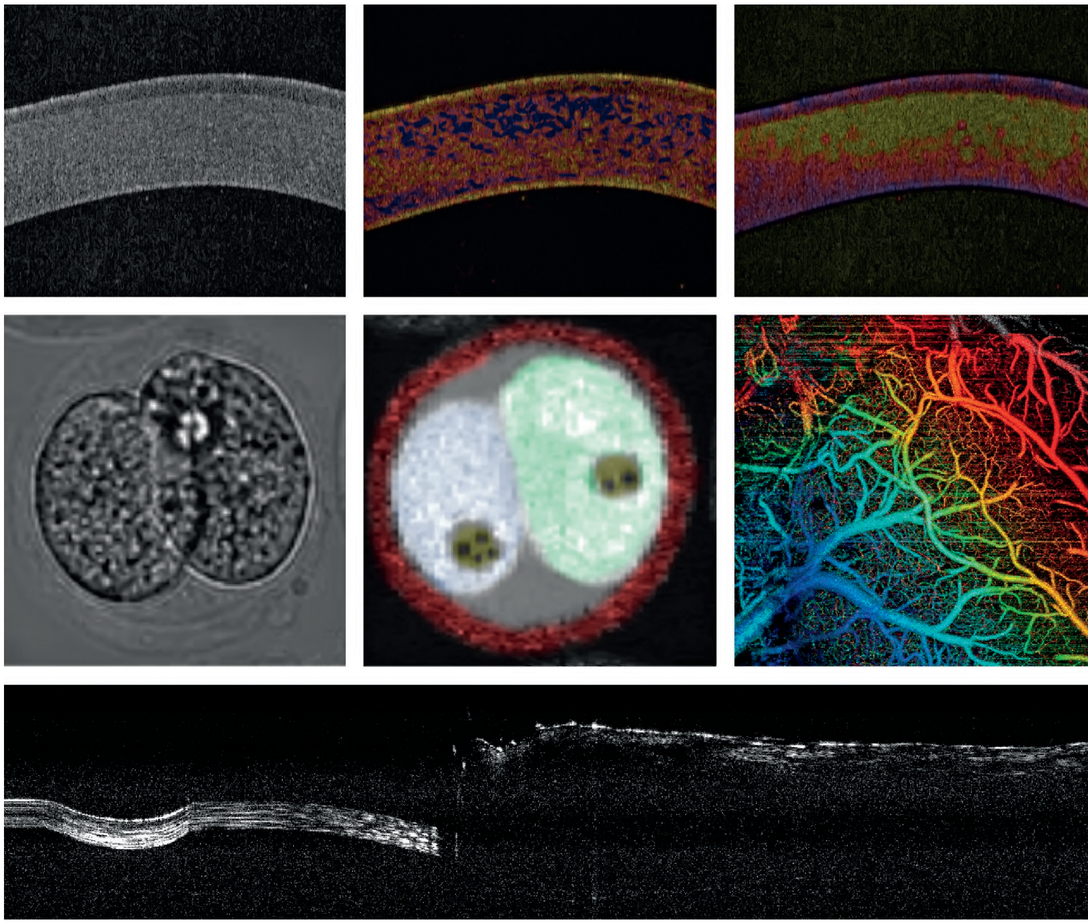
Since OCT is based on light interference (superposition of waves), the resulting images show distinctive artefacts known as speckle noise. Speckle noise can be easily observed, for instance, by illuminating a surface with a laser pointer. Light dispersed on an uneven surface undergoes interference, forming bright areas (constructive interference) and dark areas (destructive interference), resulting in a distinctive speckled pattern. Speckle noise is an undesirable element of OCT-generated images. It can be eliminated by averaging, although this can result in a reduced resolution. Fortunately, nature can also work in our favor.

In our work on a multidisciplinary project "Development of interferometric optical methods for studying the dynamics of biological systems," bringing together physicists from the Institute of Physics at the Nicolaus Copernicus University in Toruń and the PAS Institute of Physical Chemistry, biologists from the Faculty of Biology at the University of Warsaw and programmers from the Poznań University of Technology, we used OCT to study fertilized mouse eggs. Based on the natural intracellular movement of cytoplasm and organelles, as well as using measurements taken at different time intervals, we managed to average the speckle noise corresponding to dynamic changes within cells. We obtained high-quality 3D images with sufficient contrast to visualize pronuclei and the nucleoli within pronuclei, which was not possible before without invasive dyes of the sample and taking fluorescence microscopy measurements.

This research, conducted in collaboration with the PAS Institute of Experimental Biology, may find applications in assessing the developmental potential of embryos in IVF. Cytoplasm movement, which is shown in OCT-generated images as dynamic changes of patterns formed by speckles, can be used to assess the quality of processes occurring immediately after fertilization. This type of analysis will make it possible to select the most promising fertilized cells to be used in the IVF process.

Colored order

Another fascinating and promising source of contrast involves exploiting an object's polarization properties. This can be exemplified using the polarization properties of transparent plastic. If the material undergoes mechanical stress during the manufacturing process (for example, plastic being shaped into a fork), the observer can use polarization to view a color image. As a result of local stresses, the hitherto homogeneous material becomes heterogeneous. The observed colors are the result of birefringence – the optical property of a material that causes double refraction of light. An interesting example of birefringence occurs in optical fibers, as the glass rods are stretched until they are



Top:
Imagining of the human cornea with polarized OCT results in better contrast than classic OCT (left). Contrast is improved by either mapping the material's birefringence (center) or its depolarization properties (right). Measurements were taken at the OBEL Group Laboratory (University of Western Australia).

Middle:
Speckle noise can help improve contrast. Analysis of speckle noise from repeated OCT measurements (center) makes it possible to distinguish structures of the fertilized oocyte which are invisible or barely visible through a standard microscope (left). Analysis of the movement of the speckles makes it possible to visualize blood vessels in a mouse brain (right). Measurements taken at the Faculty of Physics, Astronomy and Applied Computer Science, Nicolaus Copernicus University in Toruń and at the PAS Institute of Physical Chemistry.

Bottom:
Contactless and direct measurements of a cornea deformed by a stream of air. Before the cornea returns to its original shape, we observe the patient's response in the form of closing their eye (right). Measurements taken at the Faculty of Physics, Astronomy and Applied Computer Science, Nicolaus Copernicus University in Toruń.

roughly the thickness of a human hair. In biological tissues, this occurs in some elements of tissues, such as collagen fibers or elastin. For example, muscles and tendons comprise well-organized fibrous elements, making them highly birefringent. Pathological changes (e.g. due to cancer) in these types of tissues disrupt this order, causing a localized reduction of birefringence.

Polarization-sensitive OCT provides higher contrast than classic OCT. It allows us to illustrate birefringence of fibrous structures, depolarization properties of tissues (the rate at which light polarization changes as it passes through the tissue) or the orientation of birefringent elements.

Diagnostic touch

Examination through palpation is frequently used to diagnose or self-diagnose disorders such as breast or testicular cancer by detecting changes at an early stage. Touch allows us to determine whether the body part differs mechanically from healthy tissue, for example by finding hard lumps. Since palpation is subjective, however, scientists are developing techniques of more objective analysis of the human body's biomechanical properties. The details of individual techniques vary, but the general principle remains the same. Samples are deformed by mechanical or chemical means; the changes are then examined using OCT in order to assess the object's biomechanical properties. When equal pressure is applied to the object, softer parts are deformed more noticeably than harder, more rigid parts. Optical methods such as OCT provide a quantitative description of the differences between hard and soft tissues. For example, regions affected by cancer are more rigid than healthy tissue; this means the technique can be used to determine the extent of pathological changes while removing breast tumors.

The future of contrast

Research on OCT has been ongoing for over twenty-five years and although some scholars foresaw the topic would soon become exhausted, this has not yet been the case. Teams of scientists around the globe are working on different applications of OCT in clinical studies. Strong research teams are overcoming technical limitations and conducting basic research, which may lead to breakthroughs, and – who knows – perhaps to brand-new methods of improving contrast in OCT.

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