FOCUS

ALL EARS

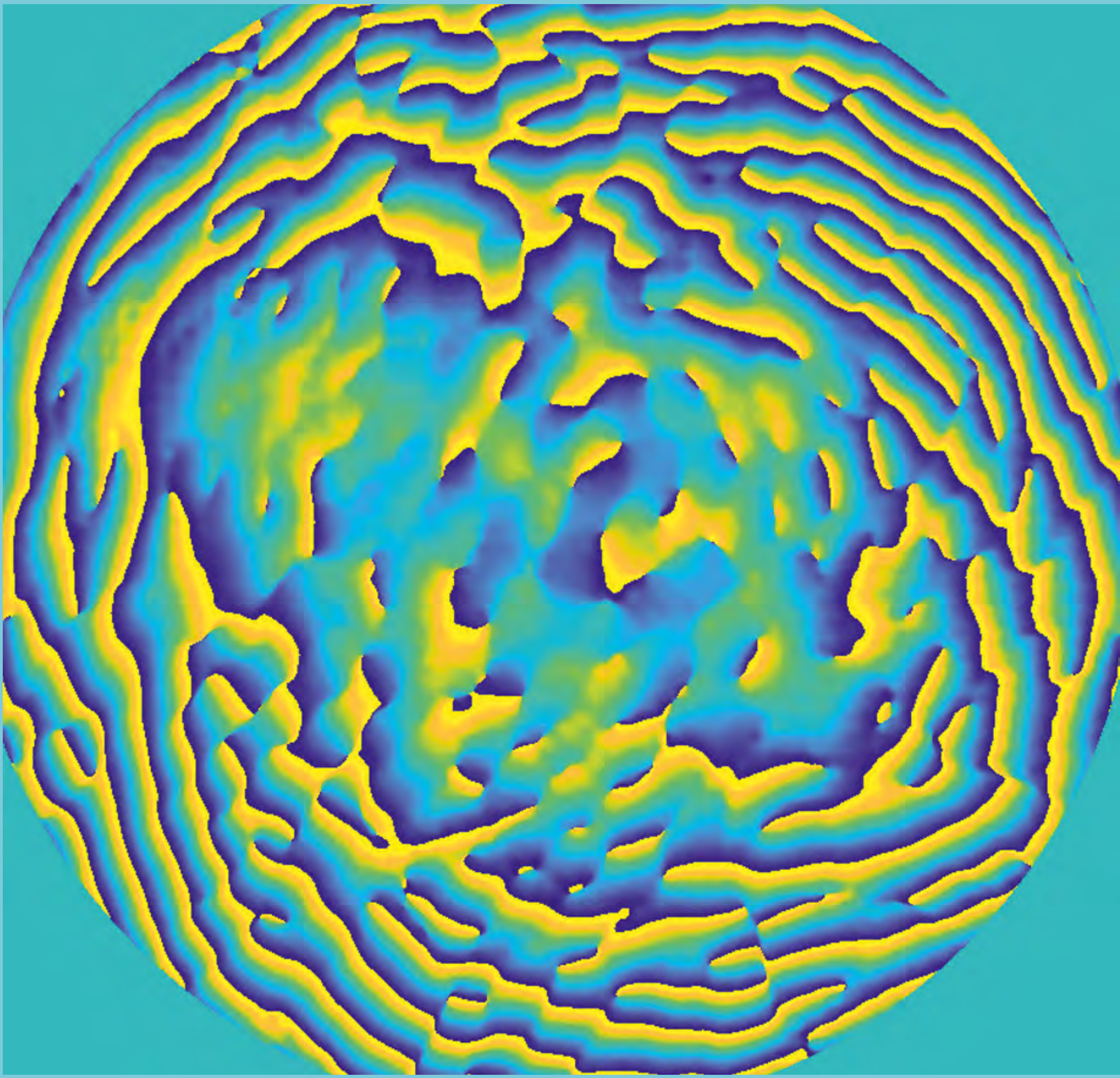


IMAGE: KAI MELDE/MPI FOR INTELLIGENT SYSTEMS

Painting with ultrasound: to construct an image with microparticles, Max Planck researchers first calculate the hologram that generates the corresponding sound profile. This simulated hologram is then used to produce an image of Picasso's "Dove of Peace" using particles.

SOUND TAKES FORM

TEXT: FELICITAS MOKLER

Ultrasound can be used manipulate tiny particles and even to arrange them in any desired patterns by using acoustic holography. This method has been developed by Peer Fischer's team of researchers from the Max Planck Institute for Intelligent Systems in Stuttgart. The physicists are already working on medical applications.

"We use ultrasound in many ways in everyday life, such as in medical imaging, in nondestructive material testing and for measuring distances when we park our car," explains Kai Melde. But the postdoc in Peer Fischer's working group at the Max Planck Institute for Intelligent Systems has a completely different use for ultrasound. He causes tiny particles to hover and transports them from one place to another with no apparent physical contact. What sounds like magic has become routine laboratory work for Kai Melde and Peer Fischer. They use a sophisticated method to modify acoustic signals to cause micron-sized particles to move and even be arranged into nearly any pattern required. This method is of interest for medical treatments with ultrasound, for analyses in materials science and for medical laboratory testing of cell cultures in petri dishes, for example.

The team led by Peer Fischer has opened up a promising new research field with this method. But, as so often happens in the field of science, that was not the original plan. Normally the scientists on his team work to develop nanobots and microbots, or they conduct research on functional materials. They arrange the tiniest of components of these materials with the aid of magnetic fields or chemical reactions to form objects such as sensors. "We hit upon the method of manipulating materials with ultrasound while searching for a way to work with biological materials as well," explains Peer Fischer.

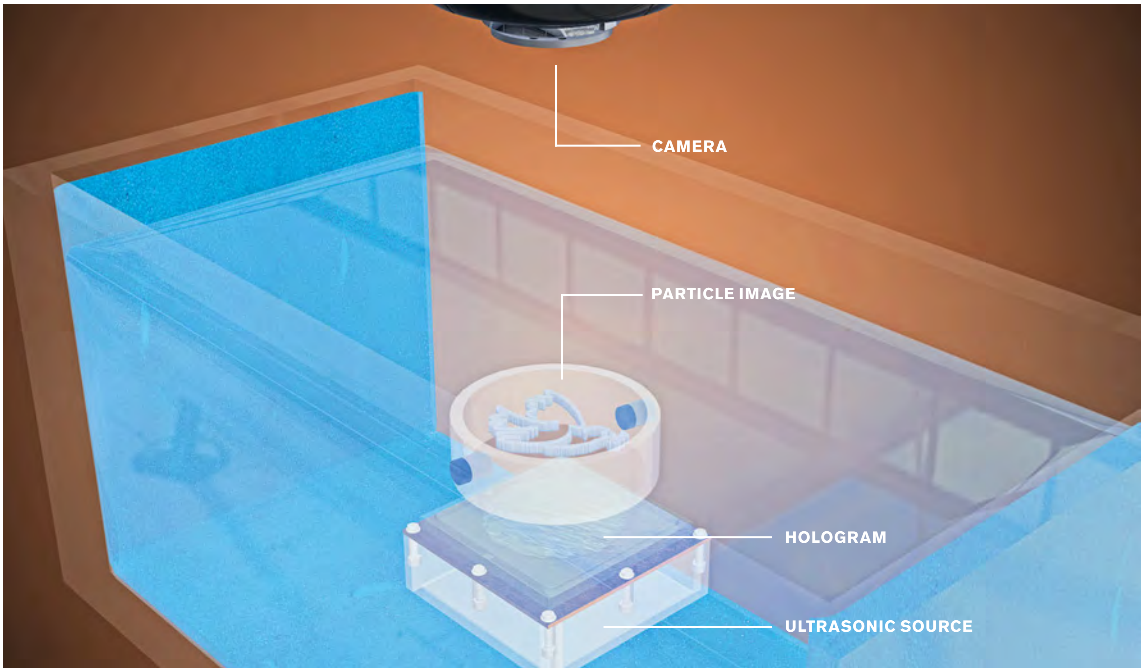
Acoustic tweezers

The actual concept of shaping sound and using it as a means of transport in the micro or nano world is not a new one. It goes back to research from the 1980s, which yielded first optical and then acoustic tweezers. Physicists use the radiation pressure of light or sound waves to trap individual microparticles in air or fluids and to position them precisely. In the simplest example of acoustic tweezers, they use a sound source to transmit pressure waves into a vessel filled with air or water. This source is called a transducer and functions like a loudspeaker – but for inaudible ultrasound. Because it is physically constrained by the vessel, a standing wave forms within the medium that is at rest at its nodes. Microparticles can be trapped at these locations. The sound acts like invisible tweezers.

If two such sources are positioned perpendicularly to each other, the standing waves are superimposed, so that the nodes and the particles trapped within them form a grid. Multiple sound sources can create even more complex patterns from a large number of nodes, which serve as pixels in an image. The phase of the individual ultrasound waves can be adjusted by individually controlling the sources. The phase determines where waves of a specific frequency reach their maximum and minimum intensity. This enables the researchers to actively control the locations where the acoustic nodes form and the microparticles accumulate. In principle, the microparticles could be arranged in any pattern by using this method. However, as the number of sound sources increases, the complexity and effort involved also increase tremendously. In addition, the resolution of the image is limited by the overall size spanned by all sources.

> **"I find the potential medical applications of acoustic holography particularly exciting, especially for ultrasound therapy."**
>
> *PEER FISCHER*

The team led by Peer Fischer and Kai Melde also included researchers from the University of Stuttgart, and together they worked out a different method to circumvent these problems. They replace the ensemble of multiple sound generators with a specially shaped plastic relief that they irradiate with sound from a single transmitter. This plastic plate is an acoustic hologram. The field of optics brought us holograms, which extend photography into the third dimension. In addition to the intensity of the light, holography also utilizes the phase of the light waves: the reflection of light off a three-dimensional object creates characteristic shifts in its peaks and troughs.



GRAPHIC & PHOTO: KAI MELDE/MPI FOR INTELLIGENT SYSTEMS

Top: Researchers place an ultrasound generator and a hologram in a water tank. These produce a sound profile in a container above them that moves particles together to form the desired pattern.

Left: Max Planck researchers in Stuttgart used Picasso's "Dove of Peace" to demonstrate for the first time that acoustic holograms can be used to generate structures from microparticles. The image shown is roughly five centimeters in diameter.

The phase of the light waves therefore transports information about the physical, i.e. spatial structure of the object, which gives holographic images their typical three-dimensional form.

Similarly, an acoustic hologram contains information about the phase of the waves, in this case sound waves. It therefore acts like thousands of tiny sound sources working together. To demonstrate how acoustic holograms can be created and used to manipulate particles, the physicists from Stuttgart arranged microparticles in a liquid to form images such as the "Dove of Peace" by Pablo Picasso. Because the subject is both highly complex and also has a fine structure, the resolution of the hologram must be correspondingly high. To duplicate the dove using particles trapped by sound, the researchers first create a phase map of the image on the computer. Then they simulate the necessary shape of a plastic relief to impose exactly this phase profile on an ultrasound wave. The thicker the material that a sound wave penetrates, the more its phase is shifted. "We use software to calculate the necessary thickness at each of the 15,000 pixels in the hologram," explains Melde. In this way, each pixel is a tiny independent sound source. Each pixel is approximately 375 microns in size. This corresponds to roughly half the wavelength of the ultrasound signal at 2MHz in water and therefore the theoretical resolution limit of the sound waves. The researchers then use a 3D printer to create the plastic relief.

⟶

For the actual experiment, they fill a chamber with water, to which they add 150-micron silicone spheres. They place the hologram beneath the chamber and irradiate it using a large ultrasonic transducer. The silicone spheres floating in the water then actually arrange themselves into a copy of Picasso's "Dove of Peace." To prevent the particles from disengaging from this shape as soon as the sound is switched off, the researchers coat them with a photochemical material. "If we irradiate these coated particles with UV light, they bond in place and the structures can then be permanently retained," explains Fischer. But creating static images is not the only capability of acoustic holograms. For example, Melde and Fischer use a different, correspondingly structured plastic relief to generate an annular wave on which they can actually get millimeter sized objects to surf.

Medical applications

Although the first experiments in acoustic holography sound a lot like clever tinkering, the method could also have a number of applications in medicine and technology. For example, acoustic holograms that can emulate multiple ultrasound sources could help simplify the inspection of materials for cracks, such as in aircraft wings. This makes use of the fact that sound propagates differently within intact material than it does in defective material. "But I find the potential medical applications of acoustic holography particularly exciting, especially for ultrasound therapy," says Fischer. Ultrasound is already in use today to destroy diseased tissue or break up kidney stones. Holograms could now be used to generate customized sound profiles that only target diseased tissue.

"However, acoustic holography also enables the use of ultrasound in the brain," explains Fischer. "This is a completely new concept, because treatments of this kind have never before been possible." This is because the thickness of the skull varies so much that it distorts an ultrasound signal to the point that it becomes unusable. A team from the Polytechnic University of Valencia in Spain recently demonstrated that a holographic plastic relief can compensate for these variations. Peer Fischer's team is working together with researchers from the Fraunhofer Institute for Biomedical Engineering to develop a medical application for this method. To holographically modulate ultrasound waves in the brain, physicians first use X-ray images to determine the variations in skull thickness and then generate a plastic relief that compensates for the differences. This method could help to remove diseased tissue, such as a tumor, from the brain.

Meanwhile, the researchers are also working on an acoustic holography method to specifically structure cells in a petri dish, without having to penetrate the culture and physically touch the cells. In this way, they hope to create artificial tumors or organoids, i.e. laboratory models of organs, with which they can improve drug testing and thereby replace testing on animals. In their experiments, the researchers are working with colorectal cancer cells; primarily because these can be easily cultivated in the laboratory, but also because artificial tumors are one of the possible applications for acoustic tweezers.

To reproduce tumors or organs, the researchers first collaborated with colleagues from the Max Planck Institute for Medical Research to find a way to organize cells into specific patterns in two dimensions. In the next step, they expanded their cellular arrangements into the third dimension, which is crucial for conclusive medical studies. "Cells behave differently in a three-dimensional environment than they do in a two-dimensional plane. And we simply need the third, spatial dimension for certain drug tests, for tumor growth experiments or for organoids," explains Fischer.

To generate two-dimensional patterns or even three-dimensional forms from biomaterial, the researchers embed the cells in a hydrogel in which they are to continue reproducing later. "The problem here is that cells consist primarily of water," explains Fischer. "So there is hardly any contrast with the surrounding hydrogel and they are therefore difficult to grasp with acoustic tweezers." The cells simply follow the movements of the sound waves. But the researchers quickly recognized that the ultrasound not only moves the particles directly through its vibrations, but also indirectly, because it can create flowing currents throughout the fluid. By carefully phasing and synchronizing the various forms of movement, they are ultimately able to position the cells in the intended configurations.

SUMMARY

Like optical holograms, acoustic holograms utilize not only the intensity of a sound wave, but also its phase. In this way, many waves can be superimposed to generate complex sound pressure profiles with which particles in a fluid can be manipulated and organized.

Max Planck researchers in Stuttgart produce acoustic holograms by first reverse-engineering how the many thousands of partial waves can be combined to yield a desired pattern and then determining the hologram that produces these individual waves.

The researchers also want to use acoustic holography to produce artificial tumors or organoids upon which realistic medical and pharmaceutical testing can then be performed. This may also make it possible to replace some animal experiments in drug development.

Kai Melde's colleague Zhichao Ma therefore used a computer to simulate how the flowing currents that are induced in the gel by the ultrasound pressure can be optimally combined with the acoustic forces acting directly on the cells to organize the cells into specific patterns. Melde then prepared the suitable hologram. Finally, the physicists positioned the resulting relief underneath the petri dish and irradiated it with ultrasound. Again, the cells organized themselves exactly as planned – in this case, forming Minerva, the Max Planck Society's logo.

If medical experts are to subsequently work with the cells, the cell cultures cannot be permitted to lose their shape as soon as the ultrasound is switched off. However, in this instance, coating the cells with a

⟶

> **"If we want to control particles in three dimensions, the sound waves must also act on them from every direction. So we need more than one sound generator."**
>
> *KAI MELDE*

The first 3D structure: Kai Melde watches as an icosahedron forms in a cubic vessel immersed in a water tank. He and his colleagues used this shape to demonstrate for the first time that two ultrasound transducers and corresponding holograms can also be used to form 3D structures. The transducers are visible as metal cylinders below the cube.

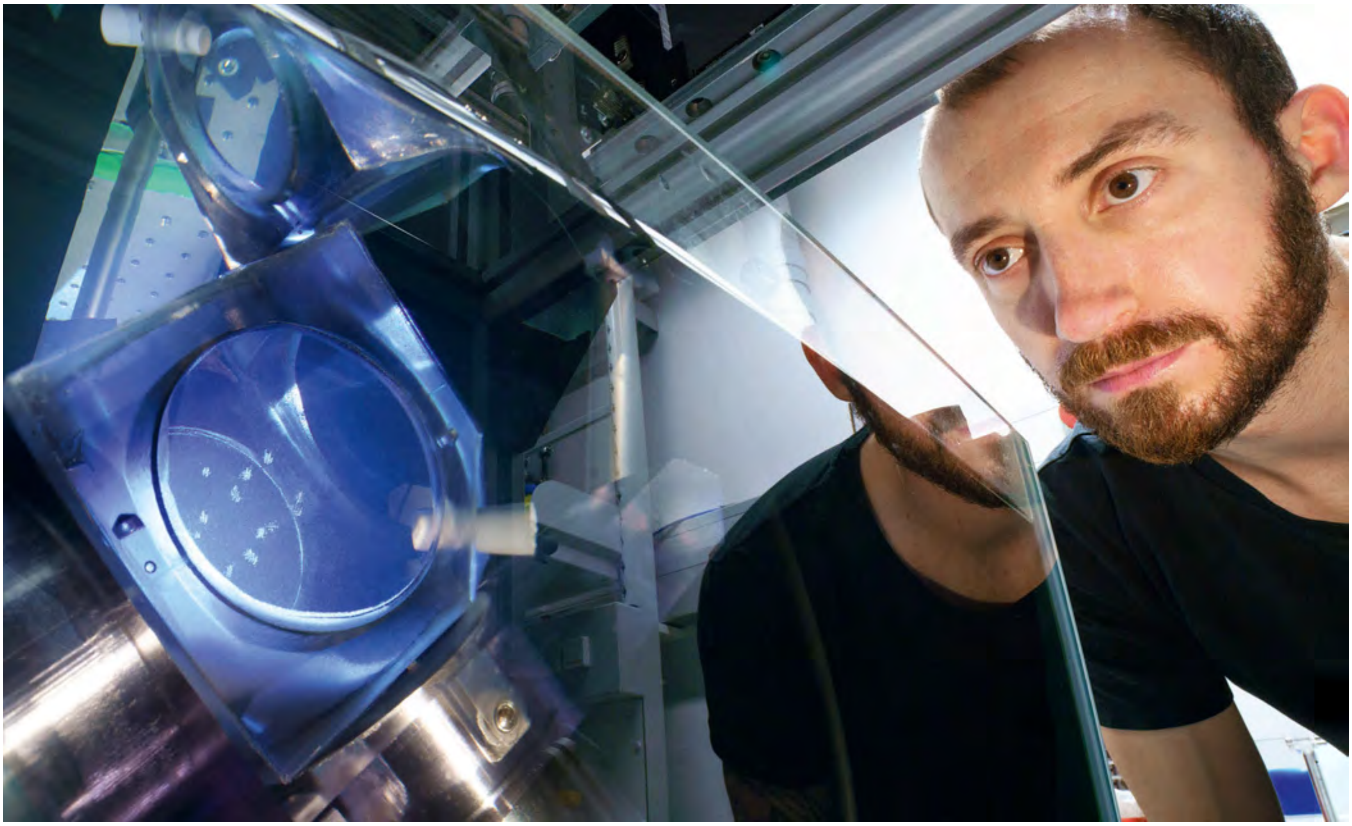


PHOTO: WOLFRAM SCHEIBLE

Sounding out a new dimension: the researchers in Stuttgart are attempting to manipulate not only the phase, i.e. the position, of the sound waves, but also their amplitude. For this purpose, they combine materials in holograms, such as various plastics (black hologram on the upper left) or plastic with white foam (to its right). However, they also incorporate air bubbles in a plastic material (hologram on the left edge of the image). This additional degree of freedom makes it easier for them to form 3D structures from particles and cells.

PHOTO: WOLFRAM SCHEIBLE

photochemical adhesive is not an option. But the physicists at the Max Planck Institute for Intelligent Systems have already solved even this problem. “We use a hydrogel that is initially a liquid but that gels after a certain period,” explains Fischer. The substance Peer Fischer and his team uses is heat-sensitive and the acoustic pressure slightly warms it. This slight rise in temperature means that, once the cells have taken on the desired configuration, the gel cools and solidifies after a few minutes. So the cells can no longer float away, but they can still multiply. And, what is also important for medical research: “We have demonstrated that the cells survive; they are unaffected by the acoustic treatment,” says Melde. “They can then be further cultivated in the configuration fixed within the gel.”

Three-dimensional cell structures

Having successfully used acoustic holography to generate cellular patterns in two dimensions, the team from Stuttgart is now working on three-dimensional structures. But the transition to the third dimension is anything but trivial. Holograms can in fact be used to generate three-dimensional sound patterns, as Peer Fischer’s team has already achieved in their experiments. However, in contrast to the previous experiments in two dimensions, particles or cells are now no longer confined to a flat surface, but rather are free to move in all directions. It is difficult to control this new-found freedom from only one direction. “If we want to control particles in three dimensions, the sound waves must also act on them from every direction. We therefore need more than one sound transducer,” explains Kai Melde. Melde and his colleagues therefore proceeded to develop concepts and ways to irradiate a contained volume with sound from two or three transducers on various sides, and thereby interconnect the particles in three-dimensional patterns.

The effort of using acoustic holography to structure cells in three dimensions could pay off in the future, such as in the design of organoids, because this method can save a lot of time without damaging cells. Once the holographic plate has been printed, the cells can be arranged in the petri dish in a matter of minutes. It would take hours or even days to produce organoids with a 3D printer, because the 3D printer can only construct a framework one point at a time from a material on which the cells could then grow. In contrast, the cells can all be configured simultaneously with an acoustic hologram.

While the researchers in their team continue working on the right method to generate three-dimensional cellular structures, Peer Fischer and Kai Melde are already sounding out the next potential uses that acoustic holography has to offer. For example, they want to set the sound profiles and therefore the particle arrangements in motion. They are therefore looking for ways to modify the holograms in near real-time. The researchers from Stuttgart are now exploring the field of acoustic holograms – which they themselves developed – in completely new directions, and it likely that in the future, they will explore many other possibilities with ultrasound.

🎧 ***www.mpg.de/podcasts/schall (in German)***

GLOSSARY

HOLOGRAPHY
This method uses not only the intensity of light or sound waves, but also their phase. Holograms therefore also contain information about the three-dimensional structure of an object.

ORGANOID
An organ-like structure made up of many cells.

PHASE
Indicates the point at which a wave is in its cycle at a specific time. Waves that share the same phase have peaks and troughs at the same location. A phase shift between waves results in an offset, which changes the resultant wave pattern when they are superimposed.