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Holographic Acoustic Tweezers

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Acoustic Tweezers use sound radiation forces to manipulate matter without contact. They provide unique characteristics when compared to the more established Optical Tweezers, such as higher trapping forces per unit input power and the ability to manipulate objects from the micrometre to the centimetre scale. They also enable the trapping of a wide range of sample materials in various media. A dramatic advancement in Optical Tweezers was the development of Holographic Optical Tweezers (HOT) which enabled the independent manipulation of multiple particles leading to applications such as the assembly of 3D micro-structures and the probing of soft matter. Now, 20 years after the development of HOT, we present the first realization of Holographic Acoustic Tweezers (HAT). We experimentally demonstrate a 40 kHz airborne HAT system implemented using two 256-emitter phased-arrays and manipulate individually up to 25 millimetric particles simultaneously. We show that the maximum trapping forces are achieved once the emitting array satisfies Nyquist sampling and an emission phase discretisation below $\pi/8$ radians. When considered on the scale of a wavelength, HAT provides similar manipulation capabilities as HOT while retaining its unique characteristics. The examples shown here suggest the future use of HAT for novel forms of displays in which the objects are made of physical levitating voxels, assembly processes in the micro-metre and millimetric scale, as well as positioning and orientation of multiple objects which could lead to biomedical applications.

Acoustic Tweezers | Contactless manipulation | Acoustic Levitation | Acoustophoresis | Displays

Introduction

In 1986, Ashkin showed that dielectric particles can be trapped in a focused laser beam¹ and that this principle also works for bacteria as well as viruses². Since then, Optical Tweezers have become a fundamental tool in biology and physics, leading to the measurement of the DNA spring constant², transport of Bose-Einstein condensates^{4,5} and trapping of cold atoms⁶. Holographic Optical Tweezers (HOT)^{7,8,9,10} further extended this functionality to enable the simultaneous manipulation of multiple particles resulting in applications such as the assembly of 3D colloidal structures¹¹, quasicrystals^{12,13} and nanowires¹⁴ as well as the probing of soft matter¹⁵.

Acoustic Tweezers use the radiation forces exerted by ultrasonic waves to trap particles^{16,17} ranging from less than $1\ \mu\text{m}$ ^{18,19} to more than $1\ \text{cm}$ ²⁰ in various media such as air²⁰, water²¹ and potentially (only proven theoretically) in biological tissue phantoms^{22,23}. Acoustic radiation forces are 5 orders of magnitude higher per unit input power than in optical trapping giving them a significant efficiency advantage and enabling low-power operation which is critical in cell manipulation applications²⁴. Consequently, Acoustic Tweezers are becoming a fundamental tool for disease diagnosis²⁵, lab-on-a-chip manipulation²⁶, centimetre scale containerless processing^{27,20} and *in-vivo* applications such as the manipulation of kidney stones²⁸.

Recent advances have enabled the dynamic positioning of acoustically trapped particles in 1-²⁷, 2-²⁰ and 3-dimensions^{29,30,31}, however, the particles were moved as a group, with no individual particle control. Acoustic radiation force devices using 3D

printed lenses have also been used to produce complex patterns of particles³², but these patterns were static and two-dimensional. Similarly, by multiplexing a focal point it was possible to manipulate in mid-air two droplets of water in 2D³³. To date, the most versatile dynamic device enables two particles to be manipulated independently in 2D using a ring of emitters in a microfluidic chamber²¹.

Being able to individually control many particles with the versatility and efficiency of Acoustic Tweezers would enable many new applications such as display spaces where levitated physical voxels form objects in 3D, or fabrication of structures ranging from the micro-scale for tissue engineering to the centimetre scale for placement of integrated circuits. Also, the inherent capability of ultrasound to act through tissue, would permit the use of HAT for complex *in-vivo* procedures in which trapped particles assume different manipulation roles, e.g. hold, orientate, release, bring together or separate.

In this paper, we explore the capabilities of HAT to dynamically manipulate multiple particles simultaneously in mid-air. We describe and evaluate a novel algorithm that for the first time enable the realization of HAT by controlling the emitted field from ultrasonic phased-arrays. For Optical Tweezers, the Digital Light Modulator (DLM) was revolutionary as it provided more than 500×500 pixels of phase control⁷. Acoustic lenses have recently been exploited to apply similar high-resolution phase modulation³², but they are static and thus not suitable for dynamic HAT. Phased-arrays are the current dynamic acoustic emitter that offers the best potential solution, e.g. emitters of up to 50×50 elements have been described in the literature³⁴, however, this acoustic array contains two orders of magnitude less elements than commonly available DLMs. We show that despite this reduced element count, it is possible to realize a HAT with

Significance

Holographic Optical Tweezers use focused light to manipulate multiple objects independently without contact. They are used in tasks such as measuring the spring-constant of DNA, the pulling force of the kinesin protein or to trap matter in exotic states. Differently, Acoustic tweezers use sound radiation forces to trap particles at a larger scale (i.e. from micrometres to centimetres). However, previous implementations did not provide individual control. We present the first realization of Holographic Acoustic Tweezers (HAT). Using an array of sound emitters, we engineer the generated sound field to manipulate multiple particles individually. This enables applications in contactless assembly both at the micrometre and centimetre scale as well as the creation of displays in which the pixels are levitating particles.

Reserved for Publication Footnotes

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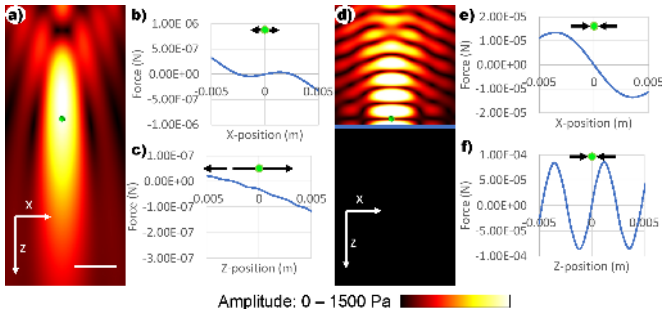


Fig. 1. Trapping over a reflective surface. (a) Pressure amplitude generated by an array focused at a single point in free space, (d) pressure amplitude when the same focal point reflects on a surface (blue line). Trapping forces in the x-direction (b,e) and z-direction (c,f) generated by the focal point. (b,c) Non-converging forces without a reflector, (e,f) converging forces in the presence of a reflector. Scale bar from (a) represents 2cm. Particle is located at the origin.

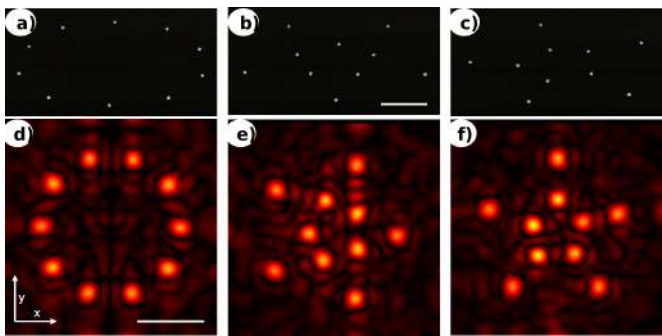


Fig. 2. Simultaneous in-plane manipulation of 10 EPS particles of 2mm diameter. The particles are trapped 2.3mm ($\lambda/4$) above a reflective surface. a) the particles start in a circle, b) odd particles move towards the centre, c) the two concentric circles of particles rotate in opposite directions. d,e,f) The simulated pressure amplitude fields generated at the reflective surface. The 16x16 array was placed parallel to the surface 13cm above it. Scale bar (b) and (d) represents 2cm.

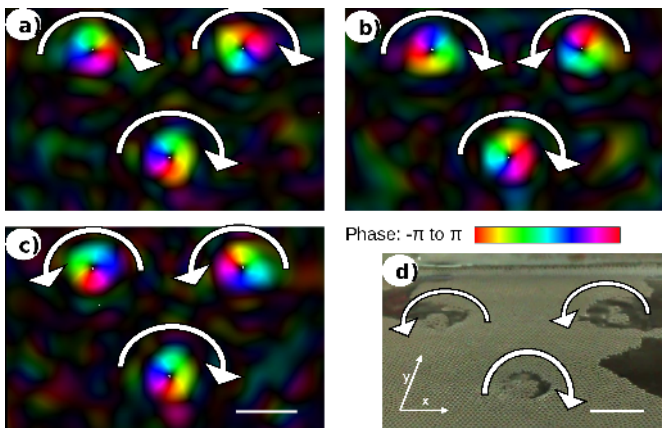


Fig. 3. Generation of vortices with independent chirality. a,b,c) simulated phase profile on a plane parallel to an array placed 15.1λ (13 cm) above it. a) all the vortices are clockwise. b) The top right vortex has changed to counter-clockwise. c) the top left vortex has also changed to counter-clockwise. d) bubbles on the surface of a water tank rotate according to the direction of the vortices from (c). Scale bar (c) and (d) represents 2cm.

independent manipulation capabilities similar to those achieved in HOT.

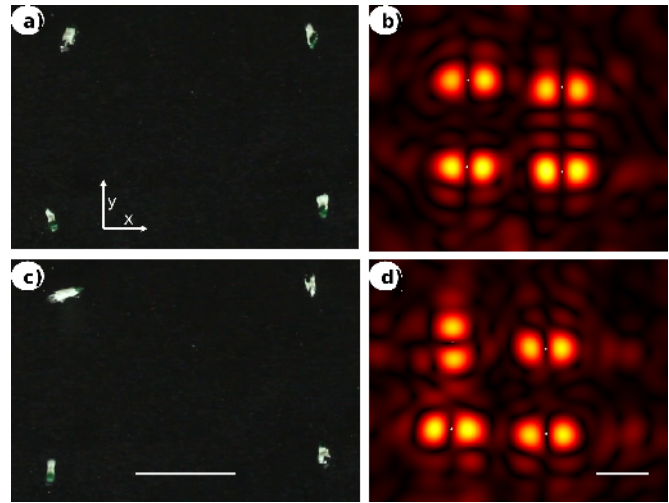


Fig. 4. Individual orientation of asymmetric particles trapped above a reflective surface with an array placed 15.1λ (13 cm) above it. a) all EPS particles aligned along the y-axis, b) one EPS particle is orientated along the x-axis. b,d) corresponding simulated pressure amplitude at the reflecting surface. Scale bar (c) and (d) represents 2cm.

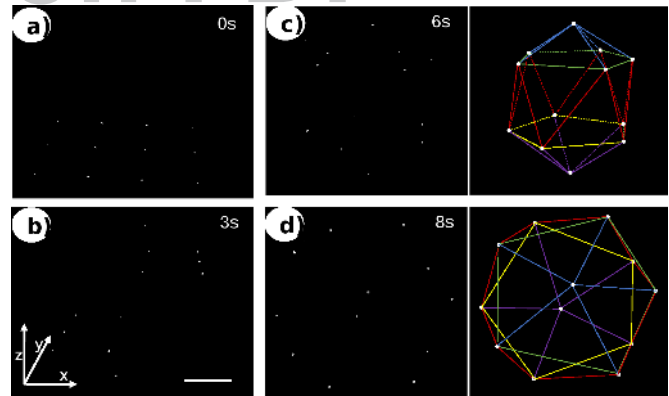


Fig. 5. Simultaneous manipulation of 12 particles starting in a planar grid and being morphed into a 3D icosahedron. a) the particles start in a single plane as a 3x4 grid. b) the particles are moved towards their target z-positions. c) the particles move to form an icosahedron. d) the icosahedron is rotated 45 degrees towards the viewer. For (c) and (d) the vertices of the icosahedron have been overlaid. Scale bar (b) represents 2cm.

An algorithm capable of realizing HAT is distinctly different from those used previously for HOT. In optics, a focus on the particle is sufficient for trapping³⁵ whereas in acoustics, only negative contrast particles (i.e. the acoustic impedance of the particle is less than that of the medium) will be trapped in this way³⁶. However, acoustic trapping in air and most particles in water-based media lead to positive acoustic contrast particles. Therefore, in practice, acoustic trapping is only achieved at the zero pressure regions of standing-waves nodes²⁷, focused vortices³⁷, twin-traps³⁰ or bottle-beams³⁰.

Here, we describe a novel Iterative Backpropagation (IB) algorithm that we use to calculate the emission phases of the array elements to realise a functional HAT. This algorithm uses a modified version of the Iterative Angular Spectrum Approach³² (IASA), IASA is itself based on the Gerchberg-Saxton (GS) algorithm³⁸. Differently from IASA and GS, IB uses propagators derived from a specific transducer model which enables us to accurately predict the acoustic field with minimum computational effort. In addition, IB permits the creation of focal points as well

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273 as the enforcement of phase dependencies between these points
274 allowing us to efficiently generate different traps (i.e. focal points,
275 twin-traps and vortices) at arbitrary positions.

276 We show that HAT can be realised using this algorithm and
277 two 256-emitter arrays with an element spacing and diameter of
278 1.2λ (1cm) operating at 40 kHz with a phase resolution of $\pi/16$
279 radians and an update rate of 90 frames per second. For in-plane
280 2D manipulation we used a single array placed 15.1λ (13cm)
281 above a sound-reflective surface, for 3D manipulation we used
282 two opposed arrays separated by 26.7λ (23cm) (Figure S1), these
283 separations were selected to provide high acoustic pressure in the
284 desired planar or volumetric manipulation region (Figure S2). We
285 use these systems to demonstrate the controlled manipulation of
286 multiple Expanded Polystyrene (EPS) spheres (1-3mm diameter)
287 (Movie S1).

288 Results

289 In-plane manipulation

290 The multi-particle manipulation capabilities of HOTs are
291 usually demonstrated with the control of various particles in a
292 single plane⁷. Here, we realize an in-plane HAT with the particles
293 initially resting on a reflective surface. This is a common practical
294 scenario, e.g. with particles resting on a microscope cover
295 slide³⁹. If a sound beam is focused on a particle situated on a
296 reflective surface, due to the interference between the incoming
297 and reflected field, a local standing wave is created with the
298 first node positioned $\lambda/4$ above the surface (Figure 1). At this
299 node, the forces in all three dimensions converge, which is the
300 requirement for stable trapping. Thus, for particles located on
301 a planar reflective surface, HAT can be realized by focusing the
302 array on the particles and manipulating these foci.

303 Multiple particles are manipulated by generating multiple
304 foci, causing the particles to be trapped in the nodes formed just
305 above the reflector. The IB algorithm (see Methods) is used to
306 generate focal points at the positions of the particles and the
307 emitter phases are dynamically controlled to move the foci and
308 hence the particles. The application of the IB algorithm ensures
309 that the pressure amplitudes at the foci are maximized and that
310 the deviation between the various points is minimized, i.e. the
311 normalized standard deviation of the focal pressure amplitude is
312 reduced by 30% when compared with the non-iterative method
313 (see Methods). In Figure 2 and Movie S2, we show the manipu-
314 lation of 10 particles in a plane $\lambda/4$ above a reflector.

315 The minimum distance achieved between the particles was
316 ≈ 1.3 cm (1.5λ) regardless of the number of trapping points (Fig-
317 ure S3). At smaller distances, the focal points merged together
318 inhibiting independent control. The Rayleigh resolution limit⁴⁰
319 for this configuration is 0.85cm ($1.22\lambda L/A$ where $L=13$ cm is the
320 focal distance and $A=16$ cm is the aperture), but this minimum
321 distance between traps can only be obtained with smaller acous-
322 tic emitters, we show the amplitude distribution of two close focal
323 points depends on the pitch of the array in Figure S4.

324 Here, at excitation signals of 10Vpp (9.5W of input power),
325 we manipulated 12 particles (Figure 2); and at 16Vpp with double
326 the number transducers (57W of input power), we manipulated 25
327 particles (Movie S3). In Figure S5, we show that when attempting
328 to use our system to generate 28 traps, the generated undesired
329 artefacts (secondary high-amplitude regions that were not defined
330 as focal points) start to become as powerful as the traps.
331 Therefore, for this system, further increase of the power will have
332 no further benefit in terms of the number of independent traps.
333 The effect of artefacts is explored in more detail in the discussion.

334 Beyond trapping: in-plane torque and orientation

335 HOT have been used to create traps with different
336 functionalities⁷, for example vortices that can transfer orbital
337 angular momentum (OAM). In acoustics, single vortex beams
338 have also been used to trap and transfer OAM^{41,42,43,44}. In HAT,

341 we can generate multiple vortices with independent chirality using
342 the IB algorithm, but this time tuned to create vortices. In Figure
343 3 and Movie S4, we generate 3 vortices above a water surface and
344 individually change their chirality in real time. The vortices were
345 separated by 10λ to correctly observe the rotation of soap bubbles
346 on the surface of the water. With our system, it was possible to
347 bring two vortices cores within ≈ 1.4 cm (1.6λ) (Figure S6) and
348 generate up to 5 discernible vortices (Figure S7).

349 In the past, it has been shown that an acoustic twin-trap can
350 orientate asymmetric particles^{45,30}. The IB algorithm presented
351 in this paper (see Methods) is capable of generating for the first
352 time multiple twin-traps at arbitrary positions and with different
353 orientations. Twin-traps and vortices can create converging forces
354 along the direction of propagation (i.e. z-axis); however, this
355 force was not enough to levitate the particles since it can be
356 more than 30 times weaker than the lateral forces⁴⁶. Hence, we
357 adopted a time-multiplexing approach between twin-traps (to
358 orientate) and focal points (to generate enough trapping force),
359 this approach has been recently demonstrated for one particle⁴⁷
360 but here we show that it can be applied for multiple particles to
361 achieve independent control of particle orientation. In Figure 4
362 and Movie S5, we show the orientation of 4 asymmetric particles
363 and change their orientations individually by rotation of the twin-
364 traps. With our current configuration, it was possible to generate
365 up to 7 twin-traps (Figure S8) with a minimum distance of $\approx 1.4\lambda$
366 between them (Figure S9).

367 3D Manipulation

368 To realize a 3D HAT we used a double-sided arrangement
369 made of two opposed arrays (16x16 elements) separated by 26.7λ
370 (23cm) to create multiple standing waves with nodes located at
371 the target trapping positions. To do so, the IB algorithm first
372 creates high intensity foci at the specified trapping positions.
373 These foci are then all shifted vertically (in the z-direction) by
374 $\lambda/2$ by the application of an additional phase delay of π radians to
375 the top array elements, thereby nodes now occur at the required
376 trapping locations. Hence, the converging forces required for
377 trapping are created and can be manipulated by dynamically
378 moving the foci. In Figure 5 and Movie S6 we show 12 particles
379 that start in a single plane, then morph into an icosahedron that
380 afterwards rotates around different axes. In Holographic Optical
381 Tweezers, this same manipulation has been shown⁴⁹, albeit on a
382 much smaller length scale.

383 In Figure S10 and 11, we use acoustic field simulations to
384 show that the trapping forces decrease linearly with the number of
385 trapped particles. More importantly, keeping the acoustic power
386 per unit area constant and reducing the pitch of the emitters
387 improves the performance of HAT in terms of trapping stiffness.
388 However, we show that, once Nyquist sampling⁵⁰ is achieved
389 (emitter pitch of $\lambda/2$), no further improvements can be obtained.
390 The HAT presented in this paper has a transducer spacing of
391 1.2λ and so is somewhat sub-optimal in this regard. We also use
392 acoustic field simulations to show that trapping strength does
393 not increase significantly for phase discretisation levels below $\pi/8$
394 radians (Figure S12). Since our system discretises phase at $\pi/16$
395 radians it is already optimal in that sense. We note that this is
396 consistent with findings from single-trap systems where a phase
397 discretisation of $\pi/5$ was found to be sufficient⁵¹.

398 Up to 27 particles have been manipulated in 3D using holo-
399 graphic optical tweezers¹¹. Despite our limited array size (i.e.
400 16x16 cm), spatial discretisation (i.e. 16x16 elements) and pres-
401 sure levels (i.e. 15Vpp to generate 2.3 Pa at 1 meter with each
402 emitter), we achieved simultaneous dynamic manipulation of 12
403 particles (Figure 5) and the partial manipulation of 25 particles
404 (Movie S3). In the last case, some particles escaped the traps as
405 they were being moved due to the increased trapping stiffness
406 required to counteract the oscillations of the particles in air.

Therefore, reaching the same number of particles as HOT would be feasible employing more powerful or smaller elements.

Discussion

We quantified the quality of the traps using the stiffness (i.e. the spatial gradient of the force), which represents the converging forces of the traps. From our simulations (Figure S10) and experiments (Figure S13) we observed that the trapping forces are inversely proportional to the number of traps created. Simulations show that the stiffness can be improved by decreasing the pitch of the emitters (Figure S10) or by increasing the emission phase resolution (Figure S12). However, when close-packed emitting elements reach a pitch smaller than $\lambda/2$ and an emission phase resolution below $\pi/8$ no further improvement can be obtained.

The IB algorithm maximises the trap quality (i.e. stiffness), rather than minimising artefacts. As a result, artefacts are often present (e.g. Figure 4.b & d, Figure S3 and S5). In general, for a low number of traps (i.e. <10) the traps have significantly larger pressure amplitude than these artefacts and thus more trapping force, so they do not cause a significant problem. However, as the number of traps increases, their trapping forces decrease, and the artefacts become increasingly powerful, this is shown qualitatively in Figure S5 and quantitatively in Figure S14. We note that with the future possibility of ultrasonic arrays with more transducers and a smaller pitch it may be possible to develop algorithms that maximize traps stiffness and also minimise artefacts.

The appearance of artefacts and ghost traps is a very pressing problem in HOT⁵², this problem also appears in our HAT system. On the one hand, secondary nodes appear along the Z-axis. The focal points are inherently elongated along the propagation direction (i.e. in an ellipsoidal shape) and this creates multiple secondary nodes separated by $\lambda/2$. The length of the focal zone (or Rayleigh length) depends on the wavelength, aperture of the array and distance from the array. In Figure S15, we show the amplitude profile of focal zones for our system depending on the number of transducers and traps, the Rayleigh length is similar for all cases (i.e. 7 cm \approx 8 nodes). We note that stronger focusing (i.e. lower F#) can be used to reduce the length of the focal zone and thus reduce the number of secondary nodes.

Undesired secondary traps can also be created in the XY plane, for example a focus at a single point can create secondary areas of high-intensity outside of the focal area following an Airy amplitude distribution⁵³. When our phased-array generates multiple focal points, it produces several of these secondary focal points (also called artefacts or ghost traps). In Figure S14 we show how the ratio between the minimum amplitude of the focal points and the maximum amplitude of the artefacts varies with the number of traps. As mentioned before, the traps are significantly stronger than the artefacts for relatively small numbers of traps (i.e. <10) but as the number of traps increase their strength reduces, until they approach that of the artefacts. For the 16x16 element array used here, the ratio is 16:1 for one focal point and decreases to 3:1 for 25 traps. Surprisingly, emitters with finer pitch lead to stronger artefacts (Figure S16.a & b) but have the advantage of generating the secondary lobes further away from the central region (Figure S16.c & d).

A trapped particle will scatter sound and affect nearby particles. However, for the particles used here (i.e. 1-2mm diameter) this effect was small. Supplementary Video 7 shows that particles of 1-2mm diameter do not affect the particles in the nodes above or below in a perceivable way. In Figure S17 we show a simulation of how particles of different diameters affect the nearby acoustic field, e.g. nodes are only displaced by 0.12mm when a 1mm diameter particle is added in the next node, this distortion becomes more pronounced as the particle size increases.

The Rayleigh criterion⁴⁰ determines how close 2 focal points can be generated without getting too distorted (e.g. start to

merge), hence we considered it to be an adequate indicator of the minimum lateral distance between the traps. In our setup, this minimum distance between traps was 1.4λ which is several times larger than the particle size.

The repositioning accuracy of the particles was $\pm 0.1\text{mm}$ ($\lambda/86$) for the in-plane and $\pm 0.5\text{mm}$ ($\lambda/17$) for the 3D manipulation. Similar levels (relative to the wavelength) of positional deviations occur in optical trapping, where the particle centre is not always at a constant distance to centre of the focus⁷. The trapped particles showed good stability over time, in Movie S8 a time lapse of 1 hour showed no noticeable deviation apart from that induced from air currents.

Optically trapped particles are often used as handles to manipulate other samples (such as DNA strands²). Similarly, we attached EPS spheres to different objects and manipulate these handles, e.g. to post a thread through a hole in a piece of fabric (Movie S1).

The demonstrated system operates in air with a wavelength of 8.6mm but, in principle, HAT can be scaled down by increasing the frequency and applied to other propagation media. For instance, a system operating at 7.5MHz in water-based solutions would have a wavelength of 200um, enabling the trapping of 20um cells (the current HAT can trap particles smaller than $\lambda/10$). The demonstrated 3D HAT employed two arrays of 256 elements, with future systems containing more and smaller elements, or higher emitter output pressure, improved capabilities can be expected. In the medical domain, arrays with twice the number of elements are already available⁵⁴. Similarly, Capacitive Micromachined Ultrasonic Transducers (CMUT) will enable the miniaturization of the systems for working at the microscale wavelength⁵⁵.

Larger systems would enable the trapping of more particles and thus allowing the creation of displays made of levitated physical voxels, these displays would have characteristics that no existing display provides. Holograms can only be viewed from specific angles and both volumetric displays or the recent photophoretic displays are based on light reflection, so they can only operate under specific lightning conditions⁵⁶.

In the supplementary methods section we describe the manual and semiautomatic approaches employed to load the particles into the system. We note that a combination of HAT with tracking methods would enable an automatic solution for inserting the particles. For example, a 3D tracking system could detect the position of the particles so that the traps are directly created at those positions.

We have presented the first demonstration of Holographic Acoustic Tweezers (HAT) that enables the individual positioning of multiple particles in 2D and 3D. These results have come 20 years after the appearance of its optical analogue (i.e. HOT). The iterative backpropagation (IB) algorithm was the key that unlocked the realization of HAT and hence it is the most novel aspect. It allowed us to create multiple functional traps using arbitrary arrangements of transducers. HAT enables the control of multiple particles individually with the unique advantages of acoustic radiation forces, i.e. scales from micro- to centimeters, support of multiple materials for samples and propagation media and high ratio of input-power to forces.

Materials and Methods

Hardware

We employed arrays of 16x16 1cm diameter 40 kHz ultrasonic transducers (Murata MA40S4S) operating in air. An FPGA (Altera Cyclone IV EP4CE6) receives the phases to be emitted from the computer using UART protocol operating at 250kbps. Mosfet Drivers (Microchip MIC4127) amplify the signals up to 15Vpp half-square waves, that are fed into the transducers. Due to the narrowband nature of the transducers the output pressure was sinusoidal⁵¹. This hardware had a phase emission resolution of $\pi/16$ radians and updated 90 times per second. The employed particles were Expanded Polystyrene (EPS) spheres of 1-3 mm diameter.

Algorithm for the HAT Calculation: Iterative Backpropagation (IB)

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To generate multiple traps, we employed Iterative Backpropagation (IB), which is a modification of the iterative IASA³² and G5³⁸ algorithms. If we have m target traps at positions $\mathbf{r}_1, \dots, \mathbf{r}_m$, we will split them into multiple control points depending on the desired type of trap. All the resulting control points c_1, \dots, c_k will have positions \mathbf{r}_j , amplitudes a_j and phases φ_j where j is between 1 and k . Amplitude and phase are represented as a single complex number c_j . The position, amplitude and phase of the control points depends on the type of trap to be generated and are described in the next paragraph. The amplitude associated with a given control point is $n_j = 1/m$ where m is the number of control points used to define a given trap. The phase of the control points starts at 0 radians but it is updated with every iteration of the algorithm. Only the phase of the first control point of a trap (φ_1) is updated; the rest of the points have a fixed phase relative to this first point and this phase pattern depends on the type of trap.

The shape of the three pressure field shapes used to create the traps (i.e. focus, vertical twin-trap and vortex) were found to be almost invariant spatially within the manipulation regions (Figure S18) This allowed us to identify a small number of features (or control points) that characterised each of these trap geometries. A focal point requires a single control point with the same position as the focus and unity amplitude. A twin-trap has control points with a separation of 1.4λ between them, the control points can be rotated around the centre of the trap to control its orientation. A vortex trap is decomposed into 8 control points with the phase pattern following an increase from 0 to 2π radians in the counter-clockwise or clockwise direction depending on the desired chirality of the vortex, the distance between these points and the centre of the trap is 1.4λ . The location and phases of control points in various traps can be seen in Figure S19.

Consider a transducer i emitting with an amplitude a_i and phase φ_i (i.e. $p_i = a_i e^{i\varphi_i}$), to produce a complex field at \mathbf{r} given by $p_{i\mathbf{r}} = p_i h_{i\mathbf{r}}$ where $h_{i\mathbf{r}}$ is the complex propagator from the position of transducer i to the point \mathbf{r} , we pre-calculate the propagators from each transducer to each control point \mathbf{r}_j . We obtain this propagator using the far field piston source model and setting the initial phase of the transducer to 0 (see Supplementary

Methods). The algorithm then proceeds by iteratively finding the phases for the transducers required to generate the target fields at the control points. If the phases between successive iterations are below a certain threshold (0.01 radians in our case) the algorithm stops, for the examples presented in the paper convergence was achieved after 200 iterations.

1. Forward propagate the transducer's complex pressure to the control points. $c_j = \sum p_i h_{i\mathbf{r}_j}$
2. Normalize the amplitude at the control points and set the phase. $c_j = n_j c_j / |c_j|$ where n_j is the relative amplitude for the point j . For all the points m which are not the first point of the trap, the phase is set in relation to the first point.
3. Time reverse the control points into the transducers. $p_i = \sum c_j \overline{h_{i\mathbf{r}_j}}$

Where $\overline{h_{i\mathbf{r}_j}}$ is the conjugate of the complex propagator.

4. Normalize the output amplitude of the transducers. $p_i = p_i / |p_i|$
- This algorithm supports the generation of focal points, twin-traps and vortices at the target positions. Furthermore, the angle of the twin-trap or the direction of the vortex is tunable by the user via the definition of the control points.

Using the above IB algorithm alone was sufficient for in-plane manipulations. However, for generating the nodes required for 3D manipulation, an additional step was used. First, as with 2D trapping, the IB algorithm is used to generate focal points at the trapping positions. Second, a π radians offset is added to all elements in the top array to shift the high intensity focus (i.e. an antinode) so that nodes are located at the target positions (Figure S20).

Using a direct time reversal method (i.e. IB with 1 iteration) provided similar results in terms of mean force magnitudes (Figure S21) but the variation between traps was larger (Figure S22). A brute-force global maximization of the Gor'kov Laplacian (trapping stiffness) for all the traps as an extension to Marzo et al. algorithm³⁰ did not produce functional traps.

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