Supplementary Materials for

Real-time frequency-encoded spatiotemporal focusing through scattering media using a programmable 2D ultrafine optical frequency comb

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Section S1. Ultranarrow linewidth fiber laser

Figure S1A shows the schematic diagram of the homemade ultranarrow linewidth continuous-wave (CW) fiber laser, named kHz laser, which can deliver an average power of up to 30 W at 1064 nm. The fiber laser system is composed of a seed oscillator, two consecutive polarization-maintaining (PM) fiber amplifiers and a final second-harmonic generation (SHG) stage. In order to generate a single longitudinal mode with an ultranarrow linewidth, the key component of the seed oscillator is a specially designed cavity with an extremely short length, fabricated using highly doping fiber optic technology (41). The laser cavity consists of two fiber Bragg gratings (FBG1 and 2): one is highly reflective (FBG1, 0.4 nm bandwidth, 99.5% reflectivity), while the other one is partially reflective (FBG2, 0.08 nm bandwidth, 60% reflectivity, PM fiber), which also serves as the output coupler. A short piece of highly ytterbium-doped phosphate fiber (YPF, 0.8 cm length) is sandwiched between these two FBGs and pumped by a single-mode fiber laser diode (LD1, 980 nm) through a wavelength-division multiplexing (WDM) coupler.

The highly doped YPF, fabricated using the rod-in-tube technique, provides a net gain up to 5.7 dB cm\(^{-1}\) (42). The seed oscillator delivers an average power of ~50 mW, at a backward pump power of 220 mW. The seed oscillator is protected from optical back reflection by a fiber isolator (ISO1), which is subsequently fed into two stages of optical amplifiers to boost up the average power.

The laser linewidth, as shown in Fig. S1B, is fitted with the Lorentzian function to estimate the spectral linewidth, which is ~36 kHz at 20 dB corresponding to a full width at half maximum (FWHM) linewidth of ~1.8 kHz (41). For high sensitivity detection, the relative intensity noise (RIN) of the laser is crucial and is characterized using a photodetector (PD) and an electrical spectrum analyzer (Fig. S1C). As can be observed, the RIN is only –133 dBc/Hz at frequencies of >2 MHz, i.e., the frequency range where we perform single-shot FEST focusing (30 – 110 MHz, see Fig. 2F). The phase noise of the fiber laser is also evaluated by an unbalanced Michelson interferometer with an optical path difference of 100 m. Fig. S1D shows the phase noise spectra of the seed oscillator and final output after the two optical amplifiers. At frequencies of >2 kHz, the phase noise is less than 1.3 µrad/Hz\(^1/2\). The discrepancy of the phase
noise between the seed oscillator and final output after the optical amplifiers can be attributed to the fluctuation of pump power and thermal diffusion in the optical amplifiers.

To match with the existing optical devices in our lab, mainly working in the visible range, the output laser beam at 1064 nm is frequency-doubled to 532 nm through SHG by a periodically poled lithium niobate (PPLN, HC Photonics) crystal, yielding a maximum power of ~7 W. It should be emphasized that the SHG is not necessary for optical systems operating in a wavelength range that covers 1064 nm.

Section S2. Full configuration of the FEST focusing system

The full configuration of the FEST focusing system is illustrated in Fig. S2A. The green laser beam after the SHG is split by two polarizing beam splitters (PBS1 and 2) into three optical paths that are used for the generations of the two-dimensional (2D) optical frequency comb (2D-OFC) beam array, the reference beam and the option beam, respectively. The power ratio between these three optical paths is controlled by rotating the half-wave plates ($\lambda/2$).

To generate the 2D-OFC beam array, an acousto-optic deflector (AODx, IntraAction AOM-802AF1) driven by a multitone radiofrequency (RF) signal is utilized to diffract (~25% efficiency) the input laser beam into a one-dimensional (1D) optical frequency comb (1D-OFC) beam array with angular dispersion in the $x$ direction (inset of Fig. S2A). Here, the $1^{\text{st}}$ diffracted order is selected. The 1D-OFC beam array is then relayed to the second deflector (AODY, IntraAction AOM-505AF1) which diffracts the 1D-OFC beam array in the $y$ direction ($-1^{\text{st}}$ diffracted order). It should be pointed out that due to the elongated apertures of the AODs, optical relays using cylindrical telescopes ($\text{CL}_{x1,x2,y1,y2}$) for independent demagnification and magnification in $x$ and $y$ directions are placed between the two deflectors. Spherical telescopes can potentially be used to relay the laser beams from AODx to AODY if AODs with circular apertures are selected. Details about how to design the multitone RF signals for the AODx,y and generate a 2D-OFC beam array without frequency overlapping in the frequency domain can be found in S3 and S4.

The generated 2D-OFC beam array with angular dispersion is then imaged onto a reflection-mode high-speed spatial light modulator (HS-SLM, Meadowlark Optics A512-P8, 512 × 512
pixels, 15 μm pixel size), through a lens (L4, 300 mm focal length) and a PBS (PBS3). Thanks to the fast response of the ferroelectric liquid crystal, this HS-SLM can operate at an average speed of >1 kHz when working in binary-phase mode (9). It should also be noted that, the speed of the SLM is not a limiting factor for wavefront shaping systems, as state-of-the-art SLMs can reach a refresh rate of >100 kHz (13). To match each sub-beam of the 2D-OFC beam array with the pixels of the HS-SLM, the HS-SLM is imaged by a camera (C1, PCO-TECH pco-edge 5.5) through a beam splitter (BS1) associated with a telescope (not shown in Fig. S2A). The inset beside C1 sketches the distribution of the 2D-OFC beam array on the HS-SLM. Depending on the physical occupancy of the sub-beam, a pixel here can encompass a binned group of HS-SLM pixels. The reflected beam array is subsequently relayed to the back aperture of an objective lens (OBJ1, Newport M-20×) through a demagnification telescope (L5 and 6, 300 and 80 mm focal lengths, respectively), which is followed by the scattering medium (SM) (and sample, if 2D imaging through the SM is to be performed). The scattered light (before the corrected phase map is applied to the HS-SLM) or spatiotemporal focus (after the corrected phase map is applied to the HS-SLM) is collected by another objective lens (OBJ2, Newport M-20×).

For wavefront measurements, the collected scattered light is combined with a reference beam through a beam splitter (BS2). The reference beam is frequency-upshifted by 40 MHz through an acousto-optic frequency shifter (AOFS, IntraAction). A PD (Thorlabs APD430A2) probes the beating signal at a targeted position. The beating signal is sent to a high-speed signal processing unit, including a data acquisition (DAQ, AlazarTech ATS9360, 12 bit, 1.8 GS/s) card and a graphics processing unit (GPU, NVIDIA GeForce GTX 1080 Ti). The FEST focusing system is controlled by a customized high throughput C++ algorithm, with details given in S7. The whole process, including the wavefront measurement and the generation of conjugated phase map, takes only ~560 μs, including 93 μs for beating signal acquisition and transferring data to GPU, 39 μs for fast Fourier transformation (FFT), 51 μs for phase calculation, 319 μs for the generation of the conjugated phase map, and 59 μs for data transfer from GPU to central processing unit (CPU). The latency time of the current C++ program can be further improved, particularly the time consumed by generating the conjugated phase map. Based on application requirements, the control system can be configured in two working modes, i.e., single or continuous measurement through manual or external triggering, respectively.
In order to provide an option for generating a spatial focus that is temporally steady, in the playback process, another beam (named as option beam in Fig. S2A) is introduced with a matched beam size through a telescope (L7 and 8, 60 and 300 mm focal lengths, respectively, yielding a magnification factor of 5). This laser beam follows the same optical path as the 2D-OPC beam array after a beam splitter (BS3). It should be pointed out that, considering the fact that the option beam has a planar wavefront while the 2D-OFC beam array is slightly diverging, careful alignment or wavefront calibration is required to generate a bright focus (43). To perform fluorescence imaging through scattering media, a vertical setup for fluorescence detection (FD) is implemented, as shown in Fig. S2B.

Section S3. Design of the driving RF signals for the 2D-OFC generation

S3.1. Requirements of the frequency map

In this work, the 2D-OFC is generated by two orthogonally oriented AODs, which are driven by two independent channels of an arbitrary waveform generator. The first AOD (AODx) that generates frequency-encoded beams along the $x$ axis operates in the frequency range of 62 – 94 MHz. The second AOD (AODY) that generates frequency-encoded beams along the $y$ axis operates in the frequency range of 32 – 64 MHz. Both AODs have the same bandwidth of $B = \sim 32$ MHz.

Without loss of generality, we assume the 2D-OFC has a dimension of $M \times N$, and in addition, the frequencies over the bandwidths of AODx,y are equally spaced along $x$ and $y$ directions, respectively. Moreover, the 1$^{\text{st}}$ and $-1^{\text{st}}$ diffracted orders are used for AODx and AODY, respectively. As a result, the optical frequency component $f(i,j)$ that locates at the $i$-th column and $j$-th row is

$$f(i,j) = f_0 + \left(62 + \frac{B}{M} (i-1) - 32 - \frac{B}{N} (j-1)\right) \text{MHz} = f_0 + \left(30 + B \left(\frac{i-1}{M} - \frac{j-1}{N}\right)\right) \text{MHz}$$

(S1)
Here, \( f_0 \) is the optical frequency of the incident light. To encode spatial information with different spectral modes, every frequency component in the 2D-OFC must have its own unique frequency. Moreover, it is also required to maximize the spacing between any two frequency components due to the following two considerations. First, the smallest frequency spacing sets the upper bound of the instantaneous linewidth of the spectral modes. Second, a longer time-domain signal is needed to resolve a smaller frequency spacing. Ideally, if we can freely adjust the frequency of each component, these \( M \times N \) components can be equally distributed to occupy the entire bandwidth of \( 2B \). In this ideal situation, the smallest frequency spacing between any two frequency components is \( 2B/(MN) \). However, we can only control the frequency components in the first column and first row by properly selecting \( M \) and \( N \). Once \( M \) and \( N \) are determined, all the remaining frequencies in the 2D-OFC are set according to Eq. (S1).

### S3.2. Choices of \( M \) and \( N \)

Equation (S1) shows that \( M \) and \( N \) cannot be the same, otherwise multiple combinations of \((i,j)\) share the same frequency for the same \( i-j \). We also note that, if \( M \) and \( N \) share a common divisor \( K (\geq 2) \), the frequency components labeled as \((M/K + 1, N/K + 1)\) have the same frequency as the first frequency component \((1,1)\), i.e.,

\[
\begin{align*}
  f(M/K + 1, N/K + 1) &= f_0 + \left(30 + B \left(\frac{M/K+1-1}{M} - \frac{N/K+1-1}{N}\right)\right) \text{MHz} = f_0 + 30\text{MHz} \quad (S2) \\
  f(1,1) &= f_0 + \left(30 + B \left(\frac{1-1}{M} - \frac{1-1}{N}\right)\right) \text{MHz} = f_0 + 30\text{MHz} \quad (S3)
\end{align*}
\]

Therefore, \( M \) and \( N \) must be coprime numbers. In the following, we show that coprime numbers of \( M \) and \( N \) are indeed the sufficient condition to guarantee that all the frequency components in the 2D-OFC are different. For any two distinct frequency components \((i,j)\) and \((k,l)\), the frequency spacing is given by

\[
\Delta f = |f(i,j) - f(k,l)| = B \left|\frac{(i-k)}{M} + \frac{(l-j)}{N}\right| = \frac{B}{M} |(i-k) + \frac{M}{N} (l-j)| \quad (S4)
\]
From Eq. (S4), it can be easily seen that, for \((i, j) \neq (k, l)\), \(\Delta f\) is always nonzero if \(M\) and \(N\) are coprime numbers, which means that \(\frac{M}{N}\) is non-integer.

Having determined that \(M\) and \(N\) must be coprime numbers, we will then discuss the smallest frequency spacing between any two frequency components for various combinations of \(M\) and \(N\), and we assume \(M < N\) for the rest of discussions.

Furthermore, with certain amount of mathematics, it can be directly shown that the smallest frequency spacing is always \(B_{MN}\). In this work, we chose \(N = M + 1\). The above analytical analysis can be further validated using numerical simulations.

**S3.3. Properties of the 2D optical frequency comb**

In this section, we discuss the properties of the 2D-OFC. There is a total number of \(M \times N\) discrete frequency components, as shown in **Fig. S3A**. The lowest frequency component is located at the bottom left corner, while the highest frequency component is located at the top right corner. We use short arrows to indicate the frequency components in ascending order. Next, we count the number of frequencies with a frequency spacing equal to \(B_{MN}\). This frequency map shows that all the short arrows pointing to the lower right direction, drawn in red, represent a frequency spacing of \(B_{MN}\). The number of these arrows is given by \((M - 1) \times (N - 1) = M(M - 1)\), where \(N = M + 1\). Besides these short arrows, a special long arrow, also drawn in red, connecting \((M, N)\) and \((1,1)\), also has a frequency spacing of \(B_{MN}\). As a result, the total number of frequencies with a frequency spacing equal to \(B_{MN}\) is \(M(M - 1) + 1\). This analytical expression is also numerically validated.

As a concrete example, for a 2D-OFC generated in this work (**Fig. 2A**), i.e., \(M = 55\), \(N = 56\), and \(B = \sim 32\) MHz, the smallest frequency spacing is \(32\) MHz/\((55 \times 56) = 10.39\) kHz. In the frequency domain, i.e., when all the frequencies are sorted in an ascending order, the number of frequencies with a frequency spacing equal to this value is 2971. This means that 96.5% of them have a frequency spacing equal to 10.39 kHz, while only 3.5% have other values, see **Figs. 2F**
and $G$. The result indicates that the frequency components are quite uniformly distributed, which is remarkable for 2D-OFC applications.

We now discuss the frequency spacings of other values. Starting from the bottom left corner, the arrow between $(1,N)$ and $(1,N-1)$ represents a frequency spacing of $\frac{B}{N}$. Given that the frequency map is symmetric, another arrow at the top right corner between $(M,2)$ and $(M,1)$ also represents a frequency spacing of $\frac{B}{N}$. Following the same strategy, it can be found that there are always two arrows at symmetric positions that represent each of the frequency spacings $\frac{B}{MN}(M-1), \frac{B}{MN}(M-2), \ldots, \frac{B}{MN}2$.

**S3.4. Discussion on other designs of 2D optical frequency combs**

An intuitive design is to use a wide band but coarse frequency spacing for the $x$ direction and a narrow band but fine frequency spacing for the $y$ direction, i.e., like a typical configuration of raster scanning. Ideally, this design can generate a 2D-OFC with an even distribution of frequency components through simple math. **Fig. S3B** shows the schematics of such a 2D-OFC with the same dimensions as the one discussed in **Fig. S3A**. Again, the lowest frequency component is located at the bottom left corner, while the highest frequency component is located at the top right corner. Long red arrows are used to indicate the frequency components in ascending order. Although both AODs can support a bandwidth of $B$, in this design, we have $B_x = B$ and $B_y = B/M$. As a result, the frequency spacing between the nearest two frequency components is $(B_x + B_y)/(MN - 1) \approx B/MN$, which is the same as the one in **Fig. S3A**. Therefore, such a standard design does not bring any advantages compared to the design used in this work in the frequency domain, and the bandwidth of the vertical direction is largely wasted. However, this design (**Fig. S3B**) has several drawbacks from an engineering perspective. First, the divergence of the sub-beams along orthogonal directions are deviated by a factor of $N$, which leads to an unbalanced rectangular shape with an $x$-$y$ ratio of $N$. This 2D-OFC pattern is undesirable for standard SLMs that typically have a square aperture. Second, the neighboring frequency components along the $y$ direction are difficult to resolve, since the divergence angle along the $y$ direction is only $\theta_y = (B / MN)(\lambda/v)$, while it is $\theta_x = (B / M)(\lambda/v)$ along the $x$ direction, where $\lambda$ is the optical wavelength of the laser beam, and $v$ is the velocity of the acoustic wave. In contrast, for the design in **Fig. S3A**, the divergence angles between
neighboring frequency components are similar along the $x$ and $y$ directions, i.e., $(B/M)(\lambda/\nu)$ and $(B/N)(\lambda/\nu)$, respectively. Third, this design is difficult to implement when the size of the 2D-OFC is large, e.g., $999 \times 1000$. It is because that the frequency resolution is typically sub-MHz for standard AODs with incident apertures of mm. Although the frequency resolution can be scaled down with an increasing incident aperture (or beam size), a large beam size, which can be up to tens of cm for a sub-kHz frequency resolution, is extremely challenging to handle for optics.

Section S4. Generation of the 2D-OFC beam array

S4.1. Theoretical analysis

As shown in Fig. S1A, the incident laser beam is successively modulated by two orthogonal AODs in the $x$ and $y$ directions, respectively. Cylindrical telescopes are employed to relay the laser beams between the AODs. Following the deflection resolution of AODs (44), we here define a 2D time-bandwidth product (TBP$_{2D}$), i.e.,

$$TBP_{2D} = TBP_x \times TBP_y = \frac{D_x B_x}{v_x} \times \frac{D_y B_y}{v_y} = \tau_x B_x \times \tau_y B_y$$

(S5)

where, $D_x$ and $D_y$ are the diameters of the laser beam entering the AODs, $B_x$ and $B_y$ are the RF bandwidths ($\sim 32$ MHz in this case), $v_x$ and $v_y$ are the velocities of the acoustic waves propagating in the AODs ($\sim 3630$ m/s in this case), $\tau_x$ and $\tau_y$ are the durations that the acoustic waves take to traverse the incident laser beams. The highest deflection resolution, or the largest number of resolvable spots when the aperture is fully filled by the laser beam (i.e., $<7$ mm diameter), is $<60$ in this case, see Fig. 2A. It is worth noting that, the AODs used in this work, which have elongated apertures, are not originally designed for laser beam deflection, as shown in the bottom inset of Fig. S2A. As a result, the deflection resolution of the current FEST focusing system is only restricted by the limited performance of the AODs, which can be potentially improved by replacing them with better ones. The state-of-the-art AODs, e.g.,
Brimrose TED20-100-50-532 and ISOMET LS110A-VIS, can provide a TBP of up to 1,000, implying considerable room for improvement.

The incident laser beam travels across the acoustic wave that propagates in the acousto-optic crystal, and the optical frequency \( f_{\text{laser}} \) of the laser beam undergoes a Doppler shift with the amount of frequency shifting equal to the acoustic frequency \( f_{\text{sound}} \) when only the 1\(^{\text{st}}\) (or \(-1\)^{\text{st}}) diffracted order is considered. In addition, the deflected laser beam exits the AOD at an angle with respect to the incident angle, i.e.,

\[
\theta = \pm \lambda f_{\text{sound}} / v
\]

Here, \( \lambda \) is the optical wavelength of the laser beam, and \( v \) is the velocity of the acoustic wave. The sign \( \pm \) means that the optical frequency can be up-shifted or down-shifted, i.e., corresponding to the 1\(^{\text{st}}\) or \(-1\)^{\text{st}} diffracted orders, respectively, depending on the orientation of the laser beam with respect to the sound field.

**S4.2. Experimental implementation**

The above theoretical analysis relies on the assumption that acousto-optic modulators are linear units. In practice, considerable third-order intermodulation always exists, which may be challenging in our case, especially where tens of frequency components are generated simultaneously. Nonetheless, this phenomenon does not present side effects on FEST: 1) if the first- and third-order intermodulation sub-beams have exactly the same frequency and propagation direction, they can be treated as one synthetic sub-beam, which does not have any effects on FEST applications; 2) if the third-order intermodulation sub-beams that have distinguishing modulation frequencies and propagation directions are detectable, they can even be treated as new synthetic beams and useful for FEST applications. To drive the AOD\(x,y\) operating at center frequencies of 50 and 80 MHz, respectively, multitone RF signals are generated by an arbitrary waveform generator (Rigol DG4162). The arrangement of the frequency components has been specifically designed to avoid frequency overlapping in the
frequency domain, see S3. In addition, the initial phases of the frequency components are also engineered to minimize the peak-to-average amplitude ratio, as shown in Fig. S4.

As the incident angle of the input laser beam is fixed, degraded diffraction efficiencies across the deflected laser beam array are expected. In practice, the diffraction efficiency usually decreases as the RF frequency moves away from the center frequency, i.e., the frequency providing the highest diffraction efficiency at this fixed incident angle. This degrading is particularly eminent for those frequencies at the edges of the multitone RF signal. Thus, the amplitudes of the frequency tones need to be readjusted to provide uniform intensities over the beam array. To this end, the amplitudes of the RF tones are separately optimized for AODx and AODy, as shown in Figs. S5 and S6, respectively. In short, a trial multitone RF signal with almost-uniform amplitudes (Figs. S5A and S6A) is first applied to the AODs, and the generated diffraction beam arrays are captured by the camera (Figs. S5B and S6B). The diffraction efficiencies are subsequently calculated from the camera images, as shown in Figs. S5C and S6C. We then divide the trial amplitudes of the RF tones by the square root of the obtained diffraction efficiencies to obtain the optimal multitone RF signal. This operation assumes that the AODs operate in the linear region. With the optimal multitone RF signals, we can generate frequency-encoded beam arrays with very uniform intensities in each direction, as shown in Figs. S5D–F and S6D–F. Finally, by driving the AODx,y simultaneously, 2D-OFC laser beam arrays with very uniform intensities are obtained, as illustrated in Fig. 2A–C. After passing through two consecutive AODs, the diffracted pattern contains ~6% of the total energy of the initial input light (see Fig. S7A).

**Section S5. Phase curvature of the 2D-OFC beam array**

The imperfect optical alignment, as well as the conditions of AODs, can introduce a phase curvature across the 2D-OFC beam array, which however is deterministic. To illustrate, we here use a 35 × 36 2D-OFC beam array, as shown in Fig. S7A, which is generated by setting the RF tones with consistent initial phases, as well as the HS-SLM. Fig. S7B shows a typical phase map measured by the FEST focusing system when no scattering medium is presented. Although such a phase curvature can be digitally compensated, it is not necessary during experiments, since the FEST focusing system has treated this phase curvature as a part of the scattering medium.
Section S6. Mathematical description of the FEST focusing system

Each sub-beam in the generated 2D-OFC beam array has a unique frequency and propagation direction. As a result, at the surface of the HS-SLM, each sub-beam is spatially separated, and its temporal light field is given by

\[ E_{m,n}(x_m, y_n, z_0, t) = V_{m,n} \exp\left[i2\pi(f_0 + f_{m,n})t + i\theta_{m,n}\right] \]  \hspace{1cm} (S7)

which is the light field as a function of space \((x_m, y_n, z_0)\) and time \(t\). Here, \(m = 1, \cdots, M\) and \(n = 1, \cdots, N\) are the labeling of each sub-beam. \(V_{m,n}\) denotes the amplitude of the sub-beam. \(f_0\) is the optical frequency of the kHz laser, and \(f_{m,n}\) is the amount of frequency shift imposed by the AOD\(x,y\). \(\theta_{m,n} = k_{m,n} \cdot (x_m, y_n, z_0)\) is the space-dependent phase term due to the angular dispersion experienced in the AOD\(x,y\). This phase term has a fixed value for each sub-beam, which can be determined through a single calibration process (S5). After the HS-SLM, these sub-beams are scrambled by a scattering medium in both space and time domains. By modeling the scattering process as a transmission matrix, the light field at the position of the photodetector \((x_D, y_D, z_D)\) becomes

\[ E_{S}(x_D, y_D, z_D, t) = \sum_{m=1}^{M} \sum_{n=1}^{N} A_{m,n} \exp(i\varphi_{m,n}) E_{m,n}(x_m, y_n, z_0, t), \]

\[ = \sum_{m=1}^{M} \sum_{n=1}^{N} A_{m,n} \exp(i\varphi_{m,n}) V_{m,n} \exp[i2\pi(f_0 + f_{m,n})t + i\theta_{m,n}] \]  \hspace{1cm} (S8)

Here, \(A_{m,n} \exp(i\varphi_{m,n})\) is the transmission matrix element, with \(A_{m,n}\) and \(\varphi_{m,n}\) representing the amplitude and phase modulations experienced in the scattering process. To extract the phase information of \(E_{S}(x_D, y_D, z_D, t)\), the time-variant speckle light is then combined with a reference beam, whose electric field is
\[ E_R(x_D, y_D, z_D, t) = V_R \exp[i2\pi(f_0 + f_r)t] \tag{S9} \]

Here, \( V_R \) denotes the amplitude of the reference beam, which oscillates at a frequency of \( f_0 + f_r \).

The intensity of the combined light becomes

\[
I(x_D, y_D, z_D, t) = [E_S(x_D, y_D, z_D, t) + E_R(x_D, y_D, z_D, t)][E_S(x_D, y_D, z_D, t) + E_R(x_D, y_D, z_D, t)]^* \\
= \sum_{m=1}^{M} \sum_{n=1}^{N} |A_{m,n}V_{m,n}|^2 + |V_R|^2 \\
+ 2 \sum_{m=1}^{M} \sum_{n=1}^{N} A_{m,n}V_{m,n} \cdot V_R \cos[2\pi(f_m - f_r)t + (\varphi_{m,n} + \theta_{m,n})] \\
+ 2 \sum_{m=1}^{M} \sum_{n=1}^{N} \sum_{m'=1}^{M} \sum_{n'=1}^{N} A_{m,n}V_{m,n} \cdot A_{m',n'}V_{m',n'} \cos[2\pi(f_m - f_{m'})t + (\varphi_{m,n} - \varphi_{m',n'})] \\
\tag{S10}
\]

In Eq. (S10), the first two terms are the direct currents (DCs) from the scattered light and reference light, respectively, which do not depend on time. The third term represents the beating signals between the scattered light and reference light, while the fourth term represents the self-beating signals among different frequency components of the scattered light. If we set \( V_R \gg A_{m,n}V_{m,n} \), Eq. (S10) can be further simplified to

\[
I(t) = I_{DC} + 2 \sum_{m=1}^{M} \sum_{n=1}^{N} A_{m,n}V_{m,n}V_R \cos[2\pi(f_m - f_r)t + (\varphi_{m,n} + \theta_{m,n})] \tag{S11}
\]

The combined light is received by a fast photodetector and is then digitized by a high-speed DAQ card. The digitized signal is finally sent to a GPU for high-throughput phase calculation. Through FFT, the signal is transformed into the frequency domain \( F(f) \) with \( M \times N \) distinct peaks located at the frequencies of \( |f_m - f_r|, m = 1, \ldots, M \) and \( n = 1, \ldots, N \). As a result, the phases \( \varphi_{m,n} \) can be calculated as

\[
\varphi_{m,n} = \text{Atan2} \left( \text{imag} \left( F(|f_m - f_r|) \right), \text{real} \left( F(|f_m - f_r|) \right) \right) - \theta_{m,n} \tag{S12}
\]
Here, \( \text{Atan2}(\bullet) \) is the four-quadrant inverse tangent operation, while \( \text{imag}(\bullet) \) and \( \text{real}(\bullet) \) are the operators that take the imaginary and real parts of a complex number, respectively.

**Section S7. C++ control program**

A customized software program for real-time data acquisition, FFT, phase calculation, conjugated phase map formation, as well as HS-SLM control is created using the C++ and the Qt framework. The beating signal is first sampled and digitized using a high-speed DAQ card and then transferred to the memory of a commercial GPU (NVIDIA GeForce GTX 1080 Ti). On the GPU, the data is first transformed into the frequency domain via FFT before a custom phase kernel extracts the desired phase values at each preselected frequency index simultaneously. Finally, the conjugated phase values are assembled into a bitmap image and transferred back into the host computer memory for display on the HS-SLM. Due to the massive parallelization offered by the large number of compute unified device architecture (CUDA) cores in a single GPU, the entire acquisition, transfer and computation process is completed in ~560 µs.

**Section S8. Effect of the laser linewidth on the wavefront measurement**

In the frequency domain, the idea of this work shares a similar concept as that of conventional optical frequency comb applications (25), but we further extend it to the spatial domain through acousto-optic diffraction. For precision single-shot wavefront measurements, the phase/frequency noise, which directly manifests in the laser linewidth, needs to be suppressed. Thus, the laser linewidth becomes essential for FEST focusing applications, especially when a dense optical frequency comb is generated to fully utilize the available RF bandwidth for frequency encoding. This section aims to describe numerical studies that can provide a qualitative understanding of how the laser linewidth influences the accuracy of wavefront measurements in a FEST focusing system. In the numerical studies, the linewidth of the CW laser is modified via a phase-diffusion model, where the spectral linewidth is treated as a key parameter (45, 46). This model generates a Lorentzian-shaped spectrum, which is a reasonable approximation of the optical spectrum of a laser beam with a limited bandwidth, e.g., the
ultranarrow linewidth laser used in this work. In the phase-diffusion model, the light field can be expressed as

\[ A(t) = A_0 \exp(i2\pi ft + i\phi(t)) \]  

(S13)

where \( A_0 \) is the amplitude, \( f \) is the optical frequency, and \( \phi(t) \) is the time-dependent small phase fluctuation with a zero mean, which is accumulated through a random fluctuation \( V_r(t) \). Then, the instantaneous frequency of the light field becomes

\[ f_i(t) = f + V_r(t) = f + \frac{1}{2\pi} \frac{d\phi(t)}{dt} \]  

(S14)

where the phase fluctuation \( \phi(t) \) is given by

\[ \phi(t) = 2\pi \int_{-\infty}^{t} V_r(k) \, dk \]  

(S15)

The random fluctuation \( V_r(t) \) can be modelled as a Gaussian white noise with a zero mean and a variance \( \sigma^2 \) that is related to the FWHM of the laser linewidth \( \Delta f \) and bandwidth \( BW \) of the simulation model, i.e.,

\[ \sigma^2 = \frac{\Delta f BW}{2\pi} \]  

(S16)

Using this phase-diffusion model, Fig. S8A shows the frequency spectra of CW lasers with varied linewidths. \( BW = 419 \) MHz is set for all situations. It can be clearly seen that the noise floor of the frequency spectrum increases with the laser linewidth. A frequency comb containing 3000 tones, ranging from \(~38\) to \(~101\) MHz, is then generated using this phase-diffusion model.
The initial phases of the frequency components are randomly assigned. The time-domain signal is then processed by using the same method as that of the FEST focusing system. Fig. S8B shows the histograms of the retrieved phase errors for different laser linewidths. Notably, the phase error becomes larger when the laser linewidth increases, e.g., the root mean square (RMS) phase error is \( \pm 0.2 \) rad for a laser linewidth of 10 kHz, i.e., approaching half of the frequency spacing; while it is \( <0.05 \) rad for a laser linewidth of 1 kHz, i.e., a similar laser linewidth used in this work (1.8 kHz).

Section S9. Accuracy and reliability of wavefront measurement using FEST technology

The long-term measurement of the absolute wavefront can be influenced by environmental vibration or thermal change, since they produce perturbations of the optical paths of the 2D-OFC beam array and reference beam, independently. In Figs. S9A and B, we showcase the phase evolutions of two sub-beams with frequencies \( f_2 \) and \( f_1 \) that are far apart in the 2D-OFC beam array (Fig. S9A, 23 \( \times \) 24 size). As shown in Fig. S9B, surprisingly, although the absolute phases with respect to the reference beam fluctuate, the relative phase between these two sub-beams is stable, i.e., the red curve in Fig. S9B. This observation is because the 2D-OFC beam array and reference beam propagate in different optical paths, while the sub-beams of the 2D-OFC beam array pass through the same optical path and fluctuate as a whole. Thus, relative phase maps are used in all FEST focusing demonstrations. It should please be noted that, in addition, although it is not an issue in this work, the environmental perturbation can be suppressed by employing stabilization systems with phase-locked loops (PLLs), which have widely applied to stabilize laser cavities. In the single-shot relative phase measurement, the result shows a root mean square (RMS) fluctuation of 4.7% (Fig. S9C), while it can be reduced to 0.5% when averaging is applied (Fig. S9D).
Fig. S1. Ultranarrow linewidth fiber laser and its basic performances. (A) Schematic diagram of the ultranarrow linewidth laser and its second-harmonic generation (SHG). FBG1,2: fiber Bragg gratings, 0.4 and 0.08 nm bandwidths, respectively. YPF: highly ytterbium-doped phosphate fiber. WDM: wavelength-division multiplexing coupler. LD1: single-mode fiber laser diode, 400 mW maximum power. LD2: multimode fiber laser diode, 9 W maximum power. LD3,4: multimode fiber laser diodes, 32 W maximum power. ISO1,2: isolators. CMS1,2: cladding-mode strippers. YF1,2: polarization-maintaining double-cladding ytterbium-doped fibers, 11/125 and 12/125 µm core/inner cladding sizes, respectively. PSC1,2: pump & signal combiners. BPF: bandpass filter. OC: optical coupler. L1: 20x objective lens. L2,3: lenses, 125 mm focal length. PPLN: periodically poled lithium niobate. DM: dichroic mirror. BD: beam dump. (B) Spectrum of the ultranarrow linewidth fiber laser, measured via the self-heterodyne method. (C) Relative intensity noise measurement. (D) Phase noise measurement. The output power of the fiber oscillator, i.e., the gray area indicated in (A), is about 50 mW, while the final output power after two consecutive optical amplifiers is >30 W at the fundamental wavelength of 1064 nm.
**Fig. S2. Full configuration of the FEST focusing system.** (A) Full schematic diagram. The FEST focusing system mainly includes three optical paths, i.e., two-dimensional (2D) optical frequency comb (2D-OFC) beam array, reference beam, and option beam. The 2D-OFC beam array is the key for high-speed wavefront measurement, and spatiotemporal focus generation after addressing the conjugated phase map to the high-speed spatial light modulator (HS-SLM). The reference beam beats with the scattered 2D-OFC beam array, giving rise to the field information. The option beam, serving as an optional playback beam, is only used for the generation of a steady spatial focus if
necessary for specific applications. L1: 20× objective lens. L2,3: lenses, 125 mm focal length. L4,5: lenses, 300 mm focal length. L6: lens, 80 mm focal length. L7: lens, 60 mm focal length. L8: lens, 300 mm focal length. CLx1,2: cylindrical lenses in the x direction. CLy1,2: cylindrical lenses in the y direction. M1,2: broadband dielectric mirrors. PPLN: periodically poled lithium niobite crystal. SHG: second-harmonic generation. SM: scattering medium. λ/2: half-wave plate. PBS1–3: polarizing beam splitters. AODx,y: acousto-optic deflectors in x and y directions. AOFS: acousto-optic frequency shifter. BS1–3: non-polarizing beam splitters. OBJ1,2: 20× objective lenses. S: high-speed shutters. FD: fluorescence detection. PD: broadband photodetector. DAQ: data acquisition. GPU: high-speed graphics processing unit. C1,2: cameras. (B) Experimental setup for fluorescence detection. The fluorescence signal is collected by inserting a dichromatic mirror (DM, Semrock FF556-SDL01-25×36), which vertically reflects the fluorescence signal to a photomultiplier tube (PMT, Hamamatsu Photonics H10721-20, 8 mm sensor diameter). An iris diaphragm is placed in front of the PMT to further suppress the excessive fluorescence background signal generated by the scattered light. BPF: bandpass filter. L9: lens. Note that, the red dotted area is light-shielded in a cage system. (Photo credit for the inset of (A): Xiaoming Wei)
**Fig. S3.** Design of the 2D-OFC spatial map when $N = M + 1$. (A) Current design. Red arrows represent frequencies separated by a spacing of $\frac{B}{MN}$ while black arrows represent frequency spacings of different values. (B) Raster scanning configuration. Long red arrows indicate the frequency components in ascending order.

**Fig. S4. Multitone RF signals.** (A) 55-tone time-domain signal with a random initial phase for each frequency. (B) 55-tone time-domain signal with a consistent initial phase for each frequency. Insets show the close-ups of the time-domain signals.
Fig. S5. Flatness optimization in the $x$ direction. Left column shows the RF signal loaded on the AODx (A), the generated 1D laser beam array (B) and the corresponding intensities of the spots across the 1D laser beam array (C) before flatness optimization. Note that, the amplitudes of (A) are originally set to be uniform, which exhibit variations after the function generator and RF amplifier. (D–F) Cases after flatness optimization.
Fig. S6. Flatness optimization in the \textit{y} direction. Top row shows the RF signal loaded on the AODy (A), the generated 1D laser beam array (B) and corresponding intensities of the spots across the 1D laser beam array (C) before flatness optimization. (D–F) Cases after flatness optimization.
Fig. S7. Typical phase curvature of the 2D-OFC beam array. (A) 2D-OFC beam array, 35 × 36 size. (B) Typical phase curvature.
Fig. S8. Effect of the laser linewidth on the FEST performance. (A) Spectra of the light field with different linewidths. The linewidth is controlled by using the phase-diffusion model. The dotted line shows the ideal spectrum without phase noise, i.e., $\sigma^2 = 0$. (B) Histograms of phase errors with different laser linewidths. The root mean square (RMS) values of the phase errors are 0.88, 8.93, 41.7 and 209 mrad for laser linewidths of 0, 0.1, 1 and 10 kHz, respectively. Note that, different bin widths are used for better visualization, i.e., 0.2, 1, 4 and 12 mrad, respectively.
Fig. S9. Relative phase measurement. (A) 2D-OFB beam array (23 × 24), where two frequency-encoded sub-beams are selected for the long-term phase measurement. (B) Evolutions of the absolute phases (with respect to the reference beam), and the relative phase between these two sub-beams. (C) Evolution of the relative phase in the single-shot measurement. (D) Evolution of the relative phase in the averaging measurement.
Fig. S10. Scanned images of the resolution target through the scattering medium using FEST focusing technology. (A–D) Images of groups 5 and 6 with corrected (A, C) and random (B, D) phase maps addressed to the HS-SLM. (E) Line-scan profiles as indicated by the blue dotted lines in (C) and (D), respectively. Note, intensities of images (A–D) have been normalized to their own maxima, while the line-scan profiles in (E) have been normalized to the maximum of (C) to show the intensity difference between the cases with and without the spatiotemporal focus. As can be observed from (E) (the blue dotted circle), element 6 of group 6 is resolved, indicating a focal spot size of less than 4.38 µm.
Movie S1. Principle of FEST focusing technology. This movie illustrates how FEST focusing works, including steps of single-shot wavefront measurement, 2D conjugated phase map generation and spatiotemporal focus formation.

Movie S2. Spatiotemporal focusing. This movie shows the spatiotemporal focus generated by FEST focusing. It was captured at a frame rate of 500 MHz.