FLC-SLM based iterative approach for formation of multiple complex structures simultaneously in 3D volume through tissue media

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Abstract

Complex structures formation and fast focusing of light inside or through turbid media is a challenging task due to refractive index heterogeneity, random light scattering and speckle noise formation. We propose a weighted mutation-assisted genetic algorithm (WMA-GA) with an R-squared metric based fitness function, that enhances the contrast, focuses light tightly and does fast convergence for both simple and complex structure formation through scattering media. As a compatible system with the binary WMA-GA, we present a fast, cost-effective, and robust wave-front shaping system design with an affordable ferroelectric liquid crystal (FLC) based binary-phase spatial light modulator (SLM). The introduced WMA-GA algorithm is integrated with the binary-phase based FLC-SLM. The developed wave-front shaping system is explored to focus light and construct multiple complex structures simultaneously in 3D volume through fresh tissue and scattering phantom. We have validated the WMA-GA in our prototype system with 120 grit, 220 grit, 450 grit, 600 grit ground glass diffusers and 323 µm, 588 µm, 852 µm thick fresh chicken tissues. The detailed results show that the proposed class of algorithm-backed integrated system converges fast with a higher contrast and enhances the signal-to-noise ratio. The WMA-GA assisted current system design can be used to make 2D/3D complex light structures inside and through real tissue media.

1 Introduction

Scattering of light or electromagnetic waves in living or non-living tissue and other disordered media is one of the primary challenges in biology, adaptive optics, deep tissue imaging, biomedical engineering and is a currently active research area in biomedical imaging, and medical science communities[1–10]. Focusing light through scattering media such as tissue has many applications in fluorescence imaging[8,11], live cell imaging, neuron excitation/imaging[12,13] and optical trapping[14]. Researchers over the globe are actively involved in overcoming the scattering problems faced in all forms of optical and other radiation based biomedical imaging[1,2,4,5,7–13]. Basic understanding of physical and biological systems instructs that the inhomogeneity of the refractive index in the medium, repeated random scattering and speckle noise due to the local interference of light causes the unavoidable distortion of the wavefront.

By modulating the incident wavefront, the effect of scattering can be countered to focus light through it[1,2,4,5,7]. Focusing light inside or through scattering media using SLM based wavefront shaping was initially demonstrated in experiment by Vellekoop et al. in 2007[15]. In that work, a step-wise sequential algorithm (SSA) was used to optimize the phase mask which was shown on the nematic liquid crystal SLM (NLC-SLM). Later, the same group in 2008 introduced the optimization of phase mask using continuous sequential algorithm (CSA) and partitioning algorithm (PA)[16]. Popoff et al. in 2010[17], introduced the transmission matrix (TM) based approach as an alternative algorithm.
to focus light through scattering media. Further, a number of research groups have demonstrated focusing light through stationary scattering media\textsuperscript{1,4,11,17–19}. In 2012, Stockbridge \textit{et al.} introduced focusing light through \textit{ex-vivo} chicken tissue which was considered as a dynamic scattering media\textsuperscript{20}. Imaging through scattering media using optical phase conjugation (OPC) was shown by Yaqoob \textit{et al.} in 2008\textsuperscript{21}. Further, digital optical phase conjugation (DOPC) was experimentally demonstrated by Cui \textit{et al.}\textsuperscript{22}, and by Hsieh \textit{et al.} in 2010\textsuperscript{23}.

During 2007 to 2012, wavefront shaping was mostly carried out using SSA, CSA, PA, transmission matrix, and DOPC techniques\textsuperscript{1,4,15–23}. In the presence of the high amount of environmental and instrumental noise, CSA and SSA have slow convergence because it is difficult to detect the variation in feedback signal due to mode-by-mode modulation, which results in initial measurement errors\textsuperscript{24}. Rising computational power in the last decade has enabled the use of machine learning and evolutionary/biological system based algorithms that require advanced hardware. It was found that optimization algorithms like genetic algorithm (GA) are well suited for the problem because of the huge solution space for the possible phase masks and this was first demonstrated by Conkey \textit{et al.} in 2012\textsuperscript{24}. Relevant studies have shown that genetic algorithms perform better in terms of enhancement compared to the previously introduced sequential algorithms (CSA, SSA), partitioning algorithm, and transmission matrix based approach, even in highly noisy environments\textsuperscript{24–26}.

Further improvements in the realm of genetic algorithm were done by introducing micro-genetic algorithm\textsuperscript{25}, genetic algorithm with signal-to-background ratio (SBR) discriminant\textsuperscript{27}, genetic algorithm with interleaved segment correction\textsuperscript{28} and four-element division GA\textsuperscript{26}. Other algorithms such as simulated annealing\textsuperscript{29}, particle swarm optimization (PSO)\textsuperscript{30,31}, gradient-assisted focusing\textsuperscript{32} and neural networks\textsuperscript{33} have also been introduced recently. Furthermore, neural network was combined with GA by Luo \textit{et al.} in 2020\textsuperscript{7}. However, all of the above algorithms were demonstrated using either a nematic liquid crystal SLM (NLC-SLM) or a digital micro-mirror device (DMD), and the realm of binary phase modulation with ferroelectric liquid crystal SLM (FLC-SLM) is still unexplored with well-suited evolution based algorithms. Despite the introduction of different types of algorithms, essential advanced hardware such as fast camera, high resolution phase-only SLM (NLC-SLM), or fast switching amplitude modulator (DMD) are still out of reach to most of the research groups due to lack of cost-effectiveness of these instruments.

Usually, digital micro-mirror devices (DMDs) have been used for fast focusing because they have a faster refresh rate ($\sim 23\text{kHz}$)\textsuperscript{34,35} and low latency. On the other hand, NLC-SLMs have high latency and low frame rate ($\sim 60\text{Hz}$)\textsuperscript{28,26,32,36}. However, DMDs can only achieve binary amplitude modulation which reduces the enhancement factor compared to the phase modulation achieved by either binary FLC-SLM (having 2 discrete phase levels) or NLC-SLM (having 256 discrete phase levels)\textsuperscript{36}. The theoretical enhancement factor of binary phase modulation with FLC-SLM is double compared to that of DMD, which can only do amplitude modulation\textsuperscript{36,37}. Furthermore, DMD based experimental setups are significantly complex. Due to its oblique reflection sensitivity, the alignment is difficult and DMDs cannot be used with high intensity pulsed lasers\textsuperscript{38,39}. For NLC-SLM, mandatory phase calibration is required, whereas FLC-SLM does not require any kind of phase calibration. Since, FLC-SLM operates in binary mode, it requires only 1 bit per pixel data transfer from PC to FLC-SLM, while NLC-SLM operates in 256 discrete levels of phase, so it requires 8 bits per pixel data transfer from PC to NLC-SLM. Theoretically, it means data transfer speed from PC to FLC-SLM is 8 times faster than NLC-SLM for a single colour channel. On the other hand, FLC-SLMs are a cost-effective alternative, provide fast binary phase modulation, do not require phase calibration, and provide more enhancement compared to DMDs. Use of FLC-SLM for focusing light in scattering media has been shown using DOPC based wavefront shaping technique\textsuperscript{36}. However, DOPC techniques have some unavoidable drawbacks. The first being that, the camera pixels and the SLM pixels must be in a near perfect match which makes alignments far more challenging\textsuperscript{22,40}. Furthermore, the SLM and camera has to be at the exact mirror conjugate
Focusing light inside or through scattering media is a result of a synergy between the feedback algorithm and the hardware. Optimization assisted by genetic algorithm depends on the crossover pattern, mutation operation and the right selection of fitness function, which have not been explored extensively in this domain. We have observed that weighted mutation plays an influential role in the genetic algorithm assisted feedback loop for enhancing the contrast and as an add-on effect, it turns out to be faster in computation compared to the standard genetic algorithm. In this context, we developed a weighted mutation assisted GA (WMA-GA) for optimizing the phase mask for fast convergence and enhancing contrast. In addition, Ferro-electric liquid crystals in FLC-SLM have a pixel switching response time of 40 µs with a refresh rate of up to 4.5 kHz at the present. In this work, we have explored the high speed features of FLC-SLM to develop the system for fast binary phase modulation and integrated it with the proposed WMA-GA for enhancing the contrast in fewer iterations and to reduce the computation time. To construct complex structures at high resolution, we have introduced an R-squared metric based fitness function in the proposed WMA-GA. The developed cost-effective system is demonstrated with FLC-SLM along with the WMA-GA algorithm to focus light and to construct multiple complex structures at different depths simultaneously in 3D volume. We have validated the algorithm and the prototype system with 120 grit, 220 grit, 450 grit, 600 grit ground glass diffuser and 323 µm, 588 µm, 852 µm thick fresh ex-vivo chicken tissue. The detailed results show that the proposed class of algorithm-backed integrated system converges faster with a higher contrast and enhances the peak-to-background ratio by a significant margin.

2 Results

2.1 Principle of proposed WMA-GA

The principle of proposed WMA-GA with the detailed computational steps is shown in flowchart (Fig. 1). The formulation of the WMA-GA as described in the flowchart has been supported by the mathematical equations (Eq. 6, 7, 8). The mathematical background of the standard GA and the development of the proposed WMA-GA is described in the following paragraph.

If an incoming light with field $E(d)$ transmits through an optical scattering media of transmission function $T(d, d')$ then the output complex field can be written as $E(d') = \sum d T(d, d') E(d)$. A transmission matrix $T$ of dimensions $M \times N$ models the wavefront scattering through disordered media. Here, $T$ is generated by a Gaussian complex random matrix. The equation for the calculation of output modes $M$ can be written as

$$E_m = \sum_n t_{mn} A_n e^{i\phi_n}$$

Where $A_n$ and $\phi_n$ are the amplitude and phase of the input mode $n$ respectively, $t_{mn}$ is a particular element of transmission matrix $T$. The standard GA starts with a population of random masks which undergo crossover followed by the mutation over iterations until the convergence criteria is met. For representing the complete output mode vector $\vec{E}_{out}$ in standard genetic algorithm, the input mode vector $\vec{E}_{in}$ can be written as a function of crossover ($r_c$) and mutation ($r_m$) as;

$$\vec{E}_{out} = T \vec{E}_{in}(r_c, r_m)$$

Here, $E_{out}$ is the complex field of the output mode and its amplitude is chosen as $A_n = 1/\sqrt{N}$, therefore the
Repeat till convergence
Step 1 (Eqn 6) : For \( W = 30 \% \), approximately 3 out of 10 off-springs are selected to mutate. Selection is done as per a uniform random distribution.

Step 2 (Eqn 7) : Selected masks undergo mutation specified by the mutation rate. Here for mutation rate \( r_m = 1/12 \), 3 pixels are undergoing mutation in the selected masks.

Step 3 (Eqn 8) : Final offsprings.

Figure 1: Flowchart of WMA-GA. The detailed step by step flowchart for the working principle of WMA-GA along with the demonstration of its weighted mutation process on the right side.

Intensity \( (I_m) \) at a particular output mode at the camera with the added noise can be written as;

\[
I_m = \frac{1}{N} \left| \sum_{n} l_{mn} e^{i\phi_n} \right|^2 + \delta \quad \text{where} \quad \delta = \Gamma \% \times \mathcal{N}(\mu, \sigma) I_o \quad (3)
\]

Here, a noise \( \delta \) is added to mimic experimental environment, \( \Gamma \) is the added noise percentage with respect to the initial average intensity \( I_o \), \( \mathcal{N}(\mu, \sigma) \) represents a random number generated from a normal distribution with mean \( \mu \) and standard deviation \( \sigma \).

We have observed in the case of binary phase modulation, that the pattern of crossover and weighted mutation plays a decisive role in optimizing the global as well as local solution. To explore its effect, we have introduced a weighting factor in the genetic algorithm which can represent a statistical sampling function for a pattern of crossover or mutation or both together. The resultant output mode vector with the statistical sampling function for weighted mutation \( (W_m) \) can be written as;

\[
\vec{E}_{\text{out}} = T \vec{E}_{\text{in}}(r_c, r_m, W_m) \quad (4)
\]

The output mode of the Eq. 4 depends on the weighted mutation and the modified algorithm is termed as ‘Weighted-mutation assisted genetic algorithm (WMA-GA)’. To generate the mask for the next iteration, a particular off-spring \( \vec{O}_{r_c, r_m} \) with parents \( \vec{P}_i \) and \( \vec{P}_j \) is generated by crossover with a uniform random binary vector \( \vec{x} \) and its conjugate \( \vec{x} \) respectively. This particular off-spring or phase mask can be written as;

\[
\vec{O}_{r_c, r_m}(r_c, \vec{P}_i, \vec{P}_j) = \vec{x} \cdot \vec{P}_i + \vec{x} \cdot \vec{P}_j \quad (5)
\]
Where, two parents $\vec{P}_i$ and $\vec{P}_j$ are randomly selected from the descending order of the phase masks ranked according to their fitness score. To generate a full set of off-springs $\mathcal{O}$, Eq. 5 has been followed for all selected parents, where $\bar{\mathcal{O}}_{\vec{P}_i, \vec{P}_j} \subseteq \mathcal{O}$. In our WMA-GA, every off-spring has not gone through mutation, but certain off-springs are selected by a weighting factor $W_m$ based on the Bernoulli sampling principle$^{42}$. For implementing the weighted mutation concept, we have adopted the Bernoulli sampling $\mathcal{B}$ to select a few off-springs from the entire set $\mathcal{O}$ with probability $W_m$ and the resultant subset of selected off-springs is denoted by $O^w$. And, it can be written as;

$$O^w = \mathcal{B}(\mathcal{O}, W_m) \subseteq \mathcal{O} \tag{6}$$

Where, $O^w \subseteq \mathcal{O}$, $W_m \in [0,1]$. Now the selected off-spring $\vec{O}_i^w$ goes through mutation, and it can be expressed as;

$$\vec{O}_M^w (r_m, \vec{z}_i) = (\vec{O}_i^w \cdot \neg \vec{z}_i (r_m)) + (\vec{z}_i (r_m) \cdot \neg \vec{O}_i^w) \tag{7}$$

Where, $\vec{z}_i (r_m)$ is a biased random binary vector [0,1] which is used to do the mutation of selected off-spring $\vec{O}_i^w$ with the current mutation rate $r_m$. Now, the new subset of off-springs or phase masks that have gone through mutation is reunited with the rest of the non-mutated phase masks, and it is defined as;

$$O_{new} = [\mathcal{O} \cap \neg O^w] \cup O^w_M (r_m, z) \tag{8}$$

The newly formed set of masks $O_{new}$ is again passed on to the SLM to measure the fitness. The above mathematical operations were implemented in simulation as well as experiment and the results are discussed in the following sections.

![Weighting factor vs enhancement plot](image)

**Figure 2: Weighting factor vs enhancement plot.** The comparison of fitness enhancement after 600 generations is shown for different values of weighting factor $W\%$.

### 2.2 Computer based simulation for verifying the performance of WMA-GA

The proposed algorithm was tested in a simulation model where different conditions and parameters are incorporated to mimic the experiment. Fig. 2 shows the comparison of fitness score enhancement with different weighting factors.
from 0% to 100% for focusing light tightly at a single spot. A general trend was observed that a lower weighting percentage results in more enhancement of the fitness function which was chosen to be the peak-to-background ratio (PBR). It shows that the standard GA performed poorly in terms of contrast enhancement. The best performing weighting percentage was lying between 0% and 25%, where the fitness score shows oscillation in nature around the maxima. Fig. 3 shows the comparison of fitness function improvement over the progress of iterations for focusing a single spot with different weighting factors $W = [0, 4, 8, 12, 14, 16, 20, 30, 50, 70, 90] \%$ and $W = 100\%$, where $W = 100\%$ represents the standard GA. With $W = 14\%$ the algorithm focused the light into a single spot the most tightly and converged approximately after 600 iterations with a fitness score of 1000. On the other hand, the standard GA progressed extremely slowly and reached a fitness score below 140 which was around 746% less than WMA-GA. Similar results were observed for focusing light at two spots (Fig. 4) where the weighting factor $W = 8\%$ performed better than the standard GA in both enhancement and fitness score. For focusing two spots, WMA-GA with $W = 8\%$ achieved up to 813% higher enhancement in just 600 iterations compared to the standard GA. Fig. 5 shows the initial intensity, the final focused single spot, and the dual spot images obtained with WMA-GA and Standard GA. Amongst both the cases, the proposed WMA-GA shows better background suppression and contrast enhancement in lesser number of iterations. The standard GA performed poorly for focusing light at both single spot and two spots cases where peak-to-background ratio does not improve even after 1000 iterations.

![Simulation results for 1 spot](image)

**Figure 3: Simulation results for focusing a single spot.** The figure shows the progress of fitness score v/s number of generations for focusing a single spot using WMA-GA with different values of weighting factor, $W = 0\%$, 4\%, 8\%, 12\%, 14\%, 16\%, 20\%, 30\%, 50\%, 70\% and 90\%. $W = 100\%$ represents the standard GA. The green colour curve on the left side show the fitness for the best performing WMA-GA with weighting factor $W = 14\%$ and the red colour curve show the standard GA. Curves on the right side show the progress of fitness for the intermediate weighting factors.
Figure 4: Simulation results for focusing two spots. The figure shows the progress of fitness score v/s number of generations for focusing two spots using WMA-GA with different values of weighting factor, $W = 0\%$, $4\%$, $8\%$, $12\%$, $16\%$, $20\%$, $30\%$, $50\%$, $70\%$ and $90\%$. $W = 100\%$ represents the standard GA. The green colour curve on the left side show the fitness for the best performing WMA-GA with weighting factor $W = 8\%$ and the red colour curve show the standard GA. Curves on the right side show the progress of fitness for the intermediate weighting factors.

Figure 5: Simulation results for focusing light through scattering media after 600 iterations. a. Focused single spot for Standard GA & WMA-GA, b. Focused two spots for Standard GA & WMA-GA. It is visible that, $W = 100\%$, i.e. the standard GA is not able to suppress background intensity compared to WMA-GA with $W = 14\%$ for single spot and $W = 8\%$ for two spots. The noise percentage added was 30\% of the initial average intensity to demonstrate the robustness of WMA-GA.

2.2.1 Fitness function and its impact on structuring light through scattering media

The fitness function in iterative optimization algorithms is an important parameter that is sensitive to the desired solution. In this work, we have noticed that the R-squared metric and the more commonly used peak-to-background ratio (PBR) as a fitness function at the region of interest (ROI) behave differently as per the complexity of the structure at the target location. Fig. 5 shows that a PBR fitness function performed well while focusing simpler structures like a single spot or dual spot. However, the PBR based fitness function was not able to resolve and equalize the intensity when the target image was a complex structure (Fig. 6a,b). To resolve complex structures, uniform intensity distribution across all the pixels at the target location is essential. In experiment, the PBR based fitness function completely fails to construct a complex structure like the alphabet letter A (see Fig. 6b). Fig. 6c,d show the histogram of the intensity distribution of the target area for both R-squared metric and PBR fitness functions. R-squared metric is a measure of variance between two datasets\textsuperscript{43} and it has been frequently used in machine learning and regression models\textsuperscript{44}. The R-squared metric is calculated over M samples and measured between two sets of
variables, camera image $I$ and the target image $S$ as follows;

$$
R^2 = 1 - \frac{\sum_{j=1}^{M}(I_j - S_j)^2}{\sum_{j=1}^{M}(I_j - \bar{I})^2}, \quad \text{where} \quad \bar{I} = \frac{1}{M} \sum_{j=1}^{M} I_j
$$

(9)

This R-squared coefficient value comes between 0 and 1. It quantifies the relationship between the movement of a dependent and an independent variable. The coefficient of 1 refers to a perfect matching among the two sets of data, and the value near 0 represents no linear relationship between the two sets of data. A more detailed analysis of the contrast enhancement and background noise suppression in the presence of varying noise percentage for standard GA and WMA-GA with PBR and R-squared fitness function has been given in supplementary section 2.

![Comparison between R-squared metric and peak-to-background (PBR) fitness functions.](image)

**Figure 6:** Comparison between R-squared metric and peak-to-background (PBR) fitness functions. a. Simulation results for focusing the letter A using PBR and R-squared metric fitness functions, b. Experimental results for focusing the letter A using PBR and R-squared metric fitness functions, c. Histogram of pixel intensity distribution in simulation for PBR and R-squared metric fitness functions, d. Histogram of pixel intensity distribution in experiment for PBR and R-squared metric fitness functions.

### 2.3 Characterization of experimental set up and formation of 2D/3D complex structures through biological tissue media

The detailed schematic of the experimental system with the various hardware building blocks, experiment tissue samples and the constructed 3D volume image is shown in Fig. 7. The system design consists of a master controller i.e the FLC-SLM hardware driver which is connected with the responders, the FLC-SLM’s micro-display unit and the arbitrary function generator which triggers both the cameras. The light from a He-Ne laser of wavelength 633 nm passes through a spatial filter and falls on the SLM. Thereafter, the modulated wavefront reflected from FLC-SLM passes through a set of optical components and falls on the scattering media. To facilitate the formation of multiple complex structures simultaneously at different depth in the 3D volume, a beam splitter is used to split the speckle field into two parts that are imaged by cameras placed at two different depths. One of the cameras is placed on a linear translation stage to visualize the 3D volume. Furthermore, a set of sequential hardware operational instructions are sent from the personal computer to the FLC-SLM display head and both the cameras to acquire the output speckle
field generated by the tissue sample.

The simulation results have been validated with the developed experimental setup, where the proposed WMA-GA algorithm was tested for its ability to focus complex 2D as well as 3D structures simultaneously. Commercial ground glass diffusers with different grit sizes (120 grit, 220 grit, 450 grit and 600 grit) have been used as a scattering media. Fresh chicken tissue samples of thickness 323 µm, 588 µm, and 852 µm have been used as a biological sample for demonstrating the robustness of the experimental setup and the WMA-GA algorithm.

Fig. 8 shows the progress of the fitness function for focusing a single spot after 700 generations for a 450 grit ground glass diffuser. The fitness function was taken as the peak-to-background ratio (PBR). The Standard GA achieved a fitness of 542 after 700 iterations while WMA-GA achieved the same in mere 75 generations. The maximum fitness score achieved by the WMA-GA with $W = 10\%$ was 1000, which is 185% higher than the standard GA. The fitness
score for intermediate weighting percentage has also been analyzed and was found to be improving with decreasing weight. It has also been observed that the algorithm is unstable below $W = 5\%$. Fig. 9 shows the initial intensity and the final focused spot images with different weighting percentages. It can be seen that a weighting factor of 10% produced a brighter spot at the focal plane which follows a similar trend observed in the simulation. Formation of single spot and complex patterns through different grit size ground glass diffusers for both standard GA and WMA-GA is shown in Fig. 10. For a single spot, WMA-GA has outperformed in intensity enhancement as well as background suppression. Standard GA is not able to focus complex structures like the alphabet letters A and O clearly while WMA-GA with $W = 10\%$ was able to form sharp A and O through all diffusers including 120 grit which is highly scattering. Fig. 10 equally shows that the background suppression of WMA-GA is superior to the standard GA by a significant margin.

The stability of the experimental setup has been demonstrated with chicken thigh tissue samples of thickness 323 $\mu$m, 588 $\mu$m and 852 $\mu$m. Fig. 11 shows the formation of complex structure through chicken tissues for the standard GA and the proposed WMA-GA with $W = 10\%$. Similar to ground glass diffuser, the WMA-GA outperforms the standard GA in complex structure formation, in both sharpness and background suppression. With the chicken tissue
Figure 10: Experimental results for focusing single spots as well as complex structures in a 2D plane using different grit size of ground glass diffusers. Comparison between WMA-GA and Standard GA is shown for a. 120 grit, b. 220 grit, c. 450 grit and d. 600 grit size.

Figure 11: Experimental results for complex structure formation in 2D space with using chicken tissues of different thickness. Comparison between WMA-GA and Standard GA is shown for chicken tissue with thickness a. 323 μm, b. 588 μm and c. 852 μm.

2.3.1 Simultaneous formation of multiple complex structures in 3D space through tissue

Multiple complex structures have been constructed simultaneously at different planes of 3D volume through ground glass diffuser of grit size 450 and fresh chicken tissue of thickness 565 μm. Fig. 12a shows the alphabet letters A and O constructed at different depths in 3D volume with a 450 grit ground glass diffuser as a scattering medium. Further, the same was also demonstrated through a real ex-vivo biological tissue. Fig. 12b shows the alphabet letters constructed at different depths in 3D volume with fresh chicken thigh tissue of thickness 565 μm. Fig. 12 shows that WMA-GA is more noise immune when forming complex structures. The axial distance between the two complex objects was 1.62 mm and the lateral distance was 266 μm.
3 Discussion

We have developed an FLC-SLM based cost-effective binary phase modulation system and, along with that, a weighted mutation-assisted genetic algorithm (WMA-GA). The fast pixel switching response time of FLC-SLM (40 µs), and a high refresh rate of 4.5 kHz makes it suitable for applications like tissue imaging, live cell imaging and photo-acoustic microscopy which require focusing through scattering media in real time. We have validated the algorithm and the prototype system as a single unit with 120 grit, 220 grit, 450 grit, 600 grit ground glass diffusers and fresh ex-vivo chicken thigh tissue of thickness 323 µm, 588 µm & 852 µm (Fig. 10, 11, 12). Our system shows better noise tolerance and achieves a higher contrast, as an additional outcome, it converges rapidly compared to the standard genetic algorithm (Fig. 3, 4, 8). This integrated system shows that it has the potential to focus light and construct multiple complex structures simultaneously at different depths in 3D volume through scattering media. Focusing light at different depths in 3D through scattering media has multiple potential applications in real-time 3D-confocal microscopy, 3D photacoustic microscopy and holography. However, the efficiency of the SLM decreases with multiple complex structures formation at different depths in 3D volume because the limited number of input modes get distributed to different planes. The system can equally be used for back reflection based focusing light in scattering media. A general trend has been observed that decreasing the weighting percentage improves the maximum achieved fitness score. In the experiment, the proposed WMA-GA shows the maximum fitness score when the weighting factor is around \( W = 10\% \). However, in simulation, the maximum fitness score comes at \( W = 14\% \). The detailed simulation and experimental observations show that the fitness function of WMA-GA is unstable and oscillating in nature when the weighting factor (\( W \)) tends to 0%. However, the best weighting value varies depending on the other parameters of the simulation model, existing noise, experimental hardware parameters and environmental conditions. Experimental noise is one of the major hindrances in enhancement of feedback based algorithms. Although the proposed algorithm performed 746% better enhancement than the standard GA in simulation, but in experiment the enhancement of the algorithm was 185% better than GA. The decreased enhancement in the experiment can be attributed to additional noise apart from the camera noise present in the experiment, such as temperature fluctuations, micron/sub-micron size dust particle movement, airflow dynamics, and mechanical vibrations. To mitigate the noise coming from the above factors in the experiment and to keep our analysis unbiased, a 4-level isolation system has been built around the prototype experimental setup. The 4-level isolation system consists of a floating table, optics-grade curtains, acrylic boxes and a transparent thin film.
Though DMDs have the highest refresh rate (up to 23 kHz) amongst all the digital light modulators, the usable frame rate is limited to a few hundred hertz due to the data transfer speed of the hardware\textsuperscript{35,36}. Despite having a lower refresh rate of 4.5 kHz, due to the limited data transfer rate, FLC-SLMs show equal potential to achieve similar results with a double enhancement factor. Camera being the slowest component in the setup, consumes more than 95% of the overall processing time. However, the delay in data transfer can be reduced drastically by using a multi-channel data transfer protocol like CoaXPress. A camera-free setup can also be developed to increase the acquisition speed to form 3D volumetric complex structure. A faster acquisition speed will further reduce the number of iterations required to reach convergence as it will nullify the noise generated due to beam shift, temperature fluctuations and the change in response of the camera sensor. The above advantages combined with its cost effectiveness makes the system more viable for real time focusing compared to DMD and NLC-SLM based setups which are expensive.

4 Materials and methods

4.1 Computer based Simulation model to mimic the experiment

The simulation was designed in open-source Python 3 programming language and NumPy was used for processing the matrices. A matrix of dimensions $200 \times 200$ was taken as the modified input mode from the FLC-SLM which corresponds to $N = 40,000$ input modes ($\vec{E}_{in}$). Another matrix of dimensions $16 \times 16$ was considered as the output wavefront which corresponds to $M = 256$ output modes ($\vec{E}_{out}$). The transmission matrix $T$ of dimensions $M \times N$ was generated using a complex Gaussian random distribution ($\mu_T = 0$ and $\sigma_T = 0.1$) to mimic the scattering of light. Further, a 30% noise ($\delta$) was added to the output mode intensity to mimic the experimental conditions (Eq. 2).

At the beginning of the algorithm, a population $P$ of random binary phase masks is generated using a discrete uniform distribution of values 0 and 255 which correspond to 0 and $\pi$ phase respectively. The genetic algorithm then evolves the population to produce the desired result at the output mode (Fig. 1). A population size of 200 was chosen as it provided a good trade-off between speed and enhancement. Crossover rate ($r_c$) was kept at the standard value of 50% while the mutation rate was picked to be a generation dependant exponentially decaying function described in\textsuperscript{24}. The initial mutation rate was fixed at 1% which decays to 0.5% exponentially with a fixed decay rate of $\Lambda = 500$. A typical choice of mutation rate in genetic algorithms is below 5%\textsuperscript{45}. Unlike in the case of NLC-SLM, binary FLC-SLM takes only two phase values, 0 and $\pi$, which implies a mutation rate ($r_m$) of 99% is the same as 1% because 0 and $\pi$ can be redefined by changing the reference (details in Supplementary Sec. 3). The proposed WMA-GA with $W = 14\%$ converged after 600 generations, while the standard GA saturated at a much lower fitness score after 1000 generations (Fig. 3, 4).

4.2 Experimental system design with binary phase based FLC-SLM for proposed WMA-GA

The built system (Fig. 7, 13) uses a 12 mW He-Ne Laser (633 nm, Newport) with 500 : 1 linearly polarized light. Laser is mounted in such a way that polarization axis of light is aligned vertically. Two flat mirrors, $M_1$ and $M_2$ are are added for laser beam alignment. Along the path, a spatial filter system (Thorlabs, KT311/M) is placed consisting of a pinhole ($\phi = 25 \ \mu m$) and objective (20X, Numerical Aperture (NA) = 0.40) for eliminating the higher-order noise in beam. Thereafter, the spatially filtered diverging beam is collimated by a lens $L_1 (f = 250 \ \text{mm})$ to get a pure flat beam profile. The resultant beam is further guided to the FLC-SLM (ForthDD, SXGA-R5) via the polarising beam splitter (PBS). The PBS is mounted in such a way that incoming vertically polarized light is directed towards the SLM which
Figure 13: 3D schematic of experimental setup. Components: 1. He-Ne laser, 2. Mirrors $M_1$ & $M_2$, 3. Spatial filter, 4. Polarizing beam splitter (PBS), 5. FLC-SLM, 6a-6b. 4F setup, 7. 1st objective, 8. Scattering media, 9. 2nd objective, 10. Lens ($f = 50\text{ mm}$), 11. 50 : 50 Beam splitter, 12. CMOS camera-1, 13. CMOS camera-2, 14. Personal computer, and 15. Arbitrary function generator.

modulates the incident wavefront in reflection mode. The FLC-SLM rotates the polarization of incident light by an angle $\pm \theta$ after reflecting from the FLC-SLM pixels. After passing through PBS, the horizontal components have a spatial phase difference of 0 and $\pi$ relatively (Fig. 7a). The modified wavefront passes through a 4F setup and enters an objective(10X, NA = 0.25) that transmits the wavefront through the scattering media. The power of the incident beam just before the tissue sample was measured and found to be 0.7 mW. A second objective (10X, NA = 0.25) is placed behind the scattering media. An 8-bit CMOS camera (Thorlabs, DCC3260C) is used for imaging the plane and providing the feedback signal for the genetic algorithm. For simultaneous construction of multiple complex structures in 3D volume, a second camera (Basler acA800-510uc) is placed on a linear translator to get feedback from different depths.

The signal from the PC to the SLM driver module is sent via a video card. Each image is a combination of 24 bit planes i.e. 24 bit information per pixel and 8 bit per channel (RGB). The hardware module of the SLM splits the RGB signal into 24 single bit black and white images. These 24 single bit images are sent and displayed on SLM screen sequentially. So conclusively, a total of $24 \times 60 = 1440$ binary images are shown on SLM screen in 1 second. Each bit plane is displayed on SLM screen for a duration of 219.02$\mu$s. The hardware driver module of the SLM provides an electrical signal output that becomes high or low in synchronization with the display of each bit plane. This signal is passed on to the function generator to generate a new signal with +3 V voltage to trigger the two CMOS cameras.

4.3 Samples used in Experiment: Ground glass with different grit sizes and Fresh chicken tissue of different thickness

A fresh chicken (weight = 2.62 kg, age = 10 weeks, measured density = 0.92 g/cm$^3$) was procured from the local market. The whole thigh of the chicken is kept in fridge for 4 hours at constant temperature of $-14^\circ$C to facilitate the slicing. A sterilized surgical knife is used to cut chicken muscles into several slices. The measured thickness of
the cut chicken tissue slices came out to be 323\(\mu\)m, 588\(\mu\)m and 852\(\mu\)m. The sliced chicken muscle was sandwiched between two microscope glass cover slips. A drop of Glycerin were added to preserve the sample and to prevent it from drying.

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**Conflict of interest**

The authors declare no conflict of interest.

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