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一种非侵入式散射介质内聚焦方法

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摘 要：针对现有非侵入式聚焦技术存在计算复杂、抗噪性能低、优化速度慢等问题,提出一种基于波前整形的非侵入式散射介质内聚焦技术。该技术利用荧光目标的线性激发特性,通过最大化荧光散斑的强度和对比度的线性组合,利用空间光调制器优化外部输入激光的波前相位,实现输入光在散射介质内的聚焦。实验结果表明,该方法实现了散射介质内对单个目标粒子的聚焦,且具有收敛速度快、抗噪性强的特点,为复杂介质内的无创成像提供了一种新的实现途径,并有望在生命科学领域得到广泛应用。

关键词：聚焦;非侵入式;波前整形;荧光散斑;散射
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A Method on Non-invasive Focusing in Scattering Medium

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Abstract: The existing non-invasive focusing technology has the problems of complex calculation, low anti-noise performance and slow optimization speed. Therefore, a wavefront shaping-based method is proposed. Based on the linear excitation of fluorescence, by maxmizing the linear combination of the intensity and contrast of the detected fluorescent speckle in an appropriate contribution, a phase-only spatial light modulator is used to optimize the input light so as to realize the focusing in the scattering medium. The experimental results show that the proposed method not only realizes deep focusing on a single particle in the scattering medium, but also has a fast convergence speed and is insensitive to noise. Moreover, it provides a new way for non-invasive deep imaging in complex medium and is expected to be used in life science.

Key words: Focusing; Non-invasive; Wavefront shaping; Fluorencent speckle; Scattering
OCIS Codes: 290.5880; 290.5850; 290.5825

0 Introduction

Propagation of light in materials with inhomogeneous refractive index, such as biological tissues, is affected by multiple scattering, resulting in complicated interference patterns, known as speckle[1], which limits the application of conventional optical method and makes it hard to focus and image in such medium. Recent years, many efforts have been devoted to developing methods such as wavefront shaping and ghost imaging so as to enable focusing and imaging objects behind or inside scattering media[2-6]. In 2007, VELLEKOOP I M[7]

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optimized the input laser speckle by maximizing the input laser intensity in the scattering medium, to focus on a specified position in the scattering medium. However, most of them are invasive since they require either the presence of a detector[8-9] or a "guide star"[10] to be placed behind or within the scattering medium. In 2012, BERTOLLOTII J et al.[11] proposed an optical method which first verified the possibility of non-invasive imaging through the scattering medium. It realized the imaging of a fluorescent object hidden behind a thin scattering layer by collected the total fluorescence using a bucket detector, without access to the region behind the scattering medium. This method was later extended to noninvasive imaging of scattering media using spatially incoherent light and ordinary cameras[12]. While, since it relied on the speckle correlation, this technique is limited by optical memory effect. Based on the concept of non-invasive detection, KATZ O et al.[13] proposed a nonlinear wavefront shaping method to achieve focusing by maximizing two-photon fluorescence intensity. In this method, the nonlinear signal from the fluorescence object is obtained, and the maximum fluorescence is achieved when the excitation light is converged as closely as possible. However, the nonlinear signal needs to use ultrafast laser source, which is not suitable for continuous wave laser imaging. Since then, different wavefront shaping technologies based on linear focusing[14-15] used in linear fluorescence microscope technology, speckle correlation[16-17], and transmission matrix[18] have emerged in succession. However, these methods tried to converge the excitation light as closely as possible by monitoring the total fluorescence intensity resulting in a bad focus. In recent years, a linear focusing method using speckle variance optimization was proposed[19-20] which can solve the problem to some extend.

In order to realize non-invasive deep focusing in biological tissue, a new wavefront shaping-based method with the linear fluorescence is proposed in this paper. The new method takes the fluorescent particles as the target deep inside the scattering medium, and realizes the focusing on the target by maximizing the linear combination of the intensity and contrast of the detected fluorescent speckle in an appropriate contribution, and modulating the wavefront of the external input laser with a phase-only Spatial Light Modulator (SLM).

1 Principle

The principle of non-invasive focusing in scattering medium is shown in Fig.1. The laser source irradiates a SLM through the Beam Splitter (BS) and produces a new wavefront E_{exc} with the phase modulation of SLM. The new wavefront reaches the Scattering Medium (SM) through the Dichroic Mirror (DM), and generates random speckles (referred as excitation speckle) to illuminate the targets which are composed of multiple fluorescent particles in the scattering medium. The excited fluorescence is diffused by the the scattering medium in the backward propagation path and generates fluorescent speckle E_{fluo} which is received by the detector through the Dichroic Mirror (DM). By maximizing the feedback of the detected fluorescent speckle and modulating the phase on SLM, a new excitation speckle is generated accordingly. Repeat the process, when the feedback reaches a stable maximum, the optimization stops and the excitation speckle forms a unique focus in the scattering medium with focusing on a single fluorescent particle. In order to observe the excitation speckle on the object plane, a monitoring camera is installed behind the scattering medium to record the speckles in the scattering medium. Note that the monitoring camera is only used as process observation, and does not involve in the optimization.

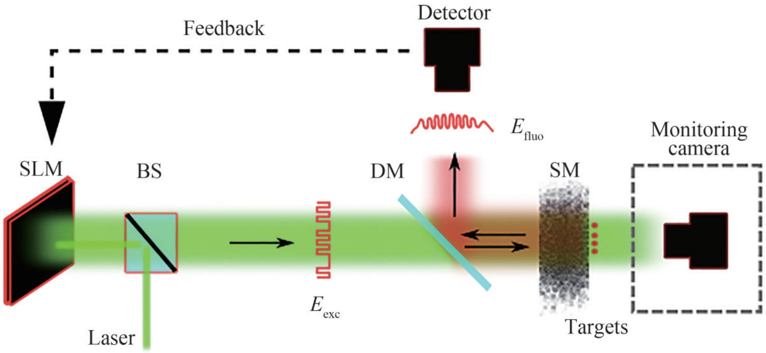


Fig. 1 Schematic diagram of non-invasive focusing in scattering media

Fluorescence, despite as being spatially and temporally incoherent light, will produce speckles when passing through complex medium. The fluorescent speckle from each single particle is formed by the incoherent superposition of the scattering responses of mutually independent polarization and spectral bands, which means the fluorescence is particle-dependent. Therefore, for a group of fluorescent particles, speckles from all the particles excited inside the medium, are also added incoherently. Thus, the detected fluorescent speckle I_{fluo} on the detector can be given by

$$I_{\text{fluo}} = \sum_{i=1}^{N} \left| T_{\text{SM}}^{i} \cdot \delta \cdot E_{\text{exc}}^{i} \right|^{2} \tag{1}$$

where, N is the number of fluorescent particles and δ is excitation response in the scattering medium. E_{exc}^{i} represents the excitation field illuminated on the i^{th} particle. T_{SM}^{i} represents the scattering effect of the medium on the i^{th} particle and its value depends on the scattering medium itself.

On one hand, due to the linear excitation characteristics of fluorescence, the intensity of fluorescent speckle is linear with the intensity of excitation light, that means the larger the intensity of excitation light, the stronger the fluorescence received by the detector. Therefore, in theory, maximizing the intensity of fluorescent speckle can make the excitation light converge closely to a certain extent, however, can not form a focus. On the other hand, since the exponential relationship between the overall contrast C of fluorescent speckle and the number N of fluorescent particles, i.e. $C = 1/\sqrt{N}$[21], when a number of particles are excited, the speckle obtained by the detector is a superimposed speckle with a very low contrast. Consequently, if one optimizes the illumination such that fewer particles are excited, the contrast should increase. In particular, the contrast reaches to the maximum when only a single particle is excited and the others receive zero intensity.

In order to form a focus on a single fluorescent particle in the scattering medium without changing the input laser intensity, it is necessary to maximize both the fluorescence intensity and the fluorescent speckle contrast. Therefore, in this paper, we use the linear combination of fluorescent speckle intensity and contrast in an appropriate contribution as feedback signal. When the value of this linear combination reaches the maximum, the fluorescence intensity and speckle contrast are optimized at the same time which allows us to focus on a single particle. The combination reads

$$F_{\text{fluo}} = \text{Mean}\left(I_{\text{fluo}}\right) + \alpha \cdot C \tag{2}$$

where, α is the contribution weight of contrast C. The value of α need to satisfy the inequality: $\alpha \cdot C \geqslant \text{Mean}\left(I_{\text{fluo}}\right)$.

2 Experimental results

According to the principle of non-invasive focusing in scattering medium as depicted in Section 1, we built the experimental setup presented in Fig. 2. The laser source is modulated by a SLM and then projected into the sample through objective 1 (Obj. 1). The excited fluorescence is backscattered and received by the detector through Obj.1 as well. The excitation speckle is observed by the monitoring camera through the objective 2 (Obj.2). Two lenses L1 and L2 form a 4f system for adjusting the size of the laser source. By maximizing the feedback of the detected fluorescent speckle and optimizing the phase of the excitation speckle, a unique focus will be formed in the scattering medium with focusing on a single fluorescent particle. Based on this, a digital simulation model in MATLAB is established and the primary simulation parameters are shown in Table 1.

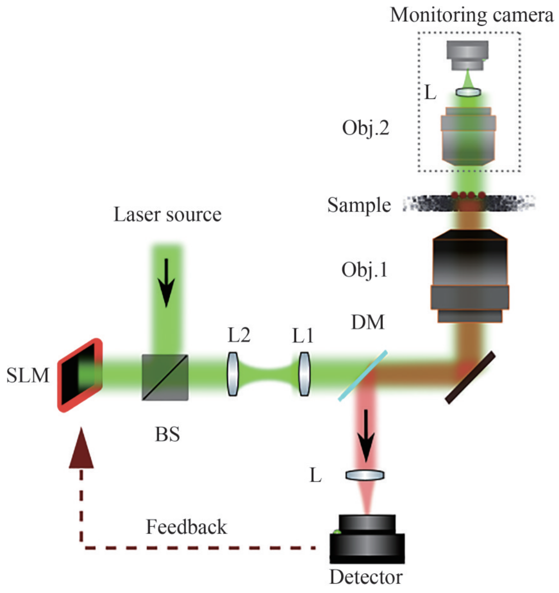


Fig. 2 The schematic of the experimental setup

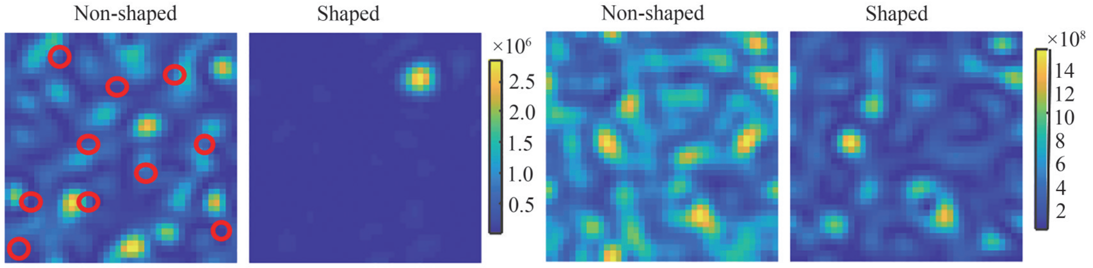
In the simulation process, the feedback is calculated by Eq.(2). The value of α need to satisfy the inequality: $\alpha \cdot C \geqslant \text{Mean}\left(I_{\text{fluo}}\right)$. Since the $\text{Mean}\left(I_{\text{fluo}}\right)/C$ is equals to 2×10^{8} in

Table 1 Primary simulation parameters

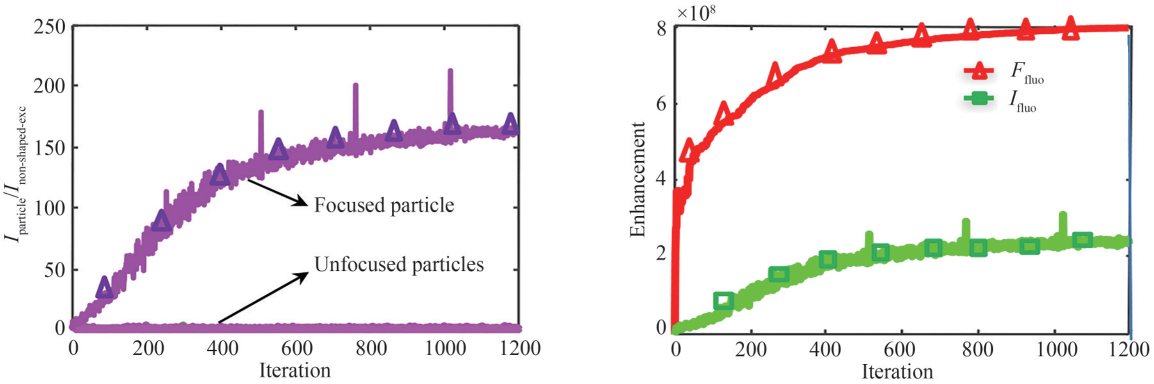
Primary simulation parameters	Parameters values
Wavelength of laser source/nm	532
Magnification of Obj.1	20×
Numerical aperture of Obj.1	1.0
Number of scattering layers	8
Distance between the scattering layers/nm	50
Focal distance of tube lens/mm	180

simulation, in order to make it to satisfy the inequality, we give α a value of 3×10^8. The wavefront of each input mode is optimized by modulating the phase of each grid on SLM using an iterative algorithm. For each iteration, we modulate the phase of the selected grid discretely increased from 0 to 2π in 5 phase steps, record the fluorescent speckles on the detector corresponding to each phase step and calculate the feedback signal. The algorithm finds the optimal phase that maximize the feedback and calculate a new optimal phase that loaded on the SLM before the next iteration. Repeat the process and optimize each grid in turn to obtain the optimal phase mask of SLM. At the end of each iteration, a new wavefront is generated and propagates through the scattering medium forming a new distribution pattern of excitation speckle and fluorescent speckle. When the fluorescent feedback reaches the optimal value, the iteration stops, and the excitation speckle focuses on a single target particle in the scattering medium.

The optimization results are shown in Fig.3. For each optimization, we run 1 200 iterations and illuminate 10 fluorescent particles as targets. The feedback F_{fluo} is estimated throughout the entire optimization process from the recorded fluorescent speckle on the detector. As shown in Fig.3(d), it increases continuously and reaches a plateau in 1 200 iterations. Accordingly, the intensity of the fluorescent speckle I_{fluo} is also optimized to the maximum. Fig.3(b) represents the evolution of the fluorescent speckle before and after optimization. It shows that maximizing the feedback not only enhances the intensity of the speckle (see Fig.3(d)) but also increases the contrast of it. Both of these two effects contribute to focus. In order to prove the focus is formed, the corresponding excitation speckle is shown in Fig.3(a), which is obtained by the monitoring camera. As we can see, the excitation speckle illuminates randomly on multiple particles in the scattering medium before optimization, while focus on a single particle after optimization. It can also be proved by observing the variation of excitation intensity irradiated on each fluorescent particle as shown in Fig. 3(c). Each curve corresponds to the enhancement of the excitation intensity distributed on each fluorescent



(a) Excitation speckle (b) Fluorescence speckle

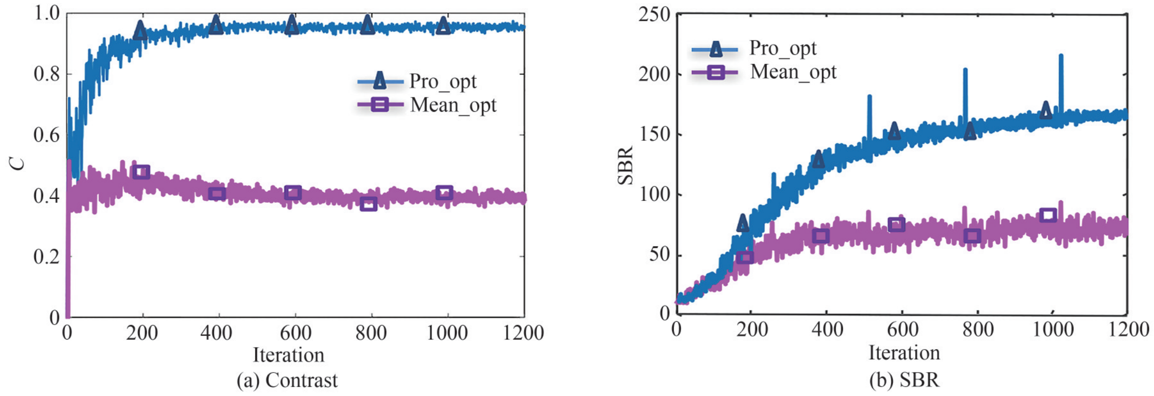


(c) Excitation on particles (d) Variation of feedback

Fig. 3 Simulation results of non-invasive focusing in scattering medium particle, which is given by $I_{\text{particle}}/I_{\text{non-shaped-exc}}$. In the entire process of 1200 iterations, the excitation intensity irradiates on the selected focused particle gradually increases and finally reaches a plateau. On the contrary, the excitation on other particles which are unfocused increases almost zero.

3 Analysis and discussion

Compared with the existing non-invasive wavefront shaping-based technologies, the proposed method in this paper can not only focus on a single particle in the scattering medium, but also enhances the contrast of the fluorescent speckle with a significant Signal-to-Background Ratio (SBR), as shown in Fig.4.



(a) Contrast (b) SBR

Fig. 4 Comparison on the performance of different methods

In Fig.4, we compare the existing algorithm that uses total fluorescence intensity as feedback with the proposed method on two aspects of fluorescence speckle contrast C and SBR. It can be seen from Fig.4(a) that the contrast of speckle is not optimized by intensity optimization method, on the contrary, it is optimized quickly by the proposed method. Besides that, the proposed algorithm tends to get a higher contrast by shaping the speckle to excite a single particle at the beginning of the optimization, thus to enhance the feedback in the maximum extent. Once a single focus is formed, the feedback enhancement mainly comes from the total fluorescence. The reason for this result is that the intensity is a scalar without spatial information, so it is impossible to distinguish the speckles from a single particle or multiple particles in different positions. Therefore, the intensity optimization method only enable the excitation speckles converge on multiple particles as closely as possible, rather than forming a focus on a single particle.

This result can also be seen from the variation of SBR of fluorescent speckle, as shown in Fig.4(b). SBR is given by the ratio of excitation intensity after and before optimization, that is

$$\mathrm{SBR}=\max\left(\left|E_{\mathrm{exc}}^{\mathrm{shaped}}\right|^2\right)\Big/\mathrm{mean}\left(\left|E_{\mathrm{exc}}^{\mathrm{nonshaped}}\right|^2\right) \tag{3}$$

where, $E_{\mathrm{exc}}^{\mathrm{shaped}}$ and $E_{\mathrm{exc}}^{\mathrm{nonshaped}}$ represent the excitation field illuminated on particles after optimization and before optimization, respectively. From Fig.4(b), we can see that the SBR does not get an improvement by the intensity optimization, which means that fluorescent speckle is the superposition of speckles from multiple fluorescent particles, rather than a single fluorescent particle. Whereas, the proposed method can significantly improve the SBR by focusing a single fluorescent particle which makes it possible for its application on deep imaging in complex media.

In addition, the noise from outside the sample is also regarded as the target signal and received by the detector, which becomes one of the factors that must be considered during the whole optimization process. In order to analyze the influence of noise, a variable “Noise Level” (NL) is defined to quantify the noise in the numerical model. Seen from Fig.5, each curve represents the value of SBR measured at different noise level. When the noise levels are 0, 0.2, 0.4, and 0.6 respectively, the SBR curves basically coincide, keep increasing and reach to a maximum, which means that the noise in this range can

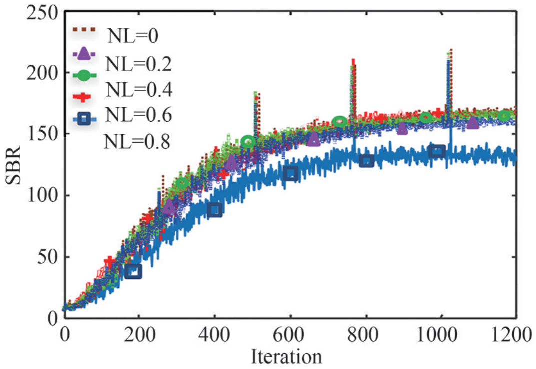
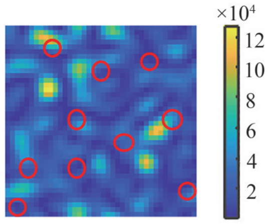
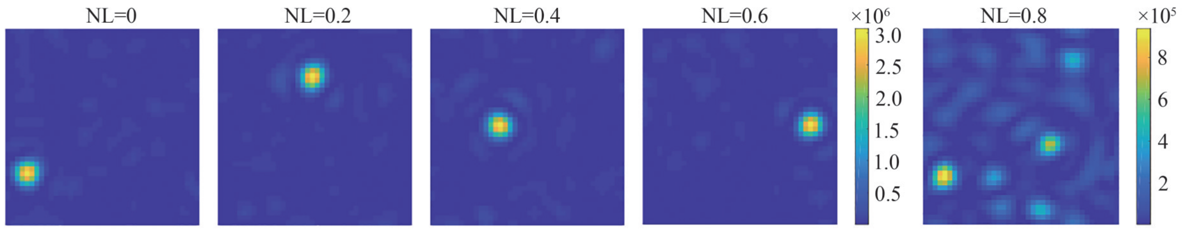


Fig. 5 The SBR of fluorescence speckle on different noise levels

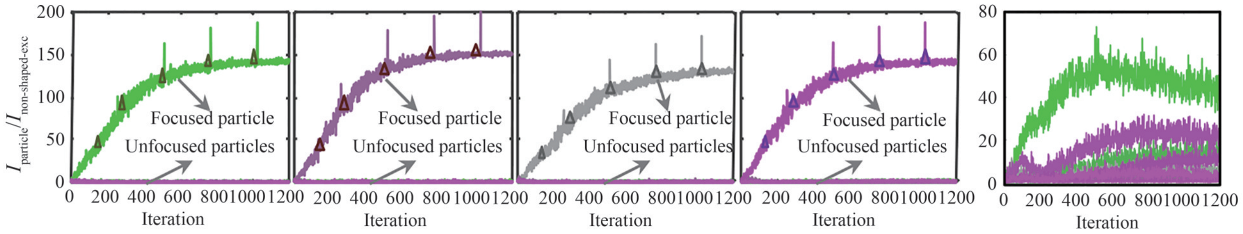
not affect the focusing performance of the algorithm. However, when the noise level is more than 0.8, the SBR performs worse. It also can be seen from Fig. 6, which shows us the excitation speckle forms a prefect focus on a single particle when NL is less than 0.6, whereas, no focus (although there is an obvious maximum point, several sub-maximum points still cannot be ignored) is formed when NL is 0.8. It can be considered that when the noise is too large, the algorithm can not focus well. Nevertheless, for fluorescence experiment, due to the high luminous efficiency and strong signal of fluorescent particles, our proposed method is always stable and feasible in noise environment. For this reason, our method has the potential to be used in the experiments with a large background noise, for example the Raman enhancement. Since the truth that Raman signal itself is weak and the speckle is submerged in the noise, it is difficult to extract the contrast of speckle at the beginning of optimization. while with the help of a filter or spectrometer, it may realize the focusing in strong noise environment, which is exactly what we are studying on.



(a) Non-shaped excitation



(b) Shaped excitation speckle



(c) Excitation onparticles

Fig. 6 Focusing ability on different noise levels

4 Conclusion

A new method on non-invasive focusing in scattering medium is proposed in this paper. The method uses the linear combination of intensity and contrast of fluorescent speckle in an appropriate contribution as feedback of the wavefront shaping, by maximizing the feedback and finding the optimal phase mask of the SLM to modulate the excitation light and converge it to form a focus in the scattering medium, so as to realize the focusing on a single particle effectively. Firstly, simulation experiments are carried out to verify the focusing ability of the algorithm in scattering medium. Secondly, by comparing with the existing methods, it is demonstrated that the proposed method is suitable for single target excitation in the case of multiple targets in the scattering medium and it has fast convergence speed and is insensitive to noise. Besides, the optimized fluorescent speckle not only reaches the maximum intensity, but also has high contrast and SBR, which provides an effective way for the application on deep imaging in complex media. What's more, this method also has the potential to be applied to label-free imaging in complex medium which is expected to be used in life science in the future.

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