Light localization properties of weakly disordered optical media using confocal microscopy: application to cancer detection

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Abstract: We have developed a novel technique to quantify submicron scale mass density fluctuations in weakly disordered heterogeneous optical media using confocal fluorescence microscopy. Our method is based on the numerical evaluation of the light localization properties of an ‘optical lattice’ constructed from the pixel intensity distributions of images obtained with confocal fluorescence microscopy. Here we demonstrate that the technique reveals differences in the mass density fluctuations of the fluorescently labeled molecules between normal and cancer cells, and that it has the potential to quantify the degree of malignancy of cancer cells. Potential applications of the technique to other disease situations or characterizing disordered samples are also discussed.

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References and links

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1. Introduction

Analyzing the structural disorder of weakly disordered optical media has many applications, in particular determining the physical properties of the samples such as mass density variations, or refractive index variation. Some typical examples of such media include polymers, thin dielectric films, cells and tissues, etc. The characterization of these disordered media becomes more complicated if the system has spatial heterogeneity involving many kinds of spatial correlation decay length scales within the sample, for example as in biological cells [1,2]. Recently, there has been a significant interest in quantifying structural disorder of cells by analyzing their light transport and localization properties [3–8]. These investigations have shown that the analyses of the light localization properties of the cells can be a useful tool to examine intracellular tumorigenicity levels. From the physics point-of-view, cells are dielectric/refractive index media with a typical length scale of ~2-10 µm, which corresponds to the mesoscopic length scale regime. Additionally, owing to the complex spatial arrangements of the basic building blocks of the cells, such as DNA, RNA, lipids, etc., the cells’ mass density (or the refractive index) media have inherent heterogeneity [1,2]. Thus, with very weak light scattering properties, cells are an ideal example of weakly disordered heterogeneous optical media. Therefore, in principle, the light transport and localization analyses of the cells can be a useful method to extract information about the physical properties of the cells. In fact, such an approach has been shown to have practical applications, such as in the detection of early carcinogenesis [5–7]. Consequently, these
developments have created new avenues for applications of “mesoscopic physics” based optical transport analysis [9–11] to understand disease processes in biological cells.

Earlier, we proposed a technique that quantifies the effective degree of structural disorder in heterogeneous biological samples in a single parameter via inverse participation ratio (IPR) analysis of the light wave eigenfunctions of these systems [3]. This approach utilizes the fact that in a weakly disordered system, the degree of structural disorder (denoted in this text as $L_{sd}$), which relates to the mass density (or, refractive index) fluctuation inside the sample, is linearly proportional to the IPR value for system [9,10]. In this study [3,4], the transmission electron microscopy (TEM) imaging technique was used to generate the ‘optical lattices’ from the cells; subsequently, light localization properties were analyzed for the control and cancerous cells. The study showed highly promising results in quantifying and differentiating nano-scale level structural disorders in biological samples.

Although this technique is highly promising, it requires the sample preparation and imaging efforts of electron microscopy. With that view in mind, we demonstrate here performing such studies with optical microscopy as an alternative to the TEM imaging. In particular, we performed the light localization analysis of biological cells using confocal fluorescence microscopy, which is one of the most widely used optical imaging techniques in life science and biomedical research. The additional advantage of using confocal fluorescence microscopy is that the structural disorder analysis can be performed for fluorescently labeled selective molecules or organelles inside the cells. For example, the present work selectively performs the structural disorder analysis of nuclear DNA using DAPI staining. In this approach, essentially two-dimensional (2D) confocal fluorescence micrographs were used to construct optical lattice systems, and the degree of structural disorder, i.e., $L_{sd}$ values were evaluated for those constructed optical media.

It should be noted that disorder analysis of optical media via light localization has been widely studied in the last few decades [9–14]. For a closed boundary disordered optical medium, the lightwave eigenfunctions are localized due to the multiple interference effects within the disordered sample. The strength of light localization in a disordered medium is quantified in terms of the IPR values [9,10]. The main length scale associated with the problem is the localization length or the scattering mean free path, which is related to the physical properties of the system, such as refractive index fluctuations and its correlation lengths. In general, the localization effect is more strongly observed in 1D and 2D systems compared to 3D systems. According to the scaling theory of localization, all optical eigenstates are localized in 1D-disordered systems, while the 2D systems have marginally localized, and 3D systems have localized/delocalized states [11,12]. As a result, the disorder analysis is preferably performed for 1D or 2D systems. Additionally, the 2D images provide a better planar visualization of the light localization strength in terms of the structural disorder. Therefore, in the present work, we have conducted the structural disorder analysis of 2D confocal images. The choice of confocal fluorescence microscopy takes advantage of the mechanism of confocal fluorescence imaging technique to construct 2D images of the sample. The pixel intensity of a 2D confocal image is obtained upon a systematic voxel-by-voxel scanning inside the sample on a horizontal thin layer of xy-plane for a fixed z-axis. The contrast of the fluorescence intensity consequently represents the targeted molecules’ mass density variation pattern on a 2D plane inside the sample.

Optical lattice matrices (i.e., the refractive index matrices) were generated using the pixel intensity values from the confocal images of the fluorescently labeled molecules, and these optical lattices were used to compute the degree of structural disorder in the molecular mass density fluctuations. To accomplish this, we employed Anderson tight binding model (TBM) Hamiltonian to the constructed 2D potential matrices (i.e., the optical lattice systems), and the eigenfunctions of the systems were obtained by solving the Maxwell’s light wave equation in a closed boundary condition. We then quantified the structural disorder of the sample in terms of an average $IPR$ value, which is calculated from the statistical analysis of all the
eigenfunctions of the system. Two biological samples with different mass density (or the refractive index) fluctuation distributions (either whole cell or labeled molecular mass density inside a cell) would result in optical lattice systems with different structural disorders (see details in the method section), and thus different average IPR values. In the present work, we compared the average IPR values of cancerous cells with control/normal cells by analyzing the spatial mass density distributions of their nuclear DNA. Specifically, in this proof of concept study, we studied normal and cancerous brain cells, namely the normal astrocyte, astrocyte progenitor (cancerous), and U87 astrocytoma cells, which represent an example of higher cancerous astrocytes. Because carcinogenesis is associated with spatial alteration/rearrangement in DNA/chromatin structure inside the nucleus, we focused on examining the degree of structural disorder in the nuclear DNA of the cells. In order to perform confocal imaging of the DNA inside the cells’ nuclei, they were dyed with DAPI fluorophore which stoichiometrically binds with the DNA molecules. By analyzing the confocal images of DAPI stained nuclei, using the IPR approach described in this work, we were able to differentiate the above mentioned normal and cancer brain cells based on different degrees of structural disorder in their nuclear DNA’s spatial mass density variations.

2. Methodology and theoretical background

In this section, we stepwise describe the methods involved in evaluating the structural disorder in nuclear DNA, defined as $L_{sd}$, using confocal imaging. The description systematically explains the methodology, starting from the construction of an optical lattice (using pixel intensities in confocal micrograph), eventually to the calculation of the IPR values.

2.1. Pixel intensity in confocal microscopy imaging in 2D

In confocal imaging, the amount of fluorescence intensity emitted from a ‘point’ (excitation center on the focal plane) inside the sample results from the fluorescing molecules inside a finite volume, ‘voxel’, around that excitation center. The excitation volume depends on the laser beam’s intensity profile used for the excitation. For a typical Gaussian-shaped laser beam, the intensity profile around the excitation center $r_{c}(x_c, y_c, z_c)$ is given as $I(r, r_c) \propto \exp\left[-2\left((x-x_c)^2 + (y-y_c)^2\right)/w_{xy}^2 - (z-z_c)^2/w_z^2\right]$, where $w_{xy}$ is the lateral width and $w_z$ is the axial width, which together determines the volume covered around the excitation center. Under optimal imaging conditions, the amount of fluorescence intensity emitted from a voxel volume is proportional to the mass density of the fluorescing molecules inside that volume [15–19]. Since, the fluorescing molecules are stoichiometrically attached to the targeted molecules in the cell, the emitted fluorescence intensity can be considered as proportional to the mass density of the fluorescently labeled molecules as well. That is to say, that the intensity detected at the confocal image plane, (i.e., the pixel values in the confocal fluorescence micrograph), is proportional of the mass density of the fluorescently labeled molecules inside a corresponding volume in the sample. Therefore, the detected confocal fluorescence image intensity can be estimated as,

$$I_{CM} = \rho \cdot dV.$$  \hspace{1cm} (1)

where $I_{CM}$ represents a pixel intensity recorded in the confocal fluorescence image at a spatial position $r(x, y)$, while $\rho$ represents the mass density of the fluorescently labeled molecules inside the corresponding small volume $dV$ in the sample.

2.2. Optical lattice construction and its physical significance

The pixel intensity values in a confocal fluorescence image are used to construct an ‘optical lattice’. As pointed out above, the variation in fluorescence intensity recorded in the confocal image depicts a spatial mass density variation of the fluorescently labeled molecules inside the cell. Additionally, it is also known that the local refractive index inside a biological
Sample is proportional to its local mass density [20–22]. Therefore, considering the mass density at any point \( r \) inside the cell of form \( \rho(r) = \langle \rho \rangle + \beta \rho'(r) \), where \( \langle \rho \rangle \) is the average mass density of the fluorescently labeled molecules (DNA in the present study) inside the sample, \( \rho'(r) \) represents the fluctuation in the mass density of the targeted molecules at the position \( r \), and \( \beta \) is a proportionality constant. Consequently, the spatial local variation of the refractive index (RI) of the fluorescently labeled molecules can be written as \( n(r) = n_0 + \alpha \rho'(r) \), where \( n_0 \) is the average refractive index of the fluorescently labeled molecules and \( \alpha \rho'(r) \) represents their spatial refractive index fluctuations. In general, the average refractive index of biological cells is \( \sim 1.38 \) and the position dependent fluctuation ranges up to \( \sim 0.02 \) and refractive index of DNA is \( > 1.48 \) and fluctuation is also higher [1,22].

Therefore, once a confocal micrograph is obtained, using its pixel intensity values a representative refractive index matrix can be constructed. The above discussed considerations suggest that the contrast of the pixel intensity values can be correlated to the spatial refractive index fluctuations of the fluorescently labeled molecular mass density variation. We defined a term \( \varepsilon(x,y) = \frac{dn(x,y)}{n_0} \) as onsite ‘optical potential’ corresponding to the pixel position \( r(x,y) \) on the 2D confocal image plane, where \( n_0 \) and \( dn(x,y) \) represent average refractive index of the fluorescently labeled molecules and its fluctuation at spatial position \( (x,y) \) inside the sample, respectively. Mathematically, one can write,

\[
\varepsilon(x,y) = \frac{dn(x,y)}{n_0} \propto \frac{dI_{CFM}(x,y)}{<I_{CFM}>}.
\]

where \( <I_{CFM}> \) represents the average intensity of the confocal image, and \( dI_{CFM}(x,y) \) represents intensity fluctuation at a pixel position \((x,y)\) in the 2D confocal image, respectively. Thus, the onsite ‘optical potential’ values, \( \varepsilon(x,y) \), are obtained from the corresponding pixel intensity values in the confocal image.

As displayed in Fig. 1, in a confocal image created by point-to-point scanning on a horizontal plane inside the sample (Figs. 1(a')–1(b)), the pixel intensity values at each point \((x,y)\), \( I_{CFM}(x,y) \), are recorded. Then the term \( dI_{CFM}(x,y)/<I_{CFM}> \) is calculated for each of the pixels in the image. Subsequently, using Eq. (2), the \( \varepsilon(x,y) \) values are calculated for all the pixel points of the confocal image. This calculation results in a matrix of the same size of the confocal image with the \( \varepsilon(x,y) \), i.e. the optical potential, values known for all points as represented in Fig. 1(c).

Since, in this process, we obtain a matrix same as the dimension of the confocal image for which the ‘optical potential’ values are known for each of its points, this matrix is simply termed an ‘optical lattice’. It should be noted that the \( \varepsilon(x,y) \) values represent the strength of the refractive index fluctuations of the fluorescently labeled molecules at those spatial
positions \((x,y)\) on a 2D plane inside the cell. Thus, an optical lattice is a representation of the labeled molecules’ spatial refractive index fluctuation pattern inside the sample.

As described later in detail, in the present work we have used DAPI fluorescence staining to label DNA molecules inside the nuclei of the cells. Therefore, the fluorescence intensity contrast observed in the confocal images in our work represents mass density of the DNA molecules inside the nuclei of the cells. Consequently, in the present study, an optical lattice obtained in such a way depicts a disordered optical system, i.e., a representative, optical refractive index of the molecular materials, corresponding to the spatial DNA mass density variations in the nuclei.

2.3. **Tight binding model (TBM) Hamiltonian, eigenfunctions, and IPR value calculations**

To determine the degree of the structural disorder, i.e., the disorder strength, of an optical lattice, generated from the confocal micrograph, we used Anderson tight binding model (TBM) Hamiltonian approach. The Anderson-TBM is a well-studied and proven to be a good model Hamiltonian in describing the disorder properties of optical systems of any geometry and disorder [9–12]. Therefore, it is suitable for biological cell characterization as well. In our study, we considered one optical state per lattice site, with the inter-lattice site hopping restricted to the nearest neighbors only. Such a Hamiltonian can be written as:

\[
H = \sum_i E_i |i\rangle \langle i| + t \sum_{\langle i,j \rangle} |i\rangle \langle j| + |j\rangle \langle i|,
\]

where \(E_i\) in Eq. (3) represents the optical potential at the space corresponding to the \(i^{th}\) number position in the lattice, \(j\) represents the nearest neighbor of the \(i^{th}\) point on the lattice site, and, \(t\) is the inter-lattice site hopping strength. The eigenfunctions of this constructed Hamiltonian is determined and further analysis is performed as described in the following section.

2.4. **Ensemble averaged IPR value and the degree of structural disorder \(L_{sd}\) calculation in terms of IPR**

The disorder strengths of the optical systems were quantified in terms of the IPR values of the systems, which is calculated by the statistical analysis of the eigenfunctions determined from the Hamiltonian matrix described in Eq. (3). An average IPR value for a lattice system is determined as a function of length scale \(L\) corresponding to the area \(L \times L\), inside the lattice system, as defined in Eq. (4) [3,4,9,10]:

\[
\langle \text{IPR}(L) \rangle_{L \times L} = \frac{1}{N} \sum_{i=1}^{N} \int_{0}^{L} \int_{0}^{L} E_i^4(x,y) dxdy.
\]

where \(E_i\) represents the \(i^{th}\) eigenfunction of a closed (bounded) system of area \(L \times L\), having \(N\) lattice points. The average symbol \(<..>_L \times L\) simply denotes that the averaging is done for the area \(L \times L\). For 2D systems, the IPR values are measured in the unit of inverse area.

To elaborate more on the calculation of an ensemble averaged IPR value for a cell (or subsequently a particular cell type) we present a brief discussion below, while a more detailed step-wise calculations are shown in the Appendix.

Consider a confocal micrograph of pixel size \(a \times a\). With \(M \times M\) pixels in a square micrograph, the size of the whole micrograph would be \(Ma \times Ma\). The micrograph is divided into arrays of small square boxes of area \(L \times L\), such that \(L = ma\), where \(m < M\) is an integer of choice. Therefore, the area of each of these small boxes inside the micrograph is \(L \times L = m^2a^2\). In such a case, the whole micrograph gets divided into \((Ma/L) \times (Ma/L) = (M/m)^2\) number of small boxes. In each of these small boxes, there are \(m \times m\) number of lattice sites (i.e., pixel points) for which the ‘optical potential’ values are known. In the next step, for every single box (of area \(L \times L\)), a Hamiltonian matrix is constructed using Eq. (3),
and the corresponding $N (= m^2)$ eigenfunctions are determined. Once all the $N$ eigenfunctions for each of these boxes are known, an average IPR value denoted by $<\text{IPR}(L)>_{L \times L}$ is calculated using Eq. (4), where $dx = dy = a$, and $L = ma$. Subsequently, the process is repeated to calculate the $<\text{IPR}(L)>_{L \times L}$ values for all the small boxes, each corresponding to the area $L \times L$, inside the sample. As a result, a spatial distribution of IPR values in the sample is obtained (see the IPR plots in next section). Note that this $<\text{IPR}(L)>_{L \times L}$ (indicating that the IPR value calculation is performed over a box of area $L \times L$ inside the sample) value constitutes the smallest unit area in a 2D IPR plot or simply the pixel of an IPR image plot. It should also be noted that for a confocal micrograph of dimension $M \times M$, the 2D IPR plot has the dimension of $Ma/m \times Ma/m$.

Using the above described process, the 2D IPR plots containing the $<\text{IPR}(L)>_{L \times L}$ distribution corresponding to each of the micrographs (i.e., confocal images) of a cell are obtained. Subsequently, an ensemble averaged IPR value, $<<\text{IPR}(L)>_{L \times L}>_{\text{ensemble}} = <\text{IPR}(L)>$ are calculated by averaging all the $<\text{IPR}(L)>_{L \times L}$ values of different micrographs of all the cells. In the present study, we analyzed 3 - 4 confocal images of 10 - 12 different cells, for each cell type. In a similar way, the $<\text{IPR}(L)>$ values are determined and analyzed for different sample lengths ($L$ values) as well.

Two different types of cellular samples with different degree of structural disorders are expected to result in different $<\text{IPR}(L)>$ values [3,4,9,10]. The $<\text{IPR}(L)>$ value corresponding to an area $L \times L$ is a measure of the strength or the degree of light localization inside the sample in that area. The degree of light localization, in term of $<\text{IPR}(L)>$ values, in a closed area ($L \times L$) measures the effective structural disorder of the sample in that area, which in turn is a function of the magnitude and spatial distribution of the mass density or refractive index fluctuations of the fluorescently labeled molecules inside the area [3].

Because biological systems are heterogeneous media, the degree of structural disorder in two biological systems are compared through their ensemble averaged $<\text{IPR}(L)>$ values (as described above) by considering an effective Gaussian color noise refractive index fluctuating system of the labeled molecules with exponentially decaying spatial correlation statistics. For such a system, it has been shown that the $<\text{IPR}(L)>$ value is proportional to the strength of the structural disorder $L_{sd}$ of the sample in an effective Gaussian model [3]. Therefore, once the $<\text{IPR}(L)>_{L \times L}$ value for each closed sample area is known, the $L_{sd}$ inside that closed area of $L \times L$ is characterized via a Gaussian color noise with an exponentially decaying spatial correlation of the refractive index fluctuations of the labeled molecular mass density. This type of Gaussian color noise is typically represented as $<dn(r)dn(r')> = <dn^2>\exp(-|r-r'|/l_c)$, where $dn$ is the fluctuation in refractive index distribution and $l_c$ is the spatial correlation decay length of the fluctuation. The choice of the Gaussian color noise model is because the theoretical framework for localization analysis is well established for a Gaussian disordered system, for both white and color noise models [9,10]. For Gaussian color noise refractive index fluctuations with short correlations length, it has been shown with extensive numerical simulations in 2D disordered system that [3],

$$<\text{IPR}(L)> \propto L_{sd} = \left( \frac{d_0}{L_c} \right)^{1/2} \times L.$$ (5)

Therefore, a change in $<\text{IPR}(L)>$ values, and thus the $L_{sd}$ values, indicates a change in the fluctuation strength of the refractive index of the molecules, a rearrangement in its spatial distribution, or the product of both the quantities for a fixed sample length $L$ (i.e., sample size $L \times L$). For the simplicity of expressing the structural disorder in terms of IPR values, we consider the proportionality constant as 1; hence, we express the $L_{sd}$ and the $<\text{IPR}>$ values as the same numerical number (i.e., $L_{sd}(<\text{IPR}(L)>) = <\text{IPR}(L)>$). A typical schematic of the steps involved in this study of comparing structural disorder in two samples using confocal fluorescence microscopy is shown in Fig. 2.
(i) The confocal images of the nuclei of two samples were obtained. (ii) Optical lattices are constructed and eigenvalues are obtained by solving the Anderson tight binding model Hamiltonian. (iii) The structural disorder of the samples are then obtained by calculating the inverse participation ratio ($IPR$) of the systems from the eigenfunctions in a Gaussian color noise model and compared.

3. Results and discussions

We studied the commercially available normal and cancerous human astrocytic cell lines. In particular, the normal astrocyte (Lonza Group Ltd, Basel, Switzerland) were used as control while the astrocyte progenitor (Applied Biological Material, Richmond, Canada) cells and the U87 astrocytoma cells (American Type Culture Collection, Rockville, MD, USA) were used as cancerous samples. The cells, grown on glass coverslips, were fixed with 4% paraformaldehyde and then stained with the fluorescent DNA binding DAPI (ThermoFisher Scientific, MA, USA) staining dyes, following a standard staining protocol.

Confocal imaging of the DAPI stained nuclei were performed with a Nikon Ti-E-A1rSI with a 63x oil objective with an NA of 1.4. A laser wavelength of 405 nm was used for the excitation of the DAPI dyes stained to the nuclear DNA. As the DAPI targeted the nuclear DNA, images were taken of cells’ nuclei with one nucleus at a time at the pixel resolution of $1024 \times 1024$ by optically sectioning thin layer by layer of the cells using standard z-stack confocal imaging of the cell.

The confocal micrographs of optical sections of cells’ nuclei were analyzed to measure the structural disorder in the nuclear DNA molecules using the methodology described in Section 2, and the results were evaluated statistically. The degrees of structural disorder ($L_{sd}$), in terms of $<IPR(L)>$ values, were calculated for different length scales ranging from $L = 0.4$ to $1.6 \mu m$. At each of the length scales, all the $<IPR>_{L \times L}$ values were ensemble averaged for $\sim 3 - 5$ confocal micrographs of a single cell’s nucleus, and 12 - 15 cells from each of the categories were analyzed. The experiment was repeated for 3 different sets.

Fig. 3. (a), (b), and (c): Representative confocal images of a normal astrocyte, an astrocyte progenitor, and a U87 astrocytoma cell nuclei, respectively. (a’), (b’) and (c’): Their corresponding $L_{sd}(IPR)$ images (2D IPR plot) at sample length $L = 0.4 \mu m$ (we have taken $L_{sd}(IPR) = <IPR>$). The scale bar in the confocal image corresponds to 5 $\mu m$. 

Fig. 2. Schematic flowchart for comparing the structural disorder using confocal micrographs.
Typical results comparing $L_{sd}$ values in all three types of cells studied in this work are shown in Fig. 3. The Figs. 3(a)–3(c) show representative confocal images of the 1) normal astrocyte, 2) astrocyte progenitor, and 3) U87 astrocytoma cells’ nuclei, respectively. The images presented in Figs. 3(a’)–3(c’) are their corresponding $L_{sd}(<IPR>)$ images or simply the 2D IPR plot, obtained at sample length $L = 0.4 \, \mu m$. These 2D IPR plots show the distribution of the $<IPR>_L$ values calculated for the sample area of $L \times L = 0.4 \times 0.4 \, \mu m^2$ inside the nuclei of the two cells.

As it can be seen in the Figs. 3(a’)–3(c’), there are more prominent hot spot (red spots) regions in the cancerous astrocyte progenitor and U87 astrocytoma cell nucleus in comparison to the normal astrocyte cell nucleus. As it can be evidently seen, the variation in the IPR values for the cancerous astrocyte progenitor and U87 astrocytoma cells are much higher in the range of 1.4 – 2, than the normal astrocyte cells. This suggests a significantly higher DNA mass density variation, in terms of magnitude and spatial arrangement, in the cancerous astrocyte cells’ nuclei compared to the normal astrocyte cells, which in turn indicates the higher degree of structural disorder in the nuclear DNA in the cancerous case.

In Fig. 4, a bar graph comparison of structural disorder between the normal and the cancerous astrocyte cell types is shown. The $L_{sd}(<IPR>)$ values were calculated at $L = 1.6 \, \mu m$, i.e., $1.6 \times 1.6 \, \mu m^2$ areas inside the sample. The $p$-values obtained from the student’s t-test (sample size > 45) were < 0.05 for all the pairs, demonstrating that the structural disorder values in the nuclear DNA of the cancerous astrocyte progenitor and U87 astrocytoma cells are significantly higher than that of the normal astrocyte cells. Similarly, the difference between $L_{sd}(<IPR>)$ values of the astrocyte progenitor and U87 astrocytoma cells are huge.

![Fig. 4. Bar plots for mean $L_{sd}(<IPR>)$ values (n = 12-15 cells, 3-5 micrographs per cell, 3 sets) for the normal astrocyte, astrocyte progenitor, and U87 astrocytoma cells nuclei at sample length $L = 1.6 \, \mu m$. Student’s t-test obtained p-value < 0.05 for each pair.](image)

Furthermore, we also examined $L_{sd}(<IPR>)$ values for all the cell types at different sample lengths $L$, ranging from 0.4 – 1.6 \, \mu m. The results are shown in Fig. 5. The results demonstrate that average structural disorder is higher for cancer cells compared to their control/non-cancerous counterpart for all the length scales studied. Furthermore, it is interesting to note in Fig. 5 that the $L_{sd}(<IPR>)$ values for the normal astrocyte cells start showing the saturating trend at higher sample lengths, which is in accordance with Eq. (3). It is interesting to point out here that the $L_{sd}(<IPR>)$ graphs for the cancerous cases would also ultimately saturate at a significantly higher sample length; a trend towards saturation can still be seen in the present plots.
Fig. 5. Structural disorder at different sample length scales \( (L) \) (sample size \( L \times L \)). \( n = 12-15 \) cells for each type of normal astrocyte, astrocyte progenitor, and U87 astrocytoma cells, where 3-5 confocal micrographs for each cell around mid-nucleus were considered for the analysis.

The higher \( L_{sd(<IPR>)} \) values for the tumorigenic astrocyte progenitor and U87 astrocytoma cells suggest more structural alterations (or spatial mass density variation) in their nuclear DNA when compared to the normal astrocyte cells. The higher structural disorder in nuclear DNA of the cancerous cells may be attributed to the unfolding of the heterochromatin inside the nuclei, which occurs during carcinogenesis [8].

At this point, it would be worth to discuss the reported works on cellular characterization related to different cancer detection method, mainly by imaging. It should be noted that histopathology is one of the most widely used preliminary approaches to detect cancerous cells and tissues using optical microscopy [23]. Biopsy samples are collected from the concerned part of the body, and stained with H&E staining (or others), and then examined under optical microscopes by an expert pathologist to distinguish cancerous and non-cancerous cases, primarily by observing the color contrast in the image [23]. Other bulk imaging techniques, such as MRI, CT scan, PET scan, etc., are also used to diagnose cancer by locating tumor inside the body [24]. While, the direct visualization of the tumor, in the images obtained using such techniques, are the first step towards examining cancer, however, further confirmation test is done using the histopathology as described above. Owing to the heterogeneous nature of the biological samples, in particular tumors, various approaches are employed in the quantification studies. As a result, the analysis of tumor heterogeneity has remained an area of significant research interest, for its potential in diseases diagnostics as well as assessment of therapeutic response, especially in regards to oncology for bulk tumor [25]. In particular, texture analysis is performed by employing different techniques to quantify spatial heterogeneity of the tumor. Some of the elementary analysis includes measuring mean, standard deviation, skewness, and kurtosis of the pixel intensity distribution. Other advanced techniques, such as entropy, co-occurrence matrix, fractal dimension based analysis are also employed to quantify the degree of heterogeneity of the tumor samples [25–28]. Entropy, which is measured from the histogram distribution of the pixel intensities of the sample’s image, provides a way of measuring and comparing spatial randomness in two samples. Similarly, co-occurrence matrix, run length matrix (RLM), etc., based analysis are used to examine the relationship between pixel intensity values of the image, such as repetition, linear dependency, etc [29,30]. Therefore, while there requires several parameters in texture analysis of heterogeneous tumor samples, continuous improvement in these measurements techniques are highly sought after as well [31]. Since the detection and analysis of malignant tumor is associated with later stages of cancer, recently there has been significant research interest in structural characterization at single cell level, which has shown potential in early cancer detection [3–8]. The structural changes at single cell level in early carcinogenesis take place at ultra-small length scales where the changes can occur either in the intracellular refractive index fluctuations or its correlations lengths or both [3–8]. While,
in such a situation, the above mentioned techniques, including entropy measurements, can still be applied for structural characterization of cells, it will require measurement of several parameters for the analysis, owing to the heterogeneity of the cells. However, using the presented light localization technique, we can characterize the system in a single parameter, the light localization strength or the \(<\text{IPR}>\) value. All the effects arising out the system’s heterogeneity are embedded in the eigenfunctions of the system, which is then quantified in term of the average \(\text{IPR} \) value, \(<\text{IPR}>\). Thus, this IPR approach introduces a new dimension to the quantification studies of cell like heterogeneous media, by quantifying the spatial heterogeneity of the fluorescently labeled molecules (or, it can be full sample) in one parameter, which is sensitive to all the types of change in RI fluctuations as well as its correlations lengths. Consequently, this technique provides an easy way to compare structural changes in two heterogeneous disordered systems.

### 4. Conclusions

In conclusion, we have developed a novel method based on light localization analysis to quantify refractive index fluctuations or mass density fluctuations of fluorescently labeled molecules, in weakly disordered heterogeneous optical system, using confocal fluorescence microscopy imaging. As an example of weakly disordered media, cells’ nuclei were studied to evaluate the potential utility of the technique for bio-medical applications, in particular, cancer detection. Our results show that the confocal fluorescence micrographs can be efficiently used to quantify refractive index fluctuations, or conversely the mass density fluctuations, in nuclear DNA using the developed technique. In particular, we studied the mass density fluctuations (or reflective index fluctuations) of DAPI fluorescently labeled DNA molecules in nuclei. Further, we showed that such analysis can be performed at the submicron level using one single parameter, that is the degree of structural disorder \(L_{sd}\) in terms of the inverse participation ratio (IPR) values. Studies were performed on commercially available normal and cancerous brain cells lines, namely the normal astrocyte as control cells, and astrocyte progenitor and U87 astrocytoma as cancerous cases. The result shows that the proposed method provides a useful numerical mean to distinguish these cell types based on the quantified mass density fluctuations in their nuclear DNA. In particular, the nuclei of the cells were chosen to compare structural disorder in the DNA, for the reason that in the case of carcinogenesis, DNA molecules inside the cells’ nuclei are known to undergo alterations, i.e., the nuclear mass density variation and rearrangement. As demonstrated in the present work, the technique can be used to measure structural disorder of selectively targeted molecules inside the cells by fluorescent labeling. In the present study, we have targeted DNA molecules using DAPI staining. Therefore, the method should potentially work for appropriate dye treated live cells as well. This method would be also useful without dye if the confocal imaging can detect sufficient mass density variations of the label-free cells. In that case, the analyzed mass density variation would correspond to the actual mass density variation of the nucleus, unlike only the DNA mass density variation studied in the present work. The way the framework of the method has been developed, it can detect structural changes in biological systems regardless of its origin, as long as the fluorescence intensity contrast in the imaged micrograph can be related to the mass density variations inside the sample. Therefore, this technique can potentially be used in a wide variety of applications. For example, cellular abnormalities or disease conditions which leads to intracellular structural changes, such as sickle cell anemia, metabolic or mechanical stress, and response of cells to therapeutic agents, are few envisaged ones. Finally, our method may also be useful for materials science of soft optical disordered media, such as polymers and thin films, in characterizing the structural disorder of the sample using the similar approach. Future work will elucidate more on the potential applicability of this technique.
Appendix: Systematic steps for IPR calculation

We describe here further explanation on calculation of IPR values discussed in the text in Section 2.

Figure 6 shows a schematic flow chart of IPR calculations and the IPR image construction starting from a confocal fluorescence micrograph. We also methodically describe the steps involved in the process. The process mainly involves five major steps, starting from acquiring the confocal images, IPR image construction, finally calculating the ensemble averaged IPR value, $<\text{IPR}>$, as follows:

**Step-1**: The desired confocal images/micrographs are acquired from the cell.

**Step-2**: Using the pixel intensity values of each micrograph, a confocal pixel intensity matrix is obtained: $I_{CFM}(x,y) = <I_{CFM}> + dI_{CFM}(x,y)$, where $<I_{CFM}>$ is the average confocal image intensity of the whole sample.

**Step-3**: From the pixel intensity matrix, an optical refractive index lattice/matrix is obtained by considering one-to-one correspondence between confocal micrographic pixel intensities and the refractive index of the fluorescently labeled molecules determined as $dn(x,y)/n_0 \propto dI_{CFM}(x,y)/<I_{CFM}>$, where $n_0$ is the average refractive index of the labeled molecules, and $dn(x,y)$ is the refractive index fluctuations of the labeled molecules at a point corresponding to the spatial position $(x,y)$ in the confocal image; $<I_{CFM}>$ and $dI_{CFM}$ $(x,y)$ are the average confocal image intensity and the intensity fluctuation at pixel $(x,y)$ on the confocal micrograph, respectively. In such a way, an optical lattice system is obtained such that for every point in the optical lattice, an “optical potential” defined as $\varepsilon(x,y) = dn(x,y)/n_0$ is known. In essence, this procedure replaces the confocal pixel intensity matrix (i.e., confocal image) with a refractive index/optical potential matrix, or, more simply, an “optical lattice” system of refractive index matrix, with varying refractive index at each point. The ‘optical lattice’ therefore depicts the refractive index variation of the labeled molecules on a 2D plane inside the sample. The disorder analysis is performed on this optical lattice.

**Step-4**: In this 2D optical lattice, we choose a sample size $L \times L$, with $m \times m$ optical lattice points, where $L = ma$, with $a$ as the confocal pixel unit (pixel dimension $a \times a$). On this lattice area, a tight binding Hamiltonian is constructed with $\varepsilon(x,y)$ ($= dn(x,y)/n_0$) as the onsite potential using Eq. (3) (in main text). Subsequently, we calculate the eigenfunctions, $E_\beta$, of the Hamiltonian.

**Step-5**: Once the eigenfunctions are known for each of the small areas $L \times L$ on the micrograph, we calculate the $<\text{IPR}>_{L \times L}$ (averaged over a small area $L \times L$ on the...
micrgraph, with $m \times m$ number of confocal pixel points in the small area) using the Eq. (4), which constitutes the unit block of the IPR image. Thus, repeating this method, we obtain a distribution of $<\text{IPR}>_{L \times L}$ values for the whole sample (confocal micrograph), thus, obtaining a 2D IPR plot (see IPR image in Fig. 3). Note that for a confocal micrograph of pixel dimension $Ma \times Ma$, we get IPR image of size $Ma/m \times Ma/m$. Similar calculations are done at different $L$ values to examine length scale dependence of disorder in the system. See also the Section 2.4, for details.

**Ensemble averaged IPR:** Once the $<\text{IPR}>_{L \times L}$ distribution is known for a micrograph, an ensemble averaged IPR value is determined: $<\text{IPR}(L)> \equiv <<\text{IPR}(L)>_{L \times L}>_{\text{ensemble}}$ by averaging all the $<\text{IPR}>_{L \times L}$ values for several micrographs of a cell, finally, for several cells of the same category. The $<\text{IPR}(L)>$ values for two samples are compared. Comparison of $<\text{IPR}(L)>$ values are also done for different length $L$ values.

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