C-CLASS microscopy allows the detection of nanoscale changes in chromatin structure

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ABSTRACT

Coherent confocal light absorption and scattering spectroscopic (C-CLASS) microscopy, which extends the principles of light scattering spectroscopy to subcellular imaging, can be used to reveal biological structures well beyond the diffraction limit. Here we show that high-resolution C-CLASS microscopy can be used to detect nanoscale changes in chromatin structure. Unlike most methods for chromatin monitoring, C-CLASS microscopy can be used label-free in live cells. Live differentiating hiPSC organoids were measured over the space of sixteen days and characteristic chromatin changes were observed.

Keywords: Light Scattering, Spectroscopy, Microscopy, Chromatin

1. INTRODUCTION

Organoids formed from human induced pluripotent stem cells (hiPSCs) could be a limitless source of functional tissue for transplantations in many organs. Unfortunately, fine-tuning differentiation protocols to form large quantities of hiPSC organoids in a controlled, scalable, and reproducible manner is quite difficult. Chromatin organization has recently been identified as an important marker of pluripotency and stem cell differentiation [1]. To pack a 2-m-long DNA strand into a nucleus that has a diameter of only a few microns, chromatin must be organized into distinct domains. Some of these domains display a more open form of chromatin, with high gene density and high gene expression, while others are more densely packed, exhibiting a closed chromatin state. Compared to differentiated cells, undifferentiated cells lack highly condensed transcriptionally inactive heterochromatin and are significantly richer in lightly packed euchromatin, which is composed of the most active portion of the genome [2]. As differentiation progresses, the amount of heterochromatin markedly increases. This has been demonstrated indirectly by histological analysis of the nucleus [3] or chromatin immunoprecipitation (ChIP) accompanied by microarray hybridization (ChIP-chip) and high-throughput sequencing analyses [4]. It is also demonstrated directly with electron microscopy–based techniques [5]. Unfortunately, all these techniques are destructive, involve extensive manipulations of the sample, and hence cannot be used in living systems for real-time differentiation monitoring. An alternative technique is coherent confocal light absorption and scattering spectroscopic (C-CLASS) microscopy, which extends the principles of light scattering spectroscopy [6,7] to subcellular imaging and can also sense the state of the chromatin organization [8, 9].

2. METHODS

The key components of the C-CLASS system are a confocal microscope, broadband light source, and spectrometer. The broadband light is delivered to the sample using an objective, with the backscattered light collected by the same objective and delivered to a spectrometer. Thus, each pixel location contains a spectrum that describes the light scattering from within the confocal volume. Conventional CLASS microscopy uses an incoherent broadband light source to illuminate the sample [10]. However, when using high-NA objectives, the informative part of the spectrum is often destroyed due to angular averaging of the scattering signal. We recently overcame this by using a coherent broadband light source. With this technique, referred to as C-CLASS microscopy [9], the informative part of the spectrum is preserved due to in-phase constructive interference, leading to significantly better spectral contrast and structural sensitivity. The ability of C-CLASS microscopy to size structures well below the diffraction limit is easily demonstrated with measurements of polystyrene microspheres. Figure 1A shows the raw spectra obtained from microspheres with diameters 350 nm and 100 nm submerged in an aqueous solution. Removing the inherent light source structure provides the spectra shown in Fig. 1B, and this can be easily validated with theoretical curves derived using Mie scattering theory.

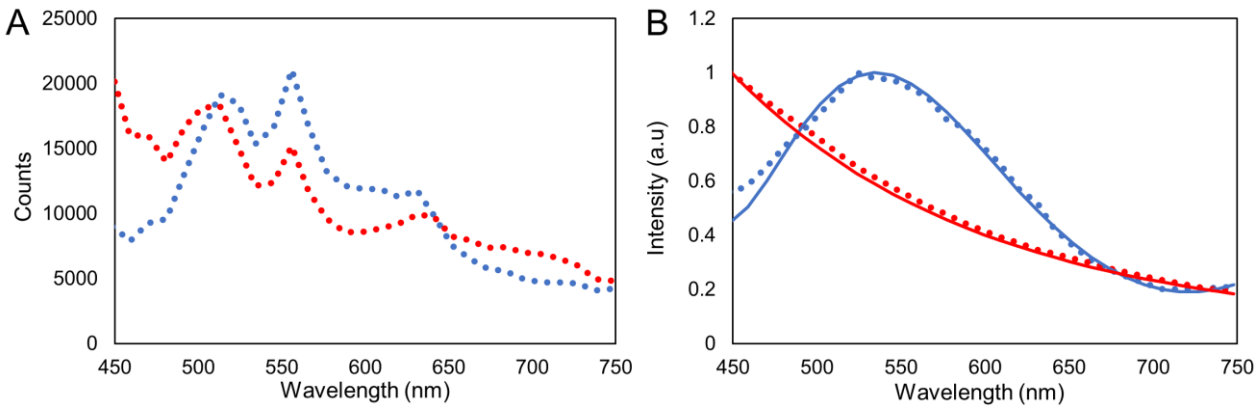

Figure 1. (A) Raw C-CLASS spectra obtained from 350 nm diameter (blue) and 100 nm diameter (red) polystyrene microspheres. **(B)** Processed spectra shown with dotted lines and the corresponding theoretical curves obtained from Mie scattering theory shown with solid lines.

In terms of chromatin structure, it is believed that chromatin displays a fractal organization [11], which facilitates easy unfolding and refolding during gene activation or repression. When associating C-CLASS spectra with the fractal organization of chromatin, it is beneficial to describe chromatin using the most general case of mass fractals bounded by surface fractals. While the explicit formula is not known, the scattering intensity can be described using the semiempirical relationship of $I \sim \lambda^{-4+X}$, with $X = 2d_m - d_s$, where d_m and d_s are the mass and surface fractal dimensions, respectively [12]. In simpler terms, the slope of the light scattering spectrum is indicative of the chromatin packing density. This is demonstrated in Fig. 2, where broadband reflectance imaging, which uses a supercontinuum laser source, provides a high contrast image that allows easy identification of the organoid nuclei. The corresponding C-CLASS image, which is taken directly after the broadband reflectance image, provides the spectral data. A processed spectrum from within the nucleus is shown in Fig. 2 using a dotted line, along with the best fit slope (solid line) obtained using a least squares minimization method. The resulting slope is indicative of the chromatin packing density.

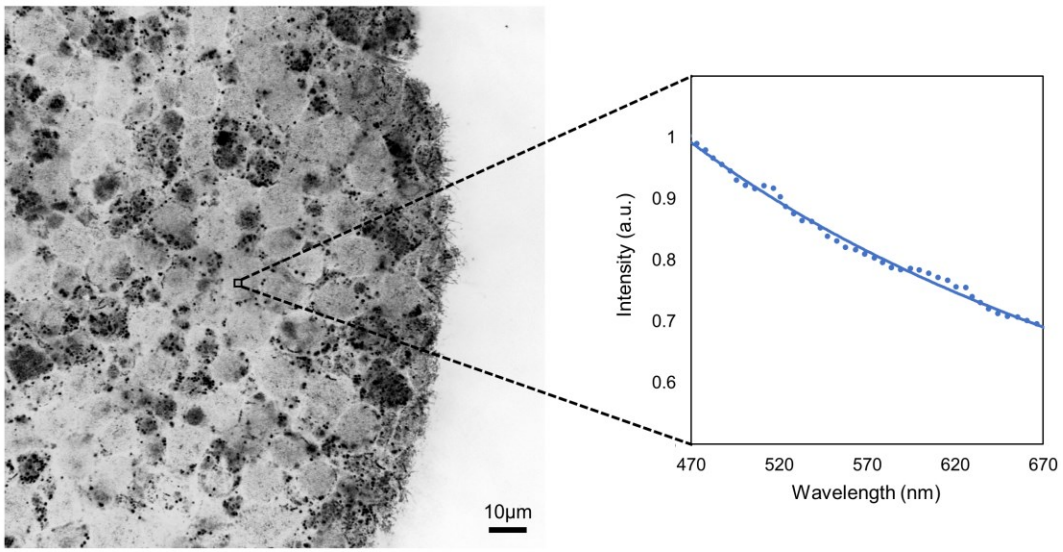

Figure 2. Broadband reflectance imaging provides a high contrast image of the organoid, with both the edge of the organoid and nuclei boundary easily identifiable. The above image is presented with inverted colors. C-CLASS microscopy provides spectral data, with the slope of the spectra indicative of the chromatin packing density. Processed spectrum is presented with dotted line and best fit slope illustrated with solid line.

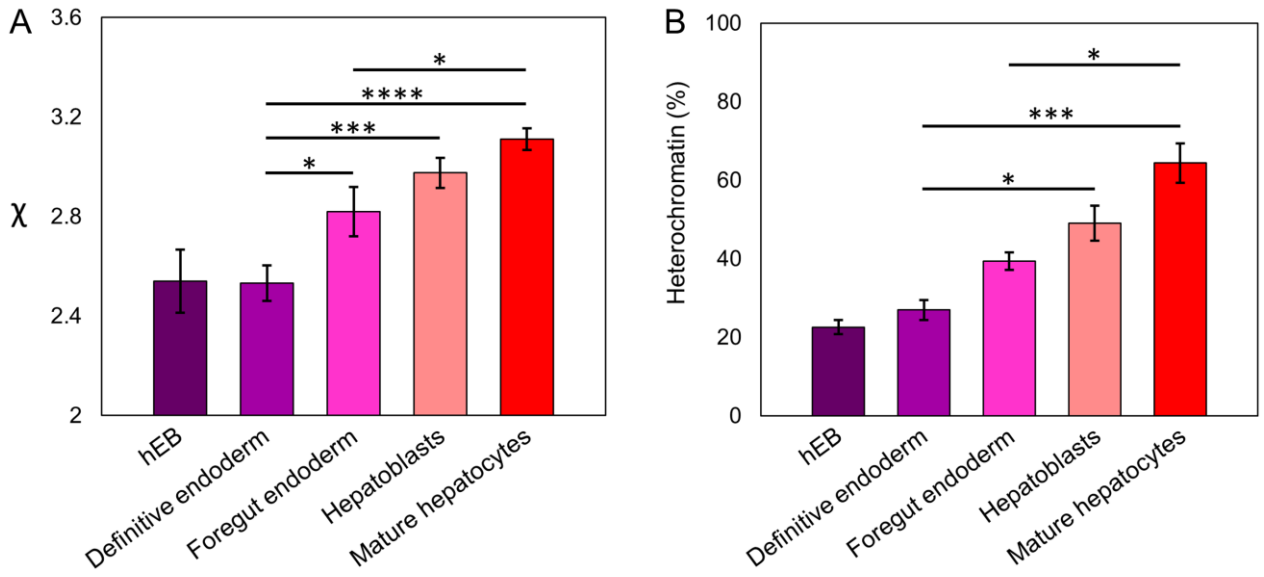

Figure 3. (A) Changes in packing parameter for hEB (n = 9), definitive endoderm (n = 19), foregut endoderm (n = 21), hepatoblasts (n = 21), and mature hepatocytes (n = 24) stages of differentiation. **(B)** Percentage of heterochromatin at hEB (n = 4), definitive endoderm (n = 4), foregut endoderm (n = 3), hepatoblasts (n = 3), and mature hepatocytes (n = 3) stages of differentiation, as calculated by TEM. Data adapted from [8] and presented as means ± SEM.

3. RESULTS

We monitored chromatin changes in live hiPSC-only organoids undergoing differentiation by collecting C-CLASS microscopy images over a period of 16 days. This 16-day period begins with human embryoid bodies (hEBs), followed by a four-stage protocol that forms definitive endoderm, foregut endoderm, hepatoblasts, and mature hepatocytes, respectively. With the progression of differentiation, the slope of the scattering spectra from nuclei within live hiPSCs showed a highly significant change. Figure 3A shows the average values of X for several organoids, calculated across the entire nucleus, at each stage of differentiation. The steady increase in packing parameter is consistent with the expected increase in the heterochromatin fraction as differentiation progresses. These results are in excellent agreement with transmission electron microscopy (TEM) measurements that we performed upon the same batches of differentiated hiPSC organoids on the same days as CLASS microscopy measurements, as illustrated in Fig. 3B.

4. CONCLUSIONS

Organoids formed with hiPSCs could be an inexhaustible source of tissue that could be used for transplantations or drug discovery [13]. While chromatin packing has been used as a reliable marker for monitoring changes in organoids, most methods are destructive, laborious, and difficult to access. An alternative is C-CLASS microscopy, which can detect nanoscale changes in chromatin packing label-free in live cells.

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