1048
doi:10.1017/S1431927622004469
Microsc. Microanal. 28 (Suppl 1), 2022

Visualization and Comparison of Cellulose Microfibers Images from Cellulose Derivatives Aerogels in CLSM and PALM Using Conventional Dyes

Susana Dianey Gallegos-Cerda[1*], Josué David Hernández-Varela[1], José Jorge Chanona Pérez[1], Carlos Alberto Huerta-Aguilar[2], Benjamín Arredondo-Tamayo[1]

[1.] Laboratorio de super resolución y nanoestructuras, Departamento de Ingeniería Bioquímica, Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional, Mexico city, Mexico.
[2.] School of Engineering and Sciences, Tecnológico de Monterrey, Puebla, Mexico
* Corresponding author: sgallegosc0100@alumno.ipn.mx

Introduction

New insights in the microscopic characterization techniques, open fields to study many samples such as biological materials at a nanometric scale without considerable damage. Particularly, plant-based materials (cellulose from plants) are normally used in the elaboration of white bond paper without further use, but they could be exploited as a resourceful material for the development of new matrixes with enhanced physicochemical properties. Aerogels emerge as a composite material that is formed off of polymer aggregates with lightweight and hyper adsorbents properties, and applications as catalysts support, electro-chemistry, thermal insulators, and pollutant removal [1].

Common techniques used for aerogels characterization without labeled procedures are XRD, FTIR, and SEM, but, when a selective analytes identification is necessary, the confocal laser scanning microscopy (CLSM) emerge as a useful tool to perform a microstructural differentiation, taking advantage of the specific label of fluorophores, where due to a wide field illumination, molecules excitation is generated and registered (Figure 1a) but high-fluorescence level create blurred images due to out-of-focus contributions [2]. However, as a new improvement in fluorescence microscopy, super-resolution microscopy (SRM) allows obtaining enhanced images to study in detail organic and inorganic samples taking advantage of the capacities and resolution below 100 nm [3].

Within SRM, the photoactivated localization microscopy (PALM), allows the specific localization of fluorophores with a resolution near 20 nm, using excitation lasers that activate switchable fluorophores, to its detection and additional reconstruction at micro and nanometric level (Figure 1b). In PALM, the number of the molecules detected depends on the labeling efficiency, high quantum yields, molecule density, and dye properties. It has been reported that photoswitchable fluorophores are the most efficient molecules to the labeling detection [3], however, here we report the use of conventional dyes, to image obtention which to the best of our knowledge are scarcely used in PALM.

Methodology and sample preparation

Aerogel preparation

Aerogels were prepared accordingly with [4], using cellulose from white bond paper and NaOH/urea solution. The obtaining functionalized pulp was used to form an aerogel in ethanol to dry at room temperature in a petri dish for 8 h (Figure 1c).

Microscopic visualization

To acquire images, a dual microscope system with CLSM and SRM module (LSM-880 and Elyra PS.1,

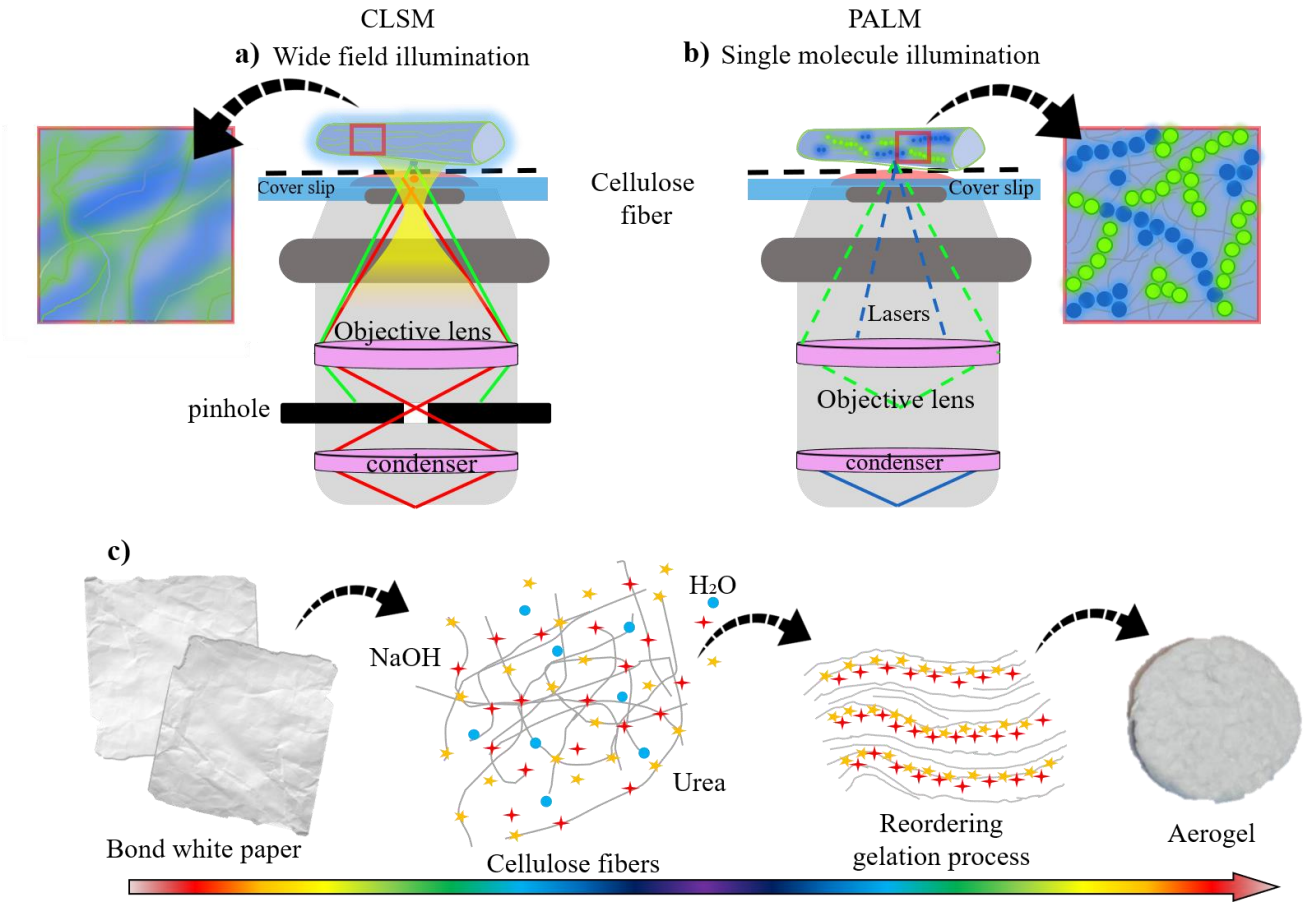

Figure 1. Schematic operating principles of a) CLSM, b) PALM and c) graphical representation of cellulose aerogel synthesis.

Zeiss, Germany) was used. Morphology visualization of an isolated cellulose fiber from the aerogel, was visualized in CLSM and compared with PALM to demonstrate the enhanced capacities of the technique, but also, to know how functionalization is carried out along the fiber. Samples were observed using a 100X 1.46 Zeiss a-Plan Apochromat oil-immersion objective DIC M27. Cellulose fiber was stained using a solution (1:1) of 0.01% of calcofluor white (Fluorescent brightness, 28, F3543, Sigma-Aldrich, USA), and a solution of 0.02% of fluorescein 5(6)-isothiocyanate (FITC) (SLBV8242, Sigma-Aldrich, USA). For CLSM images, 405 and 488 nm lasers were used to calcofluor and FITC excitation respectively. For PALM, epi-fluorescence mode and 405 and 488 nm excitation lasers were used. A time-series image recording 1000 images with intervals of 0.1 ms was acquired. Image processing was made with the Zeiss Zen 2011 software using the PALM-wizard, where blue and green colors were used for representing fluorophores intensities.

Results

Figure 2 shows CLSM and PALM images of cellulose fiber aerogel using conventional dyes. Figure 2a shows high fluorescence signals from calcofluor white (blue) and FITC (green) which appear along with the fiber, however, a selective functionalization was not recognized due to the high emission of dyes and technique capacities [5]. Figure 2b shows a PALM blurred image before adjusting the processing conditions in the software for a reconstruction (Fig. 2c). Figures 2d and 2e show the single wide-field and high-resolution reconstructions, respectively, where the high fluorescence level and selective

https://doi.org/10.1017/S1431927622004469 Published online by Cambridge University Press

molecule localization of dyes are visualized. Finally, in comparison with the CLSM, the reconstructed PALM fiber is present in Figure 2f, where isolated dots represent each dye molecule and its disposition in the fiber. This image demonstrates that cellulose fiber (blue) was functionalized with amine groups (green) once alkali/urea treatment was realized but also shows the enhanced capacities of SRM to visualize specific labeled parts. To measure a single molecule, Figure 2g and 2h were extracted from Figure 2f (red arrows) for a calcofluor white and FITC, respectively. It is observable that calcofluor white has a lower emission size (around 35 nm) than FITC in which the emission size is bigger (41 nm) related to their high quantum yields.

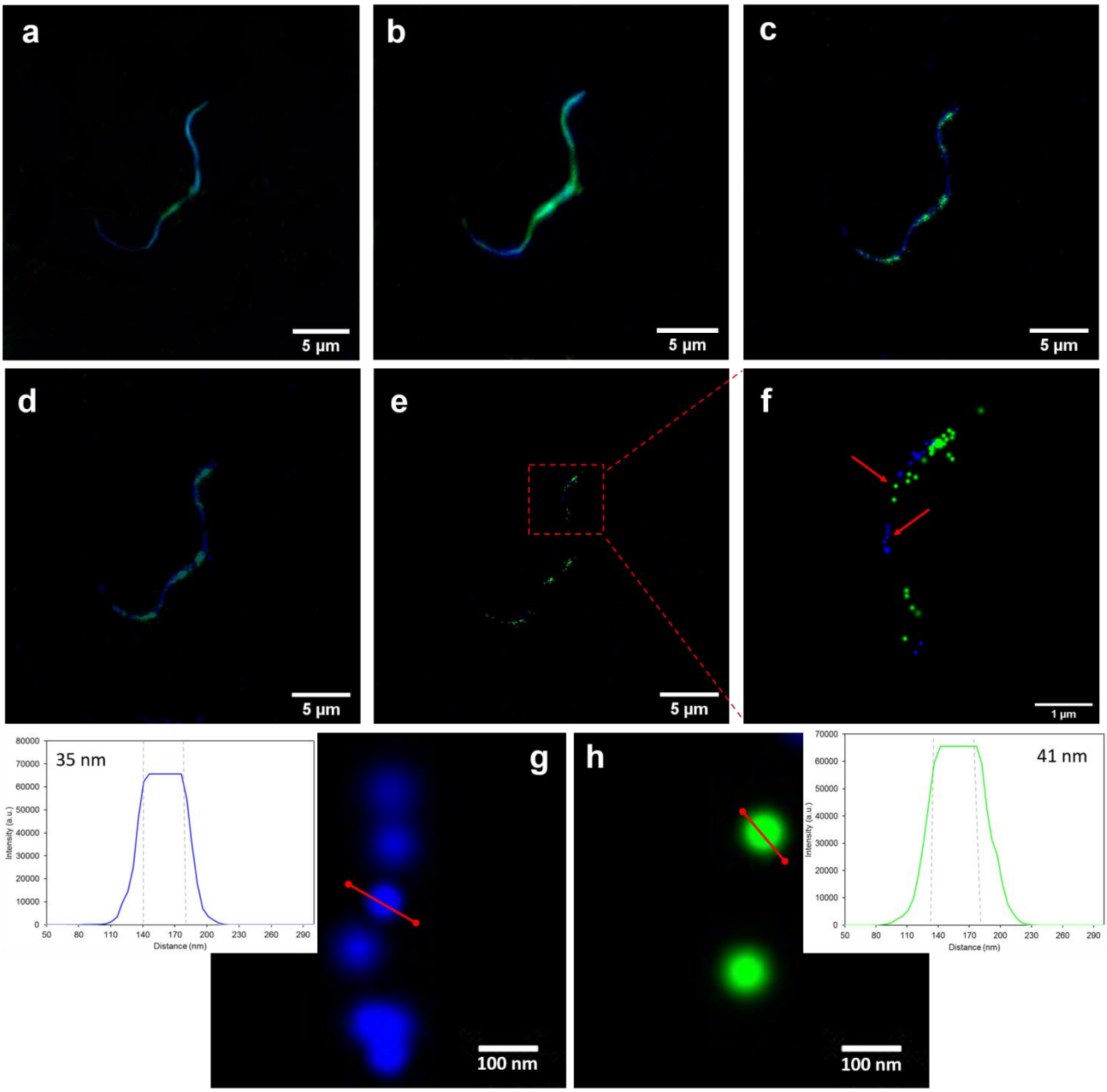

Figure 2. Cellulose fiber extracted from aerogel dye with calcofluor white (blue) and FITC (green). a) CLSM and PALM image before (b) and after (c) reconstruction. d) single wide-field image from PALM, e) high-resolution PALM image and zoom are (f) in which two single molecules were selected (g, h) for profile measurement (insets).

https://doi.org/10.1017/S1431927622004469 Published online by Cambridge University Press

Conclusion

PALM technique allows the reduction of the molecule overlapping and the molecule localization with high precision along a cellulose fiber without significant blinking reducing the excessive fluorescence noise in comparison with the CLSM. High labeling efficiencies were reached by using conventional dyes as those reached with specialized fluorophores by recording the major number of images enhancing the single-particle localization, which makes PALM an innovative technique for selective molecule localization.

References

[1] T. Budtova, Absorption and Adsorption, as Catalysts and Catalysts Support and in Electro-Chemistry When Pyrolysed, Springer Netherlands, 2019.
[2] J.B. De Monvel, S. Le Calvez, M. Ulfendahl, Biophys. J. 80 (2001) 2455–2470.
[3] J. Liesche, I. Ziomkiewicz, A. Schulz, BMC Plant Biol. 13 (2013) 226.
[4] M. Fauziyah, W. Widiyastuti, R. Balgis, H. Setyawan, Cellulose 26 (2019) 9583–9598.
[5] J. Tam, D. Merino, J. Neurochem. 135 (2015) 643–658.

https://doi.org/10.1017/S1431927622004469 Published online by Cambridge University Press