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Correlative Light and Electron Microscopy for the Study of the Structural Arrangement of Bacterial Microcrystalline Cellulose Microfibrils

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Cellulose is an abundant polymer with a crystalline structure that represents a large section of the total biomass on Earth [1,2]. The mechanical properties, biodegradability and abundant availability of cellulose makes it an ideal starting material for the production of biofuels, biocomposites and bioenergetics products [1-4]. The generation of cellulose-based products often requires both physical, chemical and biochemical processing including hydrolysis reactions to hydrolyse cellulose into cellulosic nanomaterials [3,4]. To improve the efficiency of these processes, it is crucial to understand the structure of cellulose at the micro-and nanoscale. Cellulose microfibrils are postulated to have frequent disordered regions that disrupt their crystalline structure forming "dislocations" that affect the overall structure and could influence depolymerisation reactions [5]. This structural model has been recently supported by studies involving super-resolution (SR) fluorescence imaging that showed the presence of sites with alternating high and low labelling densities along the length of bacterial cellulose microfibrils. While these observations suggest the presence of pockets of accessibility within the crystalline cellulose, their structural origin and morphology remains elusive. Insight into this question can be gained through correlative techniques, such as correlative light and electron microscopy (CLEM), which would combine the sensitivity and specificity offered by fluorescence microscopy with the nanometer resolution of transmission electron microscopy [6-9].

To investigate the crystalline structure of bacterial microcrystalline cellulose (BMCC) microfibrils and visualize putative "dislocations" along the fibril length, we have adapted and optimized a correlative light and electron microscopy (CLEM) methodology [10]. Cellulose microfibrils were aligned and correlated between three microscopy modalities, super-resolution (SR) imaging, epi-fluorescence imaging and transmission electron microscopy (TEM). To achieve optimal correlation of microfibrils between the microscopy modalities while minimizing artifacts across the microscopes four critical factors were considered: 1) To assist in locating cellulose microfibrils of interest in all microscopy modalities, samples were prepared and imaged on a formvar coated alphanumeric labelled copper finder TEM grids. 2) The samples needed to be compatible for each microscopy modality, as fluorescence imaging required for the samples to be in a hydrated state to, while for TEM imaging, samples must be compatible to the vacuum environment. 3) To locate potential disordered regions in the crystalline structure of the microfibrils, BMCC was fluorescently labelled with Cy5 and imaged. 4) Optional staining procedures for cellulose microfibrils for TEM imaging to improve image contrast were developed.

BMCC microfibril samples were prepared on the finder grid and placed on a glass coverslip (Figure 1). Following the addition of a drop of imaging buffer, a second glass coverslip was also used to sandwich the sample [10]. During SR and epi-fluorescence imaging on a Leica DMI6000 B inverted

microscope (iXon Ultra 897 EMCCD), the location of microfibrils of interest were noted. Following fluorescent imaging, the copper grid was removed from the coverslip sample and washed repeatedly. Once dry, the sample was mounted onto a TEM holder for TEM imaging. While imaging the sample in TEM, regions of interest were located and TEM image sets were taken on a JEOL JEM 1200EX TEMSCAN microscope (80kV, spot size 3) using different magnifications (10 kX- 100kX) to identify the structure of the BMCC microfibrils at the micro-and nanoscale. Optional negative staining procedures to BMCC microfibrils are being optimized to reduce electron beam damage. After images of regions of interest were acquired using multiple modal microscopy techniques, image analysis using ec-CLEM software was performed to correlate and align BMCC microfibrils to investigate the structure of fibrils, specifically in the potential disordered regions (Figure 2) [11].

Fluorescence and electron microscopy imaging of the BMCC microfibrils have been completed successively, demonstrating that the CLEM methodology is possible for BMCC fibrils and can provide more information about the structural arrangement of cellulose. SR images display the repeating high- and low-density areas of Cy5 along the fibril length and when composite with TEM images, areas of high-density Cy5 areas appear to correlate with twists (dark regions) on the BMCC fibril (Figure 2.D-F). While this methodology is still being optimized, further development of sample preparation procedures in the CLEM workflow will improve the image acquisition quality and speed for structural analysis of BMCC microfibrils. The application of multiple microscopy modalities will allow for an increase in understanding of the supermolecular organization of cellulose microfibrils which can provide beneficial information on depolymerization of cellulose and improve the production efficiency of cellulose-derived products.

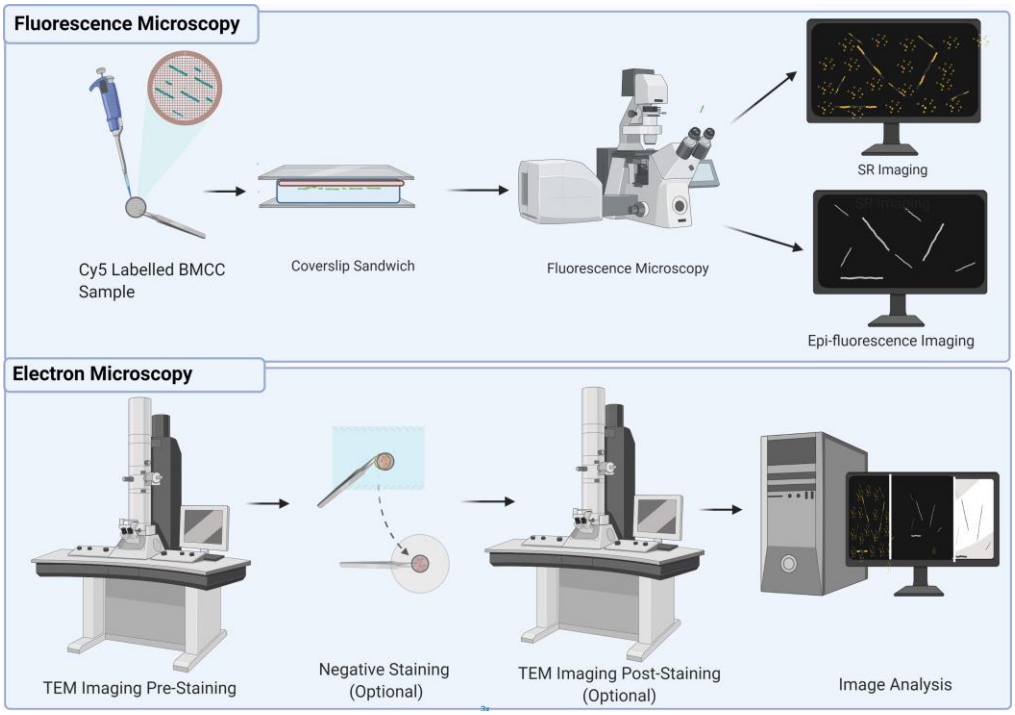

Figure 1. Overview of the correlative light and electron microscopy workflow for sample preparation, image acquisition, and data analysis (Created with BioRender.com).

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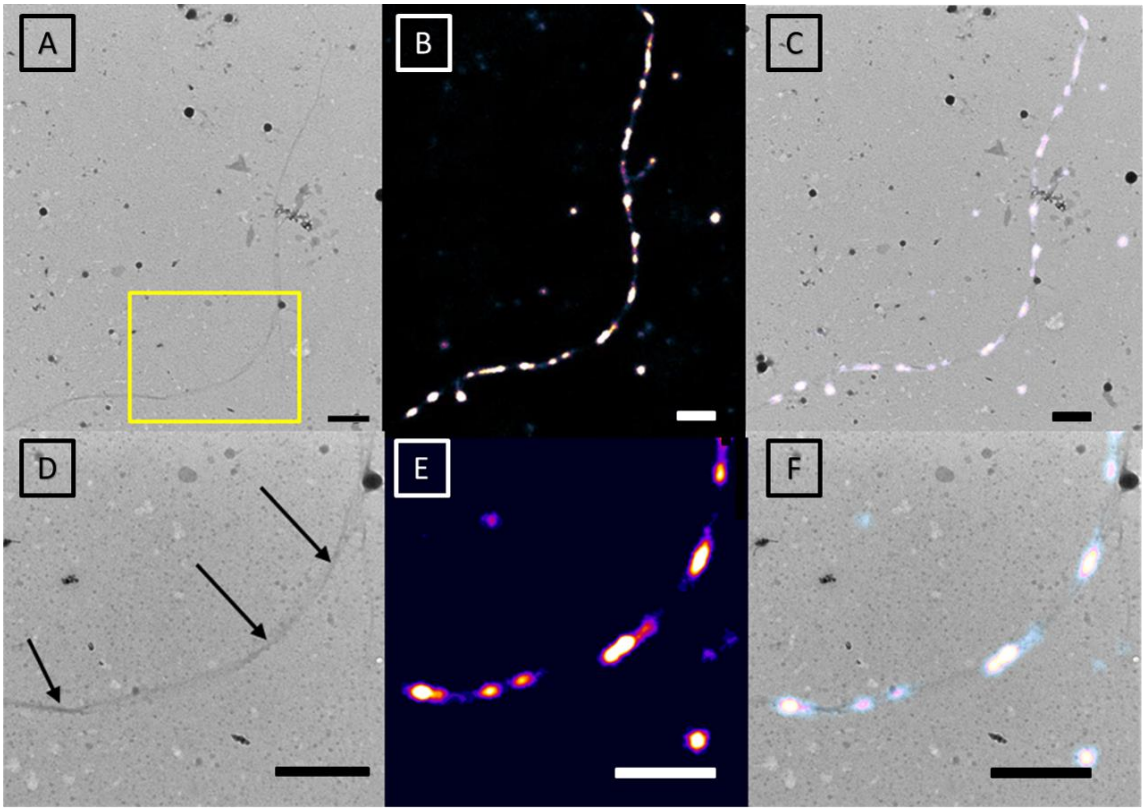

Figure 2. Multimodality microscopy images of a bacterial microcrystalline cellulose microfibril (A-C) and of a region of interest with twists along the BMCC microfibril (yellow box) (D-F). A) TEM image of the BMCC microfibril. B) SR imaging of the BMCC microfibril. C) Sr-CLEM composite image of the BMCC microfibril using SR and TEM imaging. D) TEM imaging of the region of interest on the BMCC microfibril with indicated twists (black arrows). F) SR imaging of the region of interest on the BMCC microfibril. F) sr-CLEM composite image of the region of interest on the BMCC microfibril using SR and TEM imaging. Scale bar: 1μm.

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