Meristem Microbes:

Imaging Bull Kelp (*Nereocystis luetkeana*) Blade Tissues for Microbiome Visualization

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ABSTRACT:

Canopy-forming kelps have broad ecological influence through their physical structure and biological activity, playing a foundational role in habitat creation and primary productivity. On a microscopic scale, the blades of kelps like *Nereocystis luetkeana* support dynamic microbial ecosystems. Molecular techniques have revealed the taxonomic structure of these microbiomes, yet microscopy remains underutilized for visualizing biofilm morphology and spatial arrangement. I attempted to image microbial colonization of *N. luetkeana* blades across varying tissue age, using a triple-stain fluorescent imaging protocol to target cell walls and nucleic acids. Microbiota were not successfully imaged, likely due to loss of biofilm during wash protocol. Imaging did reveal clear cell walls of *N. luetkeana*, along with unexpected nucleic structures. In the meristem and mid-blade regions, tissue cells consistently showed four discrete nucleic acid signals, despite *N. luetkeana* being a known uninucleate species. Our results highlight methodological limitations of biofilm microscopy and raise new questions about nuclear dynamics and cell division beyond the meristem in *N. luetkeana*.

INTRODUCTION:

Kelp are the foundation of diverse and biologically productive marine ecosystems that dominate many temperate coastal zones. Kelp forests, composed of macroalgae from the order Laminariales, form the base of complex food webs and shape the physical structure of these ecosystems (Schroeder et al., 2019). Kelp canopies offer habitat, refuge and nursery grounds to hundreds of marine species, while their impressive growth rate fuels the food web and contributes to blue carbon sequestration (Egan et al., 2013; Filbee-Dexter & Wernberg, 2020).

These foundational primary producers and the ecosystems they support also directly contribute to commercial fisheries and local economies, particularly in temperate regions (Springer et al., 2010). These economic benefits extend to coastline protection. The complex structure of kelp forests attenuate and buffer wave energy, reducing coastline erosion and protecting nearshore habitats (Morris et al., 2020).

Bull Kelp, *Nereocystis luetkeana,* is one of the most abundant and well-known canopy forming kelps. *N. luetkeana* is the dominant species along the rocky, wave-exposed shorelines of the North Pacific, extending from Alaska to California (Springer et al., 2010; Weigel & Pfister, 2019). Observed growing over 25 meters in a single season with blade elongation reaching up to 1.7 cm day^{-1}, bull kelp is one of the fastest growing macroalgae (Weigel & Pfister, 2019). This rapid growth creates dense canopies, creating a structurally complex habitat. These *N. luetkeana* canopy habitats directly support species of both ecological and commercial importance, including rockfish, crustaceans and shellfish (Springer et al., 2010). This impressive primary production fuels the food web in complex ways that extend beyond herbivory. Bull kelp releases approximately 16.2% of its fixed carbon as dissolved organic carbon (DOC), directly into the surrounding water (Weigel & Pfister, 2019). This DOC contributes directly to the microbial loop and detrital food webs. The combination of grazing and DOC release allows *N. luetkeana* to contribute to the food web via multiple pathways, rapidly pumping fixed carbon into their habitats.

Bull kelp are vital for canopy ecosystems, but there is another unseen ecosystem that these macroalgae support. Each blade of kelp is its own ecological environment, host to complex microbial communities. Microbes and their secretions form a biofilm that coats the blade surface. This biofilm is a complex, three dimensional habitat composed of bacteria, fungi, protists and viruses (Egan et al., 2013). Like corals and their endosymbiotic zooxanthellae, macroalgae like kelps are increasingly recognized as holobionts. Rather than existing as isolated organisms, kelp and their microbiota form an integrated biological system and function as a single ecological unit (Egan et al., 2013; Weigel & Pfister, 2019). These surface associated microbes play diverse functional roles as part of the macroalgae holobiont. Microbes that make up the biofilm release proteins and chemicals that enhance nutrient uptake, influence morphogenesis, modulate spore release and provide defense against fouling microbes and pathogens (Egan et al., 2013; Leiva et al., 2015).

In *N. luetkeana*, microbial communities are not evenly distributed along the blade. Microbial diversity, cell density and biofilm thickness all increase along the developmental gradient from the basal meristem to older apical tissues (Weigel & Pfister, 2019). Similar patterns have been observed in *Saccharina latissima*, where the increase in biofilm density on older tissue is attributed to cumulative microbial colonization over time and increased physical contact with the surrounding environment as the tissue extends (Ramírez-Puebla et al., 2022). These findings indicate that kelp blades act as selective growth environments. The initial colonization of young tissue may be stochastic, meaning the first established microbes are a random selection of the surrounding seawater microbe community (Ramírez-Puebla et al., 2022). However, the developing biofilm matures into functionally structured communities, where early colonizers fill ecological niche functions such as nitrogen cycling, metabolite exchange and antifouling (Egan et al., 2013; Bengtsson et al., 2010). These mature biofilms have microbe composition that differs significantly from surrounding seawater, and from biofilms that develop on artificial agar kelp blades (Leiva et al., 2015; Weigel & Pfister, 2021). These findings reinforce that kelp blades actively shape and sustain distinct microbial communities, which fulfill essential functions and define the macroalga as a holobiont.

While recent studies have revealed important spatial and temporal trends in *N. luetkeana* biofilms, most rely on DNA sequencing or taxonomic sorting techniques to characterize the microbiota (Ramírez-Puebla et al., 2022; Burgunter-Delamare et al., 2023). These molecular approaches offer insights into community composition, but may lack visual context regarding biofilm morphology, microbe spatial arrangement and colonization patterns across blade tissue. This creates an opportunity for microscopy-based studies, which can directly visualize biofilm structure, quantify microbial density, and capture heterogeneity in colonization across the blade surface. To explore this knowledge gap, I used staining and microscopy to visualize and compare blade surfaces across different developmental zones on a blade of *Nereocystis luetkeana*. I hypothesize that the microbial cell density and biofilm thickness will be lowest at the meristem and increase towards the apex, and that biofilm morphology will become increasingly structured along this gradient. Understanding microbial succession along the kelp blade may help clarify how these communities contribute to host health, structural integrity, and ecosystem function. This is especially important under growing anthropogenic pressures such as global warming, ocean acidification, and pollution.

METHODS:

I collected blade tissue from distinct developmental zones of a *Nereocystis luetkeana* specimen and applied a three-dye staining protocol (SR2200 for cell walls, and Cytox Green and Potomac Gold for nucleic acids) intended to highlight both host structure and microbial colonization. While microbial biofilms were not successfully retained, potentially due to fixation-related loss, imaging provided detailed views of host tissue morphology and informed improvements for future microbiome visualization techniques.

Sample Collection and Tissue Preparation

A single adult *Nereocystis luetkeana* (bull kelp) specimen was collected from the floating dock at Friday Harbor Laboratories (FHL), San Juan Island, WA. At the docks, tissue samples were collected in situ from three distinct regions of the kelp blade, using a sterile razor blade: (1) the meristematic base, located just above the stipe transition; (2) the mid-blade region, approximately 12 cm from the base; and (3) the apical tip of the blade, approximately 12 cm below the end of the base. Tissue samples were cut to approximately 1 x 0.5 cm and transferred into separate, pre-labeled 1.5 mL Eppendorf tubes.

Staining Protocol

To visualize the surface-associated microbial communities and host tissue morphology, each tissue region was stained with a three-dye combination:

- **SR2200**

  Fluorescent stain developed for labeling plant cell walls by binding to cellulose and other polysaccharides. Though designed for terrestrial plants, it effectively highlights structural features in brown algae due to their similarly polysaccharide-rich cell walls. It fluoresces in the blue to violet range with excitation ~350–410 nm (Musielak et al., 2015).

- **SYTOX Green**

  Fluorescent stain that binds to nucleic acids, allowing visualization of both eukaryotic nuclei and entire bacterial cells. Originally developed for detecting compromised cell membrane, it is widely used in microbiology and cell biology. It fluoresces in the green range with excitation ~504 nm and emission ~523 nm (Roth et al., 1997).

- **Potomac Gold**

  Fluorescent nucleic acid stain developed by the Luke Lavis lab at the Howard Hughes Medical Institute’s Janelia Research Campus. While not yet commercially available, it belongs to the Potomac family of dyes optimized for cellular imaging in live and fixed specimens. Potomac Gold binds strongly to DNA, allowing visualization of both eukaryotic nuclei and DNA-rich bacterial cells. Emission and excitation characteristics have not yet been standardized for commercial labeling, but the dye was designed for compatibility with advanced microscopy techniques (Spahn et al. 2018).

Samples were fixed in 4% paraformaldehyde (PFA) for 1 hour at room temperature with gentle agitation by Clay Adams Nutator. Following fixation, samples were washed 5 times in phosphate-buffered saline (PBS) and left in respective Eppendorf tubes with 1mL PBS. 1 μL of each stain was added to Eppendorf tubes containing tissue samples, applied sequentially, and incubated for approximately 60 minutes to facilitate permeabilization and stain uptake. After staining, samples were washed five times with PBS and mounted on slides using DOW CORNING high vacuum grease to create a sealed PBS-filled chamber beneath the coverslip, preventing compression of the kelp tissue (Figure 1). Samples were immediately taken for imaging.

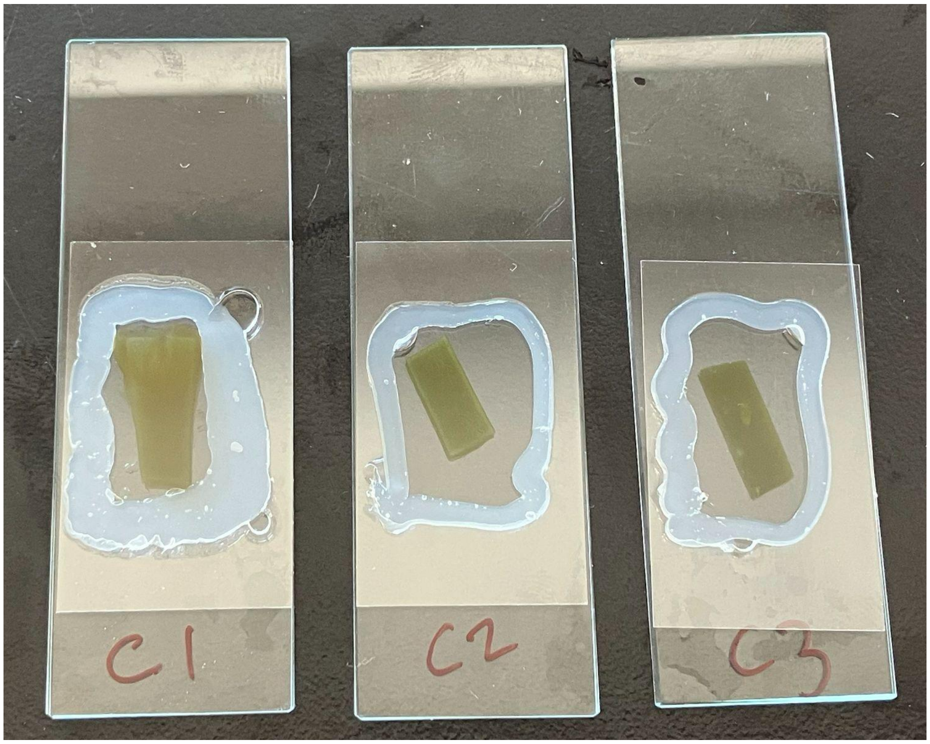

Figure 1. Mounted *Nereocystis luetkeana* blade samples from three regions: C1 (meristem), C2 (mid-blade), and C3 (apex). Tissues were stained with SR2200, SYTOX Green, and Potomac Gold, and sealed under coverslips in PBS using vacuum grease for confocal imaging.

Imaging and Analysis

Samples were imaged on a Zeiss LSM confocal microscope using fluorescence channels appropriate for each stain. Z-stack images were acquired to assess microbial density and spatial distribution across the kelp surface biofilms. Imaging parameters (e.g., laser power, gain, z-step) were kept consistent across all regions to enable qualitative and semi-quantitative comparison of microbial communities on different blade sections.

RESULTS:

No microbial cells were successfully visualized on any tissue samples. Clear imaging of cell walls and nuclei (Figures 2-7) indicate the staining protocol was successful, meaning the biofilm may have been lost during fixation and washing. Structural features of the kelp tissue were clearly captured. In meristematic and mid-blade regions, distinct cell walls and internal DNA compartments were observed. Notably, cells in these zones consistently exhibited four discrete disk-shaped nucleic acid structures. Apical tissue showed fragmented cell walls and diffuse DNA staining, aligning with tissue degradation.

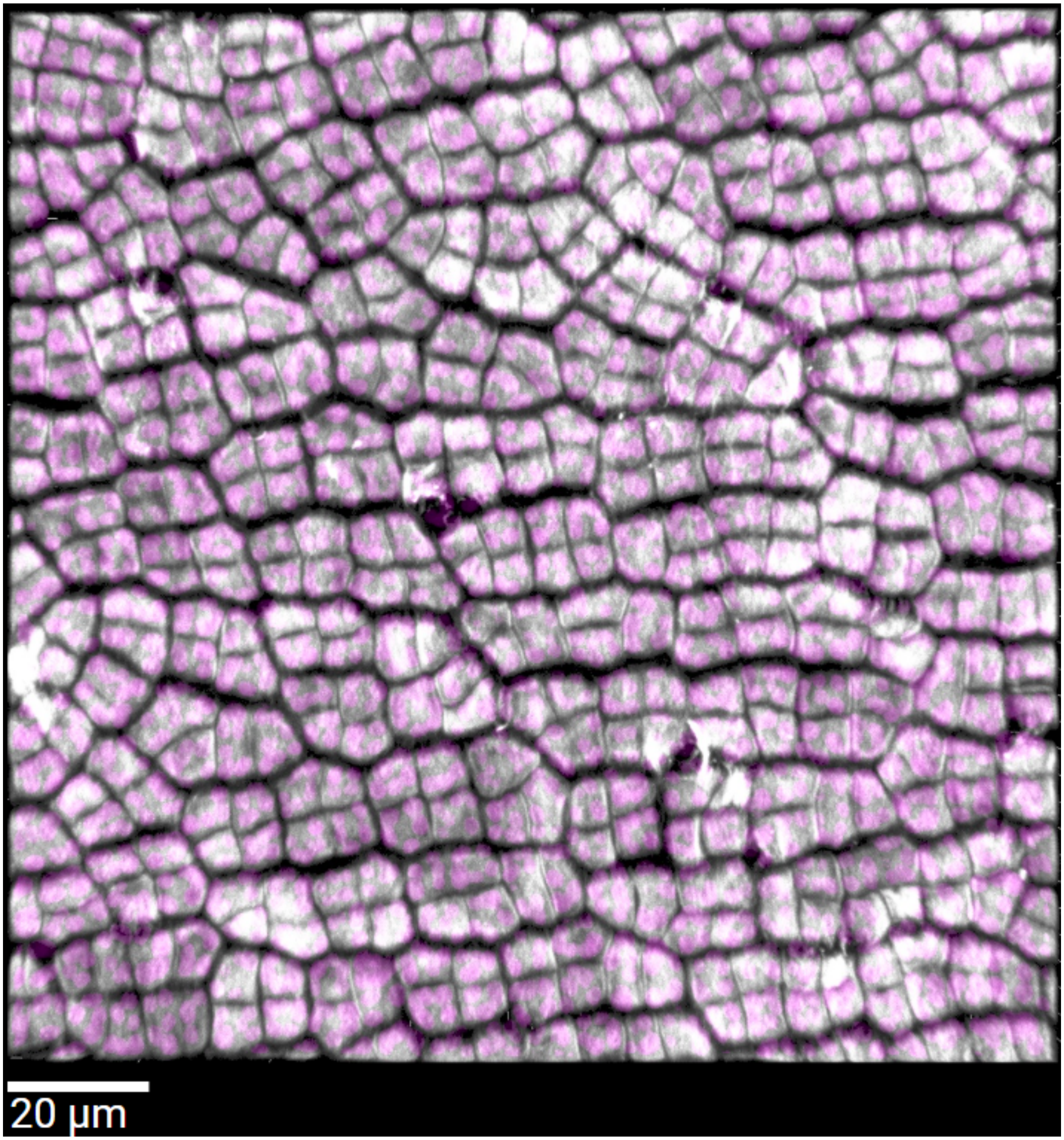

Fig. 2

Confocal fluorescence image of *Nereocystis luetkeana* meristematic blade tissue. Cell walls appear white; nucleic acid structures visualized in pink.

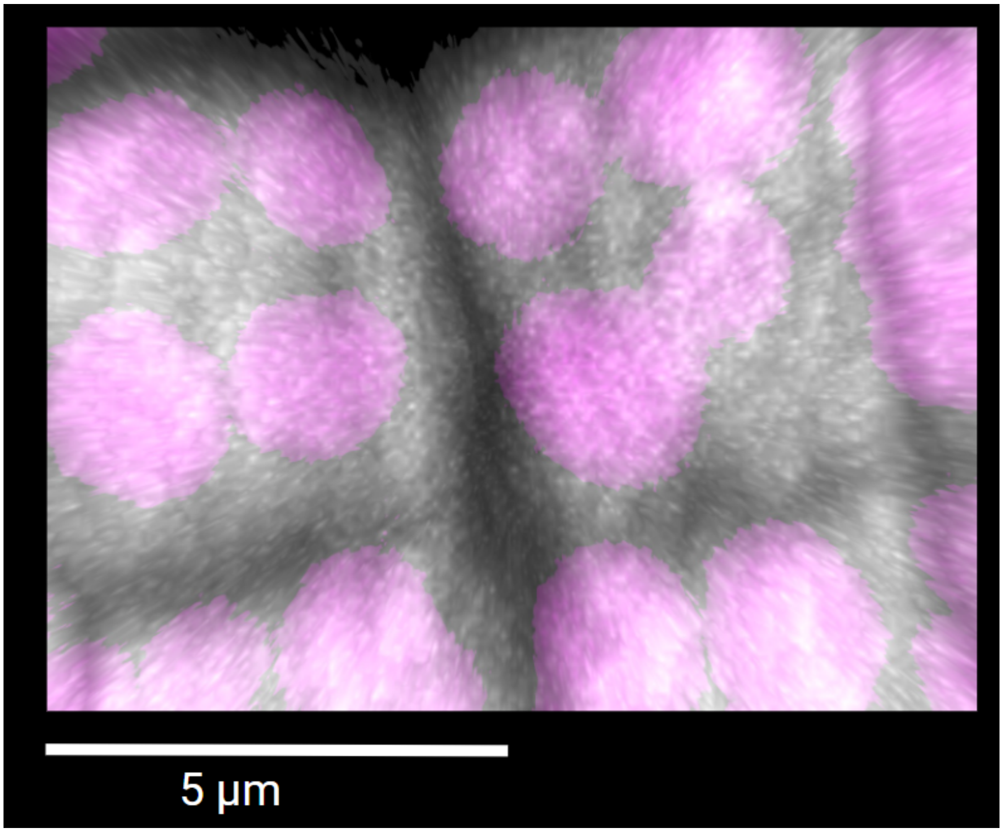

Fig. 3

Zoomed confocal image of *Nereocystis luetkeana* meristem tissue showing adjacent cells, each containing four distinct nucleic acid structures. Cell walls appear white, nucleic material in pink.

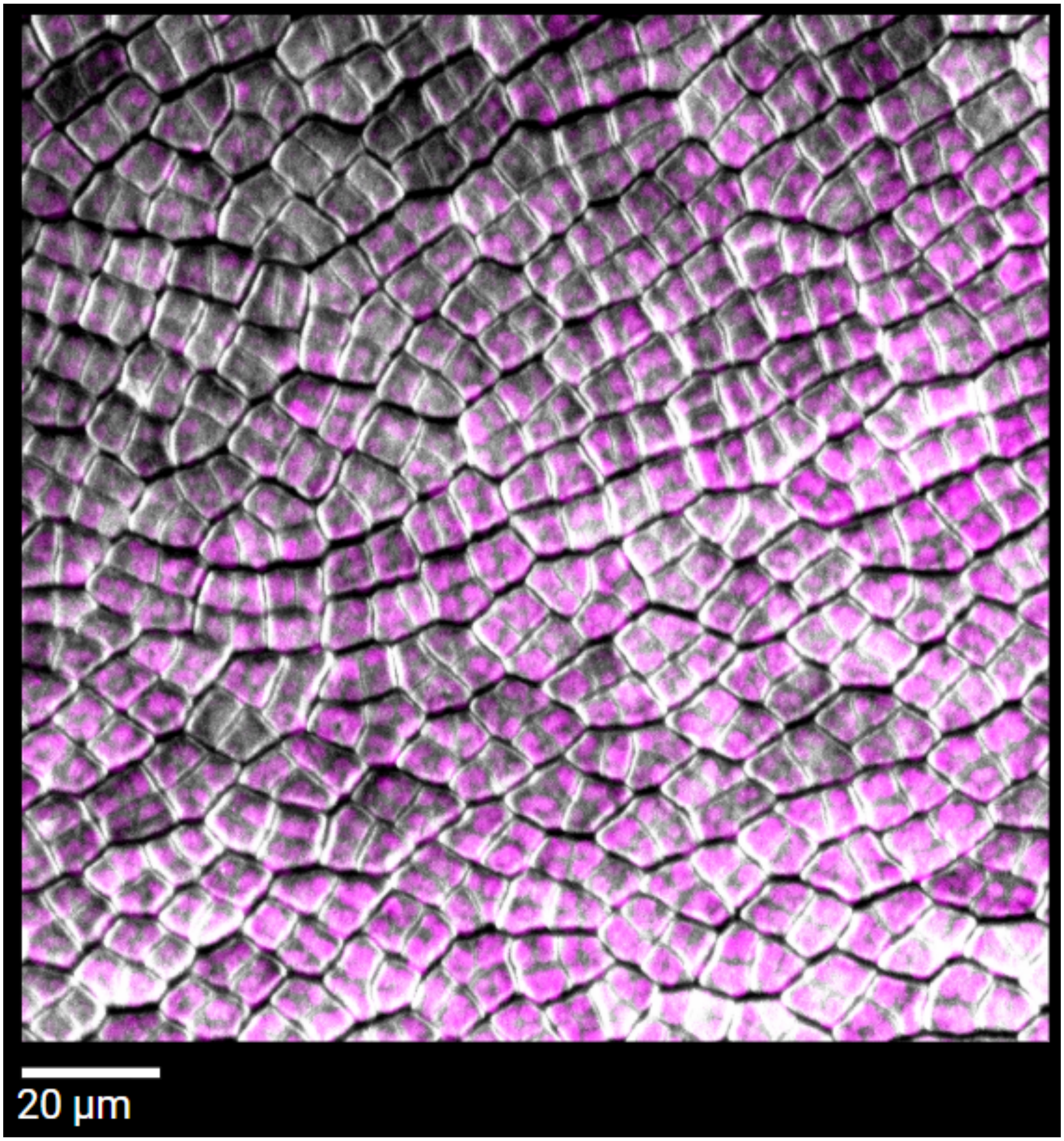

Fig. 4

Confocal image of mid-blade tissue from *Nereocystis luetkeana*, collected ~12 cm from the meristem. Cell walls appear white and nucleic acids visualized in pink. Tissue surface irregularities made Z-stack imaging more difficult, resulting in a slightly uneven focal plane.

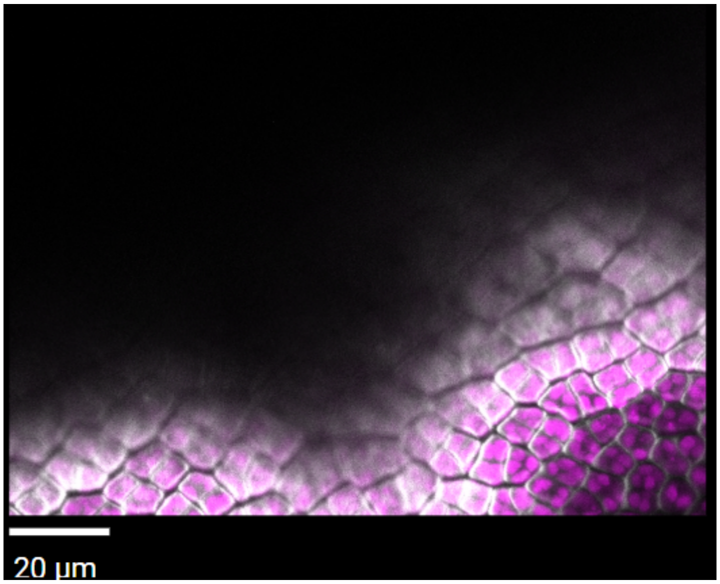

Fig. 5
Single focal plane from the mid-blade tissue of *Nereocystis luetkeana*, imaged via confocal microscopy. Cell walls appear white and nucleic acids visualized in pink. This slice highlights the uneven topography of the blade surface.

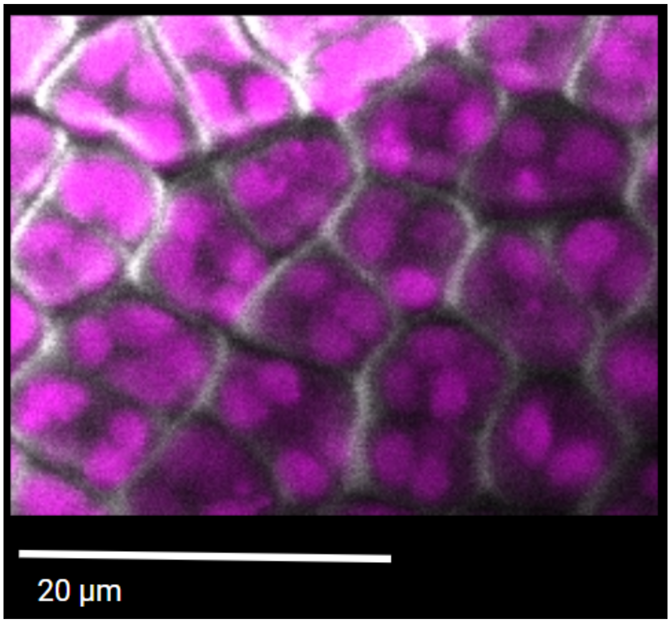

Fig. 6
Close-up of mid-blade *Nereocystis luetkeana* tissue from Figure 5. Cell walls appear white and

nucleic acids visualized in pink.

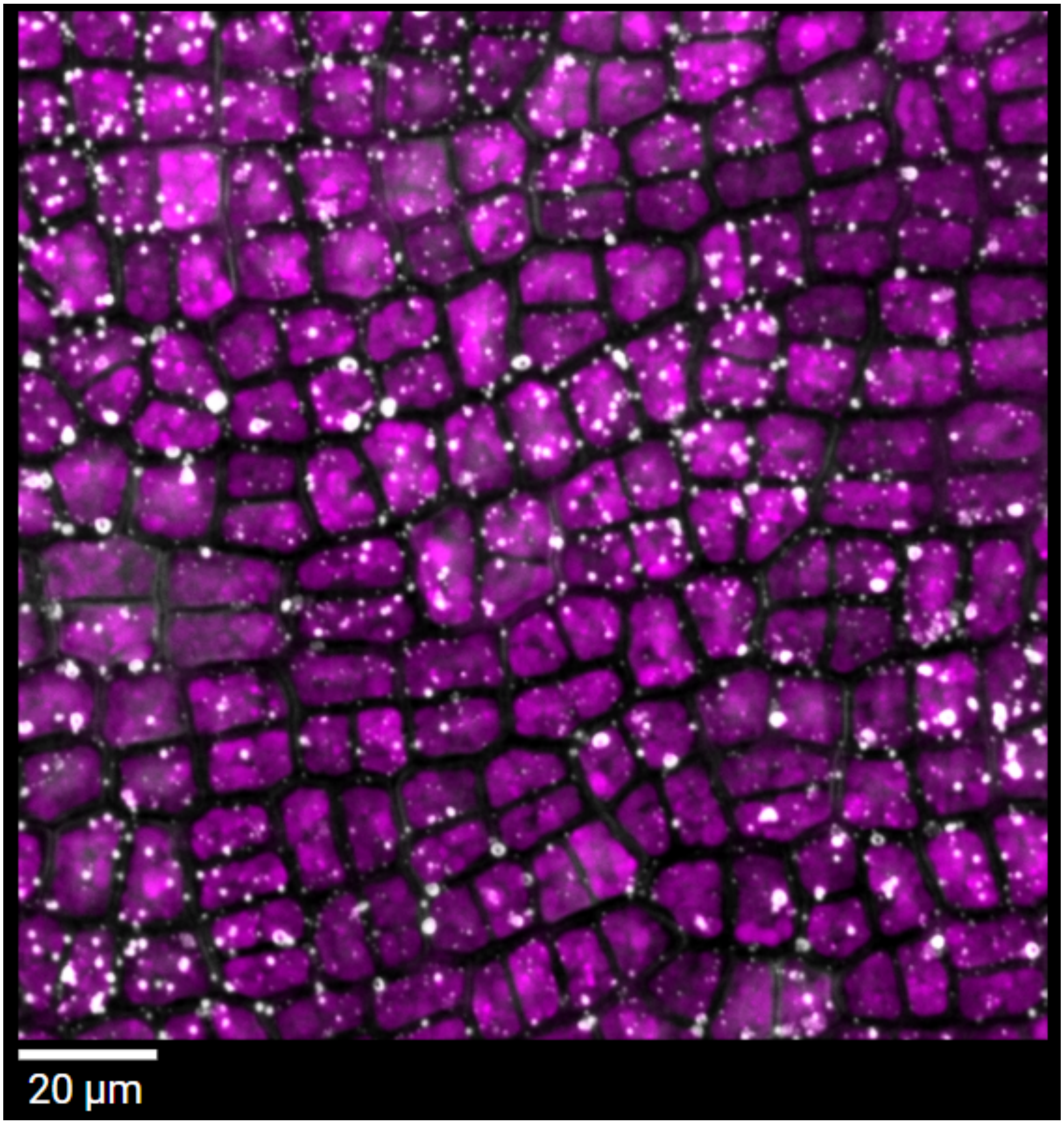

Fig. 7
Apical blade tissue of *Nereocystis luetkeana*, imaged via confocal microscopy. Cellulose appears white, nucleic acids visualized in pink. This pattern is consistent with cell degradation typical of senescing apical tissue.

DISCUSSION:

Our study aimed to visualize microbial biofilms on *Nereocystis luetkeana* blades. Despite successful staining of kelp nucleic acids and cell walls, no microbial cells were detected. I suspect that agitation from the nutator during fixation washed away the biofilm, emphasizing the need for gentler sample handling to preserve surface-bound microbiota. I was unable to gain insights into our hypothesis: the microbial cell density and biofilm thickness will be lowest at the meristem and increase towards the apex, and that biofilm morphology will become increasingly structured along this gradient.

Insights into kelp cellular biology

Mertistem and mid-blade tissue samples displayed clear cell walls and DNA compartmentalization. Individual cells consistently exhibited 4 discrete nucleic acid structures, resembling nuclei. *Nereocystis luetkeana* is known to be uninucleate (Korabik et al., 2025). An explanation for these structures could be that nuclei at a specific phase of mitosis or DNA replication, which we might expect to see in the meristem where there is rapid growth. However, these structures can also be observed in the mid-blade, well beyond the estimated meristematic zone of just a few centimeters (Weigel & Pfister, 2019). This suggests that the observed structures are not nuclei, represent abnormal nuclear division, or result from overlapping cells in the imaging plane.

Apical blade tissue showed faint or fragmented cell walls and dispersed DNA staining. The scattering of the cellulose signal could be misinterpreted as microbial biomass, emphasizing the need for caution when relying solely on structural stains to identify microbes. The faint cell walls and cellulose fragments is likely the result of cell degradation, which is consistent with blade senescence at the apex of the blade (Weigel & Pfister, 2019).

Implications and Future Directions

The visualization of these multinucleate-like structures beyond the meristem may suggest deviations from the known cell cycle. Cell division in *N. luetkeana* blades is localized to meristem at the blade base, with growth confined to this region and little evidence of mitotic activity further along the blade (Weigel & Pfister, 2019). Repeating the experiment with different kelp specimens, particularly from varied geographic locations, could help identify if our results were due to an abnormal specimen. Further studies could utilize DAPI, mitotic markers and time-lapse imaging to explore if these nuclear structures show in process of cytokinesis.

Limitations

This study faced several limitations that impacted the outcome and interpretation of results. Microbial communities were not successfully visualized, likely due to biofilm loss during agitated fixation in paraformaldehyde. Only a single *N. luetkeana* specimen was examined, meaning variability could not be assessed and our results cannot be placed within broader patterns. While fluorescence staining revealed unexpected nuclear structures, our interpretations remain speculative without molecular markers or cell cycle indicators.

Future Research

Future studies should focus on refining sampling and fixation protocols to preserve surface biofilms, setting a framework for reliable imaging of kelp-associated microbes. Utilizing specific molecular markers such as DAPI and EdU would help clarify if observed nucleic structures represent mitotic stages. Expanding sample size and comparing specimens across different geographic locations will also inform if observations are consistent across individual specimens and populations.

Acknowledgments & Attribution

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CITATIONS:

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