fluorescence remains problematic for many sample types. Excitation of nucleic acid–speci c dyes with monochromatic light (e.g., in confocal laser scanning microscopy; CLSM) can reduce such background uorescence, and CLSM is now o en used for direct cell counts and uorescence in situ hybridization (FISH) applications in environmental studies (2,6). However, many commonly used dyes require excitation in the UV range, for which confocal microscopes o en are not properly equipped. In contrast, the monomeric TO-PRO-3 iodide (TP3; 642/661 nm excitation/emission peaks) and the dimeric TOTO-3 iodide (TT3; 642/660 nm) can be excited with a standard CLSM He-Ne 633 nm laser. While not assessed in the present study, they can also be used with light microscopes that are equipped for detection of red uorescence (630–700 nm). ese cyanine dyes display a high a nity for double-stranded nucleic acids in xed cells, uorescing strongly as DNA-dye complexes but only weakly prior to DNA intercalation. TP3 is widely applied to

(2). Novel high-a nity stains, such as SYBR Green, Sytox, or Syto dyes, promise better signal-to-background ratios due to their increased fluorescence when bound to DNA (3–5). Nevertheless, the general issue of nonspeci c background

reduced uorescence. On the other hand, TP3 showed higher sensitivity to photobleaching, which agrees with published results for eukaryotic cells (13,14).

e suitability of TT3 and TP3 for detecting cells within mineral-rich samples subsequently was tested using hydrothermally degraded soils with small particle-sizes and high clay contents (from Mt. Hood, Oregon, USA) (15). When stained with DAPI or SYBR Green I (data not shown for latter), these soils showed strong nonspeci c background uorescence of mineral particles with cell-like sizes and shapes. To evaluate the suitability of TP3 and TT3, sterile soil samples were spiked with bacterial and archaeal cells prior to sood,

during sample collection, and Russell Field for particle size analysis.

Competing interests

e authors declare no competing interests.

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