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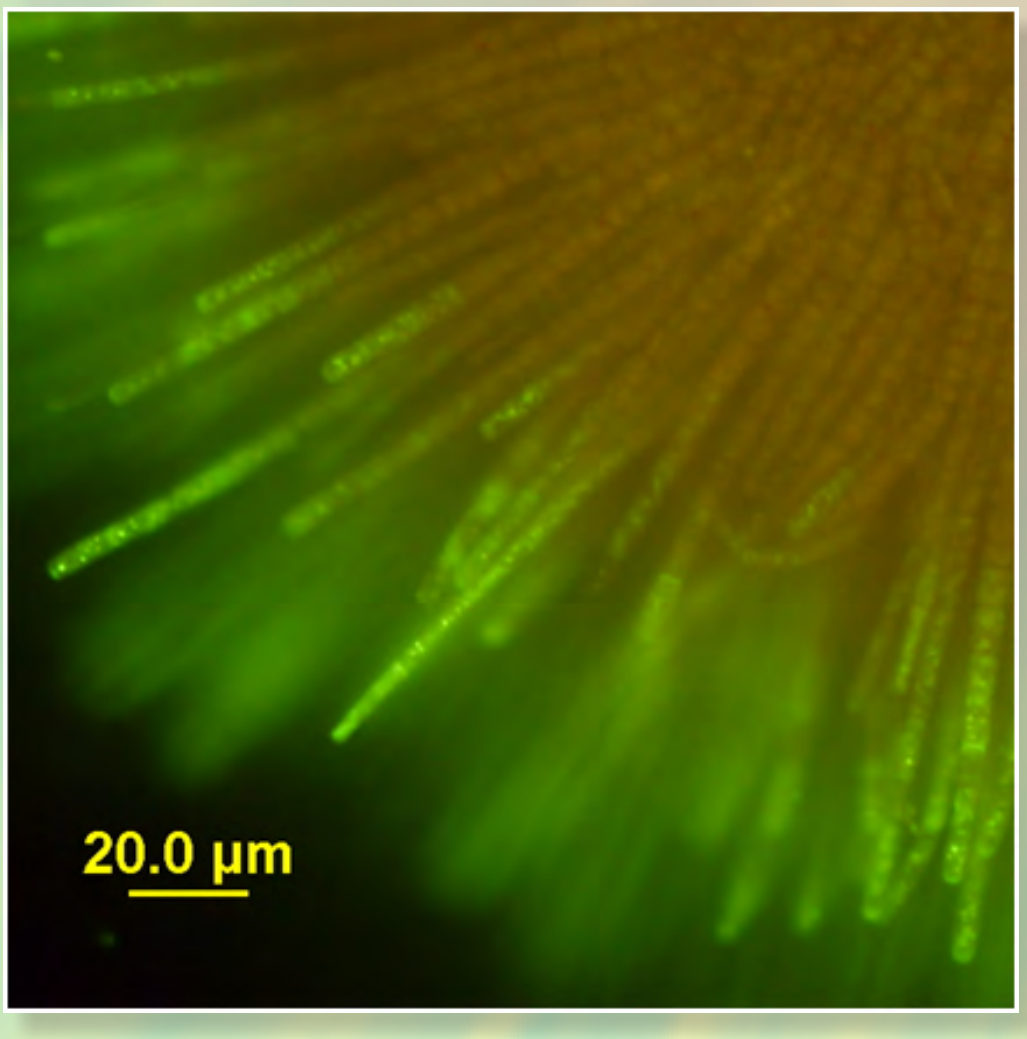
Microphotographs of Cyanobacteria Documenting the Effects of Various Cell-lysis Techniques



Open-File Report 2010–1289

U.S. Department of the Interior
U.S. Geological Survey

Cover: Microphotograph from Upper Klamath Lake, Oregon showing *Gloeotrichia echinulata*.

Microphotographs of Cyanobacteria Documenting the Effects of Various Cell-lysis Techniques

By Barry H. Rosen, Keith A. Loftin, Christopher E. Smith, Rachael F. Lane, and Susan P. Keydel

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KEN SALAZAR, Secretary

U.S. Geological Survey
Marcia K. McNutt, Director

U.S. Geological Survey, Reston, Virginia: 2011

Suggested citation:
Rosen, B.H., Loftin, K.A., Smith, C.E., Lane, R.F., and Keydel, S.P., 2010, Microphotographs of cyanobacteria documenting the effects of various cell-lysis techniques: U.S. Geological Survey Open-File Report, 2010–1289, 203 p.

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Conversion Factors

SI to Inch/Pound

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
micrometer (µm)	3.937×10^{-5}	inch (in.)
meter (m)	3.281	foot (ft)
	Area	
square millimeter (mm^2)	1.55×10^{-3}	square inch (in2)
	Volume	
liter (L)	33.82	ounce, fluid (fl. oz)
liter (L)	2.113	pint (pt)
liter (L)	1.057	quart (qt)
liter (L)	0.2642	gallon (gal)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound avoirdupois (lb)
	Pressure	
kilopascal (kPa)	0.1450	pound per square inch (lb/in^2)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F=(1.8\times°C)+32$$

Specific conductance is given in microsiemens per centimeter at 25 degrees Celsius (µS/cm at 25°C).

Concentrations of chemical constituents in water are given in parts-per-billion (ppb) which is equivalent to micrograms per liter (µg/L).

Other Abbreviations Used in this Report

ELISA	Enzyme-linked immunosorbent assay
OGRL	Organic Geochemistry Research Laboratory, USGS
sp.	The species of the genus was not determined
USGS	U.S. Geological Survey
EPA	U.S. Environmental Protection Agency
psig	Pounds per square inch gauge
%	Percentage

Microphotographs of Cyanobacteria Documenting the Effects of Various Cell-lysis Techniques

By Barry H. Rosen, Keith A. Loftin, Christopher E. Smith, Rachael F. Lane, and Susan P. Keydel

Abstract

Cyanotoxins are a group of organic compounds biosynthesized intracellularly by many species of cyanobacteria found in surface water. The United States Environmental Protection Agency has listed cyanotoxins on the Safe Drinking Water Act's Contaminant Candidate List 3 for consideration for future regulation to protect public health. Cyanotoxins also pose a risk to humans and other organisms in a variety of other exposure scenarios. Accurate and precise analytical measurements of cyanotoxins are critical to the evaluation of concentrations in surface water to address the human health and ecosystem effects. A common approach to total cyanotoxin measurement involves cell membrane disruption to release the cyanotoxins to the dissolved phase followed by filtration to remove cellular debris. Several methods have been used historically, however no standard protocols exist to ensure this process is consistent between laboratories before the dissolved phase is measured by an analytical technique for cyanotoxin identification and quantitation. No systematic evaluation has been conducted comparing the multiple laboratory sample processing techniques for physical disruption of cell membrane or cyanotoxins recovery. Surface water samples collected from lakes, reservoirs, and rivers containing mixed assemblages of organisms dominated by cyanobacteria, as well as laboratory cultures of species-specific cyanobacteria, were used as part of this study evaluating multiple laboratory cell-lysis techniques in partnership with the U.S. Environmental Protection Agency. Evaluated extraction techniques included boiling, autoclaving, sonication, chemical treatment, and freeze-thaw. Both treated and untreated samples were evaluated for cell membrane integrity microscopically via light, epifluorescence, and epifluorescence in the presence of a DNA stain. The DNA stain, which does not permeate live cells with intact membrane structures, was used as an indicator for cyanotoxin release into the dissolved phase. Of the five techniques, sonication (at 70 percent) was most effective at complete cell destruction while QuikLyse™ was least effective. Autoclaving, boiling, and sequential freeze-thaw were moderately effective in physical destruction of colonies and filaments.

Introduction

The purpose of the study is to develop standardized protocols for assessing cyanotoxins in recreational and drinking water. Only one aspect of this overall study is documented for this report; the images captured through microscopy of cells subjected to various extraction (lysing) procedures.

Several species of cyanobacteria have the ability to produce cyanotoxins (toxins) that pose a potential threat to terrestrial and aquatic life, including humans (Chorus and Bartram, 1999). Quantifying these toxins includes the extraction or cell lysis of compounds from within cyanobacterial cells where the toxins are stored; however, the effectiveness of cell lysis is not a simple or well understood procedure (Chorus and Bartram, 1999; Loftin and others, 2008). Cyanobacteria exhibit a great variety of cellular features and morphology, and while these features for example polysaccharide sheath are used to identify these organisms, they also can interfere with the commonly used approaches for extracting compounds. No literature exists, to the authors' knowledge, which demonstrates how colony and filament morphology affects the extraction of cyanotoxins. United States Environmental Protection Agency (USEPA) Region 9 (San Francisco, California) initiated this study, under the Regional Methods Program, which is being conducted by U.S. Geological Survey (USGS), in coordination with USEPA's Office of Science Policy, Office of Research and Development.

Methods

Two laboratory cultures, each consisting of an isolated cyanobacterial genus, as well as 12 field samples collected from water bodies in six different states during 2009 were used for this research. Grand Lake, Ohio, was sampled twice on two separate dates (July and September).

1. Field Samples

Twelve grab samples of various cyanobacterial cell densities were collected at the air/water interface from the locations listed in table 1 and shipped to the USGS Organic Geochemistry Research Laboratory (OGRL), Kansas Water Science Center for processing. Approximately, 8 liters (L) of sample from each site was composited, mixed, and split from an 8 L Teflon churn and then lysed by separate techniques and treatment levels at the USGS OGRL (table 2). Lysed and unlysed (control) samples were then shipped overnight on ice in small glass vials, refrigerated on arrival and examined microscopically the following day by the USGS Florida Integrated Science Center (Orlando, Fla.). The lysis or extraction treatments used are listed in table 2, along with the codes used to label each electronic image; a detailed description of the methodology and rationale for extraction procedures will be the subject of a separate publication.

2. Laboratory Cyanobacterial Cell-Lysis Techniques

Five different cyanobacterial cell-lysis techniques were used to evaluate effectiveness for disrupting cell membranes: boiling, autoclave, sonication, QuikLyse™ and freeze-thaw. Approximately, 8 L of sample from each site was composited, mixed, and split from an 8 L Teflon churn and then lysed by separate techniques and treatment levels (table 2). All cell lysis techniques had three treatment levels based on time for boiling and autoclave, power for sonication, or repetition for sequential freeze-thaw cycles. QuikLyse™ (Abraxis, LLC, Warminster, PA., USA) was used at the manufacturer recommended concentration for all samples and, in some cases, two and three times the recommended dose was used for a given sample volume to determine if reagent concentration changed effectiveness.

Boiling Technique

Fifty milliliter (mL) sample aliquots contained in 123 mL amber glass, Boston round bottles with caps loosened were placed in boiling reagent water (generated by a ThermoScientific Barnstead Nanopure® Diamond TM TOC Life Science ultrapure water treatment system-Model D11971, conductivity = 5.500 microsiemens per centimeter (µS/cm), total organic carbon less than 1 part per billion (ppb)) for 5, 15, or 30 minutes before microscopic examination at atmospheric pressure at 296 meters (m) above sea level (Google Earth 5.1, Lawrence, Kans.).

Autoclave Technique

Fifty milliliter (mL) sample aliquots contained in 123 mL amber glass, Boston round bottles with caps (Fisher Scientific, Pittsburgh, PA, USA) loosened were autoclaved (Sterilmatic, Market Forge Industries., Everett, MA, USA) for 5, 15, or 30 minutes (min) at 2.03 kPa, 120 degrees Celsius (°C), and a setting of slow before microscopic examination.

Sonication

Fifteen mL-sample aliquots in 30 mL amber glass, Boston round bottles were sonicated for 5 min using a Model 102C, 0.5 inch disruptor horn coupled to a 0.125 inch (in) tapered microtip horn (Fisher Scientific, Pittsburgh, PA, USA) connected to a digital ultrasonic dismembrator (Model 500, Fisher Scientific, Pittsburgh, PA, USA) where power was varied from 10, 35, and 70 percent (%) in separate samples. Samples were incubated for five min in an ice bath before sonication. Samples were sonicated in an ice bath with sample completely immersed below the ice bath surface inside a Branson sonifier sound enclosure (Fisher Scientific, Pittsburgh, Pa., USA). Sample temperature never exceeded 25°C. The microtip horn was placed directly in the sample and the horn was then cleaned in between samples once with methanol, once with methanol and 0.1% tetrahydrofuran (with inhibitor), once with reagent water, and finally wiped with a clean Kimwipe® (Fisher Scientific, Pittsburgh, PA, USA).

Chemical Lysis

The QuikLyse™ lysis reagents were used as recommended by the manufacturer for the “1 x treatments” (1x implies that the reagent dosages were used as recommended by manufacturer, Abraxis, LLC, 2008; Loftin and others, 2008). Separate sample aliquots (1 mL in a 2-mL amber glass vial) were also subjected to 2 and 3 times (2x and 3x, respectively) the manufacturer recommended reagent concentrations before microscopic examination.

Freeze-Thaw Technique

One freeze-thaw cycle consisted of freezing 50 mL sample aliquot in 123 mL amber glass Boston round bottles at 20°C for as many as 24 hours until frozen solid followed by a thawing step at room temperature (e.g. ranging from 25 to 30°C) for as many as 12 hours (Loftin and others, 2008). Treatment consisted of 1, 2, or 3 complete freeze-thaw cycles before microscopic examination.

3. Laboratory Cultures

Lyngbya sp. and *Phormidium* sp. initially were isolated by the Metropolitan Water District of Southern California (Izaguirre and Taylor, 2004) and subsequently maintained by USGS-Florida and were used for this research. The two genera of filamentous cyanobacteria were grown as unialgal cultures in liquid medium using standard culturing techniques (Zimmerman and Rosen, 1992).

Table 1. Sample location and taxa of the digital images in this report.

[sp., species; CA, California; FL, Florida; IA, Iowa; OH, Ohio; OR, Oregon; St., Saint]

Sample site (sample collection date)	Cyanobacteria taxa photographed	Cyanobacterial morphology
Cassidy Lake, WA (10/12/2009)	*Woronichinia naegeliana*	Colonial
	Microcystis sp.	Colonial
Spring Lake, CA (8/21/2009)	*Microcystis aeruginosa*	Colonial
	Microcystis wesenbergii	Colonial
	Woronichinia sp.	Colonial
Blackhawk Lake, IA (8/26/2009)	*Planktothrix* sp.	Filamentous
	Filamentous cyanobacteria	Filamentous
	Microcystis sp.	Colonial
Copco Reservoir, CA (9/10/2009)	*Microcystis wesenbergii*	Colonial
	Microcystis aeruginosa	Colonial
	Filamentous cyanobacteria	Filamentous
Grand Lake (Lake St. Mary), OH (7/20/2009)	*Planktothrix* sp.	Filamentous
Grand Lake (Lake St. Mary), OH (9/15/2009)	*Planktothrix* sp.	Filamentous
	Filamentous cyanobacteria	Filamentous
	Colonial cyanobacteria	Colonial
St. John's River, Jacksonville, FL (9/15/2009)	*Microcystis aeruginosa*	Colonial
Upper Klamath Lake, OR (8/21/2009)	*Aphanizomenon flos-aquae*	Filamentous
	Microcystis aeruginosa	Colonial
	Gloeotrichia echinulata	Filamentous colony
Klamath River, OR (8/21/2009)	*Microcystis aeruginosa*	Colonial
Iron Gate Reservoir, OR (8/25/2009)	*Microcystis* sp.	Colonial
Pinto Lake, CA (9/22/2009)	*Microcystis* sp.	Colonial
	Microcystis wesenbergii	Colonial
	Aphanizomenon flos-aquae	Filamentous
	Planktothrix sp.	Filamentous
Laboratory cultures		
Lyngbya DVL 1103B	*Lyngbya* sp.	Filamentous
Phormidium DVL 706A	*Phormidium* sp.	Filamentous

Table 2. Extraction procedures and codes for digital images.

[%, percent; x represents the number of times the recommended dosage was multiplied compared with manufacturer instructions]

Treatment	Identifier on Image Caption
Control (live material)	
Replicate 1	Control 1
Replicate 2	Control 2
Replicate 3	Control 3
Boiled	
5 minutes	Boiled for 5 minutes
15 minutes	Boiled for 15 minutes
30 minutes	Boiled for 30 minutes
Autoclaved	
5 minutes	Autoclaved for 5 minute
15 minutes	Autoclaved for 15 minute
30 minutes	Autoclaved for 35 minute
Sonicated (5 minutes)	
10% power	Sonicated at 10%
35% power	Sonicated at 35%
70% power	Sonicated at 70%
Freeze-thaw	
one cycle	One freeze-thaw cycle
two sequential cycles	Two freeze-thaw cycles
three sequential cycles	Three freeze-thaw cycles
QuikLyse™	
recommend concentration (1x)	QuikLyse™-1x
2x recommended concentration	QuikLyse™-2x
3x recommended concentration	QuikLyse™-3x

4. Microscopy and Staining

Samples were placed upright for a minimum of 10 min before sampling. A plastic disposable pipette was used to withdraw a sub-sample from the bottom of the sample vial and placed on an ethanol-washed glass slide. A number 1, 22 square millimeter (mm^2) glass cover slip (Thermo Fisher Scientific, USA) was placed over the sample and any excess water was blotted to prevent movement of the cyanobacteria when observed under the microscope.

Samples initially were observed and photographed using differential interference contrast microscopy using an Olympus BX-51 research microscope (Center Valley, Pa., USA) typically at 400x; a 20 micrometer (µm) bar is imbedded in most images (image identifying code-LM). The same cells were then examined and photographed under epifluorescent microscopy, with a U-MWB2: WIDE BLUE CUBE, excitation between 450-80 nanometer (nm) and emission above 515 nm and xenon – X-Cite Series 120Q as the illumination source (image identifying code-FITC). With this configuration, chlorophyll a appears as a deep red color. When other pigments are present, such as phycocyanin and phycoerythrin, the overall appearance of the cells appeared orange-red. Damaged pigments appear yellow. Images for each treatment/microscopic technique combination were captured in digitally and summarized in table 3. The material on the microscope slide was then stained with 10 microliter (µL) Sytox® Green (Invitrogen Corp., Carlsbad, Calif., USA) nucleic acid stain (S7020) – (Stock solution: 25 microgram (µg)/100 mL of 100% reagent-grade ethanol) (image identifying code-Sytox® green). Stain was applied by removing the cover slip, adding the Sytox® green, returning the cover slip, and incubating at 25 to 30°C (room temperature) for 5 min. The colonies and cells were then examined and photographed with the same epifluorescent microscope settings as above. If the stain was able to penetrate the cell membrane, as found in many of the treatments, the cells fluoresced a bright green. This was indicative that the cell membrane had lost its integrity.

Table 3. Sample inventory of digital photomicrographs.

[CA, California; FL, Florida; IA, Iowa; OH, Ohio; OR, Oregon; St., Saint; Boiled and autoclaved were time in minutes; sonicated was percent (%) of power; freeze-thaw x indicates how many times the sample was frozen and thawed; and QuikLyse™ 1x indicates the manufacturers recommended concentration, 2x is twice the concentration and 3x is three times the recommend concentration; - -, indicates no data]

Sample inventory (sample collection date)	Control			Boiled			Autoclaved			Sonicated			Freeze-thaw			QuikLyse™		
	1	2	3	5	15	30	5	15	30	10%	35%	70%	1x	2x	3x	1x	2x	3x
Cassidy Lake, WA (10/12/2009)	x	x	x	x	x	x	x	x	x	x	x	x	x	- -	x	- -	- -	- -
Spring Lake, CA (8/21/2009)	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	- -
Blackhawk Lake, IA (8/26/2009)	x	x	x	x	- -	x	x	x	x	x	x	x	x	x	x	x	- -	- -
Copco Reservoir, CA (9/10/2009)	x	x	x	x	x	x	x	- -	- -	x	x	x	x	x	x	x	- -	- -
Grand Lake (Lake St. Mary), OH (7/20/2009)	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	- -
Grand Lake (Lake St. Mary), OH (9/15/2009)	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	- -	- -
St. John's River, Jacksonville, FL (9/15/2009)	x	x	x	x	x	x	x	x	- -	x	x	x	x	x	x	x	x	- -
Upper Klamath Lake, OR (8/21/2009)	x	x	x	x	x	x	x	x	x	x	x	x	x	- -	x	x	- -	- -
Klamath River, OR (8/21/2009)	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	- -	x	x	x	- -	- -
Iron Gate Reservoir, OR (8/25/2009)	x	x	- -	x	x	x	x	x	x	x	x	x	x	x	x	x	- -	- -
Pinto Lake, CA (9/22/2009)	x	x	x	x	x	x	x	x	x	x	x	x	x	x	- -	x	- -	- -
Laboratory Culture-*Lyngbya* DVL 1103B	x	- -	- -	x	x	x	x	x	x	x	x	- -	x	x	x	x	x	x
Laboratory Culture-*Phormidium* DVL 706A	x	- -	- -	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x

[1] Cyanobacterial morphology defined in Wehr and Sheath, 2003, Van de Hoek and others, 1995.

[2] *Lyngbya* DVL 1103B and *Phormidium* DVL 706A are cultures transferred from original samples acquired in study by Izaguirre and Taylor, 2004.

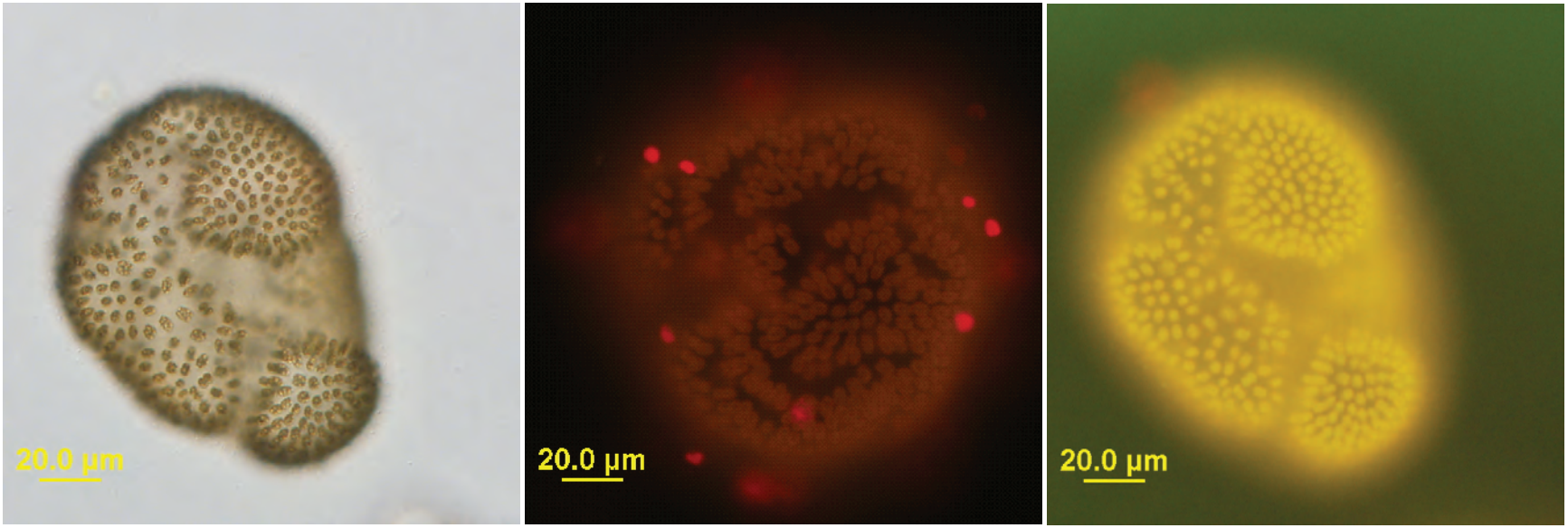


Figure 1. Cassidy Lake, WA (10/12/2009). LM-*Woronichinia naegeliana*, a colonial cyanobacterium with cells in a gelatinous matrix (synonym *Coelosphaerium*). FITC-a dull reddish color dominates the cells. The scattered bright red cells are eukaryotic algal epiphytes. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

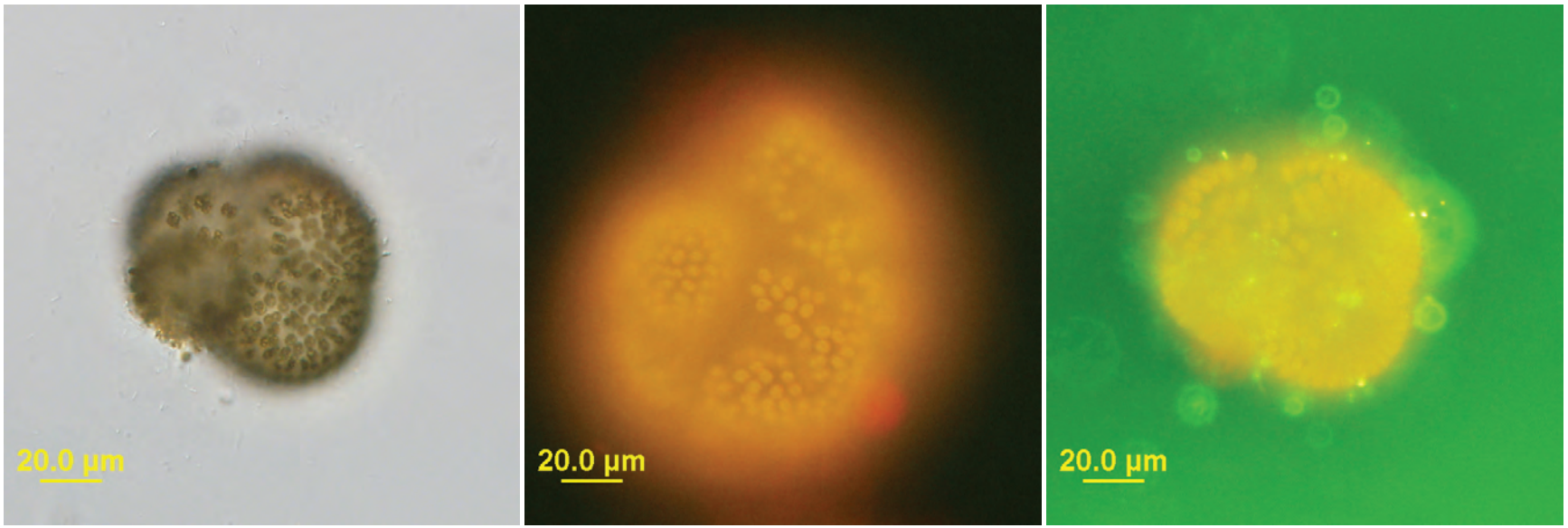


Figure 2. Cassidy Lake, WA (10/12/2009). LM-*Woronichinia naegeliana*, cells in a gelatinous matrix. FITC-a yellow-orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

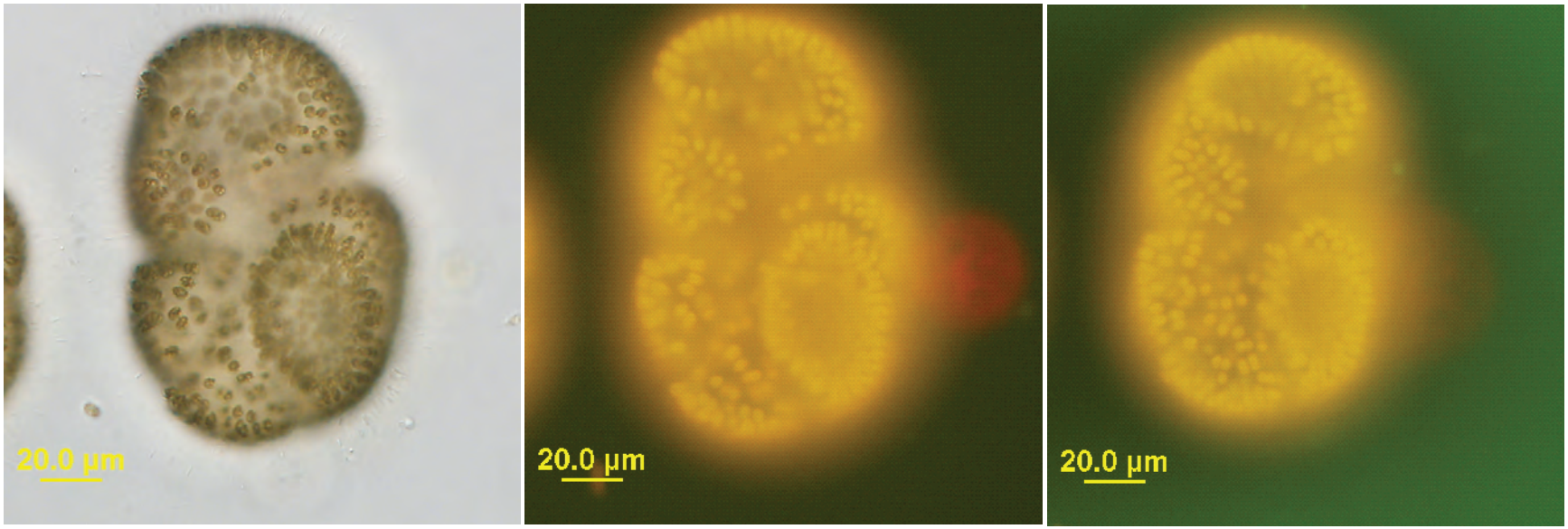


Figure 3. Cassidy Lake, WA (10/12/2009). LM-*Woronichinia naegeliana*, cells in a gelatinous matrix. FITC-a yellow-orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

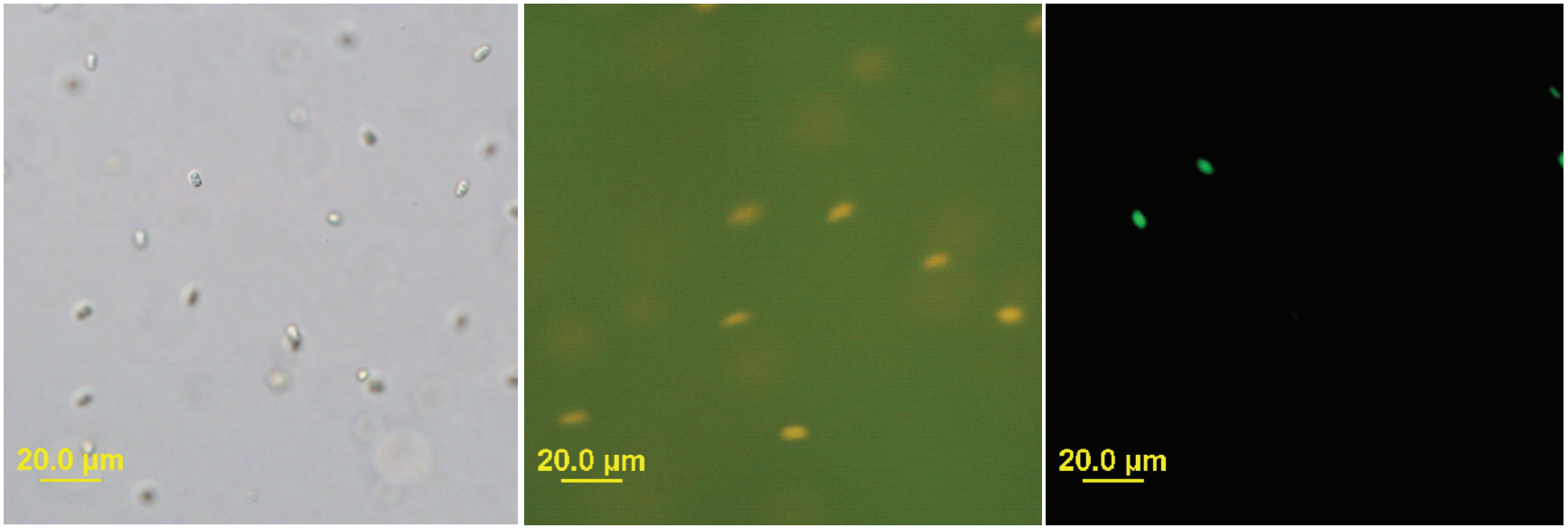


Figure 4. Cassidy Lake, WA (10/12/2009). LM-Likely the remains of a *Woronichinia naegeliana* colony, with only scattered cells and no colonies. FITC-a yellow color dominates the cells; cell shape distorted because of long photographic exposure time. Sytox® green-stain penetrated cell membranes; bright green cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

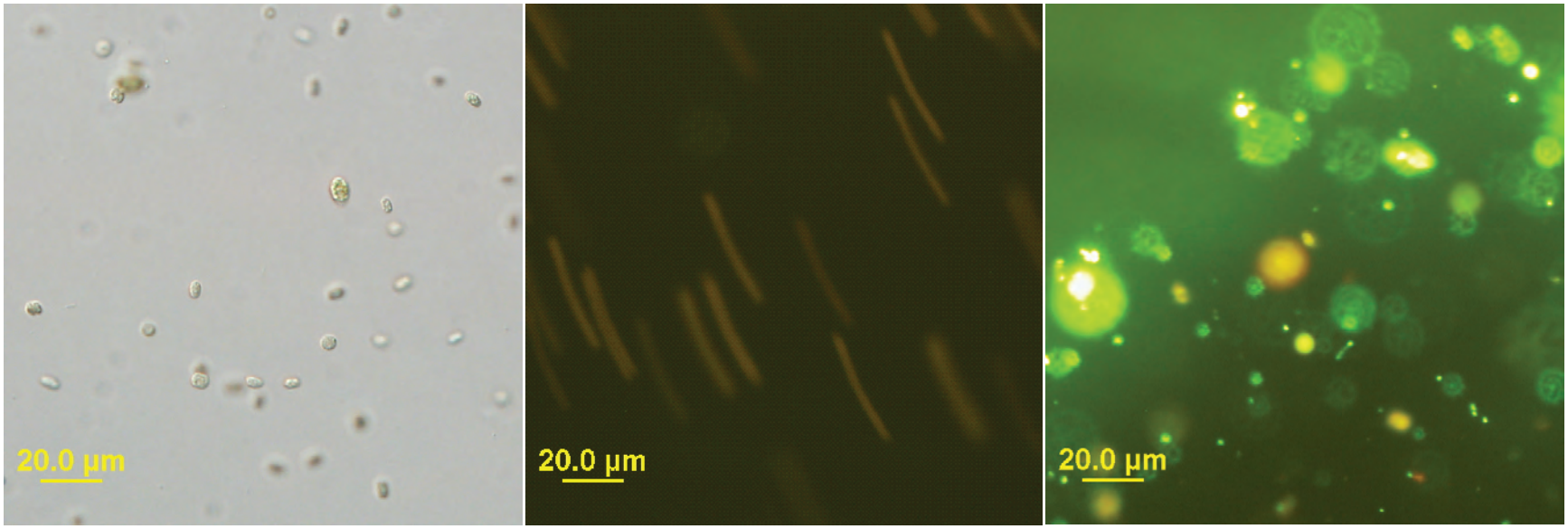


Figure 5. Cassidy Lake, WA (10/12/2009). LM-Likely the remains of a *Woronichinia naegeliana* colony, with only scattered cells and no colonies. FITC-a yellow-orange color dominates the cells; cell shape distorted because of long photographic exposure time. Sytox® green-stain penetrated cell membranes; bright green cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

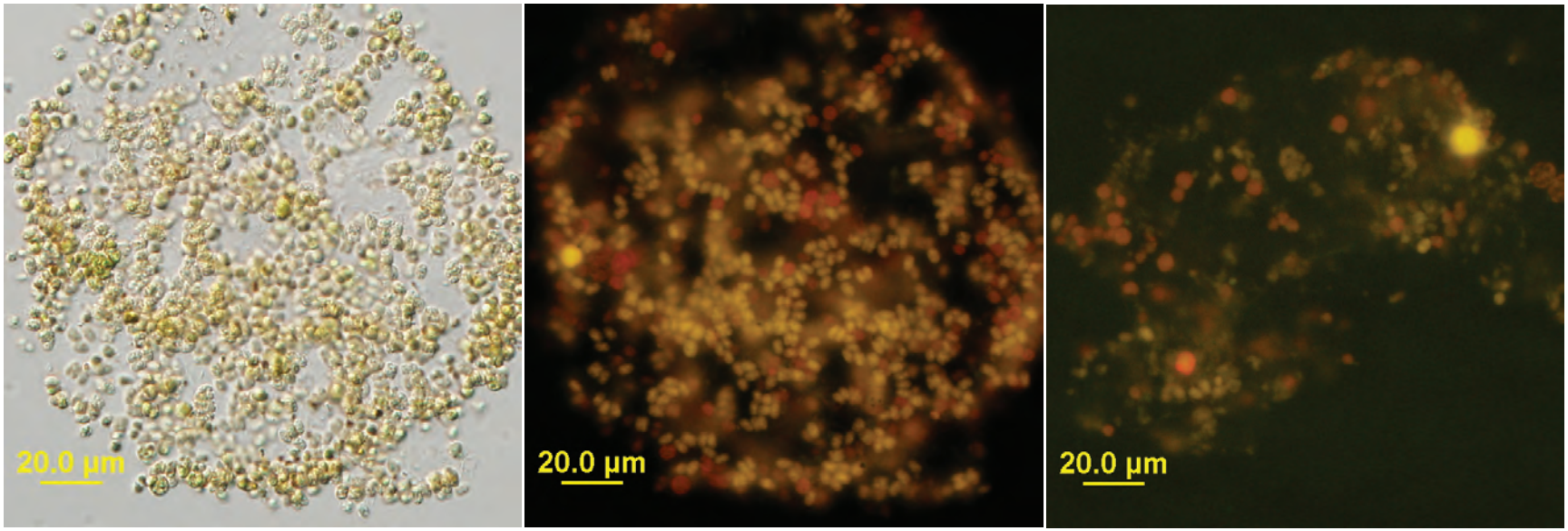


Figure 6. Cassidy Lake, WA (10/12/2009). LM-Likely the remains of a degraded colony of *Microcystis aeruginosa*. FITC-a yellow color dominates the cells of the *Microcystis* sp.; red cells are likely eukaryotic algae. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

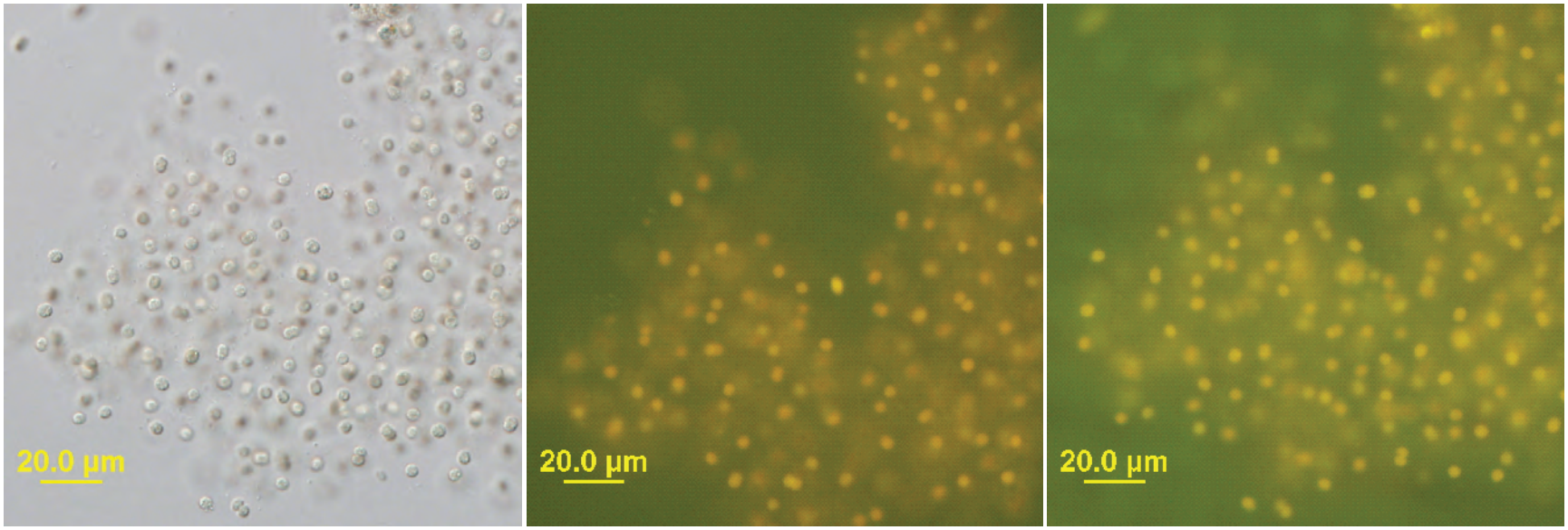


Figure 7. Cassidy Lake, WA (10/12/2009). LM-Likely the remains of a *Microcystis* sp. FITC-a yellow-orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

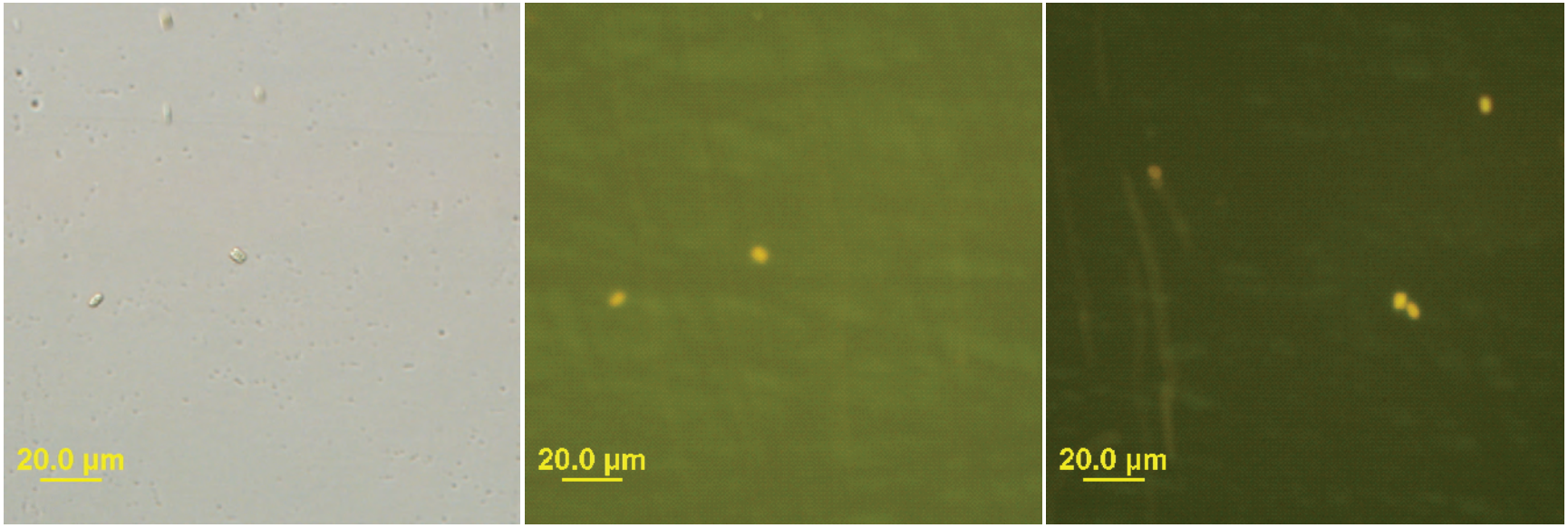


Figure 8. Cassidy Lake, WA (10/12/2009). LM-Likely the remains of a colonial cyanobacterium. FITC-a yellow-orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

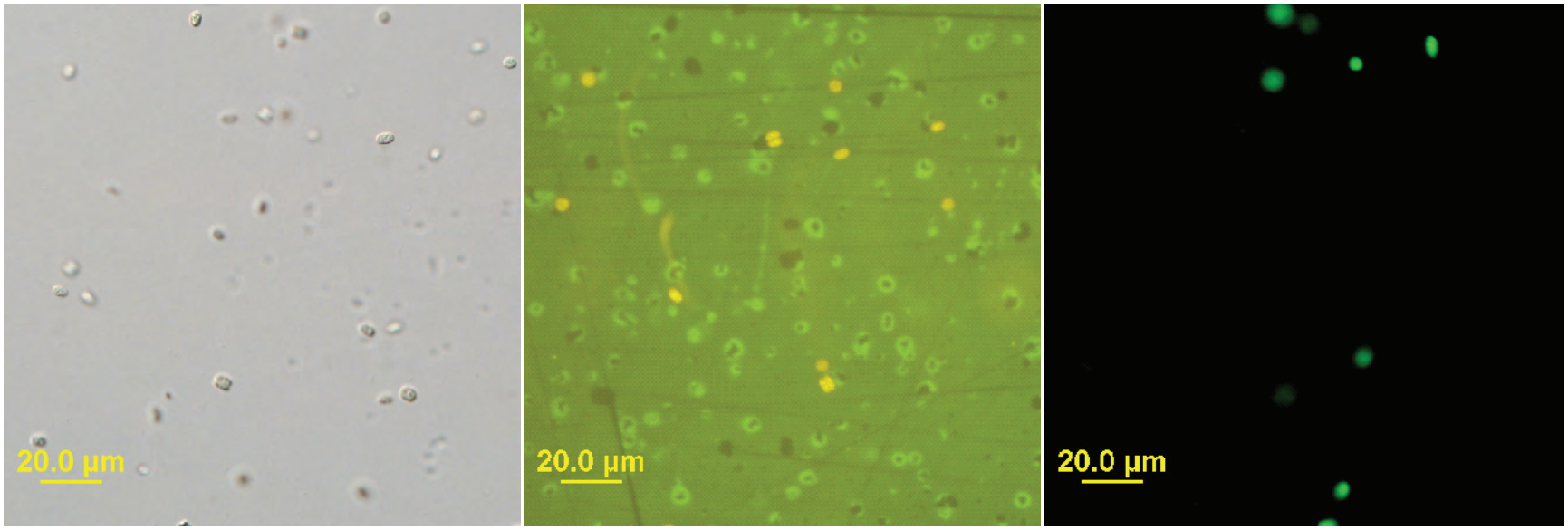


Figure 9. Cassidy Lake, WA (10/12/2009). LM-Likely the remains of a colonial cyanobacterium. FITC-a yellow-orange color dominates the cells. Sytox® green-stain penetrated cell membranes; bright green cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

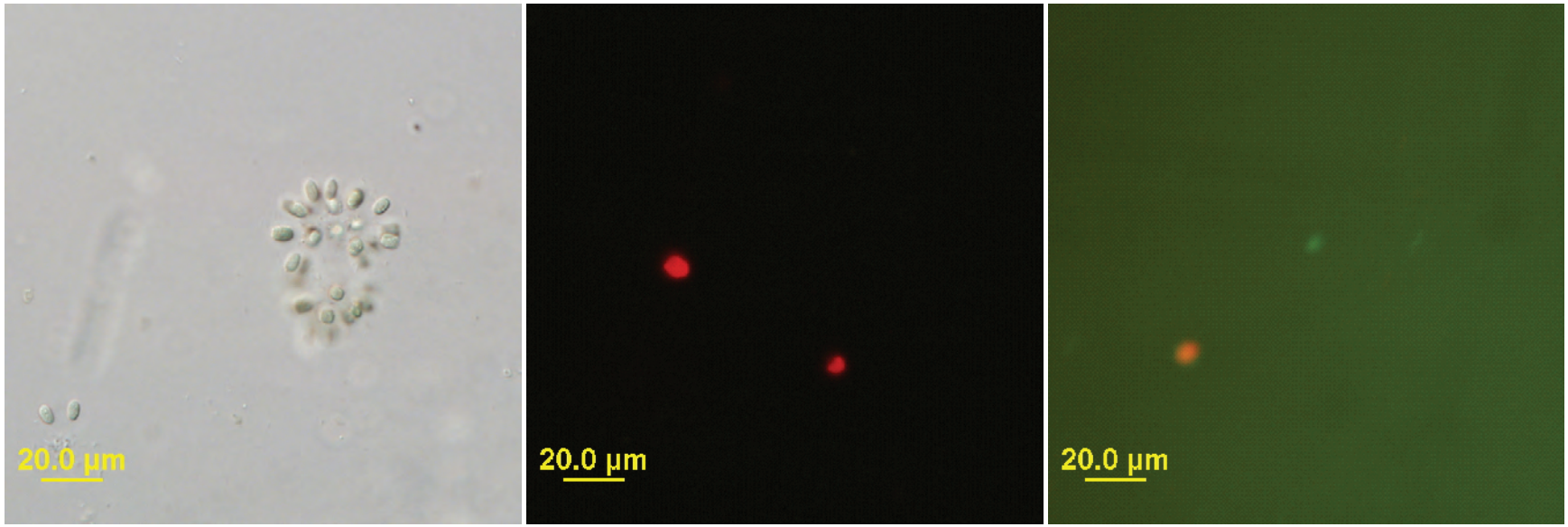


Figure 10. Cassidy Lake, WA (10/12/2009). LM-A colonial cyanobacterium. FITC-unknown organism; the cells were bright red. Sytox® green-unknown organisms; a single cell was observed and it was intact; stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

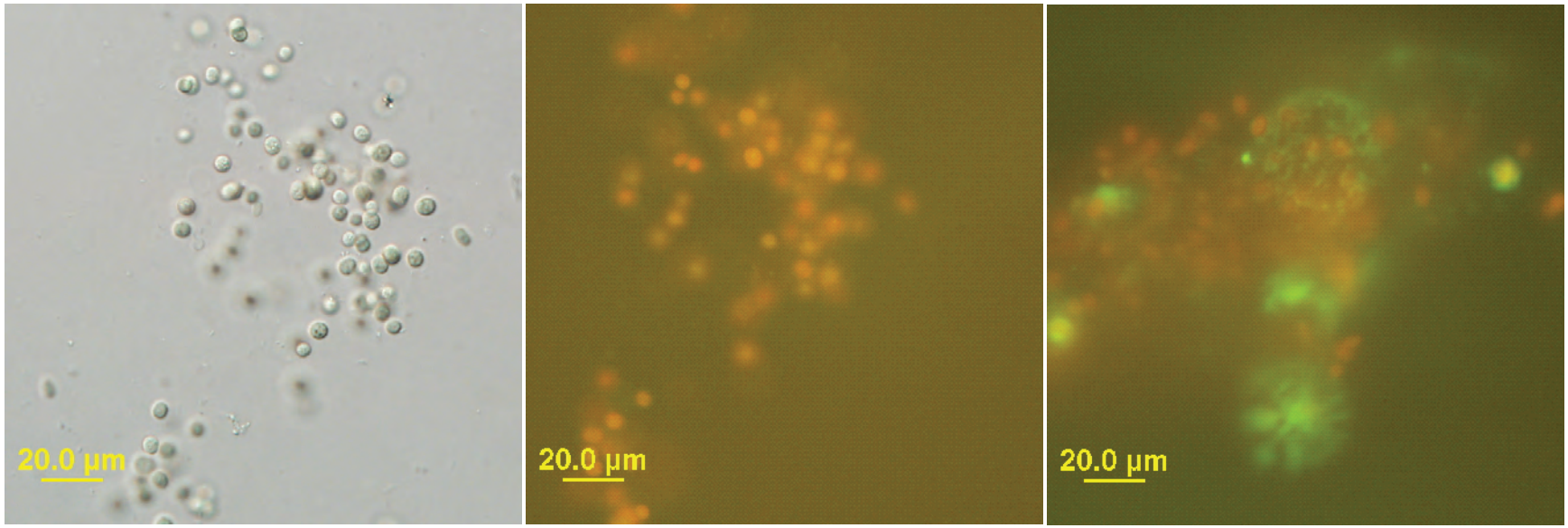


Figure 11. Cassidy Lake, WA (10/12/2009). LM-The remains of a colonial cyanobacterium. FITC-a red-orange color dominates the cells. Sytox® green-stain did not penetrated cell membranes of the cyanobacteria. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

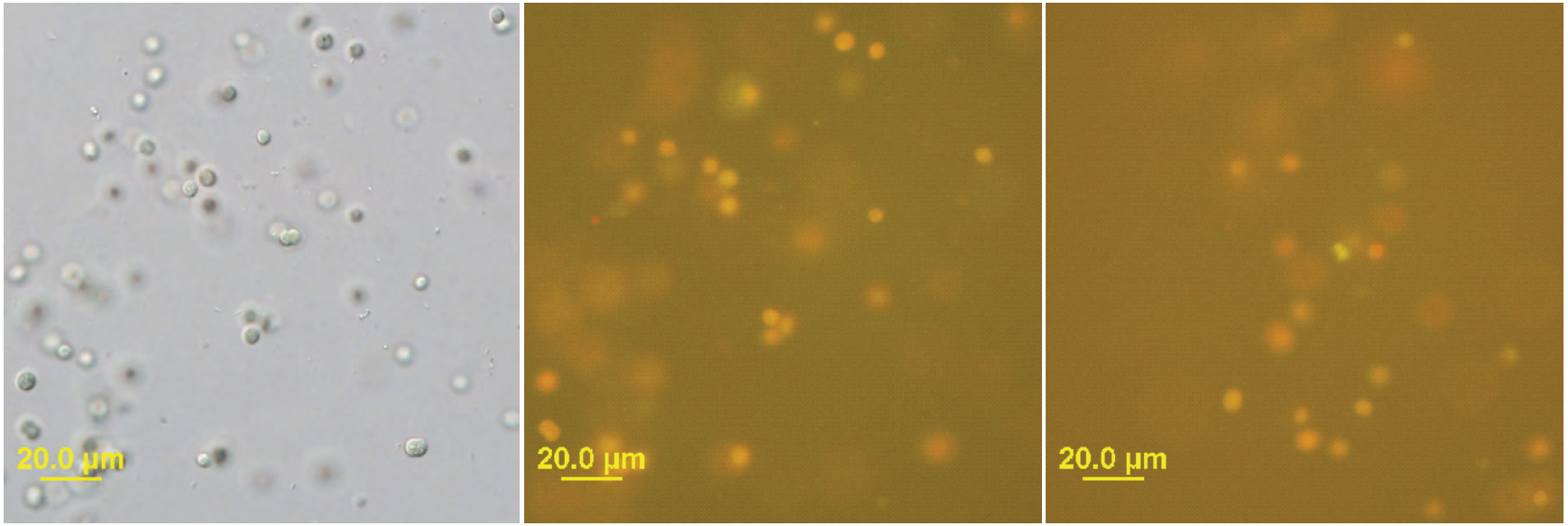


Figure 12. Cassidy Lake, WA (10/12/2009). LM-The remains of a colonial cyanobacterium. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate cell membranes of the cyanobacteria. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

Figure 13. Cassidy Lake, WA (10/12/2009). LM-The remains of colonial cyanobacteria. FITC-no image or data collected. Sytox® green-stain penetrated the cell membrane of this unknown organism; bright green cell. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

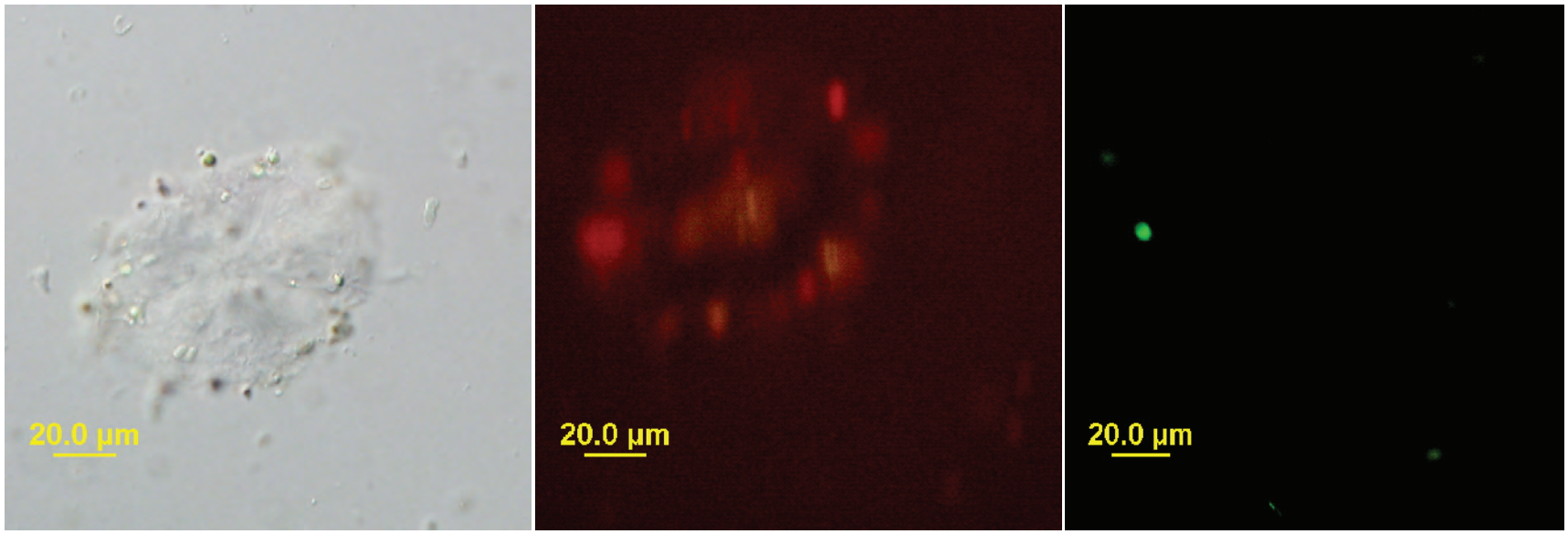


Figure 14. Cassidy Lake, WA (10/12/2009). LM-The remains of a colonial cyanobacterium; note, the mucilage that supports the cell remains. FITC-a red-orange color dominates the cells; cell shape distorted because of long photographic exposure time. Sytox® green-stain penetrated the cell membrane of this unknown organism; bright green cell. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

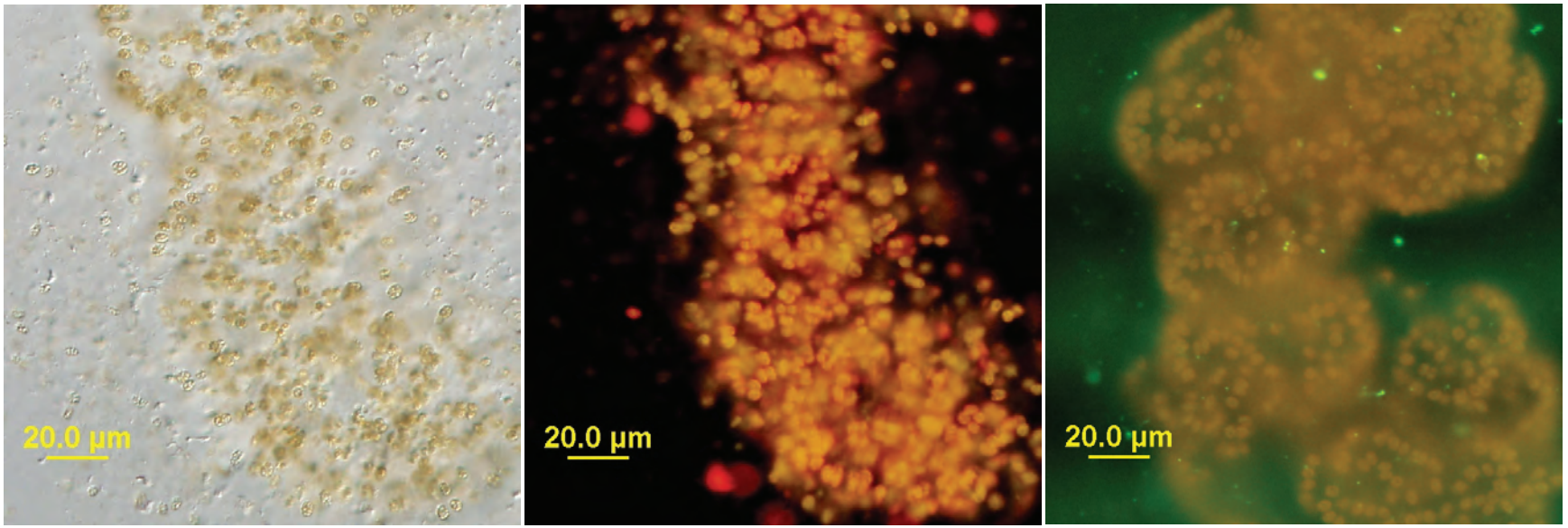


Figure 15. Spring Lake, CA (8/21/2009). LM-*Microcystis aeruginosa* colonial cyanobacterium with cells in a gelatinous matrix. FITC-a reddish-orange color dominates the cells. The scattered bright red cells are eukaryotic algal epiphytes. Sytox® green-*Woronichinia naegeliana*, stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

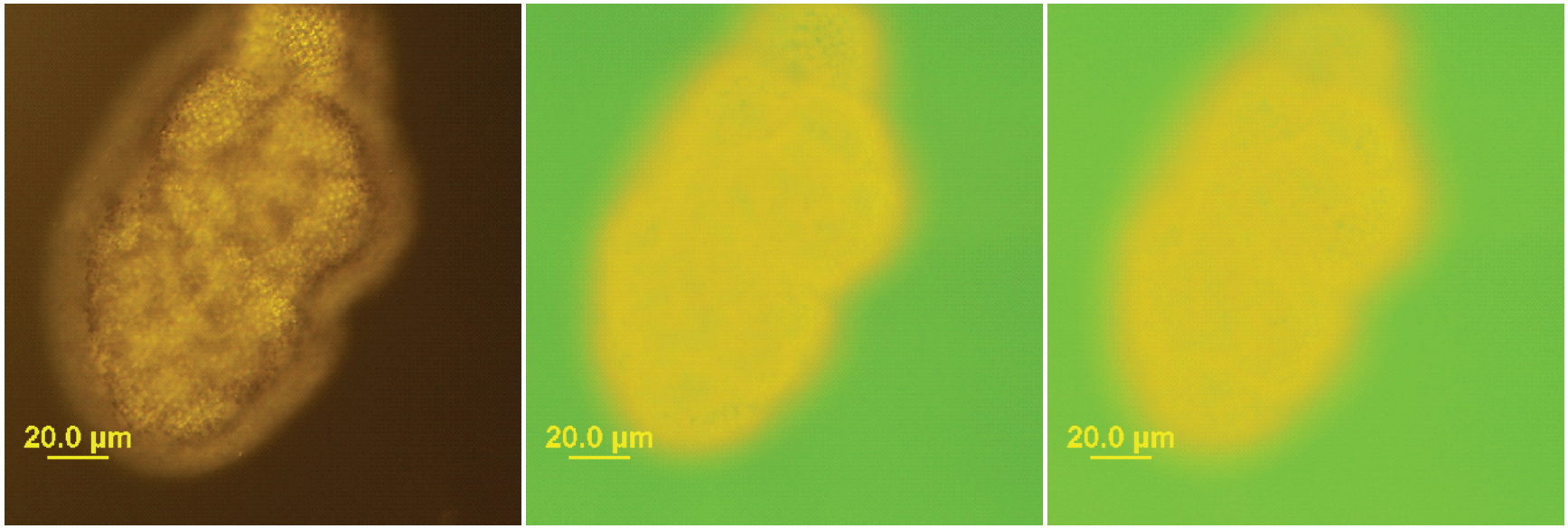


Figure 16. Spring Lake, CA (8/21/2009). LM-*Microcystis aeruginosa*. The same colony was used for all three images. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

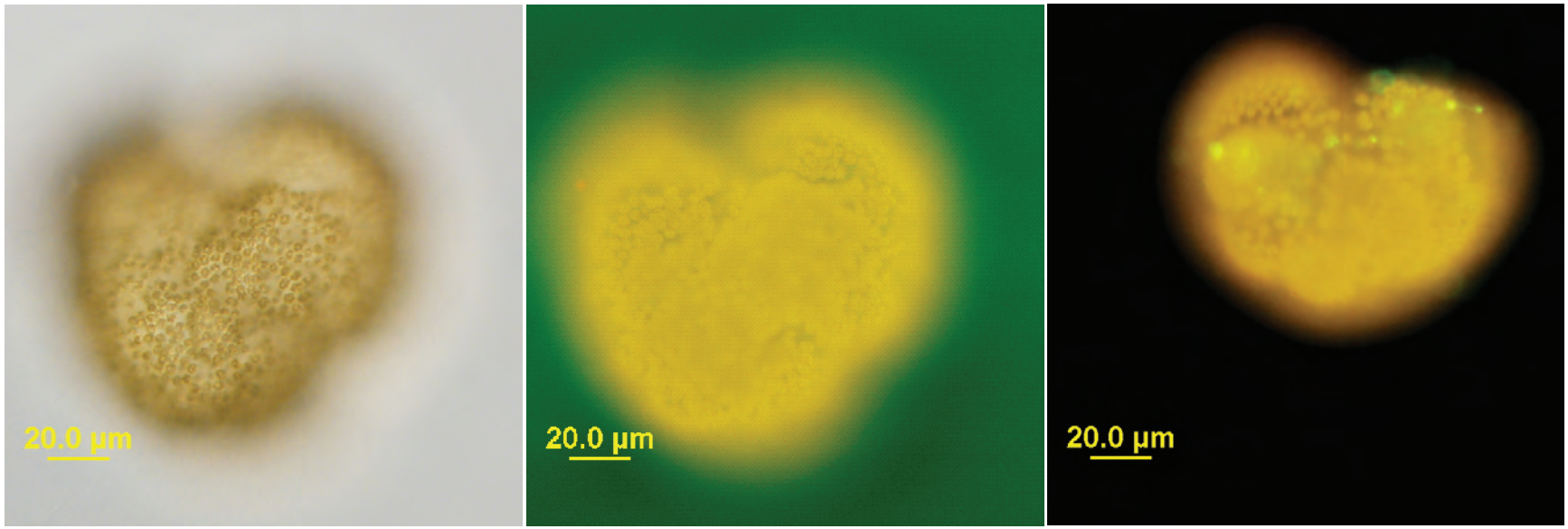


Figure 17. Spring Lake, CA (8/21/2009). LM-*Woronichinia naegeliana*. FITC-a yellow color dominates the cells. Sytox® green- stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

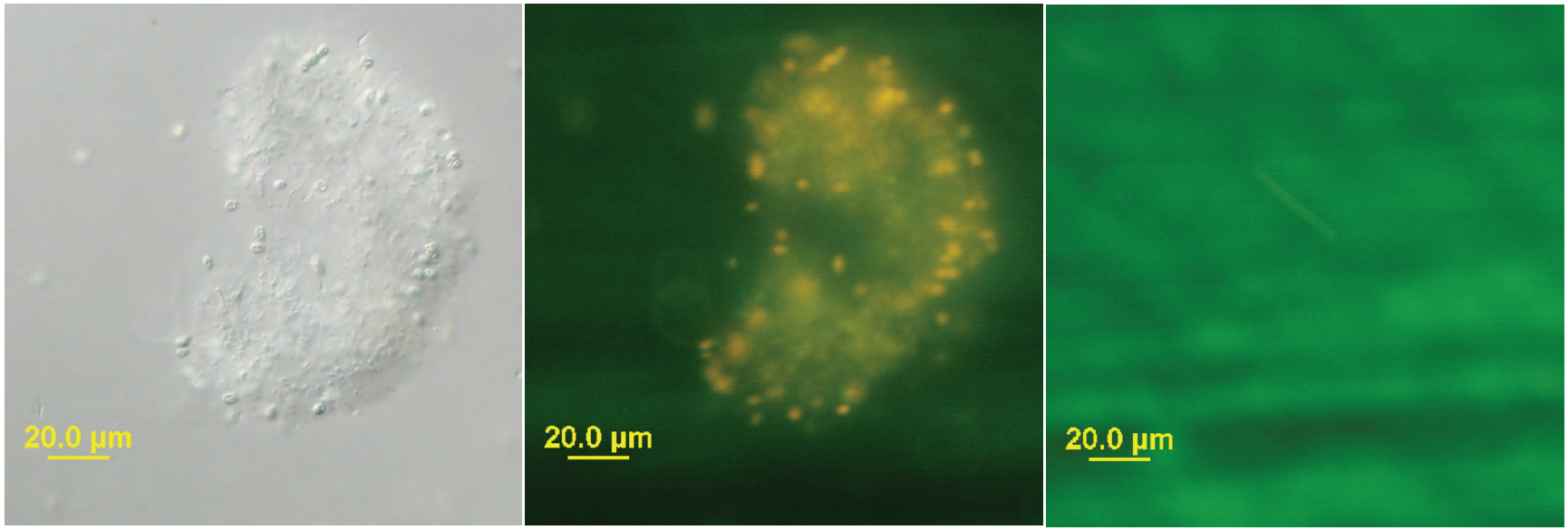


Figure 18. Spring Lake, CA (8/21/2009). LM-The remains of colonial cyanobacteria; note, the mucilage that supports the cells remains. FITC-a yellow-orange color dominates the cells. Sytox® green-nothing detectable. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

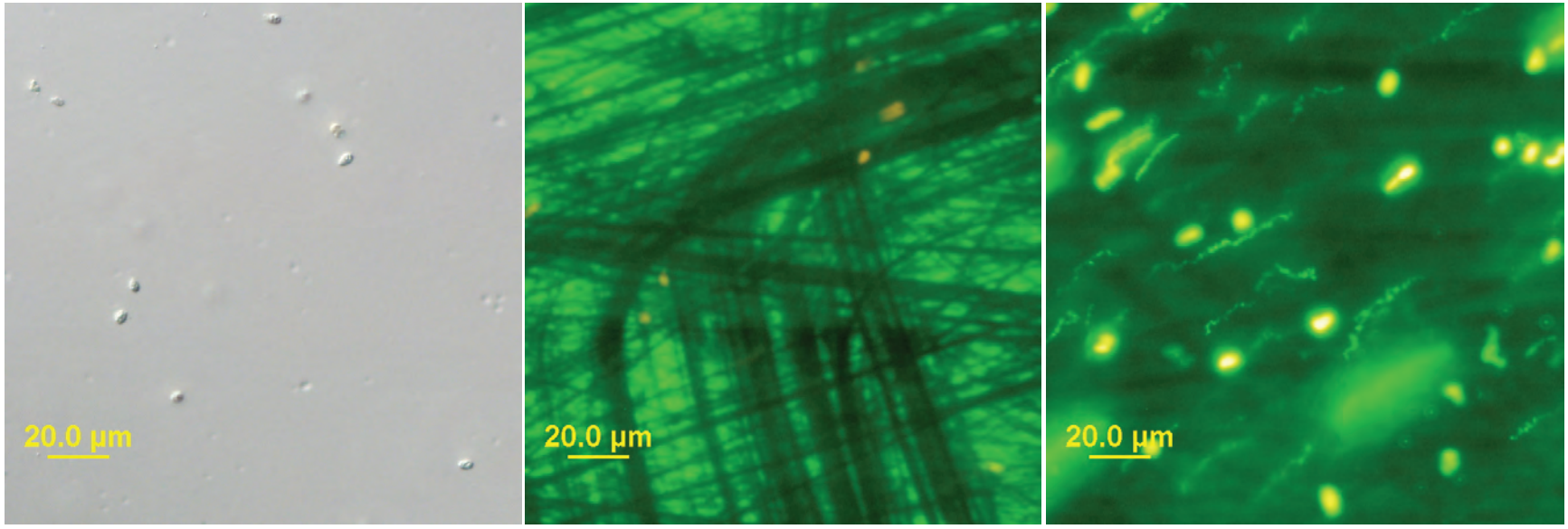


Figure 19. Spring Lake, CA (8/21/2009). LM-The remains of a colonial cyanobacterium. FITC-an orange color dominates the cells. Sytox® green-stain penetrated the cell membrane of this unknown organism; bright green cell. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

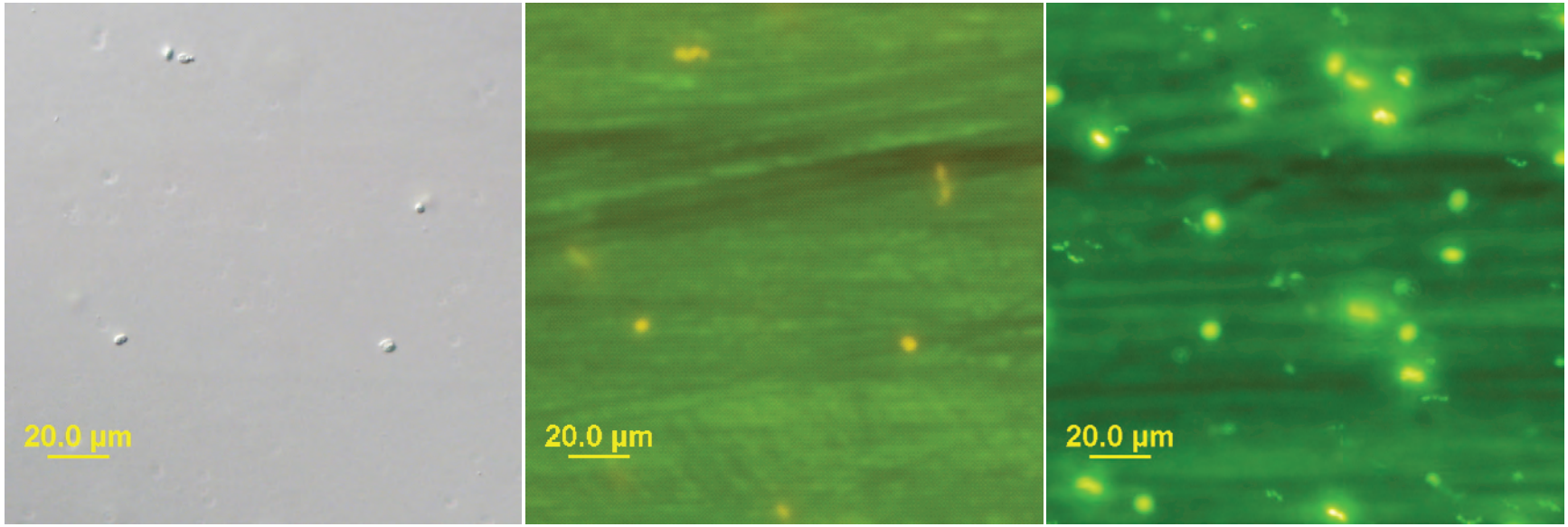


Figure 20. Spring Lake, CA (8/21/2009). LM-The remains of a colonial cyanobacterium. FITC-an orange color dominates the cells. Sytox® green-stain penetrated the cell membrane of this unknown organism; bright green cell. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

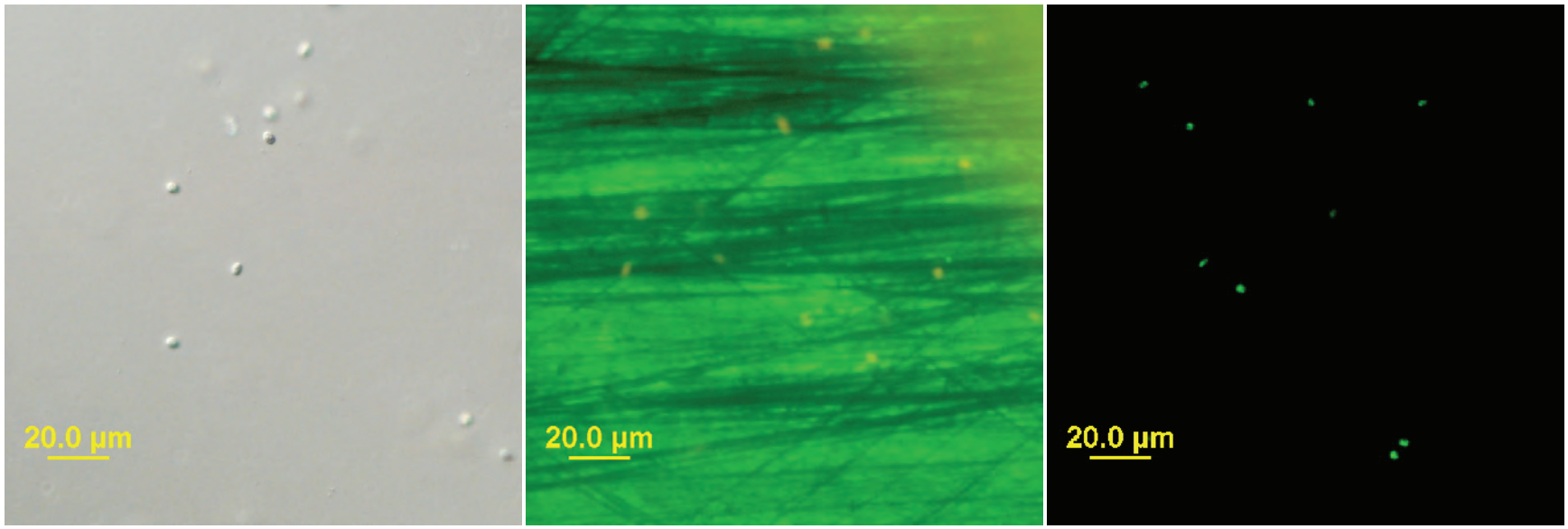


Figure 21. Spring Lake, CA (8/21/2009). LM-The remains of a colonial cyanobacterium. FITC-an orange color dominates the cells. Sytox® green-stain penetrated the cell membrane of this unknown organism; bright green cell. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

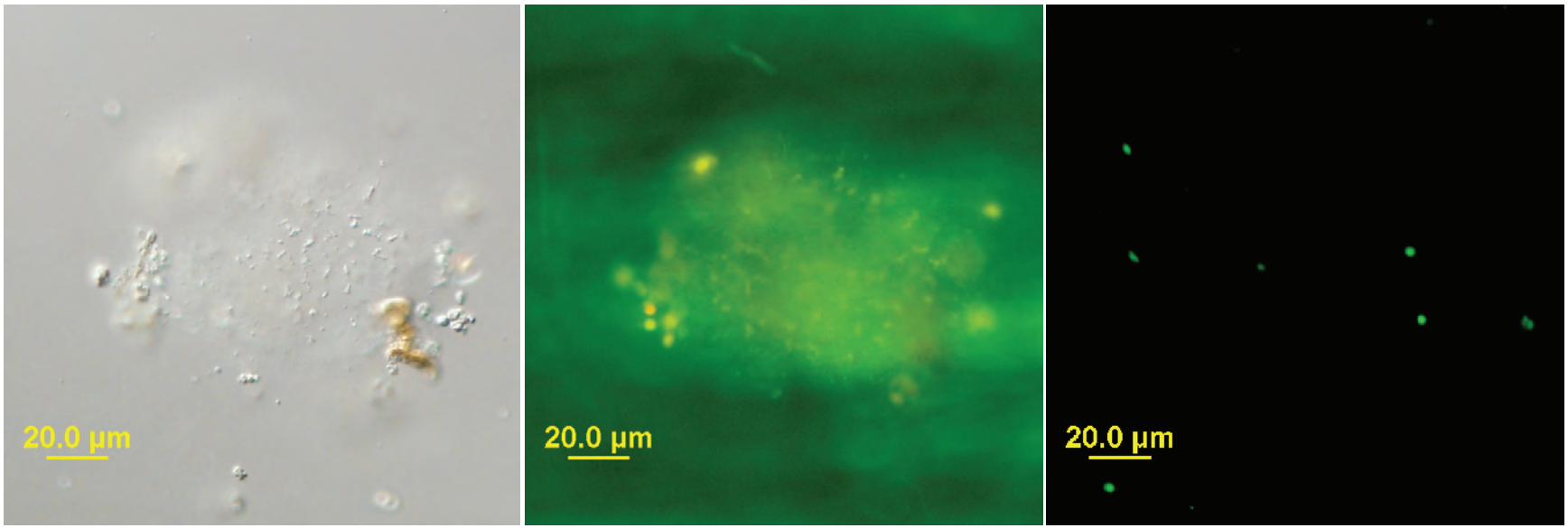


Figure 22. Spring Lake, CA (8/21/2009). LM-The remains of a colonial cyanobacterium. FITC-a yellow-orange color dominates the cells. Sytox® green-stain penetrated the cell membrane of this unknown organism; bright green cell. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

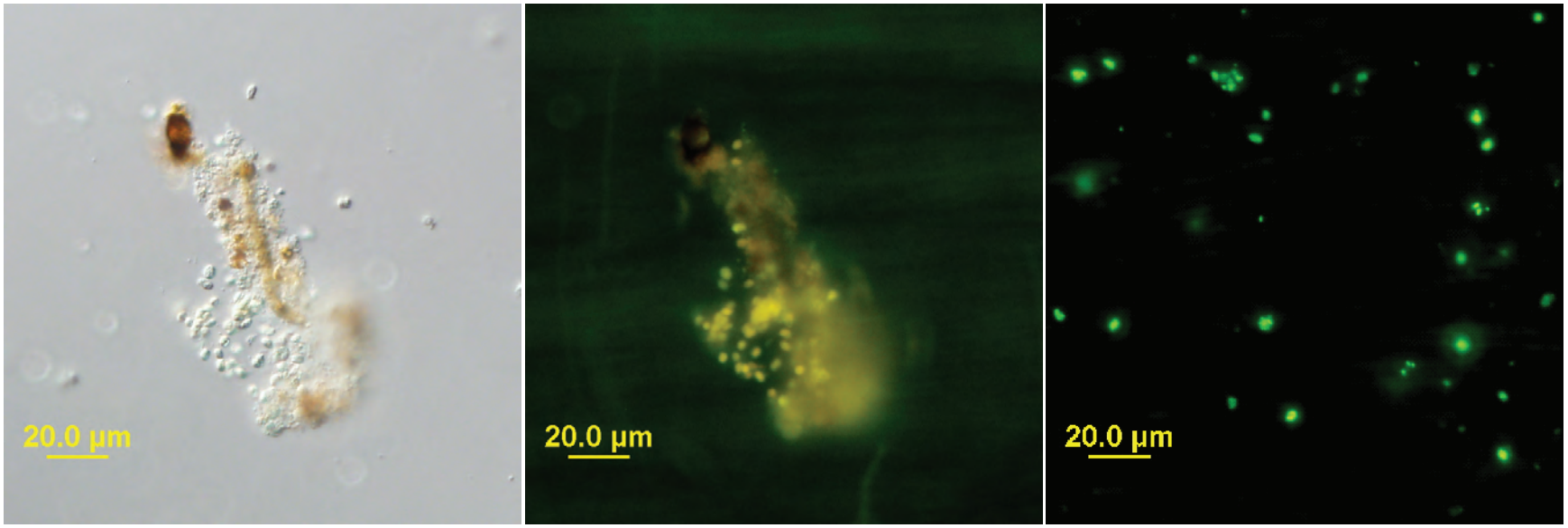


Figure 23. Spring Lake, CA (8/21/2009). LM-The remains of a colonial cyanobacterium. FITC-a yellow color dominates the cells. Sytox® green-stain penetrated cell membrane of this unknown organism; bright green cell. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

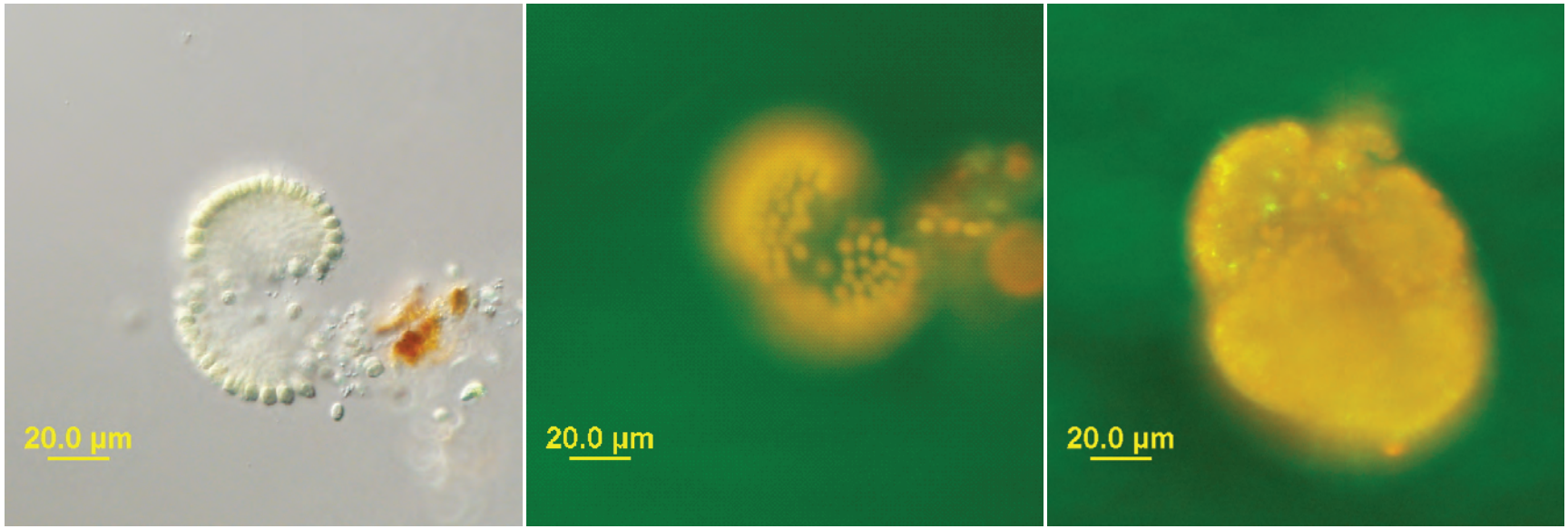


Figure 24. Spring Lake, CA (8/21/2009). LM-*Woronichinia* sp., cells in a gelatinous matrix. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

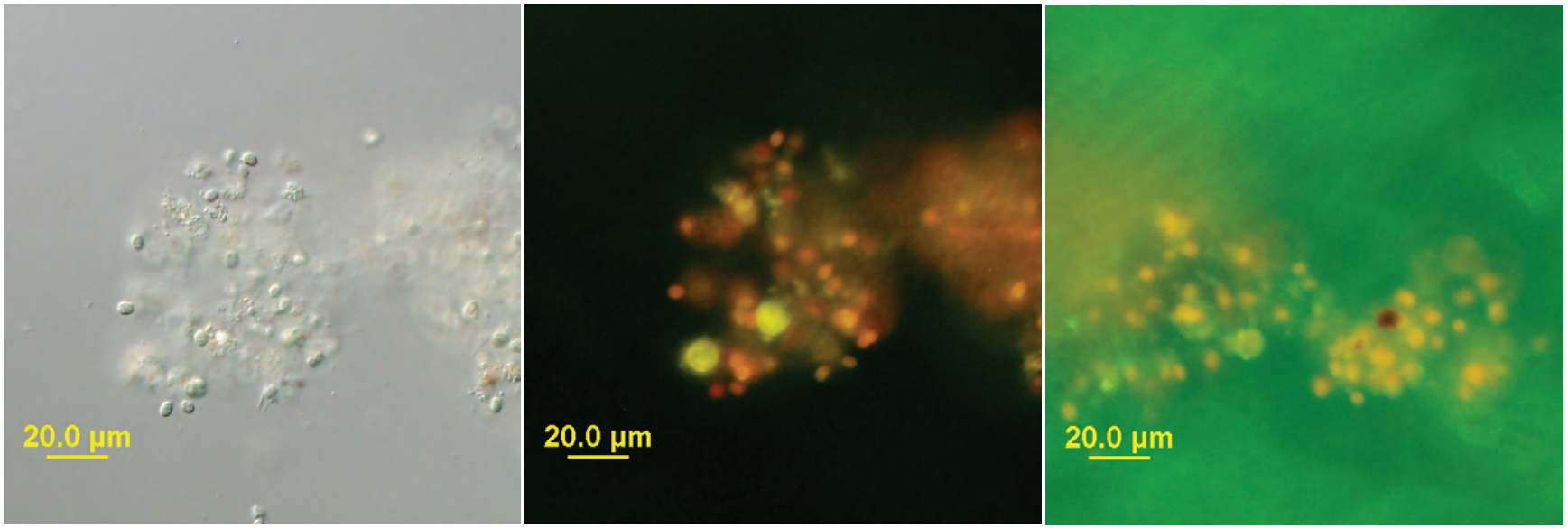


Figure 25. Spring Lake, CA (8/21/2009). LM-Likely the remains of *Woronichinia* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membranes of the cyanobacteria. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

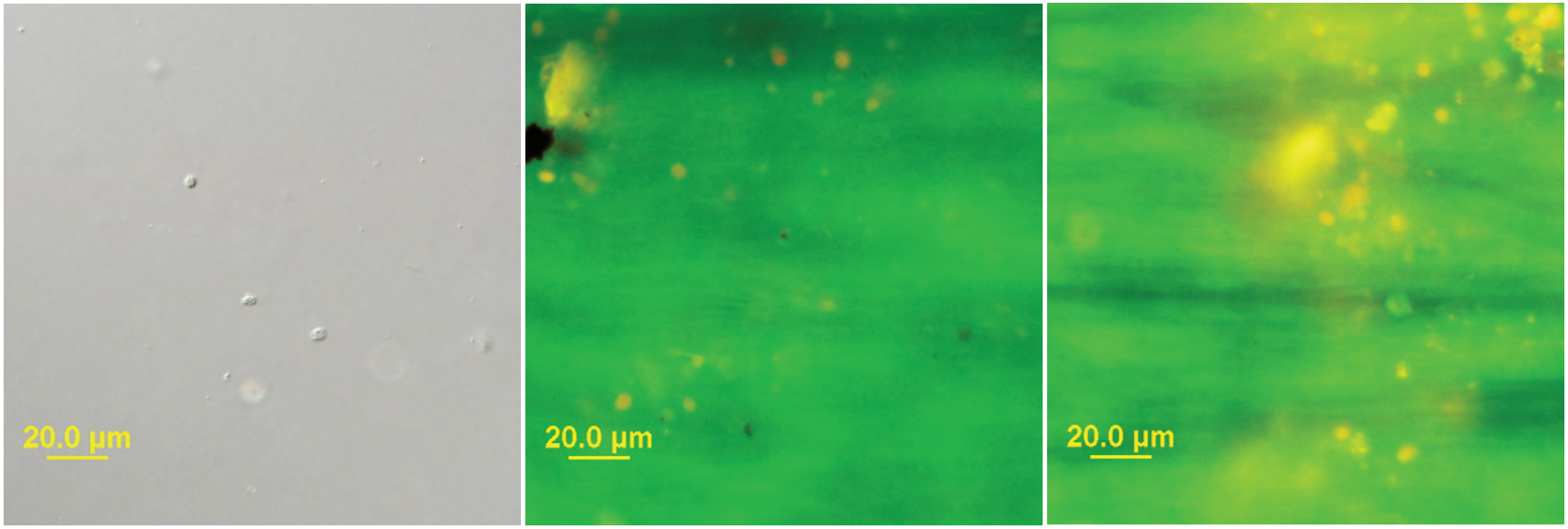


Figure 26. Spring Lake, CA (8/21/2009). LM-The remains of a colonial cyanobacterium. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membranes of the cyanobacteria. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

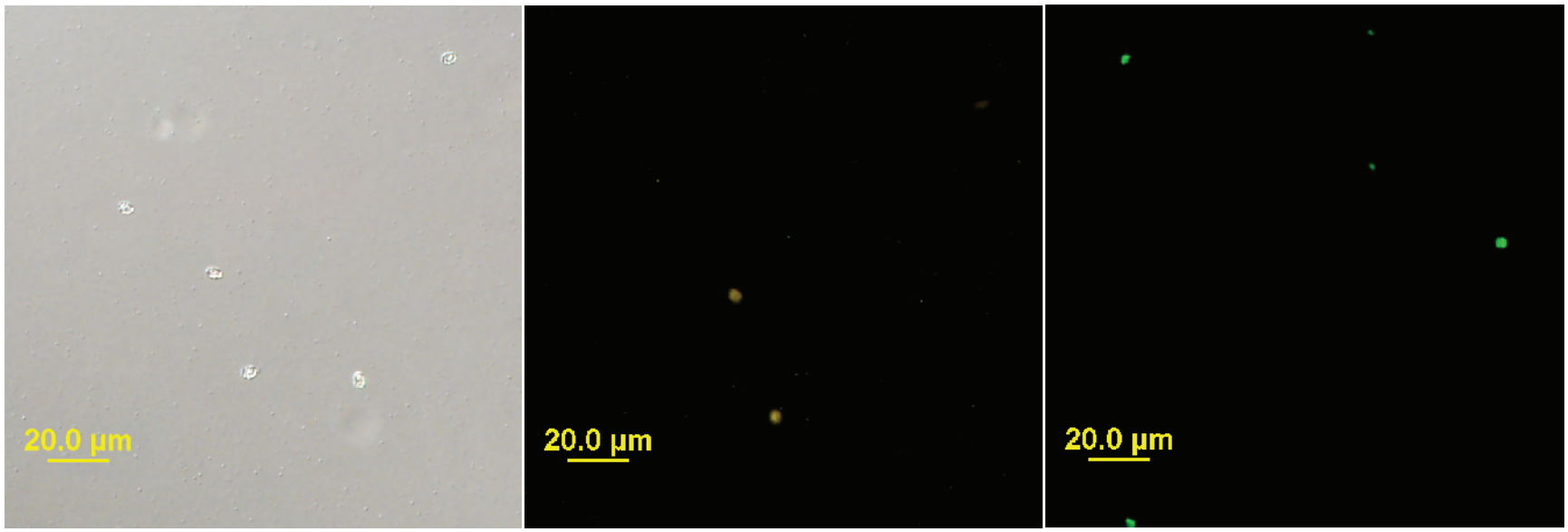
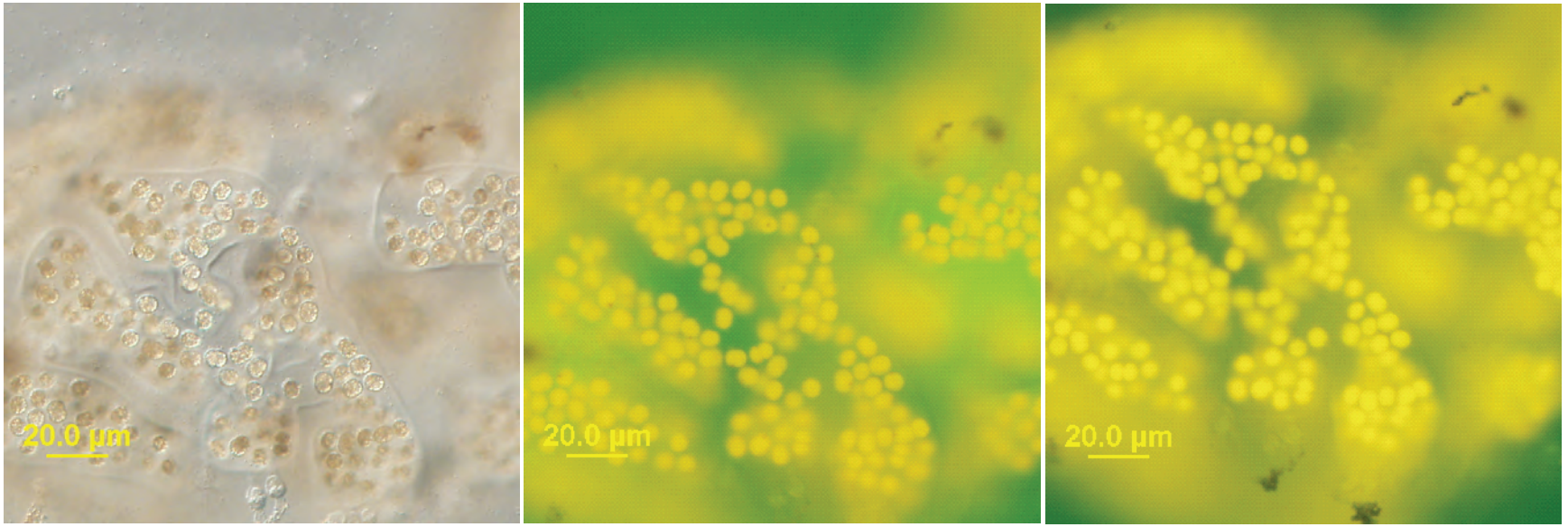


Figure 27. Spring Lake, CA (8/21/2009). LM-The remains of a colonial cyanobacterium. FITC-a yellow color dominates the cells. Sytox® green-stain penetrated the cell membranes; bright green cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.



LM FITC Sytox® green

QuikLyse™ -2x

Figure 28. Spring Lake, CA (8/21/2009). LM-*Microcystis wesenbergii*, a colonial cyanobacterium. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membranes of the cyanobacteria. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

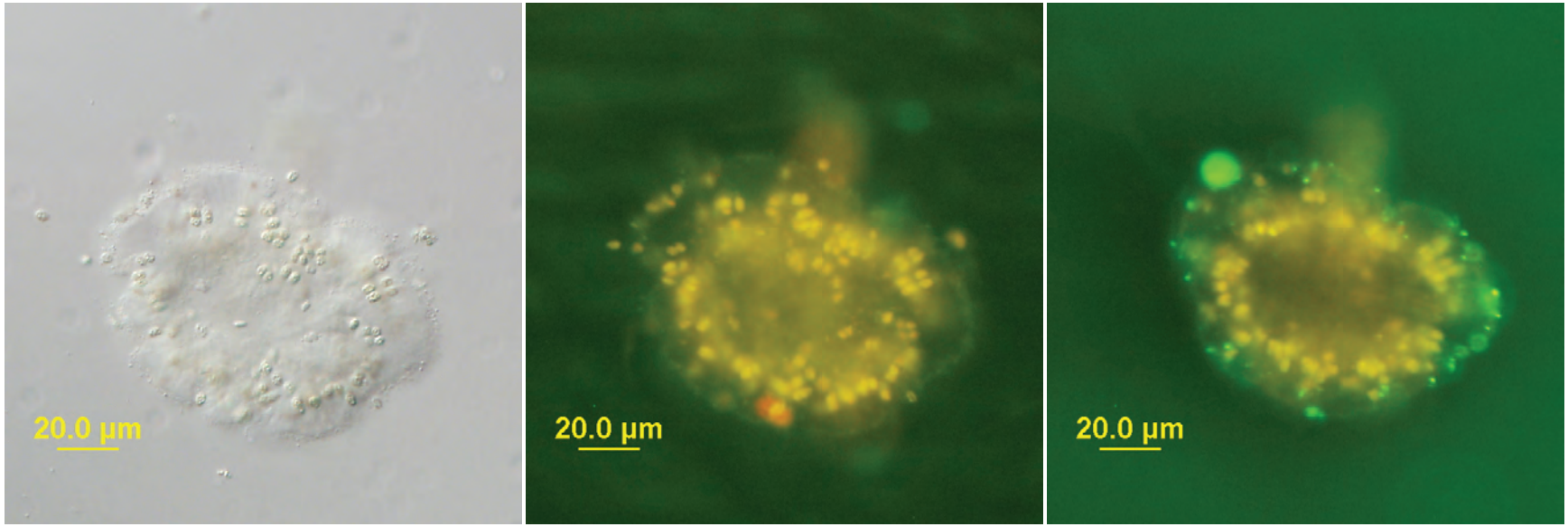


Figure 29. Spring Lake, CA (8/21/2009). LM-The remains of a colonial cyanobacterium; the mucilage that supports the cells remains. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membranes of the cyanobacteria, but did stain the bacteria associated with the mucilage. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

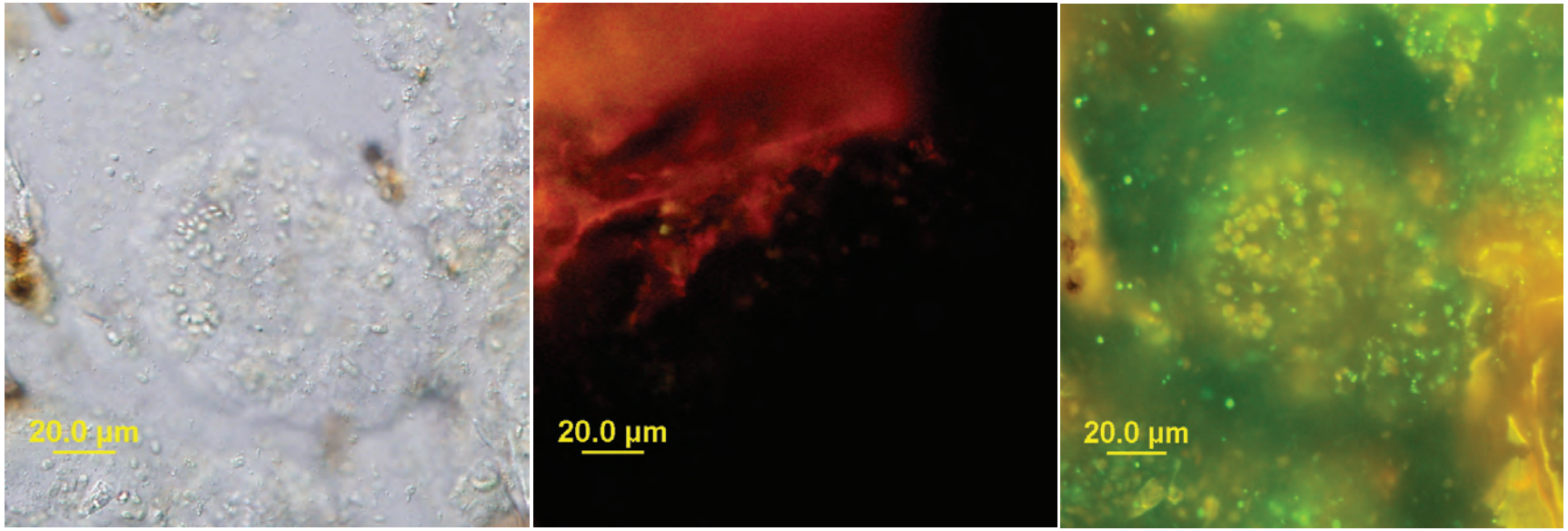


Figure 30. Spring Lake, CA (8/21/2009). LM-The remains of a colonial cyanobacterium; the mucilage that supports the cells remains. FITC-a red color dominates the cells. Sytox® green-stain did not penetrate the cell membranes of the cyanobacteria, but did stain the bacteria associated with the mucilage. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

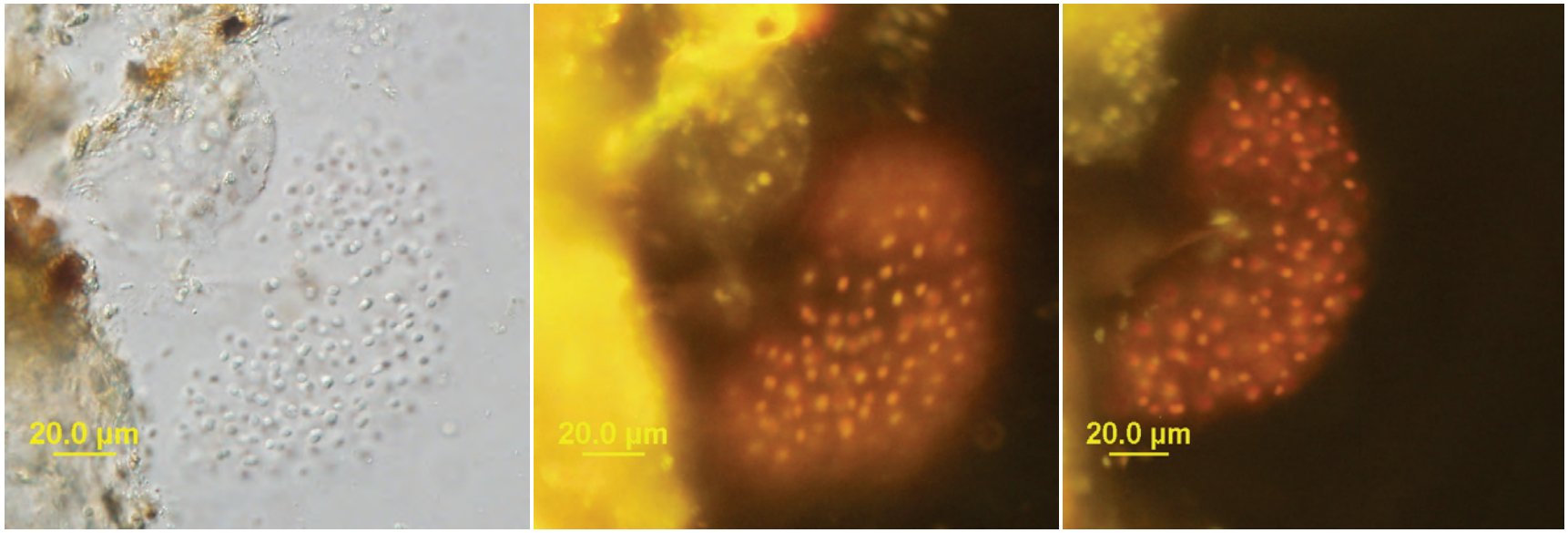


Figure 31. Spring Lake, CA (8/21/2009). LM-The remains of a colonial cyanobacterium. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membranes of the cyanobacterium. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

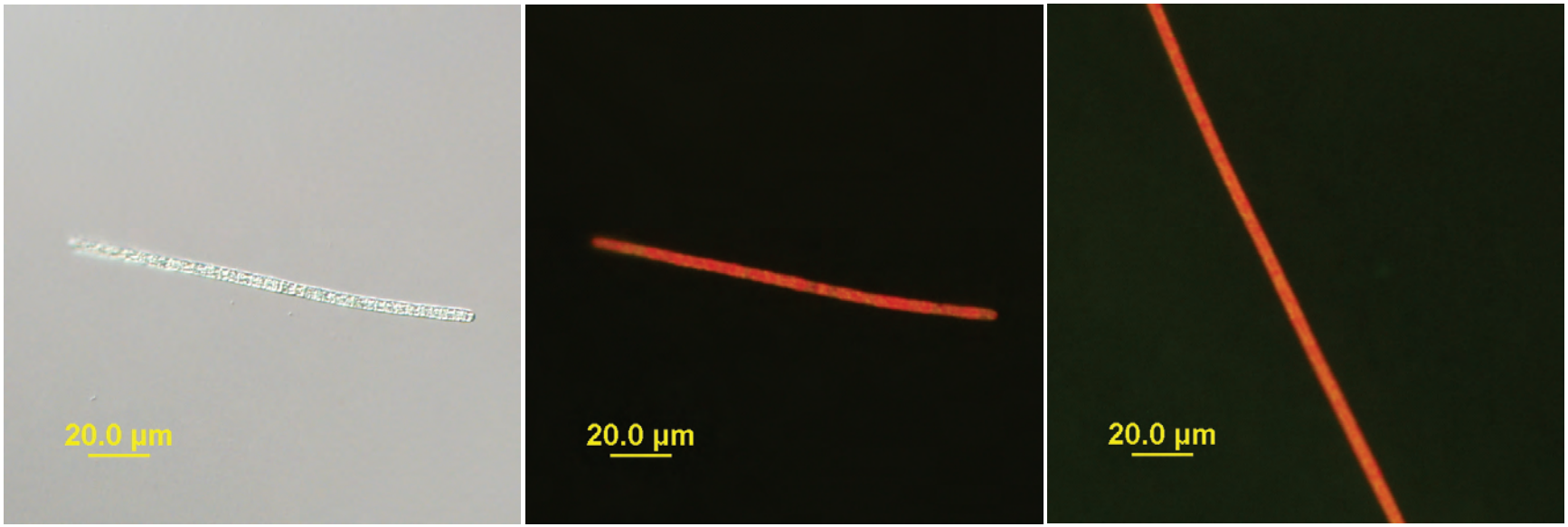


Figure 32. Blackhawk Lake, IA (8/26/2009). LM-*Planktothrix* sp., cells in a filament. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

Figure 33. Blackhawk Lake, IA (8/26/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-no images obtained. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

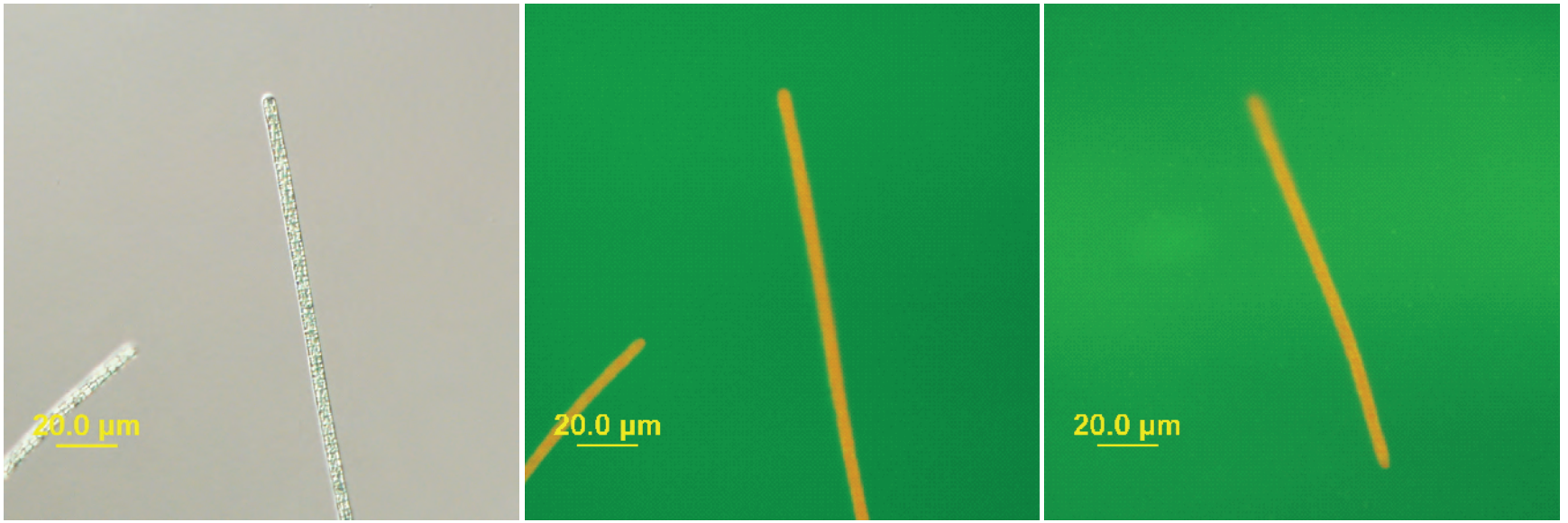


Figure 34. Blackhawk Lake, IA (8/26/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

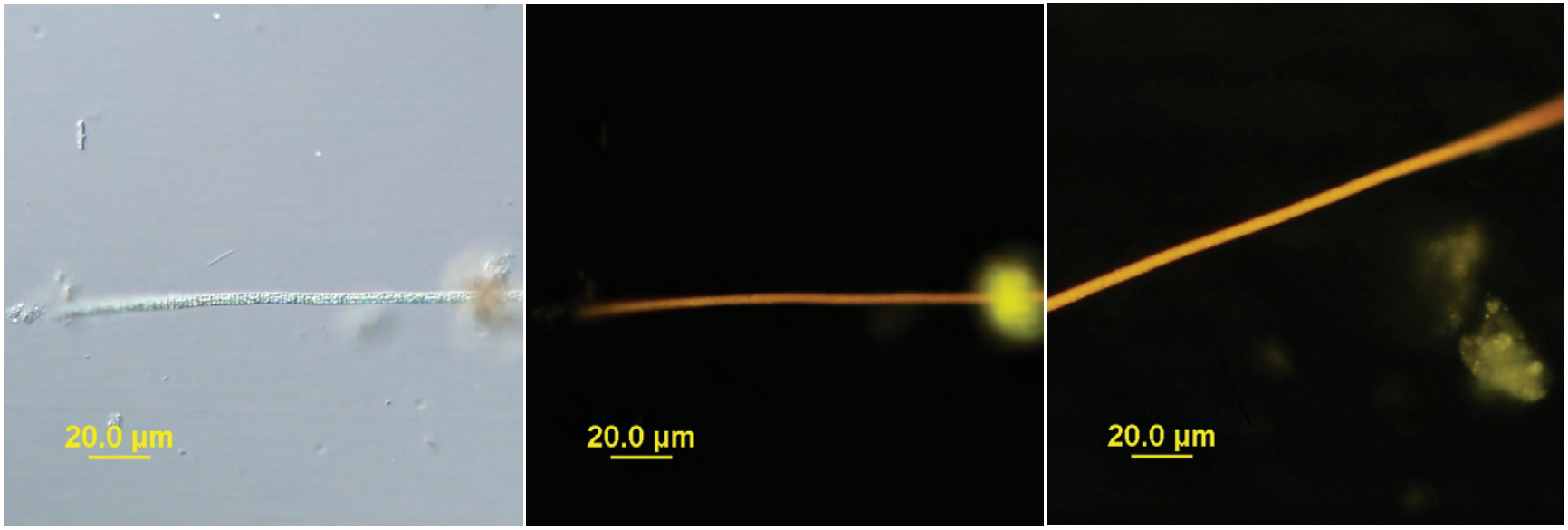


Figure 35. Blackhawk Lake, IA (8/26/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

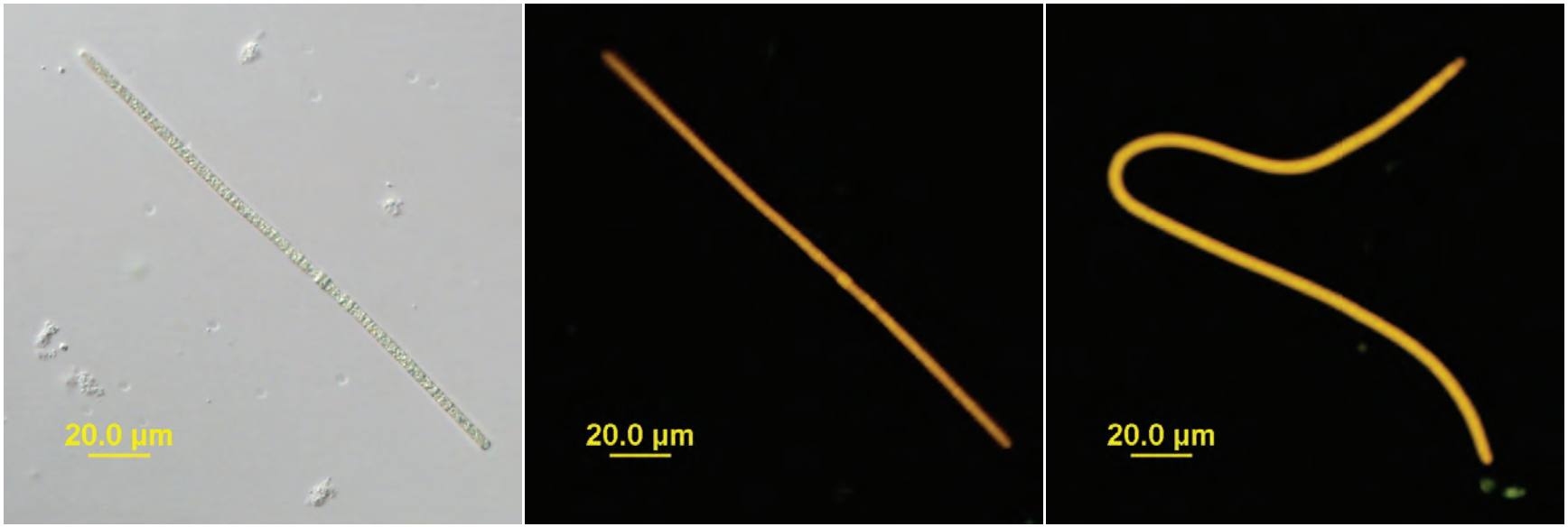


Figure 36. Blackhawk Lake, IA (8/26/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

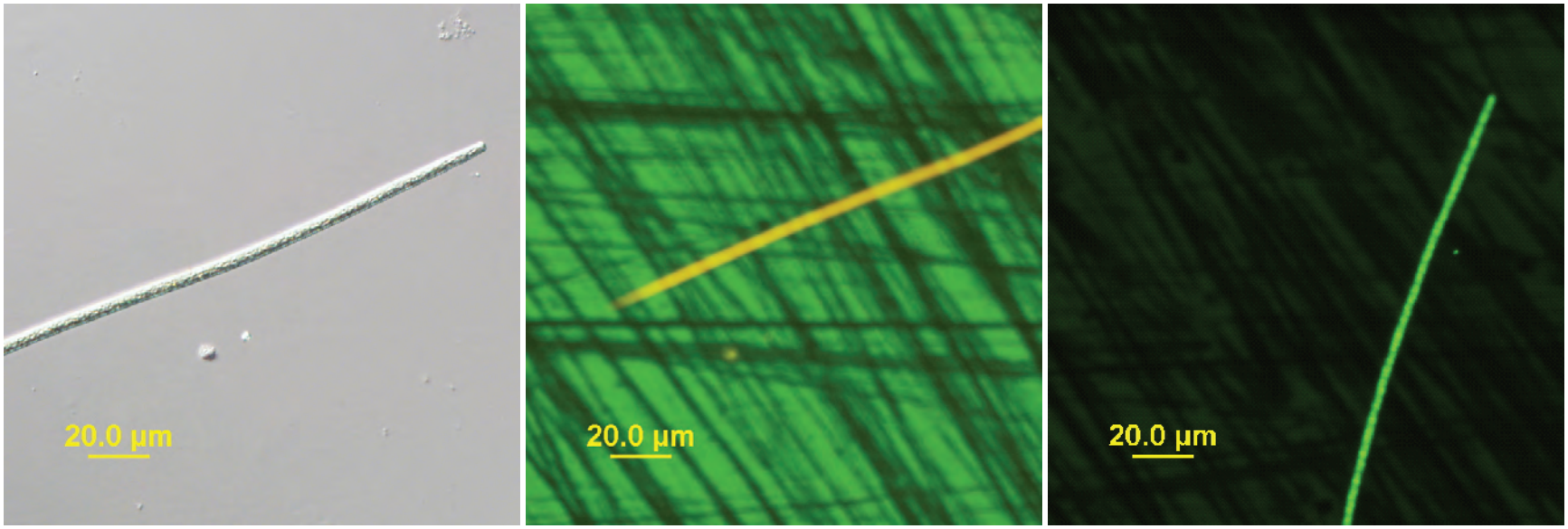


Figure 37. Blackhawk Lake, IA (8/26/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane; cells bright green . LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

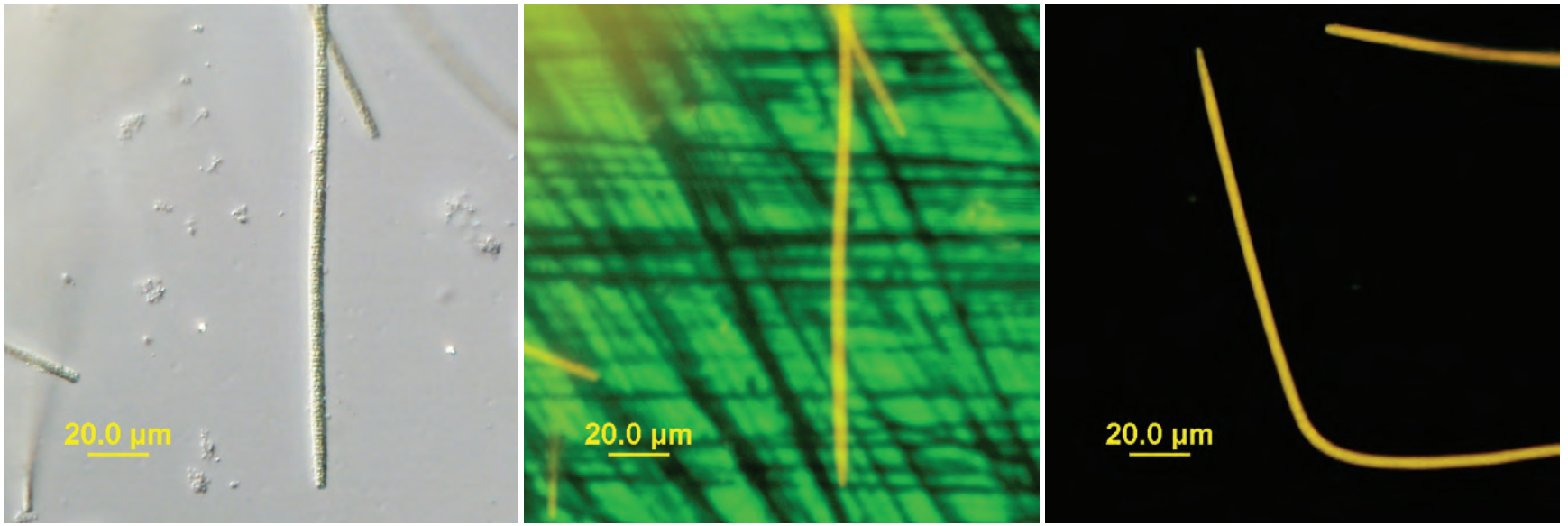


Figure 38. Blackhawk Lake, IA (8/26/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

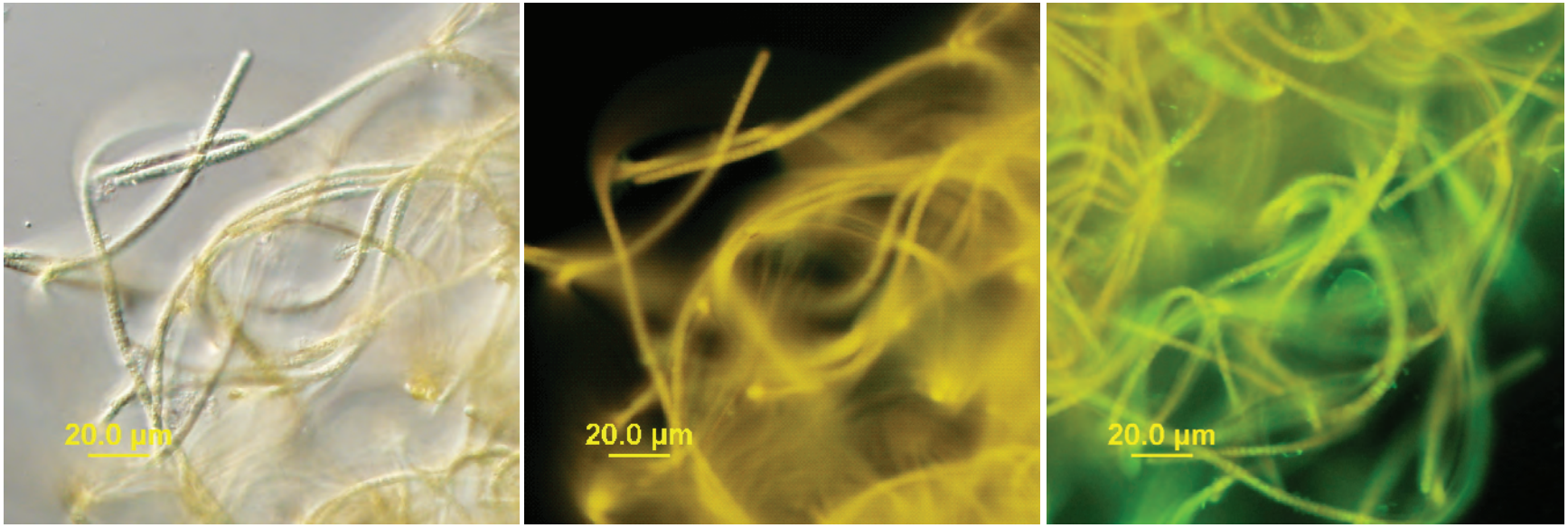


Figure 39. Blackhawk Lake, IA (8/26/2009). LM-Cyanobacterial filamentous. FITC-a yellow-orange color dominates the cells. Sytox® green-stain did penetrate the cell membranes in some of the cells in the filaments. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

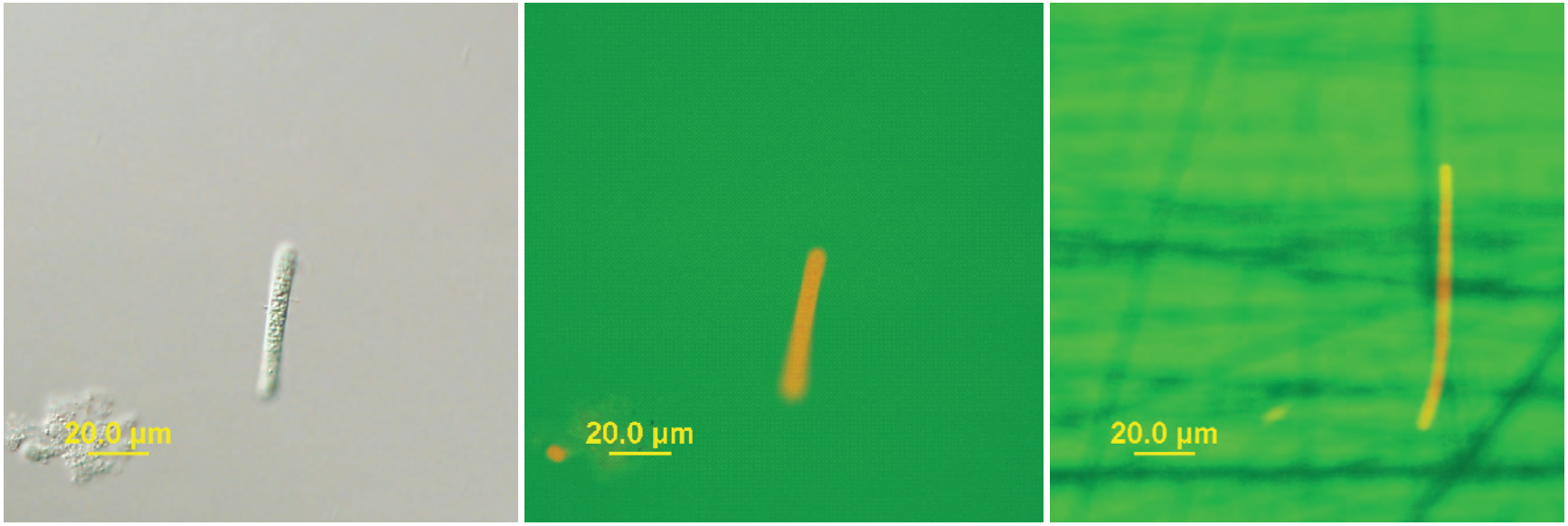


Figure 40. Blackhawk Lake, IA (8/26/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

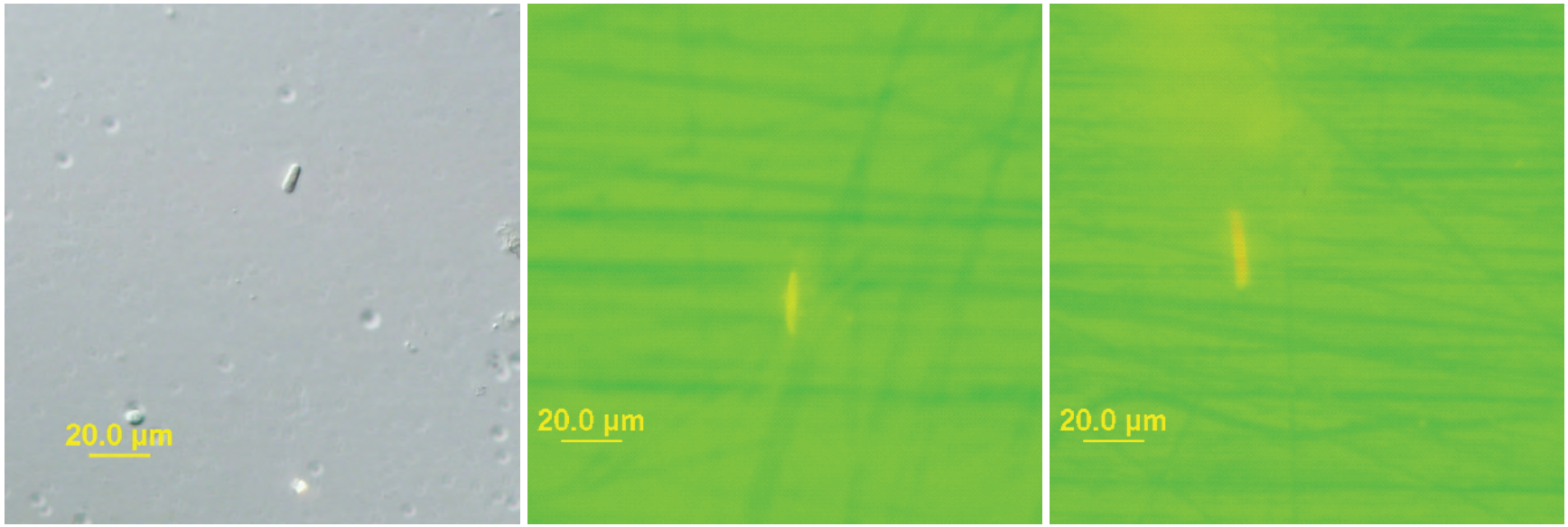


Figure 41. Blackhawk Lake, IA (8/26/2009). LM-Remains of *Planktothrix* sp. FITC-an orange-yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

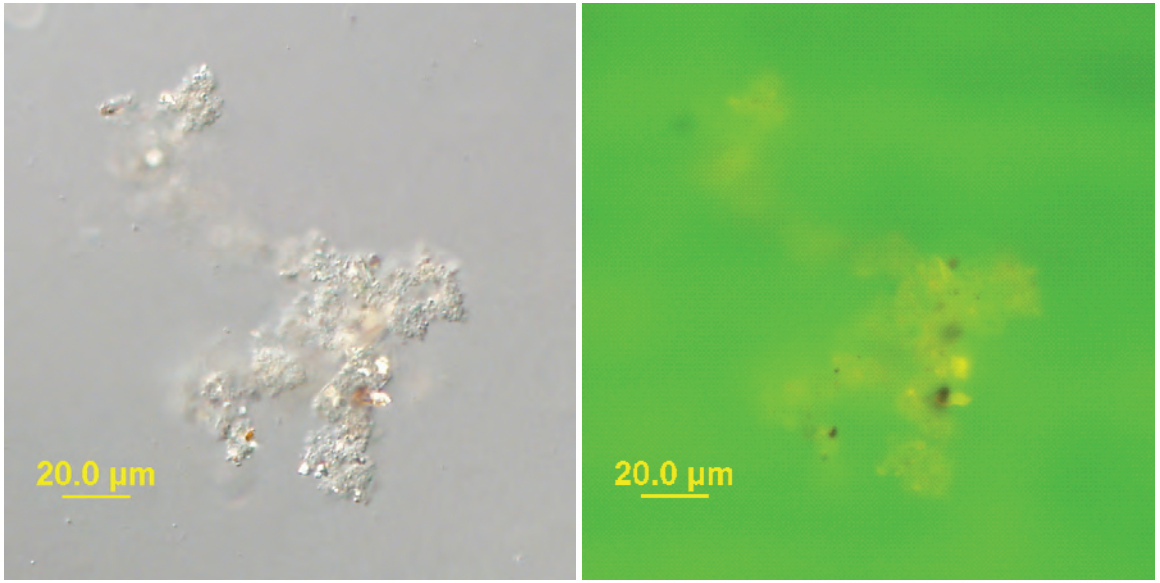


Figure 42. Blackhawk Lake, IA (8/26/2009). LM-Remains of unknown organism; no other material observed. FITC-nothing distinguishable as cells. Sytox® green-no images obtained. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

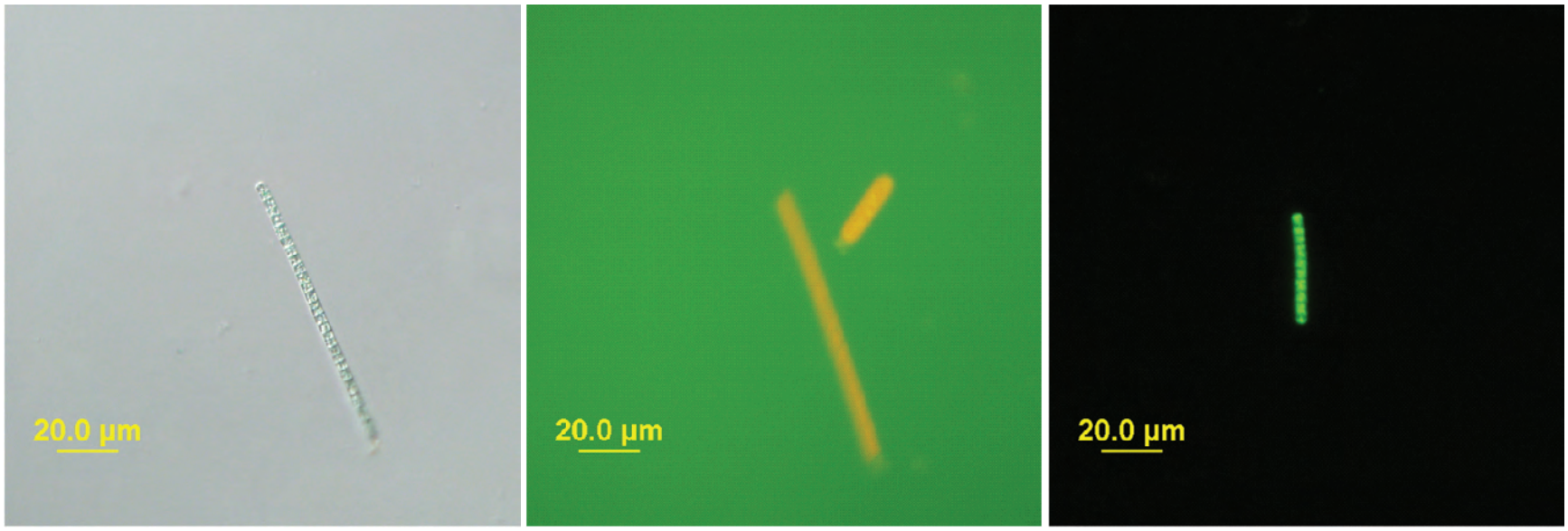


Figure 43. Blackhawk Lake, IA (8/26/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane; cells bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

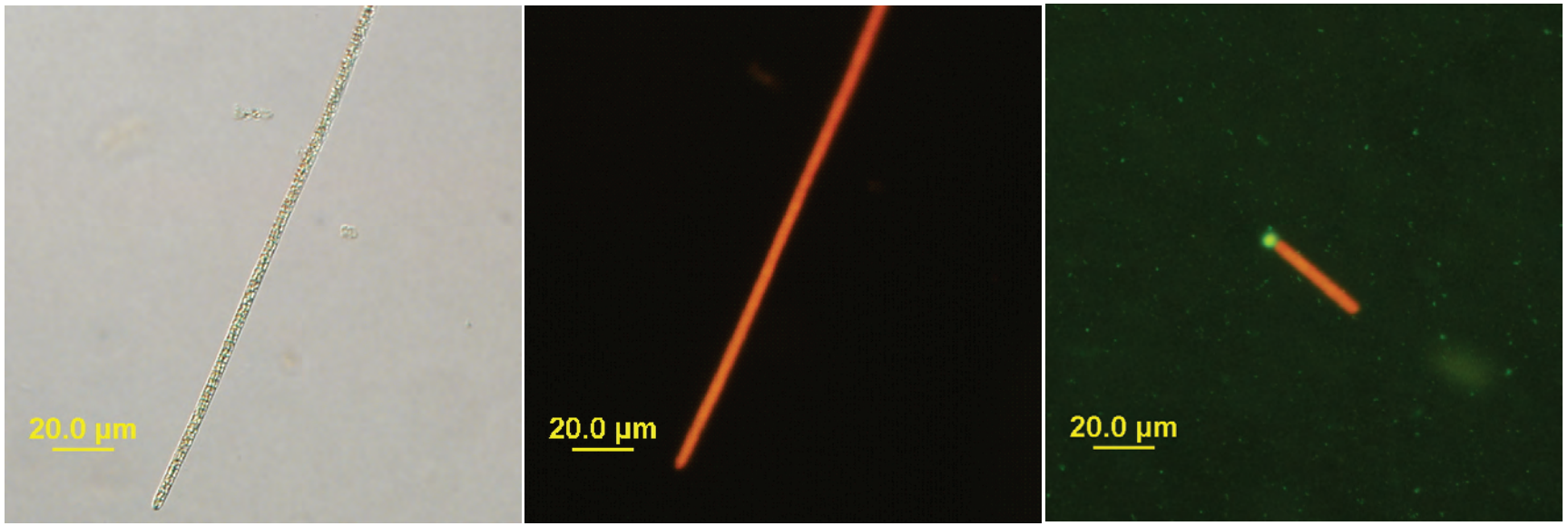


Figure 44. Blackhawk Lake, IA (8/26/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane, except end cell. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

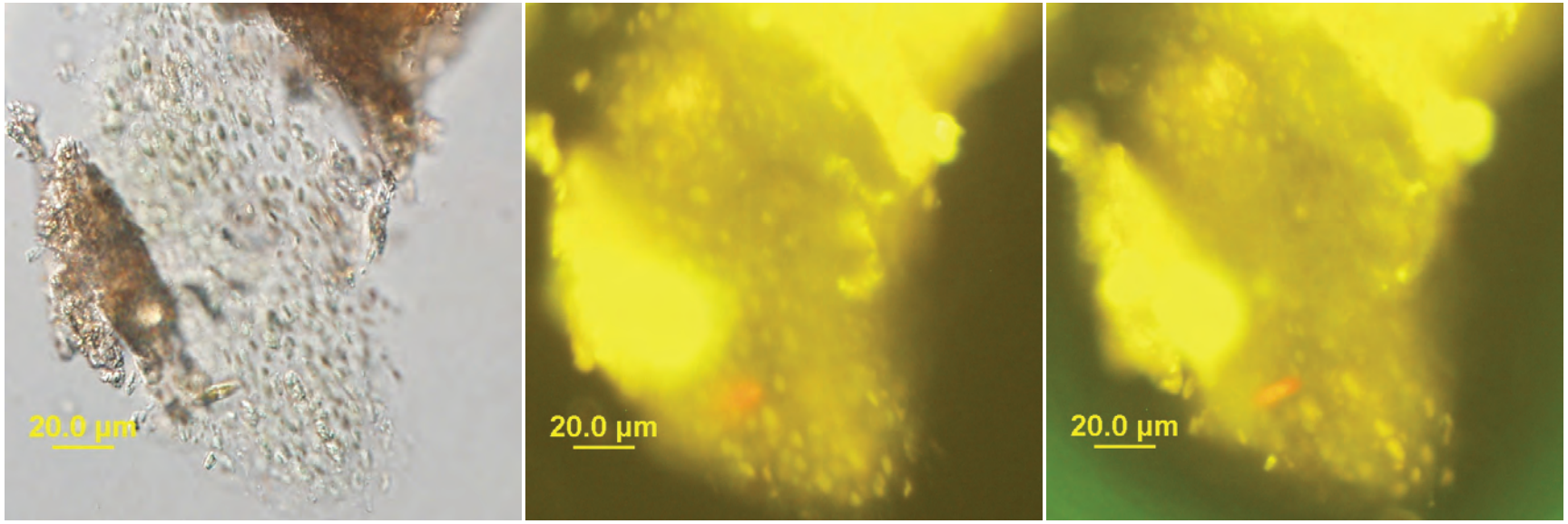


Figure 45. Blackhawk Lake, IA (8/26/2009). LM-Remains of a colonial cyanobacterium, likely *Microcystis*. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

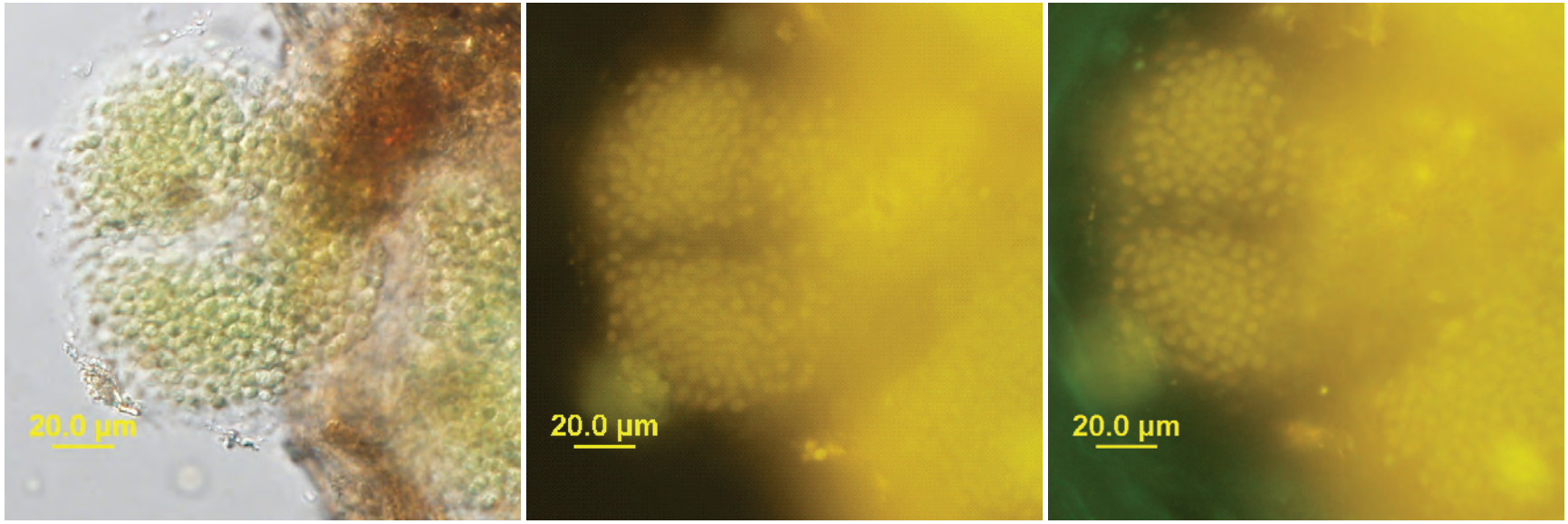


Figure 46. Blackhawk Lake, IA (8/26/2009). LM-*Microcystis* sp. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

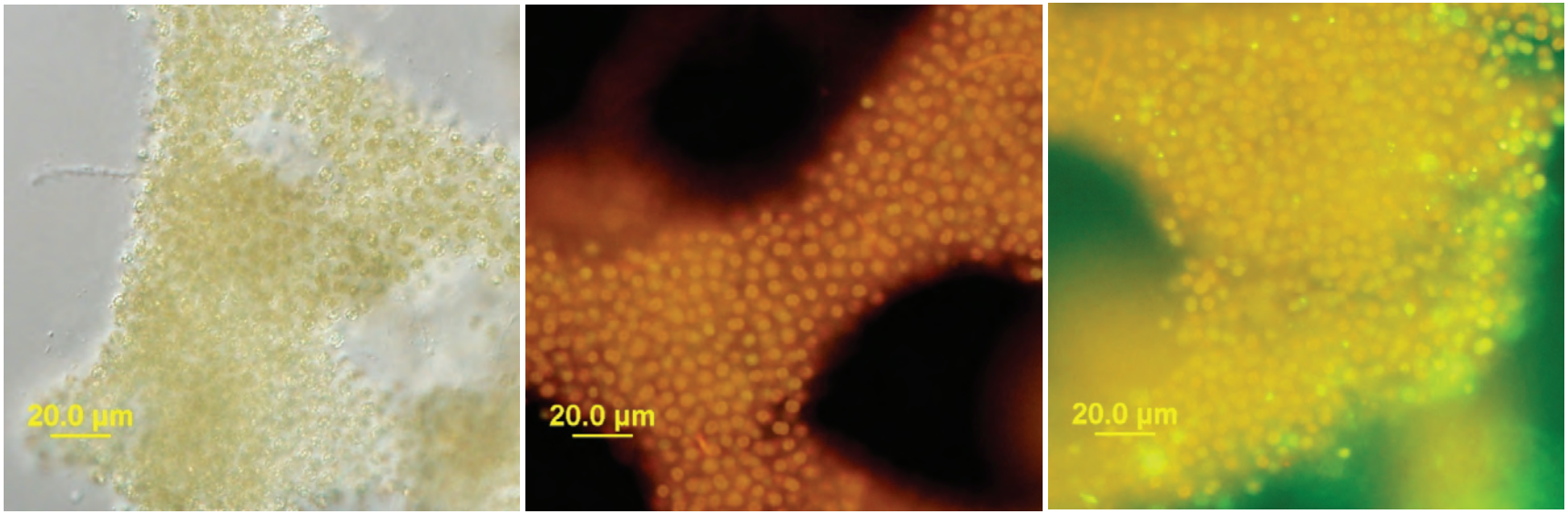


Figure 47. Copco Reservoir, CA (9/10/2009). LM-*Microcystis aeruginosa*. FITC-an orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane in some of the peripheral cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

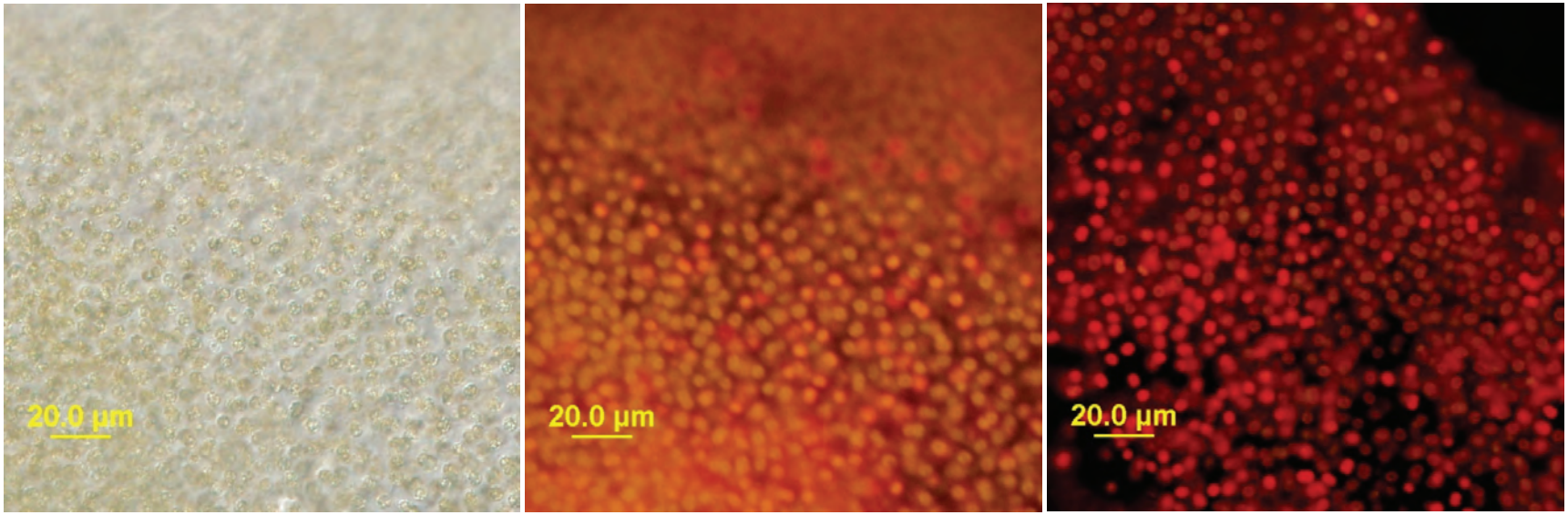


Figure 48. Copco Reservoir, CA (9/10/2009). LM-*Microcystis aeruginosa*. FITC-an orange-red color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

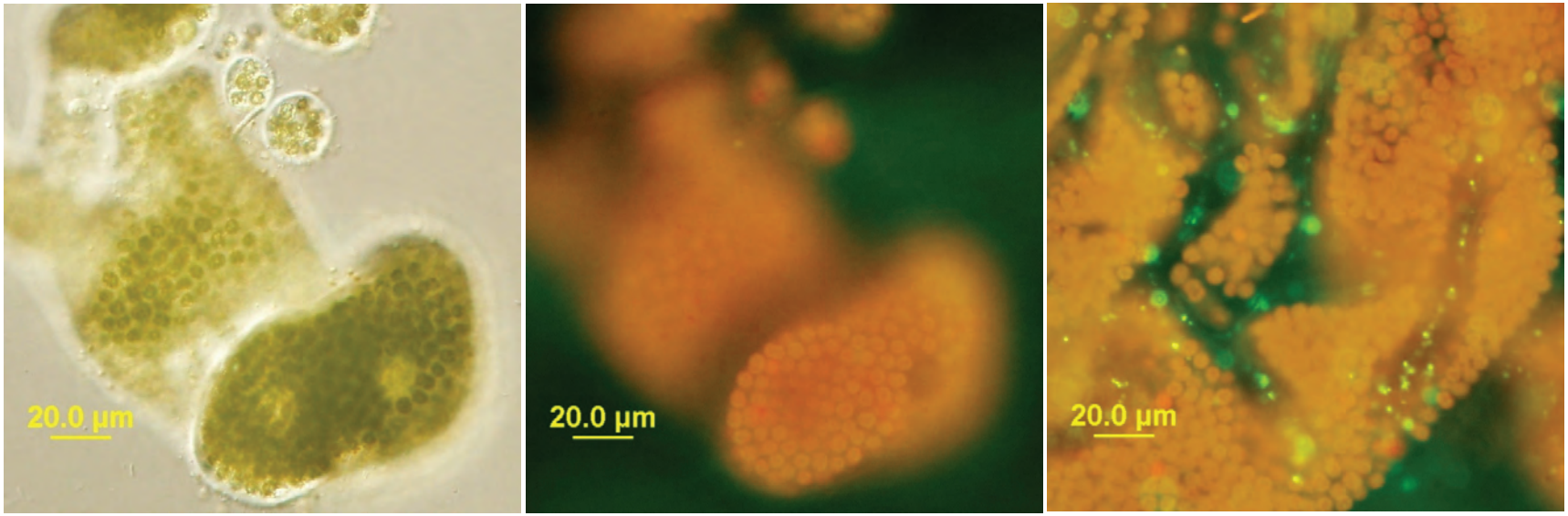


Figure 49. Copco Reservoir, CA (9/10/2009). LM-*Microcystis wesenbergii*. FITC-an orange-red color dominates the cells. Sytox® green-stain did not penetrate the cell membrane of the cyanobacteria; epiphytic bacteria did stain bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

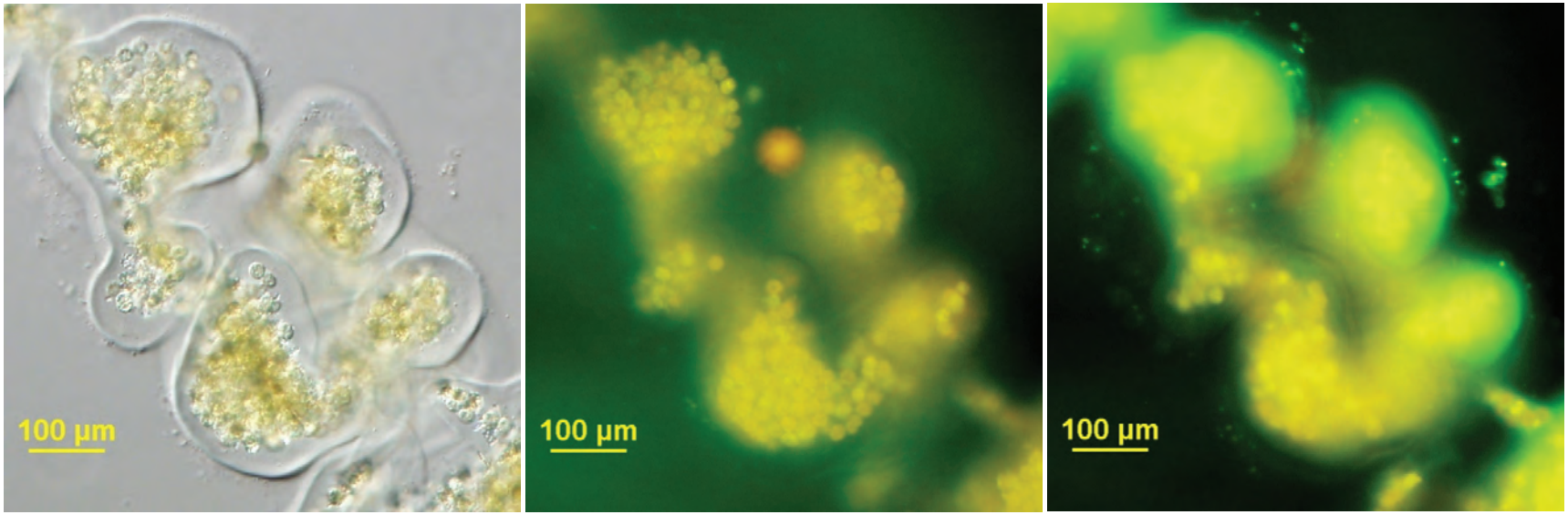


Figure 50. Copco Reservoir, CA (9/10/2009). LM-*Microcystis wesenbergii*. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membrane of the cyanobacteria; outer mucilage did stain bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

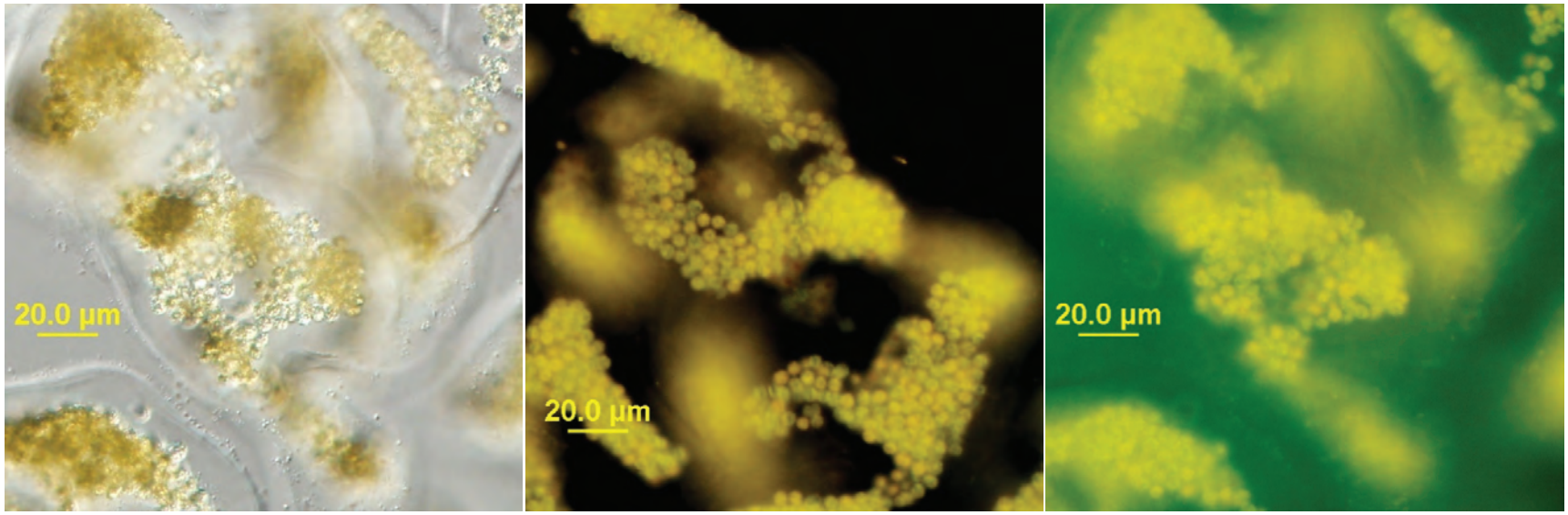


Figure 51. Copco Reservoir, CA (9/10/2009). LM-*Microcystis wesenbergii*. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membrane of the cyanobacteria; outer mucilage did stain bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

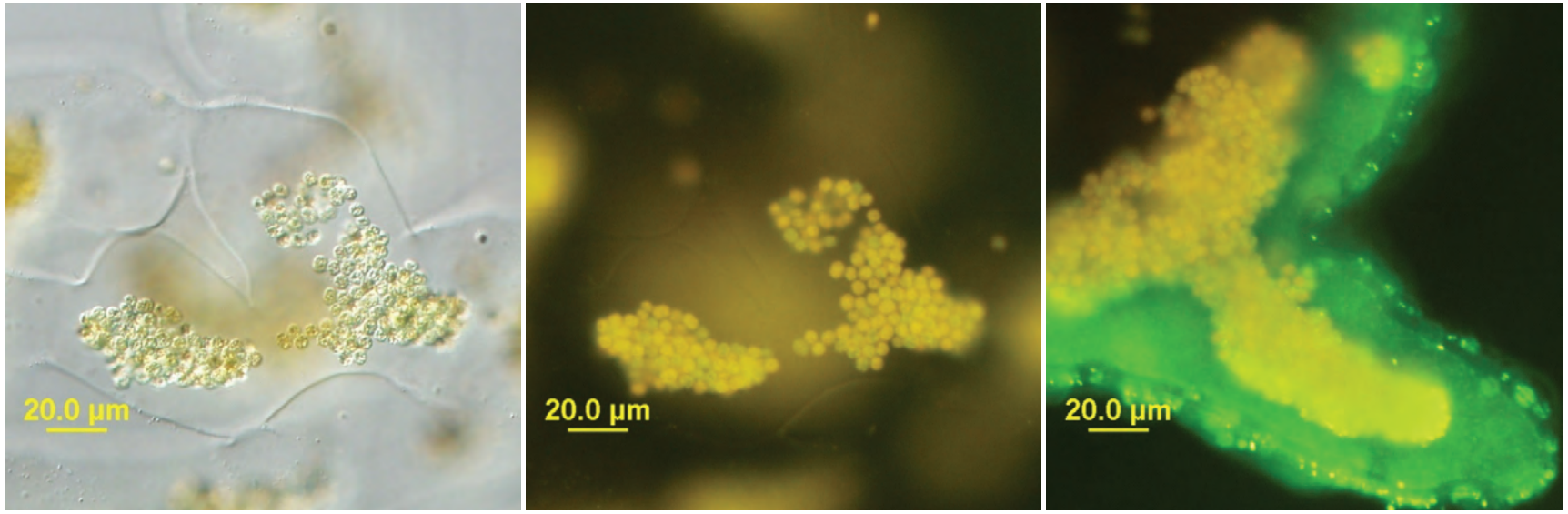


Figure 52. Copco Reservoir, CA (9/10/2009). LM-*Microcystis wesenbergii*. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membrane of the cyanobacteria; outer mucilage did stain bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

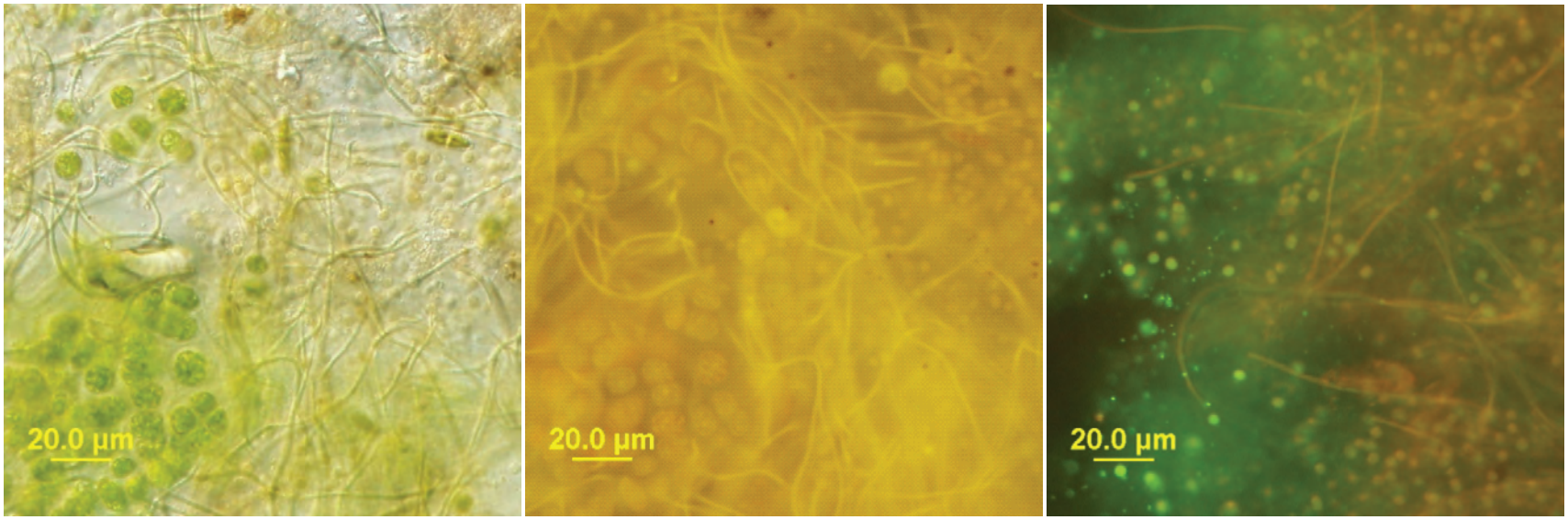


Figure 53. Copco Reservoir, CA (9/10/2009). LM-Miscellaneous cyanobacterial filaments and eukaryotic algae. FITC-a yellow-orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane of some of the colonial cyanobacterial cells, but not the unknown cyanobacterial filaments. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

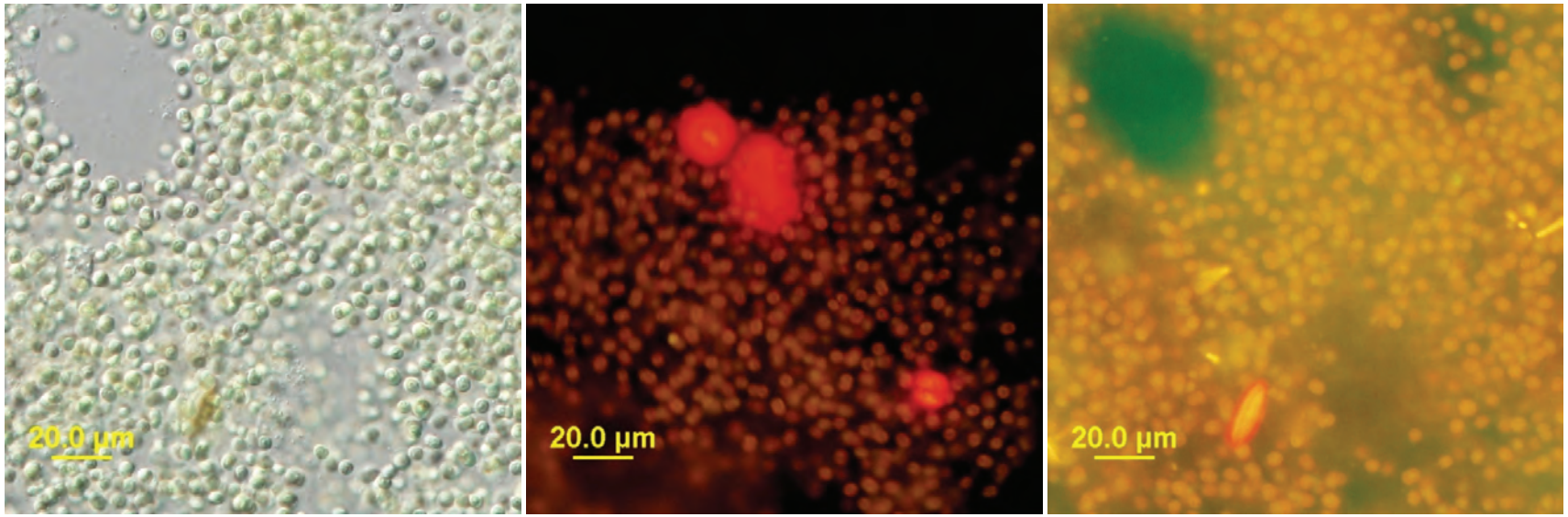


Figure 54. Copco Reservoir, CA (9/10/2009). LM-*Microcystis aeruginosa*. FITC-a red color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

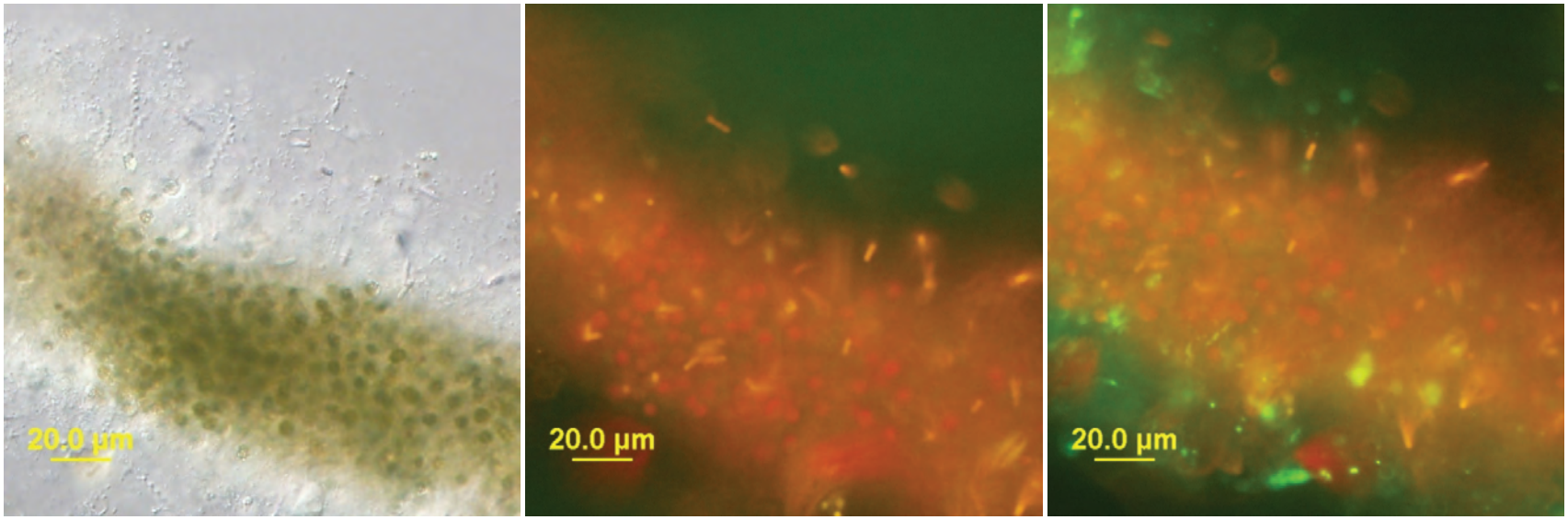


Figure 55. Copco Reservoir, CA (9/10/2009). LM-*Microcystis aeruginosa*. FITC-a red color dominates the cells. Sytox® green-stain did not penetrate the cell membrane of the cyanobacteria; epiphytes associated with the outer mucilage did stain bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

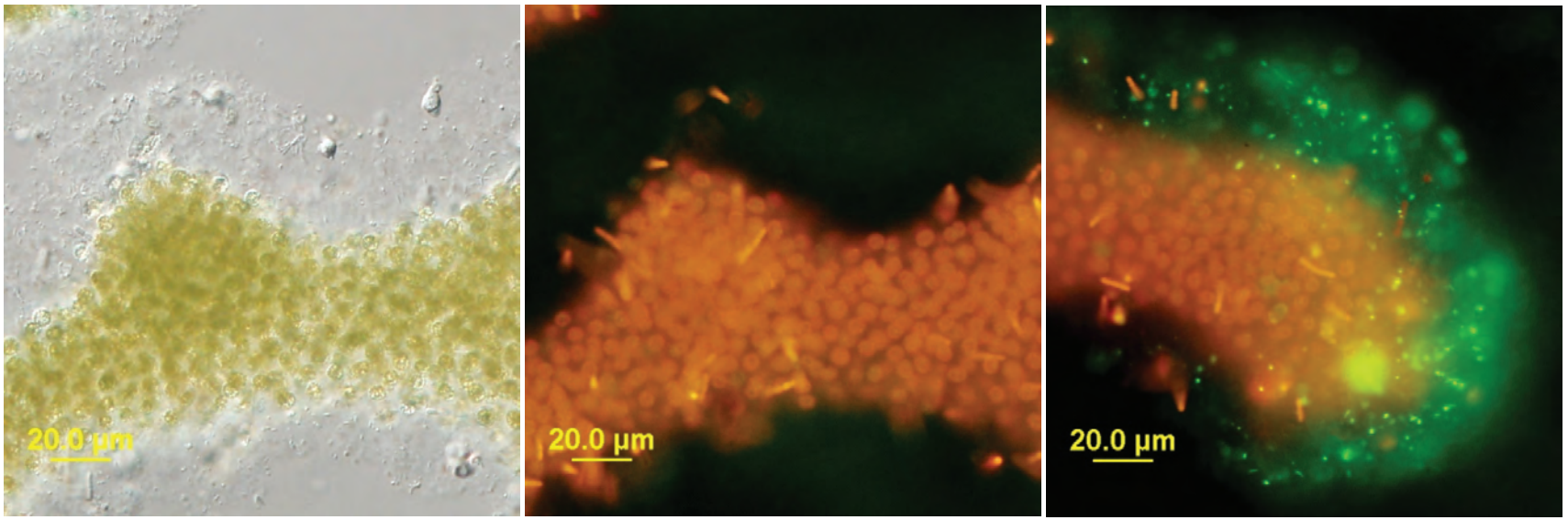


Figure 56. Copco Reservoir, CA (9/10/2009). LM-*Microcystis aeruginosa*. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane of the cyanobacteria; outer mucilage and the associated bacterial cells did stain bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

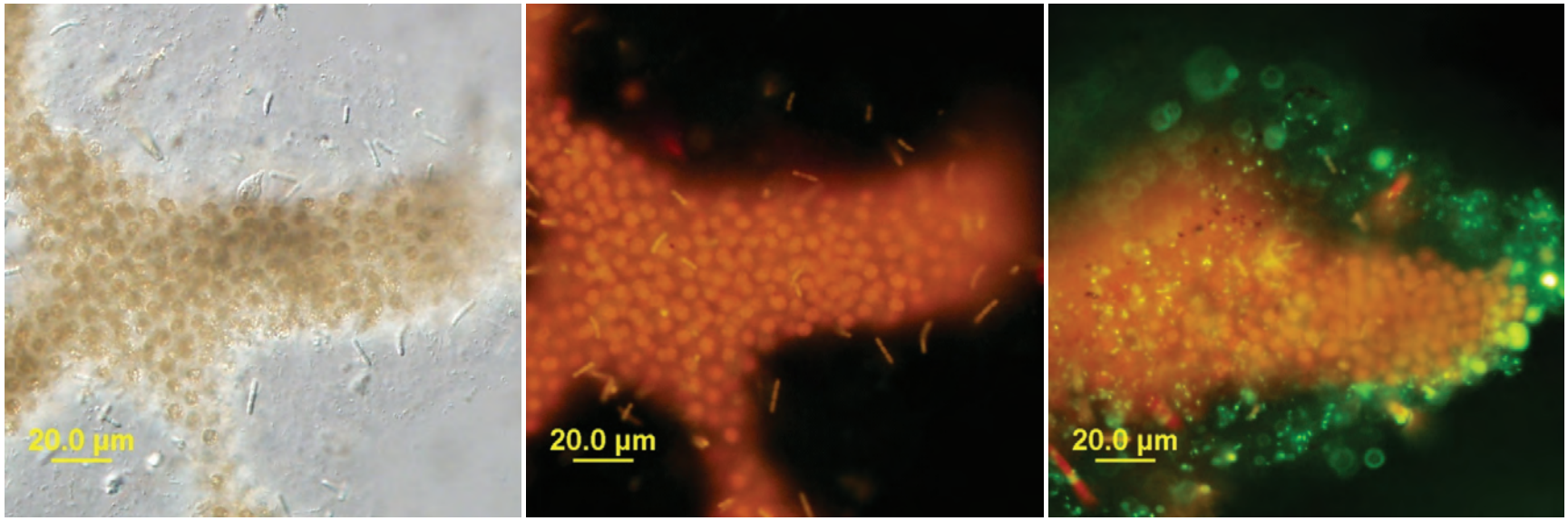


Figure 57. Copco Reservoir, CA (9/10/2009). LM-*Microcystis aeruginosa*. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane of the cyanobacteria; outer mucilage and the associated bacterial cells did stain bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

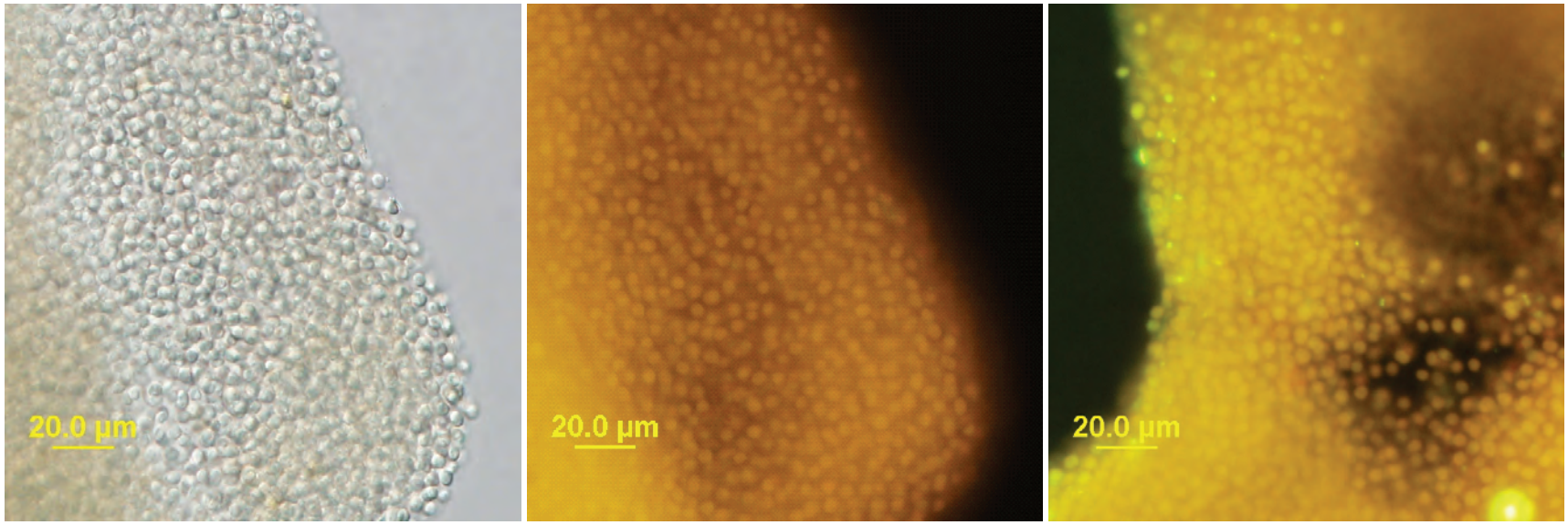


Figure 58. Copco Reservoir, CA (9/10/2009). LM-*Microcystis aeruginosa*. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

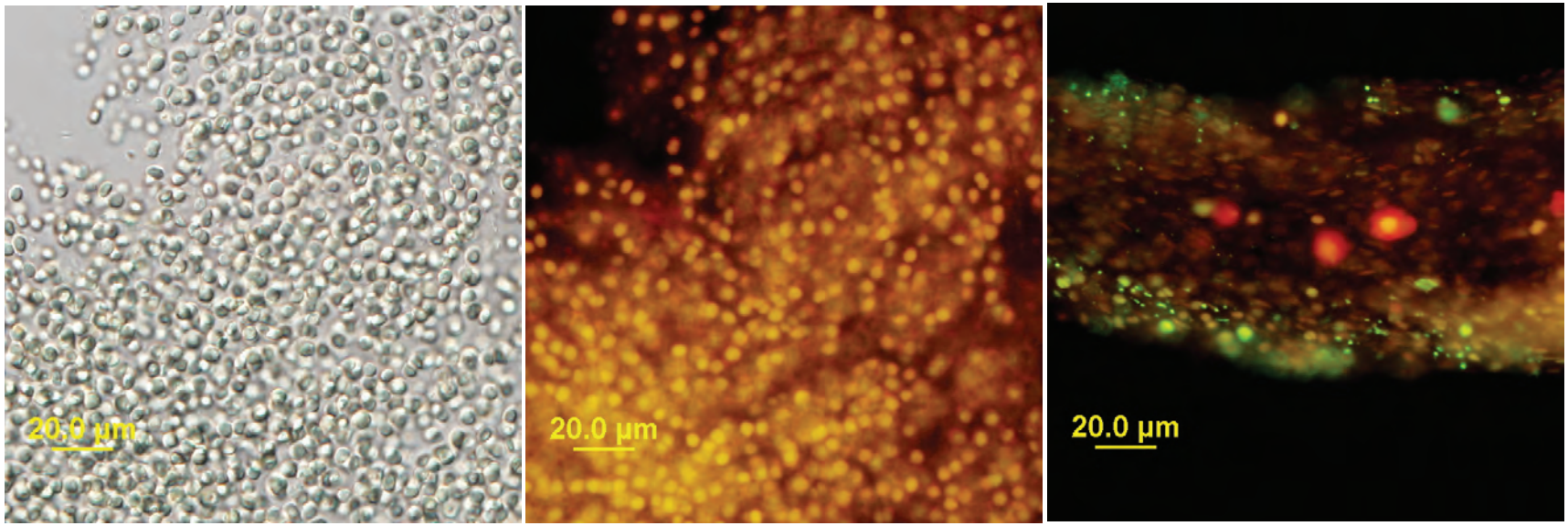


Figure 59. Copco Reservoir, CA. LM-*Microcystis aeruginosa*. FITC-*Microcystis aeruginosa*-an orange color dominates the cells. Sytox® green-*Microcystis wesenbergii*. Stain did not penetrate the cell membrane of the cyanobacteria; the associated bacterial cells did stain bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green

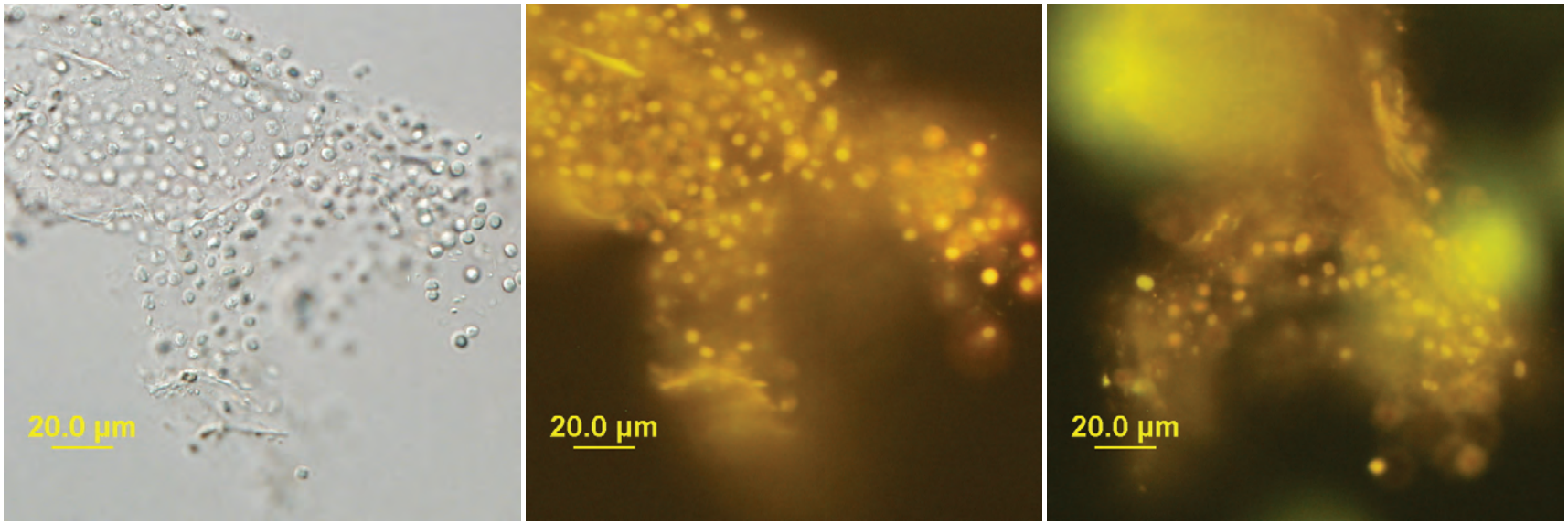


Figure 60. Copco Reservoir, CA (9/10/2009). LM-Degraded *Microcystis aeruginosa*. FITC-a yellow-orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane of the cyanobacteria. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

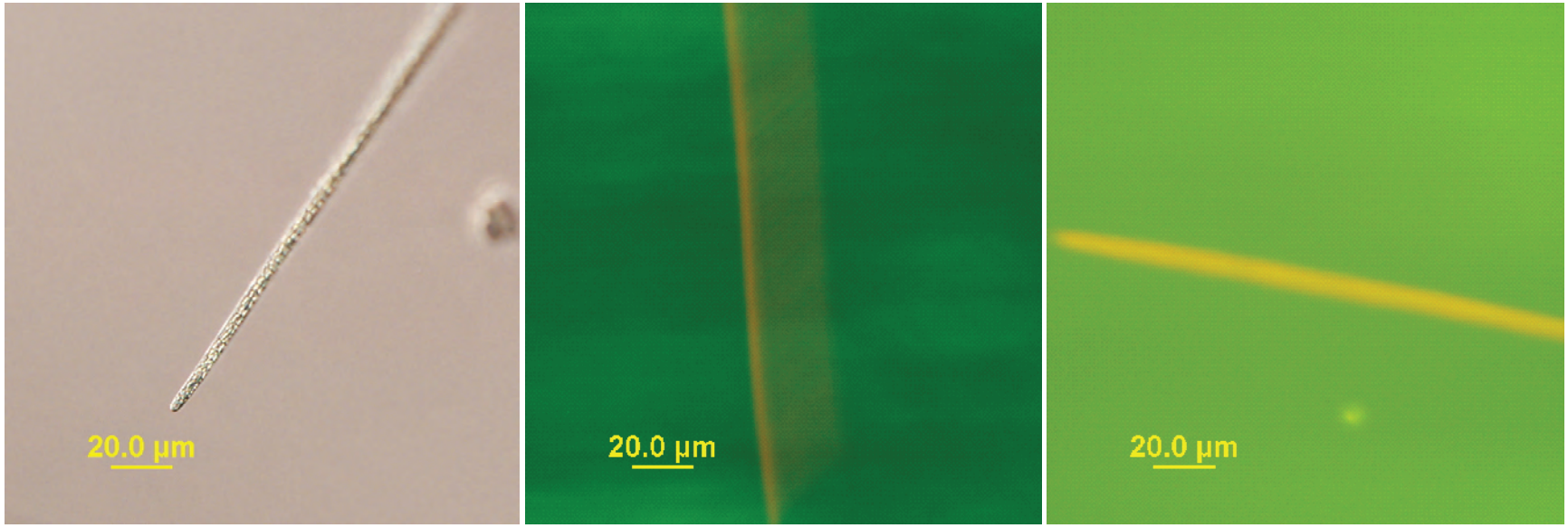


Figure 61. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

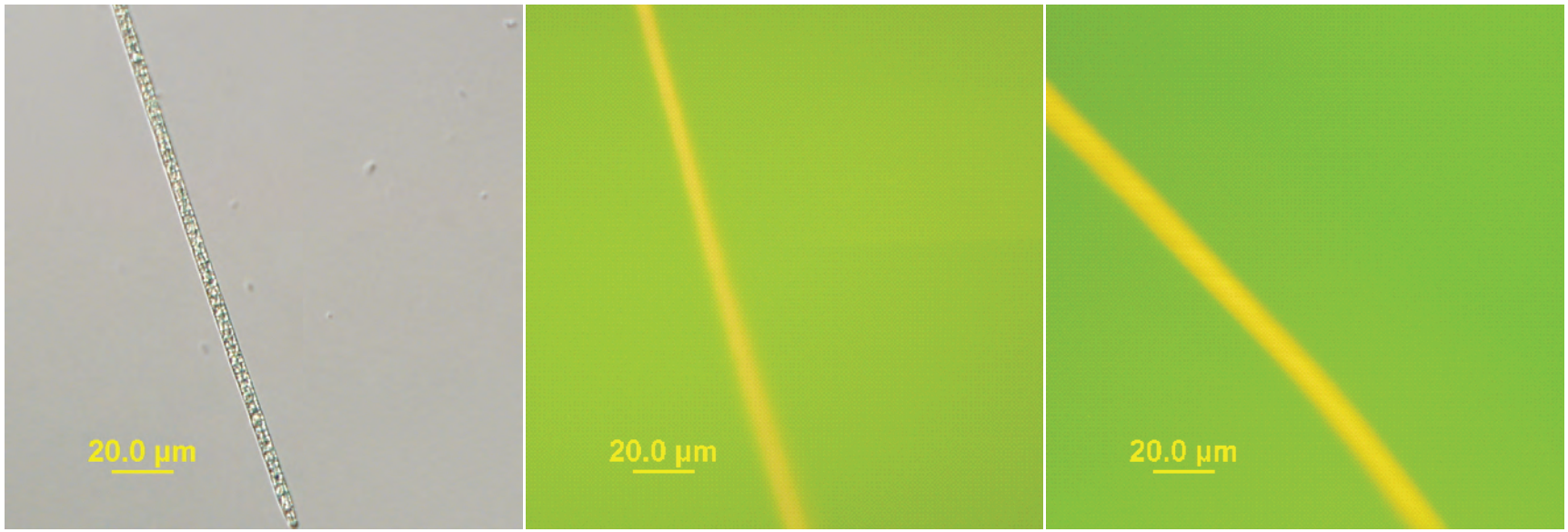


Figure 62. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

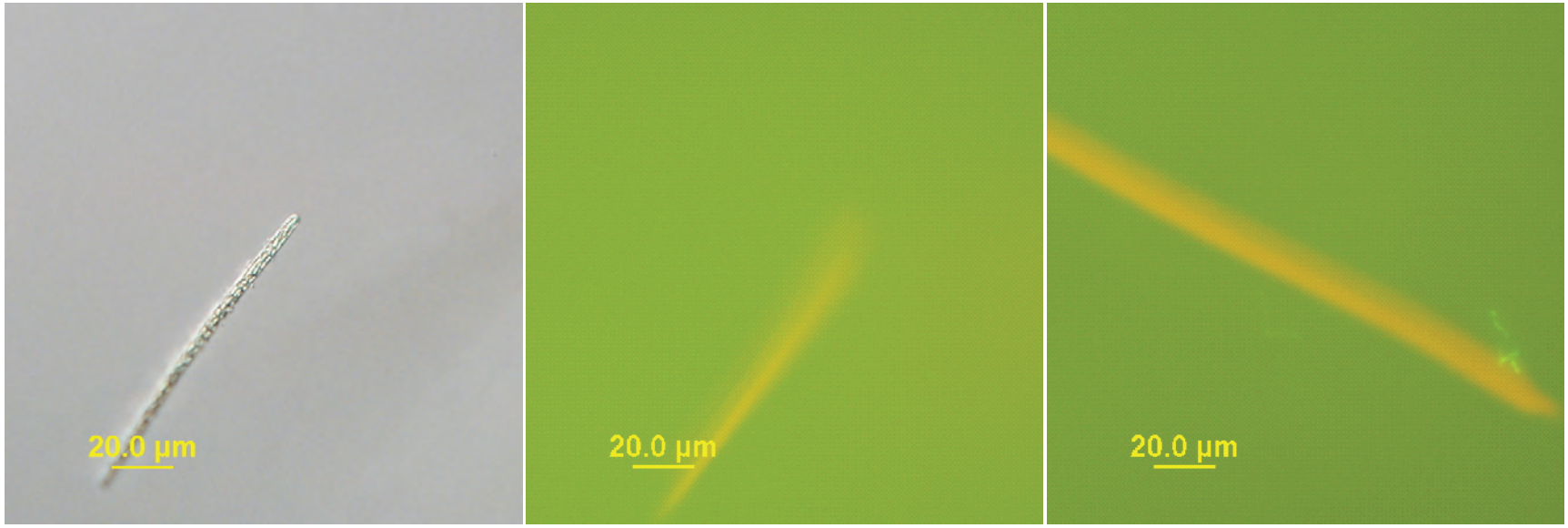


Figure 63. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

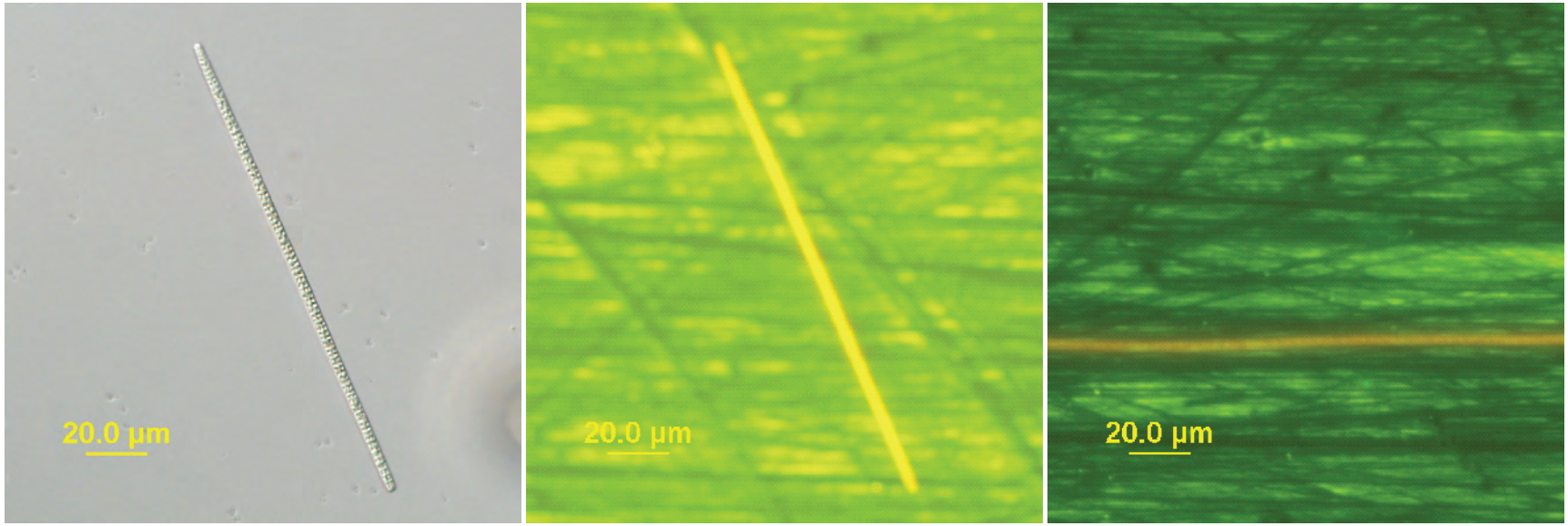


Figure 64. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

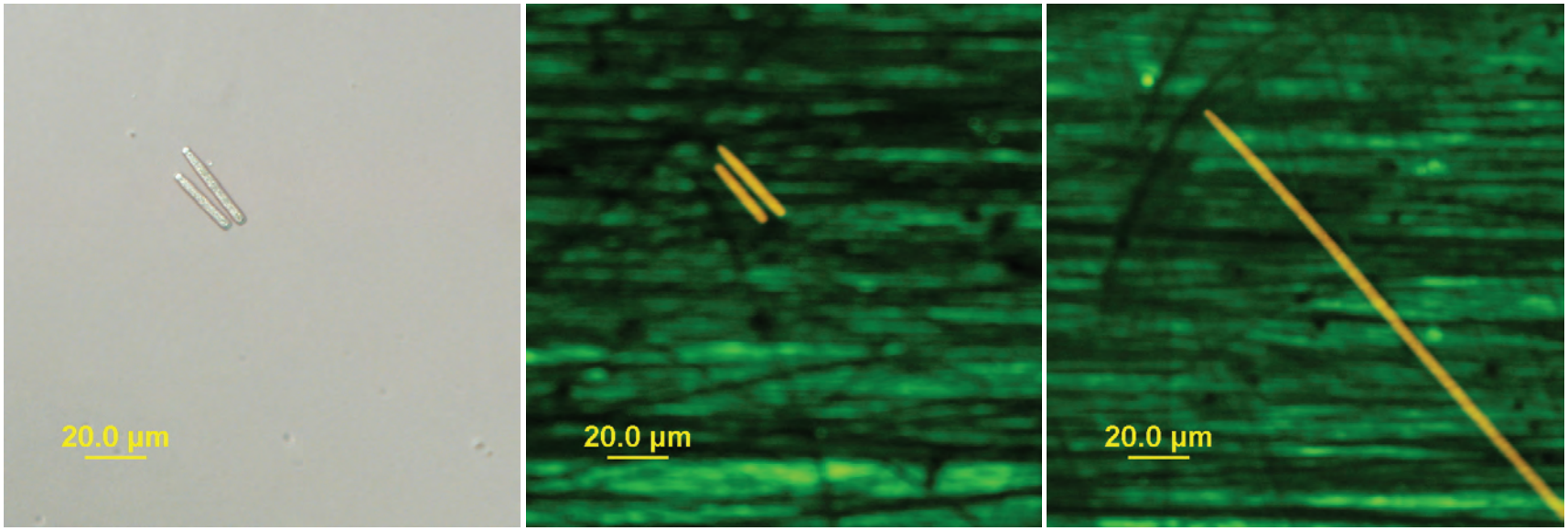


Figure 65. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

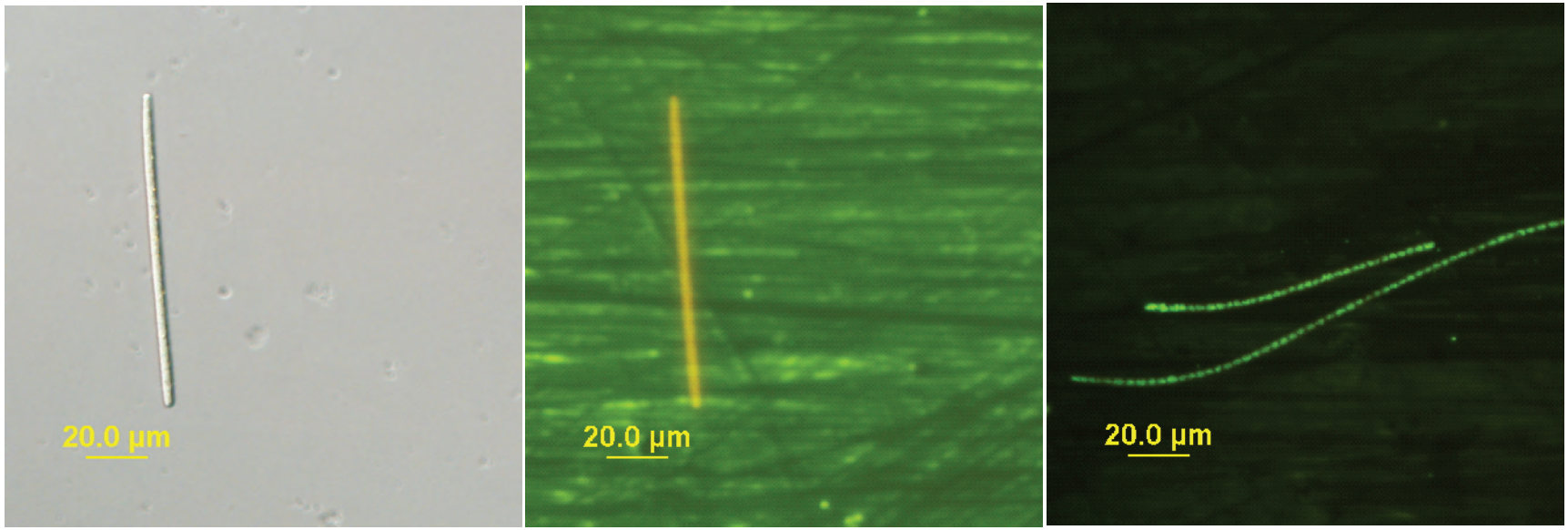


Figure 66. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane, cells bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

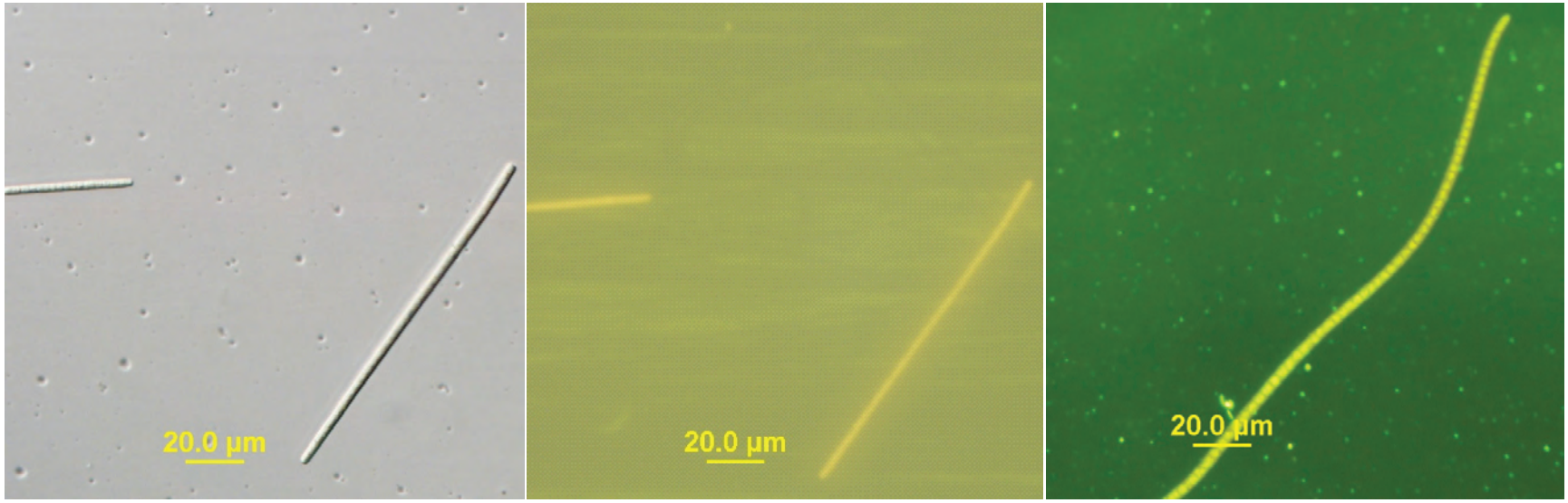


Figure 67. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane, cells yellow-green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

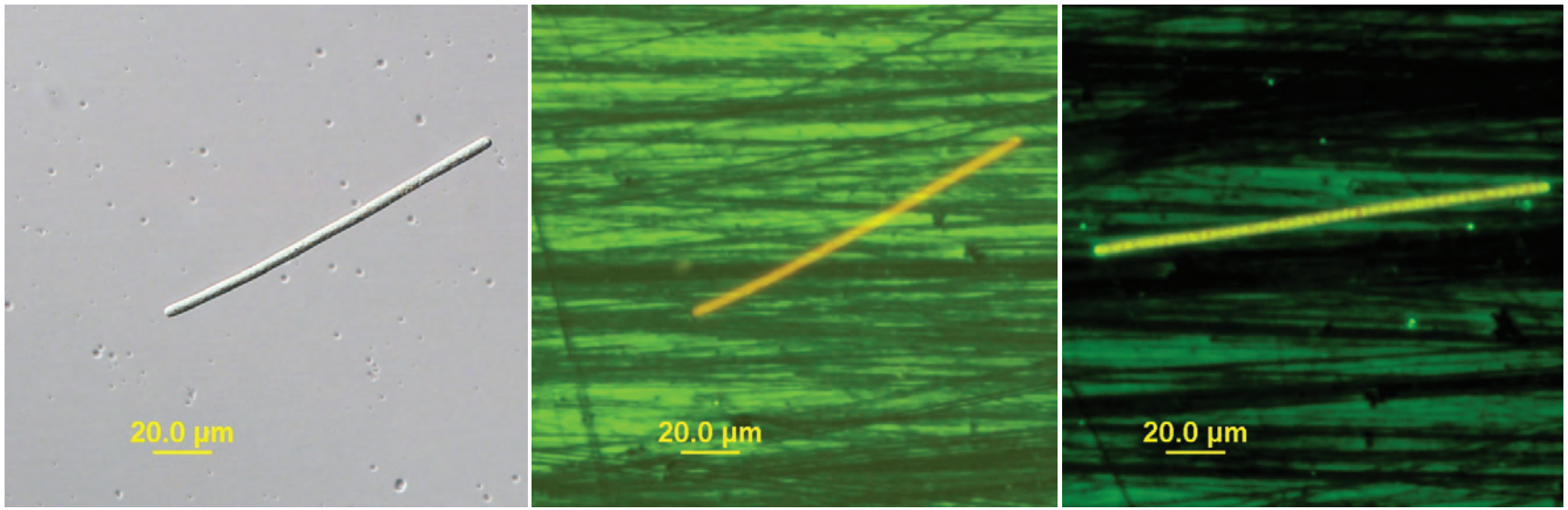


Figure 68. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane, cells yellow-green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

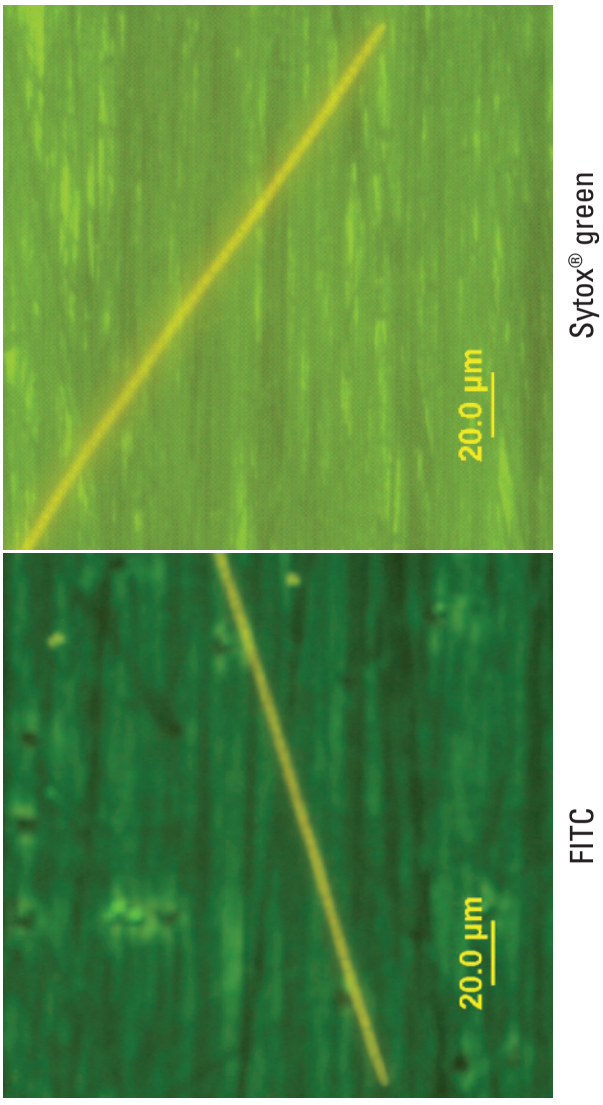


Figure 69. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-No image available. FITC-*Planktothrix* sp.; an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

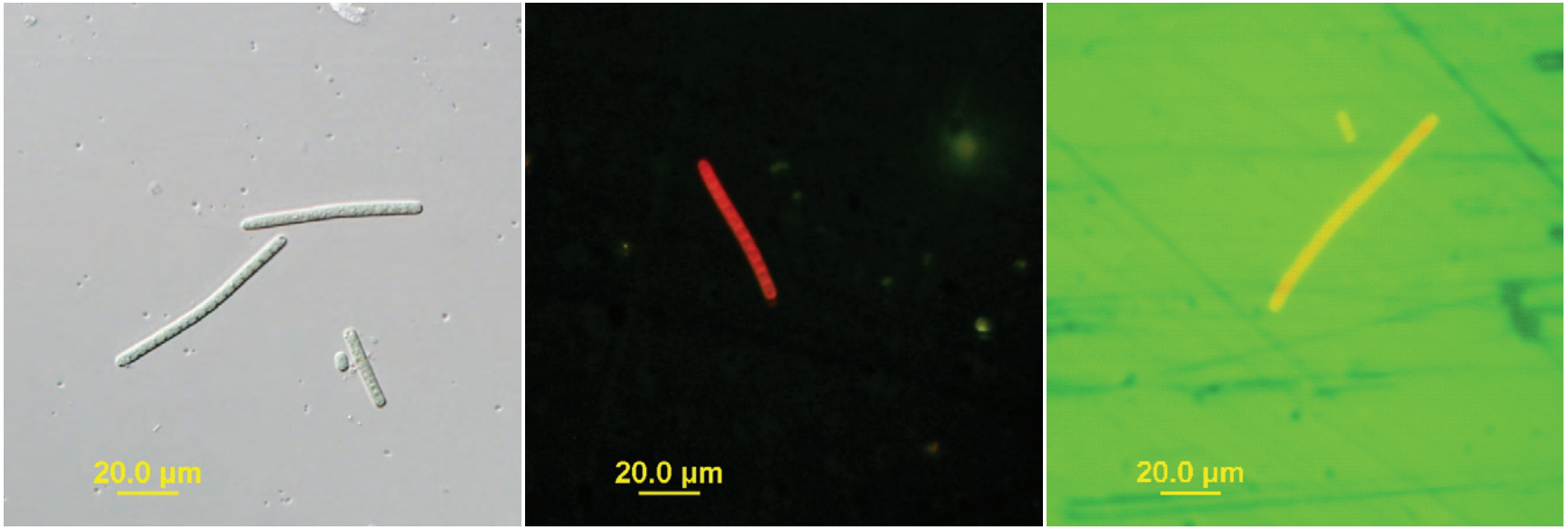


Figure 70. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-*Planktothrix* sp. FITC-a red color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

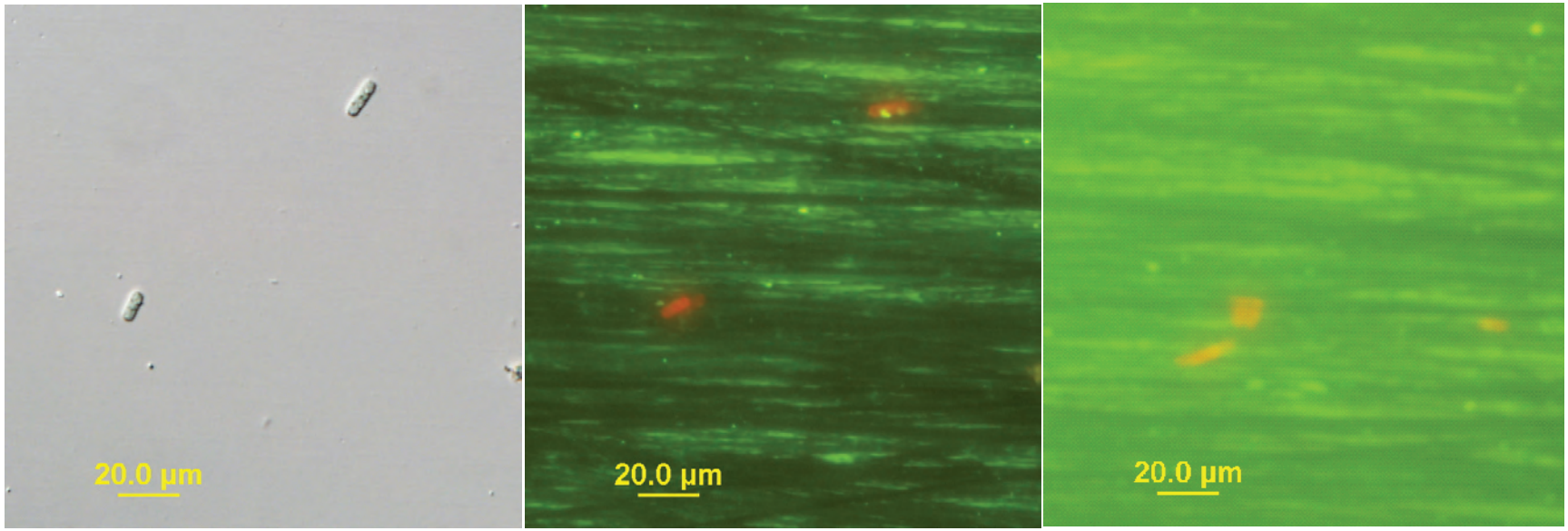
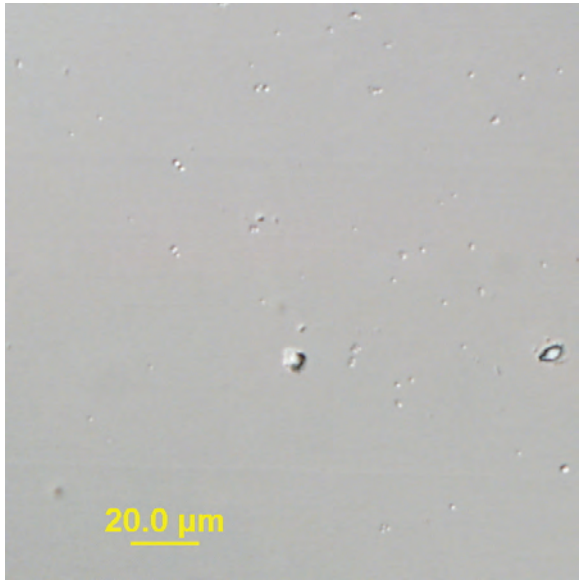


Figure 71. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-Fragments of *Planktothrix* sp. FITC-a red color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.



LM

Sonicated at 70 percent power

Figure 72. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-Nothing recognizable as cells is present. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

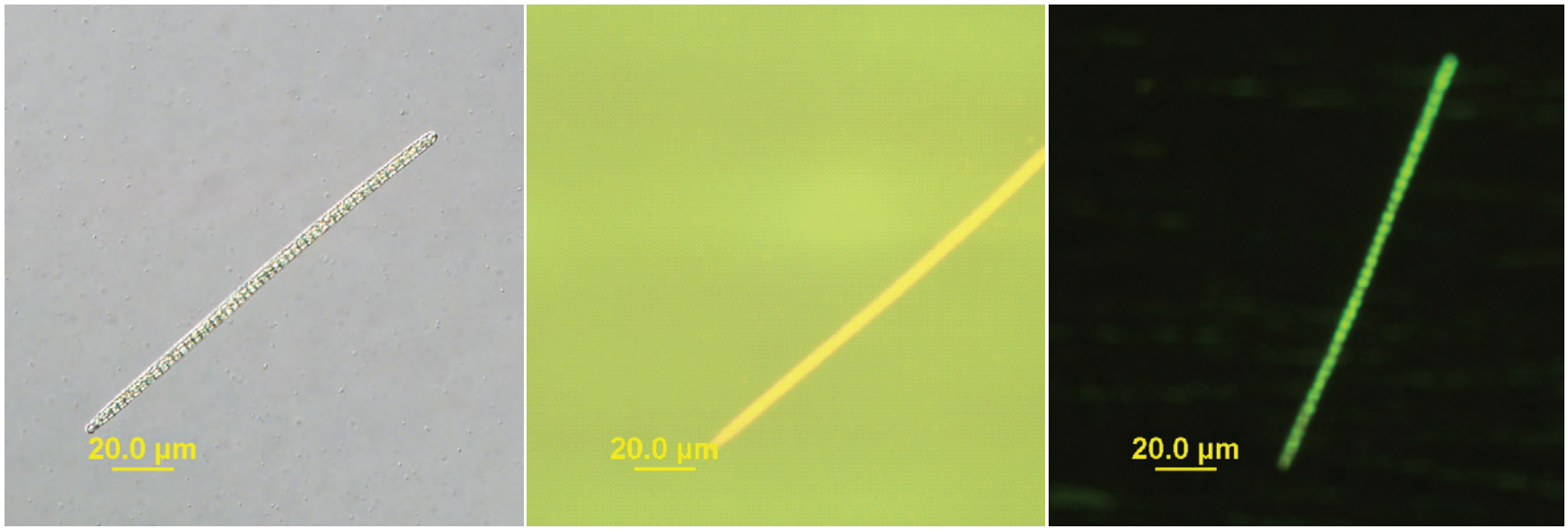


Figure 73. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-*Planktothrix* sp. FITC-an orange-yellow color dominates the cells. Sytox® green-stain did penetrate the cell membrane, cells bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

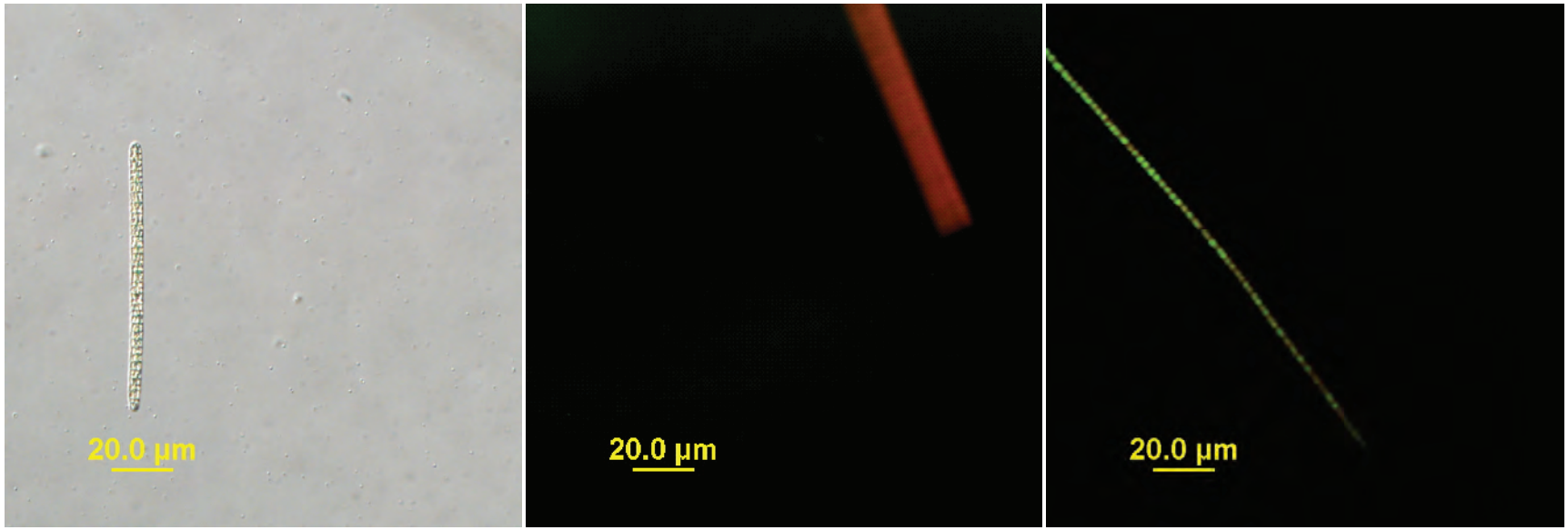


Figure 74. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-*Planktothrix* sp. FITC-red dominates the cells; this image is blurred because of the long exposure time. Sytox® green-unknown cyanobacterial filament (too thin to be the previous *Planktothrix*) stain did penetrate the cell membrane, cells bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

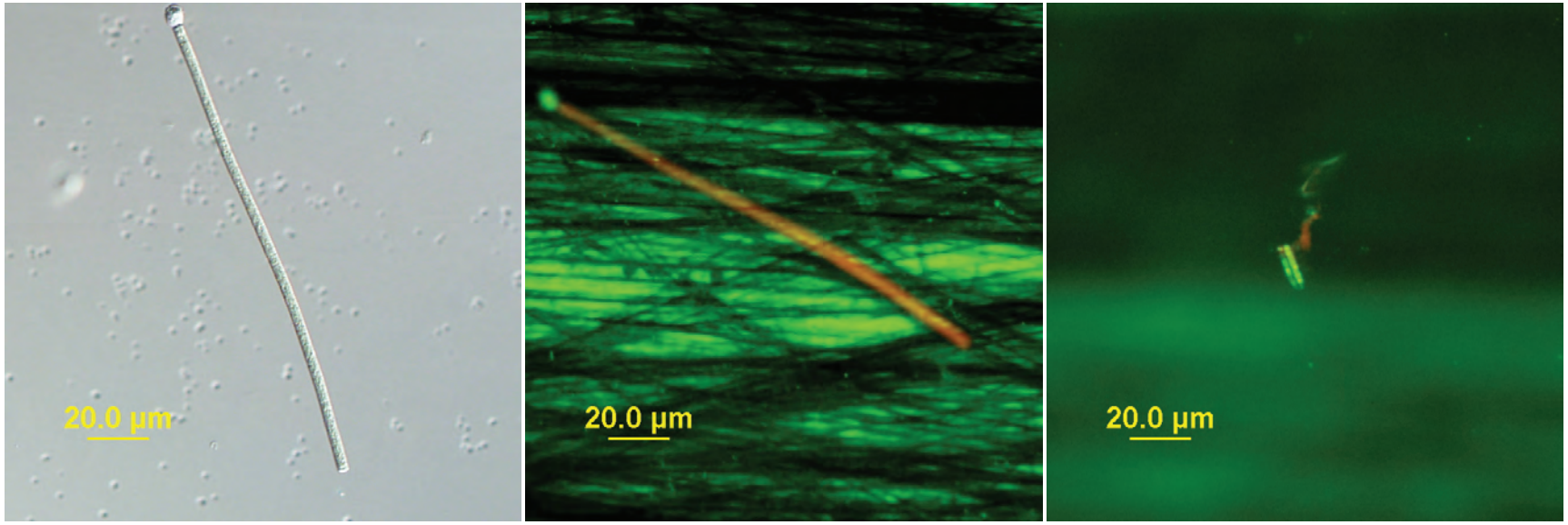


Figure 75. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells; this image is blurred because of the long exposure time. Sytox® green-too little material to describe effect. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

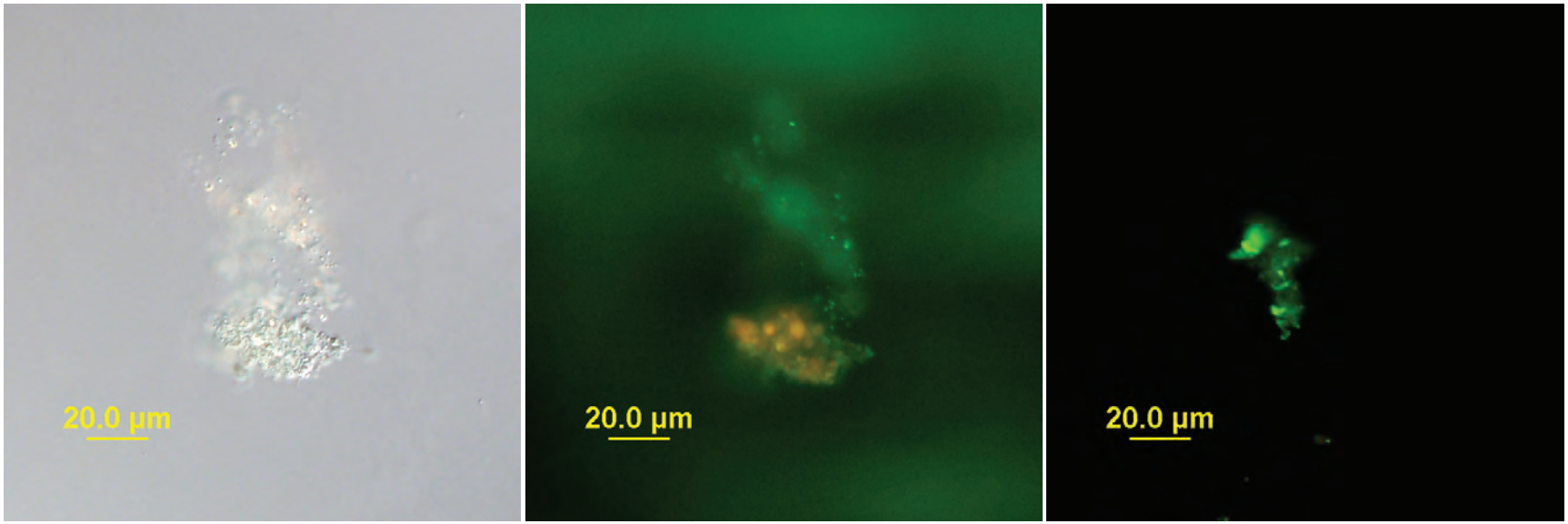


Figure 76. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-Likely the remains of a cyanobacterial colony. FITC-an orange color dominates the few cells present. Sytox® green-too little material to describe effect. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

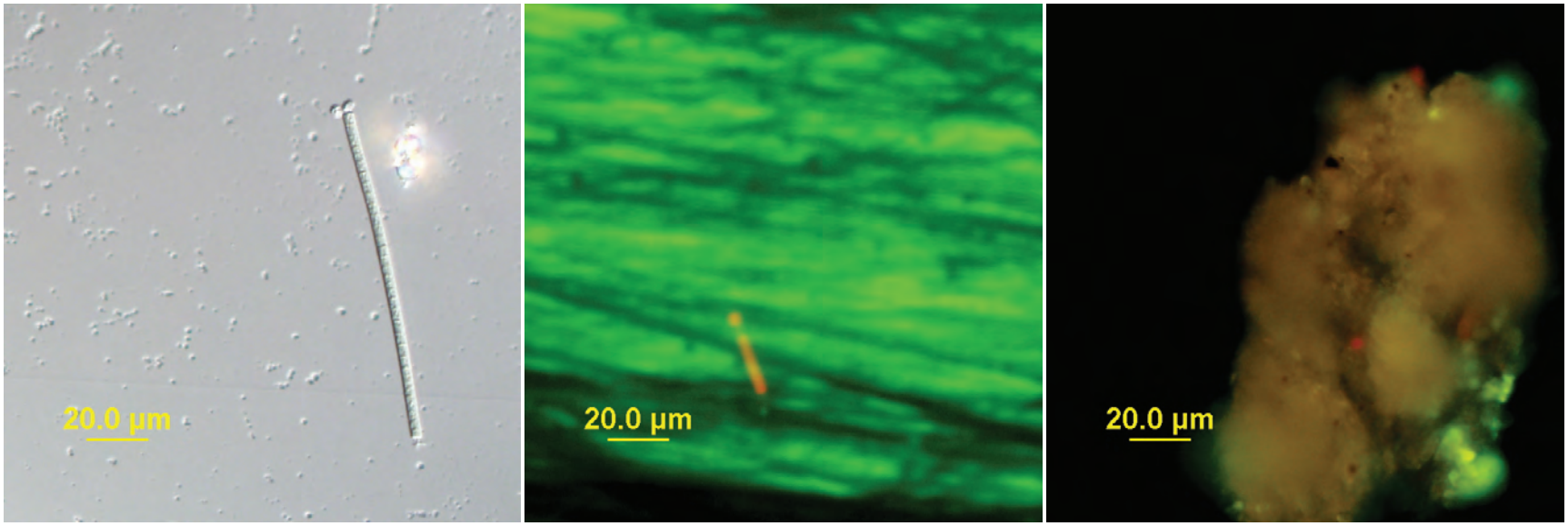


Figure 77. Grand Lake (Lake St. Mary), OH (7/20/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells; this image is blurred because of the long exposure time. Sytox® green-unknown cyanobacterial colony; stain did not penetrate the majority of the cells in the colony. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

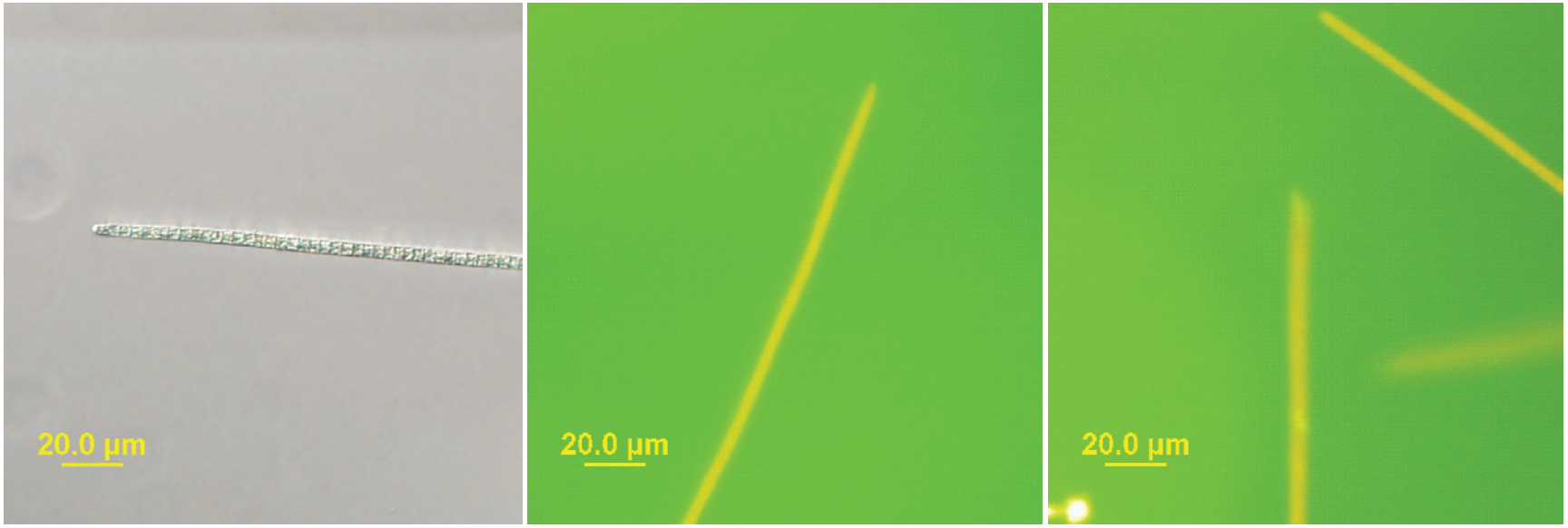


Figure 78. Grand Lake (Lake St. Mary), OH (9/15/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

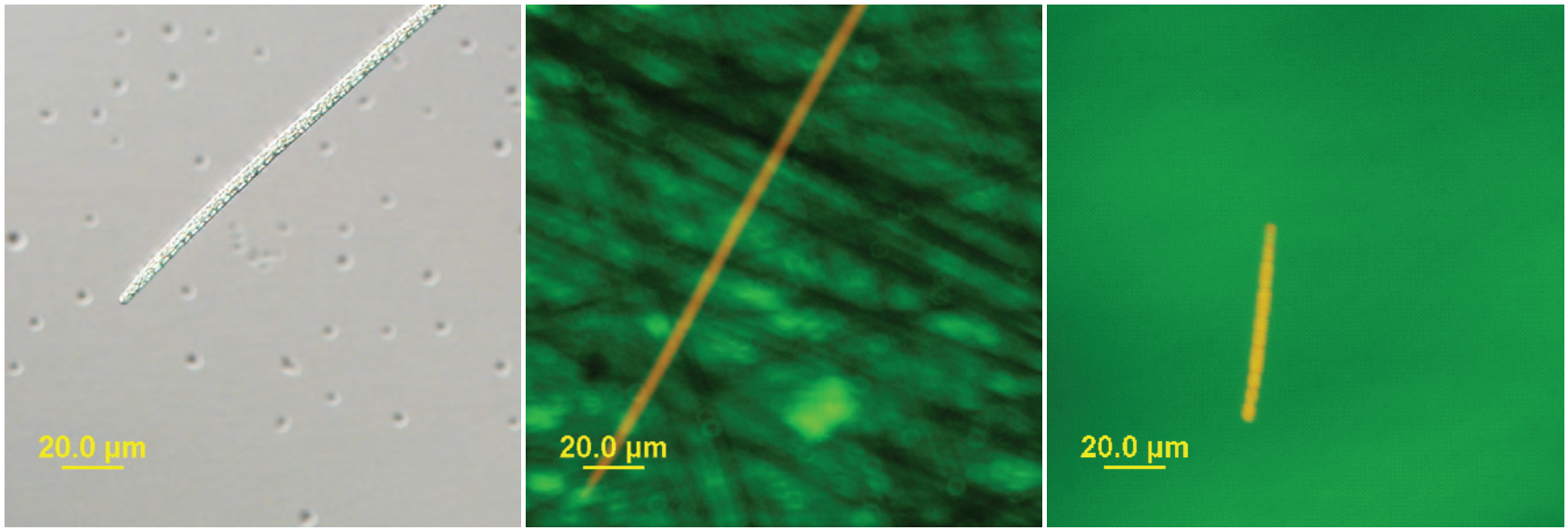


Figure 79. Grand Lake (Lake St. Mary), OH (9/15/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

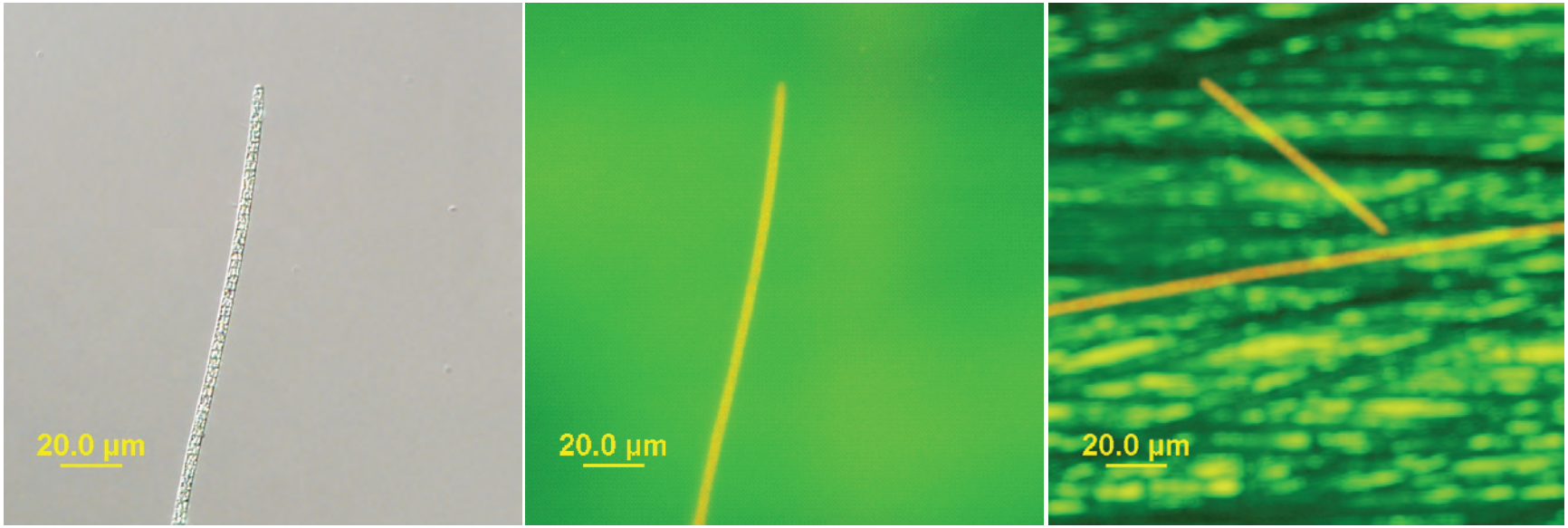


Figure 80. Grand Lake (Lake St. Mary), OH (9/15/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

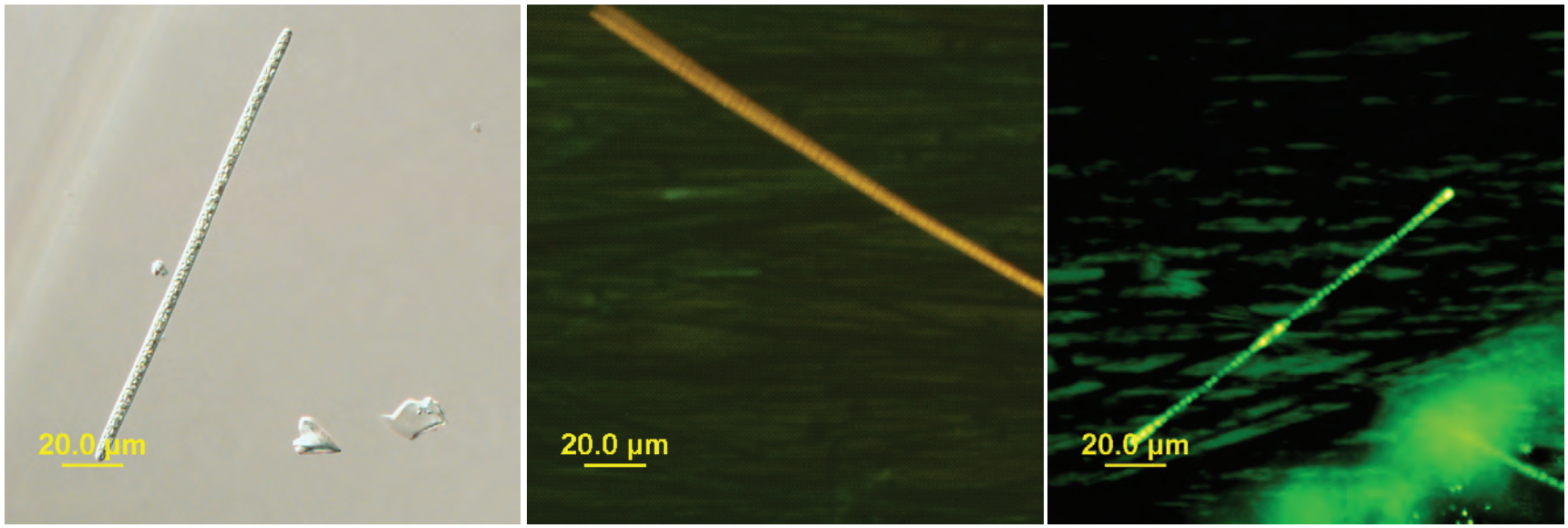


Figure 81. Grand Lake (Lake St. Mary), OH (9/15/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane, cells bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

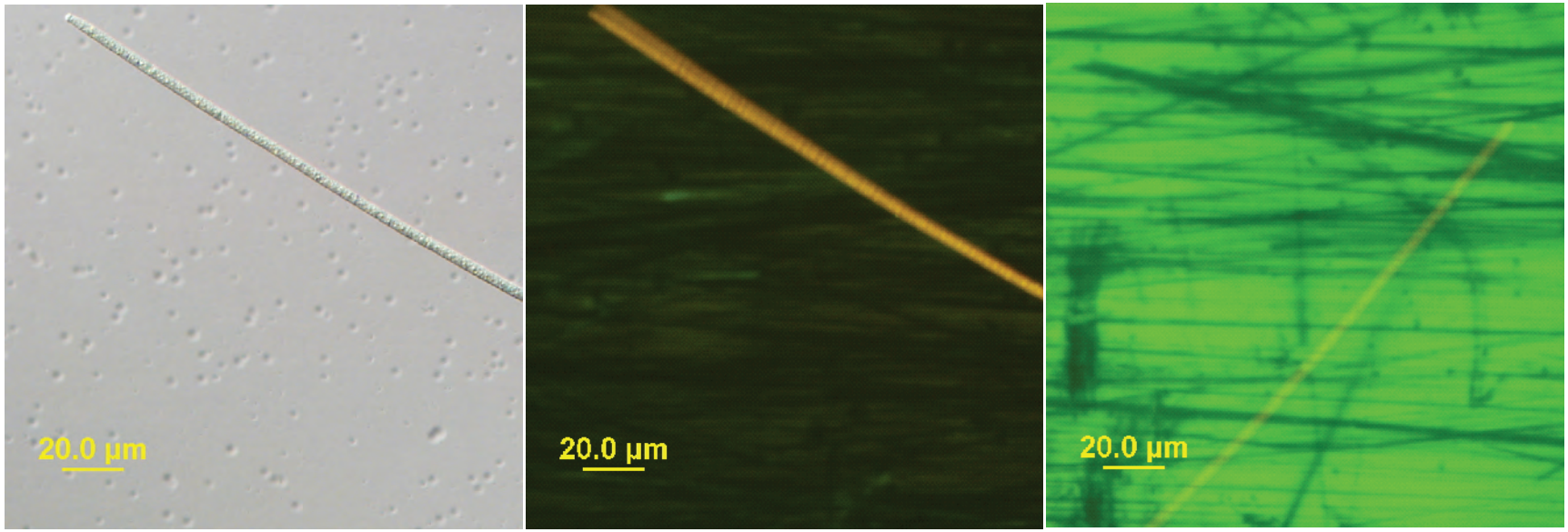


Figure 82. Grand Lake (Lake St. Mary), OH (9/15/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

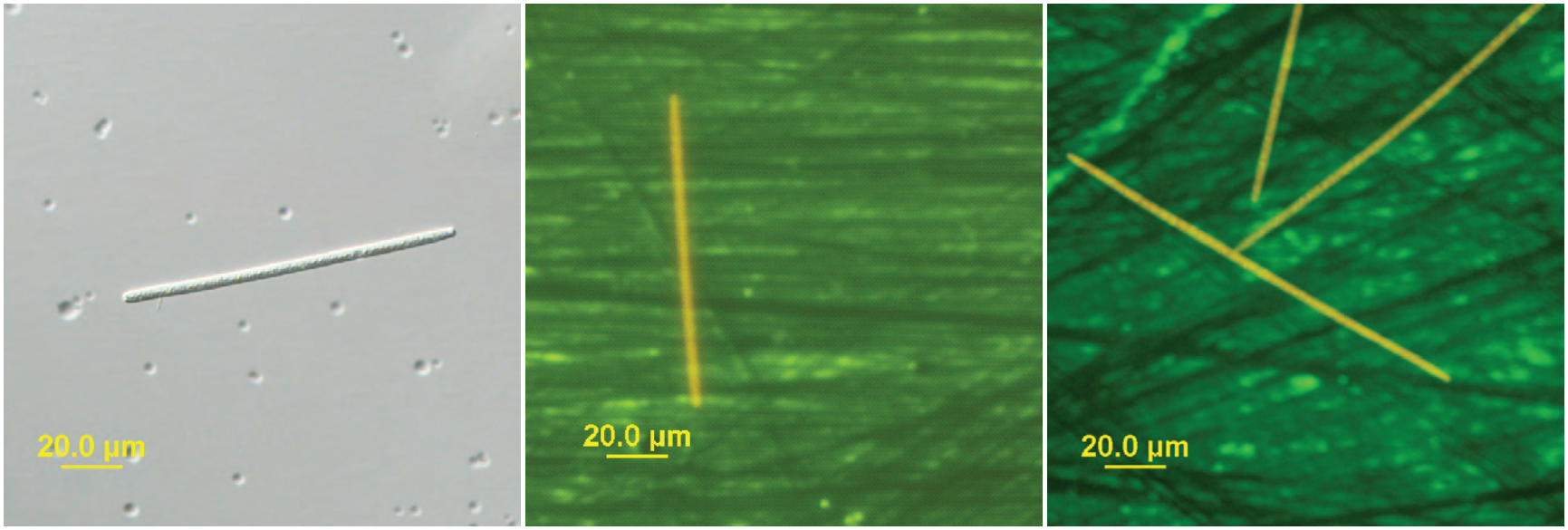


Figure 83. Grand Lake (Lake St. Mary), OH (9/15/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

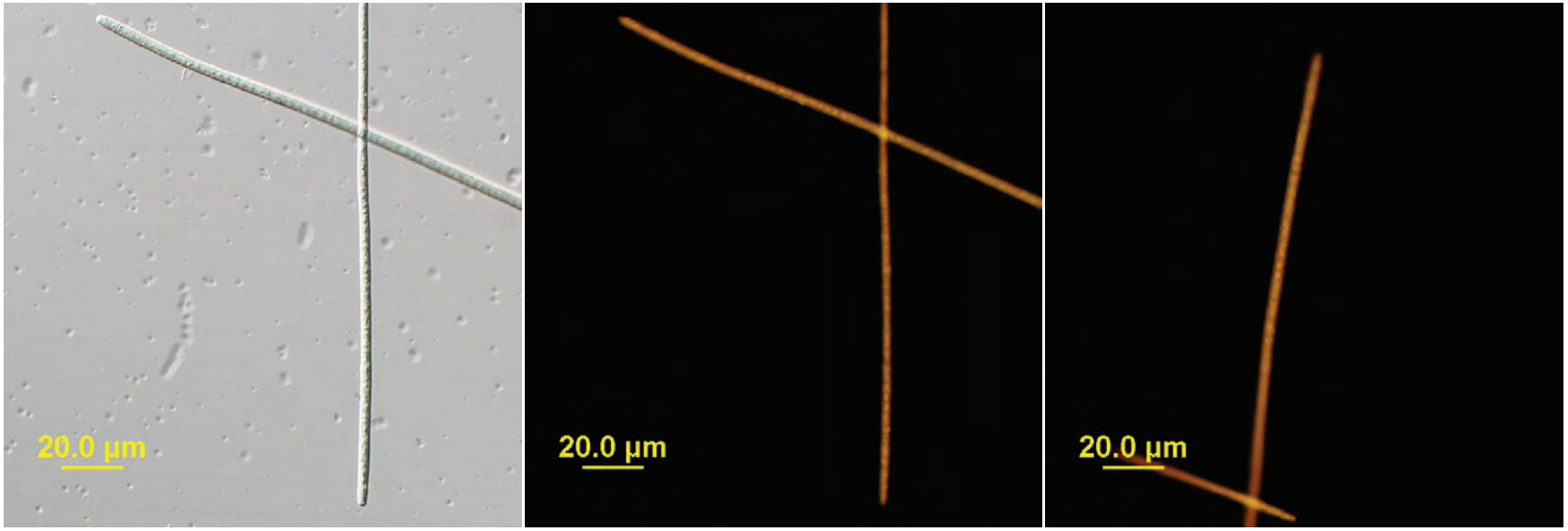


Figure 84. Grand Lake (Lake St. Mary), OH (9/15/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

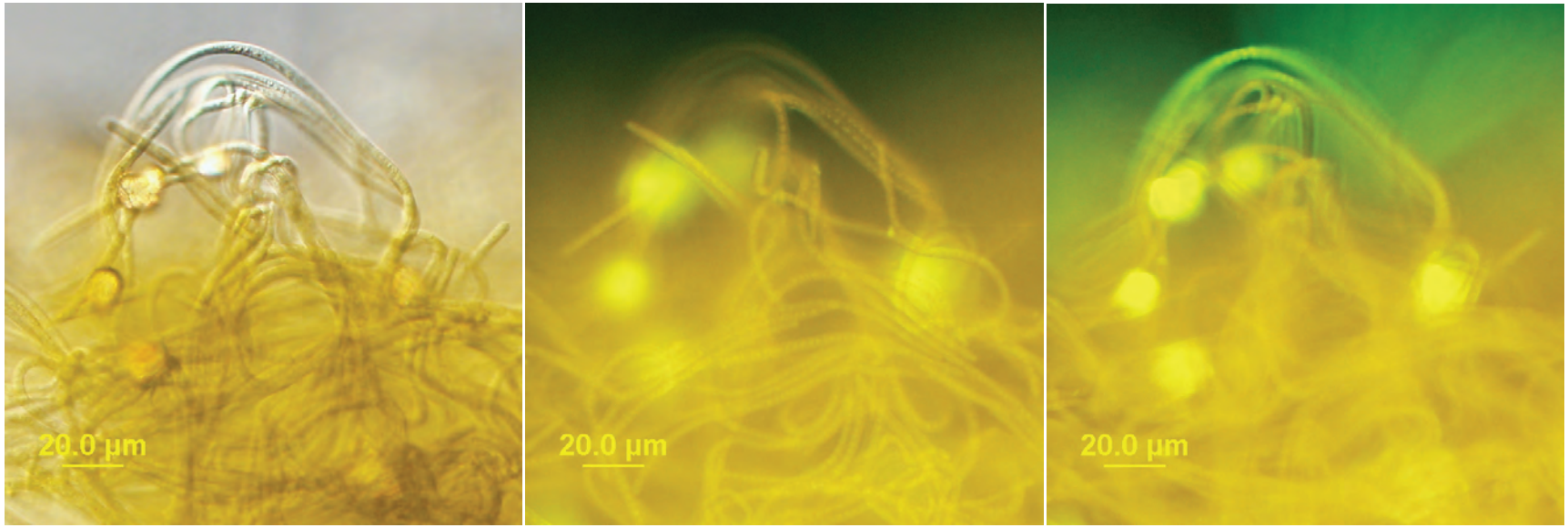


Figure 85. Grand Lake (Lake St. Mary), OH (9/15/2009). LM-A mixture of cyanobacterial filaments and eukaryotic cells. FITC-an orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane of the uppermost filament in this image. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

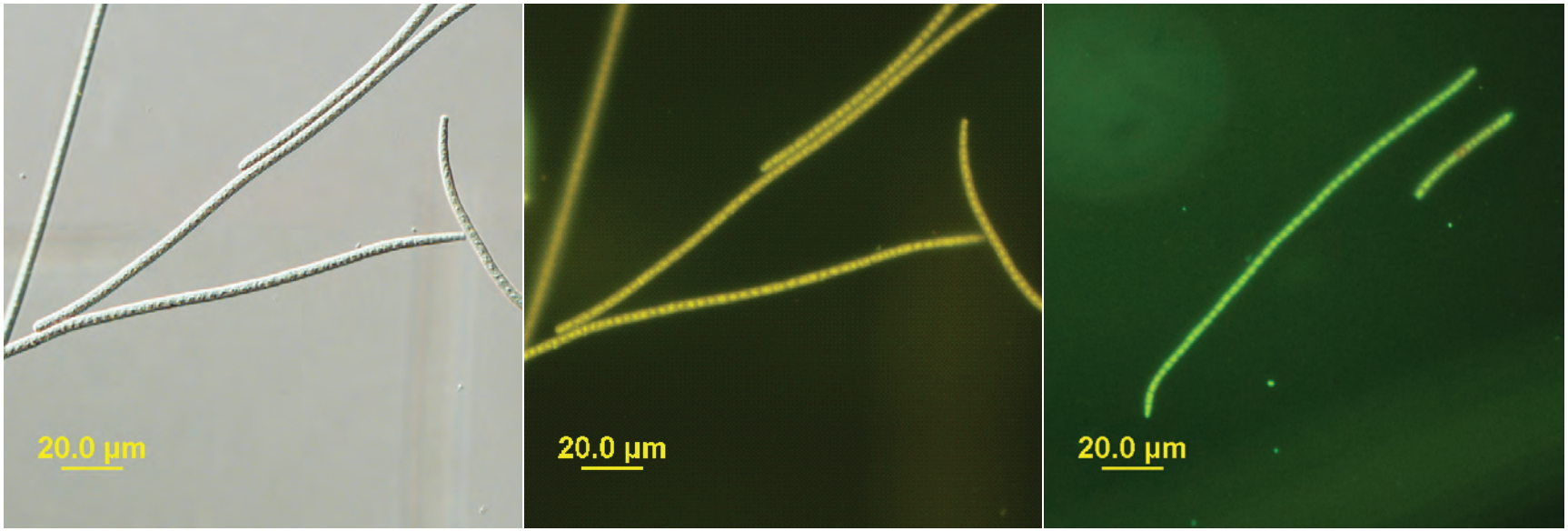


Figure 86. Grand Lake (Lake St. Mary), OH (9/15/2009). LM-*Planktothrix* sp. FITC-a yellow color dominates the cells. Sytox® green-stain did penetrate the cell membrane, cells bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

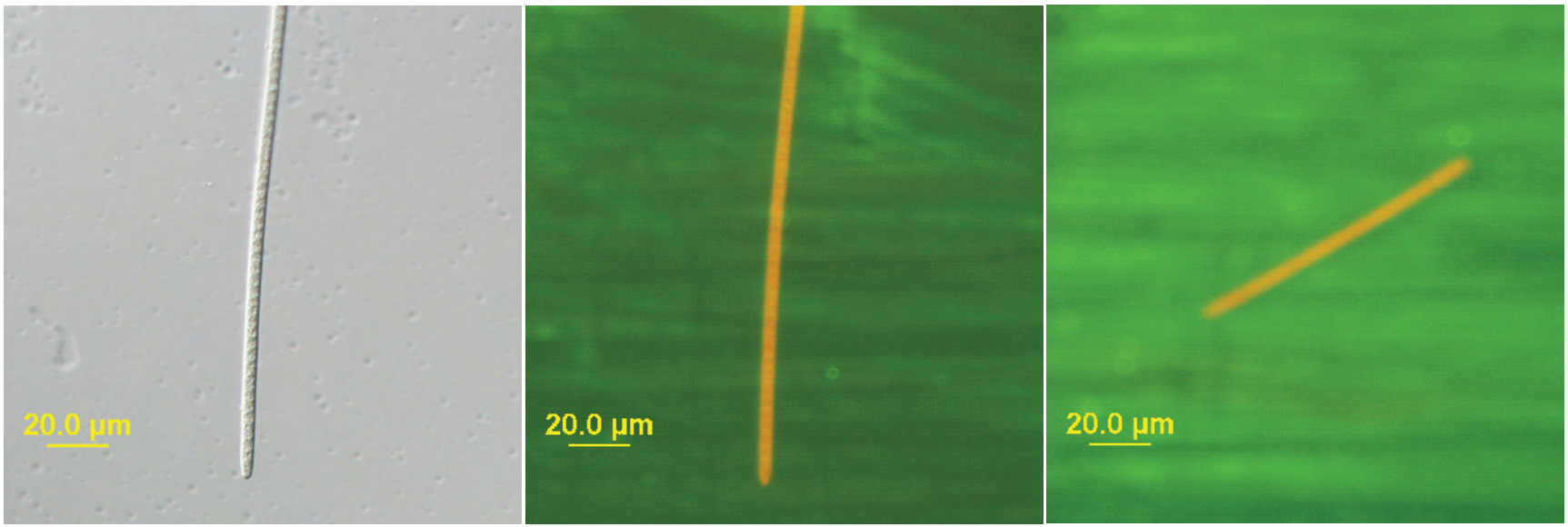


Figure 87. Grand Lake (Lake St. Mary), OH (9/15/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

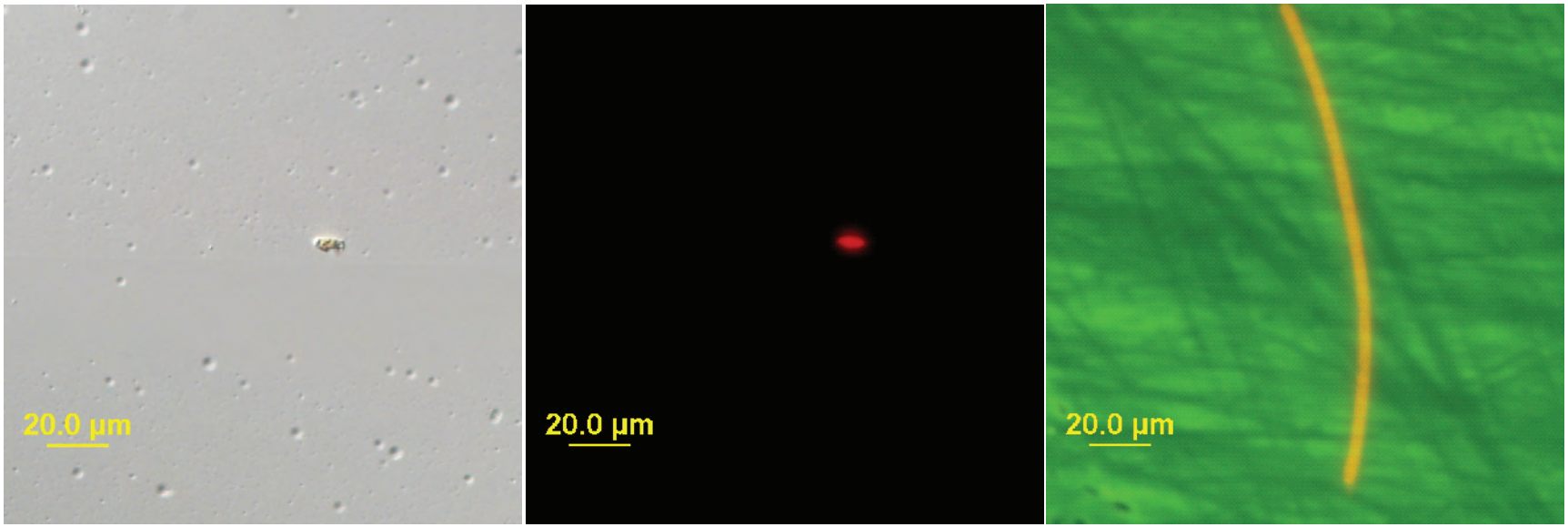


Figure 88. Grand Lake (Lake St. Mary), OH (9/15/2009). LM-Unknown fragment. FITC-a red color dominates this cell fragment. Sytox® green-stain did not penetrate the cell membrane of this *Planktothrix* filament. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green. Note: Sample sonicated at 70 percent power-no cellular material found..

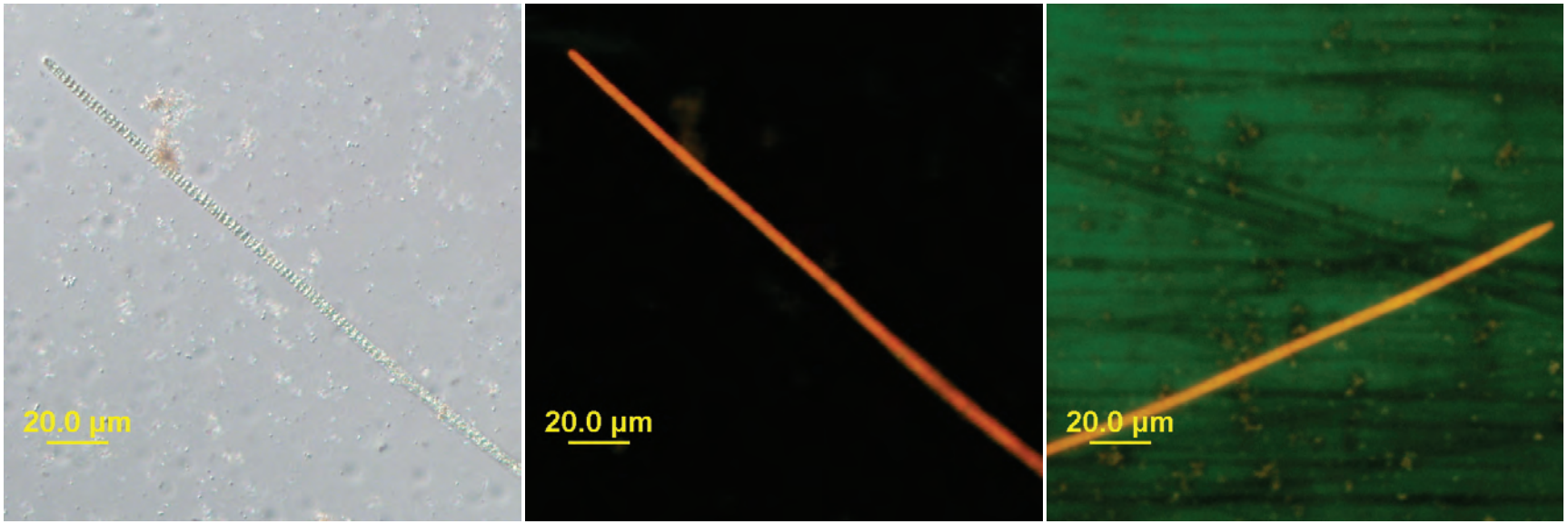


Figure 89. Grand Lake (Lake St. Mary), OH (9/15/2009). LM-*Planktothrix* sp. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

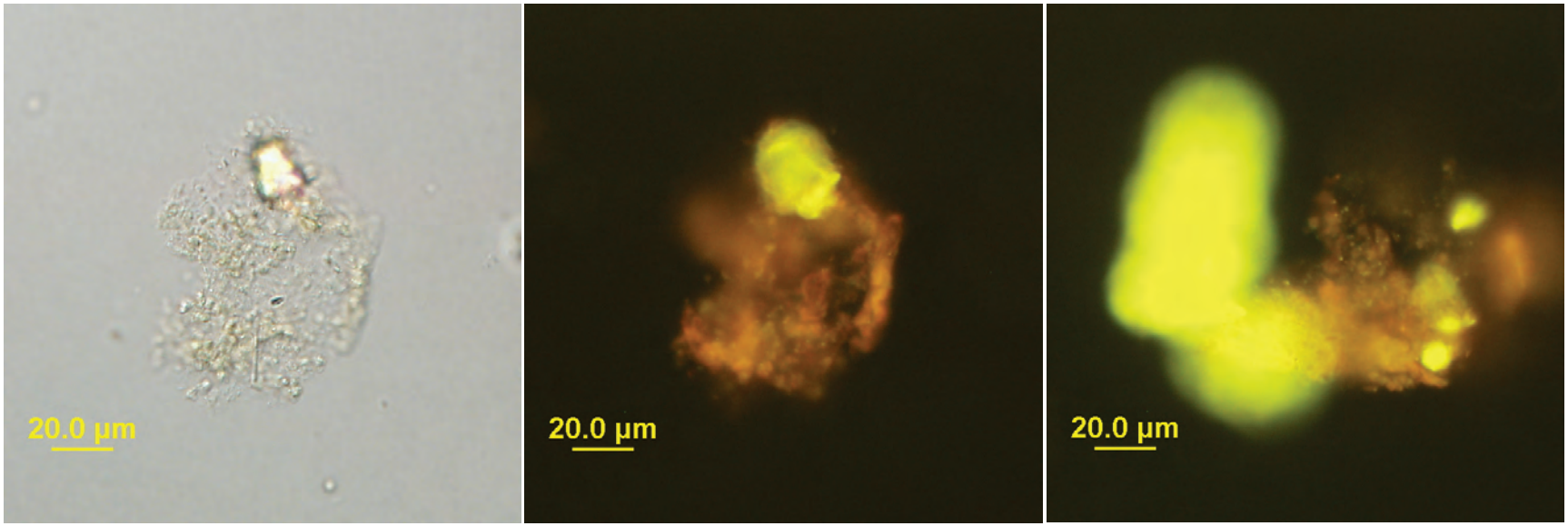


Figure 90. Grand Lake (Lake St. Mary), OH (9/15/2009). LM-Unknown cyanobacterial colony fragment. FITC-an orange color dominates this cell. Sytox® green-stain did not stain the cyanobacterial material; only the eukaryotic cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

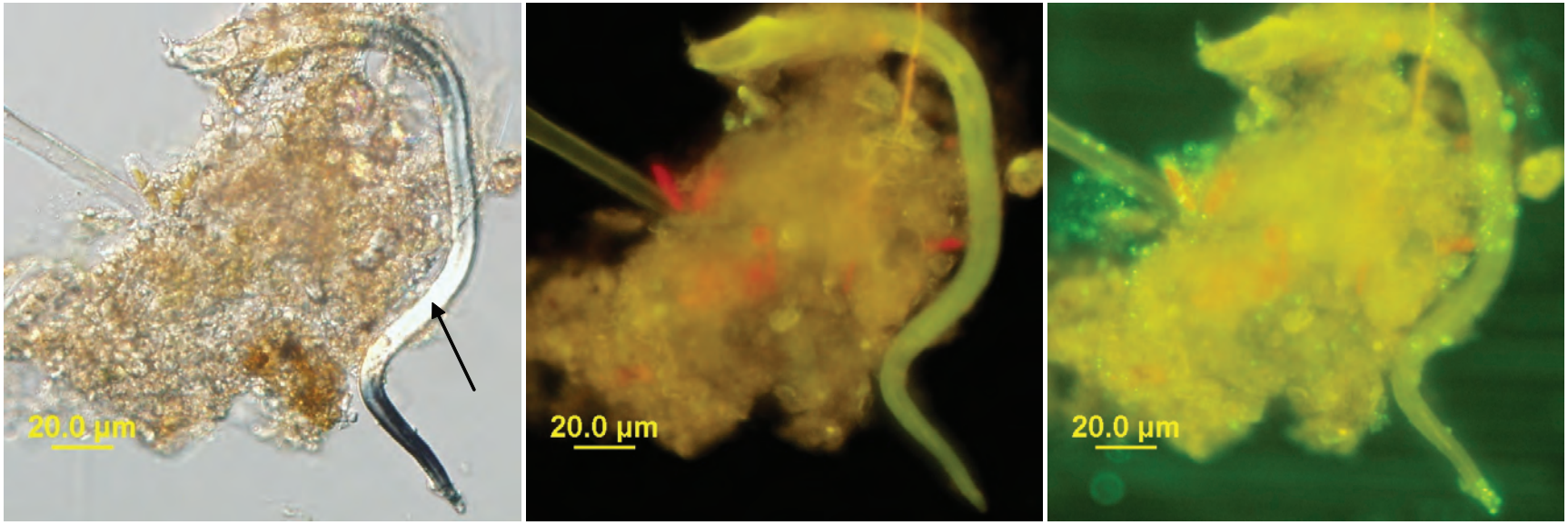


Figure 91. Grand Lake (Lake St. Mary), OH (9/15/2009). LM-Possible unknown cyanobacterial colony fragment. A nematode is evident in this sample (arrow). FITC-an orange color dominates this cell; the bright red cells are diatoms. Sytox® green-stain did not stain the cyanobacterial material; only the bacterial cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

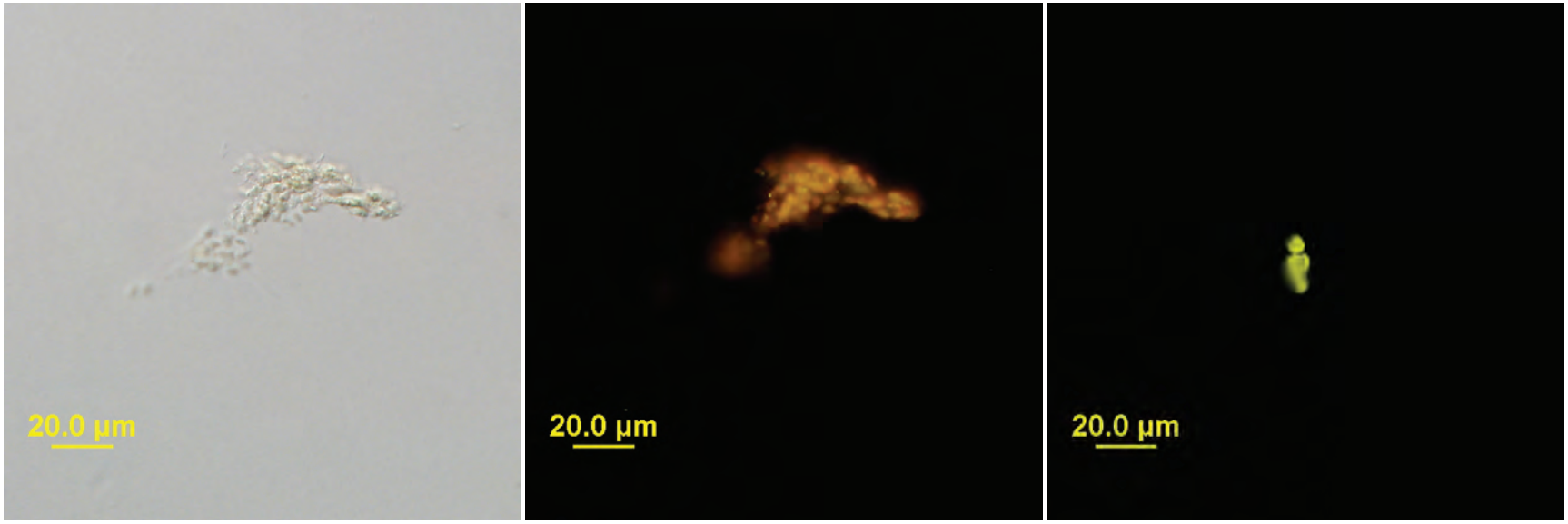


Figure 92. Grand Lake (Lake St. Mary), OH (9/15/2009). LM-Possible unknown cyanobacterial colony fragment. FITC-an orange color dominates these cells. Sytox® green-too little material to determine the effect. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

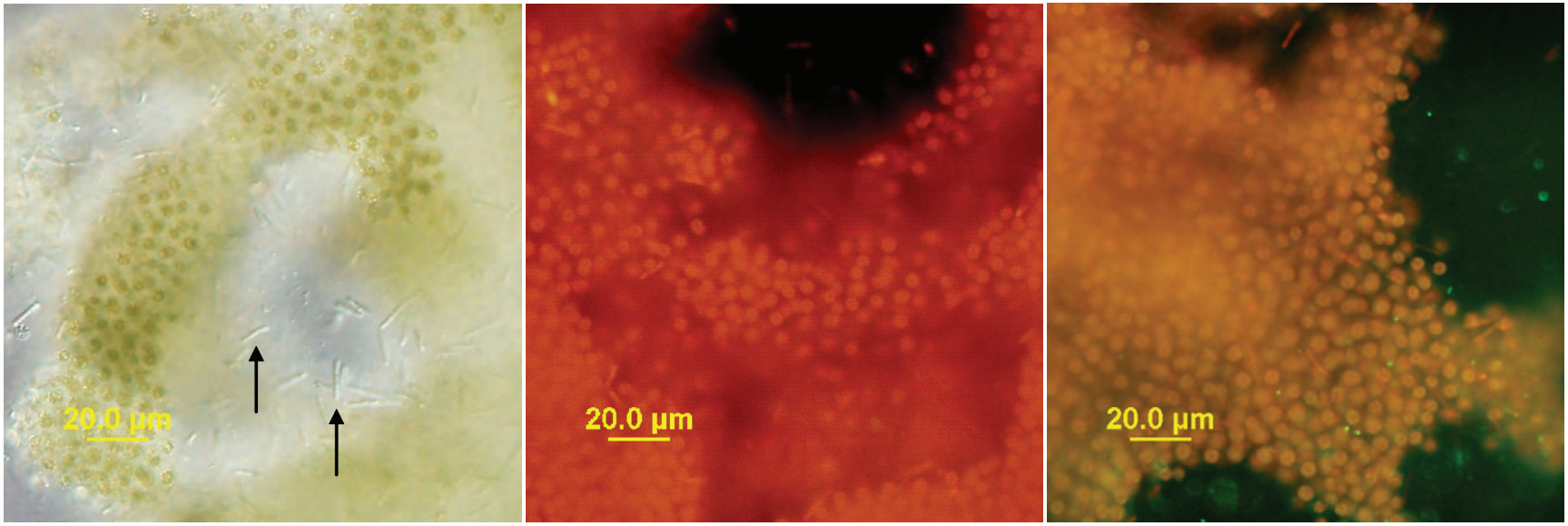


Figure 93. St. John's River, Jacksonville, FL (7/28/2009). LM-*Microcystis aeruginosa*. The small filaments (arrow) are likely the endogloeic cyanobacteria, *Pseudoanabaena mucicola* (Hindák, 2006). FITC-an orange-red color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

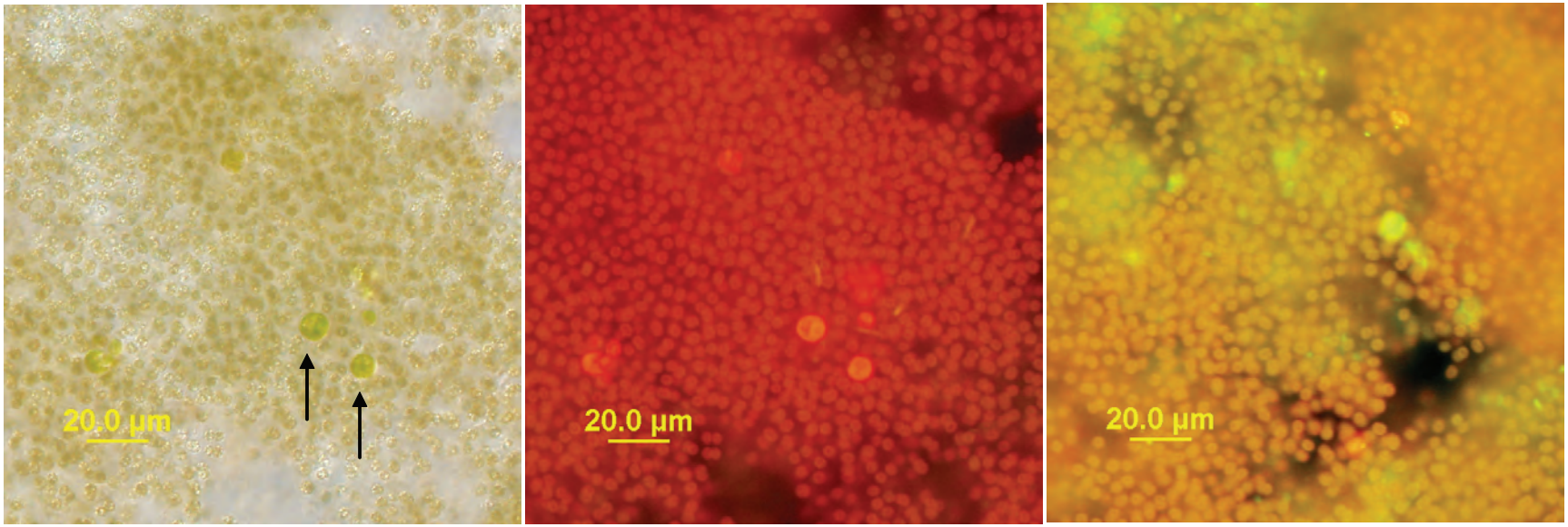


Figure 94. St. John's River, Jacksonville, FL (7/28/2009). LM-*Microcystis aeruginosa*. Eukaryotic epiphytes are green (arrow). FITC-a red color dominates the cyanobacterial cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

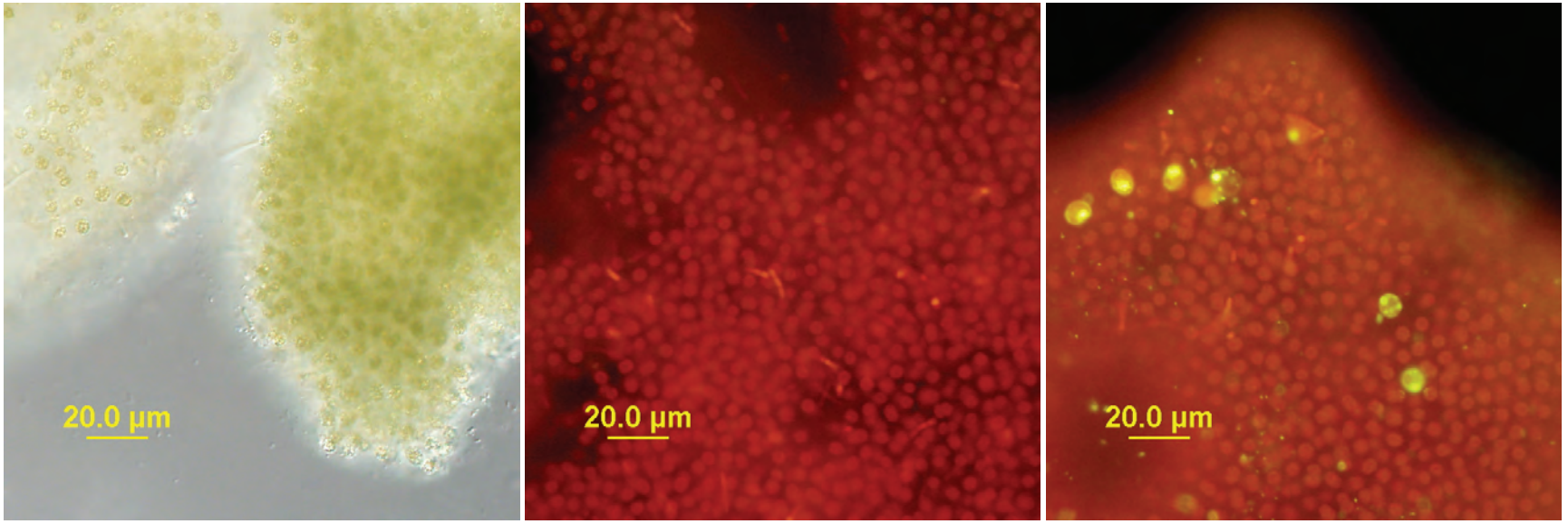


Figure 95. St. John's River, Jacksonville, FL (7/28/2009). LM-*Microcystis aeruginosa*. FITC-a red color dominates the cells. Sytox® green-stain did not penetrate the cell membrane; some of the cyanobacterial epiphytes stained bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

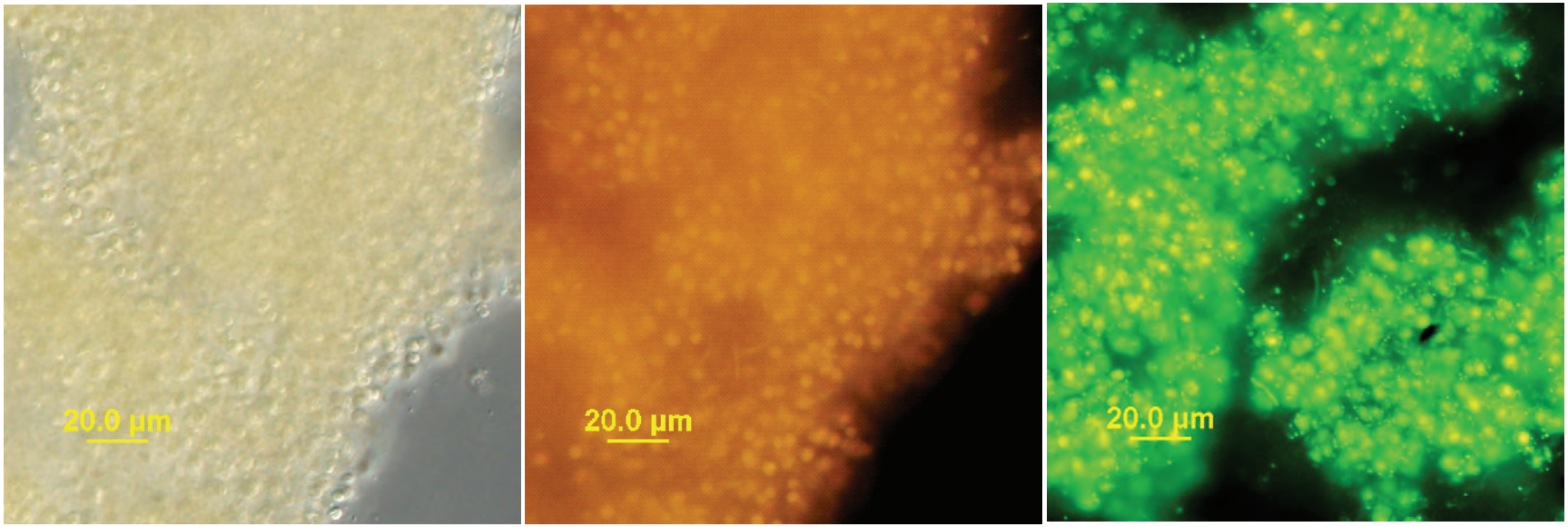


Figure 96. St. John's River, Jacksonville, FL (7/28/2009). LM-*Microcystis aeruginosa*. FITC-an orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane; bright green cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

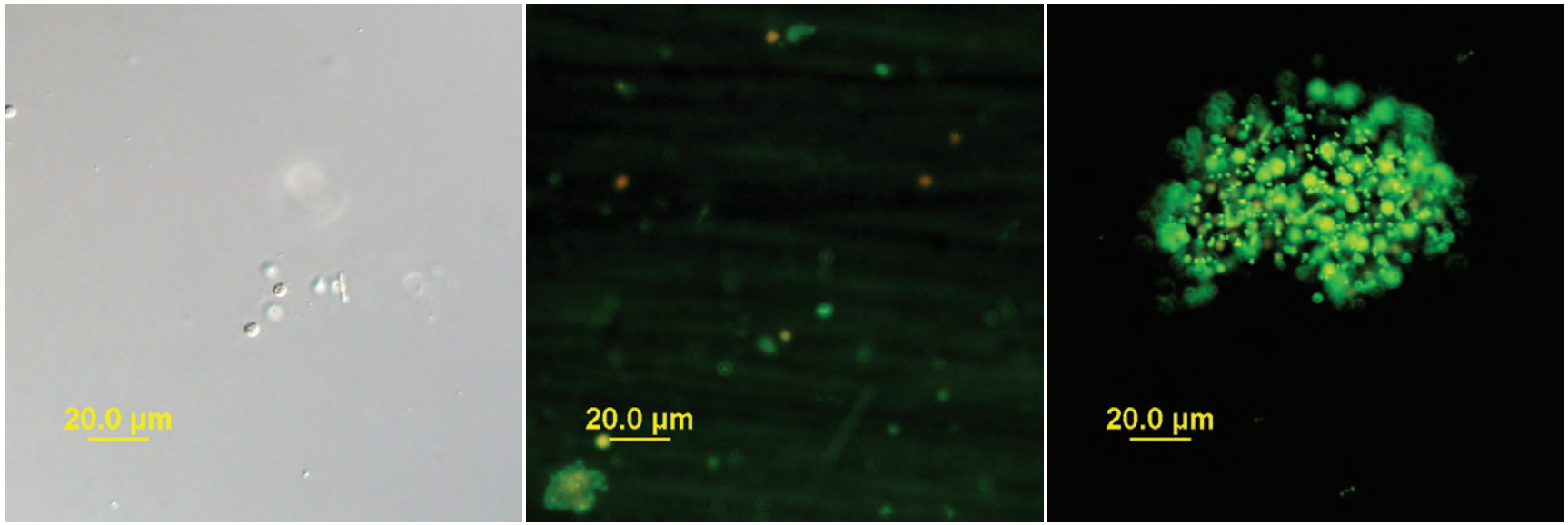


Figure 97. St. John's River, Jacksonville, FL (7/28/2009). LM-*Microcystis aeruginosa*. FITC-an orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane; bright green cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

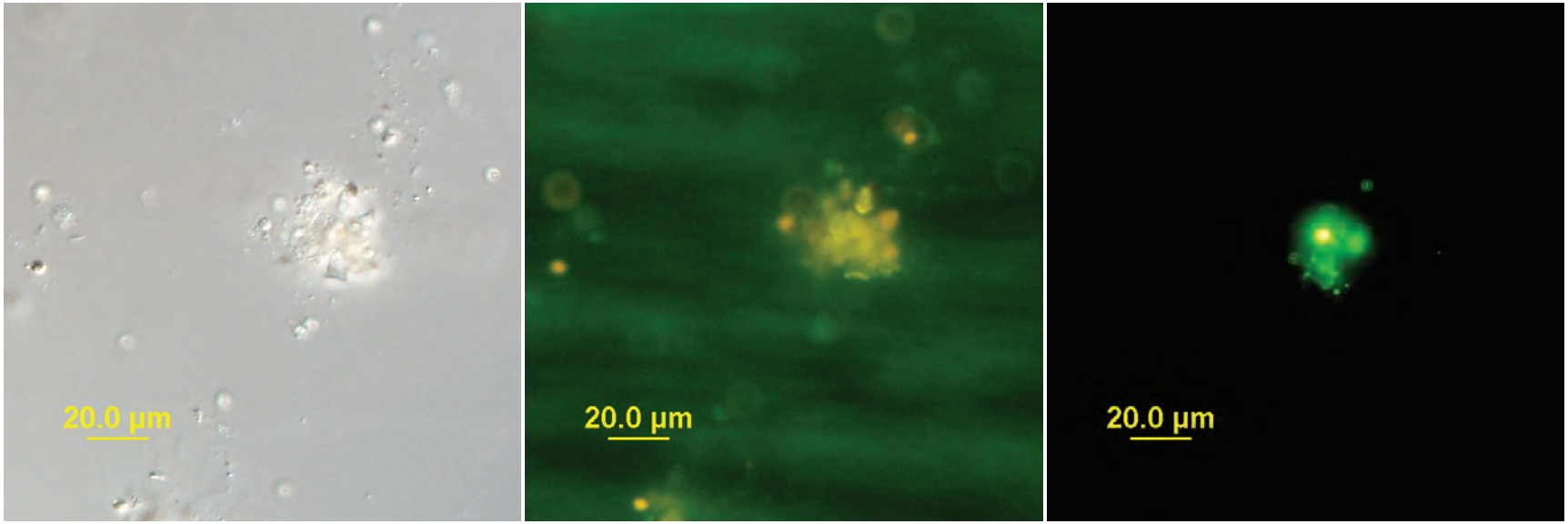


Figure 98. St. John's River, Jacksonville, FL (7/28/2009). LM-Likely the remains of a *Microcystis aeruginosa* colony. FITC-an orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane; bright green cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

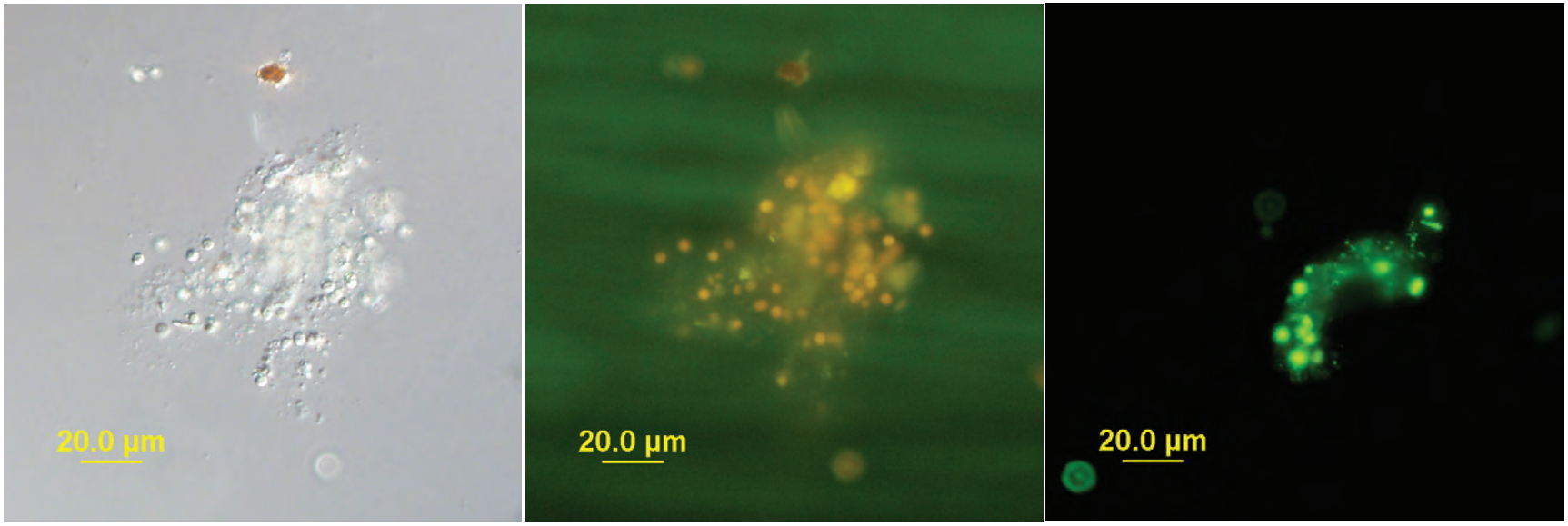


Figure 99. St. John's River, Jacksonville, FL (7/28/2009). LM-Likely the remains of a *Microcystis aeruginosa* colony. FITC-an orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane; bright green cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

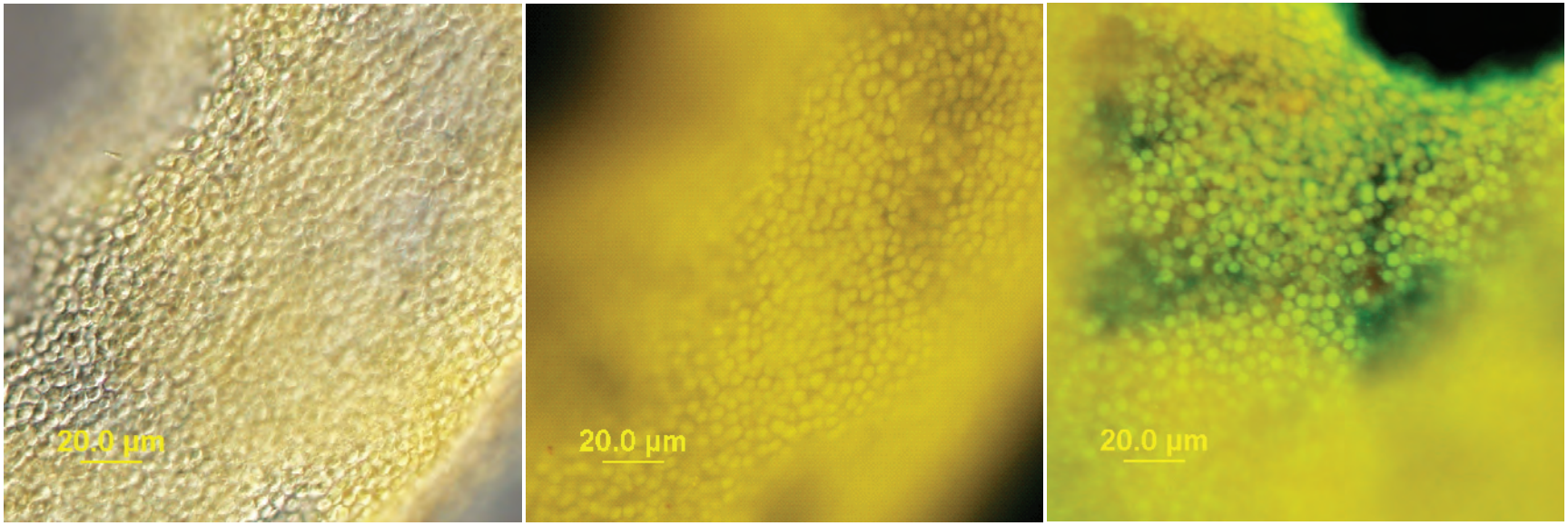


Figure 100. St. John's River, Jacksonville, FL (7/28/2009). LM-*Microcystis aeruginosa*. FITC-a yellow color dominates the cells. Sytox® green-stain did penetrate the cell membrane; bright green cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green. Note: No cells were found in the sample autoclaved for 30 minutes.

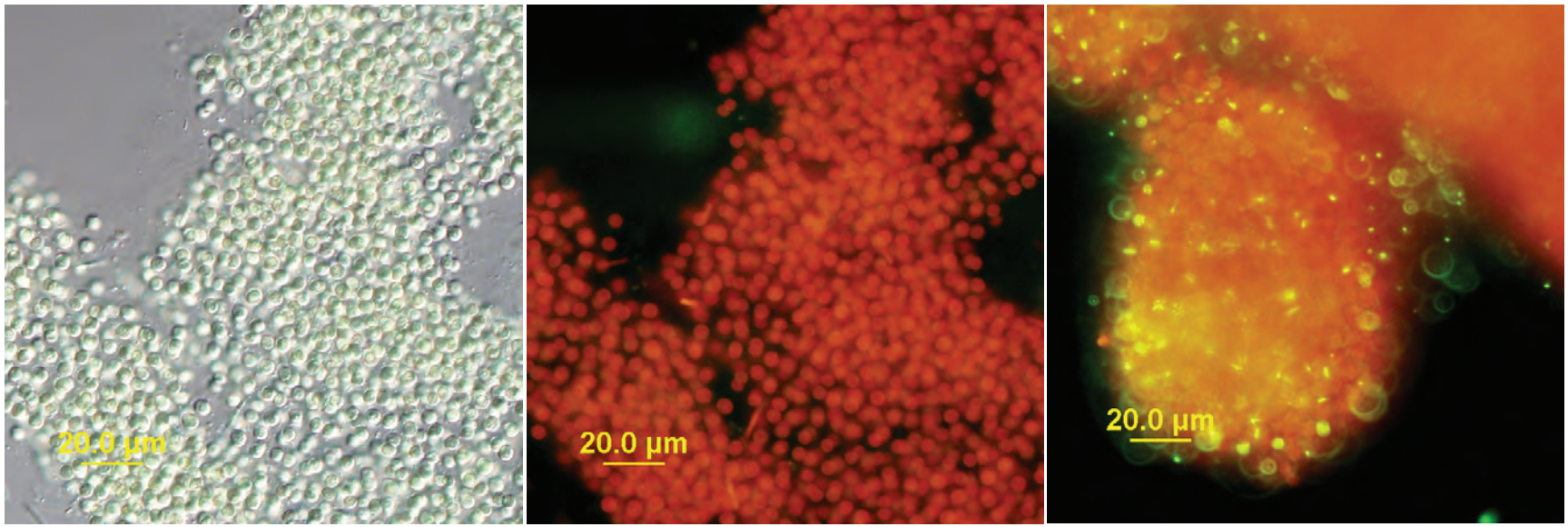


Figure 101. St. John's River, Jacksonville, FL (7/28/2009). LM-*Microcystis aeruginosa*. FITC-a red color dominates the cells. Sytox® green-stain did not penetrate the cell membrane; epiphytic bacteria bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

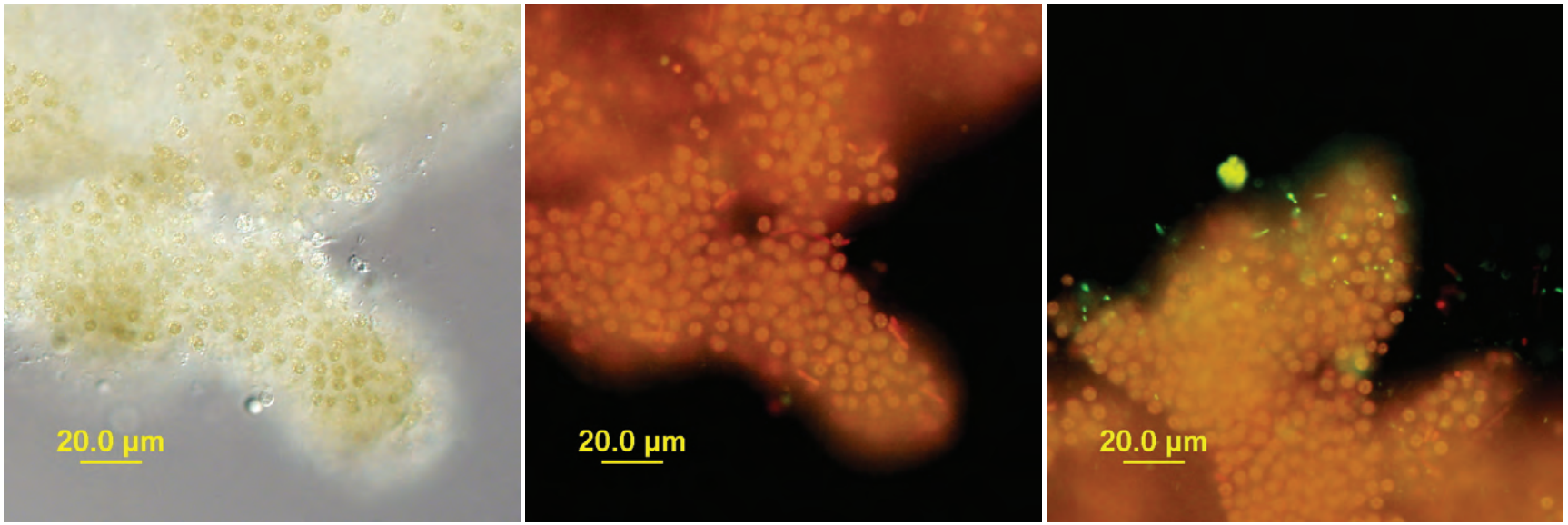


Figure 102. St. John's River, Jacksonville, FL (7/28/2009). LM-*Microcystis aeruginosa*. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membrane; epiphytic bacteria bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

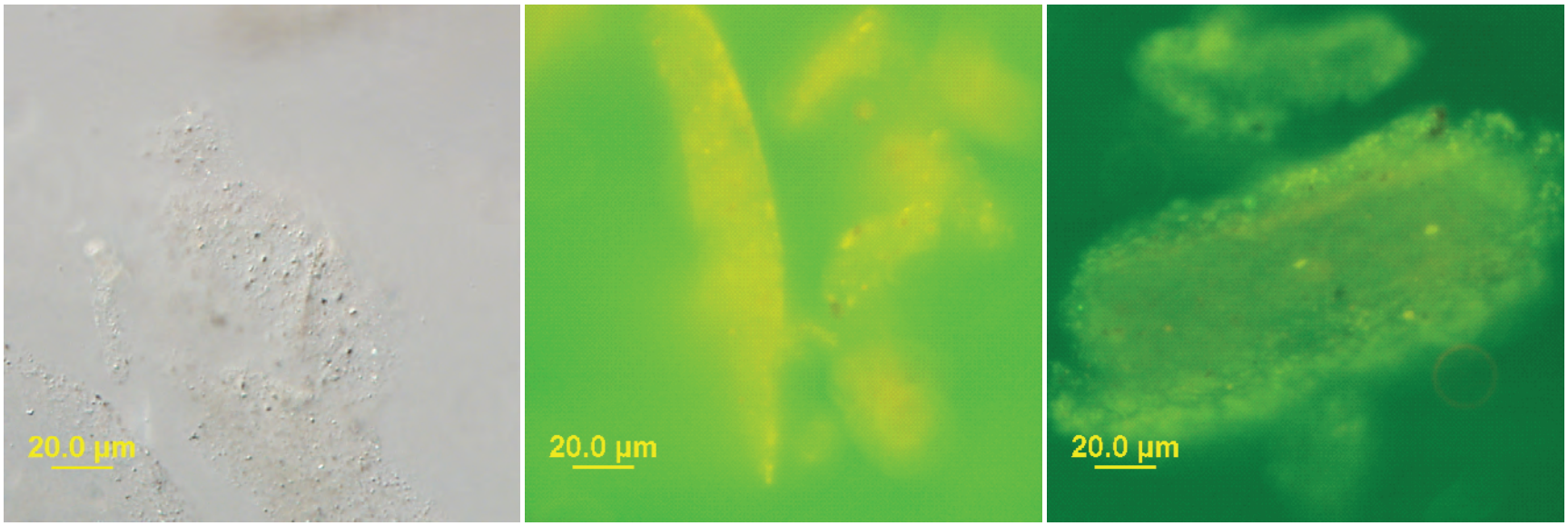


Figure 103. St. John's River, Jacksonville, FL (7/28/2009). LM-Likely the remains of a *Microcystis aeruginosa* colony. FITC-an orange color dominates, but cells cannot be distinguished. Sytox® green-slight evidence of sheath being stained. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

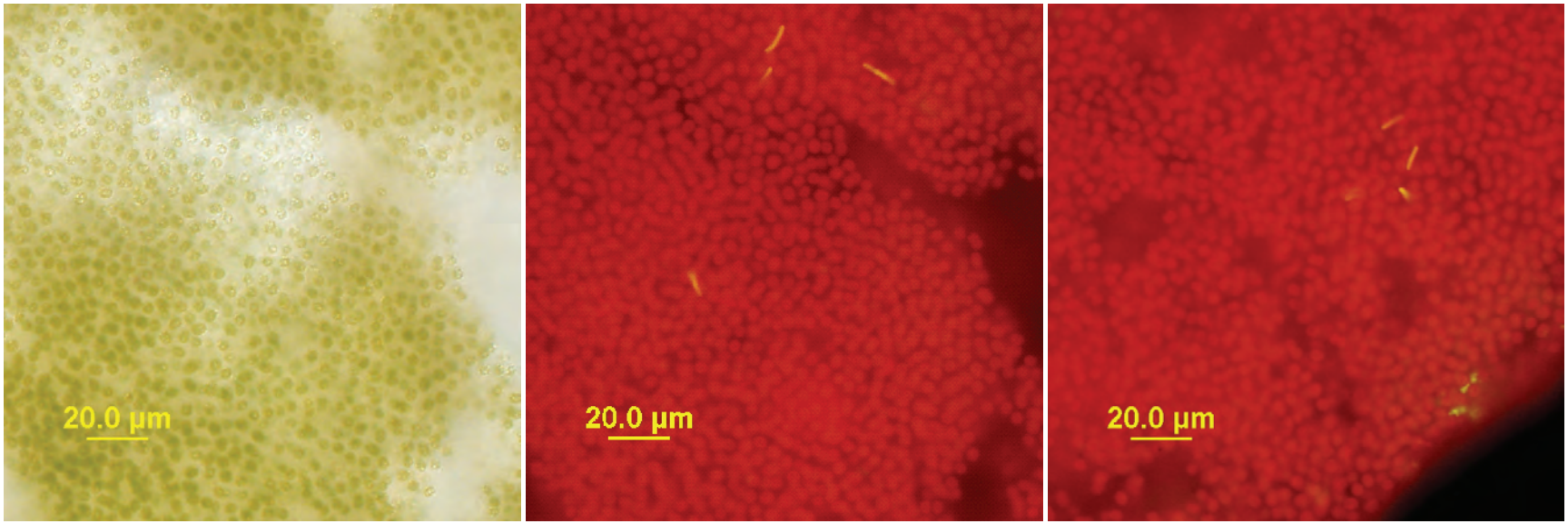


Figure 104. St. John's River, Jacksonville, FL (7/28/2009). LM-*Microcystis aeruginosa*. FITC-a red color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

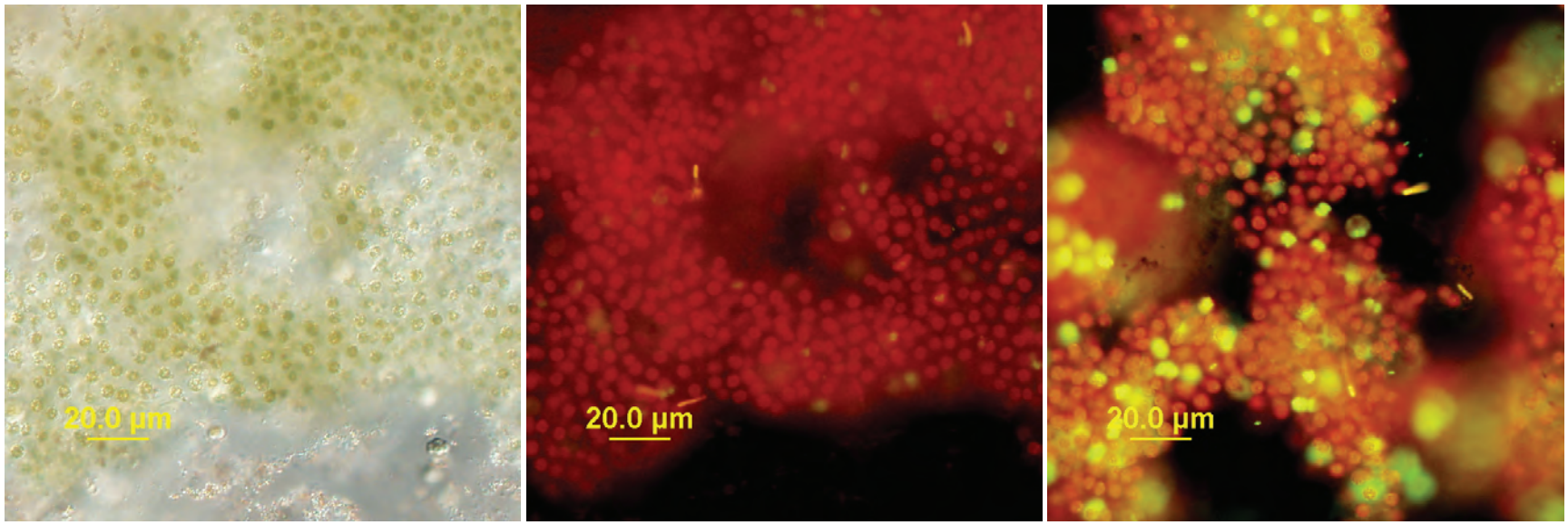


Figure 105. St. John's River, Jacksonville, FL (7/28/2009). LM-*Microcystis aeruginosa*. FITC-a red color dominates the cells. Sytox® green-stain did penetrate the cell membrane in some of the peripheral cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

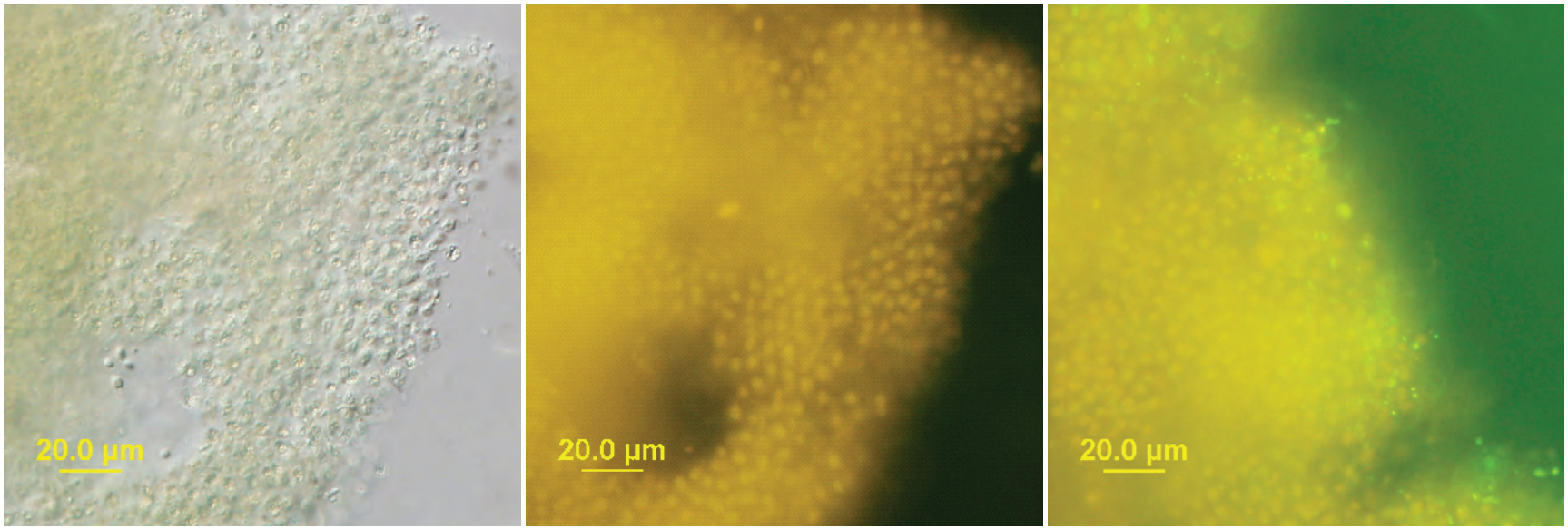


Figure 106. St. John's River, Jacksonville, FL (7/28/2009). LM-*Microcystis aeruginosa*. FITC-a yellow color dominates the cells. Sytox® green-stain did penetrate the cell membrane in some of the peripheral cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

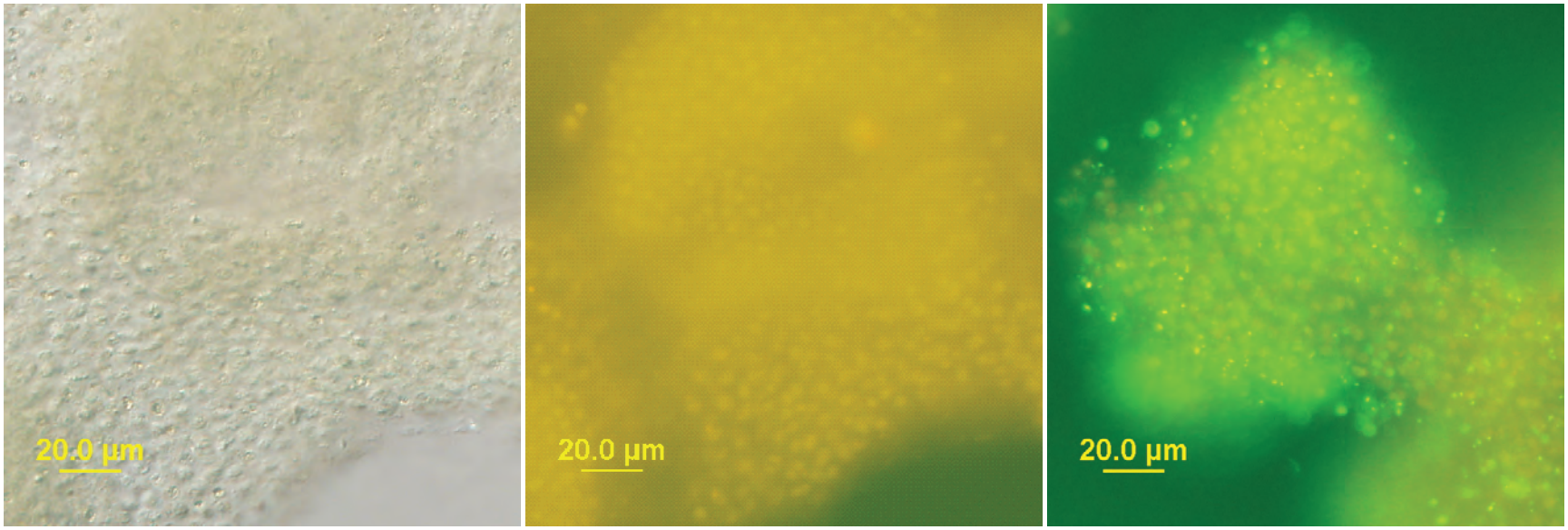


Figure 107. St. John's River, Jacksonville, FL (7/28/2009). LM-*Microcystis aeruginosa*. FITC-a yellow-orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane in some of the peripheral cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

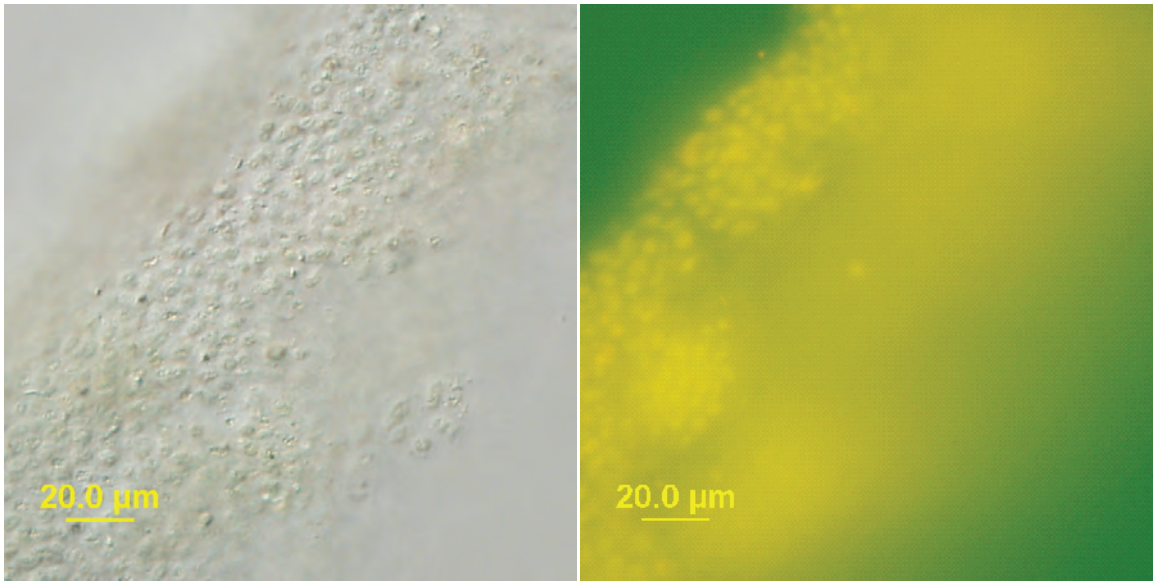


Figure 108. St. John's River, Jacksonville, FL (7/28/2009). LM-*Microcystis aeruginosa*. FITC-a yellow color dominates the cells. Sytox® green-no image available. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

Figure 109. Upper Klamath Lake, OR (8/21/2009). LM-*Aphanizomenon flos-aquae* filaments. FITC-a yellow-orange color dominates these cells. Sytox® green-no image available. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

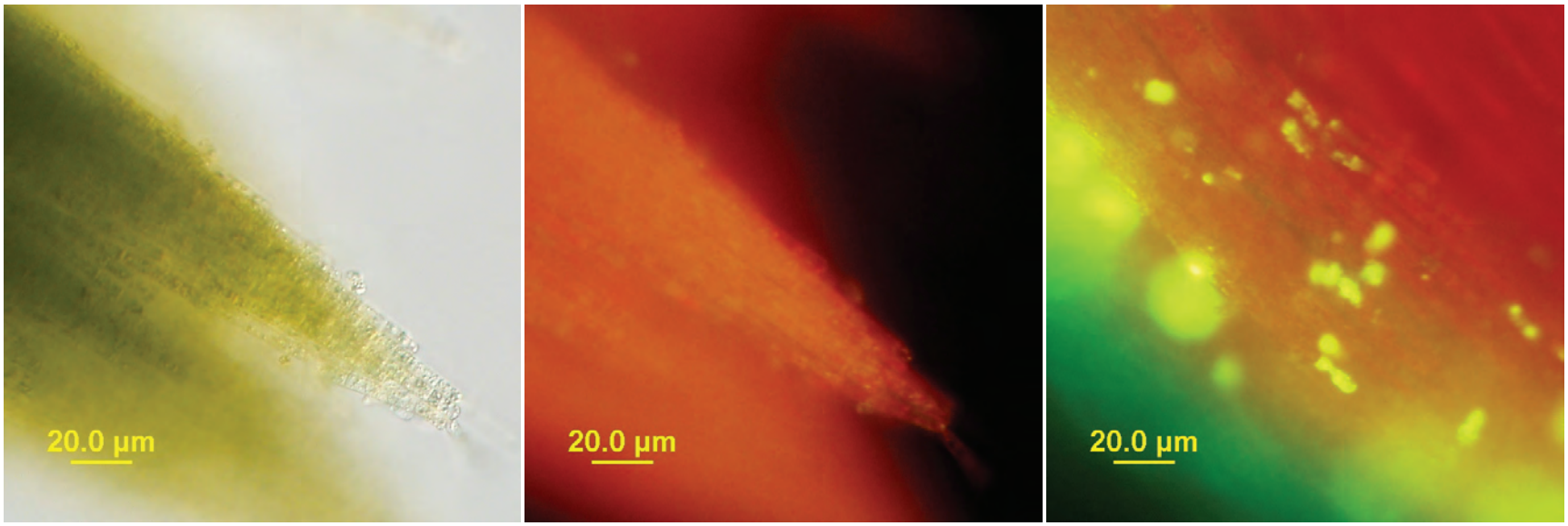


Figure 110. Upper Klamath Lake, OR (8/21/2009). LM-*Aphanizomenon flos-aquae* bundle of filaments. FITC-a red-orange color dominates these cells. Sytox® green-stain did not penetrate the cell membrane; sheath and epiphytes stained bright yellow-green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

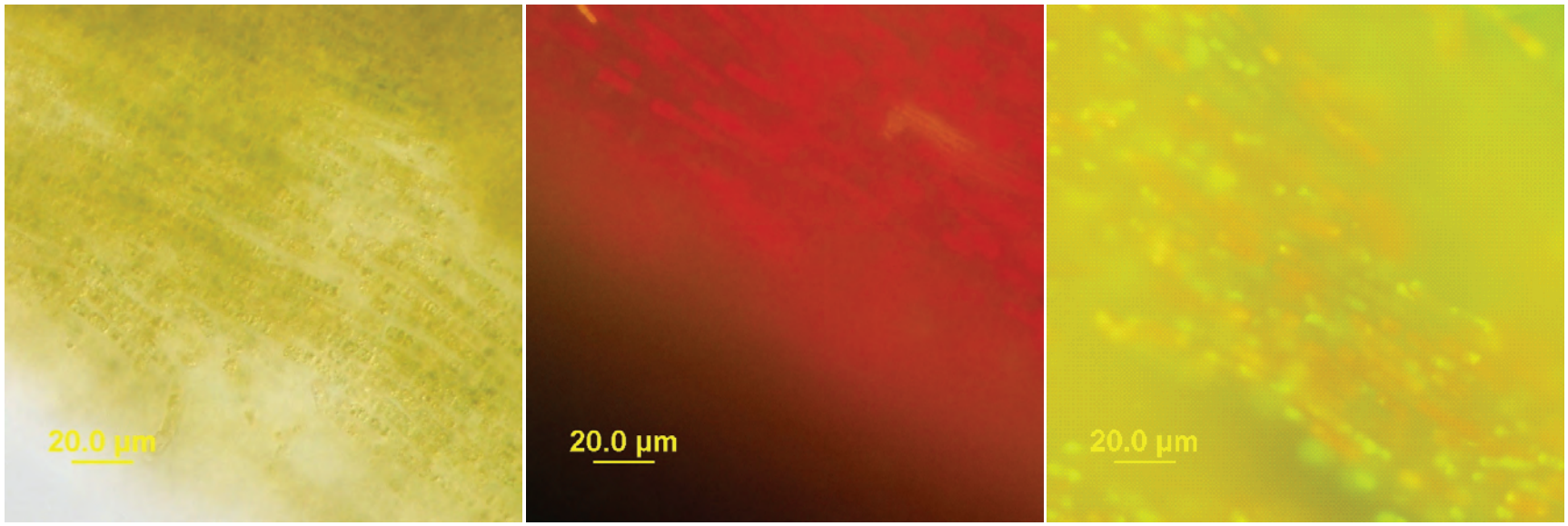


Figure 111. Upper Klamath Lake, OR (8/21/2009). LM-*Aphanizomenon flos-aquae* bundle of filaments that appear degraded compared to Control 2. FITC-a red color dominates these cells. Sytox® green-stain did penetrate the cell membrane of some cells; stained bright yellow-green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

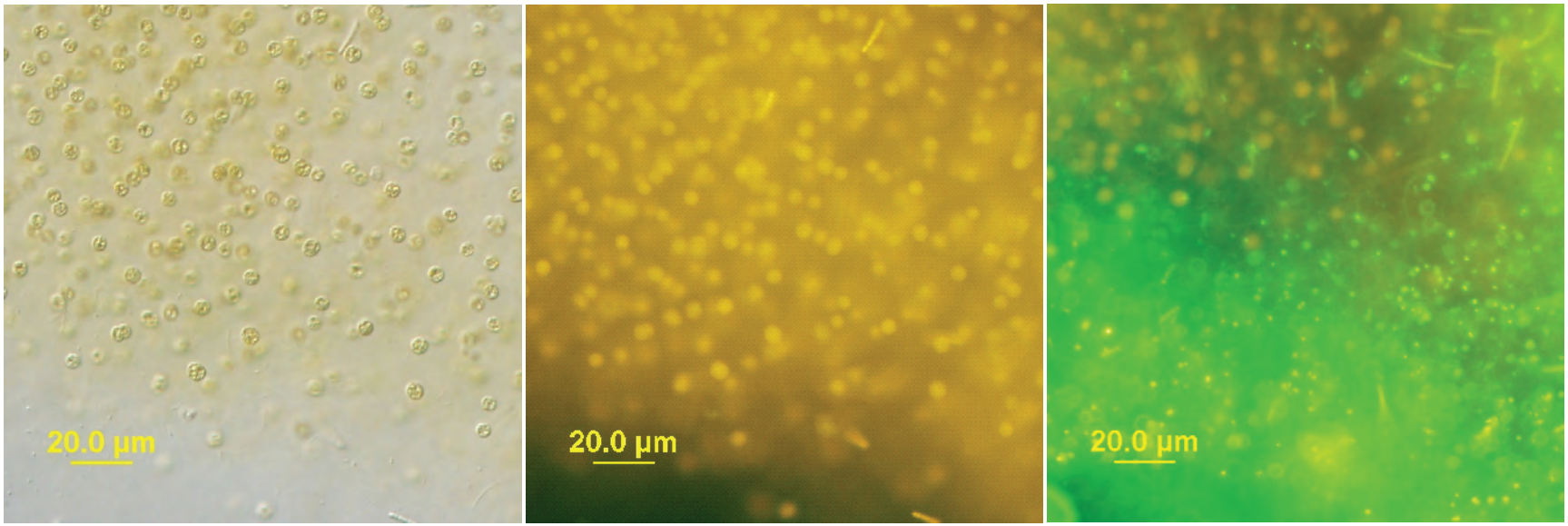


Figure 112. Upper Klamath Lake, OR (8/21/2009). LM-*Microcystis aeruginosa*. FITC-an orange color dominates the cells. Sytox® green- stain did not penetrate the cell membrane of the cyanobacteria; the sheath and epiphytic bacteria (green spots) stained bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

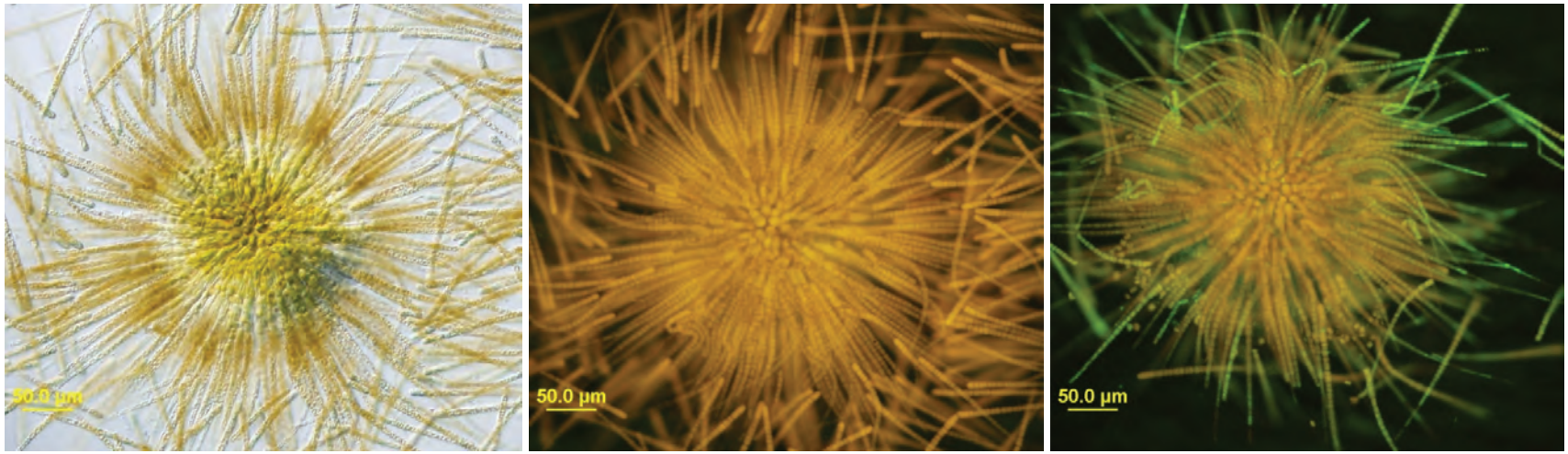


Figure 113. Upper Klamath Lake, OR (8/21/2009). LM-*Gloeotrichia echinulata*, a large (note scale bar), colony-forming filamentous cyanobacteria. FITC-an orange color dominates the cells. Sytox® green-stained the peripheral filaments, but did not penetrate to the center of this large colony. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

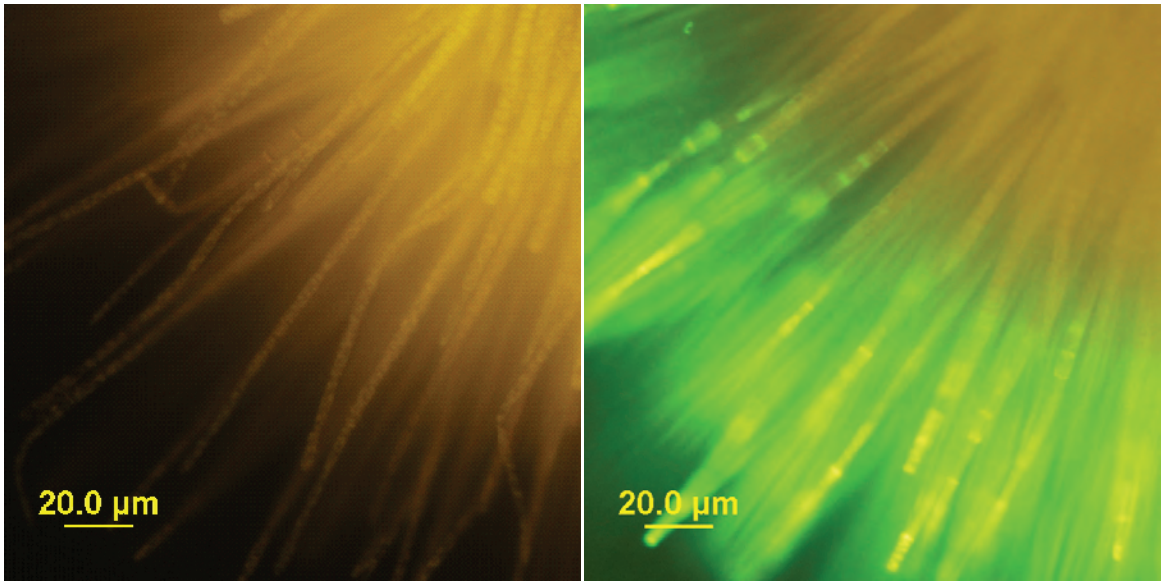


Figure 114. Upper Klamath Lake, OR (8/21/2009). LM-No image available. FITC-*Gloeotrichia echinulata*-an orange color dominates the cells. Sytox® green-stained the tips of the filaments, but did not penetrate to the center of the colony. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

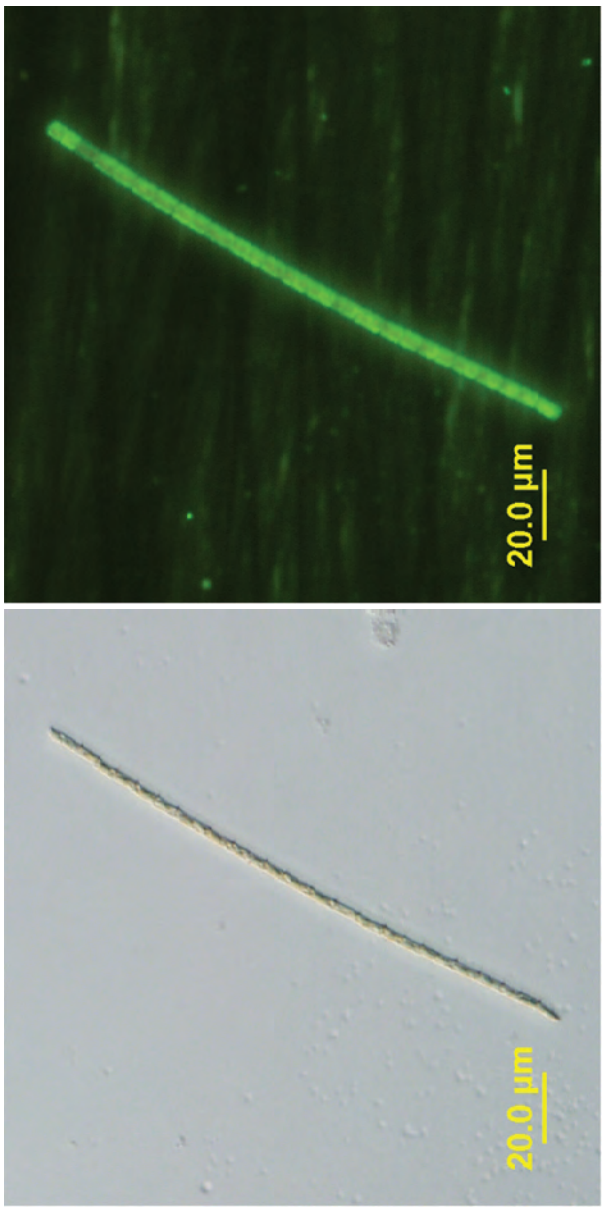


Figure 115. Upper Klamath Lake, OR (8/21/2009). LM-Unknown filamentous cyanobacteria. FITC-no image available. Sytox® green-likely *Aphanizomenon flos-aquae* filament; stain did penetrate the cell membrane; cells bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green–epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

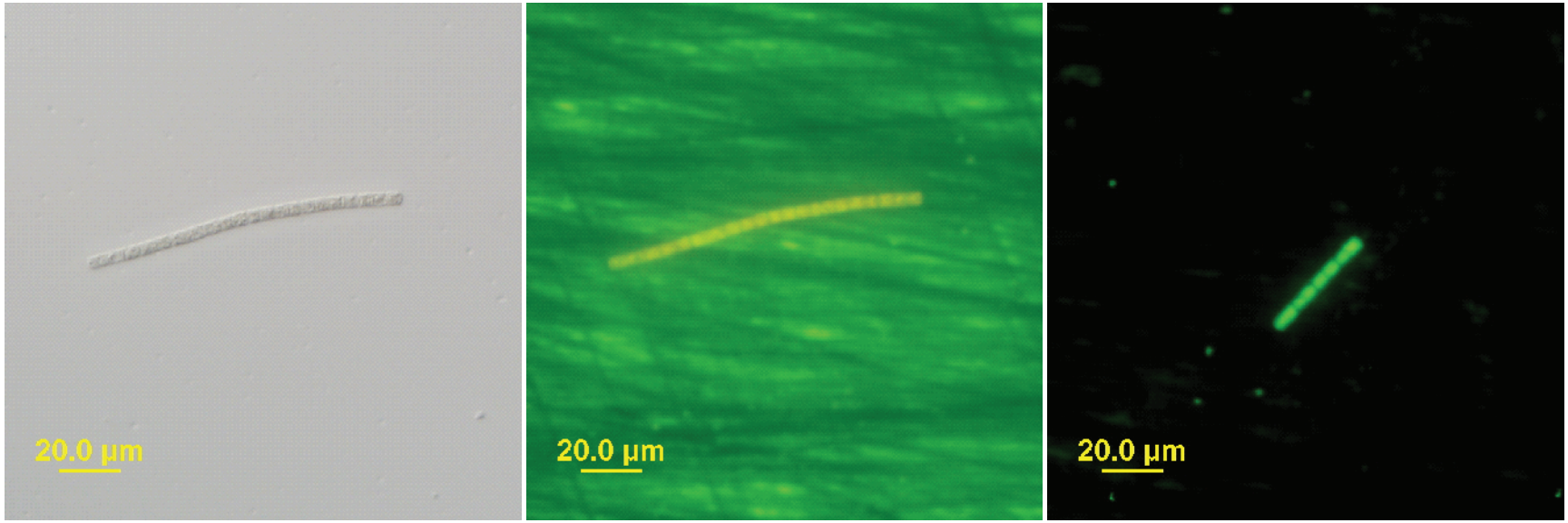


Figure 116. Upper Klamath Lake, OR (8/21/2009). LM-Likely *Aphanizomenon flos-aquae* filament. FITC-an orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane, cells bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

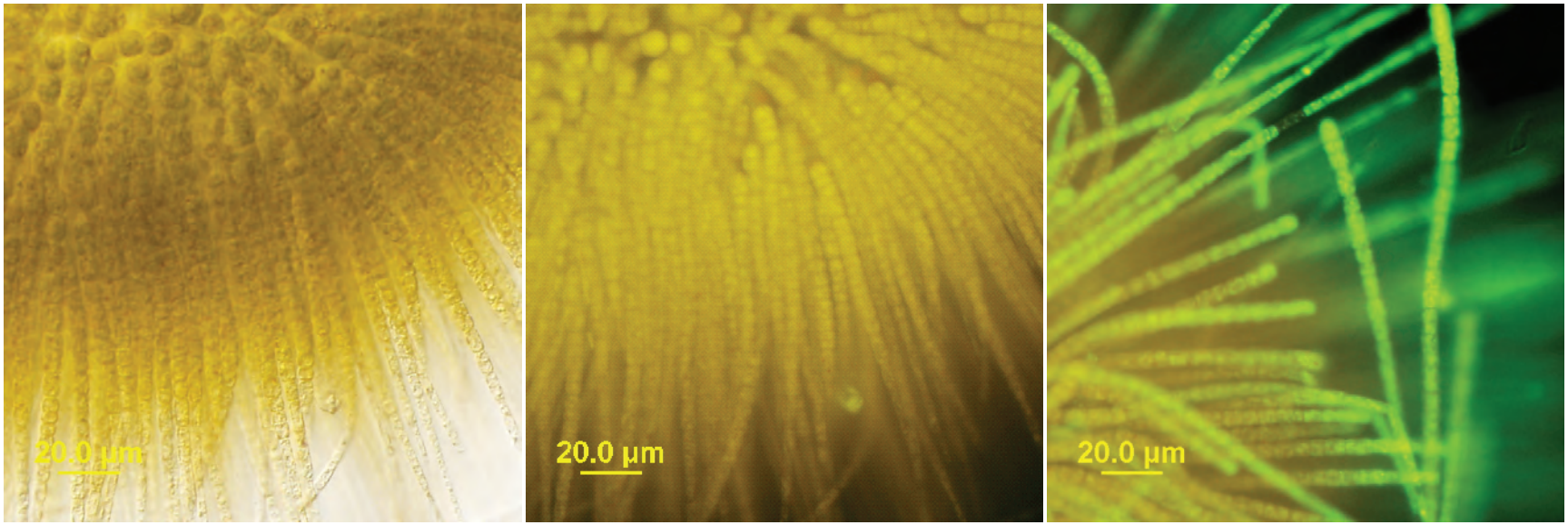
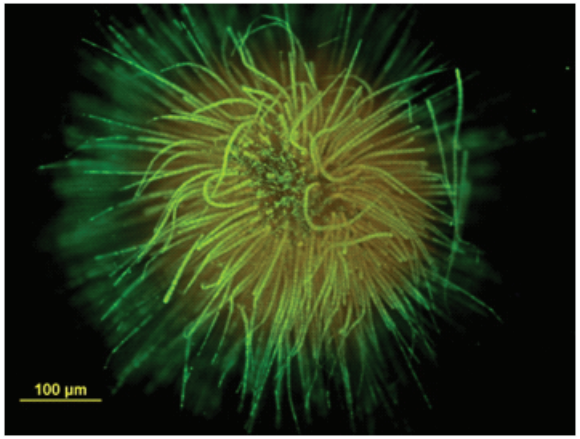


Figure 117. Upper Klamath Lake, OR (8/21/2009). LM-*Gloeotrichia echinulata*. FITC-a yellow color dominates the cells. Sytox® green-stained the peripheral filaments, but did not penetrate to the center of this large colony. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.



Sytox® green

Autoclaved for 15 minutes

Figure 118. Upper Klamath Lake, OR (8/21/2009). *Gloeotrichia echinulata*. Sytox® green-stained the peripheral filaments, but did not penetrate to the center of this large colony. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

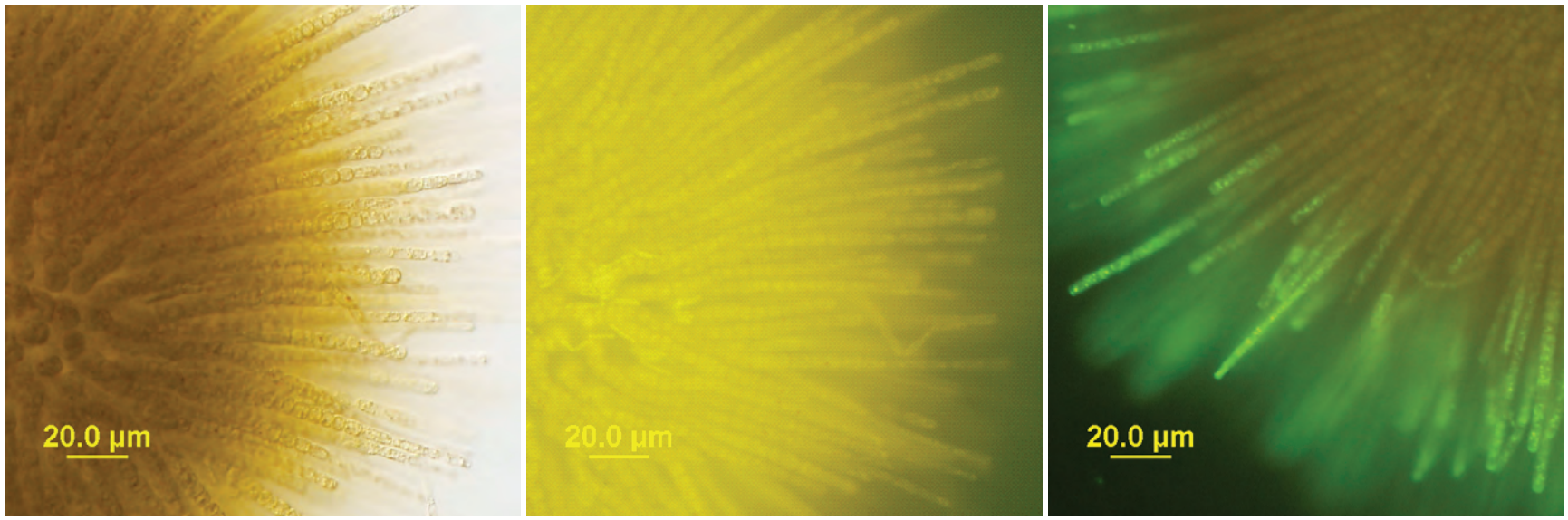


Figure 119. Upper Klamath Lake, OR (8/21/2009). LM-*Gloeotrichia echinulata*. FITC-a yellow color dominates the cells. Sytox® green-stained the tips of the filaments, but did not penetrate to the center of this large colony. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

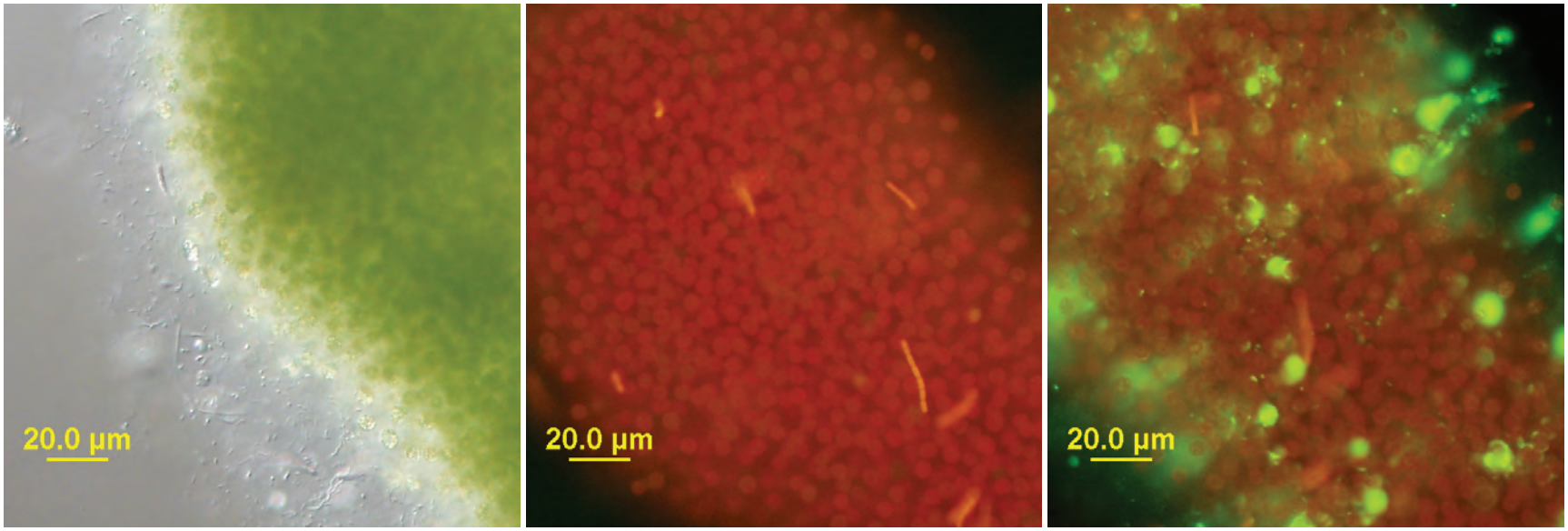
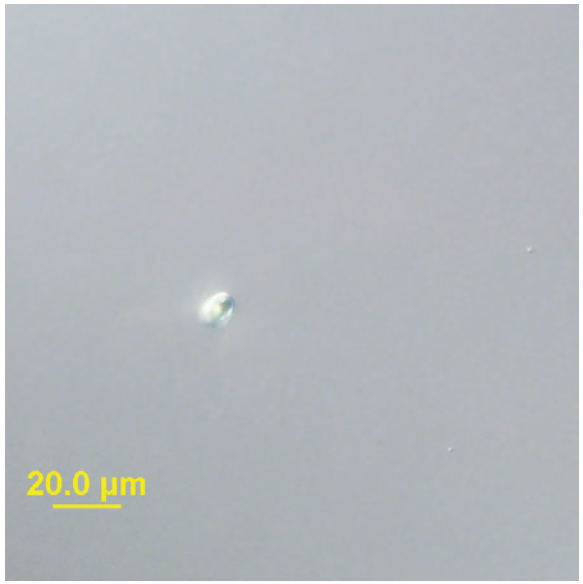


Figure 120. Upper Klamath Lake, OR (8/21/2009). LM-*Microcystis aeruginosa*. FITC-a red color dominates the cells. Sytox® green-stain did not penetrate the cell membrane; green cells were epiphytes attached to the colony. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.



LM

Sonicated at 35 percent power
and 70 percent power

Figure 121. Upper Klamath Lake, OR (8/21/2009). LM-single cell present. FITC and Sytox® green-no data. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

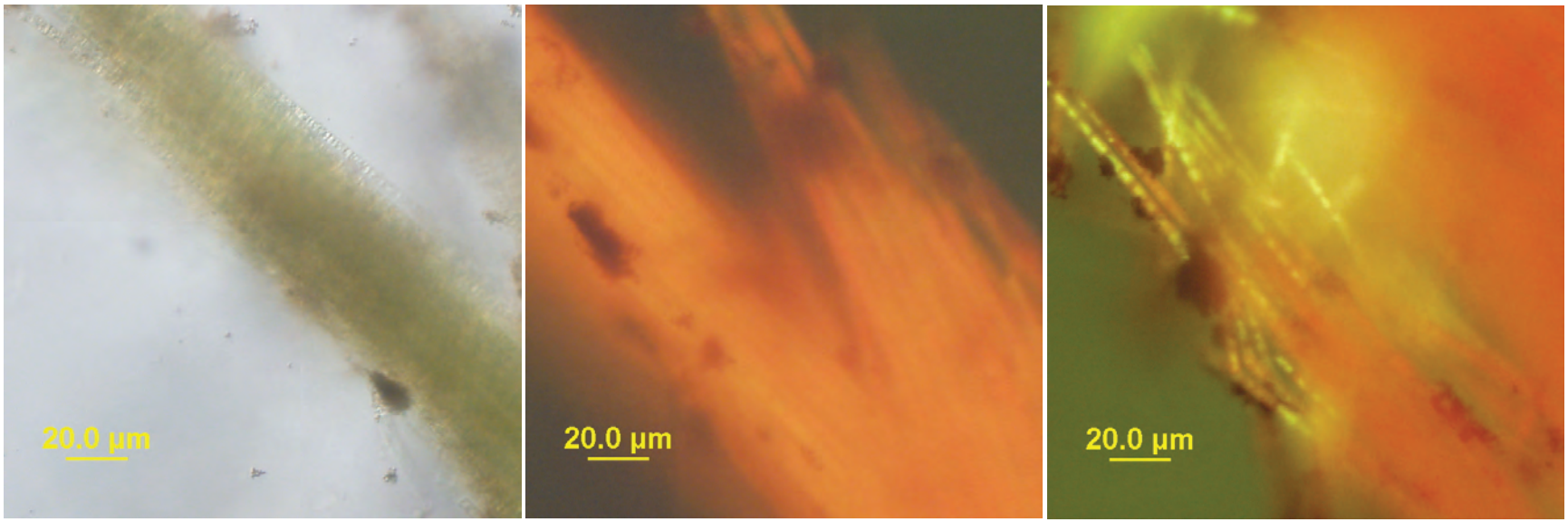


Figure 122. Upper Klamath Lake, OR (8/21/2009). LM-*Aphanizomenon flos-aquae* bundle of filaments. FITC-an orange color dominates these cells. Sytox® green-stain did not penetrate the cell membrane of the main bundle of organisms; a few filaments on the edge stained bright yellow-green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

LM

One freeze-thaw cycle

Figure 123. Upper Klamath Lake, OR (8/21/2009). LM-Likely a small fragment of *Aphanizomenon flos-aquae*. FITC and Sytox® green-no data. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

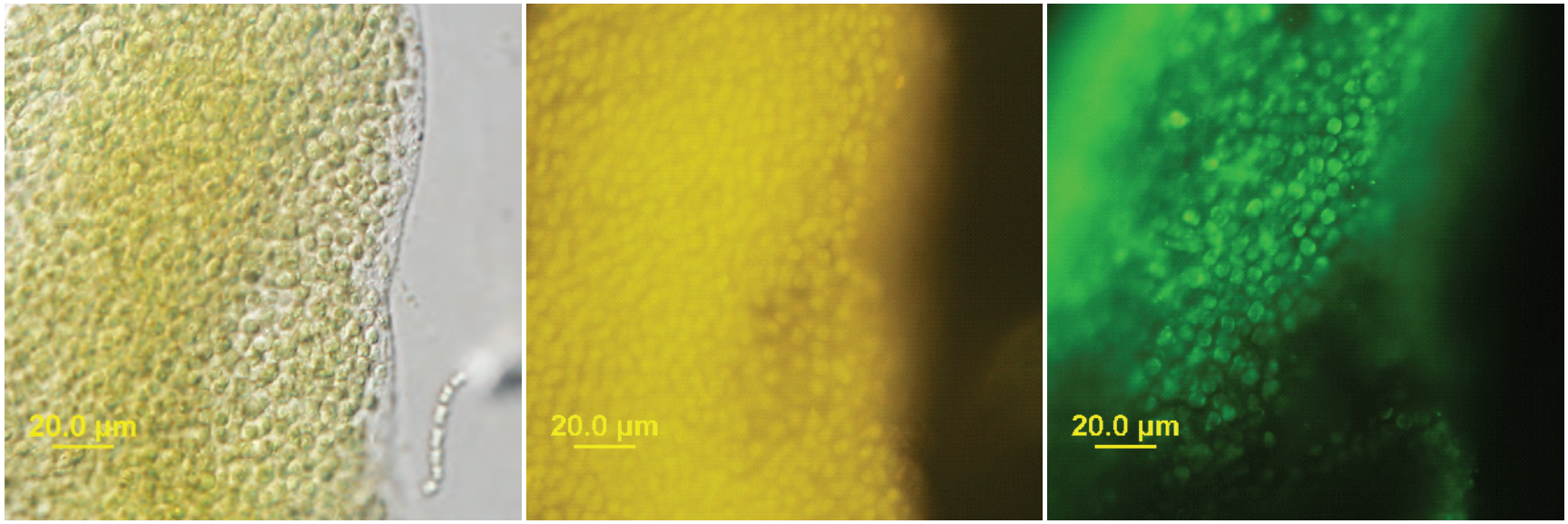


Figure 124. Upper Klamath Lake, OR (8/21/2009). LM-*Microcystis aeruginosa*. FITC-a yellow color dominates the cells. Sytox® green-stain did penetrate the cell membrane; cells were bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

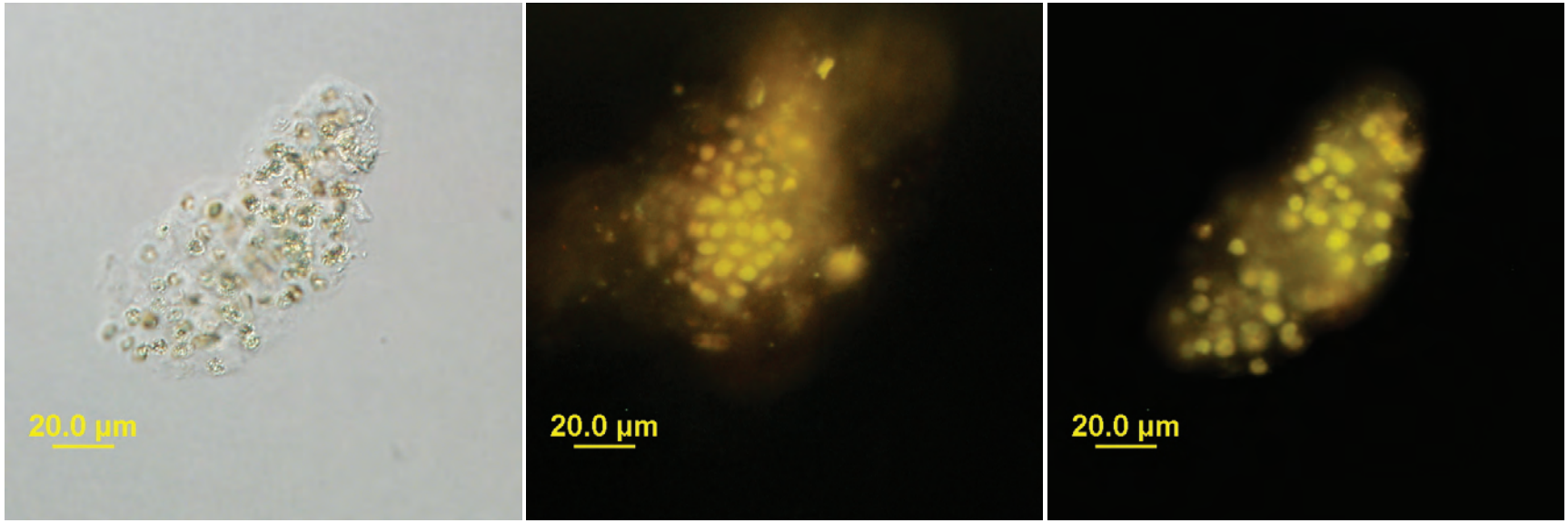


Figure 125. Klamath River, OR (8/21/2009) (only freeze-thaw material). LM-*Microcystis aeruginosa*. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

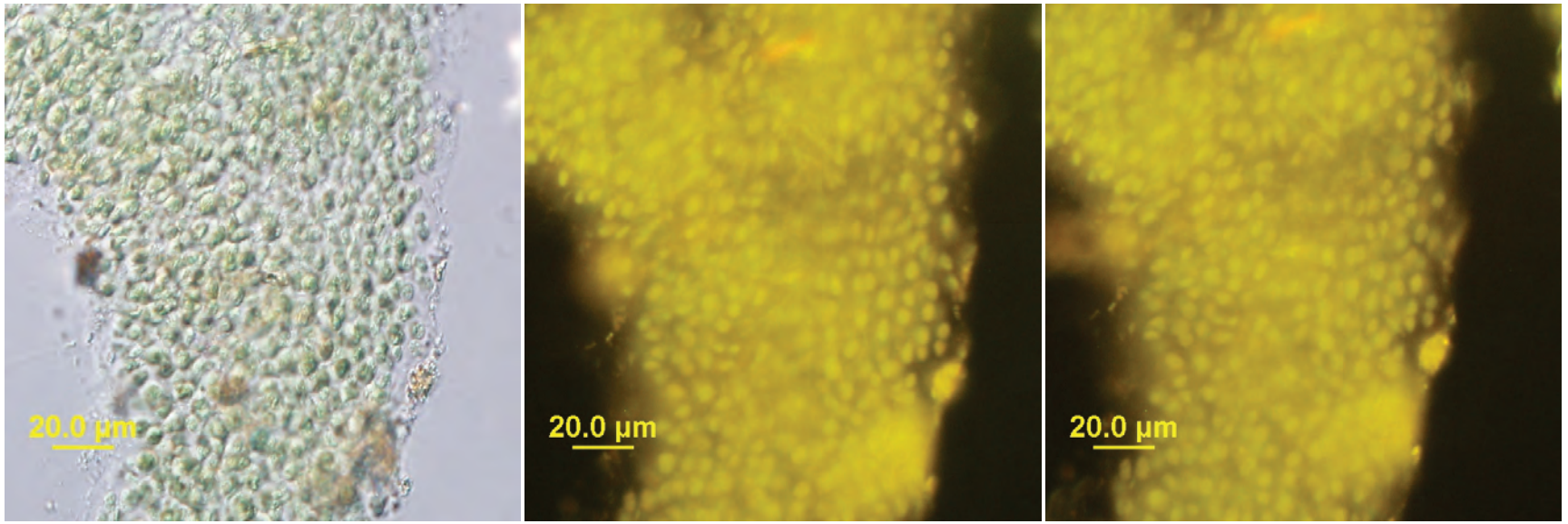


Figure 126. Klamath River, OR (8/21/2009). LM-*Microcystis aeruginosa*. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

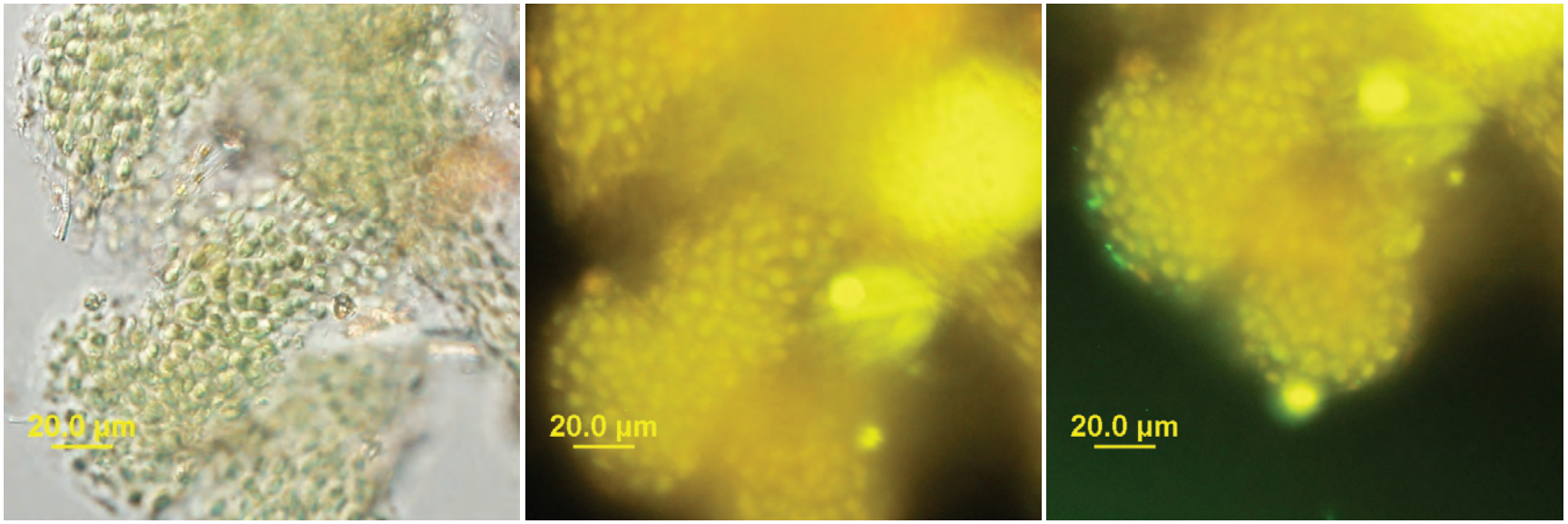


Figure 127. Klamath River, OR (8/21/2009). LM-*Microcystis aeruginosa*. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membrane of most cells in the colony; some peripheral cells appear bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

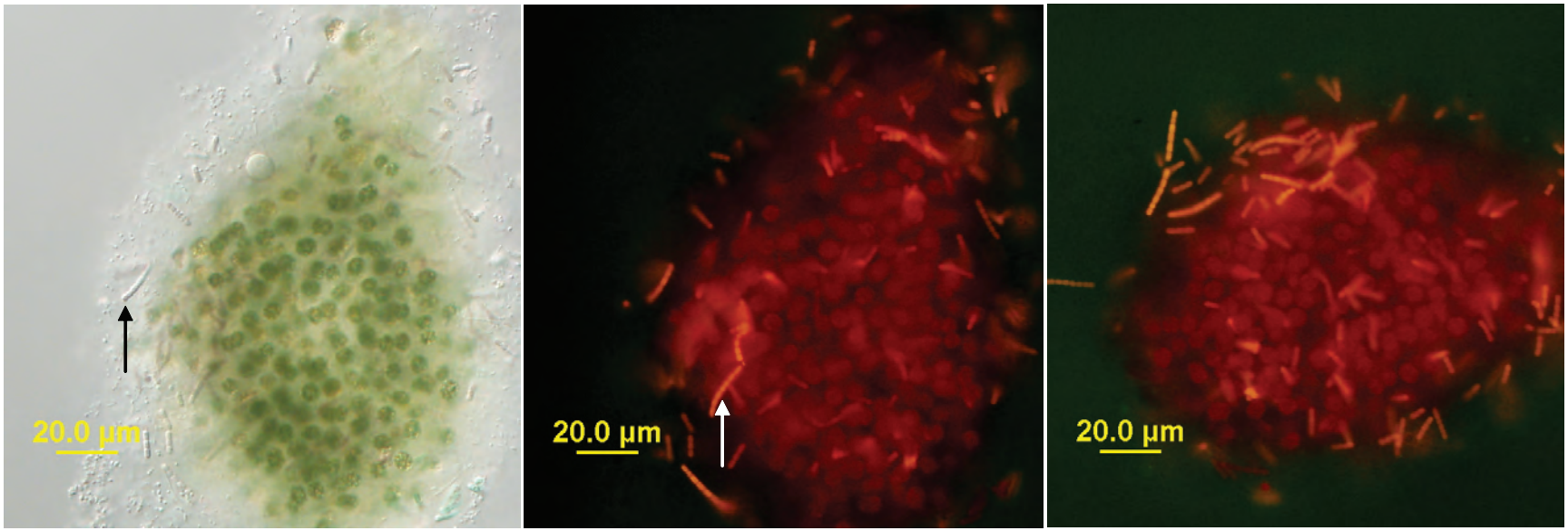


Figure 128. Iron Gate Reservoir, OR (8/25/2009). LM-*Microcystis aeruginosa*. The small filaments (arrow) are likely the endogloeic cyanobacteria, *Pseudoanabaena mucicola* (Hindák, 2006). FITC-a red color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

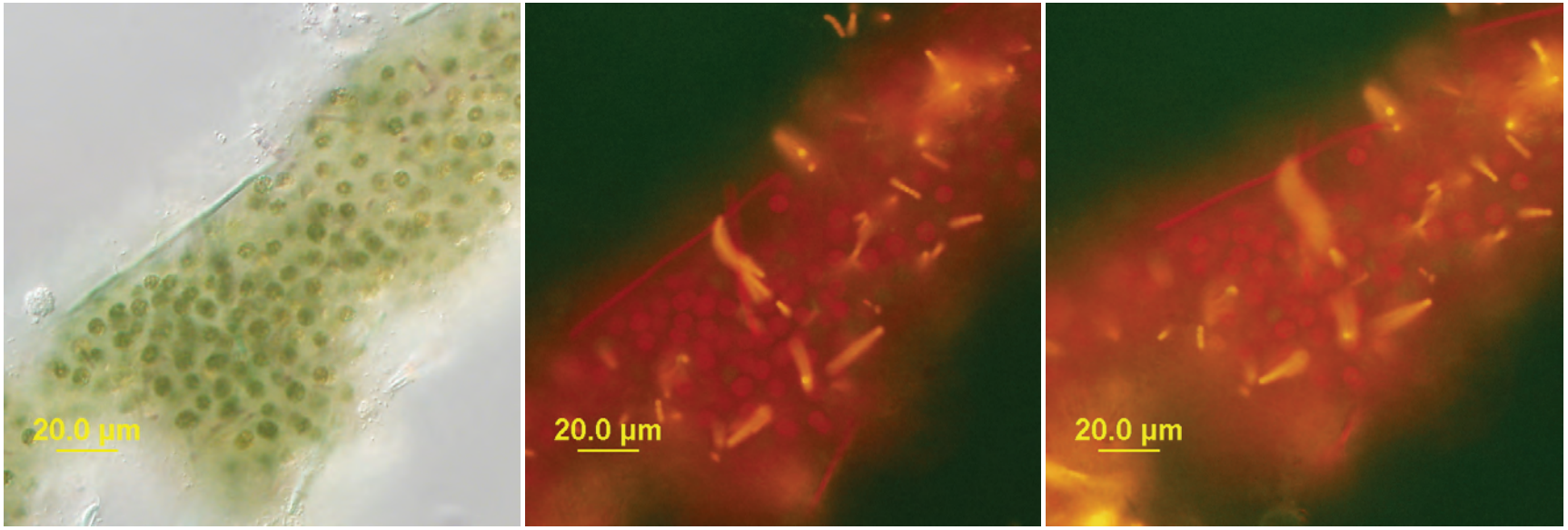


Figure 129. Iron Gate Reservoir, OR (8/25/2009). LM-*Microcystis aeruginosa*. FITC-a red color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

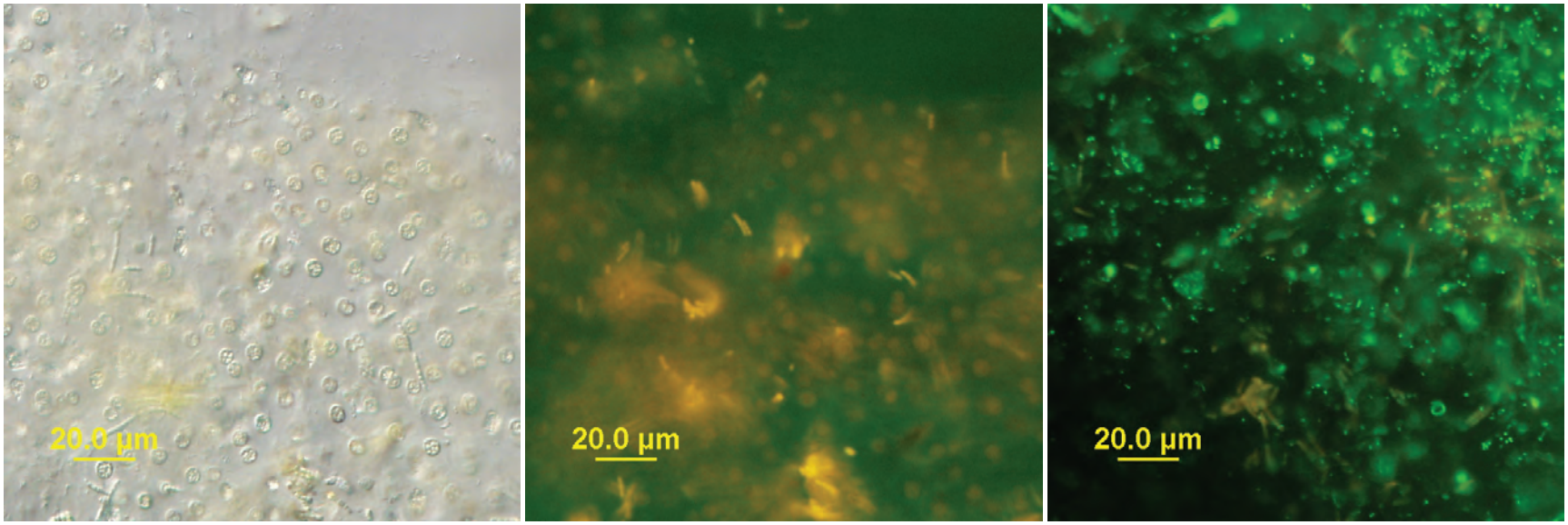


Figure 130. Iron Gate Reservoir, OR (8/25/2009). LM-*Microcystis aeruginosa*. FITC-an orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane; cells bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

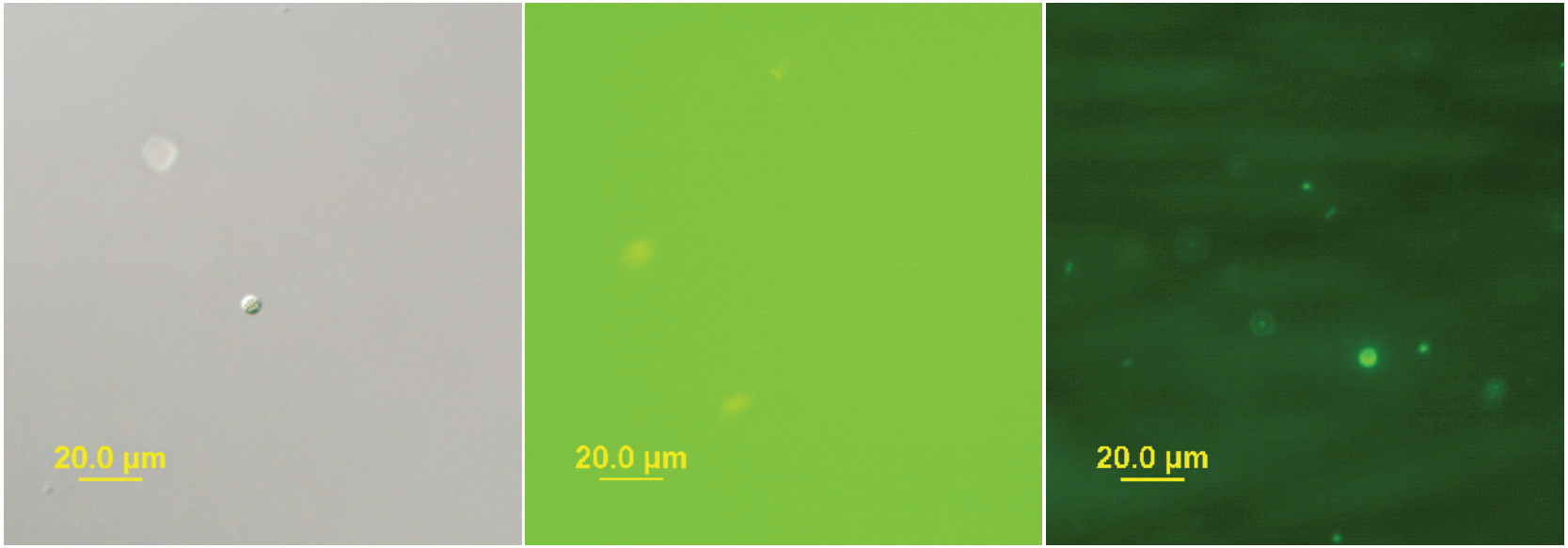


Figure 131. Iron Gate Reservoir, OR (8/25/2009). LM-Possible remains of *Microcystis aeruginosa*. FITC-an orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane; cell bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

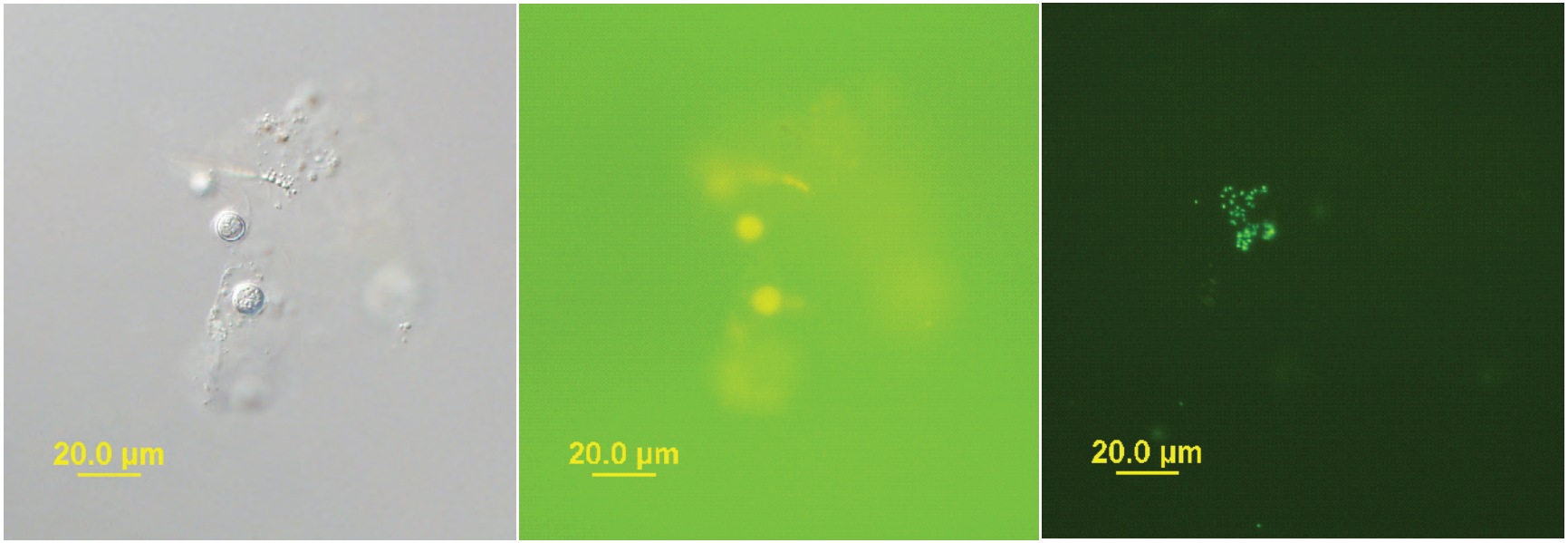


Figure 132. Iron Gate Reservoir, OR (8/25/2009). LM-Possible remains of *Microcystis aeruginosa*. FITC-an orange-yellow color dominates the cells. Sytox® green-stain did penetrate the cell membrane; cells bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

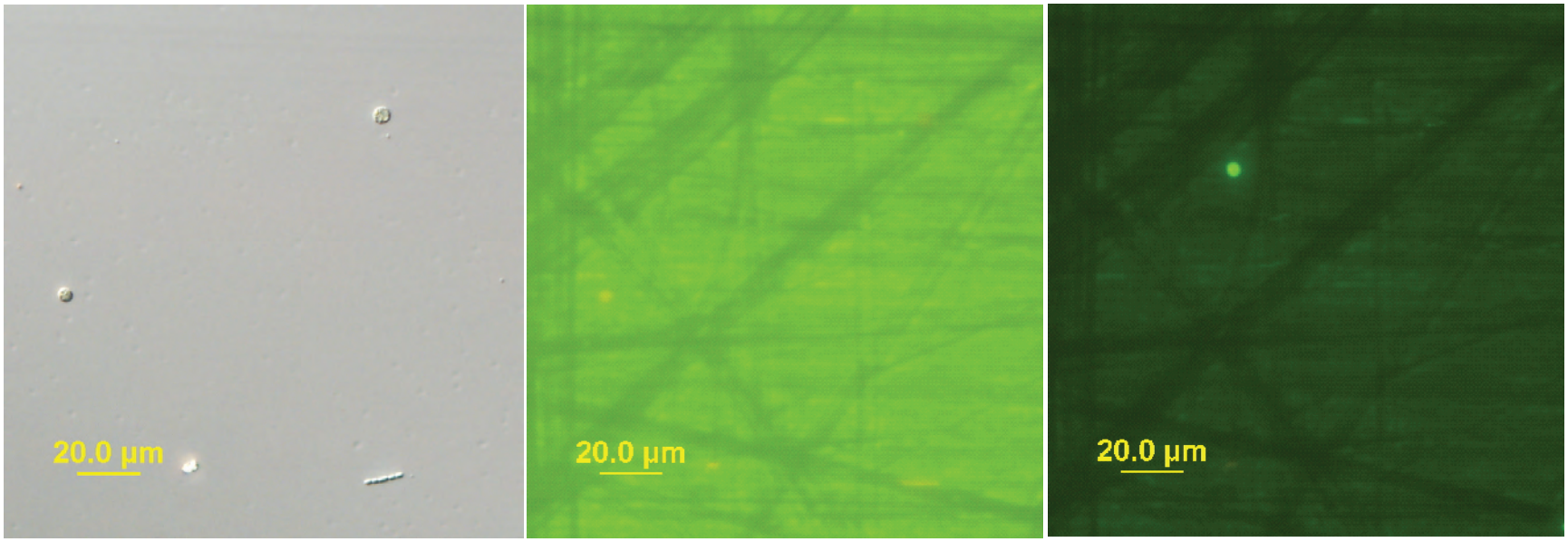


Figure 133. Iron Gate Reservoir, OR (8/25/2009). LM-Possible remains of *Microcystis aeruginosa*. FITC-an orange-yellow color dominates the cells. Sytox® green-stain did penetrate the cell membrane; cell bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

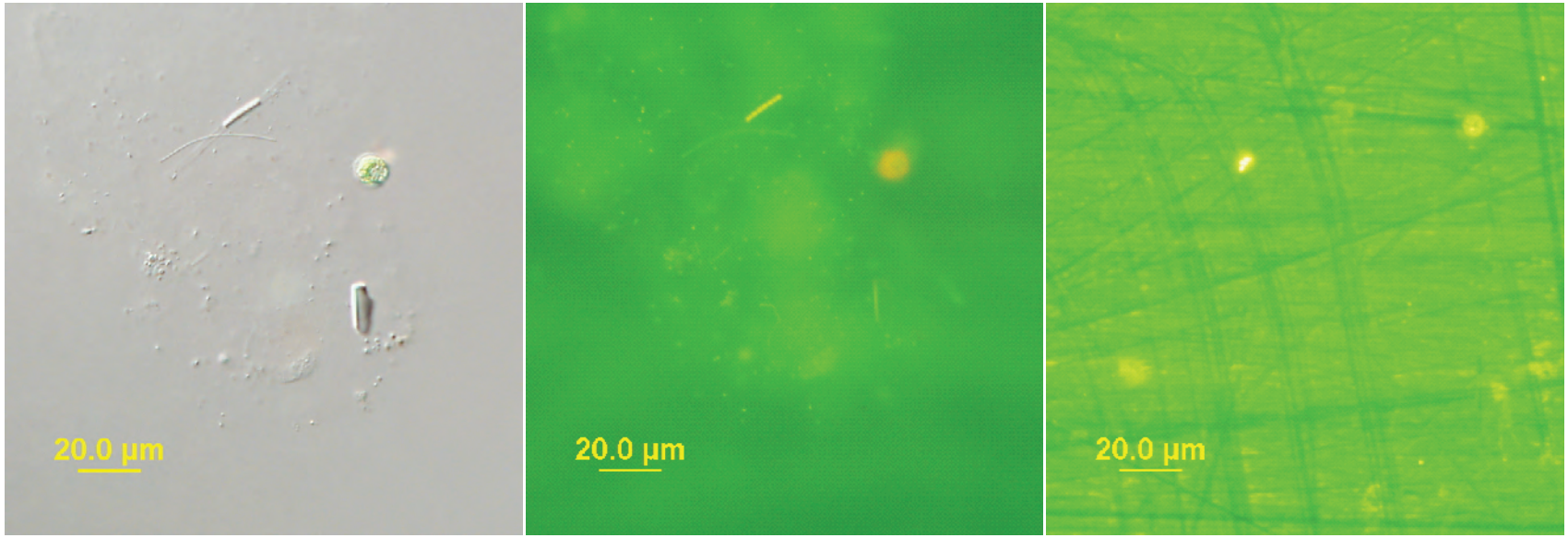


Figure 134. Iron Gate Reservoir, OR (8/25/2009). LM-Possible remains of *Microcystis aeruginosa*. FITC-no cyanobacteria cells. Sytox® green-no cyanobacteria cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

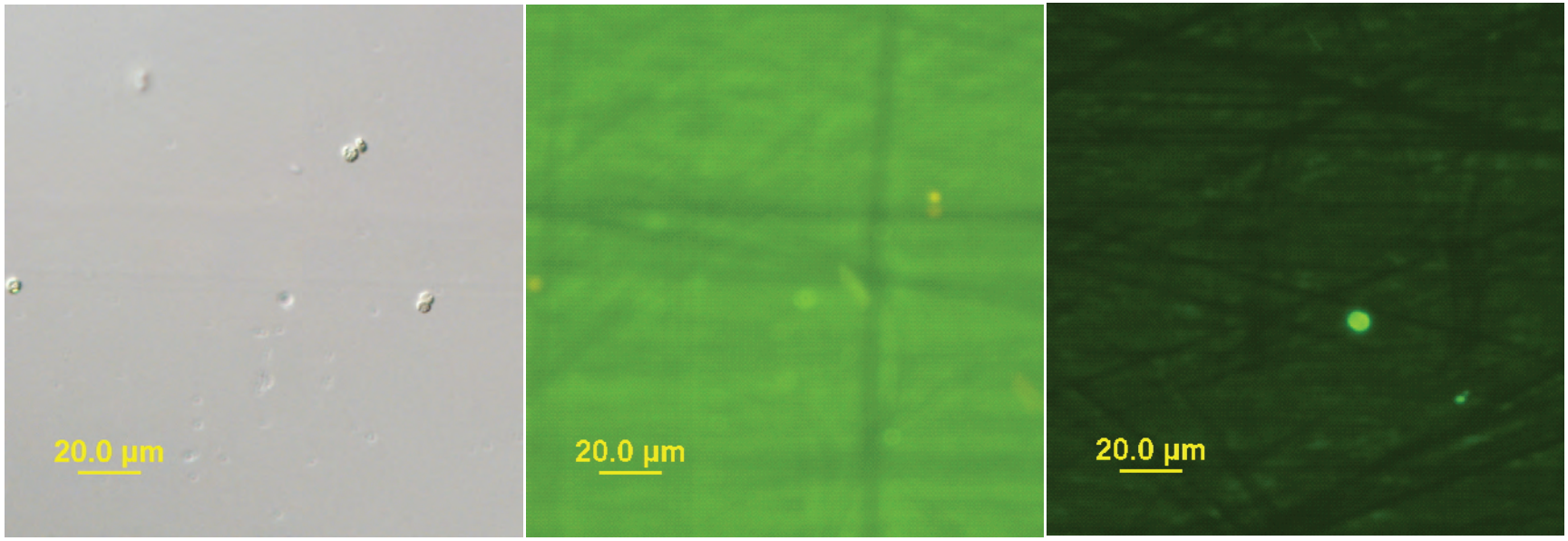


Figure 135. Iron Gate Reservoir, OR (8/25/2009). LM-Possible remains of *Microcystis aeruginosa*. FITC-an orange-yellow color dominates the cells. Sytox® green-stain did penetrate the cell membrane; cells bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

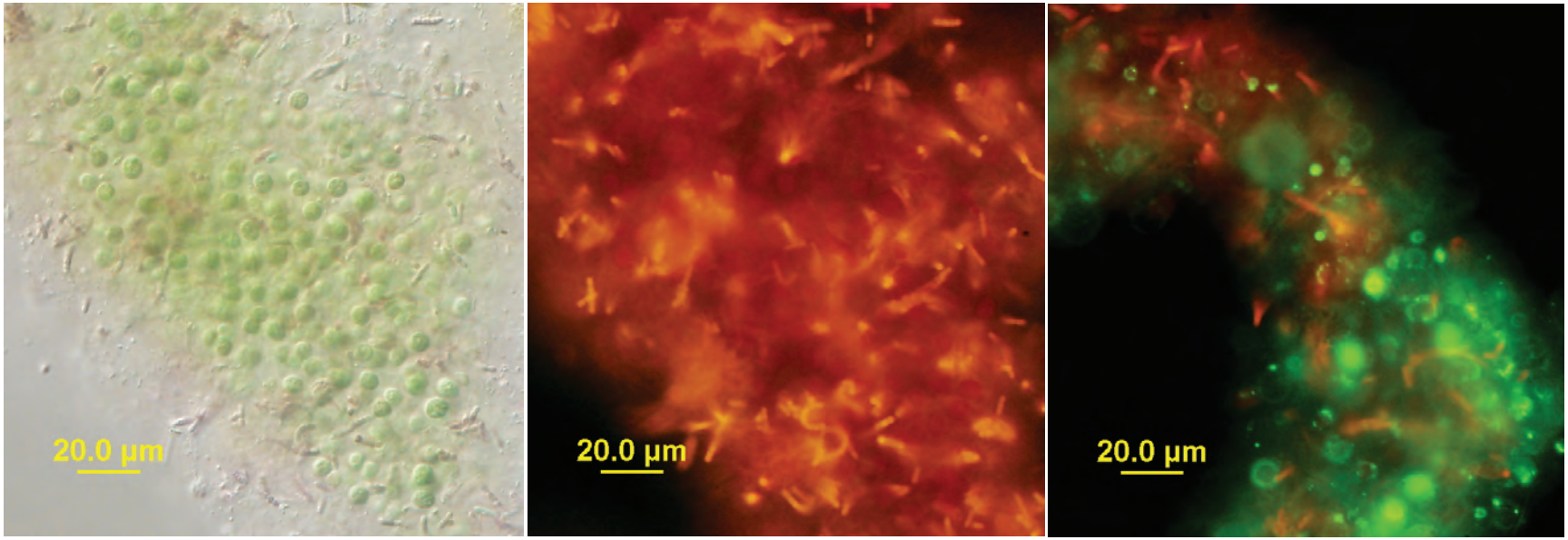


Figure 136. Iron Gate Reservoir, OR (8/25/2009). LM-*Microcystis aeruginosa*. FITC-a red color dominates the cells; orange filaments are cyanobacterial epiphyte. Sytox® green-stain did penetrate the cell membrane; some cells bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

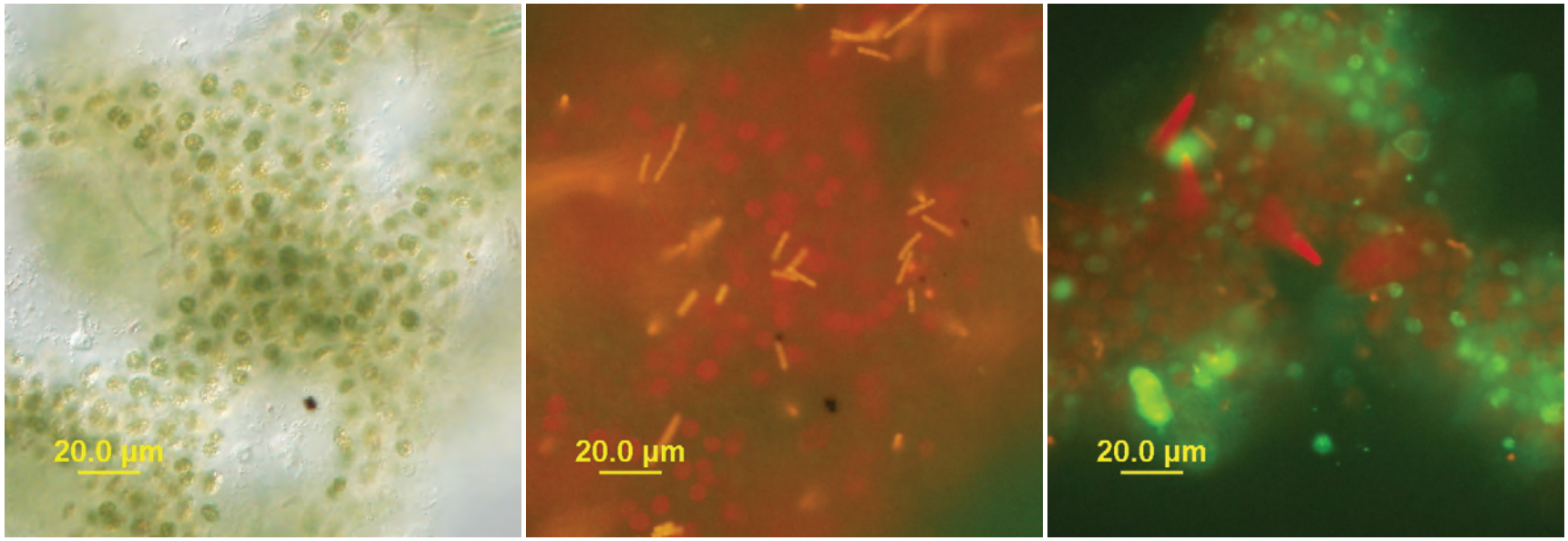


Figure 137. Iron Gate Reservoir, OR (8/25/2009). LM-*Microcystis aeruginosa*. FITC-a red color dominates the cells; orange filaments are cyanobacterial epiphyte. Sytox® green-stain did penetrate the cell membrane of the peripheral cells; cells bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

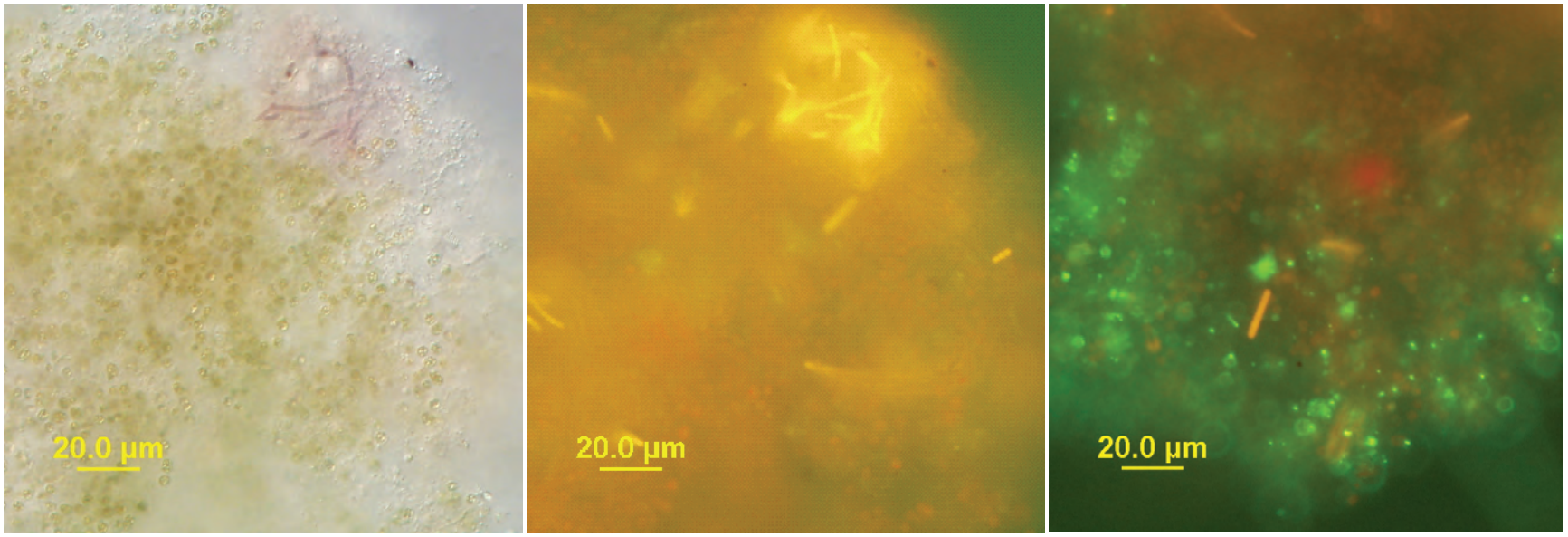


Figure 138. Iron Gate Reservoir, OR (8/25/2009). LM-*Microcystis aeruginosa*. FITC-a red color dominates the cells; yellow-orange filaments are cyanobacterial epiphyte. Sytox® green-stain did penetrate the cell membrane of the peripheral cells and epiphytic bacterial cells that are bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

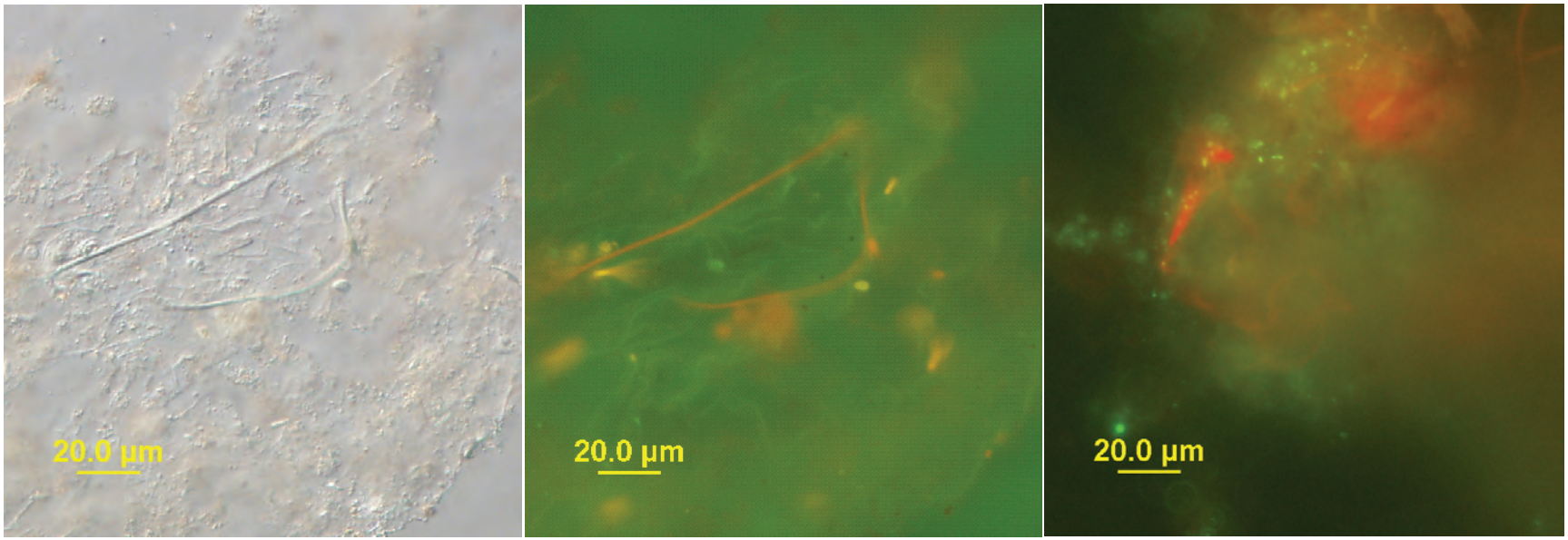


Figure 139. Iron Gate Reservoir, OR (8/25/2009). LM-Unknown filamentous cyanobacterium. FITC-an orange color dominates the cells. Sytox® green-cannot be determined. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

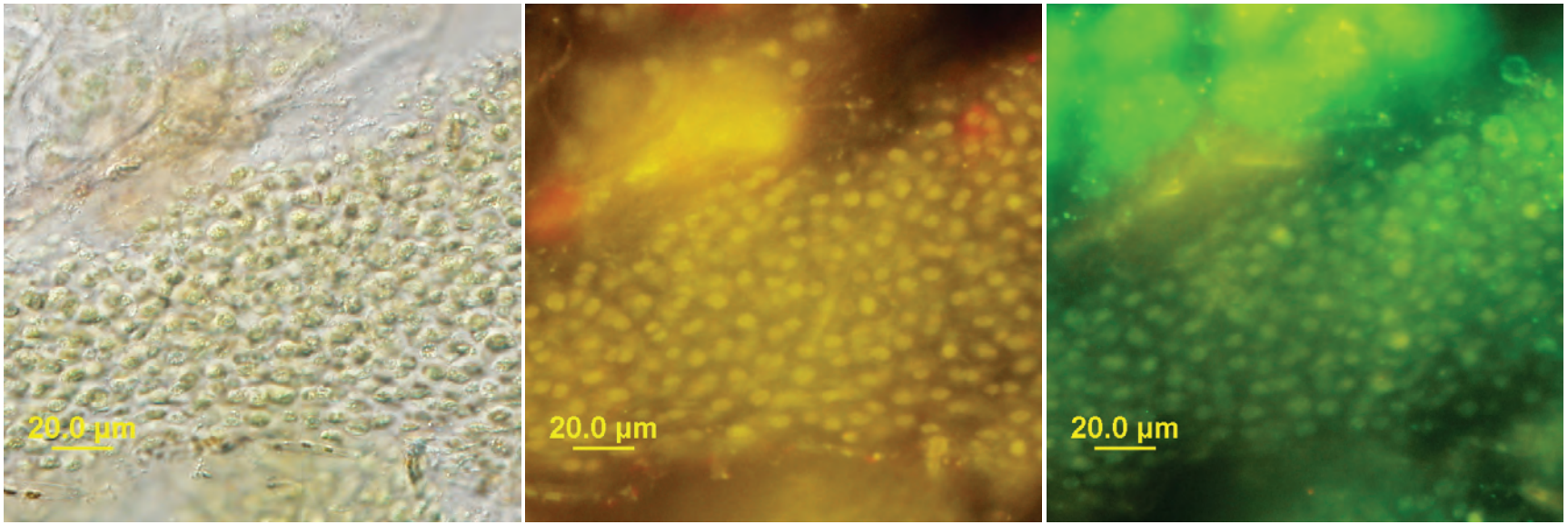


Figure 140. Iron Gate Reservoir, OR (8/25/2009). LM-*Microcystis aeruginosa*. FITC-a yellow color dominates the cells. Sytox® green-stain did penetrate the cell membrane; cells yellow-green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

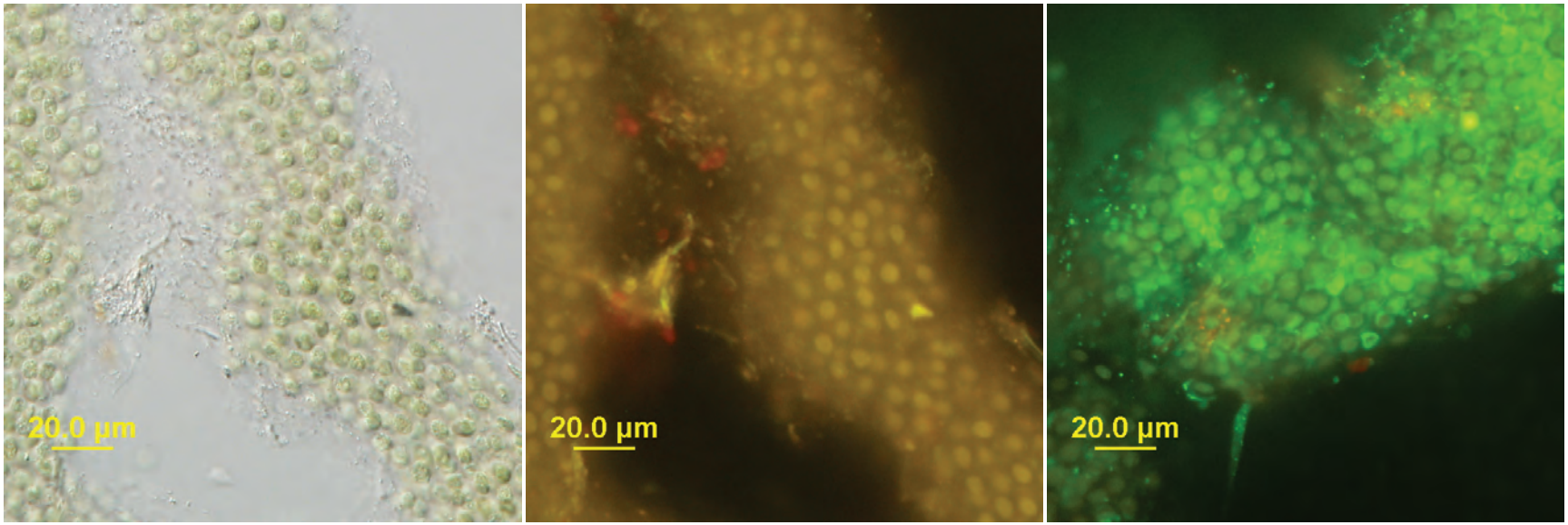


Figure 141. Iron Gate Reservoir, OR (8/25/2009). LM-*Microcystis aeruginosa*. FITC-a yellow-orange color dominates the cells. Sytox® green-stain did penetrate the cell membrane; cells and the mucilage are green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

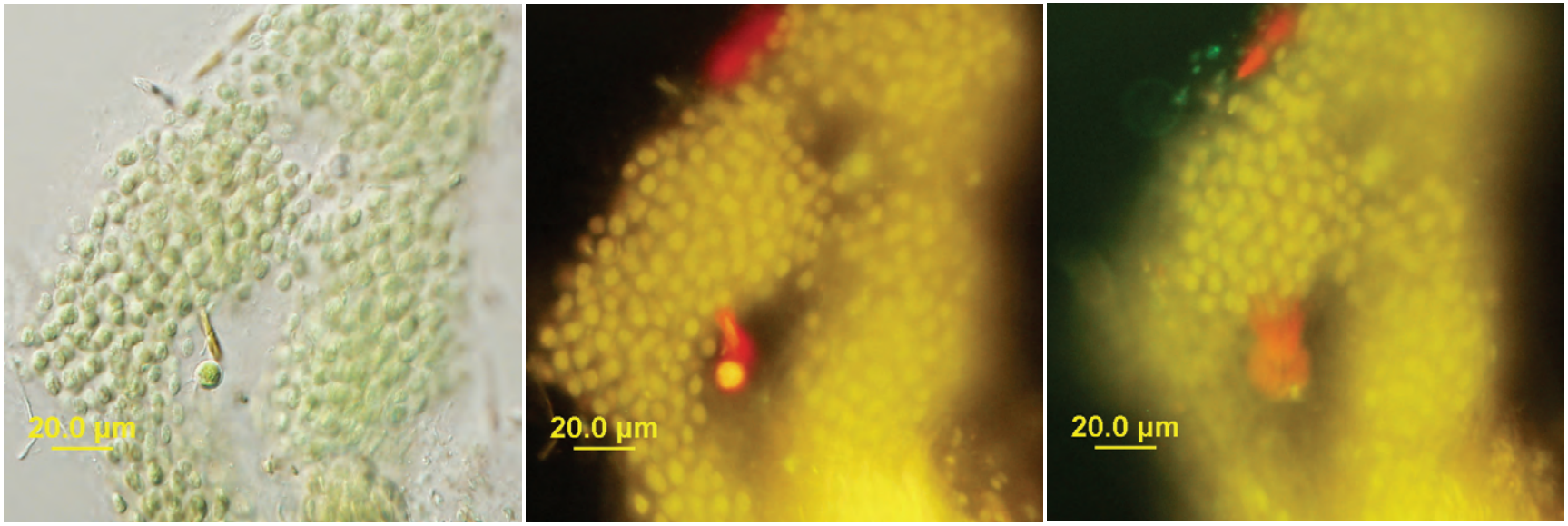


Figure 142. Iron Gate Reservoir, OR (8/25/2009). LM-*Microcystis aeruginosa*. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

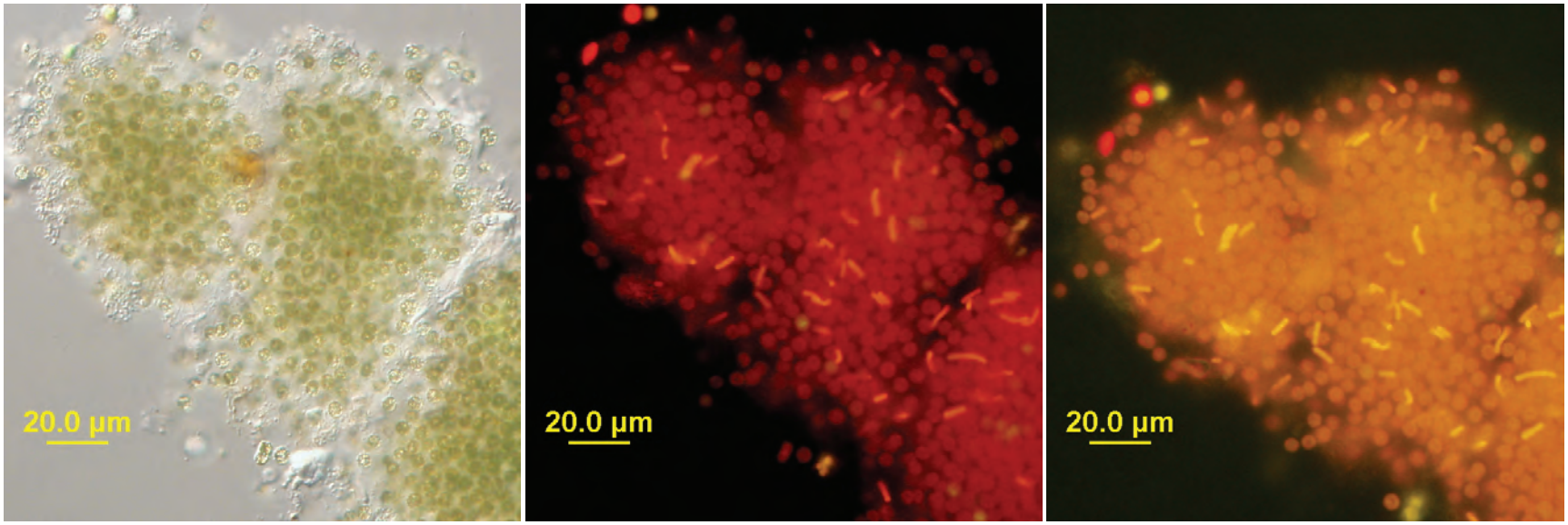


Figure 143. Pinto Lake, CA (9/22/2009). LM-*Microcystis aeruginosa*. FITC-a red color dominates the cells; orange filaments are cyanobacterial epiphytes. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

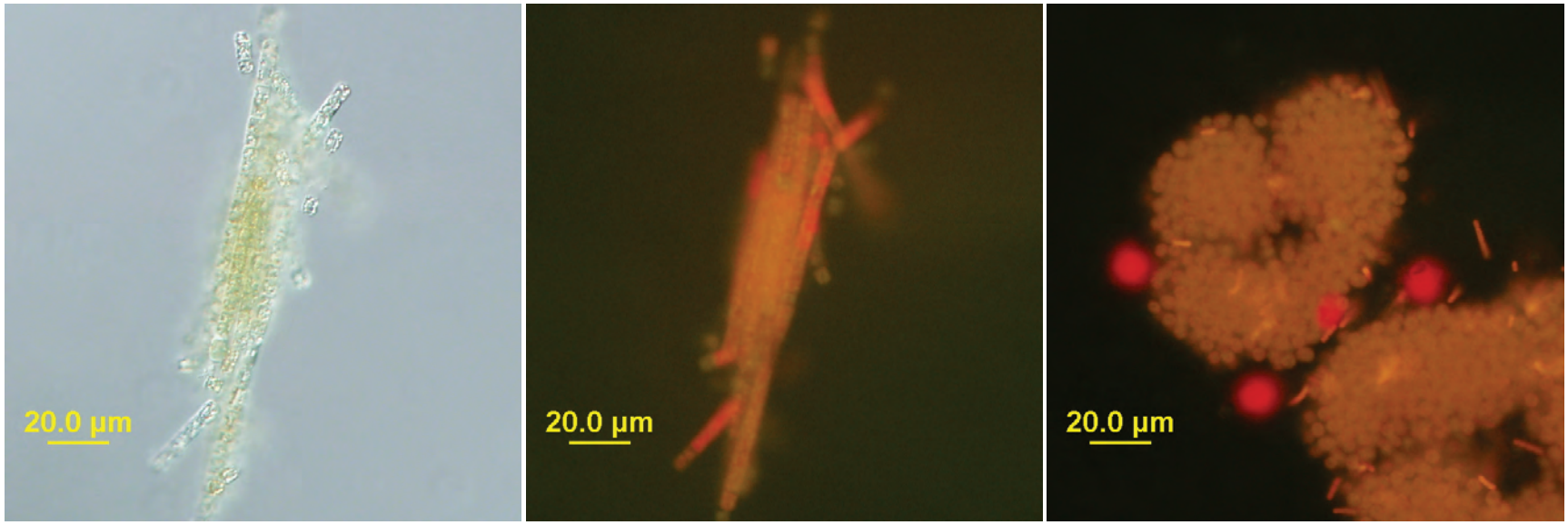


Figure 144. Pinto Lake, CA (9/22/2009). LM- *Aphanizomenon flos-aquae*. FITC-an orange color dominates the cells. Sytox® green-*Microcystis aeruginosa*; stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

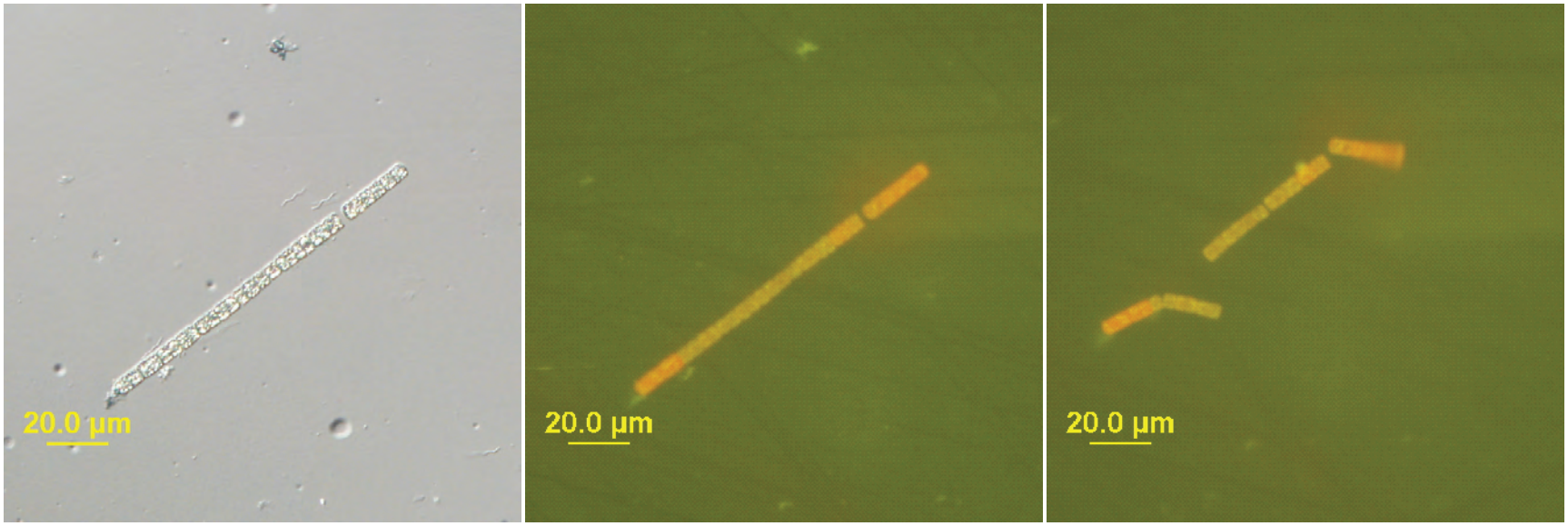


Figure 145. Pinto Lake, CA (9/22/2009). LM- *Aphanizomenon flos-aquae*. FITC-an orange color dominates the cells. Sytox® green-stain appears to have penetrated the cell membrane in some cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

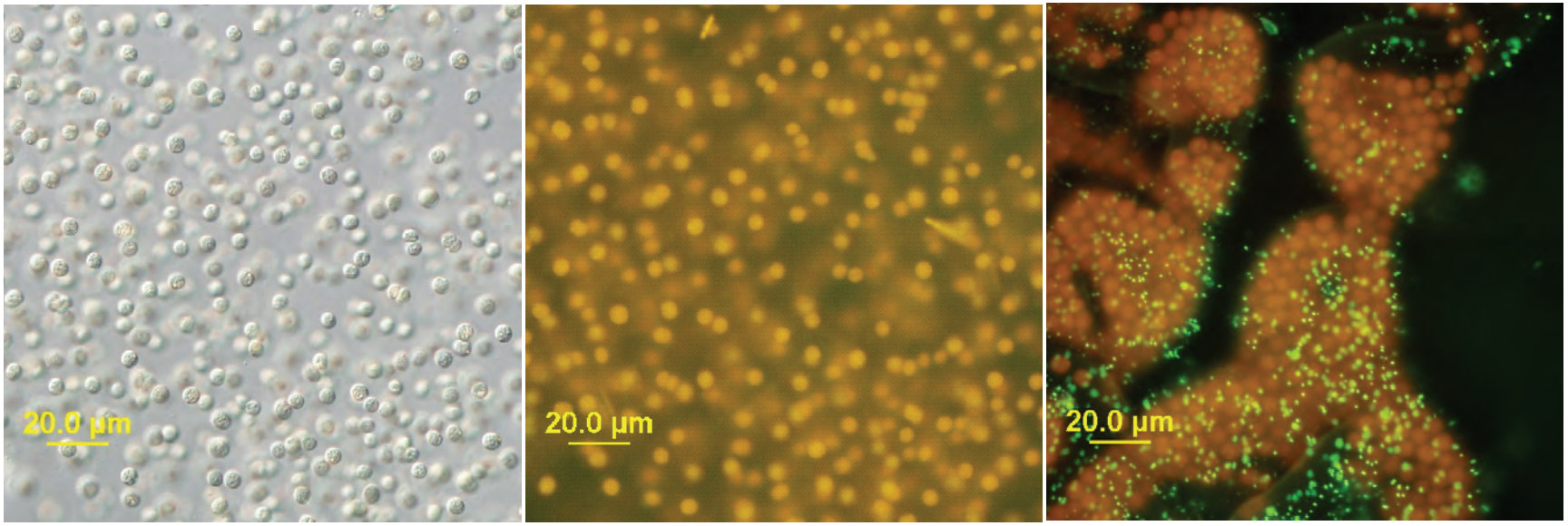


Figure 146. Pinto Lake, CA (9/22/2009). LM-*Microcystis aeruginosa*. FITC-an orange color dominates the cells. Sytox® green-*Microcystis wesenbergii*-stain did not penetrate the mucilage of the colony; bright green dots are epiphytic bacteria. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

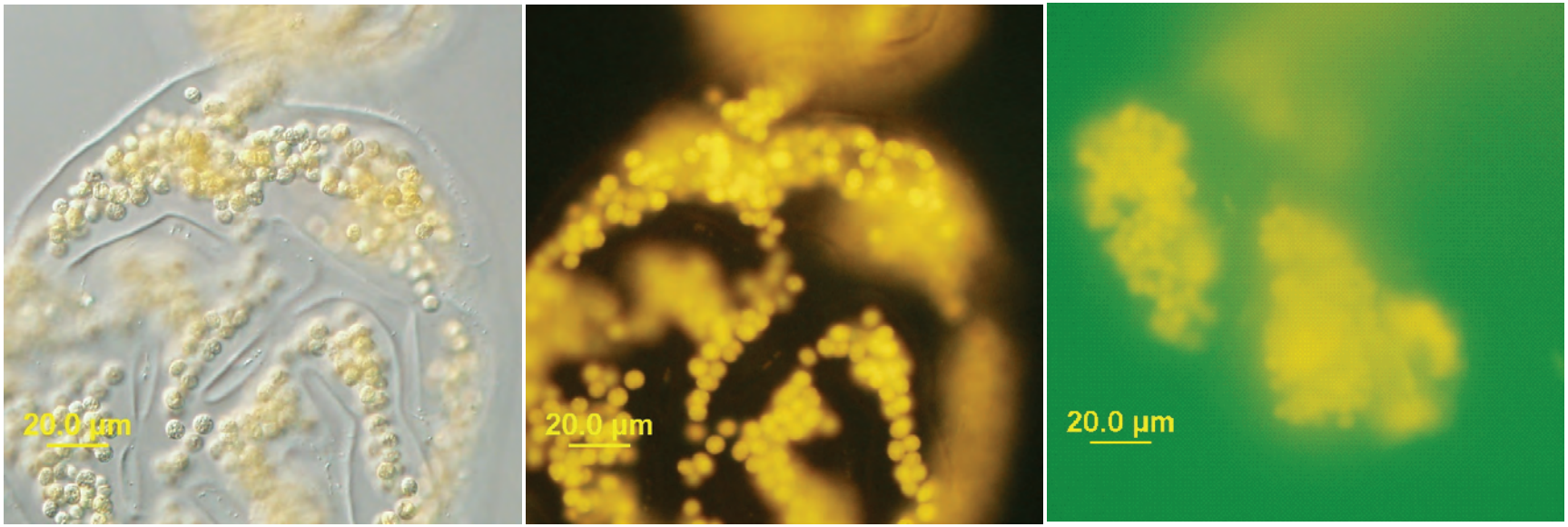
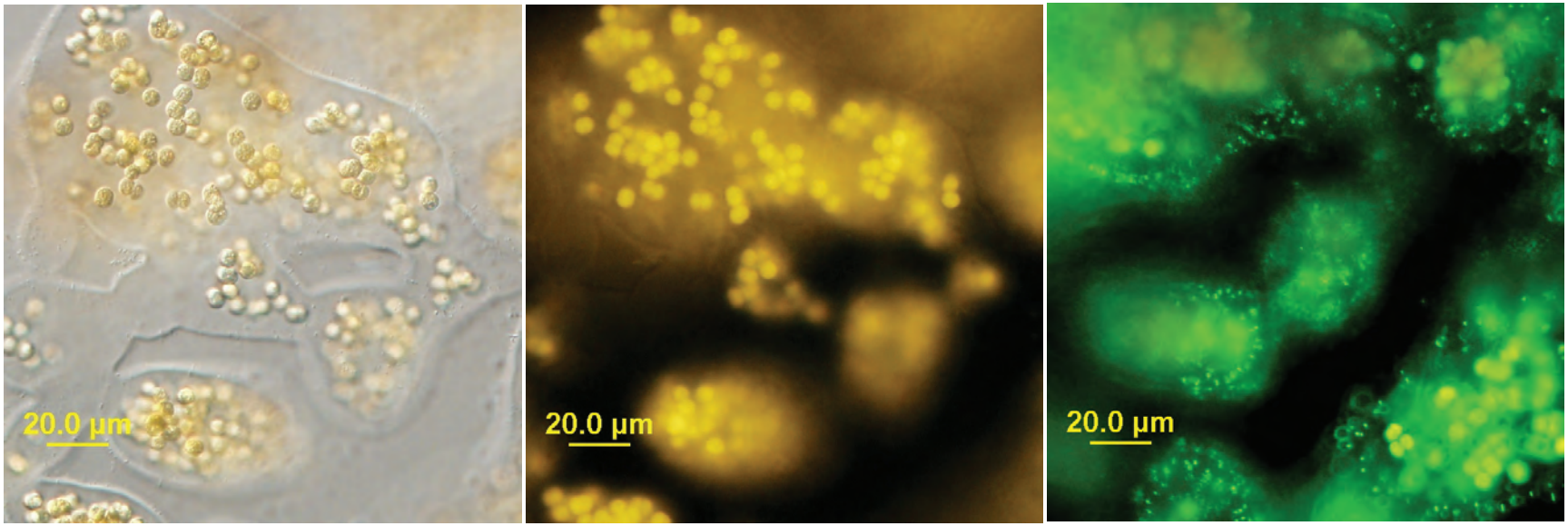


Figure 147. Pinto Lake, CA (9/22/2009). LM-*Microcystis wesenbergii*. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membranes. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.



LM FITC Sytox® green

Boiled for 30 minutes

Figure 148. Pinto Lake, CA (9/22/2009). LM-*Microcystis wesenbergii*. FITC-a yellow color dominates the cells. Sytox® green-stain did penetrate the mucilaginous cells and the cell membranes; cells bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

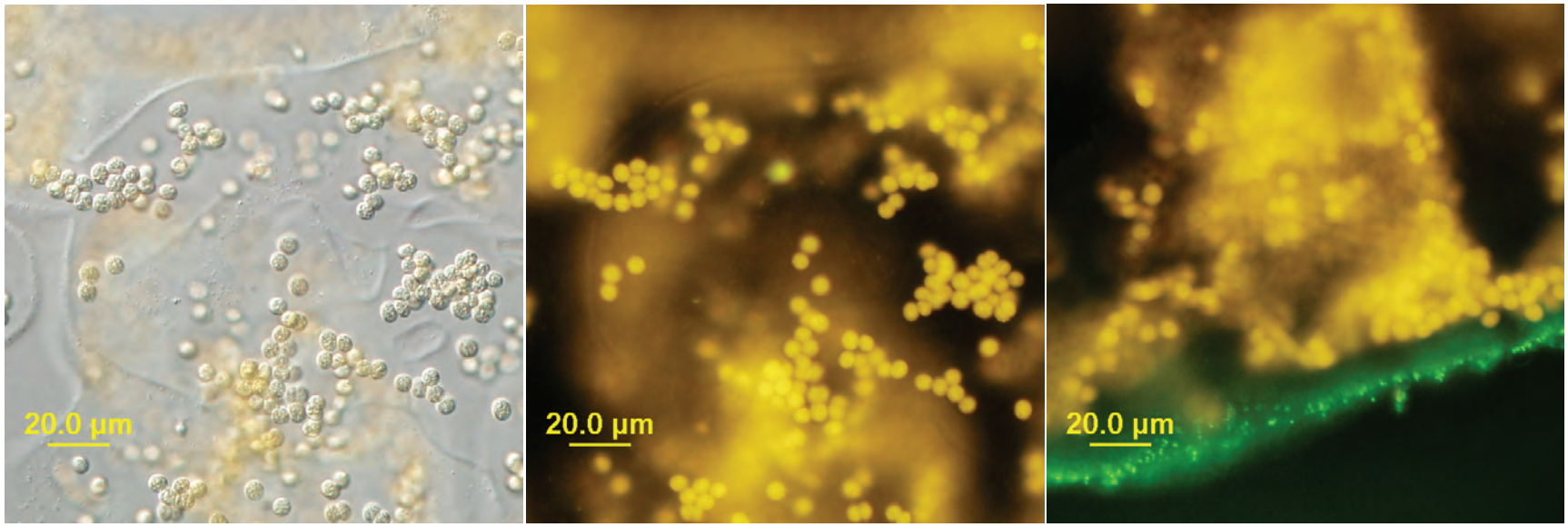


Figure 149. Pinto Lake, CA (9/22/2009). LM-*Microcystis wesenbergii*. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the mucilage of the colony; bright green dots are epiphytic bacteria and the mucilage appears to have responded to the stain. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

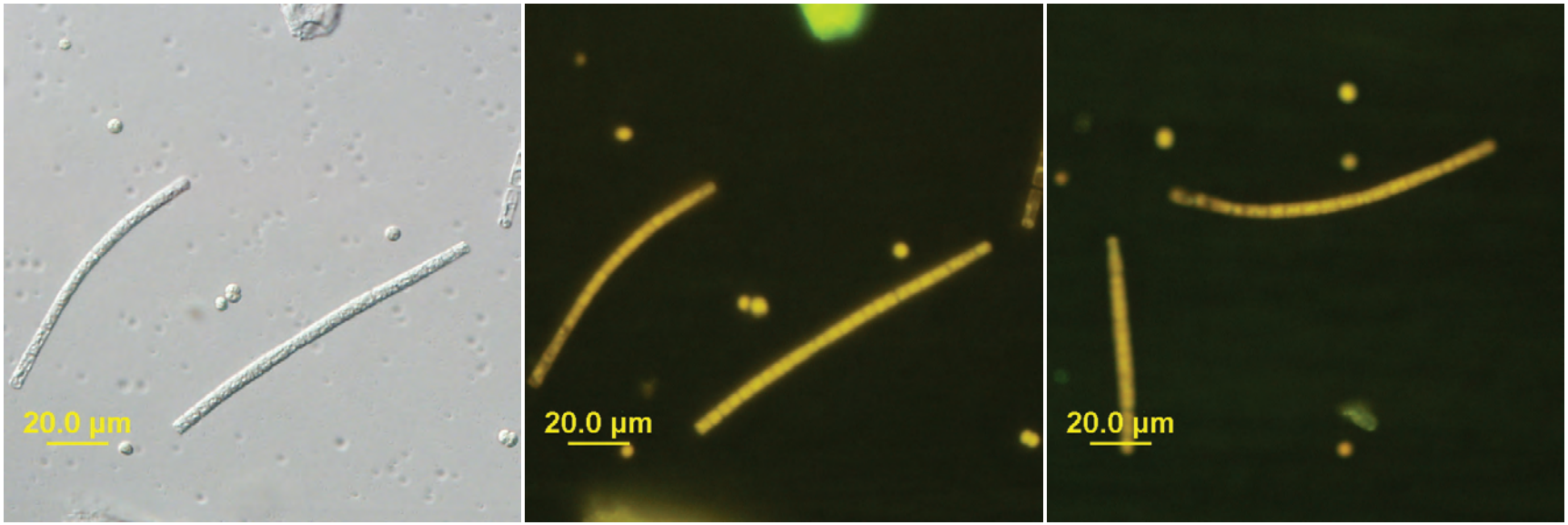


Figure 150. Pinto Lake, CA (9/22/2009). LM- *Aphanizomenon flos-aquae*. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membrane. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

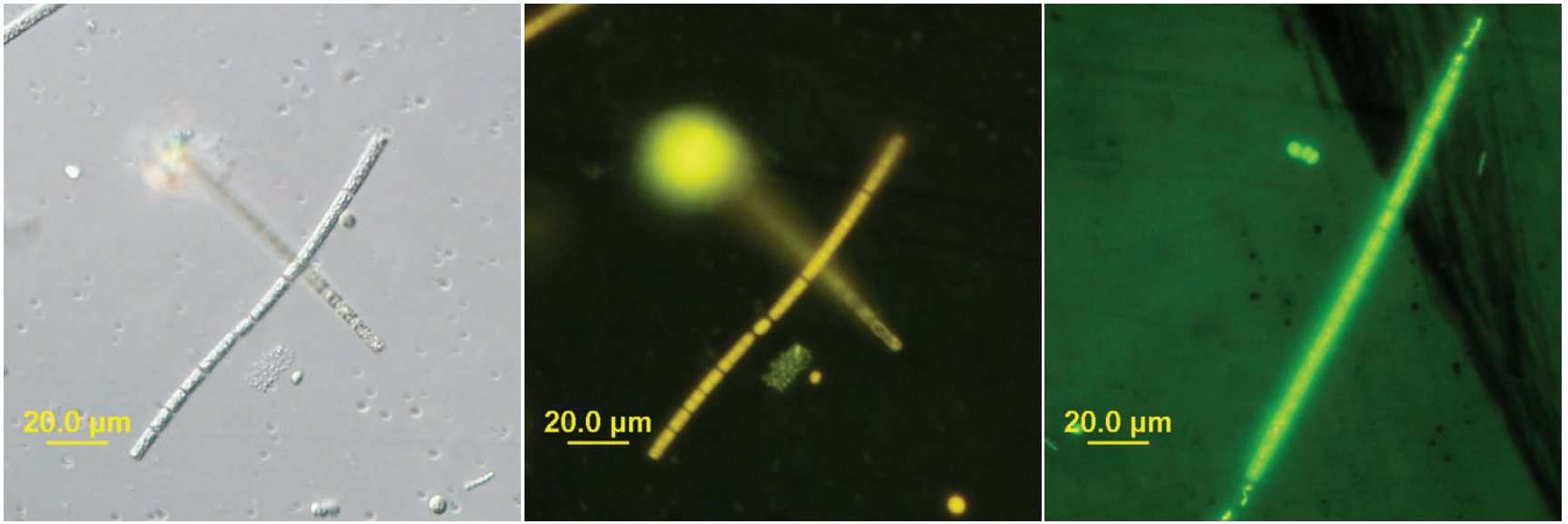


Figure 151. Pinto Lake, CA (9/22/2009). LM- *Aphanizomenon flos-aquae*. FITC-a yellow color dominates the cells. Sytox® green-stain did penetrate the cell membrane; cells bright green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

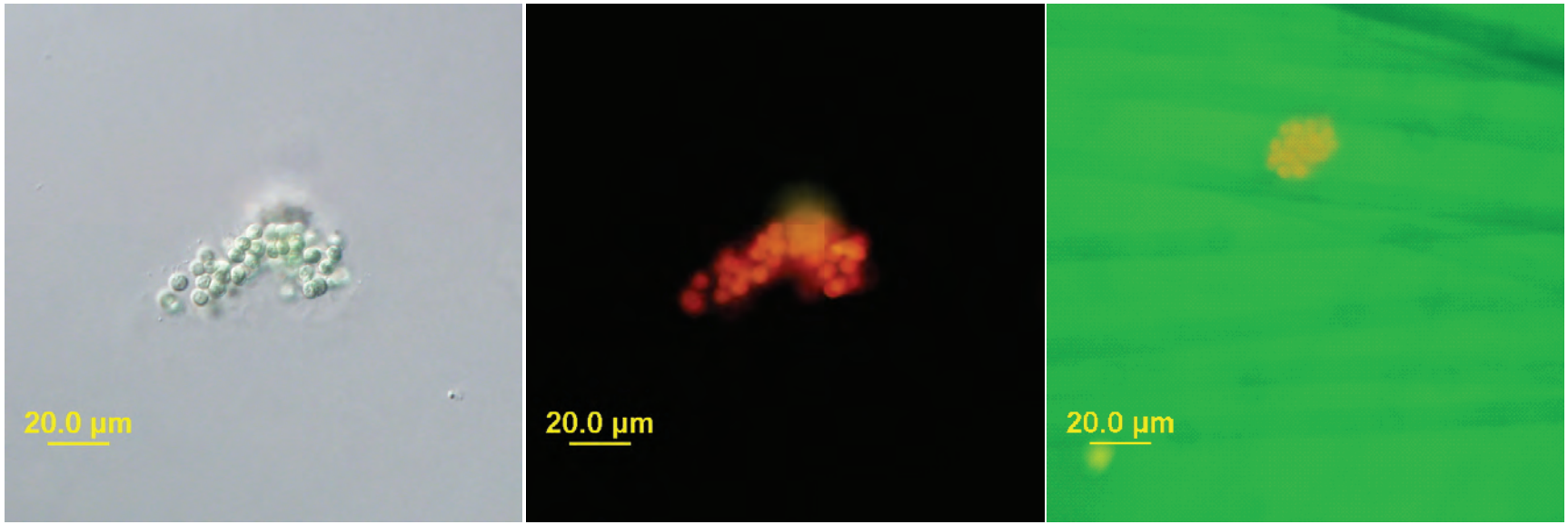


Figure 152. Pinto Lake, CA (9/22/2009). LM-*Microcystis* sp. FITC-an orange-red color dominates the cells. Sytox® green-stain did not penetrate the mucilage of the colony. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green. Note: No other sonication samples had evidence of cells.

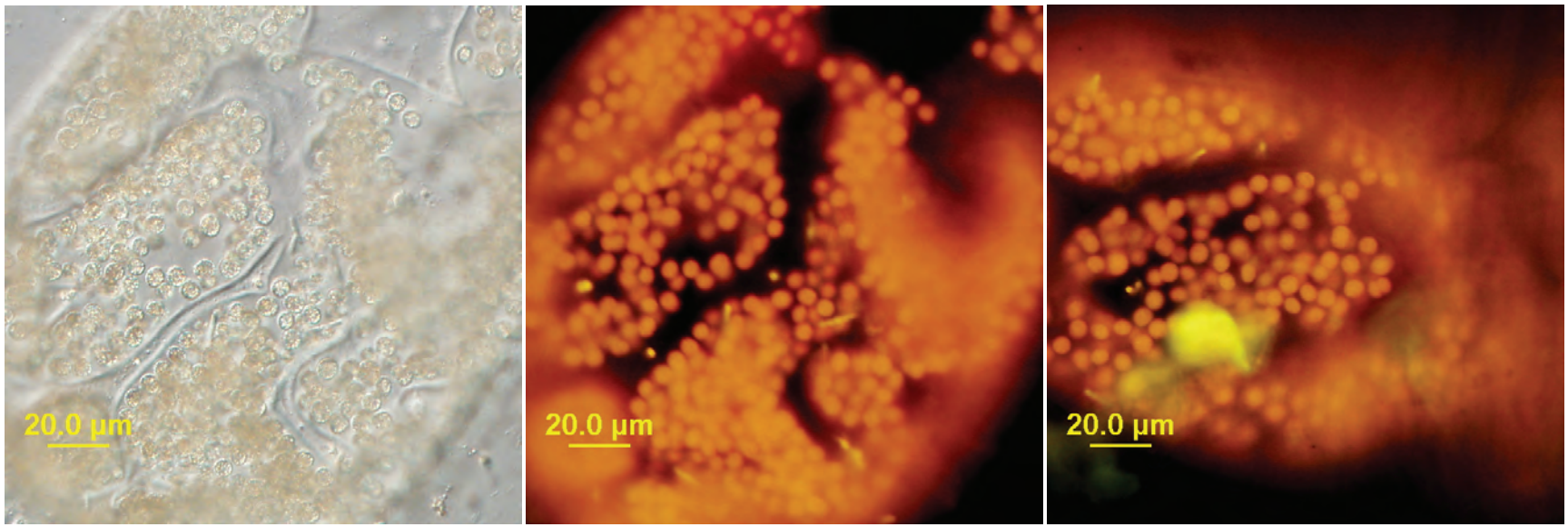


Figure 153. Pinto Lake, CA (9/22/2009). LM-*Microcystis wesenbergii*. FITC-an orange color dominates the cells. Sytox® green-stain did not penetrate the cell membranes. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

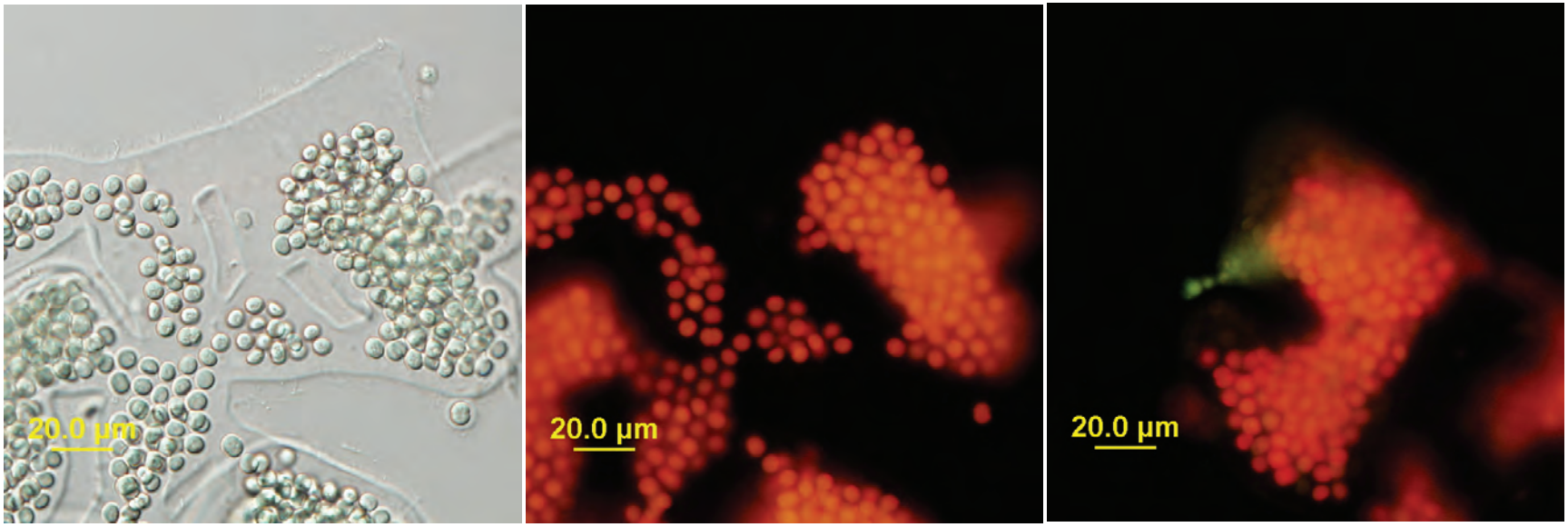


Figure 154. Pinto Lake, CA (9/22/2009). LM-*Microcystis wesenbergii*. FITC-an orange-red color dominates the cells. Sytox® green-stain did not penetrate the cell membranes. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

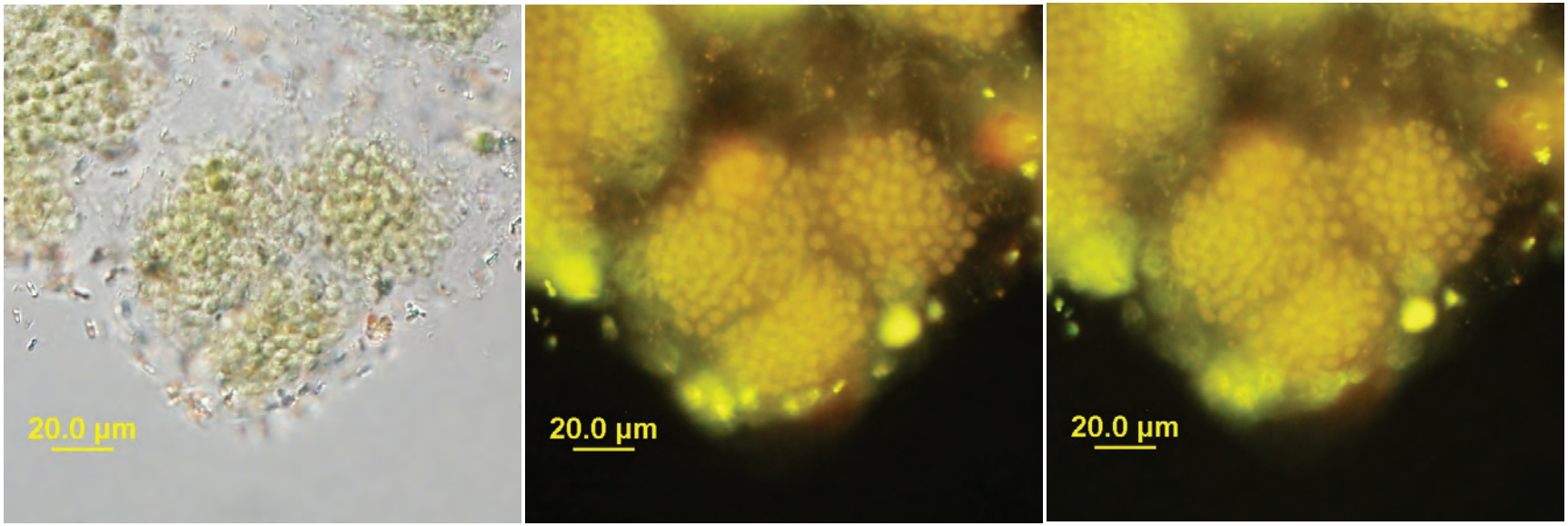


Figure 155. Pinto Lake, CA (9/22/2009). LM-*Microcystis* sp. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate the cell membranes. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

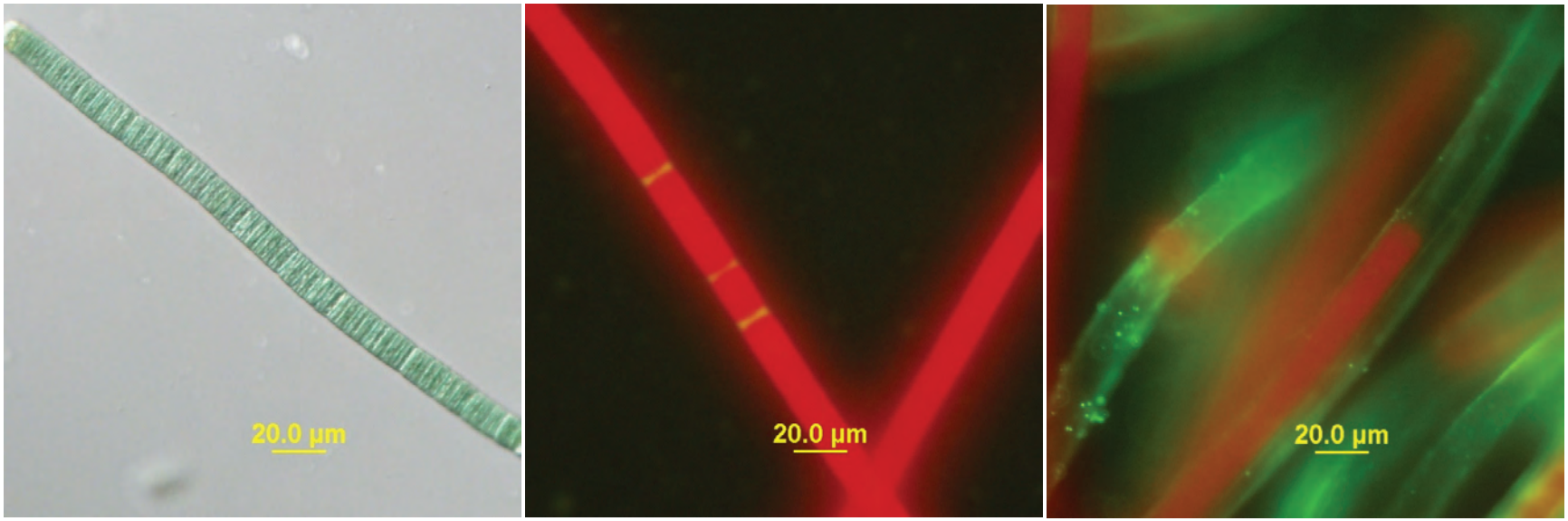


Figure 156. Laboratory culture-*Lyngbya* DVL 1103B. LM-*Lyngbya* sp. is a filamentous cyanobacteria with a mucilaginous sheath. FITC-a red color dominates the cells. Yellow is the area of filament splitting (separation discs). Sytox® green-stain was picked up by the sheath but not the cells. Bacteria appear as bright green dots on the sheath. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

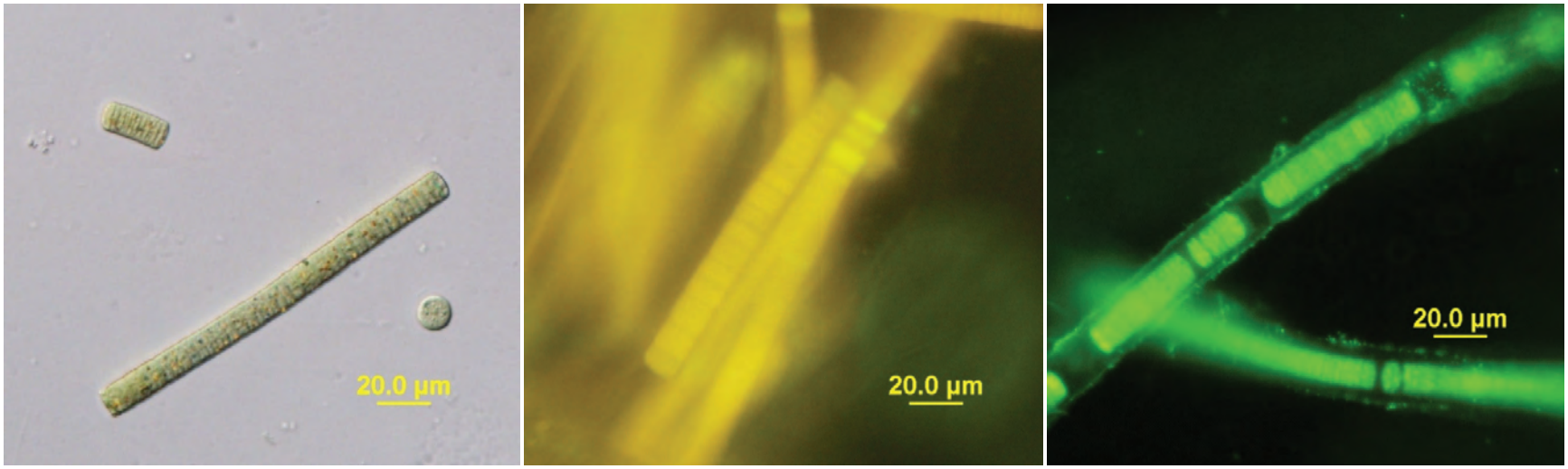


Figure 157. Laboratory culture-*Lyngbya* DVL 1103B. LM-*Lyngbya* sp. FITC-a yellow color dominates the cells. Sytox® green-stain penetrated, indicating the cell membrane was disrupted. The sheath also picked up the stain. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

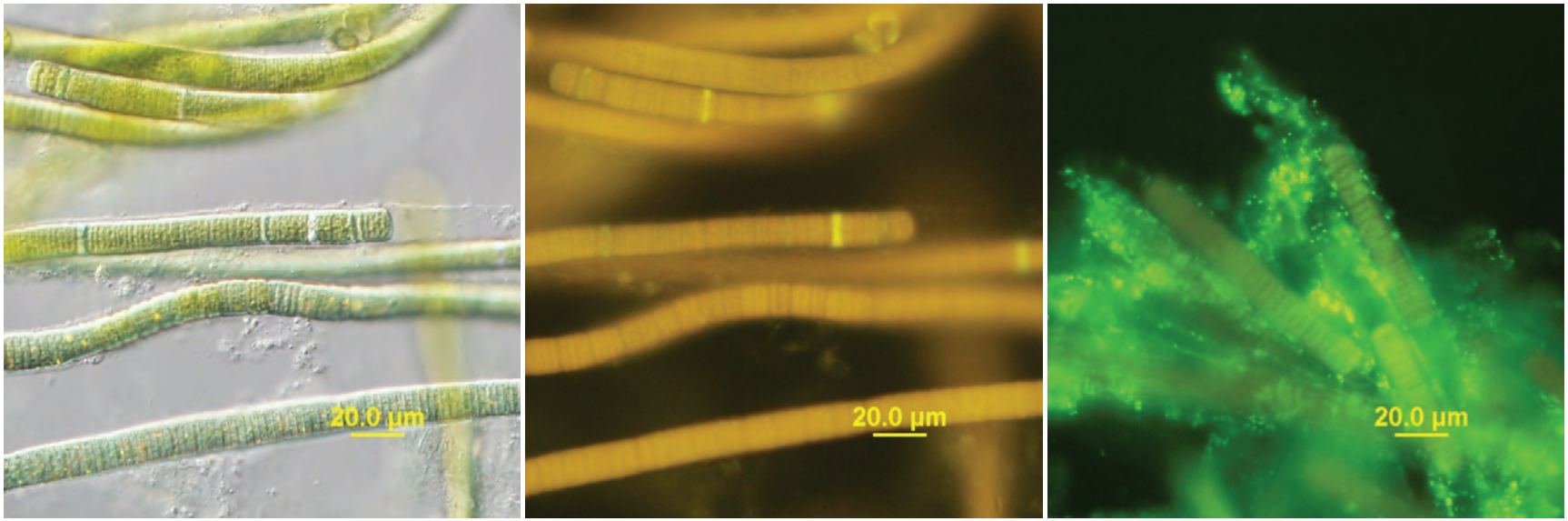


Figure 158. Laboratory culture-*Lyngbya* DVL 1103B. LM-*Lyngbya* sp. FITC-a yellow-orange color dominates the cells. Sytox® green-stain penetrated some cells, indicating the cell membrane was partially disrupted. The sheath also picked up the stain. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

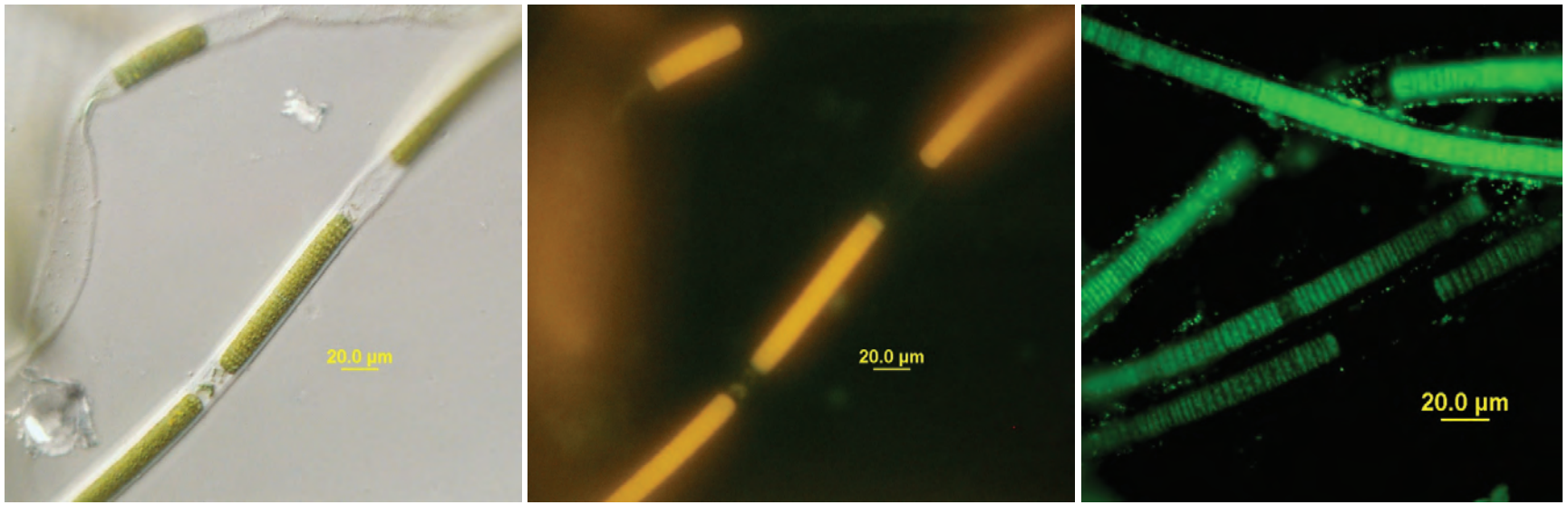


Figure 159. Laboratory culture-*Lyngbya* DVL 1103B. LM-*Lyngbya* sp. FITC-an orange color dominates the cells. Sytox® green-stain penetrated, indicating the cell membrane was disrupted. The sheath also picked up the stain. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

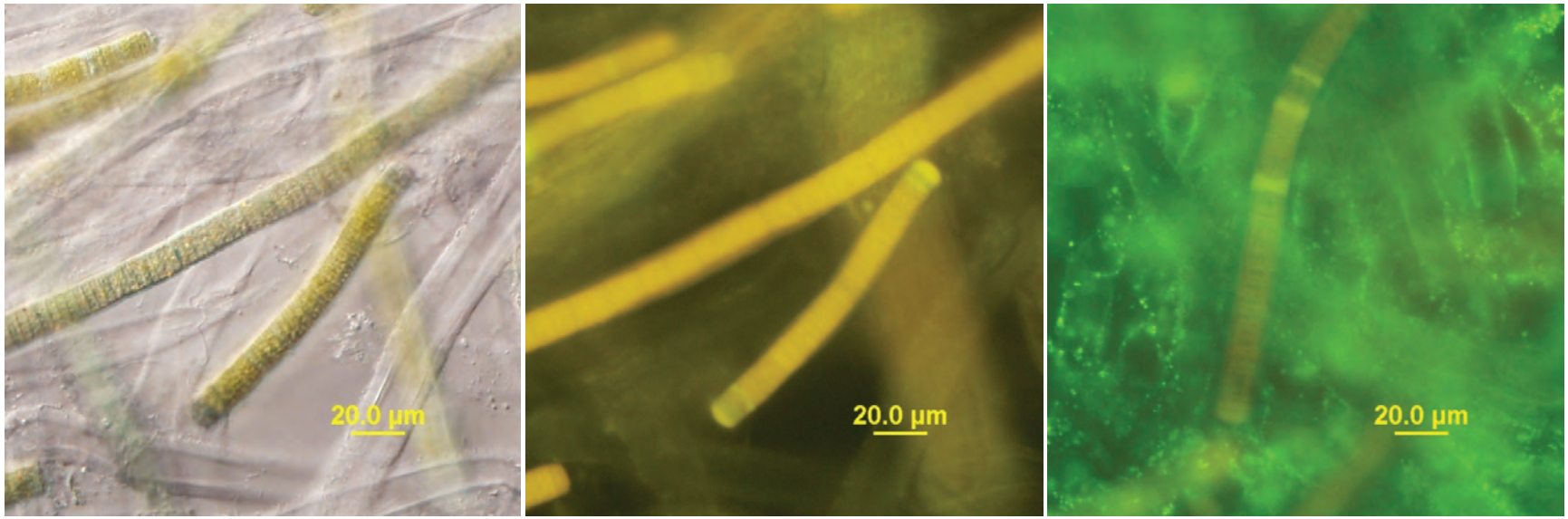


Figure 160. Laboratory culture-*Lyngbya* DVL 1103B. LM-*Lyngbya* sp. FITC-a yellow-orange color dominates the cells. Sytox® green-stain did not penetrate, indicating the cell membrane was not disrupted. The sheath also picked up the stain. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

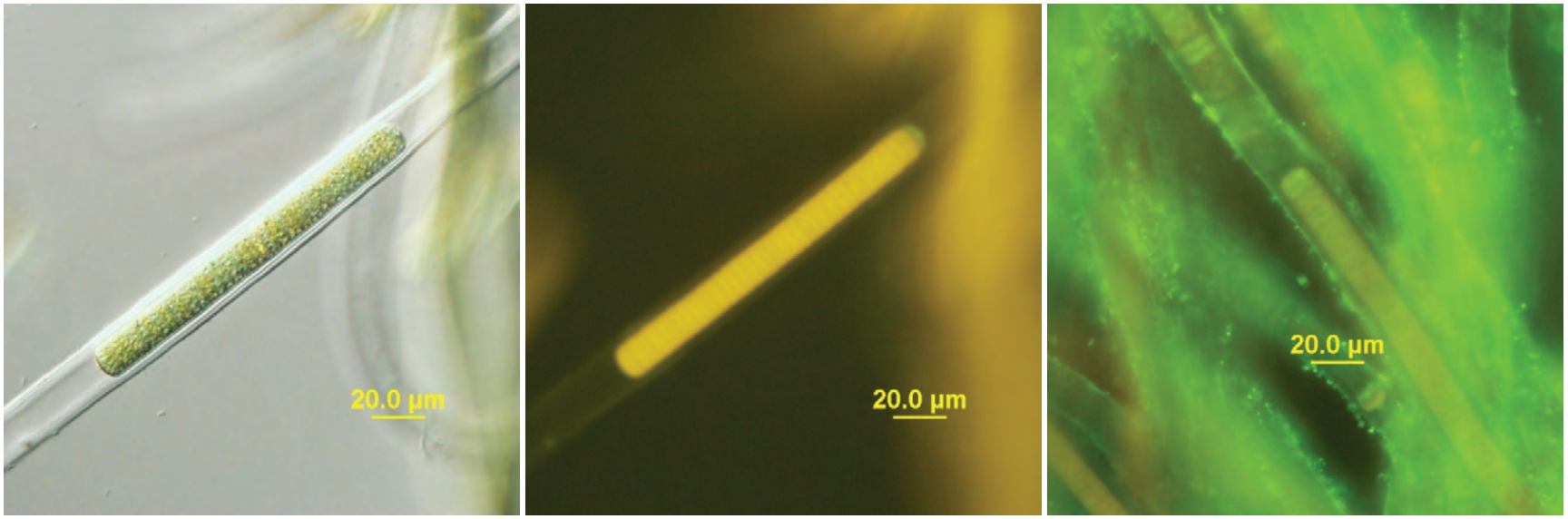


Figure 161. Laboratory culture-*Lyngbya* DVL 1103B. LM-*Lyngbya* sp. FITC-a yellow color dominates the cells. Sytox® green-stain did penetrate to some extent, indicating the cell membrane was disrupted. The sheath also picked up the stain. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

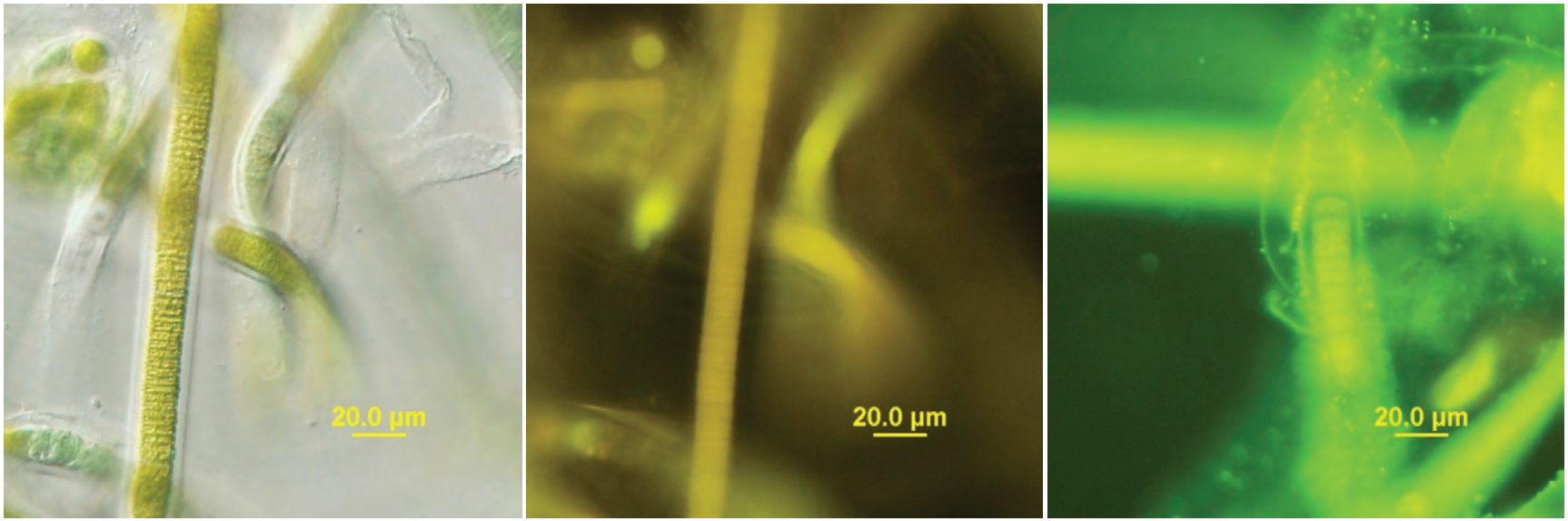


Figure 162. Laboratory culture-*Lyngbya* DVL 1103B. LM-*Lyngbya* sp. FITC-a yellow color dominates the cells. Sytox® green-stain did not penetrate, indicating the cell membrane was not disrupted. The sheath also picked up the stain. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

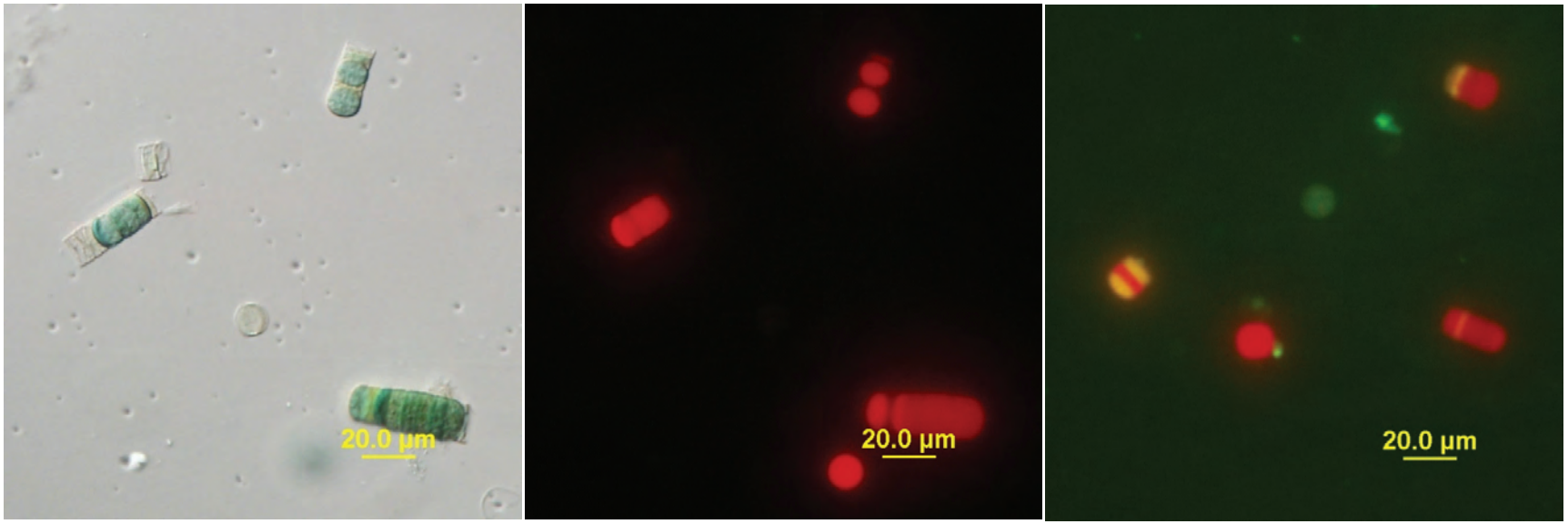
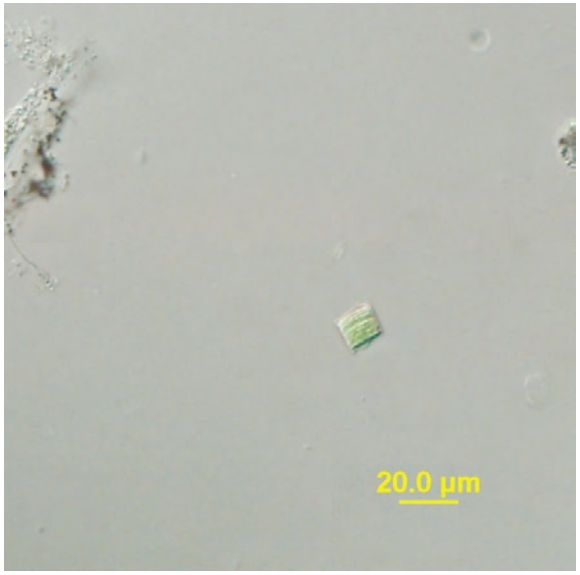


Figure 163. Laboratory culture-*Lyngbya* DVL 1103B. LM-*Lyngbya* sp. Note: the filaments were fragmented. FITC-a red color dominates the cells. Sytox® green-stain did not penetrate, indicating the cell membrane was not disrupted. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.



LM

Sonication at 35 percent power

Figure 164. Laboratory culture-*Lyngbya* DVL 1103B. LM-*Lyngbya* sp. Note: only a few fragments were found and no material for FITC or Sytox® green. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

Figure 165. Laboratory culture-*Lyngbya* DVL 1103B. LM-*Lyngbya* sp. FITC-a red color dominates the cells. Sytox® green-stain was picked up by a few cells in each filament and the tips of each filament. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

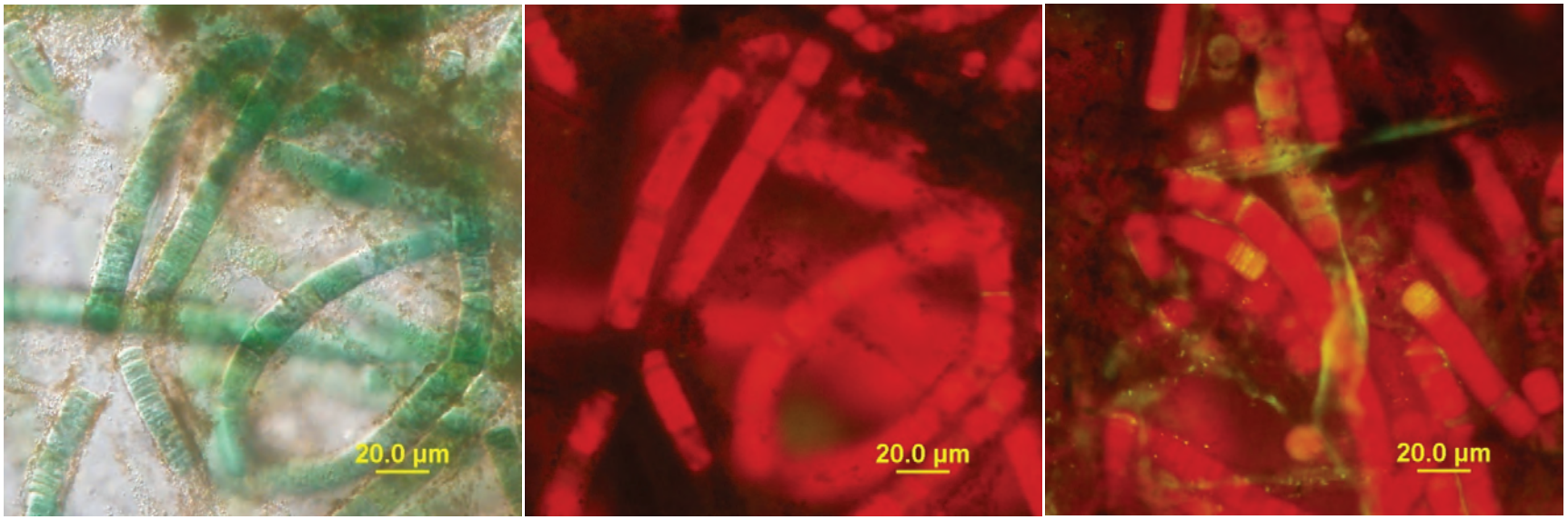


Figure 166. Laboratory culture-*Lyngbya* DVL 1103B. LM-*Lyngbya* sp. FITC-a red color dominates the cells. Sytox® green-stain was picked up by a few cells in each filament and the tips of each filament. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

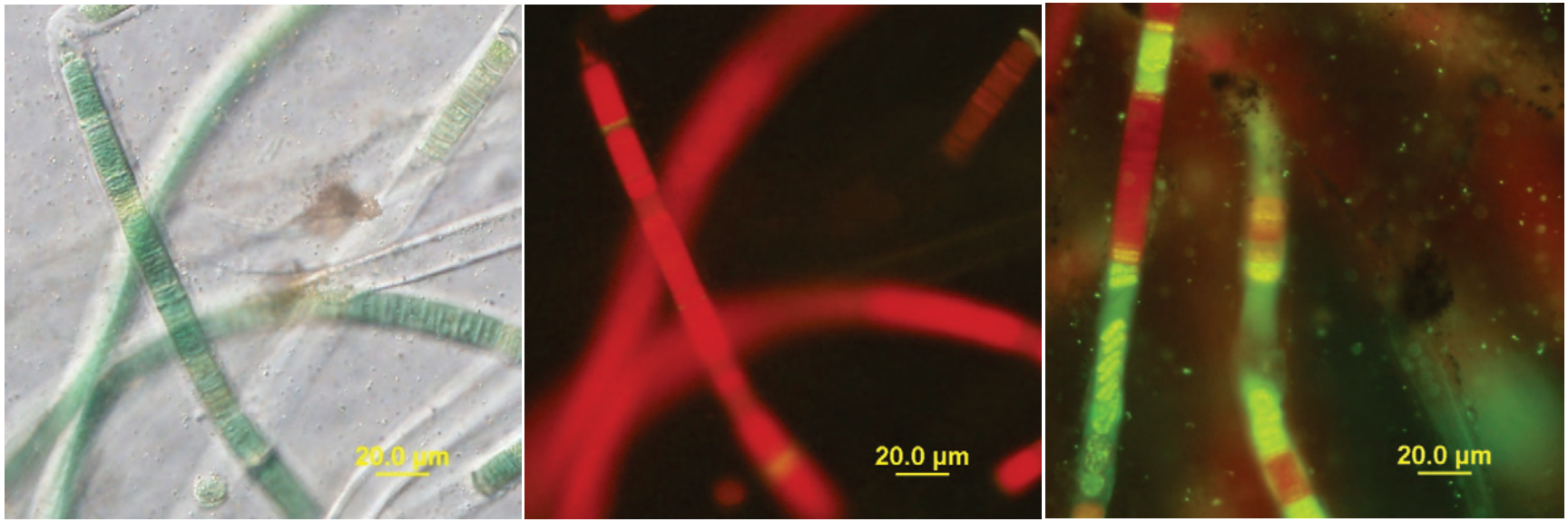


Figure 167. Laboratory culture-*Lyngbya* DVL 1103B. LM-*Lyngbya* sp. FITC-a red color dominates the cells. Sytox® green-stain was picked up by a few cells in each filament. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

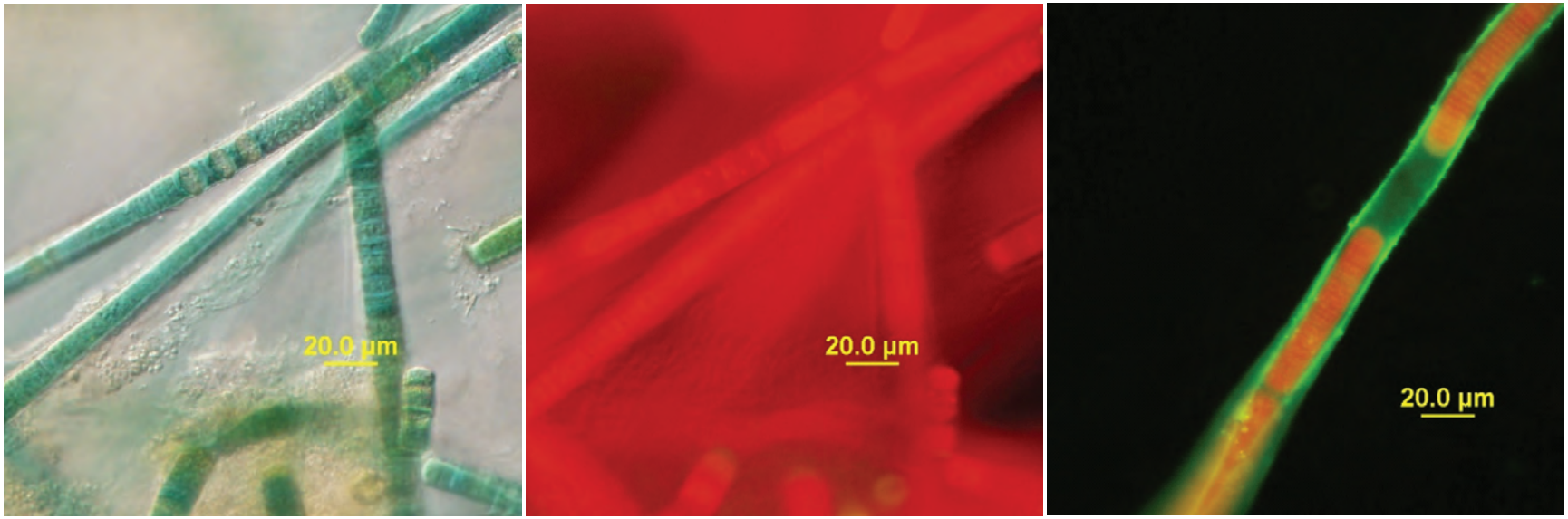


Figure 168. Laboratory culture-*Lyngbya* DVL 1103B. LM-*Lyngbya* sp. Note: the color is dominated by phycocyanin. FITC-a red color dominates the cells. Sytox® green-stain was picked up by the sheath but not the cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

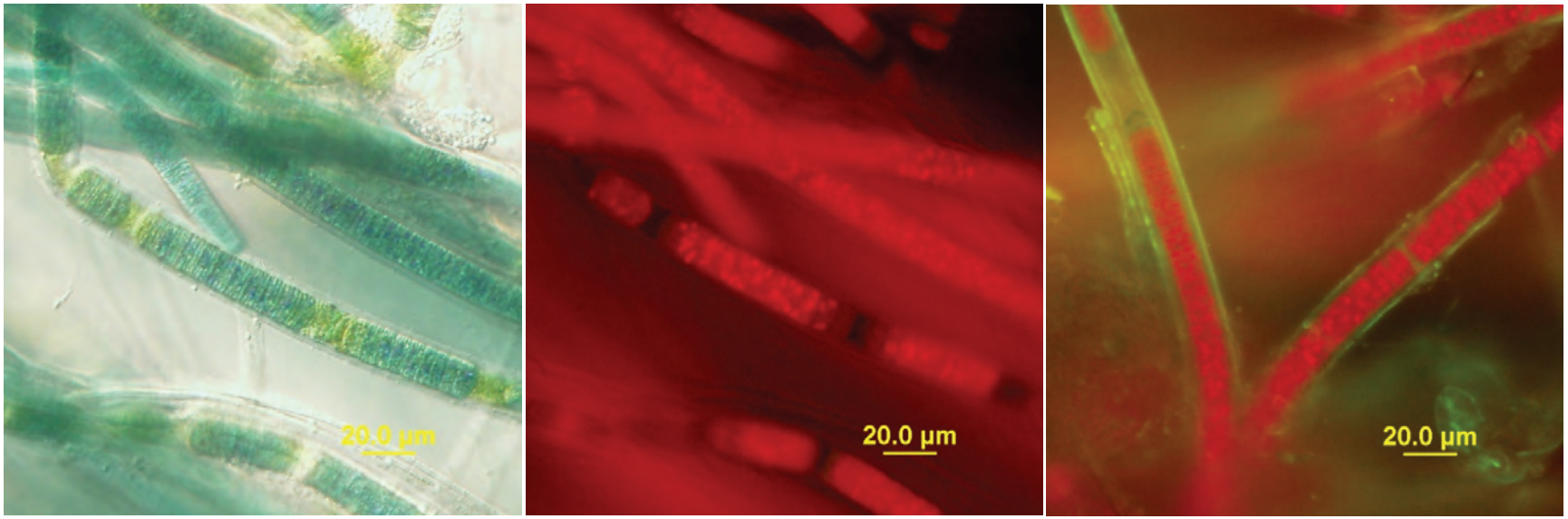


Figure 169. Laboratory culture-*Lyngbya* DVL 1103B. LM-*Lyngbya* sp. FITC-a red color dominates the cells. Note: cellular material appears to clump into particles. Sytox® green-stain was picked up by the sheath but not the cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

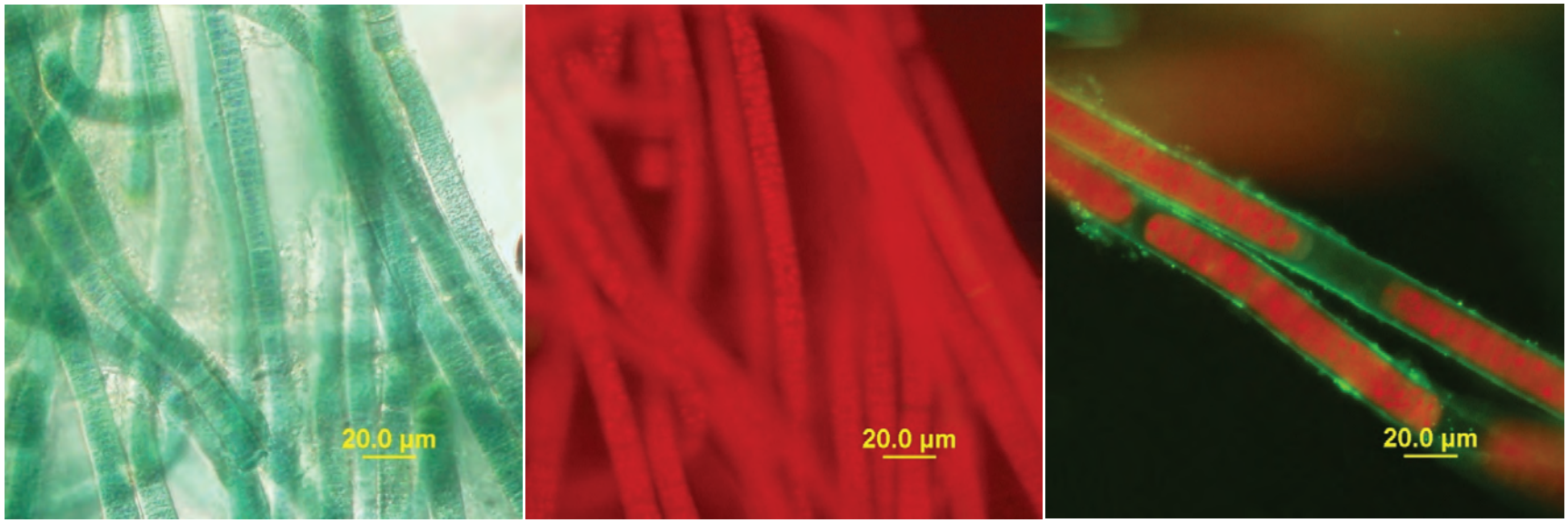


Figure 170. Laboratory culture-*Lyngbya* DVL 1103B. LM-*Lyngbya* sp. FITC-a red color dominates the cells. Note: cellular material appears to clump into particles. Sytox® green-stain was picked up by the sheath but not the cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

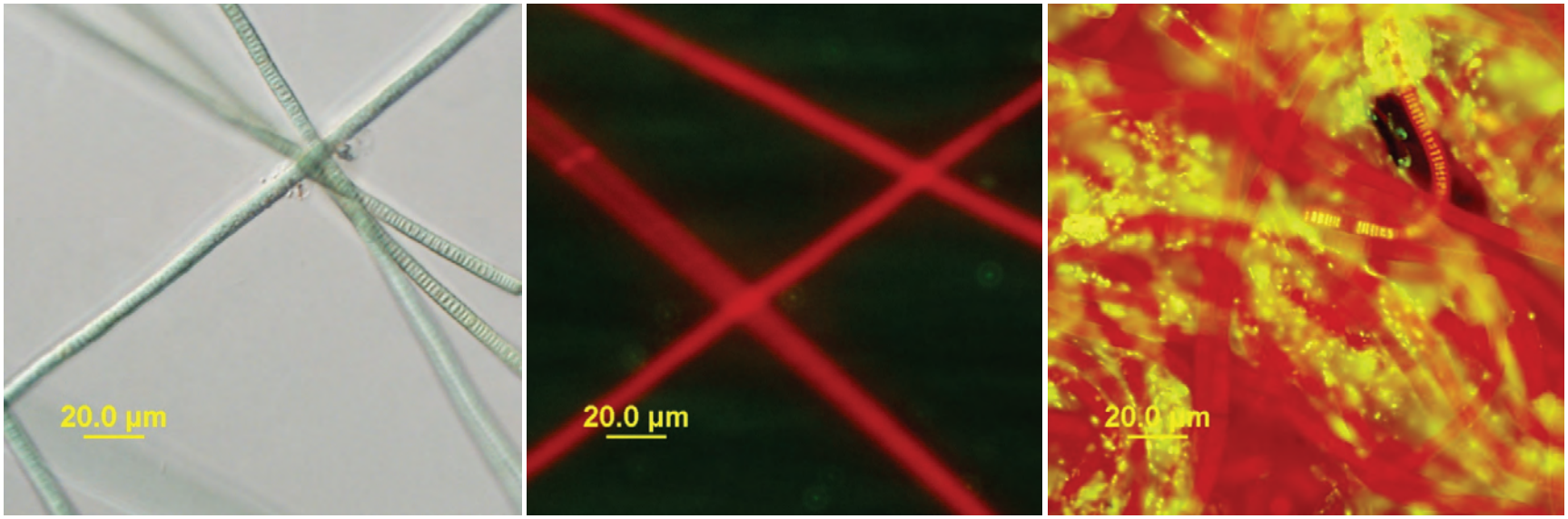


Figure 171. Laboratory culture-*Phormidium* DVL 706A. LM-*Phormidium* sp. is a filamentous cyanobacterium which may have a thin, mucilaginous sheath. Some species have no sheath. FITC-a red color dominates the cells. Sytox® green-stain was picked up by the extracellular matrix and a few cells. These cultures were not controlled for age; each culture has a mix of filaments that range from actively dividing to senescence. Bacteria appear as bright green dots on the mucilage. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

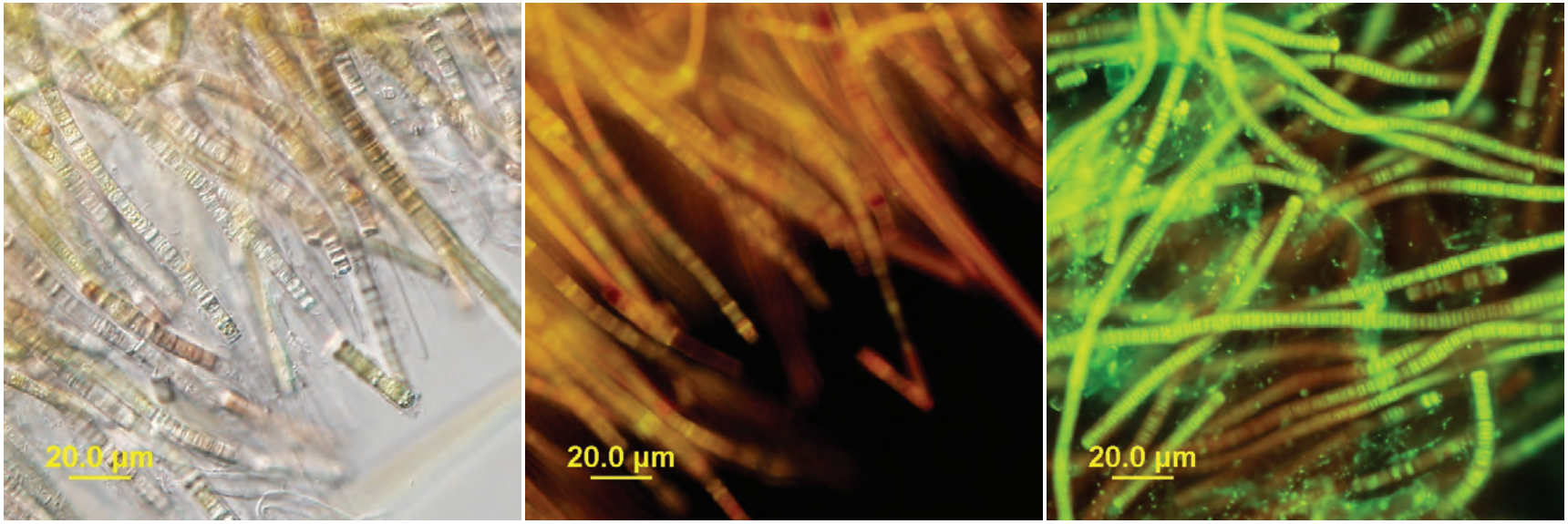


Figure 172. Laboratory culture-*Phormidium* DVL 706A. LM-*Phormidium* sp. FITC-an orange color dominates the cells. Sytox® green-stain penetrated, indicating the cell membrane was disrupted; bright green cells. Some of the filaments did not have any cells that stained, or only had some of the cells in the filament that stained. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

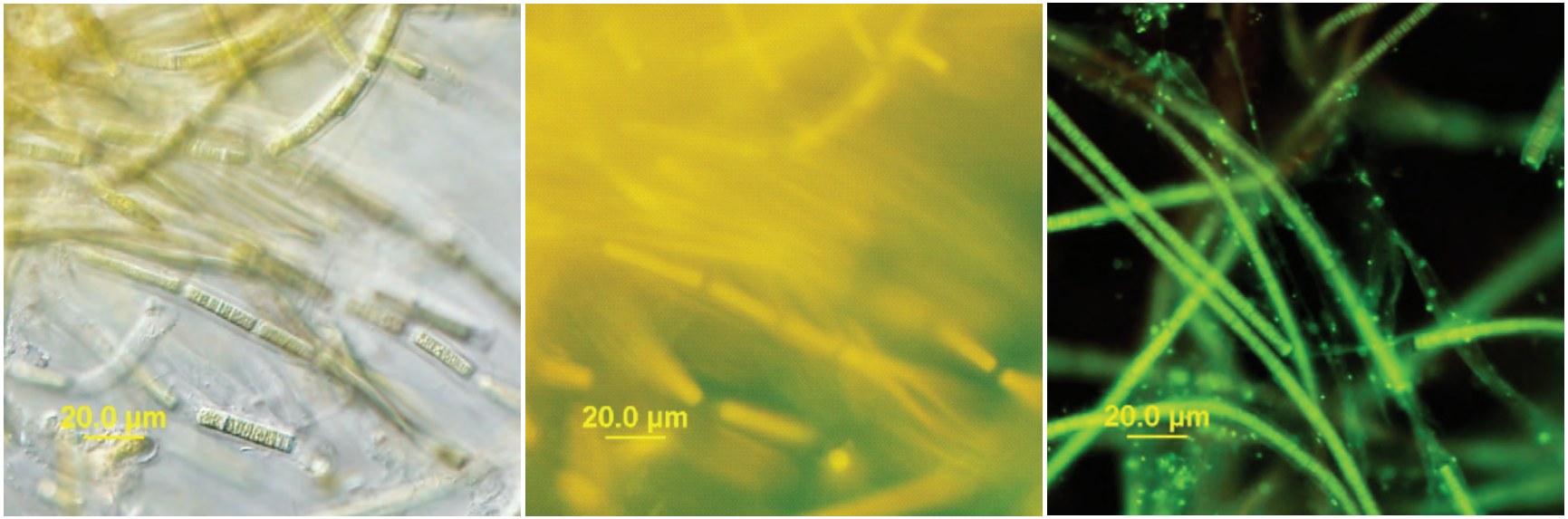


Figure 173. Laboratory culture-*Phormidium* DVL 706A. LM-*Phormidium* sp. FITC-a yellow-orange color dominates the cells. Sytox® green-stain penetrated, indicating the cell membrane was disrupted; bright green cells. Some of the filaments did not have any cells that stained, or only had some of the cells in the filament that stained. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

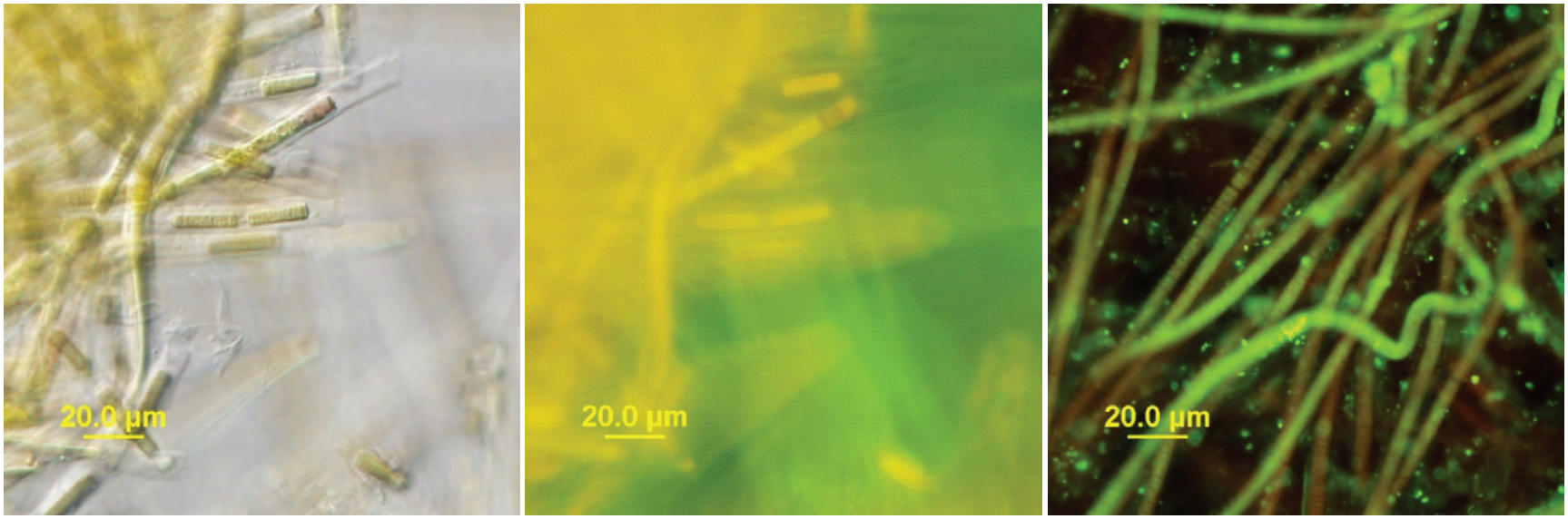


Figure 174. Laboratory culture-*Phormidium* DVL 706A. LM-*Phormidium* sp. FITC-a yellow-orange color dominates the cells. Sytox® green-stain penetrated, indicating the cell membrane was disrupted; bright green cells. Some of the filaments did not have any cells that stained, or only had some of the cells in the filament that stained. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

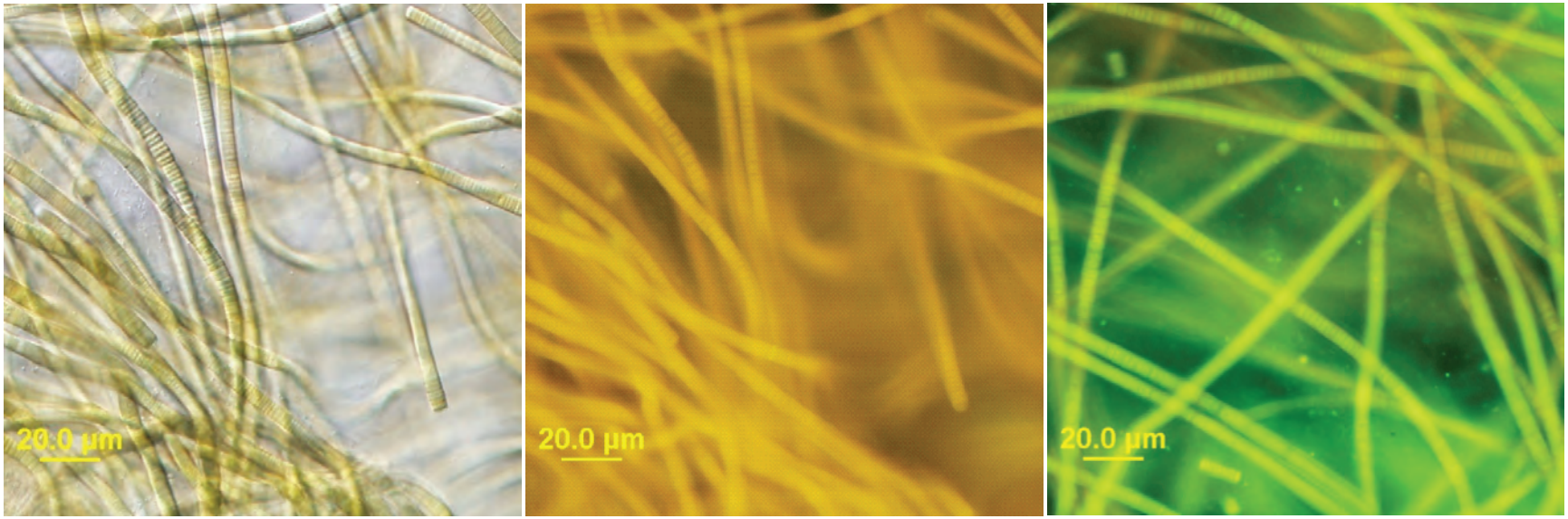


Figure 175. Laboratory culture-*Phormidium* DVL 706A. LM-*Phormidium* sp. FITC-a yellow-orange color dominates the cells. Sytox® green-stain penetrated, indicating the cell membrane was disrupted; yellow-green cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

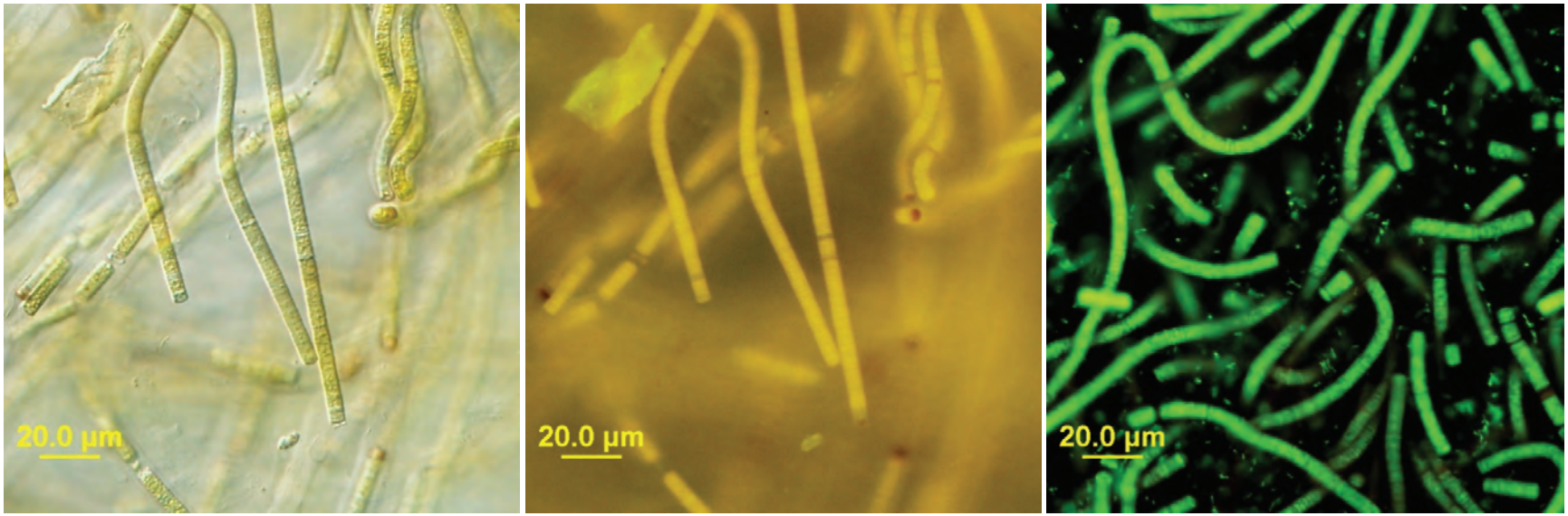


Figure 176. Laboratory culture-*Phormidium* DVL 706A. LM-*Phormidium* sp. FITC-a yellow-orange color dominates the cells. Sytox® green-stain penetrated, indicating the cell membrane was disrupted; bright green cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

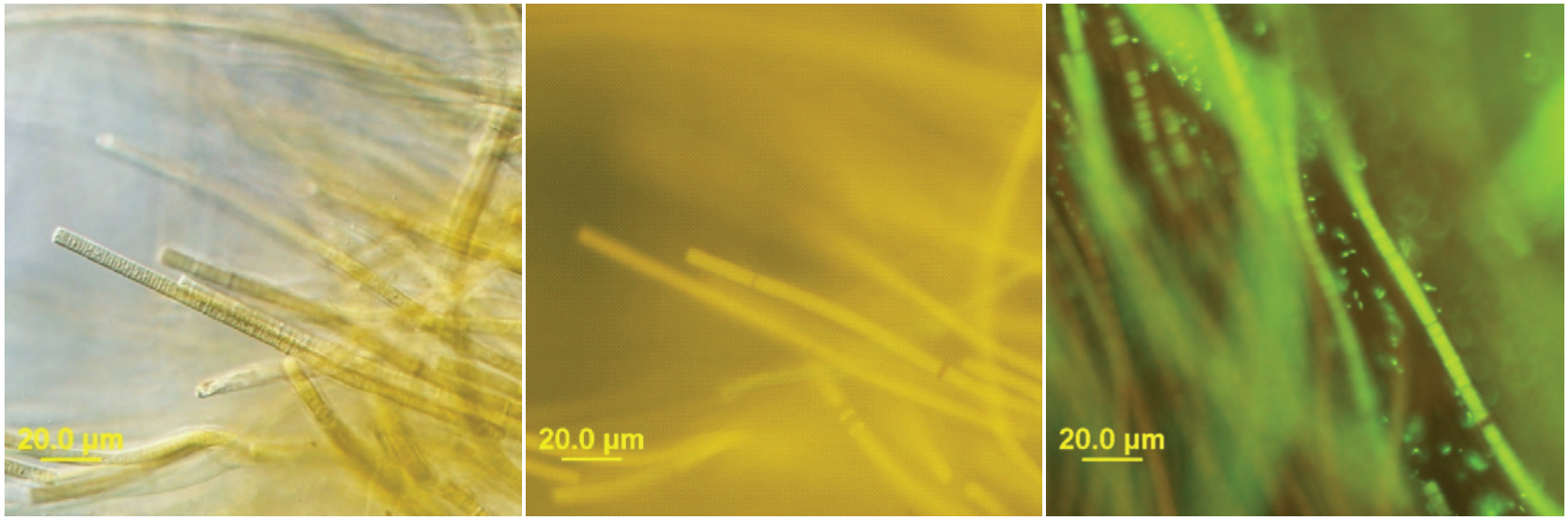


Figure 177. Laboratory culture-*Phormidium* DVL 706A. LM-*Phormidium* sp. FITC-a yellow-orange color dominates the cells. Sytox® green-stain penetrated, indicating the cell membrane was disrupted; bright green cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

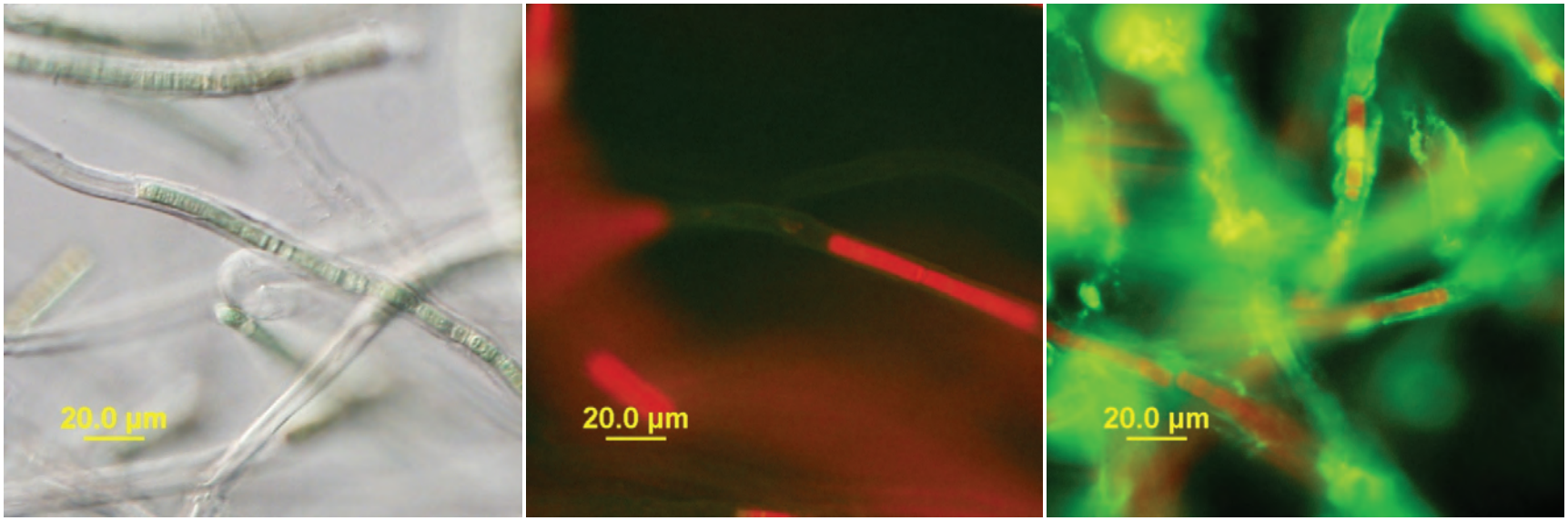


Figure 178. Laboratory culture-*Phormidium* DVL 706A. LM-*Phormidium* sp. FITC-a red color dominates the cells. Sytox® green-stained the sheath but not the cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

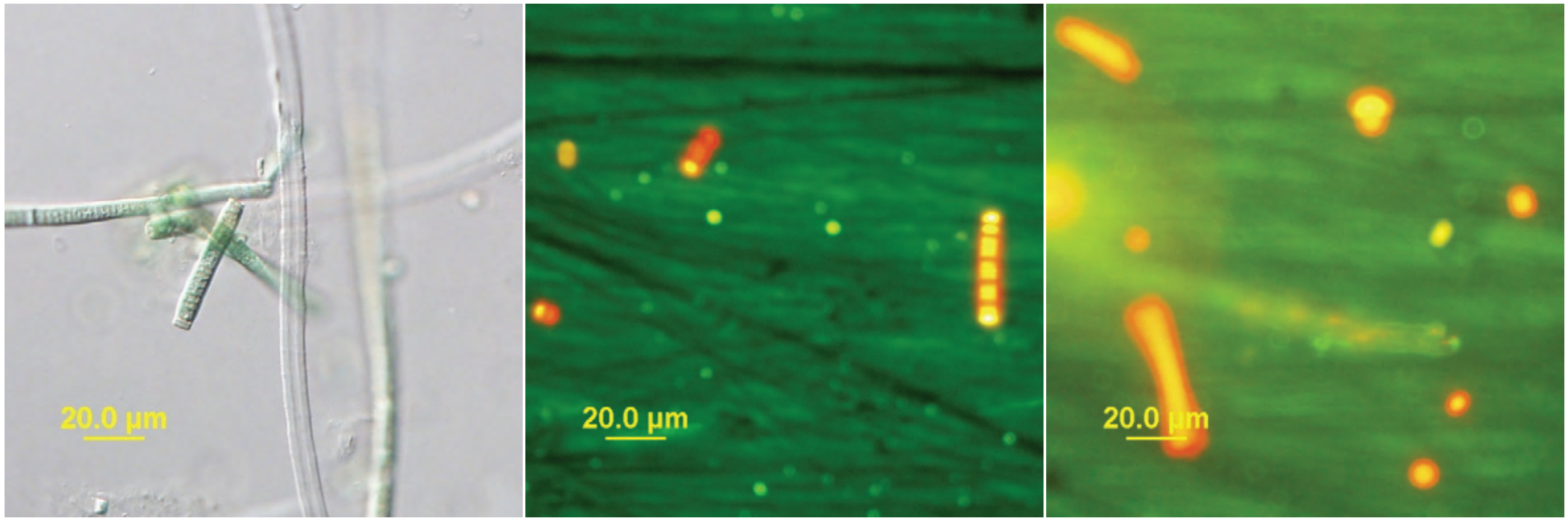
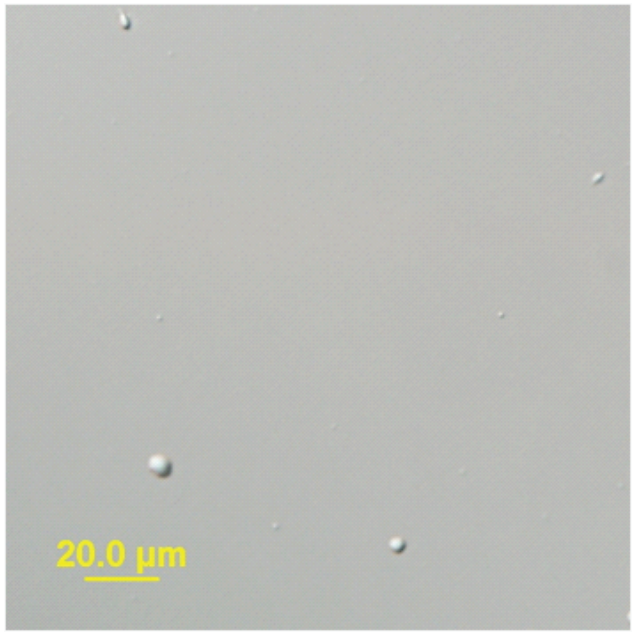


Figure 179. Laboratory culture-*Phormidium* DVL 706A. LM-*Lyngbya* sp. Note: the sheath appears to be separated from the cells. FITC-an orange color dominates the cells. Sytox® green-did not penetrate the cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.



LM-nothing discernable

Sonicated at 70 percent power

Figure 180. Laboratory culture-*Phormidium* DVL 706A. LM-Nothing discernable. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

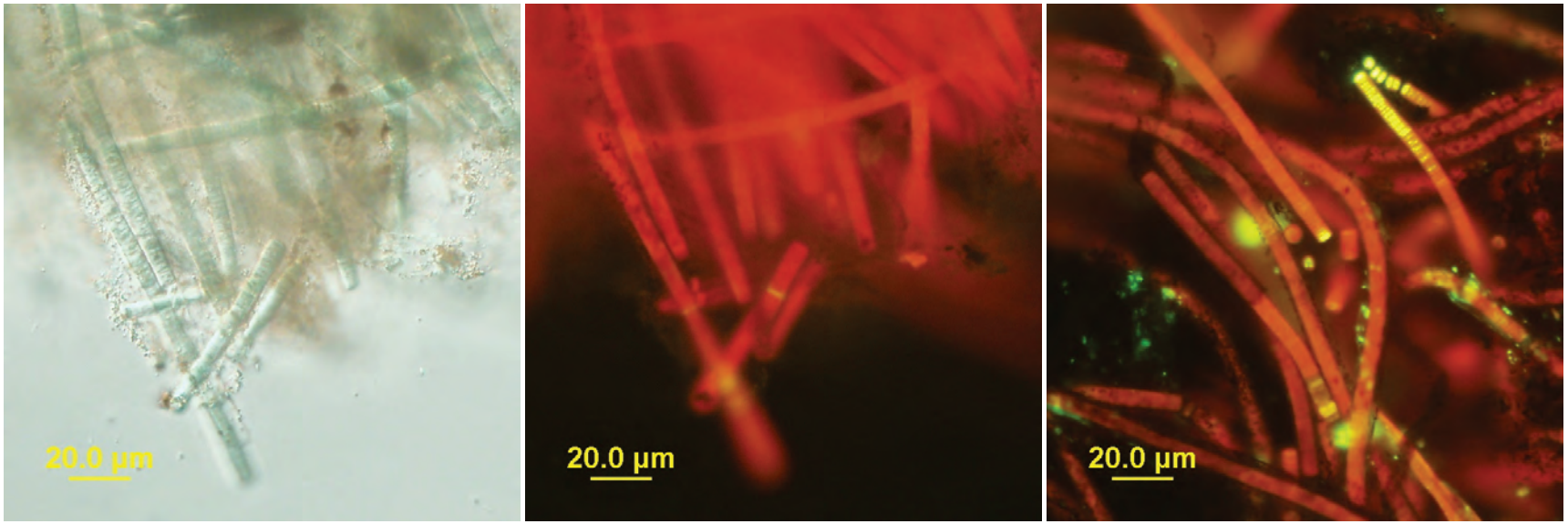


Figure 181. Laboratory culture-*Phormidium* DVL 706A. LM-*Phormidium* sp. FITC-a red color dominates the cells. Sytox® green-stain penetrated some cells, indicating the cell membrane was disrupted; bright green cells. Some of the filaments only had some of the cells in the filament that stained. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

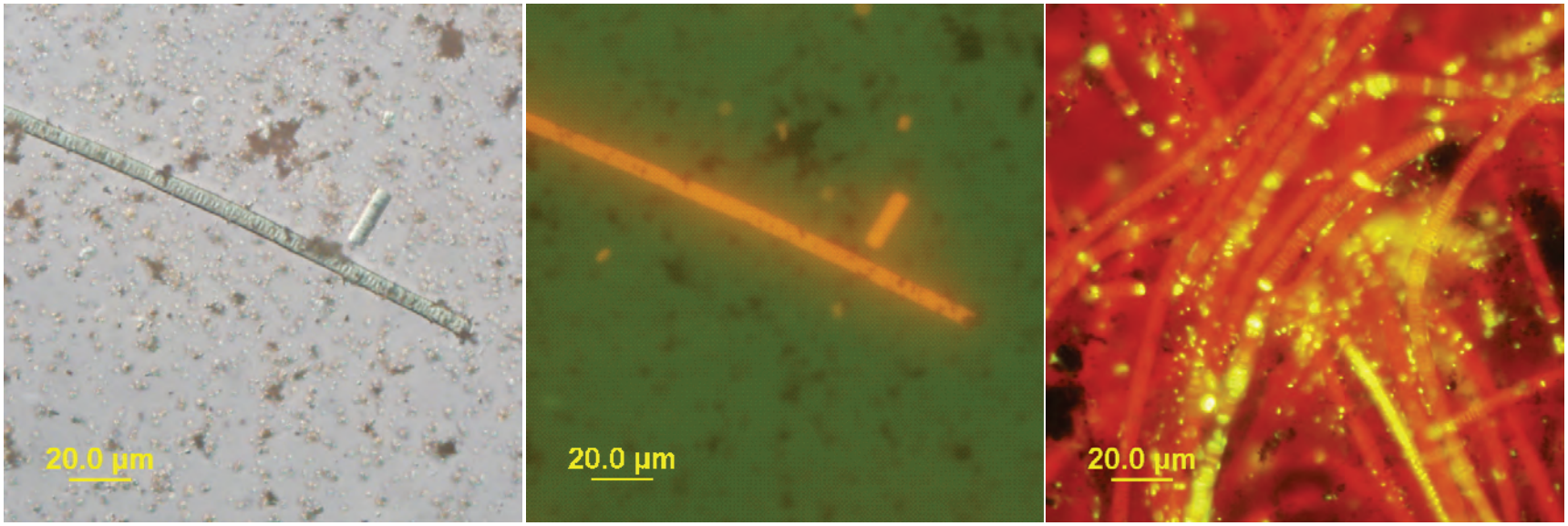


Figure 182. Laboratory culture-*Phormidium* DVL 706A. LM-*Phormidium* sp. FITC-an orange color dominates the cells. Sytox® green-stain penetrated some cells, indicating the cell membrane was disrupted; bright green cells. Some of the filaments only had some of the cells in the filament that stained. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

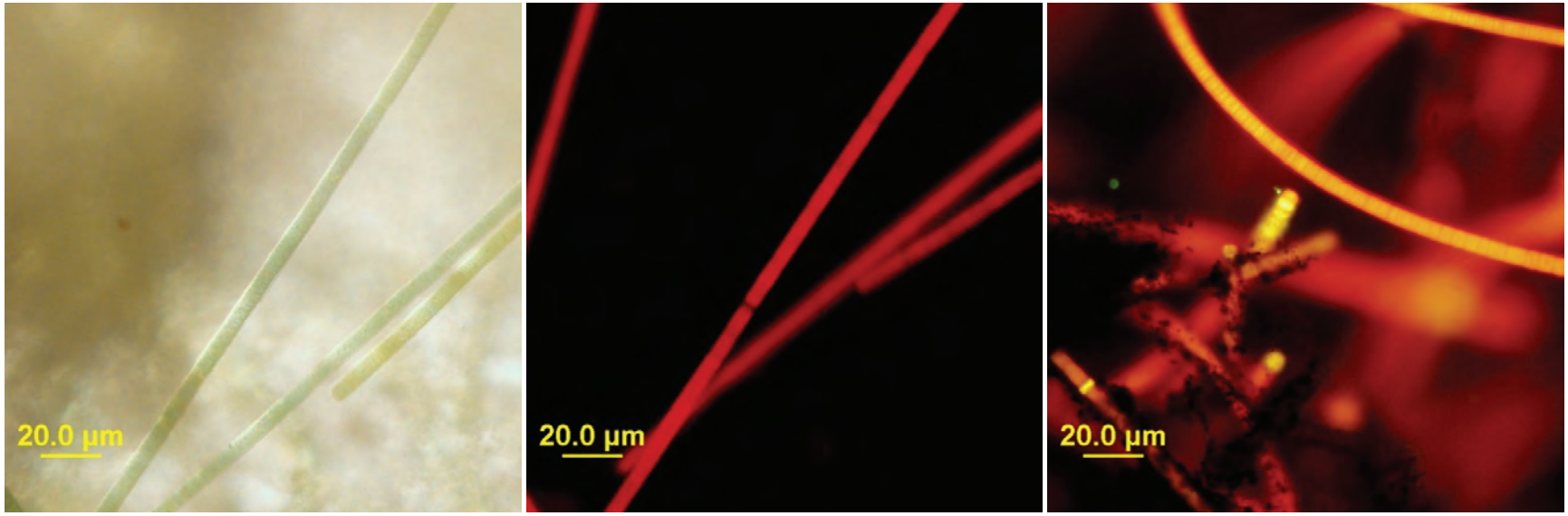


Figure 183. Laboratory culture-*Phormidium* DVL 706A. LM-*Phormidium* sp. FITC-a red color dominates the cells. Sytox® green-stain penetrated some cells, indicating the cell membrane was disrupted; bright green cells. Some of the filaments only had some of the cells in the filament that stained. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

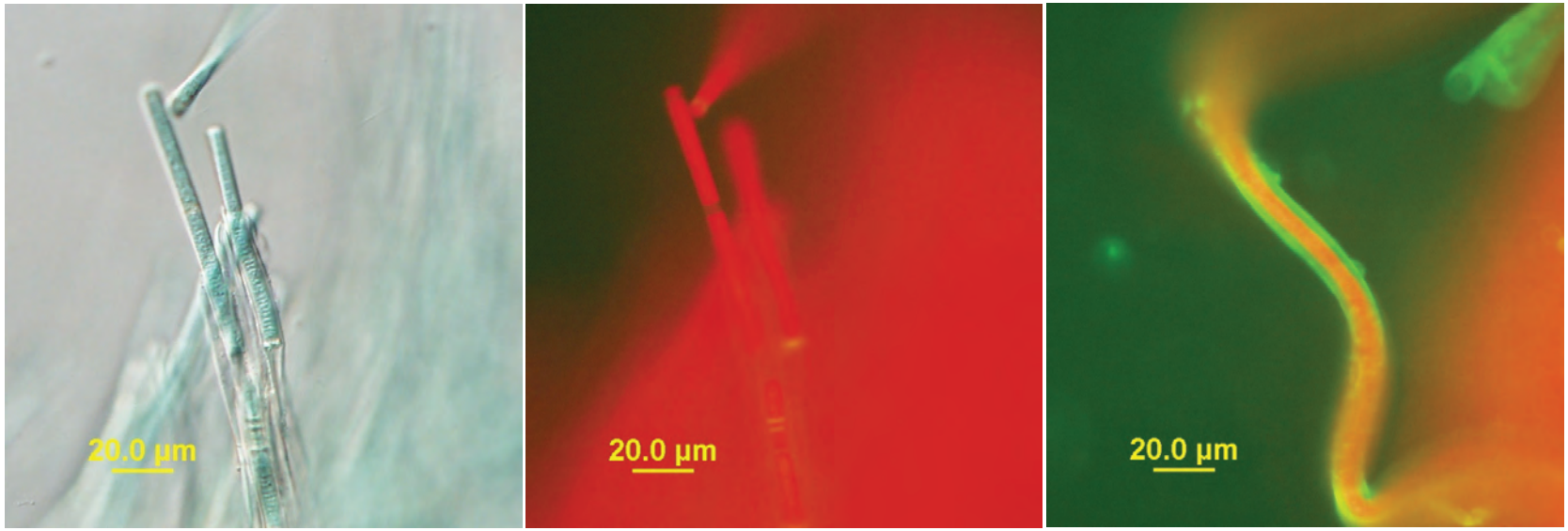


Figure 184. Laboratory culture-*Phormidium* DVL 706A. LM-*Phormidium* sp. FITC-a red color dominates the cells. Sytox® green-stain was picked up by the sheath but not the cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

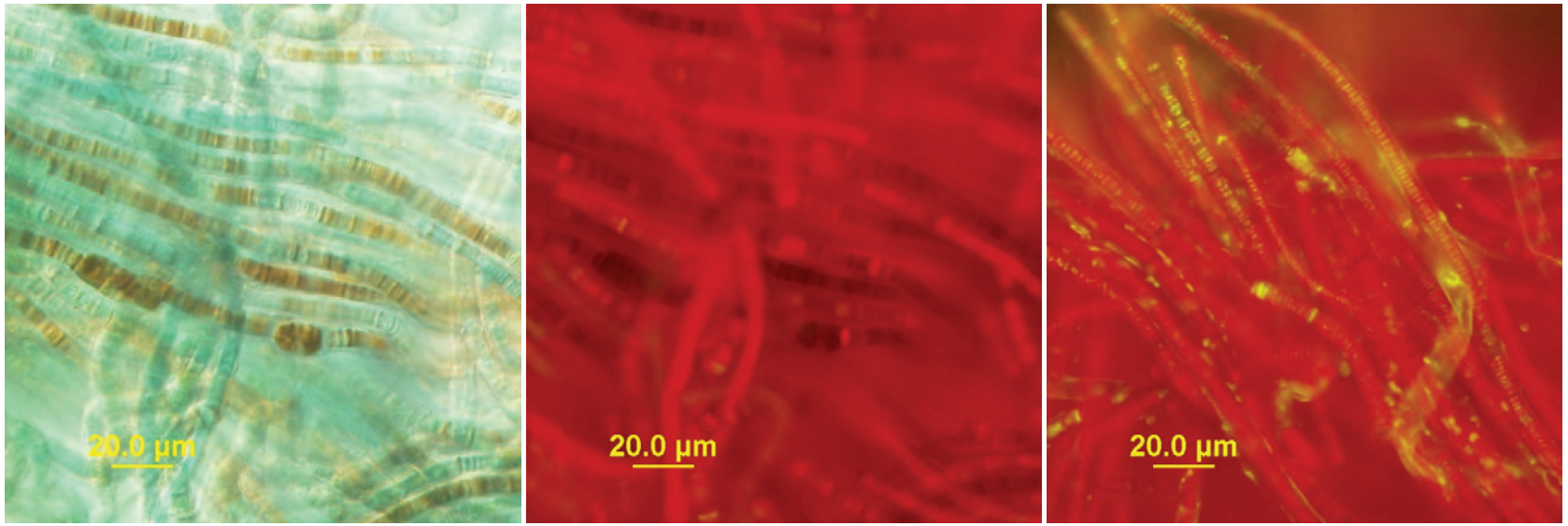


Figure 185. Laboratory culture-*Phormidium* DVL 706A. LM-*Phormidium* sp. FITC-a red color dominates the cells. Sytox® green-stain penetrated some cells, indicating the cell membrane was disrupted; bright green cells. Some of the filaments only had some of the cells in the filament that stained. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

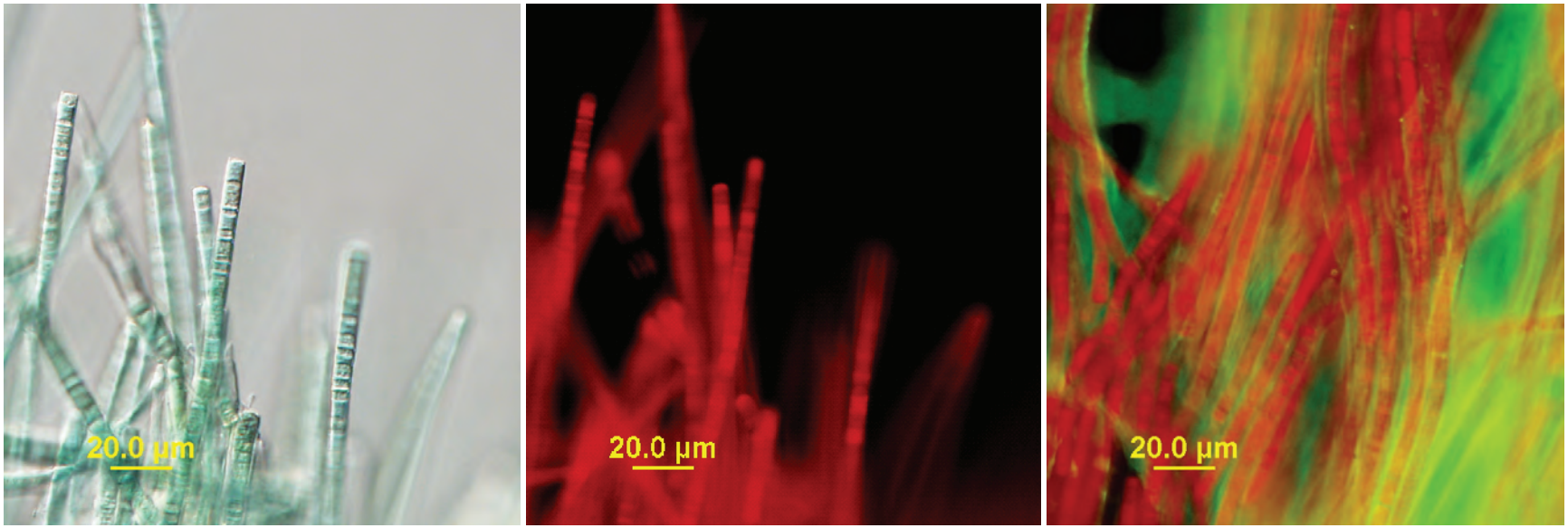


Figure 186. Laboratory culture-*Phormidium* DVL 706A. LM-*Phormidium* sp. FITC-a red color dominates the cells. Sytox® green-stain was picked up by the sheath but not the cells. LM – differential interference contrast microscopy; FITC – epifluorescent microscopy; Sytox® green – epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green.

Table 4. Summary of observations compiled from digital microphotographs of multiple cyanobacteria dominated samples exposed to five different cell-y techniques.

[CA, California; FL, Florida; IA, Iowa; OH, Ohio; OR, Oregon; St., Saint; FITC, epifluorescence microscopy; %, percent; x, when used after a number indicates the number of times a treatment was conducted (for example, 2x represents 2 times); min, minutes]

		Physical observation	FITC color	Sytox® green
Cassidy Lake, WA (10/12/2009)[1]				
Control	1	intact	red	no staining
	2	intact	yellow-orange	no staining
	3	intact	yellow-orange	no staining
Boiled	5 min	single cells	yellow-orange	bright green
	15 min	single cells	yellow-orange	bright green
	30 min	colonies degraded	yellow	no staining
Autoclaved	5 min	colonies degraded	orange	no staining
	15 min	single cells	yellow-orange	no staining
	30 min	single cells	yellow-orange	bright green
Sonicated	10%	intact	red	no staining
	35%	colonies degraded	red-orange	no staining
	70%	single cells	orange	no staining
QuikLyse™	1x	no sample	no sample	no sample
	2x	no sample	no sample	no sample
	3x	no sample	no sample	no sample
Freeze-thaw	1x	single cells	no data	bright green
	2x	no sample	no sample	no sample
	3x	single cells	red-orange	bright green
Spring Lake, CA (8/21/2009)[1]				
Control	1	intact	orange	no staining
	2	intact	yellow-orange	no staining
	3	intact	yellow-orange	no staining
Boiled	5 min	colonies degraded	orange	no data
	15 min	single cells	orange	bright green
	30 min	single cells	orange	bright green
Autoclaved	5 min	single cells	orange	bright green
	15 min	colonies degraded	orange	bright green
	30 min	colonies degraded	yellow-orange	bright green
Sonicated	10%	intact	orange	no staining
	35%	colonies degraded	orange	no staining
	70%	single cells	orange	no staining
QuikLyse™	1x	single cells	yellow	bright green
	2x	intact	yellow	no staining
	3x	no sample	no sample	no sample
Freeze-thaw	1x	colonies degraded	yellow	no staining
	2x	colonies degraded	yellow	no staining
	3x	intact	orange	no staining

Table 4. Summary of observations compiled from digital microphotographs of multiple cyanobacteria dominated samples exposed to five different cell-y techniques.—Continued

[CA, California; FL, Florida; IA, Iowa; OH, Ohio; OR, Oregon; St., Saint; FITC, epifluorescence microscopy; %, percent; x, when used after a number indicates the number of times a treatment was conducted (for example, 2x represents 2 times); min, minutes]

		Physical observation	FITC color	Sytox® green
Blackhawk Lake, IA (8/26/2009)[1]				
Control	1	intact	red	no staining
	2	intact	red-orange	no data
	3	intact	red-orange	no staining
Boiled	5 min	intact	red-orange	no staining
	15 min	no sample	no sample	no sample
	30 min	intact	yellow-orange	no staining
Autoclaved	5 min	intact	orange	bright green
	15 min	intact	orange	no staining
	30 min	intact	yellow-orange	partial
Sonicated	10%	intact	red-orange	no staining
	35%	filaments fragmented	yellow	no staining
	70%	cells destroyed	no data	no data
QuikLyse™	1x	intact	orange	bright green
	2x	no sample	no sample	no sample
	3x	no sample	no sample	no sample
Freeze-thaw	1x	intact	red-orange	no staining
	2x	colonies degraded	yellow	no staining
	3x	intact	yellow	no staining
Copco Reservoir, CA (9/10/2009)[1]				
Control	1	intact	orange	no staining
	2	intact	red-orange	no staining
	3	intact	orange	no staining
Boiled	5 min	intact	yellow	sheath only
	15 min	intact	yellow	sheath only
	30 min	colonies degraded	yellow	sheath only
Autoclaved	5 min	colonies degraded	orange	partial
	15 min	no sample	no sample	no sample
	30 min	no sample	no sample	no sample
Sonicated	10%	intact	red	no staining
	35%	intact	red-orange	no staining
	70%	intact	orange	sheath only
QuikLyse™	1x	intact	red-orange	sheath only
	2x	no sample	no sample	no sample
	3x	no sample	no sample	no sample
Freeze-thaw	1x	intact	orange	sheath only
	2x	colonies degraded	orange	sheath only
	3x	colonies degraded	yellow-orange	no staining

Table 4. Summary of observations compiled from digital microphotographs of multiple cyanobacteria dominated samples exposed to five different cell-y techniques.—Continued

[CA, California; FL, Florida; IA, Iowa; OH, Ohio; OR, Oregon; St., Saint; FITC, epifluorescence microscopy; %, percent; x, when used after a number indicates the number of times a treatment was conducted (for example, 2x represents 2 times); min, minutes]

		Physical observation	FITC color	Sytox® green
Grand Lake (Lake St. Mary), OH (7/20/2009)[1]				
Control	1	intact	orange	no staining
	2	intact	orange	no staining
	3	intact	orange	no staining
Boiled	5 min	intact	orange	no staining
	15 min	intact	orange	no staining
	30 min	intact	orange	bright green
Autoclaved	5 min	intact	orange	partial
	15 min	intact	orange	partial
	30 min	no data	orange	no staining
Sonicated	10%	intact	red	no staining
	35%	filaments fragmented	orange	no staining
	70%	cells destroyed	no data	no data
QuikLyse™	1x	intact	yellow-orange	bright green
	2x	intact	red	bright green
	3x	no sample	no sample	no sample
Freeze-thaw	1x	intact	orange	no data
	2x	colonies degraded	orange	bright green
	3x	intact	orange	no staining
Grand Lake (Lake St. Mary), OH (9/15/2009)[1]				
Control	1	intact	orange	no staining
	2	intact	orange	no staining
	3	intact	orange	no staining
Boiled	5 min	intact	orange	bright green
	15 min	intact	orange	no staining
	30 min	intact	orange	partial
Autoclaved	5 min	intact	orange	no staining
	15 min	intact	yellow	partial
	30 min	intact	yellow	bright green
Sonicated	10%	intact	orange	no staining
	35%	intact	red	no staining
	70%	cells destroyed	no data	no data
QuikLyse™	1x	intact	orange	no staining
	2x	no sample	no sample	no sample
	3x	no sample	no sample	no sample
Freeze-thaw	1x	colonies degraded	orange	no staining
	2x	colonies degraded	orange	no staining
	3x	colonies degraded	orange	no data

Table 4. Summary of observations compiled from digital microphotographs of multiple cyanobacteria dominated samples exposed to five different cell-y techniques.—Continued

[CA, California; FL, Florida; IA, Iowa; OH, Ohio; OR, Oregon; St., Saint; FITC, epifluorescence microscopy; %, percent; x, when used after a number indicates the number of times a treatment was conducted (for example, 2x represents 2 times); min, minutes]

		Physical observation	FITC color	Sytox® green
St. John's River, Jacksonville, FL (7/28/2009)[1]				
Control	1	intact	red	no staining
	2	intact	red	no staining
	3	intact	red	no staining
Boiled	5 min	colonies degraded	orange	bright green
	15 min	single cells	orange	bright green
	30 min	single cells	orange	bright green
Autoclaved	5 min	colonies degraded	orange	bright green
	15 min	colonies degraded	yellow	partial
	30 min	no sample	no sample	no sample
Sonicated	10%	intact	red	no staining
	35%	intact	orange	no staining
	70%	cells destroyed	no data	no data
QuikLyse™	1x	intact	red	no staining
	2x	intact	red	partial
	3x	no sample	no sample	no sample
Freeze-thaw	1x	colonies degraded	yellow-orange	partial
	2x	colonies degraded	yellow-orange	partial
	3x	colonies degraded	yellow-orange	no data
Upper Klamath Lake, OR (8/21/2009)[1]				
Control	1	intact	yellow-orange	no data
	2	intact	red-orange	sheath only
	3	filaments degraded	red	partial
Boiled	5 min	colonies degraded	yellow-orange	no staining
	15 min	intact	orange	partial
	30 min	intact	no data	bright green
Autoclaved	5 min	intact	orange	bright green
	15 min	intact	yellow	partial
	30 min	intact	yellow	partial
Sonicated	10%	intact	red-orange	sheath only
	35%	single cells	no data	no data
	70%	cells destroyed	no data	no data
QuikLyse™	1x	intact	orange	partial
	2x	no sample	no sample	no sample
	3x	no sample	no sample	no sample
Freeze-thaw	1x	filaments degraded	no data	no data
	2x	no sample	no sample	no sample
	3x	intact	yellow	bright green

Table 4. Summary of observations compiled from digital microphotographs of multiple cyanobacteria dominated samples exposed to five different cell-y techniques.—Continued

[CA, California; FL, Florida; IA, Iowa; OH, Ohio; OR, Oregon; St., Saint; FITC, epifluorescence microscopy; %, percent; x, when used after a number indicates the number of times a treatment was conducted (for example, 2x represents 2 times); min, minutes]

		Physical observation	FITC color	Sytox® green
Klamath River, OR (8/21/2009)[1]				
Control	1	no sample	no sample	no sample
	2	no sample	no sample	no sample
	3	no sample	no sample	no sample
Boiled	5 min	no sample	no sample	no sample
	15 min	no sample	no sample	no sample
	30 min	no sample	no sample	no sample
Autoclaved	5 min	no sample	no sample	no sample
	15 min	no sample	no sample	no sample
	30 min	no sample	no sample	no sample
Sonicated	10%	no sample	no sample	no sample
	35%	no sample	no sample	no sample
	70%	no sample	no sample	no sample
QuikLyse™	1x	no sample	no sample	no sample
	2x	no sample	no sample	no sample
	3x	no sample	no sample	no sample
Freeze-thaw	1x	colonies degraded	yellow	no staining
	2x	colonies degraded	yellow	no staining
	3x	colonies degraded	yellow	partial
Iron Gate Reservoir, OR (8/25/2009)[1]				
Control	1	intact	red	no staining
	2	intact	red	no staining
	3	no sample	no sample	no sample
Boiled	5 min	colonies degraded	orange	bright green
	15 min	single cells	orange	bright green
	30 min	single cells	orange	bright green
Autoclaved	5 min	single cells	yellow-orange	bright green
	15 min	single cells	yellow-orange	no data
	30 min	single cells	yellow-orange	bright green
Sonicated	10%	colonies degraded	red	partial
	35%	colonies degraded	red	partial
	70%	colonies degraded	orange	partial
QuikLyse™	1x	colonies degraded	orange	no data
	2x	no sample	no sample	no sample
	3x	no sample	no sample	no sample
Freeze-thaw	1x	colonies degraded	yellow-orange	partial
	2x	colonies degraded	yellow-orange	bright green
	3x	colonies degraded	yellow	no staining

Table 4. Summary of observations compiled from digital microphotographs of multiple cyanobacteria dominated samples exposed to five different cell-y techniques.—Continued

[CA, California; FL, Florida; IA, Iowa; OH, Ohio; OR, Oregon; St., Saint; FITC, epifluorescence microscopy; %, percent; x, when used after a number indicates the number of times a treatment was conducted (for example, 2x represents 2 times); min, minutes]

		Physical observation	FITC color	Sytox® green
Pinto Lake, CA (9/22/2009)[1]				
Control	1	intact	red	no staining
	2	filaments degraded	orange	no staining
	3	intact	orange	no staining
Boiled	5 min	colonies degraded	orange	no staining
	15 min	colonies degraded	yellow	no staining
	30 min	colonies degraded	yellow	bright green
Autoclaved	5 min	colonies degraded	yellow	sheath only
	15 min	intact	yellow	no staining
	30 min	intact	yellow	bright green
Sonicated	10%	colonies degraded	red-orange	no staining
	35%	cells destroyed	no data	no data
	70%	cells destroyed	no data	no data
QuikLyse™	1x	intact	orange	no staining
	2x	no sample	no sample	no sample
	3x	no sample	no sample	no sample
Freeze-thaw	1x	colonies degraded	red	no staining
	2x	colonies degraded	yellow	no staining
	3x	no sample	no sample	no sample
Laboratory Culture- *Lyngbya*[2] sp. DVL 1103B				
Control	1	intact	red	sheath only
	2	no sample	no sample	no sample
	3	no sample	no sample	no sample
Boiled	5 min	filaments degraded	yellow	bright green
	15 min	filaments degraded	yellow-orange	partial
	30 min	filaments degraded	yellow-orange	bright green
Autoclaved	5 min	filaments degraded	yellow-orange	sheath only
	15 min	filaments degraded	yellow-orange	partial
	30 min	filaments degraded	yellow-orange	partial
Sonicated	10%	filaments fragmented	red	no staining
	35%	filaments fragmented	no data	no data
	70%	no sample	no sample	no sample
QuikLyse™	1x	intact	red	partial
	2x	intact	red	partial
	3x	intact	red	partial
Freeze-thaw	1x	intact	red	sheath only
	2x	intact	red	sheath only
	3x	intact	red	sheath only

Table 4. Summary of observations compiled from digital microphotographs of multiple cyanobacteria dominated samples exposed to five different cell-y techniques.—Continued

[CA, California; FL, Florida; IA, Iowa; OH, Ohio; OR, Oregon; St., Saint; FITC, epifluorescence microscopy; %, percent; x, when used after a number indicates the number of times a treatment was conducted (for example, 2x represents 2 times); min, minutes]

		Physical observation	FITC color	Sytox® green
Laboratory Culture- *Phormidium*[2] sp. DVL 706A				
Control	1	intact	red	partial
	2	no sample	no sample	no sample
	3	no sample	no sample	no sample
Boiled	5 min	filaments degraded	yellow-orange	bright green
	15 min	filaments degraded	yellow-orange	bright green
	30 min	filaments degraded	yellow-orange	bright green
Autoclaved	5 min	intact	yellow-orange	bright green
	15 min	intact	yellow-orange	bright green
	30 min	intact	yellow-orange	bright green
Sonicated	10%	filaments degraded	red	sheath only
	35%	filaments degraded	orange	no staining
	70%	cells destroyed	no data	no data
QuikLyse™	1x	intact	red	partial
	2x	intact	orange	partial
	3x	intact	red	partial
Freeze-thaw	1x	filaments degraded	red	sheath only
	2x	filaments degraded	red	partial
	3x	filaments degraded	red	sheath only

[1]Sample collection date noted parenthetically after each sample location.

[2] *Lyngya* DVL 1103B and *Phormidium* DVL 706A are cultures transferred from original samples acquired in study by Izaguirre and Taylor, 2004.

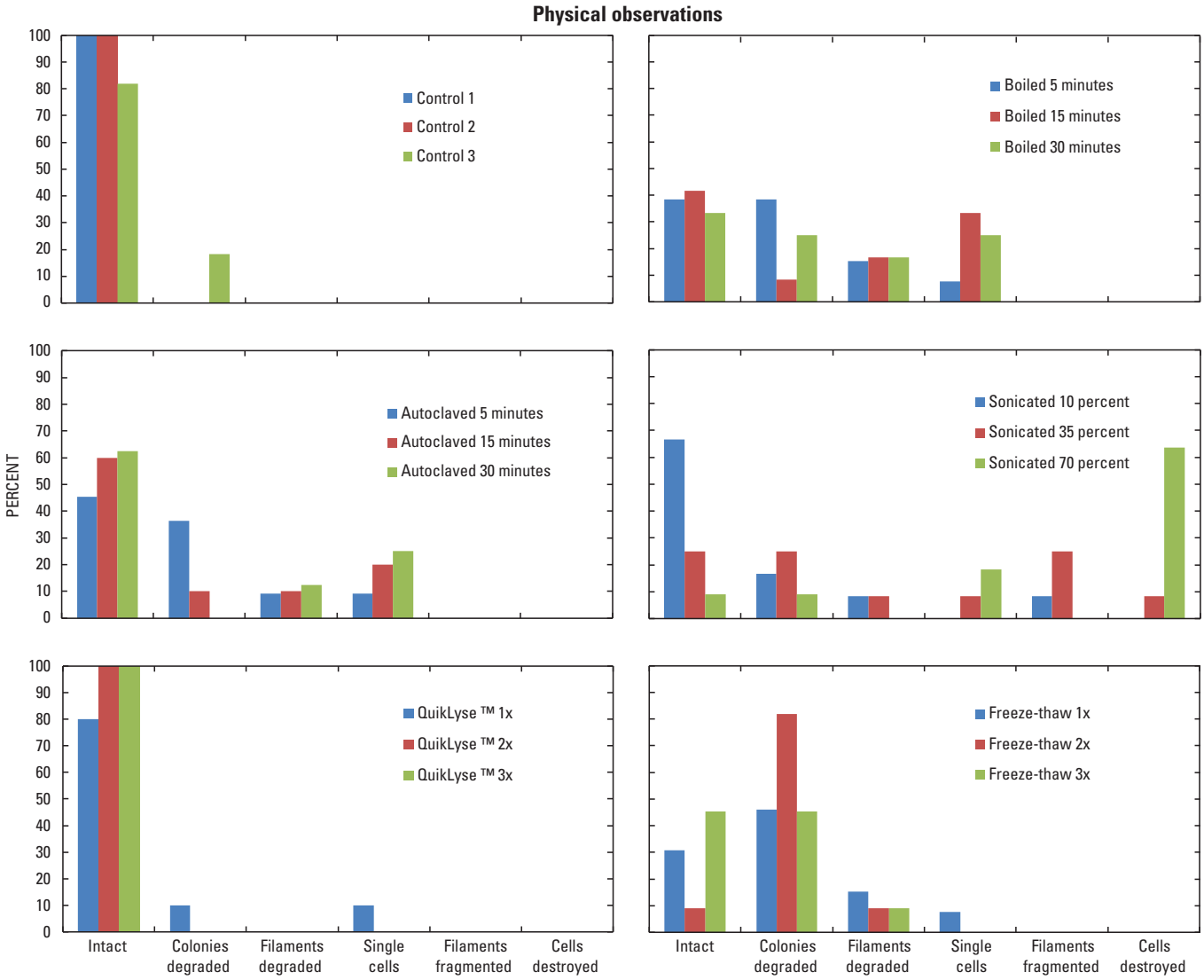


Figure 187. Summary of physical observation of cyanobacterial cell condition, epifluorescent microscopy (FITC), and epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green (Sytox® green staining) as a percentage of the total when results of all environmental samples are combined within each cell-lysis treatment.

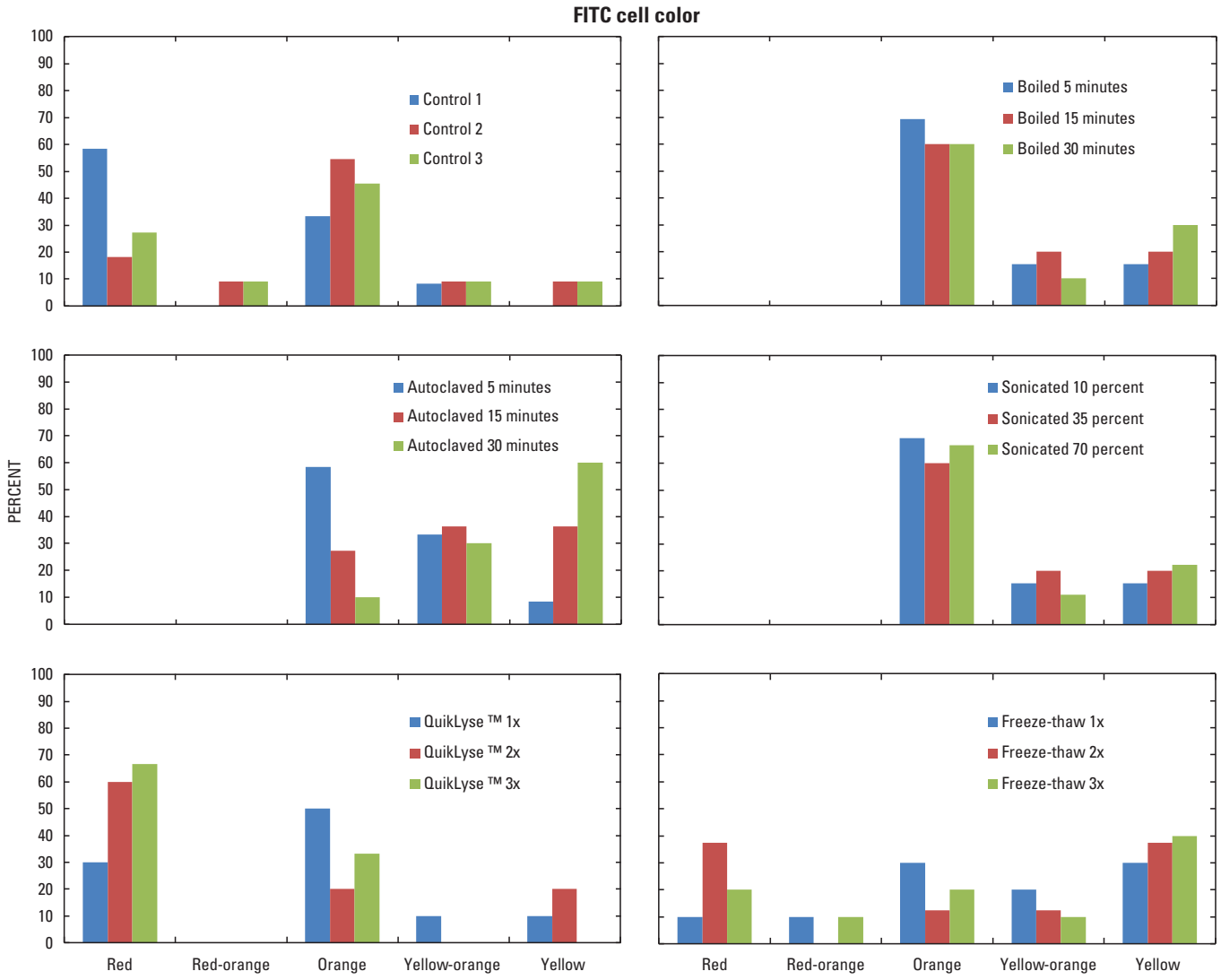


Figure 187. Summary of physical observation of cyanobacterial cell condition, epifluorescent microscopy (FITC), and epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green (Sytox® green staining) as a percentage of the total when results of all environmental samples are combined within each cell-lysis treatment.—Continued

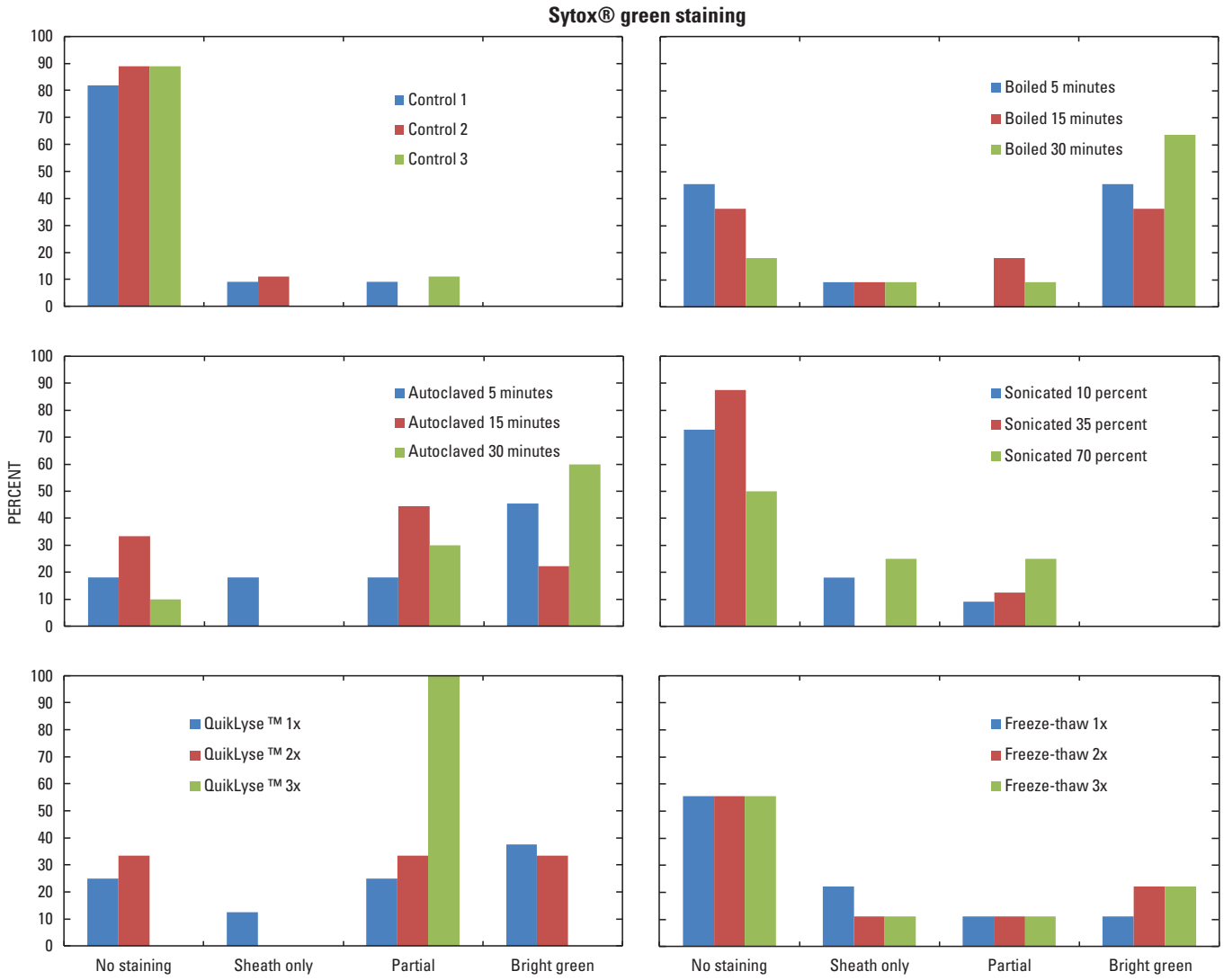


Figure 187. Summary of physical observation of cyanobacterial cell condition, epifluorescent microscopy (FITC), and epifluorescent microscopy in conjunction with the nucleic acid stain Sytox® green (Sytox® green staining) as a percentage of the total when results of all environmental samples are combined within each cell-lysis treatment.—Continued

Results

A summary of digital photomicrographs made for a given site as a function of cell-lysis technique and treatment level is represented in table 3. Digital photomicrographs of cyanobacteria are shown in figures 1 through 186 separated by site location. Images of a given cyanobacteria genus within a sample were collected by light microscopy (LM), epifluorescence (FITC), and Sytox® green stained cells by epifluorescence (Sytox® green) where complete cell destruction did not occur. A summary of observations made independent of cyanobacterial species for each sample site as a function of cell-lysis technique, treatment level and microscopic imaging technique used is represented in table 4 at the end of this report. Information in table 4 is illustrated in figure 187 by visually using bar charts independent of cyanobacteria species.

In order to summarize the results of the observed changes in the cyanobacteria because of cell lysis in all of the digital microphotographs, a simple tabulation of the observations was compiled as illustrated in figure 187. Data was categorized on the basis of physical changes to the colonies or filaments observed by LM, changes in the color of the cells observed with epifluorescent microscopy FITC, and the observations after staining with Sytox® green also observed by FITC. The observations were gathered across all treatments and all samples when possible and displayed as percentage of the total available for any given treatment.

The physical changes were subcategorized into: 1) intact; 2) colonies degraded; 3) filaments degraded; 4) single cells; 5) filaments fragmented; and 6) cells destroyed. The first subcategory, intact, indicated no change from the controls; subcategories 2 and 3 indicate that the samples, which contained either filaments or colonies of cyanobacteria, were partially degraded relative to subcategory 1, but not as severely degraded as subcategory 4 to 6. The degradation of a colony to individual cells or fragmented filaments would be less disruptive than the destruction of the cells (subcategory 6).

The epifluorescent color was subcategorized into: 1) red; 2) red-orange; 3) orange; 4) yellow-orange; and 5) yellow. Subcategory 1 and 2, are the expected colors of chlorophyll fluorescence for this setting on the microscope; subcategories 3 to 5 are indicative of degraded chlorophyll.

The Sytox® green observations were subcategorized into: 1) no staining; 2) sheath only; 3) partial (staining); and 4) bright green (staining). Subcategory 1 indicated that the cell membrane was intact and excluded the stain. Subcategory 2 appears to be a non-specific staining phenomenon that is unrelated to the treatments or extracellular genetic material. Subcategory 3 indicates that some of the cells in a treatment were stained, which indicates that the cell membrane was penetrated, while others did not. Subcategory 4 clearly indicated that cells had completely lost cell membrane integrity and stained bright green.

Control samples (table 4, figure 187) show predominately intact colonies and filaments on the basis of light microscopy, evaluation by FITC shows a distribution of cell health on the basis of red fluorescence (that is, chlorophyll), and Sytox® green staining did not reveal that genetic material was leaking from cells overall. Green fluorescence was observed in sheath material of some filamentous cyanobacteria. It is not not known from this work whether this is indicative of genetic material present in the sheath or non-specific binding to other molecules.

Of the five techniques, sonication (at 70 percent) was most effective at complete cell destruction while QuikLyse™ was least effective compared to control samples. Generally, as sonication power was increased between the three treatment levels, the percentage of cells destroyed increased. FITC results indicated that chlorophyll was degraded at all treatment levels (e.g. lack of red fluorescence). In contrast, physical observations (light microscopy) revealed little difference between control samples and those treated by QuikLyse™, however an increased number of single cells was observed after treatment indicating degradation of some colonial and filamentous cyanobacteria. Red fluorescence (that is, chlorophyll) as measured by FITC was diminished compared to controls indicating a greater proportion of chlorophyll was degraded by QuikLyse™, however increasing QuikLyse™ concentrations used above manufacturer recommendations (e.g. 2x and 3x treatment levels) did not further diminish red fluorescence in FITC results. Sytox® green staining did show more genetic material was released at the 3x treatment level though.

Autoclaving, boiling, and sequential freeze-thaw were moderately effective in physical destruction of colonies and filaments. Sequential freeze-thaw treatment appeared to have a greater or equal percentage of degraded colonies autoclaving or boiling, while sequential freeze-thaw revealed less degradation of chlorophyll. Sequential freeze-thaw also had the lowest percentage of Sytox® green staining compared to autoclaving or boiling treatments.

Acknowledgments

Funding for this study was provided by U.S. Geological Survey Toxic Substances Program and EPA DW-14-92285401. The authors are appreciative of sample collection assistance from Tim Wilheite, U.S. EPA; Linda Merchant-Masonbrink, OH EPA; Summer Burdick, Jared Botcher, and Bill Neumann, US Geological Survey; and Marisa Burghdoff, Snohomish Co. Surface Water Management.

References Cited

Abraxis, LLC, 2008, QuikLyse™ cell lysis for microcystin/nodularins ELISA microtiter plate: Product No. 529911QL, accessed May 2008 at *http://www.abraxiskits.com/moreinfo/PN529911USER.pdf*.

Chorus, I., and Bartram, J., eds., 1999, Toxic cyanobacteria in water: London, WHO, E&FN Spon, 416 p.

Google Earth 5.1, Image©2010 Digital Globe, © Google, accessed on 7/09/2010.

Hindák, F., 2006, Three planktonic cyanophytes producing water blooms in Western Slovakia: Czech Phycology, Olomouc, v. 6, p. 59–67.

Izaguirre, G. and Taylor, W.D., 2004, A guide to geosmin- and MIB-producing cyanobacteria in the United States: Water Science & Technology v. 49, no. 9, p. 19–24.

Loftin, K.A., Meyer, M.T., Rubio, F., Kamp, L., Humphries, E., Whereat, E., 2008, Comparison of two cell lysis procedures for recovery of microcystins in waters samples from Silver Lake in Dover, Delaware with microcystin producing cyanobacterial accumulations: USGS Open–File Report 2008–1341, 9 p.

Van Den Hoek, C., Mann, D.G., and Jahns, H.M., 1995, Algae, An introduction to phycology, Cambridge University Press, New York, Chap. 2.

Wehr, J.D., Sheath, R.G., eds, 2003, Freshwater algae of North America ecology and classification, A volume in the aquatic ecology series, Academic Press, Amsterdam, Chap. 3 and 4.

Zimmerman, W.J. and Rosen, B.H., 1992, Cyanobiont diversity within and among cycads of one field site: Canadian Journal of Microbiology, v. 38, no. 12, p. 1,324–1,328.

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