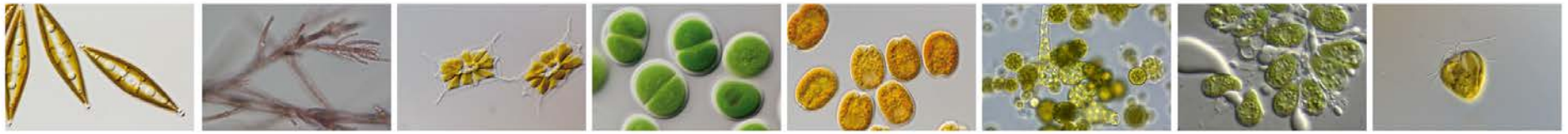

Novel Approaches to the Isolation and Maintenance of Microalgal Cultures: The CCAC Experience

C C A C
Culture Collection of Algae at the University of Cologne

Barbara Melkonian and Michael Melkonian

The History of the CCAC

1974: Foundations of an algal culture collection were laid in the Botany Department in Hamburg

1978 (MM) / 1981 (BM): Move to the Botany Department in Münster

1988: Move to the Botany Department in Cologne with 300 strains forming the basis of the „Culture Collection Melkonian" or „M-Collection"

2001: Establishment of the „Culture Collection of Algae at the University of Cologne **(CCAC)**"

2001: Membership in the World Federation for Culture Collections (number 807) and formation of a website

2011: Membership in the European Culture Collections' Organisation

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Establishment of cultures – increasing numbers of strains

1988 – 2009: Botany Department (built in 1955)
one growth chamber (ca. 2000 strains)

2009: New facilities in the „Cologne Biocenter"
four modern walk-in growth chambers

2009 – 2014: ~ 4000 strains

- 85% from freshwater/terrestrial habitats
- 15% from marine/brackish habitats

- 1615 cultures = currently publicly available
- 18% axenic cultures

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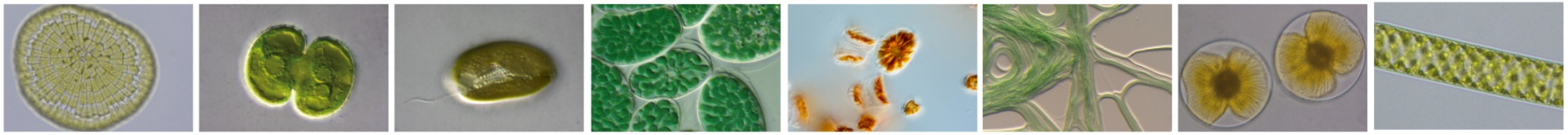

24 Algal Classes Represented

Zygnematophyceae	1054 strains
Chlorophyceae	495 strains
Euglenophyceae	384 strains
Cryptophyceae	367 strains
Dinophyceae	167 strains
Bacillariophyceae	157 strains
Prasinophytes	133 strains
Cyanobacteria	129 strains

Bias in collecting and isolating habits of the phycologist

- Chrysophyceae – Coleochaetophyceae - Eustigmatophyceae – Mesostigmatophyceae - Glaucophyceae – Haptophyceae – Klebsormidiophyceae – Pedinophyceae – Pelagophyceae – Phaeophyceae – Raphidophyceae – Rhodophyceae - Synurophyceae – Trebouxiophyceae – Ulvophyceae - Xanthophyceae -

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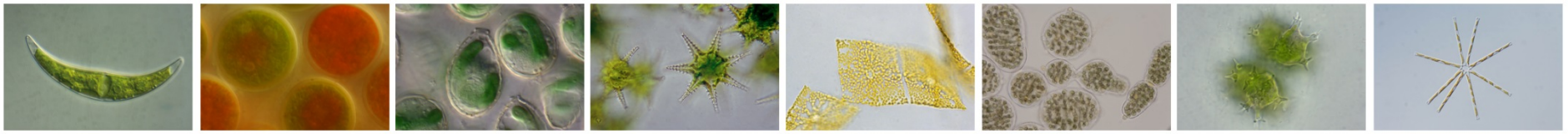

Biodiversity

Estimates of the „real" number of algal species vary tremendously

In nature: high

In culture collections: limited

Increasing the biodiversity in culture collections requires the development of new methods of isolation and cultivation

Isolation:

FACS

Fluorescence Activated Cell Sorting

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FACS: clonal and axenic cultures obtainable directly from natural samples

Flow cytometry: analysis of single cells in a flow stream

Individual particle volume, fluorescence and light scatter properties can be used for cell sorting

Chlorophyll-autofluorescence is used to discriminate algae from bacteria

Suitable method for the isolation of cells with a diameter of less than 10 µm or for very delicate cells

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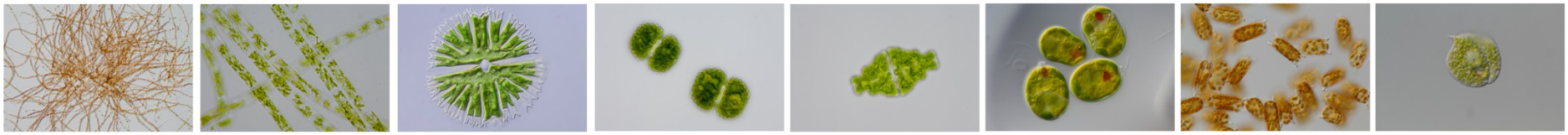

FACS: clonal and axenic cultures obtainable directly from natural samples

Pre-treatment of samples

- Filtration through 50 µm mesh-size gauze to remove large particles (dirt, but also predators and large algal cells)
- Concentration of dilute samples using membrane filters with pore size of 1.2 µm

Sorting of single cells into the wells of 96-well-microtiter plates

- Range of culture media
- Sterile-filtered water of the original sample

Isolation of 96 cells

- FACS: Approximately 30 minutes (dependent on cell density)
- Ordinary methods: longer than 30 minutes…

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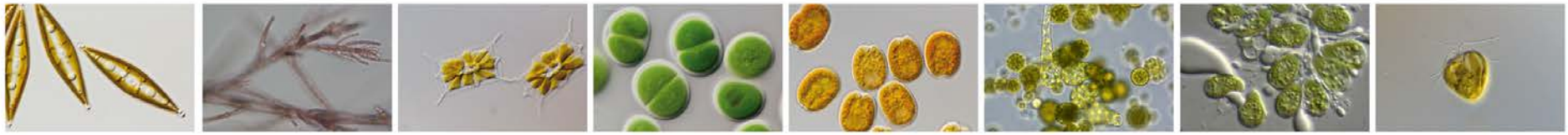

BUT: WHO DOES ALL THE WORK NOW ?

Some calculations for a 6-weeks-serial transfer

- 8.5 transfers per year x 10 minutes
  including the time for media preparation and microscopy

- Transfer of 1000 cultures = 85000 minutes per year
  = 1417 hours per year
  = 27 hours per week
  including vacation = **32 hours per week**

- Culture preparation for shipment
- Culture preparation for teaching
- Re-isolation after contamination

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AND: WHERE DO WE STORE ALL 4000 STRAINS ?

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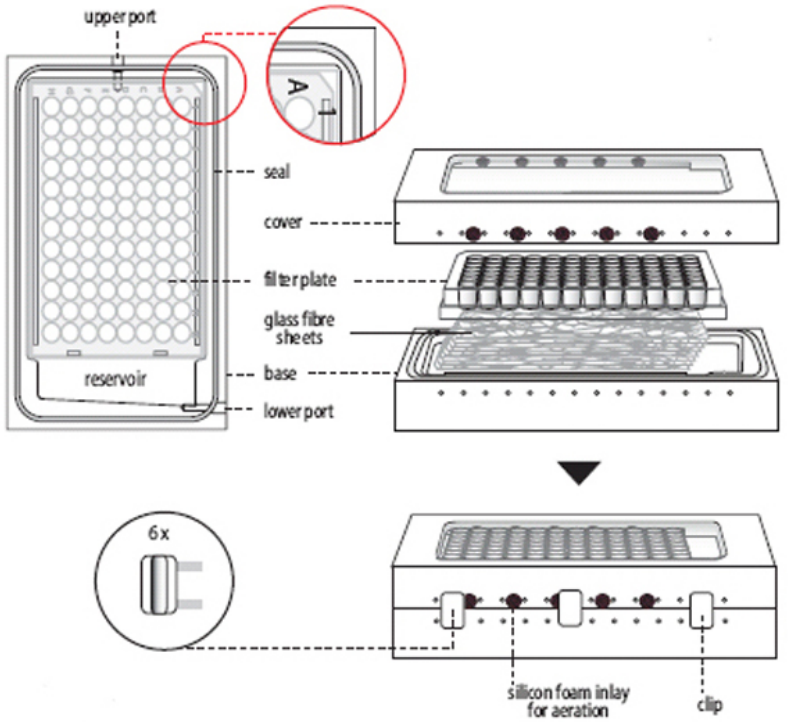
The All-In-One Solution for Microalgal Cultivation:

The Phycomat: Cultivation of microalgae on ultrathin support layers



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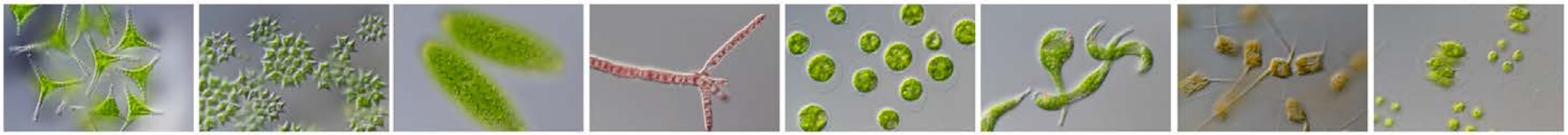

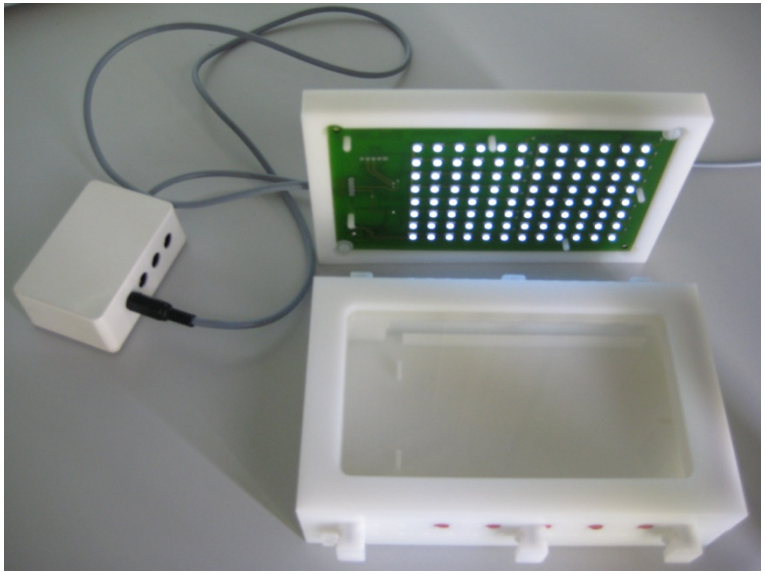

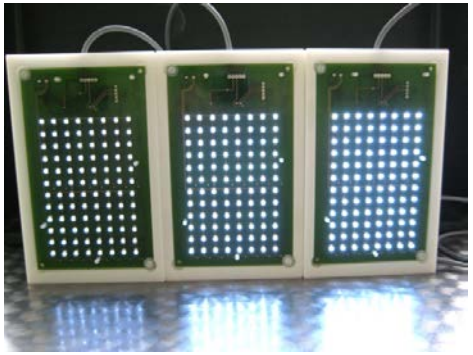

LED-lid
light-intensity = continuously adjustable
light-dark-regime = programmable

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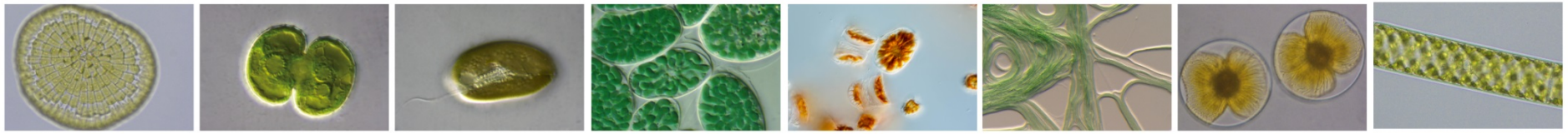

How much space is needed for 400 cultures?

2 m^2 versus 200 cm^2

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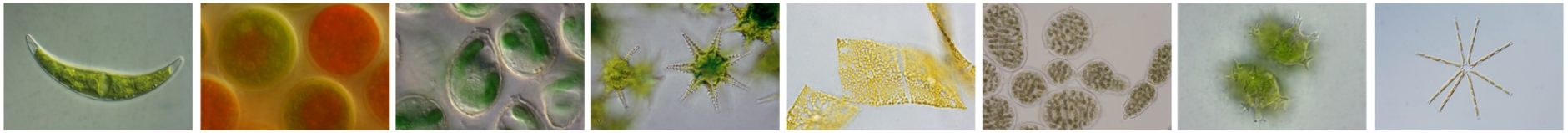

Advantages of the Phycomat

- 96 cultures can be grown in an area of 200 cm^2
- Exchange of culture medium or transfer of strains can be achieved simultaneously
- Exchange of culture medium has to be done every 3-6 months
- Transfer of cultures to a new filter-plate is required only once per year
- Minimal requirement of space, labour and cost

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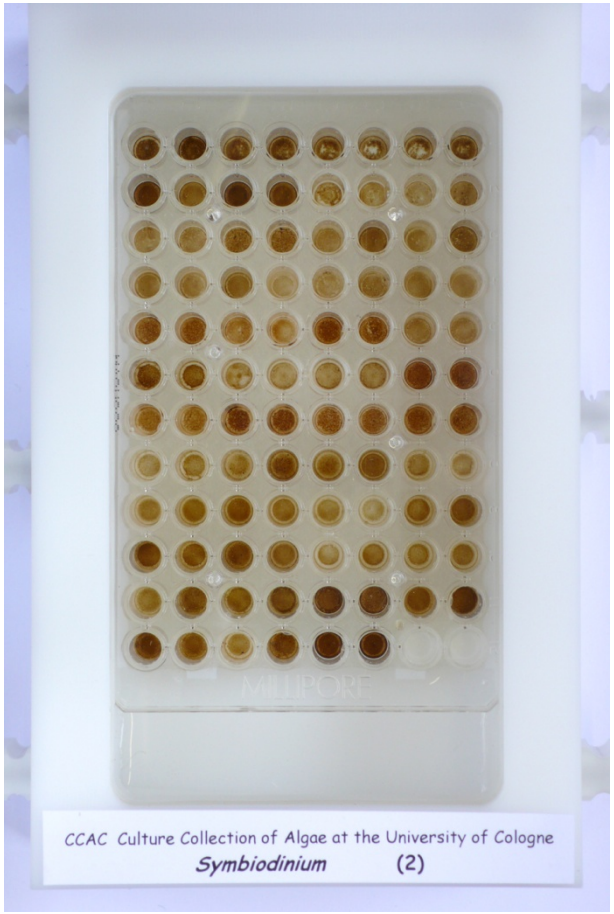

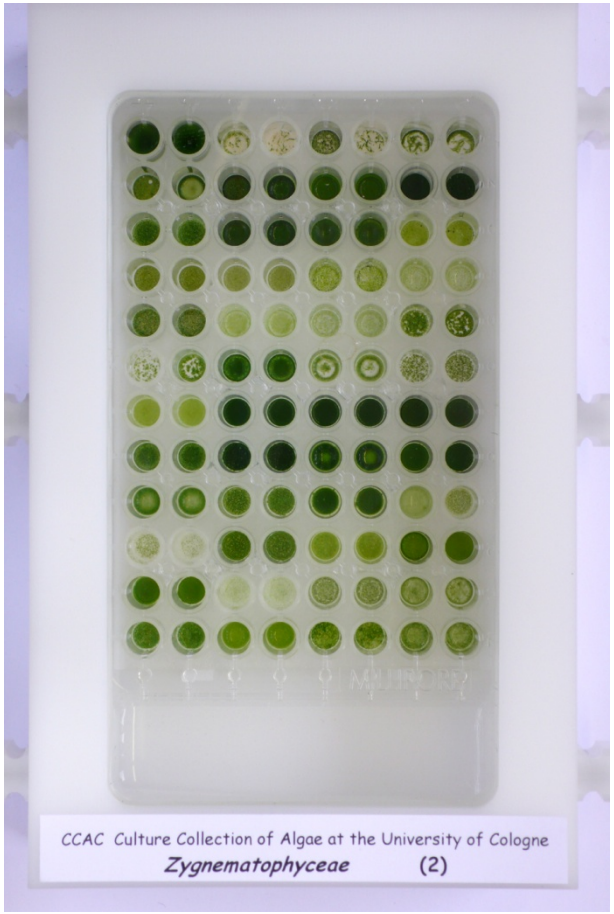

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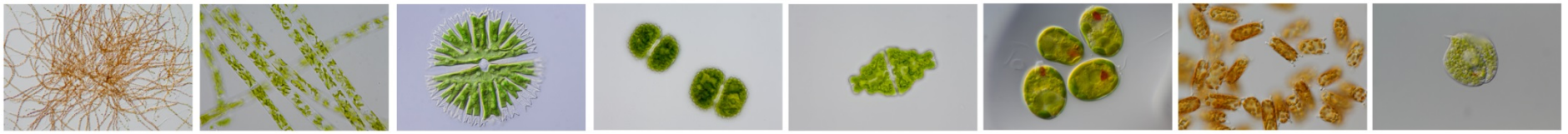

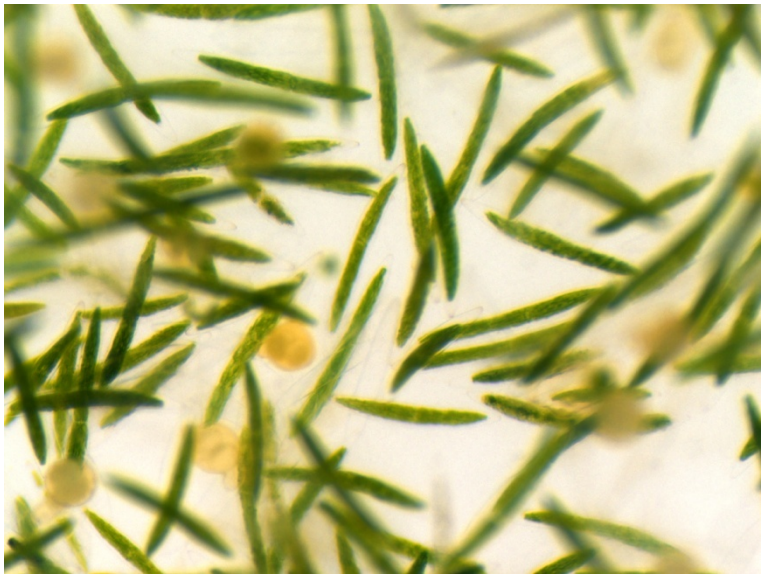

M2135 Closterium acerosum

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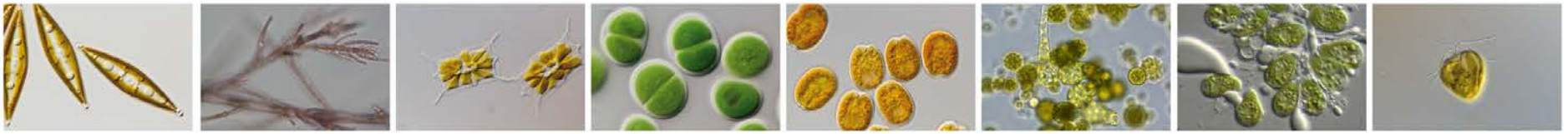

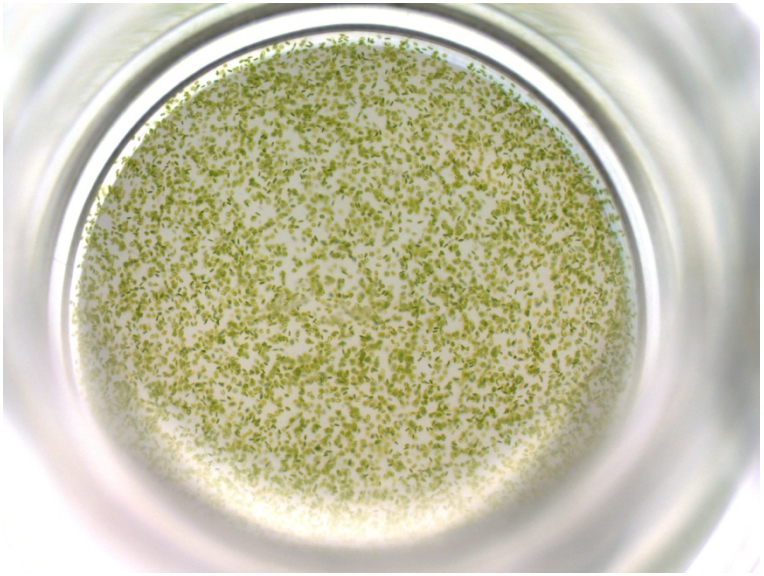

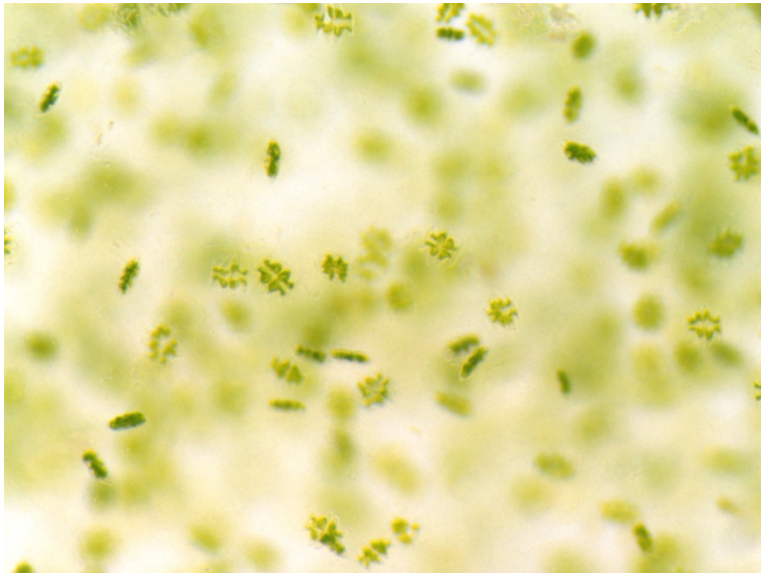

M1189 Micrasterias pinnatifida

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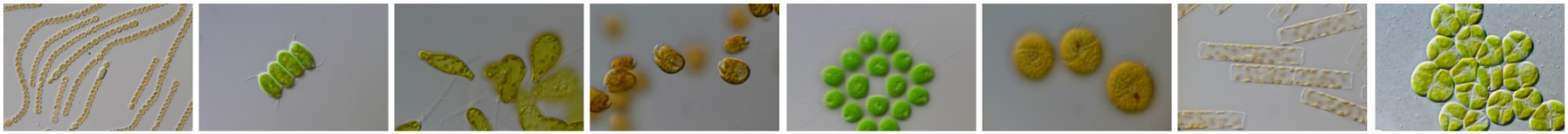

Acknowledgement

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