# In-line holographic monitoring of *Haematococcus pluvialis* growth

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We record visible light holograms of *Haematococcus pluvialis*. Back-propagation is used to obtain the light field in the entire sample volume to efficiently localize the algae. This allows to count them and to characterize their size, shape and colour. A prototype was developed that autonomously takes samples and records holograms continuously. This prototype was used in a pilot study in the laboratories of BDI - BioLife Science GmbH. The collected experience and data are currently under analysis.

General idea: Automate…
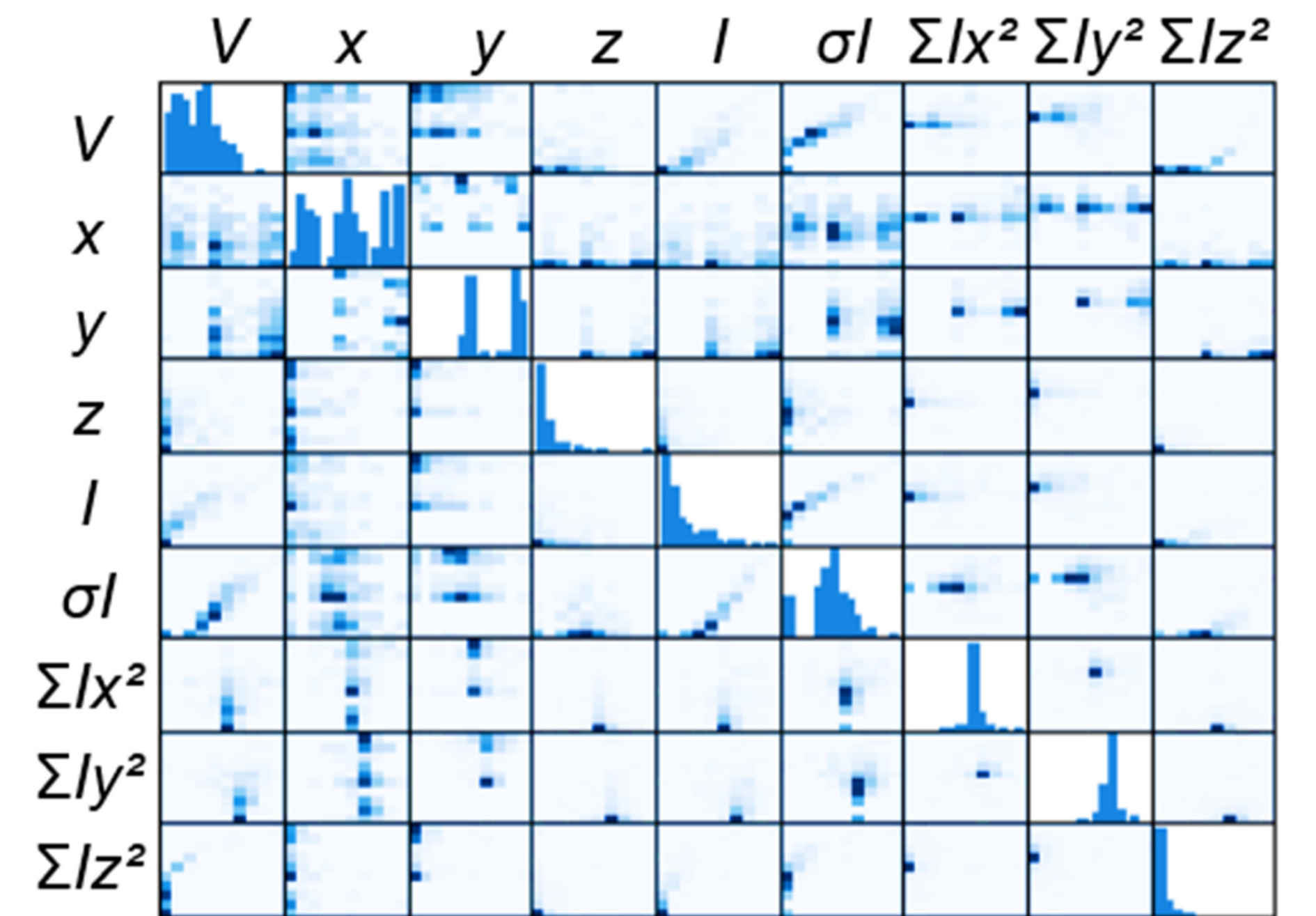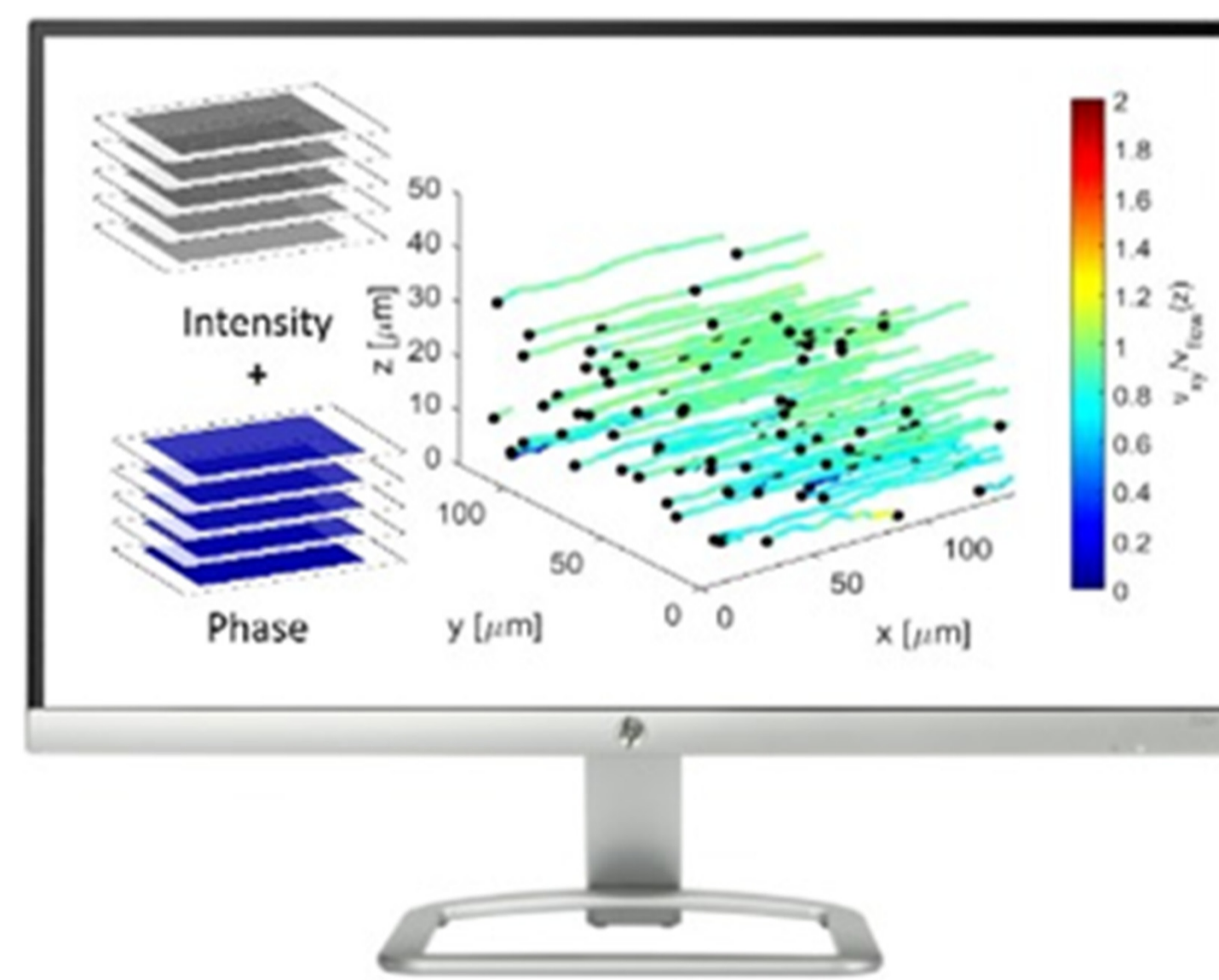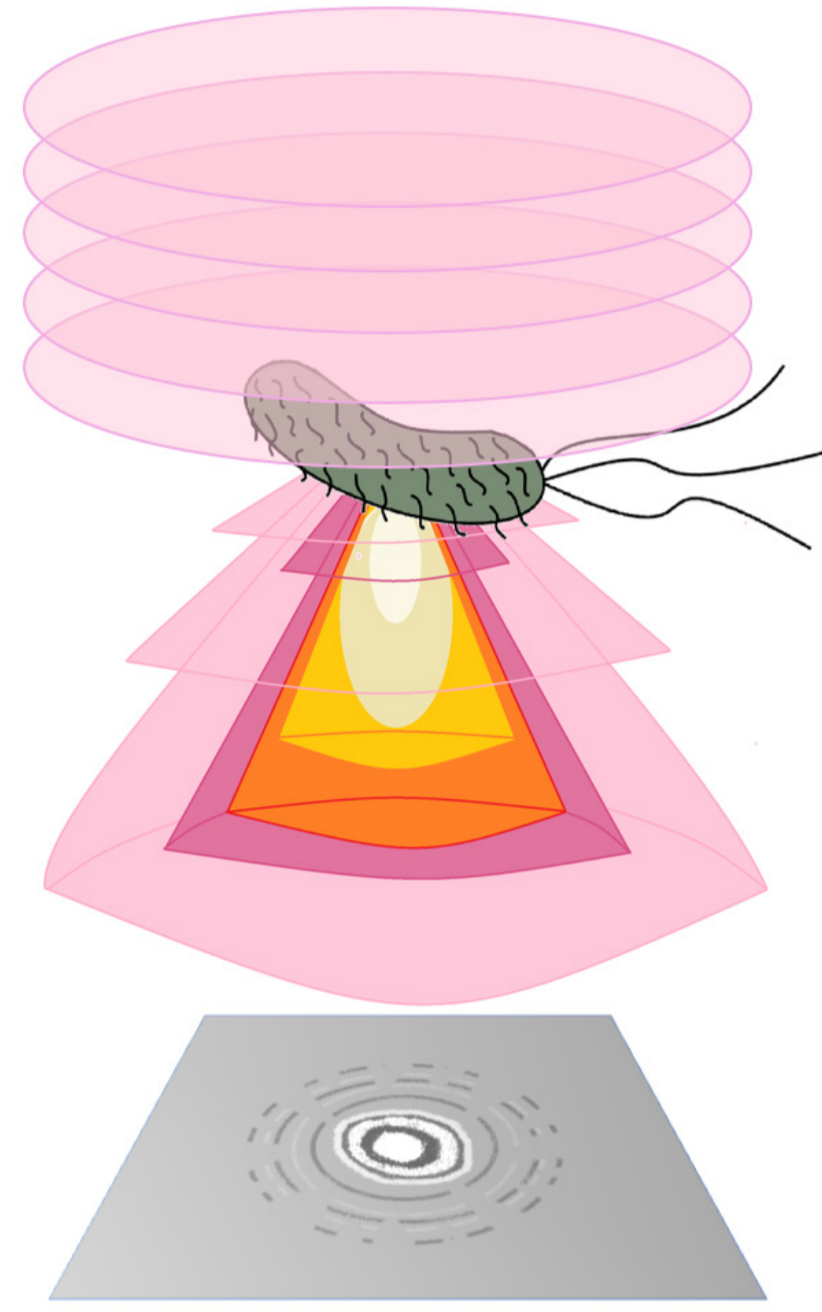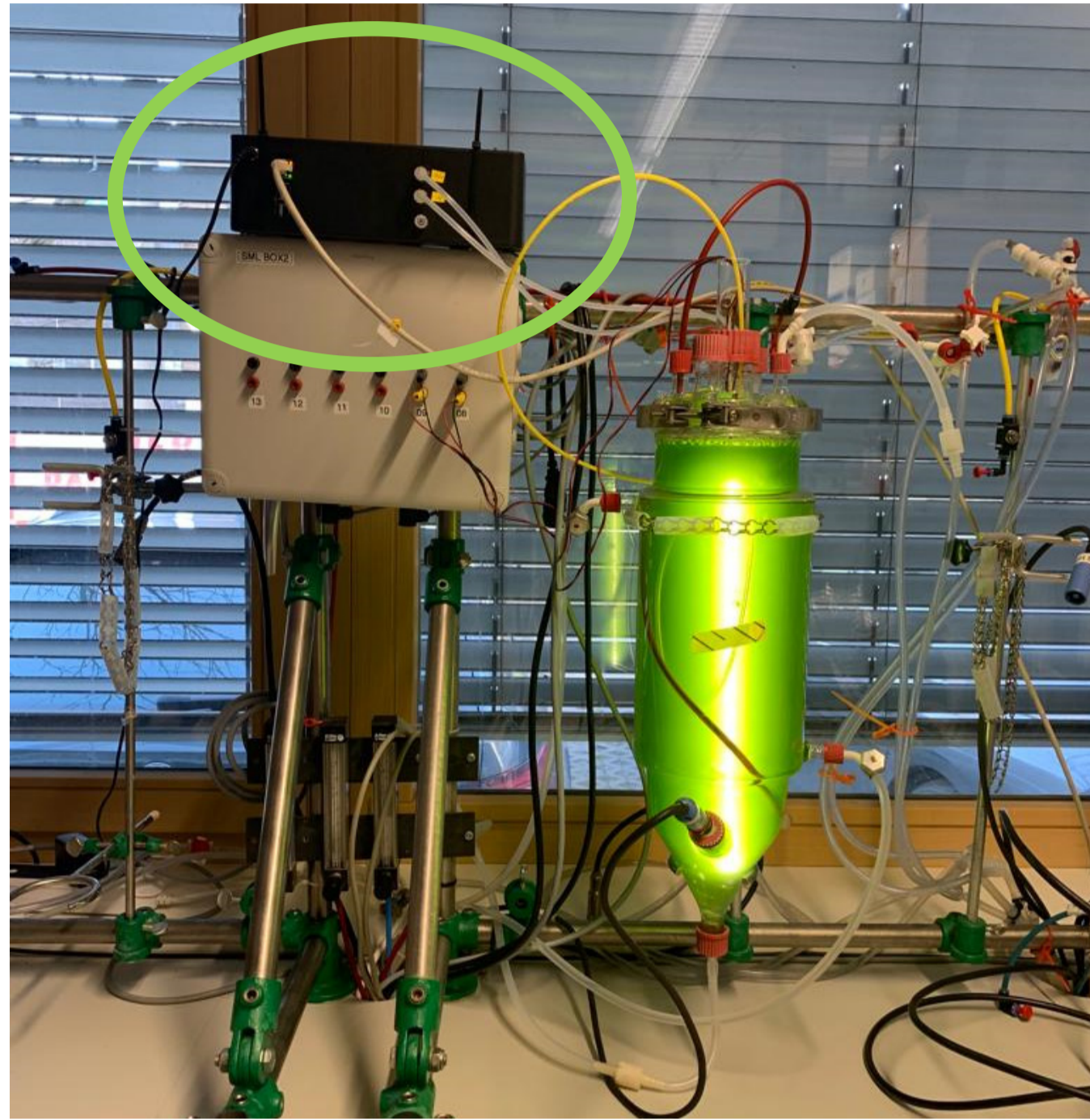
… taking samples, holograms and their analysis.
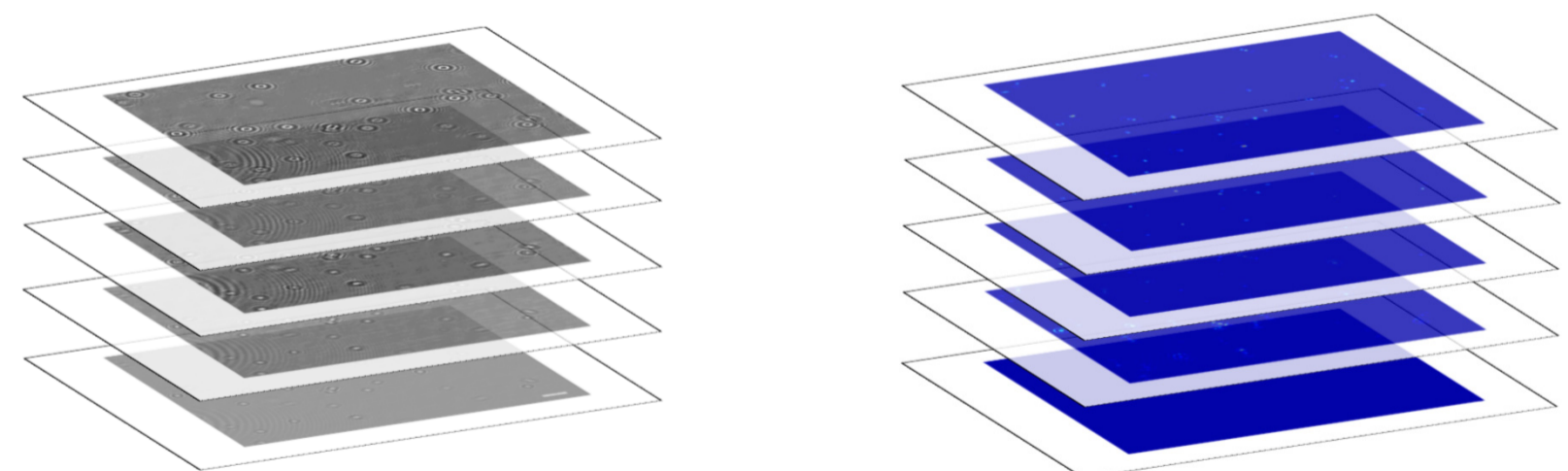
Holograms

We take defocussed micrographs

Holograms are patterns of the **interference** between **light scattered** by an object and coherent light the **reference beam**. In **in-line** holograms the illuminating beam doubles as reference beam[1].

In this study, we recorded the **interference** between light scattered by *Haematococcus pluvialis*[2] cells and the illuminating light. To get **colour** information we did so with three different **wavelengths**.

The information is in the fringes around the objects' footprints.

Prototype

The portable prototype comprises:
- A small computer
- A camera
- 3 laser diodes
- A pump

The pump takes samples, the lasers shine through them and the camera records holograms. These holograms are pre-treated on the device and sent to BOKU for analysis.

The prototype monitored the growth and reddening of *Haematococcus pluvialis* in the test facility of BDI.
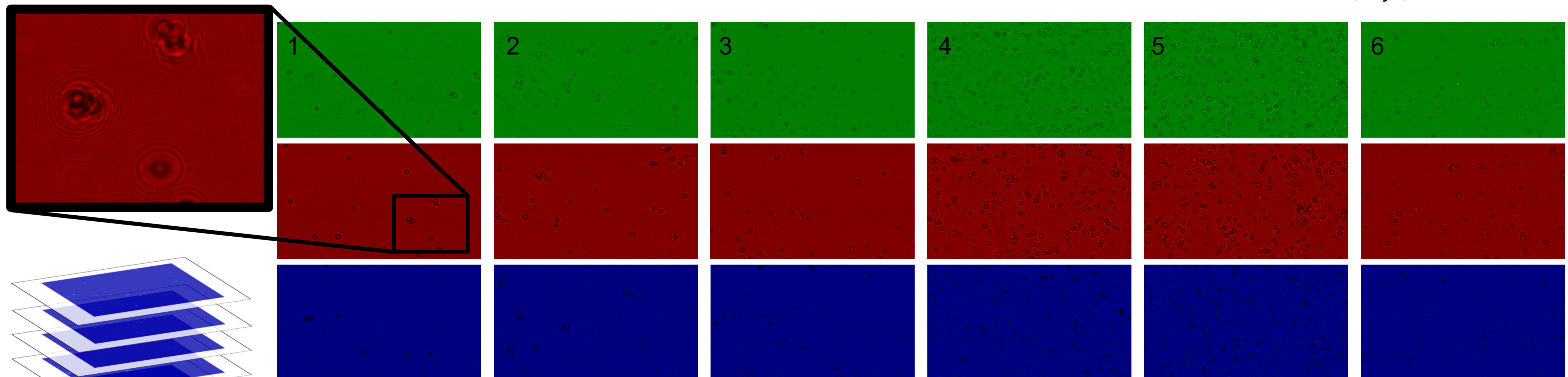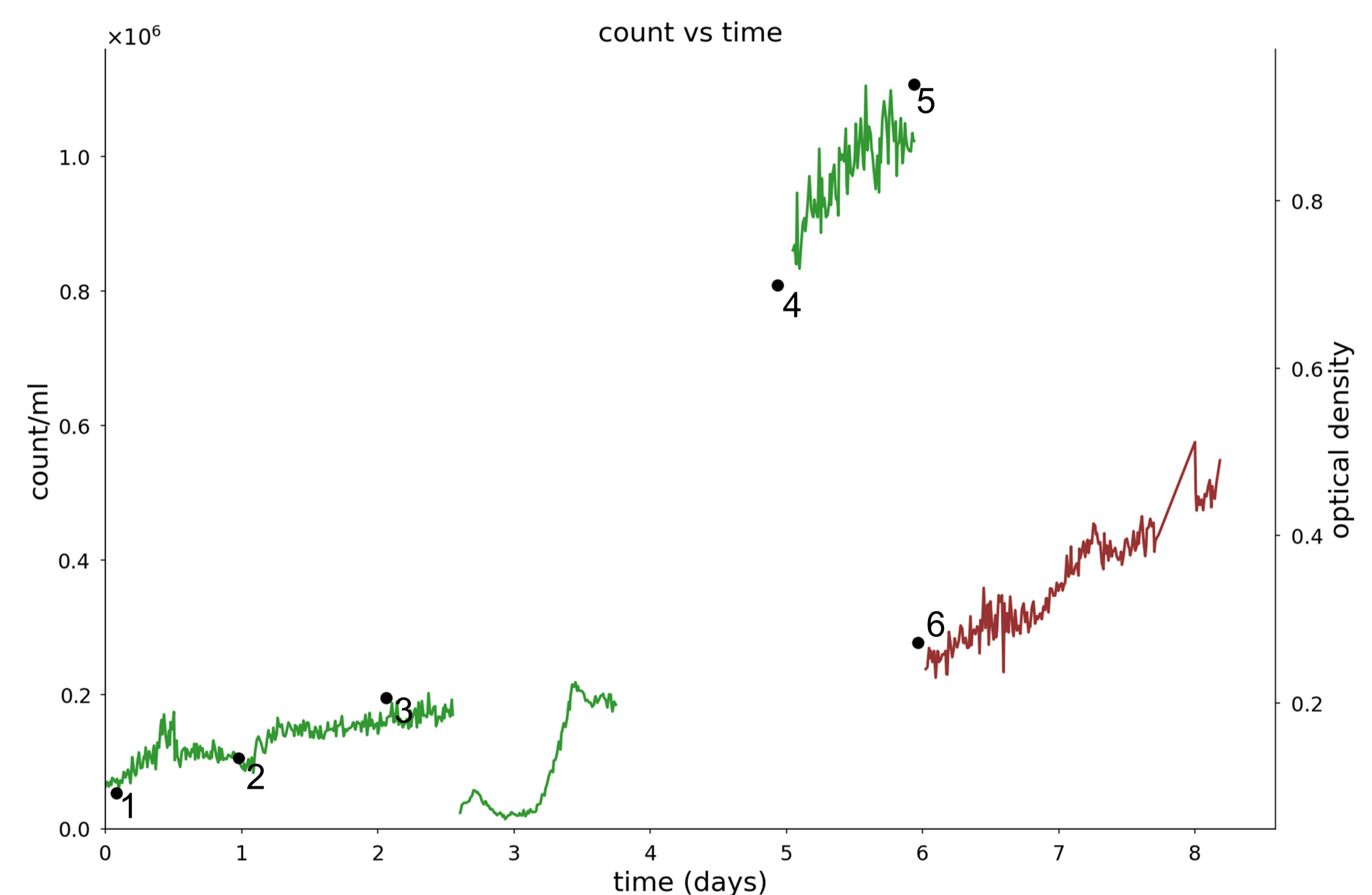
Top: Cell counts (full lines, colours refer to production phase) and OD-measurements (black points) vs growth days.
Bottom: crops of the respective color channels (700 µm wide). Missing data is caused by clogging/pumping issues.

Backpropagation

Numerical refocussing

Backpropagation means calculating the **3D scattered light field** from **single images.** This gives both **intensity** and **phase** info. These are then **combined** to give an instantaneous 3D stack of **enhanced contrast** images[1].

Preliminary results

Successful cell counts
- Automated sampling and recording of in-line color holograms.
- Automated cell counts.
- Developed the software architecture needed for continuous analysis.

There is an excellent correspondence between the automatic cell counts and manual optical density measurements.

Outlook

More details, more statistics, real-time
- Combining the colours throughout the volume.
- Characterizing each cell individually.
- Measure Astaxanthin content in each cell.
- Recognizing cell development with machine learning.
- Recognizing spoiler organisms

We founded **Holloid GmbH** to develop and offer this technology to all interested parties.

[1] BioPhys. J. 108 (2015); [2] Front Plant Sci., 7, 531 (2016)

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