

Spatial-frequency-domain hyperspectral microscopy

Lisa Uguen¹, Ronan Piedevache¹, Gaspard Russias¹, Sofian Helmer¹, Antoine Fournier², Denis Tregogat¹, and Stephane Perrin¹

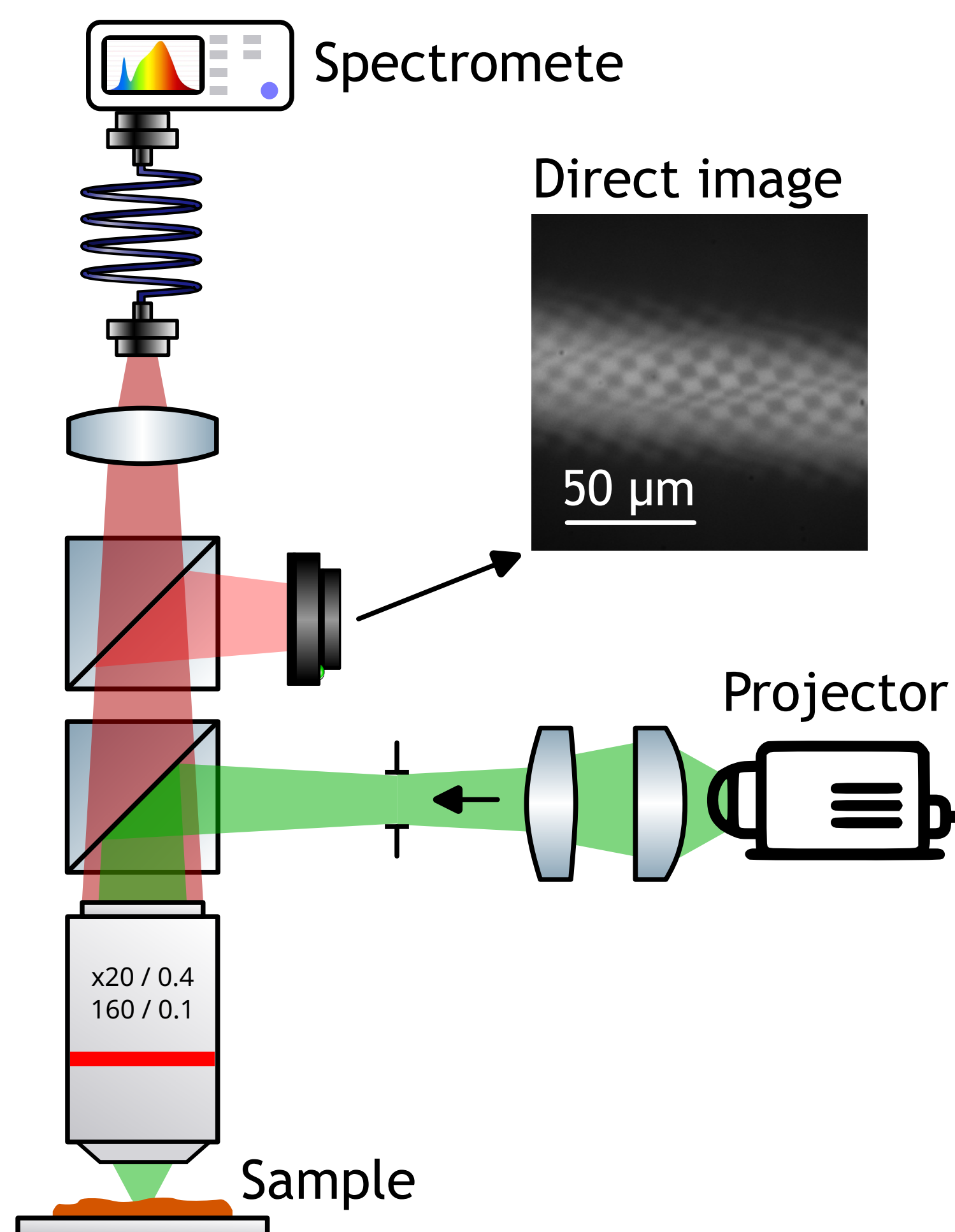
¹Department of Biophotonics, Photonics Bretagne, 4 rue Louis de Broglie, FR-22300 Lannion

²Department of Crop Phenotyping, Arvalis, 45 voie Romaine, FR-41240 Beauce-la-Romaine

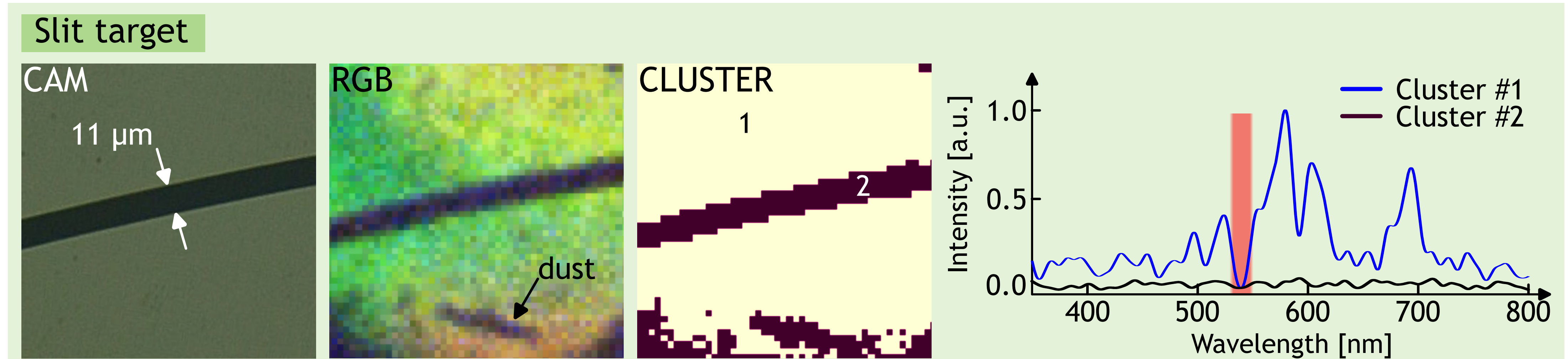
INTRODUCTION

Hyperspectral imaging allows to collect both spatial and quasi-continuous spectral information of an object (*i.e.*, an hypercube), unlike multispectral imaging where the spectrum is divided in few bands. This imaging technique is promising not only for biomedical applications, but also for vegetal sciences. Hyperspectral imagers are commonly based on punctual (*e.g.*, whiskbroom) and linear (*e.g.*, pushbroom) approaches [1], making it possible to extract the spectral distribution of macroscopic and microscopic samples. This work shows the innovating combination of single-pixel hyperspectral imaging [2] with reflective-mode optical microscopy to retrieve high-spatial-resolution hypercubes of samples in the visible and in the near-infrared ranges. Statistical algorithms enables the clustering of regions of interest of inorganic and organic samples and the identification of spectral responses of targeted cells.

METHODS

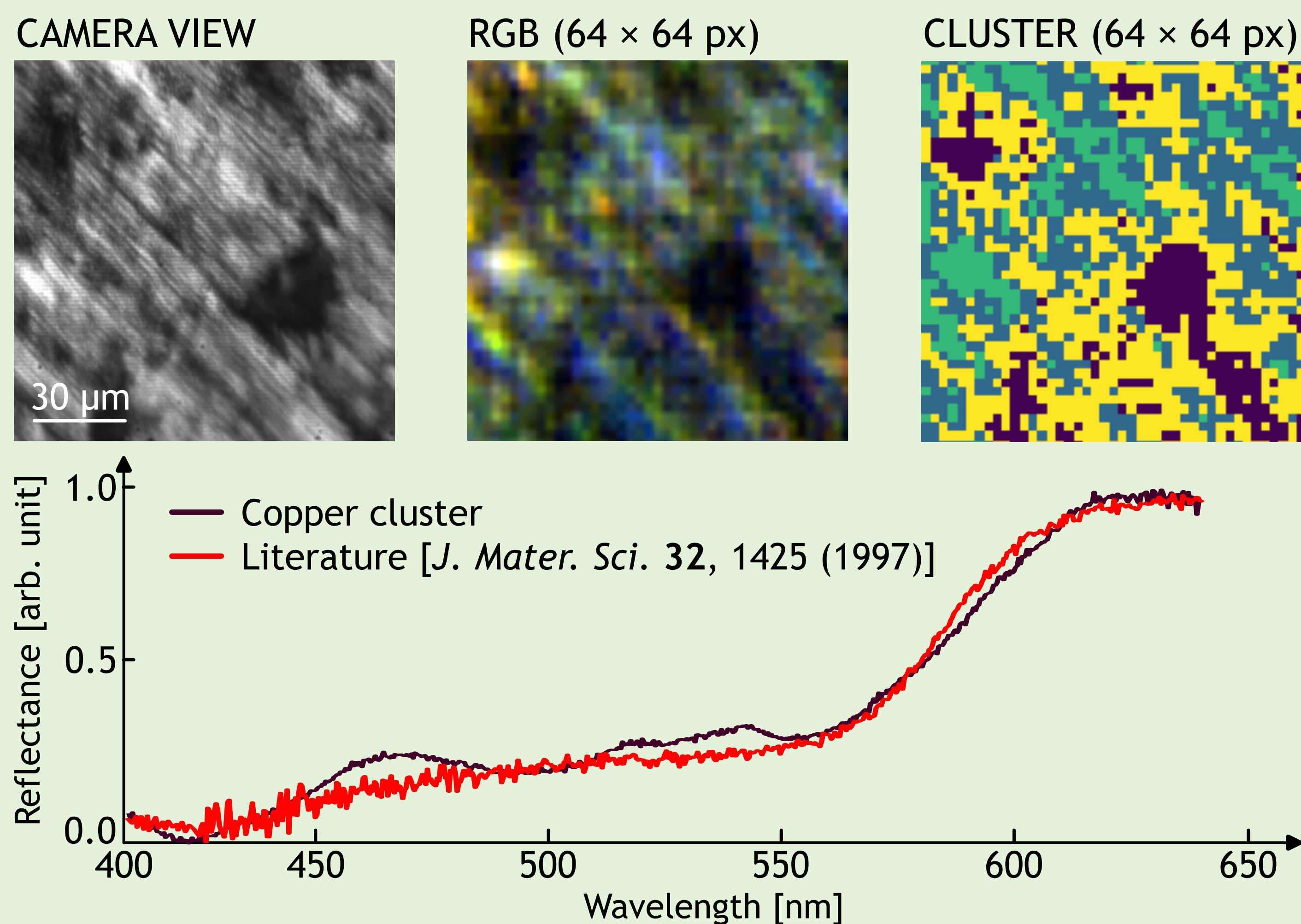


- Superimposition of a set of Hadamard patterns on the sample using a DMD-based projector
- Beam shaping of the illumination patterns to optimise the spatial-frequency projection
- Collection of the averaged spectral intensity by a VIS-NIR spectrometer (around 250 bands)
- Synchronisation of the projection patterns with the acquisition of spectra
- Implementation of an Hadamard transform algorithm [3] to retrieve the spectral distribution
- Spatial resolution of around 2.5 μm | Spectral resolution of 2.1 nm

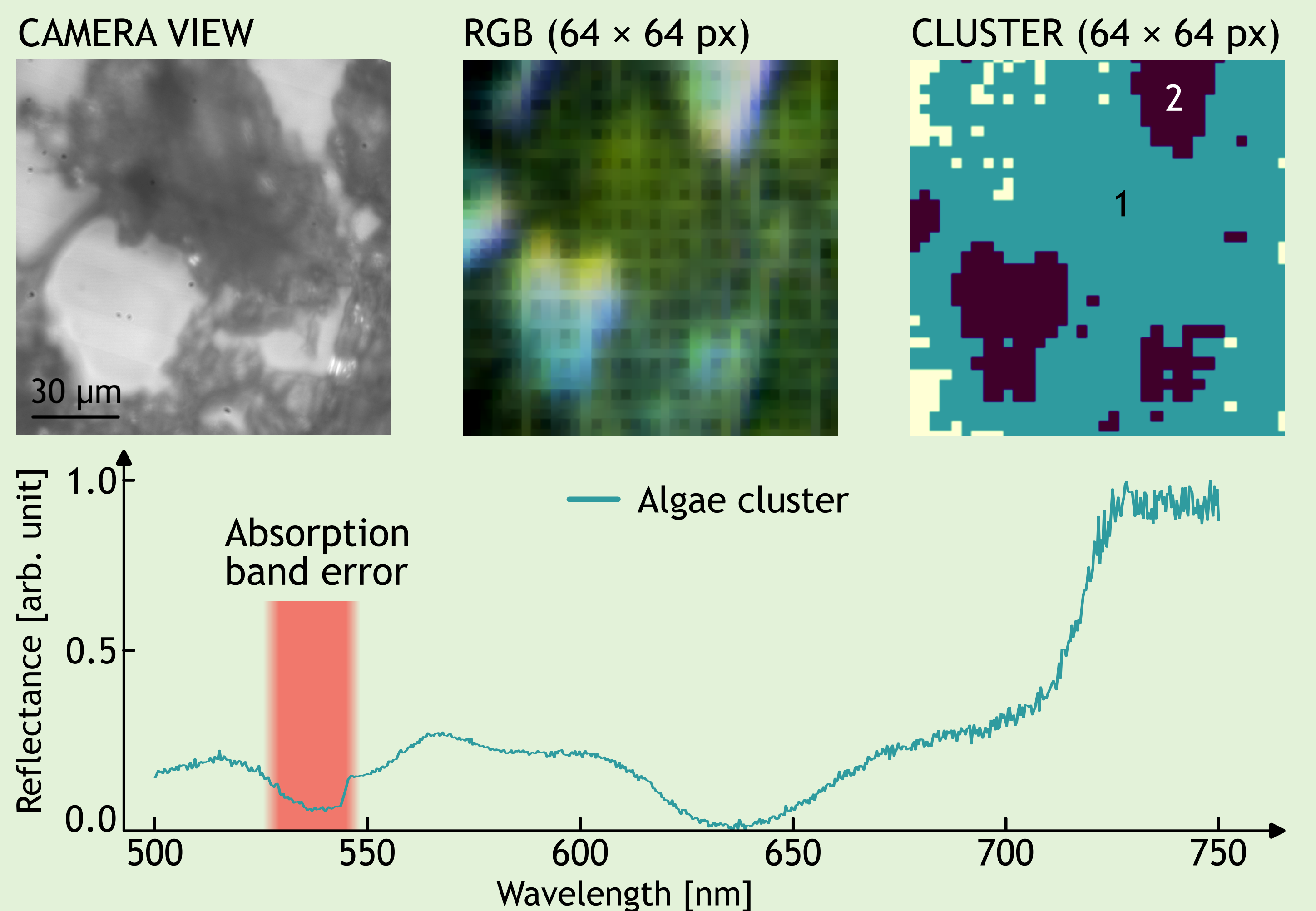


RESULTS

The spectral distribution of a PCB conductive layer was reconstructed, highlighting stripes and black defects on the surface. A clustering algorithm allows to isolate the copper area and to extract its averaged spectrum.



Green algae use the pigment chlorophyll to carry out the photosynthesis process. Through its typical reflectance profile (green- and near-infrared-light reflection), the green fraction can be clearly identified.



CONCLUSION

The innovating concept of reflective-mode hyperspectral microscopy using single-pixel imaging was demonstrated. This allows to reconstruct the continuous spectral distribution of samples with a high-spatial-resolution and, then, to identify the spectral responses of samples in the visible and in the near-infrared ranges. Spectral measurement issues will further be solved by enhancing the illumination part.

REFERENCES:

- [1] C.-I. Chang, "Hyperspectral Imaging," Springer (2013)
- [2] M. Ribes *et al.*, Sensors 20, 1132 (2020)
- [3] Q. Yi *et al.*, Opt. Express 28, 16126 (2020)