

Preface

It was morning, and the new sun sparkled gold across the ripples of a gentle sea. . . . It was another busy day beginning. . . . But way off alone, out by himself beyond boat and shore, Jonathan Livingston Seagull was practising. . . . No limits, Jonathan? he thought, and he smiled. His race to learn had begun.

– AD free excerption from “Jonathan Livingston Seagull – a story” by Richard Bach-First published in Great Britain by Turnstone Press 1972. This edition published by Element 2003

The intention behind this book is to provide a sort of starting point for those who are interested in the hard task of fully exploiting the capabilities offered by an optical microscopy fluorescence approach. It is fundamental to have some basic concepts about optical microscopy and fluorescence to understand their enormously high potential in basic and applied research. The classical spatial domain of optical microscopy is the submicron level dictated by the physics laws. Notwithstanding this we are moving to the nano-dimension, i.e., a spatial domain from 5 to 100 nm, exploiting the photophysics of the fluorescent markers being used and the technological abilities of detecting low signals and manipulating light in phase and amplitude including the utilization on nonlinear phenomena related to light–matter interactions.

When thinking about optical microscopy and the images coming from different contrast mechanisms, we mainly reason about intensity. In the last years, thanks to the rapid growth of fluorescent markers, we started thinking about spectral properties in terms of both emission and excitation. The advent of spectral microscopes and of white light laser sources enabled to improve the information content of microscopic images. At the very same time, there was an increasing demand for collecting information in terms of dynamics and of molecular interactions. To this end, there are two important methods such as fluorescence recovery after photobleaching (FRAP) that is able to provide information related to molecular diffusion and forster-fluorescence resonance energy transfer (FRET) that allows monitoring events at the nano-level. Within this scenario, spectroscopic techniques and single particle tracking approaches were developed along with an increasing knowledge about scattering phenomena affecting or “helping” image formation processes

including those nonlinear phenomena known as second and third harmonic generation processes.

Modern optical microscopy cannot prescind from integration with other data sources like those coming from the IR interactions, X-rays, and electron microscopy. For this reason, this book contains important contributions as complement to the fluorescence microscopy methods mentioned above. I personally think that this is a good collection of contributions for having the appropriate control on optical methods in fluorescence microscopy toward super-resolution methods that have their own bases on most of the topics treated here from photophysics of fluorescent molecules to point spread function engineering.

I hope the reading of this book can stimulate new projects and ideas. I have so many people to thank including you, reader, that I would like to mention my wife Teresa, my daughter Claudia, and our puppy Sissi, a fantastic Cavalier king Charles dog, for sharing myself and their time with this project that reached the end thanks to the patience of all the authors and the professionalism of Sabine Schwarz and Jutta Lindenborn. A special mention is for Prof. Antonino Zichichi, President of the Ettore Majorana Foundation and Centre for Scientific Culture in Erice, Sicily, where this book was born following the 36th Course of the Antonio Borsellino School of Biophysics. A warm thanks to all the students of the school. The IIT-Italian Institute of Technology is acknowledged for giving me an important opportunity for a new adventure in the optical microscopy field.

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