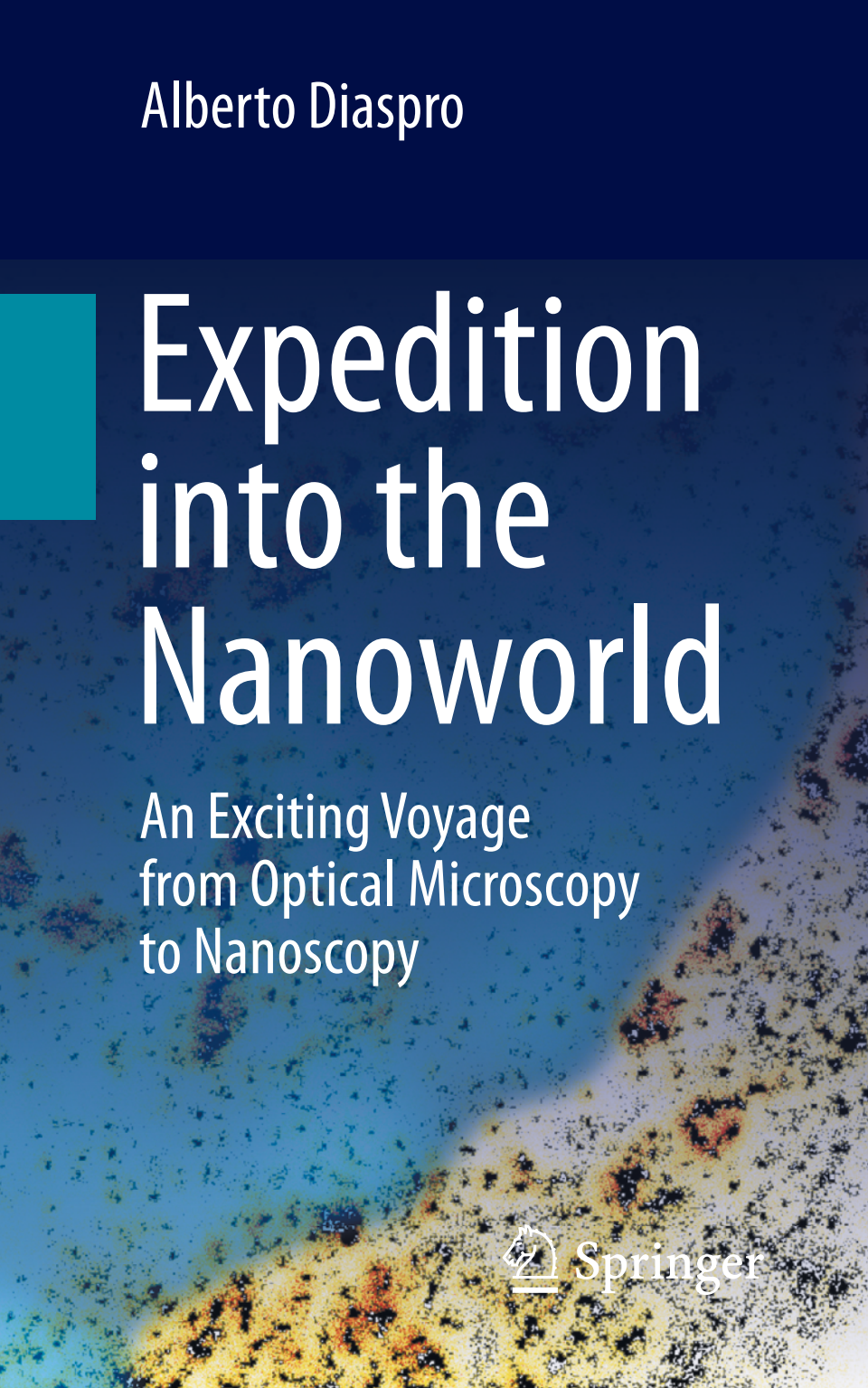
Alberto Diaspro

Expedition into the Nanoworld

An Exciting Voyage from Optical Microscopy to Nanoscopy



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Faust'o Happy Is very
verde.
Because, if you pperd…e
Now, cche vuoi? Perchè
sei Ciccio e nun ssei
Ggiuda!
Con le Bermuda, nun fa
accussi.
(F. Masala, E. Zamponi, Argentario, 1982)

Dedicated to Teresa.

Foreword

"Alberto Diaspro takes the reader on a kaleidoscopic journey of microscopy as colourful and full of beauty as science and life itself."

Stefan W. Hell
2014 Nobel Laureate "for the development of microscopy in super-resolved fluorescence."

"It is an interesting period for quality scientific divulgation in our country and we can consider ourselves lucky, given the thick curtain of misinformation that generally obscures the citizens of the planet and of Italy in particular. That's why I like to greet this popular text that has, however, much more (and, if I may, better) than other texts. In the sense that, often, scientists describe their experiences in an aseptic way and focus, as it is right, on the general goal of their effort: to explain a concept or, even, the cosmos, no matter if macro or micro. In this case, Diaspro continually and very pleasantly mixes

personal, cultural and scientific topics and themes in an admirable way, even having fun on the border of humanism and science. Contributing, in my opinion, to the reunification of what are just two sides of the same general culture, that of *sapiens*. In a continuous change of focus, never so pertinent to the subject matter, the reader enjoys himself and learns concepts, and even curiosities, that make that knowledge interesting and in some way indispensable. I want to say that, after reading, one will be surprised not to have known about those topics before and will want to know even more. A sure testimony that the mission of divulgation is accomplished."

Mario Tozzi
Italian geologist, popularizer of science
and essayist, author and TV personality
Max Planck Institute
for Biophysical Chemistry
Goettingen, DE

Acknowledgements

After the war... there was a desire to dance that made light!

F. Guccini to D. Bianchi,
Propaganda Live, La 7 broadcast, December 6th, 2019

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The "thank you" are so many, so many that they almost have to be recited, chanted aloud, because it is so, because it is right. Here they are in a row, in order, a somewhat disordered order, perhaps better to say by blocks.

Thanks to Teresa, Claudia and Irene, the most important women in my life. Thanks to little Sissi who kept me company in my morning writing at my desk. Thanks to Francesco Avola, more than a son-in-law. Thanks to Anna Mercurio, Mario Arace, Carmen Gloria and Mario Cassano, special people. Thanks to Bruno, whose student I was, and Franca Bianco for welcoming me and giving me so much more along with Betta, Francesca and Pietro. Thanks to my father, Renzo, and Franca for something they know. Thanks to Maurizio Caviglia for his way of supporting new ideas. Thanks to Antonio Borsellino for spurring me on when I wanted to stop. Thanks to Giuliano Toraldo di Francia for his lessons. Thanks to Carlo Tacchetti for allowing me to continue as a microscopist in the most difficult moments. Thanks to Enrico Gratton who is my model, I realize unequalled. Thanks to the mythical E of Cassini for being always present. Thanks to Francesco Guccini, whose songs have accompanied my studies, my research and the writing of this book. Thanks to Raffaella Zuccari who convinced Francesco Guccini to follow a forty-minute presentation on multiphoton microscopy at Via Paolo Fabbri 43. Thanks to Guendalina Zuccari and Michel Dabove for the table in Sassello and more. Thanks to Roberto Cingolani who has believed in my project on optical nanoscopy at the Italian Institute of Technology in Morego. Thanks to Paolo Bianchini, Valentina Caorsi, Davide Mazza, Giuseppe Vicidomini, Ilaria Testa, Francesca Cella Zanacchi and Emiliano Ronzitti with whom I was able to start building a group of world-renowned microscopists at the Department of Physics of the University of Genoa in Via Dodecaneso 33 between L303 and L308. A double thanks for Paolo Bianchini for sharing with me microscopy adventures… "lifetime". Thanks to the Biophysicists of L303 in the

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Thanks to Books, paper or digital, for that consultation that, since childhood, has seen the Encyclopedia Treccani opened for every question. An old habit never abandoned.

Thanks to the Internet and Wikipedia, which even within the limits of the flow of information on the net offer the possibility of acquiring information critically. It is up to us to be lazy or not. Even the Treccani is on the net.

Thanks to Music, all of it, on vinyl or CD or on the net that has always accompanied my reading or studying and my writing.

Thanks to Franz DiCioccio, excellent drummer of the Premiata Forneria Marconi (PFM), a "multimodal" musician for culture all-around. Thanks to Patrick Dijvas, PFM, a great jazz man. Thanks to Iaia De Capitani and Giovanni Caprara for making possible the jam session with the microscope. Thanks to Massimo Sideri for believing in the route from microscopy to nanoscopy.

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Thanks to Ulrico Hoepli (1847–1935), a patron, who contributed to cultural development by founding the "Biblioteca Popolare Ulrico Hoepli" in 1921, and in 1930 he donated the planetarium to the city of Milan. Thank you for that idea of "supporting institutions and initiatives of public utility or that work to promote the sciences and the arts". Thank you to those I have not mentioned but who are very much in my mind.

Thanks to you readers who have endured pages with formulas that were too simplified for some and too difficult for others, but then you got there, you got to the bottom.

Thanks for the dance! (L. Cohen, 2019)

About This Book

Some formulas and concepts that may be difficult to understand are used in the discussion. This is done intentionally by asking the reader to make a small effort to maintain rigor in a treatment that is intended to be "popular". The suggestion is to read the formulas as text, like the text of a poem. The formulas are not isolated or numbered, intentionally. Similarly, concepts that are more difficult to understand are not isolated in a box. The author's idea is that they should be harmonious with the rest of the text. After all, they help to maintain the "narrative time" of the story.

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Curious Premise

Tall, solemn, dressed in black, it seemed to me to see Granny Lucia again; [...] Oh Granny, Oh Granny! oh how beautiful she was when I was a child! tell me again, tell this wise man the news of her who seeks her lost love! [...] You sleep to my desperate cries, and the cock crows, and you won't wake up.
Giosuè Carducci, *Davanti a San Guido*, in *Rime nuove* (1906).

My grandmother Anna was a beautiful woman (Fig. 1.1). I was a curious and lively child like all my peers. When she died of cancer, an impossible promise rose from my heart and I whispered to her, "I'll understand why, Grandma dear, so you won't die anymore." It sounds a crazy promise, the next will show it was not so crazy. I didn't realize that I already was a young microscopist, at that time. I was born in Genoa, but I had spent most of my childhood in Verona. It often snowed and those flakes that covered everything in white fascinated me. One day in my stamp collection, among those detached from a blue envelope, I

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Fig. 1.1 Grandmother Anna Mercurio in Arace

found a coloured stamp with a very white and beautiful snowflake in the center (Fig. 1.2).

It was then that I realized that there are things that the eyes are not able to see, besides Santa Claus and his nine reindeers. The snowflakes, on those "Santa Lucia" days when everything was quickly covered in white around me, did not appear like that postage stamp. When I let them rest on the palm of my hand, the newly captured snowflakes became transparent and transformed into drops of water. Because of those strange combinations of life, I was

Fig. 1.2 The snowflake

given a small microscope with a cedar wood box containing slides in that winter.

However, I had ten rectangular slides on which, protected by a thinner square of glass, had been deposited the strangest things: from butterfly wings to cat hairs, from leaf veins to insect legs or thistle roots. In the package there were also five empty slides to give space to the creativity of budding microscopists. Creativity was mainly developed in finding something attractive to observe: a piece of rotten banana, a bit of spit or a few drops of blood. Stuff from Tom Sawyer and Huckleberry Finn, young boys protagonists of daring stories (Mark Twain, 1876 and 1884) or from Hardy Boys and Nancy Drew, very young amateur detectives ripped from their books and gathered in a single telefilm by Universal TV between 1977 and 1979.

There, I was ready to observe the snowflakes. Once the flakes had been captured, it was a question of orienting a small mirror that collected the light of the day and sent it to the slide on which I had dropped the snow so that I could observe it with that curved piece of glass, the lens, which made it possible to form the image observable to the eye. Focusing was fast, I was skilled in using the knobs of the microscope. I had to be fast before snowflakes could turn into water. In fact, all these operations took time and, in the meantime, the snow was melting. I learned that it was fundamental to cool the slide, perhaps leaving it immersed in the snow in the garden while I was at school. The garden of my house in via Fiume 2, the one with a beautiful willow tree in the middle, was my laboratory (Fig. 1.3).

So, conducting measurements in the cold, being quick in manual operations and knowing that the subject to observe could change over time were the first three things I learned as a promising microscopist. At home, after all, I did not have all these problems with the preparations in the cedar box. I would bring the microscope up to temperature along with the slides by hiding it under the willow tree, so that when I returned from school, before going up to the house, I could try to observe the snowflakes. The reasons for my delay in going up to the house were immediately revealed by the wet trousers at knee height on which I leaned, immersed in the snow during the observations.

The passion for the microscope remained intact over the years becoming one of the staples in my university and research career. During the third year of electrical engineering at the University of Genoa I was struck by a lesson on electromagnetic fields given by Professor Bruno Bianco. Professor Bianco explained, starting from Maxwell's equations that were the cross and delight of student engineers,

Fig. 1.3 Via Fiume 2 in Verona, the old willow tree and the bench. Drawing by Gianluigi and Lucio Perin, Verona, 6-5-1968

how visible light was an electromagnetic wave of which we could "see" the squared modulus with our eyes as with a video camera and how it was in a very restricted portion of the spectrum of energies carried by electromagnetic waves. Then, in an exciting double somersault, he explained that what was happening in space could be treated like music, with harmonics and spatial frequencies. It could be treated with high, medium and low frequencies, the same ones I used to set on my amplifier at home when I switched from the Italian singer-songwriter Francesco Guccini to the progressive sound of Pink Floyd. Call them sounds, vibrations, or images, but they can all be described in a harmonic way as nature is after all. A well detailed image of a thick spider web can be thought of as an image of high frequencies, spatial rather than temporal or musical. The high frequency sound of a violin. A beautiful photograph

of a landscape with a sunset will contain mostly medium frequencies, the discreet sound of a classical guitar. While the final scene in the fog of *Casablanca*, with its "Today perhaps we inaugurate a beautiful friendship", is dominated by low frequencies, like Jacqueline Du Pré's cello in the first movement of Elgar's cello concerto. It was called Fourier optics, spatial frequencies instead of distances. There was no mathematical constraint on treating images with the Fourier transform (Fig. 1.4), which was permissible for any phenomenon occurring in nature.[1]

At that time, I only had optical microscopy in my thoughts as a potential future researcher, that its transforming real images into microscope images that I could process in the so called Fourier domain, the subject of a book by Joseph W. Goodman that was the object of my first professional purchase, made with the proceeds of my first salary.[2]

What the eyes do not see of living things are details, because they are too fine, and molecules, because biological molecules, for the most part, do not absorb visible radiation, so they are unable to produce contrast. Biological molecules behave more or less like a crystal glass. Visible light, just think of it as the electromagnetic wave that propagates, enters the crystal glass and exits unaltered in amplitude and slowed down in time. We catch the intensity but not the slowing down, since the speed of light is quite high. It was determined by Ole Rømer, Galilei's disciple, in 1676 while he was working at the royal observatory in Paris directed by Gian Domenico Cassini, who together with Robert Hooke in 1664

[1] R.N. Bracewell, The Fourier Transform and Its Applications. (McGraw-Hill, 1986).

[2] J.W. Goodman, *Introduction to Fourier Optics, 3rd ed.*, Roberts & Company, 2005 (McGraw Hill, 1968).

Fig. 1.4 An image and its Fourier transform in two dimensions. The diagonal momentum to the right is "flipped" in the transform at the top left. The central bright spot, coordinate 0-0, collects all the "energies" in play distributed over the various frequencies

discovered Jupiter's Great Red Spot and, in about 1690, was the first to observe the differential rotation of Jupiter's atmosphere.

The fact that biological molecules do not absorb or absorb very little light in the visible region of the electromagnetic spectrum (Fig. 1.5) is reasonable if you think that we are under visible light for most of the day. If the radiation were absorbed, we would be "cooked like chickens on a spit". As soon as we move out from the visible region, for example into the ultraviolet, the radiation can be absorbed. Molecules such as DNA and a large part of proteins absorb energy in the ultraviolet and the consequent rise in temperature could deteriorate them. The sun, being outdoors, is pleasant but, as always, excessive exposure also exposes us to ultraviolet radiation which, when