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Chemical Imaging Analysis

**FREDDY ADAMS**  
**AND CARLO BARBANTE**

Comprehensive Analytical Chemistry  
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# Chemical Imaging Analysis

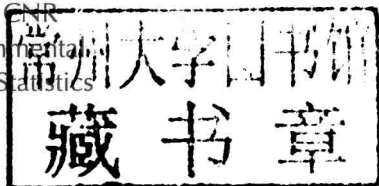
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**Freddy Adams**

Department of Chemistry  
University of Antwerp  
Wilrijk, Belgium

**Carlo Barbante**

Institute for the Dynamics of  
Environmental Processes - CNR  
and Department of Environmental  
Sciences, Informatics and Statistics  
University of Venice  
Venice, Italy



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# Series Editor's Preface

Chemical imaging is the analytical technique that couples spatial and chemical characterisation. It has been reported to be increasingly important in emerging fields of analytical chemistry such as nanotechnology. It is a basic technique for studying the composition and structure at the nanoscale. In this respect this book by Adams and Barbante is a useful addition to the two recent books on nanotechnology published in this series as volumes 59 and 66, *Analysis and Risk of Nanomaterials in Environmental and Food Samples* and *Gold Nanoparticles in Analytical Chemistry*, respectively.

This book contains 10 chapters that cover different aspects of chemical imaging analysis, which can be considered as an extension of spectroscopic analysis. The various chapters cover applications of imaging analysis to nanotechnology as well as a perfect enhancement of well-known spectroscopic techniques like mass spectrometry, X-ray, electron- and particle-based imaging techniques and optical spectroscopy imaging. When chemical imaging is applied there is the need as well for increasingly complex data evaluation tools based on reliable statistical and chemometric methods. In this sense, advanced chemometric, statistical and image analysis are being combined to enhance the chemical image data collected. This combination will facilitate the presentation and data interpretation of the results being achieved.

The book can be used as an academic text and as a reference book, both for those with more expertise as well as those entering the field of chemical imaging analysis. Overall it covers an important technique increasingly used in Analytical Chemistry with important applications to clinical, pharmaceutical and food. Finally, I would like to thank the authors of this book for their considerable time and effort in preparing these chapters. Without their engagement this reference book on chemical imaging analysis would certainly not have been possible.

**D Barceló**

IDAEA-CSIC, Barcelona and ICRA, Girona, March 9, 2015

Editor in Chief of the *Comprehensive Analytical Chemistry* Series, Elsevier



*De luminoso et opaco*

This is the Latin subtitle of the 5th volume of the treatise on optics by Franciscus Aguilonius, published in Antwerp in 1613 – and illustrated by Peter Paul Rubens – in which the fundamentals of light propagation and the role of distance in the difference in luminosity of light sources are explored. Courtesy Museum Plantin-Moretus, Antwerpen – UNESCO World Heritage.

# Preface

Newly developed technologies often provide interesting new possibilities to study things in a way that was not possible in the past. The past 20 years have witnessed spectacular progress in experimental methodology, opening the way to chemical analysis and characterisation down to the nanometre spatial level. The crossroads between imaging and spectroscopy multiplied the possibilities of spatial and imaging analysis, leading to the creation of an exciting new sub-discipline of analytical chemistry.

The purpose of this book is to provide basic information on state-of-the-art methodologies in chemical imaging analysis. Numerous methods for localised analysis depend on the use of beam techniques, based on the excitation of a particular position in or at the surface of a sample by a number of means, followed by the measurement of details during the de-excitation process. Most of these techniques make use of intense particle or X-ray beams and provide essential information down to the atomic scale, with a sensitivity and specificity that would be impossible with conventional analytical techniques.

Imaging analytical techniques are of great interest in life sciences, and bio-imaging has enabled new approaches for the detailed study of components in individual cells and cell structures. The increasing use of structural and compositional analysis has become the basis of a flexible platform for various biological studies: delivery/uptake in living organisms, high-sensitivity diagnosis, targeting with specificity and therapeutic efficacy. Spatially resolved chemical analysis also provides new opportunities for the study of heterogeneity in composition, a marking feature of many natural phenomena. Finally, 2-D and 3-D spatial analytical techniques have many interesting applications in art and conservation studies.

This book is about localised 2-D and 3-D chemical analysis, with particular emphasis on submicroscopic observation and imaging. Surface analysis does play a role in our book, but not a leading one, as the topic is best left to books exclusively devoted to this technique.

Chapter 1 starts with a general introduction. A discussion of the general features and development of the methodologies follows in Chapters 2 and 3. The peculiar place of nanotechnology in chemical imaging is discussed in Chapter 4. Chapters 5–9, the core of this book, are devoted to various beam analysis techniques covering the main chemical imaging methodologies. The general conclusions in Chapter 10 frame the subject of the book, i.e. ‘chemical imaging’, within the realm of analytical chemistry as a scientific discipline.

Mass spectrometry, covered in Chapter 5, gave a major impulse to the development of an extensive range of instrumental analytical techniques, providing unique coverage of isotopic, elemental and molecular levels on the basis of the ionisation of the sample's elemental and molecular constituents. It developed also into a number of important mass spectrometric imaging methods. Mass spectrometry requires that analytes be converted into gas-phase ions, and the ionisation process is integral to the quality of the observed spectrum. A number of ionisation methods may be used to this purpose, including but not limited to secondary ion mass spectrometry, matrix-assisted laser desorption and laser-ablation inductively coupled plasma mass spectrometry.

As for analytical techniques used in microscopic imaging, synchrotron-based X-ray fluorescence and diffraction emerge as important new methodologies for the characterisation and analysis of diverse materials. They are described in Chapter 6. The simultaneous application of fluorescence and diffraction techniques greatly enhances the study of microscopically heterogeneous materials, providing 3-D elemental and speciation information in a nondestructive manner. An element of particular importance here is that X-rays can penetrate deeply into solid samples and provide tomographic information on their inner structure.

Chapter 7 covers chemical imaging methods in electron microscopy. Over the last two decades, dramatic progress has been achieved in electron microscopy and its instrumentation. Scanning electron microscopy and electron probe microanalysis evolved into widely used imaging tools with a resolving power far superior to that of the optical microscope, while simultaneously providing elemental and structural mapping. Techniques for low-voltage microanalysis of light elements and high-sensitivity analysis at sub-nanometre lateral resolution have become available. Electron-filtered transmission electron microscopy, combining electron spectroscopic imaging and electron energy-loss spectrometry, has become a powerful tool for the study of nanoscale materials and for obtaining elemental maps with a spatial resolution better than 1 nm. The development of high-resolution Scanning Transmission Electron Microscopy enabled the direct observation of isolated atoms.

The ion beam analytical methods covered in Chapter 8 offer a combination of particle-induced X-ray emission, Rutherford backscattering, elastic recoil detection analysis and scanning transmission ion microscopy. Together they form a set of powerful characterisation and analysis tools with considerable potential for chemical imaging and surface analysis. Helium ion microscopy is instrumentally able to provide high-resolution and high-contrast images with excellent depth of field.

Fluorescence and Raman imaging, discussed in Chapter 9, have undergone a strong development due to the control of surface structures at the nanolevel as well as because of the improved understanding of the origin of localised surface plasmon resonance from both a theoretical and practical viewpoint. Super-resolution localisation microscopy provides powerful new capabilities

for probing biological structures at the nanometre level via fluorescence imaging. Methods based on single-molecule localisation and single-particle tracking have enabled the nanoscale imaging with visible light of increasingly complex biological structures.

In order to keep this book reasonably concise, we assume a basic knowledge in chemistry and physics. The basics of particularly well-established techniques are therefore not explained. The relevant information can be found in various handbooks or online. Our primary aim was to be useful to readers with some experience in one or more of the experimental areas of analytical chemistry covered in the book. Moreover, we assume that the text could be relevant to Master's and PhD students from various backgrounds (mainly physics and chemistry, but also engineering and biology).

Although there was no premeditation – we have been working on this project for quite some time – our book appears in the year 2015, designated by the United Nations as the International Year of Light and Light-based Technologies. We hope that the focus on chemical imaging analysis and its practical applications will be stimulating to researchers in this exciting new field.

We are immensely indebted to a number of people who helped us throughout this project. We are particularly grateful to Daniela Almansi for her careful English revision of the various versions of the book; and to Fabio Polo for his competence and patience in drawing all the figures, interpreting our ideas and sometimes even our moods. We also wish to acknowledge the critical revision work provided by some of our colleagues, who helped us improve the shape and target of this book; in particular, we would like to thank Petru Jitaru, Patrizia Canton and Grazia Ghermandi.

Freddy Adams particularly wishes to thank his wife Denise for her patience during the long months of preparation of this book. Carlo Barbante dedicates this work to Paola, Lorenzo and Caterina, the light and energy of his life.

**Freddy Adams, Carlo Barbante**  
May 2015





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