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Axio Hybrid User Manual

Christian Drosten



Axio Hybrid User Manual:

The bh TCSPC Handbook Dr. Wolfgang Becker, 2021-09-01 Time Correlated Single Photon Counting Modules SPC 130EMN SPC 130EMNX SPC 130IN SPC 130INX SPC 150N SPC 150NX SPC 150NXX SPC 160 SPC 160PCIE SPC 180N SPC 180NX SPC 180NXX Detectors Lasers and Peripheral Devices Simple Tau Systems Technical Principles TCSPC Applications FLIM Systems Applications in Life Sciences Clinical FLIM Applications SPCM Software SPCImage NG Data Analysis Software Time correlated single photon counting TCSPC is an amazingly sensitive technique for recording low level light signals with picosecond resolution and extremely high precision TCSPC originates from the measurement of excited nuclear states and has been used since the late 60s 775 1250 For many years TCSPC was used primarily to record fluorescence decay curves of organic dyes in solution Due to the low intensity and low repetition rate of the light sources and the limited speed of the electronics of the 70s and 80s the acquisition times were extremely long More important classic TCSPC was intrinsically one dimensional i e limited to the recording of the waveform of a periodic light signal Light sources ceased to be a limitation when the first mode locked Argon lasers and synchronously pumped dye lasers were introduced For the recording electronics the situation changed with the introduction of the SPC 300 modules of Becker multi module TCSPC systems followed in 1999 Since then the Becker Hickl TCSPC systems became bigger faster and more flexible Recent TCSPC modules like the SPC 150NX or the SPC 180 can be configured for sequential recording imaging or time tag recording by a simple software command Multi module systems like the SPC 134EM and SPC 154 can be used for scanning at unprecedented count rates and acquisition speeds Nevertheless TCSPC still has the reputation to be an extremely sluggish technique unable to record any fast changes in the fluorescence or scattering behaviour of a sample The multidimensional features of modern TCSPC are not commonly understood Thus many users do not make efficient use of their SPC modules However if appropriately used multidimensional TCSPC techniques not only deliver superior results but also solve highly sophisticated measurement problems This handbook is an attempt to help existing and potential users understand and make use of the advanced features of modern TCSPC After an introduction into the bh TCSPC devices and associated detector laser and experiment control modules the principles of advanced TCSPC techniques are described These include multidetector TCSPC multiplexed TCSPC sequential recording techniques scanning techniques parameter tag recording and multi module TCSPC techniques The next chapter describes the architecture of the bh SPC modules A chapter about detectors gives a review of detector principles and of the parameters used to characterise detectors It describes a number of detectors commonly used for TCSPC and gives advice about obtaining best performance from them The implementation of bh SPC devices is described in the next part of the handbook It includes principles and wiring diagrams for typical experiments guidelines for first system setup and advice for system optimisation It describes dead time counting loss and pile up effects detector effects and effects related to the optical system The next chapter of the handbook is dedicated to TCSPC

applications The first part of this chapter describes the measurement of fluorescence and anisotropy decay curves multispectral lifetime experiments recording of transient fluorescence lifetime phenomena and measurements of phosphorescence decay curves The second part of the chapter is dedicated to time resolved laser scanning microscopy It contains sections on a wide variety of fluorescence lifetime imaging FLIM experiments and procedures such as FLIM with various excitation principles excitation sources and detection principles high speed and time series FLIM Z stack FLIM simultaneous fluorescence and phosphorescence lifetime imaging FLIM PLIM fluorescence lifetime transient scanning FLITS and FLIM with special microscope configurations A third part contains FLIM background knowledge Signal to noise ratio acquisition time the effect of counting loss and pile up photobleaching and fluorescence depolarisation on the recorded data The book contains a large chapter on TCSPC applications most of them in Biology It contains sections on FLIM of molecular environment parameters in tissue FLIM based FRET measurements in cells autofluorescence FLIM of biological tissue plant physiology and clinical FLIM applications A section about diffuse optical tomography DOT by NIRS techniques includes breast imaging static and functional brain imaging perfusion measurement in the human brain diffuse tissue spectroscopy and small animal imaging Picosecond photon correlation fluorescence correlation spectroscopy burst integrated fluorescence lifetime techniques and photon counting histogram techniques are reviewed in the next sections The last part of the application chapter gives an review of non biological TCSPC applications like positron lifetime measurement measurement of barrier discharges remote sensing metrological applications and characterisation of detectors The application chapter also includes practical hints about optical systems detectors and other technical aspects of the applications described Another large chapter describes the SPCM operating software of the bh SPC modules It describes the various user interface configurations operation modes the system and control parameters the handling and display of the multidimensional data recorded by the modules and the associated data file structure The TCSPC Handbook also contains a chapter on the SPCImage NG fluorescence decay and FLIM data analysis software It describes the general principles of fluorescence decay analysis the calculation of fluorescence decay parameters and lifetime images by various decay models pseudo global analysis multi wavelength FLIM analysis batch processing of FLIM series and analysis of PLIM data The handbook ends with a list of more than 1200 references related to TCSPC most of them being applications of the bh SPC devices

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Table of Contents Axio Hybrid User Manual

1. Understanding the eBook Axio Hybrid User Manual
 - The Rise of Digital Reading Axio Hybrid User Manual
 - Advantages of eBooks Over Traditional Books
2. Identifying Axio Hybrid User Manual
 - Exploring Different Genres
 - Considering Fiction vs. Non-Fiction
 - Determining Your Reading Goals
3. Choosing the Right eBook Platform
 - Popular eBook Platforms
 - Features to Look for in an Axio Hybrid User Manual
 - User-Friendly Interface
4. Exploring eBook Recommendations from Axio Hybrid User Manual
 - Personalized Recommendations
 - Axio Hybrid User Manual User Reviews and Ratings
 - Axio Hybrid User Manual and Bestseller Lists
5. Accessing Axio Hybrid User Manual Free and Paid eBooks
 - Axio Hybrid User Manual Public Domain eBooks
 - Axio Hybrid User Manual eBook Subscription Services
 - Axio Hybrid User Manual Budget-Friendly Options
6. Navigating Axio Hybrid User Manual eBook Formats
 - ePub, PDF, MOBI, and More
 - Axio Hybrid User Manual Compatibility with Devices
 - Axio Hybrid User Manual Enhanced eBook Features
7. Enhancing Your Reading Experience
 - Adjustable Fonts and Text Sizes of Axio Hybrid User Manual
 - Highlighting and Note-Taking Axio Hybrid User Manual
 - Interactive Elements Axio Hybrid User Manual
8. Staying Engaged with Axio Hybrid User Manual

- Joining Online Reading Communities
- Participating in Virtual Book Clubs
- Following Authors and Publishers Axio Hybrid User Manual
- 9. Balancing eBooks and Physical Books Axio Hybrid User Manual
 - Benefits of a Digital Library
 - Creating a Diverse Reading Collection Axio Hybrid User Manual
- 10. Overcoming Reading Challenges
 - Dealing with Digital Eye Strain
 - Minimizing Distractions
 - Managing Screen Time
- 11. Cultivating a Reading Routine Axio Hybrid User Manual
 - Setting Reading Goals Axio Hybrid User Manual
 - Carving Out Dedicated Reading Time
- 12. Sourcing Reliable Information of Axio Hybrid User Manual
 - Fact-Checking eBook Content of Axio Hybrid User Manual
 - Distinguishing Credible Sources
- 13. Promoting Lifelong Learning
 - Utilizing eBooks for Skill Development
 - Exploring Educational eBooks
- 14. Embracing eBook Trends
 - Integration of Multimedia Elements
 - Interactive and Gamified eBooks

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