Advanced Techniques for Single Molecule Experiments and Their Applications

by

Mehdi Yavuz Yüce

A Thesis Submitted to the
Graduate School of Engineering
in Partial Fulfillment of the Requirements for
the Degree of

Doctor of Philosophy
in
Physics

Koc University

September 2012

Koç University

Graduate School of Sciences and Engineering

This is to certify that I have examined this copy of a doctoral dissertation by

Mehdi Yavuz Yüce

and have found that it is complete and satisfactory in all respects,
and that any and all revisions required by the final
examining committee have been made.

Committee Members:

_____

Assoc. Prof. Alper Kiraz (Advisor)

_____

Prof. Adem Levent Demirel

_____

Assoc. Prof. Özgür Esat Müstecaplıoğlu

_____

Assoc. Prof. Alper Tunga Erdoğan

_____

Assoc. Prof. Selçuk Aktürk

Date: _____

ABSTRACT

In this work, fluorescence properties and video-based tracking of single molecules were studied using time-resolved far field optical detection techniques.

A room temperature setup with both wide-field microscopy and sample-scanning confocal detection systems, and a cryostat setup for low temperature experiments were developed. The range of possible measurements includes time correlated single photon counting (TCSPC), fluorescence correlation spectroscopy (FCS), analysis of fluorescence spectra, and wide-field- and confocal microscopies. Proper operation of the setups was verified by sample data acquired from model systems. The room temperature setup was then put into use to address original topics.

As the first study, a well-known dye molecule terrylene was doped into a relatively unexplored host anthracene, and characterized using confocal detection techniques. Fluorescence lifetime, molecular orientation, detection rate at saturation, intersystem crossing rate, and photostability of terrylene were investigated on single molecule level. This dye-host combination was shown to be a promising sample for future room temperature single molecule experiments.

The second study was about single molecule tracking. A new imaging model was developed that takes into account the motion of a molecule during the exposure time of a frame. This extended imaging model better represented the actual data generation process and improved the accuracy of position estimates. It also allowed molecules' velocity to be estimated from an individual frame. The position and velocity data were further processed in a Kalman filter to recover the trajectory of a single molecule.

The last part of the thesis involved characterization of avalanche photodiode detectors (APD), which are commonly used in single molecule experiments. APDs with different brands and/or manufacturers were compared in terms of their dynamic range, dark count rate, linearity, and stability. Evaluation boards were used to study signal to noise ratio as functions of temperature and bias voltage.

ÖZET

Bu çalışmada, tek moleküllerin fulorasan özellikleri ve video-tabanlı izlenmesi zaman çözünürlüklü uzak alan optik algılama teknikleri kullanılarak incelenmiştir.

Geniş alan mikroskopisi ve örnek taramalı eşodaklı agılama yapılabilen bir oda sıcaklığı düzeneği ile düşük sıcaklık deneyleri için düşünülmüş bir kriyostat düzeneği geliştirilmiştir. Mümkün olan ölçümler arasında zaman bilgili tek foton sayımı (TCSPC), fulorasan korelasyon spektroskopisi (FCS), fulorasan tayfların analizi, ve geniş alan- ve eşodaklı mikroskopileri yer almaktadır. Düzeneklerin doğru çalıştığı model sistemlerden alınan örnek verilerle doğrulanmıştır. Sonrasında, oda sıcaklığı düzeneği orjinal araştırma konuları için kullanılmıştır.

İlk çalışma olarak iyi bilinen bir boya molekülü olan terilen nispeten az çalışılmış bir matris olan antrasin içine katkılanmış, ve eşodaklı agılama teknikleri kullanılarak karakterize edilmiştir. Terilenin fulorasan ömür süresi, moleküler yönelim, doygunluk algılama hızı, sistem arası geçiş hızı, ve fotostabilite özellikleri tek molekül düzeyinde incelenmiştir. Bu boya-matris eşlemesinin gelecekteki oda sıcalığı tek molekül deneyleri için ümit verici bir örnek olduğu gösterilmiştir.

İkinci çalışma tek molekül izlemeyle ilgili olmuştur. Molekülün bir kare zamanı içindeki hareketini dikkate alan yeni bir görüntüleme modeli geliştirilmiştir. Bu genişletilmiş model gerçek veri oluşma işlemini daha iyi temsil etmiş, ve konum tahminlerinin doğruluğunu iyileştirmiştir. Bu model aynı zamanda moleküllerin hızlarının tek bir kareden tahmin edilebilmesini de mümkün kılmıştır. Konum ve hız verileri daha sonra bir Kalman filtresinde işlenerek molekülün izlediği yol çıkarılmıştır.

Tezin son bölümü tek molekül deneylerinde sıklıkla kullanılan çığ fotodiyot algılayıcıların (APD) karakterizasyonunu içermiştir. Değişik marka ve/veya modellerdeki APDler dinamik aralık, karanlık sayı, doğrusallık, ve kararlılık yönünden karşılaştırılmıştır. Model devreler kullanılarak sinyal gürültü oranının sıcaklık ve ters gerilime bağlı değişimi incelenmiştir.

ACKNOWLEDGEMENTS

I would like to express my gratitude to Professor Kiraz for his guidance throughout this study, his honesty, and the comfortable working environment he has provided to me. With separation imminent, I wish him a peaceful life.

I would also like to thank my thesis monitoring committee members Professor Demirel and Professor Müstecaplıoğlu, and my thesis defense committee members Professor Erdoğan and Professor Aktürk for the time they spent during evaluating my progress and thesis.

My sincere thanks are extended to past and present friends, colleagues, and staff at Koç University, whose names I list alphabetically: M. Aas, R. Asif, N. Atağ, C. Aydoğmuş, A. Baş, E. Beyatlı, S. Çakmak, H. Çankaya, M. Eryürek, U. S. Gökay, M. Güler, M. Gündoğan, A. Jonas, Y. Karadağ, İ. Karakaş, B. Kaya, A. Kurt, İ. Küçükkara, M. Mestre, F. Solğun, N. Taşaltın, Y. Uysallı, N. Vardar, N. Yılmaz, H. Yılmaz, H. Yılmaz, İ. Yorulmaz, and M. Yorulmaz. I apologize in advance to those whose names were forgotten in this list.

I also thank F. Hacızade, A. Hasekioğlu, and M. A. Can from TÜBİTAK BİLGEM for their collaboration. Their continued interest has been a source of motivation for me.

This work was partially supported by the Ministry of Development under Grant No. 2009K120200, and TÜBİTAK Ph.D. scholarship BİDEB-2211.

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NOMENCLATURE

ZPT	Zero phonon transition.
PSB	Phonon side band.
SNR	Signal to noise ratio.
TIRF	Total internal reflection.
NA	Numerical aperture.
PALM	Photo-activated localization microscopy.
STORM	Stochastic optical reconstruction microscopy.
APD	Avalanche photodiode detector.
TAC	Time to amplitude converter.
PMMA	Poly (methyl methacrylate).
FCS	Fluorescence correlation spectroscopy.
DBT	7.8,15.16-dibenzoterrylene.
IRF	Instrument response function.
FSTL	Full-scale time limit.
τ_F	Fluorescence lifetime.
τ	Delay time.
$g^{(2)}(\tau)$	Normalized intensity autocorrelation function.
PVA	Poly (vinyl alcohol).
FWHM	Full width at half maximum.
SMT	Single molecule tracking.
MLE	Maximum likelihood estimator/estimation.
T	Exposure time.
(x_0,y_0)	Initial position of a moving molecule.
(x_c,y_c)	Mid-frame-time position of a moving molecule.
(v_x,v_y)	Velocity components of a moving molecule.

λ_0	Rate of the Poisson process that models photon emission from a molecule.
λ_{bg}	Rate of the Poisson process that models photon emission from background.
$g(x,y)$	Point spread function.
L	Log-likelihood function.
^	Estimator, or a particular estimate.
CRLB	Cramer Rao lower bound.
FIM	Fisher Information Matrix.
$\mathbf{x}$	State vector for Kalman filter.
$\mathbf{y}$	Measurement vector for Kalman filter.
Q	Process noise covariance matrix.
R	Measurement error covariance matrix.