Limit of the accuracy of parameter estimation for moving single molecules

Wong, Yau

2012

Wong, Y. (2012). Limit of the accuracy of parameter estimation for moving single molecules.
Doctoral thesis, Nanyang Technological University, Singapore.

<https://hdl.handle.net/10356/50783>

<https://doi.org/10.32657/10356/50783>

Limit of the Accuracy of Parameter Estimation for Moving Single Molecules

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A thesis submitted to the Nanyang Technological University
in partial fulfilment of the requirement for the degree of
Doctor of Philosophy

2012

Acknowledgements

First and foremost, I would like to express my heartfelt gratitude to my supervisor, Associate Professor Lin Zhiping, for his guidance and patience during the course of my research work.

I would also like to thank my co-supervisor Professor Raimund J. Ober of the University of Texas at Dallas, for his valuable inputs and comments during the course of my research work.

I would also like to thank the management staff of Nanyang Polytechnic for their support, without which this would not have been possible.

Last but not least, I would like to thank my wife, Phua Mui Luan, for being there for me.

This has indeed been a long, tumultuous but enjoyable journey of life!

Summary

This thesis presents the research work done in determining a benchmark from which the accuracy of the parameter estimates of the attributes of moving molecules imaged by single-molecule fluorescence microscopy can be measured against. The focus is on moving single molecules since a sound theoretical basis for determining the limit of the accuracy of the parameter estimates is lacking whereas one exists for the stationary single molecules. Since accuracy in parameter estimation is closely related to the Fisher information matrix, we have derived expressions of the Fisher information matrix showing its dependence on the trajectories of the molecules. From these Fisher information matrices, we have obtained the Cramér-Rao Lower Bound which provides us with the limit of the accuracy of the parameter estimates.

We first derived expressions of the Fisher information matrix for the case where the image of a moving molecule is acquired in a single exposure. This is subsequently extended to the case where the images of the moving molecule are acquired in a sequence of frames. The case where molecules moving in proximity to one another is also considered. Common to all three cases is that no prior knowledge of the parameters is available. Hence, we have also addressed the issue of prior knowledge of the parameters since it is known in estimation theory that prior knowledge can improve the Cramér-Rao Lower Bound.

For specific conditions and trajectories, we are able to obtain explicit expressions of the limit of the accuracy of the parameter estimates from the Fisher information matrix derived. Not only does it provide us with a convenient means of determining the benchmark, it also enables us to study the impact of important optical and physical parameters without being confounded by the influence of experimental conditions.

Through simulations, we have studied both the ideal and practical cases where images of the moving molecule are acquired with a non-pixelated detector and a pixelated detector, respectively. Comparisons are made between the two cases from which we gain valuable insights into how experimental conditions such as pixel size of the detector, readout noise of the camera, etc., affect the limit of the accuracy of the parameter estimates. Various scenarios are also considered to enhance our understanding of how the limit of the accuracy of the parameter estimates is affected by parameters that describe the trajectory of the molecule. Even though the results obtained here are essentially for the single-molecule fluorescence microscopy, they can be extended to the general problem of tracking an object using quantum limited detectors.

Notation

$\mathcal{G}$	Image detection process
$\mathcal{C}$	Non-pixelated detector
$\mathbf{I}(\theta)$	Fisher information matrix
Λ_θ	Intensity function or photon detection rate that is dependent on θ
$f_{\theta,\tau}$	Probability density function or photon distribution profile that is dependent on θ and τ , which is the time point of a detected photon
$z_\theta(\tau)$	Focus level of the object of interest
$o_\theta(\tau)$	Orientation of the object of interest
$q_{z_\theta(\tau),o_\theta(\tau)}$	Image function which is dependent on $z_\theta(\tau)$ and $o_\theta(\tau)$
M	Magnification
$(x_\theta(\tau), y_\theta(\tau))$	Time dependent trajectory of the object of interest
ϕ	Direction of movement of the object of interest
v	Speed of the object of interest
(x_0, y_0)	Starting location of the object of interest
δ	Fundamental limit
(x_c, y_c)	Coordinates of the center of the circular trajectory
R	Radius of the circular trajectory

ω	Angular velocity of the object of interest
ψ_0	Angular offset of (x_0, y_0) from the x -axis
T_p	Time period
$\mathcal{C}_p$	Pixelated detector
N_p	Total number of pixels that constitute $\mathcal{C}_p$
$\mu_{\theta,k}$	Mean of the number of detected photons at the k^{th} pixel due to the object of interest for the time interval $[t_0, t]$
β_k	Mean of the number of detected photons at the k^{th} pixel due to the background component for the time interval $[t_0, t]$
K	Prior knowledge matrix
N_f	Total number of frames in the movie sequence
t_{ue}	Duration of the exposure interval
t_{uf}	Duration of the frame interval
T_{tat}	Duration of the total acquisition time
F	Acquisition frame rate
$\delta \mathbf{I}(\theta)$	Difference matrix

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Chapter 1

Introduction

1.1 Background and Motivations

Optical microscopy, also referred to as light microscopy, is widely used in cell biology to observe the fine details of biological samples. However, the need to achieve specificity and greater contrast among the observed details give rise to fluorescence microscopy and this radically changes the way optical microscopes are used in the study of cellular structure and its functions. In general, the objects of interest are labelled with fluorescent dye/protein and are illuminated and excited with a light source at a specific wavelength range. As a result of the excitation, the fluorescent dye/protein re-emits photons at another, typically longer, wavelength, which is then detected as the signal of interest. By analyzing the acquired data, we can therefore gain greater insights that lead to a better understanding of the complex biological system.

Previously in fluorescence microscopy, cellular imaging studies using fluorescent-labelled proteins were bulk studies since a substantial amount of fluorescence from the proteins is required in order for the signal to be detectable. Hence the results obtained are the average behaviours of vast numbers of molecules thereby masking subtle individual variations within [Wei99][MF03]. However, in the study of heterogeneous systems of the living cells, such individual variations may be particularly important. With recent technical advances in fluorescence microscopy, it has extended the use of fluorescent probes attached to macromolecules to that of a single molecule [HS07, JBI⁺08]. To this end, a single dye molecule is attached to a specific location in the molecule of interest and then followed in a number of ways to report on its behaviour. It has become abundantly clear that the ability to measure the distribution of behaviour, as opposed to only the average behaviour, provides insight in cases where static or dynamic heterogeneity is present, such as in the complex biological system. In other words, it provides quantitative information on the behaviour of molecules in cells, which is seldom available through bulk studies due to averaging effects [YXR⁺06, LKK01, YFM⁺03, PHBV97]. Other than providing information on the behaviour of individual molecules, these single molecule studies also circumvent the need to synchronize the large numbers of molecules at a certain stage of a biochemical process, which is difficult and often impossible to attain [ZvH06]. Thus, in recent years, single-molecule fluorescence microscopy has become an important biological research tool in cell biology, biochemistry and biophysics and is experiencing a rapid growth in its applications [ZvH06, MF03, SSHS00, Wei99, Moe07, VAVU06].

In cellular imaging studies, other than to observe cellular structures, biologists are also interested in cell dynamics. This has evolved from snap shots of

fixed specimens to the current state where we can monitor in real time the evolution of processes in living cells. Thus, by observing and following individual particle ranging from organelles to protein molecules as they move in the cell, we can begin to unravel the mechanisms driving the motion of these cellular components [SMW⁺99, DV05, KKKP00]. To follow the position of individual particles in time, Single Particle Tracking (SPT) approaches were developed. The particle position can be obtained by either fitting an Airy or Gaussian distribution function to its intensity profile or by center of mass calculation. Repeating this procedure frame by frame, the trajectory of the particle can then be reconstructed and analyzed to extract quantitative information about the mechanism involved in the motion of the particles [QSE91, SJ97, CWG01, SDH05].

However, one of the fundamental issues in the quantitative analysis of single-molecule fluorescence microscopy data concerns the estimation accuracy of the attributes of the moving single molecule. It has been shown that in analyzing the diffusion behaviour of single molecules, localization error of the single molecule locations due to the noisy measurements, is misinterpreted as sub-diffusion in a homogeneous system that only allows normal diffusion [MFK02]. In addition, the molecular motion during the finite acquisition time also contributes to the localization error [SD05]. Therefore, it is essential to have a benchmark from which the accuracy of the parameter estimates of the molecules of interest can be measured against. This benchmark can also serve as a quantitative measure for experimentalists to optimize their experimental setups or to evaluate the various single-particle tracking algorithms commonly used in single-molecule fluorescence microscopy [CWG01].

One of the earlier works done in the characterization of the localization accuracy is by Bobroff [Bob86]. He used the chi-square statistic as the basis for

his analysis. By fitting the model instrument response to the observed data using the least squares method, the position of the particle can be determined by optimizing the fit between the model and data. Because the chi-square reflects the likelihood that a parameter is correct, it can be used to set well-defined statistical error limits on a fitted parameter. Therefore, the maximum positional deviation from the value of the fitted parameter for a specified confidence level is the limit of the accuracy of the parameter estimates or the benchmark. Similar approaches using the least-squares criterion were also adopted by Kubitscheck *et al.* [KKKP00], Thompson *et al.* [TLW02] and Mortensen *et al.* [MCSF10]. However, the use of the least-square criterion is problematic if applied to probability distributions that are not compatible with this criterion. This criterion is ideally suited to estimate parameters from data that has a Gaussian probability distribution because in that case, the least-squares estimate is a maximum likelihood estimate [kay]. It does, however, appear problematic to assume that single molecule data is in fact Gaussian distributed. In recent years, an approach based on the well-established statistical theory concerning the Fisher information matrix has been used by Ober and Ram *et al.* to characterize the localization accuracy [ORW04, RWO06b]. Using this approach, they have obtained the Cramér-Rao Lower Bound (CRLB) which provides a theoretical limit of the accuracy of parameter estimates for single molecules which cannot be surpassed by any unbiased estimator. Subsequently, they extended this approach to the case of a stationary object in the three-dimensional (3D) space [RWO]. Furthermore, they applied the approach to the problem of resolution in 3D microscopy [RAWO, CRA⁺09]. Contrast with the work done for the two-dimensional (2D) space, no closed-form expression of the limit of the localization accuracy can be found. Other than these theoretical studies, most work in 3D imaging to date

centered on the introduction of novel techniques and algorithms to enhance the accuracy of the parameter estimates, i.e., multi-focal plane microscopy, off-focus imaging, optical astigmatism, etc., [RCP⁺, SJF03, HWBZ08, RPC⁺08]. Moreover, the existing work done do not consider the limit of the accuracy.

While a sound theoretical basis has been established for the localization accuracy of stationary objects, the case of moving objects that is ubiquitous in SPT experiments has been largely neglected [DNB11]. Hence, the problem of the accuracy of estimating the parameters of a moving object is the primary focus of this thesis.

1.2 Objectives

The objectives of our work are as follows.

- To investigate the limit of the accuracy of parameter estimation where images of a moving object are acquired by a non-pixelated detector of infinite size in the absence of extraneous noise. This is to be achieved by using a statistical approach based on the Fisher information matrix since the recorded images are stochastic in nature. Various scenarios of commonly encountered situations in the conduct of the single-molecule fluorescence microscopy experiments are to be considered. These scenarios include those where the image of the moving object or two moving objects are acquired in a single exposure interval and also where the images of the moving object are acquired in a sequence of frames. Prior knowledge of object's attributes is also to be considered.
- To investigate the Fisher information matrix, from which the limit of the

accuracy of the parameter estimates is obtained, for the case where images of the moving object are acquired by a pixelated detector of finite size. It is to be based upon the same statistical approach used in the case where the images of the moving object are acquired with a non-pixelated detector. The influence of extraneous noise on the Fisher information matrix is also considered.

- To conduct simulations in order to gain insights into the influence of experimental conditions and various attributes of the moving object on the limit of the accuracy of the parameter estimates for different scenarios. Experimental conditions such as the acquisition frame rate, the exposure interval, the measurement noise of the camera, the pixel size of the detector, magnification, etc., are to be considered. Attributes of the moving object to be considered are its starting location, its speed, its direction of movement, etc.

1.3 Major Contributions

The major contributions from the research work done are as follows.

1. General expressions of the Fisher information matrix in terms of the trajectory of the object are derived for the case where the image of a moving object is acquired during a single exposure interval. In the case where an object moves in the 2D focus plane with either a linear or circular trajectory, explicit CRLB expression, which provides the limit of the accuracy of the parameter estimates, is derived. We have shown that when the image of a linearly moving object is acquired with a non-pixelated

detector, the limit of the localization accuracy of the parameter estimates obtained is twice that of a stationary object. From the simulations performed, we have shown how the extraneous noise, pixelation, parameters of the detection system and parameters of the trajectory affect the limit of the accuracy. In the case where the image of the object is acquired by a pixelated detector, the limit of the accuracy is dependent on the trade-off between the number of pixels that sample the image and the number of detected photons per pixel and these in turn are influenced by the speed of the object.

2. In estimation theory, it is known that prior knowledge of parameters can improve the CRLB. Hence, we have formulated a general expression of the Fisher information matrix in terms of the prior knowledge matrix, the image function and the object trajectory. Applying this expression to an object moving linearly in the 2D plane for two distinct cases of prior knowledge, explicit CRLB expressions are derived. From these expressions, we have shown that the improvement in the limit of the localization accuracy is dependent on the availability of the prior knowledge of either the direction of movement or speed of the object.
3. General expressions of the Fisher information matrix in terms of the trajectory of the object are derived for the case where the images of the moving object are acquired in a sequence of frames. We have shown that the Fisher information matrix is dependent on the acquisition frame rate. However, when the images are acquired by a non-pixelated detector, it is independent of the acquisition frame rate when the exposure interval is equal to the frame interval. Using an example of an object moving lin-

early in the 2D space, we have derived an explicit expression of the limit of the accuracy of the parameter estimates in terms of the acquisition frame rate, the duration of the exposure interval and the duration of the total acquisition time for the case where the images are acquired with a uniform exposure interval. This greatly simplifies the calculation of the limit of the accuracy of the parameter estimates. When the images are acquired with a pixelated detector with the exposure interval being equal to the frame interval, we have analytically shown that in the absence of extraneous noise, there exists a Fisher information matrix that provides a practical limit that is better than or at least as good as that acquired with a single frame for the same total acquisition time. Similarly, we have shown that by doubling the number of frames used in the image acquisition, a Fisher information matrix exists that provides a practical limit that is better than or at least as good as that prior to the increase in number of frames used. From simulations, we have shown how the basic experimental conditions and extraneous noise affect the limit of the accuracy. In the presence of extraneous noise, there exists an acquisition frame rate where an optimal parameter estimation accuracy is attained. This contrasts with the noise-free case where the accuracy of the parameter estimates improves monotonically with the acquisition frame rate, which is corroborated by our analytical results. Through the effect of exposure interval, we have shown that a higher readout rate of the camera system is necessary for better parameter estimation accuracy. It is also shown that for a fixed acquisition frame rate, the rate of improvement of the parameter estimation accuracy reduces as the total acquisition time increases. Finally, the choice of the resolution of the pixelated detector also has a

profound effect in achieving the best possible accuracy in the presence of extraneous noise.

4. General expressions of the Fisher information matrix in terms of the trajectories of two closely spaced objects are derived for the case where their images are acquired simultaneously in a single exposure interval. These expressions can also be easily adapted to the case where either one or both of the objects are stationary. Hence, it is a generalization of the case of two stationary objects investigated by Ram *et al.* [RWO12]. In the case where the images are acquired by a pixelated detector, we have shown through simulations that the proximity of an adjacent object has a profound effect on limit of the accuracy of the parameter estimates. When their photon detection rates are different, the object with the lower photon detection rate has a poorer limit of the accuracy of the parameter estimates. It is also poorer when compared to the case where both objects have equal photon detection rate. The spread of the image of the two objects should also be taken into account as it has been shown to significantly affect the limit of the accuracy of the parameter estimates when they are different.

The practical implications of the results obtained are as follows. In the case where images of the object are acquired by a non-pixelated detector, the limit of the accuracy of the parameter estimates obtained provides experimentalists with a quantity of what is theoretically possible. In other words, the accuracy of the parameter estimates obtained from single-molecule fluorescence experiments should not surpass this limit without its validity being questioned. When the images of the object are acquired by a pixelated detector, it provides experimentalists with a quantity of what they can reasonably expect to be the best

possible limit of the accuracy of the parameter estimates in practice. In addition, the insights gained from the simulations would enable them to optimize their experimental setups for tracking single molecules in order to achieve the best possible accuracy.

1.4 Overview of the Thesis

The rest of the thesis is organized as follows.

In Chapter 2, we consider the case where the image of a moving molecule is acquired in a single exposure interval. Expressions of the Fisher information matrix are derived for cases where the image of the moving object is acquired by a non-pixelated detector and a pixelated detector, respectively. From the expressions derived, we obtain the limit of the accuracy of the parameter estimates. Through simulations, we investigate the influence of experimental conditions and parameters of the trajectory on the limit of the accuracy of the parameter estimates.

In Chapter 3, we study the influence of prior knowledge on the CRLB of the estimates of the parameters that describe the trajectory of a single molecule. We formulate a general expression of the Fisher information matrix that takes into account the prior knowledge of the parameters. Two distinct cases of prior knowledge are considered for an object moving linearly in the 2D plane.

In Chapter 4, we extend upon the work done in Chapter 2 to the case where images of a moving molecule are acquired in a sequence of frames. The general expression of the Fisher information matrix is re-formulated to account for the data acquired in a sequence of frames for two cases. One where the images are acquired by a non-pixelated detector and another by a pixelated detector.

From the former case, we obtain expressions of the limit of the accuracy for the case where an object moves linearly in the 2D space. Subsequently, we perform simulations for a number of scenarios in order to gain insights into how experimental conditions and the extraneous noise affect the limit of the accuracy of the parameter estimates.

In Chapter 5, we consider the case where images of two closely spaced molecules, moving independently of one another, are acquired simultaneously in a single exposure interval. General expressions of the Fisher information matrix for the estimation problem that underlies the acquisition of the images of two closely spaced objects are derived. We illustrate through an example how the expression of the Fisher information matrix can be adapted for the case where one of the molecules is stationary. Subsequently, we perform simulations for a number of scenarios in order to gain insights into how experimental conditions and parameters of the trajectory affect the limit of the accuracy of the parameter estimates.

Finally, conclusions and recommendations for future work are given in Chapter 6.

For convenience of reading, proofs for theorems, corollaries, etc., are given in the Appendix at the end of the thesis.

Chapter 2

Limit of the Accuracy of Parameter Estimation for Moving Single Molecules Acquired in a Single Exposure

2.1 Introduction

It is well known that in single-molecule fluorescence microscopy, the detection process of the emitted photons from the fluorescent-labelled molecules is inherently a random phenomenon and its recorded image is stochastic in nature. Hence, this gives rise to the localization error when estimating the parameters that are relevant to the biological questions underlying the study. In the dynamic cellular and molecular processes, the molecular motion of the molecules further contributes to the localization error. Since the localization error may

lead to the misinterpretation of the acquired data [MFK02, SD05], it is therefore important to have a benchmark for which the accuracy of the parameter estimates could be measured against.

In this chapter, we apply the general framework developed in [RWO06b, ORW04] to the case of a moving point source, which is used to model a single molecule here. Since the molecule comprises a collection of point masses, such as electrons and nuclei, connected by rigid bonds, it can therefore be considered a rigid body. Like the observation of real-time production of single protein molecules in individual *Escherichia Coli* cells, the image of the moving point source is acquired in a single exposure [YXR⁺06]. Acquiring the image in a single exposure is also relevant to the method of obtaining the diffusion coefficient by Schuster *et al.* [SCvB02], which is based on the size of the single-molecule spot in each individual frame of the video sequence. The Fisher information matrix from which the performance limit that quantifies the capabilities of an optical microscope is determined is expressed in terms of the image function and object trajectory. Explicit CRLB expressions are obtained for a moving single object with the object trajectory being either linear or circular. In the case of a 2D pixelated detector, we show through simulations how extraneous noise sources, pixelation, parameters of the detector system and parameters of the trajectory affect the performance of an optical microscope. Some of the results obtained are unique to a moving point source as no counterparts exist for a stationary point source [RWO06b, ORW04]. For example, in the case of a linear trajectory, the practical limit of the accuracy for the estimation of the starting location depends not only on the acquisition time, but also on the speed of the moving point source. In the case of a circular trajectory, for certain starting points, there is a noticeable disparity between the limits of the localization

accuracy for estimating the coordinates of its center x_c and y_c when the radius of the circular trajectory is small.

The organization of this chapter is as follows. In Section 2.2, we consider an object moving in the 3D space and derive expressions of the Fisher information matrix for the case where the image is acquired by a non-pixelated detector of infinite size. We then consider an object moving in the 2D focus plane with either a linear or circular trajectory, and obtain explicit expressions for the fundamental limit of the accuracy of parameter estimation for specific image functions. In Section 2.3, we consider a pixelated detector of finite size and derive the general expression of the Fisher information matrix for two stochastic models when various types of noise are present. In Section 2.4, we investigate the effect of extraneous noise sources, pixelation, parameters of the detector system and parameters of the trajectory on the limit of the accuracy of the parameter estimates. Conclusions are presented in Section 2.5. Proofs are given in the Appendix at the end of the thesis. The work done in this chapter has been published [J1].

2.2 General Framework

In this chapter, we consider an object of interest moving in the object space, imaged by a lens system and its image captured by a detector in the detector space. The photons emitted by the fluorescent-labelled object are detected by the detector during a fixed acquisition time.

From the acquired data, using a specific estimation technique such as the maximum likelihood method, we can estimate object attributes such as the location and orientation and for a moving object, additional attributes such as its

speed, direction of movement, etc. By repeating the experiments we can then calculate their standard deviations, which are the accuracy of these estimates, from their estimated parameter values [KKKP00, CWG01, RWO06b, TLW02]. Since the values of the object attributes obtained are estimates, it is therefore essential to measure the accuracy of these estimates against a benchmark, which is the limit of the accuracy of the parameter estimates. We obtain this benchmark from the Cramér-Rao inequality [Rao65, Kay93, ZLO06, YN06], which states that the (co)variance (matrix) of any unbiased estimator $\hat{\theta}$ of an unknown vector parameter θ is bounded from below by the inverse of the Fisher information matrix $\mathbf{I}(\theta)$, i.e., $\text{Cov}(\hat{\theta}) \geq \mathbf{I}^{-1}(\theta)$. Specifically, the benchmark is obtained by taking the square root of the diagonal elements of the inverse Fisher information matrix for the underlying random process that characterizes the acquired data. It should be noted that the Fisher information matrix is independent of any estimation technique used and only depends on the statistical nature of the acquired data.

Following [RWO06b], the acquired data is modelled as a space-time random process [SM99] which we will refer to as the image detection process $\mathcal{G}$. The temporal part describes the time points of the photons detected by the detector and is modelled as a temporal Poisson process with intensity function Λ_θ . The spatial part describes the spatial coordinates of the arrival location of the detected photons and is modelled as a family of mutually independent random variables $\{\mathcal{U}_\tau\}_{\tau \geq t_0}$ with probability densities $\{f_{\theta,\tau}\}_{\tau \geq t_0}$ defined on the detector $\mathcal{C}$, where τ denotes the time point of a detected photon. The time dependence of the random variables $\{\mathcal{U}_\tau\}_{\tau \geq t_0}$ denotes the fact that the spatial distribution of the detected photons can change with time as is the case with a moving object. Although not explicitly denoted as such, the probability densities $\{f_{\theta,\tau}\}_{\tau \geq t_0}$ can

also depend on the focus level $z_\theta(\tau)$ and orientation $o_\theta(\tau)$, $\tau \geq t_0$, of the object. Throughout the thesis, we let $t_0 \in \mathbb{R}$ and $\theta \in \Theta$, where Θ denotes the parameter space that is an open subset of $\mathbb{R}^n$ with n being the dimension of θ which consists of the location and other attributes of the moving object that are to be estimated. We assume that the spatial and temporal parts of $\mathcal{G}$ are mutually independent of each other and that the probability density function $f_{\theta,\tau}$ satisfies certain regularity conditions that are necessary for the calculation of the Fisher information matrix (see [RWO06b] for details).

In the following theorem and throughout this section, we consider the case of a non-pixelated detector of infinite size, i.e. $\mathcal{C} = \mathbf{R}^2$. This idealized detector provides us with the best case scenario where all the photons from the moving object are detected and pixelation does not deteriorate the accuracy of the photon impact measurements. In addition, we assume that there are no extraneous noise sources that negatively influence the quality of the acquired data. Therefore this ideal scenario allows us to evaluate what is theoretically possible. In Section 2.4, we will consider the practical scenario that also models the experimental factors that are not considered here. Comparison of the results from the two models provides important insights to what extent the specific experimental settings, such as pixel size, array size and the noise levels adversely affect the quality of the estimates. In the following theorem, an expression for the Fisher information matrix is given for data acquired as a space-time random process. It is a slightly simplified version of a more general result presented in [RWO06b].

Theorem 2.1. [RWO06b] *Let $\mathcal{G}(\Lambda_\theta, \{f_{\theta,\tau}\}_{\tau \geq t_0}, \mathcal{C})$ be an image detection process. Assume that the photon distribution rate $\Lambda_\theta(\tau)$, $\tau \geq t_0$, is independent of the parameter vector θ . Then for $\theta \in \Theta$, the Fisher information matrix $\mathbf{I}(\theta)$ of $\mathcal{G}$*

for the time interval $[t_0, t]$ is given by

$$\mathbf{I}(\theta) = \int_{t_0}^t \int_C \frac{\Lambda(\tau)}{f_{\theta,\tau}(r)} \left(\frac{\partial f_{\theta,\tau}(r)}{\partial \theta} \right)^T \left(\frac{\partial f_{\theta,\tau}(r)}{\partial \theta} \right) dr d\tau. \quad (2.1)$$

In the remainder of the section this result will be used to derive expressions for the Fisher information for the estimation of parameters related to moving objects as observed using highly sensitive microscopy techniques. As the next step of the derivation we make the assumption that the photon distribution profile $f_{\theta,\tau}$ can be expressed as a scaled and shifted version of the image of the object since in an optical microscope, the image of an object can often be considered to be invariant with respect to shifts in the object location. In the case of a moving object, $f_{\theta,\tau}$ can then be written as $f_{\theta,\tau}(x, y) = 1/M^2 q_{z_\theta(\tau), o_\theta(\tau)}(x/M - x_\theta(\tau), y/M - y_\theta(\tau))$, $(x, y) \in \mathbb{R}^2$, $\tau \geq t_0$, where $q_{z_\theta(\tau), o_\theta(\tau)}$ denotes an image function, $M > 0$ denotes the lateral magnification and $(x_\theta(\tau), y_\theta(\tau))$, $\tau \geq t_0$, denotes the time dependent trajectory of the object. The image function $q_{z_\theta(\tau), o_\theta(\tau)}$, which is dependent on its focus level $z_\theta(\tau)$ and orientation $o_\theta(\tau)$, describes the image of an object on the detector plane at unit lateral magnification when the object is located along the z -axis in the object space and is assumed to be normalized such that $\int_{\mathbb{R}^2} q_{z_\theta(\tau), o_\theta(\tau)}(x, y) dx dy = 1$, $\tau \geq t_0$.

The following theorem provides a more concrete expression for the Fisher information matrix than that given in Theorem 2.1 by illustrating its dependence on the trajectory of the tracked object.

Theorem 2.2. (Appendix A1) Let $\mathcal{G}(\Lambda, \{f_{\theta,\tau}\}_{\tau \geq t_0}, \mathbb{R}^2)$ be an image detection process. For $\theta \in \Theta$, assume that

(A1) there exists an image function $q_{z_\theta(\tau), o_\theta(\tau)}$ such that for $M > 0$, the pho-

ton distribution profile $f_{\theta,\tau}$ of a moving object is given by $f_{\theta,\tau}(x, y) = 1/M^2 q_{z_\theta(\tau), o_\theta(\tau)}(x/M - x_\theta(\tau), y/M - y_\theta(\tau))$, $(x, y) \in \mathbb{R}^2$, $\tau \geq t_0$,

(A2) $|x_\theta(\tau)|$ and $|y_\theta(\tau)|$ are uniformly bounded for $t_0 \leq \tau \leq t$,

(A3) $\partial q_{z_\theta(\tau), o_\theta(\tau)}(x/M - x_\theta(\tau), y/M - y_\theta(\tau)) / \partial p(\tau)$ exists for $(x, y) \in \mathbb{R}^2$, $z_\theta(\tau)$, $o_\theta(\tau) \in \mathbb{R}$, $\tau \geq t_0$ where $p(\tau) := [x \ y \ z_\theta(\tau) \ o_\theta(\tau)]^T$.

Then for $\theta \in \Theta$, the Fisher information matrix $\mathbf{I}(\theta)$ of $\mathcal{G}$ for the time interval $[t_0, t]$ is given by

$$\mathbf{I}(\theta) = \int_{t_0}^t \Lambda(\tau) V_\theta^T(\tau) \left[\int_{\mathbb{R}} \int_{\mathbb{R}} \frac{\left(\frac{\partial q_{z_\theta(\tau), o_\theta(\tau)}(x, y)}{\partial p(\tau)} \right)^T \left(\frac{\partial q_{z_\theta(\tau), o_\theta(\tau)}(x, y)}{\partial p(\tau)} \right)}{q_{z_\theta(\tau), o_\theta(\tau)}(x, y)} dx dy \right] V_\theta(\tau) d\tau, \quad (2.2)$$

where $V_\theta(\tau) := \left[-\frac{\partial x_\theta(\tau)}{\partial \theta} \quad -\frac{\partial y_\theta(\tau)}{\partial \theta} \quad \frac{\partial z_\theta(\tau)}{\partial \theta} \quad \frac{\partial o_\theta(\tau)}{\partial \theta} \right]^T$, $\tau \geq t_0$.

The inner integral $\int_{\mathbb{R}} \int_{\mathbb{R}} \frac{1}{q_{z_\theta(\tau), o_\theta(\tau)}(x, y)} \left(\frac{\partial q_{z_\theta(\tau), o_\theta(\tau)}(x, y)}{\partial p(\tau)} \right)^T \left(\frac{\partial q_{z_\theta(\tau), o_\theta(\tau)}(x, y)}{\partial p(\tau)} \right) dx dy$ of the Fisher information matrix in Theorem 2.2 is essentially the same as the spatial integral of the time-invariant case [RWO06b]. Thus the time-varying case can be obtained by integrating the time-invariant result over time with weighting functions $V_\theta(\tau)$ and $V_\theta^T(\tau)$. The weighting function $V_\theta(\tau)$ is the derivative of the object trajectory with respect to the parameters concerned. The significance of this theorem is that we can now calculate the Fisher information matrix of the underlying random process that characterizes the acquired data from a moving object by assuming the image function to be stationary in the $x-y$ plane, such that its origin is located along the z -axis, i.e., the optical axis of the objective lens. To calculate the Fisher information matrix of an object moving in 3D space, we simply use the derivative of the parametric expressions

of the object trajectory and its image at the corresponding locations along the optical axis. This expression can be applied to an arbitrary trajectory in 3D space. Note that the image function used is quite general.

In the following proposition, we consider a 2D time-varying case where the image function $q_{z_\theta(\tau), o_\theta(\tau)}$ does not depend on the focus level $z_\theta(\tau)$ and the orientation $o_\theta(\tau)$ and is simply denoted as q . This leads to a further simplification of the expression for the Fisher information matrix.

Proposition 2.1. (*Appendix A2*): *Let $\mathcal{G}(\Lambda, \{f_{\theta, \tau}\}_{\tau \geq t_0}, \mathbb{R}^2)$ be an image detection process. For $\theta \in \Theta$, assume that*

(A1) *there exists a radially symmetric image function q , i.e., $q(x, y) = \tilde{q}(r^2) = \tilde{q}(x^2 + y^2)$, for a function $\tilde{q} : \mathbb{R} \rightarrow \mathbb{R}$, that does not depend on $z_\theta(\tau)$ and $o_\theta(\tau)$ such that for $M > 0$, the photon distribution profile $f_{\theta, \tau}$ of a moving object is given by $f_{\theta, \tau}(x, y) = 1/M^2 q(x/M - x_\theta(\tau), y/M - y_\theta(\tau))$, $(x, y) \in \mathbb{R}^2$, $\tau \geq t_0$,*

(A2) *$\partial q(x, y)/\partial x$ and $\partial q(x, y)/\partial y$ exist for every $(x, y) \in \mathbb{R}^2$.*

Let $(x_\theta(\tau), y_\theta(\tau))$, $\tau \geq t_0$, denote the time dependent trajectory of the object with respect to its starting location (x_0, y_0) . Then for $\theta \in \Theta$, the Fisher information matrix $\mathbf{I}(\theta)$ of $\mathcal{G}$ for the time interval $[t_0, t]$ is given by

$$\mathbf{I}(\theta) = 4\pi \int_0^\infty \frac{r^3}{\tilde{q}(r^2)} \left(\frac{\partial \tilde{q}(r^2)}{\partial(r^2)} \right)^2 dr \int_{t_0}^t \Lambda(\tau) \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix}^T \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix} d\tau. \quad (2.3)$$

The expression of $\mathbf{I}(\theta)$ in Proposition 2.1 is now separable in terms of the spatial and temporal integrals, similar to the case of a stationary object [RWO06b]. The spatial integral includes the image function and its derivative while the

temporal integral includes the photon detection rate and the derivative of the trajectory. Thus, to calculate the Fisher information matrix of an object moving in the 2D focus plane or in a relatively flat structure, we can assume that its trajectory is decoupled from its image. The significance of this expression is that it greatly simplifies the calculation of the Fisher information matrix since it is now simply a product of two entities and hence it can be easily applied to an arbitrary trajectory in the 2D focus plane provided that the image function is radially symmetric. Note that there is a major difference between the expressions of the Fisher information matrix for the time-invariant and the time-varying cases. In the former case, its Fisher information matrix is affected by the image function and the photon detection rate whereas in the latter, in addition to the image function and the photon detection rate, it is also affected by the parameters of the trajectory to be estimated.

We now illustrate the result in Proposition 2.1 by considering specific image functions that describe the image of a moving object, more specifically a moving point source. In accordance to the optical diffraction theory, the intensity distribution of an in-focus point source is best described by the Airy profile. However, due to the good approximation of its central region by the 2D Gaussian profile, the latter is often used as it simplifies the analysis [TLW02, ZZOM07, SY00]. Hence, we will consider two different image profiles, specifically a Gaussian image profile and an Airy profile, for both the linear and circular trajectories. For both trajectories, we use the expression of $\mathbf{I}(\theta)$ in Proposition 2.1 to derive general expressions for the lower bound to the best possible accuracy for the parameters to be estimated. We will also obtain an explicit analytical expression for the lower bound for a special case where the photon detection rate is assumed to be a known constant. Following [RWO06b], this lower bound is re-

ferred to as the fundamental limit of the accuracy for the particular parameter vector, or in short, the fundamental limit. The term fundamental is used to describe the fact that the model which underlies the expressions for calculating the lower bound does not take into account any deteriorating effects of the acquisition system such as pixelation of the detector and the various noise sources that typically occur in experimental settings. The fundamental limit has practical value as it provides us with a quantity of what is theoretically possible in the absence of deteriorating factors and thus serves as a benchmark for practical cases. Since the fundamental limit only takes into consideration the basic optical and stochastic phenomena that are inherent in any single-molecule experiment, it can easily be used to study the impact of the important optical and physical parameters without being confounded by the influence of extraneous parameters such as noise, detector properties, etc. In particular, comparisons with the practical limits (see Section 2.4) allow us to evaluate by how much the experimental conditions, e.g., detector array size, pixel size, readout noise level, deteriorate the theoretically best possible results.

In the following corollary, we consider the case of a linear trajectory where the object moves from a given initial position (x_0, y_0) in the direction of movement ϕ at a constant speed v . We derive the fundamental limits of the estimated parameters for the corresponding time interval $[t_0, t]$, and then specialize the results to the case where the photon detection rate of the image detection process is a known constant.

Corollary 2.1. (*Appendix A3*): Let $\mathcal{G}(\Lambda, \{f_{\theta, \tau}\}_{\tau \geq t_0}, \mathbb{R}^2)$ be an image detection process. The parametric expressions for the linear trajectory of the object for the time interval $[t_0, t]$ are given by $x_{\theta}(\tau) = x_0 + v(\tau - t_0) \cos \phi$, $y_{\theta}(\tau) = y_0 + v(\tau - t_0) \sin \phi$, $t_0 \leq \tau \leq t$. For $\tau \geq t_0$ and $M > 0$, assume that there exists a

radially symmetric image function $q(x, y)$ such that $f_{\theta, \tau}(x, y) = 1/M^2 q(x/M - x_{\theta}(\tau), y/M - y_{\theta}(\tau))$, $(x, y) \in \mathbb{R}^2$ with $q(x, y) = \tilde{q}(r^2) = \tilde{q}(x^2 + y^2)$, for a function $\tilde{q} : \mathbb{R} \rightarrow \mathbb{R}$ and let $\gamma^2 := 4\pi \int_0^\infty r^3 / \tilde{q}(r^2) (\partial \tilde{q}(r^2) / \partial(r^2))^2 dr$.

1. For $\theta = (x_0, y_0, \phi, v) \in \Theta$, the fundamental limit of the localization accuracy δ_{x_0} (δ_{y_0}) of x_0 (y_0), the fundamental limits δ_ϕ and δ_v of ϕ and v are given, respectively, by

$$\delta_{x_0} = \delta_{y_0} = \frac{1}{\gamma} \sqrt{\frac{b_3}{b_1 b_3 - b_2^2}}, \quad \delta_\phi = \frac{1}{\gamma v} \sqrt{\frac{b_1}{b_1 b_3 - b_2^2}}, \quad \delta_v = \frac{1}{\gamma} \sqrt{\frac{b_1}{b_1 b_3 - b_2^2}}, \quad (2.4)$$

where

$$b_1 = \int_0^{t-t_0} \Lambda(\tau+t_0) d\tau, \quad b_2 = \int_0^{t-t_0} \Lambda(\tau+t_0) \tau d\tau, \quad b_3 = \int_0^{t-t_0} \Lambda(\tau+t_0) \tau^2 d\tau. \quad (2.5)$$

Hence, for a 2D Gaussian image function $q(x, y) = 1/(2\pi\sigma^2) \exp(-(x^2 + y^2)/(2\sigma^2))$, $\sigma > 0$, $(x, y) \in \mathbb{R}^2$, $\gamma := 1/\sigma$. As for the Airy image function $q(x, y) = J_1^2(2\pi n_a \sqrt{x^2 + y^2}/\lambda)/(\pi(x^2 + y^2))$, $(x, y) \in \mathbb{R}^2$, $\gamma := 2\pi n_a/\lambda$, where n_a and λ denote the numerical aperture and emission wavelength respectively and J_1 denotes the first order Bessel function of the first kind.

2. If $\Lambda(\tau) = \Lambda_0$, $\tau \geq t_0$, where Λ_0 is a positive constant, then for $\theta = (x_0, y_0, \phi, v) \in \Theta$, the fundamental limit of the localization accuracy δ_{x_0} (δ_{y_0}) of x_0 (y_0), the fundamental limits δ_ϕ and δ_v of ϕ and v are given, respectively, by

$$\delta_{x_0} = \delta_{y_0} = \frac{2}{\gamma \sqrt{N}}, \quad \delta_\phi = \frac{2}{\gamma v(t-t_0)} \sqrt{\frac{3}{N}}, \quad \delta_v = \frac{2}{\gamma(t-t_0)} \sqrt{\frac{3}{N}}, \quad t > t_0, \quad (2.6)$$

where $N := \Lambda_0(t - t_0)$ denotes the expected number of detected photons for the time interval $[t_0, t]$.

From Corollary 2.1, it can be seen that for both the Airy and the Gaussian image functions, the fundamental limits of x_0 , y_0 and v are independent of θ whereas the fundamental limit of ϕ is only independent of x_0 , y_0 and ϕ . When the photon detection rate is assumed to be a constant, the expressions for the fundamental limit of the parameter estimates further simplify to expressions comprising some properties of the photon emission process of the single-molecule, parameters of the detection system and parameters of the trajectory. There are also several interesting common observations for both image functions. The fundamental limit exhibits an inverse square root dependence on the expected number of detected photons. This result is similar to the case of a stationary object [RWO06b]. As for δ_ϕ and δ_v , not only are they inversely proportional to $\sqrt{N}$, the former is also inversely proportional to the distance moved by the object of interest $v(t - t_0)$ while the latter to the acquisition time interval $(t - t_0)$. The fundamental limit of the localization accuracy δ_{x_0} or δ_{y_0} derived here is twice that of δ_{x_0} or δ_{y_0} for a stationary object [RWO06b]. Therefore, in the case of an object moving in a straight line, we are able to estimate the unknown parameters δ_ϕ and δ_v at the expense of reducing the estimation accuracy of x_0 and y_0 .

In the next corollary, we consider the case of a non-linear trajectory, specifically a circular trajectory [CSL00, DRD⁺07]. The object is assumed to start moving at (x_0, y_0) , which is angularly offset at ψ_0 degrees with respect to the x -axis. It revolves at a constant angular velocity ω at a fixed radius R about the center of its trajectory (x_c, y_c) . For the corollary that follows, we derive

the Fisher information matrix for the corresponding time interval $[t_0, t]$ using a similar approach to that of the linear trajectory. We also consider a special case where the length of the time interval $[t_0, t]$ is assumed to equal the period with respect to the angular velocity and the photon detection rate Λ of the image detection process is assumed to be a known constant.

Corollary 2.2. (*Appendix A4*): Let $\mathcal{G}(\Lambda, \{f_{\theta, \tau}\}_{\tau \geq t_0}, \mathbb{R}^2)$ be an image detection process. The parametric expressions for the circular trajectory of the object of interest for the time interval $[t_0, t]$ are given by $x_\theta(\tau) = x_c + R \cos(\omega(\tau - t_0) + \psi_0)$, $y_\theta(\tau) = y_c + R \sin(\omega(\tau - t_0) + \psi_0)$, $t_0 \leq \tau \leq t$, where (x_c, y_c) denotes the center of the circular trajectory, R , ω and ψ_0 , its radius, angular velocity and angular offset of the starting point (x_0, y_0) from the x -axis, respectively. For $\tau \geq t_0$ and $M > 0$, assume that there exists a radially symmetric image function $q(x, y)$ such that $f_{\theta, \tau}(x, y) = 1/M^2 q(x/M - x_\theta(\tau), y/M - y_\theta(\tau))$, $(x, y) \in \mathbb{R}^2$ with $q(x, y) = \tilde{q}(r^2) = \tilde{q}(x^2 + y^2)$, for a function $\tilde{q} : \mathbb{R} \rightarrow \mathbb{R}$. Let $\gamma^2 := 4\pi \int_0^\infty r^3 / \tilde{q}(r^2) (\partial \tilde{q}(r^2) / \partial (r^2))^2 dr$.

1. For $\theta = (R, x_c, y_c, \omega, \psi_0) \in \Theta$, the Fisher information matrix of $\mathcal{G}$ for the time interval $[t_0, t]$ is given by

$$I(\theta) = \gamma^2 \begin{bmatrix} \int_{t_0}^t \Lambda(\tau) d\tau & \int_{t_0}^t \Lambda(\tau) \cos \psi d\tau & \int_{t_0}^t \Lambda(\tau) \sin \psi d\tau & 0 & 0 \\ \int_{t_0}^t \Lambda(\tau) \cos \psi d\tau & \int_{t_0}^t \Lambda(\tau) d\tau & 0 & -\int_{t_0}^t \Lambda(\tau) R(\tau - t_0) \sin \psi d\tau & -\int_{t_0}^t \Lambda(\tau) R \sin \psi d\tau \\ \int_{t_0}^t \Lambda(\tau) \sin \psi d\tau & 0 & \int_{t_0}^t \Lambda(\tau) d\tau & \int_{t_0}^t \Lambda(\tau) R(\tau - t_0) \cos \psi d\tau & \int_{t_0}^t \Lambda(\tau) R \cos \psi d\tau \\ 0 & -\int_{t_0}^t \Lambda(\tau) R(\tau - t_0) \sin \psi d\tau & \int_{t_0}^t \Lambda(\tau) R(\tau - t_0) \cos \psi d\tau & \int_{t_0}^t \Lambda(\tau) R^2 (\tau - t_0)^2 d\tau & \int_{t_0}^t \Lambda(\tau) R^2 (\tau - t_0) d\tau \\ 0 & -\int_{t_0}^t \Lambda(\tau) R \sin \psi d\tau & \int_{t_0}^t \Lambda(\tau) R \cos \psi d\tau & \int_{t_0}^t \Lambda(\tau) R^2 (\tau - t_0) d\tau & \int_{t_0}^t \Lambda(\tau) R^2 d\tau \end{bmatrix},$$

where $\psi := \omega(\tau - t_0) + \psi_0$, $\tau \geq t_0$. In the case of the 2D Gaussian image

function $q(x, y) = 1/(2\pi\sigma^2) \exp(-(x^2 + y^2)/(2\sigma^2))$, $\sigma > 0$, $(x, y) \in \mathbb{R}^2$,

$\gamma = 1/\sigma$. As for the Airy image function $q(x, y) = J_1^2(2\pi n_a \sqrt{x^2 + y^2}/\lambda)/(\pi(x^2 +$

$y^2))$, $(x, y) \in \mathbb{R}^2$, $\gamma = 2\pi n_a/\lambda$, where n_a and λ denote the numerical aper-

ture and emission wavelength respectively and J_1 denotes the first order

Bessel function of the first kind.

2. Let T_p denote the length of the time interval $[t_0, t]$, i.e., $T_p := t - t_0$, and assume that T_p equals the period with respect to the angular velocity, i.e., $T_p = 2\pi/\omega$. Assume also the photon detection rate to be a known constant, i.e., $\Lambda(\tau) = \Lambda_0$, $t_0 \leq \tau \leq t$. Then the fundamental limit of δ_{x_c} (δ_{y_c}) of x_c (y_c) and the fundamental limits δ_R , δ_ω and δ_{ψ_0} of R , ω and ψ_0 are given by

$$\begin{aligned} \delta_R &= \frac{1}{\gamma \sqrt{N_{T_p}}}, \quad \delta_{x_c} = \frac{1}{\gamma} \sqrt{\frac{1}{N_{T_p}} \left(1 + \frac{3 \cos^2 \psi_0}{\pi^2 - 3} \right)}, \quad \delta_{y_c} = \frac{1}{\gamma} \sqrt{\frac{1}{N_{T_p}} \left(1 + \frac{3 \sin^2 \psi_0}{\pi^2 - 3} \right)}, \\ \delta_\omega &= \frac{2\pi}{\gamma R T_p} \sqrt{\frac{3}{N_{T_p}(\pi^2 - 3)}}, \quad \delta_{\psi_0} = \frac{1}{\gamma R} \sqrt{\frac{4\pi^2 - 3}{N_{T_p}(\pi^2 - 3)}}, \end{aligned} \tag{2.7}$$

where $N_{T_p} := \Lambda_0 T_p$ denotes the expected number of detected photons for the period T_p .

The general expression to calculate the Fisher information matrix $\mathbf{I}(\theta)$ of $\mathcal{G}$ corresponding to the time interval $[t_0, t]$ for an object with a circular trajectory is given in the first part of the above corollary. By taking the square root of diagonal elements of the inverse of Fisher information matrix, we obtain the fundamental limits of the parameter estimates. Unlike the linear trajectory case, it seems that no simple analytical expression is available for the fundamental limits in the case of a general circular trajectory.

However, in the special case where the length of the time interval $[t_0, t]$ is the period T_p with respect to the angular velocity and the photon detection rate is a known constant Λ_0 , the fundamental limit of $\theta = (R, x_c, y_c, \omega, \psi_0)$ simplifies to that shown in result 2 of Corollary 2.2 with analytical expressions given in

(2.7). These expressions are given in terms of some properties of the photon emission process of the single-molecule, parameters of the detection system and parameters of the trajectory. Similar to the case of the linear trajectory, the fundamental limits for all the five parameters are dependent on the inverse square root of the expected number of detected photons and independent of the acquisition starting time. However, the fundamental limits of the δ_{x_c} and δ_{y_c} are periodic in nature and dependent on the angular offset of the starting point ψ_0 . On the other hand, the fundamental limits δ_ω , δ_{ψ_0} and δ_R are independent of ψ_0 . Moreover, both δ_ω and δ_{ψ_0} are inversely proportional to the radius of the circular trajectory and in addition, δ_ω is inversely proportional to T_p .

2.3 Effects of Pixelation

So far we have only considered a moving object where its image is acquired by a non-pixelated detector of infinite size without extraneous noise sources. In fluorescence single-molecule microscopy, CCD cameras are commonly used for acquiring images of fluorescent-labelled molecules. The detectors of CCD cameras are of finite size and pixelated, i.e., they consist of a matrix of light sensing elements (pixels) where photoelectrons are accumulated during an exposure interval. We will henceforth refer to it as a pixelated detector of finite size $\mathcal{C}_p$ or in short, just a pixelated detector since a pixelated detector is always of finite size in practice.

As for the acquired data, it comprises the detected photons from the object of interest and noise from a variety of sources. The detected photons from the object of interest and the external background radiation introduce a Poisson signal from the object of interest and from the background component respec-

tively. Hence we let $\mathcal{G}_1(\Lambda_1, \{f_{\theta,\tau,1}\}_{\tau \geq t_0}, \mathbb{R}^2)$ denote an image detection process for the detected photons from the object of interest and $\mathcal{G}_2(\Lambda_2, \{f_{\tau,2}\}_{\tau \geq t_0}, \mathbb{R}^2)$ for the background component. Readout noise, which is characterized as a Gaussian random process, further contributes to the degradation of the images acquired. As such, we consider two stochastic models for the pixelated detector, one purely in terms of Poisson random variables while the other is in terms of Poisson and Gaussian random variables. As for the photon distribution profile $f_{\tau,2}$ of $\mathcal{G}_2$, it is assumed to be independent of the time point τ and is denoted by f_2 .

In the following theorem, we provide expressions to calculate the Fisher information matrix of the acquired data from a pixelated detector in terms of its image function, photon detection rate and object trajectory for two different scenarios: one where its acquired data comprise only Poisson random variables and the other, its acquired data comprise both Poisson and Gaussian random variables.

Theorem 2.3. *Let $\mathcal{G}_1(\Lambda_1, \{f_{\theta,\tau,1}\}_{\tau \geq t_0}, \mathbb{R}^2)$ and $\mathcal{G}_2(\Lambda_2, \{f_{\tau,2}\}_{\tau \geq t_0}, \mathbb{R}^2)$ be two independent image detection processes for the object of interest and the background component, respectively. Let the pixelated detector $\mathcal{C}_p$ be defined as a collection $\{C_1, \dots, C_{N_p}\}$ of open, disjoint subsets of $\mathbb{R}^2$ such that $\bigcup_{k=1}^{N_p} C_k = \mathcal{C}_p$, where N_p denotes the total number of pixels. For $\theta \in \Theta$, assume that*

(A1) *the photon detection rates of $\mathcal{G}_1$ and $\mathcal{G}_2$ are known,*

(A2) *there exists an image function $q_{z_\theta(\tau), o_\theta(\tau), 1}$ such that for $M > 0$, the photon distribution profile $f_{\theta,\tau,1}$ of a moving object is given by $f_{\theta,\tau,1}(x, y) = 1/M^2 q_{z_\theta(\tau), o_\theta(\tau), 1}(x/M - x_\theta(\tau), y/M - y_\theta(\tau))$, $(x, y) \in \mathbb{R}^2$, $\tau \geq t_0$, and for q_2 , which is assumed to be independent of the focus level and the orien-*

tation, the photon distribution profile f_2 of the background component is given by $f_2(x, y) = 1/M^2 q_2(x/M, y/M)$.

1. Let $\mathcal{I}_{\theta,k} = S_{\theta,k} + B_k$, $k = 1, \dots, N_p$, where $S_{\theta,k}$ and B_k are Poisson random variables from the object of interest and background component respectively. For $\theta \in \Theta$, the Fisher information matrix for $\{\mathcal{I}_{\theta,1}, \dots, \mathcal{I}_{\theta,N_p}\}$ for the time interval $[t_0, t]$ is given by

$$\mathbf{I}(\theta) = \sum_{k=1}^{N_p} \frac{1}{v_{\theta,k}} \left(\frac{\partial \mu_{\theta,k}}{\partial \theta} \right)^T \left(\frac{\partial \mu_{\theta,k}}{\partial \theta} \right), \quad (2.8)$$

where

$$\begin{aligned} v_{\theta,k} &= \frac{1}{M^2} \int_{t_0}^t \int_{C_k} \left[\Lambda_1(\tau) q_{z_{\theta}(\tau), o_{\theta}(\tau), 1} \left(\frac{x}{M} - x_{\theta}(\tau), \frac{y}{M} - y_{\theta}(\tau) \right) \right. \\ &\quad \left. + \Lambda_2(\tau) q_2 \left(\frac{x}{M}, \frac{y}{M} \right) \right] dx dy d\tau, \\ \frac{\partial \mu_{\theta,k}}{\partial \theta} &= \frac{1}{M^2} \int_{t_0}^t \Lambda_1(\tau) \int_{C_k} \left(\frac{\partial q_{z_{\theta}(\tau), o_{\theta}(\tau), 1} \left(\frac{x}{M} - x_{\theta}(\tau), \frac{y}{M} - y_{\theta}(\tau) \right)}{\partial p_1(\tau)} \right) dx dy V_{\theta}(\tau) d\tau, \\ p_1(\tau) &:= \begin{bmatrix} \frac{x}{M} - x_{\theta}(\tau) & \frac{y}{M} - y_{\theta}(\tau) & z_{\theta}(\tau) & o_{\theta}(\tau) \end{bmatrix}^T, \\ V_{\theta}(\tau) &:= \frac{\partial p_1(\tau)}{\partial \theta} = \begin{bmatrix} -\frac{\partial x_{\theta}(\tau)}{\partial \theta} & -\frac{\partial y_{\theta}(\tau)}{\partial \theta} & \frac{\partial z_{\theta}(\tau)}{\partial \theta} & \frac{\partial o_{\theta}(\tau)}{\partial \theta} \end{bmatrix}^T. \end{aligned}$$

2. Let $\mathcal{I}_{\theta,k} = S_{\theta,k} + B_k + W_k$, $k = 1, \dots, N_p$, where $S_{\theta,k}$ and B_k are Poisson random variables from the object of interest and background component respectively and W_k denotes the Gaussian random variable with mean η_k and variance $\sigma_{w,k}^2$, which models the measurement noise. For $\theta \in \Theta$, the Fisher information matrix for $\{\mathcal{I}_{\theta,1}, \dots, \mathcal{I}_{\theta,N_p}\}$ for the time interval $[t_0, t]$

is given by

$$\mathbf{I}(\theta) = \sum_{k=1}^{N_p} \left(\frac{\partial \mu_{\theta,k}}{\partial \theta} \right)^T \left(\frac{\partial \mu_{\theta,k}}{\partial \theta} \right) \times \left(\int_R \frac{\left(\sum_{l=1}^{\infty} \frac{[v_{\theta,k}]^{l-1} e^{-v_{\theta,k}}}{(l-1)!} \frac{1}{\sqrt{2\pi}\sigma_{w,k}} e^{-\frac{1}{2} \left(\frac{z-l-\eta_k}{\sigma_{w,k}} \right)^2} \right)^2}{p_{\theta,k}(z)} dz - 1 \right), \quad (2.9)$$

where $v_{\theta,k}$ and $\partial \mu_{\theta,k}/\partial \theta$ are given in (2.8). The Poisson-Gaussian mixture probability density function, $p_{\theta,k}(z)$, is given by

$$p_{\theta,k}(z) = \frac{1}{\sqrt{2\pi}\sigma_{w,k}} \sum_{l=0}^{\infty} \frac{[v_{\theta,k}]^l e^{-v_{\theta,k}}}{l!} e^{-\frac{1}{2} \left(\frac{z-l-\eta_k}{\sigma_{w,k}} \right)^2}, \quad z \in \mathbb{R}, \quad k = 1, \dots, N_p.$$

We define the square roots of the diagonal elements of the inverse of the Fisher information matrix associated with a pixelated detector of finite size as the practical limits of the accuracy for the particular parameter vector, or in short just as the practical limits. The word “practical” is used here to differentiate it from the fundamental limit which is associated with a non-pixelated detector of infinite size. Moreover, for simplicity, the term “limit of the accuracy” is used when we refer to both the fundamental and practical limits.

2.4 Simulation Results

In the following simulations, we use the results of Theorem 2.3 to show how extraneous noise sources, parameters of the detection system and parameters of the trajectory affect the practical limit of the accuracy of θ . The practical

limits will be benchmarked against their corresponding fundamental limits.

Consider a single exposure/image for the time interval $[t_0, t]$. The photon detection rate of a moving point source is assumed to be a known constant, i.e., $\Lambda_1(\tau) = \Lambda_{0,1} \in \mathbb{R}^+$, $\tau \geq t_0$ and its image function to be a Gaussian, i.e., $q_1(x, y) = 1/(2\pi\sigma^2) \exp(-((x/M - x_\theta(\tau))^2 + (y/M - y_\theta(\tau))^2)/2\sigma^2)$, $(x, y) \in \mathbb{R}^2$, $\tau \geq t_0$. The photon detection rate of the background component is also assumed to be a known constant $\Lambda_2(\tau) = \Lambda_{0,2} \in \mathbb{R}^+$, $\tau \geq t_0$, and the detected photons from the background component are assumed to be uniformly distributed. Since we are considering the 2D case where the image function is independence of $z_\theta(\tau)$ and $o_\theta(\tau)$, the column vectors $p_1(\tau)$ and $V_\theta(\tau)$ in Theorem 2.3 are reduced to $p_1(\tau) := \begin{bmatrix} x/M - x_\theta(\tau) & y/M - y_\theta(\tau) \end{bmatrix}^T$ and $V_\theta(\tau) := \begin{bmatrix} -\partial x_\theta(\tau)/\partial \theta & -\partial y_\theta(\tau)/\partial \theta \end{bmatrix}^T$. For both the linear and circular trajectories illustrated in Figure 2.1, we consider the noise-free and the noise-corrupted cases. In our context, noise-free refers to the case where only “Poisson noise” or “shot noise” [Wol97] from the object of interest is present. This noise arises due to the stochastic nature of the acquired data. As for the noise-corrupted case, it includes Poisson noise from the background component and Gaussian noise from the readout process.

2.4.1 Linear Trajectory

In the case of a linear trajectory, we assume that the object commences to move with a constant speed v from a given initial position (x_0, y_0) at an angle ϕ between the linear trajectory and the x -axis. During the acquisition, the image of the object is well within the bounds of the pixelated detector as shown in Figure 2.1a. Its parametric expressions are given by $x_\theta(\tau) = x_0 + v(\tau - t_0) \cos \phi$,

Figure 2.1: Schematic sketch of a linear trajectory and a circular trajectory

$y_\theta(\tau) = y_0 + v(\tau - t_0) \sin \phi$, $t_0 \leq \tau \leq t$. Then for $\theta = (x_0, y_0, \phi, v) \in \Theta$, $\partial \mu_{\theta,k} / \partial \theta = \begin{bmatrix} \partial \mu_{\theta,k} / \partial x_0 & \partial \mu_{\theta,k} / \partial y_0 & \partial \mu_{\theta,k} / \partial \phi & \partial \mu_{\theta,k} / \partial v \end{bmatrix}$, where

$$\begin{aligned}
 \frac{\partial \mu_{\theta,k}}{\partial x_0} &= \frac{1}{(M\sigma)^2} \int_{t_0}^t \Lambda_{0,1} \int_{C_k} \left(\frac{x}{M} - x_\theta(\tau) \right) q_1(x, y) dx dy d\tau, \\
 \frac{\partial \mu_{\theta,k}}{\partial y_0} &= \frac{1}{(M\sigma)^2} \int_{t_0}^t \Lambda_{0,1} \int_{C_k} \left(\frac{y}{M} - y_\theta(\tau) \right) q_1(x, y) dx dy d\tau, \\
 \frac{\partial \mu_{\theta,k}}{\partial \phi} &= \frac{1}{(M\sigma)^2} \int_{t_0}^t \Lambda_{0,1} v(\tau - t_0) \\
 &\quad \times \int_{C_k} \left(\left(\frac{y}{M} - y_\theta(\tau) \right) \cos \phi - \left(\frac{x}{M} - x_\theta(\tau) \right) \sin \phi \right) q_1(x, y) dx dy d\tau, \\
 \frac{\partial \mu_{\theta,k}}{\partial v} &= \frac{1}{(M\sigma)^2} \int_{t_0}^t \Lambda_{0,1} (\tau - t_0) \\
 &\quad \times \int_{C_k} \left(\left(\frac{y}{M} - y_\theta(\tau) \right) \sin \phi + \left(\frac{x}{M} - x_\theta(\tau) \right) \cos \phi \right) q_1(x, y) dx dy d\tau.
 \end{aligned} \tag{2.10}$$

To calculate the Fisher information matrix for the noise-free and noise-corrupted cases, we substitute the expressions in (2.10) into the results of Theorem 2.3. By taking the square roots of the diagonal elements of the inverse Fisher information matrix of the respective cases, we obtain their respective practical limits of $\theta = (x_0, y_0, \phi, v)$.

All the simulations are performed in the Matlab environment. A flowchart outlining the generic algorithm used for the simulations is given in Figure 2.2

whereas the specific parameter setting for each simulation is specified in the caption of the figures. The simulations conducted are for the effect of the speed of the object, the magnification, the radius of the circular trajectory and the angular offset of the starting point on the limit of the accuracy of the parameter estimates.

2.4.1.1 Effect of the Speed of the Object on the Limit of the Accuracy of the Parameter Estimates

To study the effect of the speed of the object on the practical limit of θ , we fix the acquisition time and consider a range of speeds. It can be observed in Figure 2.3a and 2.3c that the practical limits of x_0 , y_0 and v improve initially but deteriorate subsequently as the speed increases. The improvement in the practical limit is due to the increase in the number of pixels that sample the image as the speed increases. Meanwhile, fewer photons are detected per pixel because the same number of photons is now distributed over a greater number of pixels. With increasing speed, the number of pixels that sample the image increases while the number of detected photons per pixel decreases. Subsequently, this leads to the deterioration of the practical limit. The deterioration is more pronounced in the noise-corrupted case where the extraneous noise is present as compared to the noise-free case where only the Poisson signal is present. It can also be observed that there is a disparity between the practical limits of x_0 and y_0 in Figure 2.3a as the trajectory influences the practical localization limits differently. For the practical limit of ϕ , it improves monotonically as the speed increases. Hence for a fixed acquisition time, the improvement in the practical limit is dependent on the trade-off between the number of pixels that sample

Figure 2.2: Flowchart outlining the generic algorithm used in the simulations.

the image and the number of detected photons per pixel.

We now benchmark the practical limits against the fundamental limits from Corollary 2.1. From Figure 2.3, the practical limits of the accuracy of x_0 , y_0 and v first approach and then deviate from their respective fundamental limits as the speed increases. Note that their fundamental limits remain constant throughout because they are independent of the speed of the object. As for the practical limit of ϕ , it approaches its fundamental limit, which improves monotonically as the speed increases.

For relatively low speeds, such as when the particle moves at 200 nm/s, the practical limits of both x_0 and y_0 are quite large for the noise-corrupted case in comparison to the fundamental limits, the actual values of the parameters and the sizes of the pixels in object space. For example, at this speed the practical limits are larger than 243 nm while the size of an area in the object space corresponding to a pixel in the detector is only about $40 \text{ nm} \times 40 \text{ nm}$. In this particular scenario it is clear that a parameter estimate would be highly questionable. For significantly higher speeds the practical limits that also account for extraneous noise are much lower, although they never reach even single pixel precision. In contrast, for the practical limits that are computed ignoring extraneous noise sources, the predicted accuracies are of acceptable levels for speeds over around 1000 nm/s. They are not significantly above the fundamental limits. This suggests that, for this range of speeds, no significant improvements in accuracy can be achieved by changing the detector size, magnification and pixel size. This is in stark contrast to the range of speeds below 1000 nm/s. For these speeds the difference between the fundamental limit and the practical limit that excludes noise sources is rather large. Therefore changing the experimental conditions promises major improvements. However, for all speeds there

is a significant difference between the practical limits that include extraneous noise sources and those without. This suggests, that in a concrete experimental setting, the extraneous noise sources have to be significantly reduced in order to obtain estimates that have accuracies close to what is theoretically possible as specified by the fundamental limit.

2.4.1.2 Effect of the Magnification on the Limit of the Accuracy of the Parameter Estimates

We next investigate the effect of magnification on the practical limit of θ . At low magnification, the photons from the object of interest are concentrated over a small number of pixels and the projected distance moved by the object in the pixel array is short. As the magnification increases, the image magnifies and the projected distance moved increases too. This causes the photons from the object of interest to be distributed over a larger number of pixels. Thus in the noise-free case, for a fixed acquisition time, the trade-off between the number of pixels that sample the image and the number of detected photons per pixel results in the improvement of the practical limit of θ as the magnification increases. However, in the noise-corrupted case, the practical limit deteriorates as the magnification increases and, as a result, the number of detected photons from the object decreases in relation to the photons from the extraneous noise sources, as shown in Figure 2.4. It is noted that in the stationary case, the distribution of photons over the pixel array is due to the change in its image size whereas in the moving case, the distance moved by the object affects the photon distribution too.

From Figure 2.4, when the magnification increases, the practical limit of θ

Figure 2.3: Limits of the accuracy of the parameter estimates as a function of the speed of a linearly moving object. Panel (a) shows the practical limits of x_0 (—) and y_0 (---). Panel (b) shows the practical limit of ϕ and Panel (c) of v . (○) corresponds to the noise-free case and (◇) corresponds to the case where Poisson noise ($\Lambda_{0,2}$) of 2 photons/pixel/s and Gaussian noise (σ_k) of 4 e^- /pixel are present. Their corresponding fundamental limits (*) are included as the references. For the object in all plots, $\sigma = 83$ nm, magnification $M = 100$, its direction of movement $\phi = 30^\circ$, and its starting coordinates are $(x_0, y_0) = (-268.7, -268.7)$ nm. The photon detection rate $\Lambda_{0,1} = 1000$ photons/s, acquisition time is 0.2 s, pixel size is $4.03 \mu\text{m} \times 4.03 \mu\text{m}$ and the array size is 31×31 pixels.

approaches its fundamental limit for the noise-free case while it deviates from its fundamental limit for the noise-corrupted case. It should be noted that the fundamental limit of θ is independent of magnification and thus it remains constant regardless of the magnification M .

2.4.2 Circular Trajectory

For the case of a circular trajectory, we assume that the center of the circular trajectory is located at the center of the pixelated detector as shown in Figure 2.1b. Its parametric expressions are given by $x_\theta(\tau) = x_c + R \cos(\omega(\tau - t_0) + \psi_0)$, $y_\theta(\tau) = y_c + R \sin(\omega(\tau - t_0) + \psi_0)$, $t_0 \leq \tau \leq t$. For $\theta = (R, x_c, y_c, \omega, \psi_0) \in \Theta$, $\partial\mu_{\theta,k}/\partial\theta = \begin{bmatrix} \partial\mu_{\theta,k}/\partial R & \partial\mu_{\theta,k}/\partial x_c & \partial\mu_{\theta,k}/\partial y_c & \partial\mu_{\theta,k}/\partial \omega & \partial\mu_{\theta,k}/\partial \psi_0 \end{bmatrix}$, where

$$\begin{aligned}
\frac{\partial\mu_{\theta,k}}{\partial R} &= \frac{1}{(M\sigma)^2} \int_{t_0}^t \Lambda_{0,1} \\
&\quad \times \int_{C_k} \left(\left(\frac{x}{M} - x_\theta(\tau) \right) \cos \psi + \left(\frac{y}{M} - y_\theta(\tau) \right) \sin \psi \right) q_1(x, y) dx dy d\tau, \\
\frac{\partial\mu_{\theta,k}}{\partial x_c} &= \frac{1}{(M\sigma)^2} \int_{t_0}^t \Lambda_{0,1} \int_{C_k} \left(\frac{x}{M} - x_\theta(\tau) \right) q_1(x, y) dx dy d\tau, \\
\frac{\partial\mu_{\theta,k}}{\partial y_c} &= \frac{1}{(M\sigma)^2} \int_{t_0}^t \Lambda_{0,1} \int_{C_k} \left(\frac{y}{M} - y_\theta(\tau) \right) q_1(x, y) dx dy d\tau, \\
\frac{\partial\mu_{\theta,k}}{\partial \omega} &= \frac{R}{(M\sigma)^2} \int_{t_0}^t \Lambda_{0,1}(\tau - t_0) \\
&\quad \times \int_{C_k} \left(\left(\frac{y}{M} - y_\theta(\tau) \right) \cos \psi - \left(\frac{x}{M} - x_\theta(\tau) \right) \sin \psi \right) q_1(x, y) dx dy d\tau, \\
\frac{\partial\mu_{\theta,k}}{\partial \psi_0} &= \frac{R}{(M\sigma)^2} \int_{t_0}^t \Lambda_{0,1} \\
&\quad \times \int_{C_k} \left(\left(\frac{y}{M} - y_\theta(\tau) \right) \cos \psi - \left(\frac{x}{M} - x_\theta(\tau) \right) \sin \psi \right) q_1(x, y) dx dy d\tau,
\end{aligned} \tag{2.11}$$

Figure 2.4: Limits of the accuracy of the parameter estimates as a function of the magnification of a linearly moving object. Panel (a) shows the practical limits of x_0 (—) and y_0 (- - -). Panel (b) shows the practical limit ϕ and Panel (c) of v . (o) corresponds to the noise-free case and ($\diamond$) to the case where Poisson noise ($\Lambda_{0,2}$) of 2 photons/pixel/s and Gaussian noise (σ_k) of 4 e^- /pixel are present. Their corresponding fundamental limits (*) are included as reference. For the object in all plots, $\sigma = 83$ nm, its direction of movement $\phi = 30^\circ$, its speed $v = 1800$ nm/s, and its starting coordinates are $(x_0, y_0) = (-127.3, -127.3)$ nm.. The photon detection rate $\Lambda_{0,1} = 1000$ photons/s, acquisition time is 0.2 s, pixel size is $4.03 \mu\text{m} \times 4.03 \mu\text{m}$ and the array size is 31×31 pixels.

and $\psi = \omega(\tau - t_0) + \psi_0$.

Like the case of the linear trajectory, we substitute the expressions in (2.11) into the results of Theorem 2.3 to obtain the Fisher information matrix for the noise-free and noise-corrupted cases. Inverting the Fisher information matrices and taking the square root of their diagonal elements, we obtain the practical limit of $\theta = (R, x_c, y_c, \omega, \psi_0)$.

2.4.2.1 Effect of the Radius of the Circular Trajectory on the Limit of the Accuracy of the Parameter Estimates

To investigate the effect of the radius of the circular trajectory on the practical limit of θ , we fix the constant angular velocity for a range of radii and also fix the acquisition time as one period with respect to the angular velocity. As the radius of the circular trajectory increases for the noise-free case, the practical limits of R , x_c and of y_c approach their respective fundamental limits, whereas for the noise-corrupted case, they improve initially but deteriorate subsequently as shown in Figure 2.5a and 2.5b, respectively. Similar to the case of the linear trajectory, this improvement is due to the increase in the number of pixels that sample the image as the radius increases. Meanwhile, fewer photons are detected per pixel because the same number of photons are now distributed over a larger number of pixels. When the number of detected photons from the object decreases in relation to the photons from the extraneous noise sources, the practical limits deteriorate. For ω and ψ_0 , their practical limits improve as the radius of the circular trajectory increases, following the same trend of the fundamental limits.

We notice that there is also a disparity between the practical limits of x_c

and y_c , as shown in Figure 2.5b. This disparity diminishes as the radius of the circular trajectory increases. This phenomenon can also be observed in the case of the angular offset of the starting location as shown in Figure 2.6b and 2.6c. Hence, it is seen that the trajectory has a strong influence on the practical limit. As in the case of the linear trajectory, the practical limit of the accuracy for the circular case is also dependent on the trade-off between the number of pixels that sample the image and the number of detected photons per pixel.

We now benchmark the practical limits against the fundamental limits of θ from Corollary 2.2. From Figure 2.5, for the noise-corrupted case, the practical limits of R , x_c and y_c first approach and then deviate from their respective fundamental limits as the radius of the circular trajectory increases. Note that the fundamental limits of R , x_c and y_c are independent of the radius of the circular trajectory and hence they remain constant as the radius increases. As for the practical limits of ω and ψ_0 , they approach their respective fundamental limits, which improve monotonically as the radius increases.

2.4.2.2 Effect of the Angular Offset of the Starting Point on the Limit of the Accuracy of the Parameter Estimates

From Figure 2.6, the practical limits of R , ω and ψ_0 are almost independent of the angular offset of the starting point ψ_0 , and as the radius of the circular trajectory increases, they approach their respective fundamental limits which are independent of the angular offset of the starting point ψ_0 . However, the situation of the practical limits of x_c and y_c is quite different as their deviations from the respective fundamental limits are dependent on the angular offset of the starting point ψ_0 and the radius of the circular trajectory. It should be

noted that the fundamental limits of x_c and y_c are functions of the angular offset of the starting point ψ_0 .

2.5 Conclusions

In this chapter, we have investigated the performance of parameter estimation for moving single molecules imaged by fluorescence microscopy. The acquired data are modelled as a space-time random process where the detected photons are Poisson distributed. A non-pixelated detector of infinite size is first considered and we derived a general expression of the Fisher information matrix in terms of its image function and object trajectory for an object moving in the 3D space. We have shown that the Fisher information matrix can be obtained by integrating the corresponding time-invariant results with a weighting function that is associated with the derivative of the object trajectory with respect to the parameters concerned. For an object moving in the 2D focus plane, we have also shown that the Fisher information matrix is separable in terms of the spatial and temporal integrals. Furthermore, explicit CRLB expressions have been obtained when the object moves in the 2D focus plane with the object trajectory being either linear or circular and for two specific image functions: the Airy and the Gaussian image functions.

We next considered a pixelated detector of finite size. From the simulations conducted, we have obtained insights into how extraneous noise sources, pixelation, parameters of the detection system and parameters of the trajectory affect the limits of the accuracy of the estimated parameters. In the linear trajectory case, for a fixed acquisition time, the number of pixels that sample

Figure 2.5: Limits of the accuracy of the parameter estimates as a function of the radius of the circular trajectory. Panel (a) shows the practical limit of R . Panel (b) of x_c (—) and y_c (---). Panel (c) of ω and Panel (d) of ψ_0 . (o) corresponds to the noise-free case and ($\diamond$) to the case where Poisson noise ($\Lambda_{0,2}$) of 2 photons/pixel/s and Gaussian noise (σ_k) of $4 e^-$ /pixel are present. Their corresponding fundamental limits (*), which are independent of the pixel array, are included as reference. For the object in all plots, $\sigma = 83$ nm, magnification $M = 100$, angular offset of the starting point $\psi_0 = 20^\circ$, and the coordinates of x_c and y_c are (0,0). The photon detection rate $\Lambda_{0,1} = 1000$ photons/s, period $T_p = 0.2$ s, pixel size is $4.03 \mu\text{m} \times 4.03 \mu\text{m}$ and the array size is 31×31 pixels.

Figure 2.6: Limits of the accuracy of the parameter estimates as a function of the angular offset of the starting point ψ_0 of an object with a circular trajectory. Panel (a) shows the practical limit of R . Panel (b) of x_c . Panel (c) of y_c . Panel (d) of ω and Panel (e) of ψ_0 . (o) corresponds to a radius of the circular trajectory of 350 nm while (*) corresponds to a radius of 50 nm. The line style (— · —) corresponds to the noise-free case while (—) refers to that of the fundamental limit. For the object in all plots, $\sigma = 83$ nm, magnification $M = 100$, and the coordinates of x_c and y_c are (0,0). The photon detection rate $\Lambda_{0,1} = 1000$ photons/s, period $T_p = 0.2$ s, pixel size is $4.03 \mu\text{m} \times 4.03 \mu\text{m}$ and the array size is 31×31 pixels.

the image is proportional to the speed of the object while the number of detected photons per pixel is inversely proportional. Consequently, the practical limits of the parameter estimates depend on the trade-off between the number of pixels that sample the image and the number of detected photons per pixel. As for the magnification, the distribution of photons over the pixel array in the time-varying case is dependent on both the image size and the projected distance moved by the object whereas it is only dependent on the image size for the time-invariant case. In the circular trajectory case, we have shown that the disparity between the practical limits of the center coordinates x_c and y_c diminishes as the radius of the circular trajectory increases. The increase in radius also reduces the effect of the angular offset of the starting point on the practical limits. We have also discussed the meanings and practical implication of the results obtained.

Chapter 3

Influence of Prior Knowledge on the Accuracy Limit of Parameter Estimation

3.1 Introduction

In Chapter 2, we have addressed the issue of the limit of the accuracy of the parameter estimates where the image of a moving object is acquired in a single exposure interval. The benchmark, which provides the limit of the accuracy of the parameter estimates, is obtained from the Cramér-Rao inequality [Rao65, Kay93] and under the circumstances where no prior knowledge of the parameters is available. However, it is known in estimation theory that the use of prior knowledge may lead to a more accurate estimate [Kay93, CDH⁺00]. With fewer parameters to be estimated, the size of the Fisher information matrix reduces accordingly. Not only does this simplify the computation of the

CRLB, it may also lead to the reduction of the CRLB which is analogous to an improvement in the limit of the accuracy of the parameter estimates. Since we are dealing primarily with a moving object, the influence of prior knowledge of the parameters that describe the trajectory of the object on the limit of the accuracy of the parameter estimates will be the subject matter of this chapter.

The organization of this chapter is as follows. In Section 3.2, we generalize the expressions of the Fisher information matrix in Chapter 2 by incorporating the prior knowledge matrix. In Section 3.3, two different cases of prior knowledge of the parameters that describe the trajectory are considered in order to study their influence on the limit of the accuracy of the parameter estimates. Conclusions are presented in Section 3.4. Proofs are given in the Appendix at the end of the thesis.

3.2 General Framework

To account for the prior knowledge of the parameters that describe the trajectory of the object, we adopt an approach in where a prior knowledge matrix is used [CDH⁺99, CDH⁺00]. The prior knowledge matrix is denoted by K and expressed as $K = [\partial\theta/\partial\tilde{\theta}]$, $\tilde{\theta} \subseteq \theta \in \Theta$, where $\theta = (\theta_1, \theta_2, \dots, \theta_L)^T$ corresponds to all the parameters that describe the trajectory of the object of interest and $\tilde{\theta} = (\theta_1, \theta_2, \dots, \theta_H)^T$ corresponds to those to be estimated. By incorporating the prior knowledge matrix to the expression of the Fisher information matrix in Chapter 2, we obtain a general expression in the following theorem. It should be noted that the photon detection rate is assumed to be independent of θ and the emitted photons are detected by a non-pixelated detector of infinite size, i.e. $\mathcal{C} = \mathbf{R}^2$, in the absence of extraneous noise.

Theorem 3.1. *Let $\mathcal{G}(\Lambda, \{f_{\theta,\tau}\}_{\tau \geq t_0}, \mathbb{R}^2)$ be an image detection process. For $\theta \in \Theta$, assume that*

(A1) *there exists an image function $q_{z_\theta(\tau), o_\theta(\tau)}$ such that for the lateral magnification $M > 0$, the photon distribution profile of a moving object is given by $f_{\theta,\tau}(x, y) = 1/M^2 q_{z_\theta(\tau), o_\theta(\tau)}(x/M - x_\theta(\tau), y/M - y_\theta(\tau))$, $(x, y) \in \mathbb{R}^2$, $\tau \geq t_0$,*

(A2) *the time dependent trajectory of the object $(x_\theta(\tau), y_\theta(\tau))$ are uniformly bounded for $t_0 \leq \tau \leq t$,*

(A3) *$\partial q_{z_\theta(\tau), o_\theta(\tau)}(x/M - x_\theta(\tau), y/M - y_\theta(\tau)) / \partial p(\tau)$ exists for $(x, y) \in \mathbb{R}^2, z_\theta(\tau), o_\theta(\tau) \in \mathbb{R}$, $\tau \geq t_0$ where $p(\tau) := [x \ y \ z_\theta(\tau) \ o_\theta(\tau)]^T$,*

(A4) *$V_\theta(\tau) := [-\partial x_\theta(\tau)/\partial \theta \ -\partial y_\theta(\tau)/\partial \theta \ \partial z_\theta(\tau)/\partial \theta \ \partial o_\theta(\tau)/\partial \theta]^T$, $\tau \geq t_0$.*

Then for $\tilde{\theta} \subseteq \theta \in \Theta$, the Fisher information matrix $\mathbf{I}(\tilde{\theta})$ of $\mathcal{G}$ for the time interval $[t_0, t]$ is given by

$$\mathbf{I}(\tilde{\theta}) = \int_{t_0}^t \Lambda(\tau) K^T V_\theta^T(\tau) \left(\int_{\mathbb{R}} \int_{\mathbb{R}} \frac{\left(\frac{\partial q_{z_\theta(\tau), o_\theta(\tau)}(x, y)}{\partial p(\tau)} \right)^T \left(\frac{\partial q_{z_\theta(\tau), o_\theta(\tau)}(x, y)}{\partial p(\tau)} \right)}{q_{z_\theta(\tau), o_\theta(\tau)}(x, y)} dx dy \right) V_\theta(\tau) K d\tau, \quad (3.1)$$

where the prior knowledge matrix $K = \left[\frac{\partial \theta}{\partial \tilde{\theta}} \right]$.

The general expression in Theorem 3.1 allows us to calculate the Fisher information matrix for the case where prior knowledge of some or all of the parameters that describe the trajectory of the object are unavailable. When prior knowledge of all the parameters are unavailable, i.e., $\tilde{\theta} = \theta$, the prior knowledge matrix K becomes an identity matrix of size that is equivalent to the total

number of parameters that describe the trajectory. This general expression pertains to the case where an object moves in the 3D space. Since an analytical expression is unavailable, numerical methods are needed for its computation. From its inverse, we can therefore obtain the lower bound to the best possible accuracy for the unknown parameter estimates.

Like the preceding chapter, we refer to the lower bound of the unknown parameter vector as the fundamental limit, in short. The term fundamental is used to describe the fact that the model which underlies the expressions for calculating the lower bound does not take into account any deteriorating effects of the acquisition system such as pixelation of the detector and the various noise sources that typically occur in experimental settings. The fundamental limit has practical value as it provides us with a quantity of what is theoretically possible in the absence of deteriorating factors and thus serves as a benchmark for practical cases.

In the case where the object of interest moves in a 2D focus plane or in a relatively flat structure, we consider the image function $q_{z_\theta(\tau), o_\theta(\tau)}$ to be independent of the focus level $z_\theta(\tau)$ and the orientation $o_\theta(\tau)$ of the object. We further assume the image function to be radially symmetric such that $q(x, y) = \tilde{q}(r^2)$ for a function $\tilde{q} : \mathbb{R}^2 \rightarrow \mathbb{R}$. Thus, the general expression in Theorem 3.1 simplifies to an expression comprising the product of two separable integrals as shown in the following proposition.

Proposition 3.1. *Let $\mathcal{G}(\Lambda, \{f_{\theta, \tau}\}_{\tau \geq t_0}, \mathbb{R}^2)$ be an image detection process. For $\theta \in \Theta$, assume that there exists a radially symmetric image function q , i.e., $q(x, y) = \tilde{q}(r^2)$, for a function $\tilde{q} : \mathbb{R} \rightarrow \mathbb{R}$, that does not depend on $z_\theta(\tau)$ and $o_\theta(\tau)$ such that for $M > 0$, the photon distribution profile $f_{\theta, \tau}$ of a moving object is given by $f_{\theta, \tau}(x, y) = 1/M^2 q(x/M - x_\theta(\tau), y/M - y_\theta(\tau))$, $(x, y) \in \mathbb{R}^2$, $\tau \geq$*

t_0 . Then for $\tilde{\theta} \subseteq \theta \in \Theta$, the Fisher information matrix $\mathbf{I}(\tilde{\theta})$ of $\mathcal{G}$ for the time interval $[t_0, t]$ is given by

$$\mathbf{I}(\tilde{\theta}) = 4\pi \int_0^\infty \frac{r^3}{\tilde{q}r^2} \left(\frac{\partial \tilde{q}(r^2)}{\partial(r^2)} \right)^2 dr \int_{t_0}^t \Lambda(\tau) K^T \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix}^T \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix} K d\tau, \quad (3.2)$$

where the prior knowledge matrix $K = [\partial\theta/\partial\tilde{\theta}]$.

The significance of the expression in Proposition 3.1 is that it greatly simplifies the calculation of the Fisher information matrix where prior knowledge of some or all of the parameters that describe the trajectory are unavailable. Like the preceding case, numerical methods are necessary for its computation since an analytical expression is unavailable. From the Fisher information matrix, we can then obtain the fundamental limit. However, for some specific trajectories and under specific conditions, e.g., linear and circular trajectories, explicit analytical expressions of the fundamental limit can be derived from the expression in Proposition 3.1.

3.3 Influence of Prior Knowledge on the Fundamental Limit of Accuracy

To study the influence of prior knowledge on the fundamental limit, we consider two distinct cases where an object with radially symmetric image function moves linearly in the 2D focus plane. We consider the availability of the prior knowledge of some of the parameters that describe its trajectory in the following proposition.

Proposition 3.2. (Appendix A6): Let $\mathcal{G}(\Lambda, \{f_{\theta, \tau}\}_{\tau \geq t_0}, \mathbb{R}^2)$ be an image detection process. The parametric expressions for the linear trajectory of the object for the time interval $[t_0, t]$ are given by $x_\theta(\tau) = x_0 + v(\tau - t_0) \cos \phi$, $y_\theta(\tau) = y_0 + v(\tau - t_0) \sin \phi$, $t_0 \leq \tau \leq t$. For $\tau \geq t_0$ and $M > 0$, assume that there exists a radially symmetric image function $q(x, y)$ such that $f_{\theta, \tau}(x, y) = 1/M^2 q(x/M - x_\theta(\tau), y/M - y_\theta(\tau))$, $(x, y) \in \mathbb{R}^2$ with $q(x, y) = \tilde{q}(x^2 + y^2) = \tilde{q}(r^2)$, for a function $\tilde{q} : \mathbb{R}^2 \rightarrow \mathbb{R}$ and let $\gamma^2 := 4\pi \int_0^\infty r^3 / \tilde{q}(r^2) (\partial \tilde{q}(r^2) / \partial (r^2))^2 dr$. For a 2D Gaussian image function $q(x, y) = 1/(2\pi\sigma^2) \exp(-(x^2 + y^2)/(2\sigma^2))$, $\sigma > 0$, $(x, y) \in \mathbb{R}^2$, $\gamma := 1/\sigma$. As for the Airy image function $q(x, y) = J_1^2(2\pi n_a \sqrt{x^2 + y^2}/\lambda)/(\pi(x^2 + y^2))$, $(x, y) \in \mathbb{R}^2$, $\gamma := 2\pi n_a/\lambda$, where n_a and λ denote the numerical aperture and emission wavelength respectively and J_1 denotes the first order Bessel function of the first kind.

1. When prior knowledge of the speed of the object v is available, the fundamental limit of $\tilde{\theta} = (x_0, y_0, \phi) \in \Theta$ is given by

$$\delta_{x_0} = \frac{1}{\gamma} \sqrt{\frac{b_3 - \frac{b_2^2}{b_1} \cos^2 \phi}{b_1 b_3 - b_2^2}}, \quad \delta_{y_0} = \frac{1}{\gamma} \sqrt{\frac{b_3 - \frac{b_2^2}{b_1} \sin^2 \phi}{b_1 b_3 - b_2^2}}, \quad \delta_\phi = \frac{1}{\gamma v} \sqrt{\frac{b_1}{b_1 b_3 - b_2^2}}, \quad (3.3)$$

where

$$b_1 = \int_0^{t-t_0} \Lambda(\tau + t_0) d\tau, \quad b_2 = \int_0^{t-t_0} \Lambda(\tau + t_0) \tau d\tau, \quad b_3 = \int_0^{t-t_0} \Lambda(\tau + t_0) \tau^2 d\tau. \quad (3.4)$$

2. If the photon detection rate $\Lambda(\tau) = \Lambda_0 \in \mathbb{R}^+$, $\tau \geq t_0$, then the fundamental

limit of $\tilde{\theta} = (x_0, y_0, \phi) \in \Theta$ is given by

$$\delta_{x_0} = \frac{1}{\gamma} \sqrt{\frac{4 - 3 \cos^2 \phi}{N}}, \quad \delta_{y_0} = \frac{1}{\gamma} \sqrt{\frac{4 - 3 \sin^2 \phi}{N}}, \quad \delta_{\phi} = \frac{2}{\gamma v(t - t_0)} \sqrt{\frac{3}{N}}, \quad (3.5)$$

where $N := \Lambda_0(t - t_0)$ denotes the expected number of detected photons for the time interval $[t_0, t]$.

3. When prior knowledge of the direction of movement of the object ϕ is available, the fundamental limit of $\tilde{\theta} = (x_0, y_0, v) \in \Theta$ is given by

$$\delta_{x_0} = \frac{1}{\gamma} \sqrt{\frac{b_3 - \frac{b_2^2}{b_1} \sin^2 \phi}{b_1 b_3 - b_2^2}}, \quad \delta_{y_0} = \frac{1}{\gamma} \sqrt{\frac{b_3 - \frac{b_2^2}{b_1} \cos^2 \phi}{b_1 b_3 - b_2^2}}, \quad \delta_v = \frac{1}{\gamma} \sqrt{\frac{b_1}{b_1 b_3 - b_2^2}}. \quad (3.6)$$

4. If the photon detection rate $\Lambda(\tau) = \Lambda_0 \in \mathbb{R}^+$, $\tau \geq t_0$, then the fundamental limit of $\tilde{\theta} = (x_0, y_0, v) \in \Theta$ is given by

$$\delta_{x_0} = \frac{1}{\gamma} \sqrt{\frac{4 - 3 \sin^2 \phi}{N}}, \quad \delta_{y_0} = \frac{1}{\gamma} \sqrt{\frac{4 - 3 \cos^2 \phi}{N}}, \quad \delta_v = \frac{2}{\gamma(t - t_0)} \sqrt{\frac{3}{N}}. \quad (3.7)$$

From the analytical expressions in (3.5) and (3.7), we can easily see how prior knowledge of the parameters that describe the trajectory affects the fundamental limit by comparing them to the expression of the fundamental limit in Corollary 2.1. It can be seen that there is no change in the fundamental limit of either ϕ or v when either prior knowledge of v or ϕ is available. However, improvement can be observed for the fundamental localization limits albeit a difference exists between the limits of the two coordinates as shown in Figure 3.1. With prior

Figure 3.1: Fundamental localization limit as a function of the acquisition time interval for an object with linear trajectory. Panel (a) Fundamental limit of x_0 . Panel (b) Fundamental limit of y_0 . (o) corresponds to the case where all the parameters that describe the trajectory $\theta = (x_0, y_0, \phi, v)$ are unknown. (*) corresponds to the case where prior knowledge of v is available such that $\theta = (x_0, y_0, \phi)$. For the moving object, $\sigma = 84$ nm, magnification $M = 100$, its direction of movement $\phi = 30^\circ$, its speed $v = 1000$ nm/s and the photon detection rate $\Lambda_{0,1} = 1000$ photons/s.

knowledge of either v or ϕ , the fundamental localization limit is dependent on the direction of movement ϕ . It is interesting to note that the best fundamental limit of x_0 occurs when prior knowledge of v is available and the object is moving horizontally with respect to the detector. Incidentally, this is also the condition where the worst fundamental limit of y_0 occurs. When the object moves perpendicularly to the horizontal on the 2D plane, the results of the fundamental limit of x_0 and y_0 are inverted. They are only equal when the object moves diagonally across the 2D plane. When prior knowledge of ϕ is available, the outcome of the fundamental limit of x_0 and y_0 is in contrary to the preceding case where prior knowledge of v is available. Hence, from these observations, which coordinate has a better fundamental limit will depend on whether prior knowledge of ϕ or v is available, and the direction of movement

of the object.

3.4 Conclusions

In this chapter, we have provided a study of the influence of prior knowledge of the parameters that describe the trajectory on the parameter estimation accuracy. A general expression of the Fisher information matrix for the underlying detection process is formulated in terms of the image function, the trajectory and the prior knowledge matrix.

Using the explicit analytical expression of the fundamental limit for the case of a linear trajectory, we have considered the availability of the prior knowledge of each parameter in turn in order to study their influence on the performance limit. It has been shown that prior knowledge of either the speed or the direction of movement only improves the fundamental localization limit when compared to the case where prior knowledge is unavailable. In addition, the fundamental localization limit becomes dependent on the direction of movement which results in a disparity between the accuracy limit of the coordinates.

Chapter 4

Performance Bounds on Moving Single Molecules Imaged with Time Discretization

4.1 Introduction

In Chapter 2, we have addressed the problem of the accuracy of estimating the parameters of a moving molecule acquired in a single exposure or frame spanning the total acquisition time. The results obtained are only applicable to cases where the individual frame is concerned. An example is the method of determining the diffusion coefficients by Schuster *et al.* [SCvB02] where the acquisition frame rate is irrelevant, which differs from that by other research groups [SD05, LSJSS, SJ97].

However, to understand cellular and molecular processes in the dynamic biological systems, one of the ways is to optically track the moving molecules of

interest within [SSB⁺, SJ97, SRE⁺01, MSD06]. This can be achieved by acquiring images of the fluorescent-labelled molecules of interest in a sequence of frames in the single-particle tracking experiments [CWG01, CSG05]. By analyzing the acquired data from these experiments, we are able to obtain their spatiotemporal relationship [DV05, CVM07, KPK01]. However, like the case where the image is acquired in a single exposure or frame, the acquired data may be misinterpreted because of the localization error of the molecules of interest [MFK02, SD05]. Hence, the primary focus of this chapter pertains to the determination of the performance bound of moving single molecules imaged with time discretization or a sequence of frames. The results obtained here complement that in Chapter 2 as each has their specific applications. The results in this chapter are considered important because the acquisition frame rate and the exposure interval are basic experimental conditions commonly used in single-particle tracking experiments [GS00, CVM07, SK05]. Moreover, these basic experimental conditions have a direct influence on processes such as the detection and characterization of the anomalous diffusion in the plasma membrane [RSK⁺05].

To address the problem of the accuracy of estimating the parameters of a moving molecule whose images are acquired in a sequence of frames, we consider three distinct cases. An ideal case where the images of a moving object are acquired with a non-pixelated detector in the absence of extraneous noise, a practical case where a pixelated detector is used and in the presence of extraneous noise, and another where extraneous noise is absent. We consider the practical case without extraneous noise so that a differentiation between the effect of pixelation and extraneous noise can be made when compared to the ideal case and the practical case with extraneous noise, respectively. It should

be noted that some of the results obtained here are interesting. For example, the Fisher information matrix is dependent on the acquisition frame rate but in the ideal case it is independent of the acquisition frame rate when the exposure interval is equal to the frame interval. When the images are acquired with a pixelated detector in the absence of extraneous noise, by discretizing the image acquisition process with the exposure interval being equal to the frame interval, we can obtain a Fisher information matrix that provides a limit of the accuracy of the parameter estimates that is better than or at least as good as that acquired with a single frame for the same total acquisition time. Similar result can also be achieved when the number of frames used are being doubled for the same total acquisition time. However, in the presence of extraneous noise, there exists an acquisition frame rate where optimal limit of the accuracy of the parameter estimates is attained.

The organization of this chapter is as follows. In Section 4.2, we consider the ideal case and re-formulate the general expression of the Fisher information matrix in Chapter 2 to account for the acquired data from the sequence of frames. From this Fisher information matrix, we obtain an explicit expression of the limit of the accuracy of the parameter estimates where the images of a linearly moving object in the 2D space are acquired with uniform exposure intervals. In Section 4.3, we consider the case where images of the object are acquired in a sequence of frame with a pixelated detector. We show that under specific conditions, we can obtain a Fisher information matrix that provides a limit of the accuracy that is better than or at least as good as that acquired with a single frame for the same total acquisition time. Similarly, we show that by doubling the number of frames used, we can achieve similar result when compared to that prior to the increase in the number of frames used. In addition, we also

consider the effect of extraneous noise on the limit of the accuracy. In Section 4.4, we investigate the effect of the basic experimental conditions, extraneous noise and the resolution of the pixelated detector on the limit of the accuracy of the parameter estimates. Conclusions are presented in Section 4.5 and proofs are provided in the Appendix at the end of the thesis.

4.2 Fisher Information Matrix for the Ideal Case

Following Chapter 2, we consider a basic optical microscope setup where the detection of the emitted photons from the fluorescent-labelled object is inherently a random phenomenon. The only difference now is that the images of the moving fluorescent-labelled object are acquired in a sequence of frames. Since the recorded images are stochastic in nature, specific parameter estimation technique such as the maximum likelihood method is used to estimate the object's attributes. As for the accuracy of these estimates, it can be determined by calculating their standard deviations from the estimated parameter values upon repeated experiments [KKKP00, CWG01, RWO06b, TLW02]. However, in any estimation problem, it is important to have a benchmark against which the accuracy of the estimate of the desired attribute can be measured. Hence, in line with the objectives of this thesis, we again adopt the statistical approach by considering the Cramér-Rao inequality [Kay93, ZLO06, YN06] which provides a lower bound to the performance in terms of the variance from which the standard deviation is obtained. It should be reiterated that the Fisher information matrix is independent of any parameter estimation technique used and only depends on the statistical nature of the acquired data.

Assuming that the images of a moving object are acquired in a sequence

of frames $i = 1, 2, \dots, N_f$ over the total acquisition time, we will henceforth use the notation specified in Figure 4.1. It should be noted that the images are only acquired during the exposure interval of each frame $[t_{i-1}, e_i]$, $i = 1, 2, \dots, N_f$, with $e_i \leq t_i$, depending on the type of CCD camera or the acquisition mode used [MSV⁺04]. Like the preceding chapters, we assume that the photon detection rate is independent of θ and denote it as Λ and the photon distribution profile of the moving object to be given by $f_{\theta, \tau}(x, y) = 1/M^2 q_{z_\theta(\tau), o_\theta(\tau)}(x/M - x_\theta(\tau), y/M - y_\theta(\tau))$, $(x, y) \in \mathbb{R}^2$, $\tau \geq t_0$, where $q_{z_\theta(\tau), o_\theta(\tau)}$ denotes an image function, $M > 0$ denotes the lateral magnification and $(x_\theta(\tau), y_\theta(\tau))$, $\tau \geq t_0$, denotes the time dependent trajectory of the object. The image function $q_{z_\theta(\tau), o_\theta(\tau)}$ describes the image of an object on the detector plane at unit lateral magnification when the object is located along the z -axis in the object space and is assumed to be normalized such that $\int_{\mathbb{R}^2} q_{z_\theta(\tau), o_\theta(\tau)}(x, y) dx dy = 1$, $\tau \geq t_0$. Since the time point of the detected photon and its arrival location are known and are mutually independent of each other, the image acquired in each frame of the sequence is independent of one another. Thus, the Fisher information matrix of $\mathcal{G}$ for the total acquisition time $[t_0, t_{N_f}]$ can be expressed as the sum of the Fisher information matrix for each frames [Kay93], i.e., $\mathbf{I}(\theta) = \sum_{i=1}^{N_f} \mathbf{I}_i(\theta)$.

We first consider the ideal case where the images of the moving object are acquired by a non-pixelated detector of infinite size in the absence of extraneous noise. Since the photons are detected only during the exposure interval we consider a case of general exposure interval $[t_{i-1}, e_i]$ where $e_i \leq t_i$ and a special case where the exposure interval is equal to the frame interval, i.e., $e_i = t_i$, in the following theorem. We show that in order to account for the data acquired in all the frames, we not only have to consider each frame in turn but also the

Figure 4.1: Time discretization terminology and notation

totality of frames over the total acquisition time.

Theorem 4.1. (Appendix A7): Let $\mathcal{G}(\Lambda, \{f_{\theta, \tau}\}_{\tau \geq t_0}, \mathbb{R}^2)$ be an image detection process. Let the images of a moving object be acquired during each exposure interval $[t_{i-1}, e_i]$ of $i = 1, 2, \dots, N_f$ over the total acquisition time $[t_0, t_{N_f}]$. Assume that

(A1) there exists an image function $q_{z_{\theta}(\tau), o_{\theta}(\tau)}$ such that for $M > 0$, the photon distribution profile $f_{\theta, \tau}$ of a moving object is given by $f_{\theta, \tau}(x, y) = 1/M^2 q_{z_{\theta}(\tau), o_{\theta}(\tau)}(x/M - x_{\theta}(\tau), y/M - y_{\theta}(\tau))$, $(x, y) \in \mathbb{R}^2$, $\tau \geq t_0$;

(A2) $|x_{\theta}(\tau)|$ and $|y_{\theta}(\tau)|$ are uniformly bounded for $t_0 \leq \tau \leq t_{N_f}$;

(A3) $\partial q_{z_{\theta}(\tau), o_{\theta}(\tau)}(x/M - x_{\theta}(\tau), y/M - y_{\theta}(\tau)) / \partial p(\tau)$ exists for $(x, y) \in \mathbb{R}^2$, $z_{\theta}(\tau)$, $o_{\theta}(\tau) \in \mathbb{R}$, $\tau \geq t_0$ where $p(\tau) := \begin{bmatrix} x & y & z_{\theta}(\tau) & o_{\theta}(\tau) \end{bmatrix}^T$;

(A4) $V_{\theta}(\tau) := \begin{bmatrix} -\partial x_{\theta}(\tau) / \partial \theta & -\partial y_{\theta}(\tau) / \partial \theta & \partial z_{\theta}(\tau) / \partial \theta & \partial o_{\theta}(\tau) / \partial \theta \end{bmatrix}^T$, $\tau \geq t_0$.

1. In the case where $e_i \leq t_i$, for $\theta \in \Theta$, the Fisher information matrix $\mathbf{I}(\theta)$

of $\mathcal{G}$ for the total acquisition time $[t_0, t_{N_f}]$ is given by

$$\mathbf{I}(\theta) = \sum_{i=1}^{N_f} \int_{t_{i-1}}^{e_i} \Lambda(\tau) V_{\theta}^T(\tau) \left[\int_{\mathbb{R}} \int_{\mathbb{R}} \frac{\left(\frac{\partial q_{z_{\theta}(\tau), o_{\theta}(\tau)}(x, y)}{\partial p(\tau)} \right)^T \left(\frac{\partial q_{z_{\theta}(\tau), o_{\theta}(\tau)}(x, y)}{\partial p(\tau)} \right)}{q_{z_{\theta}(\tau), o_{\theta}(\tau)}(x, y)} dx dy \right] V_{\theta}(\tau) d\tau. \quad (4.1)$$

2. In the special case where $e_i = t_i$, for $\theta \in \Theta$, the Fisher information matrix

$\mathbf{I}(\theta)$ of $\mathcal{G}$ for the total acquisition time $[t_0, t_{N_f}]$ is given by

$$\mathbf{I}(\theta) = \int_{t_0}^{t_{N_f}} \Lambda(\tau) V_{\theta}^T(\tau) \left[\int_{\mathbb{R}} \int_{\mathbb{R}} \frac{\left(\frac{\partial q_{z_{\theta}(\tau), o_{\theta}(\tau)}(x, y)}{\partial p(\tau)} \right)^T \left(\frac{\partial q_{z_{\theta}(\tau), o_{\theta}(\tau)}(x, y)}{\partial p(\tau)} \right)}{q_{z_{\theta}(\tau), o_{\theta}(\tau)}(x, y)} dx dy \right] V_{\theta}(\tau) d\tau. \quad (4.2)$$

Theorem 4.1 gives expressions of the Fisher information matrix for the case where an object moves in the 3D object space. In result 1 of this theorem, the expression of the Fisher information matrix comprises the sum of the Fisher information matrix for each frame spanning the exposure interval $[t_{i-1}, e_i]$. Even though, the exposure intervals used in the image acquisition process are equal in practice, the expression in result 1 can also be applied to cases where the exposure intervals are not equal. When $e_i < t_i$, the Fisher information matrix is dependent on the acquisition frame rate and this can be best explained in physical terms. For different acquisition frame rates spanning the same total acquisition time, their respective exposure intervals are different. Since the data, which comprise the time points of the detected photons and their arrival locations, are acquired during the exposure intervals, they differ for different acquisition frame rates. However, when $e_i = t_i$ there is no break in the acquisition of data between successive frames regardless of the acquisition frame rate used.

Hence, there is no difference in the acquired data and the Fisher information matrix becomes independent of the acquisition frame rate as shown in result 2 of this theorem. The significance of the Fisher information matrix being independent of the acquisition frame rate under these circumstances is that it provides a convenient means in determining the Fisher information matrix based on the total acquisition time instead of the number of frames used. This greatly reduces the time and effort needed in determining the Fisher information matrix from which the limit of the accuracy of the parameter estimates or fundamental limit, in short, is obtained. If the accuracy of the unbiased parameter estimates of the experiment surpasses this limit, the validity of the experimental results would be questionable.

In the following corollary, we consider an object moving in a relatively flat structure [vHGR⁺06, SSH⁺03] or the 2D plane. The image function of the object of interest is considered to be independent of the focus level $z_\theta(\tau)$ and the orientation $o_\theta(\tau)$ of the object. In addition, its image function is assumed to be radially symmetric. This leads to a simplification of the expression for the Fisher information matrix.

Corollary 4.1. (*Appendix A8*): Let $\mathcal{G}(\Lambda, \{f_{\theta,\tau}\}_{\tau \geq t_0}, \mathbb{R}^2)$ be an image detection process. Let the images of an object moving in a 2D plane be acquired during in a sequence of frames $i = 1, 2, \dots, N_f$ during each exposure interval $[t_{i-1}, e_i]$ of $i = 1, 2, \dots, N_f$ over the total acquisition time $[t_0, t_{N_f}]$. The image function is assumed to be independent of the focus level $z_\theta(\tau)$ and the orientation $o_\theta(\tau)$ of the object, and is radially symmetric, i.e., $q(x, y) = \tilde{q}(r^2)$ for a function $\tilde{q} : \mathbb{R}^2 \rightarrow \mathbb{R}$.

1. In the case where $e_i \leq t_i$, for $\theta \in \Theta$, the Fisher information matrix $\mathbf{I}(\theta)$

of $\mathcal{G}$ for the total acquisition time $[t_0, t_{N_f}]$ is given by

$$\mathbf{I}(\theta) = 4\pi \int_0^\infty \frac{r^3}{\tilde{q}(r^2)} \left(\frac{\partial \tilde{q}(r^2)}{\partial r^2} \right)^2 dr \sum_{i=1}^{N_f} \int_{t_{i-1}}^{e_i} \Lambda(\tau) \left[\frac{\partial x_\theta^T(\tau)}{\partial \theta} \frac{\partial x_\theta(\tau)}{\partial \theta} + \frac{\partial y_\theta^T(\tau)}{\partial \theta} \frac{\partial y_\theta(\tau)}{\partial \theta} \right] d\tau. \quad (4.3)$$

2. In the special case where $e_i = t_i$, for $\theta \in \Theta$, the Fisher information matrix

$\mathbf{I}(\theta)$ of $\mathcal{G}$ for the total acquisition time $[t_0, t_{N_f}]$ is given by

$$\mathbf{I}(\theta) = 4\pi \int_0^\infty \frac{r^3}{\tilde{q}(r^2)} \left(\frac{\partial \tilde{q}(r^2)}{\partial r^2} \right)^2 dr \int_{t_0}^{t_{N_f}} \Lambda(\tau) \left[\frac{\partial x_\theta^T(\tau)}{\partial \theta} \frac{\partial x_\theta(\tau)}{\partial \theta} + \frac{\partial y_\theta^T(\tau)}{\partial \theta} \frac{\partial y_\theta(\tau)}{\partial \theta} \right] d\tau. \quad (4.4)$$

The results in Corollary 4.1 are significantly simpler than those in Theorem 4.1 since the problem is now restricted to a single plane. The expression of the Fisher information matrix in result 1 of this corollary is simply a product of the spatial integral and the sum of temporal integrals with respect to the frames used. The spatial integral includes the image function and its derivative whereas the temporal integrals include the photon detection rate and the derivative of the trajectory with respect to the parameter to be estimated. It can be seen from the expression that the trajectory of the object can now be assumed to be decoupled from its image function. This simplifies the calculation of the Fisher information matrix from which the fundamental limit is obtained.

When the exposure interval is equal to the frame interval, result 1 of this corollary further simplifies such that the sum of the temporal integrals reduces to a single temporal integral spanning the total acquisition time as shown in result 2 of this corollary. The expression of the Fisher information matrix is now simply a product of the spatial and temporal integrals, and is similar to the result of Proposition 2.1 of Chapter 2. Since analytical expressions of the fundamental

Figure 4.2: Schematic sketch and notation of a linear trajectory

limit cannot be derived from the Fisher information matrix, numerical methods are needed for their computation. However, for specific trajectories and under certain conditions, analytical expression of the fundamental limit can be derived. We illustrate this with an example where a fluorescent-labelled molecule moves linearly in the 2D object space.

A fluorescent-labelled molecule with a linear trajectory is considered because for a short total acquisition time, most trajectories can be reasonably assumed to be linear as shown in Figure 4.2. The fluorescent-labelled molecule is modelled as a point source since it is much smaller than the optical resolution of the imaging system. Consequently the acquired image from this molecule when in focus is a diffraction-limited spot describes by an Airy profile. However for tractability and ease of analysis, the Airy profile is often approximated with a 2D Gaussian profile since it provides a good approximation in the central region [TLW02, ZZOM07, SY00]. Hence, both image profiles will be considered in the following corollary.

Corollary 4.2. (*Appendix A9*): Let $\mathcal{G}(\Lambda, \{f_{\theta, \tau}\}_{\tau \geq t_0}, \mathbb{R}^2)$ be an image detection process. Let the trajectory of the linearly moving object be acquired during each exposure interval $[t_{i-1}, e_i]$ of $i = 1, 2, \dots, N_f$ over the total acquisition time $[t_0, t_{N_f}]$. The parametric expressions for the linear trajectory are given by

$x_\theta(\tau) = x_0 + v(\tau - t_0) \cos \phi$, $y_\theta(\tau) = y_0 + v(\tau - t_0) \sin \phi$, $t_0 \leq \tau \leq t_{N_f}$, where (x_0, y_0) is the coordinates of the starting point of the object of interest, ϕ is its direction of movement with respect to the x -axis and v is its speed. For $\tau \geq t_0$, we assume that there exists a radially symmetric image function $q(x, y) := \tilde{q}(r^2)$ for a function $\tilde{q} : \mathbb{R}^2 \rightarrow \mathbb{R}$ and let $\gamma^2 = 4\pi \int_0^\infty r^3 / \tilde{q}(r^2) (\partial \tilde{q}(r^2) / \partial r^2)^2 dr$.

1. In the case where $e_i \leq t_i$, the fundamental limit of $\theta = (x_0, y_0, \phi, v) \in \Theta$ is given by

$$\delta_{x_0} = \delta_{y_0} = \frac{1}{\gamma} \sqrt{\frac{b_3}{b_1 b_3 - b_2^2}}, \quad \delta_\phi = \frac{1}{\gamma v} \sqrt{\frac{b_1}{b_1 b_3 - b_2^2}}, \quad \delta_v = \frac{1}{\gamma} \sqrt{\frac{b_1}{b_1 b_3 - b_2^2}}, \quad (4.5)$$

where

$$\begin{aligned} b_1 &= \sum_{i=1}^{N_f} \int_{t_{i-1}}^{e_i} \Lambda(\tau) d\tau, \quad b_2 = \sum_{i=1}^{N_f} \int_{t_{i-1}}^{e_i} \Lambda(\tau) (\tau - t_0) d\tau, \\ b_3 &= \sum_{i=1}^{N_f} \int_{t_{i-1}}^{e_i} \Lambda(\tau) (\tau - t_0)^2 d\tau. \end{aligned} \quad (4.6)$$

For a 2D Gaussian image function $q(x, y) = 1/(2\pi\sigma^2) \exp(-(x^2 + y^2)/(2\sigma^2))$, $\sigma > 0$, $(x, y) \in \mathbb{R}^2$, $\gamma := 1/\sigma$. As for the Airy image function $q(x, y) = J_1^2(2\pi n_a \sqrt{x^2 + y^2}/\lambda)/(\pi(x^2 + y^2))$, $(x, y) \in \mathbb{R}^2$, $\gamma := 2\pi n_a/\lambda$, where n_a and λ denote the numerical aperture and emission wavelength respectively and J_1 denotes the first order Bessel function of the first kind.

2. In the case where the photon detection rate $\Lambda(\tau) = \Lambda_0 \in \mathbb{R}^+$, $\tau \geq t_0$, and we have uniform exposure intervals throughout the image acquisition process, i.e., $e_i - t_{i-1} = e_{i+1} - t_i := t_{ue}$, $i = 1, 2, \dots, N_f$. Hence, the

fundamental limit of $\theta = (x_0, y_0, \phi, v) \in \Theta$ is given by

$$\begin{aligned}\delta_{x_0} = \delta_{y_0} &= \frac{2}{\gamma} \sqrt{\frac{t_{ue}^2 + \frac{3}{2}t_{ue} \left(T_{tat} - \frac{1}{F}\right) + \left(T_{tat}^2 - \frac{3T_{tat}}{2F} + \frac{1}{2F^2}\right)}{\Lambda_0 F T_{tat} t_{ue} \left(t_{ue}^2 + T_{tat}^2 - \frac{1}{F^2}\right)}}, \\ \delta_{\phi} &= \frac{2}{\gamma v} \sqrt{\frac{3}{\Lambda_0 F T_{tat} t_{ue} \left(t_{ue}^2 + T_{tat}^2 - \frac{1}{F^2}\right)}}, \\ \delta_v &= \frac{2}{\gamma} \sqrt{\frac{3}{\Lambda_0 F T_{tat} t_{ue} \left(t_{ue}^2 + T_{tat}^2 - \frac{1}{F^2}\right)}},\end{aligned}\tag{4.7}$$

where $T_{tat} := t_{N_f} - t_0$ denotes the duration of the total acquisition time and $F := N_f/T_{tat}$ denotes the acquisition frame rate.

Result 1 of Corollary 4.2 pertains to the case where $e_i \leq t_i$ and no assumption is made with regard to the uniformity of the exposure intervals throughout the image acquisition process. The fundamental limit of x_0 , y_0 and v in this corollary are independent of θ whereas that of ϕ is independent of x_0 , y_0 and ϕ . Their dependencies are identical to the results in Corollary 2.1 since the only fundamental difference is the number of frames used in acquiring the images of the moving object. This is manifested in the terms b_1 , b_2 and b_3 , which comprise the sum of the temporal integrals with respect to the frames used.

When the photon detection rate is a constant and the exposure intervals are uniform throughout the image acquisition process, the fundamental limit can be expressed explicitly in terms of commonly used basic experimental conditions such as the acquisition frame rate, the duration of the exposure interval and the duration of the total acquisition time as shown in result 2 of this corollary. These explicit expressions are useful as they allow the experimentalists to easily calculate the fundamental limit of θ without the intermediate step of determining the Fisher information matrix for the underlying random process

that characterizes the acquired data. In addition, they provide a convenient means for the experimentalists to evaluate the effect of the acquisition frame rate and the exposure interval on the fundamental limit. In the special case where the exposure interval is equal to the frame interval, i.e., $t_{ue} = T_{tat}/N_f$, the fundamental limits in result 2 of Corollary 4.2 simplify to $2\sqrt{1/(\Lambda_0 T_{tat})}/\gamma$ for x_0 (y_0), $2\sqrt{3/(\Lambda_0 T_{tat})}/(\gamma v T_{tat})$ for ϕ and $2\sqrt{3/(\Lambda_0 T_{tat})}/(\gamma T_{tat})$ for v . These fundamental limits are now independent of the acquisition frame rate and are similar to result 2 of Corollary 2.1.

4.3 Effects of Pixelation and Time Discretization of the Image Acquisition Process

In this section where the images of a moving object is captured by a pixelated detector of finite size in a sequence of frames, $i = 1, 2, \dots, N_f$, we refer to this as the practical case in order to differentiate it from the ideal case where photons are detected by a non-pixelated detector of infinite size. Following [RWO06b], the pixelated detector $\mathcal{C}_p$ is defined as a collection $\{C_1, \dots, C_{N_p}\}$ of open, disjoint subsets of $\mathbb{R}^2$ such that $\bigcup_{k=1}^{N_p} C_k = \mathcal{C}_p$, where N_p denotes the total number of pixels that constitute the pixelated detector. As for the acquired data, they include detected photons from the object of interest and extraneous noise from a variety of sources. In our context, the extraneous noise comprises the detected photons from the background component and the measurement noise from the readout process. The detected photons from the object of interest and the background component are Poisson distributed whereas the measurement noise is Gaussian distributed. Hence, we refer to the detected photons from

the background component as the Poisson noise and the measurement noise as the Gaussian noise. Since the acquired data are described in terms of the photon distribution profile and the photon detection rate, which is assumed to be independent of θ , of an image detection process, following [ORW04], we let $\mathcal{G}_1(\Lambda_1, \{f_{\theta,\tau,1}\}_{\tau \geq t_0}, \mathcal{C}_p)$ and $\mathcal{G}_2(\Lambda_2, \{f_{\tau,2}\}_{\tau \geq t_0}, \mathcal{C}_p)$ denote the image detection processes that model the detected photons from the object of interest and the background component, respectively. Letting $n_{k,i}$ denote the total number of detected photons from the object of interest in the pixel C_k during the exposure interval $[t_{i-1}, e_i]$, then the total number of detected photons from the object of interest for the total acquisition time $[t_0, t_{N_f}]$ is given by $\sum_{i=1}^{N_f} \sum_{k=1}^{N_p} n_{k,i}$. Thus it can be shown that for $k = 1, 2, \dots, N_p$ and $i = 1, 2, \dots, N_f$, $n_{k,i}$ is independently Poisson distributed with mean

$$\mu_{\theta,k,i} = \int_{t_{i-1}}^{e_i} \int_{C_k} \Lambda_1(\tau) f_{\theta,\tau,1}(\tau) dr d\tau, \quad k = 1, 2, \dots, N_p, \quad i = 1, 2, \dots, N_f, \quad \theta \in \Theta. \quad (4.8)$$

Similarly, the number of detected photons from the background component in the pixel C_k during the exposure interval $[t_{i-1}, e_i]$ is independently Poisson distributed with mean

$$\beta_{k,i} = \int_{t_{i-1}}^{e_i} \int_{C_k} \Lambda_2(\tau) f_{\tau,2}(\tau) dr d\tau, \quad k = 1, 2, \dots, N_p, \quad i = 1, 2, \dots, N_f. \quad (4.9)$$

When there is no extraneous noise, we simply let $\beta_{k,i} = 0$ and the standard expression for the Fisher information matrix of a Poisson distribution [SM99] becomes,

$$\mathbf{I}(\theta) = \sum_{i=1}^{N_f} \sum_{k=1}^{N_p} \frac{1}{\mu_{\theta,k,i}} \left(\frac{\partial \mu_{\theta,k,i}}{\partial \theta} \right)^T \left(\frac{\partial \mu_{\theta,k,i}}{\partial \theta} \right), \quad \theta \in \Theta, \quad (4.10)$$

where $\mu_{\theta,k,i}$ is given in (4.8). However, when extraneous noise is present, we assume that the measurement noise at each pixel of each frame is Gaussian distributed with mean $\eta_{k,i}$ and variance $\sigma_{k,i}^2$, $k = 1, 2, \dots, N_p$, $i = 1, 2, \dots, N_f$. Thus, the Fisher information matrix corresponding to the total acquisition time $[t_0, t_{N_f}]$ can be expressed as [RWO06b]

$$\mathbf{I}(\theta) = \sum_{i=1}^{N_f} \sum_{k=1}^{N_p} \left(\frac{\partial \mu_{\theta,k,i}}{\partial \theta} \right)^T \left(\frac{\partial \mu_{\theta,k,i}}{\partial \theta} \right) \times \left(\int_{\mathbb{R}} \frac{\left(\sum_{l=1}^{\infty} \frac{[v_{\theta,k,i}]^{l-1} e^{-v_{\theta,k,i}}}{(l-1)!} \frac{1}{\sqrt{2\pi}\sigma_{k,i}} e^{-\frac{1}{2}\left(\frac{z-l-\eta_{k,i}}{\sigma_{k,i}}\right)^2} \right)^2}{p_{\theta,k(z),i}} dz - 1 \right), \quad (4.11)$$

where $v_{\theta,k,i} = \mu_{\theta,k,i} + \beta_{k,i}$ and the Poisson-Gaussian mixture probability density function $p_{\theta,k(z),i}$ is given by

$$p_{\theta,k(z),i} = \frac{1}{\sqrt{2\pi}\sigma_{k,i}} \sum_{l=0}^{\infty} \frac{[v_{\theta,k,i}]^l e^{-v_{\theta,k,i}}}{l!} e^{-\frac{1}{2}\left(\frac{z-l-\eta_{k,i}}{\sigma_{k,i}}\right)^2},$$

$$z \in \mathbb{R}, \quad k = 1, 2, \dots, N_p, \quad i = 1, 2, \dots, N_f.$$

Using the expression of the Fisher information matrix in (4.10), we can obtain the practical limit of the accuracy of the parameter estimates or practical limit in short where extraneous noise is absent. Similarly, we can obtain the practical limit where extraneous noise is present by using the expression of the Fisher information matrix in (4.11). We recall that when we refer to both the fundamental and practical limits, we use the term "limit of the accuracy". These expressions are used in subsequent simulations to investigate the effect of the pixelation, basic experimental conditions and extraneous noise on the practical

limit.

We next proceed to show analytically that when the exposure interval is equal to the frame interval, by acquiring the images of the object in a sequence of frames in the absence of extraneous noise, we can obtain a Fisher information matrix that provides a practical limit that is better than or at least as good as that acquired in a single frame spanning the same total acquisition time. We consider two cases, one where the images of the object are acquired with $N_f = 2$ frames and another where $N_f > 2$ frames are used. By using the expression of the Fisher information matrix in (4.10), we formulate the Fisher information matrix for the case where the image is acquired in a single frame spanning the total acquisition time $[t_0, t_{N_f}]$ and another where N_f frames are used for the same total acquisition time. We then take the difference of these two Fisher information matrices and show that this difference matrix, denoted by $\delta \mathbf{I}_{N_f-1}(\theta)$, is positive semidefinite. The subscript of $\delta \mathbf{I}_{N_f-1}(\theta)$ refers to the difference in the number of frames used.

Theorem 4.2. (*Appendix A10*): Let $\mathcal{G}_1(\Lambda_1, \{f_{\theta,\tau,1}\}_{\tau \geq t_0}, \mathcal{C}_p)$ be an image detection process of the emitted photons from an object of interest moving in a 2D plane in the absence of extraneous noise. The images are acquired during the exposure interval, which is equal to the frame interval, i.e., $e_i = t_i$. The photon detection rate of the object of interest is assumed to be a constant, i.e., $\Lambda_1(\tau) = \Lambda_0 \in \mathbb{R}^+$. The pixelated detector $\mathcal{C}_p$ is defined as a collection $\{C_1, \dots, C_{N_p}\}$ of open, disjoint subsets of $\mathbb{R}^2$ such that $\bigcup_{k=1}^{N_p} C_k = \mathcal{C}_p$, where N_p denotes the total number of pixels that constitute the pixelated detector. For the total acquisition time $[t_0, t_{N_f}]$, let the Fisher information matrix for the case where the images are acquired in N_f frames be denoted by $\mathbf{I}_{N_f}(\theta)$ whereas that acquired in a single frame be denoted by $\mathbf{I}_1(\theta)$. As for their dif-

ference, we denote it by $\delta \mathbf{I}_{N_f-1}(\theta) = \mathbf{I}_{N_f}(\theta) - \mathbf{I}_1(\theta)$. For $u = 0, 1, \dots, N_f - 1$, $v = 1, 2, \dots, N_f$, $k = 1, 2, \dots, N_p$, we let $a_{k,u,v} := \int_{t_u}^{t_v} \int_{C_k} f_{\theta,\tau,1}(r) dr d\tau \in \mathbb{R}^+$ and $A_{k,u,v} := \int_{t_u}^{t_v} \int_{C_k} \frac{\partial f_{\theta,\tau,1}(r)}{\partial \theta} dr d\tau \in \mathbb{R}^{1 \times n}$. For $m, i = 1, 2, \dots, N_f - 1$, let $W_{k,m,i} := a_{k,m,m+1} A_{k,i-1,i} - a_{k,i-1,i} A_{k,m,m+1} \in \mathbb{R}^{1 \times n}$.

1. When $N_f = 2$, for $\theta \in \Theta$, the difference matrix $\delta \mathbf{I}_1(\theta) = \mathbf{I}_2(\theta) - \mathbf{I}_1(\theta)$ for the total acquisition time $[t_0, t_2]$ is given by

$$\delta \mathbf{I}_1(\theta) = \Lambda_0 \sum_{k=1}^{N_p} \frac{W_{k,1,1}^T W_{k,1,1}}{a_{k,0,1} a_{k,1,2} a_{k,0,2}}. \quad (4.12)$$

2. When $N_f > 2$, for $\theta \in \Theta$, the difference matrix $\delta \mathbf{I}_{N_f-1}(\theta)$ for the total acquisition time $[t_0, t_{N_f}]$ is given by

$$\delta \mathbf{I}_{N_f-1}(\theta) = \Lambda_0 \sum_{k=1}^{N_p} \sum_{m=1}^{N_f-1} \sum_{i=1}^m \frac{W_{k,m,i}^T W_{k,m,i}}{a_{k,i-1,i} a_{k,m,m+1} a_{k,0,N_f}}. \quad (4.13)$$

$\delta \mathbf{I}_{N_f-1}(\theta)$ is the sum of $N_p N_f (N_f - 1)/2$ positive semidefinite matrices and hence it is also positive semidefinite. Thus, $\mathbf{I}_1^{-1}(\theta) - \mathbf{I}_{N_f}^{-1}(\theta)$ is positive semidefinite [Sim90]. This implies that the diagonal elements of the inverse of $\mathbf{I}_{N_f}(\theta)$ are smaller than or at most the same as the corresponding elements of the inverse of $\mathbf{I}_1(\theta)$. As a result, it shows that by acquiring the images of the object in N_f frames spanning the total acquisition time $[t_0, t_{N_f}]$, we can obtain a Fisher information matrix that provides a practical limit that is better than or at least as good as that acquired in a single frame spanning the same total acquisition time. Given the fact that a better practical limit may be obtained, it implies that the Fisher information matrix is dependent on the acquisition frame rate. This differs from the ideal case where the Fisher information matrix is indepen-

dent of the acquisition frame rate.

From Theorem 4.2, we can further show that by using twice the number of frames to acquire the images of the object for the same acquisition time, we can also obtain a Fisher information matrix that provides a practical limit that is better than or at least as good as that prior to the increase in the number of frames used.

Corollary 4.3. (*Appendix A11*): Let $\mathcal{G}_1(\Lambda_1, \{f_{\theta, \tau, 1}\}_{\tau \geq t_0}, \mathcal{C}_p)$ be an image detection process of the emitted photons from an object of interest moving in a 2D plane in the absence of extraneous noise. The images are acquired during the exposure interval, which is equal to the frame interval, i.e., $e_i = t_i$. The photon detection rate of the object of interest is assumed to be a constant, i.e., $\Lambda_1(\tau) = \Lambda_0 \in \mathbb{R}^+$. The pixelated detector $\mathcal{C}_p$ is defined as a collection $\{C_1, \dots, C_{N_p}\}$ of open, disjoint subsets of $\mathbb{R}^2$ such that $\bigcup_{k=1}^{N_p} C_k = \mathcal{C}_p$, where N_p denotes the total number of pixels that constitute the pixelated detector. For the total acquisition time $[t_0, t_{N_f}]$, let the Fisher information matrix for the case where the images are acquired in $N_f \in 2\mathbb{Z}^+$ frames be denoted by $\mathbf{I}_{N_f}(\theta)$ and those acquired in $N_f/2$ frames be denoted by $\mathbf{I}_{N_f/2}(\theta)$. As for their difference, we denote it by $\delta\mathbf{I}_{N_f/2}(\theta) = \mathbf{I}_{N_f}(\theta) - \mathbf{I}_{N_f/2}(\theta)$. Then for $\theta \in \Theta$,

$$\delta\mathbf{I}_{N_f/2}(\theta) = \Lambda_0 \sum_{k=1}^{N_p} \sum_{i=1}^{N_f/2} \frac{W_{k, 2i-1, 2i-1}^T W_{k, 2i-1, 2i-1}}{a_{k, 2i-2, 2i-1} a_{k, 2i-1, 2i} a_{k, 2i-2, 2i}}, \quad (4.14)$$

where $a_{k,u,v}$, $A_{k,u,v}$ and $W_{k,m,i}$ are defined in Theorem 4.2.

$\delta\mathbf{I}_{N_f/2}(\theta)$ in (4.14) is the sum of $N_p N_f/2$ positive semidefinite matrices and hence it is also positive semidefinite. Since Corollary 4.3 is a special case of Theorem 4.2, its result has the same implication. That is $\mathbf{I}_{N_f/2}^{-1}(\theta) - \mathbf{I}_{N_f}^{-1}(\theta)$ is

positive semidefinite. Thus, the diagonal elements of the inverse of $\mathbf{I}_{N_f}(\theta)$ are smaller than or at most the same as the corresponding elements of the inverse of $\mathbf{I}_{N_f/2}(\theta)$. As a result, it shows that by doubling the number of frames used in acquiring the images of the object over the given total acquisition time, the practical limit of the parameter estimates in the absence of extraneous noise is better than or at least as good as that prior to the doubling of the number of frames used for the same total acquisition time.

4.4 Simulation Results

In this section, we present and discuss the simulation results of the limit of the accuracy of the parameter estimates when subject to the influence of the acquisition frame rate, the exposure interval, the total acquisition time, and the resolution of the pixelated detector. In all four cases, we consider both the ideal and practical cases and for the latter, we further consider the effect of extraneous noise on the practical limit. For the simulations, we consider the images of a linearly moving object being captured in a sequence of frames at uniform exposure interval. The object is assumed to move with a constant speed v from an initial position (x_0, y_0) at a direction of movement ϕ with respect to the x -axis as shown in Figure 4.2. Its photon detection rate is assumed to be a known constant, i.e., $\Lambda_1(\tau) = \Lambda_{0,1} \in \mathbb{R}^+$, $\tau \geq t_0$, and its image function is a Gaussian distribution, i.e., $q_1(x, y) = 1/(2\pi\sigma^2) \exp(-((x/M - x_\theta(\tau))^2 + (y/M - y_\theta(\tau))^2)/(2\sigma^2))$, $(x, y) \in \mathbb{R}^2$, $\tau \geq t_0$. As for the background component, its photon detection rate is also assumed to be a known constant, i.e., $\Lambda_2(\tau) = \Lambda_{0,2} \in \mathbb{R}^+$, $\tau \geq t_0$, and the detected photons are uniformly distributed.

All the simulations in this chapter are performed in the Matlab environment. A flowchart outlining the generic algorithm used for the simulation work done is shown in Figure 4.3. The specific parameter setting for each simulation is specified in the caption of the figures.

4.4.1 Effect of the Acquisition Frame Rate on the Limit of the Accuracy of the Parameter Estimates

To investigate the effect of the acquisition frame rate on the limit of the accuracy, we fix the total acquisition time to 0.4 s and vary the number of frames used in acquiring the images of the linearly moving object. The exposure interval during which photons from the object of interest and the background component are detected is assumed to be equal to the frame interval. The limits of the accuracy of the parameter estimates are plotted against the number of frames used which is proportional to the acquisition frame rate in order to facilitate comparison between cases where the images are acquired with multiple frames and that with a single frame for the same total acquisition time.

Recalling that the practical limit is dependent on the number of pixels that sample the image and the number of detected photons per pixel, it improves monotonically with increasing number of frames used in the absence of extraneous noise as shown in Figure 4.4. This corroborates our analytical result that in the absence of extraneous noise, the practical limit obtained is better than or at least as good as that acquired with a single frame for the same total acquisition time. This phenomenon is analogous to increasing the shutter speed during photography in order to acquire a sharper image of a fast moving object. The dependency of the practical limit on the acquisition frame rate contrasts

Figure 4.3: Flowchart outlining the generic algorithm used in the simulations.

with that of the fundamental limit which is independent of the acquisition frame rate.

In the presence of extraneous noise, with increasing number of frames used,

the exposure interval becomes shorter and thus for each frame, fewer photons are detected per pixel. Since the Gaussian noise is assumed constant per pixel, it eventually overwhelmed the detected photons per pixel per frame and thus leads to the deterioration of the practical limit after an initial improvement. In physical terms, the image per frame would have appeared sharper because of the reduction in motion blur with increasing acquisition frame rate. However, due to the reduced number of detected photons and inclusion of extraneous noise, the image appears noisier and thus there is a greater amount of uncertainty in estimating the parameter which results in a poorer accuracy for the parameter estimates.

It should be noted that in practice, the acquisition frame rate used is often dictated by the shortest experimental time scale needed to observe cellular and molecular processes concerned. Hence the acquisition frame rate used may not be the optimal frame rate for the parameter setting used. Under such circumstances, the optimal acquisition frame rate is still useful as it provides an estimate of the amount of inaccuracy incurred in the experiment conducted if the acquisition frame rate used had been the optimal. Moreover, for the acquisition frame rate used, the practical limit provides a limit which the accuracy of the parameter estimates cannot surpass without the validity of the results being questionable.

An interesting observation can also be made in Figure 4.4 with regard to the difference between the practical limit of x_0 and y_0 . As more frames are used to acquire the images of the moving object, their difference diminishes.

4.4.2 Effect of the Exposure Interval on the Limit of the Accuracy of the Parameter Estimates

To investigate the effect of the exposure interval on the limit of the accuracy, we fix the total acquisition time at 0.4 s, consider an acquisition frame rate of 7.5 fps and vary the duration of the exposure interval.

It can be seen in Figure 4.5 that as the difference between the durations of the exposure and frame intervals increases, i.e., decreasing ratio of the durations of the exposure to frame interval, the fundamental limit deteriorates monotonically since fewer photons are detected for a constant photon detection rate. Similar trend for the practical limit can also be observed even though the result is dependent on the number of pixels that sample the image per frame and the number of detected photons per pixel per frame.

In the noise-corrupted case, the practical limits obtained are consistently poorer than those of the noise-free case. This is expected because the acquired images are corrupted by the extraneous noise. What is interesting to note is that the deterioration of the practical limit exacerbates with increasing difference between the durations of the exposure to frame intervals. This results in an increasing disparity between the practical limits of the noise-free and noise-corrupted cases. The exacerbation of the deterioration can be attributed primarily to the overwhelming of the detected photons per pixel of each frame by the Gaussian noise.

The practical implication of this is that a higher readout rate of the camera system is necessary not only to enable it to record specimen data at higher temporal frequency but also to ensure better parameter estimation accuracy. And in the case of the time-lapse imaging of the fluorescent-labelled molecules

where a predefined delay between each image acquired is specified, we would not expect the parameter estimation accuracy to be good when the delay is large.

In addition, we also consider a case of the effect of the number of frames used on the limit of the accuracy of the parameter estimates for different durations of the exposure interval. Three different durations of the exposure interval with respect to the duration of the frame interval are considered in the simulation. When the exposure interval is equal to the frame interval, the fundamental limit is independent of the acquisition frame rate. However, it is dependent on the acquisition frame rate when the duration of the exposure interval is less than that of the frame interval as shown in Figure 4.6. Under these circumstances, it can be seen that the fundamental limit of ϕ and v improve monotonically with increasing number of frames used whereas for the fundamental localization limit, it deteriorates after an initial improvement. However, for the range of the number of frames and the durations of the exposure interval considered, the fundamental localization limits obtained are still better than that acquired in a single frame spanning the same total acquisition time.

In the case where the images are acquired with a pixelated detector in the absence of noise, when the exposure interval is equal to the frame interval, the practical localization limit improves monotonically with increasing number of frames used. However, when the duration of the exposure interval is less than that of the frame interval, the practical localization limit deteriorates after an initial improvement when the images are acquired with a greater number of frames for a fixed total acquisition time, i.e., at high acquisition frame rate. This detrimental effect becomes discernible when the duration of the exposure time is half that of the frame interval. Compared to the fundamental localization limit, the difference in the practical localization limit between that acquired

with multiple frames and a single frame is significant. Similar phenomenon can be observed for the practical limit of ϕ and v where they improve monotonically with increasing number of frames used for a fixed total acquisition time.

4.4.3 Effect of the Total Acquisition Time on the Limit of the Accuracy of the Parameter Estimates

To investigate the effect of the total acquisition time on the limit of the accuracy of the parameter estimates, we fix the acquisition frame rate at 10 fps and consider a range of total acquisition times. In doing so, the frame interval is fixed at 0.1 s. Thus, for each 0.1 s increment in the total acquisition time, one additional frame is used in the acquisition of the images of the moving object. In the simulation that follows, we consider the exposure interval to be equal to the frame interval in order that the results obtained are not confounded by the loss of information due to the exposure interval.

In Figure 4.7, it can be seen that the fundamental and practical limits improve monotonically with increasing total acquisition time. This is expected since there is a proportional increase in the acquired information when the total acquisition time increases. The improvement in the practical limit can also be observed for the noise-corrupted case since the ratio of the number of detected photons to the amount of extraneous noise remains the same for every frame. This contrasts with the case where increasingly fewer photons are detected when the images of the moving object are acquired with a higher acquisition frame rate for a fixed total acquisition time.

It should be noted that for the parameter setting considered, when the total acquisition time is 0.1 s the practical limit is an order of magnitude poorer than

that when the total acquisition time is 0.2 s. Subsequent improvements in the practical limits with increasing total acquisition time are more gradual. This phenomenon is due to the fact that for independent observations, the variance from which the practical limit is obtained is dependent and inversely proportional to the number of frames used in the image acquisition process. The implication of this is that for a fixed acquisition frame rate, the rate of improvement of the parameter estimation accuracy reduces as the total acquisition time increases.

4.4.4 Effect of the Detector Array Resolution on the Limit of the Accuracy of the Parameter Estimates

To investigate the effect of the detector array resolution on the limit of the accuracy of the parameter estimates, we assume a square pixelated detector of a fixed size and vary the number of columns/rows of pixels that constitute the detector. The acquisition frame rate is fixed at 7.5 fps.

In the ideal case, there is no change in the fundamental limit for the range of detector array resolutions considered since the images of the linearly moving object are assumed to be acquired by a non-pixelated detector. However, in the practical case, the resolution of the detector array has a profound impact on the practical limit as shown in Figure 4.8. Hence, the trade-off between the number of pixels that sample the image and the number of detected photons per pixel is not only affected by the acquisition frame rate but also by the resolution of the detector array. Contrasting with the case of the acquisition frame rate, it is the size of the pixel that affects the number of pixels that sample the image and the number of detected photons per pixel whereas in the case of acquisi-

tion frame rate it is the time, specifically the exposure interval. In the absence of extraneous noise, the practical limit improves monotonically with increasing resolution of the detector array, i.e., decreasing pixel size. This improvement is due primarily to the increase in spatial information when the array resolution increases.

In the presence of extraneous noise, the practical limit is poor at low detector array resolution because of the lack of spatial information. However, as the resolution of the detector array increases, the practical limit improves before it subsequently deteriorates at higher array resolution. This deterioration at higher detector array resolution is due to the overwhelming of the limited number of detected photons per pixel by the Gaussian noise since fewer photons from the object of interest are detected per pixel due to the reduced pixel size. Since the use of detector array with a higher resolution does not always lead to better parameter estimation accuracy in the presence of extraneous noise, proper care must be exercised in selecting a detector of the right resolution for the experiment to be conducted in order to achieve the best possible parameter estimation accuracy.

4.5 Conclusions

In this chapter, we have addressed the accuracy issue of parameter estimation when images of a moving object are acquired in a sequence of frames. We have shown that the Fisher information matrix for the underlying random process that characterizes the acquired data is dependent on the acquisition frame rate except in the ideal case when the exposure and frame intervals are equal. Under these ideal conditions, the Fisher information matrix can simply be determined

Figure 4.4: Limits of the accuracy of the parameter estimates as a function of the number of frames used in acquiring the images of a linearly moving object. Panel (a) shows the limit of the accuracy of x_0 (—) and y_0 (---). Panel (b) shows the limit of the accuracy of ϕ and Panel (c) of v . The marker ($\square$), ($\circ$) and ($*$) correspond to the noise-corrupted, noise-free and ideal (non-pixelated) cases, respectively. In the noise-corrupted case, the Poisson noise ($\Lambda_{0,2}$) is 2 photons/pixel/s and the Gaussian noise (σ_k) is $2 e^-/\text{pixel}$. For the object, $\sigma = 84$ nm, its speed $v = 500$ nm/s, its direction of movement $\phi = 30^\circ$, its starting coordinates are $(x_0, y_0) = (-141.4, -141.4)$ nm with respect to the origin of the $x - y$ axes that passes through the center of the pixelated detector. The total acquisition time is 0.4 s. The photon detection rate $\Lambda_{0,1} = 2000$ photons/s, magnification $M = 100$, the pixel size is $6.8 \mu\text{m} \times 6.8 \mu\text{m}$ and the detector array size is 19×19 pixels.

Figure 4.5: Limits of the accuracy of the parameter estimates as a function of the ratio of the duration of the exposure interval to that of the frame interval when acquiring the images of the linearly moving object with an acquisition frame rate of 7.5 fps. Panel (a) shows the limit of the accuracy of x_0 (—) and y_0 (— · —). Panel (b) shows the limit of the accuracy of ϕ and Panel (c) of v . The marker ($\square$), ($\circ$) and ($*$) correspond to the noise-corrupted, noise-free and ideal (non-pixelated) cases, respectively. In the noise-corrupted case, the Poisson noise ($\Lambda_{0,2}$) is 2 photons/pixel/s and the Gaussian noise (σ_k) is $2 e^-/\text{pixel}$. For the object, $\sigma = 84$ nm, its speed $v = 500$ nm/s, its direction of movement $\phi = 30^\circ$, its starting coordinates are $(x_0, y_0) = (-141.4, -141.4)$ nm with respect to the origin of the $x - y$ axes that passes through the center of the pixelated detector. The total acquisition time is 0.4 s. The photon detection rate $\Lambda_{0,1} = 2000$ photons/s, magnification $M = 100$, the pixel size is $6.8 \mu\text{m} \times 6.8 \mu\text{m}$ and the detector array size is 19×19 pixels.

Figure 4.6: Limits of the accuracy of the parameter estimates as a function of the number of frames used in acquiring the images of a linearly moving object in the absence of extraneous noise for various durations of the exposure interval. Panel (a) shows the limit of the accuracy of x_0 . Panel (b) shows the limit of the accuracy of y_0 . Panel (c) shows the limit of the accuracy of ϕ and Panel (d) of v . The line style (—) and (— · —) correspond to cases where the images are acquired with a non-pixelated detector and a pixelated detector, respectively. The marker (*), (o) and (◊) correspond to the case where the duration of the exposure interval is equal to that of the frame interval, the duration of the exposure interval is $0.75\times$ that of the frame interval and the duration of the exposure interval is $0.5\times$ that of the frame interval, respectively. For the object, $\sigma = 84$ nm, its speed $v = 1000$ nm/s, its direction of movement $\phi = 30^\circ$, and its starting coordinates are $(x_0, y_0) = (-141.4, -141.4)$ nm with respect to the origin of the $x - y$ axes that passes through the center of the detector, its photon detection rate $\Lambda_{0,1} = 2000$ photons/s. The total acquisition time is 0.4 s, magnification $M = 100$, the pixel size is $6.8 \mu\text{m} \times 6.8 \mu\text{m}$ and the array size is 19×19 pixels.

Figure 4.7: Limits of the accuracy of the parameter estimates as a function of the total acquisition time for the case where the images of the linearly moving object are acquired with an acquisition frame rate of 10 fps. Panel (a) shows the limit of the accuracy of x_0 (—) and y_0 (---). Panel (b) shows the limit of the accuracy of ϕ and Panel (c) of v . The marker ($\square$), ($\circ$) and ($*$) correspond to the noise-corrupted, noise-free and ideal (non-pixelated) cases, respectively. In the noise-corrupted case, the Poisson noise ($\Lambda_{0,2}$) is 2 photons/pixel/s and the Gaussian noise (σ_k) is $2 e^-$ /pixel. For the object, $\sigma = 84$ nm, its speed $v = 500$ nm/s, its direction of movement $\phi = 30^\circ$, its starting coordinates are $(x_0, y_0) = (-141.4, -141.4)$ nm with respect to the origin of the $x - y$ axes that passes through the center of the pixelated detector. The photon detection rate $\Lambda_{0,1} = 2000$ photons/s, magnification $M = 100$, the pixel size is $6.8 \mu\text{m} \times 6.8 \mu\text{m}$ and the detector array size is 19×19 pixels.

Figure 4.8: Limits of the accuracy of the parameter estimates as a function of the detector array size for the case where the images of a linearly moving object are acquired with an acquisition frame rate of 7.5 fps. Panel (a) shows the limit of the accuracy of x_0 (—) and y_0 (— · —). Panel (b) shows the limit of the accuracy of ϕ and Panel (c) of v . The marker ($\square$), ($\circ$) and ($*$) correspond to the noise-corrupted, noise-free and ideal (non-pixelated) cases, respectively. In the noise-corrupted case, the Poisson noise ($\Lambda_{0,2}$) is 2 photons/pixel/s and the Gaussian noise (σ_k) is $2 e^-$ /pixel. For the object, $\sigma = 84$ nm, its speed $v = 500$ nm/s, its direction of movement $\phi = 30^\circ$, its starting coordinates are $(x_0, y_0) = (-141.4, -141.4)$ nm with respect to the origin of the $x - y$ axes that passes through the center of the pixelated detector. The total acquisition time is 0.4 s. The photon detection rate $\Lambda_{0,1} = 2000$ photons/s, magnification $M = 100$ and the detector size is $129.2 \mu\text{m} \times 129.2 \mu\text{m}$.

based on the total acquisition time regardless of the number of frames used. Using an example of a linearly moving object, we have also shown that an explicit expression of the fundamental limit can be derived when its images are acquired with a uniform exposure interval. When the images are acquired by a pixelated detector in the absence of extraneous noise, we have shown analytically that when the exposure interval is equal to the frame interval, by using more frames, we can obtain a Fisher information matrix that provides a practical limit that is better than or at least as good as that acquired with a single frame for the same total acquisition time. We have also shown that by doubling the number of frames used, we can also obtain similar result when compared to that prior to the increase in number of frames used.

Through simulations, we have provided insights into how the basic experimental conditions and extraneous noise affect the limit of the accuracy. It has been shown that in the absence of extraneous noise, increasing the acquisition frame rate improves the parameter estimation accuracy. However, in the presence of extraneous noise, there exists an acquisition frame rate where optimal practical limit is attained. Since the readout rate of the camera system is related to the difference between the durations of the exposure and frame interval, a high readout rate is necessary to improve the parameter estimation accuracy. It is also shown that the rate of improvement of the parameter estimation accuracy reduces as the total acquisition time for a fixed acquisition frame rate increases. Finally the resolution of the detector needs to be matched to the required parameter estimation accuracy as the use of detector of higher resolution does not always lead to better parameter estimation accuracy in the presence of extraneous noise.

Chapter 5

Limit of the Accuracy of Parameter Estimation for Two Closely Spaced Moving Single Molecules

5.1 Introduction

In the preceding chapters, the fluorescent-labelled molecule is modelled as a solitary point source. However, in the study of biomolecular interactions using single-molecule fluorescence microscopy, the fluorescent-labelled molecules are often in proximity to one another [YXR⁺06, XT98, SMY00]. These interactions, which typically take place at nanometer-scale distances, were previously precluded by the Rayleigh's resolution limit. However, with the advent of photon detection technology and fluorescent labelling method in re-

cent years, this limit can be surpassed even with optical microscopy [QDWS04, GHS04, LRJH05, RWO06a]. The current experimental approaches in the study of biomolecular interactions in single-molecule fluorescence microscopy can be broadly classified into two categories. One involves the temporal separation of the photon emissions from the molecules [BPS⁺06, RBZ06, HGM06] and blinking [LRJH05, LAW⁺06]. The acquired image typically contains signal from only one of the molecules. In the other set of approaches, it involves the acquisition of signals from both molecules simultaneously [SY00, RWO06a, CRA⁺09], which is the approach that we are considering in this chapter. Regardless of the approaches taken, localization error in estimating the parameters of the interacting molecules still persists. Moreover in the approach that we are considering, the confounding effect of signals from both sources further affects the parameter estimation and consequently its accuracy. In a recent paper by Ram *et al.* [RWO12], they have framed the accuracy issue of the parameter estimation of the two experimental approaches as a resolution problem between two stationary sources in proximity. In other words, the accuracy of the estimated distance between two stationary sources. By using the Cramér-Rao inequality [Kay93, ZLO06, YN06, Rao65], they interpreted the CRLB of the distance parameter as a measure of resolvability of the two stationary point sources. Furthermore, they showed that the distance estimation problem is in fact related to the localization accuracy problem. The distance estimation problem has also been investigated by other groups in the past where they adopted a simplified model such that the acquired data is described as a deterministic signal corrupted by additive noise [Hel64, Smi05, SM04]. However, in biomolecular interactions, molecular motions often occur and they contribute to the error in the parameter estimation. Thus, in this chapter, we consider the case of two

closely spaced molecules that move independently of one another and determine the limit of the accuracy of the parameter estimates that describes their trajectories. The results to be presented here also differ from those in Chapter 2 since the acquired image comprises signals from both sources instead of a single source.

To address the problem of the accuracy of estimating the parameters of two closely spaced molecules, we specifically consider two distinct cases. An ideal case where the images of the two objects are acquired with a non-pixelated detector of infinite size and in the absence of extraneous noise, and a practical case where a pixelated detector of finite size is used. In the practical case, we further consider two stochastic models, one without extraneous noise and another with extraneous noise. We extend upon our work done in Chapter 2 and derive a general expression of the Fisher information matrix from which the limit of the accuracy of the parameter estimates is obtained. Even though the expression derived is based on the condition of moving objects, it can easily be adapted to the case where either one or both of the objects are stationary.

The organization of this chapter is as follows. In Section 5.2, we derive general expressions of the Fisher information matrix for the estimation problem that underlies the acquisition of the images of two closely spaced molecules that move independently. We consider the ideal and practical cases. Through an example, we illustrate how the expression of the Fisher information matrix can be adapted to the case where only one of the molecules is moving. In Section 5.3, we perform simulations for a number of scenarios in order to gain insights into how the movement of the molecules in proximity affects the limit of the accuracy of the parameter estimates. Effect of differing photon detection rates and photon distribution profiles of the two closely spaced molecules on the limit

of the accuracy of the parameter estimates are also considered. Conclusions are presented in Section 5.4. Proofs are given in the Appendix at the end of the thesis.

5.2 General Framework

We again consider a basic optical microscope setup where the emission and detection of the photons from the fluorescent-labelled objects is inherently a random phenomenon. The acquired data comprise signals from both objects and are modelled as a spatio-temporal random process (refer to Section 2.2 for details). It consists of the time points and the spatial coordinates of the detected photons. Using estimation technique such as the maximum likelihood method, attributes of the objects such as their locations, orientations, separation distance, etc., can be estimated and the measure of their accuracies is the standard deviation [KKKP00, CWG01, RWO06b, TLW02]. Thus the question with regard to what is the best possible standard deviation of the parameter estimates for a given data set irrespective of the estimation technique used arises. Like the preceding chapters, this can be obtained by taking the square root of the Cramér-Rao Lower Bound, which is obtained from the inverse of the Fisher information matrix.

5.2.1 Fisher Information Matrix for the Ideal Case

We first consider the ideal case where the images of the two closely spaced fluorescent-labelled objects are acquired simultaneously with a non-pixelated detector of infinite size, i.e., $\mathcal{C} = \mathbb{R}^2$, and in the absence of extraneous noise. This

idealized detector provides us with the best case scenario where all the photons emitted by the two fluorescent-labelled molecules are detected upon reaching the detector plane. Thus, we can evaluate what is theoretically possible without the confounding effect of extraneous noise. Following [RWO12] and our work done in Chapter 2, we assume that the photon detection rate is independent of the unknown parameter vector θ such that

$$\Lambda(\tau) = \Lambda_1(\tau) + \Lambda_2(\tau), \quad \tau \geq t_0, \quad (5.1)$$

where $\Lambda_i(\tau)$, $i = 1, 2$, are the photon detection rates of the two objects, respectively. As for the photon distribution profile, it is the sum of the weighted photon distribution profiles of the respective objects and is expressed as

$$f_{\theta,\tau}(r) = \epsilon_1 f_{\theta,\tau,1}(r) + \epsilon_2 f_{\theta,\tau,2}(r), \quad \tau \geq t_0, \quad \theta \in \Theta, \quad (5.2)$$

where $\epsilon_i = \Lambda_i(\tau)/\Lambda(\tau)$, $i = 1, 2$, $\tau \geq t_0$, and $f_{\theta,\tau,i}(r)$, $i = 1, 2$, are the photon distribution profiles of the two objects, respectively. To derive a general expression of the Fisher information matrix in terms of the trajectories of the objects in the following theorem, we assume a specific functional form for the photon distribution profiles such that $f_{\theta,\tau,i}(r) = 1/M^2 q_{z_{\theta,i}(\tau), o_{\theta,i}(\tau), i}(x/M - x_{\theta,i}(\tau), y/M - y_{\theta,i}(\tau))$, $i = 1, 2$, $r = (x, y) \in \mathbb{R}^2$, $\tau \geq t_0$, where $q_{z_{\theta,i}(\tau), o_{\theta,i}(\tau), i}$ denotes the image function, $M > 0$ denotes the lateral magnification and $(x_{\theta,i}(\tau), y_{\theta,i}(\tau))$, $i = 1, 2$, denote the time-dependent trajectories of the two objects, respectively. The image function $q_{z_{\theta,i}(\tau), o_{\theta,i}(\tau), i}$ describes the image of the respective objects on the detector plane at unit lateral magnification.

Theorem 5.1. (*Appendix A12*) Let $\mathcal{G}(\Lambda, \{f_{\theta,\tau}\}_{\tau \geq t_0}, \mathcal{C})$ be an image detection

process where Λ and $f_{\theta,\tau}$ are defined in (5.1) and (5.2), respectively. Assume that for $\theta \in \Theta$,

(A1) $f_{\theta,\tau,i}(r) = 1/M^2 q_{z_{\theta,i}(\tau), o_{\theta,i}(\tau), i}(x/M - x_{\theta,i}(\tau), y/M - y_{\theta,i}(\tau))$, $i = 1, 2$,
 $r = (x, y) \in \mathbb{R}^2$, where $q_{z_{\theta,i}(\tau), o_{\theta,i}(\tau), i}$ denotes the image function, $M > 0$
denotes the lateral magnification and $(x_{\theta,i}(\tau), y_{\theta,i}(\tau))$, $i = 1, 2$, denote the
time-dependent trajectories of the two objects, respectively;

(A2) $p(\tau) := \left[\frac{x}{M} - x_{\theta,1}(\tau) \quad \frac{y}{M} - y_{\theta,1}(\tau) \quad z_{\theta,1}(\tau) \quad o_{\theta,1}(\tau) \quad \frac{x}{M} - x_{\theta,2}(\tau) \quad \frac{y}{M} - y_{\theta,2}(\tau) \quad z_{\theta,2}(\tau) \quad o_{\theta,2}(\tau) \right]^T$.

Then the Fisher information matrix of $\mathcal{G}$ corresponding to the acquisition time interval $[t_0, t]$ is given by

$$\mathbf{I}(\theta) = \frac{1}{M^2} \int_{t_0}^t \int_{\mathcal{C}} V_{\theta}^T(\tau) \frac{1}{w_{3D}} \left[\frac{\partial w_{3D}}{\partial p(\tau)} \right]^T \left[\frac{\partial w_{3D}}{\partial p(\tau)} \right] V_{\theta}(\tau) dx dy d\tau, \quad (5.3)$$

where

$$w_{3D} = \sum_{i=1}^2 \Lambda_i(\tau) q_{z_{\theta,i}(\tau), o_{\theta,i}(\tau), i} \left(\frac{x}{M} - x_{\theta,i}(\tau), \frac{y}{M} - y_{\theta,i}(\tau) \right), \quad \tau \geq t_0, \quad \theta \in \Theta,$$

$$V_{\theta}(\tau) = \left[\frac{-\partial x_{\theta,1}(\tau)}{\partial \theta} \quad \frac{-\partial y_{\theta,1}(\tau)}{\partial \theta} \quad \frac{\partial z_{\theta,1}(\tau)}{\partial \theta} \quad \frac{\partial o_{\theta,1}(\tau)}{\partial \theta} \quad \frac{-\partial x_{\theta,2}(\tau)}{\partial \theta} \quad \frac{-\partial y_{\theta,2}(\tau)}{\partial \theta} \quad \frac{\partial z_{\theta,2}(\tau)}{\partial \theta} \quad \frac{\partial o_{\theta,2}(\tau)}{\partial \theta} \right]^T. \quad (5.4)$$

The expression of the Fisher information matrix in Theorem 5.1 pertains to a general case where two objects move independently in the 3D object space. The size of the Fisher information matrix is dependent on that of the weighting function $V_{\theta}(\tau)$, which is determined by the parameters to be estimated. Even though the expression is derived for two closely spaced objects moving independently of one another, it can easily be adapted to cases where either one or both of the objects are stationary, which are also commonly encountered in the study

of biomolecular interaction. To adapt to such cases, the time-dependent trajectory of the object concerned is simply replaced with its location coordinates. Since an analytical expression of the Fisher information matrix is not available in this instance, numerical methods are needed for its computation from which the limit of the accuracy of the parameter estimates is obtained from its inverse. The significance of the result in Theorem 5.1 lies in its generality of applications. It is applicable to various types of trajectories, to both the time-vary and time-invariant cases, and also when both objects are far apart.

In the following proposition, we consider the case where the two objects move independently in a relatively flat structure [vHGR⁺06, SSH⁺03] or the 2D object plane. The image functions of both objects are also considered to be independent of their focus level $z_\theta(\tau)$ and the orientation $o_\theta(\tau)$. In addition, they are assumed to be radially symmetric, which is typical in single-molecule fluorescence microscopy [ZZOM07]. Thus, this leads to a simplification of the expression for the Fisher information matrix.

Proposition 5.1. *(Appendix A13) Let $\mathcal{G}(\Lambda, \{f_{\theta,\tau}\}_{\tau \geq t_0}, \mathcal{C})$ be an image detection process where Λ and $f_{\theta,\tau}$ are defined in (5.1) and (5.2), respectively. It is assumed that the image functions of both objects are independent of their focus level $z_\theta(\tau)$ and orientation $o_\theta(\tau)$ such that $f_{\theta,\tau,i}(r) = 1/M^2 q_i(x/M - x_{\theta,i}(\tau), y/M - y_{\theta,i}(\tau))$, $i = 1, 2$, $r = (x, y) \in \mathbb{R}^2$, $\tau \geq t_0$. It is also assumed that the image functions are radially symmetric, i.e., $q_i(x, y) = \tilde{q}_i(r^2)$ for a function $\tilde{q}_i : \mathbb{R}^2 \rightarrow \mathbb{R}$, $i = 1, 2$. Then for $\theta \in \Theta$, the Fisher information matrix of $\mathcal{G}$ corresponding to the acquisition time interval $[t_0, t]$ is given as follows.*

$$\mathbf{I}(\theta) = \int_{t_0}^t \int_{\mathcal{C}} \frac{V_\theta^T(\tau)}{w_{2D}} \begin{bmatrix} W_{11} & W_{12} \\ W_{12}^T & W_{22} \end{bmatrix} V_\theta(\tau) dx dy d\tau, \quad (5.5)$$

where

$$\begin{aligned}
 w_{2D} &= \sum_{i=1}^2 \Lambda_i(\tau) q_i \left(\frac{x}{M} - x_{\theta,i}(\tau), \frac{y}{M} - y_{\theta,i}(\tau) \right), \\
 V_{\theta}(\tau) &= \begin{bmatrix} \frac{-\partial x_{\theta,1}(\tau)}{\partial \theta} & \frac{-\partial y_{\theta,1}(\tau)}{\partial \theta} & \frac{-\partial x_{\theta,2}(\tau)}{\partial \theta} & \frac{-\partial y_{\theta,2}(\tau)}{\partial \theta} \end{bmatrix}^T, \\
 W_{kl} &= \Lambda_k(\tau) \Lambda_l(\tau) \\
 &\times \begin{bmatrix} \frac{\partial q_k(\frac{x}{M} - x_{\theta,k}(\tau), \frac{y}{M} - y_{\theta,k}(\tau))}{\partial x} \frac{\partial q_l(\frac{x}{M} - x_{\theta,l}(\tau), \frac{y}{M} - y_{\theta,l}(\tau))}{\partial x} & 0 \\ 0 & \frac{\partial q_k(\frac{x}{M} - x_{\theta,k}(\tau), \frac{y}{M} - y_{\theta,k}(\tau))}{\partial y} \frac{\partial q_l(\frac{x}{M} - x_{\theta,l}(\tau), \frac{y}{M} - y_{\theta,l}(\tau))}{\partial y} \end{bmatrix}, \\
 &\quad k, l = 1, 2, \theta \in \Theta, \tau \geq t_0.
 \end{aligned} \tag{5.6}$$

The result in Proposition 5.1 pertains to the case where both objects move independently in the 2D plane and that their image functions are radially symmetric. Since the movement of the two objects has been restricted to a single plane, the expression of the Fisher information matrix becomes simpler than that in the preceding case where the two objects move in the 3D space. The expression is further simplified based on the assumption that their image functions are radially symmetric. Under this assumption, the matrix W_{kl} reduces to a diagonal matrix. Similar to the result in Theorem 5.1, the expression here can also be easily adapted to the case where either one or both of the objects are stationary by simply replacing the trajectory of the object concerned with its location coordinates. As for the size of this Fisher information matrix, it is dictated by the number of parameters to be estimated. Hence, we have the biggest matrix dimension when both objects are moving and the smallest when both are stationary. This is based on the assumption that there is no prior knowledge for any of the parameters to be estimated. It should be noted that

the expression in Proposition 5.1 is also applicable to the case where the two objects are far apart such that their images are distinct since no assumption is made with regard to the distance between them. Even though the expression of the Fisher information matrix is simpler now, numerical methods are still needed for its computation since no analytical expression is available. By taking the square root of the diagonal elements of the inverse Fisher information matrix, we can obtain the limit of the accuracy of the parameter estimates or the fundamental limit as defined in the previous chapter. The significance of this proposition is similar to that of Theorem 5.1 except that it pertains to the case where the two closely spaced objects lie on the focal plane of the objective lens.

Since the expression of the Fisher information matrix can easily be adapted to the case where either one or both of the objects are moving, we illustrate its versatility through two examples in the following corollary. We first consider an example where both objects move independently in the focal plane of the objective lens. This is followed by another example where only one of the objects is moving. In the following corollary, we assume that their trajectories are linear since for a short acquisition time interval, they can be reasonably approximated with a linear function.

Corollary 5.1. (*Appendix A14*) *Let $\mathcal{G}(\Lambda, \{f_{\theta,\tau}\}_{\tau \geq t_0}, \mathcal{C})$ be an image detection process where Λ and $f_{\theta,\tau}$ are defined in (5.1) and (5.2), respectively.*

1. *When the trajectories of both objects are linear in the focal plane of the objective lens, we let the parametric expressions of the trajectories for the acquisition time interval $[t_0, t]$ be given by $x_{\theta,i}(\tau) = x_i + v_i(\tau - t_0) \cos \phi_i$, $y_{\theta,i}(\tau) = y_i + v_i(\tau - t_0) \sin \phi_i$, $i = 1, 2$, where $(x_i, y_i) \in \mathbb{R}^2$, $i = 1, 2$, denote the starting locations of the two objects, ϕ_i , $i = 1, 2$, are their respective*

directions of movement with reference to the x -axis, and v_i , $i = 1, 2$, are their respective speeds. Then for $\theta = (x_1, y_1, \phi_1, v_1, x_2, y_2, \phi_2, v_2) \in \Theta$, the Fisher information matrix corresponding to the acquisition time interval $[t_0, t]$ is given by

$$\mathbf{I}(\theta) = \int_{t_0}^t \int_{\mathcal{C}} \frac{1}{w_{2D}} \begin{bmatrix} W_{11} & W_{11}R & W_{12} & W_{12}P \\ (W_{11}R)^T & R^T W_{11}R & R^T W_{12} & R^T W_{12}P \\ W_{12} & (R^T W_{12})^T & W_{22} & W_{22}P \\ (W_{12}P)^T & (R^T W_{12}P)^T & (W_{22}P)^T & P^T W_{22}P \end{bmatrix} dx dy d\tau, \quad (5.7)$$

where

$$w_{2D} = \sum_{i=1}^2 \Lambda_i(\tau) q_i \left(\frac{x}{M} - x_{\theta,i}(\tau), \frac{y}{M} - y_{\theta,i}(\tau) \right),$$

$$R = \begin{bmatrix} -v_1(\tau - t_0) \sin \phi_1 & (\tau - t_0) \cos \phi_1 \\ v_1(\tau - t_0) \cos \phi_1 & (\tau - t_0) \sin \phi_1 \end{bmatrix}, \quad P = \begin{bmatrix} -v_2(\tau - t_0) \sin \phi_2 & (\tau - t_0) \cos \phi_2 \\ v_2(\tau - t_0) \cos \phi_2 & (\tau - t_0) \sin \phi_2 \end{bmatrix},$$

$$W_{kl} = \Lambda_k(\tau) \Lambda_l(\tau)$$

$$\times \begin{bmatrix} \frac{\partial q_k(\frac{x}{M} - x_{\theta,k}(\tau), \frac{y}{M} - y_{\theta,k}(\tau))}{\partial x} \frac{\partial q_l(\frac{x}{M} - x_{\theta,l}(\tau), \frac{y}{M} - y_{\theta,l}(\tau))}{\partial x} & 0 \\ 0 & \frac{\partial q_k(\frac{x}{M} - x_{\theta,k}(\tau), \frac{y}{M} - y_{\theta,k}(\tau))}{\partial y} \frac{\partial q_l(\frac{x}{M} - x_{\theta,l}(\tau), \frac{y}{M} - y_{\theta,l}(\tau))}{\partial y} \end{bmatrix},$$

$$k, l = 1, 2, \tau \geq t_0, \theta \in \Theta.$$

2. When one of the objects moves linearly while the other remains stationary, we let $(x_{\theta,1}(\tau), y_{\theta,1}(\tau)) = (x_1, y_1)$, which is the coordinates of the location of the stationary object. Its photon distribution profile is then expressed as $f_{\theta,1}(r) = 1/M^2 q_1(x/M - x_1, y/M - y_1)$. As for the moving object, the parametric expressions of its trajectory and photon distribution profile are as given in result 1 of this corollary. Then for $\theta = (x_1, y_1, x_2, y_2, \phi_2, v_2) \in \Theta$, the Fisher information matrix corresponding

to the acquisition time interval $[t_0, t]$ is given by

$$\mathbf{I}(\theta) = \int_{t_0}^t \int_{\mathcal{C}} \frac{1}{w_{2D}} \begin{bmatrix} W_{11} & W_{12} & W_{12}P \\ W_{12} & W_{22} & W_{22}P \\ (W_{12}P)^T & (W_{22}P)^T & P^T W_{22}P \end{bmatrix} dx dy d\tau. \quad (5.8)$$

From result 1 of this corollary, when both objects are moving and prior knowledge of the parameters is unavailable, we obtain a 8×8 matrix for the Fisher information matrix. However, to adapt result 1 of this corollary to the case where one of the objects is stationary, we replace the trajectory of object 1 with its location coordinates, i.e., $(x_{\theta,1}(\tau), y_{\theta,1}(\tau)) = (x_1, y_1)$, and thus its time-varying image function becomes time-invariant. Hence, matrix R , which comprises the speed and direction of movement parameters of object 1 disappears and result 2 of this corollary can simply be obtained by deleting the second row and column of the Fisher information matrix in result 1. The size of the Fisher information matrix reduces from a 8×8 matrix to a 6×6 matrix. Given the fact that the Fisher information matrix is a symmetric matrix, its computation is simplified even though an analytical expression of the Fisher information matrix is unavailable. It should be noted when both objects are stationary, it reduces to the case investigated by Ram *et al.* [RWO12].

5.2.2 Fisher Information Matrix for the Practical Case

We next consider the practical case where images of the two moving objects are acquired simultaneously by a pixelated detector of finite size, which is denoted by $\mathcal{C}_p$. In this case, we consider two stochastic models: one where extraneous noise is absent and another where it is present. Using the former model, it

allows us to study the effect of pixelation on the limit of the accuracy of the parameter estimates without the confounding effect of extraneous noise.

For the acquired data, it comprises detected photons from the two objects and extraneous noise from a variety of sources. In this chapter, we consider the extraneous noise to be the detected photons from the background component and the measurement noise from the readout process. The detected photons from the two objects and the background component are Poisson distributed and we refer to the latter as the Poisson noise. The measurement noise, which is Gaussian distributed, is referred to as the Gaussian noise.

We let $\mathcal{G}_s(\Lambda_{\theta,s}, \{f_{\theta,\tau,s}\}_{\tau \geq t_0}, \mathcal{C}_p)$ and $\mathcal{G}_b(\Lambda_b, \{f_{\tau,b}\}_{\tau \geq t_0}, \mathcal{C}_p)$ denote the image detection process for photons from the two objects and the background component, respectively. Similar to the ideal case, the photon distribution rate $\Lambda_{\theta,s}$ of $\mathcal{G}_s$ is assumed to be independent of the parameter vector θ and is denoted by Λ_s . As for the photon distribution profile $f_{\tau,b}$ of $\mathcal{G}_b$, it is assumed to be independent of the time point τ and is denoted by f_b . The pixelated detector $\mathcal{C}_p$ is modelled as a collection $\{C_1, \dots, C_{N_p}\}$ of open disjoint subsets of $\mathbb{R}^2$ such that $\bigcup_{k=1}^{N_p} C_k = \mathcal{C}_p$, where N_p denotes the total number of pixels that constitute the pixelated detector. Assuming that n_k denotes the total number of detected photons from both objects in the pixel C_k during the acquisition time interval $[t_0, t]$, then the total number of detected photons from both objects for the acquisition time $[t_0, t]$ is given by $\sum_{k=1}^{N_p} n_k$. Then it can be shown that for $k = 1, 2, \dots, N_p$, n_k is independently Poisson distributed with mean [RWO06b]

$$\mu_{\theta,k} = \int_{t_0}^t \int_{C_k} \Lambda_s(\tau) f_{\theta,\tau,s}(x, y) dx dy d\tau, \quad k = 1, 2, \dots, N_p, \quad \theta \in \Theta, \quad (5.9)$$

where $\Lambda_s(\tau) = \Lambda_1(\tau) + \Lambda_2(\tau)$, $\tau \geq t_0$, and $f_{\theta,\tau,s}(x, y) = \epsilon_1(\tau)f_{\theta,\tau,1}(x, y) + \epsilon_2(\tau)f_{\theta,\tau,2}(x, y)$, $(x, y) \in \mathcal{C}_p$, $\theta \in \Theta$, $\tau \geq t_0$. The notations Λ_1 , Λ_2 and $f_{\theta,\tau,1}$, $f_{\theta,\tau,2}$ denote the photon detection rates and the photon distribution profiles of the two objects, respectively, and $\epsilon_i(\tau) = \Lambda_i(\tau)/\Lambda_s(\tau)$, $i = 1, 2$, $\tau \geq t_0$. Expressing (5.9) in terms of the photon detection rates and the photon distribution profiles of the respective objects

$$\mu_{\theta,k} = \int_{t_0}^t \int_{C_k} (\Lambda_1(\tau)f_{\theta,\tau,1}(x, y) + \Lambda_2(\tau)f_{\theta,\tau,2}(x, y)) dx dy d\tau, \quad k = 1, 2, \dots, N_p, \quad \theta \in \Theta. \quad (5.10)$$

Similarly, the number of detected photons from the background component in the pixel C_k during the acquisition time interval $[t_0, t]$ is independently Poisson distributed with mean

$$\beta_k = \int_{t_0}^t \int_{C_k} \Lambda_b(\tau)f_b(x, y) dx dy d\tau, \quad k = 1, 2, \dots, N_p. \quad (5.11)$$

In the stochastic model where there is no extraneous noise, we simply let $\beta_k = 0$ and the standard expression for the Fisher information matrix of a Poisson distribution [SM99] becomes,

$$\mathbf{I}(\theta) = \sum_{k=1}^{N_p} \frac{1}{\mu_{\theta,k}} \left(\frac{\partial \mu_{\theta,k}}{\partial \theta} \right)^T \left(\frac{\partial \mu_{\theta,k}}{\partial \theta} \right), \quad \theta \in \Theta, \quad (5.12)$$

where $\mu_{\theta,k}$ is as given in (5.10).

In the stochastic model where extraneous noise is present, we use (5.11) for the Poisson noise and assume a mean η_k and variance $\sigma_{w,k}^2$, for the Gaussian noise at k^{th} pixel, $k = 1, 2, \dots, N_p$. Thus, the Fisher information matrix corre-

sponding to the acquisition time interval $[t_0, t]$ can be expressed as [RWO06b]

$$\mathbf{I}(\theta) = \sum_{k=1}^{N_p} \left(\frac{\partial \mu_{\theta,k}}{\partial \theta} \right)^T \left(\frac{\partial \mu_{\theta,k}}{\partial \theta} \right) \times \left(\int_R \frac{\left(\sum_{l=1}^{\infty} \frac{[v_{\theta,k}]^{l-1} e^{-v_{\theta,k}}}{(l-1)!} \frac{1}{\sqrt{2\pi}\sigma_k} e^{-\frac{1}{2} \left(\frac{z-l-\eta_k}{\sigma_{w,k}} \right)^2} \right)^2}{p_{\theta,k}(z)} dz - 1 \right), \quad (5.13)$$

where $v_{\theta,k} = \mu_{\theta,k} + \beta_k$ and the Poisson-Gaussian mixture probability density function $p_{\theta,k}(z)$ is given by

$$p_{\theta,k}(z) = \frac{1}{\sqrt{2\pi}\sigma_k} \sum_{l=0}^{\infty} \frac{[v_{\theta,k}]^l e^{-v_{\theta,k}}}{l!} e^{-\frac{1}{2} \left(\frac{z-l-\eta_k}{\sigma_{w,k}} \right)^2}, \quad z \in \mathbb{R}, \quad k = 1, 2, \dots, N_p.$$

Using the expression of the Fisher information matrix in (5.12), we can obtain the limit of the accuracy of the parameter estimates for the model without extraneous noise by taking the square root of the diagonal elements of the inverse Fisher information matrix. Similarly, we can obtain the limit of the accuracy of the parameter estimates for the model with extraneous noise from the inverse of (5.13). We again refer to the limit of the accuracy of the parameter estimates obtained when a pixelated detector is used as the practical limit. Using these expressions, we can then gain important insights to what extent the experimental conditions and the noise levels adversely affect the quality of the estimates.

5.3 Simulation Results

To gain insights into how the movement of two closely spaced molecules and the movement of only one of the two molecules affect the limit of the accuracy of the parameter estimates under various conditions, we consider a number of scenarios. In the first scenario, we consider two molecules moving towards one another. In the second scenario, we consider one of the molecules to be stationary while the other moves towards it. In both scenarios, the imaging condition is considered to be analogous to that of the Rayleigh's criterion, i.e., identical, self-luminous in-focus point sources emitting unpolarized incoherent light [MW06, RWO06a]. As for the third scenario, we consider different photon detection rates for the two molecules and lastly we consider different spreads of the photon distribution profile for the two molecules. For the last two scenarios, we consider one of the molecules to be moving towards the other, which is stationary. Since fluorescent-labelled molecules are usually much smaller than the optical resolution of the optical microscope used in acquiring their images, we can reasonably assume that they are point sources. Thus the acquired image consists of the diffraction-limited spots from both point sources and they overlap when the two point sources are in proximity to one another. This overlapping of images inevitably has an effect on the limit of the accuracy of the parameter estimates since the detected photons from the respective molecules cannot be easily differentiated. For generality, we refer to these point sources as objects in the following simulations. Like all previous simulation work done, the simulations here are performed in the Matlab environment. It shares the same generic algorithm as shown in Figure 2.2

Figure 5.1: Schematic sketch where both objects are moving (a) and where one object is moving while the other remains stationary (b)

5.3.1 Two Linearly Moving Objects

In this scenario, where there are two linearly moving objects, their starting locations are denoted by (x_1, y_1) and (x_2, y_2) , respectively, as shown in Figure 5.1a. They move towards each other at the same speed during the acquisition time interval. By considering a range of separation distances for the starting locations of the two moving objects, we investigate how the movement of two closely spaced objects affect the limit of the accuracy of the parameter estimates. The range of separation distances considered is from 400 nm in the object space to 200 nm. At 400 nm apart, the images of the two objects are distinct whereas at 200 nm, they are barely discernible. The parametric expressions of the trajectories for the acquisition time interval $[t_0, t]$ are defined as $x_{\theta,i}(\tau) = x_i + v_i(\tau - t_0) \cos \phi_i$, $y_{\theta,i}(\tau) = y_i + v_i(\tau - t_0) \sin \phi_i$, $i = 1, 2$, $t_0 \leq \tau \leq t$, where ϕ_i , $i = 1, 2$, are the direction of movement and v_i , $i = 1, 2$, are the speed of the two objects, respectively. The photon detection rates of both objects are assumed to be equal and constant, i.e., $\Lambda_1(\tau) = \Lambda_2(\tau) = \Lambda_0 \in \mathbb{R}^+$, $\tau \geq t_0$. The background component is also assumed to be a constant, i.e., $\Lambda_b(\tau) = \Lambda_{0,b} \in \mathbb{R}^+$, $\tau \geq t_0$. and the detected photons to be uniformly distributed. Since the Gaussian profile provides a good approximation in the central region of the Airy profile [TLW02, ZZOM07, SY00], for tractability and ease of analysis, we use the Gaussian profile instead of the Airy profile for an in-focus object. Hence,

the image functions are expressed as

$$q_i(x, y) = \frac{1}{2\pi\sigma_i^2} \exp \left(-\frac{\left(\frac{x}{M} - x_{\theta,i}(\tau)\right)^2 + \left(\frac{y}{M} - y_{\theta,i}(\tau)\right)^2}{2\sigma_i^2} \right), \quad i = 1, 2, (x, y) \in \mathbb{R}^2, \tau \geq t_0. \quad (5.14)$$

For identical objects, their standard deviations are equal, i.e., $\sigma_1 = \sigma_2 = \sigma$.

Then for $\theta = (x_1, y_1, \phi_1, v_1, x_2, y_2, \phi_2, v_2) \in \Theta$,

$$\frac{\partial \mu_{\theta,k}}{\partial \theta} = \begin{bmatrix} \frac{\partial \mu_{\theta,k}}{\partial x_1} & \frac{\partial \mu_{\theta,k}}{\partial y_1} & \frac{\partial \mu_{\theta,k}}{\partial \phi_1} & \frac{\partial \mu_{\theta,k}}{\partial v_1} & \frac{\partial \mu_{\theta,k}}{\partial x_2} & \frac{\partial \mu_{\theta,k}}{\partial y_2} & \frac{\partial \mu_{\theta,k}}{\partial \phi_2} & \frac{\partial \mu_{\theta,k}}{\partial v_2} \end{bmatrix}, \quad (5.15)$$

where

$$\begin{aligned} \frac{\partial \mu_{\theta,k}}{\partial x_i} &= \int_{t_0}^t \int_{C_k} \frac{\Lambda_0}{M^2 \sigma^2} \left(\frac{x}{M} - x_{\theta,i}(\tau) \right) q_i(x, y) dx dy d\tau, \\ \frac{\partial \mu_{\theta,k}}{\partial y_i} &= \int_{t_0}^t \int_{C_k} \frac{\Lambda_0}{M^2 \sigma^2} \left(\frac{y}{M} - y_{\theta,i}(\tau) \right) q_i(x, y) dx dy d\tau, \\ \frac{\partial \mu_{\theta,k}}{\partial \phi_i} &= \int_{t_0}^t \int_{C_k} \frac{\Lambda_0 v_i (\tau - t_0)}{M^2 \sigma^2} \left(\left(\frac{y}{M} - y_{\theta,i}(\tau) \right) \cos \phi_i - \left(\frac{x}{M} - x_{\theta,i}(\tau) \right) \sin \phi_i \right) q_i(x, y) dx dy d\tau, \\ \frac{\partial \mu_{\theta,k}}{\partial v_i} &= \int_{t_0}^t \int_{C_k} \frac{\Lambda_0 (\tau - t_0)}{M^2 \sigma^2} \left(\left(\frac{x}{M} - x_{\theta,i}(\tau) \right) \cos \phi_i + \left(\frac{y}{M} - y_{\theta,i}(\tau) \right) \sin \phi_i \right) q_i(x, y) dx dy d\tau, \\ &\quad i = 1, 2, \quad k = 1, 2, \dots, N_p, \quad (x, y) \in \mathbb{R}^2, \quad \tau \geq t_0. \end{aligned} \quad (5.16)$$

By substituting the above expressions into (5.12) and (5.13), we obtain the Fisher information matrices for cases where there is no extraneous noise and where there is extraneous noise, respectively. Taking the square root of the diagonal elements of the respective inverse Fisher information matrices, we obtain the practical limit of $\theta = (x_1, y_1, \phi_1, v_1, x_2, y_2, \phi_2, v_2) \in \Theta$.

Since the two objects are identical and move at the same speed towards each other, their corresponding limits of the accuracy of the parameter estimates are equal as shown in Figure 5.2. By reducing the separation distance of their starting points, we are effectively increasing the amount of overlapping of the

Figure 5.2: Limits of the accuracy of the parameter estimates as a function of the separation distance for the starting points of the two objects as they moved linearly towards each other. Panel (a) shows the limit of the accuracy of x_1 (—), y_1 (---), x_2 (—) and y_2 (---). Panel (b) shows the limit of the accuracy of ϕ_1 and ϕ_2 and Panel (c) of v_1 and v_2 . The markers ($\circ$) and ($\times$) correspond to object 1 and 2, respectively, in the absence of extraneous noise whereas ($\diamond$) and ($+$) correspond to object 1 and 2, respectively, in the presence of extraneous noise. The extraneous noise consists of Poisson noise ($\Lambda_{0,b}$) of 2 photons/pixel/s and Gaussian noise (σ_k) of $4 e^-/\text{pixel}$. The markers ($*$) and ($\square$) correspond to the fundamental limit of object 1 and 2, respectively. For all plots, the coordinates of the starting point of object 1 is $(-87, -87)$ nm in the object space with respect to the origin of the $x-y$ axes that passes through the center of the detector. The objects move at $v_1 = v_2 = 500$ nm/s. The direction of movement $\phi_1 = 30^\circ$ and $\phi_2 = 210^\circ$. The photon detection rate $\Lambda_0 = 2000$ photons/s, $\sigma = 84$ nm, the magnification $M = 100$, the acquisition time interval is 0.2 s, the pixel size is $6.8 \mu\text{m} \times 6.8 \mu\text{m}$ and the array size is 21×21 pixels.

two images prior to their movements and this leads to the deterioration of the fundamental and practical limits. The deterioration of the fundamental limit can be easily explained in physical terms. The fundamental limit is good when the starting points of the two moving objects are far apart, i.e., 400 nm, because for the fixed distance travelled and the parameter settings used, their images overlap towards the latter part of their movements. In other words, their images are distinct for most part of their movements and the detected photons are generally distinguishable. However, when their starting points are located closer to one another, for the same distance travelled, there will be more overlapping of their images for the duration of travel. At a separation distance of 200 nm, object speed of 500 nm/s and an acquisition time interval of 0.2 s, their images superimposed at the end of their travel. Hence, this results in the greatest number of detected photons being indistinguishable which leads to the poorest fundamental limit for the range of separation distances considered. Thus the overlapping of the images, which contributes to the deterioration of the fundamental limit, is dependent on the distance between the starting points of the two objects, their distance travelled and their direction of movement. This is distinctly different from [RWO12] where the superposition of the stationary images is solely dependent on the separation distance of the two objects.

When the images are acquired with a pixelated detector, the practical limit also deteriorates monotonically with the reduction of the separation distance. Since the practical limit is a trade-off between the number of pixels that sample the image and the number of detected photons per pixel, not only are there fewer pixels that sample the image when the starting points of the objects are closer, there are also a greater number of detected photons from both objects per pixel and they are indistinguishable. With the inclusion of extraneous noise,

the deterioration of the practical limit is exacerbated.

5.3.2 One Stationary and One Linearly Moving Object

In this scenario where an object moves linearly towards a stationary object, the stationary object is arbitrarily located at (x_1, y_1) in the object space while the starting point of the moving object (x_2, y_2) is located 200 nm to its right at a relative angle of 30° as shown in Figure 5.1b. The parametric expressions of the trajectory of the moving object for the acquisition time interval $[t_0, t]$ are defined as $x_{\theta,2}(\tau) = x_2 + v_2(\tau - t_0) \cos \phi_2$, $y_{\theta,2}(\tau) = y_2 + v_2(\tau - t_0) \sin \phi_2$, $t_0 \leq \tau \leq t$, where ϕ_2 is the direction of movement and v_2 is its speed. The photon detection rates of the two identical objects and that of the background component are the same as those in the preceding scenario. Their photon distribution profiles are also assumed to be Gaussian such that one is time-invariant while the other is time-varying.

$$\begin{aligned} q_1(x, y) &= \frac{1}{2\pi\sigma_1^2} \exp\left(-\frac{\left(\frac{x}{M} - x_1\right)^2 + \left(\frac{y}{M} - y_1\right)^2}{2\sigma_1^2}\right), \quad (x, y) \in \mathbb{R}^2, \\ q_2(x, y) &= \frac{1}{2\pi\sigma_2^2} \exp\left(-\frac{\left(\frac{x}{M} - x_{\theta,2}(\tau)\right)^2 + \left(\frac{y}{M} - y_{\theta,2}(\tau)\right)^2}{2\sigma_2^2}\right), \quad (x, y) \in \mathbb{R}^2, \tau \geq t_0. \end{aligned} \quad (5.17)$$

For identical objects, their standard deviations are equal, i.e., $\sigma_1 = \sigma_2 = \sigma$.

Then for $\theta = (x_1, y_1, x_2, y_2, \phi_2, v_2) \in \Theta$,

$$\frac{\partial \mu_{\theta,k}}{\partial \theta} = \begin{bmatrix} \frac{\partial \mu_{\theta,k}}{\partial x_1} & \frac{\partial \mu_{\theta,k}}{\partial y_1} & \frac{\partial \mu_{\theta,k}}{\partial x_2} & \frac{\partial \mu_{\theta,k}}{\partial y_2} & \frac{\partial \mu_{\theta,k}}{\partial \phi_2} & \frac{\partial \mu_{\theta,k}}{\partial v_2} \end{bmatrix}, \quad (5.18)$$

where

$$\begin{aligned}
\frac{\partial \mu_{\theta,k}}{\partial x_1} &= \int_{t_0}^t \int_{C_k} \frac{\Lambda_0}{M^2 \sigma^2} \left(\frac{x}{M} - x_1 \right) q_1(x, y) dx dy d\tau, \\
\frac{\partial \mu_{\theta,k}}{\partial y_1} &= \int_{t_0}^t \int_{C_k} \frac{\Lambda_0}{M^2 \sigma^2} \left(\frac{y}{M} - y_1 \right) q_1(x, y) dx dy d\tau, \\
\frac{\partial \mu_{\theta,k}}{\partial x_2} &= \int_{t_0}^t \int_{C_k} \frac{\Lambda_0}{M^2 \sigma^2} \left(\frac{x}{M} - x_{\theta,2}(\tau) \right) q_2(x, y) dx dy d\tau, \\
\frac{\partial \mu_{\theta,k}}{\partial y_2} &= \int_{t_0}^t \int_{C_k} \frac{\Lambda_0}{M^2 \sigma^2} \left(\frac{y}{M} - y_{\theta,2}(\tau) \right) q_2(x, y) dx dy d\tau, \\
\frac{\partial \mu_{\theta,k}}{\partial \phi_2} &= \int_{t_0}^t \int_{C_k} \frac{\Lambda_0 v_2(\tau - t_0)}{M^2 \sigma^2} \left(\left(\frac{y}{M} - y_{\theta,2}(\tau) \right) \cos \phi_2 - \left(\frac{x}{M} - x_{\theta,2}(\tau) \right) \sin \phi_2 \right) q_2(x, y) dx dy d\tau, \\
\frac{\partial \mu_{\theta,k}}{\partial v_2} &= \int_{t_0}^t \int_{C_k} \frac{\Lambda_0(\tau - t_0)}{M^2 \sigma^2} \left(\left(\frac{x}{M} - x_{\theta,2}(\tau) \right) \cos \phi_2 + \left(\frac{y}{M} - y_{\theta,2}(\tau) \right) \sin \phi_2 \right) q_2(x, y) dx dy d\tau,
\end{aligned}$$

$$k = 1, 2, \dots, N_p, \quad (x, y) \in \mathbb{R}^2, \quad \tau \geq t_0.$$

(5.19)

Adopting a similar approach to the preceding scenario, we obtain the practical limit of $\theta = (x_1, y_1, x_2, y_2, \phi_2, v_2) \in \Theta$ by taking the square root of the diagonal elements of the inverse Fisher information matrix.

In this simulation, we can observe how the movement of an object affects the limit of the accuracy of the stationary object and vice versa. As the speed of the approaching object increases for a fixed acquisition time interval, the limit of the accuracy of the parameter estimates of the stationary object deteriorates monotonically as shown in Figure 5.3a. This deterioration is due to the influence of the detected photons from the moving object as the amount of overlap increases. As a greater number of detected photons become indistinguishable, the limit of the accuracy of the parameter estimates becomes poorer. In the presence of extraneous noise, it leads to further deterioration of the practical limit.

As for the moving object, other than the fundamental limit of ϕ_2 , all other fundamental limits deteriorate monotonically with increasing speed of the ob-

ject. When the images are acquired with a pixelated detector, it can be seen in Figure 5.3b, c and d that the practical limit is poorest when the speed of the object is 200 nm/s even though the amount of overlapping of images is at the minimum for the range of speeds considered. This is the result of the trade-off between the number of the pixels that sample the image and the number of detected photons per pixel since for a short distance travelled, there is minimal information acquired. As the speed of the object increases for a fixed acquisition time interval, the practical limit improves but deteriorates subsequently due to the influence of the detected photons from the stationary object. Thus, the results suggest that not only does the practical limit depend on the trade-off between the number of pixels that sample the image and the number of detected photons per pixel, it is also dependent on the influence of the adjacent object.

However, when extraneous noise is present, specifically the measurement noise, not only is the practical limit poorer than that without extraneous noise, the deterioration is also more pronounced at higher speed. This is due to fewer detected photons per pixel since the number of photons are now spread over a longer path while the measurement noise remains constant for each pixel. Thus the deterioration of the practical limit is exacerbated. It can also be observed that the limit of the accuracy of x_2 and y_2 are significantly poorer than that of x_1 and y_1 and this is due primarily to the detrimental effect of motion.

5.3.3 Different Photon Detection Rates and Spreads of Image of the Two Objects

Thus far the imaging conditions used in our simulations are analogous to those of Rayleigh's criterion where the two objects are considered to be in-focus point

Figure 5.3: Limits of the accuracy of the parameter estimates as a function of the speed of an object moving linearly towards the stationary object. Panel (a) shows the limit of the accuracy of x_1 (—) and y_1 (---) and Panel (b) of x_2 (—) and y_2 (---). Panel (c) shows the limit of the accuracy of ϕ_2 and Panel (d) of v_2 . The markers (*), ($\circ$) and ($\diamond$) correspond to the ideal case, the practical case where extraneous noise is absent and where extraneous noise is present, respectively. The extraneous noise consists of Poisson noise ($\Lambda_{0,b}$) of 2 photons/pixel/s and Gaussian noise (σ_k) of $4 e^-$ /pixel. For all plots, the coordinates of the stationary object in the object space is $(-87, -87)$ nm with respect to the origin of the $x - y$ axes that passes through the center of the detector. For the moving object, its starting point is located at $(86.2, 13)$ nm with respect to the origin. The direction of movement $\phi_2 = 210^\circ$. The photon detection rate $\Lambda_0 = 2000$ photons/s, $\sigma = 84$ nm, the magnification $M = 100$, the acquisition time interval is 0.2 s, the pixel size is $6.8 \mu\text{m} \times 6.8 \mu\text{m}$ and the array size is 21×21 pixels.

sources that emit unpolarized, incoherent light and have equal intensity. However, in practice, the intensities of the fluorescent-labelled objects may be different [MF03, SMY00] or the spread of the image may differ due to the two objects moving in slightly different planes [TVN95, ST97]. Hence to investigate how different image intensities or image spreads of the two objects affect the limit of the accuracy of the parameter estimates, we consider the two scenarios separately. For both scenarios, we assume that one of the objects is moving towards the other, which is stationary.

In the scenario where the two objects have unequal intensities, we assume that the photon detection rate of the moving object to be twice that of the stationary object, i.e., $\Lambda_2 = 2\Lambda_1$. Under these circumstances, the limit of the accuracy of the parameter estimates has similar trend to that in Figure 5.3 except for their values, which differ. Contrast to that in Figure 5.3, the limit of the accuracy of x_2 , y_2 , ϕ_2 and v_2 are better. On the contrary, the limit of the accuracy of x_1 and y_1 are poorer than that in Figure 5.3 as the speed increases. This is because now there are more detected photons from the moving object confounding those of the stationary object.

In the scenario where the images of the two objects are of different spreads, we assume that the standard deviation of the Gaussian profile of the moving object is 1.5 times that of the stationary object, i.e., $\sigma_2 = 1.5\sigma_1$. Under these circumstances, it can be observed in Figure 5.5 that the limit of the accuracy of x_2 , y_2 , ϕ_2 and v_2 are poorer than their corresponding limits in Figure 5.3 where both images are of the same spread. By having a bigger spread without any increase in the photon detection rate, there are effectively fewer detected photons per area. This results in a weaker signal and thus the limit of the accuracy of x_2 , y_2 , ϕ_2 and v_2 are poorer. This situation is exacerbated in the

presence of extraneous noise because the weak signal will be overwhelmed by the extraneous noise. As for the limit of the accuracy of x_1 and y_1 , they are poorer at lower speeds but better at higher speeds when compared to the corresponding limits of the accuracy where both image spreads are equal in Figure 5.3a. This is because with a bigger spread for the moving object, its influence can be felt further away. Thus it influences the result of the stationary object even when the object has moved a short distance for the fixed acquisition time. Since there are fewer detected photons per area from the moving object because of the bigger spread, the photons of the stationary object dominate when the images are superimposed in the acquired image. Hence, this results in a better limit of the accuracy at higher speed than that where the images are of the same spread.

5.4 Conclusions

In this chapter, we have investigated how the movement of two closely spaced molecules affect the limit of the accuracy of parameter estimation in single-molecule fluorescence microscopy experiments. Since the photon emission from the fluorescent-labelled molecule is inherently a random phenomenon, we adopted a stochastic framework and modelled the acquired data as a spatio-temporal random process. We derived general expression of the Fisher information matrix showing its dependence on the trajectories of the two closely spaced objects. We illustrate through an example how this expression can easily be adapted for a specific case where one of the objects is stationary while the other moves linearly. Hence, the expression of the Fisher information matrix derived in this chapter is for a general case whereas that by Ram *et al.* [RWO12] is a special

Figure 5.4: Limits of the accuracy of the parameter estimates as a function of the speed of an object moving linearly towards the stationary object in the scenario where the photon detection rates of the two objects are different. Panel (a) shows the limit of the accuracy of x_1 (—) and y_1 (— · —) and Panel (b) of x_2 (—) and y_2 (— · —). Panel (c) shows the limit of the accuracy of ϕ_2 and Panel (d) of v_2 . The markers (*), (o) and (◇) correspond to the ideal case, the practical case where the extraneous noise is absent and where it is present, respectively. The extraneous noise consists of Poisson noise ($\Lambda_{0,b}$) of 2 photons/pixel/s and Gaussian noise (σ_k) of $4 e^-$ /pixel. For all plots, the coordinates of the stationary object in the object space is $(-87, -87)$ nm with respect to the origin of the $x - y$ axes that passes through the center of the detector. For the moving object, its starting point is located at $(86.2, 13)$ nm with respect to the origin. The direction of movement $\phi_2 = 210^\circ$. The photon detection rate $\Lambda_1 = 2000$ photons/s, $\Lambda_2 = 4000$ photons/s, $\sigma = 84$ nm, the magnification $M = 100$, the acquisition time interval is 0.2 s, the pixel size is $6.8 \mu\text{m} \times 6.8 \mu\text{m}$ and the array size is 21×21 pixels.

Figure 5.5: Limits of the accuracy of the parameter estimates as a function of the speed of an object moving linearly towards the stationary object in the scenario where the standard deviations of the two objects are different. Panel (a) shows the limit of the accuracy of x_1 (—) and y_1 (---) and Panel (b) of x_2 (—) and y_2 (---). Panel (c) shows the limit of the accuracy of ϕ_2 and Panel (d) of v_2 . The markers (*), (o) and (◇) correspond to the ideal case, the practical case where the extraneous noise is absent and where it is present, respectively. The extraneous noise consists of Poisson noise ($\Lambda_{0,b}$) of 2 photons/pixel/s and Gaussian noise (σ_k) of $4 e^-$ /pixel. For all plots, the coordinates of the location of the stationary object in the object space is $(-87, -87)$ nm and for the moving object, it is $(86.2, 13)$ nm with respect to the origin of the $x-y$ axes that passes through the center of the detector. The direction of movement $\phi_2 = 210^\circ$, the photon detection rate $\Lambda_0 = 2000$ photons/s, $\sigma_1 = 84$ nm, $\sigma_2 = 126$ nm, the magnification $M = 100$, the acquisition time interval is 0.2 s, the pixel size is $6.8 \mu\text{m} \times 6.8 \mu\text{m}$ and the array size is 21×21 pixels.

case of two stationary objects. Since no explicit expression of the Fisher information matrix is available, numerical methods are needed for its computation from which the fundamental limit of the parameter estimates is obtained. We also consider the practical case where the image is acquired with a pixelated detector and in the presence of extraneous noise.

In the simulations that followed, we have considered four scenarios to gain insights into how the movement of the closely spaced objects, their photon detection rates and photon distribution profiles affect the limit of the accuracy of the parameter estimates. Since the acquired image comprises the diffraction-limited images of both objects, we have shown how the overlapping of their images in addition to the movement of the object or objects affect the limit of the accuracy of the parameter estimates. As for the practical limit of the parameter estimates, not only does it depend on the trade-off between the number of pixels that sample the image and the number of detected photons per pixel, it also depends on the influence of the adjacent object. When both objects have different intensities, not only does the object with a lower intensity has a poorer limit of the accuracy, it is also poorer than that of the corresponding object with the same intensity in the case of two closely spaced objects with equal intensity. We have also shown that the image spread of the two object has a significant effect on the limit of the accuracy when they are different.

Chapter 6

Conclusions and Recommendations for Future Work

6.1 Conclusions

In this thesis, we have addressed the problem of the accuracy of estimating the parameters of moving objects in the context of the single-molecule fluorescence microscopy. Both the theoretical and practical aspects are considered and the results obtained complement the theoretical work done on the stationary single molecules by various research groups. Since accuracy in parameter estimation is closely related to the Fisher information matrix, we have derived expressions of Fisher information matrix showing its dependence on the trajectories of the molecules. When images are acquired in a sequence of frames for an ideal case, the Fisher information matrix can be determined based on the total

acquisition time regardless of the number of frames used when the exposure and frame intervals are equal. Various scenarios common in single-molecule fluorescence microscopy are also considered and for specific trajectories under certain conditions, explicit expressions of the CRLB which provides us with the fundamental limit are derived. These explicit expressions circumvent the need to determine the Fisher information matrix and provide a convenience means of determining the benchmark for use by experimentalists in the conduct of their single-molecule fluorescence microscopy experiments. The fundamental localization limit derived is twice that of the stationary object in the absence of prior knowledge of the parameters that describe the trajectory. However, it improves and approaches that of the stationary object when prior knowledge of either the speed or direction of movement is available but with disparity between the limits of their coordinates. From the simulations conducted, we have gained the following insights.

1. The speed of the object has a profound effect on the practical limit and so does the magnification used. Contrast with that of a stationary object, not only does the practical limit depend on the images size, it is also dependent on the projected distance moved which is related to the speed of the object.
2. A better practical limit can be obtained with a higher acquisition frame rate in the absence of extraneous noise. However, there exists an acquisition frame rate where the optimal parameter estimation accuracy is attained in the presence of extraneous noise. It has also been shown through the effect of exposure interval that a higher readout rate of the camera system is necessary for better parameter estimation accuracy. As to the

choice of the resolution of the pixelated detector to use in the presence of extraneous noise, there also exists a resolution where optimal parameter estimation accuracy can be attained.

3. When two objects are in proximity, it results in the deterioration of the practical limits of their parameter estimates. The intensity and spread of the image of the objects also have a profound effect on the practical limits. As such, the object with the lower intensity or greater spread of the image will always be poorer.

We hope that the insights gained from these simulations will enable the experimentalists to optimize their experimental setups in order to achieve the best possible accuracy. It should be noted that even though the results obtained here are for single-molecule fluorescence microscopy, they can be extended to the general problem of tracking an object using quantum limited detectors.

6.2 Recommendations

Based on the research work presented in this thesis, some recommendations for future work are given as follows.

1. In Chapter 3 we considered the influence of prior knowledge of the parameter on the accuracy limit of parameter estimation. In Chapter 4 we considered the images of the moving object to be acquired in a sequence of frames and in Chapter 5, we considered the images of two closely spaced objects to be acquired in a single exposure. Hence, it is only natural that a general expression of the Fisher information matrix be formulated

that allows the images of two closely spaced objects to be acquired in a sequence of frames and also to take into account the prior knowledge of the parameters concerned. Furthermore, the case of a finite-size should also be considered. This generalized expression of the Fisher information matrix is useful as it is also related to the Fisher information matrix for the localization accuracy problem [RWO12]. In other words, when the two objects are far apart, the Fisher information matrix becomes equivalent to that of two independent localization accuracy problem. The foreseeable challenge of culminating these conditions into a generalized solution is the complexity of the expression which is likely to make it cumbersome for use in practice. Hence, approximations may be necessary in order that a general expression can be derived that provides a reasonable limit of the accuracy that does not compromise the validity of the results obtained.

2. It would also be beneficial, in terms of completeness, to document and compare the approach described here against various existing techniques in terms of (i) the accuracy of the estimation procedures, (ii) the stability of the computation processes, (iii) the modelling of the noise and errors, and (iv) the strength and weakness of each method. Other than through simulations, practical image data from experiments should also be used to substantiate the simulation results obtained.
3. In single-particle tracking experiments, the trajectories of the objects are usually reconstructed from images from the sequence of frames used in their acquisition. The reconstructed trajectories are exploited to compute the mean-squared displacement that characterizes the dynamics of the probe particles. However, the accuracy of the mean-squared displacement

estimation from which the diffusion coefficient is obtained is affected by the limited spatial resolution and statistical uncertainty of the photon detection process. Hence it will be useful to have a theoretical limit of the accuracy of the diffusion coefficient.

Author's Publications

Journal Papers

- [J1] Y. Wong, Z. Lin and R.J. Ober, "Limit of the accuracy of parameter estimation for moving single molecules imaged by fluorescence microscopy," IEEE Transactions of Signal Processing, vol. 59, pp. 895–911, 2011.
- [J2] Y. Wong, Z. Lin and R.J. Ober, "Performance bounds on moving single molecules imaged with time discretization," submitted to Optics Express.

Conference Papers

- [C1] Y. Wong, Z. Lin and R.J. Ober, "Effect of Time Discretization on the Limit of the Accuracy of Parameter Estimation for Moving Single Molecules Imaged by Fluorescence Microscopy," Proceedings of 2012, 9th IEEE International Symposium on Biomedical Imaging (ISBI 2012), pp. 1228–1231, Barcelona, Spain, May 2012.
- [C2] Y. Wong, Z. Lin and R.J. Ober, "Accuracy of the Estimation of Properties of Moving Single Molecules," presented in the Focus on Microscopy Conference, Singapore, April 2012.

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Appendices

A1 Proof of Theorem 2.2

Let $\tilde{p}(\tau) := [\tilde{x}_\theta(x, \tau) \ \tilde{y}_\theta(y, \tau) \ \tilde{z}_\theta(\tau) \ \tilde{o}_\theta(\tau)]^T = [x/M - x_\theta(\tau) \ y/M - y_\theta(\tau) \ z_\theta(\tau) \ o_\theta(\tau)]^T$, $x, y, \tau \in \mathbb{R}$, $\theta \in \Theta$, and $V_\theta(\tau) = \partial \tilde{p}(\tau) / \partial \theta = [-\partial x_\theta(\tau) / \partial \theta \ -\partial y_\theta(\tau) / \partial \theta \ \partial z_\theta(\tau) / \partial \theta \ \partial o_\theta(\tau) / \partial \theta]^T$, where $V_\theta(\tau)$ is independent of x and y . The photon distribution profile $f_{\theta, \tau}$, in terms of the entries of $\tilde{p}(\tau)$, can then be rewritten as

$$f_{\theta, \tau}(x, y) = \frac{1}{M^2} q_{\tilde{z}_\theta(\tau), \tilde{o}_\theta(\tau)}(\tilde{x}_\theta(x, \tau), \tilde{y}_\theta(y, \tau)), \quad x, y \in \mathbb{R}. \quad (6.1)$$

We first show that the existence of $\partial q_{z_\theta(\tau), o_\theta(\tau)}(x/M - x_\theta(\tau), y/M - y_\theta(\tau)) / \partial p(\tau)$ implies the existence of $\partial q_{\tilde{z}_\theta(\tau), \tilde{o}_\theta(\tau)}(\tilde{x}_\theta(x, \tau), \tilde{y}_\theta(y, \tau)) / \partial \tilde{p}(\tau)$. Using the chain rule, we have

$$\begin{aligned} \frac{\partial q_{z_\theta(\tau), o_\theta(\tau)}\left(\frac{x}{M} - x_\theta(\tau), \frac{y}{M} - y_\theta(\tau)\right)}{\partial p(\tau)} &= \frac{\partial q_{\tilde{z}_\theta(\tau), \tilde{o}_\theta(\tau)}\left(\tilde{x}_\theta(x, \tau), \tilde{y}_\theta(y, \tau)\right)}{\partial \tilde{p}(\tau)} \frac{\partial \tilde{p}(\tau)}{\partial p(\tau)} \\ &= \frac{\partial q_{\tilde{z}_\theta(\tau), \tilde{o}_\theta(\tau)}\left(\tilde{x}_\theta(x, \tau), \tilde{y}_\theta(y, \tau)\right)}{\partial \tilde{p}(\tau)} \text{Diag}\left[\frac{1}{M}, \frac{1}{M}, 1, 1\right]. \end{aligned}$$

Hence,

$$\frac{\partial q_{\tilde{z}_\theta(\tau), \tilde{o}_\theta(\tau)}(\tilde{x}_\theta(x, \tau), \tilde{y}_\theta(y, \tau))}{\partial \tilde{p}(\tau)} = \frac{\partial q_{z_\theta(\tau), o_\theta(\tau)}\left(\frac{x}{M} - x_\theta(\tau), \frac{y}{M} - y_\theta(\tau)\right)}{\partial p(\tau)} \text{Diag} \begin{bmatrix} M, M, 1, 1 \end{bmatrix}.$$

Therefore, $\partial q_{\tilde{z}_\theta(\tau), \tilde{o}_\theta(\tau)}(\tilde{x}_\theta(x, \tau), \tilde{y}_\theta(y, \tau)) / \partial \tilde{p}(\tau)$ exists. Consequently, the partial derivative of $f_{\theta, \tau}(x, y)$ with respect to θ can be expressed as follows

$$\begin{aligned} \frac{\partial f_{\theta, \tau}(x, y)}{\partial \theta} &= \frac{1}{M^2} \frac{\partial q_{\tilde{z}_\theta(\tau), \tilde{o}_\theta(\tau)}(\tilde{x}_\theta(x, \tau), \tilde{y}_\theta(y, \tau))}{\partial \tilde{p}(\tau)} \frac{\partial \tilde{p}(\tau)}{\partial \theta} \\ &= \frac{1}{M^2} \frac{\partial q_{\tilde{z}_\theta(\tau), \tilde{o}_\theta(\tau)}(\tilde{x}_\theta(x, \tau), \tilde{y}_\theta(y, \tau))}{\partial \tilde{p}(\tau)} V_\theta(\tau), \end{aligned} \quad (6.2)$$

Substituting (6.1) and (6.2) into the expression of $\mathbf{I}(\theta)$ in Theorem 2.1 and simplifying gives

$$\begin{aligned} \mathbf{I}(\theta) &= \frac{1}{M^2} \int_{t_0}^t \Lambda(\tau) V_\theta^T(\tau) \left[\int_{\mathbb{R}} \int_{\mathbb{R}} \frac{1}{q_{\tilde{z}_\theta(\tau), \tilde{o}_\theta(\tau)}(\tilde{x}_\theta(x, \tau), \tilde{y}_\theta(y, \tau))} \right. \\ &\quad \times \left. \left(\frac{\partial q_{\tilde{z}_\theta(\tau), \tilde{o}_\theta(\tau)}(\tilde{x}_\theta(x, \tau), \tilde{y}_\theta(y, \tau))}{\partial \tilde{p}(\tau)} \right)^T \frac{\partial q_{\tilde{z}_\theta(\tau), \tilde{o}_\theta(\tau)}(\tilde{x}_\theta(x, \tau), \tilde{y}_\theta(y, \tau))}{\partial \tilde{p}(\tau)} dx dy \right] V_\theta(\tau) d\tau. \end{aligned}$$

Since $dx = M d\tilde{x}_\theta$, $dy = M d\tilde{y}_\theta$ and the assumption of $|x_\theta(\tau)|$ and $|y_\theta(\tau)|$ being uniformly bounded for $\theta \in \Theta$, $t_0 \leq \tau \leq t$, the above expression becomes

$$\begin{aligned} \mathbf{I}(\theta) &= \int_{t_0}^t \Lambda(\tau) V_\theta^T(\tau) \left[\int_{\mathbb{R}} \int_{\mathbb{R}} \frac{1}{q_{\tilde{z}_\theta(\tau), \tilde{o}_\theta(\tau)}(\tilde{x}_\theta, \tilde{y}_\theta)} \right. \\ &\quad \times \left. \left(\frac{\partial q_{\tilde{z}_\theta(\tau), \tilde{o}_\theta(\tau)}(\tilde{x}_\theta, \tilde{y}_\theta)}{\partial \tilde{p}(\tau)} \right)^T \frac{\partial q_{\tilde{z}_\theta(\tau), \tilde{o}_\theta(\tau)}(\tilde{x}_\theta, \tilde{y}_\theta)}{\partial \tilde{p}(\tau)} d\tilde{x}_\theta d\tilde{y}_\theta \right] V_\theta(\tau) d\tau. \end{aligned} \quad (6.3)$$

Replacing the dummy variables $\tilde{x}_\theta$ with x , and $\tilde{y}_\theta$ with y in the above expression, respectively, and recalling that $\tilde{z}_\theta(\tau) = z_\theta(\tau)$, and $\tilde{o}_\theta(\tau) = o_\theta(\tau)$, we have $\tilde{p}(\tau) = p(\tau)$ in (6.3) and hence obtain the desired result given in (2.2). $\square$

A2 Proof of Proposition 2.1

As the image function q is independent of $z_\theta(\tau)$ and $o_\theta(\tau)$, $p(\tau)$ and $V_\theta(\tau)$ in Theorem 2.2 reduce to $p := \begin{bmatrix} x & y \end{bmatrix}^T$ and $V_\theta(\tau) := \begin{bmatrix} -\partial x_\theta(\tau)/\partial\theta & -\partial y_\theta(\tau)/\partial\theta \end{bmatrix}^T$, respectively, and the corresponding expression of $\mathbf{I}(\theta)$ becomes

$$\mathbf{I}(\theta) = \int_{t_0}^t \Lambda(\tau) \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial\theta} \\ \frac{\partial y_\theta(\tau)}{\partial\theta} \end{bmatrix}^T \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{1}{q(x, y)} \begin{bmatrix} \left(\frac{\partial q(x, y)}{\partial x} \right)^2 & \frac{\partial q(x, y)}{\partial x} \frac{\partial q(x, y)}{\partial y} \\ \frac{\partial q(x, y)}{\partial x} \frac{\partial q(x, y)}{\partial y} & \left(\frac{\partial q(x, y)}{\partial y} \right)^2 \end{bmatrix} dx dy \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial\theta} \\ \frac{\partial y_\theta(\tau)}{\partial\theta} \end{bmatrix} d\tau, \theta \in \Theta. \quad (6.4)$$

With the assumption of the image profile $q(x, y)$ being radially symmetric and condition (A2), it can be readily shown that

$$\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{1}{q(x, y)} \frac{\partial q(x, y)}{\partial x} \frac{\partial q(x, y)}{\partial y} dx dy = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{4xy}{\tilde{q}(x^2 + y^2)} \left(\frac{\partial \tilde{q}(x^2 + y^2)}{\partial(x^2 + y^2)} \right)^2 dx dy = 0 \quad (6.5)$$

since $(\partial \tilde{q}(x^2 + y^2)/\partial(x^2 + y^2))^2$ is also radially symmetric, and that

$$\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{1}{q(x, y)} \left(\frac{\partial q(x, y)}{\partial x} \right)^2 dx dy = \int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{4x^2}{\tilde{q}(x^2 + y^2)} \left(\frac{\partial \tilde{q}(x^2 + y^2)}{\partial(x^2 + y^2)} \right)^2 dx dy. \quad (6.6)$$

Using the polar coordinate system, where $x = r \cos \phi$ and $y = r \sin \phi$, $\phi, r \in \mathbb{R}$, and with some algebraic manipulations, we have

$$\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{4x^2}{\tilde{q}(x^2 + y^2)} \left(\frac{\partial \tilde{q}(x^2 + y^2)}{\partial(x^2 + y^2)} \right)^2 dx dy = 4\pi \int_0^{\infty} \frac{r^3}{\tilde{q}(r^2)} \left(\frac{\partial \tilde{q}(r^2)}{\partial r^2} \right)^2 dr. \quad (6.7)$$

Similarly, it can be shown that

$$\int_{-\infty}^{\infty} \int_{-\infty}^{\infty} \frac{1}{q(x, y)} \left(\frac{\partial q(x, y)}{\partial y} \right)^2 dx dy = 4\pi \int_0^{\infty} \frac{r^3}{\tilde{q}(r^2)} \left(\frac{\partial \tilde{q}(r^2)}{\partial r^2} \right)^2 dr. \quad (6.8)$$

Substituting (6.5) - (6.8) into (6.4) gives

$$\begin{aligned} \mathbf{I}(\theta) &= \int_{t_0}^t \Lambda(\tau) \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix}^T \begin{bmatrix} 4\pi \int_0^\infty \frac{r^3}{\tilde{q}(r^2)} \left(\frac{\partial \tilde{q}(r^2)}{\partial r^2} \right)^2 dr & 0 \\ 0 & 4\pi \int_0^\infty \frac{r^3}{\tilde{q}(r^2)} \left(\frac{\partial \tilde{q}(r^2)}{\partial r^2} \right)^2 dr \end{bmatrix} \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix} d\tau \\ &= 4\pi \int_0^\infty \frac{r^3}{\tilde{q}(r^2)} \left(\frac{\partial \tilde{q}(r^2)}{\partial r^2} \right)^2 dr \cdot \int_{t_0}^t \Lambda(\tau) \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix}^T \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix} d\tau. \end{aligned}$$

□

A3 Proof of Corollary 2.1

1) Since $\gamma^2 = 4\pi \int_0^\infty r^3 / \tilde{q}(r^2) (\partial \tilde{q}(r^2) / \partial r^2)^2 dr$, the expression of $\mathbf{I}(\theta)$ in Proposition 2.1 can be rewritten as

$$\mathbf{I}(\theta) = \gamma^2 \int_{t_0}^t \Lambda(\tau) \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix}^T \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix} d\tau. \quad (6.9)$$

The linear trajectory of the object of interest for the time interval $[t_0, t]$ is given by

$$x_\theta(\tau) = x_0 + v(\tau - t_0) \cos \phi, \quad y_\theta(\tau) = y_0 + v(\tau - t_0) \sin \phi, \quad t_0 \leq \tau \leq t,$$

where (x_0, y_0) is the starting location of the object, ϕ is the direction of movement, i.e., the angle between the linear trajectory and the x -axis and v is the constant speed of the object. Then for $\theta = (x_0, y_0, \phi, v)$,

$$\begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix} = \begin{bmatrix} 1 & 0 & -v(\tau - t_0) \sin \phi & (\tau - t_0) \cos \phi \\ 0 & 1 & v(\tau - t_0) \cos \phi & (\tau - t_0) \sin \phi \end{bmatrix}, \quad \tau \geq t_0.$$

Hence,

$$\begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix}^T \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix} = \begin{bmatrix} 1 & 0 & -v(\tau - t_0) \sin \phi & (\tau - t_0) \cos \phi \\ 0 & 1 & v(\tau - t_0) \cos \phi & (\tau - t_0) \sin \phi \\ -v(\tau - t_0) \sin \phi & v(\tau - t_0) \cos \phi & v^2(\tau - t_0)^2 & 0 \\ (\tau - t_0) \cos \phi & (\tau - t_0) \sin \phi & 0 & (\tau - t_0)^2 \end{bmatrix}. \quad (6.10)$$

By substituting (6.10) into (6.9), partitioning the matrix obtained and considering the limits of integration from 0 to $t - t_0$, $\mathbf{I}(\theta)$ can be expressed as

$$\mathbf{I}(\theta) = \gamma^2 \left[\begin{array}{c|c} b_1 B_1(\theta) & b_2 B_2(\theta) \\ \hline b_2 B_2^T(\theta) & b_3 B_3(\theta) \end{array} \right], \quad (6.11)$$

where b_1 , b_2 and b_3 are given in (2.5) and

$$B_1(\theta) = \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}, \quad B_2(\theta) = \begin{bmatrix} -v \sin \phi & \cos \phi \\ v \cos \phi & \sin \phi \end{bmatrix}, \quad B_3(\theta) = \begin{bmatrix} v^2 & 0 \\ 0 & 1 \end{bmatrix}.$$

As $b_i \neq 0$, for $i = 1, \dots, 3$ and $B_1(\theta)$ is the 2×2 identity matrix I_2 , the inverse of the partitioned matrix $\mathbf{I}^{-1}(\theta)$ [Kra02] is given by

$$\mathbf{I}^{-1}(\theta) = \frac{1}{\gamma^2} \begin{bmatrix} C_1 & C_2 \\ C_2^T & C_3 \end{bmatrix}, \quad (6.12)$$

where

$$C_1 = \{b_1 B_1(\theta)\}^{-1} + \{b_1 B_1(\theta)\}^{-1} \{b_2 B_2(\theta)\} D^{-1} \{b_2 B_2(\theta)\}^T \{b_1 B_1(\theta)\}^{-1},$$

$$C_2 = -\{b_1 B_1(\theta)\}^{-1} \{b_2 B_2(\theta)\} D^{-1},$$

$$C_3 = D^{-1},$$

$$D = b_3 B_3(\theta) - \{b_2 B_2(\theta)\}^T \{b_1 B_1(\theta)\}^{-1} \{b_2 B_2(\theta)\}.$$

Some simple algebraic manipulations give

$$D = \frac{b_1 b_3 - b_2^2}{b_1} \begin{bmatrix} v^2 & 0 \\ 0 & 1 \end{bmatrix}, \quad (6.13)$$

$$C_3 = D^{-1} = \frac{b_1}{b_1 b_3 - b_2^2} \begin{bmatrix} \frac{1}{v^2} & 0 \\ 0 & 1 \end{bmatrix}, \quad (6.14)$$

$$C_1 = \frac{b_3}{b_1 b_3 - b_2^2} I_2. \quad (6.15)$$

By substituting (6.14) and (6.15) into the inverse Fisher information matrix $\mathbf{I}^{-1}(\theta)$ in (6.12) and taking the square root of its diagonal elements, we obtain the desired result given in (2.4). For the case of the 2D Gaussian image function, $4\pi \int_0^\infty r^3 / \tilde{q}(r^2) (\partial \tilde{q}(r^2) / \partial (r^2))^2 dr = 1/\sigma^2$ [ORW04]. Hence by replacing γ with $1/\sigma$ in (2.4), we obtain the fundamental limit of the accuracy of θ . Similarly for the Airy profile, we replace γ with $2\pi n_a/\lambda$ [ORW04].

2) For the special case where the photon detection rate is constant, i.e., $\Lambda(\tau) = \Lambda_0$, $\tau \geq t_0$, $\Lambda_0 \in \mathbb{R}^+$, the integrals of the photon detection rate with respect to time for the expressions in (2.5) become

$$b_1 = \Lambda_0(t - t_0), \quad b_2 = \frac{\Lambda_0}{2}(t - t_0)^2, \quad b_3 = \frac{\Lambda_0}{3}(t - t_0)^3, \quad t > t_0.$$

Substituting the above expressions into result 1 of this corollary and letting $N = \Lambda_0(t - t_0)$, we obtain the desired result given in (2.6). $\square$

A4 Proof of Corollary 2.2

1) Since $\gamma^2 = 4\pi \int_0^\infty r^3/\tilde{q}(r^2) (\partial\tilde{q}(r^2)/\partial(r^2))^2 dr$, the expression of $\mathbf{I}(\theta)$ in Proposition 2.1 can be rewritten as

$$\mathbf{I}(\theta) = \gamma^2 \int_{t_0}^t \Lambda(\tau) \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix}^T \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix} d\tau. \quad (6.16)$$

The parametric expressions of the moving object with circular trajectory are given by

$$x_\theta(\tau) = x_c + R \cos(\omega(\tau - t_0) + \psi_0), \quad y_\theta(\tau) = y_c + R \sin(\omega(\tau - t_0) + \psi_0), \quad t_0 \leq \tau \leq t,$$

where (x_c, y_c) is the center, R is the radius of the circle, ω is the constant angular velocity of the object and ψ_0 is the angular offset of the starting point (x_0, y_0) from the x -axis. Given that the unknown parameter vector is $\theta = (R, x_c, y_c, \omega, \psi_0)$, then we can readily express

$$\begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix}^T \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix} = \begin{bmatrix} 1 & \cos \psi & \sin \psi & 0 & 0 \\ \cos \psi & 1 & 0 & -R(\tau - t_0) \sin \psi & -R \sin \psi \\ \sin \psi & 0 & 1 & R(\tau - t_0) \cos \psi & R \cos \psi \\ 0 & -R(\tau - t_0) \sin \psi & R(\tau - t_0) \cos \psi & R^2(\tau - t_0)^2 & R^2(\tau - t_0) \\ 0 & -R \sin \psi & R \cos \psi & R^2(\tau - t_0) & R^2 \end{bmatrix}, \quad (6.17)$$

where $\psi = \omega(\tau - t_0) + \psi_0$.

Substituting (6.17) into (6.16), we obtain the desired Fisher information matrix

given in result 1 of Corollary 2.2.

For the case of the 2D Gaussian image function, $4\pi \int_0^\infty r^3/\tilde{q}(r^2) (\partial\tilde{q}(r^2)/\partial(r^2))^2 dr = 1/\sigma^2$ [ORW04] and by replacing γ with $1/\sigma$, we obtain its Fisher information matrix $\mathbf{I}(\theta)$. Similarly for the Airy profile, we obtain its Fisher information matrix by replacing γ with $2\pi n_a/\lambda$ [ORW04].

2) Assuming $\Lambda(\tau) = \Lambda_0$, $t_0 \leq \tau \leq t$, and $t = t_0 + T_p$ where $T_p = 2\pi/\omega$, we can further simplify the Fisher information matrix $\mathbf{I}(\theta)$ in (2.7) after evaluating the following expressions.

$$\int_{t_0}^t \Lambda(\tau) d\tau = \int_{t_0}^{t_0+T_p} \Lambda_0 d\tau = \Lambda_0 T_p, \quad (6.18)$$

$$\int_{t_0}^t \Lambda(\tau)(\tau - t_0) d\tau = \int_{t_0}^{t_0+T_p} \Lambda_0(\tau - t_0) d\tau = \Lambda_0 \frac{T_p^2}{2}, \quad (6.19)$$

$$\int_{t_0}^t \Lambda(\tau)(\tau - t_0)^2 d\tau = \int_{t_0}^{t_0+T_p} \Lambda_0(\tau - t_0)^2 d\tau = \Lambda_0 \frac{T_p^3}{3}. \quad (6.20)$$

Next,

$$\int_{t_0}^t \Lambda(\tau) \cos \psi d\tau = \int_{t_0}^{t_0+T_p} \Lambda_0 \cos(\omega(\tau - t_0) + \psi_0) d\tau = 0, \quad (6.21)$$

since it is the integration over one period of a cosine. Similarly, $\int_{t_0}^t \Lambda(\tau) \sin \psi d\tau = 0$.

Using integration by parts and with some simplifications, we also have

$$\int_{t_0}^t \Lambda(\tau) R(\tau - t_0) \sin \psi d\tau = -R\Lambda_0 \frac{T_p}{\omega} \cos \psi_0, \quad (6.22)$$

$$\int_{t_0}^t \Lambda(\tau) R(\tau - t_0) \cos \psi d\tau = R\Lambda_0 \frac{T_p}{\omega} \sin \psi_0. \quad (6.23)$$

Substituting (6.18)-(6.23) into (2.7) and making use of $\omega = 2\pi/T_p$, we have

$$\begin{aligned} \mathbf{I}(\theta) &= \gamma^2 \begin{bmatrix} \Lambda_0 T_p & 0 & 0 & 0 & 0 \\ 0 & \Lambda_0 T_p & 0 & R\Lambda_0 \frac{T_p^2}{2\pi} \cos \psi_0 & 0 \\ 0 & 0 & \Lambda_0 T_p & R\Lambda_0 \frac{T_p^2}{2\pi} \sin \psi_0 & 0 \\ 0 & R\Lambda_0 \frac{T_p^2}{2\pi} \cos \psi_0 & R\Lambda_0 \frac{T_p^2}{2\pi} \sin \psi_0 & R^2 \Lambda_0 \frac{T_p^3}{3} & R^2 \Lambda_0 \frac{T_p^2}{2} \\ 0 & 0 & 0 & R^2 \Lambda_0 \frac{T_p^2}{2} & R^2 \Lambda_0 T_p \end{bmatrix} \\ &= \gamma^2 N_{T_p} \left[\begin{array}{c|c} 1 & 0 \\ \hline 0 & \mathbf{I}_1(\theta) \end{array} \right], \end{aligned}$$

where $N_{T_p} = \Lambda_0 T_p$ and

$$\mathbf{I}_1(\theta) := \left[\begin{array}{cc|cc} 1 & 0 & R\frac{T_p}{2\pi} \cos \psi_0 & 0 \\ 0 & 1 & R\frac{T_p}{2\pi} \sin \psi_0 & 0 \\ \hline R\frac{T_p}{2\pi} \cos \psi_0 & R\frac{T_p}{2\pi} \sin \psi_0 & R^2 \frac{T_p^2}{3} & R^2 \frac{T_p}{2} \\ 0 & 0 & R^2 \frac{T_p}{2} & R^2 \end{array} \right].$$

Hence $\mathbf{I}^{-1}(\theta)$ is given by

$$\mathbf{I}^{-1}(\theta) = \frac{1}{\gamma^2 N_{T_p}} \left[\begin{array}{c|c} 1 & 0 \\ \hline 0 & \mathbf{I}_1^{-1}(\theta) \end{array} \right]. \quad (6.24)$$

Adopting the same approach to inverting the 4×4 matrix in (6.11), which has a similar structure as $\mathbf{I}_1(\theta)$ here, we can readily obtain

$$\mathbf{I}_1^{-1}(\theta) = \begin{bmatrix} 1 + \frac{3}{(\pi^2-3)} \cos^2 \psi_0 & \frac{3}{(\pi^2-3)} \sin \psi_0 \cos \psi_0 & \frac{-6\pi}{(\pi^2-3)RT_p} \cos \psi_0 & \frac{3\pi}{(\pi^2-3)R} \cos \psi_0 \\ \frac{3}{(\pi^2-3)} \sin \psi_0 \cos \psi_0 & 1 + \frac{3}{(\pi^2-3)} \sin^2 \psi_0 & \frac{-6\pi}{(\pi^2-3)RT_p} \sin \psi_0 & \frac{3\pi}{(\pi^2-3)R} \sin \psi_0 \\ \frac{-6\pi}{(\pi^2-3)RT_p} \cos \psi_0 & \frac{-6\pi}{(\pi^2-3)RT_p} \sin \psi_0 & \frac{12\pi^2}{(\pi^2-3)R^2T_p^2} & \frac{-6\pi^2}{(\pi^2-3)R^2T_p} \\ \frac{3\pi}{(\pi^2-3)R} \cos \psi_0 & \frac{3\pi}{(\pi^2-3)R} \sin \psi_0 & \frac{-6\pi^2}{(\pi^2-3)R^2T_p} & \frac{4\pi^2-3}{(\pi^2-3)R^2} \end{bmatrix}. \quad (6.25)$$

Substituting $\mathbf{I}_1^{-1}(\theta)$ in (6.25) into (6.24) and taking the square root of its diagonal elements, we obtain the fundamental limits of the accuracy of $\theta = (R, x_c, y_c, \omega, \psi_0)$ as given in (2.7). $\square$

A5 Proof of Theorem 2.3

1) Using condition (A1), the mean of the number of detected photons at the k^{th} pixel due to the object of interest for the time interval $[t_0, t]$ is given by [RWO06b]

$$\mu_{\theta,k} = \int_{t_0}^t \int_{C_k} \Lambda_1(\tau) f_{\theta,\tau,1}(x, y) dx dy d\tau, \quad \theta \in \Theta, \quad k = 1, \dots, N_p.$$

The above expression can then be expressed in terms of its image function

$q_{z_\theta(\tau), o_\theta(\tau), 1}$ as

$$\mu_{\theta,k} = \frac{1}{M^2} \int_{t_0}^t \int_{C_k} \Lambda_1(\tau) q_{z_\theta(\tau), o_\theta(\tau), 1} \left(\frac{x}{M} - x_\theta(\tau), \frac{y}{M} - y_\theta(\tau) \right) dx dy d\tau. \quad (6.26)$$

The mean of the number of detected photons at the k^{th} pixel due to the background component for the time interval $[t_0, t]$ is given by [RWO06b]

$$\begin{aligned}\beta_k &= \int_{t_0}^t \int_{C_k} \Lambda_2(\tau) f_2(x, y) dx dy d\tau \\ &= \frac{1}{M^2} \int_{t_0}^t \int_{C_k} \Lambda_2(\tau) q_2\left(\frac{x}{M}, \frac{y}{M}\right) dx dy d\tau, \quad \theta \in \Theta, k = 1, \dots, N_p.\end{aligned}\quad (6.27)$$

From [RWO06b], $v_{\theta,k} = \mu_{\theta,k} + \beta_k$. Substituting (6.26) and (6.27) into $v_{\theta,k}$ gives

$$\begin{aligned}v_{\theta,k} &= \frac{1}{M^2} \int_{t_0}^t \int_{C_k} \left[\Lambda_1(\tau) q_{z_{\theta}(\tau), o_{\theta}(\tau), 1}\left(\frac{x}{M} - x_{\theta}(\tau), \frac{y}{M} - y_{\theta}(\tau)\right) \right. \\ &\quad \left. + \Lambda_2(\tau) q_2\left(\frac{x}{M}, \frac{y}{M}\right) \right] dx dy d\tau.\end{aligned}\quad (6.28)$$

The partial derivative of $\mu_{\theta,k}$ with respect to θ is expressed as

$$\frac{\partial \mu_{\theta,k}}{\partial \theta} = \frac{1}{M^2} \frac{\partial}{\partial \theta} \left[\int_{t_0}^t \int_{C_k} \Lambda_1(\tau) q_{z_{\theta}(\tau), o_{\theta}(\tau), 1}\left(\frac{x}{M} - x_{\theta}(\tau), \frac{y}{M} - y_{\theta}(\tau)\right) dx dy d\tau \right]. \quad (6.29)$$

Interchanging the operation of differentiation with that of integration for (6.29) and adopting a similar approach used in Theorem 2.2, the derivative of $\mu_{\theta,k}$ with respect to θ can be expressed as

$$\frac{\partial \mu_{\theta,k}}{\partial \theta} = \frac{1}{M^2} \int_{t_0}^t \Lambda_1(\tau) \int_{C_k} \left[\frac{\partial q_{z_{\theta}(\tau), o_{\theta}(\tau), 1}\left(\frac{x}{M} - x_{\theta}(\tau), \frac{y}{M} - y_{\theta}(\tau)\right)}{\partial p_1(\tau)} \right] dx dy V_{\theta}(\tau) d\tau. \quad (6.30)$$

Substituting (6.28) and (6.30) into $\mathbf{I}(\theta) = \sum_{k=1}^{N_p} \frac{1}{v_{\theta,k}} \left(\frac{\partial \mu_{\theta,k}}{\partial \theta} \right)^T \frac{\partial \mu_{\theta,k}}{\partial \theta}$ [SM99], we obtain the Fisher information matrix $\mathbf{I}(\theta)$ in (2.8) for the pixelated detector $\mathcal{C}_p$ where the detected photons from the object of interest and background component are independently Poisson distributed.

2) For the case comprising Poisson and Gaussian random variables, we can sub-

stitute the mean η_k and variance $\sigma_{w,k}^2$ of the Gaussian random variable, (6.28) and (6.30) into the expression of $\mathbf{I}(\theta)$ in (2.9) [RWO06b]. $\square$

A6 Proof of Proposition 3.2

By letting $\gamma^2 = 4\pi \int_0^\infty r^3/\tilde{q}(r^2) (\partial\tilde{q}(r^2)/\partial r^2)^2 dr$, the expression of the Fisher information matrix in Proposition 3.1 can be further simplified and rewritten as

$$\mathbf{I}(\tilde{\theta}) = \gamma^2 \int_{t_0}^t \Lambda(\tau) K^T \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix}^T \begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix} K d\tau, \quad (6.31)$$

where the prior knowledge matrix $K = [\partial\theta/\partial\tilde{\theta}]$, $\tilde{\theta} \subseteq \theta \in \Theta$.

When the image of the moving object is in focus, the diffraction-limited spot is described by an Airy profile and thus for $q(x, y) = J_1^2(2\pi n_a \sqrt{x^2 + y^2}/\lambda)/(\pi(x^2 + y^2))$, $(x, y) \in \mathbb{R}^2$, $\gamma := 2\pi n_a/\lambda$ [ORW04], where n_a and λ denote the numerical aperture and emission wavelength respectively and J_1 denotes the first order Bessel function of the first kind. When the image profile is approximated with the 2D Gaussian profile $q(x, y) = 1/(2\pi\sigma^2) \exp(-(x^2 + y^2)/(2\sigma^2))$, $\sigma > 0$, $(x, y) \in \mathbb{R}^2$, then $\gamma := 1/\sigma$ [ORW04].

The parametric expressions for the linear trajectory of the object of interest for the time interval $[t_0, t]$ are given by $x_\theta(\tau) = x_0 + v(\tau - t_0) \cos \phi$, $y_\theta(\tau) = y_0 + v(\tau - t_0) \sin \phi$, $t_0 \leq \tau \leq t$, where (x_0, y_0) is the starting location of the object, ϕ is the direction of movement, i.e., the angle between the linear trajectory and the x -axis and v is the speed of the object. Then for

$$\theta = (x_0, y_0, \phi, v) \in \Theta,$$

$$\begin{bmatrix} \frac{\partial x_\theta(\tau)}{\partial \theta} \\ \frac{\partial y_\theta(\tau)}{\partial \theta} \end{bmatrix} = \begin{bmatrix} 1 & 0 & -v(\tau - t_0) \sin \phi & (\tau - t_0) \cos \phi \\ 0 & 1 & v(\tau - t_0) \cos \phi & (\tau - t_0) \sin \phi \end{bmatrix}, \quad \tau \geq t_0. \quad (6.32)$$

1) When prior knowledge of v is available, the corresponding prior knowledge matrix for the unknown parameter vector $\tilde{\theta} = (x_0, y_0, \phi) \in \Theta$ is given by $K = \begin{bmatrix} I_3 & 0 \end{bmatrix}^T$, where I_3 is a 3×3 identity matrix and 0 is a 1×3 null vector. Using the fact that the matrix multiplication is an associative operation, we can first reduce the matrix size of (6.31) before subjecting the expression to further algebraic manipulations such that

$$\mathbf{I}(\tilde{\theta}) = \gamma^2 \begin{bmatrix} b_1 & 0 & -vb_2 \sin \phi \\ 0 & b_1 & vb_2 \cos \phi \\ -vb_2 \sin \phi & vb_2 \cos \phi & v^2 b_3 \end{bmatrix}, \quad (6.33)$$

where $b_1 = \int_0^{t-t_0} \Lambda(\tau + t_0) d\tau$, $b_2 = \int_0^{t-t_0} \Lambda(\tau + t_0) \tau d\tau$ and $b_3 = \int_0^{t-t_0} \Lambda(\tau + t_0) \tau^2 d\tau$. For $b_i \neq 0$, $i = 1, 2, 3$, an inverse exists for (6.33). Thus if the photon detection rate is a function of time, i.e., $\Lambda(\tau)$, $\tau \leq t_0$, by taking the square roots of the diagonal elements of the inverse Fisher information matrix of (6.33), we obtain

$$\delta_{x_0} = \frac{1}{\gamma} \sqrt{\frac{b_3 - \frac{b_2^2}{b_1} \cos^2 \phi}{b_1 b_3 - b_2^2}}, \quad \delta_{y_0} = \frac{1}{\gamma} \sqrt{\frac{b_3 - \frac{b_2^2}{b_1} \sin^2 \phi}{b_1 b_3 - b_2^2}}, \quad \delta_\phi = \frac{1}{\gamma v} \sqrt{\frac{b_1}{b_1 b_3 - b_2^2}}, \quad (6.34)$$

which is result 1 of this proposition.

2) If the photon detection rate $\Lambda(\tau) = \Lambda_0 \in \mathbb{R}^+$, $\tau \geq t_0$,

$$b_1 = \Lambda_0(t - t_0), \quad b_2 = \frac{\Lambda_0}{2}(t - t_0)^2, \quad b_3 = \frac{\Lambda_0}{3}(t - t_0)^3, \quad t > t_0. \quad (6.35)$$

Substituting the above expressions into (6.34), we obtain result 2 of this proposition.

3) In the case where prior knowledge of ϕ is available, the corresponding prior knowledge matrix of the unknown parameter vector $\tilde{\theta} = (x_0, y_0, v) \in \Theta$ is given by

$$K^T = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}. \quad (6.36)$$

Substituting (6.36) and (6.32) into (6.31),

$$\mathbf{I}(\tilde{\theta}) = \gamma^2 \begin{bmatrix} b_1 & 0 & b_2 \cos \phi \\ 0 & b_1 & b_2 \sin \phi \\ b_2 \cos \phi & b_2 \sin \phi & b_3 \end{bmatrix}. \quad (6.37)$$

Similar to the preceding case, if the photon detection rate is a function of time, i.e., $\Lambda(\tau)$, $\tau \leq t_0$, then the fundamental limit of $\tilde{\theta} = (x_0, y_0, v) \in \Theta$ for the time interval $[t_0, t]$ is given as follows.

$$\delta_{x_0} = \frac{1}{\gamma} \sqrt{\frac{b_3 - \frac{b_2^2}{b_1} \sin^2 \phi}{b_1 b_3 - b_2^2}}, \quad \delta_{y_0} = \frac{1}{\gamma} \sqrt{\frac{b_3 - \frac{b_2^2}{b_1} \cos^2 \phi}{b_1 b_3 - b_2^2}}, \quad \delta_v = \frac{1}{\gamma} \sqrt{\frac{b_1}{b_1 b_3 - b_2^2}}, \quad (6.38)$$

which is result 3 of this proposition.

4) If the photon detection rate $\Lambda(\tau) = \Lambda_0 \in \mathbb{R}^+$, $\tau \geq t_0$, we substitute the expressions of b_1 , b_2 and b_3 derived in result 2 into the above expressions and obtain result 4 of this proposition. $\square$

A7 Proof of Theorem 4.1

1. Since the image acquired in each individual frame of the sequence of frames is assumed to be independent of one another, the Fisher information matrix of $\mathcal{G}$ for the total acquisition time $[t_0, t_{N_f}]$ can be expressed as $\mathbf{I}(\theta) = \sum_{i=1}^{N_f} \mathbf{I}_i(\theta)$, where $\mathbf{I}_i(\theta)$ is the Fisher information matrix of $\mathcal{G}$ for the exposure interval $[t_{i-1}, e_i]$. By applying the expression of the Fisher information matrix derived in Theorem 2.2 to each individual frame of the sequence and summing them, we obtain the following expression.

$$\begin{aligned} \mathbf{I}(\theta) = & \sum_{i=1}^{N_f} \int_{t_{i-1}}^{e_i} \Lambda(\tau) V_{\theta}^T(\tau) \\ & \times \left[\int_{\mathbb{R}} \int_{\mathbb{R}} \frac{\left(\frac{\partial q_{z_{\theta}(\tau), o_{\theta}(\tau)}(x, y)}{\partial p(\tau)} \right)^T \left(\frac{\partial q_{z_{\theta}(\tau), o_{\theta}(\tau)}(x, y)}{\partial p(\tau)} \right)}{q_{z_{\theta}(\tau), o_{\theta}(\tau)}(x, y)} dx dy \right] V_{\theta}(\tau) d\tau. \quad (6.39) \end{aligned}$$

2. When the exposure interval is equal to the frame interval, i.e., $e_i = t_i$, by applying the property of the integral - additivity of integration on intervals

to result 1 of this theorem, we obtain

$$\begin{aligned} \mathbf{I}(\theta) &= \int_{t_0}^{t_{N_f}} \Lambda(\tau) V_\theta^T(\tau) \\ &\times \left[\int_{\mathbb{R}} \int_{\mathbb{R}} \frac{\left(\frac{\partial q_{z_\theta(\tau), o_\theta(\tau)}(x, y)}{\partial p(\tau)} \right)^T \left(\frac{\partial q_{z_\theta(\tau), o_\theta(\tau)}(x, y)}{\partial p(\tau)} \right)}{q_{z_\theta(\tau), o_\theta(\tau)}(x, y)} dx dy \right] V_\theta(\tau) d\tau. \end{aligned} \quad (6.40)$$

□

A8 Proof of Corollary 4.1

1. When the image function is independent of $z_\theta(\tau)$ and $o_\theta(\tau)$ of the object of interest, the column vectors $V_\theta(\tau)$ and $p(\tau)$ in Theorem 4.1 reduce to $\begin{bmatrix} -\partial x_\theta(\tau)/\partial\theta & -\partial y_\theta(\tau)/\partial\theta \end{bmatrix}^T$ and $\begin{bmatrix} x & y \end{bmatrix}^T$, respectively. Together with the assumptions that the image function is radially symmetric, i.e., $q(x, y) = \tilde{q}(r^2)$ where $\tilde{q} : \mathbb{R}^2 \rightarrow \mathbb{R}$, and recalling the derivations in Proposition 2.1, the Fisher information matrix can be made separable in terms of the spatial and temporal integrals. Thus, we obtain the following expression.

$$\mathbf{I}(\theta) = 4\pi \int_0^\infty \frac{r^3}{\tilde{q}(r^2)} \left(\frac{\partial \tilde{q}(r^2)}{\partial r^2} \right)^2 dr \sum_{i=1}^{N_f} \int_{t_{i-1}}^{e_i} \Lambda(\tau) \left[\frac{\partial x_\theta^T(\tau)}{\partial\theta} \frac{\partial x_\theta(\tau)}{\partial\theta} + \frac{\partial y_\theta^T(\tau)}{\partial\theta} \frac{\partial y_\theta(\tau)}{\partial\theta} \right] d\tau. \quad (6.41)$$

2. When the exposure interval is equal to the frame interval, i.e., $e_i = t_i$, by applying the property of the integral - additivity of integration on intervals to result 1 of this corollary, we obtain

$$\mathbf{I}(\theta) = 4\pi \int_0^\infty \frac{r^3}{\tilde{q}(r^2)} \left(\frac{\partial \tilde{q}(r^2)}{\partial r^2} \right)^2 dr \int_{t_0}^{t_{N_f}} \Lambda(\tau) \left[\frac{\partial x_\theta^T(\tau)}{\partial\theta} \frac{\partial x_\theta(\tau)}{\partial\theta} + \frac{\partial y_\theta^T(\tau)}{\partial\theta} \frac{\partial y_\theta(\tau)}{\partial\theta} \right] d\tau. \quad (6.42)$$

□

A9 Proof of Corollary 4.2

1. Since we let $\gamma^2 = 4\pi \int_0^\infty r^3 / \tilde{q}(r^2) (\partial \tilde{q}(r^2) / \partial r^2)^2 dr$, the expression of the Fisher information matrix in Corollary 4.1 can then be rewritten as

$$\mathbf{I}(\theta) = \gamma^2 \sum_{i=1}^{N_f} \int_{t_{i-1}}^{e_i} \Lambda(\tau) \left[\frac{\partial x_\theta^T(\tau)}{\partial \theta} \frac{\partial x_\theta(\tau)}{\partial \theta} + \frac{\partial y_\theta^T(\tau)}{\partial \theta} \frac{\partial y_\theta(\tau)}{\partial \theta} \right] d\tau. \quad (6.43)$$

The parametric equations of a linear trajectory for the total acquisition time $[t_0, t_{N_f}]$ are given by $x_\theta(\tau) = x_0 + v(\tau - t_0) \cos \phi$, $y_\theta(\tau) = y_0 + v(\tau - t_0) \sin \phi$, $t_0 \leq \tau \leq t_{N_f}$. Hence for $\theta = (x_0, y_0, \phi, v) \in \Theta$,

$$\begin{aligned} \frac{\partial x_\theta(\tau)}{\partial \theta} &= \begin{bmatrix} 1 & 0 & -v(\tau - t_0) \sin \phi & (\tau - t_0) \cos \phi \end{bmatrix}, \\ \frac{\partial y_\theta(\tau)}{\partial \theta} &= \begin{bmatrix} 0 & 1 & v(\tau - t_0) \cos \phi & (\tau - t_0) \sin \phi \end{bmatrix}. \end{aligned} \quad (6.44)$$

Substituting the partial derivatives of the trajectory with respect to θ from (6.44) into (6.43), the Fisher information matrix becomes

$$\mathbf{I}(\theta) = \gamma^2 \begin{bmatrix} b_1 & 0 & -b_2 v \sin \phi & b_2 \cos \phi \\ 0 & b_1 & b_2 v \cos \phi & b_2 \sin \phi \\ -b_2 v \sin \phi & b_2 v \cos \phi & b_3 v^2 & 0 \\ b_2 \cos \phi & b_2 \sin \phi & 0 & b_3 \end{bmatrix},$$

where $b_1 = \sum_{i=1}^{N_f} \int_{t_{i-1}}^{e_i} \Lambda(\tau) d\tau$, $b_2 = \sum_{i=1}^{N_f} \int_{t_{i-1}}^{e_i} \Lambda(\tau) (\tau - t_0) d\tau$ and $b_3 = \sum_{i=1}^{N_f} \int_{t_{i-1}}^{e_i} \Lambda(\tau) (\tau - t_0)^2 d\tau$. Since the expression of the Fisher information matrix is similar to that in Corollary 2.1, we adopt the same approach in

determining its inverse. By taking the square root of its diagonal elements from the inverse of the Fisher information matrix, we obtain the following expressions.

$$\begin{aligned}\delta_{x_0} = \delta_{y_0} &= \frac{1}{\gamma} \sqrt{\frac{b_3}{b_1 b_3 - b_2^2}}, & \delta_\phi &= \frac{1}{\gamma v} \sqrt{\frac{b_1}{b_1 b_3 - b_2^2}}, \\ \delta_v &= \frac{1}{\gamma} \sqrt{\frac{b_1}{b_1 b_3 - b_2^2}},\end{aligned}\tag{6.45}$$

where

$$\begin{aligned}b_1 &= \sum_{i=1}^{N_f} \int_{t_{i-1}}^{e_i} \Lambda(\tau) d\tau, & b_2 &= \sum_{i=1}^{N_f} \int_{t_{i-1}}^{e_i} \Lambda(\tau) (\tau - t_0) d\tau, \\ b_3 &= \sum_{i=1}^{N_f} \int_{t_{i-1}}^{e_i} \Lambda(\tau) (\tau - t_0)^2 d\tau.\end{aligned}\tag{6.46}$$

2. When the photon detection rate $\Lambda(\tau) = \Lambda_0 \in \mathbb{R}^+$, $\tau \geq t_0$, and the exposure intervals are uniform throughout the image acquisition process, i.e., $[t_{i-1}, e_i] = [t_i, e_{i+1}]$, $i = 1, 2, \dots, N_f$, then b_1 , b_2 and b_3 in result 1 of this corollary can be expressed in terms of the photon detection rate Λ_0 , number of frames N_f , duration of the exposure interval $t_{ue} = e_i - t_{i-1}$ and

duration of the total acquisition time $T_{tat} = t_{N_f} - t_0$ as follows.

$$b_1 = \sum_{i=1}^{N_f} \int_0^{e_i - t_{i-1}} \Lambda_0 d\tau = \sum_{i=1}^{N_f} \int_0^{t_{ue}} \Lambda_0 d\tau = N_f \Lambda_0 t_{ue}, \quad (6.47)$$

$$\begin{aligned} b_2 &= \sum_{i=1}^{N_f} \int_0^{e_i - t_{i-1}} \Lambda_0 (\tau + t_{i-1} - t_0) d\tau \\ &= \Lambda_0 \sum_{i=1}^{N_f} \left(\int_0^{t_{ue}} \tau d\tau + \int_0^{t_{ue}} (t_{i-1} - t_0) d\tau \right) \\ &= \Lambda_0 N_f \frac{t_{ue}^2}{2} + \Lambda_0 t_{ue} \sum_{i=1}^{N_f} (t_{i-1} - t_0), \end{aligned} \quad (6.48)$$

and

$$\begin{aligned} b_3 &= \sum_{i=1}^{N_f} \int_0^{e_i - t_{i-1}} \Lambda_0 (\tau + t_{i-1} - t_0)^2 d\tau \\ &= \Lambda_0 \sum_{i=1}^{N_f} \left(\int_0^{t_{ue}} \tau^2 d\tau + \int_0^{t_{ue}} 2\tau(t_{i-1} - t_0) d\tau + \int_0^{t_{ue}} (t_{i-1} - t_0)^2 d\tau \right) \\ &= \Lambda_0 N_f \frac{t_{ue}^3}{3} + \Lambda_0 t_{ue}^2 \sum_{i=1}^{N_f} (t_{i-1} - t_0) + \Lambda_0 t_{ue} \sum_{i=1}^{N_f} (t_{i-1} - t_0)^2. \end{aligned} \quad (6.49)$$

Since $t_{i-1} - t_0 = (i-1)T_{tat}/N_f$, then $\sum_{i=1}^{N_f} (t_{i-1} - t_0)$ and $\sum_{i=1}^{N_f} (t_{i-1} - t_0)^2$ in (6.48) and (6.49), respectively, can be expressed in terms of T_{tat} and N_f as follows.

$$\sum_{i=1}^{N_f} (t_{i-1} - t_0) = T_{tat} \frac{(N_f - 1)}{2}, \quad \sum_{i=1}^{N_f} (t_{i-1} - t_0)^2 = T_{tat}^2 \frac{(N_f - 1)(2N_f - 1)}{6N_f}. \quad (6.50)$$

Substituting the identities in (6.50) into (6.48) and (6.49), we can express

$$\begin{aligned} b_2 &= \Lambda_0 N_f \frac{t_{ue}^2}{2} + \Lambda_0 t_{ue} T_{tat} \frac{(N_f - 1)}{2}, \\ b_3 &= \Lambda_0 N_f \frac{t_{ue}^3}{3} + \Lambda_0 t_{ue}^2 T_{tat} \frac{(N_f - 1)}{2} + \Lambda_0 t_{ue} T_{tat}^2 \frac{(N_f - 1)(2N_f - 1)}{6N_f}. \end{aligned} \quad (6.51)$$

By substituting expressions in (6.47) and (6.51) into result 1 of this corollary, and expressing them in terms of commonly used basic experimental conditions such as the acquisition frame rate $F = N_f/T_{tat}$, the duration of the total acquisition time T_{tat} and the duration of the exposure interval t_{ue} , we obtain following expressions.

$$\begin{aligned} \delta_{x_0} = \delta_{y_0} &= \frac{2}{\gamma} \sqrt{\frac{t_{ue}^2 + \frac{3}{2}t_{ue}(T_{tat} - \frac{1}{F}) + (T_{tat}^2 - \frac{3T_{tat}}{2F} + \frac{1}{2F^2})}{\Lambda_0 F T_{tat} t_{ue} (t_{ue}^2 + T_{tat}^2 - \frac{1}{F^2})}}, \\ \delta_\phi &= \frac{2}{\gamma v} \sqrt{\frac{3}{\Lambda_0 F T_{tat} t_{ue} (t_{ue}^2 + T_{tat}^2 - \frac{1}{F^2})}}, \\ \delta_v &= \frac{2}{\gamma} \sqrt{\frac{3}{\Lambda_0 F T_{tat} t_{ue} (t_{ue}^2 + T_{tat}^2 - \frac{1}{F^2})}}. \end{aligned} \quad (6.52)$$

□

A10 Proof of Theorem 4.2

1. For the convenience of exposition, we first consider pixel k . The photon detection rate is assumed to be a constant, i.e., $\Lambda^{(1)}(\tau) = \Lambda_0 \in \mathbb{R}^+$ and the exposure interval to be equal to the frame interval, i.e., $e_i = t_i$. When the images are acquired with N_f frames ($N_f > 1$) over the total acquisition time $[t_0, t_{N_f}]$ in the absence of extraneous noise, the Fisher information

matrix can be obtained by substituting (4.8) into (4.10). By interchanging the differentiation operator with the integration operator, the Fisher information matrix for pixel k , denoted by $\mathbf{I}_{N_f,k}(\theta)$, can be expressed as follows.

$$\mathbf{I}_{N_f,k}(\theta) = \Lambda_0 \sum_{i=1}^{N_f} \frac{\left(\int_{t_{i-1}}^{t_i} \int_{C_k} \frac{\partial f_{\theta,\tau,1}(r)}{\partial \theta} dr d\tau \right)^T \left(\int_{t_{i-1}}^{t_i} \int_{C_k} \frac{\partial f_{\theta,\tau,1}(r)}{\partial \theta} dr d\tau \right)}{\int_{t_{i-1}}^{t_i} \int_{C_k} f_{\theta,\tau,1}(r) dr d\tau}. \quad (6.53)$$

Recalling $a_{k,u,v} = \int_{t_u}^{t_v} \int_{C_k} f_{\theta,\tau,1}(r) dr d\tau \in \mathbb{R}^+$ and $A_{k,u,v} = \int_{t_u}^{t_v} \int_{C_k} \frac{\partial f_{\theta,\tau,1}(r)}{\partial \theta} dr d\tau \in \mathbb{R}^{1 \times n}$ from Theorem 4.2, where the first entry of the subscript of $a_{k,u,v}$ and $A_{k,u,v}$ refers to the k^{th} pixel and the subsequent two subscripts refer to the temporal limits of integration, the Fisher information matrix for the case where $N_f = 2$ can then be written as

$$\mathbf{I}_{2,k}(\theta) = \Lambda_0 \sum_{i=1}^2 \frac{A_{k,i-1,i}^T A_{k,i-1,i}}{a_{k,i-1,i}} = \Lambda_0 \left(\frac{A_{k,0,1}^T A_{k,0,1}}{a_{k,0,1}} + \frac{A_{k,1,2}^T A_{k,1,2}}{a_{k,1,2}} \right). \quad (6.54)$$

Similarly, when the image is acquired in a single frame over the total acquisition time $[t_0, t_2]$,

$$\mathbf{I}_{1,k}(\theta) = \Lambda_0 \left(\frac{A_{k,0,2}^T A_{k,0,2}}{a_{k,0,2}} \right). \quad (6.55)$$

By applying the property of integral-additivity of integration on intervals to the above expression, the row vector $A_{k,0,2}$ can be expressed as $A_{k,0,2} = A_{k,0,1} + A_{k,1,2}$. Thus, after the expansion of the above expression, it is

written as follows.

$$\mathbf{I}_{1,k}(\theta) = \Lambda_0 \left(\frac{A_{k,0,1}^T A_{k,0,1}}{a_{k,0,2}} + \frac{A_{k,0,1}^T A_{k,1,2}}{a_{k,0,2}} + \frac{A_{k,1,2}^T A_{k,0,1}}{a_{k,0,2}} + \frac{A_{k,1,2}^T A_{k,1,2}}{a_{k,0,2}} \right). \quad (6.56)$$

To express (6.54) into the form above, we multiply its numerator and denominator with the scalar quantity $a_{k,0,2}$ and expand $a_{k,0,2} = a_{k,0,1} + a_{k,1,2}$ such that

$$\mathbf{I}_{2,k}(\theta) = \Lambda_0 \left(\frac{a_{k,0,1} A_{k,0,1}^T A_{k,0,1}}{a_{k,0,1} a_{k,0,2}} + \frac{a_{k,1,2} A_{k,0,1}^T A_{k,0,1}}{a_{k,0,1} a_{k,0,2}} + \frac{a_{k,0,1} A_{k,1,2}^T A_{k,1,2}}{a_{k,1,2} a_{k,0,2}} + \frac{a_{k,1,2} A_{k,1,2}^T A_{k,1,2}}{a_{k,1,2} a_{k,0,2}} \right). \quad (6.57)$$

Thus, by taking the difference between $\mathbf{I}_{2,k}(\theta)$ and $\mathbf{I}_{1,k}(\theta)$, i.e., $\delta \mathbf{I}_{1,k}(\theta) = \mathbf{I}_{2,k}(\theta) - \mathbf{I}_{1,k}(\theta)$, $\delta \mathbf{I}_{1,k}(\theta)$ is expressed as follows.

$$\delta \mathbf{I}_{1,k}(\theta) = \Lambda_0 (d_{11} + d_{12} + d_{21} + d_{22}). \quad (6.58)$$

where

$$\begin{aligned} d_{11} &= d_{22} = 0, \\ d_{12} &= \frac{a_{k,1,2} A_{k,0,1}^T A_{k,0,1}}{a_{k,0,1} a_{k,0,2}} - \frac{A_{k,0,1}^T A_{k,1,2}}{a_{k,0,2}}, \\ d_{21} &= \frac{a_{k,0,1} A_{k,1,2}^T A_{k,1,2}}{a_{k,1,2} a_{k,0,2}} - \frac{A_{k,1,2}^T A_{k,0,1}}{a_{k,0,2}}. \end{aligned} \quad (6.59)$$

By summing d_{12} and d_{21} and making their denominators common with the scalar quantities $a_{k,0,1}$ and $a_{k,1,2}$, $\delta \mathbf{I}_{1,k}(\theta)$ becomes

$$\delta \mathbf{I}_{1,k}(\theta) = \frac{\Lambda_0}{a_{k,0,1}a_{k,1,2}a_{k,0,2}} \left(a_{k,1,2}^2 A_{k,0,1}^T A_{k,0,1} - a_{k,0,1}a_{k,1,2} A_{k,0,1}^T A_{k,1,2} - a_{k,0,1}a_{k,1,2} A_{k,1,2}^T A_{k,0,1} + a_{k,0,1}^2 A_{k,1,2}^T A_{k,1,2} \right). \quad (6.60)$$

Using the identity $b^2 X^T X - bc(X^T Y + Y^T X) + c^2 Y^T Y = (bX - cY)^T (bX - cY)$, where b, c are the scalar quantities and X and Y are the row vectors, expression (6.60) is expressed concisely as

$$\begin{aligned} \delta \mathbf{I}_{1,k}(\theta) &= \frac{\Lambda_0}{a_{k,0,1}a_{k,1,2}a_{k,0,2}} (a_{k,1,2}A_{k,0,1} - a_{k,0,1}A_{k,1,2})^T (a_{k,1,2}A_{k,0,1} - a_{k,0,1}A_{k,1,2}) \\ &= \Lambda_0 \frac{W_{k,1,1}^T W_{k,1,1}}{a_{k,0,1}a_{k,1,2}a_{k,0,2}}, \end{aligned} \quad (6.61)$$

where $W_{k,1,1} = a_{k,1,2}A_{k,0,1} - a_{k,0,1}A_{k,1,2}$.

Recalling that the above derivation is for pixel k , we now sum up (6.61) for N_p pixels that constitute the pixelated detector and obtain result 1 of this theorem.

2. For $N_f > 2$, the Fisher information matrix is

$$\begin{aligned} \mathbf{I}_{N_f,k}(\theta) &= \Lambda_0 \sum_{i=1}^{N_f} \frac{A_{k,i-1,i}^T A_{k,i-1,i}}{a_{k,i-1,i}} \\ &= \Lambda_0 \left(\frac{A_{k,0,1}^T A_{k,0,1}}{a_{k,0,1}} + \frac{A_{k,1,2}^T A_{k,1,2}}{a_{k,1,2}} + \dots + \frac{A_{k,N_f-1,N_f}^T A_{k,N_f-1,N_f}}{a_{k,N_f-1,N_f}} \right). \end{aligned} \quad (6.62)$$

Adopting the approach used in the case of $N_f = 2$, we multiply the numerator and denominator of each term in the above expression with $a_{k,0,N_f}$.

Since the scalar quantity $a_{k,0,N_f} = a_{k,0,1} + a_{k,1,2} + \dots + a_{k,N_f-1,N_f}$, the above expression is then expressed as

$$\begin{aligned}
\mathbf{I}_{N_f,k}(\theta) = \Lambda_0 & \left(\frac{a_{k,0,1} A_{k,0,1}^T A_{k,0,1}}{a_{k,0,1} a_{k,0,N_f}} + \frac{a_{k,1,2} A_{k,0,1}^T A_{k,0,1}}{a_{k,0,1} a_{k,0,N_f}} + \dots + \frac{a_{k,N_f-1,N_f} A_{k,0,1}^T A_{k,0,1}}{a_{k,0,1} a_{k,0,N_f}} \right. \\
& + \frac{a_{k,0,1} A_{k,1,2}^T A_{k,1,2}}{a_{k,1,2} a_{k,0,N_f}} + \frac{a_{k,1,2} A_{k,1,2}^T A_{k,1,2}}{a_{k,1,2} a_{k,0,N_f}} + \dots + \frac{a_{k,N_f-1,N_f} A_{k,1,2}^T A_{k,1,2}}{a_{k,1,2} a_{k,0,N_f}} \\
& + \dots \\
& + \frac{a_{k,0,1} A_{k,N_f-1,N_f}^T A_{k,N_f-1,N_f}}{a_{k,N_f-1,N_f} a_{k,0,N_f}} + \frac{a_{k,1,2} A_{k,N_f-1,N_f}^T A_{k,N_f-1,N_f}}{a_{k,N_f-1,N_f} a_{k,0,N_f}} + \dots \\
& \left. + \frac{a_{k,N_f-1,N_f} A_{k,N_f-1,N_f}^T A_{k,N_f-1,N_f}}{a_{k,N_f-1,N_f} a_{k,0,N_f}} \right). \tag{6.63}
\end{aligned}$$

As for acquiring the image with a single frame for the total acquisition interval $[t_0, t_{N_f}]$, the Fisher information matrix is

$$\mathbf{I}_{1,k}(\theta) = \Lambda_0 \left(\frac{A_{k,0,N_f}^T A_{k,0,N_f}}{a_{k,0,N_f}} \right). \tag{6.64}$$

Since the row vector $A_{k,0,N_f}$ can be expressed as $A_{k,0,N_f} = A_{k,0,1} + A_{k,1,2} +$

$\dots + A_{k,N_f-1,N_f}, \mathbf{I}_{1,k}(\theta)$ can be rewritten as

$$\begin{aligned} \mathbf{I}_{1,k}(\theta) = \Lambda_0 & \left(\frac{A_{k,0,1}^T A_{k,0,1}}{a_{k,0,N_f}} + \frac{A_{k,0,1}^T A_{k,1,2}}{a_{k,0,N_f}} + \dots + \frac{A_{k,0,1}^T A_{k,N_f-1,N_f}}{a_{k,0,N_f}} \right. \\ & + \frac{A_{k,1,2}^T A_{k,0,1}}{a_{k,0,N_f}} + \frac{A_{k,1,2}^T A_{k,1,2}}{a_{k,0,N_f}} + \dots + \frac{A_{k,1,2}^T A_{k,N_f-1,N_f}}{a_{k,0,N_f}} \\ & + \dots \\ & \left. + \frac{A_{k,N_f-1,N_f}^T A_{k,0,1}}{a_{k,0,N_f}} + \frac{A_{k,N_f-1,N_f}^T A_{k,1,2}}{a_{k,0,N_f}} + \dots + \frac{A_{k,N_f-1,N_f}^T A_{k,N_f-1,N_f}}{a_{k,0,N_f}} \right). \end{aligned} \quad (6.65)$$

Taking the difference between $\mathbf{I}_{N_f,k}(\theta)$ and $\mathbf{I}_{1,k}(\theta)$, i.e., $\delta \mathbf{I}_{N_f-1,k}(\theta) = \mathbf{I}_{N_f,k}(\theta) - \mathbf{I}_{1,k}(\theta)$, $\delta \mathbf{I}_{N_f-1,k}(\theta)$ is expressed as follows.

$$\begin{aligned} \delta \mathbf{I}_{N_f-1,k}(\theta) = \Lambda_0 & (d_{11} + d_{12} + \dots + d_{1N_f} \\ & + d_{21} + d_{22} + \dots + d_{2N_f} \\ & + \dots \\ & + d_{N_f1} + d_{N_f2} + \dots + d_{N_fN_f}). \end{aligned} \quad (6.66)$$

where for $h, j = 1, 2, \dots, N_f$,

$$d_{hj} = \begin{cases} 0, & h = j, \\ \frac{a_{k,j-1,j} A_{k,h-1,h}^T A_{k,h-1,h}}{a_{k,h-1,h} a_{k,0,N_f}} - \frac{A_{k,h-1,h}^T A_{k,j-1,j}}{a_{k,0,N_f}}, & h \neq j. \end{cases} \quad (6.67)$$

Summing d_{hj} and d_{jh} , $h \neq j$, $h, j = 1, 2, \dots, N_f$, the expression is then

manipulated to achieve a common denominator such that

$$\begin{aligned} d_{hj} + d_{jh} = & \frac{a_{k,j-1,j}^2 A_{k,h-1,h}^T A_{k,h-1,h}}{a_{k,j-1,j} a_{k,h-1,h} a_{k,0,N_f}} - \frac{a_{k,j-1,j} a_{k,h-1,h} A_{k,h-1,h}^T A_{k,j-1,j}}{a_{k,j-1,j} a_{k,h-1,h} a_{k,0,N_f}} \\ & - \frac{a_{k,h-1,h} a_{k,j-1,j} A_{k,j-1,j}^T A_{k,h-1,h}}{a_{k,j-1,j} a_{k,h-1,h} a_{k,0,N_f}} + \frac{a_{k,h-1,h}^2 A_{k,j-1,j}^T A_{k,j-1,j}}{a_{k,j-1,j} a_{k,h-1,h} a_{k,0,N_f}}. \end{aligned} \quad (6.68)$$

Applying the identity $b^2 X^T X - bc(X^T Y + Y^T X) + c^2 Y^T Y = (bX - cY)^T(bX - cY)$ to each $d_{ij} + d_{ji}$, $i \neq j$, group of terms, we obtain $N_f(N_f - 1)/2$, $N_f \geq 2$, symmetric matrices. Thus the difference matrix $\delta \mathbf{I}_{N_f-1,k}(\theta)$ is expressed as follows.

$$\begin{aligned} \delta \mathbf{I}_{N_f-1,k}(\theta) = & \Lambda_0 \left(\sum_{i=1}^1 \frac{(a_{k,1,2} A_{k,i-1,i} - a_{k,i-1,i} A_{k,1,2})^T (a_{k,1,2} A_{k,i-1,i} - a_{k,i-1,i} A_{k,1,2})}{a_{k,i-1,i} a_{k,1,2} a_{k,0,N_f}} \right. \\ & + \sum_{i=1}^2 \frac{(a_{k,2,3} A_{k,i-1,i} - a_{k,i-1,i} A_{k,2,3})^T (a_{k,2,3} A_{k,i-1,i} - a_{k,i-1,i} A_{k,2,3})}{a_{k,i-1,i} a_{k,2,3} a_{k,0,N_f}} \\ & + \dots \\ & \left. + \sum_{i=1}^{N_f-1} \frac{(a_{k,N_f-1,N_f} A_{k,i-1,i} - a_{k,i-1,i} A_{k,N_f-1,N_f})^T (a_{k,N_f-1,N_f} A_{k,i-1,i} - a_{k,i-1,i} A_{k,N_f-1,N_f})}{a_{k,i-1,i} a_{k,N_f-1,N_f} a_{k,0,N_f}} \right). \end{aligned} \quad (6.69)$$

Using an intermediary index m to account for the number of summations,

(6.69) can be expressed concisely as

$$\delta \mathbf{I}_{N_f-1,k}(\theta) = \Lambda_0 \sum_{m=1}^{N_f-1} \sum_{i=1}^m \frac{W_{k,m,i}^T W_{k,m,i}}{a_{k,i-1,i} a_{k,m,m+1} a_{k,0,N_f}}, \quad N_f \geq 2, \quad (6.70)$$

where $W_{k,m,i}$ is defined before as

$$W_{k,m,i} = a_{k,m,m+1} A_{k,i-1,i} - a_{k,i-1,i} A_{k,m,m+1}. \quad (6.71)$$

Recalling that the above derivation is for pixel k , we now sum up (6.70) for N_p pixels that constitute the pixelated detector and obtain result 2 of this theorem. $\square$

A11 Proof of Corollary 4.3

Given that the exposure interval is equal to the frame interval, i.e., $e_i = t_i$, the Fisher information matrix of $\mathcal{G}$ for the total acquisition time $[t_0, t_{N_f}]$ can be expressed as

$$\mathbf{I}(\theta) = \sum_{i=1}^{N_f} \mathbf{I}_i(\theta) = \mathbf{I}_1(\theta) + \mathbf{I}_2(\theta) + \dots + \mathbf{I}_{N_f}(\theta). \quad (6.72)$$

By doubling the number of frames in acquiring the images for the same total acquisition time, we are effectively halving the frame interval. Thus, we let $\mathbf{I}'_i(\theta) = \mathbf{I}'_{i,1}(\theta) + \mathbf{I}'_{i,2}(\theta)$, $i = 1, 2, \dots, N_f$, where the temporal limits of integration for $\mathbf{I}'_{i,1}(\theta)$ and $\mathbf{I}'_{i,2}(\theta)$ are equal. We let the frame interval for $\mathbf{I}'_{i,1}(\theta)$ and $\mathbf{I}'_{i,2}(\theta)$ be denoted by $[t_{2i-2}, t_{2i-1}]$ and $[t_{2i-1}, t_{2i}]$, respectively. For the frame interval of $\mathbf{I}'_i(\theta)$ to be equal to the sum of the frame intervals of $\mathbf{I}'_{i,1}(\theta)$ and $\mathbf{I}'_{i,2}(\theta)$, we denote it by $[t_{2i-2}, t_{2i}]$. Hence, the Fisher information matrix of the i^{th} frame $\mathbf{I}'_i(\theta)$ for the frame interval $[t_{2i-2}, t_{2i}]$ can be expressed as

$$\mathbf{I}'_i(\theta) = \Lambda_0 \sum_{k=1}^{N_p} \frac{\left(\int_{t_{2i-2}}^{t_{2i}} \int_{C_k} \frac{\partial f_{\theta, \tau, 1}(r)}{\partial \theta} dr d\tau \right)^T \left(\int_{t_{2i-2}}^{t_{2i}} \int_{C_k} \frac{\partial f_{\theta, \tau, 1}(r)}{\partial \theta} dr d\tau \right)}{\int_{t_{2i-2}}^{t_{2i}} \int_{C_k} f_{\theta, \tau, 1}(r) dr d\tau}, \quad (6.73)$$

whereas $\mathbf{I}'_{i,1}(\theta)$ for the frame interval $[t_{2i-2}, t_{2i-1}]$ is expressed as

$$\mathbf{I}'_{i,1}(\theta) = \Lambda_0 \sum_{k=1}^{N_p} \frac{\left(\int_{t_{2i-2}}^{t_{2i-1}} \int_{C_k} \frac{\partial f_{\theta,\tau,1}(r)}{\partial \theta} dr d\tau \right)^T \left(\int_{t_{2i-2}}^{t_{2i-1}} \int_{C_k} \frac{\partial f_{\theta,\tau,1}(r)}{\partial \theta} dr d\tau \right)}{\int_{t_{2i-2}}^{t_{2i-1}} \int_{C_k} f_{\theta,\tau,1}(r) dr d\tau}, \quad (6.74)$$

and $\mathbf{I}'_{i,2}(\theta)$ for frame interval $[t_{2i-1}, t_{2i}]$ is expressed as

$$\mathbf{I}'_{i,2}(\theta) = \Lambda_0 \sum_{k=1}^{N_p} \frac{\left(\int_{t_{2i-1}}^{t_{2i}} \int_{C_k} \frac{\partial f_{\theta,\tau,1}(r)}{\partial \theta} dr d\tau \right)^T \left(\int_{t_{2i-1}}^{t_{2i}} \int_{C_k} \frac{\partial f_{\theta,\tau,1}(r)}{\partial \theta} dr d\tau \right)}{\int_{t_{2i-1}}^{t_{2i}} \int_{C_k} f_{\theta,\tau,1}(r) dr d\tau}. \quad (6.75)$$

Hence,

$$\mathbf{I}'(\theta) = \mathbf{I}'_{1,1}(\theta) + \mathbf{I}'_{1,2}(\theta) + \mathbf{I}'_{2,1}(\theta) + \mathbf{I}'_{2,2}(\theta) + \dots + \mathbf{I}'_{N_f,1}(\theta) + \mathbf{I}'_{N_f,2}(\theta). \quad (6.76)$$

By taking the difference between $\mathbf{I}'_{i,1}(\theta) + \mathbf{I}'_{i,2}(\theta) - \mathbf{I}_i(\theta)$, $i = 1, 2, \dots, N_f \in 2\mathbb{Z}^+$, for N_p pixels that constitute the pixelated detector, we can simply apply result 1 of Theorem 4.2. Thus for the difference between (6.76) and (6.72), i.e., $\mathbf{I}'(\theta) - \mathbf{I}(\theta)$, for N_p pixels, we obtain the following expression.

$$\delta \mathbf{I}_{N_f/2}(\theta) = \Lambda_0 \sum_{k=1}^{N_p} \sum_{i=1}^{N_f/2} \frac{W_{k,2i-1,2i-1}^T W_{k,2i-1,2i-1}}{a_{k,2i-2,2i-1} a_{k,2i-1,2i} a_{k,2i-2,2i}}. \quad (6.77)$$

□

A12 Proof of Theorem 5.1

Since the photon detection rate is assumed to be independent of the unknown parameter vector θ , we use the following expression of the Fisher information

matrix in Theorem 2.1 for our derivation.

$$\mathbf{I}(\theta) = \int_{t_0}^t \int_C \frac{\Lambda(\tau)}{f_{\theta,\tau}(r)} \left(\frac{\partial f_{\theta,\tau}(r)}{\partial \theta} \right)^T \left(\frac{\partial f_{\theta,\tau}(r)}{\partial \theta} \right) dr d\tau, \quad r \in \mathbb{R}, \theta \in \Theta. \quad (6.78)$$

Substituting the photon detection rate $\Lambda(\tau)$ and the photon distribution profile $f_{\theta,\tau}(r)$ from (5.1) and (5.2), respectively, into (6.78), the expression of the Fisher information matrix for the acquisition time interval $[t_0, t]$ can be rewritten as

$$\begin{aligned} \mathbf{I}(\theta) &= \int_{t_0}^t \int_C \frac{\Lambda(\tau)}{\epsilon_1 f_{\theta,\tau,1}(r) + \epsilon_2 f_{\theta,\tau,2}(r)} \left[\frac{\partial(\epsilon_1 f_{\theta,\tau,1}(r) + \epsilon_2 f_{\theta,\tau,2}(r))}{\partial \theta} \right]^T \left[\frac{\partial(\epsilon_1 f_{\theta,\tau,1}(r) + \epsilon_2 f_{\theta,\tau,2}(r))}{\partial \theta} \right] dr d\tau \\ &= \int_{t_0}^t \int_C \frac{1}{\Lambda_1(\tau) f_{\theta,\tau,1}(r) + \Lambda_2(\tau) f_{\theta,\tau,2}(r)} \left[\frac{\partial(\Lambda_1(\tau) f_{\theta,\tau,1}(r) + \Lambda_2(\tau) f_{\theta,\tau,2}(r))}{\partial \theta} \right]^T \\ &\quad \times \left[\frac{\partial(\Lambda_1(\tau) f_{\theta,\tau,1}(r) + \Lambda_2(\tau) f_{\theta,\tau,2}(r))}{\partial \theta} \right] dr d\tau, \\ &\quad r \in \mathbb{R}, \theta \in \Theta. \end{aligned} \quad (6.79)$$

Applying condition (A1) to the above expression, we express it in terms of the image functions. Taking the partial derivative of condition (A2) with respect to the unknown parameter vector θ , i.e., $\partial p(\tau)/\partial \theta = V_\theta(\tau)$, and applying the chain rule of differentiation to the above expression, we obtain the following expression.

$$\mathbf{I}(\theta) = \frac{1}{M^2} \int_{t_0}^t \int_C V_\theta^T(\tau) \frac{1}{w_{3D}} \left[\frac{\partial w_{3D}}{\partial p(\tau)} \right]^T \left[\frac{\partial w_{3D}}{\partial p(\tau)} \right] V_\theta(\tau) dx dy d\tau, \quad (6.80)$$

where

$$\begin{aligned} w_{3D} &= \sum_{i=1}^2 \Lambda_i(\tau) q_{z_{\theta,i}(\tau), o_{\theta,i}(\tau), i} \left(x/M - x_{\theta,i}(\tau), y/M - y_{\theta,i}(\tau) \right), \quad \tau \geq t_0, \theta \in \Theta, \\ V_\theta(\tau) &= \left[\frac{-\partial x_{\theta,1}(\tau)}{\partial \theta} \quad \frac{-\partial y_{\theta,1}(\tau)}{\partial \theta} \quad \frac{\partial z_{\theta,1}(\tau)}{\partial \theta} \quad \frac{\partial o_{\theta,1}(\tau)}{\partial \theta} \quad \frac{-\partial x_{\theta,2}(\tau)}{\partial \theta} \quad \frac{-\partial y_{\theta,2}(\tau)}{\partial \theta} \quad \frac{\partial z_{\theta,2}(\tau)}{\partial \theta} \quad \frac{\partial o_{\theta,2}(\tau)}{\partial \theta} \right]^T. \end{aligned} \quad (6.81)$$

□

A13 Proof of Proposition 5.1

When the image functions of both objects are independent of $z_\theta(\tau)$ and $o_\theta(\tau)$,

the column vectors $p(\tau)$ and $V_\theta(\tau)$ in Theorem 5.1 reduce to

$$\begin{bmatrix} x/M - x_{\theta,1}(\tau) & y/M - y_{\theta,1}(\tau) & x/M - x_{\theta,2}(\tau) & y/M - y_{\theta,2}(\tau) \end{bmatrix}^T \text{ and } \begin{bmatrix} -\partial x_{\theta,1}(\tau)/\partial\theta & -\partial y_{\theta,1}(\tau)/\partial\theta & -\partial x_{\theta,2}(\tau)/\partial\theta & -\partial y_{\theta,2}(\tau)/\partial\theta \end{bmatrix}^T, \text{ respectively. Hence,}$$

$$\mathbf{I}(\theta) = \frac{1}{M^2} \int_{t_0}^t \int_{\mathcal{C}} \frac{V_\theta^T(\tau)}{w_{2D}} \begin{bmatrix} \frac{\partial w_{2D}}{\partial(\frac{x}{M} - x_{\theta,1}(\tau))} \\ \frac{\partial w_{2D}}{\partial(\frac{y}{M} - y_{\theta,1}(\tau))} \\ \frac{\partial w_{2D}}{\partial(\frac{x}{M} - x_{\theta,2}(\tau))} \\ \frac{\partial w_{2D}}{\partial(\frac{y}{M} - y_{\theta,2}(\tau))} \end{bmatrix} \begin{bmatrix} \frac{\partial w_{2D}}{\partial(\frac{x}{M} - x_{\theta,1}(\tau))} \\ \frac{\partial w_{2D}}{\partial(\frac{y}{M} - y_{\theta,1}(\tau))} \\ \frac{\partial w_{2D}}{\partial(\frac{x}{M} - x_{\theta,2}(\tau))} \\ \frac{\partial w_{2D}}{\partial(\frac{y}{M} - y_{\theta,2}(\tau))} \end{bmatrix}^T V_\theta(\tau) dx dy d\tau, \quad (6.82)$$

where

$$w_{2D} = \sum_{i=1}^2 \Lambda_i(\tau) q_i \left(\frac{x}{M} - x_{\theta,i}(\tau), \frac{y}{M} - y_{\theta,i}(\tau) \right), \quad \tau \geq t_0, \theta \in \Theta. \quad (6.83)$$

Given that $\partial(x/M - x_{\theta,i})/\partial x = 1/M$ and $\partial(y/M - y_{\theta,i})/\partial y = 1/M$ for $i = 1, 2$, the above expression can then be rewritten as

$$\begin{aligned} \mathbf{I}(\theta) &= \int_{t_0}^t \int_{\mathcal{C}} \frac{V_{\theta}^T(\tau)}{w_{2D}} \\ &\times \begin{bmatrix} \Lambda_1(\tau) \frac{\partial q_1(\frac{x}{M} - x_{\theta,1}(\tau), \frac{y}{M} - y_{\theta,1}(\tau))}{\partial x} \\ \Lambda_1(\tau) \frac{\partial q_1(\frac{x}{M} - x_{\theta,1}(\tau), \frac{y}{M} - y_{\theta,1}(\tau))}{\partial y} \\ \Lambda_2(\tau) \frac{\partial q_2(\frac{x}{M} - x_{\theta,2}(\tau), \frac{y}{M} - y_{\theta,2}(\tau))}{\partial x} \\ \Lambda_2(\tau) \frac{\partial q_2(\frac{x}{M} - x_{\theta,2}(\tau), \frac{y}{M} - y_{\theta,2}(\tau))}{\partial y} \end{bmatrix} \begin{bmatrix} \Lambda_1(\tau) \frac{\partial q_1(\frac{x}{M} - x_{\theta,1}(\tau), \frac{y}{M} - y_{\theta,1}(\tau))}{\partial x} \\ \Lambda_1(\tau) \frac{\partial q_1(\frac{x}{M} - x_{\theta,1}(\tau), \frac{y}{M} - y_{\theta,1}(\tau))}{\partial y} \\ \Lambda_2(\tau) \frac{\partial q_2(\frac{x}{M} - x_{\theta,2}(\tau), \frac{y}{M} - y_{\theta,2}(\tau))}{\partial x} \\ \Lambda_2(\tau) \frac{\partial q_2(\frac{x}{M} - x_{\theta,2}(\tau), \frac{y}{M} - y_{\theta,2}(\tau))}{\partial y} \end{bmatrix}^T V_{\theta}(\tau) dx dy d\tau. \end{aligned} \quad (6.84)$$

From the above expression, we partition the 4×4 matrix and express it as follows.

$$\mathbf{I}(\theta) = \int_{t_0}^t \int_{\mathcal{C}} \frac{V_{\theta}^T(\tau)}{w_{2D}} \begin{bmatrix} W_{11} & W_{12} \\ W_{12}^T & W_{22} \end{bmatrix} V_{\theta}(\tau) dx dy d\tau, \quad (6.85)$$

where

$$\begin{aligned} W_{kl} &= \Lambda_k(\tau) \Lambda_l(\tau) \\ &\times \begin{bmatrix} \frac{\partial q_k(\frac{x}{M} - x_{\theta,k}(\tau), \frac{y}{M} - y_{\theta,k}(\tau))}{\partial x} \frac{\partial q_l(\frac{x}{M} - x_{\theta,l}(\tau), \frac{y}{M} - y_{\theta,l}(\tau))}{\partial x} & \frac{\partial q_k(\frac{x}{M} - x_{\theta,k}(\tau), \frac{y}{M} - y_{\theta,k}(\tau))}{\partial x} \frac{\partial q_l(\frac{x}{M} - x_{\theta,l}(\tau), \frac{y}{M} - y_{\theta,l}(\tau))}{\partial y} \\ \frac{\partial q_k(\frac{x}{M} - x_{\theta,k}(\tau), \frac{y}{M} - y_{\theta,k}(\tau))}{\partial y} \frac{\partial q_l(\frac{x}{M} - x_{\theta,l}(\tau), \frac{y}{M} - y_{\theta,l}(\tau))}{\partial x} & \frac{\partial q_k(\frac{x}{M} - x_{\theta,k}(\tau), \frac{y}{M} - y_{\theta,k}(\tau))}{\partial y} \frac{\partial q_l(\frac{x}{M} - x_{\theta,l}(\tau), \frac{y}{M} - y_{\theta,l}(\tau))}{\partial y} \end{bmatrix}, \\ &k, l = 1, 2, \tau \geq t_0, \theta \in \Theta. \end{aligned} \quad (6.86)$$

We next consider each element of the Fisher information matrix in (6.85). By letting $u_j := x/M - x_{\theta,j}(\tau)$ and $v_j := y/M - y_{\theta,j}(\tau)$, $j = k, l$, the image function $q_j(x/M - x_{\theta,j}(\tau), y/M - y_{\theta,j}(\tau))$ can be succinctly expressed as $q_j(u_j, v_j)$. As

for the derivative of u_j and v_j with respect to x and y , they are $\partial u_j/\partial x = \partial v_j/\partial y = 1/M$, respectively. Since the image functions $q_j(u_j, v_j)$, $j = k, l$, are assumed to be radially symmetric, i.e., $q_j(u_j, v_j) = \tilde{q}_j(u_j^2 + v_j^2)$, $j = k, l$ for a function $\tilde{q} : \mathbb{R}^2 \rightarrow \mathbb{R}^2$, it can be readily shown that for $k, l = 1, 2$,

$$\begin{aligned} & \int_{t_0}^t \int_{\mathcal{C}} \frac{\Lambda_k(\tau) \Lambda_l(\tau) V_{\theta}^T(\tau)}{\sum_{i=1}^2 \Lambda_i(\tau) q_i\left(\frac{x}{M} - x_{\theta,i}(\tau), \frac{y}{M} - y_{\theta,i}(\tau)\right)} \\ & \times \frac{\partial q_k\left(\frac{x}{M} - x_{\theta,k}(\tau), \frac{y}{M} - y_{\theta,k}(\tau)\right)}{\partial x} \frac{\partial q_l\left(\frac{x}{M} - x_{\theta,l}(\tau), \frac{y}{M} - y_{\theta,l}(\tau)\right)}{\partial y} V_{\theta}(\tau) dx dy d\tau, \end{aligned} \quad (6.87)$$

can be rewritten as

$$\int_{t_0}^t \int_{\mathcal{C}} \frac{\Lambda_k(\tau) \Lambda_l(\tau) V_{\theta}^T(\tau)}{\sum_{i=1}^2 \Lambda_i(\tau) \tilde{q}_i(u_i^2 + v_i^2)} \frac{\partial \tilde{q}_k(u_k^2 + v_k^2)}{\partial u_j} \frac{\partial \tilde{q}_l(u_l^2 + v_l^2)}{\partial v_j} V_{\theta}(\tau) du_j dv_j d\tau. \quad (6.88)$$

Applying the chain rule of differentiation,

$$\begin{aligned} \frac{\partial \tilde{q}_k(u_k^2 + v_k^2)}{\partial u_j} &= \frac{\partial \tilde{q}_k(u_k^2 + v_k^2)}{\partial (u_k^2 + v_k^2)} \cdot \frac{\partial (u_k^2 + v_k^2)}{\partial u_j} \\ &= 2u_k \frac{\partial \tilde{q}_k(u_k^2 + v_k^2)}{\partial (u_k^2 + v_k^2)}. \end{aligned} \quad (6.89)$$

Similarly, $\partial \tilde{q}_l(u_l^2 + v_l^2)/\partial v_l = 2v_l \partial \tilde{q}_l(u_l^2 + v_l^2)/\partial (u_l^2 + v_l^2)$. Thus expression (6.88) can be rewritten as

$$\int_{t_0}^t \int_{\mathcal{C}} \frac{\Lambda_k(\tau) \Lambda_l(\tau) V_{\theta}^T(\tau)}{\sum_{i=1}^2 \Lambda_i(\tau) \tilde{q}_i(u_i^2 + v_i^2)} 4u_k v_l \frac{\partial \tilde{q}_k(u_k^2 + v_k^2)}{\partial (u_k^2 + v_k^2)} \frac{\partial \tilde{q}_l(u_l^2 + v_l^2)}{\partial (u_l^2 + v_l^2)} du_j dv_j d\tau = 0, \quad (6.90)$$

since $\partial \tilde{q}_j(u_j^2 + v_j^2)/\partial(u_j^2 + v_j^2)$, $j = \{k, l\}$ are also radially symmetric. Hence we obtain the following expression.

$$\mathbf{I}(\theta) = \int_{t_0}^t \int_{\mathcal{C}} \frac{V_{\theta}^T(\tau)}{w_{2D}} \begin{bmatrix} W_{11} & W_{12} \\ W_{12}^T & W_{22} \end{bmatrix} V_{\theta}(\tau) dx dy d\tau, \quad (6.91)$$

where

$$\begin{aligned} w_{2D} &= \sum_{i=1}^2 \Lambda_i(\tau) q_i \left(\frac{x}{M} - x_{\theta,i}(\tau), \frac{y}{M} - y_{\theta,i}(\tau) \right), \\ V_{\theta}(\tau) &= \begin{bmatrix} \frac{-\partial x_{\theta,1}(\tau)}{\partial \theta} & \frac{-\partial y_{\theta,1}(\tau)}{\partial \theta} & \frac{-\partial x_{\theta,2}(\tau)}{\partial \theta} & \frac{-\partial y_{\theta,2}(\tau)}{\partial \theta} \end{bmatrix}^T, \\ W_{kl} &= \Lambda_k(\tau) \Lambda_l(\tau) \\ &\times \begin{bmatrix} \frac{\partial q_k(\frac{x}{M} - x_{\theta,k}(\tau), \frac{y}{M} - y_{\theta,k}(\tau))}{\partial x} \frac{\partial q_l(\frac{x}{M} - x_{\theta,l}(\tau), \frac{y}{M} - y_{\theta,l}(\tau))}{\partial x} & 0 \\ 0 & \frac{\partial q_k(\frac{x}{M} - x_{\theta,k}(\tau), \frac{y}{M} - y_{\theta,k}(\tau))}{\partial y} \frac{\partial q_l(\frac{x}{M} - x_{\theta,l}(\tau), \frac{y}{M} - y_{\theta,l}(\tau))}{\partial y} \end{bmatrix}, \\ &\quad k, l = 1, 2, \theta \in \Theta, \tau \geq t_0. \end{aligned} \quad (6.92)$$

□

A14 Proof of Corollary 5.1

1. When both objects are moving linearly in the 2D object space, the unknown parameter vector $\theta = (x_1, y_1, \phi_1, v_1, x_2, y_2, \phi_2, v_2) \in \Theta$. Given that the parametric expressions of the trajectories for the acquisition time interval $[t_0, t]$ are $x_{\theta,i}(\tau) = x_i + v_i(\tau - t_0) \cos \phi_i$, $y_{\theta,i}(\tau) = y_i + v_i(\tau - t_0) \sin \phi_i$, $i = 1, 2$, $\tau \geq t_0$, $\theta \in \Theta$, using the result in Proposition 5.1, $V_{\theta}(\tau)$ can be

expressed as

$$V_{\theta}(\tau) = \begin{bmatrix} I_2 & R & 0 & 0 \\ 0 & 0 & I_2 & P \end{bmatrix}, \quad (6.93)$$

where

$$\begin{aligned} I_2 &= \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix}, \quad R = \begin{bmatrix} -v_1(\tau - t_0) \sin \phi_1 & (\tau - t_0) \cos \phi_1 \\ v_1(\tau - t_0) \cos \phi_1 & (\tau - t_0) \sin \phi_1 \end{bmatrix}, \\ P &= \begin{bmatrix} -v_2(\tau - t_0) \sin \phi_2 & (\tau - t_0) \cos \phi_2 \\ v_2(\tau - t_0) \cos \phi_2 & (\tau - t_0) \sin \phi_2 \end{bmatrix}. \end{aligned} \quad (6.94)$$

Thus, substituting (6.93) into Proposition 5.1

$$V_{\theta}^T(\tau) \begin{bmatrix} W_{11} & W_{12} \\ W_{12}^T & W_{22} \end{bmatrix} V_{\theta}(\tau) = \begin{bmatrix} W_{11} & W_{11}R & W_{12} & W_{12}P \\ R^T W_{11} & R^T W_{11}R & R^T W_{12} & R^T W_{12}P \\ W_{12}^T & W_{12}^T R & W_{22} & W_{22}P \\ P^T W_{12}^T & P^T W_{12}^T R & P^T W_{22} & P^T W_{22}P \end{bmatrix}. \quad (6.95)$$

Since W_{11} , W_{22} and W_{12} are diagonal matrices for the case of radially symmetric image functions, $W_{11} = W_{11}^T$, $W_{22} = W_{22}^T$ and $W_{12} = W_{12}^T$.

Thus, we obtain following expression.

$$\mathbf{I}(\theta) = \int_{t_0}^t \int_{\mathcal{C}} \frac{1}{w_{2D}} \begin{bmatrix} W_{11} & W_{11}R & W_{12} & W_{12}P \\ (W_{11}R)^T & R^T W_{11}R & R^T W_{12} & R^T W_{12}P \\ W_{12} & (R^T W_{12})^T & W_{22} & W_{22}P \\ (W_{12}P)^T & (R^T W_{12}P)^T & (W_{22}P)^T & P^T W_{22}P \end{bmatrix} dx dy d\tau, \quad (6.96)$$

where

$$\begin{aligned}
 w_{2D} &= \sum_{i=1}^2 \Lambda_i(\tau) q_i \left(\frac{x}{M} - x_{\theta,i}(\tau), \frac{y}{M} - y_{\theta,i}(\tau) \right), \\
 R &= \begin{bmatrix} -v_1(\tau - t_0) \sin \phi_1 & (\tau - t_0) \cos \phi_1 \\ v_1(\tau - t_0) \cos \phi_1 & (\tau - t_0) \sin \phi_1 \end{bmatrix}, \quad P = \begin{bmatrix} -v_2(\tau - t_0) \sin \phi_2 & (\tau - t_0) \cos \phi_2 \\ v_2(\tau - t_0) \cos \phi_2 & (\tau - t_0) \sin \phi_2 \end{bmatrix}, \\
 W_{kl} &= \Lambda_k(\tau) \Lambda_l(\tau) \\
 &\times \begin{bmatrix} \frac{\partial q_k(\frac{x}{M} - x_{\theta,k}(\tau), \frac{y}{M} - y_{\theta,k}(\tau))}{\partial x} \frac{\partial q_l(\frac{x}{M} - x_{\theta,l}(\tau), \frac{y}{M} - y_{\theta,l}(\tau))}{\partial x} & 0 \\ 0 & \frac{\partial q_k(\frac{x}{M} - x_{\theta,k}(\tau), \frac{y}{M} - y_{\theta,k}(\tau))}{\partial y} \frac{\partial q_l(\frac{x}{M} - x_{\theta,l}(\tau), \frac{y}{M} - y_{\theta,l}(\tau))}{\partial y} \end{bmatrix}, \\
 &\quad k, l = 1, 2, \tau \geq t_0, \theta \in \Theta.
 \end{aligned}$$

2. When one of the objects moves linearly while the other remains stationary, the unknown parameter vector reduces to $\theta = (x_1, y_1, x_2, y_2, \phi_2, v_2) \in \Theta$. Since $x_{\theta,1}(\tau) = x_1$, $y_{\theta,1}(\tau) = y_1$, $x_{\theta,2}(\tau) = x_2 + v_2(\tau - t_0) \cos \phi_2$, $y_{\theta,2}(\tau) = y_2 + v_2(\tau - t_0) \sin \phi_2$, $\tau \geq t_0$, $\theta \in \Theta$, using the result in Proposition 5.1, $V_\theta(\tau)$ can be expressed as

$$V_\theta(\tau) = \begin{bmatrix} I_2 & 0 & 0 \\ 0 & I_2 & P \end{bmatrix}, \quad (6.97)$$

where I_2 and P are as given in (6.94).

As R is now an empty matrix, we can thus delete the second row and second column of expression (6.95) and obtain the following expression.

$$V_\theta^T(\tau) \begin{bmatrix} W_{11} & W_{12} \\ W_{12}^T & W_{22} \end{bmatrix} V_\theta(\tau) = \begin{bmatrix} W_{11} & W_{12} & W_{12}P \\ W_{12}^T & W_{22} & W_{22}P \\ P^T W_{12}^T & P^T W_{22} & P^T W_{22}P \end{bmatrix}. \quad (6.98)$$

Since object 1 is assumed to be stationary, its trajectory is replaced with its location coordinates, i.e., $(x_{\theta,1}(\tau), y_{\theta,1}(\tau)) = (x_1, y_1)$ and its image function $q_1(x/M - x_{\theta,1}(\tau), y/M - y_{\theta,1}(\tau))$ in W_{kl} , $l, k = 1, 2$, becomes $q_1(x/M - x_1, y/M - y_1)$. Like result 1 of this corollary, the transpose of the diagonal matrix is the diagonal matrix itself. Thus, we obtain the following expression.

$$\mathbf{I}(\theta) = \int_{t_0}^t \int_{\mathcal{C}} \frac{1}{w_{2D}} \begin{bmatrix} W_{11} & W_{12} & W_{12}P \\ W_{12} & W_{22} & W_{22}P \\ (W_{12}P)^T & (W_{22}P)^T & P^T W_{22}P \end{bmatrix} dx dy d\tau. \quad (6.99)$$

□