EFFICIENT ACQUISITION AND LEARNING OF FLUORESCENCE MICROSCOPY DATA MODELS

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ABSTRACT

This paper presents a method to efficiently acquire fluorescence microscopy datasets, to allow for higher spatial and temporal resolution, and with less damage from photobleaching. Our proposal is to restrict acquisition to regions where we expect to find an object. Given that the objects are continuously moving, we must have an accurate model to describe their motion to predict their future locations. We outline a system for learning and applying this motion model, demonstrate its application in a case study, and summarize results from more complex applications.

Index Terms— Fluorescence, microscopy, tracking, state space methods, Monte Carlo methods

1. INTRODUCTION

Fluorescence microscopy is one of the most popular tools for live-cell imaging. As the trend in biology tends more and more towards automated systems for high-throughput applications, the amount of image data acquired with this technique is growing rapidly. To observe a cellular process over a sustained period, we take a time series of images, where each image is known as a frame. This paper describes an efficient way to acquire such images, in which we obtain the required information without acquiring the entire field of view.

The first motivation for this work is to reduce photobleaching and phototoxicity. In fluorescence microscopy, images are acquired by shining excitation light on the specimen to activate fluorescence. However, this can damage the fluorescent signal (photobleaching) [1], as well as the cell itself (phototoxicity) [2]. Thus, the duration over which we can view a cellular process is limited because eventually photobleaching destroys the fluorescent signal or, less commonly, phototoxicity destroys the cell itself. By reducing the total area acquired in each frame, we reduce the overall exposure to excitation light, hence reducing photobleaching and phototoxicity.

The second motivation is to enhance resolution. In laser scanning confocal microscopy, images are acquired line-by-line, pixel-by-pixel [3]. We can achieve significant time savings by only imaging those regions where we expect to find an object. These time savings could be used to increase the frame rate, or to acquire the selected regions at a higher spatial resolution.

Although algorithms relating to efficient image compression or image enhancement are well studied, the efficient acquisition of these images is not. In [4], the authors designed an algorithm to reduce the number of pixels sampled in a 2D or 3D image when using a laser scanning confocal microscope. They observed that a large portion of scanning time is spent on low fluorescence regions, which presumably contain little useful information. The approach is then to begin by scanning the field at a low resolution. Each scanned value is examined, and if found to be significant, the area around it is scanned at a higher resolution. The process is repeated iteratively.

Here, we want to observe a large number of tiny moving objects over a sustained period. Instead of taking an image of the entire field of view, the approach is to acquire only those regions where we expect to find an object. Therefore, we need to develop a model to describe the objects' motion, and continually refine this model to more accurately predict the locations where objects will be found. In [5], the authors provide algorithms for modeling objects' motions for the purposes of tracking, and although not used directly, their work helped inspire our approach.

Section 2 outlines the framework used for developing the motion models, and the process of the actual algorithm is described in Section 3. Section 4 presents a case study of a simple system, while Section 5 outlines some characteristics of more complex models.

2. TRACKING FRAMEWORK

The fundamental part of this work is to learn the motion model for each object, and thus, here, we describe the framework in which we do this. We have taken the same approach as used in [5] for object tracking algorithms.

2.1. State Space Model

The state space model assumes that all the necessary information about a system can be summarized by a set of state variables. For a simple case of object tracking, these state variables could be, for example, the 3D coordinates of the centroids of every object. A more advanced model could also include the size, shape, type of every object, etc.

This system is governed by two fundamental state space equations [6]. Equation (1) describes the present state in terms of the previous state, and (2) describes the observed variables in terms of the state variables:

$$x(t+1) = F(t)x(t) + \nu(t), \quad (1)$$

$$z(t) = H(t)x(t) + \mu(t). \quad (2)$$

Equation (1) shows the state at time t, $x(t)$, evolving to the state at time $t+1$, $x(t+1)$, as governed by the state transition matrix

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$F(t)$. The equation implicitly assumes a linear system, although a more general form can be used for nonlinear systems. The state noise $\nu(t)$ reflects that the model will not perfectly predict the state transitions.

Equation (2) maps the state variables $x(t)$ to the measurement variables $z(t)$. $H(t)$ is the observation matrix (if $H(t)$ were a diagonal matrix then the system would be fully observable), and $\mu(t)$ is the measurement noise. This paper assumes perfect measurement.

2.2. Application to Motion Modeling

Our method of modeling the objects' motion follows that of [6], as described in this section. Learning the motion model of an object is equivalent to learning $F(t)$ and the properties of $\nu(t)$ for the object. Because we assume stationary object dynamics, $F(t)$ can be represented simply as F, and the state noise has a constant covariance Q. Every object in the specimen potentially moves under a different motion model. Therefore, we have state transition matrices $F_1, ..., F_m$ and covariances $Q_1, ..., Q_m$ for the m objects of interest. Learning the motion models equates to learning these matrices.

Two restrictions are imposed on the model. The first is that covariance matrices are diagonal (making each dimension independent). The second is that F is restricted to one of the three motion models from [5] to cover most motions observed in practice. These three models are a random walk, F_{RW}, a first-order linear extrapolation, F_{FLE}, and a second-order linear extrapolation, F_{SLE}, and require knowledge of the object's position in up to three successive frames. Therefore, in 2D, we define the state vector as:

$$x(t) = \begin{bmatrix} x_t & y_t & x_{t-1} & y_{t-1} & x_{t-2} & y_{t-2} \end{bmatrix}^\top,$$

where (x_t, y_t) represent the 2D coordinates of the object at time t. The state transition matrices for each motion model are:

$$F_{RW} = \begin{bmatrix} 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \end{bmatrix},$$

$$F_{FLE} = \begin{bmatrix} 2 & 0 & -1 & 0 & 0 & 0 \\ 0 & 2 & 0 & -1 & 0 & 0 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \end{bmatrix},$$

$$F_{SLE} = \begin{bmatrix} 3 & 0 & -3 & 0 & 1 & 0 \\ 0 & 3 & 0 & -3 & 0 & 1 \\ 1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 \end{bmatrix}.$$

The first two rows of these matrices compute the object coordinates for the next time frame. For example, if using the FLE model:

$$x_{t+1} = 2x_t - x_{t-1} + N(0, \sigma_x), \quad (3)$$

$$y_{t+1} = 2y_t - y_{t-1} + N(0, \sigma_y). \quad (4)$$

The third row shows that x_t in the current state becomes x_{t-1} in the subsequent state, and so forth.

3. ALGORITHM OUTLINE

Problem statement. Our goal here is to learn the motion model for each object. The input to the system is the time series of images (frames), and the set of possible motion models that could describe the objects. For each of these motion models, and for each object, the system outputs the relative likelihood that a given motion model describes a given object. As our knowledge of each object's motion becomes more refined, the efficiency of acquisition improves.

Assumptions. We assume that objects are perfectly detected provided that the appropriate region is acquired. This is in contrast to [6], which considers the possibility that an object goes undetected due to background noise, or, alternatively, that background clutter is falsely detected as an object. These considerations will be taken into account in future work.

A second assumption is that each object of interest occupies a single pixel, thus avoiding size and shape considerations. Future algorithms will model the possibly deformable sizes and shapes of objects along with their motion.

Finally, although our system does allow for nonstationary object dynamics, all experiments so far assume that these dynamics are stationary. That is, we assume that the motion models are not changing over time.

Algorithm. The system does not make hard decisions about which motion model an object is operating under, but instead associates a probability with each possible model. We begin by assigning initial probabilites to each model. For example, we could assume that the three models F_{RW}, F_{FLE}, F_{SLE}, are all equally likely. The covariance of the state noise is also a model parameter. We could initially assume that the possible values of the variance (either in the x-direction or the y-direction) are all equally likely, with some upper bound (such as the diameter of the cell). The algorithm then refines these probabilities as it cycles through the following steps (a pseudo code is given in Algorithm 1):

1. Predict distribution of objects: Based on our current beliefs about the motion model, we calculate the likelihood of finding each object in any given pixel in the subsequent frame.
2. Acquire pixels according to some policy: For example, we may choose to acquire the smallest number of pixels that give a 95% probability of capturing each object.
3. Having acquired these pixels, we observe where each object was actually located (or whether an object was not located in any of the acquired pixels). This information is used to update the motion models.
4. If we allow for nonstationary object dynamics then we must now update our belief about the motion models to reflect that they may have changed.

4. CASE STUDY

We now explain these steps in more detail with a simple case study. We assume that we are only acquiring a single object, and that the motion is known in advance to be the random walk model. Hence, the only unknown variable is the covariance. For the sake of clear diagrams, we will further assume that the object moves only in one dimension, meaning that the covariance consists of a single variable.

Algorithm 1 Input: M, the set of all possible motion models, I_{1-N}, a set of N frames, $f_{x_1}(x)$, the distribution of the object's location in the first frame. Output: $f_M(m)$, the probability that the object follows motion model m.

initialize $f_M(m)$, the prior likelihood of each motion model
for $t = 1$ to N **do**
  compute $f_{x_{t+1}}(x|m)$ for all $m \in M$
  $f_{x_{t+1}}(x) = \int_M f_{x_{t+1}}(x|m) f_M(m) dm$
  choose smallest set of pixels D s.t. $\int_D f_{x_{t+1}}(x)dx = 0.95$
  acquire pixels in D in I_{t+1}
  if object found **then**
    $f_{x_{t+1}}(x) = 1$ at object location, 0 elsewhere
  else
    $f_{x_{t+1}}(x) = 0$ for all $x \in D$
  end if
  $f_M(m) = \Pr(x_{t+1}|m) f_M(m)$ for all $m \in M$
end for
return $f_M(m)$

4.1. Initial Conditions

We assume the initial position of the object is known. As stated above, the only unknown variable is the variance of the object's motion, or, equivalently, the standard deviation σ. Our initial assumption is that this standard deviation lies between 0 and 10 with equal probability. The true σ (initially unknown by our system) is set to 1.

4.2. Prediction of the Distribution of the Object

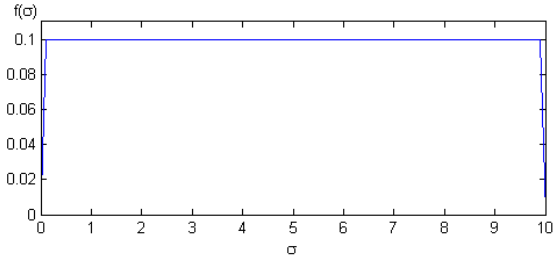
Because the object follows a random walk, its expected location in the subsequent frame is the same as in the current frame, but it is still subject to the Gaussian state noise from (1). The problem is that the system does not know the standard deviation of this Gaussian, which would be required to compute the distribution of the object's expected location. However, the system does maintain the probability of any given standard deviation being the true standard deviation. Equation (5) shows how we compute the distribution of the object's position, $f_x(x)$, when the standard deviation σ is only known as a probability distribution $f_\sigma(\sigma)$. $f_x(x|\sigma)$ refers to the expected object distribution when σ is known, and is thus simply a Gaussian of mean 0 and standard deviation σ;

$$f_x(x) = \int_\sigma f_x(x|\sigma) f_\sigma(\sigma) d\sigma. \quad (5)$$

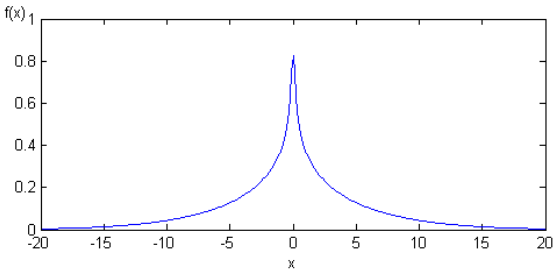
Figure 1 shows (a) an example of our intial prediction of the standard deviation (equal likelihood everywhere up to 10), (b) the object's expected distribution given this initial prediction as computed by (5), and (c) the object's expected distribution if we knew the true standard deviation of 1.

Figure 2 shows the first four iterations of the algorithm. We see that as the knowledge of σ becomes more precise, the distribution of the object's expected location also becomes more precise.

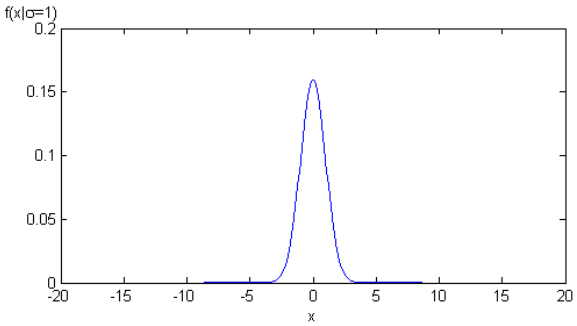
The assumption thus far is that the object's original position is known. However, sometimes we fail to acquire the object in one frame, but must still predict its location in the next frame. Figure 3 shows an example where the object's current location is only known probabilistically, and the subsequent object distribution is the convolution of this current distribution and the random walk function.



(a) Initial likelihood of object standard deviation.



(b) Expected object distribution when standard deviation is unknown.



(c) Expected object distribution if standard deviation is known to be 1.

Fig. 1. The effect of our knowledge of the object standard deviation on the expected object distribution.

4.3. Acquisition of Pixels According to Policy

Suppose that our aim is to capture the object in each frame with 95% probability. Thus we select the pixels with the highest probability of containing the object, selecting just enough so that the cumulative probability of acquring the object is 95%.

4.4. Update of the Motion Model

On the basis of this acquisition, we now update our estimate of σ as follows:

$$f_{new}(\sigma) = \frac{\Pr(d \in D|\sigma) f_{old}(\sigma)}{\Pr(d \in D)}.$$

In this equation, D refers to the displacement of the object between the two frames. If the object was acquired in both frames then D is a single number representing the actual measured displacement of the object. However, if the object was not acquired, then the actual displacement of the object is unknown and thus D is the set of all possible values of displacement.

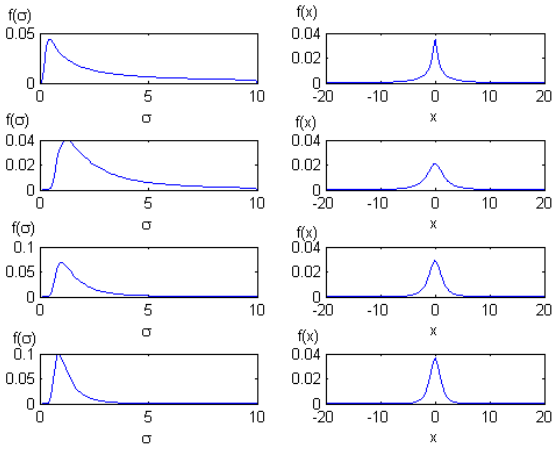


Fig. 2. Four iterations of the algorithm. The left side reflects our knowledge of σ; the right side shows the expected object distribution.

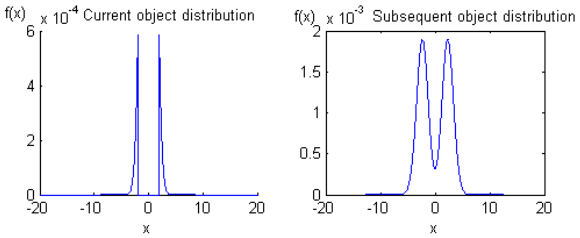


Fig. 3. Expected object distribution in subsequent frame when object position is only known probabilistically in current frame.

5. HIGHER-ORDER MODELS

When we consider higher-order models, we must also maintain distributions of the objects' past positions. In the second-order linear extrapolation, with state transition matrix F_{SLE}, the update equation for a single dimension is given as:

$$x_{t+1} = 3x_t - 3x_{t-1} + x_{t-2} + N(0, \sigma).$$

Thus, we need to maintain estimates of x_t, x_{t-1} and x_{t-2}. For the frames when the object is observed, this estimate will be an exact point. However, for frames when the object was missed, the position of the object must be represented probabilistically.

This added complexity means that it takes longer to learn σ than for the random walk model. Figure 4 shows the rate at which σ is learnt for each of the three motion models. It can be seen that the lower-order models are faster to converge to the true value of σ.

Even once σ has been learnt, the higher-order models still require a larger average acquisition region. This is because when an object is missed in any given frame, the resulting uncertainty about the object's motion is propagated for longer when using a higher-order model. If the object must be captured in 90% of frames in a 1-dimensional setting, then an RW model requires an average acquisition region of length 3.78σ, an FLE model requires an average acquisition region of length 4.48σ, and the SLE requires 6.97σ.

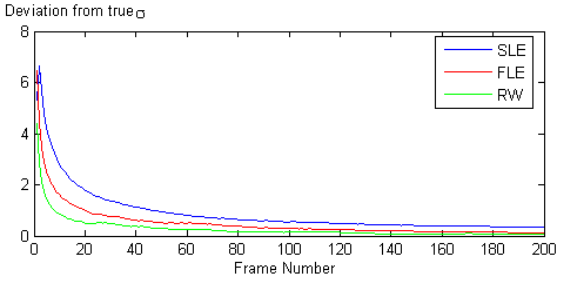


Fig. 4. The rate at which σ is learnt under each of the motion models

6. CONCLUSION

We outlined an efficient way of acquiring fluorescence microscopy images through modeling the motion of the objects and acquiring only in regions likely to contain objects. The reduced acquisition time can reduce photobleaching due to a lessening of light exposure, or can lead to an increase in the frame rate or spatial resolution of the images. We have described a general process that is applicable to a wide range of motion models.

The case study shows how the system can be applied to a simple example. Extension to more dimensions, multiple objects, and more complicated motion models, is simple and intuitive. The limiting factor in these more advanced systems is the increased computational complexity. The main challenge of future work will be to ascertain what simplifications can be made to maintain a manageable computational load yet without adversely affecting system behavior.

7. ACKNOWLEDGEMENTS

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8. REFERENCES

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