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Measurement Errors in Fluorescence Microscopy Image Registration

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***Abstract*—Image registration is an important processing step in fluorescence microscopy, for example in tracking or super-resolution methods. Precision localization of single fluorescent molecules from a quantum limited photon detection process, subject to Gaussian readout noise, is key to the use of single molecule microscopy. It is therefore important to know the effect that registration has on the accuracy of localizing a single molecule. Here we demonstrate a suitable approach to image registration that accounts for point-wise errors in localizing the control points typically used in fluorescence microscopy. This allows expressions for the localization errors caused by the registration process to be derived, showing dependence on the number of control points and their associated photon counts.**

I. INTRODUCTION

Image registration is the process of overlaying two or more images of the same scene [1], or equivalently is the process of establishing the geometric transformations between two or more data sets such that they can be viewed in a single coordinate system. These images could arise from different times (multitemporal), different viewpoints (multiview), or different sensors (multimodal).

In the fluorescence microscopy setting we are concerned with feature-based registration where the features used for matching are points in the image where pair correspondence is certain. In this case they are known as *control points* (CPs). It is common that these points are created with the use of fiduciary markers, e.g. fluorescent beads [2]. Using fiduciary markers to perform image registration is an important pre-processing step when correcting for drift between successive frames (multitemporal) e.g. [2], or combining a pair of different colored monochromatic images captured through different sensors (multimodal) e.g. [3], [4]. Here we review some results from [5] and [6] and include some new results related to an approximation made on the distributional properties of CP measurement errors.

We consider an image to capture a subset of the space $\mathbb{R}^d$, $d = 2$ or 3. Given two image spaces $\mathcal{I}_1 \subseteq \mathbb{R}^d$ and $\mathcal{I}_2 \subseteq \mathbb{R}^d$, say, registration is concerned with estimating the mapping $T : \mathcal{I}_1 \rightarrow \mathcal{I}_2$. It is typical to consider T to be an *affine* transformation e.g. [7], [8]. In this circumstance, for $x \in \mathcal{I}_1$, $T(x) = Ax + s$ where $A \in \mathbb{R}^{d \times d}$ is a square invertible matrix and $s \in \mathbb{R}^d$ is a translation vector. Registration involves using the CP locations in $\mathcal{I}_1$ and their corresponding mapped positions in $\mathcal{I}_2$ to find T.

In general, due to noisy signals, the location of the fiduciary markers in both images can not be measured exactly and instead are perturbed by random errors. Traditional least squares estimators are therefore inappropriate [9] and we are instead presented with an errors-in-variables (EIV) problem. Further to this localization accuracy depends on the brightness of the light emitting object (see [10], [11], [12], [13], [14]) and hence each fiduciary marker is localized with varying degrees of accuracy. This presents us with a heteroscedastic EIV model and the traditional EIV methods of total least squares (TLS) [15] and the equivalent generalized least squares (GLS) [16] give inconsistent estimators. With this problem in mind, two key questions arise. Firstly; what is the procedure for estimating A and s that correctly accounts for the measurement errors in localizing the CPs? Secondly; how accurately can we determine the transformation and hence what are the effects of registration on the localization accuracy of a single molecule?

For the second of these questions it becomes useful to define a new measure of registration error called the localization registration error (LRE) [5], [6]. Localizing a single molecule in $\mathcal{I}_1$ typically has its own errors associated with it. The LRE measures the combined effect of this localization error and the registration error to give the localization error of the feature registered in the second image, and is of importance to researchers [3], [4].

The paper proceeds as follows. In Section 2 we formulate the image registration problem and take time to consider the distributional properties of the measurement errors in localizing a fiduciary marker in a microscopy experiment. In Section 3 we show how this form can be used with previously published results to give an expression for the loss in localization accuracy of a single molecule induced by the image registration process, which is shown to be a function of the number of control points and the photon counts associated with them.

II. FORMULATING THE PROBLEM

Suppose K CPs are located in $\mathcal{I}_1 \subseteq \mathbb{R}^d$ at true locations $\{x_{1,k} \in \mathcal{I}_1, k = 1, ..., K\}$, and in $\mathcal{I}_2 \subseteq \mathbb{R}^d$ at true locations

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$\{x_{2,k} \in \mathcal{I}_2, k = 1,...,K\}$, such that $x_{2,k} = T(x_{1,k}) = Ax_{1,k} + s$, $k = 1,...,K$, where $A \in \mathbb{R}^{d\times d}$ and $s \in \mathbb{R}^d$. In reality the positions of the CPs cannot be known exactly and must instead be measured. Consequently we observe the CP locations as $\{y_{1,k} \in \mathcal{I}_1, k = 1,...,K\}$ and $\{y_{2,k} \in \mathcal{I}_2, k = 1,...,K\}$, where $y_{j,k} = x_{j,k} + \epsilon_{j,k}$, $k = 1,...,K$, $j = 1,2$. The term $\epsilon_{j,k} \in \mathbb{R}^d$ is a random variable known as the measurement error. Each measurement error is assumed zero mean and to have individual symmetric positive definite covariance matrix $\Omega_{j,k}$. It is assumed that all measurement errors are pairwise independent across the CPs.

We define the $\mathbb{R}^{d\times K}$ matrices $X_j \equiv [x_{j,1},...,x_{j,K}]$, $Y_j \equiv [y_{j,1},...,y_{j,K}]$ and $\mathcal{E}_j \equiv [\epsilon_{j,1},...,\epsilon_{j,K}]$, $j = 1,2$, and further define the *stacked* $\mathbb{R}^{2d\times K}$ matrices $X \equiv \left[X_1^T, X_2^T\right]^T$, $Y \equiv \left[Y_1^T, Y_2^T\right]^T$ and $\mathcal{E} \equiv \left[\mathcal{E}_1^T, \mathcal{E}_2^T\right]^T$. With this notation the system of equations can be conveniently represented as the single matrix equation

$$Y = \Lambda X_1 + \alpha \mathbf{1}_K^T + \mathcal{E}, \tag{1}$$

where ^{T} is the matrix transpose, $\alpha = [\mathbf{0}^T, s^T]^T$, $\Lambda = [I_d, A^T]^T$ and $\mathbf{1}_K$ is a column vector of length K with every element taking the value 1. Maximum likelihood (ML) estimators for parameters A and s are considered in the iid case in [16] and in the general heteroscedastic case in [17].

Given estimators $\hat{A}$ and $\hat{s}$ it is important to know the effect the registration procedure will have on the localization of a single molecule. In [5] and [6] is defined the localization registration error (LRE). First let us define the difference between the estimated and true values of the transform parameters as $\Delta A \equiv \hat{A} - A$ and $\Delta s \equiv \hat{s} - s$. Suppose we are interested in registering a single molecule in $\mathcal{I}_1$ with true position $x_{1,F} \in \mathcal{I}_1$, in the second image the true position of this feature is $x_{2,F} \in \mathcal{I}_2$, with $x_{2,F} = Ax_{1,F} + s$. However, as with the CPs, the position of the feature in $\mathcal{I}_1$ is actually measured to be at $y_{1,F} = x_{1,F} + \epsilon_{1,F}$, where $\epsilon_{1,F}$ is a measurement error with zero mean and covariance $\Omega_{1,F}$. Therefore our estimator for the position of the feature in $\mathcal{I}_2$ is $\hat{A}y_{1,F} + \hat{s}$. The error associated with localizing the feature (single molecule) in $\mathcal{I}_2$ is given by the LRE.

Definition II.1. *For a feature in $\mathcal{I}_1$ with true and measured locations $x_{1,F}$ and $y_{1,F} = x_{1,F} + \epsilon_{1,F}$ respectively, the localization registration error (LRE) ℓ_F is defined as the difference between the true position and the registered position, i.e.*

$$\begin{aligned} \ell_F &\equiv x_{2,F} - (\hat{A}y_{1,F} + \hat{s}) \\ &= Ax_{1,F} + s - (\hat{A}y_{1,F} + \hat{s}) \\ &= -A\epsilon_{1,F} - \Delta A\epsilon_{1,F} - \Delta A x_{1,F} - \Delta s. \end{aligned}$$

A. Measurement errors

To use the results in [5] it is necessary to show that the covariance matrices for the errors in localizing the fiduciary markers (the columns of $\mathcal{E}$ in (1)), while different, can all be treated as scalar multiples of a common matrix.

Let $\mathcal{C} \subset \mathbb{R}$ represent the detector and let the pixel array $\{C_1,...,C_{N_P}\}$ be a collection of N_P disjoint sets such that $\mathcal{C} = \bigcup_{m=1}^{N_P} C_m$. Let $\theta = (u,v)$ be the object space location of a light emitting source in a microscopy imaging experiment, emitting photons as an inhomogeneous Poisson process with time dependent rate $\Lambda(\cdot)$. Taking into account the effects of noise, the photon count during an interval of $[t_0, t_0+t]$ at each pixel is the sum of the number of photons due to the imaged object and the number of photons introduced by background noise and the readout noise. Let $\mathcal{P}_{\theta,m}$ denote the total photon count at the mth pixel during the interval $[t_0, t_0+t]$. We write

$$\mathcal{P}_{\theta,m} = S_{\theta,m} + B_m + W_m,$$

where $S_{\theta,m}$ is the photon count at the mth pixel due to the object located in the object space at positional vector θ. We have $S_{\theta,m} \overset{\mathrm{d}}{=} \text{Poisson}(\mu_{\theta,m,t})$ (where $\overset{\mathrm{d}}{=}$ means "equal in distribution"), with

$$\mu_{\theta,m,t} = \int_{t_0}^{t_0+t}\int_{C_m} \Lambda_\theta(\tau) f_\theta(r)\mathrm{d}r\mathrm{d}\tau, \quad m = 1,...,N_P, \tag{2}$$

where the coordinates on the detector of the detected photons are assumed to be iid with known distribution $f_\theta(r)$, $r \in \mathbb{R}^2$. Random variable $B_m \overset{\mathrm{d}}{=} \text{Poisson}(\beta_{m,t})$ is the count at the mth pixel due to scattering, and $W_m \overset{\mathrm{d}}{=} N(\varphi_m, \sigma_m^2)$ is the count at the mth pixel due to readout noise. We can write (2) as $\mu_{\theta,m,t} = \lambda_t h_\theta(m,t)$ where $\lambda_t = \int_{t_0}^{t_0+t}\Lambda_\theta(\tau)\mathrm{d}\tau$ is the time average Poisson rate for the light emitting object and $h_\theta(m,t) = \int_{C_m} f_\theta(r)\mathrm{d}r$ is the probability that an emitted photon will be collected in pixel $m = 1,...,N_P$. We assume that the Poisson background noise and Gaussian readout noise is homogeneous across all pixels and the distributions known.

In [10] the following is given for the Fisher information matrix for the location parameter θ of a light emitting object in a microscopy setup

$$I(\theta) = \sum_{m=1}^{N_P}\left[\frac{\partial\mu_{\theta,m,t}}{\partial u}\right]\left[\frac{\partial\mu_{\theta,m,t}}{\partial v}\right]^T \times \int_{\mathbb{R}} \frac{\left(\sum_{l=1}^{\infty}\frac{[\nu_\theta(m)]^{l-1}\mathrm{e}^{-\nu_\theta(m)}}{(l-1)!}\frac{1}{\sqrt{2\pi}\sigma_m}\mathrm{e}^{-\frac{1}{2}\left(\frac{z-l-\eta_m}{\sigma_m}\right)^2}\right)^2}{p_{\theta,m}(z)}\mathrm{d}z - 1 \tag{3}$$

where for $z \in \mathbb{R}$

$$p_{\theta,m}(z) \equiv \frac{1}{\sqrt{2\pi}\sigma_m}\mathrm{e}^{-\frac{1}{2}\left(\frac{z-l-\eta_m}{\sigma_m}\right)^2}\sum_{l=0}^{\infty}\frac{[\nu_\theta(m)]^l \times \mathrm{e}^{-\nu_\theta(m)}}{l!}$$

with $\nu_{\theta,m} \equiv \mu_{\theta,m} + b_m t$. The inverse of the Fisher information matrix, $I^{-1}(\theta)$, is the Cramér-Rao lower bound [18] for the covariance matrix of the error in object space of localizing an isolated point source emitting photons as an inhomogeneous Poisson process in the presence of background and readout noise, which in turn is shown in [10] to be a reasonable estimate for the true covariance matrix. We now consider an approximation to (3) that will be valid for fiduciary markers.

The sum of two Poisson distributed random variables is itself Poisson and hence

$$S_{\theta,m} + B_m \overset{\mathrm{d}}{=} \text{Poisson}(\mu_\theta(m,t) + \beta(m,t)).$$

Fiduciary markers used in fluorescence microscopy experiments typically have a high signal to noise ratio, hence we consider $\mu_\theta(m,t)$ to be large enough such that the normal approximation to the Poisson distribution is valid. We approximate

$$S_{\theta,m} + B_m \stackrel{\mathrm{d}}{=} N(\mu_\theta(m,t) + \beta(m,t), \mu_\theta(m,t) + \beta(m,t)).$$

Using the fact that if $X \stackrel{\mathrm{d}}{=} N(\mu_X, \sigma_X^2)$ and $Y \stackrel{\mathrm{d}}{=} N(\mu_Y, \sigma_Y^2)$, then $X + Y \stackrel{\mathrm{d}}{=} N(\mu_X + \mu_Y, \sigma_X^2 + \sigma_Y^2)$, we make the suitable approximation that

$$S_{\theta,m} + B_m + W_m \stackrel{\mathrm{d}}{=} N(\rho_{\theta,m}, \varsigma_{\theta,m}^2).$$

where $\rho_{\theta,m} = \mu_\theta(m,t) + \beta(m,t) + \eta_m$ and $\varsigma_{\theta,m}^2 = \mu_\theta(m,t) + \beta(m,t) + \sigma_m^2$. The probability mass function (pmf) for the total photon count $\mathcal{P}_{\theta,m}$ in the mth pixel is therefore approximated by

$$p_{\theta,m}(z) = \frac{1}{\sqrt{2\pi}\varsigma_{\theta,m}} \mathrm{e}^{-\frac{1}{2\varsigma_{\theta,m}^2}(z-\rho_{\theta,m})^2}.$$

By the mutual independence of $\mathcal{P}_{\theta,1}, ..., \mathcal{P}_{\theta,N_P}$, the pmf of the pixelated image $\mathcal{I}_\theta = \{\mathcal{I}_{\theta,1}, ..., \mathcal{I}_{\theta,N_P}\}$ is just the product of the pmf for $\mathcal{P}_{\theta,m}$, $m = 1, ..., N_P$, that is, $p_{\mathcal{P}_\theta} = \prod_{m=1}^{N_P} p_{\theta,m}(z_m)$. Therefore if $\{z_1, ..., z_{N_P}\}$ is a realization of $\{\mathcal{P}_{\theta,1}, ..., \mathcal{P}_{\theta,N_P}\}$, then the log-likelihood function $\mathcal{L}(\theta|z_1, ..., z_{N_P})$ is given by

$$\mathcal{L}(\theta|z_1, ..., z_{N_P}) = \ln(p_{\mathcal{I}_\theta}(z_1, ..., z_{N_p})) = \sum_{m=1}^{N_P} \ln(p_{\theta,m}(z_m)),$$

and its partial derivative with respect to θ is given by

$$\frac{\partial \mathcal{L}(\theta|z_1, ..., z_{N_P})}{\partial \theta} = \sum_{m=1}^{N_P} \left[\frac{1}{p_{\theta,m}(z_m)} \frac{\partial p_{\theta,m}(z_m)}{\partial \theta} \right].$$

By chain-rule

$$\frac{\partial p_{\theta,m}(z_m)}{\partial \theta} = \frac{\partial p_{\theta,m}(z_m)}{\partial \rho_{\theta,m}} \frac{\partial \rho_{\theta,m}}{\partial \theta} + \frac{\partial p_{\theta,m}(z_m)}{\partial \varsigma_{\theta,m}} \frac{\partial \varsigma_{\theta,m}}{\partial \theta},$$

and it follows that

$$\frac{\partial \mathcal{L}(\theta|z_1, ..., z_{N_P})}{\partial \theta} = \sum_{m=1}^{N_P} \frac{\partial \mu_\theta(m,t)}{\partial \theta} \zeta_{\theta,m}(z_m)$$

where $\zeta_{\theta,m}(z_m) = \frac{(z_m - \rho_{\theta,m})}{\varsigma} + \frac{(z_m - \rho_{\theta,m})^2}{2\varsigma_{\theta,m}^4} - \frac{1}{2\varsigma_{\theta,m}^2}$. We now give two important results. Using the identities for the moments of a normally distributed random variable then

$$E\{\zeta_{\theta,m}(z_m)\} = 0 \tag{4}$$

$$E\{[\zeta_{\theta,m}(z_m)]^2\} = \frac{1}{2}\varsigma_{\theta,m}^{-4} + \varsigma_{\theta,m}^{-2}. \tag{5}$$

From definition the Fisher information matrix $I(\theta)$ for location parameter θ is equal to

$$\sum_{m=1}^{N_P} \sum_{n=1}^{N_P} \left[\frac{\partial \mu_\theta(m,t)}{\partial \theta} \right]^T \left[\frac{\partial \mu_\theta(n,t)}{\partial \theta} \right] \times E\{\zeta_{\theta,m}(z_m)\zeta_{\theta,n}(z_n)\}.$$

Making use of the results (4) and (5), and the pairwise independence of $\{\zeta_{\theta,m}(z_m), m = 1, ..., N_P\}$ we conclude

$$E\{\zeta_{\theta,m}(z_m)\zeta_{\theta,n}(z_n)\} = (\frac{1}{2}\varsigma_{\theta,m}^{-4} + \varsigma_{\theta,m}^{-2})\delta_{mn}$$

and thus

$$I(\theta) = \sum_{m=1}^{N_P} \left[\frac{\partial \mu_\theta(m,t)}{\partial \theta} \right]^T \left[\frac{\partial \mu_\theta(m,t)}{\partial \theta} \right] \left(\frac{1}{2}\varsigma_{\theta,m}^{-4} + \varsigma_{\theta,m}^{-2} \right).$$

We are concerned with the 2D spacial parameter $\theta = (u, v)$. Using the identity $\mu_\theta(m,t) = \lambda_t h_\theta(m)$ then $\frac{\partial \mu_\theta(m,t)}{\partial u} = \lambda_t \frac{\partial h_\theta(m,t)}{\partial u}$ and $\frac{\partial \mu_\theta(m,t)}{\partial v} = \lambda_t \frac{\partial h_\theta(m,t)}{\partial v}$, giving

$$I(\theta) = \lambda_t^2 \sum_{m=1}^{N_P} \left[\frac{\partial h_\theta(m,t)}{\partial \theta} \right]^T \left[\frac{\partial h_\theta(m,t)}{\partial \theta} \right] \times \left(\frac{1}{2}\varsigma_{\theta,m}^{-4} + \varsigma_{\theta,m}^{-2} \right).$$

Reminding ourselves that $\varsigma_{\theta,m}^2 = \mu_\theta(m,t) + \beta(m,t) + \sigma_m^2$, then

$$\frac{1}{2}\varsigma_{\theta,m}^{-4} + \varsigma_{\theta,m}^{-2} = \frac{1}{2[\mu_\theta(m,t)]^2} \left(1 - 2\frac{\beta(m,t) + \sigma_m^2}{\mu_\theta(m,t)} + ... \right) + \frac{1}{\mu_\theta(m,t)} \left(1 - \frac{\beta(m,t) + \sigma_m^2}{\mu_\theta(m,t)} + ... \right).$$

Let $q_{m,t} \equiv \frac{\beta(m,t) + \sigma_m^2}{\mu_\theta(m,t)}$ then

$$\frac{1}{2}\varsigma_{\theta,m}^{-4} + \varsigma_{\theta,m}^{-2} \approx \frac{1}{\mu_\theta(m,t)}(1 + q_{m,t} + \mathcal{O}(q_{m,t}^2))$$

for large $\mu_\theta(m,t)$.

If $\mu_\theta(m,t) \gg \beta(m,t) + \sigma_m^2$, i.e. the signal to noise ratio is high, then $\frac{1}{2}\varsigma_{\theta,m}^{-4} + \varsigma_{\theta,m}^{-2} \approx \frac{1}{\mu_\theta(m,t)} = \frac{1}{\lambda_t h_\theta(m,t)}$ giving

$$I(\theta) = \lambda_t \sum_{m=1}^{N_P} \frac{1}{h_\theta(m)} \left[\frac{\partial h_\theta(m,t)}{\partial \theta} \right]^T \left[\frac{\partial h_\theta(m,t)}{\partial \theta} \right].$$

Therefore $N^{-1}\mathcal{M}^2 J$ is a suitable approximation to the covariance matrix for image space errors in localizing the fiduciary markers, where N is the number of photons collected at the detector for that object (an estimate of λ_t), $\mathcal{M}$ is the system magnification and

$$J = \left[\sum_{m=1}^{N_P} \frac{1}{h_\theta(m)} \left[\frac{\partial h_\theta(m,t)}{\partial \theta} \right]^T \left[\frac{\partial h_\theta(m,t)}{\partial \theta} \right] \right]^{-1}$$

is a symmetric positive definite matrix that can be computed from experimental parameters including photon wavelength, numerical aperture and the point spread function of the optical system.

We assume the image registration formulation of Section II and model (1) with the use of K fiduciary markers for the CPs. The matrix J and system magnification $\mathcal{M}$ are specific to the image and hence labeled J_j and $\mathcal{M}_j$, respectively, $j = 1, 2$. Suppose $N_{j,k}$ photons are detected at the detector for fiduciary marker k in $\mathcal{I}_j$, $k = 1, ..., K$, $j = 1, 2$. The measurement errors $\epsilon_{j,k}$, $k = 1, ..., K$, $j = 1, 2$, are therefore assumed to have covariance $\Omega_{j,k}$ of the form $\Omega_{j,k} = (1/N_{j,k})\Omega_{j,0}$, where $\Omega_{j,0} \equiv \mathcal{M}_j^2 J_j$ is a symmetric positive definite matrix and universal for all CPs in $\mathcal{I}_j$. This gives the covariance matrix of $\epsilon_k \equiv [\epsilon_{1,k}^T, \epsilon_{2,k}^T]^T$ as the block diagonal matrix $\Omega_k = \text{diag}\{(1/N_{1,k})\Omega_{1,0}, (1/N_{2,k})\Omega_{2,0}\}$.

Consider performing drift correction for tracking or super-resolution purposes by registering two images taken by the same sensor at different times (multitemporal). In this case, provided the brightness of the marker remains constant in the time between captures, then we assume $N_{1,k} \approx N_{2,k}$. With this assumption we have the situation where the covariance matrices Ω_k $k = 1, ..., K$ are scalar multiples of

$$\Omega_0 = \begin{bmatrix} \Omega_{1,0} & 0 \\ 0 & \Omega_{2,0} \end{bmatrix} \qquad (6)$$

with $1/N_{1,k}$ providing the scaling factor.

The ML estimators $\hat{A}$ and $\hat{s}$ of A and s, respectively, for models of type (1) where the covariance matrices Ω_k, $k = 1, ..., K$ are of form (6) are considered in [5] and dealt with in full in [6]. Further to this distributional results are derived for the ML estimators $\hat{A}$ and $\hat{s}$.

III. Localization registration error results

In [5] and [6] the following 2D model was considered.

Assumption (i). We model the CP measurement errors $\epsilon_k \overset{\text{d}}{=} N_4(\mathbf{0}, \Omega_k)$ with $\Omega_{j,k} = \sigma_{j,k}^2 I_2 = (1/N_{j,k})\mathcal{M}_j^2 \zeta_j I_2$ where $N_{j,k}$ is the photon count at the detector associated with CP k in $\mathcal{I}_j$, $k = 1, ..., K$, $j = 1, 2$. $\mathcal{M}_j$ is the known system magnification associated with $\mathcal{I}_j$. Scalar ζ_j is a known function of the point spread function, photon wavelength and numerical aperture. In multimodal registration $\mathcal{M}_j$ and ζ_j will be different for each image, while in multitemporal registration they will be identical for both images. Ω_0 is of form (6) where $\Omega_{j,0} = \mathcal{M}_j^2 \zeta_j I_2$.

Assumption (ii). Consider the CP true positions $\{x_{1,k}, k = 1, ..., K\}$ to be K realizations of a random variable $\mathcal{X} \in \mathbb{R}^2$ with mean zero and covariance $\kappa^2 I_2$, and let associated photon counts be non-zero, finite and independent of CP positions.

Assumption (iii). The affine transformation parameter $A = SR$ represents a scaling $S = \varsigma I_2$, $\varsigma \in \mathbb{R}^+$, combined with a unitary rotation or reflection (or a combination of both) R, i.e. $R^T R = R R^T = I_2$.

Assuming the localization error of the feature (single molecule) has covariance $\Omega_{1,F} = (\mathcal{M}_1^2 \zeta_1 / N_{1,F}) I_2$, where $N_{1,F}$ is the photon count associated with the feature (molecule) imaged in $\mathcal{I}_1$, the expression for the (m, n)th element of the LRE covariance in [5] is corrected in [6] to

$$[\Omega_\ell]_{mn} \approx \varsigma \frac{\mathcal{M}_1^2 \zeta_1}{N_{1,F}} + K^{-1} \left(\varsigma^2 \frac{\mathcal{M}_1^2 \zeta_1}{\bar{N}_1} + \frac{\mathcal{M}_2^2 \zeta_2}{\bar{N}_2} \right) \left(1 + \left(\frac{r_F}{\kappa} \right)^2 \right) \delta_{mn}. \qquad (7)$$

where r_F is the radial distance of the feature (single molecule) to the center of the image and $\bar{N}_j = (1/K) \sum_{k=1}^K N_{j,k}$ is the mean photon count for the CPs in $\mathcal{I}_j$, $j = 1, 2$. The covariance matrix Ω_ℓ is given with respect to image space $\mathcal{I}_2$. To express LRE covariance with respect to the object space coordinates we use $\mathcal{M}_2^{-2} \Omega_\ell$.

IV. Conclusion

The first term in (7) is just the localization accuracy of the single molecule in the first image (scaled by ς). The second term is the contribution from the registration process. This shows that we always lose localization accuracy in the registration process, however asymptotically we approach the localization accuracy in the first image. Hence, by increasing the number of control points and/or the number of photons captured for each control point we are able to manage the localization loss and confine it to a quantifiable amount. [6] gives an in depth analysis along with simulations verifying the results.

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References

[1] B. Zitová and J. Flusser, "Image registration methods: a survey," *Image and Vision Computing*, vol. 21, pp. 977 — 1000, 2003.

[2] M. Bates, B. Huang, G. T. Dempsey, and X. Zhuang, "Multicolor super-resolution imaging with photo-switchable fluorescent probes," *Science*, vol. 317, pp. 1749 — 1753, 2007.

[3] L. Stirling Churchman, Z. Ókten, R. S. Rock, J. F. Dawson, and J. A. Spudich, "Single molecule high-resolution colocalization of Cy3 and Cy5 attached to macromolecules measures intramolecular distances through time," *PNAS*, vol. 102, pp. 1419 — 1423, 2005.

[4] I. Koyama-Honda, K. Ritchie, R. Iion, H. Murakoshi, R. S. Kasai, and A. Kusumi, "Fluorescence imaging for monitoring the colocalization of two single molecules in living cells," *Biophysical Journal*, vol. 88, pp. 2126 — 2136, 2005.

[5] E. A. K. Cohen and R. J. Ober, "Image registration error analysis with applications in single molecule microscopy," *Proceedings of the IEEE International Symposium for Biomedical Imaging*, pp. 996 — 999, 2012.

[6] E. A. K. Cohen and R. J. Ober, "Analysis of point based image registration errors with applications in single molecule microscopy," *Under Review. Unpublished*, 2012.

[7] B. Ma, M. H. Moghari, R. E. Ellis, and P. Abolmaesumi, "Estimation of optimal fiducial target registration error in the presence of heteroscedastic noise," *IEEE Transactions on Medical Imaging*, vol. 29, pp. 708 — 723, 2010.

[8] M. H. Moghari and P. Abolmaesumi, "Distribution of target registration error for anisotropic and inhomogeneous fiducial localization error," *IEEE Transactions on Medical Imaging*, vol. 28, pp. 799 — 813, 2009.

[9] R. B. Davies and B. Hutton, "The effect of errors in the independent variables in linear regression," *Biometrika*, vol. 62, pp. 383 — 391, 1975.

[10] R. J. Ober, S. Ram, and E. S. Ward, "Localization accuracy in single-molecule microscopy," *Biophysical Journal*, vol. 86, pp. 1185 — 1200, 2004.

[11] S. Ram, E. S. Ward, and R. J. Ober, "Resolution beyond rayleighs criterion: a new resolution measure with applications to single molecule microscopy," *Proceedings National Academy of Science USA*, vol. 103, pp. 4457 — 4462, 2006.

[12] S. Ram, E. S. Ward, and R. J. Ober, "A stochastic analysis of distance estimation approaches in single molecule microscopy: quantifying the resolution limits of photon-limited imaging systems," *Multidimensional Systems and Signal Processing*, vol. DOI 10.1007/s11045-012-0175-6, 2012.

[13] Z. Shen and S. B. Andersson, "Bias and precision of the fluoroBrancroft algorithm for single particle localization in fluorescence microscopy," *IEEE Transactions on Signal Processing*, vol. 59, pp. 4041 — 4046, 2011.

[14] 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 on Signal Processing*, vol. 59, pp. 895 — 911, 2011.
[15] S. Van Huffel, *The total least squares problem : computational aspects and analysis*, SIAM Philadelphia, 1991.
[16] L. J. Gleser, "Estimation in a multivariate "errors in variable" regression model: large sample results," *The Annals of Statistics*, vol. 9, pp. 24 — 44, 1981.
[17] N. N. Chan and T. K. Mak, "Heteroscedastic errors in a linear-functional relationship," *Biometrika*, vol. 71, pp. 212 — 215, 1984.
[18] H. Cramér, *Mathematical Methods of Statistics*, Princeton University Press, 1946.