A REGISTRATION ERROR ESTIMATION FRAMEWORK FOR CORRELATIVE IMAGING

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ABSTRACT

Correlative imaging workflows are now widely used in bio-imaging and aims to image the same sample using at least two different and complementary imaging modalities. Part of the workflow relies on finding the transformation linking a source image to a target image. We are specifically interested in the estimation of registration error in point-based registration. We propose an application of multivariate linear regression to solve the registration problem allowing us to propose a framework for the estimation of the associated error in the case of rigid and affine transformations and with anisotropic noise. These developments can be used as a decision-support tool for the biologist to analyze multimodal correlative images.

Index Terms— bio-imaging, error estimation, image registration, point clouds, correlative microscopy

1. INTRODUCTION

Correlative microscopy has become ubiquitous in bio-medical research. The technique consists in observing the same sample under different complementary imaging modalities in order to gain more insight. The most known association is undoubtedly correlative light-electron microscopy, but it is possible to combine other imaging modalities [1]. The process includes a registration step in which the images of the different modalities are overlaid by estimating the transformation linking them. In this work, we only consider point-based registration requiring a set of matched points, either fiducial points [2], anatomical or other natural landmarks [3] or cloud of points, which can be used in this context after a segmentation step by representing a contour or a surface by a cloud of points [4]. All these usages can be modeled under a unique paradigm, that we will refer to as point-based registration in the following, and we will name points used for the registration as fiducial points, as opposed to points not used for the registration, called points of interest (POIs). The localization of fiducial points is prone to error, due to imaging resolution, method of localization or of sampling....

This will create a registration error for all points of the image [5, 6, 7], having consequences on the overlaying of points of interest. There is a clear need in the bio-medical community to estimate errors in registration, as reflected by the reporting done in biological papers using correlative approaches (see Fig.1). In correlative imaging, usual intensity metrics to assess the quality of the registration are usually not directly applicable due to the discrepancy of contents and scales. This is why generic cross-validation methods like leave-one-out (LOOCV) are usually used to estimate the registration error as global values, based on fiducial points [8]. In [4], error map representation based on the local average expected target registration error was proposed, using the statistical framework for rigid registration developed in [6]. The case of rigid transformations and estimated error distribution has been developed in [5]. Unfortunately the rigid model can be too specific and fail to account for more general sample deformations. An error-in-variable model solving for affine transformations has been developed in [7]. However due to their assumption of heteroscedastic noise it is necessary to estimate the noise distribution of each fiducial point which may not be possible from a practical point of view. The main contribution of our work is the error estimation framework provided for both rigid and affine registration, by modeling the problem as a multivariate regression problem. The developed asymptotic expressions of the covariance matrix of the registration errors allow us to give different graphical representation of local accuracy: as confidence ellipses at 95%, or as estimation of the average error. Finally we demonstrate the application of these methods using simulations and real data.

2. METHODS

2.1. General affine registration problem modeling

In our framework, in order to find the transformation linking a source image and a target image of respective dimension $r$ and $m$ (that are typically 2 or 3 for 2D or 3D images), each fiducial point of the source image is assigned a corresponding point on the target image. We denote by $X$ the matrix containing the coordinates of the $n$ observed fiducial points.
Fig. 1. Error representation in correlative imaging. (a) target (electron microscopy) with fiducials used; (b) registered source (fluorescence microscopy in red, bright field in inverted gray); (c) overlay of registered source and target, showing discrepancy of POIs (black and red); (d) LOOCV information as in [8], only on fiducials; (e) Average expected error map as in [4]; (f) Confidence ellipses at 95% for POIs as we propose. Zoom for full resolution.

Let $x_i \in \mathbb{R}^r$ ($i = 1, \ldots, n$) of the source image, stacked in rows so that $X$ is a $n \times r$ matrix. We similarly denote by $Y$ the matrix of size $n \times m$ containing the $n$ observed fiducial points $y_i \in \mathbb{R}^m$ of the target image. Let $Z = [I \ X]$ be the matrix of size $n \times (r + 1)$ where all components of the first column are 1. If the transformation between $X$ and $Y$ was affine, we would have exactly $Y = Z\beta$ for some matrix $\beta$ of size $(r + 1) \times m$. In practice of course this relation is just an approximation of the true transformation and a modeling error has to be added. Another error, the so-called localization error, comes from the resolution limit of the images and the possible presence of noise, that make the coordinates of the fiducial points inaccurate and so the theoretical relation – even it was true – imprecise for the observed fiducial points. However, $X$ and $Y$ gather the observed locations of fiducial points (and not the theoretical locations of them) and thus already include the localization errors. For this reason, we do not deal with an errors-in-variables model as studied in [7]. We consider the following model relating $Y$ and $X$:

$$Y_{(n \times m)} = \left[ \begin{array}{c} Y \\ (r+1) \times m \end{array} \right] + \epsilon_{(n \times m)} \right),$$

where $\epsilon$ is a random matrix error of size $n \times m$. Accordingly, each row $\epsilon_i$ of $\epsilon$ is a vector of dimension $m$ corresponding to the random modeling error associated to the $i$-th pair of observed fiducial points $(x_i, y_i)$. We assume that the $\epsilon_i$’s are independent, centered and follow the same multidimensional Gaussian distribution in $\mathbb{R}^m$ with unknown variance $\Sigma$ of size $m \times m$, i.e. $\epsilon_i \sim \mathcal{N}_m(0, \Sigma)$ for all $i = 1, \ldots, n$. We do not assume any particular constraints on $\beta$ and $\Sigma$ apart, for the latter, to be a well-defined covariance matrix. This means that our setting includes as particular cases: i) rigid transformation, the case where the affine transformation reduces to a translation combined with a rotation, as considered for instance in [6, 5, 4]; ii) isotropic errors, when $\Sigma$ is proportional to the identity matrix; and iii) uncorrelated errors in each direction, the case when $\Sigma$ is diagonal. Our general setting thus accounts for a general affine transformation and a general error that can be anisotropic and correlated along the different directions of the image. In this setting, $\beta$ and $\Sigma$ are unknown. Our goal is then to be able to predict the location $y_0$ of a POI in the target image associated to $x_0$ in the source image, and to get a confidence region for the registration error, here the error associated to this prediction. We provide in the following sections analytic expressions of these confidence regions, making their computation fast and reliable. We consider the general affine model first and then discuss the more constrained rigid model.

### 2.2. Error estimation for an affine model

The affine model (1) is a multivariate linear regression model, see for instance [9, Chapter 7]. The maximum likelihood estimators of $\beta$ and $\Sigma$ are respectively $\hat{\beta} = (Z' Z)^{-1}Y$ and $\hat{\Sigma} = \hat{\epsilon}\hat{\epsilon}/n$, where $\hat{\epsilon} = Y - Z\hat{\beta}$ is the residuals matrix and where we recall that $Z$ denotes the matrix $[I \ X]$. Once the model is fitted, we can predict the position $y_0 \in \mathbb{R}^m$ in the target image of a point of interest $x_0 \in \mathbb{R}^r$ observed in the source image. Let $z_0 = (1, x_0)' \in \mathbb{R}^{r+1}$, then according to the affine model (1), $y_0 = \hat{\beta}'z_0 + \epsilon_0$ for an error $\epsilon_0 \sim \mathcal{N}_m(0, \Sigma)$ independent of all $\epsilon_i$’s, $i = 1, \ldots, n$, meaning that $\epsilon_0$ is independent of $\epsilon$. The prediction of $y_0$ is then $\hat{y}_0 = \hat{\beta}'z_0$ and the prediction error is:

$$y_0 - \hat{y}_0 = (\beta - \hat{\beta})'z_0 + \epsilon_0.$$ 

This error is distributed as a centered Gaussian distribution in $\mathbb{R}^m$ with variance $(1 + z_0' (Z' Z)^{-1}z_0) \Sigma$. Plugging the estimate $\hat{\Sigma}$, we obtain that the confidence ellipsoidal region $\mathcal{E}(y_0)$ of $y_0$ at the significance level $\alpha$ is given by the inequality, see [9]: for all $y \in \mathcal{E}(y_0)$, 

$$\frac{(y - \hat{\beta}'z_0)' \left( \frac{n}{n - r - 1} \hat{\Sigma} \right)^{-1} (y - \hat{\beta}'z_0)}{1 + z_0' (Z' Z)^{-1}z_0} \leq \left( \frac{m(n + r - 1)}{n - r - m} \right) F_{m,n-r-m}(1-\alpha),$$

where $F_{m,n-r-m}(1-\alpha)$ denotes the $(1-\alpha)^{th}$ percentile of a Fisher’s law with parameters $m$ and $n - r - m$. By construction, $\mathcal{E}(y_0)$ has a probability $1 - \alpha$ to contain the unknown location $y_0$. This confidence region is available analytically, making its computation fast and accurate, and importantly, it depends on the source location $x_0$ through $z_0$. 
2.3. Error Estimation under the constraints of the rigid model

Rigid models are considered in [6] and [5], among others. They correspond to the particular case of (1) where \( r = m \) and \( \beta' = [t R_\theta] \) is composed of a translation vector \( t \in \mathbb{R}^m \) and of a rotation matrix \( R_\theta \) of size \( m \times m \). Here \( \theta \) is the parameter of size \( m(m-1)/2 \) characterizing the rotation. The transformation matrix \( \beta \) of a rigid motion thus involves \( m(m+1)/2 \) free parameters against \( m(m+1) \) in the affine case. As in the affine case we assume that each error \( \epsilon_i \) independently follows a \( N_m(0, \Sigma) \) and a maximum likelihood procedure can be used to estimate the unknown parameters \( \beta \) (under the above constraints) and \( \Sigma \). In the general case, this optimization problem amounts to find \( t, \theta \) and \( \Sigma \) that maximize

\[
\begin{align*}
    n \log \left( \frac{1}{\sqrt{(2\pi)^m |\Sigma|}} \right) \\
    - \frac{1}{2} \sum_{i=1}^{n} [y_i - (R_\theta x_i + t)]' \Sigma^{-1} [y_i - (R_\theta x_i + t)].
\end{align*}
\]

This problem, discussed in [5], does not admit a closed-form solution and some numerical optimization procedure are needed to solve it in the general case. However, in the particular case where the errors are assumed to be isotropic, meaning that \( \Sigma \) is proportional to the identity matrix, this problem reduces to a constrained least squares optimization problem known as the orthogonal Procrustes problem. An analytic solution in this setting is known, see [10, 11]. In our implementation, we use the latter solution in the isotropic case, while we use a numerical solver in the general case. For a rigid model, the prediction of the target point

\[
    y_0 = R_\theta x_0 + t + e_0
\]

where \( e_0 \) is an estimation error is equal to \( R_\theta y_0 + t \) and \( y_0 \) the maximum likelihood estimates.

The computation of the confidence ellipses for the rigid model is shifted above 95% because the distribution of the registration. Table 1 compares the distribution of the mean area of the prediction ellipses gives an indication of the mean area of the prediction ellipses for the rigid and affine cases. For a rigid model, the prediction of the target point in the general case, this optimization problem amounts to find \( t, \theta \) and \( \Sigma \) that maximize

\[
    n \log \left( \frac{1}{\sqrt{(2\pi)^m |\Sigma|}} \right) \\
    - \frac{1}{2} \sum_{i=1}^{n} [y_i - (R_\theta x_i + t)]' \Sigma^{-1} [y_i - (R_\theta x_i + t)].
\]

In the general case, the asymptotic approximation of the variance of the registration error in the rigid case writes \( J_e \Sigma_{\hat{\theta}, \hat{\theta}} J_e' + \Sigma \), where \( \Sigma_{\hat{\theta}, \hat{\theta}} = I(t, \theta; \Sigma; X) \), where \( I \) is the block matrix

\[
    I(t, \theta; X) = \left( \begin{array}{c} I_{tt} \ I_{t\theta_1} \cdots \ I_{t\theta_q} \\ I_{t\theta_1}^T \ I_{\theta_1} \ \cdots \ I_{\theta_q} \\ \vdots \quad \vdots \quad \vdots \\ I_{t\theta_q}^T \ I_{\theta_q} \end{array} \right).
\]

The computation of \( I \) is approximated by linearisation in [5] when \( m = 3 \) and under the hypothesis that \( \theta \) is small, but exact formulas can be derived. Specifically, we have

\[
    I_{tt} = n \Sigma^{-1}, \\
    I_{t\theta_k} = \sum_{i=1}^{n} \Sigma^{-1} \partial_k R_\theta x_i, \quad k = 1, \ldots, q, \\
    I_{\theta k} = \left[ \sum_{i=1}^{n} x_i' \partial_k R_\theta \Sigma^{-1} \partial_l R_\theta x_i \right]_{l=1, \ldots, q}.
\]

In our implementation, we use these exact formulas. In the end, the asymptotic approximation of the variance of the registration error in the rigid case writes \( J_e \Sigma_{\hat{\theta}, \hat{\theta}} J_e' + \Sigma \), where \( \Sigma_{\hat{\theta}, \hat{\theta}} = I(t, \theta; \Sigma; X) \). Based on this variance, asymptotic confidence ellipsoidal region can be constructed, exactly as carried out in the affine case.

3. RESULTS

3.1. Accuracy of confidence region and of registration

In addition to test on real data as demonstrated on fig.1, simulations were performed with rigid and affine transformations and 10, 25 and 100 fiducial points. Here we present the results for 95\textsuperscript{th} percentile of the used Fisher’s law, but any \( \alpha \) could be used. When we compute a 95\% prediction ellipse then theoretically the estimated point lies within the ellipse 95\% of the time. Simulations are repeated 10 000 times. Prediction ellipses are computed for 100 points of interest drawn from a uniform distribution in the square of size 1024x1024 pixels. Fiducial points were drawn from a Gaussian distribution centered at the point (256, 256) with isotropic variance equal to 500 in each direction. The coverage rate is defined as the number of times the noisy point of interest lies within the computed prediction ellipse, divided by the number of iterations. This statistic is used to check whether the computed confidence ellipses are correct. The mean area of the prediction ellipses gives an indication of the size of the estimated error and allows to assess the quality of the registration. Table 1 compares the distribution of the coverage rate of 95\% prediction ellipses computed with the analytic methods of the rigid and affine models under a rigid or affine transformation. Coverage rates distribution lies at 95\% with high precision and accuracy. Distribution of coverage rates for the rigid model is shifted above 95\% because the
model assumes an asymptotic minimum variance. The distribution converges towards 95% when the number of fiducial points is increased. The affine model can still produce good estimations with rigid transformation and is more robust than the rigid model. We use the same variant of LOOCV method as described in [8]. In this flavor, the prediction area is the disk whose radius is the 95th quantile of the measured errors. Results presented in figure 2 show that with our method the coverage rate is correctly constant at 95% and the mean area of predicted ellipses increase with the distance to the nearest fiducial point. The LOOCV method underestimates the registration error since the registration error is estimated to be the same at any location. Our confidence ellipses (Fig.1(f)) can be a useful indication for the biologist in order to associate unknown structures (here q-dots used for demonstration as POIs, when other structures were used as fiducials), and complementary of the error maps proposed (Fig.1(e)).

Table 1. Distribution of the coverage rate of 95% prediction ellipses (Cov%, target value is 95%) for rigid and affine models (model) computed with analytic method under rigid and affine transformations (Transfo) using 10 and 100 fiducial points

| Transfo | Model  | Cov% n=10 mean/STD | Cov% n=100 mean/STD |
|---------|--------|--------------------|---------------------|
| rigid   | rigid  | 99.35 / 0.08       | 95.68 / 0.21        |
| rigid   | affine | 94.98 / 0.14       | 94.57 / 0.22        |
| affine  | rigid  | 3.53 / 17.51       | 2.00 / 14.07        |
| affine  | affine | 95.05 / 0.21       | 94.62 / 0.34        |

3.2. Testing the influence of attenuation bias in our biomedical context

As explained in section 2.1, our observations X and Y in model (1) include the localization error, and assume that both fiducials and POIs observations contains noise. If we change the paradigm such that X does not include a localization error (which could indeed be negligible in case of very different scale), we are back to an errors-in-variable problem and we could suffer from the attenuation bias. In a microscopy context, localization are often sought to have a subpixelic resolution. Taking into account a broader range of case, assuming a 3 pixels radius of error in the localization sounds a reasonable assertion for fiducial points in the image with less resolution. Results presented in table 2 clearly indicate there is a bias when we assume both noise since computed 99% confidence intervals for the mean coverage rate does not includes 95% which is the theoretical value. However the bias never exceeded 1% deviation from the theoretical value. We conclude the attenuation bias in our context is negligible.

4. DISCUSSION AND CONCLUSION

In this article we have developed a framework for both 2D and 3D, rigid and affine, registration error estimation under anisotropic and isotropic conditions with no other input than the fiducials measured position. The closest work for error estimation of affine registration [7] was assuming an heteroscedastic noise and then the a priori estimation of the noise in each fiducial point. We demonstrate through simulations that our method outperformed the classical cross-validation registration error estimation using the left-one-out approach. We provide an implementation of registration and error estimation as an ICY plugin [13]. This method provides analytic registration error estimation through prediction ellipses which is a visual and intuitive way of assessing the physical matching of two unknown structures and the registration quality. Furthermore our source code is released and available at https://github.com/anrcrocoval/ec-clem with video example on real data. The method has been used on real data for biological studies [14, 15]. Other registration methods can be used to account for non-linear deformations in biological samples [16]. Due to the non-linear property, registration error is difficult to estimate, but our method could be directly extended to a local affine registration framework to take into account local deformation.

Table 2. Distribution of coverage rates (min, mean, STD, max, 99% confidence interval for mean coverage rate) for 95% prediction ellipses obtained by simulation (model affine/transformation affine) including attenuation bias under the condition of microscopy for 10, 25 and 100 fiducial points

| n     | min  | mean | STD | max  | CI 99% of mean |
|-------|------|------|-----|------|----------------|
| 10    | 94.54| 94.94| 0.13| 95.31| [94.90, 94.97] |
| 25    | 94.61| 95.07| 0.21| 95.48| [95.02, 95.13] |
| 100   | 94.08| 94.68| 0.28| 95.33| [94.61, 94.75] |
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