

Singular value decomposition of multiframe polarized fluorescence microscopes

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1 Introduction

In these notes we will develop the continuous models for several multiframe polarized fluorescence microscopes. For each design we will calculate the spatio-angular point spread function, optical transfer function, and singular system consisting of the singular values, object-space singular functions, and data-space singular functions.

All of the microscopes we will consider are imaging fields of oriented fluorophores. To a good approximation any field of oriented fluorophores can be represented by a member of the set $\mathbb{U} = \mathbb{L}_2(\mathbb{R}^3 \times \mathbb{S}^2)$ —square-integrable functions that assign a scalar value to each position and orientation.

We will be considering multiframe microscopes that capture multiple images of the same object (we assume that the object is static over the imaging time). The data for the n th frame can be represented by a member of $\mathbb{V}_n = \mathbb{L}_2(\mathbb{R}^2)$ —square-integrable functions that assign a scalar value to each point in a 2-dimensional Euclidean space. If the microscope collects N frames, then all of the data can be represented by a member of the larger set $\mathbb{V} = \mathbb{L}_2(\mathbb{R}^{2N})$ —square-integrable functions that assign a scalar value to each point in a $2N$ -dimensional Euclidean space. We can say that the complete data space \mathbb{V} is built by taking the *orthogonal direct sum* of the data space for each frame \mathbb{V}_n

$$\mathbb{V} = \bigoplus_{n=1}^N \mathbb{V}_n. \quad (1)$$

Notice that we are assuming that data space is continuous—we are ignoring the effects of finite pixels and a finite field-of-view.

We can model any linear relationship between object space and data space using an integral transform

$$g_n(\mathbf{r}_d) = [\mathcal{H}f]_n(\mathbf{r}_d) = \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^3} d\mathbf{r}_o h_n(\mathbf{r}_d; \mathbf{r}_o, \hat{\mathbf{s}}_o) f(\mathbf{r}_o, \hat{\mathbf{s}}_o), \quad n = 1, 2, \dots, N, \quad (2)$$

where $g_n(\mathbf{r}_d) \in \mathbb{V}_n$ is the data for the n th frame, $f(\mathbf{r}_o, \hat{\mathbf{s}}_o) \in \mathbb{U}$ is the object, and $h_n(\mathbf{r}_d; \mathbf{r}_o, \hat{\mathbf{s}}_o) \in \mathbb{U} \times \mathbb{V}_n$ is the point response function of the imaging system for the n th frame.

In these notes we'll only be considering shift-invariant microscopes, so we can simplify the model to

$$g_n(\mathbf{r}_d) = [\mathcal{H}f]_n(\mathbf{r}_d) = \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^3} d\mathbf{r}_o h_n(\mathbf{r}_d - \mathbf{r}_o, \hat{\mathbf{s}}_o) f(\mathbf{r}_o, \hat{\mathbf{s}}_o), \quad n = 1, 2, \dots, N. \quad (3)$$

In these notes we will ignore magnification—in the previous notes we showed that we can make a change of variables that puts a system with magnification in the form of a system without magnification. In other words, we can drop the primes that indicated magnified quantities in previous note sets. We will also restrict ourselves to the paraxial approximation and drop the (p) superscripts.

2 Calculating the singular value decomposition

In this section we will lay out the steps to calculate the singular value decomposition of a multiframe polarized fluorescence microscope. We will follow Section 2A of [1] closely and find the data-space singular functions first.

In Patrick's notes we found the object-space singular functions first, but both approaches will give identical results and the eigenvalue problem is smaller for the data-space singular functions.

The forward operator for the system is given by

$$[\mathcal{H}f]_n(\mathbf{r}_d) = \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^3} d\mathbf{r}_o h_n(\mathbf{r}_d - \mathbf{r}_o, \hat{\mathbf{s}}_o) f(\mathbf{r}_o, \hat{\mathbf{s}}_o), \quad n = 1, 2, \dots, N, \quad (4)$$

and the adjoint operator for the system is given by

$$[\mathcal{H}^\dagger \mathbf{g}](\mathbf{r}_o, \hat{\mathbf{s}}_o) = \sum_{j=1}^N \int_{\mathbb{R}^2} d\mathbf{r}_d h_n(\mathbf{r}_d - \mathbf{r}_o, \hat{\mathbf{s}}_o) g(\mathbf{r}_d). \quad (5)$$

We will also need the data-space to data-space operator

$$[\mathcal{H}\mathcal{H}^\dagger \mathbf{g}]_n(\mathbf{r}_d) = \sum_{n'=1}^N \int_{\mathbb{R}^2} d\mathbf{r}'_d k_{nn'}(\mathbf{r}_d - \mathbf{r}'_d) g_{n'}(\mathbf{r}'_d), \quad (6)$$

where

$$k_{nn'}(\mathbf{r}_d - \mathbf{r}'_d) = \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^2} d\mathbf{r}_o h_n(\mathbf{r}_d - \mathbf{r}_o, \hat{\mathbf{s}}_o) h_{n'}(\mathbf{r}_d - \mathbf{r}_o, \hat{\mathbf{s}}_o). \quad (7)$$

To find the data-space singular functions and singular values, we need to solve the following eigenequation

$$[\mathcal{H}\mathcal{H}^\dagger \mathbf{v}_{\rho,j}]_n(\mathbf{r}_d) = \mu_{\rho,n} \mathbf{v}_{\rho,j}(\mathbf{r}_d). \quad (8)$$

where $\mathbf{v}_{\rho,j}(\mathbf{r}_d)$ are the data-space eigenfunctions and $\mu_{\rho,j}$ are the eigenvalues. Each $\mathbf{v}_{\rho,j}(\mathbf{r}_d)$ is an $N \times 1$ vector where each element is a function of \mathbf{r}_d . The eigenfunctions $\mathbf{v}_{\rho,j}(\mathbf{r}_d)$ are indexed by a continuous two-dimensional vector index $\boldsymbol{\rho}$ associated with the transverse directions and a discrete index j associated with the angular directions. Since the imaging system is linear shift invariant, the lateral part of the data-space eigenfunctions will be complex exponentials. We still need to solve for the angular part, so the data-space eigenfunctions will be in the form

$$\mathbf{v}_{\rho,j}(\mathbf{r}_d) = \mathbf{V}_j(\boldsymbol{\rho}) e^{i2\pi \boldsymbol{\rho} \cdot \mathbf{r}_d}. \quad (9)$$

Inserting Eqs. 9 and 7 into 6 yields

$$[\mathcal{H}\mathcal{H}^\dagger \mathbf{v}_{\rho,j}]_n(\mathbf{r}_d) = e^{i2\pi \mathbf{r}_o \cdot \boldsymbol{\rho}} \sum_{n'=1}^N K_{nn'}(\boldsymbol{\rho}) [\mathbf{V}_j(\boldsymbol{\rho})]_{n'}, \quad (10)$$

where

$$K_{nn'}(\boldsymbol{\rho}) = \int_{\mathbb{R}^2} d\mathbf{r}'_d k_{nn'}(\mathbf{r}'_d) e^{-i2\pi \boldsymbol{\rho} \cdot \mathbf{r}'_d}. \quad (11)$$

Comparing Eqs. 9 and 10 yield the eigenvalue problem

$$\mathbf{K}(\boldsymbol{\rho}) \mathbf{V}_j(\boldsymbol{\rho}) = \mu_{\rho,j} \mathbf{V}_j(\boldsymbol{\rho}), \quad (12)$$

where $\mathbf{K}(\boldsymbol{\rho})$ is an $N \times N$ matrix containing the elements $K_{nn'}(\boldsymbol{\rho})$. To simplify the calculation of the elements $K_{nn'}(\boldsymbol{\rho})$ we notice that we need to take the Fourier transform (Eq. 11) of the autocorrelation of the spatio-angular point spread function (Eq. 7). We can use the autocorrelation theorem to relate the elements of $\mathbf{K}(\boldsymbol{\rho})$ to the spatio-angular optical transfer function as

$$K_{nn'}(\boldsymbol{\rho}) = \sum_{l=0}^{\infty} \sum_{m=-l}^l H_{l,n}^m(\boldsymbol{\rho}) H_{l,n'}^m(\boldsymbol{\rho}). \quad (13)$$

Once we have solved the $N \times N$ eigenvalue problem in Eq. 12 for each value of $\boldsymbol{\rho}$, we can build the complete data-space eigenfunctions using Eq. 9. Finally, we can calculate the object-space eigenfunctions from the data-space eigenfunctions using

$$[\mathcal{H}^\dagger \mathbf{v}_{\boldsymbol{\rho},j}](\mathbf{r}_o, \hat{\mathbf{s}}_o) = \sqrt{\mu_{\boldsymbol{\rho},j}} u_{\boldsymbol{\rho},j}(\mathbf{r}_o, \hat{\mathbf{s}}_o). \quad (14)$$

Plugging in Eq. 5 and solving for $u_{\boldsymbol{\rho},j}(\mathbf{r}_o, \hat{\mathbf{s}}_o)$ yields

$$u_{\boldsymbol{\rho},j}(\mathbf{r}_o, \hat{\mathbf{s}}_o) = \frac{1}{\sqrt{\mu_{\boldsymbol{\rho},j}}} e^{i2\pi \boldsymbol{\rho} \cdot \mathbf{r}_o} \sum_{n=1}^N [\mathbf{V}_j(\boldsymbol{\rho})]_n \sum_{l=0}^{\infty} \sum_{m=-l}^l H_{l,n}^m(\boldsymbol{\rho}) y_l^m(\hat{\mathbf{s}}_o). \quad (15)$$

As we'd expect from Patrick's notes, we can rewrite the object-space singular functions as a complex exponential multiplied by a linear combination of spherical harmonics

$$u_{\boldsymbol{\rho},j}(\mathbf{r}_o, \hat{\mathbf{s}}_o) = U_j(\boldsymbol{\rho}, \hat{\mathbf{s}}_o) e^{i2\pi \boldsymbol{\rho} \cdot \mathbf{r}_o}, \quad (16)$$

where

$$U_{\boldsymbol{\rho},j}(\mathbf{r}_o, \hat{\mathbf{s}}_o) = \frac{1}{\sqrt{\mu_{\boldsymbol{\rho},j}}} \sum_{n=1}^N [\mathbf{V}_j(\boldsymbol{\rho})]_n \sum_{l=0}^{\infty} \sum_{m=-l}^l H_{l,n}^m(\boldsymbol{\rho}) y_l^m(\hat{\mathbf{s}}_o). \quad (17)$$

In summary, these are the steps to calculate the singular system for a polarized multiframe microscope.

1. Calculate the spatio-angular point spread function $h_n(\mathbf{r}_d - \mathbf{r}_o, \hat{\mathbf{s}}_o)$ for $n = 1, 2, \dots, N$.
2. Calculate the spatio-angular optical transfer function $H_{l,n}^m(\boldsymbol{\nu})$ for $n = 1, 2, \dots, N$.
3. Calculate the elements of the matrix $\mathbf{K}(\boldsymbol{\rho})$ using $K_{nn'}(\boldsymbol{\rho}) = \sum_{l=0}^{\infty} \sum_{m=-l}^l H_{l,n}^m(\boldsymbol{\rho}) H_{l,n'}^m(\boldsymbol{\rho})$.
4. Solve the eigenvalue problem $\mathbf{K}(\boldsymbol{\rho}) \mathbf{V}_j(\boldsymbol{\rho}) = \mu_{\boldsymbol{\rho},j} \mathbf{V}_j(\boldsymbol{\rho})$ at each value of $\boldsymbol{\rho}$. Store the eigenvalues $\mu_{\boldsymbol{\rho},j}$ and eigenvectors $\mathbf{V}_j(\boldsymbol{\rho})$.
5. Calculate the data-space singular functions using $\mathbf{v}_{\boldsymbol{\rho},j}(\mathbf{r}_d) = \mathbf{V}_j(\boldsymbol{\rho}) e^{i2\pi \boldsymbol{\rho} \cdot \mathbf{r}_d}$.
6. Calculate the object-space singular functions using

$$u_{\boldsymbol{\rho},j}(\mathbf{r}_o, \hat{\mathbf{s}}_o) = \frac{1}{\sqrt{\mu_{\boldsymbol{\rho},j}}} e^{i2\pi \boldsymbol{\rho} \cdot \mathbf{r}_o} \sum_{n=1}^N [\mathbf{V}_j(\boldsymbol{\rho})]_n \sum_{l=0}^{\infty} \sum_{m=-l}^l H_{l,n}^m(\boldsymbol{\rho}) y_l^m(\hat{\mathbf{s}}_o). \quad (18)$$

Under the paraxial approximation we will be able to perform steps 1 and 2 analytically. We could perform step 3 analytically, but I don't think this step will yield much insight so I will do it numerically. Step 4 can be performed analytically for $N \leq 4$ ($N > 4$ yields a quintic equation which has no solution in terms of radicals), but I will solve it numerically in all of the cases we'll consider. Steps 5 and 6 can be carried out numerically.

Outside of the paraxial approximation we will only be able to write the spatio-angular point spread function in terms of an integral, so we will perform all steps numerically. In these notes we'll only consider the paraxial case, though.

3 Polarized epi-illumination with unpolarized epi-detection

We will start by restricting our analysis of epi-illumination microscopes to in-focus objects. This means that our object space is $\mathbb{L}_2(\mathbb{R}^2 \times \mathbb{S}^2)$.

3.1 Point response function

In the previous notes we showed that the excitation point response function for polarized epi-illumination is given by

$$h_{\text{exc}}^{\hat{\mathbf{z}}}(\hat{\mathbf{s}}_o; \hat{\mathbf{p}}) = y_0^0(\hat{\mathbf{s}}_o) - \frac{1}{\sqrt{5}} \tilde{A} y_2^0(\hat{\mathbf{s}}_o) + \sqrt{\frac{3}{5}} \tilde{B} \{[(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})^2 - (\hat{\mathbf{p}} \cdot \hat{\mathbf{y}})^2] y_2^2(\hat{\mathbf{s}}_o) - 2(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})(\hat{\mathbf{p}} \cdot \hat{\mathbf{y}}) y_2^{-2}(\hat{\mathbf{s}}_o)\}, \quad (19)$$

where

$$\tilde{A} \equiv \cos^2(\alpha/2) \cos(\alpha), \quad (20a)$$

$$\tilde{B} \equiv \frac{1}{12}(\cos^2 \alpha + 4 \cos \alpha + 7), \quad (20b)$$

and $\alpha \equiv \arcsin(\text{NA}/n_o)$.

We also showed that the point response function for unpolarized epi-detection is given by

$$h_{\text{det}}(\mathbf{r}_o; \hat{\mathbf{s}}_o) = [a^2(r_o) + 2b^2(r_o)] y_0^0(\hat{\mathbf{s}}_o) + \frac{1}{\sqrt{5}} [-a^2(r_o) + 4b^2(r_o)] y_2^0(\hat{\mathbf{s}}_o), \quad (21)$$

where

$$a(r_o) = \frac{J_1(2\pi\nu_o r_o)}{\pi\nu_o r_o}, \quad b(r_o) = \frac{\text{NA}}{n_o} \left[\frac{J_2(2\pi\nu_o r_o)}{\pi\nu_o r_o} \right], \quad (22)$$

and

$$\nu_o \equiv \frac{\text{NA}}{\lambda}, \quad \text{NA} = n_o \sin \alpha. \quad (23)$$

The excitation and detection processes are incoherent, so to find the complete point response function we can multiply the excitation and detection point response functions which gives

$$h(\mathbf{r}_o, \hat{\mathbf{s}}_o; \hat{\mathbf{p}}) = h_{\text{exc}}^{\hat{\mathbf{z}}}(\hat{\mathbf{s}}_o; \hat{\mathbf{p}}) h_{\text{det}}(\mathbf{r}_o, \hat{\mathbf{s}}_o) = \frac{1}{\tilde{N}} \sum_{l=0}^{\infty} \sum_{m=-l}^l h_l^m(\mathbf{r}_o; \hat{\mathbf{p}}) y_l^m(\hat{\mathbf{s}}_o), \quad (24)$$

where $\tilde{N} = \frac{\tilde{A}}{10} + \frac{1}{2}$ is a normalization constant and the terms in the series are given by

$$h_0^0(r_o) = \left[\frac{\tilde{A}}{10} + \frac{1}{2} \right] a^2(r_o) + \left[-\frac{2\tilde{A}}{5} + 1 \right] b^2(r_o), \quad (25)$$

$$h_2^{-2}(r_o; \hat{\mathbf{p}}) = \left[\frac{9\sqrt{15}}{70} a^2(r_o) + \frac{3\sqrt{15}}{35} b^2(r_o) \right] \tilde{B}[(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})^2 - (\hat{\mathbf{p}} \cdot \hat{\mathbf{y}})^2], \quad (26)$$

$$h_2^0(r_o) = \left[-\frac{\sqrt{5}\tilde{A}}{14} + \frac{\sqrt{5}}{10} \right] a^2(r_o) + \left[-\frac{11\sqrt{5}\tilde{A}}{35} + \frac{2}{\sqrt{5}} \right] b^2(r_o), \quad (27)$$

$$h_2^2(r_o; \hat{\mathbf{p}}) = -2 \left[\frac{9\sqrt{15}}{70} a^2(r_o) + \frac{3\sqrt{15}}{35} b^2(r_o) \right] \tilde{B}(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})(\hat{\mathbf{p}} \cdot \hat{\mathbf{y}}), \quad (28)$$

$$h_4^{-2}(r_o; \hat{\mathbf{p}}) = \left[-\frac{3\sqrt{5}}{70} a^2(r_o) + \frac{6\sqrt{5}}{35} b^2(r_o) \right] \tilde{B}[(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})^2 - (\hat{\mathbf{p}} \cdot \hat{\mathbf{y}})^2], \quad (29)$$

$$h_4^0(r_o) = \frac{3\tilde{A}}{35} [a^2(r_o) - 4b^2(r_o)], \quad (30)$$

$$h_4^2(r_o; \hat{\mathbf{p}}) = -2 \left[-\frac{3\sqrt{5}}{70} a^2(r_o) + \frac{6\sqrt{5}}{35} b^2(r_o) \right] \tilde{B}(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})(\hat{\mathbf{p}} \cdot \hat{\mathbf{y}}), \quad (31)$$

and all other h_l^m terms in the series are zero. Finally, the complete forward model for this class of microscope is given by

$$g_n(\mathbf{r}_d) = \int_{\mathbb{S}^2} d\hat{\mathbf{s}}_o \int_{\mathbb{R}^3} d\mathbf{r}_o h(\mathbf{r}_d - \mathbf{r}_o, \hat{\mathbf{s}}_o; \hat{\mathbf{p}}_n) f(\mathbf{r}_o, \hat{\mathbf{s}}_o), \quad n = 1, 2, \dots, N. \quad (32)$$

Notice that we have used a single function $h(\mathbf{r}_d - \mathbf{r}_o, \hat{\mathbf{s}}_o; \hat{\mathbf{p}}_n)$ to describe the complete imaging system, and we have shifted the index n to the illumination polarization. A typical choice of frames is given by $N = 4$ and

$$\hat{\mathbf{p}}_1 = \hat{\mathbf{x}}, \quad \hat{\mathbf{p}}_2 = \frac{1}{\sqrt{2}}(\hat{\mathbf{x}} + \hat{\mathbf{y}}), \quad \hat{\mathbf{p}}_3 = \hat{\mathbf{y}}, \quad \hat{\mathbf{p}}_4 = \frac{1}{\sqrt{2}}(\hat{\mathbf{x}} - \hat{\mathbf{y}}). \quad (33)$$

The point response function of the microscope now contains seven terms in the $l = 0, 2$, and 4 bands. Each term is radially symmetric which means that we expect a radially symmetric point spread function for any angular distribution of fluorophores. Notice that the h_l^0 terms do not depend on the polarizer orientation, while the h_l^{-2} and h_l^2 do depend on the polarizer orientation. This is because the fluorophore distributions corresponding to the h_l^0 terms are rotationally symmetric about the optic axis, while the other terms are not rotationally symmetric.

3.2 Optical transfer function

The optical transfer function for this microscope is given by

$$H_l^m(\nu; \hat{\mathbf{p}}) = \frac{1}{\tilde{M}} \sum_{l'=0}^{\infty} \sum_{m'=-l'}^{l'} H_{l'}^{m'}(\nu; \hat{\mathbf{p}}) \delta(l - l', m - m'), \quad (34)$$

where $\tilde{M} = \left[\frac{\tilde{A}}{10} + \frac{1}{2} \right] + \left(\frac{\text{NA}}{n_o} \right) \left[-\frac{2\tilde{A}}{10} + \frac{1}{2} \right]$ is a normalization constant, and

$$H_0^0(\nu) = \left[\frac{\tilde{A}}{10} + \frac{1}{2} \right] A(\nu) + \left[-\frac{2\tilde{A}}{5} + 1 \right] B(\nu), \quad (35)$$

$$H_2^{-2}(\nu; \hat{\mathbf{p}}) = \left[\frac{9\sqrt{15}}{70} A(\nu) + \frac{3\sqrt{15}}{35} B(\nu) \right] \tilde{B}[(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})^2 - (\hat{\mathbf{p}} \cdot \hat{\mathbf{y}})^2], \quad (36)$$

$$H_2^0(\nu) = \left[-\frac{\sqrt{5}\tilde{A}}{14} + \frac{\sqrt{5}}{10} \right] A(\nu) + \left[-\frac{11\sqrt{5}\tilde{A}}{35} + \frac{2}{\sqrt{5}} \right] B(\nu), \quad (37)$$

$$H_2^2(\nu; \hat{\mathbf{p}}) = -2 \left[\frac{9\sqrt{15}}{70} A(\nu) + \frac{3\sqrt{15}}{35} B(\nu) \right] \tilde{B}(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})(\hat{\mathbf{p}} \cdot \hat{\mathbf{y}}), \quad (38)$$

$$H_4^{-2}(\nu; \hat{\mathbf{p}}) = \left[-\frac{3\sqrt{5}}{70} A(\nu) + \frac{6\sqrt{5}}{35} B(\nu) \right] \tilde{B}[(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})^2 - (\hat{\mathbf{p}} \cdot \hat{\mathbf{y}})^2], \quad (39)$$

$$H_4^0(\nu) = \frac{3\tilde{A}}{35} [A(\nu) - 4B(\nu)], \quad (40)$$

$$H_4^2(\nu; \hat{\mathbf{p}}) = -2 \left[-\frac{3\sqrt{5}}{70} A(\nu) + \frac{6\sqrt{5}}{35} B(\nu) \right] \tilde{B}(\hat{\mathbf{p}} \cdot \hat{\mathbf{x}})(\hat{\mathbf{p}} \cdot \hat{\mathbf{y}}), \quad (41)$$

all other H_l^m terms in the series are zero, and

$$A(\nu) = \frac{2}{\pi} \left[\arccos \left(\frac{\nu}{2\nu_o} \right) - \frac{\nu}{2\nu_o} \sqrt{1 - \left(\frac{\nu}{2\nu_o} \right)^2} \right] \Pi \left(\frac{\nu}{2\nu_o} \right), \quad (42)$$

$$B(\nu) = \frac{1}{\pi} \left(\frac{\text{NA}}{n_o} \right)^2 \left[\arccos \left(\frac{\nu}{2\nu_o} \right) - \left[3 - 2 \left(\frac{\nu}{2\nu_o} \right)^2 \right] \frac{\nu}{2\nu_o} \sqrt{1 - \left(\frac{\nu}{2\nu_o} \right)^2} \right] \Pi \left(\frac{\nu}{2\nu_o} \right). \quad (43)$$

References

- [1] Anna Burvall, Harrison H. Barrett, Christopher Dainty, and Kyle J. Myers. Singular-value decomposition for through-focus imaging systems. *J. Opt. Soc. Am. A*, 23(10):2440–2448, Oct 2006.