Using dominant eigenvalue analysis to predict formation of alternans in the heart

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Ventricular fibrillation at the whole heart level is often preceded by the alternation of action potential duration (APD), i.e., alternans, at the cellular level. As proven in many experiments, traditional approaches based on the slope of the restitution curve have not been successful in predicting alternans formation. Recently, a technique has been theoretically developed based on dominant eigenvalue analysis to predict alternans formation in isolated cardiac myocytes. Here, we aimed to demonstrate that this technique can be applied to predict alternans formation at the whole heart level. Optical mapping was performed in Langendorff-perfused hearts from New Zealand white rabbits (n = 4), which were paced at decreasing basic cycle lengths to introduce APD alternans. In each heart, the basic cycle length corresponding to the local onset of alternans, B onset, was determined and two regions of the heart were identified at B onset, one region which exhibited alternans (1:1 alt) and one which did not (1:1). Corresponding two-dimensional eigenvalue (λ) maps were generated using principal component analysis by analyzing action potentials after short perturbations from the steady state, and mean eigenvalues (λ̅) were calculated separately for the 1:1 and 1:1 alt regions. We demonstrated that λ̅ calculated at B onset was significantly different (p < 0.05) between the two regions. Our results suggest that this dominant eigenvalue technique can be used to successfully predict the local alternans formation in the heart.

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I. INTRODUCTION

Alternans is a beat-to-beat alternation in the amplitudes of T waves on an electrocardiogram (ECG), which has been attributed to alternation of the action potential duration (APD) at the cellular level [1]. Alternans has been established as a precursor to cardiac arrhythmias, such as ventricular fibrillation [1–4], which is one of the leading causes of death in the United States [5].

When tissue is paced periodically at large basic cycle length (BCL), a normal (or 1:1) response is elicited, while alternans usually occurs at small BCLs. Traditionally, the onset of alternans has been linked to the slope of the restitution curve, i.e., the nonlinear relationship between the APD and the preceding diastolic interval (DI) is equal to 1 [6]. However, several experimental studies show that alternans can occur at slopes less than 1 [7–10], or be absent for slopes greater than 1 [8,11,12]. This failure of the restitution curve to predict the onset of alternans in isolated myocytes has been attributed to several causes, such as the presence of short-term memory that significantly complicates the dynamics of cardiac tissue [7–9,13–16], or neglect of the intracellular calcium cycling that can play a major role in developing APD alternans [13,17,18]. Another reason could be the complex spatiotemporal formation of APD alternans in the heart where spatial complexity plays an important role. It has been demonstrated that APD alternans has a local onset in the heart both spatial and temporal, i.e., alternans develops in a small region of the heart, and then occupies the entire surface as the BCL decreases [18,19]. Several attempts have been made to incorporate complex cardiac dynamics into the restitution relation and to improve the prediction of alternans at both the single-cell and tissue levels [16,19–23], but the problem remains unsolved. The intrinsic complexity associated with the nonuniqueness of the restitution curve and its construction remains a major flaw, and therefore facilitates the search for more reliable alternative approaches for alternans prediction.

The stability of a complex dynamical system, e.g., the heart in our case, is associated with its eigenvalues [24]. Specifically, alternans results from a period-doubling bifurcation, which occurs when a negative eigenvalue approaches −1 [24]. When the underlying model of a cardiac action potential is explicitly available, eigenvalue calculation is straightforward [24] allowing determination of the system’s stability [25,26]. On the other hand, stability analysis based on experimental data is more difficult to perform, for several reasons, e.g., the appropriate state variables governing the dynamical system are unknown and approximations to the state space may consist of quantities that cannot be readily measured [25,26]. Recently, a technique has been proposed to estimate the stability of the system based on the calculation of the most dominant eigenvalues from a time series of readily measurable quantities [27]. This technique is based on the principle that when a dynamical system is mildly perturbed from its steady state, only a small number of dominant eigenvalues are associated with it and the most dominant eigenvalues can be found via principal component analysis (PCA) [28]. This technique was validated numerically in [27] where dominant eigenvalues were calculated from time series of action potentials obtained using a Shiferaw-Fox model of a single cardiac myocyte [29,30].
repolarization, respectively. The value \((\text{NaHCO}_3 = 12.6 \text{ mM}, \text{mannitol} = 34 \text{ mM})\). The aorta was quickly immersed in cardioplegic solution (glucose 280 mM, KCl 13.44 mM, \(\text{CaCl}_2 = 1.8 \text{ mM}\), \(\text{MgCl}_2 = 1.0 \text{ mM}\), NaHPO\(_4\) 1.2 mM, NaHCO\(_3\) 12.6 mM, mannitol 34 mM). The hearts were quickly removed and immersed with sodium pentobarbital (75 mg/kg intravenously). After thoracotomy, the hearts were quickly removed and immersed in cardioplegic solution (glucose 280 mM, KCl 13.44 mM, \(\text{CaCl}_2 = 1.8 \text{ mM}\), \(\text{MgCl}_2 = 1.0 \text{ mM}\), NaHPO\(_4\) 1.2 mM, NaHCO\(_3\) 12.6 mM, mannitol 34 mM). The aorta was quickly cannulated and retrogradely perfused with warm \((36 \pm 1 \text{ °C})\) oxygenated Tyrode’s solution (\(\text{NaCl} = 130 \text{ mM}\), \(\text{CaCl}_2 = 1.8 \text{ mM}\), KCl 4 mM, \(\text{MgCl}_2 = 1.0 \text{ mM}\), NaHPO\(_4\) 1.2 mM, NaHCO\(_3\) 24 mM, glucose 5.5 mM) under constant pressure (70 mm Hg). The Tyrode’s solution’s \(pH\) was maintained at 7.4 with adjustments made with HCl. The hearts were immersed in a chamber and superfused with the same Tyrode’s solution. Blebbistatin \((10 \mu\text{mol/l})\) was added to the Tyrode’s solution to reduce motion artifacts.

A bolus of 5 ml of the voltage-sensitive dye Di-4-ANEPPS \((10 \mu\text{mol/l})\) was injected. This dye was excited with the use of a diode-pumped continuous-excitation green laser \((532\text{ nm}, 1 \text{ W}, \text{Shanghai Dream Laser Technology Co, China})\). A charge-coupled device camera (CA-D1-0128T, DALSA, Waterloo, Ontario, Canada) was used to record fluorescence intensity from the right ventricle (RV) of the heart. Movies were acquired at 600 frames per second with a spatial resolution of 64 \(\times\) 64 pixels. The background fluorescence was subtracted from each frame. In addition, spatial \((3 \times 3 \text{ pixels})\) and temporal \((5 \text{ pixels})\) conical convolution filters were used.

B. Pacing protocol

External stimuli \((5 \text{ ms duration, twice the threshold})\) were applied to the base of the heart using a down-sweep pacing protocol [16] in which steady-state BCLs, \(B^{SS}\), were progressively reduced from 300 ms to 130 ms in 10 or 20 ms decrements. The following steps were applied at each BCL, and are represented in Fig. 1(a) for the specific case of \(B^{SS} = 240 \text{ ms}\):

I) 100 stimuli were applied at \(B^{SS}\) to achieve a steady state (SS). II) Eight stimuli were delivered at \(B^{SS}\) to measure the SS response (filled circles). III) One additional stimulus (long perturbation, LP) was applied at a longer BCL \(B^{LP} = B^{SS} + 10 \text{ ms (open circle). IV) Four stimuli were applied at } B^{SS} \text{ to return to SS after the LP (open squares). V) One additional stimulus (short perturbation, SP) was applied at a shorter BCL } B^{SP} = B^{SS} − 10 \text{ ms (open circle). VI) Four stimuli were applied at } B^{SS} \text{ to return to SS after the SP (filled squares). }

Optical mapping movies \((5 \text{ s duration})\) were acquired to capture action potentials during SS, or after the SP \([\text{filled circles and squares in Fig. 1(a), respectively}]\) at each BCL.

C. APD and alternans measurement

At each pixel, optical action potentials were analyzed, and the maximum change of the depolarization front of the action potential \(v\) was calculated. This value \((dv/dt)_{\text{max}}\) is indicated by a dot in Fig. 1(b). The APDs were measured from \((dv/dt)_{\text{max}}\) to different percentages of action potential repolarization. For instance, Fig. 1(b) shows APD\(^90\) and APD\(^50\) measurements, i.e., the duration when the action potential is at 90% and 50% repolarization, respectively.

At each pixel, the magnitude of the steady-state APD alternans was calculated for responses measured at SS (step II of the pacing protocol) as \(\Delta \text{APD} = \langle \text{APD}_{\text{odd}} \rangle − \langle \text{APD}_{\text{even}} \rangle\), where \(\langle \text{APD}_{\text{odd}} \rangle\) and \(\langle \text{APD}_{\text{even}} \rangle\) are mean values of APDs calculated from odd and four even beats at SS, respectively. The threshold for alternans was set to 5 ms [19,31]. The phase of APD alternans was negative for short-long APD sequences (represented by blue) and positive for long-short APD sequences (represented by red).

Two-dimensional (2D) APD and corresponding \(\Delta \text{APD}\) (alternans) maps were constructed for the epicardial surfaces of the heart based on SS responses at each BCL. In the heart, the local spatial onset of alternans, \(B_{\text{onset}}\), was defined as the BCL at which at least 10% of the surface of the heart was occupied by alternans [19]. Two spatial regions of the heart were defined at \(B_{\text{onset}}\): the 1:1 alt region, where alternans was present, and the 1:1 region, which exhibited 1:1 behavior. These two regions were back-projected to all previous BCLs, and the mean values
and standard errors for all parameters were calculated and averaged separately for these two regions.

**D. Eigenvalue calculation**

The eigenvalue calculation was fully described in [27]. Briefly, we assume that the essential dynamics of the heart can be approximately described using only a few dominant eigenvectors \( v \), when the system is near a steady state:

\[
y_n = y_* + \lambda_1^n v_1 + \lambda_2^n v_2 + \cdots + \lambda_m^n v_m, \quad n = 1, \ldots, N, \quad m = 1, \ldots, M \leq N,
\]

where \( y_n \) is a vector consisting of the \( n \)th APD measurement observed at each \( n \)th beat, \( y_* \) is a vector of steady-state APDs, and \( N = 4 \) for our specific pacing protocols (step VI) as we consider only four responses after the SP. Therefore, to use the proposed approach, we need to complete the following major tasks at each pixel of our optical mapping movies: (1) to construct a pseudostate vector \( y_n \) of the heart using four APD measurements after SP (step VI of the pacing protocol); (2) to compute the dominant eigenvalues using PCA.

We decided to compare the dominant eigenvalues using either \( M = 4 \) (\( \lambda_{[4]} \); APD\(_{90}^0\), APD\(_{70}^0\), APD\(_{50}^0\), APD\(_{30}^0\)) or \( M = 2 \) (\( \lambda_{[2]} \); APD\(_{90}^0\), APD\(_{50}^0\)) APD measurements from \( N = 4 \) action potentials after the SP. Therefore, at each pixel, two different matrices \( Z_{[4]} \) and \( Z_{[2]} \) were constructed at each BCL as follows:

\[
Z_{[4]} = \begin{bmatrix}
APD_{90}^0 - APD_*^0 & \cdots & APD_{30}^0 - APD_*^0 \\
\vdots & \ddots & \vdots \\
APD_{4}^0 - APD_*^0 & \cdots & APD_{30}^0 - APD_*^0
\end{bmatrix}
\]

and

\[
Z_{[2]} = \begin{bmatrix}
APD_{90}^0 - APD_*^0 & APD_{50}^0 - APD_*^0 \\
\vdots & \ddots & \vdots \\
APD_{4}^0 - APD_*^0 & APD_{50}^0 - APD_*^0
\end{bmatrix}
\]

where \( APD_k^n \) and \( APD_*^n \) indicate the APD measured at \( k \% \) of repolarization from either the \( n \)th beat after the SP (step VI of the pacing protocol), or the SS response (mean of eight APDs at step II of the pacing protocol).

PCA was then performed on \( Z_{[4]} \) and \( Z_{[2]} \) to determine the most dominant eigenvalues \( \lambda_{[4]} \) and \( \lambda_{[2]} \) respectively for each pixel, at each BCL [27]. Since some of the eigenvalues were complex valued, only the most negative (or zero) real parts of the eigenvalues were used for further analysis. The most negative value of the eigenvalues was restricted to \(-2\).
FIG. 3. (Color) (a) A representative example of a 2D ΔAPD map demonstrating discordant APD alternans at BCL of 150 ms (top) and corresponding 2D $\lambda_{4}$ map (bottom). Blue (red) color in ΔAPD map indicates positive (negative) phases of discordant alternans. (b) The correlation between the amplitude of alternans, ΔAPD, and the value of $\lambda_{4}$, for both positive and negative phases of discordant APD alternans.

2D $\lambda_{4}$ and $\lambda_{2}$ maps were constructed for each BCL, similar to 2D ΔAPD maps. The $B_{\text{onset}}$ measurements and information about the two regions of the heart, 1:1 and 1:1alt, were taken from the 2D ΔAPD maps and projected to 2D eigenvalue maps. The eigenvalues $\lambda_{4}$ and $\lambda_{2}$ were used for eigenvalue histogram generation for each BCL, separately for 1:1 and 1:1alt regions. Similarly, 2D $\lambda_{2}$ maps were used to generate $\lambda_{2}$ values.

E. Statistical analysis

The mean values and standard errors for all parameters were calculated and averaged separately for 1:1 and 1:1alt regions of the heart. Group data are presented as the mean ± standard error. We performed statistical comparisons between the two regions in the heart using a two-sample $t$ test, and between different rabbits using analysis of variance (ANOVA) (Origin Software, Northampton, MA). Values of $p<0.05$ were considered statistically significant.

III. RESULTS

The spatiotemporal evolution of alternans in the RV of the heart is presented in Fig. 2(a) where 2D ΔAPD (alternans) maps are shown for different values of BCLs. In Fig. 2(a), the white color corresponds to 1:1 behavior, while the red and blue colors show alternans with different phases. Note that the local onset of APD alternans occurred at $B_{\text{onset}} = 170$ ms [see the red box in Fig. 2(a)] and alternans occupied the entire surface of the heart as the BCL was progressively decreased. Note the formation of spatially discordant alternans (SDA) at lower BCL. The local onset of alternans in the heart has
FIG. 5. Mean eigenvalue \( \tilde{\lambda}_{4} \) as a function of BCLs, corresponding to Fig. 4, calculated separately for 1:1 (filled circles) and 1:1alt (open circles) regions. The local onset of alternans, \( B^{\text{onset}} = 170 \) ms, is marked with the dashed line. * denotes statistical significance \((p<0.05)\).

been described in our previous studies \([19]\). The representative traces from a single pixel at \( B^{\text{onset}} \) illustrating 1:1 behavior (black) and alternans (blue and red) are shown in Fig. 2(b).

Figure 2(c) represents the spatial distribution of dominant negative eigenvalues \( \lambda_{4} \) that were calculated using the \( Z_{4} \) matrix, for the same values of BCLs as the 2D APD maps in Fig. 2(a). Note that only nonpositive \( \lambda_{4} \) are present and are restricted to the \([-2,0]\) range. The local onset of APD alternans, \( B^{\text{onset}} = 170 \) ms, is shown in Fig. 2(c) as a red box. Note the visual correlation between the APD alternans and \( \lambda_{4} \), especially at small BCLs.

Figure 3 shows the 2D \( \Delta \)APD and corresponding \( \lambda_{4} \) maps [panel (a)] and the correlation between the amplitude of the SDA with different phases (red and blue) and \( \lambda_{4} \) [panel (b)]. The data are taken from a different representative experiment for which the \( B^{\text{onset}} = 180 \) ms, and SDA occurs at a BCL of 150 ms. Note that \( \lambda_{4} \) has a normal distribution around the value of \(-1\) for both phases of SDA, suggesting that the phase of alternans does not affect the eigenvalue.

Figure 4(a) shows the definitions of 1:1 and 1:1alt regions of the heart for the 2D \( \Delta \)APD maps from the experiment illustrated in Fig. 2(a). Specifically, at the spatial local onset of alternans, i.e., at BCL \( B^{\text{onset}} = 170 \) ms, the region without alternans (1:1) and the region with alternans (1:1alt) are defined (see the black dashed outlines) and back-projected on the previous BCLs. Figure 4(b) shows corresponding 2D \( \lambda_{4} \) maps calculated using the \( Z_{4} \) matrix. The 1:1 and 1:1alt regions on the 2D \( \lambda_{4} \) maps are taken from the 2D \( \Delta \)APD maps to visually illustrate the spatial distribution of eigenvalues in these regions up to \( B^{\text{onset}} \). Figure 4(c) shows the histograms of eigenvalues \( \lambda_{4} \) separately for the 1:1 (black) and 1:1alt (red) regions at different BCLs. Note the pronounced narrowing of the histogram and clustering around a mean value of \(-0.84 \pm 0.01\) as we approach \( B^{\text{onset}} \) in the 1:1alt region. Similar but less pronounced behavior occurs in the 1:1 region.

FIG. 6. Comparison between eigenvalues \( \tilde{\lambda}_{4} \) (open circles) and \( \tilde{\lambda}_{2} \) (closed circles) calculated separately for 1:1 region (a) and 1:1alt region (b). The local onset of alternans, \( B^{\text{onset}} = 170 \) ms, is marked with the dashed line. (c) Average eigenvalues \( \langle \tilde{\lambda}_{4} \rangle \) and \( \langle \tilde{\lambda}_{2} \rangle \) from all \( n = 4 \) experiments calculated separately for 1:1 (filled bar) and 1:1alt (open bar) regions. * indicates statistical significance \((p<0.05)\).
with some clustering around the mean value of $-0.73 \pm 0.01$ as we approach $B^\text{reset}$.

The mean values of the eigenvalues $\lambda_{[4]}$ from Fig. 4(b) are shown in Fig. 5 for different BCLs for both 1:1 (filled circles) and 1:1$_{alt}$ (open circles) regions. Note that the mean eigenvalue $\bar{\lambda}_{[4]}$ decreases as BCL decreases for both regions, although the differences are not statistically significant between 1:1 and 1:1$_{alt}$ regions until the onset of alternans, i.e., $B^{\text{reset}} = 170$ ms. At $B^{\text{reset}}$, the mean eigenvalues $\bar{\lambda}_{[4]}$ are statistically different ($p < 0.05$) between the two regions (indicated by * in Fig. 5). These results indicate that the eigenvalues $\bar{\lambda}_{[4]}$ can predict the local onset of APD alternans in the heart.

We further evaluated whether the eigenvalues $\hat{\lambda}_{[2]}$ calculated using the matrix $Z_{[2]}$, i.e., two values of APD, can be used to predict the local onset of alternans in the heart. As a representative example, we used an experiment shown in Fig. 2. Figure 6 illustrates the mean eigenvalues $\lambda_{[2]}$ (filled circles) and $\bar{\lambda}_{[2]}$ (open circles) as functions of the BCL for both for 1:1 [panel (a)] and 1:1$_{alt}$ [panel (b)] regions. Our results indicate that for both regions, the two methods give statistically similar eigenvalues at all BCLs.

Next, we evaluated whether the mean eigenvalues $\bar{\lambda}_{[2]}$ and $\hat{\lambda}_{[2]}$ calculated from all our experiments ($n = 4$) can predict the local onset of alternans. The values of $\bar{\lambda}_{[2]}$ and $\hat{\lambda}_{[2]}$ calculated at the onset of alternans, $B^{\text{reset}}$, are shown in Fig. 6(c) separately for the 1:1 (filled) and 1:1$_{alt}$ (open) regions. Note that the mean eigenvalues $\bar{\lambda}_{[2]}$ were significantly different between the 1:1 (−0.76 ± 0.02) and 1:1$_{alt}$ (−0.84 ± 0.01, $p < 0.05$) regions. On the other hand, the mean eigenvalues $\hat{\lambda}_{[2]}$ did not reach statistical significance (1:1: $-0.82 \pm 0.03$, and 1:1$_{alt}$: $-0.76 \pm 0.04$, $p$ is not significant).

IV. DISCUSSION AND CONCLUSIONS

Our results demonstrate that the recently developed technique that estimates the stability of the system based on calculation of the most dominant eigenvalues from a time series of readily measurable quantities can be applied to predict alternans formation at the whole heart level. Specifically, we demonstrated that the mean dominant eigenvalues decrease as the BCL decreases, and approach the theoretically predicted value of $-1$ for both 1:1 and 1:1$_{alt}$ regions of the heart. At $B^{\text{reset}}$, however, the eigenvalues were statistically different between the 1:1 and 1:1$_{alt}$ regions, allowing us to predict local onset of alternans in the heart. We also demonstrated that eigenvalue calculations that were based on four APD measurements ($\lambda_{[4]}$) were more robust against experimental noise than those based on two ($\lambda_{[2]}$).

When a dynamical system is perturbed from its stable state, usually a small number of eigenvalues are associated with the induced transient dynamics. When the underlying model is known, these eigenvalues can be determined analytically or numerically using finite-difference methods [26]. However, underlying models may not be available. Even if they are available, these models may not describe the dynamics completely. In such a case, the eigenvalues, which govern the stability of the system, may be derived by measuring the output responses of these systems after perturbing them from their stable states [27]. Specifically, this work demonstrates that the transition to alternans can be predicted in a numerical model of isolated cardiac myocytes. However, in a more general sense, this work is based on the theorem of state-space reconstruction, which is valid for both simple dynamical systems (i.e., isolated cardiac myocytes) as well as complex spatiotemporal systems (i.e., hearts). For example, Sugihara et al. [32] recently demonstrated that the state-space reconstruction technique could be applied to both the simple Lorenz oscillator and to complex ecological systems.

Instabilities in the heart are associated with ventricular fibrillation, which is a chaotic spatiotemporal organization of electrical activity in the heart. Ventricular fibrillation is preceded by APD alternans at the whole heart level, which in turn originates from APD alternans at the cellular level. It has been previously demonstrated that the formation of APD alternans in the heart is a complex spatiotemporal process characterized by a local spatial onset of alternans [19,31,33]. We must also take into account that the resolution of our optical mapping system allows us to record an average electrical activity from a multitude of cardiac myocytes in one pixel. Therefore, all eigenvalue calculations that were performed in this study for individual pixels already contain averaged electrical activity from individual cells. Despite this complexity, we demonstrated that the dominant eigenvalue approach can be used to predict local alternans formation in the heart.

Stability analysis using eigenvalues of physiological systems has been done previously by studying their physical analogs like the swinging heart analyzed by the forced spring pendulum [34] and the respiratory control system [35]. This dominant eigenvalue technique provides an alternative technique to assess the likelihood of alternans formation in the heart since the eigenvalue associated with it approaches $-1$ as we reach $B^{\text{reset}}$. It may be used with changes to guide the development of a method to detect fibrillatory tendencies in the heart by considering the durations of different segments of the ECG, instead of the APDs, as significant state-space variables governing the stability of the heart.

There are several limitations of this study that we would like to point out. First, we stated that our aim is to use the dominant eigenvalue technique to predict alternans formation at the whole heart level. Indeed, the optical mapping experiments were performed in the whole isolated rabbit heart, but actually we recorded electrical activity only from the epicardial surface of the heart, not the entire heart. In addition, the APD measurements and dominant eigenvalue calculation were ultimately performed at the level of pixels. While the eigenvalue analysis is based on local measurements, we construct the spatial patterns of alternans and their corresponding eigenvalues from recordings at multiple pixels. Other challenges may come from strong heterogeneities in the heart. In that case, analyses of local pixels may not reveal the true dynamical state of the heart and one should resort to more rigorous, global analysis.

One more limitation of our study is that our experiments were designed to use only $N = 4$ stimuli after perturbation. This placed an upper limit on the number of APD measurements that can be used for eigenvalue calculations, i.e., $m = 1, \ldots, M \leq N$. Therefore, we decided to compare eigenvalues that are calculated using either four ($\lambda_{[4]}$) or two ($\lambda_{[2]}$) APD measurements. Our results demonstrate that
although both $\lambda_{[4]}$ and $\lambda_{[2]}$ are similar for all BCLs, only $\lambda_{[4]}$ was able to achieve statistical significance between the 1:1 and 1:1alt regions at $B_{\text{onset}}$. We believe that this result indicates that using more APD measurements increases the robustness of the technique by reducing the effect that noise may have on the eigenvalue calculation, at least when applied to experimental data. However, more experiments are necessary to provide more detailed analysis of the optimal dimensionality of the state space and to further optimize the technique.

Due to the above-mentioned experimental limitations, we may not be able to accurately estimate the dominant eigenvalues at each pixel, or the calculated eigenvalues may not accurately represent the dynamic state of the corresponding pixel. To more accurately estimate the eigenvalues, we may need to more carefully control the experiments and/or to resort to more rigorous numerical methods.

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