Method of unconfounding orientation and direction tunings in neuronal response to moving bars and gratings

Jun Zhang

Department of Psychology, University of Michigan, Ann Arbor, Michigan 48109

When an oriented bar or grating is drifted across the receptive field of a cortical neuron at various orientations, the tuning function reflects both, and thus confounds the orientation (ORI) and the direction-of-motion (DIR) selectivity of the cell. Since ORI (or DIR), by definition, has a period of 180(or 360) deg/cycle, a popular method for separating these two components, due to Wörgötter and Eysel [Biol. Cybern. **57**, 349 (1987)], is to Fourier decompose the neuron's response along the angular direction and then identify the first and the second harmonic with DIR and ORI, respectively (the SDO method). Zhang [Biol. Cybern. **63**, 135 (1990)] pointed out that this interpretation is misconceived—all odd harmonics (not just the first harmonic) reflect the DIR component, whereas all even harmonics (including the second harmonic) contain contributions from both DIR and ORI. Here, a simplified procedure is proposed to accomplish the goal of unconfounding ORI and DIR. We first construct the sum of all odd harmonics of the overall tuning curve, denoted ODDSUM, by calculating the difference in the neuronal response to opposite drifting directions. Then we construct ODDSUM+|ODDSUM| and identify it with DIR (here |·| denotes the absolute value). Subtracting DIR, that is ODDSUM+|ODDSUM|, from the overall tuning curve gives ORI. Our method ensures that (i) the reconstructed DIR contains only one, positive peak at the preferred direction and can have power in all harmonics, and (ii) the reconstructed ORI has two peaks separated by 180° and has zero power for all odd harmonics. Using this procedure, we have unconfounded orientation and direction components for a considerable sample of macaque striate cortical cells, and compared the results with those obtained using Wörgötter and Eysel's SDO method. We found that whereas the estimate of the peak angle of ORI remains largely unaffected, Wörgötter and Eysel's method considerably overestimated the relative strength of ORI. To conclude, a simple method is provided for appropriately separating the orientation and directional tuning in a neuron's response that is confounded as a result of the use of drifting oriented stimuli. © 2005 Optical Society of America

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1. INTRODUCTION

When oriented stimuli, such as bars or gratings, are drifted across a neuron's receptive field at various angles to probe the neuron's response property, the tuning curve obtained will reflect both the orientation selectivity and direction-of-motion selectivity of the neuron. Since these two properties arise from very different underlying processes, it would be desirable to separate ("unconfound") these selectivities in the neuron's response. However, unless one makes *a priori* assumptions about the functional form of the orientational and directional tuning, it is difficult to decompose the overall tuning curve into one representing orientation and another representing direction.

A. SDO Analysis
A method that has become increasing popular among neurophysiologists is SDO analysis proposed by Wörgötter and Eysel based on the technique of Fourier analysis.[1] Observing that the orientation component and the direction component, by definition, have periods of 180 and 360 deg/cycle, respectively, these authors suggested quite originally to Fourier analyze the overall tuning curve using the fundamental period of $360°(2\pi)$, and then to identify the first harmonic to be the directional component and the second harmonic to be the orientation component. They dubbed this method SDO analysis, where S refers to

the zeroth harmonic (DC component) in the Fourier decomposition, D refers to the first harmonic identified as the directional tuning because of its 360° periodicity, and O refers to the second harmonic identified as the orientational tuning because of its 180° periodicity. Let $R(\theta)$ denote a cell's overall (i.e., orientational plus directional) tuning curve. It represents the cell's response, in terms of spike rate, to the polar angle θ of a drifting bar or grating that might activate the cell through either an orientation or a direction mechanism, or both. The decomposition used in the SDO analysis can be formally written as

$$R(\theta) = S + D(\theta) + O(\theta).$$

Performing Fourier transform of $R(\theta)$ along the angular dimension, the coefficients of the first two harmonics are given by ($l = 1, 2$)

$$\eta_l = \frac{1}{\pi}\sum_k R(\theta_k)\cos(l\,\theta_k), \quad \zeta_l = \frac{1}{\pi}\sum_k R(\theta_k)\sin(l\,\theta_k).$$

According to Wörgötter and Eysel,[1] the first harmonic (η_1, ζ_1) is identified with the direction component $D(\theta)$ and the second harmonic (η_2, ζ_2) with the orientation component $O(\theta)$. For the former, the peak angle θ_d and the strength r_d are given by

$$\theta_d = \arctan(\zeta_1/\eta_1), \quad r_d = r_1 = \sqrt{(\zeta_1)^2 + (\eta_1)^2}, \qquad (1)$$

whereas for the latter, the peak angle θ_o and the strength r_o are calculated as

$$\theta_o = \arctan(\zeta_2/\eta_2), \quad r_o = r_2 = \sqrt{(\zeta_2)^2 + (\eta_2)^2}. \qquad (2)$$

The SDO analysis has been applied to several data sets by the original authors,[2–4] and has since become quite popular.[5–19] Two main advantages of the SDO method are that (i) it does not appear to make any *a priori* assumptions about the shapes of the direction and the orientation tuning curves (which we will show below not to be the case), and (ii) it is relatively easy and straightforward to implement. Many researchers used this method to determine, in particular, the contribution of the orientation component to a neuron's overall response. For example, Weliky and Katz[9] reported that orientation selectivity in primary visual cortex (V1) of ferrets was substantially weakened, as determined by extracellular single-unit recording, by chronic electric stimulation of the optic nerve during their early, postnatal visual development. Their claim was based on an assessment of orientation tuning of V1 neurons in both the artificially stimulated and the unstimulated control animals using high-contrast bars that drifted at various orientations across the receptive fields of recorded neurons. Central to Weliky and Katz's data analysis was the use of an orientation selectivity index (OSI), given as the amplitude of the second harmonic divided by the average firing value (zeroth harmonic or DC term), for quantifying the degree of orientation selectivity (see Figs. 2 and 3 of Ref. 9).

The SDO method is equivalent to the vector sum method used by other researchers for analyzing a circular distribution of neuronal responses.[20–24] There, the preferred direction θ_d is calculated as the vectorial sum of $R(\theta)$ at each angle θ with the preferred orientation θ_o as the vectorial sum of $R(\theta)$ but with its angles being doubled because of its 180° periodicity. In complex number notation

$$\exp(i\theta_d) \propto \sum_k R(\theta_k)\exp(i\theta_k)$$

$$= \sum_k [R(\theta_k)\cos(\theta_k) + iR(\theta_k)\sin(\theta_k)] \propto \eta_1 + i\zeta_1,$$

$$\exp(i2\theta_o) \propto \sum_k R(\theta_k)\exp(i2\theta_k)$$

$$= \sum_k [R(\theta_k)\cos(2\theta_k) + iR(\theta_k)\sin(2\theta_k)] \propto \eta_2 + i\zeta_2.$$

Therefore, the SDO method can also be seen as a formal description of the vector sum method, a descendant of "circular analysis" (see, e.g., Ref. 25).

B. Critique of SDO Analysis

Despite its popularity, there is a severe defect in SDO analysis, as first pointed out by Zhang.[26] Specifically, the identification of the first harmonic of the Fourier series with the direction component (to be abbreviated as DIR below) and its second harmonic with the orientation component (to be abbreviated as ORI below) was mathematically unwarranted. In the Fourier decomposition of the overall tuning curve $R(\theta)$, there are more than just the first two harmonics (in addition to the zeroth harmonic or DC component which we absorb into ORI). It turns out that ORI, having a period of 180 deg/cycle, contributes to all even harmonics (not just the second harmonic), whereas DIR, having a period of 360 deg/cycle, contributes to both even and odd harmonics (not just the first harmonic).[27] In other words, all the odd harmonics reflect the DIR component, whereas all the even harmonics contain contributions not just from ORI but also from DIR. In that regard, the problem with SDO analysis is twofold: (1) it ignores the contribution of DIR to the second harmonic, and (2) it ignores all higher-order (>2) harmonics[28] in the Fourier decomposition of $R(\theta)$. Even if the latter (i.e., ignoring higher harmonics) may be justified as simply obtaining a first approximation to solving the unconfounding problem, the former (i.e., ignoring the contribution of DIR to the second harmonic) will even affect, unavoidably, such first approximation to ORI.

The deficiency of the SDO method becomes apparent if one is reminded of the following mathematical fact: Any periodic function is made up of a *series* of Fourier harmonics, not just the first (fundamental) harmonic; the only function that does not carry harmonics of order ≥ 2 in its Fourier decomposition is the sinusoidal function (sine or cosine). In other words, the only situation when the SDO analysis would work (that is, successfully decompose the tuning curve into an ORI and a DIR component) is when the cell's tuning curve has the form[29]:

$$R(\theta) = r_0 + \sum_{l=1}^{2} [\eta_l \cos(l\theta) + \zeta_l \sin(l\theta)].$$

Equivalently, the above expression can be cast as [using Eqs. (1) and (2)]

$$R(\theta) = r_0 + r_d \cos(\theta - \theta_d) + r_o \cos[2(\theta - \theta_o)],$$

where both the DIR component (second term, with strength r_d) and the ORI component (third term, with strength r_o) must be sinusoidally tuned, not merely with respective periods of 360 and 180 deg/cycle. Therefore, to the extent that directional tuning is not sinusoidal, the Fourier decomposition of the DIR component *by itself* will contain first-, second-, and all higher-order harmonics. That neurons in V1 have much narrower direction tuning than would be accounted for by a sinusoidal function has been well established (see, e.g., Ref. 30).

The proper procedures of applying Fourier analysis to unconfound orientational and directional contributions were discussed in detail in Ref. 26. The highlight of that mathematical analysis was to show that, assuming additivity of the ORI and DIR components, all odd harmonics of the Fourier series are contributed to by DIR, whereas all even harmonics contain contributions from *both* DIR and ORI. DIR may contribute to all harmonics because it is of the same periodicity as $R(\theta)$, whereas ORI contributes only to the even harmonics because it has twice the periodicity of $R(\theta)$. In Ref. 26, Zhang proposed first to use odd harmonics individually to estimate both the peak and the bandwidth of DIR, and then to subtract out DIR's contamination of the even harmonics before finally estimat-

ing ORI using the "corrected" even harmonics. The only assumption made there was that ORI and DIR are linearly additive (an assumption implicitly made as well by Wörgötter and Eysel in Ref. 1), the validity of which can be checked against the data within the framework itself.

C. Improved Method

The procedure outlined in Ref. 26, however, has a drawback: Unlike the SDO method, it is difficult to implement because it involves regression analysis of the peak angle of a periodic function from all of its individual harmonics. This practical inconvenience prompted us to look for an easily implemented method to separate the orientation and direction tunings while still correcting for the latter's contamination in the estimate of the former.

We note that Li *et al.*[31] also realized that SDO analysis inappropriately truncated the Fourier series to the first two harmonics, and proposed to identify the sum of all odd harmonics as DIR and the sum of all even harmonics as ORI. If this were the case, then DIR need not be as broadly tuned as a sinusoidal function while still *not* contaminating the even harmonics. However, as we will demonstrate, the proposal in Ref. 31 amounts to assuming that DIR has the shape of an antisymmetric function with both a positive peak and a negative peak separated by 180°; this is unrealistic in view of empirical findings about directional selectivity in V1 cells that seldom manifest the negative inhibitory peak.[30]

As an improvement over Ref. 31, we now propose to rectify this antisymmetric sum-of-odd-harmonics to get rid of its negative lobe and retain only the positive one, and to identify the resulting single-peaked function as the directional tuning. Specifically, after Fourier decomposing the overall tuning curve $R(\theta)$, we will form the sum of all its odd harmonics, denoted below as $G(\theta)$ (also called ODDSUM). But, instead of identifying $G(\theta)$ as the direction component, as Ref. 31 did, we define it as[32]

$$\text{DIR}(\theta) = G(\theta) + |G(\theta)|, \qquad (3)$$

where $|\cdot|$ denotes the absolute value. Equivalently,

$$\text{DIR}(\theta) = \begin{cases} 2G(\theta) & \text{if } G(\theta) \geqslant 0 \\ 0 & \text{if } G(\theta) < 0 \end{cases}.$$

So our proposed method differs from the method of Ref. 31 in the presence of the $|G(\theta)|$ term in Eq. (3), which causes $\text{DIR}(\theta)$ to have a positive peak only. The contamination of DIR by the even harmonics, from our current model, is given by $|G(\theta)|$, a function with a period of 180 deg/cycle, and can be removed easily since $G(\theta)$ is computed straightforwardly as

$$G(\theta) = \frac{1}{2}[R(\theta) - R(\theta + \pi)]. \qquad (4)$$

The orientation component $\text{ORI}(\theta)$, which is taken to include the DC value as well, can then be constructed based on the linearity assumption, namely, that $R(\theta)$ is a linear sum of DIR and ORI:

$$\text{ORI}(\theta) = R(\theta) - \text{DIR}(\theta) = R(\theta) - G(\theta) - |G(\theta)|. \qquad (5)$$

In a nutshell, our proposed method decomposes $R(\theta)$ into a 360°-periodic directional component $\text{DIR}(\theta)$ and a 180°-periodic orientation component $\text{ORI}(\theta)$ according to

$$\text{DIR}(\theta) = \frac{1}{2}[R(\theta) - R(\theta + \pi) + |R(\theta) - R(\theta + \pi)|], \qquad (6)$$

$$\text{ORI}(\theta) = \frac{1}{2}[R(\theta) + R(\theta + \pi) - |R(\theta) - R(\theta + \pi)|]. \qquad (7)$$

This method was first presented in an abstract form (Ref. 33) to which R. L. De Valois contributed as a coauthor.

2. METHODS

The cells being analyzed in this report were taken from a previous study (Ref. 34) intended for a different purpose. Briefly, in that study, macaque monkeys (*Macaca mulatta* and *M. fascicularis*) were anesthetized and paralyzed following surgery while electrocardiogram, electroencephalogram, blood temperature, and expired CO_2 were monitored continuously. Single cell recordings were made within the central 5° visual angle, and action potentials (spikes) were recorded with a temporal resolution of 1 ms. Visual stimuli were generated and controlled by a Sun/TAAC image processor (Sun Microsystems, Mountain View, Calif.), and presented on an NEC monitor (Nippon Electric, Tokyo) with a spatial resolution of 1024 × 900 pixels, a 66 Hz refresh rate, and a mean luminance of 70 cd/m^2. Drifting sinusoidal gratings were used to determine the cell's spatial and temporal frequency as well as orientational/directional tunings. Those cells were subsequently tested with other stimuli for a different purpose (hereby omitted) as reported in Ref. 34, their response to drifting sinusoidal gratings, which served as initial characterization of cells in Ref. 34, will be analyzed in detail in this report.

A. Rationale for Our Procedures

For a given cell, its response (in terms of average spike rate) to a sinusoidal grating drifting at angle θ is denoted $R(\theta)$. A series of drifting angles, $\theta_k (k=1,2,\ldots,N)$, in equal steps of $360°/N$, were used; here we assume N to be an even natural. For instance, $N=12$ indicates that the drifting angles are 30° apart. The resulting $R(\theta)$, where θ takes on the sampling values of $\theta_k = k/N \times 360°$, will be referred to as the overall tuning curve; it contains both a directional (DIR) and an orientational (ORI) component. Since $R(\theta)$ is 360°-periodic, one can decompose it into the Fourier series

$$R(\theta) = \eta_0 + \sum_{l=1}^{N/2-1} [\eta_l \cos(l\theta) + \zeta_l \sin(l\theta)] + \eta_{N/2}, \qquad (8)$$

where $\eta_l, \zeta_l (l=1,2,\ldots,N/2-1)$ are the Fourier coefficients given by

$$\eta_l = \frac{1}{\pi} \sum_{k=1}^{N} R(\theta_k) \cos(l\theta_k), \quad \zeta_l = \frac{1}{\pi} \sum_{k=1}^{N} R(\theta_k) \sin(l\theta_k),$$

with

$$\eta_0 = \frac{1}{\pi}\sum_{k=1}^{N} R(\theta_k), \quad \eta_{N/2} = \frac{1}{\pi}\sum_{k=1}^{N} (-1)^k R(\theta_k).$$

It is Fourier's theorem that guarantees the existence and uniqueness of Eq. (8) for any periodic function $R(\theta)$.

Consider the partial sum of all odd harmonics, $G(\theta)$ (also called ODDSUM), defined as

$$G(\theta) = \sum_{l=1,3,\dots} [\eta_l \cos(l\theta) + \zeta_l \sin(l\theta)]. \tag{9}$$

It follows from Eq. (8) that $G(\theta)$ can be computed simply by taking the difference of the values of the overall tuning curve $R(\cdot)$ at opposite drifting directions θ and $\theta+\pi$; see Eq. (4). Obviously $G(\theta+\pi)=-G(\theta)$, that is, $G(\theta)$ is an antisymmetric function within any 180° subinterval, and will have both a positive peak/lobe and a negative peak/lobe in the full period of 360°. Consequently, the function $|G(\theta)|$, which contains (at least) two positive peaks/lobes, is 180°-periodic, since $|G(\theta+\pi)|=|G(\theta)|$. In its Fourier decomposition under the fundamental period of 360 deg/cycle, only even harmonics are present:

$$|G(\theta)| = \sum_{l=2,4,\dots} [\alpha_l \cos(l\theta) + \beta_l \sin(l\theta)], \tag{10}$$

where, for $l=2, 4, \dots$

$$\alpha_l = \frac{1}{\pi}\sum_{k=1}^{N} |G(\theta_k)|\cos(l\theta_k), \quad \beta_l = \frac{1}{\pi}\sum_{k=1}^{N} |G(\theta_k)|\sin(l\theta_k).$$

Our method reconstructs the direction tuning curve $\mathrm{DIR}(\theta)$ according to Eq. (3) and the orientation tuning curve $\mathrm{ORI}(\theta)$ according to Eq. (5). Such construction has the property that the odd harmonics of $\mathrm{DIR}(\theta)$ are those of $G(\theta)$, while the even harmonics of $\mathrm{DIR}(\theta)$ are those of $|G(\theta)|$:

$$\mathrm{DIR}(\theta) = \sum_{l=1,3,\dots} [\eta_l \cos(l\theta) + \zeta_l \sin(l\theta)]$$
$$+ \sum_{l=2,4,\dots} [\alpha_l \cos(l\theta) + \beta_l \sin(l\theta)]. \tag{11}$$

Such a form not only makes DIR contain only one positive peak but also ensures that all of its odd harmonics are the odd harmonics in the Fourier decomposition of $R(\theta)$. It follows, from Eqs. (5) and (10), that

$$\mathrm{ORI}(\theta) = \eta_0 + \sum_{l=2,4,\dots} [\eta_l' \cos(l\theta) + \zeta_l' \sin(l\theta)], \tag{12}$$

where

$$\eta_l' = \eta_l - \alpha_l, \quad \zeta_l' = \zeta_l - \beta_l, \quad \text{for } l=2,4,\dots.$$

That is, $\mathrm{ORI}(\theta)$ indeed contains even harmonics only. It is not difficult to show that Eqs (11) and (12) are equal to Eqs. (6) and (7), respectively.

B. Estimate of Peak and Strength of the Tuning function
Our procedures for computing peak tuning and strength of orientation and direction components can be implemented as follows:

Step 1. Calculate

$$\eta_1 = \frac{1}{\pi}\sum_{k=1}^{N} R(\theta_k)\cos(\theta_k), \quad \zeta_1 = \frac{1}{\pi}\sum_{k=1}^{N} R(\theta_k)\sin(\theta_k);$$

$$\eta_2 = \frac{1}{\pi}\sum_{k=1}^{N} R(\theta_k)\cos(2\theta_k), \quad \zeta_2 = \frac{1}{\pi}\sum_{k=1}^{N} R(\theta_k)\sin(2\theta_k).$$

Step 2. Calculate

$$\alpha_2 = \frac{1}{2\pi}\sum_{k=1}^{N} |R(\theta_k) - R(\theta_k + \pi)|\cos(2\theta_k),$$

$$\beta_2 = \frac{1}{2\pi}\sum_{k=1}^{N} |R(\theta_k) - R(\theta_k + \pi)|\sin(2\theta_k).$$

Step 3. Calculate

$$\eta_2' = \eta_2 - \alpha_2, \quad \zeta_2' = \zeta_2 - \beta_2.$$

Step 4. The peak angle θ_d and the strength r_d of DIR can be estimated as

$$\theta_\mathrm{d} = \arctan(\zeta_1/\eta_1), \quad r_\mathrm{d} = \sqrt{(\zeta_1)^2 + (\eta_1)^2},$$

which is the same as Eq. (1); and the peak angle θ_o and the strength r_o of ORI can be estimated as

$$\theta_\mathrm{o} = \arctan(\zeta_2'/\eta_2'), \quad r_\mathrm{o} = \sqrt{(\zeta_2')^2 + (\eta_2')^2}. \tag{13}$$

The relative strength γ of the orientation tuning to direction tuning of a cell is indexed by

$$\gamma = r_\mathrm{o}/r_\mathrm{d}.$$

Remark: With reference to Eqs. (1) and (2), the estimate for DIR remains the same, while our current method
provides a correction term for estimating ORI (both its peak angle and strength).

3. RESULTS

For illustrative purposes, let us apply our method to a typical cell with step-by-step explanations (see Fig. 1). The response of this cell to a grating drifting at a series of angles is plotted in Fig. 1(a), where the drifting angles are in increments of 15°. This is the overall tuning curve $R(\theta)$. Our first step is to calculate the sum of odd harmonics $G(\theta)$ by using Eq. (4), that is, by shifting the overall tuning curve by 180° and then subtracting it from the original tuning curve. The result is shown as Fig. 1(b). By our construction, $G(\theta)$ (denoted ODDSUM) is antisymmetric, with both a positive peak and a negative peak. Next, we take the absolute value $|G(\theta)|$ *pointwise*, that is, at each θ value, and plot $|G(\theta)|$ in Fig. 1(c). Note that $|G(\theta)|$ (i.e., |ODDSUM|) necessarily has a period of 180° Next, we construct $\mathrm{DIR}(\theta)$ by adding $G(\theta)$ and $|G(\theta)|$, i.e., superimposing the graph of Fig. 1(b) with the graph of Fig. 1(c). This gives rise to the graph in Fig. 1(e), namely the direction component $\mathrm{DIR}(\theta)$. For comparison, we also present the sum of all even harmonics (plus the DC term) as a graph in Fig. 1(d). This, however, is *not* the orientation component $\mathrm{ORI}(\theta)$, because it contains the contamination from
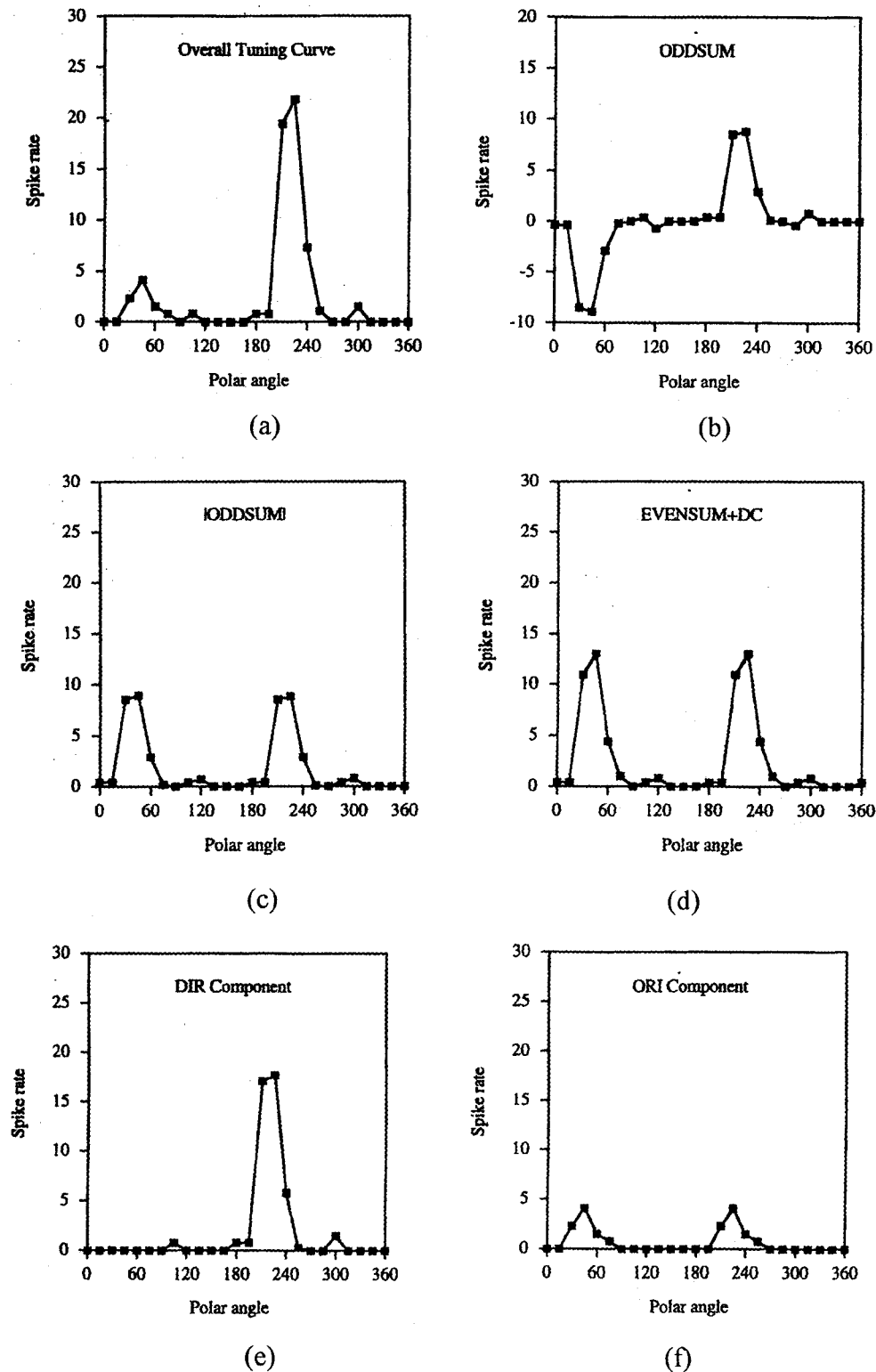
Fig. 1.   Illustration of the method of separating the DIR and ORI components, using a typical cell. (a) Overall tuning curve $R(\theta)$ of the cell, plotting its spike rate response (in ordinate) as a function of drifting angles θ (in abscissa) of a sinusoidal grating stimulus. Drifting angles are in 15° increments. (b) ODDSUM, denoted $G(\theta)$ in the text, is the sum of all odd Fourier harmonics of $R(\theta)$. It can be easily calculated by shifting $R(\theta)$ by 180°, then subtracting it from the unshifted $R(\theta)$, and dividing by 2. Note the antisymmetric shape of ODDSUM, and the presence of both the positive and the negative peak in it. (c) |ODDSUM|, denoted $|G(\theta)|$ in the text, is the point-by-point absolute value of ODDSUM, obtained by flipping all negative values of the curve in (b). (d) The sum of all even Fourier harmonics of $R(\theta)$ plus the zeroth harmonic (the DC term). (e) Recovered DIR component, by adding the graph in (b) to the graph in (c). (f) Recovered ORI component, by subtracting DIR [graph in (e)] from $R(\theta)$ [graph in (a)]. Note that the graphs in (b) and (d) sum to the graph in (a); this is the Fourier decomposition of the cell's overall tuning curve. Note also that the graphs in (e) and (f) sum to the graph in (a); this is the decomposition of the cell's tuning curve into DIR and ORI. These graphs clearly show that ODDSUM $\neq$ DIR, EVENSUM $\neq$ ORI, contrary to the claim of Li *et al.*[31]

the even harmonics of $DIR(\theta)$, modeled here as $|G(\theta)|$. Subtracting the graph of Fig. 1(c) from the graph of Fig. 1(d) yields the graph of Fig. 1(f), which is taken to be the orientation component $ORI(\theta)$ (including the DC term). Note that the orientation component is much smaller in magnitude compared with the sum of all even harmonics (denoted EVENSUM) in Fig. 1(d), both of period 180°, due to directional contamination shown as Fig. 1(c).

Our procedures are applied to 149 cells recorded in the primary visual cortex of the monkey (see Section 2). Figure 2 shows the results of 12 sample cells, with the ordinate representing the polar angles of the drifting grating, and the abscissa the spike rates. In each panel, the cell's overall tuning curve $R(\theta)$ is represented by squares connected by solid lines. The recovered direction component $DIR(\theta)$ is represented by diamonds connected by dotted lines. The recovered orientation component $ORI(\theta)$ is represented by circles connected by dashed lines. One can easily observe that $DIR(\theta)$ is single-peaked with a period of 360°, whereas $ORI(\theta)$ is double-peaked with a period of 180°. For each panel, the sum of $DIR(\theta)$ and $ORI(\theta)$ equals the overall tuning curve, $R(\theta)$.

The strength r_d of the directional tuning can be characterized by the amplitude r_1 of the first harmonic in the Fourier expansion of $DIR(\theta)$ using a fundamental frequency of 360 deg/cycle, given by Eq. (1). Likewise, the strength r_o of the orientational tuning can be characterized by the amplitude of the first harmonic in the Fourier expansion of $ORI(\theta)$ using the fundamental frequency of 180 deg/cycle. However, this does not equal the amplitude r_2 of the second harmonic in the Fourier decomposition of $R(\theta)$, given by Eq. (2); in fact the whole point of this paper is to correct this misunderstanding arising from Ref. 1. The strength of the orientation tuning, after the correction term, should be r_o given by Eq. (13). The ratio $\gamma = r_o/r_d$, which measures the relative strength of ORI and DIR, can be calculated. Figure 3 plots, in the form of a scatter diagram, Fig. 3(a), and in the form of a histogram, Fig. 3(b), the distribution of the relative strengths, in log coordinates, for the population of 149 cells, when the orientation components are calculated using either the method advanced in this paper (solid bars, "with correction") or the SDO method (stitched bar, "without correction"). The plots clearly show that the strength of ORI is overestimated by SDO analysis: The second harmonic (and all even harmonics) indeed tend to be contributed by DIR as well.

To appreciate why SDO analysis overestimated the orientation tuning, we plot, in Fig. 4(a), the second harmonic of the recovered direction tuning $DIR(\theta)$. It is clear that for almost all cells, $DIR(\theta)$ contains significant power in the second harmonic in addition to the first harmonic. This reaffirms our claim that DIR contaminates all even harmonics (including the second harmonic) in the Fourier decomposition of the cell's overall tuning curve. In fact this contamination seems to grow in magnitude as the strength of its first harmonic grows, which makes sense for any single-peaked, narrowband tuning function $DIR(\theta)$. In Fig. 4(b), the strength r_o of ORI is plotted (in the ordinate) against the magnitude r_2 of the second harmonic of the overall tuning curve (in the abscissa). The latter is, in most cases, larger than the former, indicating

a contribution (contamination) to the second harmonic from DIR.

We then examine whether our proposed correction to the even harmonics has a significant impact on the estimate of the orientation peak angle. For each cell, we compare two methods of estimating the peak angle of ORI, one as $\arctan(\eta_2/\zeta_2)$ directly using the SDO method ("without correction") versus the other estimated from our proposed method as $\arctan(\eta_2'/\zeta_2')$ ("with correction"). The scatter diagram of the two estimates for the population of 149 cells is presented as Fig. 5. As shown, for the majority of the cells, our correction does not appear to affect the estimate of the peak angle.

The lack of a systematic difference in estimating the peak angle, coupled with a clear improvement in estimating the relative strength, could only mean that for most cells, the orientation peak and the direction peak co-align or are perpendicular to each other. In Fig. 6, we examine the relationship between the peak angles of the directional tuning and the orientational tuning. Indeed, in over half the cells, the orientation peak and the direction peak differ by less than ±30°; here perfect coincidence of the two peaks ($\Delta=0$) means that the preferred orientation and the preferred direction are orthogonal.

4. DISCUSSION

When an oriented stimulus (such as a bar or a grating) is drifted across the receptive field,[35] a cortical cell will unavoidably respond to both the directional aspect and the orientational aspect of the stimulus. Unconfounding the cell's direction tuning and orientation tuning is not an easy task because, in general, the cell's tuning curve $R(\theta)$ may be a complex function of its component tuning curves:

$$R(\theta) = \mathcal{F}(ORI(\theta), DIR(\theta)).$$

Here, the functional form $\mathcal{F}(\cdot, \cdot)$ specifies how the orientational tuning mechanism and the directional tuning mechanism interact. The difficulty, and the challenge of an unconfounding method, is that neither the shapes of the tuning curves $ORI(\theta)$, $DIR(\theta)$ themselves,[36] nor the functional form of their interaction $\mathcal{F}(\cdot, \cdot)$ is known *a priori*. Without their being properly specified, the problem is underconstrained and hence ill-posed; a formal solution requires at the very least an explicit account of the interaction between orientation and direction tunings to give rise to the overall spike rate of a cell.

The basic premise of SDO analysis, as well as the current approach, is that the 360°-periodic direction component DIR and a 180°-periodic orientation component ORI are linearly additive in forming the tuning curve $R(\theta)$

$$R(\theta) = ORI(\theta) + DIR(\theta). \qquad (14)$$

Because of this, applying Fourier transform (which is a linear, integral transform) to both sides of Eq. (14) suggests that each Fourier harmonic of $R(\theta)$ is equal to the
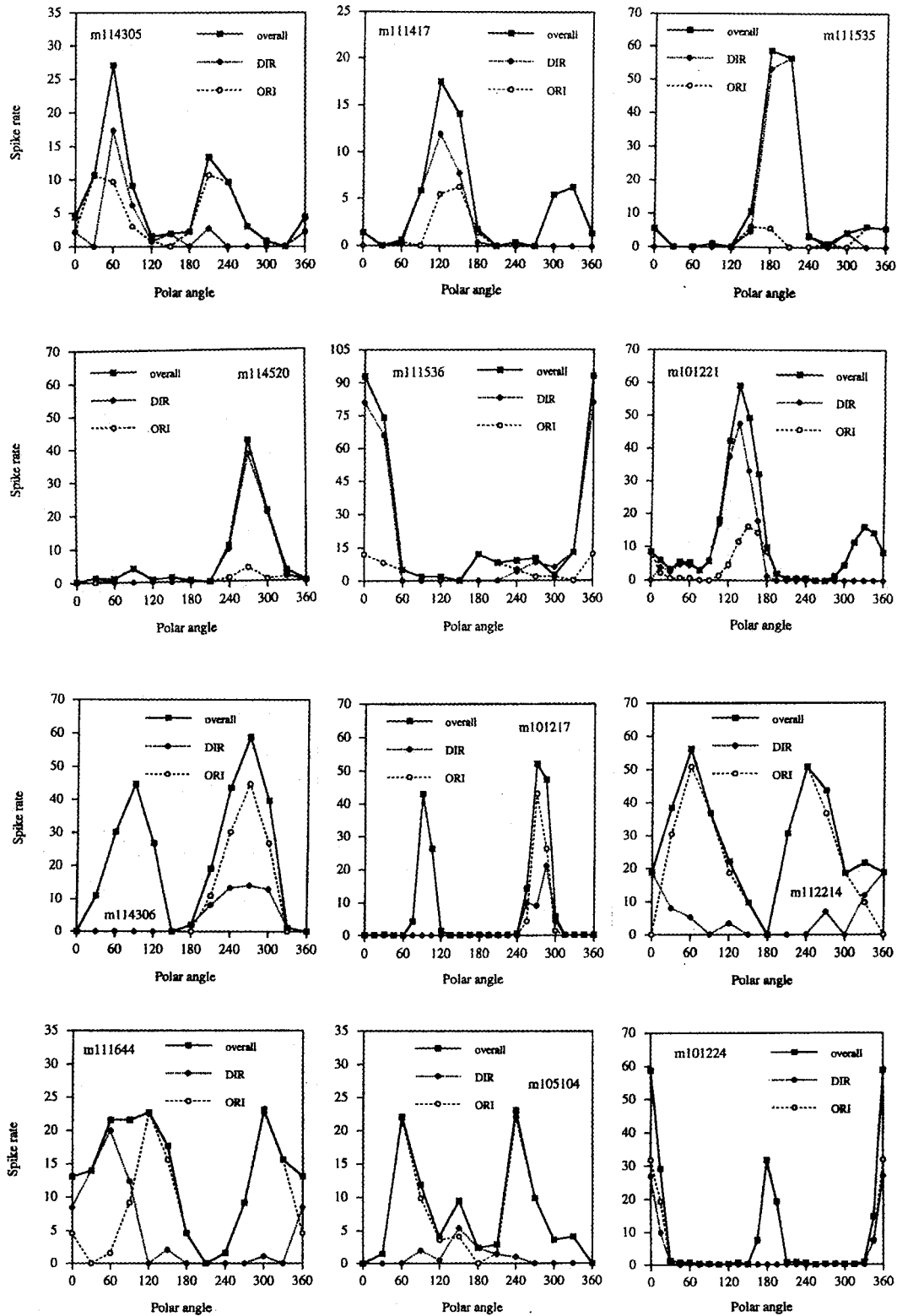
Fig. 2.   Twelve sample cells, with decomposed DIR and ORI components. The ordinate represents a cell's response, the abscissa the drifting angles. The overall tuning curves are plotted as solid lines with solid square symbols representing the data points. The DIR (or ORI) component is plotted as dotted (or dashed) lines with diamond (or circle) symbols. Note that the DIR and ORI curves sum to the overall tuning curve for each cell. The alphanumeric string starting with "m" is a cell's identification number.

sum of the corresponding harmonic of $\mathrm{ORI}(\theta)$ and of $\mathrm{DIR}(\theta)$, which in general have the Fourier decompositions

$$\mathrm{DIR}(\theta) = d_0 + d_1 \cos(\theta - \theta_{\mathrm{d}}) + d_2 \cos[2(\theta - \theta_{\mathrm{d}})] + \cdots$$

$$\mathrm{ORI}(\theta) = o_0 + o_1 \cos[2(\theta - \theta_{\mathrm{o}})] + o_2 \cos[4(\theta - \theta_{\mathrm{o}})] + \cdots .$$

Here, θ_{d} and θ_{o} represent, respectively, the peak angle of the directional and orientational tuning functions, and

the coefficients $d_1, d_2,\ldots$ and $o_1, o_2,\ldots$ depend on their tuning widths and strengths. For simplicity, we make the following operational definitions of the direction and orientation tuning strengths: $r_d = d_1$, $r_o = o_1$ [but of course $d_1 = r_1 = (\eta_1^2 + \zeta_1^2)^{1/2}$ while $o_1 \neq r_2 = (\eta_2^2 + \zeta_2^2)^{1/2}$]. Under Eq. (14), the Fourier decomposition of the experimentally measured neuronal tuning curve $R(\theta)$, given by Eq. (8), is related to these coefficients by

Strength of ORI and DIR Components
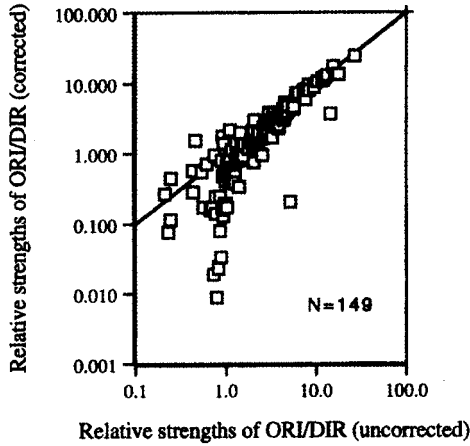
(a)

### Relative Strength of ORI/DIR Components
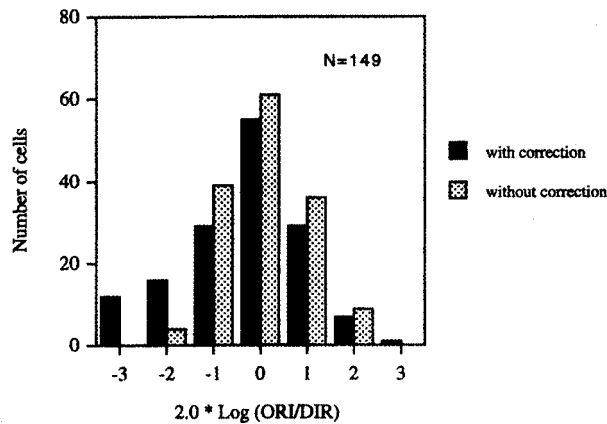
(b)

Fig. 3. Relative strength of ORI/DIR, when directional contamination of even harmonics has been corrected (the current method) or uncorrected (the SDO method). (a) The scatter plot of relative strengths of the population of 149 neurons, before correction (as abscissa) and after correction (as ordinate) by our method. Each square represents a cell. Note the overestimation of the orientation strength by SDO analysis. (b) The histogram of the distribution of the relative strengths in this population. Note the leftward shift of the distribution when corrections have been performed, again demonstrating the overestimation of the strength of the orientation component by SDO analysis.

Amplitude of DIR Component
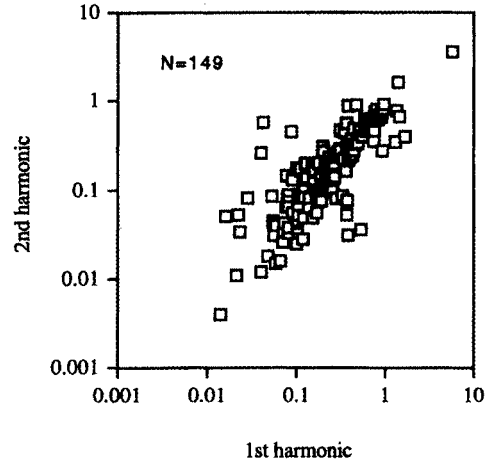
(a)

Comparing ORI Component and Second Harmonic
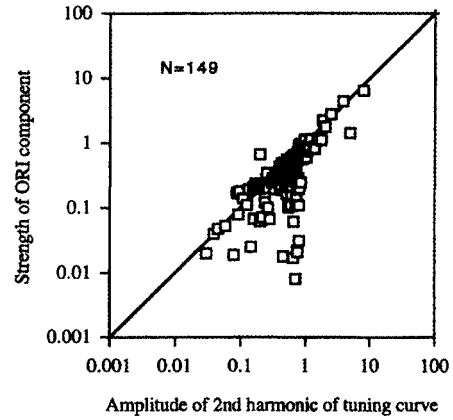
(b)

Fig. 4. Overestimation of ORI strength as a result of DIR contamination of the second harmonic. (a) Scatter plot of the amplitude of DIR's second harmonic (ordinate, in logarithmic scale) versus the amplitude of its first harmonic (abscissa, in logarithmic scale). Note the positive correlation between the two amplitudes. (b) Scatter plot of the amplitude of the second harmonic of the overall tuning curve (before correction, abscissa) versus the amplitude of the second harmonic of the ORI component (after correction, ordinate), both in logarithmic scale. Note most data points fall below the positive diagonal. In both (a) and (b), each square represents a cell, and the amplitudes are all normalized by the DC component.

$$\eta_k = d_k \cos(k\,\theta_d), \quad \zeta_k = d_k \sin(k\,\theta_d), \quad k = 1,3,5,\ldots,$$

$$\eta_k = d_k \cos(k\,\theta_d) + o_{k/2} \cos(k\,\theta_o),$$

$$\zeta_k = d_k \sin(k\,\theta_d) + o_{k/2} \sin(k\,\theta_o), \quad k = 2,4,6,\ldots,$$

$$r_0 = d_0 + o_0.$$

From the above the relationship of the amplitude r_2 of the second harmonic is

$$(r_2)^2 = (o_1)^2 + (d_2)^2 + 2o_1d_2\cos[2(\theta_d - \theta_o)].$$

Clearly, the magnitude of r_2 depends not only on o_1, which indeed equals r_o and characterizes the strength of orientation tuning, but also on d_2, which is related to direction tuning, and on $\Delta = |\theta_d - \theta_o|$, which is the separation of the orientational and direction peak angles. When r_2 is used (instead of o_1) as a measure of the strength of orientation selectivity, for example, the OSI in Ref. 9, the estimate of the degree of orientation selectivity will not be accurate.
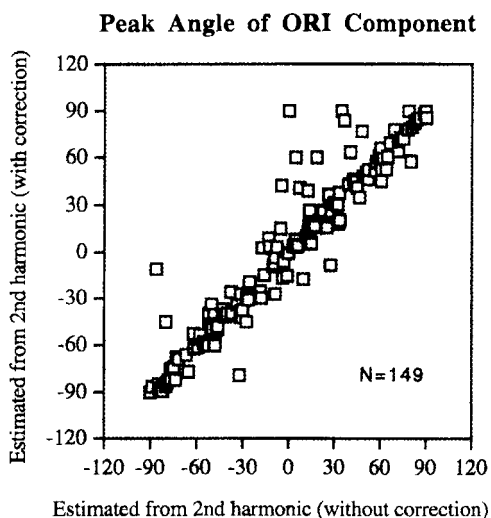
Peak Angle of ORI Component

Fig. 5. Comparison of the distributions of peak angles before (as abscissa) and after (as ordinate) correction for DIR contamination of the second harmonic. Each square represents a cell. Generally speaking, there does not appear to be a systematic difference between the peak angles estimated by the SDO method (before correction) and by our method (after correction).

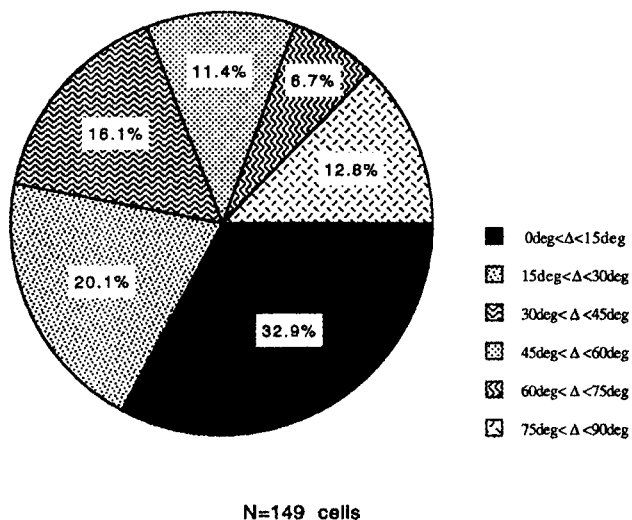## Relationship Between ORI and DIR Peaks

N=149 cells

Fig. 6. Distribution of the difference between the peak angles of DIR and ORI components. The absolute value of difference in the two peak angles, Δ, for each cell, is calculated and segregated into six categories, ranging from almost perpendicular ($\Delta=0$) to almost colinear ($\Delta=90°$), with a precision of ±15° on both sides.

If $\theta_d = \theta_o$ or $\Delta = 0$, that is, when the preferred direction is orthogonal to the preferred orientation, the magnitude of r_2 will represent an overestimate of the degree of orientation selectivity; if $\theta_d = \theta_o \pm 90°$ or $\Delta = 90°$, that is, when the preferred direction is aligned with the preferred orientation, the magnitude of r_2 will represent an underestimate.

In this context, the deficiency of Wörgötter and Eysel's original analysis can be seen as not properly considering higher harmonics for DIR beyond the first order when DIR is not as broadly tuned as a sinusoidal function. Regardless of how DIR combines with ORI to give rise to the overall response of a cell, the claim that "second harmonic ↔ orientation component" cannot, in general, be justified. It is not a matter of an individual researcher's assumptions about the underlying processes generating orientational and direction selectivities; it is a sheer mathematical fact. In reality, when elongated bars were used, higher-order (≥3) harmonics were consistently observed;[2,3,26,31] this had presented a considerable problem for one-to-one correspondence between a functional component (e.g., DIR or ORI) and a Fourier component (i.e., first or second harmonics). In fact, the presence of nonvanishing higher-order Fourier harmonics even led Wörgötter and Eysel (Ref. 37, p. 655) to postulate an independent quadruple-lobed tuning mechanism (in addition to direction and orientation tuning); but even so, it leaves the third-, fifth-, and other higher-order Fourier components unexplained (and unexplainable).[38]

Li et al.,[31] in an effort to account for those higher harmonics, proposed that the sum of all odd harmonics corresponds to DIR and the sum of all even harmonics corresponds to ORI. This proposal is equivalent to assuming that the direction tuning DIR(θ) takes on the antisymmetric form of $G(\theta)$, that is, cells are tuned excitatory in their preferred direction but tuned inhibitory in the opposite/null direction while maintaining an elevated level of spike rate for all other directions between: $G(\theta + 180°) = -G(\theta)$. It is unrealistic to assume DIR(θ)=$G(\theta)$ from the observed directional selectivity of V1 cells (see, e.g., Ref. 30). To avoid this undesirable property, our remedy here is to provide an additional term for DIR(θ), namely, that of $|G(\theta)|$, so that DIR(θ) contains only the positive lobe with the positive peak. As a result, all odd harmonics of DIR(θ) come from $G(\theta)$ and all even harmonics come from $|G(\theta)|$. This is a specific, but nonparametric assumption we made about the distribution of even harmonics (and hence about the tuning curve) of DIR; in all other aspects, we follow the analysis of Zhang (Ref. 26) in treating the higher (>1)-order harmonics. We hope our current method provides a reasonable middle ground in both standing on sound logic with correct mathematics and still being simple and feasible in practical implementation.

To conclude, we have provided a feasible method to appropriately decompose the direction and orientation components in a neuron's response to moving oriented stimuli. Application of this method to a population of V1 neurons demonstrated that the SDO method significantly underestimates the strength of the orientation component while leaving the estimate of the orientation peak angle largely unaffected. Therefore, any previous conclusions regarding the strength of orientation tuning derived from

the SDO analysis may have to be reexamined after rectifying the methodological problem as highlighted here.[39]

REFERENCES AND NOTES

1. F. Wörgötter and U. T. Eysel, "Quantitative determination of orientational and directional components in the response of visual cortical cells to moving stimuli," Biol. Cybern. **57**, 349–355 (1987).
2. F. Wörgötter, T. Muche, and E. T. Eysel, "Correlations between directional and orientational tuning of cells in cat striate cortex," Exp. Brain Res. **83**, 665–669 (1991).
3. F. Wörgötter and U. T. Eysel, "Topographical aspects of intracortical excitation and inhibition contributing to orientation specificity in area 17 of the cat visual cortex," Eur. J. Neurosci. **3**, 1232–1244 (1991).
4. F. Wörgötter, O. Gründel, and U. T. Eysel, "Quantification and comparison of cell properties in cat's striate cortex determined by different types of stimuli," Eur. J. Neurosci. **2**, 928–941 (1990).
5. D. O'Carroll, "Feature-detecting neurons in dragonflies," Nature (London) **362**, 541–543 (1993).
6. B. Chapman and M. P. Stryker, "Development of orientation selectivity in ferret visual-cortex and effects of deprivation," J. Neurosci. **13**, 5251–5262 (1993).
7. D. Malonek, R. B. H. Tootell, and A. Grinvald, "Optical imaging reveals the functional architecture of neurons processing shape and motion in owl monkey area MT," Proc. R. Soc. London, Ser. B **258**, 109–119 (1994).
8. A. Shmuel and A. Grinvald, "Functional organization for direction of motion and its relationship to orientation maps in cat area 18," J. Neurosci. **16**, 6945–6964 (1996).
9. M. Weliky and L. C. Katz, "Disruption of orientation tuning in visual cortex by artificially correlated neuronal activity," Nature (London) **386**, 680–685 (1997).
10. A. Schmidt, J. Engelage, and H. J. Bischof, "Single cell responses from the optic tectum of the zebra finch (*Taeniopygia guttata castanotis Gould*)," J. Comp. Physiol. A **185**, 69–79 (1999).
11. F. Sengpiel, P. Stawinski, and T. Bonhoeffer, "Influence of experience on orientation maps in cat visual cortex," Nat. Neurosci. **2**, 727–732 (1999).
12. T. Yousef, T. Bonhoeffer, D.-S. Kim, U. T. Eysel, É. Tóth, and Z. F. Kisvárday, "Orientation topography of layer 4 lateral networks revealed by optical imaging in cat visual cortex (area 18)," Eur. J. Neurosci. **11**, 4291–4308 (1999).
13. J. Sharma, A. Angelucci, and M. Sur, "Induction of visual orientation modules in auditory cortex," Nature (London) **404**, 841–847 (2000).
14. K. Krug, C. J. Akerman, and I. D. Thompson, "Responses of neurons in neonatal cortex and thalamus to patterned visual stimulation through the naturally closed lids," J. Neurophysiol. **85**, 1436–1443 (2001).
15. A. Schmidt and H. J. Bischof, "Neurons with complex receptive fields in the stratum griseum centrale of the zebra finch (*Taeniopygia guttata castanotis Gould*) optic tectum" J. Comp. Physiol., A **187**, 913–924 (2001).
16. C. J. Akerman, D. Smyth, and I. D. Thompson, "Visual experience before eye-opening and the development of the retinogeniculate pathway," Neuron **36**, 869–879 (2002).
17. F. Sengpiel and F. T. Bonhoeffer, "Orientation specificity of contrast adaptation in visual cortical pinwheel centres and iso-orientation domains," Eur. J. Neurosci. **15**, 876–886 (2002).
18. C. Monier, F. Chavane, P. Baudot, L. J. Graham, and Y. Frégnac, "Orientation and direction selectivity of synaptic inputs in visual cortical neurons: a diversity of combinations produces spike tuning," Neuron **37**, 663–680 (2003).
19. T. H. Schwartz, "Optical imaging of epileptiform events in visual cortex in response to patterned photic stimulation," Cereb. Cortex **13**, 1287–1298 (2003).
20. A. G. Leventhal, K. G. Thompson, D. Liu, Y. Zhou, and S. J. Ault, "Concomitant sensitivity to orientation, direction, and color of cells in layers 2, 3, and 4 of monkey striate cortex," J. Neurosci. **15**, 1808–1818 (1995).
21. T. D. Shou, A. G. Leventhal, K. G. Thompson, and Y. F. Zhou, "Direction biases of X-type and Y-type retinal ganglion-cells in the cat," J. Neurophysiol. **73**, 1414–1421 (1995).
22. Y. F. Zhou, A. G. Leventhal, and K. G. Thompson, "Visual deprivation does not affect the orientation and direction sensitivity of relay cells in the lateral geniculate nucleus of the cat," J. Neurosci. **15**, 689–698 (1995).
23. A. G. Leventhal, Y. C. Wang, M. T. Schmolesky, and Y. F. Zhou, "Neural correlates of boundary perception," Visual Neurosci. **15**, 1107–1118 (1998).
24. A. Shmuel and A. Grinvald, "Coexistence of linear zones and pinwheels within orientation maps in cat visual cortex," Proc. Natl. Acad. Sci. U.S.A. **97**, 5568–5573 (2000).
25. E. Batschelet, *Circular Statistics in Biology* (Academic, 1981).
26. J. Zhang, "How to unconfound the directional and orientational information in visual neuron's response," Biol. Cybern. **63**, 135–142 (1990).
27. Note that here and below the designation of "first" and "second" harmonics refers to using a fundamental period of 360 deg/cycle in Fourier analysis. If one were to Fourier decompose, say ORI, using a fundamental period of 180 deg/cycle, then the second harmonic mentioned above should be renamed the first harmonic (in describing a periodic function with a period of 180 deg/cycle).
28. In fact, higher-order harmonics were consistently present even in these authors' own data; see Ref. 2. However they were dismissed based on the percentage of total power they contributed. This was misguided, because any single-peaked DIR will have decreasing power in its higher harmonics. In fact, Zhang[26] showed that under quite mild restriction, the strength of the kth harmonic of DIR is proportional to $\sin(k\alpha)/k$, with α characterizing the bandwidth. So the systematic presence of powers with decreasing strength in higher harmonics cannot be explained away simply as noise; rather it reflects narrow tuning curves of DIR.
29. Sinusoidal tuning may describe directional selectivity in ganglion cells; the reader is directed to S. G. He *et al.*, "Distinguishing direction selectivity from orientation selectivity in the rabbit retina," Visual Neurosci. **15**, 439–447 (1998). This is where the vector sum method (and also the SDO analysis) would work well. In estimating the orientation (and by analogy, direction) tuning, the vector sum or SDO method is equivalent to calculating the least-squares fit to a sinusoidal curve, as pointed out by N. V. Swindale, "Orientation tuning curves: empirical

description and estimation of parameters," Biol. Cybern. **78**, 45–56 (1998).

30. R. L. De Valois, E. W. Yund, and N. Hepler, "The orientation and direction selectivity of cells in macaque visual cortex," Vision Res. **22**, 531–544 (1982).

31. B. Li, Y. Wang, and Y. C. Diao, "Quantification of directional and orientational selectivities of visual neurons to moving stimuli," Biol. Cybern. **70**, 282–290 (1994).

32. The reason we do not identify DIR(θ) as [ODDSUM(θ) +|ODDSUM(θ)|]/2 is because all odd harmonics of DIR(θ) are required to be exactly the same as all odd harmonics of $R(\theta)$. The reason we do not write DIR(θ) as ODDSUM(θ) +λ|ODDSUM(θ)| for some $0 < \lambda < 1$ is because we want to completely cancel its negative lobe/peak.

33. J. Zhang and R. L. De Valois (1998), "Unconfounding orientation and direction tuning in cortical neuron's response," Invest. Ophthalmol. Visual Sci. **39**, s324 (1998).

34. R. L. De Valois and N. P. Cottaris, "Inputs to directionally selective simple cells in macaque striate cortex," Proc. Natl. Acad. Sci. U.S.A. **95**, 14488–14493 (1998).

35. Note that our analysis applies only to the use of drifting oriented stimuli. When, for instance, cells are driven by flashing oriented stimuli, the use of the circular variance measure in analogy to Eq. (2) to characterize orientation tuning would not be subject to our criticism described here; the reader is directed to Ringach *et al.*, "Dynamics of orientation tuning in macaque primary visual cortex," Nature **387**, 281–284 (1997) and Ringach *et al.*, "Orientation selectivity in macaque V1: diversity and

laminar dependence," J. Neurol. Sci. **22**, 5639–5651 (2002). Also, our analysis will not apply if the stimulus is a drifting random dot pattern because, under certain conditions, the cell's directional tuning would exhibit a bifurcation of peaks, as shown by Skottun *et al.*, "On the direction selectivity of cortical neurons to drifting dot patterns," Visual Neurosci. **11**, 885–897 (1994).

36. For an approach using a particular parametric form for orientation and direction tuning curves, the reader is directed to Swindale *et al.*, "The spatial pattern of response magnitude and selectivity for orientation and direction in cat visual cortex," Cereb. Cortex **13**, 225–238 (2003), and the reference by Swindale cited in Ref. 29.

37. F. Wörgötter and U. T. Eysel, "Axial responses in visual cortical cells: Spatial-temporal mechanisms quantified by Fourier components of cortical tuning curves," Exp. Brain Res. **83**, 656–664 (1991).

38. If indeed such higher-order tuning mechanism exists, then the correct way of testing it is to assume the quadruple-lobed mechanism $Q(\theta)$ to have a period of 90° and hence Fourier decomposable as $Q(\theta) = q_0 + q_1 \cos[4(\theta - \theta_q)] + q_2 \cos[8(\theta - \theta_q)] + \cdots$. One can then proceed as before and relate (η_4, ζ_4) to d_4, o_2, and q_1.

39. This includes, for example, Ref. 9, which reported the weakening of orientation selectivity in the primary visual cortex (V1) of ferrets after chronic electric stimulation of the optic nerve during their early, postnatal visual development.