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Estimation of Parameters Subject to Order Restrictions on a Circle With Application to Estimation of Phase Angles of Cell Cycle Genes

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Abstract

Motivated by a problem encountered in the analysis of cell cycle gene expression data, this article deals with the estimation of parameters subject to order restrictions on a unit circle. A normal eukaryotic cell cycle has four major phases during cell division, and a cell cycle gene has its peak expression (phase angle) during the phase that may correspond to its biological function. Because the phases are ordered along a circle, the phase angles of cell cycle genes are ordered unknown parameters on a unit circle. The problem of interest is to estimate the phase angles using the information regarding the order among them. We address this problem by developing a circular version of the well-known isotonic regression for Euclidean data. Because of the underlying geometry, the standard pool adjacent violator algorithm (PAVA) cannot be used for deriving the circular isotonic regression estimator (CIRE). However, PAVA can be modified to obtain a computationally efficient algorithm for deriving the CIRE. We illustrate the CIRE by estimating the phase angles of some of well-known cell cycle genes using the unrestricted estimators obtained in the literature.

Keywords

Circular isotonic regression; Order restrictions on circle; Phase angles; Pool adjacent violator algorithm; Restricted maximum likelihood estimators; von Mises distribution

1. INTRODUCTION

In some applications, researchers are interested in estimating angular parameters when they are intrinsically ordered around a unit circle. For instance, some biologists are interested in determining the phases of cell cycle genes (e.g., Whitfield et al. 2002), which are functionally ordered around a circle. Similarly, some are interested in determining phase regulation of circadian genes (Akashi et al. 2006; Nakamura et al. 2008), or a gynecologist may be interested in studying the effect of stress on the duration of follicular phase in premenopausal women (Xiao et al. 1998). Problems such as these can be formulated as a problem of estimating a vector $\boldsymbol{\varphi} = (\varphi_1, \varphi_2, \dots, \varphi_q)'$ of angular parameters for which the components are ordered around a unit circle. There exists more than 50 years of literature for the case when $\boldsymbol{\varphi} \in R^q$, the Euclidean space, and its components are constrained by inequalities (see Robertson, Wright, and Dykstra 1998; Silvapulle and Sen 2005; Van Eeden, 2006). However, no literature exists for the case

when the components of $\boldsymbol{\varphi}$ are ordered points on a unit circle—the focus of this article. As demonstrated here, the existing methodology for R^q cannot be trivially extended to the current problem.

This article is motivated by a problem encountered in the analysis of cell cycle gene expression data. A normal eukaryotic cell goes through four phases—namely, G_1 , S , G_2 , and M phases during cell division (Fig. 1). Each phase has a well-defined function and, within each phase, a collection of genes (called “cell cycle genes”) plays an important role. For instance, some genes are associated with DNA replication during the S phase, whereas some are with mitosis during the M phase. Cell biologists are interested in determining the phase associated with a cell cycle gene (Whitfield et al. 2002) that corresponds to its peak expression during the cell cycle.

Typically at the start of the experiment all cells are synchronized by arresting them at a specific phase of cell cycle. This establishes the pole of the circle. Cells are then released to measure messenger RNA (mRNA) gene expressions at various times using microarrays as the cells go through the cell cycle. The phase angle φ of a gene is the angle corresponding to its peak expression relative to the pole. As a result of their biological functions, the phase angles of cell cycle genes are expected to be ordered on a circle. For instance, the cyclins CCNE1, CCNA2, and CCNB1 have peak mRNA expression in the G_1/S , G_2 , and G_2/M phases, respectively (Pines and Hunter 1989, 1990; Whitfield et al. 2002). The DNA metabolism gene RRM2 peaks during the early S phase (Bjorklund et al. 1990), whereas the gene VEGFC peaks during the M/G_1 phase (Datta and Sokhansanj 2007). Hence, if cells are arrested in the G_1/S boundary, then we may expect the simple order $0 \leq \varphi_{\text{CCNE1}} \leq \varphi_{\text{RRM2}} \leq \varphi_{\text{CCNA2}} \leq \varphi_{\text{CCNB1}} \leq \varphi_{\text{VEGFC}} \leq 2\pi$.

Biologically, the order among cell cycle genes should be rotation invariant and, hence, invariant of the location of the pole. Components of $\boldsymbol{\varphi}$ are said to follow a rotation-invariant simple order around a unit circle, denoted by $\varphi_1 < \varphi_2 < \dots < \varphi_p < \varphi_1$, if for all $i = 1, \dots, p$, φ_i “follows” φ_{i-1} and is “followed by” φ_{i+1} (in the counter clockwise direction), where $\varphi_0 \equiv \varphi_p$ and $\varphi_{p+1} \equiv \varphi_1$. This rotation-invariant simple order may be expressed as the union of simple orders $\{0 \leq \varphi_1 \leq \varphi_2 \leq \dots \leq \varphi_p \leq 2\pi\}$, $\{0 \leq \varphi_2 \leq \varphi_3 \leq \dots \leq \varphi_p \leq \varphi_1 \leq 2\pi\}$, ..., $\{0 \leq \varphi_p \leq \varphi_1 \leq \varphi_2 \leq \dots \leq \varphi_{p-1} \leq 2\pi\}$. The estimator satisfying the rotation-invariant simple order can be obtained by deriving the estimator under each of the individual simple order restrictions and by selecting the one that has the smallest value of the objective function in the minimization problem defined in Section 2. Thus, it is enough to develop the methodology for obtaining the order-restricted estimators under $\{0 \leq \varphi_1 \leq \varphi_2 \leq \dots \leq \varphi_p \leq 2\pi\}$, which is the focus of this article. The methodology can then be applied to each of the other subsets that make up the order restriction $\varphi_1 < \varphi_2 < \dots < \varphi_p < \varphi_1$ to obtain the rotation-invariant simple order estimate.

Liu et al. (2004) developed a nonlinear regression model called the “random periods model” for estimating the phase angle of a cell cycle gene (see Supplementary Materials for details). Because they do not impose the order restriction among the phase angles, their unrestricted estimates $\hat{\boldsymbol{\varphi}} = (\hat{\varphi}_1, \hat{\varphi}_2, \dots, \hat{\varphi}_q)'$ may not necessarily follow the desired order restriction.

Analogous to R^q , the objective of this article is to construct a “circular” isotonic regression estimator of $\boldsymbol{\varphi}$ using the unrestricted estimator $\hat{\boldsymbol{\varphi}}$ by solving a suitable minimization problem described in Section 2. Recall that similar to normal distribution for R^q data, the von Mises distribution is commonly used for describing circular data (Mardia and Jupp 2000). For independently and normally distributed data, the isotonic regression estimator yields the restricted maximum likelihood estimator (RMLE) of the population means. Similarly we observe that the circular isotonic regression estimator (CIRE), introduced in Section 2, is the RMLE of $\boldsymbol{\varphi}$, if the components of $\hat{\boldsymbol{\varphi}}$ are independently distributed according to von Mises distribution. We demonstrate that the standard pool adjacent violators algorithm (PAVA)

(Robertson, Wright, and Dykstra 1988), used for deriving the isotonic regression estimator when $\boldsymbol{\varphi} \in R^q$, is not suitable in the current context. In that section we also discuss the existence and uniqueness of the CIRE. An algorithm for computing the CIRE is described in Section 3. It is based on PAVA, but is tailored for circular data. As in standard PAVA, the algorithm is applied sequentially and the solution at each stage depends on the geometry of the unrestricted estimates wrapped around the circle. The algorithm investigates the geometry of the estimates at each stage and updates the estimates accordingly. For some configurations, the solution is given by PAVA, for some the solution is given by the boundary points (0 or 2π), whereas for others the estimates are suitably pooled. Using simulation studies, we evaluate the performance of the CIRE in Section 4, and in Section 5 we illustrate the CIRE. Concluding remarks along with open research problems are discussed in Section 6. Proofs are in the Appendix and some supplementary details are in the accompanying supplementary materials.

2. ISOTONIC REGRESSION AND PAVA

2.1 PAVA for Euclidean Space Data: A Brief Review

Suppose $\hat{\boldsymbol{\varphi}} = (\hat{\varphi}_1, \hat{\varphi}_2, \dots, \hat{\varphi}_q)'$ is an unrestricted estimator of a population parameter $\boldsymbol{\varphi} \in A \subseteq R^q$, where $A = \{(\varphi_1, \varphi_2, \dots, \varphi_q)' \in R^q: \varphi_1 \leq \varphi_2 \leq \dots \leq \varphi_q\}$. Then the isotonic regression estimator is a point on A that is “closest” to $\hat{\boldsymbol{\varphi}}$, where the distance is the weighted Euclidean norm

$\sum_{i=1}^q w_i(\hat{\varphi}_i - \varphi_i)^2$ and w_i are suitable weights. Thus, the isotonic regression estimator is a

solution to the following minimization problem: $\min_{\boldsymbol{\varphi} \in A} \sum_{i=1}^q w_i(\hat{\varphi}_i - \varphi_i)^2$. Because the distance is convex and is a well-defined norm, the solution is unique and is determined by the orthogonal projection of $\hat{\boldsymbol{\varphi}}$ onto A using PAVA (Robertson, Wright, and Dykstra 1988). According to PAVA, whenever two adjacent unrestricted estimates violate the hypothesized inequality, then such estimates are replaced by their weighted average. Note that for any two real numbers x , y (with $x \leq y$), their weighted average always lies between x and y . This is known as the “Cauchy mean value” property (Robertson and Wright 1980) and it plays a crucial role when using PAVA for independent R^q data.

2.2 Notations and Some Underlying Geometry

Many of the basic concepts used for R^q data are not valid for circular data. For instance, the arithmetic mean of angular data may not be physically meaningful. Suppose two birds are flying east at angles, $\eta_1 = 0.52$ radians (30°) and $\eta_2 = 5.76$ radians (330°), respectively. Then the arithmetic mean is $\bar{\eta} = \pi$ radians (i.e., 180°), suggesting that the birds on the average are actually flying westward, which refutes common sense. Instead, angular mean is 0 radians (i.e., 0° ; see Fig. S-1 in supplementary materials).

More generally, suppose $S_g = \{\theta_{g1}, \theta_{g2}, \dots, \theta_{gn_g}\}$ are a random sample of n_g observations from a population with a mean direction that is φ_g , $g = 1, 2, \dots, q$. Then the sample mean direction of S_g (see Mardia and Jupp 2000) is given by

$$\text{Ave}(S_g) = \begin{cases} \tan^{-1}(A/B), & \text{if } A > 0, B > 0 \\ \pi + \tan^{-1}(A/B), & \text{if } B < 0 \\ 2\pi + \tan^{-1}(A/B), & \text{if } A < 0, B > 0 \end{cases}, \quad (1)$$

where $A_g = \sum_{j=1}^{n_g} \sin(\theta_{gj})$, $B_g = \sum_{j=1}^{n_g} \cos(\theta_{gj})$, and the resultant length is $r_g = \sqrt{A_g^2 + B_g^2}$, which is a measure of concentration (inverse of dispersion) of a dataset.

In the previous example, 0 does not lie between 0.52 and 5.76, and hence the angular mean does not always satisfy the Cauchy mean value property. As we see later, this causes difficulties

in using PAVA. Throughout this article the term “PAVA” refers to the usual PAVA, with the exception that means are computed using (1), rather than the weighted arithmetic means.

Just as the arithmetic mean is not a suitable measure of average, the Euclidean distance $(\eta_1 - \eta_2)^2$ between a pair of angles η_1, η_2 is not a suitable measure of distance for angular data. Suppose $(\eta_1, \eta_2, \eta_3) = (0, \pi, 2\pi)$, then $(\eta_1 - \eta_3)^2 > (\eta_1 - \eta_2)^2$, suggesting that 0 is closer to π than to 2π , which is counterintuitive. A natural distance for angular data is the “angular distance” $d(\eta_1, \eta_2) = 1 - \cos(\eta_1 - \eta_2)$ (Mardia and Jupp 2000). This measure is actually proportional to the Euclidean distance between $\mathbf{a}_1$ and $\mathbf{a}_2$, where $\mathbf{a}_i = (\cos \eta_i, \sin \eta_i)'$, $i = 1, 2$, because $(\mathbf{a}_1 - \mathbf{a}_2)'(\mathbf{a}_1 - \mathbf{a}_2) = 2(1 - \cos(\eta_1 - \eta_2))$. Thus, the angular distance between $\boldsymbol{\alpha} = (\alpha_1, \alpha_2, \dots, \alpha_q)'$ and $\boldsymbol{\beta} = (\beta_1, \beta_2, \dots, \beta_q)'$ is $d(\boldsymbol{\alpha}, \boldsymbol{\beta}) = \sum_{i=1}^q \{1 - \cos(\alpha_i - \beta_i)\} = 2\|\boldsymbol{\alpha} - \boldsymbol{\beta}\|^2$, where $\|\boldsymbol{\delta}\|^2 = \sum_{i=1}^q \sin^2(\delta_i/2)$. Note that for $\lambda > 0$, $\|\lambda \boldsymbol{\delta}\|^2 \neq \lambda^2 \|\boldsymbol{\delta}\|^2$; hence, $\|\boldsymbol{\delta}\|^2$ is not a norm.

Let $\mathbf{Ave}(\mathbf{S}) = (\text{Ave}(S_1), \dots, \text{Ave}(S_q))'$ be the vector of sample mean directions. Then the distance between $\boldsymbol{\alpha}$ and $\mathbf{Ave}(\mathbf{S})$ is called the sum of circular error (SCE) and is defined by

$$\begin{aligned} \text{SCE}(\mathbf{Ave}(\mathbf{S}), \boldsymbol{\alpha}) &= \sum_{g=1}^q \sum_{j=1}^{n_g} d(\theta_{gj}, \alpha_g) \\ &= \sum_{g=1}^q r_g (1 - \cos(\text{Ave}(S_g) - \alpha_g)) \end{aligned} \quad (2)$$

2.3 CIRE and RMLE

Let $C = \{\mathbf{x} \in [0, 2\pi]^q: 0 \leq x_1 \leq x_2 \leq \dots \leq x_q \leq 2\pi\}$ denote the simple order set. Then the problem of CIRE is to determine $\boldsymbol{\varphi} \in C$ that minimizes (2). Equivalently, the problem is to determine $\boldsymbol{\varphi} \in C$, which is “closest” to the unrestricted estimator $\mathbf{Ave}(\mathbf{S})$. Because $\|\boldsymbol{\delta}\|^2$ is not a norm, hence, unlike the isotonic regression in the Euclidean case, it may not be possible to derive the CIRE using an orthogonal projection. Also, the CIRE is not unique, as noted in Example S-2 in the supplementary materials. This, together with the fact that the Cauchy mean value property is not satisfied by angular data, prompts us to develop an alternate strategy.

Suppose that θ_{gj} , $g = 1, 2, \dots, q$, $j = 1, 2, \dots, n_g$ are independently distributed according to von Mises distribution, represented by $M(\varphi_g, \kappa)$, with mean direction φ_g and known concentration parameter κ . Then the log-likelihood function is given by

$$\begin{aligned} l(\boldsymbol{\theta}, \boldsymbol{\varphi}) &= l(\theta_{gj}, \varphi_g; g=1, \dots, q, j=1, \dots, n_g) \\ &= \sum_{g=1}^q \left\{ n_g \log 2\pi + \kappa \sum_{j=1}^{n_g} \cos(\theta_{gj} - \varphi_g) - n_g \log I_0(\kappa) \right\} \\ &= \text{Constant} + \kappa \sum_{g=1}^q r_g \cos(\text{Ave}(S_g) - \varphi_g). \end{aligned} \quad (3)$$

From (3), the unrestricted maximum likelihood estimator (UMLE) of φ_i , $i = 1, 2, \dots, q$, is $\hat{\varphi}_i = \text{Ave}(S_i)$ (see Mardia and Jupp 2000). Hence, in the rest of this article we shall denote the unrestricted estimator of φ_i by $\hat{\varphi}_i$ instead of $\text{Ave}(S_i)$. Note that determination of the CIRE is equivalent to maximization of (3) under the constraint $\boldsymbol{\varphi} \in C$, and hence when θ_{gj} are independently distributed according to von Mises distribution, the CIRE is also the RMLE. The von Mises distribution is briefly reviewed in the supplementary materials. Throughout this

article, the word “pooling” refers to averaging the angles using (1). Also, we shall denote the PAVA estimator by $\hat{\Phi}_{PAVA}$ and the CIRE by $\hat{\Phi}$.

2.4 Existence and the Uniqueness of the CIRE

We now provide a characterization for CIRE, which serves as the building block for the computational algorithm described in Section 3.

Theorem 1—If $\hat{\Phi} \in C$, then $\tilde{\Phi}$ exists. Furthermore, if $\hat{\Phi} \notin A$, then $\tilde{\Phi}$ is defined by the blocks

$$0 = \tilde{\varphi}_{s(0)} < \tilde{\varphi}_{s(1)} < \cdots < \tilde{\varphi}_{s(m)} < \tilde{\varphi}_{s(m+1)} = 2\pi, \quad (4)$$

where $\{(i)\}_{i=0}^{m+1}$ is a partition of $\{1, \dots, q\}$, $S(i) = \{\theta_{gj}, g \in (i), j = 1, \dots, n_g\}$ and $\tilde{\varphi}_g = \tilde{\varphi}_{S(i)} = Ave(S(i))$ with resultant length $r_{S(i)}$ for any $g \in (i)$ and $1 \leq i \leq m$.

Although the CIRE may not be unique (Ex. S-1), we demonstrate in Theorem 2 that it is almost surely unique under some conditions. In practice, if multiple solutions are realized, then one may select the most plausible solution for the application.

Theorem 2—If the unrestricted estimators are independent and are continuous random variables, then the CIRE is almost surely unique.

2.5 Some Properties of the CIRE and PAVA

Because of the challenges described in the previous section, it may not always be possible to derive the CIRE using PAVA. The focus of this section is to derive some properties of CIRE and PAVA for circular data that would be useful for the algorithm described in Section 3. In the following, we let $\varphi_g^\pi = (\varphi_g - \pi) \pmod{2\pi}$. Also, for a pair of angles φ_g and $\varphi_{g'}$, $\text{arc}(\varphi_g, \varphi_{g'})$ denotes the simplex corresponding to the smallest arc joining them.

We consider two motivating examples. In Example 1(a) the CIRE is derived using PAVA because the Cauchy mean value property is satisfied here. In Example 1(b) the Cauchy mean value property is not true, and hence PAVA cannot be applied directly. However, if we rotate the unrestricted estimates from $\hat{\varphi}_g$ to $\tilde{\varphi}_g^\pi$, then the Cauchy mean value property holds in the new orientation, and hence we apply PAVA. The resulting estimates are then retransformed to the original orientation. Such situations are characterized in Proposition 1, which suggests that the location of $\hat{\Phi}$ is essential to identify the indices that can be pooled for obtaining the CIRE.

Example 1

- a. Suppose $n_1 = n_2 = n_3 = 1$ and $\hat{\Phi} = (0.50, 3.20, 0.75)$. The violation of the inequality occurs between $\hat{\varphi}_2$ and $\hat{\varphi}_3$. Because the Cauchy mean value property holds in this case, $\hat{\Phi}_{PAVA} = (0.50, 2.475, 2.475)' = \hat{\Phi}$.
- b. Suppose $n_1 = n_2 = n_3 = 1$ and $\hat{\Phi} = (0.50, 6.00, 1.75)$. Once again, $\hat{\varphi}_2$ and $\hat{\varphi}_3$ violate the inequality, and $\hat{\Phi}_{PAVA} = (0.50, 0.73, 0.73)'$ with $SCE = 0.947$, but the Cauchy mean value property is violated here. Rotating $\hat{\Phi}$ by π , we have $\hat{\Phi}^\pi = (3.64, 2.86, 4.89)'$. Now $\tilde{\varphi}_2^\pi$ and $\tilde{\varphi}_3^\pi$ violate the order restriction; therefore, $\tilde{\varphi}_{PAVA}^\pi = (3.25, 3.25, 4.89)'$. By rotating $\tilde{\varphi}_{PAVA}^\pi$ back to the original orientation, we have $(\tilde{\varphi}_{PAVA}^\pi)^\pi = \tilde{\varphi} = (0.108, 0.108, 1.75)'$, with $SCE = 0.1514$, which is less than the SCE of $\hat{\Phi}_{PAVA}$.
- a. Suppose that $0 \notin \text{arc}(\hat{\varphi}_g, \tilde{\varphi}_g)$ and $0 \notin \text{arc}(\hat{\varphi}_{g+1}, \tilde{\varphi}_{g+1})$, then

$$\widehat{\varphi}_g > \widehat{\varphi}_{g+1} \Rightarrow \tilde{\varphi}_g = \tilde{\varphi}_{g+1}.$$

- b. Suppose $\pi \notin \text{arc}(\widehat{\varphi}_g, \tilde{\varphi}_g)$ and $\pi \notin \text{arc}(\widehat{\varphi}_{g+1}, \tilde{\varphi}_{g+1})$, then

$$\widehat{\varphi}_g^\pi > \widehat{\varphi}_{g+1}^\pi \Rightarrow \tilde{\varphi}_g = \tilde{\varphi}_{g+1}.$$

Although Proposition 1 provides an important characterization for implementing PAVA, as noted in Example 2, from one step of PAVA to the next, the arc $(\widehat{\varphi}_{g^*}, \varphi_{g^*})$ can change for the pooled value $\widehat{\varphi}_{g^*}$ obtained by pooling original observations even though $\forall g, 0 \notin \text{arc}(\widehat{\varphi}_g, \tilde{\varphi}_g)$. Hence, PAVA may not result in the CIRE in such cases.

Example 2—Suppose $n_1 = n_4 = 10, n_2 = n_3 = 1, \theta_{gj} = \widehat{\varphi}_g, j = 1, \dots, 10, g = 1, 4$, and $\widehat{\varphi} = (3.1, 5.2, 1.6, 3.9)$. Then, $\varphi_{\text{PAVA}} = (3.086, 3.086, 3.086, 3.90)$ and $\varphi = (3.1, 3.922, 3.922, 3.922)$, with $0 \notin \text{arc}(\widehat{\varphi}_g, \varphi_g), g = 1, 2, 3, 4$.

Depending upon the geometry of the data, the CIRE can take values on the boundary such as 0 or 2π and not the angular mean (Ex. S-2). In Proposition 2, we provide the conditions under which φ_g takes such values and not the angular mean.

- a. If $\pi \leq \widehat{\varphi}_g < 2\pi$ and $0 \in \text{arc}(\widehat{\varphi}_g, \tilde{\varphi}_g)$, then $\tilde{\varphi}_g = \tilde{\varphi}_{g-1}$ where $\tilde{\varphi}_0 = 0$.
b. If $0 < \widehat{\varphi}_g \leq \pi$ and $0 \in \text{arc}(\widehat{\varphi}_g, \tilde{\varphi}_g)$, then $\tilde{\varphi}_g = \tilde{\varphi}_{g+1}$ where $\tilde{\varphi}_{q+1} = 2\pi$.

From these results, we now identify situations where PAVA results in a CIRE.

Theorem 3: If $0 \notin \text{arc}(\widehat{\varphi}_g, \widehat{\varphi}_{g'})$ for all $g, g' \in \{1, \dots, q\}$ such that $\tilde{\varphi}_g = \tilde{\varphi}_{g'}$, then PAVA yields the CIRE.

Corollary 1: If, for all $g = 1, \dots, q, \widehat{\varphi}_g \in [\eta_1, \eta_2]$ such that $\eta_1 \geq 0, \eta_2 \leq 2\pi, \eta_2 - \eta_1 \leq \pi$, then PAVA yields the CIRE.

Proposition 3 proves that we can obtain the CIRE by pooling the original observations step by step if it is known which inequalities $\varphi_i \leq \varphi_{i+1}$ the CIRE verifies as equalities.

Proposition 3—Suppose that for a sample of angles $\widehat{\varphi}_i, i = 1, 2, \dots, q$, it is known that $\tilde{\varphi}_g = \varphi_{g+1}$. Let $\widehat{\varphi}_{i^*}, i = 1, 2, \dots, g, g+2, \dots, q$, be another sample in R^{q-1} defined as follows:

$$\begin{aligned} \widehat{\varphi}_{g^*} &= \text{Ave}(\theta_{ij}, i=g, g+1; j=1, \dots, n_i), n_{g^*} = n_g + n_{g+1}, \\ \widehat{\varphi}_{i^*} &= \widehat{\varphi}_i, \forall i \neq g. \text{ Then } \tilde{\varphi}_i = \tilde{\varphi}_{i^*}, \forall i \in \{1, \dots, g, g+2, \dots, q\}. \end{aligned}$$

3. AN ALGORITHM TO CALCULATE THE CIRE

Based on the characterization of CIRE provided in Theorem 1, a simple-minded strategy would enumerate all possible solutions by pooling adjacent indices and/or suitably assigning 0 or 2π (Proposition 2), and then selecting that solution with the smallest SCE. Although such a strategy appears to be a simple task, the computational effort increases exponentially with the number of parameters, because there are 2^{q+1} possible combinations of blocking adjacent indices. The proposed algorithm is computationally efficient, especially as the number of parameters increases, and results in the true CIRE. We have developed a freely downloadable SAS program.

We first provide a detailed explanation of the algorithm before summarizing all the steps. A flowchart is provided in the supplementary materials. The proposed algorithm is based on the characterization provided in Theorem 1 using a pair of indices (s, p) , with $0 \leq s < p \leq q + 1$ that verifies

$$\begin{aligned} 0 &\leq \dots \leq \tilde{\varphi}_s < \frac{\pi}{2} < \tilde{\varphi}_{s+1} \leq \dots \leq \tilde{\varphi}_{p-1} < \frac{3\pi}{2} < \tilde{\varphi}_p \\ &\leq \dots \leq 2\pi \end{aligned} \quad (5)$$

and that defines three separate sets of indices as follows:

$$\begin{aligned} G_1^{(s,p)} &= \{g: g \leq s \text{ with } \tilde{\varphi}_g \in [0, \pi/2]\}, \\ G_2^{(s,p)} &= \{g: s < g < p \text{ with } \tilde{\varphi}_g \in [\pi/2, 3\pi/2]\}, \quad \text{and} \\ G_3^{(s,p)} &= \{g: g \geq p \text{ with } \tilde{\varphi}_g \in [3\pi/2, 2\pi]\}. \end{aligned}$$

Our algorithm examines all possible selections of pairs (s, p) and, for each (s, p) choice, the best solution $\tilde{\Phi}^{(s,p)}$, if it exists, is obtained. Then the final solution is given by the pair (s, p) for which $\tilde{\Phi}^{(s,p)}$ has the smallest SCE.

We now describe the process of determining $\tilde{\Phi}^{(s,p)}$ for each pair (s, p) . The idea is to consider only those poolings that may lead to the best solution.

For each $G_i^{(s,p)}$, $i = 1, 2, 3$, the out-of-range angles are identified as follows: (a) $g \in G_1^{(s,p)}$ but $\hat{\varphi}_g \notin [0, \pi)$, (b) $g \in G_2^{(s,p)}$ but $\hat{\varphi}_g \notin [\pi/2, 3\pi/2]$, and (c) $g \in G_3^{(s,p)}$ but $\hat{\varphi}_g \notin (\pi, 2\pi]$. If there are no such angles, then Corollary 1 can be applied within each set to obtain $\tilde{\Phi}^{(s,p)}$. On the other hand, for an out-of-range angle $\hat{\varphi}_g$, we know $\hat{\varphi}_g \neq \tilde{\varphi}_g^{(s,p)}$; therefore, it is pooled with adjacent angles or is set equal to 0 or 2π to derive $\tilde{\Phi}^{(s,p)}$. This is done using an efficient sequential examination designed specifically for each of the three sets. For simplicity, in the following we eliminate the superscript (s, p) for $\tilde{\varphi}_g$ and denote $\varphi_0 = 0$ and $\varphi_{q+1} = 2\pi$.

For $G_1^{(s,p)}$ we begin with $\hat{\varphi}_s$ and end with $\hat{\varphi}_1$. For indices such that $\hat{\varphi}_g \notin [0, \pi)$, the pooling actions in $G_1^{(s,p)}$ depends on whether $3\pi/2 < \hat{\varphi}_g < 2\pi$ or $\pi < \hat{\varphi}_g \leq 3\pi/2$. In the former case using Proposition 2, we set $\tilde{\varphi}_g = \tilde{\varphi}_{g-1}$. In the latter case, three possible pooling actions are considered:

1. If $\tilde{\varphi}_g \neq \hat{\varphi}_{g+1}$, necessarily $\hat{\varphi}_g$ must be pooled with angles having smaller indices until the new pooled angle satisfies $\tilde{\varphi}_g^* \in [0, \pi/2)$.
2. If $\tilde{\varphi}_{g-1} \neq \hat{\varphi}_g$, necessarily $\hat{\varphi}_g$ must be pooled with angles having larger indices until the new pooled angle satisfies $\tilde{\varphi}_g^* \in [0, \pi/2)$.
3. If $\tilde{\varphi}_g = \hat{\varphi}_{g+1} = \tilde{\varphi}_{g-1}$, necessarily $\hat{\varphi}_g$ must be pooled with $\hat{\varphi}_{g-1}$ and $\hat{\varphi}_{g+1}$.

For the other two sets, a similar strategy is designed. For indices in $G_2^{(s,p)}$, we begin with $\hat{\varphi}_{s+1}$ and we end with $\hat{\varphi}_{p-1}$. If $\hat{\varphi}_g \notin [\pi/2, 3\pi/2]$, then we explore three possibilities (not necessarily disjoint):

1. $\tilde{\varphi}_g \neq \tilde{\varphi}_{g+1}$. In this case $\tilde{\varphi}_{gs} = \dots = \tilde{\varphi}_g$, where

$$\text{Sup} \{p \leq l < g : \text{Ave}(T) \in (3\pi/2, 2\pi], T = \{\theta_{ij}; j=l, \dots, g; i=1, \dots, n_i\}\}.$$

If such a gs does not exist, then this solution is rejected. Otherwise, new observations are generated as follows: $\widehat{\varphi}_{gs}^* = \dots = \widehat{\varphi}_g^* = \text{Ave}(T)$.

2. $\tilde{\varphi}_{g-1} \neq \tilde{\varphi}_g$. In this case

$$\begin{aligned}\tilde{\varphi}_g &= \dots = \tilde{\varphi}_{gi}, \text{ gi} = \inf\{p > l : \text{Ave}(T) \in [\pi/2, 3\pi/2], \\ T &= \{\theta_{ij}; j=g, \dots, l; i=1, \dots, n_i\}\}.\end{aligned}$$

If such a gi does not exist, then this solution is rejected. Otherwise, new observations are generated as follows: $\widehat{\varphi}_g^* = \dots = \widehat{\varphi}_{gi}^* = \text{Ave}(T)$.

3. $\tilde{\varphi}_g = \tilde{\varphi}_{g+1} = \tilde{\varphi}_{g-1}$. If $g = s + 1$ or $g = p - 1$, then this solution is rejected. Otherwise, new observations are generated as follows:

$$\begin{aligned}\widehat{\varphi}_g^* &= \widehat{\varphi}_{g-1}^* = \widehat{\varphi}_{g+1}^* = \text{Ave}(\theta_{ij}; i=g, g-1, g+1; j \\ &= 1, \dots, n_i)\end{aligned}$$

For indices in $G_3^{(s,p)}$, we begin with $\hat{\varphi}_p$ and end with $\hat{\varphi}_q$. For indices such that $\hat{\varphi}_g \notin (\pi, 2\pi]$, the pooling actions in $G_1^{(s,p)}$ depend on whether $0 < \hat{\varphi}_g < \pi/2$ or $\pi/2 < \hat{\varphi}_g < \pi$.

If $0 < \hat{\varphi}_g < \pi/2$, then set $\tilde{\varphi}_g = \tilde{\varphi}_{g+1}$. Because, in this case, $\tilde{\varphi}_g \in (3\pi/2, 2\pi]$, we therefore have $0 \in \text{arc}(\hat{\varphi}_g, \varphi_g)$ and so the equality follows from Proposition 2(b). New observations are generated as follows: If $\hat{\varphi}_{g+1} = 2\pi$, then $\widehat{\varphi}_g^* = 2\pi$; otherwise,

$$\widehat{\varphi}_g^* = \widehat{\varphi}_{g+1}^* = \text{Ave}(\theta_{ij}; i=g, g+1; j=1, \dots, n_i).$$

On the other hand, if $\pi/2 < \hat{\varphi}_g < \pi$, then we explore three possibilities (not necessarily disjoint):

1. $\tilde{\varphi}_g \neq \tilde{\varphi}_{g+1}$. In this case, $\tilde{\varphi}_{gs} = \dots = \tilde{\varphi}_g$, where $gs =$

$$\begin{aligned}\text{Sup } \{p \leq l < g : \text{Ave}(T) \in (3\pi/2, 2\pi], T = \{\theta_{ij}; j=l, \dots, g; i \\ = 1, \dots, n_i\}\}.\end{aligned}$$

If such a gs does not exist, then this solution is rejected. Otherwise, new observations are generated as follows: $\widehat{\varphi}_{gs}^* = \dots = \widehat{\varphi}_g^* = \text{Ave}(T)$.

2. $\tilde{\varphi}_{g-1} \neq \tilde{\varphi}_g$. In this case,

$$\begin{aligned}\tilde{\varphi}_g &= \dots = \tilde{\varphi}_{gi}, \text{ gi} = \inf\{q \geq l : \text{Ave}(T) \in (3\pi/2, 2\pi], \\ T &= \{\theta_{ij}; j=g, \dots, l; i=1, \dots, n_i\}\}.\end{aligned}$$

If such a gi does not exist, then set $\widehat{\varphi}_i^* = 2\pi \forall i \geq g$. Otherwise, new observations are generated as follows:

$$\widehat{\varphi}_g^* = \dots = \widehat{\varphi}_{gi}^* = \text{Ave}(T).$$

3. $\tilde{\varphi}_g = \tilde{\varphi}_{g+1} = \tilde{\varphi}_{g-1}$. If $g = p$ or $g = q$, then this solution is rejected. Otherwise, new observations are generated as follows:

$$\begin{aligned}\widehat{\varphi}_g^* &= \widehat{\varphi}_{g-1}^* = \widehat{\varphi}_{g+1}^* \\ &= \text{Ave}(\theta_{ij}; i=g, g-1, g+1; j=1, \dots, n_i).\end{aligned}$$

This procedure guarantees that $\forall g$, $\widehat{\varphi}_g^*$ is “in range” and satisfies the sufficient conditions of Corollary 1. For each (s, p) , we apply PAVA on these new “observations” $\widehat{\varphi}^*$ within each of the three sets. Proposition 3 guarantees that $\tilde{\varphi}^{(s,p)}$, if it exists, is the one obtained by concatenating those solutions that satisfy (5) and minimize the SCE in each set. Then the CIRE is one with smallest SCE within all acceptable $\tilde{\varphi}^{(s,p)}$. In the event of multiple local solutions, we select the one that is practically most plausible. The partition $[0, \pi/2)$, $[\pi/2, 3\pi/2]$, $(3\pi/2, 2\pi]$ has been selected because it exploits the results of Section 2.

We now summarize the algorithm. A step-by-step illustration is provided in the supplementary materials.

Step 0. Apply PAVA to obtain $\tilde{\varphi}_{PAVA}$ and set $\text{SCE} = \text{SCE}(\tilde{\varphi}_{PAVA})$, $\tilde{\varphi} = \tilde{\varphi}_{PAVA}$.

Step 1. Select a pair of indices (s, p) , such that $0 \leq s < p \leq q + 1$. Let $G_1^{(s,p)} = \{g: g \leq s\}$, $G_2^{(s,p)} = \{g: s < g < p\}$, $G_3^{(s,p)} = \{g: g \geq p\}$.

Step 2. Three sets of new subvectors are generated from $\hat{\varphi}$ separately in each group $G_i^{(s,p)}$, $i = 1, 2, 3$ defined in Step 1. This is done by sequentially examining the indices. As we have previously described, those indices that are “out of range” are pooled with other indices, following Proposition 2, and indices “in range” remain unchanged.

Step 3. Corresponding to $G_i^{(s,p)}$, $i = 1, 2, 3$, for each new subvector obtained in Step 2, apply PAVA using Corollary 1. Now select only those PAVA-transformed subvectors with components that satisfy (5) and, among these, select the one that minimizes the SCE in each set. Then $\tilde{\varphi}^{(s,p)}$ is obtained by concatenating the best solutions from each set. If $\text{SCE}(\tilde{\varphi}^{(s,p)}) < \text{SCE}$, then set $\text{SCE} = \text{SCE}(\tilde{\varphi}^{(s,p)})$ and $\tilde{\varphi} = \tilde{\varphi}^{(s,p)}$.

Step 4. Go to Step 1 and select the next acceptable pair (s, p) . The algorithm concludes when all the pairs (s, p) have been examined.

4. SIMULATION STUDIES

4.1 Design of the Simulation Experiment

We generated q independent von Mises random variables $\hat{\varphi} = (\hat{\varphi}_1, \hat{\varphi}_2, \dots, \hat{\varphi}_q)'$ with corresponding mean directions given by $\varphi = (\varphi_1, \varphi_2, \dots, \varphi_q)'$ and a common κ . Hence, $\hat{\varphi}$ is the UMLE and the CIRE $\tilde{\varphi}$ is also the RMLE. Motivated by quadratic risk for Euclidean space data, the mean circular error (MCE) of an estimator $\hat{\eta} = (\hat{\eta}_1, \hat{\eta}_2, \dots, \hat{\eta}_q)'$ for a parameter φ is

defined as $\text{MCE}(\hat{\eta}) = \sum_{i=1}^q E(1 - \cos(\hat{\eta}_i - \varphi_i))/q$. Note that $\text{MCE}(\hat{\varphi}) = 1 - (I_1(\kappa))/(I_0(\kappa))$, where $I_n(z)$ is the modified Bessel function of order n , and hence $\text{MCE}(\hat{\varphi})$ depends only on κ .

Because as $\kappa \rightarrow 0$ the von Mises distribution approaches the uniform distribution on a circle, and as $\kappa \rightarrow \infty$ it approaches the normal distribution, we considered $\kappa = 0.5, 1, 10$ to understand the impact of κ on the performance of RMLE. We also considered three patterns of q , namely, 3, 5, and 10. Although simulation studies for a variety of patterns of φ were conducted, in the interest of space, we present the results for $\varphi_1 = \varphi_2 = \dots = \varphi_q$ (denoted by φ_0), the most interesting case for RMLE. Results are summarized in Figure 2, with ordinates $\text{MCE}(\hat{\varphi})$, $\text{MCE}(\tilde{\varphi})$ and abscissa $\varphi_0 \in [0, 6.2]$, with an increment of 0.1. The solid line represents $\text{MCE}(\hat{\varphi})$. The values of $\text{MCE}(\varphi)$ are represented by either longer dashed lines ($q = 3$), shorter dashed lines ($q = 5$), or dotted lines ($q = 10$). Each MCE value was computed using 5,000 simulation runs.

4.2 Results of the Simulation Experiment

As expected, for a given κ , $\text{MCE}(\hat{\phi})$ is constant in ϕ_0 and q , but $\text{MCE}(\tilde{\phi})$ is not. For larger values of κ and q , $\text{MCE}(\phi)$ is uniformly smaller than $\text{MCE}(\hat{\phi})$ for all ϕ_0 . As q increases, the performance of RMLE is better even for very small values of κ . For example, when $q = 10$ and $\kappa = 5.0$, $\text{MCE}(\phi) \leq \text{MCE}(\hat{\phi})$ (Fig. 2). However, this is not true for very small q and κ . For instance, if $q = 3$ and $\kappa = 0.5$, then $\text{MCE}(\phi) > \text{MCE}(\hat{\phi})$ for $1.75 \leq \phi_0 \leq 4.5$. Perhaps $\tilde{\phi}$ performs poorly as $\kappa \rightarrow 0$ because the von Mises distribution in this case converges to uniform distribution on a circle, which is not informative about ϕ_0 . In our applications, based on the results of Liu et al. (2004), we expect $\kappa > 10$. Hence, we expect RMLE to perform well in practice.

5. ILLUSTRATION

We illustrate our methodology using the unrestricted estimates of the phase angles obtained by Liu et al. (2004) for data by Whitfield et al. (2002) labeled “Thythy3” in the database www.cyclebase.org/. Liu et al. (2004) estimated the phase angles of CCNE1, RRM2, CCNA2, CCNB1, and VEGFC as 0.56, 5.36, 3.55, 2.67, and 2.66 radians, respectively. These unrestricted estimates do not satisfy $0 \leq \phi_{\text{CCNE1}} \leq \phi_{\text{RRM2}} \leq \phi_{\text{CCNA2}} \leq \phi_{\text{CCNB1}} \leq \phi_{\text{VEGFC}} \leq 2\pi$.

In addition to the CIRE, for comparison purposes we also implemented the standard PAVA on the unrestricted estimates. Results are summarized in Table 1. As expected, the PAVA estimate has a larger *SCE* than the CIRE.

From Liu et al. (2004) we know that the approximate period of the cell cycle is about 15 hours 24 minutes. Thus, each radian represents approximately 2 hours 27 minutes. According to Gauthier et al. (2008) (www.cyclebase.org/), for the data discussed in this section, the *S* phase lasts approximately 3 hours 33 minutes, the *G₂* phase is about 3 hours 5 minutes, the *M* phase is about 1 hour 32 minutes, and the *G₁* phase is about 7 hours 14 minutes. The phases and the duration of time, along with the cumulative time from the *S* phase are summarized in Table 2. Using these estimates we converted our phase angle estimates into cell cycle phases. Thus in Table 1, corresponding to each estimation procedure, we provide the estimated phase angles as well as the estimated phases of each gene. In column 2 we provide the phase of each gene as determined by Gauthier et al. (2008) (denoted as *G*), Datta and Sokhansanj (2007) (denoted by *S*), and those by Whitfield et al. (2002) (denoted by *W*). For some genes, Gauthier et al. (2008) did not provide their estimates of the phase, and in such cases *G* is missing. Results are displayed graphically in Figure 3.

Consistent with earlier findings reported in column 2, all estimation procedures assigned CCNE1 to the *S* phase. However, the three estimation procedures differed on the phase assignment of RRM2, with the CIRE’s assignment agreeing with that in column 2. A surprising result was noted with respect to CCNA2. Gauthier et al. (2008), Datta and Sokhansanj (2007), and Whitfield et al. (2002) assigned this gene to the *G₂* phase, whereas CIRE assigned CCNA2 to the *M* phase. Based on the expression profile of CCNA2 (Fig. S-5 in the supplementary materials) across three periods (a total of about 46 hours), it appears that the peak expression of CCNA2 is more likely to be during the *M* phase than during the *G₂* phase. Thus, based on the mRNA data of Whitfield et al. (2002) and the unrestricted phase angle estimates of Liu et al. (2004), our phase assignment of CCNA2 seems to be more reasonable than that of Gauthier et al. (2008), Datta and Sokhansanj (2007), and Whitfield et al. (2002). The result of PAVA for gene RRM2 appears to be inconsistent with CIRE. PAVA assigns RRM2 to the *M* phase whereas the CIRE agrees with Gauthier et al. (2008) and Whitfield et al. (2002).

Using the strategy described in the Introduction, the phase angle estimates under the rotation-invariant simple order restriction for the five genes, CCNE1, RRM2, CCNA2, CCNB1 and VEGFC are 6.10, 6.10, 2.95, 2.95 and 2.95, respectively.

6. CONCLUDING REMARKS AND OPEN PROBLEMS

This article takes the first step towards statistical inference on angular parameters when they are ordered around a unit circle. We have developed the theory and methodology for computing the circular isotonic regression estimators when the components of the angular parameter ϕ are constrained by a simple order restriction on a unit circle.

There are several problems that remain to be addressed and serve as topics for future research. For instance, it would be useful to explore theoretical properties of the proposed estimation procedures along the lines of Rueda, Salvador, and Fernández (1997). Other interesting research questions include the determination of standard errors for the CIRE and the associated confidence intervals using resampling procedures such as those explored in Rueda, Menéndez, and Salvador (2002) for R^q data.

A cell biologist may be interested in testing the hypothesis that a subset of cell cycle genes followed a particular order around the circle during cell division. Before deriving the suitable test statistic and its null distribution, an important aspect of this problem is the formulation of suitable null and alternative hypotheses.

As commonly done for Euclidean space data, it would be useful to develop methods for estimating angular parameters by combining data from multiple laboratories/experiments, while accounting for variation among experiments. For example recently three different laboratories (Rustici et al. 2004; Oliva et al. 2005; Peng et al. 2005) conducted a total of ten different yeast cell cycle experiments under two different platforms. The experimental conditions among the 10 experiments are not necessarily identical. Consequently, it may not be reasonable to assume that the concentration parameters of the phase angles from such experiments are the same (and known). It would be useful to derive rotation-invariant simple order restricted estimators using data from such heterogeneous experiments.

In summary, we believe there are several theoretical problems in this line of research that may have applications to cell biology, and possibly other applied fields.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

APPENDIX: PROOFS

For $\mathbf{u} = (u_1, u_2, \dots, u_q)'$, $\hat{\boldsymbol{\phi}} = (\hat{\phi}_1, \hat{\phi}_2, \dots, \hat{\phi}_q)'$, we define $G(\hat{\boldsymbol{\phi}}, \mathbf{u}) = \sum_{g=1}^q r_g d(u_g, \hat{\phi}_g)$. Let $C = \{\mathbf{x} \in [0, 2\pi]^q: 0 \leq x_1 \leq x_2 \leq \dots \leq x_q \leq 2\pi\}$ denote the simple order set and let $C_J = \{\mathbf{x} \in [0, 2\pi]^q: x_i = x_{i+1} \text{ if } i \in J, \text{ and } x_i < x_{i+1} \text{ if } i \in I-J\}$ denote a face C . The “relative interior” of C is $C^I = \{\mathbf{x} \in [0, 2\pi]^q: 0 < x_1 < x_2 < \dots < x_q < 2\pi\}$ with the boundary of C being $C^B = C - C^I = \bigcup_{j \in I} C_j$. First we prove the following lemma which will be useful for proving the other results.

A.1 Lemma 1

Let $\mathbf{u} = (u_1, u_2, \dots, u_q)'$, $\mathbf{v} = (v_1, v_2, \dots, v_q)'$, and $\hat{\boldsymbol{\phi}} = (\hat{\phi}_1, \hat{\phi}_2, \dots, \hat{\phi}_q)'$. Suppose, for $i = 1, 2, \dots, q$, u_i, v_i , and $\hat{\phi}_i \in [0, 2\pi]$, then the following holds:

- a. if for all $i = 1, 2, \dots, q$,

$$\pi \geq \hat{\phi}_i - v_i \geq \hat{\phi}_i - u_i \geq 0 \text{ or } -\pi \leq \hat{\phi}_i - v_i \leq \hat{\phi}_i - u_i \leq 0, \text{ then } G(\hat{\boldsymbol{\phi}}, \mathbf{v}) \geq G(\hat{\boldsymbol{\phi}}, \mathbf{u}).$$

- b. If for all $i = 1, 2, \dots, q$,

$$\pi \leq \hat{\phi}_i - v_i \leq \hat{\phi}_i - u_i \leq 2\pi \text{ or } -\pi \geq \hat{\phi}_i - v_i \geq \hat{\phi}_i - u_i \geq -2\pi, \text{ then } G(\hat{\boldsymbol{\phi}}, \mathbf{v}) \geq G(\hat{\boldsymbol{\phi}}, \mathbf{u}).$$

- c. In both cases (a) and (b), $G(\hat{\boldsymbol{\phi}}, \mathbf{v}) = G(\hat{\boldsymbol{\phi}}, \mathbf{u}) \Leftrightarrow \mathbf{v} = \mathbf{u}$

Proof

- a. Proof follows from the fact that $\cos(x)$ decreases in x if $x \in [-\pi, \pi]$.
- b. Proof follows from the fact that $\cos(x)$ increases in x if $x \in [\pi, 2\pi]$ or $x \in [-2\pi, -\pi]$.
- c. Clearly, if $\mathbf{u} = \mathbf{v}$, then $G(\hat{\boldsymbol{\phi}}, \mathbf{v}) = G(\hat{\boldsymbol{\phi}}, \mathbf{u})$. To prove the converse, suppose there exists an index i such that $u_i \neq v_i$ and $\cos(\hat{\phi}_i - v_i) < \cos(\hat{\phi}_i - u_i)$. This implies that
- $$\sum_{g=1}^q r_g \cos(\hat{\phi}_i - v_i) < \sum_{g=1}^q r_g \cos(\hat{\phi}_i - u_i) \Rightarrow G(\hat{\boldsymbol{\phi}}, \mathbf{v}) < G(\hat{\boldsymbol{\phi}}, \mathbf{u}), \text{ which is a contradiction. Hence, it is necessary that } \mathbf{u} = \mathbf{v}.$$

A.2 Proof of Theorem 1

The problem is to find the minimum of a continuous function in a closed and convex set in R^q . From standard results in real analysis we know that the function has an upper bound and attains it. We first prove that the minimum is attained on the boundary of the convex cone C . Let $x_0 = 0$ and $x_{q+1} = 2\pi$. Assume that the minimum $\hat{\boldsymbol{\phi}}$ is attained in C^I and that $\hat{\boldsymbol{\phi}} \notin C$. Let $\mathbf{v} = \hat{\boldsymbol{\phi}} - \hat{\boldsymbol{\phi}}$. Then we demonstrate that none of the following three possibilities can be true:

- a. $-\pi < v_i < \pi, \forall i = 1, \dots, q$.
- b. There exists an index i such that $v_i = \hat{\phi}_i - \hat{\phi}_i > -\pi$.
- c. There exists an index i such that $v_i = \hat{\phi}_i - \hat{\phi}_i < -\pi$.

If (a) is true then there exists a λ between 0 and 1 such that $\mathbf{h} = \hat{\boldsymbol{\phi}} + \lambda \mathbf{v} \in C^B$. From Lemma 1, we have $G(\hat{\boldsymbol{\phi}}, \mathbf{h}) < G(\hat{\boldsymbol{\phi}}, \hat{\boldsymbol{\phi}})$, but this contradicts the fact that $\hat{\boldsymbol{\phi}}$ is the CIRE. Suppose (b) is true. Then we have $\hat{\phi}_{i+1} - \hat{\phi}_i > \hat{\phi}_i - \hat{\phi}_i > \pi$. Now consider a new point $b_g = \hat{\phi}_g$ if $g \neq i$, else $b_g =$

$\tilde{\varphi}_{i+1}$, then clearly $\mathbf{b} \in C^B$ and from Lemma 1 we must have $G(\hat{\varphi}, \mathbf{b}) < G(\hat{\varphi}, \tilde{\varphi})$, which again contradicts the fact that $\hat{\varphi}$ is the CIRE. Similar to (b), we can also demonstrate that (c) cannot be true. Next we prove that $\varphi_{S(i)} = \text{Ave}(S(i))$. We begin by showing that

$$\tilde{\varphi}_{S(i-1)} \leq \text{Ave}(S(i)) \leq \tilde{\varphi}_{S(i+1)} \quad (\text{A.1})$$

If (A.1) were false, then it is necessary that one of the following four statements is true:

- a. $\text{Ave}(S(i)) < \tilde{\varphi}_{S(i-1)}$ and $\tilde{\varphi}_{S(i)} - \text{Ave}(S(i)) < \pi$.
- b. $\text{Ave}(S(i)) > \tilde{\varphi}_{S(i+1)}$ and $\tilde{\varphi}_{S(i)} - \text{Ave}(S(i)) > \pi$.
- c. $\text{Ave}(S(i)) < \tilde{\varphi}_{S(i-1)}$ and $\tilde{\varphi}_{S(i)} - \text{Ave}(S(i)) > \pi$.
- d. $\text{Ave}(S(i)) > \tilde{\varphi}_{S(i+1)}$ and $\tilde{\varphi}_{S(i)} - \text{Ave}(S(i)) < \pi$.

In the following we demonstrate that none of these four statements can be true. Suppose (a) is true. Define $b_g = \tilde{\varphi}_{S(i-1)}$ if $g \in S(i)$, else define $b_g = \varphi_g$, then we have $b \in C$. Now, applying Lemma 1, we have $G(\hat{\varphi}, \mathbf{b}) < G(\hat{\varphi}, \hat{\varphi})$, which is a contradiction. Using a similar argument and the same vector $\mathbf{b}$, we can prove that (b) cannot be true either. Letting $e_g = \tilde{\varphi}_{S(i+1)}$, if $g \in S(i)$, else letting $e_g = \varphi_g$, then we have $e \in C$. Using an argument similar to those used in (a) and (b), we also prove that cases (c) and (d) do not hold. If $g \in S(i)$, then let $\tilde{\varphi}_g = \text{Ave}(S(i))$; otherwise, let $\tilde{\varphi}_g = \varphi_g$ then

$$\begin{aligned} \sum_{g=1}^q r_g d(\tilde{\varphi}_g, \tilde{\varphi}_g) &= \sum_{g \in S(i)} r_g - r_{S(i)} \cos(\text{Ave}(S(i)) - \text{Ave}(S(i))) \\ &\quad + \sum_{j \neq i} \left| \sum_{g \in S(j)} r_g - r_{S(j)} \cos(\text{Ave}(S(j)) - \tilde{\varphi}_{S(j)}) \right| \\ &\leq \sum_{g \in S(i)} r_g - r_{S(i)} \cos(\text{Ave}(S(i)) - \tilde{\varphi}_{S(i)}) \\ &\quad + \sum_{j \neq i} \left| \sum_{g \in S(j)} r_g - r_{S(j)} \cos(\text{Ave}(S(j)) - \tilde{\varphi}_{S(j)}) \right| \\ &= \sum_{g=1}^q r_g d(\tilde{\varphi}_g, \tilde{\varphi}_g) = \min_{\varphi \in C} \sum_{g=1}^q r_g d(\tilde{\varphi}_g, \varphi_g). \end{aligned}$$

b From (A.1) we have $\tilde{\varphi} \in C$; hence, $\tilde{\varphi} = \tilde{\varphi}$ and $\text{Ave}(S(i)) = \tilde{\varphi}_{S(i)}$.

A.3 Proof of Theorem 2

Let L_J be the biggest subspace contained on a face C_J of C . Then the minimization problem $\arg \min_{\varphi \in L_J} G(\hat{\varphi}, \varphi)$ has a unique solution (Mardia and Jupp 2000). Suppose that there are a total of r different subspaces ($L_i, i = 1, \dots, r$) associated with faces of C denoted by $G_i(\hat{\varphi}) \min_{\varphi \in L_i} G(\hat{\varphi}, \varphi), i = 1, 2, \dots, r$. Then $\Pr[\exists i, i' \in \{1, \dots, r\} \text{ with } G_i(\hat{\varphi}) = G_{i'}(\hat{\varphi})] = \Pr[\cup_{i,i'} (G_i(\hat{\varphi}) = G_{i'}(\hat{\varphi}))] \leq \sum_{i,i'} \Pr[G_i(\hat{\varphi}) = G_{i'}(\hat{\varphi})] = 0$. The sum has a finite number of summands and the sets inside the brackets have probability zero because they are Lebesgue measure zero sets and hence the result follows.

A.4 Proof of Proposition 1

We prove (a), the proof of (b) follows similarly. If $\tilde{\varphi}_g < \tilde{\varphi}_{g+1}$, then consider the following possibilities: (i) $\hat{\varphi}_g \leq \varphi_g$, (ii) $\hat{\varphi}_g > \varphi_g$, $\hat{\varphi}_g \leq \varphi_{g+1}$, and (iii) $\varphi_g > \varphi_g$, $\hat{\varphi}_g \geq \varphi_{g+1}$. We prove that none of these three are true. Suppose (i) is true. Then $\hat{\varphi}_g \leq \varphi_g \Rightarrow \varphi_{g+1} < \varphi_g \leq \varphi_g < \varphi_{g+1}$ and $\varphi_g - \varphi_{g+1} < \varphi_{g+1} - \hat{\varphi}_{g+1} < \pi$.

The last inequality in the previous expression is a consequence of $0 \notin \text{arc}(\tilde{\varphi}_{g+1}, \hat{\varphi}_{g+1})$. Define $b_i = \varphi_{g+1}$, if $i = g$, else define $b_i = \varphi_i$, then $b \in C$. Now, applying Lemma 1, we have $G(\hat{\varphi}, b) < G(\hat{\varphi}, \varphi)$, which is a contradiction.

Suppose (ii) is true; then, $\tilde{\varphi}_g < \hat{\varphi}_g < \tilde{\varphi}_{g+1}$. Letting $b_i = \hat{\varphi}_g$, if $i = g$ else letting $b_i = \varphi_i$ and using a similar argument as in (i), we conclude that (ii) cannot be true either. Finally, suppose (iii) is true. Then we have $\varphi_g \geq \varphi_{g+1} > \varphi_g$ and $0 < \hat{\varphi}_g - \varphi_{g+1} < \hat{\varphi}_g - \varphi_g < \pi$. The last inequality is as result of the fact that $0 \notin \text{arc}(\varphi_g, \hat{\varphi}_g)$. Let $b_i = \varphi_{g+1}$, if $i = g$, else let $b_i = \varphi_i$, then again from Lemma 2.1 we have $G(\hat{\varphi}, b) < G(\hat{\varphi}, \varphi)$, which is a contradiction.

A.5 Proof of Proposition 2

We prove (b), the proof of (a) follows similarly. Suppose that $\pi \leq \hat{\varphi}_g < 2\pi$, $0 \in \text{arc}(\hat{\varphi}_g, \tilde{\varphi}_g)$ and $\varphi_g > \varphi_{g-1}$, then $0 < \hat{\varphi}_g - \varphi_{g-1} < \hat{\varphi}_g - \varphi_g < \pi$. Let $b_i = \varphi_{g-1}$, if $i = g$, else let $b_i = \varphi_i$, then $b \in C$. Applying Lemma 1, we have $G(\hat{\varphi}, b) < G(\hat{\varphi}, \varphi)$, which is a contradiction.

A.6 Proof of Theorem 3

The proof of Theorem 3 follows by applying Proposition 1 (a) sequentially to each pair of indices in a reverse order.

A.7 Proof of Corollary 1

The result follows from Theorem 2.9 as

$$\begin{aligned} \eta_1 &\geq 0, \eta_2 \leq 2\pi, \eta_2 - \eta_1 \leq \pi \\ &\Rightarrow 0 \notin \text{arc}(\eta_1, \eta_2) \Rightarrow 0 \notin \text{arc}(\widehat{\varphi}_g, \widehat{\varphi}_{g'}) \forall g, g' \\ &\in \{1, \dots, q\}. \end{aligned}$$

A.8 Proof of Proposition 3

If $\tilde{\varphi}_g = \tilde{\varphi}_{g+1}$, then

$$\begin{aligned} \min_{\varphi \in C} \sum_{i=1}^q r_i d(\widehat{\varphi}_i, \varphi_i) &= \min_{\varphi \in C, \varphi_g = \varphi_{g+1}} \sum_{i=1}^q r_i d(\widehat{\varphi}_i, \varphi_i) \\ &= \min_{\varphi \in C, \varphi_g = \varphi_{g+1}} \sum_{i=1}^q \sum_{j=1}^{n_g} d(\theta_{ij}, \varphi_i) \\ &= \min_{\varphi \in C, \varphi_g = \varphi_{g+1}} \sum_{i=1, i \neq g+1}^q r_i^* d(\widehat{\varphi}_{i^*}, \varphi_i), \end{aligned}$$

where $\hat{\varphi}_{g^*} = \text{Ave}(\theta_{ij}, i = g, g+1; j = 1, \dots, n_i)$, $n_{g^*} = n_g + n_{g+1}$, $\hat{\varphi}_{i^*} = \hat{\varphi}_i \forall i \neq g, g+1$, $r_{g^*} =$ The resultant length of the angles $(\theta_{ij}, i = g, g+1; j = 1, \dots, n_i)$, $r_{i^*} = r_i, \forall i \neq g, g+1$. Hence the result follows.

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Figure 1.

Phases of a normal cell division cycle. The dark lines describe the boundaries of the phases and the arrows correspond to the angle of peak expression of genes, *Gene 1* and *Gene 2*. The outside arrow points in the direction of the cell cycle.

Figure 2.
 $MCE(\varphi)$ and $MCE(\hat{\varphi})$, for a 3-, 5-, and 10-dimensional parameter when all coordinates are equal and for κ values 0.5, 1, and 10.

Figure 3. (a) Unrestricted estimates from Liu et al. (2004). (b) PAVA. (c) CIRE. Dotted arrows point to the estimates and the solid lines are the boundaries between phases. Angles are in radians reported in Table 1. Phase angle estimates are in parentheses.

Table 1
Estimates of phase angles and phase classifications from different procedures.

Gene	Phase*	Unrestricted Estimates		PAVA		CIRE	
		Phase	Angle	Phase	Angle	Phase	Angle
CCNE1	$G_1/S(W, S)$	S	0.56 radians 1 hr 12 min	S	0.56 radians 1 hr 12 min	S	0
RRM2	$S(G, W, S)$	G_1	5.36 radians 13 hr 8 min	M	3.27 radians 8 hr 1 min	S	0
CCNA2	$G_2(G, W, S)$	G_1	3.55 radians 8 hr 42 min	M	3.27 radians 8 hr 1 min	M	2.95 radians 7 hr 14 min
CCNB1	$G_2/M(W) M(S)$	G_2	2.67 radians 6 hr 32 min	M	3.27 radians 8 hr 1 min	M	2.95 radians 7 hr 14 min
VEGFC	$M(G) M/G_1(W, S)$	G_2	2.66 radians 6 hr 31 min	M	3.27 radians 8 hr 1 min	M	2.95 radians 7 hr 14 min
Sum of Circular Error (SCE)					1.8902		0.8048

NOTE: All angles are estimated from the beginning of the S phase.

* G, Gauthier; W, Whitfield; S, Datta and Sokhansanj.

Table 2

Duration of time spent in each phase of the cell cycle in the double thymidine block experiment of Whitfield et al. (2002)

Phase	Duration of Time in the Phase	Time Interval
<i>S</i>	3 hr 33 min	0–3 hr 33 min
<i>G₂</i>	3 hr 05 min	3 hr 33 min–6 hr 38 min
<i>M</i>	1 hr 32 min	6 hr 38 min–8 hr 10 min
<i>G₁</i>	7 hr 14 min	8 hr 10 min–15 hr 24 min

NOTE: Data provided by N. Gauthier.