

# SoCal: supervised genotype calling via ellipsoid separation for Affymetrix SNP microarray

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In this article, I present SoCal, a supervised genotype calling algorithm for Affymetrix SNP microarray. For each SNP, SoCal first efficiently identify ellipsoidal decision regions for each genotype from reference genotype calls, and then uses these regions to classify future SNPs into different genotypes. Using only a small portion of training genotype calls from the HapMap Project, SoCal achieves an accuracy of 97.5% during validation.

## 1 Introduction

Accurate genotyping of SNPs is essential to discovering true signals in association studies. Although next generation sequencing technology provides cheap whole-genome sequences for genotyping SNPs, SNP microarray is still a cost-effective genotyping technology for many specific association studies. In an Affymetrix SNP microarray, oligonucleotide probes are used to match and bind DNA fragments containing biallelic SNPs. Then a fluorescence scanner scans the microarray to quantify perfect match and mismatch of these fragments. Most genotype calling procedures for SNP microarray consists of two steps. In the first step, raw information from microarray is summarized to obtain the intensities,  $\theta_A$  and  $\theta_B$ , of the two alleles, denoted by A and B, of each SNP. In the second step, SNPs are classified into genotype AA, AB, or BB based on the allele intensities they generate. The focus of this article is on the second step of the genotype calling procedure—genotype calling using summarized allele intensities.

For a specific SNP, if a sample has genotype AA or BB, the allele intensity,  $\theta_A$  or  $\theta_B$ , will be higher respectively. If a sample has genotype AB, the intensities,  $\theta_A$  and  $\theta_B$ , will be similar. If one plots  $\log(\theta_A)$  versus  $\log(\theta_B)$  of a SNP for a number of samples, normally 3 ellipsoidal clusters can be observed, one for each genotype, as shown in Figure 1. Many genotype calling algorithms use model-based unsupervised clustering to identify these clusters and then assign genotypes to each cluster. Although these methods are applicable to a wide range of microarrays, they don't take advantage of genotype calls that are already available. Also, these methods use the EM algorithm to estimate model parameters, which is sensitive to starting parameters and slow to converge. To utilize reference genotype calls, Rabbie et al. proposed the RLMM algorithm, a supervised genotype calling method that uses reference genotype calls to form decision regions by fitting bivariate Gaussian distributions on observed allele intensities for each genotype.

These Gaussian decision regions are then used to call SNPs with unknown genotype. However, fitting a Gaussian distribution is known to be sensitive to outliers, which, for SNP microarrays, can be caused by genomic structural variations.

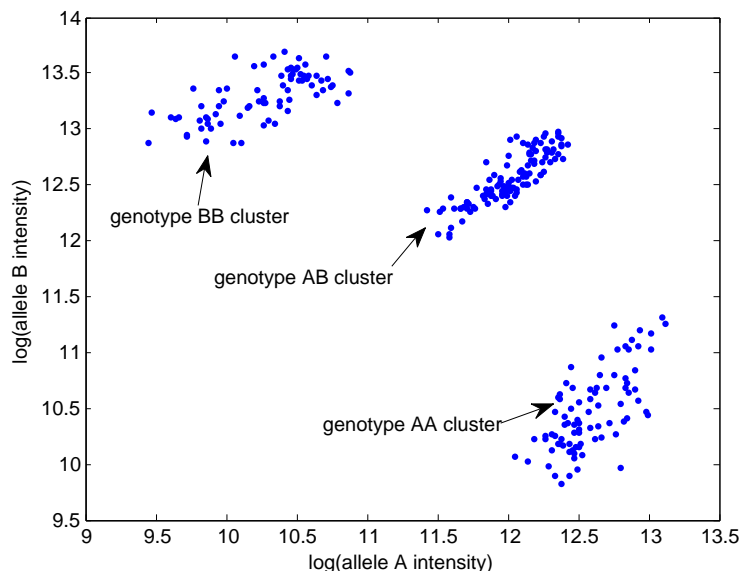


Figure 1: Genotype clusters obtained from Affymetrix SNP array allele intensity values

In this article, I present SoCal, a supervised genotype calling algorithm for Affymetrix SNP microarrays. Instead of fitting Gaussian distributions on allele intensities with reference genotype calls, SoCal efficiently finds ellipsoidal decision regions for each genotype of a SNP by solving a conic programming problem. SoCal can control the effect of outliers by assigning different weights to each of the criteria for finding the ellipsoids—separation ratio, ellipsoid volume, and number of enclosed points. After SoCal finds the ellipsoidal decision regions for each genotype of a SNP, it uses them to call the same SNP with unknown genotype.

Using reference genotype calls from the HapMap Project as training and validation data, SoCal achieved 99.71% accuracy at a call rate of 95% during leave-one-out cross-validation. Furthermore, SoCal shows more robustness than the RLMM method when there are outliers in the training data.

## 2 Methods

### 2.1 Overview of SoCal's genotype calling procedure

SNP allele intensities are first summarized from raw microarray data using SNPRMA, which removes non-biological effect from the data. After this step, SoCal calls genotypes in two steps. In the first step, SoCal finds ellipsoidal decision regions for each of the

genotype of a SNP using reference genotype calls. In the second step, SoCal classifies SNPs with unknown genotypes using minimum distance classification.

## 2.2 Pattern separation by ellipsoid

An ellipsoid  $\mathcal{E} \subseteq \mathbb{R}^n$  can be expressed as  $\mathcal{E} = \{x \in \mathbb{R}^n | (x - c)^T E (x - c) \leq 1\}$ , where  $c$  is the center of the ellipsoid, and  $E$  a positive definite matrix denoting the shape and orientation of the ellipsoid. Let  $\{a_i\}$  be the points to be included in an ellipsoid, and  $\{b_j\}$  be the points to be excluded, the problem of ellipsoidal separation is to find  $c$  and  $E$  such that  $(a_i - c)^T E (a_i - c) \leq 1 \forall i$  and  $(b_j - c)^T E (b_j - c) > 1 \forall j$ .

## 2.3 Forming ellipsoidal decision regions for each genotype

Let  $G = \{AA, AB, BB\}$  be the set of genotypes of a SNP, and  $J_{AA}, J_{AB}, J_{BB}$  the index set of samples with the corresponding genotype. Let  $X = \{(\log(\theta_A), \log(\theta_B))_i | i = 1, \dots, |J_{AA}| + |J_{AB}| + |J_{BB}|\}$  be the set of log transformed allele intensities of all the samples, and  $X_{AA} = \{x_j | x_j \in X, j \in J_{AA}\}$ ,  $X_{AB} = \{x_j | x_j \in X, j \in J_{AB}\}$ ,  $X_{BB} = \{x_j | x_j \in X, j \in J_{BB}\}$  the set of log transformed allele intensities from samples having the corresponding genotype.

To find the ellipsoid that includes  $X_{AA}$  and excludes  $X_{AB} \cup X_{BB}$ , one sets  $\{a_i\} = X_{AA}$  and  $\{b_j\} = X_{AB} \cup X_{BB}$ , and solves the following conic programming problem. For the sake of space, detailed derivation of the problem formulation is not presented here.

$$\begin{aligned} & \text{minimize} && -\beta_1 k + \beta_2 \text{trace}(T) + \beta_3 \|u - \mathbb{1}\|_1 \\ & \text{subject to} && (1, a_i)^T \tilde{E} (1, a_i) \leq u_i \quad \forall i \\ & && (1, b_j)^T \tilde{E} (1, b_j) \geq k \quad \forall j \\ & && \tilde{E} = \begin{bmatrix} s & v^T \\ v & F \end{bmatrix} \geq 0 \\ & && \begin{bmatrix} F & I \\ I & T \end{bmatrix} \geq 0 \end{aligned}$$

In the problem formulation above  $\beta_i > 0$  are the weights assigned to each criteria of finding the ellipsoid—separation ratio, ellipsoid volume, and number of enclosed points. In SoCal,  $\beta_1, \beta_2, \beta_3$  are empirically set to 1,  $10^4$ , and  $10^2$  respectively.

Let  $\tilde{E}^* = \begin{bmatrix} s & v^T \\ v & F \end{bmatrix}$  be the optimal solution to the problem above. The separating ellipsoid  $\mathcal{E}^*$  is defined as  $\mathcal{E}^* = \{x \in \mathbb{R}^n | (x - c^*)^T E^* (x - c^*) \leq 2(1 + k)\}$ , where  $c^* = -F^{-1}v$ ,  $E^* = \frac{F}{(1 - s + c^{*T} F c^*)}$ .

To find the ellipsoid that includes  $X_{AB}$  and excludes  $X_{AA} \cup X_{BB}$ , one sets  $\{a_i\} = X_{AB}$  and  $\{b_j\} = X_{AA} \cup X_{BB}$ , and solves the above conic programming problem. The same procedure also applies to finding the ellipsoid that includes  $X_{BB}$  and excludes  $X_{AA} \cup X_{AB}$ .

## 2.4 Rescuing missing genotype clusters

If a SNP has moderate minor allele frequency (MAF), the genotype clusters of that SNP are well defined, and SoCal obtains three ellipsoidal decision regions for that SNP, one for each genotype (Figure 2a). However, if a SNP has lower MAF, some genotype cluster may not be well defined. For these SNPs, SoCal estimates the ellipsoid for the missing genotype cluster using the ellipsoids for the other two genotypes through simple geometric transformations (Figure 2b).

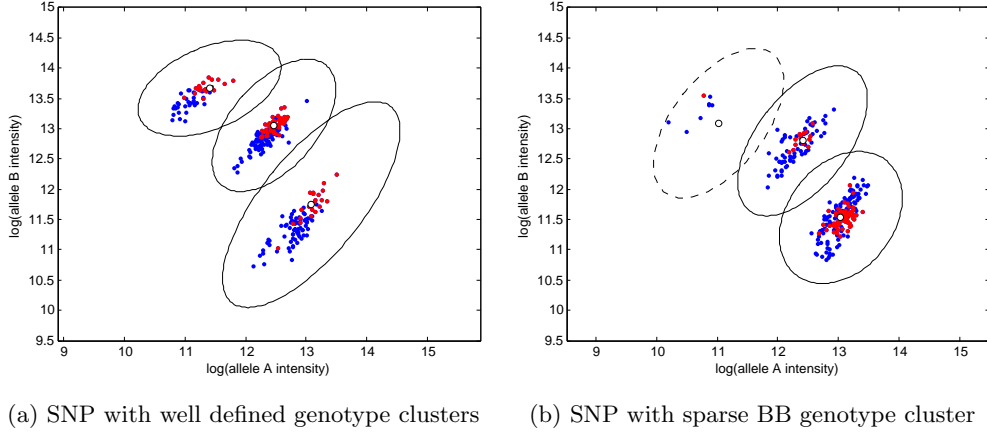


Figure 2: Each dot in the plots above represents a sample, with samples having HapMap reference genotype calls marked red. The ellipsoids were obtained using only the reference calls.

### 2.4.1 Missing genotype AA or BB cluster

If genotype  $AA$  cluster of a SNP has less than 3 reference calls, SoCal first finds the ellipsoids for genotype  $AB$  and  $BB$  clusters, and then estimates that for genotype  $AA$  cluster through simple geometric transformations.

Let  $\mathcal{E}_{AB} = \{x \in \mathbb{R}^n | (x - c_{AB})^T E_{AB} (x - c_{AB}) \leq 1\}$  and  $\mathcal{E}_{BB} = \{x \in \mathbb{R}^n | (x - c_{BB})^T E_{BB} (x - c_{BB}) \leq 1\}$  be the ellipsoids obtained for genotype  $AB$  and  $BB$  clusters, and  $n_{AB}$ ,  $n_{BB}$  the unit vectors pointing in the direction of the major axis of the corresponding ellipsoid. SoCal estimates the center of  $\mathcal{E}_{AA}$ , the ellipsoid for genotype  $AA$  cluster, by reflecting  $c_{BB}$ , the center of  $\mathcal{E}_{BB}$ , across the major axis of  $\mathcal{E}_{AB}$ . To estimate the orientation of  $\mathcal{E}_{AA}$ , SoCal first determines the angle between  $n_{AB}$  and  $n_{BB}$ , and then applies a rotation matrix of that angle on  $E_{AB}$ .

Formally, let  $\mathcal{E}_{AA} = \{x \in \mathbb{R}^n | (x - c_{AA})^T E_{AA} (x - c_{AA}) \leq 1\}$  be the estimated ellipsoid for genotype  $AA$  cluster, and  $\alpha$  the angle between  $n_{AB}$  and  $n_{BB}$ , then  $c_{AA} = -c_{BB} + 2c_{AB} + 2n_{AB}((c_{BB} - c_{AB})^T n_{AB})$ , and  $E_{AA} = R^T E_{AB} R$ , where  $R$  is a rotation matrix of angle  $\alpha$ .

If genotype  $BB$  cluster is missing, the center and orientation of the ellipsoid for that cluster is estimated in a similar way. Formally, let  $\mathcal{E}_{BB} = \{x \in \mathbb{R}^n | (x - c_{BB})^T E_{BB} (x -$

$c_{BB}) \leq 1\}$  be the estimated ellipsoid for genotype  $BB$  cluster, and  $\alpha$  the angle between  $n_{AB}$  and  $n_{AA}$ , then  $c_{BB} = -c_{AA} + 2c_{AB} + 2n_{AB}((c_{AA} - c_{AB})^T n_{AB})$ , and  $E_{BB} = R^T E_{AB} R$ , where  $R$  is a rotation matrix of angle  $-\alpha$ .

#### 2.4.2 Missing genotype AB cluster

Although SNPs with genotype  $AB$  cluster missing were not observed in HapMap reference genotype calls, for completeness, for these SNPs SoCal first obtains,  $\mathcal{E}_{AA}$  and  $\mathcal{E}_{BB}$ , the ellipsoids for genotype  $AA$  and  $BB$  cluster, and then estimates the center of  $\mathcal{E}_{AB}$ , the ellipsoid for the missing cluster, using the mid-point between the centers of  $\mathcal{E}_{AA}$  and  $\mathcal{E}_{BB}$ . The orientation of  $\mathcal{E}_{AB}$  is obtained by applying a rotation to the ellipsoid with the minimum volume among  $\mathcal{E}_{AA}$  and  $\mathcal{E}_{BB}$ .

Formally, let  $\mathcal{E}_{AB} = \{x \in \mathbb{R}^n | (x - c_{AB})^T E_{AB} (x - c_{AB}) \leq 1\}$  be the estimated ellipsoid for genotype  $AB$  cluster, and  $\alpha$  the angle between  $n_{AA}$  and  $n_{BB}$ , then  $c_{AB} = (c_{AA} + c_{BB})/2$ , and  $E_{AB} = R^T \hat{E} R$ , where  $\hat{E}$  is the matrix of the ellipsoid with the minimum volume among  $\mathcal{E}_{AA}$  and  $\mathcal{E}_{BB}$ , and  $R$  a rotation matrix of angle  $\pm\alpha/2$ . The sign of the angle of rotation is dependent on the choice of ellipsoid on which rotation is applied—positive for  $\mathcal{E}_{AA}$  and negative for  $\mathcal{E}_{BB}$ .

### 2.5 Genotype calling

After the ellipsoidal decision regions,  $\mathcal{E}_g = \{x \in \mathbb{R}^n | (x - c_g)^T E_g (x - c_g) \leq 1\}, \forall g \in \{AA, AB, BB\}$  of a SNP are obtained, SoCal uses them to classify SNPs with unknown genotypes using minimum distance classification.

If a sample has allele intensity  $\theta_A$  and  $\theta_B$  at SNP  $n$ , SoCal first computes  $D_g = \sqrt{(x - c_g)^T E_g (x - c_g)}$ , where  $x = (\log(\theta_A), \log(\theta_B))$ , for each  $g \in \{AA, AB, BB\}$ . SoCal then calls the genotype,  $\mathcal{G}$ , of that sample at SNP  $n$  as the genotype having the minimum  $D_g$ , that is,  $\mathcal{G} = \arg \min_{g \in \{AA, AB, BB\}} D_g$ . SoCal defines  $\lambda = 1 - D_{\mathcal{G}} / (D_{AA} + D_{AB} + D_{BB})$  to quantify the confidence of each genotype call.

## 3 Materials

The microarray used for evaluation in this project was the Affymetrix GeneChip Human Mapping 50K Xba Array, which contains 58,960 SNPs. Raw microarray data for 270 samples was obtained from HapMap FTP. And reference genotype calls were obtained from HapMap using HapMart.

After removing strand-ambiguous SNPs and SNPs not present on HapMart from the microarray, 16,387 SNPs were left. Figure 3 shows the minor allele frequency distribution for these SNPs.

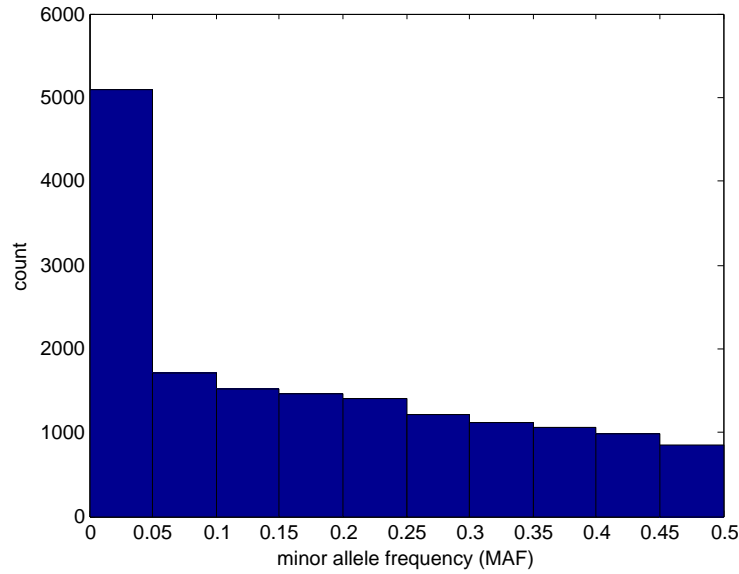


Figure 3: Minor allele frequency distribution for the 16,387 SNPs.

From the 16,387 SNPs, 4,064 SNPs with two genotype clusters having less than 3 reference genotype calls were further removed from the microarray. Among these SNPs, 3,596 are monomorphic SNPs. In total, 12,323 SNPs were left for evaluation. On average, each of these SNPs has 83 reference genotype calls.

## 4 Results

### 4.1 Cross-validation with HapMap reference calls

To evaluate the accuracy of SoCal, I compared the genotype calls made by SoCal with the reference calls from HapMap through leave-one-out cross-validation. For each SNP, I used one sample from the reference set as validation data and the rest as training data. I repeated this process until all the samples in the reference set were used as validation data exactly once.

Table 1 shows the genotype calls made by SoCal and those available on HapMap during leave-one-out cross-validation. At a call rate of 100%, SoCal made 1,081,319 calls in total, out of which 1,069,857 were concordant with HapMap calls, achieving a concordance rate of 98.94%.

| HapMap/SoCal | AA      | AB      | BB      | No Call |
|--------------|---------|---------|---------|---------|
| AA           | 360,289 | 2,282   | 1,058   | 0       |
| AB           | 2,667   | 341,012 | 2,257   | 0       |
| BB           | 851     | 2,347   | 368,556 | 0       |

Table 1: At a call rate of 100%, SoCal achieved 98.94% concordance rate in the leave-one-out cross-validation with HapMap reference calls.

By increasing the threshold for the confidence score  $\lambda$ , SoCal can achieve higher concordance rate at the cost of making fewer calls. At  $\lambda \geq 0.863$  and call rate of 95%, SoCal made 1,028,258 calls in total, out of which 1,025,242 were concordant with HapMap calls, achieving a concordance rate of 99.71%. Table 2 shows the detailed comparison between SoCal and HapMap calls at a call rate of 95%.

| HapMap/SoCal | AA      | AB      | BB      | No Call |
|--------------|---------|---------|---------|---------|
| AA           | 348,221 | 390     | 298     | 14,720  |
| AB           | 710     | 319,394 | 775     | 25,057  |
| BB           | 410     | 427     | 357,627 | 13,290  |

Table 2: At a call rate of 95%, SoCal achieved 99.71% concordance rate in the leave-one-out cross-validation with HapMap reference calls.

Figure 4 shows the concordance rate of SoCal by varying the call rate. At a fixed high concordance rate, SoCal can achieve very high call rate. This result is comparable to the results reported by many state-of-the-art genotype calling methods.

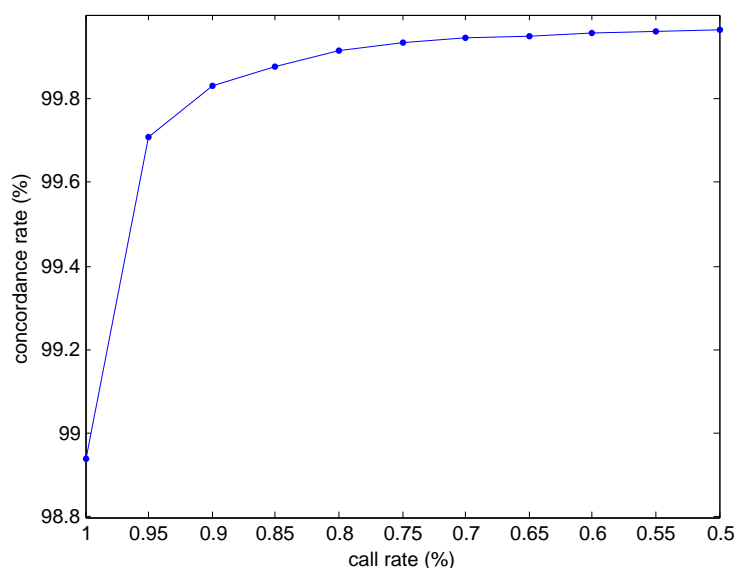


Figure 4: Concordance rate of SoCal in the leave-one-out cross-validation with HapMap reference calls as a function of call rate.

## 4.2 Comparison with CRLMM calls

As another way of evaluating the accuracy of SoCal, I compared the genotype calls made by SoCal and those made by CRLMM, a widely used state-of-the-art genotype calling method for SNP microarrays.

I first trained SoCal using all the samples with HapMap reference calls, and then I made genotype calls on the rest of the samples not in the reference set. I excluded the training samples when comparing SoCal with CRLMM, and compared these two methods only at samples not in the training samples.

Table 3 shows the detailed comparison between SoCal and CRLMM at a call rate of 100%. In total, SoCal made 2,245,891 calls, out of which 2,134,868 were concordant with those made by CRLMM, achieving a concordance rate of 95.10%. The high concordance rate between SoCal and CRLMM implies that SoCal has the potential to become an alternative genotype caller.

| CRLMM/SoCal | AA      | AB      | BB      | No Call |
|-------------|---------|---------|---------|---------|
| AA          | 781,903 | 23,244  | 10,405  | 0       |
| AB          | 22,340  | 564,280 | 22,533  | 0       |
| BB          | 7,730   | 24,771  | 788,685 | 0       |

Table 3: At a call rate of 100%, SoCal achieved 95.10% concordance rate with the calls made by CRLMM. Training samples for SoCal were excluded in the comparison.



### 4.3 Comparison with RLMM in the presence of outliers

I investigated how robust SoCal is when there are outliers in the training data. For comparison, I implemented the RLMM algorithm, which independently fits bivariate Gaussian distributions on the log transformed allele intensities of each genotype cluster and then classifies SNPs with unknown genotype into the distribution having minimum Mahalanobis distance.

For accurate comparison, I selected a subset of 3,442 SNPs that have more than 10 reference calls for each of the genotype cluster from the set of 12,323 SNPs used for evaluation in the previous section. To simulate outliers, for each SNP, I first estimated  $\mu_g$ , the mean of the log transformed allele intensities of each genotype cluster, and then drew one outlier for each genotype cluster from the Gaussian distribution  $N(\mu_g, \gamma I)$ , where  $I$  is the identity matrix and  $\gamma$  a positive constant controlling the variance of the distribution—by increasing  $\gamma$ , one increases the effect of outliers. In total, I simulated 3 outliers for each SNP, one for each genotype cluster.

As an example, Figure 5 shows the ellipsoidal decision regions obtained by SoCal and the level curves of the Gaussian decision regions obtained by RLMM before and after an outlier is introduced to the genotype AA cluster. Before an outlier is introduced, both SoCal and RLMM can make accurate genotype calls. However, after an outlier is introduced into the genotype AA cluster, the variance of the Gaussian decision region obtained by RLMM is significantly affected, making the classification much less accurate. On the other hand, because SoCal not only puts penalty on outliers but also jointly uses data from other genotype clusters to form the decision region, it can still classify most SNPs into correct genotypes.

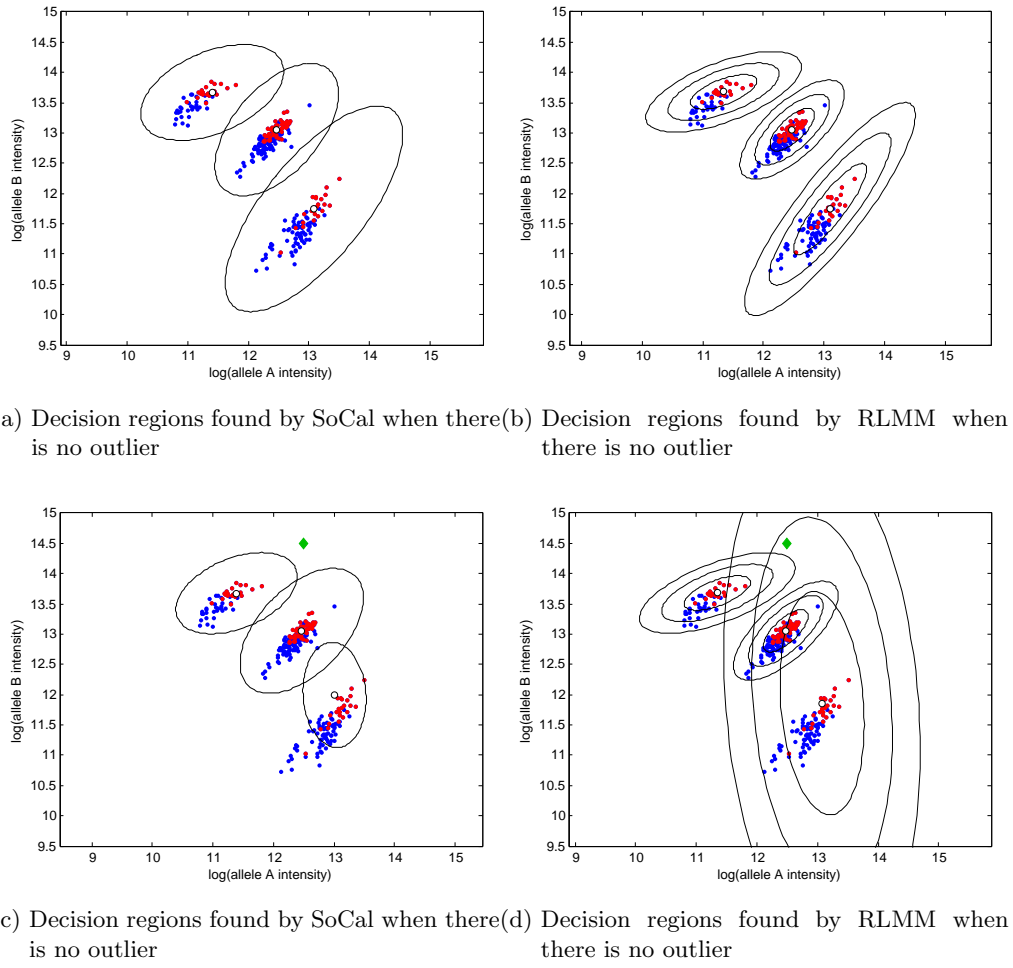


Figure 5: Each dot in the plots above represents a sample, with samples having HapMap reference genotype calls marked red. The ellipsoids were obtained using only the reference calls.

Figure 6 shows the decrease in concordance rate of SoCal and RLMM at call rate of 100% in leave-one-out cross-validation with HapMap reference genotype calls as the variance of simulated outliers varies from 0 to 10. Clearly, the concordance rate of SoCal decreases much more slowly than does the RLMM method. Thus, SoCal is in general more robust to outliers than RLMM.

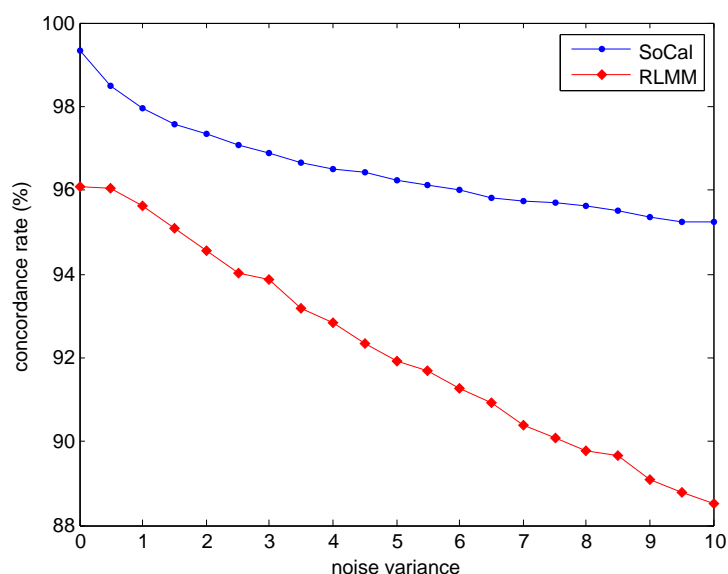


Figure 6: Concordance rate of SoCal and RLMM in the leave-one-out cross-validation with HapMap reference calls as a function of outlier variance.

#### 4.4 Software implementation

SoCal is implemented in Python. To solve the conic programming problem of finding separating ellipsoids, SoCal uses CVXOPT. Source code of SoCal is available at [https://github.com/huwenboshi/wqe/tree/master/genotype\\_caller](https://github.com/huwenboshi/wqe/tree/master/genotype_caller).

## 5 Discussion

SoCal demonstrates the possibility of using ellipsoids as decision regions for classifying SNPs into genotypes. In experiments involving HapMap data, SoCal is able to achieve 97.5% genotype calling accuracy using only a small portion of reference genotype calls.

SoCal is different from many other genotype calling algorithms in different aspects. First, SoCal is a supervised genotype caller that uses reference genotype calls to form decision regions and then call genotypes for future SNPs. Second, SoCal is efficient - the problem of finding separating ellipsoids can be formulated as a conic programming problem and solved in polynomial time, and is guaranteed to find a global optimum. Third, the decision regions of SoCal can be used repeatedly. Once the decision regions of a SNP for a particular SNP array are found, future genotype calls can repeatedly use these regions without having to first train a genotype caller.

SoCal can be improved and extended in many ways. First, for a SNP with low minor allele frequencies, the ellipsoid learned directly from one genotype may not be the best ellipsoid for classification because of limited amount of data. Instead, one can use

the ellipsoids learned for other genotypes, which have more data, to infer the ellipsoid for the genotype with relatively less data. Second, SoCal can be extended to detect copy number variations in samples. Individual samples with copy number variations may have extra A's or B's at a SNP location. And if one plots the allele intensities ( $I_A, I_B$ ) for these individuals, these data point will appear as outliers. SoCal can use the ellipsoids learned from reference genotype calls to detect outliers and then call copy number variations accordingly.

In summary, SoCal is an efficient and accurate genotype caller for Affymetrix SNP arrays. It can also be extended and improved to have more and better functionalities.

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