Segmentation and intensity estimation of microarray images using a gamma-t mixture model

Jangsun Baek, Young Sook Son and Geoffrey J. McLachlan

Bioinformatics 2007, Vol. 23 no. 4, pages 458-465

Seminar in bioinformatics

Triinu Kõressaar

TARTU

2007

Simultaneously performed image segmentation and intensity estimation

Two-component mixture model

background intensity

foreground intensity

=> Intensity measurement is a bivariate vector

(red and green intensities)

Background intensity component – bivariate gamma distribution

Foreground intensity component - bivariate t-distribution

Segmentation methods can be grouped for instance whether a parametric distribution of the pixel intensity is assumed or not:

1. Nonparametric segmentation

* no particular type of distribution on intensities is assumed (e.g fixed circle segmentation, adaptive circle segmentation, seeded region growing method)

2. Parametric segmentation

* distribution for intensity is specified up to a vector of unknown parameters (e.g bivariate normal distribution, scaled bivariate normal distribution, exponential distribution, uncorrelated bivariate t distribution)

next..

Description of image segmentation and intensity estimation method

Presentation of the stable parameter estimation result of the proposed method using synthetic data

Presentation of the segmentation and estimation results from applying the method to two real experimental microarray image datasets

Comparision of the results with those from other methods

Summary of the method

microarray image analysis:

1. automatic gridding
2. model-based clustering of pixels
3. intensity estimation

Data

a pair of unsigned ***16-bit images*** (.tiff) -> transformation of images (square root or logarithms) for:

- preventing very bright pixels from dominating
- makeing the work with images computationally more efficient

Automatic gridding

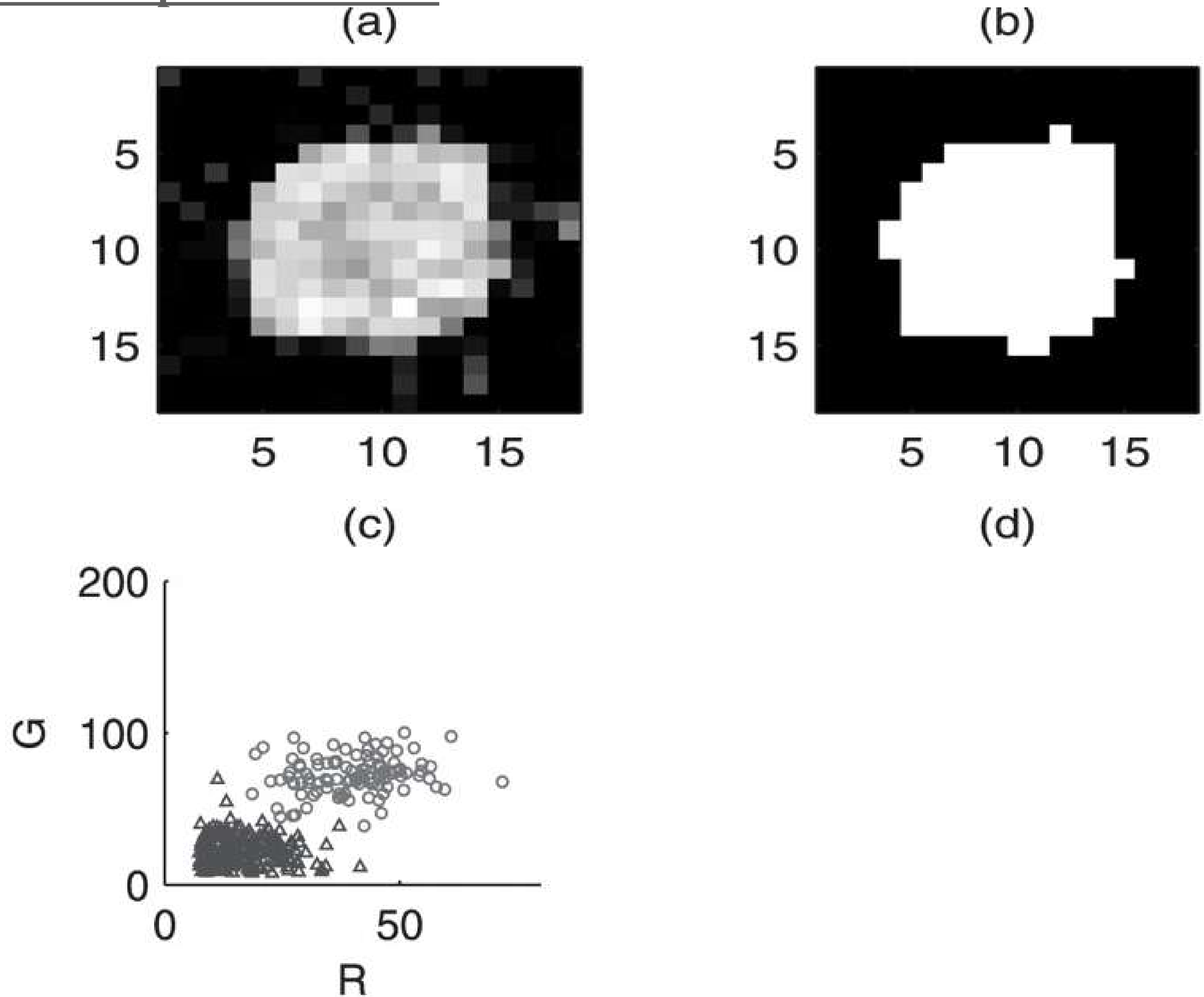
Identifying blocks and positioning rows and columns of spots within each block

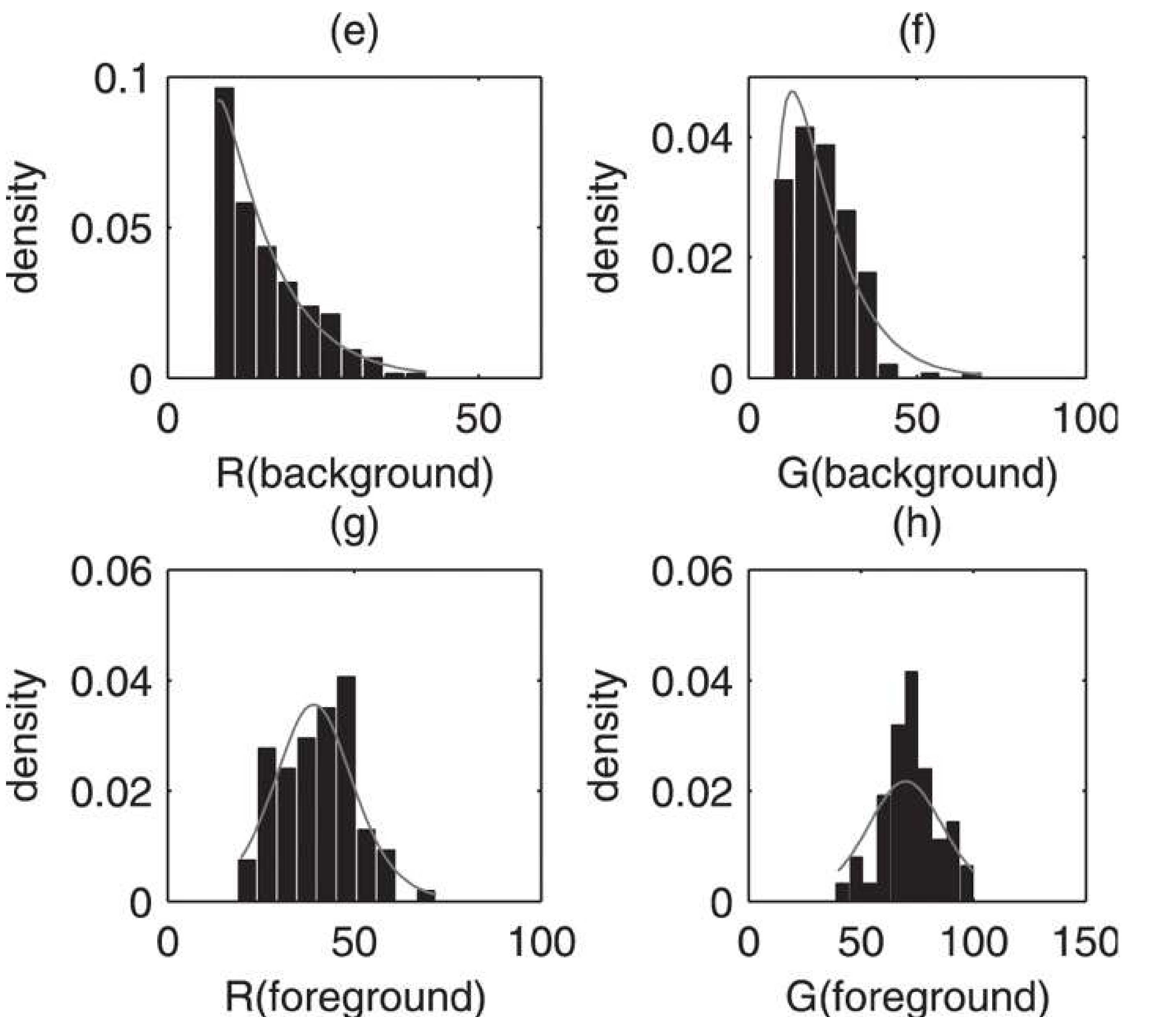
Using the combined image, projecting the intensities by summing up across the pixels in each row and each column

Smoothing the projections using robust loess (bandwith – width of a typical spot) -> series of peaks and valleys

Grid – drawing a line in each valley

Distribution of pixel intensities





(e)
0.1
0.05
0
density
0
50
R(background)
(f)
0.04
0.02
0
density
0
50
100
G(background)
(g)
0.06
0.04
0.02
0
density
0
50
100
R(foreground)
(h)
0.06
0.04
0.02
0
density
0
50
100
150
G(foreground)

Kolmogorov-Smirnov goodness-of-fit test:

for checking the validity of the proposed distributions

Table 1. Mean P-values for goodness-of-fit test

	R_b	G_b	R_f	G_f
Exponential	0.2264	0.0119	0.0232	0.0279
	(0.1405)	(0.0036)	(0.1031)	(0.1276)
Normal	0.0012	0.0635	0.6913	0.5888
	(0.0014)	(0.0777)	(0.7380)	(0.6847)
Gamma/t	0.2325	0.2144	0.7726	0.7269
	(0.1501)	(0.1243)	(0.8237)	(0.8046)

R_b, R_f: red intensity in background and foreground; G_b, G_f: green intensity in background and foreground. Mean P-values for the segmented data by the method of Li *et al.* (2005) are in the parentheses.

Image segmentation and intensity estimation

Density function of observed intensities:

$f(y;\ \Psi)=\pi_1 f_1(y;\ \Theta) + \pi_2 f_2(y;\mu,\Sigma,\nu)$, where $\Psi=\{\Theta,\ \mu,\ \Sigma,\ \nu,\ \pi_\iota\}$,

f_1 and f_2 are the p.d.f for background and foreground pixel intensity distributions, respectively

π_i – probaility that pixel belongs to the *i*th component

EM algorithm to obtain the maximum likelihood estimates of the parameters in Ψ

$\hat{}\tau_{ij}$ – the estimate of posterior probability that y_j belongs to the *i*th component of the mixture

Rectangle containing a spot – foreground at the center, background surrounding the foreground -> neighboring pixels in each segment must belong to the same class

Final step of EM: nonparametric kernel estimate $\hat{}\tau^*_{ij}$ ($\hat{}\tau_{ij}$ is multiplied with weight), weight is dependent on *h*

h – bandwidth – how many neighboring pixels' information is needed to estimate the posterior probability of the pixel to be classified

Step for choosing the *h*: different weights to the pixels at x_{ij}'s which are within $x_i \pm 1.96h$ according to their closeness to x_i under the 95% confidence level; smoothing up to first nearest pixel $h \approx 0.51$

Pixels are segmented according to $\hat{\tau}^*_{ij}$:

*j*th pixel is classified as background if $\hat{\tau}^*_{ij} \geq 0.5$

as foreground if $\hat{\tau}^*_{ij} < 0.5$

blank and low-expressed spots – they are flagged

BIC – Bayesian Information Criterion

m – the number of components in the mixture model

m=1 -> no foreground, only background

$BIC_1 < BIC_2$ -> all pixels are treated as background

spot is flagged as a blank

intensity of spot is _not_ estimated

m=2 -> foreground + background

*if spot is _not_ flagged as blank, BIC_2 is _not_ significantly less than BIC_1

-> are there two groups in the spot rectangle?

If $0 \le BIC_1 - BIC_2 \le \delta|BIC_1|$ for $0 < \delta << 1$ -> spot is flagged as having low expression (the pixels of uncertain classification); intensity of spot _is_ estimated

high-intensity artifact regions

Detected on the foreground in the valid spots

For each channel the intensities of the pixels segmented as foreground are rearranged in ascending order

Q_{3R} and Q_{3G} - 3rd quartile of the foreground pixel intensities of R and G

IQR_R and IQR_G - interquartile ranges of the same foreground pixel intensities

Pixel whith intensities R_i and G_i is classified as a high-intensity artifact if $R_i > Q_{3R} + 3xIQR_R$ and $G_i > Q_{3G} + 3xIQR_G$.

High intensity artifacts are excluded from the foreground when the foreground intensity is estimated

Intensity estimation

Spot intensity is estimated by maximum likelihood

The mean intensity of foreground ≥ the mean intensity of background

Estimation of intensity for each spot - log ratio of background-corrected R and G intensities ($\log_2(\hat{\phi}^*_1/\hat{\phi}^*_2)$)

Results

1. Simulated data::segmentation

Spot rectangle is 17x17 pixels

Background: dist. - gamma(α_i,β_i,γ_i), i=1,2, where α_1=1, β_1=0.1, γ_1=7, α_2=1, β_2=0.1, γ_2=7; $\mu_b=(\mu_{b1},\mu_{b2})'=(17,30)$'

Foreground: two independent t distributions - location parameters $\mu_{fi} = \mu_{bi} + 20$, DF υ_i=20, dispersion parameters σ^2_i= 100.

($x_{1(ij)}$, $x_{2(ij)}$) - coordinate for the (i, j)th pixel in the spot rectangle located at ith column and jth row of the rectangle lattice, where $x_{1(ij)}$, $x_{2(ij)}$=1, 2, . . . , 17.

Intensity of the (i, j)th pixel is randomly generated from the foreground distribution if $(x_{1(ij)}-9)^2+(x_{2(ij)}-9)^2 \leq 6^2$, and from the background distribution otherwise.

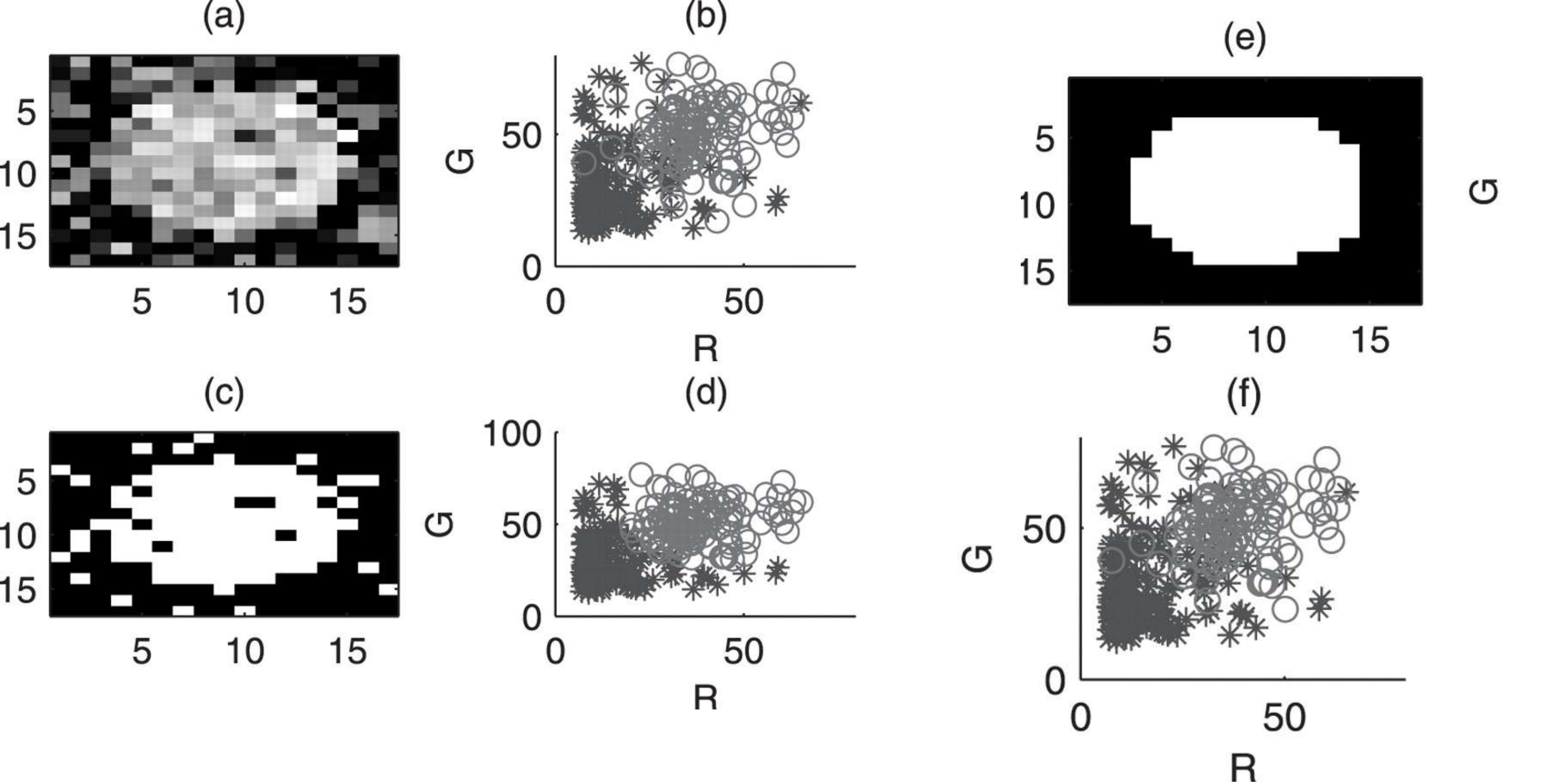


Fig. 2. (**a** and **b**): true image and scatter plot of the intensities indicating their true classes; (**c** and **d**): image and scatter plot of the segmented intensities based on non-smoothed posterior probability $\hat{\tau}_{ij}$; (**e** and **f**): image and scatter plot of the segmented intensities based on $\hat{\tau}^*_{ij}$. The circle in the scatter plot corresponds to the foreground, and * to the background.

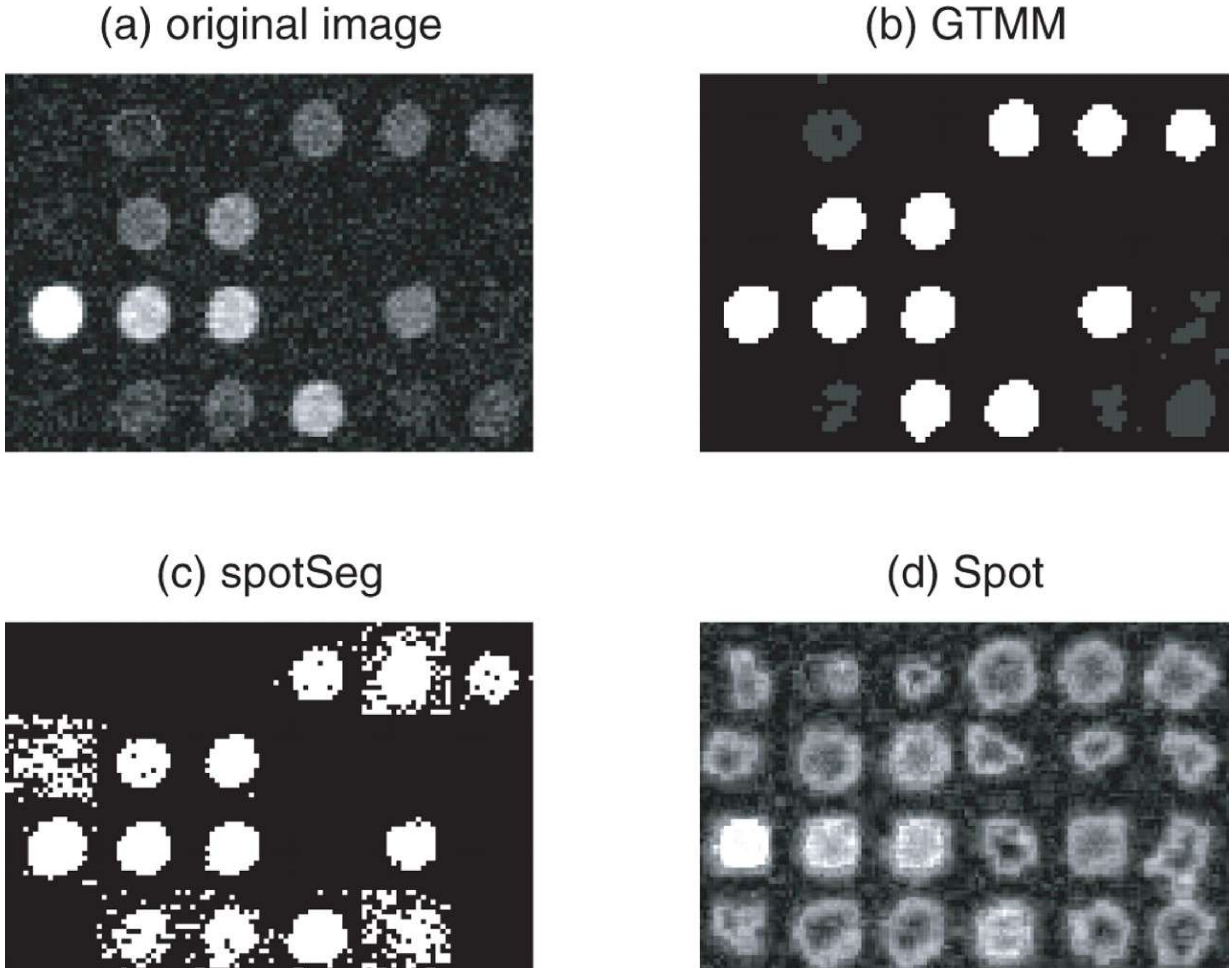
Results:: 1. Simulated data:: Intensity analysis

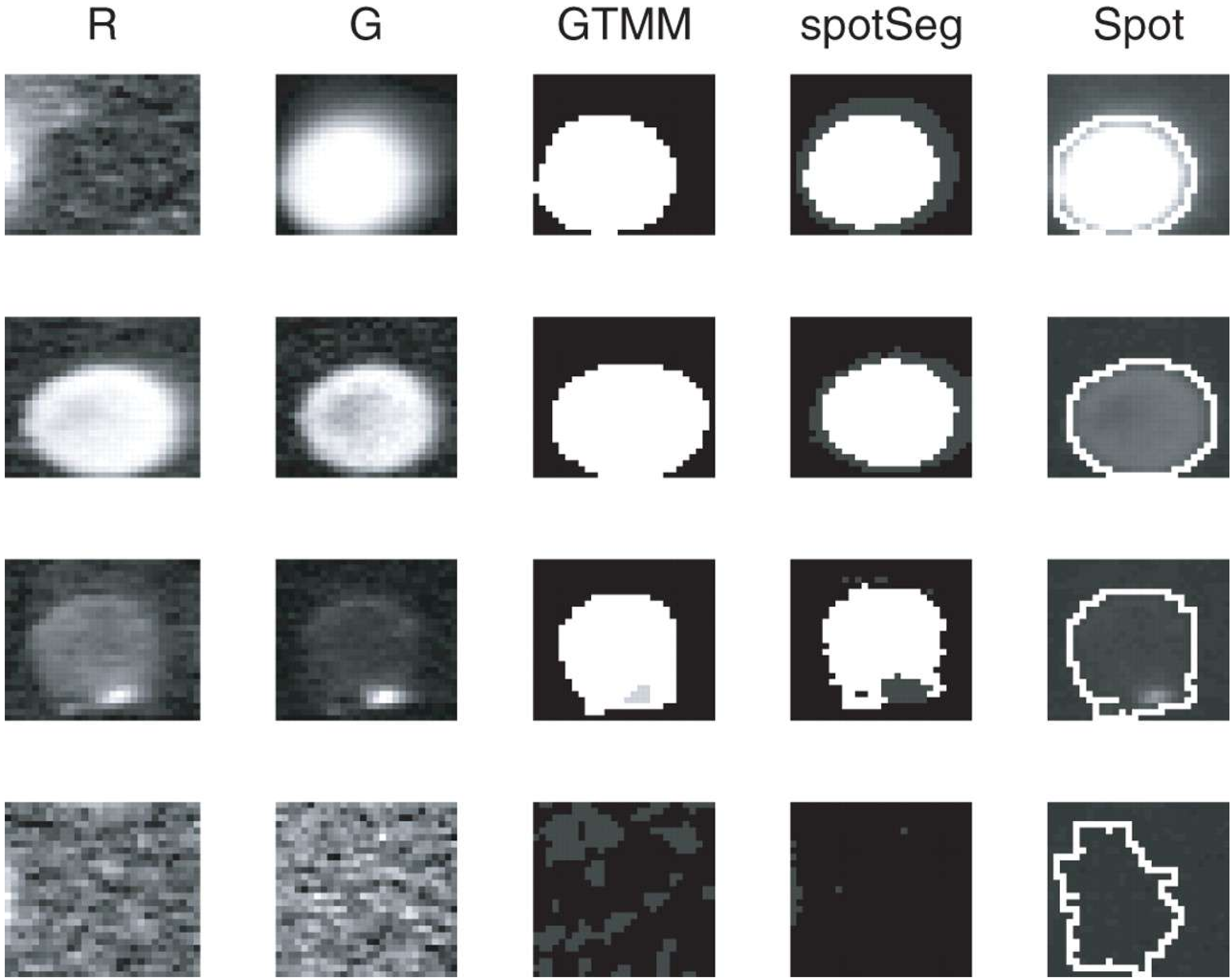
Table 2. The mean of the parameter estimates and the P-value for the t-test on the parameter estimate, obtained from 100 synthetic spot data

Δ		μ_{b1}	μ_{b2}	μ_{f1}	μ_{f2}	ϕ_1	ϕ_2	$\log_2(\phi_1/\phi_2)$
	θ_0	17.00	30.00	57.00	50.00	40.00	20.00	1.00
20	$\hat{\theta}$	17.21	30.23	57.07	50.01	39.86	19.78	1.01
	p	0.00	0.02	0.31	0.90	0.15	0.09	0.19
	θ_0	17.00	30.00	77.00	60.00	60.00	30.00	1.00
30	$\hat{\theta}$	16.91	30.06	77.04	59.98	60.13	29.91	1.01
	p	0.19	0.53	0.58	0.75	0.19	0.51	0.22
	θ_0	17.00	30.00	97.00	70.00	80.00	40.00	1.00
40	$\hat{\theta}$	17.00	29.99	97.00	70.01	80.00	40.03	1.00
	p	0.944	0.894	0.97	0.85	0.99	0.83	0.93

μ_{b1}, μ_{b2}: mean red and green intensity in background; μ_{f1}, μ_{f2}: mean red and green intensity in foreground; ϕ_1: $\mu_{f1} - \mu_{b1}$; ϕ_2: $\mu_{f2} - \mu_{b2}$; θ_0: true parameter value; $\hat{\theta}$: mean of the parameter estimates; p: P-value for $H_0 : E(\hat{\theta}) = \theta_0$.

Results::Real datasets:: GTMM, Spot software and spot-Segmentation




R
G
GTMM
spotSeg
Spot

Conclusion

Gamma distribution for the background intensity:

- is very flexible in its shape (asymmetric exponential type to symmetric normal type)
- is bivariate by taking the R and G intensities to be independent in the background

Bivariate t distribution for the foreground intensity:

- provides a longer-tail alternative to the normal distribution
- less affected by atypical observations

Conclusion

EM algorithm to estimate the pixels' posterior probabilities, a nonparametric kernel smoothing technique that utilizes the neighborhood information in forming the posterior probabilities for the final segmentation.

Model constrains the mean intensity for the foreground to be greater than that for the background.