

Methods of Comparing Digital PCR Experiments

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Introduction

The outcome of digital PCR (dPCR) experiments are mean copies per partition (λ). Results are derived from the measured data, an ordered (in one or two dimensions) sequence of positive partitions. The usual analysis involves assumption the template molecules are Poisson distributed among partitions. On this premise, already proposed approaches, based on the confidence intervals (Dube et al., 2008) or uncertainty quantification (Bhat et al., 2009), allow a comparison of experiments.

GLM testing framework

Generalized Linear Models (GLM) are linear models for data, where the response variables may have non-normal distributions (as binomially distributed positive partitions in digital PCR experiments). We propose a model reflecting relationships in results of digital PCR as given by:

$$\log Y = \beta^T X \quad (1)$$

where Y are counts, X are experiments names (categorical data) and β are linear model coefficients for every experiment. Moreover, $\exp \beta = \lambda$. Estimated means copies per partitions obtained from the model are compared each other using multiple t-test (Bretz et al., 2010).

Multiple testing (MT) framework

The dPCR experimentes are compared pairwise using the uniformly most powerful (UMP) ratio test (Fay, 2010). Furthermore, computed p-values are adjusted using Benjamini Hochberg correction (Benjamini and Hochberg, 1995) to control family-wise error rate.

The UMP ratio test has following null-hypothesis:

$$H_0 : \frac{\lambda_1}{\lambda_2} = 1 \quad (2)$$

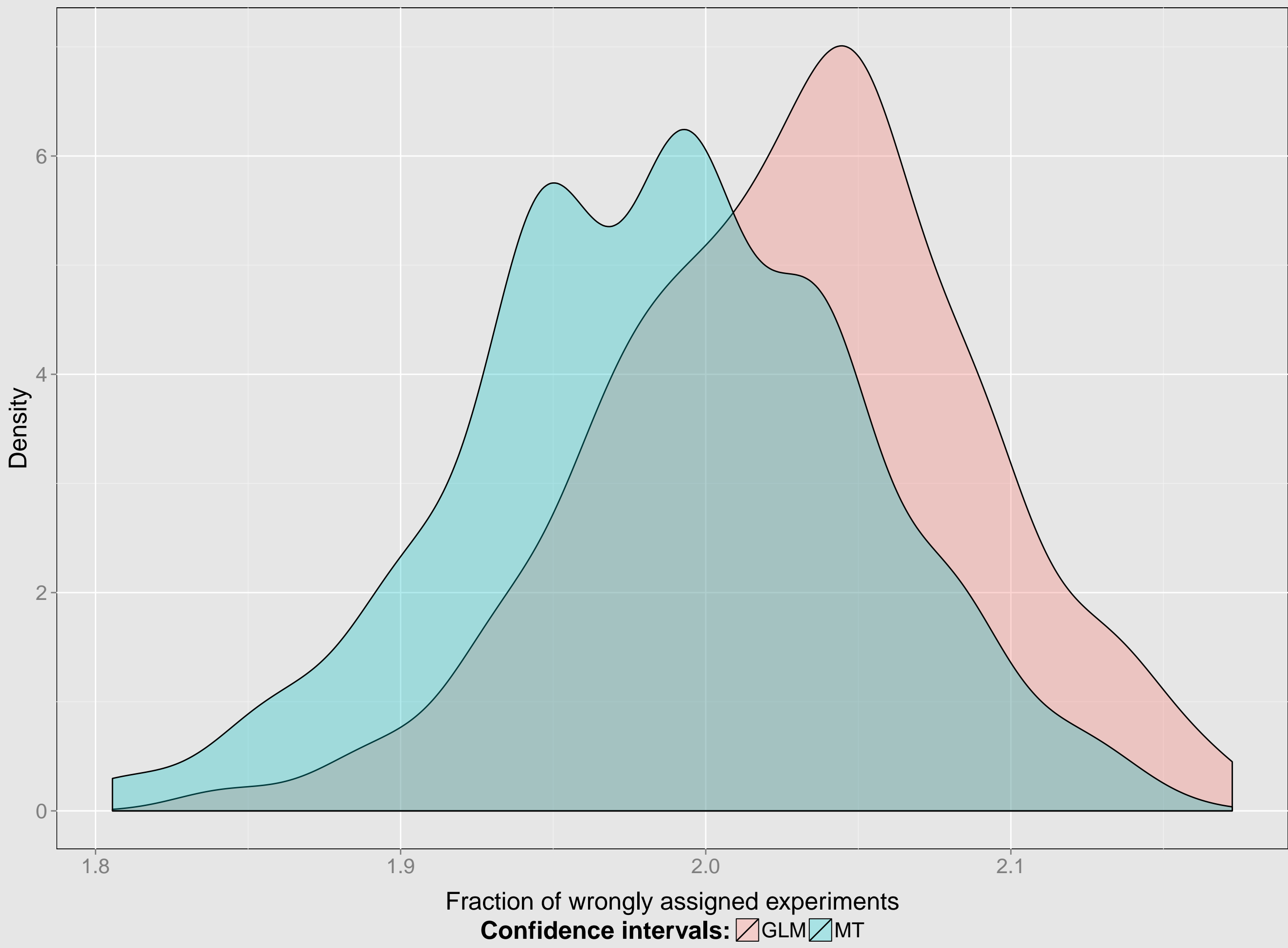
The Wilson's confidence intervals (Brown et al., 2001) are calculated independently for every dPCR experiment and adjusted using Dunn – Šidák correction, where:

$$\alpha_{\text{adj}} = 1 - (1 - \alpha)^{\frac{1}{T}} \quad (3)$$

Such intervals are wider then usual, but ensure that confidence intervals simultaneously contain true value of lambda.

Comparision of frameworks

Two approaches presented above were compared in a simulation approach over 150 000 simulated array dPCR experiments. Each simulation contained six reactions. Three of them had roughly the same amount of molecules per plate and other three had experiments with 10 to 50 molecules more. Experiments were compared using GLM and MT frameworks.



On average, 2.03 and 1.98 reactions were assessed to a wrong group by respectively GLM and MT.

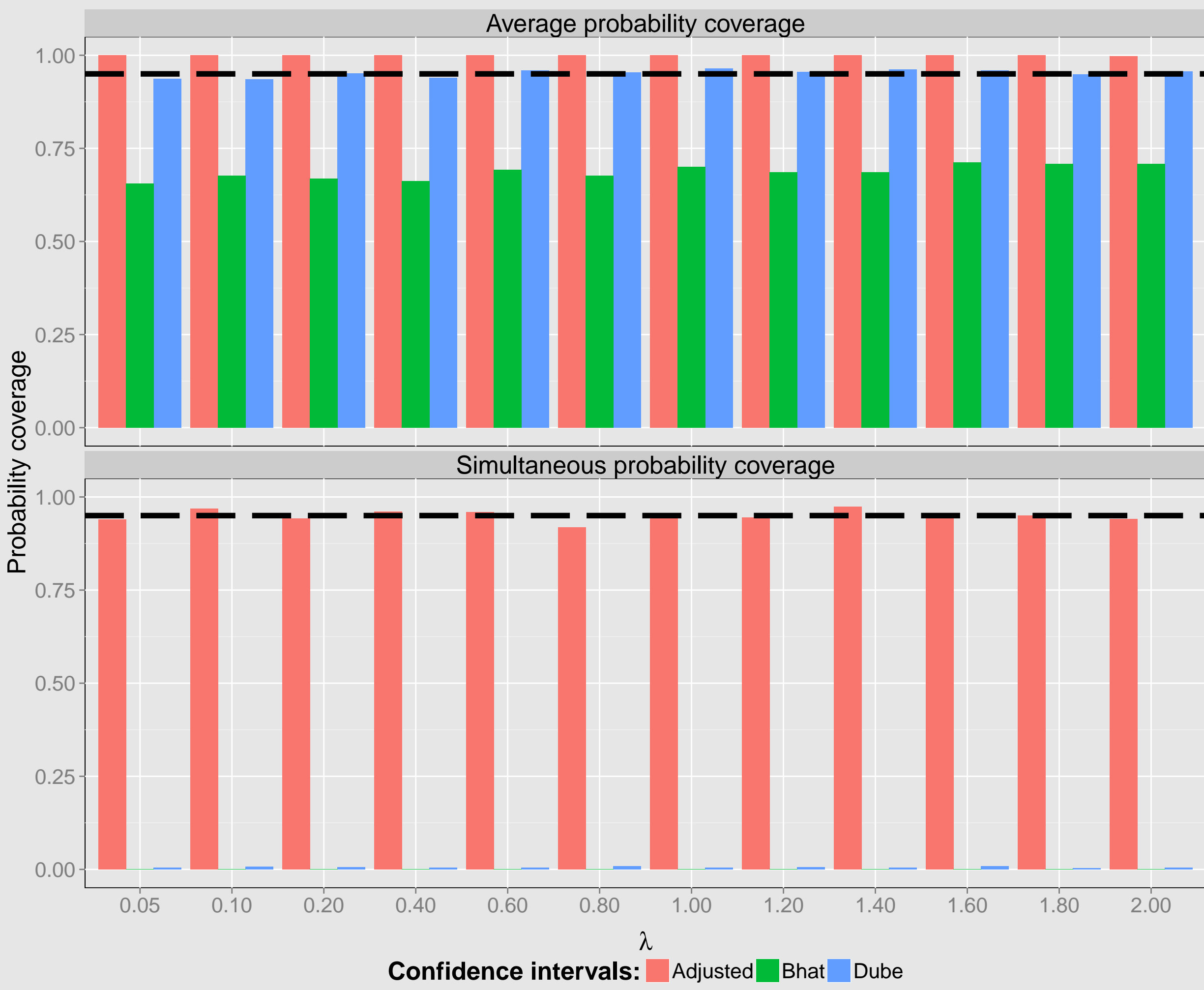
A single GLM comparison took roughly 183 times longer than MT (on average 1.10 seconds versus 0.006 seconds on the Intel i7-2600 processor). The difference growths with the number of experiments and number of partitions (data not shown).

Probability coverage of confidence intervals

Average coverage probability is the proportion of the time that the interval contains the true value of λ .

In the example below, we simulated 1×10^6 droplet dPCR experiments (2×10^4 droplets each) for each level of λ (1.2×10^7 experiments total). We computed average probability coverage of CI obtained by three methods: Dube's (Dube et al., 2008), Bhat's (Bhat et al., 2009) and by MT ($\alpha = 0.95$).

To assess simultaneous coverage probability, we randomly divided experiments into 2000 groups (500 experiments each) for each possible value of λ . We counted frequency of groups in which all confidence intervals contain the true value of λ .



The dashed black line marks 0.95 border.

Method name	Type of coverage	Value
Adjusted	Average probability coverage	1.00
Bhat	Average probability coverage	0.69
Dube	Average probability coverage	0.95
Adjusted	Simultaneous probability coverage	0.95
Bhat	Simultaneous probability coverage	0.00
Dube	Simultaneous probability coverage	0.01

Summary

- Both GLM and MT frameworks are able to accurately assess λ and on this ground assign dPCR experiments to different groups.
- The MT approach is much faster than GLM and can be used in the analysis of huge droplet dPCR data sets.
- The adjusted confidence intervals computed by MT guarantee stable 0.95 simultaneous coverage probability, which offers more reliable comparison of technical repeats.

Availibility and funding

pcRuniveRsum:

<http://michbur.github.io/pcRuniveRsum/>



dpcR R package:

<http://cran.r-project.org/web/packages/dpcR/>

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Simulations

We performed all in-silico experiments using the novel digital PCR data simulation (Jacobs et al., 2014).

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