False Discovery Rates: A New Deal

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Abstract

We introduce a novel Empirical Bayes approach for performing large-scale hypothesis testing, including estimating False Discovery Rates (FDRs), and estimating effect sizes. Compared with existing commonly-used approaches to FDR analysis, the method has two key differences. First, it assumes that the distribution of the actual (unobserved) effects being tested is unimodal, with a mode at 0. This "unimodal assumption" (UA), which is common in other related contexts, but very different from assumptions usually made in FDR analyses, yields more accurate estimates of FDR than existing methods, provided the UA holds. Second, the method takes as it input two numbers for each test (an effect size estimate, and corresponding standard error), rather than the one number usually used (p value, or z score). Provided these two numbers are available, using them allows our method to better account for variations in measurement precision across tests. It also facilitates the estimation of actual effect sizes, and our approach provides "shrunken" interval estimates (credible regions) for each effect in addition to measures of "significance". To provide a bridge between interval estimates and signicance measures we introduce the term "local false sign rate" to refer to the probability of getting the sign of an effect wrong, and argue that it is a superior measure of significance than the local FDR because it is both more generally applicable, and can be more robustly estimated. Our methods are implemented in an R package `ashr` available from `http://github.com/stephens999/ashr`.

Introduction

Since its introduction in 1995 by Benjamini and Hochberg [1], the "False Discovery Rate" (FDR) has quickly established itself as a key concept in modern statistics, and the primary tool by which most practitioners handle large-scale multiple testing in which the goal is to identify the non-zero "effects" among a large number of imprecisely-measured effects.

Here we consider an Empirical Bayes (EB) approach to FDR. This idea is, of course, far from new: indeed, the notion that EB approaches could be helpful in handling multiple comparisons predates introduction of the FDR (e.g. [2]). More recently, EB approaches to the FDR have been extensively studied by several authors, especially B. Efron and co-authors [3–7]; see also [8–11] for example. So what is the "New Deal" here? We introduce two simple ideas that are new (at least compared with existing widely-used FDR pipelines) and can substantially affect inference. The first idea is to *assume that the distribution of effects is unimodal*. This yields a very simple, fast, and stable computer implementation, as well as improving inference of FDR when the unimodal assumption is correct. The second idea is to use two numbers – effect sizes, and their standard errors – rather than just one – p values, or z scores – to summarize each measurement. This idea allows variations in measurement precision to be better accounted for, and avoids a

problem with standard pipelines that poor-precision measurements can inflate estimated FDR.

In addition to these two new ideas, we highlight a third idea that is old, but which remains under-used in practice: the idea that it may be preferable to focus on estimation rather than on testing. In principle, Bayesian approaches can naturally unify testing and estimation into a single framework – testing is simply estimation with some positive prior probability that the effect is exactly zero. However, despite ongoing interest in this area from both frequentist [12] and Bayesian [13,14] perspectives, in practice large-scale studies that assess many effects almost invariably focus on testing significance and controlling the FDR, and not on estimation. To help provide a bridge between FDR and estimation we introduce the term "local false sign rate" (lfsr), which is analogous to the "local false discovery rate" (*lfdr*) [6], but which measures confidence in the *sign* of each effect rather than confidence in each effect being non-zero. We show that in some settings, particularly those with many discoveries, the *lfsr* and *lfdr* can be quite different, and emphasise benefits of the *lfsr*, particularly its increased robustness to modelling assumptions.

Our methods are implemented in an R package, `ashr` (for **a**daptive **sh**rinkage in **R**), available at `http://github.com/stephens999/ashr`. (The names comes from the fact that EB methods in general, and – due to the UA – our EB method in particular, is a form of shrinkage estimation, and the shrinkage is adaptive to both the amount of signal in the data and the measurement precision of each observation. This will be discussed further in Xing and Stephens (in preparation).)

Methods

Model Outine

Here we describe the simplest version of our method, and briefly discuss embellishments we have also implemented.

Suppose that we are interested in the values of J "effects" $\beta = (\beta_1, \ldots, \beta_J)$. For example, in a typical genomics application that aims to identify differentially expressed genes, β_j might be the difference in the mean (log) expression of gene j in two conditions. In contexts where FDR methods are applied, interest often focuses on identifying "significant" non-zero effects; that is, in testing the null hypotheses $H_j : \beta_j = 0$. Here we tackle both this problem, and the more general problem of estimating, and assessing uncertainty in, β_j.

Assume that the available data are estimates $\hat{\beta} = (\hat{\beta}_1, \ldots, \hat{\beta}_J)$ of the effects, and corresponding (estimated) standard errors $\hat{s} = (\hat{s}_1, \ldots, \hat{s}_J)$. Our goal is to compute a posterior distribution for β given the observed data $\hat{\beta}, \hat{s}$, which by Bayes theorem can be written as

$$p(\beta|\hat{\beta}, \hat{s}) \propto p(\beta|\hat{s})p(\hat{\beta}|\beta, \hat{s}). \tag{1}$$

For $p(\beta|\hat{s})$ we assume that the β_j are independent from a unimodal distribution g. This unimodal assumption (UA) is a key assumption that distinguishes our approach from previous EB approaches to FDR analysis. A simple way to implement the UA is to assume that g is a

mixture of a point mass at 0 and a mixture of *zero-mean* normal distributions:

$$p(\beta|\hat{s},\pi) = \prod_j g(\beta_j;\pi), \tag{2}$$

$$g(\cdot;\pi) = \pi_0\delta_0(\cdot) + \sum_{k=1}^{K} \pi_k N(\cdot;0,\sigma_k^2), \tag{3}$$

where $N(\cdot;\mu,\sigma^2)$ denotes the density of the normal distribution with mean μ and variance σ^2. Here the mixture proportions $\pi = (\pi_0,\ldots,\pi_K)$ are hyperparameters, which are non-negative and sum to one, and are to be estimated, while the mixture component standard deviations $\sigma_1,\ldots,\sigma_K$ represent a large and dense grid of *fixed* positive numbers spanning a range from very small to very big (so K is fixed and large). (We encourate the reader to think of this grid as becoming infinitely large and dense, as a non-parametric limit, although of course in practice we use a finite grid – see Implementation Details.)

For the likelihood $p(\hat{\beta}|\beta,\hat{s})$ we assume

$$p(\hat{\beta}|\beta,\hat{s}) = \prod_j N(\hat{\beta}_j;\beta_j,\hat{s}_j^2). \tag{4}$$

Here, in additional to some conditional independence assumptions, we are effectively assuming that the number of observations used to compute $\hat{\beta}_j,\hat{s}_j$ are sufficiently large to justify a normal approximation.

This simple model features both the key ideas we want to emphasise in this paper: the UA is encapsulated in (3) while the different measurement precision of different observations is encapsulated in the likelihood (4) – specifically, observations with larger standard error will have a flatter likelihood, and therefore have less impact on inference. However, this simple model also has several additional assumptions that can be relaxed. Specifically,

1. The use of a mixture of zero-mean normals (3) also implies that g is symmetric about 0; more flexibility can be obtained by replacing the mixture of normals with mixtures of uniforms [see (11)].

2. The model (2) assumes that the effects are identically distributed, independent of their standard errors $\hat{s}$. We can relax this to allow for a relationship between these quantities [see (12)].

3. The likelihood (4) effectively assumes that the number of observations used to compute $\hat{\beta}_j,\hat{s}_j$ are sufficiently large to justify a normal approximation. We can generalize this likelihood using a t likelihood [see (13)].

These embellishments are detailed in Detailed Methods. Of course there remain limitations that are harder to relax, most notably the independence and conditional independence assumptions encapsulated in our model (which are also made by most existing EB approaches to this problem). Correlations among tests certainly arise in practice, either due to genuine correlations in the system of study, or due to unmeasured confounders, and their potential to impact results of an FDR analysis is important to consider whatever analysis methods are used: see [15, 16] for relevant discussion.

Fitting the model

In words, the model above assumes that the effects β_j are independent and identically distributed from a mixture of zero-centered normal distributions, and each observation $\hat{\beta}_j$ is a noisy measurement of β_j with standard error $\hat{s}_j$. Together, these assumptions imply that the observations $\hat{\beta}_j$ are also independent observations, each from a mixture of normal distributions:

$$p(\hat{\beta}|\hat{s},\pi) = \prod_j [\sum_{k=0}^{K} \pi_k N(\hat{\beta}_j; 0, \sigma_k^2 + \hat{s}_j^2)], \tag{5}$$

where we define $\sigma_0 := 0$.

The usual EB approach to fitting this model would involve two simple steps:

1. Estimate the hyperparameters π by maximizing the likelihood $L(\pi)$, given by (5), yielding $\hat{\pi} := \arg\max L(\pi)$.

2. Compute quantities of interest from the conditional distributions $p(\beta_j|\hat{\beta},\hat{s},\hat{\pi})$. For example, the evidence against the null hypothesis $\beta_j = 0$ can be summarized by $p(\beta_j \neq 0||\hat{\beta},\hat{s},\hat{\pi})$.

Both steps 1 and 2 are very straightforward: $\hat{\pi}$ can be obtained using a simple EM algorithm [17], and the conditional distributions $p(\beta_j|\hat{\beta}_j,\hat{s}_j,\hat{\pi})$ are analytically available, each a mixture of a point mass on zero and K normal distributions. (The simplicity of the EM algorithm in step 1 is due to our use of a fixed grid for σ_k in (3), instead of estimating σ_k, which may seem more natural but is not straightforward when $\hat{s}_j$ varies among j. This simple device may be useful in other applications.)

Here we slightly modify this usual procedure: instead of obtaining $\hat{\pi}$ by maximizing the likelihood, we maximise a penalized likelihood [see (18)], where the penalty encourages $\hat{\pi}_0$ to be as big as possible whilst remaining consistent with the observed data. We introduce this penalty because in FDR appllications it is considered desirable to avoid underestimating π_0 so as to avoid underestimating the FDR.

The local False Discovery Rate and local False Sign Rate

As noted above, the posterior distributions $p(\beta_j|\hat{\beta},\hat{s},\hat{\pi})$ have a simple analytic form. In practice it is common, and desirable, to summarize these distributions to convey the "significance" of each observation j. One natural measure of the significance of observation j is its "local FDR" [6], which is the probability, given the observed data, that effect j would be a false discovery, if we were to declare it a discovery. In other words it is the posterior probability that β_j is actually zero:

$$lfdr_j := \Pr(\beta_j = 0|\hat{\beta},\hat{s},\hat{\pi}). \tag{6}$$

The *lfdr*, like most other measures of significance (e.g. p values and q values), is rooted in the hypothesis testing paradigm which focuses on whether or not an effect is exactly zero. This paradigm is popular, despite the fact that many statistical practitioners have argued that it is often inappropriate because the null hypothesis $H_j : \beta_j = 0$ is often implausible. For example,

Tukey ([18]) argued that "All we know about the world teaches us that the effects of A and B are always different – in some decimal place – for any A and B. Thus asking 'Are the effects different?' is foolish." Instead, Tukey suggested ([19], p32,) that one should address

> ...the more meaningful question: "is the evidence strong enough to support a belief that the observed difference has the correct sign?"

Along the same lines, Gelman and co-authors [14, 20] suggest focussing on "type S errors", meaning errors in sign, rather than the more traditional type I errors.

Motivated by these suggestions, we define the "local False Sign Rate" for effect j, $lfsr_j$, to be the probability that we would make an error in the sign of effect j if we were forced to declare it either positive or negative. Specifically,

$$lfsr_j := \min[p(\beta_j \geq 0|\hat{\beta}, s), p(\beta_j \leq 0|\hat{\pi}, \hat{\beta}, s)]. \tag{7}$$

To illustrate, suppose that

$$\begin{aligned} p(\beta_j < 0|\hat{\beta}, s, \hat{\pi}) &= 0.95, \\ p(\beta_j = 0|\hat{\beta}, s, \hat{\pi}) &= 0.03, \\ p(\beta_j > 0|\hat{\beta}, s, \hat{\pi}) &= 0.02. \end{aligned}$$

Then from (7) $lfsr_j = \min(0.05, 0.98) = 0.05$ (and, from (6), $lfdr_j = 0.03$). This *lfsr* corresponds to the fact that, given these results, our best guess for the sign of β_j is that it is negative, and the probability that this guess is wrong would be 0.05.

As our notation suggests, $lfsr_j$ is intended to be compared and contrasted with $lfdr_j$: whereas small values of $lfdr_j$ indicate that we can be *confident that β_j is non-zero*, small values of $lfsr_j$ indicate that we can be *confident in the sign of β_j*. Of coure, being confident in the sign of an effect logically implies that we are confident it is non-zero, and this is reflected in the fact that $lfsr_j \geq lfdr_j$ (this follows from the definition because both the events $\beta_j \geq 0$ and $\beta_j \leq 0$ in (7) include the event $\beta_j = 0$). In this sense, as a measure of "significance", *lfsr* is more conservative than *lfdr*. More importantly, as we illustrate in Results, *lfsr* can be substantially more robust to modelling assumptions than *lfdr*.

From these "local" measures of significance, we can also compute average error rates over subsets of observations $\Gamma \subset \{1, \ldots, J\}$. For example,

$$\widehat{\text{FDR}}(\Gamma) := (1/|\Gamma|) \sum_{j \in \Gamma} lfdr_j. \tag{8}$$

estimates the FDR we would obtain if we were to declare all tests in Γ significant. And

$$q_j := \widehat{\text{FDR}}(\{k : lfdr_k \leq lfdr_j\}) \tag{9}$$

provides a measure of significance analogous to Storey's q value [21].

Related work

Previous approaches focussed on FDR

Among previous methods that explicitly consider the FDR and related quantities, our work here seems most naturally compared with the EB methods of [6] and [11] (implemented in the R packages `locfdr` and `mixfdr` respectively) and with the widely-used methods from [21] (implemented in the R package `qvalue`), which although not formally an EB approach, shares some elements in common.

There are two key differences between our approach and all of these three existing methods. First, whereas these existing methods summarize the information on β_j by a single number – either a z score (`locfdr` and `mixfdr`), or a p value (`qvalue`) – we instead work with two numbers $(\hat{\beta}_j, \hat{s}_j)$. Here we are building on [22], who develops Bayesian tests for individual null hypotheses using these two numbers, using the normal approximation 4. Using two numbers instead of one clearly has the potential to be more informative, and indeed, results later (Figure 4) illustrate how it can improve performance by taking better account of variation in measurement precision among observations.

Second, our unimodal assumption (UA) that the effects are unimodal about zero is quite different from assumptions made by `qvalue`, `locfdr` or `mixfdr`. Indeed, `locfdr` assumes that all z scores near 0 are null (Efron calls this the Zero Assumption; ZA), which implies that under the alternative hypothesis the distribution of z scores has *no mass at 0*; this contrasts strikingly with the UA, which implies that this distribution has its peak at 0! Similarly, `qvalue` assumes that all p values near 1 are null, which is the same as the ZA because p values near 1 correspond to z scores near 0. And although `mixfdr` does not formally make the ZA, we have found that in practice, with default settings, the results often approximately satisfy the ZA (due, we believe, to the default choice of penalty term β described in [11]). Thus, not only do these existing methods not make the UA, they actually make assumptions that are, in some sense, as different from the UA as they can be.

Given that the UA and ZA are so different, it seems worth discussing why we generally favor the UA. Although the UA will not apply to all situations, we believe that it will often be reasonable, especially in FDR-related contexts that have traditionally focussed on rejecting the null hypotheses $\beta_j = 0$. This is because if "$\beta_j = 0$" is a plausible null hypothesis, it seems reasonable to expect that "β_j very near 0" is also plausible. Further, it seems reasonable to expect that larger effects become decreasingly plausible, and so the distribution of the effects will be unimodal about 0. To paraphrase Tukey, "All we know about the world teaches us that large effects are rare, whereas small effects abound." We emphasise that the UA relates to the distribution of *all* effects, and not only the *detectable* effects (i.e. those that are significantly different from zero). It is very likely that the distribution of *detectable* non-zero effects will be multimodal, with one mode for detectable positive effects and another for detectable negative effects, and the UA does not contradict this.

In further support of the UA for FDR applications, we note that almost all analogous work in sparse regression models make the UA for the regression coefficents - common choices of uni-modal distribution being the spike and slab, Laplace, t, normal-gamma, normal-inverse-gamma, or horseshoe priors [23]. These are all less flexible than the approach we take here, which provides for general uni-modal distributions, and it may be fruitful to apply our methods to

the regression context; indeed see [24] for work in this vein. The UA assumption on regression coefficients is directly analagous to our UA here, and so its widespread use in the regression context supports its use here.

Alternatively, we could motivate the UA by its effect on point estimates, which is to "shrink" the estimates towards the mode - such shrinkage is desirable from several standpoints for improving estimation accuracy. Indeed most model-based approaches to shrinkage make parametric assumptions that obey the UA (e.g. [25]).

Finally, the UA also has a considerable practical benefit: it yields simple algorithms that are both computationally and statistically stable. We illustrate these features in Results.

Other work

There is also a very considerable literature that does not directly focus on the FDR problem, but which involves similar ideas and methods. Among these, a paper about deconvolution [26] is most similar, methodologically, to our work here: indeed, this paper includes all the elements of our approach outlined above, except for the point mass on 0 and corresponding penalty term. This said, the focus is very different: [26] focuses entirely on estimating g, whereas our primary focus is on estimating β_j. Also, they provide no software implementation. More generally, the related literature is too large to review comprehensively, but relevant key-words include "empirical bayes", "shrinkage", "deconvolution", "semi-parametric", "shape-constrained", and "heteroskedastic". Some pointers to recent papers in which other relevant citations can be found include [27–29]. Much of the literature focusses on the homoskedastic case (i.e. $\hat{s}_j$ all equal) whereas we allow for heteroskedasticity. And much of the recent shrinkage-oriented literature focuses only on point estimation of β_j, whereas for FDR-related applications measures of uncertainty are essential. Several recent papers consider more flexible non-parametric assumptions on g than the UA assumption we make here. In particular, [29, 30] consider the unconstrained non-parametric maximum likelihood estimate (NPMLE) for g. These methods may provide alternatives to our approach in settings where the UA assumption is considered too restrictive. However, the NPMLE for g is a discrete distribution, which will induce a discrete posterior distribution on β_j, and so although the NPMLE may perform well for point estimation, it seems possible it will not adequately reflect uncertainty in β_j, and some regularization on g may be necessary. Indeed, one way of thinking about the UA is as a way to regularize g.

Results

We compare results of `ashr` with existing FDR-based methods implemented in the R packages `qvalue` (v2.0 from Bioconductor), `locfdr` (v1.1-7 from `https://cran.r-project.org/src/contrib/Archive/locfdr/`), and `mixfdr` (v1.0, from `https://cran.r-project.org/src/contrib/Archive/mixfdr/`). In all our simulations we assume that the test statistics follow the expected theoretical distribution under the null, and we indicate this to `locfdr` using `nulltype=0` and to `mixfdr` using `theonull=TRUE`. Otherwise all packages were used with default options.

Effects of the Unimodal Assumption

Here we consider the effects of making the UA. To isolate these effects we consider the simplest case, where every observation has the same standard error, $s_j = 1$ and all methods are provided that information. That is, $\hat{\beta}_j|\beta_j \sim N(\beta_j, 1)$ and $\hat{s}_j = s_j = 1$. In this case the z scores $z_j := \hat{\beta}_j/\hat{s}_j = \hat{\beta}_j$, so modelling the z scores is the same as modelling the $\hat{\beta}_j$, and so the only difference between our method and methods like `locfdr` and `mixfdr` are in how they estimate g.

To briefly summarize the results in this section:

1. The UA can produce very different inferences compared with the ZA made by existing methods.

2. The UA can yield conservative estimates of the proportion of true nulls, π_0, and hence conservative estimates of *lfdr* and FDR.

3. The UA results in a stable procedure, both numerically and statistically, and is somewhat robust to deviations from unimodality.

The UA and ZA can produce different inferences

To illustrate the different inferences from the UA and ZA we show results for a single dataset simulated with the true effects $\beta_j \sim N(0, 1)$ (so with $s_j = 1$, $\hat{\beta}_j \sim N(0, 2)$). Note that none of the effects are truly null, but nonetheless there are many p values near 1 and z scores near 0 (Figure 1). We used each of the methods `qvalue`, `locfdr`, `mixfdr` and `ashr` to decompose the z scores ($z_j = \hat{\beta}_j$), or their corresponding p values, into null and alternative components. The results (Figure 1) illustrate the clear difference between the existing methods and our method. The effects of the ZA made by `qvalue` and `locfdr` are visually clear, producing a "hole" in the alternative z score distribution around 0. Although `mixfdr` does not formally make the ZA, its decomposition exhibits a similar hole. In contrast, due to the UA, the alternative z score distribution for `ashr` is required to have a mode at 0, effectively "filling in" the hole. (Of course the null distribution also has a peak at 0, and the local fdr under the UA is still smallest for z scores that are far from zero – i.e. large z scores remain the "most significant".)

Figure 1 may also be helpful in understanding the interacting role of the UA and the penalty term (18) that attempts to make π_0 as "large as possible" while remaining consistent with the UA. Specifically, consider the panel of Figure 1 that shows `ashr`'s decomposition of z scores, and imagine increasing π_0 further. This would increase the null component (dark blue) at the expense of the alternative component (light blue). Because the null component is $N(0, 1)$, and so is biggest at 0, this would eventually create a "dip" in the light-blue histogram at 0. The role of the penalty term is to push the dark blue component as far as possible, right up to (or, to be conservative, just past) the point where this dip appears. In contrast the ZA pushes the dark blue component until the light-blue component *disappears* at 0. See `https://stephens999.shinyapps.io/unimodal/unimodal.Rmd` for an interactive demonstration.

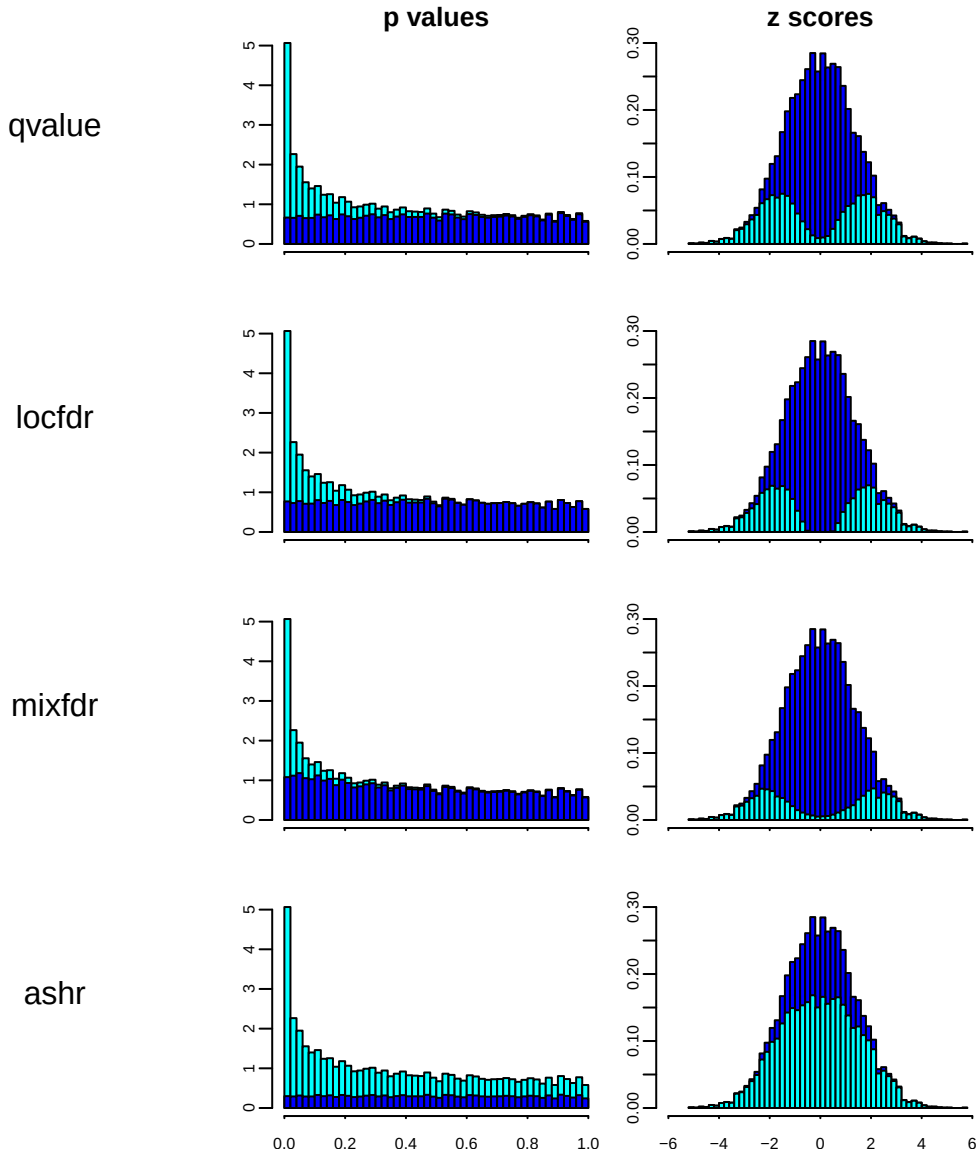


Figure 1. Illustration that the unimodal assumption (UA) in `ashr` can produce very different results from existing methods. The figure shows, for a single simulated dataset, the way different methods decompose p values (left) and z scores (right) into a null component (dark blue) and an alternative component (cyan). In the z score space the alternative distribution is placed on the bottom to highlight the differences in its shape among methods. The three existing methods (`qvalue`, `locfdr`, `mixfdr`) all effectively make the Zero Assumption, which results in a "hole" in the alternative z score distribution around 0. In contrast the method introduced here (`ashr`) makes the Unimodal Assumption – that the effect sizes, and thus the z scores, have a unimodal distribution about 0 – which yields a very different decomposition. (In this case the `ashr` decomposition is closer to the truth: the data were simulated under a model where all of the effects are non-zero, so the "true" decomposition would make everything cyan.)

The UA can produce conservative estimates of π_0

The illustration in Figure 1 suggests that the UA will produce smaller estimates of π_0 than the ZA. Consequently `ashr` will estimate smaller *lfdr*s and FDRs than existing methods that make the ZA. This is desirable, provided that these estimates remain conservative: that is, provided that π_0 does not underestimate the true π_0 and *lfdr* does not underestimate the true *lfdr*. The penalty term (18) aims to ensure this conservative behaviour. To check its effectiveness we performed simulations under various alternative scenarios (i.e. various distributions for the non-zero effects, which we denote g_1), and values for π_0. The alternative distributions are shown in Figure 2a, with details in Table 2. They range from a "spiky" distribution – where many non-zero β are too close to zero to be reliably detected, making reliable estimation of π_0 essentially impossible – to a much flatter distribution, which is a normal distribution with large variance ("big-normal") – where most non-zero β are easily detected making reliable estimation of π_0 easier. We also include one asymmetric distribution ("skew"), and one clearly bimodal distribution ("bimodal"), which, although we view as generally unrealistic, we include to assess robustness of `ashr` to deviations from the UA.

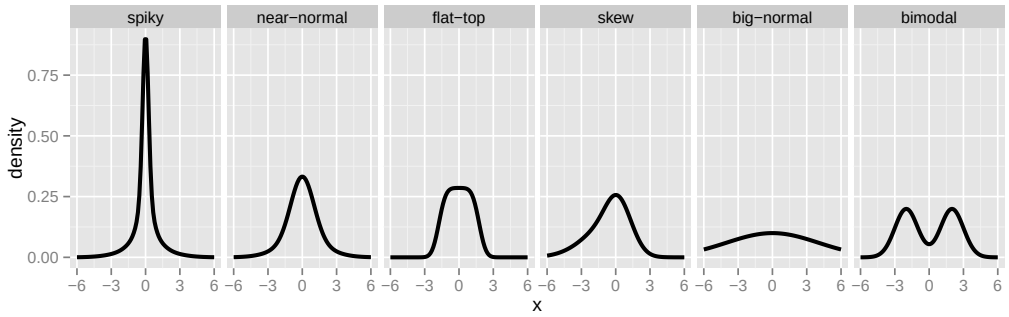
For each simulation scenario we simulated 100 independent data sets, each with $J = 1000$ observations. For each data set we simulated data as follows:

1. Simulate $\pi_0 \sim U[0, 1]$.
2. For $j = 1, \ldots, J$, simulate $\beta_j \sim \pi_0\delta_0 + (1 - \pi_0)g_1(\cdot)$.
3. For $j = 1, \ldots, J$, simulate $\hat{\beta}_j|\beta_j \sim N(\beta_j, 1)$.

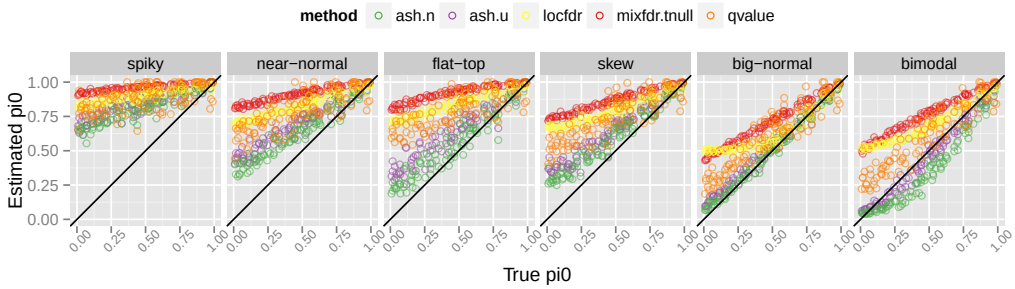
Figure 2b compares estimates of π_0 from `qvalue`, `locfdr`, `mixfdr` and `ashr` (y axis) with the true values (x axis). For `ashr` we show results for g_1 modelled as a mixture of normal components ("ash.n") and as a mixture of symmetric uniform components ("ash.u"). (Results using the asymmetric uniforms, which we refer to as "half-uniforms", and denote "ash.hu" in subsequent sections, are here generally similar to ash.u and omitted to avoid over-cluttering figures.) The results show that `ashr` provides the smallest more accurate, estimates for π_0, while remaining conservative in all scenarios where the UA holds. When the UA does not hold ("bimodal" scenario) the `ashr` estimates can be slightly anti-conservative. We view this as a minor concern in practice, since we view such a strong bimodal scenario as unlikely in most applications where FDR methods are used. (In addition, the effects on *lfsr* estimates turn out to be relatively modest; see below).

The lfsr is more robust than lfdr

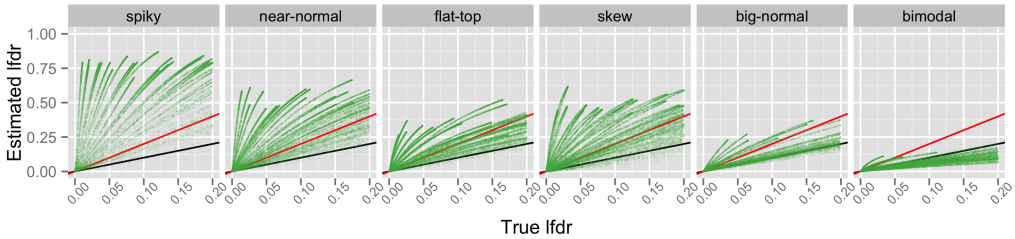
The results above show that `ashr` can improve on existing methods in producing smaller, more accurate, estimates of π_0, which will lead to more accurate estimates of FDR. Nonetheless, in many scenarios `ashr` continues to substantially over-estimate π_0 (see the "spiky" scenario for example). This is because these scenarios include an appreciable fraction of "small non-null effects" that are essentially indistinguishable from 0, making accurate estimation of π_0 impossible. Put another way, and as is well known, π_0 is not identifiable: the data can effectively provide an upper bound on plausible values of π_0, but not a lower bound (because the data



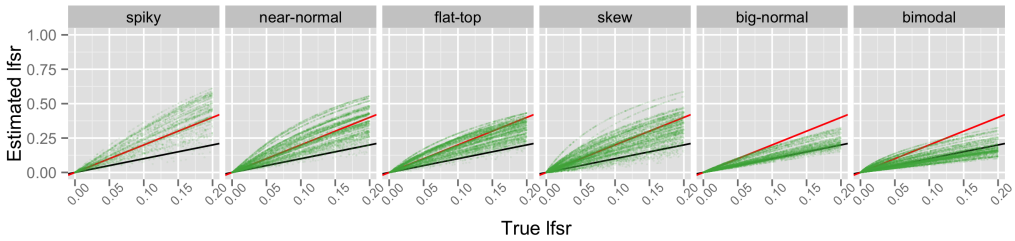
(a) Densities of non-zero effects, g_1, used in simulations.



(b) Comparison of true and estimated values of π_0. When the UA holds all methods yield conservative (over-)estimates for π_0, with `ashr` being least conservative, and hence most accurate. When the UA does not hold ("bimodal" scenario) the `ashr` estimates are slightly anti-conservative.



(c) Comparison of true and estimated *lfdr* from `ashr` (ash.n). Black line is $y = x$ and red line is $y = 2x$. Estimates of *lfdr* are conservative when UA holds, due to conservative estimates of π_0.



(d) As in c), but for *lfsr* instead of *lfdr*. Estimates of *lfsr* are consistently less conservative than *lfdr* when UA holds, and also less anti-conservative in bimodal scenario.

Figure 2. Results of simulation studies (constant precision $s_j = 1$).

cannot rule out that everything is non-null, but with miniscule effects). To obtain conservative behaviour we must estimate π_0 by this upper bound, which can be substantially larger than the true value.

Since FDR-related quantities depend quite sensitively on π_0, the consequence of this overestimation of π_0 is corresponding overestimation of FDR (and *lfdr*, and q values). To illustrate, Figure 2c compares the estimated *lfdr* from ash.n with the true value (computed using Bayes rule from the true g_1 and π_0). As predicted, *lfdr* is overestimated, especially in scenarios which involve many non-zero effects that are very near 0 (e.g. the spiky scenario with π_0 small) where π_0 can be grossly overestimated. (Of course other methods will be similarly affected by this: those that more grossly overestimate π_0, will more grossly overestimate *lfdr* and FDR/q-values.)

The key point we want to make here is estimation of π_0, and the accompanying identifiability issues, become substantially less troublesome if we use the local false sign rate *lfsr* (7), rather than *lfdr*, to measure significance. This is essentially because *lfsr* is less sensitive to the estimate of π_0. To illustrate, Figure 2d compares the estimated *lfsr* from ash.n with the true value: although the estimated *lfsr* continue to be conservative, overestimating the truth, the overestimation is substantially less pronounced than for the *lfdr*, especially for the "spiky" scenario. Further, in the bi-modal scenario, the anti-conservative behaviour is less pronounced in *lfsr* than *lfdr*.

Note that, compared with previous debates regarding testing, this section advances an additional reason for focussing on the sign of the effect, rather than just testing whether it is 0. In previous debates authors have argued against testing whether an effect is 0 because it is *implausible that effects are exactly 0*. Here we add that *even if one believes that some effects may be exactly zero*, it is still better to focus on the sign, because generally *the data are more informative about that question* and so inferences are more robust to, say, the inevitable misestimation of π_0. To provide some intuition, consider an observation with a z score of 0. The *lfdr* of this observation can range from 0 (if $\pi_0 = 0$) to 1 (if $\pi_0 = 1$). But, assuming a symmetric g, the *lfsr* > 0.5 whatever the value of π_0, because the observation $z = 0$ says nothing about the sign of the effect. Thus, are two reasons to use the *lfsr* instead of the *lfdr*: it answers a question that is more generally meaningful (e.g. it applies whether or not zero effects truly exist), and estimation of *lfsr* is more robust.

Given that we argue for using *lfsr* rather than *lfdr*, one might ask whether we even need a point mass on zero in our analysis. Indeed, one advantage of the *lfsr* is that it makes sense even if no effect is exactly zero. And, if we are prepared to assume that no effects are exactly zero, then removing the point mass yields smaller and more accurate estimates of *lfsr* when that assumption is true (Figure 6a). However, there is "no free lunch": if in fact some effects are exactly zero then the analysis with no point mass will tend to be anti-conservative, underestimating *lfsr* (Figure 6b). We conclude that *if* ensuring a "conservative" analysis is important then one must allow for a point mass at 0.

Numerical Stability

The EM algorithm, which we use here to fit our model, is notorious for convergence to local optima. However, in this case, over hundreds of applications of the procedure, we observed no obvious serious problems caused by such behaviour. To quantify this, we ran `ashr` 10 times on each of the 600 simulated datasets above using a random initialization for π, in addition

running it using our default initialization procedure (see Implementation details). We then compared the largest log-likelihood achieved across all 11 runs with the log-likelihood achieved by the default run. When using a mixture of normals (ash.n) the results were extremely stable: 96% showed a negligible log-likelihood difference (< 0.02), and the largest difference was 0.8. When using mixtures of uniforms (ash.u,ash.hu) results were slightly less stable: 89% showed a negligible log-likelihood difference (< 0.02), and 6% of runs showed an appreciable log-likelihood difference (> 1), with the largest difference being 5.0. However, perhaps suprisingly, even for this largest difference results from the default run (e.g. the *lfsr* values, and the posterior means) were in other ways virtually indistinguishable from the results from the run with the highest log-likelihood. See `http://github.com/stephens999/ash/blob/master/dsc-robust/summarize_dsc_robust.rmd` for further details.

The UA helps provide reliable estimates of g

An important advantage of our EB approach based on modelling the effects β_j, rather than p values or z scores, is that it can estimate the *size* of each effect β_j. Specifically, it provides a posterior distribution for each β_j, which can be used to construct interval estimates for β_j and address question such as "which effects exceed T", for any threshold T. Further, because the posterior distribution is, by definition, conditional on the observed data, interval estimates based on posterior distributions are also valid Bayesian inferences for any subset of the effects that have been selected based on the observed data. This kind of "post-selection" validity is much harder to achieve in the frequentist paradigm. In particular the posterior distribution solves the (Bayesian analogue of the) "False Coverage Rate" problem posed by [12] which [6] summarizes as follows: "having applied FDR methods to select a set of nonnull cases, how can confidence intervals be assigned to the true effect size for each selected case?". [6] notes the potential for EB approaches to tackle this problem, and [13] consider in detail the case where the non-null effects are normally distributed.

The ability of the EB approach to provide valid "post-selection" interval estimates is extremely attractive in principle. But its usefulness in practice depends on reliably estimating the distribution g. Estimating g is a "deconvolution problem", which are notoriously difficult in general. Indeed, Efron emphasises the difficulties of implementing a stable general algorithm, noting in his rejoinder "the effort foundered on practical difficulties involving the perils of deconvolution... Maybe I am trying to be overly nonparametric ... but it is hard to imagine a generally satisfactory parametric formulation..." ([6] rejoinder, p46). Our key point here is that the UA greatly simplifies the deconvolution problem. While not meeting Efron's desire for an entirely general nonparametric approach, we believe that the UA can handle many cases of practical interest.

To illustrate this, Figure 3 compares the estimated g from `ashr` with that from `mixfdr` which does not make the UA (and which models g as a mixture of J normal distributions, with $J = 3$ by default). The greater reliability of estimates afforded by the UA is immediately apparent. In particular the estimated cdf from `mixfdr` often has an almost-vertical segment at some non-zero location, indicative of a concentration of density in the estimated g at that location. The UA prevents this kind of "irregular" behaviour, effectively requiring g to be somewhat smooth. While the UA is not the only way to achieve this, we find it an attractive, simple and effective

approach.

Interestingly, even in the "bimodal" scenario `ashr` is visually more accurate than `mixfdr`: although `mixfdr` is capable, in principle, of fitting the multiple modes of g, it does not do this well here. Possibly the noise level here is sufficiently large to make reliable estimation of the multiple modes difficult. Indeed, in multi-modal simulations where the multiple modes are sufficiently well-spaced to be clearly visible in the observed $\hat{\beta}$, `mixfdr` fits these modes (`http://github.com/stephens999/ash/blob/master/dsc-shrink/check_mixfdr_lownoise.rmd`). Of course, we would not advocate the UA in settings where multi-modality is clearly visible in the observed $\hat{\beta}$.

We note one caveat on the accuracy of estimated g: due to the penalty term (18) `ashr` tends to systematically overestimate the mass of g near zero. On careful inspection, this is apparent in Figure 3: the estimated cdf is generally below the true cdf just to the left of zero, and above the true cdf just to the right of zero. Averaging the cdf over many replicates confirms this systematic effect (Figure 3b), and applying our methods without the penalty term removes this systematic effect, although at the cost of sometimes under-estimating π_0 (Figure 3c).

Calibration of posterior intervals

To quantify the effects of errors in estimates of g we examine the calibration of the resulting posterior distributions (averaged over 100 simulations in each Scenario). Specifically we examine the empirical coverage of nominal lower 95% credible bounds for a) all observations; b) significant negative discoveries; c) significant positive discoveries. We examine only lower bounds because the results for upper bounds follow by symmetry (except for the one asymmetric scenario). We separately examine positive and negative discoveries because the lower bound plays a different role in each case: for negative discoveries the lower bound is typically large and negative and limits how big (in absolute value) the effect could be; for positive discoveries the lower bound is positive, and limits how small (in absolute value) the effect could be. Intuitively, the lower bound for negative discoveries depends on the accuracy of g in its tail, whereas for positive discoveries it is more dependent on the accuracy of g in the center.

The results are shown in Table 1. Most of the empirical coverage rates are in the range 0.92-0.96 for nominal coverage of 0.95, which we view as adequate for practical applications. The strongest deviations from nominal rates are noted and discussed in the table captions.

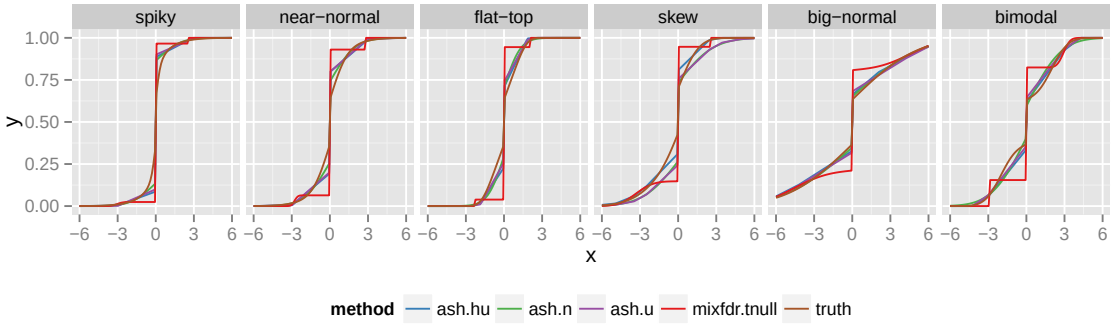
Differing measurement precision across units

We turn now to the second important component of our work: allowing for varying measurement precision across units. The key to this is the use of a likelihood, (4) or (13), that explicitly incorporates the measurement precision (standard error) of each $\hat{\beta}_j$.

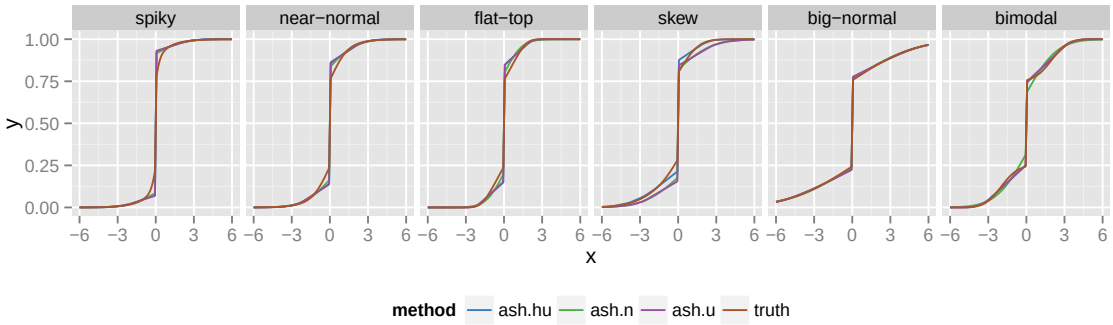
To illustrate, we conduct a simulation where half the measurements are quite precise (standard error $s_j = 1$), and the other half are very poor ($s_j = 10$). In both cases, we assume that half the effects are null and the other half are normally distributed with standard deviation 1:

$$p(\beta) = 0.5\delta_0(\beta) + 0.5N(\beta; 0, 1). \tag{10}$$

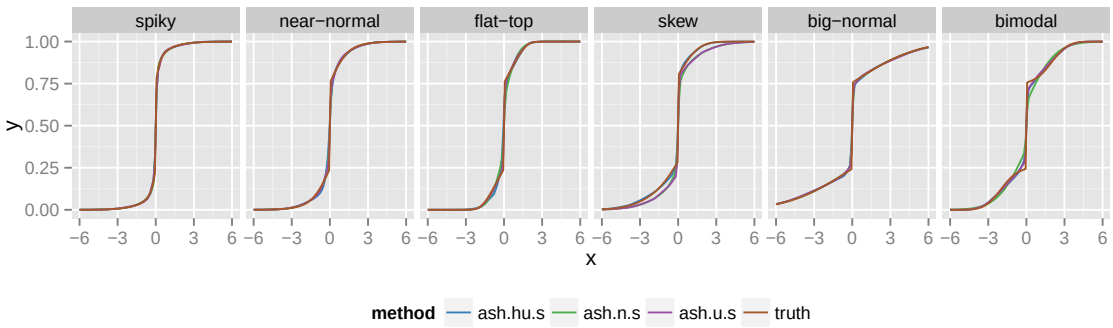
In this setting, the poor-precision measurements ($s_j = 10$) tell us very little, and any sane analysis should effectively ignore them. However, this is not the case in standard FDR-type analyses



(a) Example estimated cdfs for single data sets compared with truth. The unimodal assumption made by the ash methods effectively regularizes estimates compared with `mixfdr`.



(b) Average estimated cdfs across ~ 10 data sets compared with truth; methods here use penalty (18) so π_0 is systematically overestimated.



(c) Average estimated cdfs across ~ 10 data sets compared with truth; methods here do not use penalty (18) so π_0 is not systematically overestimated. Systematic differences from the truth in "skew" and "bimodal" scenarios highlight the effects of model mis-specification.

Figure 3. Comparisons of estimated cdfs of g and true cdf of g. See Figure 2b for simulation scenarios.

	spiky	near-normal	flat-top	skew	big-normal	bimodal
ash.n	0.90	0.94	0.95	0.94	0.96	0.96
ash.u	0.87	0.93	0.94	0.93	0.96	0.96
ash.hu	0.88	0.93	0.94	0.94	0.96	0.96

(a) All observations. Coverage rates are generally satisfactory, except for the extreme "spiky" scenario. This is due to the penalty term (18) which tends to cause over-shrinking towards zero. Removing this penalty term produces coverage rates closer to the nominal levels for uniform and normal methods (Table 3). Removing the penalty in the half-uniform case is not recommended (see Appendix).

	spiky	near-normal	flat-top	skew	big-normal	bimodal
ash.n	0.93	0.94	1.00	0.94	0.95	0.98
ash.u	0.86	0.88	0.93	0.91	0.94	0.94
ash.hu	0.87	0.87	0.92	0.93	0.94	0.94

(b) "Significant" negative discoveries. Coverage rates are generally satisfactory, except for the uniform-based methods in the spiky and near-normal scenarios, and the normal-based method in the flat-top scenario. These results likely reflect inaccurate estimates of the tails of g due to a disconnect between the tail of g and the component distributions in these cases. For example, the uniform methods sometimes substantially underestimate the length of the tail of g in these long-tailed scenarios, causing over-shrinkage of the tail toward 0.

	spiky	near-normal	flat-top	skew	big-normal	bimodal
ash.n	0.94	0.94	0.94	0.86	0.95	0.96
ash.u	0.93	0.93	0.93	0.84	0.95	0.95
ash.hu	0.92	0.92	0.93	0.92	0.95	0.95

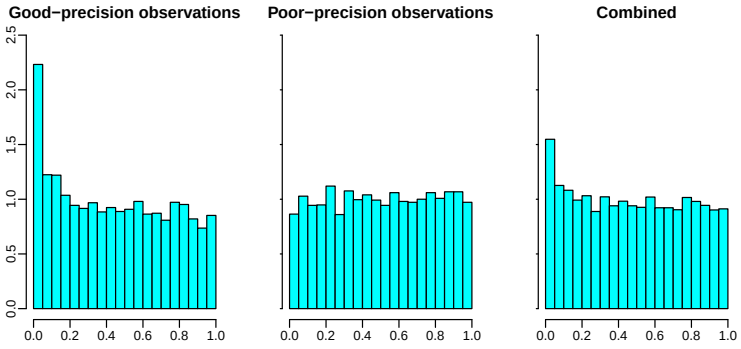
(c) "Significant" positive discoveries. Coverage rates are generally satisfactory, except for the symmetric methods under the asymmetric ("skew") scenario.

Table 1. Table of empirical coverage for nominal 95% lower credible bounds

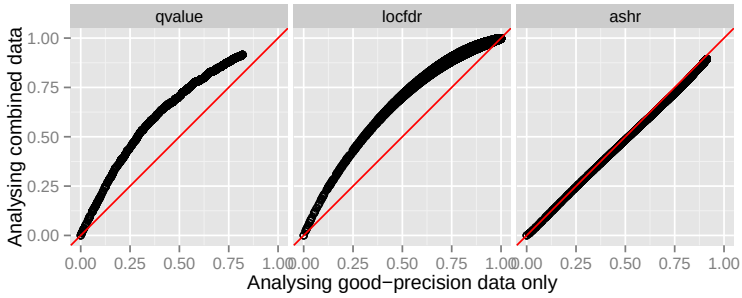
(Figure 4). This is because the poor measurements produce p values that are approximately uniform (Figure 4a), which, when combined with the good-precision measurements, dilute the overall signal (e.g. they reduce the density of p values near 0). This is reflected in the results of FDR methods like `qvalue` and `locfdr`: the estimated error rates (q-values, or *lfdr* values) for the good-precision observations increase when the low-precision observations are included in the analysis (Figure 4b). In contrast, the results from `ashr` for the good-precision observations are unaffected by including the low-precision observations in the analysis (Figure 4b).

Reordering of significance, and the "p value prior"

Another consequence of accounting for differences in measurement precision is that `ashr` may re-order the significance of the observations compared with the original p values or z scores. This is illustrated, using the same simulation as above, in Figure 5 (left panel). We see that poor precision measurements are assigned a higher *lfsr* than good precision measuments that



(a) Density histograms of p values for good-precision, poor-precision, and combined observations



(b) Comparison of results of different methods applied to good-precision observations only (x axis) and combined data (y axis). Each point shows the "significance" (q values from `qvalue`; *lfdr* for `locfdr`; *lfsr* for `ashr`) of a good-precision observation under the two different analyses.

Figure 4. Simulation illustrating how, for existing FDR methods, poor-precision observations can contaminate signal from good-precision observations. The top panel (a) illustrates that when p values from good-precision observations (left) and from poor-precision observations (center) are combined (right), they produce a distribution of p values with less overall signal - and so, by conventional methods, will give a higher estimated FDR at any given threshold. The bottom panel (b) illustrates this behaviour directly for the methods `qvalue` and `locfdr`: the q-values from `qvalue` and the *lfdr* estimates from `locfdr` are higher when applied to all data than when applied to good-precision observations only. In contrast the methods described here (`ashr`) produce effectively the same results (here, the lfsr) in the good-precision and combined data analyses.

have the same p value. The intuition is that, due to their poor precision, these measurements contain very little information about the sign of the effects (or indeed any other aspect of the effects), and so the *lfsr* for these poor-precision measurements is always high.

The potential for Bayesian analyses to re-order the significance of observations, and specifically to downweight imprecise observations, was previously discussed in [31]. However, Wakefield [22] showed that, under a certain prior assumption, the Bayesian analysis produces the same ranking of significance as p values (or their z scores). He named this prior the "p-value prior" because it can be thought of as the implicit prior assumption that is being made if we rank the significance of observations by their p value. Wakefield's p-value prior assumes that the less precise effect estimates correspond to larger true effects, and specifically that they scale proportional to the standard errors s_j. More specifically still, it assumes a normal prior for the non-zero β_j with mean 0 and variance Ks_j^2 for some constant K. Here we observe that Wakefield's result extends to our mixture of (zero-mean) normal priors. Specifically, if, instead of assuming that β_j is independent of s_j as we have up to now, we assume that $z_j = \beta_j/s_j$ is independent of s_j, and drawn from a mixture of zero-mean normal distributions, then the *lfsr* computed by ash.n provides the *same ranking* of observations as the z scores and p values, as is illustrated in Figure 5, right panel. (This result does not hold when using the mixtures of uniforms prior, ash.u.)

This p-value prior assumes that the z scores $z_j = \beta_j/s_j$ are identically distributed, independent of s_j. This is essentially the assumption made, implicitly or explicitly, by existing methods – like `locfdr`, `mixfdr` and `qvalue`– that model the z_j or p_j directly. In contrast, we have assumed up to now that the β_j are independent of s_j. We can set both these assumptions within a more general framework, which allows that β_j/s^α is independent of s_j for some α [Equation (12))]. Setting $\alpha = 0$ implies that β_j is independent of s_j, as we have assumed up to now, and $\alpha > 0$ implies that observations with larger standard error tend to have larger effects (in absolute value). This latter assumption may often be qualitatively plausible: for example, in gene expression studies the standard error for gene j depends partly on the variance of its expression among samples, and genes with a larger variance may tend to be less tightly regulated and so be amenable to a larger shift in expression between conditions (i.e. larger effect β_j). On the other hand, there is no particular reason to expect that either $\alpha = 1$ or $\alpha = 0$ will be the optimal choice. Indeed, optimal choice of α will depend on the actual relationship between β_j and s_j, which will be dataset-specific. Framing the problem in this way – that is, as comparing different modelling assumptions for β_j, rather than as comparing "modelling β_j" vs "modelling z_j" (or "modelling p_j") – has the important advantage that likelihood-based methods can be used to select α. For example, following the logic of the EB approach it would be natural to select α by maximum likelihood. Since α is a one-dimensional parameter, this can be achieved by a 1-d grid search, which has been implemented in our software by C. Dai.

Discussion

We have presented an Empirical Bayes approach to large-scale multiple testing that emphasises two ideas. First, we emphasise the potential benefits of using two numbers ($\hat{\beta}$, and its standard error) rather than just one number (a p value or z score) to summarize the information on each

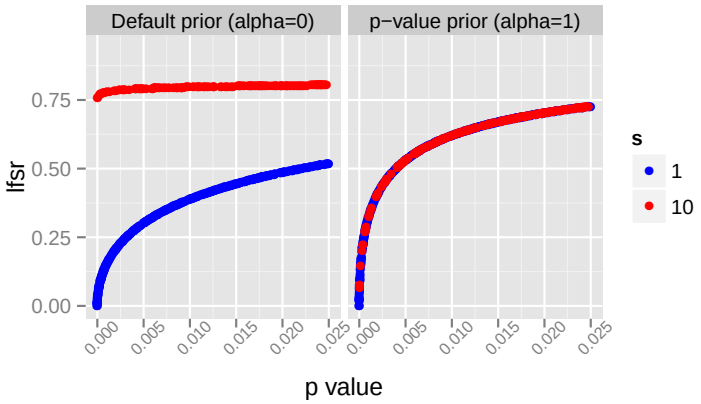


Figure 5. Figure illustrating affects of prior assumptions on re-ordering of significance. Left panel shows results under our "default prior" which assumes that effects β_j are identically distributed, independent of s_j. Right panel shows results under the "p-value prior", which assumes that z scores β_j/s_j are identically distributed, independent of s_j.

test. While requiring two numbers is slightly more onorous than requiring one, in many settings these numbers are easily available and if so we argue it makes sense to use them. Second, we note the potential benefits – both statistical and computational – of assuming that the effects come from a unimodal distribution, and provide flexible implementations for performing inference under this assumption. We also introduce the "false sign rate" as an alternative measure of error to the FDR, and illustrate its improved robustness to errors in model fit, particularly mis-estimation of the proportion of null tests, π_0.

Multiple testing is often referred to as a "problem" or a "burden". In our opinion, EB approaches turn this idea on its head, treating multiple testing as an *opportunity*: specifically, an opportunity to learn about the prior distributions, and other modelling assumptions, to improve inference and make informed decisions about significance (see also [2]). This view also emphasises that, what matters in multiple testing settings is *not* the number of tests, but the *results* of the tests. Indeed, the FDR at a given fixed threshold does not depend on the number of tests: as the number of tests increases, both the true positives and false positives increase linearly, and the FDR remains the same. (If this intuitve argument does not convince, see [21], and note that the FDR at a given p value threshold does not depend on the number of tests m.) Conversely, the FDR *does* depend on the overall distribution of effects, and particularly on π_0 for example. The EB approach captures this dependence in an intuitive way: if there are lots of strong signals then we infer π_0 to be small, and the estimated FDR (or *lfdr*, or *lfsr*) at a given threshold may be low, even if a large number of tests were performed; and conversely if there are no strong signals then we infer π_0 to be large and the FDR at the same threshold may be high, even if relatively few tests were performed. More generally, overall signal strength is reflected in the estimated g, which in turn influences the estimated FDR.

Two important practical issues that we have not addressed here are correlations among tests, and the potential for deviations from the theoretical null distributions of test statistics.

These two issues are connected: specifically, unmeasured confounding factors can cause both correlations among tests and deviations from the theoretical null [15, 16]. And although there are certainly other factors that could cause dependence among tests, unmeasured confounders are perhaps the most worrisome in practice because they can induce strong correlations among large numbers of tests and profoundly impact results. Approaches to deal with unmeasured confounders can be largely divided into two types: those that simply attempt to correct for the resulting inflation of test statistics [32, 33], and those that attempt to infer counfounders using clustering, principal components analysis, or factor models [16,34–36], and then correct for them in computation of the test statistics (in our case, $\hat{\beta}, \hat{s}$). When these latter approaches are viable, they provide perhaps the most satisfactory solution, and are certainly a good fit for our framework. Alterntively, our methods could also be modified to allow for test statistic inflation, perhaps by incorporating the inflation into the likelihood (4) using $\hat{\beta}_j|\hat{s}_j \sim N(0, \lambda_1\hat{s}_j + \lambda_2)$, where λ_1, λ_2 are to be estimated. However, this immediately raises issues of identifiability and we do not pursue the idea further here.

Another important practical issue is the challenge of small sample sizes. For example, in genomics applications researchers sometimes attempt to identify differences between two conditions based on only a handful of samples in each. In such settings the normal likelihood approximation (4) will be inadequate. And, although the t likelihood (13) partially addresses this issue, it is also, it turns out, not entirely satisfactory. The root of the problem is that, with small sample sizes, raw estimated standard errors $\hat{s}_j$ can be horribly variable. In genomics it is routine to address this issue by applying EB methods [37] to "moderate" (i.e. shrink) variance estimates, before computing p values from "moderated" test statistics. We are currently investigating how our methods should incorporate such "moderated" variance estimates to make it applicable to small sample settings.

Our approach involves compromises between flexibility, generality, and simplicity on the one hand, and statistical efficiency and principle on the other. For example, in using an EB approach that uses a point estimate for g, rather than a fully Bayes approach that accounts for uncertainty in g, we have opted for simplicity over statistical principle. And in summarizing every test by two numbers and making a normal or t approximation to the likelihood, we have aimed to produce generic methods that can be applied whenever such summary data are available – just as `qvalue` can be applied to any set of p values for example – although possibly at the expense of statistical efficiency compared with developing multiple tailored approaches based on context-specific likelihoods. Any attempt to produce generic methods will involve compromise between generality and efficiency. In genomics, many analyses – not only FDR-based analyses – involve first computing a series of p values before subjecting them to some further downstream analysis. An important message here is that working with two numbers $(\hat{\beta}_j, \hat{s}_j)$, rather than one ($p_j$ or z_j), can yield substantial gains in functionality (e.g. estimating effect sizes, as well as testing; accounting for variations in measurement precision across units) while losing only a little in generality. We hope that our work will encourate development of methods that exploit this idea in other contexts.

Detailed Methods

Embellishments

More flexible unimodal distributions

Using a mixture of zero-centered normal distributions for g in (3) implies that g is not only unimodal, but also symmetric. Furthermore, even some symmetric unimodal distributions, such as those with a flat top, cannot be well approximated by a mixture of zero-centered normals. Therefore, we have implemented a more general approach based on

$$g(\cdot;\pi) = \sum_{k=0}^{K} \pi_k f_k(\cdot), \tag{11}$$

where f_0 is a point mass on 0, and f_k $(k = 1, \ldots, K)$ are pre-specified component distributions with one of the following forms:

(i) $f_k(\cdot) = N(\cdot; 0, \sigma_k^2)$, ("ash.n")

(ii) $f_k(\cdot) = U[\cdot; -a_k, a_k]$, ("ash.u")

(iii) $f_k(\cdot) = U[\cdot; -a_k, 0]$ and/or $U[\cdot; 0, a_k]$, ("ash.hu")

where $U[\cdot; a, b]$ denotes the density of a uniform distribution on $[a, b]$. (In (iii) we include both components in the mixture (11), so a grid of values $a_1, \ldots, a_K$ defines $2K+1$ mixture component densities, and π is a $2K + 1$ vector that sums to 1.) The simplest version (3) corresponds to (i). Replacing these with uniform components (ii)-(iii) only slightly complicates calculations under the normal likelihood (4), and greatly simplifies the calculations under the t likelihood (13) introduced below. The use of uniform components here closely mirrors [26]. (In fact our implementation can handle *any* prespecified uniform or normal distributions for f_k provided they are all from the same family; however, we restrict our attention here to (i)-(iii) which imply a unimodal g.)

Moving from (i) to (iii) the representation (11) becomes increasingly flexible. Indeed, using a large dense grid of σ_k^2 or a_k, (i)-(iii) can respectively approximate, with arbitrary accuracy,

(i) any scale mixture of normals, which includes as special cases the double exponential (Laplace) distribution, any t distribution, and a very large number of other distributions used in high-dimensional regression settings.

(ii) any symmetric unimodal distribution about 0.

(iii) any unimodal distribution about 0.

The latter two claims are related to characterizations of unimodal distributions due to [38] and [39]; see [40], p158. In other words, (ii) and (iii) provide fully non-parametric estimation for g under the constraints that it is (ii) both unimodal and symmetric, or (iii) unimodal only.

Although our discussion above emphasises the use of large K, in practice modest values of K can provide reasonable performance. The key point is that the value of K is not critical provided it is sufficiently large, and the grid of σ_k or a_k values suitably chosen. See Implementation for details of our software defaults.

Dependence of effects on standard errors

Equation (2) assumes that the β_j all come from the same distribution g, independent of $\hat{s}_j$. This can be relaxed to allow the distribution of β_j to depend on $\hat{s}_j$ using

$$\frac{\beta_j}{\hat{s}_j^\alpha} \,|\hat{s}_j \sim g(\cdot;\pi) \tag{12}$$

for any α. Setting $\alpha = 0$ yields (2), and setting $\alpha = 1$ corresponds to assuming that the $t_j = \beta_j/\hat{s}_j$ have a common distribution. This case is of special interest: it effectively corresponds to the "p value prior" in [22] and is, implicitly, the assumption made by existing FDR methods that rank tests by their p values (or z or t scores). See Results for further discussion.

The model 12 for general α can be fitted using the algorithm for $\alpha = 0$. To see this, define $b_j := \beta_j/\hat{s}_j^\alpha$, and $\hat{b} := \hat{\beta}_j/\hat{s}_j^\alpha$. Then $\hat{b}_j$ is an estimate of b_j with standard error $\hat{s}'_j := \hat{s}_j^{1-\alpha}$. Applying the algorithm for $\alpha = 0$ to effect estimates $\hat{b}_1, \ldots, \hat{b}_J$ with standard errors $\hat{s}'_1, \ldots, \hat{s}'_J$ yields a posterior distribution $p(b_j|\hat{s}_j, \hat{b}_j, \hat{\pi}, \alpha)$, which induces a posterior distribution on $\beta_j = b_j\hat{s}_j^\alpha$.

Replace normal likelihood with t likelihood

We generalize the normal likelihood (4) by replacing it with a t likelihood:

$$\hat{\beta}_j \,|\, \beta_j, \hat{s}_j \sim T_\nu(\beta_j, \hat{s}_j) \tag{13}$$

where $T_\nu(\beta_j, \hat{s}_j)$ denotes the distribution of $\beta_j + \hat{s}_j T_\nu$ where T_ν has a standard t distribution on ν degrees of freedom, and ν denotes the degrees of freedom used to estimate $\hat{s}_j$ (assumed known, and for simplicity assumed to be the same for each j). The normal approximation (4) corresponds to the limit $\nu \to \infty$. This generalization does not complicate inference when the mixture components f_k in (11) are uniforms; see Implementation below. When the f_k are normal the computations with a t likelihood are considerably more difficult and we have not implemented this combination.

Equation (13) is, of course, motivated by the standard asymptotic result

$$(\hat{\beta}_j - \beta_j)/\hat{s}_j \sim T_\nu. \tag{14}$$

However (14) does not imply (13), because in (14) $\hat{s}_j$ is random whereas in (13) it is conditioned on. In principle it would be preferable, for a number of reasons, to model the randomness in $\hat{s}_j$; we are currently pursuing this improved approach (joint work with M.Lu) and results will be published elsewhere.

Non-zero mode

An addition to our software implementation, due to C.Dai, allows the mode to be estimated from the data by maximum likelihood, rather than fixed to 0.

Implementation Details

Likelihood for π

We define the likelihood for π to be the probability of the observed data $\hat{\beta}$ conditional on $\hat{s}$: $L(\pi) := p(\hat{\beta}|\hat{s}, \pi)$, which by our conditional indendence assumptions is equal to the product $\prod_j p(\hat{\beta}_j|\hat{s}, \pi)$. [One might prefer to define the likelihood as $p(\hat{\beta}, \hat{s}|\pi) = p(\hat{\beta}|\hat{s}, \pi)p(\hat{s}|\pi)$, in which case our definition comes down to assuming that the term $p(\hat{s}|\pi)$ does not depend on π.]

Using the prior $\beta_j \sim \sum_{k=0}^{K} \pi_k f_k(\beta_j)$ given by (11), and the normal likelihood (4), integrating over β_j yields

$$p(\hat{\beta}_j|\hat{s}, \pi) = \sum_{k=0}^{K} \pi_k \tilde{f}_k(\hat{\beta}_j) \tag{15}$$

where

$$\tilde{f}_k(\hat{\beta}_j) := \int f_k(\beta_j) N(\hat{\beta}_j; \beta_j, \hat{s}_j^2)\, d\beta_j \tag{16}$$

denotes the convolution of f_k with a normal density. These convolutions are straightforward to evaluate whether f_k is a normal or uniform density. Specifically,

$$\tilde{f}_k(\hat{\beta}_j) = \begin{cases} N(\hat{\beta}_j; 0, \hat{s}_j^2 + \sigma_k^2) & \text{if } f_k(\cdot) = N(\cdot; 0, \sigma_k^2), \\ \frac{\Psi((\hat{\beta}_j - a_k)/\hat{s}_j) - \Psi((\hat{\beta}_j - b_k)/\hat{s}_j)}{b_k - a_k} & \text{if } f_k(\cdot) = U(\cdot; a_k, b_k), \end{cases} \tag{17}$$

where Ψ denotes the cumulative distribution function (c.d.f.) of the standard normal distribution. If we replace the normal likelihood with the t_ν likelihood (13) then the convolution for f_k uniform the convolution is still given by (17) but with Ψ the c.d.f. of the t_ν distribution function. (The convolution for f_k normal is tricky and we have not implemented it.)

Penalty term on π

To make *lfdr* and *lfsr* estimates from our method "conservative" we add a penalty term $log(h(\pi; \lambda))$ to the log-likelihood $\log L(\pi)$ to encourage over-estimation of π_0:

$$h(\pi; \lambda) = \prod_{k=0}^{K} \pi_k^{\lambda_k - 1} \tag{18}$$

where $\lambda_k \geq 1 \, \forall k$. The default is $\lambda_0 = 10$ and $\lambda_k = 1$, which yielded consistently conservative estimation of π_0 in our simulations (Figure 2b).

Although this penalty is based on a Dirichlet density, we do not interpret this as a "prior distribution" for π: we chose it to provide conservative estimates of π_0 rather than to represent prior belief.

Problems with removing the penalty term in the half-uniform case

It is straightforward to remove the penalty term by setting $\lambda_k = 1$ in (18). We note here an unanticipated problem we came across when using no penalty term in the half-uniform case (i.e. $f_k(\cdot) = U[\cdot; -a_k, 0]$ and/or $U[\cdot; 0, a_k]$ in (11)): when the data are nearly null, the estimated

g converges, as expected and desired, to a distribution where almost all the mass is near 0, but sometimes all this mass is concentrated almost entirely just to one side (left or right) or 0. This can have a very profound effect on the local false sign rate: for example, if all the mass is just to the right of 0 then all observations will be assigned a very high probability of being positive (but very small), and a (misleading) low local false sign rate. For this reason we do not recommend use of the half-uniform with no penalty.

EM algorithm

With this in place, the penalized log-likelihood for π is given by:

$$\log L(\pi) + \log h(\pi) = \sum_{j=1}^{n} \log(\sum_{k=0}^{K} \pi_k l_{kj}) + \sum_{k=0}^{K} (\lambda_k - 1) \log \pi_k \tag{19}$$

where the $l_{kj} := \tilde{f}_k(\hat{\beta}_j)$ are known. This can be maximized using an EM algorithm [17], whose one-step updates are:

$$w_{kj} = \pi_k l_{kj} / \sum_{k'} \pi_{k'} l_{k'j} \tag{20}$$

$$n_k = \sum_j w_{kj} + \lambda_k - 1 \quad \text{[E Step]} \tag{21}$$

$$\pi_k = n_k / \sum_{k'} n_{k'} \quad \text{[M step]} \tag{22}$$

Note that π_k can be interpreted as the prior probability that β_j arose from component k, and l_{kj} is the likelihood for β_j given that it arose from component k, so w_{kj} is the posterior probability that β_j arose from component k, given $\hat{\beta}, \hat{s}, \pi$. (The w_{kj} are sometimes referred to as the "responsibilities".) Thus n_k is the expected number of β_j that arose from component k, plus pseudo-counts $\lambda_k - 1$ from the penalty term. We used the elegant R package `SQUAREM` [41] to accelerate convergence of this EM algorithm.

Initialization

By default we initialize our EM algorithm with $\pi_k = 1/n$ for $k = 1, \ldots, K$, with $\pi_0 = 1 - \pi_1 - \cdots - \pi_K$. (In all our simulations here $K << n$ so this initializes with most mass on π_0.) Our rationale for initializing "near the null" like this is that we expect that strong signal in the data can quickly draw the EM algorithm away from the null (in a single iteration), but weak signal in the data cannot quickly draw the algorithm towards the null.

In addition, once the EM algorithm has converged, we check that the (penalized) log-likelihood attained is higher than that achieved by the global null solution $\pi_0 = 1; \pi_k = 0 (k > 0)$. If not then we replace the EM solution with the global null solution. This aims to guard against errors due to convergence to a local optimum when the data are consistent with the global null.

Conditional distributions

Given $\hat{\pi}$, we compute the conditional distributions

$$p(\beta_j|\hat{\pi},\hat{\beta},s) \propto g(\beta_j;\pi)L(\beta_j;\hat{\beta}_j,\hat{s}_j). \tag{23}$$

Each posterior is a mixture on $K+1$ components:

$$p(\beta_j|\hat{\pi},\hat{\beta},s) = \sum_{k=0}^{K} w_{kj}p_k(\beta_j|\hat{\beta}_j,\hat{s}_j) \tag{24}$$

where the posterior weights w_{kj} are computed as in (20) with $\pi = \hat{\pi}$, and the posterior mixture component p_k is the posterior on β_j that would be obtained using prior $f_k(\beta_j)$ and likelihood $L(\beta_j;\hat{\beta}_j,\hat{s}_j)$. All these posterior distributions are easily available. For example, if f_k is uniform and L is t_ν then this is a truncated t distribution. If f_k is normal and L is normal, then this is a normal distribution.

Choice of grid for σ_k, a_k

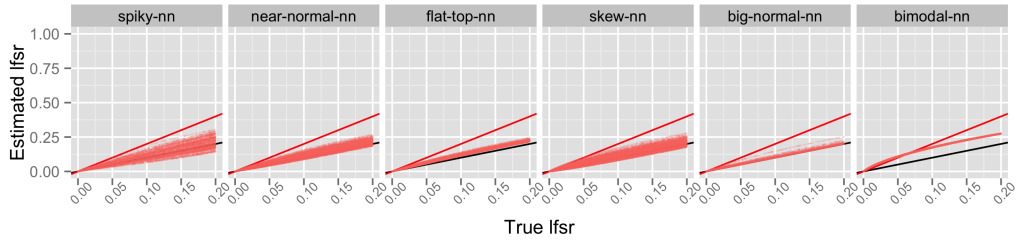
When f_k is $N(0,\sigma_k)$ we specify our grid by specifying: i) a maximum and minimum value $(\sigma_{\min},\sigma_{\max})$; ii) a multiplicative factor m to be used in going from one gridpoint to the other, so that $\sigma_k = m\sigma_{k-1}$. The multiplicative factor affects the density of the grid; we used $m = \sqrt{2}$ as a default. We chose $\sigma_{\min}$ to be small compared with the measurement precision ($\sigma_{\min} = min(\hat{s}_j)/10$) and $\sigma_{\max} = 2\sqrt{\max(\hat{\beta}_j^2 - \hat{s}_j^2)}$ based on the idea that $\sigma_{\max}$ should be big enough so that $\sigma_{\max}^2 + \hat{s}_j^2$ should exceed $\hat{\beta}_j^2$. (In rare cases where $\max(\hat{\beta}_j^2 - \hat{s}_j^2)$ is negative we set $\sigma_{\max} = 8\sigma_{\min}$.)

When the mixture components f_k are uniform, we use the same grid for the parameters a_k as for σ_k described above.

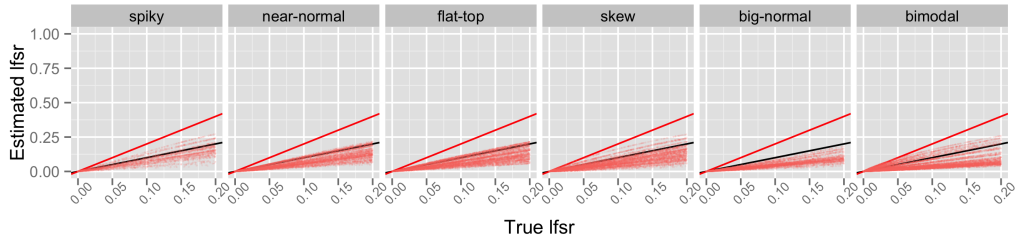
Our goal in specifying a grid was to make the limits sufficiently large and small, and the grid sufficiently dense, that results would not change appreciably with a larger or denser grid. For a specific data set one can of course check this by experimenting with the grid, but these defaults usually work well in our experience.

Scenario	Alternative distribution, g_1
spiky	$0.4N(0,0.25^2) + 0.2N(0,0.5^2) + 0.2N(0,1^2), 0.2N(0,2^2)$
near normal	$2/3N(0,1^2) + 1/3N(0,2^2)$
flattop	$(1/7)[N(-1.5,.5^2) + N(-1,.5^2) + N(-.5,.5^2) +$ $N(0,.5^2) + N(0.5,.5^2) + N(1.0,.5^2) + N(1.5,.5^2)]$
skew	$(1/4)N(-2,2^2) + (1/4)N(-1,1.5^2) + (1/3)N(0,1^2) + (1/6)N(1,1^2)$
big-normal	$N(0,4^2)$
bimodal	$0.5N(-2,1^2) + 0.5N(2,1^2)$

Table 2. Summary of simulation scenarios considered



(a) Comparison of true and estimated *lfsr* when data are simulated with no point mass at zero ($\pi_0 = 0$), and also analyzed by `ashr` with no point mass on 0 (and mixture of normal components for g). Black line is $y = x$ and red line is $y = 2x$. The results illustrate how estimates of *lfsr* can be more accurate in this case. That is, assuming there is no point mass can be beneficial if that is indeed true.



(b) Comparison of true and estimated *lfsr* when data are simulated with point mass at zero (drawn uniformly from [0,1] in each simulation), but analyzed by `ashr` with no point mass on 0 (and mixture of normal components for g). Black line is $y = x$ and red line is $y = 2x$. The results illustrate how estimates of *lfsr* can be anti-conservative if we assume there is no point mass when the truth is that there is a point mass.

Figure 6. Illustration of effects of excluding a point mass from the analysis.

	spiky	near-normal	flat-top	skew	big-normal	bimodal
ash.n.s	0.95	0.95	0.95	0.95	0.96	0.96
ash.u.s	0.94	0.95	0.95	0.94	0.96	0.96
ash.hu.s	0.88	0.92	0.92	0.92	0.92	0.93

(a) All observations

	spiky	near-normal	flat-top	skew	big-normal	bimodal
ash.n.s	0.95	0.95	0.99	0.93	0.95	0.97
ash.u.s	0.89	0.91	0.89	0.91	0.94	0.94
ash.hu.s	0.89	0.92	0.91	0.94	0.95	0.94

(b) "Significant" negative discoveries.

	spiky	near-normal	flat-top	skew	big-normal	bimodal
ash.n.s	0.94	0.94	0.92	0.88	0.95	0.94
ash.u.s	0.94	0.93	0.93	0.88	0.95	0.95
ash.hu.s	0.32	0.61	0.53	0.54	0.79	0.82

(c) "Significant" positive discoveries.

Table 3. Table of empirical coverage for nominal 95% lower credible bounds for methods *without* the penalty term).

Acknowledgements

Statistical analyses were conducted in the R programming language [42], Figures produced using the `ggplot2` package [43], and text prepared using LaTeX. Development of the methods in this paper was greatly enhanced by the use of the `knitr` package [44] within the RStudio GUI, and git and github. The `ashr` R package is available from `http://github.com/stephens999/ashr` and includes contributions from Chaoxing (Rick) Dai, Mengyin Lu, and Tian Sen.

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Figure Legends

Tables

Supporting Information Legends

Supplementary material can be found in **Supplementary Information S1.**