A Bayesian model for single cell transcript expression analysis on MERFISH data

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Abstract

Motivation: Multiplexed error-robust fluorescence in-situ hybridization (MERFISH) is a recent technology to obtain spatially resolved gene or transcript expression profiles in single cells for hundreds to thousands of genes in parallel. So far, no statistical framework to analyze MERFISH data is available.

Results: We present a Bayesian model for single cell transcript expression analysis on MERFISH data. We show that the model successfully captures uncertainty in MERFISH data and eliminates systematic biases that can occur in raw RNA molecule counts obtained with MERFISH. Our model accurately estimates transcript expression and additionally provides the full probability distribution and credible intervals for each transcript. We further show how this enables MERFISH to scale towards the whole genome while being able to control the uncertainty in obtained results.

Availability and implementation: The presented model is implemented on top of Rust-Bio (Koester, 2016) and available open-source as MERFISHTools (https://merfishtools.github.io). It can be easily installed via Bioconda (Gruning et al., 2018). The entire analysis performed in this paper is provided as a fully reproducible Snakemake (Koester and Rahmann, 2012) workflow via Zenodo (https://doi.org/10.5281/zenodo.752340).

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1 Introduction

The investigation of gene or transcript expression at single cell resolution is of increasing importance in various areas of biological and medical research. In particular, it is used to study heterogeneous tissues like brain or tumors (Darmanis et al., 2015; Patel et al., 2014) and it enables the determination of cell types and states (Trapnell, 2015). Based on RNA sequencing (Eberwine et al., 2014; Navya, 2014), fluorescence microscopy (Femino, 1998; Lyubimova et al., 2013) and mass spectrometry (Angelo et al., 2014; Giesen et al., 2014), various technologies and protocols to quantify gene, transcript or protein expression in single cells have emerged. Among these, in situ methods offer the possibility to preserve spatial information at cellular or even subcellular level. Currently, only fluorescence microscopy based approaches (Femino, 1998; Lubeck et al., 2014; Nilsson et al., 1994) offer information about the position of
transcript expression on MERFISH data. We show that this model work, we use this knowledge to build a Bayesian model for gene or error rates, we derive the probabilities for the different events that can happen in the calling process. These constitute an urn model for each transcript. By combining these urns into a joint model over all transcripts, the likelihood of transcript expression given the observed counts can be calculated. (Fig. 1).

Chen et al. (2015) presented a new approach that allows the measurement of hundreds to thousands of different transcripts at the same time, while retaining their coordinates within the cell. In contrast to previous approaches that use multiple colors to measure multiple different transcripts at the same time (Lubeck et al., 2014), MERFISH uses a binary code to represent RNA species. First, a unique binary word (the encoding) of length n is assigned to each gene or transcript of interest (see Fig. 1a). Then, specific readout probes and corresponding fluorescent labels are designed for n rounds of hybridization and bleaching. Therefore, probes are designed such that a 1-bit at position i in the binary word corresponds to a fluorescence signal in the ith hybridization round. When imaging each of the n hybridization rounds, RNA molecules can be localized by fluorescent spots, and the on/off pattern of a spot over the n hybridization rounds yields a binary word (the readout) again. By matching the latter against the designed encoding, individual RNA molecules can be assigned to transcripts or genes, and expression can be quantified. Since both the hybridization and the fluorescence measurement are error-prone, individual bits in the readout can differ from the encoding. To enable the matching to deal with such errors, Chen et al. (2015) propose to use a binary code with a given minimum Hamming distance (Hamming, 1950) between any pair of words. With a Hamming distance of 2, 1-bit errors can be detected. With a Hamming distance of 4, 1-bit errors can also be corrected, by assigning to the closest encoding.

More than one error in a particular readout can lead to missed calls and misidentification, which both are the source for systematic biases in MERFISH data. Indeed, Chen et al. (2015) already report that about 20% of the molecules are missed when using the first version of the MERFISH protocol. A remarkable property of MERFISH is that the average probability for accidentally reading a 1-bit instead of a 0-bit (0-1 error) and vice versa (1-0 error) can easily be determined (Chen et al., 2015; Moffitt et al., 2016). In this work, we use this knowledge to build a Bayesian model for gene or transcript expression on MERFISH data. We show that this model enables the correction of the systematic biases that occur within raw counts. In addition, the model reports full posterior expression distributions for each transcript along with credible intervals. In line with recent reports (Halsey et al., 2015), this enables a thorough exploration of the uncertainties in downstream analyses, which is exemplified in Supplementary Section S6.

2 Materials and methods

We strive to obtain a posterior estimate of transcript (or gene) expression in each single cell that is free of biases (Fig. 1). Here, the unit of expression shall be RNA molecule counts. Using the known rates for 0-1 and 1-0 errors, we derive probabilities for exact calls (i.e. the event that an RNA molecule is identified correctly and no error occurred), corrected calls (correct identification, but 1-bit error corrected), exact miscalls (RNA molecule is assigned to the wrong transcript), corrected miscalls (RNA molecule is wrongly assigned although a 1-bit error has been corrected) and dropouts (RNA molecule is missed because the binary readout is not recognized at all). These probabilities define an urn model that allows the calculation of the probability to observe a certain distribution of calls, miscalls and dropouts for a given transcript expression using a multinomial distribution. By combining such urns into a joint model over all transcripts, the joint likelihood of transcript expressions can be calculated. To ensure computational feasibility, we first estimate the maximum likelihood solution using the EM algorithm, and then calculate individual likelihoods per transcript while fixing miscalls and expressions of other transcripts to the maximum likelihood estimates. Bayes’ theorem yields corresponding posterior probabilities from which the maximum a posteriori expression along with credible intervals can be calculated.

2.1 Readout probabilities

With MERFISH, each transcript is labeled with a different set of encoding probes. In each of N performed hybridization rounds, different encoding probes are targeted with fluorescent labels. The encoding probes are designed such that for each transcript, there are
m unique fluorescence signals in N hybridization rounds. This read- 
out can be seen as a binary word. Chen et al. (2015) present two 
encoding schemes. With MHD4, the binary words (say, encoding) 
assigned to each transcript have length N = 16 (i.e. number of hy-
bridization rounds), contain m = 4 1-bits and the minimum Hamming distance between two words is 4. With MHD2, words 
have length N = 14 with m = 4 1-bits and a minimum Hamming di-
stance of 2. Technically, the readout for each RNA molecule in a cell 
is determined using fluorescence microscopy and subsequent compu-
tational recognition and assignment of the fluorescence signals to 
binary words (the readouts). In the following we will use the terms 
encoding and transcript interchangeably, since every transcript of 
tation of 2. Technically, the readout for each RNA molecule in a cell 
in the binary word, we denote with \(p_{0,k}\) the probability to acciden-
tally obtain a 1-bit instead of a 0-bit (0-1 error rate) and with \(p_{1,k}\) 
the probability to obtain a 0-bit instead of a 1-bit (1-0 error rate). It 
is worth noting that our model is agnostic of the underlying process 
as long as the measured outcome is a binary word and error rates 
are known. Hence, it will be applicable to any future versions of 
MERFISH as long as these properties stay the same. The error rates 
\(p_{0}\) and \(p_{1}\) can be estimated from MERFISH data, see Supplementary 
Section S4. In the following, we derive probabilities for different 
readout events.

After readouts have been obtained, they can be compared to the 
designed encodings in order to determine from which transcript they 
come. With MHD4, it is possible to correct for 1-bit errors during 
this calling process, with MHD2, it is only possible to detect, but 
not correct 1-bit errors (Chen et al., 2015). Obviously, these calls 
can be incorrect and incomplete, which motivates the comprehen-
sive description of the underlying uncertainties as it will be done in 
the following.

Let \(N\) be the number of bits in the used encoding and \(m \leq N\) be 
the number of 1-bits. First, we strive to calculate the probability to 
obtain a readout that is called as a certain target transcript. This can 
be an exact call with hamming distance \(d = 0\) to the target transcript 
or a corrected call with hamming distance \(d = 1\). We denote with \(t\) 
the binary encoding of the target transcript and with \(s\) the binary 
encoding of the real origin of the readout (i.e. the source). The prob-
ability is denoted as

\[
\xi_{d,t,s} = \xi_{d,t,s,N}
\]

with \(\xi\) being the recurrence

\[
\xi_{d,t,s,k} = \begin{cases} 
\xi_{d,t-1,s,k-1} + (1 - p_{1,k}) \xi_{d,t-1,s-1,k-1} \\
\xi_{d-1,t-1,s,k} + (1 - p_{0,k}) \xi_{d-1,t-1,s-1,k-1} \\
0 & \text{if } s_k = t_k = 1, k > 0, d \geq 0 \\
0 & \text{if } s_k = t_k = 0, k > 0, d \geq 0 \\
p_{0,k} \xi_{d-1,t-1,s-1,k-1} + (1 - p_{0,k}) \xi_{d-1,t-1,s-1,k-1} \\
p_{1,k} \xi_{d-1,t-1,s-1,k-1} + (1 - p_{1,k}) \xi_{d-1,t-1,s-1,k-1} \\
0 & \text{if } s_k = 1 \neq t_k, k > 0, d \geq 0 \\
0 & \text{if } s_k = 0 \neq t_k, k > 0, d \geq 0 \\
1 & \text{if } k = 0, d = 0 \\
0 & \text{if } d < 0.
\end{cases}
\]

In the recurrence equation, we calculate the probability for the prefix of 
length \(r\). If \(s_k = t_k\), any bit flip (the first term of the respective sums) 
increases the Hamming distance to the target transcript, whereas no 
bit flip keeps the previous distance (the second term of the sum). In 
contrast, if \(s_k \neq t_k\), a bit flip keeps the previous Hamming distance 
(because it will flip the source bit to the correct bit of the target 
encoding), while no bit flip increases the Hamming distance to the 
target transcript.

The function \(\xi\) can be used to calculate probabilities for the dif-
ferent events that can occur to the readout of a certain transcript. 
Let \(e\) be the encoding for an arbitrary transcript and \(r\) be the 
obtained readout. Further, let \(\delta(r, e) = d\) denote the event that the 
Hamming distance between any readout \(r\) and any encoding \(e\) is 
\(d \in N\). The probability that the transcript is called correctly with 
Hamming distance 0 (an exact call) is

\[
\Pr(\delta(r, e) = 0) = \xi_{0,t,e}.
\]

The probability for a corrected call (Hamming distance 1) of the 
transcript of origin is

\[
\Pr(\delta(r, e') = 1) = \xi_{1,t,e}.
\]

Note that in both cases the source and target transcript are the 
same, because we calculate the probability for a correct call. The 
next type of event that can happen to a transcript is that it is mis-
called as another transcript. Let \(e'\) be the encoding of the latter. 
Then, we can calculate the probability for an exact miscall of \(e'\) as

\[
\Pr(\delta(r, e) = 0) = \xi_{0,t,e'}
\]

and the probability for a corrected miscall of \(e'\) as

\[
\Pr(\delta(r, e') = 1) = \xi_{1,t,e'}.
\]

We consider above probabilities for all \(e'\) that have a reasonable 
Hamming distance to \(e\) and denote this set of encodings as neigh-
bors \(N_e\). With the error probabilities known so far, a maximum 
Hamming distance of 4 is a reasonable threshold because the prob-
ability would essentially be zero beyond this [as previously also 
shown by Chen et al. (2015)]. Finally, the probability for a dropout 
event (i.e. the transcript is neither called correctly nor miscalled 
because the readout contains too many errors) is the sum of all remai-
ning combinations of bit flips that haven’t been covered by above 
events. Alternatively, the result can be obtained by summing call 
and miscall probabilities:

\[
\Pr(\forall e' \neq e : \delta(r, e') > 0) = 1 - \sum_{e' \in N_e} \Pr(\delta(r, e') = 0) + \Pr(\delta(r, e') = 1).
\]

### 2.2 Estimating transcript expression

In the following, we consider the transcript (or gene) expression as 
the real, but unknown, number of RNA molecules present in a single 
cell. We first define parameters, latent and observed variables of the 
model.

Let \(E\) be the set of encodings given by a MERFISH codebook. 
The MERFISH protocol suggests to keep a small set \(E \subset C\) of encod-
ings as misidentification probes, i.e. they are not assigned to any 
transcript. Any call of a misidentification probe can be considered as 
an artifact. There are two reasons for such an artifact. First, it can 
be generated by a miscall from an expressed transcript. Second, it 
can be generated out of nothing, by interpreting microscopy or hy-
bridization noise as a real signal. To model the latter, we consider 
‘noise’ to be a special transcript with encoding \(e_n = 0^N\), which will 
be later treated in a special way.

We denote with \(n = |E|\) the number of real transcripts that 
are measured in the experiment and with \(\eta' = |E|\) the number of 
all measured encodings (including misidentification probes). Let 
\(X \in \mathbb{N}^{n+1}\) be the vector of expressions of all transcripts and the noise.
source. Let $M^d \in \mathbb{N}^{d \times (d+1)}$ with $d \in \{0, 1\}$ be the matrices of miscalls with Hamming distance 0 and 1, with $M^d_{e,e'}$ denoting the number of miscalls from encoding $e$ to encoding $e'$. Finally, let $K^d \in \mathbb{N}^d$ with $d \in \{0, 1\}$ be the vector of observed counts with Hamming distance 0 and 1 to the called encoding (e.g. $K^0_e$ are the observed exact counts that have been assigned to the transcript represented by the binary word $e$). While $K$ is an observed variable, $M$ is a latent variable that we will have to estimate along with the expressions $X$. See Figure 1d for a graphical representation of the model.

For each transcript with encoding $e \in E \setminus \hat{E}$, we now define an urn model that represents the different events that can occur to obtained readouts. Imagine that the urn contains differently colored balls for exact calls, corrected calls, miscalls to every neighbor $e' \in N_e$ and dropouts. Their relative quantities are defined by the readout probabilities from Section 2.1. The numbers of draws from the urn is then equivalent to the expression of the transcript and the distribution of drawn balls is equivalent to the observed and latent events that happen in the experiment. More formally we can write

$$\Pr(K^0_e, K^1_e \mid X, M^0, M^1) = h(k^0_e, k^1_e, M^0_{e,e}, M^0_{e,e'}, \ldots, M^1_{e,e}, M^1_{e,e'}, \ldots, M^1_{e',e}, X_e)$$

with $h(\ldots; X_e)$ being the multinomial probability mass function with event probabilities

$$\Pr(\delta(e, e) = 0), \Pr(\delta(e, e) = 1), \ldots, \Pr(\delta(e, e) = k^0_e), \Pr(\delta(e, e) = k^1_e), \ldots, \Pr(\delta(e, e) = 1),$$

and $X$ trials. The support (i.e. the drawn events) consists of the correct exact and inexact calls $k^0_e$ and $k^1_e$, the miscalls to neighboring encodings, and the dropout events $\lambda_e$. Thereby it is $k^0_e = K^0_e - \sum_{e' \in N_e \setminus \{e\}} M^0_{e,e'}$ and $k^1_e = K^1_e - \sum_{e' \in N_e \setminus \{e\}} M^1_{e,e'}$, i.e. the correct calls are the observed counts minus miscalls from other transcripts or the artificial noise source (see above). For the dropout events $\lambda_e$ we take the number of trials $X_e$ minus the sum of the other events. Note that miscalls computed by the urn for one transcript reoccur in the $x$ terms of neighboring transcripts. While this ensures that the real processes in a MERFISH experiment are properly modeled, it also gives rise to additional computational challenges outlined in Supplementary Section S3.

For the artificial noise source, we define a slightly different urn. While we do not have directly assigned counts for the noise, we can use the misidentification probes as evidence. As there are having two events for correct and inexact calls ($k^0_e$ and $k^1_e$), we define the two events for each misidentification probe, i.e. $k^0_e, k^1_e$ for each $e \in \hat{E}$. We use the corresponding probabilities $\Pr(\delta(e, e) = 0), \Pr(\delta(e, e) = 1)$ for miscalling $e$ from noise as event probabilities and keep the rest of the urn model as defined before.

Now we can define a joint model for the likelihood of transcript expression as

$$\Pr(K^0, K^1 \mid X) = \sum_{M^0, M^1} \prod_{e \in E \setminus \hat{E}} \Pr(K^0_e, K^1_e \mid X, M^0, M^1).$$

By keeping miscalls $M^0, M^1$ fixed and keeping $X$ fixed for every transcript except a particular one $e$, we can approximate the posterior probability distribution for expression $X_e$ as

$$\Pr(X_e = x \mid K^0, K^1) \approx \frac{\Pr(X_e = x)h(x)}{\sum_x \Pr(X_e = x)h(x)}$$

with

$$h(x) := \Pr(K^0, K^1 \mid X_e = x, X_{e'} = X_{e'}', \forall e' \neq e, M^0, M^1).$$

Depending on the experiment, reasonable prior probabilities could be negative binomial or Poisson distributed. However, MERFISH so far is applied to subsets of all available genes or transcripts. Their expression distribution can be heterogeneous in single cells and strongly affected by the investigated conditions. Finding appropriate priors is, among other tasks, subject to future research (see Supplementary Section S7). For now, we consider flat prior probabilities, which is conservative in the sense that (a) the resulting probability mass functions will be less sharp and (b) no further assumptions about the experiment are made. From the posterior probabilities, we can report the maximum a posteriori probability (MAP) and the 95% credible interval.

### 3 Results

We strive to evaluate the benefits of using the presented model on both simulated and real MERFISH data. For this, we will first show that the obtained expression estimates are, in contrast to raw counts, unbiased. Since there exists no real MERFISH dataset where the true molecule counts are known on a large scale, we show this on simulated data. On real data, we will show that the presented model enables improved correlation among the same genes across datasets.

#### 3.1 Available data

Currently, two versions of the MERFISH protocol are available: Chen et al. (2015) published version 1 with a reported average 0-1 error rate of $p_0 = 4\%$ and a 1-0 error rate of $p_1 = 10\%$. Later, Moffitt et al. (2016) published version 2 with significantly improved average error rates of $p_0 = 0.5\%$ and a $p_1 = 1\%$. To assess the accuracy of our method, we simulate MERFISH data for both protocols. Note that we estimated higher error rates as reported for protocol 1 (see Supplementary Section S4). However, we will use the reported lower error rates for our simulations to avoid an exaggeration of the benefits. Further, we use real data from protocol version 1 published by Chen et al. (2015): The **MHD4 dataset** measures 130 primary transcripts (plus 10 controls) on human fibroblast cells (IMR90), using two codebooks (i.e. the binary words assigned to the transcripts) with a pairwise Hamming distance of at least 4. The **MHD2 dataset** measures 983 primary transcripts (plus 16 controls) on the same cell line, using a codebook with a pairwise Hamming distance of at least 2.

#### 3.2 Example output

Figure 2 exemplifies the output of our model for two genes of the MHD4 dataset. Since the model reports credible intervals and the entire probability distribution for each estimate, the certainty of results can be properly assessed even in single cells. In most cases, the raw counts obtained by MERFISH are lower than our posterior estimates. This is consistent with the tendency of underestimation reported by Chen et al. (2015).
3.3 Evaluation on simulated data

To validate the estimates of the Bayesian model, we simulated MERFISH counts for different codebooks and the two protocol versions. Transcript expressions were drawn from Poisson distributions with different means. For each drawn count, the corresponding binary word from the codebook was mutated according to the reported average rates of 0-1 and 1-0 errors of the used protocol version. In addition we simulated noise by adding 50% of the total counts as 0-only binary words and mutating them in the same way. This is equivalent to expecting \( \frac{1}{3} \) of the investigated cell to be covered by transcripts. Finally, the mutated binary words were matched against the codebook, yielding artificial raw counts that are subject to the same miscall and dropout effects as real MERFISH data. Figure 3a–c and Supplementary Figure S1d–f (see Supplementary Section S1) provide results for protocol version 1 with the codebooks from the MHD4 and MHD2 datasets described above. The simulation is able to reproduce the reported underestimation bias of raw counts. In contrast, the posterior estimates reported by our Bayesian model do not show this bias and provide a more accurate estimate of the true expression. This is also robust with respect to uncertainty in the 0-1 and 1-0 error rates (see Supplementary Fig. S3). With protocol version 2, as error rates are much smaller, biases are expected to be less severe. In fact, when using the MHD4 codebook with protocol version 2 (MHD4v2), they disappear even when only considering the raw counts (Supplementary Fig. S1a–c). The corresponding credible intervals predicted by our model are narrow, which reflects the increased certainty in the data. While bias is not an issue with MHD4v2, there is still uncertainty in the data and our model properly captures this in the credible intervals (Supplementary Fig. S2c). The improved error rates of protocol version 2 offer the opportunity to use a more aggressive encoding in order to scale towards the whole genome. To illustrate this, we

![Graphs showing results](https://example.com/graphs.png)

Fig. 2. The presented Bayesian model can estimate probability distributions, maximum a-posteriori estimates (MAP) and credible intervals of transcript expression. The figure shows example probability mass functions (PMF, black dots) of the genes FLNC (a, b) and PRKCA (c) in three single cells from the MHD4 dataset. The raw total count (number of readouts assigned to the gene) is shown as dashed, and the raw exact count (number of readouts where no bit-correction was necessary) is shown as dotted gray lines. See Supplementary Section S2 for the general distribution of exact versus corrected counts in the MHD4 dataset.

![Graphs showing results](https://example.com/graphs.png)

Fig. 3. The presented Bayesian model is an accurate estimator of gene expression. We simulated MERFISH data under protocol version 1 (MHD4 codebook) and protocol version 2 (MHD2v2 codebook). (a) Prediction error as violin plots for different mean expressions using posterior estimates (maximum a posteriori probability, right violin half) and raw counts (left violin half) with MHD4 encoding. Data is shown in hexagonal bins with intensities corresponding to bin counts (i.e. a 2D histogram). (d, e, f) Corresponding results for MHD2v2 encoding.
Bayesian model for single-cell gene or transcript expression analysis on MRFISH data. Using simulated and real data, we showed that the model provides expression estimates that are free of systematic biases seen with raw MRFISH counts. Reported estimates from our model are complemented by credible intervals and the entire probability distribution, which allows to obtain a complete picture of the uncertainties as it was, e.g. demanded by Halsey et al. (2015). In the Supplementary Section S6 we exemplify how this information can be used to control the false discovery rate of differential expression analysis. The presented model provides, for the first time, a framework to assess gene or transcript expression with MRFISH, while properly handling and summarizing uncertainty in the data, even when investigating a single cell. In combination with the improved accuracy of version 2 of the MRFISH protocol (Moffitt et al., 2016) our model even enables to increase the number of measured transcripts by an order of magnitude, thereby scaling MRFISH towards the whole genome.

Uncertainties in MRFISH data stem from errors made during hybridization, fluorescence microscopy and imaging. All these accumulate in the 0-1 and 1-0 error rates that are the main parameter of our Bayesian model (they can be estimated from the data). Given that MRFISH is a new technology, it is not yet possible to quantify how the error rates would differ between labs and microscopes. Since our model takes these rates into account, the provided unbiased estimates of transcript expressions together with information about the uncertainty will help to provide reproducible results across labs and machines, even if error rates turn out to differ significantly.

We have shown that the current version of our model is an accurate predictor for Poisson distributed transcript expressions, although it uses a flat prior. Future work will entail the evaluation of suitable prior probability distributions (e.g. negative binomial), that enable to (a) introduce prior knowledge about expected mean and overdispersion or (b) infer these parameters from the data. Given that MRFISH is a targeted approach, we also plan to support setting priors per gene or group of genes. By this, knowledge about perturbations that are applied to the cells (e.g. the knockout of a gene) can be incorporated into the Bayesian inference.

The MHD4 dataset published by Chen et al. (2015) exhibits an additional, protocol-specific type of bias: a caveat in the MRFISH image analysis causes increased dropout rates for encodings with a large Hamming distance to the most abundant encoding. This problem has been eliminated in version 2 of the MRFISH protocol (Moffitt et al., 2016) (personal communication). Due to certain choices in our implementation of the presented Bayesian model we are currently only able to partially eliminate this type of bias. In Supplementary Section S5, we analyze the bias in detail and outline our plans to improve our implementation accordingly.

With MRFISH protocol version 2, the throughput has been improved considerably, enabling to measure hundreds of thousands of cells (Moffitt et al., 2016). This necessitates the efficient data exchange between the different steps of MRFISH analysis. Instead of introducing a custom binary file format, we plan to use an existing generic approach for binary and compressed data encoding, like Apache Arrow (https://arrow.apache.org). This will ensure interoperability with other tools as well as scripting languages typically used for post processing (e.g. Python and R).

With single-cell expression analysis, it is reasonable to consider cells to be in different states and transcript expression to be dynamic over time (e.g. when investigating cell differentiation). Here, an important tool is to label individual cells with a pseudotime, that represents at which point in time of a dynamic process each cell appears

3.4 Evaluation on real data

The MHD4 and MHD2 datasets published by Chen et al. (2015) share 107 genes. If both experiments would have perfectly measured all transcripts from these genes on exactly the same cells, we would expect both experiments to yield exactly the same counts. In reality, the experiments have of course been conducted on different cells. Nevertheless, under the assumption that biological conditions have been the same, the mean expression of each gene within both datasets should be the same. We used this property as a proxy for missing gold standard data, and calculated for both raw counts and the posterior estimates yielded by our model how accurately the posterior estimates yielded by our Bayesian model how accurately the posterior estimates yielded by our model are for each of the expected number of protein coding genes in the human genome. Figure 3d-f shows that even the improved error rates of MRFISH protocol version 2 cannot avoid biased raw counts for such a codebook (this is also the case for the regular MHD2 codebook in combination with protocol version 2). Again, our Bayesian model removes the bias, thereby enabling an almost whole-genome scale analysis of MRFISH data.

### Discussion

MRFISH has been identified as a major advance in spatial transcriptomics (Shalek and Satija, 2015). In this work, we presented a generated a codebook with a pairwise hamming distance of at least 2, binary words of length $N = 16$, and $m = 8$ 1-bits (MHD2v2, see Section 2). Such a codebook permits to measure 12,870 transcripts between the datasets compared to using raw counts. Bayesian model provides a significantly improved agreement between the two datasets. Linear regression is depicted by the line. (Fig. 4. Each dot represents the mean estimate across all cells in the two datasets. SMAPE denotes the symmetric mean absolute percentage error for a prediction between the two datasets. Linear regression is depicted by the line. (a) Posterior estimates, (b) raw counts
to be. Campbell and Yau (2016) have previously shown how uncertainties in gene expression can be incorporated into a Bayesian model for pseudotime inference. Future work might entail the integration of the uncertainty information provided by our model into such frameworks, such that reliable pseudotime estimates can be calculated for MERFISH data.

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Author contributions

J.K. developed the model, designed and conducted the analysis, implemented MERFISH tools and wrote the manuscript. M.B. and X.S.L. supervised the research and contributed to the manuscript.

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