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Statistical Methods for Fluorescence Lifetime Measurements

ABSTRACT

The probability distribution of fluorescence lifetime measurements represents a nonlinear distortion of a mixture of exponential distributions. We aim at improving the entire estimation procedure by (1) a good choice of the tuning parameter of the experiment that optimizes information bounds and (2) an adequate estimator taking into account the nonlinear distortion. As a result the data acquisition time can be reduced up to 90% in comparison to methods that are used at present.

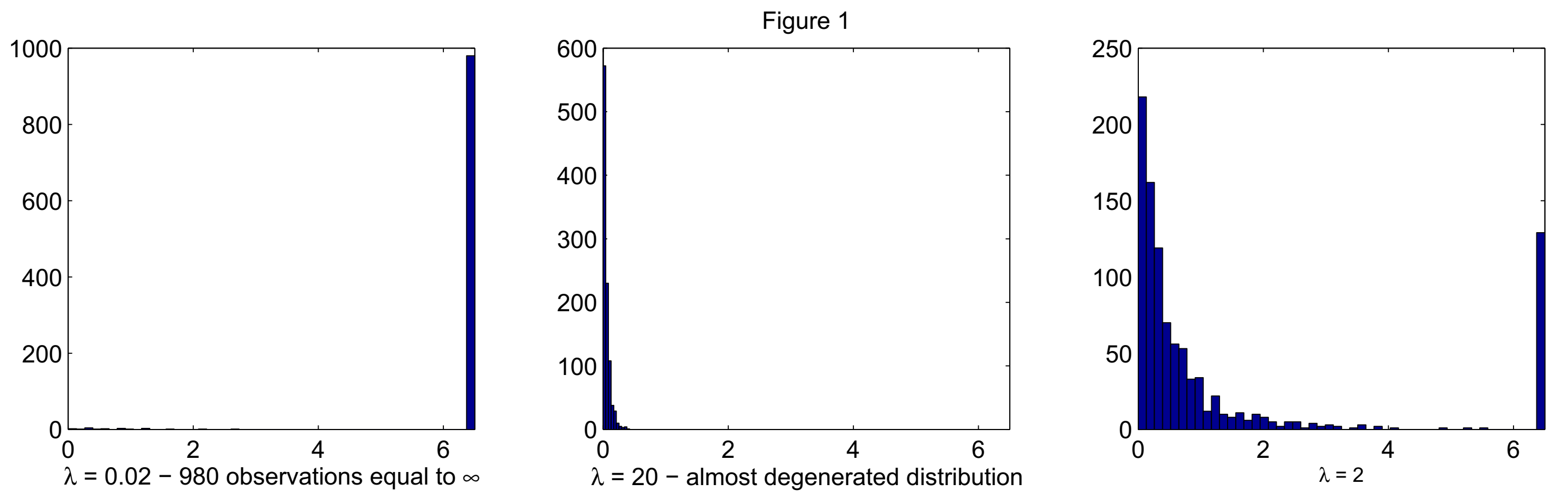
1 Statistical Model

GOAL Recover the parameter $\theta = (\alpha_1, \ldots, \alpha_{K-1}, \nu_1, \ldots, \nu_K)^T$ of the multi-exponential density $f_\theta(y) = \sum_{k=1}^{K} \alpha_k \nu_k \mathrm{e}^{-\nu_k y}$, with exponential parameters $\nu_k > 0$ and mixing weights $(\alpha_1, \ldots, \alpha_K)^T$ in the unit simplex. The order of the mixture K is supposed to be fixed and known.

DATA Let $Y_1, Y_2, \ldots$ be i.i.d. random variables with density f_θ representing the arrival times of fluorescent photons on the detector. An observation Z is the minimum of a packet of random size of these Y_i, that is $Z = \min\{Y_1, \ldots, Y_N\}$, where N has Poisson distribution $\mathrm{Poi}(\lambda)$. The density of the observation Z is $g_\theta(z) = \lambda f_\theta(z) \mathrm{e}^{-\lambda F_\theta(z)}$.

2 Effect of laser intensity λ on the data distribution

In the experimental set-up the user can choose the Poisson parameter λ by tuning the laser intensity. Thus, the question about an optimal value of λ arises. Figure 1 illustrates the influence of different choices of λ on the distribution of the data Z for the underlying density $f_\theta(y) = \mathrm{e}^{-y}$.

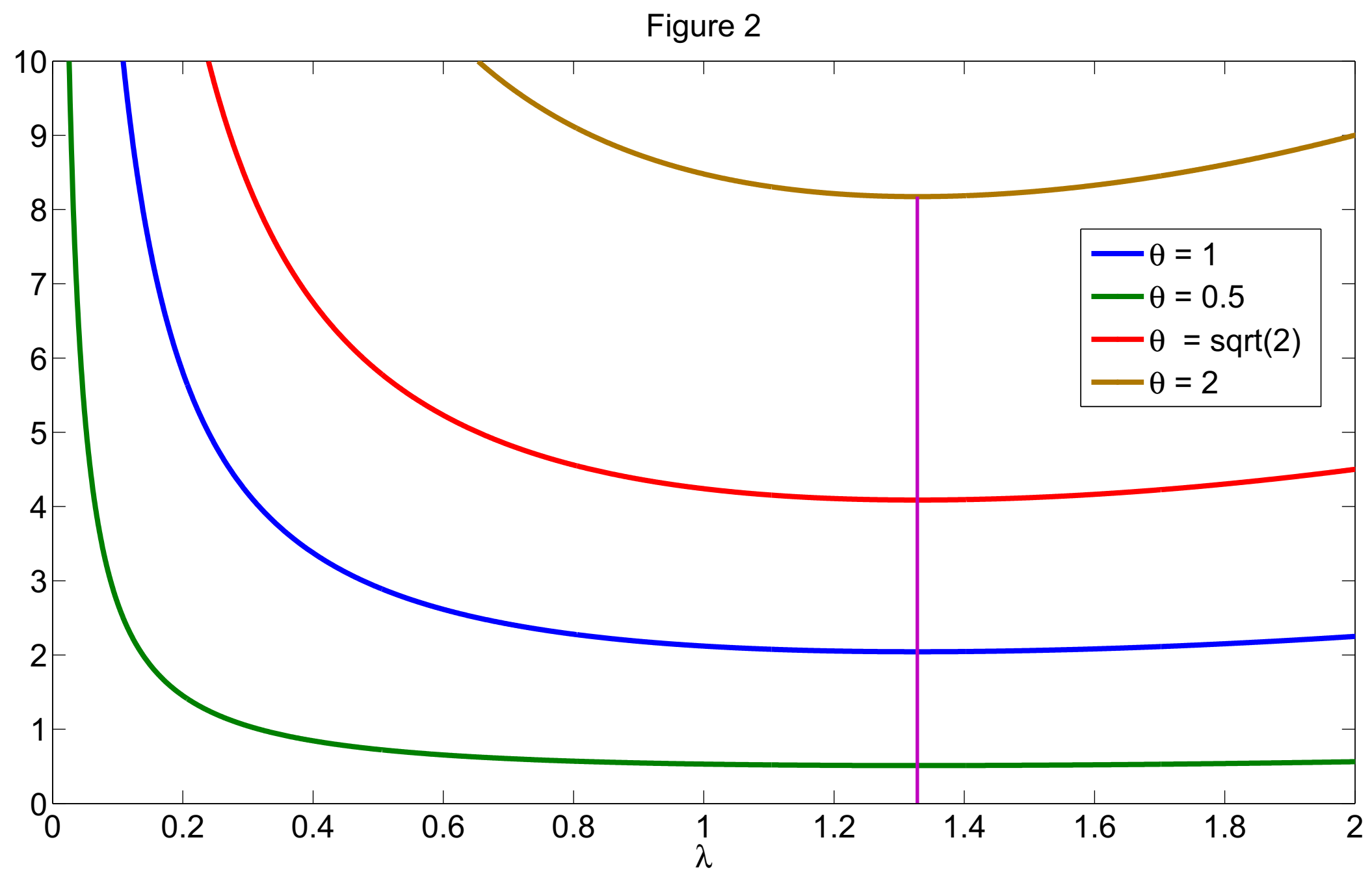


2.1 Low intensity λ

A study of the Cramér-Rao bound CR_θ of the parameter θ as a function of the intensity λ shows that it is not favorable to choose a small λ since $CR_\theta(\lambda_0, \theta_0) = O\left(\frac{1}{\lambda}\right)$, as $\lambda \to 0$.

2.2 Exponential case

Let f_θ be the exponential density ($K = 1$). Then one can show that the value of λ_0 minimizing the Cramér-Rao bound $CR_\theta(\lambda_0, \theta_0)$ does not depend on θ_0, that is $CR_\theta(\lambda_0, \theta_0) = \theta_0^2 CR_\theta(\lambda_0, 1)$. Figure 2 illustrates this result by showing the Cramer-Rao bound as a function of λ for different choices of the exponential parameter. A numerical minimization of the Cramér-Rao bound yields the point of minimum $\lambda_{\text{opt}} \approx 1.32$. The consequence for the experimental set-up is that a laser intensity with $\lambda_{\text{opt}} = 1.32$ is optimal whatever the value of the exponential parameter.



2.3 Multi-exponential case

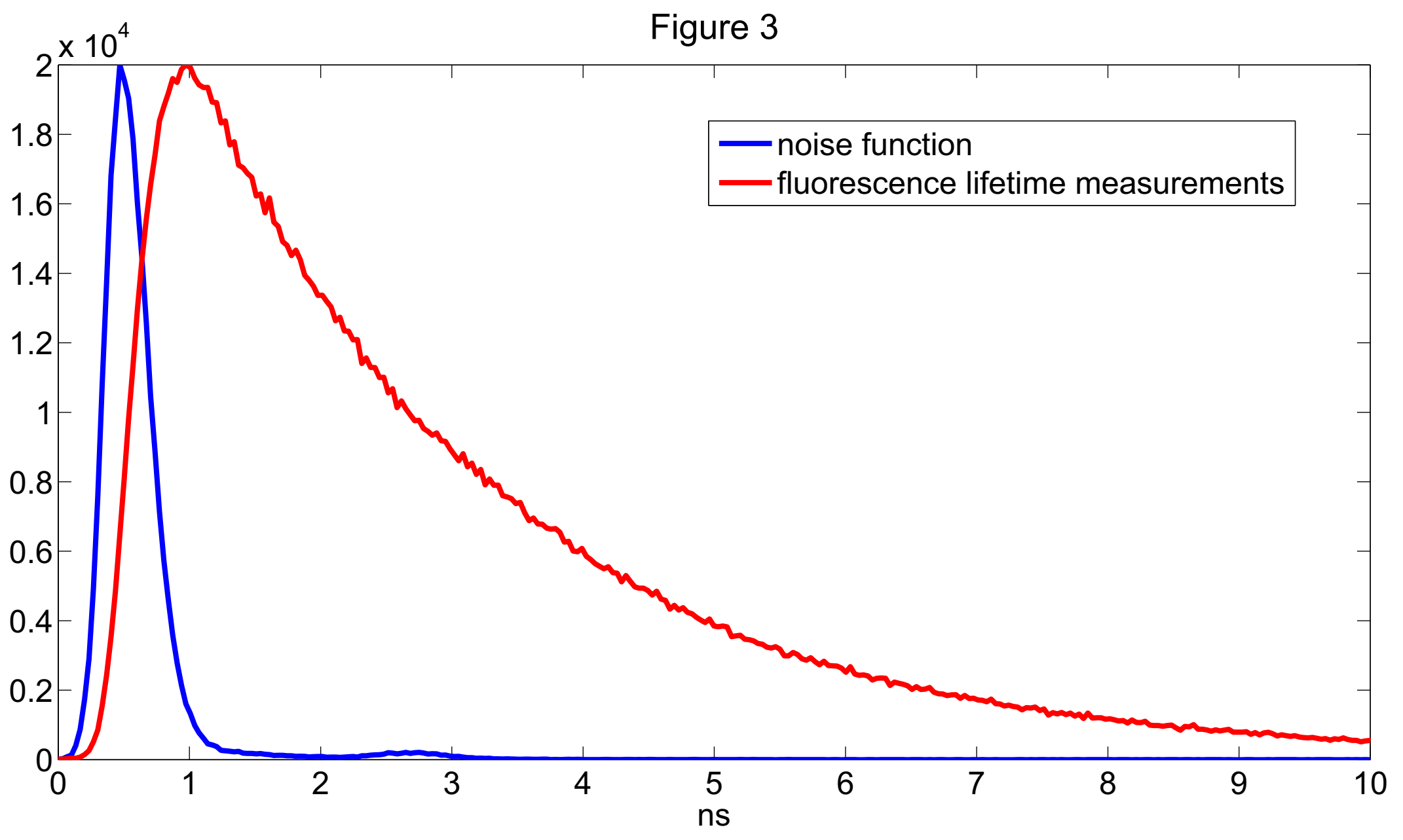
In the multi-exponential case ($K \geq 2$), the minimum of the Cramér-Rao bound depends on the choice of parameters θ. However, simulations reveal that λ in the range of $[1, 2]$ remains a very good choice.

3 Novel Parameter Estimator

Due to the involved form of the density g_θ of the observations, standard estimators as the maximum likelihood estimator are intractable. We propose to use an estimator which is an approximation of the classical maximum likelihood estimator (MLE) that is easily computed by applying the EM-algorithm. Furthermore, the estimator is easily adapted to other distributions than the multi-exponential. For instance, additive noise can be taken into account which is necessary for application on real fluorescence measurements.

4 Application to real measurements

To estimate parameters from real fluorescence lifetime measurements the multi-exponential model is extended by including additive noise. Figure 3 shows real fluorescence data and the noise function due to the measuring instrument. Data were obtained at a laser intensity corresponding to $\lambda = 0.166$. Hence, about 8% of the arrival times are the minimum of two or more photon arrival times. So there is a nonnegligible pile-up effect. The number of measured arrival times is $n = 1,743,811$ and there is a single exponential component, $K = 1$. In this experiment the lifetime of the molecule is known to be $\tau = 1/\nu = 2.54$ ns. An estimator of the exponential parameter that does not take into account the pile-up effect yields the value $\tilde{\tau} = 2.40$ ns which is significantly shorter than the expected value. Applying the novel approximated MLE provides the estimated value $\hat{\tau} = 1/\hat{\nu} = 2.5393$ ns. We draw the conclusion that the new estimator is well adapted to the pile-up model and handles additive noise correctly.



5 Reduction of acquisition time

In a simulation study we compare the performance of the new approximated MLE to the 'standard estimation method'. That is, for a fixed multi-exponential density with 3 components we simulate many data sets with a low intensity ($\lambda = 0.05$) for the standard method and with an elevated intensity ($\lambda = 1.5$) for the new method. Then the empirical bias and variances of the estimators are evaluated and the results are gathered in the table below. We observe that the approximated MLE attains the same estimation quality as the standard method with 10 times less observations. That is the data acquisition time can be reduced up to 90% in comparison to methods that are used at present.

$K = 3, \alpha_1 = 0.4, \alpha_2 = 0.3, \nu_1 = 0.1, \nu_2 = 0.5, \nu_3 = 2$

sample size		1,000		5,000		10,000		50,000	
standard	α_1	0.0226	(0.0267)	0.0163	(0.0123)	0.0232	(0.0084)	0.0131	(0.0016)
method	α_2	0.0828	(0.0212)	0.0746	(0.0110)	0.0585	(0.0083)	0.0133	(0.0028)
$\lambda = 0.05$	ν_1	0.0089	(0.0017)	$3.95\mathrm{e}^{-5}$	(0.0004)	0.0011	(0.0002)	$1.76\mathrm{e}^{-5}$	$(3.86\mathrm{e}^{-5})$
	ν_2	0.5778	(0.5275)	0.1911	(0.1483)	0.0953	(0.0904)	0.0096	(0.0181)
	ν_3	7.4744	(7442)	2.1364	(259)	1.7785	(345)	0.1343	(0.3094)
approx.	α_1	0.0185	(0.0097)	0.0059	(0.0017)	0.0019	(0.0008)	0.0004	(0.0001)
MLE	α_2	0.0544	(0.0074)	0.0129	(0.0020)	0.0064	(0.0012)	0.0010	(0.0002)
$\lambda = 1.5$	ν_1	0.0018	(0.0003)	0.0006	$(4.79\mathrm{e}^{-5})$	0.0002	$(2.23\mathrm{e}^{-5})$	0.0001	$(4.55\mathrm{e}^{-6})$
	ν_2	0.0844	(0.0820)	0.0112	(0.0165)	0.0072	(0.0085)	0.0035	(0.0017)
	ν_3	0.5706	(4.5160)	0.0737	(0.0937)	0.0418	(0.0424)	0.0101	(0.0077)

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