Appendix A: estimation of a 95% confidence interval for EQD_{RT}

Here, we describe a method to estimate a 95% confidence interval for EQD_{RT}. In this example we consider a maximum likelihood estimation of the SiHa cell survival data to provide parameter estimates and a confidence region for those parameters. We use these data to calculate EQD_{RT} and its confidence interval for a treatment with 4 Gy irradiation immediately (t_{int} = 0 h) followed by hyperthermia at 42°C. As fraction dose for the reference (radiation-only) schedule, 2 Gy is used.

While the maximum likelihood estimation in the main manuscript has seven free parameters for the SiHa and HeLa data, we limit ourselves in this example to maximum likelihood estimation of the SiHa cell survival data with only two free parameters (α_{41}/α_{37} and β_{41}/β_{37}), for easier interpretation and visualization. To this end, we perform an initial maximum likelihood estimation with all parameters, followed by a second maximum likelihood estimation in which we fix all parameters at their maximum likelihood estimate except α_{41}/α_{37} and β_{41}/β_{37}.

The 95% confidence region in maximum likelihood estimation

In maximum likelihood estimation, the 95% confidence region can be established based on the likelihood ratio test. Suppose we have the parameter set $\theta = (\alpha_{41}/\alpha_{37}, \beta_{41}/\beta_{37})$, and suppose that $\hat{\theta}$ represents the parameter set that maximizes the likelihood, then the 95% confidence region is bounded by the set of parameter values for θ that satisfy the equality

$$\log[L(\theta)] - \log\left[L\left(\hat{\theta}\right)\right] = \chi_2^2(0.95)/2\,, \qquad (1)$$

where χ_2^2 is the chi-squared distribution with two degrees of freedom and L(θ) is the likelihood function [1]. In other words, θ represents that set of parameter values for which the log-likelihood differs by $\chi_2^2(0.95)/2$ from the log-likelihood at the maximum likelihood estimate. This can be visualized as a contour in the (log-)likelihood surface (dashed black line, Figure A1).

Univariate description of the 95% confidence region

Confidence regions are not commonly reported, even though a confidence region based on the likelihood ratio test would provide detailed insight in the dependency between both parameters. Generally, only the univariate confidence intervals are reported, which approximate the confidence region by a rectangle (dashed red lines, Figure A1).

The most straightforward way to estimate a confidence interval for EQD_{RT} using only the univariate 95% confidence intervals of α_{41}/α_{37} and β_{41}/β_{37} (Table A1) would be to generate four sets of parameter values, based on the extreme values of these univariate confidence intervals (red dots, Figure A1), and calculate the corresponding EQD_{RT} (Table A2). The extreme values then provide an estimate for the 95% confidence interval for EQD_{RT}. For the example, this results in EQD_{RT} = 7.43 (7.09 – 7.76) Gy.

However, comparing these sets of parameter values to the actual 95% confidence region (middle dashed contour, Figure A1), it is clear that two parameter sets fall well outside the 95% confidence region, and thus represent parameter values that are unlikely. This is confirmed by the fact that the difference with the maximum likelihood for each of the four sets of parameter values (Table A2) is substantially larger than $\chi_2^2(0.95)/2 = 3.0$. The reason is that the true 95% confidence region as

depicted by the dashed contour (Figure A1) is not well approximated by a rectangle. This approximation is particularly poor when two variables are strongly correlated, as is the case here. The resulting 95% confidence interval for EQD_{RT} is therefore likely to be an overestimation of the true 95% confidence interval.

Table A1. Maximum likelihood estimates and univariate 95%CI for maximum likelihood estimation with only α_{41}/α_{37} and β_{41}/β_{37} as free parameters.

	α_{37}	α_{37}/β_{37}	α_{41}/α_{37}	β_{41}/β_{37}	μ	ΔS	ΔH	SD
Estimate	0.39	17.9	1.73	0.42	0.027	392.1	147908	0.27
(95% CI)	(fixed)	(fixed)	(1.69-1.78)	(0.30-0.55)	(fixed)	(fixed)	(fixed)	(fixed)

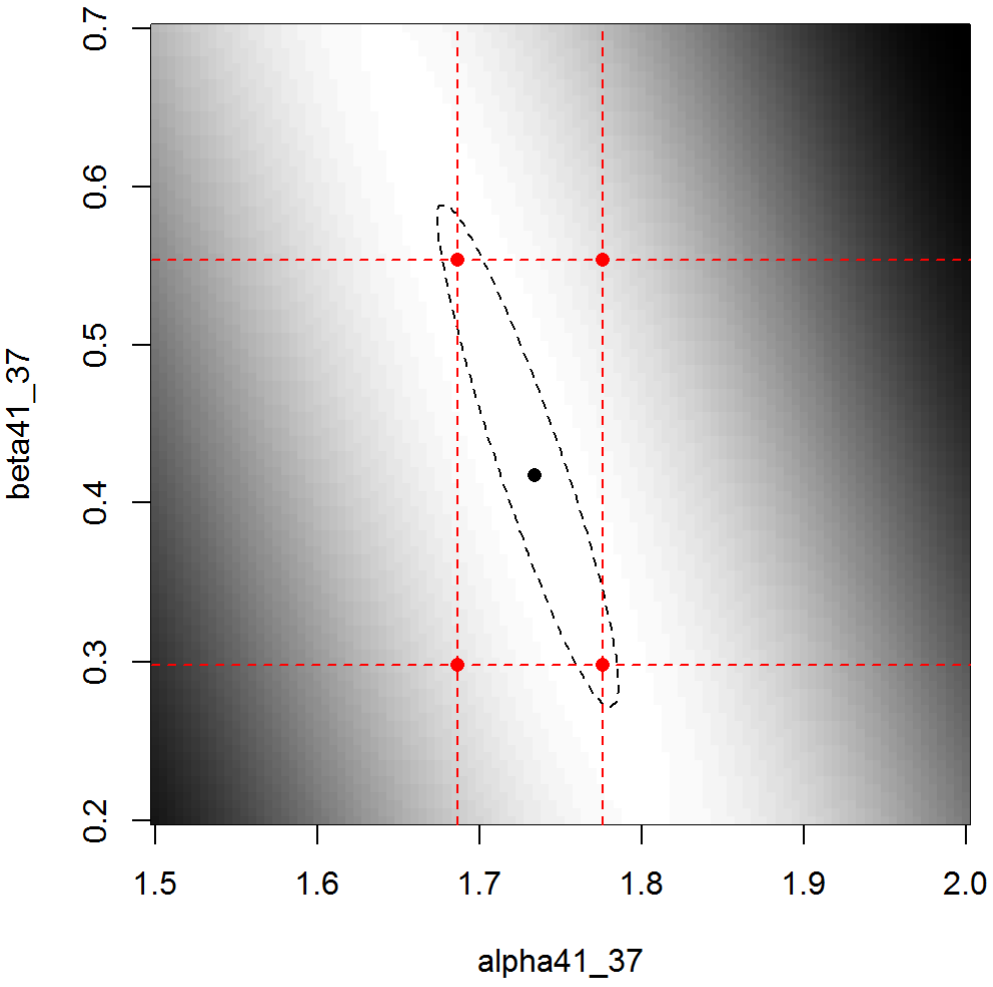

Figure A1. Likelihood surface (white = highest likelihood) for the SiHa data, with parameters α_{37}, α_{37}/β_{37}, τ, ΔS, ΔH, σ fixed at their most likely values. Shown are the maximum likelihood estimate for α_{41}/α_{37} and β_{41}/β_{37} (black dot), univariate 95% confidence interval (red dashed lines), bivariate 95% confidence region (dashed black contour), and the four sets of parameter values obtained when combining the extreme values of the univariate confidence intervals (red dots).

Table A2. Four scenarios based on the extreme values of the univariate 95% confidence intervals

α_{37}	α_{37}/β_{37}	α_{41}/α_{37}	β_{41}/β_{37}	μ	ΔS	ΔH	SD	ΔLog-likelihood*	EQD_{RT}
0.39	17.9	1.687	0.298	0.027	392.1	147908	0.27	51.4	7.09
0.39	17.9	1.687	0.554	0.027	392.1	147908	0.27	2.0	7.30
0.39	17.9	1.776	0.298	0.027	392.1	147908	0.27	2.0	7.55
0.39	17.9	1.776	0.554	0.027	392.1	147908	0.27	52.3	7.76

* $\Delta\text{Log} - \text{likelihood} = \log[L(\theta)] - \log\left[L\left(\hat{\theta}\right)\right]$

Multivariate normal approximation of the 95% confidence region

Using the full covariance matrix (Table A3), the 95% confidence region can be approximated more accurately. The estimates and covariance matrix describe a multivariate normal distribution, of which the 95-percentile is an ellipsoid. This ellipse (dashed red contour, Figure A2) provides an approximation of the true 95% confidence region (dashed black contour, Figure A2).

The axes of the ellipse are in the direction of the eigenvectors of the covariance matrix. These eigenvectors can be obtained through principle component analysis. The length of the axes of the ellipsoid are related to the eigenvalues, and the chi-squared distribution. For the i-th axis, the length of the axis (l_i) can be calculated using

$$l_i = 2 \cdot \sqrt{\left(\lambda_i \cdot \chi_p^2(0.95)\right)}, \tag{2}$$

where λ_i is the i-th eigenvalue and χ_p^2 is the chi-squared distribution with p degrees of freedom [2].

For our example, four sets of parameters can be calculated that lie on the ellipse in the direction of the principle components of the covariance matrix (red arrows, Figure A2) using the formulae

$$\begin{aligned}
\theta_1 &= \hat{\theta} + \sqrt{\left(\lambda_1 \cdot \chi_2^2(0.95)\right)} \cdot V_1 \\
\theta_2 &= \hat{\theta} - \sqrt{\left(\lambda_1 \cdot \chi_2^2(0.95)\right)} \cdot V_1 \\
\theta_3 &= \hat{\theta} + \sqrt{\left(\lambda_2 \cdot \chi_2^2(0.95)\right)} \cdot V_2 \\
\theta_4 &= \hat{\theta} - \sqrt{\left(\lambda_2 \cdot \chi_2^2(0.95)\right)} \cdot V_2
\end{aligned}, \tag{3}$$

where V_1 and V_2 are the eigenvectors of the covariance matrix (Table A3).

Calculating EQD_{RT} for the four sets of parameter values in our example (Table A4), and again taking the extreme values of EQD_{RT} we obtain EQD_{RT} = 7.43 (7.29 – 7.58) Gy. This confidence interval is approximately 40% smaller than the one calculated using the univariate confidence intervals and more realistic as the four sets of parameter values are closer to the actual 95% confidence region ($\Delta\text{Log} - \text{likelihood} \approx \chi_2^2(0.95)/2$ for all scenarios).

More generally, for a maximum likelihood estimation using N free parameters, the $2 \cdot N$ points at the 95% confidence region boundary in the directions of the principle components can be obtained using

$$\left.\begin{aligned}\theta_{2i-1} &= \hat{\theta} + \sqrt{\left(\lambda_i \cdot \chi^2_N(0.95)\right)} \cdot V_i \\ \theta_{2i} &= \hat{\theta} - \sqrt{\left(\lambda_i \cdot \chi^2_N(0.95)\right)} \cdot V_i\end{aligned}\right\} \quad i = 1, \ldots, N. \tag{4}$$

As the maximum likelihood estimations in the main manuscript contain seven free parameters for the SiHa and Hela analysis, 14 sets of parameter values were calculated to be used for the estimation of EQD_{RT} 95% confidence intervals.

Table A3. Covariance matrix for maximum likelihood estimation with only α_{41}/α_{37} and β_{41}/β_{37} as free parameters, and its eigenvalues (V) and eigenvectors (λ)

	α_{41}/α_{37}	β_{41}/β_{37}
α_{41}/α_{37}	0.000523	-0.00139
β_{41}/β_{37}	-0.00139	0.00429

V_1	V_2
-0.312	-0.950
0.950	-0.312

λ_1	λ_2
$4.75 \cdot 10^{-3}$	$6.73 \cdot 10^{-5}$

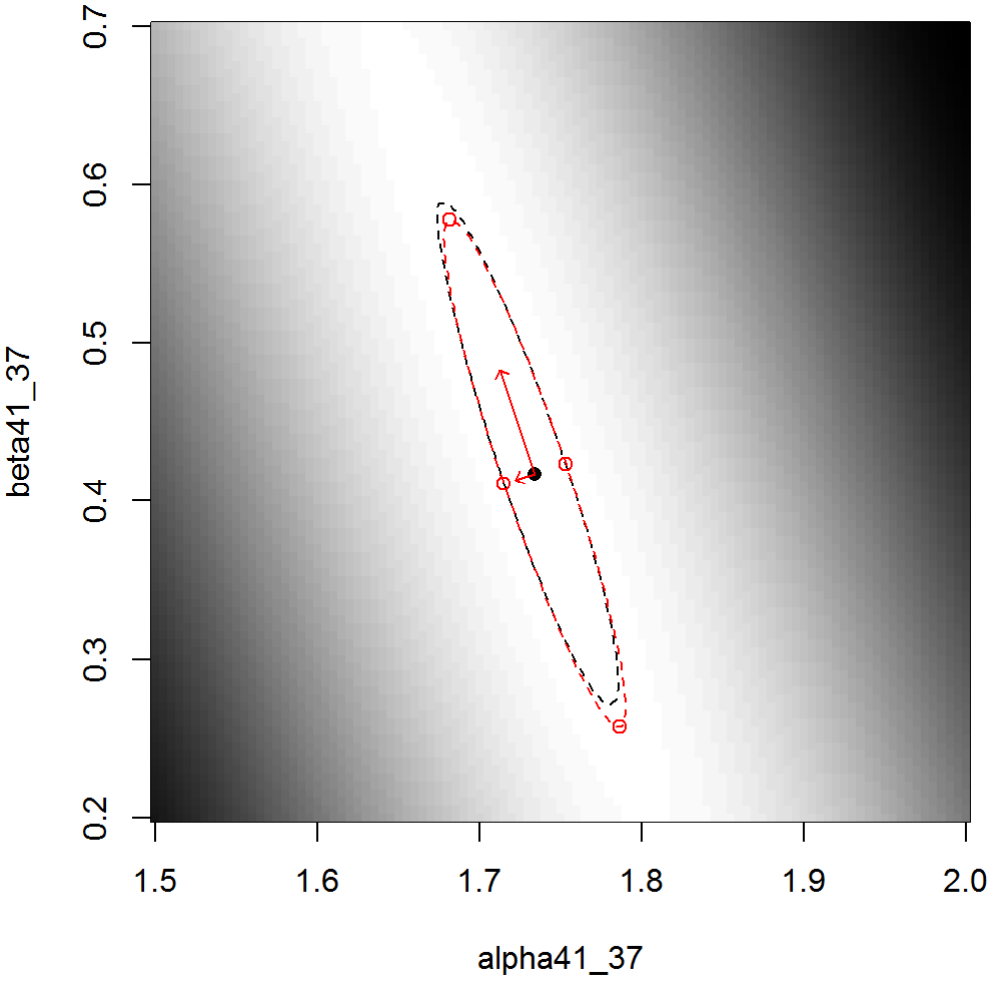

Figure A2. Likelihood surface (white = highest likelihood) for the SiHa data, with parameters α_{37}, α_{37}/β_{37}, τ, ΔS, ΔH, σ fixed at their most likely values. Shown are the maximum likelihood estimate for α_{41}/α_{37} and β_{41}/β_{37} (black dot), bivariate 95% confidence region based on the likelihood ratio test (dashed black contour), approximation of the 95% confidence region using the covariance matrix

(dashed red contour), the eigenvectors of the covariance matrix (red arrows) and the four parameter sets obtained from the principle component analysis of the covariance matrix (open red dots).

Table A4. Four scenarios based on the principle component analysis of the covariance matrix

α_{37}	α_{37}/β_{37}	α_{41}/α_{37}	β_{41}/β_{37}	μ	ΔS	ΔH	SD	ΔLog-likelihood*	EQD_{RT}
0.39	17.9	1.681	0.578	0.027	392.1	147908	0.27	2.6	7.29
0.39	17.9	1.787	0.257	0.027	392.1	147908	0.27	3.7	7.58
0.39	17.9	1.715	0.411	0.027	392.1	147908	0.27	3.0	7.33
0.39	17.9	1.753	0.424	0.027	392.1	147908	0.27	3.0	7.53

* $\Delta\text{Log} - \text{likelihood} = \log[L(\theta)] - \log\left[L\left(\hat{\theta}\right)\right]$

[1] Bolker BM. Likelihood and All that. Ecol Model Data R, Oxfordshire: Princeton University Press; 2008, p. 169–221.

[2] Johnson RA, Wicherin DW. The multivariate normal distribution. Appl Multivar Stat Anal. 6th ed., Upper Saddle River: Pearson Prentice Hall; 2007, p. 149–209.

Appendix B: *in vitro* analysis of SiHa cells, additional tables & figures

This appendix contains figures showing the correlation matrix for the model parameters (Figure B1), 2D slices of the log-likelihood surface (Figure B2), and tables with the covariance matrix (Table B1) and the 14 parameter sets generated to calculate 95% confidence intervals for EQD_{RT} (Table B2).

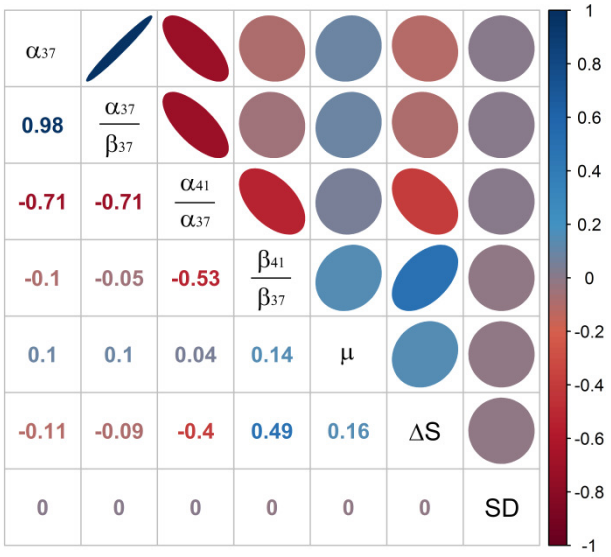

Figure B1. Correlation matrix for maximum likelihood estimation of the SiHa data.

Table B1. Covariance matrix for maximum likelihood estimation of the SiHa data.

	α_{37}	α_{37}/β_{37}	α_{41}/α_{37}	β_{41}/β_{37}	μ	ΔS	σ
α_{37}	1.35E-04	2.22E-02	-3.92E-04	-8.77E-05	7.50E-06	-2.62E-04	0.00E+00
α_{37}/β_{37}	2.22E-02	3.83E+00	-6.60E-02	-7.20E-03	1.28E-03	-3.76E-02	0.00E+00
α_{41}/α_{37}	-3.92E-04	-6.60E-02	2.26E-03	-1.95E-03	1.36E-05	-4.00E-03	0.00E+00
β_{41}/β_{37}	-8.77E-05	-7.20E-03	-1.95E-03	6.01E-03	7.49E-05	8.04E-03	0.00E+00
μ	7.50E-06	1.28E-03	1.36E-05	7.49E-05	4.54E-05	2.21E-04	0.00E+00
ΔS	-2.62E-04	-3.76E-02	-4.00E-03	8.04E-03	2.21E-04	4.49E-02	0.00E+00
σ	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	1.25E-05

Table B2. The 14 parameter sets used to calculate the 95% confidence intervals of EQD_{RT}.

α_{37}	α_{37}/β_{37}	α_{41}/α_{37}	β_{41}/β_{37}	μ	ΔS	σ	ΔH	ΔLog-likelihood*
0.344	10.571	1.860	0.431	0.024	392.155	0.273	147907.8	60.86
0.429	25.256	1.608	0.404	0.029	392.009	0.273	147907.8	11.69
0.387	17.907	1.822	0.258	0.023	391.293	0.273	147907.8	6.19
0.386	17.920	1.646	0.577	0.031	392.872	0.273	147907.8	11.43
0.388	17.915	1.801	0.175	0.026	392.139	0.273	147907.8	9.72
0.385	17.912	1.667	0.660	0.027	392.026	0.273	147907.8	5.87
0.381	17.915	1.794	0.435	0.043	392.085	0.273	147907.8	6.90
0.391	17.912	1.674	0.400	0.010	392.079	0.273	147907.8	7.23
0.392	17.913	1.730	0.416	0.045	392.082	0.273	147907.8	6.42

0.381	17.914	1.738	0.419	0.008	392.083	0.273	147907.8	7.76
0.386	17.914	1.734	0.418	0.027	392.082	0.286	147907.8	6.50
0.386	17.914	1.734	0.418	0.027	392.082	0.259	147907.8	7.64
0.392	17.914	1.735	0.418	0.025	392.082	0.273	147907.8	7.05
0.381	17.914	1.733	0.417	0.028	392.082	0.273	147907.8	7.02

*$\Delta\mathrm{Log}-\mathrm{likelihood}$ is the difference in likelihood of the listed parameter set with the maximum likelihood estimate and should ideally be $\chi^2_7(0.95)/2 = 7.03$, with deviations being present because the likelihood-ratio based 95% confidence region deviates from the ellipsoidal approximation.

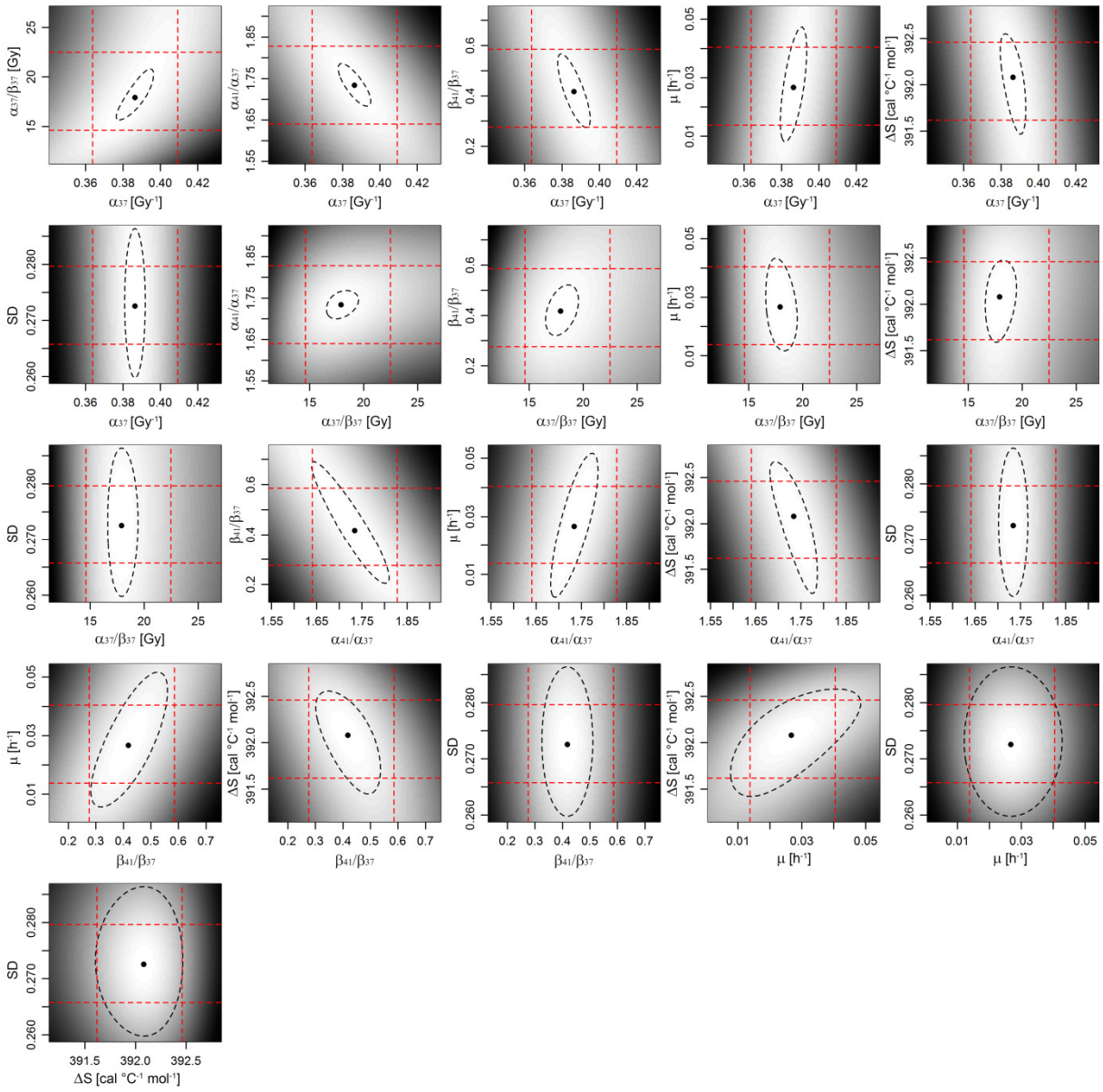

Figure B2. 2D slices of the likelihood surface, with the remaining values fixed at their maximum likelihood estimates. Shown are the maximum likelihood estimate (black dot), univariate 95% confidence intervals (red dashed lines) and the boundary of the 95% confidence region (black dashed contour).

Appendix C: *in vitro* analysis of HeLa cells, additional tables & figures

This appendix contains figures showing the survival data for the HeLa experiments as functions of dose, temperature and time interval (Figure C1), the correlation matrix for the model parameters (Figure C2), 2D slices of the log-likelihood surface (Figure C3), and tables with the covariance matrix (Table C1) and the 14 parameter sets generated to calculate 95% confidence intervals for EQD_{RT} (Table C2).

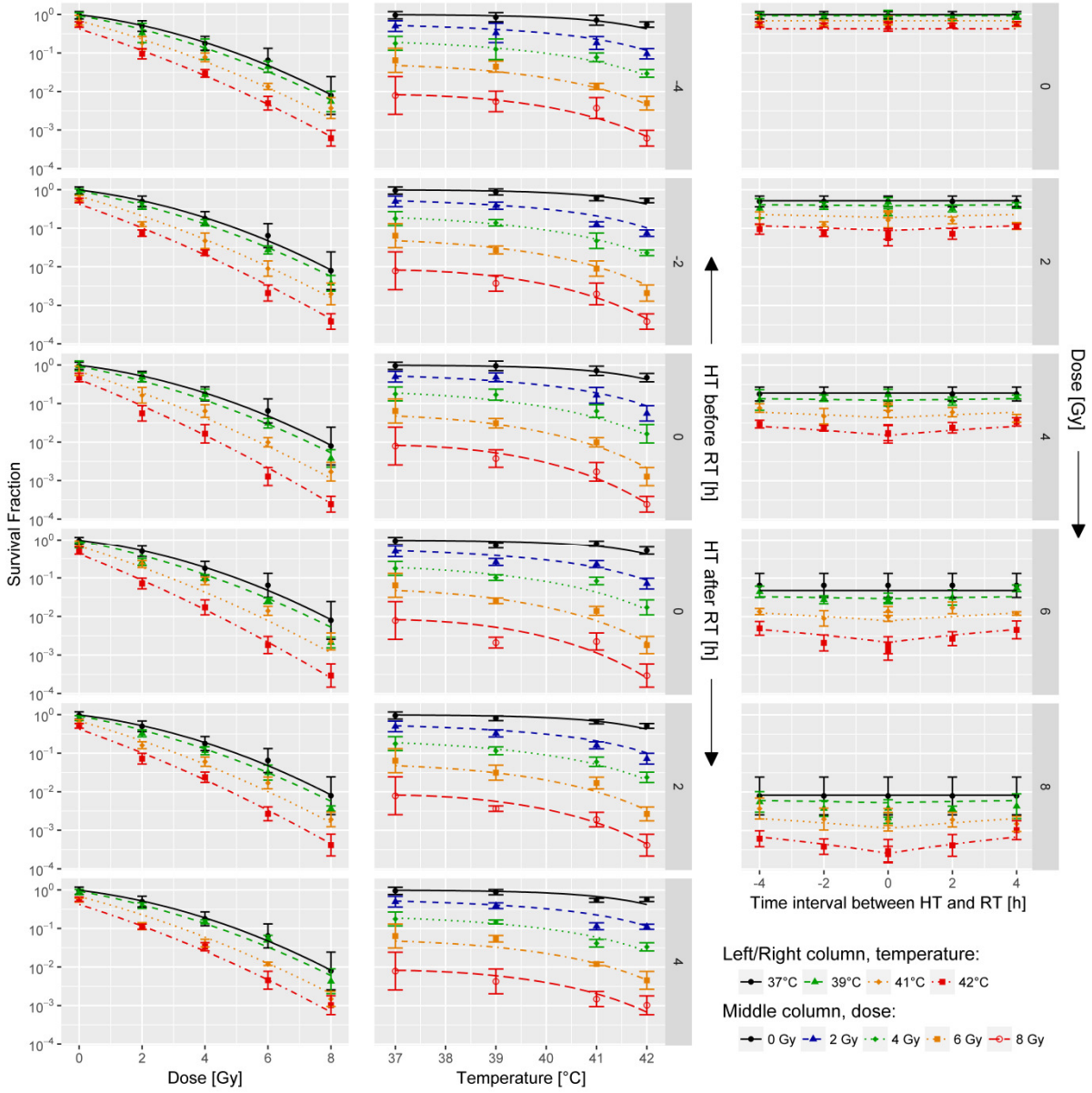

Figure C1. Three different views on the HeLa survival data: survival fraction as a function of radiation dose (left column), temperature (middle column) and time interval (right column). Shown are the mean measured survival (data points) with 95% CI of the mean (error bars) and the model from Eq. 7 (lines). RT, radiotherapy; HT, hyperthermia.

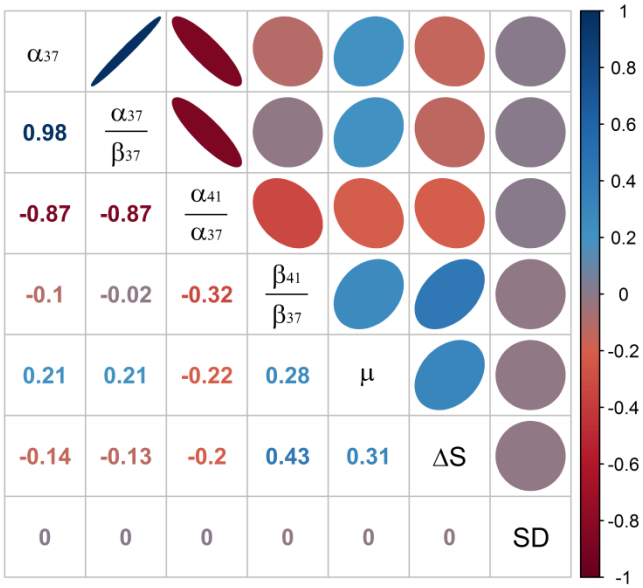

Figure C2. Correlation matrix for maximum likelihood estimation of the HeLa data.

Table C1. Covariance matrix for maximum likelihood estimation of the HeLa data.

	α_{37}	α_{37}/β_{37}	α_{41}/α_{37}	β_{41}/β_{37}	μ	ΔS	σ
α_{37}	2.86E-04	1.10E-02	-2.12E-03	-8.94E-05	2.63E-05	-3.22E-04	0.00E+00
α_{37}/β_{37}	1.10E-02	4.36E-01	-8.29E-02	-6.11E-04	1.05E-03	-1.12E-02	1.00E-07
α_{41}/α_{37}	-2.12E-03	-8.29E-02	2.08E-02	-2.41E-03	-2.40E-04	-4.00E-03	0.00E+00
β_{41}/β_{37}	-8.94E-05	-6.11E-04	-2.41E-03	2.70E-03	1.09E-04	3.04E-03	0.00E+00
μ	2.63E-05	1.05E-03	-2.40E-04	1.09E-04	5.70E-05	3.15E-04	0.00E+00
ΔS	-3.22E-04	-1.12E-02	-4.00E-03	3.04E-03	3.15E-04	1.83E-02	-2.00E-07
σ	0.00E+00	1.00E-07	0.00E+00	0.00E+00	0.00E+00	-2.00E-07	3.26E-05

Table C2. The 14 parameter sets used to calculate the 95% confidence intervals of EQD_{RT}.

α_{37}	α_{37}/β_{37}	α_{41}/α_{37}	β_{41}/β_{37}	μ	ΔS	σ	ΔH	ΔLog-likelihood*
0.167	2.523	3.028	0.578	0.047	432.421	0.292	160105.6	72.49
0.292	7.476	2.077	0.576	0.059	432.302	0.292	160105.6	15.46
0.232	5.026	2.752	0.469	0.044	431.866	0.292	160105.6	6.73
0.228	4.973	2.354	0.685	0.062	432.857	0.292	160105.6	9.13
0.231	5.030	2.701	0.444	0.056	432.451	0.292	160105.6	7.94
0.228	4.969	2.404	0.710	0.050	432.271	0.292	160105.6	6.34
0.238	4.985	2.479	0.484	0.042	432.351	0.292	160105.6	7.58
0.221	5.014	2.626	0.670	0.064	432.371	0.292	160105.6	6.65
0.225	5.000	2.554	0.578	0.029	432.362	0.292	160105.6	8.22
0.234	4.999	2.551	0.576	0.076	432.361	0.292	160105.6	6.13
0.230	4.999	2.553	0.577	0.053	432.361	0.314	160105.6	6.25
0.230	4.999	2.553	0.577	0.053	432.361	0.271	160105.6	7.99
0.235	4.999	2.553	0.577	0.052	432.361	0.292	160105.6	7.05

0.225	5.000	2.552	0.577	0.054	432.361	0.292	160105.6	7.02

*$\Delta\text{Log}-\text{likelihood}$ is the difference in likelihood of the listed parameter set with the maximum likelihood estimate and should ideally be $\chi^2_7(0.95)/2 = 7.03$, with deviations being present because the likelihood-ratio based 95% confidence region deviates from the ellipsoidal approximation.

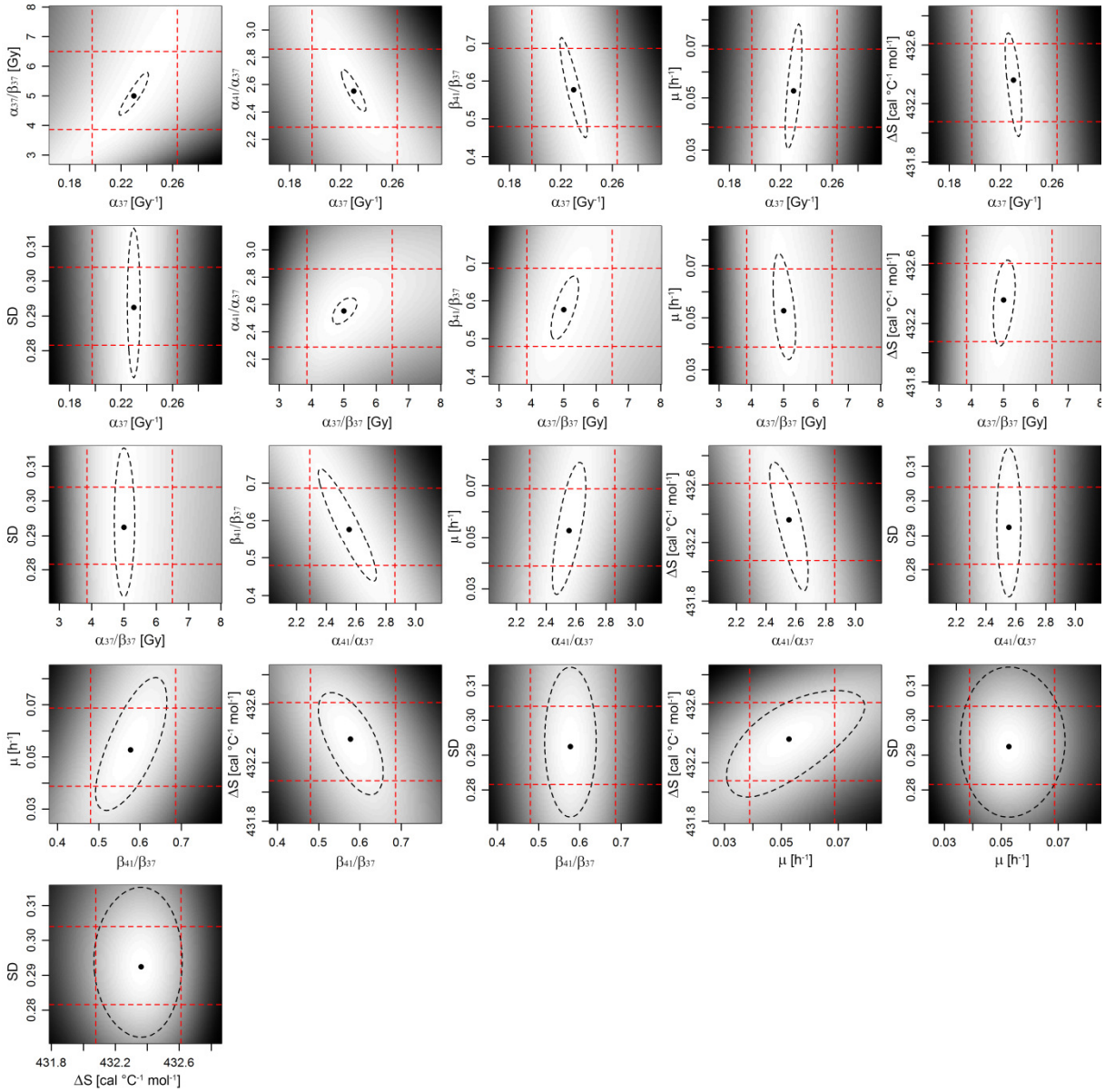

Figure C3. 2D slices of the likelihood surface, with the remaining values fixed at their maximum likelihood estimates. Shown are the maximum likelihood estimate (black dot), univariate 95% confidence intervals (red dashed lines) and the boundary of the 95% confidence region (black dashed contour).