Supplementary Material for "Demonstration of Neural Networks to Reconstruct Temperatures from Simulated Fluorescent Data towards use in Bio-Microfluidics"

S1 Temperature Map Equations and Grayscale Fit Equations

This section provides the series of equations used to create several temperature maps, and the fitting parameters used to related fluorescent intensity to grayscale and finally to temperature. For each of the temperature map equations, $T_1 = 300K$ and $T_2 = 312K$ and these temperature maps where then used with the 2nd order fit to the data in Figure S1 to create the different channels of fluorescent images.

S1.1 XvsY Temperature map equation

$$T(x,y) = T_1 + \frac{2(T_2 - T_1)}{\pi} \sum_{n=1}^{\infty} \frac{(-1)^{n+1} + 1}{n} \times sin\left(\frac{n\pi x}{L}\right) \frac{sinh\left(\frac{n\pi y}{L}\right)}{sinh\left(\frac{n\pi W}{L}\right)} \quad (S.1)$$

S1.2 RvsZ Temperature map equations

$$T(r,z) = T_1 + \frac{q'''L^2}{2k}\left[2\left(\frac{z}{L}\right) - \left(\frac{z}{L}\right)^2\right] + \sum_{n=1}^{\infty} a_n I_0(\lambda_n r) sin(\lambda_n z) \quad (S.2)$$

$$a_n = \frac{-2}{(\lambda_n L) I_0(\lambda_n r_0)}\left[(T_1 - T_2) + \frac{q'''}{n\lambda_n^2}\right] \quad (S.3)$$

$$\lambda_n = \frac{(2n-1)\pi}{2L} \quad (S.4)$$

S1.3 Fitting parameters

Table S1 presents the original spectral band intensity from Ref [1]. The fluorescent intensity within each of 5 nm-wide bands were summed, at which point the maximum fluorescent intensity was assigned a grayscale value of 65,535 (300 K in Channel 4), the lowest was assigned a grayscale value of 0 (312 K in Channel 1), and the other values were linearly interpreted between these points. The data in Figure S1 is the

Channel #	Min	Max
1 (576.3 to 581.1 nm)	15,779*	23,668
2 (596.6 to 601.4 nm)	76,714	128,342
3 (616.7 to 621.5 nm)	280,983	480,996**
4 (630.0 to 634.8 nm)	394,296	193,072
5 (650.2 to 654.9 nm)	130,972	652,389

Table S1 Spectra intensity values for each band, and the slope and intercept of the linear fit based on a 290-380 K temperature range. A grayscale value of 0 assigned to spectral intensity *, and a grayscale value of 65,535 assigned to spectral intensity **.

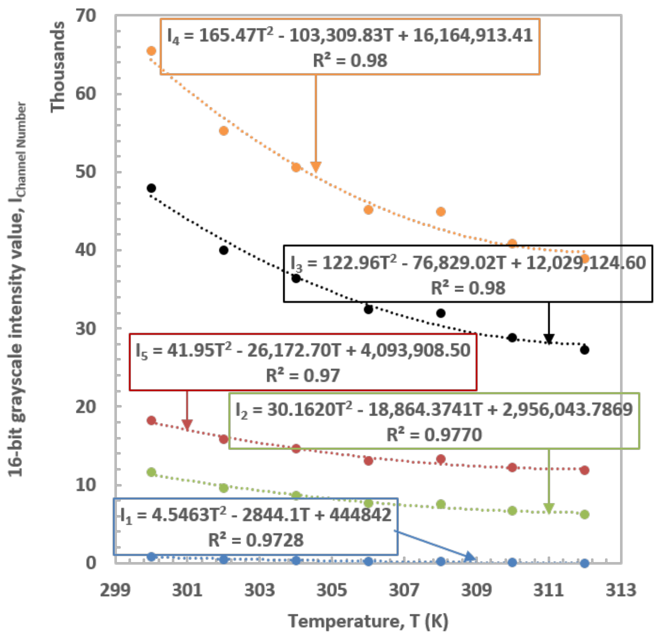

Fig. S1 Relationship between grayscale intensity value and the temperature, for each of the 5 channels that would represent different wavelength regions of the fluorescent image. This data is based on Ref [1] where the maximum fluorescent intensity was assigned a 65,535 value for grayscale intensity and the minimum fluorescent intensity was assigned a 0 value for grayscale intensity. The 2nd order polynomial fits with their coefficients are presented, as well as the R-squared coefficient of determination for each channel.

result of this process and is the basis for creating simulated fluorescent images from the temperature distribution from Sections S1.1-S1.2.

S2 Error Functions

This section details the form of the error functions used in training and validating the neural networks. These include standard error functions used in neural network training as well as the final Log Cosh hybrid chosen for the current research.

S2.1 Mean Squared Error, MSE

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(X_i - \hat{X}_i)^2 \tag{S.5}$$

S2.2 Root Mean Squared Error, RMSE

$$RMSE = \sqrt{\frac{\sum_{i=1}^{m}\sum_{j=1}^{n} e_{ij}^2}{m * n}} \tag{S.6}$$

S2.3 Mean Absolute Error, MAE

$$\text{Forward loss} = \frac{1}{n}\sum_{i=1}^{n}|X_i - \hat{X}_i| \tag{S.7}$$

$$\text{Backward Loss} = \frac{\text{sign}(X_i - \hat{X}_i)}{n} \tag{S.8}$$

S2.4 Log Cosh Error, LC

$$\text{Forward loss} = \log(\cosh((X_i - \hat{X}_i)^{12}) \tag{S.9}$$

$$\text{Backward loss} = \frac{\sinh((X_i - \hat{X}_i)^{12})(X_i - \hat{X}_i)^{11}}{(\cosh((X_i - \hat{X}_i)^{12}))} \tag{S.10}$$

S2.5 Log Cosh - Mean Absolute Hybrid Error, LCH

$$\text{Forward Loss} = \log(\cosh((X_i - \hat{X}_i)^{12}) \tag{S.11}$$

$$\text{Backward Loss} = \frac{\text{sign}(X_i - \hat{X}_i)}{n} \tag{S.12}$$

S3 Hyper-parameter tuning

This section details the impact of varying different hyperparameters as we tuned the neural network for optimal results. Although the 300-312 K temperature range was used during the majority of the analysis in the main paper, there was one exception where the temperature range used in fitting the fluorescent intensities and grayscale values was linearly expanded from 290-380 K (see Figure S2). This was during the tuning of the CNN and U-net hyperparameters to determine the network structure to obtain the lowest error. For this case, the same 16 bit intensity values of Table S1 and Figure S1 were used, but the x-axis values ranged from 290-380 K, and the grayscale-to-temperature fit was a linear fit. Reasons for this change are in the main paper, but are focused on providing a set of data where there are no non-linearities to potentially cause problems and to make the ±0.1 K white Gaussian noise less impactful over a larger temperature range.

Tables S2-S4 present the 3 rounds of tuning the AvCNN network to minimize the average RMSE values. Each variation in the parameter variation was run five times, with the minimum, maximum, and average RMSE values.

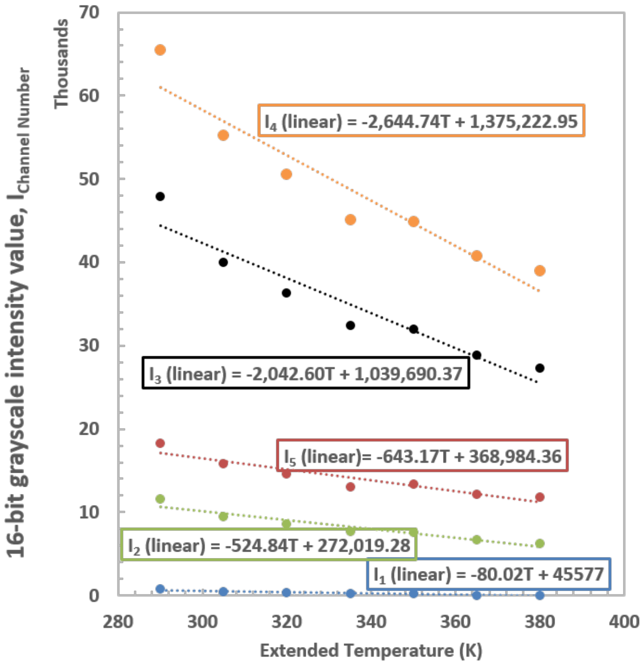

Fig. S2 Linearly expanded temperature and linearly fit intensity.

Parameter	Max_{RMSE} (K)	Min_{RMSE} (K)	Avg_{RMSE} (K)
Baseline (B)	1.04	0.63	0.82
LR		(B = 1×10^{-4})	
1×10^{-3}	9.71	2.61	5.31
5×10^{-4}	2.21	1.37	1.81
2×10^{-4}	1.24	0.57	0.90
8×10^{-5}	1.12	0.50	0.93
6×10^{-5}	1.02	0.70	0.83
1×10^{-5}	1.17	0.56	0.95
4×10^{-5}	1.70	0.86	1.33
C/P Layers		(B = 4/3)	
4/4	1.65	0.88	1.35
3/3	1.18	0.75	0.88
2/2	1.28	0.46	0.89
3/2	1.54	0.71	0.92
5/2	1.48	0.60	0.82
8/2	0.91	0.65	0.82
FS		(B = 3x3)	
5x5	1.07	0.62	0.77
7x7	0.87	0.61	0.75
Sc by CS	1.58	0.29	0.84
Sc by CSH	0.92	0.45	0.67
Sc by ICSH	1.10	0.48	0.80
Sc by ICS	1.04	0.51	0.78
MS		(B = 128)	
64	1.07	0.34	0.75
256	0.79	0.59	0.69
32	2.05	0.54	0.99
512	2.59	0.39	1.03

Table S2 First round of parameter tuning. B = Baseline, C = Convolution, CS = Convolution size, P = Pooling, Sc = Scaled, LR = Learn rate, FS = Filter size, MS = Minibatch size, CSH = Convolution size (high base value), ICSH = Inverse convolution size (high base value), ICS = Inverse convolution size. In this instance scaling means that filter sizes increase as convolution sizes increase (where a high base value means that all filters sizes are increased, inverse scaling means that filter sizes decrease as convolution sizes increase).

Parameter	Max_{RMSE} (K)	Min_{RMSE} (K)	Avg_{RMSE} (K)
Baseline (B)	1.04	0.63	0.82
Num of I		(B = 1)	
5	1.22	0.57	0.90
10	1.12	0.44	0.78
DP per SI		(B = 20 x 20)	
12	0.52	1.19	0.91
15	0.36	0.98	0.67
10	0.62	1.33	0.90
30	0.73	1.48	1.30
TD C-C		(B = 5)	
5	0.61	1.12	0.81
10	0.83	2.60	1.60
15	2.38	0.84	1.61
TD range I-I		(B = 0)	
5	0.90	1.31	1.16
10	0.56	1.00	0.80
25	1.53	1.91	1.67
25[a]	1.39	2.89	2.05
ME		(B = 30)	
50	0.59	0.38	0.49
70	0.75	0.54	0.61
60	0.72	0.54	0.62
Misc.[b]		(B = none)	
10i,+1/-1,40e	1.10	0.27	0.55
10i,+0/-0,40e	0.25	0.63	0.40
10i,+0/-0,20e	0.37	1.40	0.90
10i,+5/-5,40e	1.00	0.28	0.58
10i,+0/-5,40e	1.19	0.30	0.92

Table S3 **Second** round of parameter tuning. I = Images, DP = Data points, SI = Sub-image, TD = Temperature difference, C-C = Center of temperature range of one image to the center of temperature range for another image, ME = Max Epoch, I-I = Image to image. [a]Using 20 images. [b]10i,+1/-1,40e means: 10 images, +1 difference in maximum and -1 minimum temperatures between each image, 40 training epochs.

Parameter	Max_{RMSE} (K)	Min_{RMSE} (K)	Avg_{RMSE} (K)
LR		(B = 1×10^{-4})	
1×10^{-4}	0.27	0.51	0.33
1×10^{-3}	0.45	0.84	0.68
C/P Layers		(B = 4/3)	
5/2	0.45	0.84	0.68
7/2	0.24	0.85	0.44
6/2	0.35	0.74	0.55
7/2	0.31	1.16	0.78
7/2[a]	0.31	0.86	0.53
6/3	0.33	0.84	0.65
Extra layer	0.48	1.00	0.73
MS		(B = 128)	
64	0.40	1.11	0.72
256	0.34	0.69	0.50
512	0.34	0.77	0.55

Table S4 **Third** Round of Parameter Tuning. NB = New baseline, FS = Filter size, MS = Minibatch size. [a]Differencing operation.

Parameter (value)	Max_{RMSE} (K)	Min_{RMSE} (K)	Avg_{RMSE} (K)
Baseline	1.04	0.63	0.82
LR (1×10^{-4})	0.27	0.51	0.33
C/P Layers (7/2)	0.24	0.85	0.44
MS (256)	0.34	0.69	0.50
FS (Sc by CSH)	0.92	0.45	0.67
Num of I (10)	1.12	0.44	0.78
DP per SI (15)	0.36	0.98	0.67
TD C-C (5)	0.61	1.12	0.81
TD range I-I (10)	0.56	1.00	0.80
ME (50)	0.59	0.38	0.49
Misc.[a](10i,+0/-0,40e)	0.25	0.63	0.40

Table S5 Summary of parameter tuning process for CNN compared to initial baseline. A three-round tuning process was used and supplementary data details the parameters used for each stage of the process. C = Convolution, CS = Convolution size, P = Pooling, Sc = Scaled, LR = Learn rate, FS = Filter size, MS = Minibatch size, CSH = Convolution size (high base value), I = Images, DP = Data points, SI = Sub-image, TD = Temperature difference, C-C = Center of one image to the center of another image, ME = Max Epoch, I-I = Image to image.
Baseline parameters were LR = 1×10^{-4}, C/P Layers = 4/3, FS = 3x3, MS = 128, Num of I = 1, DP per SI = 20x20, TD C-C = 5, TD range I-I = 0, ME = 30.
[a]10i,+1/-1,40e means: 10 images, +1 difference in maximum and -1 minimum temperatures between each image, 40 training epochs.

S3.1 Impact of Sub-image Size on NN performance

This section details the tests performed to determine the sub-image size that would result in the lowest error based on the different error functions used. Results are presented for the AvCNN, PtPCNN, and U-net networks.

Sub-image Size	Error Func	Max_{RMSE} (K)	Min_{RMSE} (K)	Avg_{RMSE} (K)
25x25	MSE	3.54×10^{11}	21.85	7.09×10^{10}
25x25	MAE	5.80	5.79	5.80
25x25	LC	6.00	5.80	5.86
25x25	LCM	5.83	5.79	5.81
15x15	MSE	1.84×10^{14}	1.40×10^{12}	9.51×10^{13}
15x15	MAE	305.72	5.78	68.23
15x15	LC	6.04	5.80	5.88
15x15	LCM	11.87	5.78	8.22
7x7	MSE	17.26	5.98	11.09
7x7	MAE	5.84	5.71	5.75
7x7	LC	110.67	5.70	26.73
7x7	LCM	5.84	6.71	5.75
5x5	MSE	7.11	5.72	6.20
5x5	MAE	5.81	5.65	5.71
5x5	LC	5.85	5.41	5.68
5x5	LCM	5.83	5.67	5.72

Table S6 Summary of the tests from using the CNN to predict every pixel in the entire sub-image. Four different sub-image sizes were used. The CNN was trained using four different error functions. MSE = Mean Squared Error, MAE= Mean Average Error, LC = Log Cosh Error, LCM = Log Cosh Hybrid.

Internal Sub-image	Error Func	Avg_{RMSE}
5x5	MAE	5.77
5x5	LC	5.78
5x5	LCM	5.72
3x3	MAE	5.62
3x3	LC	5.52
3x3	LCM	5.96

Table S7 The average RMSE from calculating the temperature of an internal sub-image of a 15x15 image. MSE = Mean Squared Error, MAE= Mean Average Error, LC = Log Cosh Error, LCM = Log Cosh Hybrid.

Sub-image Size	Error Func	Max_{RMSE} (K)	Min_{RMSE} (K)	Avg_{RMSE} (K)
32x32	MSE	4.47×10^{10}	36.22	4.33×10^{10}
32x32	MAE	30.76	5.97	15.19
32x32	LC	5.82	5.80	5.81
32x32	LCM	6.53	5.89	6.17
16x16	MSE	1.08×10^{11}	7.88×10^{6}	3.61×10^{10}
16x16	MAE	5.93	5.84	5.89
16x16	LC	5.82	5.76	5.80
16x16	LCM	6.12	5.89	5.97

Table S8 The RMSE from the U-net predicting the temperature of every pixel in the sub-image

Sub-image Size	Error Func	Max_{RMSE} (K)	Min_{RMSE} (K)	Avg_{RMSE} (K)
33x33	MSE	11.41	5.77	8.53
33x33	MAE	1.56	1.31	1.41
33x33	LC	5.71	3.05	4.57
33x33	LCM	3.39	1.54	2.37
17x17	MSE	5.88	5.75	5.785
17x17	MAE	1.20	1.08	1.12
17x17	LC	1.75	0.59	1.09
17x17	LCM	1.42	1.01	1.18

Table S9 The RMSE from the U-net predicting the temperature of the center pixel of the sub-image

S4 Visualizing Temperature Results for Different Temperature Maps

To supplement the discussion in the main paper about the impact of the frequency of particular temperatures and grayscale intensities in Channel 1, the histograms for the Circles and XvsY temperature maps are presented in Figure S3 and S4, respectively. Like the RvsZ histogram, the Circles histogram shows that the occurrences of temperature and intensities are rarely below 5,000 counts, while the XvsY histograms have a lot of data below this threshold. This is likely one reason why the XvsY predicted temperatures have higher RSME values.

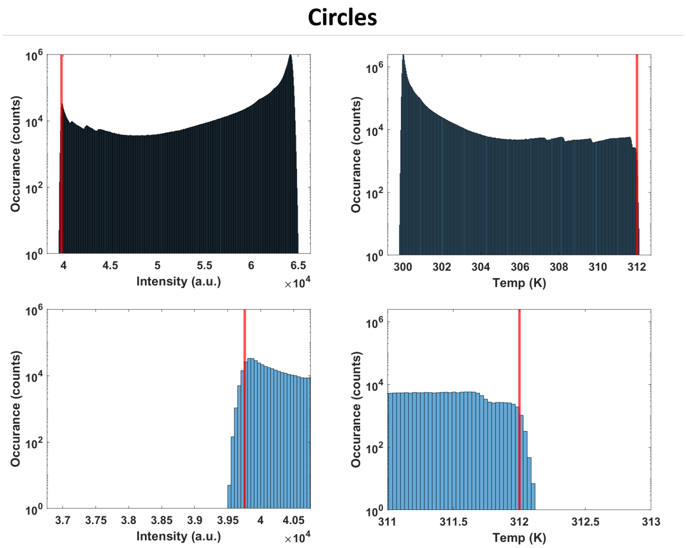

Fig. S3 Histograms of the temperature and fluorescent intensity values (Channel 4) for the Circles temperature map (top). Zoomed in section of both histograms near 312 K. The red line represents the temperature at 312 K and the matching intensity (bottom).

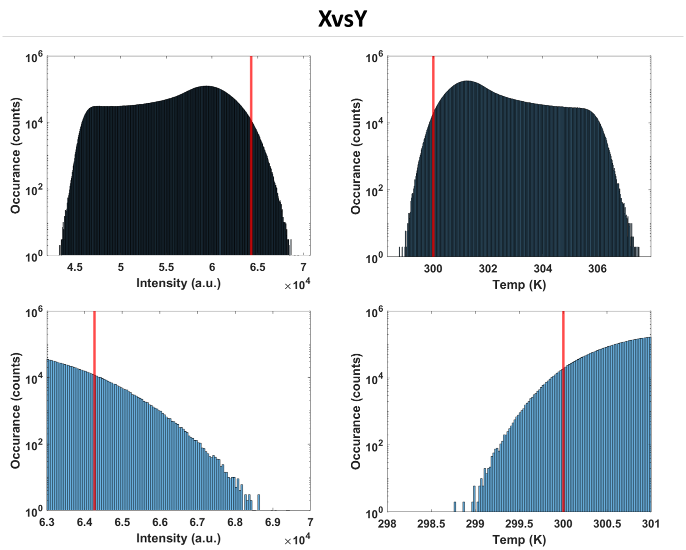

Fig. S4 Histograms of the temperature and fluorescent intensity values (Channel 4) for the XvsY temperature maps (top). Zoomed in section of both histograms near 312 K. The red line represents the temperature at 312 K and the matching intensity (bottom).

S5 Multi-parameter curve fitting results

This section provides the results of fitting two intensity channels (Channel 4 and 5) to the temperature for all three temperature maps (Figure S5).

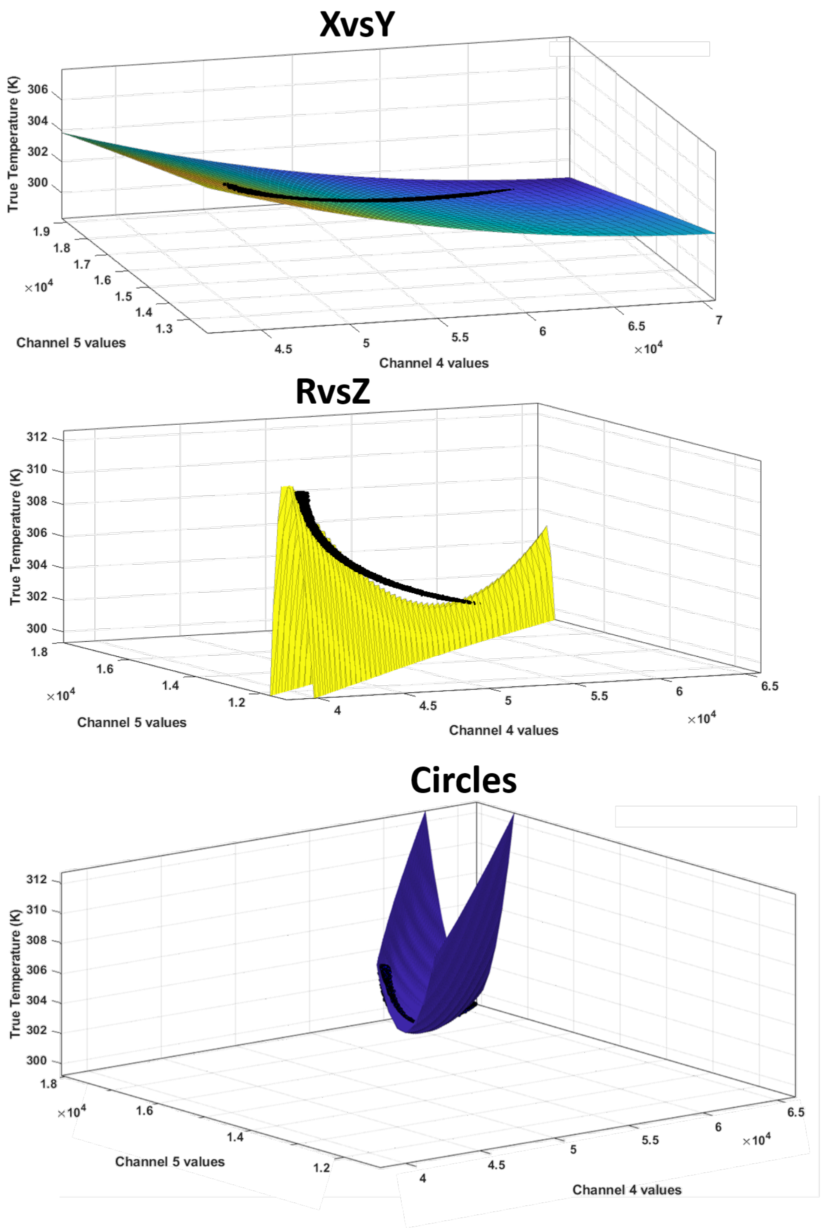

Fig. S5 2-parameter curve fits for all three temperature maps.

S6 5MCNN error during training

This section provides the results of the Log Cosh Hybrid error function as the 5MCNN network was trained on the XvsY temperature map, resulting in an RMSE of ±0.23 K (Figure S6).

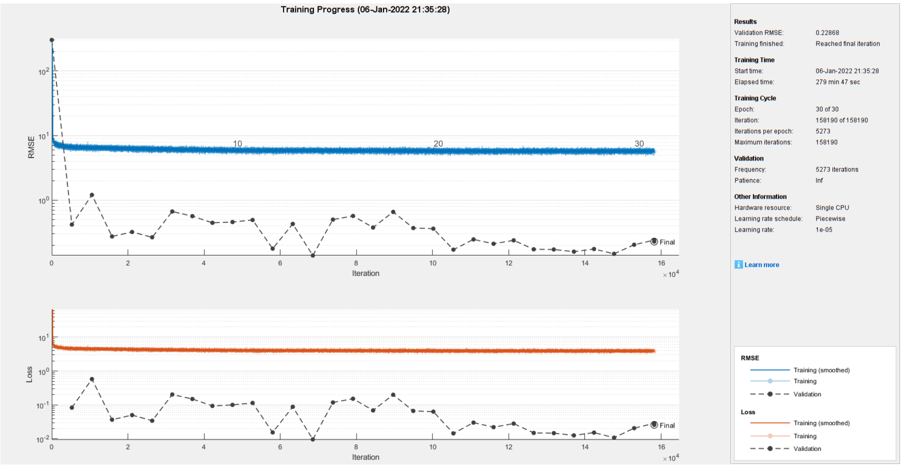

Fig. S6 Error plot of 5MCNN on XvsY.

References

[1] Munro, T., Liu, L., Glorieux, C. & Ban, H. CdSe/ZnS quantum dot fluorescence spectra shape-based thermometry via neural network reconstruction. *Journal of Applied Physics* **119** (21), 214903 (2016) .