



Research article

Optical and embedded ML driven computational optimization of portable micro-volume spectrophotometer for biochemical analysis in resource-limited environment

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Abstract: Comprehensive biochemical analysis is an essential part of clinical diagnostics, pharmaceutical development, and biomedical research. The gold-standard technique for such analysis is spectrophotometry, which has demonstrated excellent reliability, quantitative accuracy, and broad biomolecular sensitivity. Commercial micro-volume spectrophotometers, which are instrumental to applications with limited sample volumes, are often restricted to well-equipped laboratories due to the high cost and portability limitations especially in resource-limited settings. To address this gap, we propose a proof-of-concept, 3D-printed micro-volume spectrophotometer that integrates precision optics, microfluidic sample handling (8–15 μL), combined with spectral reconstruction and machine learning-based concentration estimation algorithm in a compact and affordable format. Preliminary validation of the proposed system based on bovine serum albumin (BSA) protein quantification via Biuret assay demonstrates strong agreement with commercial spectrophotometers ($R^2 > 0.91$). With a production cost of approximately \$36 and rapid measurement, the architecture provides a framework that can be extended to other absorbance-based biochemical assays ensuring accessible spectrophotometric systems for point-of-care and resource-limited applications, overcoming traditional limits of cost, sample volume, and portability.

Keywords: tiny ML; portable spectrophotometry; regression AI; embedded neural networks; point of care devices; optoelectronics

Supplementary material

This supplementary material provides representative raw datasets used to generate trend comparison and statistical figures (Figures 6, 8, 9, 10, and 11) along with the trained neural network parameters. The data correspond to representative samples used for spectral reconstruction, interpolation analysis, model performance validation and Bland-Altman plots. These datasets enable reproducibility of the presented plots and computational results.

Table S1. Representative absorbance dataset used for Figure 6 (direct absorbance mode comparison).

| Wavelength (nm) | Absorbance (AU) LAB | Absorbance (AU) SPPM3.3 | RMSE (AU) |
|-----------------|---------------------|-------------------------|-----------|
| 375 | 0.198 | 0.133 | 0.048 |
| 475 | 0.029 | 0.022 | |
| 525 | 0.068 | 0.123 | |
| 625 | 0.295 | 0.341 | |

Table S2. Representative absorbance dataset used for Figures 8 and 9 (interpolation mode comparison).

| Wavelength (nm) | Absorbance (AU) LAB (Measured) | Absorbance (AU) LAB (Calculated) | Absorbance (AU) SPPM3.3 (Calculated) |
|-----------------|--------------------------------|----------------------------------|--------------------------------------|
| 375 | 0.198 | 0.198 | 0.133 |
| 400 | 0.051 | 0.125 | 0.071 |
| 425 | 0.017 | 0.072 | 0.038 |
| 450 | 0.012 | 0.039 | 0.024 |
| 475 | 0.029 | 0.029 | 0.022 |
| 500 | 0.044 | 0.042 | 0.059 |
| 525 | 0.068 | 0.068 | 0.123 |
| 550 | 0.110 | 0.104 | 0.176 |
| 575 | 0.174 | 0.155 | 0.229 |
| 600 | 0.214 | 0.219 | 0.285 |
| 625 | 0.295 | 0.295 | 0.341 |
| 650 | 0.328 | 0.381 | 0.399 |
| 675 | 0.334 | 0.476 | 0.459 |
| 700 | 0.322 | 0.578 | 0.520 |

| | | |
|--------------------------------------------------|--------|--------|
| RMSE (AU) in bounded range (< 650 nm) | 0.0322 | 0.0484 |
| R ² score in bounded range (< 650 nm) | 0.9075 | 0.7916 |

Table S3. Representative absorbance dataset used for Figures 10 and 11(machine learning mode comparison).

| Wavelength (nm) | Absorbance (AU) LAB (Measured) | Absorbance (AU) LAB (Predicted) | Absorbance (AU) SPPM3.3 (Predicted) |
|-----------------|-----------------------------------|------------------------------------|----------------------------------------|
| 375 | 0.198 | 0.177 | 0.261 |
| 400 | 0.051 | 0.051 | 0.013 |
| 425 | 0.017 | -0.008 | 0.048 |
| 450 | 0.012 | -0.014 | 0.054 |
| 475 | 0.029 | 0.032 | 0.035 |
| 500 | 0.044 | 0.037 | 0.067 |
| 525 | 0.068 | 0.069 | 0.081 |
| 550 | 0.110 | 0.137 | 0.060 |
| 575 | 0.174 | 0.175 | 0.191 |
| 600 | 0.214 | 0.243 | 0.258 |
| 625 | 0.295 | 0.294 | 0.334 |
| 650 | 0.328 | 0.333 | 0.360 |
| 675 | 0.334 | 0.346 | 0.357 |
| 700 | 0.322 | 0.330 | 0.365 |

| | | |
|----------------------------------------|--------|--------|
| RMSE (AU) in complete range | 0.160 | 0.0364 |
| R ² score in complete range | 0.9823 | 0.9089 |

Table S4. Trained neural network specifications.

| | |
|---------------------|------------------------------------------------------------------------------------------------------------|
| Inputs | 4 absorbance readings at wavelengths 375, 475, 525, 625 nm. |
| Outputs | 15 outputs. 14 absorbance readings in the range 375-700nm at 25nm increment. Concentration estimate. |
| Dataset | 5 classes of protein concentrations (0.8%, 0.6%, 0.4%, 0.2%, and 0%) |
| Model | Feed forward neural network using Levenberg–Marquardt algorithm. |
| Layers | 1 layer with 6 hidden neurons. |
| Activation Function | Hyperbolic tangent sigmoid. |



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