

# A look under the hood of Lunatic

## Introduction

Lunatic is the concentration liberator for next-gen protein and nucleic acid quantification. From 2  $\mu$ L of sample, Lunatic delivers results with a full-spectrum spectrophotometer, a wide absorbance range from 0.03 to 275 OD, and unique analysis skills from a library of apps (Figure 1). Lunatic is a plate-based system that determines the concentration of up to 96 samples in as little as 5 minutes. Lunatic can be integrated with a liquid handler to automate a workflow and really speed things up. An optional 21 CFR Part 11 package is available for Lunatic if you need to ensure compliance.

Lunatic performs high-speed UV/Vis spectral analysis with just 2  $\mu$ L of sample in fixed pathlength microcuvettes. The unique microfluidic circuits molded into Lunatic plates ensure that there's no cross-contamination or evaporation. Worried about contaminants in your sample affecting your results? Lunatic's Unmix algorithms allow you to quantify nucleic acids or proteins in the presence of a variety of common impurities and buffer components, so you can move forward with confidence.

This technical note describes in more detail how Lunatic leverages state-of-the-art optics, signal processing, and purpose-built applications to determine the concentration of proteins and nucleic acids using the well-known principles of UV/Vis spectrophotometry.

## Fundamentals of UV/Vis spectrophotometry

When light shines through a liquid, some of it is absorbed based on the components of the liquid, their concentration, and the optical path length through the sample. The relationship between absorptivity, concentration, and path length is expressed with the Beer-Lambert law:

$$\text{Absorbance} = \epsilon \times c \times l$$



Figure 1: Lunatic: The next-gen UV/Vis reader. Lunatic is a plate-based system and reads up to 96 samples in 5 minutes.

In this equation,  $\epsilon$  is the extinction coefficient,  $c$  is the concentration, and  $l$  is the optical path length. Applying this law allows us to determine a sample's concentration from its absorbance. Proteins and nucleic acids have well-known absorbance spectra with characteristic peaks in the UV range at 280 and 260 nm, respectively. UV/Vis spectrophotometers measure the intensity of light at specific wavelengths before and after transmission through a sample with a known optical path length (Figure 2). These light intensities are then used to determine the sample absorbance spectrum.

When a UV/Vis spectrophotometer like Lunatic determines the absorbance spectrum of a sample, we can use this spectrum with the known path length and the sample's characteristic extinction coefficient to determine its concentration using a rearrangement of the Beer-Lambert law:

$$c = \frac{\text{Absorbance}}{\epsilon \times l}$$

UV/Vis spectrophotometry is one of the oldest and most well-accepted methods of directly quantifying proteins and nucleic acids. Lunatic takes this technique and pushes it to the next level with innovative optics, well-controlled consumables, and honed analysis techniques.

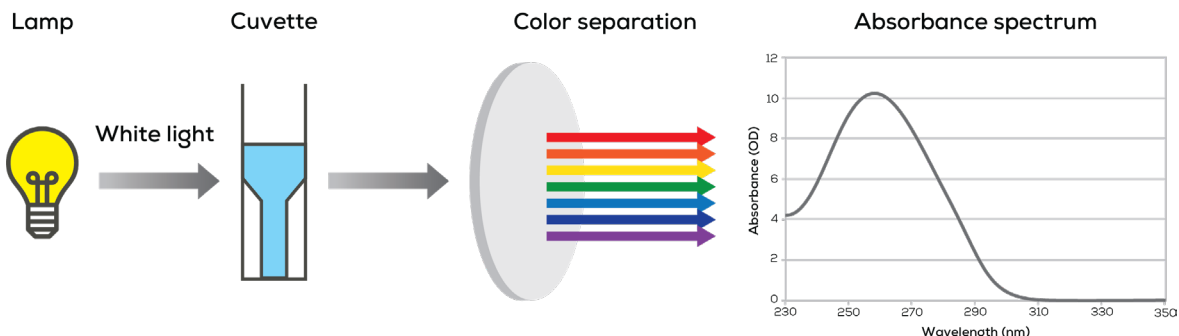


Figure 2: In this basic schematic of a UV/Vis spectrophotometer, white light shines through a sample in a fixed pathlength cuvette. The sample absorbs different wavelengths of the light based on its composition. An optical component, like a prism or diffraction grating, separates the light into individual wavelengths which are detected and turned into a UV/Vis absorbance spectrum.

## See the light

The heart of every Lunatic is the unique optical design (Figure 3). An efficient, long-lived xenon flashlamp serves as the light source. Each flashlamp lasts for around 2 million samples, or 7 years with typical usage, and produces intense white light across the UV/Vis spectrum with low power consumption.

Lunatic has a vertically-oriented light path, which provides a small footprint and high-speed reads. A fiber optic cable directs the light from the xenon flashlamp through a pinhole to create a narrow beam. A lens focuses the light so the focal plane goes through the center of each microcuvette for maximum sensitivity. The Lunatic system uses a reference spectrophotometer in the same configuration as the sample spectrophotometer. It uses reference measurements to compensate for any small variation in the lamp’s spectral output over time. This allows for more precise determination of sample absorbance and a more reliable instrument. Once the light has passed through a microcuvette it enters the spectrophotometer block.

Lunatic features robust, compact polychromatic spectrophotometer blocks (Figure 4). The proprietary design improves upon the traditional Czerny-Turner configuration by combining the functions of separate optical components into a single module. Thus, Lunatic takes up less space and does not need regular adjustments to the alignment. Unlike monochromator-based UV/Vis instruments, Lunatic measures the entire UV/Vis spectrum at once with

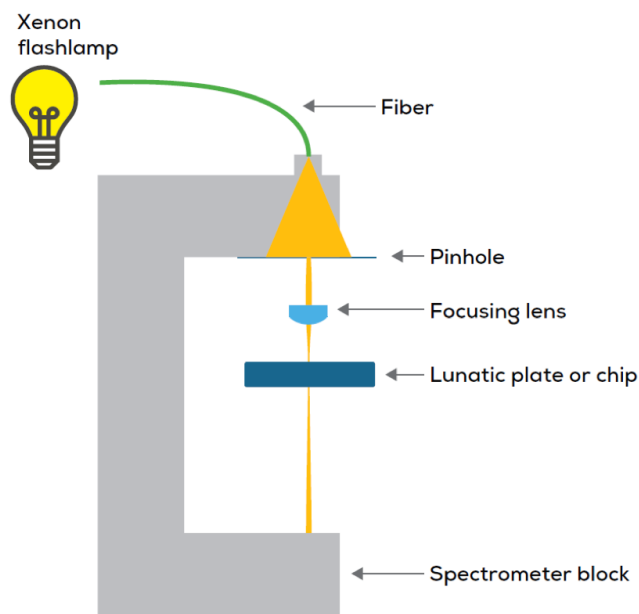


Figure 3: In Lunatic, an optical fiber carries light from the xenon flashlamp through a pinhole and focusing lens to concentrate light on the sample-containing plate or chip. Then the light enters the spectrophotometer block for color separation and detection.

no moving filters or grids. As a result, Lunatic is low-maintenance and gets results fast.

Inside the spectrometer block, a cylindrical lens collimates entering light towards the diffraction grating, which then separates the colors and reflects the spectrum to a mirrored surface of the lens. The mirror focuses and reflects the spectrum onto the detector. The photodiode array (PDA) of the detector senses the light and passes the signal through a low-noise analog-to-digital converter. Finally, Lunatic processes that signal into an absorbance spectrum from 230 to 750 nm.

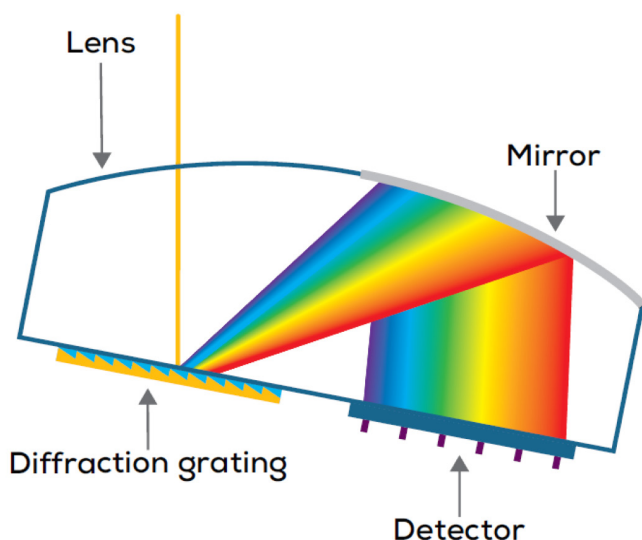


Figure 4: Lunatic’s compact, robust spectrophotometer block has no moving parts so it can’t get misaligned. Light entering the spectrophotometer passes through a cylindrical lens and hits the diffraction grating, which reflects the beam and separates the colors. The mirror reflects the spectrum to the detector.

Every Lunatic instrument undergoes rigorous factory calibration using spectra from mercury and xenon lamps, as well as holmium and didymium glass. Each instrument is quality-checked and calibrated using samples with absorbance peaks across the UV/Vis spectrum. The design and factory checks make sure every Lunatic has a linear absorbance range from 0.03–275 OD as well as precise and accurate wavelength detection and absorbance measurements (Table 1).

Lunatic’s unique and innovative design builds and improves upon the well-understood principles of UV/Vis spectrophotometry to provide accurate, sensitive,

and precise UV/Vis spectra, fast. Lunatics are further optimized by factory calibration and rigorous quality control. Lunatic UV/Vis spectrophotometers are compact, robust, long-lived, and low-maintenance so you can rely on them for nucleic acid and protein quantification.

### Strips and plates

Lunatic measures absorbances from 0.03–275 OD with 2 µL of sample across the full UV/Vis spectrum from 230–750 nm. Every measurement starts by loading a sample into the one-of-a-kind Lunatic consumable, which makes the wide dynamic range and low sample volumes possible (Figure 5).

Lunatic strips have proprietary microfluidic circuits molded from a low-absorbance cyclic olefin copolymer (COC) plastic to temporarily hold samples with no risk of cross-contamination or evaporation. Built into these circuits are the fixed pathlength microcuvettes that are used for the measurements. Each strip contains 16 microfluidic circuits and is mounted onto polystyrene frames as a set of 6 for Lunatic plates (Figure 5). The polystyrene and COC plastic are halogen-free; reusable metal plates are also available for the Lunatic system.

Lunatic plates have the same dimensions as standard 96-well microplates. This means that Lunatic can easily be integrated with liquid handlers and other lab automation to enable hands-off, high-throughput analysis.

Specification	Lunatic
Dimensions	37 cm W x 46 cm D x 33 cm H; 21 kg
Operative Voltage	24 VDC, 30 W (max)
Light source	Xenon flashlamp
Detectors	UV/Vis polychromatic spectrophotometer
Wavelength range/accuracy	230–750 nm   $\pm 0.2$ nm
Spectral resolution	2–2.5 nm (FWHM at Hg 253.7 nm)
Absorbance precision/accuracy (1 cm quartz cuvette) <1 OD	+/- 0.005 OD   +/- 0.01 OD
Absorbance precision/accuracy (1 cm quartz cuvette) 1–2 OD	+/- 0.5% CV   +/- 1%
Approval	CE, FCC, CSA

Table 1: Lunatic specifications

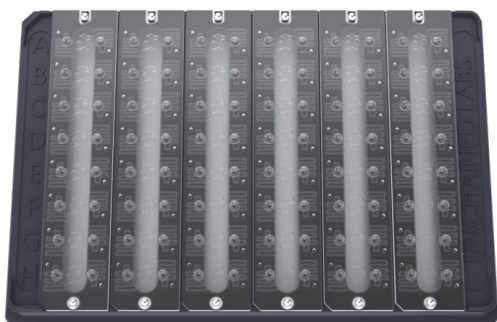


Figure 5: Strips containing 16 individual microfluidic circuits are mounted on frames to make Lunatic plates.

There are 2 types of Lunatic strips: regular and high. Regular strips have a single microcuvette with a 0.5 mm path length, enabling a dynamic range from 0.03–40 OD (Figure 6). High Lunatic strips have 2 microcuvettes in each circuit, measuring 0.1 and 0.7 mm (Figure 7). The 2 different sized microcuvettes make the wide dynamic range from 0.03–275 OD possible; the 0.1 mm cuvette provides a short enough path length to enable a high maximum detectable absorbance. Both types of strip have the same lower limit of detection.

Each circuit contains 5 main features: the input well, storage channel, microcuvette(s), overflow reservoir, and vent hole (Figure 7). Samples are deposited into the input well and drawn into the storage channel by capillary action. A sample can be left in the storage channel for up to 2 hours without detectable evaporation. During a read, Lunatic attaches a pump to the vent hole to move the sample from the storage channel into the microcuvette(s).

Lunatic detects the change in transmission through a microcuvette during pumping to prevent overfilling. As further protection against overfilling, each microfluidic circuit has an overflow reservoir.

For High Lunatic strips, the sample is pumped through two microcuvettes that together read across a 0.03 to 275 OD range in each circuit (Figure 8).

Lunatic strips are compatible with most buffers used in life sciences research and development. However, no technology used for determining concentration is valid for every type of sample. Unchained Labs has characterized the effects of some common detergents and solvents to determine what concentrations may affect Lunatic’s results (Table 2). Even minor changes in buffers and components can impact the properties of a sample, so researchers

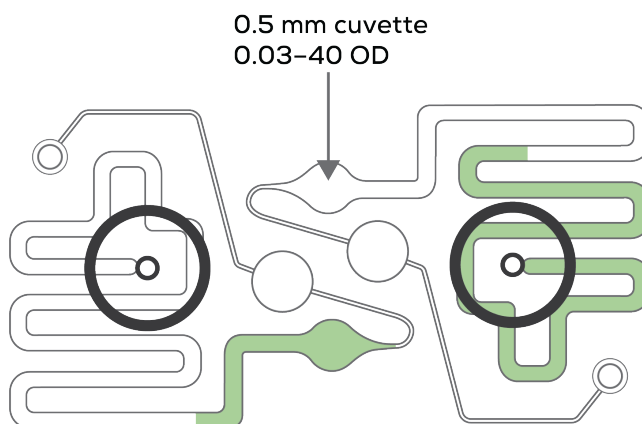


Figure 6: Illustration of Lunatic microfluidic circuits with 1 microcuvette and its dynamic range.

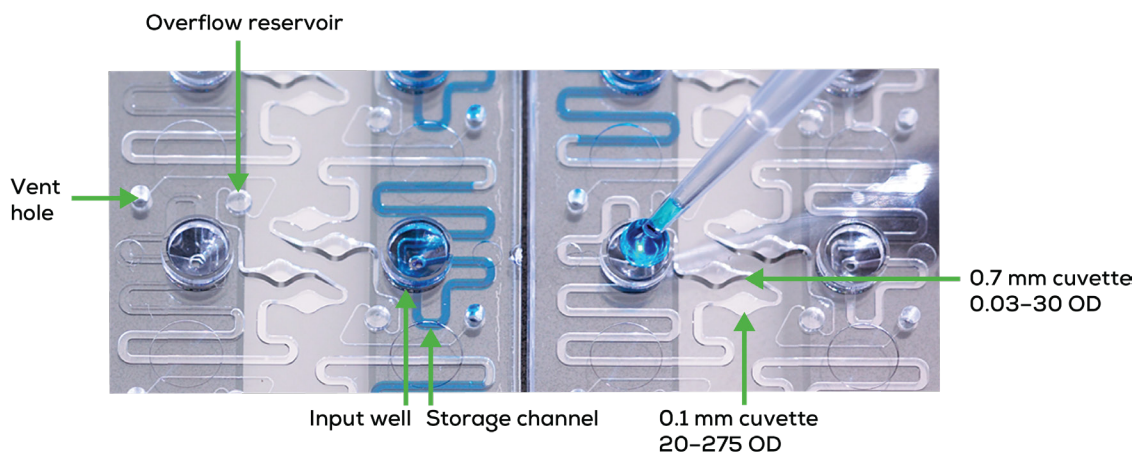


Figure 7: Close-up view of High Lunatic microfluidic circuits with 2 different path length microcuvettes and their dynamic ranges.

must determine if these effects are acceptable for their specific purposes.

Both regular and High Lunatic strips are rigorously tested with low and high concentrations of organic and inorganic and UV/Vis-absorbing compounds

Component	Maximum Concentration
Tween 80	10%
Triton X-100	0.01%
Tween 20	2%
NP-40	0.01%
SDS	0.5%
CHAPS	20%
RIPA	0%
Brij 97	0.125%
Brij 35	10%
CTAB	0.06%
DMSO	100%
Methanol	20%
Ethanol	20%

Table 2: Circuit compatibility table. This table serves as a guide, as minor changes in buffers and components can change the properties of a sample.

to guarantee high standards of strip performance (Table 3).

## Lunatic measurements, step-by-step

Once you load a sample plate into Lunatic, it completes 5 steps to make a measurement. Lunatic can measure 96 samples in about 5 minutes.

### 1. Empty beam measurement

Lunatic determines the intensity spectrum of light emitted by the xenon flashlamp through air with the sample spectrophotometer and a reference spectrophotometer. Lunatic uses the empty beam measurements to determine the exact spectral output of the xenon flashlamp for every experiment.

### 2. Automatic positioning

For optimal spectral measurements, Lunatic positions the plate so the focal point of the xenon flashlamp’s light passes through the center of each micro-cuvette. Lunatic determines the intensity of light transmitted through an empty microcuvette while making small adjustments to the plate position and repeats this process 2 more times. Based on these 3 measurements, Lunatic automatically optimizes the correct plate position.

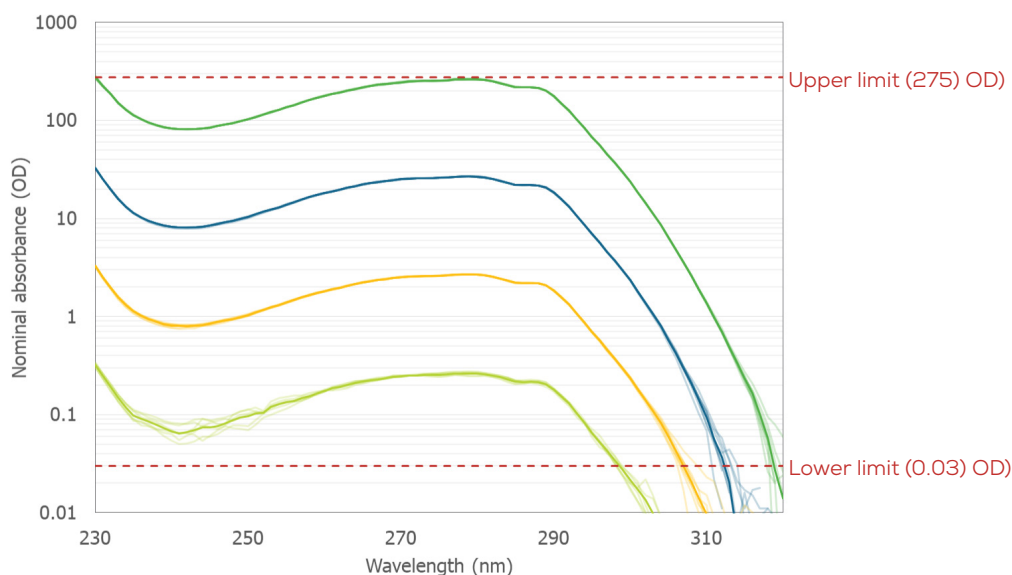


Figure 8: Lunatic determined absorbance spectra of 10-fold serially-diluted aqueous L-tryptophan (E1% = 271.5) in octuplicate with a High Lunatic plate. Light-colored lines are individual measurements and darker lines show averaged absorbances. The y-axis is on a logarithmic scale to show Lunatic’s nearly 10,000-fold dynamic range.

Lunatic strip specifications	Lunatic strip	High Lunatic strip
Measurement cuvettes per sample	1 microcuvette	2 microcuvettes
Path length(s)	0.5 mm	0.1 & 0.7 mm
Recommended sample volume	2 $\mu$ L	
Sample retention time	Up to 2 hours	
Measurement range OD 10mm ng/ $\mu$ L dsDNA mg/mL average protein mix	0.03–40 OD 1.5 – 2000 ng/ $\mu$ L 0.03–40 mg/mL	0.03–275 OD 1.5 – 13750 ng/ $\mu$ L 0.03 – 275 mg/mL
Precision	<1 OD: +/- 0.01 OD st dev >1 OD: +/- 1.5% CV	<1 OD: +/- 0.01 OD st dev 1–200 OD: +/- 1% CV

Table 3: Lunatic strip specifications.

### 3. Empty microcuvette measurement

Lunatic measures the intensity spectrum of light transmitted through the empty microcuvette,  $I_{empty}$ , to monitor the filling of the microcuvette and determine sample absorbance spectra. By measuring an intensity spectrum for each empty microcuvette, Lunatic removes any contribution the surrounding plastic may have on the spectrum, so you can be confident the resulting absorbances belong to the sample alone.

### 4. Sample pumping

After measuring the empty microcuvette, Lunatic connects a pump to the vent hole of the microfluidic circuit and cycles the pump at 22.5 mbar while monitoring the change in transmitted light intensity in the microcuvette. With this intelligent fill detection, Lunatic sees when the microcuvette has been filled and stops pumping, reducing measurement times.

### 5. Filled microcuvette measurement

Finally, Lunatic measures the intensity spectrum transmitted through the filled microcuvette,  $I_{filled}$ .

Lunatic repeats steps 3–5 for each of the userspecified microfluidic circuits, acquiring an empty and a full intensity spectrum. The software then uses these intensity spectra to determine the sample absorbance spectrum. Lunatic’s polychromatic detector measures all wavelengths across the UV/Vis spectrum simultaneously. All 5 measurement steps are optimized to give high quality spectral data in the shortest possible time.

## Determining sample absorbance

Lunatic’s detector is a PDA of 512 light-sensitive silicon photodiodes that each generate a current when exposed to light. These currents are monitored and used to determine the intensity of light falling on each diode. When the white light from the xenon flashlamp is separated by the diffraction grating, different wavelengths fall on different photodiodes in the array. By knowing the location of the diode in the array and the light intensity falling on the array, Lunatic determines light intensity at each wavelength. By comparing the light intensity transmitted through the empty and full cuvettes, Lunatic determines the sample absorbance spectrum.

### Dark current correction

Like all semi-conductors, the silicon photodiodes of Lunatic’s PDA generate a tiny current, even when no light falls on the detector. This is known as “dark current.” Lunatic monitors the dark current and saves the value from just before the flashlamp is triggered to measure each well. It automatically subtracts the dark current contribution from the intensity spectra.

### Auto-calibration

For every measurement Lunatic compares the beam measurements from the reference and sample spectrophotometers. Lunatic uses the comparison to relate the signal from the sample spectrophotometer to the xenon lamp’s spectral output. Lunatic adapts to changes in the lamp’s output spectra in real-time,

so it operates effectively over a long lifespan with minimal maintenance requirements.

## Diode-to-wavelength conversion

When an intensity spectrum hits the detector, different wavelengths of light fall on different diodes in the array. Lunatic measures the intensity of light falling on each diode but needs to convert the position of the diode in the array to a corresponding wavelength. The conversion is done by auto-calibrating the detector signal to the known xenon spectrum. Since Lunatic determines this relationship immediately before each experiment the resultant absorbance spectra are exceptionally reliable.

## Stray light correction

Stray light is any light measured by a UV/Vis detector that is not of the chosen wavelength. Stray light tends to have the biggest influence in the UV range, which is also the most important range for nucleic acid and protein quantification. Lunatic spectrophotometers are assembled to maximize linearity by minimizing the influence of common sources of stray light, like internal reflections and 2<sup>nd</sup> order diffraction. UV/Vis spectrophotometers that do not correct for stray light have lower accuracy, especially for high absorbance samples.<sup>1</sup>

To boost accuracy even more, each Lunatic instrument undergoes a factory calibration using high absorbance samples with peaks across the UV/Vis spectrum. The results of the calibration are used to create an individualized stray light profile which is applied to all spectra. This combination of assembly and calibration results in a near-zero impact of stray light so Lunatic remains linear from its lowest to its highest detectable absorbance.

## Transforming intensity into absorbance

Lunatic calculates a sample absorbance spectrum from the intensity spectra of the empty microcuvette,  $I_{empty}$ , and the filled microcuvette,  $I_{filled}$ , using the following equations:

$$Absorbance = -\log\left(\frac{I_{filled}}{I_{empty}}\right)$$

$$Nominal\ Absorbance = \frac{10\ mm}{cuvette\ path\ length\ (mm)} \times Absorbance$$

Lunatic represents absorbance spectra with nominal path lengths of 1 cm so they can easily be compared to other cuvette-based UV/Vis spectrophotometers. Since absorbance values are directly proportional to the optical path length, converting to the nominal path length is a simple multiplication operation. Nominal sample absorbances are used for all subsequent calculations and analysis applications. For most apps, the resultant absorbance spectra are smoothed using Savitzky-Golay filtering, but unsmoothed absorbance spectra can also be accessed in the software.

High Lunatic strips have 2 cuvettes, so each sample gets 2 distinct absorbance spectra. Since the smaller microcuvette is best for samples with high absorbances and the large microcuvette best for lower absorbances, Lunatic combines them into 1 spectrum. It uses the values from the smaller microcuvette when the nominal absorbance is >30 OD and the larger microcuvette when the nominal absorbance is <20 OD. For values between 20 and 30 OD, Lunatic averages the values from both cuvettes for more robust results.

Optimized measurement steps and signal processing means Lunatic reads absorbance spectra across a wide absorbance range and the full UV/Vis spectrum fast and reliably. Lunatic's analysis software delivers results across a wide range of possibilities, including raw absorbance spectra, deconvoluted signals, or customized algorithms.

## There's an app for that A260 for nucleic acid

Nucleic acid samples can be checked for both concentration and quality with Lunatic using a

variety of apps and methods (Table 4). Nucleic acids have an absorption maximum at 260 nm (A260) and the spectrographic reading at this wavelength is the most common method for detecting dsDNA, ssDNA, RNA and oligonucleotides in a solution. Based on Beer-Lambert law, an A260 reading of 1.0 in a 10 mm pathlength cuvette is equivalent to ~50 ng/μL dsDNA, ~33 ng/μL ssDNA, or ~40 ng/μL RNA. Therefore, concentration ( $c$ ) can be calculated from A260 using the following equation:

$$c = A260 \times F$$

where  $F$  is the concentration factor corresponding to the nucleic acid of interest. The description of each Lunatic app identifies which concentration factor it uses and the equivalent nucleic acid (dsDNA, ssDNA, or RNA).

After nucleic acid purification, residual cellular contaminants like proteins or sample preparation reagents frequently remain in the solution and can interfere with A260 measurement, leading to incorrect results. They can also cause issues in downstream processes, like PCR or sequencing. Both protein and DNA absorb UV light but have different absorbance curves. Nucleic acids have an absorbance peak at 260 nm, while proteins absorb strongly at 280 nm. Therefore, the purity of a DNA sample can be calculated by examining the ratio of the two absorbance values. A260/A280 values of 1.7–1.8 indicate “clean” DNA; good quality RNA will have a A260/A280 ratio >2.0. Lower values may indicate significant protein, phenol, or other aromatic compound contamination.

The A260/A280 ratio by itself is not a complete representation of DNA purity. Other additives or contaminants, like EDTA, guanidine, or carbohydrates, have low 280 nm absorption but absorb UV light around 230 nm. Furthermore, some proteins with few aromatic amino acid residues have little absorbance at 280 nm, while all proteins have a clear absorption peak at 190–230 nm due to their peptide bonds. This

makes the A260/A230 ratio a valuable indicator of the presence of protein or other impurities in a nucleic acid sample. Therefore, absorbance readings at 230 and 280 nm provide a better estimate of contaminants that may be present in nucleic acid samples. The ratio of A260/A230 should be ≥1.8 for pure nucleic acid samples.

Nucleic acid apps on Lunatic are purpose-built to assess purity and concentration from UV/Vis absorbance spectra.

## A280 for protein

Lunatic has built-in apps for quantifying protein in multiple conditions (Table 5). Protein concentration can be determined by measuring UV absorbance at 280 nm (A280) and calculating the concentration using the extinction coefficient of the protein in the Beer-Lambert equation. This method is well-suited for quantifying purified proteins. Lunatic uses the following equation to calculate protein concentration from A280:

$$c = \frac{A280 \times 10}{E1\%}$$

where E1% is an extinction coefficient that can be determined empirically by measuring A280 of a 1% solution of a protein, or 1 gram of protein in 100 mL of solvent.

UV absorbance of proteins depends on the number of aromatic amino acids (Trp, Tyr and Phe) and Cys–Cys disulfide bonds in its amino acid sequence. Post-translation modifications and protein conformation have an impact as well, as can buffer type, ionic strength, and pH. When determining protein concentration by A280, the best approach is to empirically derive the extinction coefficient for the protein of interest, or search for published protein extinction coefficients. Alternatively, if the amino acid sequence of the protein to be measured is known, the theoretical molar extinction coefficient can be calculated using the equation:

$$\epsilon = 5500 \times Trp + 1490 \times Tyr + 125 \times Cys$$

where  $\epsilon$  is the molar extinction coefficient and  $Trp$ ,  $Tyr$ , and  $Cys$  are the number of tryptophan, tyrosine, and cysteines, respectively.

Lunatic uses E1% rather than molar extinction coefficients for its protein absorbance calculations. E1% can be calculated from the molar extinction coefficient ( $\epsilon$ ) and the molecular weight ( $MW$ ) of a protein as follows:

$$E1\% = \frac{\epsilon \times 10}{MW}$$

Protein apps on Lunatic makes quantification a snap at high or low concentration.

## Unmix

Unmix applications exploit the full potential of UV/Vis spectral measurements, allowing specific concentration determination of extracted nucleic acids, PCR amplicons, or proteins in complex samples.

Tuned to the UV/Vis capabilities of Lunatic, Unmix applications uncover the presence of absorbing contaminants by assessing their particular contribution within the measured UV/Vis spectrum. Unmix algorithms are trained to look for impurities commonly found in different biological techniques. As a result, the measured absorbance spectrum is deconvoluted into multiple spectra of individual components, allowing specific quantification of the biomolecule of interest as well as evaluating the quality and purity of the sample. For more specific descriptions of each Unmix app, see the blue app names in [Tables 4 & 5](#).

## Standard curve assays

Protein concentration quantification by standard curves of colorimetric protein assays, such as the BCA, Bradford, and Lowry assays, can easily be performed with the Lunatic using the corresponding applications. These methods are commonly used for quantification of protein solutions and cell lysates.

The starting point is a set of reference samples with known concentrations. The Lunatic system measures the absorption spectra and builds a standard curve to relate reference concentration and the measured OD at a certain wavelength.

When samples of unknown concentration are measured, the OD at the reference wavelength is compared to the standard curve to calculate the concentration of the unknown sample.

## Homebrew

Solving the challenges of life sciences sometimes requires specifically tailored analysis. Creating your own applications for analysis by Lunatic is easy with the Homebrew toolkit. Put the full UV/Vis spectrum to work by creating outputs with algebraic, arithmetic, or statistical functions and variables that are built-in like absorbance values, or user-defined. Choose what kind of background correction is most relevant for each analysis. From determining average and standard deviation of the concentration of replicates to figuring out the drug incorporation efficiency of an antibody-drug conjugate, Homebrew has the tools to build custom solutions.

## Background correction options

Lunatic apps provide several options to correct for any background absorbance. Turbidity correction uses the known relationship between wavelength and Rayleigh scattering to apply wavelength-specific correction to the absorbance spectrum. By examining a sample's absorbance spectrum from 320–400 nm, Lunatic determines a unique turbidity profile and optimizes background correction based on the results.

Single-point background correction lets you select a wavelength and subtract the absorbance at that wavelength from the entire spectrum, as is common for other UV/Vis spectrophotometers. Intelligent single point correction tells the software to find a wavelength with no absorbance to use for background correction, ensuring absorbance values are always positive. Homebrew background correction choices include turbidity, single-point, and intelligent single-point, but also has a few other options. Homebrew can apply correction equal to the average absorbance in a wavelength range, typically used for colored samples with a well-defined transparent wavelength range. Or you can choose to do no background correction at all.

## Nucleic acid apps

Name	Description
dsDNA (Turbidity)	Best used for purified dsDNA. Uses the total absorbance at 260 nm with a concentration factor of 50 (dsDNA equivalence) and sample background (turbidity) correction to calculate dsDNA concentration (ng/μl).
dsDNA (Single point)	Best used for purified dsDNA. Uses the total absorbance at 260 nm with a concentration factor of 50 (dsDNA equivalence) and single wavelength (340 nm or custom) background correction to calculate dsDNA concentration (ng/μl).
ssDNA (Turbidity)	Best used for purified ssDNA. Uses the total absorbance at 260 nm with a concentration factor of 33 (ssDNA equivalence) and sample background (turbidity) correction to calculate ssDNA concentration (ng/μl).
ssDNA (Single point)	Best used for purified ssDNA. Uses the total absorbance at 260 nm with a concentration factor of 33 (ssDNA equivalence) and single wavelength (340 nm or custom) background correction to calculate ssDNA concentration (ng/μl).
DNA (Tissue/blood)	Used to quantify human/mammalian DNA isolated from tissue and blood. Unmix algorithms subtract sample background (turbidity) and impurities (RNA, thiocyanate salts, EDTA, phenol, azide) from the total absorbance spectrum to isolate the contribution from DNA, then use a concentration factor of 50 (dsDNA equivalence) to calculate DNA concentration (ng/μl).
DNA (Plant)	Used to quantify DNA isolated from plant extracts. Unmix algorithms subtract sample background (turbidity) and impurities (RNA, thiocyanate salts, EDTA, phenol, citrate) from the total absorbance spectrum to isolate the contribution from DNA, then use a concentration factor of 50 (dsDNA equivalence) to calculate DNA concentration (ng/μl).
DNA (Purified PCR)	Used to quantify purified PCR products. Unmix algorithms subtract sample background (turbidity) and impurities (dNTPs, EDTA, betaine, thiocyanate salts, PCR tube leachables) from the total absorbance spectrum to isolate the contribution from DNA, then use a concentration factor of 50 (dsDNA equivalence) to calculate DNA concentration (ng/μl).
Nucleic Acids (DNA equiv.)	Used to quantify total nucleic acids isolated from any source. Unmix algorithms subtract sample background (turbidity) and impurities (protein, thiocyanate salts, EDTA, phenol, azide, detergents) from the total absorbance spectrum to isolate the contribution from nucleic acids, then use a concentration factor of 50 to calculate nucleic acid concentration (dsDNA equivalent).
Labeled dsDNA	Uses the total absorbance at 260 nm with a concentration factor of 50 to calculate dsDNA concentration (ng/μl). Uses the specific dye absorbance wavelength and molar extinction coefficient to determine incorporation of dyes (pmol/μl) and degree of labeling (DoL).
Labeled ssDNA	Uses the total absorbance at 260 nm with a concentration factor of 33 to calculate ssDNA concentration (ng/μl). Uses the specific dye absorbance wavelength and molar extinction coefficient to determine incorporation of dyes (pmol/μl) and degree of labeling (DoL).
ssDNA Cy3/Cy5	Used to quantify ssDNA probes labeled with Cy3 and/or Cy5 (pmol/μl). Unmix algorithms subtract sample background (turbidity) and impurities (protein, thiocyanate salts, EDTA, phenol, azide, citrate) from the total absorbance spectrum to isolate the contribution from ssDNA, Cy3 and Cy5 dyes, using a concentration factor of 33 to calculate ssDNA concentration (ng/μl).
RNA (Turbidity)	Best used for purified RNA. Uses the total absorbance at 260 nm with a concentration factor of 40 (RNA equivalence) and sample background (turbidity) correction to calculate RNA concentration (ng/μl).
RNA (Single point)	Best used for purified RNA. Uses the total absorbance at 260 nm with a concentration factor of 40 (RNA equivalence) and single wavelength (340 nm or custom) background correction to calculate RNA concentration (ng/μl).
RNA (Any source)	Used to quantify RNA isolated from any source. Unmix algorithms subtract sample background (turbidity) and impurities (DNA, protein, thiocyanate salts, phenol, azide) from the total absorbance spectrum to isolate the contribution from RNA, then use a concentration factor of 40 (RNA equivalence) to calculate RNA concentration (ng/μl).

Name	Description
RNA (FFPE)	Used to quantify RNA isolated from FFPE specimens. Unmix algorithms subtract sample background (turbidity) and impurities (protein, thiocyanate salts, EDTA, phenol, xylene, azide, citrate, detergents) from the total absorbance spectrum to isolate the contribution from RNA, then use a concentration factor of 40 (RNA equivalence) to calculate RNA concentration (ng/μl).
Nucleic Acids (RNA equiv.)	Used to quantify total nucleic acids isolated from any source. Unmix algorithms subtract sample background (turbidity) and impurities (protein, thiocyanate salts, EDTA, phenol, azide, detergents) from the total absorbance spectrum to isolate the contribution from nucleic acids, then use a concentration factor of 40 to calculate nucleic acid concentration (RNA equivalent).
Labeled RNA	Uses the total absorbance at 260 nm with a concentration factor of 40 to quantify RNA concentration (ng/μl). Uses the specific dye absorbance wavelength and molar extinction coefficient to determine incorporation of dyes (pmol/μl) and degree of labeling (DoL).
RNA Cy3/Cy5	Used to quantify RNA probes labeled with Cy3 and/or Cy5 (pmol/μl). Unmix algorithms subtract sample background (turbidity) and impurities (protein, thiocyanate salts, EDTA, phenol, azide, citrate) from the total absorbance spectrum to isolate the contribution from RNA, Cy3 and Cy5 dyes, using a concentration factor of 40 to calculate RNA concentration (ng/μl).
Oligo DNA Sequence	Uses the total absorbance at 260 nm with the sequence-dependent concentration factor to calculate DNA oligonucleotide concentration (ng/μl and pmol/μl).
Oligo RNA Sequence	Uses the total absorbance at 260 nm with the sequence-dependent concentration factor to calculate RNA oligonucleotide concentration (ng/μl and pmol/μl).
Oligo Molar E	Uses the total absorbance at 260 nm with the concentration factor determined by molar extinction coefficient and molecular weight to calculate DNA and RNA oligonucleotide concentrations (ng/μl and pmol/μl).
ADC Dual Wvl	Used to calculate concentration of an antibody and a conjugated payload from absorbance at 280 nm and at a second user-defined wavelength with user-defined molar extinction coefficients. Antibody concentration, drug concentration, and drug-antibody ratio (DAR) are reported.

Table 4: Nucleic acid app names and descriptions. Apps with blue names are Unmix applications.

## Protein apps

Name	Description
Protein (Turbidity)	Best used for purified proteins. Uses the total absorbance at 280 nm with user-defined E1% extinction coefficients and sample background (turbidity) correction to calculate protein concentration (mg/ml).
Protein (Single point)	Best used for purified proteins. Uses the total absorbance at 280 nm with user-defined E1% extinction coefficients and single wavelength (340 nm or custom) background correction to calculate protein concentration (mg/ml).
Protein (Lysates)	Used to quantify total protein (mg/ml) from mixed samples such as cell lysates. Unmix algorithms subtract the sample background (turbidity) and impurities (nucleic acids, azide, detergents, polysaccharides, generic protease inhibitors) from the total absorbance spectrum to isolate the contribution from protein, with user-defined E1% extinction coefficients.
IgG (High concentration)	Used to quantify highly concentrated, full-length IgG samples (>200 mg/ml) with absorption maxima above the 275 OD spectral limit. Unmix algorithms extrapolate the spectral shape to calculate accurate protein concentrations, using a fixed E1% value of 13.7.
IgG (His-tagged)	Used to quantify His-tagged IgG (mg/ml), purified from IMAC Ni-columns with imidazole-containing buffers. Unmix algorithms subtract sample background (turbidity) and imidazole from the total absorbance spectrum to isolate the contribution from protein, with user-defined E1% extinction coefficients.
Labeled Protein	Uses the total absorbance at 280 nm with user-defined E1% extinction coefficients to calculate protein concentration (mg/ml). Incorporation of dyes (pmol/μl) and degree of labeling (DoL) are determined using the specific dye absorbance wavelength and molar extinction coefficient.

Name	Description
660nm Assay	Uses the 660 nm Protein Assay or similar (OD 660 nm) to quantify total protein concentration.
BCA Assay	Uses the BCA protein assay (OD 562 nm) to quantify total protein concentration.
Bradford Assay	Uses the Bradford protein assay (OD 595 nm) to quantify total protein concentration.
Lowry Assay	Uses the Lowry protein assay (OD 650 nm) to quantify total protein concentration.
MS Injection volume	Intended for use with proteomic peptide samples after trypsin digest for a LC-MS/MS system. Peptide concentration ( $\mu\text{g}/\mu\text{l}$ ) is quantified using the total absorbance at 280 nm and a default E1% value of 10, and is used with the user-defined injection amount ( $\mu\text{g}$ ) to calculate the desired injection volume ( $\mu\text{l}$ ).

Table 5: Protein app names and descriptions. Apps with blue names are Unmix applications.

## Other apps

Name	Description
Plasma QC	Used for plasma quality assessment. Unmix algorithms analyze the total absorbance spectrum to isolate and quantify sample turbidity, bilirubin, hemoglobin, and protein, using the concentration factor for each component with peak absorbance values and a default E1% of 10.
UV/Vis	Used for full UV/Vis spectral measurements and reports OD values for up to 3 wavelengths. Intelligent single point background correction is applied.
Raw Absorbance	Used for unprocessed, full UV/Vis spectral measurements with no applied blank or background corrections.
Culture OD600	Used to determine the optical density of bacterial or cell cultures at 600 nm.
Standard Curve	Uses a set of reference standards with known concentrations to determine the concentration of a sample based on a user-defined standard curve.

Table 6: Other app names and descriptions. Apps with blue names are Unmix applications.

## Conclusion

By improving on traditional UV/Vis spectrophotometry principles, Lunatic takes protein and nucleic acid quantification and quality checks to the next level. The combination of innovative optical design, microfluidic-based chips with fixed pathlength cuvettes, robust signal processing, and a range of purpose-built apps means Lunatic will provide state-of-the-art precise and accurate results. Lunatic delivers results from 230–750 nm and from 0.03–275 OD. It is a high-throughput, plate-based system that determines the concentration of up to 96 samples in about 5 minutes. Lunatic is complete with built-in applications that make it easy to measure nucleic acids or proteins from any source.

## References

1. UV-VIS Spectroscopy and Its Applications, H Perkampus, Germany: Springer Berlin Heidelberg, 2013.



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