Advancing Process Analytical Technologies

Exploring NIR for Robust High-Concentration Protein Analysis

IFPAC Annual Meeting 2026

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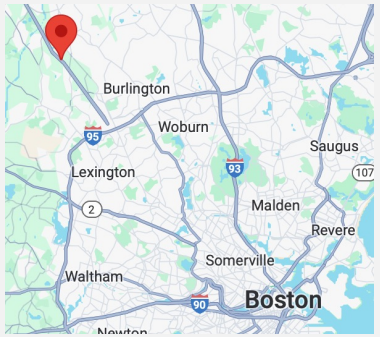
nirrin
technologies

Intros

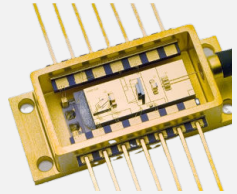
About Nirrin Technologies

- Founded in 2019
- HQ just north of Boston, MA



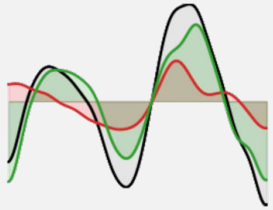
Technical expertise

Tunable Near Infrared Lasers



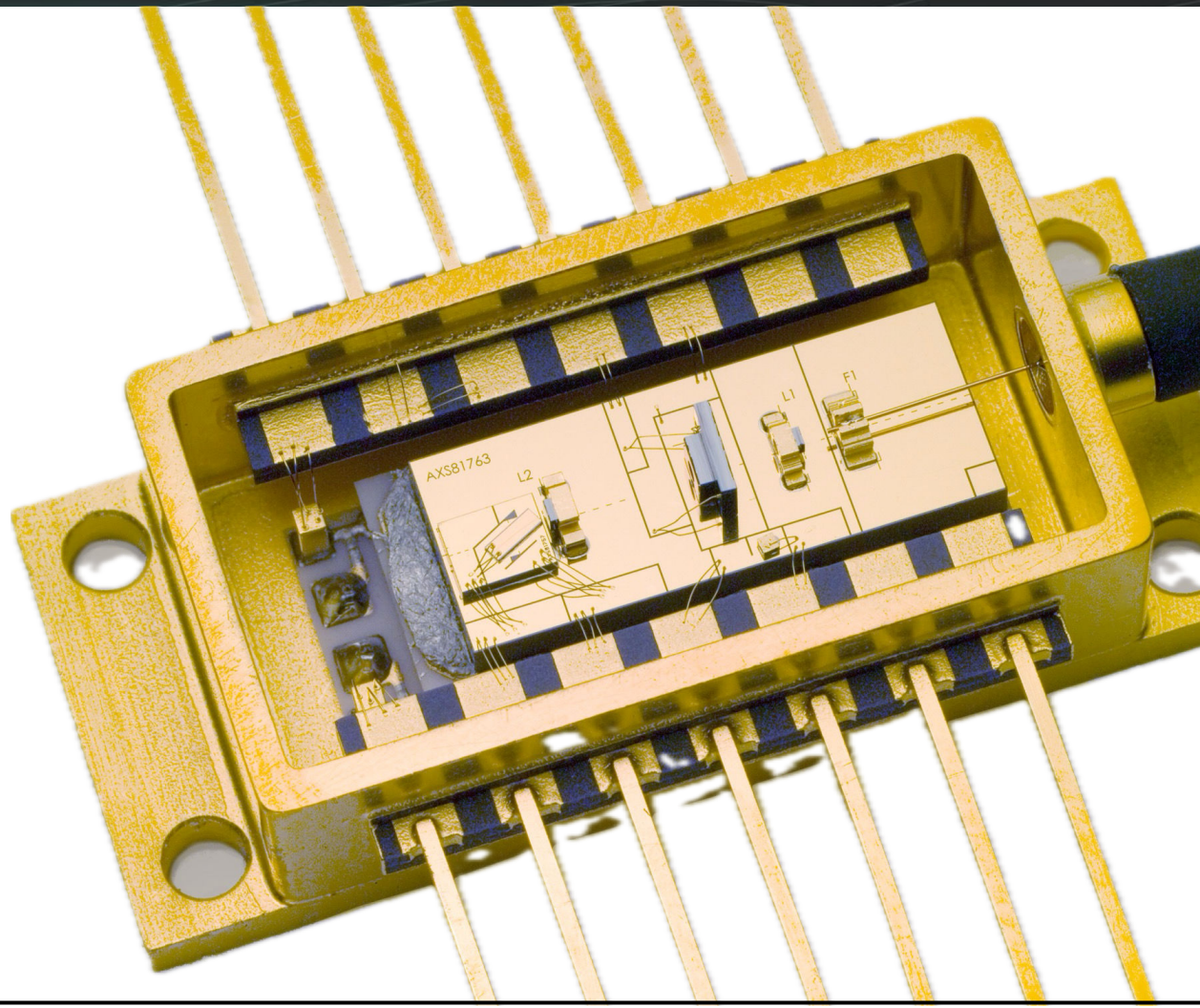
Engineering and manufacturing

Analytics



Talk agenda

1. The Shift to High-Concentration Biologics
2. UV-Vis Operating Regime at High Concentration
3. Spectroscopic Shift: Combination-Band NIR
4. From Univariate to Spectrally-Resolved Beer–Lambert
5. Real-World Deployment & UF/DF
6. Operational Impact & Future Directions



High-Concentration Biologics Are Redefining Analytical Requirements

~30% of approved mAbs are formulated at **≥100 mg/mL**

Many exceed **150–200 mg/mL** for subcutaneous delivery

Shawn Wang, Yifei Yan, Kin Ho, US FDA-approved therapeutic antibodies with high-concentration formulation: summaries and perspectives, *Antibody Therapeutics*,.

Process & Analytical Implications

- Reduced measurement tolerance (±1–2% matters)
- Increased scattering & viscosity effects
- Greater UF/DF and formulation sensitivity
- Narrow operating window for UV-Vis at high concentration

The UV-Vis Operating Regime at High Concentration

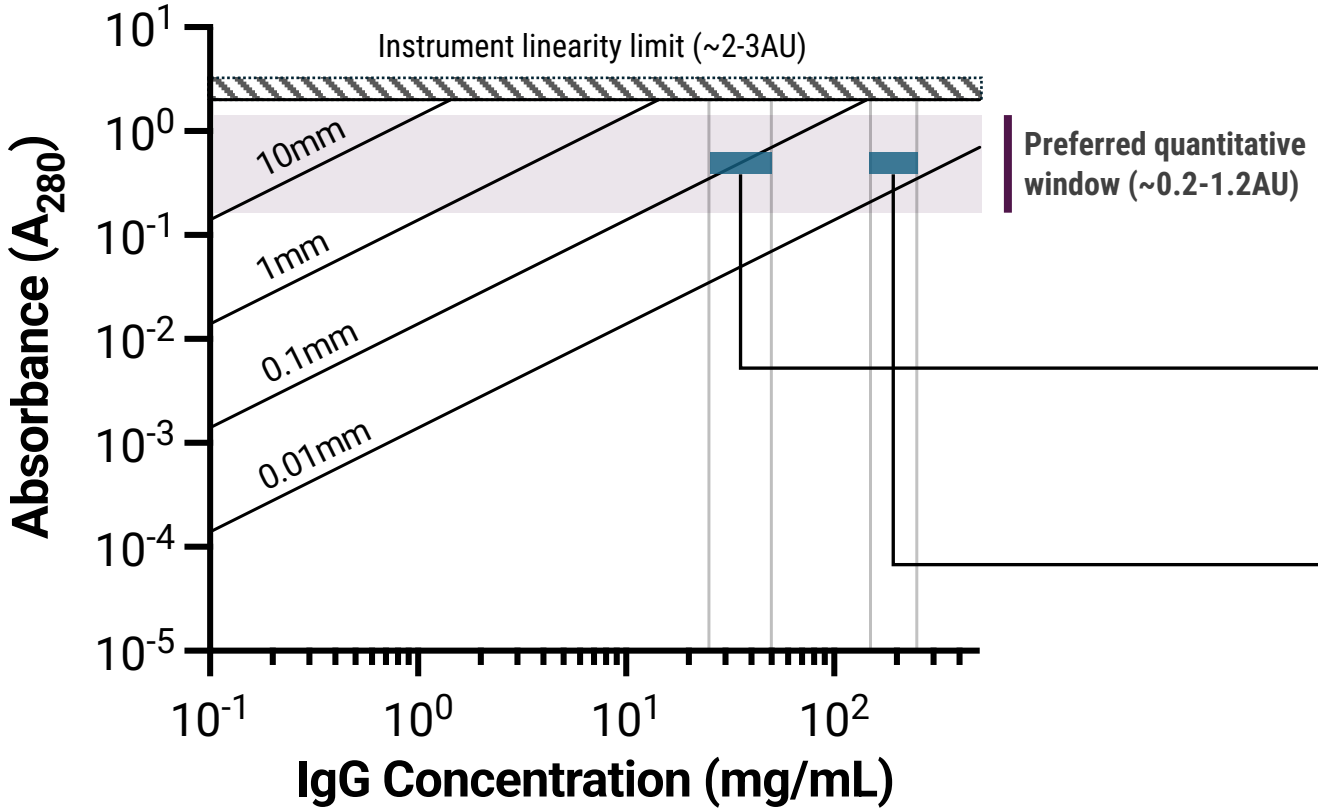


Figure: Iso-pathlength lines for typical IgG (ε≅1.4 mg/mL⁻¹·cm⁻¹)

$$A(\lambda_{280}) = \varepsilon_{protein}(\lambda_{280}) \cdot c_{protein} \cdot l$$

Dynamic pathlength extends linear range

Classic conditions (25-50 mg/mL)

- ~70um - 400um required pathlength

New regime (150-250 mg/mL)

- Required pathlength < 100um
- Measurement becomes geometry-, fouling- and scattering- sensitive

The Spectroscopic Shift:
From Electronic to Vibrational Absorption

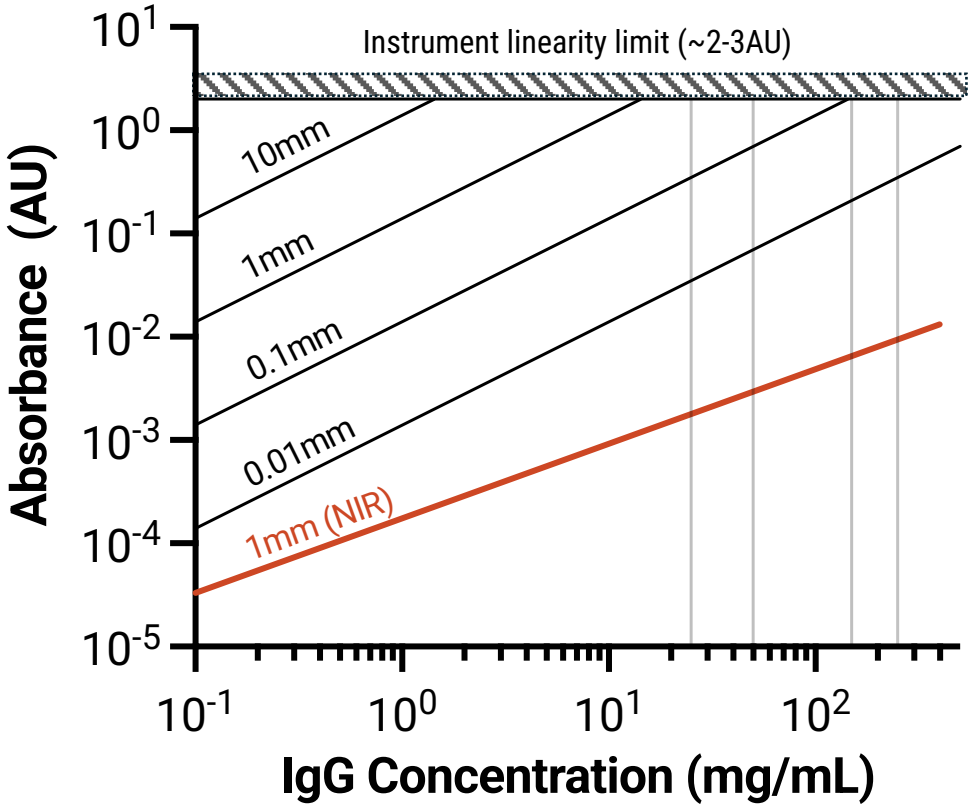


Figure: UV iso-pathlength lines (black) for typical IgG ($\varepsilon_{280} \cong 1.4$ mg/mL^{-1}·cm^{-1}) and NIR (**red $\varepsilon_{2290} \cong 3.3E\text{-}03$ mg/mL^{-1}·cm^{-1}**) demonstrating Lower absorptivity preserves linear regime at high protein concentration.

UV (280 nm)	NIR (1200–2400 nm)
π→π* electronic transitions	O–H, N–H, C–H vibrational modes
High extinction coefficient	Much lower absorptivity (400X lower ε)
Short pathlength required	mm scale fixed-pathlength viable
Aromatic residues only	Whole-molecule vibrational fingerprint

Challenges of Conventional NIR in Aqueous Bioprocessing

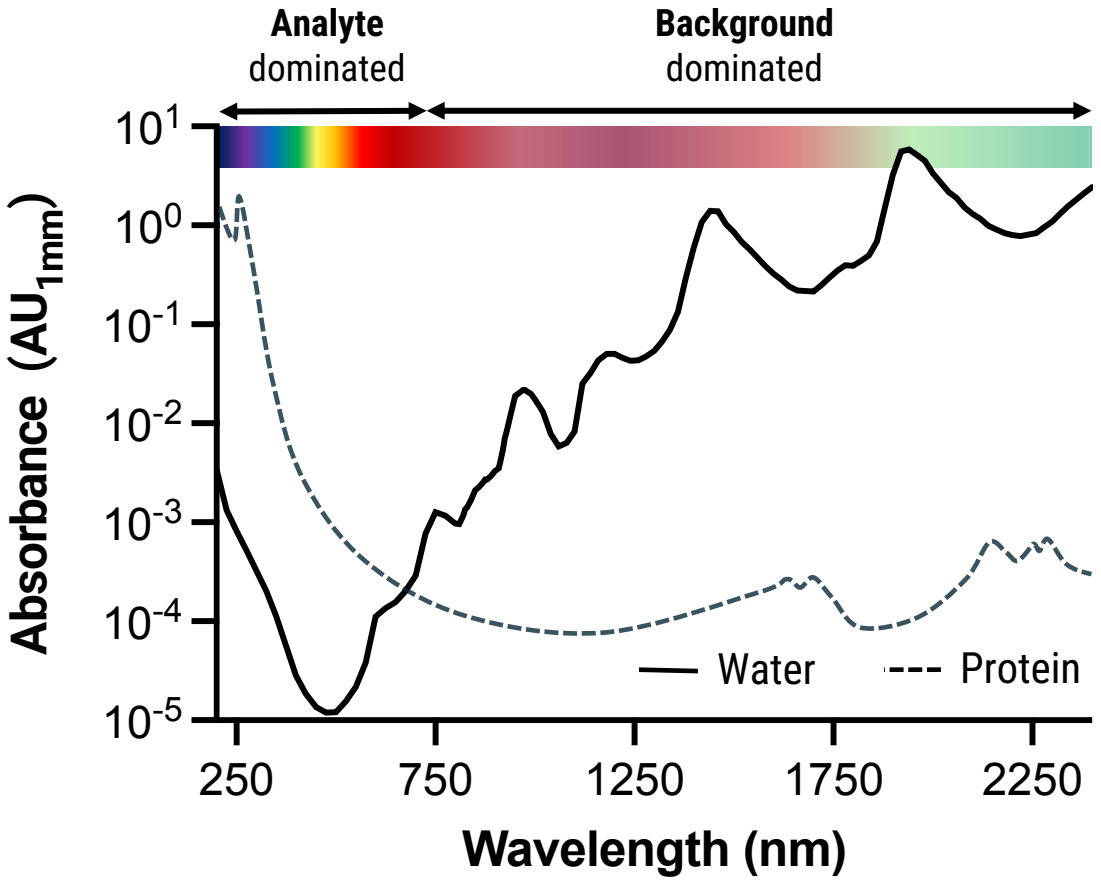


Figure: Representative protein absorbance magnitudes for illustration

UV-Vis: Analyte-dominated regime

- Protein absorption >> water
- Direct Beer–Lambert quantitation
- High signal amplitude

NIR: Background-dominated regime

- Water absorption dominates spectrum
- Protein appears as small perturbation
- Quantitation relies on differential sensitivity

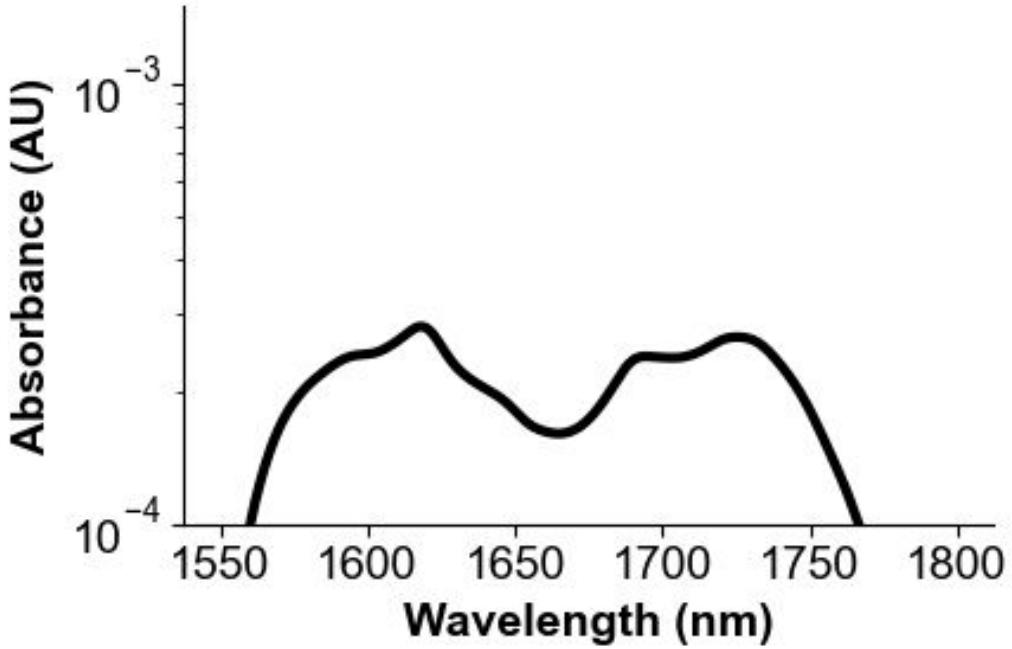
Enhancing Sensitivity in the NIR Region

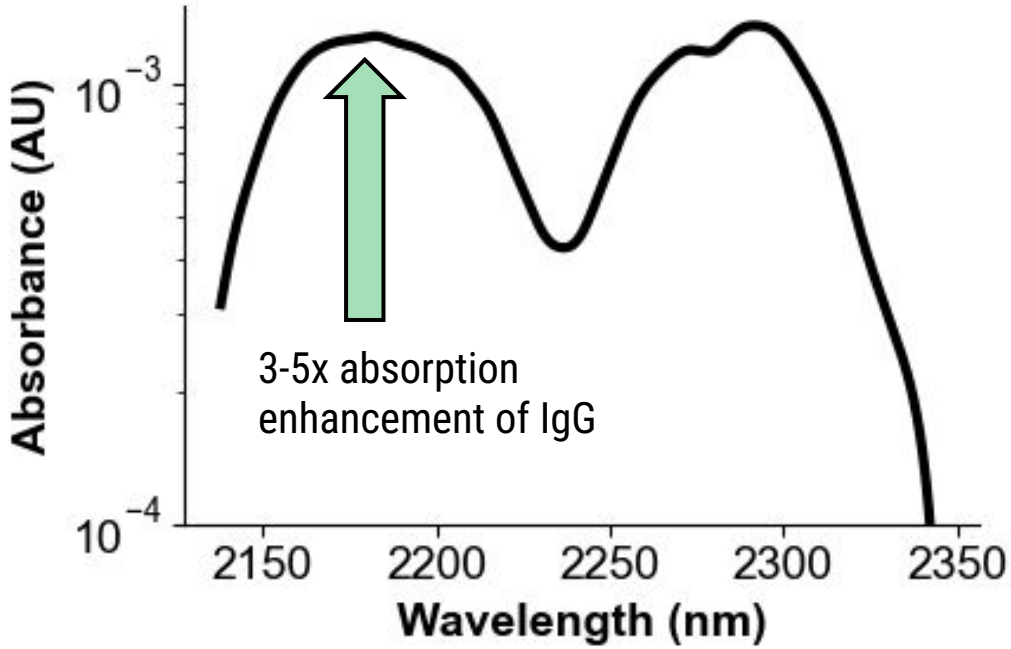
Overtone region (~1550-1800nm)

- Lower effective absorptivity
- Smaller concentration-dependent response

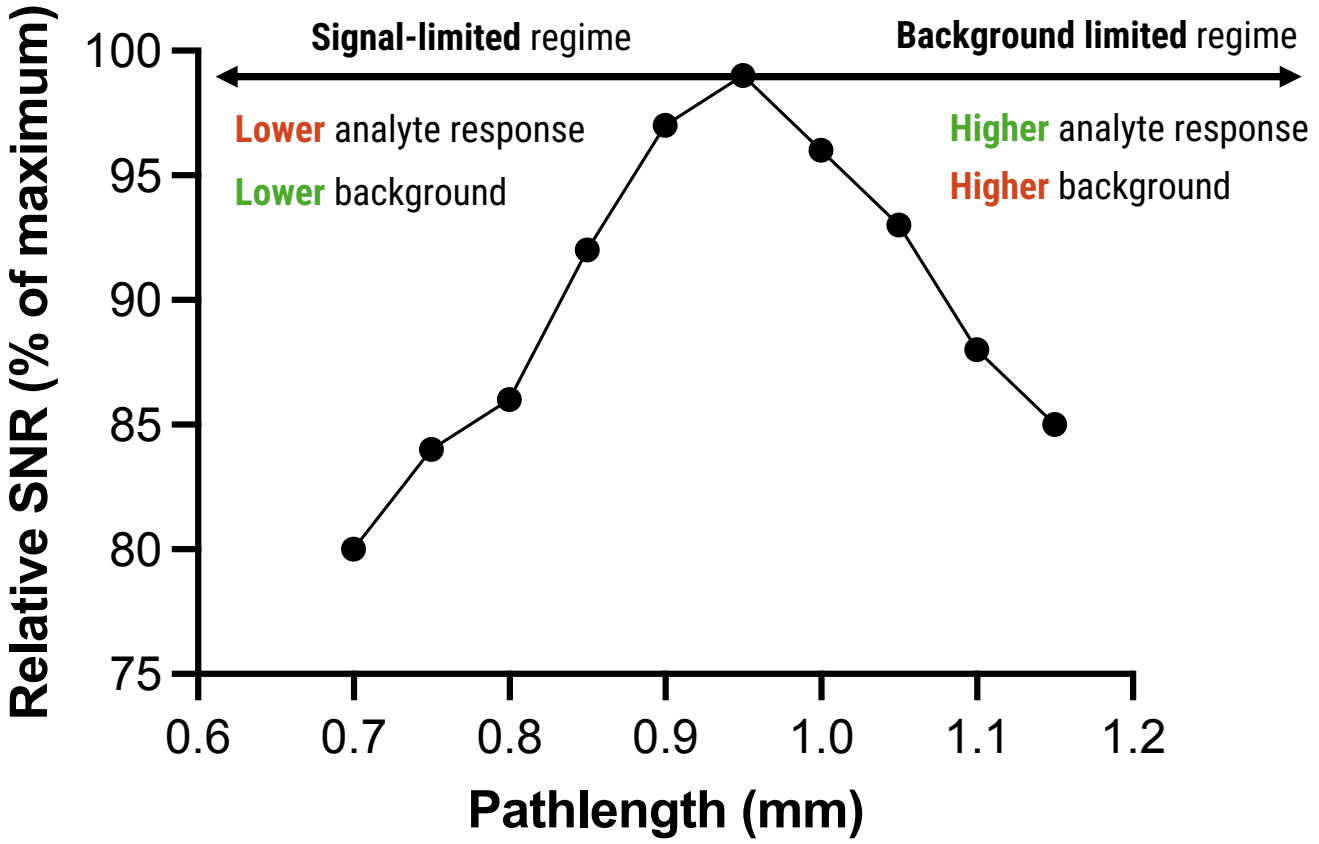
Combination region (~2100-2350nm)

- Higher effective absorptivity
- Greater concentration-dependent response





Pathlength Optimization in a Background-Dominated Regime



Quantitative Protein Measurement in a Coupled Aqueous Matrix

Physical Regime Established

- Linear absorbance regime at high concentration
- Combination-band region selected for maximal sensitivity
- Optimal pathlength identified for SNR

Remaining Technical Challenges

- Overlapping spectral components (protein + water + buffer)
- Temperature-induced spectral variability
- Wavelength stability requirements
- Robust concentration estimation without extinction assumptions

From Univariate to Spectrally Resolved Beer–Lambert

Classical UV

$$A(\lambda_{280}) = \varepsilon_{protein}(\lambda_{280}) \cdot c_{protein} \cdot l$$

- Uses discrete or limited wavelengths for concentration inference
- Requires known absorptivity per wavelength
- Often assumes constant concentration during geometry adjustment
- Minimal spectral overlap

Combination-band NIR

$$A(\lambda) = A_{measured}(\lambda) - A_{background}(\lambda)$$

$$A(\lambda) = \underbrace{\varepsilon_p(\lambda)\, l\, c_p}_{\text{protein}} + \underbrace{\varepsilon_b(\lambda)\, l\, c_b}_{\text{buffer}} + \underbrace{\sum_j \phi_j(\lambda)\beta_j}_{\text{nuisance base}}$$

(path, temp, scatter)

Full-spectrum fitting

Physically constrained multi-wavelength Beer-Lambert fitting

$$\mathbf{A}(\lambda_1 \ldots \lambda_n) = \mathbf{Ec}$$

Where:

A = absorbance vector across wavelengths
E = matrix of basis spectra (water, protein, buffer)
c = concentration vector

$$\hat{\boldsymbol{c}} = \mathrm{argmin}_{\mathrm{c}} \left\| \mathbf{A}_{signal} - \mathbf{Ec} \right\|$$

High-Precision Tunable Laser Spectroscopy (HPTLS)

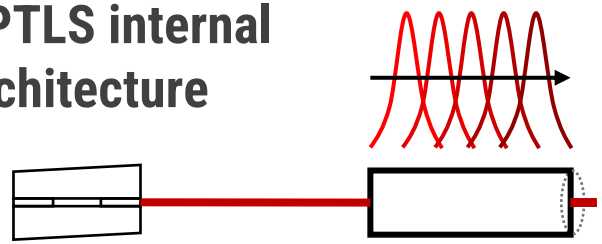
Architecture for Stable Spectral Basis Separation

HPTLS design element	Impact
Targeted combination-band region	Maximizes concentration-dependent contrast
Narrow-line tunable laser (sub-nm)	Resolves overlapping basis functions
Absolute wavelength referencing	Prevents basis misalignment
~20 μAU repeatability	Preserves sub-milli-absorbance perturbations
Millimeter fixed pathlength	Maintains optimal SNR regime

HPTLS enables physically constrained basis separation in a background-dominated regime

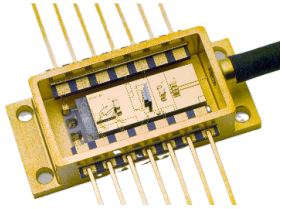
Transferable NIR Quantitation Across Bioprocess Formats

HPTLS internal architecture



Nirrin gain chip
(generates light in the combination NIR)

Tunable external cavity
Sweep through discreet wavelengths (0.01nm resolution) to generate “continuous” spectrum



- MEMS butterfly packaged
- Small and robust

Bioprocess Form Factors

At-line

15µL drop

- **QC spot check** (DS/DP concentration verification)
- **Formulation screening / final formulation confirmation**
- **Fast turnaround** (no dilution, fixed pathlength)

In-line

µL/min-L/min flow

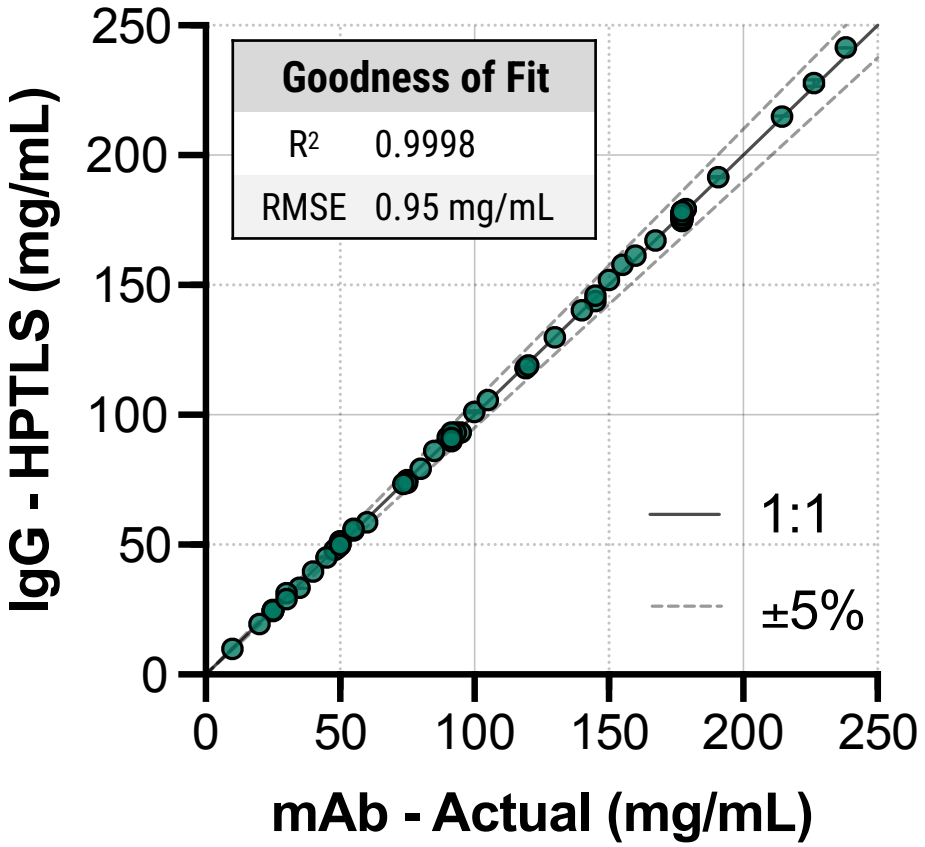
- **UFDF** (endpoint or trajectory)
- **Pool / hold-tank transfers**
- **Closed, sterile measurements**

In-situ

12mm insertion

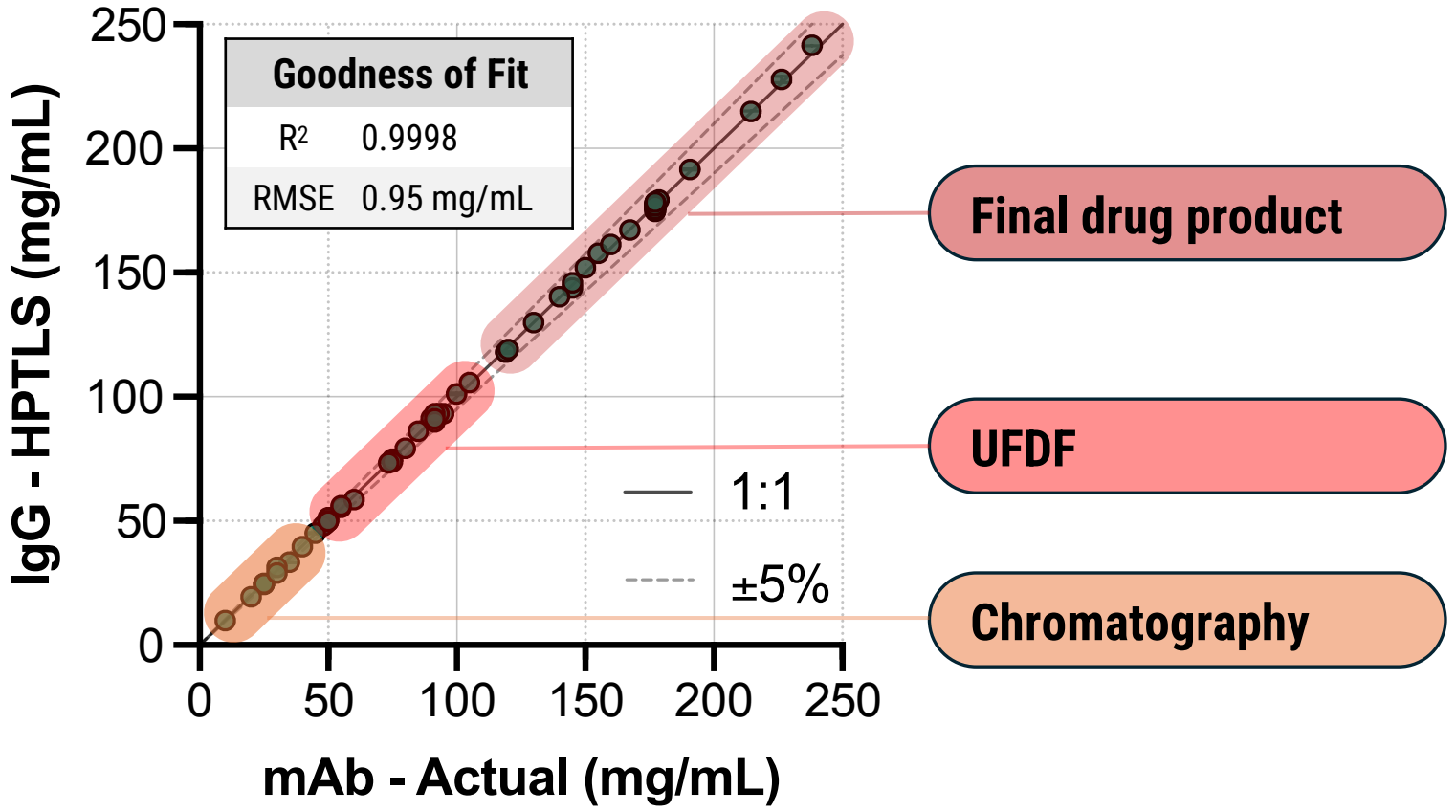
- **Designed for vessel integration**
- **Compatible with standard bioprocess fittings**

Broad At-Line Dynamic Range IgG Quantitation Across Aqueous Environments



Reference concentration determined by orthogonal methods (SoloVPE, gravimetric, HPLC)

Broad At-Line Dynamic Range IgG Quantitation Across Aqueous Environments



Reference concentration determined by orthogonal methods (SoloVPE, gravimetric, HPLC)

Major takeaways

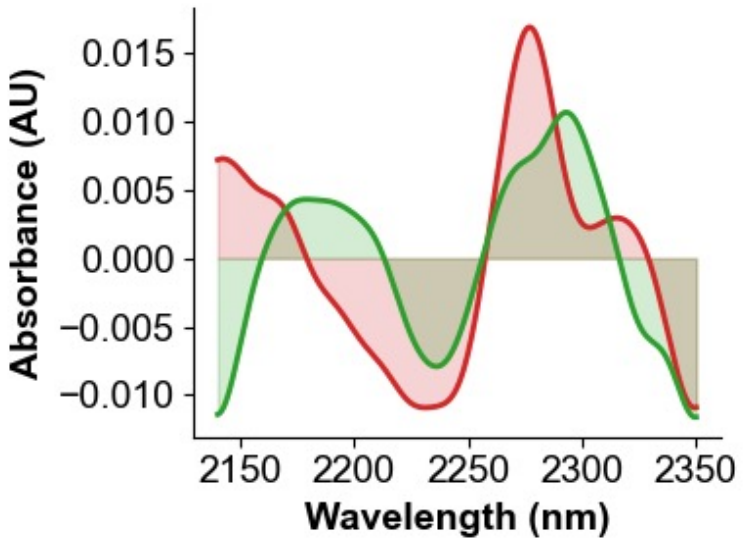
- Broad linear dynamic range (10 - 250 mg/mL)
- Fixed 1 mm pathlength (no dynamic pathlength adjustment)
- General IgG basis (NIST reference)
- Robust in varied aqueous formulations
- No dilution required

Spectral Fits and Residual Analysis of 50 mg/mL Formulation

Model basis contributions

Buffer contribution

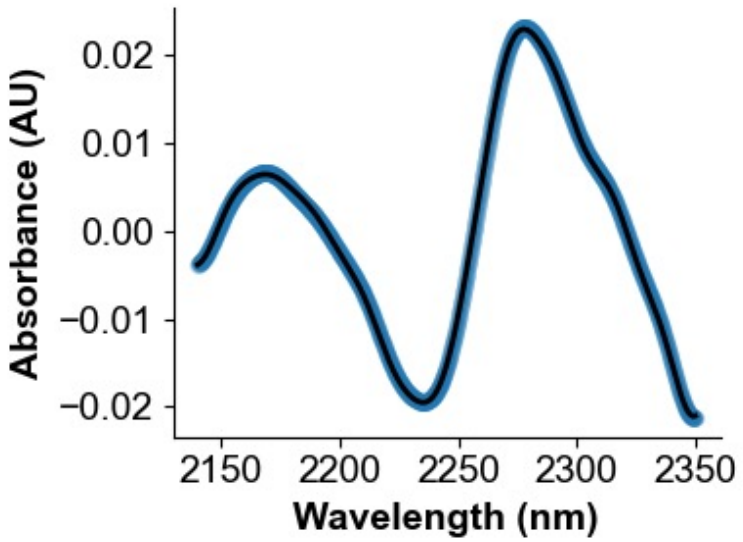
Protein contribution



Measured vs Model Overlay

Measured spectrum

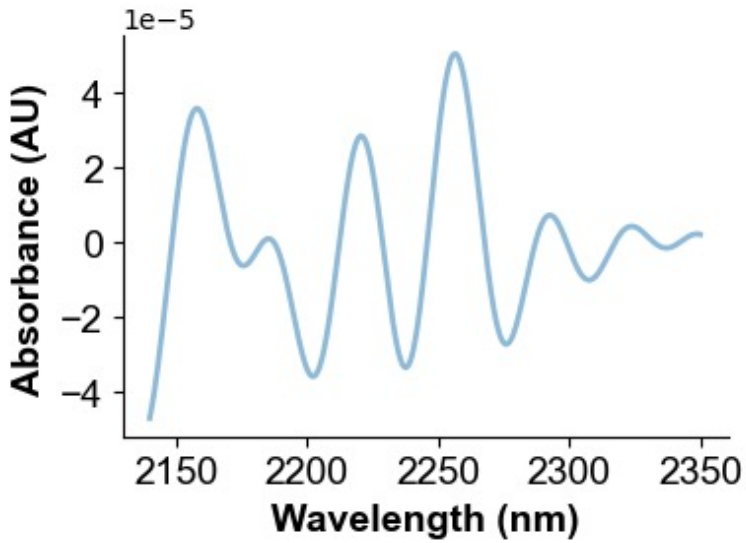
Model fit (buffer + protein)



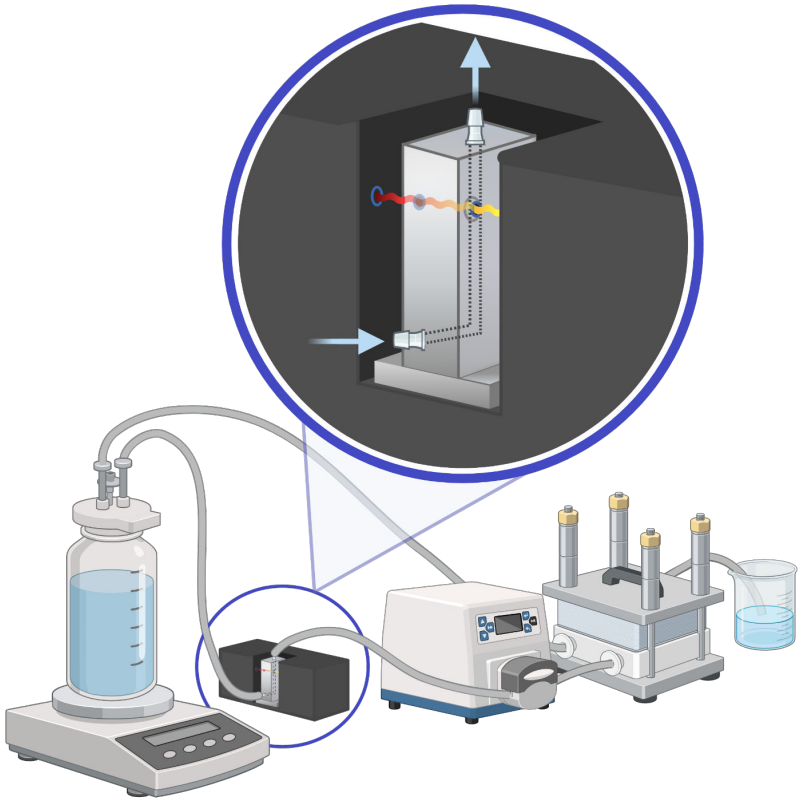
Model Residual

Residual RMS = 20.3µAU

~1000X smaller than total absorbance



Integration of HPTLS Flow Cell in UFDF Skids

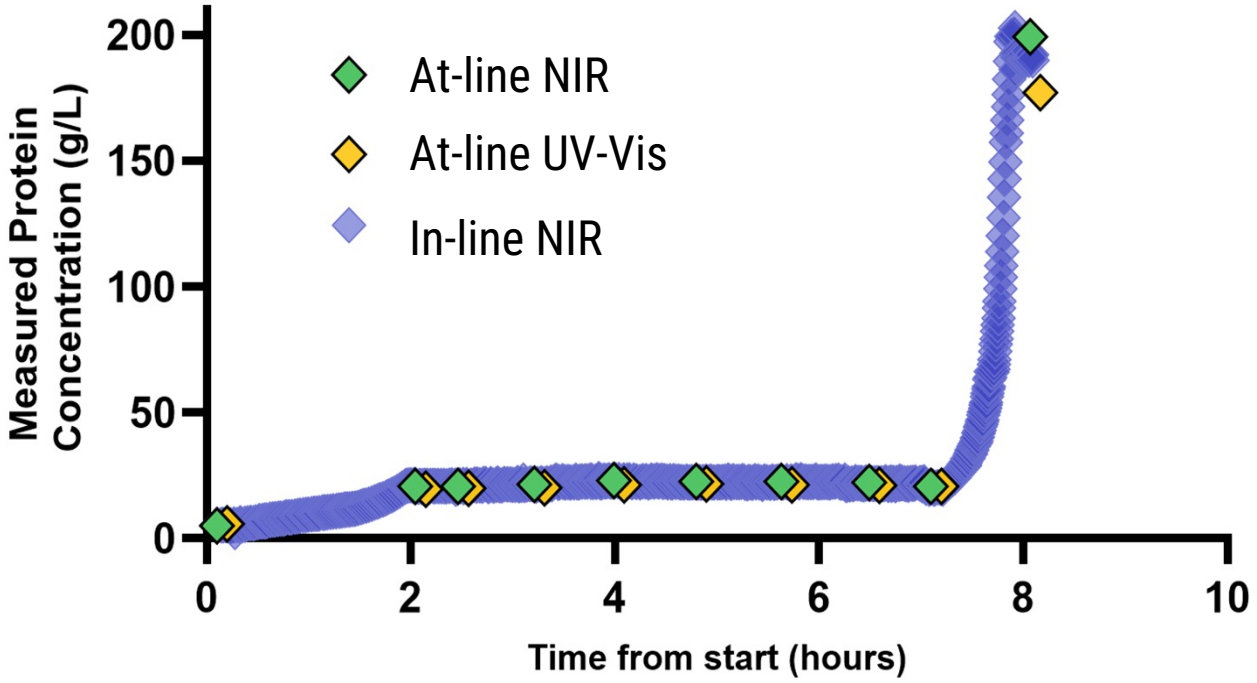


- Integrated in-line upstream of filter (pre-filtration position)
- Compatible with CIP/SIP, autoclave, or gamma-sterile tubing sets
- Modular flow-cell design for sterile bioprocess environments
- Fixed ~1 mm optical pathlength maintained during flow

Real-Time Protein Quantitation During UFDF Concentration Ramps

Real implementation

HPTLS flow cell connected to Akta Flux™ by Cytiva



Key takeaways

- Demonstrated broad linear dynamic range (5 - 200 mg/mL) in flow
- General IgG basis (NIST reference)
- Robust in varied aqueous formulations

Insights

- Fixed 1 mm pathlength maintains stable optical geometry during concentration ramps
- No pathlength adjustment required as absorbance increases

Conclusions and Future Work

What Combination-Band NIR Enables

- Fixed optical geometry across unit operations
- Broad linear range without dilution
- Transferable spectral basis across form factors
- Robust performance in background-dominated regimes
- Simplified PAT qualification pathway

Next Steps Toward Scalable PAT

- Extend deployment to in-situ vessel monitoring
- Expanded therapeutic validation dataset
- Integration with control systems (closed-loop potential)
- Ongoing method validation & regulatory alignment