

Label-Free Native Fluorescence Detection Results for Slab-Gel & Microchip Electrophoresis Separation Devices

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Outline

- ❑ Advantages of Label-free Detection methods in the deep UV
- ❑ New LOD results for CE, μ Cap LC, MCE, slab-gel
- ❑ Sensitivity and specificity using multi-band **L**aser Induced **N**ative **F**luorescence (LINF) detection
- ❑ Enabling Technologies: Deep UV Lasers & Detectors

Advantages of Deep UV Excitation

- ❑ Excites **native fluorophors** within biological and organic molecules without the need for dye tags
- ❑ Allows **simultaneous** detection of untagged & tagged compounds with the same deep UV laser
- ❑ **Strong absorption** in biological and organic molecules
- ❑ **High native fluorescence quantum efficiency**, approaching that of dye tags
- ❑ Native fluorescence emission spectra provide information on **chemical identity** of unknown compounds

Advantages of Tag-less Detection

- ❑ Enables detection of a wide range of otherwise **unknown compounds** in a sample
- ❑ Enables orthogonal peak identification using **retention time and emission spectra**
- ❑ Eliminates issues of tagging (slow kinetics and incomplete reactions, sample denaturation, tag shelf lifetime, small volumes or low concentrations, etc.)
- ❑ Minimizes sample handling
- ❑ Provides high sensitivity and **low LODs**
- ❑ Eliminates interference with downstream methods (GC-MS, etc.)
- ❑ Allows post column detection in capLC without post column derivatization

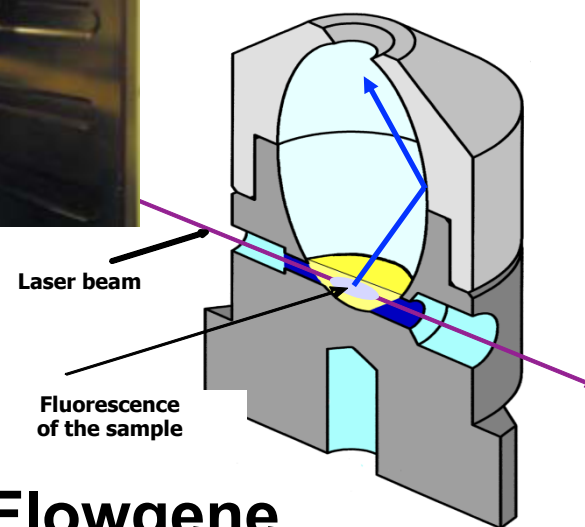
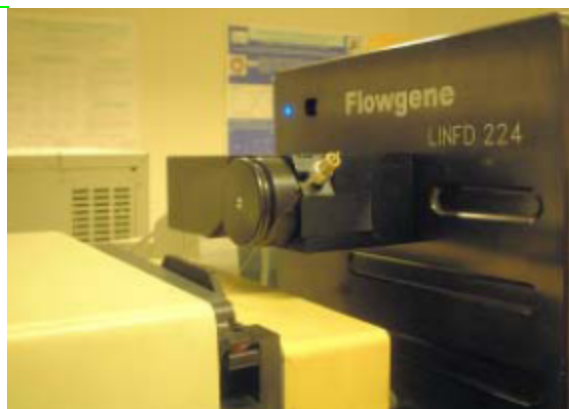
Deep UV Native Fluorescence Detectors for CE, μ CapLC, MCE, & Slab-gels

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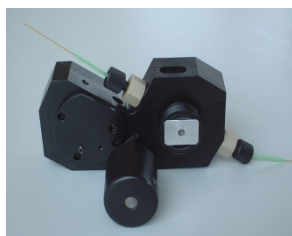
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Flowgene LINF Detector: 224nm CE/ μ CapLC



Flowgene
Elliptical CapLC
Detection Cell



CE Head



HPLC Head



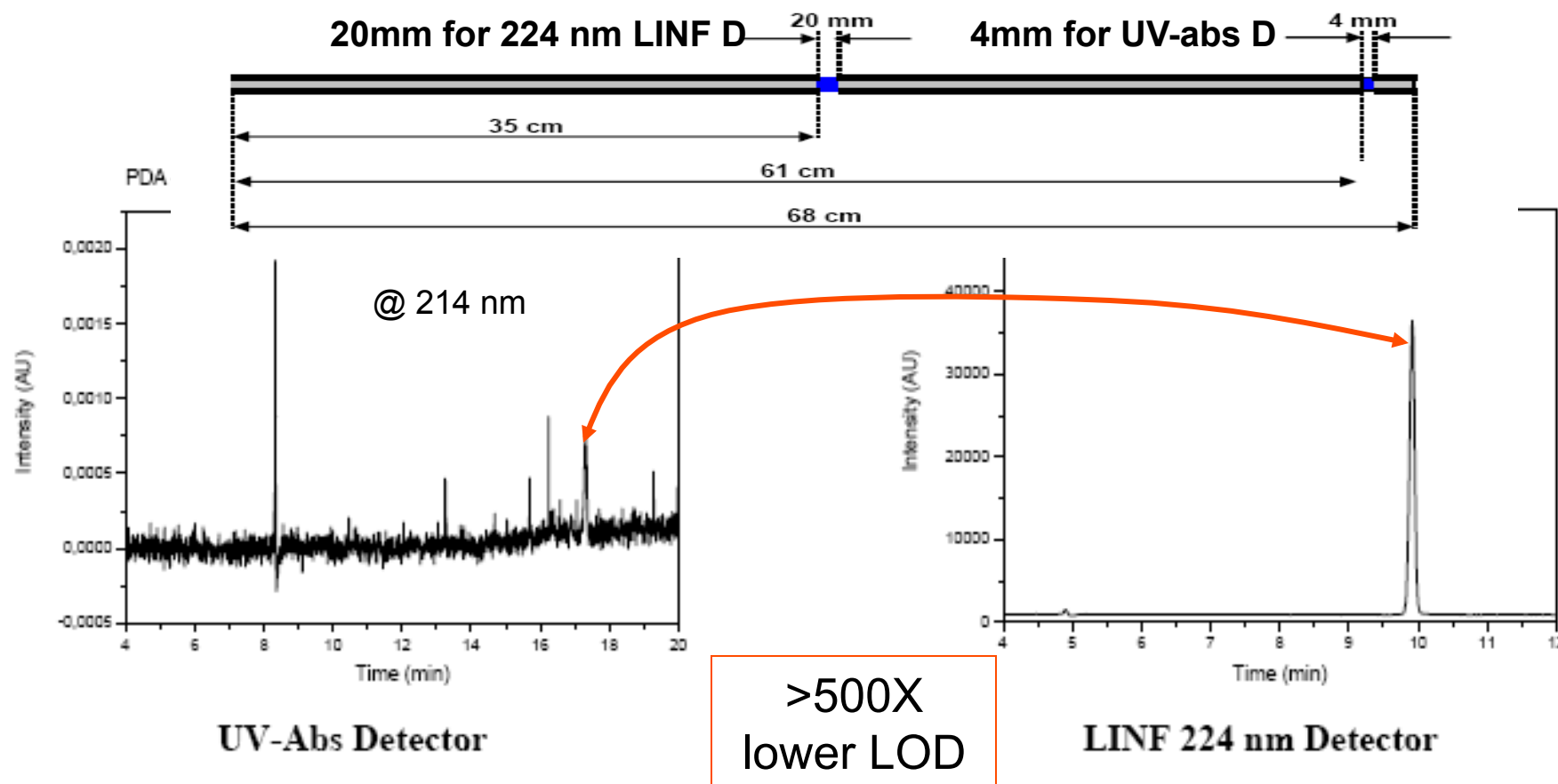
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CE LOD Comparison



UV-abs (PDA) detector in tandem with 224 nm LINF detector

Beckman P/ACE 2100 CE with 2 capillary windows at 10 μ M Ibuprofen, 20kV



$$S/N : 6.81 \times 10^{-4} / 2.210 \times 10^{-4} = 3$$

$$36551/24 = 1523$$

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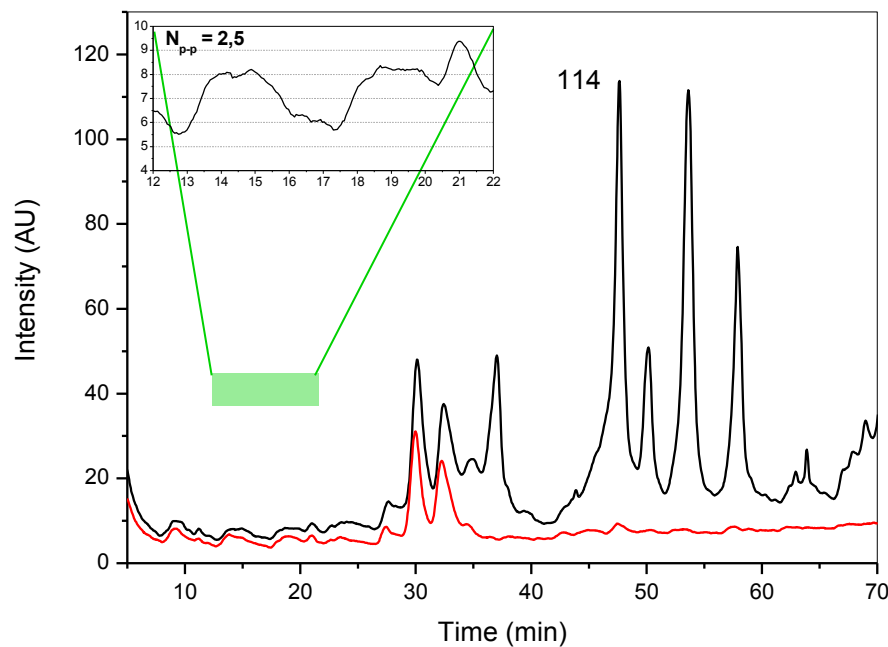
Data from C. Morin, J. Viellard, and P.L. Desbene, Rouen University, 55, rue Saint-Germain, F-27000 Evreux, FR

HPLC LOD Comparison

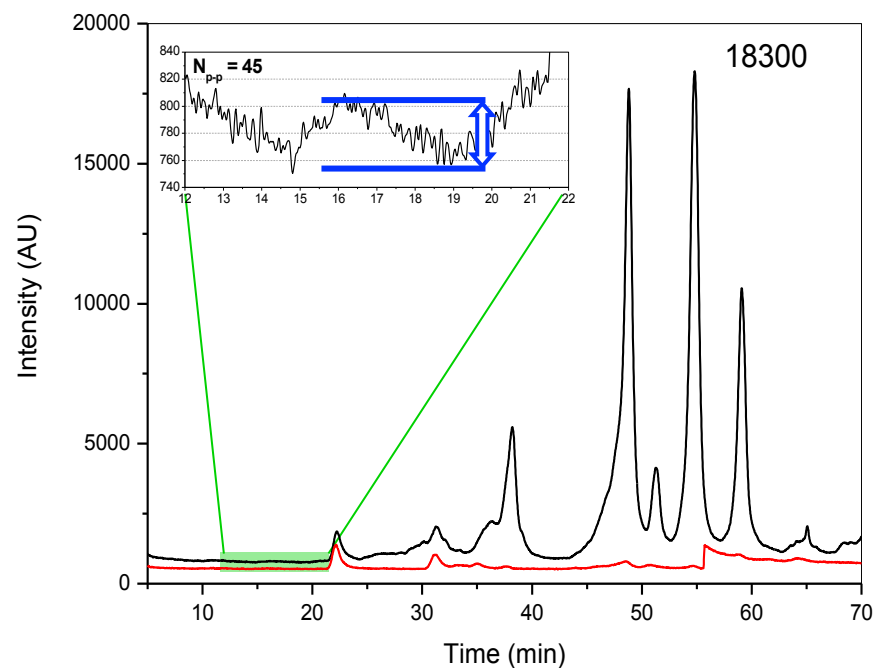
(PDA in tandem with 224nm LINF detector)



Biotech/Kontron HPLC, protein mix from wheat, 220um ID, 15uL injection,



PDA Detector S/N=46 at 214 nm



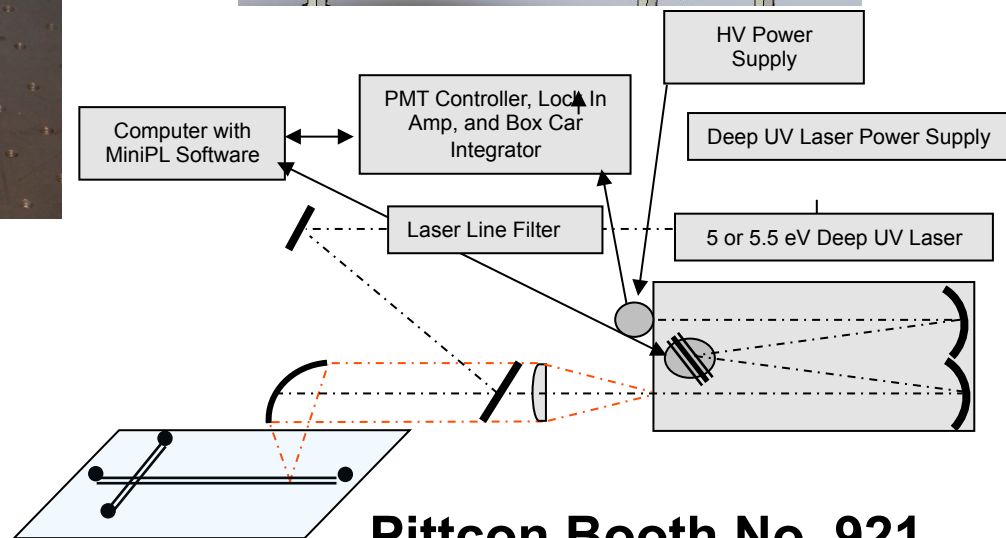
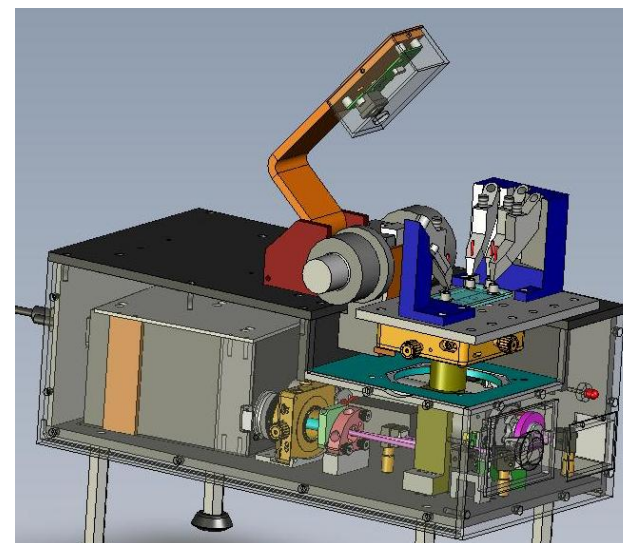
**224 nm Laser Detector S/N=2060
+ dramatically reduced baseline**

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Recent LOD Results with 224nm HeAg Laser

Material		
Neurotransmitters	Lepanis/Nov 06	
Serotonin (5-HT)	75am/40nM	
Dopamine (DA)	300am/40nM	
Norepinephrine(NA)	330am/44nM	
Octopamine (OA)	85am/11nM	
Aromatic Amino Acids	DeVandiere/Jan 08	Bonnin/Nov 06
Tryptophan	2fm/2nM	0.71 pm/35 nM
Tyrosine		0.8 pm/39 nM
Phenylalanine		360 pm/182 uM

DUV LINF MCE Detector

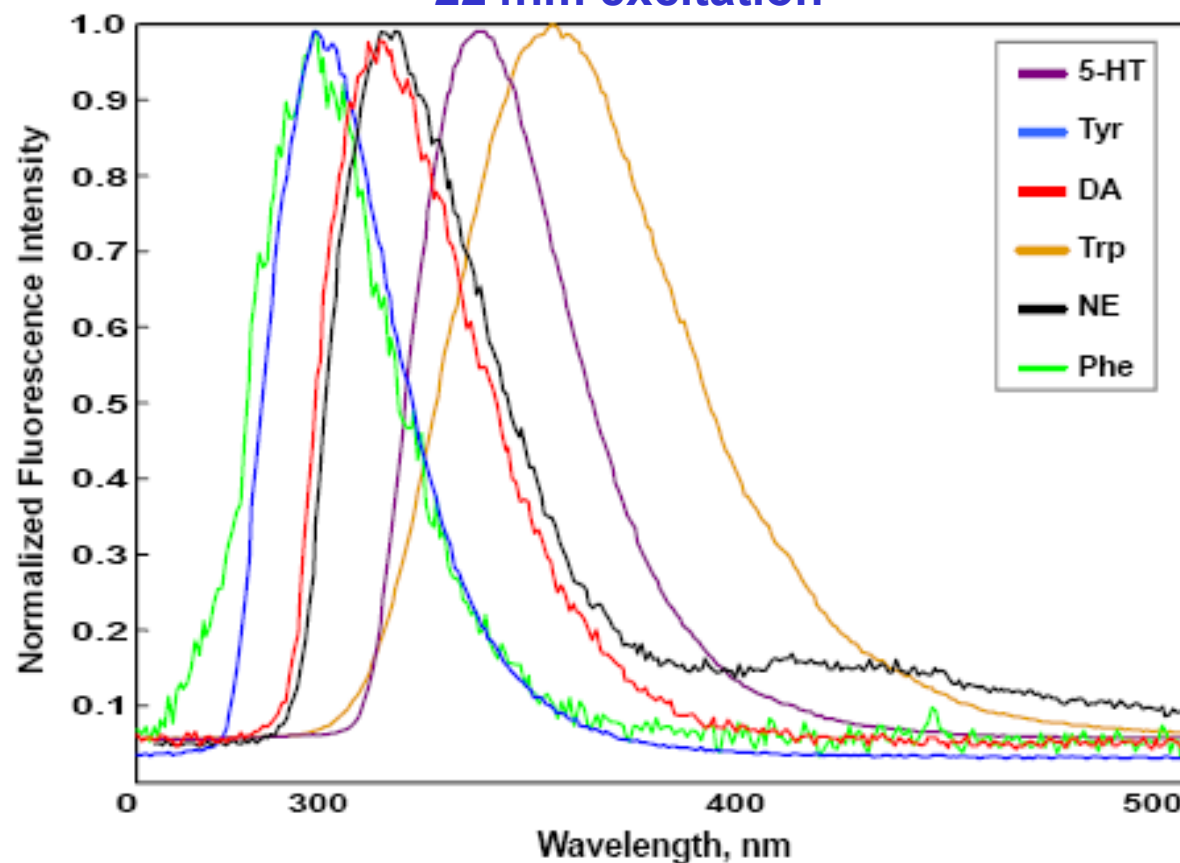


Simultaneous Multi-Band LINF Scanner for Slab-Gel Electrophoresis

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Emission of Biomolecules

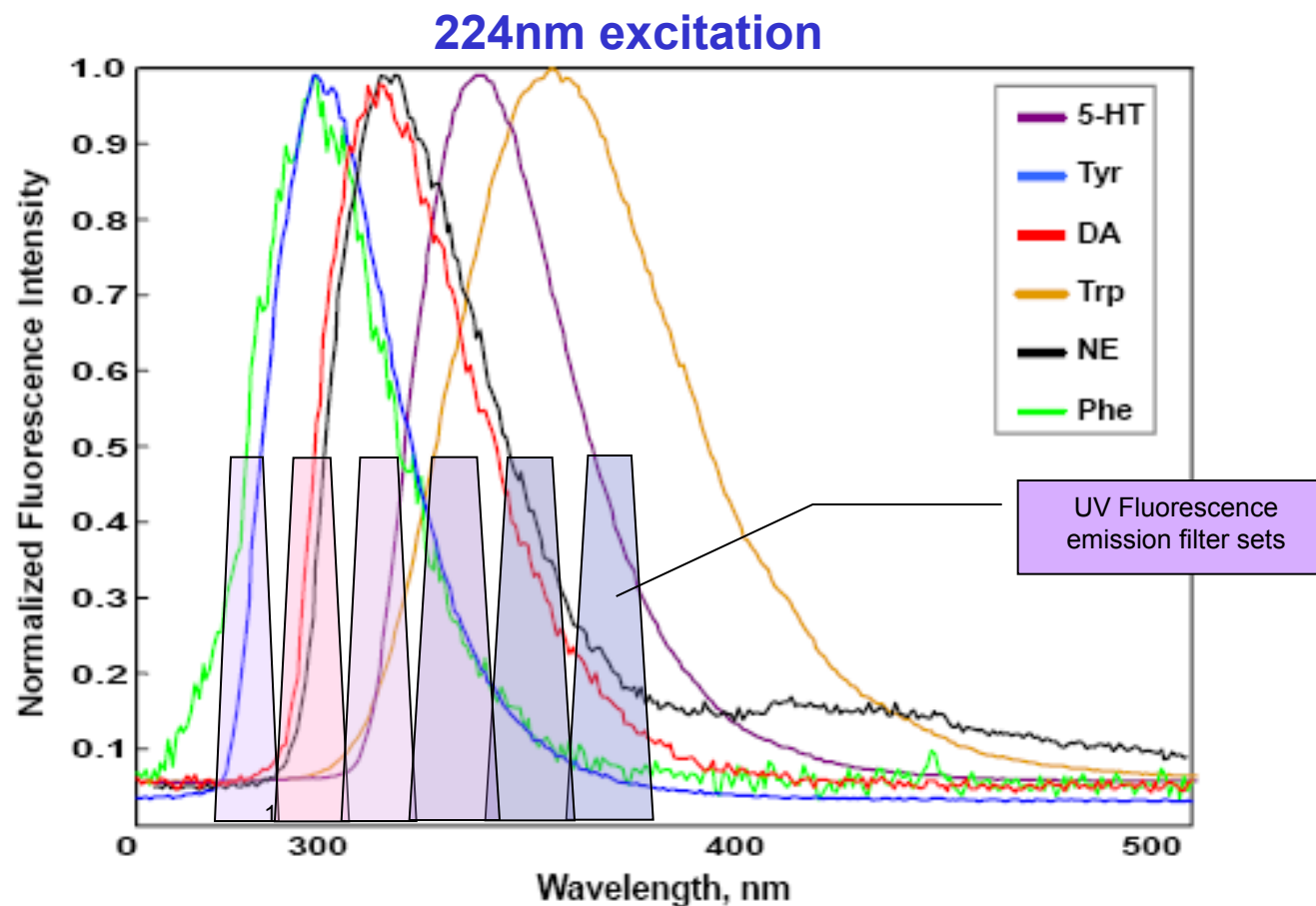
224nm excitation



Lepainis, T., C. Scanlan, S. Rubakhin, and J. Sweedler, "A multichannel native fluorescence detection systems for capillary electrophoretic analysis of neurotransmitters in single neurons", Anal. Bioanal. Chem, Springer Verlag, 10 August 2006

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LINF Marker Bands for Biomolecules



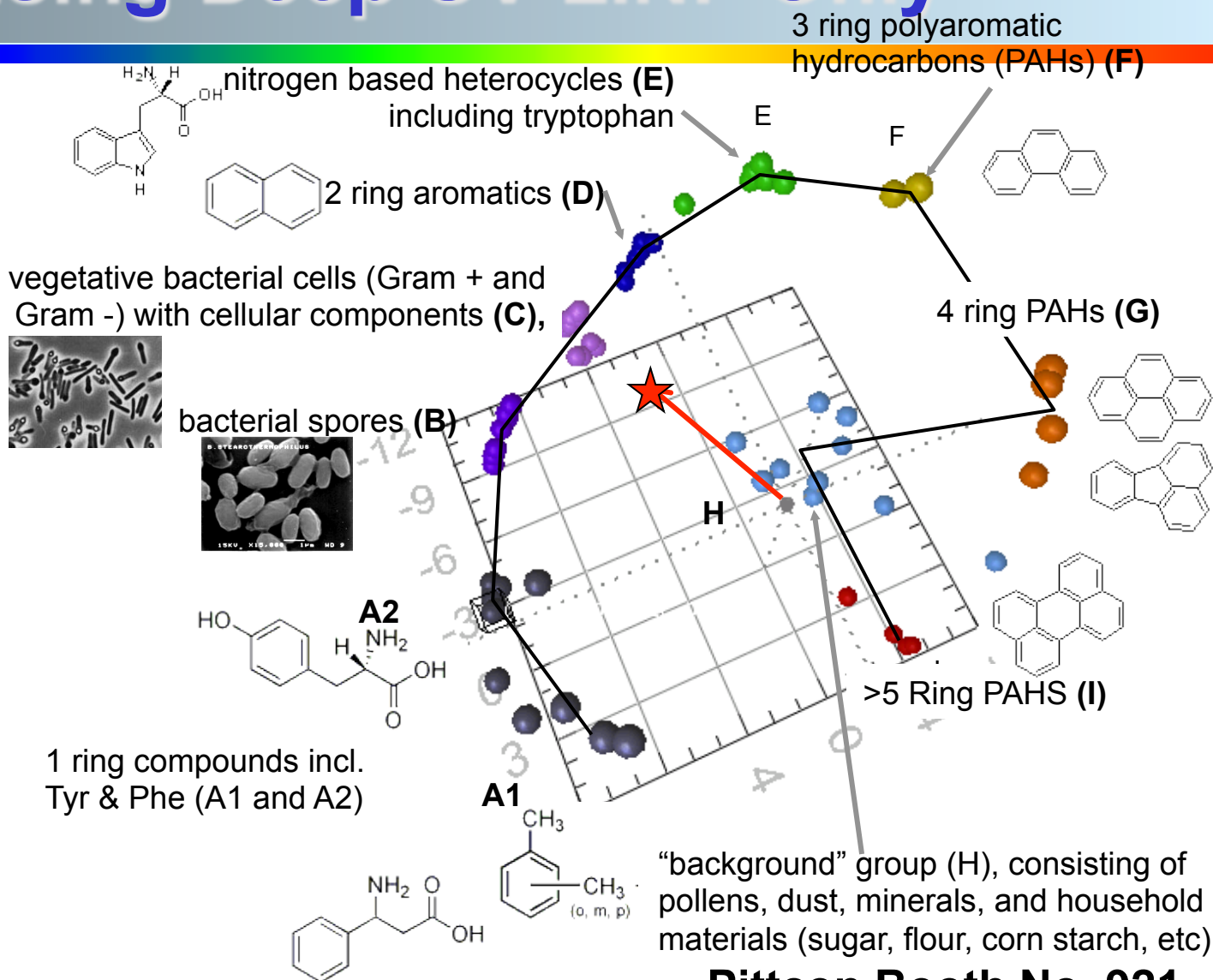
Lepainis, T., C. Scanlan, S. Rubakhin, and J. Sweedler, "A multichannel native fluorescence detection systems for capillary electrophoretic analysis of neurotransmitters in single neurons", Anal. Bioanal. Chem, Springer Verlag, 10 August 2006

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Chemical Differentiability using Deep UV LINF Only

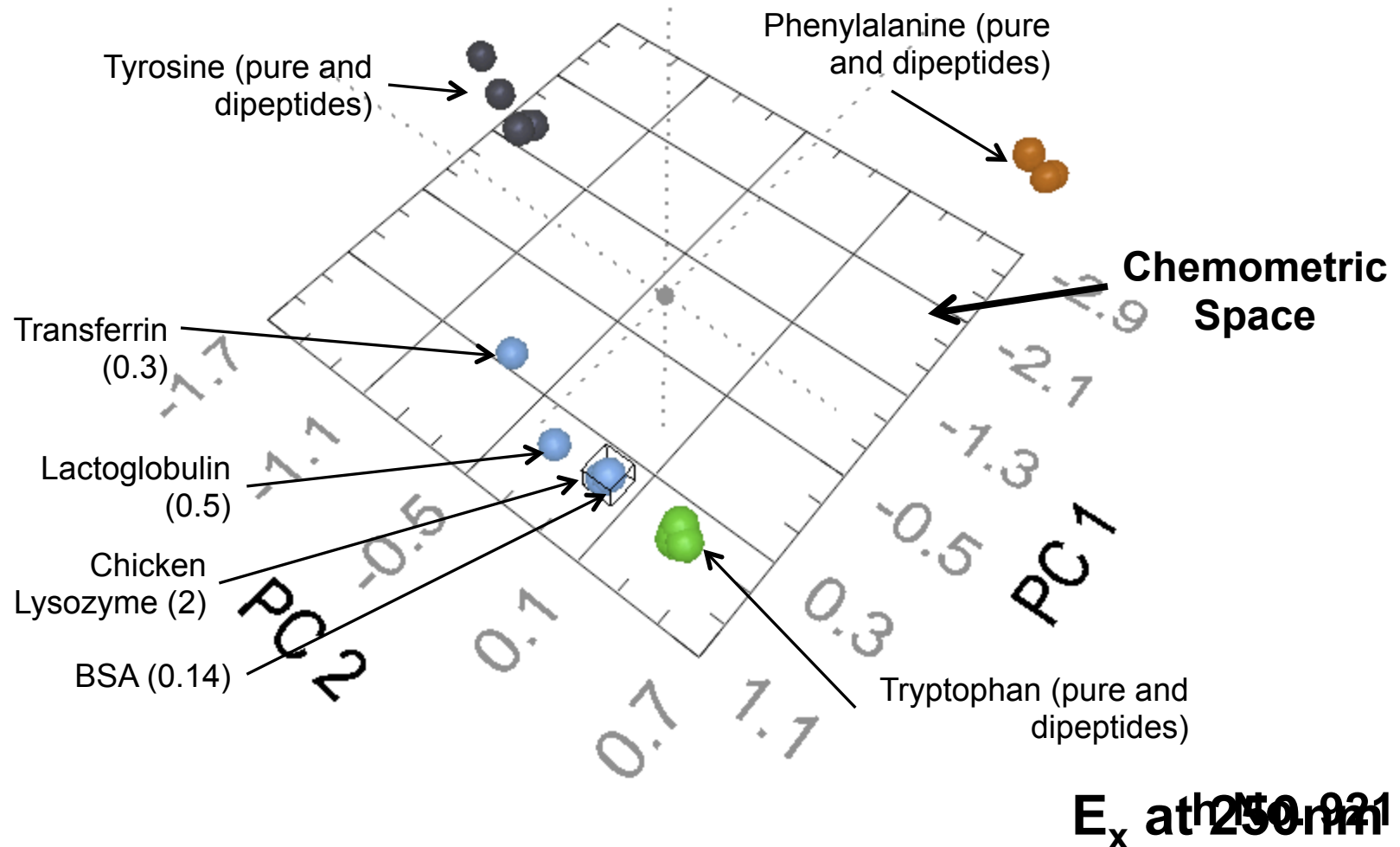


A single laser pulse determines the location of an unknown substance in this chemometric space

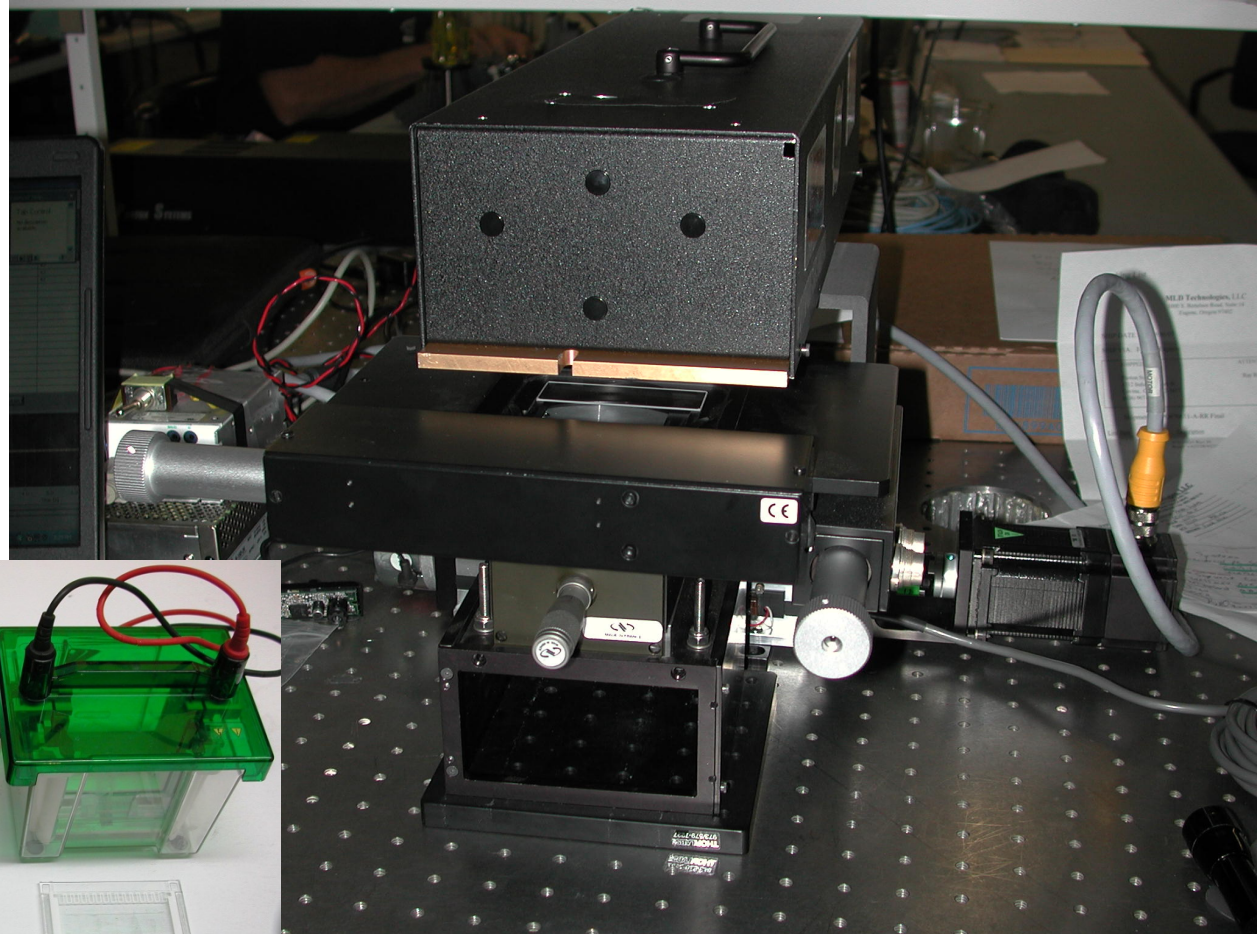


Native Fluorescence Protein Differentiation

Differentiability of native proteins is primarily based on the ratio of Tyrosine:Trptophan (Y:W) residues and the location of the Tryptophan in the protein structure.



DUV LINF Slab-Gel Electrophoresis Scanner



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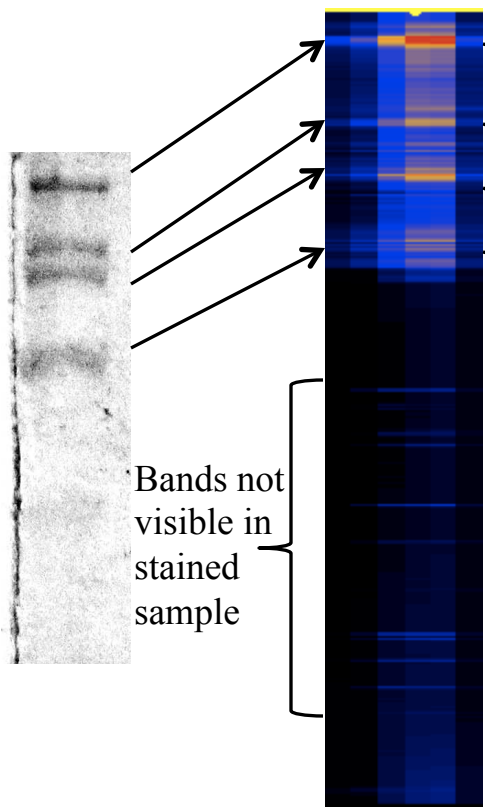
LINF Detection & Classification of Proteins on Gel Plane Electrophoretic Separation



Loaded: 1 μ g Protein/Band

224nm UV-LINF

(run prior to Commassie Staining
Shown on Left)

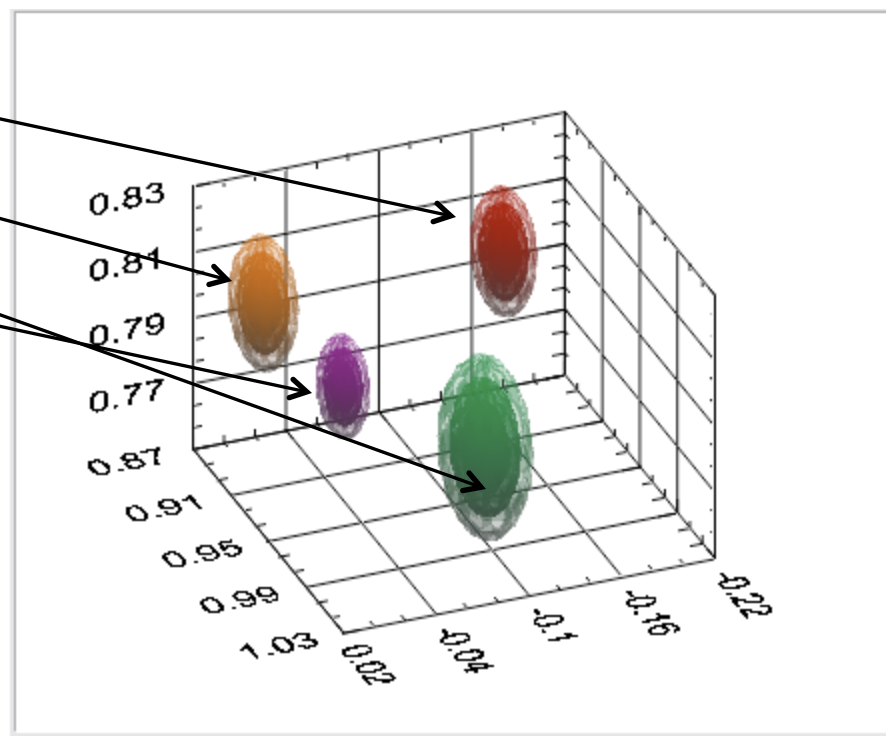


Bands not
visible in
stained
sample

Fluorescence Bands: 280 300 320 340 360 380

Chemometrics of UV-LINF

Myosin, β -Galactosidase, Phosphorylase b, BSA

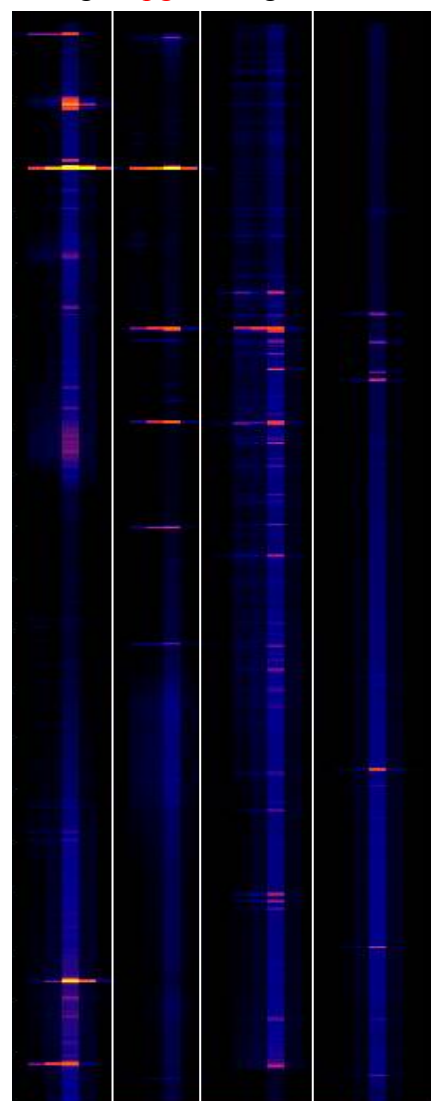


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LINF Detection & Classification of Proteins on Slab-Gel Electrophoretic Separation



CSA 10ng CSA/CL 10pg CL 10ng SB



LOD < 1 femtomole for LINF detection of proteins in gel plane electrophoresis - No tags

1 Fluorescence Intensity Map for Chicken Albumin - CSA (10ng), a Mixture of Chicken Albumin and Chicken Lysozyme - CSA/CL (10pg), Chicken Lysozyme - CL (10ng) and Sample Buffer (SB).

Each lane is comprised of 6 fluorescence channels centered at 280, 300, 320, 340, 360, and 400nm.

The colors depict fluorescence intensity where black → blue → purple → red → yellow is the order of increasing photon counts. The peak (band 1-CSA) is ~583k photons in a single 100us laser pulse. The background ranges from 20k photon for the mixture and SB and ~50k photons for the nanogram samples of CSA and CL.

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A horizontal bar with a color gradient from black on the left to red on the right, passing through blue, green, and yellow. A black arrow points to the left from the black end of the bar.

Enabling Technology:

- Deep UV Lasers**
- Large dynamic range detectors**
- Chemometric and operating software**

Deep UV Lasers < 300nm

Present Lasers

- ❑ *Freq. Doubled Ion (Ar & Kr)
(Ar-229, 238, 244, 248, 257, 264nm)
(Kr-206nm)*
- ❑ *Excimer (ArF @193nm, KrF @ 248nm)*
- ❑ *Freq. Doubled XeCl (308nm) pumped
dye laser (208->250nm)*
- ❑ *Freq. Doubled 3rd & 4th Harmonic
Nd:YAG pumped Ti-sapphire (IndigoS-
193-225nm)*
- ❑ *3rd, 4th, or 5th Harmonic Nd:YAG (532,
355, 266, 213nm) with or w/o Raman
shifters*
- ❑ *Freq. Doubled 337nm Nitrogen
pumped dye (225nm to >250nm)*

New Lasers

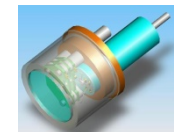
- ❑ *Transverse Excited Hollow
Cathode*
- ❑ *224.3 nm (HeAg)*
- ❑ *248.6 nm (NeCu)*



Future Sources

*Composition tunable E-beam
pumped external cavity wide
bandgap semiconductor (AlGaN)*

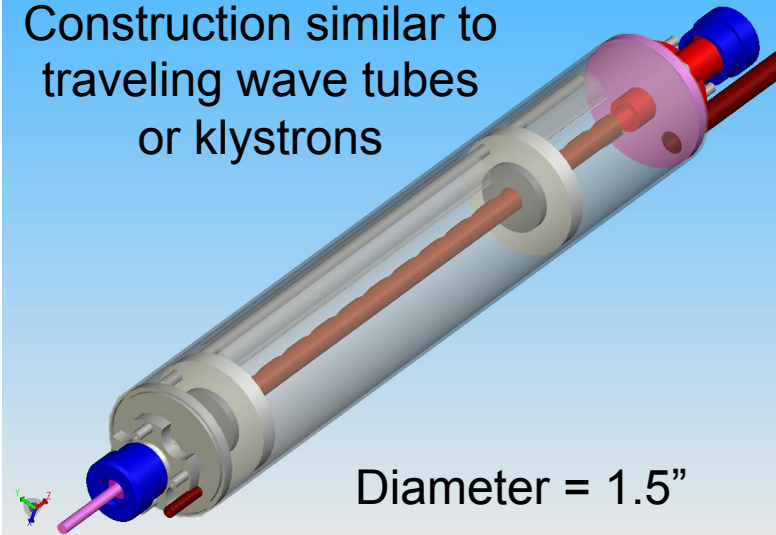
200nm to 365nm



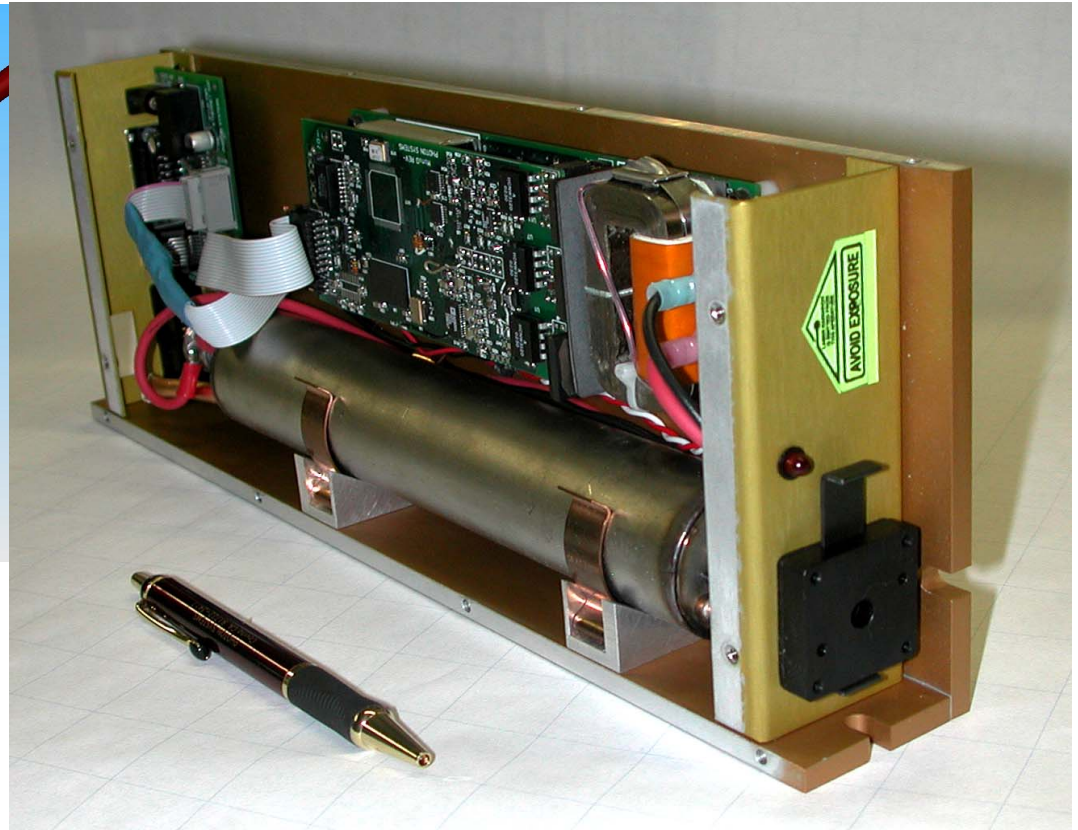
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224nm HeAg or 248nm NeCu Lasers

Construction similar to
traveling wave tubes
or klystrons



Diameter = 1.5"

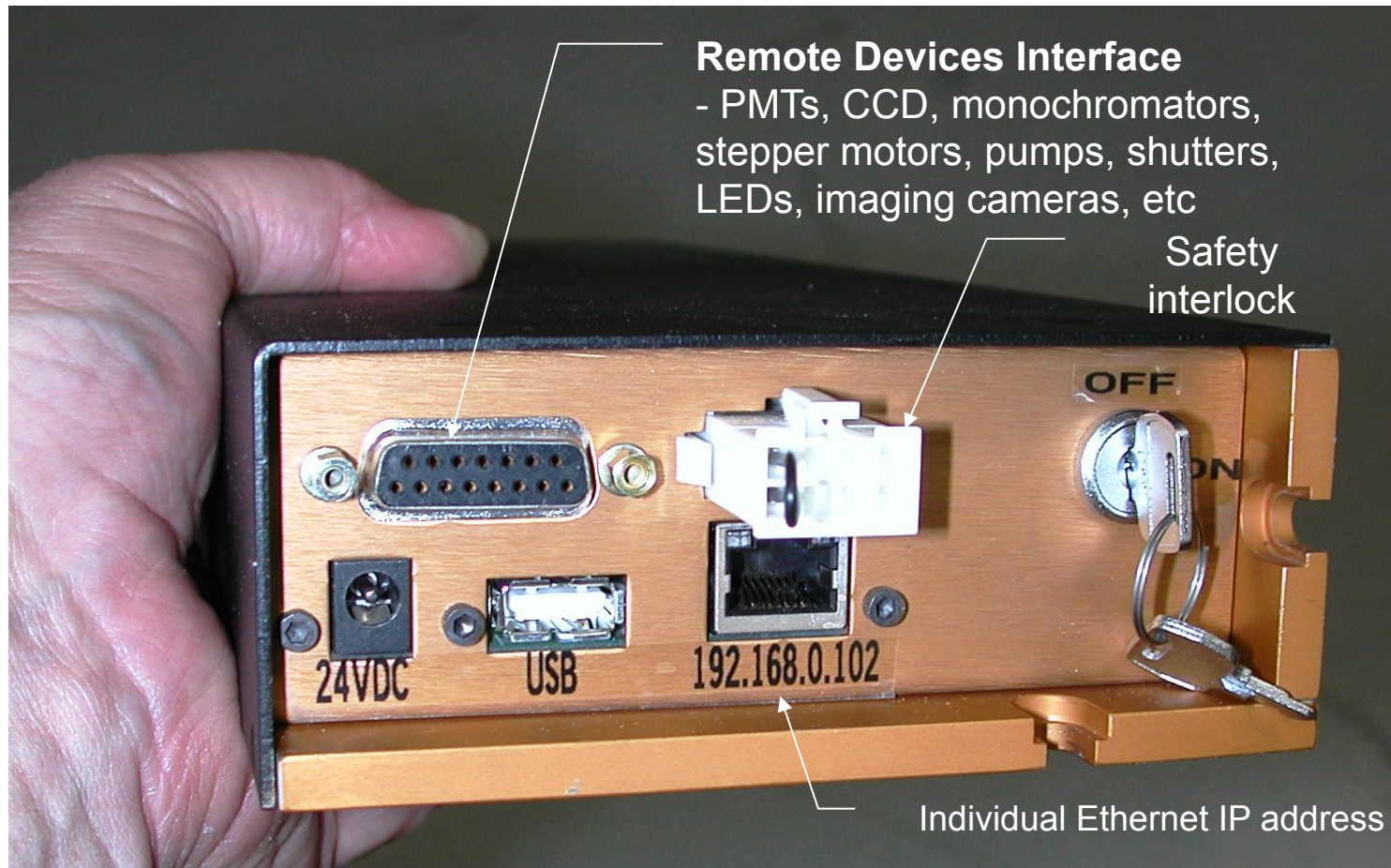


U.S. Patents: 6,287,869; 6,693,994
+ applications

- Built-in laser power/energy meter
- digital oscilloscope for laser pwr & drive I
- digital control of PRF, PW, amplitude

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DUV Laser Interface

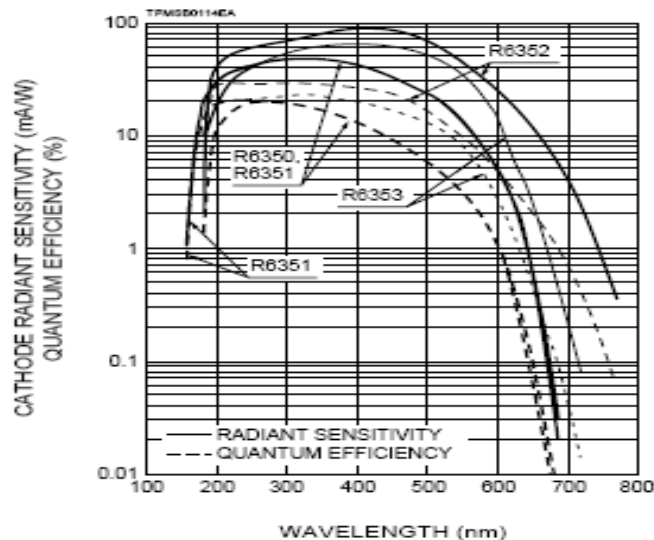
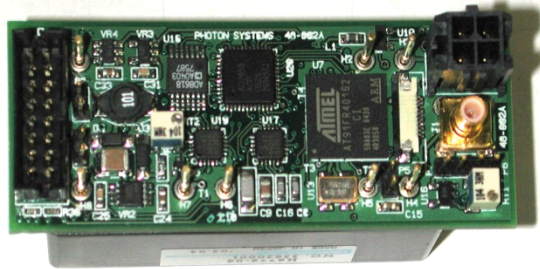


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Multi-band Deep UV Detectors

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PMT with Gated Boxcar Integrator



- ❑ **10 decades of linear detection range** (4 decades of calibrated capacitors and 6 decades of calibrated PMT gain)
- ❑ **Synchronizes detection** with deep UV laser soft pulse.
- ❑ **Digitally selectable start/finish** signal integration
- ❑ **Integrates PMT-generated photoelectrons** into AGC computer selected capacitors of 47pf, 470pf, & 4700pf
- ❑ **Digitizes capacitor charge** into detection “counts” at the end of integration period.
- ❑ **Computes incident photons**, independent of gain and capacitor setting, based on on-board calibrated capacitance and look-up-table of absolute PMT gain versus PMT voltage
- ❑ **32 bit, 75 Mips processor with** 2M RAM and 256K flash
- ❑ **16 bit A/D** with 16 bit resolution
- ❑ **Automatic calibration** and test
- ❑ **USB or Ethernet** interface
- ❑ **LabView** control

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Conclusions

- Deep UV detection methods that combine both native and tagged fluorescence offer a the ability to detect a wide range of unknown compounds with am LODs and known compounds with zm LODs in the same separation
- Simultaneous multi-band detection enables classification of unknown compounds as an orthogonal method to retention time
- Multiple tags can be employed simultaneously together with native fluorescence to provide a broad range of capability in a single separation

Questions ?

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