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The new NanoDrop™ *micro-volume spectrometer for DNA analytics*

Erich Heiden
Area Manager EEMEA
Core UC; µ UV; NMR

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Agenda

- Nucleic Acid Workflow
- The NanoDrop concept
- NanoDrop Lite
- NanoDrop 8000
- NanoDrop One and Acclaro™ software
- NanoDrop for protein and cell growth
- NanoDrop in the workflow

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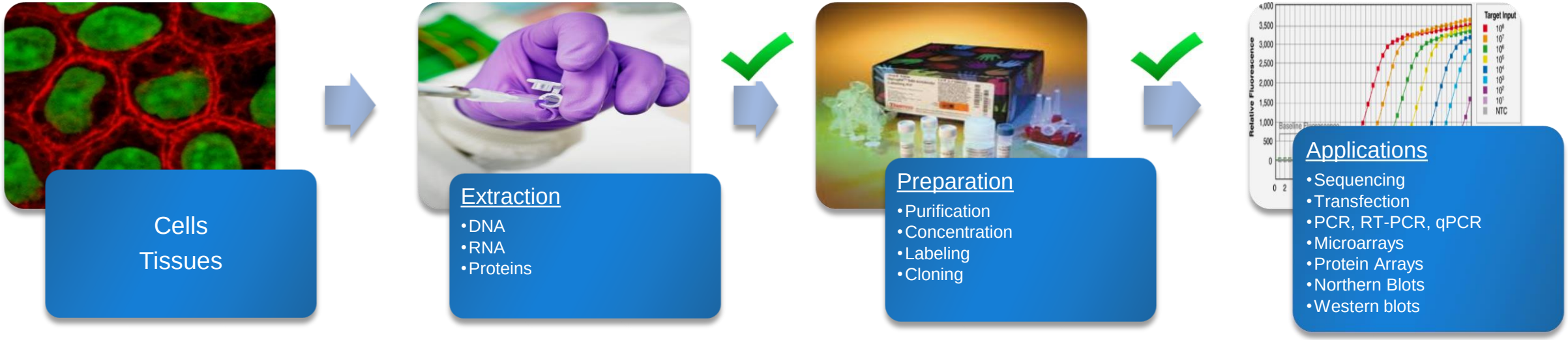
How do life scientists use NanoDrop to evaluate samples?



- Sample evaluation is a key step at multiple points during many life science workflows.
- Samples result from extraction and purification steps. Residual contaminants can be introduced during this process.
- Samples are used in complex and costly downstream experiments. The success of the experiment depends in part to the quality of the sample.

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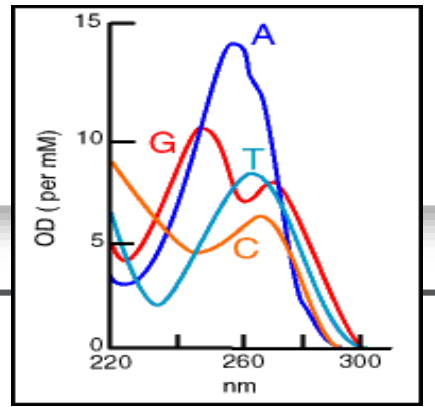
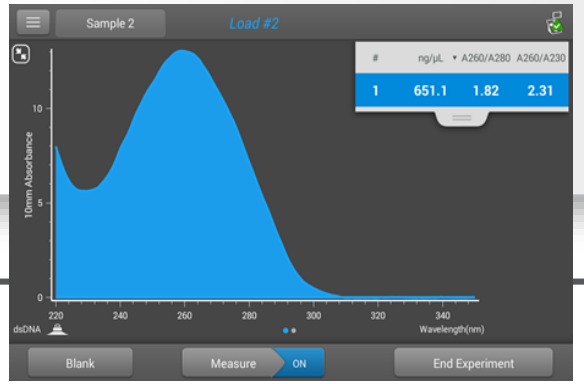
Nucleic Acid concentration from A_{260} (UV measurement)

- Nucleic Acids are made of five bases, A, T, G ,C and U
- A nucleic acid spectrum is a “blending” of all nucleotide spectra in the sample
  - *Beer- Lambert equation:* $\mathbf{A_{260} = c * \varepsilon * b \Rightarrow c = (A_{260} * \varepsilon) / b}$
- Everything that absorbs around 260 nm will contribute to A_{260} value: dsDNA, ssDNA, free oligos, dNTPs, RNA, proteins(absorb at 280nm), some contaminants
- A_{280} and Spectra deliver information about sample purity & quality





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Basic Contaminant Measurement: A_{260}/ A_{280}

- Contaminants (e.g. Protein , Phenol) are often found in NA samples
  - Extraction kits (Trizol)/ Phenol)
  - From a “dirty” sample
- Protein and Phenol absorb at 280nm, and can affect the A_{260} measurement

---

- **Good**:
  - A_{260}/A_{280} > 1.8 signals minimal amounts of protein and residual Phenol
- **Better**:
  - Add A_{260}/A_{230} between 1.8 – 2.2 to ensure no residual salts (e.g. Guanidine HCl)
  - Familiarity with the “right” spectrum shape can reveal excess salts and proteins
- **Best** (more on this later!)

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The Thermo Scientific NanoDrop pedestal technology

- ✓ Pipette 1 -2 µL of sample directly onto the measurement surface

- ✓ Close the arm and sample is drawn into a liquid column
- ✓ Optical fibers transmit light from a Xenon flash lamp through sample column; sample absorbance is measured

- ✓ After measurement, sample is simply wiped away
- ✓ No Carryover

By moving the pedestals, absorbance is measured at multiple path-lengths to achieve a very wide dynamic range (2-27,500ng/ µL for dsDNA). No dilutions needed

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The NanoDrop Product Line Today - Summary

Absorbance

- DNA/Protein quantitation
- Single sample
- Most basic

- DNA/Protein quantitation
- Other absorbance studies
- Single sample
- Full Spectra

- DNA/Protein quantitation
- Other absorbance studies
- Up to 8 samples at a time
- Full Spectra

Fluorescence

- FLR label quantitation
- Other fluorescence studies
- Single sample

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NanoDrop-Lite

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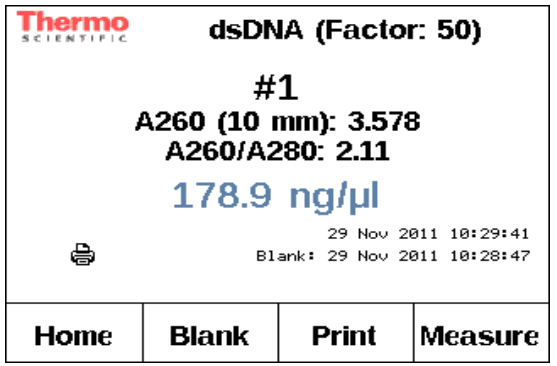
NanoDrop Lite UV-Vis Spectrophotometer

- **Compact design, fits on any bench, fits small budgets**
- **Designed for routine measurements of NA and proteins**



- Small color screen with built-in methods
- Uses LEDs to measures absorbance at 260 and 280 nm
  - Purified Nucleic Acid A260, A260/A280 purity ratio
  - Purified Proteins A260
- Fixed wavelengths, no spectral output
- Dynamic range 4 – 1,500 ng/µL dsDNA
- Last 500 measurements saved locally, save data to USB device
- Optional docking station style printer, prints cryogenic tube labels

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Why choose the ND-Lite?

For routine measurements of purified DNA, RNA and proteins (A_{260} & A_{280})

- Augment Full-spectrum NanoDrop products
- Value-priced “first NanoDrop” when moving to Micro-volume UV quant of DNA

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The NanoDrop Product Line

NanoDrop-8000

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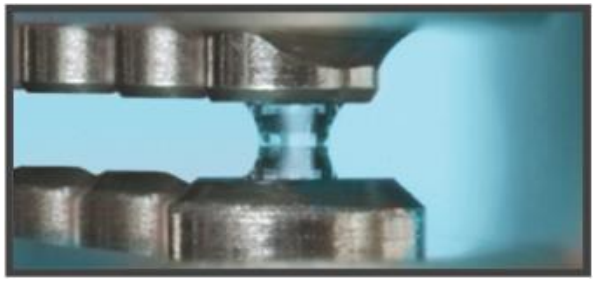
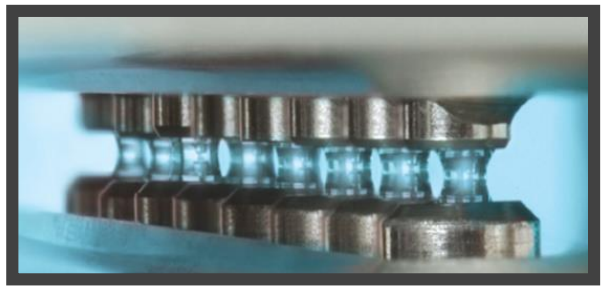
NanoDrop 8000 UV-Vis Spectrophotometer

- **Designed for labs with higher throughput needs**
  - **Biopharma, biobanking, CROs and CMOs**



- Full-spectrum UV-Vis absorbance (220-750nm)
- Dynamic range: 2 - 3,700 ng/mL (dsDNA)
  0.15 - 100mg/mL (BSA)
- Single Sample or 8-Sample modes
- 8 samples simultaneously in 20 seconds
- PC software for instrument control and data analysis



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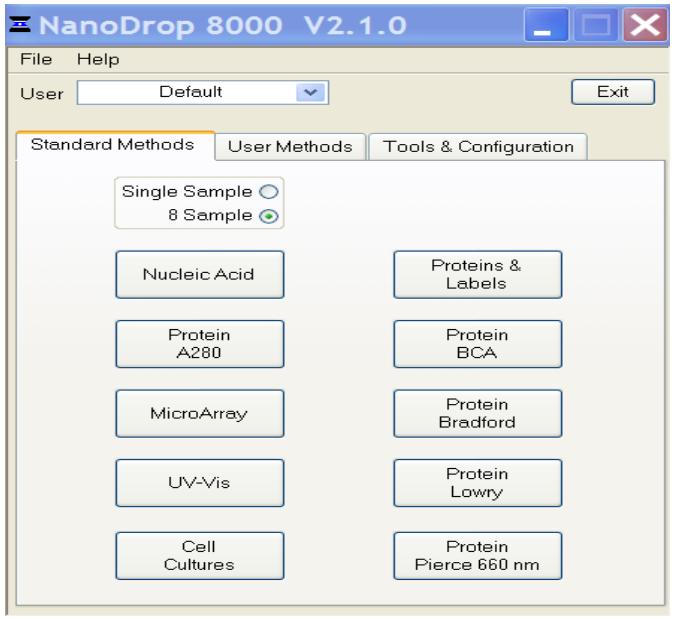
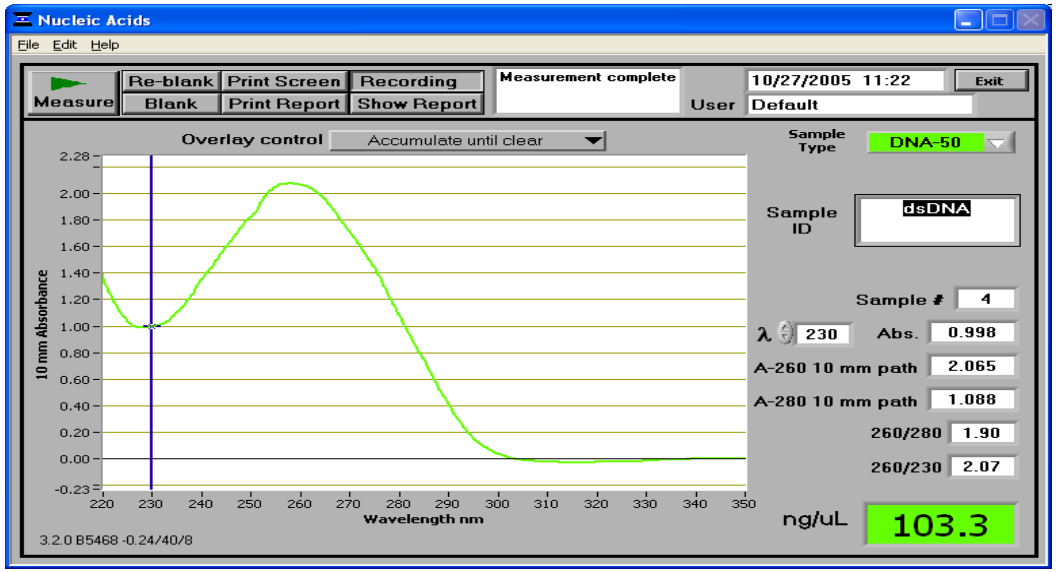
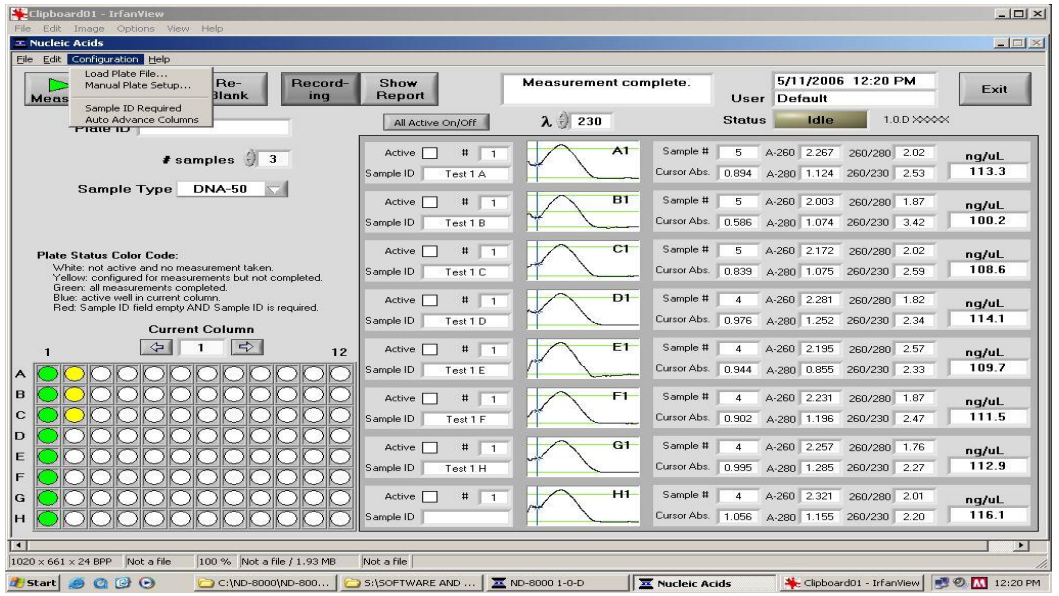
NanoDrop 8000 Software







- Designed for life science labs,
- Software offers preconfigured modules
  - Nucleic Acid, Proteins, Colorimetric Protein
  - Build your own methods, including Factor, Standards, and Oligos
- Measure labeled nucleic acids and proteins using MicroArray and Proteins & Labels methods

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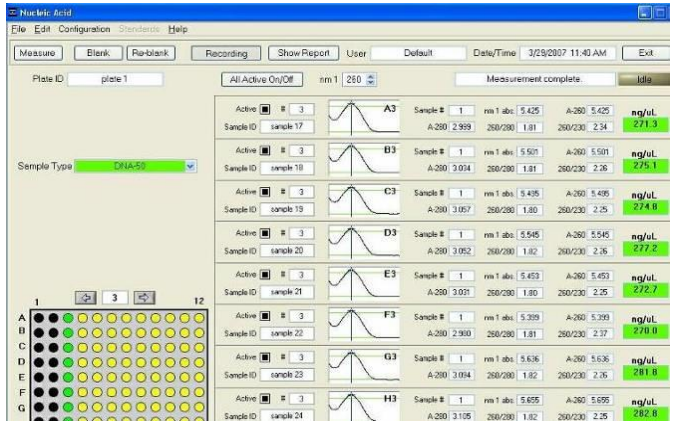
NanoDrop 8000: Sample Position Illuminator for 96 well plates

- This guide lights the respective samples to be assayed next.
- The Sample Position Illuminator correlates directly to the color-coded samples on the software screen.
- The Column Auto-advance feature identifies the next samples awaiting measurement.



- Can analyze 96 samples with full spectral data in <6min
-

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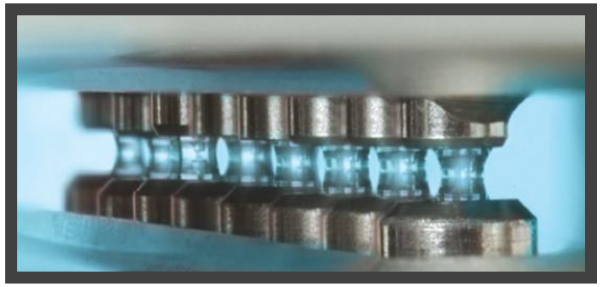
Why choose the NanoDrop-8000?

For medium-throughput measurements of small-volume DNA, RNA and proteins in plate-based workflows



- Higher-throughput with only 1-2µl
- Full-spectrum analysis
  - Know your Purity ratios at 230 nm and 280 nm
  - Measure NA, Proteins and labeled samples
- PC software is intuitive and flexible
  - User Methods
  - Report generation, save, and print

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The NanoDrop Product Line

NanoDrop-3300

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NanoDrop 3300 Fluorospectrometer

- Fluorescence measurements from 1-2 uL of sample

Strengths

- Minimal sample required, no waste
- Flexibility –can use multiple fluorescence kits/vendors
- “Out of the box” applications

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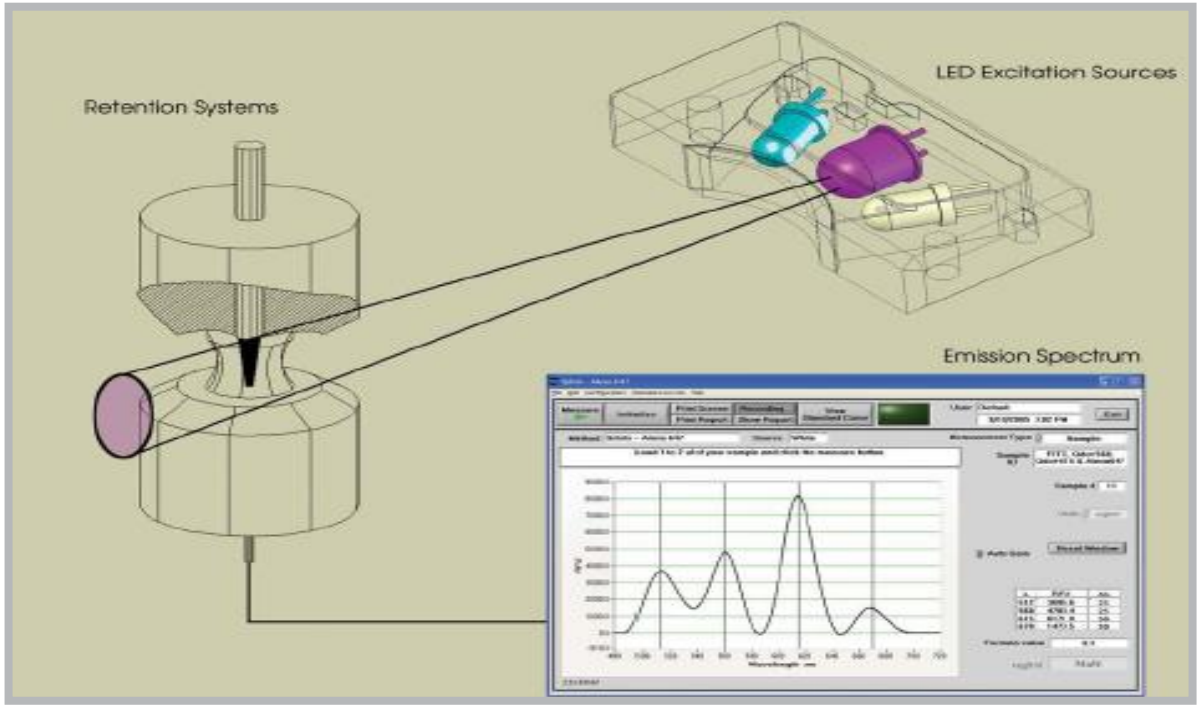
NanoDrop 3300 System Overview

- Fluorescence captured at 90˚ angle
- Excitation by one of three fixed LEDs
  - UV 365nm
  - blue 470nm
  - white 460nm-650nm
- CCD Detector records emission wavelengths between 400nm-750nm
- 1-2 μL sample
- **Communication, power via USB**
- Fluorescence signal output into a custom software interface



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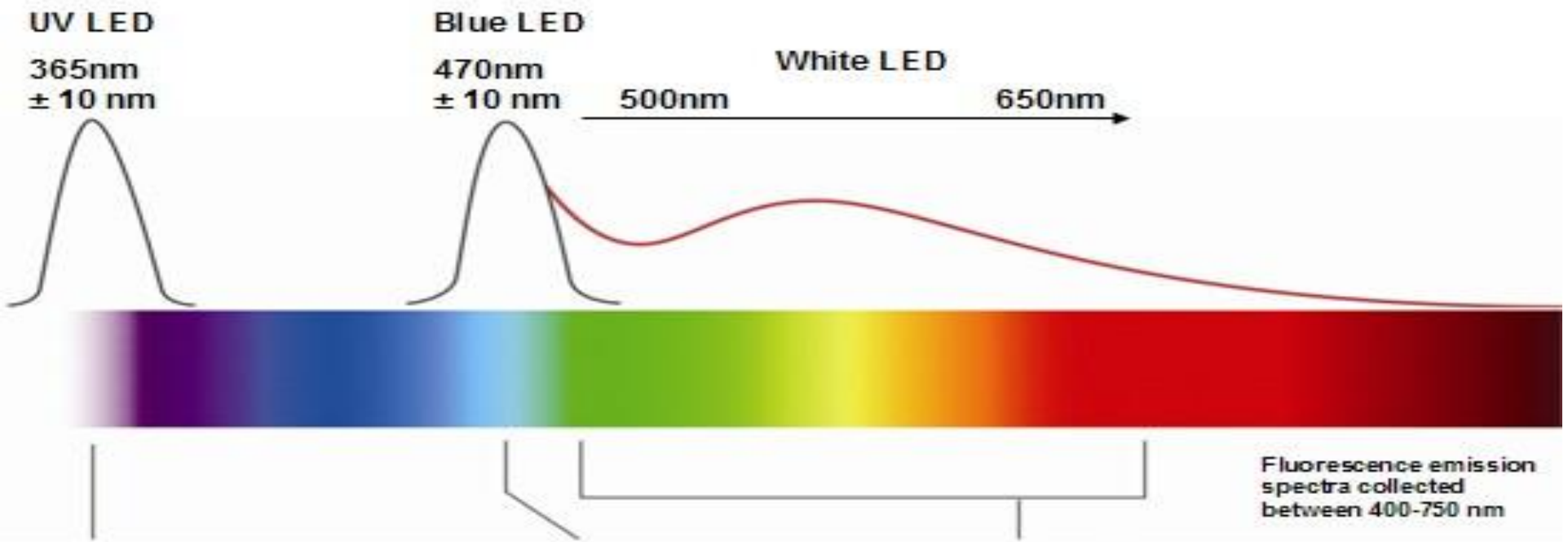
NanoDrop 3300 Excitation Source Overview



UV LED

- GFP wt Em509
- Quinine Sulfate Em450
- Hoechst 33258 Em450
- 4-MU Em450
- Q Dots Various

BLUE LED

- GFP wt Em509
- EGFP Em509
- FITC-FAM Em515
- Alexa 488 Em520
- PicoGreen Em525
- RiboGreen Em535
- Alexa 555 Em565
- B-Phycoerythrin Em575
- Q Dots Various

WHITE LED

- Cy3, Alexa555 Em565
- Alexa 568 Em600
- Cy5, Alexa647 Em667
- Sulforhodamine 101 Em600
- 5-CTMR Em570
- Q Dots Various
- TET Em535
- HEX Em555

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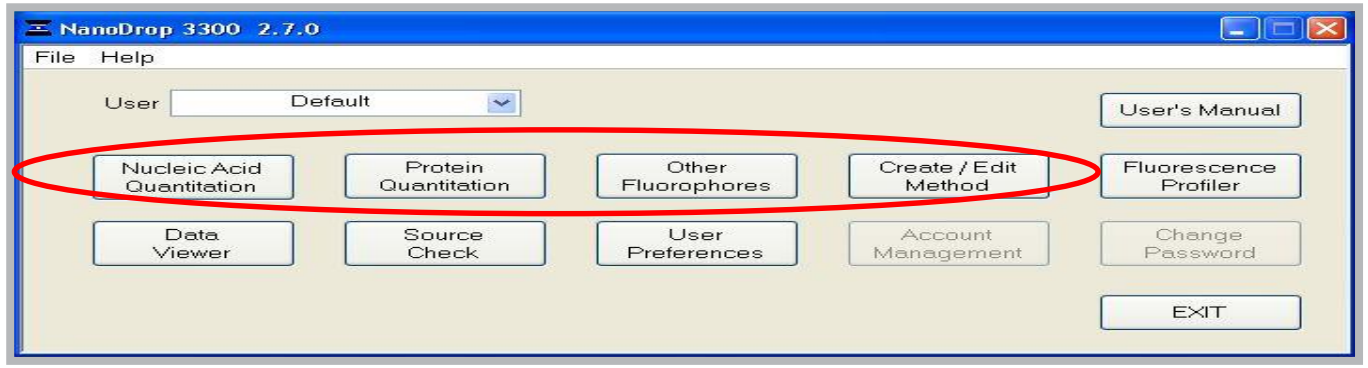
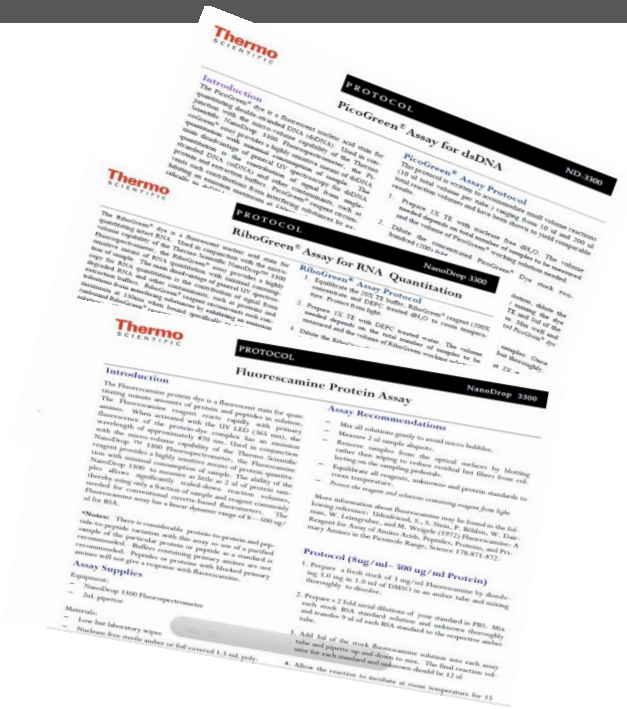
ND 3300 Applications –Protocols available





Nucleic Acid

- PicoGreen® assay dsDNA
- RiboGreen® assay RNA
- Hoechst 33258 dsDNA
- Sybr Green I
- Quant-ItTM DNA HS
- Quant-ItTM DNA BR

Proteins

- Quant-iTTM Protein assay
- FluoroProfile
- Fluoraldehyde OPA
- Fluorescamine

Other Samples

- 4-Methyl Umbelliferone
- Cy3/Alexa555
- Cy5/Alexa647
- Fluorescein-FITC-FAM
- Quinine Sulfate
- Dylights- 405,488,549,633,649, 680

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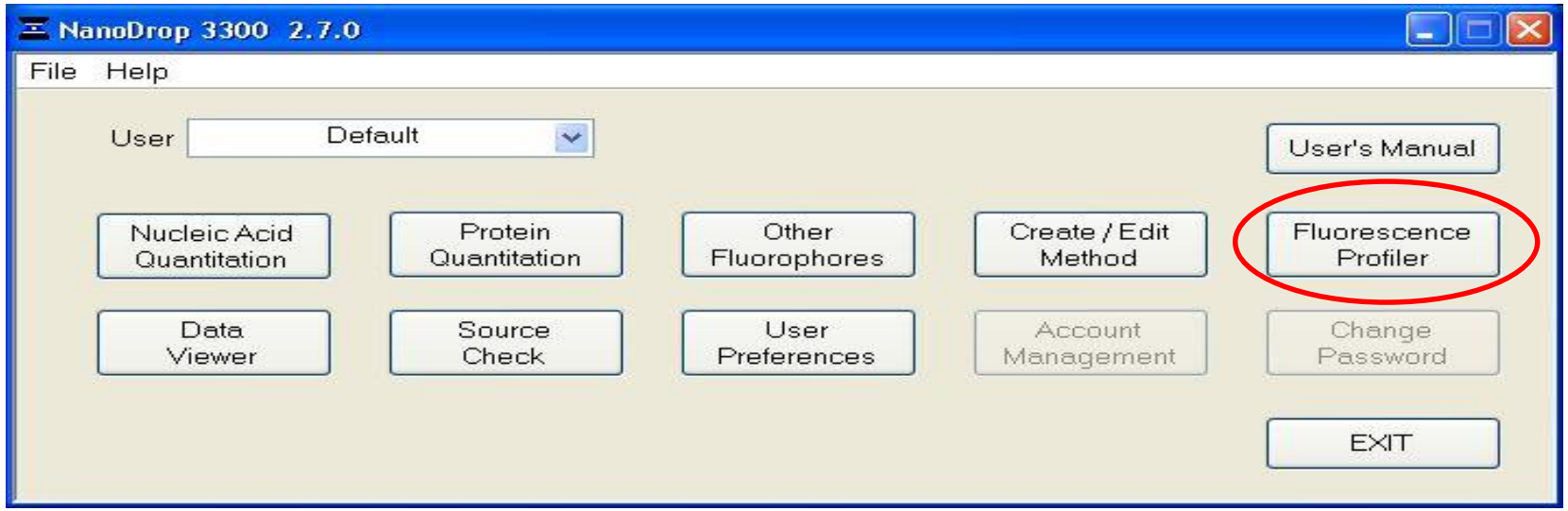
Fluorescence Profiler



- Method generation tool
  - Determines the fluorescence maxima
  - Suggests best excitation
  - Direct method saving into the method list
    - Able to save multiple methods from one measurement

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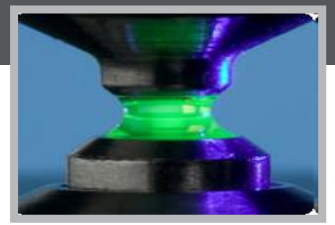
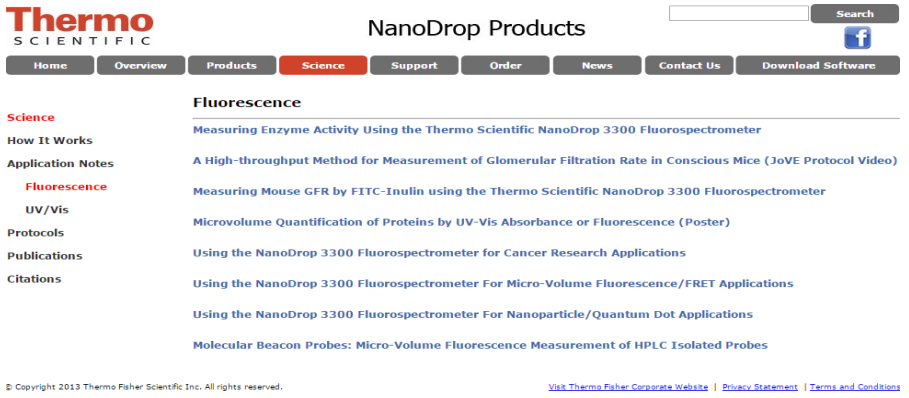
NanoDrop 3300 Applications



- **Nucleic acid quantitation**
- **Protein quantitation**
- **Molecules with inherent fluorescence (GFP)**
- **Fluorescent Probes**

- Forensics
- FRET
- Molecular Beacons
- Immunochemistry
- Microgenomics
- Histocompatability

- 4-Methyl Umbelliferone
- Cy3/Alexa555
- Cy5/Alexa647
- Fluorescein-FITC-FAM
- Quinine Sulfate
- Evitag-UV



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The NanoDrop Product Line

NEW , unique NanoDrop One/ One^{C}

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Introducing: NanoDrop One and NanoDrop One^{c}

- Will replace the current NanoDrop 2000/2000c
- Offer unique features that address user pain points
- Maintain clear leadership position of NanoDrop products demonstrating
  - Commitment to **innovation**
  - Commitment to **customer satisfaction** with compelling, relevant features

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The NanoDrop One Value Proposition

- **The NanoDrop promise**
  - Fast and easy answers from microvolumes
  - Now even better

- ***Acclaro*™ Sample Intelligence**
  - Contaminant identification using *data analysis* algorithms, supports downstream success
  - On-demand *embedded technical support* answers context-sensitive questions
  - Embedded camera with digital image analysis ensures *measurement integrity*

- **A high quality experience**
  - New modern design with high resolution touchscreen
  - Local control software with workflow-centric design
  - Enhanced connectivity, extended data management options

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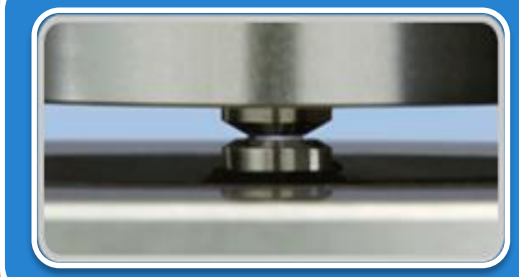
NanoDrop One: The NanoDrop promise enhanced

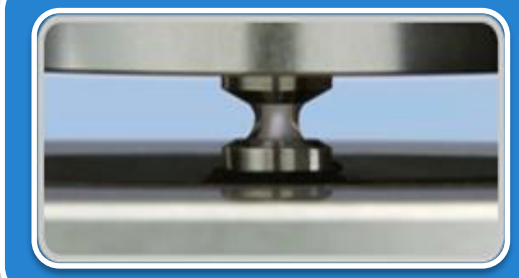
Fast and easy to use

- NanoDrop One - even *faster* with Auto-Measure
- NanoDrop One - even *easier* with local control and touch screen interface



Microvolume measurements

- No need for slides or cuvettes, pipette directly on pedestal
- No waste of precious sample, measurements with 1-2 µL



No need for dilutions

- NanoDrop One - five auto-range pathlengths 0.03-1.0 mm, more accurate for concentrated samples
- NanoDrop One - extended dynamic range 2-27,500 ng/µL dsDNA, *better accuracy*

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Five auto-adjust pathlengths

Beer-Lambert equation

$$A = c * \varepsilon * b$$

Shorter pathlengths (**b**) >> higher concentrations (**c**) can be measured

NanoDrop One offers five pathlengths, 0.03 -1.0 mm

- Higher dynamic range, no dilutions needed
- More accurate results for concentrated samples
- Can measure up to 400mg/mL IgG

NanoDrop 1000
2 pathlength

0.2 mm

max conc.=3,750 ng/μL dsDNA

NanoDrop 2000/c
4 pathlengths

0.05 mm

max conc.=15,000 ng/μL dsDNA

NanoDrop One/One^{c}
5 pathlenths

0.03 mm

max conc.=27,500 ng/μL dsDNA

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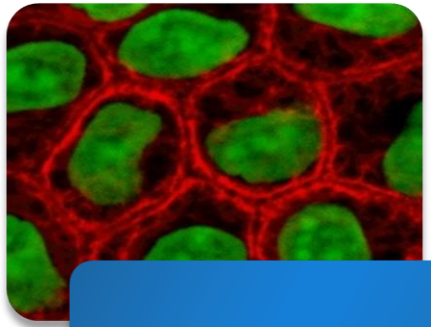
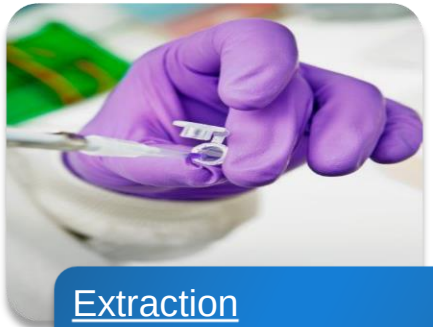
How do life scientists use UV-Vis to evaluate samples?

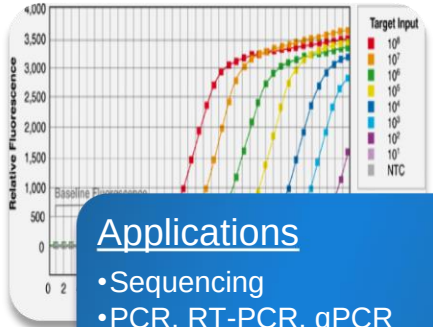


Cells
Tissues



Extraction
- DNA
- RNA
- Proteins

Preparation
- Purification
- Concentration
- Labeling
- Cloning



Applications
- Sequencing
- PCR, RT-PCR, qPCR
- Microarrays
- Protein Arrays
- Northern Blots
- Western blots

- Sample evaluation is a key step at multiple points during many life science workflows.
- Samples result from extraction and purification steps. Residual contaminants can be introduced during this process. Small yields, final sample is precious.
- Samples are used in complex and costly downstream experiments. The success of the experiment depends in part to the quality of the sample.

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Pain points in life-science sample evaluation

Instrument not designed for me

- UV-Vis spectrophotometers are not made for “my kind” of samples
- Require training, cleaning cuvettes, and manual calculations
- Time consuming

Unknown concentration

- How do I know I am within dynamic range?
- Dilutions introduce errors

Samples are precious

- I have to use half of my sample to measure its concentration
- I wish I could evaluate my samples more than once but I don’t have enough

Sample quality is critical

- How do I know if my sample is good enough to use in my next experiment?
- Contaminated or inaccurately quantified samples lead to experimental failures

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VoC indicates customers are concerned about sample quality

Bioinformatics survey

- 90% of customers look at spectra
- Customers look at purity ratios
- Customers concerned about contaminants in NA samples
-

Protein in DNA (RNA) samples	83%
Ethanol	72%
Phenol	57%
Guanidine	37%
DNA in protein	83%

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The NanoDrop One Value Proposition

- **The NanoDrop promise**
  - Fast and easy answers from microvolumes
  - Now even better
- ***Acclaro*™ Sample Intelligence**
  - Contaminant identification using *data analysis* algorithms, supports downstream success
  - On-demand *embedded technical support* answers context-sensitive questions
  - Embedded camera with digital image analysis ensures *measurement integrity*
- **A high quality experience**
  - New modern design with high resolution touchscreen
  - Local control software with workflow-centric design
  - Enhanced connectivity, extended data management options

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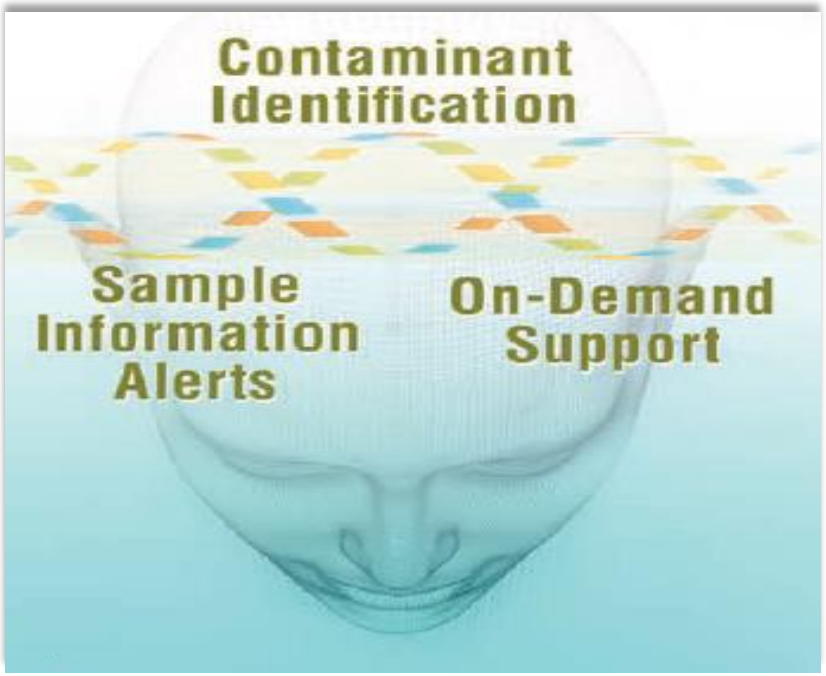
Acclaro Sample Intelligence Technology

- ***Identifies contaminants*** in sample using powerful data analysis algorithms
- ***Ensures measurement integrity*** with embedded sensor and digital image analysis that monitors for broken columns and bubbles
- ***Delivers on-demand*** technical support with information alerts and guided troubleshooting



Acclaro = "to make clear"

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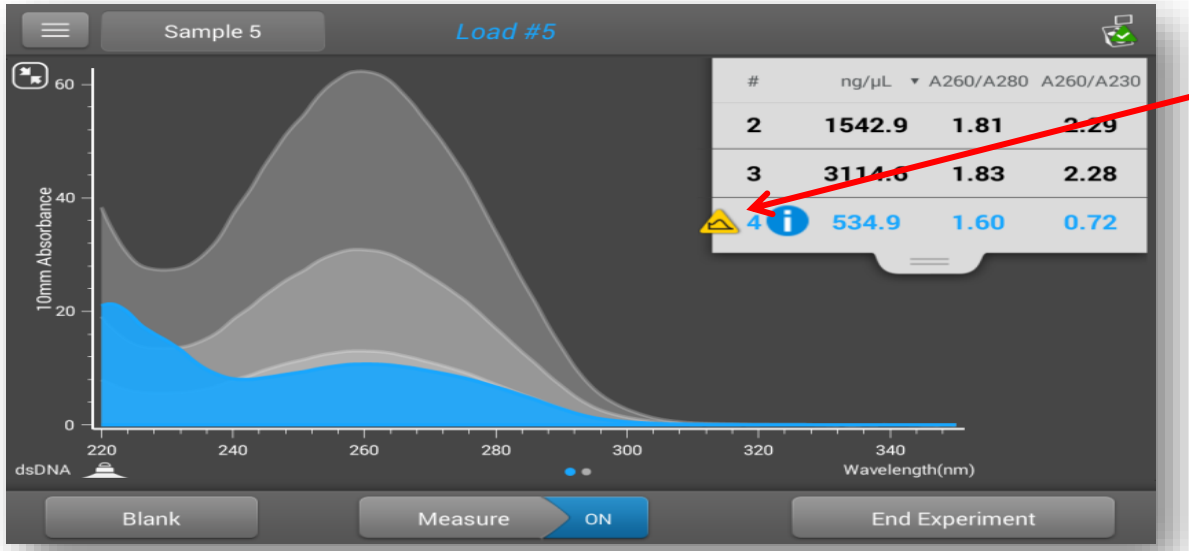
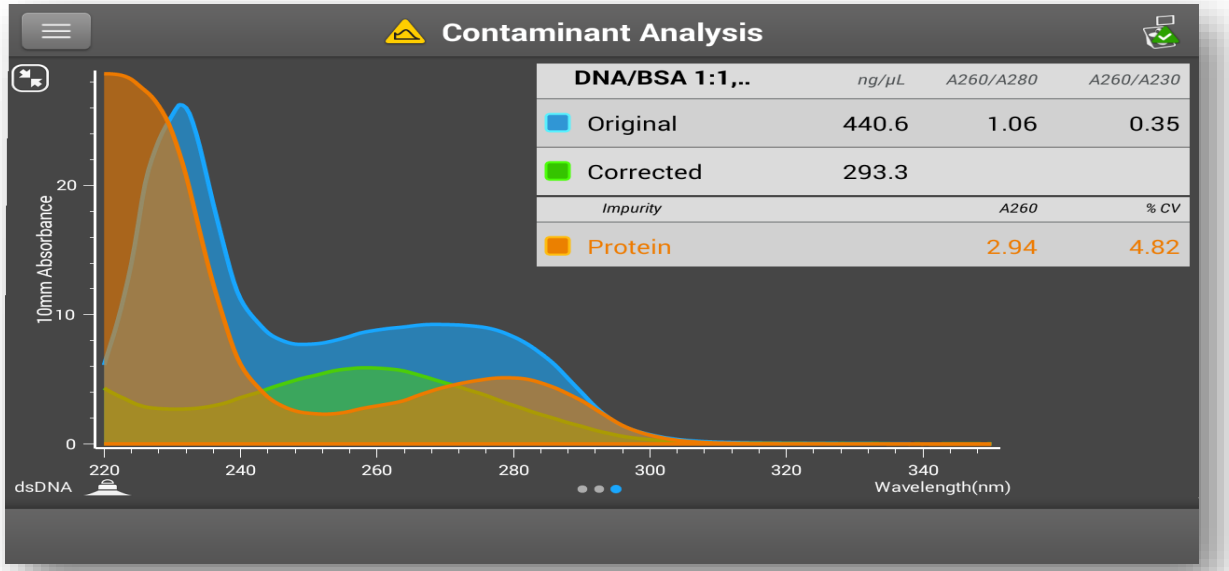
Contaminant identification



Acclaro flags dsDNA sample #4



Protein in the sample contributes to the A260 and inflates dsDNA concentration. Protein contribution is subtracted from the original value to give the corrected concentration.

Acclaro identifies:

Nucleic Acid contaminants:

- Protein, phenol, guanidine HCl, guanidinium SCN

Protein contaminants:

- Nucleic acids and phenol

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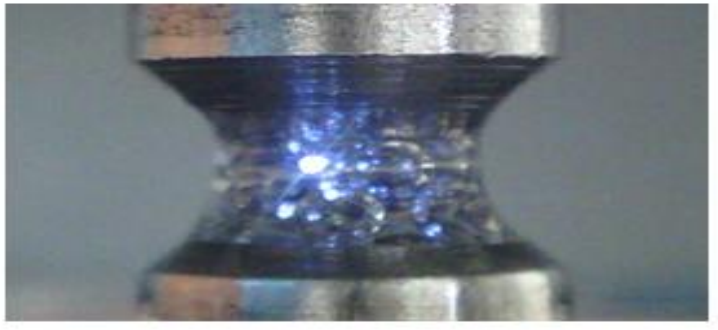
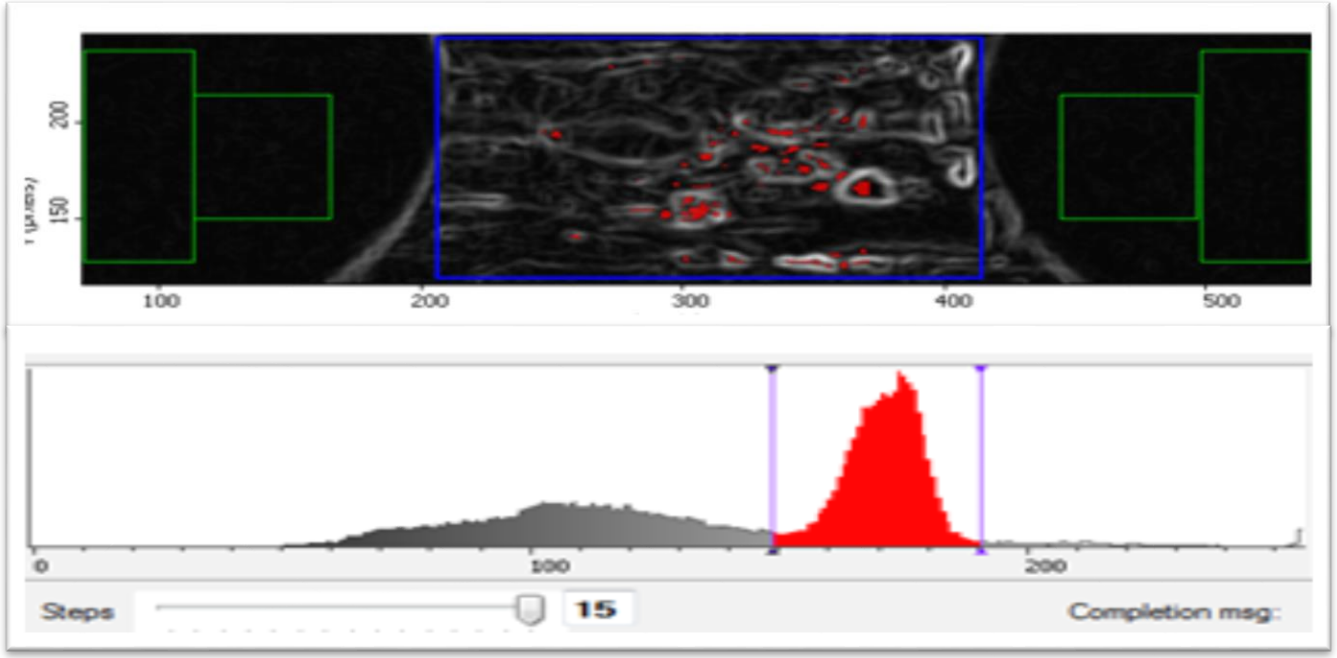
Ensure measurement Integrity

- Image of the sample column collected by embedded sensor is digitally analyzed
- Broken column:
  - Cancel measurement
- Bubbles in sample:
  - the air in bubbles diffracts light, the spectrum quality is degraded and the calculated concentration is inaccurate





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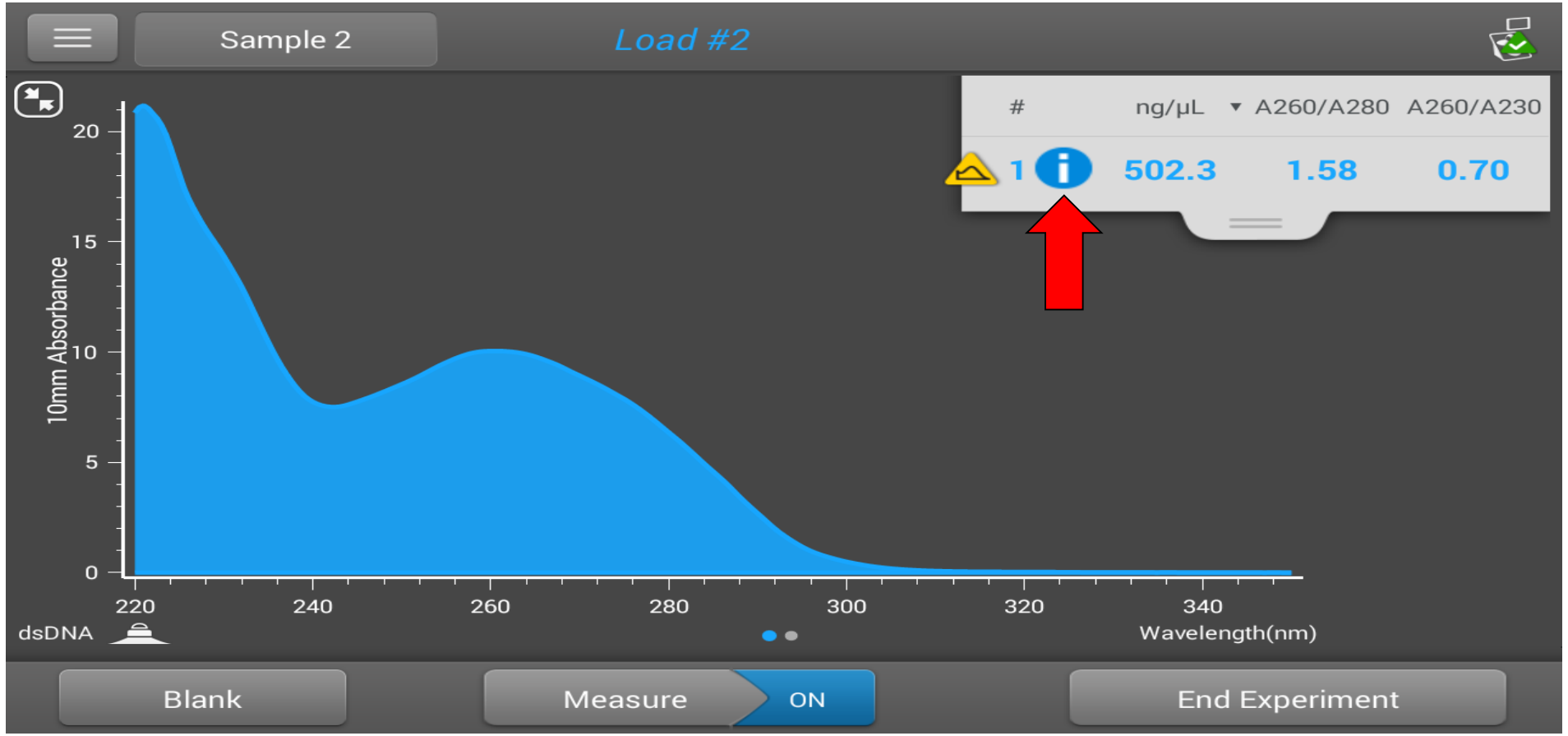
User Alert: Bubbles Detected



What do I do now???

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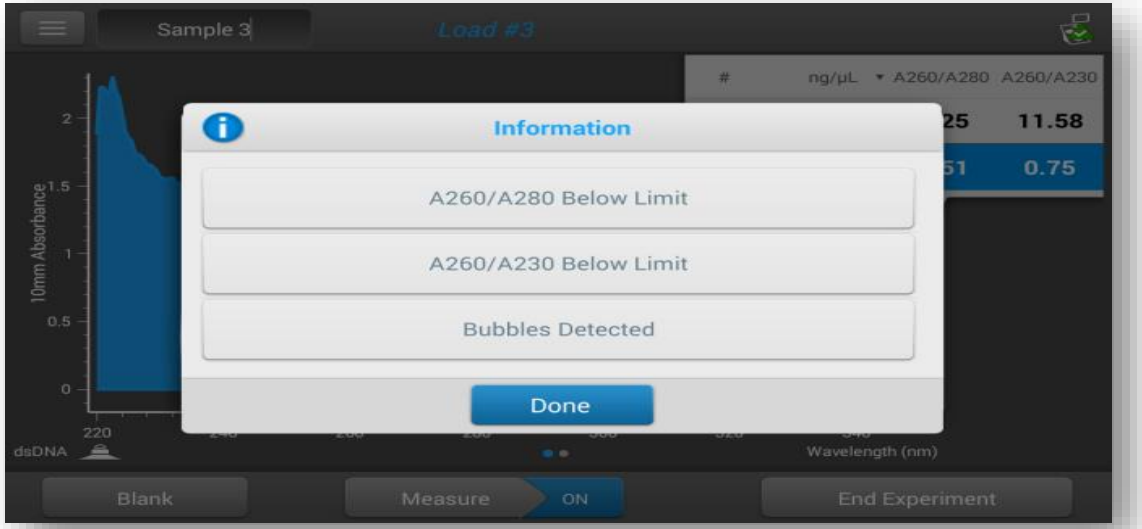
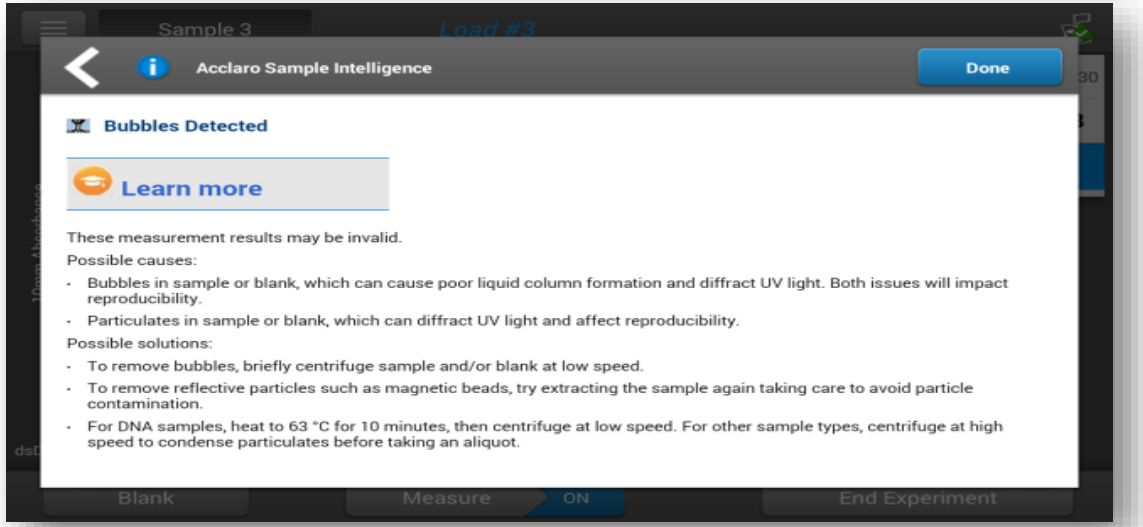
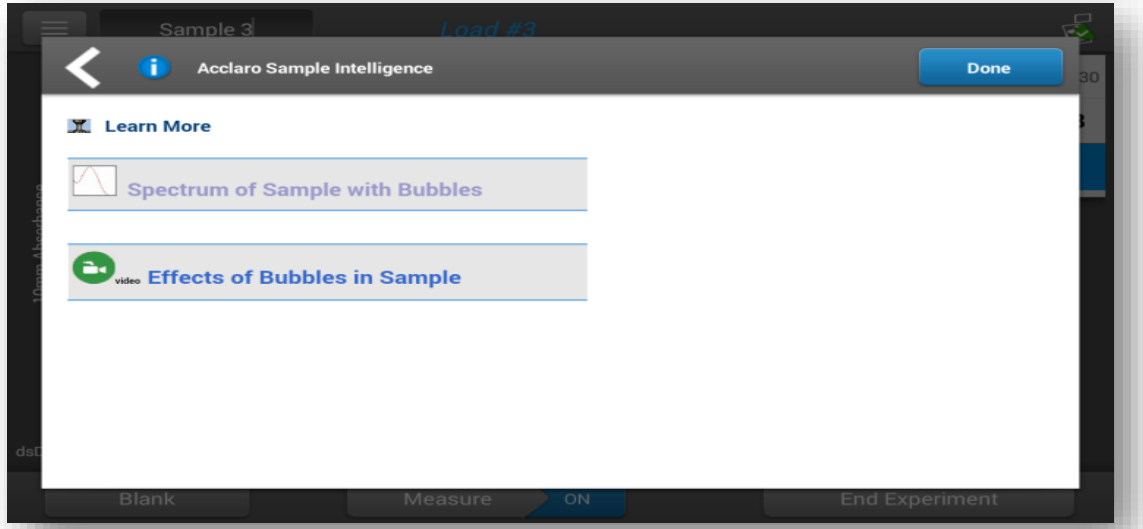
Learn more with Acclaro…

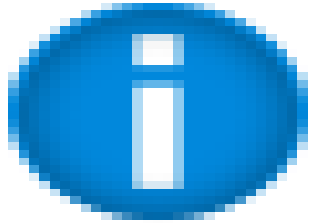






Information Alerts

- Based on the most common questions received by ND tech support
  - Educates user on the science with short video animations
  - Presents common causes of particular problem, recommends solutions
  - Guides through troubleshooting with support tools
- Deliver information on sample quality and provide guided troubleshooting
- Topics covered are:
  - Purity ratios: when A260/A280 or A260/A230 ratios are outside acceptable limits for a pure DNA or RNA sample
  - Purity ratios: when A260/A280 is outside acceptable limits for protein sample (A280)
  - Effects of dirty pedestals
  - Blanking issues
  - Baseline correction



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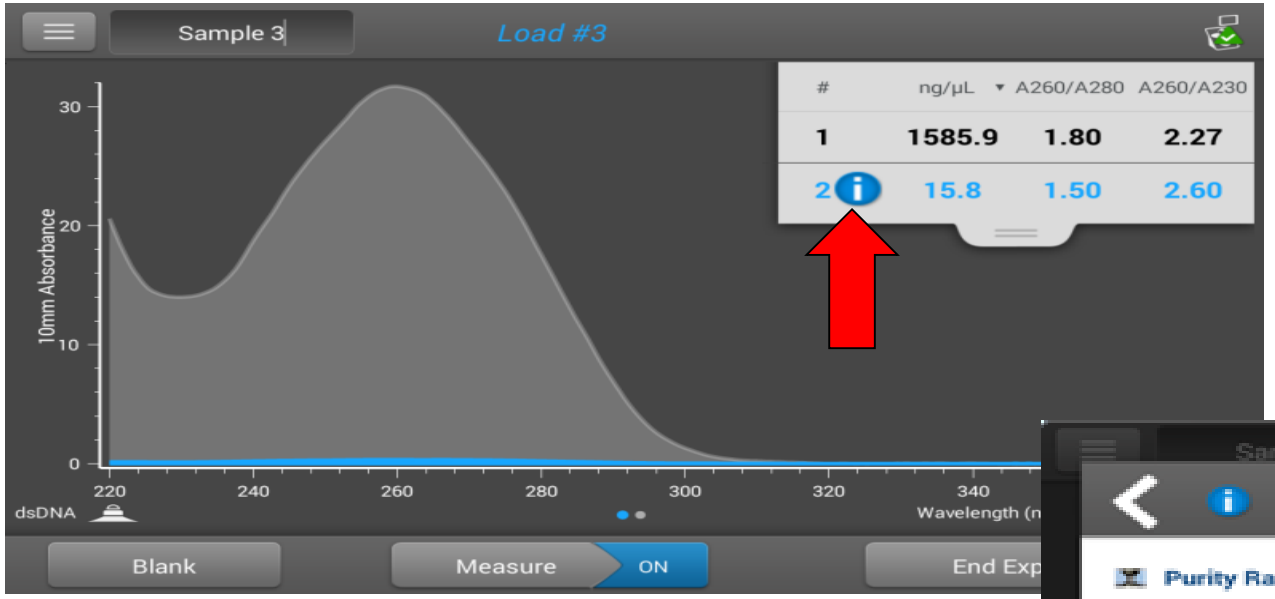
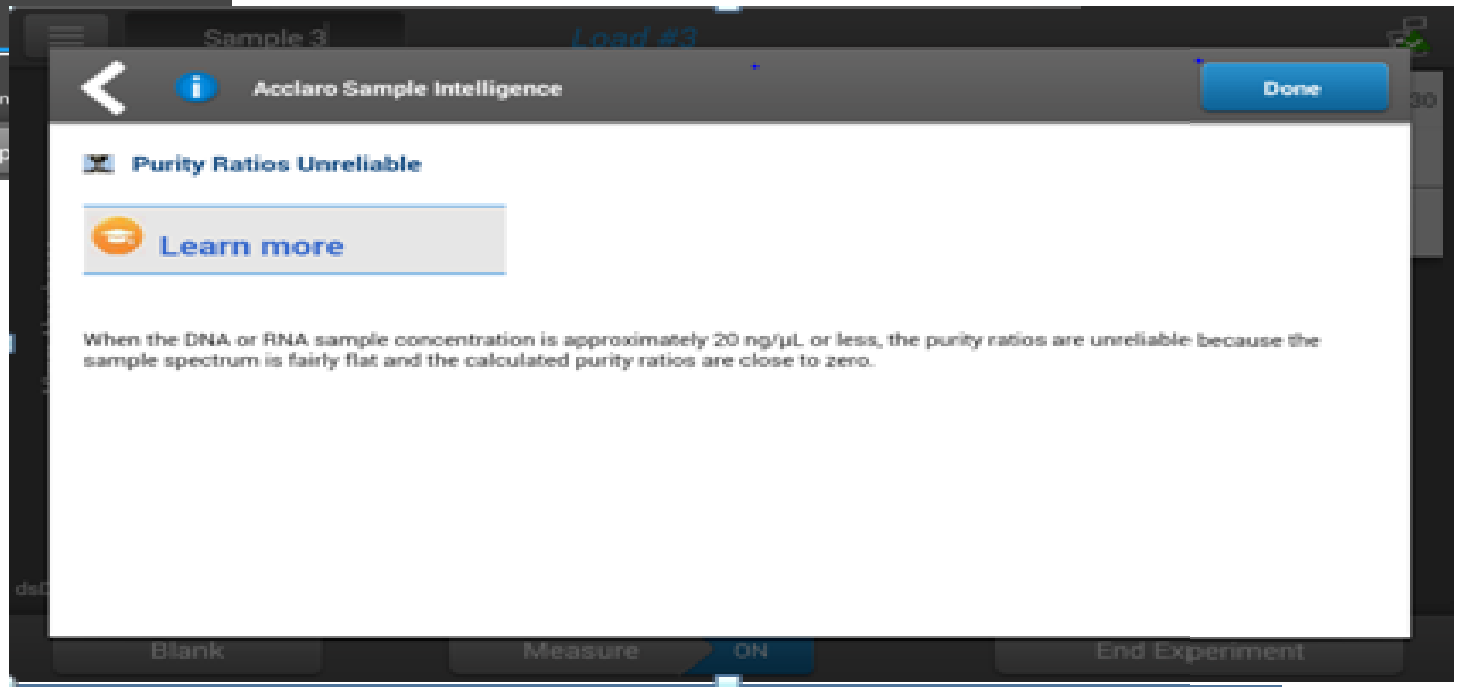
User Alert: Purity ratios unreliable



When DNA&RNA sample concentration is <20ng/µL the purity ratios are not reliable



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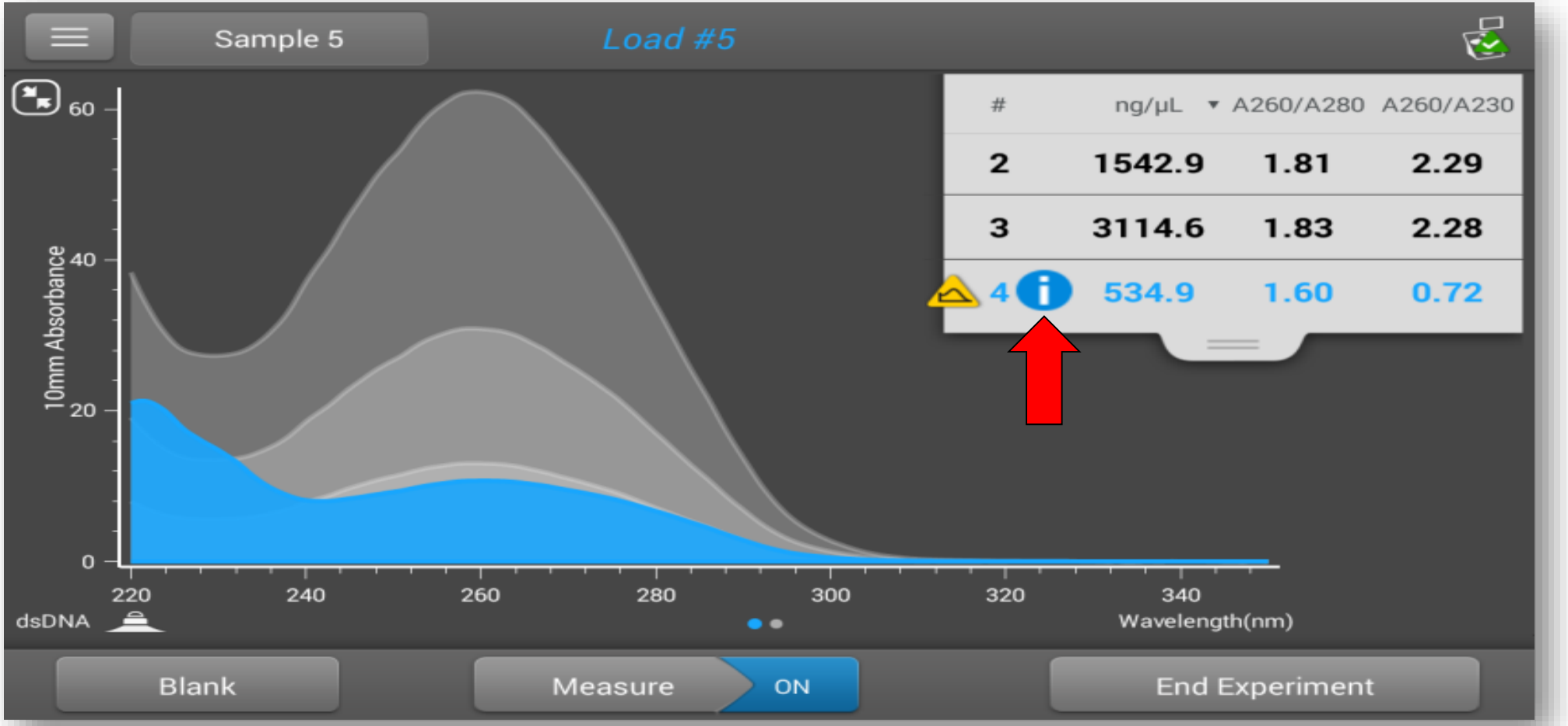
User Alert: Purity Ratio Out of Range



What do I do now???

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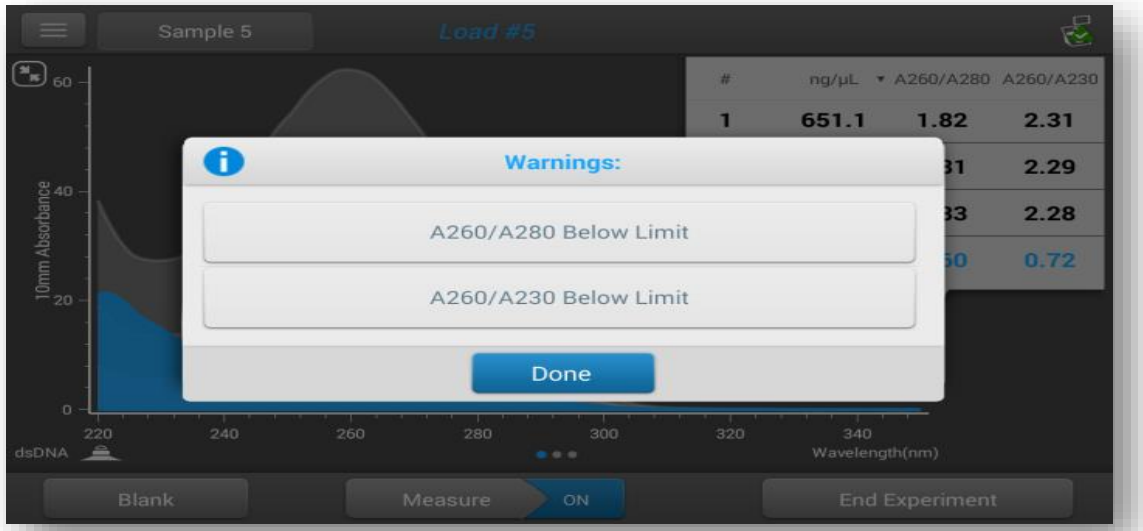
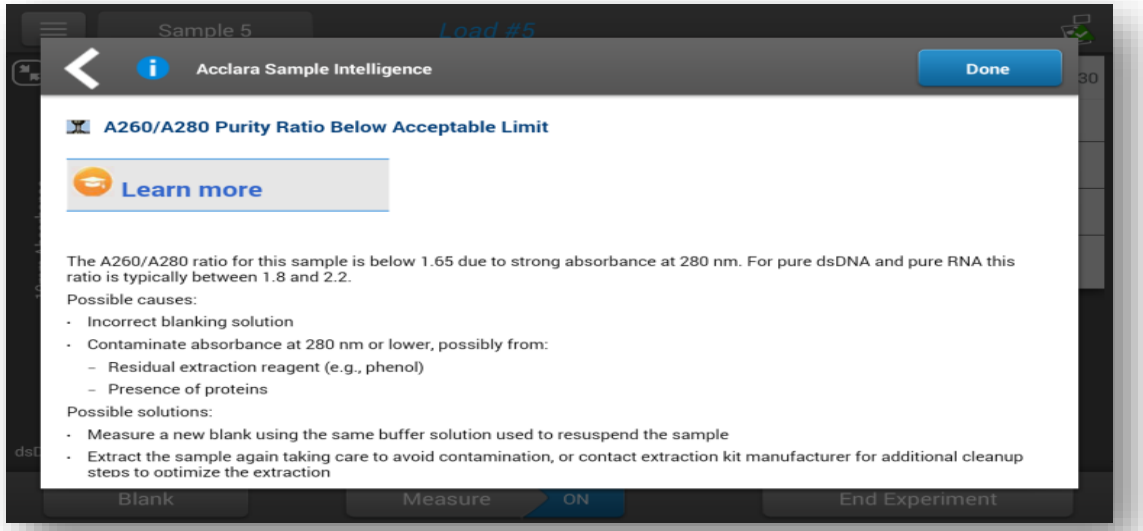
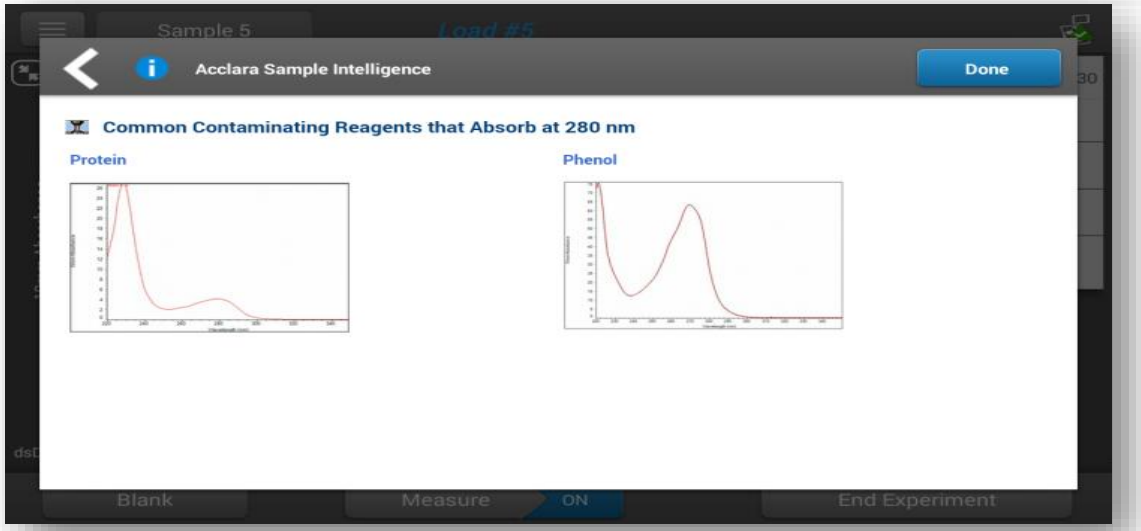
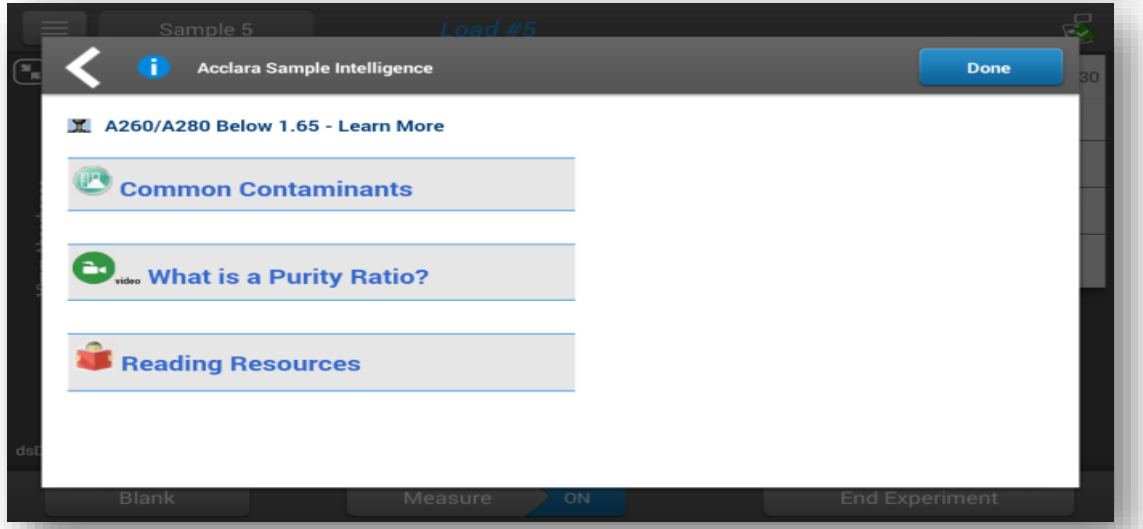
Learn more with Acclaro…









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Acclaro Sample Intelligence technology

Take-aways:

✓*Acclaro provides users with information about sample quality so they can make informed decisions on the suitability of the sample for use in downstream applications*

✓*Sample quality is crucial for success in downstream experiments*

- Successful experiments lead to publications, patents, PhDs, even tenure
- Successful experiments avoid lengthy troubleshooting, costly delays, and having to repeat expensive procedures

NanoDrop One customers perform successful experiments

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The NanoDrop One Value Proposition

- **The NanoDrop promise**
  - Fast and easy answers from microvolumes
  - Now even better

- ***Acclaro*™ Sample Intelligence**
  - Contaminant identification using *data analysis* algorithms, supports downstream success
  - On-demand *embedded technical support* answers context-sensitive questions
  - Embedded camera with digital image analysis ensures *measurement integrity*

- **A high quality experience**
  - New modern design with high resolution touchscreen
  - Local control software with workflow-centric design
  - Enhanced connectivity, extended data management options

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Modern design, looks like no other

- Distinctive design promises a high quality experience
- Local Control reduces footprint, streamlines workflows
- High resolution touchscreen interface
  - tilts for good visibility whether user is standing or sitting
  - slides to fit the space and accommodate R-and L-handed users
  - is compatible with lab gloves
- Glass disk is lit when instrument is ON and ready to use
  - Flashes during measurement
- New: power switch

Design Patent Pending!

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Run with Local Control, PC compatible

- Local Control
  - Streamlined workflow guides users through measurement
  - Applications-based software
  - Print data using Dymo printer
- PC software
  - For data viewing, analysis, saving, and printing
  - Can explore all the Acclaro support tools
  - User can create new custom methods on PC then run them on LC
- Enhanced connectivity, extended data management options
  - Wi-Fi connects you to network,
  - Bluetooth for mouse and keyboard
  - 3 USB-A ports

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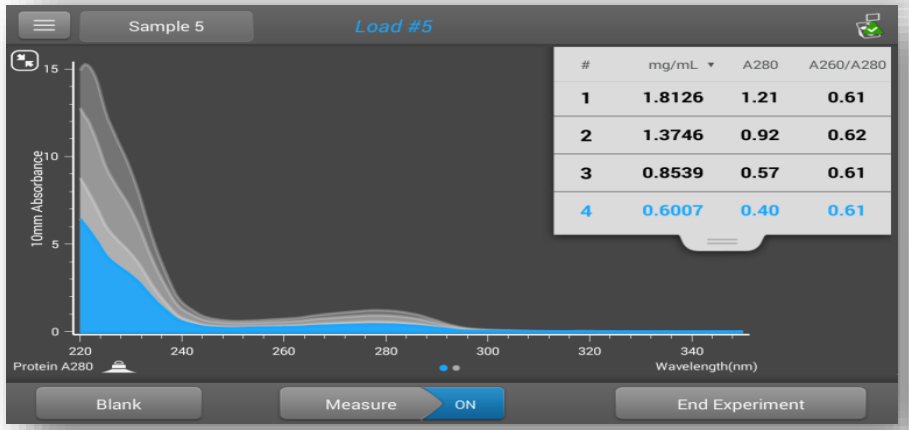
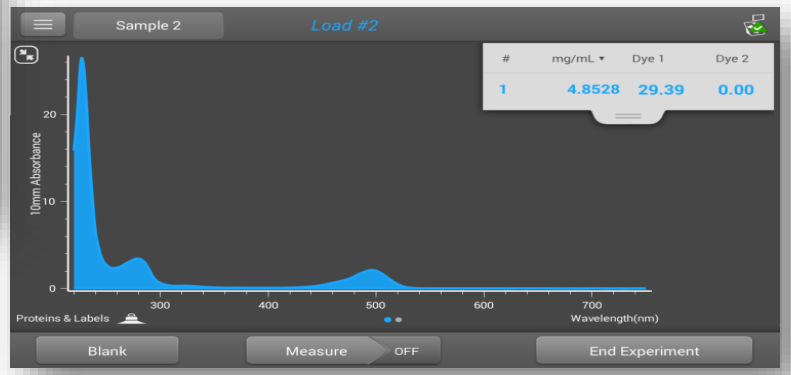
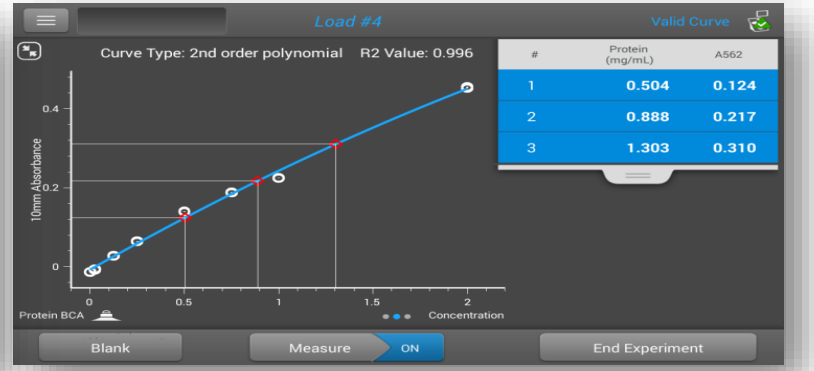
New protein capabilities

83% of our customers quantify proteins with a spectrophotometer at least once per week



- Protein A205 (new)
  - for proteins without Trp, Tyr
- Protein A280
  - Protein Editor in Settings - create and save your “special protein” (new)
- Proteins and Labels
- Protein colorimetric assays





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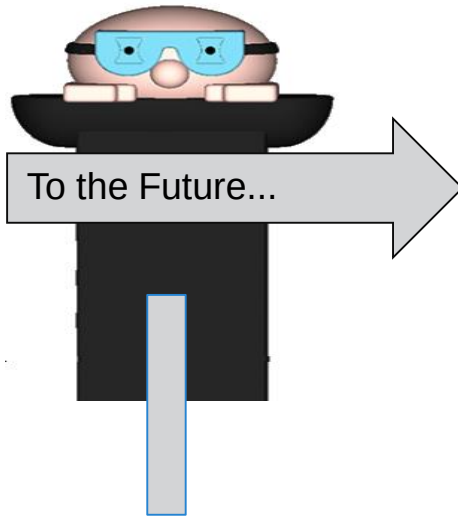
Beyond NA and Proteins Applications

Software anticipated at FCS

- Nucleic Acids Applications
- Proteins Applications
- OD600 Applications
  - Enter conversion factor to convert OD600 value to #cells/mL(new)
- Custom Methods
  - Formula method without standard curve
- PC Viewer Software
- Ethernet tether NDOne to computer
- Sensor diagnostics

- Very easy to upgrade Local Control software
  - Download from our website on USB and update LC



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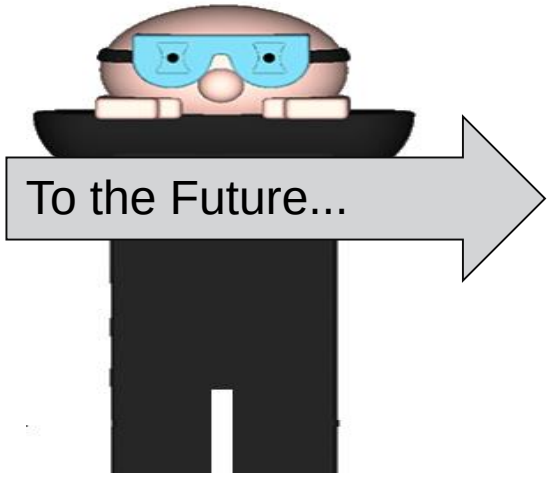
Future releases coming by end of 2016

- UV-Vis “Scan” method
- UV peak find, 3-pt net
- Acclaro Blank evaluation
- Acclaro Baseline correction
- Custom Methods – standard curve
- Custom Methods - advanced features
- Kinetics (5 stages, three wavelengths)
- WiFi connectivity - send data to one or more computers; send data to network
- Translations – Spanish, Portuguese, Japanese, Chinese, German, French, Polish, English, Korean
- IQ/OQ



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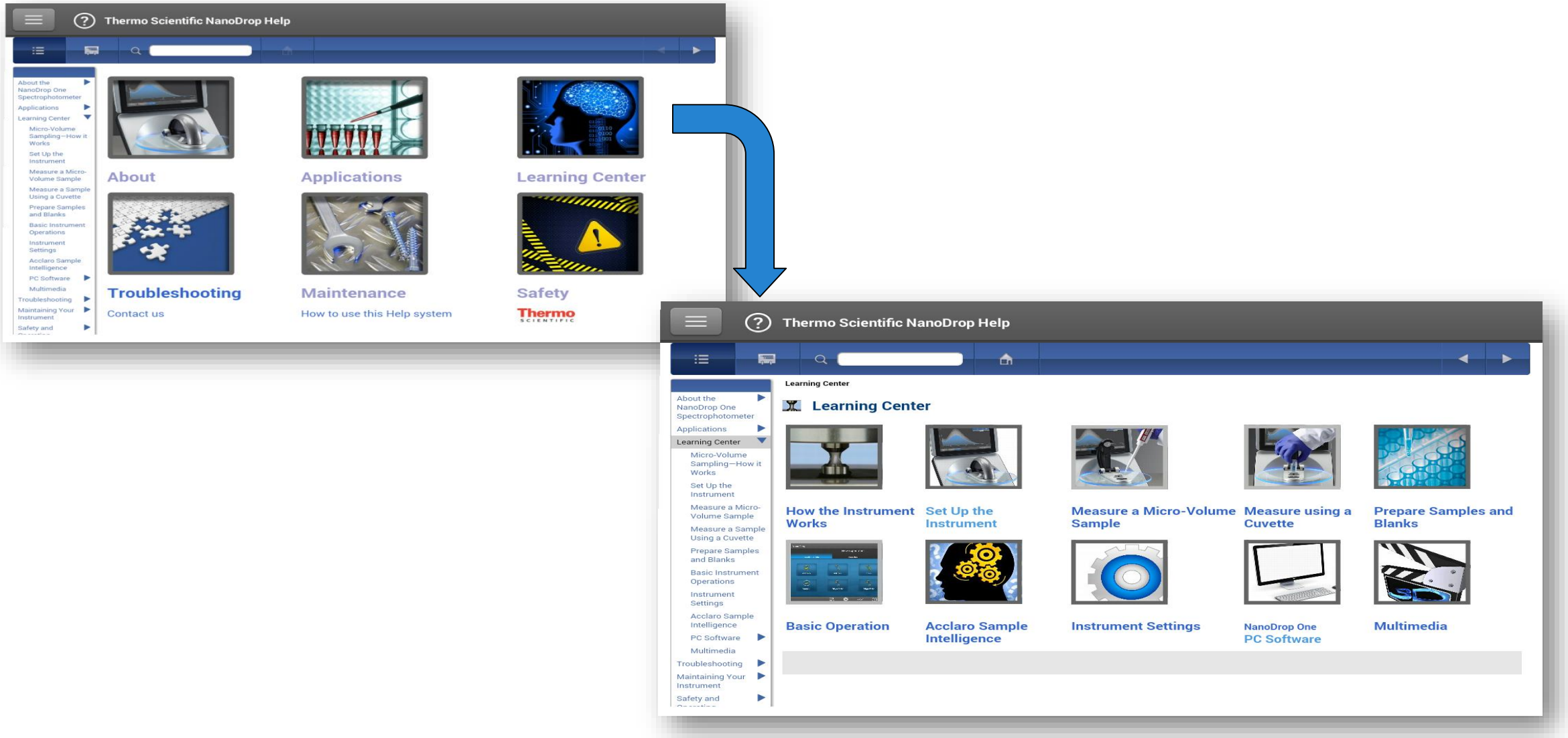
NanoDrop One Help System



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NanoDrop Product availability

The Nano Drop range

And now

Questions??

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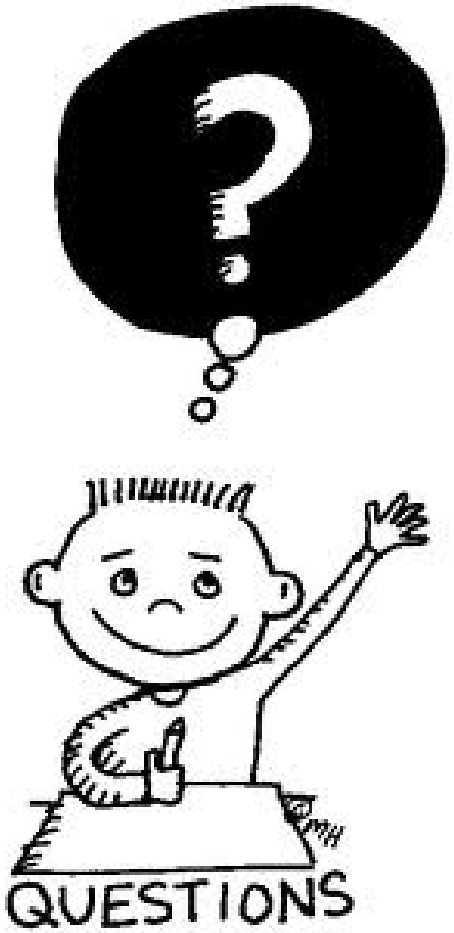
Thank you!



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