WTW
a xylem brand

Photometry Compendium

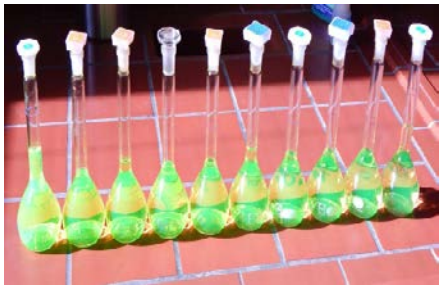
in cooperation with G.I.T. LABORATORY JOURNAL

Special Photometric Procedures and Multi-step Measurements

- General Overview of Measurement & programming principles with photoLab® 7000 Series
- Programming of Chlorophyll method: step by step
- Wine quality factors: Colour intensity and nuance
- Overview of sugar determination principle with enzymatic test kits: Glucose, Sucrose, Fructose

Programms and Programming:

- **Concentrations measurements:**
  Programmed methods for commercial test kits
  User calibration curves e.g. for Uranine



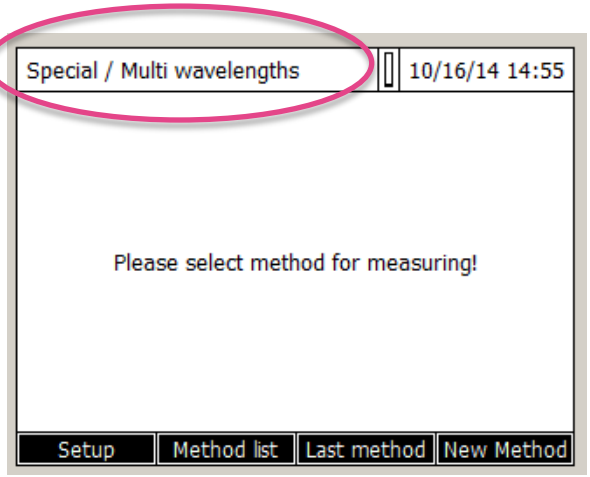
- **Multi-step measurements:**
  Chlorophyll methods for environmental analytics, Food (nutrition), R&D…

- **Enzymatic test kits**
  available from various manufacturers,
  complex handling eased by (user-defined) programs

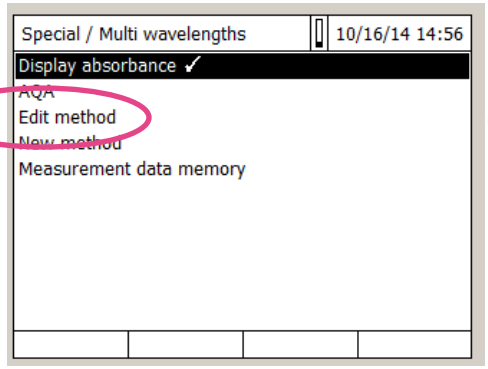
- **Multi-wavelength measurements**
  e.g. wine colour

- **Extense Programming options**

Programming with photoLab® 7000

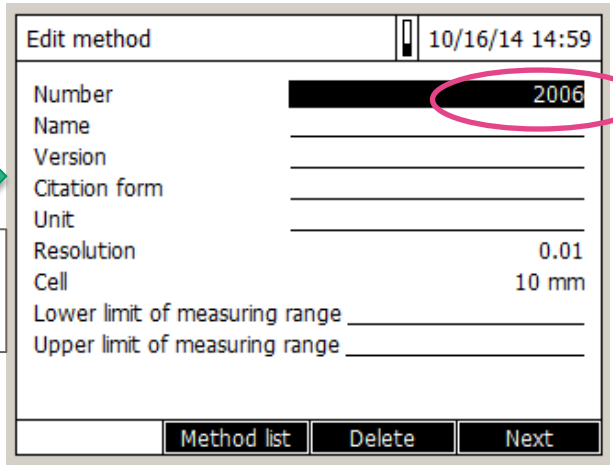


Method numbers 2xxx: the next available number is offered automatically



Edit existing user method: **F1 key**

New method **F4 key**



Operators, Variables, Conditions…

R = Result

K = Variable in formula, to be defined

A = Absorption A_{xxxnm}

Same absorbance at different steps
=> Index to be entered for second and more absorption measurements at same wavelength: A_{xxxnm_2}; A_{xxxnm_3}…

F-Keys for easy operation:

Operators via F2

Operators			
+	-	·	/
(	)	^	Pi
sin	arcsin	sinh	arsinh
cos	arccos	cosh	arcosh
tan	arctan	tanh	artanh
log	ln	10^	e

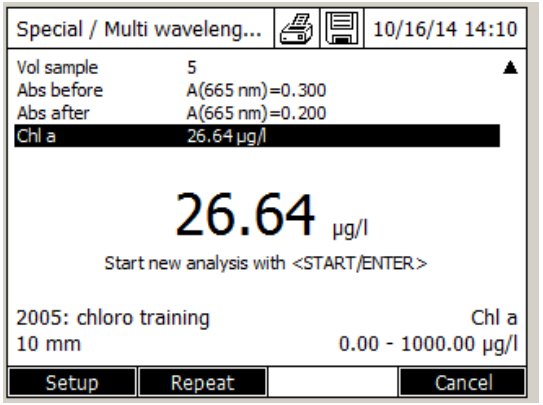
F1: Back

F4: Next

Variables via F3 (Axxxnm, K1, K2…) **F4: Next**

Chlorophyll measurement is needed in many applications:

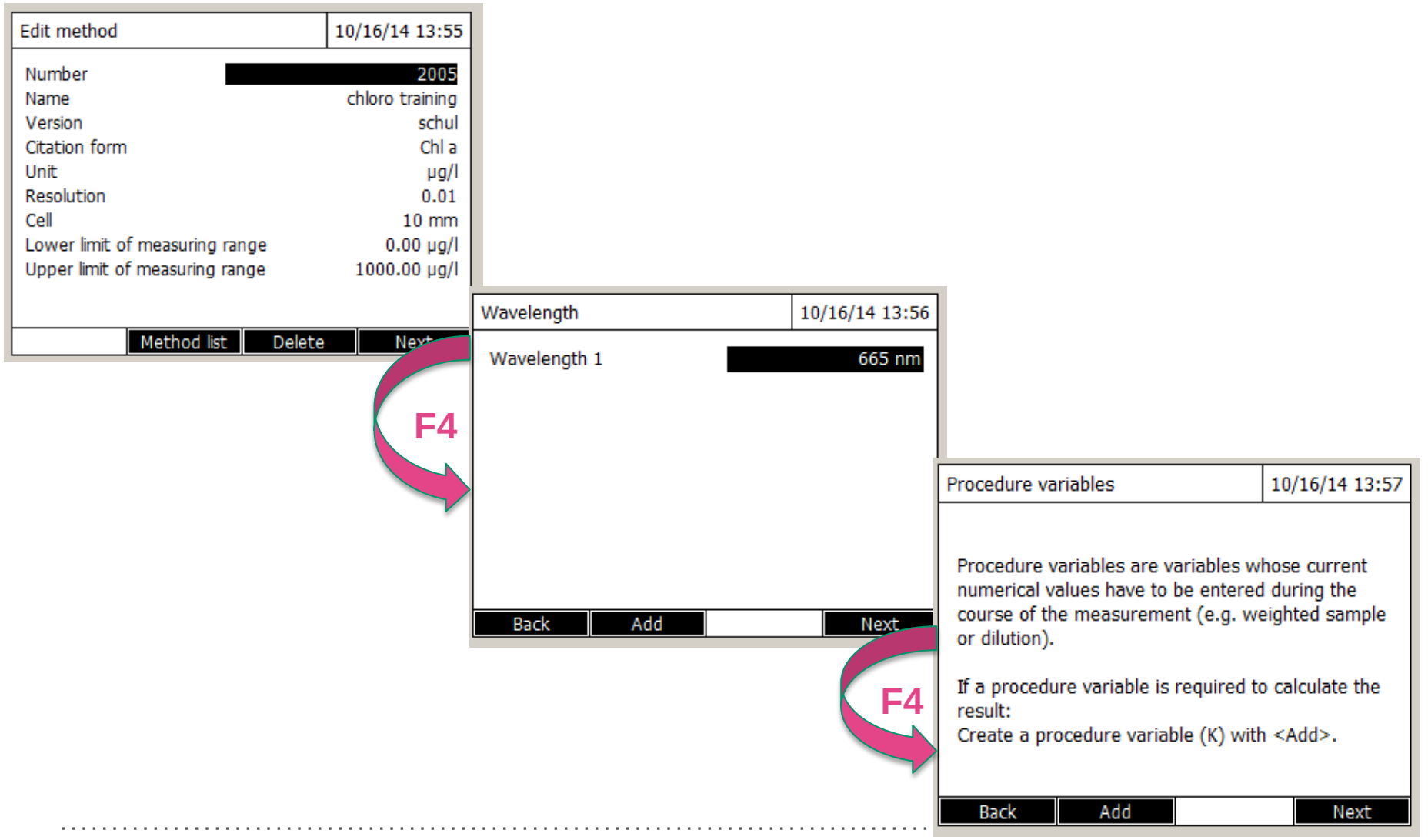
- Environmental Analysis
- Algae content in food & beverage industry
- Required procedures vary according to application field!



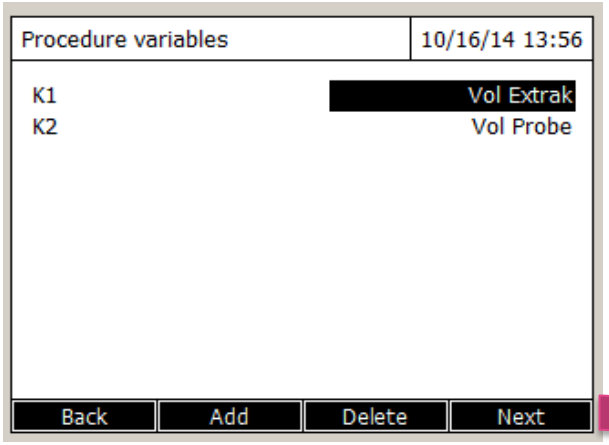
Easy programming according to application requirements

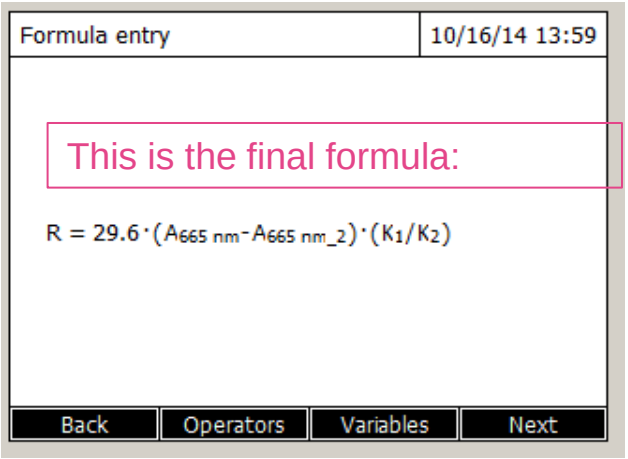
- Sample preparation according to standard methods:
  Chl is extracted, and a small volume of the extract is measured photometrically
- The determination of the Chlorophyll is done by measuring the difference of the absorbance before and after adding HCl.
- The final formula of Chlorophyll take sample and extraction volume into the automatic calculation.

Programming Chlorophyll Measurement



Programming Chlorophyll Measurement



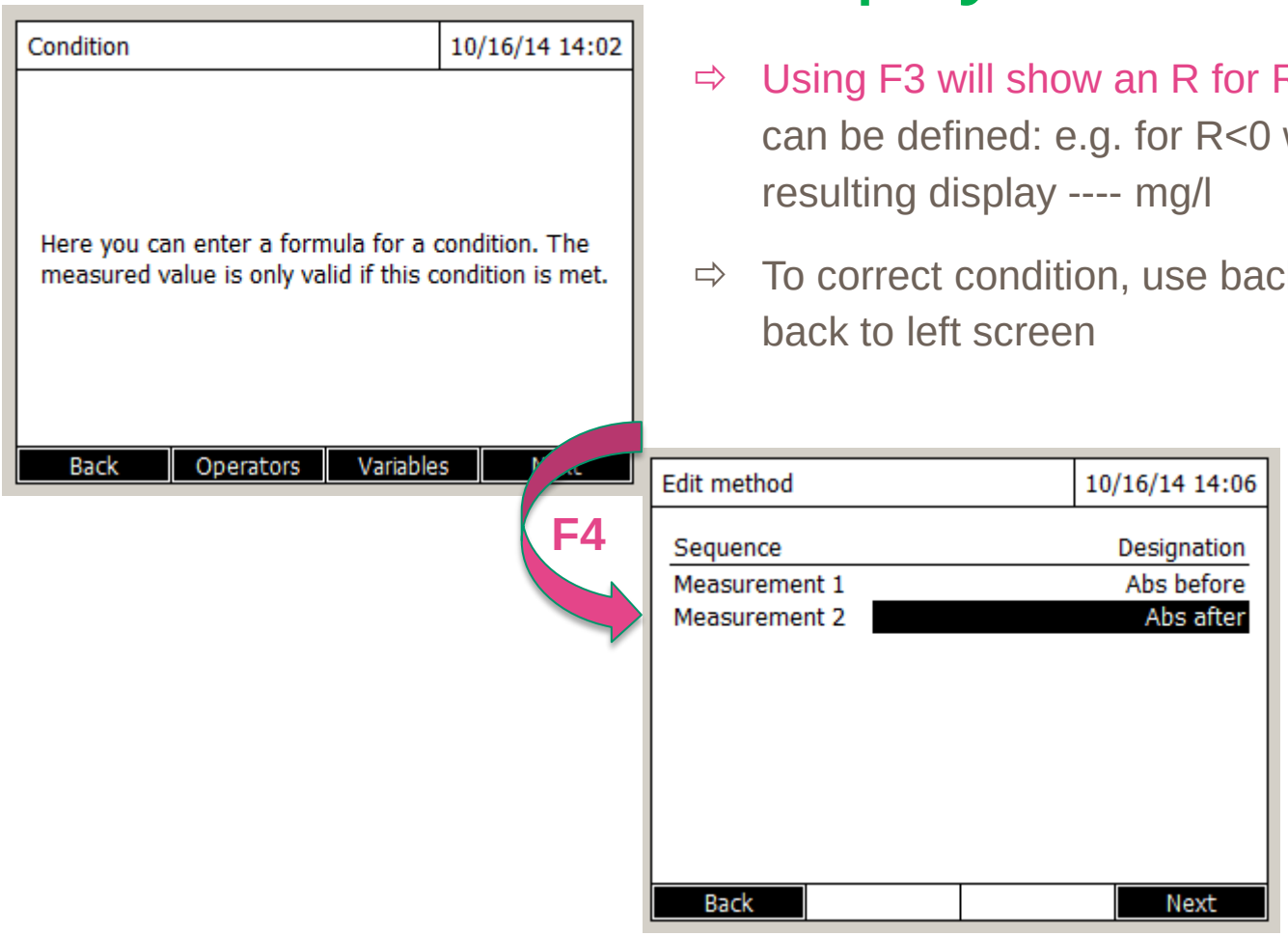


F4

Here, it is important to indicate the sequence of **measurement steps by assigning an index to the absorbance measurement (2, 3…).**

Subsequently a clear description to each single step can be entered for user-guidance on display.

Programming Chlorophyll Measurement



- ⇨ Using F3 will show an R for Result which can be defined: e.g. for R<0 wrong the resulting display ---- mg/l
- ⇨ To correct condition, use back space until back to left screen

Wine Colour:
An Important Quality Factor

Colour Measurement in Beverage / Wine Industry …

… is important for:

- Wine growers
- Third party bottlers' income quality control.

Photometric colour measurement offers:

- Accuracy and comparability (versus human eye, comparator …)
- Reproducibility

Bottlers need several routine testings besides coulor:

- Testing Water parameters, sugar content, …
- Turbidity (in many other beverages)
- COD before disposing water to municipal wastewater plant

Wine Colour = **Multi-λ** measurement

Wine characterization by colour

Colour Intensity = Sum

Absorbance measurement @ λ 420nm, 520nm and 620nm

Abs_{420} for yellow/orange/ocker

Abs_{520} for red

Abs_{620} for blue (not always included)

$R = Abs_{420} + Abs_{520} + Abs_{620}$

$R = Abs_{420} + Abs_{520}$

Colour nuance

Ratio of brown/red nuances

$R = Abs_{420} / Abs_{520}$

< 0,8 violet, 0,8-1,2 red, > 1,2 orange

Image source:perrotwein@bluewin.ch

Determinations of Sugar (Glu…) with enzymtic test kits, e.g. with r-biopharm

Understanding Enzymtic Test Kits

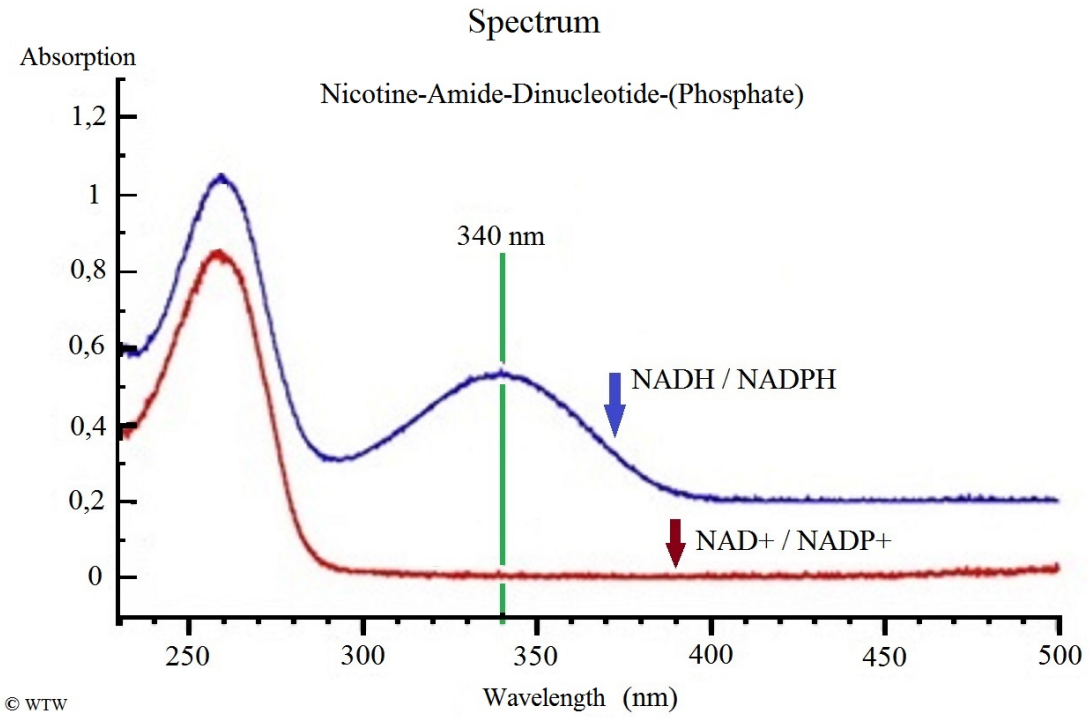
Sugar (D-Glu, D-Fru, Sucrose etc.) are important parameters in Wine, Food & Beverage

- Test kits are based on **enzymatic reactions** for many substances
- Enzymes are specific to chemical reactions and substances (substrates)
- Some tests offer multi-step measurements for determinations of individual sugar molecule concentration such as Sucrose, Glu and Fru
- Enzymatic test kits are gradually replacing other standard methods
- Are accepted by many International Organisations in Food & Beverage
- Enzymes „co-operate“ with metabolistic „energy provider“ (ATP) and Co-enzymes offering a Redox system, here NAD^+/NADH and $NADP^+$/NADPH

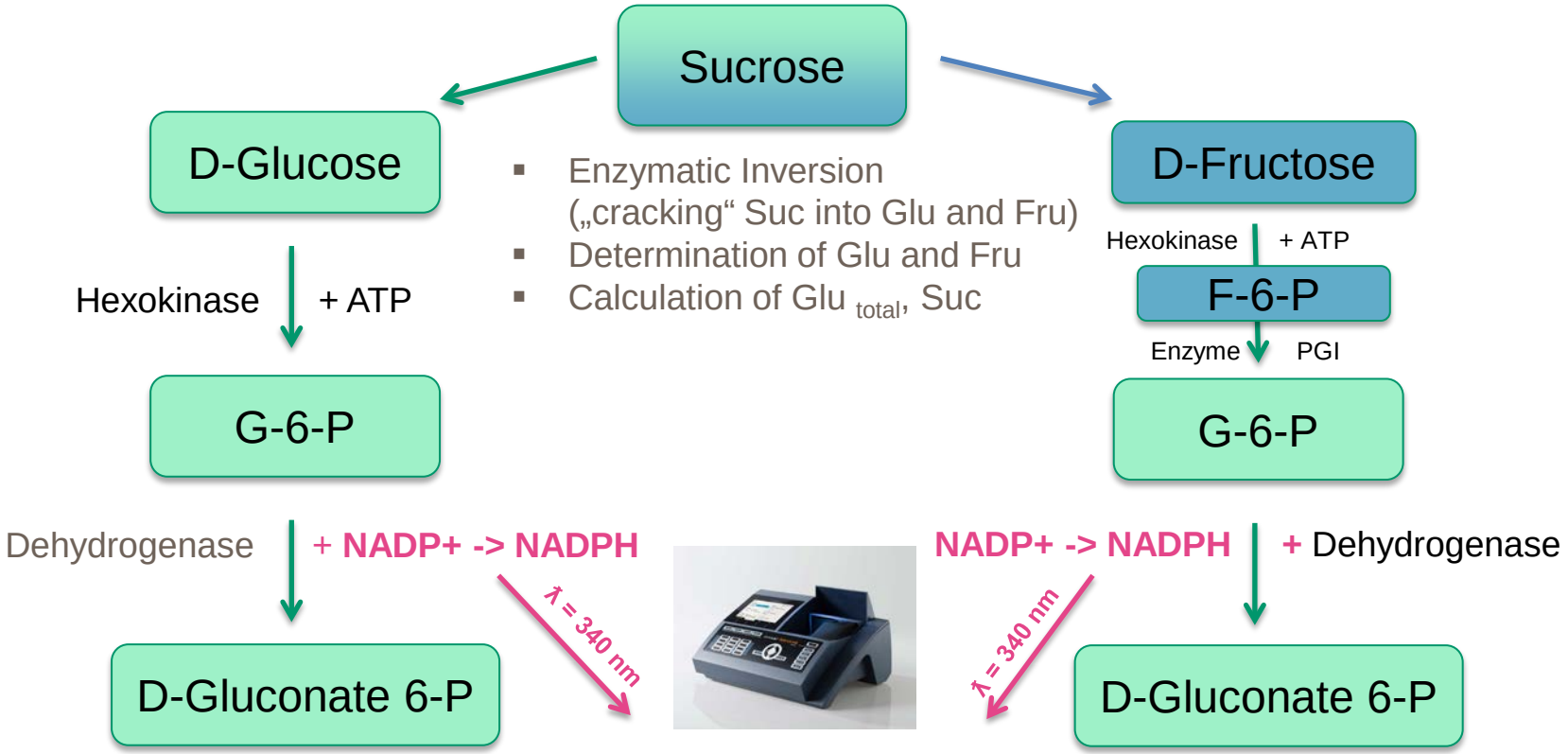
The Hub: NAD+/NADH co-enzyme

NAD+/NADH and NADP+/NADPH is a standard step in enzymatic reactions.

In reduced form, these redox systems show a peak at 340 nm.



Scheme of Suc, Glu, Fru determination



1 NADP is equivalent to 1 D-Glu / 1 D-Fru

Glu_{total}: Σof Glu_{free} and Glu_{bound}

Suc / Fru: Glu_{free} determination before and after „cracking“ of Sucrose

Enzymatic Test Kits …

…. provide:

- all necessary information on substances to enter formula
- all enzymes needed
- all co-enzymes such as $NADP^+$, ATP etc.
- AQA material

The transfer of reaction into a simplified photometric routine may need several steps and formulae.