Practicals in Food Analysis

Department of Bioscience and Agro-Food and Environmental Technology

University of Teramo

Degree Course Class LM-70: Food Science and Technology

Optional course (4CFU)

Dr. Annalisa Scroccarello

2nd chapter
Calibration and external calibration curve

Practicals in food analysis

How the course is organized: MOTTI LABORATORY.

Calendar draft:
People online will attend only the frontal classes.
Bring your own laptop with Excel installed every time.

- **26/10/2025_Practicals Unit 1** - Integration of a colorimetric assay on a paper-based sensor and use of the external calibration curve (external calibration curve)

- **09/11/2025_Practicals Unit 2** - Paraoxon-methyl determination in Honey Samples through internal calibration (in-matrix calibration)

- **23/11/2025_Practicals Unit 3** - Electrochemical determination of phenolic compounds via standard addictions method (standard addiction method)

- **14/12/2025_Practicals Unit 4** - Aflatoxin extraction from food supplements coupled to HPLC-MS/MS analysis. The internal standard method (Internal standard)

- **21/12/2025_Practicals Unit 5** – to define

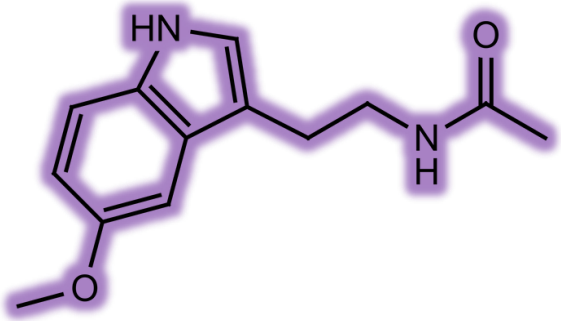
(for practicals in LAB. MOTTI. bring lab coat).

Classes schedule:
https://unite.prod.up.cineca.it/calendarioPubblico/linkCalendarioId=68c3f84e7f9c2a004b69daf3

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DI TERAMO

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COLORIMETRIC PAPER-BASED KIT FOR THE SELECTIVE DETERMINATION OF MELATONIN



Calibration curve refresh

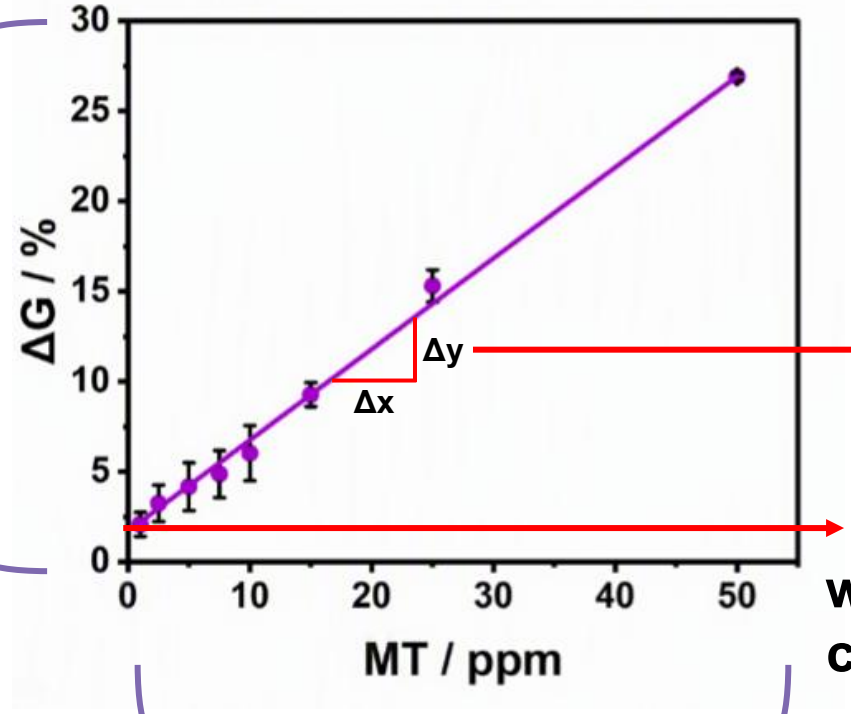
Concentration

$C = k\,S$

Signal

$y = mx + q$

y = S
x = C



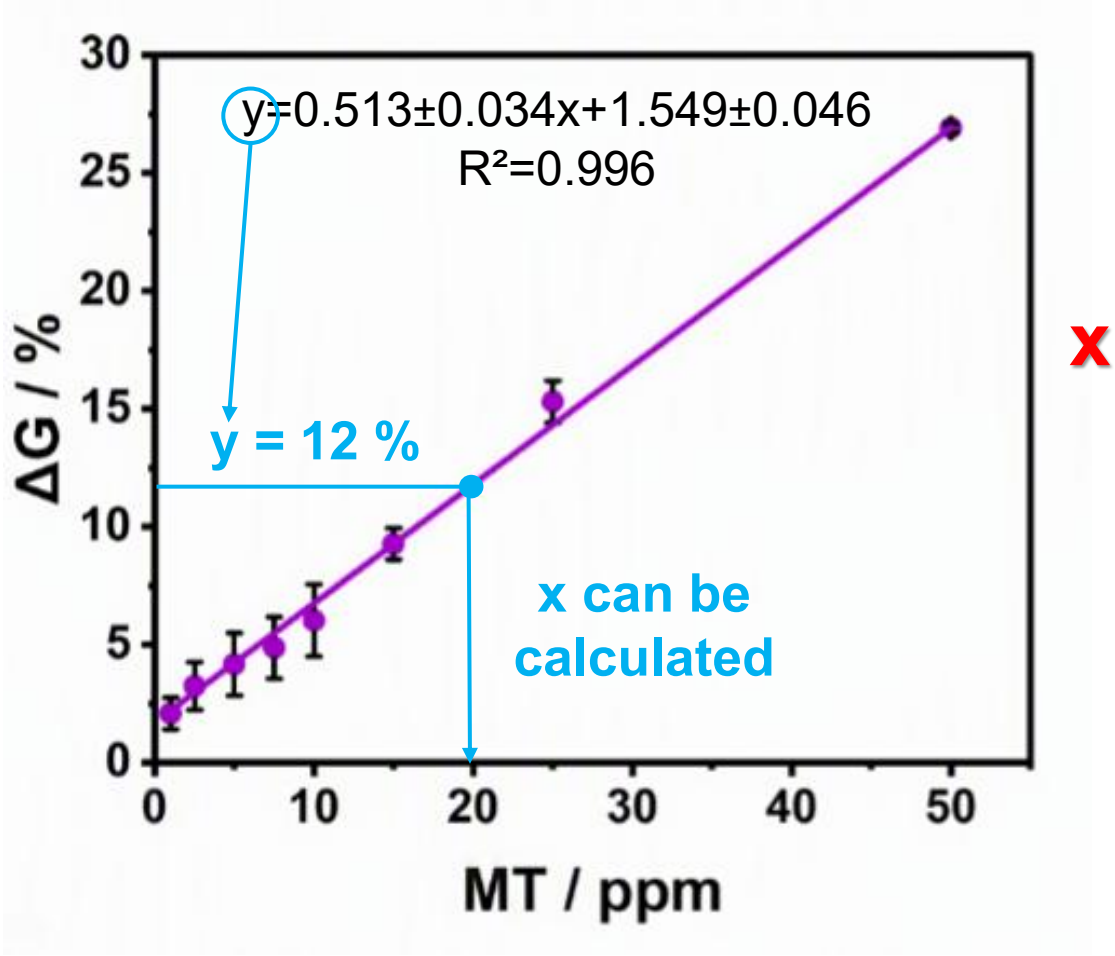
m = slope
Δy / Δx

q = intercept where the curve cross the Y axis

Calibration curve refresh

Standard solution to build the calibration points

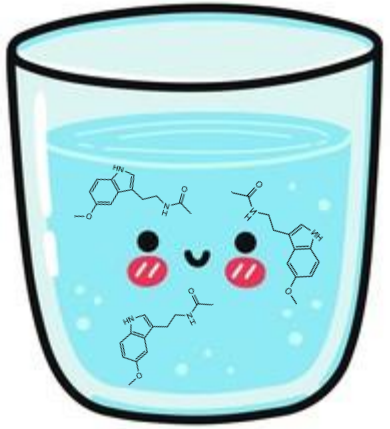
Unknown sample quantification using curve equation

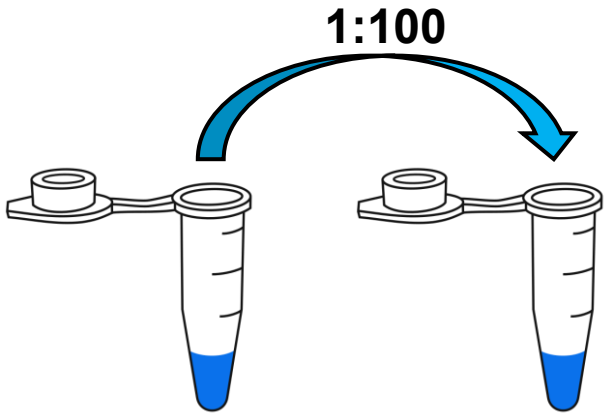


$x = (y - q)/m$

External calibration: when is used?

- Samples with a simple matrix (ex. tap water)
- Matrix and interferents undergo dilution effect
- Commercial standard exist (or compounds similar to the analyte)







External calibration: when is used?

Melatonin in water





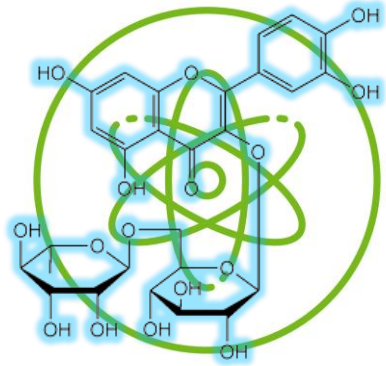
Paraoxon in honey





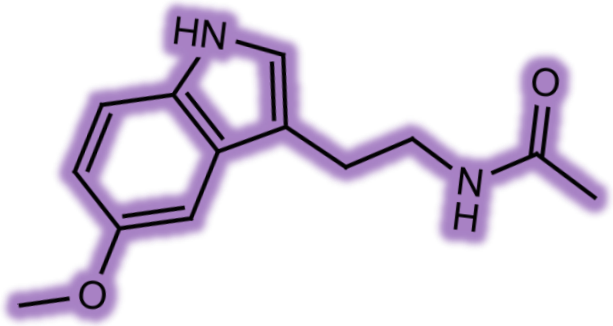


Rutin in tea

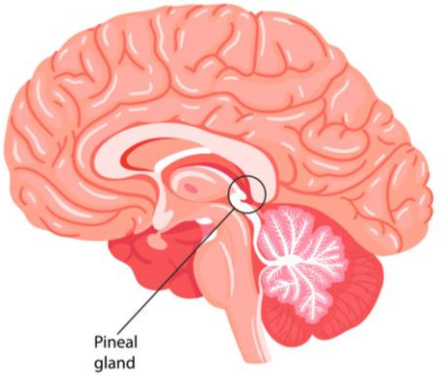




What is melatonin?



Hormone produced by pineal gland





Regulates sleep-wake cycle





Used for insomnia treatment, has been associated with antioxidant and anti-inflammatory properties



What is melatonin?

Plenty of integrators on the market



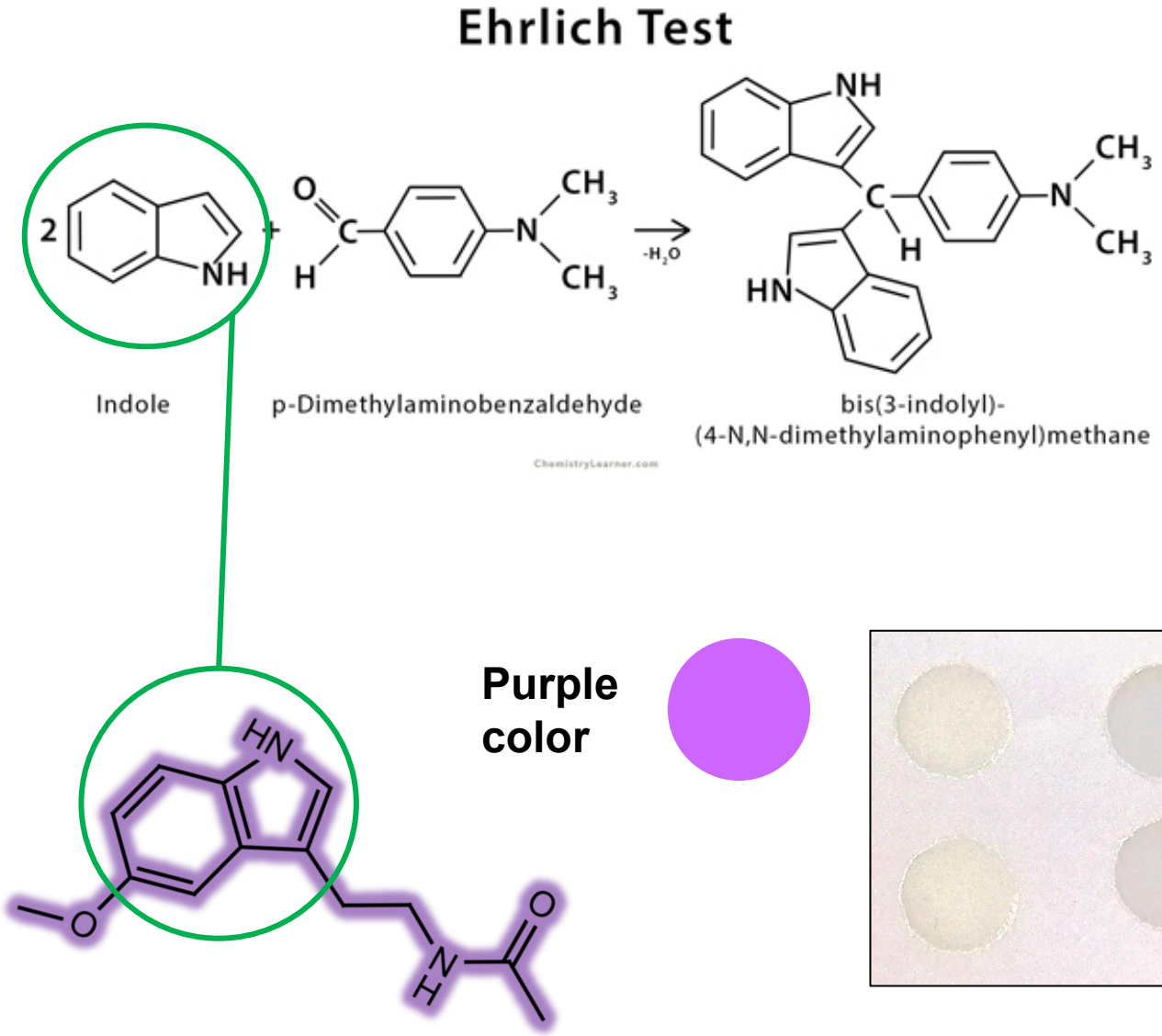
Regulation and monitoring are also important, because…

Adverse effects could occur, as

- **Depression in predisposed patients**
- **Ovulation inhibition**
- **Increase in aggressivity**

Some studies underline the correlation between prolonged melatonin assumption and those side effects

Colorimetric detection of melatonin



Materials, apparatus and reagents

Reagents: **Melatonin** (MW:*232.28 g/mol*); **HCl**; **4-Dimethylaminobenzaldehyde** (Ehrlich's reagent) (MW:149.19 g/mol); MilliQ water (in Squeeze bottle), supplements (**Melatonina Pura Junior** and **Melatonina Equilibria**) **.**





Materials: Squeeze bottle, chromatographic paper, Eppendorfs, rack, Gilson pipette, tips, falcon (15 mL), volumetric flask.

***Apparatus*:** analytical balance, thermal plate, HPRT thermal printer, CO_2 laser, own smartphone, please download the app Color Picker from the Google/Apple Store (Icon Google play ; Icon apple store)





Safety Sheet of the used reagents

Melatonin

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Not a hazardous substance or mixture according to Regulation (EC) No. 1272/2008.

2.2 Label elements

No hazard pictogram, no signal word, no hazard statement(s), no precautionary statement(s) required.

Not a hazardous substance!

Safety Sheet of the used reagents

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SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Skin sensitisation, (Sub-category 1B) — H317: May cause an allergic skin reaction.

2.2 Label elements

Labelling according Regulation (EC) No 1272/2008

Pictogram



Signal word — Warning

The life science business of Merck operates as MilliporeSigma in the US and Canada

Hazard Statements	
H317	May cause an allergic skin reaction.
Precautionary Statements	
P261	Avoid breathing dust.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.
P302 + P352	IF ON SKIN: Wash with plenty of water.
P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.
Supplemental Hazard Statements	none

Ehrlich reagent

May cause allergic reaction, pay attention!



Personal protective equipment

Safety Sheet of the used reagents

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2.2 Label elements

Labelling (REGULATION (EC) No 1272/2008)

Hazard pictograms :



Signal word : Danger

Hazard statements :

H290	May be corrosive to metals.
H314	Causes severe skin burns and eye damage.
H335	May cause respiratory irritation.

Precautionary statements :

Prevention:

P234	Keep only in original packaging.
P261	Avoid breathing mist or vapours.
P271	Use only outdoors or in a well-ventilated area.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.

Response:

P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

HCl

Dangerous substance!



Personal protective equipment

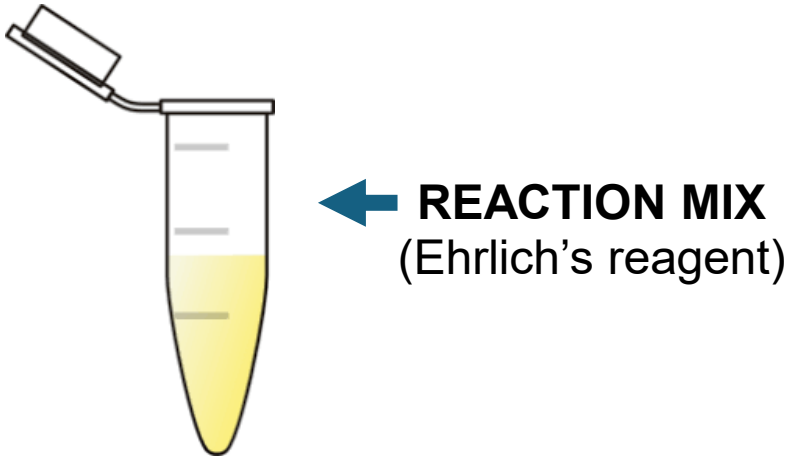
1) Melatonin standard and Ehlrich reagent preparation



- **STANDARD SOLUTION:** Prepare 2 mL of 1000 ppm Melatonin standard solution **in water**.

  Give an aliquot of the standard solution to each lab group.

- **REACTION MIX:** Prepare in final volume of 1 mL a 4 M HCL from (12 M HCL stock), 2 M of 4-Dimethylamino benzaldehyde (Ehrlich's reagent) from the stock of 5M and add compete with water to reach final volume of 1mL.



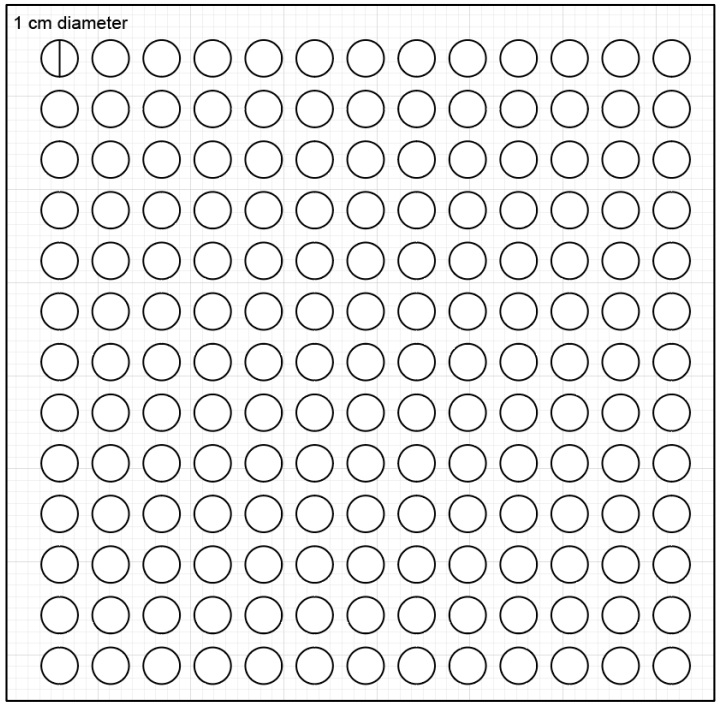
REACTION MIX
(Ehrlich's reagent)

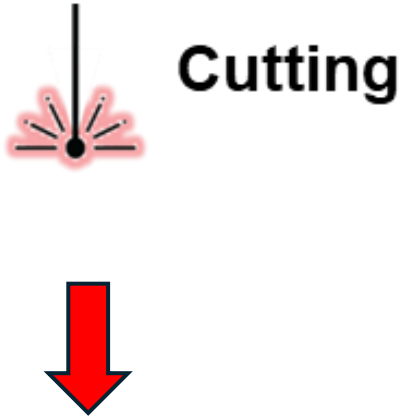
2) Colorimetric strip and paper analytical device manufacturing

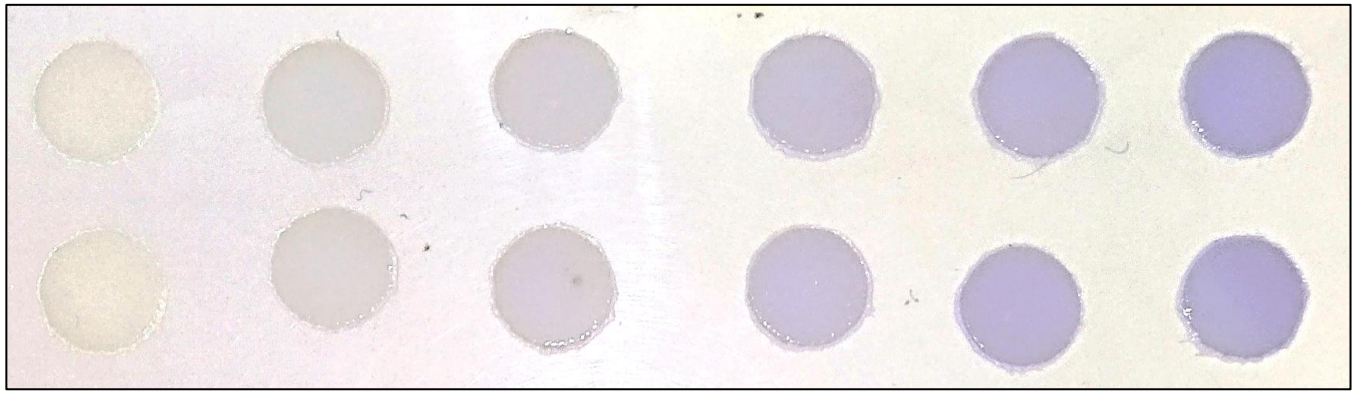
1. Cut the chromatographic paper into 20 circles (named strips) sized 1 cm x 1 cm.

2. Place the papers circles on the plastic support.

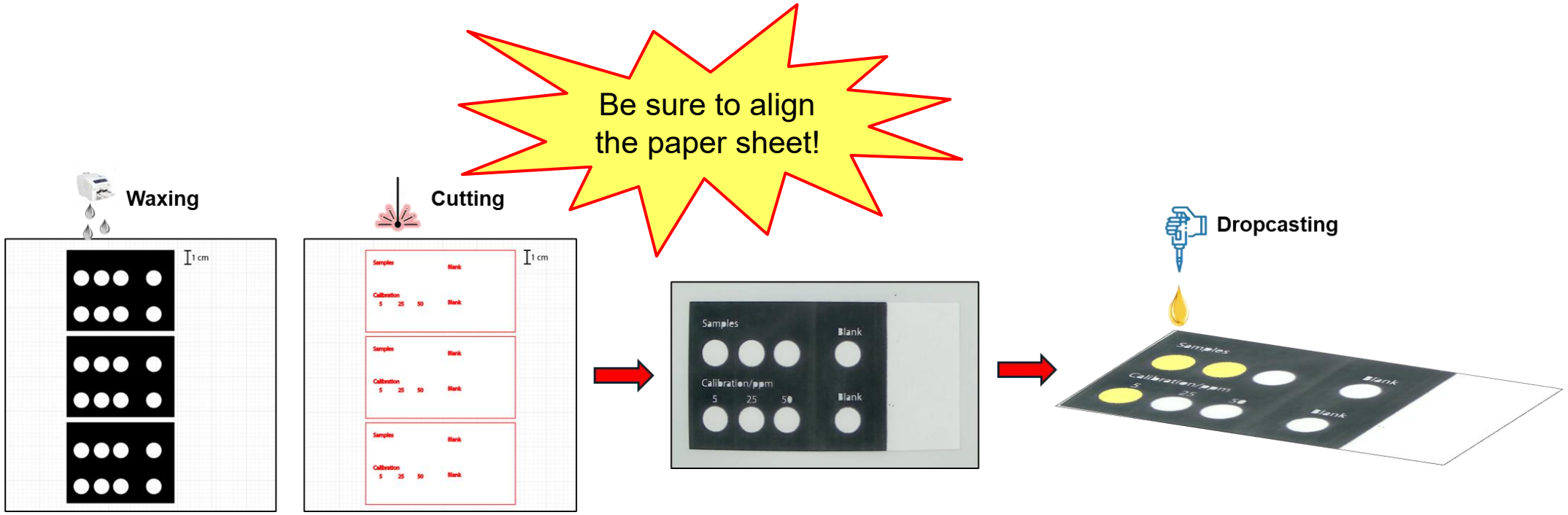
3. Drop-cast 10 µL of Ehrlich's reagent (Reaction mix) onto each strip and allow it to dry for 10 minutes at room temperature.







3) Colorimetric Paper Based Device (PAD) fabbrication

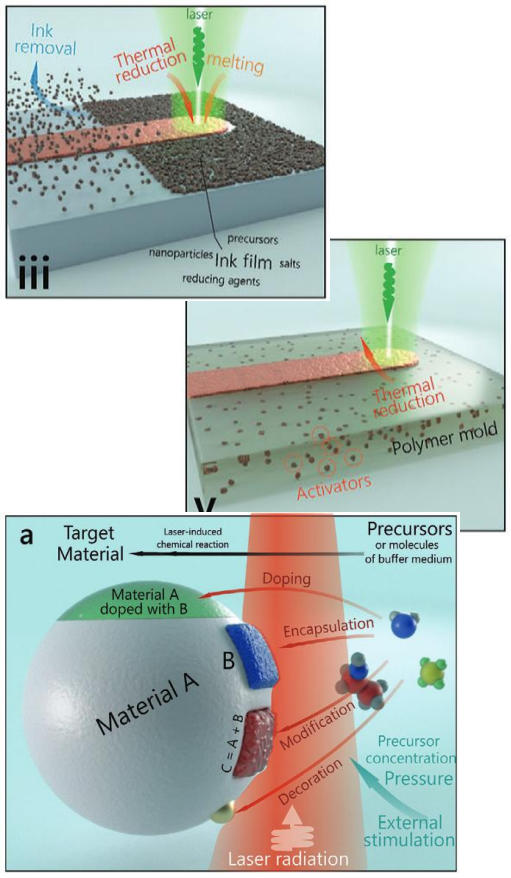


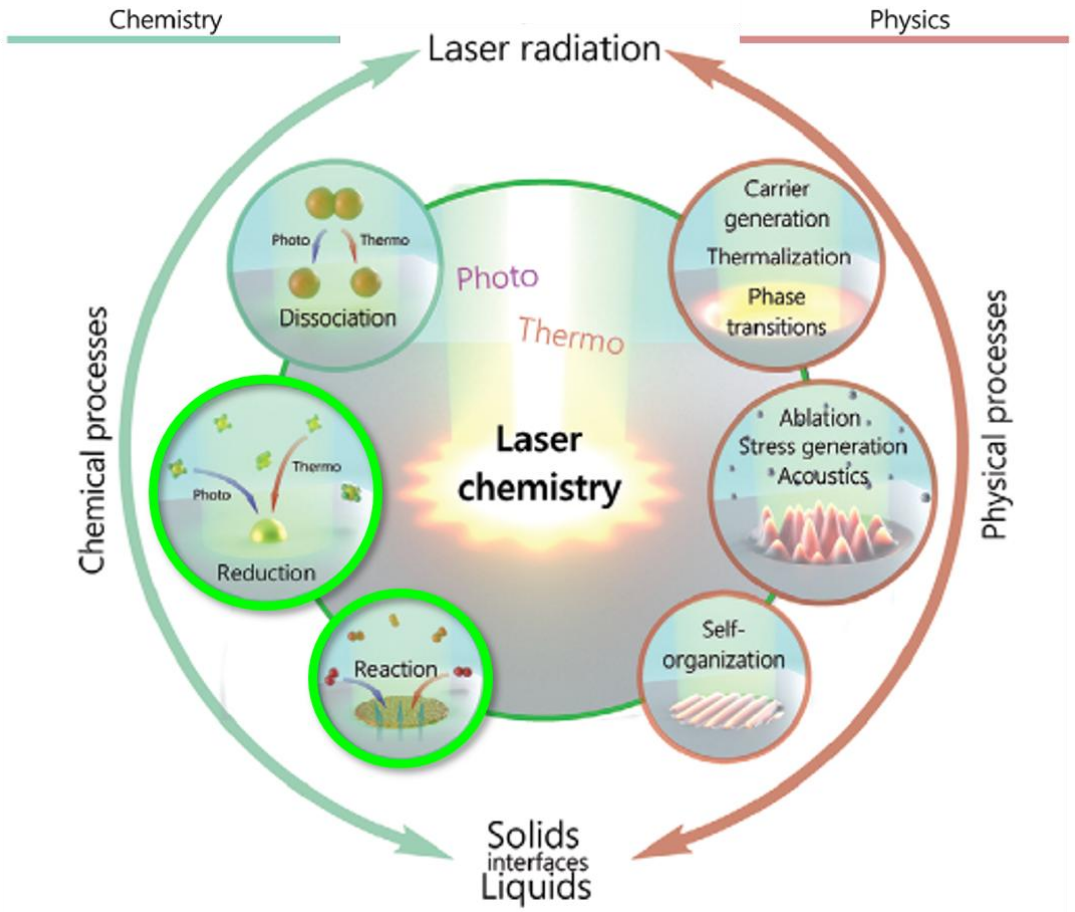
1. Print the hydrophobic patterns of the device using the HPRT Thermal printer

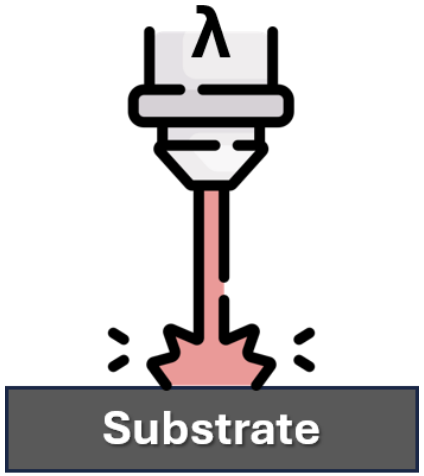
2. Cut the shape and writhing of the device using the CO_2 laser plotter

3. Dropcast the reagent mix in the circle sposts

CO2 Laser principles







SOLUTION PREPARATION

NaCl
MW. 58.443 g/mol

Prepare a 50 mM solution in 25 mL.

You have to calculate the gram of NaCl needed to prepare that solution.

4) Calibration curve standard solutions calculation

- From the 1000 ppm Melatonin stock solution, prepare melatonin solutions concentrated at 50, 30, 20, 10, 5 ppm.
- Each solution should be prepared in 1 mL of final volume in water.
- Perform the dilution calculation for each solution.

[C] ppm	50	30	20	10	5	Blank (µL)
Melatonin (µL)						////////
Water (µL)						

$$C1*V1=C2*V2$$

5) Sample extraction and dilution

Sample 1, **Melatonina Pura Junior**, is a dietary supplement specifically formulated for children and contains 1mg/mL of melatonin.
Sample 2, **Melatonina Equilibria**, provided 1 mg per tablet, intended for sleep-regulation applications.

- Samples are used in their commercial forms (1 mL for sample 1 and one tablet for sample 2) and dissolved in 10 mL of distilled water. To achieve complete solubilization and homogeneity, stir for 1 minute, then let it extract statically for 10 minutes. **Which kind of extraction is?**.
- Filter only the extract of Sample 2 by using a syringe filter (PTFE): **(i)** screw the filter to the syringe; **(ii)** place the syringe filter on the 50 mL falcon; **(iii)** load the extract in the syringe; **(iv)** perform the filtration.
- Perform the calculation to prepare the dilutions described below:

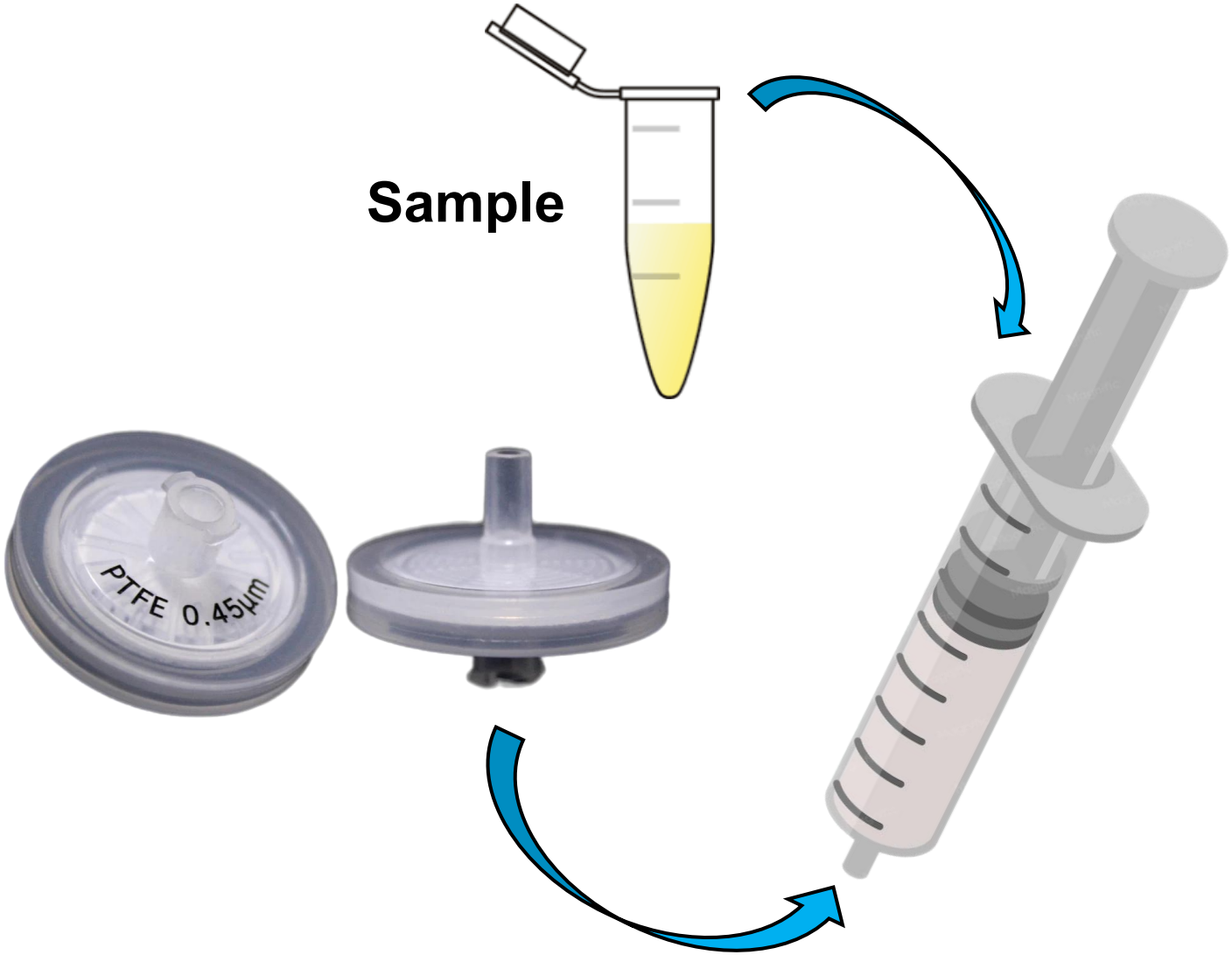
  Dilute the samples 1:2, 1:4, 1:10 in a final volume of 1000 µL.

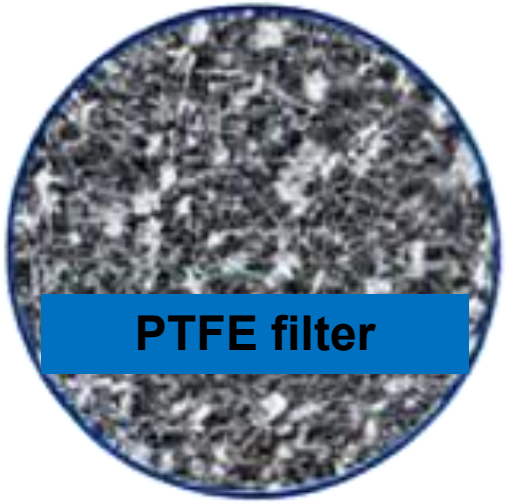


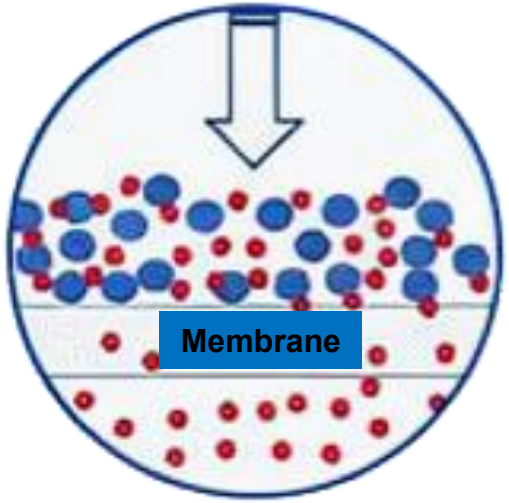
DILUTIONS	1:2	1:4	1:10	Blank
Extract (µL)				////////
Water (µL)				



PTFE filter extraction







7) The dose response curve

1) STANDARD

2) ANALYTE

3) DOSE RESPONSE CURVE

4) SAMPLE

5) MATRIX

6) INTERFERENTS

8) The dose response curve

SEE THE FOLLOWING VIDEO ON EXTERNAL CALIBRATION

https://www.youtube.com/watch?v=pYG9M1YMlvU&t=1s

External standard calibration:

When preparing independent standard curves from the samples → you create a series of separate, known-concentration standard solutions independently from the unknown samples

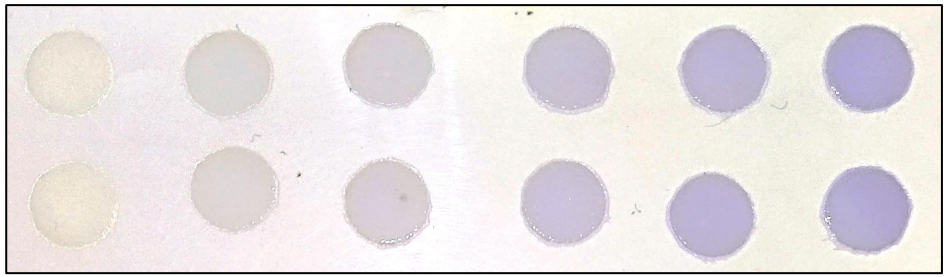
When I can use the extrenal standard calibration:

- **When sample matrices are simple and consistent (**low unpredictability**)**
- **When the samples undergoes to dilution effects concerning the potential matrix and interfering compounds**
- **When the composition of standards closely matches the sample, and during analysis I have low background interference or low matrix effect.**

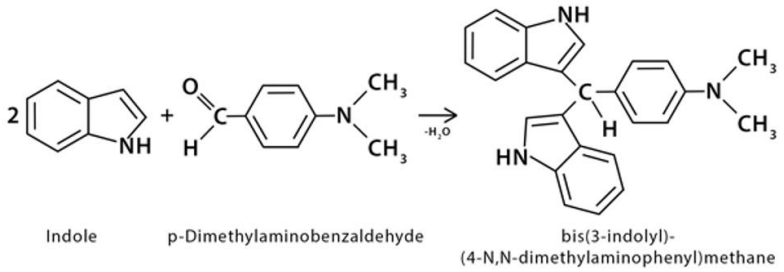
9) The analytical signal

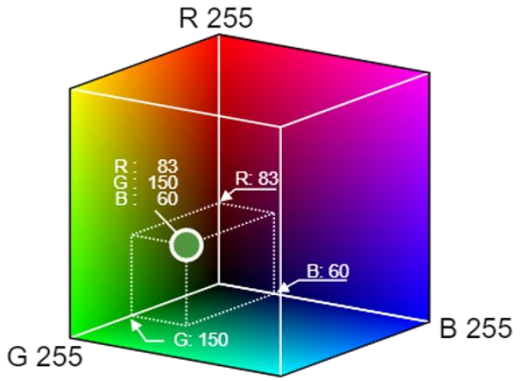


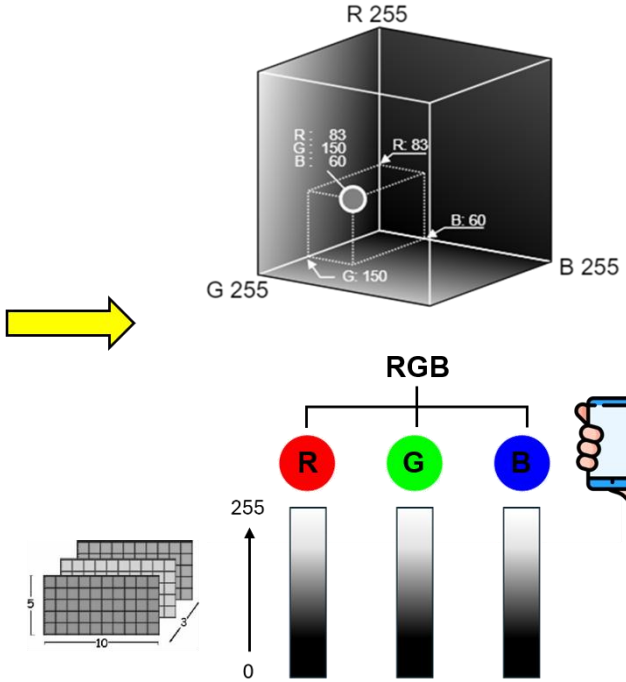
Color variation from blank to violet



$$A + B \longrightarrow AB$$

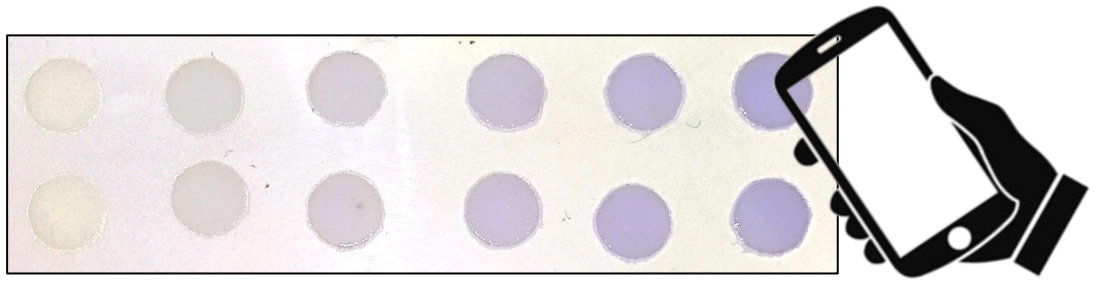


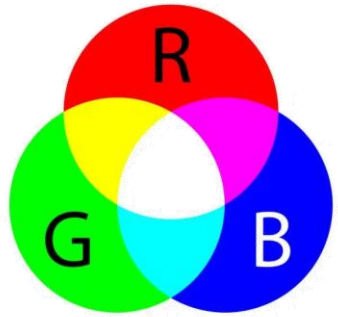




10) The analytical signal

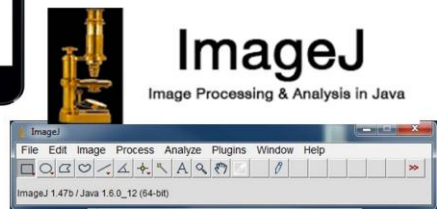
Colorimetric readout analysis



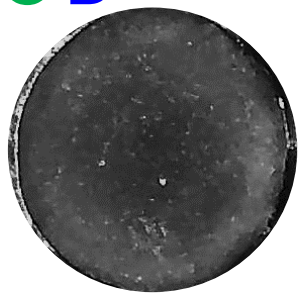


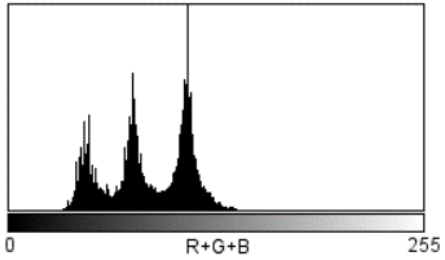


Google Play



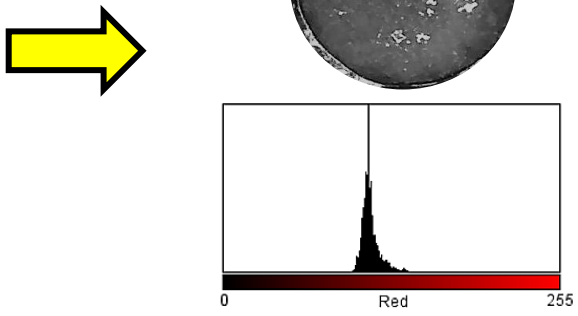
R G B





Count: 132192
Mean: 85.191
StdDev: 26.054

Min: 30
Max: 177
Mode: 110 (6566)



R

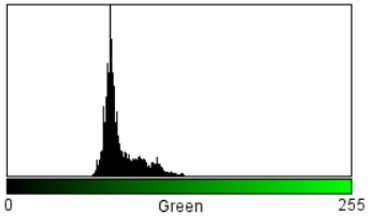


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StdDev: 7.450

Min: 96
Max: 172
Mode: 110 (6143)

G



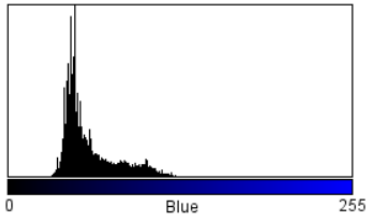


Count: 44064
Mean: 83.949
StdDev: 14.490

Min: 61
Max: 177
Mode: 76 (4141)

B





Count: 44064
Mean: 59.850
StdDev: 20.508

Min: 30
Max: 170
Mode: 49 (3039)

11) The analytical signal

Colorimetric readout analysis

Unknown

