BMOL2201/6201 PRACTICAL 2

Micropipettes, Spectral Scan, Standard Curve and Protein Determination

PM	AM	Outline
2:05	10:05	Introductory talk
2:15	10:15	**(A) Using Micropipettes** (Tutor demonstration)
2:20	10:20	(Go to iLearn and complete Pre-lab Quiz if reqd.)
2:25	10:25	**(B) Spectral scan to determine λ_{max}**
		(C) Standard absorbance curve and Protein determination by Bradford method
4:20	12:20	Clean up
4:30	12:30	Upload Prac 2 data file and complete Prac Quiz
4:55	12:55	Prepare to leave lab

Practical 2 Aims

A. Understand how to use a micropipette (demo).

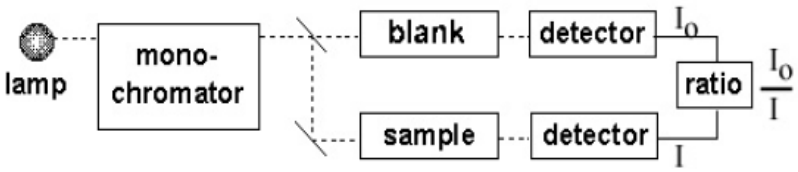
B. Determine the absorbance maximum (λ_{max}: lambda max) value for given protein solution, treated with Bradford reagent.

C. Using the Bradford method, generate a standard curve at λ_{max} for a series of known protein concentrations and use this to determine the protein concentration of the unknown solution and an abnormal protein, gelatin.

- *Related Lecture*:
  - Lecture 3: Amino Acids - 2

Measuring the absorbance of a solution

- A Spectrometer passes a series of wavelengths (denoted by Greek alphabet, lambda: λ) of light through a solution of a substance (sample) and solvent only (blank).



- For each wavelength the ratio of the intensity of light passing through the blank (I_0) to the intensity of light passing through the sample (I) is measured

- This ratio of intensities is reported as **Absorbance (*A*)**:

$$A = \log_{10} \frac{I_0}{I}$$

***A* has <u>NO</u> units**
A = 0 means that no light has been absorbed
A = 1 means 90% of the light at that wavelength has been absorbed
(i.e. sample 10% intensity of blank)

Measuring the absorbance of a solution (contd.)

• Many biomolecules exhibit unique absorbance spectra.

• The spectrometers we use can be set in **2 main modes:**

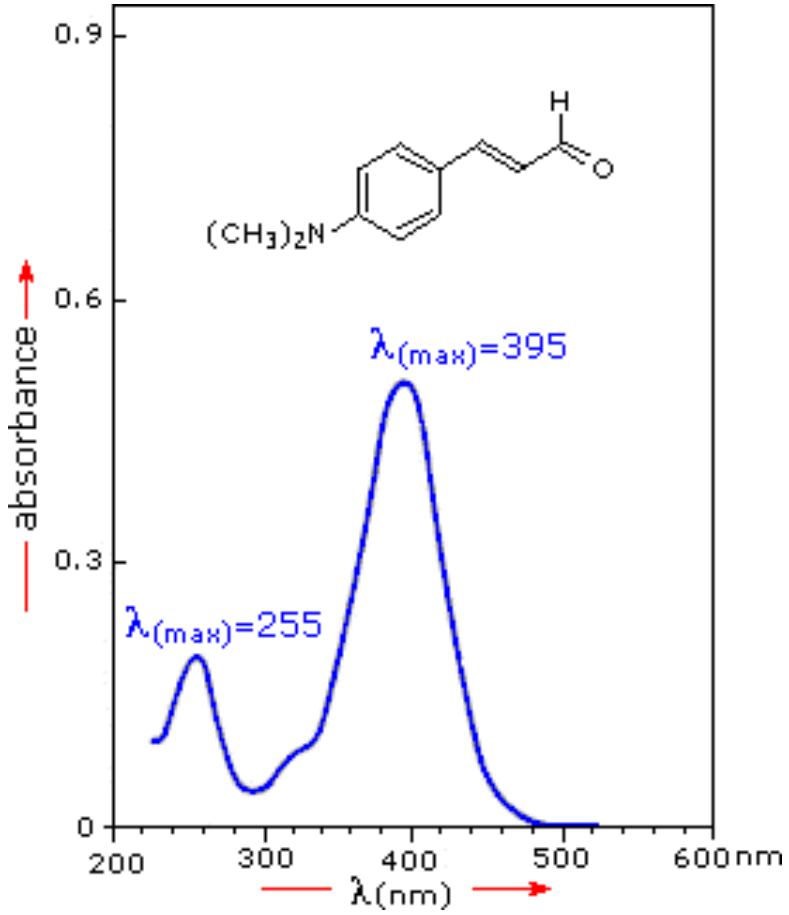
'Scan' mode

−Measures the full spectrum from a low to a high wavelength, that we set.

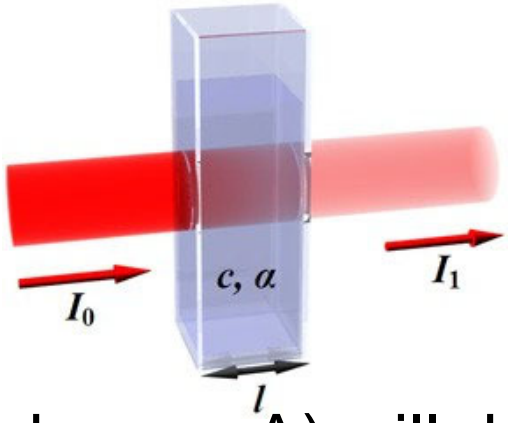
−We can use the cursor to scroll to view absorbance at a particular wavelength.

'Fixed' mode

−Measures the absorbance at a fixed wavelength (or wavelengths), normally at the maximum absorbance: λ_{max} (either specified or determined in scan mode) – we use the Greek alphabet **lambda** for wavelength.



Beer-Lambert's Law



- The amount of the light absorbed (absorbance, A) will depend on how many molecules it interacts with
  - ❖ **concentration (c)** of the sample (Beer's Law)
  - ❖ distance travelled by the light as it passes through the sample (**pathlength l**) (Lambert's Law)

$$A = \varepsilon\, c\, l$$

At a given wavelength, *A* is proportional to *c* for any substance if Beer-Lambert's law is obeyed. Also, l = 1 cm.

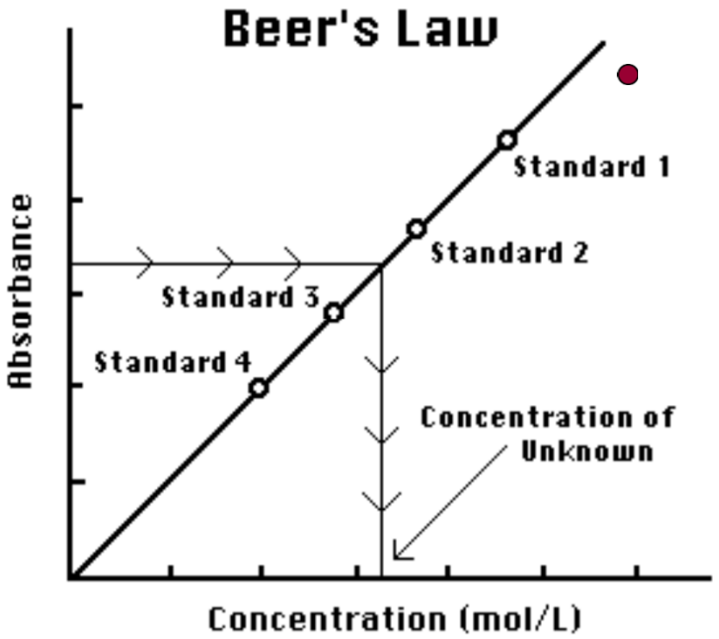
Beer's Law is used extensively

- For relating **concentration** to the optical measurement of '**absorbance**'
  - If we knew the absorbance values for known concentrations of a substance, we can then determine an ***unknown concentration*** of the same substance.
- With spectrophotometry, it can be used to distinguish and compare different molecules in solution:
  - Identify different molecules
  - Follow the course of a separation experiment
  - Follow the course of a chemical reaction

Deviations from Beer's Law

- **Instrument variation**
  - not a major problem with today's instruments
  - more important factor: cuvette (the vessel in which the samples are measured) variation

- **Sample**
  - pH
  - electrolytes
  - adsorption onto wall of cuvette
  - aggregation in solution
  - fluorescence
  - very high absorption coefficient
  - **high concentrations!**



- **Beer's Law basically applicable for very dilute solutions**
- **Reliable when A < 1.0**

To summarise

1. We need to measure λ_{max}
2. We need a series of dilute solutions
3. We can then determine unknown concentrations

- More importantly, we need to measure out small volumes.
- Our next step will be to understand what is dilution
- And then how to use micropipettes to measure out tiny volumes!!

Recap: Prefixes

Standard prefixes for the SI units of measure

Multiples	**Prefix name**		**deca**	**hecto**	**kilo**	**mega**	**giga**	**tera**	**peta**	**exa**	**zetta**	**yotta**
	Prefix symbol		da	h	k	M	G	T	P	E	Z	Y
	Factor	10^0	10^1	10^2	10^3	10^6	10^9	10^{12}	10^{15}	10^{18}	10^{21}	10^{24}
Fractions	**Prefix name**		**deci**	**centi**	**milli**	**micro**	**nano**	**pico**	**femto**	**atto**	**zepto**	**yocto**
	Prefix symbol		d	c	m	μ	n	p	f	a	z	y
	Factor	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-6}	10^{-9}	10^{-12}	10^{-15}	10^{-18}	10^{-21}	10^{-24}

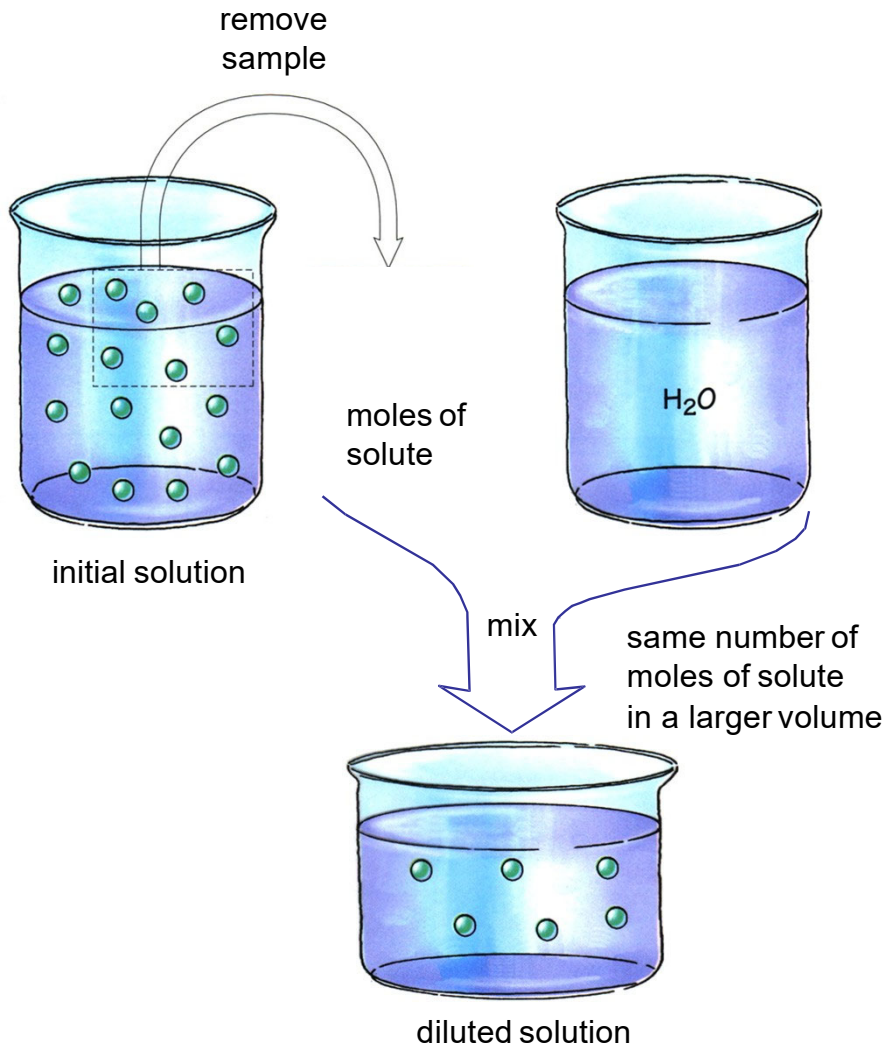
- Frequently used in Biochemistry:
  - Centi- (1/100) centimetre (cm)
  - **Milli- (1/1000) millilitre (mL)**
  - **Micro- (1/1000 000) microlitre (μL)**
  - Nano- (1/1000 000 000) nanometre (nm)

You will be using millilitre and microlitre volumes of liquid in this Prac. **1000 μL = 1 mL**.

What is dilution?

- ***Concentration*** is a measure of solute-to-solvent ratio
  - **Concentrated** solution has lots of solute, which can be accurately weighed.
  - ***Dilute*** solutions have *little solute* (are more watery) and it is quite difficult to weigh out such small quantities accurately.
- So, for accurate experiments, we just make **concentrated** solutions first – called **stock** solutions.
- We then make a dilute solution by accurately measuring out a quantity of this stock solution and then add the appropriate amount of solvent (usually water).

Making a Dilute Solution



Timberlake, Chemistry 7th Edition, page 344

How much do we need to remove?

$$c_{initial} \cdot v_{initial} = c_{final} \cdot v_{final}$$

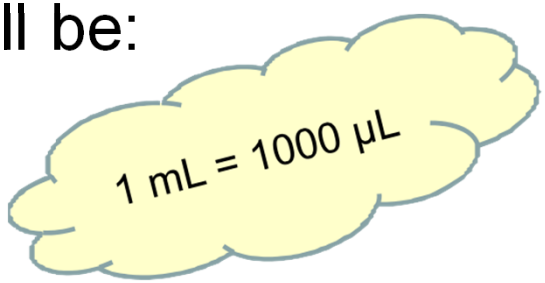
- For a 10 times dilution, remove **1 ml of stock** and make up to **10 ml solution** (i.e. by adding ***9 ml of solvent***).
- Note: concentration is inversely proportional to volume:

$$c_{initial}/c_{final} = v_{final}/v_{initial}$$

- Frequently, you will need **microlitres (µL)** of solution to be pipetted out.
- Also the std. solution is quite concentrated, say 100 µg/mL. If you pipette 50 µL, the amount of solute will be:

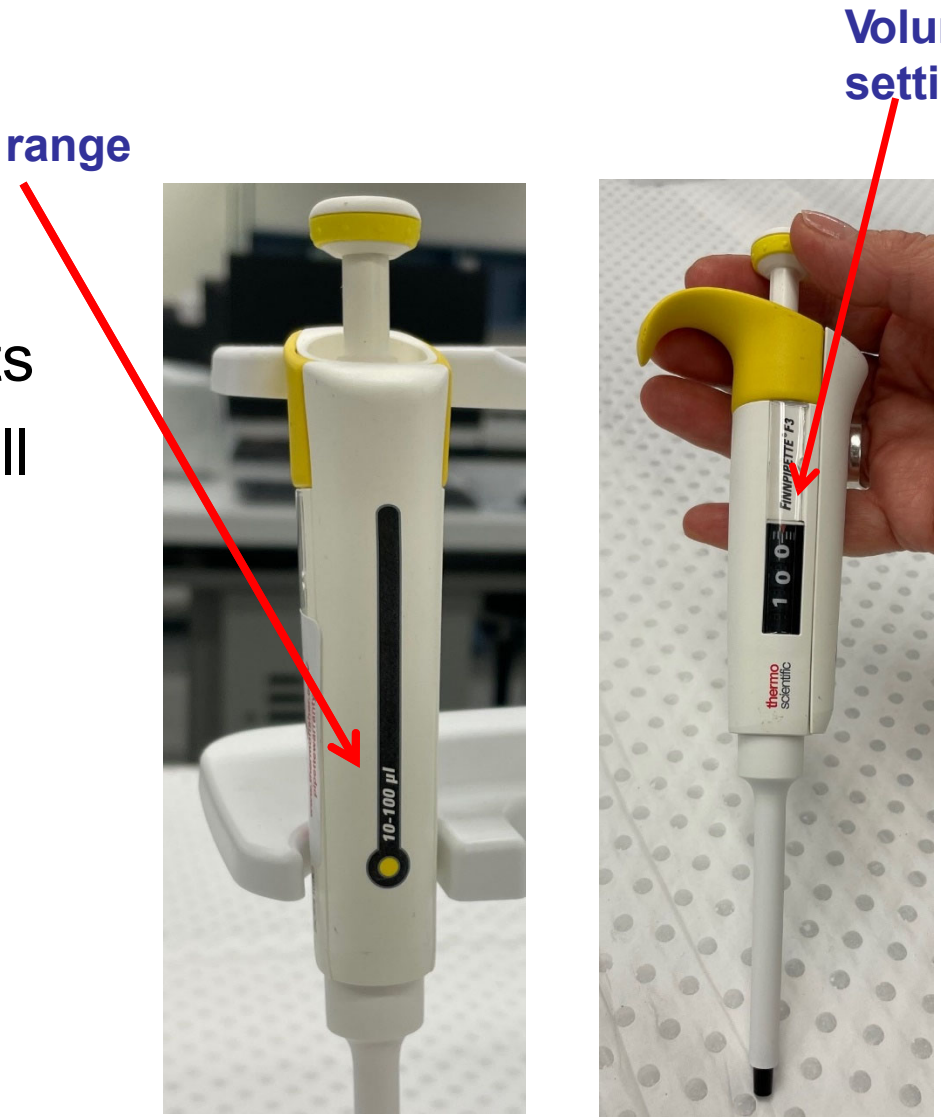
(100 µg/1000 µL) x 50 µL

= 5000/1000 µg = 5 µg.

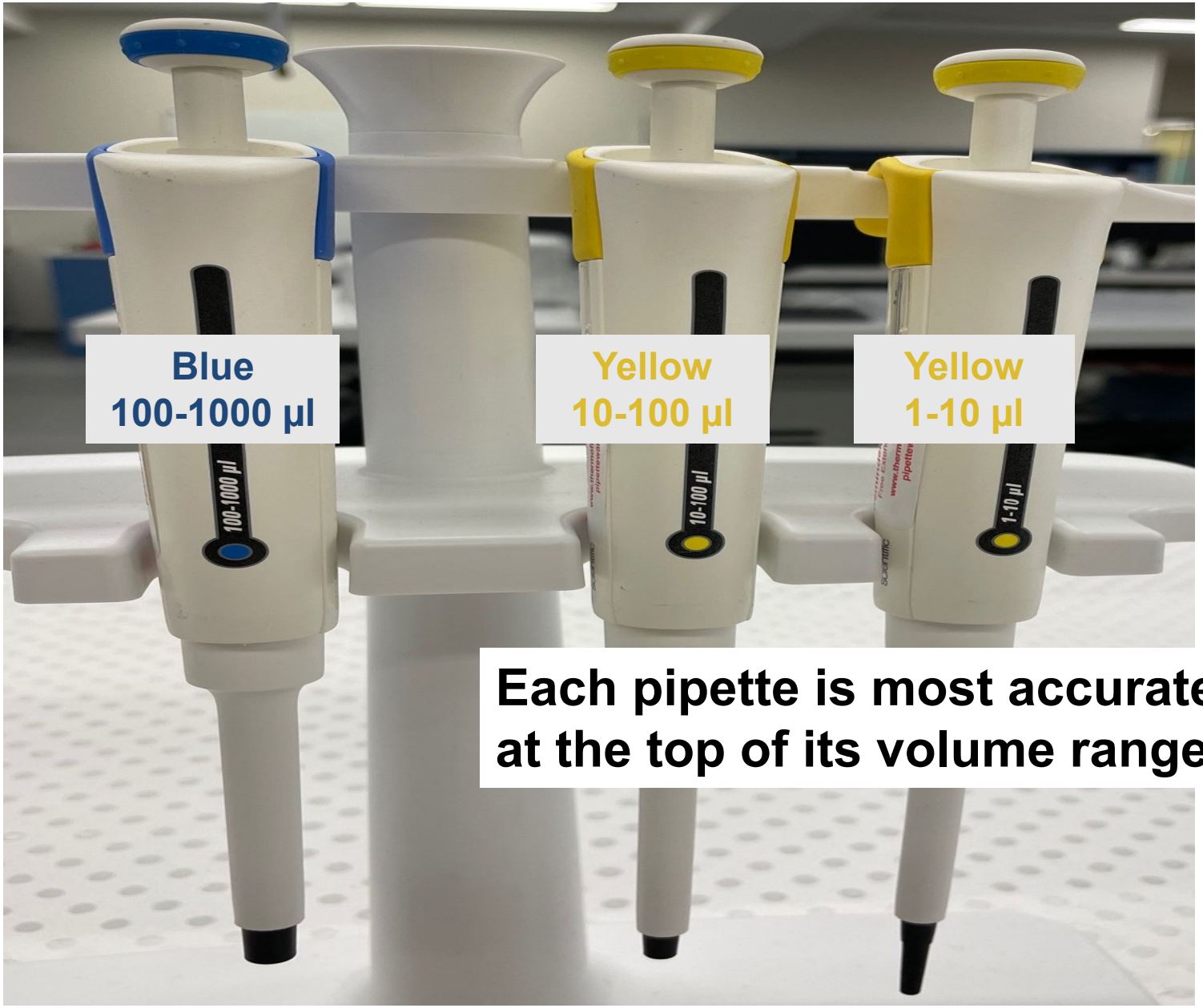


A. Using a Micropipette!

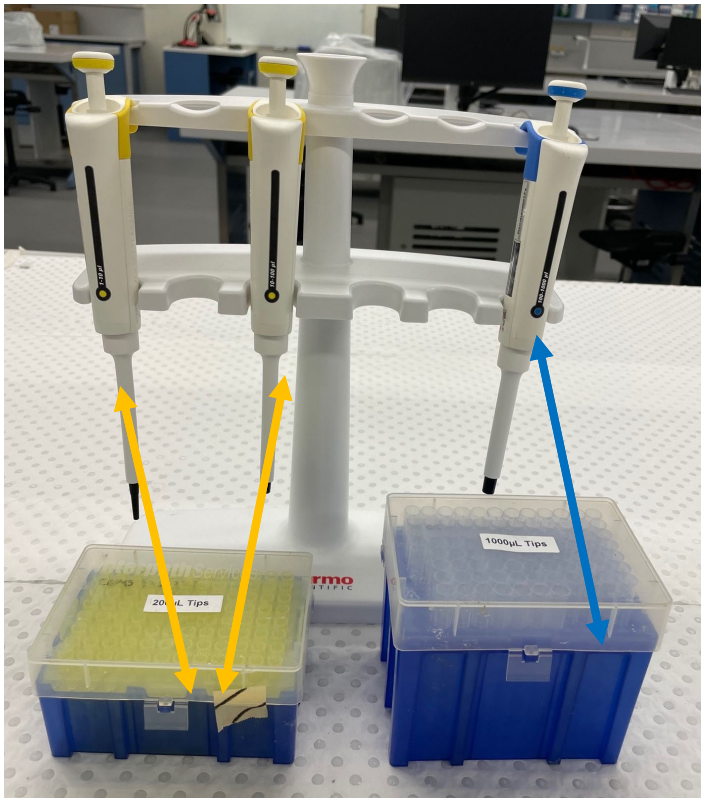
- New Pipettes!
- High precision instruments
- Vital for pipetting out small volumes
- Check the volume range first!
- You can set the required volume.



Different Micropipettes – plunger ring colour!



Micropipette: which tip do I use?



- See the micropipette video (iLearn) on how to pipette out small volumes

When all the pipetting is done, eject the used tip into the waste bin by pressing down here:

Tips while using micropipettes

- **Never change the volume setting without releasing the lock** – release the lock pulling the plunger head UP.

- **Never turn the volume setting above or below the volume range** listed on the side – this will break the pipette.

- **Never place the pipette on the workbench, with liquid in the tip,** to prevent chemical burns and contamination.
- ***Please watch the video carefully***!

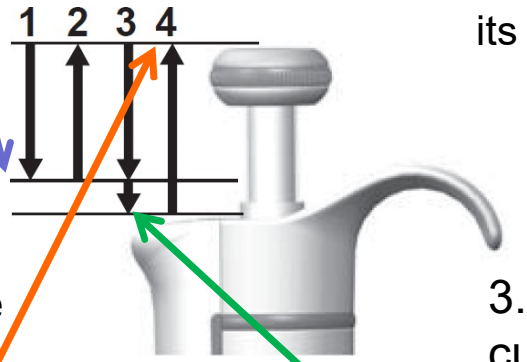
A. Using a Micropipette:

- Set the volume required and
- Fit the correct tip.

<u>$$ Pipettes are expensive $$</u>
<u>NEVER</u> wind a pipette above or below the volume range
<u>NEVER</u> put a pipette down when there is liquid in the tip
<u>ALWAYS</u> use the correct tip

1. Press the knob to the **first stop** – *do not force the knob down further.*

2. Dip the tip into the reagent keeping the knob down and slowly release it to its **normal position.**



Watch the micropipette video now!

4. Lift the pipette out and gently release the knob to the **starting position**. If repeating the pipetting, continue steps 1-4. for another reagent, change the tip.

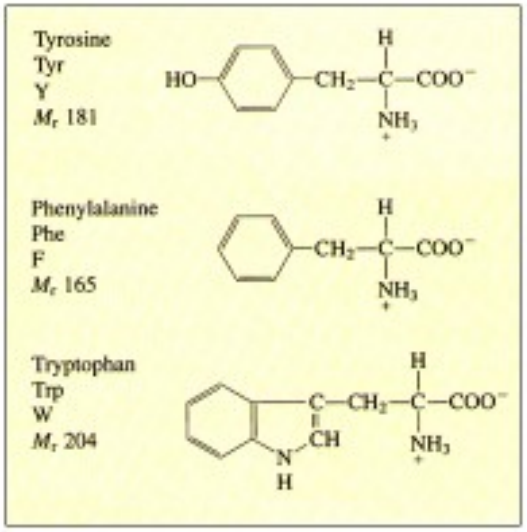
3. Deliver the liquid into a cuvette or beaker by pushing the knob down gently to the **first stop** and then to **second stop**, to empty the tip.

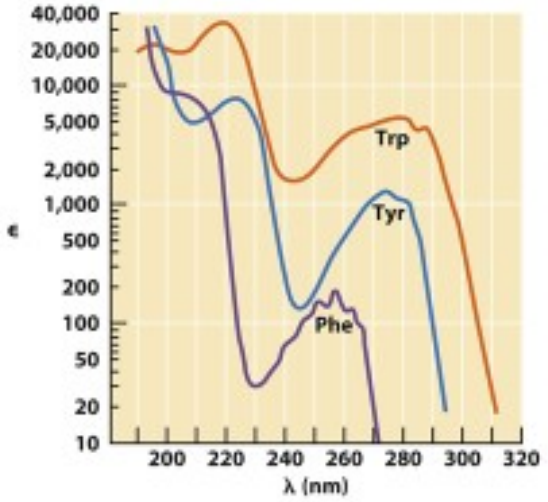
Lecture 3

- We know that proteins absorb light.
- However, in biological samples, their concentration is so low that hardly any light is absorbed.
- This affects our ability to measure their concentration reliably.

Aromatic amino acids absorb light!

- Tyrosine,
- Phenylalanine
- Tryptophan

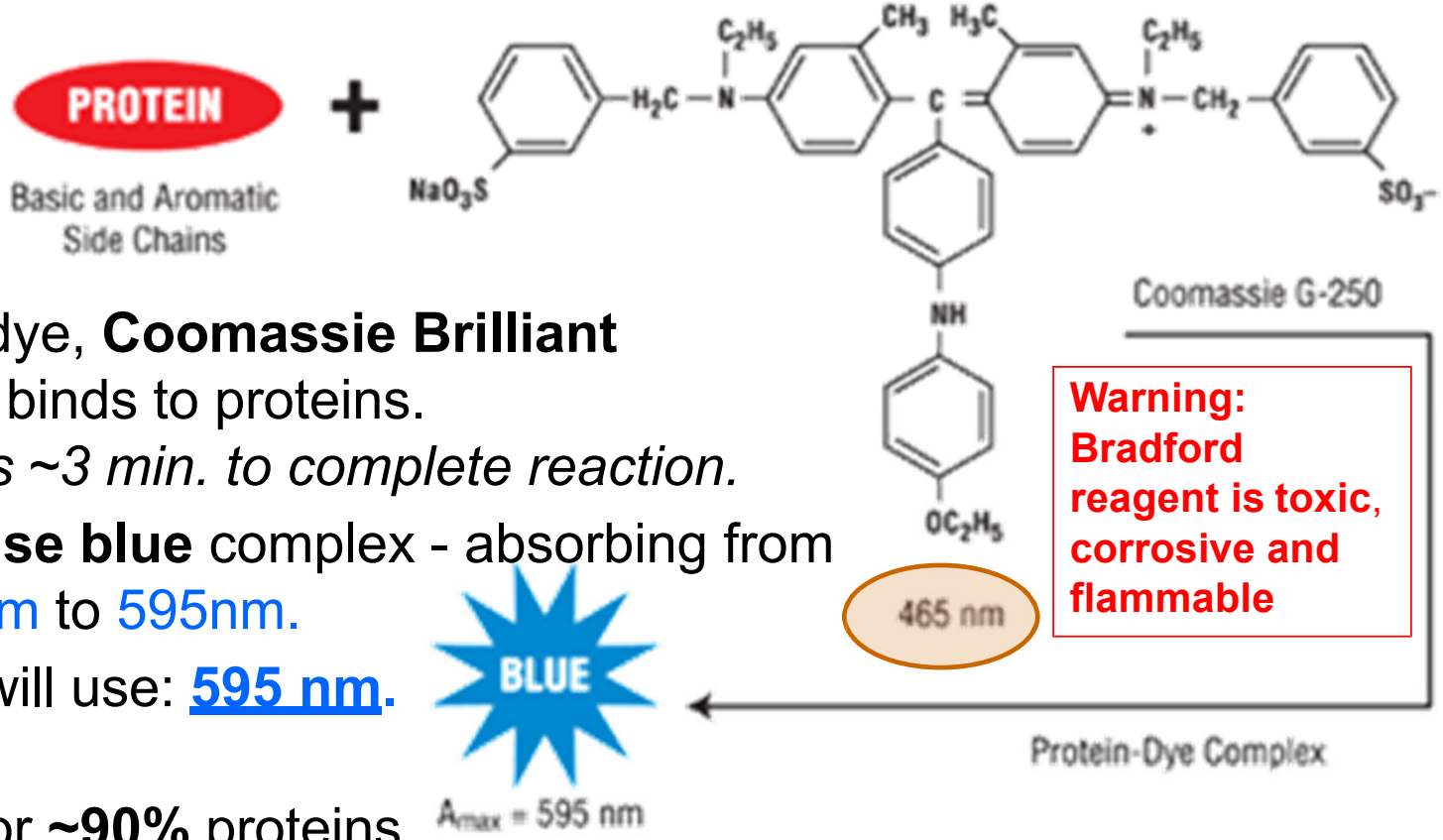




Therefore, most proteins absorb light near **280 nm**.

(VVP5: 5.2)

Protein determination by the Bradford method



- The dye, **Coomassie Brilliant Blue** binds to proteins.
- *Takes ~3 min. to complete reaction.*
- **Intense blue** complex - absorbing from 465nm to 595nm.

➢We will use: **595 nm.**

Warning: Bradford reagent is toxic, corrosive and flammable

- OK for **~90%** proteins
- Residues involved are arginine (R), histidine (D), lysine (K), tyrosine (Y), tryptophan (W) and phenylalanine (F)
- Very sensitive method for protein estimation.

Hazard statement(s)
H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.
H371 May cause damage to organs.

Precautionary statement(s)
P260 Do not breathe dust or mist.
P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.
P234 Keep only in original container.

Spills/Leaks
Contain spillage if safe to do so, and then soak up with inert absorbent material and place in container for disposal according to local regulations. Do not let product enter drains.

CAS-No. : 7664-38-2

Bradford Reagent

DANGER

MACQUARIE UNIVERSITY, SCIENCE, NORTH RYDE 2113. PH 9850 7112

First Aid

If inhaled, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact, Take off contaminated clothing and shoes immediately, wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

In case of eye contact
Flush eyes with water for at least 15 minutes.
Consult a physician.

If swallowed
Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water.
Consult a physician.

Additional information is listed in the Material Safety Data Sheet

Bradford reagent is toxic and may cause damage to organs.

Measuring absorbance: using the BioMate 3S: UV-Vis Spectrophotometer

- Operated via the lab PC
- Vision*Lite* software is on the desktop
- The default application is “Scan” – we use this for **Part B.**
- We will change the default setting to “Fixed” for **Part C.**
- **Watch the spectrophotometer video now!**

Before you start the experiment

- (Lab Safety video: *view this if you did not attend Prac 1*)

Prac 2 relevant videos guides for setting up and using equipment:

- Micropipette video
- Spectrophotometer video

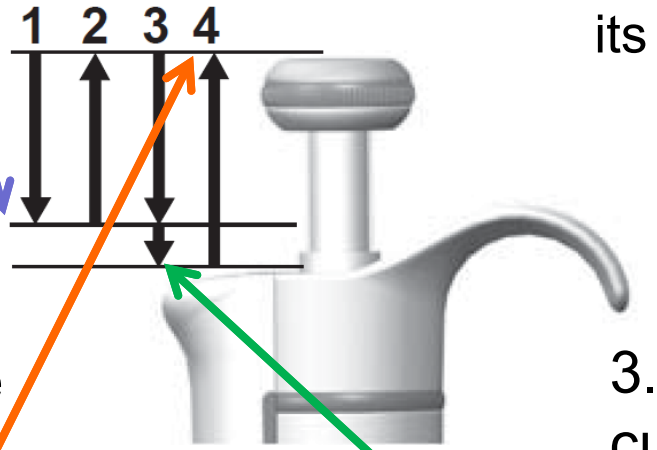
- (Waste disposal video: *view only if you did not attend Prac 1*)

A. Using a Micropipette: Tutor demo

- Set the volume required and
- Fit the correct tip.

$$ Pipettes are expensive $$
NEVER wind a pipette above or below the volume range
NEVER put a pipette down when there is liquid in the tip
ALWAYS use the correct tip

1. Press the knob to the **first stop** – *do not force the knob down further*



2. Dip the tip into the reagent keeping the knob down and slowly release it to its **normal position.**

3. Deliver the liquid into a cuvette or beaker by pushing the knob down gently to the **first stop** and then to **second stop**, to empty the tip.

4. Lift the pipette out and gently release the knob to the **starting position**. If repeating the pipetting, continue steps 1-4. for another reagent, change the tip.

B. Procedure: Step 0

What to do - all students

1. You need **covered shoes**!
2. No **bags** on lab bench – place **in under-bench storage**
3. **Tie** up your **hair**, if applicable – **<u>tuck long hair into coat</u>**
4. **Button up** your **lab coat**
5. Put on **safety glasses**.
6. No food or drink please!
7. No laptops during experiment.






Need help?

- Lab Safety video

B. Procedure: Step 1: Getting started

Each team to collect one tray and fill it as follows:

Student 1 (gloves on)

A. Collect from the backbench and put into the tray:

1. a **cuvette holder**
2. 10 cuvettes
3. Bradford Reagent
4. 5 mL Pipette (green ring)
5. **100-1000 µl pipette** (blue ring)
6. **10-100 µl pipette** (yellow ring)
7. **Tips for these pipettes**
8. **A tube rack**

Student 2 (gloves on)

A. Collect from the backbench and put into the tray:

1. a **wash bottle**
2. a box of **Kimwipes**
3. a **liquid waste container**
4. a **used tip container**
5. a **whiteboard marker**
6. a **Parafilm square container**

- Take the tray back to your desk
- The rest of the solutions are on your desk – mostly in the esky (icebox).

B. Procedure: Step 2

Student 1 (no gloves)

1. Login in with your student ID and go to iLearn (those who have not done their pre-lab can do it now).
2. Download and open Prac2 excel file from iLearn, **save on the Desktop** and fill in your desk number and team details on the first worksheet.

Two teams share the spectrophotometer by switching the connecting **USB cable** – pl. be patient. You may call your tutor to help.

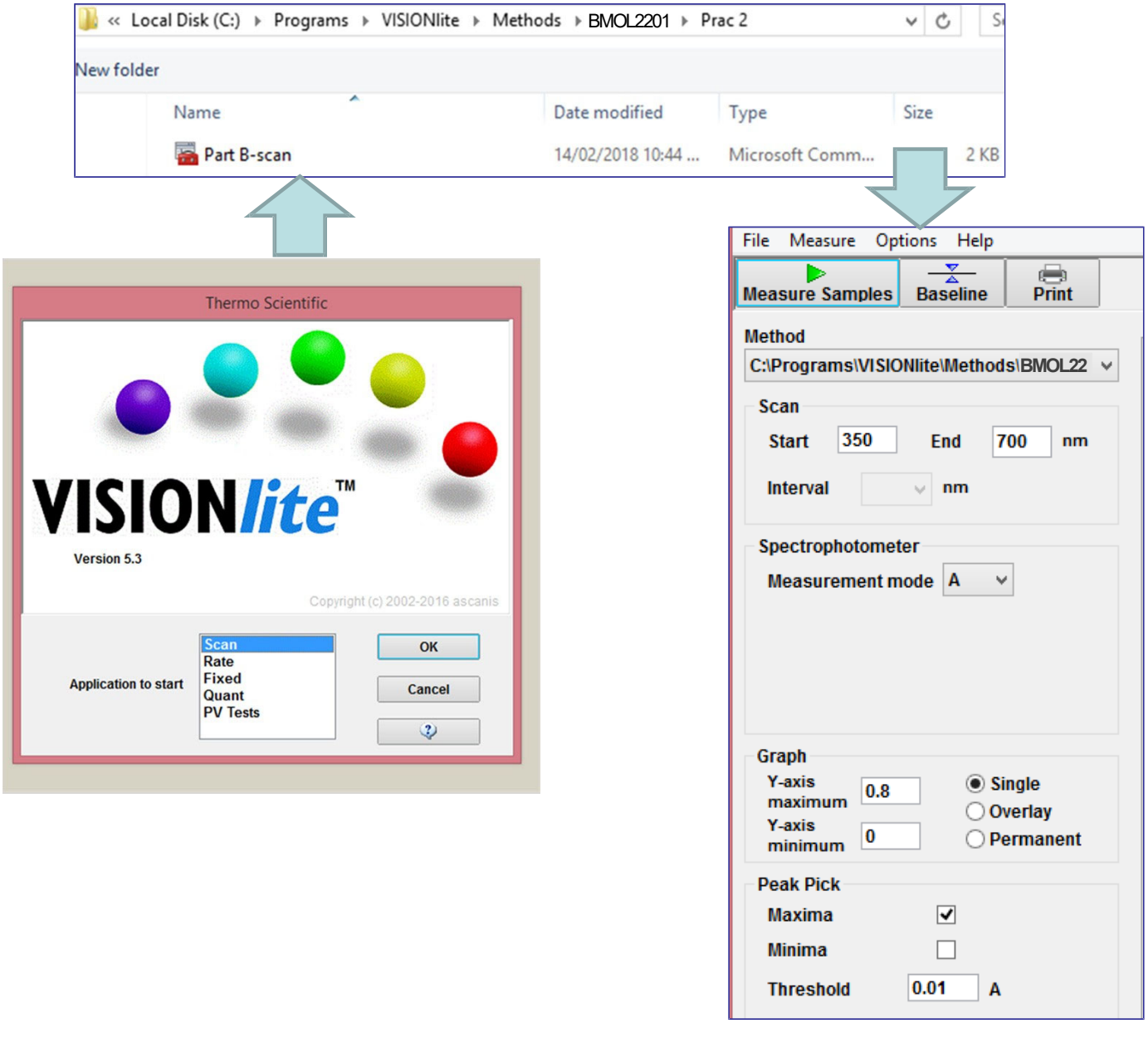
3. Double click on Vision*Lite* software on the Deskop and load the default **Scan** application. (*Check:* the spectrophotometer screen should display **REMOTE MODE** – if not restart the software).
4. Go to

   File→Open Method→BMOL2201→Prac2→Part B-scan

   from the menu bar, to load parameters (refer next slide).
5. Click **Baseline** (at the top). After a few mins, you should have "**A = 0.000**" at the top right hand side.

Notes:

- ***Baseline correction** is done without any cuvettes in the spectrophotometer, i.e. in an **empty state – always good to check first.***
- *It is only done once during the prac.*


« Local Disk (C:) ▸ Programs ▸ VISIONlite ▸ Methods ▸ BMOL2201 ▸ Prac 2
New folder
Name
Date modified
Type
Size
Part B-scan
14/02/2018 10:44 ...
Microsoft Comm...
2 KB
Thermo Scientific
VISIONlite™
Version 5.3
Copyright (c) 2002-2016 ascanis
Application to start
Scan
Rate
Fixed
Quant
PV Tests
OK
Cancel
File
Measure
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Help
Measure Samples
Baseline
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Method
C:\Programs\VISIONlite\Methods\BMOL22
Scan
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Measurement mode
A
Graph
Y-axis maximum
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Y-axis minimum
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Single
Overlay
Permanent
Peak Pick
Maxima
Minima
Threshold
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A

B. Procedure: Step 3

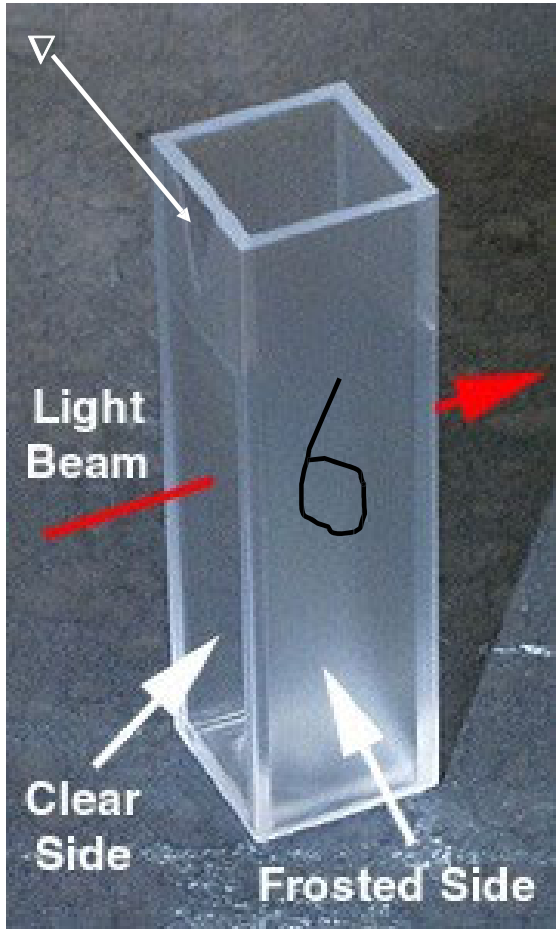
Student 2 (gloves on)

Need help?

1. Take 2 cuvettes and label them '**B**' and '**0**' *on the frosted side*, with the marker provided.
2. Use a suitable pipette and tip to add **150 µl** of the BSA standard solution (in the esky) to the bottom of cuvette **0**. *Put the tip in the used tip container. Put the BSA standard solution back in the esky.*
3. With another pipette and tip, add **3 ml** or **3000 µl** **Bradford reagent** into **both cuvettes**. Cover the top of cuvette **0** with the plastic side of the parafilm square, invert 4-6 times to mix, then wait **3 min** for the chemical reaction to complete. Wipe the sides of the cuvettes with a clean wipe.
4. After the baseline correction is complete, place cuvette **B** in the slot marked '**B**' and cuvette **0** in the slot '**1**' in the spectrophotometer chamber, with the clear sides facing the holes in each slot.

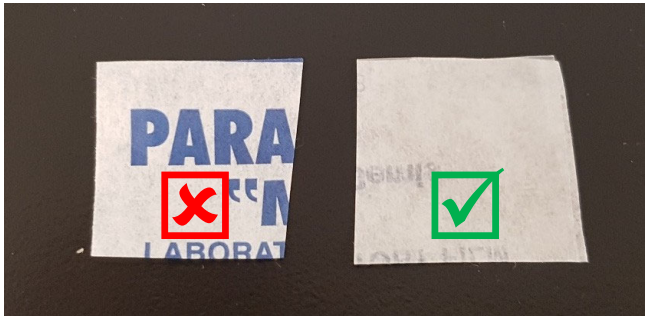
- Micropipette video
- Spectrophoto-meter video

The cuvette is what solutions go in!



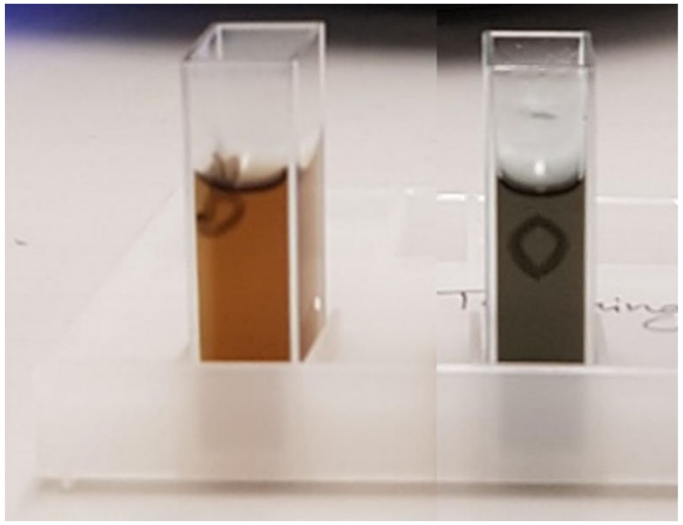
- The ***cuvette*** is a small tube-like container with straight sides and a **1 cm x 1 cm** square cross section.
- It holds **solutions** for **spectroscopic measurement**.
- It has **two clear sides** (**one marked with a ∇**) through which a beam of light is passed for measuring absorbance.
- *The other two sides are frosted for holding the cuvette and for labelling*, with a **non-permanent marker**.
- You will add the required liquid(s) into the cuvette using **a micropipette**.

Mixing solutions in the cuvette



- To mix the contents of a cuvette, you need to use **a protective covering**.
- Use **parafilm** pieces (aka “paraffin squares” – shown above)
- The parafilm has **two sides – one of paper and a plastic film on the other.**
- ***Place the plastic side on top of the cuvette*** *for mixing – do not peel it off.*

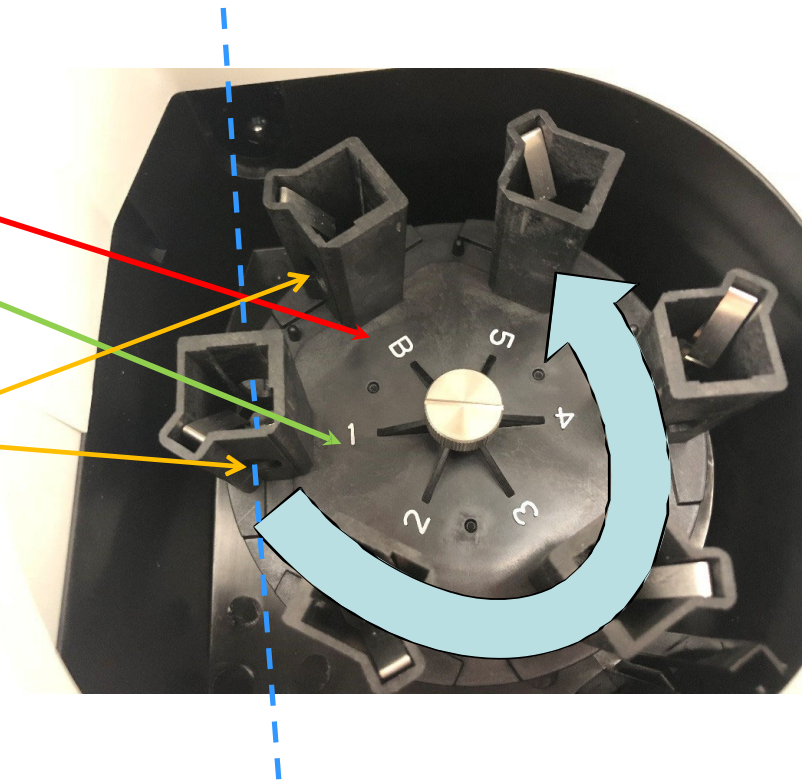
The solutions look like this



- The **Blank**, marked **B** remains brownish – contains only Bradford reagent!
- The protein solution, marked **0**, turns bluish.
- We need to put these into the spectrophotometer to measure the absorbance values.

Where to put the cuvettes? Open the lid to see the spectrophotometer's 6 cell/cuvette chamber:

- **Samples go here**
  - **B** for **Blank**
  - **1-5** for **samples**
- The transparent side of the cuvette should be against the holes for light to pass through the sample



B. Procedure: Step 4

Student 1 (no gloves)

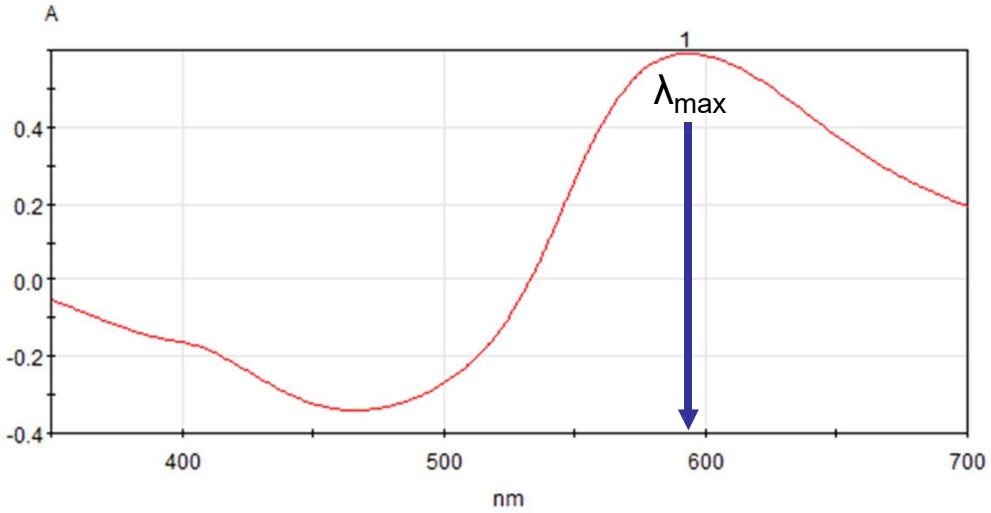
1. Click **Measure Sample** (at the top) and the sample information window will open up.
   a. Fill in your name/initials as operator and click to select the **Measure Blank** option.
   b. Against location 1, type in **0** and add **Spectral Scan** as description.
   c. Click **Measure**.
2. After the *spectral scan graph* is complete, click **Close** in the sample information window.
3. Click on **autoscale** at the bottom of the graph.
4. Go to the **Prac2 Data file** and **record the value of λ_{max}** on the ***B Spectral scan* w**orksheet and save.

B. Procedure: Step 5

Student 1 (no gloves)

1. Go back to **VISION*lite*** and then, select **File→Print Preview** *via* the menu bar. The preview will open in another window.
2. In the *B Spectral Scan* worksheet, go to **Insert→Screen Shot→Screen Clipping**. Select the screen clipping image shown – this will show the Print Preview window.
3. Select the **graph *with the mouse*** from the **print preview window** – you can choose the exact rectangle you want to extract.
4. This will automatically **paste an image of the graph** into the Excel file.
5. **Save the Excel file**. Check that you see the graph properly. (If not, delete the image and **repeat steps 1.-3.** and save the Excel file.)
6. Go back to **VISION*lite*** and then click **Clear** (at the bottom of the graph).

Assumptions while using Beer's Law

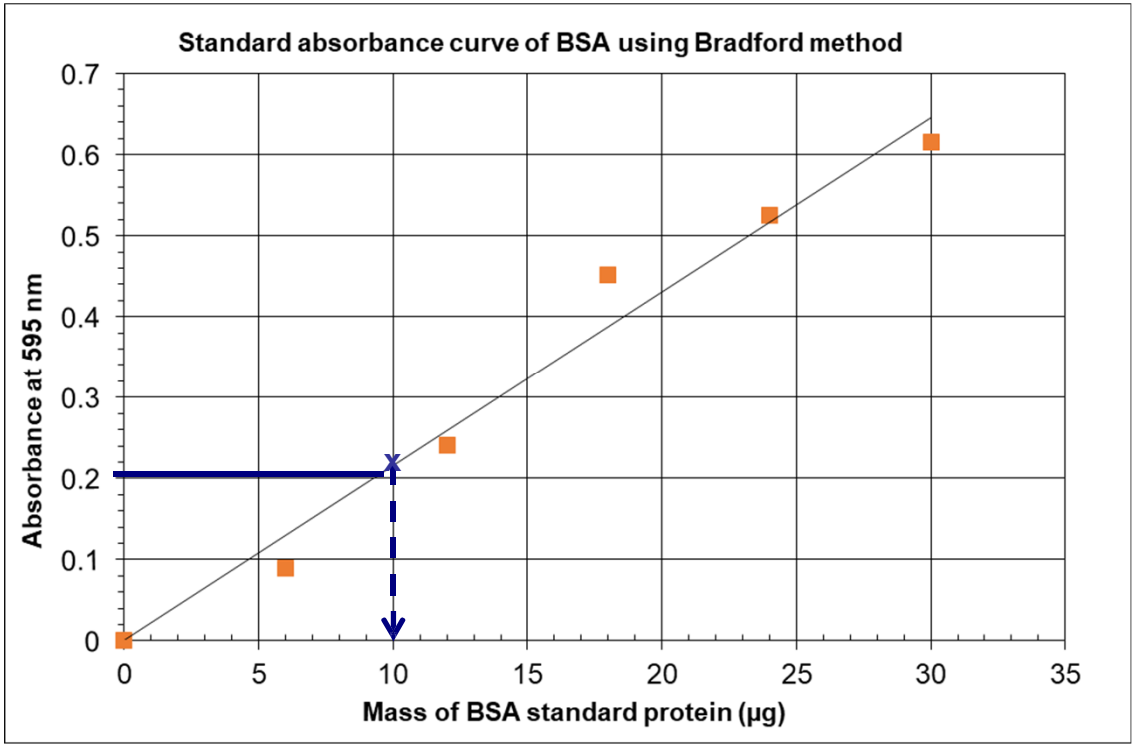


Example graph for protein treated with Bradford reagent

- The experiment to determine concentrations is conducted at a wavelength where the substance shows **maximum absorbance**: λ_{max}, (lambda max; see graph above), to minimise experimental error.
- **Assumptions made:**
  - **only the substance of interest** absorbs at the experimental wavelength.
  - the solutions are **dilute enough** to obey Beer's Law.

Standard Absorbance Curve

- **Used to determine the concentration of an unknown solution, when the max. absorbance wavelength (λ_{max}) is known.**
- Measure **single wavelength absorbance** for a series of solutions with a **range of known concentrations,** made by **diluting** specific amounts of the **stock solution**, using a ***micropipette***.
- **Plot absorbance vs. concentration**
- The graph should be **linear,** enabling a **straight line** to be drawn.
- To find concentration of an **unknown solution**, **measure its absorbance and read off the graph!**



Unknown A_{595} = 0.22

Unknown mass = 10 µg

B. Procedure: Step 6

Student 2 (gloves on)

- ***Leave cuvette B in the Blank position for Part C***.
- Remove cuvette **0** in slot **1** and **tip the solution** carefully into the liquid waste container.
- Place the used cuvette in the appropriate collection tray, on the back bench.

Need help?

- **Waste disposal video**

End of Part B - Students to swap places now.

C. Procedure: Step 1a

Student 1 (gloves on)

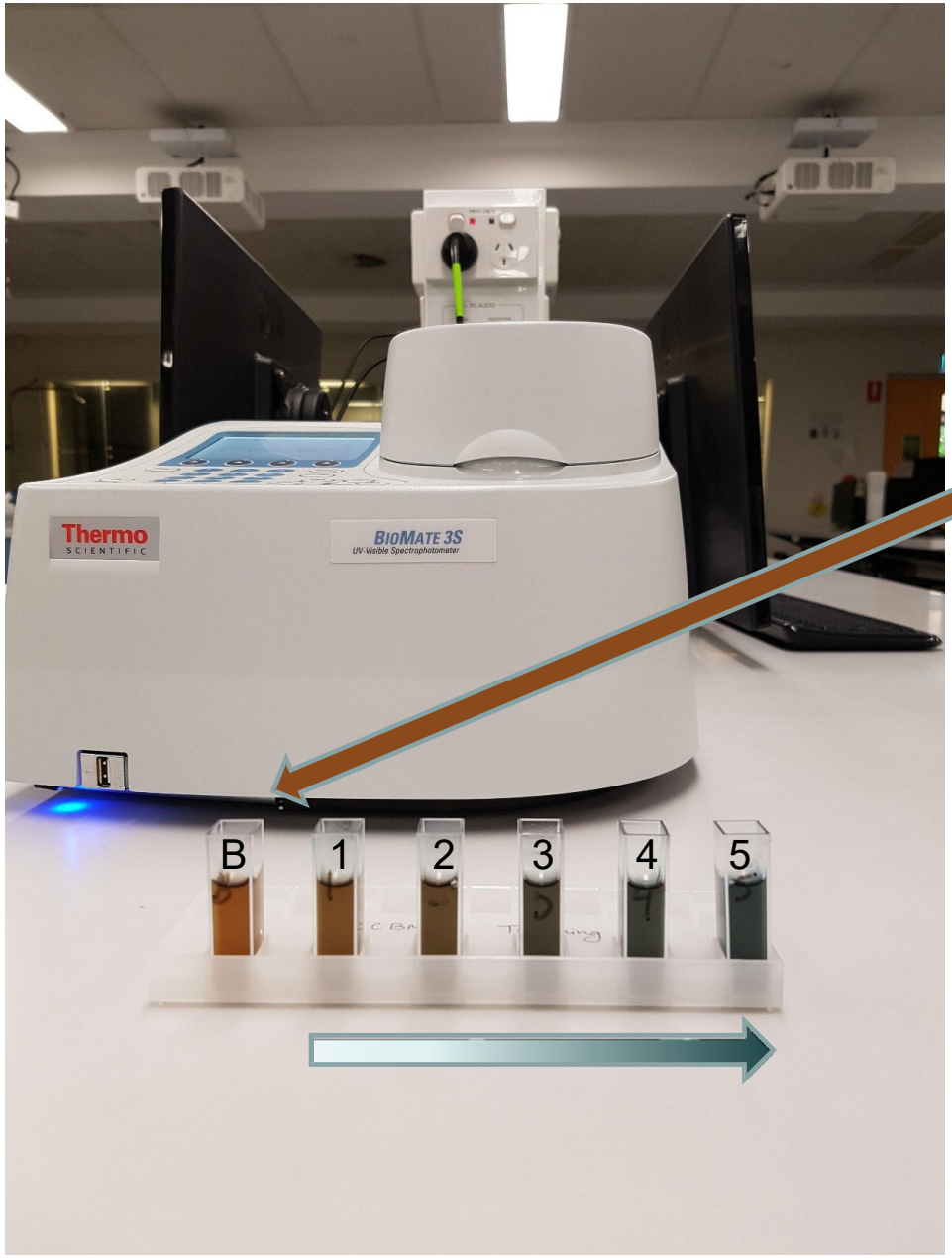
1. Label the remaining **8** clean cuvettes **1, 2, …8** on the frosted side only, using the marker provided and place them in the cuvette stand.
2. Set up cuvettes **1-5** for the Bradford assay **standard curve**, as in **Table 1** on the Prac 2 excel file: tab "C. Std Curve & Unknowns". Use **a suitable micropipette and the same tip** for pipetting out BSA stock solution as stated in Table 1. *Put the tip in the used tip container. Put the BSA stock solution back in the esky.*
3. ***With the same micropipette and a fresh tip,*** set up cuvettes **6 and 7** with the **'unknown' BSA** solution (see **Table 2**). *Put the tip in the used tip container and the unknown solution back in the esky.*
4. ***With a fresh tip,*** add **gelatin** to cuvette **8** (see **Table 2**). *Put the tip in the used tip container.* Leave the gelatin on the bench.

C. Procedure: Step 1b

Student 1 (gloves on)

5. Use **a <u>second suitable micropipette</u>** and **a fresh tip** to add **3000 µl (3 ml) Bradford reagent** into **<u>all the cuvettes</u>**.
6. **Mix each solution for 1 min** with the <u>plastic side</u> of the parafilm square, and **wait for 3 min.** for the reaction to complete. (see slide 40)
7. Wipe clean and place cuvettes **1-5** into positions or **slots 1-5** in the spectrophotometer, with the clear sides facing the holes in each slot. The **blank solution from Part B (cuvette B)** should already be in the slot marked **B** (see slide 41).

- ***The chamber will now have all six positions filled.***

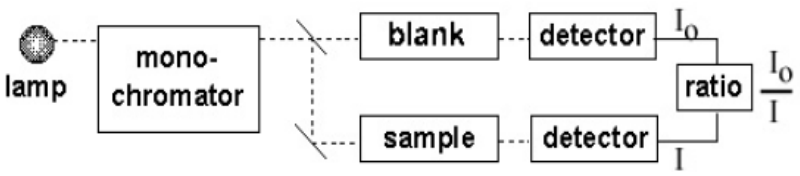


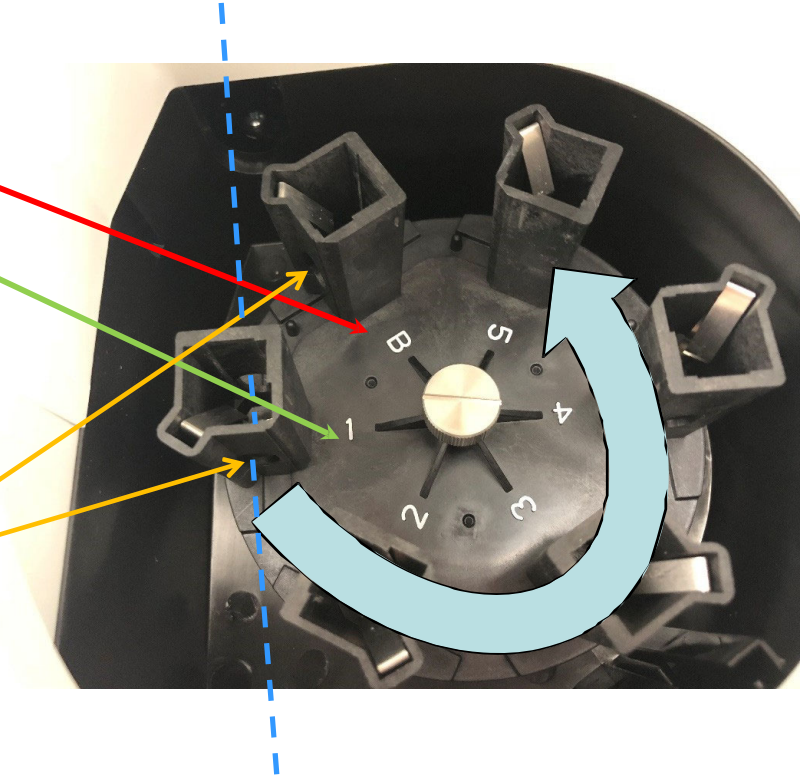
The standard solutions for Part C look like this

- The blank (cuvette B) contains only Bradford reagent and stays brown.
- The standard solutions (as in Table 1; cuvettes 1-5) get bluer as the amount of protein increases.

Where to put the cuvettes? Open the lid to see the spectrophotometer's 6 cell/cuvette chamber:

- **Recall:** we need a **reference or blank** solution to measure absorbance
  - ➢ **B** for **Blank**
  - ➢ **1-5** for **samples**





- The transparent side of the cuvette should be against the holes for light to pass through the sample

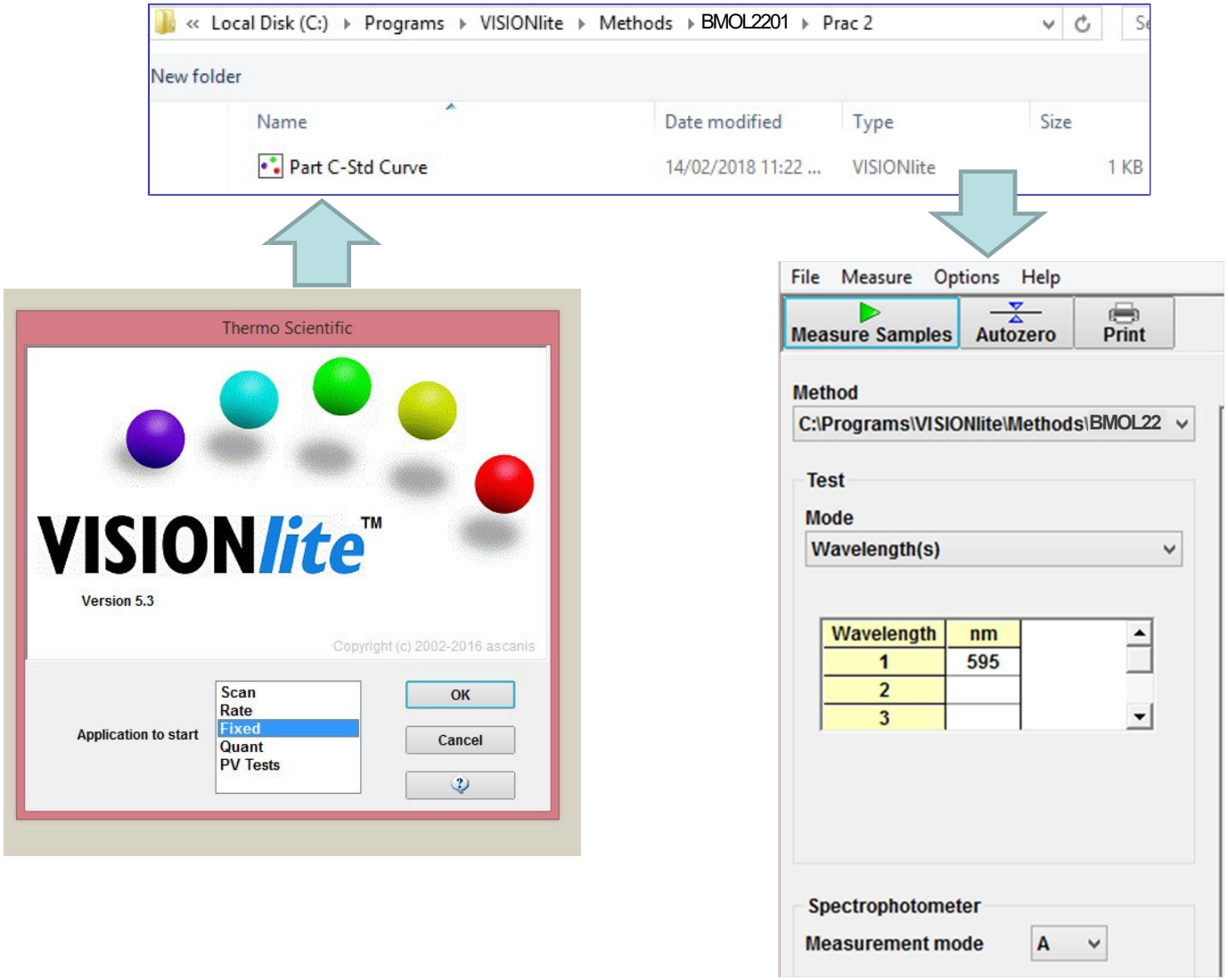
C. Procedure: Step 2a

Student 2 (no gloves)

1. In the **VISIONlite** window, go to **File→Change application**
2. Select **FIXED** from the **start** options and click OK. {If it loads **SCAN** application by default, repeat Step 1}.
3. The spectrophotometer screen should display **REMOTE MODE**. {If not, close the VISIONlite program and open it again with the selection FIXED}.
4. Select the following options *via* the menu bar:

   File → Open Method → BMOL2201 → Prac2 → Part C-Std Curve

   to load the pre-set parameters (refer next slide).


« Local Disk (C:) ▸ Programs ▸ VISIONlite ▸ Methods ▸ BMOL2201 ▸ Prac 2
New folder
Name
Date modified
Type
Size
Part C-Std Curve
14/02/2018 11:22 ...
VISIONlite
1 KB
Thermo Scientific
VISIONlite™
Version 5.3
Copyright (c) 2002-2016 ascanis
Application to start
Scan
Rate
Fixed
Quant
PV Tests
OK
Cancel
File
Measure
Options
Help
Measure Samples
Autozero
Print
Method
C:\Programs\VISIONlite\Methods\BMOL22
Test
Mode
Wavelength(s)
Wavelength
nm
1
595
2
3
Spectrophotometer
Measurement mode
A

C. Procedure: Step 2b

Student 2 (no gloves)

5. To measure the absorbance, click **Measure Samples** (at the top of the screen) and sample information window will open up.
6. Type in **Std Curve-<u>your name</u>** as the name of the **Result File**, your name as the **operator** and type in **1** against **Location 1**.
7. Click **Fill down.** This should populate all the locations with the respective cuvette numbers (from 1 to 5).

C. Procedure: Step 3

Student 2 (no gloves)

1. In the Vision*Lite* window, click **Measure**. When the measurement is complete, the **sample information** window will pop up again.

Student 1 (gloves on)

2. **Remove** the cuvettes carefully from **slots 1 to 5 only - leave cuvette B** (blank solution) **in slot B.**
3. **Wipe** and **place** cuvettes **6, 7 and 8** in **slots 1, 2 and 3.**

Student 2 (no gloves)

4. In Vision*Lite*'s **sample information** window, type **6** in **Location 1**, **7** against **Location 2** and **8** in **Location 3**.
5. Click **Measure** again. When the measurement is complete, sample information window pops up again. Click **Close**.

C. Procedure: Step 4

Student 2 (no gloves)

- Go to **File→Export Results** and save results as **partC.csv** file on the desktop first and then copy it to USB or OneDrive (we have done this for you!)

C. What you need to do

1. Double click on the **partC.csv** file, to open it with the Excel program. **Select and copy** the **Absorbance values** for **samples 1-5,** in the column labelled **"595nm."**
2. Go to the ***Prac 2 Excel file*** '**C Std Curve & Absorbance**' worksheet and **paste** the **copied values** into Table **1**. Then c**opy** the **Absorbance values** for **samples 6-8** into **Table 2**. **Save** the Excel file.
3. Go back to **Table 1** and **calculate the amount of protein** for cuvettes **1-5**, using the **concentration (200 μg/mL)** and the **volume** of the **BSA standard solution** added to each cuvette.

(Hint: 200 μg in 1 ml – so how many μg in 30 μl?, etc.)

Procedure: Step 5 – Part C

Student 2 (no gloves)

1. Save the Excel file. **The graph should update itself**.
2. Use the mouse to move the coloured lines (*as you did in Prac 1*). From the **Absorbance values** recorded for **cuvettes 6-8**, to determine the amount of protein in these samples from the graph. **Record your values in Table 2**. **Save** the Excel file.
3. **Calculate and record the concentration of protein** in the **'unknown'** BSA solution (average of values from #6 and #7), and **the apparent concentration** of protein in the 200µg/mL **gelatin** solution (#8). Save the Excel file.

C. Procedure: Step 6a – Packing up

Student 1 and Student 2 (gloves on)

1. **Wipe your bench clean with a Kimwipe.**
2. **Tip the solution** from all the cuvettes into the **liquid waste container.**
3. **Take the tray back to the back bench.**
4. **Tip the liquid waste into** the **liquid waste disposal container**.
5. Empty the **used tips** into their **respective trays/containers**.
6. **Wash** the following **using the water taps** and put them in the drying trays next to the sink:
   a. the liquid waste container and
   b. the used tip container.
7. Place **all used cuvettes** in the **collection tray**.

C. Procedure: Step 6b – Packing up

Each team to return the following to their respective collection locations

Student 1 (gloves on)

1. a **cuvette holder**
2. **5 mL pipette (green pipette)**
3. **100-1000 µl pipette (blue pipette)**
4. **10-100 µl pipette** (yellow ring)
5. **Tips for these pipettes**
6. **A tube rack**

Student 2 (gloves on)

1. a **wash bottle**
2. a box of **Kimwipes**
3. a **whiteboard marker**
4. a **Parafilm square container**

- Leave the tray on the backbench.

Procedure: Step 7

All team members

- *Clean up your workspace and return your tray.*
  - *Check the spectrophotometer for that last cuvette left behind.*
  - *Get your tutor to check your place after returning your trays – thanks*
- Complete all the calculations in the Data file and save the excel file on the desktop.
- Email the file to all team members, just in case.
- Complete the Prac Quiz individually as described in the next slide.
- You may use your laptops now, if there are no chemicals around.
- Submission due at the end of your practical session.
- *Remember to logout of your account on the computer!*
- *Take your USB drive with you!*

Additional viewing from JoVE

https://www.jove.com/science-education/5688/photometric-protein-determination

Submitting your data and Results

- Each student to login to iLearn individually and follow the instructions for completing the Prac.

- **Upload your 'Prac 2 data file' individually to ilearn.**
- – Check your Prac 2 data file carefully to see if it is complete.
- – When you are 100% happy, upload your file to iLearn.
  - DO NOT EMAIL US YOUR DATA FILE AS ILEARN DOES NOT ALLOW US TO UPLOAD IT FOR YOU
  - IF YOU NEED TO RE-SUBMIT CONTACT PHANI/ELSA/SHOBA, TO RESET YOUR SUBMISSION TO DRAFT
- – You will then get access to **Prac 2 Quiz**
- – Complete the Prac Quiz individually before you leave the lab!
- – ***We need your data file and your completed Quiz for grading!***