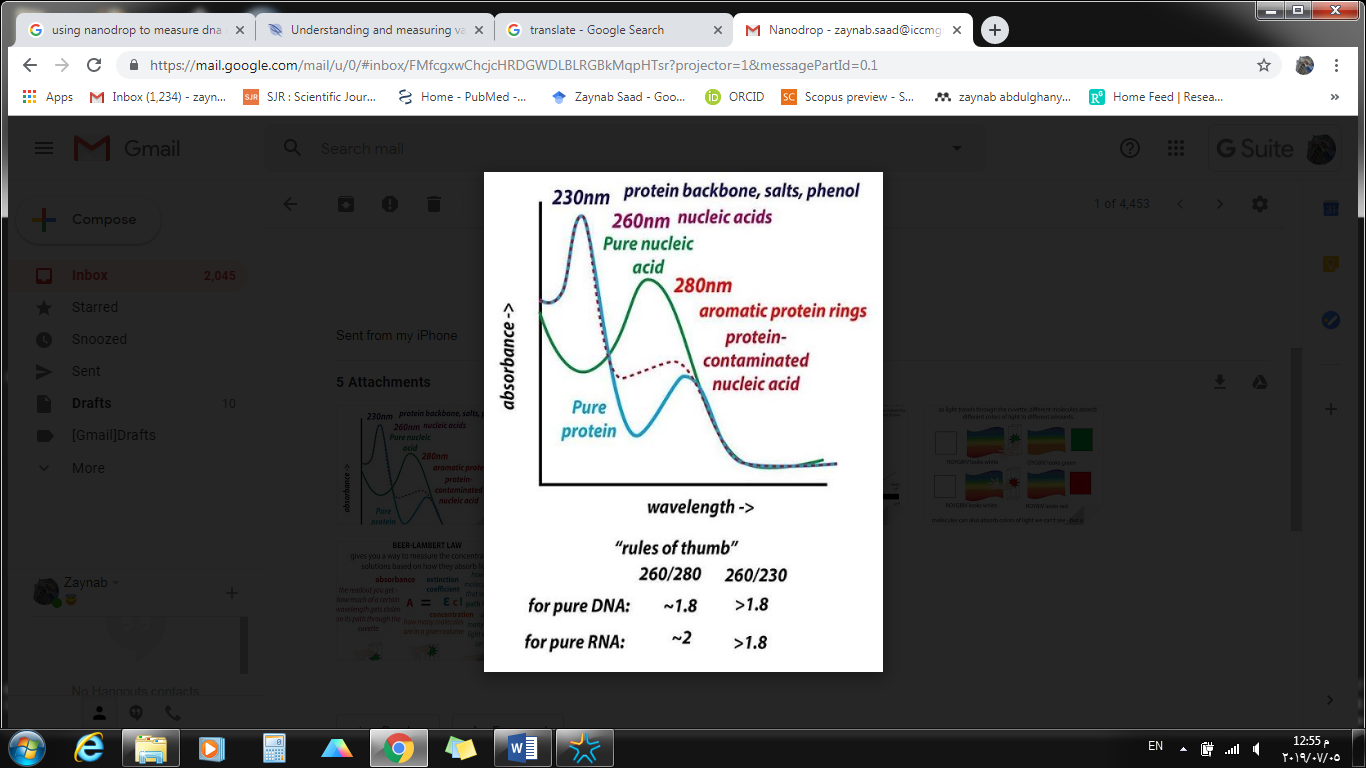
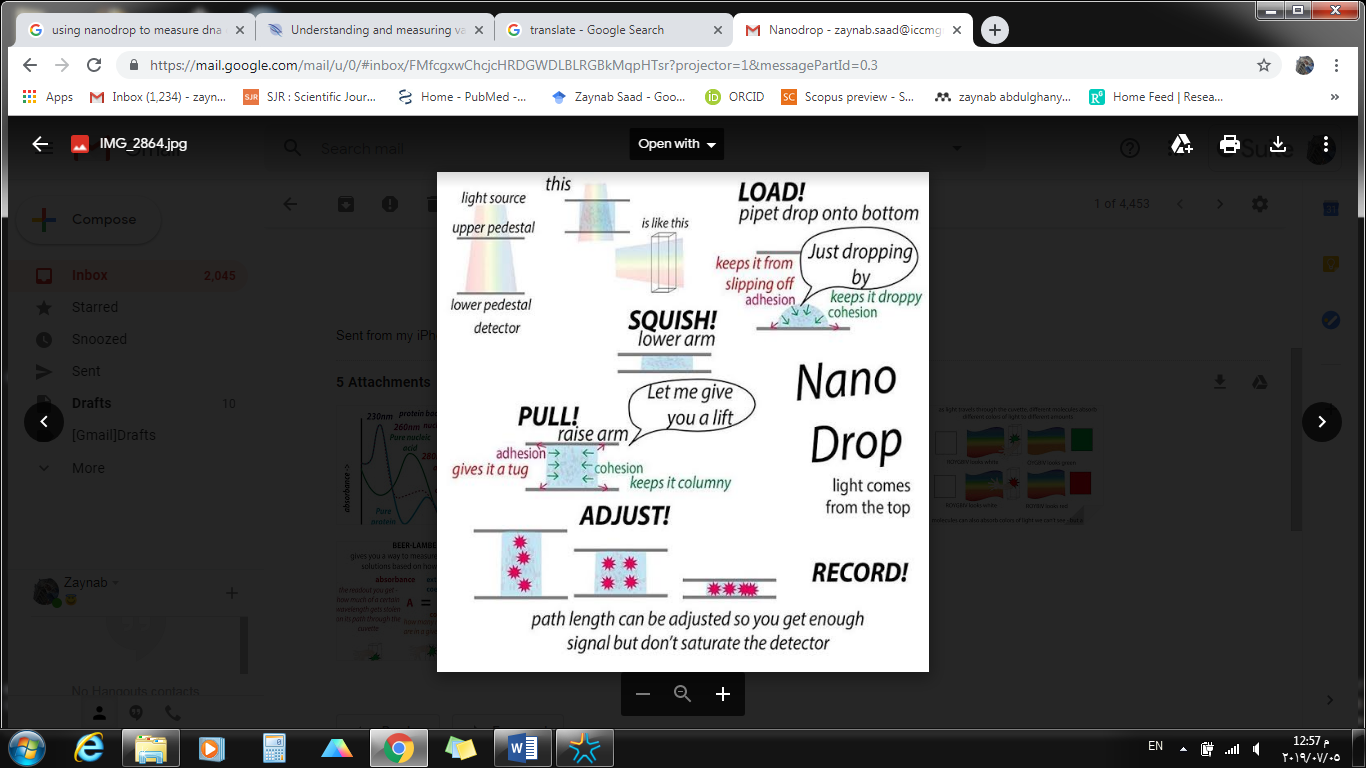
Do you check your spectrograph peaks? Or just you look at the highest one and assume the purification job is done?

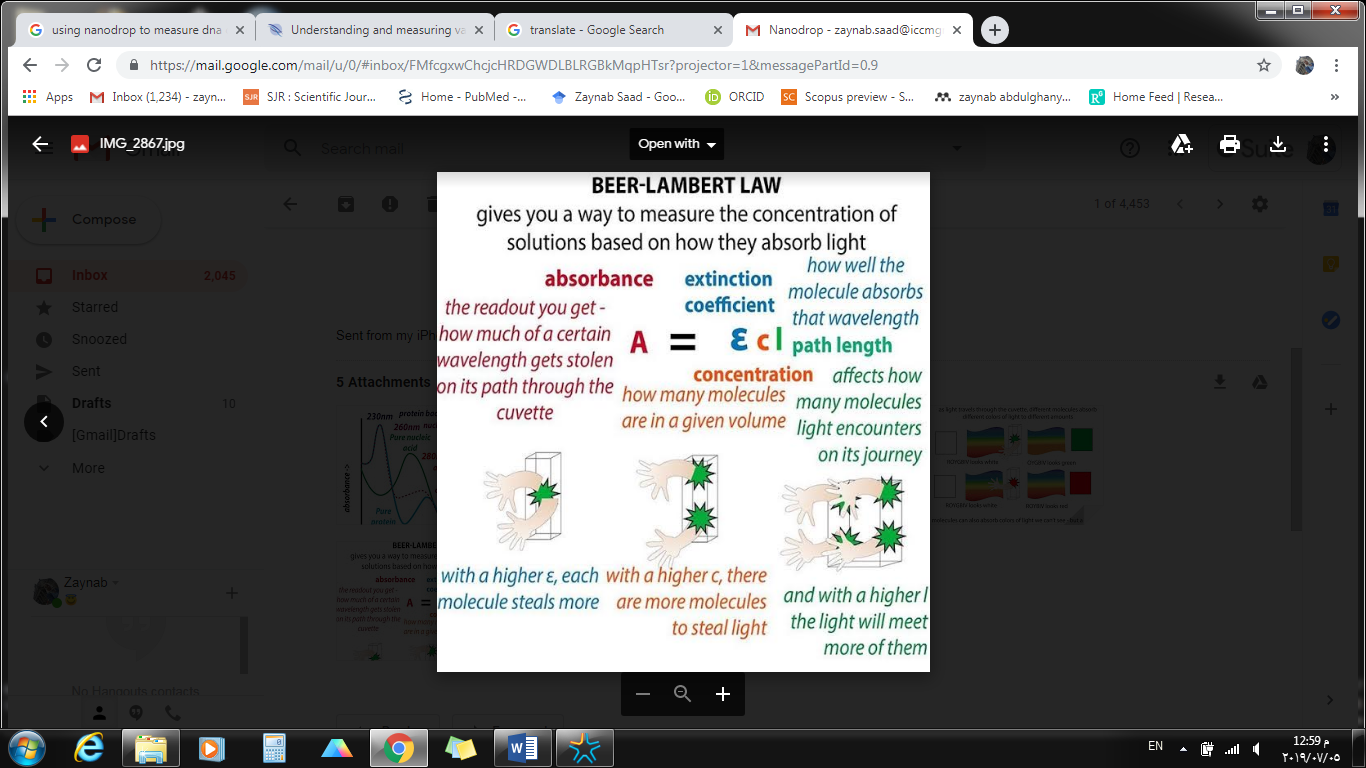
* The peaks might tell you concentration, but the rest spectrum tell you about contamination
* And that contamination might be causing inflation leading to DNA/RNA/protein concentrations overestimation
* Spectroscopy such as with a Nanodrop spectrophotometer is often used to measure concentration of molecules like DNA, RNA and protein by taking advantage of different molecules tendency to absorb certain wavelengths of light to different extents but a lot of information about purity can be gained by looking at where they shouldn’t be absorbing much light
* The height of the peak you usually focus on for a particular molecule is where it absorbs best. This corresponds to how much concentration is there
* While ratios between peaks can tell you about how pure that sample is
* Light electromagnetic radiation EMR is little packets of energy traveling in waves
* Different colors have different wavelengths of light with different energies
* Different color absorbs different wavelengths of light to different extents, and this can be quantified by a number called the extinction coefficient which tell us how well a molecule absorbs light of a particular wavelength



* Pay attention to surface tension
* Spectrophotometers shine light through a liquid containing a dissolved solute you want to measure the concentration. It knows what it shines in, then it measures what comes out the other side. What it directly measures is transmission (what was allowed to pass through) and it subtract that from 1 to get the absorbance (what didn’t pass through because molecules stole it)
* The nanodrop spectrophotometer has a little pedestal you put a drop of liquid on >>>> Then you lower machines arm>>> it contacts the liquid the pull up a little pit, it pulls on the liquid. It dose this because of surface tension
* Surface tension occurs because the molecules of the liquid like each other more than they like in the air, so they try to stay together and maximize the liquid – liquid interactions while minimizing their combined air exposure.
* When you put the drop on, surface tension causes it to remain drop-like. But when you lower the arm of the machine it squish it down, some of the water molecules stick to the top surface. And when the arm pulls back up, these molecules get lifted and the other water molecules don’t want to leave their combined molecules behind as a result a column of liquid forms



* This column is just like the column of liquid in the cuvette except its much smaller and it's held up by surface tension instead of being sealed by glass/ plastic. And the light travels through from above and is recorded below
* Another differences about this column is that the path lengths adjustable (the column can be pulled up higher or squished) so it can adjust for different concentrations.. e.g. if your sample is too concentrated it will see this squish down to shorten the path length so the light doesn't meet any more, or if it is dilute it can pull up to make sure lots of molecules get hit by light. The nanodrop software then has to correct this by using Beer's law to get the concentration



* It's easier to get columns to form for DNA or RNA than proteins because proteins often weaken the surface tension ( not all parts of proteins like water) so it's harder to get continuous water networks to stay as you pull up the column.. Column breaks
* But there's strength in numbers, so when loading protein usually load 2µl and 1.5µl for nucleic acids
* **A260/A280 ratio**

Pure RNA has an A260/A280 ratio of 2.0, therefore if a DNA sample has an A260/A280 ratio of greater than 1.8 this could suggest RNA contamination. However, due to the similarity in absorption profiles of RNA and DNA, probably the most accurate way to determine RNA contamination is to run the sample on an agarose gel where the RNA will clearly been seen migrating ahead of the DNA.  RNA can easily be removed by adding RNase A during purification.

pure DNA preparations have an A260/A280 ratio of greater than or equal to 1.8.

When the A260/A280 ratio is determined for a range of different DNA/protein mixtures it has been shown that the ratio is relatively insensitive to the addition of protein to pure nucleic acid.  However the utility of the ratio becomes apparent when DNA is prepared from tissue or blood.  These samples have a protein content that greatly exceeds nucleic acid on a weight basis and so in these cases the approximate purity of dsDNA preparations and contamination by protein may be estimated by determination of the ratio of absorbance at 260nm and 280nm.

Several factors can influence the accuracy of the A260/A280 ratio.  For example, readings from very dilute samples will have very little difference between the absorbance at 260 and 280nm leading to inaccurate ratios.  The type(s) of protein present will also have an effect.  Absorbance in the UV range by proteins is primarily the result of aromatic ring structures.  Proteins are composed of 22 different amino acids of which only three contain aromatic side chains.  Thus the amino acid sequence of proteins would be expected to influence the ability of a protein to absorb light at 280 nm.  For example bovine serum albumin (BSA) has an extinction coefficient value of 0.7 for a 1 mg/ml solution at 280nm, while streptavidin has an extinction coefficient of 3.4, absorbing almost five times as much light at 280nm at the same concentration.

It is also important to note the phenol and other contaminants can also absorb at 280 nm and can affect the ratio calculation. Phenol absorbs with a peak at 270nm and has an A260/A280 ratio of 2. Nucleic acid preparations uncontaminated by phenol should have an A260/A280 ratio of around 1.8.  As well as affecting the ratio calculations, contamination by phenol can significantly contribute to overestimation of DNA concentration and inhibit downstream reactions.

The pH of the solution can also affect the A260/A280 ratio, with acidic solutions having a lower ratio of up to 0.2–0.3 and alkaline solutions having an increased ratio by a similar amount.

Due to their different absorption spectra, the nucleotide composition of the bases present in DNA will have different A260/A280 ratios.

* **A260/A230 ratio**

This ratio is used as a secondary measure of nucleic acid purity. The A260/A230 ratio values for pure samples are often higher than the respective A260/A280 ratio values. Strong absorbance around 230nm can indicate that organic compounds or chaotropic salts are present in the purified DNA.  A ratio of 260nm to 230nm can help evaluate the level of salt carryover in the purified DNA. The lower the ratio, the greater the amount of salt present. As a guideline, the A260/A230 ratio should be greater than 1.5, ideally close to 1.8.  Urea, EDTA, carbohydrates and phenolate ions all have absorbance near 230nm. The TRIzol®reagent which can be used for DNA extraction, although more commonly used for RNA preparation is a phenolic solution which absorbs in the UV spectrum at 230 nm and around 270nm.  Guanidine HCL which is used in the extraction of DNA will absorb at 230nm while guanidine isothiocyanate, used for RNA isolations will absorb at 260nm.

* reading at 320nm will indicate if there is turbidity in the solution, another indication of possible contamination. Therefore, taking a spectrum of readings from 230nm to 320nm is most informative.