Cube

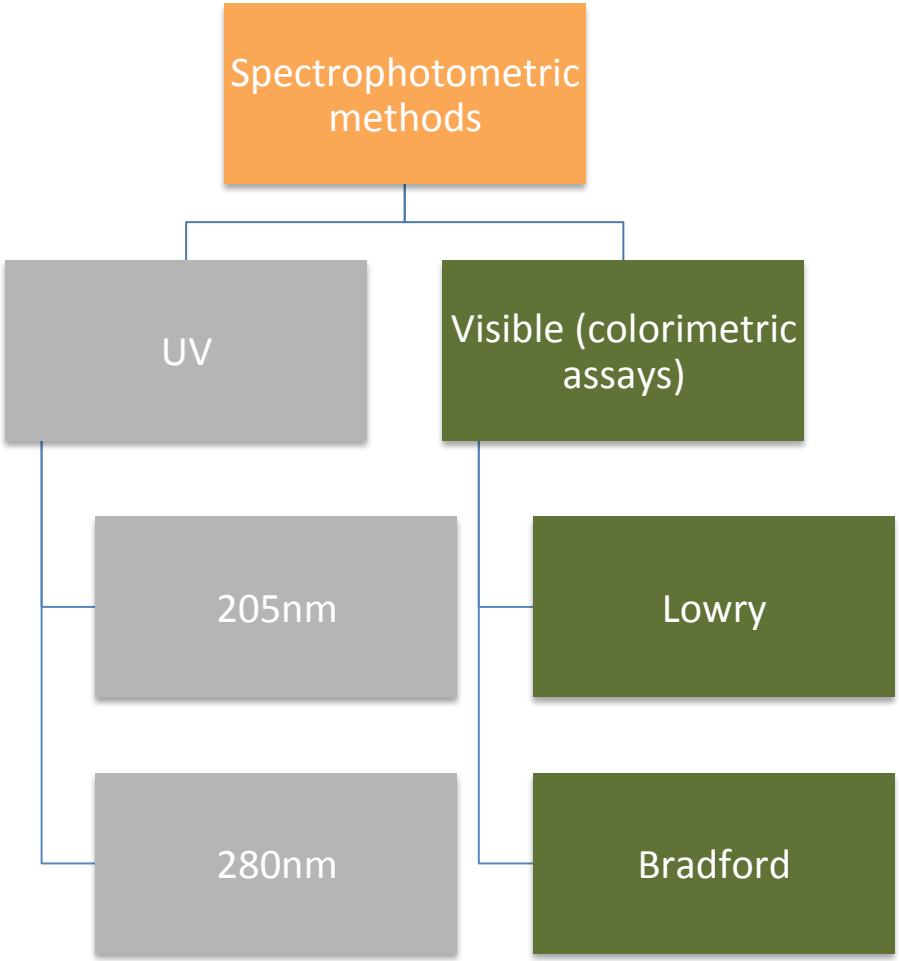
Computational Systems Biology

Quantitative Methoden in der Molekularbiologie

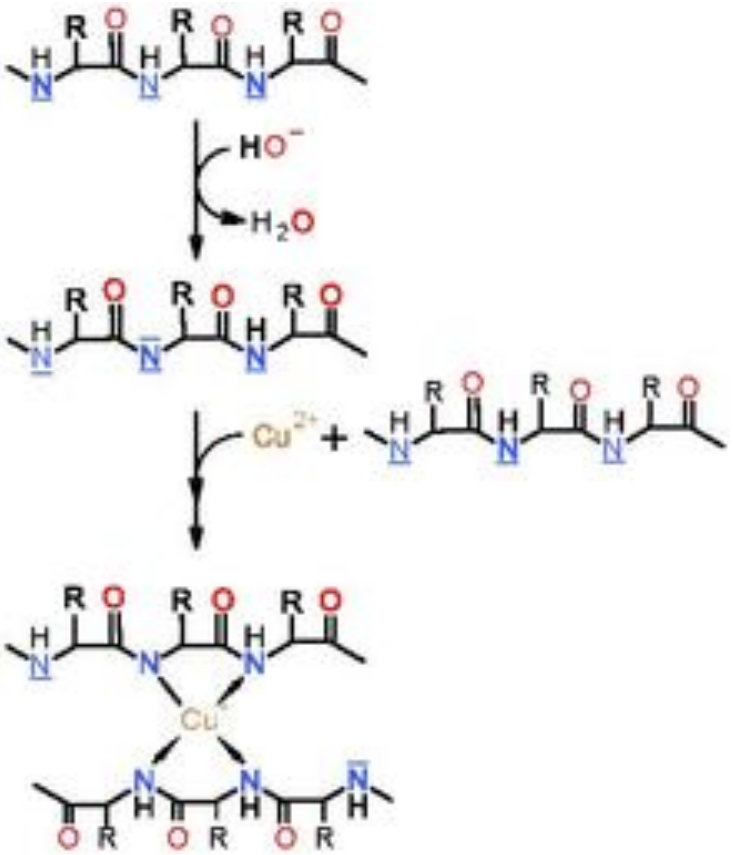
5. Protein quantification: spectrophotometric methods and standard curve

WS 2015/16

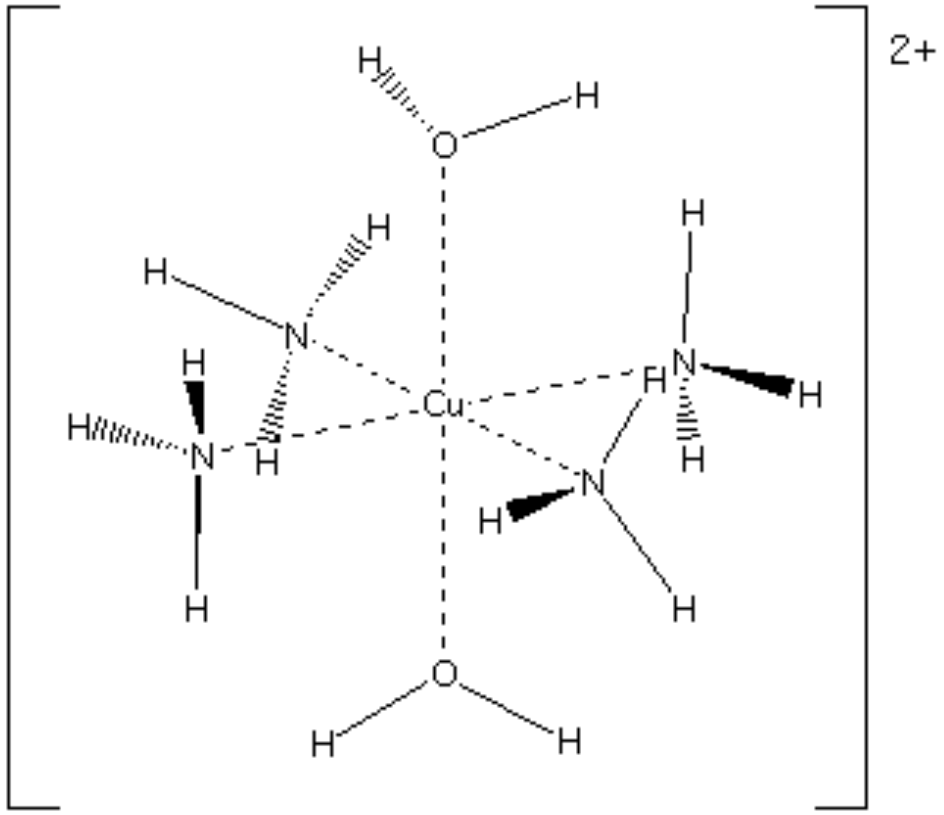
Outline


Spectrophotometric methods
UV
Visible (colorimetric assays)
205nm
280nm
Lowry
Bradford

Biuret complex



Other coloured copper complexes

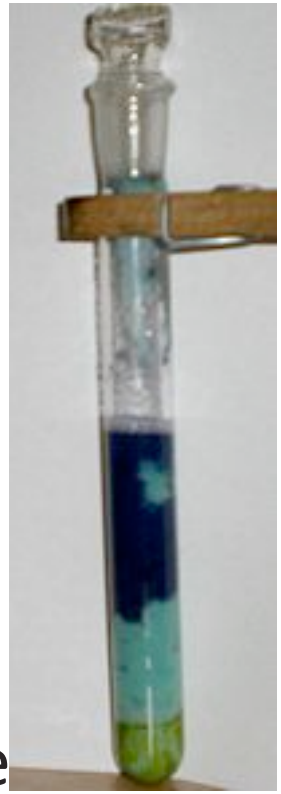


Ammonia

Tris buffer

…

Lowry assay (1)



Biuret chromophore:

- copper ion complex + peptide bonds
- reduced copper in alkaline solution

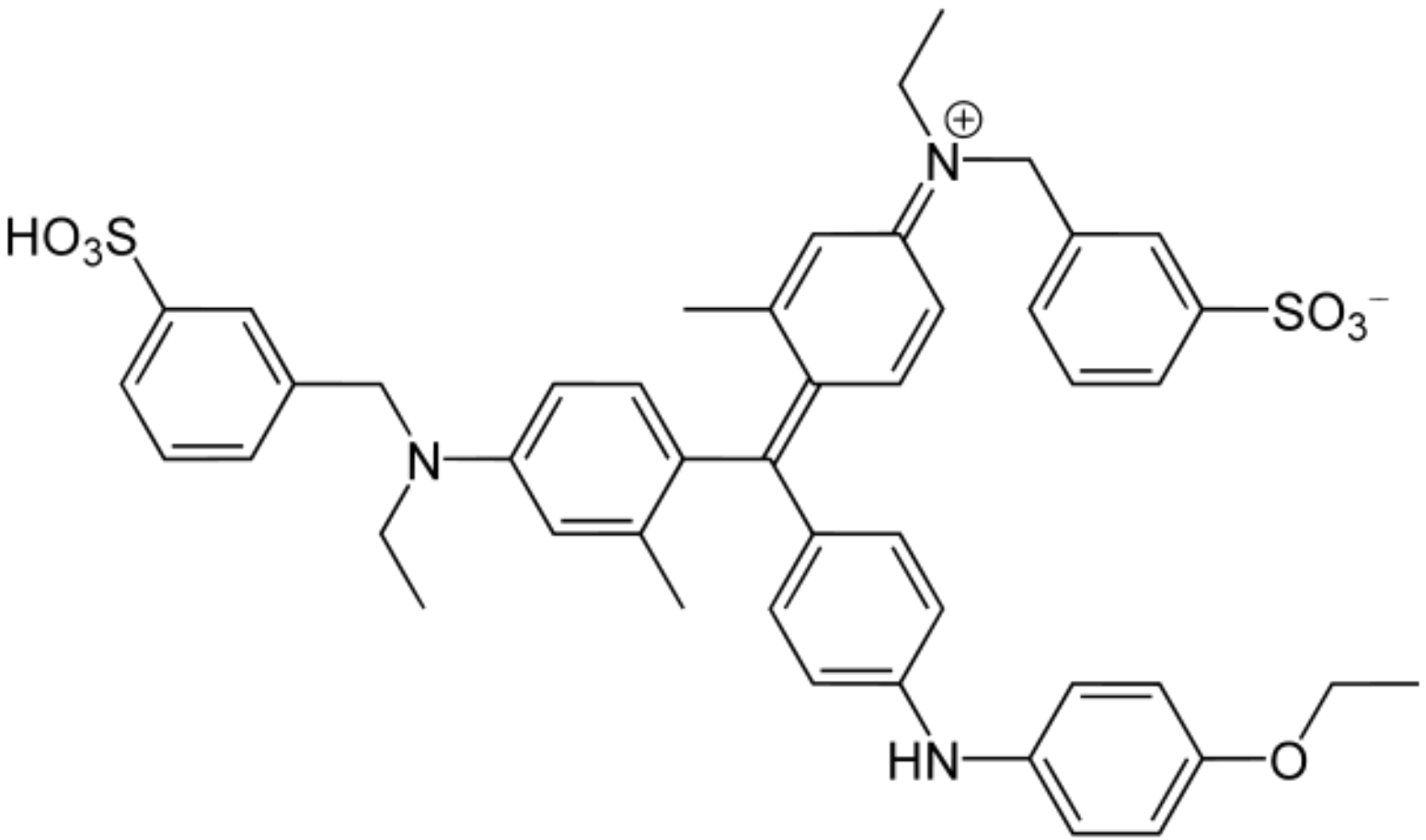
Reduction of the Folin-Ciocalteu reagent:

- With Cu^{+} (product of first reaction) + tyrosine tryptophane residues
- blue: detectable 500-750 nm

Lowry assay (2)

- Several modifications of the method allowed insensitivity to interference by detergents.
- Although Bradford is as sensitive as Lowry, it has the advantage that it‘s able to detect protein concentration in solutions that contain SDS, that would prevent the Bradford assay.
- Time consuming compared to other assays like Bradford.
- Disadvantage: dependence from presence and abundance of certain aminoacids (tyrosine and tryptophane), which are essential for the reaction.

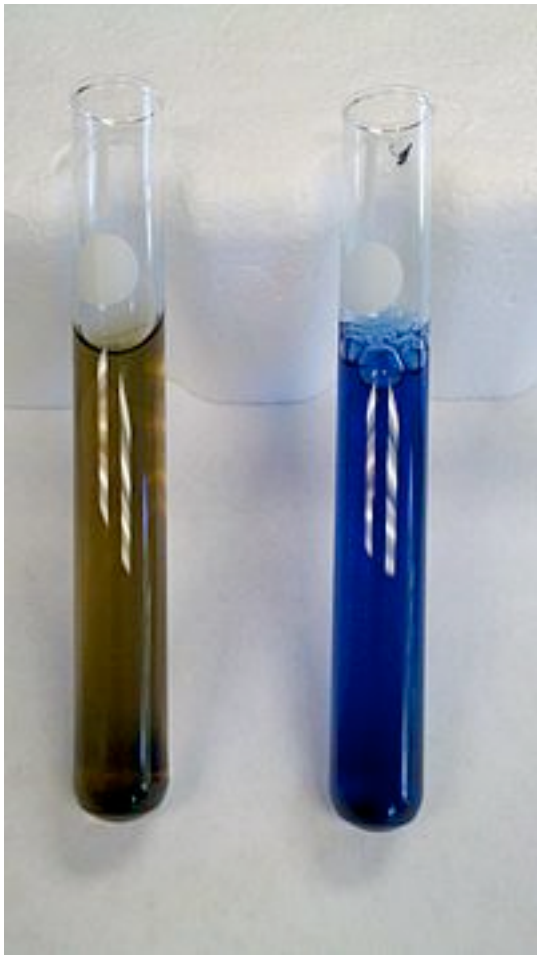
Coomassie Brilliant Blue G-250



Dye forms

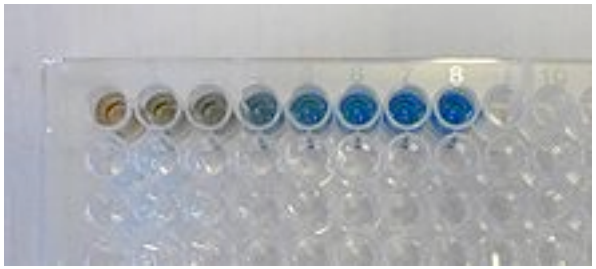
Cationic: green/red balance

Anionic: blue



Bradford assay (1)

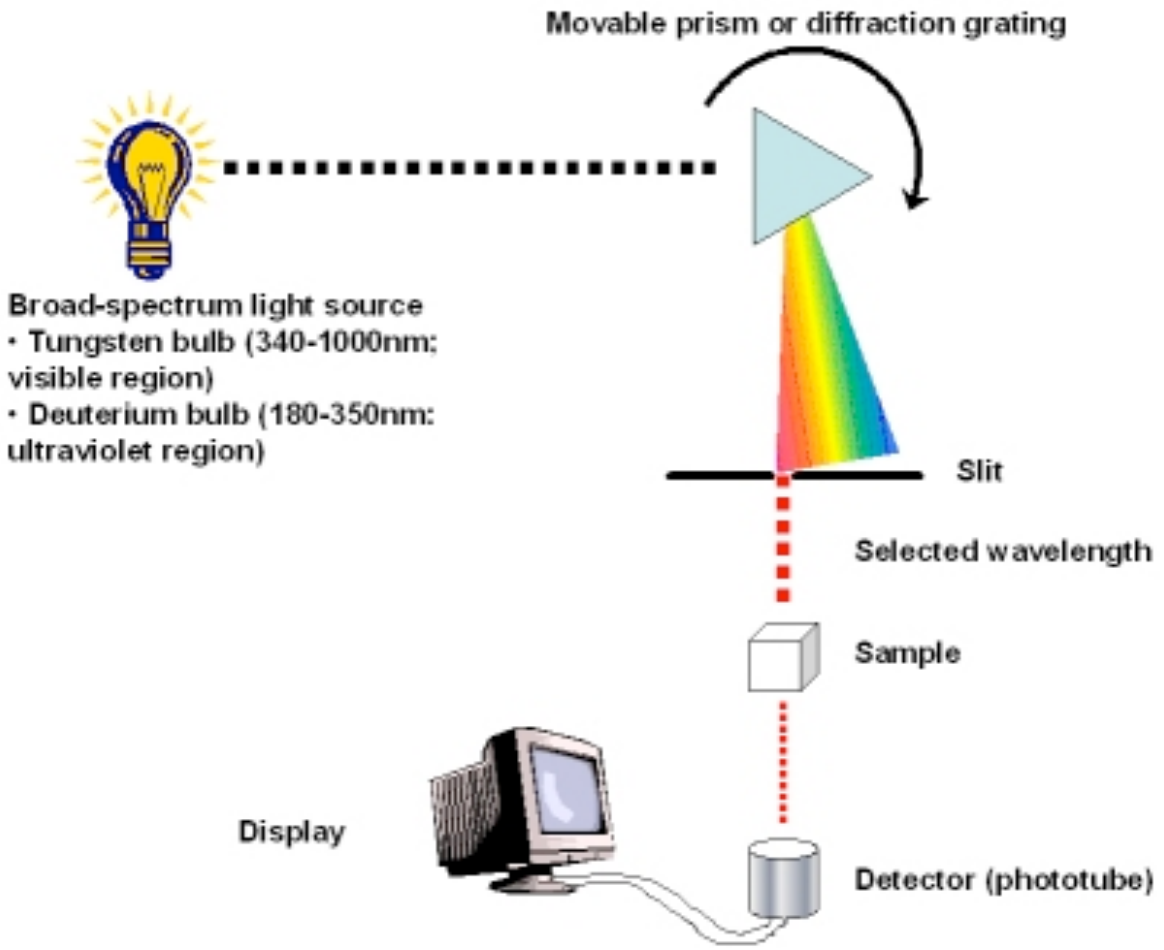
- Coomasssie Brilliant Blue G-250 Dye (CBBG) denatures the protein and makes hydrophobic pockets accessible.
- The binding of the CBBG results in a peak shift from 470 nm when free to 595 nm when loaded with the protein used in the assay.
- There are mainly two formats; micro assay and the macro assay for different concentrations of the protein used.
- For high throughput adaptations the use of a microwell plate is advised to process the samples as rapidly as possible.



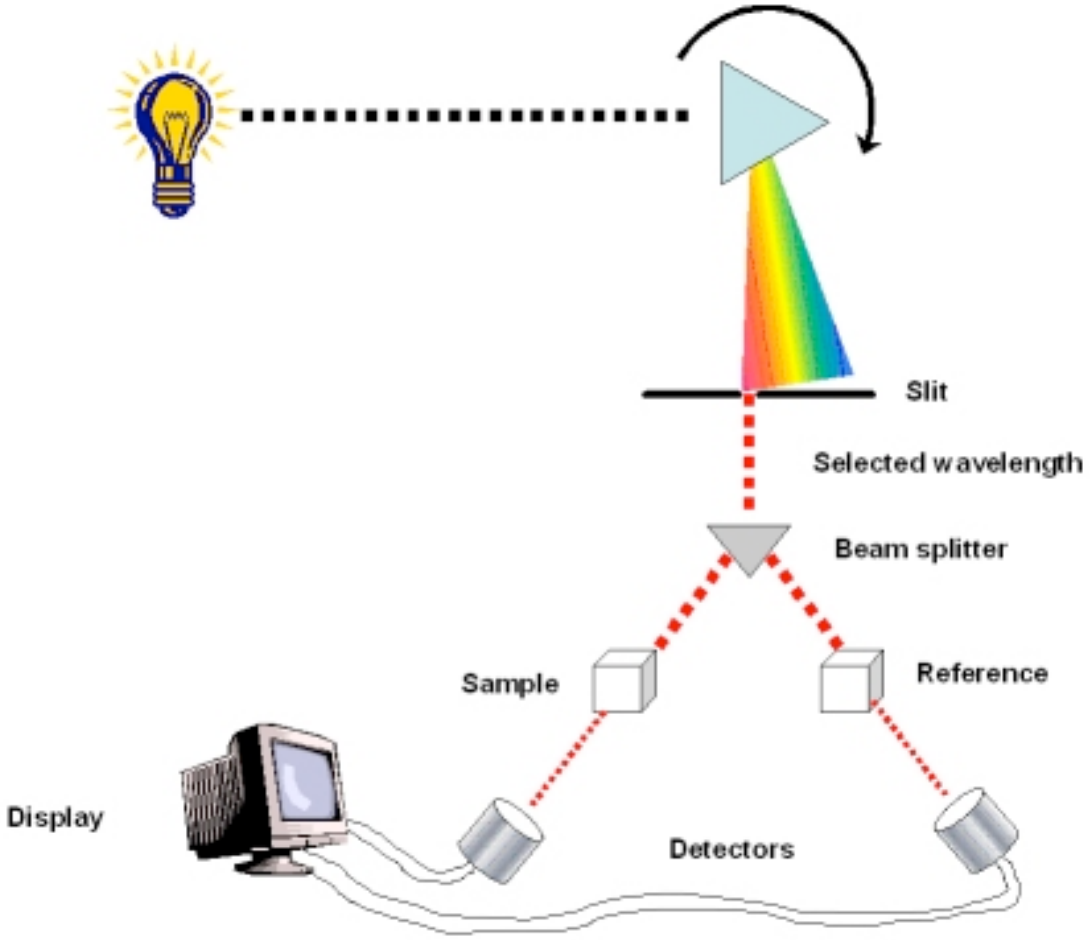
Bradford assay (2)

- The standard protein used for the Bradford assay is BSA (Bovine Serum Albumin), but BSA has a higher response than other proteins in the assay and as a substitute can IgG or lysozyme be used.
- Some proteins tend to precipitate when CBBG is added, therefore specific pH may be needed to solubilize the protein.
- Has a non linear response in a wide concentration interval.
- SDS binds strongly with protein and with CBBG!

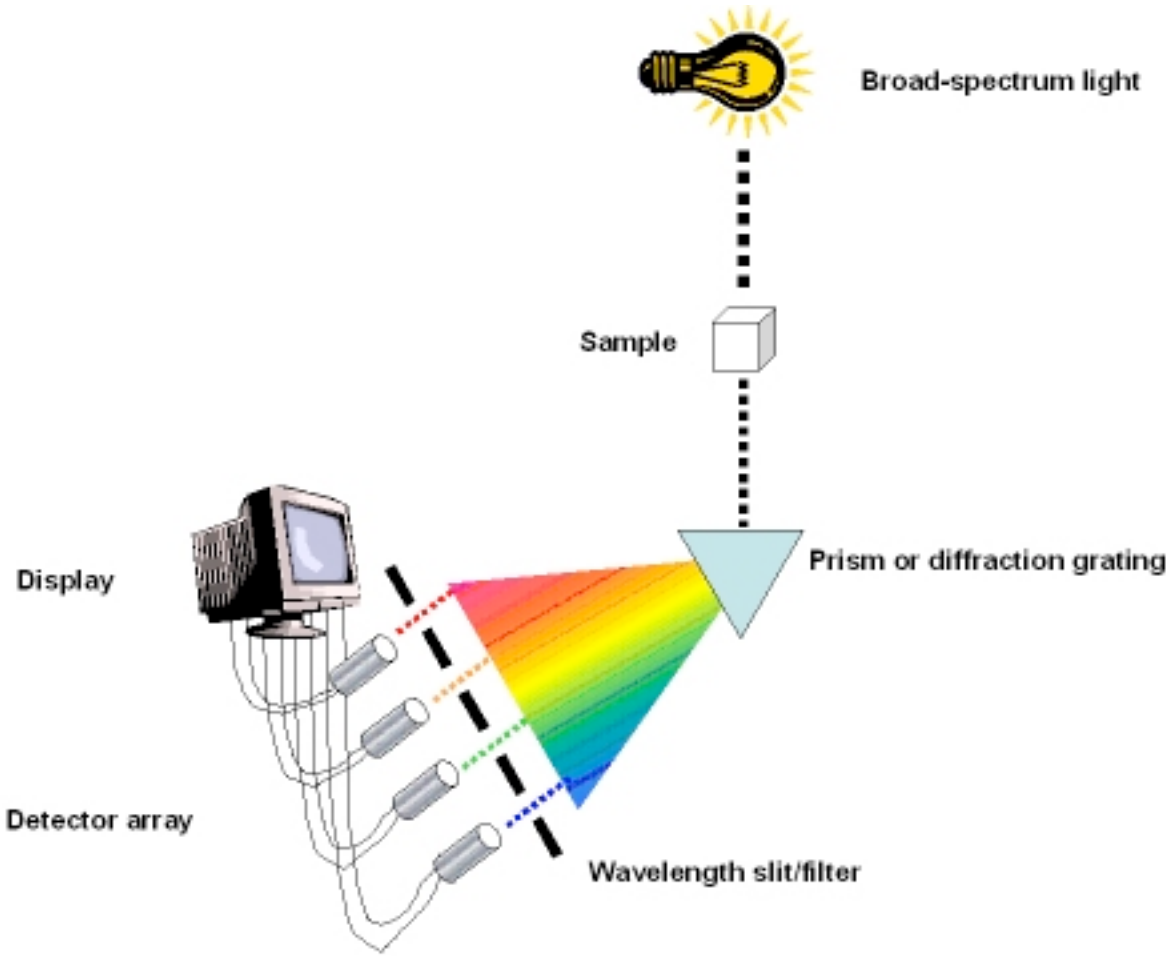
Spectrophotometers: single beam



Spectrophotometers: dual beam



Spectrophotometers: detector array



Spectrophotometers

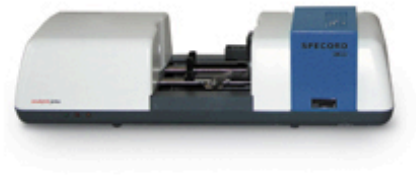
Overview of all UV/Vis spectrophotometers

Double-beam photometers

- SPECORD® 50, 200, 210, 250 PLUS

Dissolution systems

- SPECORD® 200, 210 PLUS



Diode-array Spectrophotometers

- SPECORD® S 600

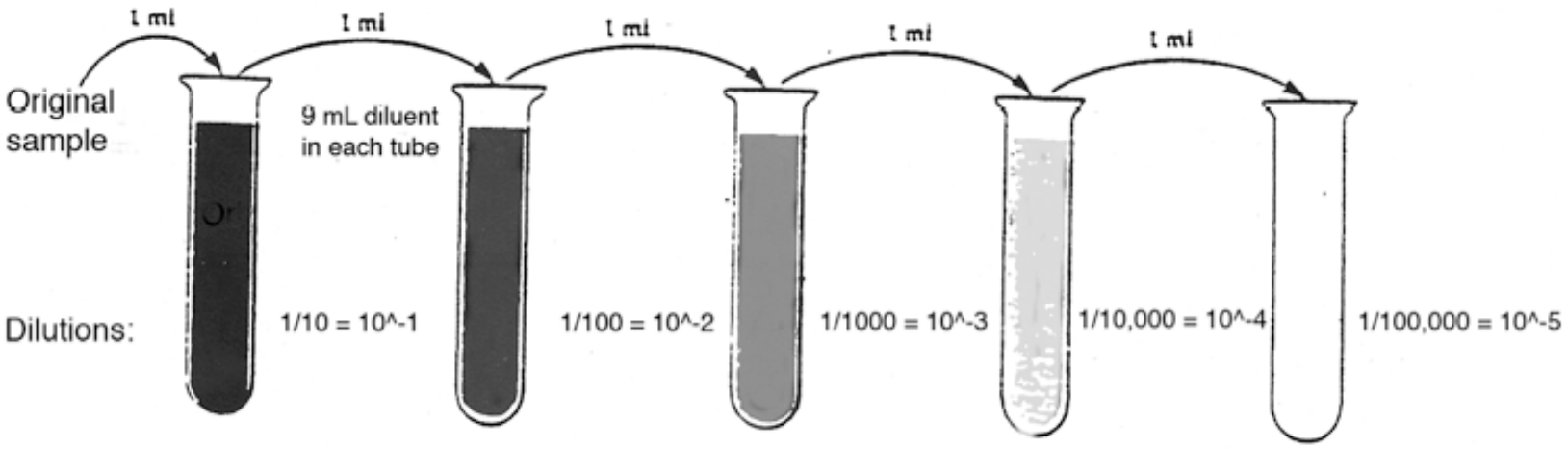
UV/Vis Accessories

- View accessories page

http://www.analytik-jena.de/en/analytical-instrumentation/products/uvvis-spectrophotometers.html

The standard curve

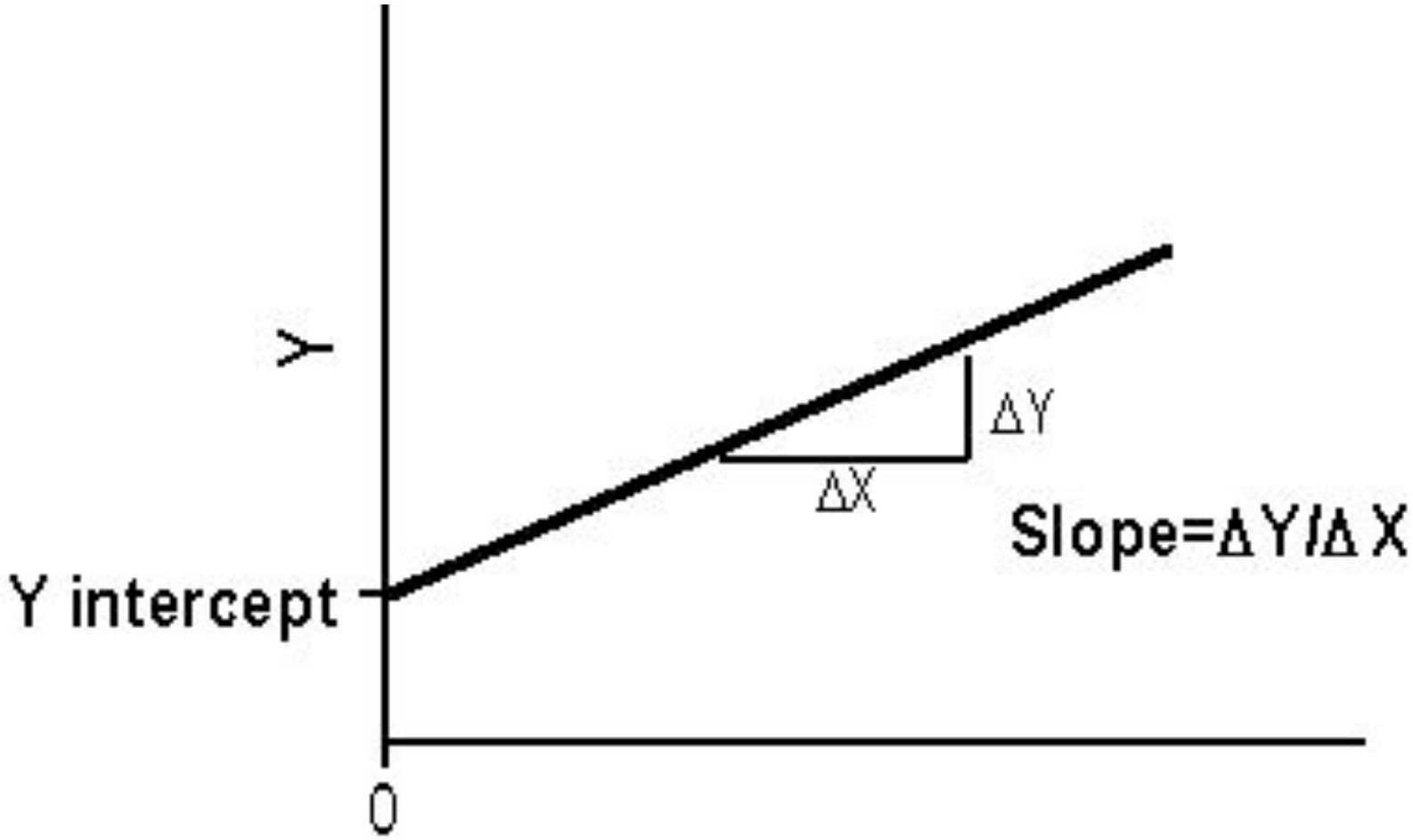
Dilution series



Dilution series

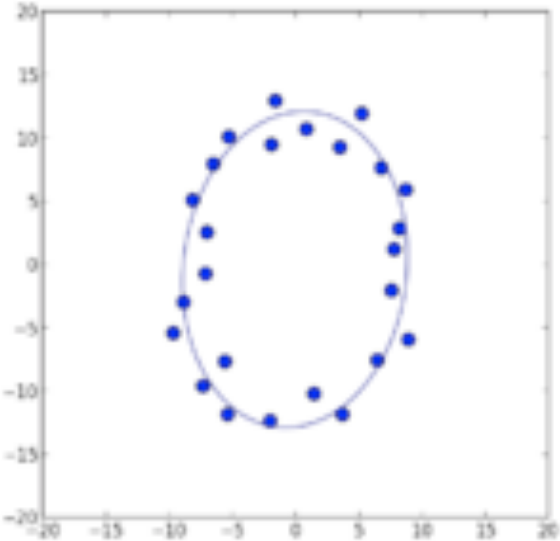
Volume BSA (uL)	Absorbance at 595 nm
1	.008
2	.015
4	.028
6	.037

Standard curve: linear model

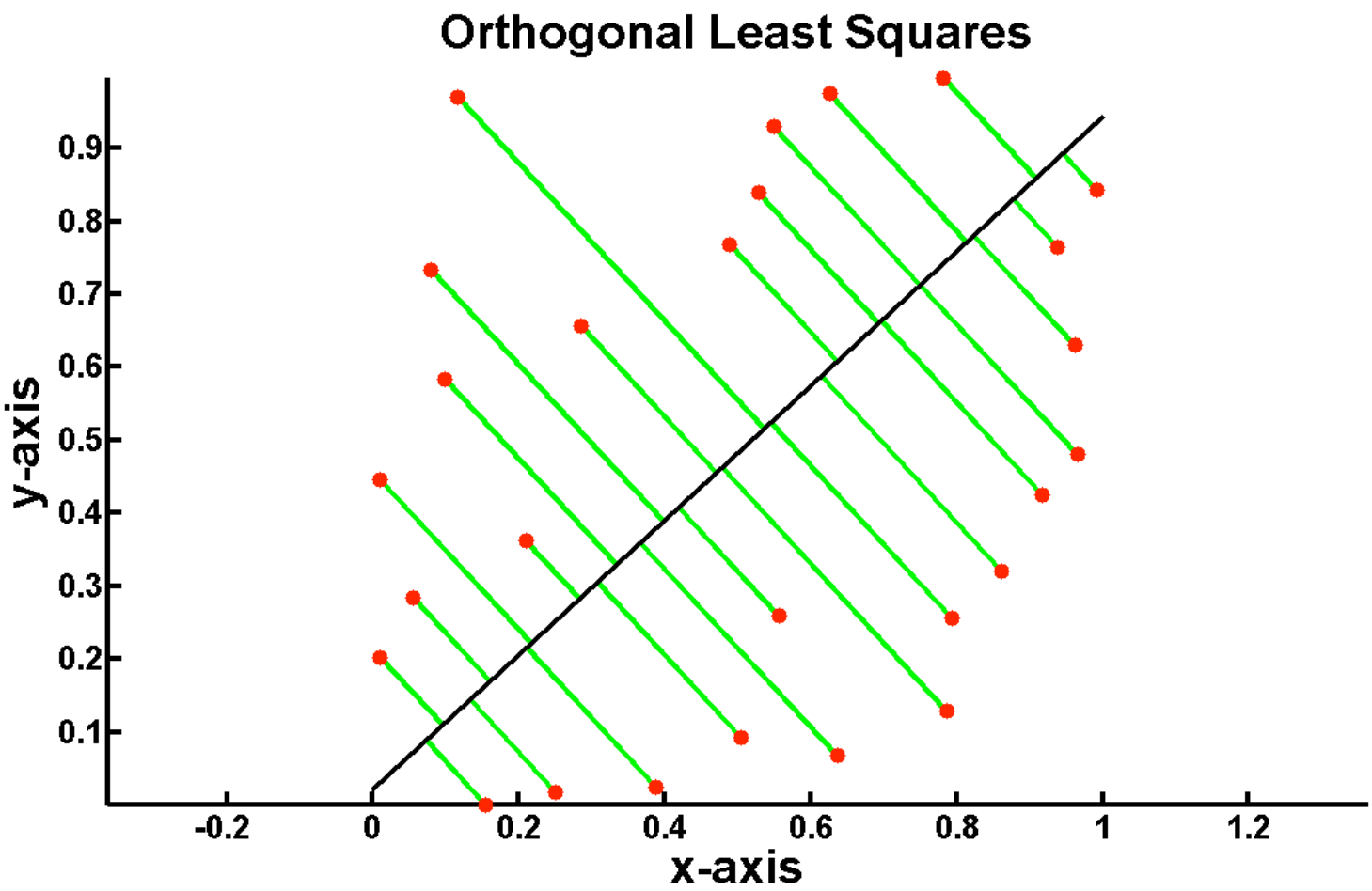


Least squares approximation

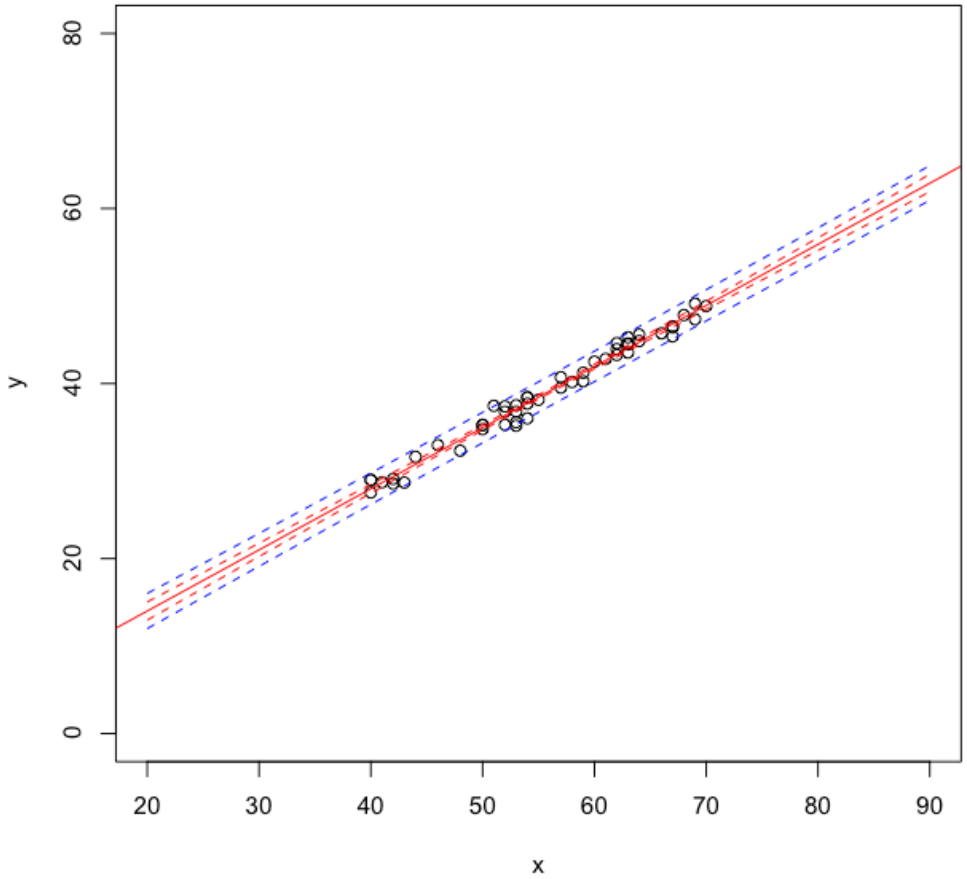
First applied to the trajectories of celestial bodies (Legendre, Gauss)



Least squares approximation



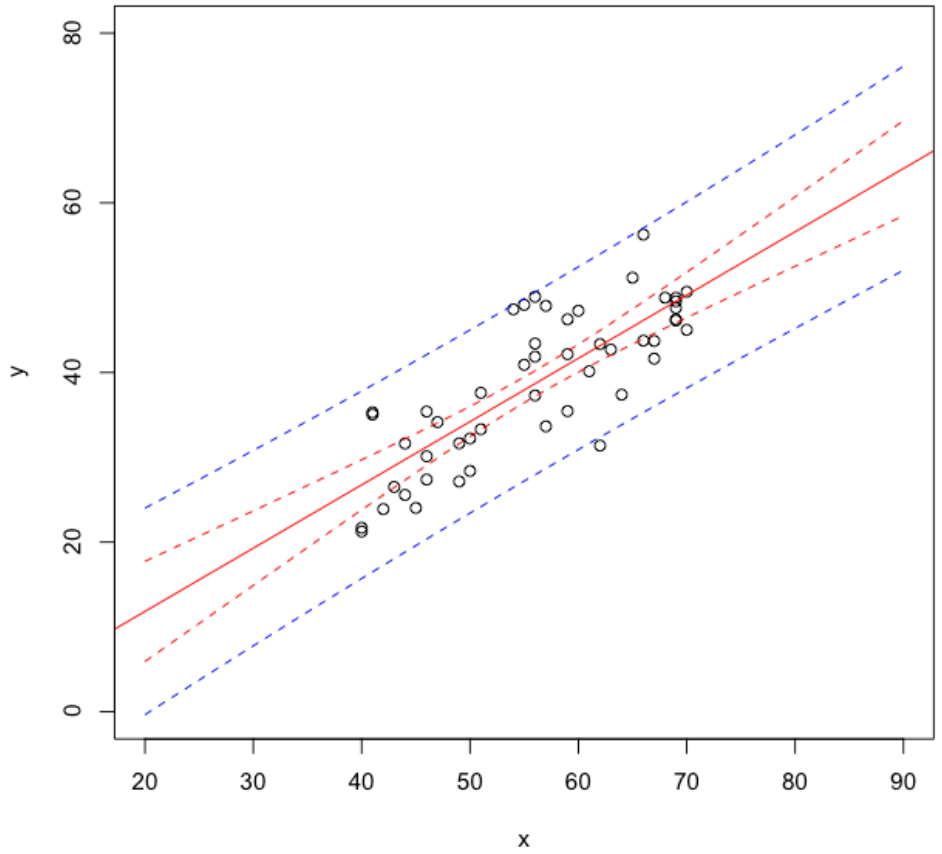
Confidence and prediction intervals



Confidence

Prediction

Confidence and prediction intervals



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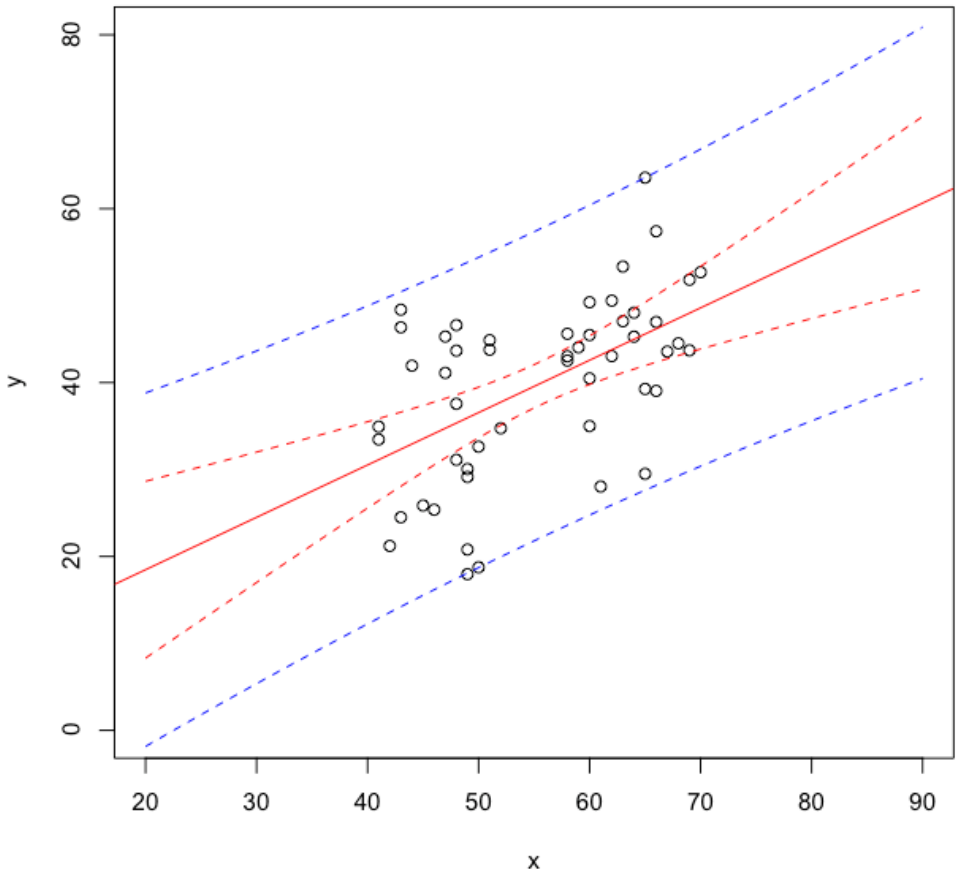

Confidence

Prediction