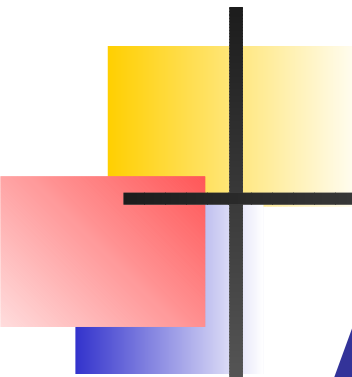




Absorption Corrections in Protein Crystallography

W. Armour & G. Evans

Diamond Light Source



Introduction.

What are we trying to achieve?

- We aim to develop methods to apply analytical absorption corrections to macromolecular diffraction data.
- These methods should allow for the whole experimental sample to be treated and not just the crystal sample.



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- These methods should allow for the whole experimental sample to be treated and not just the crystal sample.

Why are we trying to achieve this?

- This will allow data collection strategies optimized for collection rates and coverage to be used, rather than ones designed to minimize absorption effects.



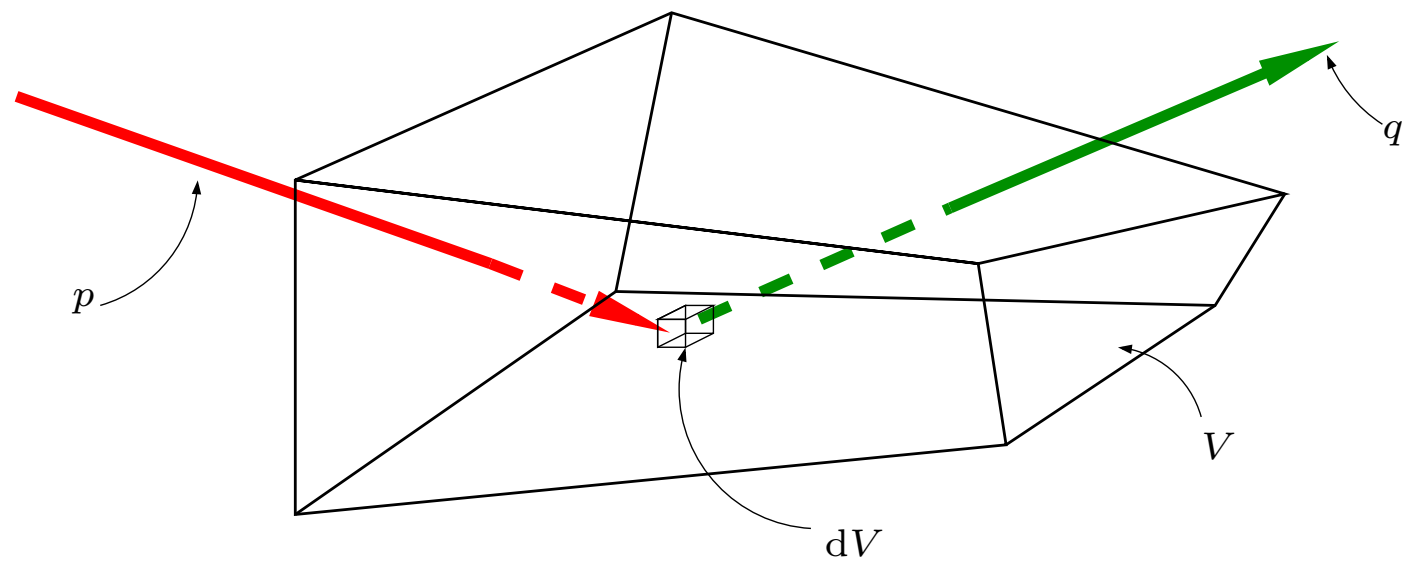
The Problem.

- To calculate the transmission coefficient for a crystal we consider a small diffracting element of the crystal which we call dV .
- The diffracting volume (dV) will contribute to the diffracted beam intensity. The contribution will be proportional to

$$e^{-\mu(p+q)} dV$$

- where μ is the absorption coefficient of the crystal and p and q are the lengths of the incident and diffracted beams.

The Problem.



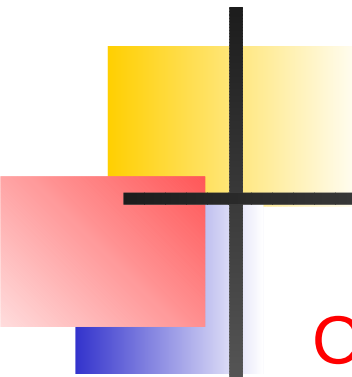


The Problem.

- The fraction by which the total intensity is reduced is thus given by

$$T = \frac{1}{V} \iiint_V e^{-\mu(p+q)} dV$$

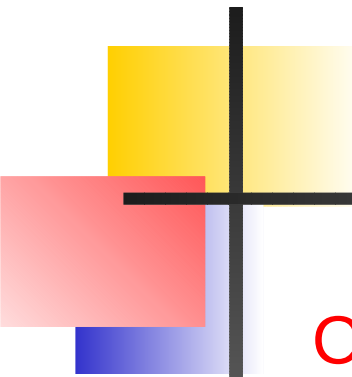
- T is the transmission coefficient and V is the crystal volume.



Current Solutions.

Clarke and Reid

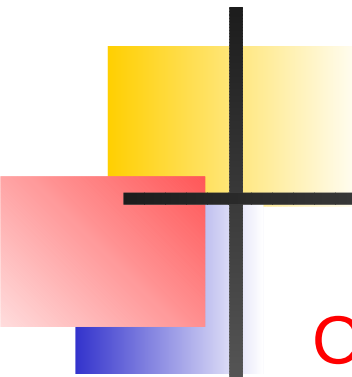
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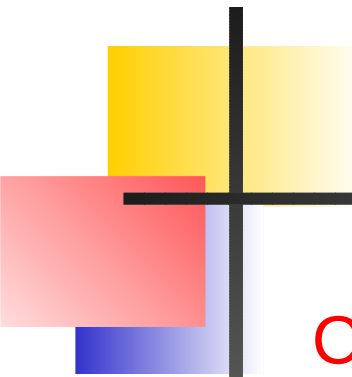
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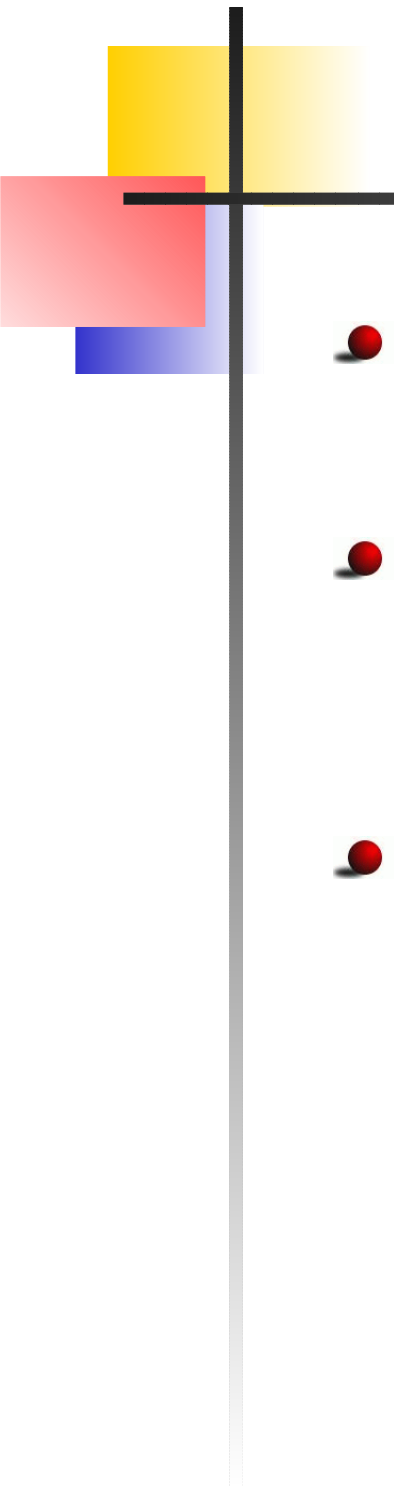
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This does not allow for and surrounding material that absorbs but does not diffract.



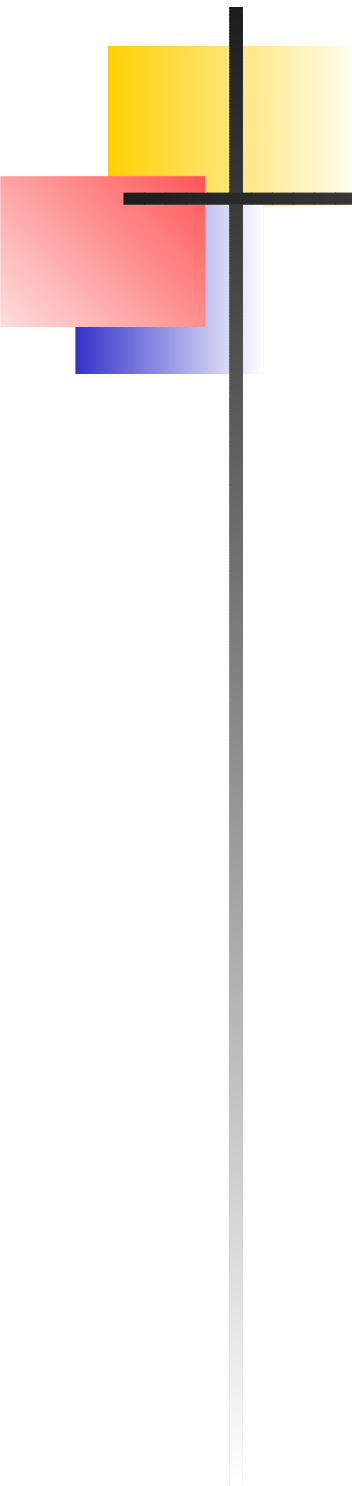
Solving the Integral.

- So solving the transmission integral is only half the problem.
- We must also address the mother liquor in the loop/capillary... since this can also have a significant contribution to absorption.



Solving the Integral.

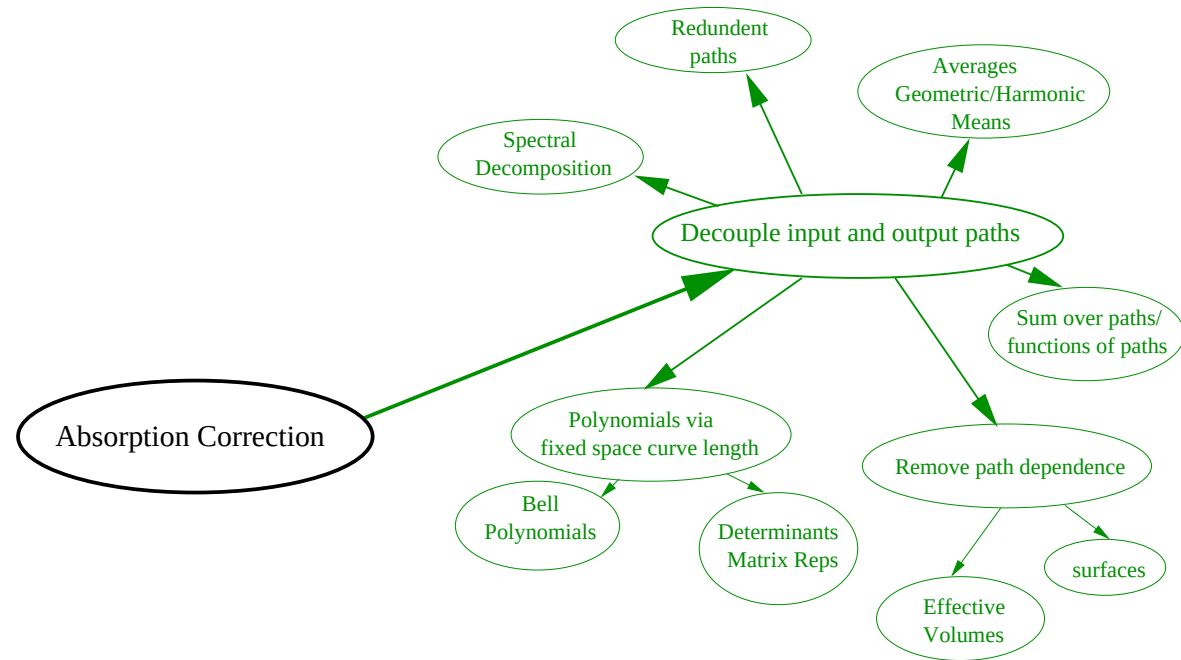
- So solving the transmission integral is only half the problem.
- We must also address the mother liquor in the loop/capillary... since this can also have a significant contribution to absorption.
- This becomes very hard when diffraction takes place because one input "ray" has many output rays associated with it.



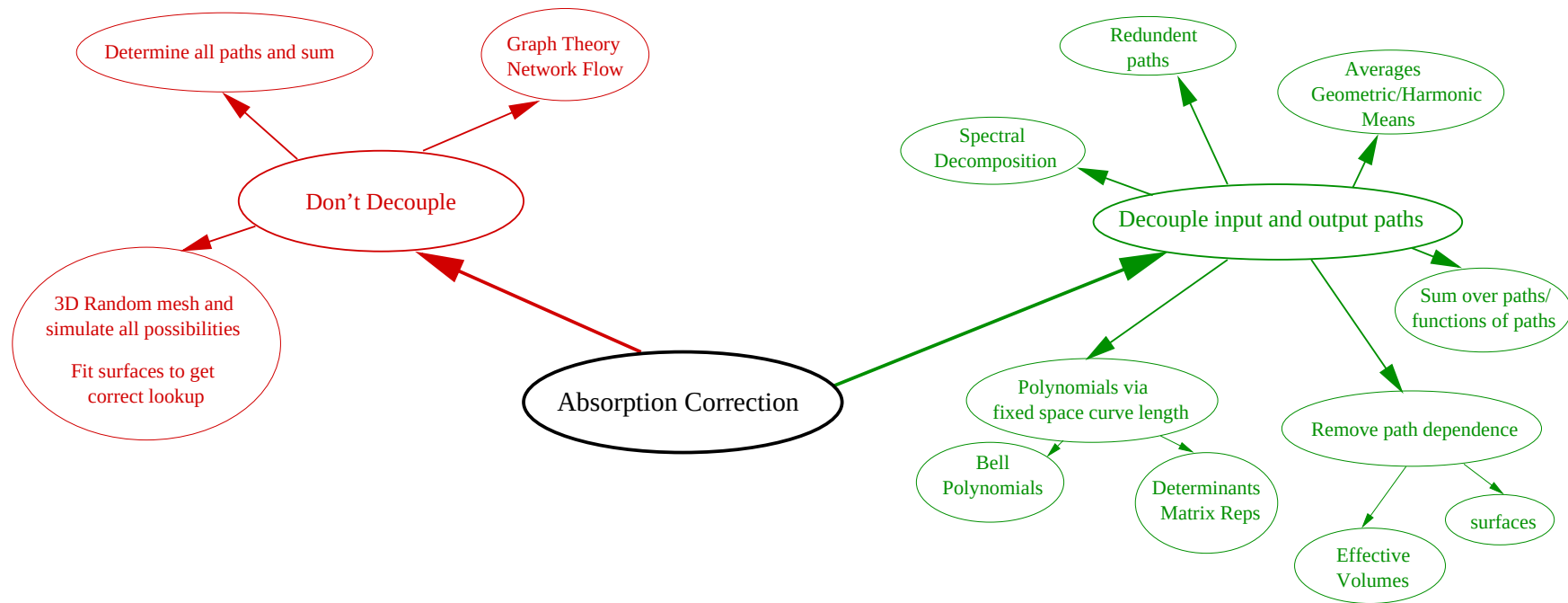
A Road to a Solution.

Absorption Correction

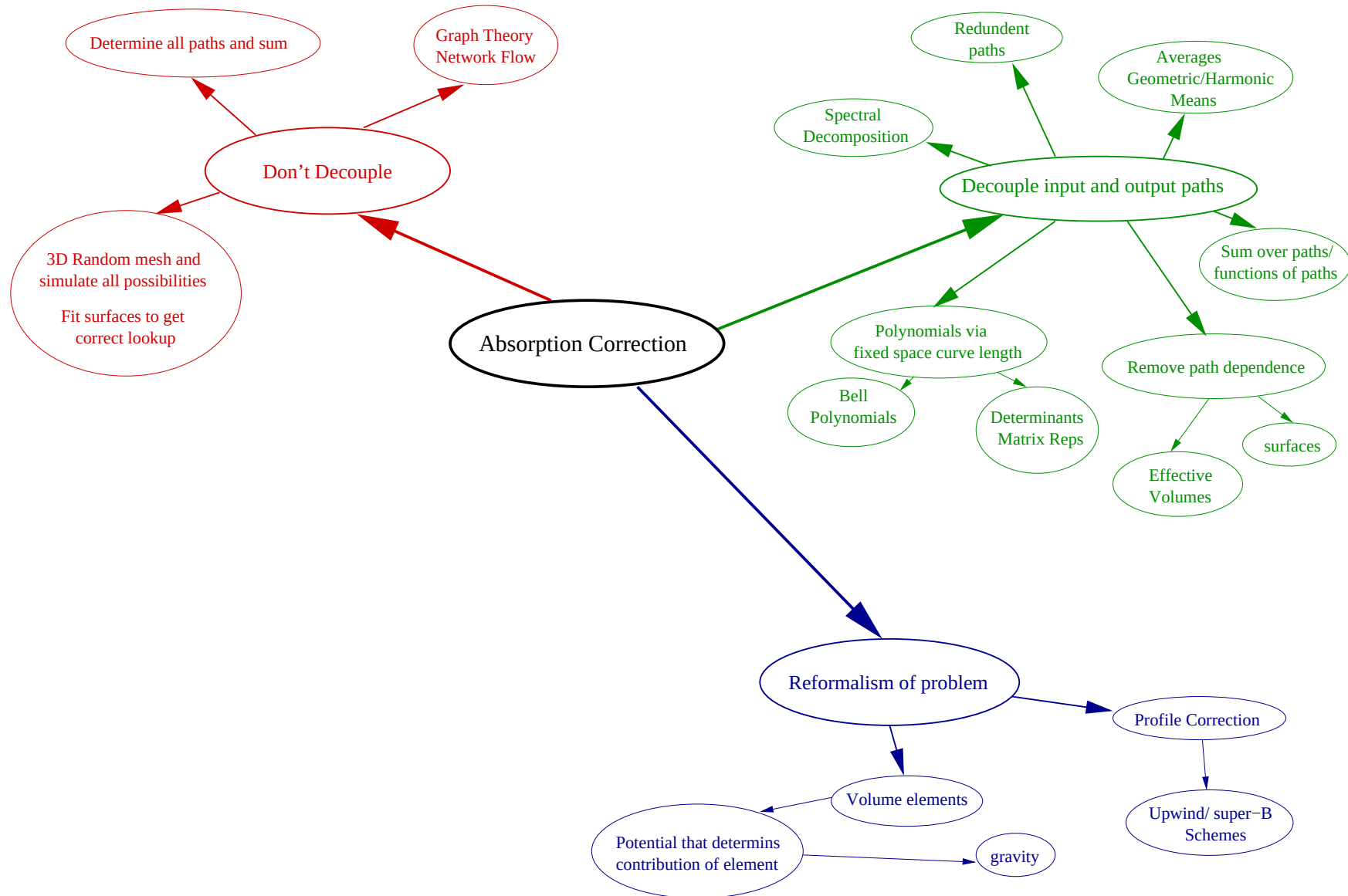
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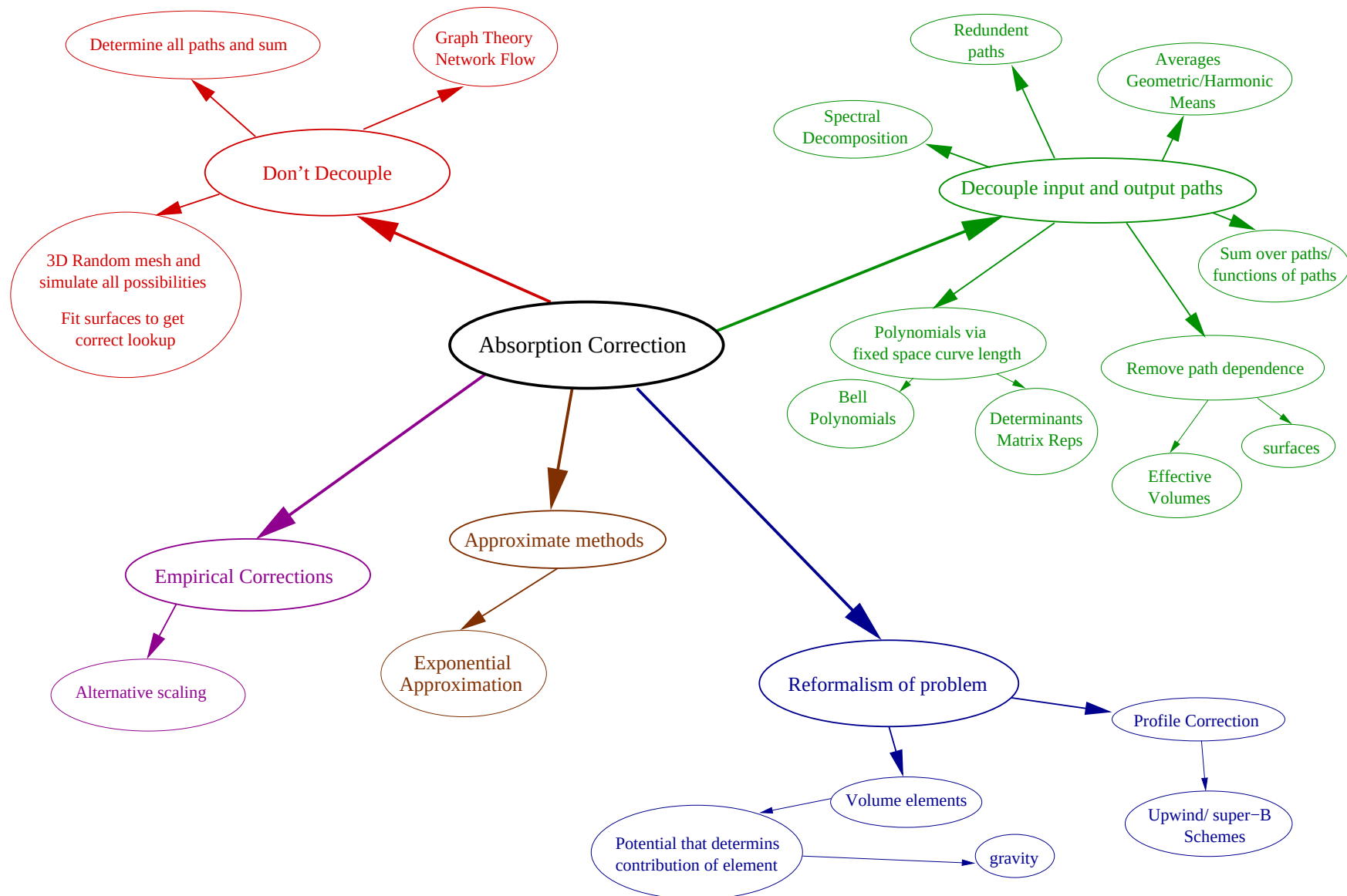
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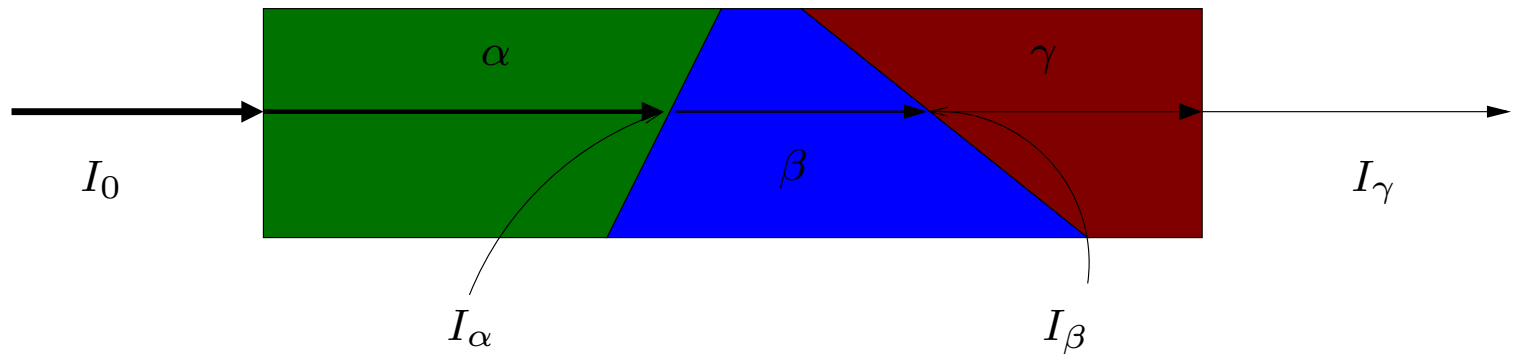


A Road to a Solution.



Tools.

Consider the following collection of objects.



- The fraction of light transmitted through the first object (α) along path x_α^i is:

$$\frac{I_\alpha}{I_0} = e^{-\mu_\alpha x_\alpha^i} = T_\alpha^i$$

Tools.

- This is easily generalized:

$$\frac{I_{\alpha}}{I_0} = e^{-\mu_{\alpha} x_{\alpha}^i} = T_{\alpha}^i$$

$$\frac{I_{\beta}}{I_{\alpha}} = e^{-\mu_{\beta} x_{\beta}^i} = T_{\beta}^i$$

$$\frac{I_{\gamma}}{I_{\beta}} = e^{-\mu_{\gamma} x_{\gamma}^i} = T_{\gamma}^i$$

Tools.

- We can also construct a transmission coefficient for multiple objects

$$\frac{I_\gamma}{I_0} = e^{-\mu_\alpha x_\alpha^i} e^{-\mu_\beta x_\beta^i} e^{-\mu_\gamma x_\gamma^i} = T_{\alpha\beta\gamma}^i$$

- Generalizing this to m different objects all having different absorption coefficients and different path lengths we have

$$T_{out}^i = T_{\alpha\beta\gamma\dots}^i = \prod_{j=1}^m T_j^i$$

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$$T_{out}^i = T_{\alpha\beta\gamma\dots}^i = \prod_{j=1}^m T_j^i$$

- Hence we have proved that along any given path transmission coefficients are multiplicative.

Tools 2.

Consider the transmission integral.

$$T = \frac{1}{V} \iiint_V e^{-\mu(p+q)} dV$$

- We can approximate the integral by a sum over n different paths.

$$\tilde{T} = \frac{1}{n} \sum_{i=1}^n e^{-\mu(p_i+q_i)}$$

- This can be expanded to include other other absorbing material by using our previous generalization of the transmission coefficient.

Tools 2.

- If we have m different regions and n different sample paths:

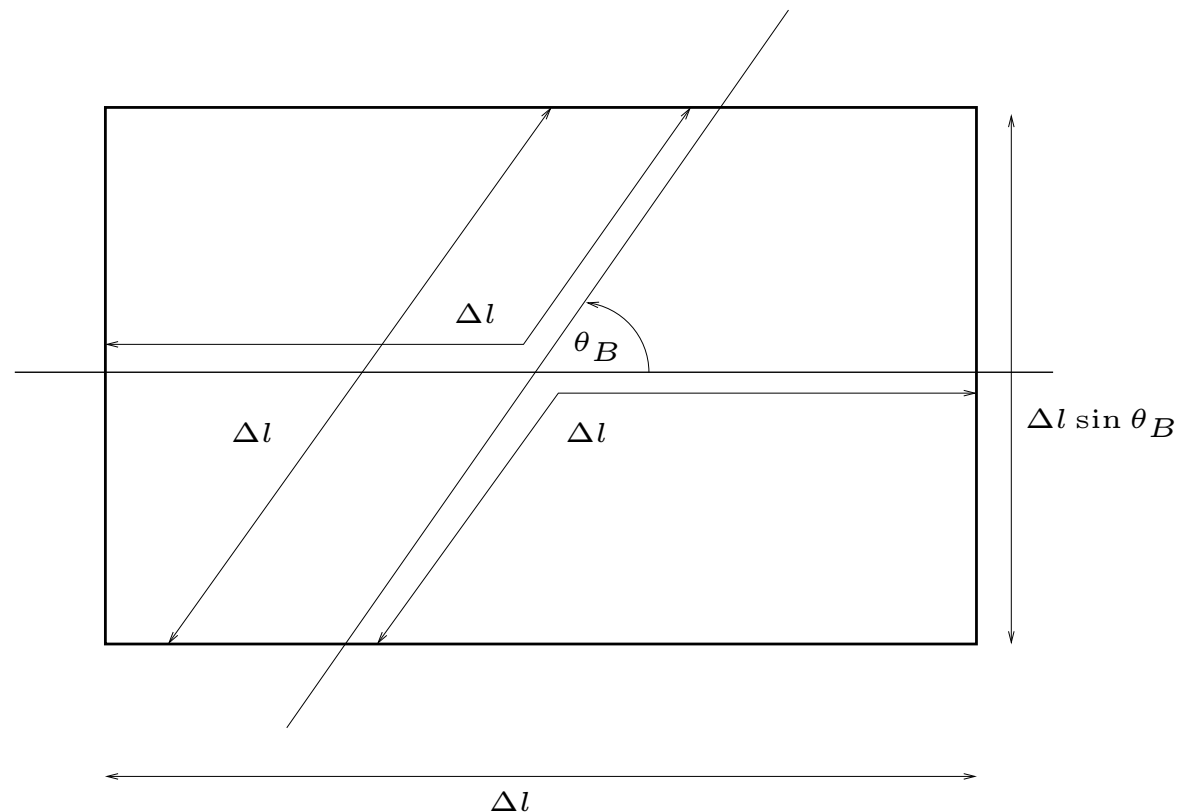
$$\tilde{T} = \frac{1}{n} \sum_{i=1}^n \prod_{j=1}^m e^{-\mu_j x_j^i} = \frac{1}{n} \sum_{i=1}^n \exp \left[\sum_{j=1}^m -\mu_j x_j^i \right]$$

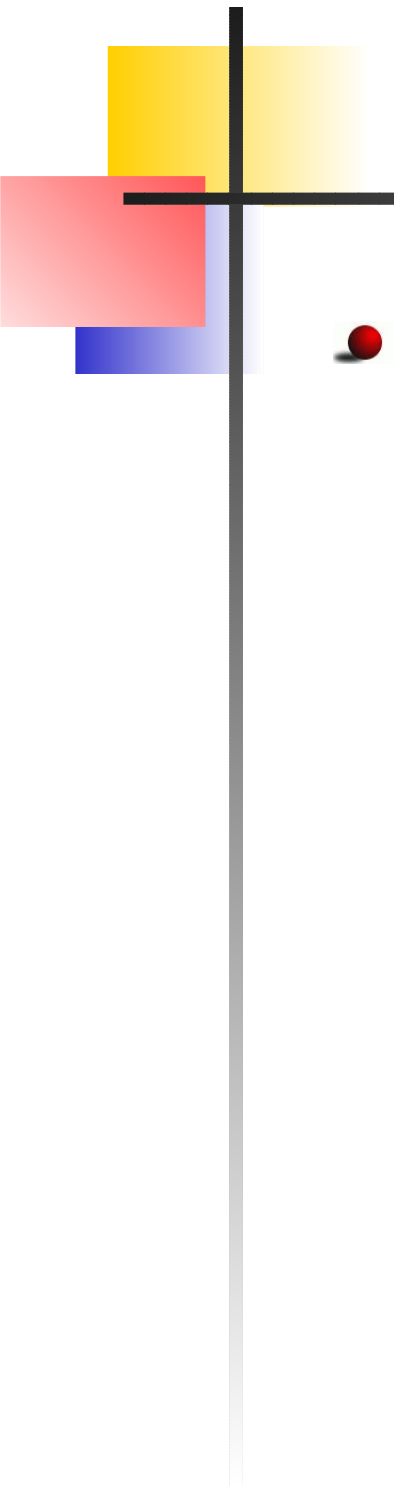
- Note that we could have also bounded the paths in our integral by the surface of the solution and integrated over the crystal volume. This is incompatible with Howells polyhedra and all subsequent methods based on them.

Tools 3.

Discretization continued...

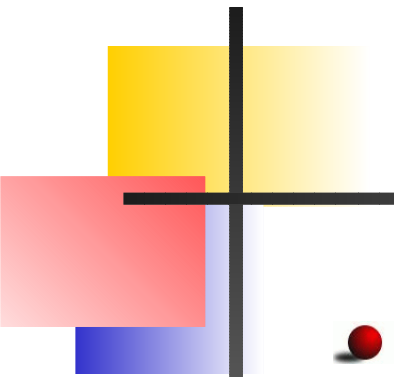
- We could divide our loop/crystal sample up into discretized blocks.





Tools 3.

- Choosing the block dimensions in such a manner gives equal length paths (Δl) for all paths through the block, e.g.
 1. A diffracted ray passing through the block from a lower block
 2. A ray that is diffracted in the block
 3. A ray that passes through the block



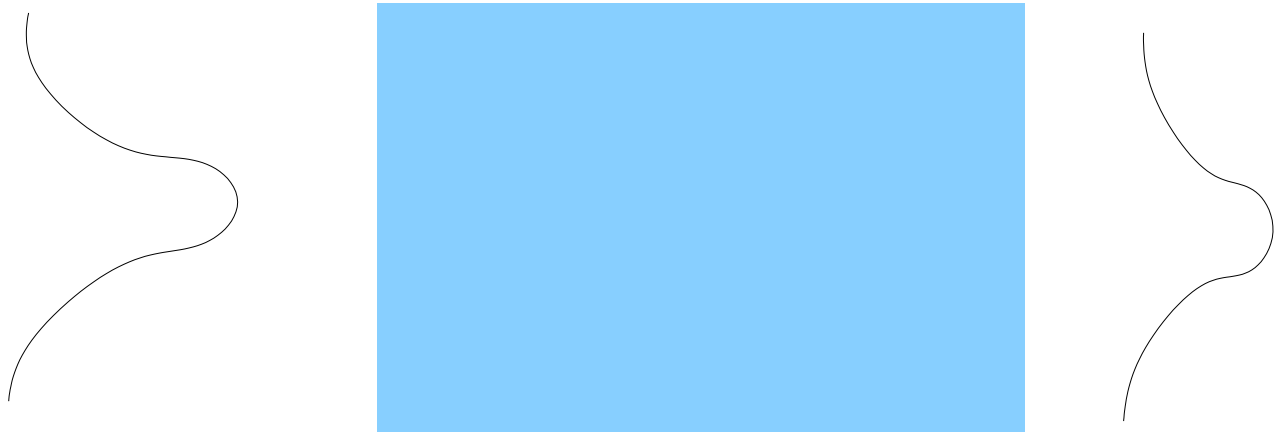
Tools 3.

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 1. A diffracted ray passing through the block from a lower block
 2. A ray that is diffracted in the block
 3. A ray that passes through the block
- Hence the transmission factor for a given block is constant:

$$\begin{aligned} T(\Delta l) &= e^{-\mu \Delta l} \\ \Rightarrow T(\Delta l)^n &= T(n\Delta l) \end{aligned}$$

Tools 4.

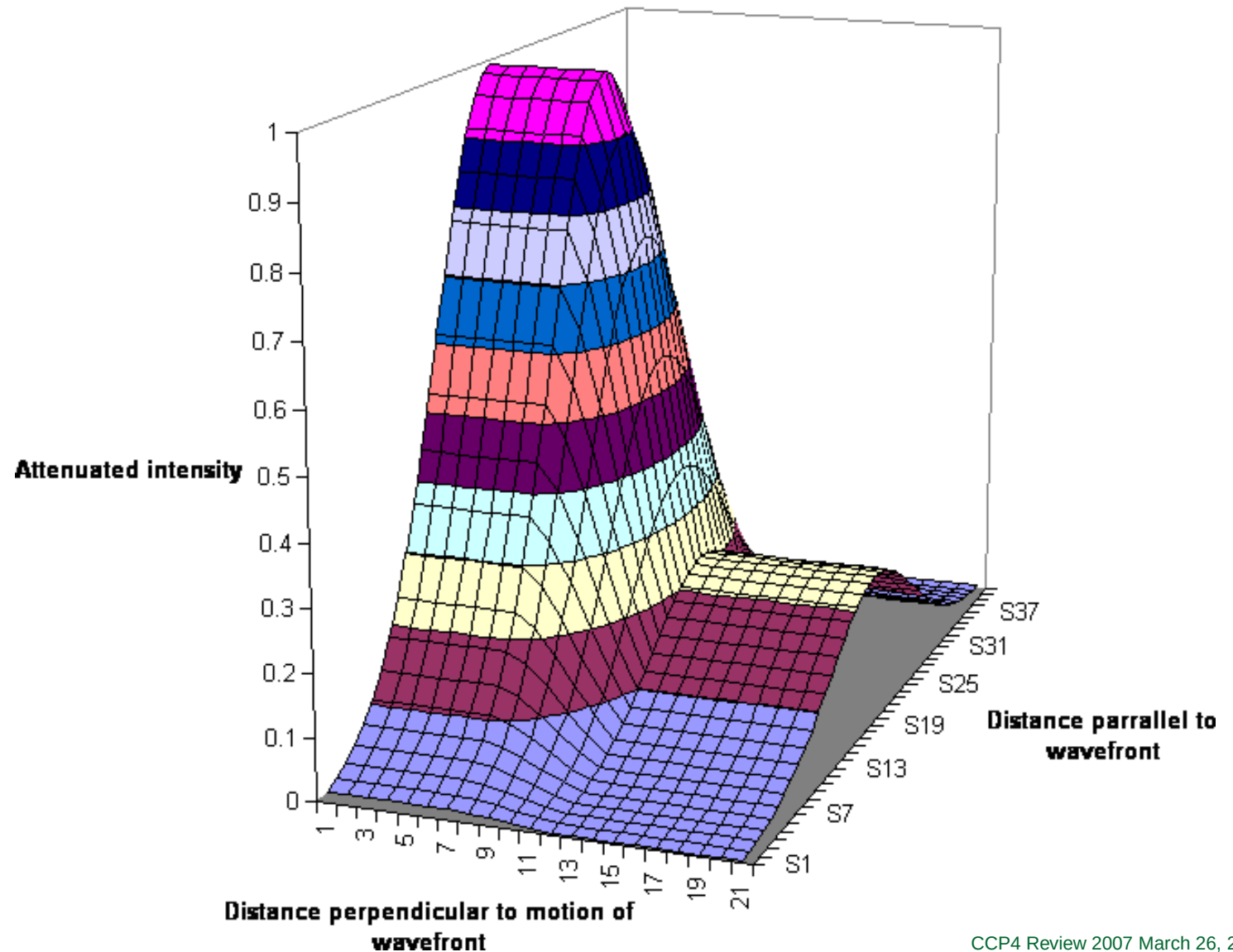
- Imagine a Gaussian intensity profile traveling through a rectangular block:



- The intensity is changed due to the absorption that the profile experiences.

Tools 4.

Attenuation of intensity of a gaussian through an absorbing rectangle



Tools 4.

- We could also imagine a flat profile traveling through a block that has a suitably shaped end:



- From the point of view of the resulting intensity profile the two ways of arriving at its destination are interchangeable.
- Also note that the input and output profiles are interchangeable for absorbing media.

Tools 5.

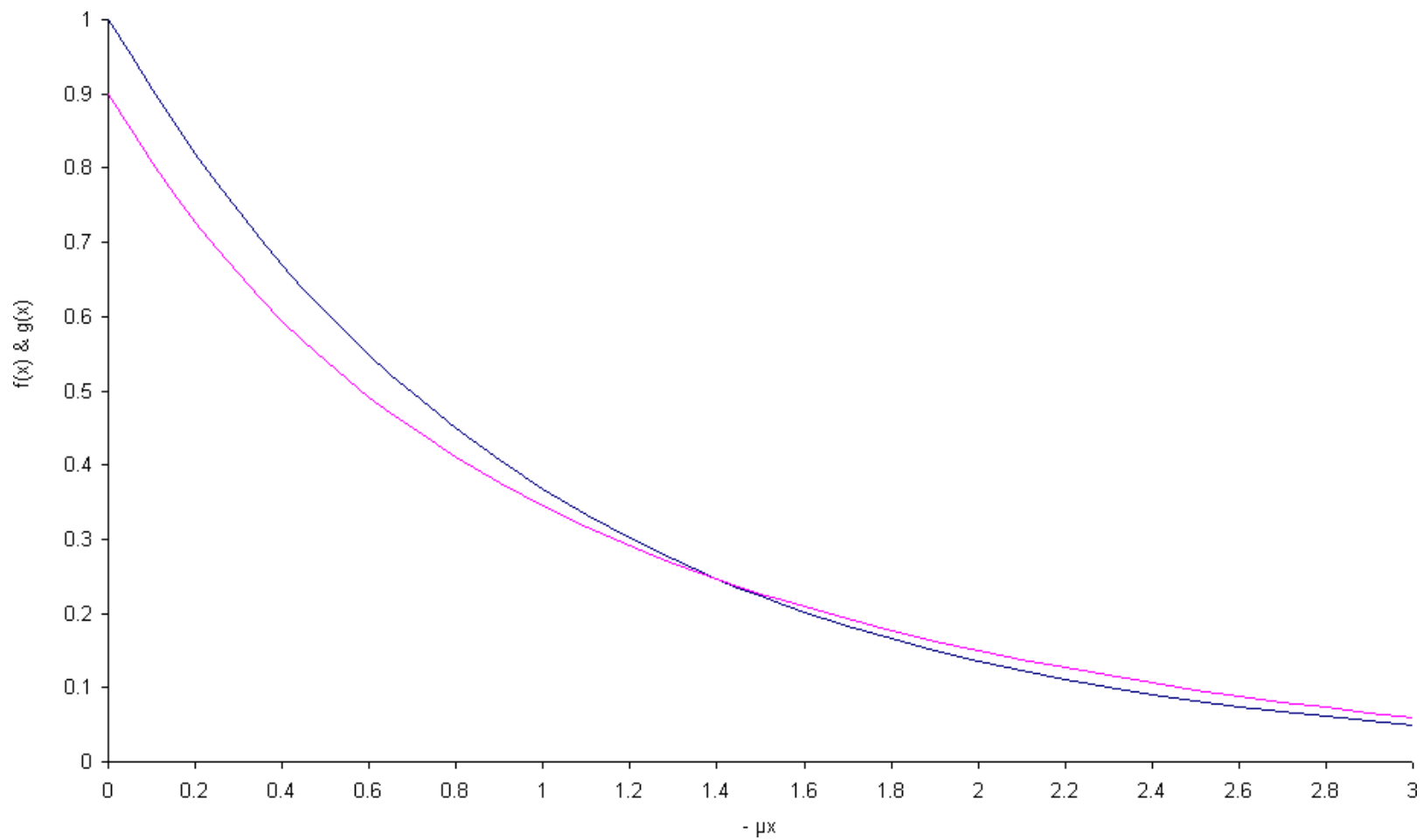
- We could replace the exponential function that describes the decay in intensity by a function that looks similar over a given range.

$$f(x) = e^{-\mu x}$$

$$g(x) = \frac{A}{(x + B)^C} - D$$

- For a given A, B, C, D $f(x) \approx g(x)$ over a certain range.
- The values of the coefficients could be determined by e.g. Taylor expanding the functions then equating expansion coefficients, or, curve fitting over a specified interval.

Tools 5.



Approximate Methods.

- We could approximate our discretized equation for $\tilde{T}$ by expanding the exponential function to first order:

$$\begin{aligned}\tilde{T} &= \frac{1}{n} \sum_{i=1}^n \exp \left[\sum_{j=1}^m -\mu_j x_j^i \right] \\ &= 1 - \frac{1}{n} \sum_{i=1}^n \sum_{j=1}^m \mu_j x_j^i\end{aligned}$$

- By resumming the sum we have:

$$\tilde{T} = 1 - \sum_{j=1}^m \mu_j \tilde{x}_j$$



Approximate Methods.

- Here, $\tilde{x}_j$ is the arithmetic mean path length in the j^{th} region, given by:

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Approximate Methods.

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- Doing this removes the need for complicated integration and only requires sampling paths in different regions.
- However this method is only accurate for short wavelengths and/or small crystals.
- The associated error is highly non-linear and depends on many different contributions.



Series Summations.

- If we assume the following:
 1. The crystal is bounded by planes.
 2. The crystal is formed from a regular periodic lattice of diffracting points.
 3. The crystal is divided by critical lines (as with Howells polyhedra)
- Using series summation we can define an equation that accounts for diffraction along any given path.
- Input parameters are the Bragg angle, the gradients of the bounding planes and the distance along the ray.

Series Summations.

- Our equation looks like:

$$A_{ray} = \tilde{A} \left(\frac{1 - M^{i_c+1}}{1 - M} \right) + \tilde{N} \left(\frac{N - N^{n-i_c+1}}{1 - N} \right)$$

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Series Summations.

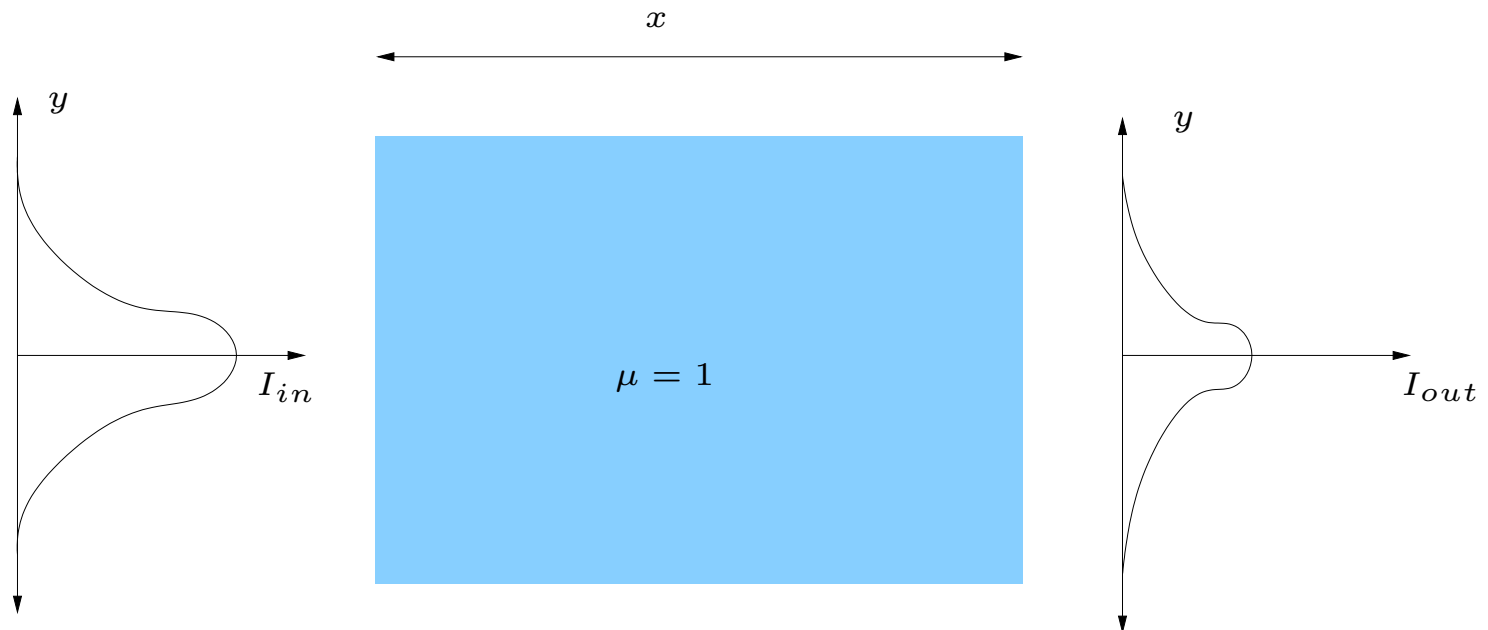
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- Because transmission coefficients are multiplicative along any given path we can easily extend this formula so that it takes account of the non-diffracting material that a ray passes through as it enters the loop/capillary...
- Since diffraction needs to be defined by a "one-to-many" function we can only estimate the absorption that the associated outgoing rays experience.

Profile Correction.

- In essence we wish to know the results of the experiment as if it were performed through media that has no absorption, i.e. $\mu = 0$
- Lets look at a very simplistic example:



Profile Correction.

If...

$$I_{in} = e^{-y^2}$$

$$I_{out} = I_{in}e^{-x} = e^{-y^2-x}$$

$$I_{corrected} = \epsilon(x)I_{out} = e^x e^{-y^2} e^{-x} = I_{in}$$

$$\Rightarrow \epsilon(x) = e^x$$

- This is a very simplistic example that demonstrates the idea.
- The challenge is to include diffraction.

Profile Correction.

Numerically...

- By using Tools 3 and a suitable indexing scheme we can derive equations that describe the transmitted intensities for a diffracted and transmitted ray for the ij^{th} block.

$$I_{ij}^t = [(1 - F^2)A_b]^j I_0$$

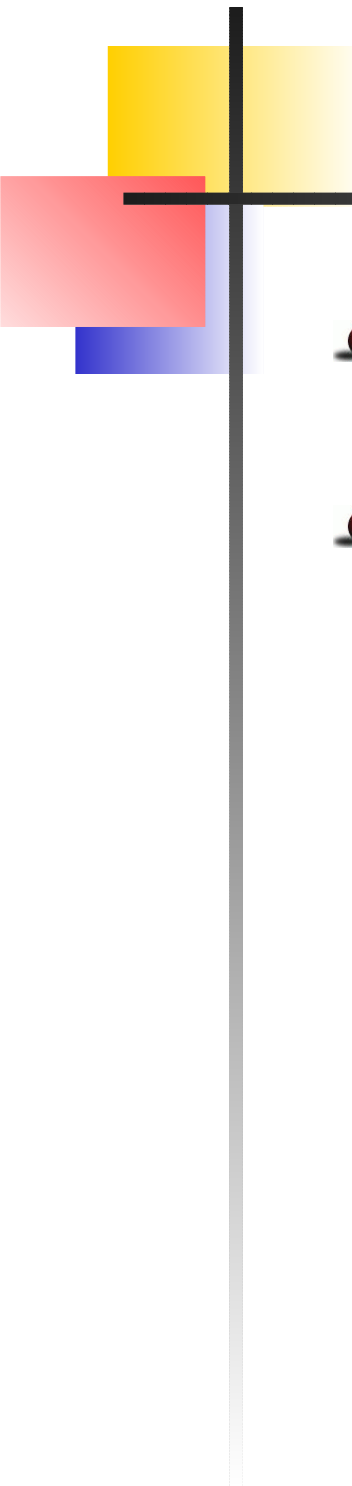
$$I_{ij}^d = A_b F^2 [(1 - F^2)A_b]^{j-1} I_0 + A_b I_{i-i,j}^d$$

Profile Correction.

Numerically...

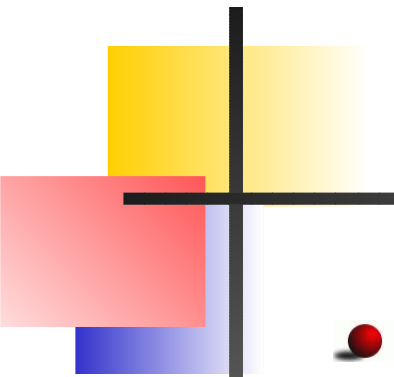
- Using recursion and doing the sum we find the transmission coefficient for the diffracted ray that only depends on the input and output surfaces:

$$T_{ij}^d = \frac{F^2(A_b^j - A_b^{i+j})(1 - F^2)^{j-1}}{1 - A_b}$$



Profile Correction.

- Using this equation we can produce an accurate numerical profile correction.
- We can also account for any non-diffracting material surrounding the crystal

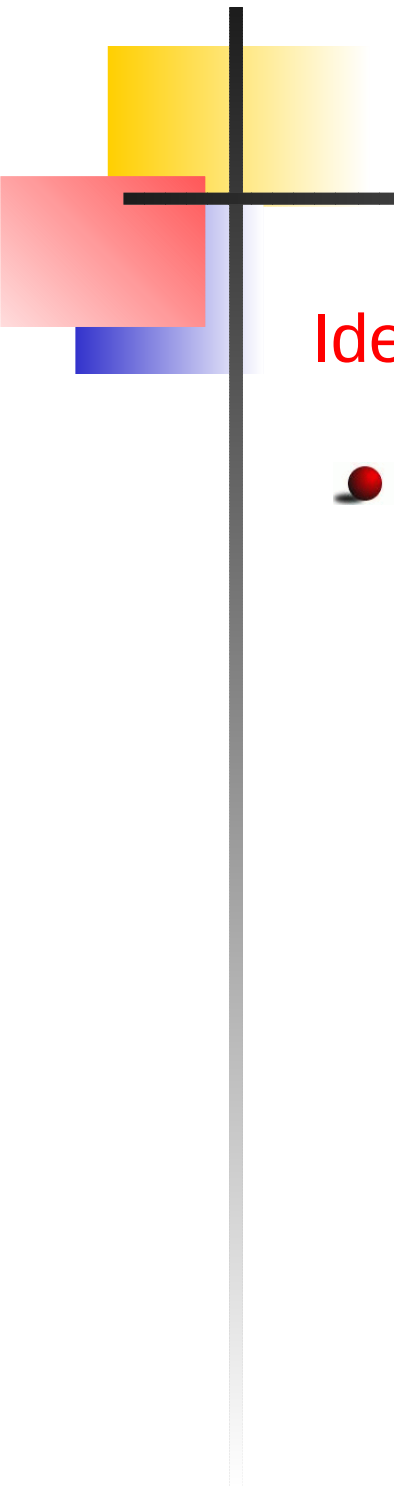


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The downside...

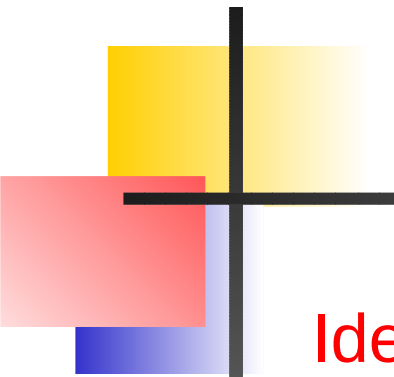
- This method is computationally expensive.
- A prior knowledge of the diffracting power of the crystal F^2 is required. A way to overcome this might be to normalize and work on a relative scale?



Spherical Waves.

Idea...

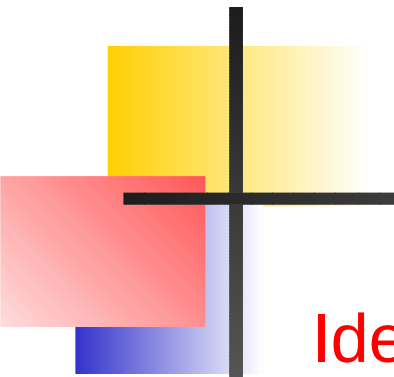
- We treat the crystal as an object that emits a spherical wave from every point within.



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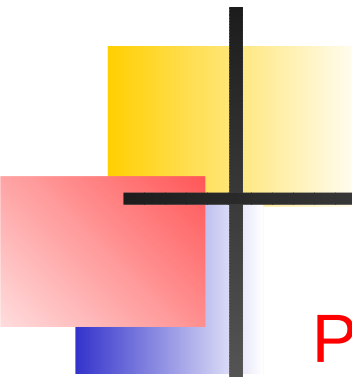
- We treat the crystal as an object that emits a spherical wave from every point within.
- Measure the energy density at the bounding surface (for example the surface of the droplet). Since this decays $\propto 1/r^2$ we can use Tools 5 to get an approximate function that looks like an exponential decay.



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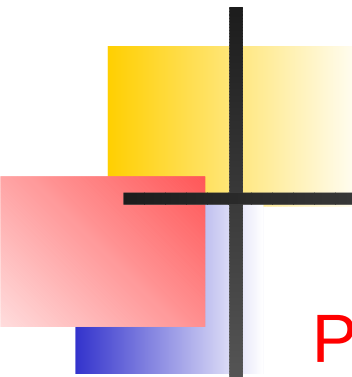
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- Multiply the input and output surfaces that are cut out by the beam and then normalize.



Spherical Waves.

Problems...

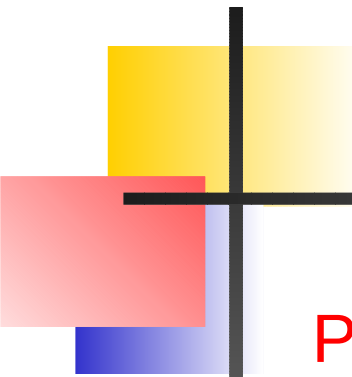
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- How do we measure the energy density at the surface? $\Rightarrow$ Look at the intersection of the wave and surface (i.e. equate the equations).



Spherical Waves.

Problems...

- Mixing between off axis paths. $\Rightarrow$ introduce a Bragg dependent phase. But! this might not provide a sharp enough cut-off.
- How do we measure the energy density at the surface? $\Rightarrow$ Look at the intersection of the wave and surface (i.e. equate the equations).
- How do we combine the waves in a meaningful manner? $\Rightarrow$ Hard. But the starting equation need only describe the intersection of the wave and surface where the wave is emitted from any given point relative to the surface. Providing there is no mixing of off axis paths we need only integrate over the crystal volume or do the sum.

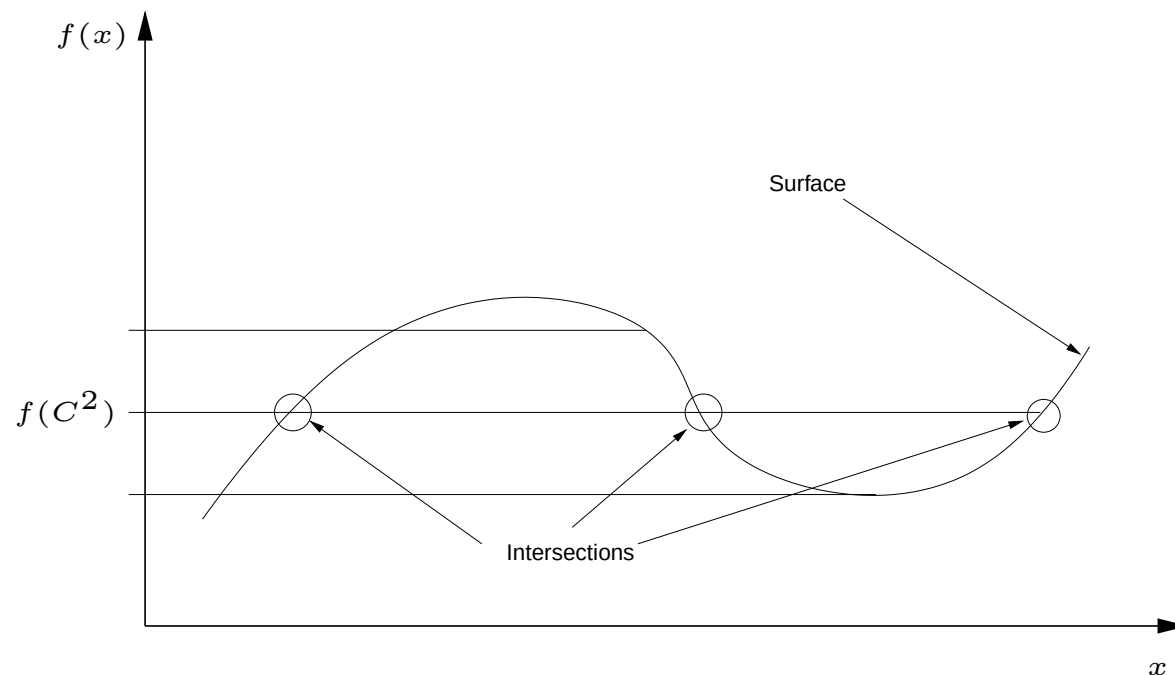


Other Ideas.

- Previously we considered Riemann integration. There are other ways...
- Numerical, e.g. Runge-Kutta methods.
- Lebesgue integration.
- Daniell integration.
- Darboux integration.
- Haar integration.

Other Ideas.

- Represent the transmission coefficient by a general Maclaurin series and determine the coefficients by counting intersections or looking at densities related to the distance around a given contour $\Rightarrow$ Lebesgue Integration.





Other Ideas.

- Tensor/Matrix methods. LU decompose, identities...

$$\det M = e^{\ln \det M} = e^{\text{Tr} \ln M}$$



Other Ideas.

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- Transformation of the integral. Schwarz-Christoffel, Jacobian, Wick rotate???

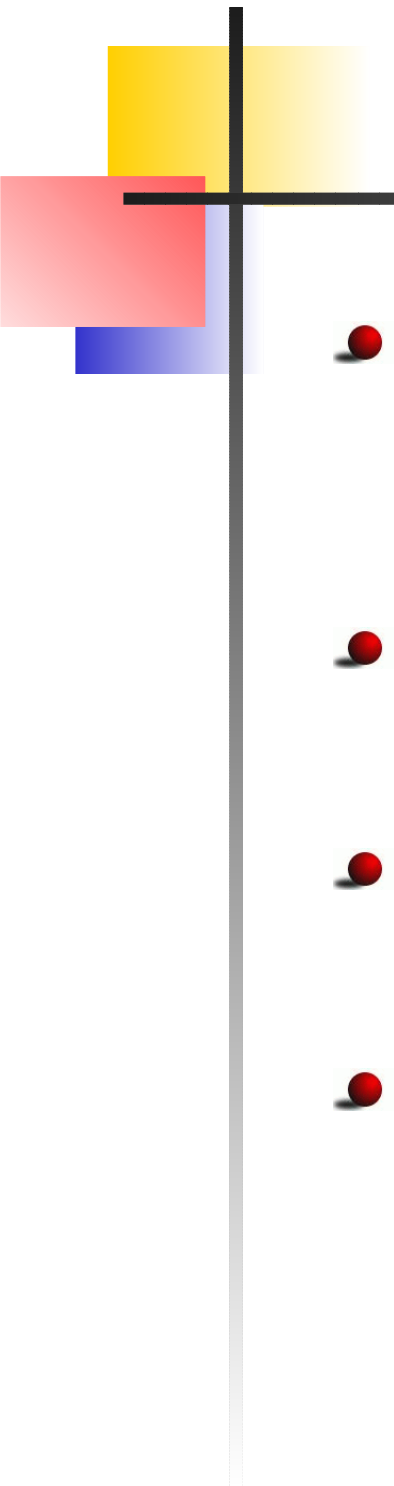


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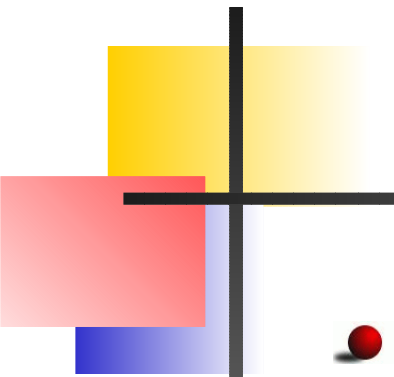


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- External potentials and equality of volume blocks.



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- Probabilistic methods, the transmission coefficient can be viewed as a measure of probability.
- External potentials and equality of volume blocks.
- Graph theory and Network flow.