IMPEDANCE SPECTROSCOPY

• Antibody sensors

• Biomimetic membrane sensors

• Nucleic acid sensors
  [lab-on-a-chip]
Would have liked an antibody based ChemFET
Does not give a reliable response

A more complex analysis of the antibody-antigen electrostatic interactions needed.
Impedance spectroscopy can be used to analyse protein–charged analyte interactions.
Simple equivalent circuit of a charged interface
Impedance Spectroscopy

\[ V_{\text{exc}} = V_i \sin(\omega t) \]

Scan frequency, \( \omega \),
[milliHertz to MegaHertz range]

\[ \frac{V_{\text{test}}}{V_{\text{ref}}} = \frac{Z_{\text{test}}}{Z_{\text{ref}}} = \frac{Z_{\text{test}}}{R_{\text{ref}}} \]

\[ Z_{\text{test}} = \frac{V_{\text{test}} R_{\text{ref}}}{V_{\text{exc}} - V_{\text{test}}} \]

Test System,
\( Z_{\text{test}} \)

\[ V_{\text{test}} = V_o \sin(\omega t + \varphi) \]

Reference resistor, \( R_{\text{ref}} \)

Measure amplitude, \( V_o \), and phase, \( \varphi \), as a function of the scanned frequency, \( \omega \).

Calculate real, \( Z_{\text{Re}} \), and imaginary, \( Z_{\text{Im}} \), parts of the impedance of the test system
Signal – magnitude and phase

Signal e.g. voltage

Magnitude of signal 1

Magnitude of signal 2

Phase difference

Signal 1

Signal 2

Time
Voltages and impedances represented as complex numbers

**Complex number**

\[ x + jy \]

\( j = \) the imaginary number \( \sqrt{-1} \) (square root of minus one)

Impedance, \( Z = x + jy \)

Mathematicians use \( i \) to represent \( \sqrt{-1} \)
Engineers use \( j \)
Voltages represented as complex numbers

\[ V = V_o \cos(\omega t + \phi) = V_o \cos(2\pi ft + \phi) \]

In complex arithmetic

\[ \exp(j\theta) = \cos(\theta) + j\sin(\theta) \]

Therefore

\[ V = \Re[V_o \exp(j\omega t + j\phi)] \]

Usually written (remembering in calculations that it is the real part of a complex number)

\[ V = V_o \exp(j\omega t + j\phi) = V_o \exp(j\omega t)\exp(j\phi) \]
Impedances represented as complex numbers

- Resistance, $Z_R = R$  \hspace{1cm} (R + j0)
- Capacitance, $Z_C = -j/C\omega$  \hspace{1cm} (0 - j/C\omega)
- Inductance, $Z_L = jL\omega$  \hspace{1cm} (0 + jL\omega)
- Constant Phase Element, $Z_{CPE} = \sigma(j\omega)^{-\alpha}$

$Z = \text{Impedance (ohms)}$

$\omega = \text{Radial frequency} \equiv 2\pi f$

$f = \text{frequency (Hz \equiv s}^{-1})$
Impedance Spectroscopy

Impedances in series

\[ Z_{\text{equiv}} = Z_1 + Z_2 \]

Impedances in parallel

\[ \frac{1}{Z_{\text{equiv}}} = \frac{1}{Z_1} + \frac{1}{Z_2} \]

\[ Z_{\text{equiv}} = \frac{Z_1 Z_2}{Z_1 + Z_2} \]
Simple equivalent circuit of a charged interface

- Charge Transfer Resistance ($R_{ct}$)
- Double Layer Capacitance ($C_d$)
- Bulk Solution Resistance ($R_\Omega$)
- Surface charge ($\sigma$) in $C m^{-1}$
Impedance Spectroscopy

\[ Z_d = -\frac{j}{\omega C_d} \]

\[ Z_{ct} = R_{ct} \]

\[ Z_\Omega = R_\Omega \]

\[ Z_{equiv} = \frac{R_{ct} + R_\Omega + jC_d R_{ct} R_\Omega \omega}{1 + jC_d R_{ct} \omega} \]

\[ Z_{equiv} = \frac{R_{ct} + R_\Omega - C_d R_{ct}^2 R_\Omega \omega^2}{1 + C_d R_{ct}^2 \omega^2} + j \left( \frac{C_d R_{ct} R_\Omega + C_d R_{ct}^2 + C_d R_{ct} R_\Omega \omega}{1 + C_d R_{ct}^2 \omega^2} \right) \omega \]
Impedance Spectroscopy

\[ V_{exc} = V_i \sin(\omega t) \]

Scan frequency, \( \omega \), [milliHertz to MegaHertz range]

\[ Z_{test} = \frac{V_{exc} R_{ref}}{V_{exc} - V_{test}} \]

Reference resistor, \( R_{ref} \)

Test System, \( Z_{test} \)

\[ V_{test} = V_o \sin(\omega t + \varphi) \]

Measure amplitude, \( V_o \), and phase, \( \varphi \), as a function of the scanned frequency, \( \omega \).

Calculate real, \( Z_{Re} \), and imaginary, \( Z_{Im} \), parts of the impedance of the test system.
Impedance Spectroscopy

\[ V_{in} = V_i \sin(\omega t) \]

Test System

\[ V_{out} = V_o \sin(\omega t + \phi) \]

Reference resistor

Fit real, \( Z_{Re} \), and imaginary, \( Z_{Im} \), parts of the impedance of the test system to an equivalent circuit.
Impedance Spectroscopy

Cole-Cole Plots

\[ Z_{\text{total}} = \frac{R_{ct} + R_{\Omega} + jC_d R_{ct} R_{\Omega} \omega}{1 + jC_d R_{ct} \omega} \]
Impedance Spectroscopy

$-Z_{\text{imag}} / (\text{ohms} \times 10^4)$

$Z_{\text{real}} / (\text{ohms} \times 10^4)$

Base of a root canal
Non-linear Regression and Impedance Spectroscopy
Impedance Spectroscopy

Frequency Response Analyser

~30 cm
Impedance Spectroscopy

Set frequency scan, $\omega$, [milliHertz to MegaHertz range] and voltage $V_i$

Display output signal magnitude, $V_o$, phase, $\varphi$, $Z_{\text{real}}$ and $Z_{\text{imag}}$

Send magnitude, $V_o$, phase, $\varphi$, $Z_{\text{real}}$ and $Z_{\text{imag}}$ to a PC

Test system monitoring port ($V_{\text{test}}$)

Excitation signal port ($V_{\text{exc}}$)

$$V_{\text{exc}} = V_i \sin(\omega t)$$

$$V_{\text{test}} = V_o \sin(\omega t + \varphi)$$

Reference resistor
## Impedance Spectroscopy
### Frequency Response Analyser

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Magnitude</th>
<th>Phase</th>
<th>$Z_{\text{real}}$</th>
<th>$Z_{\text{imag}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mHz</td>
<td>7.2</td>
<td>0.21</td>
<td>7.04</td>
<td>1.50</td>
</tr>
<tr>
<td>10 MhZ</td>
<td>5.3</td>
<td>0.33</td>
<td>5.01</td>
<td>1.72</td>
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<tr>
<td>100 MhZ</td>
<td>3.5</td>
<td>0.47</td>
<td>3.12</td>
<td>1.59</td>
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<tr>
<td>1 hZ</td>
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<tr>
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<td>0.05</td>
<td>0.70</td>
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## Impedance Spectroscopy

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<td>real1</td>
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<tr>
<td>10</td>
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How do we get the parameters?
FITTING EXPERIMENTAL DATA TO A MODEL

• LINEAR REGRESSION

• NON-LINEAR REGRESSION
LINEAR REGRESSION

\[ y = a + bx + cx + dx + \ldots \quad \text{\(y\) dependent variable} \]

\[ y = a + bx + cx^2 + dx^3 + \ldots \quad \text{\(x, z\) independent variables} \]

\[ y = a + bx + cz \]

Minimise sum of weighted residuals squared

\[
\chi^2 = \sum_{i=1}^{n} \left( \frac{y_{i,\text{exper}} - y_{i,\text{theor}}}{\sigma_i} \right)^2
\]

straight line

\[
\chi^2 = \sum_{i=1}^{n} \left( \frac{y_i - a - bx_i}{\sigma_i} \right)^2
\]
LINEAR REGRESSION

Quadratic, i.e. an analytical function that may be differentiated, e.g. for a straight line

\[ \chi^2 = \sum_{i=1}^{n} \left( \frac{y_i - a - bx_i}{\sigma_i^2} \right)^2 \]

\[ \frac{\partial \chi^2}{\partial a} = -2 \sum_{i=1}^{n} \left( \frac{y_i - a - bx_i}{\sigma_i^2} \right) \]

\[ \frac{\partial \chi^2}{\partial b} = -2 \sum_{i=1}^{n} \left( \frac{y_i - a - bx_i}{\sigma_i^2} \right) x_i \]

At the minimum

\[ \frac{\partial \chi^2}{\partial a} = 0, \quad \frac{\partial \chi^2}{\partial b} = 0 \]

\[ a = \frac{1}{\Delta} \left( \sum_{i=1}^{n} \frac{x_i^2}{\sigma_i^2} \sum_{i=1}^{n} \frac{y_i^2}{\sigma_i^2} - \sum_{i=1}^{n} \frac{x_i}{\sigma_i^2} \sum_{i=1}^{n} \frac{x_i y_i}{\sigma_i^2} \right) \]

\[ b = \frac{1}{\Delta} \left( \sum_{i=1}^{n} \frac{1}{\sigma_i^2} \sum_{i=1}^{n} x_i y_i - \sum_{i=1}^{n} \frac{x_i}{\sigma_i^2} \sum_{i=1}^{n} \frac{y_i}{\sigma_i^2} \right) \]

\[ \Delta = \sum_{i=1}^{n} \frac{1}{\sigma_i^2} \sum_{i=1}^{n} \frac{x_i^2}{\sigma_i^2} - \left( \sum_{i=1}^{n} \frac{x_i}{\sigma_i^2} \right)^2 \]
LINEAR REGRESSION
ESTIMATION OF ERRORS IN BEST ESTIMATES

1. chi-square at the minimum
2. Curvature (‘Width’) of the chi square surface at the minimum

Curvature

= reciprocal of the second differential

For a single parameter:

\[
\frac{1}{\frac{\partial^2 \chi^2}{\partial \theta^2}}
\]
2. Curvature (‘Width’) of the chi square surface at the minimum

\[
\text{Curvature} = \frac{1}{\frac{\partial^2 \chi^2}{\partial \theta^2}}
\]
3. Correlation between parameters, e.g. for a straight line

Curvature equation must accommodate all parameter
Bringing all three aspects together:

First create a Hessian Matrix (n unknown variables, $\theta$)

$$
\begin{bmatrix}
\frac{\partial^2 \chi^2}{\partial \theta_1^2} & \frac{\partial^2 \chi^2}{\partial \theta_1 \partial \theta_2} & \frac{\partial^2 \chi^2}{\partial \theta_1 \partial \theta_3} & \cdots & \frac{\partial^2 \chi^2}{\partial \theta_1 \partial \theta_n} \\
\frac{\partial^2 \chi^2}{\partial \theta_2 \partial \theta_1} & \frac{\partial^2 \chi^2}{\partial \theta_2^2} & \frac{\partial^2 \chi^2}{\partial \theta_2 \partial \theta_3} & \cdots & \frac{\partial^2 \chi^2}{\partial \theta_2 \partial \theta_n} \\
\frac{\partial^2 \chi^2}{\partial \theta_3 \partial \theta_1} & \frac{\partial^2 \chi^2}{\partial \theta_3 \partial \theta_2} & \frac{\partial^2 \chi^2}{\partial \theta_3^2} & \cdots & \frac{\partial^2 \chi^2}{\partial \theta_3 \partial \theta_n} \\
\cdots & \cdots & \cdots & \cdots & \cdots \\
\frac{\partial^2 \chi^2}{\partial \theta_n \partial \theta_1} & \frac{\partial^2 \chi^2}{\partial \theta_n \partial \theta_2} & \frac{\partial^2 \chi^2}{\partial \theta_n \partial \theta_3} & \cdots & \frac{\partial^2 \chi^2}{\partial \theta_n^2}
\end{bmatrix} = \bar{H}
$$
LINEAR REGRESSION
ESTIMATION OF ERRORS IN BEST ESTIMATES

Bringing all three aspects together:

Invert the Hessian Matrix (H) to obtain the Variance-covariance matrix (C)

\[
\mathbf{C} = \mathbf{H}^{-1}
\]

Corresponds to simple inversion if only one parameter variance

\[
\sigma^2 = \frac{1}{\left(\frac{\partial^2 \chi^2}{\partial \theta^2}\right)}
\]
\[
\overline{H} = \begin{vmatrix}
\frac{\partial^2 \chi^2}{\partial a^2} & \frac{\partial^2 \chi^2}{\partial a \partial b} \\
\frac{\partial^2 \chi^2}{\partial b \partial a} & \frac{\partial^2 \chi^2}{\partial b^2}
\end{vmatrix}
\]

\[
\overline{H}^{-1} = \begin{vmatrix}
\frac{\partial^2 \chi^2}{\partial b^2} & -\frac{\partial^2 \chi^2}{\partial a \partial b} \\
\frac{\partial^2 \chi^2}{\partial a^2} & \frac{\partial^2 \chi^2}{\partial a^2} - \left(\frac{\partial^2 \chi^2}{\partial a \partial b}\right)^2 \\
-\frac{\partial^2 \chi^2}{\partial b \partial a} & \frac{\partial^2 \chi^2}{\partial b \partial a} - \left(\frac{\partial^2 \chi^2}{\partial a \partial b}\right)^2 \\
\frac{\partial^2 \chi^2}{\partial a^2} & \frac{\partial^2 \chi^2}{\partial b^2} - \left(\frac{\partial^2 \chi^2}{\partial a \partial b}\right)^2
\end{vmatrix}
\]
LINEAR REGRESSION
ESTIMATION OF ERRORS IN BEST ESTIMATES

Variance-covariance matrix, \( \mathbf{C} \)

\[
\begin{pmatrix}
\text{var}_1 & \text{cov}_{12} & \ldots & \text{cov}_{1n} \\
\text{cov}_{21} & \text{var}_2 & \ldots & \text{cov}_{2n} \\
\vdots & \vdots & \ddots & \vdots \\
\text{cov}_{n1} & \text{cov}_{n2} & \ldots & \text{var}_n
\end{pmatrix}
\]

Standard deviation of the \( i \)th estimated parameter, \( \sigma_i = \sqrt{\text{var}_i} \)

Correlation coefficient between parameters,

\[
\rho_{ij} = \frac{\text{cov}_{ij}}{\sqrt{\text{var}_i \cdot \text{var}_j}}
\]

\( \rho_{ij} = 0 \) no correlation

\( \rho_{ij} = 1 \) total correlation
Variance

\[ \sigma_x^2 = \sum_{i=1}^{n} \frac{(x_i - \bar{x})^2}{n-1}, \quad \bar{x} = \sum_{i=1}^{n} \frac{x_i}{n}, \]

Standard deviation

\[ \sigma_x = \sqrt{\sum_{i=1}^{n} \frac{(x_i - \bar{x})^2}{n-1}} \]

Covariance

\[ \sigma_{xy}^2 = \sum_{i=1}^{n} \frac{(x_i - \bar{x})(y_i - \bar{y})}{n-1}, \quad \bar{x} = \sum_{i=1}^{n} \frac{x_i}{n}, \quad \bar{y} = \sum_{i=1}^{n} \frac{y_i}{n} \]

Correlation coefficient

\[ \rho_{xy} = \frac{\sigma_{xy}^2}{\sigma_x \sigma_y} \]
Correlation coefficient

\[ \rho_{xy} = \frac{\sigma_{xy}^2}{\sigma_x \sigma_y} = 1 \]

\[ \rho_{xy} = \frac{\sigma_{xy}^2}{\sigma_x \sigma_y} = 0 \]
LINEAR REGRESSION
ESTIMATION OF ERRORS IN BEST ESTIMATES

Variance-covariance matrix, $\bar{C}$

$$
\begin{pmatrix}
\text{var}_1 & \text{cov}_{12} & \ldots & \text{cov}_{1n} \\
\text{cov}_{21} & \text{var}_2 & \ldots & \text{cov}_{2n} \\
\vdots & \vdots & \ddots & \vdots \\
\text{cov}_{n1} & \text{cov}_{n2} & \ldots & \text{var}_n
\end{pmatrix}
$$

Standard deviation, $\sigma = \sqrt{\text{var}}$

Correlation coefficient between parameters, 
\[ \rho_{ij} = \frac{\text{cov}_{ij}}{\sqrt{\text{var}_i \cdot \text{var}_j}} \]

- $\rho_{ij} = 0$ no correlation
- $\rho_{ij} = 1$ total correlation
LINEAR REGRESSION
ESTIMATION OF ERRORS IN BEST ESTIMATES

Hessian matrix, \( \bar{H} \), for a linear regression on \( y = ax + b \) [analytical solution]

\[
\begin{vmatrix}
\frac{\partial^2 \chi^2}{\partial a^2} & \frac{\partial^2 \chi^2}{\partial a \partial b} \\
\frac{\partial^2 \chi^2}{\partial b \partial a} & \frac{\partial^2 \chi^2}{\partial b^2}
\end{vmatrix}
\]

\[ \bar{C} = \bar{H}^{-1} \]

\[
\sigma_a = \sqrt{\frac{1}{\Delta} \sum_{i=1}^{n} \frac{x_i^2}{\sigma_i^2}}
\]

\[
\sigma_b = \sqrt{\frac{1}{\Delta} \sum_{i=1}^{n} \frac{x_i^2}{\sigma_i^2}}
\]

\[
\Delta = \sum_{i=1}^{n} \frac{1}{\sigma_i^2} \left( \sum_{i=1}^{n} \frac{x_i^2}{\sigma_i^2} - \left( \sum_{i=1}^{n} \frac{x_i}{\sigma_i} \right)^2 \right)
\]
If standard deviations of data points NOT known
(unweighted regression)

Replace all $\sigma$ by 1; the variance-covariance matrix becomes

$$
\underline{C} = \sqrt{\frac{SS}{n-p}} \; \underline{H}^{-1}
$$

$n = \text{number of points}, \; p = \text{number of estimated parameters}, \; ss = \text{sum of squares of residuals}

$$
ss = \sum_{i=1}^{n} (y_i - a - bx_i)^2 \quad \text{for a straight line}
$$

$\underline{C}$ is the variance-covariance matrix

$\underline{H}^{-1}$ is the inverse of the Hessian matrix, $\underline{H}$

$\underline{H}$ is the Hessian matrix, whose elements are now the second differentials of the sum of squares, $ss$, with respect to all pairs of parameters
Regression Model

\[ Z_{\text{total}} = \frac{\left( R_{ct} + \frac{\sigma}{\omega^n} - \frac{j\sigma}{\omega^n} \right) \left( -j \right)}{R_{ct} + \frac{\sigma}{\omega^n} - \frac{j\sigma}{\omega^n} - \frac{j}{C_d \omega}} + R_{\Omega} \]

\[ Z_{\text{Re}} \]

\[ R_{ct}, R_{\Omega}, C_d, \sigma \text{ and } n \]
FITTING EXPERIMENTAL DATA TO A MODEL

• LINEAR REGRESSION

• NON-LINEAR REGRESSION
NON-LINEAR REGRESSION

The dependent variable cannot be expressed as a linear combination of coefficients and dependent variables,

\[ Z_{total} = \left( R_{ct} + \frac{\sigma}{\omega^n} - \frac{j\sigma}{\omega^n} \right) \left( -j \frac{C_d\omega}{j} \right) + R_\Omega \]

\[ \frac{R_{ct} + \frac{\sigma}{\omega^n} - \frac{j\sigma}{\omega^n} - \frac{j}{C_d\omega}}{R_{ct} + \frac{\sigma}{\omega^n} - \frac{j\sigma}{\omega^n} - \frac{j}{C_d\omega}} \]

\( Z_{real} \) and \( Z_{imag} \) are the dependent variables, \( \omega \) is the independent variables

Parameters to be estimated: \( R_{ct}, R_\omega, \sigma, n \) and \( C_d \)

We still minimise sum of weighted residuals squared

\[ \chi^2 = \sum_{i=1}^{n} \left( \frac{Z_{real,i,exper} - Z_{real,i,theor}}{\sigma_i^2} \right)^2 + \sum_{i=1}^{n} \left( \frac{Z_{imag,i,exper} - Z_{imag,i,theor}}{\sigma_i^2} \right)^2 \]

BUT NO GENERAL ANALYTICAL SOLUTION
Coupling via evanescent field

- **Prism**: $n_{prism}$
- **Air Gap**: 1.0
- **Waveguide**: 1.6 – 1.8
- **Substrate**: 1.52

$n$:

- 1.5 – 2.0
\[
\begin{align*}
\kappa_0 n_{\text{prism}} \sin(\varphi) &= \kappa_{\text{z,core}} \rho = \kappa_{\text{c}} (n_{\text{eff}}) \rho \\
\kappa_0 \sqrt{\zeta^2_{\text{core}} - \langle n_{\text{eff}}^2 \rangle \rho}^{1/2} &= \varphi \rho + \gamma_{\text{superstrate}} + \gamma_{\text{substrate}} + T_{\text{prism}} \\
T_{\text{prism}} &= \sin(\gamma_{\text{superstrate}}) \cos(\gamma_{\text{prism}}) e^{-2in_{\text{superstrate}}}
\end{align*}
\]

\[
T_{\text{prism}} = \begin{cases} 
\tan^{-1} \left( \frac{\zeta_{x, \text{superstrate}}}{\zeta_{x, \text{core}}} \right) & \text{TE mode} \\
\tan^{-1} \left( \frac{\zeta_{x, \text{superstrate}}}{\zeta_{x, \text{core}} n_{\text{superstrate}}^2} \right) & \text{TM mode}
\end{cases}
\]

with similar terms for \( \gamma_{\text{substrate}} \) and \( \gamma_{\text{prism}} \)

\[
\begin{align*}
\zeta_{x, \text{superstrate}} &= \sqrt{k_0 \langle n_{\text{eff}}^2 \rangle \rho - k_0 n_{\text{superstrate}}^2} \\
\zeta_{x, \text{core}} &= \sqrt{k_0 \langle n_{\text{eff}}^2 \rangle \rho - k_0 n_{\text{core}}^2} \\
\zeta_{x, \text{substrate}} &= \sqrt{k_0 \langle n_{\text{eff}}^2 \rangle \rho - k_0 n_{\text{substrate}}^2} \\
\zeta_{x, \text{prism}} &= \sqrt{k_0 \langle n_{\text{eff}}^2 \rangle \rho - k_0 n_{\text{prism}}^2}
\end{align*}
\]

Measured parameter, \( n_{\text{core}} \)
NON-LINEAR REGRESSION

\[ \chi^2 \]

\[ \theta \]

Non-linear surface

Linear surface
NON-LINEAR REGRESSION

No general equation. Make initial estimates (in all dimensions – picture below in just one dimension) and move systematically over the surface to the minimum.
Steepest Descent

Based on the idea that a local minimum is reached if one always moves in the direction \( \frac{\nabla F}{|\nabla F|} \), where \( \nabla F \) is the \textbf{gradient} of the function \( F(x_1, x_2 \ldots x_p) \).

Pictures from Subramaniam Ganapathy and Yi Wu
Nelder and Mead Simplex Method
(1 dimension – first steps)

Step 1 – initial estimate of $\theta$
Step 2 – calculate $\chi^2$

Step 3 – initial estimate of accuracy of initial guess: - step size, $\Delta$

Step 4 – add and subtract $\Delta$ to and from initial estimate

Step 5 – calculate, $\chi^2$, at the initial estimate plus and minus $\Delta$
Step 6 – determine minimum value
Nelder and Mead Simplex Method
(Initial simplex in 2 dimension)
Nelder and Mead Simplex Method
(Initial simplex in 2 dimension)

mid – point of average of all points excluding the worst

Line through worst point and average of other points
Nelder and Mead Simplex Method

**REFLECTION**

Default new trial point
Nelder and Mead Simplex Method

EXPANSION

If $f_3$ is a new minimum move further down the surface.
If \( f_3 \) is a worse point, look at a smaller step.
If a simple contraction does not improve matters bring all points nearer to current minimum.
Nelder and Mead Simplex Method

Calculate initial $P_i$ and $y_i$
Determine $h$, calculate $\bar{P}$
Form $P = (1+\alpha)\bar{P} - \alpha P_h$
Calculate $y^*$

- **Reflection coefficient, $\alpha$**

  - is $y^* < y_i$ ?
    - No
    - is $y^* > y_i$, $i \neq h$ ?
      - Yes
      - No
      - Form $P^{**} = (1+\gamma)P^* - \gamma\bar{P}$
      - Calculate $y^{**}$
      - is $y^{**} < y_i$ ?
        - Yes
        - Replace $P_h$ by $P^{**}$
        - No
        - Replace $P_h$ by $P^*$
        - Has minimum been reached ?
          - Yes
          - Exit
          - No

- **Contraction coefficient, $\gamma$**

  - Yes
  - No
  - is $y^* > y_i$, $i \neq h$ ?
    - Yes
    - No
    - is $y^* > y_h$ ?
      - Yes
      - Replace $P_h$ by $P^*$
      - Replace all $P_i$'s by $(P_i + P)/2$
      - No
      - Replace $P_h$ by $P^{**}$
      - Has minimum been reached ?
        - Yes
        - Exit
        - No

- **Expansion coefficient, $\beta$**

  - Yes
  - No
  - Replace $P_h$ by $P^{**}$
  - Has minimum been reached ?
    - Yes
    - Exit
    - No
Nelder and Mead Simplex Method
(Initial simplex in 3 dimension)
• Make good initial estimate
• Repeat the regression with new initial estimates at $2\theta_{min} - \theta_i$
Multiple minima
No general equation. Approximate values can be obtained if the chi-square surface is close to a quadratic about the minimum.

\[ \chi^2 \]

Non-linear surface

Linear approximation about the minimum

\[ \theta \]
NON-LINEAR REGRESSION
ESTIMATION OF ERRORS IN BEST ESTIMATES

No general equation. Approximate values can be obtained if the chi-square surface is close to a quadratic surface about the minimum – then use

\[
\overline{C} = \overline{H}^{-1}
\]

\[
\overline{H} = \begin{bmatrix}
\frac{\partial^2 \chi^2}{\partial \theta_1^2} & \frac{\partial^2 \chi^2}{\partial \theta_1 \partial \theta_2} & \cdots & \frac{\partial^2 \chi^2}{\partial \theta_1 \partial \theta_n} \\
\frac{\partial^2 \chi^2}{\partial \theta_2 \partial \theta_1} & \frac{\partial^2 \chi^2}{\partial \theta_2^2} & \cdots & \frac{\partial^2 \chi^2}{\partial \theta_2 \partial \theta_n} \\
\cdots & \cdots & \cdots & \cdots \\
\frac{\partial^2 \chi^2}{\partial \theta_n \partial \theta_1} & \frac{\partial^2 \chi^2}{\partial \theta_n \partial \theta_2} & \cdots & \frac{\partial^2 \chi^2}{\partial \theta_n^2}
\end{bmatrix}
\]

Chi-square may not be differentiable and numerical differentiation will then be required (\(\delta \sim 10^{-3} \text{ or } 10^{-4}\))

\[
\frac{\partial \chi^2(p)}{\partial \phi_i} \Rightarrow \frac{\chi^2((1+\delta/2)p_i) - \chi^2((1-\delta/2)p_i)}{p_i \delta}
\]

\[
\frac{\partial^2 \chi^2(p)}{\partial \phi_i \partial \phi_j} \Rightarrow
\]

\[
\frac{\chi^2((1+\delta/2)p_i,(1+\delta/2)p_j) - \chi^2((1-\delta/2)p_i,(1+\delta/2)p_j) - \chi^2((1+\delta/2)p_i,(1-\delta/2)p_j) + \chi^2((1-\delta/2)p_i,(1-\delta/2)p_j)}{p_i p_j \delta^2}
\]
ESTIMATION OF ERRORS IN BEST ESTIMATES
CORRELATION

\[ y = abx = cx \]

\[ y = \frac{abx}{b + x} \]
WHICH IS THE BEST MODEL?

\( \chi^2_A \); \( C_d \), \( R_{ct} \), \( R_\Omega \) and \( Z_w \)

\( \chi^2 \) reduced model

\( \chi^2_B \); \( C_{d,w} \), \( R_{ct,w} \), \( CPE_w \), \( R_P \), \( C_{d,P} \), \( R_\Omega \), \( C_{d,C} \), \( R_{ct,C} \) and \( CPE_C \)

\( \chi^2 \) fuller model
WHICH IS THE BEST MODEL?

\[ \chi^2 = \sum_{i=1}^{n} \frac{(y_{i,\text{exper}} - y_{i,\text{theor}})^2}{\sigma_i^2} \]

is a measure of the goodness of fit.

Checking which of the two chi squares \( \chi^2_{\text{reduced model}} \) or \( \chi^2_{\text{fuller model}} \) is the smaller is not adequate.
WHICH IS THE BEST MODEL?
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\[ \chi^2 = \sum_{i=1}^{n} \left( \frac{y_{i, \text{exper}} - y_{i, \text{theor}}}{\sigma_i^2} \right)^2 \]

is a measure of the goodness of fit.

Checking which of the two chi squares \( \chi^2_{\text{reduced model}} \) or \( \chi^2_{\text{fuller model}} \) is the smaller is not adequate.

We need a statistical test with which to compare \( \chi^2_{\text{reduced model}} \) and \( \chi^2_{\text{fuller model}} \).
WHICH IS THE BEST MODEL?

\( \chi^2_{\text{reduced model}} \) and \( \chi^2_{\text{fuller model}} \)

divided by the degrees of freedom are variances

\[
\chi^2 = \sum_{i=1}^{n} \left( \frac{y_{i,\text{exper}} - y_{i,\text{theor}}}{\sigma_i^2} \right)^2 / \left( N_{\text{data}} - n_{\text{parameters}} \right)
\]

\[
\sigma_x^2 = \sum_{i=1}^{n} \frac{(x_i - \bar{x})^2}{n-1}, \quad \bar{x} = \sum_{i=1}^{n} x_i / n,
\]
WHICH IS THE BEST MODEL?

Use an F-test

$$F = \frac{\text{variance1}}{\text{variance2}}$$

There are tables of the probabilities that

$$F = \frac{X_1^2/\nu_1}{X_2^2/\nu_2}$$

would be as large as it is if the denominator distribution with observed $X_1^2/\nu_1$
actually has a smaller reduced chi-square than that of the denominator distribution with the observed $X_2^2/\nu_2$. The degrees of freedom of the two distributions are $\nu1$ and $\nu2$. 
The $F$ hypothesis test is defined as:

$H_0$: \hspace{1cm} \sigma_1^2 = \sigma_2^2$

$H_a$: \hspace{1cm} \sigma_1^2 < \sigma_2^2 \quad \text{for a lower one-tailed test}$

\hspace{2cm} \sigma_1^2 > \sigma_2^2 \quad \text{for an upper one-tailed test}$

\hspace{2cm} \sigma_1^2 \neq \sigma_2^2 \quad \text{for a two-tailed test}$

Test Statistic: \hspace{1cm} F = \frac{s_1^2}{s_2^2}$

where $s_1^2$ and $s_2^2$ are the sample variances. The more this ratio deviates from 1, the stronger the evidence for unequal population variances.
WHICH IS THE BEST MODEL?

- Calculate the $\chi^2$ for both the fuller model ($\chi^2_{\text{fuller}}$) and the reduced model ($\chi^2_{\text{reduced}}$).

- Calculate variance of the fuller model

$$\text{var}_{\text{fuller}} = \frac{\chi^2_{\text{fuller}}}{df_{\text{fuller}}} = \frac{\chi^2_{\text{fuller}}}{N_{\text{data}} - n_{\text{fuller}}}$$

- Calculate the variance of the ‘extra $\chi^2$’

$$\text{var}_{\text{extra}} = \frac{\chi^2_{\text{reduced}} - \chi^2_{\text{fuller}}}{df_{\text{reduced}} - df_{\text{fuller}}} = \frac{\chi^2_{\text{reduced}} - \chi^2_{\text{fuller}}}{(N_{\text{data}} - n_{\text{reduced}}) - (N_{\text{data}} - n_{\text{fuller}})}$$

$$= \frac{\chi^2_{\text{reduced}} - \chi^2_{\text{fuller}}}{n_{\text{fuller}} - n_{\text{reduced}}}$$

$N_{\text{data}} = \text{number of data points}$, $n = \text{number of estimated parameters}$

$df = \text{degrees of freedom}$

$= \text{number of data points} - \text{number of estimated parameters}$
WHICH IS THE BEST MODEL?

\textbf{F-test}

- Ratio the ‘extra’ variance and the fuller model variance

\[
F = \frac{\text{var}_{\text{extra}}}{\text{var}_{\text{fuller}}} = \frac{\chi^2_{\text{reduced}} - \chi^2_{\text{fuller}}}{n_{\text{fuller}} - n_{\text{reduced}}} = \frac{\chi^2_{\text{fuller}}}{N_{\text{data}} - n_{\text{fuller}}} = \left(\frac{\chi^2_{\text{reduced}} - \chi^2_{\text{fuller}}}{n_{\text{fuller}} - n_{\text{reduced}}}\right)\left(\frac{N_{\text{data}} - n_{\text{fuller}}}{\chi^2_{\text{fuller}}}\right)
\]

- This ratio is termed an \textit{F-ratio}.

Use statistical tables or a statistical computer program library to obtain the probability, given the calculated \(F\) value, for degrees of freedom, \(n_{\text{fuller}} - n_{\text{reduced}}\) and \(N_{\text{data}} - n_{\text{fuller}}\), that the fuller model is a better fit than the reduced model.
Impedance spectroscopy and sensors

Requirement for smaller instrumentation
Impedance Spectroscopy Instrumentation

Frequency Response Analyser (FRA)

Analog Devices AD5933
1 Msps, 12 Bit Impedance Converter Network Analyzer

- ~30 cm
- ~6 mm

FRA price range
£3000 - £15000

~£4 per chip
~£80 per chip plus evaluation board
Impedance spectroscopy and sensors for biological and medical applications
Differences between 'wet' equivalent circuits and classical electrical circuits
Impedance Spectroscopy

\[ Zn^{2+} + 2e^- \rightleftharpoons Zn \]
Impedance Spectroscopy

\[ \text{Hg}_2^{2+} + 2\text{e}^- \rightleftharpoons 2\text{Hg} \]
Impedance Spectroscopy

![Graph showing impedance spectroscopy](image)

- Kinetic control
- Mass transfer control
- Decreasing $\omega$

Mathematical expressions:
- $Z_{lm}$
- $R_\Omega$
- $R_\Omega + \frac{R_{ct}}{2}$
- $R_\Omega + R_{ct}$
- $Z_{Re}$
Simple equivalent circuit of a charged interface
Impedance Spectroscopy

WARBURG IMPEDANCE

<table>
<thead>
<tr>
<th>Double Layer Capacitance</th>
<th>Bulk Solution Resistance</th>
<th>Charge Transfer (electrode) Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_d$</td>
<td>$R_\Omega$</td>
<td>$R_{ct}$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mass transfer controlled impedance (Warburg impedance)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{ct}$ $Z_w$ $R_\Omega$</td>
</tr>
</tbody>
</table>
Impedance Spectroscopy

**WARBURG IMPEDANCE**

\[
Z_w = \frac{\sigma}{\omega^{1/2}} - \frac{j\sigma}{\omega^{1/2}}
\]

\[
\sigma = \frac{RT}{n^2 F^2 A \sqrt{2}} \left[ \frac{1}{C_o \sqrt{D_o}} + \frac{1}{C_R \sqrt{D_R}} \right]
\]

- Number of electrons transferred
- Electrode area
- Diffusion coefficient of oxidant
- Concentration of oxidant
- Concentration of reductant
- Diffusion coefficient of reductant
Warburg impedance is a special case of a CPE where $\alpha = 1/2$

$$Z_{CPE} = \frac{Q}{(j\omega)^\alpha}$$

$$Z_W = Z_{CPE} = \frac{Q}{(j\omega)^{1/2}} = \frac{\sqrt{2}\sigma}{(j\omega)^{1/2}} = \frac{\sigma}{\omega^{1/2}} - \frac{j\sigma}{\omega^{1/2}}$$
Impedance Spectroscopy

CONSTANT PHASE ELEMENT

• Surface Roughness
• A Distribution of Reaction Rates
• Varying Thickness or Composition
• Non-uniform Current Distribution
Impedance Spectroscopy

\[-Z_{\text{imag}} / (\text{ohms} \times 10^4)\]

\[Z_{\text{real}} / (\text{ohms} \times 10^4)\]

Base of a root canal
Impedance Spectroscopy
Impedance Spectroscopy of Antibody-Protein Interactions

O. Ouerghi, A. Touhami, N. Jaffrezic-Renaulta, C. Marteleta, H. Ben Ouada & S. Cosnier

Antigen (human IgG) added

- ▲ 100 ng/ml
- △ 50 ng/ml
- ○ 10 ng/ml

Capture antibody (anti-human IgG) on gold

Bioelectrochemistry, 56 (1-2), 15 May 2002, pp 131-133
Bio-functionalized Pt nanoparticles based electrochemical impedance immunosensor for human cardiac myoglobin (cMb)
Fig. 6  Impedance measurements of IgG-modified silicon samples before and after exposure to anti-IgG and anti-IgM. (a) n-type, 0.1 Ω cm Si substrate. (b) p-type, 0.12 Ω cm Si substrate.
• EIS studies on biomembranes and biomimetic membrane sensors may be found in Section 8, Biomimetic membranes

• EIS studies on nucleic acids and on DNA sensors may be found in Section 9, Lab-on-a-Chip