Supplementary Material (ESI) for Lab on a Chip


# Supplementary Material 1

# Impedance Model for the Maxwell-Garnett Mixing Equation

The Maxwell-Garnett mixing equation (eq. 3) allows to calculate the complex permittivity of a suspension of spherical particles occupying a volume fraction $\phi \ll 1$:

$$\bar{\epsilon}_{\text{mix}} = \bar{\epsilon}_m \frac{1 + 2\phi f_{\text{CM}}}{1 - \phi f_{\text{CM}}} \approx \bar{\epsilon}_m \left(1 + 3\phi f_{\text{CM}}\right) \tag{1.1}$$

The complex permittivity $\bar{\epsilon}$ in turn is reversely related to the impedance (eq. 5 in the main text):

$$Z = \frac{\kappa}{i\omega\bar{\epsilon}} \tag{1.2}$$

where the cell constant $\kappa$ depends on the geometrical arrangement of the measuring electrodes. The simplest case occurs when there is no particle present at all, then $\phi = 0$ and we have:

$$\bar{\epsilon}_{\text{mix}} = \bar{\epsilon}_m \Rightarrow Z_0 = \frac{\kappa}{i\omega\bar{\epsilon}_m} \tag{1.3}$$

Eq. 1.1 and 1.2 taken together suggest that a discrete model for the Maxwell-Garnett mixing equation should exist. And indeed, several combinations of discrete elements satisfying eq. 1.1 and eq. 1.2 are in use for modeling purposes in impedance spectrometry [2–4]. For the comparison with the dielectrophoretic response, however, we need a model that does not lump together properties of the medium with properties of the cell, and that depends explicitly on the particle permittivity $\bar{\epsilon}_p$ rather than some specific property such as membrane capacitance. In this way, we can combine the discrete element analysis freely with sophisticated particle models such as the multi-shell models.

## 1. IMPEDANCE MODEL FOR THE MAXWELL-GARNETT MIXING EQUATION

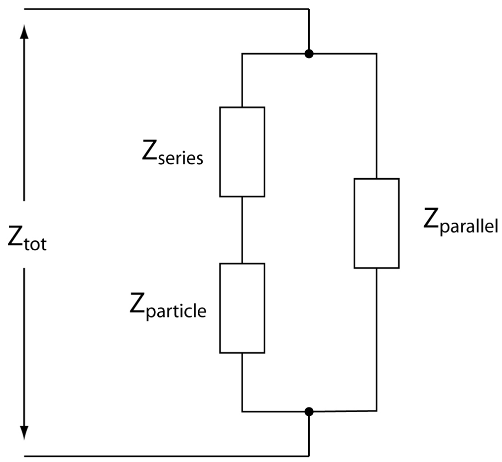


**Figure 1.1:** Layout of the equivalent circuit for the Maxwell-Garnett mixing equation. The current path through the cell is reflected by $Z_{series}$ and $Z_{particle}$, whereas the current path around the cell corresponds to $Z_{parallel}$. Adapted from [3].

We shall adapt the basic layout of the equivalent circuit for the Maxwell-Garnett mixing equation given in [3] to accommodate for an arbitrary particle permittivity. The layout of this equivalent circuit is shown in Fig. 1.1. The $Z_{parallel}$ impedance reflects the current path around the particle, whereas $Z_{series}$ impedance together with the particle impedance $Z_{particle}$ correspond to the current path leading through the cell.

The total impedance of the circuit in Fig. 1.1 is obtained by the standard addition theorems of parallel and series resistors:

$$Z_{tot} = \frac{Z_{parallel} \cdot (Z_{series} + Z_{particle})}{Z_{parallel} + (Z_{series} + Z_{particle})} \tag{1.4}$$

$Z_{tot}$ can be expressed in terms of $Z_0$ and the Clausius-Mossotti factor by combining eq. 1.1, eq. 1.2 and eq. 1.3:

$$Z_{tot} = \frac{\kappa}{i\omega\bar{\epsilon}_{mix}} = \frac{\kappa}{i\omega \cdot \bar{\epsilon}_m (1 + 3\phi f_{\mathrm{CM}})} = \frac{Z_0}{1 + 3\phi f_{\mathrm{CM}}} \tag{1.5}$$

The values of the impedances $Z_{parallel}$, $Z_{series}$ and $Z_{particle}$ in Fig. 1.1 can be found by imposing three particular values for the complex permittivity of the particle: $\bar{\epsilon}_p = 0$, $\bar{\epsilon}_p = \infty$, and $\bar{\epsilon}_p = \bar{\epsilon}_m$.

These three values give rise to simple values for $f_{CM}$ (eq. 8 in the main text):

$$f_{\mathrm{CM}}(\epsilon_p = 0) = \frac{0 - \bar{\epsilon}_m(\omega)}{0 + 2\bar{\epsilon}_m(\omega)} = -\frac{1}{2} \tag{1.6}$$

$$f_{\mathrm{CM}}(\epsilon_p \to \infty) = \frac{\infty - \bar{\epsilon}_m(\omega)}{\infty + 2\bar{\epsilon}_m(\omega)} = 1 \tag{1.7}$$

$$f_{\mathrm{CM}}(\epsilon_p = \epsilon_m) = \frac{\bar{\epsilon}_m(\omega) - \bar{\epsilon}_m(\omega)}{\bar{\epsilon}_m(\omega) + 2\bar{\epsilon}_m(\omega)} = 0 \tag{1.8}$$

The particular values for $\bar{\epsilon}_p$ also lead to simplified expressions for $Z_{tot}$. For instance, when $\bar{\epsilon}_p = 0$, we have $Z_{particle} = \infty$ so that current can pass only through $Z_{parallel}$. So the case of $\bar{\epsilon}_p = 0$ directly yields $Z_{parallel}$:

$$\begin{aligned} Z_{parallel} &= Z_{tot}(\epsilon_p = 0) = \frac{Z_0}{1 - \frac{3}{2}\phi} = \\ &= \frac{1}{1 - \frac{3}{2}\phi} \cdot \frac{\kappa}{i\omega\bar{\epsilon}_m} \end{aligned} \tag{1.9}$$

For $\bar{\epsilon}_p \to \infty$, the impedance $Z_{particle}$ tends to 0, so that it represents a shunt. $Z_{tot}$ is then obtained from $Z_{series}$ in parallel with $Z_{parallel}$. Since $Z_{parallel}$ is already given by eq. 1.9, we can obtain $Z_{series}$:

$$Z_{tot}(\epsilon_p \to +\infty) = \frac{Z_{parallel} \cdot Z_{series}}{Z_{parallel} + Z_{series}} = \frac{Z_0}{1 + 3\phi} \Rightarrow \tag{1.10}$$

$$\begin{aligned} Z_{series} &= \frac{Z_{tot}(\epsilon_p \to +\infty) \cdot Z_{parallel}}{Z_{parallel} - Z_{tot}(\epsilon_p \to +\infty)} \\ &= Z_0 \frac{(1 + 3\phi)^{-1} \left(1 - \frac{3}{2}\phi\right)^{-1}}{\left(1 - \frac{3}{2}\phi\right)^{-1} - (1 + 3\phi)^{-1}} = \frac{1}{1 + 3\phi - 1 + \frac{3}{2}\phi} = \frac{2}{9\phi} \cdot Z_0 \\ &= \frac{2}{9\phi} \cdot \frac{\kappa}{i\omega\bar{\epsilon}_m} \end{aligned} \tag{1.11}$$

Finally, for deriving $Z_{particle}$, we impose an additional constraint. We require $Z_{particle}$ to be solely a function of $\bar{\epsilon}_p$, not $\bar{\epsilon}_m$. Admitting that $Z_{particle}$ arises from $\bar{\epsilon}_p$ via some cell constant $\kappa_p$, we have:

$$Z_{particle} = \frac{\kappa_p}{i\omega\bar{\epsilon}_p} = Z_0 \cdot \frac{\kappa_p}{\kappa} \cdot \frac{\bar{\epsilon}_m}{\bar{\epsilon}_p} \tag{1.12}$$

The value of $\kappa_p$ can be found for the particular case where $\bar{\epsilon}_p = \bar{\epsilon}_m$. $Z_{tot}$ in that case is equal to $Z_0$, since the particle has the same electric properties as the surrounding medium. Taking into account also $Z_{particle} = Z_0 \cdot \frac{\kappa_p}{\kappa}$ for $\bar{\epsilon}_p = \bar{\epsilon}_m$, we have:

## 1. IMPEDANCE MODEL FOR THE MAXWELL-GARNETT MIXING EQUATION

$$
\begin{aligned}
&Z_{tot} = Z_0 = \frac{Z_{parallel} \cdot (Z_{series} + Z_{particle})}{Z_{parallel} + (Z_{series} + Z_{particle})} \Rightarrow \\
&(Z_{series} + Z_{particle}) = \frac{Z_0 \cdot Z_{parallel}}{Z_{parallel} - Z_0} = Z_0 \frac{\left(1 - \frac{3}{2}\phi\right)^{-1}}{\left(1 - \frac{3}{2}\phi\right)^{-1} - 1} = \\
&Z_0 \frac{1}{1 - 1 + \frac{3}{2}\phi} = Z_0 \cdot \frac{2}{3\phi} \Rightarrow \\
&Z_{particle} = Z_0 \cdot \frac{2}{3\phi} - Z_{series} = Z_0 \left(\frac{2}{3\phi} - \frac{2}{9\phi}\right) = Z_0 \cdot \frac{4}{9\phi} = Z_0 \frac{\kappa_p}{\kappa} \Rightarrow \\
&\kappa_p = \kappa \cdot \frac{4}{9\phi} \qquad (1.13)
\end{aligned}
$$

Plugging eq. 1.13 back into eq. 1.12, we obtain finally for $Z_{particle}$:

$$Z_{particle} = \frac{4}{9\phi} \cdot \frac{\kappa}{i\omega\bar{\epsilon}_p} \qquad (1.14)$$

It remains to check that $Z_{series}$, $Z_{parallel}$ and $Z_{particle}$ together with eq. 1.2 are able to reproduce the complex permittivity given by the approximative Maxwell-Garnett mixing equation (eq. 1.1). The total impedance of the circuit in Fig. 1.1 is obtained by plugging the expressions for $Z_{series}$, $Z_{parallel}$ and $Z_{particle}$ into eq. 1.4:

$$
\begin{aligned}
Z_{tot} &= \kappa \cdot \frac{\left(1 - \frac{3}{2}\phi\right)^{-1} (i\omega\bar{\epsilon}_m)^{-1} \cdot \left(\frac{2}{9\phi} (i\omega\bar{\epsilon}_m)^{-1} + \frac{4}{9\phi} (i\omega\bar{\epsilon}_p)^{-1}\right)}{\left(1 - \frac{3}{2}\phi\right)^{-1} \cdot (i\omega\bar{\epsilon}_m)^{-1} + \frac{2}{9\phi} (i\omega\bar{\epsilon}_m)^{-1} + \frac{4}{9\phi} (i\omega\bar{\epsilon}_p)^{-1}} \\
&= \frac{\kappa}{i\omega\bar{\epsilon}_m} \cdot \frac{2 (i\omega\bar{\epsilon}_m)^{-1} + 4 (i\omega\bar{\epsilon}_p)^{-1}}{9\phi \cdot (i\omega\bar{\epsilon}_m)^{-1} + 2\left(1 - \frac{3}{2}\phi\right) (i\omega\bar{\epsilon}_m)^{-1} + 4\left(1 - \frac{3}{2}\phi\right) (i\omega\bar{\epsilon}_p)^{-1}} \\
&= \frac{\kappa}{i\omega\bar{\epsilon}_m} \cdot \frac{2\bar{\epsilon}_p + 4\bar{\epsilon}_m}{9\phi \cdot \bar{\epsilon}_p + 2\left(1 - \frac{3}{2}\phi\right)\bar{\epsilon}_p + 4\left(1 - \frac{3}{2}\phi\right)\bar{\epsilon}_m} \\
&= \frac{\kappa}{i\omega\bar{\epsilon}_m} \cdot \frac{2\bar{\epsilon}_p + 4\bar{\epsilon}_m}{2\bar{\epsilon}_p + 4\bar{\epsilon}_m + \left(9 - 2 \cdot \frac{3}{2}\right)\phi \cdot \bar{\epsilon}_p - 4 \cdot \frac{3}{2}\phi \cdot \bar{\epsilon}_m} \\
&= \frac{\kappa}{i\omega\bar{\epsilon}_m} \cdot \frac{\bar{\epsilon}_p + 2\bar{\epsilon}_m}{\bar{\epsilon}_p + 2\bar{\epsilon}_m + 3\phi \cdot \bar{\epsilon}_p - 3\phi \cdot \bar{\epsilon}_m} = \frac{\kappa}{i\omega\bar{\epsilon}_m} \frac{1}{1 + 3\phi \cdot \frac{\bar{\epsilon}_p - \bar{\epsilon}_m}{\bar{\epsilon}_p + 2\bar{\epsilon}_m}} \\
&= \frac{\kappa}{i\omega\bar{\epsilon}_m} \cdot \frac{1}{1 + 3\phi \cdot f_{CM}} \qquad (1.15)
\end{aligned}
$$

where we have used the definition of the Clausius-Mossotti factor (eq. 8 in the main text, i.e. $f_{CM} = \frac{\bar{\epsilon}_p - \bar{\epsilon}_m}{\bar{\epsilon}_p + 2\bar{\epsilon}_m}$) in the last step.

The total impedance is related to the effective complex permittivity $\bar{\epsilon}_{mix}$ by the general relation between the impedance and the complex permittivity (eq. 1.2):

$$Z_{tot} = \frac{\kappa}{i\omega\bar{\epsilon}_{mix}} \tag{1.16}$$

Comparing eq. 1.16 and eq. 1.15, we obtain for $\epsilon_{mix}$:

$$\bar{\epsilon}_{mix} = \bar{\epsilon}_m \cdot (1 + 3\phi \cdot f_{CM}) \tag{1.17}$$

Eq. 1.17 corresponds to eq. 1.5, showing that indeed the equivalent circuit shown in Fig. 1.1, with $Z_{parallel}$, $Z_{series}$ and $Z_{particle}$ given by eq. 1.9, eq. 1.11 and eq. 1.14 respectively, is equivalent to the approximated Maxwell-Garnett mixing equation (eq. 1.1). The full Maxwell-Garnett mixing equation is of course only approximatively fulfilled, but since it is itself an approximation for $\phi \ll 1$, the additional Bernoulli approximation in eq. 1.1 does not add much additional imprecision. In this sense, the equivalent circuit presented here is a reliable approximation for the Maxwell-Garnett mixing equation, as long as $\phi \ll 1$ is respected.

# Supplementary Material 2

# Impedance Model for Single Shell Model

In its simplest approximation, a biological cell can be described by two impedance elements in series[2, 3]: the membrane impedance $Z_{mem}$ , in series with cytoplasmic impedance $Z_{cyt}$. So we have:

$$Z_{cell} = Z_{mem} + Z_{cyt} \tag{2.1}$$

An original approach to the estimation of $Z_{mem}$ and $Z_{cyt}$ is given in [3]: the cell, of homogeneous height, is projected onto its cross section, and the current path length in the regions of different permittivities is used to obtain the impedance. However, it is then difficult to distinguish between the true radius of the cell and the apparent radius obtained when fitting the model to experimental data[3].

In Supplementary Material 1, we have obtained an expression for the apparent particle cell constant for a particle occupying a volume fraction $\phi$ in a larger measurement volume of cell constant $\kappa$. This apparent cell constant is given by (eq. 1.13):

$$\kappa_p = \kappa \cdot \frac{4}{9\phi}$$

Since this cell constant arises from the current distribution around the cell, its use should avoid the problems with the effective radius encountered in[3].

Since from now on, we focus specifically on the single-shell model for biological cells, we shall use the nomenclature $\kappa_{cell} = \kappa_p$. Using $\kappa_{cell}$, the addition relation for the impedances given by eq. 2.1 can be transformed into a corresponding relation for the complex permittivities:

$$\begin{aligned} Z_{cell} &= \frac{\kappa_{cell}}{i\omega\bar{\epsilon}_{cell}} = \frac{\kappa_{cell}}{i\omega\bar{\epsilon}_{mem,\text{ effective}}} + \frac{\kappa_{cell}}{i\omega\bar{\epsilon}_{cyt,\text{ effective}}} \Rightarrow \\ \bar{\epsilon}_{cell} &= \frac{\bar{\epsilon}_{mem,\text{ effective}} \cdot \bar{\epsilon}_{cyt,\text{ effective}}}{\bar{\epsilon}_{mem,\text{ effective}} + \bar{\epsilon}_{cyt,\text{ effective}}} \end{aligned} \tag{2.2}$$

We allow for effective values since the volume fractions occupied by the membrane and cytoplasm are very different.

The complex permittivity $\bar{\epsilon}_{cell}$ of the cell is also given by the multi-shell mixing equation (eq. 9 in the main text), repeated here for convenience:

$$\bar{\epsilon}_{\text{effective}} = \bar{\epsilon}_{\text{outer}} \cdot \frac{\left(\frac{r_{\text{outer}}}{r_{\text{inner}}}\right)^3 + 2\frac{\bar{\epsilon}_{\text{inner}} - \bar{\epsilon}_{\text{outer}}}{\bar{\epsilon}_{\text{inner}} + 2\bar{\epsilon}_{\text{outer}}}}{\left(\frac{r_{\text{outer}}}{r_{\text{inner}}}\right)^3 - \frac{\bar{\epsilon}_{\text{inner}} - \bar{\epsilon}_{\text{outer}}}{\bar{\epsilon}_{\text{inner}} + 2\bar{\epsilon}_{\text{outer}}}} \tag{2.3}$$

For the single shell model, $\bar{\epsilon}_{\text{inner}} = \bar{\epsilon}_{cyt}$ and $\bar{\epsilon}_{\text{outer}} = \bar{\epsilon}_{cyt}$. We are now looking for the link between the intrinsic permittivity values $\bar{\epsilon}_{cyt}$ and $\bar{\epsilon}_{mem}$ and the corresponding effective values, $\bar{\epsilon}_{cyt,\,\text{effective}}$ and $\bar{\epsilon}_{mem,\,\text{effective}}$, respectively.

To find these values, we apply two approximations to eq. 2.3: First, the membrane is very thin compared to the cell body, so that $r_{outer} = r_{inner} + \Delta r$, with $\Delta r \ll r_{inner}$. The term $\left(\frac{r_{\text{outer}}}{r_{\text{inner}}}\right)^3$ can therefore be approximated by the linearized expression $1 + \frac{3\Delta r}{r}$. Second, the plasma membrane generally not only has lower conductivity, but also a lower real permittivity than the cytoplasm[1, 2], so that at all frequencies, $|\bar{\epsilon}_{mem}| \ll |\bar{\epsilon}_{cyt}|$.

Using $\left(\frac{r_{\text{outer}}}{r_{\text{inner}}}\right)^3 \approx 1 + \frac{3\Delta r}{r}$ we can develop eq. 2.3:

$$\begin{aligned}
\bar{\epsilon}_{\text{cell}} &\approx \bar{\epsilon}_{\text{mem}} \cdot \frac{\left(1 + \frac{3\Delta r}{r}\right) + 2\frac{\bar{\epsilon}_{\text{cyt}} - \bar{\epsilon}_{\text{mem}}}{\bar{\epsilon}_{\text{cyt}} + 2\bar{\epsilon}_{\text{mem}}}}{\left(1 + \frac{3\Delta r}{r}\right) - \frac{\bar{\epsilon}_{\text{cyt}} - \bar{\epsilon}_{\text{mem}}}{\bar{\epsilon}_{\text{cyt}} + 2\bar{\epsilon}_{\text{mem}}}} \\
&= \bar{\epsilon}_{\text{mem}} \cdot \frac{3\bar{\epsilon}_{\text{cyt}} + \frac{3\Delta r}{r} \cdot (\bar{\epsilon}_{\text{cyt}} + 2\bar{\epsilon}_{\text{mem}})}{3\bar{\epsilon}_{\text{mem}} + \frac{3\Delta r}{r}(\bar{\epsilon}_{\text{cyt}} + 2\bar{\epsilon}_{\text{mem}})} \\
&= \bar{\epsilon}_{\text{mem}} \cdot \frac{\bar{\epsilon}_{\text{cyt}} + \frac{\Delta r}{r} \cdot (\bar{\epsilon}_{\text{cyt}} + 2\bar{\epsilon}_{\text{mem}})}{\bar{\epsilon}_{\text{mem}} + \frac{\Delta r}{r}(\bar{\epsilon}_{\text{cyt}} + 2\bar{\epsilon}_{\text{mem}})} \\
&= \bar{\epsilon}_{\text{mem}} \cdot \frac{\bar{\epsilon}_{\text{cyt}} \cdot \left(1 + \frac{\Delta r}{r}\right) + 2\frac{\Delta r}{r}\bar{\epsilon}_{\text{mem}}}{\bar{\epsilon}_{\text{mem}}\left(1 + \frac{2\Delta r}{r}\right) + \frac{\Delta r}{r} \cdot \bar{\epsilon}_{\text{cyt}}} \\
&\approx \bar{\epsilon}_{\text{mem}} \cdot \frac{\bar{\epsilon}_{\text{cyt}} + 2\frac{\Delta r}{r}\bar{\epsilon}_{\text{mem}}}{\bar{\epsilon}_{\text{mem}} + \frac{\Delta r}{r} \cdot \bar{\epsilon}_{\text{cyt}}}
\end{aligned} \tag{2.4}$$

We have $|\bar{\epsilon}_{mem}| \ll |\bar{\epsilon}_{cyt}|$, and since also $\frac{\Delta r}{r} \ll 1$, we can neglect the term $2\frac{\Delta r}{r}\bar{\epsilon}_{\text{mem}}$ in the numerator of eq. 2.4. We obtain:

$$\bar{\epsilon}_{\text{cell}} \approx \bar{\epsilon}_{\text{mem}} \cdot \frac{\bar{\epsilon}_{\text{cyt}}}{\bar{\epsilon}_{\text{mem}} + \frac{\Delta r}{r} \cdot \bar{\epsilon}_{\text{cyt}}} = \frac{\left(\bar{\epsilon}_{\text{mem}} \cdot \frac{r}{\Delta r}\right) \cdot \bar{\epsilon}_{\text{cyt}}}{\left(\bar{\epsilon}_{\text{mem}} \cdot \frac{r}{\Delta r}\right) + \bar{\epsilon}_{\text{cyt}}} \tag{2.5}$$

Eq. 2.5 is written such as to allow direct comparison with the corresponding addition equation for the complex permittivities obtained from the discrete circuit approximation (eq. 2.2). Indeed, by direct comparison of eq. 2.5 with eq. 2.2 we obtain for the effective permittivities for the cell membrane and cytoplasm:

## 2. IMPEDANCE MODEL FOR SINGLE SHELL MODEL

$$\bar{\epsilon}_{mem,\text{ effective}} = \bar{\epsilon}_{\text{mem}} \cdot \frac{r}{\Delta r} \tag{2.6}$$

$$\bar{\epsilon}_{cyt,\text{ effective}} = \bar{\epsilon}_{\text{cyt}} \tag{2.7}$$

Finally, from the general relation between the impedance and complex permittivity, we obtain the membrane and cytoplasmic equivalent impedance, noting that $\Delta r$ is simply the membrane thickness $d$:

$$Z_{mem} = \frac{d}{r} \cdot \frac{\kappa_{cell}}{i\omega\bar{\epsilon}_{mem}} \tag{2.8}$$

$$Z_{cyt} = \frac{\kappa_{cell}}{i\omega\bar{\epsilon}_{cyt}} \tag{2.9}$$

# Bibliography

[1] P. Gascoyne, J. Satayavivad, and M. Ruchirawat, *Microfluidic approaches to malaria detection*, Acta Tropica, 89 (2004), pp. 357–369. 7

[2] S. Gawad, K. Cheung, U. Seger, A. Bertsch, and P. Renaud, *Dielectric spectroscopy in a micromachined flow cytometer: theoretical and practical considerations*, Lab On A Chip, 4 (2004), pp. 241–251. 1, 6, 7

[3] J. Gimsa and D. Wachner, *A unified resistor-capacitor model for impedance, dielectrophoresis, electrorotation, and induced transmembrane potential*, Biophys. J., 75 (1998), pp. 1107–1116. 2, 6

[4] T. Sun, N. Green, and H. Morgan, *Analytical and numerical modeling methods for impedance analysis of single cells on-chip*, Nano, 3 (2008), pp. 55–63. 1