Manual to OxyTis - English version Aug-24-2012

The program calculates oxygen partial pressures in a tissue volume, for a given set of input data. OxyTis comes in two versions, OxyTisS and OxyTis. The first version, OxyTisS, is a simplified version with only limited applicability, but it can be downloaded free of charge from http://www.baloh.nl. The second, full version, OxyTis, has many more features and can be ordered from the same website.

This manual is an extension to the help and information in the program itself. The manual will be updated for new features and can be downloaded freely from the above website. Some features only apply to the full version.

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1   Introduction

1.1   Layout

OxyTis calculates oxygen partial pressures in a tissue volume with parallel capillaries (red in the figure). The cross section of the volume perpendicular to the capillaries (x, y) has to be identical for any level. In the program, this cross section is called a **plane**, while the capillaries run in the **longitudinal** direction (z).

In the simplified version, the cross section has to be either a circle or a rectangle – tissue volumes of cylinder and block, respectively. Tissue properties have to be uniform, but capillary data can be different for every capillary, as elucidated in the data input section.

The calculation method is based on the theoretical treatments of Hoofd[1),2)], extended for myoglobin in the tissue according to Hoofd & Kreuzer[3)]. To understand how the program works, it is essential that this combined treatment is shortly introduced. For the oxygen pressures in a plane, at location $\bar{r}$:
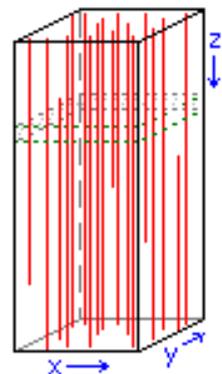
$$(1) \qquad P + P_F S = \tfrac{1}{4} ÐO2 \left\{ \Phi(\bar{r}) - \sum_{i=1}^{N} \frac{A_i}{\pi} \ln\left(\frac{|\bar{r} - \bar{r}_i|^2}{r_{ci}^{\;2}} \right) \right\}$$

where P is the oxygen partial pressure, S the corresponding myoglobin saturation, and $\bar{r}_i$ the capillary location of the ith capillary in the plane – there are N capillaries. The input parameters P_F, ÐO2 and r_{ci} are handled in the data input section, the terms $\Phi(\bar{r})$ and A_i are calculated by the program. Important here are the terms A_i, the **supply areas** of the capillaries: A_i is the size of the region in the plane supplied of oxygen by the ith capillary. In the classical Krogh-Erlang model, the first model of tissue oxygenation, this area is circular, leading to the Krogh-Kety cylinder model. Here, the area's shape is indeterminate, only its size is relevant (though there are methods to determine its shape also).

The amount of oxygen consumed in this area A_i equals the amount of oxygen delivered from the capillary. Then, capillary oxygen loss can be tracked along its longitudinal path, requiring only the firstly encountered capillary oxygen pressures for a complete calculation of the tissue oxygen situation.

[1] *Reference: Hoofd L (1995). Calculation of oxygen pressures in tissue with anisotropic capillary orientation. I: Two-dimensional analytical solution for arbitrary capillary characteristics. Math. Biosci. 129: 1-23.*

[2] *Reference: Hoofd L (1995). Calculation of oxygen pressures in tissue with anisotropic capillary orientation. II: Coupling of two-dimensional planes. Math. Biosci. 129: 25-39.*

[3] *Reference: Hoofd L, Kreuzer F (1979). A new mathematical approach for solving carrier-facilitated steady state diffusion problems. J. Math. Biol. 8: 1-13. 23.*

1.2   Input data

Input files are plain text files. Normally, such files have an extension (last part of the filename) .txt and can be made and edited by most database and word processor programs and simple edit programs such as the Windows[®] program Notepad.

 - Each line starting with an at-sign (@) is considered a comment line, no further processing is done.
 - The first data section has 12 lines with data, as listed below. Each line contains one or two data items and may be followed by any comment or elucidation, all separated by a comma, a semicolon, and/or or one or more blanks. To avoid ambiguity, the comment or elucidation should start with a letter or an at-sign. Note: numerical values are in computer notation: fractions indicated by a decimal point (not a comma) and power values by a consecutive letter e or E and the power value; e.g. 1.23e3 meaning $1.23 \times 10^3 = 1230$. In the listing below, example values are given for rat heart[4].

 - Line 1:   the unit where distances are expressed in   e.g. – µm
 - Line 2:   a character indicating the shape of a plane. This can be C [Circle] or R [Rectangle]
 - Line 3:   for a circle, its radius   e.g. – 90
                 for a rectangle, its width and height   e.g. – 177 140
     In the full version, these may be followed by values of inner regions of different oxygen consumption, see Line 8. For the circle, smaller radii, for the rectangle, non-overlapping width/height pairs.
 - Line 4:   the distance, up to the border of the plane, of a region to be excluded from histogram calculations. It is unknown whether there are capillaries just outside the volume, or, contrary, void regions, and that cannot be taken into account by the program, leading to locally uncertain results.
                                                                                                                          e.g. – 0
     In the full version, this may be followed by a longitudinal distance to the initial plane also to be excluded from histogram calculations. The influence of border zones can easily be checked by varying their size – there is a menu option for that.
 - Line 5:   the unit where oxygen partial pressures are expressed in   e.g. – kPa
 - Line 6:   the average **Extraction Pressure**, EP (Capillary Barrier). This is a pericapillary oxygen pressure drop accounting for transport phenomena in and just around the capillary[5]. Values can be estimated using literature[6,7] or the formula in Appendix 4.1.   e.g. – 0.7
 - Line 7:   myoglobin data: **Facilitation Pressure** (P_F) and P_{50} of the Mb saturation curve. P_F is calculated from $P_F = D_{Mb}c_{Mb}/Þ_{O_2}$, where D_{Mb} is the myoglobin diffusion coefficient, c_{Mb} the concentration of myoglobin in the tissue, and $Þ_{O_2}$ the oxygen permeability of the tissue; see next entry. P_{50} is the oxygen pressure where the myoglobin is 50% saturated. Note, that the data have to be given in terms of the pressure unit of Line 5 (in the example, kPa).   e.g. – 2,0.7
 - Line 8:   **Oxygen drop parameter**, the oxygen consumption of the tissue divided by its oxygen permeability. For tissue, this permeability is better known as 'Krogh's diffusion coefficient', but actually is not a diffusion coefficient. It can be calculated from $Þ_{O_2} = D_{O_2} \times \alpha_{O_2}$, where D_{O_2} is the oxygen diffusion coefficient and α_{O_2} is the oxygen solubility in the tissue. The term 'permeability' is not uniquely defined, so check literature values for the correct dimensions or use the above equation. The dimension of the oxygen drop parameter is pressure per area and in the program it is indicated by ÐO2   e.g. – .0253

In the full version, a series of values can be given corresponding with the zones of Line 3, in the same order. In the program, all zone values can be inspected or edited through the » box preceding ĐO2 in the data form.

- Line 9:   the longitudinal size of the volume, along the capillaries   e.g. – 500
- Line 10: a **Transfer factor**, indicating the oxygen transfer by the capillaries. It is equal to the average capillary blood flow (volume per time! – Appendix 4.5) divided by the tissue's oxygen diffusion coefficient D_{O_2} resulting in a dimension of length expressed in the unit of Line 1. e.g. – 10.56
- Line 11: hemoglobin data: **oxygen capacity pressure** (PCap) and P_{50} of the saturation curve. The PCap is the blood oxygen capacity expressed in pressure units, so divided by the oxygen solubility. P_{50} is the oxygen pressure where the hemoglobin is 50% saturated.   e.g. – 844,4.93
- Line 12: hemoglobin saturation curve Hill factor, n-Hill. This value can be derived from the slope of the saturation curve at P_{50}: slope= n-Hill/(4P_{50}), according to the Hill model. The Hill model describes most saturation curves quite well above ~10% saturation; the program applies a modification that is also valid for low values- see Appendix 4.2.   e.g. – 2.69
- After these 12 lines of basic data follows a capillaries section. This starts with another basic line:
- Line 13: the number of capillaries. This includes all capillaries present anywhere in the volume, also capillaries that do not extend over the full longitudinal distance (Line 9)
- followed by a data line for each capillary, a series of values separated by a comma, a semicolon, and/or or one or more blanks. The data may be different for each capillary but must be in the units of Line 1 and Line 5; in order:
- - capillary coordinates in the plane – for a rectangle, relative to the bottom left corner, for a circle, relative to its centre. First the horizontal coordinate, then the vertical coordinate in the plane
- - capillary radius; this should be the (average) distance from the capillary centre to where the tissue begins.
- - capillary oxygen pressure in the first plane where the capillary appears, e.g., the oxygen pressure of the supplying arteriole (or end-capillary PO_2 for negative capillary flow)
- - longitudinal start and end of the capillary; e.g., for a longitudinal tissue distance of 800 µm, the capillary might start (originate from an arteriole) at z= 200 µm and end (drain into a venule) at z= 500 µm. Note: the start value has to be smaller than the end value also for countercurrent capillaries.
- - flow multiplication factor. If this value is 1, the capillary flow is equal to the average value used to calculate the Transfer factor in Line 10. If it is 2, the flow is twice that, and so on.
- - capillary hematocrit multiplication factor. If this value is 1, the capillary oxygen capacity is equal to the average value used to calculate PCap in Line 11. If it is 2, it is twice that, and so on.
- - extraction pressure multiplication factor. If this value is 1, the capillary extraction pressure is derived from the overall value of Line 6. If it is 2, it is twice that, and so on.

Required are the coordinate values for any capillary and the radius and pressure for the first capillary. Defaults are taken for all trailing values as follows:
- - for the first capillary, start and end are defaulted to 0 and full longitudinal distance (Line 9) respectively
- - also for the first capillary, flow factor, hematocrit factor and extraction pressure factor are defaulted to 1
- - for all following capillaries, the values are taken of the preceding capillary.

So, smartly organizing the input file can save its amount of entry data, by grouping capillaries with similar properties. E.g., when a group of capillaries has similar data except for its coordinates, only for the first in the group radius, pressure, … have to be explicitly given. Note, however, that non-trailing values are not defaulted; so, if a pressure is given, also the radius has to be given explicitly as it precedes the pressure.

If multiple datasets use the same general data, there is an option to read only capillary data. In that case, the first 12 lines can be skipped from the file, thus containing only capillary data.

[4] *Reference: Hoofd L, Olders J, Turek Z (1990). Oxygen pressures calculated in a tissue volume with parallel capillaries. In: Oxygen Transport to Tissue XII, Advances in Experimental Medicine & Biology. 277: 21-29.*

[5] *Reference: Hoofd L (1992). Updating the Krogh model, assumptions and extensions. In: Oxygen Transport in Biological Systems. Modelling of pathways from environment to cell, Soc. Exper. Biol. Seminar Ser. 51, eds. S. Egginton, and H.F. Ross, Cambridge University Press, Cambridge, U.K.: p. 207.*

[6] *Reference: Bos C, Hoofd L, Oostendorp T (1995). Mathematical model of erythrocytes as point-like sources, Math. Biosci. 125: 165-189.*

[7] *Reference: Bos C, Hoofd L, Oostendorp T (1996). The effect of separate red blood cells on capillary tissue oxygenation calculated with a numerical model. IMA Journal of Mathematics Applied in Medicine & Biology. 13(4): 259-274.*

1.3   Statistics

1.3.1   Histograms

Some of the results of OxyTis are in the form of a histogram. Gathering results into a histogram implies not storing the results themselves, but only counting how many there are in between certain boundaries. Here, the boundaries are equally spaced according to a class width. For example, for a class width of 5, the boundary values are 0, 5, 10, 15, 20, and so on. When there are 93 results in class 10-15, 93 results came out larger than 10 and smaller than (or equal to) 15. An example is in the figure to the right – note the horizontal axis, showing the class number; not the PO_2.
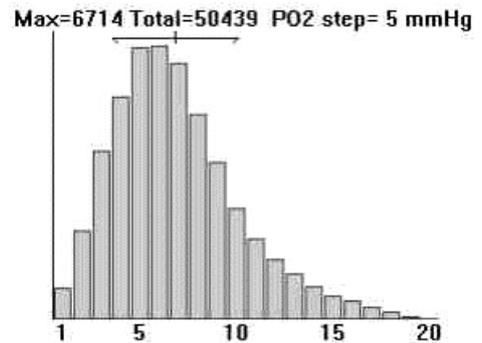
The histograms in OxyTis may have two additional classes:
 - a class below zero; here, the number of anoxic regions is counted
 - an additional class for values that were higher than the highest boundary. This might occur for small class width; OxyTis limits the number of classes to 50.
In addition to the histogram, mean value and standard deviation (SD) of the mean are calculated and included in the results (assuming anoxic regions to have an oxygen pressure of zero). These values are informational only, since the tissue oxygen results are far from normally distributed.

1.3.2   Longitudinal plots

Capillary pressures and capillary supply areas vary along the longitudinal path of the volume. Since there can be many capillaries, it is of no use to plot these values individually. Instead, a mean value and a standard deviation of the mean (SD) are calculated for every plane, and lines are plotted representing this mean, mean+SD and mean−SD. Note: SD=0 for a single capillary. Also note, that the supply area mean value is the inverse of the **capillary density** provided there is no supply limitation for anoxic regions.

2   Calculations

2.1   Basic

For any new or changed case, all program calculations are reset and have to be redone explicitly. The basis for all these calculations is equation (1), and in particular the capillary supply areas A_i. These are calculated from the capillary pressures. For the entry plane, plane zero, these pressures are those from the input data, whereas for any following planes these have to be calculated from the capillary's oxygen loss. When clicking 'Basic' in the 'Calculations' menu, the number of planes to follow the initial plane is asked for. This number should not be too small, since the program utilizes a discrete-step approximation for the oxygen loss calculations that is increasingly inaccurate for increasing step size.

If the number is zero or the number-of-planes dialog is aborted, only the initial plane is calculated and the program assumes there is only this plane – ignoring the input data of Lines 8-12. All further calculations will be valid for this plane only.

2.2   Supply limitation

The calculation method considers PO_2s as continuous values with no special meaning of the value zero. Consequently, the method may calculate negative values for PO_2. In fact, this means, that not enough oxygen can reach these locations resulting in anoxic tissue – and the results are to be interpreted in that way. Such anoxic regions no longer consume oxygen, but it is difficult to account for that, and the simplified version does not. So, calculations resulting in anoxic tissue will be inaccurate, increasingly for larger amounts of anoxic tissue.

For the capillaries, however, it is possible to incorporate a correction for this non-consumed and thus non-delivered oxygen, by limiting its supply to what can be maximally sustained by the actual capillary PO_2, which can be toggled through clicking 'Supply limitation' in the 'Options' menu. Limiting the capillary oxygen loss ignores the fact that other, neighbouring capillaries partially take over oxygen supply, so still will lead to inaccurate results.

2.3   Oxygen values

The basic calculations result in values for supply areas and capillary oxygen pressures in any plane, which can be inspected or output as explained below. Two more calculations can be done.
 - calculation of tissue PO_2s – and corresponding Mb saturations – in a plane. Click 'Oxygen field'.
   Calculations will be done in a rectangular grid in the plane, according to a grid distance asked for.
   Choose a grid distance fine enough to resolve the field; e.g., 5 µm for the above example. In the Output chapter under 'Show window' is an exemplary figure.

- calculation of tissue PO_2s – and corresponding Mb saturations – in the entire volume, but these results will not be stored per location, as for the plane calculation, but gathered into histograms for PO_2 and saturation. Click 'Histograms'. Calculations will be done for a rectangular grid identical for each plane according to a grid distance asked for – this distance may be different from the former one. The figure under 'Statistics – Histograms' has an example.

3   Output

Data and results can be listed, inspected and stored from the main window and two additional windows, the Results window and the Show window.

3.1   Main window

The main window shows the plane lay-out, a cross section with all capillaries and the border zone. From here, basic data can be inspected (and edited – but this may reset calculations!) and data and results can be stored into a file through the menu entry 'Save…': These output files are plain text files and consequently can be imported in almost any other processing program.

The 'Print' and 'Save as' entries print and store the Basic data, respectively, including capillary data.

3.2   File output

The main window's 'Save' entry offers the following possibilities.
 - Basic data: the basic data and capillaries, resulting in a file that can be used as later input for OxyTis
 - Supply area: a table of supply areas for each capillary and each plane
 - PO2 in capillary: a table of capillary oxygen pressures for each capillary and each plane
 - PO2 in plane: a table of tissue oxygen pressures for each grid point in the selected plane
 - Tissue PO2 Histogram: tabulated histogram and cumulative histogram PO_2 values
 - Tissue MbO2 Histogram: tabulated histogram and cumulative histogram Mb saturation values
Note, that the elements in the tables are separated by Tabulation stops.

Caveat: output depends on what situation was calculated, number of planes and/or grid values, so some output files might grow quite large.

A plane's Mb saturation values can be calculated from the PO_2-in-plane data in a spread sheet.

3.3   Results window

The entries in the menu 'Results' call a separate window with text in tabulated form. It has its own menu options, in particular, 'to Clipboard', which copies its contents as text to the Windows' Clipboard, from where it can be pasted into any text processing program. Also, the other entries are for the contents only. Note, that the contents may be limited to avoid too many window or print data.

3.4   Show window

The entries in the menu 'Show' call a separate window with output and visualization in graphical form. Also this window has its own menu options, acting on the contents only. The entry 'to Clipboard' copies the contents to the Windows' Clipboard now in graphical form, from where it can be pasted into any pictures processing program. The picture's dimensions are those of the window, so first resize it adequately. Some more about the entries:

'Capillaries' shows the layout as in the Main window, to enable printing, copying or saving
'Saturation curves' shows – crudely – the Hb and Mb saturation curves over the PO_2 range applied
'Supply areas' and 'Capillary pressures' show mean and mean±SD along the longitudinal axis, so, how these values vary along the capillaries
'Oxygen field' and 'Mb Saturations' give a 3-dimensional view of these quantities against the coordinates of the actual plane. An example is in the figure to the right.
'Oxygen Histogram' and 'MbO2 Histogram' show these histograms in graphical form. If there are anoxic regions, there will be an extra column to the left; if values are too large to fit in 50 histogram classes, there will be an extra column to the right.
The figure under 'Statistics – Histograms' has an example.
Graphics are elementary; for better graphical output, use the data files saved from the Main window as processed in an appropriate computer program.
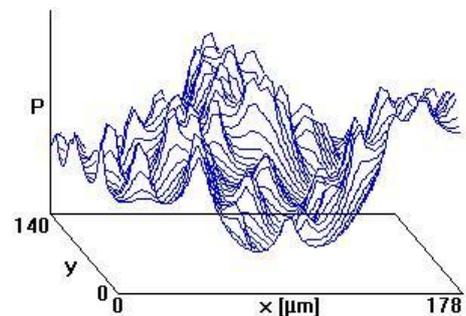
4   Concluding remarks

OxyTis applies a semi-analytical, semi-numerical approach. In particular, longitudinal diffusion is ignored. Consult the specific literature about the validity of the various assumptions. The results can be considered reliable in most situations, with a particular exception in case of significant anoxic tissue.

The program does not handle what happens in arterioles or venules, or capillaries not in the longitudinal direction. The user has to decide about capillary values, in particular, entry PO_2, to make realistic situations. Here, the advantage of the semi-analytical approach is, that calculations are very fast so that many calculations for different (try-out) situations can be compared.

Supply areas can be negative, indicating that oxygen flows into the capillary instead of being supplied. This phenomenon is called **shunting**. In fact, there will already be shunting when the supply area is smaller than the capillary cross sectional area.

Since tissue oxygenation can be locally very different, for a realistic tissue situation one should take a region encompassing sufficient capillaries and tissue regions, e.g. 50 capillaries.

Or, the program can be used to study varying input data in a specific situation, E.g., one might visualize what happens in a Krogh cylinder when varying capillary hematocrit, or compare supply from a centrally located capillary with capillaries located at the border.

Appendix

4.1   Extraction Pressure

The capillary oxygen pressure P_{cap} is considered to be related to the capillary's hemoglobin saturation S in the erythrocytes (red) through chemical equilibrium. However, before the oxygen reaches the tissue at the capillary rim (violet lines), it first has to be released from the hemoglobin and subsequently transported through the erythrocyte, the capillary plasma and the capillary wall, resulting in an oxygen drop below P_{cap}. This drop will depend on whether the rim location is close to or far from an erythrocyte, but that variation smoothes out quickly so that most of the

surrounding tissue experiences a certain constant capillary PO_2 below P_{cap}.(visualized by the brown lines). This value is called Extraction Pressure (EP). For example, if the capillary PO_2 is 8 kPa and the EP is 1.5 kPa, the tissue 'sees' a capillary PO_2 of 6.5 kPa, i.e., tissue oxygenation can be calculated as if the capillary PO_2 were 6.5 kPa without internal capillary pressure gradients.
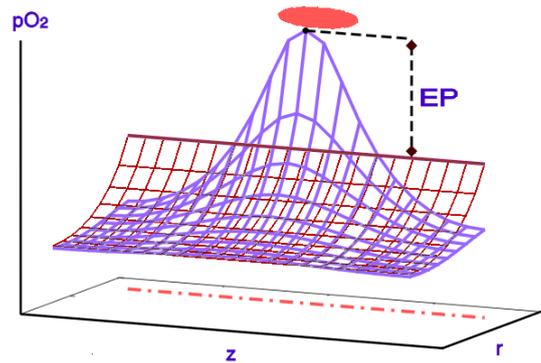
The Extraction Pressure phenomenon has been extensively studied in mathematical models[6),7)] and depends on capillary properties in a complicated way. However, it is proportional to the capillary's net supply area A_i' (so, excluding the capillary area πr_{ci}^2):

(2) $EP_i = c_0 A_i' EP$

where EP_i is the Extraction Pressure of the ith capillary, c_0 is a factor determined by the program and EP is the value given in Line 6 of the basic data. From the mathematical models, formulas have been developed to estimate EP, the best one, implemented in OxyTis, being quite complicated[8)]. A relatively simple, not-too-bad approximation for the value of Line 6 is:

$$EP = \frac{\text{Đ}O2\ A}{8\pi N} \left\{ 1 + \left(\frac{1}{Hct} - 1\right) \frac{c_1}{(c_2 + Pe^2)(c_3 + Hct)} \right\} \qquad \text{where}$$

$$Pe = \frac{\Delta z\ v_{RBC}}{2\ D_{O2}} \quad \text{is the \textbf{Peclet number}}$$

and A is the area of the plane, N is the number of capillaries. Hct is the hematocrit, Δz is the spacing between two red blood cells (the length of the plasma layer in between) and v_{RBC} their velocity, all for an average capillary. D_{O2} is the diffusion coefficient of oxygen, and the constants c_1, c_2, c_3 are to be fitted from numerical results; rough estimates for rat heart[7)]:

      $c_1 = 85$ $c_2 = 130$ $c_3 = 0.1$

For better estimates see the references.

[8)]   *Reference: Bos (1997). Mathematical Modeling of Oxygen Transport from Capillaries to Tissue., Chapter 9. Dissertation Thesis, University of Nijmegen.*

4.2   Extended Hill model

The Hill model probably is the simplest model to describe the hemoglobin's oxygen saturation or oxygen dissociation curve:

$$S = \frac{P^n}{P^n + P_{50}^{\ n}}$$

where S is the fractional saturation, P the oxygen partial pressure, P_{50} the 50% saturation pressure, and n the Hill coefficient (termed n-Hill in OxyTis). The Hill model mostly works remarkably well but underestimates S for low oxygen pressures; see the blue line in the figure. However, low capillary PO_2s are rare in normal tissue. An advantage of the Hill model is, that it characterizes the blood in terms of only two parameters. To avoid problems in those situations where capillary PO_2 is low, a formula was used derived from the MWC model, as a modified Pauling model[4),9)]:

$$S = \frac{t^3x + 3t^4x^2 + 3t^3x^3 + x^4}{1 + 4t^3x + 6t^4x^2 + 4t^3x^3 + x^4}$$
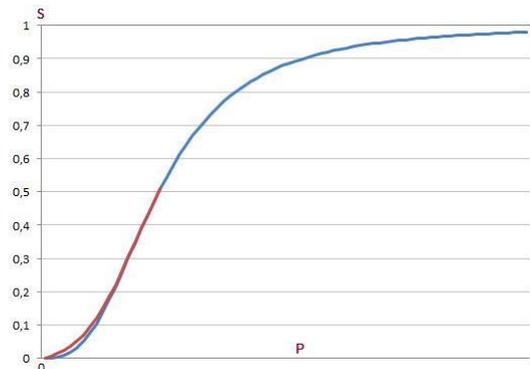
where $x = P/P_{50}$ and t is a parameter linked to the Hill coefficient:

$$n = \frac{4+4t^3}{1+4t^3+3t^4}$$

This equation is used below 50% saturation and the Hill equation above 50%, so that the entire curve is described adequately; see the red line in the figure.

Exemplary values for the respective parameters are
  Man: $n = 2.70 \Rightarrow t = 0.49376$ $P_{50} = 3.55$ kPa = 26.6 mm Hg
 Rat: $n = 2.69 \Rightarrow t = 0.49563$ $P_{50} = 4.93$ kPa = 37 mm Hg

[9] *Reference: Bouwer S Th Hoofd L Kreuzer F (2001). Reaction rates of oxygen with hemoglobin measured by non-equilibrium facilitated oxygen diffusion through hemoglobin solutions. Biochimica et Biophysica Acta. 1525(1-2): 108-117.*

4.3   Flux lines and Supply area shapes

   The oxygen flux J_{O_2} can be obtained from the gradient in driving pressure, equation (1):

$$J_{O_2} = \mathbb{P}_{O_2} \nabla (P + P_F S)$$

and by following its direction, the oxygen path after leaving the capillary can be constructed, as shown by the arrowed lines in the figure (modified from [10]). By following all the possible fluxes for one capillary, the shape of the capillary's supply area can be found; as indicated by the gray shading in the figure.
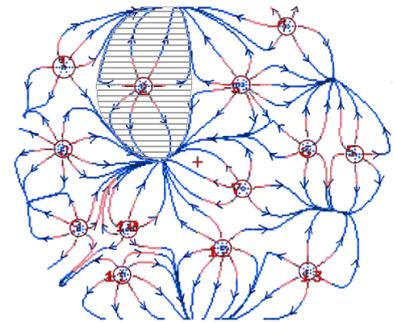
[10] *Reference: Hoofd L Turek Z Olders J (1989). Calculation of oxygen pressures and fluxes in a flat plane perpendicular to any capillary distribution. In: Oxygen Transport to Tissue XI, Advances in Experimental Medicine & Biology. 248: 187-196.*

4.4   Supply limitation

   Limiting the supply area is to prevent negative oxygen partial pressures, $P \geq 0$. So, the maximum supply area A_{max} is determined for an isolated capillary having $P = 0$ at the border of its supply area, where the minimum value is expected. In that case, the supply area is circular, $A_{max} = \pi R_{max}^2$ and equation (1) can be applied using $\bar{r}_i = 0$, $\Phi(\bar{r}) = $ Constant$+r^2$ and $P = 0$ at $r = R_{max}$:

$$P + P_F S = \frac{1}{4}\text{Đ}O2 \left\{ r^2 - R_{max}^2 - R_{max}^2 \ln\left(\frac{r^2}{R_{max}^2}\right) \right\}$$

At the capillary rim, $r = r_c$, the tissue's $P = P_{rim}$ can be found from the capillary oxygen pressure P_c using EP as explained above, equation (2):

$$P_{rim} = P_c - c_0 (\pi R_{max}^2 - \pi r_c^2) \, EP$$

yielding a direct relation between P_c and R_{max}.

4.5   Blood flow

   Capillary blood flow F is a volume per time but blood flow in literature often is found as volume per time per tissue volume Q. Conversion:

$$F = Q \times L / CD$$

where L is the average capillary length and CD the capillary density in a typical plane.