# Limitations of Traditional Models for Perfusion

In my previous review, I expressed major concerns regarding the lack of substantial new contribution to the field presented in the manuscript. Unfortunately, this opinion still stands because the authors appear to have done - without resorting to hyperbole - nothing, to address these concerns. In fact there appears to be very little changes of substance made in this revision overall. On the other hand, several equations appear to have changed but are unmarked - with multiple reviewers, keeping track of changes can be complicated. Please use coloured text to mark the changes clearly.

To reiterate my concerns, I believe the claims that the community is unaware of the voxel-sizes dependency of the tracer kinetics model is incorrect. These are well-documented in several publications. Let me provide a few quick examples:

"If we think of perfusion as the amount of blood flowing per unit time into (or equivalently out of) a voxel of tissue, we run into a difficulty with scaling. Consider a cubic voxel of tissue with linear dimension d cm. Let the uniform rate of blood flowing into this voxel be V ml/min. Assuming tissue has unit density the perfusion could be expressed as 100 $V/d^3$ in units of ml/min/100g. However, if we choose instead, a voxel of linear dimension 2d, then the total blood flow into this larger voxel would be 4V ml/min since the inflow of blood scales as the surface area. The only assumption required by this scaling is that the flow pattern be spatially equivalent over the distance 2d. (It could be isotropic, unidirectional, or bidirectional in one orientation-the geometry is unimportant as long as flow is not spatially varying). The perfusion in the larger voxel then becomes 100 X $4V/(2d)^3$ which is one-half the perfusion in the smaller voxel. Thus, a definition of perfusion based on blood flow into (or out of) a voxel does not produce a well-defined differential quantity and is inappropriate for quantitatively mapping perfusion."
(Henkelman, 1990, MRM)

The situation described above is equivalent to that described in figure 2 of the submitted manuscript. The author goes on to say:

"If, instead, we consider the classical measurement of perfusion, then a total number of counts, N, will be deposited in (or equivalently removed from) a voxel of tissue of linear dimension d cm in the course of the experiment. Assuming unit density tissue, the classical perfusion can be expressed as $N/d^3$. If we had chosen a voxel with twice the linear dimension, 2d, then the total counts deposited would be 8N (assuming spatial uniformity) and the perfusion would be $8N/(2d)^3 = N/d^3$. In this case, the definition provides a well-defined differential quantity. Quantitative mapping or imaging of perfusion as terminal deposition is a meaningful objective whereas measuring perfusion as flow into a voxel is an impossible exercise because the answer depends on voxel size."

This line of thinking is consistent with my original comments regarding oxygen extraction. If the concern is that the above warnings are too dated, a more recent reminder has been given:

"From a physics perspective, the normalisation of fluxes and flows to tissue volume appears unusual: these quantities normally scale with the surface through which they flow … Normalisation to volume would therefore not produce a well-defined local quantity but rather one that is dependent on voxel size. This is not the case in physiological flow, as the capillary bed is organized so that the inflow of arterial blood into a region of interest is proportional to the volume of the region it is meant to feed. The fundamental assumption underlying those definitions is therefore that all blood flow entering a region is 'feeding flow', i.e. it all passes through the capillary bed of the region before being evacuated. If some of the measured flow passes through the region 'unused', i.e. the flow in larger arteries, veins or arterio-venous shunts, the measured quantities will show a dependence on voxel size."
(Sourbron & Buckley, 2013, NMR Biomed)

Other investigators have used large-scale explicit reconstruction of the cerebromicrovasculature combined with fluid simulation to show this effect:

"Here, we show that the CBF measurement depends on the size of the processed virtual voxel, i.e., the size of the cubic box. From evaluating CBF using different voxel sizes, we have been able to analyze its size dependence. The total blood perfusion flux Q in a given voxel is directly evaluated from summing the input contributions of all the vascular segments that are also equal to the output ones since blood is incompressible. It obviously increases when sampling more and more vessels so that it is significantly different when varying the voxel-box size… Finally, it is interesting to mention that the filtration velocity (or Darcy flux), which is considered in porous media, is also a flow per surface unit (and not per volume unit) consistent with our finding for the brain perfusion."
(Guibert et al., 2013, JCBFM)

And while the second cause of perfusion overestimation stated by the authors (the interdependency of the flow between adjacent voxels) had been given a thorough treatment which proposed a new quantification method via the tracer-kinetic-field theory (Sourbron 2014), which, despite having been cited, is questionably described as "a two compartment model … as a definition of local perfusion".

These are but a few quick examples from the literature, and a more comprehensive and objective survey should be conducted by the authors.

Although it is not clearly conveyed in the submitted manuscript, I am sure the authors would agree with me that at least the theoreticians of the perfusion community are well-aware of the existing limitations. That leads to the question, why are these models continuing to be used in high-resolution studies? Voxel-wise quantification is incorrectly performed not because of a lack of awareness, but due to the lack of a usable alternative method. I agree this is an area requiring urgent attention, and should be pursued as a priority. However, the authors should also be aware

that in order to convince the practitioners in the field, one has to provide an alternative method and convincingly *show*, through suitable evidence, to support its validation. This is the source of my main reservation – that neither a new method (of determining the theoretical measure Ps or the radius associated with it) nor new data have been provided in the present work.

On a minor note, I think your estimates of perfusion in the real dataset may be around a thousand times larger than the accepted average range.