

# <sup>1</sup> 3DQLayers: Volumetric Layer Based Analysis for Quantitative Renal MRI

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## Software

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## <sup>7</sup> Summary

<sup>8</sup> Quantitative Magnetic Resonance Imaging (qMRI) provides informative measurements of the  
<sup>9</sup> structure and function of an organ where each volumetric pixel (voxel) provides a measure of the  
<sup>10</sup> physical properties of the underlying tissue. Traditionally, analysis of MR images is performed  
<sup>11</sup> by first segmenting the organ and its constituent tissue types, which for the kidneys involves  
<sup>12</sup> separating them into the cortex and medulla, before calculating the average measurement  
<sup>13</sup> within each tissue. The process of segmenting renal tissue types is typically manual, making it  
<sup>14</sup> time consuming and prone to inaccuracies.

<sup>15</sup> An alternative to voxel-based analysis in MRI is the layer model which divides the organ into  
<sup>16</sup> ordered surfaces. For the kidney, this involves generating layers based on the distance of each  
<sup>17</sup> voxel between the outer and inner surface of the kidney. From this, the gradient of change in  
<sup>18</sup> qMRI measures between the cortex and medulla of the kidney can be computed to evaluate  
<sup>19</sup> pathological and physiological aspects of the kidney. Here, 3DQLayers, an open-source Python  
<sup>20</sup> software package to automatically define and interrogate 3D renal layers is presented.

## <sup>21</sup> Statement of need

## <sup>22</sup> Background

<sup>23</sup> The kidneys are structurally and functionally complex organs in the abdomen responsible for  
<sup>24</sup> the removal of waste products and excess fluid from the blood to produce urine ([Lote, 2012](#)).  
<sup>25</sup> Each kidney is separated into the cortex which forms the outer layer of the kidney and the  
<sup>26</sup> medulla in the inner part which is arranged in a series of small pyramids ([Hall, 2015](#)), as shown  
<sup>27</sup> in [Figure 1](#). The kidney maintains homeostasis through filtration, reabsorption, secretion, and  
<sup>28</sup> maintenance of the cortico-medullary gradient (CMG), meaning a method to assess changes in  
<sup>29</sup> physiology from the cortex to the medulla is key.

<sup>30</sup> Conventional MRI primarily assesses signal intensity in a voxel in arbitrary units, however  
<sup>31</sup> Quantitative MRI (qMRI) goes beyond conventional MRI by instead providing voxel-wise  
<sup>32</sup> measurements with numerical significance in physical units, based on the tissues underlying  
<sup>33</sup> properties. For example, qMRI of relaxation times with parameters which carry information  
<sup>34</sup> about the local microstructure, or those of how readily water can diffuse through the tissue,  
<sup>35</sup> and the rate at which blood perfuses the tissue. To interpret quantitative images, regions of  
<sup>36</sup> interest (ROIs) for the kidney cortex and medulla are defined and statistical analysis performed  
<sup>37</sup> on the voxels within each ROI. Segmenting such ROIs manually is time consuming, and prone  
<sup>38</sup> to intra- and inter-reader variation.

<sup>39</sup> The group of Pruijm proposed an alternative to voxel based ROI analysis of tissue termed  
<sup>40</sup> the Twelve Layer Concentric Object (TLCO) method ([Li et al., 2020](#); [Milani et al., 2017](#);

41 Piskunowicz et al., 2015) where users delineate the inner and outer boundaries of the kidney  
42 to generate twelve equidistant layers between the renal pelvis and the surface of the kidney.  
43 The outer layers represent the cortex and the inner layers the medulla, with the gradient across  
44 the central layers computed to estimate the CMG. Since this layer-based analysis only requires  
45 segmentation of the boundaries of the kidney, rather than the cortex and medulla within, it  
46 is quicker and more repeatable. An analogy to this is the development of the layer-based  
47 analysis tools applied in the brain for neuroimaging including BrainVoyager (Goebel, 2012),  
48 CBSTools/Nighres (Bazin et al., 2014; J. Huntenburg et al., 2017; J. M. Huntenburg et al.,  
49 2018), FreeSurfer (Fischl, 2012), and FSL (Jenkinson et al., 2012).

50 However, the TLCO software is closed-source and has some limitations. It requires manual  
51 delineation of the outside and inside surfaces of the kidney, divides the kidney into the same  
52 number of layers irrespective of the size of the kidney, and can only be performed on a single  
53 slice cutting through the kidneys on their longest axis (coronal-oblique) which is not always  
54 desirable (Bane et al., 2020). Due to the spatial distribution of kidney pathology, there is a  
55 need to acquire multi-slice images for full 3D coverage of the kidney to increase the number of  
56 voxels sampled and gain a better understanding of the heterogeneity of the kidney. Recently,  
57 an automated-TLCO method has been proposed (Ishikawa et al., 2022) to determine the CMG  
58 from the water signal of a Dixon scan, however this work again has limitations of not being  
59 full kidney coverage, including the renal pelvis, and highlights the difficulty in analysing small  
60 kidneys with a fixed number of layers.

61 The motivation of 3DQLayers was to address these limitations of TLCO to provide an open-  
62 source Python package to automatically define 3D, multi-slice layers in the kidney of known  
63 thickness for quantitative-depth based analysis across a range of kidney MRI data, enabling its  
64 use in large renal MRI trials to address clinical questions.

## 65 Methods

66 3DQLayers is an open-source Python package building on the ideas within TLCO, with the  
67 fundamental difference that the layers are defined based on a voxels' distance from the surface  
68 of the kidney in millimetres rather than the proportion of the kidney. As such, the input to  
69 3DQLayers is a whole kidney mask, which can be automatically generated from a structural  
70 image e.g. here using a U-net applied to T<sub>2</sub>-weighted images (Daniel et al., 2021; Daniel,  
71 2024).

72 The pipeline for defining the layers from the whole kidney mask is outlined in Figure 2. Pre-  
73 processing steps first fill in the holes in the kidney mask caused by cysts, as the surface of a  
74 cyst is not characteristic of the surface of the kidney. Next, the voxel-based representation of  
75 the mask is converted to a smoothed mesh-based representation of the kidneys, the distance  
76 from the centre of each voxel to the surface of the mesh is calculated to produce a depth  
77 map (Dawson-Haggerty, 2023). Tissue adjacent to the renal pelvis that is not representative  
78 of the medulla is then excluded from layer-based analysis. This is achieved by automatically  
79 segmenting the renal pelvis then calculating the distance from each voxel to the renal pelvis as  
80 described above. Those voxels closer than a specified threshold, typically 10 mm, are excluded  
81 from the depth map. Finally, a layer image is generated by quantising the depth map to a  
82 desired layer thickness, typically 1 mm.

83 The layer image and quantitative images are resampled to the same spatial resolution using  
84 NiBabel (Brett et al., 2023), to allow each layer to be used as an ROI to interrogate each  
85 qMRI image with statistical measures (e.g. median, standard deviation and kurtosis) across  
86 the depth of the kidney. The gradient of the central layers can be calculated to estimate the  
87 CMG in qMRI metrics. These metrics can be computed for the left and right kidney separately,  
88 or analysed in a combined manner. Additionally, if the renal cortex and medulla ROIs are  
89 available, the distribution of tissue types across layer depth can be explored. As the layers  
90 are generated from a structural image rather than the quantitative map, using 3DQLayers  
91 stipulates no requirements on quantitative map acquisition, unlike TLCO.

92 An object-oriented interface is provided to allow end users to simply generate layers and apply  
93 these to qMR images. [Documentation](#) is provided to guide users through installation via PyPI,  
94 conda or from [source code on GitHub](#); it also includes tutorials and an API reference. An  
95 automated test suite with high coverage provides users with confidence in the stability of  
96 3DQLayers and that there will be no unexpected changes to results unless highlighted in the  
97 change-log.

## 98 Usage Examples

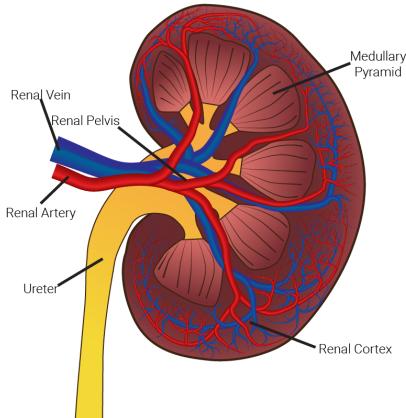
99 [Figure 3](#) shows the use of 3DQLayers to measure different gradients of  $R_2^*$  in a healthy volunteer  
100 with normal renal function and a patient with impaired renal function (an estimated glomerular  
101 filtration rate (eGFR) of above 90 ml/min/1.73m<sup>2</sup> measured from blood samples is considered  
102 in the healthy range ([Stevens et al., 2006](#))). This replicates results shown using TLCO, with a  
103 lower gradient in patients compared to healthies, however 3DQLayers controls for kidney size  
104 resulting in the gradient being measured in quantitative units of Hz/mm rather than Hz/layer  
105 as in TLCO, thus increasing generalisability.

106 [Figure 4](#) shows how 3DQLayers can be used in combination with cortex and medulla tissue ROIs  
107 to analyse the distribution of voxel counts of each tissue as a function of layer depth of the  
108 kidney. Here cortex and medulla ROIs are initially generated using a Gaussian mixture model to  
109 segment a T<sub>1</sub>-weighted structural image followed by manual ROI correction. From this, average  
110 renal cortical thickness can be defined from the depth at which the voxel distribution crosses  
111 from cortex to medulla. Cortical thickness has been hypothesised as a potential biomarker of  
112 renal disease ([Yamashita et al., 2015](#)).

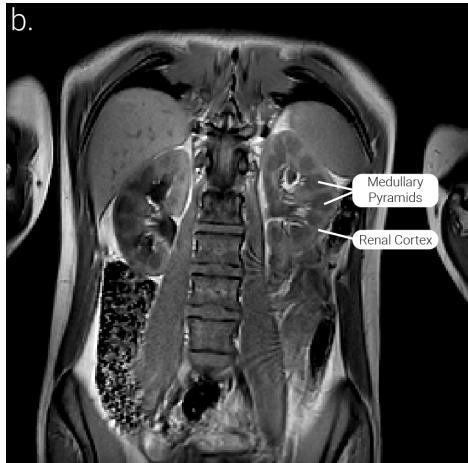
113 3DQLayers can also be used to analyse ex-vivo kidneys imaged outside the body. [Figure 5](#) shows  
114 example quantitative maps acquired from a kidney removed for transplant but subsequently  
115 deemed unsuitable and the associated layer profiles. [Figure 6](#) compares the results of tissue ROI  
116 based analysis and layer-based analysis in fifteen transplant kidneys. A significant correlation  
117 between outer layers and the cortex, and inner layers and the medulla was shown across all  
118 quantitative mapping techniques and a significant correlation between cortico-medullary ratio  
119 and layer gradient was shown for T<sub>1</sub>, T<sub>2</sub>, T<sub>2</sub><sup>\*</sup> and Magnetisation Transfer Ratio (MTR)  
120 mapping.

121 **Figures**

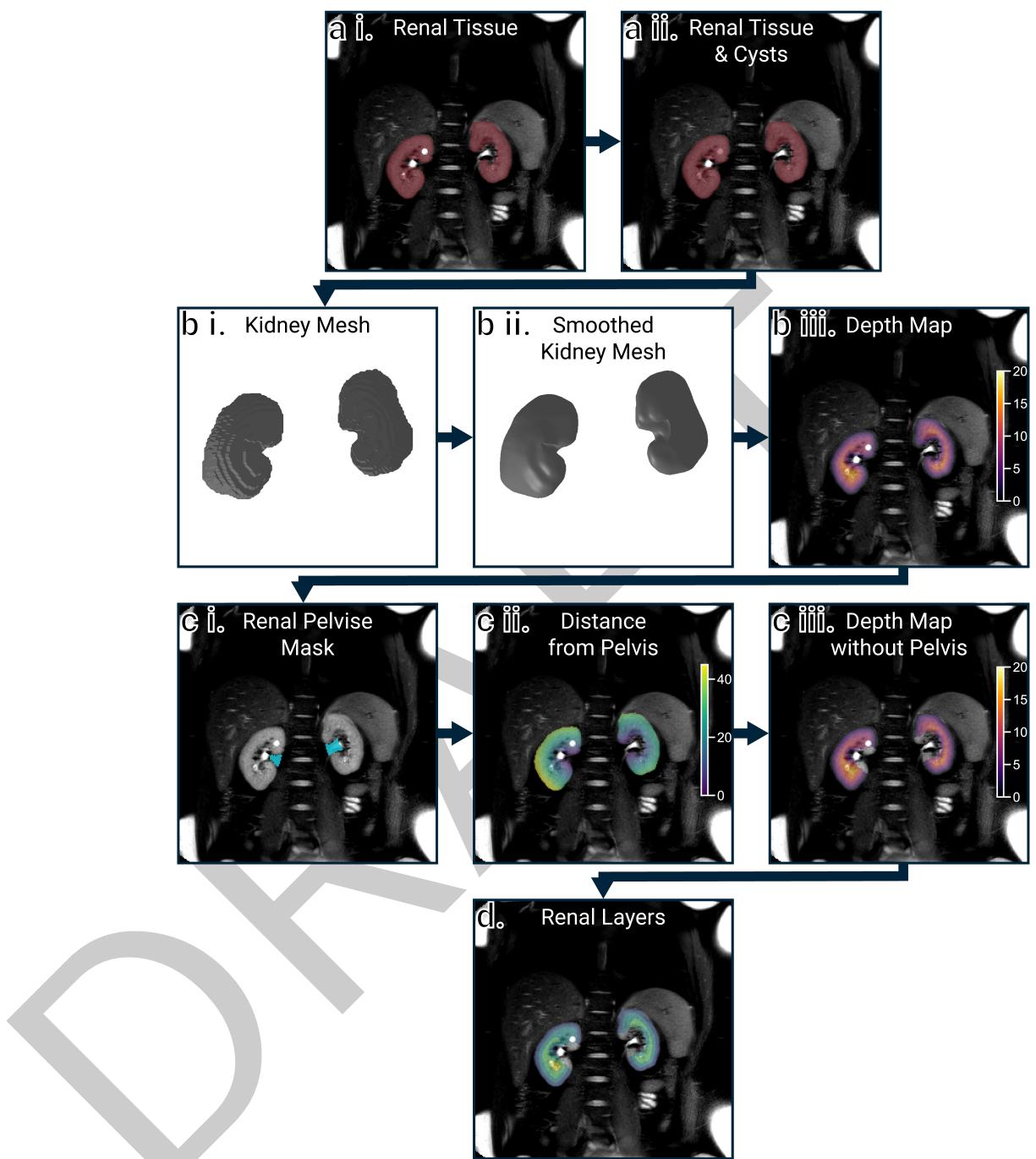
a.



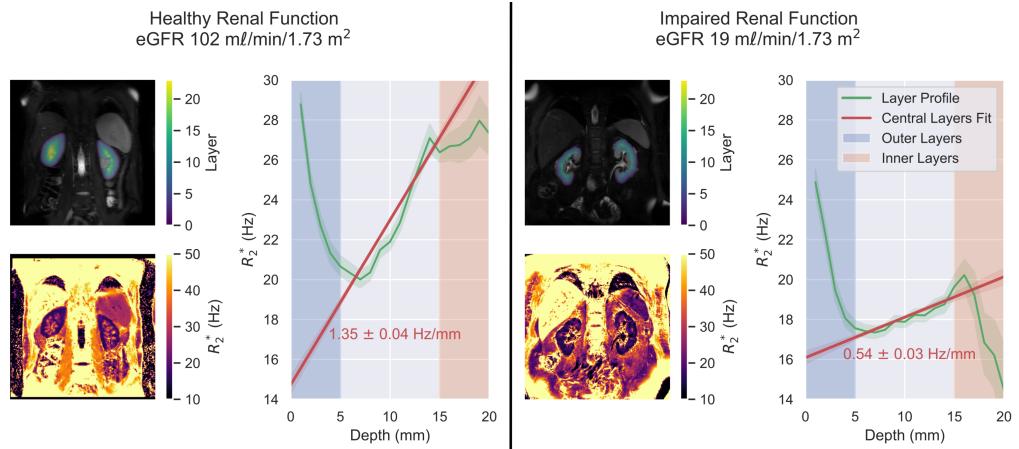
b.



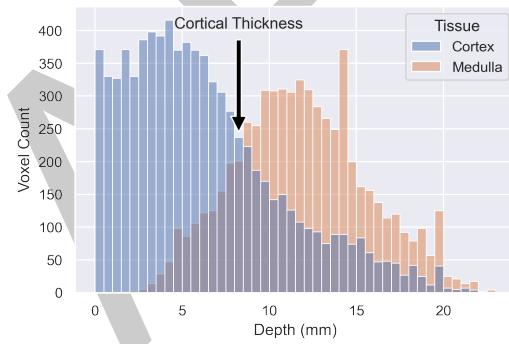
**Figure 1:** a) A schematic of the kidneys showing the renal cortex and medullary pyramids. b) A T<sub>1</sub>-weighted structural MR image of the abdomen showing the kidneys with the renal cortex appearing as a light band on the outer edge of the kidney and the medullary pyramids as darker patches on the inner portion of the kidneys.



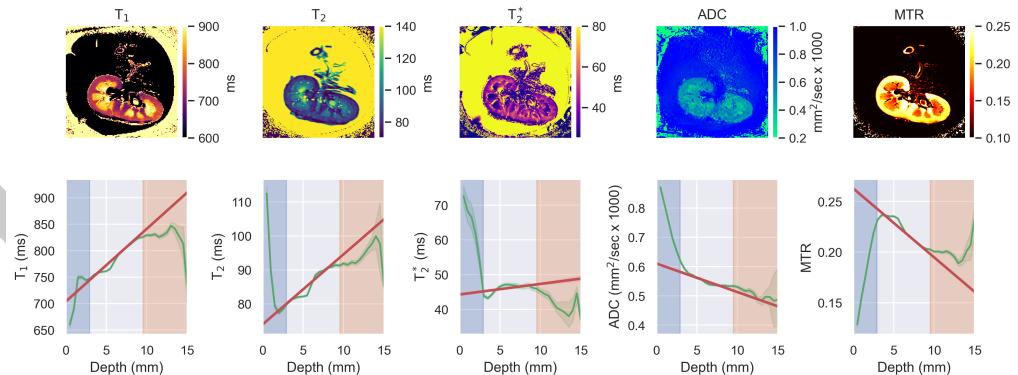
**Figure 2:** The mask automatically computed using a U-net from the T<sub>2</sub>-weighted structural MR image (a i) has any cysts filled (a ii) and is converted into a smooth mesh representing the renal surface (b i and ii). The distance (in mm) of each voxel to the surface of the mesh is then calculated to generate a depth map (b iii). The renal pelvis is segmented (c i) and any tissue within 10 mm (c ii) of the pelvis is excluded from the depth map (c iii). The tissue is then grouped into layers of a desired thickness, here shown as 5 mm renal layers for illustrative purposes (d).



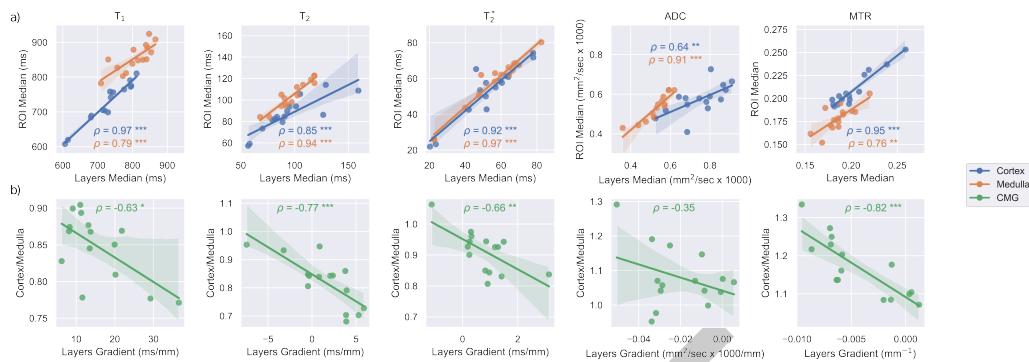
**Figure 3:** Layers,  $R_2^*$  maps, layer profiles, and central layer gradients for the left and right kidneys combined measured using 3DQLayers. Examples are shown for a subject with normal renal function and a patient with impaired renal function. Shading around profiles shows the 95% confidence interval within each layer.



**Figure 4:** Exploring the distribution of tissue types through the kidney to measure cortical thickness.



**Figure 5:** Example quantitative maps and associated layer profiles when 3DQLayers is applied to ex-vivo transplant kidneys. Uncertainty shading shows the 95% confidence interval of each layer.



**Figure 6:** Agreement between tissue ROI-based measures and analogous layer-based measures shown for fifteen ex-vivo transplant kidneys for each qMRI alongside the Pearson's correlation coefficient ( $\rho$ ). \* represents a  $p$ -value between 0.05 and 0.01, \*\* between 0.01 and 0.001, and \*\*\*  $< 0.001$ . a) Plots the median within each tissue ROI (cortex or medulla semi-automatically defined) against the equivalent layers (outer layers and inner layers respectively as highlighted in Figure 5) b) Shows the cortico-medullary ratio (calculated by dividing the median within the cortex ROI by the median within the medullary ROI) against central layer gradient profiles calculated using 3DQLayers.

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