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# **Ultrasound Localization Microscopy of the human kidney allograft on a clinical ultrasound scanner**

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*Running Headline: Non-invasive and quantitative imaging tool on the human kidney graft*

## **Word Count:**

- Abstract: 188
- Introduction: 453
- Methods: 108
- Results: 295
- Discussion: 579

1 **ABSTRACT**

2 Chronic kidney disease is a major medical problem, causing more than a million deaths each  
3 year in the world. Peripheral renal microvascular damage is found in most chronic kidney  
4 diseases, yet noninvasive and quantitative diagnostic tools are still lacking. Ultrasound  
5 Localization Microscopy (ULM) can assess tissue microvasculature with unprecedented  
6 resolution.

7 In this work, we studied the feasibility of ULM in human kidney allografts with a standard low  
8 frame rate ultrasound scanner. The acquisition parameters were derived from Contrast-  
9 Enhanced Ultrasound (CEUS) examinations by increasing the duration of the recorded clip at  
10 the same plane. ULM images were compared with Color Doppler, Advanced Dynamic Flow  
11 (ADF), and Superb Microvascular Imaging (SMI) with a contrast agent. Despite some  
12 additional limitations due to movement and saturation artifacts, ULM identified vessels 2 to 4  
13 times thinner compared with Doppler modes; i.e. the mean ULM smallest analyzable vessel  
14 cross section is  $0.3 \pm 0.2$  mm in 7 patients. Additionally, ULM was able to provide quantitative  
15 information on blood velocities in the cortex area.

16 In this proof-of-concept study, ULM was shown to be a promising imaging technique for the  
17 qualitative and quantitative assessment of microvessels.

18

**Keywords:** Ultrasound Localization Microscopy, super resolution, renal transplantation, renal  
ultrasound imaging

19

## 20 INTRODUCTION

21 Chronic kidney disease (CKD) causes more than one million deaths worldwide each year [1].  
22 This pathology is affected by acute kidney injury, age, and comorbid conditions [2,3] and is  
23 associated with an increase in hospital mortality, long-term development of renal failure, and  
24 end-stage kidney disease. The observation of the renal microvascular rarefaction can be used  
25 to monitor CKD progression [4,5]. Although pathophysiological mechanisms are still under  
26 investigation, several studies have shown a loss of microvascular density linked to an acute  
27 kidney injury [6-8].

28 Current imaging techniques for the non-invasive quantification of renal microvascular changes  
29 are still lacking [5]. Indeed, magnetic resonance imaging (MRI) [9], computed tomography  
30 (CT) [10], and ultrasound (US) [11] have been used to assess changes in renal microcirculation  
31 but all these perfusion methods provide indirect quantification without direct access to the  
32 microvasculature. Ultrasound has the advantage of safety, non-invasiveness, portability,  
33 affordability, and ease of use. Several approaches, such as Doppler imaging and Contrast-  
34 Enhanced Ultrasound (CEUS), have been explored in animals and humans [11]. However, none  
35 of these techniques offers a sufficiently high spatial resolution to assess microvessels, mainly  
36 due to the acoustic diffraction [12].

37 The concept of Ultrasound Localization Microscopy (ULM) imaging was recently introduced  
38 [12] and improves the resolution of the vascular system way beyond the acoustic diffraction  
39 limit [13]. ULM is achieved by combining microbubbles, constitutive of ultrasound contrast  
40 agents [14], ultrafast ultrasound imaging, [14], and post-processing steps: filtering that extracts  
41 signals from microbubbles circulating in the vessels [15], sub-pixel localization of individual  
42 microbubbles [16], frame to frame pairing of microbubbles for temporal tracking of their paths  
43 in blood vessels and final image reconstruction by the accumulation of microbubbles count. In  
44 previous studies, ULM has been successfully tested for in vivo microvessels imaging in rat  
45 brains [14], rat kidneys [14], mouse kidneys [4], and in several human organs [17, 18].  
46 However, these studies required research on ultrafast ultrasound scanners which are not  
47 common in hospitals. Other studies have been performed with conventional clinical scanners  
48 [19], but none in the field of kidney vasculature.

49 Our objective was to test the hypothesis that the kidney microcirculation sensitivity of ULM on  
50 a low frame rate clinical machine could image the micro-vascularization of kidney transplant  
51 recipients (KTRs). There was no modification to the imaging system and the post-processing  
52 steps were performed directly on the clips recorded by the scanner. Therefore, this technique

53 will be easy to replicate without having to develop new hardware. In this article, we describe  
54 the imaging protocol and post-processing steps needed to perform ULM on any existing setup.  
55 Furthermore, we provide a side-by-side comparison of ULM with state-of-the-art Doppler  
56 modes. Finally, we investigated the relationship between vessel velocities and their distances  
57 to the kidney capsule within the cortex.

## 58 **METHODS**

59 The French College of Radiology ethics committee approved this study (n° IRB: CRM-2112-  
60 218). The work described here has been carried out by The Code of Ethics of the Declaration  
61 of Istanbul. All methodologies are detailed in the Supplementary Methods, including subject  
62 inclusion, contrast agent injection optimization (Supplementary Figures S1), data acquisition,  
63 data optimization, data processing (Supplementary Figures S2), vessel diameter measurements  
64 (Supplementary Figures S3, Supplementary Table S1), and kidney capsule segmentation for  
65 quantitative speed analysis (Supplementary Figures S4).

66 In total, the study included 49 KTRs, 35 of whom were used to optimize the method. The results  
67 on 7 patients are reported in this article (details in Supplementary Methods).

68 **RESULTS**

69 **ULM density and directivity maps**

70 Interlobar vessels, arcuate vessels, cortical radial vessels, and part of the medullary organization  
71 are visible on ULM density maps (Figure 1a, 1b). Smaller structures are not clearly detectable  
72 by clinical ULM at a low frame rate. ULM directivity maps, i.e. upward flows in red and  
73 downward flows in blue, highlighted cortical radial veins and arteries more precisely (Figure  
74 1c, 1d), especially in the upper regions where the vessels are aligned in the axial direction  
75 (Figure 1e, 1f). The medullary vasa recta can be seen but are not as clear as the cortical vessels.

76 **Comparison of ULM with Doppler modes**

77 Interlobar vessels, arcuate vessels, and cortical radial vessels are more visible with ULM  
78 (Figure 2d) than with other Doppler techniques (Figure 2a-c). In addition, in this same figure,  
79 the medullary organization is visible by the ULM density map in patient 1 whereas it is not with  
80 other Doppler modes (Figure 2). Five imaged vessel diameters have been estimated for each  
81 technique by manual cross-section (Supplementary Figures S3) in 7 patients (Table. 1). The  
82 average of the smallest vessel in the 7 patients measured  $0.3 \pm 0.2$  mm for ULM,  $0.8 \pm 0.3$  for  
83 Superb Microvascular Imaging (SMI),  $1.2 \pm 0.4$  for Advanced Dynamic Flow (ADF) and  $1.3 \pm$   
84  $0.5$  for Color Doppler; i.e. 2 to 4 times thinner with ULM than with usual Doppler techniques.

85 **Quantitative speed analysis in the cortex area**

86 ULM speed maps (Figure 3a, 3b) and their further analysis on upper cortex tracks (Figure 3c,  
87 3d) indicate an increase in velocity as the vessels move away from the kidney capsule in two  
88 patients (Figure 3c, 3d, Supplementary Figures S4). The averaged velocities of 7 patients who  
89 underwent the same capsule segmentation also allow observing this tendency (Figure 3e).

## 90 **DISCUSSION**

91 In this study, we have shown the feasibility of ULM in kidney transplant recipients using a  
92 conventional low frame rate clinical ultrasound scanner. We demonstrated that with very minor  
93 adjustments to the examination procedure and simple post-processing tools, we were able to  
94 improve the resolution of vascular imaging. We showed that ULM density and directivity maps  
95 revealed the different renal structures and that ULM maps reached 2 to 4 times thinner vessel  
96 diameter than the conventional high-resolution Doppler modes. Furthermore, we have shown  
97 that velocities in cortical regions depend on their distance from the renal capsule: ULM is  
98 therefore sensitive enough to detect changes in microvasculature enabling quantitative analysis.  
99 These results suggest that ULM could be used in clinical routine to detect microvascular  
100 alterations in various kidney diseases [4-8]. In the case of fibrosis or interstitial nephropathy,  
101 for example, ULM could be performed instead of pathological analysis by biopsy, thus avoiding  
102 risks of bleeding, pain or too small sample size. Changes in kidney microvascularization in  
103 addition to morphology can be quantified, as it has already been done in rats [4] and mice [5]:  
104 thus, ULM could serve as a new in vivo companion biomarker for the diagnosis of chronic  
105 kidney diseases, given that there is no gold standard in imaging yet. Moreover, considering that  
106 clinical ultrasound scanners are widely available, such investigation could be replicated on  
107 diverse organs without having to develop new equipment.

108 Nevertheless, several limitations can be identified in this proof-of-concept of clinical ULM in  
109 human kidney allograft. First, acquisitions in 35 patients were exploited for optimization  
110 leading to a limited number of patients with complete data (details in Supplementary Methods).  
111 Besides, the layout of the kidney allografts and the singularity of each patient forced us to carry  
112 out segmentations of kidney capsules, upper cortex area (Supplementary Figures S4), and cross-  
113 sections by hand (Supplementary Methods, Supplementary Figures S3). In addition,  
114 conventional Doppler imaging and ULM are not exactly in the same plane because the probe is  
115 hand-held and the examinations are minutes apart. Quantitative estimations are therefore biased  
116 and should be taken as indicative.

117 In addition, since ULM is based on the tracking of microbubbles between images, the maximum  
118 velocities observable by ULM are low because of our low frame rate: this confines our  
119 sensitivity to large vessels. Ultrafast imaging can improve the estimation of the velocities, but  
120 it is not easily accessible in clinics.

121 Finally, the superficial (heterotopic) position of the renal graft is an advantage for this  
122 technique, because the graft is less mobile. ULM could be applied to the native kidney but not  
123 without difficulty. The depth of the organ makes it less accessible and more impacted by

124 breathing: apnea is therefore necessary to be able to perform ULM on the native kidney, which  
125 limits the duration of the clips and therefore the richness of the ULM mapping. In obese  
126 patients, the depth of the organ due to the thickness of the subcutaneous tissue makes it less  
127 accessible. Nevertheless, we still managed to obtain ULM mapping on 2 obese and 3  
128 overweight patients (Supplementary Table S2).

129 In the future, imaging a native kidney in patients with kidney diseases will be needed to identify  
130 the different ULM biomarkers. The ULM's performance could also be improved by using a US  
131 probe with a higher frequency to increase the spatial resolution, increasing the signal-to-noise  
132 ratio with upgraded signal processing techniques [16], and using a 3D probe [20] that could  
133 allow a much faster and more reliable diagnosis.

134



135 **DISCLOSURE**

136 O.C. and V.H., hold patents in the field of ultrasound localization microscopy (EP4011299A1).

137 O.C. and V.H. are founders and shareholders of the ResolveStroke startup.

138 Other authors have nothing to declare.

139 We would also like to declare that the Aplio i800 (Canon MS, Nasu, Japan) ultrasound scanner

140 from which the data was obtained was loaned to the Department of Nephrology and Kidney

141 Transplantation, Necker University Hospital (APHP).

142 In addition, we want to mention the funding of Bracco at CRIN as a teaching aid.

143	<b>LIST OF SUPPLEMENTARY MATERIALS</b>
144	<b>Supplementary Methods</b>
145	<b>Supplementary Figures</b>
146	<b>Supplementary Tables</b>
147	<b>Supplementary References</b>

148 **FIGURE TITLES AND LEGENDS**

149 **Figure. 1. Ultrasound Localization Microscopy (ULM) density and directivity maps. (a)**  
150 ULM density map of two renal lobes vasculature in the first patient. “C” is for renal cortex,  
151 “M” for medullary organization, “1” for interlobar vessels, “2” for arcuate vessels, “3” for  
152 interlobular vessels (cortical radial vessels) and “4” for vasa recta. **(b)** ULM density map of  
153 three renal lobes in the second patient. **(c)** ULM directivity map of the first patient. **(d)** ULM  
154 directivity map of the second patient. **(e)** and **(f)** Close-up of the two patients ULM directivity  
155 maps.

156

157 **Figure. 2. Smallest imageable vessel diameter measured in Ultrasound Localization**  
158 **Microscopy and in Doppler classical modes in patient 1. (a)** Super Microvascular Imaging,  
159 **(b)** Color Doppler, **(c)** Advanced Dynamic Flow, **(d)** Ultrasound Localization Microscopy. The  
160 white dotted center marks indicate the smallest imageable blood vessel determined by the cross-  
161 section diameter measurement on this specific image. **(e)** Statistical differences between all  
162 vessels cross sectioned of all 7 patients in each imaging technique. **(f)** Statistical differences  
163 between the smallest imageable vessel for each 7 patients in each imaging technique. Student's  
164 t test was performed to quantify the differences between vessel cross sections with a 95%  
165 confidence level. The significance of the results is as follows: ns =  $P > 0.05$ , \* =  $P \leq 0.05$ , \*\* =  
166  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ , \*\*\*\* =  $P \leq 0.0001$ .

167

168 **Figure. 3. ULM speed maps quantitative analysis. (a)** ULM speed maps of the first patient.  
169 ULM density map has been encoded with the velocity of each track. **(b)** ULM speed maps of  
170 the second patient with same encoding. **(c)** ULM tracks only in the cortex with kidney capsule  
171 area indicated in yellow line in patient 1. **(d)** ULM tracks only in the cortex with kidney capsule  
172 area indicated in yellow line in patient 2. **(e)** Distance to the capsule as a function of the mean  
173 speed in 7 patients. The x-axis corresponds to the mean velocity values of all tracks present in  
174 the cortex area at a certain distance of the capsule. White dot indicates the median in all 7  
175 patients, and color dots indicate the mean tracks velocity of each patient at this capsule distance.

176

177

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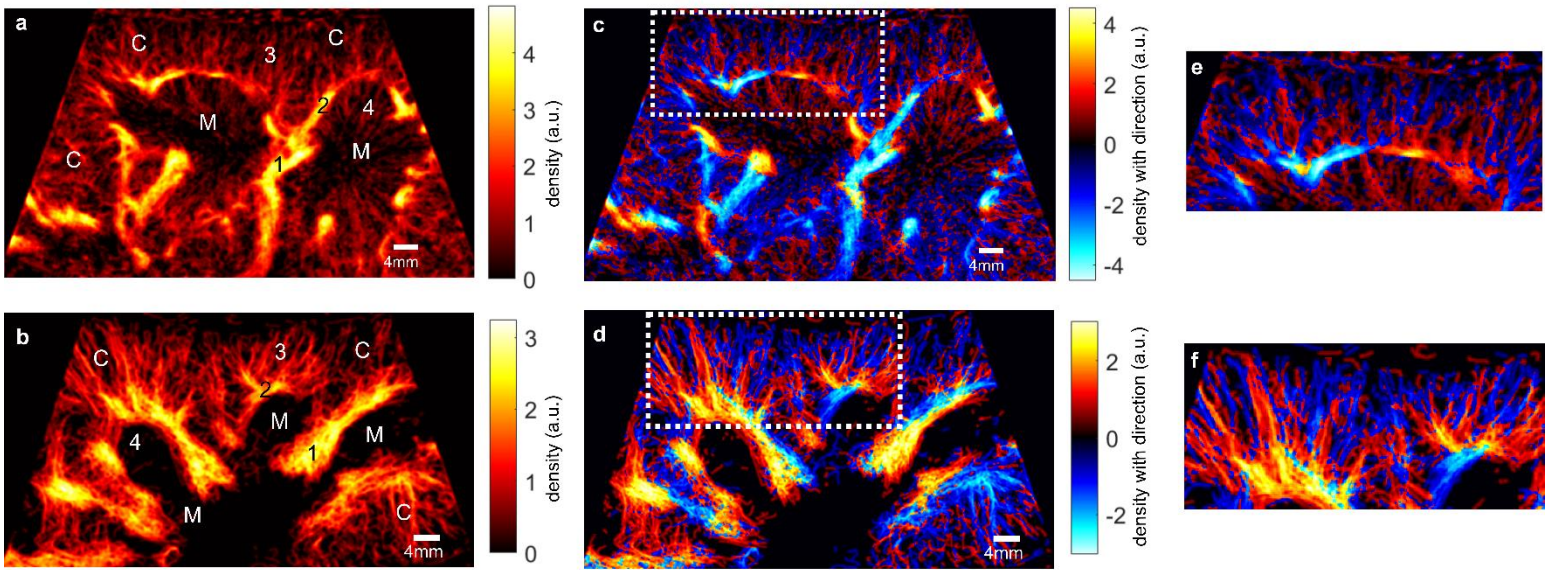
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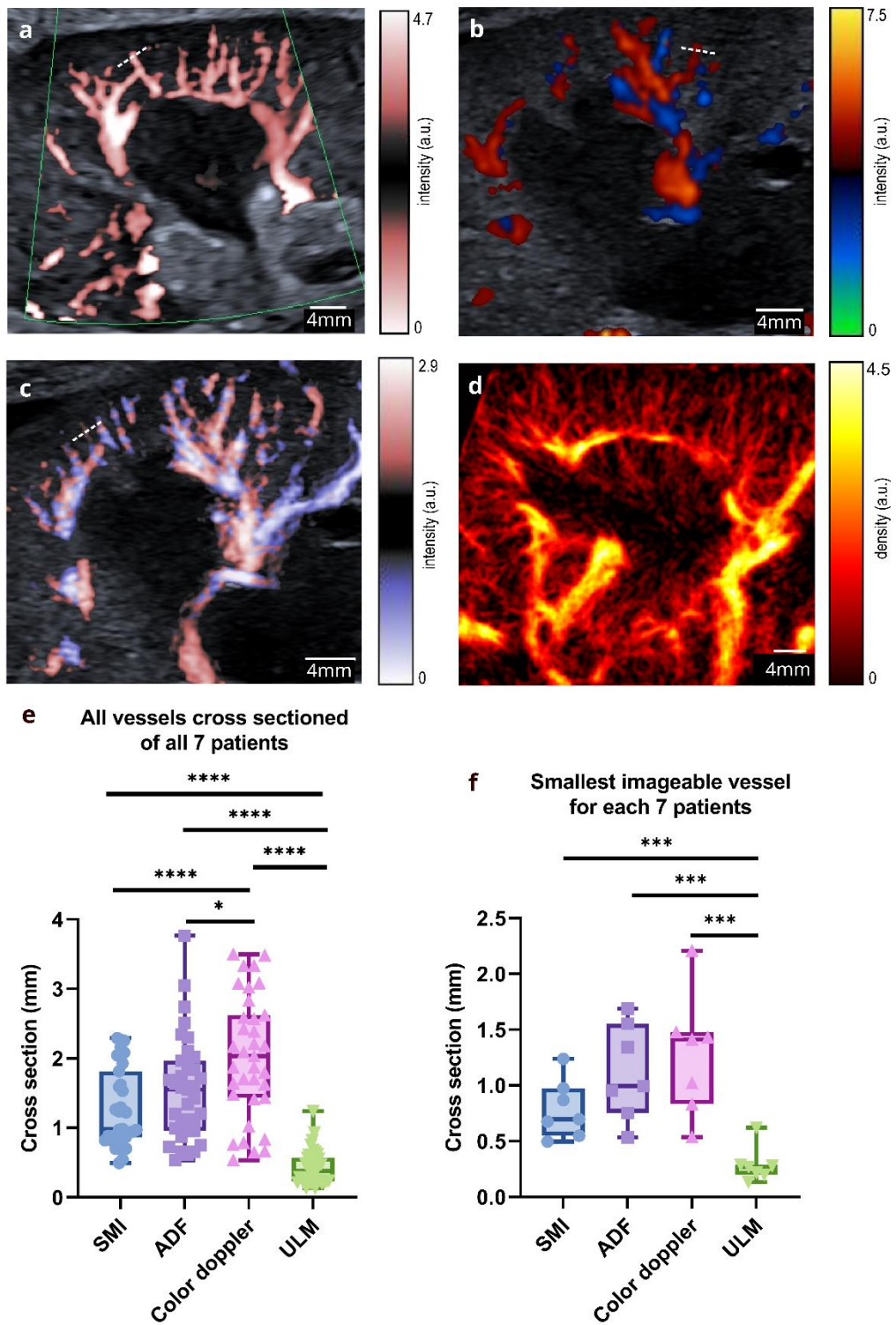
237 **ACKNOWLEDGEMENTS**

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## FIGURES

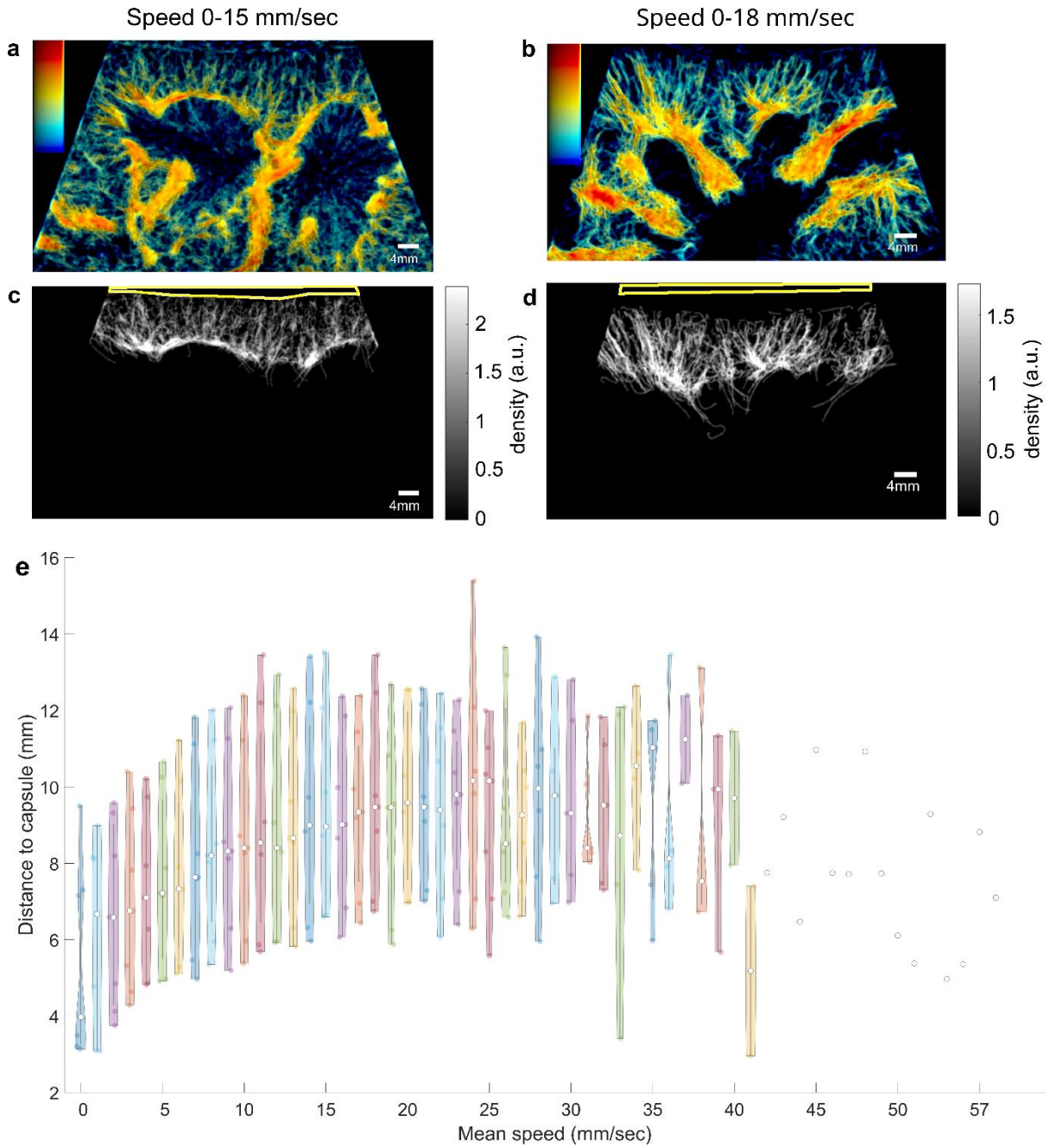


**Figure 1. Ultrasound Localization Microscopy (ULM) density and directivity maps.** (a) ULM density map of two renal lobes vasculature in the first patient. “C” is for renal cortex, “M” for medullary organization, “1” for interlobar vessels, “2” for arcuate vessels, “3” for interlobular vessels (cortical radial vessels) and “4” for vasa recta. (b) ULM density map of three renal lobes in the second patient. (c) ULM directivity map of the first patient. (d) ULM directivity map of the second patient. (e) and (f) Close-up of the two patients ULM directivity maps.



**Figure. 2. Smallest imageable vessel diameter measured in Ultrasound Localization Microscopy and in Doppler classical modes in patient 1.** (a) Super Microvascular Imaging, (b) Color Doppler, (c) Advanced Dynamic Flow, (d) Ultrasound Localization Microscopy. The white dotted center marks indicate the smallest imageable blood vessel determined by the cross-section diameter measurement on this specific image. (e) Statistical differences between all vessels cross sectioned of all 7 patients in each imaging technique. (f) Statistical differences between the smallest imageable vessel for each 7 patients in each imaging technique. Student's t test was performed to quantify the differences between vessel cross sections with a 95% confidence level. The significance of the results is as follows: ns =  $P > 0.05$ , \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ , \*\*\* =  $P \leq 0.001$ , \*\*\*\* =  $P \leq 0.0001$ .





**Figure 3. ULM speed maps quantitative analysis.** (a) ULM speed maps of the first patient. ULM density map has been encoded with the velocity of each track. (b) ULM speed maps of the second patient with same encoding. (c) ULM tracks only in the cortex with kidney capsule area indicated in yellow line in patient 1. (d) ULM tracks only in the cortex with kidney capsule area indicated in yellow line in patient 2. (e) Distance to the capsule as a function of the mean speed in 7 patients. The x-axis corresponds to the mean velocity values of all tracks present in the cortex area at a certain distance of the capsule. White dot indicates the median in all 7 patients, and color dots indicate the mean tracks velocity of each patient at this capsule distance.

<b>Imaging technique</b>	<b>Mean (<math>\pm</math> SD) of the smallest imageable vessel diameter among the 7 patients (mm)</b>	<b>Mean (<math>\pm</math> SD) of the 7 patients' vessel diameter (mm)</b>
ULM*	0.3 $\pm$ 0.2	0.4 $\pm$ 0.2
SMI** + contrast agent	0.8 $\pm$ 0.3	1.3 $\pm$ 0.6
ADF***	1.2 $\pm$ 0.4	1.5 $\pm$ 0.7
Color Doppler	1.3 $\pm$ 0.5	2.0 $\pm$ 0.9

**Table. 1. Cross-section measurements for each imaging technique in 5 vessels per patient on 7 patients.** We noted the mean ( $\pm$  standard deviation) of the smallest imageable vessel diameter among the 5 vessels of each 7 patients and the mean ( $\pm$  standard deviation) of the 5 vessels of every 7 patients' diameter.

\*Ultrasound Localization Microscopy (ULM), \*\*Superb Microvascular Imaging (SMI), \*\*\*Advanced Dynamic Flow (ADF).

## 246 **SUPPLEMENTARY MATERIAL**

### 247 **Supplementary Methods**

248 From February to August 2020, 49 kidney transplant recipients older than 18 years were  
249 included. Patients were those who were previously referred for ultrasound examination in our  
250 adult radiology department at Necker University Hospital. In clinical routine, these subjects  
251 receive several postoperative ultrasounds: on day 1, then on month 3, on month 12, and  
252 annually. They also receive ultrasound scans in case of graft dysfunction to explore a surgical  
253 or medical complication. The examination includes B-mode, classical Dopplers (Color  
254 Doppler, Superb Microvascular Imaging, and Advanced Dynamic Flow), and pulsed Doppler  
255 acquisitions. An ultrasound acquisition with Sonovue® microbubbles injection (Bracco) is also  
256 performed to explore hypo- or avascular areas and necrosis: thus, no additional injection was  
257 necessary for this study and we used the same type of acquisition to perform the ULM. In  
258 addition, to avoid motion artifacts in the acquisitions, patients were supine and breathing  
259 slowly.

260 We started to optimize the CEUS mode embedded in the clinical scanner on the first 15 patients  
261 to have satisfactory ULM images. To perform ULM, we used an Aplio i800 (Canon MS, Nasu,  
262 Japan) and an i8CX1 convex abdominal probe (3 MHz). Probes used to perform conventional  
263 Doppler techniques were either i8CX1 (3MHz) or i11LX3 (7MHz). The dynamic range and  
264 gain were adapted to the ultrasound machine, which allowed better discrimination of  
265 microbubbles and facilitated their localization. Because of the superficial position of the renal  
266 graft in the iliac fossa, we were able to reduce the imaging depth to explore between 4 and 10  
267 cm, resulting in a maximum clip time of 1 to 3 minutes. In this way, we could increase the  
268 frame rate (between 14 and 64Hz). Data were collected in DICOM format and all dynamic  
269 clips were stored anonymously on a hard disk. From 2 to 4 clips were stored per patient resulting  
270 in a total of 142 clips for the 49 patients. All data analyses were performed at the Biomedical  
271 Imaging Laboratory by members of the PPM (Physiology Pathology of the Microcirculation)  
272 team, specialist in ULM for over 5 years.

273 After optimization of the probe's positioning on 5 patients, we searched for time-window with  
274 the optimal number of microbubbles on 15 patients. The injection of a bolus of 1.2 mL of  
275 microbubbles, followed by an injection of 10 ccs of saline, was repeated twice, as in the clinical  
276 routine. The optimal number of microbubbles, i.e. to have isolated ones, was reached during

277 the late venous phase, i.e. on average between 45 and 192 seconds after injection. The  
278 difference in microbubbles concentration between a too-early phase (too many microbubbles),  
279 an optimum phase (many distinct microbubbles), and a delayed phase (disappearing  
280 microbubbles) was observed in CEUS acquisitions (Supplementary Figures S1). We used a low  
281 mechanical index ( $=0.07$ ) to exploit the non-linear properties of microbubbles [S1] by limiting  
282 their destruction. The examination duration then depends almost exclusively on the natural  
283 lifetime of the microbubbles in the blood compartment.

284 In short, from the 49 included KTRs, 35 were used for the optimization of CEUS mode, probe's  
285 positioning, and microbubbles optimal number targeting. The remaining 14 were used to  
286 perform ULM, and among them, 7 were excluded because of respiratory movements: results  
287 on the remaining 7 are presented in this study.

288 To do ULM images, clips were divided into blocks of 200 frames each: a clip of 173 seconds  
289 at 22 Hz corresponding to 3812 consecutive frames was thus divided into 20 blocks. ULM was  
290 achieved with classical steps on each block: filtering, localization of microbubbles, tracking,  
291 and track accumulation. Filtering was already done by the CEUS mode embedded in the  
292 ultrasound system (Supplementary Figures S2a): bandpass filters with cutoff frequencies from  
293 0.5 to 8.5Hz have been added to enhance the moving microbubbles. Localization has been  
294 realized thanks to a 2-D Gaussian filter with a standard deviation of 1 pixel (i.e. from 0.07 to  
295 0.17mm) and targeting of the regional maximums (Supplementary Figures S2b). Tracking was  
296 performed with the Hungarian algorithm method [S2] using a maximum distance between the  
297 microbubbles of 1 to 2.8mm and a minimum track duration varying from 0.08 to 0.4 seconds  
298 (Supplementary Figures S2c). Finally, tracks accumulation of the 8 to 35 blocks, allowed us to  
299 obtain a vascular density map of the kidney (Supplementary Figures S2d).

300 We keep the same pixel size for ULM maps as the original grid (from 0.07 to 0.17mm). We  
301 measured five vessels' diameters in Doppler modes and ULM with the cross-section technique  
302 [S3, S4] (Supplementary Figures S3) in every 7 patients: we thus have an estimation of the  
303 mean of these thirty-five vessels' diameters, their standard deviation, and an estimation of the  
304 average of the 7 smallest vessels for each technique. The intensity of the red component was  
305 used to estimate diameter on ADF, SMI, and color Doppler (color of segmented vessels).

306 It is important to specify that these measurements are not resolution measurements but  
307 measurements of the vessels' diameters, which can give us an idea of the resolution achieved  
308 by each of the ultrasound techniques. Pixel size of each ultrasound techniques, also called  
309 spatial resolution, have been described in Supplementary Table S1.

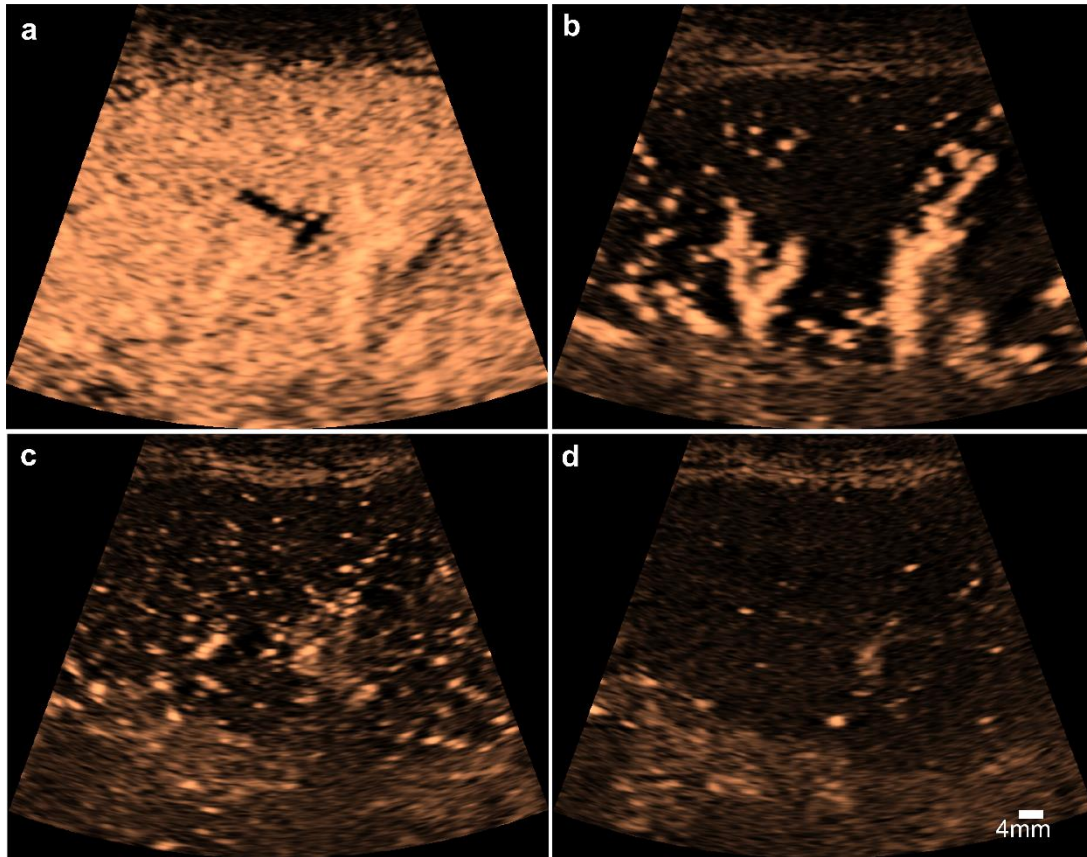
310 Velocity encoding was performed and overlaid on the density map. Directions were encoded in  
311 red when tracks go towards the probe, and in blue when they go away from it. To perform a  
312 quantitative ULM analysis, we manually segmented the kidney capsule and upper cortex area  
313 to investigate a potential correlation between vessel velocity and its distance to the capsule  
314 (Supplementary Figures S4). This analysis was performed only on tracks present in the upper  
315 cortex for two reasons: the lower kidney capsule was not visible on acquisitions, and to avoid  
316 aliasing bias present in the bigger vessel by ULM. Indeed, max speed detected by ULM varies  
317 from 2cm/sec to 6cm/sec whereas biggest kidney vessels speed is normally around 100cm/sec  
318 [S5].

319

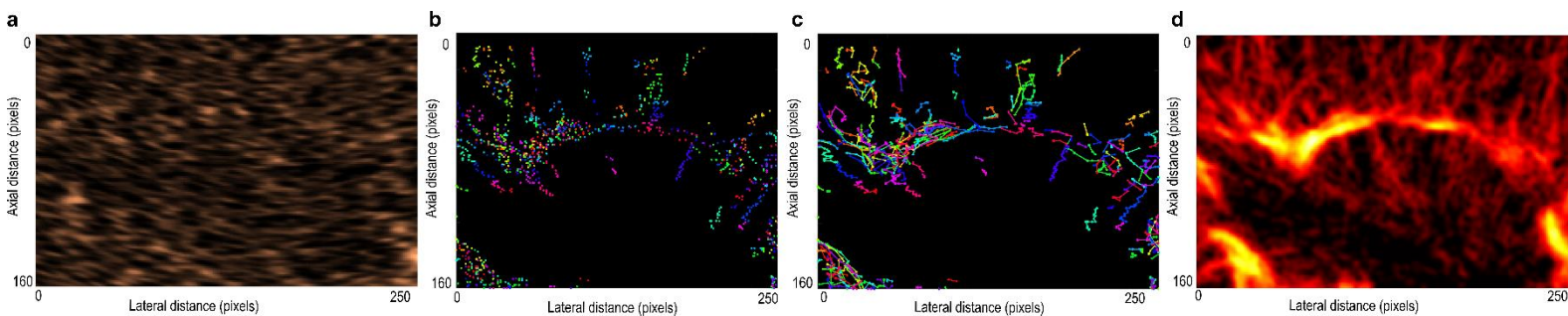
320 All image processing was made with MATLAB (Mathworks).

321 Statistical analyses were performed with Graphpad Prism 9 software. Student's t test was  
322 performed to quantify the differences between vessel cross sections with a 95% confidence  
323 level. The significance of the results is as follows: ns =  $P > 0.05$ , \* =  $P \leq 0.05$ , \*\* =  $P \leq 0.01$ ,  
324 \*\*\* =  $P \leq 0.001$ , \*\*\*\* =  $P \leq 0.0001$ .

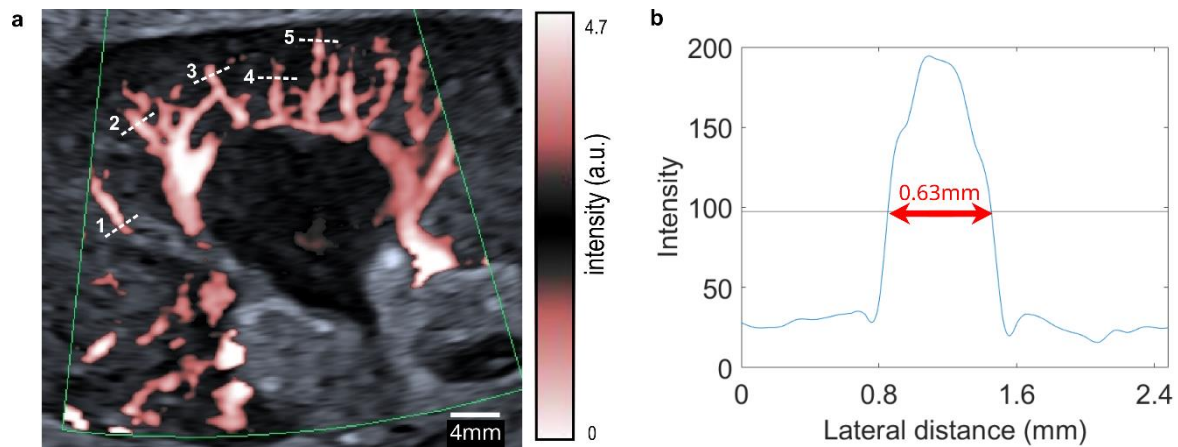
325 SRQR reporting guidelines were applied [S6].



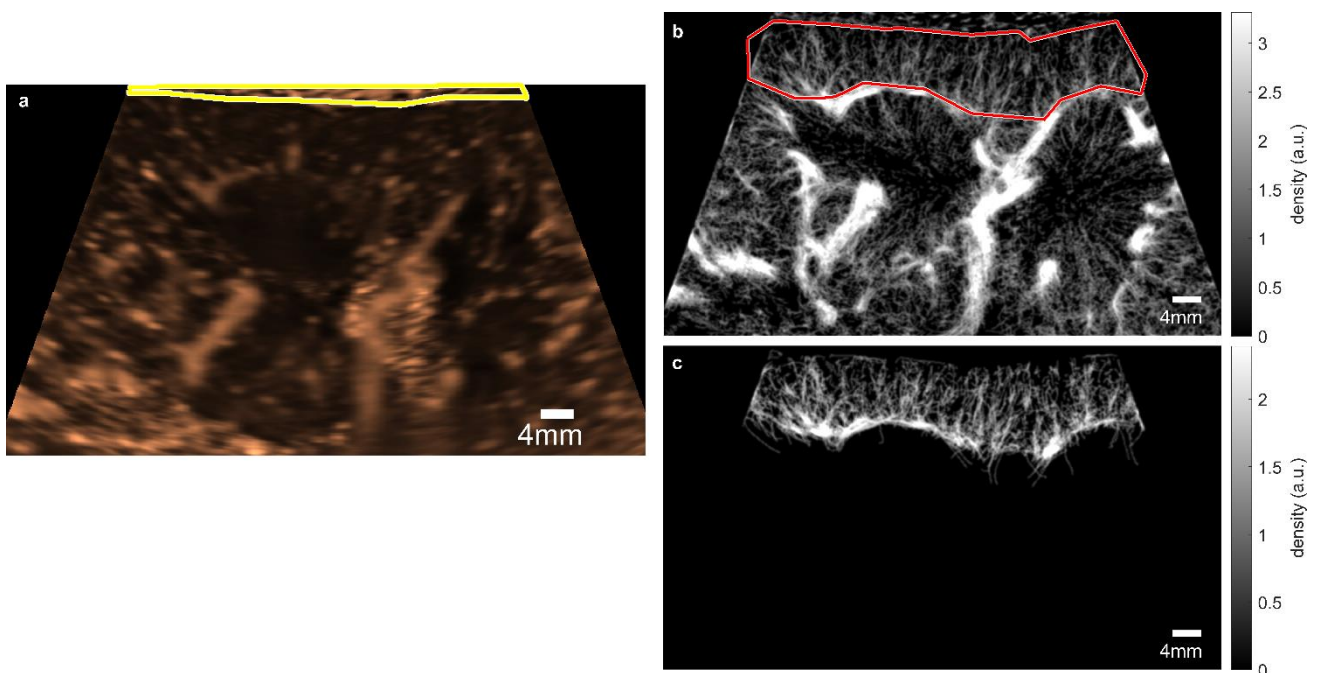
**Supplementary Figure. S1. Difference in microbubbles concentration in patient 1.** (a) Contrast Enhanced Ultrasound acquisitions in clinical practice. (b) Microbubbles early arrival. (c) Late venous phase (optimal phase). (d) Very delayed phase. The colormap comes from the ultrasound scanner without specification (arbitrary unit). The scale bar is the same as in d for all 4 images.



**Supplementary Figure.S2. Framework of ULM image formation in zoomed patient 1.** (a) Contrast Enhanced Ultrasound (CEUS) acquisition with a clutter filter integrated in the clinical echograph. The color map comes from the ultrasound scanner without specification (arbitrary unit). (b) Localization of microbubbles thanks to a 2D gaussian filter in one bloc (arbitrary colors). (c) Tracking thanks to Hungarian algorithm in the same bloc (arbitrary colors). (d) ULM density map resulting from the accumulation of twenty blocs (density colormap from 0 to 4.5 in arbitrary units).



**Supplementary Figure.S3. Example of cross-section measurement in Superb Microvascular Imaging (SMI), with contrast agent, acquisition in patient 1. (a)** SMI, with contrast agent, image with five cross-sectioned vessels indicated with dotted white lines. The color map comes from the ultrasound scanner without specification (arbitrary unit). **(b)** Diameter measured as the width at half the maximum intensity (of the red component), in the third manually cross-sectioned vessel. Red arrow indicates the width at half the maximum intensity.



**Supplementary Figure.S4. Kidney capsule and upper cortex manual segmentation on patient 1. (a)** Capsule segmentation made on temporal mean of the first block of CEUS acquisition in patient 1. Kidney capsule is indicated with a yellow line. The color map comes from the ultrasound scanner without specification (arbitrary unit). **(b)** Upper cortex segmentation made on ULM density map. Cortex mask is drawn with a red line. **(c)** Resulting cortex tracks from the upper cortex segmentation. If at least one point of a track was present in the upper cortex mask, track was preserved.

328 **Supplementary Tables**

<b>Patients n°</b>	<b>Pixel resolution (mm)</b>			
	<b>CEUS</b>	<b>ADF</b>	<b>SMI</b>	<b>Color Doppler</b>
<b>1</b>	0.12	0.07	0.10	0.07
<b>2</b>	0.07	0.15	0.15	0.18
<b>4</b>	0.12	0.15	0.07	0.18
<b>10</b>	0.14	0.08	0.13	0.15
<b>11</b>	0.17	0.18	0.17	0.17
<b>13</b>	0.15	0.18	0.13	0.17
<b>19</b>	0.10	0.13	0.15	0.13

**Supplementary Table S1. Pixel resolution of each ultrasound modes in each patient.**

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Patient number	Etiology of initial renal failure	Sonovue Dose (mL)	Time frame of the transplant ation	Dilation of the pyelo-caliceal cavities	SRT (ms)	RI interlobar arteries	Nb of grefon veins	Nb of grefon arteries	Creatinin ( $\mu\text{mol/L}$ )	Graft depth (mm)	BMI ( $\text{kg/m}^2$ )	Age (y)	Sex
1	Cystinosis	1.2	11 days	No	<200	0.72 - 0.82	1	1	105	9	31	33	F
2	Valve of the posterior urethra	1.2	12 hours	No	<200	0.41 - 0.52	1	1	180	15	24	19	M
3	TSB + ADPKD	1.2	3 days	No	<200	0.82 - 0.89	1	2	203	36	25	36	F
4	MPGN	1.2	17 days	No	<200	0.62 - 0.65	1	1	130	24	21	19	M
5	Diabetic nephropathy	1.2	24 hours	No	<200	0.76 - 0.92	1	2	111	27	28	55	M
6	Nephropathy indeterminate	1.2	6 months 3 days	No	<200	0.67 - 0.72	1	2	200	14	32	28	M
7	Berger's disease	1.2	10.5 years	No	<200	0.75 - 0.85	1	1	380	15	29	68	M

331  
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**Supplementary Table S2. Patient characteristics.**

TSB: Tuberos sclerosis of Bourneville; ADPKA: Autosomal Dominant Polycystic Kidney Disease; IR: Resistance Index; SRT: Systolic Rise Time; MPGN: Membranoproliferative glomerulonephritis.

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