1 **Protocol for Imaging and 3D reconstruction of bilateral kidney reflux**
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3 Level of expertise needed:
4 A: Basic
5 B: Moderate
6 C: Advanced
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8 1. Anesthetize female mouse (5 min). Expertise: A
9   - Place female mouse in induction chamber with 2.5% of inhaled Isoflurane with
10   2.5 L/min of O_2 for 5 minutes or until well sedated/anesthetized. Monitor heart
11   rate, respiratory rate and paw pinch reflex.
12 2. Remove fur on the lower back adjacent to the kidneys and on the lower abdomen
13   adjacent to the bladder (5 min). Expertise: A
14   - Apply and lather in a pea size amount of hair removal lotion over the lower back
15   (to eventually visualize the kidneys) and lower ventral abdomen (to eventually
16   visualize the bladder). Let stand for 1 minute and gently remove fur with gauze.
17 3. Catheterize the urethra using PE10 tubing (7 min). Expertise: B
18   - Under dissecting microscope, use fine forceps to gently introduce a lubricated
19   end of PE10 tubing approximately 1cm through the mouse urethra into the
20   bladder (being carful not to perforate the bladder or to create a false tract).
21   - Securely tape the catheter to the procedure table to avoid movement out of the
22   bladder.
23 4. Ultrasound measurement of kidney lengths (10 min). Expertise: C
24   - Place ultrasound gel on the back of the mice (over areas in which fur was
25   removed) being carful to introduce no air into the gel.
26   - Use 710B ultrasound probe with the VEVO 770 machine in the fundamental
27   mode to visualize the kidneys and measure and record the distance between
28   upper and lower poles as well as the absolute position of each upper and lower
29   pole (for proper positioning of the 3D imaging to follow).
30   - Place the ultrasound probe over the approximate midpoint of the kidneys.
31 5. Initialize the 3D imaging mode (5 minutes). Expertise: C
32   - While still in fundamental mode, select the 3D imaging option and initialize the
33   3D motor.
34   - Set the upper and lower limits of the field to be scanned (use the "lowest" lower
35   pole and "highest" upper pole between the two kidneys).
36   - Set the number of frames to be acquired at each step or level (50 frames per
37   level).
38   - Set the step size distance between levels (100µm)
39   - Select subharmonic mode.
40 6. Begin 3D scanning (10-12 min). Expertise: C
41   - Start acquiring the images.
42   - Simultaneously inject microbubbles (30µl bolus and 10µl/min thereafter until
43   150 µl total volume).
44   - Scan starts at the lower poles and finishes at the upper poles.
45 7. Confirm correct bladder catheter placement (5 min). Expertise: B
46   - After scan is completed, carefully turn mice to a supine position.
47   - Change to fundamental mode and image the bladder.
48   - Change to subharmonic mode to visualize microbubbles in the bladder
49   (confirming correct catheter placement).
50 8. Perform 3D rendering of acquired images (15 min). Expertise: C

51   •   Select "render" using Vevo 770 3D software to generate a 3D image
52   (automatically aligns and stacks the layers rendering the 3D image). An editing
53   cube also automatically appears around the entire scanned region allowing for
54   measurements as outlined below.
55   •   Rotate the image and select "measurement" to generate long axis, wide axis, and
56   depth axis measurements within each renal pelvis (Supplemental Figure).
57   •   To generate a volume of bubbles within the renal pelvis, use editing arrows
58   within the cube to "cut" into and visualize individual layers. Trace around the
59   outer boundary of the renal pelvis in each layer (Supplemental Figure). The
60   program will then align the layers summate the measurements into a total volume
61   of the renal pelvis (Supplemental Figure).