Video Article

Phase Contrast Magnetic Resonance Imaging in the Rat Common Carotid Artery

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Abstract

Phase contrast magnetic resonance imaging (PC-MRI) is a noninvasive approach that can quantify flow-related parameters such as blood flow. Previous studies have shown that abnormal blood flow may be associated with systemic vascular risk. Thus, PC-MRI can facilitate the translation of data obtained from animal models of cardiovascular diseases to pertinent clinical investigations. In this report, we describe the procedure for measuring blood flow in the common carotid artery (CCA) of rats using cine-gated PC-MRI and discuss relevant analysis methods. This procedure can be performed in a live, anesthetized animal and does not require euthanasia after the procedure. The proposed scanning parameters yield repeatable measurements for blood flow, indicating excellent reproducibility of the results. The PC-MRI procedure described in this article can be used for pharmacological testing, pathophysiological assessment, and cerebral hemodynamics evaluation.

Video Link

The video component of this article can be found at https://www.jove.com/video/57304/

Introduction

Magnetic resonance imaging (MRI) is a versatile approach that provides detailed information on internal body structures and physiology, and is increasingly being used for clinical diagnosis and in preclinical animal studies. Animal models are vital for better understanding of considerable clinical implication [1]. As animal models considerably differ from humans with respect to anesthesia requirements and physiological parameters, optimization of MRI procedures for such animals assumes importance.

Phase contrast MRI (PC-MRI) is a specialized type of MRI that uses the velocity of flowing spins to quantify flow-related parameters such as blood flow. With PC-MRI, mapping flow patterns in the major arteries using animal models can help shed light on cardiovascular pathologies [2]. Moreover, PC-MRI can non-invasively monitor the inherent alternations in blood flow in pathophysiological conditions [3]. These observations suggest that PC-MRI is a valuable approach that can be used in animal models of human cardiovascular diseases.

In this report, we describe a method for the quantification of blood flow in the common carotid artery (CCA) of rats. The two CCAs supply the head and neck with oxygenated blood, and the carotid artery disease is a major cause of stroke. Therefore, detecting the early pathology in the CCA is pivotal. This procedure has a duration of approximately 15 min and can be potentially applied to conditions with hemodynamics alterations, such atherosclerosis or stroke.

Protocol

The Institutional Care and Use Committees (IACUC) of the China Medical University approved all procedures.

1. Animal Preparation and Monitoring

1. Leave all magnetically susceptible objects such as wallets, keys, credit card, *etc.* outside the scanner room prior to commencing animal preparation for MRI scanning.
2. Initially anesthetize the rat (2-month-old male Sprague-Dawley (SD) rat, 280–350 g) in an induction box using a mixture of 5% isoflurane (ISO) and oxygen (2 L/min) for 3–5 min, as necessary.
3. When the animal is recumbent and exhibits no response to a tail or toe pinch, discontinue ISO administration and transfer the animal to the scanning room.
4. Place the rat in the MRI bed in a head-first prone position, and deliver 2-3% ISO through a nose cone device for maintaining anesthesia.
5. Monitor respiration by placing a respiratory pillow sensor under the animal's torso.
6. Connect the sensor to a respiratory system and check for respiration rate between 40-50 beats per minute (bpm).

7. For cine-gated PC-MRI acquisition, place one electrode each on the right forepaw and the left hind paw, respectively (**Figure 1a**).
8. Twist the electrocardiography (ECG) cables together.
9. Use a head holder with ear bars and a bite bar to secure the animal to restrict head movement.
10. Use a warm air heating system or gauze pads to maintain body temperature while in the magnet.
11. Ensure that the R-wave is clear on the ECG monitor (**Figure 1b**), and place the animal in the scanner. There is no need to place the surface coil on the top of the animal's neck as images are acquired by the volume coil.

2. MRI Acquisition

1. Use 2–3% ISO to maintain anesthesia during the entire imaging procedure. Continuously monitor physiological responses and keep as constant as possible.
2. Start the MRI scans once the animal is placed inside the scanner and continues to be physiologically stable. In this study, use a 7 T small animal MRI system with a gradient strength of 630 mT/m, but other field strengths of small animal MRI systems can be used.
3. Select the *"Localizer"* sequence from the console monitor of the MRI scanner and acquire scout images along all three orientations using any fast image acquisition sequence, *e.g.*, the fast spin echo, to create coronal, axial, and sagittal images. The purpose of these scout images is to determine the imaging planes.
4. Ensure that the center of the animal's head and neck is at the center of the magnet. If necessary, adjust the animal's position until the correct position is reached. If the animal is repositioned, repeat scanning to obtain scout images.
5. Select the *"Time-of-flight (TOF) angiogram"* sequence from the console monitor of the MRI scanner and acquire a 2D TOF angiogram first to ascertain the precise anatomical location of the CCA. Use the following scanning parameters: repetition time (TR)/ echo time (TE)=22/4.87 ms, flip angle = 90°, field of view (FOV) = 40 × 40 mm^2, matrix size = 256 × 256, slice thickness 0.6 mm, with the number of excitation (NEX) = 1.
   NOTE: The name of the TOF sequence could be vender-specific. The user can insert these parameters in the console monitor.
6. Ensure that the saturation band is "on" and is placed on the top to avoid interference from venous signals.
   NOTE: For the saturation band, it usually comes with the TOF sequence. If the saturation band does not show on the monitor, please notify the service person.
7. After locating the CCA using the TOF angiogram, target the image plane of the PC-MRI to the center of the CCA and orient such that the slice is perpendicular to the direction of blood flow (**Figure 2a**).
8. Ensure that both respiration and ECG gating are connected to the MRI system, showing the clear signal on the monitor computer (**Figure 1b**), and set the trigger module to be "on" in the *"Trigger Mode"* from the console monitor of the MRI scanner.
9. Confirm that the animal's physiological responses are stable before initiating the PC-MRI scan from the monitoring computer (**Figure 1b**). Verify that gating selections are "on" in both the monitor computer and the console monitor of MRI scanner.
   Note: The physiology monitoring system used in this study is provided by the vender. For most animal scanners, the similar physiology monitoring systems are provided and vender-specific.
10. Select the sequence of PC-MRI sequence from the console monitor of the MRI scanner and perform gated PC-MRI scans using the following parameters: TR/TE=15.55/4.51 ms (minimum TR and TE), flip angle=30°, FOV = 40 × 40 mm^2, matrix size = 192 × 192, slice thickness = 2 mm, velocity encoding (VENC) = 120 cm/s, with NEX=8. Unidirectional VENC is acquired in the through-plane direction.
    Note: The scan time is approximately 8.5 min, but the actual scan time may be slightly different among animals due to the variation in cardiac cycles.
11. Repeat steps 2.6–2.9 of image acquisition if the region of interest (ROI) is to be changed to another location in the CCA, such as at the bifurcation [4].
12. Remove the animal from the scanner and return it to its recovery cage when the scan is complete.
13. Warm the animal with a heating lamp to maintain body temperature. Keep the lamp at least 15 cm away from the animal to prevent overheating.
14. When the animal starts to move and exhibits a response to a tail or toe pinch, turn off the heating lamp.

3. Data Processing

1. Save MRI data in Digital Imaging and Communications in Medicine (DICOM) format or any other vender-specific format. Generate cine series with two types of images: a magnitude image (anatomy image) and a phase image (**Figure 2b**).
   NOTE: In some scanners, the third type of image, which could be the magnitude image × phase image or the complex-difference (the complex subtraction between the two acquisitions with different velocity-encoding gradients), is generated. The third image is vendor-dependent.
2. Pre-process the image data. Covert the phase image into the velocity map and correct the phase-offset error [5].
   NOTE: The phase image has an arbitrary MR unit of signal intensity instead of true velocity values, but the MR signal intensity is linearly proportional to velocity. The maximum MRI signal from the phase image is typically assigned as the value of VENC, and the minimum signal is assigned the opposite value of VENC. See supplemental code file 1 for an example of the Matlab script and press the button of "*Run*".
3. Delineate the ROI carefully by tracing the boundary of the CCA. As the artery may dilate and construct during the various cardiac phases, delineate ROIs for each time frame. Calculate the blood flow by integrating over the artery ROI, *i.e.*, velocity × area. The resulted blood flow of each artery was in the units of mL/s. See supplemental code file 2 for an example of the Matlab script and press the button of "*Run*".

Representative Results

Correct slice geometry is pivotal for ensuring the success of the PC-MRI experiment. The accurate image plane positioning yields a "round" artery shape (**Figure 3a**), and as angulation increases, *i.e.*, when it is less perpendicular to the artery, the resulting artery geometry becomes ovoid, leading to larger partial volume effects (**Figure 3b**). Severe partial volume effects would lead to the overestimation of blood flow [6,7]. Therefore, we advocate re-positioning of the image plane if the artery shape is ovoid.

Intra-scan reproducibility of time course changes in blood flow within one cardiac cycle from a representative rat is displayed in **Figures 4**. As can be seen, blood flow reaches its maximum during the systolic phase and returns to baseline during the diastolic phase for both sections. **Figure 5a** and **5b** show a Bland-Altman plot and a scatter plot, respectively, between two blood measurements in the same session, demonstrating a good correlation between measurements (R^2=0.7, P < 0.001). With the proposed scanning parameters, blood flow achieves repeatable measurements, demonstrating excellent reproducibility of the results. This characteristic could prove beneficial in testing pharmacological effects on major arteries [8,9].

As PC-MRI is a noninvasive approach to measure blood flow, it can be advantageous in protocols that require longitudinal monitoring. **Figure 6** displays the time course for one cardiac cycle in an animal scanned at ages 2 and 4 months and shows that blood flow in the CCA is significantly age-dependent, suggesting rapid development in rats. These quantitative assessments of blood flow are essential for a better understanding of the circulatory system and may, therefore, become a potentially useful tool in preclinical studies on stroke and atherosclerosis.

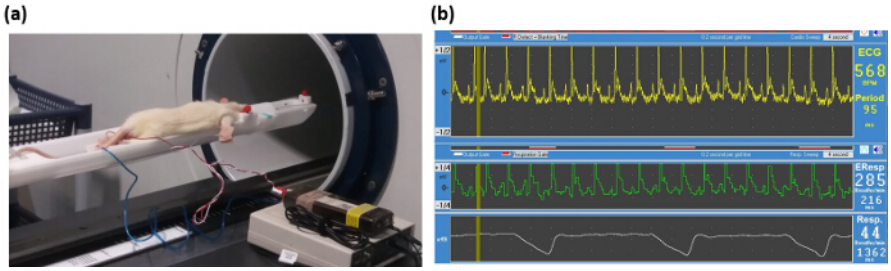

Figure 1: Animal monitoring. (**a**) ECG electrodes are placed on the right forepaw and the left hind paw, and the respiratory pillow sensor is placed under the animal's torso. (**b**) The ECG and respiratory signals are clearly visible on the monitor. Please click here to view a larger version of this figure.

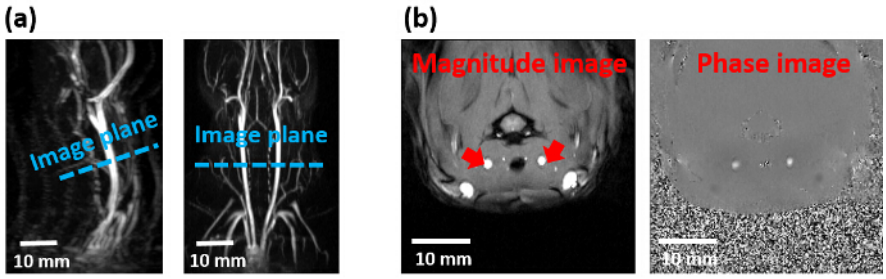

Figure 2: Illustration of the position of PC-MRI scans and representative images. (**a**) Slice positioning on the reconstructed sagittal and coronal views from the TOF angiogram. The blue line indicates image plane at the level of the midpoint of the CCA. (**b**) Magnitude and phase images from one time-frame of the cine-series images from a representative animal. Red arrows indicate CCA location. Please click here to view a larger version of this figure.

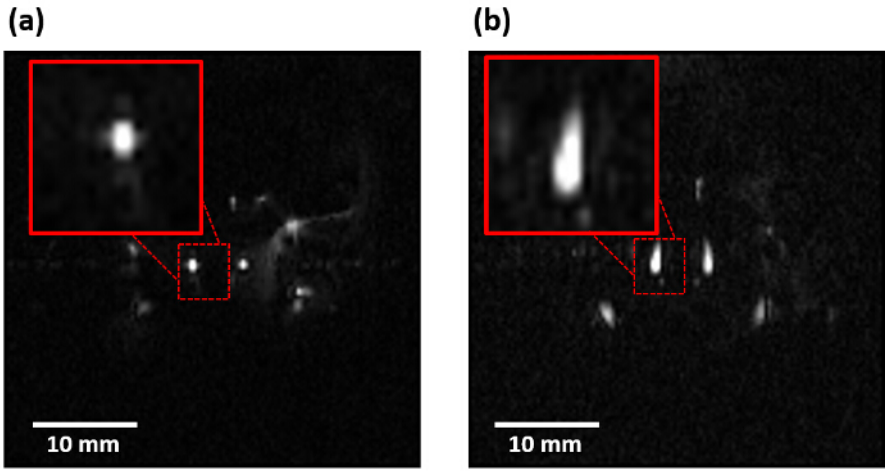

Figure 3: Representative magnitude images. (**a**) Imaging plane is perpendicular to the artery and (**b**) imaging plane is non-perpendicular to the artery. The shape of artery changes from round to ovoid if the imaging plane is not perpendicular to the artery. The area containing the CCA is amplified in the red box. Please click here to view a larger version of this figure.

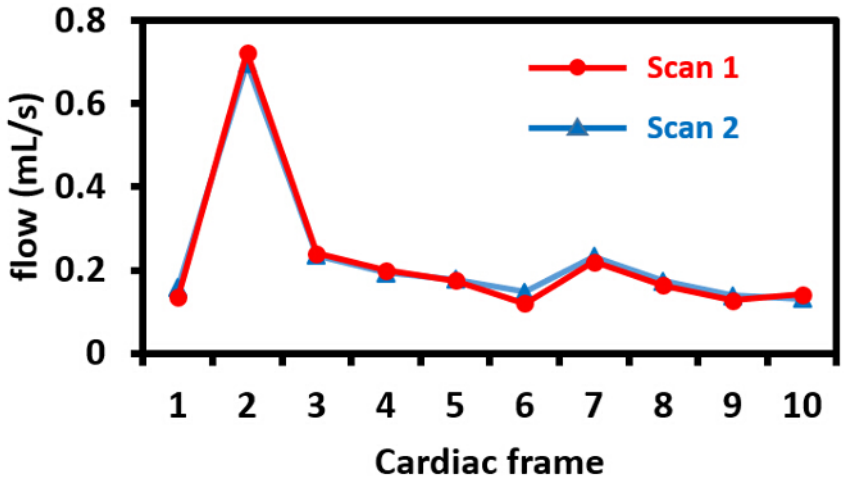

Figure 4: Intra-scan test of blood flow from a representative rat. Please click here to view a larger version of this figure.

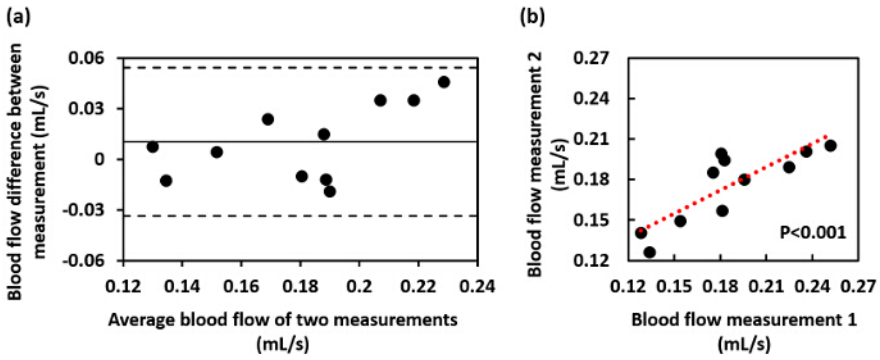

Figure 5: Intra-section reproducibility of CCA blood flow measurements. (**a**) Bland-Altman plot comparing two blood flow measurements acquired between sections. The solid line represents the mean difference between two measurements while dashed lines depict the 95% confidence interval. (**b**) Scatter plot of the two blood flow measurements. Please click here to view a larger version of this figure.

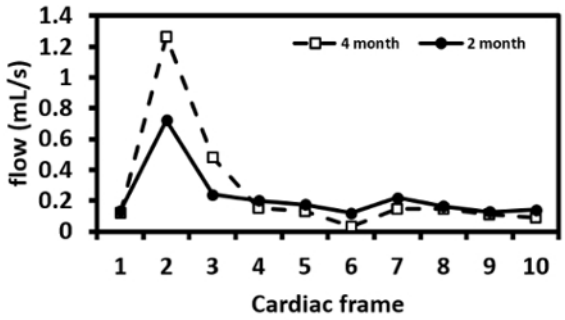

Figure 6: Longitudinal scan in a 2-month and 4-month old animal showing age-dependent changes in blood flow in the CCA.

Discussion

PC-MRI is a comprehensive approach for non-invasive and longitudinal evaluation of blood flow. We present a protocol for performing PC-MRI of the rat CCA. This procedure is easy to perform in any animal MRI scanner and demonstrates good reproducibility.

The PC-MRI technique has gained increasing popularity in human [10,11] as well as animal studies [4,12]. Among these studies, results of Peng *et al.*[4] is of particular interest due to the similarity of their approach, but the major difference in the current work is the use of the spatial resolution of 0.21 mm, compared with that of 0.31 mm in the aforementioned report. The limited spatial resolution significantly reduces the scan time, but the resultant partial volume effect may bias the flow quantification, especially for smaller vessels [6,7]. Since measurement accuracy is on the list of priorities, a spatial resolution of 0.21 mm with longer scanning time is suggested in future animal studies.

Non-gated PC-MRI is being used as an alternate method of blood flow measurement in many human studies due to its considerably lesser scanning time [6,13,14,15]. However, non-gated PC-MRI is not suggested in animals used for pre-clinical testing as the heart rate of rats can be as

high as 400 bpm, leading to the fast alternation between systolic and diastolic phases. Non-gated PC-MRI may miss crucial information during the systolic phase, resulting in relatively lower flow values and higher variations [7]; therefore, it can only be used for arriving at rough estimations in animals used for pre-clinical testing.

Many scanning parameters are linked to precise quantifications for PC-MRI data, and VENC is one of them. VENC underestimation causes phase aliasing [16] but a higher VENC value will lead to deterioration in image quality [17]. We used a VENC value of 120 cm/s, which is appropriate for normal adult SD rats. When changes in vascular tone are expected, such as different species [4], the VENC value should be optimized such that better images or assessments can be acquired.

Particular attention should be paid to important steps of the protocol to obtain a reliable result. First, to avoid resonant circuits and MRI resonant frequency corruption by the ECG signal, it is suggested to twist the ECG cables together. Second, the majority of small animal MRI scanners incorporate a circulating warm water circuit to maintain the animal's body temperature while in the magnet. However, the flowing water introduces noise and, therefore, interferes with the ECG signal. Thus, in this gated PC-MRI study, we suggest using a warm air heating system or gauze pads instead of using the warm water circulation system to improve the gating quality.

It should be noted that, in this work, only a 2D PC-MRI sequence was employed for data acquisition. The basic technique of cine-gated 2D PC-MRI has emerged as a promising tool for blood flow quantification due to the advantages of reduced scanning time and easy to be implemented in standard scanners. However, data acquired by the 2D PC-MRI technique is limited due to the lack of volumetric acquisitions and reliable streamline tracking, thereby missing some important information such as turbulent flow. Time-resolved 3D PC-MRI pulse sequences with more state-of-the-art techniques such as accelerated cine PC-MRI with compressed sensing and parallel imaging [18,19] should be implemented in future rat CCA experiments. This improvement will enable the provision of insights into spatial aspects of both velocity distribution and flow structures. Nevertheless, the animal preparation and monitoring protocols presented in this report are still applicable in these 4D PC-MRI techniques.

In conclusion, we demonstrate a simple and reliable procedure that measures blood flow in the rat CCA using non-invasive PC-MRI. Further applications of this imaging method include testing of pharmacological effects, pathophysiological assessment, and evaluation of cerebral hemodynamics.

Disclosures

There is nothing to disclose.

Acknowledgements

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References

1. Zakszewski, E., Schmit, B., Kurpad, S., & Budde, M. D. Diffusion imaging in the rat cervical spinal cord. *J Vis Exp.* (98) (2015).
2. Wise, R. G., Al-Shafei, A. I., Carpenter, T. A., Hall, L. D., & Huang, C. L. Simultaneous measurement of blood and myocardial velocity in the rat heart by phase contrast MRI using sparse q-space sampling. *J Magn Reson Imaging.* **22** (5), 614-627 (2005).
3. Skardal, K., Espe, E. K., Zhang, L., Aronsen, J. M., & Sjaastad, I. Three-Directional Evaluation of Mitral Flow in the Rat Heart by Phase-Contrast Cardiovascular Magnetic Resonance. *PLoS One.* **11** (3), e0150536 (2016).
4. Peng, S. L. *et al.* Phase-contrast magnetic resonance imaging for the evaluation of wall shear stress in the common carotid artery of a spontaneously hypertensive rat model at 7T: Location-specific change, regional distribution along the vascular circumference, and reproducibility analysis. *Magn Reson Imaging.* **34** (5), 624-631 (2016).
5. Yu, H. Y., Peng, H. H., Wang, J. L., Wen, C. Y., & Tseng, W. Y. Quantification of the pulse wave velocity of the descending aorta using axial velocity profiles from phase-contrast magnetic resonance imaging. *Magn Reson Med.* **56** (4), 876-883 (2006).
6. Peng, S. L. *et al.* Optimization of phase-contrast MRI for the quantification of whole-brain cerebral blood flow. *J Magn Reson Imaging.* **42** (4), 1126-1133 (2015).
7. Peng, S. L., Shih, C. T., Huang, C. W., Chiu, S. C., & Shen, W. C. Optimized analysis of blood flow and wall shear stress in the common carotid artery of rat model by phase-contrast MRI. *Sci Rep.* **7** (1), 5253 (2017).
8. Bozgeyik, Z., Berilgen, S., Ozdemir, H., Tekatas, A., & Ogur, E. Evaluation of the effects of sildenafil citrate (viagra) on vertebral artery blood flow in patients with vertebro-basilar insufficiency. *Korean J Radiol.* **9** (6), 477-480 (2008).
9. Swampillai, J., Rakebrandt, F., Morris, K., Jones, C. J., & Fraser, A. G. Acute effects of caffeine and tobacco on arterial function and wave travel. *Eur J Clin Invest.* **36** (12), 844-849 (2006).
10. Neff, K. W., Horn, P., Schmiedek, P., Duber, C., & Dinter, D. J. 2D cine phase-contrast MRI for volume flow evaluation of the brain-supplying circulation in moyamoya disease. *AJR Am J Roentgenol.* **187** (1), W107-115 (2006).
11. Stalder, A. F. *et al.* Quantitative 2D and 3D phase contrast MRI: optimized analysis of blood flow and vessel wall parameters. *Magn Reson Med.* **60** (5), 1218-1231 (2008).
12. Dall'Armellina, E. *et al.* Improved method for quantification of regional cardiac function in mice using phase-contrast MRI. *Magn Reson Med.* **67** (2), 541-551 (2012).
13. Peng, S. L., Ravi, H., Sheng, M., Thomas, B. P., & Lu, H. Searching for a truly "iso-metabolic" gas challenge in physiological MRI. *J Cereb Blood Flow Metab.* **37** (2), 715-725 (2017).
14. Liu, P. *et al.* Quantitative assessment of global cerebral metabolic rate of oxygen (CMRO2) in neonates using MRI. *NMR Biomed.* **27** (3), 332-340 (2014).
15. Xu, F., Ge, Y., & Lu, H. Noninvasive quantification of whole-brain cerebral metabolic rate of oxygen (CMRO2) by MRI. *Magn Reson Med.* **62** (1), 141-148 (2009).

16. Lotz, J., Meier, C., Leppert, A., & Galanski, M. Cardiovascular flow measurement with phase-contrast MR imaging: basic facts and implementation. *Radiographics.* **22** (3), 651-671 (2002).
17. Pelc, N. J., Herfkens, R. J., Shimakawa, A., & Enzmann, D. R. Phase contrast cine magnetic resonance imaging. *Magn Reson Q.* **7** (4), 229-254 (1991).
18. Kim, D. *et al.* Accelerated phase-contrast cine MRI using k-t SPARSE-SENSE. *Magn Reson Med.* **67** (4), 1054-1064 (2012).
19. Valvano, G. *et al.* Accelerating 4D flow MRI by exploiting low-rank matrix structure and hadamard sparsity. *Magn Reson Med.* **78** (4), 1330-1341 (2017).

