**Supplementary material**

**Creation of 3-dimensional myocardial flow reserve images**

Parametric PET rest and stress volumetric images, as created from ‘Carimas v2.9’, were further processed with a software package ‘PET Image’ partially developed in-house in IDL version 8.3 (Exelis Visual Information Solutions, Berkshire, UK). The purpose was to establish a volumetric image, containing the calculated myocardial flow reserve (MFR) for later fusion with CT-images. The Carimas v2.9 software package only displays the polar map of MFR values, whereas a volumetric model is not available.

In Carimas v2.9, we used an identical contouring mask for the analysis of both rest and stress studies, whenever possible. In cases where identical mask were not appropriate between the two studies, we created the masks to fit each study separately.

In the software ‘PET Image’, images of the rest and stress studies were manually checked for misalignment (Figure S1a) as this would result in seriously impaired images when calculating the MFR by dividing the stress image by the rest image (Figure S1b). In fact, MFR was calculated by applying the equation:

$$MFR=\frac{Stress}{Rest+0.0001}$$

The addition of ‘0.0001’ in the denominator was applied to ascertain values above 0. The resulting MFR value, which physiologically ranges 2-4, would not be affected by such a small inaccuracy in the denominator.

The alignment of the two studies was carried out semi-automatically by a build-in iterative algorithm applying an image resolution of 128\*128\*128. Only rigid movement in the x, y and z-axes was allowed, not rotation. For the program not to take voxel values into account in the alignment, we converted the rest and stress studies to dichotomous images, each study containing only the values zero and one. This was done by truncating each voxel value above 0.000001 to the value ‘1’ (Figure S2).

Though images were aligned, a perfect match of the studies was not always possible. Therefore edge effects similar to that demonstrated in Figure S1d still occurred as seen from Figure S3, though appearing less devastating after alignment of the studies.

To avoid these edge effects, the dichotomous masks from Figure S2 were used for ‘cutting of the non-overlapping edges’. Before calculating the MFR, the volumetric *stress* image was multiplied by the dichotomous mask from the *rest* image, as to ‘cut off’ non-overlapping volumes of the studies. Similarly, the volumetric *rest* image was multiplied by the *stress* dichotomous mask (Figure S4).

Finally, the rest and the stress study had a perfect overlay and the volumetric MFR image without edge effects was generated (Figure S5).

{Figure S1}

**Figure S1 Checking images for misalignment** The rest study (a) and the stress study (b). A combined view (c) indicated that rest study (upper right and lower left) was misaligned with the stress study (upper left and lower right), which resulted in hampering ‘edge effects’ when the myocardial flow reserve was calculated (d). Notice the extreme supra physiological values of MFR, colored in dense red, as a result of dividing by denominator values close to zero.

 {Figure S2}

**Figure S2 Truncated images for semi-automatized registering** Rest images (greyscale, upper right and lower left part of images) and stress images (also greyscale, upper left and lower right part of images), before (a) and after (b) the automatized alignment was performed.

 {Figure S3}

**Figure S3 Myocardial flow reserve after alignment** Still supra physiological values occurred at the edges due to non-overlapping volumes of the rest and stress studies, from which it was calculated.

 {Figure S4}

**Figure S4 Images were cut to only include overlapping volumes** Rest (a) and stress (b) images, ready for myocardial flow reserve calculation after multiplying by the dichotomous mask from the opposite examination.

 {Figure S5}

**Figure S5 Final volumetric image of myocardial flow reserve** Without the edge effects and ready for fusion with CT.

**Supplementary tables**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Component | Factor level | Estimate | 95% CI | P-value |
| Observer | 1 – reference |  |  |  |
|  | 2 | -0.007 | -0.017;0.004 | 0.22 |
| Measurement | 1 – reference |  |  |  |
|  | 2 | -0.005 | -0.015;0.005 | 0.31 |
| Constant |  | 1.09 | 1.02;1.17 | <0.001 |
| Patient variance |  | 0.050 | 0.029;0.086 |  |
| Vascular territory variance |  | 0.021 | 0.012;0.039 |  |
| Segment variance |  | 0.035 | 0.021;0.059 |  |
| Residual variance |  | 0.005 | 0.003;0.007 |  |

***Table S1*** *Variance components analysis of segment-based rest measurements. CI, confidence interval.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Component | Factor level | Estimate | 95% CI | P-value |
| Observer | 1 – reference |  |  |  |
|  | 2 | 0.030 | -0.030;0.089 | 0.33 |
| Measurement  | 1 – reference |  |  |  |
|  | 2 | -0.024 | -0.068;0.020 | 0.29 |
| Constant |  | 2.75 | 2.48;3.00 | <0.001 |
| Patient variance |  | 0.750 | 0.543;1.037 |  |
| Vascular territory variance |  | 0.143 | 0.087;0.234 |  |
| Segment variance |  | 0.257 | 0.212;0.313 |  |
| Residual variance |  | 0.071 | 0.055;0.092 |  |

***Table S2*** *Variance components analysis of segment-based stress measurements. CI, confidence interval.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Component | Factor level | Estimate | 95% CI | P-value |
| Reader | 1 – reference |  |  |  |
|  | 2 | 0.045 | -0.015;0.104 | 0.14 |
| Measurement | 1 – reference |  |  |  |
|  | 2 | -0.018 | -0.055;0.020 | 0.36 |
| Constant |  | 2.65 | 2.36;2.95 | <0.001 |
| Patient variance |  | 0.934 | 0.614;1.421 |  |
| Vascular territory variance |  | 0.160 | 0.094;0.272 |  |
| Segment variance |  | 0.247 | 0.176;0.347 |  |
| Residual variance |  | 0.088 | 0.063;0.122 |  |

***Table S3*** *Variance components analysis of segment-based myocardial flow reserve measurements. CI, confidence interval.*