Automated myocardial perfusion from coronary X-ray angiography

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ABSTRACT

The purpose of our study is the evaluation of an algorithm to determine the physiological relevance of a coronary lesion as seen in a coronary angiogram. The aim is to extract as much as possible information from a standard coronary angiogram to decide if an abnormality, percentage of stenosis, as seen in the angiogram, results in physiological impairment of the blood supply of the region nourished by the coronary artery. Coronary angiography, still the golden standard, is used to determine the cause of angina pectoris based on the demonstration of an important stenose in a coronary artery. Dimensions of a lesion such as length and percentage of narrowing can at present easily be calculated by using an automatic computer algorithm such as Quantitative Coronary Angiography (QCA) techniques resulting in just anatomical information ignoring the physiological relevance of the lesion. In our study we analyze myocardial perfusion images in standard coronary angiograms in rest and in artificial hyperemic phases, using a drug *e.g.* papaverine intracoronary. Setting a Region of Interest (ROI) in the angiogram without overlying major vessels makes it possible to calculate contrast differences as a function of time, so called time-density curves, in the basal and hyperemic phases. In minimizing motion artifacts, end diastolic images are selected ECG based in basal and hyperemic phase in an identical ROI in the same angiographic projection. The development of new algorithms for calculating differences in blood supply in the region as set are presented together with the results of a small clinical case study using the standard angiographic procedure.

Keywords: Quantitative Coronary Angiography, myocardial perfusion, Computer Aided Diagnosis

1. INTRODUCTION

Coronary angiography is up till now the most important modality to diagnose coronary abnormalities particularly stenoses causing the clinical syndrome angina pectoris. See Fig. 1 for an example of a right coronary angiogram (RCA). In clinical cardiology the estimation of the percentage stenosis in a coronary artery is in the standard clinical practice still mostly based on visual assessment leading to large inter and intra observer variability in reading coronary arteriograms .[1–3] Image analysis using computer assistance does result in a more consistent judgment, however, this approach is mainly based upon static geometric parameters such as length of the stenosis and percentage of diameter and area reduction of a single segment in the stenosed artery.[4,5] In 70-90% of the coronary angiograms there are stenoses to justify the diagnosis angina pectoris, however, in the 10-30% of the coronary angiograms there are slight or no abnormalities at all in the epicardial vessels although the patients do have real anginal complaints.[6] This group of patients may benefit from additional diagnostic procedures demonstrating disturbances in the micro circulation of the myocardium. These disturbances are mostly caused by the reduction of blood supply based on atherosclerotic changes of the epicardial coronary vessels but not always. In that case secondary to specific heart diseases are affecting the microcirculation such as hypertension and/or diabetes. However, if the disturbances are not secondary to a known clinical syndrome, the syndrome is called syndrome X. The microcirculation of the myocardium is responsible for optimizing the functional capacity of the myocardial cells. The extent of myocardial perfusion to nourish the myocardial cells is regulated on demand and activated by local and general factors: exercise, stress and many others. The imbalance of demand and supply causes complaints such angina pectoris or heart failure.

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The anatomical basis of the microcirculation consists of a proximal and distal compartment in which the prearterioles in the proximal compartment play an important role in optimizing the perfusion pressure in the arterioles. These regulation is the combined action of neural (sympathetic), humoral and local vasoactive factors. In contrast the arterioles are sparsely innervated resulting in a regulation mostly depending on local factors in particular the oxygen concentration. That is why these arterioles play a very important role in the regulation of blood flow in the myocardium to ensure equilibrium in supply of nutrients and the washout of waste products. Through this mechanism the coronary flow is regulated in proportion to the need of the myocardium for oxygen,

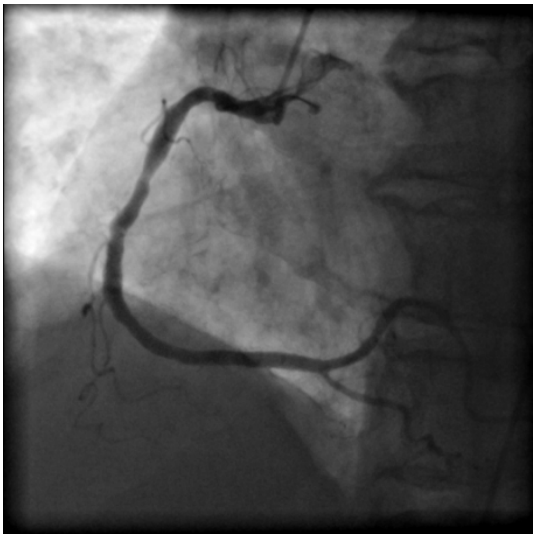


Figure 1. Example of an angiogram of a right coronary artery in right anterior oblique view, the apparent stenosis is (visually) estimated to be 50%. In standard practice this is considered too less for interventional follow-up. However, in case the arterioles are maximally dilated, the stenosis can become flow limiting.

which is closely related to the delivered amount of cardiac work. In the absence of diseased epicardial vessels there is hardly any resistance to the flow in these vessels in contrast to the micro vascular component. So the microvascular vessels determine the amount of blood the myocardium will get in a factor three to six times the basal amount depending on the demand. This is called Coronary Flow Reserve (CFR).[7,8] Coronary flow reserve depends mostly on the possibility of the arterioles to dilate. Micro vascular dysfunction is mostly a combination of factors: vascular based vessel wall thickening or abnormal reaction on regulating substances, and myocardial such as myocardial wall thickening in hypertension. In case of syndrome X there is no conclusive anatomical substrate in the coronary angiograms to explain a reduced CFR. However, ultrasound studies show a high percentage of vessel wall abnormalities causing a reduced release of vasoactive hormones leading to a decrease in vasodilatation of the arterioles. Diagnostic tests are necessary when multi angulated angiograms, to reduce the chance missing asymmetric lesions, give no critical lumen changes explaining the complaints of the clinical syndrome angina pectoris. Spasm of the epicardial vessels should be excluded by provocative *e.g.* ergonovine intracoronary during angiography. When both procedures are negative micro vascular dysfunction has to be excluded by measuring coronary flow reserve. The CFR is measured by pharmacologic intervention to get maximal vasodilatation, in humans three compounds are used, i.e. intravenous dipyridamole or adenosine or intracoronary papaverine or adenosine. There are several methods to measure the CFR: invasively or non-invasively. The non-invasive methods are mostly using radioactive materials (nuclear medicine), the invasive techniques consist of mostly expensive catheters for example Doppler catheters to measure flow difference in the epicardial vessels in basal conditions and after pharmacologic interventions. Doppler flow measurement has an extra problem because of epicardial vessel dilatation resulting often in a change of position causing a change of the angle used as cosines in the flow calculations. Quantitative angiography is to our opinion a useful method to evaluate the extent of possible dilatation of the arterioles after pharmacologic intervention as substitute of exertion in an easy way without extra catheters or procedures in connection of a normal standard coronary

angiography. This paper is organized as follows. In the next section we describe our approach in detail. Then we describe the data and present results. We finalize with a discussion and conclusions.

2. METHODS

2.1 CFR

The relation between aortic root pressure and coronary flow is quite complex and has been studied by many authors and this has resulted in a variety of approaches and models.[9] Consensus has been established that the vasodilation of the arterioles regulates the flow through the myocard. The visual or computer assisted assessment of the severity of a stenosis, such as visible in Fig. 1, influences the flow only if its resistance is of the same order of magnitude as the resistance by the myocard. Therefore, a more functional, physiological measure is to be preferred over geometric parameters characterizing the severity of a stenosis.[10] The functional CFR measurement provides information about the perfusion of the heart muscle.[18] In a Region-of-Interest (ROI) in the angiogram (without an overlying major blood vessel) the contrast is measured as a function of time (the so-called time-density curve). With CFR the measured local maximum contrast density represents the vascular volume and the measured local contrast arrival time is inversely proportional to flow.[11] The required hyperemic state of exercise can be induced artificially by the intracoronary injection of a vasodilator drug *e.g.* papaverine. In this hyperemic state, contrary to the basal state, the arterioles are maximally dilated, thus the normal increase of the blood flow to the physical limits is set by the sizes of the epicardial coronary arteries and especially the partially occluded segments. The CFR can be visualized in a functional image with the color coded image values proportional to the increase in blood flow in the pertinent part of the heart muscle. Areas with less blood flow increase do show up dark, no essential difference in comparison with the basal state, indicating effects of impaired blood flow due to stenosed segments in the coronary arteries and possible (partial) infarction. Although good results with the CFR method have been reported,[12–14] in clinical practice the procedure is demanding, especially the correction of the background contributions is difficult because of the dynamics of the contracting heart. The requirement is that the two image sequences, basal and hyperemic, should be registered exactly the same. However, movements due to patient respiration cannot be eliminated completely. In order to avoid misregistration artifacts, pacing of the heart together with ECG triggered contrast injection and image acquisition did appear to be mandatory. As the measurement of the absolute CFR is difficult,[15] alternatives such as the Relative Coronary Flow Reserve (RCFR)[10] and the Fractional Flow Reserve (FFR)[16] have been developed. Dual energy subtraction[17] has been proposed to overcome the motion artifacts. The main advantage of the FFR method is that all measurements are made during maximum arteriolar vasodilatation. On the other hand the method is not that easy incorporated in daily clinical routine because this method is time consuming and expensive due to the extra required catheter for the intra-coronial flow measurement. In this paper we present in the next section a less demanding approach which is less time-consuming and less demanding in procedure.

2.2 Model

In this section we model the integration over a ROI positioned on the myocard in the angiogram. We assume that within the ROI there are no overlying major bloodvessels. The integration *i.e.* the summation of the (log of the) pixel intensities inside the ROI together with the angiogram being a radiographic projection, provides for a 3D volume measurement of the amount of contrast agent contained in the myocard. We assume with neglect of possible diffusion that the injected contrast completely replaces the blood. The myocard can be considered to be a reservoir which holds temporarily the contrast agent for a certain mean transit time $\tau_{transit}$. Let $P(x, y, t)$ denote the pixel intensities at time t as a function of the two spatial coordinates x and y. The angiogram $P(x, y, t)$ is a projection along the z-axis of the 3D distribution of contrast agent which is a function of the spatial coordinates x, y, z and t. The result of the integration is proportional to the concentration contrast material under the ROI. A schematic diagram of the myocard model is shown in Fig. 2, the inflow of contrast at the arterial side is $I_{in}(t)$, the outflow $I_{out}(t)$ is at the venal side. The result of the integration over the reservoir is given by the contrast measurement $M(t)$:

$$M(t) = \begin{cases} 0, & \text{if } t \leq \tau_{inject} \\ \int\int_{ROI} P(x, y, t) dx dy, & \text{if } t > \tau_{inject} \end{cases} \quad (1)$$

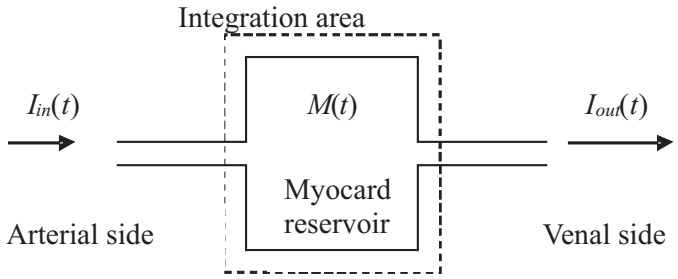


Figure 2. Schematic diagram of the ROI integration resulting in the contrast measurement $M(t)$ in the myocard reservoir.

Conservation of mass leads to the relation between the measured contrast and blood flow:

$$\frac{d}{dt}M(t) = I_{in}(t) - I_{out}(t) \tag{2}$$

After a certain time τ_{peak} the contrast concentration saturates as the whole contrast bolus is contained in the myocard. The contrast concentration starts to diminish after the mean transit time $\tau_t ransit$. An idealized contrast curve is shown in Fig. 3. According to (2), the rising slope of the $M(t)$ curve of Fig. 3 is proportional

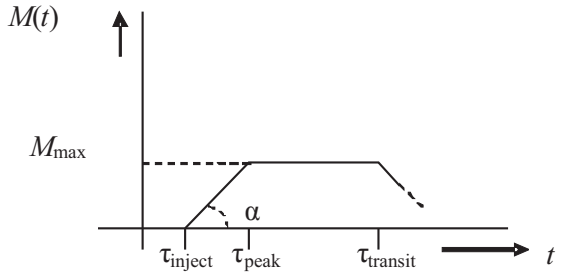


Figure 3. The idealized contrast $M(t)$ and the describing parameters. Of special interest is the slope α as it is related to flow.

to flow. From first principles, we obtain the following solution to the differential equation (2):

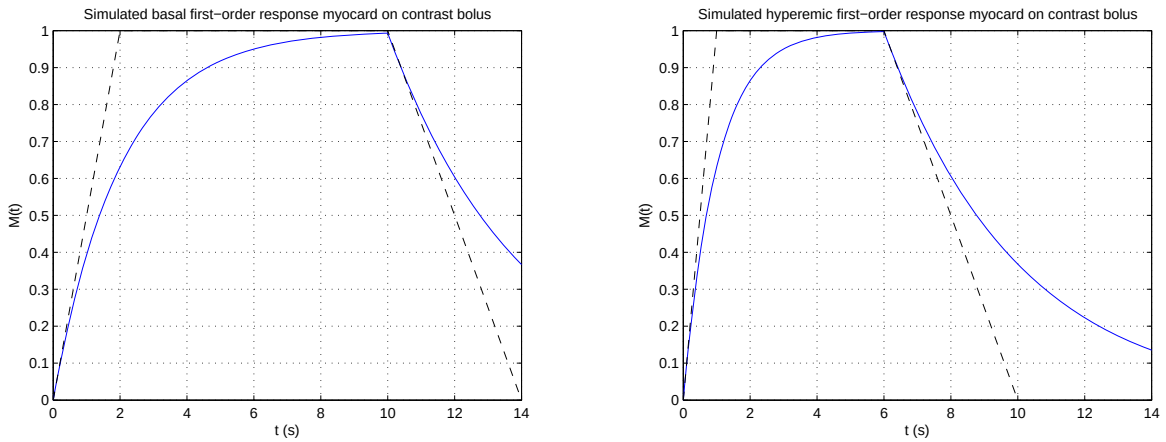
$$M(t) = \left(1 - exp(-\frac{t}{\tau_{rise}})\right) u(t) - \left(1 - exp(-\frac{t - \tau_{transit}}{\tau_{decay}})\right) u(t - \tau_{transit}) \tag{3}$$

with $u(t)$ the unit step function. In Fig. 10 we show two simulated first-order responses of the myocard to a contrast bolus injection.

In practice, we compare the (ratio) of the rising slope in basal and hyperemic conditions. There is a small time - offset between the moment of injection and the start of the image sequence. Therefore, we have to synchronize the two runs with respect to each other.

2.3 Image Handling

The images analyzed in this paper are acquired with an Axiom Artis dFC single plane C-arm system with a dynamic flat detector for cardiology from Siemens Medical Solutions. The frame rate is 15 frames / s. During the acquisition, the ECG is recorded simultaneously. In this paper we have chosen for the vascular bed of the right coronary artery for reasons of less overlying larger bloodvessels, see *e.g.* Fig. 1. The method we propose consists of the following steps: The basal and the hyperemic runs are typically acquired at the end of the patient study. The hyperemic state is evoked by intracoronary injection of 8 mg papaverine and flushed with contrast



(a) basal state (b) hyperemic state

Figure 4. Simulated first-order responses of the myocard on a contrast bolus, basal (a) state with parameters $\tau_{rise} = 2$ s, $\tau_{transit} = 10$ s and $\tau_{decay} = 4$ s;and hyperemic (b) state with parameters $\tau_{rise} = 1$ s, $\tau_{transit} = 6$ s and $\tau_{decay} = 4$ s.

agent. Overall 6F catheters are used. The image data is archived on CD. With a computer script[19] the Dicom files are converted to 8 bit 512×512 bitmap format for further processing by Matlab. The converted Dicom files are read into Matlab together with the ECG data. From the ECG data the R-peaks are automatically detected and the end-diastolic images are selected in each heartbeat. For this we select the image at *e.g.* 80% of the R-R interval after the previous R-peak. See Fig. 10 for an example of an ECG signal with detected R-peaks and the selected diastolic images. Our first image is slightly before the second R - peak after the start of the run. In this way we obtain a set of in the order of 10 - 15 images for the basal and the hyperemic flow situation. See Fig. 10 for an example of selected images corresponding to Fig. 10. Fig. 10 shows the ECG signal of the consecutive hyperemic run, together with the detected R-peaks and the selected end-diastolic images. Fig. 10 displays these hyperemic images.

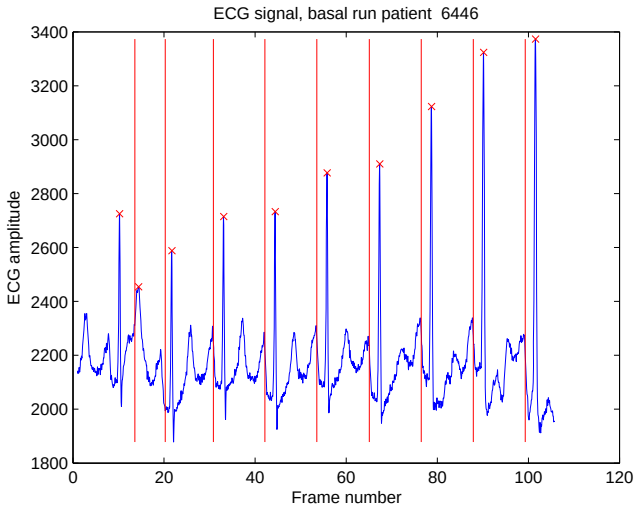


Figure 5. Example of a patient ECG signal, indicated are the detected R-peaks and the selected diastolic images.

Because we are going to compare the two runs with respect to slope of the time-density curve, we must ensure the same contrast flow situation to start with in both runs. There is a variable time-offset between the start of the image acquisition and the manual injection of the contrast material. Coronary flow is primarily during

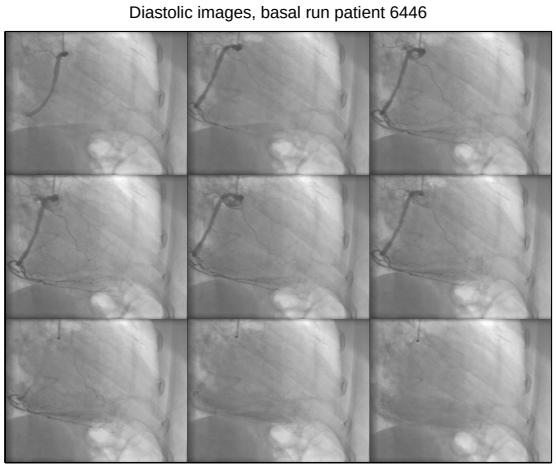


Figure 6. The selected basal diastolic images corresponding to Fig. 5.

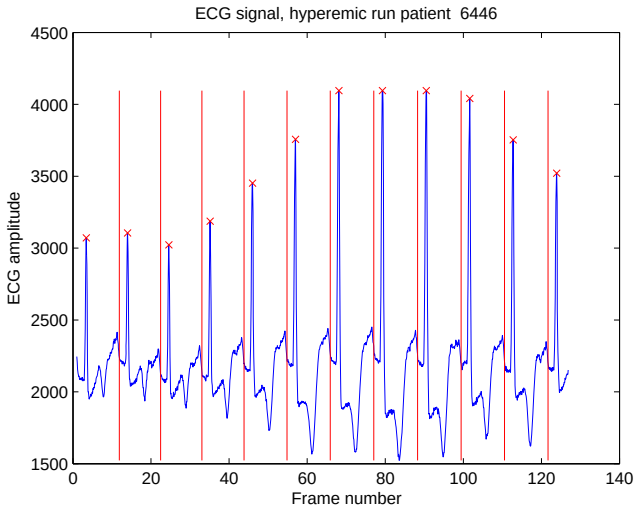


Figure 7. The ECG signal of the hyperemic run, indicated are the detected R-peaks and the selected diastolic images.

diastole, so the first images with visible contrast are not necessarily in the same relative heart cycle in the two runs. We then can either manually select a polygon shaped ROI or have the pertinent myocard automatically detected and select the ROI accordingly. For the selection an image is chosen in the middle of the sequence. This is done because both the larger vessels and the myocard are visible and we can manually place the ROI such to avoid to overlay the larger vessels. Fig. 9 shows the manual selected ROIs at end - diastolic phase of 18 patients.

2.4 Automated ROI selection

The automated ROI selection is preferably based on an image near the end of the sequence where the myocard is visible. The image segmentation can be simplified by logarithmic subtraction of a background so-called mask image, *i.e.* an image of the same ECG phase selection, acquired before appearing contrast. In our case applying a threshold to the image histogram after mask subtraction appeared to be sufficient for identifying the ROI. In this way the ROI does not need to be contiguous, major blood vessels are automatically excluded, if the heart beat is regular. Figure 10a shows an enhanced log-subtracted image of patient 6446 from the basal run,

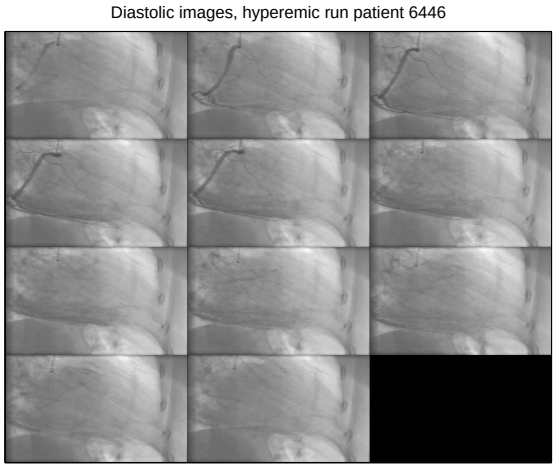
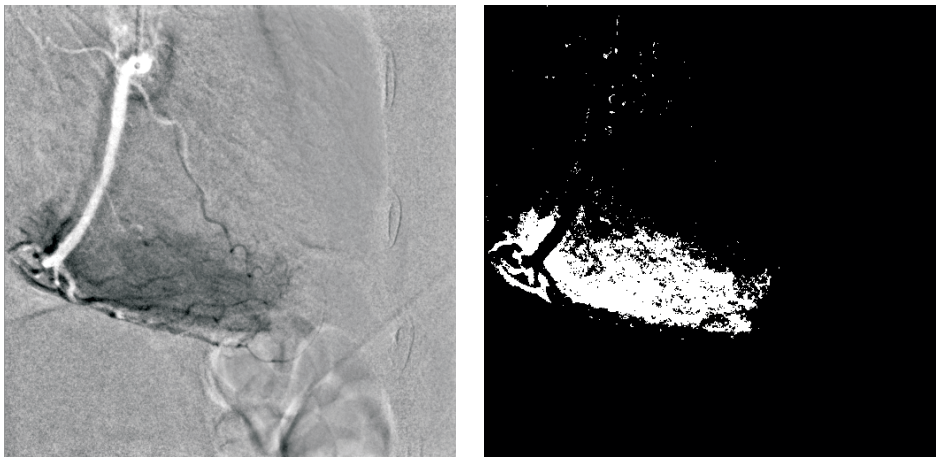


Figure 8. The selected hyperemic diastolic images corresponding to Fig. 7.

Figure 9. Manual selected end - diastolic myocardial ROIs of 18 patients.

the coloring of the myocard is clearly visible, leading to the ROI shown in Fig. 10(b). The advantage is that in such an automatic selected ROI only the projected myocardial muscle is taken into account without larger and medium size bloodvessels. On the other hand, the contrast from the vessels is only a minor contribution to the integration over the ROI. The intensity is integrated over the ROI for each of the images at the same ECG phase. Also from the hyperemic run the selected images are measured with the same ROI. The contrast is computed as function over time from both runs, normalized through division by the area of the applied ROI



(a) enhanced log - subtracted image (b) resulting binary ROI

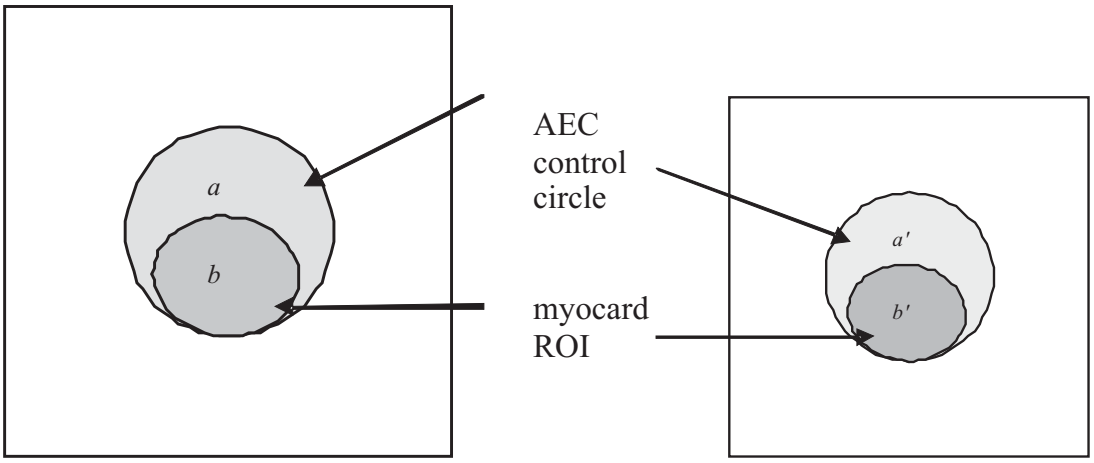
Figure 10. On the left (a) an enhanced log - subtracted image. *i.e.* image 76 - 19 of the basal run of patient 6446. On the right (b) the resulting binary ROI based on histogram thresholding.

and the comparison between normal and hyperemic run can be made. As the final step, we have to take into account the Automatic Exposure Control (AEC) of the diagnostic X-ray system.

2.5 AEC correction

The function of the AEC of a diagnostic X-ray system is to ensure the proper exposure for the consecutive image acquisition. This system component is of crucial importance for obtaining good image quality by setting appropriate values for kVp and mA and s. In the cardio application the X-ray exposures are typically pulsed with duration of 5 - 8 ms at 15 frames / s. There is a wide variation in patient mass and size, the AEC ensures about the same image intensity for each patient. As our purpose is to measure the response of the myocard over time and not the response of the AEC on the changed contrast, we have set up measurements to identify the AEC behavior.[21] The ideal AEC for quantitative measurements would keep the exposure factors constant after the start of the run. We were not able to manually set or overrule the AEC. On the other hand, it was our purpose to keep the standard protocol. Not much literature about AEC in general could be found and also the information from the vendor was not conclusive. From our experiments[21] we conclude that the AEC control loop is steered by the average image intensity over a circle inside the image matrix of 50% diameter. Fortunately the kVp setting appeared not to change; only the mA was adapted in our case with only small contrast dynamics. The appearing image contrast strongly depends on kVp; for changing kVp the quantitative measurements become more complicated. We now have three situations:

1. 1. If the selected myocard ROI is totally outside the AEC control circle, the exposures are not adjusted and the myocard response is adequately measured. No correction is needed.
2. 2. If the selected myocard ROI is completely inside the AEC control circle, the system adapts the mA in order to keep the average image intensity constant. The contrast within the myocard ROI with respect to the background intensity inside the AEC control circle is increased this way and we compensate for this effect by a weighting factor. The measured myocard intensity contribution is weighted by the ratio of the average intensity inside the AEC control circle excluding the myocard ROI region before contrast injection and after contrast injection, see Fig. 11.
3. 3. If the selected myocard ROI is partly inside and partly outside the AEC control circle, we have only to compensate the contribution from the part inside the circle.



(a) AEC circle a (b) AEC circle b

Figure 11. On the left (a) the image matrix with the AEC control circle before contrast injection, average image intensity in the myocard ROI equal to a, average image intensity over the AEC circle excluding the myocard ROI equal to b. On the right (b) the image matrix with the AEC control circle after contrast injection, average image intensity in the myocard ROI equal to $a^{'}$, average image intensity over the AEC circle excluding the myocard ROI equal to $b^{'}$. Weighting factor to compensate the measured $b^{'}$ equals $a/a^{'}$.

3. RESULTS

We have performed the analysis described in the previous section on patient 6446. Fig. 12 shows the obtained results for the case of a manually selected ROI. The results with an automated ROI selection are similar. There is hardly any difference is response between basal and hyperemic flow. This indicates that there is no CFR,

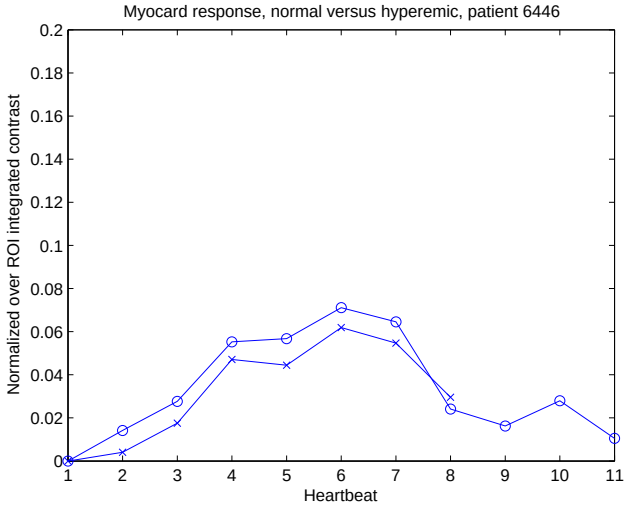
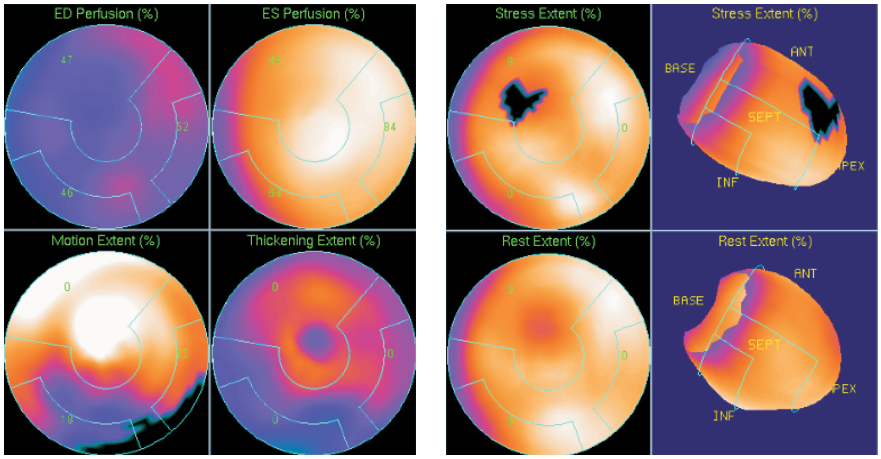


Figure 12. The basal ROI integrated time-density indicated with $'x'$ and the hyperemic ROI integrated time-density indicated with $'o'$. There is no difference in myocard response between basal and hyperemic run as function over time, indication no CFR.

apparently the preaterioles are already maximally dilated also in the basal situation. In this case the RCA can be flow limiting and possible should be taken care off. Of patient 6446 also a nuclear medicine SPECT myocard perfusion scan was made. The rest image is shown in Fig. 13(a) and the corresponding exercise image is shown in Fig. 13(b). The nuclear medicine rest study shows up quite normal; however, the stress study reveals a small



(a) (b)

Figure 13. On the left the result of the rest study with 527 MBq Tc-99m of patient 6446; on the right the result of the stress study with 530 MBq Tc-99m.

ischemic area near the anterior septum. The angiographic findings including the extra papaverine image sequence of the right dominant patient 6446 are confirmed by the nuclear medicine study.

4. CONCLUSIONS

Our purpose is in the automated evaluation of the patho-physiological relevance of lesions, stenoses, in the coronary arteries as seen in an angiogram. We aim to extract as much as possible quantitative information about the physiological condition of the heart from standard angiographic image sequences. Coronary angiography is able to reveal the location and the projected diameter and length of the lesion. There is no information about the clinical, pathophysiological consequences of the stenosis. In this paper our aim is to assess the clinical significance of a lesion. We therefore analyze the myocardial perfusion as revealed in standard angiographic image sequences. In[20]20 we have presented the first results of our approach. In the present paper we report a case study about a patient that has been catheterized following the standard protocol and from whom also a scintigraphic perfusion scan was made. The angiographic image sequences consist of 8 bit images with a resolution of 512×512 pixels at a frame rate of 15 images / s. The frame lengths are somewhat longer than usual in order to include the registration of the perfusion. Our approach is simple and can be highly automated, is less time-consuming, less difficult and cheap as compared with other methods, above all there are no extra hazards for the patient.

Time density curves are obtained over the myocardium separated from the overlying major coronary vessels in case a ROI was manually placed. Automatic ROI selection appears also feasible; almost all bloodvessels can be excluded from the ROI. Because of the motion of the coronary arteries and the local myocardium we use the ECG signal to select images at the same (diastolic) phase.

The results depend to some extend on the X-ray exposure control dynamics, *i.e.* we want to measure the response of the vascular bed in the myocardium and not the response of the X-ray exposure control. Preferably we acquire the images with a fixed kVp and mA during the acquisition of the image sequence. This appeared not to be possible, and we have realized a compensation scheme. Because we compare the two flow situations basal and hyperemic which we measure identically, our approach to obtain the relative CFR is rather robust. The obtained relative perfusion images from the case study show no CFR, a conclusion which is confirmed by the nuclear medicine study. This holds promise for clinical application. In the past we have studied myocardial perfusion with CFR methods from the literature such as for example.[12] These methods are demanding with respect to the procedure in the catheterization laboratories. We now use the standard protocol with slightly longer image sequences, only one or two runs, with comparable results. Though the proposed method looks promising, further clinical evaluation with a larger patient series, supported by in vitro experiments, is needed for further improvement and validation.

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REFERENCES

[1] K.M. Detre, E. Wright, M.L. Murphy, T. Takaro, "Observer agreement in evaluating coronary angiograms," Circulation 52(6), 979-986 (1975).

[2] L.M. Zir, S.W. Miller, R.E. Dinsmore, J.P. Gilbert, J.W. Hawthorne, "Interobserver variability in coronary angiography," Circulation 53(4), 627-632 (1976).

[3] E.J. Topol, S.E. Nissen, "Our preoccupation with coronary luminology," Circulation 92(8), 2333-2342 (1995).

[4] J.H.C. Reiber, P.W. Serruys, C.J. Kooijman, W. Wijns, C.J. Slager, J.J. Gerbrands, J.C.H. Schuurbiers, A. den Boer, P.G. Hugenholtz, " Assessment of short-, medium-, and long-term variations in arterial dimensions from computer-assisted quantitation of coronary cineangiograms," Circulation 71(2), 280-288 (1985).

[5] J.H.C. Reiber, P.W. Serruys, *Quantitative Coronary Arteriography*, Kluwer Academic Publishers, 1991.

[6] R.O. Cannon, P.G. Camici, S.E. Epstein, "Pathophysiological dilemma of syndrome X," Circulation 85(3), 883-892 (1992).

[7] R.L. Kirkeeide, K.L. Gould, L. Parsel, "Assessment of coronary stenoses by myocardial perfusion imaging during pharmacologic coronary vasodilation. VII. Validation of coronary flow reserve as a single integrated functional measure of stenosis severity reflecting all its geometric dimensions," J. Am. Coll. Cardiol. 7(1), 103-113 (1986).

[8] K.L. Gould, R.L. Kirkeeide, M. Buchi, "Coronary flow reserve as a physiologic measure of stenosis severity," J. Am. Coll. Cardiol. 15(2), 459-474 (1990).

[9] F.J. Klocke, R.E. Mates, J.M. Canty, A.K. Ellis, "Coronary pressure-flow relationships, controversial issues and probable implications," Circ. Res. 56(3), 310-323 (1985).

[10] K.L. Gould, K. Lipscomb, "Effects of coronary stenoses on coronary flow reserve and resistance," Am. J. Cardiol. 34, 48-55 (1974).

[11] J.T. Cusma, E.J. Toggart, J.D. Folts, W.W. Peppler, N.J. Hangiandreou, C.S. Lee, C.A. Mistretta, "Digital subtraction angiographic imaging of coronary flow reserve," Circulation 75(2), 461-472 (1987).

[12] R.A. Vogel, "The radiologic assessment of coronary blood flow parameters," Circulation 72(3), 460-465 (1985).

[13] V. Legrand, G.B. Mancini, E.R. Bates, J.M. Hodgson, M.D. Gross, R.A. Vogel, "Comparative study of coronary flow reserve, coronary anatomy and results of radionuclide exercise tests in patients with coronary artery disease," J.Am. Coll. Cardiol. 8(5), 1022-1032 (1986).

[14] G.B. Mancini, S.B. Simon, M.J. McGillem, M.T. LeFree, H.Z. Friedman, R.A. Vogel, " Automated quantitative coronary arteriography: morphologic and physiologic validation in vivo of a rapid angiographic method," Circulation 75(2), 452-460 (1987).

[15] M.J. Kern, A. Lerman, J. -W. Bech, B. de Bruyne, E. Eeckhout, W.F. Fearon, S.T. Higano, M.J. Lim, M. Meuwissen, J.J. Piek, N.H.J. Pijls, M. Siebes, J.A.E. Spaan, "Physiological assessment of coronary artery disease in the cardiac catheterization laboratory: a scientific statement from the American heart association committee on diagnostic and interventional cardiac catheterization, council on clinical cardiology," Circulation 114(12), 1321-1341 (2006).

[16] N.H.J. Pijls, B. van Gelder, P. van der Voort, K. Peels, F.A.L.E. Bracke, H.J.R.M. Bonnier, M.I.H. El Gamal, "Fractional flow reserve: a useful index to evaluate the influence of an epicardial coronary stenosis on myocardial blood flow," Circulation 92(11), 3182-3193 (1995).

[17] S. Molloi, A. Ersahin, J. Tang, J. Hicks, C.Y. Leung, "Quantification of volumetric coronary blood flow with dual energy subtraction angiography," Circulation 93(10), 1919-1927 (1996).

[18] M. Schrijver, *Angiographic image analysis to assess the severity of coronary stenoses*, PhD thesis, University of Twente, The Netherlands, 2002.

[19] G.A. ten Brinke, *Quantitative analysis of angiographic sequences*, MSc thesis, University of Twente, The Netherlands, 2005.

[20] C.J. Storm, C.H. Slump, "CAD of myocardial perfusion," Proc. SPIE Medial Imaging: Computer-Aided Diagnosis, vol. 6513, pp. 65132D, 2007.

[21] C.J. Storm, C.H. Slump, "Estimation of X-ray parameters in digital coronary angiography for compensation of myocardial perfusion measurement," Proc. SPIE Medical Imaging: Physics of Medical Imaging, vol. 6913, pp. 6913L-11, 2008.