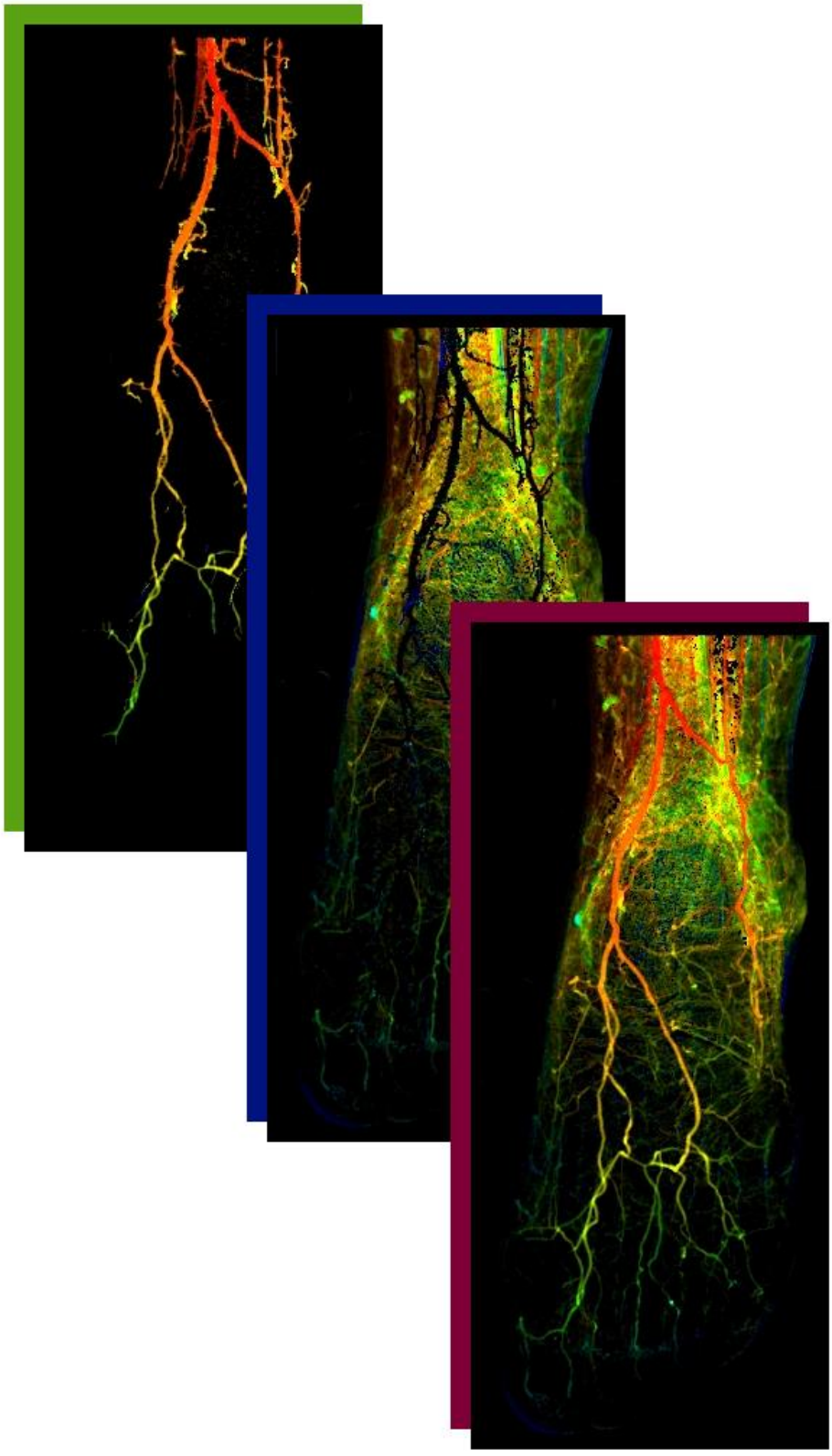
2D perfusion angiography in patients with critical limb ischemia



A study into reproducibility, variability and vessel extraction

Master thesis

2D perfusion angiography in patients with critical limb ischemia

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Master thesis of:
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Summary

Critical limb ischemia (CLI) is a serious condition caused by end stage peripheral arterial disease in which the viability of the limb is at risk. Endovascular therapy has become the treatment of choice, because of its minimal invasive nature and high technical success rate. The aim of revascularization is to increase oxygen supply to the wound so that tissue perfusion, oxygen and nutrient levels become sufficiently high to enable wound healing. Unfortunately, the success of endovascular revascularizations is largely unpredictable, as demonstrated by the high rates of delayed wound healing and repeated interventions. Therefore, an objective method to assess the adequacy of revascularization and subsequent tissue perfusion following endovascular treatment is needed.

2D perfusion angiography (2DPA) is a post processing technique which uses digital subtraction angiography (DSA) images to quantify density changes caused by propagation of contrast medium. Within a certain observer determined region of interest (ROI), pre- and post-interventional density change over time can be visualized as time density curves (TDC). The currently available literature supports the feasibility of 2DPA in CLI patients, because it enables quantification and comparison of pre- and post-interventional perfusion results. Several important aspects need to be researched further to enable and optimize clinical use of 2DPA, among others research into reproducibility, separation of the macro- and microcirculation to enable assessment of tissue perfusion alone, and reduction of the influence of motion artifacts. This thesis aims to study the first two, namely reproducibility and vessel separation.

Because literature is lacking on reproducibility and observer agreement, both are studied in this thesis. In eleven patients, reproducibility was investigated by comparison of two perfusion acquisitions, which were obtained under equal patient and acquisition settings. Initial reproducibility was only 64%. Reproducibility of three out four unreproducible TDC pairs was achieved by removal of the frames before contrast arrival, and inclusion of the same number of frames. This resulted in a reproducibility of 91%. Optimal analyzing conditions and the effect on reproducibility need to be researched further.

To determine observer variability, two ROIs were researched, one including the complete foot with exclusion of the digits, and one limited to the wound area. For intra-observer variability, one observer drew each ROI five times in each of the ten included acquisitions. For inter-observer variability, two observers drew each ROI in twenty acquisitions. Both analyses showed excellent agreement for the ROI including the foot. Variability of the ROI including the wound area was high, because interpretation of the wound area and location appeared to differ within and between observers. Therefore, using the ROI including the foot is preferred over the ROI limited to the wound area.

To improve tissue perfusion assessment using the ROI of the whole foot, a method to extract the arteries from perfusion images was presented. Successful separation resulted in a typical microvascular and arterial perfusion curve, which was demonstrated by two clinical cases. Clinical validation studies must confirm the added value of this extraction method.

List of abbreviations

2DPA	2D perfusion angiography
ABI	Ankle-brachial index
AP	Anterior-posterior
ATA	Anterior tibial artery
ATP	Posterior tibial artery
AUC	Area under the curve
CTA	Computed tomography angiography
DSA	Digital subtraction angiography
eGFR	Estimated glomerular filtration rate
FWHM	Full whit at half maximum
ICC	Intra-class correlation coefficient
LAT	Lateral
LTT	Leg transit time
MRA	Magnetic resonance angiography
MTT	Mean transit time
NRMSE	Normalized root mean square error
PAD	Peripheral artery disease
PD	Peak density
PTA	Percutaneous transluminal angioplasty
REPEAT	Reproducibility and rEliability of Perfusion angiography and prEdiction of wound heAling in criTical limb ischemia
RMSE	Root mean square error
ROI	Region of interest
TDC	Time density curve
TOA	Time of arrival
TTP	Time to peak
VAS	Visual analogue scale
WIR	Wash-in rate

Table of contents

1

General introduction

Critical limb ischemia

Critical limb ischemia (CLI) is defined as chronic ischemic rest pain, and ulcers or gangrene caused by proven peripheral artery disease (PAD) in the limb.[1,2] Due to narrowing or occlusion of arteries, perfusion pressure decreases which results in an insufficient supply of oxygen and nutrients to the foot.[1] Oxygen and nutrient shortage causes among others endothelial dysfunction, disturbed hemorheology, decreased oxygenation and inflammation.[1] These effects result in rest pain and impaired wound healing[1,3].

Peripheral artery disease can be classified using different classification methods. Most commonly used are Fontaine and Rutherford classifications[4,5] (Table 1.1). Fontaine grade III and IV describe CLI.[5] The PAD lesion itself can be classified by TASC II criteria[6], see Appendix A.

Table 1.1 - Classification of peripheral arterial disease by Fontaine and Rutherford.[4,5]

Fontaine grade	Rutherford category	Clinical symptoms
I	**0**	Asymptomatic, incomplete vessel obstruction
IIa	**1**	Claudication at a distance > 200m
IIb	**2 – 3**	Claudication at a distance < 200m
III	**4**	Rest pain
IV	**5 – 6**	Necrosis and/or gangrene of the limb

The number of patients with CLI is increasing, due to among others increasing rates of diabetes mellitus and the aging population.[7–9] In a European population of 1 million, there are approximately 500 to 1000 new cases of CLI, every year.[1] Thereby, prognosis for survival and salvage of the limb of CLI patients is poor. After one year, 25% of CLI patients have died[1], mainly caused by a cardiac or cerebrovascular event[10]. In CLI patients unsuitable for revascularization, mortality rates increase to 40%.[11] After one year, 30% of the patients will have a major amputation and in only 25% of CLI patients CLI will be resolved.[1]

The main risk factors associated with the development of PAD are diabetes and smoking.[1,12] In addition, patients who continue smoking have a poorer outcome compared to patients who quit smoking with higher mortality rates and lower amputation-free survival.[13,14] Other important risk factors are male gender, age, race, hypertension, hypercholesterolemia and dyslipidemia[1,12].

Many CLI patients suffer from ischemic rest pain[1,15–17] reflected by a high mean visual analogue scale (VAS) pain score of 5.1[17] (there is no normative value for VAS[18]). Besides pain, patients suffer from physical disfunction.[1,15,16] The quality of life deteriorates with increasing Fontaine grades, caused by increased pain, immobility, and decreased ability of self-care.[15,16,19]

CLI is diagnosed by physical examination, functional measurement and imaging. Clinical presentation of foot ulcers and gangrene differs between wounds caused by arterial insufficiency and wounds caused by diabetes (neuropathic or ischemic, Table 1.2).[1] Functional measurement consists of ankle-branchial index (ABI, < 0.9)[1,12], ankle pressure (≤ 50-70 mmHg)[1,20], toe pressure (≤ 50 mmHg)[1,20], and transcutaneous oxygen pressure (≤ 30 mmHg)[1]. Imaging is used to diagnose PAD and to determine suitable treatment for the lesion. Angiography is considered the current gold standard to visualize arterial lesions.[1,12] Other available imaging methods are duplex ultrasonography, magnetic resonance angiography (MRA) and computed tomographic angiography (CTA)[1,12].

Table 1.2 - Symptoms of neuropathic and ischemic ulcers.[1]

Neuropathic ulcer	**Ischemic ulcer**
Painless	Painful
Normal pulses	Absent pulses
Loss of sensation and vibration	Variable sensory findings
Dry, warm foot	Cold foot
Red appearance	Pale, cyanotic appearance

Treatment of CLI

Treatment of CLI aims to relieve ischemic pain, heal (ischemic) ulcers and prevent amputation.[1,21] The main treatment options for CLI are revascularization, life-style changes, pharmacological treatment and amputation.[1,20] In combination with cardiovascular risk management, revascularization is the treatment of choice[1,20,22], because this may result in re-established blood inflow to the foot[1] and thus in restored perfusion of the tissue at risk. Cardiovascular risk reduction can consist of medical therapy with antiplatelets, β-blockers and statins, and risk factor modification, such as smoking cessation and lipid-lowering therapy.[23] Pharmacological treatment alone is more likely to be successful in asymptomatic PAD[1,24] and primary amputation is less desirable, but unfortunately necessary in some cases[1,20], mainly in patients with severe comorbidity or end stage arterial disease[1,20].

Revascularization consists of bypass surgery or endovascular revascularization.[11] In bypass surgery, blood is redirected through a graft; a prosthetic graft or an autologous vein. Whenever available, an autologous vein is preferred over a prosthetic graft, because of superior results.[25,26] In endovascular revascularization, inflow is re-established with balloon angioplasty, also known as percutaneous transluminal angioplasty (PTA). A deflated balloon is inserted and passed into the stenosis. Inflation of the balloon results in expansion of the lumen.

The choice of treatment in CLI patients is not easy and depends on different patient and procedure characteristics.[11] Short term results show a small preference for endovascular treatment, because of lower 30-day morbidity and lower 12-months reintervention rates.[27] Other studies found no short term differences in patient outcome between bypass surgery and endovascular revascularization.[28–32] However, after two years, bypass surgery is preferred over endovascular revascularization, because of higher amputation-free and overall survival.[33] In patients who have no comorbidities, a life expectancy greater than 2 years and availability of a usable vein, bypass surgery is preferred over endovascular revascularization.[11,33] However, most CLI patients have multiple comorbidities[1,27,31,34] and are therefore often unsuitable for bypass surgery[27,28,35]. Consequently, endovascular revascularization procedure rates have increased[36] and endovascular revascularization has more often become the first treatment of choice[37], especially in patients with below the knee lesions[38]. Therefore, this thesis focuses on endovascular revascularization.

Prediction of patient outcome

In case of unsatisfying endovascular treatment outcome, secondary amputation is often unavoidable. Most secondary amputations are required within the first months after treatment.[38–40] Insight in treatment success may prevent the need for secondary amputation, because additional therapy that potentially saves the limb can be started earlier. Therefore, treatment success needs to be determined prior to or as soon as possible after treatment, but at least within

weeks. Clinical improvement itself, especially wound healing, can take months to years. 12 months after revascularization, only 54% of CLI patients had complete wound healing[21] and only 43% of patients had clinical improvement to a level of Fontaine grade II[41].

Some authors advocate the application of the angiosome concept[42] to determine the wound related artery to guide their revascularization procedure[43,44]. The angiosome concept divides the foot in specific angiosomes (Figure 1.1). These are 3D tissue regions supplied by a specific artery.[42] The foot is divided into six angiosomes; three angiosomes are nourished by the posterior tibial artery (ATP), two by the peroneal artery and one by the anterior tibial artery (ATA).[45,46] However, available literature is inconclusive about treatment success by treatment of the wound related artery[47,48]. Moreover, the angiosome concept is developed in healthy subjects.[42] In case of vascular morbidities, the vascular anatomy adjusts to overcome the oxygen and nutrient deficiency, by the formation of collaterals and microcirculatory changes.[49,50] For that reason, the angiosome concept may not assign the correct artery as the wound related artery in patients suffering from CLI. Additionally, it can be impossible to revascularize the wound related artery (direct revascularization). Neither technically successful revascularization on DSA is a good outcome parameter, because any increase in blood flow to the foot will only induce clinical improvement when this increases tissue perfusion[51].

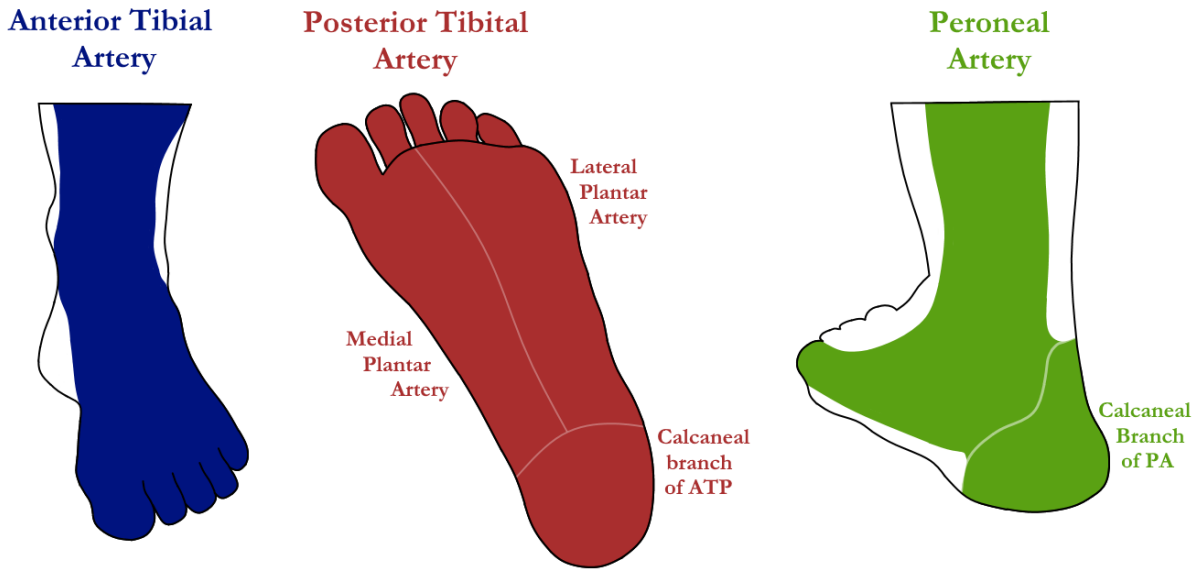


Figure 1.1 - Six angiosomes of the foot. Left: angiosomes nourished by the anterior tibial artery. Middle: angiosomes nourished by the posterior tibial artery (ATP). Right: angiosome nourished by the peroneal artery (PA).

Currently, the most important endpoint of angioplasty is wound blush on DSA images.[52] Wound blush is the contrast opaqueness of the vessels around the wound, obtained directly after endovascular revascularization.[53] Presence of wound blush after endovascular treatment is associated with higher rates of limb salvage.[53] However, wound blush is judged by the interventionist and is therefore a qualitative predictor of clinical outcome[49,53,54]. An objective tool to quantify wound blush might be two-dimensional perfusion angiography (2DPA).

2DPA is a post processing technique which uses digital subtraction angiography (DSA) images and allows quantitative analysis of changes in tissue perfusion following interventions. Perfusion-angiography is real-time and can therefore be performed directly in the interventional suite.

2D perfusion angiography

Description of technique

X-ray digital subtraction angiography has been used for decades to guide endovascular interventions. DSA has a high temporal and spatial resolution which enables excellent vascular imaging. By looking at the propagation of iodinated contrast medium during DSA acquisition, the interventionist obtains a qualitative measure of blood flow and subjective measure of blood velocity traveling through the vasculature. 2DPA is an automatic conversion of a standard DSA image using post processing software, which provides quantitative information of tissue perfusion by quantifying density changes within a certain operator determined region of interest (ROI). Pre- and post-intervention density changes over time can be visualized as time density curves (TDC) in the same graph[55]. Objective parameters such as contrast arrival time, wash-in rate, area under the curve, mean transit time and time to peak can be determined. In addition, every parameter can be visualized by a color-coded image (Figure 1.2) where each pixel represents its time-dependent value of the selected perfusion parameter.

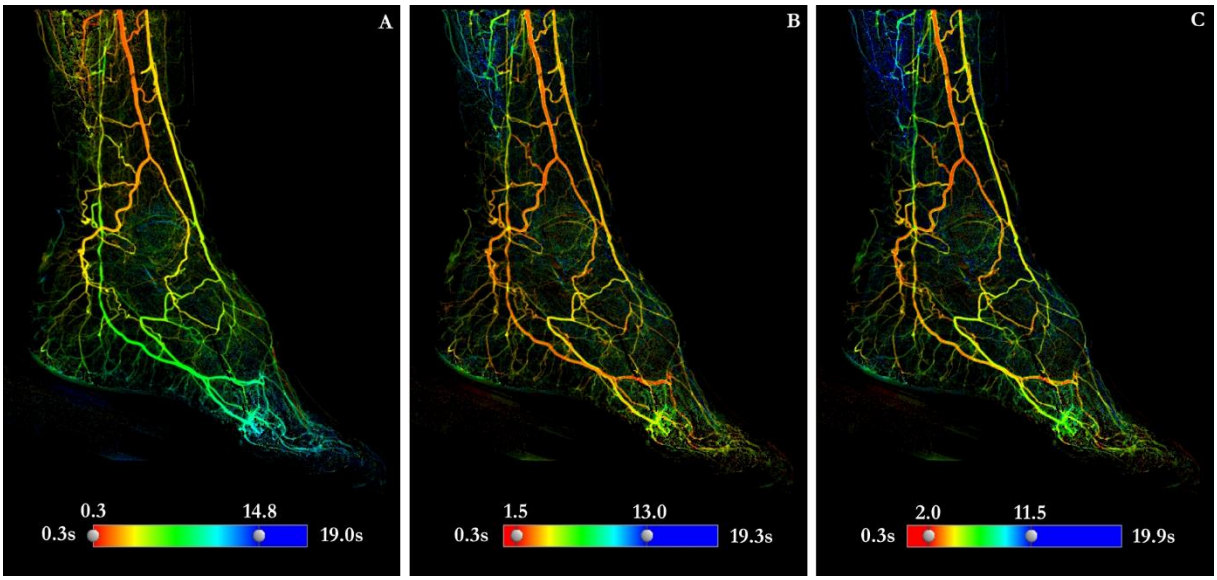


*Figure 1.2 – Color-coded perfusion images in which the color of the pixel represents the pixel's perfusion parameter value. Color-coded images of the **(A)** time of arrival, **(B)** time-to-peak, and **(C)** mean transit time.*

Image acquisition

2DPA can be performed with the standard imaging system already available in the interventional suite without the requirement of additional acquisitions. For example, the Azurion system (Philips Medical Systems, Best, The Netherlands) has a standard perfusion protocol available. In this protocol, kVp and mAs are kept constant within acquisitions to enable comparison of densities within the acquisition. Therefore, the technique is fast without the need for additional radiation or contrast is needed[55].

Post-processing of perfusion images

Perfusion images obtained by the Azurion system are analyzed with the 3D Interventional Work Spot (version R1.2.0, Philips Medical Systems, Best, The Netherlands). The first four frames are

used for image optimization and not used to compose the perfusion images or the time density curve. The TDC is determined from the selected ROI. Density values are averaged for all pixels within the ROI over all frames and visualized over time, resulting in the time density curve. The y-axis indicates density [-] and the x-axis time [s]. The TDC is a representation of blood flow in the ROI. The upward trend represents blood inflow and the downward trend blood outflow (Figure 1.3). A steep trend corresponds to fast in- or outflow and a gentle upward trend corresponds to slow in- or outflow.

Perfusion parameters

Several perfusion parameters can be derived from the TDC. In many studies, these parameters are insufficiently defined, which makes comparison impossible. Therefore, the following definitions are proposed to obtain standardization of perfusion parameters (Figure 1.3).

1. In patient changes of the time of arrival (TOA) give an indication of flow changes proximal to the ROI. The TOA [s] is the time between the first frame included in the TDC and the frame at which contrast is first detected.
2. Peak density (PD) gives an indication of the maximum blood volume in the ROI. The PD is defined as the density at the first frame that is followed by four subsequent frames with a density ≤101%:
   $$PD = y(t) \qquad \text{if: } y(t+n) \leq 1.01 \cdot y(t) \qquad [1]$$
   with y(t) the density at frame (t) with n = [1, 2, 3, 4].
3. The time to peak (TTP) indicates the time blood takes to travel into the tissue. TTP [s] is the time between the TOA and the time at which PD is achieved.
4. Wash-in rate (WIR) gives an indication of the blood flow rate in the ROI most likely dependent on the arterial inflow and peripheral resistance. The WIR [1/s] is the wash-in slope between two frames after TOA and two frames before PD is achieved.
5. To assess the time blood takes to travel through the tissue and into the capillaries, the difference between the wash-in and wash-out rate is used in previous studies[56]. However, contrast outflow is often partially acquired. Therefore, the full width at half maximum (FWHM) is proposed for travel time assessment. The FWHM [s] of the time density curve is assessed using the well-defined FWHM criterion.
6. The area under the curve (AUC) is an indicator of the total amount of volume that travels through the evaluated tissue. The AUC is standardized by calculating the area under the curve from the TOA to the end time of the shortest acquisition of the given patient to ensure the AUC is calculated over the same period of time independent from acquisition time.
7. The mean transit time (MTT) gives an indication of the average time blood needs to travel through the tissue. MTT [s] is the time between the centroid of the TDC and the TOA.

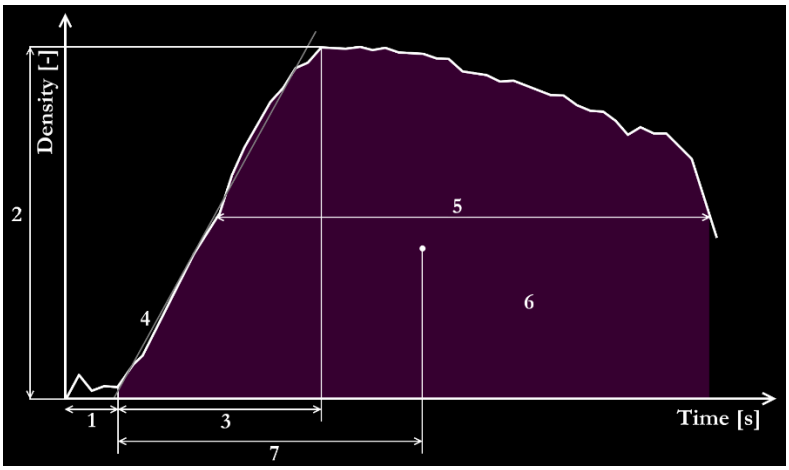


Figure 1.3 – Perfusion parameters derived from the time density curve. 1: time of arrival, 2: peak density, 3: time to peak, 4: wash-in rate, 5: full width at half maximum, 6: area under the curve, 7: mean transit time

Application of 2D perfusion angiography

Initially, 2DPA was developed for cerebral artery imaging to quantify the intracranial circulation[57,58] and perfusion[59,60]. 2DPA is studied for other clinical applications as well, among others to quantify perfusion reduction in patients with hepatocellular carcinoma who are treated with transarterial chemoembolization to reduce tumor perfusion.[61] Recently, 2DPA of the foot has been introduced for quantitative assessment of foot perfusion. Previous reports have shown the feasibility of 2DPA to identify changes following endovascular intervention in patients with peripheral arterial disease[55,56,62,63].

In the feasibility study and technical note by Jens et al., 18 CLI patients scheduled for below-the-knee angioplasty were included[55]. The authors demonstrated increased AUC after successful intervention while curves were unchanged after failed revascularization attempts, indicating feasibility of 2DPA for assessment of perfusion following intervention.

In 2015, Murray et al. performed a retrospective single-center study including 24 vascular interventions in 21 patients with Fontaine III (n=10) and IV (n=14)[62]. In 9 patients, above-the-knee lesions were treated, in 11 patients, below-the-knee lesions and in 4 patients above- and below-the-knee lesions were treated. Pre- and post-intervention perfusion parameters using 2DPA were compared with ROIs at the hind- and forefoot. The area under the (time-density) curve of the ROI at the hindfoot was the only parameter found to be significantly different following angioplasty.

Hinrichs et al. evaluated the feasibility to quantify blood flow using 2D perfusion angiography measurement proximally and distally of a target lesion[63]. The retrospective single-center study included 21 patients with Fontaine stage IIb (n=19) or III (n=2). The study included a total of 24 vascular interventions of which 20 interventions were performed above-the-knee (3 iliac and 17 superficial femoral artery) and 4 interventions were performed below-the-knee (1 anterior tibial, 1 posterior tibial and 2 in the fibular artery). 2DPA parameters were correlated to changes in the ankle-brachial index. A significant increase in ankle-brachial index was observed after interventional treatment. Only the difference in pre- and post-intervention TTP showed a significant correlation with the difference in pre-interventional and post-interventional ABI. However, in the scope of this study, blood flow of the treated vessel was measured and not tissue perfusion.

Kim et al. investigated tissue perfusion parameters in the foot in 31 PAD patients (Fontaine IIb, III and IV)[56]. In 16 patients of this cohort 18 percutaneous angioplasty procedures were performed and pre- and post-intervention perfusion parameters were recorded. The ROI was drawn at the level of the medial malleolus over the most robust tibial or peroneal artery. The following parameters were measured and correlated to the ankle-brachial index: TOA, PD, TTP, WIR, AUC, MTT and leg transit time (LTT). It was observed that the analyzed parameters tend to reflect improvements in blood flow in a predictive manner. Measurements of travel time decreased while measurements of volume increased after successful intervention. In addition, a significant correlation was found between worsening of the ABI and increased LTT. Moreover, a trend towards prolonged TOA and TTP was observed. When comparing pre- and post-interventional perfusion parameters, all except for the area under the curve, changed significantly.

Reekers et al. performed a prospective observational study in 89 consecutive patients with CLI[51]. Of the pre- and post-intervention datasets that were compared, 10% was not usable due to motion artifacts. 2DPA demonstrated an increase in volume flow (increased area under the curve and maximal peak density) in the foot after successful angioplasty of the below-the-knee vessels. Additionally, the authors investigated the functionality of the microcirculation to identify sub-types of patients with CLI. In 12 patients where no below-the-knee revascularization options were available, 4 mg of tolazoline was administered. Perfusion angiography acquisitions were obtained before and immediately after administration of tolazoline. After tolazoline administration, seven patients showed faster propagation of contrast and 5 patients did not show a response to the pharmacological stimulation. All these 5 patients had diabetes, whereas of the 7 responders 3 patients had diabetes. These findings suggest that perfusion angiography in combination with a pharmacological stimulation might give important functional information of the microcirculation and can be used to identify different phenotypes of CLI patients.

The currently available literature indicates that the technique is feasible and that it makes quantification and comparison of pre- and post-interventional perfusion possible.

Outline

Despite feasibility of 2DPA in CLI patients is implied, literature on reproducibility and observer variability is lacking. Therefore, the first aim of this thesis is to study reproducibility and intra- and inter-observer variability. In case 2DPA appears to be reproducible and the reliable ROI contains large vessels, these vessels can distort the ability of 2DPA to assess tissue perfusion. Therefore, the second aim of this thesis is to develop a method to extract large vessels from perfusion images.

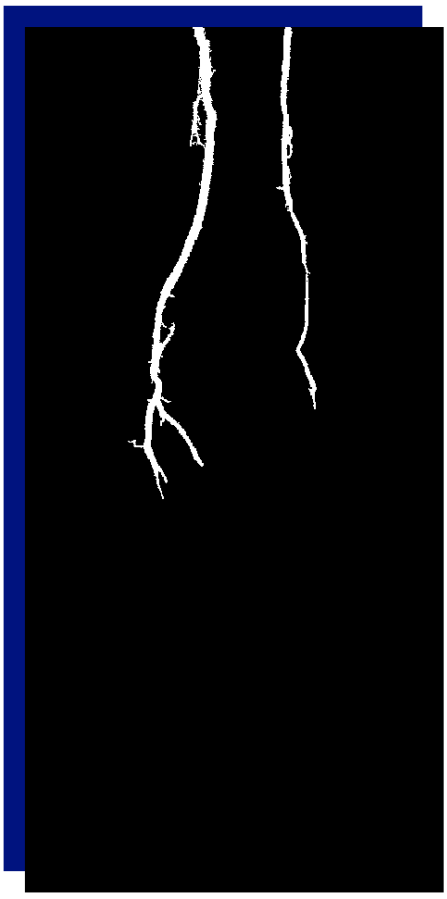
In Chapter 2, reproducibility of 2DPA is studied. To determine reproducibility, two perfusion acquisitions, which are obtained under equal patient and acquisition circumstances, are compared.
In Chapter 3, intra- and inter-observer variability of 2DPA is studied. Variability of two regions of interest is investigated in lateral and anterior-posterior perfusion acquisitions. Intra-observer agreement is studied by comparing TDCs and perfusion parameters of five repeated measurements drawn by the same observer on ten acquisitions. Inter-observer agreement is tested by comparing TDCs acquired from twenty acquisitions by two observers.
In Chapter 4, a vessel extraction method is presented to enable perfusion assessment of regions of interest containing large vessels. The extraction method is illustrated by two cases.

In Chapter 5, the findings of this thesis are summarized and discussed. This chapter focuses on insights important for clinical use of 2DPA. In addition, future perspectives regarding 2DPA are discussed.

Besides the performed studies as described above, this thesis involved clinical activities as well. The personal development that evolved from these clinical responsibilities, is described in the *Verantwoording*.

2

Reproducibility of 2D perfusion angiography in patients suffering from critical limb ischemia



Introduction

Quantitative assessment of foot perfusion may be the answer to determine the success rate of endovascular treatment in patients with critical limb ischemia. 2D perfusion angiography quantifies the amount of contrast over time, which gives insight in foot perfusion. The first clinical results demonstrate the feasibility of 2DPA in pre- and post-treatment assessment of foot perfusion in CLI patients[55,56,62,63].

An important step toward clinical use is confirming reproducibility and observer variability of 2DPA. However, data on these analyses regarding 2DPA in CLI patients are lacking. As a first step, reproducibility of 2DPA needs to be studied. 2DPA is hypothesized to be reproducible when all circumstances are equal, wherein e.g. the same position of the same patient, equal acquisition and table settings, and the same contrast agent and injection settings, are pursued. The aim of this chapter is to study reproducibility and test this hypothesis.

Methods

The St. Antonius Hospital started a clinical trial with among others the objective to determine the reproducibility of 2DPA in the foot of patients suffering from critical limb ischemia. The REPEAT-study (Dutch trial registry: NTR6615) is a single-center observational study approved by the medical research ethics committee. A total of fifty patients are to be included.

In- and exclusion criteria

Patients who were diagnosed with CLI, had non-healing ulcer(s) or gangrene (Fontaine IV) and were scheduled for below-the-knee endovascular treatment, were eligible to participate in the REPEAT-study. In addition, patients were required to be older than 18 years and did not have or had adequately treated inflow disease. Exclusion criteria were severe renal failure (eGFR < 30 mL/1.73 m^2), distal embolization after previous treatment of inflow disease, scheduled major amputation (above the ankle), inability to place the foot in a foot rest, severe allergy for the used contrast medium, or pregnancy. All participants gave written informed consent.

Image acquisition

Motion artifacts distort the image quality[51,55,62] of 2DPA images. Therefore, during intervention, the index foot was placed in a foot rest to minimize these artifacts. Intra-arterial access into the common femoral artery was obtained using the Seldinger technique. The sheath was placed at the level of the popliteal artery. Either one of two contrast agents was used, iodixanol 320 mg I/mL (Visipaque, GE Healthcare, Cork, Ireland) or iobitridol 300 mg I/mL (Xenetix 300, Guerbet, Villepinte, France). A total of 9 mL contrast medium was injected by a power injector with a flow-rate of 3 mL/s. The perfusion protocol of the Azurion system (Philips Medical Systems, Best, the Netherlands) was used, in which kVp and mAs remain constant to ensure equal density ranges. 2DPA images were acquired with a frame rate of 3 frames per second. Data analyses were performed on a 3D Interventional Work Spot (version R1.2.0, Philips Medical Systems, Best, the Netherlands).

To study reproducibility, two pre-interventional, lateral 2DPA images with the same acquisition settings were acquired. Equal foot position at the start of both acquisitions was pursued using the foot rest. To ensure complete wash-out of the iodinated contrast agent, the DSAs were acquired five minutes apart from each other. The TDC of the first acquisition (t=0 min) is defined as TDC_1 and of the second acquisition (t=5 min) is defined as TDC_2.

After acquisition, an experienced radiologist scored patient movement as non-present, minor or major movement and indicating frame numbers containing movement. Minor movement was defined as patient movement after which image series were expected to be usable for perfusion assessment. Major movement was defined as patient movement after which image series were expected to be unusable for perfusion assessment. The radiologist was blinded for color-coded perfusion images, because perfusion results might influence movement scores.

Assessment of reproducibility

To test reproducibility, an ROI was drawn around the complete color-coded image in the first acquisition of each patient. The Interventional Work Spot automatically copied this ROI to the patient's second color-coded image. DSA pixels with no density, i.e. no change with regard to the reference frame, are not included in the TDCs average density. By this method, reproducibility was tested for 2DPA results as suggested by Philips Medical Systems (Best, the Netherlands). No frame selection was performed. The first ninety frames (or less in case of shorter acquisition) were used to generate perfusion images and TDCs.

The error between TDC_1 and TDC_2 was assessed by the root mean square error (RMSE). The magnitude of RMSE is influenced by the density range, which differs substantially between patients. To compare the results between patients, the RMSE was normalized by the maximum density of TDC_1 and TDC_2, resulting the normalized root mean square error (NRMSE, Equation 2). TDC_1 and TDC_2 were considered reproducible when the RMSE is smaller than or equal to 10% of this maximum value, thus when NRMSE ≤ 0.10.

$$NRMSE = \frac{\sqrt{\sum(TDC_1 - TDC_2)^2/n}}{TDC_{max}} \qquad [2]$$

where TDC_{max} is the maximum density of TDC_1 and TDC_2, and n is the number of datapoints, i.e. frames.

Before calculation of the NRMSE, all datapoints before the patient's time of arrival were removed from the dataset for two reasons. First, TOA is in a clinical setting quite sensitive for external factors, e.g. the absence or presence of contrast in the sheath has a large influence on TOA. Second, a possible time shift between two identical curves has a large influence on the NRMSE. The NRMSEs were determined using Matlab software (version 2017b, MathWorks Inc., Natick, Massachusetts, United States).

The perfusion parameters of the TDCs are presented in Chapter 1. To determine whether the parameters of paired pre-interventional acquisitions of a patient are equal, several statistic tests were performed. Data samples were too small to test normality. Therefore, WIR, AUC and MTT were tested by the Wilcoxon signed rank test, because the differences were symmetrically distributed. Symmetrical distribution was determined based on histogram assessment. The other

parameters, TOA, PD, TTP and FWHM, were tested with the Sign test. All tests hold the null hypothesis that TDC_1 and TDC_2 were equal. The null hypothesis was rejected when $p < 0.05$.

Correlations between reproducibility, used contrast agent, and clinical patient characteristics were assessed by Spearman's rank correlation coefficient. Correlation between reproducibility, movement in the first acquisition, and movement in the second acquisition was assessed by logistic regression. T-tests, correlations and the logistic regression were performed using SPSS Statistics (version 25, IBM Corp., Armonk, New York, United States).

Results

Study population

Eleven patients were included (9 men) with a median age of 74 years (range 55-89 years). Eight patients had diabetes and the median toe pressure was 36 mmHg (range 20-78 mmHg). Five patients suffered from rest pain with a median VAS-score of 7 (range 2-8) and seven suffered from night pain with a median VAS-score of 8 (range 6-10). A total of fifteen ulcers were included. Seven wounds were located on a digit (two first, one second, three third, one fourth and none on the fifth digit) and two on the metatarsal level, one at the first and one at the fifth metatarsal level. One patient suffered from a non-healing heel ulcer and one patient suffered from three ulcers on her instep. The median wound size was 12×10 mm (length range 4-150 mm, and width range 1-100 mm). In the first four patients, iodixanol-320 was used. Hereafter, the contrast agent was changed to iobitridol-300, which was used in the subsequent seven patients.

Reproducibility analyses

Both pre-interventional lateral 2DPA acquisitions were analyzed. NRMSEs are presented per patient in Table 2.1. P-values of the perfusion parameters are presented in Table 2.2. In Figure 2.1, reproducible TDCs are shown with their color-coded images. In Figure 2.2, non-reproducible TDCs and color-coded images are shown. TDC of all patients are presented in Appendix B, Figure B.1 – B.11.

Table 2.1 – Reproducibility between TDC_1 and TDC_2, used contrast agent and scored movement per patient. NRMSE = normalized root mean square error. Minor: minor movement, which was defined as patient movement after which image series were expected to be useable for perfusion assessment. Major: major movement, which was defined as patient movement after which image series were expected to be unusable for perfusion assessment.

Patient	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Contrast	Iodixanol-320				Iobitridol-300						
NRMSE	0.15	0.05	0.11	0.06	0.05	0.03	0.06	0.07	0.05	0.20	0.30
Motion TDC_1	No	No	No	Minor	Minor	Minor	Major	Minor	No	Minor	Major
Motion TDC_2	No	No	Minor	No	Minor	Minor	Major	Minor	Minor	Minor	Major

Table 2.2 - Means and mean differences of perfusion parameters between TDC_1 and TDC_2.

	TOA	**PD**	**TTP**	**WIR**	**FWHM**	**AUC**	**MTT**
***p*-value**	1.00	0.23	1.00	0.25	N/A	0.48	0.28

TOA = Time of Arrival, PD = Peak Density, TTP = Time to Peak, WIR = Wash-in Rate, FWHM = Full Width Half Maximum, AUC = Area under the Curve, MTT = Mean Transit Time, N/A = not available

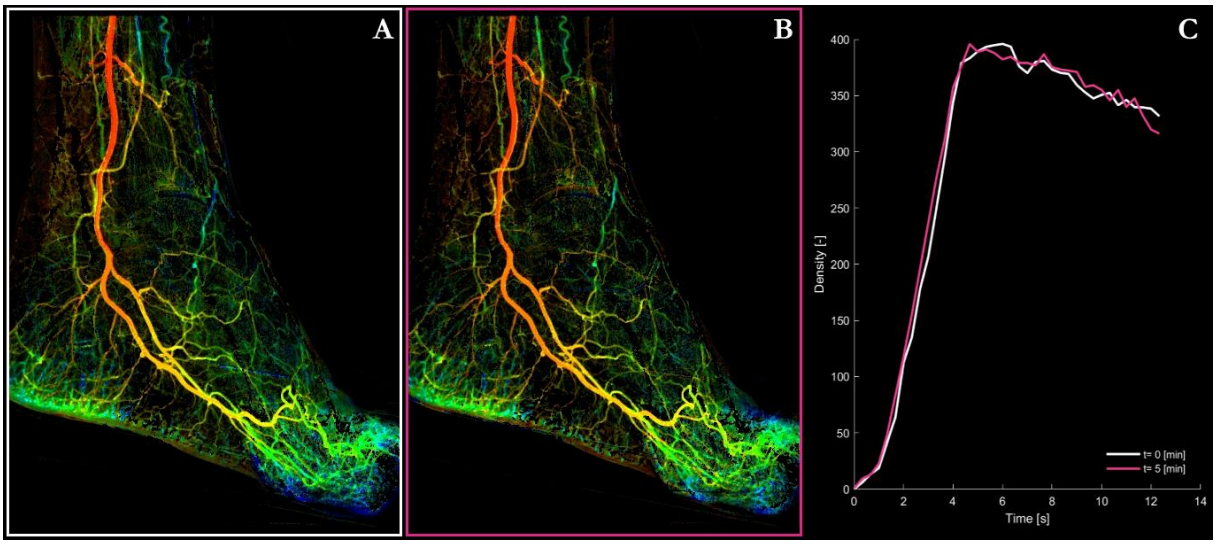


Figure 2.1 – Reproducible lateral perfusion acquisitions of patient #6. The patient's wound was located on his third digit. The color-coded images represent the time of arrival. ***(A)*** *First pre-interventional perfusion image at t=0 min.* ***(B)*** *Second pre-interventional perfusion image at t=5 min.* ***(C)*** *Time-density curves of Whole Image ROI as drawn in* ***(A)*** *and* ***(B)****.*

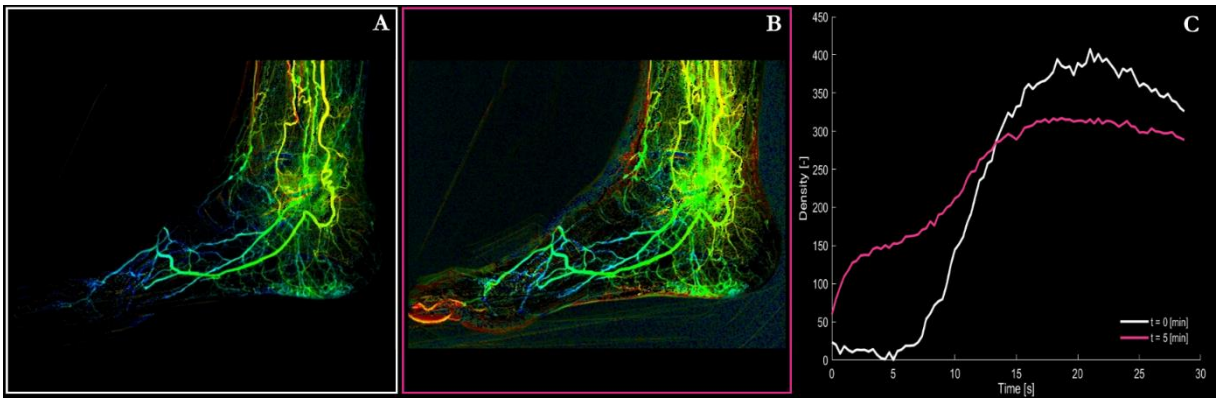


Figure 2.2 – Unreproducible lateral perfusion acquisitions of patient #10. The patient's wound was located on his lateral malleolus. The perfusion images represent the time of arrival. ***(A)*** *First pre-interventional perfusion image at t=0 min.* ***(B)*** *Second pre-interventional perfusion image at t=5 min.* ***(C)*** *Time-density curves of Whole Image ROI as drawn in* ***(A)*** *and* ***(B)****.*

In total, seven pairs of TDCs (64%) were reproducible. Of the patients in whom iodixanol-320 was used, two out of four (50%) showed to be reproducible. Of the seven patients in whom iobitridol-300 was used, five (71%) had reproducible TDCs. In two patients (#7 and #11), major movement occurred on both acquisitions. In patient #7, two events of major movement were seen within the same frame range in TDC_1 and TDC_2. In patient #11, movement was seen throughout the complete run and no separate frame ranges could be identified. In seven patients, minor movement was seen in eleven out of their fourteen acquisitions. In five patients, no movement was seen in seven out of their ten acquisitions.

FWHM could not be determined in any of the TDCs, because density did not decrease to or lower than half maximum. This is further discussed in the general discussion (Chapter 5). For all other perfusion parameters, the null hypothesis is accepted, and parameters are found to be equal between TDC_1 and TDC_2.

No significant correlations were found between reproducibility and patient movement (p=0.75), used contrast agent (p=0.53), or other relevant patient characteristics, such as diabetes mellitus (p=0.24), toe pressure (0.62), and rest (p=0.17) and night pain (p=0.60).

Requirements for obtaining reproducible time density curves

By reviewing the unreproducible TDCs, insight was gained in effects influencing reproducibility between TDC_1 and TDC_2. Beside acquisition and protocol parameters, frame selection and in- or exclusion of the background in the ROI influenced reproducibility.

In patient #3 and #10, TDC_2 showed fast density increase quickly after acquisition start. Density became constant after several frames. Some frames later, density increased again, probably with an equal trend as normal density increase caused by inflow. However, peak density and the plateau was lower than in TDC_1 (Figure 2.2 C, patient #10). Reviewing the X-ray images, intensity changes were seen right after acquisition start. This was probably caused by automatic image optimization after acquisition of the first frame, resulting in a density change. Removal of the frames before contrast arrival overcame this problem and improved TDC_2 (Figure 2.3 A, patient #10). Thereby, reproducibility was achieved (patient #3 NRMSE = 0.07, patient #10 NRMSE = 0.06). Due to removal of all frames before contrast arrival, no correction for TOA was applied for NRMSE determination. In patient #1, no intensity changes were seen on the X-ray images. However, TDC_2 appeared to improve as well by removal of the frames before contrast arrival (Figure 2.3 B-C). The TDC pair became reproducible as well with NRMSE = 0.06. Reproducibility improved to 91% when frame selection was applied in the unreproducible TDC pairs.

A second insight gained regarding frame selection was the influence of the number of included frames. The TDC appeared to be determined on the average density of the complete included acquisition. Therefore, the number of included frames influenced the densities of the complete TDC.

By defining the ROI, it was assumed no density changes would occur in the background. However, in patient #10, density changes were seen in the background (Figure 2.2 B, blue background). Thereby, in some patients, movement of e.g. the arm moved the sterile drape, which resulted in density changes in the background as well. In total, thirteen perfusion images (59%) showed a distorted background. These contrast differences in the background highly influenced the TDC, because numerous low-density pixels were included instead of excluded. This substantially lowered the mean density and thus TDC values.

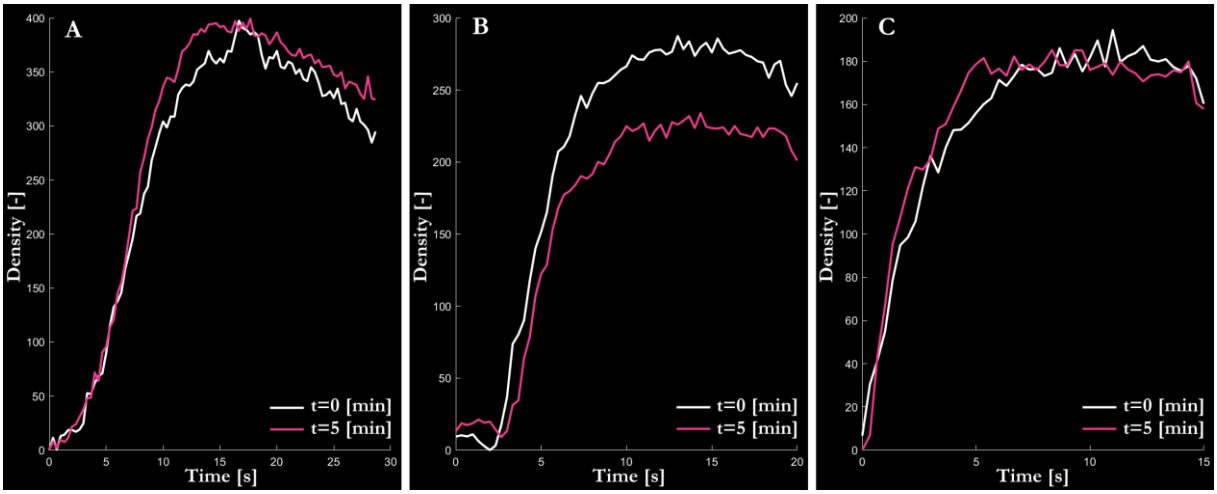


*Figure 2.3 - **(A)** Time density curves (TDCs) of patient #10 after exclusion of the frames before contrast arrival (original TDCs in Figure 2.2C), **(B)** TDCs of patient #3 before frame selection, **(C)** TDCs of patient #3 after frame selection.*

Discussion

In this chapter, the reproducibility of 2DPA was studied in eleven CLI patients. Only 64% of TDC pairs were reproducible when no manual adjustments were made. Reproducibility increased by applying frame selection and exclusion of the background from the ROI. All six determined perfusion parameters were reproducible.

No previous literature was found on reproducibility of 2DPA. Therefore, the findings of the current study are an important step towards a broader understanding of 2DPA reproducibility and eventually clinical use.

The current findings indicate the importance of frame selection. 2DPA analyses should be performed from the frame just before contrast arrival and onward. The frames before contrast arrival might distort the perfusion image, and thus the TDC and its parameters. A second finding regarding frame selection is that the number of included frames influences the TDC. When comparing two acquisitions, the same number of frames should be included to ensure reliable comparison between TDCs. Further research into reproducibility of 2DPA needs to confirm these findings in all included patients. Thereby, automated frame selection should be enabled to ensure correct analyses of the perfusion results.

In the current study, the ROI included the complete perfusion image, because no density changes were expected to occur in the background. This assumption is contradicted, because in thirteen of the 22 acquisitions, density changes occurred in the background and distorted the results. To overcome these distortions, the background should be excluded from the ROI and only consist of anatomical structures of interest.

One patient with unreproducible TDCs (Figure B.11) suffered from Parkinson's disease and therefore, moved during both complete acquisitions. Movement is a known problem in 2DPA[51,55,62] and will be further discussed in Chapter 5.

The perfusion parameters of TDC_1 and TDC_2 demonstrate no significant difference (Table 2.2), which indicates that different TDC pairs do not necessarily change the parameters significantly. An explanation may be that artifacts or small errors are included in the NRMSE, but do not necessarily influence the overall trend of the TDC. Therefore, the influence of artifacts and small errors may be limited on perfusion parameters. This should be confirmed in further research in which more patients need to be studied.

In addition, correlations were determined between reproducibility and patient movement, used contrast agent and patient characteristics. No significant correlations were found. However, the researched population was small with only eleven included patients. When 2DPA turns out to be unreproducible during future research in a larger population, correlation analyses should be reperformed to determine the cause of unreproducible results.

After inclusion of four patients, the study protocol was modified, and the used contrast agent was changed from iodixanol-320 to iobitridol-300. During interim analysis, two out of four TDC pairs (50%) appeared to be reproducible. By visual assessment, the fourth TDC pair showed the same trend as seen in one of the unreproducible TDC pairs, namely a delayed arterial outflow (Figure B.3 and B.4). Therefore, a literature search was performed to gain insight in the influence of the contrast agent on blood flow in small vessels.
Jung et al. studied the influence of the high-viscous contrast agent iopentol-350[64] (viscosity = 12.0 mPa·s at 37^0C) on the microcirculation. After injection of this high-viscous contrast agent, a drop in capillary erythrocyte velocity was seen[64]. These effects on capillary erythrocyte velocity were not seen after injection of iopentol-150 (viscosity = 1.7 mPa·s at 37^0C) [64].

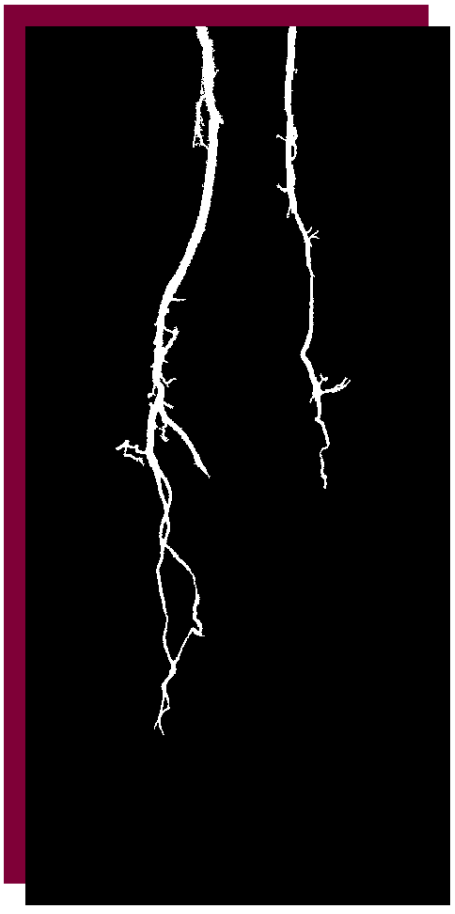
The observed arterial outflow delays of patient #3 and #4 can be explained by the used contrast agent, which has a high viscosity of 11.8 mPa·s at 37^0C[65]. Due to the viscosity, the capillary erythrocyte velocity decreased after the first acquisition, which on its turn decreased arterial outflow in the second acquisition. This effect was expected to occur in patient #2 (Figure B2) but not seen in patient #1 (Figure 2.3 B).
Upon this observation, it was decided to change from iodixanol-320 to iobitridol-300, which is the hospital's standard contrast agent used in angiography and has a viscosity of 6.0 mPa·s at 37^0C[66]. Initially, iodixanol-320 was used, because this was expected to minimize pain and cramp in the calf muscles[55]. Therefore, this change included the risk of more motion artifacts, caused by pain after intravascular injection.

After changing to iobitridol-300, the delay in arterial outflow as seen in Figure B3 and B4 was not seen in the subsequent patients, and reproducibility improved from 50% to 71%. However, two insights put the decision to change to iobitridol-300 under discussion. First, the frame selection improved both unreproducible TDC pairs in which iodixanol-320 was used. Second, iobitridol-300 may cause pain resulting in patient movement. Therefore, the relation between used contrast agent and patient movement was tested by determining Spearman's rank correlation coefficient. Significant strong correlations were found; $\rho = 0.61$ ($p = 0.04$) for the first acquisition and $\rho = 0.76$ ($p < 0.01$) for the second acquisition. Therefore, the use of iodixanol-320 should be reconsidered for the remainder of the study.

In conclusion, without frame selection, reproducibility of 2DPA was only 64%. However, reproducibility increased to 91% by frame selection in the unreproducible TDCs alone. Frame selection consisted of removal of the frames before contrast arrival and inclusion of the same number of frames in both analyzed TDCs. In future research, the influence of frame selection should be studied in all patients, as well as the sensitivity of perfusion parameters to TDC changes. As a second finding, the background should be excluded from the ROI. Thereby, using iodixanol-320 in the remainder of the REPEAT-study must be reconsidered.

3

Inter- and intra-observer variability of 2D perfusion angiography in critical limb ischemia



Introduction

In 2DPA, density per unit time is determined within a certain observer determined region of interest (ROI). The density over time can be visualized by a time-density curve (TDC). Subsequently, perfusion parameters can be derived from the obtained TDCs. Among others, the size and location of the drawn ROI potentially influence the TDC and perfusion parameters. For clinical use of 2DPA, it is crucial to ensure that equal ROIs results in equal perfusion results, and to determine variability between observers. Therefore, the aim of this chapter is to determine intra- and inter-observer variability of 2DPA.

Different theories about ROI location and size are applied in perfusion assessment in CLI patients. The two most commonly used are based on two theories. In the first theory, the foot is seen as an end organ, and the non-healing wound is only a symptom of the microcirculatory pathology of the complete foot.[51] From this assumption, the theory arises that improvement of perfusion of the whole foot is necessary for wound healing, and the ROI used in 2DPA should therefore include the entire foot. Based on this theory, Reekers introduced an ROI which includes the foot, but excludes the toes, because movement of the toes often results in motion artifacts[51]. In Reekers ROI, the influence of the larger inflow arteries (macrocirculation) is assumed to be negligible.
In the second theory assumes that regional perfusion disturbances prevent the ulcer from healing and therefore, the wound related artery should be revascularized. Therefore, local perfusion around the wound should be assessed to determine treatment success. Therefore, the 2DPA ROI should contain the wound area without any large conducting vessels disturbing the perfusion results.

Methods

A second objective of the REPEAT-study is to determine observer variability of 2DPA in the foot. In Chapter 2, the REPEAT-study is introduced with its in- and exclusion criteria and image acquisition method, which were used for variability analyses.

Acquisition selection

Per patient, a total of seven 2DPA acquisitions were obtained, four in lateral (LAT) view and three in anterior-posterior (AP) view. Of these acquisitions, two acquisitions per patient were selected (Table 3.1). Ten acquisitions were selected to investigate intra-observer variability and twenty to investigate inter-observer variability. Acquisitions with motion artifacts were excluded, which was scored by an experienced radiologist (Chapter 2). Both pre- and post-interventional acquisitions were used and randomly selected for lateral and AP acquisitions.

Table 3.1 – Numbers of included 2DPA acquisitions for variability analyses. The same acquisitions were used for both Reekers and Wound ROI.

	# of patients	Total # of acq.	# of AP acq.	# of LAT acq.	# of pre acq.	# of post acq.
Intra-observer variability	5	10	5	5	5	5
Inter-observer variability	10	20	10	10	10	10

Acq. = acquisition, AP = anterior-posterior, LAT = lateral, pre = pre-intervention, post = post-intervention

Region of interest

Two ROIs are studied for intra- and inter-observer variability. As demonstrated in Chapter 2, the background of the perfusion image should be excluded, because density changes may distort perfusion results. ROIs were drawn according to the two presented theories, i.e. the method proposed by Reekers et al.[51] (Reekers ROI) and the area around the wound (Wound ROI). Both ROIs were drawn using the free-hand ROI tool of the 3D Interventional Work Spot (version R1.2.0., Philips Medical Systems, Best, The Netherlands).
To ensure the ROIs were drawn under the same conditions, a manual was composed and used by the observers for both intra- and inter-observer variability analyses (Appendix C). For Reekers ROI, the proximal boundary was defined as a horizontal line through the tibiotalar joint and the distal boundary was drawn so that the metatarsal arteries lay within the ROI. In order to standardize the Wound ROI, the ROI needed to be drawn one centimeter around the wound in all directions. The observer was informed about the wound location by a photograph of the patient's wound.
After drawing the ROI, a snapshot of the ROI and its location on the foot, and the datapoints of the TDCs were exported.

Intra-observer variability

For intra-observer variability analyses, one experienced observer (AJ) drew Reekers and Wound ROI on ten 2DPA acquisitions. These measurements were repeated five times. Between each measurement, there was a minimum time-interval of 24 hours to prevent recall bias of ROI size and location.

Inter-observer variability

To gain insight in inter-observer variability, two observers (AJ & SH) drew Reekers and Wound ROI on twenty 2DPA acquisitions.

Statistics

For both variabilities, agreement was assessed by the normalized root mean square error between TDC_1 and TDC_2 (Chapter 2, Equation 2). For intra-observer analyses, the five ROIs in each acquisition were compared with each other, resulting in 10 comparisons per acquisition for each ROI. Intra- and inter-observer variability was considered reliable when the NRMSE is smaller than or equal to 10% of the maximum density, thus when NRMSE ≤ 0.10. Before calculation of the NRMSE, the datapoints before TOA were removed from the dataset for the same reasons as mentioned in Chapter 2.

For intra-observer variability assessment, two additional statistics were determined. To compare intra-observer agreement between acquisition direction, the NRMSEs of AP acquisitions were compared with NRMSEs of lateral acquisitions. This was done by the McNemar's test. Second, the intraclass correlation coefficient (ICC) of the perfusion parameters was determined. Perfusion parameters are defined in Chapter 1. For ICC, the two-way mixed-effects model was used, because the scores of one rater were not necessarily comparable to the scores of a larger group of

observers.[67] Absolute agreement was selected because one observer's repeated measurements should be consistent.[67] In addition, single rater type was selected, because one observer's measurement is taken into account in the clinical application of 2DPA.[67] ICC values smaller than 0.50 implied poor agreement, values equal to 0.50, and between 0.50 and 0.75 imply moderate agreement, ICC values equal to 0.75, and between 0.75 and 0.90 implied good agreement and ICC values equal to and larger than 0.90 implied excellent agreement.[67]

NRMSEs and ICCs were determined using Matlab software (version 2017b, MathWorks Inc., Natick, Massachusetts, United States). McNemar's test was performed using SPSS Statistics (version 25, IBM Corp., Armonk, New York, United States).

Results

Eleven CLI patients were included in the REPEAT-study. Seventy-six perfusion acquisitions were acquired. After movement scoring, one patient was excluded, because of major movement on all 2DPA acquisitions. Sixty acquisitions were suitable for perfusion analyses, twenty were selected as described above. From nine patients, one lateral and one AP acquisition were included. In one patient, only one lateral acquisition was included, because this was the only suitable 2DPA acquisition. Therefore, from one patient, an additional AP acquisition was included.

In two patients had two wounds, thus two Wound ROIs were drawn. In one patient, the wounds were located on the third and fourth digit. Therefore, they overlapped on the lateral acquisition and only one Wound ROI was drawn on this acquisition.

Intra-observer variability

To asses intra-observer variability, one observer (AJ) drew 115 ROIs. Of these ROIs, fifty were Reekers ROIs and 65 were Wound ROIs.

The determined NRMSEs are presented in Appendix D. All NRMSEs of Reekers ROI are smaller than or equal to 0.10, thus all ROIs are considered reproducible. The mean error was 0.02 (range 0.00-0.08). The mean error per wound ranged from 0.01-0.05. For Wound ROIs, mean NRMSE was 0.08 (range 0.01-0.40). The mean error per wound ranged from 0.02-0.19. Of the 130 determined NRMSEs, 91 NRMSEs were considered reproducible. In four AP acquisitions and in one lateral acquisition, all Wound ROIs were reproducible. 6 Wound ROIs differed from all four other ROIs.

Comparison between AP and lateral acquisitions was performed for Wound ROI only, because all Reekers ROI were reproducible. In seventy AP acquisitions, 57 (81%) ROIs were reproducibly drawn, and in sixty lateral acquisitions, only 34 (57%). Reproducibility differed significantly between AP and lateral acquisitions ($p<0.01$), in favor of AP acquisitions. In Appendix E Figure E.1 and E.2, reproducible TDCs of Reekers ROI and Wound ROI are shown. Unreproducible Wound ROI and TDCs of one patient are presented in Figure E.3.

Intraclass correlation coefficients for the perfusion parameters and their p-values are presented in Table 3.2. Full width at half maximum could not be determined, because densities did not decrease to or lower than half maximum, which will be further discussed in Chapter 5. Eleven out of twelve ICC values indicate excellent intra-observer agreement. Wound ROI's WIR indicates good agreement.

Table 3.2 – Intra-class correlation coefficients and their p-values of Reekers and Wound ROI drawn by one observer (AJ)

	Reekers ROI		Wound ROI	
	ICC	**p-value**	**ICC**	**p-value**
TOA	1.00	<0.001	0.99	<0.001
PD	0.99	<0.001	0.97	<0.001
TTP	0.95	<0.001	0.93	<0.001
WIR	0.99	<0.001	0.84	<0.001
FWHM	N/A	N/A	N/A	N/A
AUC	0.99	<0.001	0.98	<0.001
MTT	1.00	<0.001	0.99	<0.001

TOA = Time of Arrival, PD = Peak Density, TTP = Time to Peak, WIR = Wash-in Rate, FWHM = Full Width Half Maximum, AUC = Area under the Curve, MTT = Mean Transit Time, N/A = not available.

Inter-observer variability

For inter-observer assessment, each observer drew 43 ROIs, twenty Reekers ROIs and 23 Wound ROIs, which resulted in a total of 86 drawn ROIs.

In Table 3.3 and 3.4, all NRMSEs are presented for Reekers ROI and Wound ROI, respectively. In Appendix F Figure F.1 and F.2, reproducible TDCs of Reekers and Wound ROIs are shown. Unreproducible TDCs of both ROIs are presented in Figure F.3 and F.4. Nineteen Reekers ROIs (95%) were reproducible between both observers with a mean error of 0.03 (range 0.01-0.13). Eight Wound ROIs (35%) were reproducible with a mean error of 0.15 (range 0.02-0.43).

Table 3.3 – Normalized root mean square errors of Reekers ROIs drawn by two observers in ten patients. Reekers ROIs were drawn in anterior-posterior (AP) and lateral (LAT) acquisitions (acq.). In one patient #7, only the lateral acquisition was suitable for assessment. Therefore, two different AP acquisitions of patient #4 were assessed.

Patient	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**	**#9**	**#10**
AP acq.	0.03	0.01	0.07	0.01 0.01	0.06	0.01	-	0.02	0.09	0.09
LAT acq.	0.01	0.02	0.01	0.02	0.03	0.01	0.13	0.02	0.01	0.01

Table 3.4 – Normalized root mean square errors of Wound ROIs drawn by two observers in ten patients, around twelve wounds. Wound ROIs were drawn in anterior-posterior (AP) and lateral (LAT) acquisitions (acq.). In patient #7, only the lateral acquisition was suitable for assessment. Therefore, two different AP acquisitions of patient #4 were assessed. Patient #1 and #8 had two ulcers and thus two Wound ROIs were drawn. In patient #1, wound regions overlapped in the lateral acquisitions, therefore, only one Wound ROI was drawn in lateral acquisition.

Patient	**#1**		**#2**	**#3**	**#4**	**#5**	**#6**	**#7**	**#8**		**#9**	**#10**
Wound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
AP acq.	0.08	0.24	0.13	0.12	0.06 0.07	0.17	0.02	-	0.11	0.29	0.19	0.09
LAT acq.	0.24		0.02	0.15	0.21	0.06	0.14	0.43	0.05	0.25	0.25	0.18

Discussion

The aim of this study was to determine intra- and inter-observer variability of 2DPA in the foot. Intra-observer agreement was excellent for Reekers ROI and good for Wound ROI. For Reekers ROI, all TDCs were considered equal and parameter comparisons showed excellent agreement. All wound ROIs agreed in five out of ten acquisitions. On the other hand, for six wounds one ROI differed from the four other ROIs. The ICCs of TOA, PD, TTP, AUC and MTT showed excellent agreement and ICC of WIR showed good agreement.
Inter-observer agreement was excellent for Reekers ROI and moderate for Wound ROI. 95% of Reekers ROI were reproducibly drawn by both observers. Only 35% of Wound ROIs were equally drawn.

To our knowledge, inter-observer variability for 2DPA was researched in only one other study. Maschke et al. studied inter-observer variability in transjugular intrahepatic portosystemic shunts for treatment of severe complications of portal hypertension.[68] Two radiologists agreed on ROI placement of eight ROIs in four different locations. ROIs were drawn in fifteen patients. These results were compared with the same ROIs of another pair of radiologists. ICC values of PD, TTP and AUC ranged from 0.88-0.99[68]. Applying our ICC categories to these ICCs, Maschke et al. found good to excellent agreement.
Direct comparison of these findings with the inter-observer agreement found in the current study is not possible, because different outcome measures were used (ICC in Maschke et al. and NRMSE in the current study). Indirect comparison shows that inter-observer agreement determined in the current study is as well excellent for Reekers ROI. However, determined inter-observer agreement of Wound ROIs is lower.
Variability between perfusion results was caused by differences in ROI sizes and/or locations. When ROIs were equally drawn, perfusion results demonstrated to be equal. Wound ROIs were probably more sensitive for these errors, because wound location and size needed to be translated from the patient's photograph to perfusion images. This appeared difficult, because reference points from the wound photo were not visible on X-ray or perfusion images. For Reekers ROI, no translation was necessary, because reference points are visible on X-ray or perfusion images.
In Maschke et al., ROIs were drawn in agreement between two observers. Therefore, the influence of location interpretation between the two observer pairs was overcome and excellent agreement was ensured. For clinical use of 2DPA, variability between ROI size and/or location must be minimized by clearly defined ROIs. Thereby, automated ROI placement would be ideal to overcome observer variability. Such an automation method needs to be developed and studied in future research.

To our knowledge, no prior studies researched intra-observer variability of 2DPA, neither in arterial, cerebral or any other diseases. Therefore, the current study is an important step into clinical validation of 2DPA. ICCs of intra-observer agreement were excellent for Reekers ROI and good to excellent for Wound ROI. However, several Wound ROIs were considered unreliable based on NRMSEs. This indicates that 10% mean difference between two TDCs not necessarily influence the perfusion parameters to significantly change. For clinical use of 2DPA, it is important to understand which extend of difference between pre- and post-interventional TDCs and perfusion parameters are relevant, which should be addressed in future research.

Reekers ROI is demonstrated to be a more consistent ROI than Wound ROI, both within and between observers. However, Reekers ROI includes the conducting arteries which might limit the ability of 2DPA to assess tissue perfusion. To gain insight in the influence of the macrocirculation on the TDC, separation of the macro- and microcirculation is needed. In Chapter 4, a method for extraction of the macrocirculation is presented.

Reviewing the perfusion parameters, PD appeared to be wrongly determined for several Wound ROIs. TDCs of wound areas often have an interrupted upward trend (Figure E.2). With the current definition of PD (Chapter 1, Equation 1), PD is assigned to the interruption of the upward trend, because in these curves, the density of four subsequent frames is smaller or equal to 101% of the density of the analyzed frame. The lower ICC of WIR (0.84) is probably caused by inconsequent determination of PD, because WIR is calculated based on TOA and PD. To overcome this drawback for Wound ROIs, PD should be determined by analyzing the TDC from the last frame to the first frame. In this way, the last peak will be selected instead of the first.

Variability of Wound ROIs was significantly higher in lateral acquisitions. An explanation might be that the ease to define the wound location is influenced by acquisition direction. Another explanation might be that many wounds located on the digits were included, which overlap on lateral acquisitions. Therefore, these acquisitions might be more sensitive to ROI shifts. Therefore, AP acquisitions should be analyzed when the wound areas are examined despite the aforementioned discouragement of using Wound ROIs.

In conclusion, intra- and inter-observer agreement is excellent for Reekers ROI, because of well-defined ROI boundaries. Lower intra- and inter-observer agreement of Wound ROIs is caused by differences in ROI location and/or size. Based on these findings, Reekers ROI would be preferred over Wound ROI. However, the Reekers ROI includes the conducting arteries which may distort tissue perfusion results. Therefore, an extraction method for the arteries is presented in the next chapter.

4

2D perfusion angiography to assess tissue perfusion in critical limb ischemia endovascular interventions: a technical note

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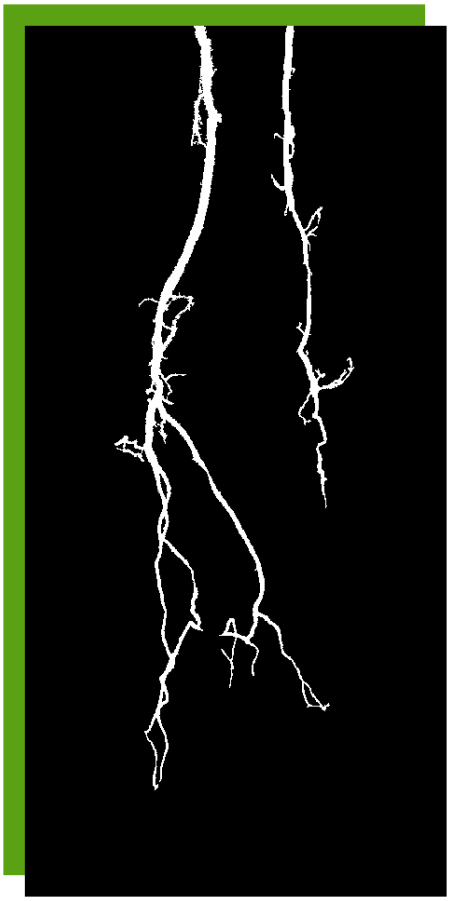

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Abstract

Treatment of critical limb ischemia requires a multi-disciplinary approach in which revascularization plays an important role. Because of its minimal invasive nature and high technical success rates, endovascular approach has become the treatment of choice. The aim of revascularization is to increase oxygen supply to the wound so that oxygen levels become sufficiently high to enable wound healing. It would be ideal to have a tool in the angio suite that predicts clinical outcome after endovascular procedures. 2D perfusion angiography (2DPA) is a post processing software tool that uses digital subtraction angiography images to calculate perfusion during revascularization procedures. Although studies have shown that 2DPA is able to demonstrate perfusion changes during endovascular revascularization procedures there are some limitations to this technique. To evaluate perfusion, a region of interest is drawn over the foot or wound. Since 2DPA produces a 2D reconstruction of a 3D structure there is overprojection of large conducting vessels over the tissue of interest which will influence perfusion results. This article describes perfusion angiography and focuses on a technique to overcome the 2D-3D mismatch by performing vessel extraction. The results are illustrated by two cases.

Keywords: Critical limb ischemia, peri-interventional outcome assessment, perfusion angiography, vessel extraction

Introduction

In literature, 2DPA is described as a feasible technique to compare pre- and post-interventional perfusion in CLI patients.[51,55,56,62,69] In this thesis, reproducibility is studied and described and observer agreement is demonstrated for the approach practiced by Reekers et al., who regard the whole foot as an end organ[51]. A non-healing wound, for instance on the heel, is merely a symptom of diffuse microcirculatory disease of the whole foot. Therefore, endovascular treatment should improve perfusion of the foot, which 2DPA aims to quantify.

However, perfusion angiography is a 2D representation of 3D anatomy and therefore, a complete foot analysis as proposed by Reekers includes the conducting arteries as well. These large arteries could influence perfusion results significantly rendering 2DPA to be of less use for evaluation of the microcirculation and tissue perfusion. Extraction of the large conducting vessels, which are not involved in oxygen and nutrient exchange, could improve the assessment of tissue perfusion. Theoretically, separation of the macro- and microcirculation should result in an arterial curve and a tissue perfusion curve (Figure 4.1). After injection of contrast (Figure 4.1 A), arterial inflow is achieved with some delay. The arterial phase is characterized by a steep inflow, reaches its maximum fast and steep outflow[70] (Figure 4.1 B). The perfusion curve of the microcirculation is further delayed, less steep and widened in comparison to the arterial curve (Figure 4.1 C). TDCs of the macro- and microcirculation resulting from the extraction method presented in this chapter are expected to show similar characteristics. Results of the extraction method are illustrated by two cases.

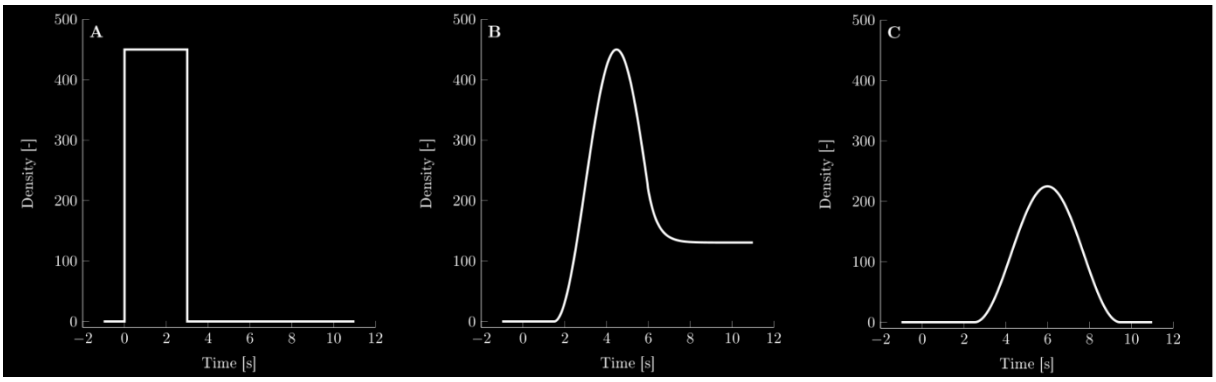


Figure 4.1 – Theoretical expected perfusion curves with arbitrary contrast density. ***(A)*** *Step function representing an input bolus of 9 mL at a constant flow rate of 3 mL/s administered by the contrast pump.* ***(B)*** *Arterial input function delayed in time with respect to bolus administered by the pump.* ***(C)*** *Microvascular perfusion curve delayed in time with respect to the arterial perfusion curve.*

Methods

Vessel extraction

Arteries were extracted from the DSA images using Matlab software (version R2017b, MathWorks Inc., Natick, Massachusetts, United States). To distinguish arteries from the capillaries a region growing algorithm was applied. For every major artery (anterior tibial artery, posterior tibial artery and/or peroneal artery) visible, an initial starting point was automatically defined, based on the time-averaged pixel intensity of the most cranial pixel row of the image (Figure 4.2 B). Every region was then iteratively grown by comparing the neighboring pixels with connectivity four to the region (Figure 4.3). The neighboring pixel closest to the mean of the region was subsequently considered as part of the artery and added to the region. The algorithm halted when the intensity of the region mean and new pixel became larger than a predefined threshold value. To increase the robustness of the algorithm, the extraction of every frame was used as starting point for the next frame. The selected pixels of the final frame contained all the present arteries and were analyzed as the macrocirculation. All other pixels were analyzed as the microcirculation.

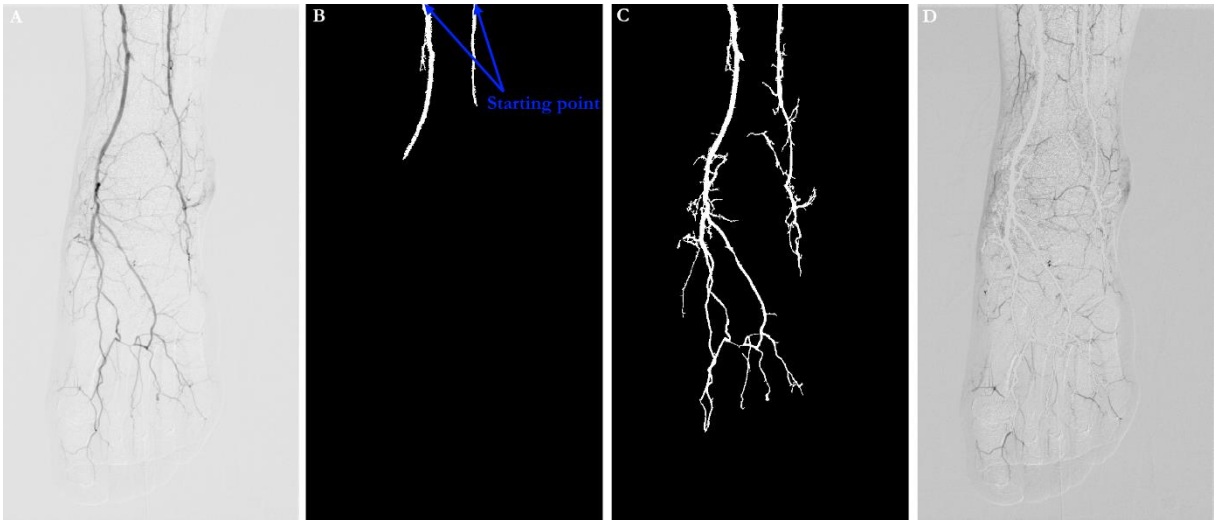


Figure 4.2 – Vessel extraction method. ***(A)*** *original DSA image* ***(B)*** *starting point selection based on time-averaged pixel intensity of the most cranial pixel row* ***(C)*** *vessel selection after applying the region growing algorithm to several frames* ***(D)*** *DSA image after extraction of the macrocirculation; pixel values of the first DSA frame are applied to the macrocirculation pixels.*

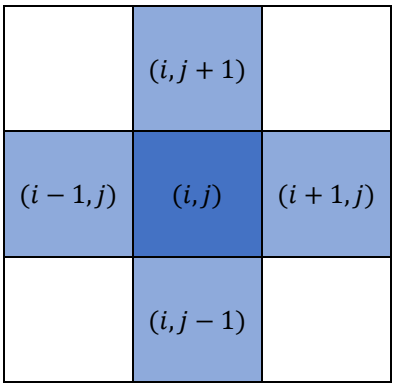


Figure 4.3 – Neighboring with connectivity 4 and (i, j) the current pixel to be evaluated.

Validation of the algorithm

After development of the extraction method, the proposed approach was validated by analyzing two available pre-treatment perfusion angiograms of the same patient. Both perfusion angiograms were acquired with identical table and acquisition settings. Subsequently, the algorithm was used to obtain the microcirculation and macrocirculation time density curves from both scans. The correspondence between the two original TDCs, microcirculation TDCs and between the macrocirculation TDCs were assessed by determining the normalized root mean square error (Chapter 2, Equation 2). Figure 4.5 shows the original, microcirculation and macrocirculation TDCs. The NRMSE demonstrated very small errors between both original pre-treatment perfusion images (NRMSE = 0.05), both reconstructed microcirculation images (NRMSE = 0.03) and both reconstructed macrocirculation perfusion images (NRMSE = 0.04). In future research, reproducibility of the extraction method needs to be validated in a larger study population.

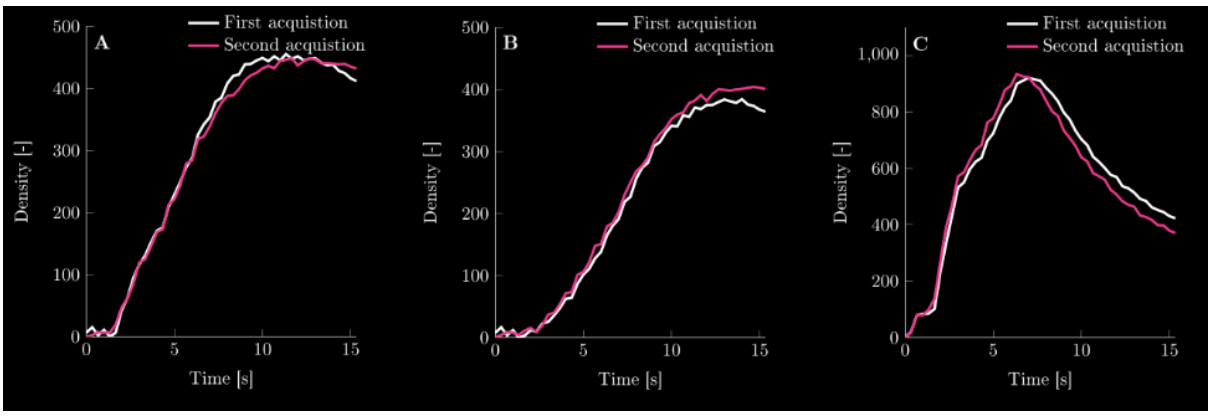


*Figure 4.5 – **(A)** Time density curves of two pre-treatment acquisitions demonstrating almost perfect correspondence (NRMSE = 0.05). The proposed vessel extraction method was applied to both acquisitions **(B)** Time density curves of the microcirculation demonstrating almost perfect correspondence (NRMSE = 0.03). **(C)** Time density curves of the macrocirculation demonstrating almost perfect correspondence (NRMSE = 0.04).*

Case example 1

A 72-year old man visited our outpatient clinic with two necrotic wounds on his left foot, one on his third digit (12×12 mm) and one on his forth digit (4×3 mm). The patient had type 2 diabetes and suffered from rest pain (VAS-score 7) and severe night pain (VAS-score 9). Pre-intervention ABI was high, 0.88, probably as a result of the patient's diabetes. On diagnostic computed tomography angiography (CTA), no significant iliac or femoral lesions were identified. However, below-the knee lesions among which long occlusions of the posterior (ATP) and anterior tibial artery (ATA) were identified. Therefore, below-the-knee endovascular intervention was indicated.

Via antegrade puncture, common femoral artery access was obtained. Pre-interventional DSA images were obtained, demonstrating posterior and anterior tibial artery occlusion and a subtotal stenosis in the distal fibular artery. The fibular artery was successfully treated with percutaneous transluminal angioplasty (PTA). Revascularization attempts of the ATP and ATA were not successful. Pre- and post-intervention 2DPA images were acquired.

At 3-month follow-up, ABI improved to 0.9 and wound status improved as well. The small wound on digit IV healed completely and the other, larger wound on digit III improved substantially to a size of 2×2 mm. Rest pain decreased with 74% to a VAS-score of 2 and night pain completely resolved. At 6-month follow-up the second wound healed as well, and rest pain further resolved.

The vessel extraction method was applied to the obtained perfusion acquisitions. In Figure 4.6, pre- and post-interventional 2DPA images are shown for the original, micro- and macrocirculation images. The corresponding TDCs are shown. In table 4.1, the perfusion parameters are presented. All 2DPA images and TDCs show improved inflow and perfusion. Improvement of the PD and AUC is seen in all three TDCs. TOA is improved in the original and macrocirculation TDCs, TTP in original and microcirculation TDCs and WIR and MTT are only improved in the microcirculation TDC. On the macrocirculation TDC, delayed outflow is seen, which is expressed in the increased FWHM as well. On the original and macrocirculation color-coded images, the worsened status of the ATA can be clearly seen.

Table 4.1 – Perfusion parameters of the original, microcirculation and macrocirculation TDCs. Arrows indicate if in- or decrease of the parameter is needed for improvement.

	Original		**Microcirculation**		**Macrocirculation**	
	Pre	Post	Pre	Post	Pre	Post
TOA (↓)	1.3	0.3	1.3	2.3	1.3	0.3
PD (↑)	277	325	196	246	669	799
TTP (↓)	9.7	6	13.7	13	5.3	5.7
WIR (↑)	33	31	15	17	161	142
FWHM (↓)	N/A	N/A	N/A	N/A	14.0	14.3
AUC (↑)	12 325	16 651	6 287	8 697	23 925	30 010
MTT (↓)	11.0	11.1	12.4	10.7	9.3	9.4

Pre = pre-intervention parameters, post = post-intervention parameters. TOA = time of arrival, PD = peak density, TTP = time to peak, WIR = wash-in rate, AUC = area under the curve, MTT = mean transit time. N/A = not available because of no after-peak density decrease to or below half of the peak-density.

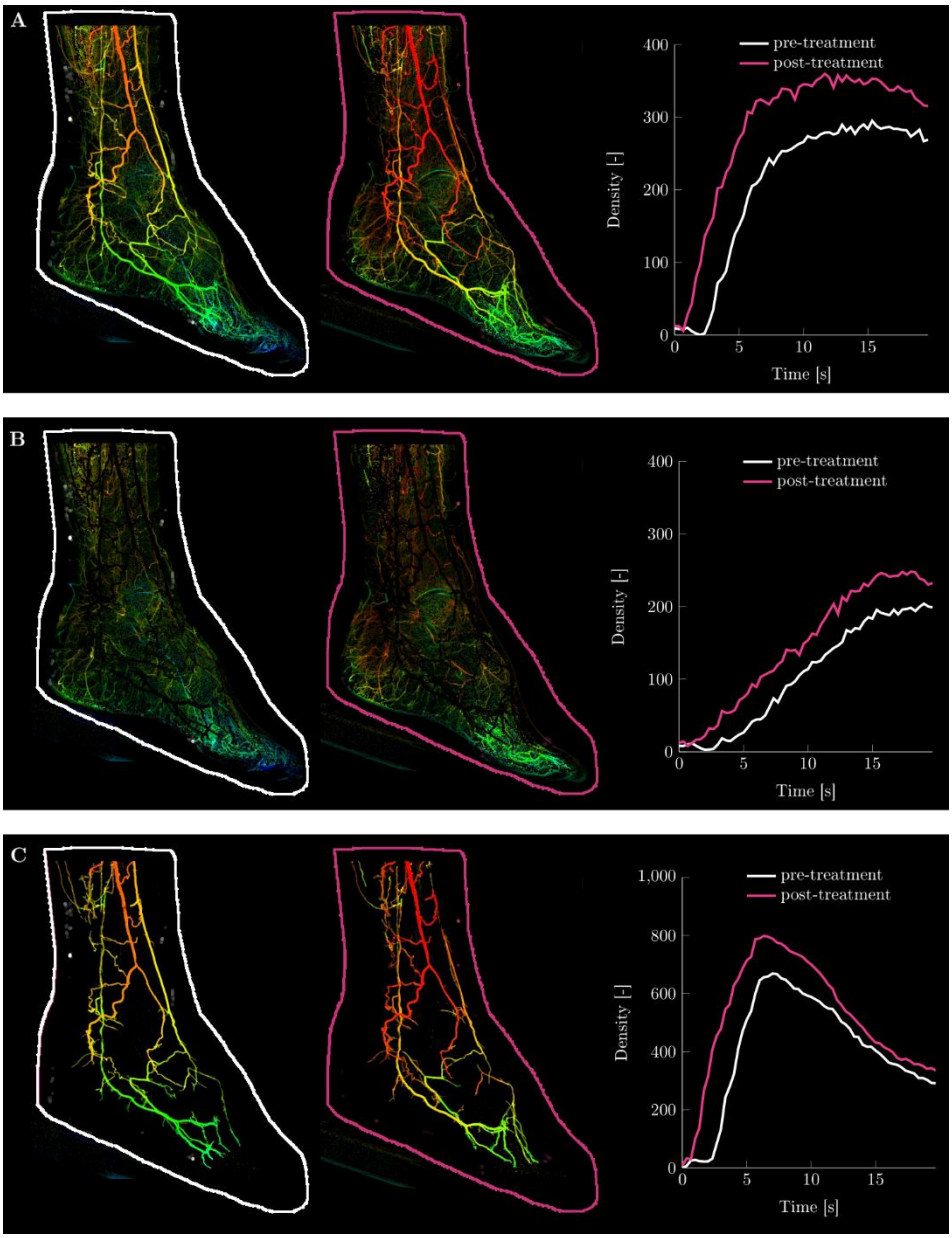


Figure 4.6 – Analysis of a lateral angiographic projection. ***(A)*** *Original pre-intervention and post-intervention perfusion angiogram.* ***(B)*** *Microcirculation pre-intervention and post-intervention perfusion angiogram. The time density curves show a clear capillary perfusion.* ***(C)*** *Macrocirculation pre-intervention and post-intervention perfusion angiogram. The time density curves show a clear arterial perfusion response.*

Case example 2

An 81-year old man presented at our outpatient clinic with a non-healing wound of 30×30mm on his left lateral malleolus and severe night pain (VAS-score 10). There was no relevant medical history. The pre-interventional MRA showed no iliac or femoral stenoses. The ATP, ATA and peroneal artery were occluded. Despite these abnormalities, the ABI was relatively high at 0.6, which was probably inaccurate. Due to worsening of the wound and presence of below-the-knee occlusions, below-the-knee endovascular intervention was indicated.

On pre-intervention DSA images, the identified lesions were similar to the diagnostic MRA. The ATP and fibular artery were successfully treated by endovascular revascularization. The ATA was not treated, due to the occlusion length and absence of pedal outflow. Pre- and post-intervention 2DPA images were acquired.

At 3-months follow-up the wound healed completely, night pain resolved, and ABI normalized.

In figure 4.7, pre-and post-interventional 2DPA images and their corresponding TDCs are shown for the original, micro- and macrocirculation images. Table 4.2 summarizes the perfusion parameters. Improved inflow and perfusion are seen on original, microcirculation and macrocirculation 2DPA images and TDCs. Except for TOA, all parameters are improved in the original, microcirculation and macrocirculation TDCs. In all three pre-interventional TDCs, a peak is seen between nine to eleven seconds, most likely corresponding to a movement artefact. Image quality, among which patient movement, was scored by an experienced interventional radiologist as minor.

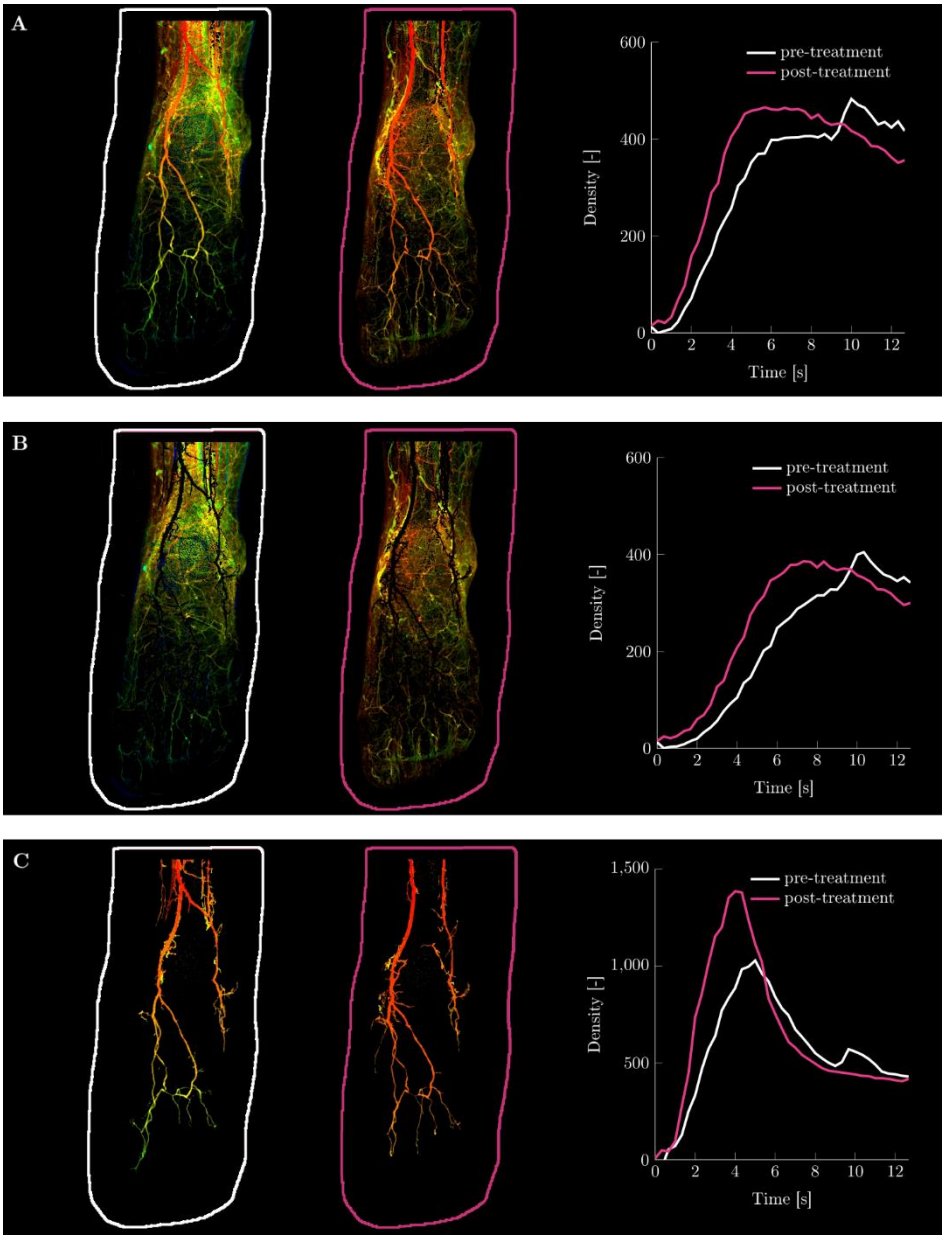


Figure 4.7 – Analysis of an anterior-posterior angiographic projection. ***(A)*** *Original pre-intervention and post-intervention perfusion angiogram. At the end of the pre-intervention curve a clear motion artefact can be observed.* ***(B)*** *Microcirculation pre-intervention and post-intervention perfusion angiogram. The time density curves show a clear capillary perfusion.* ***(C)*** *Macrocirculation pre-intervention and post-intervention perfusion angiogram. The time density curves show a clear arterial perfusion response.*

Table 4.2 – Perfusion parameters of the original, microcirculation and macrocirculation TDC. Arrows indicate if in- or decrease of the parameter is needed for improvement.

	Original		Microcirculation		Macrocirculation	
	Pre	Post	Pre	Post	Pre	Post
TOA (↓)	0.3	0.7	1	1	0.3	0.7
PD (↑)	403	461	351	386	1,028	1,385
TTP (↓)	6	4,3	9.3	6	4.3	3
WIR (↑)	84	92	45	74	283	477
FWHM (↓)	N/A	N/A	N/A	N/A	5.7	4.4
AUC (↑)	11 732	13 245	8 113	9 861	21 168	24 351
MTT (↓)	7.7	6.8	7.7	6.9	6.3	5.3

Pre = pre-intervention parameters, post = post-intervention parameters. TOA = time of arrival, PD = peak density, TTP = time to peak, WIR = wash-in rate, AUC = area under the curve, MTT = mean transit time. N/A = not available because of no after-peak density decrease to or below half of the peak-density.

Discussion

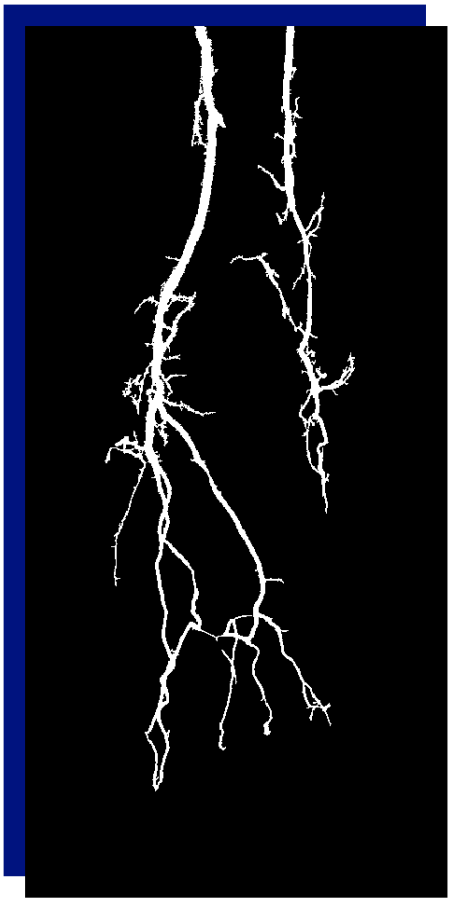
The two presented clinical cases show a typical microvascular (Figure 4.1 C, 4.6 B and 4.7 B) and arterial (Figure 4.1 B 4.6 C and 4.7 C) perfusion curve[71]. Large vessel extraction with subsequent separation of the micro- and macrocirculation is technically feasible and reduces the summation effect in 2DPA. Moreover, separation of the macro- and microcirculation allows for determining perfusion parameters of the microcirculation alone. In addition, new perfusion parameters such as peak-to-peak time can be introduced. This perfusion parameter describes the time between the micro- and macrocirculation to reach maximum intensity and can be interpreted as peripheral circulation time. The peak-to-peak time has already been shown to be a clinically relevant parameter in detecting carotid artery stenoses[71] and might serve as an objective perfusion parameter to predict delayed wound healing. Another indicative perfusion parameter which comes available with the separation of the micro- and macrocirculation that might be an indicative parameter for delayed wound healing is the width of the microcirculatory perfusion curve which can be quantified by the full width at half maximum (FWHM). Post-stenotic widening of the flow curve, known as "tardus parvus", is commonly observed by Doppler ultrasonography[72] and is observed in perfusion time-attenuation curves of MR imaging and CT perfusion studies as well[70,71,73]. Widening of the flow curve might indicate reduced microcirculatory inflow. Therefore, the presence of this phenomena in the microcirculation perfusion curve might be an indicative parameter for delayed wound healing as well.

Finally, it is interesting to explore the combination of our approach described above with the tolazoline administration as reported by Reekers et al. to assess the functionality of the microcirculation[51]. Combining these two approaches might be key for pre-interventional differentiation of patient sub-types with clinical identical CLI in those who might or might not benefit from revascularization attempts.

In conclusion, large vessels were successfully extracted from perfusion images, resulting in typical arterial and perfusion curves. Extraction of the macrocirculation is expected to enable better quantitative assessment of the microcirculation in CLI patients which will be the subject of future research.

5

General discussion and future perspectives



General discussion

The aim of this thesis was to investigate reproducibility and observer variability of 2D perfusion angiography, and to assess feasibility of extraction of the arteries. To this end, clinical data from the prospective, single arm REPEAT-study was used. Initial reproducibility of 2DPA was 64%. Reproducibility was achieved in three out of four unreproducible TDC pairs by removal of the frames before contrast arrival and inclusion of the same number of frames. Reproducibility after frame selection needs to be confirmed for all TDC pairs in future research. Intra- and inter-observer agreement of 2DPA demonstrated to be excellent for the ROI as proposed by Reekers et al.[51] Variability in Wound ROIs was relatively high, because wound locations were interpreted differently within and between observers. However, Reekers ROI also includes the large conducting arteries. Therefore, a method was developed to extract the large vessels from the perfusion images. Extraction was successful, resulting in typical arterial and tissue perfusion curves.

During the current studies, several important insights were obtained. First, as discovered during the reproducibility study, frame selection should be applied, in which frames before contrast arrival must be removed and when comparing two acquisitions, the number of frames must be equal. Second, the ROI must only include anatomical structures to prevent density changes in the background from distorting 2DPA results. Last, reconsideration about the used contrast agent in the REPEAT-study is needed, because iodixanol-320 (used in patient #1 to #4) appears to have only limited influence on reproducibility and iobitridol-300 (used in patient #5 to #11) might increase patient movement. Therefore, use of iodixanol-320 is proposed for the remainder of the REPEAT-study.

In literature regarding 2DPA in CLI patients, pre- and post-interventional differences are only determined by differences in the perfusion parameters alone.[51,56,62,63] In this thesis, reproducibility of two TDCs is determined by both comparison of the error between TDC_1 and TDC_2 and by the perfusion parameters. Comparing two curves using statistics appeared to be challenging. The two-sample Kolmogorov-Smirnov (KS) test is often used to compare two distributions, but was unsuitable for comparing TDCs, because the KS test assesses whether the underlying probability distributions differ[74]. When comparing TDCs, difference in distribution does not necessarily indicate unreproducible TDCs. Second, the coefficient of determination (R^2) is often used to compare the goodness-of-fit of regression models[75,76], however not suitable to test reproducibility of the TDCs. By determination of R^2, the predicted outcomes are tested for equality to the observed outcomes. When comparing TDCs, the goodness-of-fit of TDC_2 compared to TDC_1 is as important as the goodness-of-fit of TDC_1 compared to TDC_2.
In the current study, the normalized root mean square error is used to test reproducibility, the error between both curves needs to be quantified. This statistic expresses the magnitude of the error between two distributions, which is normalized to be able to compare results between patients with different density scales. However, no magnitude of the error is known for reproducible or unreproducible TDCs. Therefore, the assumption is made that the root mean square error may deviate by 10% of the maximum value of TDC_1 and TDC_2. This assumption was based on both visual assessment of the curves and expert discussions. In future research, the NRMSE to distinguish reproducible TDC pairs from unreproducible TDC pairs needs to be validated, e.g. by comparing the differences between pre- and post-interventional perfusion results of successfully and unsuccessfully treated CLI patients.

Additionally, patient movement is well-known to distort 2DPA results.[51,55,62] In this thesis, major movement artifacts showed to influence reproducibility. Although minor movement was visible on several DSA acquisitions, these movements did not influence reproducibility of 2DPA. Thereby,

no correlation was found between reproducibility and movement, however, this was only tested in a small study population. Visual assessment of the curves shows no substantial differences between TDCs with or without minor movement (Appendix B, e.g. Figure B4 and B.8), which may indicate that the overall sensitivity of 2DPA for patient movement is lower than described in literature, where up to 10% of perfusion datasets were not usable due to motion artifacts.[51] Nevertheless, it is important to realize that contrast inflow and movement both cause density increase. Therefore, distinction between contrast inflow and movement may not be possible based on TDC analyses alone. Accordingly, false positive density changes caused by motion artifacts should be automatically identified.
To overcome the influence of movement on 2DPA results, the following procedural steps must be performed. First, prevention of any movement should be pursued. With the current foot limited movement is still possible. Both the heel and the toes must be fixed to obtain the best results. However, this might be challenging due to the patients' wounds. Second, use of anterior-posterior acquisitions might be preferred over lateral acquisitions, because movement of the toes in cranial direction influences AP acquisitions less than lateral acquisitions. Third, build-in motion correction algorithms would be an ideal solution to resolve motion artifacts. Unfortunately, these algorithms are currently insufficient to overcome distortions caused by patient movement and should be further developed.

Furthermore, absolute values of 2DPA should not be compared between patients. While comparing two acquisitions, the current algorithm adjusts absolute density values based on *both* images. As an effect of this algorithm, comparing perfusion acquisition A with two different acquisitions, B and C, the absolute values vary between both datasets of A. However, the trend in both datasets of A is equal, i.e. the differences between datapoint #1, #2, #3, etc. are equal. While interpreting the results of 2DPA, the observer needs to be aware of this algorithm. Therefore, on the Interventional Work Spot, the y-values should be expressed as percentages of the maximum density difference of both acquisitions.

In addition, during performance of the study protocol, the workflow to acquire perfusion images appeared to be suboptimal. First, storing of table and acquisition settings was inconvenient, because settings were easily overwritten, but identical settings are necessary to be able to compare pre- and post-interventional perfusion results. Moreover, it is currently not possible to store multiple table positions, which is useful when lateral and AP acquisitions are obtained. Second, automated position matching between pre- and post-interventional acquisitions is not possible with the current system, which is needed in case of position changes between pre- and post-interventional acquisitions. Third, drawing ROIs on the current Interventional Work Spot has some drawbacks as well. It is not possible to save or copy ROIs within or between patients. Adjustment of free-hand ROIs is not possible either. For successful use in a clinical setting, these drawbacks of the current 2DPA system need to be addressed to increase ease of use.

Moreover, new insights were obtained regarding the perfusion parameters. In 2DPA of the foot, full width at half maximum (FWHM) can often not be determined, because densities do not decrease lower than or to half maximum. This is caused by a combination of two effects. First, complete outflow of contrast takes quite long in these patients, because arterial outflow is often prolonged in patients who suffer from arterial disease. Current perfusion software includes only ninety frames for determination of the TDC, which appears to be too short to achieve complete arterial outflow in this patient population. By inclusion of more frames, complete contrast outflow might be included in the TDC. However, this might increase the risk of movement. Therefore, FWHM might be a parameter which is clinically not possible to determine in CLI patients. In future clinical research, this absence of density decrease should be confirmed or contradicted.

Last, the macrocirculation was extracted from 2DPA images to enable perfusion assessment of the whole foot without the influence of the large arteries. First results show that the method successfully separates the macrocirculation from the microcirculation, which should be validated in future research. In addition, further automation of the current method is needed, because the current separation method is only semi-automated. Therefore, application to individual patients is time consuming, and therefore not easily applied in real time during revascularization procedures.

Future perspectives

Prior to introduction for broad clinical use, application of 2DPA measurements requires clinical validation in larger prospective studies using clinically relevant outcomes. To this end, the REPEAT-study was initiated. Once it has been demonstrated that 2DPA results are consistent and reproducible, further studies can be performed to investigate the clinical applicability of 2DPA in evaluating adequacy of endovascular therapy.

In addition, many other applications of 2DPA are probably possible, such as identification of different sub-types of patients. Identification of these sub-types could play a crucial role in predicting which patients will benefit from revascularization and which patients will not. Separation of the macrocirculation and microcirculation might help achieving this goal, because it enables selective microvascular perfusion assessment.

Last, new insights obtained by this approach, may eventually be translated to perfusion MR imaging and/or perfusion CTA. If possible, this would allow for non-invasive assessment of revascularization options and probability of wound healing without the need for DSA imaging.

Conclusion

In conclusion, 2DPA is a promising technique that allows quantitative assessment of perfusion changes following interventions in patients with CLI. However, optimization of the current system is needed to ensure ease of use during clinical practice. Reproducibility of 2DPA should be researched further. Based on inter-observer reliability analyses, Reekers ROI is preferred over Wound ROI. To assess tissue perfusion alone while using Reekers ROI, a vessel extraction method is successfully developed, which makes analyses of the microcirculation feasible. Clinical validation studies, which are currently conducted, have to confirm the added value of 2DPA and of this extraction method.

Verantwoording

Tijdens mijn afstuderen heb ik mij niet alleen ontwikkeld op wetenschappelijk gebied, maar ook op klinisch en persoonlijk gebied. In deze verantwoording bespreek ik deze ontwikkelingen: op klinisch gebied heb ik mij voornamelijk ontwikkeld door mijn verantwoordelijkheden omtrent de REPEAT-studie en op persoonlijk gebied heb ik voornamelijk mijn communicatie met begeleiders verder ontwikkeld.

Klinische ontwikkeling

In de eerste weken van mijn afstuderen hebben verschillende betrokkenen van de REPEAT-studie me geleerd hoe patiënten worden geselecteerd en geïnformeerd, hoe de procedure wordt uitgevoerd en hoe het follow-up traject eruitziet. In januari ben ik verantwoordelijk geworden voor de studie en allereerst begonnen met het zelfstandig includeren van patiënten.

In de eerste twee maanden wilde er slechts één patiënt deelnemen aan de studie terwijl ik ongeveer vijf geschikte patiënten heb geïnformeerd. Dit was reden om het patiëntinformatiegesprek te evalueren met mijn medisch begeleider. Allereerst bleek het om een lastige patiëntenpopulatie te gaan. Patiënten hadden vaak veel comorbiditeiten en wilden daarom niet nog meer 'gedoe aan hun lijf'. Daarnaast kwam naar voren dat ik de patiënt te goed wilde informeren. Ik wilde de patiënt de studie volledig laten begrijpen en daarnaast inzicht geven in de precieze gevolgen. In mijn ogen was dit voor de patiënt noodzakelijk om een weloverwogen keuze te maken. Echter bleken niet alle patiënten in deze uitleg geïnteresseerd en mijn uitleg bleek vaak te uitgebreid en complex. Door dit evaluatiemoment zag ik in dat ik de inhoud van het gesprek bepaalde op basis van mijn eigen behoeftes. Als ik namelijk in de situatie van patiënt zou verkeren, zou ik alles over de studie willen weten. Daarom betekent een goed patiëntinformatiegesprek voor mij persoonlijk een duidelijke en volledige uitleg over de studie. De behoefte van de patiënt bleek echter anders te liggen, omdat veel patiënten alleen interesse hadden in de directe extra handelingen die van hen werden verwacht. Wanneer ik ruimte liet voor verdiepende vragen en verwees naar het patiëntinformatieformulier, bleken maar weinig patiënten meer over de studie te willen weten dan dat ik in het kort had verteld. Het korter en bondiger insteken van het gesprek bleek een positief effect te hebben op het aantal patiënten dat wilde deelnemen aan de studie. Uiteindelijk heb ik in totaal acht patiënten kunnen includeren in een periode van zeven maanden. Het includeren van patiënten heeft mij niet alleen inzicht gegeven vanuit welke behoeftes ik het patiëntinformatiegesprek voerde, ook heb ik steeds meer inzicht gekregen in de patiëntenpopulatie en op welke manier zij worden behandeld. In de eerste maanden van mijn afstuderen was er veel overleg nodig met mijn medisch begeleiders over de geschiktheid van kandidaten. Naarmate ik meer ervaring kreeg in het selecteren en includeren van patiënten, werd ik steeds zekerder over mijn inschatting omtrent de geschiktheid van mogelijke kandidaten. Hierdoor was er steeds minder overleg met mijn begeleiders nodig en was het voldoende om de geschiktheid van kandidaten te controleren en randgevallen te bespreken. Ook merkte ik dat mijn begeleiders steeds meer vertrouwden op mijn oordeel over de geschiktheid van kandidaten.

Nadat de patiënt het toestemmingsformulier had ondertekend, zorgde ik er tijdens de ingreep voor dat de perfusie opnamen volgens studieprotocol werden uitgevoerd. Allereerst moest de patiënt op een bepaalde manier op tafel komen te liggen. In het begin vond ik het lastig om de laboranten op te dragen hoe zij dit moesten doen, omdat zij in mijn ogen meer verstand van zaken hadden. Echter zag ik na enkele procedures in dat zij over het algemeen meer verstand van zaken

hebben op de angiokamer, maar wanneer het de ingreep volgens studieprotocol betrof, bleek ik meer verstand van zaken te hebben. Daarnaast zag ik tijdens de procedures het belang van de juiste uitvoering steeds beter in, waardoor ik ook inzag dat het voor de studie juist belangrijk was dat ik de laboranten vertelde hoe het protocol moest worden uitgevoerd. Hierdoor kon ik gemakkelijker aangeven wat de laboranten moesten doen.
Een tweede uitdaging tijdens de uitvoering van het studieprotocol was de tijd die er moest worden gewacht. Als onderdeel van het protocol moest er namelijk eenmaal vijf minuten en eenmaal tien minuten worden gewacht. In deze tijd kon de behandelend arts weinig andere werkzaamheden verrichten doordat hij steriel moest blijven. Dit zorgde echter bijna alle keren voor ongeduld van de arts. Het was aan mij om niet toe te geven en pas na tien minuten toestemming te geven voor de opnames. Dit bleek voornamelijk lastig wanneer het programma op de angiokamer erg vol was. De kennis dat deelname van de patiënt niet zinvol was wanneer ik toegaf, heeft me geholpen om voet bij stuk te houden. Van beide situaties heb ik geleerd dat ik voor mijn kennis en verantwoordelijkheden ga staan wanneer ik weet waar ik het voor doe en er het belang van inzie.

Na de ingreep, is het belangrijk de follow-up van patiënten goed te organiseren. Dit om de beelden die we voor de studie hebben verkregen, te kunnen relateren aan de klinische uitkomst van de patiënt. Het was mijn verantwoordelijkheid deze afspraken te plannen en de follow-up gesprekken uit te voeren. De organisatie van deze gesprekken bleek voornamelijk een uitdaging doordat afspraken vaak werden geannuleerd wanneer patiënten geen klachten meer hadden. Dit omdat het niet bekend was dat het om een studie gerelateerde afspraak ging. Dit heb ik zoveel mogelijk proberen te voorkomen door de behandelend arts bij de eerste post-interventie afspraak te informeren over de follow-up afspraak die op komst was en door regelmatig te controleren of alle afspraken daadwerkelijk in het systeem bleven staan. Dit bleek voldoende te zijn, ik heb namelijk geen follow-up momenten gemist. Echter was ik af en toe het overzicht kwijt, waardoor ik een aantal keer na heb moeten vragen of afspraken nu wel of niet waren ingepland (deze orders waren voor mij niet zichtbaar in het EPD). In het vervolg zou ik taken waar veel tijd overheen gaat bijhouden in aparte documenten, zodat ik op ieder moment na kan gaan of en wanneer afspraken zijn aangevraagd en ingepland.
Een tweede taak die bij de follow-up van geïncludeerde patiënten kwam kijken, was de uitvoering van het follow-up gesprek. Omdat het om aanvullende vragen ging, voerde ik dit gesprek zelfstandig uit. Doordat ik meerdere keren had meegelopen op de poli vaatchirurgie, merkte ik al tijdens het eerste follow-up gesprek dat het vooral belangrijk is om mijn vragen duidelijk te stellen en zo min mogelijk aan de interpretatie van de patiënt over te laten. Dit om te voorkomen dat patiënten antwoord geven op een hele andere vraag. Een aantal weken geleden werd dit belang onderstreept toen een patiënt voor zijn follow-up afspraak kwam, maar ook klachten had aan zijn contralaterale been. Doordat ik in mijn vraagstelling duidelijk naar het studie gerelateerde, en dus genezen been vroeg, kreeg ik de antwoorden die nodig waren voor het onderzoek.

Enkele patiënten werden in het St. Antonius Ziekenhuis behandeld na te zijn doorverwezen vanuit het Rivierenland Ziekenhuis in Tiel. Controleafspraken van deze patiënten vonden weer in het Rivierenland Ziekenhuis plaats. Indien deze patiënten wilden deelnemen aan de studie, was het voor hen erg prettig wanneer hun follow-up in het Rivierenland kon plaatsvinden. Ondanks dat enkele vaatchirurgen uit het St. Antonius ook in het Rivierenland werken, was deze samenwerking nog niet geregeld. Daarom was de taak aan mij om dit te regelen. In overleg met mijn medisch begeleider heb ik contact gezocht met de juiste personen in het Rivierenland en was samenwerking snel geregeld. Hierdoor was aan mij slechts de taak een kort protocol op te stellen, door te geven wanneer welke patiënt voor een follow-up gesprek moest komen en na afloop van de follow-up gegevens op te vragen.

Persoonlijke ontwikkeling

Aan het begin van mijn afstudeerstage gaf een van mijn medisch begeleiders direct aan niet altijd even goed bereikbaar te zijn. Daarnaast zijn er veel verschillende begeleiders betrokken bij dit project. Om van mijn afstuderen een succes te maken, was goede communicatie daarom vanaf het begin een belangrijk aandachtspunt.

Doordat mijn medisch begeleider direct aangaf matig bereikbaar te zijn, was het vanaf het begin duidelijk dat ik volhardend moest zijn wanneer ik zijn hulp nodig had. Terugkijkend op de afgelopen maanden heb ik weinig problemen ervaren met zijn bereikbaarheid. Dit komt aan de ene kant doordat ik volhardend ben geweest wanneer ik mijn begeleider moest spreken, maar ook doordat ik twee vaardigheden verder heb ontwikkeld. Allereerst ben ik beter geworden in het onderscheiden van situaties waarin veel tijd is voor overleg en situaties waarin weinig tijd is. Inzicht in de situatie van mijn begeleider heeft me hier voornamelijk bij geholpen. Het was al snel duidelijk dat zijn matige bereikbaarheid geen onwil was, maar dat de kliniek simpelweg voorging. Wanneer mijn begeleider een 'klinische dag' had, was er weinig tijd en was er in mijn ogen alleen ruimte voor dringende vragen. Om mijn minder dringende en uitgebreidere vragen te kunnen stellen, zorgde ik ervoor dat ik inzicht kreeg in de planning van mijn begeleider en dus wanneer hij op de angiokamer stond en wanneer niet. Hierdoor lukte het vrijwel altijd mijn vragen binnen een week uitgebreid te bespreken. Als tweede heb ik geleerd om duidelijk te communiceren en dit af te stemmen op de situatie. Op drukke, klinische dagen zorgde ik ervoor dat ik mijn vragen kort en duidelijk formuleerde, zodat mijn vraag snel helder was en mijn begeleider wist wat ik van hem verwachtte. Op momenten dat er meer tijd was, kon ik situaties uitgebreider schetsen en was er meer ruimte voor informeel contact en inhoudelijke discussies.

Op het gebied van communicatie heb ik een tweede uitdaging ervaren doordat ik door verschillende medici begeleid werd. Hierdoor was afstemming tussen mijn begeleiders erg belangrijk. Wat deze afstemming allereerst lastig maakte, was dat de klinische planning van twee begeleiders een week van tevoren bekend werd, terwijl de agenda's van mijn andere begeleiders juist weken van tevoren volstroomden. Dit veroorzaakte in het begin wat problemen met het plannen van afspraken met meerdere begeleiders, maar uiteindelijk bleek dit vaak wel los te lopen door mijn begeleiders tijdig te herinneren aan de geplande afspraak. Meer richting het einde van mijn afstuderen loste ik dit vaak op door afzonderlijk met mijn begeleiders te overleggen. Om hun ideeën op elkaar afgestemd te houden, heb ik samenvattende mails gestuurd waarin ik mijn begeleiders vroeg commentaar direct met elkaar te bespreken. Dit om te voorkomen dat ik af moest blijven stemmen tussen verschillende begeleiders. Door op deze manier met mijn begeleiders te communiceren, leverden deze overleggen weinig vertraging op.

Conclusie

Tijdens mijn afstuderen heb ik mij op verschillende gebieden verder ontwikkeld, waarin ik verscheidene zaken meeneem in mijn verdere loopbaan. Doordat ik mijn inzicht in de situatie van mijn gesprekspartner verder heb ontwikkeld, leerde ik ook om mijn communicatie beter af te stemmen op de situatie van mijn gesprekspartner. Dit heb ik zowel ervaren in mijn communicatie met begeleiders als in mijn communicatie met patiënten.

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Appendices

Appendix A: TASC II criteria

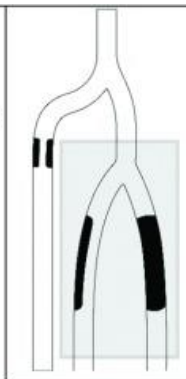
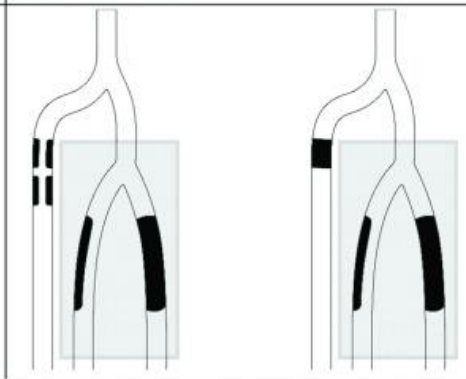
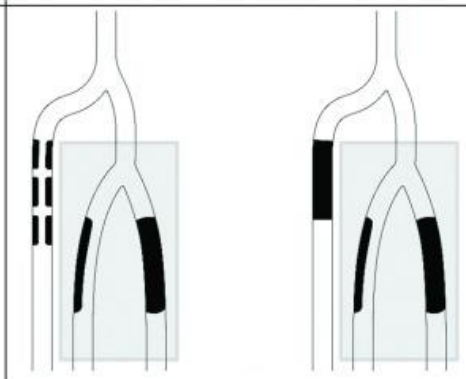
TASC A lesions Single focal stenosis, ≤5 cm in length, in the target tibial artery with occlusion or stenosis of similar or worse severity in the other tibial arteries.	
TASC B lesions Multiple stenoses, each ≤5 cm in length, or total length ≤10 cm or single occlusion ≤3 cm in length, in the target tibial artery with occlusion or stenosis of similar or worse severity in the other tibial arteries.	
TASC C lesions Multiple stenoses in the target tibial artery and/or single occlusion with total lesion length >10 cm with occlusion or stenosis of similar or worse severity in the other tibial arteries.	
TASC D lesions Multiple occlusions involving the target tibial artery with total lesion length >10 cm or dense lesion calcification or non-visualization of collaterals. The other tibial arteries occluded or dense calcification.	

Figure A.1 – Trans-Atlantic Inter-Society Consensus for the Management of Peripheral Arterial Disease (TASC II) classification of below-the-knee lesions. The unshaded area represents the target lesions and the area within the gray rectangle represents non-target lesions. In the represented situation, the anterior tibial artery is the target lesion.[6]

Appendix B: pre- and post-interventional time density curves

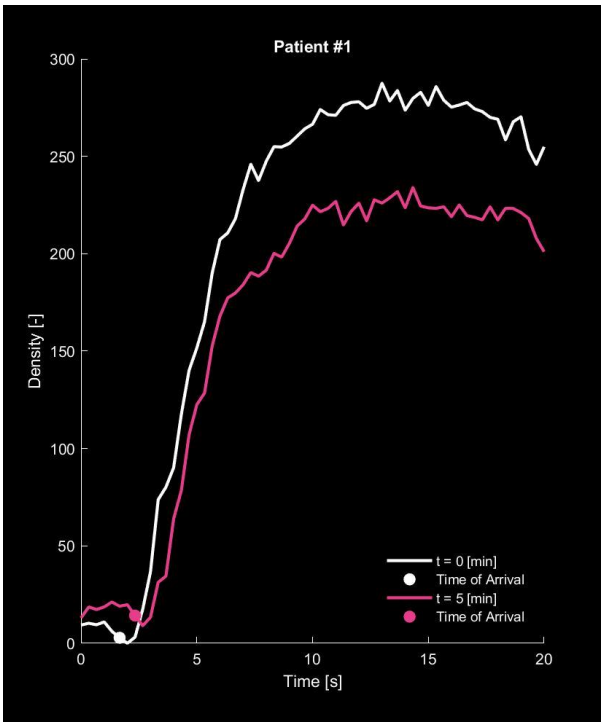


Figure B.1 – TDC_1 and TDC_2 of patient #1, NRMSE = 0.15

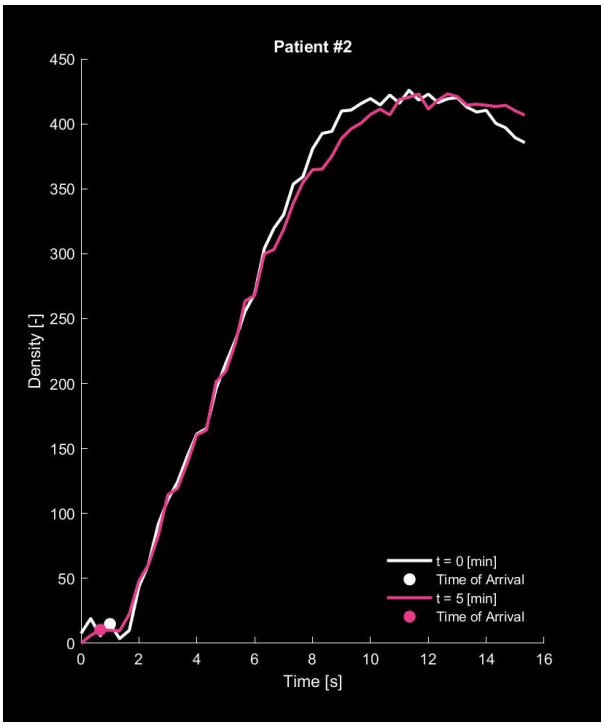


Figure B.2 – TDC_1 and TDC_2 of patient #2, NRMSE = 0.05

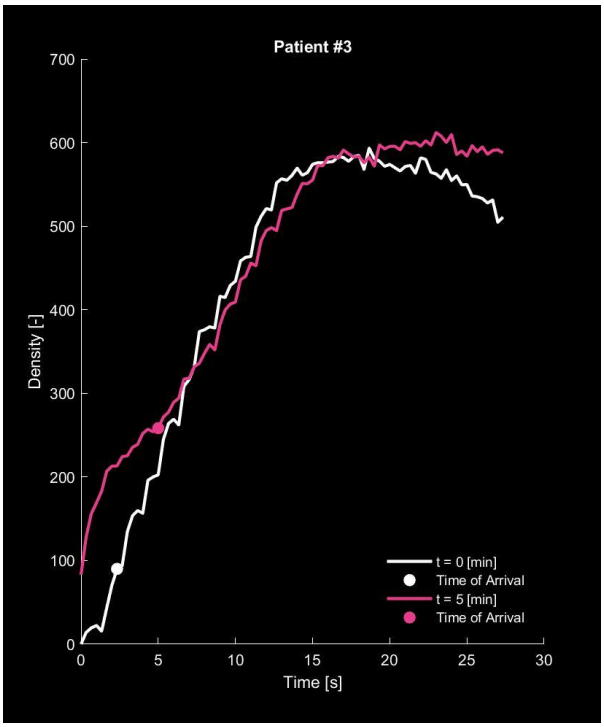


Figure B.3 – TDC_1 and TDC_2 of patient #3, NRMSE = 0.11

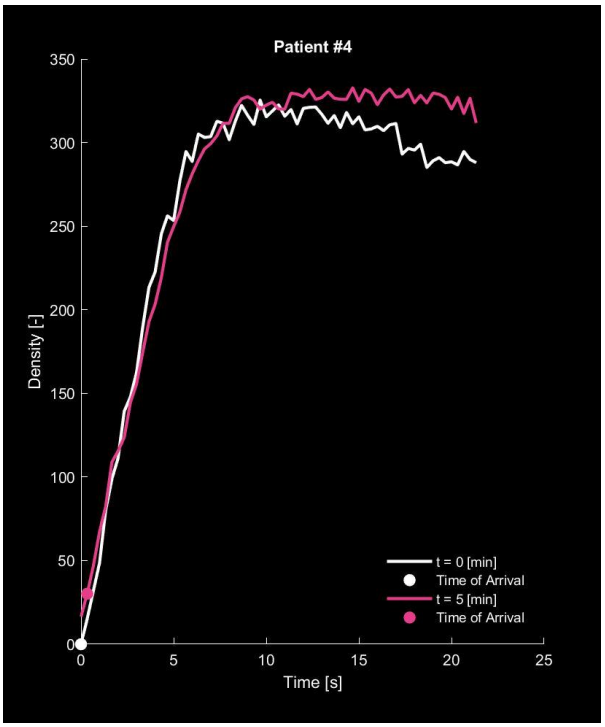


Figure B.4 – TDC_1 and TDC_2 of patient #4, NRMSE = 0.06

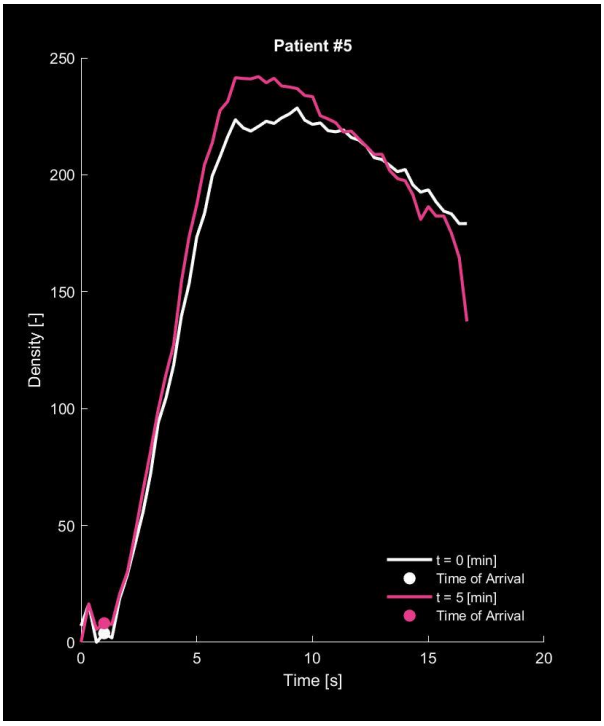


Figure B.5 – TDC_1 and TDC_2 of patient #5, NRMSE = 0.05

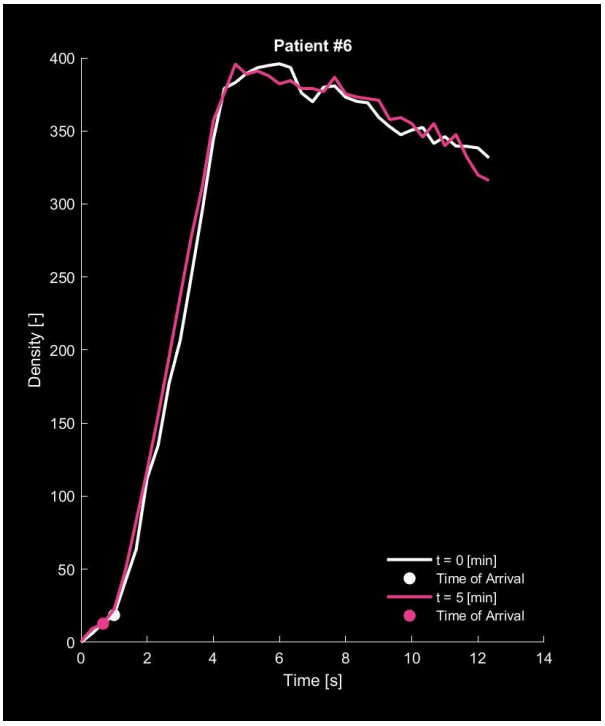


Figure B.6 – TDC_1 and TDC_2 of patient #6, NRMSE = 0.03

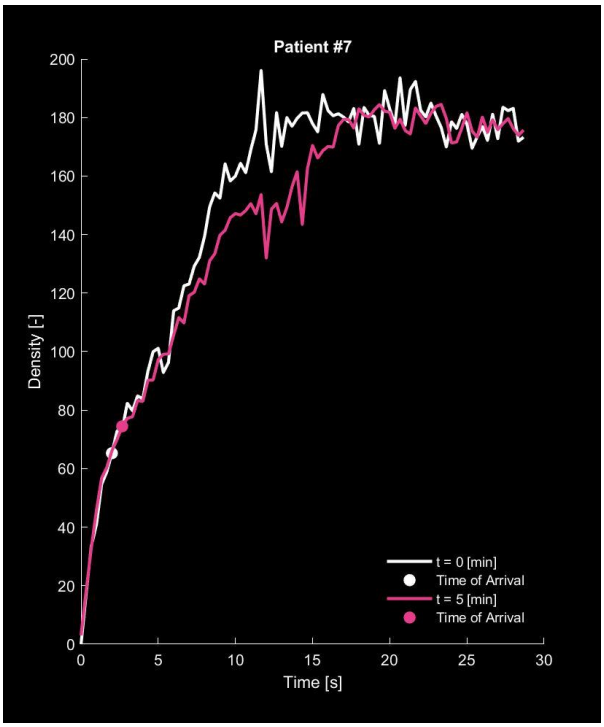


Figure B.7 – TDC_1 and TDC_2 of patient #7, NRMSE = 0.06

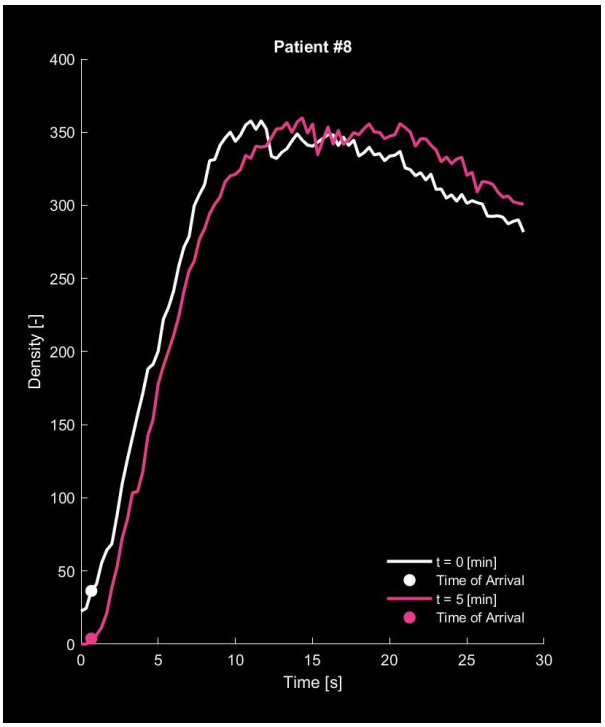


Figure B.8 – TDC_1 and TDC_2 of patient #8, NRMSE = 0.07

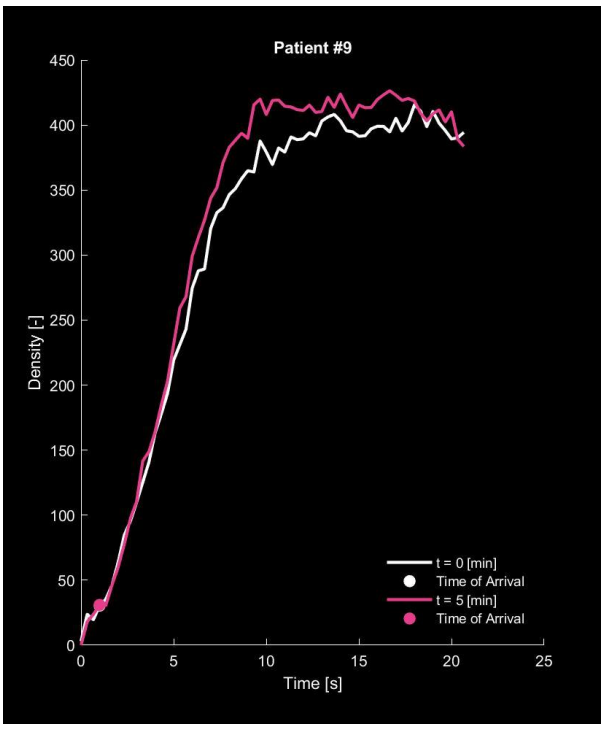


Figure B.9 – TDC_1 and TDC_2 of patient #9, NRMSE = 0.05

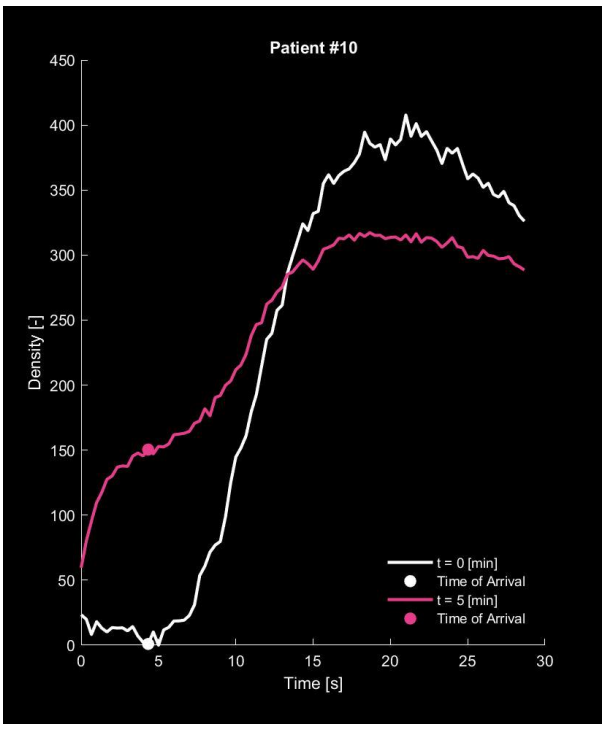


Figure B.10 – TDC_1 and TDC_2 of patient #10, NRMSE = 0.20

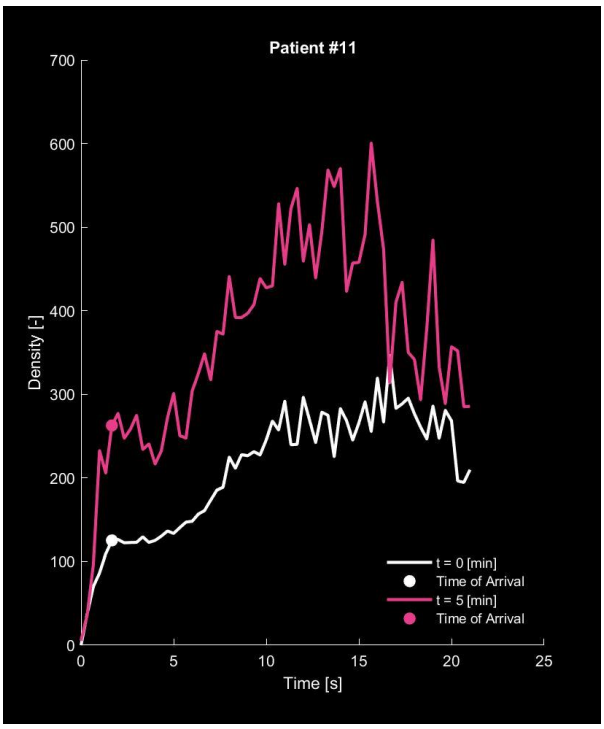
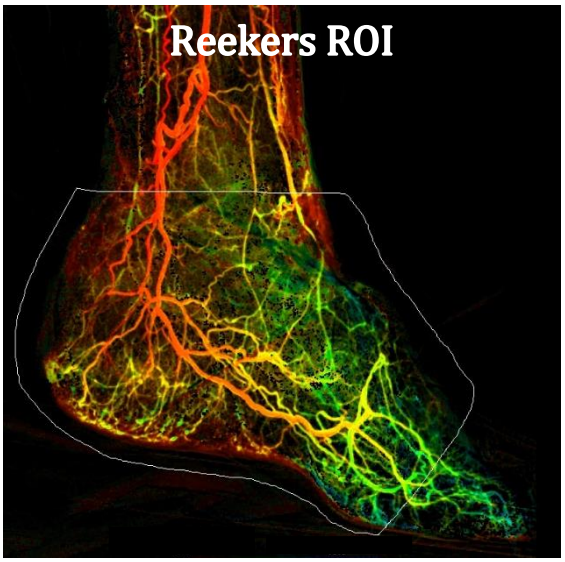


Figure B.11 – TDC_1 and TDC_2 of patient #11, NRMSE = 0.30

Appendix C: ROI manual

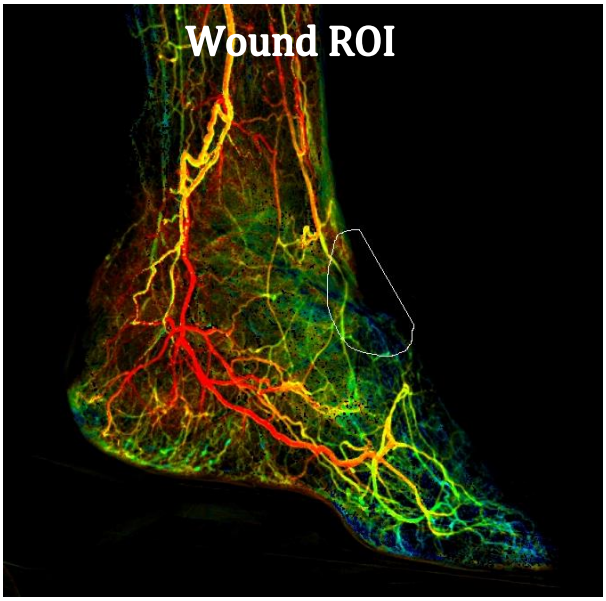
Reekers ROI

- Reekers ROI includes the ankle and foot, but no toes
- Use the freehand ROI tool
- Proximal boundary: draw a horizontal line at the transition of lower leg into ankle. If visible, use the most distal point of the tibiotalar joint.
- Distal boundary: draw a straight line so that the metatarsal arteries lay in the ROI
- Minimize the presence of non-foot background ROI



Wound ROI

- Use the wound photo to determine the wound location
- Use the freehand ROI tool
- Draw the ROI a centimeter around all wound edges



Appendix D: NRMSE for intra-observer variability

Table D.1 – Normalized root mean square errors for five Reekers ROIs drawn by one observer (AJ) in patient #1.

	Anterior-posterior view					Lateral view				
	ROI 1	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**	**ROI 1**	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**
ROI 1	-	0.02	0.03	0.07	0.06	-	0.01	0.01	0.01	0.01
ROI 2	0.02	-	0.04	0.08	0.07	0.01	-	0.01	0.01	0.00
ROI 3	0.03	0.04	-	0.05	0.04	0.01	0.01	-	0.00	0.01
ROI 4	0.07	0.08	0.05	-	0.01	0.01	0.01	0.00	-	0.01
ROI 5	0.06	0.07	0.04	0.01	-	0.01	0.00	0.01	0.01	-

Table D.2 – Normalized root mean square errors for five Reekers ROIs drawn by one observer (AJ) in patient #3.

	Anterior-posterior view					Lateral view				
	ROI 1	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**	**ROI 1**	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**
ROI 1	-	0.03	0.03	0.02	0.04	-	0.02	0.00	0.01	0.00
ROI 2	0.03	-	0.01	0.01	0.00	0.02	-	0.01	0.01	0.01
ROI 3	0.03	0.01	-	0.00	0.01	0.00	0.01	-	0.01	0.00
ROI 4	0.02	0.01	0.00	-	0.01	0.01	0.01	0.01	-	0.01
ROI 5	0.04	0.00	0.01	0.01	-	0.00	0.01	0.00	0.01	-

Table D.3 – Normalized root mean square errors for five Reekers ROIs drawn by one observer (AJ) in patient #4.

	Anterior-posterior view					Lateral view				
	ROI 1	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**	**ROI 1**	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**
ROI 1	-	0.00	0.02	0.03	0.02	-	0.03	0.03	0.03	0.04
ROI 2	0.00	-	0.02	0.04	0.02	0.03	-	0.06	0.05	0.06
ROI 3	0.02	0.02	-	0.01	0.01	0.03	0.06	-	0.00	0.01
ROI 4	0.03	0.04	0.01	-	0.02	0.03	0.05	0.00	-	0.01
ROI 5	0.02	0.02	0.01	0.02	-	0.04	0.06	0.01	0.01	-

Table D.4 – Normalized root mean square errors for five Reekers ROIs drawn by one observer (AJ) in patient #8.

	Anterior-posterior view					Lateral view				
	ROI 1	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**	**ROI 1**	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**
ROI 1	-	0.02	0.04	0.04	0.04	-	0.00	0.01	0.03	0.01
ROI 2	0.02	-	0.03	0.02	0.02	0.00	-	0.00	0.02	0.01
ROI 3	0.04	0.03	-	0.01	0.01	0.01	0.00	-	0.02	0.00
ROI 4	0.04	0.02	0.01	-	0.01	0.03	0.02	0.02	-	0.02
ROI 5	0.04	0.02	0.01	0.01	-	0.01	0.01	0.00	0.02	-

Table D.5 – Normalized root mean square errors for five Reekers ROIs drawn by one observer (AJ) in patient #10.

	Anterior-posterior view					Lateral view				
	ROI 1	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**	**ROI 1**	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**
ROI 1	-	0.04	0.01	0.02	0.05	-	0.01	0.02	0.01	0.00
ROI 2	0.04	-	0.04	0.02	0.01	0.01	-	0.03	0.01	0.01
ROI 3	0.01	0.04	-	0.02	0.05	0.02	0.03	-	0.02	0.02
ROI 4	0.02	0.02	0.02	-	0.03	0.01	0.01	0.02	-	0.01
ROI 5	0.05	0.01	0.05	0.03	-	0.00	0.01	0.02	0.01	-

Table D.6 – Normalized root mean square errors for five Wound ROIs drawn by one observer (AJ) in patient #1.

Wound 1										
	Anterior-posterior view					**Lateral view**				
	ROI 1	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**	**ROI 1**	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**
ROI 1	-	0.04	0.14	0.13	0.07	-	0.14	0.11	0.09	0.07
ROI 2	0.04	-	0.14	0.13	0.07	0.14	-	0.03	0.05	0.08
ROI 3	0.14	0.14	-	0.01	0.08	0.11	0.03	-	0.02	0.04
ROI 4	0.13	0.13	0.01	-	0.07	0.09	0.05	0.02	-	0.02
ROI 5	0.07	0.07	0.08	0.07	-	0.07	0.08	0.04	0.02	-
Wound 2										
ROI 1	-	0.10	0.24	0.23	0.11					
ROI 2	0.10	-	0.21	0.21	0.03					
ROI 3	0.24	0.21	-	0.02	0.20					
ROI 4	0.23	0.21	0.02	-	0.20					
ROI 5	0.11	0.03	0.20	0.20	-					

Table D.7 – Normalized root mean square errors for five Wound ROIs drawn by one observer (AJ) in patient #3.

	Anterior-posterior view					**Lateral view**				
	ROI 1	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**	**ROI 1**	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**
ROI 1	-	0.03	0.04	0.02	0.03	-	0.06	0.05	0.23	0.06
ROI 2	0.03	-	0.02	0.01	0.01	0.06	-	0.09	0.22	0.05
ROI 3	0.04	0.02	-	0.02	0.02	0.05	0.09	-	0.26	0.11
ROI 4	0.02	0.01	0.02	-	0.01	0.23	0.22	0.26	-	0.20
ROI 5	0.03	0.01	0.02	0.01	-	0.06	0.05	0.11	0.20	-

Table D.8 – Normalized root mean square errors for five Wound ROIs drawn by one observer (AJ) in patient #4.

	Anterior-posterior view					**Lateral view**				
	ROI 1	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**	**ROI 1**	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**
ROI 1	-	0.04	0.02	0.01	0.06	-	0.02	0.01	0.10	0.13
ROI 2	0.04	-	0.03	0.05	0.07	0.02	-	0.02	0.08	0.11
ROI 3	0.02	0.03	-	0.03	0.05	0.01	0.02	-	0.10	0.13
ROI 4	0.01	0.05	0.03	-	0.04	0.10	0.08	0.10	-	0.03
ROI 5	0.06	0.07	0.05	0.04	-	0.13	0.11	0.13	0.03	-

Table D.8 – Normalized root mean square errors for five Wound ROIs drawn by one observer (AJ) in patient #8.

Wound 1										
	Anterior-posterior view					**Lateral view**				
	ROI 1	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**	**ROI 1**	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**
ROI 1	-	0.01	0.04	0.02	0.01	-	0.31	0.33	0.30	0.40
ROI 2	0.01	-	0.03	0.02	0.01	0.31	-	0.04	0.03	0.15
ROI 3	0.04	0.03	-	0.05	0.04	0.33	0.04	-	0.04	0.11
ROI 4	0.02	0.02	0.05	-	0.01	0.30	0.03	0.04	-	0.15
ROI 5	0.01	0.01	0.04	0.01	-	0.40	0.15	0.11	0.15	-
Wound 2										
ROI 1	-	0.04	0.02	0.04	0.06	-	0.14	0.19	0.16	0.15
ROI 2	0.04	-	0.03	0.04	0.02	0.14	-	0.14	0.04	0.05
ROI 3	0.02	0.03	-	0.05	0.05	0.19	0.14	-	0.15	0.15
ROI 4	0.04	0.04	0.05	-	0.03	0.16	0.04	0.15	-	0.02
ROI 5	0.06	0.02	0.05	0.03	-	0.15	0.05	0.15	0.02	-

Table D.10 – Normalized root mean square errors for five Wound ROIs drawn by one observer (AJ) in patient #10.

	Anterior-posterior view					**Lateral view**				
	ROI 1	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**	**ROI 1**	**ROI 2**	**ROI 3**	**ROI 4**	**ROI 5**
ROI 1	-	0.05	0.09	0.06	0.06	-	0.08	0.12	0.04	0.03
ROI 2	0.05	-	0.05	0.04	0.01	0.08	-	0.15	0.08	0.09
ROI 3	0.09	0.05	-	0.03	0.05	0.12	0.15	-	0.12	0.11
ROI 4	0.06	0.04	0.03	-	0.03	0.04	0.08	0.12	-	0.06
ROI 5	0.06	0.01	0.05	0.03	-	0.03	0.09	0.11	0.06	-

Appendix E: figures of intra-observer variability

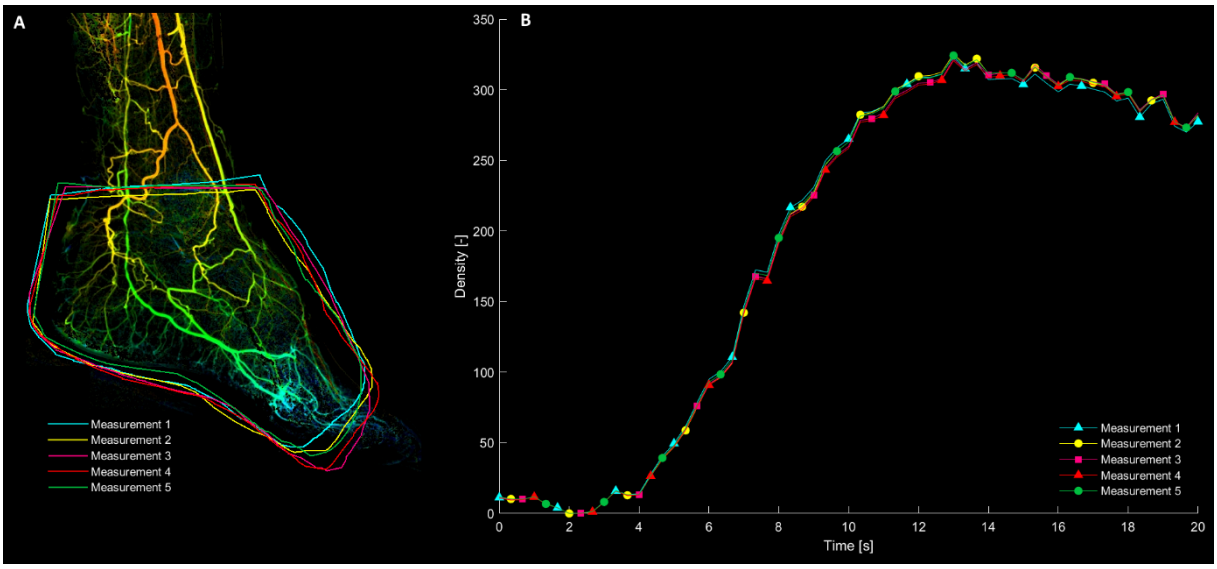


Figure E.1 – Reproducible Reekers ROIs drawn by one observer (AJ) in patient #1. ***(A)*** *lateral perfusion image with Reekers ROIs. The perfusion image represents time of arrival.* ***(B)*** *TDCs corresponding to Reekers ROIs drawn in* ***(A)***

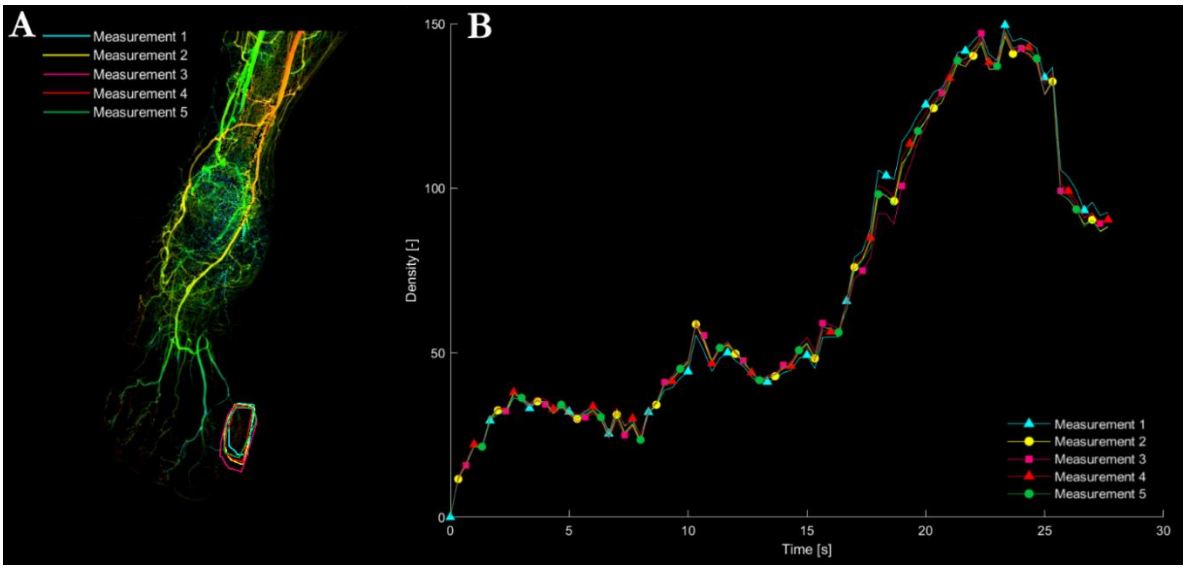


Figure E.2 –Reproducible Wound ROIs drawn by one observer (AJ) in patient #3. ***(A)*** *AP perfusion image with Wound ROIs. The perfusion image represents time of arrival.* ***(B)*** *TDCs corresponding to Wound ROIs drawn in* ***(A)***

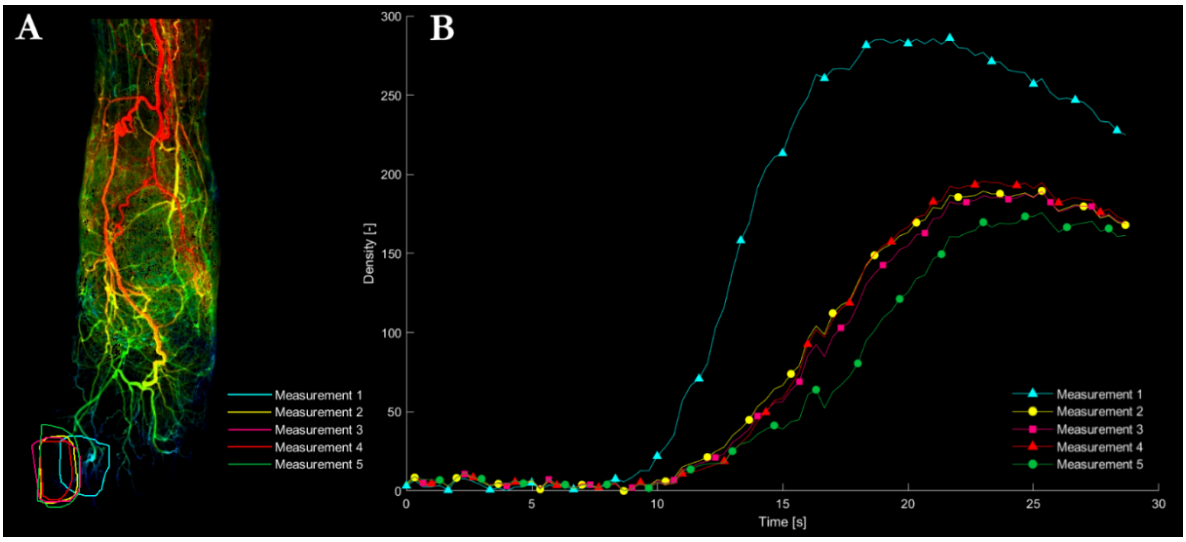


Figure E3 – Unreproducible Wound ROIs drawn by one observer (AJ) in patient #8. ROI 1 and 5 differ from all four other ROIs. ROI 2, 3 and 4 are equal to each other. ***(A)*** *AP perfusion image with Wound ROIs. The perfusion image represents time of arrival.* ***(B)*** *TDCs corresponding to Wound ROIs drawn in* ***(A)***

Appendix F: figures of inter-observer variability

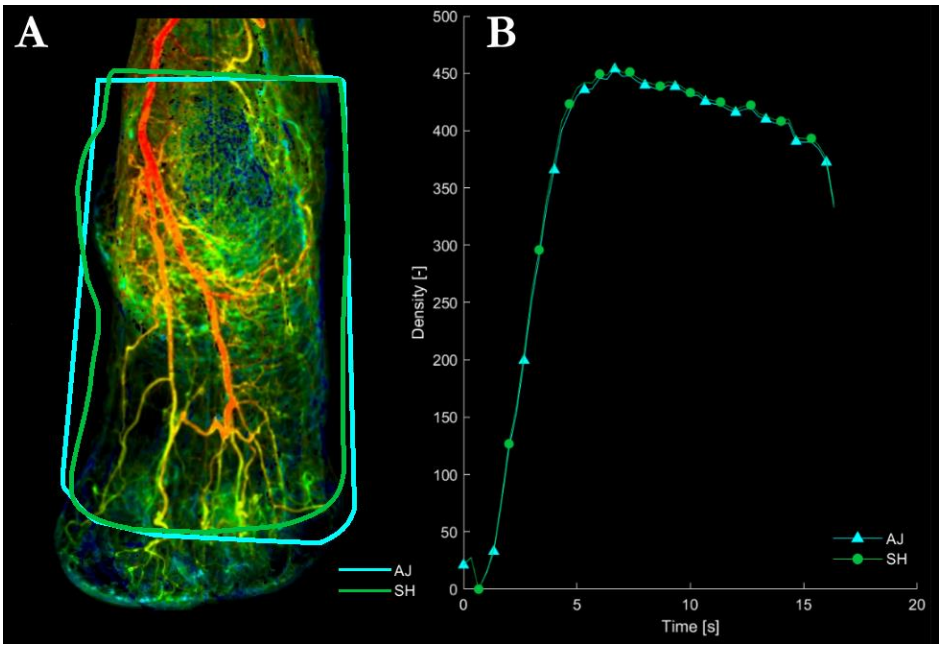


Figure F.1 – Reproducible Reekers ROIs drawn by two observers (AJ and SH) in patient #6. ***(A)*** *AP perfusion image with Reekers ROIs. The perfusion image represents time of arrival.* ***(B)*** *TDCs corresponding to Reekers ROIs drawn in* ***(A)***

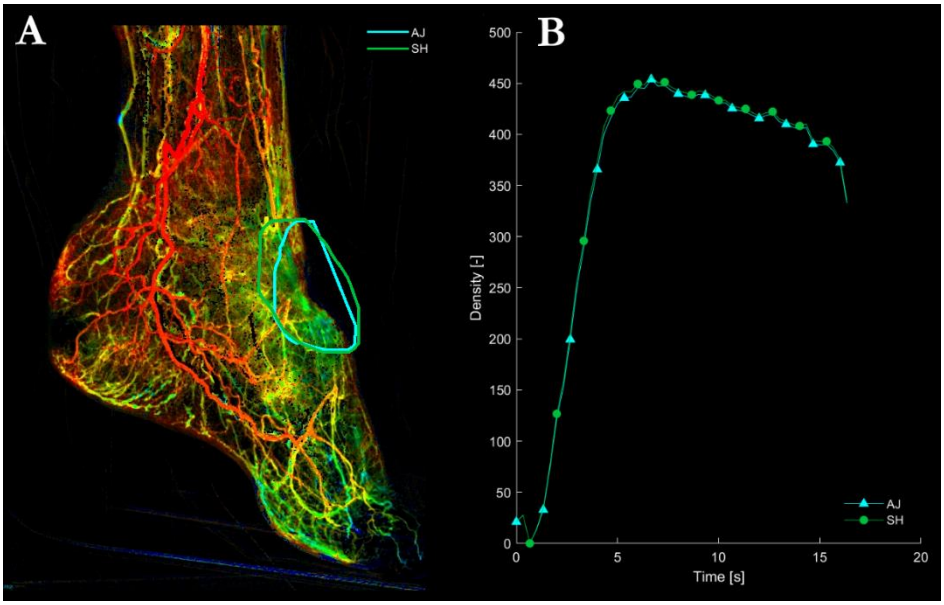


Figure F.2 – Reproducible Wound ROIs drawn by two observers (AJ and SH) in patient #8. ***(A)*** *lateral perfusion image with Wound ROIs. The perfusion image represents time of arrival.* ***(B)*** *TDCs corresponding to Wound ROIs drawn in* ***(A)***

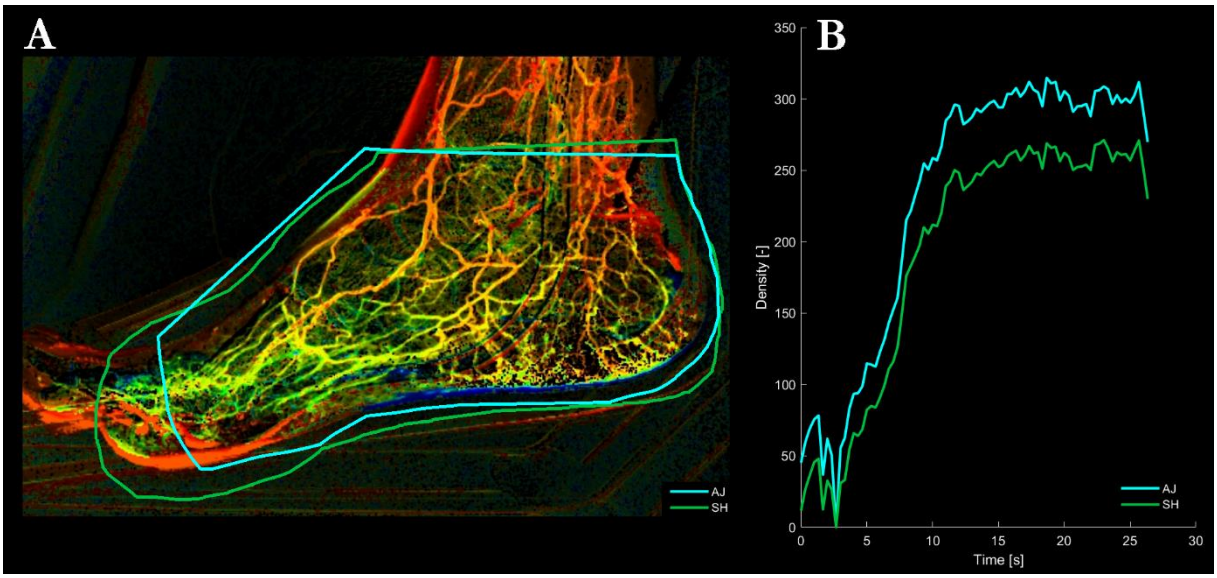


Figure F.3 – Unreproducible Reekers ROIs drawn by two observers in patient #7. ***(A)*** *lateral perfusion image with Reekers ROIs. The perfusion image represents time of arrival.* ***(B)*** *TDCs corresponding to Reekers ROIs drawn in* ***(A)***

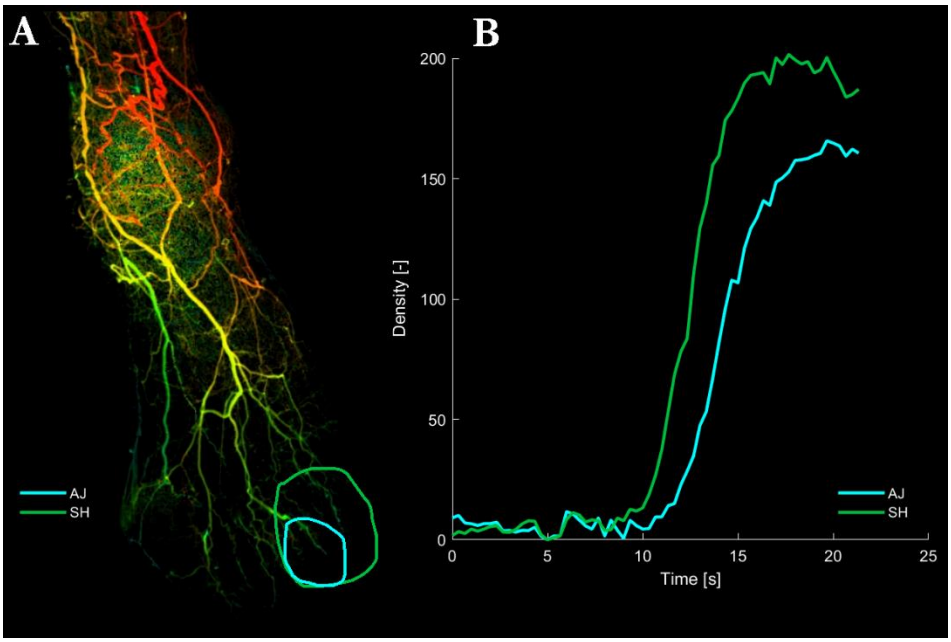


Figure F.4 – Unreproducible Wound ROIs drawn by two observers in patient #1. ***(A)*** *AP perfusion image with Wound ROIs. The perfusion image represents time of arrival.* ***(B)*** *TDCs corresponding to Wound ROIs drawn in* ***(A)***