A new validation concept for in silico tools using X-ray micro computed tomography images of pharmaceutical formulations

Inaugural-Dissertation

zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Heinrich-Heine-Universität Düsseldorf

vorgelegt von

Sebastian Bollmann
aus Bocholt

Düsseldorf, März 2022

aus dem Institut für Pharmazeutische Technologie und Biopharmazie
der Heinrich-Heine-Universität Düsseldorf

Gedruckt mit der Genehmigung der
Mathematisch-Naturwissenschaftlichen Fakultät der
Heinrich-Heine-Universität Düsseldorf

Berichterstatter:

1. Prof. Dr. Dr. h.c. Peter Kleinebudde

2. Prof. Dr. Jörg Breitkreutz

Tag der mündlichen Prüfung: 24.05.2022

Contents

List of abbreviations

API	Active Pharmaceutical Ingredient
CA	Cellular Automata
CCD	Charge-Coupled Device
CT	Computer Tomography
DEM	Discrete Element Method
DM-T	Dissolution Method Tab
DoE	Design of Experiment
DS-M	Dissolution Simulation Module
EMA/EMEA	European Medicines Agency
FaSSIF	Fasted State Simulated Intestinal Fluid
FDA	American Food and Drug Administration
FEM	Finite Element Method
F-T	Formulation Tab
GB	Gigabyte
HPLC	High Performance Liquid Chromatography
ICH	International Council of Harmonisation
ID	Identification number
IVIVC	*In Vivo* - *In Vitro* Correlation
JP	Japanese Pharmacopoeia
MDT	Mean Dissolution Time
MRT	Mean Residence Time
PAC-M	Particle Arrangement and Compaction Module
PAT	Process Analytical Technology
Ph. Eur.	European Pharmacopoeia
QbD	Quality by Design
RAM	Random-Access Memory
S-T	Simulation Tab
TD-M	Tablet Designer Module
TIFF	Tagged Image File Format
USP	United States Pharmacopoeia
XμCT	X-ray Micro-Computed Tomography

1 Introduction

1.1 Formulation development in the pharmaceutical industry

1.1.1 Background

Pharmaceutical formulation development is the process of designing and manufacturing an administrable dosage form for an active pharmaceutical ingredient (API). The pharmaceutical formulation should deliver the API in the correct concentration at a desired time and location in the body. To accomplish that, there are many different pharmaceutical vehicles and routes of administration available (Leuenberger and Leuenberger, 2016).
Two decades ago, the pharmaceutical formulation development was more an art than science (Leuenberger and Lanz, 2005). Since then, there has been a considerable change. New concepts and tools have been introduced to optimise the formulation development and new dosage forms, excipients, and devices have been developed to support this evolution. This transformation is still ongoing as there are some industries, such as the aircraft industry, which use more high-tech approaches because some fundamental processes of pharmaceutical formulations are poorly understood (Leuenberger and Leuenberger, 2016; Leuenberger and Lanz, 2005).

1.1.2 Quality by design

The concept of Quality by Design (QbD) was first introduced by Juran (1992). The idea was to build quality into the product from the start of the development. In 2004, the American Food and Drug Administration (FDA) started the Process Analytical Technology (PAT) initiative to optimise the pharmaceutical development (FDA, 2004). Additionally, Woodcock (2004) presented the basic concept of pharmaceutical quality: a high-quality product is free of contamination and reliably delivers the labelled therapeutic benefit to the consumer.
The FDA initiative and the basic concept were the starting point for the implementation of QbD within the pharmaceutical industry. Since then, the concept has been revised several times by different authorities. Nowadays, the concept has a key function in several guidelines for the pharmaceutical industry such as those from the International Council of Harmonisation (ICH) (ICH, 2009; Yu et al., 2014). The objectives of QbD are achieving product quality specifications based on the clinical performance, increasing process-ability, robustness, and homogeneity by enhancing design, understanding and control of products and processes, increasing product development and manufacturing efficiencies, and improving the root cause analysis as well as the post-approval change management (Yu et al., 2014). Therefore, science based knowledge of the product, processes and risks is mandatory. Several different approaches and tools were introduced to achieve quality by design for various purposes. Mishra et al. (2018) reviewed QbD approaches in the current pharmaceutical set-up. Some individual components of the QbD-based pharmaceutical development will be introduced in more detail in the following sections.

1.1.3 Design of experiment

The Design of experiment (DoE) approach is commonly used for empirical evaluations of processes with several important parameters and was introduced first by Fisher (1925). It allows to change several parameters instead of only one within each experiment. This reduces the experimental effort. The quantitative effects of (potentially) critical material or process attributes on the quality attributes of the intermediate or product can be determined by the systematic variation between the lower and upper limits of the factor settings. Additionally, it is possible to identify interactions of different factors. Statistical analysis of the results can increase the information content and reduce the minimum number of experiments required. Providing a good regression model, the response surface can be used for the optimisation by applying a fitted regression model to the experimental determined data to predict any factor combination within the factor space (Politis et al., 2017; Eriksson et al., 2000).
In addition to optimisation, screening and robustness testing are objectives which can be targeted using DoEs. The design and number of trials is defined by the goal. The less experimental effort is used, the lower the resolution of the DoE is (Eriksson et al., 2000).

1.2 Dissolution testing of pharmaceutical formulations

1.2.1 Background

In the 1960s, pharmaceutical scientists started to develop dissolution approaches to characterise the individual variables in solid dosage forms with increasing chemical and mathematical precision. The United States Pharmacopoeia (USP) introduced the first dissolution monograph in 1970 (Dressman and Krämer, 2005). Since then, several approaches for different drug formulations were introduced in the USP, the European Pharmacopoeia (Ph. Eur.) and the Japanese Pharmacopoeia (JP). The approaches and requirements of the three pharmacopoeias are nearly identical due to the international harmonisation (Dressman and Krämer, 2005; Limberg and Potthast, 2013).
During the early 1970s, dissolution testing was used in the context of *in vivo - in vitro* correlation (IVIVC). Nowadays, dissolution testing is a fundamental routine for the product quality control and formulation development. It guides the formulation development to select a reliable formulation for the *in vivo* testing. The dissolution testing is required for almost all solid oral dosage forms as a quality control before a product is allowed to be introduced or a batch is permitted to be released to the market. The product must meet all specifications to allow the batch release. Another important aspect is the comparison of dissolution profiles with respect to product equality, especially for post-approval changes. However, the IVIVC models are still being investigated in order to be introduced with the legitimacy of the authorities (Dressman and Krämer, 2005; Limberg and Potthast, 2013).
More than two decades ago, the concept of biorelevant dissolution media such as Fasted State Simulated Intestinal Fluid (FaSSIF) was introduced by Dressman et al. (1998);

Dressman and Reppas (2000). Since then, several biorelevant dissolution media and adapted approaches have been introduced to improve the information obtained from dissolution testing (Bhagat, 2014). The dissolution testing evolved from a simple and traditional quality control to a surrogate of an *in vitro* bioequivalence test (Shah, 2001). Gray et al. (2009) reviewed the challenges and future potential of the dissolution testing using the USP apparatus 1 and 2. It was pointed out, that it is mandatory to increase the knowledge of the variability sources and the drug releasing mechanisms. Additionally, the IVIVC and biorelevance have to become a routine to transform the dissolution test into a performance test. This development has been advanced in recent years, so that various biorelevant approaches are now available (Ranjan and Jha, 2021). Since this work focuses on classical dissolution testing as a quality control parameter, the new dissolution approaches and developments are not presented in detail in this work. A detailed background and history of classical dissolution testing is available in Dressman and Krämer (2005).

1.2.2 Experimental set-up

The most common set-ups for dissolution testing of solid oral dosage forms are the basket (Apparatus 1) or the paddle (Apparatus 2) set-up (Dressman and Krämer, 2005; Ph. Eur. 10.0, 2020). The stirring is often moderate (100 rpm of the basket, 50-75 rpm of the paddle). 500 - 1000 mL of an aqueous buffer (37°C) within the pH range of 1.2 - 6.8 is usually used as dissolution medium (Dressman and Krämer, 2005). The pH value of the buffer is dependent on the discrimination ability between batches which are equivalent and inequivalent (Limberg and Potthast, 2013). For quality control, dissolution samples are analysed at 15 minute intervals (immediate-release) or at hourly intervals (extended release) until at least 85 % of the labelled content has been released. For poorly water soluble drugs, the pH can be changed to the basic milieu or surfactants can be added (micellar solution) to achieve sink conditions. However, both changes can cause issues in the High Performance Liquid Chromatography (HPLC), which is the standard analysis tool for dissolution samples (Dressman and Krämer, 2005).
For the development of new formulations, dissolution profiles are commonly used to get more data and knowledge about the tested formulation and to optimise the formulation in line with the concept of QbD. However, dissolution testing is a destructive methodology which cannot be performed in- or on-line. Therefore, it is not possible to integrate dissolution testing in PAT concepts and consequently, surrogates for these tests need to be developed (Limberg and Potthast, 2013). For example, Galata et al. (2021) implemented NIR spectra, compression force and particle size distribution as surrogates to calculate the real-time release of tablets within Apparatus 2 through machine learning algorithms.
Although formulation science has advanced many different systems to overcome obstacles of solubility and dissolution rate, the commonly used dissolution testing approaches have not changed. The drug delivery systems are discriminated by the simple apparatus 1 or 2 (well-stirred, medium-rich environment) which does not reflect the biorelevant

performance. However, the development of an accurate in vitro surrogate of the gastrointestinal tract is still ongoing in spite of decades of research (McAllister, 2010). In addition, further research on surrogates is essential to establish real-time dissolution testing as an appropriate method in industry. Since the review of McAllister (2010), no considerable changes were introduced to the pharmacopoeias or guidance for industry.

1.2.3 Data analysis

For the data analysis of dissolution testing, commonly the cumulative drug release in percent is plotted against time. There are three different levels of data analysis possible with the dissolution data. The chosen sampling set-up determines which data analysis levels are available.
The lowest analysis level uses only a single or few data points which is usually used for the quality control within the pharmacopoeias. For example, setting the criteria that over 80 % of theophylline has to be released after 45 minutes for an immediate release tablet (US Pharmacopeial Convention, 2018a). For extended-release diclofenac sodium tablets, the drug release should be between 22 % and 42 % after 2 hours, 34 % and 61 % after 4 hours, 52 % and 82 % after 8 hours and no less than 73 % after 16 hours to meet the specifications of Test 3 (US Pharmacopeial Convention, 2018b). This is a quick method to analyse if a batch meets the specifications but does not provide any further information of the dissolution. The level of knowledge is low as only individual points are considered.
Statistical parameter such as the Mean Dissolution Time (MDT) or Mean Residence Time (MRT) form the second level of analysis. They provide further information on the dissolution as they consider all data points. It is possible to statistically compare dissolution profiles or to use the data for IVIVC of dissolution profiles. This information is useful for the pharmaceutical development of modified release dosage forms for oral use. There are several approaches available to calculate those parameters (Podczeck, 1993) However, this analysis is only reliable, if a sufficient number of samples were taken to describe the dissolution profile.
The highest data analysis level is represented by plots such as introduced by Higuchi (Higuchi, 1961, 1963) or Korsmeyers-Peppas (Korsmeyer et al., 1983; Peppas, 1985) which determine the kinetics and provide information about the drug releasing mechanism. They also allow for the comparison of dissolution profiles. The Higuchi model is applied to the dissolution of water soluble and poorly soluble drugs incorporated in a matrix formulation. The drug release is described by Fick's law based on the diffusion. It is dependent on the square root of the time (Costa and Sousa Lobo, 2001).
The Korsmeyers-Peppas model is commonly used if the release mechanism of a polymeric dosage form is not well known or more than one type of releasing mechanism could be involved. Only the data points up to 60 % drug release should be included to determine the value of the exponent n. If n is 0.5, the Fickian diffusion is the releasing mechanism. For values between 0.5 and 1.0, an anomalous transport is assumed while for n equal to 1.0 a Case-II transport is declared. If n is above 1.0, a super Case-II

transport is assumed. The model has been modified by different scientists to take the lag time or the burst effect into account (Costa and Sousa Lobo, 2001).
There are many more models available for dissolution testing such as the Weibull model, Hixon-Crowell model, Baker-Lonsdale model, and Hopfenberg model (Costa and Sousa Lobo, 2001). The understanding of the release mechanism is essential to develop new formulations in line with the QbD concept. The data can be used for IVIVC. The IVIVC is divided in three levels as well: level A is a point-to point relationship between the in vitro and in vivo data, level B is based on statistical parameters, and level C is based on single parameters, as described by Limberg and Potthast (2013).

1.2.4 Comparison of dissolution profiles

The comparison of dissolution profiles is important to ensure a reliable method transfer between different laboratories and to compare different batches or products regarding their equivalence. The f_2 similarity factor is the often used approach to compare dissolution profiles (Costa and Sousa Lobo, 2001). This methodology was introduced by Moore and Flanner (1996) and compares a reference profile with a test profile. The original approach used the f_1 difference factor (Eq. 1) and f_2 similarity factor (Eq. 2). The f_1 value had to be lower than 15 and the f_2 above 50. The FDA and the European Medicines Agency (EMA) waived the f_1 value to simplify the methodology (Costa and Sousa Lobo, 2001). It is recommended to use only one sampling time point after 85 %

$$f_1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} * 100 \tag{1}$$

Equation 1: f_1 as the difference factor of the calculated dissolution profiles, R_t as cumulative percentage dissolved of the reference and, T_t as cumulative percentage dissolved of the tested profile.

$$f_2 = 50 * log\left(\frac{100}{\sqrt{1 + \frac{1}{n}\sum_{t=1}^{n}(R_t - T_t)^2}}\right) \tag{2}$$

Equation 2: f_2 as the similarity factor of the calculated dissolution profiles, R_t as cumulative percentage dissolved of the reference and, T_t as cumulative percentage dissolved of the tested profile.

dissolution because the f_2 factor is most sensitive here (Shah et al., 1998).
Before the f_2 factor was introduced, several other methodologies were presented: Chow and Fanny (1997) introduced a dissolution difference measurement and similarity testing by comparing the parameters based on fitted one-degree auto-regression time series models. Sathe et al. (1996) used a selected mathematical model to fit the profiles and then determined the parameters for the dissolution difference measurement and similarity testing. Tsong et al. (1996) determined the multivariate 'Mahalanobis' distance to calculate the difference and similarity of dissolution data sets and Shah et al. (1987)

applied multivariate analysis of variance to test the difference and similarity of dissolution profiles. All approaches have in common, that they are not as applicable as the f_2 factor (Shah et al., 1998).
Costa and Sousa Lobo (2001) reviewed the different approaches to compare different dissolution profiles and criticised that the f_2 similarity factor is more of a convenience rather than a scientific fact based criterion and suggested to use the Rescigno index (bioequivalence index for plasma concentrations as function of time) instead.
The models and statistical parameters described in 1.2.3 can also be used to compare dissolution profiles.

1.3 In silico tools

1.3.1 Background

The term "in silico" was first mentioned in the scientific literature in the early 1990s by Sieburg (1990) and Danchin et al. (1991). It was used in the style of *in vitro* or *in vivo* to describe experiments performed on a computer. A software tool is used to predict the outcome of real experiments from certain input parameters. The use of *in silico* tools started in the research area of genetics. Nowadays, *in silico* approaches are common in nearly all disciplines to support research and development. The complexity of the software packages varies considerably. For example, there are 2D (Laaksonen et al., 2009) and 3D (Puchkov et al., 2013) models available for dissolution testing. The focus of this work was on *in silico* tools for dissolution testing of pharmaceutical formulations.

1.3.2 Requirements

In general, a fundamental understanding of all relevant processes within the experiment is required which should be simulated *in silico*. Additionally, the prediction can only provide reliable data, if both the compounds are suitable for the approach and the processes can be fully and correctly described by equations, rules and/or models. Therefore, the applicability of *in silico* tools in different fields and technologies is heterogeneous (Leuenberger and Leuenberger, 2016; Modi et al., 2012).
The dissolution of pharmaceutical formulations depends on several different processes which are not yet fully understood. Additionally, several variability sources have not been investigated sufficiently to predict all kinds of dissolution profiles. Nevertheless, it has been tried to predict the dissolution of new systems as accurately as possible to support the development. A deeper understanding of the variability sources, the formulations and their drug releasing mechanisms and the implementation of biorelevant conditions would enable to design pharmaceutical drug delivery systems with a specific in vivo performance (Gray et al., 2009).

1.3.3 Advantages and Drawbacks

In silico tools provide the possibility to accelerate the research and development and thereby save resources and money (Leuenberger and Leuenberger, 2016). Especially in the early stages of developments, the *in silico* tools can support the selection of promising candidates or approaches to avoid future failure (Modi et al., 2012; Mirzaei, 2020). However, the requirements for the applicability are a disadvantage as it is only possible to describe well understood systems. Additionally, the calculation time increases for complex systems considerably (Leuenberger and Leuenberger, 2016). Therefore, the size of the compartment or system could be limited as the simulation time may exceed the experimental time. The calculation time with a given software is hardware dependent and for some *in silico* experiments, high-end hardware is mandatory. If a task can be divided into subtasks, high-end hardware can be used to run calculations of smaller systems in parallel, which reduces the simulation time.

1.3.4 Approaches

General There are several different *in silico* tools available. The tools are either based on the Discrete Element Method (DEM), Finite Element Method (FEM) or Cellular Automata (CA) (Leuenberger and Leuenberger, 2016). Some software packages are combining different calculation approaches. DEM and FEM are numeric approaches which solve complex systems of differential equations. These calculations are limited to processes which can be accurately described by differential equations. The hardware requirements are usually high as a large memory is necessary and the computation is time intensive. The solver uses the hard disk if the RAM is exceeded. The access rate of the solver to the hard disk is considerably slower than to the RAM which decreases the performance of the solver. The hardware determines the number of particles which can be simulated (Leuenberger and Leuenberger, 2016) (rule of thumb 2 - 2.5 GB RAM/ 1 million elements).
CA were introduced to describe complex phenomena which could not be explained by differential equations (Wolfram, 2002). CA are based on simple rule sets which can differ for different compounds. Therefore, processes such as swelling, which cannot be sufficiently described by differential equations, can be described with CA. The hardware requirements are lower compared to DEM and FEM because less system memory is required. Therefore, more particles can be simulated within a shorter calculation time using identical hardware (Leuenberger and Leuenberger, 2016). A few software tools are presented as examples in the following paragraphs.

DDDplus DDDPlus™(Dose Disintegration and Dissolution) by Simulations Plus Inc. (Lancaster, CA, USA) is a software program based on differential equations. The tool is divided into three different tabs: formulation (F-T), dissolution method (DM-T) and simulation (S-T) (Njoku et al., 2019; Almukainzi et al., 2015; Njoku et al., 2020).
The F-T defines the physicochemical properties of the API and formulation parameters such as release mechanism (e.g. immediate release or controlled release) and

manufacturing characteristics (e.g. compression force, diameter). The composition can be based on excipients within the included database or self-defined excipients. The user has to define the function of the compounds within the composition and the dissolution model of the compounds. A calibration coefficient and excipient-specific coefficient can be used to fit the model to the measured data. Physicochemical properties of a new API (e.g. solubility, pKa, diffusion coefficient, logP) can be predicted from its chemical structure using the integrated ADMET predictor module (Simulations Plus, Lancaster, CA, USA) (Njoku et al., 2019).
The DM-T sets all the parameters for the dissolution method such as the volume and type of medium, the rotation speed and the apparatus. If a surfactant is used, a surfactant solubility model can be built to estimate the API solubility versus the surfactant concentration within the media (Njoku et al., 2019).
The S-T is calculating the drug releasing profiles from the given data sets. The simulation length can be chosen according to the experimental design (Njoku et al., 2019). The software was successfully applied to determine formulation strategies (Njoku et al., 2020) and to calculate the in vitro release pattern of montelukast sodium and glyburide (Almukainzi et al., 2015).

Cellular 2D model Laaksonen et al. (2009) introduced a 2D CA model to predict dissolution profiles. The model was able to predict realistic dissolution profiles of binary mixtures representing surface erosion, matrix release or membrane controlled release. The used grid was 126 x 126 pixels with a tablet cross-section of a round tablet (101 pixels in diameter). The model was simple to apply and had low hardware requirements. However, the model was not able to predict the behaviour of single particles and always assumes a disk shape of the tablet. The processes of the dissolution testing were considerably simplified. The model was tested only against other models and not against experimental data which considerably reduced its impact. However, the authors stated no limitations for shapes, releasing mechanisms, lag time or burst effects.

F-CAD The software package F-CAD (CINCAP, Basel, Switzerland) is based on a 3D CA model and differential equations. The package consists of three different modules: The tablet designer module (TD-M), the particle arrangement and compaction module (PAC-M) and the dissolution simulation module (DS-M). The TD-M allows to design any shape of the desired drug delivery system. The PAC-M uses the designed shape as matrix to build virtual pharmaceutical dosage forms. It is mandatory to specify all the APIs and excipients (can be stored in a database), their type (e.g. swelling compound or non-soluble compound) and their respective target masses. So far, the particle size is not directly determined, but indirectly defined by adjacent voxels of the identical ID. The density of the compounds is required to calculate the respective volume within the matrix. The compounds can be either randomly distributed within the matrix or seeds can be distributed and subsequently grown to the target mass (assuming spherical particles). The DS-M calculates the drug release or disintegration of the dosage form

created in the PAC-M. The mandatory input data depends on the types of excipients used and are defined in a task-file, as are the parameters of the dissolution method (Puchkov et al., 2013). Kimura et al. (2013) showed that F-CAD was able to successfully calculate the disintegration of tablets containing mefenamic acid.
F-CAD was used in this work as it has an interface to load uncompressed Tagged Image File Format (TIFF) as a virtual matrix in the PAC-M. This feature provides the opportunity to use processed X-ray micro-computed tomography images of pharmaceutical dosage forms as virtual matrices. Therefore, the predictions of these virtual matrices can be compared to the experimental data of the imaged pharmaceutical dosage forms. So far, other tools does not provide this feature.

1.3.5 Current use of in silico tools in the pharmaceutical development

The pharmaceutical formulation development is based on experiments carried out using DoEs. The existing *in silico* tools are used to reduce the number of experiments to support formulation development. The software packages are not able to predict the performance of pharmaceutical formulations without experimental data and fittings (Njoku et al., 2019; Leuenberger and Leuenberger, 2016). Since 2004, the pharmaceutical industry has started to close the gap of *in silico* development with other industries. While aircrafts and cars can be completely developed *in silico*, a complete *in silico* development of pharmaceutical formulations is not yet possible. The 'holy grail' would enable full *in silico* formulation development of a pharmaceutical drug delivery system based on accurate predictions of in vitro and in vivo formulation performances (Njoku et al., 2019; Leuenberger and Leuenberger, 2016; Leuenberger and Lanz, 2005) which has not been possible to date. Until all relevant processes can be accurately predicted, the *in silico* tools will be used to reduce the number of experiments for optimisation purpose within DoEs.

1.3.6 Validation of in silico tools in the pharmaceutical development

The validation of *in silico* tools for the pharmaceutical formulation development is done by fitting the simulation data to experimental data. Some software packages use input data of the individual compounds to predict the performance of the composition. However, a calibration factor or parameter fitting is mandatory to obtain suitable results (Njoku et al., 2019; Puchkov et al., 2013). This allows for the optimisation of a formulation within a defined factor space of a DoE. A performance prediction for a full *in silico* development is not possible by using this validation approach. So far, there is no validation approach that assesses the performance of an *in silico* tool for a complete *in silico* formulation development.

1.4 X-ray micro-computed tomography in the pharmaceutical context

1.4.1 Background

X-ray micro-computed tomography (XμCT) enables the non-destructive three dimensional imaging of samples. The first commercial CT system for medical scanning was developed by Hounsfield in the early 1970s (Hounsfield, 1973; Ambrose, 1973). In 1981 the first XμCT scanning device was released by Sato (Sato et al., 1981). The X-ray beam projects the sample onto a detector (e.g. charge-coupled device detector (CCD detector), transmission measurement) to obtain a 2D X-ray image. The procedure is repeated from multiple angles so that the data can subsequently be reconstructed to a 3D image (Elliott and Dover, 1982; Wiedey and Kleinebudde, 2017). The most common X-ray beam geometries are the conical beam or the synchrotron beam. The XμCT scanning device in this work used a conical X-ray beam which will be explained in detail in the following sections.

Figure 1 depicts a scheme of the XμCT set-up. In principle, a computer tomograph consists of three functional units. An X-ray source generates the X-ray beam, a sample holder which places the sample within the XμCT scanning device and a detector which measures the transmission of the X-ray beam. In the medical field, the sample is commonly static while the X-ray source and detector rotate around the sample. In contrast to the medical set-up, in the machine used for this work the sample itself rotates in the XμCT scanning device while the X-ray source and the detector are static. The conical shape of the X-ray beam magnifies the projection of the sample onto the detector. Therefore, the magnification can be varied within a certain range, as the scale is determined by the position of the sample between X-ray source and detector. The number of pixels within the detector determines the resolution of the resulting image. The voxel size, which is the common scale in literature, depends on both the pixel size of the detector and the ratio of the distances between X-ray source/sample and sample/detector.

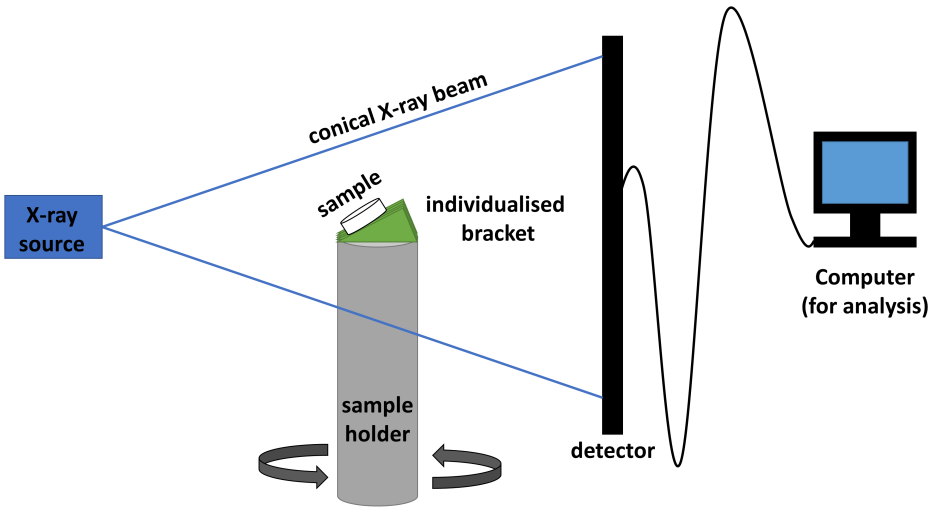

Figure 1: The schematic set-up of a XμCT scanning device.

The number of projections determines the rotation angle (360°/projections).
As the detector captures the transmission of X-rays, the image depends on the partial absorption of X-rays and the resulting radiation energy decrease by passing through the sample. McCullough (1975) described the absorption behaviour of X-rays within a given material. The attenuation coefficient results from the effective atomic number, the density, the atomic mass and the energy of the photon. As the used constants (Avogadro's number, the Klein-Nishina-coefficient and a dimensionless constant) are the same for a specific material, they can be differentiated in XμCT images if either the elemental composition and/or the density of the materials are sufficiently different.
Figure 2 depicts the principle of the filtered back-projection which is one possibility to reconstruct XμCT measurements. The original object (Figure 2 a) is imaged from 256 different angles. The resulting sinogramm is displayed in Figure 2 b. The algorithm converts the sinogram back into an image by applying the inverse Fourier transform. The result is projected back to the angle θ to obtain the final image. Therefore, interpolation steps are necessary. If the back-projection is not filtered, Figure 2 c is the result. The image is blurred and noisy. The filtered back projection (Figure 2 d) results

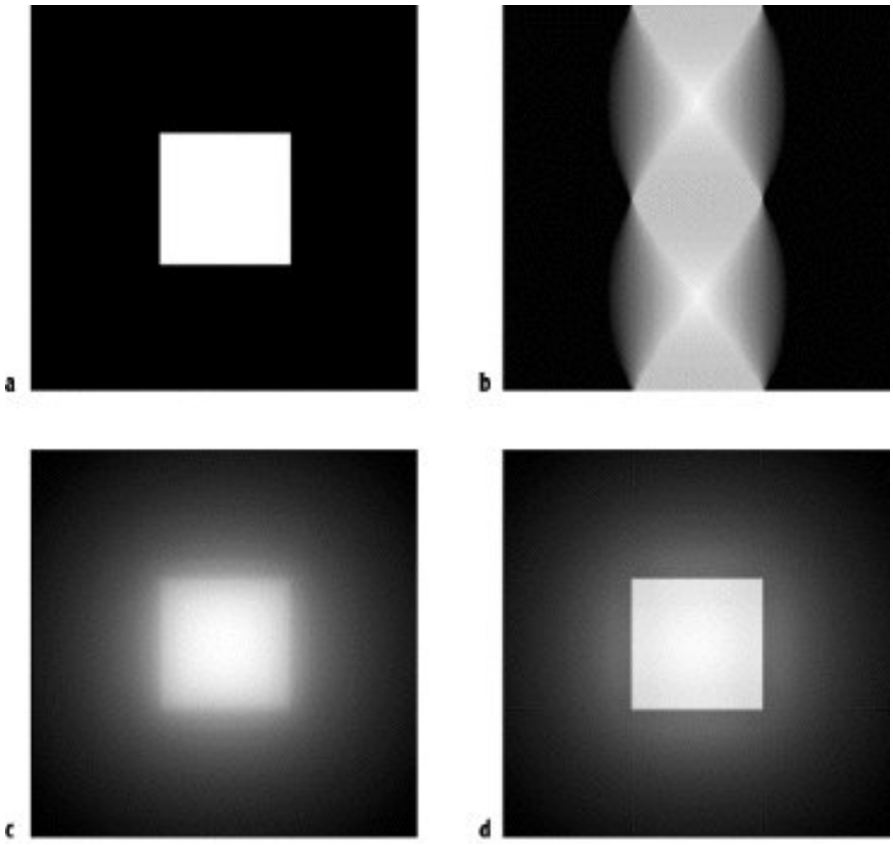

Figure 2: Material from: Ulrich Kilian, Lexikon der Physik Bd. 2, published 1998 by Spektrum Akademischer Verlag, reproduced with permission of SNCSC (Spektrum Akademischer Verlag, Lexikon der Physik, 1998): The filtered back-projection. a) depicts an original image of a simulated squared object, b) the corresponding sinogramm, c) the back-projected image without filtering and d) the filtered back-projection.

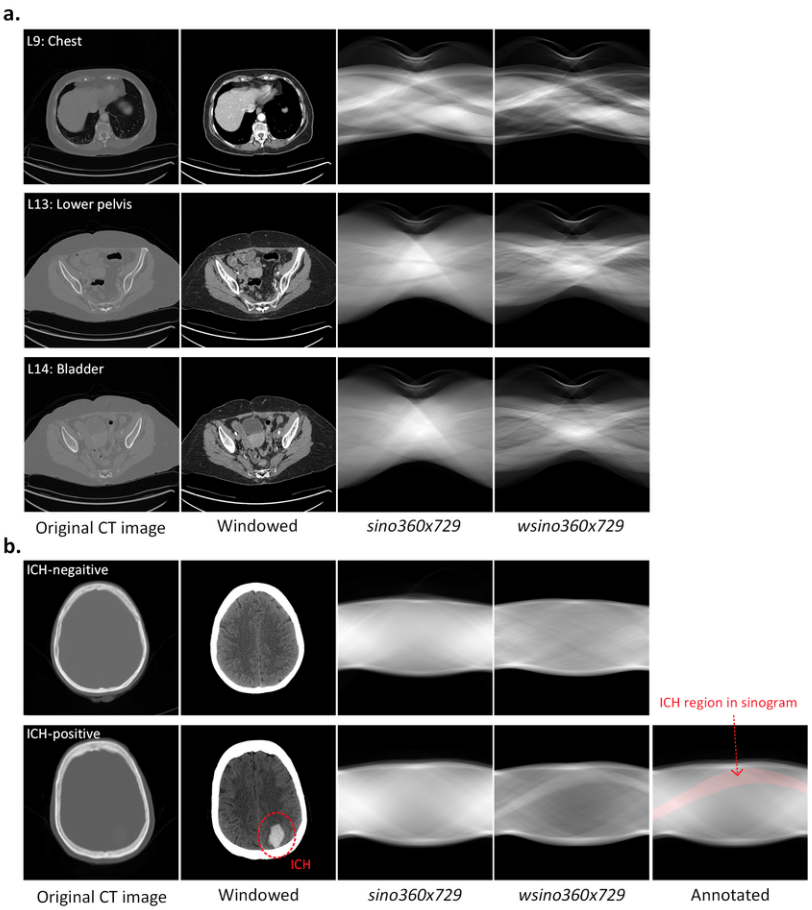

Figure 3: Examples of XμCT images and their sinogramms published by Lee et al. (2019) (https://creativecommons.org/licenses/by/4.0/).

in a sharp image of the object. However, the image is still noisy, as the back projection amplifies high-frequency content. The impact of this effect is reduced in more complex XμCT images because of a lower contrast between object and background. Figure 3 illustrates examples of complex XμCT images and their sinogramms. As the last row of figure 3 b. depicts, characteristic regions within an image can be detected within the sinogramm.

Geometry, volume and/or surface XμCT measurements have often been used to determine the geometry, volume and/or surface parameters of pharmaceutical intermediates or dosage forms, especially if the geometry differs from the simple tablet or capsule shape. 3D-printing has pushed the field of personalised medicine forwards and has resulted in the development of many diverse geometries for individual dosage forms. Analytical testing of these new dosage forms was often not feasible using standard methodologies of the USP or Ph. Eur.. XμCT scanning of these drug delivery devices has been established to determine volume and surface parameters of the complex geometries (Gioumouxouzis et al., 2018; Smith et al., 2018; Korte and Quodbach, 2018).

Coating thickness A second application of XμCT imaging is the determination of the coating thickness of tablets (Russe et al., 2012) and pellets (Li et al., 2014). Since this is a critical quality attribute for functional coatings with a potential impact on safety and efficacy, a PAT-tool is desirable to control the coating process in-line compliant to the QbD approach. XμCT measurements are not a suitable candidate for a PAT-tool for various reasons (e.g. long measurement and analysis times), but they provide a reliable off-line reference for the development of PAT-tools (Radtke et al., 2019). For example, XμCT measurements have been used as references for Terahertz spectroscopy (Russe et al., 2012), full-field optical coherence tomography (Li et al., 2014) and near-infrared spectroscopy (Kim and Woo, 2021).

Micro-structure The analysis of micro-structures of pharmaceutical dosage forms can be divided into two parts. The first and most described one is the pore structure and system analysis or density distribution of intermediates such as ribbons (Wiedey and Kleinebudde, 2017), 3D prints (Markl et al., 2017), or tablets (Markl et al., 2018). In contrast to mercury porosimetry, it is possible to analyse and characterise the entire pore within a particle or agglomerate and it's structure instead of only detecting the smallest diameter. However, the resolution of XμCT measurements is not sufficient to investigate small pores (smaller than approximately 1 - 20 μm) as the voxel edge size limits the applicability while mercury porosity is sensitive for pores in the scale of low nanometre ranges (Markl et al., 2018). If the solid fraction or porosity of the sample is to be investigated, a second approach is to use the grey value of a discrete area as a marker in low resolution scans, for example to characterise density distribution patterns within tablets (Sinka et al., 2004) or ribbons (Miguélez-Morán et al., 2009).
The second part is the investigation of the API and excipient(s) distribution within pharmaceutical formulations, to link these results to the performance of the dosage form in quality control tests. Dissolution profiles and disintegration times especially have been addressed by combining the information of the geometry, pore systems and distribution of compounds (Zhang et al., 2021).

1.4.2 Artefacts

There are several well described artefacts which can occur in XμCT images. Some characteristic artefacts are presented in the following paragraphs:

Noise As the CCD detector converts an analogue signal into a digital one, a random signal alternation is added to the target signal. This noise can be reduced by averaging several images and using image processing, if necessary. However, it will always be present in the images.

Scanning device dependent artefacts Ring artefacts result from the different sensitivity of single detector pixels. The impact on the image quality is high, especially for subsequent segmentation processes (Vidal et al., 2005; Kak and Slaney, 1988). If the

densities of two materials are considerably different, the occurrence of ring artefacts at the horizontal planar interfaces is likely. The ring artefacts can be reduced by slight variations of the detector position. The different positions have to be considered for the reconstruction of the image. As the artefacts are often present at the horizontal planar interfaces, the sample should be placed at a 45° angle. With the use of reconstruction software, a horizontal reconstruction and thereby a top view of the sample is possible. The spot size of the X-ray source limits the spatial resolution of the device. Therefore, a point spread function is mandatory, potentially causing blurring of the edges of the reconstructed image. If the rotation step is too large, stripes can appear as a result of an under sampling.

Physics based artefacts As low energy X-rays are absorbed faster than those with high energy when transmitting through material, the X-ray beam is hardening up (Brooks and Di Chiro, 1976). This causes both a cupping effect (edges of an object appear brighter than the center) and streaks and dark bands (in between dense objects). The effect cannot be completely removed but metal filters (copper or aluminium) and mathematical algorithms can reduce the impact. Another artefact caused by a similar phenomenon is photon starvation. It appears, if certain particles (e.g. small metal particles) absorb the X-rays so strongly that too few X-rays hit the detector at this position. This leads to characteristic streaks within the image (Mori et al., 2013). There are some mathematical concepts to reduce streaking but the best method of avoiding photon starvation is to remove metal parts of objects, whenever possible.

Object-based artefacts The object should be placed statically for XμCT measurements, as movement of the object can cause artefacts like blurring or stripes (Hsieh, 2003). Static sample placement is especially challenging for elastic or free liquid containing objects. As there are almost no possibilities to reduce the artefacts using algorithms, the most promising approach is to develop optimised sample holders to reduce the dynamics of the sample.
Another object-based artefact is caused by incomplete projections (e.g. scanning an object which is either too large or was poorly centred). This leads to shading near the edge of the truncation. These artefacts can be avoided by proper centring or extension of the measurement field (Hsieh, 2003).
If the object contains details such as pores which are smaller than the voxel size, a voxel contains a mixture of compounds and the measured X-ray absorption may not reflect any of the compounds within the voxel. This partial volume effect can cause image misinterpretation and misallocations during the subsequent image processing. This effect is not always avoidable as some samples contain details smaller than the highest possible resolution of the scanning device.

Reconstruction based artefacts Data approximation of the reconstruction algorithm can cause blurring at the edge slices of the reconstructed image stack. The most common algorithm used in cone-beam geometries is the filtered back-projection which assumes approximately parallel X-rays. As a cone-beam has no parallel X-rays, data approximation from mid-slices to edge-slices is needed to overcome slight deviations caused by the algorithm. (Tuy, 1983; Schulze et al., 2011).

A detailed background, description and illustration with example images of the XμCT measurement artefacts was given by Hsieh (2003).

1.4.3 Requirements for image quality

Requirements for the image quality strongly depend on the examination objectives. In general, the highest available image quality of the system should be used. However, the negative impact of artefacts (refer to section 1.4.2) depends on the objectives. While a moderate cupping effect will probably not affect a pore structure analysis as the binary thresholding approach will remove the artefact, it will presumably impact the differentiation between API and excipient in the segmentation process as the contrast between API and excipient is usually lower than that between solid fraction and air.
In this work, the goal was a quantitative image segmentation to distinguish between API, excipient(s) and air. Therefore, images as shown in Figures 4 and 5 would not

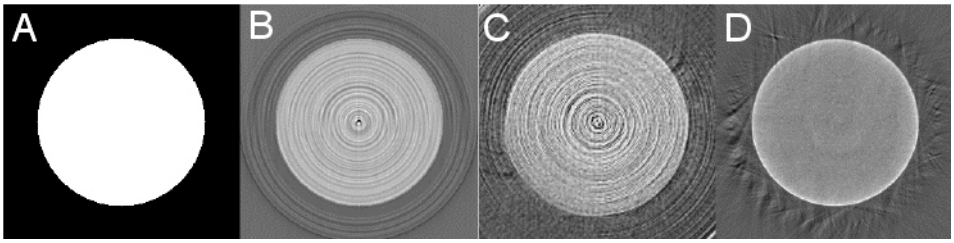

Figure 4: Republished with permission of IOP Publishing Ltd, from: A CCD-based optical CT scanner for high-resolution 3D imaging of radiation dose distributions: Equipment specifications, optical simulations and preliminary results; 3.2. Detector performance, Simon Doran, Physics in medicine and biology, volume 46, 2001 (Doran et al., 2001); permission conveyed through Copyright Clearance Center, Inc.: only image C of the montage is relevant for this work. It shows an example for strong ring artefacts which would disturb the segmentation process.

fulfil the requirements as the artefacts would lead to misallocations during the segmentation process. In contrast, the image depicted in Figure 6 meets the requirements as there is no misallocation expected due to measurement artefacts.

1.4.4 Advantages and disadvantages

The 3D images obtained by non-destructive XμCT imaging allows for the analysis of micro-structures or geometries of objects without changing or destroying the sample. On the one hand, it allows for the analysis of objects which are expensive and/or unique

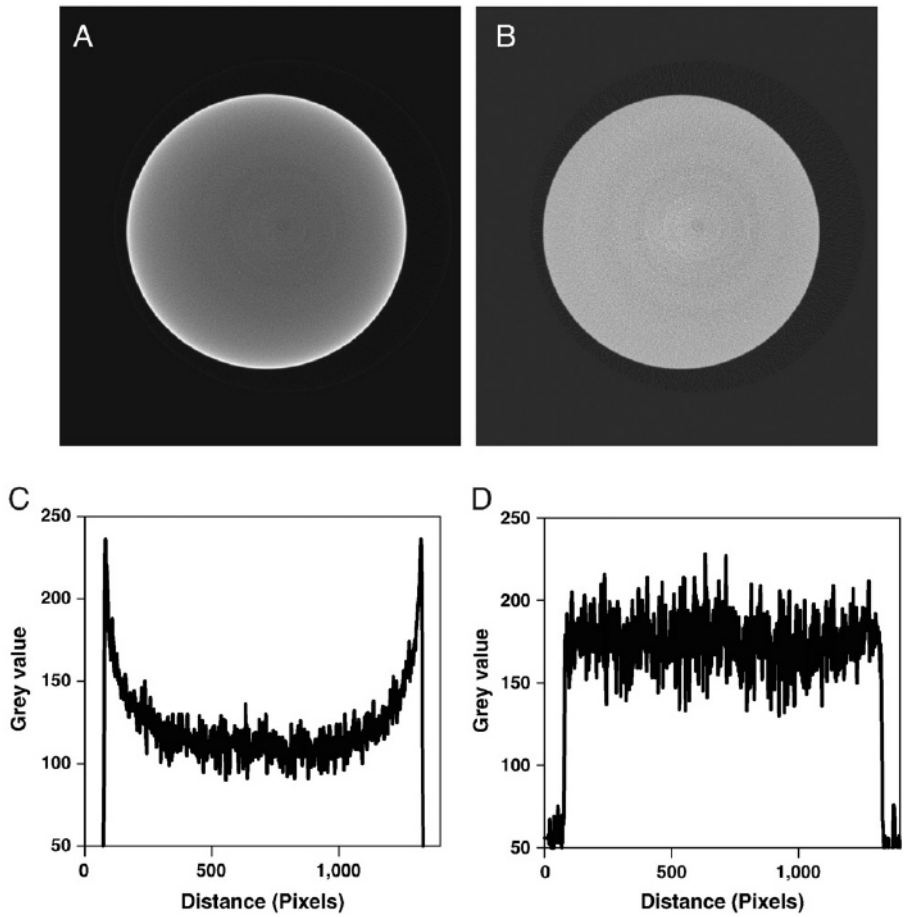

Figure 5: Reprinted from Journal of Experimental Marine Biology and Ecology, 396, Ronan C. et al., Quantification of porosity in Acropora pulchra (Brook 1891) using X-ray micro-computed tomography techniques, 4, 2010, (Roche et al., 2010) with permission from Elsevier.: A: Image with medium ring artefacts and strong cupping effect, B: cupping effect corrected image (image processing used), C: grey values along a transect from A, D: grey values along a transect from B.

which should not be destroyed. On the other hand, it is possible to compare an object before and after an experiment to examine the changes of the object caused by the experimental set-up. Additionally, the behaviour of an object within an experiment can be predicted, depending on the analysed structure. The prediction can be verified by performing the experiment with the imaged sample.

In spite of the possibilities, a common issue of XμCT image analysis is the comparison of multiple images as the grey values differ. Often it is not possible to scan all samples in a single run and even if the identical parameters for the scanning and reconstruction are applied, the grey values can be not comparable. Therefore, the measurements are not reproducible. The grey values are also affected by artefacts and the micro-structures of the sample. The solution for this issue is either the use of a calibration object in each measurement or each image has to be processed on its own and the final results are statistically analysed. The resolution limits the analysis of too large objects or structures smaller than the voxel size. Additionally, XμCT imaging is time-consuming

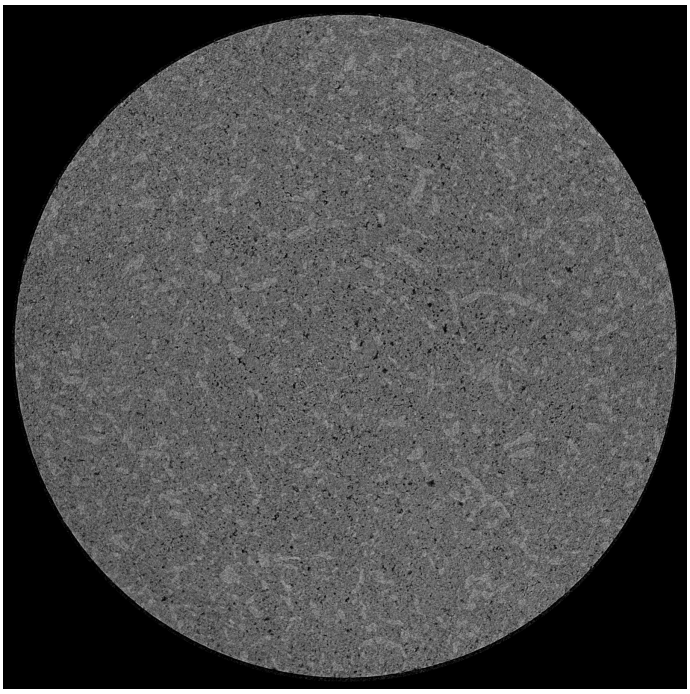

Figure 6: Example for a good quality XμCT image, cross-section 183 of tablet 5 (batch T25E75)

and the access to scanning devices is usually limited as an XμCT scanner is costly. As the grey value and image quality depends on the positioning of the sample and the chosen parameters, the methodology is user dependent. If a manual image analysis is performed, the user dependency is considerably increased and the reproducibility is not given.

1.5 Image processing

1.5.1 Background

A pixel is a "picture element" indexed by x and y or column and row from the origin of the image. It is the smallest constituent digital image element (commonly a square) containing a numerical value representing the basic image information at this grid point at a given spatial resolution and quantisation level. This is usually the colour or intensity response as a small sample point of electromagnetic waves within the image (Solomon and Breckon, 2011). A voxel is the 3D version of a pixel (x, y, z or length, height and depth). As 3D images containing voxels were used in this work, the following sections will always used the term voxel. If the same operations are used with 2D images, the term voxel has to be replaced by pixel.
An image is a digitised 2D or 3D analogue signal stored as a grid of either pixels or voxels while the information content of the image may vary considerably (Solomon and Breckon, 2011). The image can be interpreted mathematically either as a continuous function within a designed space or a matrix with x * y (* z) values. Therefore, all mathematical operations such as addition, subtraction, multiplication and division can be applied. Commonly, differential equations are used for image processing approaches.

The visual perception is an important sense of humans as most information received of their surroundings is captured visually. The importance can be pointed out by some examples: scientific results are usually reported in written texts and supported by images rather than emitting smell or audible tones, most people document their private life or special moments with photos, almost all warning systems contain a visual compound and photos are used to think of important persons, things or places. The percentage of information using visual pathways has been estimated at ca. 90 - 95% for a human without any sensory impairment. There are many optical devices available to generate images of objects which are either too small, large or far away for the naked eye. Additionally, devices expand the visible spectrum of the electromagnetic spectrum (ca. 380 nm - 780 nm) to different areas such as X-ray (ca. 5 pm - 10 nm) infrared (ca. 780 nm - 1 mm) or ultraviolet light (ca. 10 nm - 380 nm). However, many images need to be processed in the scientific field to measure features or extract structures. All operations which change something within the raw image are called image processing. Beside the analysis and information extraction, image processing can enhance the subjective visual perception and image quality (Ross and Russ, 2010). In spite of the wide visual flow of information, the ability to interpret scientific images is generally poor because there is a difference between scientific images and the real world (images). It is important to understand the differences in the information which both types of images can provide and to know the biases introduced by our vision systems. Additionally, the information extracted from an image strongly depend on the viewer. Therefore, images are an inefficient way to communicate as they offer much space for misinterpretations. The human visual perception compares colours relatively and does not determine absolute values as computers do. The image processing methods work with the absolute values as well (Ross and Russ, 2010). Additionally, every image is compared to memorised images to find similarities as the evolution developed our visual perception to identify friends or enemies. Since recognition has to be fast, our brain only checks if both some features have been found and no characteristics have been identified that are not present in the memorised image. If these criteria are met, the person or object is identified as a memory. Everyone has experienced a misidentification based on the first view. As scientists have to identify new and unknown things, the interpretation of the images is much more difficult. Figures 7 to 9 illustrate the difference between the human visual perception and the perception of the computer. In Figure 7, square A appears black and square B seems to be white. Our mind tells us, that there is a chessboard and a shadow of the cylinder. For the computer both squares have the identical colour as their grey values are the same. Therefore, the computer would assign both squares to the same class while the scientist would rather assign them to different classes. In Figure 8, the central square is always in the identical colour. However, it is much more obvious in the second row as the black border helps us to distinguish the inner and outer part. Unfortunately, there are no black borders in the raw images which can help us to identify such similarities. In Figure 9, there seems to be a spiral but a closer look will reveal only circles of different sizes. The placement of the structures

leads to the optical illusion and a misinterpretation of the image. There are many more examples available to depict the potential of visual misinterpretation of images. Therefore, suitable image processing is mandatory to extract the information from the scientific images (Ross and Russ, 2010).
A detailed background on the differences of visual perception and computer perception and the resulting consequences for the image processing was provided by Ross and Russ (2010) in "The Image Processing Handbook". Some approaches of the image processing will be presented in the following sections.

1.5.2 Goals of image processing

The goals of image processing approaches can be divided into three main topics: in-painting, recognition and reconstruction. The in-painting of images is used to recover lost or destroyed parts. The viewer of the image should not be able to recognise differences to the original intact image. In-painting can either be used to restore old images, to remove objects from images or to compress images to save storage capacity. The recognition of images is used to analyse features or pattern of images. The image is segmented to extract the information. The targeted information can be the number, size, dimensions, location, orientation or pattern of objects. In this work, the aim was to get the number of voxels representing either API or excipient within the correct pattern. The last main topic is the image reconstruction. Converting the analogue signal into a digital one alongside all of the adjustments to the signal (e.g. enhancing the image contrast by adjusting the intensity values) are considered as image reconstruction. The image processing done before the segmentation in this work is an image reconstruction

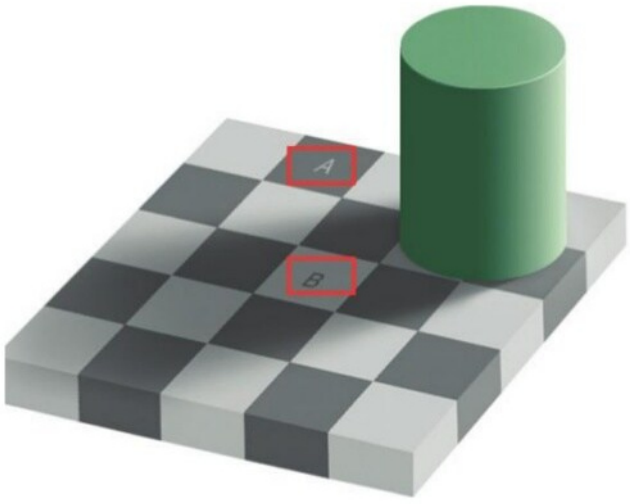

Figure 7: Republished with permission of Taylor & Francis Group LLC - Books, from The image processing handbook, Russ, John C., edition number 6 and 2010] (Ross and Russ, 2010); permission conveyed through Copyright Clearance Center, Inc.: Impact of expectations and surroundings on the visual interpretation of brightness. The squares marked with A and B seem to be black and white although they have actually the identical shade of grey.

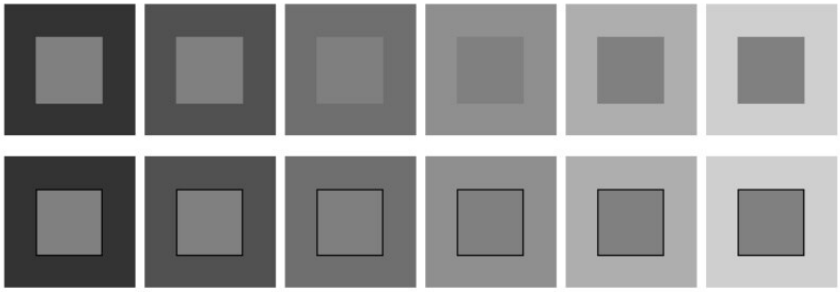

Figure 8: Republished with permission of Taylor & Francis Group LLC - Books, from The image processing handbook, Russ, John C., edition number 6 and 2010] (Ross and Russ, 2010); permission conveyed through Copyright Clearance Center, Inc.: Lighter and darker surroundings of the identical shade of grey coloured square affecting the visual comparison (top row). The black border in the bottom row reduce the effect.

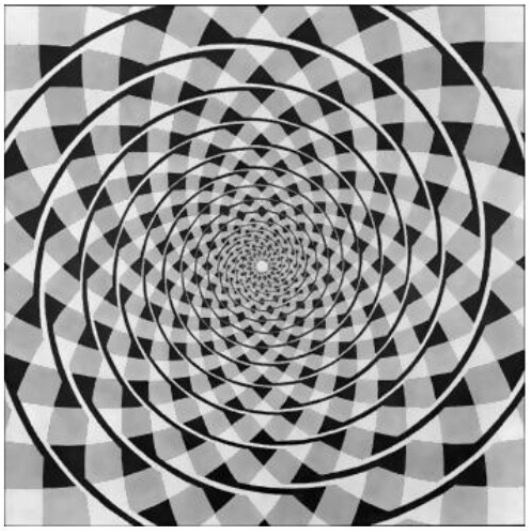

Figure 9: Republished with permission of Taylor & Francis Group LLC - Books, from The image processing handbook, Russ, John C., ed. 6, 2010 (Ross and Russ, 2010); permission conveyed through Copyright Clearance Center, Inc.: Fraser's spiral. The circular rings seem to be tilted and looks like a spiral.

applied to simplify the subsequent segmentation. However, the goal of reconstruction can also be allow for or improve the visual perception of a human observer.

1.5.3 Requirements and rules

Although there are many image processing methodologies available which considerably impact both the image data and interpretation, the evaluation of image processing methods is rarely reported in scientific publications. Commonly, no image processing methodologies or just the (incomplete) final applied image processing pathway is published (Promentilla et al., 2009; Lak et al., 2009; Wu et al., 2016; Akseli et al., 2013). This makes a transfer of image processing pathways or approaches to similar images

nearly impossible. Additionally, published data cannot be reproduced to confirm the results and conclusions drawn from the experiments. Since many images in scientific literature are inappropriately processed or manipulated, the unpublished image processing approaches are problematic. The manipulations are often hard to recognise and many scientists are not aware of appropriate image processing. Therefore, many images are unconsciously processed inappropriately (Farid, 2008; Bik et al., 2016). A transparent publication of image processing methodologies may reduce the number of unconsciously inappropriate processed images.
As there are many ways to manipulate images, ethics regarding the requirements and rules of image processing are mandatory. Cromey (2010) published an ethical guideline for scientific image processing in the biological field. The ethics are generally transferable to other scientific disciplines. The following paragraph briefly summarises the most important rules and requirements valid for all images or either qualitative or quantitative image analysis based on Cromey (2010) ethics:

Ethics based on Cromey (2010) Digital images can be compromised by inappropriate manipulations. Scientific digital images should be acquired using appropriate experiments. The images should not intend to obscure information or to deceive the viewer to suppress alternative interpretations. Images have to be handled like numeric data as images represent the numerical sampling of the object obtained by the data acquisition system. These systems all have limitations and errors resulting from the physics and the construction of the device. Commonly, the user can change the settings of the data acquisition system. A supersaturation or too aggressive black level within the images have to be avoided during the image acquisition as information of the image are lost irrecoverably. Therefore, the full range of the grey scale should be used to get the best scientific results.
Image processing approaches should only be applied to copies of the unprocessed raw image. The original raw image file has to be stored safely as this is the only way to compare the final processed image with the original data. Many journals state in their instructions for authors that the original raw image has to be provided by request. Scientists need to ensure the data integrity of the raw images over time to allow for the re-analysis of their images, if their research is questioned in the future. Additionally, the raw image is the strongest protection against allegation of misconduct. Many raw images are acquired in a manufacturer's proprietary file format containing important meta data. However, it is often mandatory to convert the image into a universal image format. TIFF is the recommended file format because no information is lost and it provides a wide range of bit depths.
Simple adjustments such as a slight and reasonable change in brightness and contrast affecting the entire image are commonly acceptable. Auto-adjustment tools should be avoided as they tend to over-process images and it is almost impossible to retrace which processes have been applied to the image. All adjustments affecting the intensity values are inappropriate for a subsequent intensity quantification but can ease the feature extraction, if no original information is eliminated, obscured or misrepresented. Even

a slight supersaturation can be adequate for the segmentation but it has to be clearly reported.
It is commonly acceptable to crop an image to remove irrelevant sections of the image. It is inappropriate if any information is removed which could change the context or interpretation of the remaining data.
If digital images should be compared, they should be acquired under identical conditions and processed in the same way. The conditions and applied image processing methodologies should be clearly described in the methods section. The same is true, if several images are grouped together in a montage. If there are compelling reasons for different conditions or post-processing approaches and the images should be compared and/or grouped together, it must be clearly described in the methods and figure description to avoid misinterpretations.
Manipulations of specific image areas which are not performed on the entire image are questionable. There are only a few exceptions where this may be appropriate but it always has to be reported. Almost all specific manipulations are inappropriate and have to be avoided.
The use of digital filters has to be differentiated. The ethics based on Cromey (2010) were written for biological images (e.g. fluorescence microscopy) where an intensity quantification is often applied to analyse cells. In this field, digital filters should be avoided as they are mathematical functions which change the intensity values. Additionally, they can introduce artefacts and remove the background of the images. The tissues in the background of an image may contain important information and ideas for other researchers. In general, data embellishment is a kind of misrepresentation and can be misleading.
However, digital filters can be important and valid tools in a different context. If intensity quantification is not the objective but a feature extraction is to be performed, filters are important tools to prepare the segmentation process as a homogeneous object on a homogeneous background is easier to detect and extract (Solomon and Breckon, 2011). But as Cromey (2010) stated, if a digital filter is applied, it should be reported including the software version, the filter name and all used parameters. If the code of the algorithm is available, it should be provided as well.
Cloning and copying objects either from different locations within an image or other images is inappropriate with only one exception. It is valid to build an image montage if it is clear and obvious that the parts are based on different images and it is clearly described which part was derived from which image. If retouching tools, cloning or copying are used in a different context, it indicates an inappropriate image processing for scientific images.
Intensity quantifications should only be performed on unprocessed or uniformly processed images. Furthermore, a calibration using a known standard should be applied. All image processing approaches affecting the intensity values should be avoided if possible or be carefully described.
Lossy compressions have to be avoided as raw data within the images is removed and

lost forever. It is recommended to use TIFF as format for images. A famous example for lossy compressions is the image file format JPEG which is used by software like Adobe Acrobat or Microsoft PowerPoint to compress included images, even if the included images are TIFF images.
Magnification and resolution are important parameters. The dimensions of pixels and voxels can be equal but they do not have to be. As the scale of all dimensions has to remain the same for the correct interpretation and it is impossible to know the final published image size, a scale bar should be included to represent the image scale. Measurements of objects close to the diffraction-limited resolution of the instrument are probably inaccurate. Therefore, it is important to know the resolution. An undersampling of image can introduce aliasing artefacts and has to be avoided. Oversampling does not lead to artefacts but can reduce the contrast. Therefore, a moderate oversampling (2.5 - 3 times) is recommended.
Changing the image size in voxels is a critical step and has to be carefully performed. Commonly, it is necessary to increase or decrease the number of voxel to use the images in different software packages or to fit them to the page size. The decrease of voxels should be applied using a power of two to avoid interpolations. If the voxel number is increased, the intensity values are interpolated if the image is not only placed in the centre and framed by the added uniform voxels. All of these changes have to be carefully described as they can dramatically chance the interpretation of images.

1.5.4 Quality assessment

Background Image quality assessment is important to rate image processing approaches. It targets either the perceptual quality of images or the correct classification/ feature extraction. As the objective of the image processing can vary considerably, the quality assessment approaches do as well. However, four different approaches can be distinguished: the subjective image quality measure and three objective image quality assessments called full reference, reduced reference or no reference quality measure (Wang and Bovik, 2006). As videos are image series with time as an additional dimension, the quality assessment can be performed for videos as well.

Subjective measure In the subjective quality measurement, a human rates the image quality based on the subjective visual perception. A mean opinion score can be calculated to rate the image quality. The approach is accurate but time consuming and expensive as it has to be done for each image manually (Wang and Bovik, 2006). Zhai et al. (2008) performed a subjective image quality assessment for videos to rate the impact of encoder type, video content, bit rate, frame size and frame rate. In this work, a qualitative subjective quality measure was performed for the most promising image processing pathways to verify the calculated pathway performance.

Full reference measure The full reference measure requires the original image which is not always available. The final processed image is compared to the full original image in terms of similarity or fidelity (Wang and Bovik, 2006). Larson and Chandler (2010) proposed two different strategies of the human visual system to analyse the image quality compared to the original image: detection of visible differences for high-quality images and recognisability of the image content for low-quality images. Therefore, Larson and Chandler (2010) developed two different approaches to access high- and low-quality images.

Reduced reference measure If only certain extracted features of an image are available, a reduced reference measure is possible. The extracted information of the original image is used in models to rate the image quality of the processed images (Wang and Bovik, 2006). As an example, Ma et al. (2011) developed a reduced reference measure based on the mathematical analysis of discrete cosine transform coefficient distributions.

No reference measure Since the system for evaluating the image quality often does not have access to the reference image, a no reference measure is desired. However, it is complex and difficult to evaluate the image quality without comparison with a reference because, in contrast to the human brain, the computer does not typically have information stored in memories that simplify and thus enable the interpretation of the image (Wang and Bovik, 2006). Exemplary, Panetta et al. (2013) introduced two new approaches for no reference quality measures of colour images. In this work, the concept of desirability was used to rate the final processed images.

1.5.5 Bleach correction

Background A bleach correction is a common methodology used for biological fluorescence microscopy to overcome photo-bleaching (Miura, 2020; Füreder-Kitzmüller et al., 2005; Markham and Conchello, 2001). The 3D image stack of XμCT images can contain a similar artefact. The slices in the middle of the image stack appear brighter than the slices at the edges of the image stack. The difference in grey value of two compounds within a slice remains identical but the absolute grey values changes. As our eyes compare the colour of voxels relative to its neighbours, human beings are able to distinguish two compounds both in the middle of the stack and at the edges of the stack. However, the absolute difference can lead to misallocations during the segmentation process, if the absolute grey value of compound B in the middle slices is similar to the absolute grey value of compound A at the edge slices.
A bleach correction normalises the absolute grey values to reduce an artificial grey value gradient. The grey values of each slice of the image stack can be normalised either to one slice of the image stack or to a reference image slice (Burri, 2018; Miura, 2010a,b). There are several principles available to correct the gradient in 3D images.

Simple ratio A widely used technique to reduce photo-bleaching is a double normalisation using the ratio of the mean intensities (Miura, 2020). It has been introduced as a macro for ImageJ in 2004 (Rietdorf, 2004). It recalculates the grey values according to the equation 3 (Miura, 2020, 2010a).

$$I_i^c(x,y) = \frac{\bar{I}_0 - I_b}{\bar{I}_i - I_b}(I_i(x,y) - I_b) \tag{3}$$

Equation 3: I_i^c as the corrected intensity, I_b as background intensity, $\bar{I}_0$ as mean intensity of the first slice and $\bar{I}_i$ as mean intensity of the ith slice. (Miura, 2020)

Exponential fitting Another approach to correct the grey value gradient within an image stack is an exponential decay curve fit. It estimates the bleach ratio at each frame to calculate the intensity. The background intensity has to be estimated as well. The example approach implemented in ImageJ recalculates the grey values according to the equations 4 and 5 (Miura, 2020).

$$\bar{I}_i'(x,y) = ae^{-bi} + c \tag{4}$$

Equation 4: $\bar{I}_i'$ as mean intensity and a, b and c as estimated variables. The estimated background c is subtracted from the original image (Miura, 2020).

$$I_i^c(x,y) = \frac{a' + c'}{a'e^{-b'i} + c'}(I_i(x,y) - c) \tag{5}$$

Equation 5: I_i^c as the corrected intensity and a', b' and c' as estimated variables (Miura, 2020).

Histogram matching A histogram matching modifies the voxel values of an image to meet the histogram shape of a reference image. This methodology is favourable for a subsequent segmentation process, as it homogenises the different grey value histograms of the image stack slices but can lead to issues during an intensity quantification (Miura, 2020, 2010a). As a quantitative segmentation was the target of this work, the histogram matching was the applied bleach correction approach. The used methodology which is implemented in ImageJ calculates the cumulative distribution function of the histogram with Equation 6.

$$CDF_i(p) = \sum_{x=0}^{p} H_i(x) \tag{6}$$

Equation 6: CDF_i as cumulative distribution function, p as pixel value and H_i as histogram (Miura, 2020).

$$p' = CDF_0^{-1}(CDF_i(p)) \tag{7}$$

Equation 7: p' as the updated pixel value, CDF_i as cumulative distribution function and CDF_0^{-1} as inverse cumulative distribution function of the reference image (Miura, 2020).

1.5.6 Background correction

Background Several different phenomena in images are called background. They all are unwanted because they impact the intensity quantification or the feature extraction. Most phenomena can be reduced by choosing an appropriate experimental setup. However, a compensation can be misleading for an intensity quantification as it affects the intensities (e.g. histogram clipping or high detector offset).

Low offset value A low offset value can lead to a high black level. This reduces the visual contrast and can hide weak structures. An appropriate offset and an adjustment of the light source can be used to avoid this issue. During image processing, it can be eliminated by measuring the average intensity within a certain area outside the sample. This average intensity is subtracted from each voxel within the image. The result depends on the position and size of the area for the average intensity. Only if the lighting is even, the methodology can be used before an intensity quantification. This example is implemented in the *Image math* macro of ImageJ (Rueden and Schindelin, 2019).

Unspecific signal Unspecific signals can disturb both the feature extraction and intensity quantification. If the unspecific signals cannot be suppressed by the experimental setup, they can be reduced or removed by many different image processing methodologies. Two examples will be presented: the rolling ball and the convoluted background subtraction.
The rolling ball approach is based on the feature size. A radius of the rolling ball is determined which should be equal to the largest non-background object. The rolling ball cuts off any signal above the peak width corresponding to the ball size (Schindelin, 2015). Therefore, this methodology is inappropriate for a subsequent intensity quantification.
The convoluted background subtraction uses a blurred copy of the original image as an invisible background and subtracts the artificial background from the original image (Brocher, 2015). If the blurring radius is chosen too small, features might be lost. This methodology is not suitable for intensity quantifications, either.

Uneven lighting A common background artefact is an uneven lighting which leads to a gradient within images. This effect is present in XμCT images as well. While the gradient between the different slices of the 3D stack can be corrected with the bleach correction, the gradient within each slice can be corrected with the lighting correction. Different methodologies are available to correct an uneven lighting.

The flat-field correction requires a background image to be taken before the sample is imaged. The image containing the sample is divided by the background image. This removes the uneven lighting but reduces the brightness of the image considerably. A subsequent multiplication with the mean intensity of the background image restores the brightness level. ImageJ provides the possibility to process these images semi-automatically (Rueden and Schindelin, 2014).
If a background image is not available, a pseudo-flat-field correction can be applied. A pseudo background image is created by a strongly blurred copy of the original image (Gaussian blur filter with high sigma). The subsequent procedure is equal to the flat-field correction (Brocher, 2014). The pseudo-flat-field correction is not appropriate for a subsequent intensity quantification as it uses artificial assumptions which change the data set.
The function *"Remove Background"* uses a global polynomial of low degree to correct for uneven lighting. It is applied over the entire image and subtracts the calculated values of the polynomial function from the original image. It assumes that the overall image values are constant (Münch, 2019).

1.5.7 Digital image filters

Background Digital image filters are used to reduce noise within images or to make structures more homogeneous so that features can be extracted. There are many different digital filters available. They can either blur or sharpen the image while either preserving the edges or not. Commonly, filters are mathematical functions which change the image data (Chandel and Gupta, 2013). Therefore, careful use is mandatory in the scientific fields as filters can lead to manipulated images with wrong informational content. Filters are inappropriate for intensity quantifications or images for presentational use (e.g. publications or presentations).
Some general filtering concepts exist and some of these concepts will be shortly introduced in the following paragraphs.

Low-pass filter A low-pass filter reduces voxel values above a certain threshold value while voxel values lower than the threshold value stay nearly unchanged. Low-pass filters can often cause blurring of the images. An example for a low-pass filter is a Gaussian function with a large σ value (Solomon and Breckon, 2011).

High-pass filter A high-pass filter decreases the voxel values below a defined threshold value while voxel values above the threshold remain almost the same. High-pass filters often sharpen images. An example for a high-pass filter is the unsharp masking (Ramponi et al., 1996).

Band-pass filter A band-pass filter lowers the voxel values outside a defined range while the voxel values within the range stay almost unchanged. An example for a band-pass filter is the inverse Fourier filter (Solomon and Breckon, 2011).

Global filter If a global filter process is used, the value of the processed voxel is independent from it's surrounding ones. For example, a constant value is added to each voxel to increase the brightness of the image.

Local filter If a local filter is applied, the value of the processed voxel depends on the values of the voxels in it's proximity. A defined window is used to determine the relevant area and all voxel values within this window are used to calculate the new value of the targeted voxel. An easy example of a local filtering is the windowed mean filtering. A window size is defined (e.g. 3 x 3 x 3 voxel). The mean value of all voxels within the window around the target voxel is calculated and defines the new voxel value.
In spite of it's name, the non-local means filter which was used in this work (Buades et al., 2011; Behnel and Wagner, 2013) belongs to the local filters. The name is misleading and semi-local would have been a more accurate term (Buades et al., 2011).

Linear filter As the name implies, linear filters apply linear functions to calculate the new voxel value. Commonly, linear filters are implemented as local filters. Examples of linear filters are the mean, linear smoothing or Gaussian blur filter.

Non-linear filter Non-linear filters apply non-linear functions to calculate the new voxel value. As linear filters, non-linear filters are commonly implemented as local filters. Examples of non-linear filters are the median or the bilateral filter.

1.5.8 Contrast enhancement

Background The adjustment of the contrast can improve the visual perception of images. However, it can be misleading as it may change the relative distance between different grey values. If intensity measurements are required, a contrast adjustment has to be avoided. A contrast adjustment potentially increases measurement artefacts or introduces new artefacts into the image which have to be corrected afterwards (Rabin et al., 2011; Bovik, 2010). Therefore, it should be handled with care. However, it can improve the feature extraction. Although a supersaturation of white voxels within the image should be avoided while using the contrast enhancement, it may improve the feature extraction. Nevertheless, a supersaturation can lead to misallocations during the feature extraction if the number of white voxels exceeds the voxel number of the targeted feature. Contrast enhancement approaches can generally be divided into linear and non-linear methodologies. There are many different contrast enhancement approaches available as well as subclasses of the linear and non-linear approaches (Bovik, 2010). In general, all approaches should be applied to the entire image and not only to certain regions.

Linear contrast enhancement In a linear contrast enhancement, the grey value histogram of an image is stretched linearly to increase the contrast. The simplest example is a histogram normalisation where the darkest voxel is assigned to the value black, the brightest one to the value white and the intermediate grey values are distributed linearly between these values. Therefore, gaps between the grey values within the new histogram are unavoidable. However, in contrast to non-linear contrast enhancements, the relative intensity and the shape of the histogram are left unchanged as the dependency on grey value and intensity remains. The histogram normalisation implemented in ImageJ was used in this work as linear contrast enhancement (Schindelin, 2017). Other examples are the additive image offset and the creation of an image negative (Bovik, 2010).

Non-linear contrast enhancement Non-linear contrast enhancement increases the contrast by changing the dependency of grey value and intensity to stretch the image histogram. Commonly, the contrast increase is higher than when using a linear contrast enhancement. However, the altered dependency of grey value and intensity may lead to a misinterpretation of images, but it can simplify feature extraction. Histogram equalisation is a simple example for a non-linear contrast enhancement. It uses the cumulative grey value histogram which is acquired by consecutively adding sequential intensities from the original histogram to flatten the histogram. It is a special case of a histogram shaping approach (Bovik, 2010; Ross and Russ, 2010). As the original histograms of different images vary, the cumulative histograms do too and the impact of the equalisation can be considerably distinguish for different images. Therefore, the direct comparison of equalised images is invalid. The histogram equalisation implemented in ImageJ was applied in this work as contrast enhancement (Schindelin, 2017).
Another non-linear contrast enhancement is the gamma adjustment. It re-maps the voxel intensity. The edge values of the histogram are only slightly affected while the ones in the middle are considerably altered. A gamma value lower than 1.0 increases the intensity values while a gamma value above 1.0 decreases the intensity values of the histogram. Rueden (2019) implemented the gamma adjustment applied in this work.

1.5.9 Segmentation

Background A segmentation extracts features from images. It changes the image data considerably as different voxel values are merged to groups with resulting identical attributes. As for all image processing steps before, there is a broad range of segmentation approaches since the targeted features vary strongly (e.g. cells, sand corns, powder particles, air bubbles, bones, tissues ...). The segmentation is mandatory to analyse characteristics of features such as length, size or number. It allows to convert a complex image into a meaningful and more simple accessible one. Segmentation approaches can be divided in local (targeting only specific parts of an image) and global segmentations (aiming the entire image). Segmentation methodologies are

either based on the detection of similarity or discontinuity (Kaur and Kaur, 2014). A segmentation can be binary (black and white) or contain multi levels depending on the image and the analysing goal.
Kaur and Kaur (2014) reviewed different segmentation approaches, their advantages and disadvantages and divided them into the following categories.

Threshold The simplest way to segment images is the binary thresholding. The voxels are assigned to black or white depending on their intensity value. Commonly, the features are brighter than the background. A thresholding can be applied manually or automated (Kaur and Kaur, 2014; Solomon and Breckon, 2011). An automated thresholding is preferred as it user-independent and faster. In this work, various binary thresholding algorithms were used. Examples are Li's minimum cross entropy (Li and Lee, 1993; Li and Tam, 1998) and Otsu's threshold clustering (Otsu, 1975). In principle, multilevel thresholdings work like binary thresholdings. Instead of dividing the histogram with one threshold in two fractions, the histogram is divided in three or more fractions using several thresholds.

Edge based Edge based segmentation methodologies use the rapid intensity change within an image as detection criterion. They either use the first or second derivative of the intensity to detect the edges. The detected edges are connected to form the boundaries of the feature. Some approaches are based on the grey value histogram while others use the gradient. Examples of edge detection approaches are the sobel or canny edge detection (Kaur and Kaur, 2014; Solomon and Breckon, 2011).

Region based Region based segmentation approaches segment the image into various regions with similar characteristics. Two general approaches can be distinguished: Region growing approaches distribute seeds and grow them. The growth is controlled by the voxel connectivity. The second method is the region splitting and merging. The image is divided in various regions and those with similar characteristics are merged (Kaur and Kaur, 2014; Solomon and Breckon, 2011). The statistical region merging is an example of a region based segmentation approach (Rueden, 2014).

Clustering based Clustering based segmentation techniques segment images into clusters of voxels with similar characteristics. The clustering can be implemented either hierarchically or partitionally. Soft clustering approaches like the fuzzy c-means clustering may assign voxels to more than one cluster. This clustering is more flexible and robust against noise but does not provide an exact division. In contrast to soft clustering, hard clustering such as the k-means clustering assign each voxel to exact one cluster (Kaur and Kaur, 2014; Solomon and Breckon, 2011). The k-means clustering was applied in this work. It iteratively minimises the squared error objective function. The result is dependent on the prior defined number of k clusters (Kaur and Kaur, 2014; Solomon and Breckon, 2011; Sacha, 2019; Jain, 2010).

Watershed based Approaches based on watershed interpret the gradient of the grey value histogram topologically. The basins with holes in their minima are represented by the intensity. The water spills from the minima. If the water reaches the edge of a basin, the bordering basins are merged. Dams constructed by dilation maintain the separation between different basins and represent the borders of regions (Kaur and Kaur, 2014). The watershed algorithm was implemented by Schindelin (2011) in ImageJ.

Partial differential equation based Partial differential equation based approaches use mathematical models to segment images. The calculation speed of these approaches is high but a sufficiently precise model is required for an accurate segmentation. Commonly, either a non-linear isotropic diffusion or a non-quadratic variation restoration are used (Kaur and Kaur, 2014). E.g. Ecabert et al. (2008) presented a model based segmentation for CT - images of the human heart.

Artificial neural network based Artificial neural networks use learning strategies of the human brain to segment images. Nodes are weightily connected to make the right decision. The approach is widely used to analyse medical images. However, to obtain reliable results, a sufficient amount of training data and time is required (Kaur and Kaur, 2014). The trainable Weka segmentation provides various classifiers and attributes for segmenting images and is implemented in ImageJ (Arganda-Carreras, 2019).

1.6 Aims of the thesis

As the number of *in silico* tools to support and accelerate the formulation development in the pharmaceutical industry is increasing, an approach is mandatory to rate and compare the performance of these tools. The methodology should be able to validate *in silico* tools which aim at a full *in silico* development of pharmaceutical dosage forms which comply with the quality by design approach. The approach should be based on processed 3D-images of pharmaceutical dosage forms. Therefore, the methodology as well as the image processing have to be robust, reproducible, comprehensible and performable by everyone without limitations of software licenses. The concept should be transferable to the research and development of the pharmaceutical industry. It should be ensured, that different software packages can be rated and/or compared using reference data sets as the authorities need to prove and to rate the tools as well. Therefore, the approach should be broadly applicable to different *in silico* tools and reflect different real pharmaceutical formulations.

The detailed goals of this thesis were as follows:

- To generate suitable 3D XμCT images of pharmaceutical dosage forms in a widely used image format ensuring data integrity and transparency for the purpose of the use in *in silico* tools.
- To clearly and concisely evaluate the XμCT image processing for an *in silico* tool for different tablet batches containing binary mixtures. Different levels of specialisation should be investigated to decide which batches could be grouped for the image processing.
- To analyse and rank the performance of the image processing pathways and the final processed XμCT images.
- To use suitable final processed XμCT images to analyse and rate the performance of an exemplary *in silico* tool.
- To develop an approach to reduce the range of image processing parameters for images with similar properties and characteristics. The methodology should predict likely high performing image processing pathways to accelerate future image processing.
- To investigate the transfer and applicability of the developed concept from tablets to oral films.

1.7 Outline of the thesis

In this thesis, a new validation concept for *in silico* tools is introduced. The concept includes the production of pharmaceutical dosage forms, the acquisition of 3D XμCT images, the 3D image processing and it's performance assessment as well as the comparison of predicted *in silico* data and experimental data to rate the software performance.
Section 2 deals with whether the evaluation of image pre-processing methods using a standardised segmentation approach can fulfil the requirements of a software package which predicts dissolution profiles. Therefore, twelve different tablet batches were produced, imaged by XμCT measurements and characterised. The image pre-processing pathways were rated with the concept of desirability. Although no overall pathway for all batches was found, promising pathways were identified for individual or groups of batches.
After evaluating the image pre-processing, the evaluation of different segmentation methodologies was next. In section 3, the highest performing pathways of the image pre-processing evaluation were used to investigate the performance of different segmentation approaches. The images were prepared to comply with the software requirements. Since some issues have been identified in the pre-processing evaluation, the performance rating was slightly adjusted and different densities for diclofenac were compared to optimise the results. For each batch and group of batches, high-performing pathways were identified. The images complied with the software and were uploadable. The determined image density was introduced for compounds with a small particle size as a work around for the issues occurring when the helium density was used.
Since the evaluation of image processing pathways was time intensive and therefore a critical disadvantage for the new validation concept, it was desired to find an approach to reduce the evaluation time without a considerable loss in performance. A simple methodology using the coefficient of variation is presented in section 4 to predict a narrow window of likely high performing image processing parameters for similar images which should be processed with the identical objective. The reduced evaluation led to high performing pathways for images of new tablet batches within a calibrated range and were transferable to new tablet formulations containing different compounds with similar properties as well.
In section 5, the different final processed images of the evaluation described in section 3 were used to analyse both the sensitivity of the software package to different pathway performances and the software performance. Therefore, both virtual matrices obtained by the final processed images and virtual matrices designed by the software itself were used to predict the dissolution of three different batches. The results were compared to the experimental dissolution of the imaged tablets. The chosen software performed high, if the dissolution was considerably determined by the dissolution rate of the API whereas the performance was poor for diffusion-based matrix dissolution and slowly eroding matrices, which results in a dissolution dominated by the diffusion speed. The performance of the virtual tablets designed by the software itself was comparably low. Therefore, the introduced concept was able to verify the prediction of the software. It

would be possible to validate an *in silico* tool with the presented concept.
The subsequent step after introducing the new validation concept of *in silico* tools was to check the transferability of the methodology from tablets to another pharmaceutical dosage form. Section 6 illustrates the concept transfer to oral films and investigated possible issues occurring due to the different properties of oral films. Artefacts of the XμCT measurements of the films which disturbed the usage within the concept were identified by confocal Raman microscopy.
Section 7 discusses the performance of the entire concept and conclusions from this work presented in several segments in different scientific publications. Additionally, starting points for future research and an outlook are presented to conclude this work.

References

Akseli, I., Abebe, A., Sprockel, O., Cuitiño, A.M., 2013. Mechanistic characterization of bilayer tablet formulations. Powder Technology 236, 30–36.

Al-Tabakha, M.M., Alomar, M.J., 2020. In Vitro Dissolution and in Silico Modeling Shortcuts in Bioequivalence Testing. Pharmaceutics 12, 45.

Almukainzi, M., Okumu, A., Wei, H., Löbenberg, R., 2015. Simulation of In Vitro Dissolution Behavior Using DDDPlus™. AAPS PharmSciTech 16, 217–221.

Ambrose, J., 1973. Computerized transverse axial scanning (tomography): Part 2. Clinical application. The British Journal of Radiology 46, 1023–1047.

Amini, E., Kurumaddali, A., Bhagwat, S., Berger, S.M., Hochhaus, G., 2021. Optimization of the Transwell® System for Assessing the Dissolution Behavior of Orally Inhaled Drug Products through In Vitro and In Silico Approaches. Pharmaceutics 13, 1109.

Arganda-Carreras, I., 2019. Weka. URL: `https://github.com/fiji/Trainable_Segmentation/blob/80f130c332a71be7823a09c7dbea5c29e7b105f3/src/main/java/trainableSegmentation/Weka_Segmentation.java`. (10. September 2020).

Behnel, P., Wagner, T., 2013. Non-Local Means Denoising. URL: `https://raw.githubusercontent.com/thorstenwagner/ij-nl-means/master/src/main/java/de/biomedical_imaging/ij/nlMeansPlugin/NLMeansDenoising_.java`. (05. March 2020).

Bhagat, N.B., 2014. A Review on Development of Biorelevant Dissolution Medium. Journal of Drug Delivery and Therapeutics 4, 140–148.

Bik, E.M., Casadevall, A., Fang, F.C., Sibley, L.D., 2016. The Prevalence of Inappropriate Image Duplication in Biomedical Research Publications. mBio 7, e00809–16.

Bovik, A.C., 2010. Handbook of Image and Video Processing. Academic Press, San Diego, CA, USA.

Brocher, J., 2014. Pseudo flat field correction. URL: `https://github.com/biovoxxel/BioVoxxel_Toolbox-old-/blob/master/Pseudo_flat_field_correction.java`. (05. March 2020).

Brocher, J., 2015. Convoluted Background Subtraction. URL: `https://github.com/biovoxxel/BioVoxxel_Toolbox-old-/blob/master/Convoluted_Background_Subtraction.java`. (05. November 2021).

Brooks, R.A., Di Chiro, G., 1976. Beam hardening in x-ray reconstructive tomography. Phys Med Biol 21, 390–8.

Buades, A., Coll, B., Morel, J.M., 2011. Non-Local Means Denoising. Image Processing On Line 1, 208–212.

Burri, O., 2018. Parallel histogram matching. URL: `https://gist.github.com/lacan/3de16eb24f954399b763445070fe4bfc`. (03. March 2020).

Chandel, R., Gupta, G., 2013. Image Filtering Algorithms and Techniques: A Review. International Journal of Advanced Research in Computer Science and Software Engineering 3, 198–202.

Chow, S.C., Fanny, Y.K., 1997. Statistical comparison between dissolution profiles of drug products. Journal of Biopharmaceutical Statistics 7, 241–258.

Costa, P., Sousa Lobo, J.M., 2001. Modeling and comparison of dissolution profiles. European Journal of Pharmaceutical Sciences 13, 123–133.

Cromey, D.W., 2010. Avoiding Twisted Pixels: Eethical Guidelines for the Appropriate Use and Manipulation of Scientific Digital Images. Science and Engineering Ethics 16, 639–667.

Danchin, A., Médigue, C., Gascuel, O., Soldano, H., Hénaut, A., 1991. From data banks to data bases. Research in Microbiology 142, 913–916.

Doran, S.J., Koerkamp, K.K., Bero, M.A., Jenneson, P., Morton, E.J., Gilboy, W.B., 2001. A CCD-based optical CT scanner for high-resolution 3D imaging of radiation dose distributions: equipment specifications, optical simulations and preliminary results. Physics in Medicine & Biology 46, 3191–3213.

Dressman, J.B., Amidon, G.L., Reppas, C., Shah, V.P., 1998. Dissolution Testing as a Prognostic Tool for Oral Drug Absorption: Immediate Release Dosage Forms. Pharmaceutical Research 15, 11–22.

Dressman, J.B., Krämer, J., 2005. Pharmaceutical Dissolution Testing. Taylor and Francis, Boca Raton, FL, USA.

Dressman, J.B., Reppas, C., 2000. In vitro–in vivo correlations for lipophilic, poorly water-soluble drugs. European Journal of Pharmaceutical Sciences 11, S73–S80.

Ecabert, O., Peters, J., Schramm, H., Lorenz, C., von Berg, J., Walker, M.J., Vembar, M., Olszewski, M.E., Subramanyan, K., Lavi, G., et al., 2008. Automatic Model-Based Segmentation of the Heart in CT Images. IEEE Transactions on Medical Imaging 27, 1189–1201.

Elliott, J.C., Dover, S., 1982. X-ray microtomography. Journal of Microscopy 126, 211–213.

Eriksson, L., Johansson, E., Kettaneh-Wold, N., Wikstrom, C., Wold, S., 2000. Design of Experiments – Principles and Applications. Umetrics AB, Umea Learnways AB, Stockholm, Schweden.

Farid, H., 2008. Digital image forensics. Scientific American 298, 66–71.

FDA, 2004. Guidance for Industry PAT — A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance. URL: `https://www.fda.gov/media/71012/download`. (12. May 2020).

Fisher, R., 1925. Statistical methods for research workers. Oliver and Boyd, Edinburgh and London, UK.

Füreder-Kitzmüller, E., Hesse, J., Ebner, A., Gruber, H.J., Schütz, G.J., 2005. Non-exponential bleaching of single bioconjugated Cy5 molecules. Chemical Physics Letters 404, 13–18.

Galata, D.L., Könyves, Z., Nagy, B., Novák, M., Mészáros, L.A., Szabó, E., Farkas, A., Marosi, G., Nagy, Z.K., 2021. Real-time release testing of dissolution based on surrogate models developed by machine learning algorithms using NIR spectra, compression force and particle size distribution as input data. International Journal of Pharmaceutics 597, 120338.

Gioumouxouzis, C.I., Baklavaridis, A., Katsamenis, O.L., Markopoulou, C.K., Bouropoulos, N., Tzetzis, D., Fatouros, D.G., 2018. A 3D printed bilayer oral solid dosage form combining metformin for prolonged and glimepiride for immediate drug delivery. European Journal of Pharmaceutical Sciences 120, 40–52.

Gray, V., Kelly, G., Xia, M., Butler, C., Thomas, S., Mayock, S., 2009. The Science of USP 1 and 2 Dissolution: Present Challenges and Future Relevance. Pharmaceutical Research 26, 1289–1302.

Higuchi, T., 1961. Rate of Release of Medicaments from Ointment Bases Containing Drugs in Suspension. Journal of Pharmaceutical Sciences 50, 874–875.

Higuchi, T., 1963. Mechanism of sustainedaction medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. Journal of Pharmaceutical Sciences 52, 1145–1149.

Hounsfield, G.N., 1973. Computerized transverse axial scanning (tomography): Part 1. Description of system. The British Journal of Radiology 46, 1016–1022.

Hsieh, J., 2003. Computed Tomography: Principles, Design, Artifacts, and Recent Advances. SPIE Press, Bellingham, WA, USA. volume 114.

ICH, 2009. ICH Harmonised tripartite guideline. Pharmaceutical development Q8(R2). URL: `https://www.ich.org/page/quality-guidelines`. (12. May 2020).

Jain, A.K., 2010. Data clustering: 50 years beyond K-means. Pattern Recognition Letters 31, 651–666.

Juran, J.M., 1992. Juran on Quality by Design: The New Steps for Planning Quality into Goods and Services. Simon and Schuster, New York, NY, USA.

Kak, A.C., Slaney, M., 1988. Principles of Computerized Tomographic Imaging. IEEE Press, New York, NY, USA. volume 33.

Kaur, D., Kaur, Y., 2014. Various Image Segmentation Techniques: A Review. International Journal of Computer Science and Mobile Computing 3, 809–814.

Kim, B., Woo, Y.A., 2021. Optimization of in-line near-infrared measurement for practical real time monitoring of coating weight gain using design of experiments. Drug Development and Industrial Pharmacy 47, 72–82.

Kimura, G., Puchkov, M., Leuenberger, H., 2013. An Attempt to Calculate In Silico Disintegration Time of Tablets Containing Mefenamic Acid, a Low Water-Soluble Drug. Journal of Pharmaceutical Sciences 102, 2166–2178.

Korsmeyer, R.W., Gurny, R., Doelker, E., Buri, P., Peppas, N.A., 1983. Mechanisms of solute release from porous hydrophilic polymers. International Journal of Pharmaceutics 15, 25–35.

Korte, C., Quodbach, J., 2018. 3D-Printed Network Structures as Controlled-Release Drug Delivery Systems: Dose Adjustment, API Release Analysis and Prediction. AAPS PharmSciTech 19, 3333–3342.

Laaksonen, T.J., Laaksonen, H.M., Hirvonen, J.T., Murtomäki, L., 2009. Cellular automata model for drug release from binary matrix and reservoir polymeric devices. Biomaterials 30, 1978–1987.

Lak, M., Fleck, G., Azar, D., Engel, M.S., Kaddumi, H.F., Neraudeau, D., Tafforeau, P., Nel, A., 2009. Phase contrast X-ray synchrotron microtomography and the oldest damselflies in amber (Odonata: Zygoptera: Hemiphlebiidae). Zoological Journal of the Linnean Society 156, 913–923.

Larson, E.C., Chandler, D.M., 2010. Most apparent distortion: full-reference image quality assessment and the role of strategy. Journal of Electronic Imaging 19, 011006.

Lee, H., Huang, C., Yune, S., Tajmir, S.H., Kim, M., Do, S., 2019. Machine Friendly Machine Learning: Interpretation of Computed Tomography Without Image Reconstruction. Scientific Reports 9, 1–9. URL: `license:https://creativecommons.org/licenses/by/4.0/`.

Leuenberger, H., Lanz, M., 2005. Pharmaceutical powder technology — from art to science: the challenge of the FDA's Process Analytical Technology initiative. Advanced Powder Technology 16, 3–25.

Leuenberger, H., Leuenberger, M.N., 2016. Impact of the digital revolution on the future of pharmaceutical formulation science. European Journal of Pharmaceutical Sciences 87, 100–111.

Li, C., Zeitler, J.A., Dong, Y., Shen, Y.C., 2014. Non-Destructive Evaluation of Polymer Coating Structures on Pharmaceutical Pellets Using Full-Field Optical Coherence Tomography. Journal of Pharmaceutical Sciences 103, 161–166.

Li, C.H., Lee, C.K., 1993. Minimum cross entropy thresholding. Pattern Recognition 26, 617–625.

Li, C.H., Tam, P.K.S., 1998. An iterative algorithm for minimum cross entropy thresholding. Pattern Recognition Letters 19, 771–776.

Limberg, J., Potthast, H., 2013. Regulatory status on the role of in vitro dissolution testing in quality control and biopharmaceutics in Europe. Biopharmaceutics and Drug Disposition 34, 247–253.

Ma, L., Li, S., Zhang, F., Ngan, K.N., 2011. Reduced-Reference Image Quality Assessment Using Reorganized DCT-Based Image Representation. IEEE Transactions on Multimedia 13, 824–829.

Markham, J., Conchello, J.A., 2001. Artefacts in restored images due to intensity loss in three-dimensional fluorescence microscopy. Journal of Microscopy 204, 93–98.

Markl, D., Strobel, A., Schlossnikl, R., Bøtker, J., Bawuah, P., Ridgway, C., Rantanen, J., Rades, T., Gane, P., Peiponen, K.E., et al., 2018. Characterisation of pore structures of pharmaceutical tablets: A review. International Journal of Pharmaceutics 538, 188–214.

Markl, D., Zeitler, J.A., Rasch, C., Michaelsen, M.H., Müllertz, A., Rantanen, J., Rades, T., Bøtker, J., 2017. Analysis of 3D Prints by X-ray Computed Microtomography and Terahertz Pulsed Imaging. Pharmaceutical Research 34, 1037.

McAllister, M., 2010. Dynamic Dissolution: A Step Closer to Predictive Dissolution Testing? Molecular Pharmaceutics 7, 1374–1387.

McCullough, E.C., 1975. Photon attenuation in computed tomography. Medical Physics 2, 307–320.

Miguélez-Morán, A.M., Wu, C.Y., Dong, H., Seville, J.P., 2009. Characterisation of density distributions in roller-compacted ribbons using micro-indentation and X-ray micro-computed tomography. European Journal of Pharmaceutics and Biopharmaceutics 72, 173–182.

Mirzaei, M., 2020. Lab-in-Silico: An International Journal. Lab-in-Silico 1, 1–2.

Mishra, V., Thakur, S., Patil, A., Shukla, A., 2018. Quality by design (QbD) approaches in current pharmaceutical set-up. Expert Opinion on Drug Delivery 15, 737–758.

Miura, K., 2010a. Bleach Correction. URL: `https://raw.githubusercontent.com/fiji/CorrectBleach/CorrectBleach_-2.0.2/src/main/java/emblcmci/BleachCorrection.java`. (03. March 2020).

Miura, K., 2010b. Histogram matching. URL: `https://github.com/fiji/CorrectBleach/blob/CorrectBleach_-2.0.2/src/main/java/emblcmci/BleachCorrection_MH.java`. (03. March 2020).

Miura, K., 2020. Bleach correction ImageJ plugin for compensating the photobleaching of time-lapse sequences. F1000Research 9. 1494.

Münch, B., 2019. Remove Background. URL: `https://imagej.net/Xlib`. (05. March 2020).

Modi, S., Hughes, M., Garrow, A., White, A., 2012. The value of in silico chemistry in the safety assessment of chemicals in the consumer goods and pharmaceutical industries. Drug Discovery Today 17, 135–142.

Moore, J.W., Flanner, H.H., 1996. Mathematical Comparison of Dissolution Profiles. Pharmaceutical Technology 20, 64–74.

Mori, I., Machida, Y., Osanai, M., Iinuma, K., 2013. Photon starvation artifacts of X-ray CT: their true cause and a solution. Radiological Physics and Technology 6, 130–141.

Njoku, J., Amaral Silva, D., Mukherjee, D., Webster, G., Löbenberg, R., 2019. In silico Tools at Early Stage of Pharmaceutical Development: Data Needs and Software Capabilities. AAPS PharmSciTech 20.

Njoku, J.O., Mukherjee, D., Webster, G.K., Löbenberg, R., 2020. Amorphous Solid Dispersions in Early Stage of Formulation Development: Predicting Excipient Influence on Dissolution Profiles Using DDDPlus. Dissolution Technologies 27, 6–14.

Otsu, N., 1975. A Threshold Selection Method from Gray-Level Histograms. Automatica 11, 285–296.

Panetta, K., Gao, C., Agaian, S., 2013. No reference color image contrast and quality measures. IEEE Transactions on Consumer Electronics 59, 643–651.

Peppas, N., 1985. Analysis of Fickian and non-Fickian drug release from polymers. Pharmaceutica Acta Helvetiae 60, 110–111.

Ph. Eur. 10.0, 2020. 2.9.3. Dissolution test for solid dosage forms. European Directorate for the Quality of Medicines and HealthCare, Council of Europe, Strasbourg, France.

Podczeck, F., 1993. Comparison of in vitro dissolution profiles by calculating mean dissolution time (MDT) or mean residence time (MRT). International Journal of Pharmaceutics 97, 93–100.

Politis, S., Colombo, P., Colombo, G., M. Rekkas, D., 2017. Design of experiments (DoE) in pharmaceutical development. Drug Development and Industrial Pharmacy 43, 889–901.

Promentilla, M.A.B., Sugiyama, T., Hitomi, T., Takeda, N., 2009. Quantification of tortuosity in hardened cement pastes using synchrotron-based X-ray computed microtomography. Cement and Concrete Research 39, 548–557.

Puchkov, M., Tschirky, D., Leuenberger, H., 2013. 7 - 3-D cellular automata in computer-aided design of pharmaceutical formulations: mathematical concept and F-CAD software. Woodhead Publishing, Cambridge, UK. pp. 155–201.

Rabin, J., Delon, J., Gousseau, Y., 2011. Removing Artefacts From Color and Contrast Modifications. IEEE Transactions on Image Processing 20, 3073–3085.

Radtke, J., Wiedey, R., Kleinebudde, P., 2019. Effect of coating time on inter-and intra-tablet coating uniformity. European Journal of Pharmaceutical Sciences 137, 104970.

Ramponi, G., Strobel, N.K., Mitra, S.K., Yu, T.H., 1996. Nonlinear unsharp masking methods for image contrast enhancement. Journal of Electronic Imaging 5, 353–366.

Ranjan, A., Jha, P.K., 2021. Recent Advances in Dissolution Testing and Their Use to Improve In Vitro–In Vivo Correlations in Oral Drug Formulations. Journal of Pharmaceutical Innovation 16, 1–16.

Rietdorf, J., 2004. Bleach Correction macro for ImageJ. URL: `https://www.embl.de/eamnet/html/bleach_correction.html`. (04. November 2021).

Roche, R.C., Abel, R.A., Johnson, K.G., Perry, C.T., 2010. Quantification of porosity in Acropora pulchra (Brook 1891) using X-ray micro-computed tomography techniques. Journal of Experimental Marine Biology and Ecology 396, 1–9.

Ross, L., Russ, J.C., 2010. The Image Processing Handbook. 6 ed.. CRC press, Boca Raton, FL, USA.

Rueden, C., 2014. Statistical Region Merging. URL: `https://github.com/fiji/Statistical_Region_Merging/blob/Statistical_Region_Merging-2.0.1/src/main/java/SRM_.java`. (12. November 2021).

Rueden, C., 2019. Gamma. URL: `https://github.com/imagej/ImageJA/blob/232620e0a3b0fd33bb22083aaadb2c26b1a31fc6/src/main/java/ij/plugin/filter/ImageMath.java`. (05. March 2020).

Rueden, C., Schindelin, J., 2014. Calculator Plus. URL: `https://github.com/fiji/Calculator_Plus/blob/Calculator_Plus-2.0.1/src/main/java/Calculator_Plus.java`. (05. November 2021).

Rueden, C., Schindelin, J., 2019. ImageMath. URL: `https://github.com/imagej/ImageJA/blob/531cc60aa90f0d2833383edd3cdcd518d91c3a65/src/main/java/ij/plugin/filter/ImageMath.java`. (05. November 2021).

Russe, I.S., Brock, D., Knop, K., Kleinebudde, P., Zeitler, J.A., 2012. Validation of Terahertz Coating Thickness Measurements Using X-ray Microtomography. Molecular Pharmaceutics 9, 3551–3559.

Sacha, J., 2019. k-means clustering. URL: `https://github.com/ij-plugins/ijp-toolkit/blob/master/src/main/java/net/sf/ij_plugins/clustering/KMeansClusteringPlugin.java`. (05. March 2020).

Sathe, P.M., Tsong, Y., Shah, V.P., 1996. In-vitro Dissolution Profile Comparison: Statistics and Analysis, Model Dependent Approach. Pharmaceutical Research 13, 1799–1803.

Sato, T., Ikeda, O., Yamakoshi, Y., Tsubouchi, M., 1981. X-ray tomography for microstructural objects. Applied Optics 20, 3880–3883.

Schindelin, J., 2011. EDM. URL: `https://github.com/imagej/ImageJA/blob/232620e0a3b0fd33bb22083aaadb2c26b1a31fc6/src/main/java/ij/plugin/filter/EDM.java`. (10. September 2020).

Schindelin, J., 2015. BackgroundSubtracter. URL: `https://github.com/imagej/ImageJA/blob/531cc60aa90f0d2833383edd3cdcd518d91c3a65/src/main/java/ij/plugin/filter/BackgroundSubtracter.java`. (05. November 2021).

Schindelin, J., 2017. Enhance Contrast. URL: `https://github.com/imagej/ImageJA/blob/232620e0a3b0fd33bb22083aaadb2c26b1a31fc6/src/main/java/ij/plugin/ContrastEnhancer.java`. (05. March 2020).

Schulze, R., Heil, U., Groβ, D., Bruellmann, D., Dranischnikow, E., Schwanecke, U., Schoemer, E., 2011. Artefacts in CBCT: a review. Dentomaxillofacial Radiology 40, 265–273.

Segregur, D., Mann, J., Moir, A., Karlsson, E.M., Dressman, J., 2022. Biorelevant in vitro Tools and in silico Modeling to Assess pH-Dependent Drug-drug Interactions for Salts of Weak Acids: Case Example Potassium Raltegravir. Journal of Pharmaceutical Sciences 111, 517–528.

Shah, V.P., 2001. Dissolution: A Quality Control Test vs. A Bioequivalence Test. Dissolution Technologies 8, 6–7.

Shah, V.P., Tsong, Y., Sathe, P., Liu, J.P., 1998. In Vitro Dissolution Profile Comparison—Statistics and Analysis of the Similarity Factor, f2. Pharmaceutical Research 15, 889–896.

Shah, V.P., Yamamoto, L.A., Schuirman, D., Elkins, J., Skelly, J.P., 1987. Analysis of in Vitro Dissolution of Whole vs. Half Controlled-Release Theophylline Tablets. Pharmaceutical Research 4, 416–419.

Sieburg, H., 1990. Physiological Studies in Silico. Studies in the Sciences of Complexity 12, 321–342.

Sinka, I., Burch, S., Tweed, J., Cunningham, J., 2004. Measurement of density variations in tablets using X-ray computed tomography. International Journal of Pharmaceutics 271, 215–224.

Smith, D.M., Kapoor, Y., Klinzing, G.R., Procopio, A.T., 2018. Pharmaceutical 3D printing: Design and qualification of a single step print and fill capsule. International Journal of Pharmaceutics 544, 21–30.

Solomon, C., Breckon, T., 2011. Fundamentals of Digital Image Processing: A Practical Approach with Examples in Matlab. John Wiley & Sons, Chichester, UK.

Spektrum Akademischer Verlag, Lexikon der Physik, 1998. gefilterte Rückprojektion. URL: `https://www.spektrum.de/lexikon/physik/gefilterte-rueckprojektion/5647`. (20. January 2022).

Tsong, Y., Hammerstrom, T., Sathe, P., Shah, V.P., 1996. Statistical Assessment of Mean Differences between Two Dissolution Data Sets. Drug Information Journal 30, 1105–1112.

Tuy, H.K., 1983. An Inversion Formula for Cone-Beam Reconstruction. SIAM Journal on Applied Mathematics 43, 546–552.

US Pharmacopeial Convention, 2018a. USP 41 NF 36. United States Pharmacopeial Convention, North Bethesda, MD, USA. pp. 4031–4038.

US Pharmacopeial Convention, 2018b. USP 41 NF 36. United States Pharmacopeial Convention, North Bethesda, MD, USA. pp. 1258–1260.

Viceconti, M., Pappalardo, F., Rodriguez, B., Horner, M., Bischoff, J., Tshinanu, F.M., 2021. In silico trials: Verification, validation and uncertainty quantification of predictive models used in the regulatory evaluation of biomedical products. Methods 185, 120–127.

Vidal, F.P., Létang, J.M., Peix, G., Cloetens, P., 2005. Investigation of artefact sources in synchrotron microtomography via virtual X-ray imaging. Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms 234, 333–348.

Wang, Z., Bovik, A.C., 2006. Modern Image Quality Assessment. Synthesis Lectures on Image, Video, and Multimedia Processing 2, 1–156.

Wiedey, R., Kleinebudde, P., 2017. The Density Distribution in Ribbons from Roll Compaction. Chemie Ingenieur Technik 89, 1017–1024.

Wolfram, S., 2002. A New Kind of Science. Wolfram Media Inc., Champaign, IL, USA. p. 1197.

Woodcock, J., 2004. The Concept of Pharmaceutical Quality. American Pharmaceutical Review 7, 10–15.

Wu, L., Wang, L., Wang, S., Xiao, T., Chen, M., Shao, Q., York, P., Singh, V., Yin, X., Gu, J., Zhang, J., 2016. Three dimensional structural insight of laser drilled orifices in osmotic pump tablets. European Journal of Pharmaceutical Sciences 93, 287–294.

Yu, L.X., Amidon, G., Khan, M.A., Hoag, S.W., Polli, J., Raju, G.K., Woodcock, J., 2014. Understanding Pharmaceutical Quality by Design. The AAPS Journal 16, 771–783.

Zhai, G., Cai, J., Lin, W., Yang, X., Zhang, W., Etoh, M., 2008. Cross-Dimensional Perceptual Quality Assessment for Low Bit-Rate Videos. IEEE Transactions on Multimedia 10, 1316–1324.

Zhang, S., Stroud, P.A., Zhu, A., Johnson, M.J., Lomeo, J., Burcham, C.L., Hinds, J., Blakely, K.A.F., Walworth, M.J., 2021. Characterizing the Impact of Spray Dried Particle Morphology on Tablet Dissolution Using Quantitative X-Ray Microscopy. European Journal of Pharmaceutical Sciences 165, 105921.

2 Evaluation of different pre-processing methods of X-ray micro computed tomography images

Pretext

The subsequent research article has been published in 2021 by the journal Powder Technology in volume 381 on pages 539-550.
Sebastian Bollmann contributed some ideas to the concept of the study. He mainly designed the study and did all of the experimental work. Sebastian Bollmann performed main parts of the data analysis and wrote the manuscript. Peter Kleinebudde contributed the main ideas for the study and supported the study design with his remarks. He supported the data analysis with his comments and advice and was responsible for revising the manuscript.

Table 1: Evaluation of the authorship:

Author	Idea	Study design	Experimental	Evaluation	Manuscript
	%	%	%	%	%
Sebastian Bollmann	20	70	100	70	80
Peter Kleinebudde	80	30	0	30	20

Reference

Bollmann, S. & Kleinebudde, P. (2021). Evaluation of different pre-processing methods of X-ray micro computed tomography images. In Powder Technology (Vol. 381, pp. 539–550). Elsevier BV.
DOI und Link: https://doi.org/10.1016/j.powtec.2020.11.074

Abstract

Adequate images of real tablets provide the possibility to validate *in silico* software tools like F-CAD. The goal of this work was to evaluate the influence of different pre-processing methods of X-ray micro-computed tomography images of 12 different tablet batches.
The images were processed with the open source software ImageJ. Different bleach- and lightning corrections were applied. The influence of a filtering process and two different contrast enhancement methods were evaluated. The images were segmented with the k-means algorithm and F-CAD determined the recovery rates.
All results were analysed in terms of their desirability. Suitable pathways were found for each binary mixture. The mean recovery rates for all batches were approximately 100 %. However, it was not possible to find a suitable program procedure to cover all binary mixtures simultaneously. Nevertheless, it was possible to confirm the possibility of a suitable validation method for *in silico* tools.

3 Evaluation of different segmentation methods of X-ray micro computed tomography images

Pretext

The subsequent research article has been published in 2021 by the International Journal of Pharmaceutics in volume 606 (article number 120880).
Sebastian Bollmann and Peter Kleinebudde developed the concept in collaboration. Sebastian Bollmann mainly designed the study and did all of the experimental work. Sebastian Bollmann did considerable parts of the data analysis and wrote the manuscript. Peter Kleinebudde supported the study design and evaluation with his comments and advice and was responsible for revising the manuscript.

Table 2: Evaluation of the authorship:

Author	Idea %	Study design %	Experimental %	Evaluation %	Manuscript %
Sebastian Bollmann	50	80	100	80	80
Peter Kleinebudde	50	20	0	20	20

Reference

Bollmann, S. & Kleinebudde, P. (2021). Evaluation of different segmentation methods of X-ray micro computed tomography images. In International Journal of Pharmaceutics (Vol. 606, p. 120880). Elsevier BV.
DOI und Link: https://doi.org/10.1016/j.ijpharm.2021.120880

Abstract

In silico tools for the prediction of disintegration and/or dissolution of tablets can be validated using adequate images of real pharmaceutical formulations. X-ray micro-computed tomography images of 12 different tablet batches prepared from binary mixtures of API and excipient were used. The goal of this work was to compare different segmentation methods to improve the results and processing time of an evaluation of pre-processing methods. The open source software ImageJ was utilised for the image processing. Different threshold algorithms were applied as well as different cluster numbers for the k-means clustering. The pathways were analysed regarding their desirability which was calculated from the recovery rates and their ratios. It was possible to identify suitable pathways for each single batch as well as for combinations of several batches. The recovery rates of the best pathways were always approximately 100 %. It was possible to confirm the correctness of the image processing by visual perception. The image processing could be improved and sped up.

4 Predictive selection rule of favourable image processing methods for X-ray micro-computed tomography images of tablets

Pretext

The subsequent research article has been published in 2021 by the International Journal of Pharmaceutics in volume 610 (article number 121207).
Sebastian Bollmann mainly developed the concept and study design. He did all of the experimental work, most of the evaluation and wrote the manuscript. Peter Kleinebudde supported the study concept, design and evaluation with his comments and advice and was responsible for revising the manuscript.

Table 3: Evaluation of the authorship:

Author	Idea %	Study design %	Experimental %	Evaluation %	Manuscript %
Sebastian Bollmann	70	70	100	90	80
Peter Kleinebudde	30	30	0	10	20

Reference

Bollmann, S. & Kleinebudde, P. (2021). Predictive selection rule of favourable image processing methods for X-ray micro-computed tomography images of tablets. In International Journal of Pharmaceutics (Vol. 610, p. 121207). Elsevier BV.
DOI und Link: https://doi.org/10.1016/j.ijpharm.2021.121207

Abstract

Adequately processed X-ray micro-computed tomography images of real pharmaceutical formulations provide the possibility to validate *in silico* tools for the prediction of disintegration and/or dissolution. However, the evaluation of suitable image processing pathways is time consuming. The objective of this study was to prove the transferability of image processing methods and to develop an approach to select probable favourable image processing approaches for data sets with similar properties to accelerate the evaluation process. Therefore, data from a previously performed evaluation of image processing approaches and parameters were used to analyse the robustness of the image processing by statistical resampling and to develop a predictive rule set. The rule set was verified by both one new ratio of API and excipient within and outside of the ratios used to develop the rule. The rule was applied to images of a binary mixture with new compounds with similar determined image properties to prove the transferability of the rule set. It was possible to identify robust image processing pathways with narrow ranges of input parameters. The prediction of the image processing pathways led to high desirabilities which were confirmed by visual verification for ratios

within the calibrated range. The transfer to the new binary mixture was successful and confirmed as well.

5 A New Validation Methodology for In Silico Tools Based on X-ray Computed Tomography Images of Tablets and a Performance Analysis of One Tool

Pretext

The subsequent research article has been published in 2021 by the journal Pharmaceutics in volume 13 (article number 1488).
Sebastian Bollmann and Peter Kleinebudde developed the concept in collaboration. Sebastian Bollmann carried out all the experimental work of the study, which was mostly designed and evaluated by himself, and wrote the manuscript. Peter Kleinebudde supported the study design and evaluation with his comments and advice and was responsible for revising the manuscript.

Table 4: Evaluation of the authorship:

Author	Idea %	Study design %	Experimental %	Evaluation %	Manuscript %
Sebastian Bollmann	50	90	100	90	80
Peter Kleinebudde	50	10	0	10	20

Reference

Bollmann, S. & Kleinebudde, P. (2021). A New Validation Methodology for In Silico Tools Based on X-ray Computed Tomography Images of Tablets and a Performance Analysis of One Tool. In Pharmaceutics (Vol. 13, Issue 9, p. 1488). MDPI AG.
DOI und Link: https://doi.org/10.3390/pharmaceutics13091488

Abstract

In silico tools which predict the dissolution of pharmaceutical dosage forms using virtual matrices can be validated with virtual matrices based on X-ray micro-computed tomography images of real pharmaceutical formulations. Final processed images of 3 different tablet batches were used to check the performance of the *in silico* tool F-CAD. The goal of this work was to prove the performance of the software by comparing the predicted dissolution profiles to the experimental ones and to check the feasibility and application of the validation concept for *in silico* tools. Both virtual matrices based on X-ray micro-computed tomography images and designed by the software itself were used. The resulting dissolution curves were compared regarding their similarity to the experimental curve. The kinetics were analysed with the Higuchi and Korsmeyers–Peppas plot. The whole validation concept as such was feasible and worked well. It was possible to identify prediction errors of the software F-CAD and issues with the virtual tablets designed within the software.

6 Evaluation of the transferability of an image analysis approach of X-ray micro-computed tomography images for the application with a new validation concept for in silico tools

Pretext

The subsequent research article has been published in 2022 by the Journal of Drug Delivery Science and Technology in volume 70 (article number 103163).
Sebastian Bollmann mainly developed the concept of the study. He carried out considerable parts of the experimental work of the study, which was mainly designed and evaluated by himself, and wrote most of the manuscript. Björn Fischer contributed comments and advice for the concept and design of the study and carried out the experimental part of the confocal Raman spectroscopy, which was also evaluated by him. He wrote the section on confocal Raman spectroscopy within the manuscript and revised it. Peter Kleinebudde supported the development of the study concept and design with his comments and advice and was responsible for revising the manuscript.

Table 5: Evaluation of the authorship:

Author	Idea %	Study design %	Experimental %	Evaluation %	Manuscript %
Sebastian Bollmann	70	70	80	70	70
Björn Fischer	20	15	20	30	10
Peter Kleinebudde	10	15	0	0	20

Reference

Bollmann, S., Fischer, B. & Kleinebudde, P. (2022). Evaluation of the transferability of an image analysis approach of X-ray micro-computed tomography images for the application with a new validation concept for in silico tools. In Journal of Drug Delivery Science and Technology (Vol. 70, p. 103163). Elsevier BV.
DOI und Link: https://doi.org/10.1016/j.jddst.2022.103163

Abstract

It is possible to validate *in silico* tools for predicting quality attributes such as dissolution by using processed X-ray micro-computed tomography (XµCT) images of real pharmaceutical formulations as virtual matrices. Different batches of oral films were produced and imaged with XµCT. Over 30 million virtual matrices were evaluated and assessed. The highest performing image processing pathways were used for visual verification. The goal of this work was to assess the transferability of the concept of XµCT image based virtual matrices from tablets to oral films and to evaluate issues and limitations of the transferred concept to test feasibility. In principle, the images were

suitable for usage in the validation concept but air bubbles in the oral films caused artefacts in the XμCT images which were identified by confocal Raman microscopy. The final processed images represented the raw images including the artefacts. Therefore, the virtual matrices based on films with air bubbles did not represent the real oral films and could not be used for the *in silico* calculations. The images of oral films without air bubbles were appropriate.

7 Discussion of the entire results and outlook

This work developed a new concept which allowed for the validation, understanding and improvement of *in silico* tools. The concept was successfully used to evaluate the performance of an *in silico* tool. It was possible to either verify or falsify the predicted dissolution profiles of the software package F-CAD. Feedback was given to the developer of the software package who used the provided information to release software updates to resolve the identified issues. The results presented in section 5 will be used for upcoming updates. However, this work was only a starting point for a reliable validation methodology since more data is required from different pharmaceutical dosage forms to improve the approach so that a robust and reliable validation methodology can be developed. In addition, the presented concept can be a useful tool to investigate in detail the performances of formulations in quality control tests such as dissolution testing. The concept has so far only been tested with simple systems (binary mixtures of API and excipient, air as the third phase). Therefore, further investigations are imperative.

The concept described in this work was only applicable, if the contrast between the API and exipient(s) in the image was sufficient. Therefore, both the image acquisition and processing presented in sections 2 and 3 were crucial steps of the new approach. Since the densities and atomic composition of many pharmaceutical excipients and APIs are similar, the described method can usually not be used to distinguish all compounds within a pharmaceutical dosage form that has been introduced to the market. Therefore, specifically designed model formulations should be used with the presented approach to address different properties and characteristics. However, if a single compound could be clearly distinguished, its performance within a more complex formulation could be analysed.

The resolution of the images was also an important factor. The particle size of the API and excipient should be four times larger than the voxel size. This is especially critical for micronised powder grades which limits their usage. A work-around using the image density was presented in sections 3 and 4 for particles smaller than or equal to the resolution. This approach should be avoided whenever possible as the true particle distribution within the pharmaceutical dosage form is only predicted. In research, the image density could be a useful alternative to obtain data on hypotheses about the performance of quality control tests of the imaged dosage forms or the used software tools. Subsequently, conclusions could be drawn from observed phenomena and issues in formulations to be identified. Therefore, it could be a helpful aid in the development of formulations but should not be used to validate *in silico* tools.

Image processing determined the quality of the final virtual matrix. Therefore, a careful and detailed evaluation of the image processing pathways should be performed and presented. Image processing should be automated as far as possible to reduce user bias. As described in section 1.5.4, the quality assessment of image processing pathways is not straightforward. The desirability has been used in the entire work as a tool to judge the quality of virtual matrices. However, the desirability calculation has

to be improved to also take into account the distribution pattern of the compounds with respect to the raw image. The visual verification revealed that the information of the expected pattern from the raw image is essential to avoid the high performance of pathways reflecting the correct recovery rates of the individual compounds with an obviously wrong distribution pattern of the compounds. Therefore, the quality assessment should be changed from an approach without any references to the full or reduced reference method. The sensitivity analysis presented in section 5 confirmed the conclusions drawn from the visual verification that the desirability calculation has to be adjusted, as there were virtual matrices with lower desirability performing higher than those with higher desirability. This shows, that the desirability did not reflect all of the important factors in judging the quality of the virtual matrices. Nevertheless, the trend towards high desirability and high performance was clearly observed. Therefore, the desirability parameters used were generally reliable to evaluate the virtual matrices but some information was missing. If the distribution pattern is taken into account, this may solve the issue of some incorrectly high desirabilities.
The sensitivity of the investigated software increased when the impact of the diffusion on the dissolution was increased. This indicates that the image processing was more critical for some investigated system than for others. As depicted in sections 2 and 3, the evaluation of image processing pathways was time intensive. The sensitivity of different *in silico* tools has to be investigated to check whether the criticality of image processing depends either on the investigated systems or on the software tools. This knowledge could be useful to reduce the evaluation effort for specific pharmaceutical dosage forms. In section 4, a simplification of the image processing pathway evaluation was presented, based on the results of similar samples analysed in previous evaluations. This is another way to reduce the evaluation effort. However, the presented approach depends on both the similarity of the samples and the results of the pathway performances in previous evaluations. As described in section 1.5.3, scientific publications often lack the description of image processing methods and their pathway evaluation. This impedes the transferability of image processing pathways and limits the usage of image processing pathway predictions such as the presented coefficient of variation. The transferability of image processing pathways was given for different formulations with similar coefficients of variation. Since the approach was only valid in a calibrated range, a detailed evaluation of several formulations with different ratios for calibration is still mandatory. Although the obligatory detailed evaluation for the calibration set limits the feasibility of this approach, the transferability may accelerate future evaluations with fewer resources, justifying the invested effort. The approach has to be further investigated to improve the reduction of the evaluation space and minimise the required effort. An open access repository containing the results of the image processing pathway performances of the entire evaluation, including the associated pathways and raw images, could considerably accelerate future evaluations based on the identical evaluation system. In addition, the transparency of the image processing would be given. This protects the author from accusations of misconduct. However, a large stor-

age capacity would be required, as XμCT images are data intensive. It is questionable who would provide such a platform.
In this work, only a selection of available image processing approaches has been used to evaluate their performances. An open access repository or fully published evaluations, as presented in sections 2 and 3, are important since it is impossible to evaluate all image processing approaches. The number of available approaches forces each scientist to select only some of the approaches. All methodologies have their strengths and weaknesses and different scientists may be able to improve the image processing results presented or the probability of success by using different approaches.
It was possible to distinguish different levels of specification for image processing approaches. The most accurate approach would be to determine the highest performing image processing pathway for each individual image. However, it would not be feasible to expend so much effort. Sections 2, 3, and 5 have demonstrated that a ratio-independent evaluation could be appropriate for any combination of API and excipient. If the performance of these pathways is not sufficient, a batch-specific evaluation is recommended. A more general approach was insufficient because the performance of the pathways was low. The compromise of effort and specificity was a feasible and applicable solution. However, more data is required to substantiate these results.
The rationale for the image processing effort was presented in section 5. The use of appropriately prepared images to rate the performance of an *in silico* tool demonstrated the importance of such a validation concept for *in silico* tools: the performance of F-CAD was high for tablets whose dissolution profiles depended on the dissolution rate of the API. However, for tablets where diffusion through a matrix dominated over the dissolution rate, the software predicted different kinetics and dissolution profiles. Since it is desirable to predict correct dissolution profiles without experimental dissolution tests in order to design robust and suitable pharmaceutical formulations completely *in silico* according to the QbD approach, the predictions have to be compared with experimental results. If the software results have to be fitted to the experimental data until the prediction matches the experimental data, it is not possible to develop formulations fully *in silico*. The presented concept made it possible to compare the prediction of the software with the experimental data of a dosage form, since the virtual matrix, with prudent image processing, accurately reflects the real dosage form used in the experiment. In case of F-CAD, the virtual matrices designed by the software package were not comparable to the real dosage forms due to different particle and pore structure. Therefore, the design of virtual matrices by the software has to be adapted to allow full *in silico* formulation development. The calculation of the diffusion through a matrix was also revealed to be incorrect. The kinetics obtained showed that the assumed mechanism was wrong and the calculation has to be corrected. However, the dissolution rate of the API was predicted correctly. The concept made it possible to identify strengths and weaknesses of F-CAD and to give advice to the software developer as to where the models or assumptions need to be improved.
F-CAD is not a simple CA tool. It combines CA calculations with differential equa-

tions. Therefore, some parameters of the compounds have to be determined. Some parameters, such as helium density, are easy to determine, but others, like the swelling behaviour of compacted compounds, are not. The applicability of F-CAD is limited by some parameters that are difficult to determine. In addition, sometimes measured parameters have to be adjusted to obtain reliable results (e.g. the contact angle, as the contact angle of the mixture of two compounds may differ from that of the individual compound, which has to be entered). In summary, the applicability of F-CAD is limited while the performance is high only in some cases of the simple binary mixtures.
The transferability of the concept from tablets to oral films was presented in section 6. The transfer was possible in principle. However, in this case, the raw image quality was an issue. The properties of oral films differed considerably from those of tablets. It was more difficult to acquire high-quality images because the oral films were considerably thinner. In this work, the images of the oral films contained stronger measurement artefacts, which partially interfered with the image processing. The images were not appropriate to compare the *in silico* data with the experimental data because the artefacts of incorporated air bubbles of the oral films led to obvious misinterpretations of the data. Image processing was not the issue as the artefacts were clearly visible in the raw images. Confocal Raman microscopy revealed that the XμCT images were sensitive to air bubbles within the oral films and mimicked a Pickering emulsion-like system in the images. The final processed images reflected the raw images and thus the artificial system. This depicts that the raw images have to be carefully reviewed for plausibility. It has to be proven that the raw images used for validation reflect the real pharmaceutical dosage form and that they are not artificial images based on measurement artefacts. These results increase the obligatory effort to obtain suitable images for the presented validation concept. If the concept is used for oral films, it is recommended to improve the imaging methodology and to avoid air bubbles within the oral films. Air bubbles should also be avoided in other dosage forms such as film coatings of coated tablets to prevent the described phenomenon. Dosage forms that contain desired air bubbles, such as foamed oral films, cannot be used with XμCT images in the developed concept. However, 3D images acquired by another non-destructive imaging approach may be appropriate for the presented concept.
In spite of the mandatory high effort, the advantage of the presented concept lies in the possibility for future usage by the authorities. Since it would be possible to securely store the raw images and final processed images as well as the corresponding experimental data of the imaged pharmaceutical dosage forms over a long period of time, it would be possible to build a validation set for *in silico* tools. A company intending to release a software package as *in silico* tool for the development of pharmaceutical dosage forms could be benchmarked against this reference data set by the authorities. This would allow for an informed decision on the applicability of the software tool. In addition, the effort for image processing would be considerably reduced, as it has to be carried out only once for each system and dosage form. If new systems are introduced, the existing data sets can be expanded without affecting the old data sets. It would be

possible to permit only specific pharmaceutical dosage forms for software packages, for example only predicting dissolution profiles based on the dissolution rate of APIs for F-CAD. In this way, the reference data sets obtained through specially designed model formulations could be selected to target specific mechanisms or critical parameters of different dosage forms and all virtual matrix-based *in silico* tools would be comparable and verifiable for the authorities. However, the authorities would require some experts to design the model formulations, perform the proper image processing to generate optimal virtual matrices and perform the real experiments. This requires some expensive investments at the beginning which could be covered by the obligatory purchase of the data sets relevant for the approval of the software, similar to the chemical reference standards. In order to justify the high investment and guarantee a working overall concept, the presented concept has to be investigated in more detail by different scientists using different software packages and pharmaceutical dosage forms.
Commonly, *in silico* dissolution testing tools are used to compare the data from virtually designed dosage forms with experimental data, assuming that the virtual dosage form represents the experimental one (Njoku et al., 2019; Almukainzi et al., 2015; Puchkov et al., 2013) or to fit the predictive model of the *in silico* tool to the experimental data for further optimisation (Amini et al., 2021). Sometimes, attempts are also made to predict the *in vivo* data or bioequivalence based on the simulated dissolution behaviour (Segregur et al., 2022; Al-Tabakha and Alomar, 2020). This also requires model fittings and the assumption of representative virtual dosage forms. Since a reliable *in vivo* prediction is based on a correct *in vitro* prediction, a correct dissolution simulation is essential.
However, no reliable validation approaches for these tools have been introduced so far. Viceconti et al. (2021) described essential requirements for reliable *in silico* tools. In short, clearly defined objectives, risk assessments, verification of code, calculation and performance, validation of approaches including uncertainty quantification and applicability are imperative (Viceconti et al., 2021). These requirements cannot be met by retrospective studies, which are sometimes applied to confirm the performance of an *in silico* tool after the fact (Al-Tabakha and Alomar, 2020). Therefore, prospective approaches are mandatory. The new approach presented in this work allows for prospective validation of *in silico* tools used for pharmaceutical quality control testing, as well as other predictions based on these tests, such as bioequivalence predictions. In addition, the assumption of representative virtual dosage forms is replaced by the knowledge of a valid virtual dosage form if image processing and verification is done judiciously. Uncertainty determination is also possible through this concept. Thus, for the first time, the concept presented in this paper implements some of the basic requirements described by Viceconti et al. (2021). This forms the basis for future introduction of full *in silico* development of pharmaceutical dosage forms with regulatory approval.

Since this work is to be understood as a starting point, the subsequent logical step would be to investigate different *in silico* tools with this concept. After using several

different *in silico* tools for one performance parameter, the data set should be extended to include different dosage forms in order to evaluate the limitation of the concept in terms of usable pharmaceutical dosage forms. Since only tablet dissolution was used as an example in this work, the transferability to *in silico* tools for predicting other performance parameters such as disintegration or tablet breaking force has to be investigated. The applicability of the concept to more complex formulations than binary mixtures also needs to be assessed. If enough data is available, artificial intelligence could be beneficial to improve and accelerate image processing and analysis. Since images that reflect the plausible raw image and can be uploaded to the software are the goal, the processes for obtaining these images can be adapted or changed by new technologies or approaches such as artificial neural networks without affecting the concept itself. Of course, it will take at least several years before this concept can be safely applied by authorities. However, until the concept is proven and appropriable for validation by authorities, it can support the development and improvement of *in silico* tools. It has an impact on the development of *in silico* tools, as this concept provides useful information for software developers of *in silico* tools regarding their performance. The presented concept allows for the comparison of different calculation approaches, finding prediction issues in existing *in silico* tools and identifying helpful modifications of models. The software developer of F-CAD has been able to fix some bugs through updates by the feedback of the last years and still received some hints for future updates. Therefore, the concept should be investigated further to fully exploit the potential of non-destructive 3D imaging of pharmaceutical dosage forms.

8 Summary

This thesis addresses a new concept to validate *in silico* tools for full *in silico* formulation development and to investigate and improve either the performance of the *in silico* tool or the pharmaceutical dosage form. The basis of this concept is the non-destructive 3D imaging of pharmaceutical dosage forms using XμCT measurements.
The image processing of the obtained 3D images was evaluated and presented in detail. Both the effort required for the application of this concept and some crucial conditions for the applicability of the methodology were depicted. A compromise between effort and specification of the image processing could be found, which led to high performances of the image processing. Quantitative segmentation was achieved and the required virtual matrices representing real pharmaceutical dosage forms were obtained by using the desirability to evaluate the pathway performances of image processing. However, the desirability calculation should be optimised as visual verification was mandatory due to high performing pathways that contained the correct amount of each compound with an incorrect distribution pattern.
The coefficient of variation determined for grey values of images was an appropriate method to reduce the evaluation effort for image processing pathways within calibrated ranges. This could accelerate future image processing evaluations of similar samples.
The concept identified incorrect calculations of the diffusion process in a dissolution

simulation of different tablet batches of an example *in silico* tool. It was also shown that the virtual matrices designed by the software did not reflect real pharmaceutical dosage forms. In spite of these issues, the software was able to correctly predict the dissolution of tablets when the dissolution profile was based on the dissolution rate of the API. Feedback from these experiments has been and will be used to develop software updates to resolve identified issues. In addition to the ability to improve *in silico* tools or pharmaceutical formulations, these experiments demonstrate that the concept is capable of validating *in silico* tools. It was possible to benchmark the performance of the *in silico* tool by comparing the dissolution of virtual matrices representing real pharmaceutical dosage forms based on processed XμCT images with the experimental results of the imaged dosage form. Thus, a direct comparison of different *in silico* tools or the approval assessment by authorities would also be possible with this concept.
In principle, the concept can be transferred from tablets to oral films. However, the images of the oral films were disturbed by artefacts. Air bubbles within the films indicated a Pickering emulsion-like system in the raw images. This could be disproved by confocal Raman microscopy. The processed images and the resulting virtual matrices reflected these artefacts. This emphasises the importance of checking the plausibility of the non-destructive 3D images before subsequent image processing.
If the raw images are plausible and an appropriate image processing pathway has been applied, a virtual matrix representing the real pharmaceutical dosage form could be used to validate, improve or develop *in silico* tools or formulations to predict the performance of pharmaceutical dosage forms in quality control tests. However, this work is only a starting point and further investigations of the new concept are imperative to evaluate its applicability and limitations in more detail.

9 List of original publications

1. S. Bollmann and P. Kleinebudde
   Evaluation of different pre-processing methods of X-ray micro computed tomography images
   Powder Technology 381 (2021) 539–550.

2. S. Bollmann and P. Kleinebudde
   Evaluation of different segmentation methods of X-ray micro computed tomography images
   International Journal of Pharmaceutics 606 (2021) 120880.

3. S. Bollmann and P. Kleinebudde
   Predictive selection rule of favourable image processing methods for X-ray micro-computed tomography images of tablets
   International Journal of Pharmaceutics 610 (2021) 121207.

4. S. Bollmann and P. Kleinebudde
   A New Validation Methodology for In Silico Tools Based on X-ray Computed Tomography Images of Tablets and a Performance Analysis of One Tool
   Pharmaceutics 13 (2021) 1488.

5. S. Bollmann, B. Fischer and P. Kleinebudde
   Evaluation of the transferability of an image analysis approach of X-ray micro-computed tomography images for the application with a new validation concept for *in silico* tools
   Journal of Drug Delivery Science and Technology 70 (2022) 103163.

10 Contribution to conferences

10.1 Oral presentations

1. S. Bollmann and P. Kleinebudde
   Processing of X-ray micro-computed tomography images for the software package F-CAD
   12th Annual Meeting of the Pharmaceutical Solid State Research Cluster, Leuven, Belgium

2. S. Bollmann and P. Kleinebudde
   Evaluation of different pre-processing methods of X-ray micro-computed tomography images
   12th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology 12 May 2021

3. S. Bollmann and P. Kleinebudde
   Pre-processing of XμCT images for the segmentation process
   13th Annual Meeting of the Pharmaceutical Solid State Research Cluster, Düsseldorf, Germany

10.2 Poster presentations

1. S. Bollmann and P. Kleinebudde
   Evaluation of different segmentation methods of X ray micro-computed tomography images
   3rd European Conference on Pharmaceutics 25th 26th March 2019 Bologna, Italy

2. S. Bollmann and P. Kleinebudde
   Experimental vs. *in silico* dissolution testing
   PARTEC International Congress on Particle Technology 09th 11th April 2019 Nürnberg , Germany

11 Danksagung

Zu allerst bedanke ich mich herzlich bei meinem Doktorvater, Herrn Prof. Dr. Dr. h.c. Kleinebudde, dafür, dass ich überhaupt die Möglichkeit bekommen habe, diese Arbeit anfertigen zu können. Es war für mich eine spannende und lehrreiche Zeit und ich werde mich noch lange an so manches Gespräch mit ihm erinnern. Er war zu jeder Zeit ansprechbar und hat die nötige Hilfestellung oder Motivation gefunden. Ich hoffe, dass wir noch so manches Gespräch im Anschluss dieser Arbeit führen werden, denn es war immer etwas Interessantes dabei. Ich habe mich während der Promotion nicht nur beruflich und wissenstechnisch, sondern auch persönlich weiter entwickeln können und er hat mir den dafür nötigen Raum gegeben. Selbst für verrückte Ideen, wie einen Limerick in einer Publikation unterzubringen, war er zu haben. Auch wenn es das Limerick durch den Gutachterprozess nicht in die final veröffentlichte Version geschafft hat, so soll es doch an dieser Stelle stellvertretend für die gemeinsamen Auf und Abs Erwähnung finden:
There is a novel validation
methodology for simulation
Artefacts scrimmage
within the image
How incongruous for a validation.
Ebenso herzlich möchte ich mich bei Herrn Prof. Dr. Breitkreutz für die Übernahme des Korreferat bedanken. Ohne ihn wäre diese Arbeit ebenso wenig zu Stande gekommen. Neben den fachlichen Gesprächen konnten wir uns auch immer sehr gut über Fußball unterhalten und die Höhen und Tiefen unseres Vereins teilen. Ich würde mich freuen, solche Gespräche auch in Zukunft weiter führen zu können - am liebsten über tolle Spiele mit dem richtigen Ergebnis. Aber auch so manches Gespräch in der Mensa wird definitiv gut in Erinnerung bleiben.
Einen großen Anteil am Zustandekommen dieser Arbeit hatte auch Dr. Maxim Puchkov. Vielen Dank dafür, dass ich die Software F-CAD zu Forschungszwecken gestellt bekommen habe. Darüber hinaus hatte ich 2 schöne Wochen in Basel, in denen er und sein Team mir nicht nur die fachlichen Dinge zur Software vermittelt, sondern auch die schöne Stadt Basel näher gebracht haben. Neben den spannenden fachlichen Gesprächen und Diskussionen danke ich auch für die angenehmen Gespräche, die darüber hinausgegangen sind.
Einen riesen Dank muss ich meinem Bruder Thomas Bollmann aussprechen. Er hat sich während seines Studiums die Zeit genommen, stundenlang nach Fehlern in meinen Skripten zu suchen oder über die Umsetzbarkeit meiner Ideen in Skripte zu sprechen. Ohne diese Hilfestellungen wäre ich mit Sicherheit nicht mit einem Laptop durch die Promotionszeit gekommen. Neben den immensen Materialschäden, die ich aus Frust sicherlich verursacht hätte, wären natürlich auch nicht ansatzweise diese Datenmengen und -auswertungen zu Stande gekommen. Vielen Dank für deine Geduld mit mir.
Mein Dank gilt ebenfalls Dr. Raphael Wiedey und Dr. Julian Quodbach für die anfängliche Unterstützung mit dem Micro-CT und den stetigen fachlichen Austausch,

um die Bildqualität zu verbessern. Sie haben mir dabei geholfen, eine gute Grundlage für meine Versuche zu schaffen. Dabei fehlte es auch zum Glück nicht am nötigen Galgenhumor, um mit diesem Gerät arbeiten zu können.
Ebenso danke ich Dr. Svenja Niese und Hellen Windolf dafür, dass meine Ideen und abstrakten Zeichnungen von Probenhalterungen in brauchbare CAD-Dateien und letztlich in reale Halterungen umgesetzt wurden. Zum Glück hattet ihr auch die nötige Geduld, wenn ich mal wieder eine Halterung zerstört hatte oder recht spontan eine andere Halterung haben wollte.
Ein großer Dank geht auch an Anja Göbel, die mir mit Ratschlägen und Erfahrungen zu oralen Filmen zur Seite gestanden hat. In diesem Zusammenhang möchte ich mich auch bei Felix Reichel und Yu-Lin Ho für ihren Einsatz und die Arbeit im Rahmen des Wahlpflichtpraktikums bedanken. Die erhobenen Daten zu oralen Filmen konnten in einer Publikation mit einfließen und haben damit auch ein Stück zum Erfolg meiner Arbeit beigetragen.
Weiterhin danke ich Dr. Björn Fischer für die Zusammenarbeit, um die Messartefakte in den oralen Filmen eindeutig belegen zu können. Ohne diese Zusammenarbeit wären die Daten zu den oralen Filmen nicht ansatzweise so viel Wert gewesen.
Ein sehr großes Danke geht auch an meine Testleser Jerome Hansen, Hanna Plappert und Mariele Fligge. Ohne euch wäre die Arbeit sicherlich deutlich schwerer zu lesen und zu verstehen gewesen. Neben der Testlesertätigkeit habt ihr mir auch so die Zeit am Institut immer wieder versüßt. Mariele Fligge hat es immer wieder geschafft, mich aufzumuntern, wenn meine Laune im Keller gewesen ist. Außerdem werde ich die gemeinsamen Pausen vermissen. Jerome Hansen hat immer wieder den Feierabend oder das Wochenende gebührlich eingeläutet. Auch diese gemütliche Runde wird mir fehlen, da man so locker und fröhlich in die Freizeit starten konnte. Hanna Plappert hat durch ihre Tollpatschigkeit immer wieder dazu beigetragen, dass ich meine eigenen Unzulänglichkeiten nicht mehr so schlimm wahrgenommen habe und das beste daran war, dass wir deshalb immer wieder herzlich miteinander lachen und eine lustige Zeit miteinander verbringen konnten.
Ich bedanke mich bei Dorothee Eikeler, Dorothee Hetkämper-Flockert, Andrea Michel, Simone Mönninghoff-Pützer, Lisa Man, Karin Matthée, Annemarie Schmitz, Dr. Klaus Knop und Stefan Stich für die fachlichen und organisatorischen Unterstützungen. Mir wurde durch euch so mancher Schiffbruch an den scharfen universitären Klippen erspart und fachliche Arbeit ermöglicht. Darüber hinaus gab es so manches gutes Gespräch oben drauf.
Selbstverständlich möchte ich mich auch bei den besten Bürokollegen bedanken. Sebastian Pohl hat die meiste Zeit mit mir zusammen gelitten. Er musste sich zahlreiche Formeln, Ideen und Berechnungen von mir ansehen und sich in ein Thema denken, dass ihm nicht so sonderlich leicht gefallen ist. Das hat er mir umgekehrt jedoch ebenso zurückgezahlt. Daneben hatten wir viele sehr lustige Momente und viel Spaß im Büro - so viel, dass der ganze Flur Sebastians Lachen hörte. Behalte unbedingt dieses durchdringende Lachen! Hanna Ponsar war immer die weibliche Stimme der Vernunft in

unserem Büro und hat neben dem fachlichen Austausch unserem Blödsinn zur rechten Zeit Einhalt geboten, ohne dabei eine Spaßbremse zu sein. Auch mit ihr hatten wir sehr viele lustige Momente und wurden immer wieder zu etwas mehr Ordnung gemahnt.
Als sich diese Konstellation nach fast 3 Jahren auflöste, wurden die Plätze durch Martin Lück und Hanna Plappert eingenommen. Es war zwar eine vergleichsweise kurze gemeinsame Zeit im Büro, die durch die gegebenen Umstände auch noch etwas erschwert war, aber dennoch hatten wir jede Menge Spaß zusammen. Ihr wart würdige Nachfolger und habt kein zu großes Loch für mich hinterlassen, nachdem zuerst mit Hanna Ponsar und dann Sebastian Pohl meine langjährigen Bürokollegen ausgeflogen waren. Ich freue mich auf zukünftige Treffen und Gespräche mit euch.
Ebenfalls möchte ich mich bei Jhinuk Rahman-Yildir für die interessante gemeinsame Arbeit und die darüberhinausgehenden Gespräche bedanken. Nachdem wir im Studium schon irgendwie zusammen gelacht und gelitten haben, hat sich dies in der Promotion fortgesetzt.
Selbstverständlich möchte ich mich auch bei Arne Schulzen, Julia Matros, Sabrina Berkenkemper, Dr. Oscar Arndt, Dr. Illias El Aita, Dr. Vincent Lenhart, Dr. Annika Wilms und allen anderen (ehemaligen) Mitgliedern des Instituts bedanken, die mir die Zeit am Institut angenehm gestaltet haben. Es wäre zu viel, alle namentlich aufzuführen, bitte seht es mir nach. Ohne ein so gutes Team und Umfeld wäre mir die Arbeit deutlich schwerer gefallen. Ich werde viele schöne Momente aus der Zeit mitnehmen. Unvergessen sind die gemeinsamen Kongresse und Abende, die so manche lustige Anekdote zustande gebracht haben. Ohne diese ganzen schönen Erlebnisse wäre die gesamte Zeit deutlich weniger Wert und anstrengender gewesen. Ihr alle habt mich mitgeprägt und werdet ganz bestimmt in Erinnerung bleiben. Es war für mich eine besondere Zeit mit euch, danke dafür.
Natürlich bedanke ich mich auch herzlich bei meiner Familie und meinen Freunden für die Unterstützung in dieser Zeit. Meine Freunde sowie meine Brüder haben mich durch gemeinsame Unternehmungen immer wieder mit dem Kopf aus der Arbeit geholt und somit Platz und Raum für neue Ideen geschaffen. Meine Eltern und Großeltern haben mir den Rücken frei gehalten, wo sie nur konnten - auch wenn das mal bedeutet hat, dass man sich nicht treffen konnte. So konnte ich mich auf die Promotion konzentrieren. Ohne euch wären weder mein Studium noch die Promotion möglich gewesen. Vielen Dank für all die Möglichkeiten, die ich dank euch hatte.
Zu guter Letzt bedanke ich mich vor allem auch bei der meiner Freundin Laura Witt dafür, dass sie all die Strapazen des Freuens und Leidens mitgemacht hat. Sie hat es geschafft, mich aus den Löchern zu holen, wenn gerade nicht viel zusammen lief und sich geduldig mit mir gezeigt, wenn ich abends lange an der Universität gewesen bin, gereizt war oder auf dem Sofa noch Protokolle korrigiert habe. Sie hat mich aber auch immer wieder auf den Boden der Tatsachen zurückgeholt, wenn es bei mir gut lief. Sie ist und war einfach mein Kompass, der mich sicher dorthin führt, wo ich mich wohlfühle und ich zu Hause bin. Ich bin ihr dankbar dafür, dass sie mir den Rücken frei gehalten und mir in stressigen Phasen so manche Last abgenommen hat. An dieser Stelle möchte

ich auch ihrer Familie für die durchgängige Unterstützung und Hilfe danken, denn ohne sie wäre das Unterfangen auch deutlich erschwert worden.

12 Appendix

Since many different scripts were used in this work, it would way exceed this work to provide all of them as appendix. All scripts are stored on the servers of the university and accessible on request.

13 Erklärung

Ich versichere an Eides Statt, dass die Dissertation von mir selbständig und ohne unzulässige fremde Hilfe unter Beachtung der „Grundsätze zur Sicherung guter wissenschaftlicher Praxis an der Heinrich-Heine-Universität Düsseldorf“ erstellt worden ist.

Münster, 04.06.2022 

Ort, Datum Unterschrift