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# In-situ monitoring of pharmaceutical and specialty chemicals crystallization processes using endoscopy–stroboscopy and multivariate image analysis

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A B S T R A C T

This contribution presents the proof of concept of endoscopy–stroboscopy based in situ low-cost imaging of crystallization processes. This low-cost sensor currently is widely spread in the field of medical diagnosis of human vocal chords and this work presents its application in the context of pharmaceutical and chemical crystallization process monitoring. The model compounds used in this study are the active pharmaceutical ingredient (API) flufenamic acid and citric acid.

Since the acquired images are colored, the second aim of the paper is to evaluate the principal component (PCA) based multivariate image analysis (MIA) as a color to gray scale transformation method, and to compare it to the National Television System Committee (NTSC) standard, which uses fixed weights.

It was found that particle color, transparency, size and shape related information based on visual inspection is feasible using the endoscope–stroboscope system. The MIA results show that in the case of transparent particles the red, green, blue channels contribute equally to the total information content of color images. The acquisition price of the ATMOS endoscope is similar to that of a laboratory turbidity probe, feature which is relevant for the widespread use of this sensor.



*Keywords:* Process analytical technology; Imaging; Endoscopy; Pharmaceutical crystallization; Polymorphic transformation; Multivariate image analysis; Flufenamic acid; Citric acid

## 1. Introduction

Since the 2004 Food and Drug Administration (FDA) initiative the evaluation of in situ process analytical sensors (PAT) that focus on crystallization process monitoring and control has been significantly intensified (Nagy et al., 2008). The integration of several PAT sensors in the control loops is implemented using model free (Nagy et al., 2011; Abu Bakar et al., 2009, 2010) and model-based strategies (Nagy, 2009). In the class of PAT tools relevant for the monitoring of crystallization processes belong the spectroscopy methods, which monitor the liquid concentration; an exception is the FT-Raman spectroscopy, which is also capable to deliver information related to the crystal structure of the solids, thus to the polymorphic forms (Hu et al., 2005). The second class of PAT sensors is represented by those which monitor the solid phase. From complexity point of view the entry level representative is the turbidity probe, which gives reflective property related solid concentration information (Harner et al., 2009). More complex sensors are laser reflectance based and provide chord length information related to the crystal size in slurries, e.g. the focused beam reflectance FBRM (Kee et al., 2011) and the 3D ORM (Heinrich et al., 2011).

The in situ imaging sensors used for crystallization process monitoring have received significant attention in the last decade. An early attempt to capture the crystal characteristics during operation used a flow-through cell placed below a microscope (Matthews and Rawlings, 1998). Imaging sensor development activities have been reported by companies such as DuPont, USA (Scott et al., 2001), Lasentec/Mettler Toledo,

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Switzerland (Kempkes et al., 2008), MTS-Düsseldorf, Germany (Qu et al., 2006) Perdix, The Netherlands (Li et al., 2008; Kadam et al., 2011), and Sartorius Stedim Biotech, Germany (Bluma et al., 2009; Höpfner et al., 2010) which propose probe based solutions. Recently, also universities have been involved in the design of such imaging probes (Presles et al., 2010; Khalil et al., 2010, 2012). Other monitoring options are the flow-through cell imaging devices (Ferreira et al., 2011; Kempkes et al., 2010b) such as the XPT from Prozesstechnik AG, Switzerland (Arnold et al., 2011) and Qicpic from Sympatec, Germany (Borchert and Sundmacher, 2011). Non-contact, externally illuminated imaging solutions may use continuous (Simon et al., 2009a), pulsated (De Anda et al., 2005a) or laser sheet (Brown and Ni, 2011a,b, 2012) light sources.

Initially, the imaging sensors have been used for the visual observation of nucleation, growth, dissolution and aggregation processes (Barrett and Glennon, 2002; Dharmayat et al., 2006). Other visual observation based applications were reported for the monitoring of polymorphic transformation processes (O'Sullivan et al., 2003; Thirunahari et al., 2011). Several research articles published by federal authorities (Wu et al., 2011) or companies also report visual observation based process monitoring implementations (Argentine et al., 2009; Gillon et al., 2006; Leyssens et al., 2011).

In order to extract quantitative information from the captured images several projects focused on image segmentation of non-overlapping (De Anda et al., 2005b; Sarkar et al., 2009) and overlapping (Larsen and Rawlings, 2008) crystals. Once the crystals are identified as objects the two-dimensional size, surface area and several shape descriptors can be calculated. Usually, the quantified information is used to determine growth rates in two dimensions and to validate population balance models (Caillet et al., 2007; Ma et al., 2007; Puel et al., 1997). Related to the capabilities of the imaging based crystal size monitoring, Larsen and Rawlings (2008) have shown that using image analysis it is possible to adequately track the nucleated and seed crystals, and Zhou et al. (2009) have concluded that the monosodium glutamate crystal size can be tracked within 8% error. The complexity of tuning the image analysis methods is highlighted by Zhou et al. (2011) who propose a simplex gradient free optimization method for the optimal identification of image processing settings. Since the imaging methods discussed above capture the two-dimensional projection of three-dimensional particles, recently new developments for three-dimensional imaging have also been reported (Wang et al., 2008; Kempkes et al., 2009, 2010a; Darakis et al., 2010; Khanam et al., 2011; Sandler, 2011; Soppela et al., 2011).

The practical applicability of imaging sensors as quantitative analytical methods is challenging due to the fact that usually crystallization processes are operated at high solid concentrations; e.g. above 5% particle overlapping hinders the accurate particle size determination as discussed by Larsen et al. (2006, 2007). Additionally, often the changing image background and particles out of focus increase the segmentation difficulty. Alternative image analysis strategies to particle segmentation are based on texture analysis, e.g. using gray (Simon et al., 2009a) or color intensity (Simon et al., 2010a,b) as metrics or on fractal analysis (Velazquez-Camilo et al., 2010). The purpose of these methods is to identify image descriptors and to generate time series trends which can be uniquely linked to a particular process state or event (e.g. clear liquid, nucleation, agglomeration, solid density change, secondary nucleation detection). Promising results for solid concentration (Caciano de Sena et al., 2011) and crystal growth rate estimation (Brown and Ni, 2011a) based on mean intensity calculations using external bulk video imaging have been recently reported.

A further application of imaging sensors is their integration in monitoring and control loops to decrease product characteristics variability and deal with operation uncertainties. Recent advances in this direction have been made by integrating imaging based information into the framework of statistical control charts to ensure optimal transition between seed generation and conditioning steps (Simon et al., 2010a,b).

This work presents an alternative to the existing sensors, and namely the proof of concept of endoscopy–stroboscopy in situ imaging sensor. The low-cost, endoscopy based method was recently proposed for nucleation detection purposes by Simon et al. (2009b). Since the light source employed in the previous study is continuous, the moving particles appear as traces; nevertheless, it can be used for nucleation detection because the sensor is able to detect the appearance of new particles. In order to "freeze" the particles and to obtain shape related information in this work the endoscope is updated with a stroboscopic or pulsating light source. Compared to other commercially available imaging sensors a distinct feature of this endoscope system is that the acquired images are color; thus, the second aim of the paper is to evaluate the information content in the red, green, blue channels using the multivariate image analysis method. Besides the principal components, the MIA returns the loadings which weight the original color channels. Therefore, these weights are compared to the weights used by the National Television System Committee (NTSC) standard for the transformation of color to gray scale images.

## 2. The experimental setup and image analysis method

### 2.1. *The Karl-Storz endoscope sensor equipped with a custom stroboscopic light*

The custom stroboscopic light source was developed using a light emitting diode (LED) and the pulsating light was conducted to the Karl-Storz endoscope probe (3.8 mm diameter) described in the previous work (Simon et al., 2009b) using a fiber optic. Images were recorded using a capture card which delivers 25 images/s with a size of 720 pixels × 576 pixels and the resolution of this setup is 8 μm/pixel. This resolution was determined by taking the picture of a printed grid of known size. The light intensity and frequency settings are set via a Labview software and a National Instruments input–output communication interface. In order to obtain equally exposed frames and to ensure adequate illumination of the pictures usually the light source is synchronized with the camera. In our case this synchronization was carried out in open-loop fashion by setting the light pulsation frequency to the frame rate of the camera, e.g. 40 ms, as shown in Fig. 1. The other parameter, the duration of the pulsation was found to be optimal between 1 and 3 ms. Note that with the decrease of the duration of the pulsation the image exposure is also influenced that is why usually a powerful LED light is required.

The images of citric acid and flufenamic acid crystals mixture were taken at the ETH labs using an EasyMax platform from Mettler Toledo equipped with a 100 mL vessel. The slurry was stirred at 200 rpm and was kept at room temperature.