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## Confocal Fluorescence Microscopy as a Tool for Assessment of Photoluminescent Pigments Print on Polyester Fabric

*Konfokalna fluorescenčna mikroskopija kot orodje za ovrednotenje tiska fotoluminiscenčnih pigmentov na poliestrni tkanini*

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### Abstract

The size and distribution of the photoluminescent pigment particles within the selected binder may affect the quality and appearance of the final print significantly. Yet, the techniques for precise evaluation of size distribution of the pigment particles within a 3D fabric space are rather limited, based on their intrinsic fluorescent properties. The presented work demonstrates a simple screen-printing process for the sustainable application of three different types of commercial fluorescent pigments on polyester (PES) fabric, using polydimethylsiloxane (PDMS) as a binder. A comprehensive toolbox was used to compare and study different commercial photoluminescent pigments and their corresponding prints, by means of size distribution and concentration effect of emission intensity, including Confocal Fluorescence Microscopy (CFM) and Scanning Electron Microscopy (SEM) in combination with complementary spectroscopic techniques, i.e. Energy Dispersive X-ray Spectroscopy (EDX) and Ultraviolet-visible (UV-vis) spectroscopy. The focus is on CFM utilised as a non-destructive tool, used for the evaluation of photoluminescent pigments' spatial distribution within printing pastes, as well as on/within the PES fabrics.

Keywords: Photoluminescent pigments, polyester fabric, confocal fluorescent microscopy, spectroscopy

### Izvleček

Velikost in porazdelitev fotoluminiscenčnih delcev pigmenta v izbranem vezivu lahko bistveno vpliva na kakovost in videz končnega tiska. Vendar pa so tehnike za natančno oceno porazdelitve velikosti delcev pigmenta v 3-D prostoru tkanine na podlagi njihovih notranjih fluorescenčnih lastnosti precej omejene. Predstavljeno delo opisuje preprost postopek sitotiska za trajnostno nanašanje treh različnih vrst komercialnih fluorescenčnih pigmentov na poliestrno (PES) tkanino z uporabo polidimetilsiloksana (PDMS) kot veziva. Uporabili smo kombinacijo orodij, s katerimi smo primerjali in proučevali različne komercialne fotoluminiscenčne pigmente in njihove ustrezne tiske s porazdelitvijo velikosti in učinka koncentracije na intenzivnost emisij, vključno s konfokalno fluorescenčno mikroskopijo (CFM) in vrstično elek-

tronsko mikroskopijo (SEM) v kombinaciji z disperzno rentgensko spektroskopijo (EDX) in UV-vis spektroskopijo. Glavni poudarek je bil na CFM, ki se uporablja kot nedestruktivno orodje za oceno prostorske porazdelitve fotoluminescenčnih pigmentov v tiskarskih vezivih in na PES-tkanini ali v njej.

Ključne besede: fotoluminescenčni pigmenti, poliestrna tkanina, konfokalna fluorescenčna mikroskopija, spektroskopija

## 1 Introduction

Textile materials with minimum utility are far off today's people's needs and expectations; to this end, functional textiles, among which the photoluminescent textiles belong, have attracted significant attention in the textiles consumers' end-user segment [1]. Photoluminescent textiles are obtained either by fixing pigments in the fibre or onto the polymer fibrous surface using chemical binders, by coating or printing diverse patterns onto textile fabrics [2]. The photoluminescent pigments as "glow-in-the-dark" powder particles are used as textile pigments for creating high-resolution patterns [3], as an alternative to LEDs, electro-luminescent wires and optical fibres for designing light-emitting fabrics, decoration, military facilities, communication and transportation, fire emergency systems, etc. One of the interesting, and cost-acceptable applications of luminescent pigments is the protection of the original products' originality [4]. In general, photoluminescent pigments are immobilised onto textile fabrics by means of printing as the most affordable and simple procedure, fostering its use by 80% of printed merchandise [5]. Recently, the aqueous pigment-binder spray-coating technique was used as a facile method for production of coated garments hosting luminescent pigments [6].

A photoluminescent pigment consists mainly of crystals of aggregated elements and photonic traps, where, upon light source exposure, the crystals stay excited and keep discharging light, being supported by photonic traps (rare elements), which extend their phosphorescence time until the entire exhaustion of the stored light photons. Besides their chemical composition, the dimensions of photoluminescent pigments and their distribution within binders, influence the print quality and final appearance of printed material. Techniques used for precise assessment of pigments' size and pattern distribution when printed on fabric (s.c. *in situ* evaluation) are rather limited. Instead, the segmental and micro destructive sampling is mainly utilised, followed by extensive laboratory analysis [7]. In art works and cultural heritage examination of photoluminescent pigments' distribution, several *in situ* identification techniques are

in use, limited mostly to single point analysis such as vibrational spectroscopy, laser-induced breakdown spectroscopy and x-ray spectroscopy for atomic-level analysis, as well as diffuse reflectance imaging, fluorescence and fluorescence lifetime imaging [7], etc. The latter are not yet reported as relevant for evaluation of printed textiles. When only particles are considered (excluding the printed fabric), Dynamic Light Scattering (DLS) methodology is applicable for nano pigments, preferably for monodisperse and, ideally, circular particles, and, as such, it is not completely applicable for the fairly heterogeneous (photoluminescent) pigment used in textile printing. The precise microscopic tools (such as SEM and TEM microscopy) give distribution of patterns easily; however, the obtained information is limited to relatively small surfaces being examined, as well as costs of equipment and the examination itself. When commercial pigments with unknown composition and properties (particles' sizes and their distribution, excitation wavelength, emission time, etc.) are utilised, together with additives (binders, brighteners), the complexity of pigments' evaluation may increase exponentially. In the presented work, a facile procedure for screen-printing of luminescent pigments on polyester (PES) fabrics is presented, using three types of commercial luminescent pigments and polydimethylsiloxane (PDMS) as a binder. Commercial pigments and their respective prints on PES fabrics were examined by complementary microscopic (SEM and CFM) and spectroscopic (EDX and UV-vis) techniques. Special focus in this research work is the utilisation of CFM as a non-destructive tool for simultaneous examination of the morphological and optical properties of photoluminescent pigments, and respective fabric prints in a 3D spatial manner. CFM is already used for micro particles [8–10] and auto fluorescent pigments' assessment [11], and recently our group introduced it for assessment of pigment prints on cotton fabric [12], wherein this work presents the translation of a similar procedure to PES fabric. Nonetheless, examination of the spatial distribution of photoluminescent pigments is of high relevance, since the same was found to affect the propagation and external emission of light highly, through mechanisms

such as charge diffusion, re-absorption, or changes in the mean free path of the emitted photons [13].

## 2 Materials and methods

### 2.1 Materials

PES fabric in plain weave was used, with a mass of 165 g/m<sup>2</sup>, thickness of 0.505 mm, warp density of 19 threads/cm and weft density of 21 threads/cm. Polydimethylsiloxane (PDMS), a two-part liquid elastomer kit, Sylgard 184 (Dow Corning, USA), that consists of a pre-polymer base and crosslinking curing agent in the ratio 10:1, was used as a binder. Three (violet, blue and yellow-green) commercially available photoluminescent pigments Sirius (Samson, Slovenia) were utilised in the printing procedure.

### 2.2 Fabrication of luminescent samples by screen-printing

Before printing, the PES fabric was washed at 40 °C for 30 min, using a solution of 2 g/L of standard neutral non-ionic washing agent, without optical bleacher, water rinsed, and dried at ambient temperature. Individual luminescent pigment in concentrations: 1%, 5%, 10% and 30 wt.% (per weight of PDMS) was admixed in a PDMS binder for 5 min, using a high-speed paddle-stirring apparatus, to acquire homogeneous pigments' distribution. The prepared dispersions were applied onto PES fabric according to the flat screen-printing procedure using a semiautomatic printing table (Johannes Zimmer, Austria). In order to achieve an even coverage over the entire sample, a PES 125 mesh was used, as well as the following optimal parameters: A roll-rod diameter of 15 mm, speed of 1 m/min, max magnet pressure, and 2 application layers. To cross-link the PDMS, printed PES fabrics were dried for 48 hours at ambient temperature.

### 2.3 Scanning electron microscopy (SEM) and energy dispersive X-ray (EDX) spectroscopy

Morphological analysis and elemental composition of three commercial photoluminescent pigments were accomplished by the SEM-EDX system, using a Zeiss Gemini Supra 35 VP Microscope (Carl Zeiss NTS GmbH, Germany), equipped with an X-ray energy dispersion spectrometer (EDX, Oxford Instruments, model Inca 400). Pigments in powder form were positioned onto the holder by double carbon tapes, and sputter coated with Palladium (Pd). 500 X magnification and 1.00 kV setting were applied.

### 2.4 Confocal fluorescent microscopy (CFM)

Square pieces of (non)printed PES fabric were positioned on a transparent glass holder above the 20 x (dry) objective of an inverted CFM Leica TCS SP5 II, equipped with an LAS AF software program. The photoluminescent pigments were excited with an argon laser ( $\lambda_{\text{ex}} = 458 \text{ nm}$ ), while the obtained signal was detected by two hybrid detectors, with a pre-set emission range from 500 nm to 550 nm. High-resolution images (1024 pixels x 1024 pixels) were obtained by image adjusted light gain and 8 x line averaging. Each sample was depicted at several positions, in order to check the printing uniformity in the x-y direction, while in the z-direction (sample thickness), the confocal mode with 10  $\mu\text{m}$  stack thickness was applied, and assessed additionally by the depth (colour) coding function. By moving the focal plane in the z direction, up to 30 optical slices were obtained, and combined further in a 3D image stack for digital processing. The bright field images were captured in parallel, to depict the pigment-lean areas of printed PES fabrics, using a Dodt detector. The images from both the fluorescence and bright field channels were used in split or overlaid mode for further assessment by the ImageJ program, an image processing program, designed for scientific multidimensional images, in particular, the z-project function (for tracking of pigments' distribution within the PES fabric). In parallel, the pigments'-PDMS binder dispersion was also coated onto the thin glass. The particle analysis plug-in (Image J) was employed for the assessment of the size and distribution of pigments within the PDMS binder on the glass. Pure PES fabric and PDMS were analysed as controls.

### 2.5 UV-Vis spectroscopy: luminescence decay assessment

A qualitative assessment of the luminescence decay of the PES samples, printed with a combination of PDMS and individual photoluminescence pigment in different concentrations, was performed by initial, 5 min exposure of samples under UV light with a wavelength of 366 nm, within a UV chamber. Afterward, samples were exposed immediately to deep dark within the chamber, and after 20 seconds and 1 min, the photos were taken. For relative quantification of time-dependent luminescence decay, the same samples were also evaluated on a Tecan Infinite M200 Pro microplate reader in luminescence mode, without using emission filters. Printed PES fabrics were again excited under the same conditions as for

the qualitative assessment, and loaded immediately into the instrument, to commence measurement. Each decay curve was normalised by its initial intensity. Before testing, all printed PES fabrics were exposed to darkness for a 2-day period, to attenuate any afterglow illumination completely.

### 3 Results and discussion

The SEM micrographs on Figure 1 (left) depict luminescent pigment particles with irregular shape and large heterogeneity in terms of size in all three types, which we anticipate to be a consequence of their conventional solid-state bulk synthesis, sintering and powdering procedures [14]. The morphologically distinct regions, as well as large size scattering (i.e. the presence of fine powder particles up to merged aggregates) are present in all pigments. This is expected to affect the luminescent properties, influencing the amount of light absorbed, and, consequently, the emission intensity. In contrast to SEM, the CFM

resolved a larger number of particles due to the lower magnification used. Moreover, CFM has the advantage of generating high contrast images through optical sectioning, resulting from the superposition of sharp features from the focal plane and blurry features coming out of focus [15]. The CFM images (Figure 1, middle) depict particles embedded into the binder, thus, excluding the necessity of examination of pigment powders, the latter having a shortage of presenting real state spatial distribution within the applied printing binder. The CFM data (Figure 1, right) demonstrates wide size distribution within all pigments in a range of 3–80  $\mu\text{m}$ , which is reported for commonly used pigments containing Eu-doped strontium aluminates [16]. In the yellow-green pigment, the smallest fraction ( $d \sim 3 \mu\text{m}$ ) is missing, the same being dominant in the other two (blue and violet) pigments, which can be one of the factors contributing to the highest glowing intensity over the other two types at the same concentration. The size distribution of the violet pigment differs significantly when compared to the other two pigments in terms

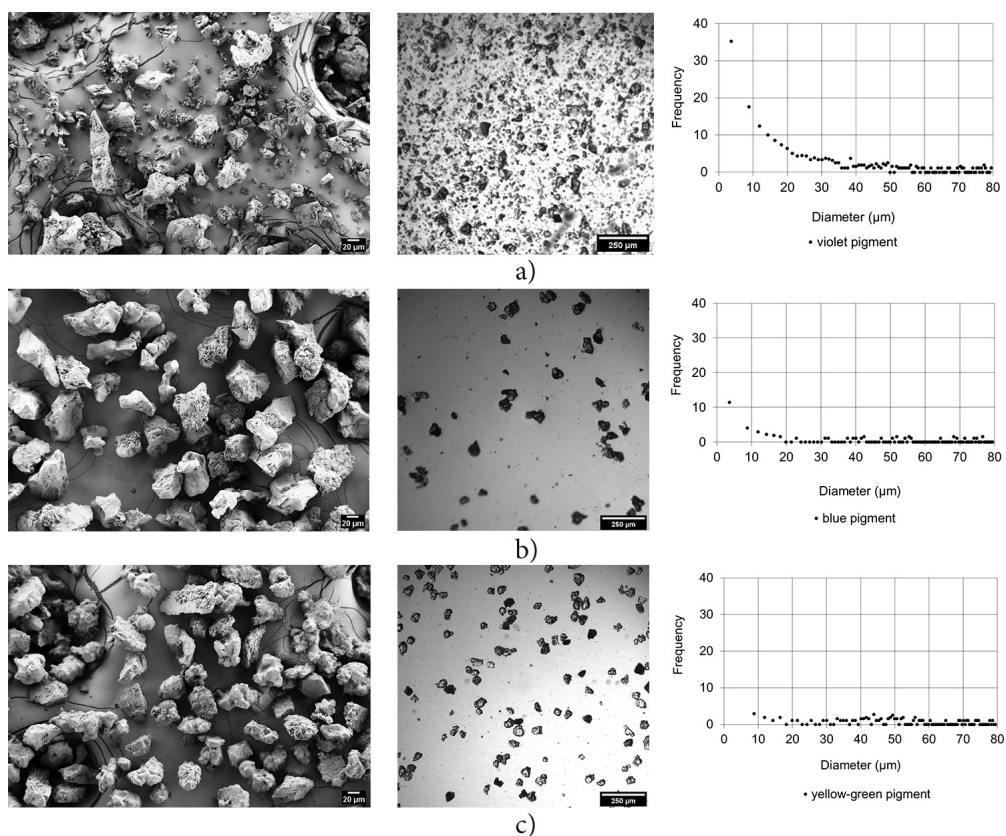


Figure 1: SEM micrographs (left), CFM micrographs (middle) and size distribution extracted (right) from CFM images of a) violet, b) blue and c) yellow-green luminescent pigments. The scale bar in the CFM images corresponds to 250  $\mu\text{m}$ .



Table 1: EDX data of violet, blue and yellow-green photoluminescent pigments

Violet pigment			Blue pigment			Yellow-green pigment		
Element	Weight percentage	Atomic percentage	Element	Weight percentage	Atomic percentage	Element	Weight percentage	Atomic percentage
<b>O K</b>	34.82	65.54	<b>O K</b>	31.68	73.21	<b>O K</b>	34.82	65.54
<b>Al K</b>	17.72	19.78	<b>Mg K</b>	3.69	5.62	<b>Al K</b>	17.72	19.78
<b>Sr L</b>	34.28	11.78	<b>Cl K</b>	1.84	1.92	<b>Sr L</b>	34.28	11.78
<b>Pd L</b>	5.10	1.44	<b>Sr L</b>	29.76	12.56	<b>Pd L</b>	5.10	1.44
<b>Eu M</b>	8.08	1.45	<b>Ta M</b>	17.67	3.61	<b>Eu M</b>	8.08	1.45
			<b>W M</b>	15.36	3.09			

of count, which is approximately three times higher for particles in the 3-30  $\mu\text{m}$  range, yet the presence of larger, irregular particles in this pigment is expected to decrease the luminescence intensity [17].

The EDX data, gathered in Table 1, identify the presence of the rare earth element europium (Eu), in the yellow-green and violet pigments. Eu is known as a photoluminescent pigments' dopant [18]. Besides Eu, the yellow-green pigment contains strontium (Sr), aluminium (Al) and oxygen (O), which suggest the presence of strontium aluminates, also having phosphorescent properties, long afterglow at room temperature, and broad spectrum distribution of luminescence from the blue to green region. In the case of the violet pigment, the calcium aluminate elements were identified, presumably as mixed compounds rather than a particular phase. As relatively diverse compositional profiles were identified for each pigment, we can speculate on the combined effect of composition, and size/distribution profile of pigments, the latter seeking further investigation involving fractionation and comparison of effects of by size-resolved components.

As promising for identification of pigments' size and distribution within the PDMS binder, the CFM was used further for the investigation of the 3D spatial distribution of pigments when printed on PES fabric. Indeed, CFM permits depiction and localisation of the fluorescing compounds unambiguously at light microscopy resolution [8], offering a non-destructive method for in-depth imaging, without demanding preparation [19], sometimes even without the need of additional fluorescent labelling [20, 21] which is also the case for PES fabric. This is due to the point by point illumination of samples, and rejection of out of focus light, which enables acquisition of z- stack (thickness) images and construction of 3D micrographs [22, 23]. The same imaging parameters were

applied for both pigments and PES fabrics. Under a specified excitation wavelength ( $\lambda_{\text{ex}} = 458 \text{ nm}$ ), the particles were visualised clearly and distinguished from the PES fabric (Figure 2), which itself exhibited a fluorescent signal tracked by a parallel detector. Image collects the maximum intensity signal from the consecutive images. Interestingly, the increase in pigment concentration from 1 up to 10 wt.% does not supplement the fluorescence signal significantly; the same can also be observed in the fluorescence emission photographs (Figure 4). Relatively homogeneous distribution of pigments was observed from the maximum intensity image, like within single pigment analysis, which points-out the suitability of the selected pigment-binder-printing procedure, allowing homogeneous distribution. At the same concentration (5%), the yellow-green pigment provides the largest filler pores' blocking effect, due to the domination of large particle fractions over the other pigments.

Printed pigments were observed within an inspected thickness ( $< 300 \mu\text{m}$ ), and not through the whole fabric thickness, as the thickness value is in the function of an objective aperture. To visualise the distribution of pigments in the z axis better (thickness), a colour-coded projection is presented in Figure 3, demonstrating the inclusion of printed particles in deeper regions of the PES fabric. Bonding of pigments on individual PES yarns is pronounced in the case of the blue pigment (Figure 3a), which is not the case with the yellow-green pigment (Figure 3b), where the presence of fibrous, fibre-like glowing impurities and significantly different pigment particles by means of structure and size are observed.

For the macroscopic investigation of printed PES fabrics, the same were illuminated with UV at 254 nm and 366 nm, and further depicted (Figure 4) and evaluated by means of afterglow decay kinetic (Figure 5).

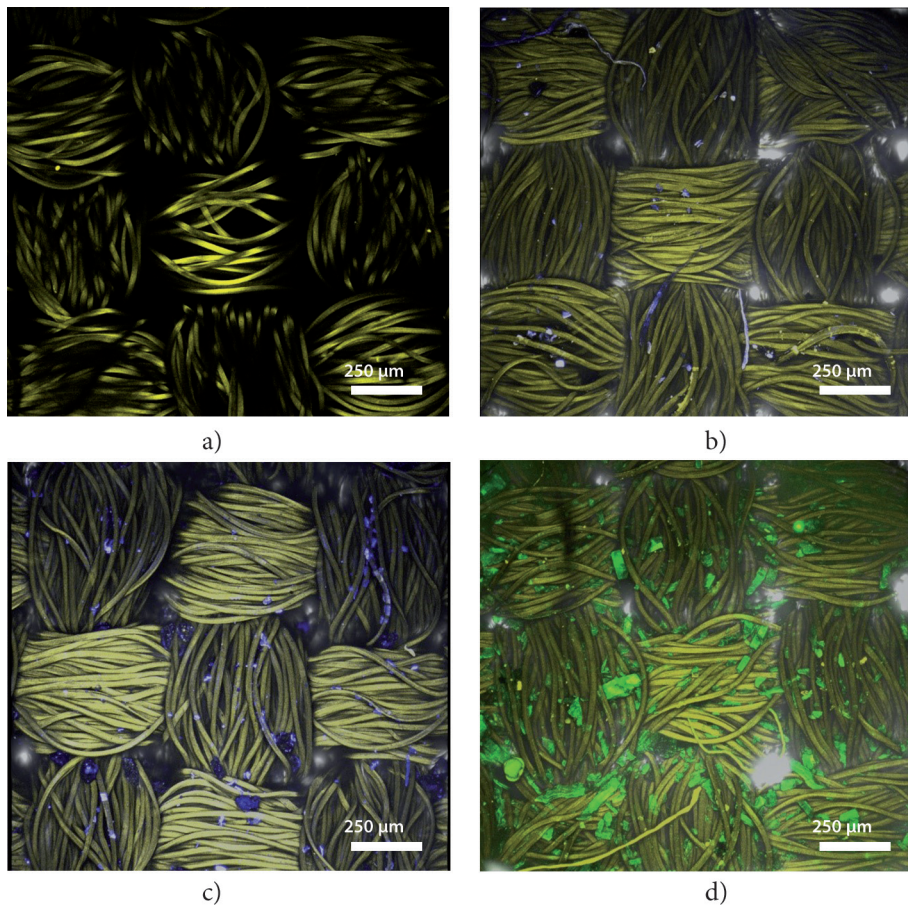


Figure 2: CFM micrographs of PES fabric a) without (reference) and with 5% of b) violet, c) blue and d) yellow-green pigment; obtained by fabric excitation by argon laser at  $\lambda_{ex} = 458 \text{ nm}$  and signal accumulation by two hybrid detectors, with a pre-set emission range from 500 nm to 550 nm range. The white scale bar corresponds to 250  $\mu\text{m}$  in all CFM micrographs.

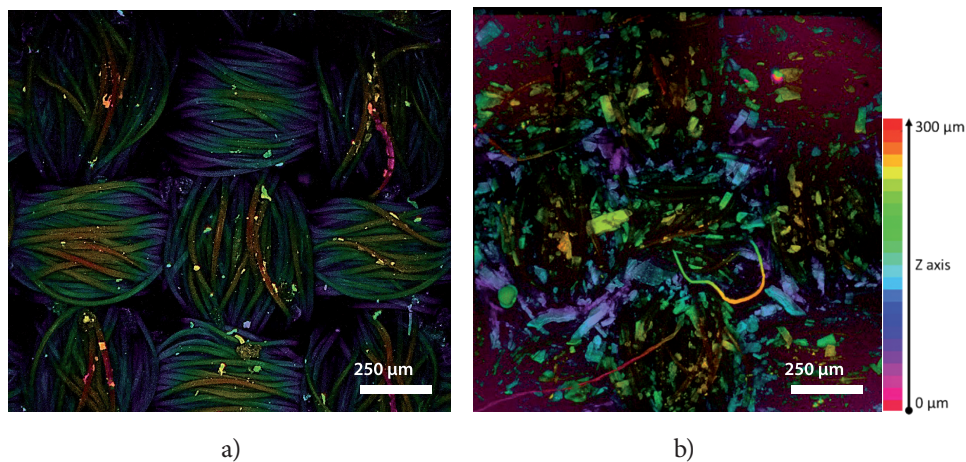


Figure 3: CFM micrographs of PES fabric with 5% of a) blue and b) yellow-green pigment; obtained by fabric excitation by argon laser at  $\lambda_{ex} = 458 \text{ nm}$  and signal accumulation by two hybrid detectors, with a pre-set emission range from 500 nm to 550 nm. The white scale bar corresponds to 250  $\mu\text{m}$  in all CFM micrographs. The colour scale bar refers to the image colours presenting the relative z depth, where glowing particles are positioned.

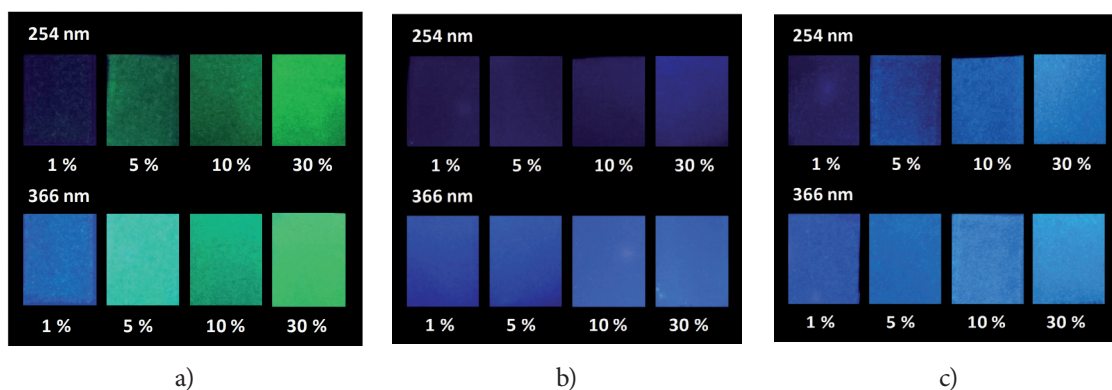


Figure 4: Photographs of screen-printed PES with 1%, 5%, 10% and 30 % of a) violet; b) blue and c) yellow-green pigments in PDMS; excited within a UV chamber at 254 nm and 366 nm for 5 min

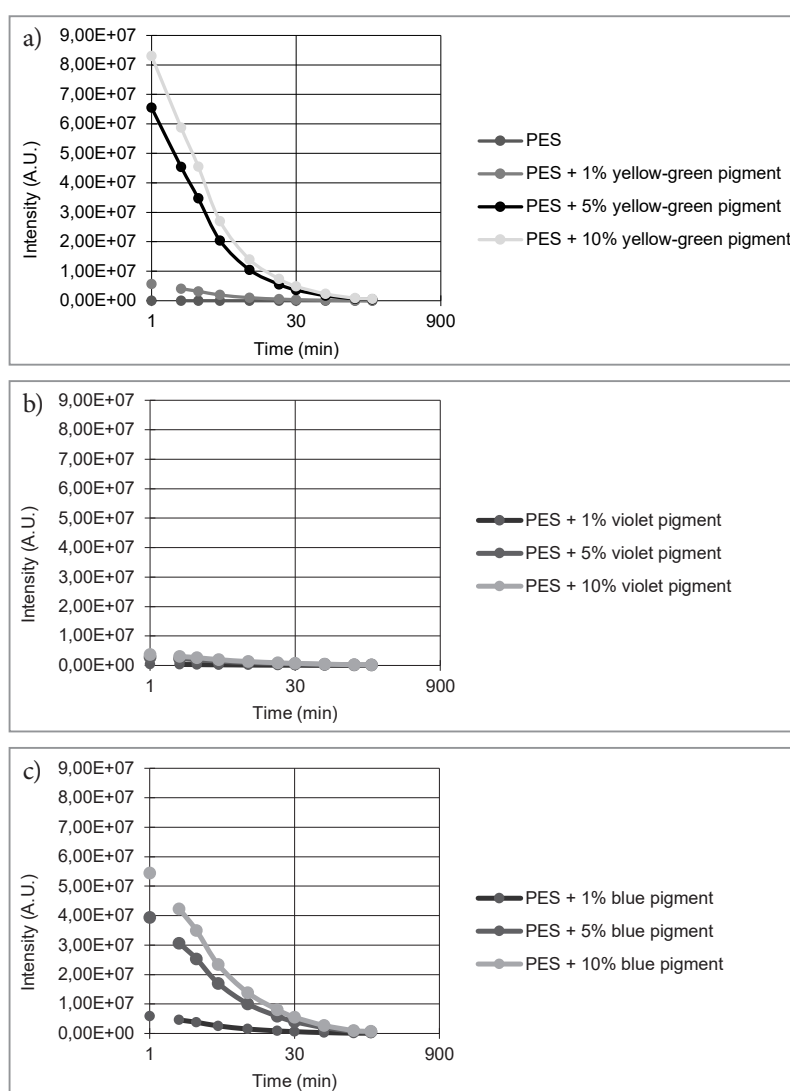


Figure 5: Luminescence decay curves of screen-printed PES with 1%, 5% and 10 % of a) violet, b) blue and c) yellow-green pigments in a 180 min period



From the photographs in Figure 4, an expected increase in luminescence intensity could be observed, with the increase in pigment's concentration (irrespective of pigment type), while, among different pigments, the intensity decreased in the order yellow-green > blue > violet at the same concentrations. Luminescence decay curves, presented in Figure 5 were obtained from the relatively wide time span measurement, i.e. from 1 up to 180 min. The presence of rare earth aluminates activated by Eu brought the highest emission intensity in the yellow-green pigment, as well as the longest afterglow, compared to the other two pigments.

## 4 Conclusion

Three commercial photoluminescent pigments were evaluated by complementary microscopic and spectroscopic techniques, before and after being screen-printed onto PES fabric by use of a PDMS binder. Herein, we identify CFM as an advanced, non-destructive visualisation tool for pigments' assessment by means of their size distribution, as well as 3D (spatial) distribution, within a binder and PES textile fabric. The proposed assessment procedure can be applied further in analysis related to the conservation of artefacts. In this preliminary work we speculate on the potential size and distribution effect of particles onto their glowing intensity, and it's complementing with the effect of their composition. Yet, further investigation and analysis are needed (e.g., including pre-fractionation of pigments) before providing a solid statement for our findings.

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