Non-destructive differentiation of treated and non-treated wood using fluorescence lifetime imaging microscopy

Eine zerstörungsfreie Unterscheidung von behandeltem und unbehandeltem Holz mittels bildgebender Fluoreszenzabklingzeitmessung

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Kurzfassung

Holz wird häufig mit Lacken oder Lasuren auf der Oberfläche behandelt. Für einen nachhaltigen Umgang und eine hohe stoffliche Verwertungsquote muss das Altholz in Altholzkategorien sortiert werden. Die zerstörungsfreie und automatisierbare FD-FLIM-Methode könnte diesen Prozess verbessern. Das Fluoreszenzspektrum und die phasenabhängige Fluoreszenzabklingzeit von mit Acryllack und Holzschutzlasur behandelten Lärchen- und Eichenholzproben werden ermittelt und ausgewertet. Die Ergebnisse zeigen, dass die verschiedenen behandelten Holzoberflächen anhand ihrer Fluoreszenzlebensdauer bei den Anregungswellenlängen 405 nm und 445 nm unterschieden werden können.

Abstract

Wood is often processed using varnish or glaze on the surface. For a sustainable handling of the renewable material and a high material recycling rate, post consumer wood has to be sorted by post consumer wood categories. The non-destructive and automatable FD-FLIM method could improve this process. The fluorescence spectrum and the phase dependent fluorescence lifetime of larch and oak samples treated with acrylic coat and preservative glaze are determined and evaluated. The results demonstrate the different treated wood surfaces can be differentiated by their fluorescence lifetime at 405 nm and 445 nm excitation wavelengths.

1 Introduction

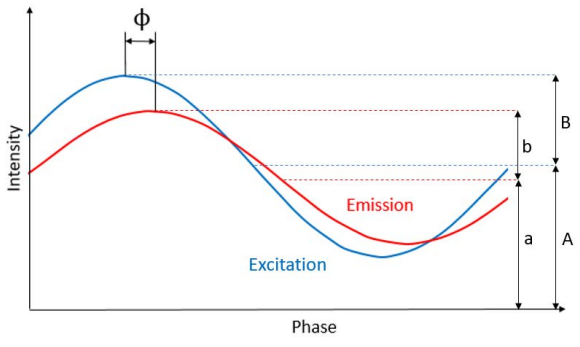
Compared to other renewable raw materials, wood is particularly environmentally friendly due to the possibility of cascade use. To exploit the full potential of cascade utilization of wood in the future, material recycling of post consumer wood should be given preference over energetic use [1]. But, wood is often processed on the surface before utilization. For instance, glazes, paints, oils or plastic coatings are added for a long service life or optical reasons. Thus, post consumer wood can contain contaminants and pollutants or contaminants, which are components that are non-wood related and can affect the technical properties of wood. A distinction can be made between mechanically and non-mechanically removable contaminants. Mechanically separable contaminants include metal parts, glass or asphalt. These can be removed through a sieve or a magnetic separator after the wood is shredded for example. Binding agents, wood preservatives, acrylic varnish or adhesives cannot be separated mechanically and are thus non removeable. When considering varnishes and glazes, there are different effects on the wood. Glazes are absorbed by the wood and are usually translucent on the surface. Varnishes build a solid layer of paint on the wood surface. Both treatments are harmful to the environment if not disposed and further processed properly. To ensure a proper disposal and that pollutants are removed from the wood cycle, a post consumer wood directive has been providing instructions on the recycling of post consumer wood in Germany since 2002. According to regulations [2], the accumulating post consumer wood is divided into 4 categories: category A1 being untreated post-consumer wood; A2 being wood treated neither with halogen-organic substances nor with wood preservatives; A3 being wood contaminated with halogenated organic components; A4 being with pollutants contaminated post-consumer wood, whereby only A1 wood can be used for a materially recycling. However, a lot of recycling companies have a mixed wood pile containing A1 to A3. An automated sorting of treated and non-treated wood could increase the percentage of material recycling significantly. Currently, there is no automated sorting technology integrated into the process to extract the A1 wood from an A1-A3 pile. The volume of additionally recyclable post consumer wood is estimated to be 8 million tons per year [3, 4].

ISBN 978-3-8007-5835-7

Nowadays, research shows that the identification of wood via the near-infrared (NIR) spectrum is possible in theory. The disadvantage hereby is that the NIR spectrum of wood is superimposed with the absorption band of water caused by the variable moisture content [4]. The method of X-ray fluorescence is also unsuitable for wood sorting, as only pollutants such as heavy metals with a high atomic number can be detected [5]. As a result, the post-consumer wood is currently sorted manually or not at all, which is contrary to the principle of sustainability.
Previously, in [6] it was demonstrated that wood species can be distinguished using the FD-FLIM (frequency domain fluorescence lifetime imaging microscopy) method, which is primarily used in the context of biochemistry or biomedicine [7]. Based on a potential impact of a wood treatment on the fluorescence characteristics of a wood sample, it is now intended to analyse whether the FD-FLIM method can also differentiate between non-treated and with non-mechanically removable contaminants treated wood. The optical measuring technique of the FD-FLIM offers the benefit of not having to modify or even damage the sample. Thus, the FD-FLIM method is evaluated for wood identification. Therefore, samples are prepared with varnish and glaze and the measurement data of the fluorescence spectrum and the fluorescence lifetime are evaluated.

2 Theory of fluorescence

Fluorescent materials, including wood, respond to excitation having a characteristic fluorescence signal [8]. The excitation can be done by a Fourier transformed test function like a sinusoidal or rectangular oscillation at a defined modulation frequency ω. A modulated excitation is needed, when measuring the fluorescence lifetime using the FD-FLIM measurement method. There are different possibilities to provide the excitation using a light source. Lasers and high-power electronics are synchronized with the detection unit, which drives the laser through a gate signal. Since the excitation varies in intensity but never breaks off completely, excited electrons are always present, the so-called "steady-state population". The fluorescence signal follows the sinusoidal/rectangular excitation with a phase shift ϕ. Additionally, the fluorescence signal is attenuated in its amplitude and shifted in its average value. In **Figure 1**, the amplitude of the excitation B, the amplitude of the fluorescence emission b, the average value of the excitation A and the average value of the fluorescence emission a as well as the phase shift ϕ can be obtained.



Using the defined modulation frequency ω, the phase shift ϕ can be used to calculate the phase-dependent fluorescence decay time τ_{Ph} as shown in [8]:

$$\tau_{Ph} = \tan(\phi)\,/\omega \qquad (1)$$

3 Experimental Setup

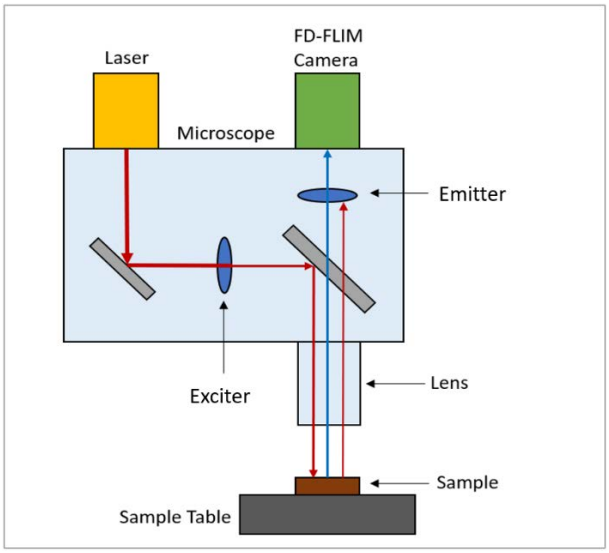
3.1 Experimental setup spectrometer

To analyse the fluorescence spectrum of treated and un-treated wood, the HAMAMATSU C10083CAH Minispectrometer is used. The samples are excited by OMICRON PhoxX® diode lasers having a wavelength of 405 nm, 445 nm or 488 nm. The excitation light signal is directed to the wood sample via the beam input of the PSM-1000 microscope from CASCADE MICROTECH. To narrow the bandwidth of the excitation laser light, an optical filter is included to the excitation light path. Reflections and stray light are blocked by an optical band pass filter, which is inserted to the fluorescence emission path. The spectrum is recorded by the spectrometer in the range of 320 nm to 1000 nm with a resolution of 1.0 ± 0.2 nm.

3.2 Experimental setup FD-FLIM

To analyse the fluorescence lifetime characteristics of treated and un-treated wood a PCO FD-FLIM camera and the OMICRON lasers are assembled to the PSM 1000 microscopes (see **Figure 2**). The selected laser is controlled by the camera and provides a sinusoidally intensity modulated excitation signal. Again, the three wavelengths of 405 nm, 445 nm or 488 nm are used. The light signal is directed to the wood sample via the beam input of the microscope. The time-shifted fluorescence response signal is captured by a PLAN APOCHROMAT ELWD 10x objective from the manufacturer MOTIC and subsequently detected by the FD-FLIM camera, which is attached to the microscope. Additionally, the optical filters described in chapter 3.1 are used to narrow the band width of the excitation light and block reflections and stray light in the emission path. The measurement data is transferred from the camera to the computer via a USB interface. NIKON's NIS-Elements software processes the data and calculates the fluorescence lifetimes of the wood samples.

ISBN 978-3-8007-5835-7



3.3 Sample preparation

Two samples are prepared to obtain the effect of varnish and stain on the fluorescence signal and lifetime. An untreated piece of larch wood and an untreated piece of oak wood, each with the dimensions 18.5x3x2cm, are each divided into three equally sized areas by surface marks. One of these three surfaces was coated twice with the shiny acrylic clear coat from ADLER, the second surface was coated twice with the ADLER PULLEX transparent glaze and the third was left untreated (see **Figure 3**).

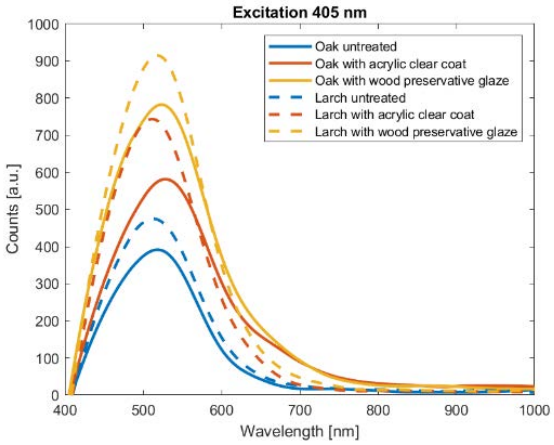
4 Data evaluation

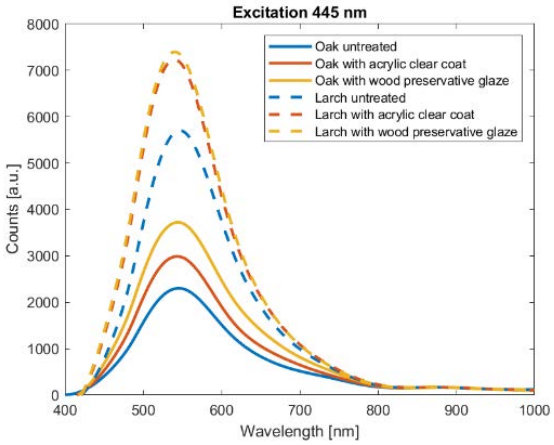
Each individual wood surface was measured four times by the spectrometer. Furthermore, 16 fluorescence lifetime measurements on every sector were performed using the FD-FLIM method. The average of the four spectra is plotted in a histogram. A Gaussian analysis is performed to evaluate the fluorescence lifetime from the 16 FD-FLIM measurements. Therefore, the measurement data of the phase dependent fluorescence lifetime is imported into the software MATLAB in the form of TIF (Tagged Image Information) files. The created program determines the maxima of the lifetime and calculates the standard deviation assuming a gaussian normal distribution. Based on the maximum of the phase dependent fluorescence lifetime and the corresponding standard deviation, a histogram is plotted. The generated graphs are stored in PNG format and the values of the maxima and the standard deviations are saved in a csv file format.

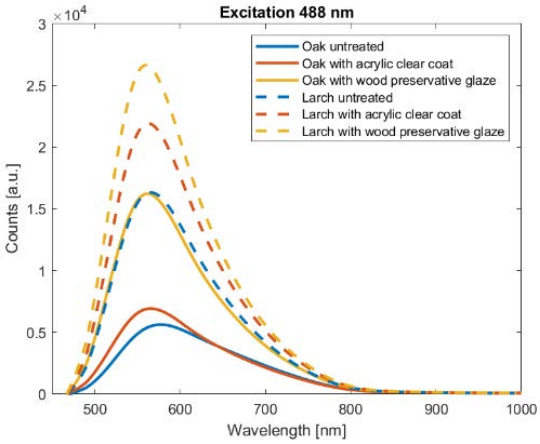
5 Results

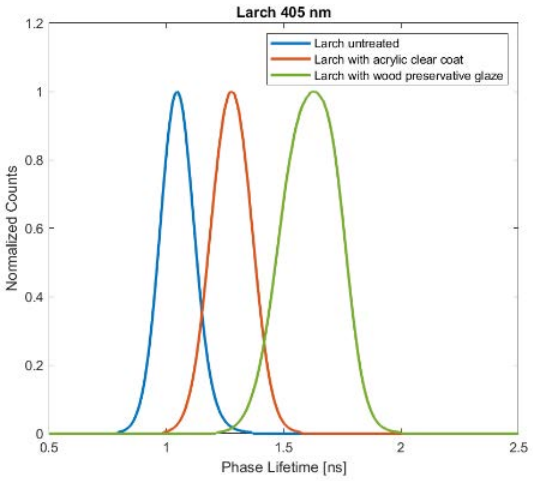
5.1 Analysis of the spectra

The **Figures 4, 5** and **6** show the average of the spectra of the individual wood surfaces in relation to the excitation wavelength.

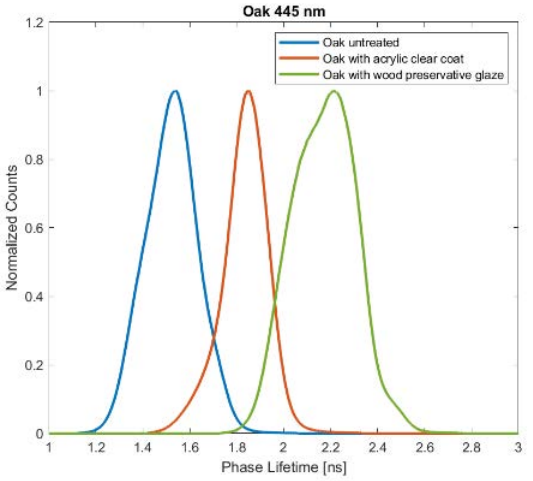






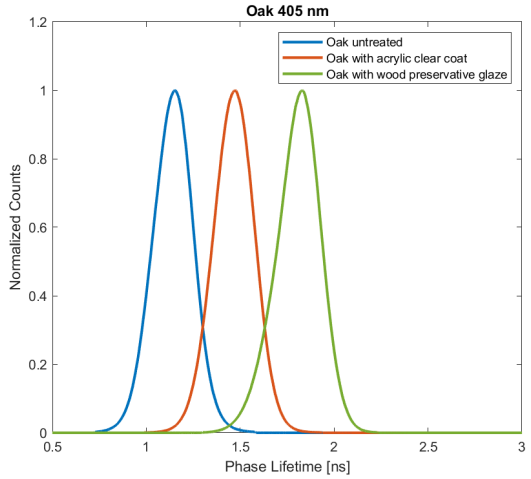


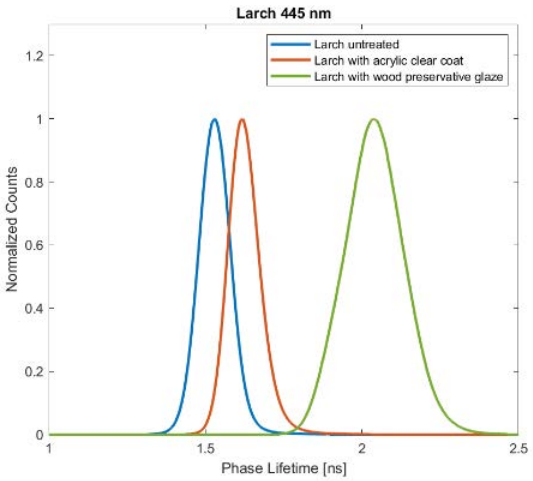
The fluorescence spectra show very similar curves having a clear maximum for all wood coatings. At the same excitation wavelength, oak has a higher intensity in the fluorescence spectrum than larch. It is significant that for all wood coatings and all excitation wavelengths, the glazed surface exhibits the highest fluorescence intensity. The acrylic coated wood surfaces show a lower intensity than the glazed surfaces. The untreated wood surfaces consistently display the lowest fluorescence intensity in the fluorescence spectra. However, a distinction between untreated and treated wood cannot be made due to the fluorescence spectra. But, the graphs indicate that the coating treatments have an effect on the fluorescence emission.

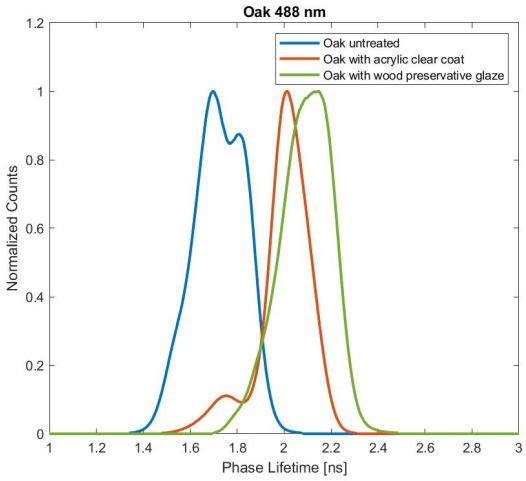


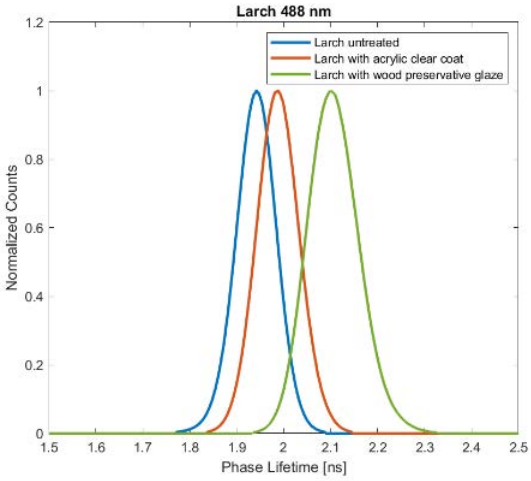
5.2 Analysis of the phase lifetime

In the **Figures 7 to 12**, the results of the Gaussian analysis of the phase dependent fluorescence lifetime measurements from the individual wood surfaces are presented in dependence of the excitation wavelengths. The corresponding values of the average (M PLT) and the standard deviation (S PLT) are listed in **Table 1**.









Wood sample	Excitation [nm]	M PLT [ns]	S PLT [ns]
Oak untreated	405	1.13	0.05
	445	1.52	0.08
	488	1.72	0.07
Oak with acrylic clear coat	405	1.46	0.06
	445	1.83	0.07
	488	2.00	0.06
Oak with wood preservative glaze	405	1.81	0.07
	445	2.16	0.07
	488	2.09	0.07
Larch untreated	405	1.05	0.01
	445	1.53	0.04
	488	1.94	0.04
Larch with acrylic clear coat	405	1.27	0.04
	445	1.62	0.04
	488	1.99	0.04
Larch with wood preservative glaze	405	1.61	0.08
	445	2.03	0.05
	488	2.11	0.04

Table 1 Values of the average (M PLT) and the standard deviation (S PLT) of treated and non-treated wood

The higher the value of the excitation wavelength, the longer the phase dependent decay time. Regardless of the excitation wavelength, oak has a shorter fluorescence lifetime than larch. It is noticeable that for all wood coatings and all excitation wavelengths, the glazed surface has the longest fluorescence lifetime. The acrylic coated wood surfaces show a shorter lifetime than the glazed surfaces and the untreated wood surfaces always show the shortest lifetime whichever wavelength is used. These results indicate that the treatments of the wooden surfaces have a characteristic effect on the fluorescence lifetime, independent of the excitation wavelength.

With regard to the mean values and standard deviations of the fluorescence lifetimes determined from 16 measurements, the differently treated surfaces can be distinguished in the range of one standard deviation in the case at an excitation wavelength of 405 nm and 445 nm. Only using the excitation wavelength of 488 nm the values are overlapping within one standard deviations.

Thus, the investigations on the dependence of the fluorescence lifetime of the treatment of the wooden surface were successful. The results show that the treated and untreated wood can be differentiated by means of their fluorescence lifetime at an excitation wavelength of 405 nm or 445 nm.

6 Conclusion

The intention of the investigations was to evaluate the FD-FLIM method as a new technology to identify treated wood. For this purpose, an oak sample and a larch sample were subdivided into three areas and treated with acrylic clear coat or wood preservative glaze, or left untreated. The fluorescence spectra and phase dependent fluorescence

ISBN 978-3-8007-5835-7

lifetime were determined for the individual sections using excitation wavelengths of 405 nm, 445 nm, and 488 nm. The evaluation of the spectra indicate that the curves have a similar pattern, but the florescence intensity varies systematically in relation to the treatment. The evaluation of the phase lifetime also demonstrates that the individual treatments have a characteristic effect on the fluorescence signal in form of a prolongation of the lifetime. Using the excitation wavelengths at 405 nm and 445 nm, the differently treated wood surfaces can be differentiated within one standard deviation. Thus, the results prove the high potential of the FD FLIM method for a non-destructive differentiation of treated and non-treated wood.

To get a better understanding of the influence a treatment has on the fluorescence lifetime, further wood samples and coatings have to be tested. By measuring the acrylic coatings and the glazes on a neutral underground, it could also be possible to analyse the correlation between fluorescence lifetime and treatment method. If post consumer wood is observed, in contrast to the samples examined here, it is often not fully covered with the coating due to aging. Therefore, further samples have to be analysed in order to define the detection limits of the FD-FLIM method.

7 Acknowledgement

This project is funded by BMBF FKZ: 033RK097A. We would also like to show our gratitude to Gerhard Holst and the PCO AG for sharing their pearls of wisdom with us during the course of this research.

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