TIME-RESOLVED LUMINESCENCE IMAGING AND APPLICATIONS

Prof. Ismail Mekkaoui Alaoui
Department of Physics, Faculty of Sciences Semlalia
Cadi Ayyad University
BP. 2390 Marrakech 40000, Morocco.
E. mail.mekkaoui@ucam.ac.ma

Abstract.
The registration of luminescence emission spectra at different decay times from the excitation pulse is called time-resolved (gated) luminescence spectroscopy. This technique makes it possible to distinguish between short and long decay components. In particular, a long decay emission (phosphorescence) can be separated from the background signal, which most often consists of short decay fluorescence and scattering; because measurements do not occur until a certain time has elapsed from the moment of excitation. Time-resolved spectroscopy finds applications in many areas such as molecular phosphorescence, fingerprint detection, immunoassay, enzyme activity measurements, etc. In this presentation we will focus on fingerprint detection with time-resolved luminescence imaging using Eu(III) and Tb(III). These two chemicals react with Ruhemann's Purple, RP (RP is the reaction product of ninhydrin with amino acid glycine), and are suitable for this purpose. They have a relatively long luminescence decay of millisecond-order. Instrumentation and fingerprint samples developed by this technique will be presented and discussed.

keywords: Luminescence, time-resolved, imaging, laser, fingerprints.

1. Introduction

The detection of latent fingerprints by laser (Menzel, 1989), or more generally, luminescence detection of fingerprints provides great sensitivity. Fingerprint treatments such as rhodamine 6G staining and ninhydrin/zinc chloride are now routine. What has kept laser fingerprint detection from becoming a truly universal technique is that many surfaces fluoresce very intensely under the laser illumination, overwhelming the fingerprint luminescence. Such surfaces were until recently not amenable to the current routine procedures such as those mentioned above. To mitigate this deficiency, time-resolved luminescence imaging has been explored (Mitchell and Menzel, 1989).

Two general chemical strategies were explored. One involved the use of transition metal complexes that yield charge transfer phosphorescence (long-lived luminescence) with microsecond-order lifetimes. These complexes would be used as staining dyes for smooth surfaces much like rhodamine 6G, or be incorporated into dusting powders (Menzel, 1988). Alternatively, for porous surfaces such as paper, ninhydrin treatment followed by treatment with rare earth salts, involving the lanthanides Eu^{3+} or Tb^{3+}, an analog to the ninhydrin/zinc chloride treatment, was investigated (Alaoui, 1992). The formed rare earth-RP complexes exhibit luminescence lifetimes of millisecond order (Alaoui and Menzel, 1993). Some of the above transition metal complexes are amenable to excitation with blue-green light (the customary Ar-laser output), others respond to near-ultraviolet excitation (also obtainable with Ar-lasers). Such wavelength switching, although not difficult, is clumsy nonetheless. Worse, for time-resolved imaging of luminescence of microsecond order lifetime, the laser chopping requires devices such as electro-optic modulators. These are not only expensive, but demand delicate optical alignment and careful gain and bias adjustment. For luminescence of millisecond-order lifetimes, the laser chopping is much easier since a cheap and easily usable mechanical light chopper suffices.

2. Time Resolved Luminescence Imaging

2.1. SENSITIZED LUMINESCENCE AND INTRAMOLECULAR ENERGY TRANSFER

Sensitized luminescence is the process whereby an element having no appreciable absorption band in the visible or ultraviolet (UV) spectrum is made to emit appreciable radiation upon excitation in this region as a result of energy transfer from the absorbing ligand with which it is complexed. The rare earth salts showing luminescence (Eu^{3+} and Tb^{3+}) are known for their

narrow and weak absorption bands in the UV region coupled with emission bands which have narrow half-widths in the visible region. The radiative transitions of these elements are weak because the absorption of the ion itself is very low. But it can be enhanced, when (Eu^{3+} or Tb^{3+}) is bonded to appropriate organic ligands, via intramolecular energy transfer from the organic ligands (good absorbers) to the rare earth ions (good luminescers) when excited with the right excitation (Weissman, 1942). Rare earth-RP complexes show emission enhancement of the rare earth ions. So far, the only excitations that lead to energy transfer from the organic ligands, RPs, to the Eu^{3+} or Tb^{3+} are in the near UV range (200-400 nm) (Alaoui, 1995). Eu-RP complexes are readily prepared in solution. If such solutions were effective for fingerprint staining, akin to rhodamin 6G, then one should be able to use this chemical strategy for fingerprints on smooth and porous surfaces alike. This could reduce instrumentation cost and also make for ease of instrumentation operation. We need to state that some fingerprint reagents, such as 1,2-indanedione, (Joullie and Petrovskaia, 1998) fluoresce under Ar laser and develop fingerprints without requiring time resolved luminescence imaging (Alaoui and all, 2005).

2.2. PRINCIPLE OF TIME-RESOLVED LUMINESCENCE

The basic principle of the technique is as follows. The beam of an Ar-laser is chopped by a mechanical light chopper or electro-optic modulator so that laser pulses with sharp cut-off illuminate the article under scrutiny. The article is viewed with an imaging device (CCD camera) synchronized to the laser pulses such that it turns on shortly after laser cut-off and turns off shortly before onset of the next laser pulse. The imaging device is operated in this way because the offending background fluorescences have short lifetimes (ns order), i.e., the background fluorescence decays very quickly after laser pulse cut-off. If fingerprint treatments can be developed such that much longer luminescence lifetimes result, then the imaging device will only detect this long-lived luminescence and will suppress the background. The principle of the time-resolved luminescence imaging is sketched in Figure 1. Time-resolved spectroscopy finds other applications in many areas (Diamandis, 1988).

The system used in time-resolved luminescence imaging consists of a laser, modulator, microscope, camera, signal trigger discriminator, microcomputer. An argon-ion laser modulated by a mechanical chopper excites the samples. The sample luminescence is detected after the background fluorescence has died. The image is taken by the CCD (charge coupled device) camera through a microscope. The camera is connected to a micro-

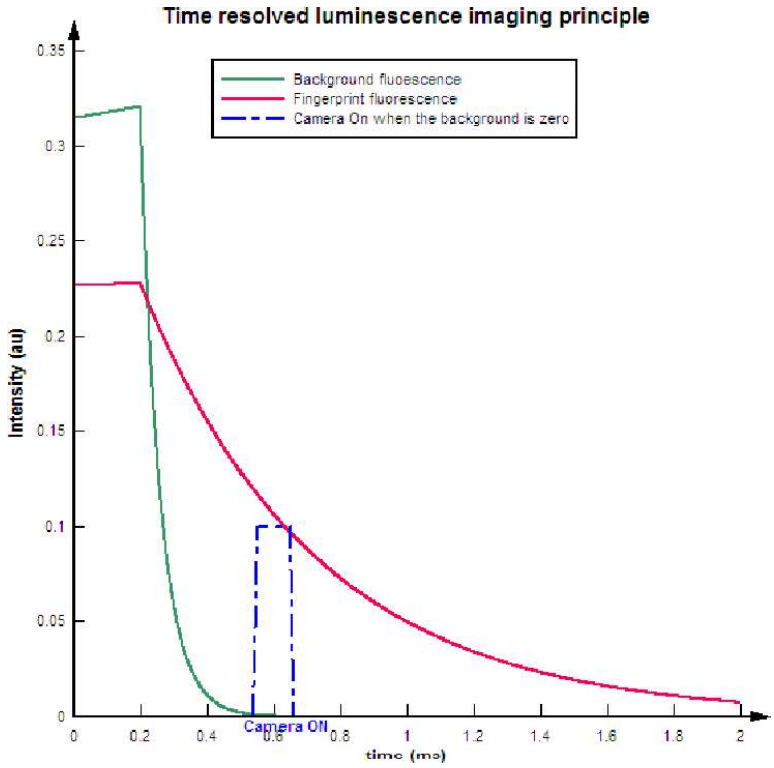

Figure 1. Principle of time-resolved luminescence imaging

computer. The image taken by the camera is sent to the monitor and can be manipulated or stored or printed.

2.3. LASER MODULATIONS

CW argon-ion lasers are used as excitation of our samples. They deliver continuous wave signals. For time-resolved luminescence purposes, we need some method of switching off and on (modulation) the excitation of the samples as desired depending on the luminescence decay time and also on the background decay lifetime. Two ways of laser modulation were used depending on the decay time range of the compounds. Mechanical light chopper for relatively long lifetimes (of milliseconds order), and electro-optic modulation for relatively short lifetime decays (of nanosecond or microseconds orders). For Eu-RP compounds (3 ms), a mechanical chopper is sufficient when operating at 169 Hz (6 ms).

3. Application in fingerprints development

3.1. FINGERPRINTS STAINING

The stability of the RP-Eu^{3+} complex when conserved at low temperature inspired us to use them for fingerprints staining. Fingerprints were deposited on surfaces that can be stained (aluminum cans, plastics, tapes, etc.). Eu-RP complexes were used to stain these surfaces. The surfaces were chosen to show high background fluorescence, and the time-resolved luminescence imaging system was used to suppress the background. The results are promising: A fingerprint from a soft drink can (a highly fluorescent surface) stained with Eu-nitrate-RP solution has been developed as shown in figure 2. We can see a high background luminescence signal (left image) overwhelming the fingerprint luminescence signal, while, when the image is gated (right), the fingerprint signal is showing up.

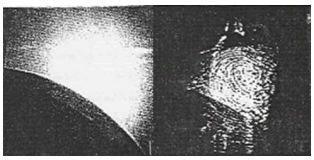

Figure 2. Fingerprint, on a coke can, developed by time-resolved luminescence technique: ungated camera (left), gated camera (right)

3.2. FINGERPRINT DEVELOPMENT

Fingerprints were deposited on porous surfaces (i.e., paper, cardboard) and were then treated with Ninhydrin and analogs. Some of the samples were left at ambient humidity and temperature, and some of the samples were incubated at about 50^oC and 60% relative humidity. The samples left at ambient conditions were slower to develop. The samples were then sprayed with solutions of $EuCl_3.6H_2O$. Under UV light, the red luminescence from the fingerprint was generally comparable to that of the rest of the surface with only slight enhancement of luminescence. The usage of the time resolved luminescence imaging system to picture the fingerprints was not always possible, because something quenches the energy transfer from the RP to Eu or possibly enhances the emission of unreacted Eu (paper absorbs strongly in the near-UV and fluoresces as well as a result). We concluded that either the problem comes from the surface itself or that the chloride salt is simply insufficient in many instances. Then we started to use different

kind of papers and different Eu anions. Some samples were developed in this way, but still more work is needed in this area.

Acknowledgements

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