E. Roland Menzel,[1] Ph.D. and Keith E. Mitchell,[2] M.S.

Intramolecular Energy Transfer in the Europium-Ruhemann's Purple Complex: Application to Latent Fingerprint Detection

ABSTRACT: The reaction product of ninhydrin with amino acids reacts further with europium chloride hexahydrate ($EuCl_3 \cdot 6H_2O$) to form an organo-rare earth complex which exhibits Eu^{3+} luminescence at 615 nm with a lifetime of 0.4 ms. This emission is sensitized by intramolecular ligand to europium resonance energy transfer. Benzo(*f*)ninhydrin is better suited to this energy transfer process than ninhydrin and yields more intense Eu^{3+} emission. The emission is effectively excited by argon-ion laser near-ultraviolet (UV) light, which can be modulated such that time-resolved imaging can reveal amino acids on strongly fluorescent surfaces which would otherwise not be tractable. This is applied to the detection of latent fingerprints.

KEYWORDS: criminalistics, fingerprints, ninhydrin, benzo(*f*)ninhydrin, 5-methoxyninhydrin, europium chloride hexahydrate, fluorescence, time-resolved imaging, lasers, proximity-focused microchannel plate image intensifier

Ninhydrin (*I*, Fig. 1), discovered by Ruhemann in 1910 [*1*], has long been used as a reagent for the assay of amino acids. The reaction of ninhydrin with an amino acid yields a purple product, known as Ruhemann's Purple (*II*, Fig. 1) (RP). The utility of ninhydrin in an unusual application which falls in the category of surface analytical chemistry, namely the detection of latent fingerprints, was first reported in 1954 [*2*]. In this application, ninhydrin reacts with amino acids present in the palmar sweat residue that comprises a latent fingerprint. Fingerprints thus develop as purple marks visible to the eye.

In the late 1970s the detection of latent prints via laser-excited fluorescence began to be explored [*3,4*]. At present, several procedures are routinely used in the growing number of laser-equipped law enforcement agencies. Among them are detection of latent fingerprints via their inherent fluorescence [*3*], dusting with fluorescent powders [*4*] for relatively fresh prints on smooth surfaces, staining with fluorescent dyes [*5*] for prints on smooth surfaces, and treatment with ninhydrin followed by zinc chloride ($ZnCl_2$) [*6,7*] for prints on porous surfaces, mainly paper, but also cardboard, wood, and so forth. At times, cadmium salt treatment is used instead of zinc salt treatment. The $ZnCl_2$ post-ninhydrin treatment, together with laser examination, very much increases the detect-
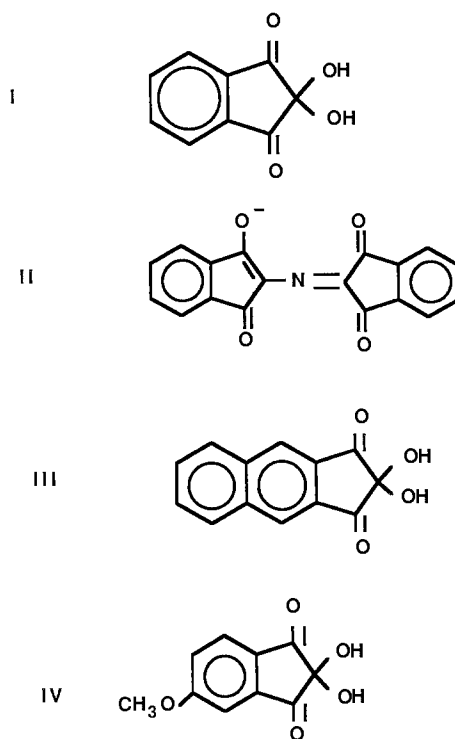
FIG. 1—*Structures of ninhydrin* (I), *Ruhemann's Purple* (II), *benzo(f)ninhydrin* (III), *and 5-methoxyninhydrin* (IV).

ability of latent fingerprints. The procedure should be useful for detection of amino acids generally. An important virtue of the ninhydrin/$ZnCl_2$ procedure from a practicality perspective is that the conventional ninhydrin treatment can be performed first, and, if it fails, it can be followed by laser examination. The structure of the complex formed when amino acid is treated by ninhydrin/CdI_2 has been determined [8]. Two water molecules and one I^- are involved in the (distorted) octahedral coordination of the Cd^{2+}, and RP acts as a tridentate ligand.

Unfortunately, poor results are often obtained on many types of surface ubiquitous at crime scenes (for example, brown paper, cardboard, wood) by the conventional ninhydrin treatment. These surfaces are not amenable to the ninhydrin/$ZnCl_2$ laser procedure either because of excessive background fluorescence.

This paper deals with a post-ninhydrin treatment designed to make such surfaces amenable to laser examination. It involves rare earth salts which form coordination compounds with RP. The purpose of investigating treatments with rare earth salts was not to generate fluorescence from the organic ligand, as in the case of the zinc complex. Rare earths have high atomic number, so that the resulting spin-orbit coupling is expected to quench such fluorescence. Instead, the intent was to explore the possibility of generating rare earth luminescence, which typically has a long lifetime. This would make it possible to suppress background fluorescences, which for the above difficult surfaces have short lifetimes [9], by time-resolved imaging. The existence of some luminescent organo-rare earth complexes has been known for a long time [10].

Materials and Methods

Fingerprints were deposited on white paper and treated with ninhydrin in the following fashion. Ninhydrin was dissolved (to saturation) in methanol. One part of solution was then diluted with four parts (by volume) of 1,1,2-trichlorotrifluoroethane. The resulting solution was (lightly) sprayed onto the paper, using a chromatographic sprayer. The solvent system is designed for high volatility to prevent bleeding of fingerprint ridge detail, and, on paper, the running of ink that might be present on a document. The paper was incubated for 1 h at 40°C and 60% ambient humidity. Humidity is essential to the developed. Prints were then sprayed with solutions of rare earth salts that were prepared in the same manner as the ninhydrin solution. The prints were then incubated under the aforementioned conditions for 15 min. Again, ambient humidity is essential to the formation of metal-RP complexes. The fingerprint marks were then examined for luminescence. This examination used three light sources, namely an ultraviolet (UV) lamp, an argon-ion-laser operating in the blue-green and also the near-UV (Coherent Innova 90-6), and an argon-ion-laser-pumped dye laser (Coherent 599) operating between 560 and 610 nm. Rare earth-RP coordination compounds were also prepared in solution as follows. Ninhydrin was dissolved in methanol, and the amino acid glycine was added. The blue solution formed upon reaction was then titrated with rare earth salts, dissolved in methanol, to form the complexes.

To facilitate luminescence observation with minimal background fluorescence interference, we spotted solutions on to thin-layer chromatographic (TLC) plates and left these to dry, thus mimicking the actual fingerprint situation. TLC glass plates coated with silica gel without UV indicator were chosen as substrate because they show virtually no luminescence.

Spectral measurements used standard photon counting techniques. Lifetime measurements used standard boxcar averager techniques.

Time-resolved imaging measurements were made with an argon-ion-laser operating in the near-UV or an argon-ion-laser pumped dye laser. The laser beam was chopped by a mechanical light chopper operating at 250 Hz. The chopped beam was transmitted by an optical fiber to illuminate the sample (about 5-cm-diameter illumination area). The imaging employed a gated proximity focused microchannel plate image intensifier. Images observed on the output phosphor screen of the image intensifier were photographed.

Results and Discussion

Purple fingerprint marks on paper obtained by the ninhydrin treatment were sprayed with methanol/1,1,2-trichlorotrifluoroethane solutions of salts (chloride hexahydrates) of Ce^{3+}, Nd^{3+}, Sm^{3+}, Eu^{3+}, and Tb^{3+}. Only the Eu-RP and Tb-RP complexes showed rare earth luminescence. Since the Eu-RP luminescence was the stronger one, the remainder of this paper will focus on Eu-RP. Purple fingerprint marks changed color to orange once europium chloride hexahydrate ($EuCl_3 \cdot 6H_2O$) was applied (magenta for very heavily developed prints). The anion of the salt should be of little consequence if complexation of RP with transition metals is any guide at all. Indeed, the results obtained with europium (III) nitrate hexahydrate did not differ significantly from those obtained with europium (III) chloride hexahydrate. Since orange compounds tend to absorb in the blue and green, ninhydrin/$EuCl_3 \cdot 6H_2O$-treated prints were subjected to examination under blue-green laser light (visual observation through orange laser safety goggles transmitting at wavelengths longer than about 550 nm in a darkened room). Only weak fingerprint luminescence (originating from the ligand) was observed, not surprisingly because of the aforementioned spin-orbit coupling fluorescence quenching mechanism. Under illumi-

nation by UV lamp or an argon-ion-laser operating in the near-UV, bright orange fin-gerprint luminescence was visible. However, there was also intense orange background luminescence because of $EuCl_3 \cdot 6H_2O$ deposition on the paper surrounding the finger-print. Thus, adequate fingerprint luminescence contrast with the background is not ob-tained if excessive amounts of $EuCl_3 \cdot 6H_2O$ are sprayed onto the paper. Under dye laser illumination at about 579 nm and visual observation through a red filter which transmits at wavelengths longer than about 600 nm, fingerprint luminescence was also seen, but it was faint compared to that seen under UV excitation. To interpret these observations, absorption, luminescence, and excitation spectra were measured on solution and TLC spot samples. Luminescence lifetime measurements on TLC spots were made as well. All measurements were made at room temperature.

Solution Luminescence and Excitation Spectra

Europium Chloride Hexahydrate—Luminescence spectral measurements were made under tunable dye laser excitation. Samples were held in standard 1-cm fluorescence cuvettes. Luminescence at 592 and 615 nm were observed, the latter being substantially more intense than the former. The full width at half maximum of the 592- and 615-nm luminescences was about 10 nm. The excitation spectrum corresponding to the 615-nm emission revealed peaks at 579 and 592 nm. The solution emission under variable dye laser illumination was also examined visually. This revealed information which we will have occasion to come back to later. The 615-nm emission from the $EuCl_3 \cdot 6H_2O$ solution was sharply defined when the laser was tuned through the 579-nm transition. The intensity, however, was low even though the dye laser power was fairly high (about 0.5 W), and the solution was nearly saturated. When the dye laser was tuned to 592 nm, the observed 615-nm emission was very much fainter and the excitation maximum was not nearly as well-defined as for excitation at 579 nm.

Europium-RP Complex—The results were very similar to those obtained with $EuCl_3 \cdot 6H_2O$. The principal emission was at 615 nm and the excitation optimum at 579 nm, but not as sharply defined as for $EuCl_3 \cdot 6H_2O$.

Solution Absorption Spectra

Europium Chloride Hexahydrate—The compound forms a colorless methanol solution. The absorption spectrum in the 570- to 620-nm region showed nothing. This is in line with the earlier-mentioned visual observations on the 615 nm emission under the dye laser and is consistent with the fact that 4f-4f transitions are essentially parity forbidden. This will be further addressed later in connection with the energy levels of Eu^{3+}. In the near UV, a sharp absorption at 396 nm was found.

Europium-RP—No transitions in absorption were seen in the 570- to 620-nm region. The complex showed a very intense and broad absorption band peaked at 500 nm. Even at 580 nm there still is significant absorbance as a result of this organic ligand band. In the near UV, a broad absorption peaked at 370 nm was found.

TLC Spot Luminescence Spectra

RP and $EuCl_3 \cdot 6H_2O$ were spotted onto a TLC plate to partially overlap such that three regions were obtained, namely $EuCl_3 \cdot 6H_2O$ alone, Eu-RP, and RP alone. Under UV (argon-ion-laser) illumination, both the $EuCl_3 \cdot 6H_2O$ and Eu-RP sample regions revealed luminescences at 579, 592, and 615 nm, with a peak height ratio of about 1:4:20. The full widths at half maximum were 3, 10, and 10 nm for the 579-, 592-, and 615-nm emissions, respectively. Intensities were substantially higher for the complex. No lumi-nescence was obtained from the RP sample region.

In addition to the laser light, the broad-band fluorescence of the rhodamine 6G in the dye laser exits the laser as well. This fluorescence is efficiently scattered from the TLC plate into the measuring monochromator. To eliminate this interfering scattered light, TLC plate luminescence measurements under dye laser excitation used a boxcar averager, with the dye laser chopped by a mechanical light chopper operating at 169 Hz and with the boxcar gate set at 0.5 ms after laser cutoff. As with the solution samples the principal emission was at 615 nm, with the excitation maximum at 579 nm not as sharply defined for Eu-RP as for the Eu salt. The luminescence lifetime of the 615-nm Eu-RP emission, measured with the boxcar averager, was 0.4 ms, a lifetime typical of what one expects from Eu^{3+} emission.

Europium Complexation with Benzo(f)ninhydrin

The obtained spectral data make it clear the $EuCl_3 \cdot 6H_2O$ post-ninhydrin treatment yields an europium-Ruhemann's Purple complex and that luminescence from this complex originates from the Eu^{3+} ion. The data obtained with UV excitation show that the luminescence is substantially more efficient from the complex than from $EuCl_3 \cdot 6H_2O$. Under dye laser excitation, the surroundings of latent prints showed a background, in areas where $EuCl_3 \cdot 6H_2O$ was sprayed on, which was no more intense than the background of the bare paper. This, together with the absorption spectral data, suggests that the fingerprint luminescence arises not only from direct excitation of the europium ion of the complex, but primarily by resonance, or thermally assisted near-resonance, energy transfer from the ligand to the europium ion of the complex. Ligand to rare earth intramolecular energy transfer is known to occur in a number of organo-rare earth compounds [11]. To further explore this interpretation, we investigated europium-Ruhemann's Purple complex formation involving the ninhydrin analog benzo(f)ninhydrin (III, Fig. 1). This compound, available from Aldrich under the name 2,2-dihydroxy-1H-benz(f)indene-1,3(2H)-dione (Catalog 31,078-6), is known to react well with fingerprints, and it forms a fluorescent complex with zinc. It also shows a ligand absorption that is red-shifted considerably from that of the Ruhemann's Purple of ninhydrin [12]. If the resonance or near-resonance ligand to rare earth energy transfer concept is correct, then this analog should yield more intense luminescence in the europium complex than that obtained using ninhydrin.

To test this scenario, benzo(f)ninhydrin in solution was reacted with glycine. The characteristic green reaction product was obtained. When $EuCl_3 \cdot 6H_2O$ was added, the product changed color to become red. As with ninhydrin, absorption spectra were obtained and luminescence was examined with dye laser excitation in the yellow-orange. The europium-complex showed a broad absorption peaked at about 545 nm that tailed off into the red up to nearly 620 nm. Considerably more absorbance remained at 580 nm than with ninhydrin. The complex formed with benzo(f)ninhydrin also showed very strong absorbance in the near-UV, with peaks at about 390 and 330 nm. Under yellow-orange dye laser illumination, the results were qualitatively the same as those obtained with ninhydrin. However, the luminescence of the europium complex was substantially more intense than that of the corresponding ninhydrin complex, both in solution and in TLC dry spots, and this is consistent with the idea of ligand to Eu^{3+} resonance energy transfer. Under near-UV excitation, very much more intense europium emission was obtained as compared to the corresponding ninhydrin/$EuCl_3 \cdot 6H_2O$ case.

Europium Complexation with 5-methoxyninhydrin

As a final check on the validity of the ligand to europium energy transfer concept, glycine/5-methoxyninhydrin/$EuCl_3 \cdot 6H_2O$ solution and TLC spot samples were examined.

5-methoxyninhydrin (*IV*, Fig. 1) is a recently reported [*13*] ninhydrin analog that reacts about as well with amino acids as ninhydrin, forming a purple product. More importantly, subsequent $ZnCl_2$ treatment yields an orange coordination compound which fluoresces very much more intensely than the corresponding RP complex. This is indicative of reduced nonradiative decay, and implies that ligand to europium energy transfer should be more efficient than in Eu-RP. Indeed, stronger emission under 579-nm dye laser excitation was obtained on solution and TLC spot samples for 5-methoxyninhydrin/ $EuCl_3 \cdot 6H_2O$ than ninhydrin/$EuCl_3 \cdot 6H_2O$. The emission was not as intense as with benzo(*f*)ninhydrin/$EuCl_3 \cdot 6H_2O$, however. Under UV argon-ion-laser excitation, similar results were obtained.

Time-Resolved Imaging

For purposes of time-resolved imaging, dye laser excitation of fingerprints treated with ninhydrin, benzo(*f*)ninhydrin, or 5-methoxyninhydrin and then with $EuCl_3 \cdot 6H_2O$ was not found to be effective. However, near-UV argon-ion-laser excitation yielded good results, as shown in Fig. 2. In this figure, photograph A (room light) shows two fingerprints on a TLC plate. One of them (right) was treated with 5-methoxyninhydrin and $EuCl_3 \cdot$ $6H_2O$. The other (left) was then deposited on top by pressing a finger stained with 3,3′-diethyloxadicarbocyanine iodide (DODC) against the TLC plate. Photograph B (luminescence under UV argon-ion-laser excitation) shows the image intensifier output with the imaging gate set in the laser-on-portion of the chopping period. With this gating, the result is the same as that one would obtain with the standard mode of laser examination. The observed DODC print fluorescence was so strong that it grossly overexposed the image intensifier and the photographic film, thus obliterating all fingerprint ridge detail. Photograph C shows the image intensifier output with the imaging gate set in the laser-off-portion of the chopping period. The luminescence of the 5-methoxyninhydrin/ $EuCl_3 \cdot 6H_2O$-treated print remained readily visible, whereas the fluorescence (of short lifetime) of the DODC stained print was almost completely eliminated. Indeed, the ridge detail of the 5-methoxyninhydrin/$EuCl_3 \cdot 6H_2O$-treated print located below the DODC stained print was recovered. Although neither the substrate nor the superposition of the DODC print over the rare earth treated print (Fig. 2) represent a realistic crime scene situation, we chose this sample configuration for presentation because it demonstrates the background suppression capability of time-resolved imaging in a very obvious way. We have obtained similar, albeit not as spectacular, results for latent prints on paper [*14*].

Eu$_9^{3+}$ Energy Levels

Calculated Eu^{3+} free-ion energy levels were reported by Ofelt in 1963 [*15*]. The spectroscopy of Eu^{3+} in lanthanum chloride ($LaCl_3$) was reported the same year by DeShazer and Dieke [*16*]. Their paper presents energies, Stark splittings, fluorescence intensities, and transition selection rules. The states relevant to the Eu^{3+} emission and excitation spectral data of this paper are the $^7F_0$ ground state, the $^7F_1$ state calculated to be at 374 cm^{-1}, the $^7F_2$ state calculated to be at 1036 cm^{-1}, and the $^5D_0$ state calculated to be at 17 374 cm^{-1} [*15*]. The agreement between calculated and observed energies for the $^7F_1$ and $^7F_2$ states is very good [*16*]. The observed energy of the $^5D_0$ state is 17 267 cm^{-1} [*16*], slightly lower than the calculated value. The emissions reported in the present paper are interpreted as being transitions from the $^5D_0$ to the $^7F_{0,1,2}$ levels. This interpretation is consistent with the data of Ref *16* in terms of energies, intensities, and line widths.

Energies—If we take the energies of the $^5D_0$, $^7F_2$, and $^7F_1$ states to be, respectively, 17 267, 1036, and 374 cm^{-1}, then the wavelengths of transitions from $^5D_0$ to $^7F_2$, $^7F_1$, and
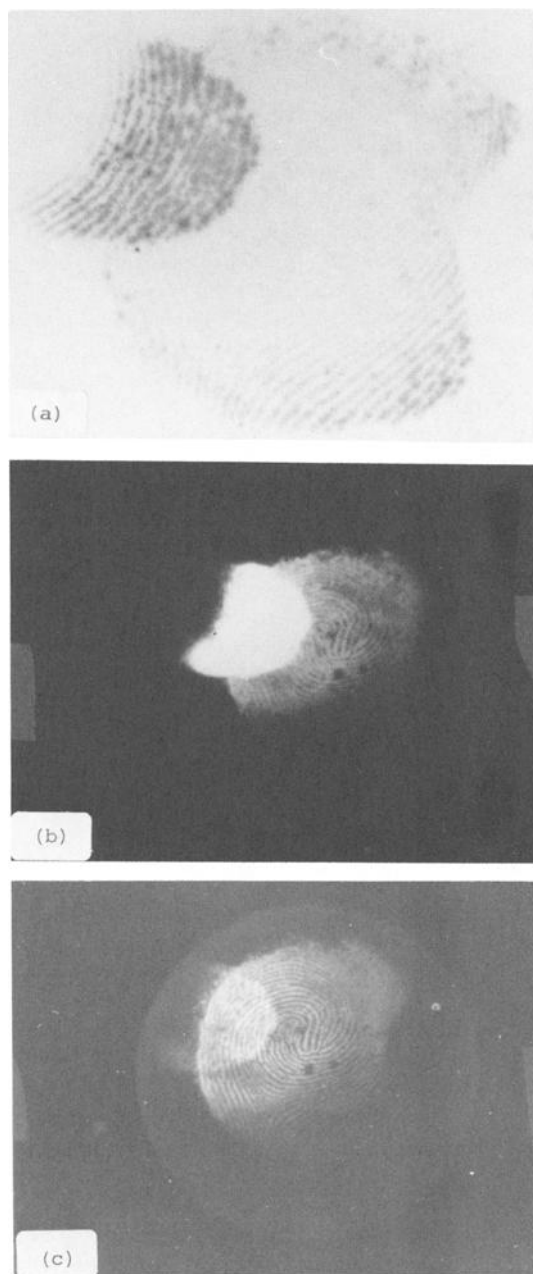
FIG. 2—(a) *Room-light photograph of two fingerprints on a TLC plate. Right print was developed by 5-methoxyninhydrin/EuCl₃·6H₂O. Left print, a DODC stain, was superimposed onto a portion of the right print.* (b) *Photograph of image intensifier output (UV Ar-laser excitation and luminescence imaging) with intensifier gate in the on-portion of laser beam chopping period.* (c) *Photograph of intensifier output with gate in the off-portion of laser beam chopping period.*

the $^7F_0$ ground state are computed to be 579, 592, and 616 nm, in excellent agreement with the measured values of this paper (which are accurate to 1 nm). The ligand-europium energy transfer under near-UV excitation involves a dense group of europium states between 25 000 and 30 000 cm^{-1} [16].

Intensities—The $^5D_0 \rightarrow {}^7F_0$ transition is forbidden. The $^5D_0 \rightarrow {}^7F_1$ and $^5D_0 \rightarrow {}^7F_2$ transitions are allowed. The low temperature results of DeShazer and Dieke indicate a higher intensity of the $^5D_0 \rightarrow {}^7F_2$ transition compared with the $^5D_0 \rightarrow {}^7F_1$ transition. Our observations parallel these intensity results; the 579-nm emission was found to be weak compared to the 592- and 615-nm emissions (see TLC Spot Luminescence Spectra section), and the 615-nm emission was stronger than the 592-nm emission. The forbidden nature of the $^5D_0 \rightarrow {}^7F_0$ transition is also in line with the absence of $EuCl_3 \cdot 6H_2O$ solution absorption in the 570- to 620-nm region. That the solution excitation spectral results of this paper indicate stronger excitation of the 615-nm emission at 579 nm than at 592 nm, even though the 579-nm transition is forbidden, is interpreted in terms of the magnetic dipole nature of the $^5D_0 \rightarrow {}^7F_1$ transition [16] and the fact that at room temperature the $^7F_1$ state is only poorly populated thermally ($\Delta E/kT \approx 1.8$).

Line Widths—The Stark splittings reported by DeShazer and Dieke for $^7F_1$ and $^7F_2$ are approximately 50 cm^{-1}. Since Eu^{3+} in the present instance (solutions, TLC spots, fingerprints) is not in a uniform environment (as in the $LaCl_3$ crystal case), line-broadening, rather than well-defined Stark splitting, is to be expected. The larger widths (10 nm) of the 615- and 592-nm TLC spot emissions, compared to that of the 579-nm emission (3 nm), are in line with the respective assignments as $^5D_0$ to (Stark-broadened) $^7F_2$ and $^7F_1$, and $^7F_0$ (no Stark-broadening).

Imaging System

Figure 3 shows a block diagram of our time-resolved imaging system. The central component is a Hamamatsu V3036 u proximity-focused microchannel plate image intensifier, which, for convenient imaging, is mounted on a photographic camera with the intensifier photocathode at the camera's image plane. The gating scheme for background fluorescence suppression is shown in Fig. 4, and the electrical diagram for the operation of the image intensifier (in the gate-on mode) is shown in Fig. 5. The photocathode is reverse biased with a battery, so that the intensifier is off unless a negative gating pulse is applied. Additional instrumentation details are reported elsewhere [17].

Conclusions

The intramolecular energy transfer and Eu^{3+} emission scheme for the Eu-RP complex is shown in Fig. 6. The fingerprint luminescence under UV argon-ion-laser excitation is weak compared, for instance, with that of ninhydrin/$ZnCl_2$-treated prints under blue-green argon-ion-laser excitation. Even so, given the sensitivity of microchannel plate image intensifiers and the background suppression possible because of the very long luminescence lifetimes of the Europium complexes formed with RP and its analogs, one can look forward to hitherto unobtained sensitivity in detecting latent prints on porous surfaces which show very strong background fluorescence. The approach should be useful generally when detection of amino acids on strongly fluorescent substrates is of interest.

If the red shift in absorption of the amino acid reaction product with ninhydrin versus benzo(*f*)ninhydrin is any guide at all, then excellent overlap between the ligand absorption and the 579 nm transition of Eu^{3+} should be obtained with naphtho(*f*)ninhydrin. Substituents akin to the 5-methoxy group might further improve the energy transfer efficiency. The syntheses of such compounds and their use for fingerprint detection are currently under investigation. We are also examining other rare earths to obtain higher luminescence intensities.
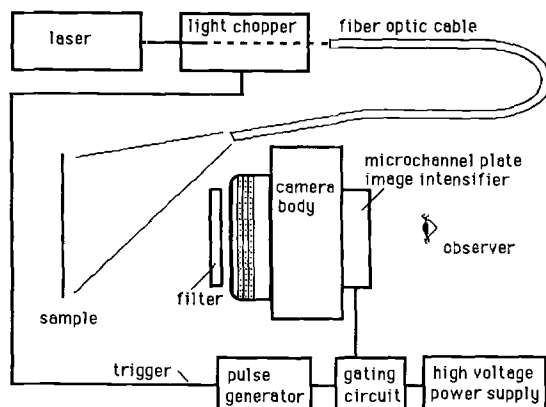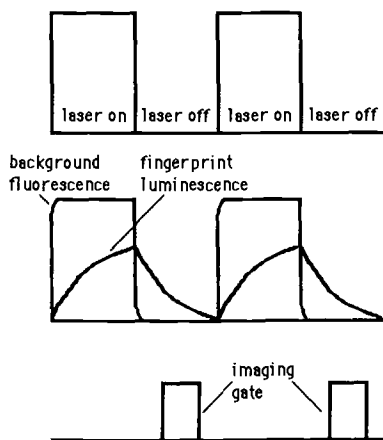
FIG. 3—*Gated imaging system.*

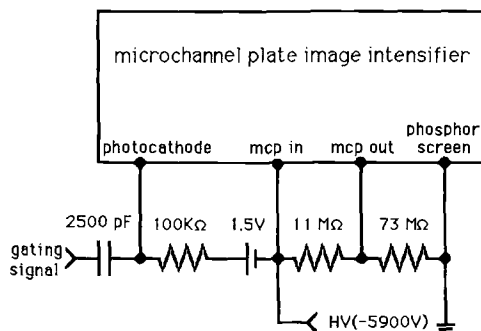FIG. 4—*Gating scheme for suppression of background.*

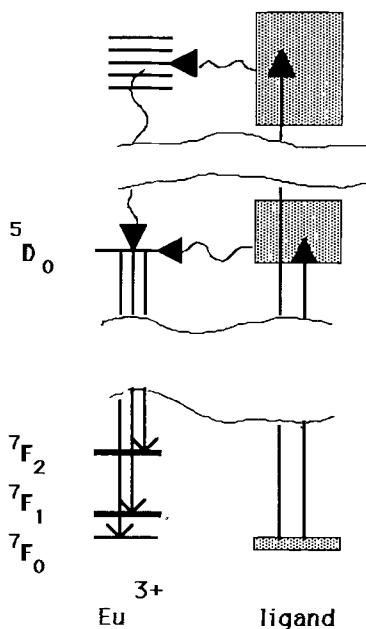
FIG. 5—*Electrical circuit for image intensifier operation.*

FIG. 6—*Intramolecular ligand-to-EuQ^{3+} energy transfer scheme. Solid arrows up denote laser pumping. Solid arrows down denote EuQ luminescence. Waved arrows represent intramolecular ligand-to-Eu energy transfer.*

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Address requests for reprints or additional information to
Dr. E. R. Menzel
Physics Department
Texas Tech University
Box 4270
Lubbock, TX 79409