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THIN INDIUM AND TIN FILMS FOR IMMUNOASSAY APPLICATIONS

A team from APL and The Johns Hopkins University School of Hygiene and Public Health has recently discovered that thin layers of indium and tin, coated with appropriate antigens (or antibodies), will respond visibly in white light when exposed to solutions containing a matching antibody (or antigen). The characteristics of the indium films and the tin films responsible for producing optimum visual responses in this immunoassay process are not yet well understood. The primary focus of the current project is to produce thin films of indium and tin (and their associated oxides and alloys) with controlled thicknesses and known microstructures suitable for immunoassay testing. Emphasis is on correlating fundamental thin-film properties with the strength and quality of the immunoassay visual response. All immunoassay evaluations have been conducted with the antigen human immunoglobulin (IgG), a protein found in the blood.

INTRODUCTION

The indium film immunoassay process is a simple, rapid, and potentially low-cost method, using prescribed antibody–antigen reactions, for identifying pathogens, toxins, and chemical agents. First reported by Giaever in the early 1970s,[1] this novel technique is based on two fundamental principles: (a) that no more than a monolayer of a protein will adhere to the indium film layer on a solid substrate (e.g., glass or plastic), and (b) that no other layers of protein will adhere to the coated surface except by a specific (e.g., antibody–antigen) reaction. The bonding of a second (or third) protein layer produces a darkening effect or change in the optical transmission properties of a protein-coated indium slide when a specific reaction has occurred.[2]

In the immunoassay process shown schematically in Fig. 1, drops of an affinity-purified antibody (capture antibody) solution are placed on an indium slide (a suitable transparent substrate, such as glass, coated with a thin indium film nominally 5 to 25 nm thick). After an incubation period, the antibody solution is washed off, leaving on the slide spots of reactive antibody (protein) that are visible in transmitted white light. Next, in a technique known as masking, the slides are placed in a solution containing an irrelevant protein that bonds to the indium film in the previously uncoated areas, thus making the whole slide optically uniform. If the masked slide is then placed in a solution containing an antigen (or if the solution containing the antigen is dropped on the slide), the antigen will bind to the capture antibody, provided the capture antibody is specific for that antigen. If enough antigen–antibody bonding occurs—that is, if enough of the specific antigen is present—the spots will reappear, since the area

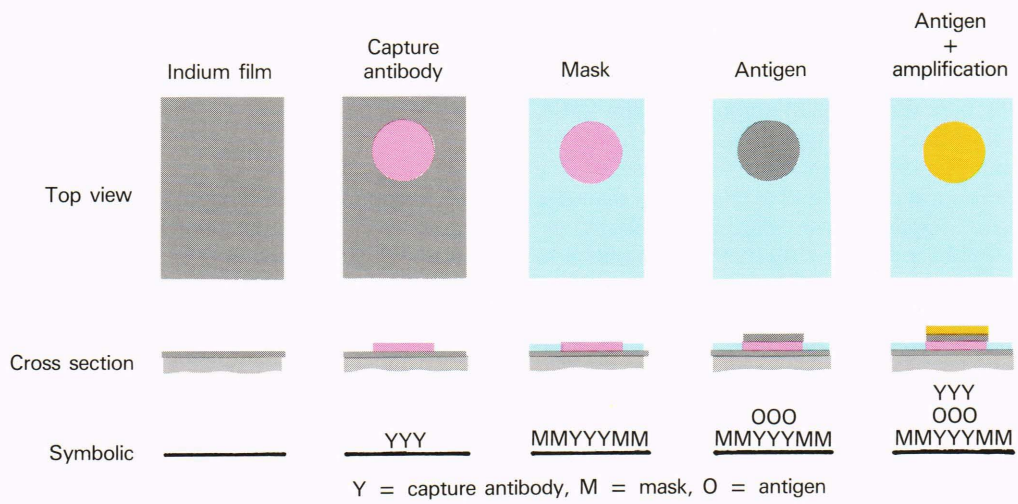

Figure 1—Schematic and symbolic representations of the indium film immunoassay process.

will contain two layers of protein and will be darker than the surrounding background. If, as frequently happens, there is insufficient protein, the reaction can be enhanced by the addition of another antibody, referred to as the second antibody or as amplification, to create three layers of protein. The second antibody is commonly the same as the capture antibody, and the method of surrounding the antigen by two layers of antibody is called the sandwich technique.[2]

FILM PREPARATION AND CHARACTERIZATION

Critical to the immunoassay spot intensity (contrast between the reaction spot and the background), and hence to the ultimate sensitivity of the detection process, are the characteristics of the host film, such as structure, morphology, and surface composition.[3] In an attempt to better understand the properties of thin indium films that make them suitable for biological immunoassay applications, a systematic deposition and characterization program was undertaken. Particular attention was focused on the deposition parameters—such as substrate temperature and rate and angle of deposition—that directly contribute to film grain size, structure, and surface morphology. Several hundred thin films of indium, in addition to Sn, Ti, Au, and other metals and dielectric films of approximately the same thickness and/or optical transmission, were prepared by vapor deposition. Deposition rates ranged from 0.01 to 0.5 nm/s. Films deposited at 0.03 nm/s were among the most successful in producing a strong immunoassay response. These thin indium films (5 to 100 nm thick) were vapor-deposited on controlled-temperature substrates (-180, $+25$, and $+100$°C) at a chamber background pressure of 2.67×10^{-8} Pa. The substrates (25 mm long × 8 mm wide × 1.25 mm thick) were chemically cleaned alumino-borosilicate glass. The films were evaporated from a resistance-heated tungsten boat using 99.99+% pure source material. After deposition, the films were allowed to reach room temperature under vacuum. The samples were then brought to atmospheric pressure with dry nitrogen gas and immediately transferred to controlled storage (a nitrogen dry box or desiccated container) to await further processing and/or analysis. Typical analysis included the determination of thickness, optical density, surface morphology, and immunoassay response using human immunoglobulin (IgG).

Figure 2 illustrates, for typical samples from the 0.03-nm/s room-temperature indium and tin series, the dependence of optical density and average grain diameter on film thickness. Optical density data were collected using an ESECO transmission densitometer. Film thickness was determined during deposition by a quartz-crystal rate monitor. Confirming thickness measurements and rate estimates on thicker films were also made using optical interference techniques and the deposition time. Surface morphology was observed using an ISI-40 scanning electron microscope (SEM) at 30 keV without sample overcoating. The surface grain structure of a 25-nm-thick indium film is illustrated in Fig. 3. Similar photomicrographs for films of other thicknesses can be found in a previous publication.[4] Average grain diameter was determined from the SEM photomicrographs using a Zeiss MOP-3 digital morphometric analyzer. For the 0.03-nm/s room-temperature film series, the best immunoassay results (those with the greatest contrast between the antigen–antibody double protein layer and the background) occurred at film thicknesses between 10 and 25 nm when the nucleating grains appeared to be of uniform size, with an average grain diameter of about 0.14 μm. When similar morphology was obtained at other deposition temperatures and rates (while maintaining similar optical transmission), the immunoassay response was again maximized.

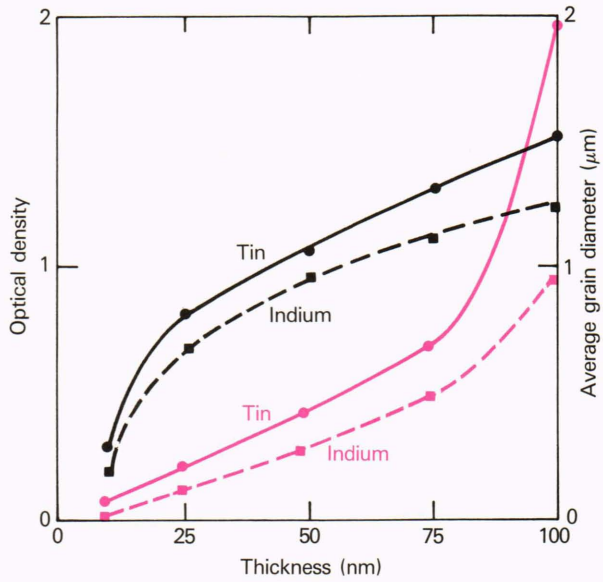

Figure 2—Relative optical density (black curves) and average grain diameter (red curves) as functions of film thickness for thin indium and tin films deposited at 25°C substrate temperature. In the thickness range of immunoassay response (10–25 nm), there is very little difference between indium and tin, either in optical transmission or in grain size.

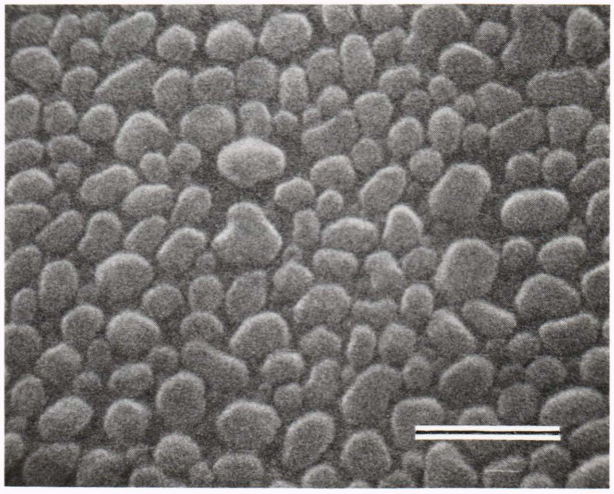

Figure 3—SEM photomicrograph of a 25-nm-thick indium film deposited on glass at 25°C substrate temperature. The marker bar equals 0.5 μm.

Thin tin films showed similar physical characteristics, as illustrated in Figs. 2 and 4. In both the indium and tin systems, films deposited at substrate temperatures near liquid nitrogen temperature (−180°C) have extremely small grain size, even in films several tens of nanometers thick. The immunoassay response on these small-grained (<0.05 μm) films was extremely poor. There was little difference in grain size or structure between films deposited at room temperature and films made at 100°C. The indium and tin film morphology reported in this study is comparable to that recently observed by Bertran et al.[5]

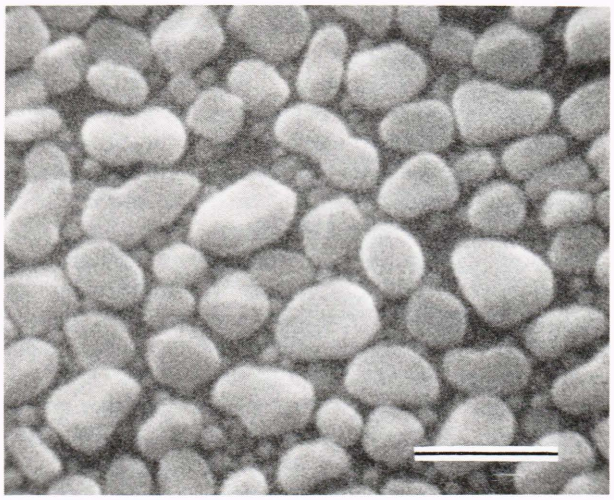

Figure 4—SEM photomicrograph of a 25-nm-thick tin film deposited on glass at 25°C substrate temperature. The marker bar equals 0.5 μm.

IMMUNOASSAY TESTING

The results of the immunoassay testing for several film samples are shown in Table 1. All samples were tested using a standardized assay (as described in the introduction) for the detection of the antigen human IgG. To test the sensitivity of the reaction, several dilutions of

Table 1—IgG immunoassay results on indium and tin films.

Sample Series	*Thickness (nm)*	*Treatment*	*Visual Readings*[a] *Capture Antibody Dilution (μg/ml)* 40	20	10	5
APL indium (8706 Series)	15	Capture antibody[b]		++++	+++	±
		Mask[c]		±	—	—
		Antigen + amplification[d]		++++	+++	++
	20	Capture antibody		++++	++	±
		Mask		±	—	—
		Antigen + amplification		+++	+++	++
Commercial control	e	Capture antibody		++++	++	+
		Mask		±	—	—
		Antigen + amplification		++++	++	++
APL tin (8608 Series)	13.5	Capture antibody	+++		+	±
		Mask	+		—	—
		Antigen + amplification	++		+	±
	16.5	Capture antibody	+++		+	—
		Mask	±		—	—
		Antigen + amplification	+++		++	+
Commercial control	e	Capture antibody	+++		+	—
		Mask	—		—	—
		Antigen + amplification	+++		++	+

[a] Immunoassay visual response code: ++++ extremely strong spot, +++ very strong spot, ++ strong spot, + clearly visible spot, ± suspicious spot, — no spot.
[b] Capture antibody: goat-antihuman IgG (gamma), affinity-purified, KPL Stock No. 01-10-02, at concentrations of 40, 20, 10, and 5 μg/ml in saline.
[c] Masking protein: normal goat serum (5%) plus 100 μg/ml of Aldolase in saline.
[d] Antigen: human IgG, Calbiochem Stock No. 401105, 25 μm/ml in tris/saline. Amplification: capture antibody, 20 μg/ml in tris/saline.
[e] Thickness and deposition parameters unknown. On the basis of transmission, thickness is estimated to be between 12.5 and 17.5 nm.

the capture antibody were used. The footnotes to Table 1 give details of the capture antibody, the masking protein, the antigen, and the amplification antibody. The incubation period for the capture antibody was 30 min. Both the masking operation and the initial antibody solution exposure were 60 min in duration. The amplification took 30 min. At each stage of the assay, a slide was washed and dried to demonstrate the effect of that operation or procedure. The response was evaluated at each stage by inspecting the slide in transmitted white light and subjectively assigning a response code relating the intensity of the spot to its background. The response codes are also described in the footnotes to Table 1.

Figure 5 compares typical APL indium samples, nominally 15 nm thick, with a commercial control. The APL films are darker, perhaps indicating a slightly thicker film, but the strength of the response appears comparable to that obtained using the control substrate. The APL films were deposited on glass; the commercial vendor used a transparent organic substrate. Three indium or tin slide samples were required to record permanently the major steps in the immunoassay process. The samples for the commercial film assay were each cut from the same plastic substrate sheet. The APL film assay was performed with three similar substrates from the same vacuum-deposition run. The softness and thinness of the indium film layer are indicated by the various scratches on the film surface. It appears that the film on the hard glass substrate is more prone to scratching (or at least the scratches are more visible) than a similar film on plastic. Solution-drying rings are also more apparent on the glass substrate. Preliminary Auger analysis indicated that the APL indium film surface is much freer of organic contamination than the commercial samples.

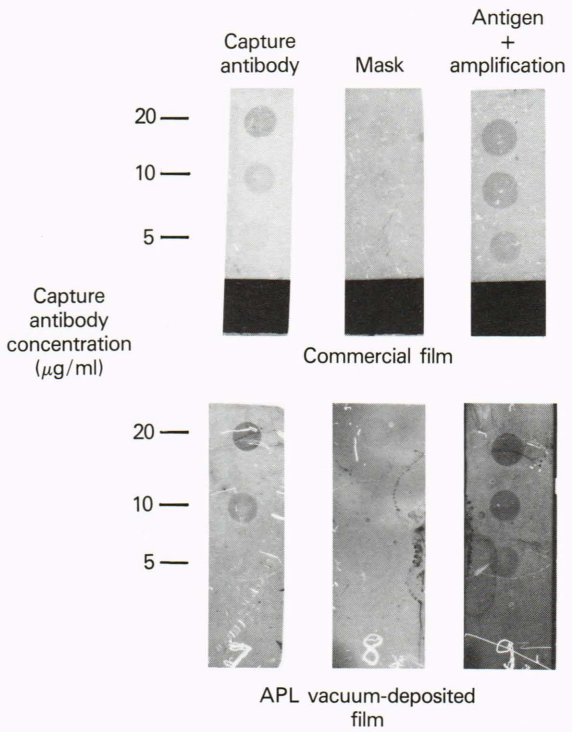

Figure 5—Photograph of IgG immunoassay on commercial and APL-produced indium thin-film test slides. Nominal film thickness is 15 nm.

SUMMARY AND FUTURE PLANS

Indium films with controlled morphology have been prepared under known conditions. Under immunoassay testing with human IgG, these films have yielded results at least equivalent to films produced by Giaever and more recently by a commercial firm. In addition, we believe that our research represents the first time that the immunoassay process has been demonstrated with tin films. The response of tin, compared with indium, is similar for films of comparable thickness and grain size. Although that is significant and new in itself, the real importance of the tin discovery lies in the future possibilities of combining indium and tin in both their metal and oxide forms. Indium–tin alloys are well known in the metallurgical world and form a class of materials whose material and structural properties can vary widely with changes in composition. Thus, a new class of thin metal films has potential for use in the immunoassay process. Such thin films could be used in the standard transmission mode in a manner similar to that used with either of their individual constituent elements. Note that reflection-mode analysis is also possible with any of these metal or metal alloy films.

Both the indium and the tin films tested to date probably contain an appreciable oxide constituent. Control of the oxide content could lead to thicker metal oxide films with larger grain sizes than are achievable in the current metal films; because of the greater percentage of oxide, such thicker films would have greater transparency. Although both indium oxide and tin oxide can be prepared as thick transparent conducting layers, a commercially important and well-characterized transparent conductor is indium–tin oxide (ITO). We consider that the demonstration of both tin and indium as effective immunoassay hosts has set the stage for the possible use of ITO. We believe that ITO, or a similar compound, offers exciting possibilities for thick transparent films of large grain size, while retaining many of the attributes of a metallic conductor. ITO could also be used as an intermediate host substrate to set the proper surface morphology and grain size and then a very thin indium or tin film could be deposited on its surface as a layer for antibody–antigen binding.

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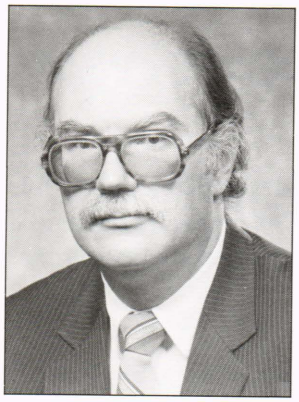
THE AUTHORS



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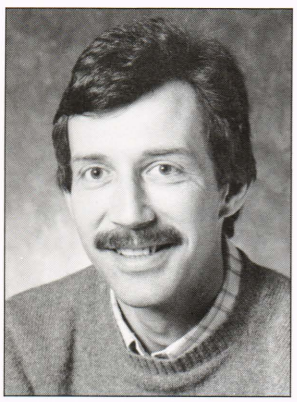

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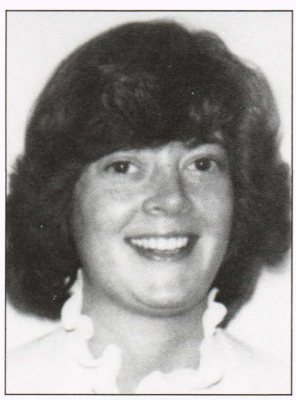

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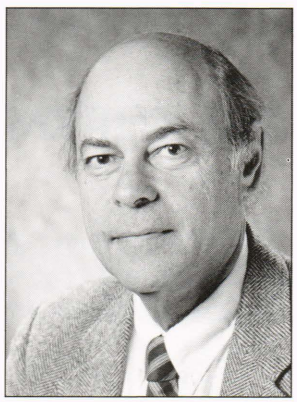

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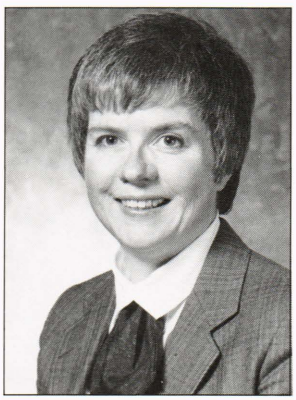

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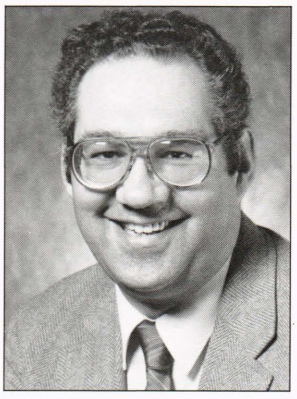

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