Hyperspectral retinal imaging for micro- and nanoplastics detection: a conceptual and methodological framework

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Appendix A. Detailed Phantom Recipe.

1. Protocol Sheet (100 mL batch scale)

A. Vitreous-equivalent gel (clear layer, 3–5 mm)

Ingredients

- 0.5 g low-melt agarose (0.5% w/v) [1,4]
- 1.0 mL Intralipid 20% (1% v/v final) [2]
- Distilled water to 100 mL
- (Optional) 20 mg sodium azide (0.02% w/v) for preservation

Steps

1. Heat 90 mL deionized (DI) water to boiling, add agarose gradually. Stir until clear[1,4]
2. Cool to 45°C.
3. Add 1.0 mL Intralipid and mix gently.[2,3]
4. Top up to 100 mL with DI water.
5. Degas under vacuum (30–60 s).
6. Pour into mold, thickness 3–5 mm. Chill at 4°C for 30 min.

B. Retina-equivalent gel (scattering + absorbing layer, ~200–300 µm)

Ingredients

- 5.0 g gelatin (5% w/v) [3,5]
- 2.0 mL Intralipid 20% (2% v/v final; adjust 2–5%) [2,3]
- Hemoglobin solution (stock 10 g/L): 2 mL → final 0.2 g/L [1,4]
- Melanin powder: 0.02 g (0.02% w/v) OR India ink: 20 µL [5]
- Distilled water to 100 mL

Steps

1. Heat 80 mL DI water to 45 °C. Add gelatin, stir until dissolved [3,5].
2. Add Intralipid, hemoglobin, and melanin/ink sequentially with gentle mixing [2,5].
3. Adjust to 100 mL.
4. Degas under vacuum.
5. Cast thin films using glass spacers (200–300 µm). Chill at 4 °C until set (~20 min).
6. If embedding particles, pour half-thickness layer first, let semi-gel (~5 min at 25–30 °C), deposit particle suspension, then overlay with remaining warm phantom.

C. RPE/backing layer (absorbing)

Ingredients

- 1.5 g agarose (1.5% w/v) [1,4]
- 0.05 g melanin powder (0.05% w/v) OR 50 µL India ink [5]
- Distilled water to 100 mL

Steps

1. Dissolve agarose in 80 mL boiling DI water.
2. Cool to 45 °C, add pigment.
3. Adjust to 100 mL, mix.
4. Pour thin layer (50–100 µm) at bottom of mold. Let set (~10 min).

Assembly (Figure 1)

1. Pour RPE layer in mold, set.
2. Add retina-equivalent layer (200–300 µm), embed particles if desired.
3. Overlay vitreous-equivalent layer (3–5 mm).
4. Chill complete phantom at 4 °C for 1 hr before imaging [1,5].

Storage: Seal in airtight box at 4 °C. Stable for 1–2 weeks. Add sodium azide for longer storage.

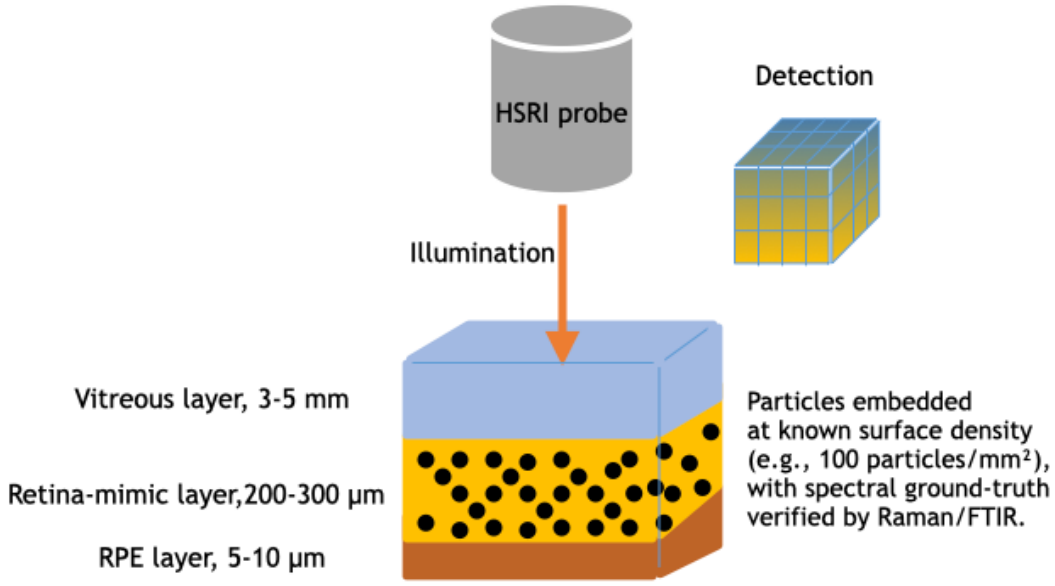

Figure 1. Schematic of multilayer phantom for HSRI validation. Three layers: vitreous-equivalent gel (3–5 mm, light blue), retina-mimic (200–300 µm, pink-orange, with embedded particles), and RPE (50–100 µm, dark brown). HSRI probe shown above with illumination and spectral detection. Particles embedded at known density (e.g., 100/mm²), validated by Raman/FTIR.

2. Worked Particle-Spiking Examples

Let's assume a stock suspension of **1 µm polystyrene beads** at **10^6 particles/mL**.

- Desired surface density: **100 particles/mm²** over 1 cm² (100 mm²).
- Total needed = 100 × 100 = **10,000 particles**.
- Stock = 10^6 particles/mL = 1,000 particles/µL.
- Required volume = 10,000 ÷ 1,000 = **10 µL** pipetted evenly over 1 cm².

If using **100 nm nanoplastics** at **10^9 particles/mL**:

- Same goal (10,000 particles over 1 cm²).
- Stock = 10^9 particles/mL = 1,000,000 particles/µL.
- Required volume = 10,000 ÷ 1,000,000 = **0.01 µL** (impractical).

  → Dilute 1:1000 (to 10^6/µL = 1000/µL) and pipette **10 µL**.

If using **10 µm beads** at **10^5 particles/mL**:

- Stock = 100 particles/µL.
- Goal = 10,000 particles.
- Required volume = 100 µL onto 1 cm² area.

Tip: Always sonicate suspensions 1–2 min before pipetting to avoid aggregation [3,5].

References

1. Mustari A, Nishidate I, Wares MA, et al. Agarose-based tissue mimicking optical phantoms for diffuse reflectance spectroscopy. J Vis Exp 2018;138:57578 .
2. Lepore M, Delfino I. Intralipid-based phantoms for the development of new optical diagnostic techniques . Open Biotechnol J 2019;13:163-72.
3. Ntombela L, Adeleye B, Chetty N. Low-cost fabrication of optical tissue phantoms for use in biomedical imaging. Heliyon 2020;6:e03602.
4. Kim M, Im S, Park I, et al. Fabrication of agar-based tissue- mimicking phantom for the technical evaluation of biomedical optical imaging systems. Curr Appl Phys 2024;61:80-5.
5. Stergar J, Hren R, Milanič M. Effects of phantom microstructure on their optical properties. J Biomed Opt 2024;29:093502.