Technology Transition Workshop| *Alexandre Beaudoin & Brian Dalrymple*

History and Evolution of Oil Red O

Did You Know?

"Fingerprints found at crime scenes lead to more suspects and generate more evidence in court than all other forensic techniques combined." *

* **Interpol European Expert Group on Fingerprint Identification (IEEGFI). Methods for Fingerprint Identification Part 1. *Interpol Fingerprint* [Online] 2004. http://www.interpol.int/public/Forensic/fingerprints/WorkingParties/IEEGFI/ieegfi.asp**

Technology Transition Workshop NIJ National Institute of Justice

Treatment on Porous Surfaces

- **Ninhydrin**
- **1,8-diazafluoren-9-one (DFO)**
- **1,2-indanedione**
- **Magnetic powder**
- **Physical developer**

Treatment on Wet Porous Surfaces

- **Multimetal deposition**
- **Physical developer**

Technology Transition Workshop

Now, Oil Red O (ORO) Can Be Added

- **Non-Destructive Technique**
  - **Not time consuming**
  - **Affordable chemicals**
  - **Simplicity of application**
  - **No need to monitor development during process**
  - **Does not render the paper weak and easy to damage**
  - **Small quantity of glassware needed**

Technology Transition Workshop NIJ National Institute of Justice

History

- **Need: Easier technique for wet porous surface for field tests**
- **Biology background: What is one of the better lipid stains used for cell membranes?**
- **Development: Find out what the best concentration is, how to darken ORO, how many times should it be soaked, need for a buffer, etc.**
- **Final recipe: 2004 in JFI (Journal of Forensic Identification)**

Technology Transition Workshop

Evolution of Formula

- **Since the first recipe, the ORO stain solution hasn't changed**
- **At the end of 2007, a new buffer solution was developed (more stable for a longer time)**
- **In 2008, Australian article in JFI that confirms the actual ORO stain solution contains the perfect concentration of all the components used**

Technology Transition Workshop NIJ National Institute of Justice

What is ORO?

- **ORO is a lysochrome (soluble lipid stain)**
- **Diazo dye (–N=N–)**
- **ORO is lipid-specific and sensitive to all lipids**
- **Color: red**
- **Water solubility: insoluble**
- **Solubility in ethanol: moderate**

Technology Transition Workshop

What is ORO?

- **A more vivid red color then Sudan III and Sudan IV**
- **Used in biology to stain lipoproteins recovered after electrophoresis separation on acetate cellulose**
- **Used in electron microscopy**
- **Used in detection of lipids in histology (muscular and hepatic tissues, blood vessels)**

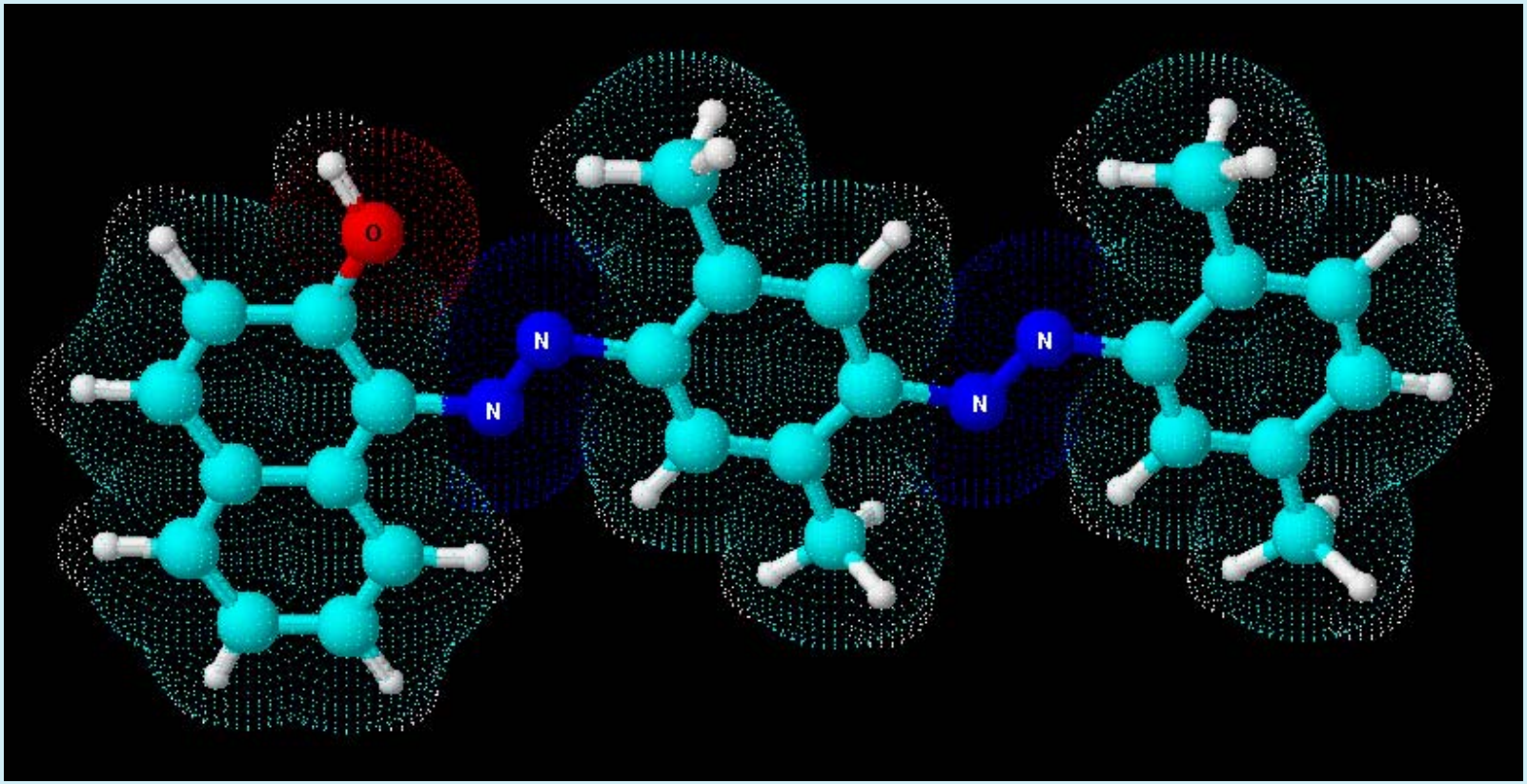
Technology Transition Workshop NIJ National Institute of Justice

What is ORO?

Technology Transition Workshop

NIJ National Institute of Justice

Oil Red O Molecule



Technology Transition Workshop

NIJ National Institute of Justice

Materials and Methods

Three steps:

1. **Coloration**
2. **Neutralization**
3. **Drying**

Technology Transition Workshop

Materials and Methods

Preparation of Solutions

- **Stain Solution**
  1. **Weigh out 1.54 g of ORO and dissolve it in 770 ml of methanol.**
  2. **Dissolve 9.2 g of NaOH (sodium hydroxide) in 230 ml of water and add it to the above solution.**
  3. **Mix and filter, then store in a brown bottle away from light.**

Technology Transition Workshop

Materials and Methods

Preparation of Solutions

- **pH 7 Buffer Solution**
  1. **Add 101.55 g of $NaH_2PO_4 \cdot H_2O$ (sodium phosphate monobasic monohydrate) to 1 L of water and shake until it is dissolved.**
  2. **Add 338.79 g of $Na_2HPO_4 \cdot 7H_2O$ (sodium phosphate dibasic heptahydrate) to 1 L of water and shake until it is dissolved.**
  3. **Mix the two solutions.**
  4. **Add enough water to increase volume to 4 L.**

Materials and Methods

Procedure

1. **Immerse the document in the stain solution and shake using a titer plate shaker for 60 to 90 minutes.**
   - **Usually, strong fingerprints give good results after only 5 to 10 minutes**
   - **The 60 to 90 minutes immersion in ORO is to be sure that no weak fingerprints are missed**
   - **Even if you leave the paper in the staining solution longer, you won't have a better result**
   - **The titer plate shaker is optional… as long as you keep the paper immersed in ORO**

Technology Transition Workshop NIJ National Institute of Justice

Materials and Methods

Procedure (continued)

2. **Remove the document from the ORO stain solution and drain.**
3. **Immerse the document in the buffer solution to adjust the pH.**
   - **The Oil Red O solution is around pH 8**
   - **If the paper is not neutralized, it becomes fragile and easily breakable**

Technology Transition Workshop NIJ National Institute of Justice

Materials and Methods

Procedure (continued)

4. **Rinse the document with distilled water (optional) and dry.**
   - **Drying can take place in the open air or in an oven at 50˚C to accelerate the process**
5. **Photographs of the prints are mandatory, as usual with any fingerprint laboratory development process.**

Technology Transition Workshop

Materials and Methods

Procedure (Short Version)

1. **Immerse document in ORO stain solution and shake for 60 to 90 minutes.**
2. **Remove and drain document.**
3. **Immerse document in buffer solution.**
4. **Rinse document in distilled water (optional) and dry.**
5. **Photograph developed prints.**

Technology Transition Workshop

NIJ National Institute of Justice

Principle of ORO

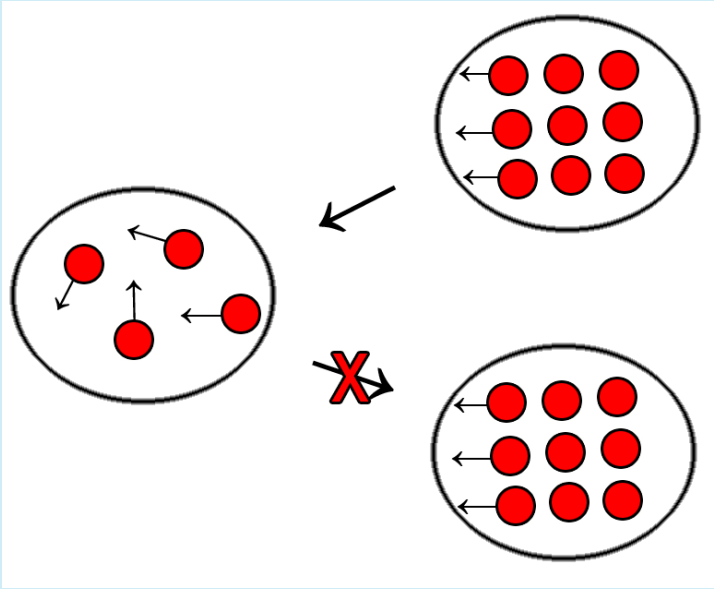
- **ORO is an hydrophobic and lipophilic dye**
- **Therefore, it will tend to take “refuge” in lipids, like those in fingerprint residue**

Technology Transition Workshop

Principle of ORO

Entropy

- **The second law of thermodynamics states that energy tends to disperse spontaneously if it is not constrained (example: car tire)**
- **Entropy is the measure of this dispersion**
- **Usually, in nature, energy tends to disperse, resulting in an increase of entropy**



Technology Transition Workshop NIJ National Institute of Justice

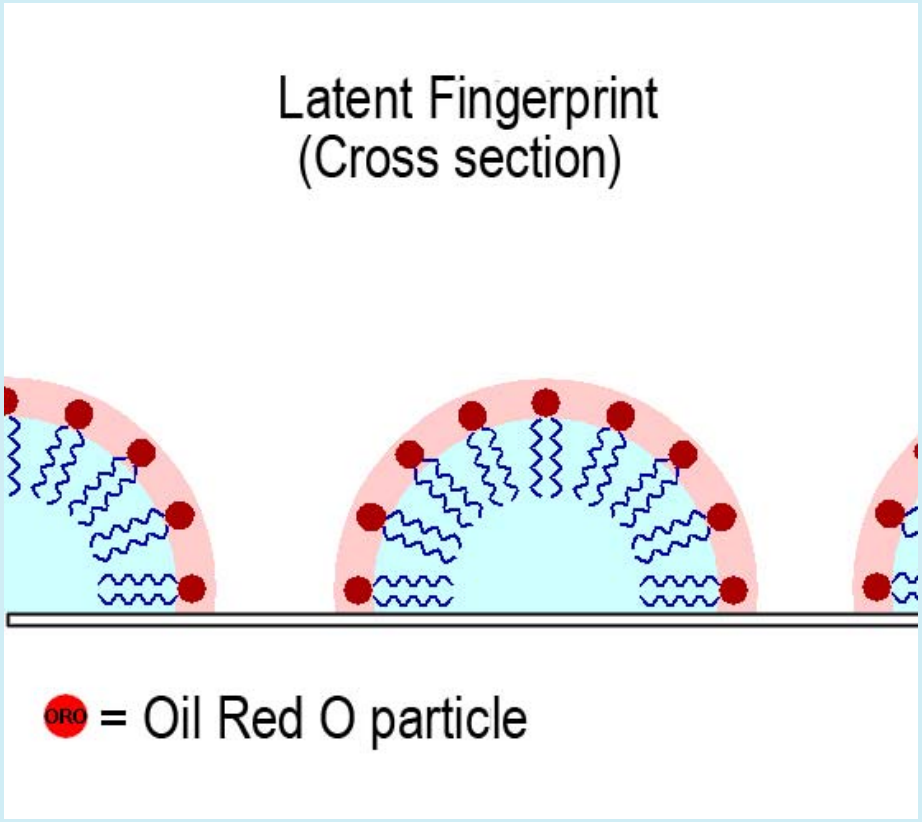
Principle of ORO

Increase of Entropy

- **The staining with ORO depends on increasing the system entropy**
- **The hydrophobic dye is dissolved into a reagent containing some water, and is “afraid” of water**
- **When we put this staining solution in contact with fingerprints, the dye will penetrate into the lipids to take more space (natural tendency is for increase in entropy)**

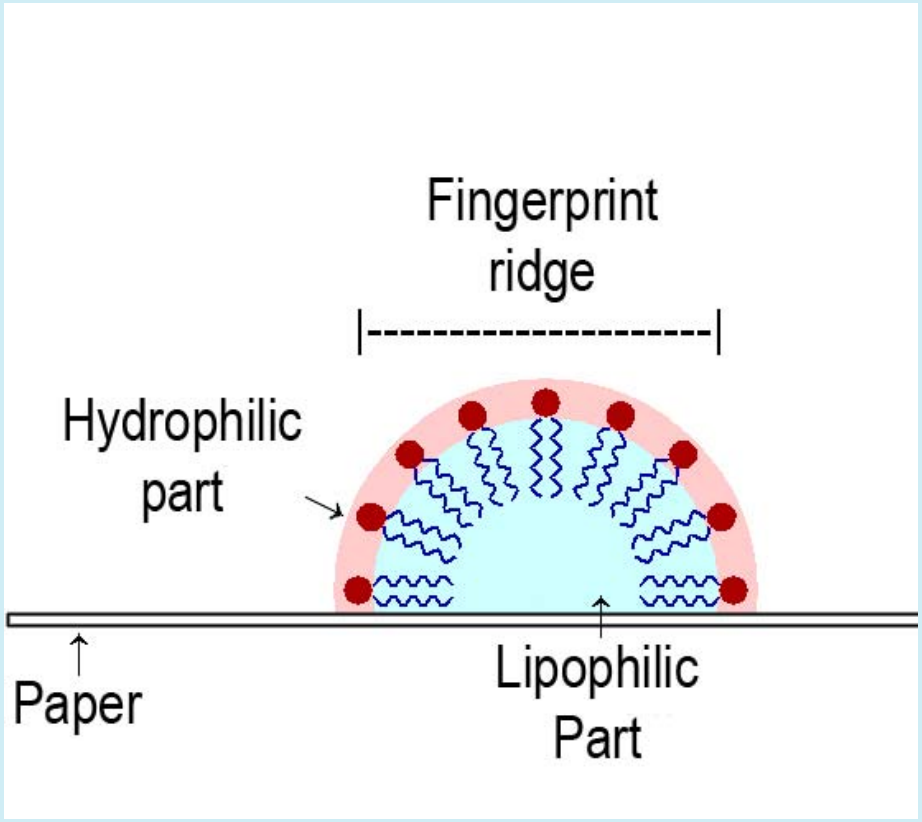
Technology Transition Workshop NIJ National Institute of Justice

Staining of Fingerprints with ORO



Technology Transition Workshop

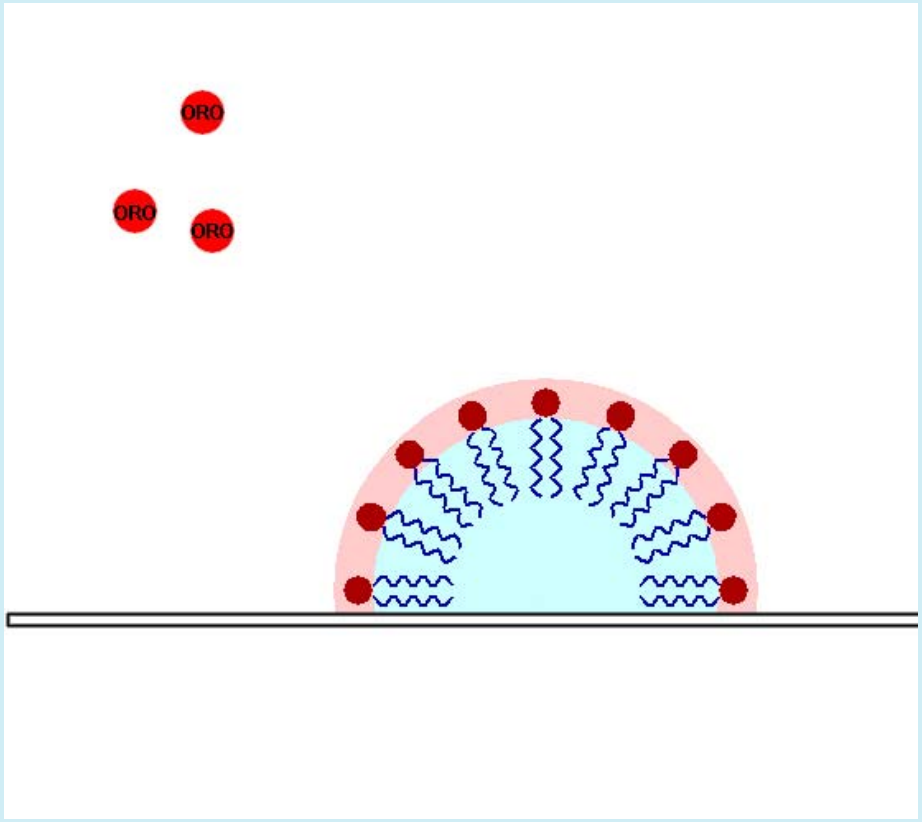
Staining of Fingerprints with ORO



Technology
Transition Workshop

NIJ National Institute of Justice

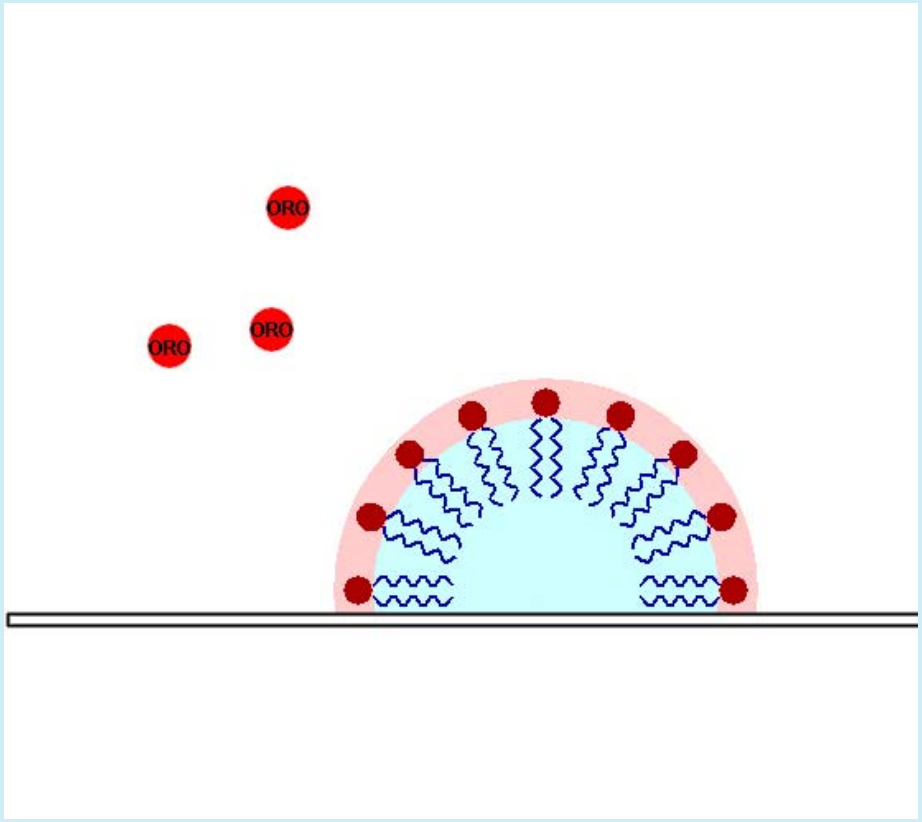
Staining of Fingerprints with ORO



Technology Transition Workshop

NIJ National Institute of Justice

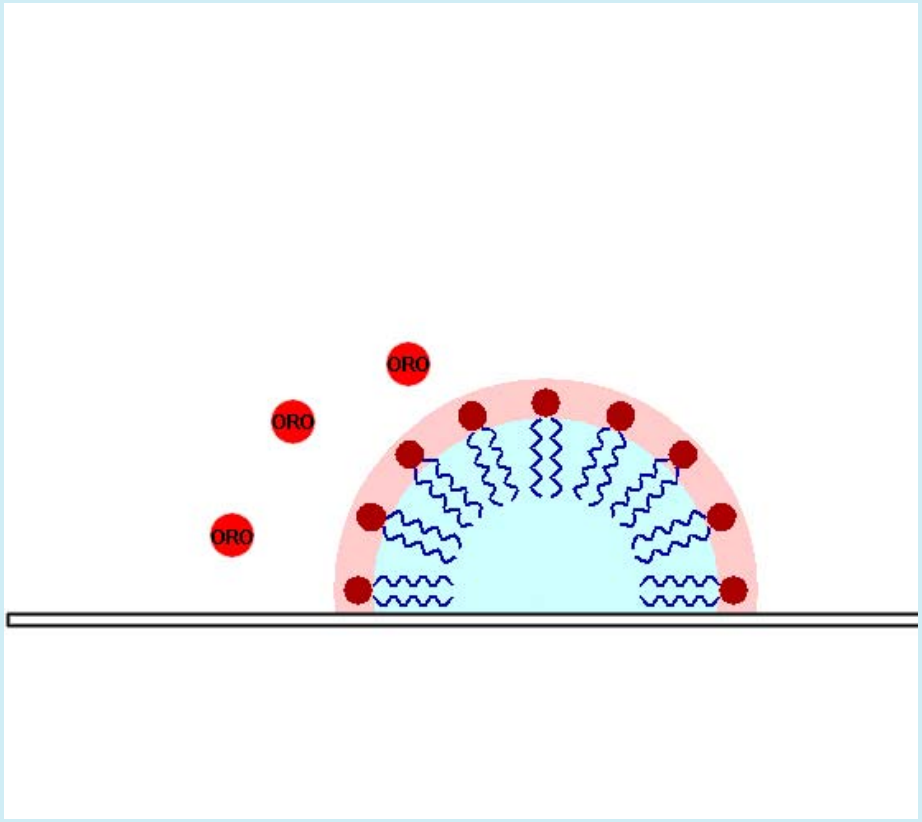
Staining of Fingerprints with ORO



Technology
Transition Workshop

NIJ National Institute of Justice

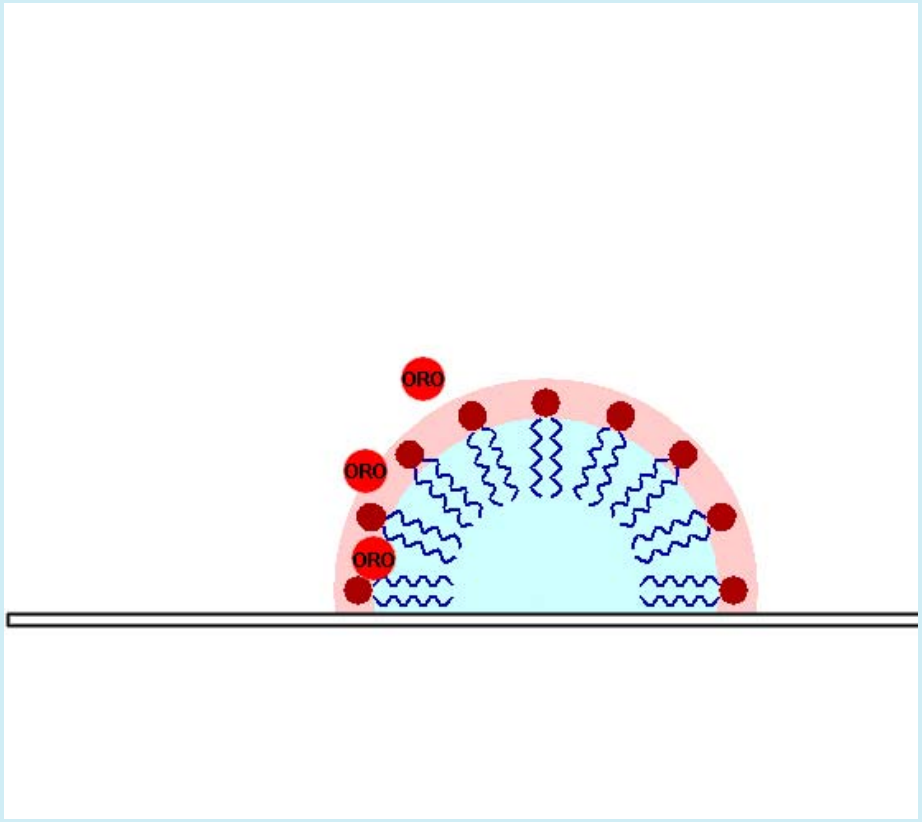
Staining of Fingerprints with ORO



Technology Transition Workshop

NIJ National Institute of Justice

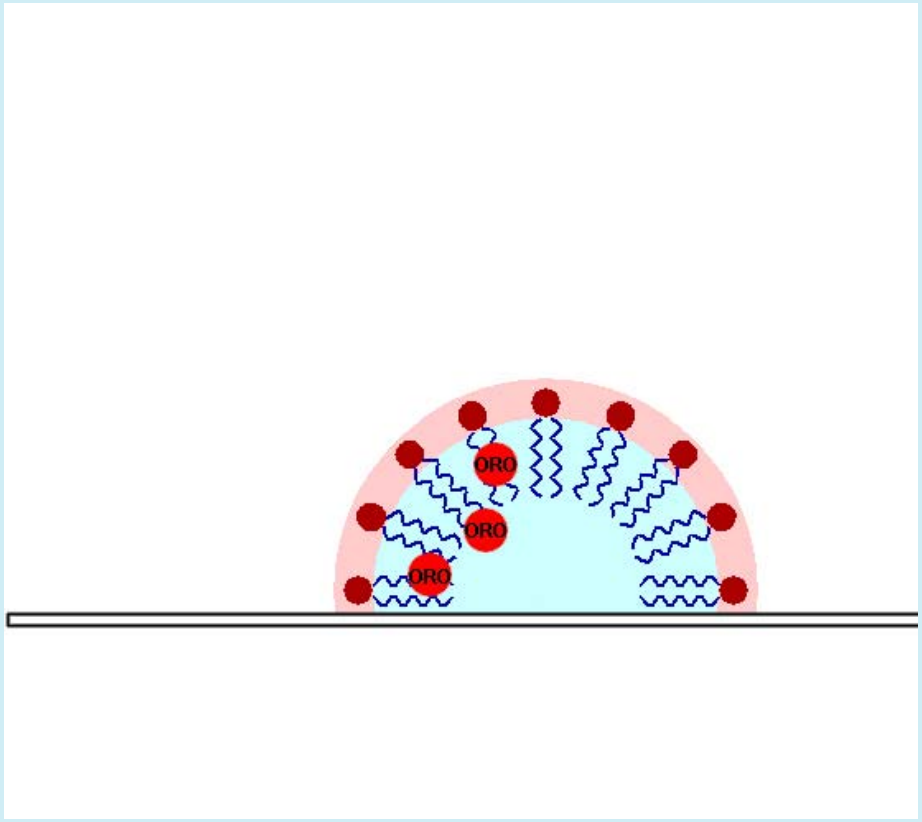
Staining of Fingerprints with ORO



Technology
Transition Workshop

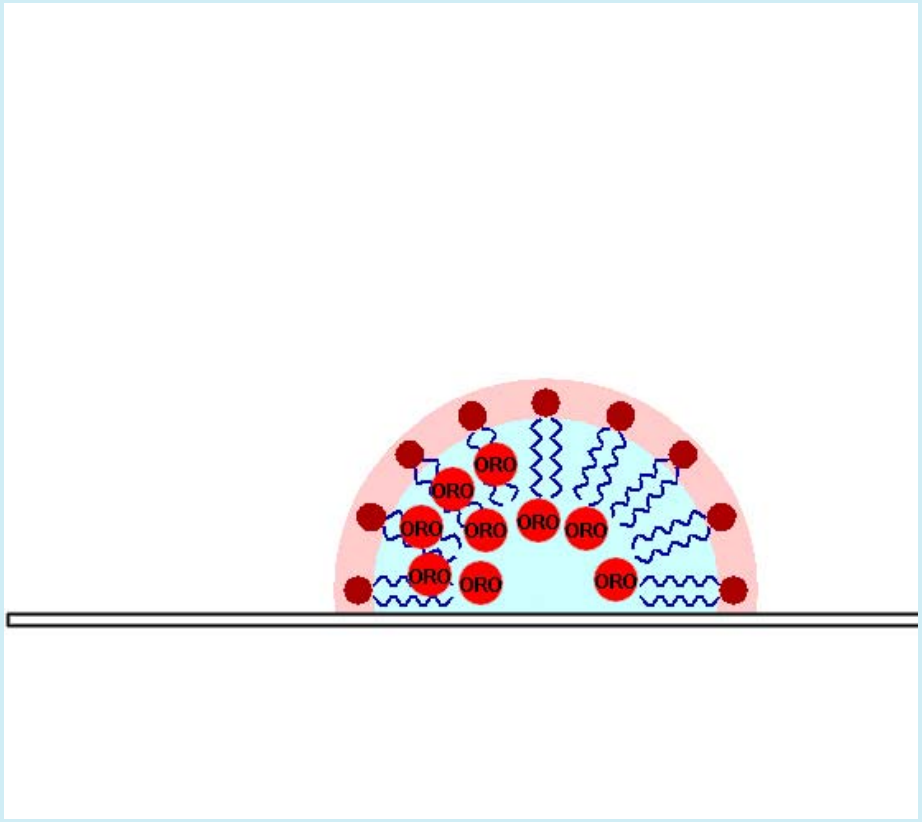
NIJ National Institute of Justice

Staining of Fingerprints with ORO



Technology Transition Workshop

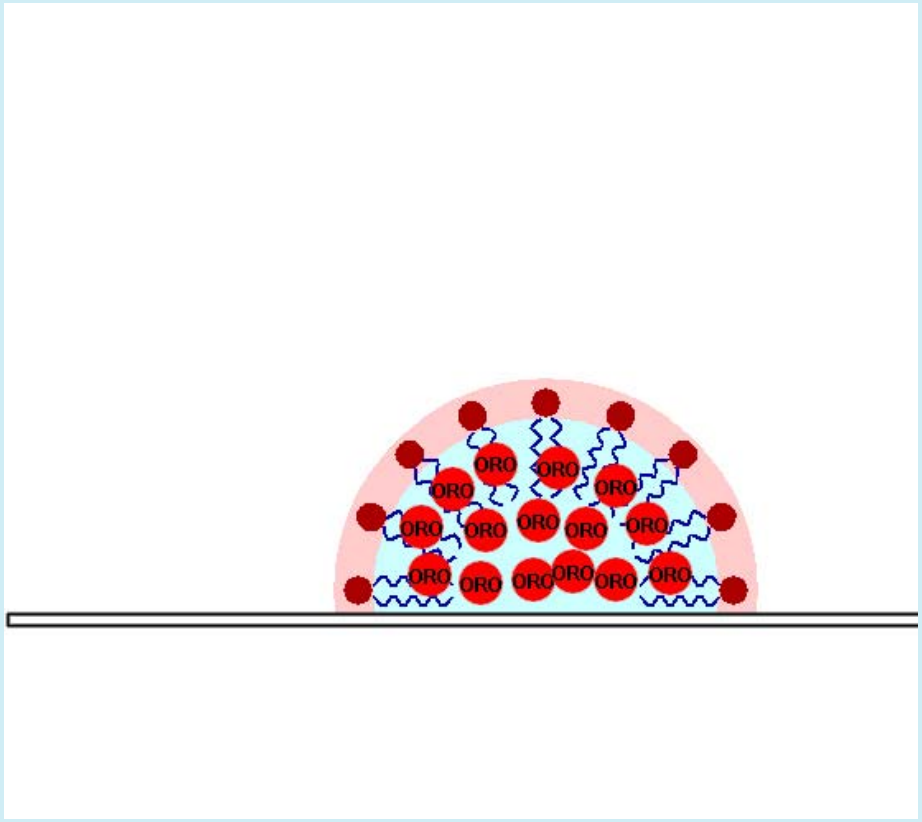
Staining of Fingerprints with ORO



Technology
Transition Workshop

NIJ National Institute of Justice

Staining of Fingerprints with ORO



Technology Transition Workshop

When Should ORO Be Used?

- **ORO should be used on dry or wet porous surfaces**
- **ORO should be integrated into your sequence on dry porous surfaces after ninhydrin/indanedione and before physical developer**
- **Dry porous surface sequence should be:**
  - **DFO-NIN-ORO-PD**
  - **IND-ORO-PD**

Technology Transition Workshop NIJ National Institute of Justice

ORO After DFO

Treatment with DFO

Technology Transition Workshop

ORO After DFO

Treatment with DFO followed by ORO

Technology Transition Workshop

Important Notice

- **Ninhydrin, DFO and indanedione shouldn't be used with petroleum ether as the carrier**
- **Why?**
  - **Because petroleum ether is also used for lipid extraction in some biological techniques**
  - **If the petroleum ether dissolves the lipids, then ORO can't stain lipidic fingerprint residues, because there won't be any left on the paper**

Technology Transition Workshop NIJ National Institute of Justice

Important Notice

- So, we should use a safer carrier
- Now that CFC113 is prohibited, we suggest you use HFE-7100 fluids as the carrier
- See research done by Rajtar (2000) for more details
  - Rajtar PE, 3M Novec Engineered Fluid HFE-7100, A New Carrier Solvent for use in Ninhydrin and DFO Formulations, *Fingerprint World*. 2000; 26(102): 143-152.

Technology Transition Workshop NIJ National Institute of Justice

When Should ORO Be Used?

- **ORO should be integrated into your sequence on wet porous surfaces before physical developer**
- **Wet porous surface sequence should be:**
  - **ORO-PD**

Technology Transition Workshop

Physical Developer After ORO

Enough or not enough contrast?

Technology Transition Workshop

Physical Developer After ORO

Enough or not enough contrast?

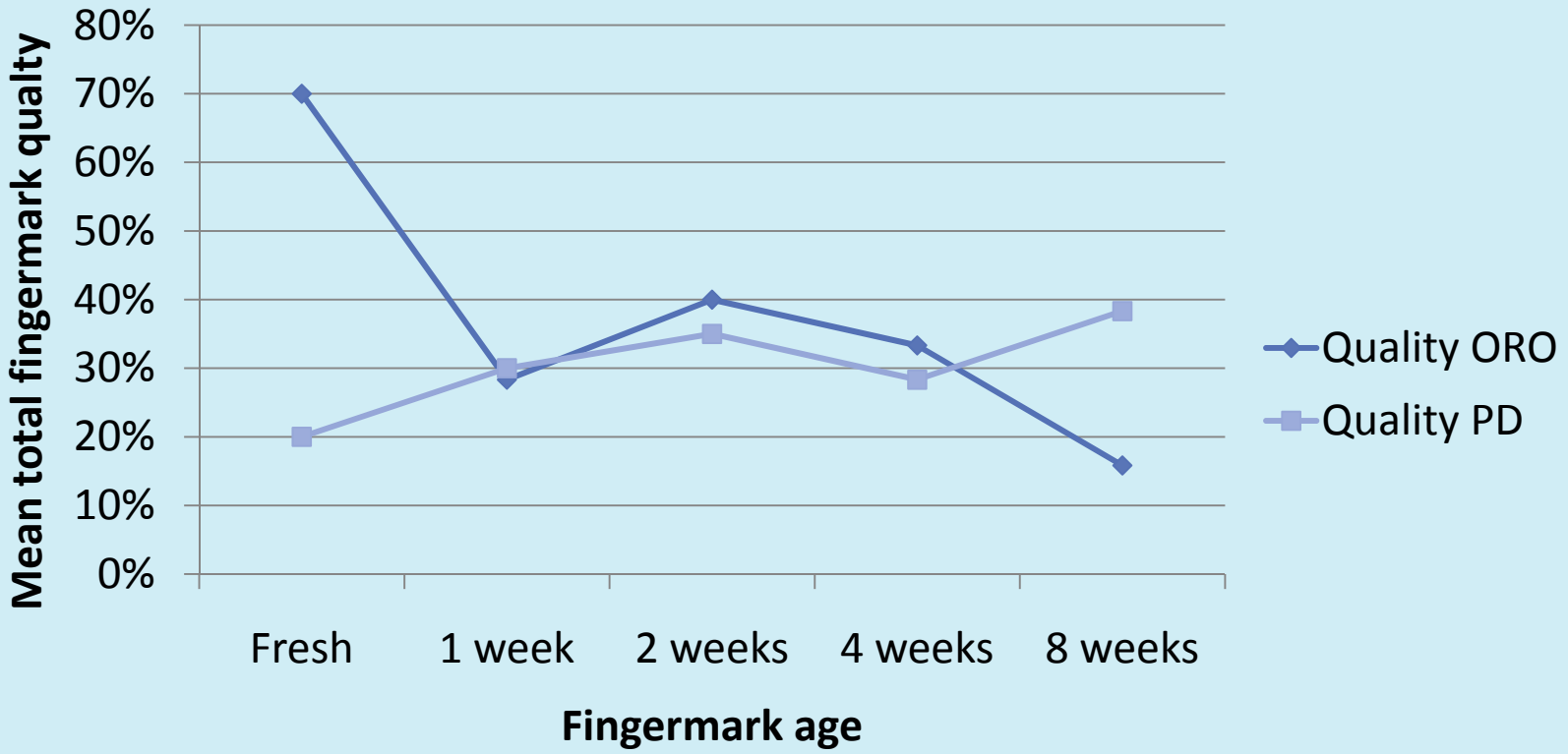
Technology Transition Workshop

Australian Validation

- **Group of Australian researchers from the University of Technology, Sydney**
- **ORO formulation is the best for fingerprints**
- **ORO and PD should be used in sequence for best results**

Technology Transition Workshop

Why Using ORO And PD In Sequence Is The Best Thing To Do…

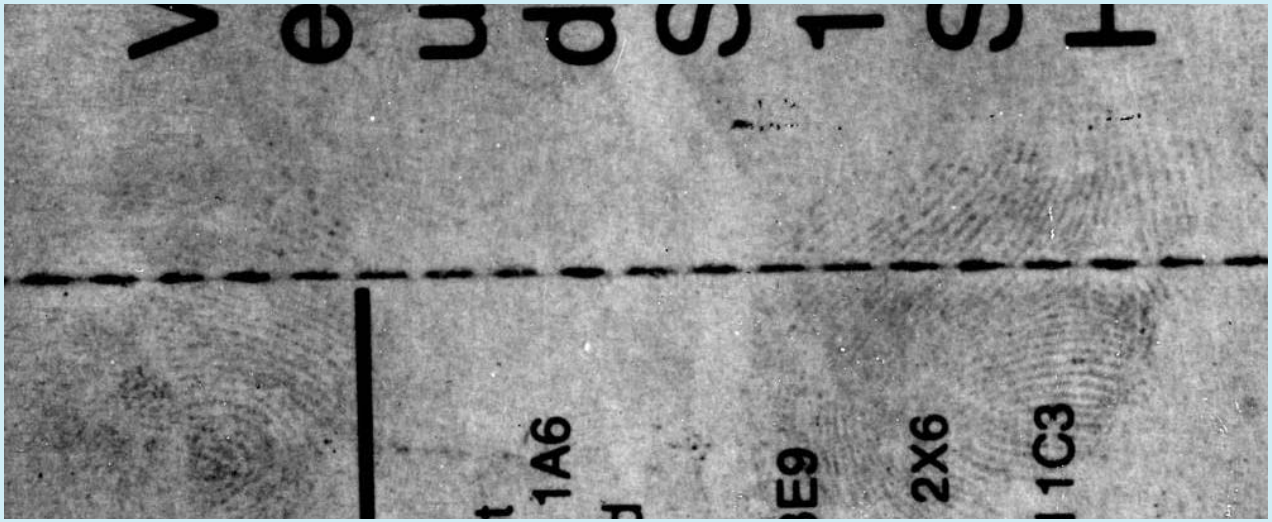


Technology Transition Workshop

NIJ National Institute of Justice

Cold Case – 21 Years Later…

- **Treated with DFO and ninhydrin without results**
- **Treated with ORO – two fingerprints**



Technology Transition Workshop

Conclusions

- **Oil Red O:**
  - **Is a simple technique for wet porous surfaces and dry porous surfaces**
  - **Is consistently superior to physical developer for cases less than 30 days old**
  - **Can be added to the treatment sequence after ninhydrin/indanedione and before the physical developer on dry porous surfaces**
  - **Should be used before physical developer on wet porous surfaces**

Technology Transition Workshop NIJ National Institute of Justice

Questions?

Technology
Transition Workshop

NIJ National Institute of Justice

Contact Information

Alexandre Beaudoin

Alexandre Beaudoin Criminalistique

465 Dalpe, Vercheres, QC J0L 2R0, Canada

514-660-6944

Alexandre.beaudoin@gmail.com

Brian Dalrymple

PO Box 296, Orillia, ON L3V 6J6, Canada

705-835-0227

info@briandalrymple.com

Note: All images are courtesy of Alexandre Beaudoin.

Technology Transition Workshop