# Fixation and staining (Item No.: P1440701)

## Curricular Relevance

**Area of Expertise:** Biologie

**Education Level:** Klasse 7-10

**Topic:** Biologie der Zelle, Mikroskopie

**Subtopic:** Grundlagen der Mikroskopie und Arbeitstechniken

**Experiment:** Fixieren und Färben

| Difficulty | Preparation Time | Execution Time | Recommended Group Size |
|---|---|---|---|
| Easy | 10 Minutes | 30 Minutes | 1 Student |

**Additional Requirements:**

- Distilled water
- Insect wing or leg or similar
- Onion skin or similar
- Piece of muscle or similar

**Experiment Variations:**

**Keywords:**

## Task and equipment

## Information for teachers

**Information**
Biological specimens are subject to alteration and rapidly show signs of decay once they are isolated from a living organism. If a relatively natural condition is to be retained, the proteins of the protoplasm must be turned into a "frozen state" or denatured. This process is referred to as fixation. Fixations are used to prepare permanent microscopic slides. Before these methods are applied, the specimens must be cut or prepared depending on the material they consist of in order to become translucent

**Information on obtaining materials**
All objects described in this manual are basically suited to be prepared as permanent microscopic slides. Information on obtaining materials is therefore to be derived from the various instructions. Very thin and hence good preparations of the specimens can be made by using a microtome and then preserving them as described below. These cutting instruments are usually not available for educational purposes. For this reason, thin sections and tear preparations presented in this manual must be made by hand. The students will be successful only after having some practice. Permanent microscopic slides should therefore only be made by students who possess experience in microscopy.

**Information on practical performances**
The objects described here and in the student worksheet are to be understood as exemplary. There are various commercially available chemicals, some of which are, however, poisonous or even carcinogenic. Denaturation with ethanol presents a rather simple fixation method which simultaneously accounts for dewatering. The withdrawal of water proceeds in a series of increasing alcohol concentrations (70-100%). If we had placed the specimens directly into water-free alcohol, the drastic concentration difference would have induced tissue shrinkage and aberrations. The exposure times indicated refer to thinly cut and small specimens. In case of larger specimens, alcohol should invariably be used in abundance and the exposure time should be extended to several hours.

**Safety information**

- Attention! Count the number of scalpel blades at the end of lessons in order to prevent accidents that also might occur afterwards!

- Ethanol and isopropanol are highly flammable. Extinguish all open flames!
- Wear protective glasses!

**Hazard and Precautionary statements**

| | |
|---|---|
| Ethanol: | |
| H225: | Highly flammable liquid and vapour. |
| P210: | Keep away from heat/sparks/open flames/hot surfaces. - No smoking. |
| Isopropanol: | |
| H225: | Highly flammable liquid and vapour. |
| H319: | Causes serious eye irritation. |
| H336: | May cause drowsiness or dizziness. |
| P210: | Keep away from heat/sparks/open flames/hot surfaces. - No smoking. |
| P233: | Keep container tightly closed. |
| P305 + P351 + P338: | IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. |
| Rotihistol: | |
| H226: | Flammable liquid and vapour. |
| H315: | Causes skin irritation. |
| H317: | May cause an allergic skin reaction. |
| H304: | May be fatal if swallowed and enters airways. |
| H400: | Very toxic to aquatic life. |
| H410: | Very toxic to aquatic life with long lasting effects. |
| P210: | Keep away from heat/sparks/open flames/hot surfaces. - No smoking. |
| P280: | Wear protective gloves/protective clothing/eye protection/face protection. |
| P273: | Avoid release to the environment. |
| P301 + P310: | IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. |
| P303 + P361 + P353: | IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. |
| P333 + P313: | If skin irritation or rash occurs: Get medical advice/attention. |

1. Fixation and dewatering without staining

The students should have all materials at their disposal right from the beginning and also be familiar with the subsequent embedding in Canada balm. It is reasonable to use insect parts (fly wings) for initial trials. These materials are rather dry already, but mere traces of water would ruin the preparations in the long term.

2. Fixation plus staining

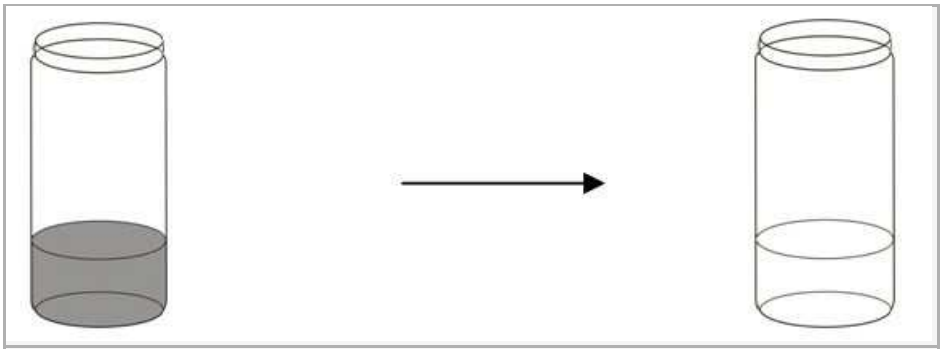
The preparation of thin specimens will be particularly important if this method is applied. Small organisms (Daphnia etc.) are appropriate to be used as primary, tender zoological specimens. However, it is possible to fully dehydrate small pieces of tissue (muscle) as well. This process will make them relatively hard and rigid. Tear preparations or sections are made before they are embedded in Canada balm.

# Fixation and staining (Item No.: P1440701)

## Task and equipment

### Task

Try out two different fixation methods!



### Equipment

| Position No. | Material | Order No. | Quantity |
|---|---|---|---|
| 1 | Tweezers,straight,pointed,120mm | 64607-00 | 1 |
| 2 | Dissecting needle, pointed | 64620-00 | 1 |
| 3 | Beaded rim glass, 30 x 50 mm | 33624-01 | 6 |
| 4 | Reagent bottle,scr.cap,cl.,30ml | 46190-00 | 6 |
| 5 | Dissecting needle, lancet-shaped | 64621-00 | 1 |
| 6 | Funnel, plastic, dia.50mm | 36890-00 | 1 |
| 7 | Pipettor,bulb,3 valves, 10ml max. | 47127-01 | 1 |
| 8 | Graduated pipette 10 ml | 36600-00 | 1 |
| 9 | Labels for microscopic slides, 120/pkg | 64703-00 | 1 |
| 10 | Chemicals set for TESS advanced Microscopy | 13290-10 | 1 |

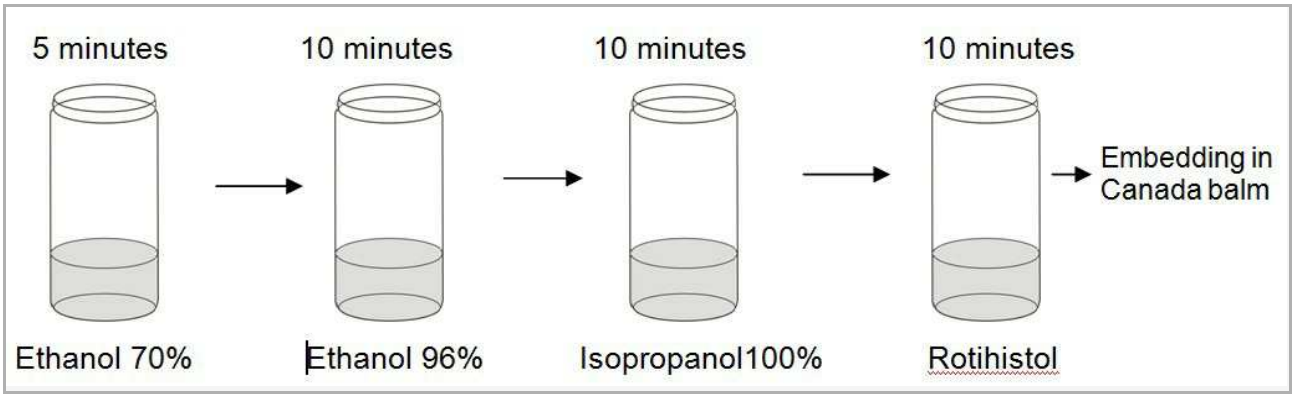
# Set-up and procedure

**Information**
Biological specimens are subject to alteration and rapidly show signs of decay once they are isolated from a living organism. If a relatively natural condition is to be retained, the proteins of the protoplasm must be turned into a "frozen state" or denatured. This process is referred to as fixation. Fixations are used to prepare permanent microscopic slides. Before these methods are applied, the specimens must be cut or prepared depending on the material they consist of in order to become translucent.

**Methods and observations**
Fixation method 1: Fixation and dewatering without staining

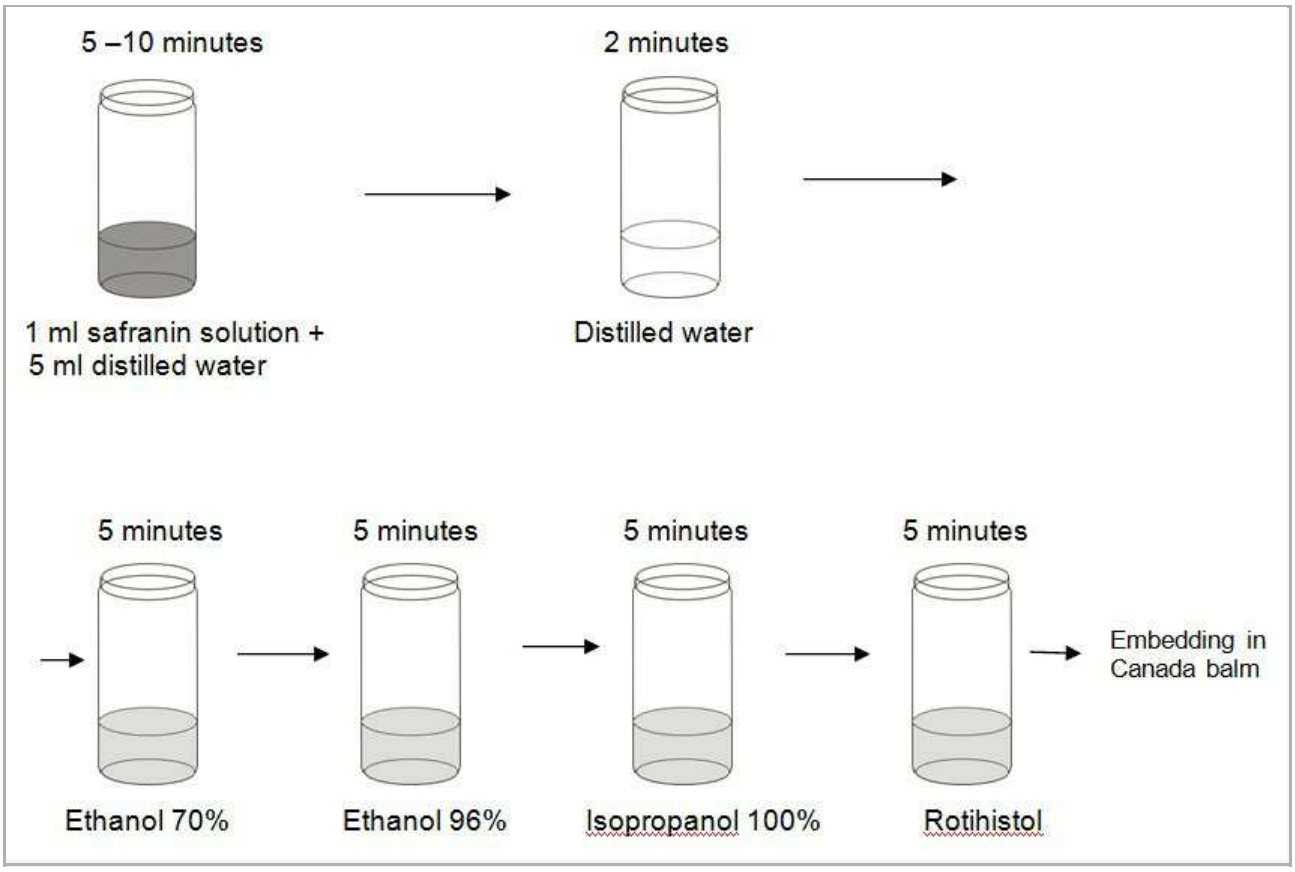
This method is particularly suited for insect parts containing chitin (legs and wings), and thin wood or cork sections which already possess an optically distinct structure. The alcohol withdraws water from the specimen, so that it can be subsequently embedded in Canada balm and will not undergo a process of natural decay.

- Snap-cap vials containing the substances mentioned in serial order are provided, filled up to approx. one quarter.
- The vials remain capped until dewatering commences. This is to prevent evaporation of the substances.
- Forceps are used to transfer the specimen from vial to vial.




Fixation method 2: Fixation plus staining

This method is suited for zoological or botanical specimens which are not yet distinctively structured optically. Preparation (cutting, tearing, or similar) may proceed either before fixation or afterwards, prior to embedding in Canada balm.


5 –10 minutes
2 minutes
1 ml safranin solution +
5 ml distilled water
Distilled water
5 minutes
5 minutes
5 minutes
5 minutes
Embedding in
Canada balm
Ethanol 70%
Ethanol 96%
Isopropanol 100%
Rotihistol

# Report: Fixation and staining

## Result - Observations

Write down your observations.

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