Ray's Fluid Thioglycollate Method*

A Standard Practical Guide for Oyster Sentinel Participants

*as demonstrated by Sammy M. Ray

Preparing Dermo tubes:

1. Add 20 gm NaCl[a] to 1L of deionized (DI) water.
2. Add 29.0 gm of thioglycollate[b] to the water, heat on low temperature and mix to dissolve.
3. Dispense 10 ml of medium into screw cap culture tubes. (Have caps on loosely for autoclaving.)
4. Autoclave for 15 min.
5. Allow the tubes to cool then tighten the caps.
6. Store the tubes in the dark at room temperature until needed.

Suggested Vendors:

a. Sigma 55886-500G, 500 grams of sodium chloride

b. Fluka 70157 – 500G, 500 grams of Thioglycollate Broth

Preparing antibiotics:

Stock Nystatin[c] (=Mycostatin)

1. Add 9ml of DI water to the 5 million unit vial and shake well.

2. Add 2.5 ml of the re-hydrated nystatin into each of 4 vials. Date and label as Nystatin Stock 1, 2, 3 and 4.

3. Freeze until needed. (Good for at least a year.)

Chloromycetin/Nystatin Working Solution:

1. Add 4.5 ml of DI water to a 1 gm vial of Chloromycetin[d] (=Chloramphenicol) and shake well.

2. Add the re-hydrated Chloromycetin to the Nystatin Stock vial.

3. Add 17.5 of DI water to the Chloromycetin/Nystatin mix.

4. Date and label the vial as Chlor/Nystatin Working Solution.

5. Refrigerate.

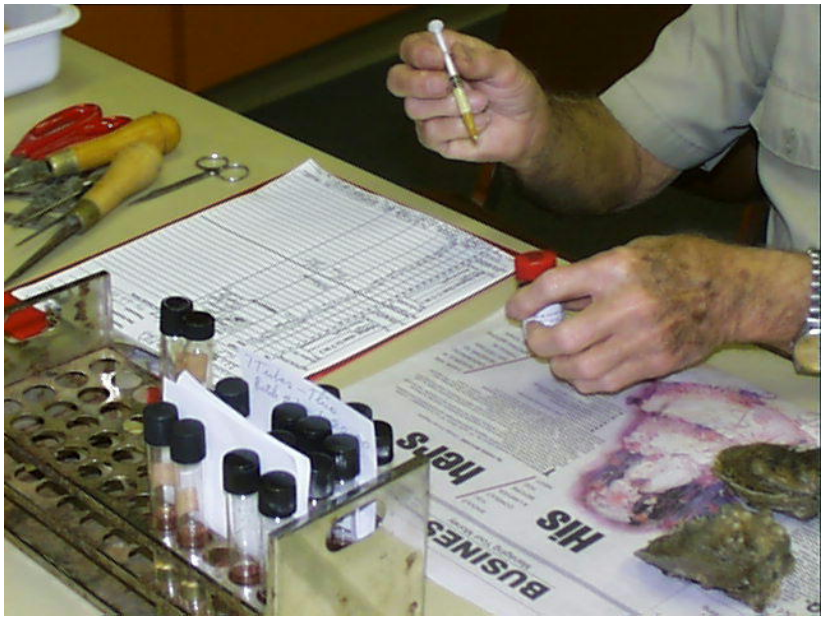
Suggested Vendors:

c. Sigma N6261 - 5MU, 5 Million unit vial of Nystatin

d. Sigma C3738 – 5G, 5 grams of Chloramphenicol Succinate Sodium Salt

Inoculation of tubes:

1. Shake the Chloromycetin/Nystatin Working Solution well.
2. Add 0.05 ml of the Chloromycetin/Nystatin Working Solution to each Dermo tube and mix by inverting the tube.
3. Place oyster ~ 5mm^2 piece of anterior mantle tissue into Dermo tube. (Be sure the tissue is in the fluid!)
4. Store in the dark at room temperature for about a week.



Preparation of Lugol's working solution and staining of samples:

1. Prepare 50 ml of working solution by adding 40 ml of distilled or deionized water to 10ml of 1N Iodine[e] stock solution.
2. With an innoculating needle, carefully remove the oyster tissue from the tube and place it on a glass slide.
3. Add 2-3 drops of the Lugol's working solution to the tissue.
4. Spread and macerate the tissue with a blunt probe to get a thin, well-stained preparation.
5. Cover the tissue with a cover slip. Push on the cover slip to get a flattened preparation.
6. Remove excess Lugol's with absorbent paper.

Suggested Vendors:

e. Fisher S178 – 500, 500 ml of 1N Iodine Solution

Reading of samples:

1. Determine level of parasitism using the Mackin (1962) 0-5 scale, as modified by Craig et al. (1989). Scan the slide under low magnification (e.g., 40x) then switch to 100x magnification if necessary.

2. Use the following photomicrographs as standards as needed. Note that the field of view shown in the following slides is intended to represent the entire sample.

References:

Craig, A., E. N. Powell, R. R. Fay & J. M. Brooks. 1989. Distribution of *Perkinsus marinus* in gulf coast oyster populations. *Estuaries*. 12:82-91.

Mackin, J. G. 1961. Oyster disease caused by *Dermocystidium marinum* and other microorganisms in Louisiana. *Publ. Inst. Mar. Sci.* 7:132-299.

Dermo Code:

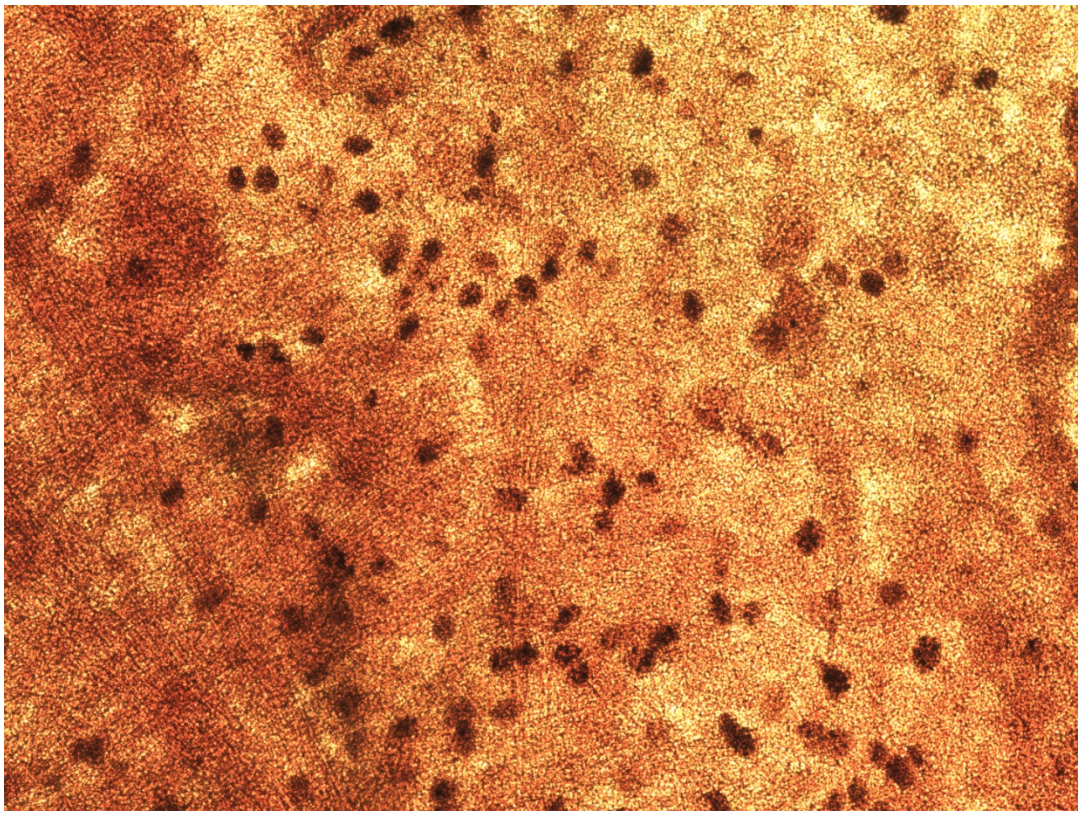
0.00

Description:

No hypnospores present

Comment:

Note the numerous Brown Cells , which are NOT Dermo



Dermo Code:

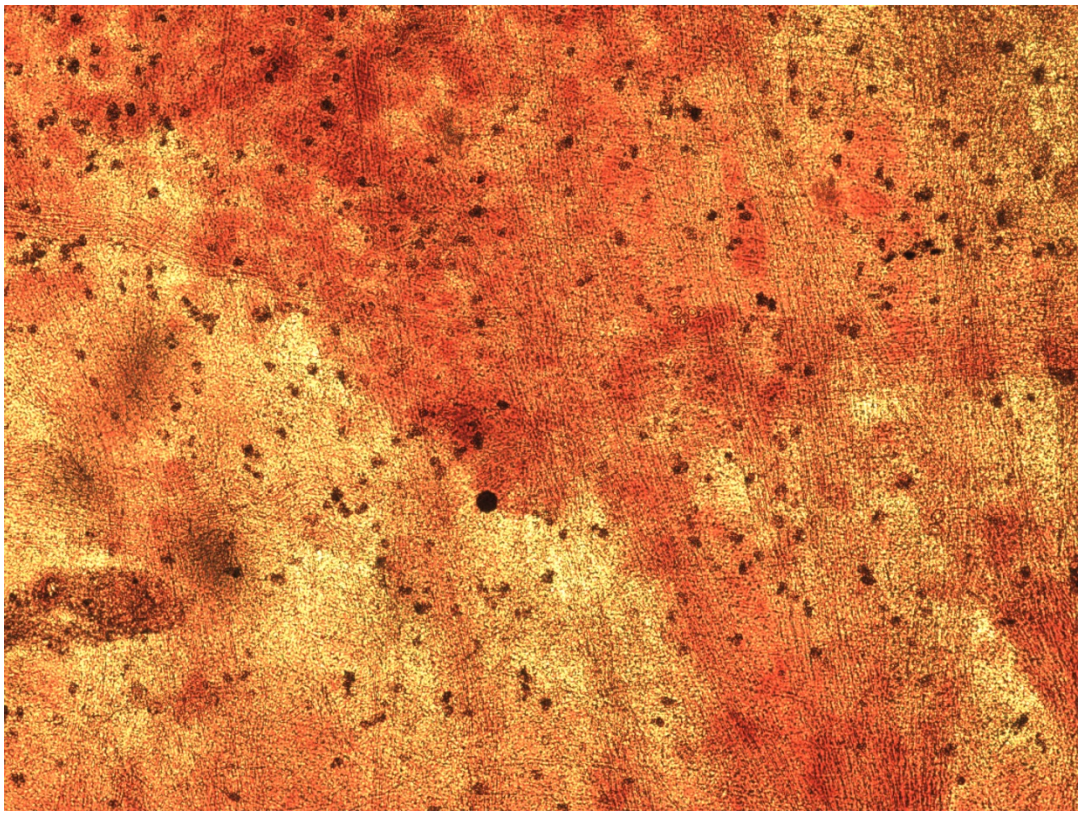
0.33

Description:

1-10 hypnospores

Comment:

Note the single Dermo cell among the many Brown Cells



Dermo Code:

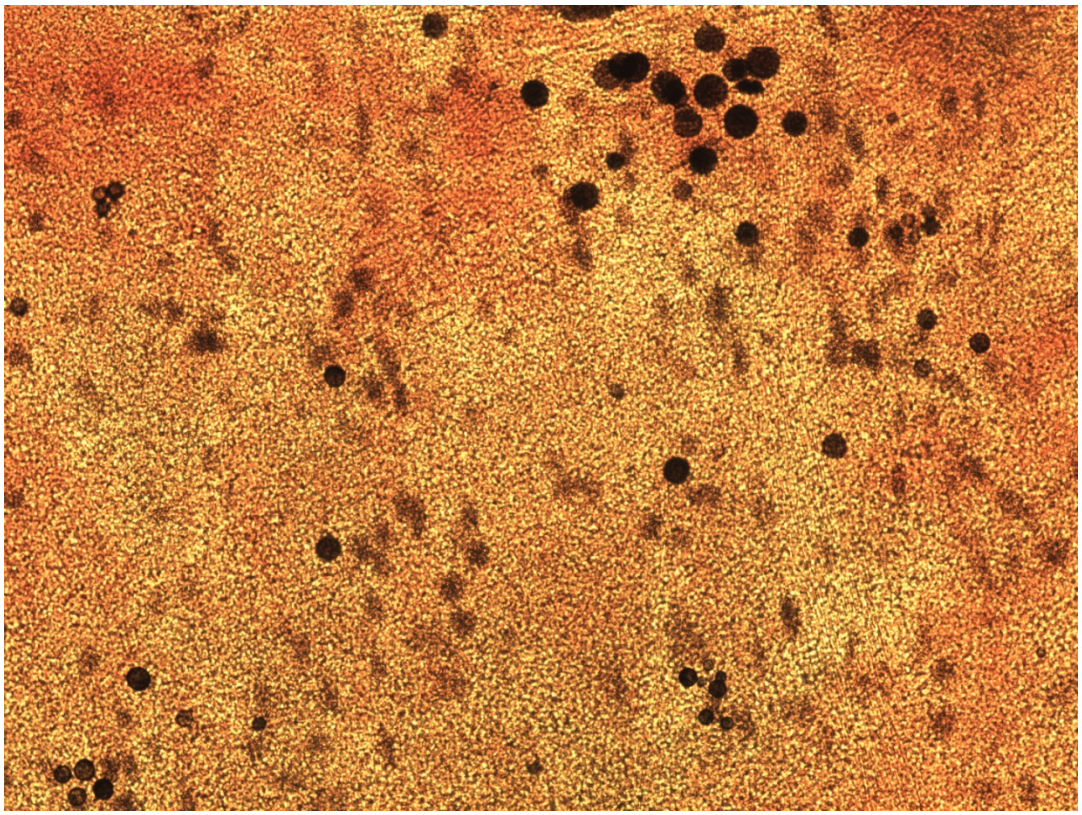
0.67

Description:

11-74 hypnospores

Comment:

Note the dark-stained spherical Dermo cells among the Brown Cells



Dermo Code:

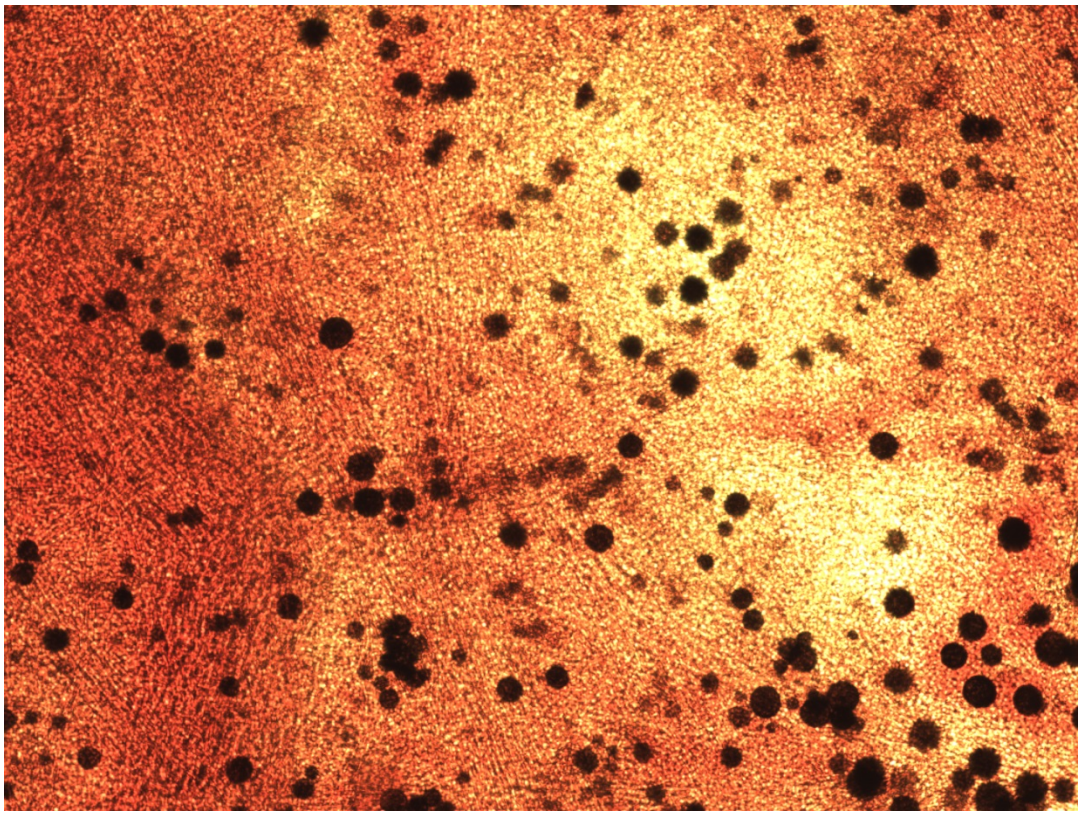
1.00

Description:

75-125 hypnospores

Comment:

Note the many Dermo cells among the many Brown Cells

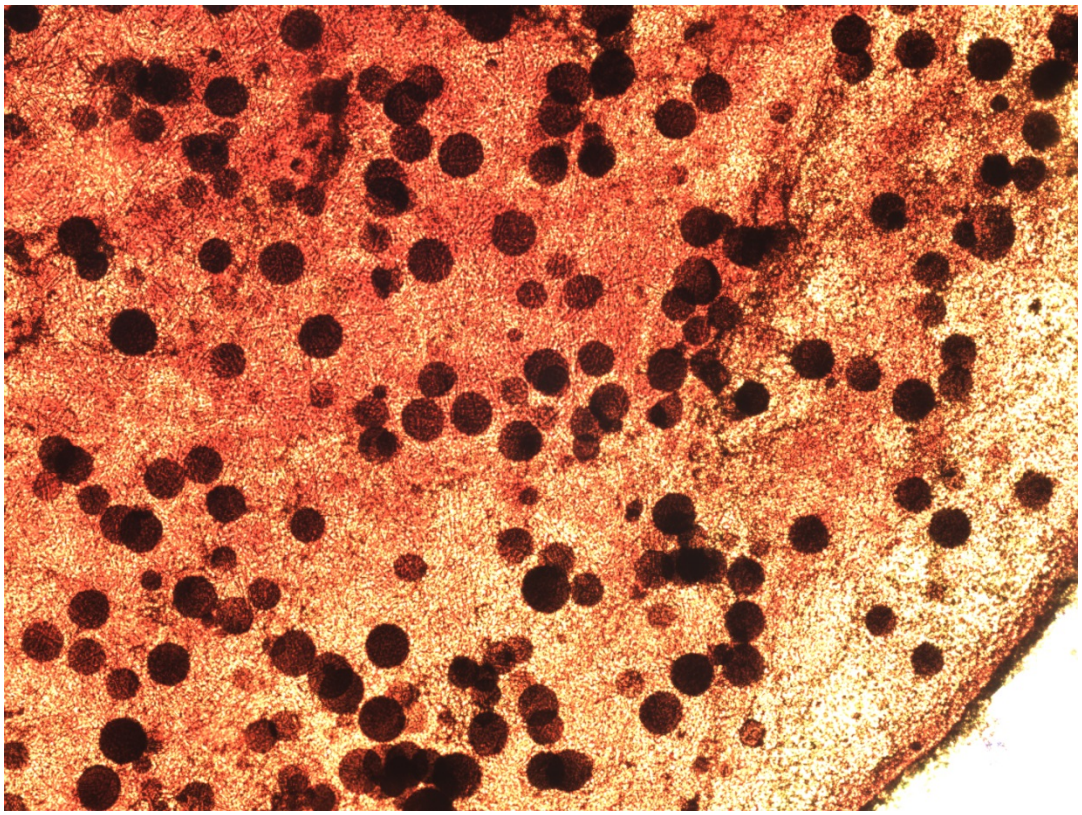


Dermo Code:

1.33

Description:

>125 hypnospores but much less than 25% of tissue is hypnospores. Do not count beyond 125 hypnospores



Comment:

Note the slight variation in hypnospore size and staining intensity

Dermo Code:

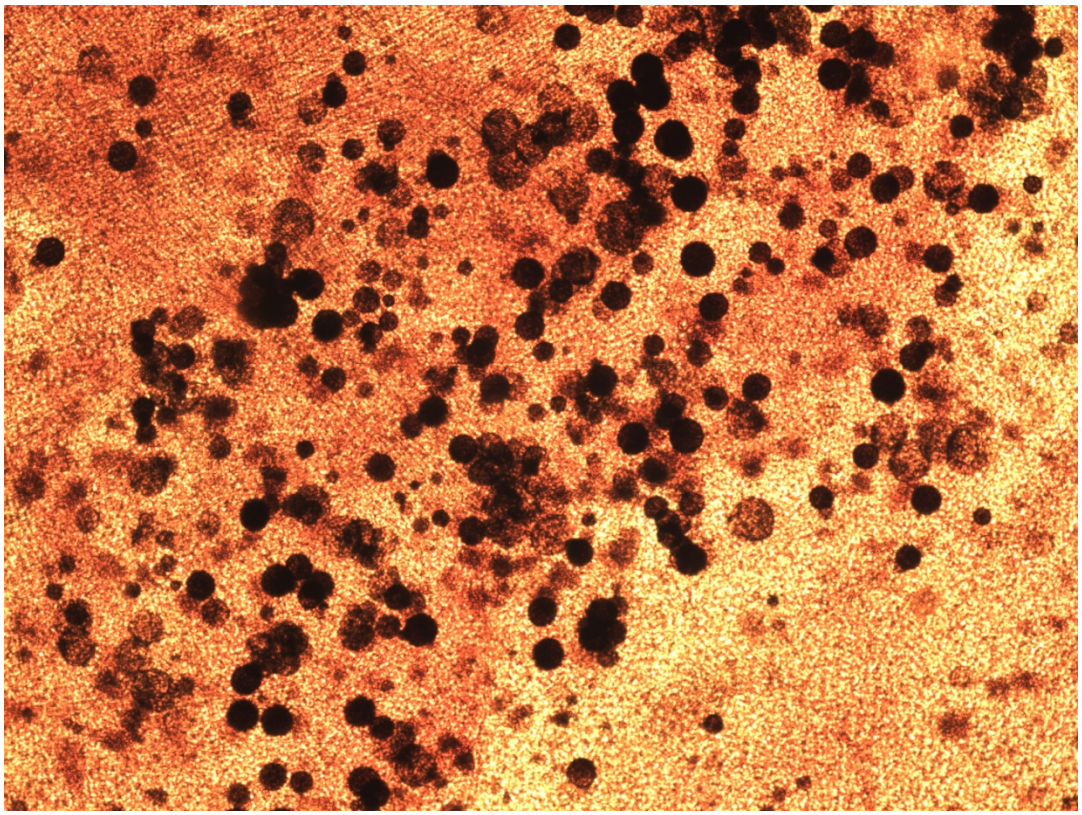
1.67

Description:

<25% of tissue is hypnospores

Comment:

Starting with code 1.67, dermo codes are based on percent coverage not numbers

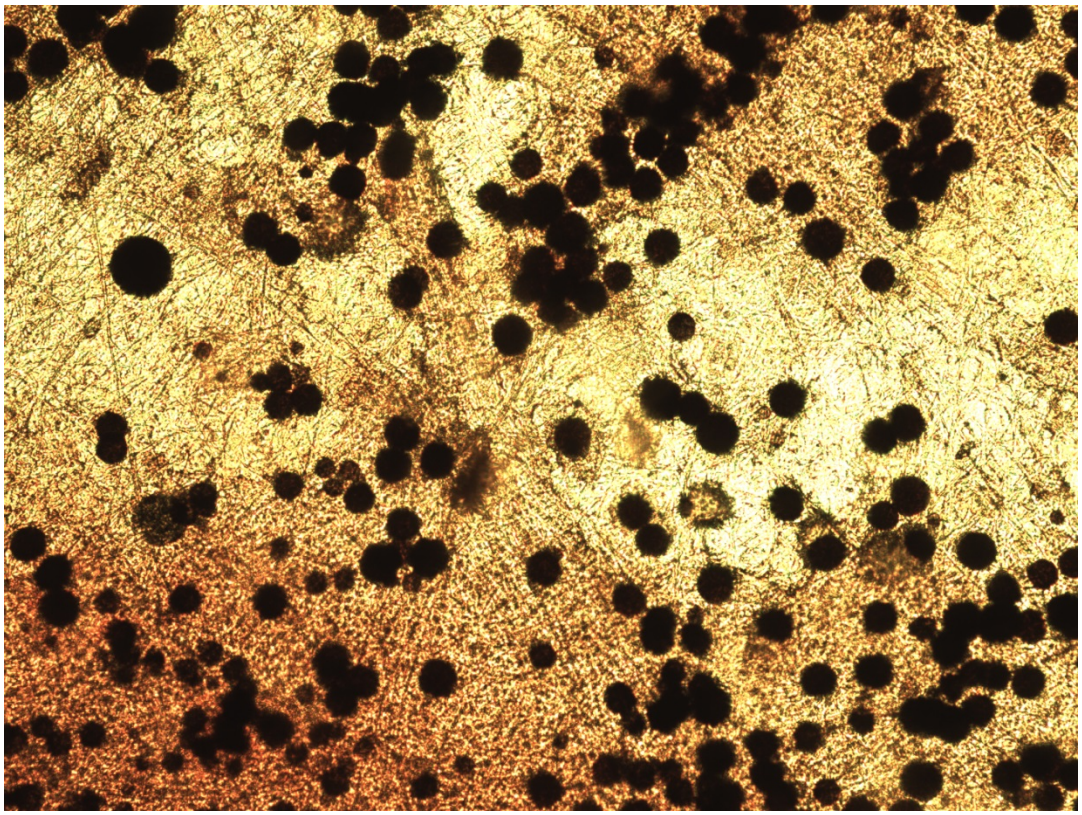


Dermo Code:

2.00

Description:

25% of tissue is hypnospores



Comment:

It is difficult to judge an exact percent coverage such as this.

It helps to judge the sample in relationship to the codes below and above.

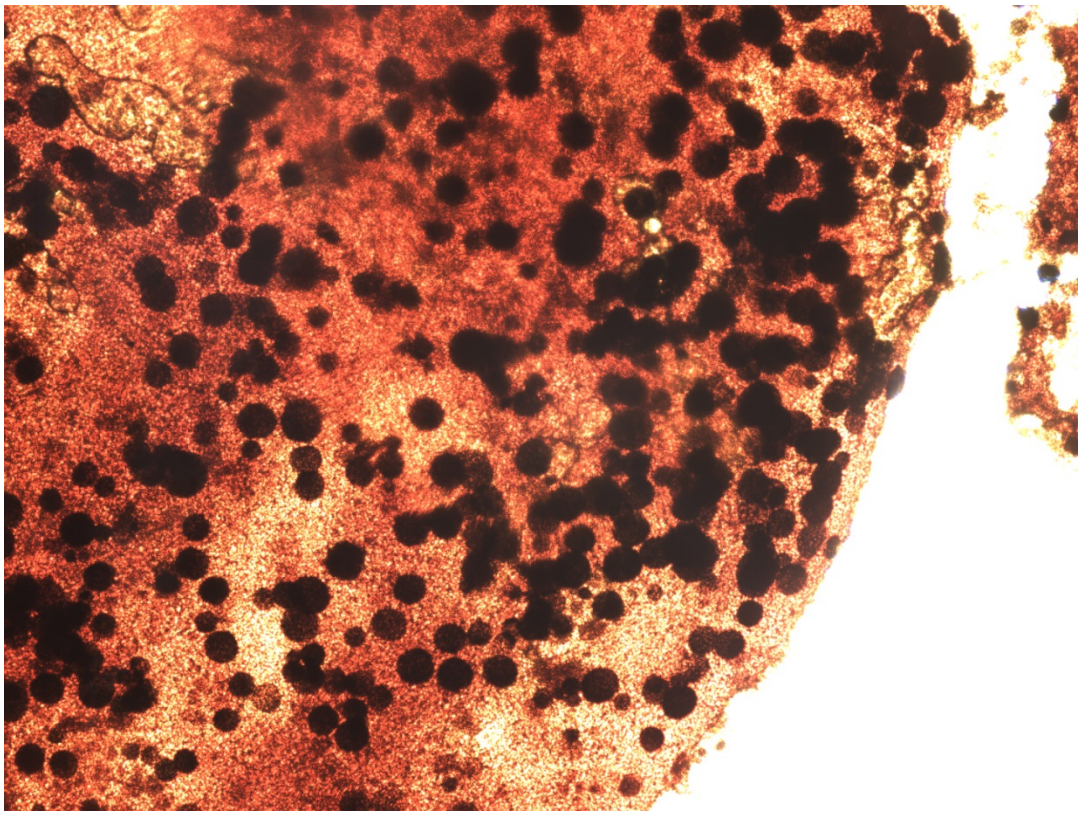
Note also that dermo is patchy and that the percent coverage must be judged for the entire sample.

Dermo Code:

2.33

Description:

>25% but much less that 50% of tissue is hypnospores



Comment:

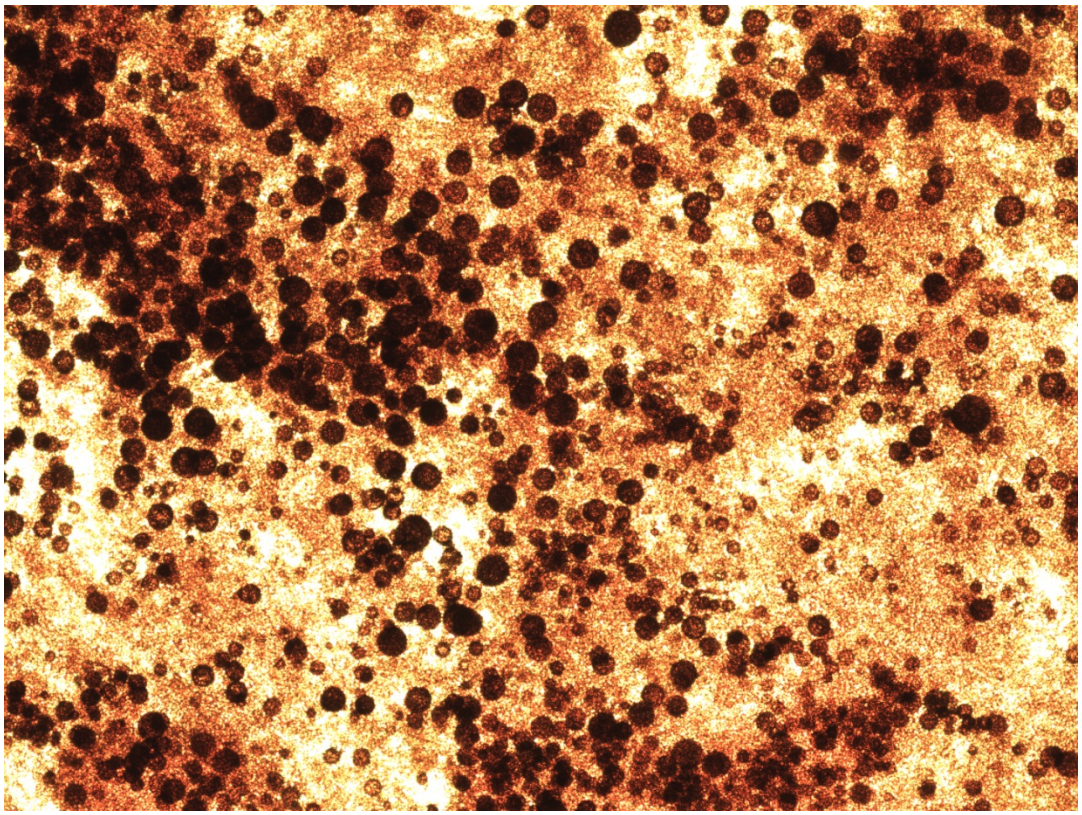
Note the patchy distribution of the hypnospores

Dermo Code:

2.67

Description:

>25% but <50% of tissue is hypnospores



Comment:

Some areas are >50% covered, whereas other areas are <25% covered.

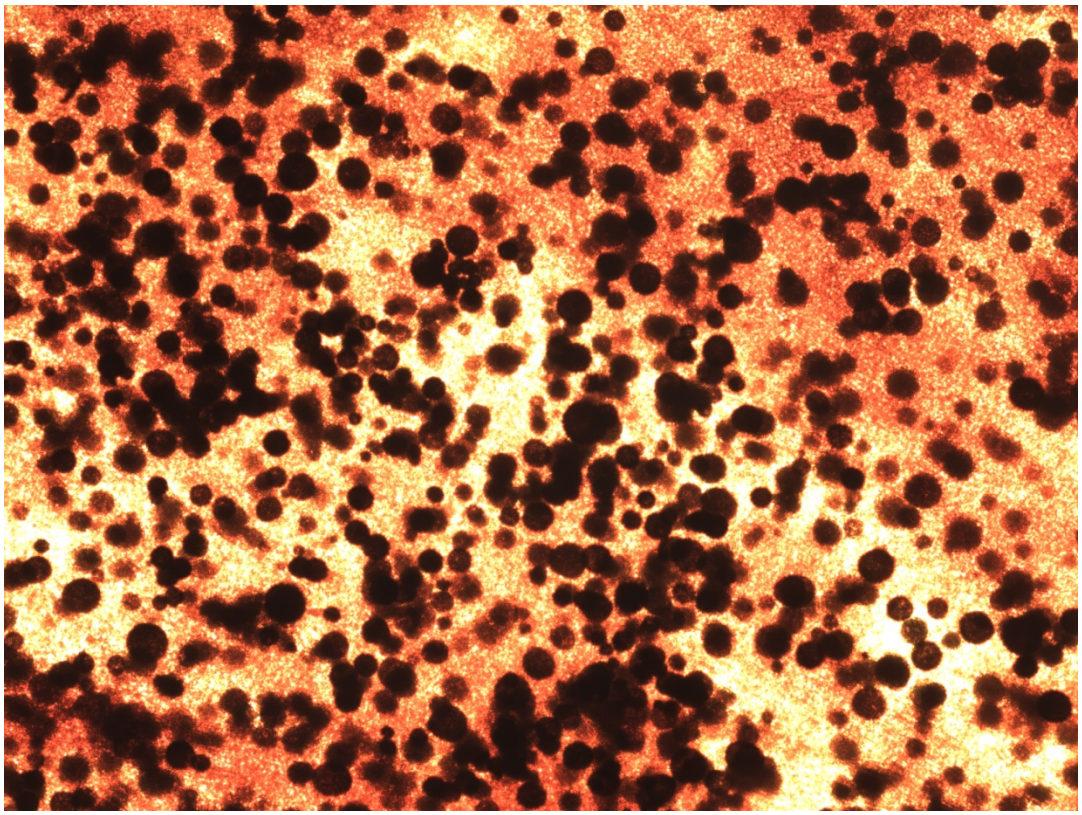
A judgment is made on the average percent coverage of the whole sample.

Dermo Code:

3.00

Description:

50% of tissue is hypnospores



Comment:

It is difficult to judge an exact percent coverage such as this.

It helps to judge the sample in relationship to the codes below and above.

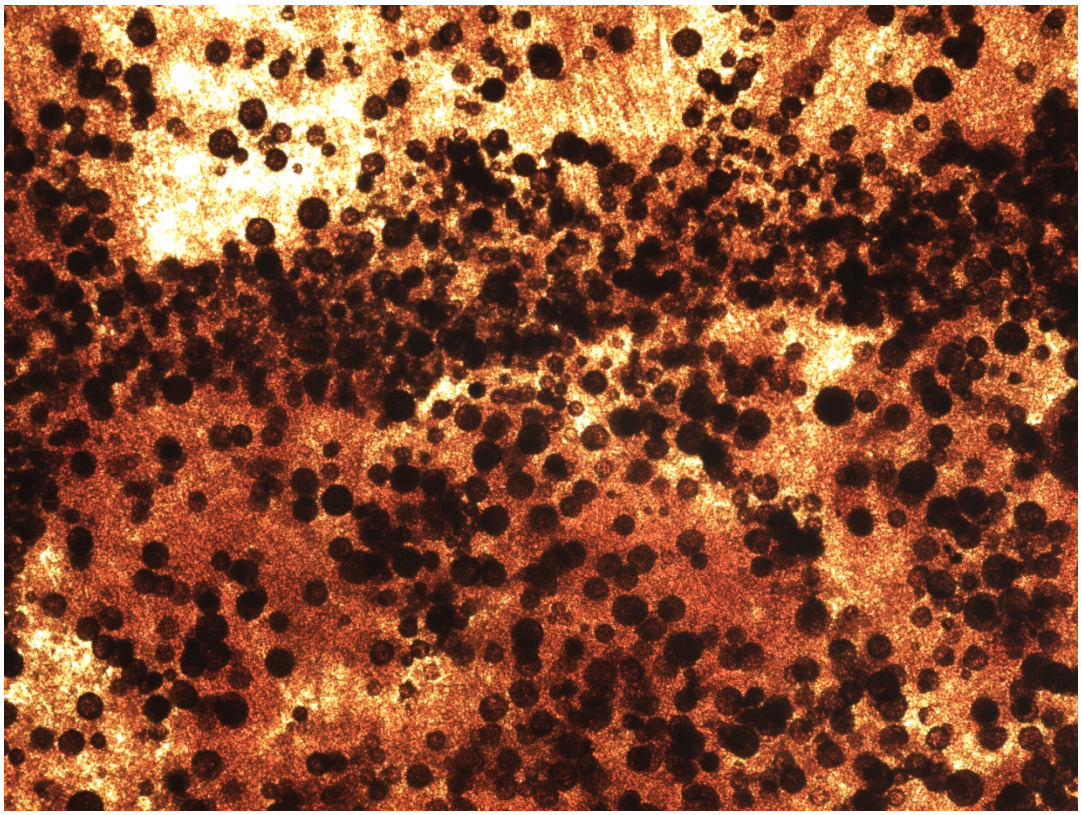
Note also the variation in hypnospore size which affects percent coverage.

Dermo Code:

3.33

Description:

>50% but *much less* than 75% of tissue is hypnospores



Comment:

Note the patchiness of hypnospore distribution

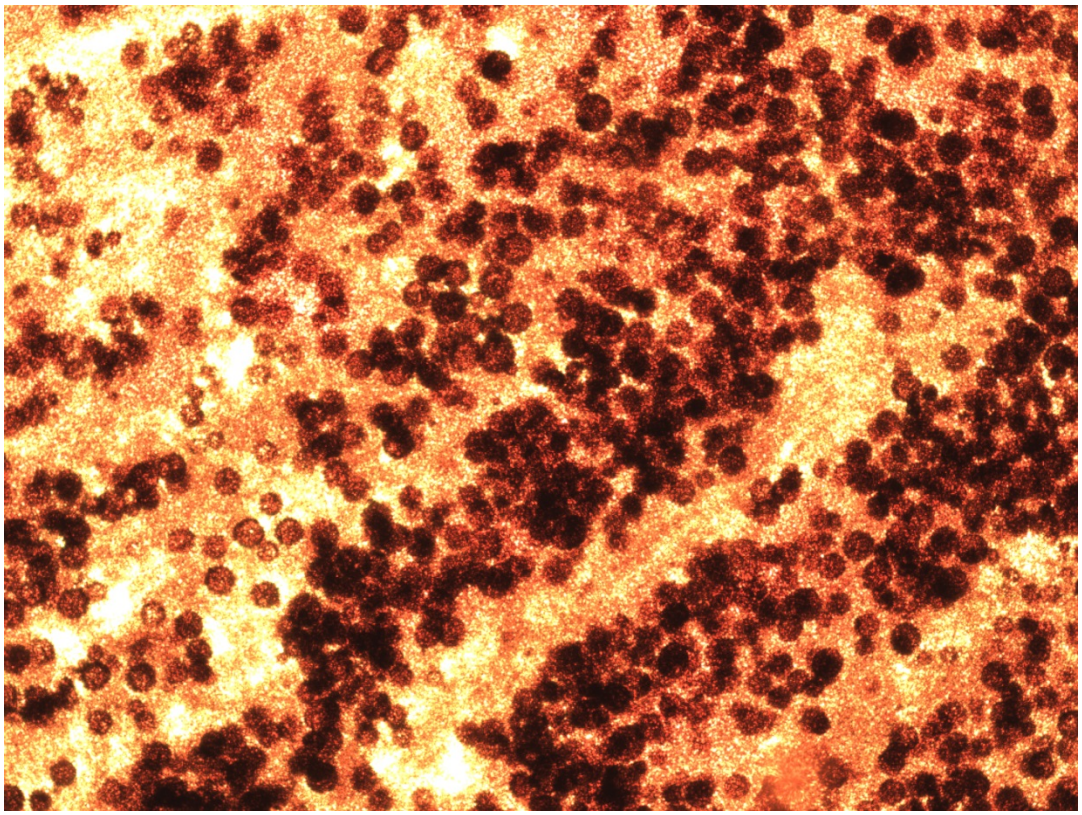
Determination of disease code must be made for the entire sample which in this case includes dense and sparse patches

Dermo Code:

3.67

Description:

>50% but <75% of tissue is hypnospores



Comment:

Note the patchiness of hypnospore distribution

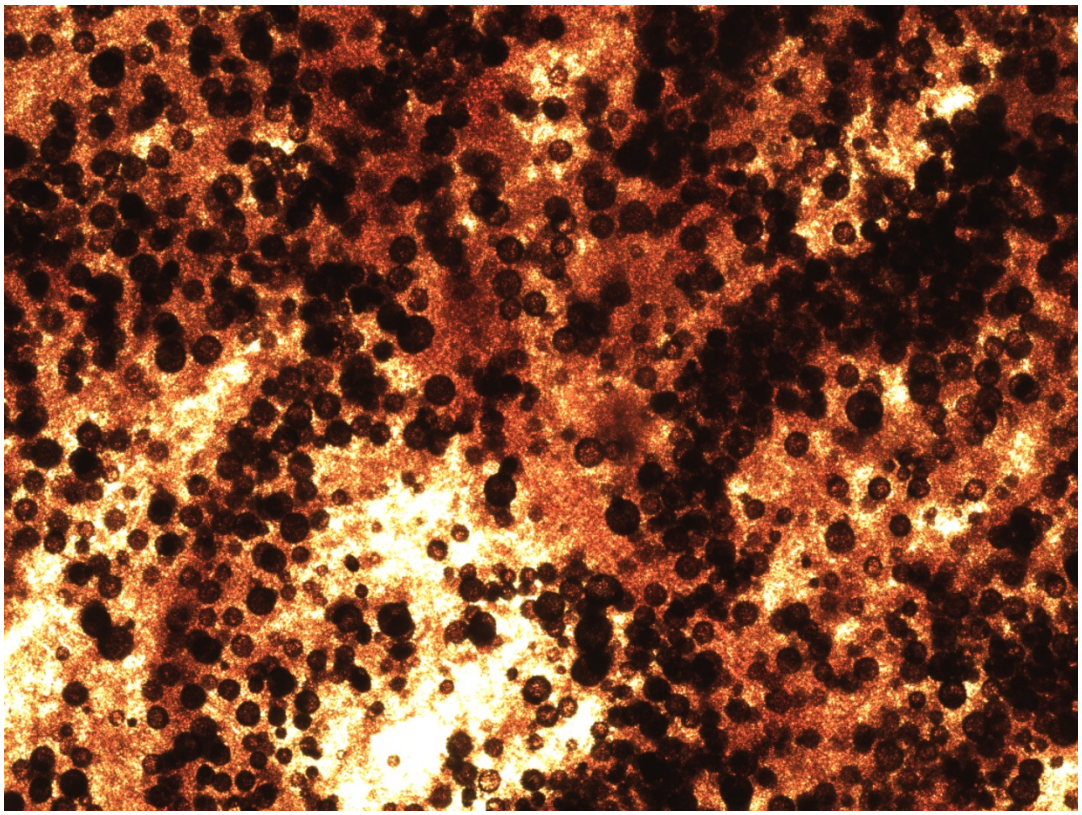
Determination of disease code must be made for the entire sample, which in this example includes dense and sparse patches

Dermo Code:

4.00

Description:

75% of tissue is hypnospores



Comment:

Note the patchiness of hypnospore distribution.

It is difficult to judge an exact percent coverage such as this.

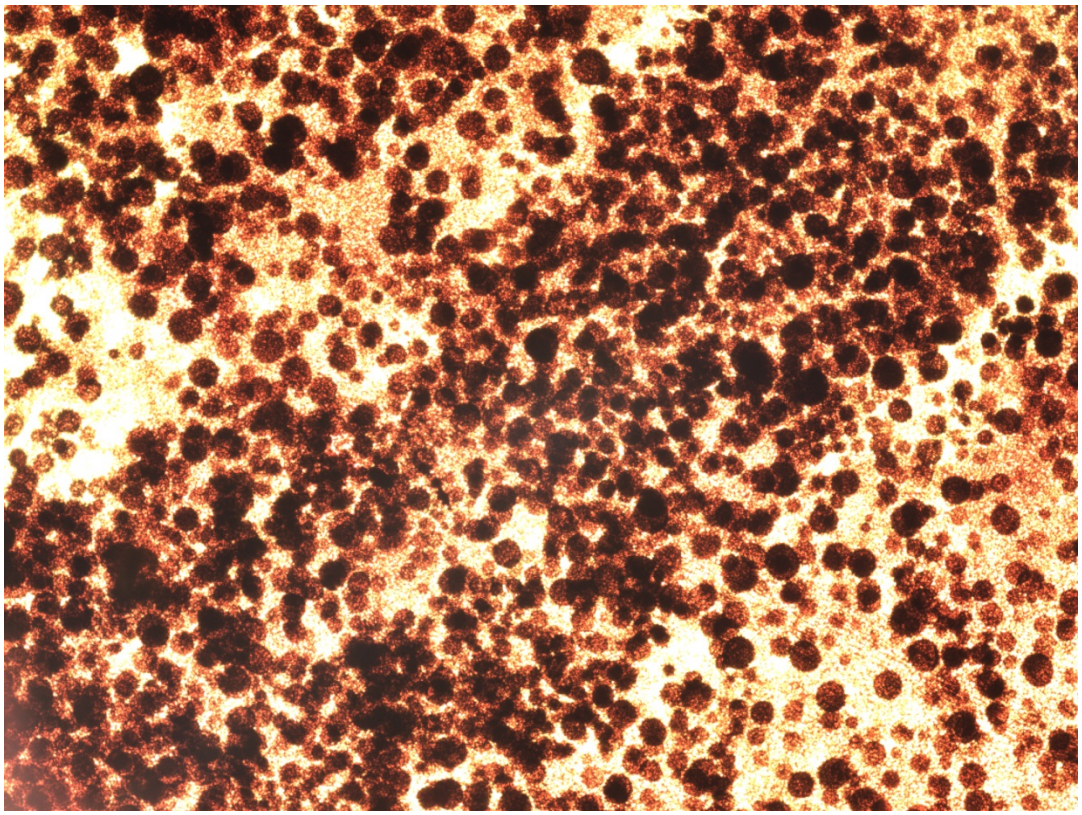
It helps to judge the sample in relationship to the codes below and above.

Dermo Code:

4.33

Description:

>75% but *much less* than 100% of tissue is hypnospores



Comment:

This sample has a fairly even distribution of hypnospores, which however are of varying size and staining intensity

Dermo Code:

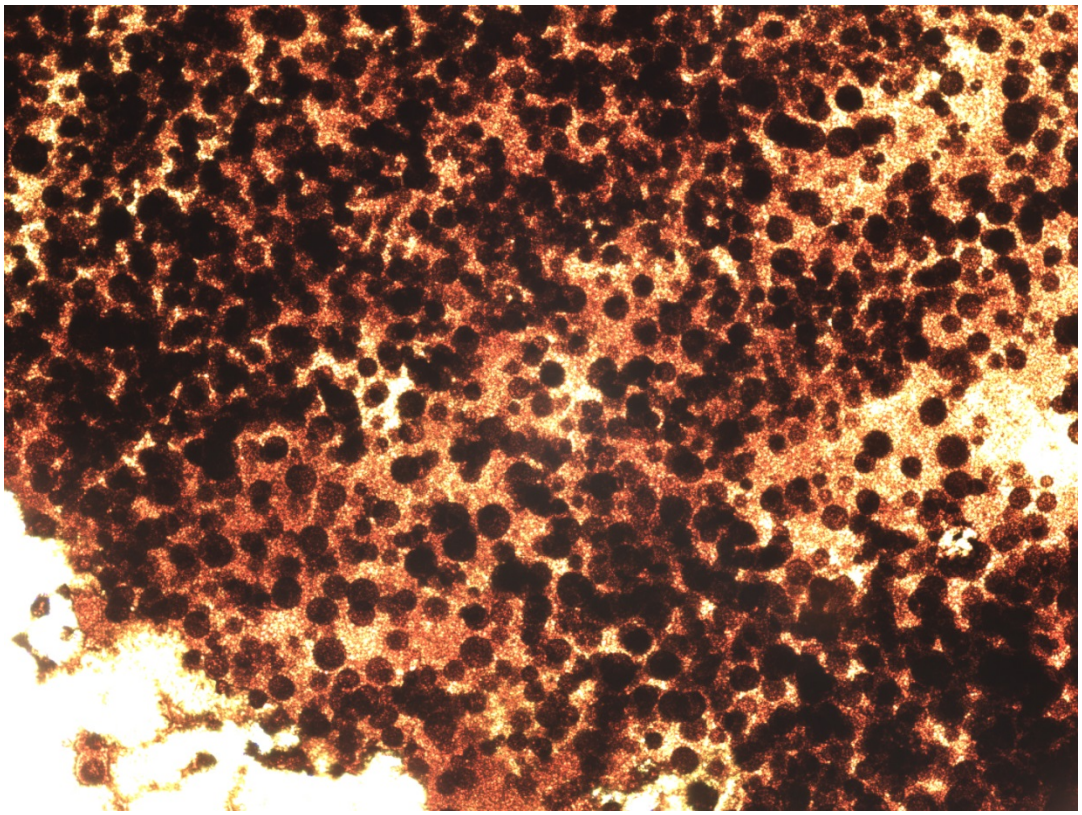
4.67

Description:

>75% tissue is hypnospores but some oyster tissue is still visible

Comment:

Note the heavy coverage by dermo but with oyster tissue still visible

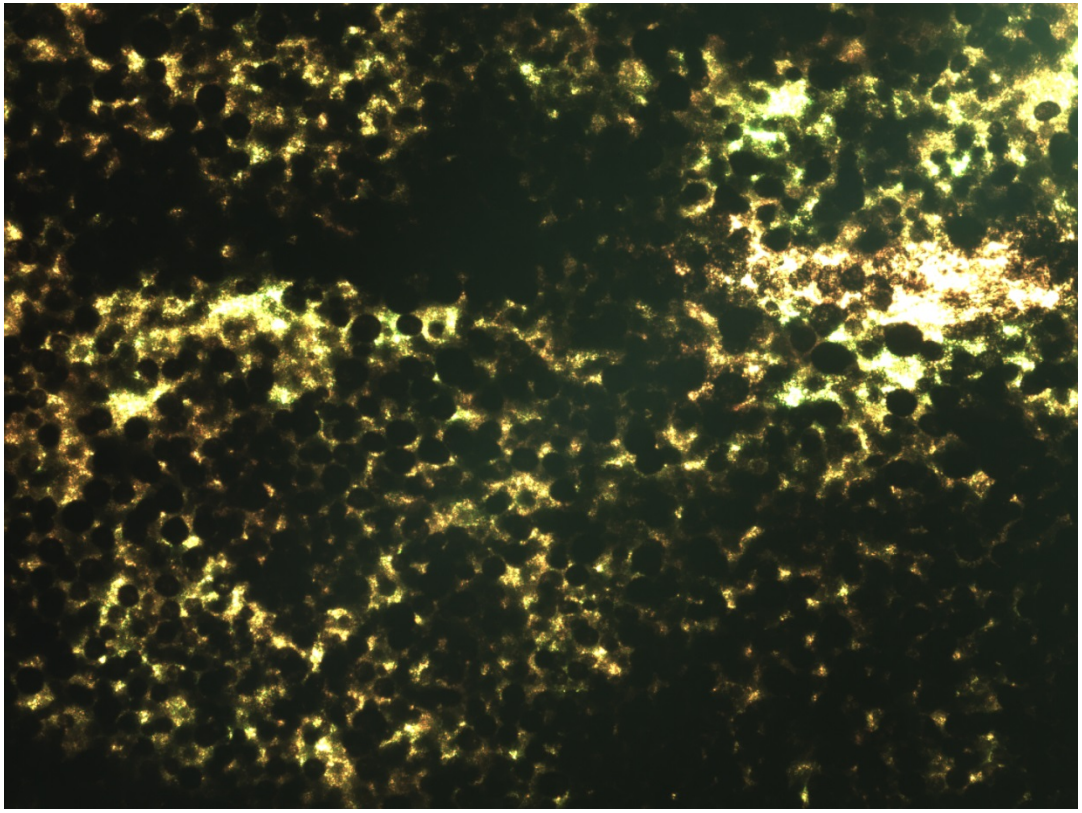


Dermo Code:

5.00

Description:

Nearly 100% of tissue is hypnospores



Comment:

Note that the oyster tissue is nearly totally obscured by hypnospores

Data entry:

Go to

www.oystersentinel.cs.uno.edu

Enter data

Check data entry

Submit data

The web site will display:

Percent Infection as

(no. infected/no. assayed) x 100

Infection Intensity as

sum of disease codes/no. infected

Weighted Prevalence as

sum of disease codes/no. assayed

Check the web site to see if data were properly posted