Der Biofilm verschwindet nach Fixierung mit Formalin (Dapi)

markiert die Nukleinsäuren blau

Carnoy Formalin

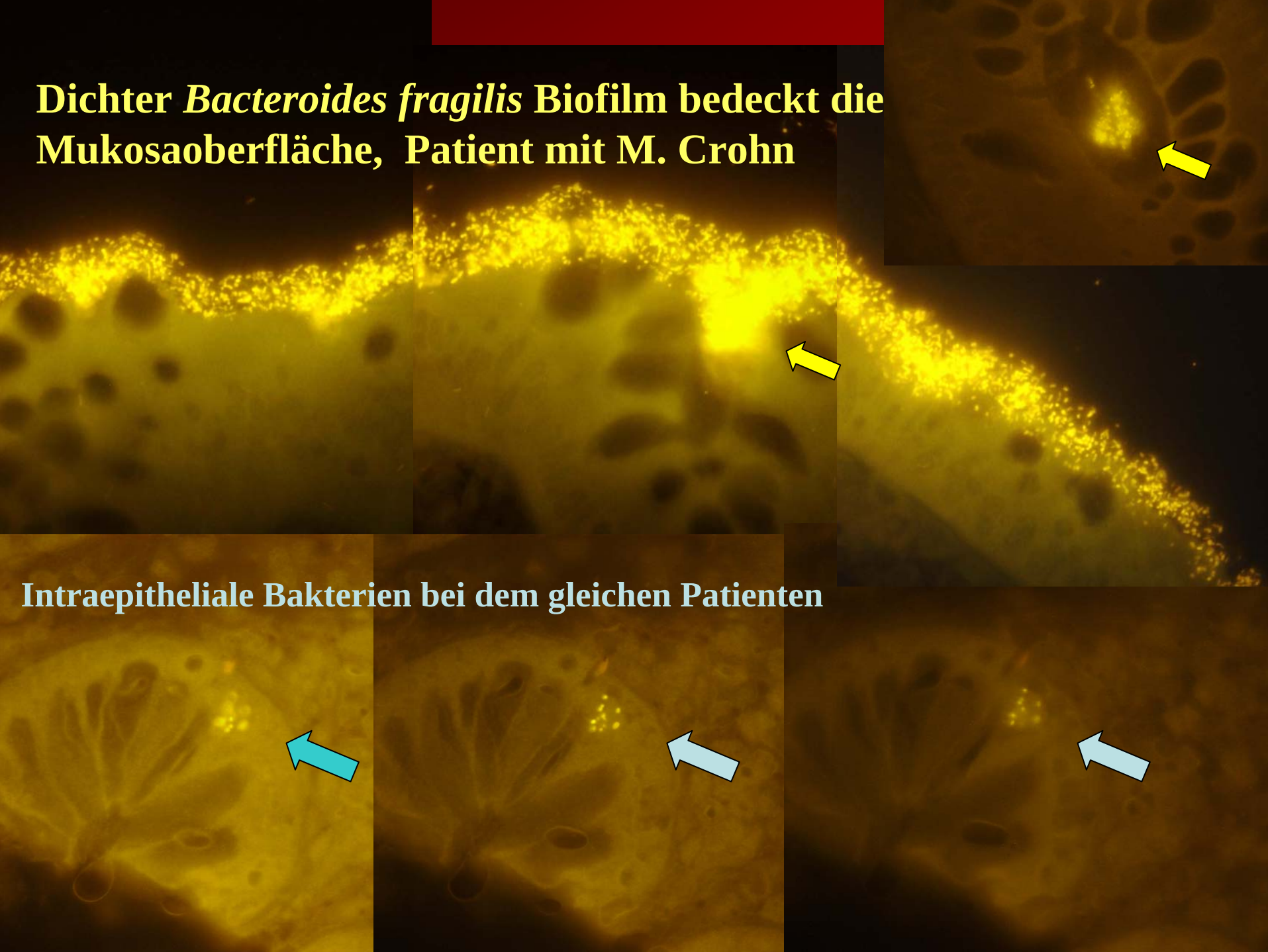
Der gleiche Patient und die gleiche Lokalisation, M. Crohn

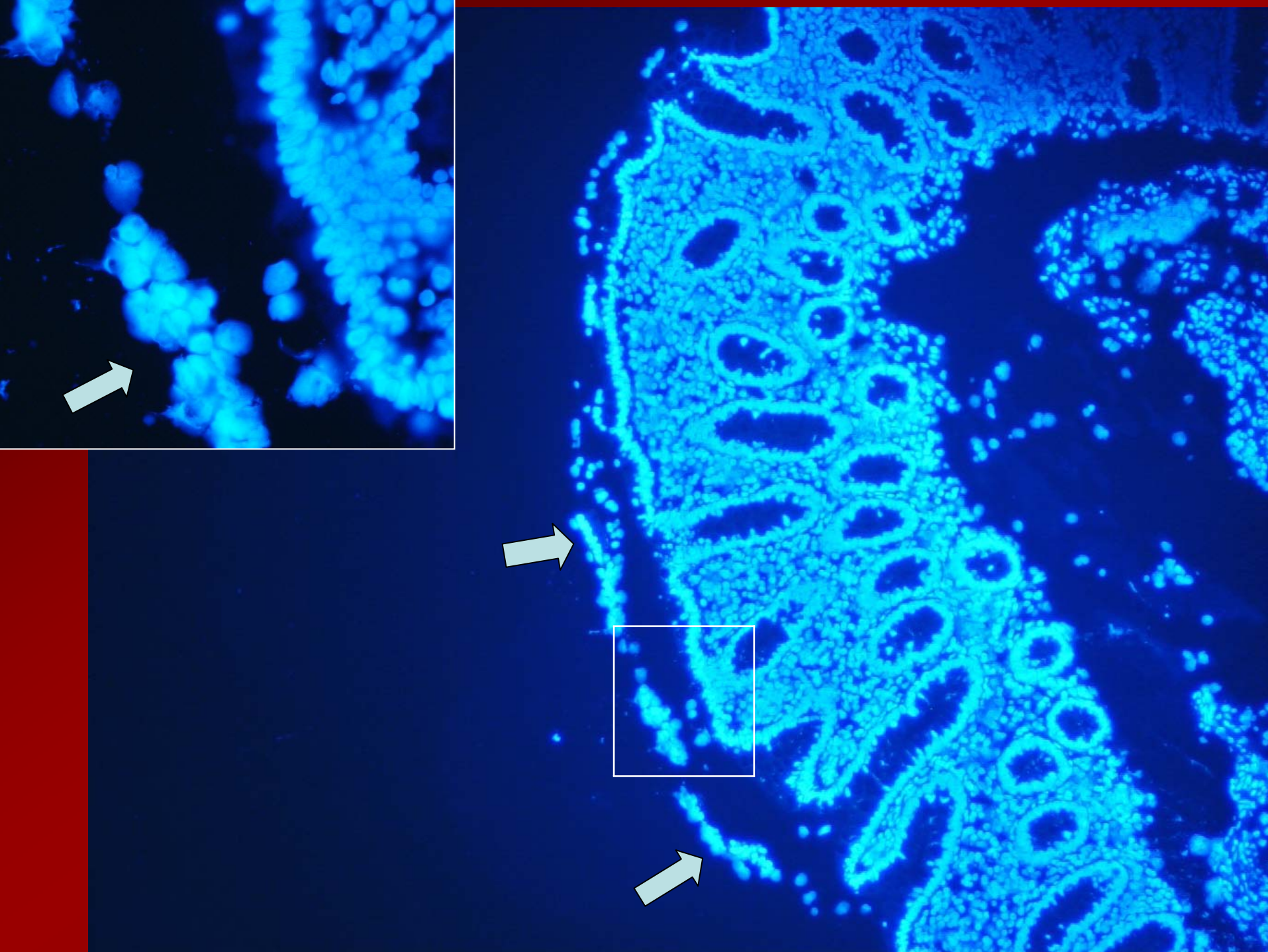
FISH Analyse des mukosalen Biofilms

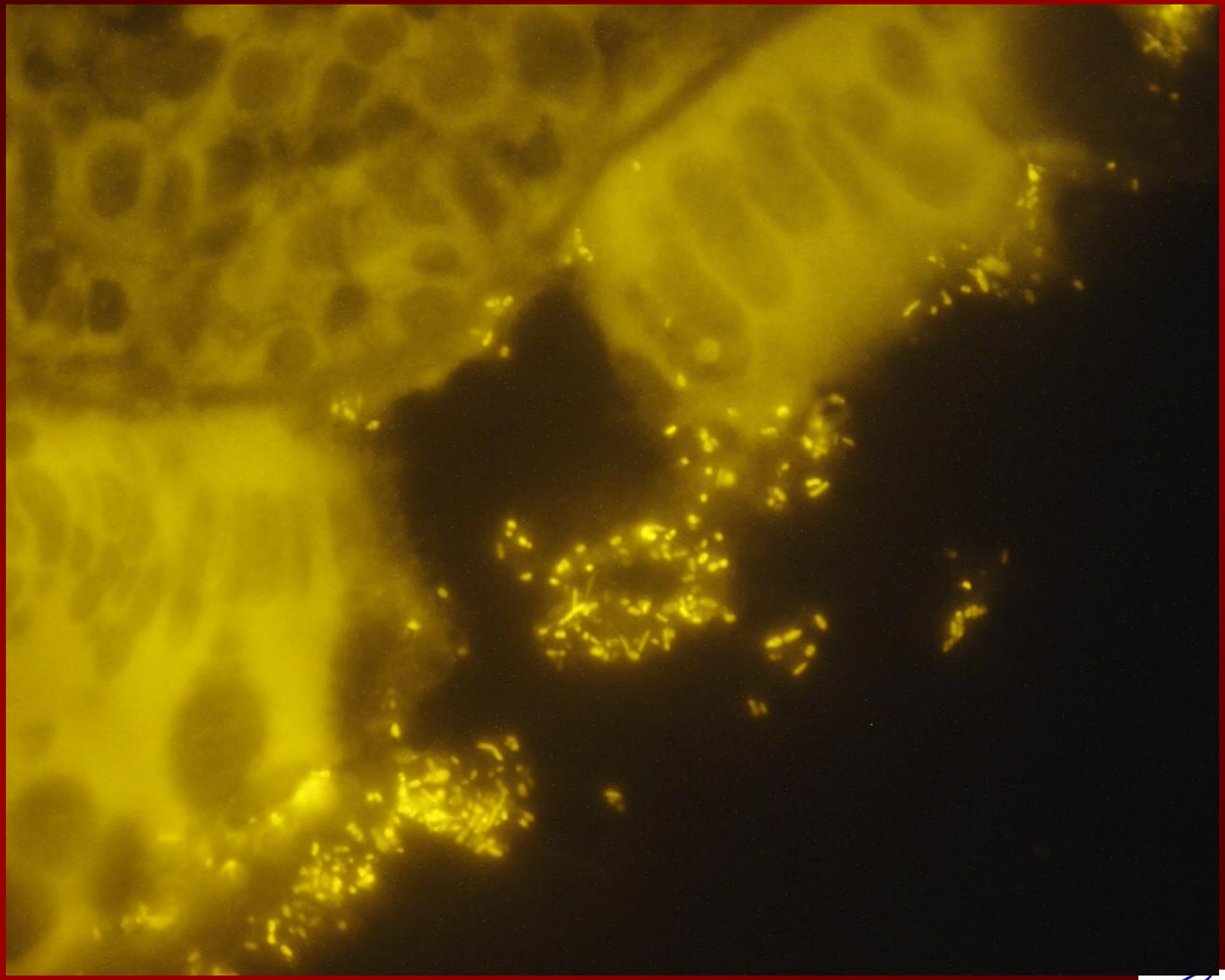
Eub338
Alf1b
Beta42a
Gam42a
Ebac
Ec1531
Y16s-69
Srb385
Sgd
Hpy-1
Arc1430
HGC
LGC
Sfb
Erec
Lach
Ehal
Chis150
Clit135
Lab158
Strc493
Enc131
Efaec
Ato291
Cor653
Ecyl
Phasco
Veil
Rbro, Rfla
UroA, UroB
Ser1410
Bif164
CF319a
Bac303
Bfra602
Bdis656
Fprau
Dss658
Arch915

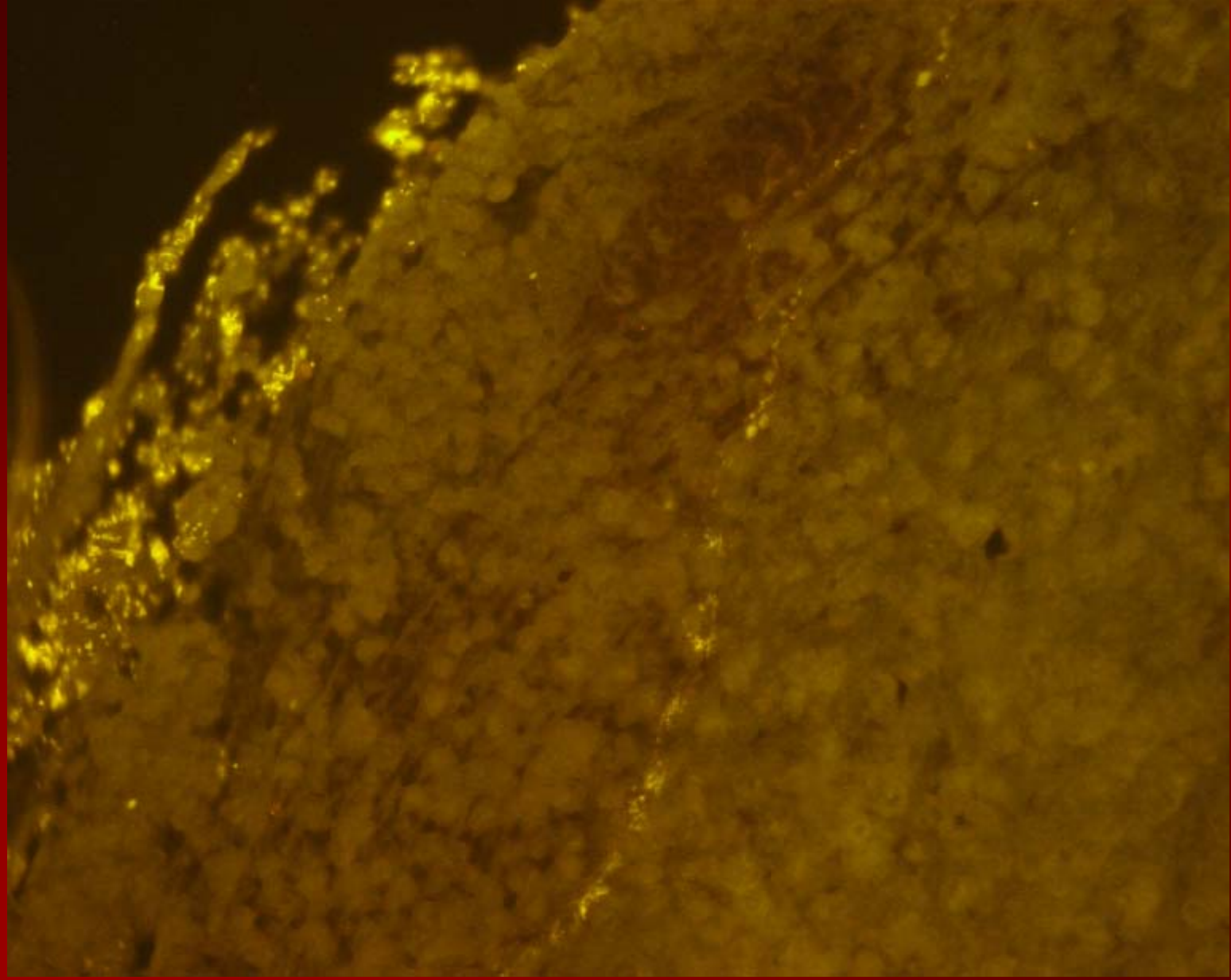
mit r-RNA komplimentären Sonden


gesunde
Dickdarm-Wand

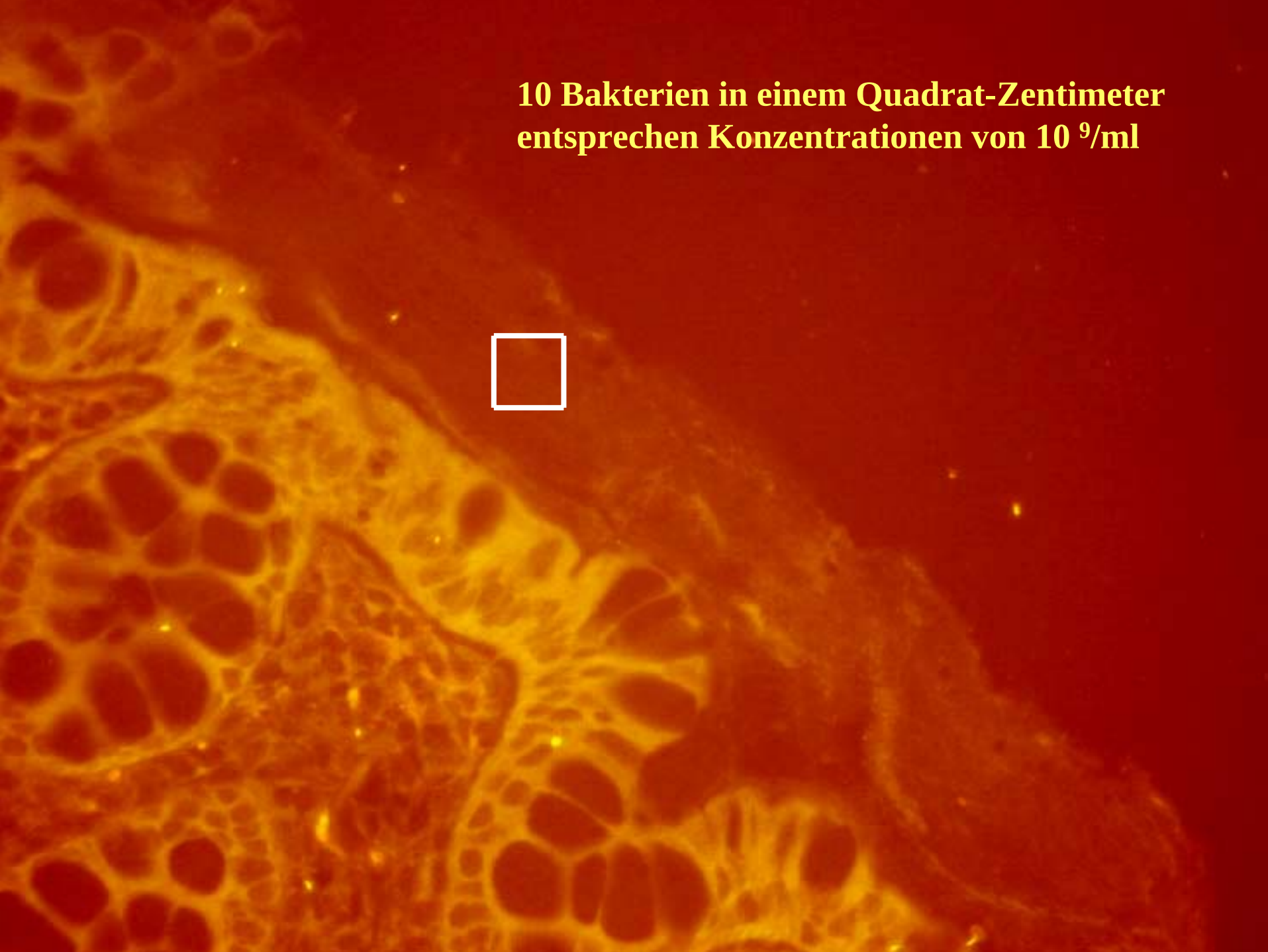

Dichter *Bacteroides fragilis* Biofilm bedeckt die Mukosaoberfläche, Patient mit M. Crohn
Intraepitheliale Bakterien bei dem gleichen Patienten

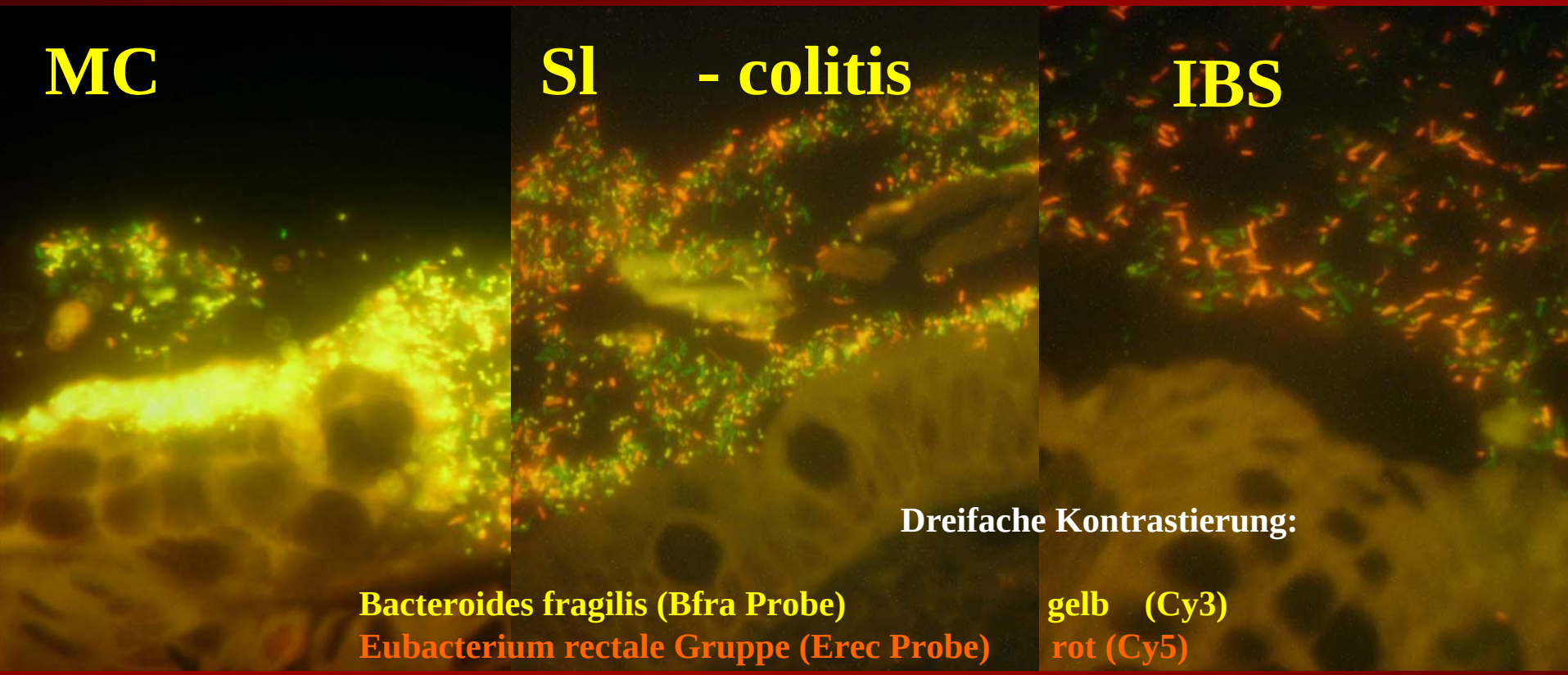






MC Eine Straße von Bacteroides-Infiltration

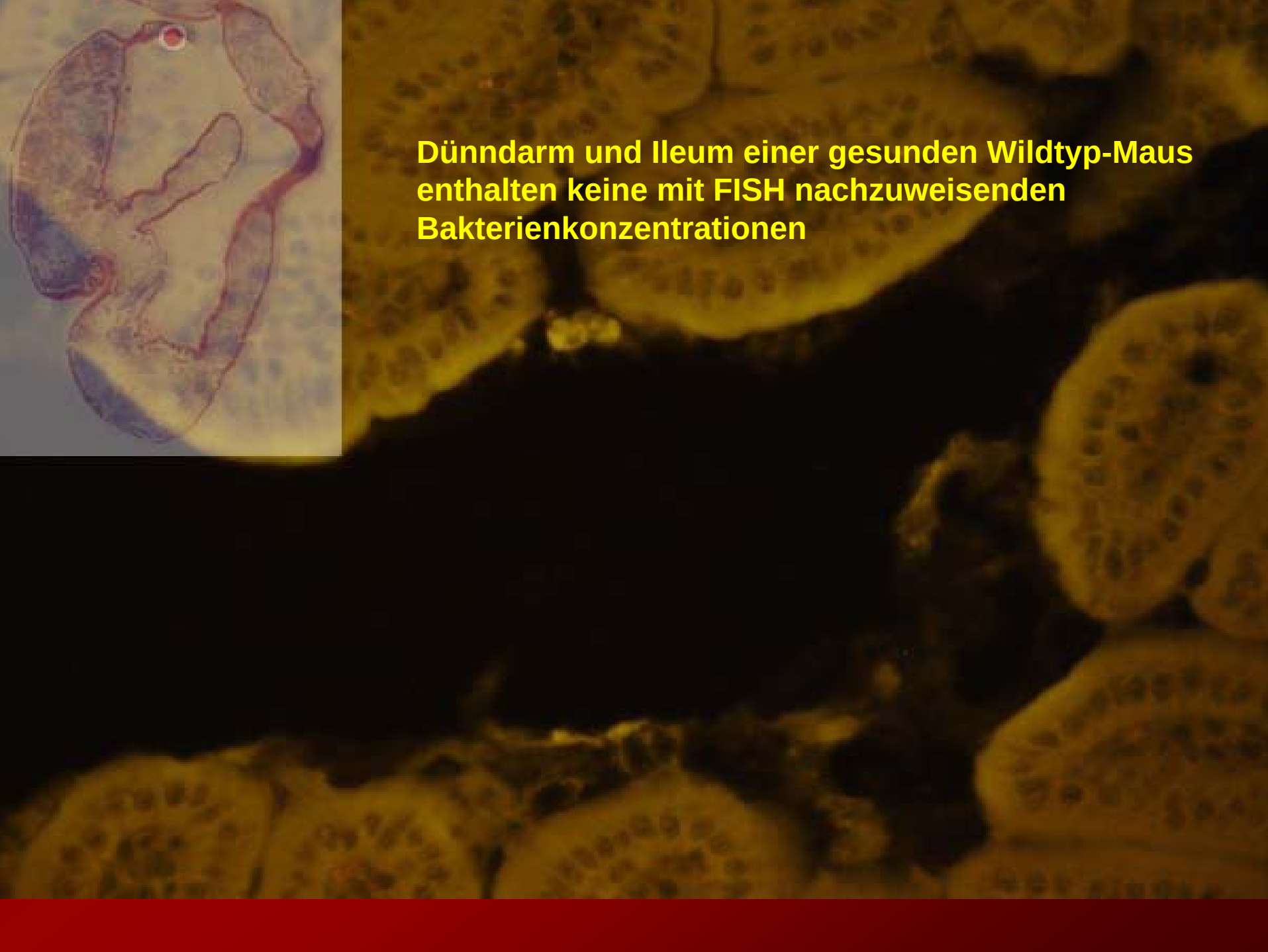

10 Bakterien in einem Quadrat-Zentimeter
entsprechen Konzentrationen von 10 9/ml



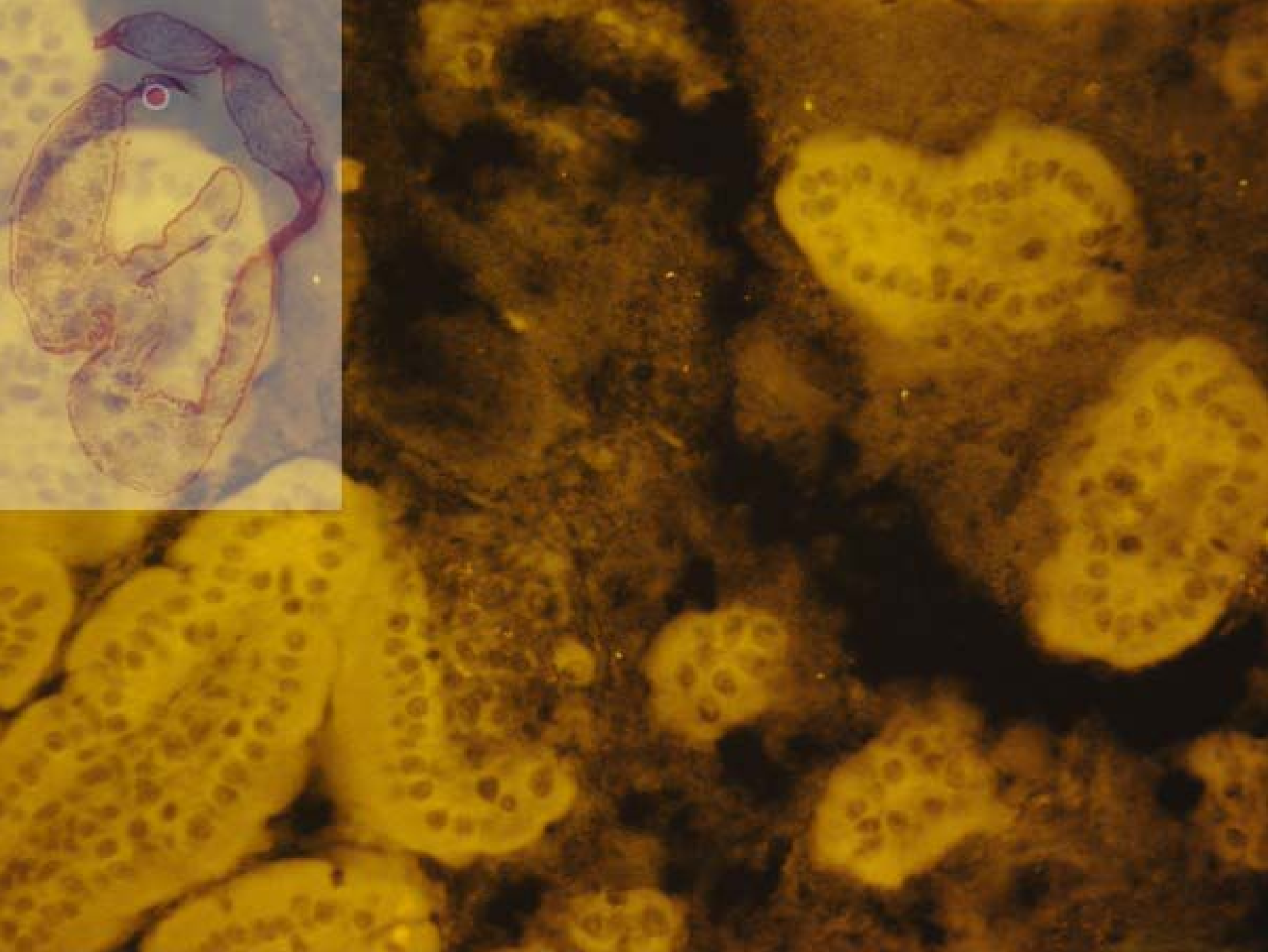
Dreifache Kontrastierung:

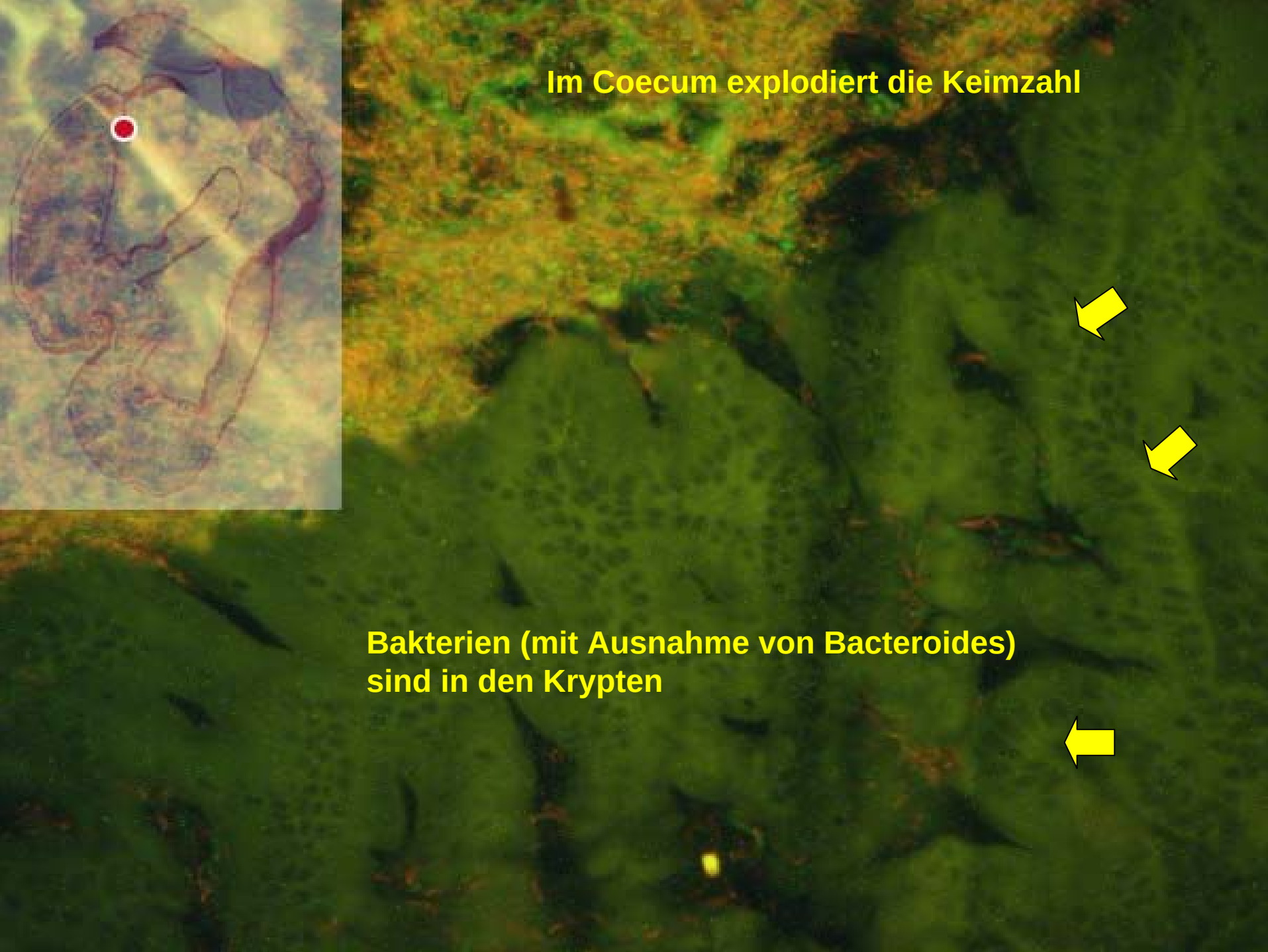
Bacteroides fragilis (Bfra Probe)	**gelb (Cy3)**
Eubacterium rectale Gruppe (Erec Probe)	**rot (Cy5)**
Alle anderen Bakterien (Eub338)	**grün (FITC)**

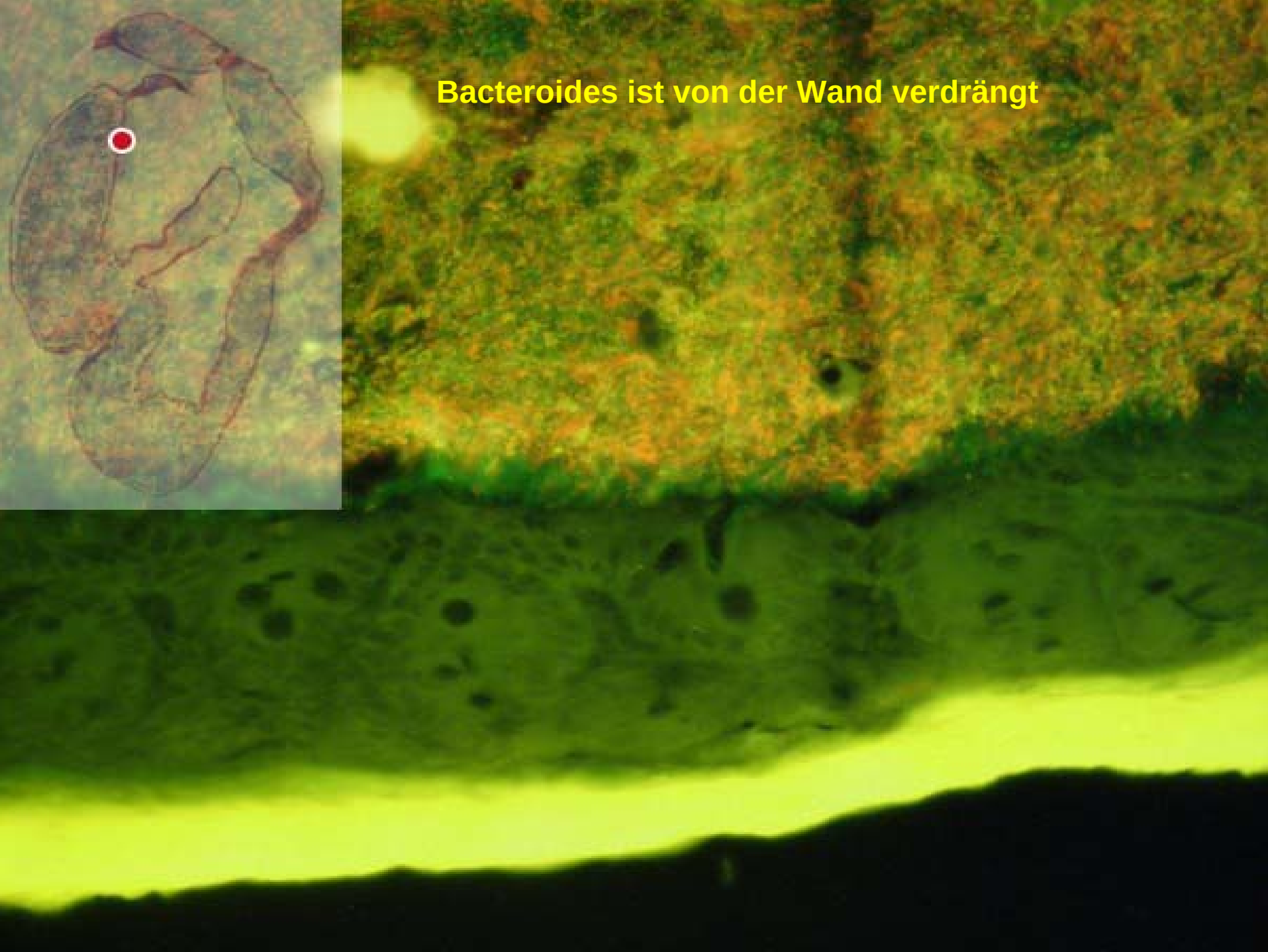
Prozent von Patienten mit 10^9 B/ml		MC 98%	UC 94%	Slc 78%	IBS 62%	Kontr. 16%
Anteil am Biofilm	Bfra	60%	30%	31%	14%	16%
	Erec	10%	5%	18%	48%	32%

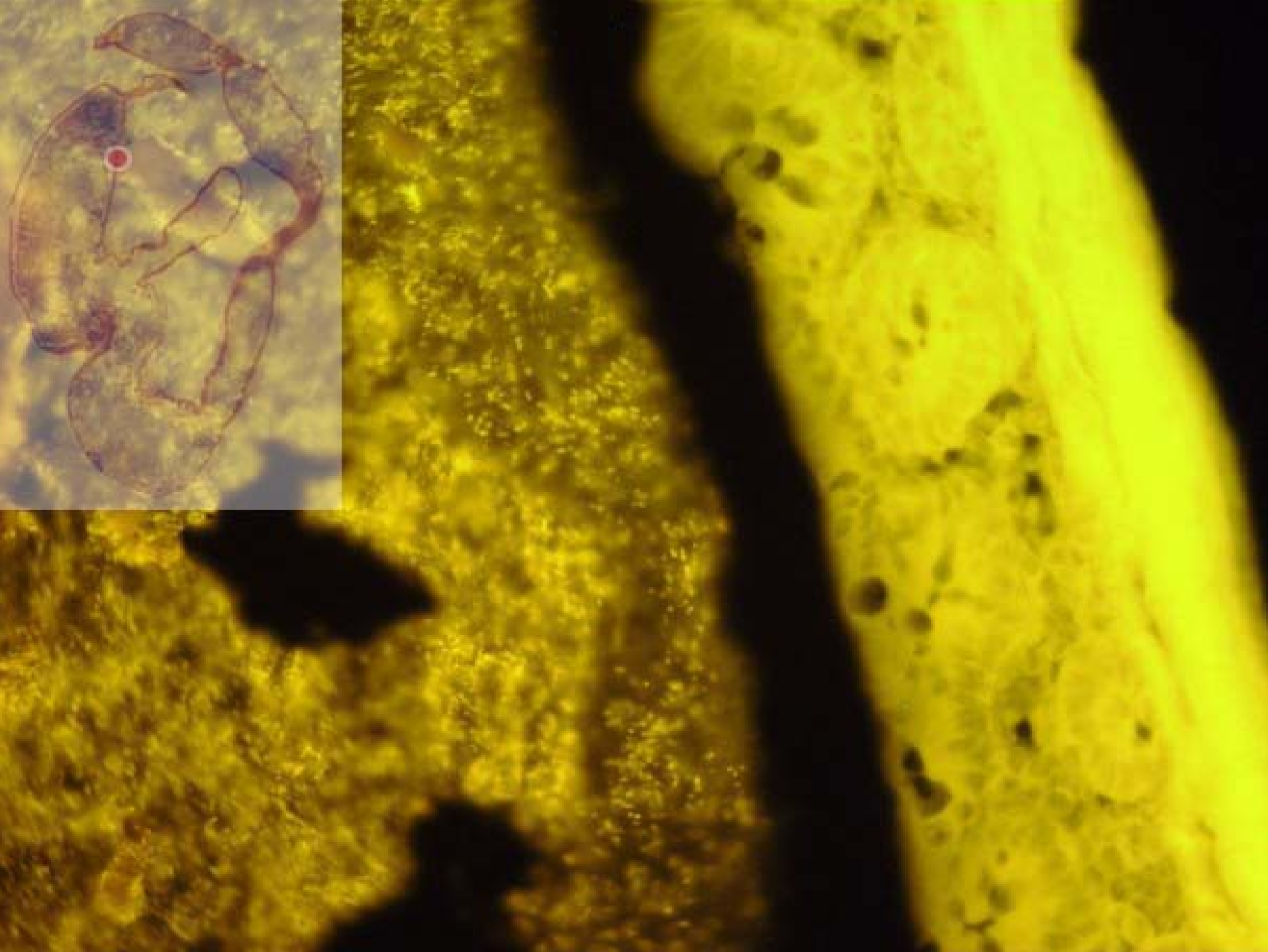


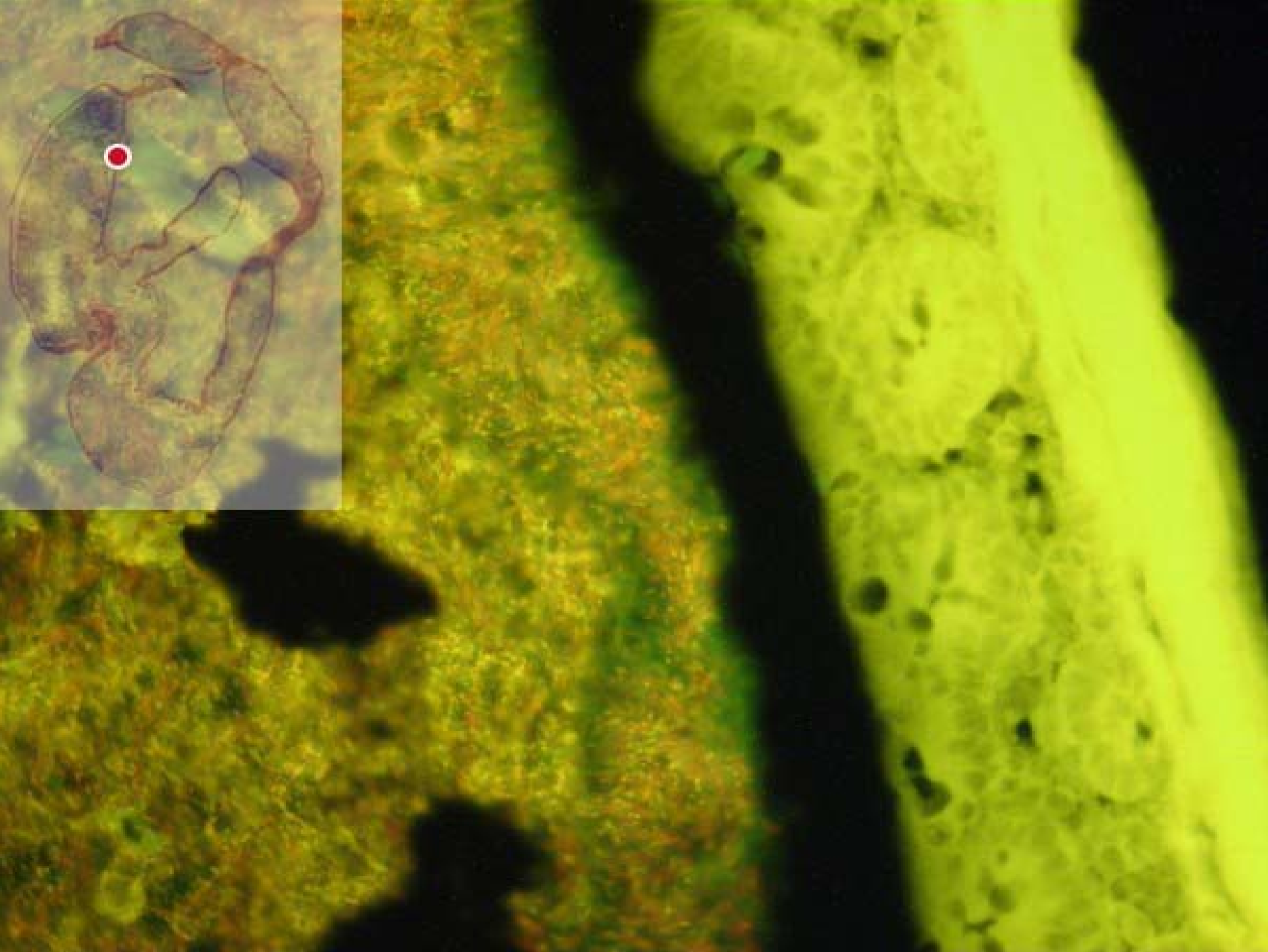
Dünndarm und Ileum einer gesunden Wildtyp-Maus enthalten keine mit FISH nachzuweisenden Bakterienkonzentrationen

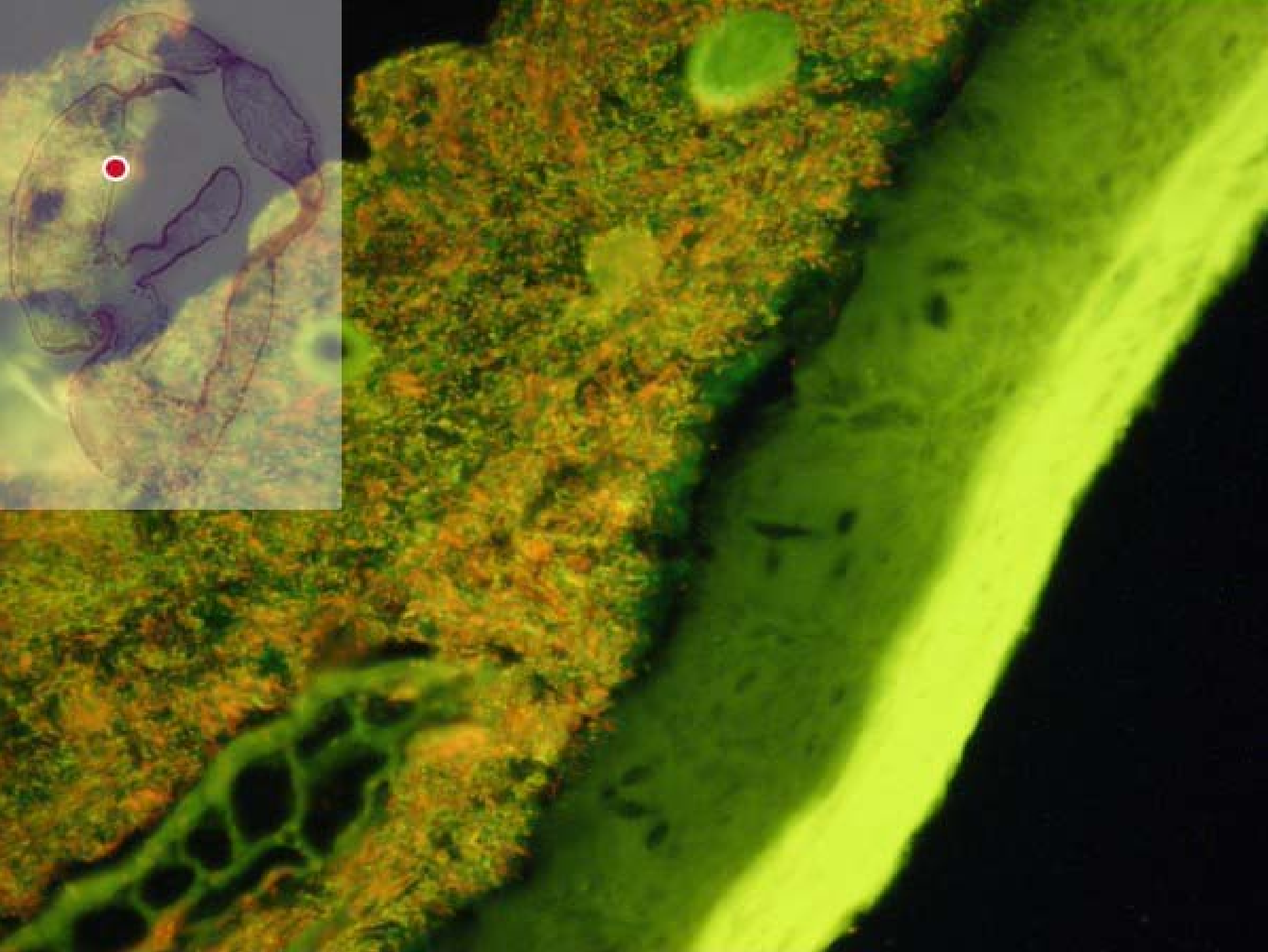


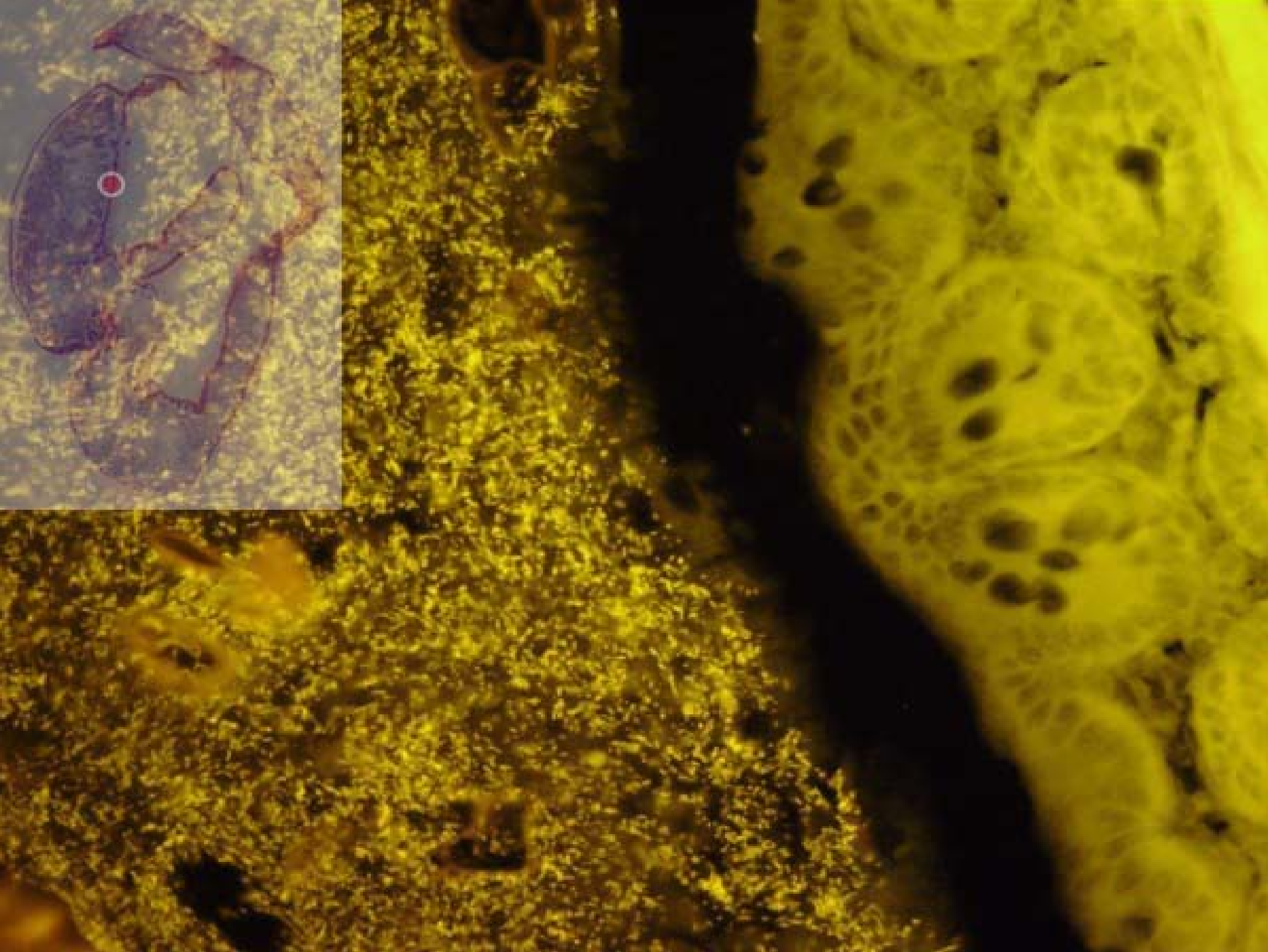

Im Coecum explodiert die Keimzahl
Bakterien (mit Ausnahme von Bacteroides)
sind in den Krypten

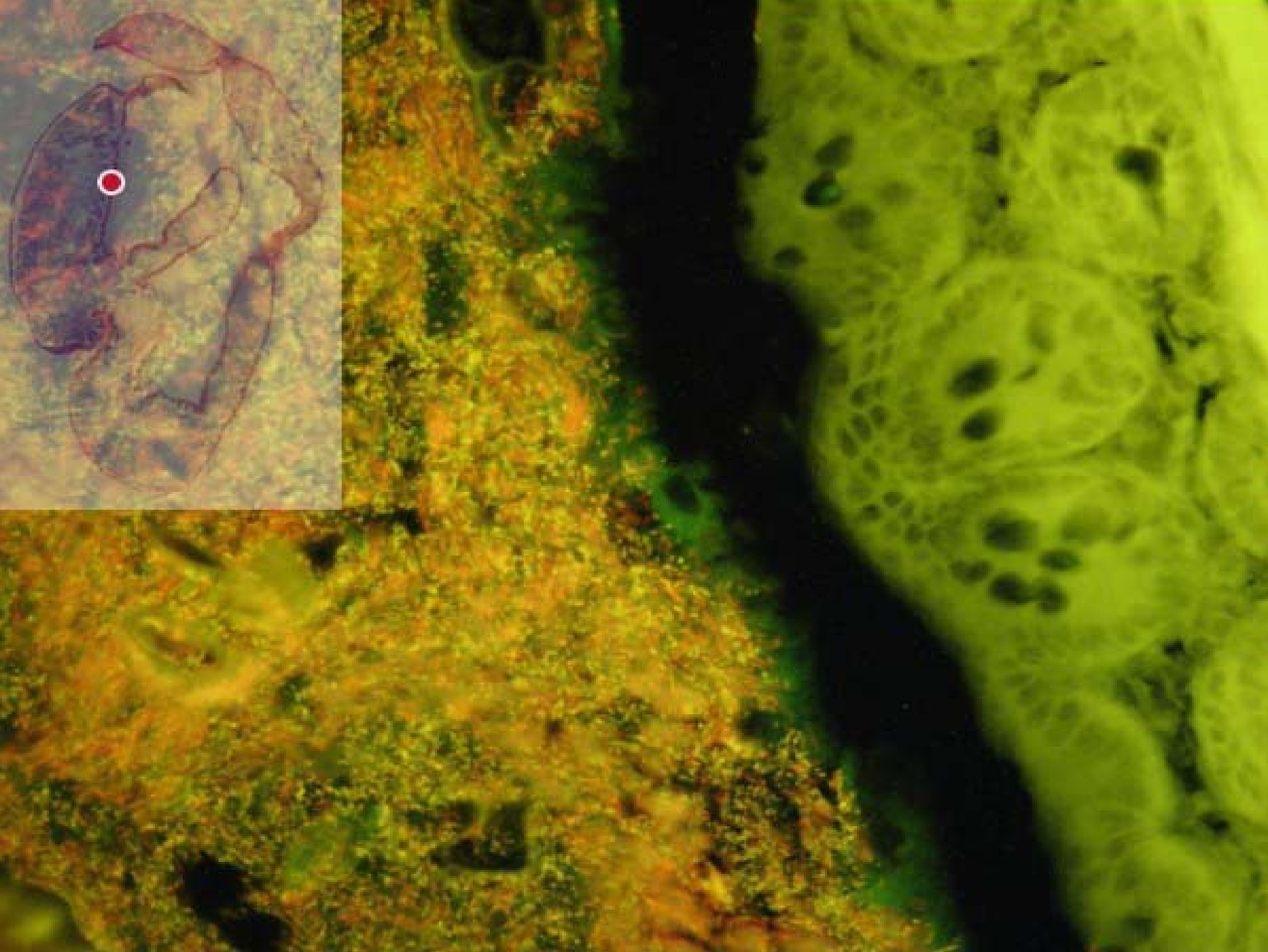

Bacteroides ist von der Wand verdrängt

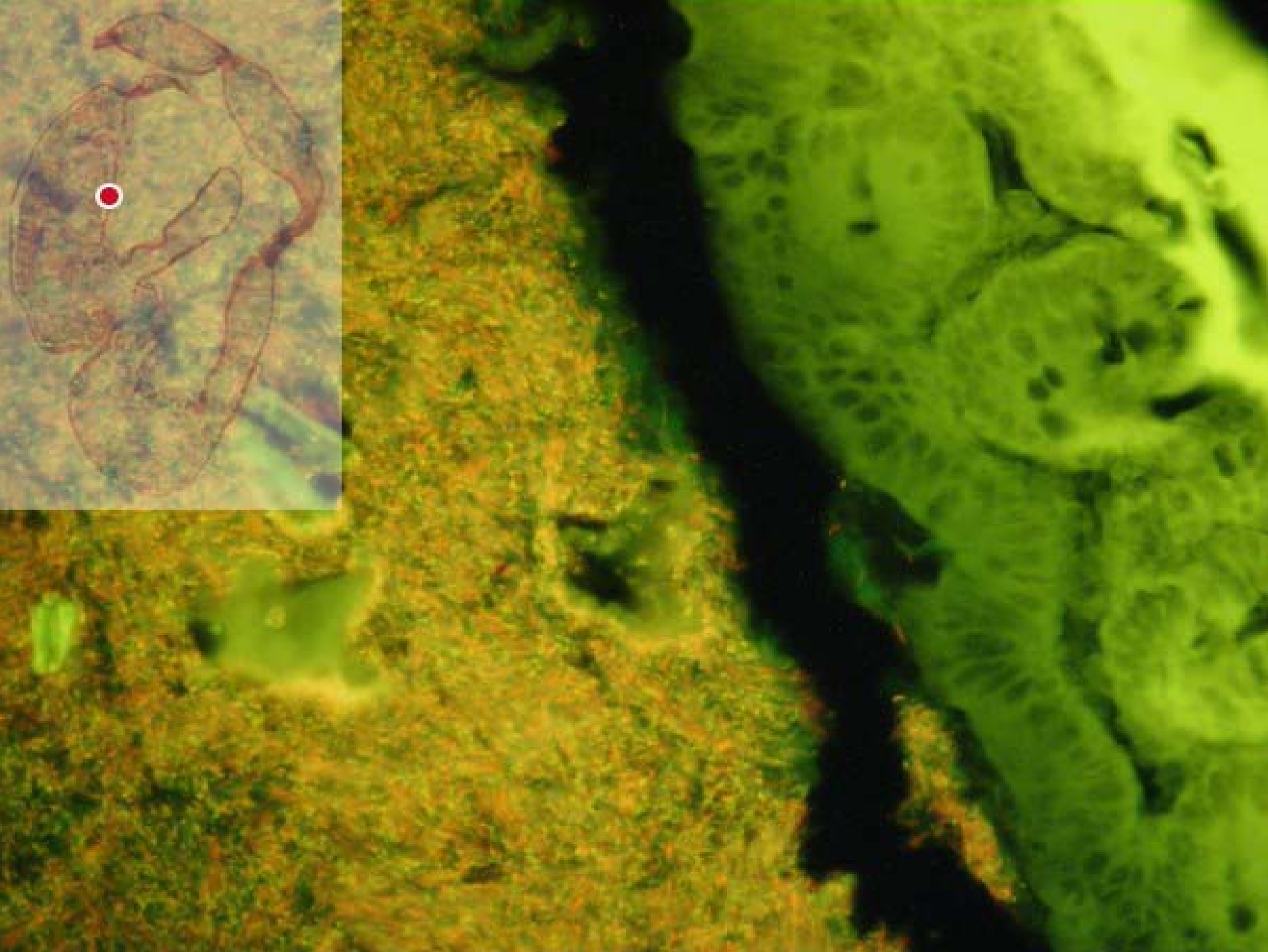


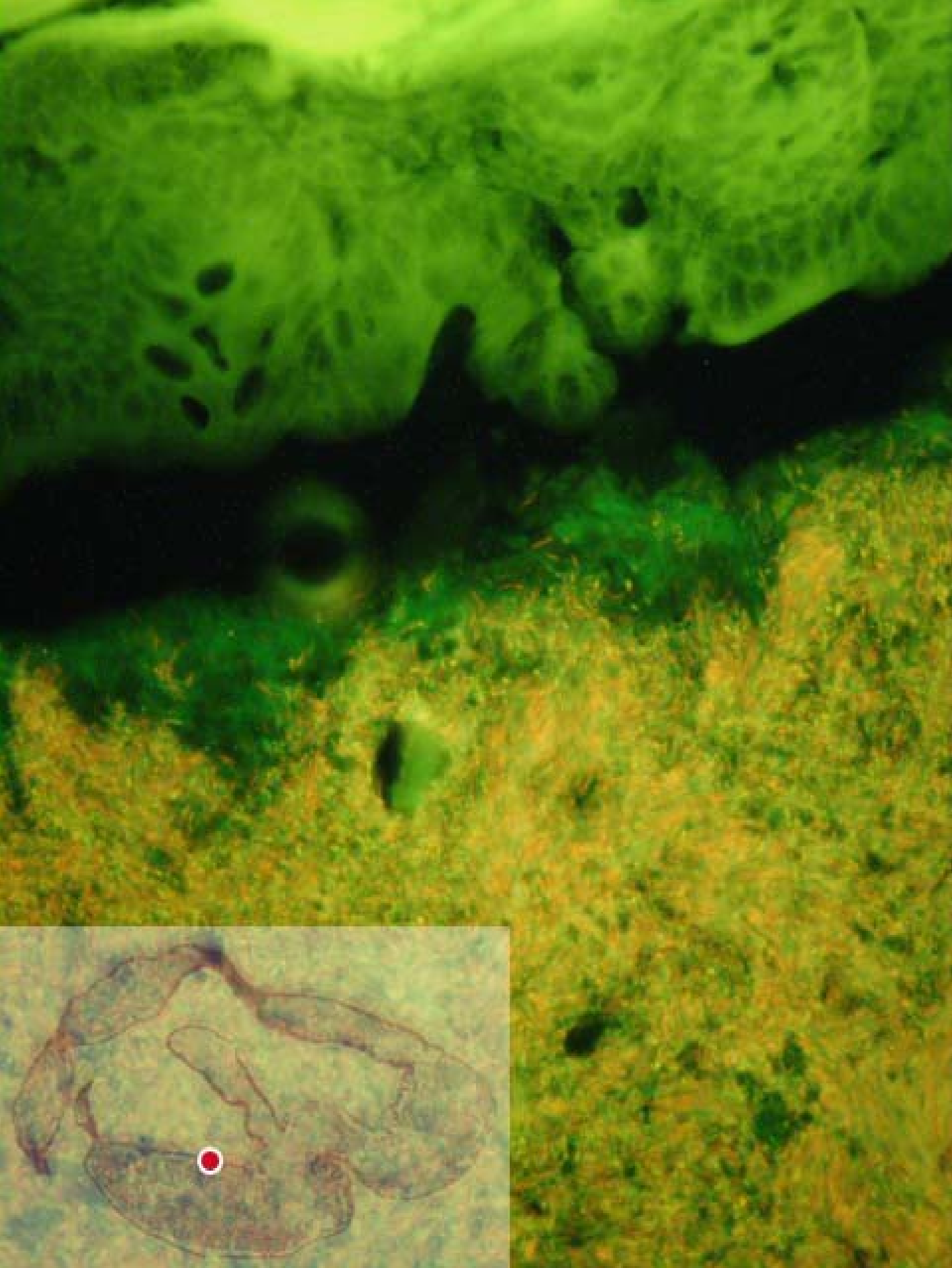


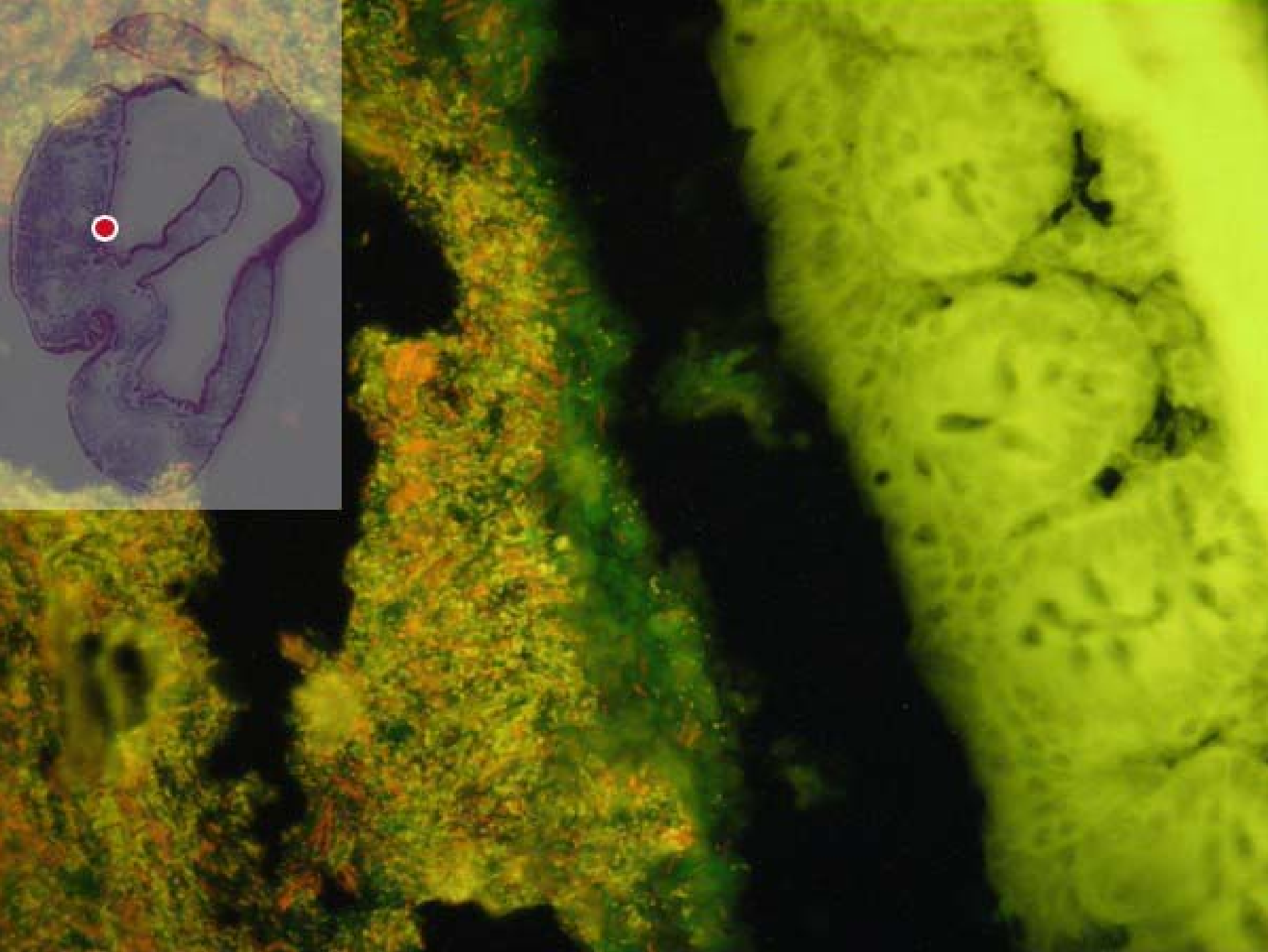


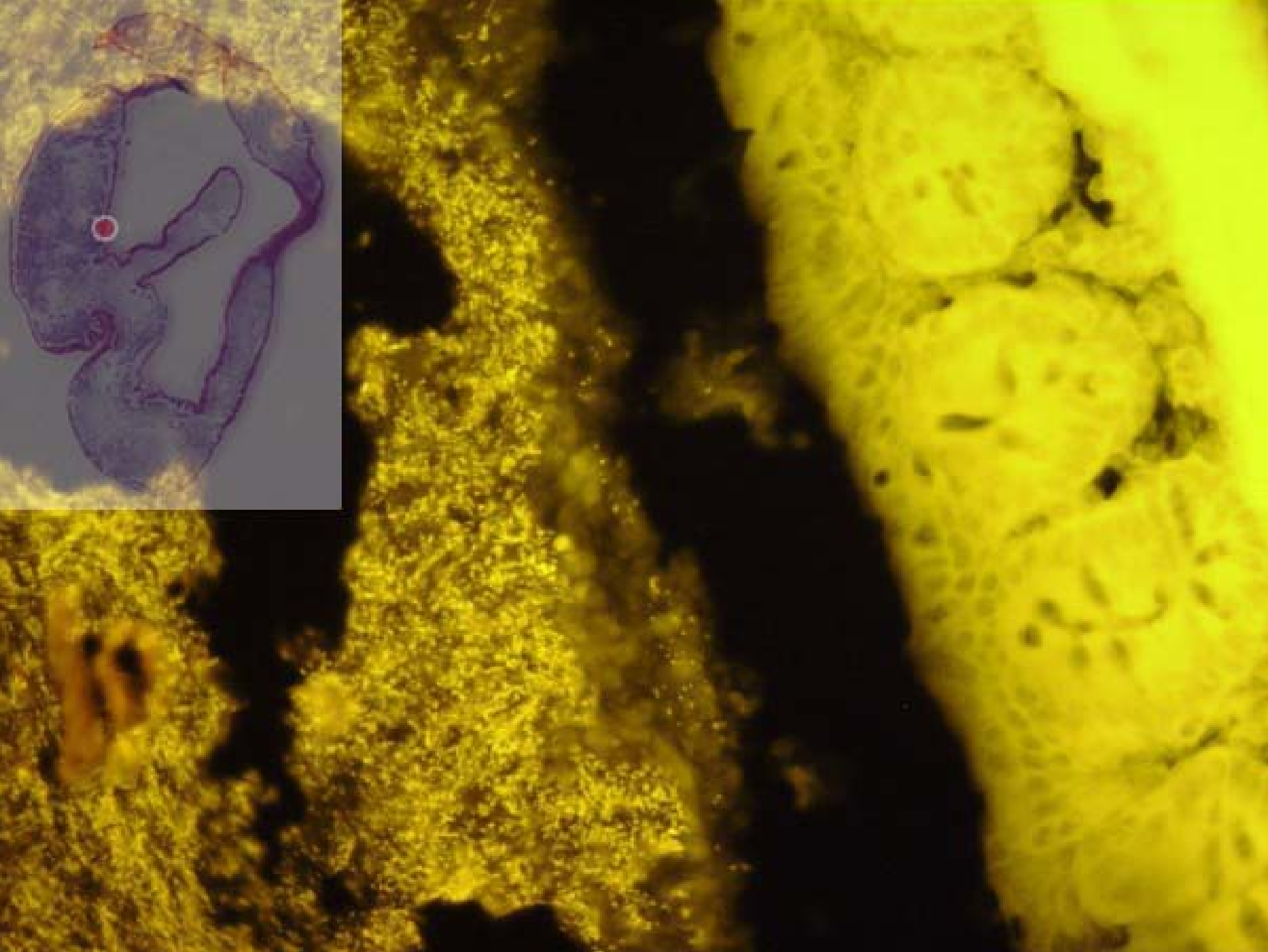


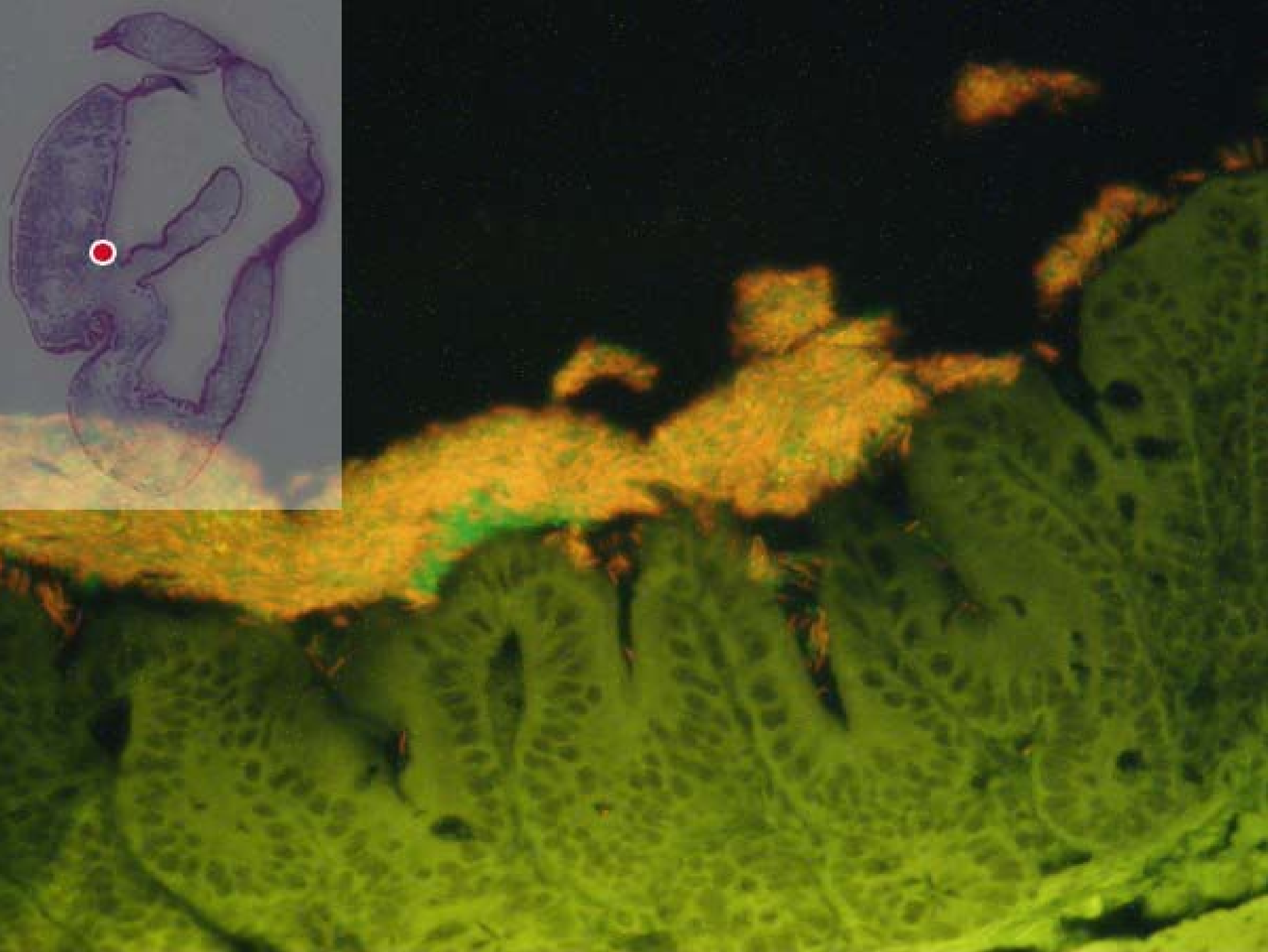


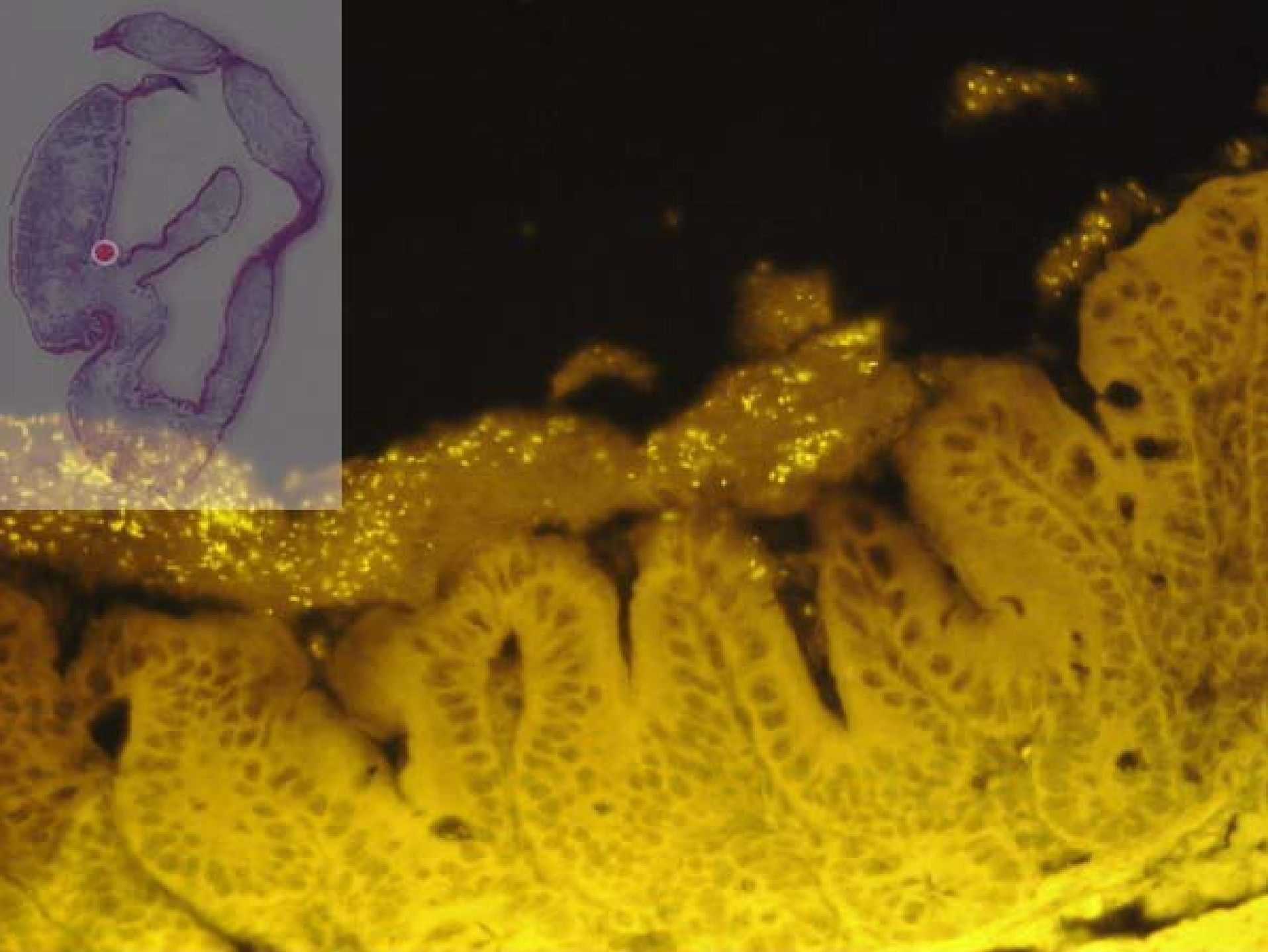


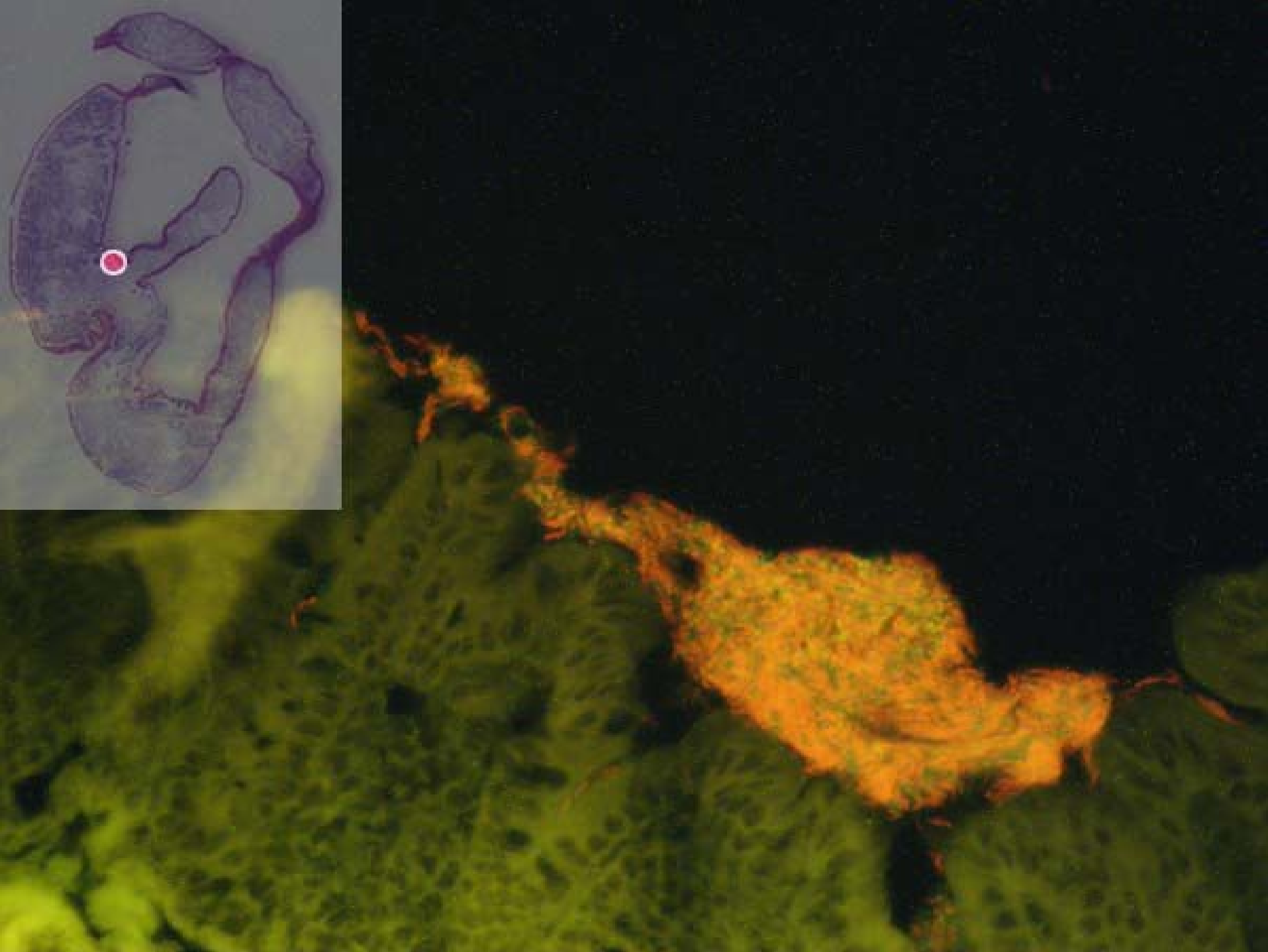


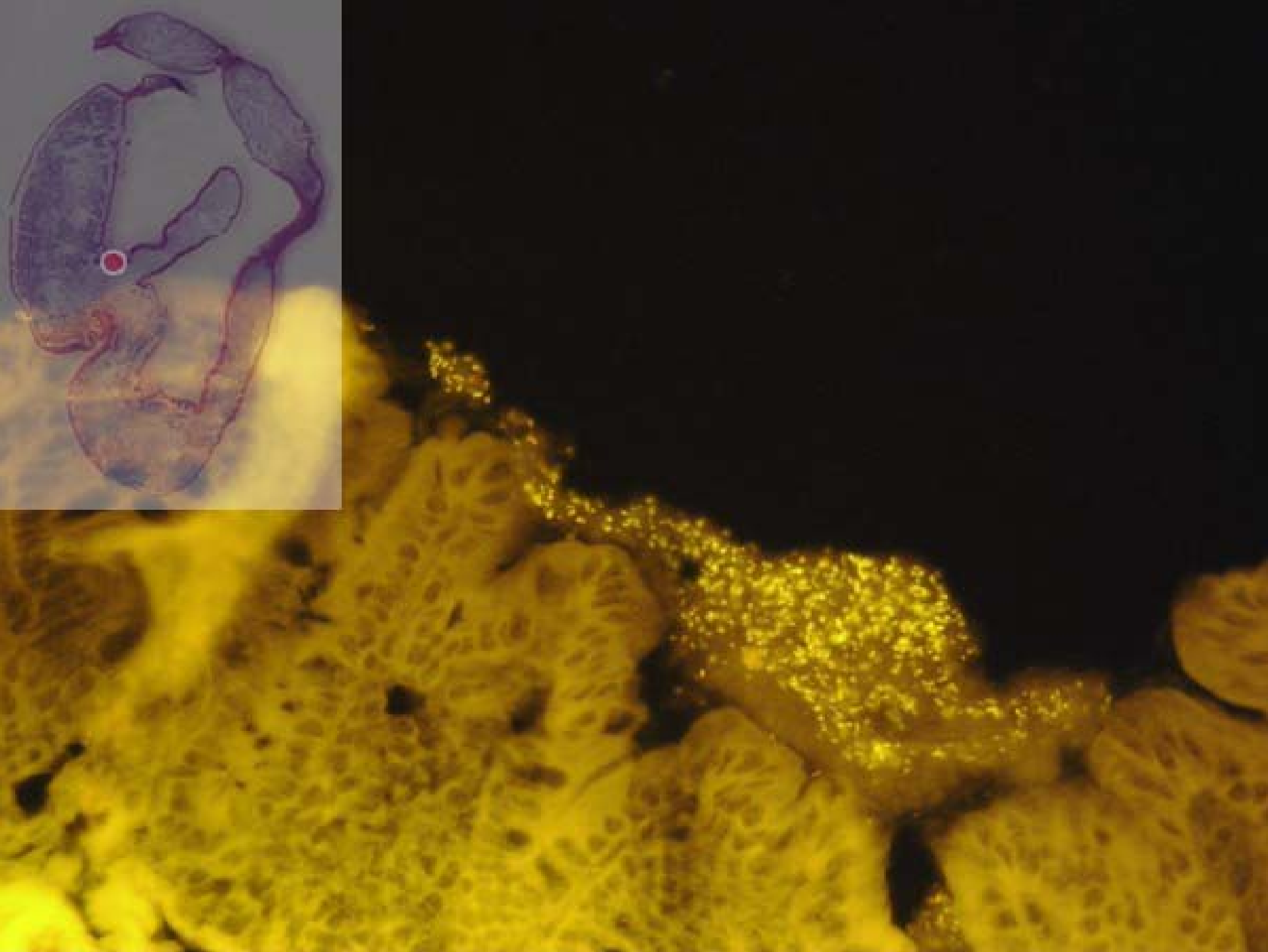


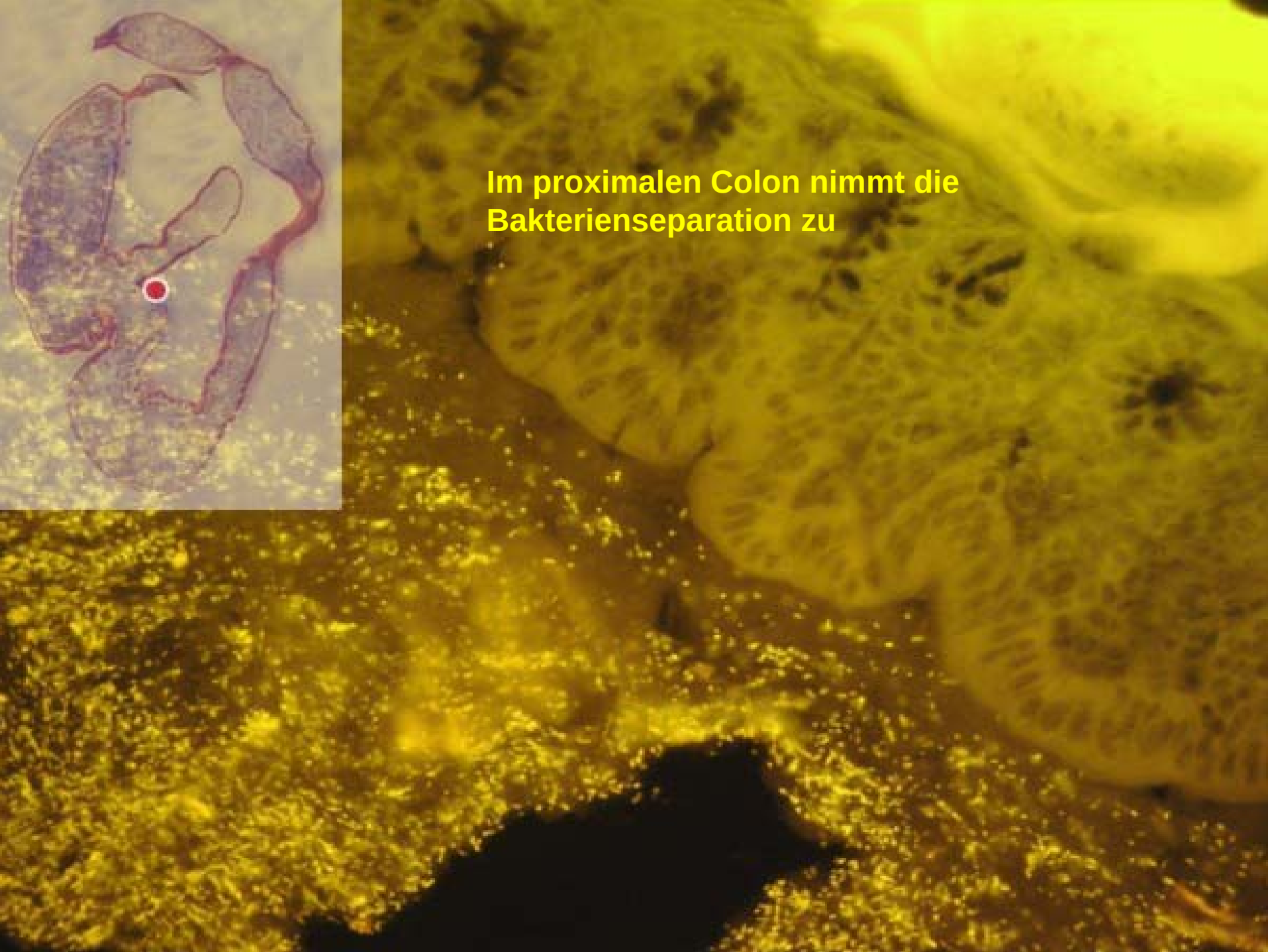




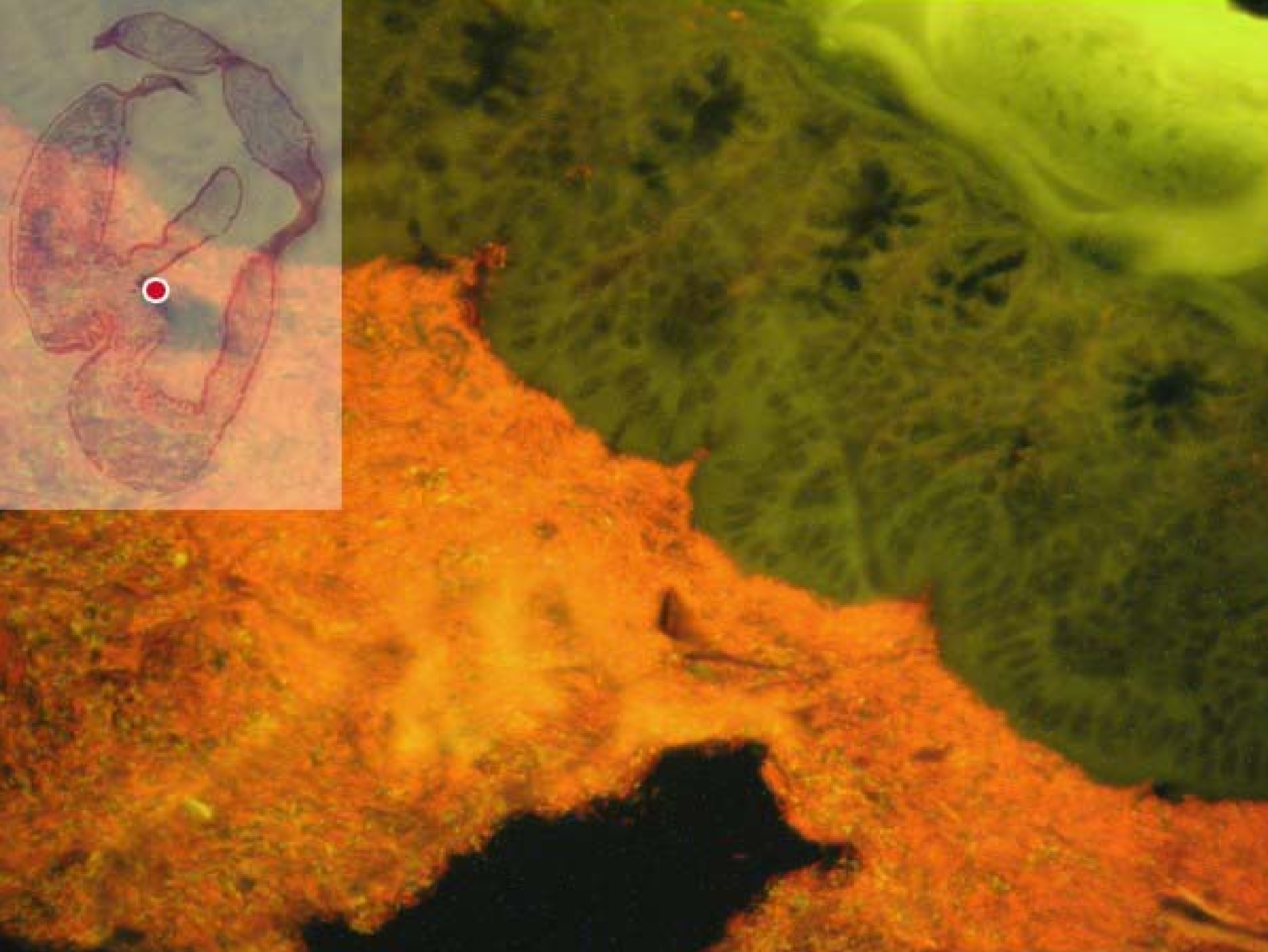


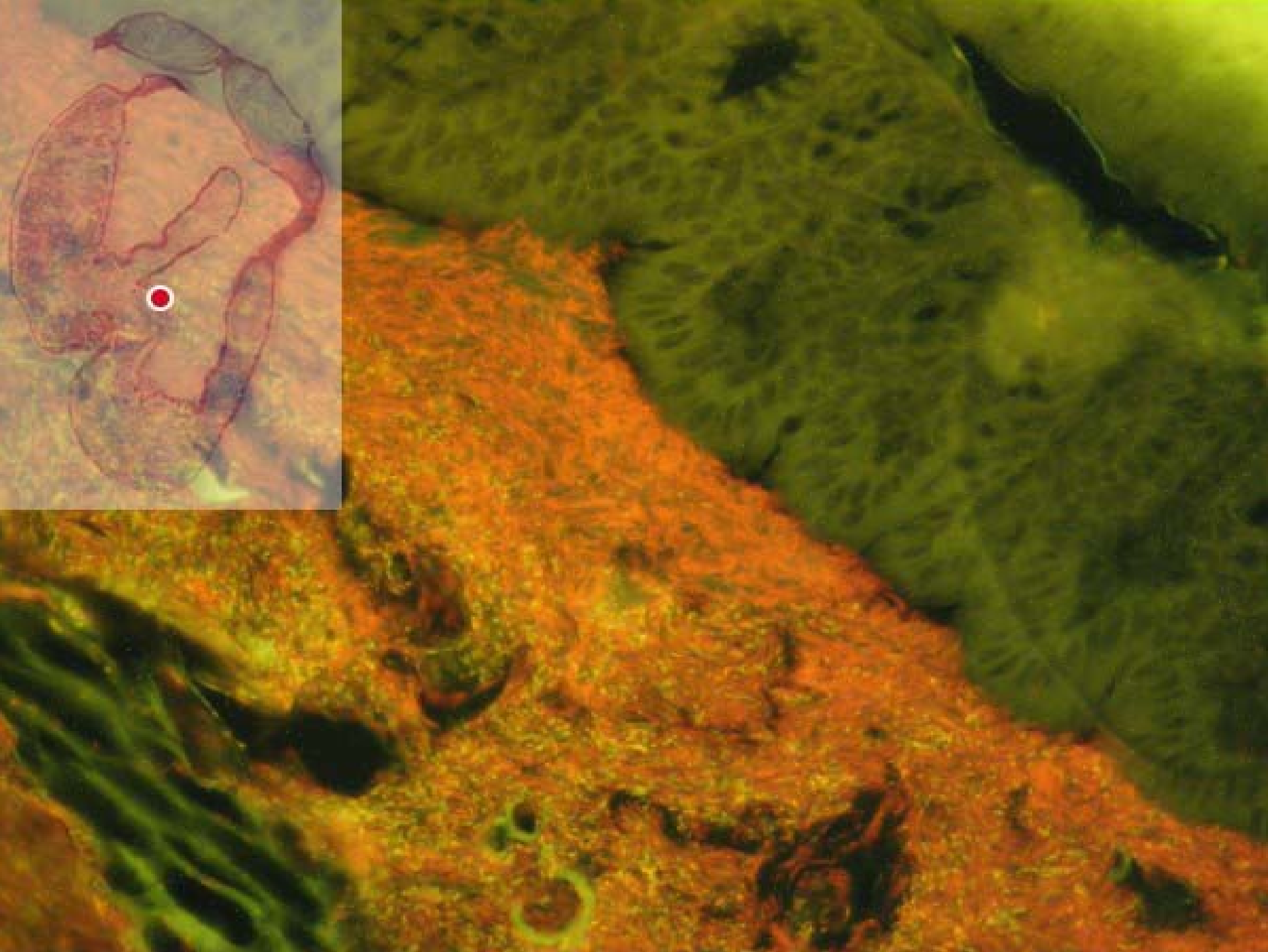


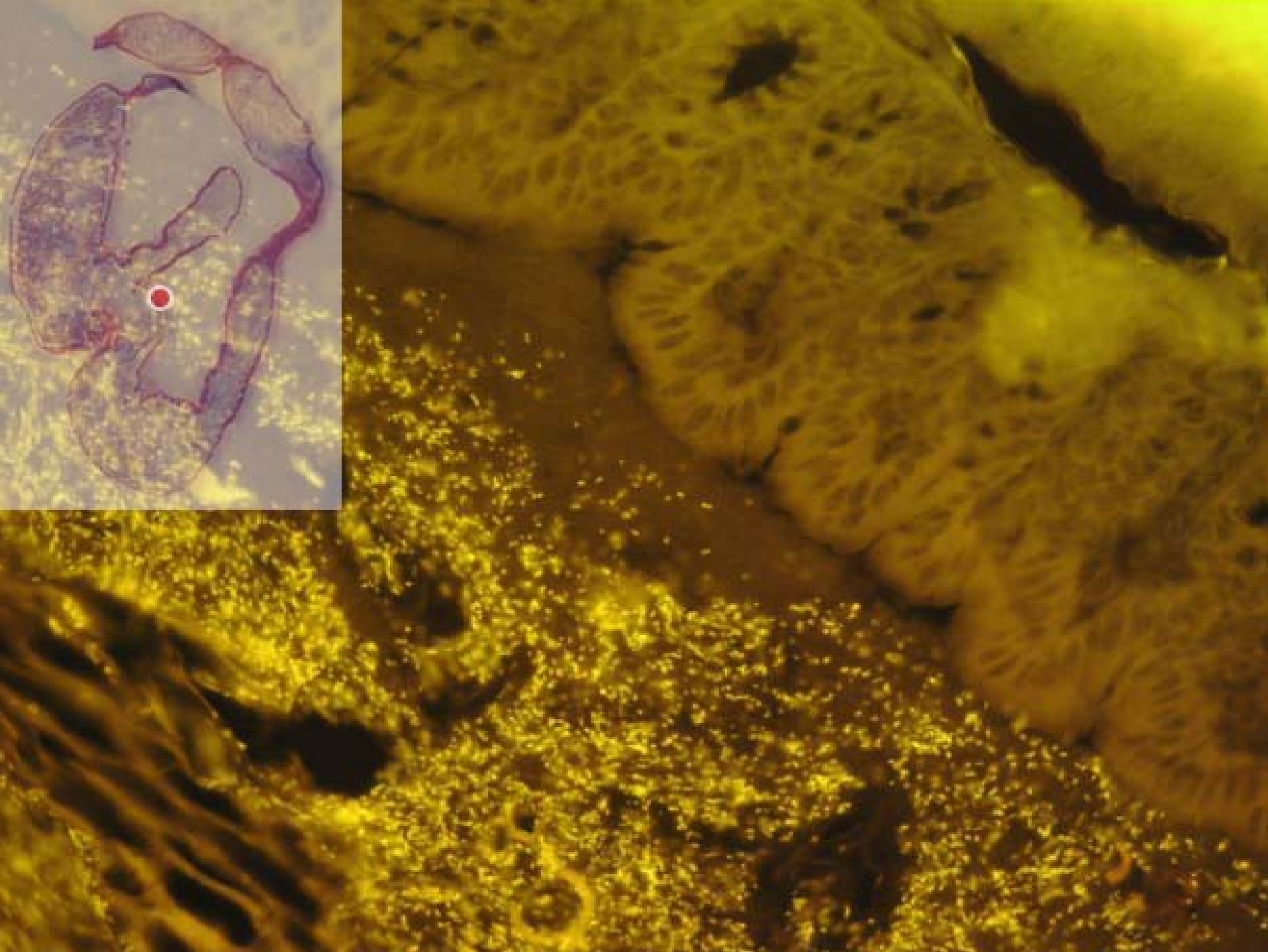


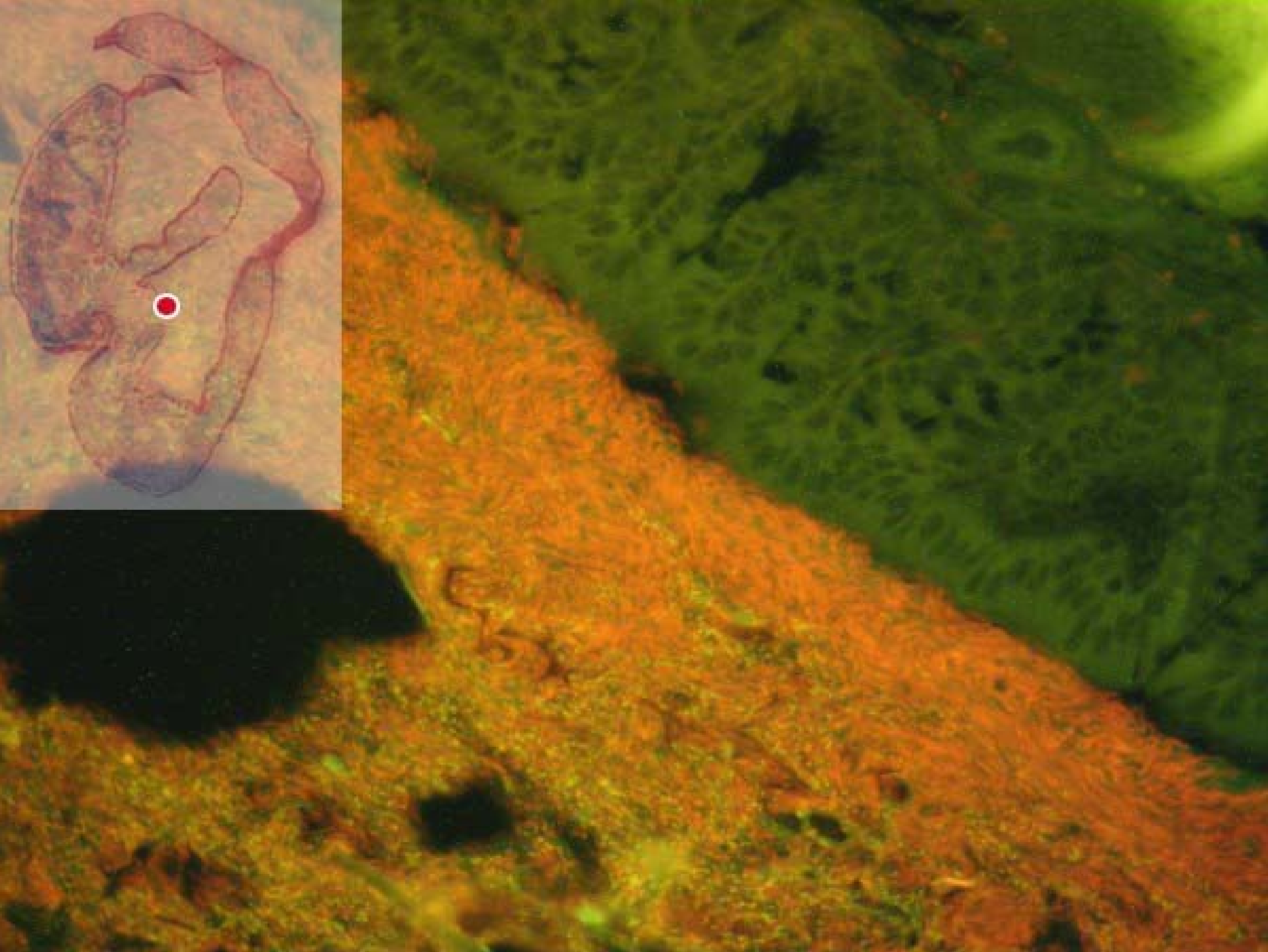


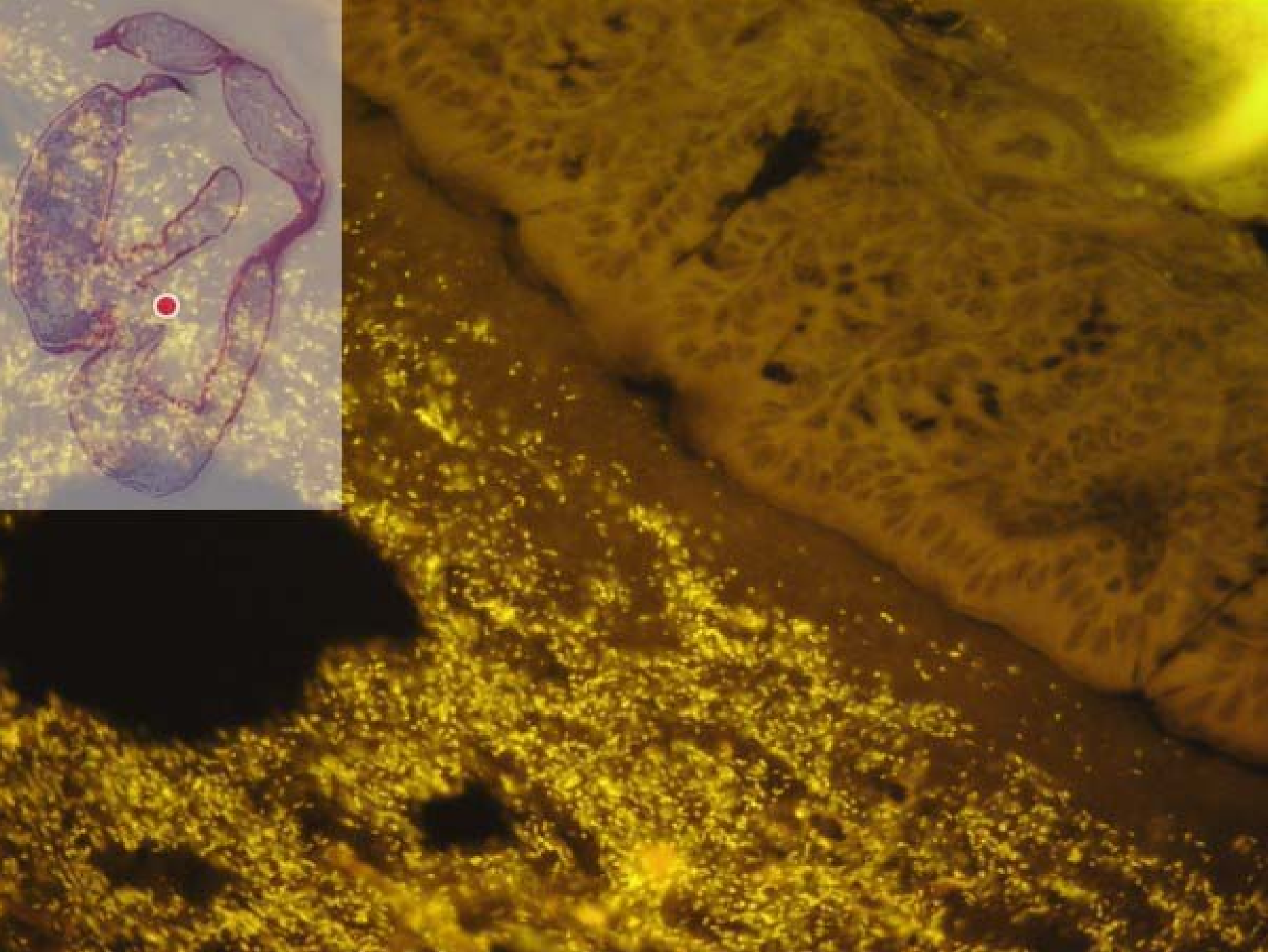
Im proximalen Colon nimmt die Bakteriensepara

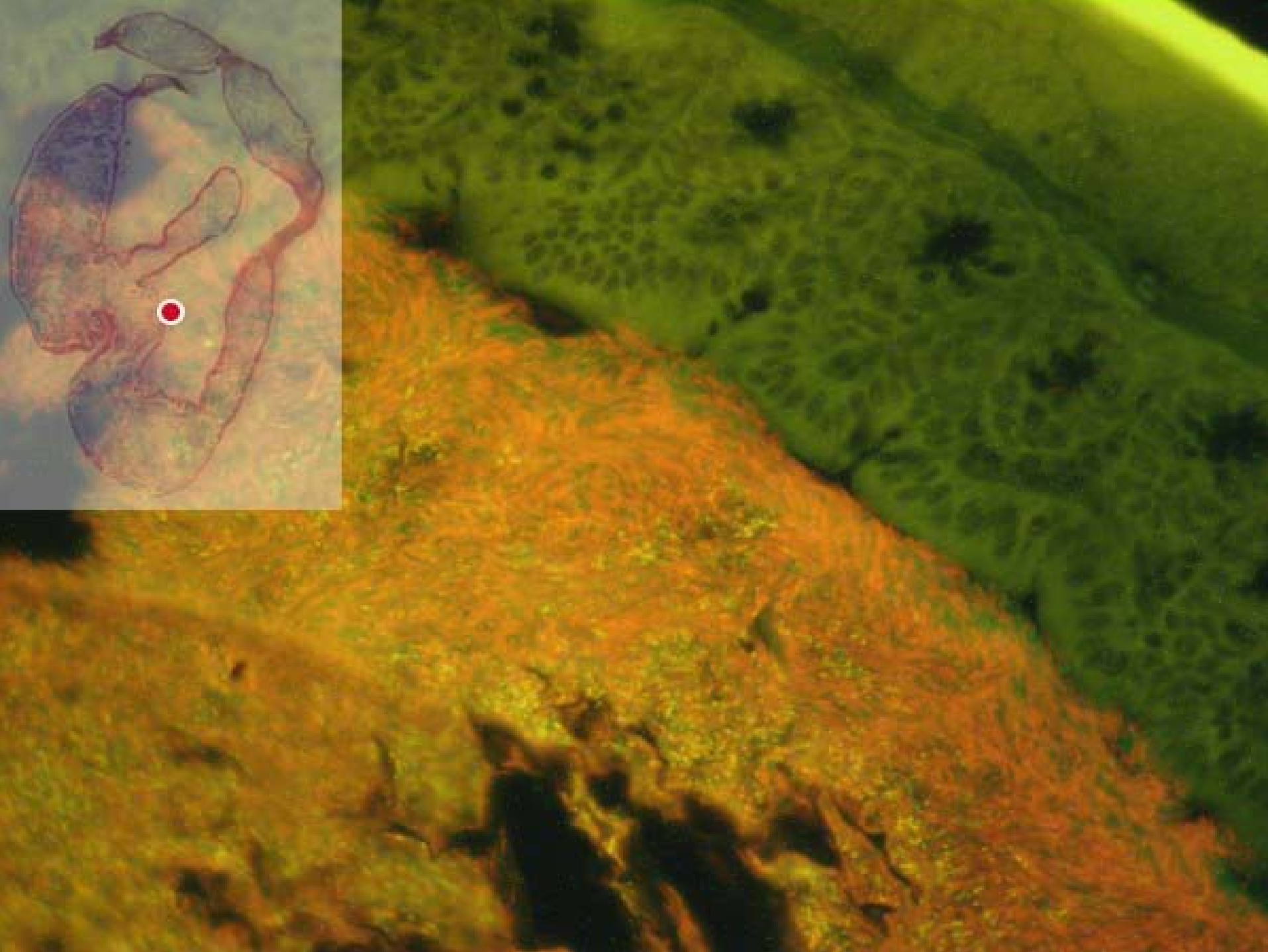


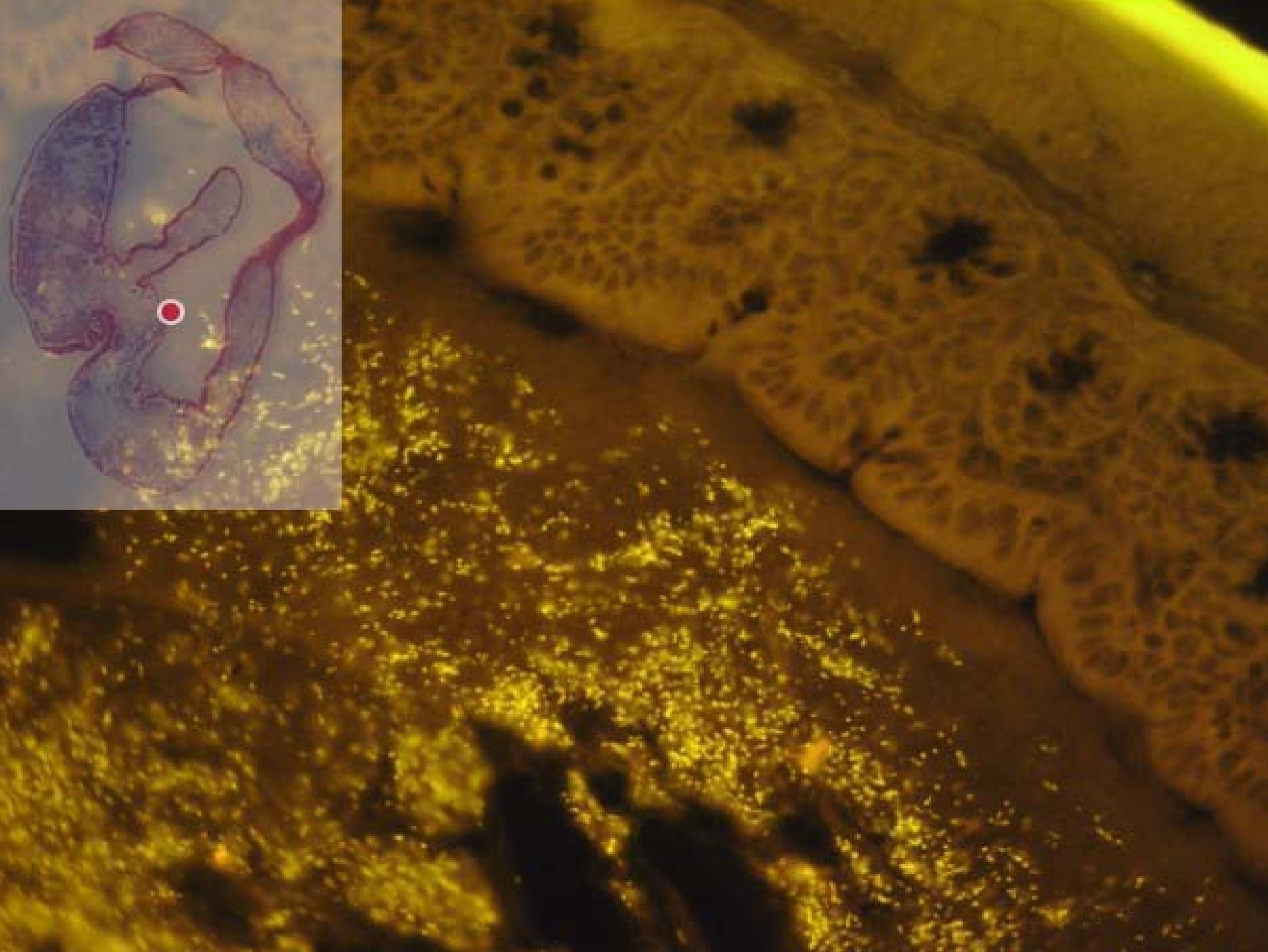


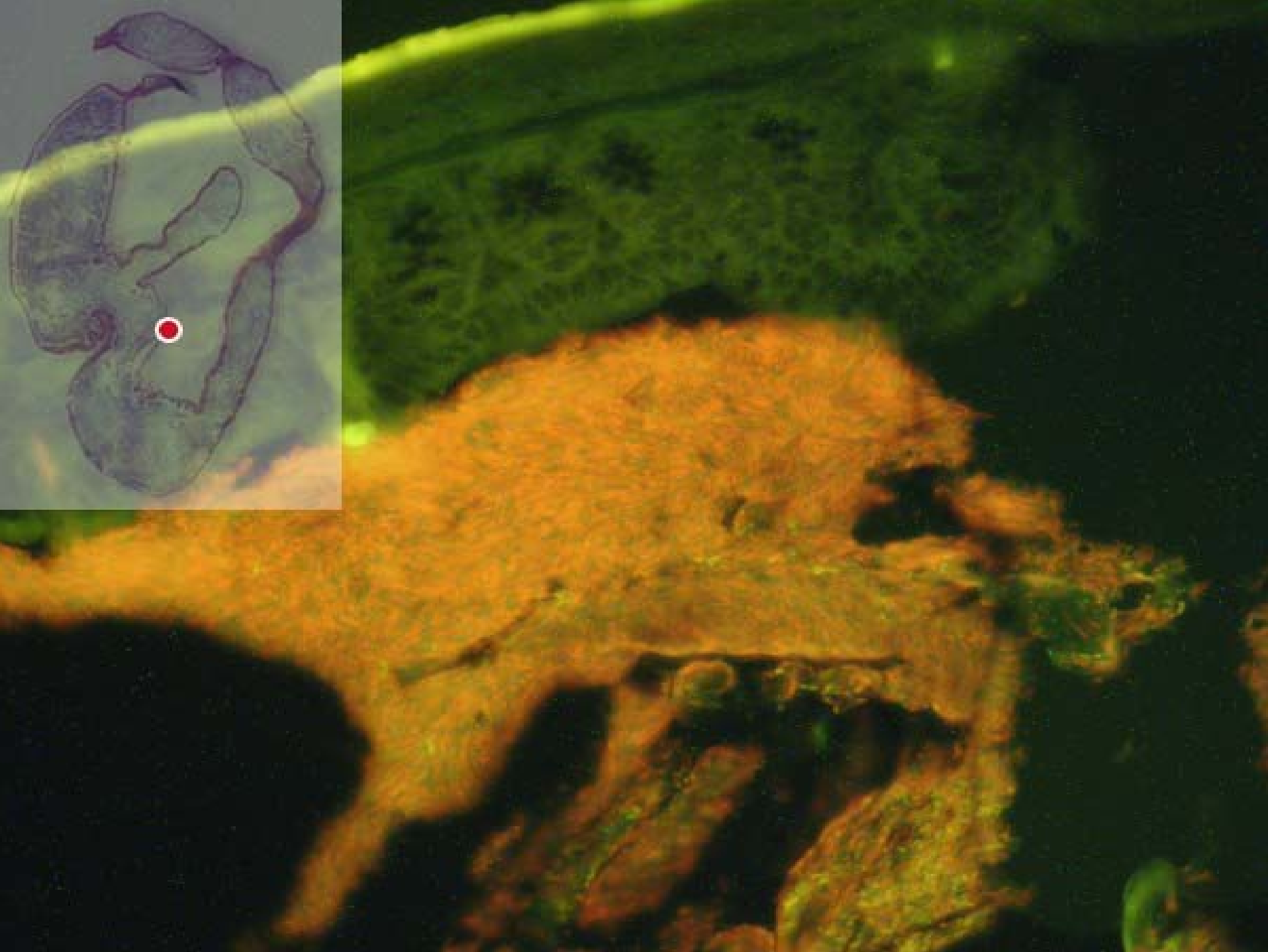


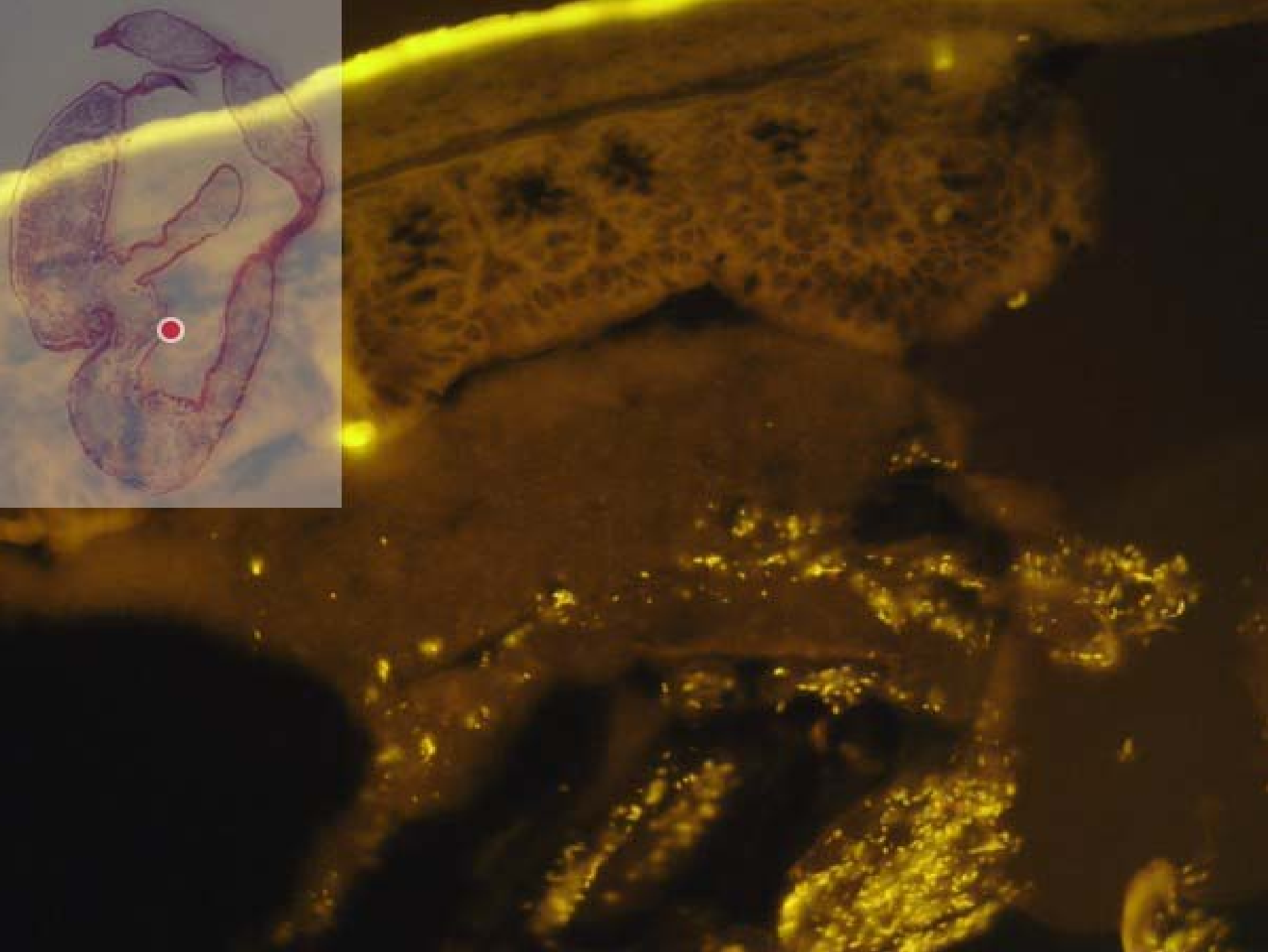


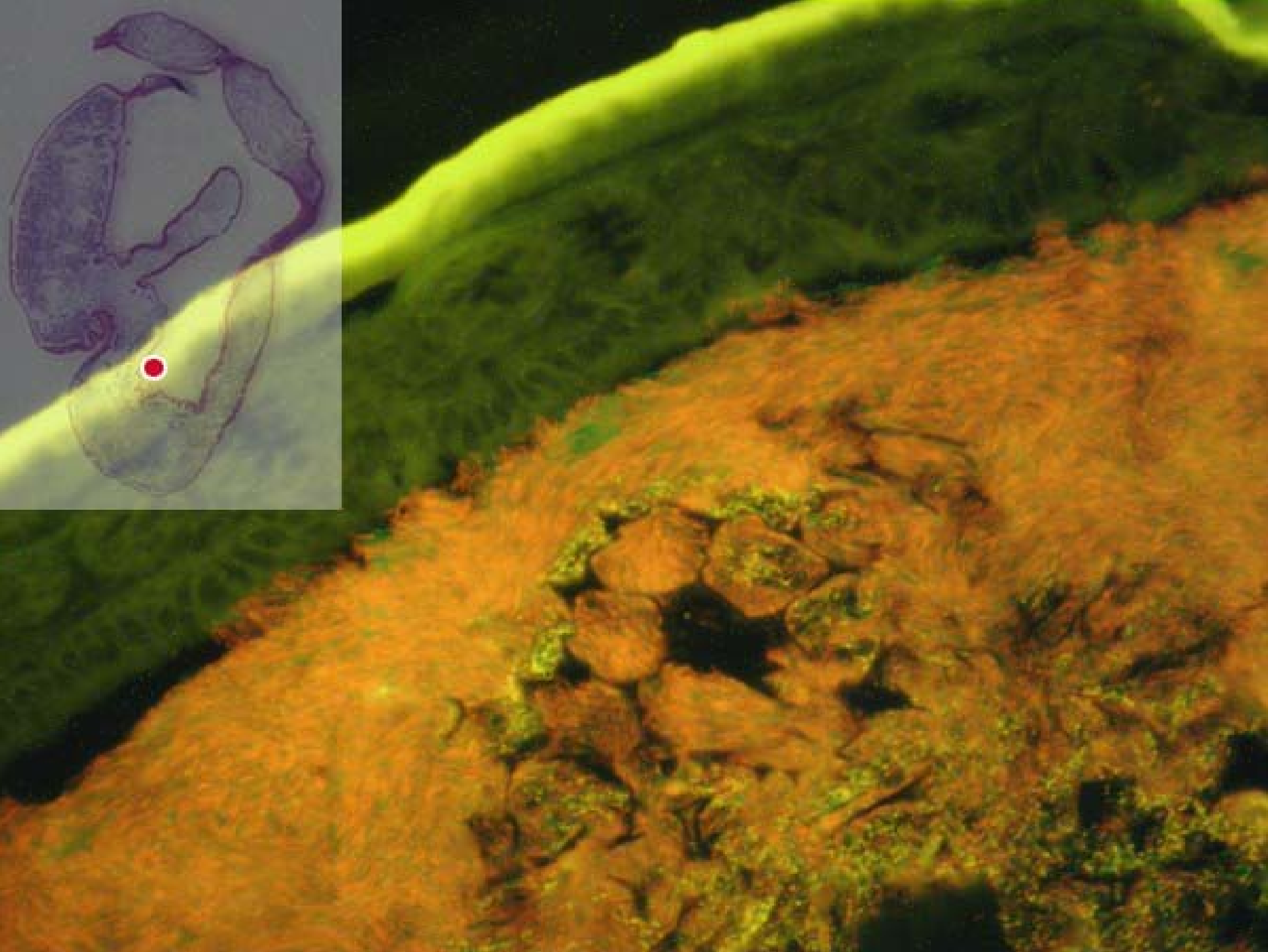


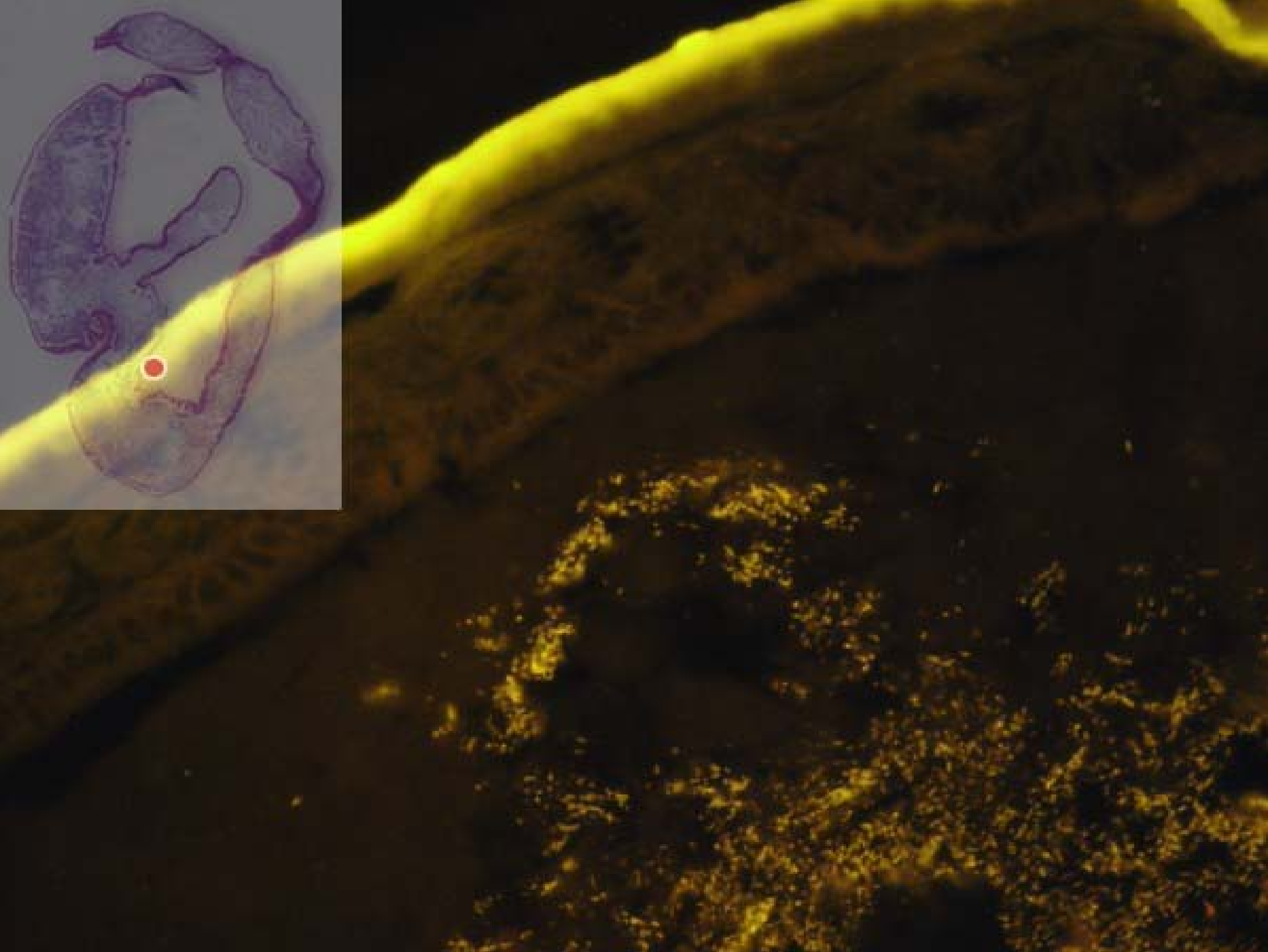


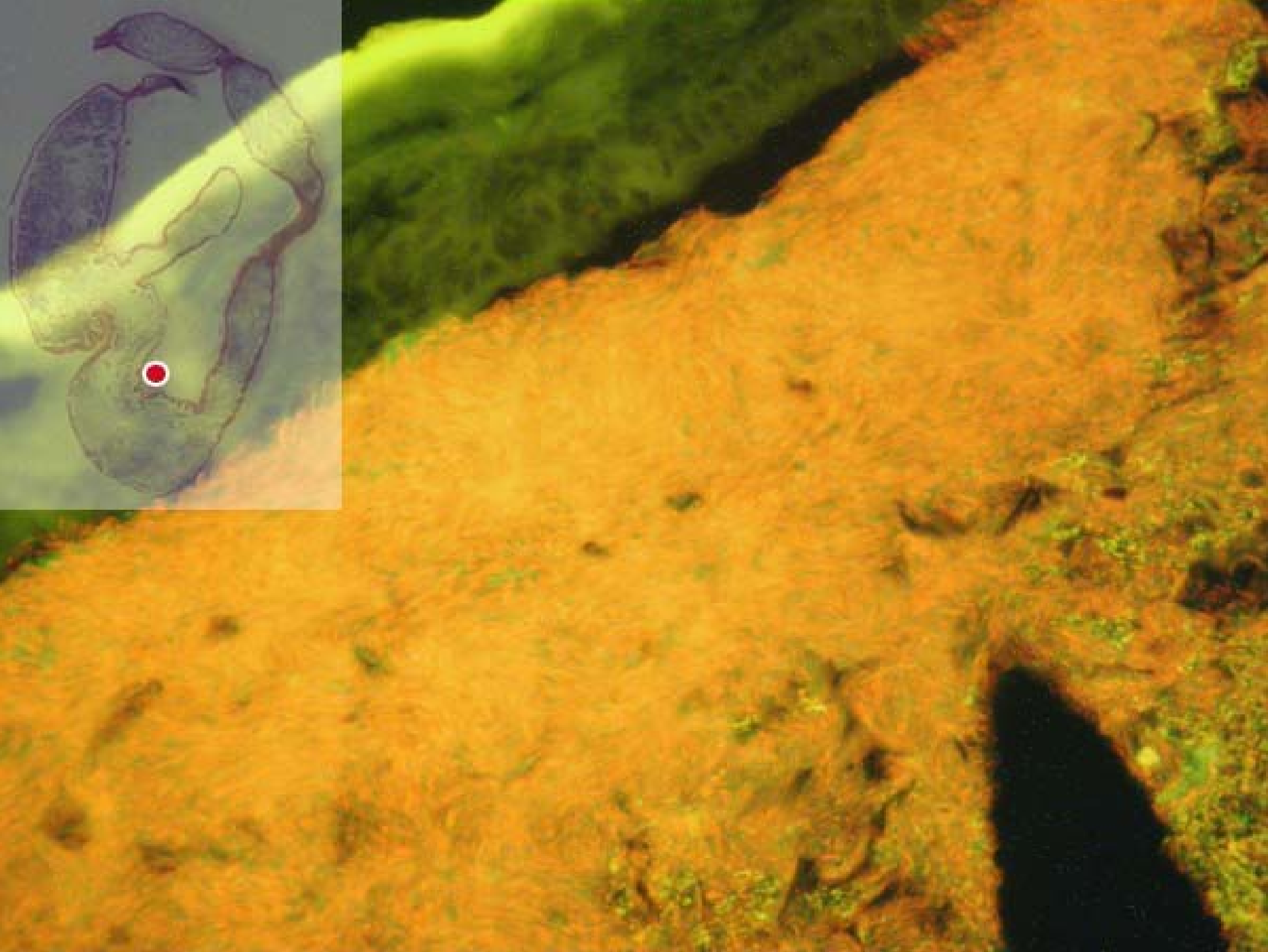


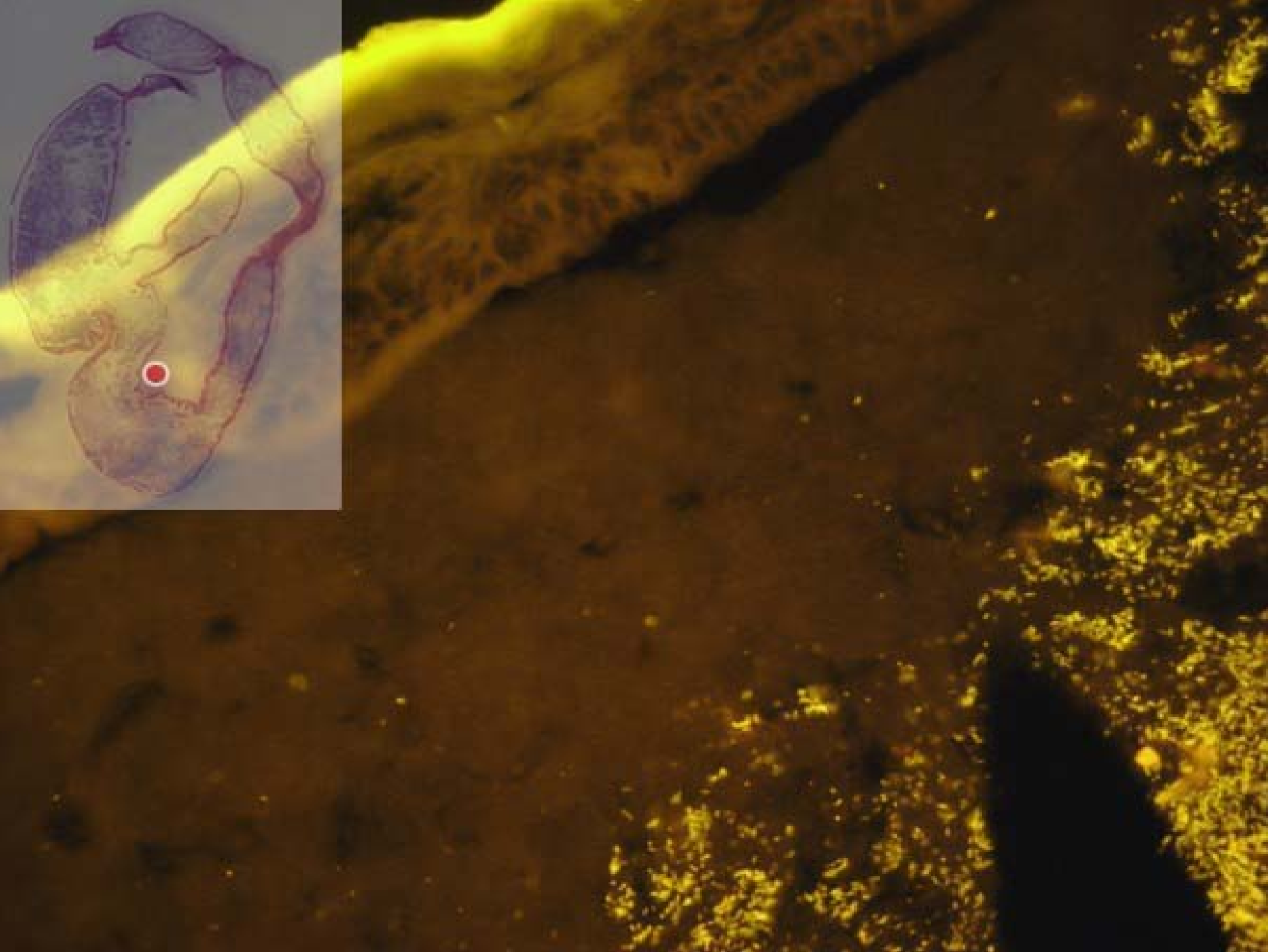


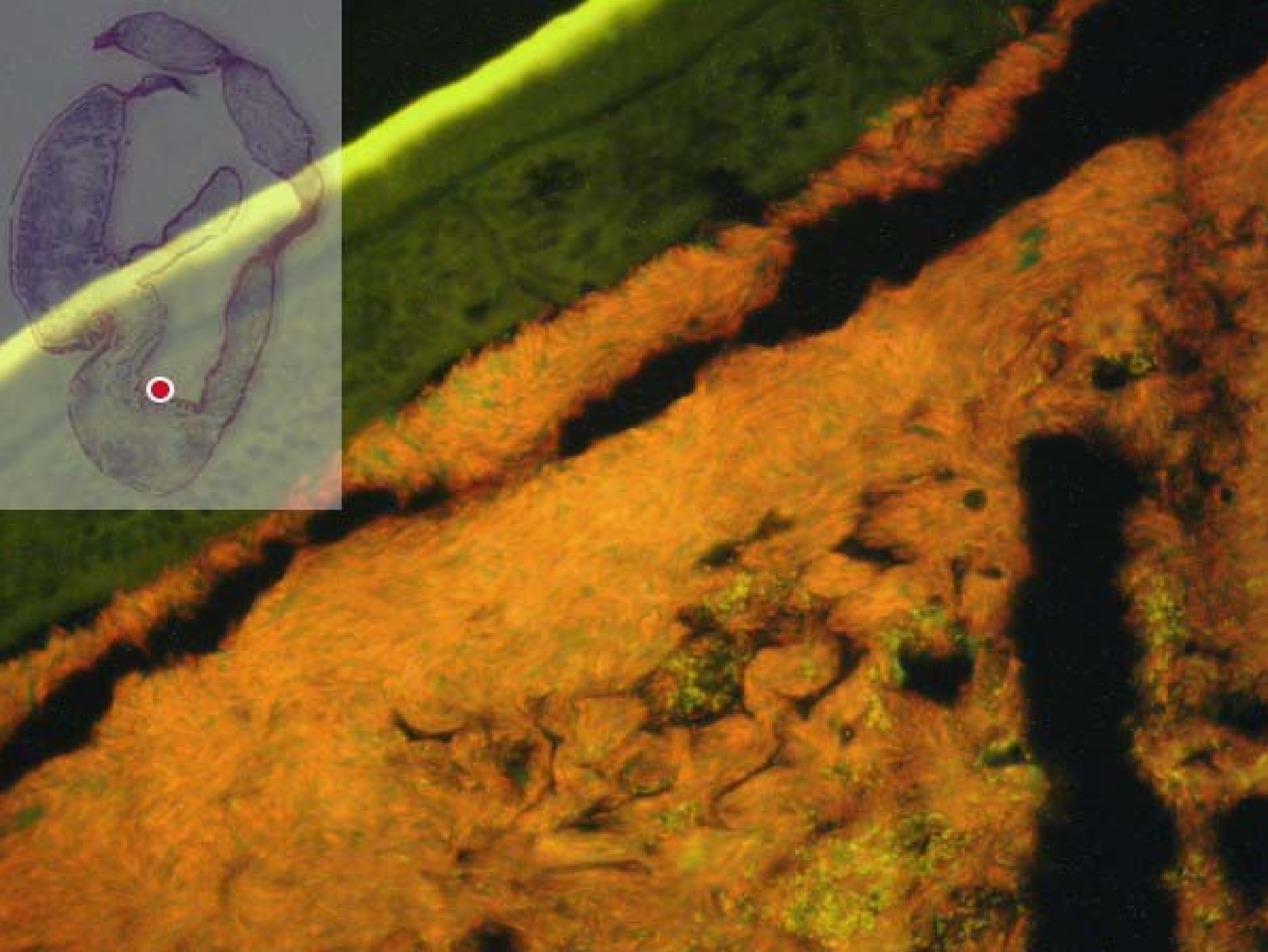


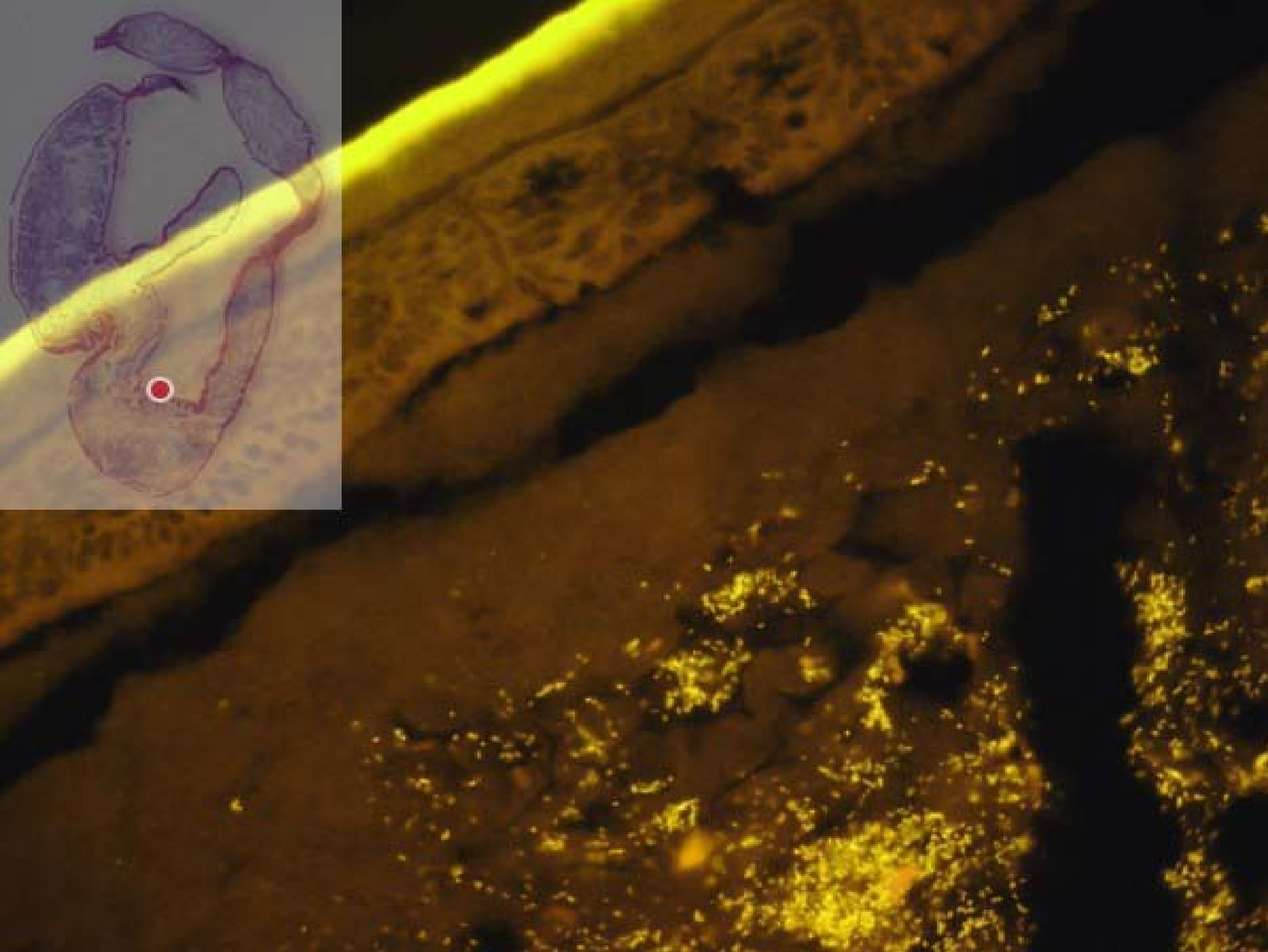


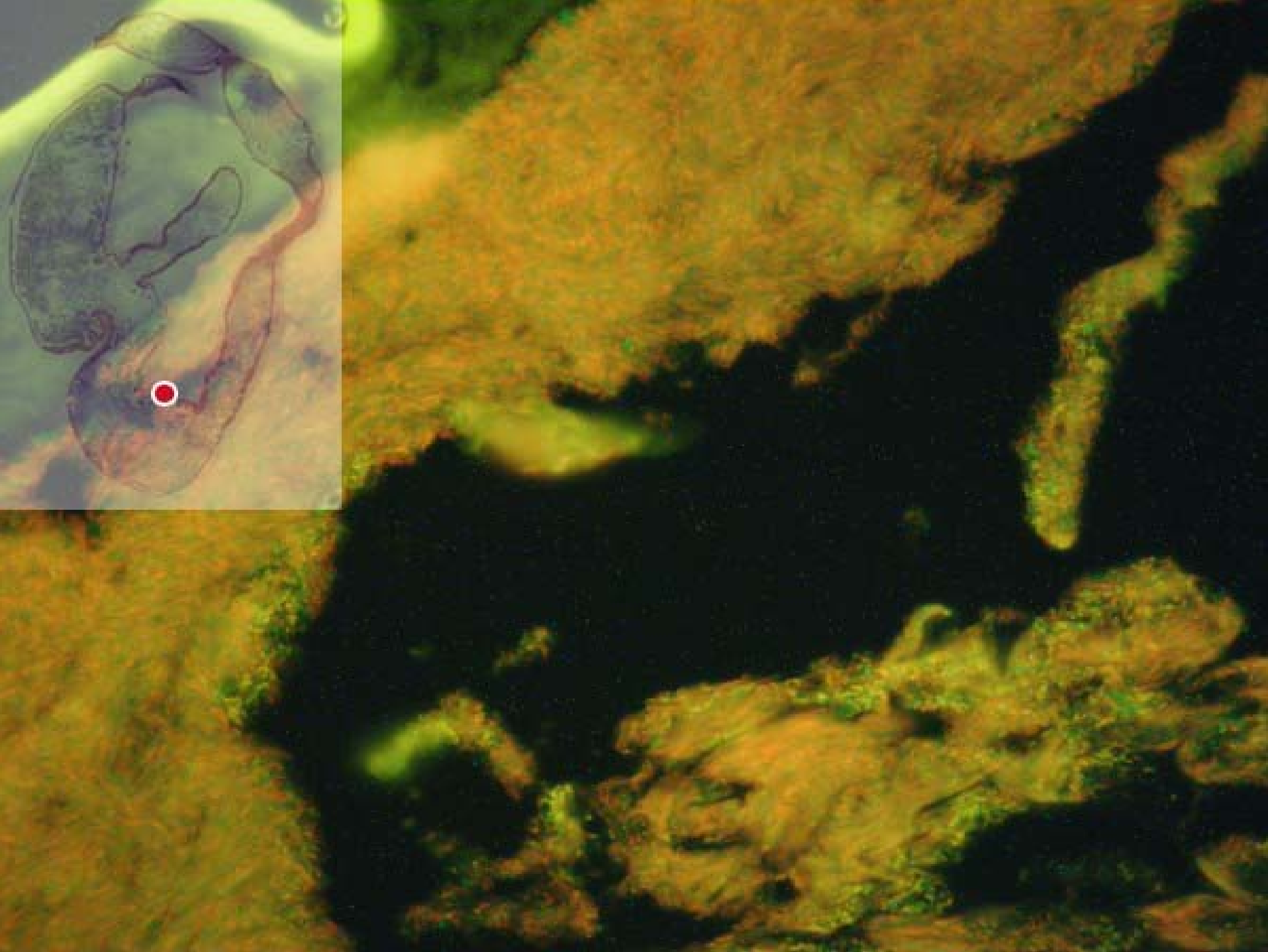


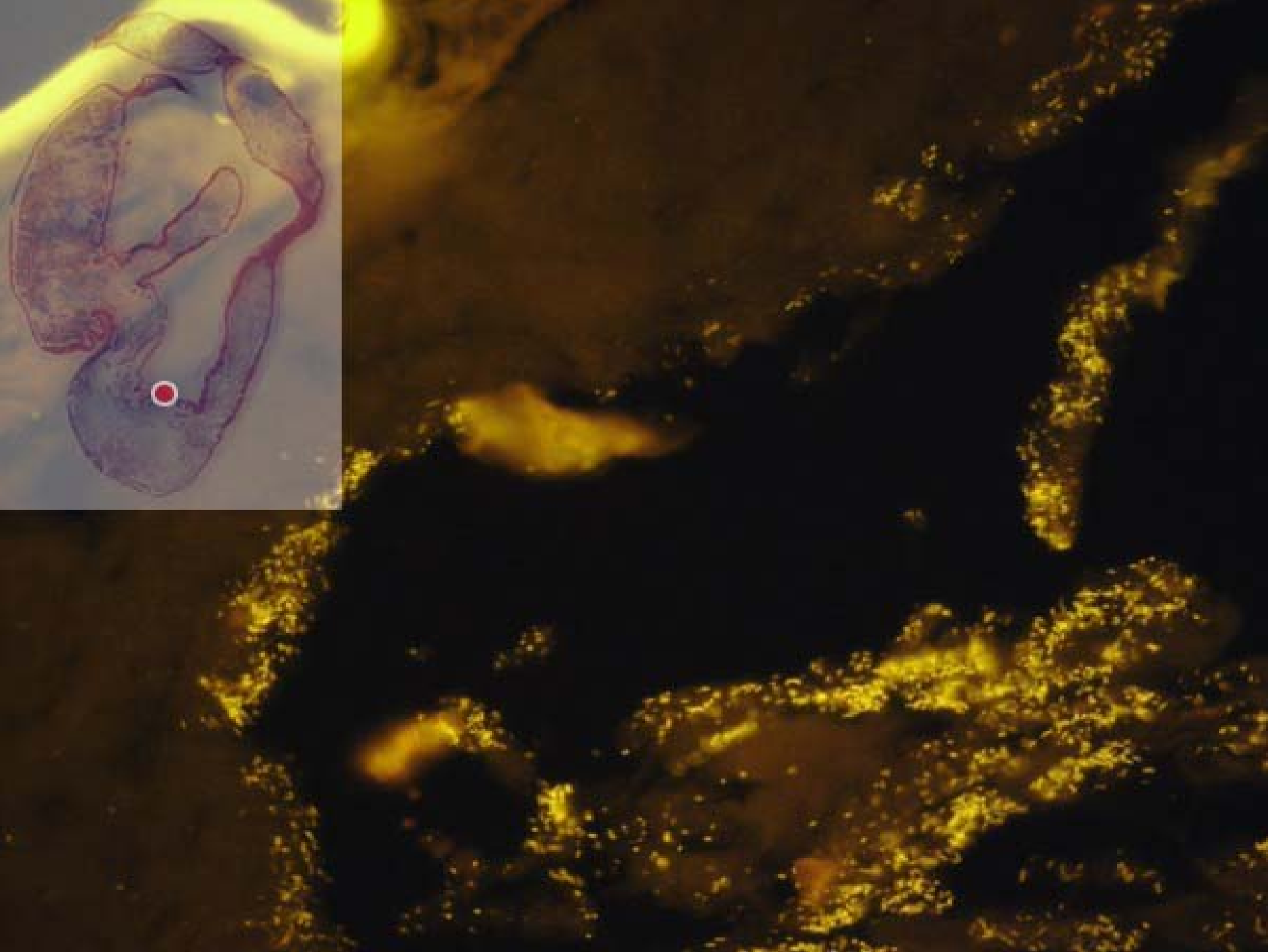


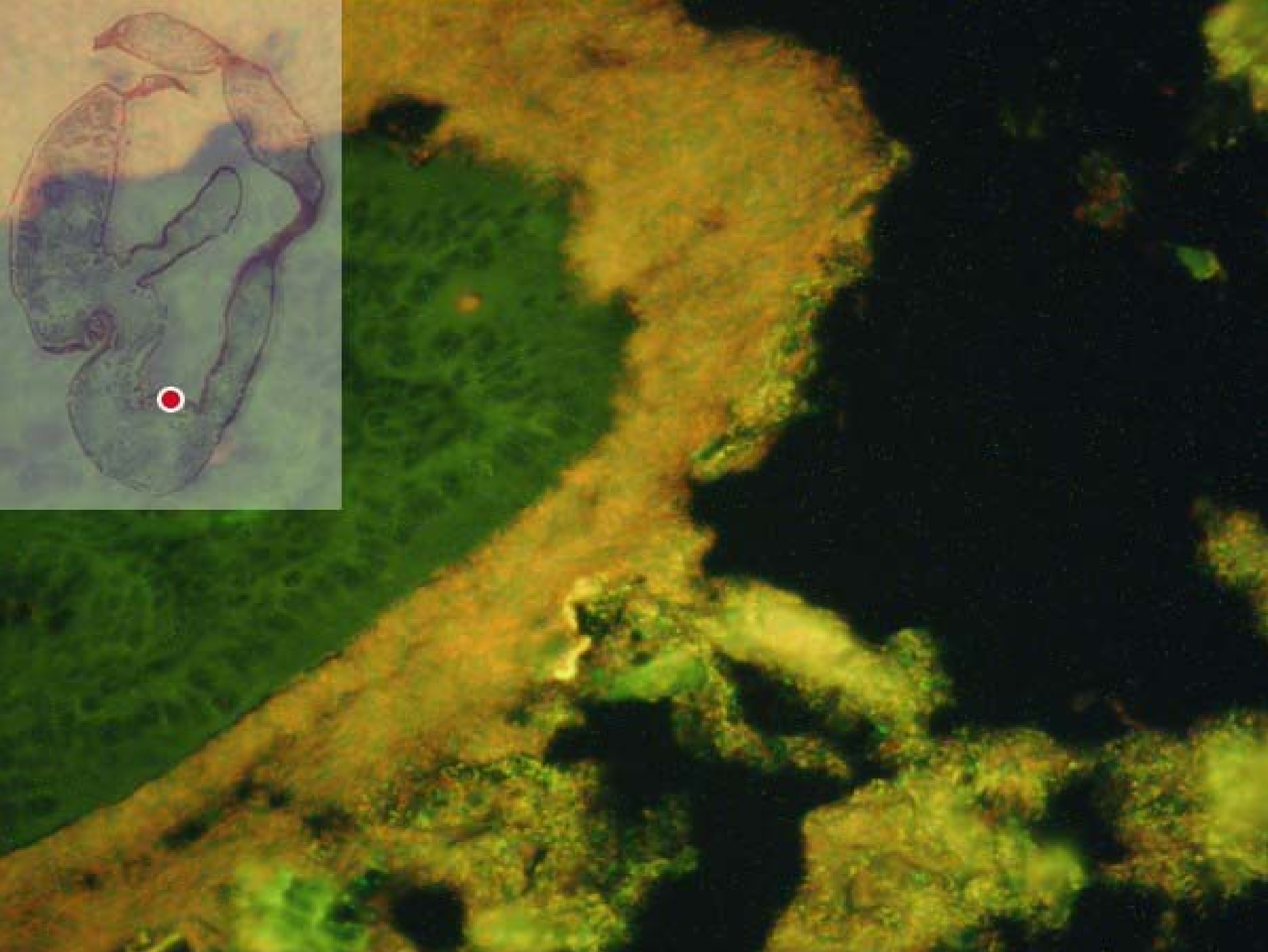


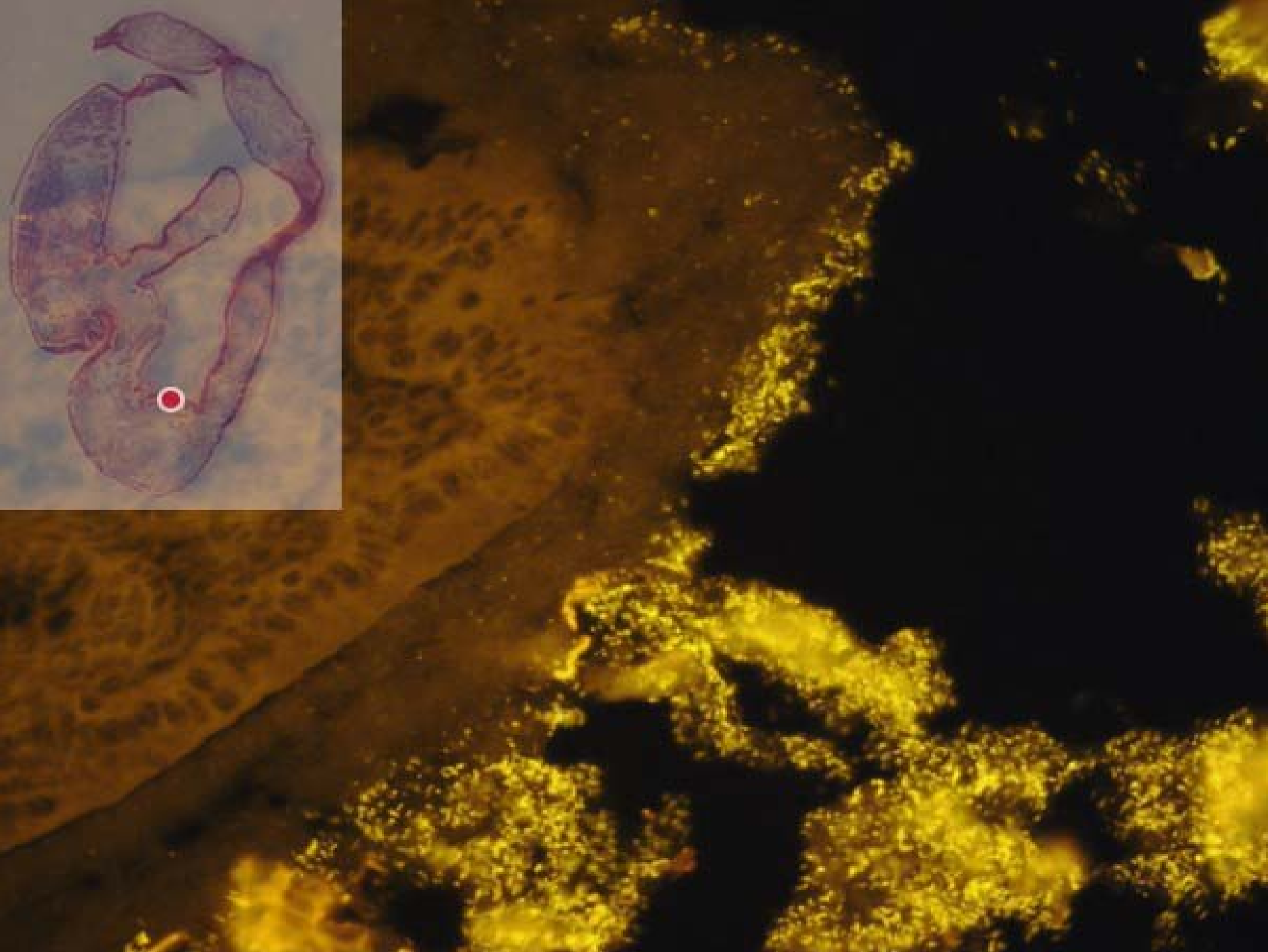


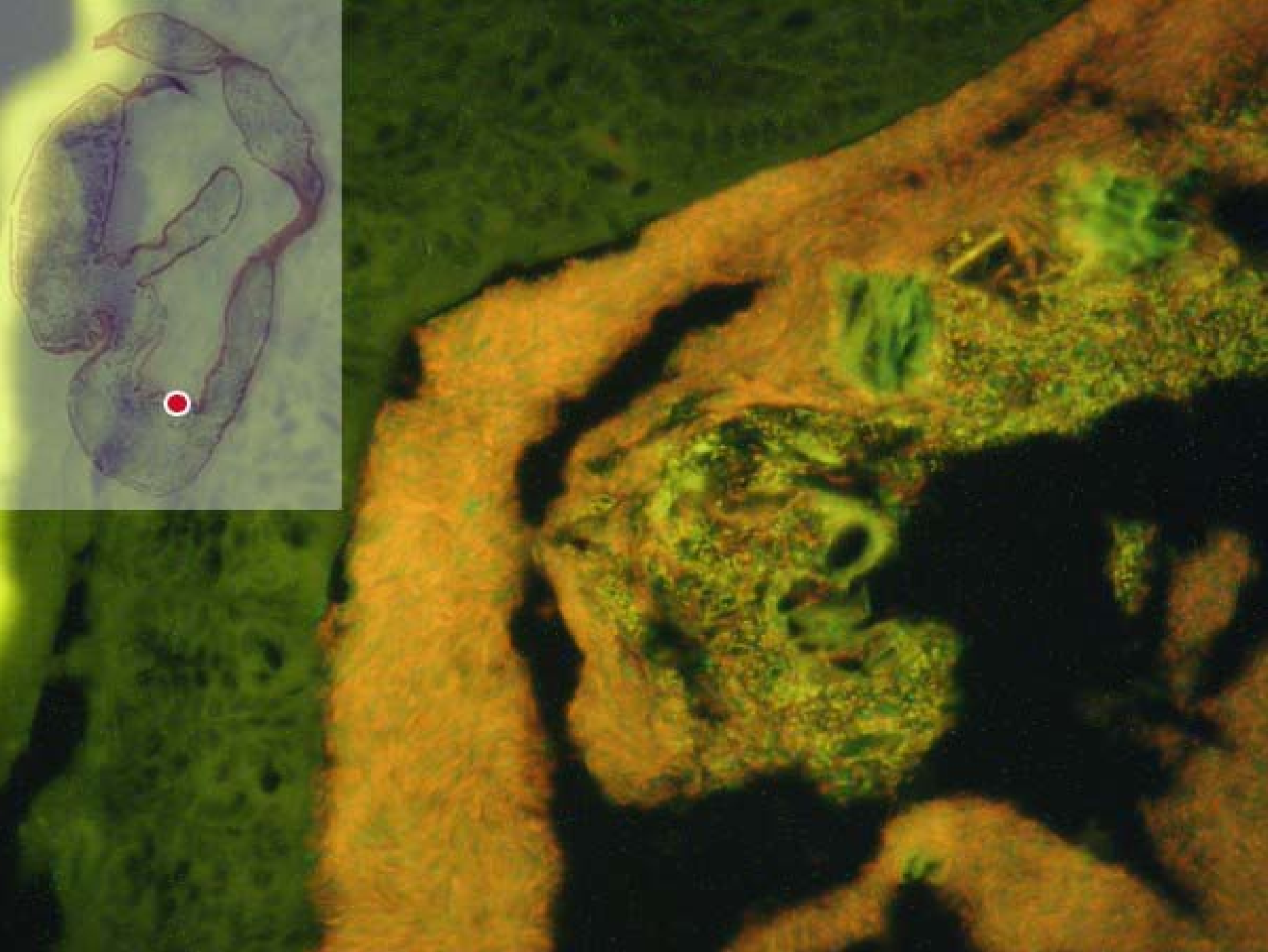


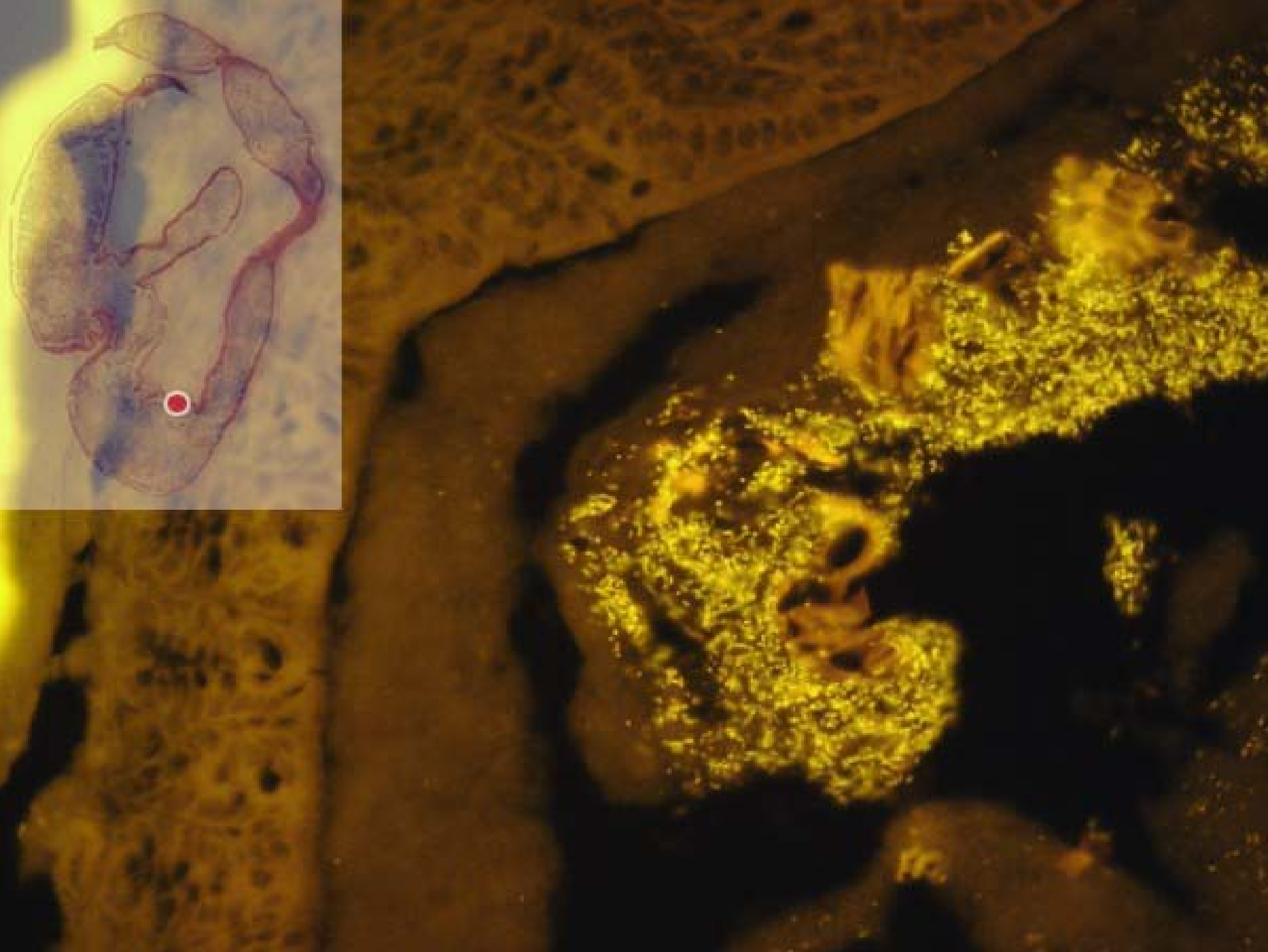


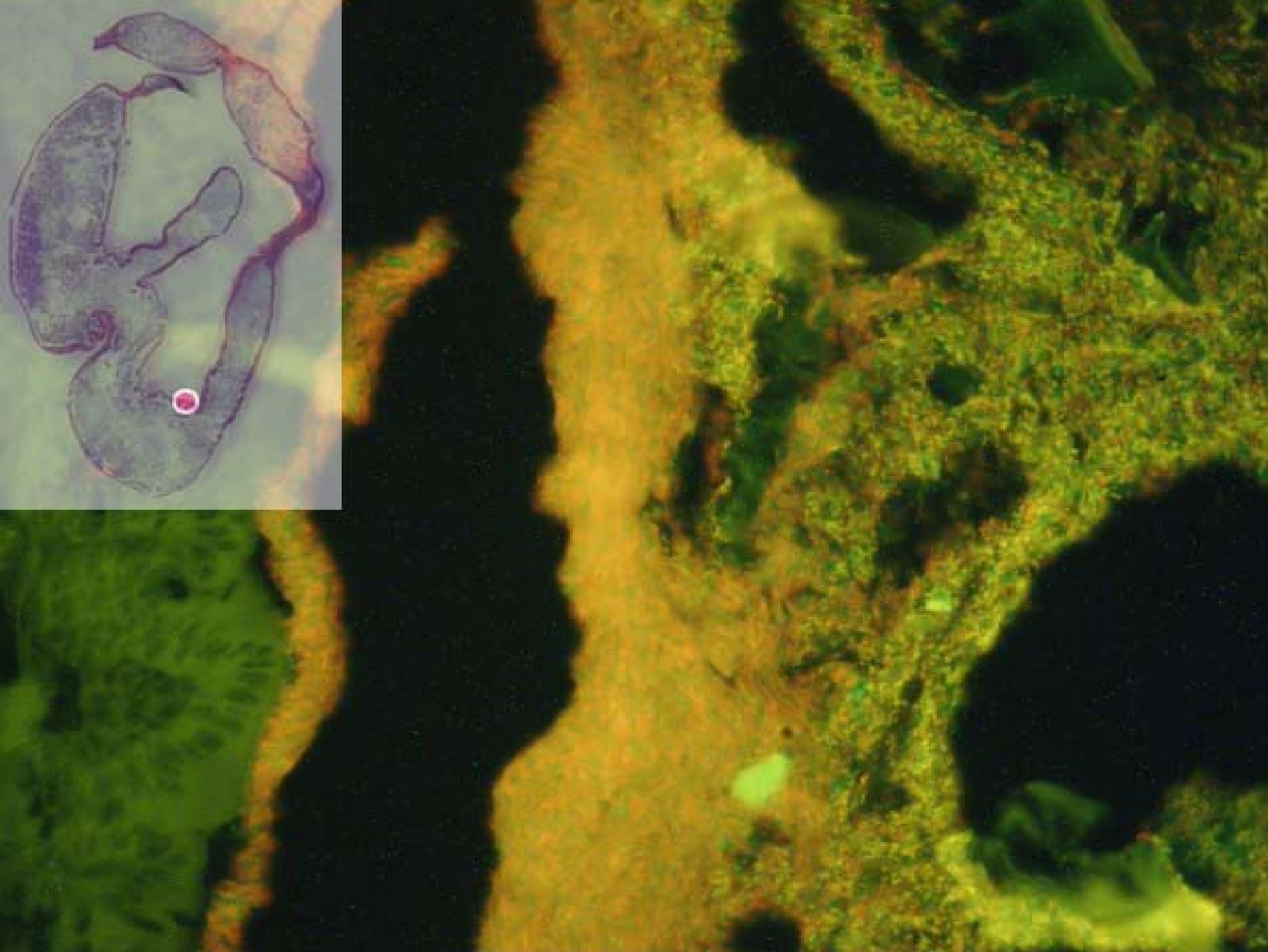


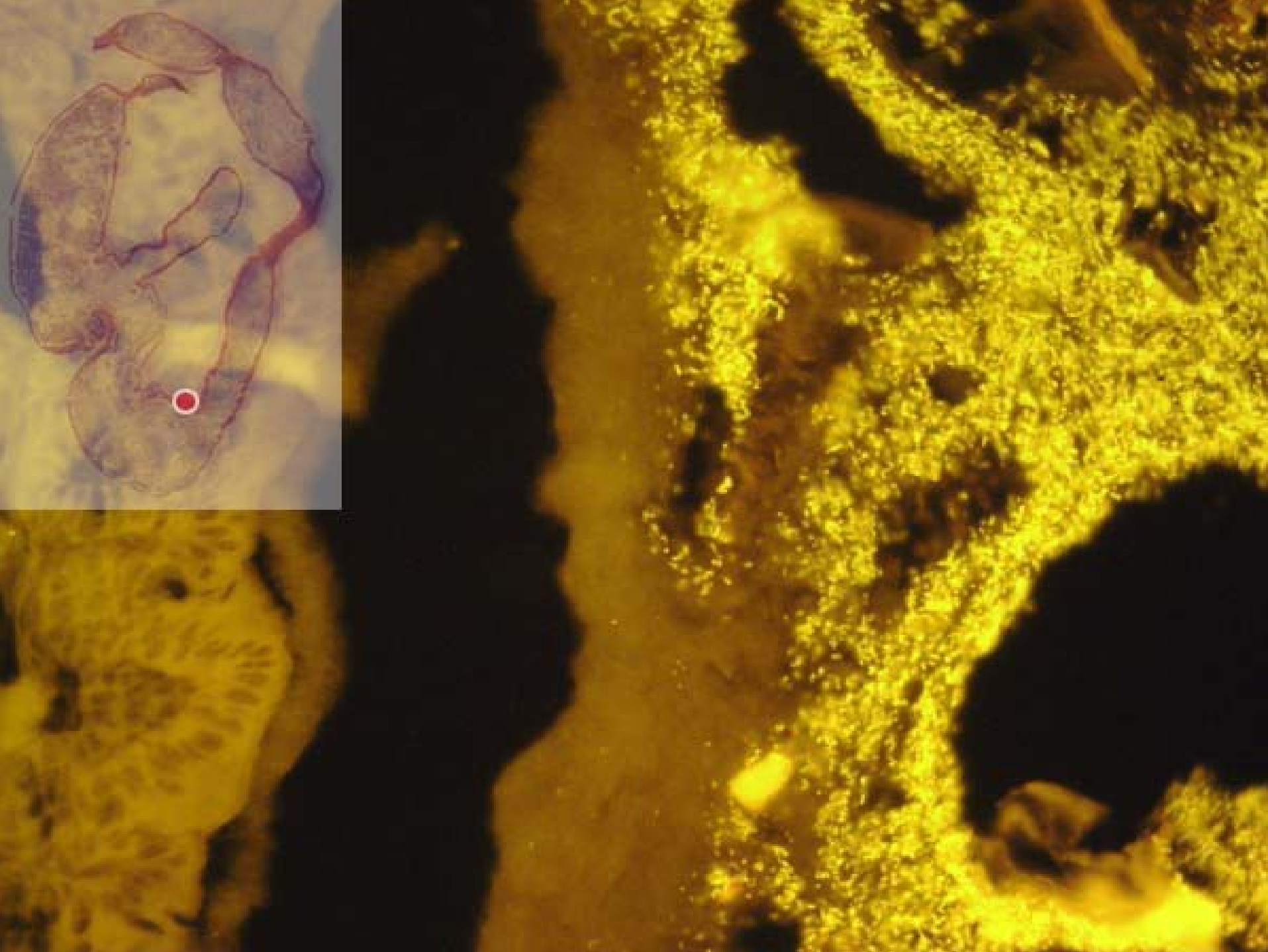


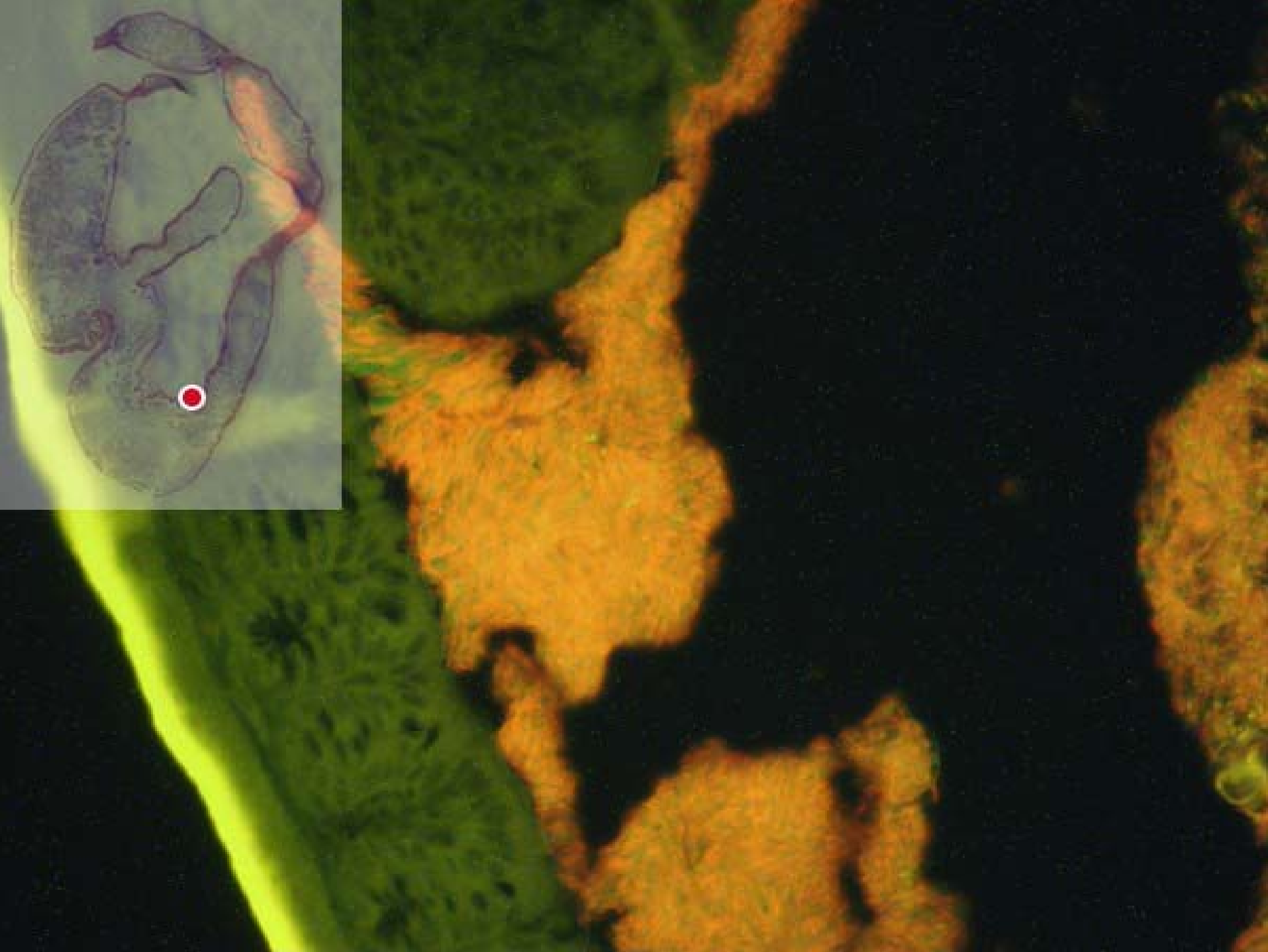


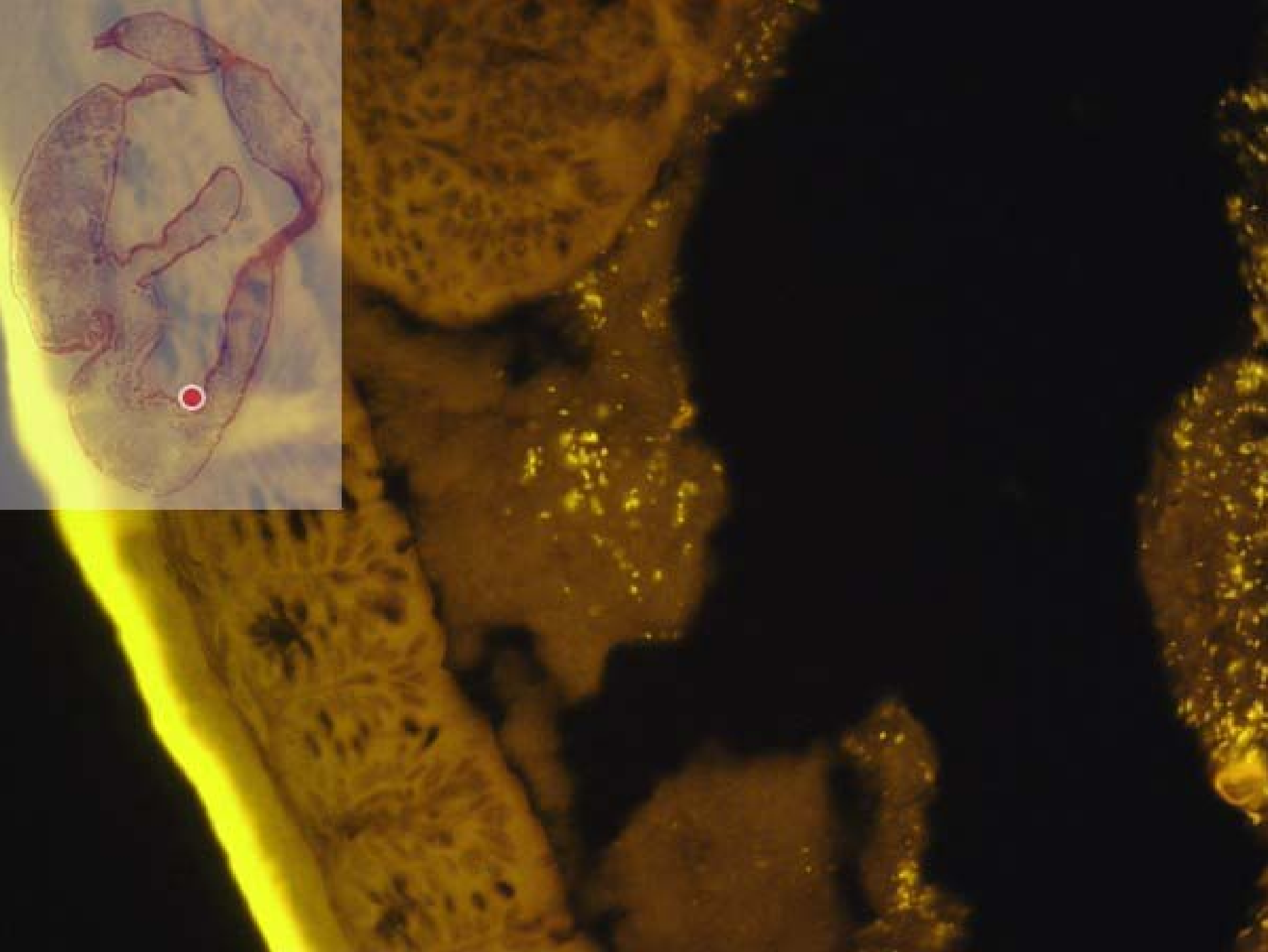


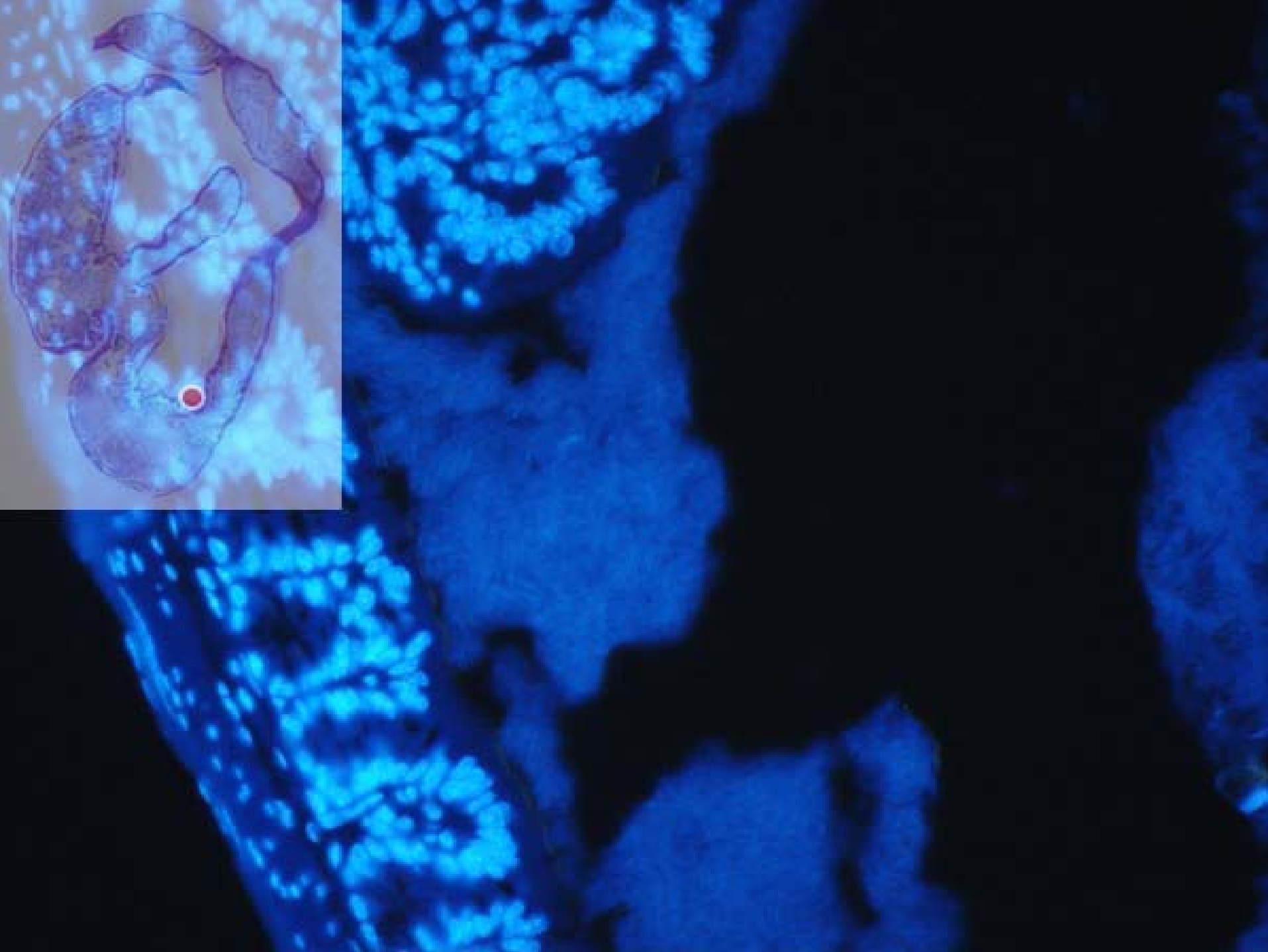


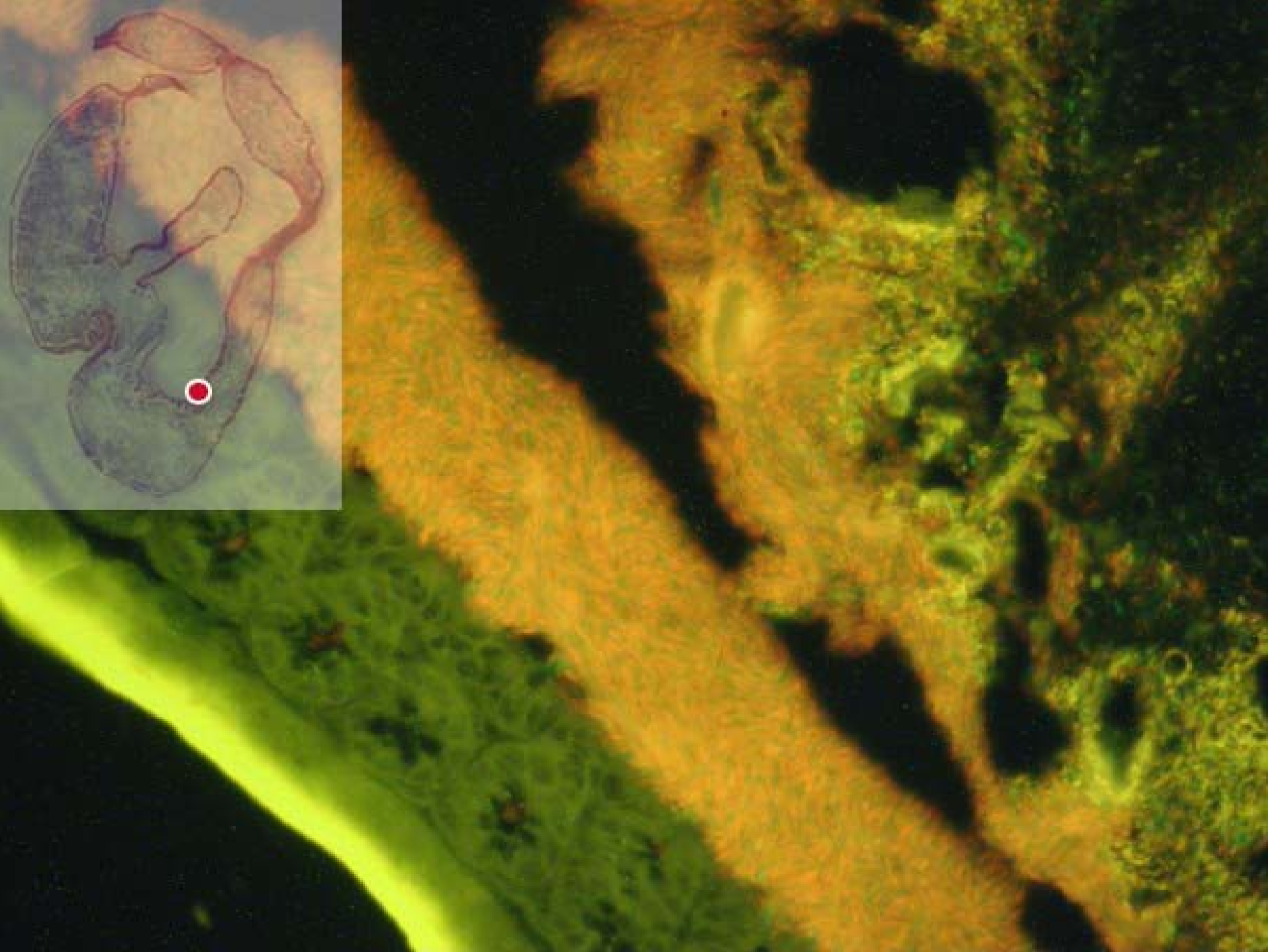


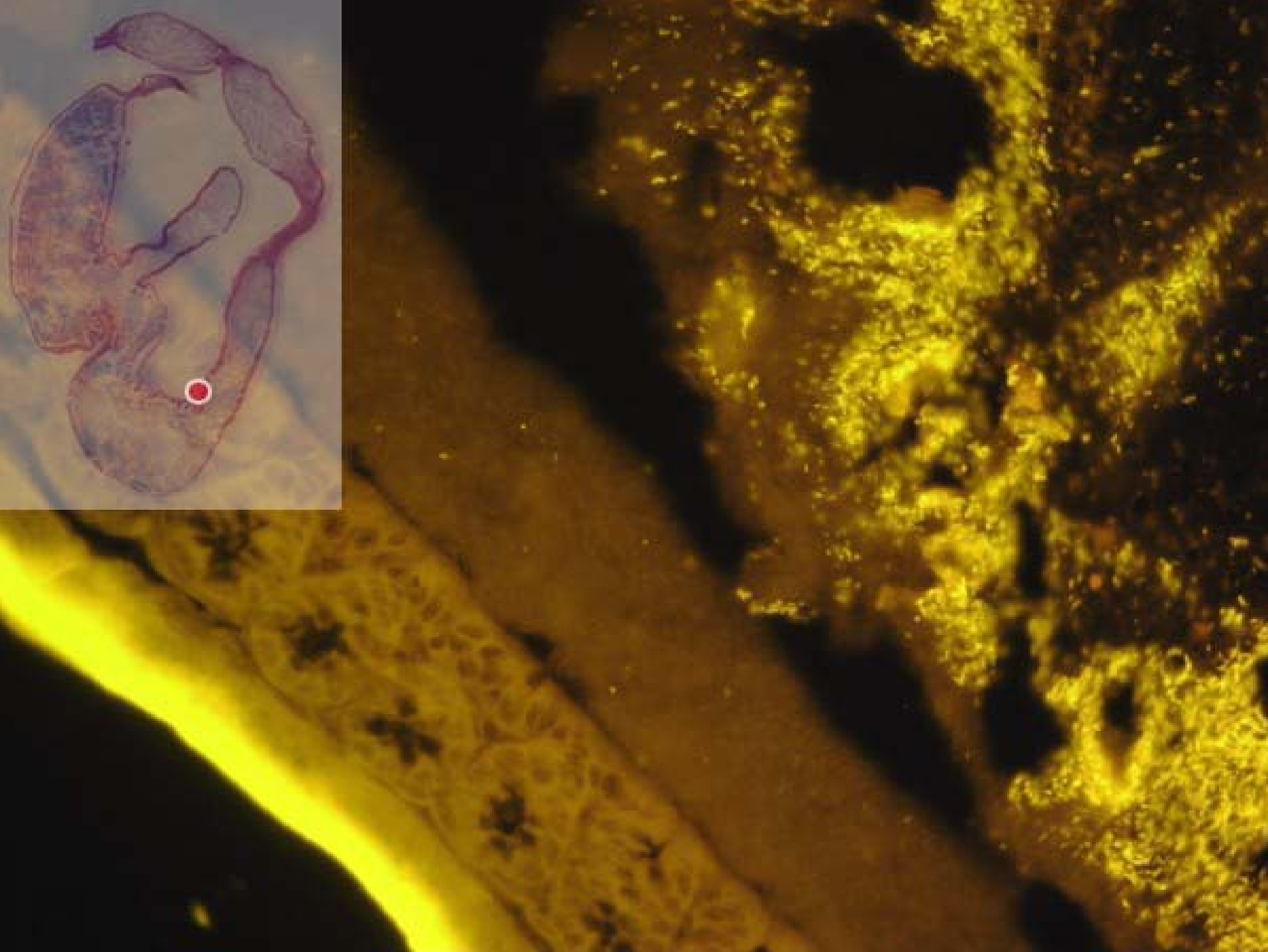


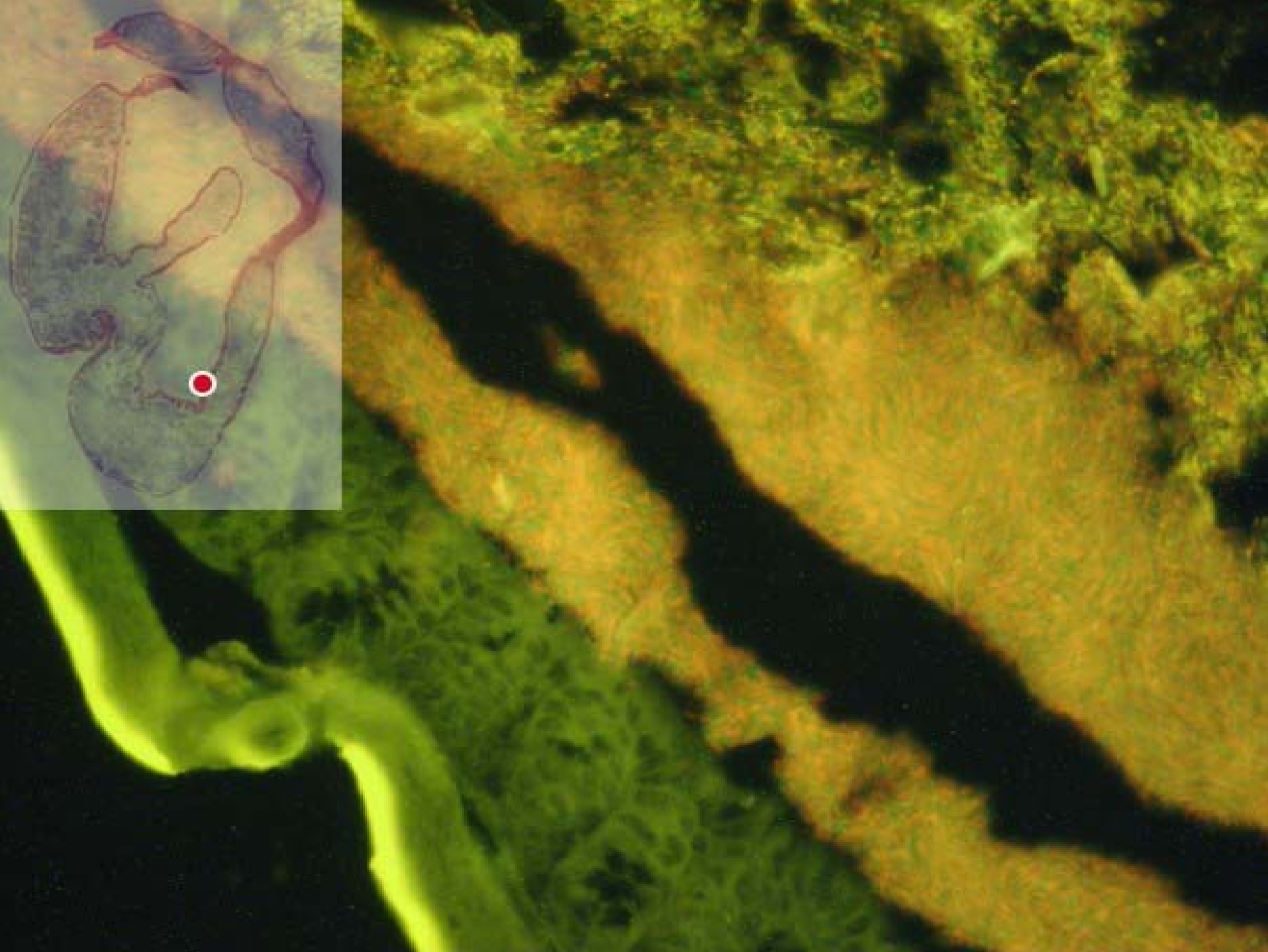


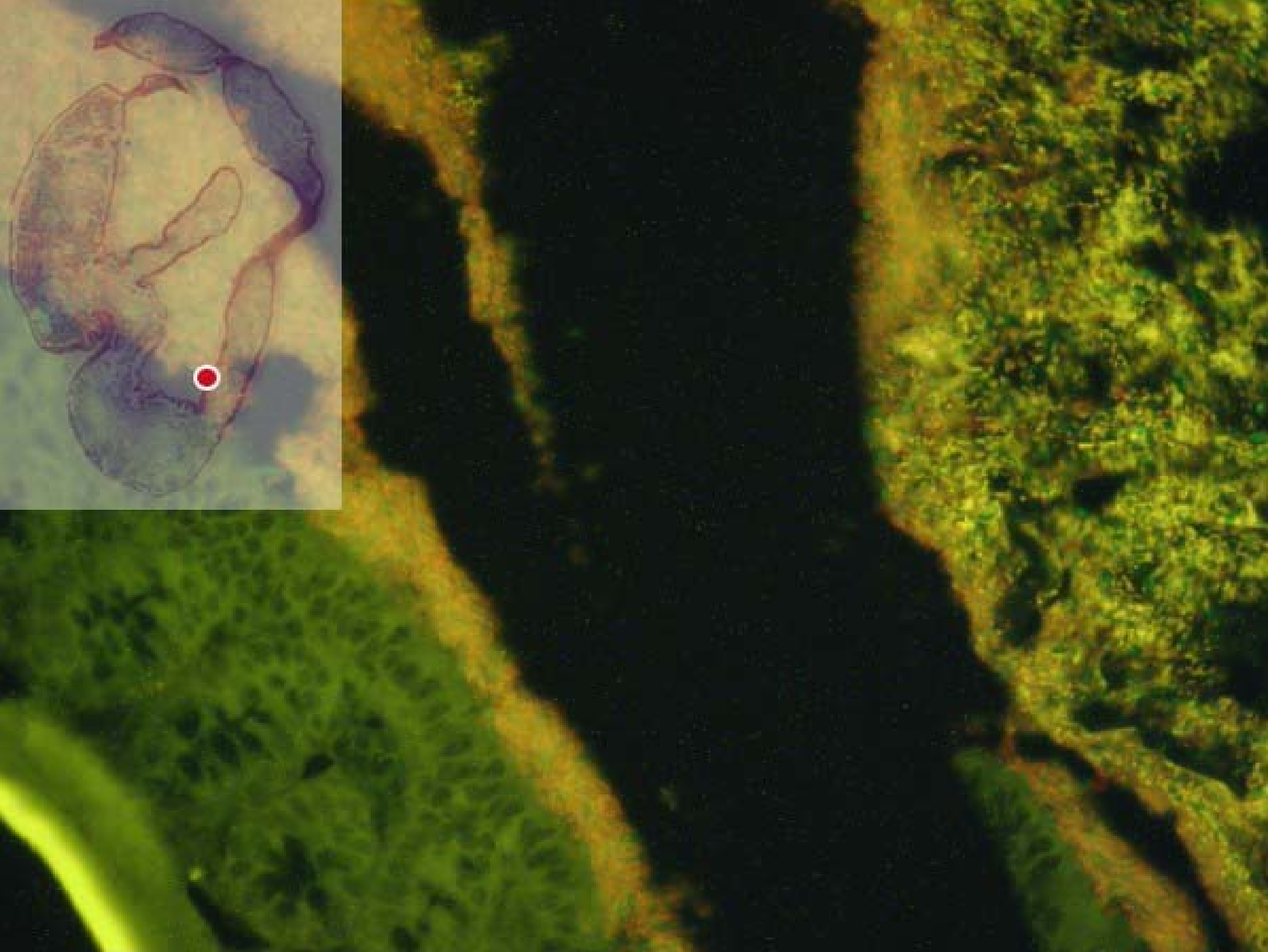


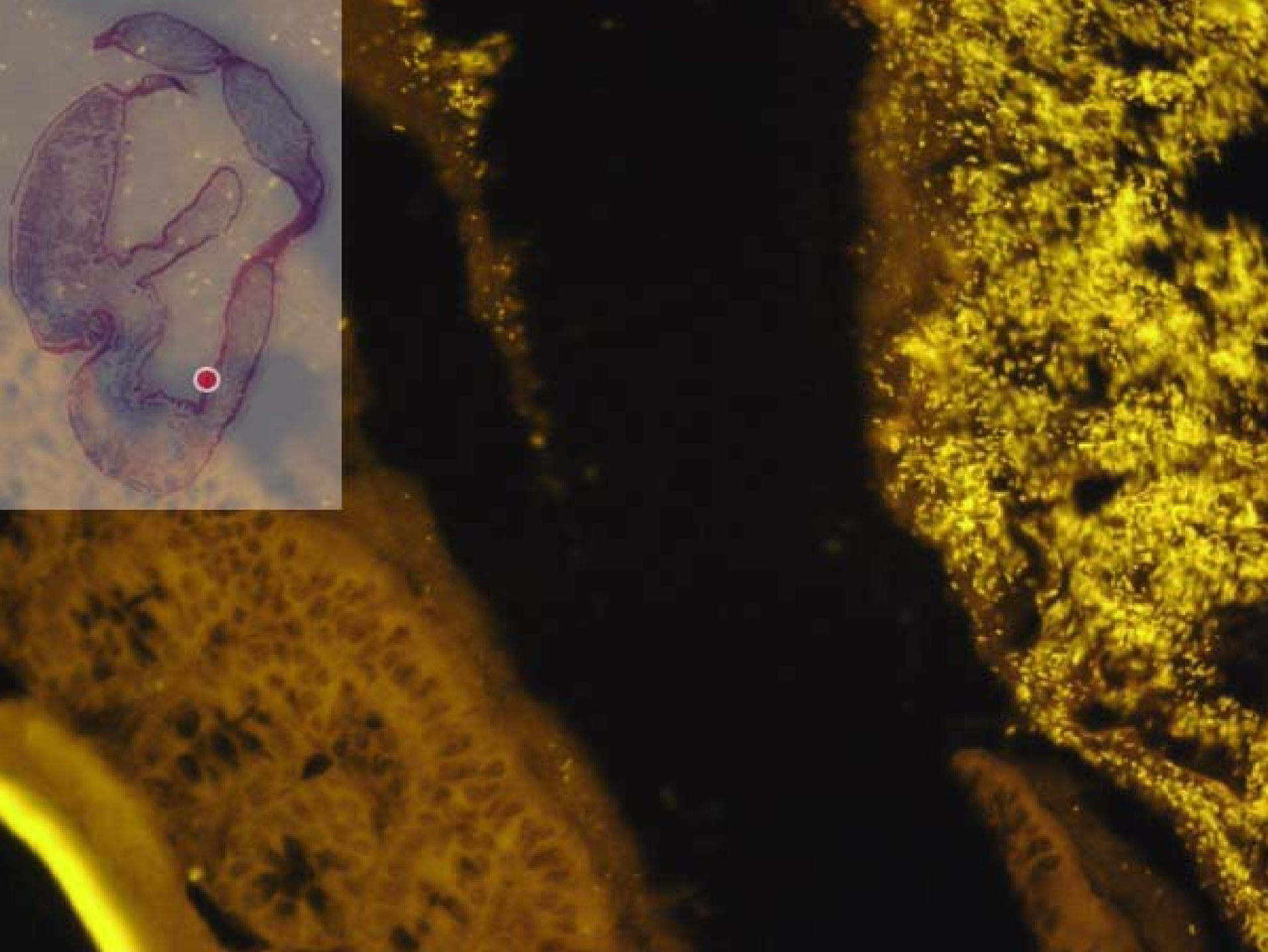






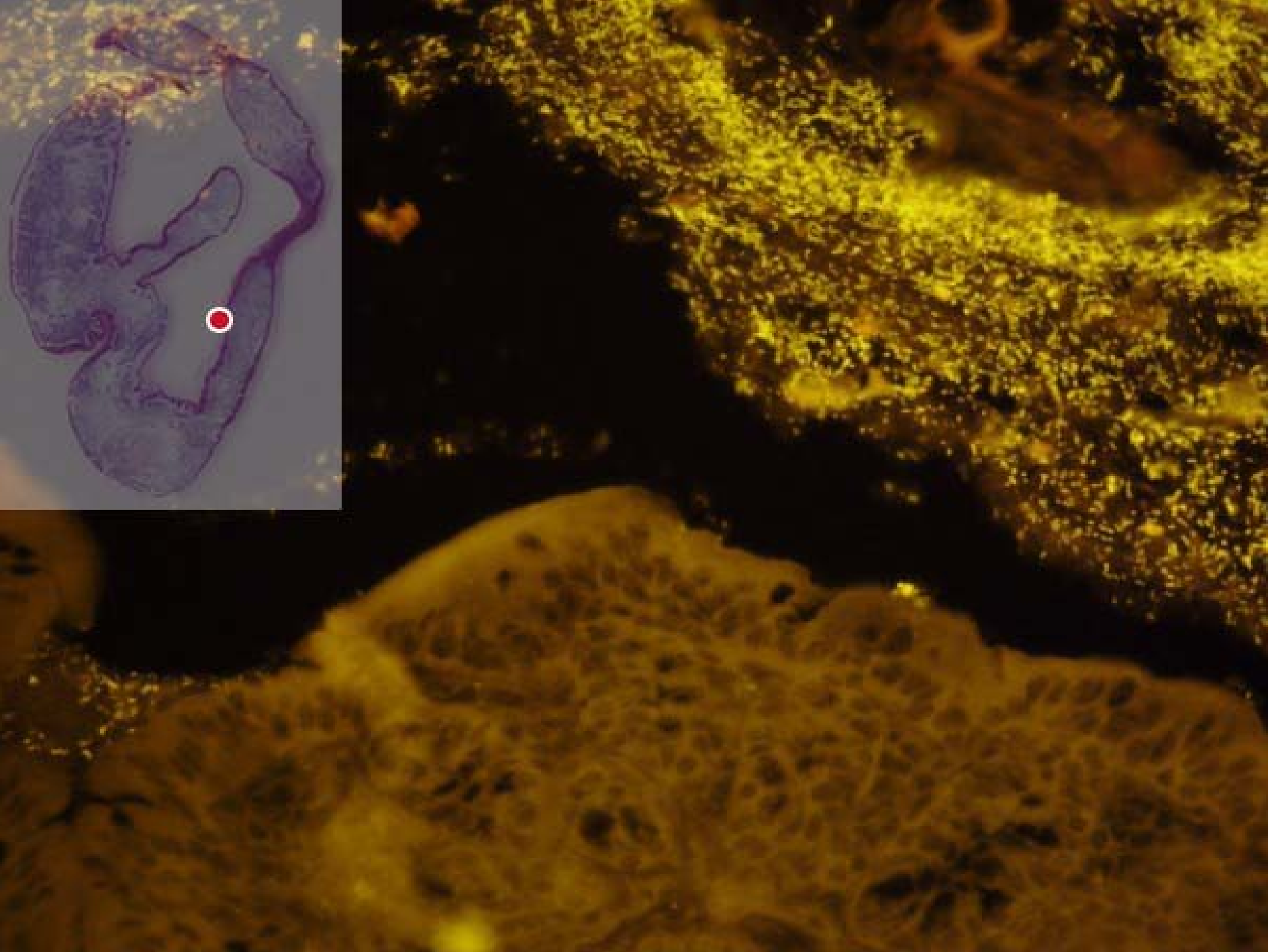


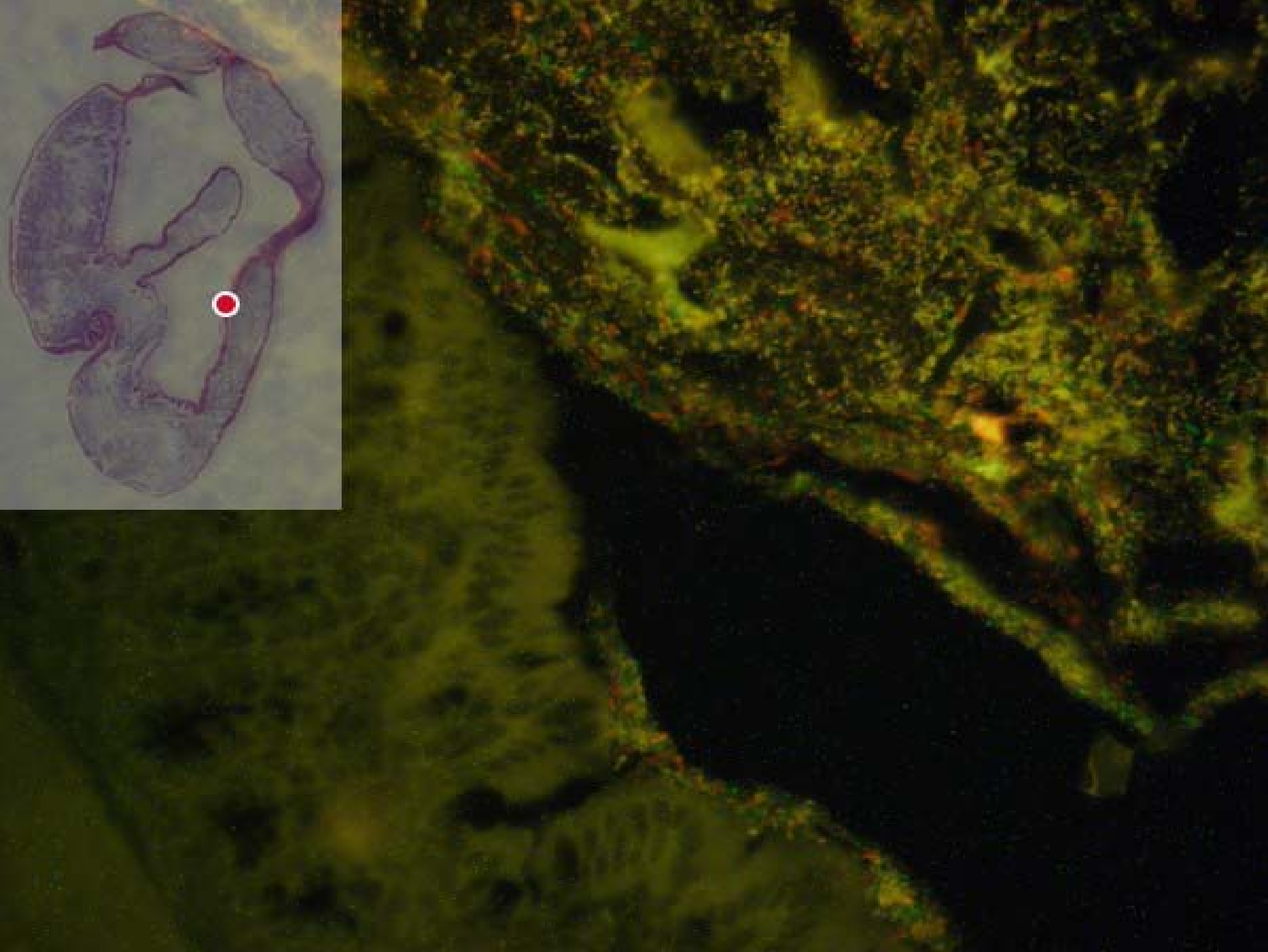


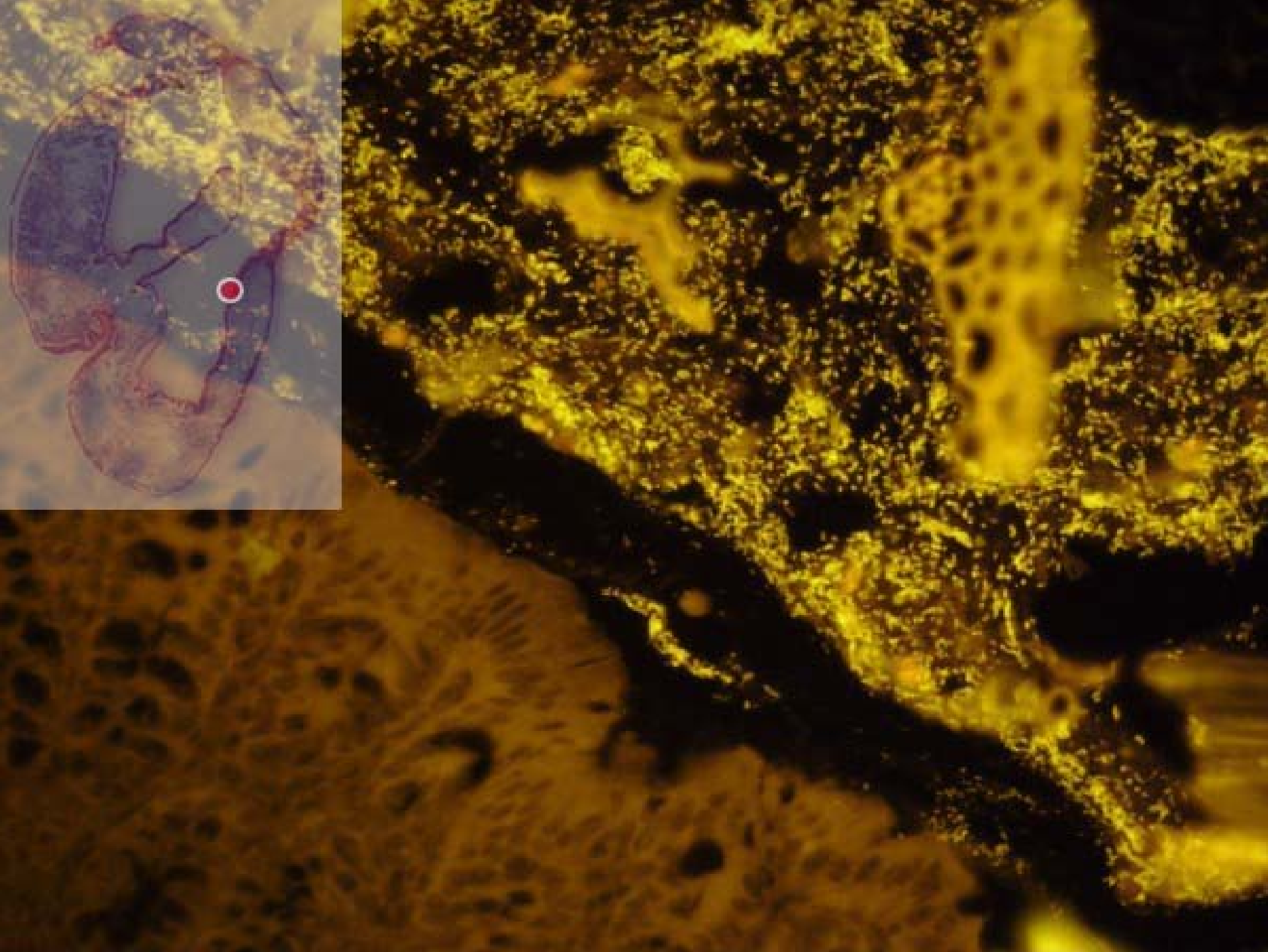


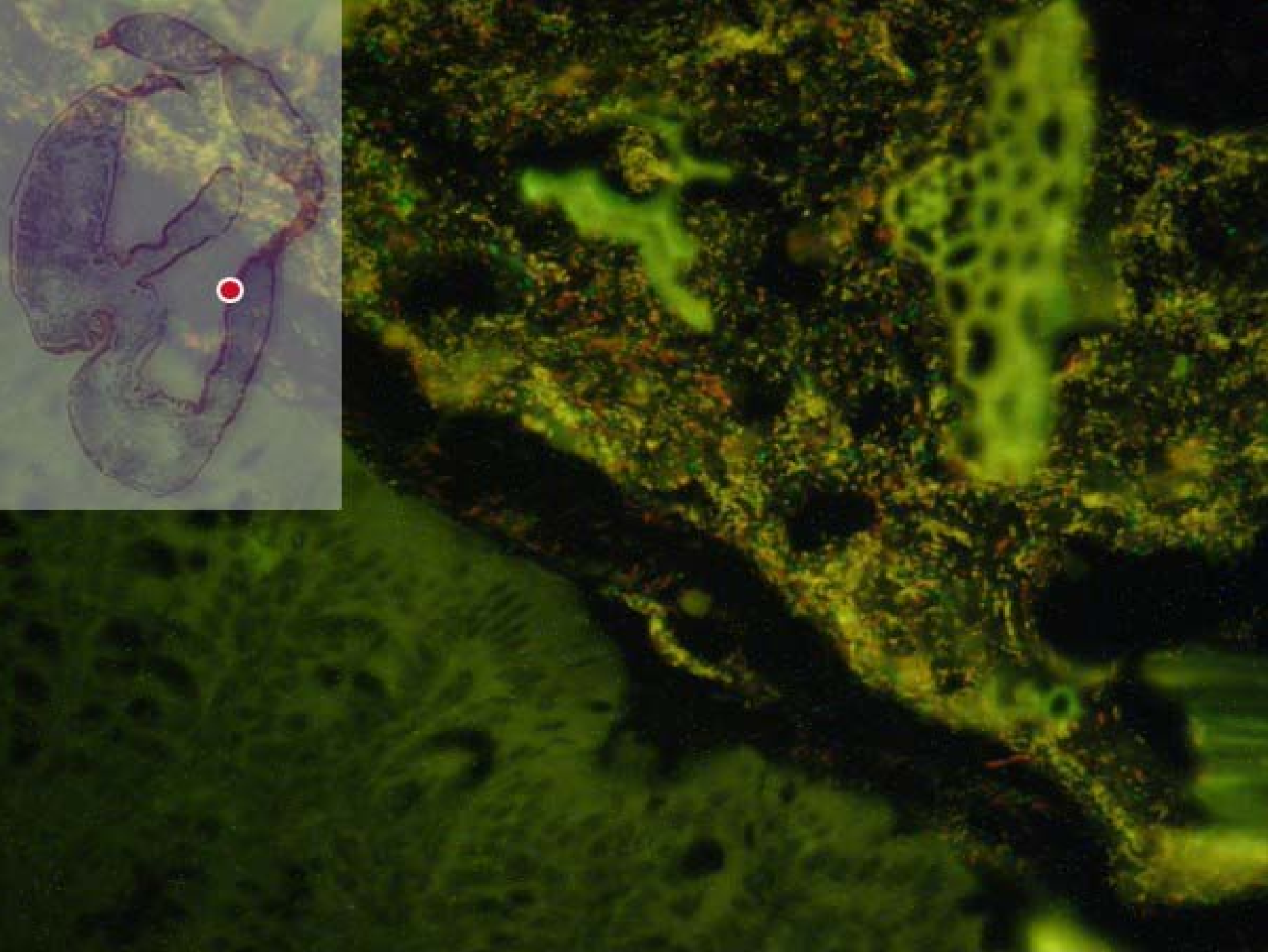
Zusammensetzung der Sperrschicht

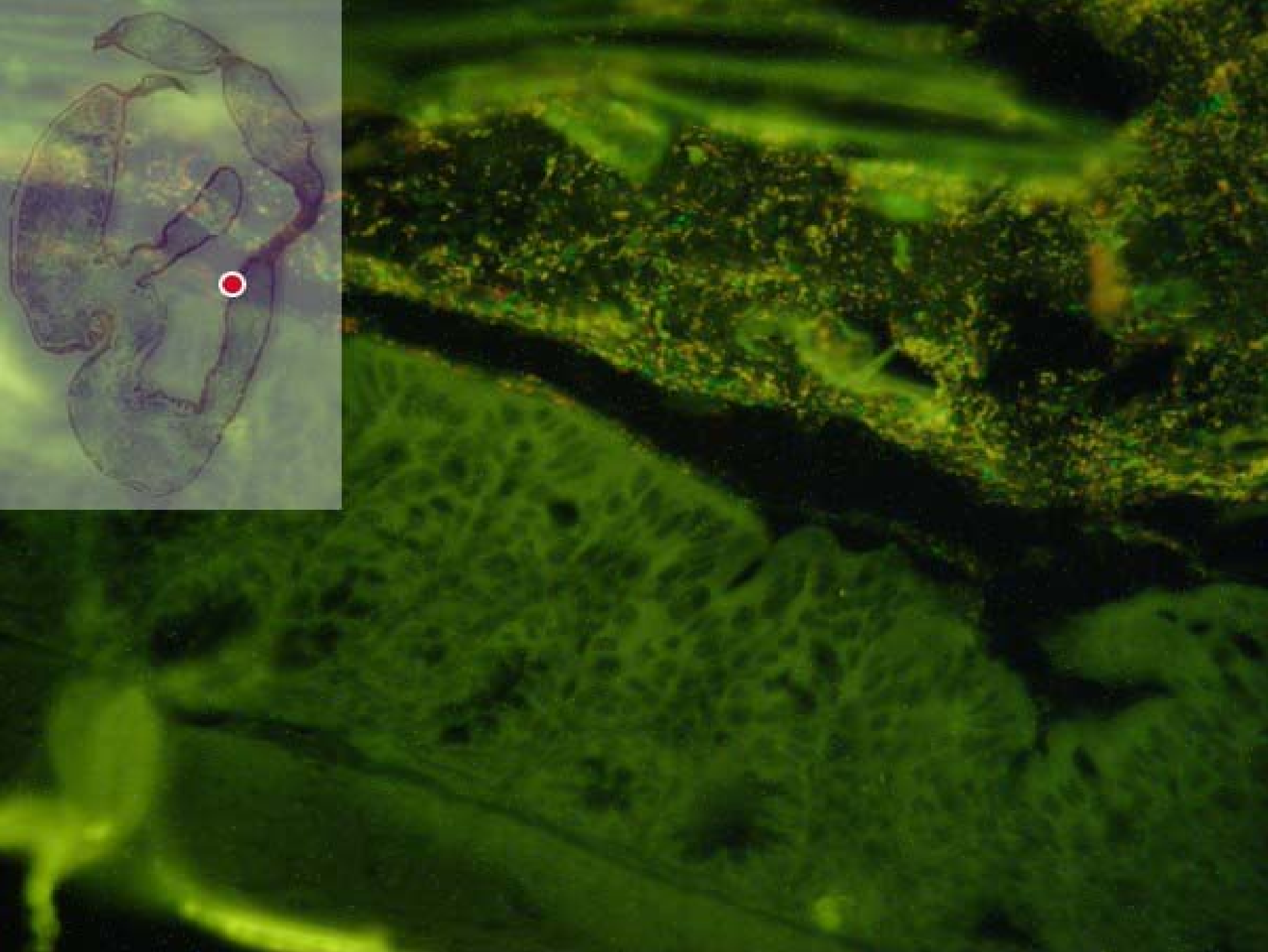
Bacteroides,
Enterobacteriaceae,
Clostridium difficile,
Veillonella
haben keinen Kontakt mit der Darmwand

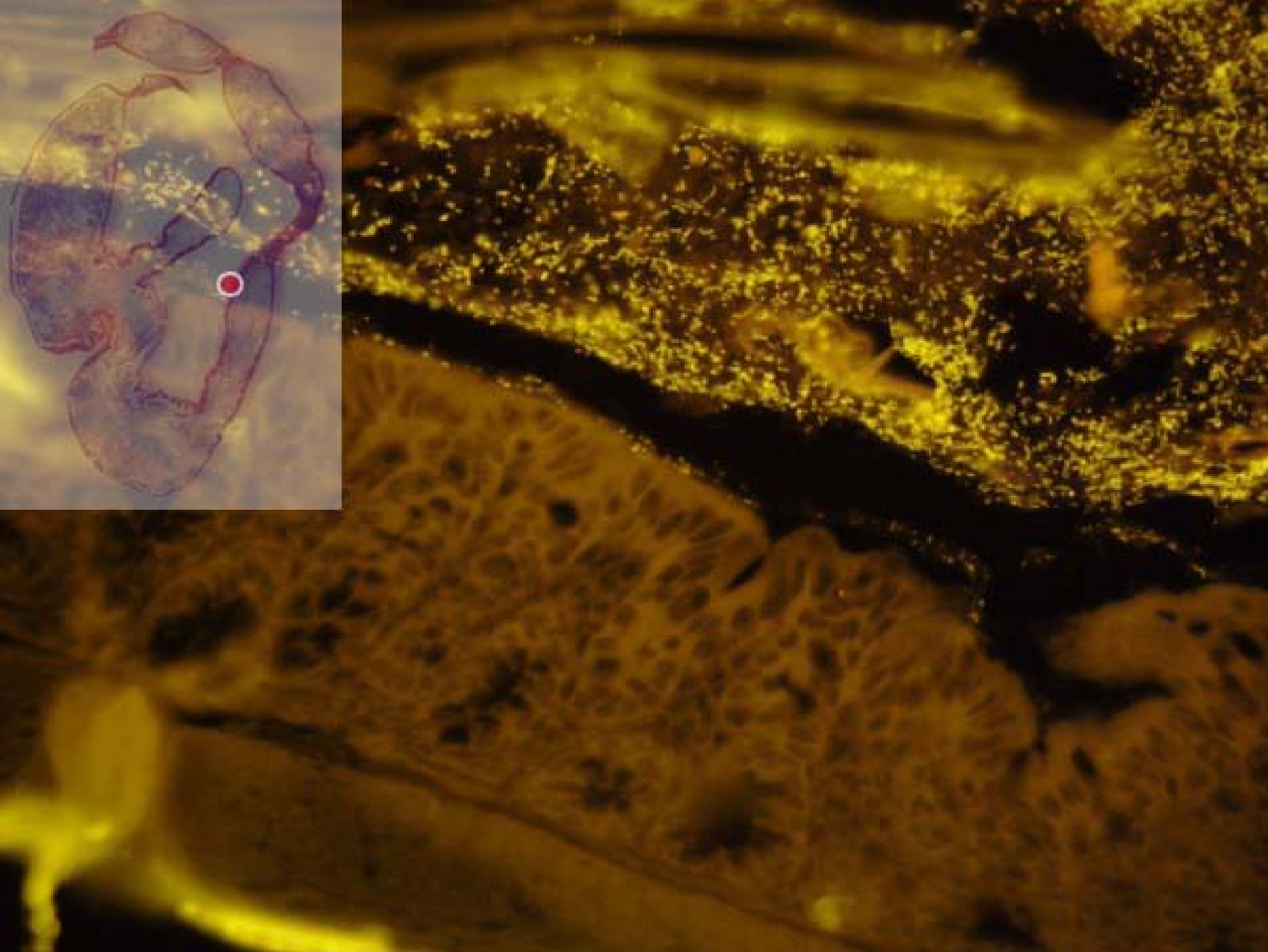


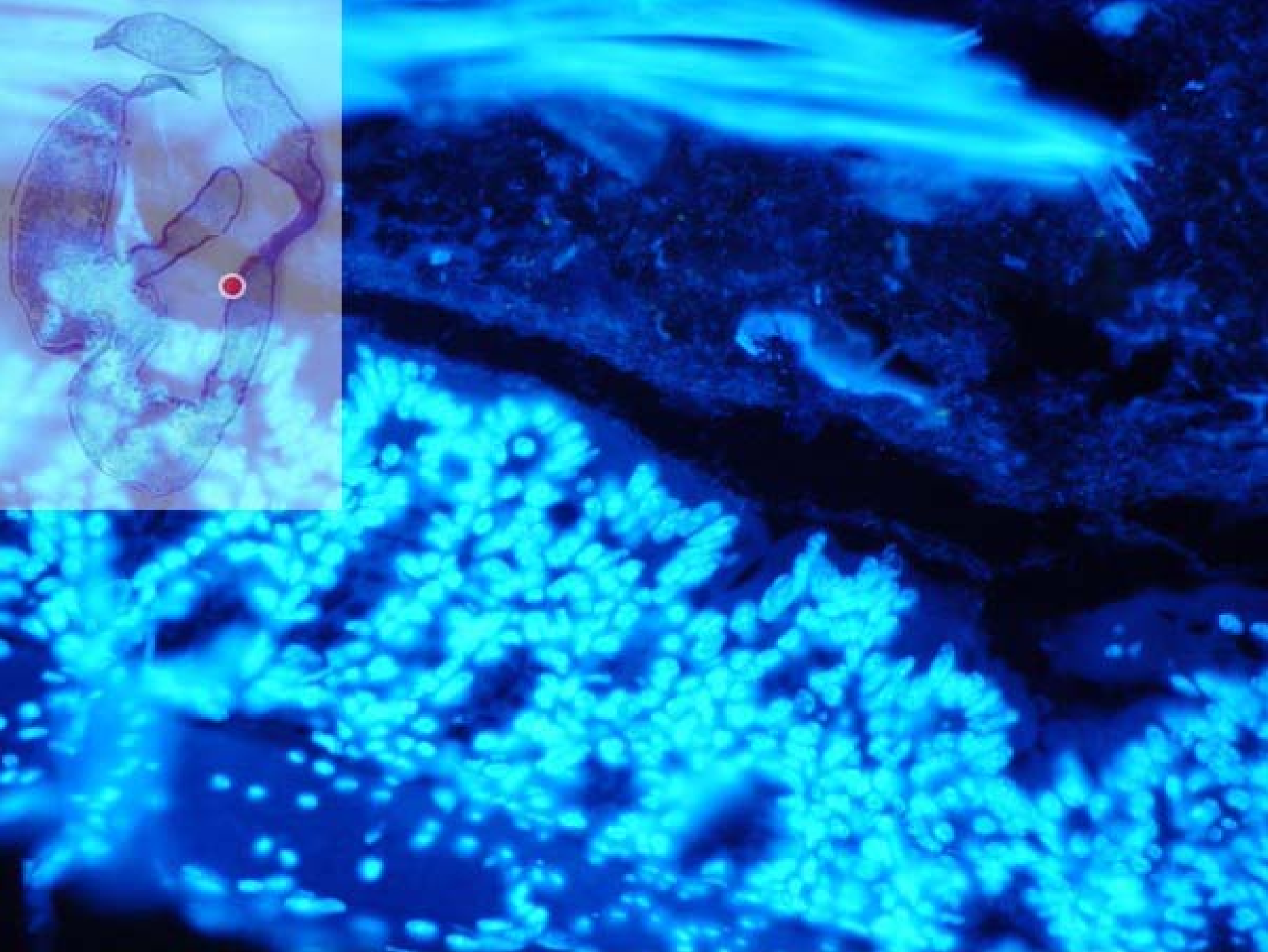


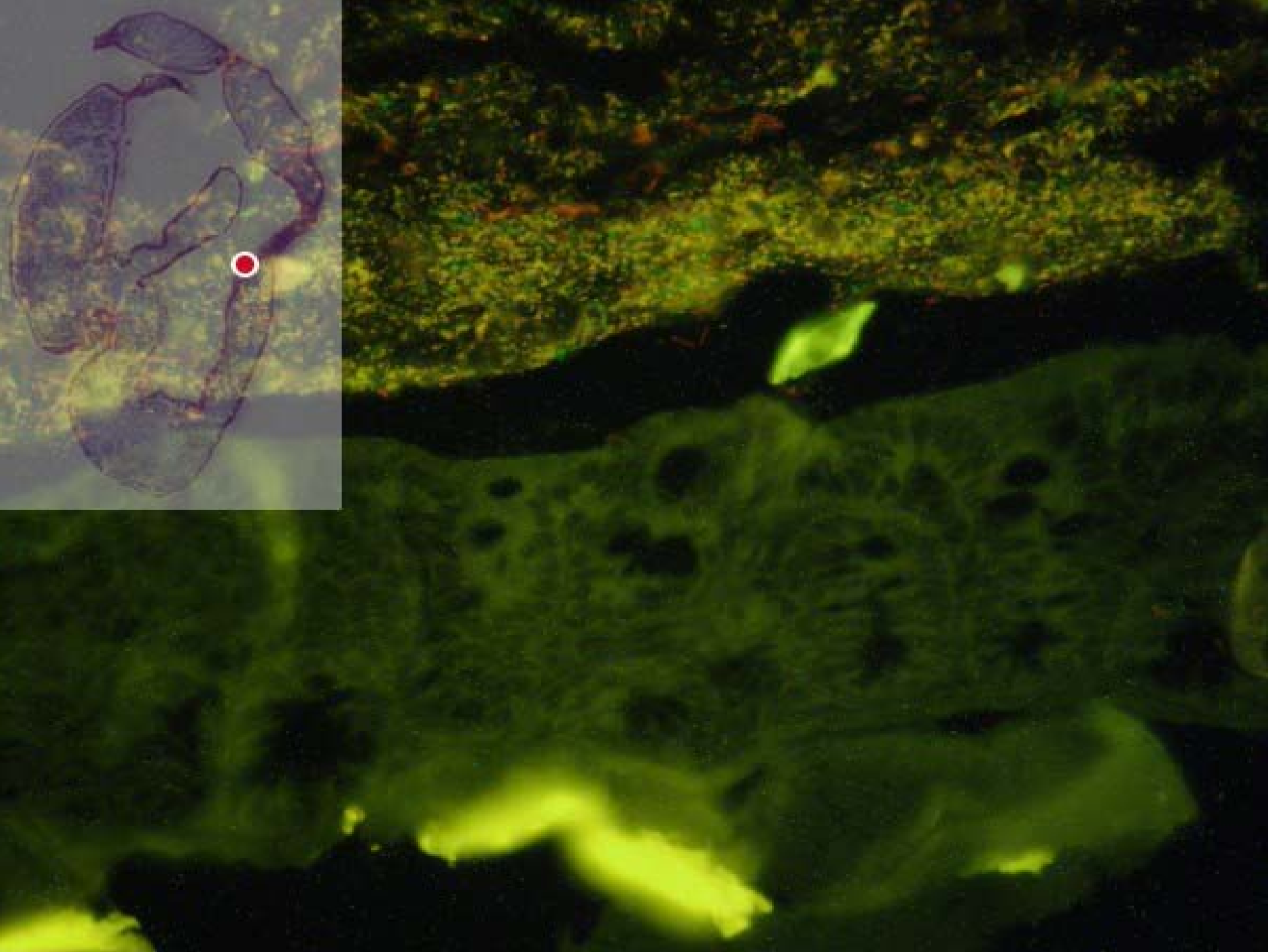


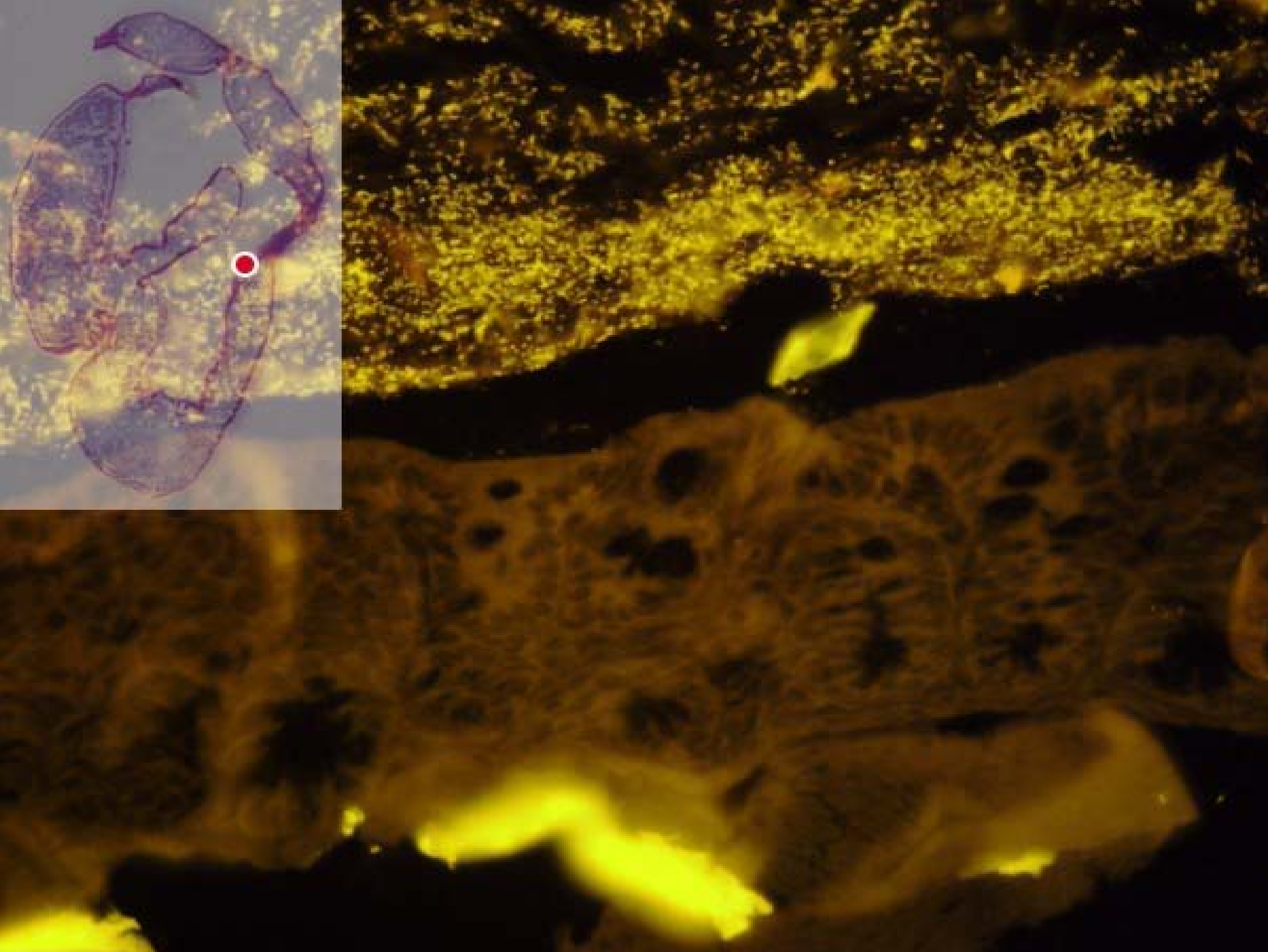


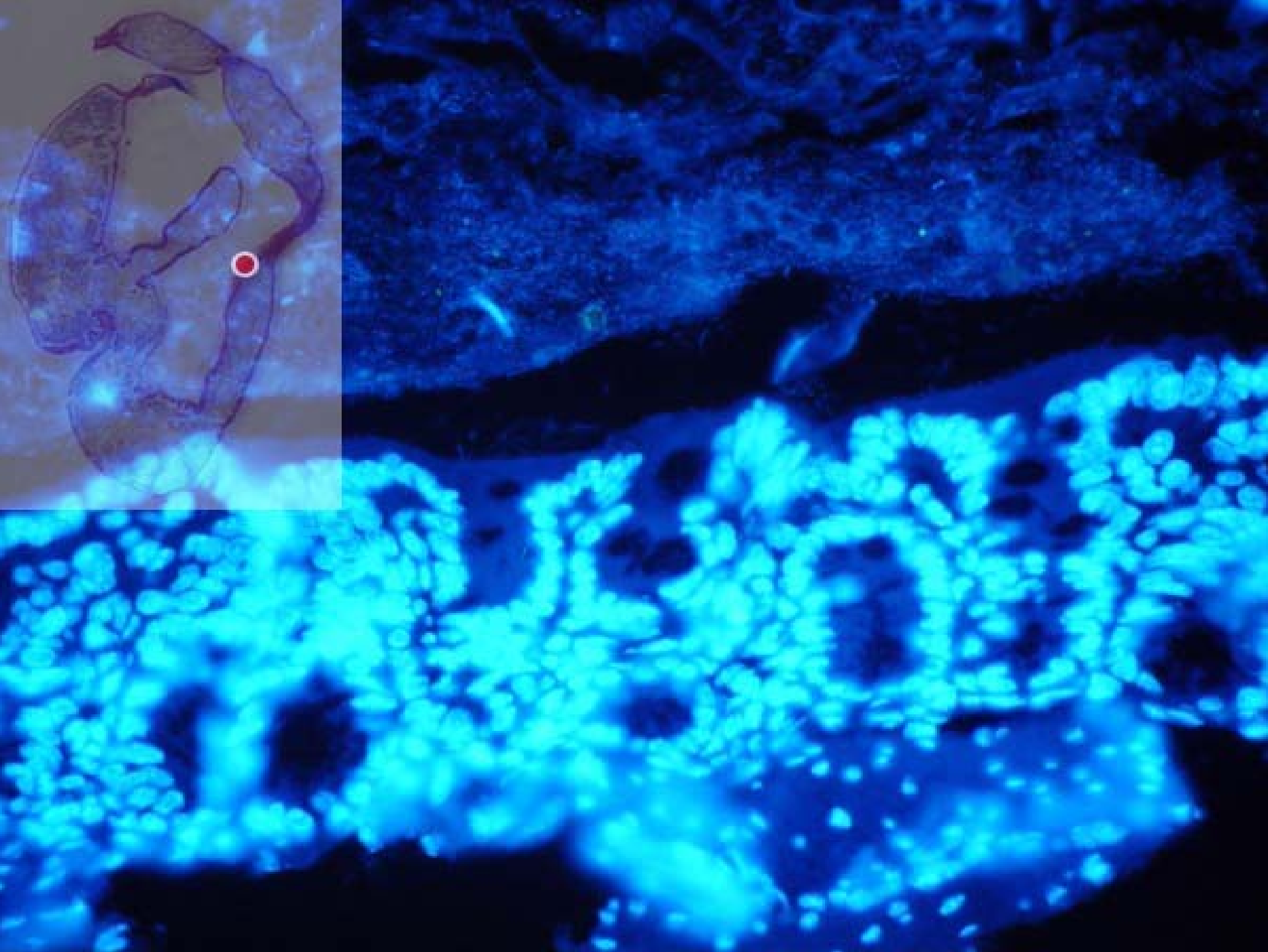


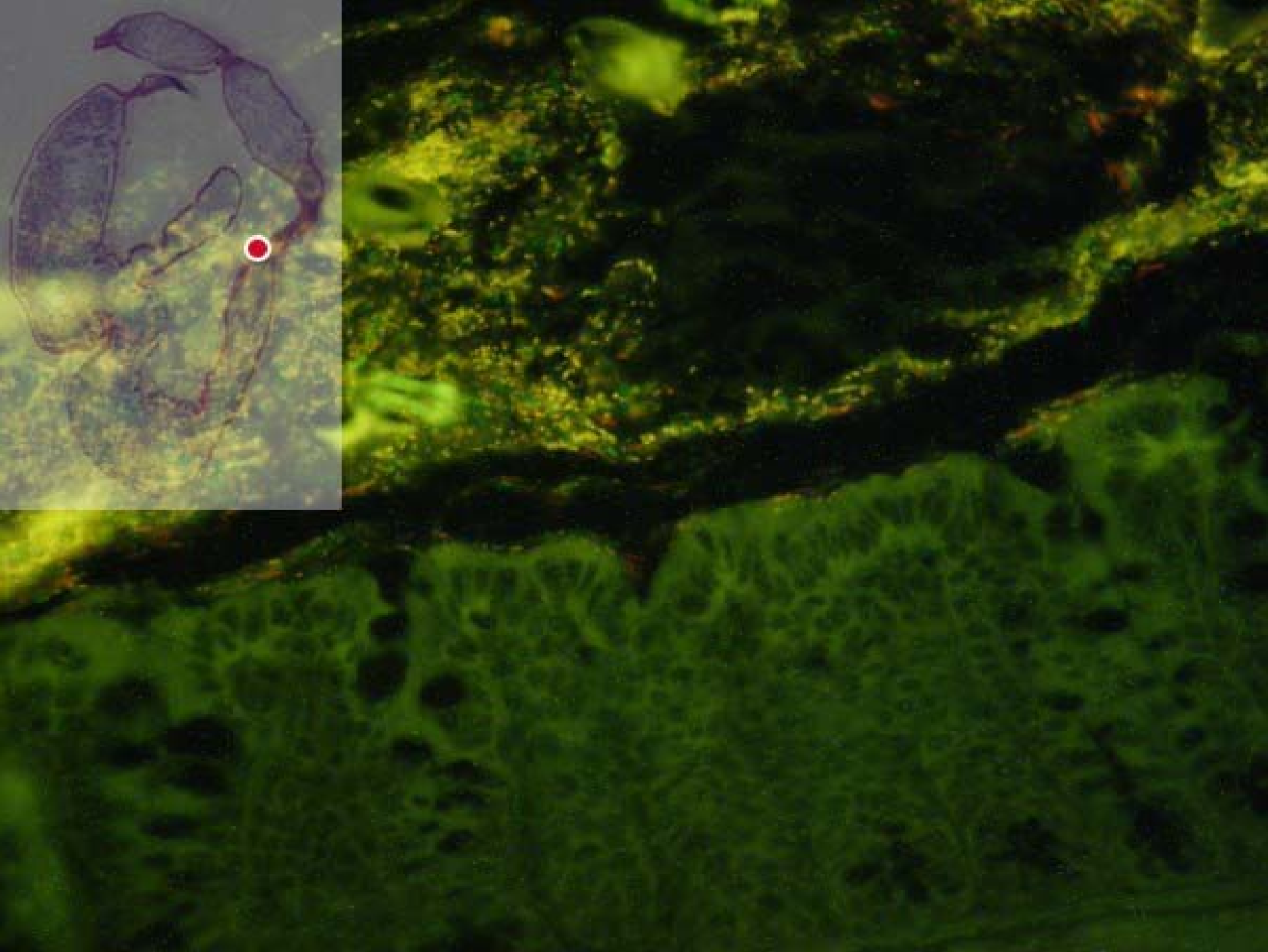


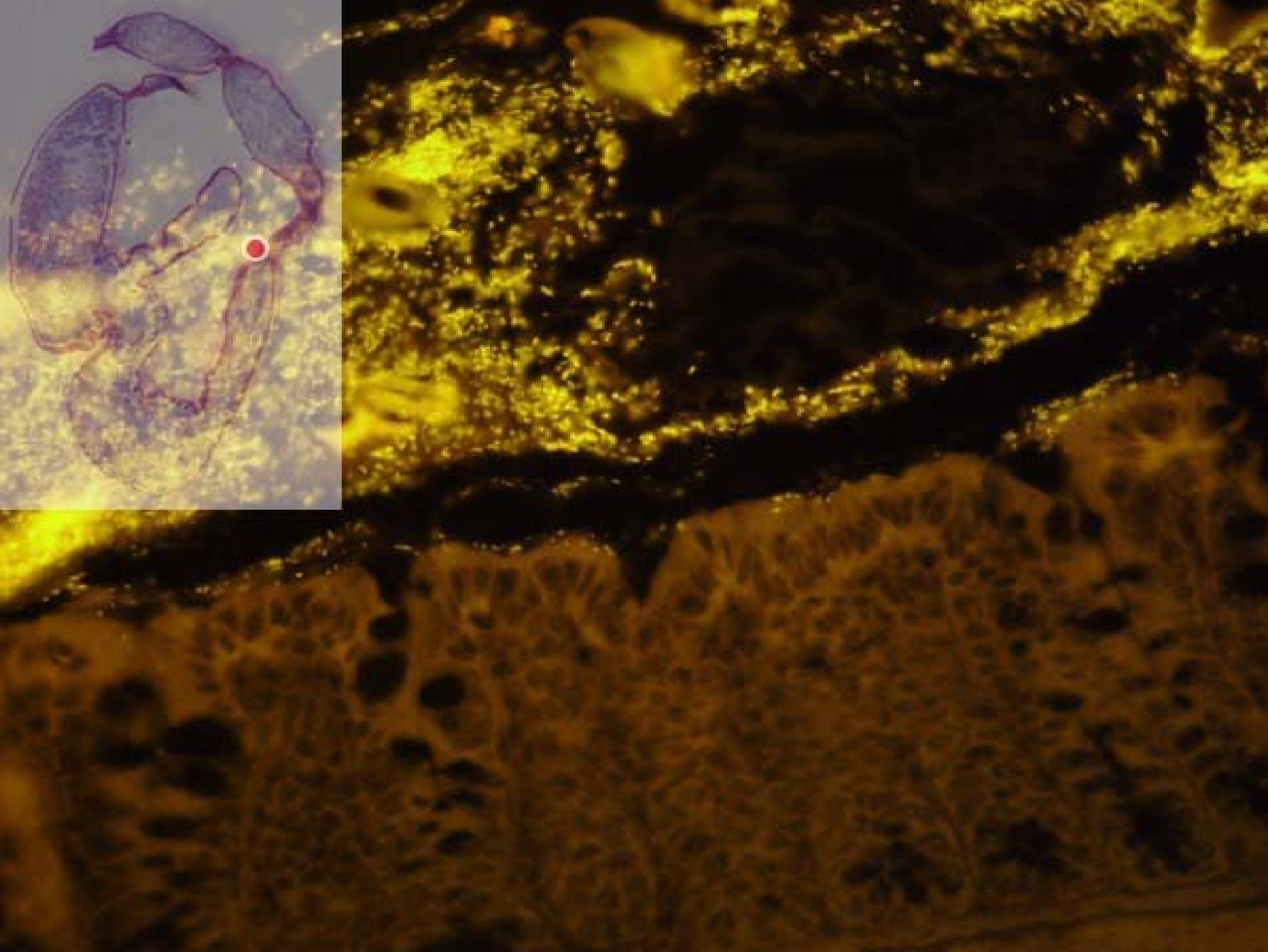


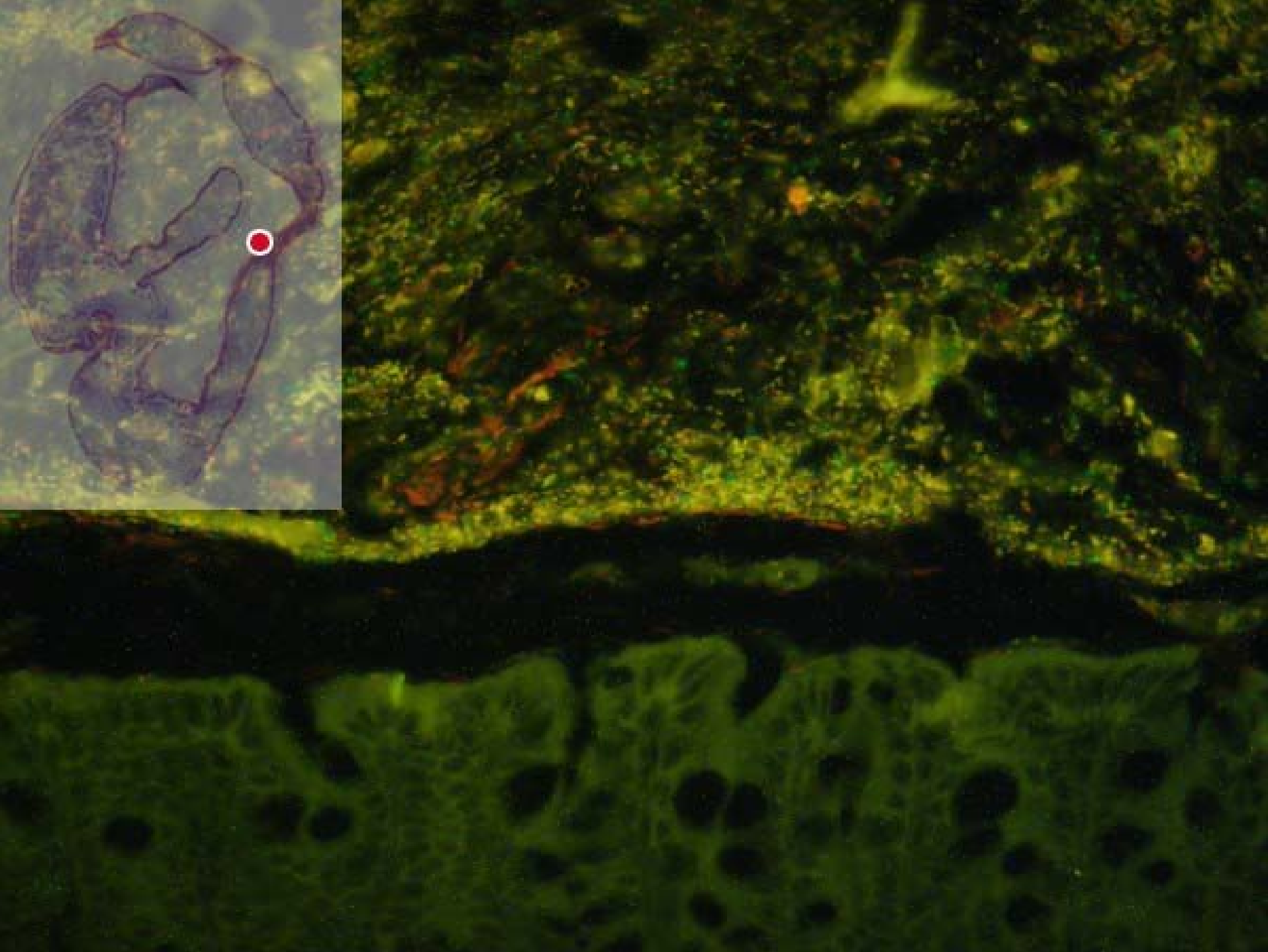


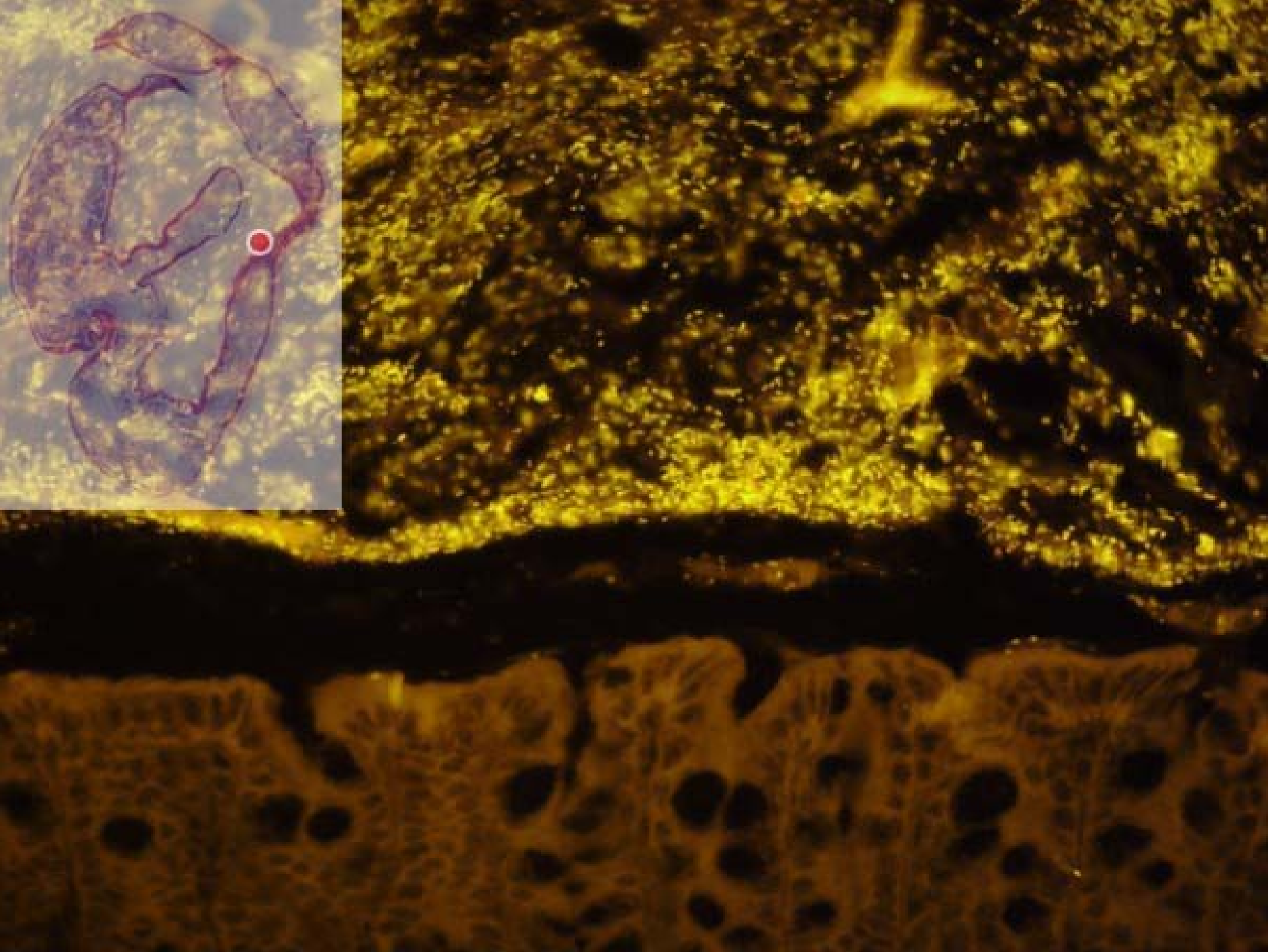


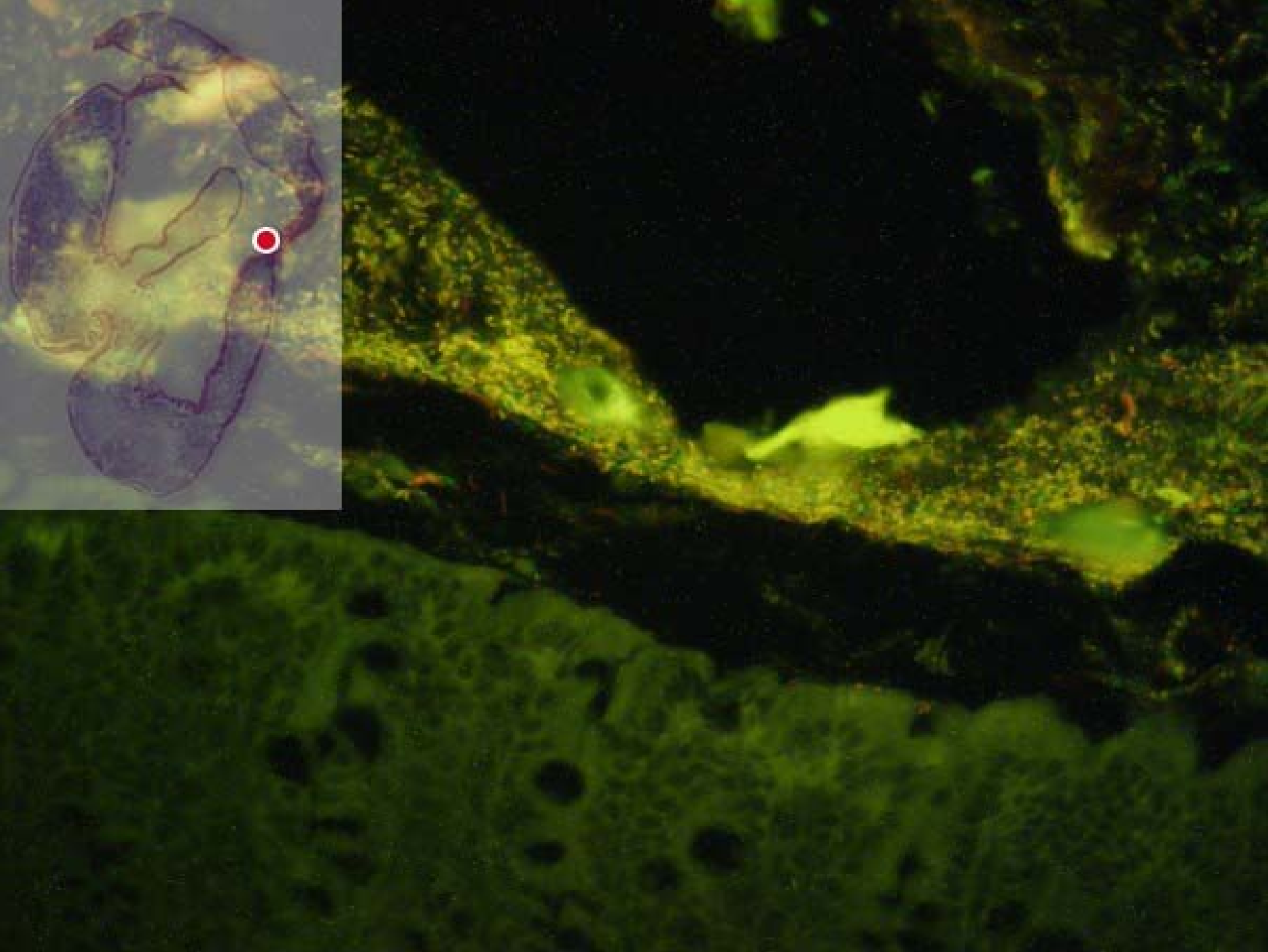


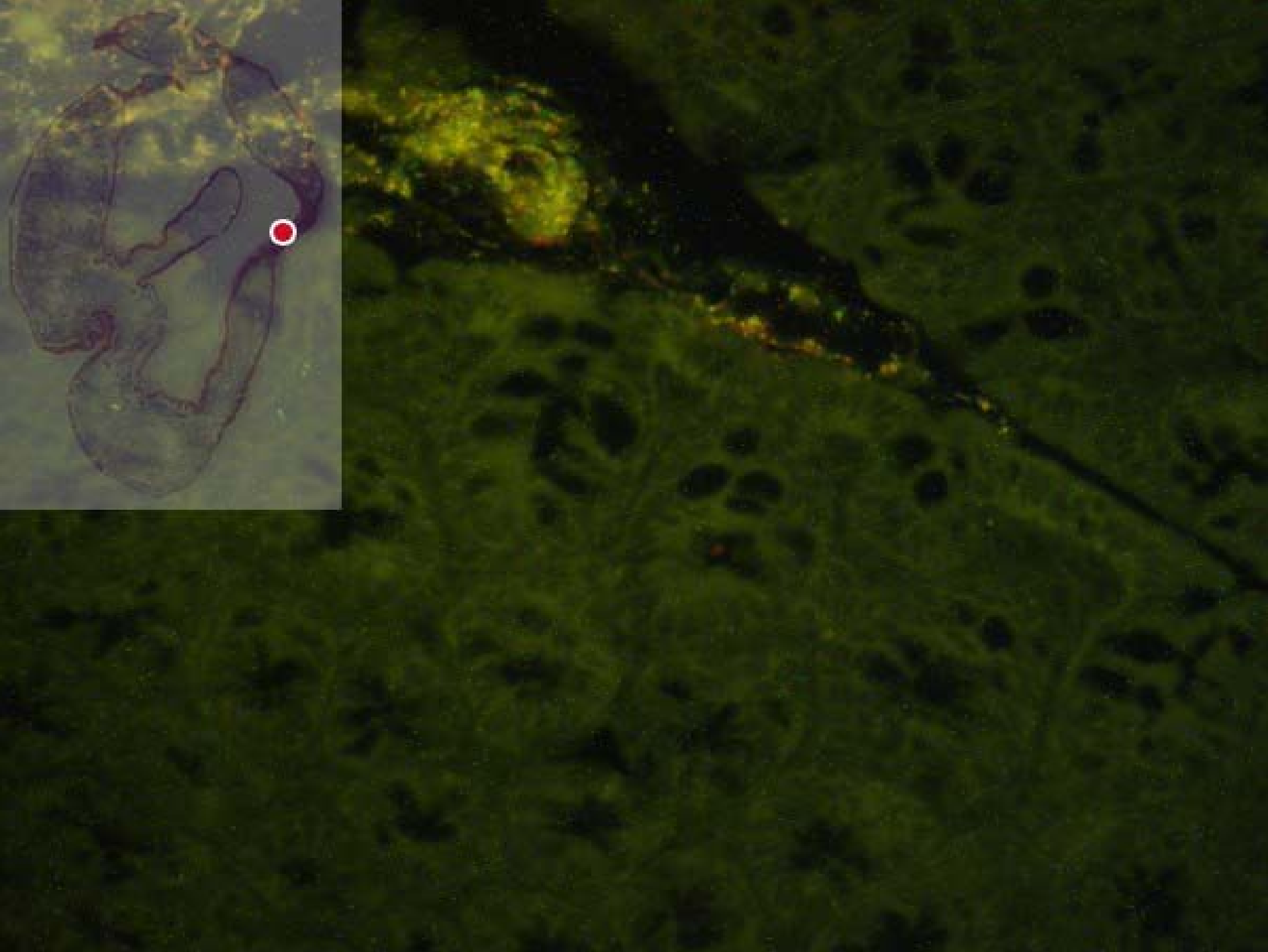


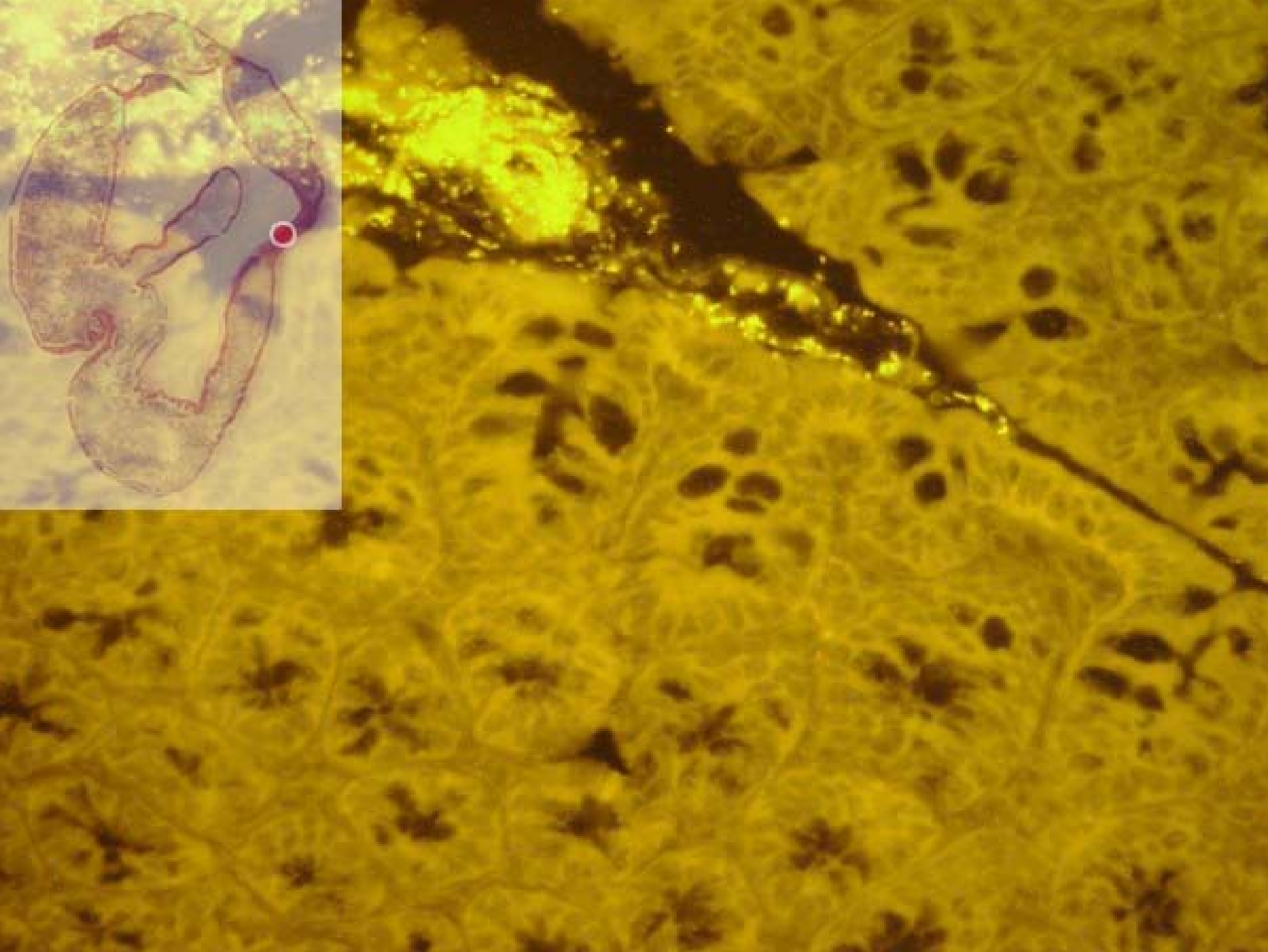


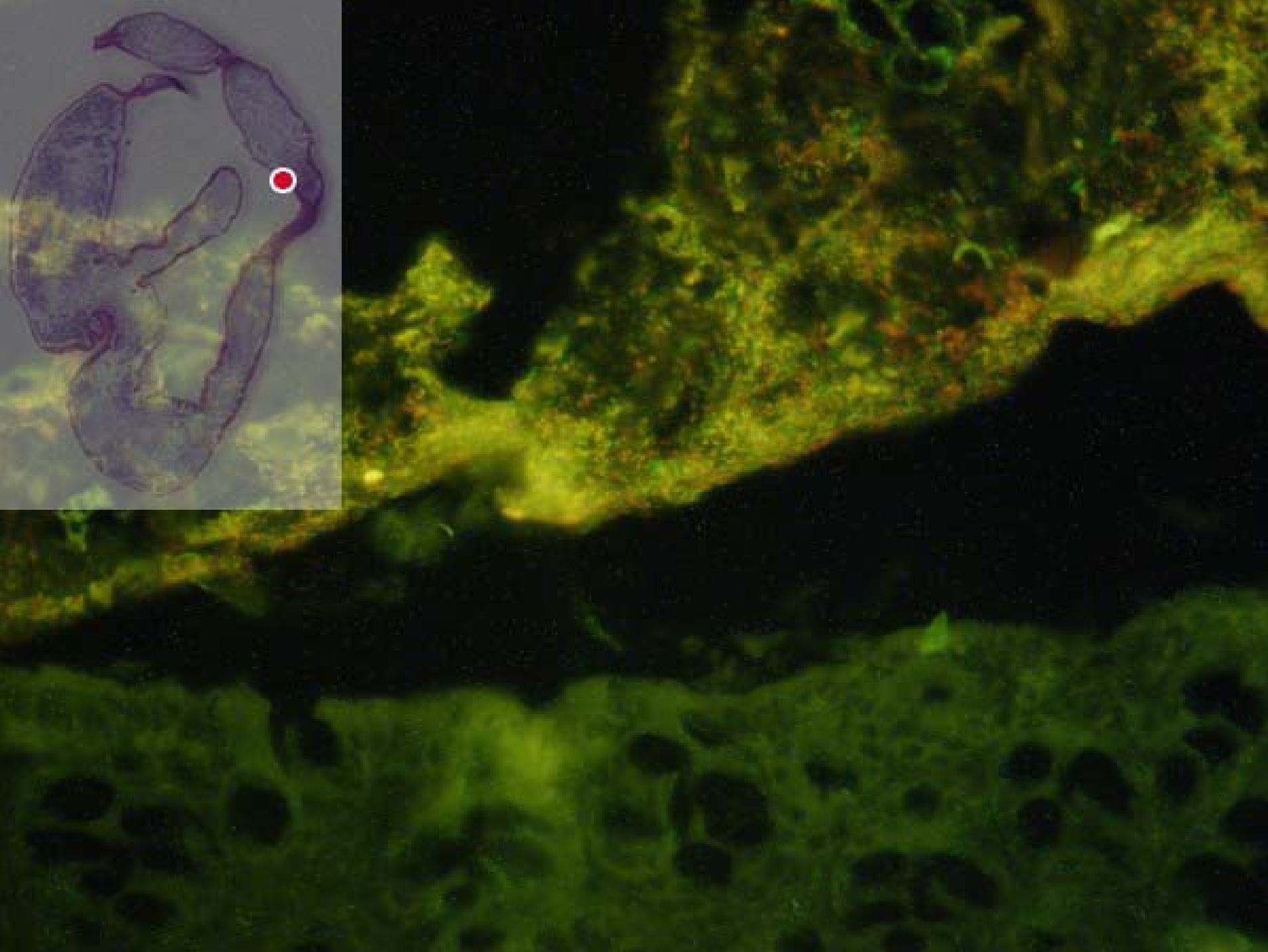


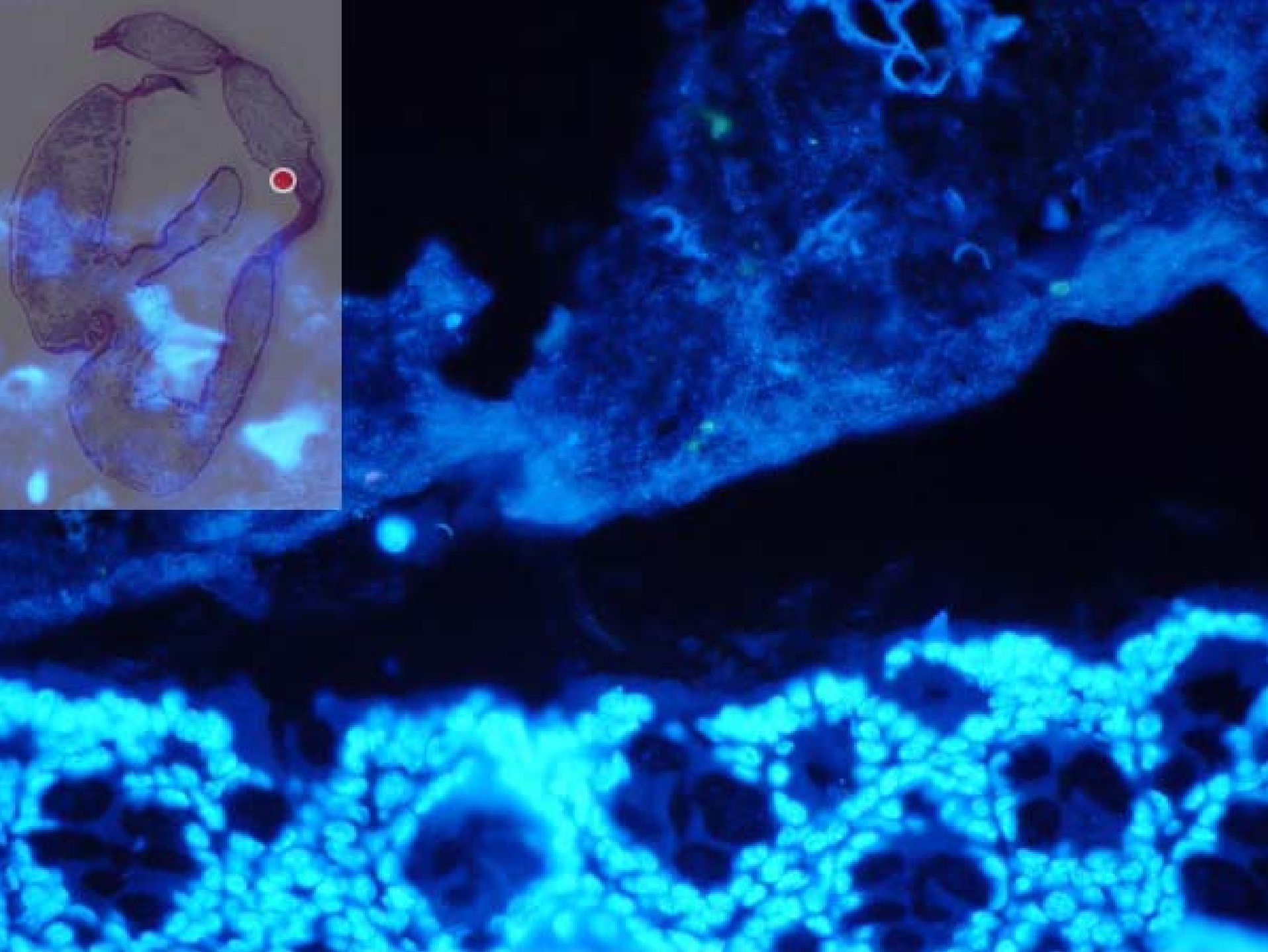


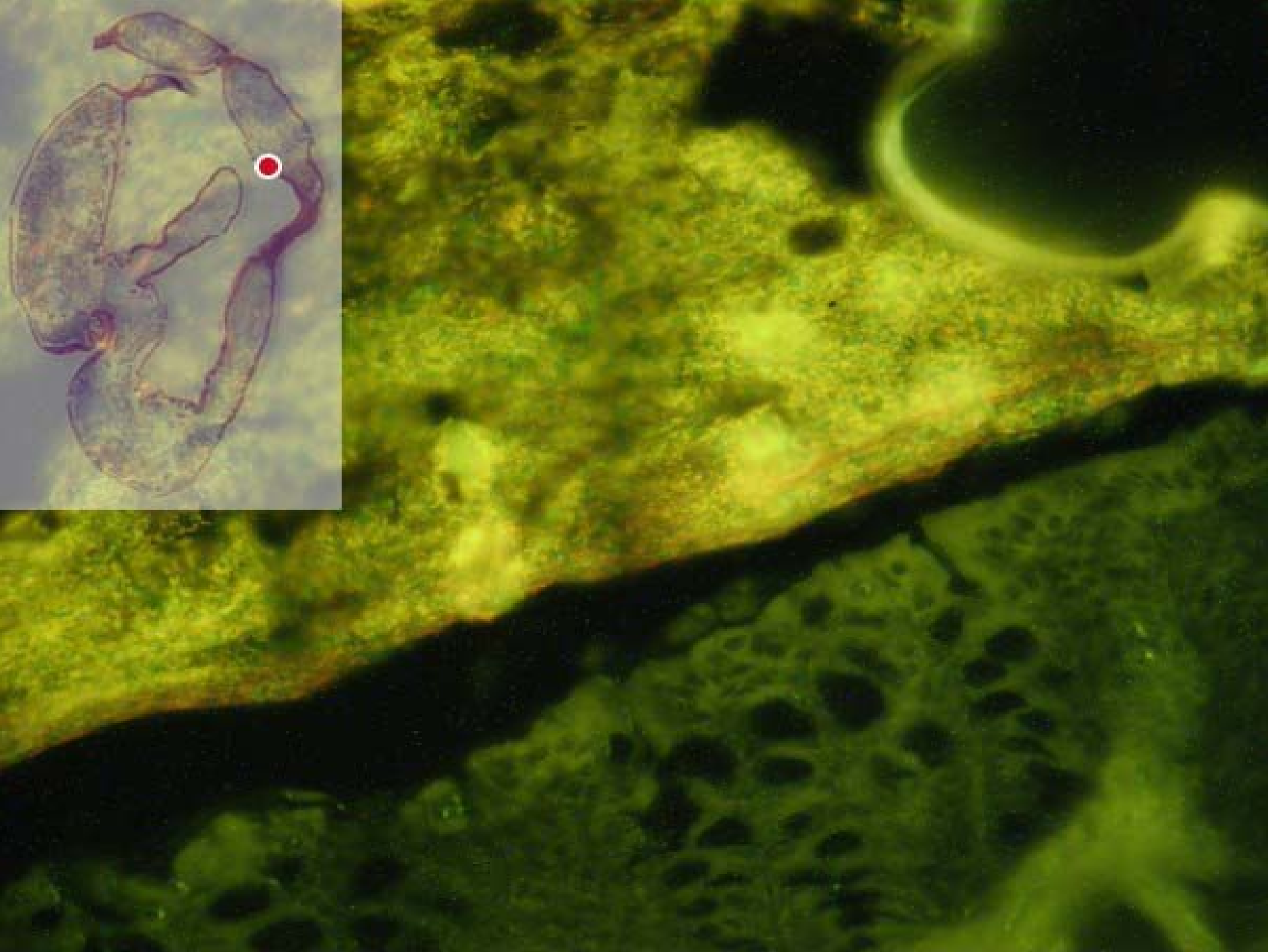


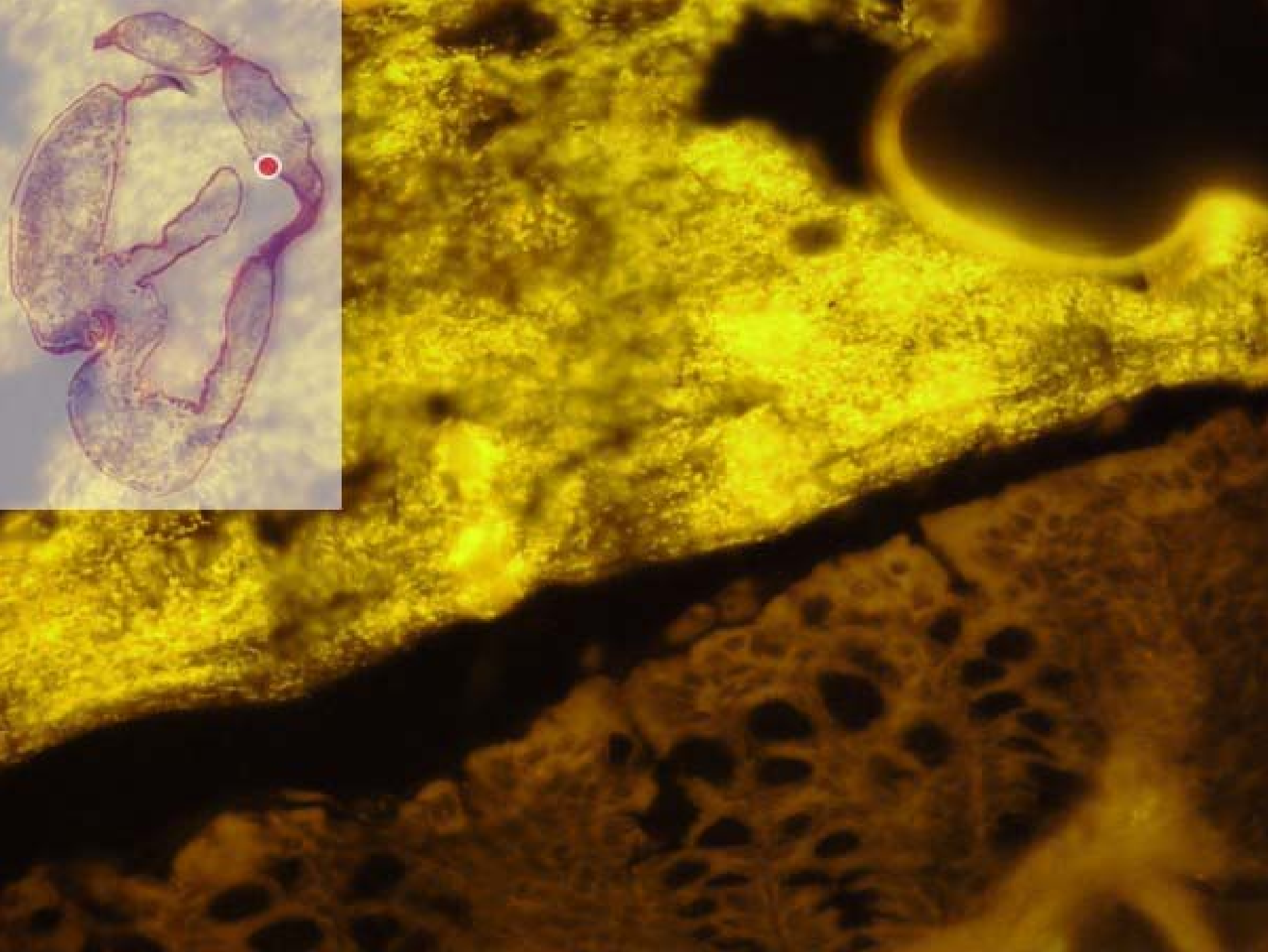


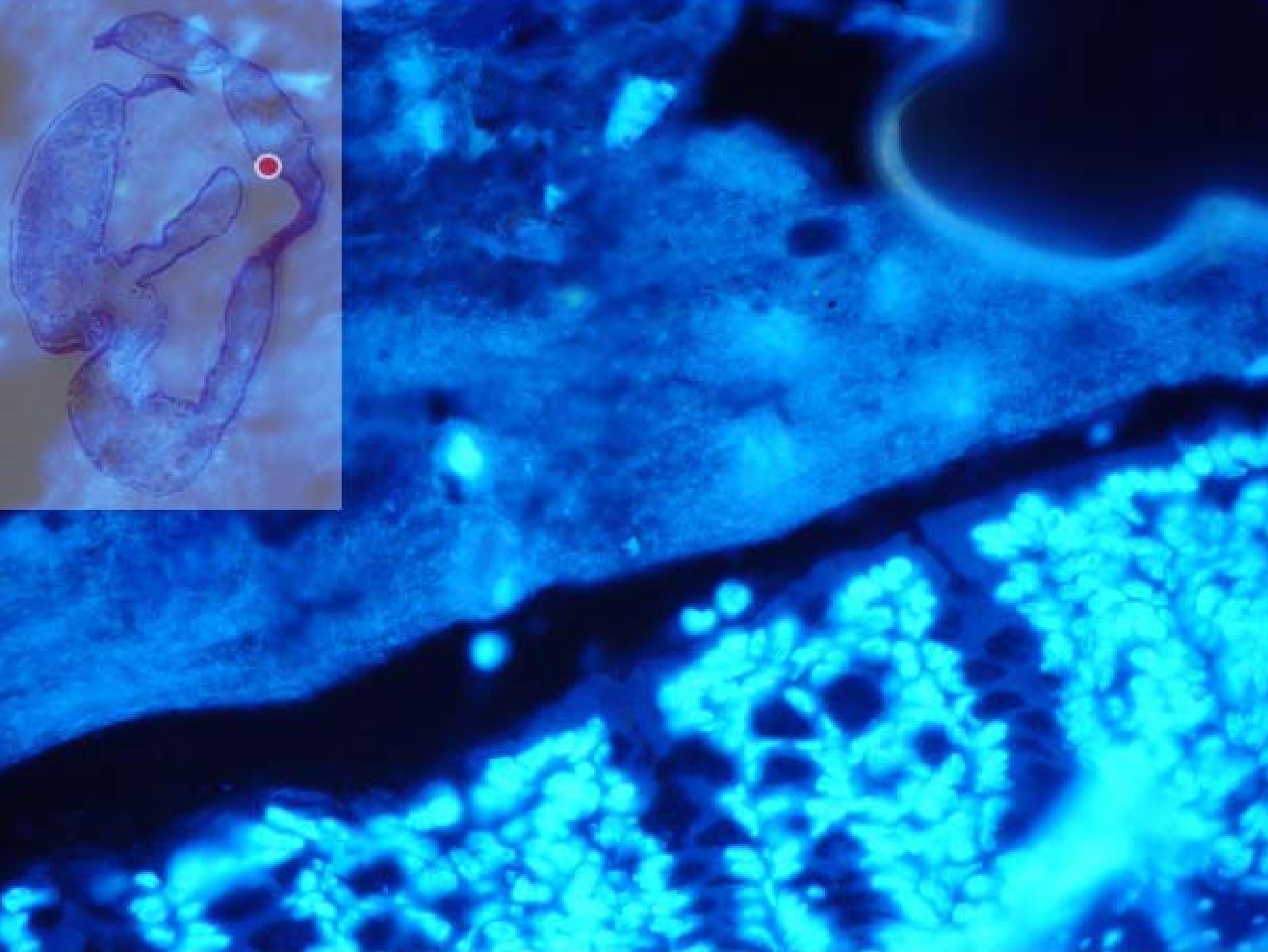


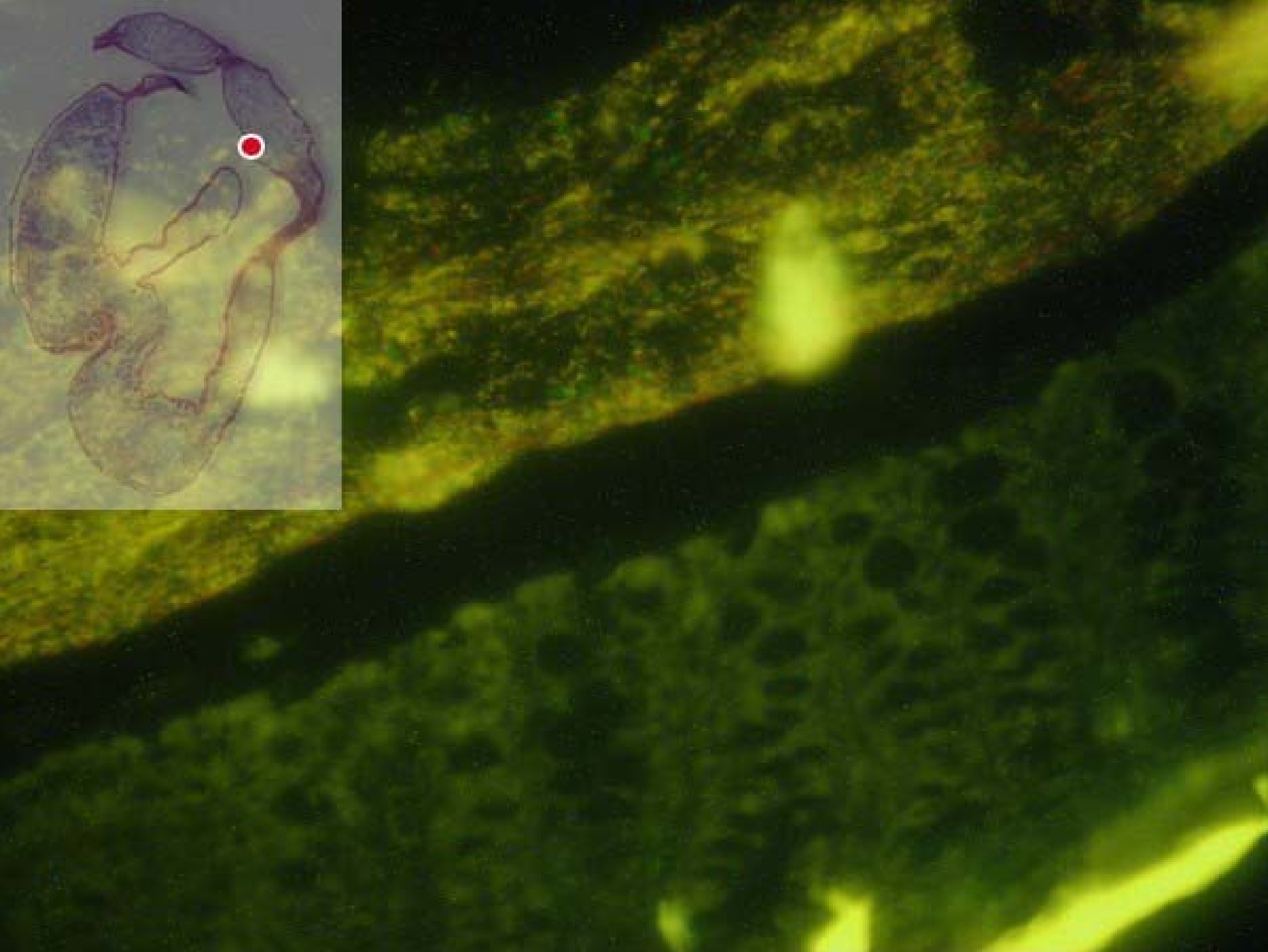


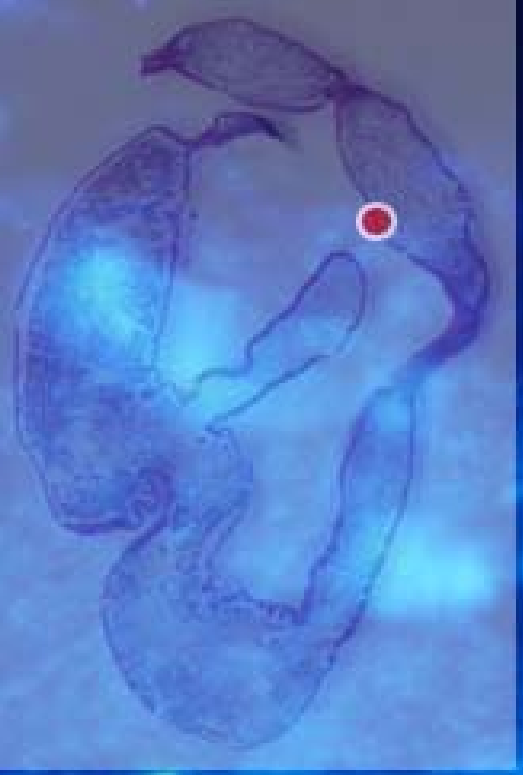


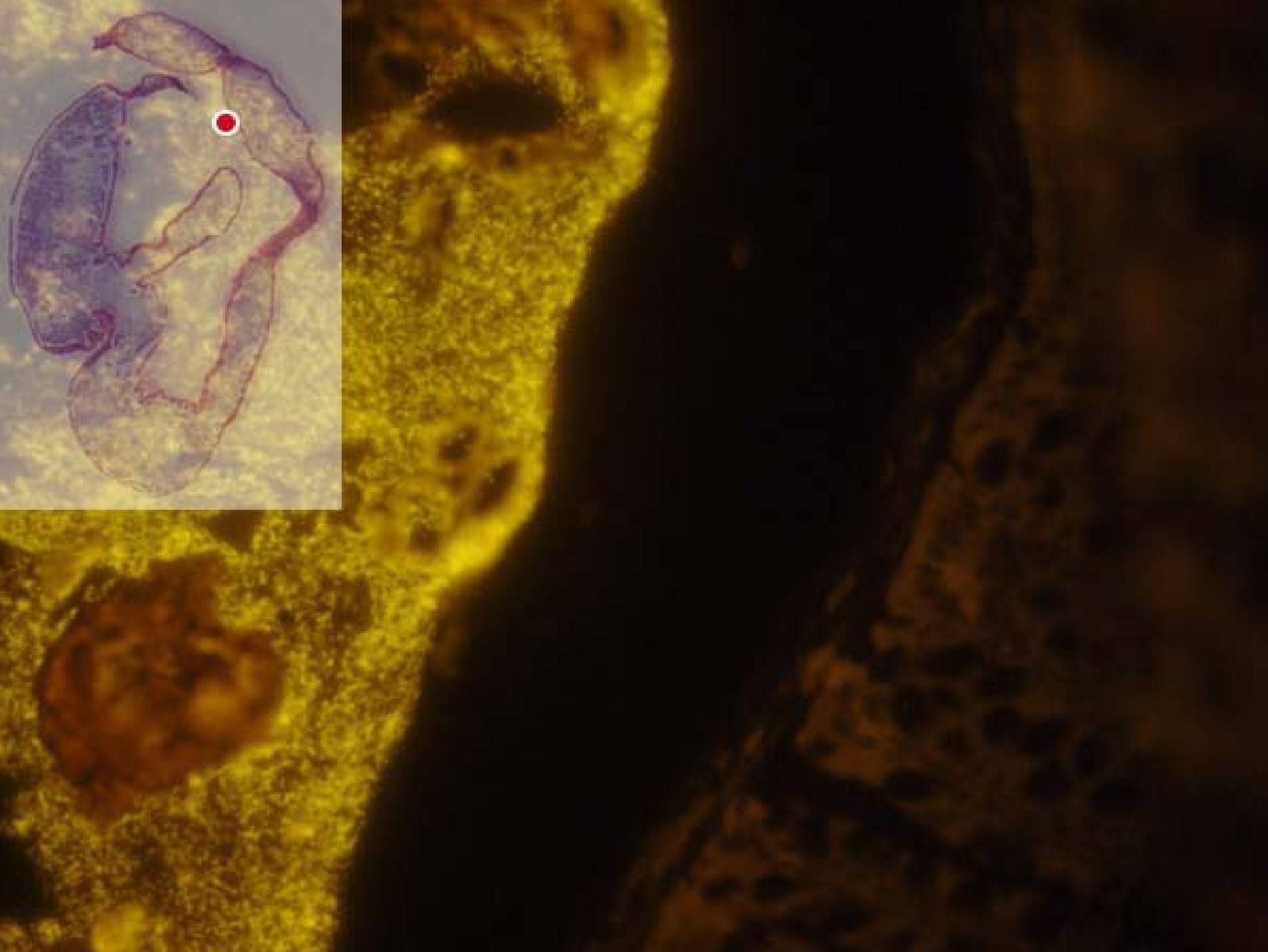


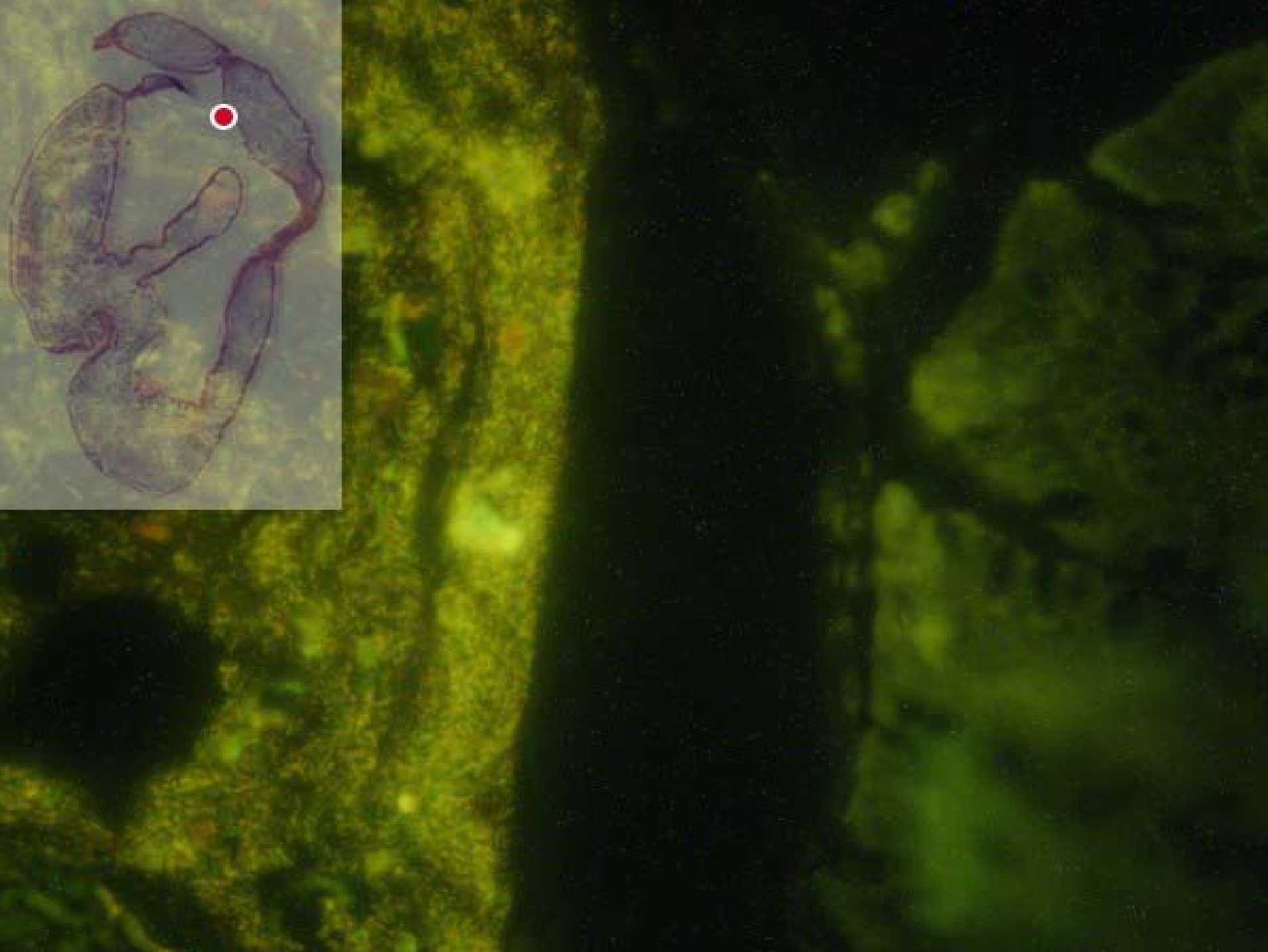


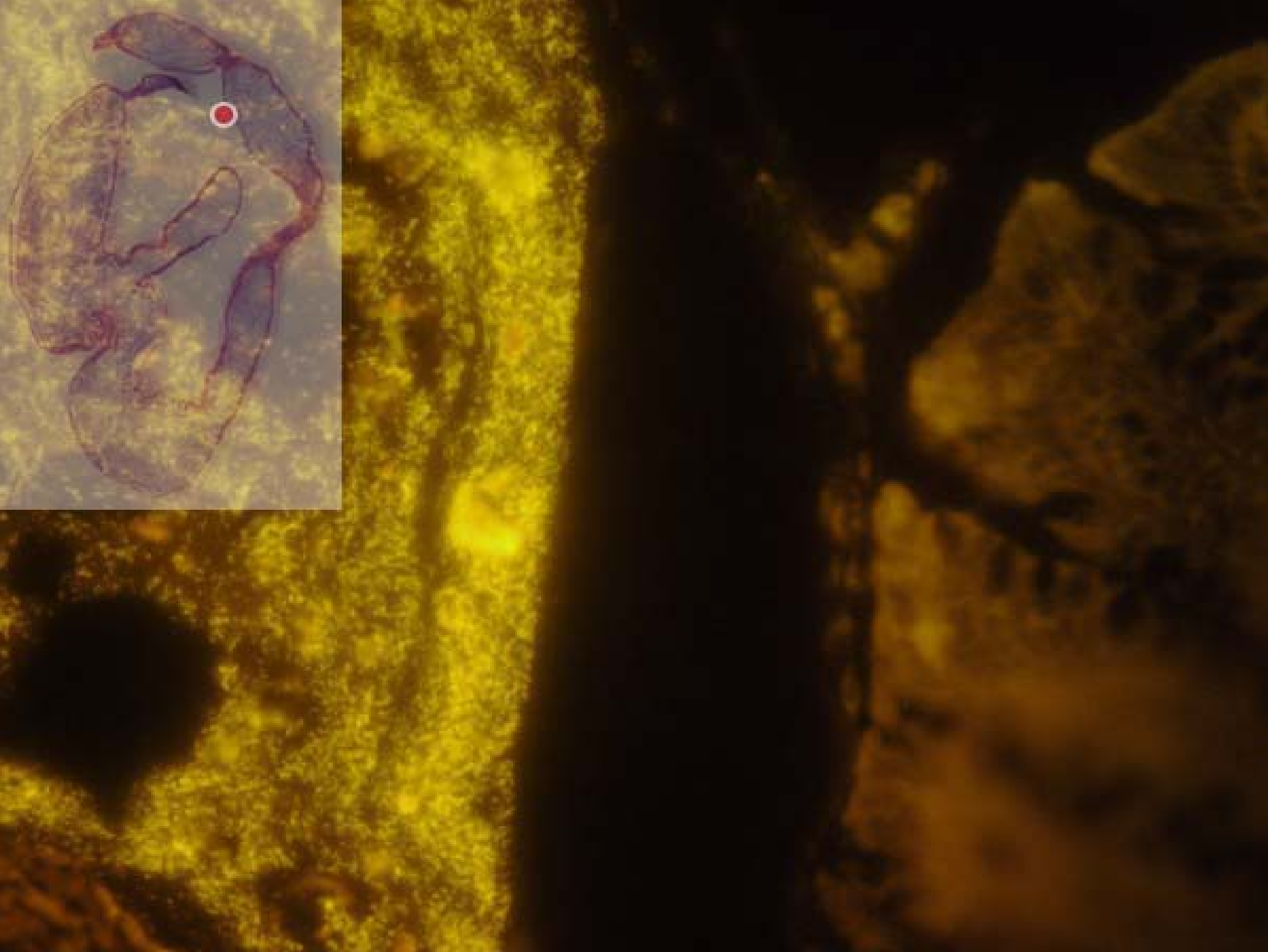


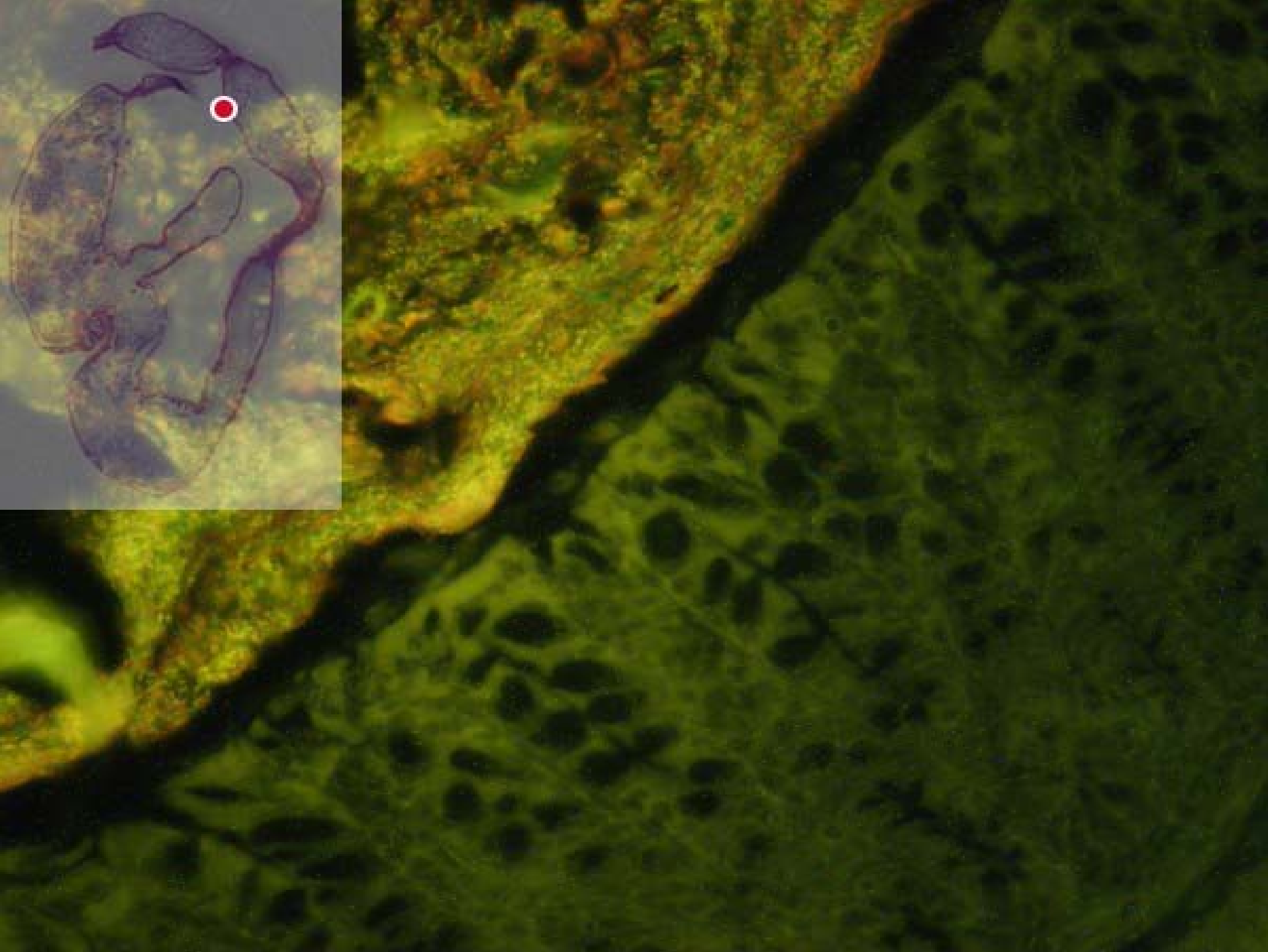


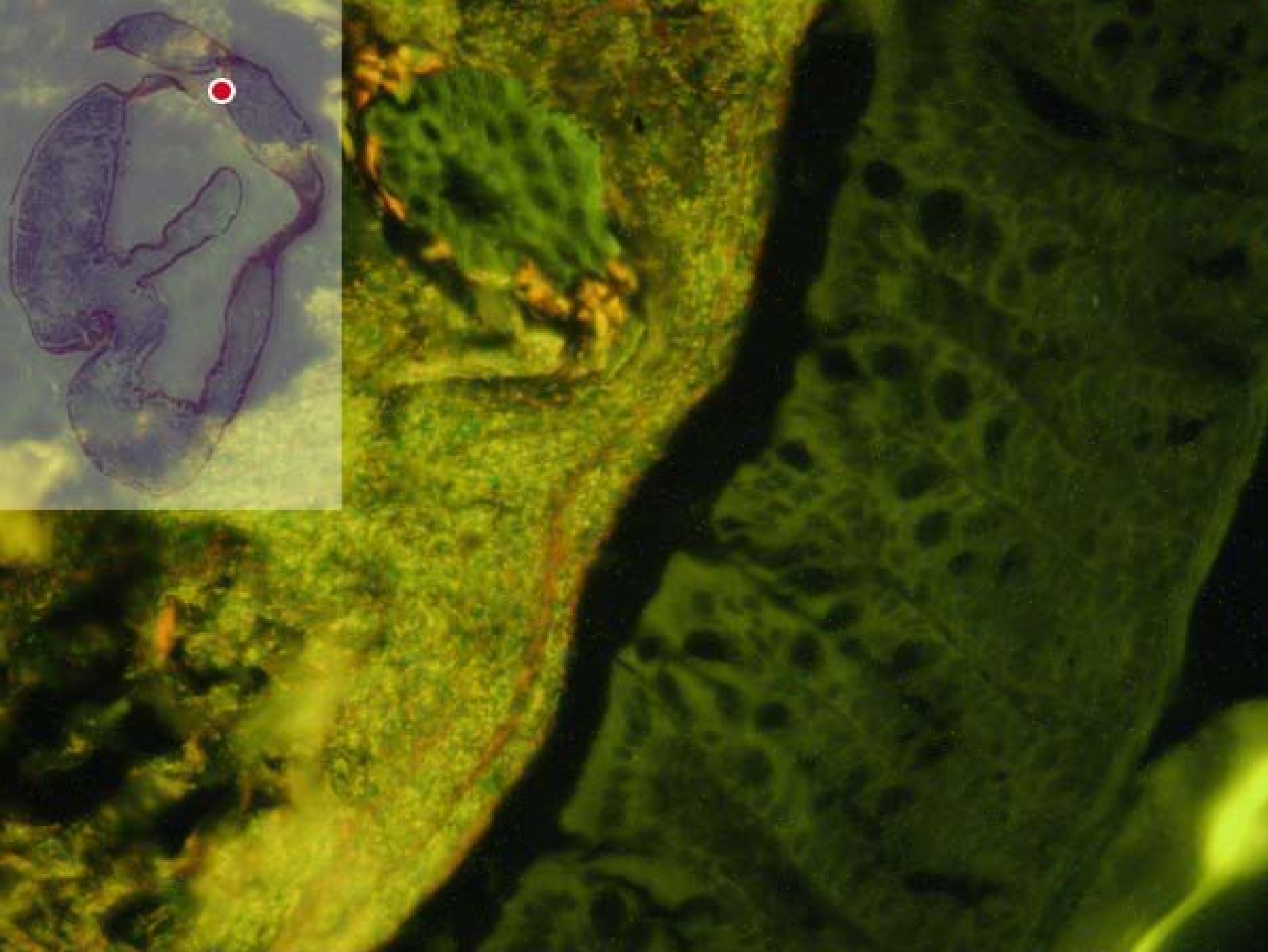


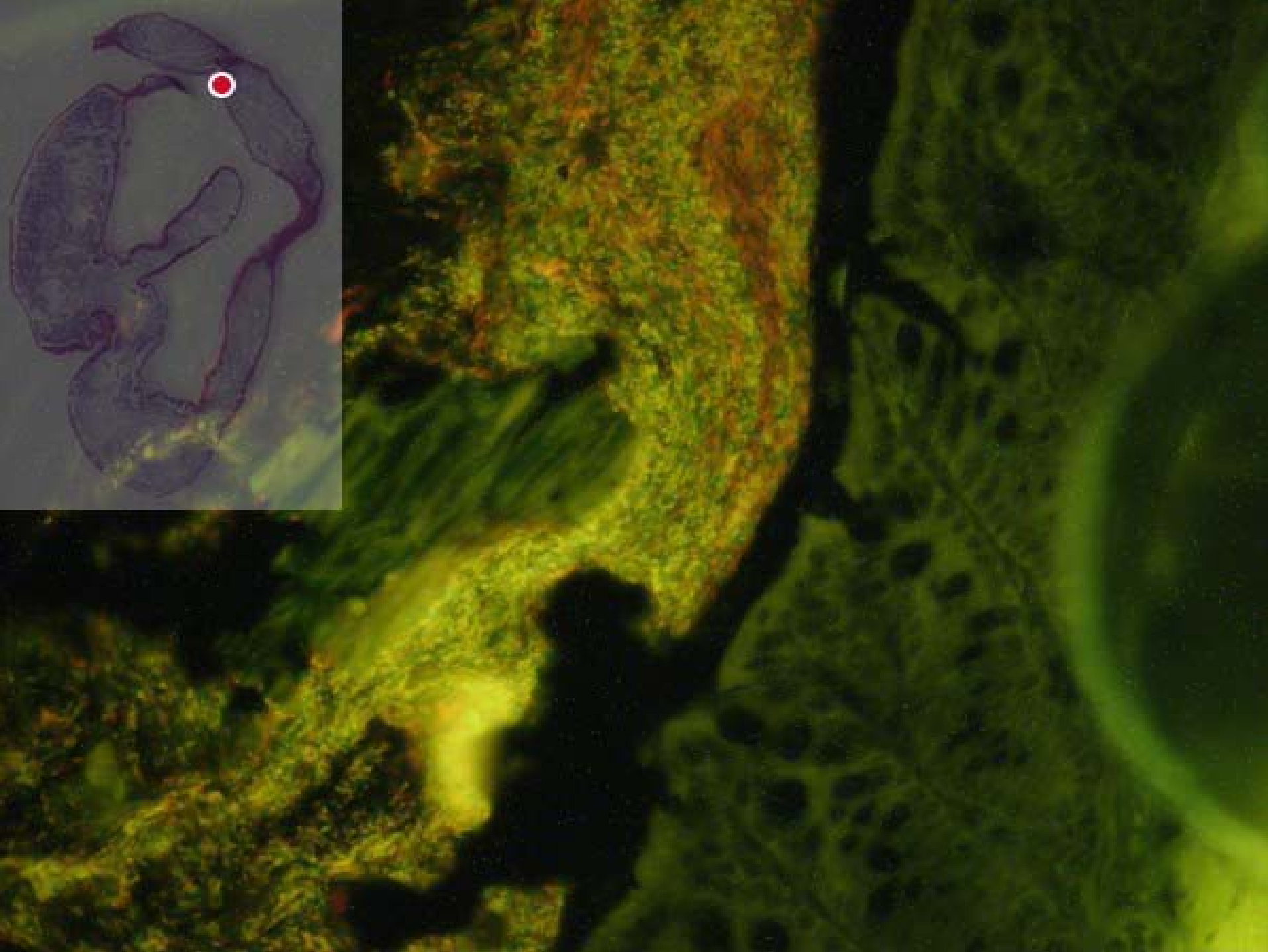


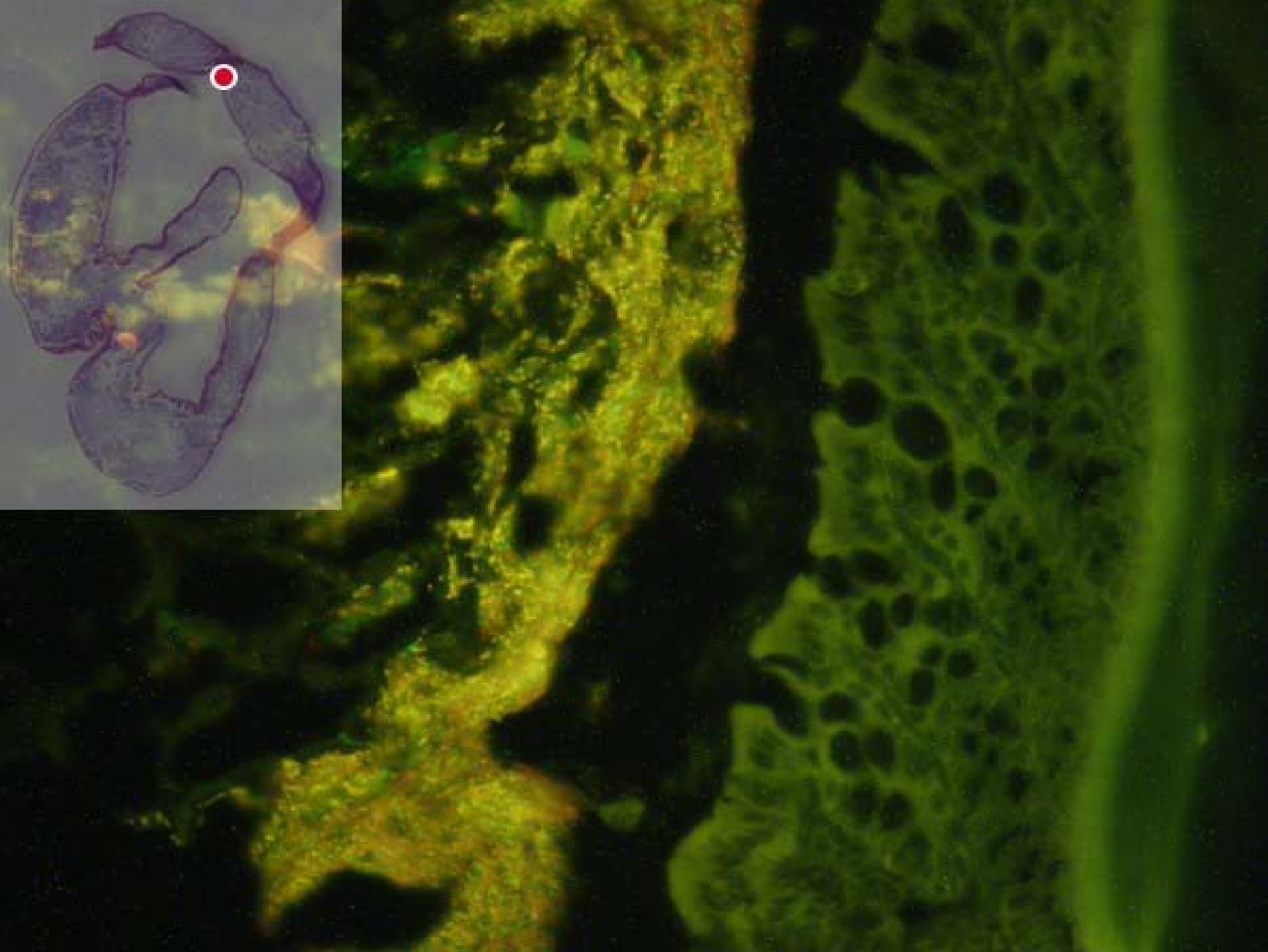


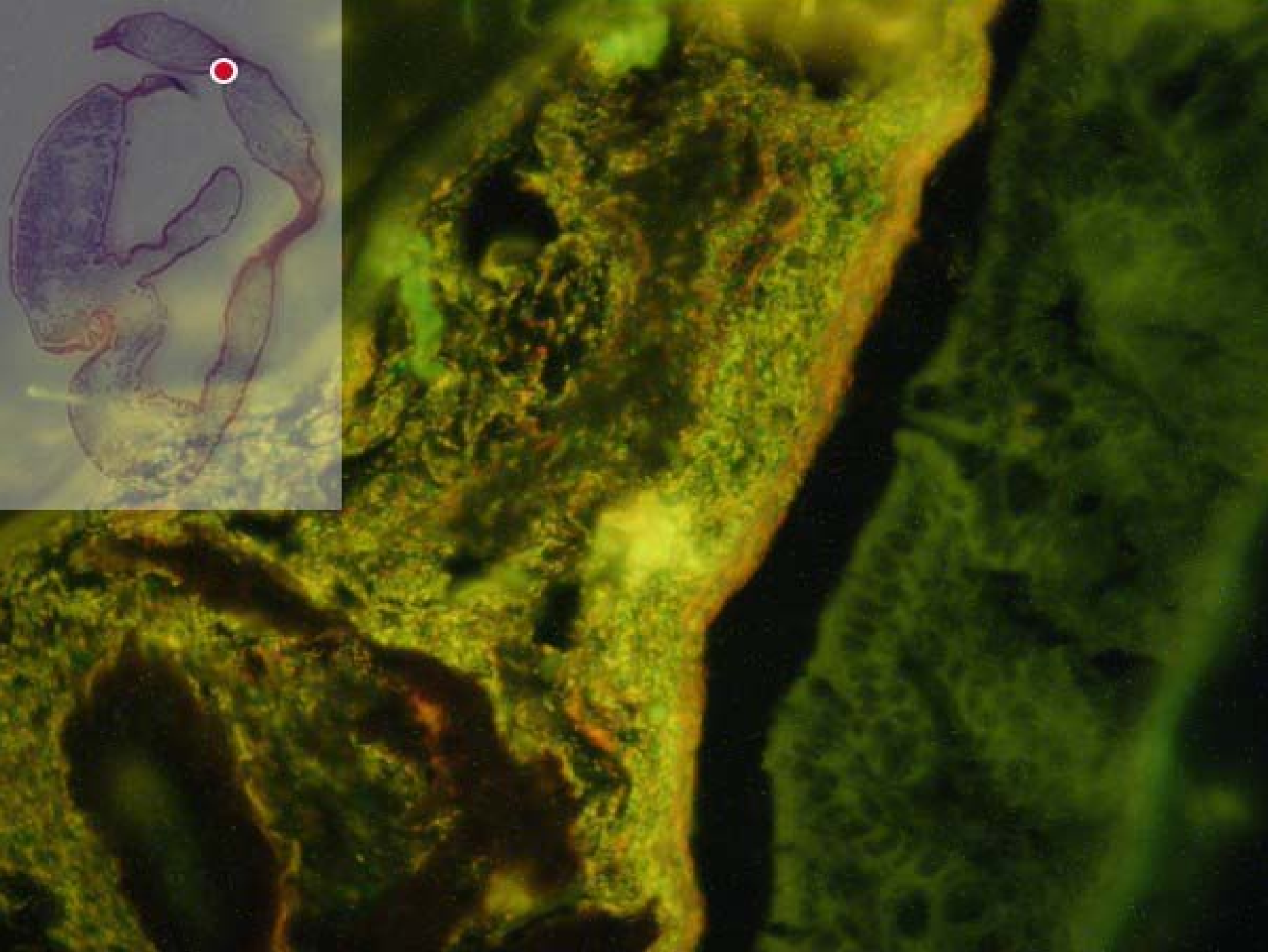


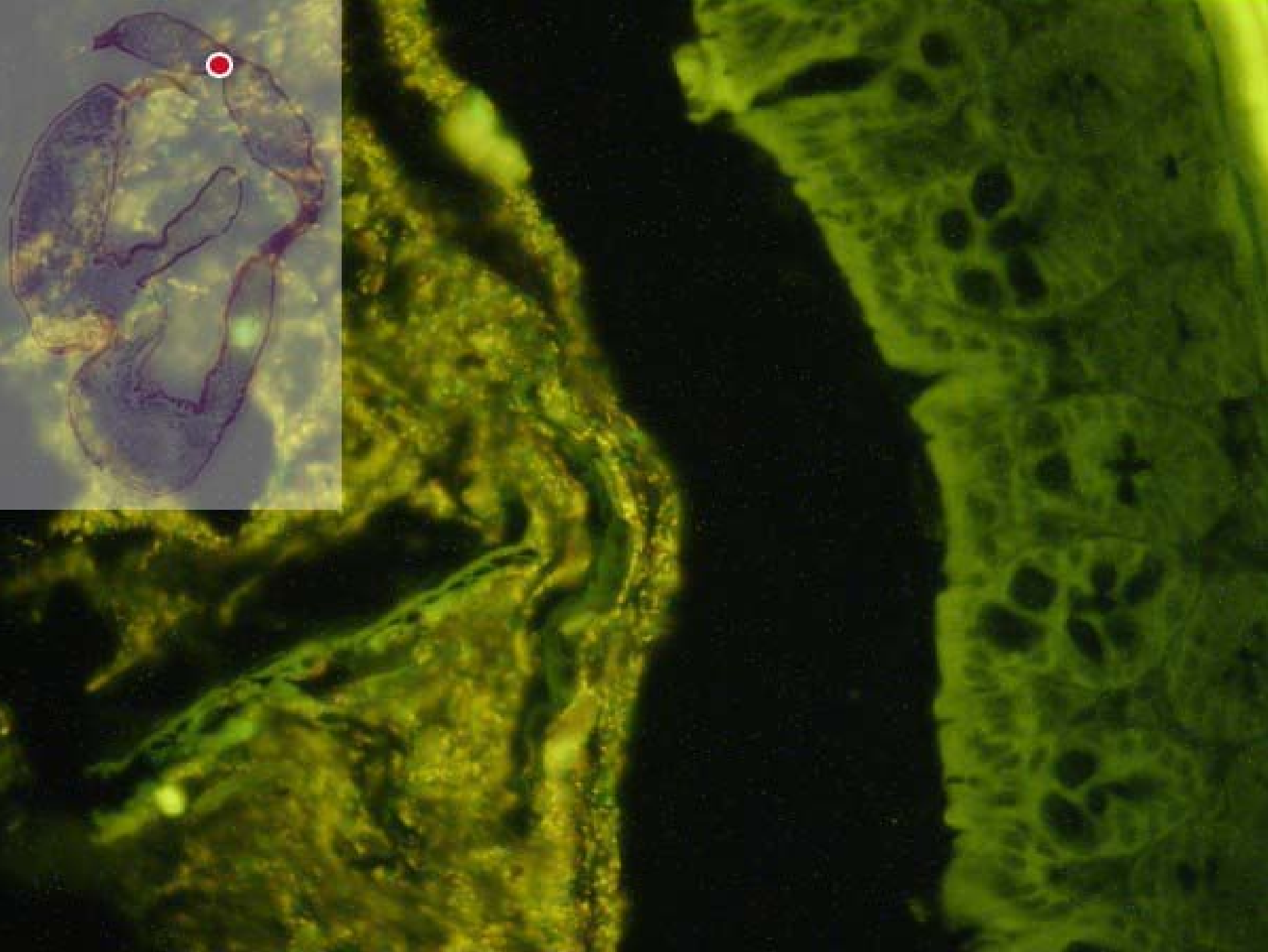


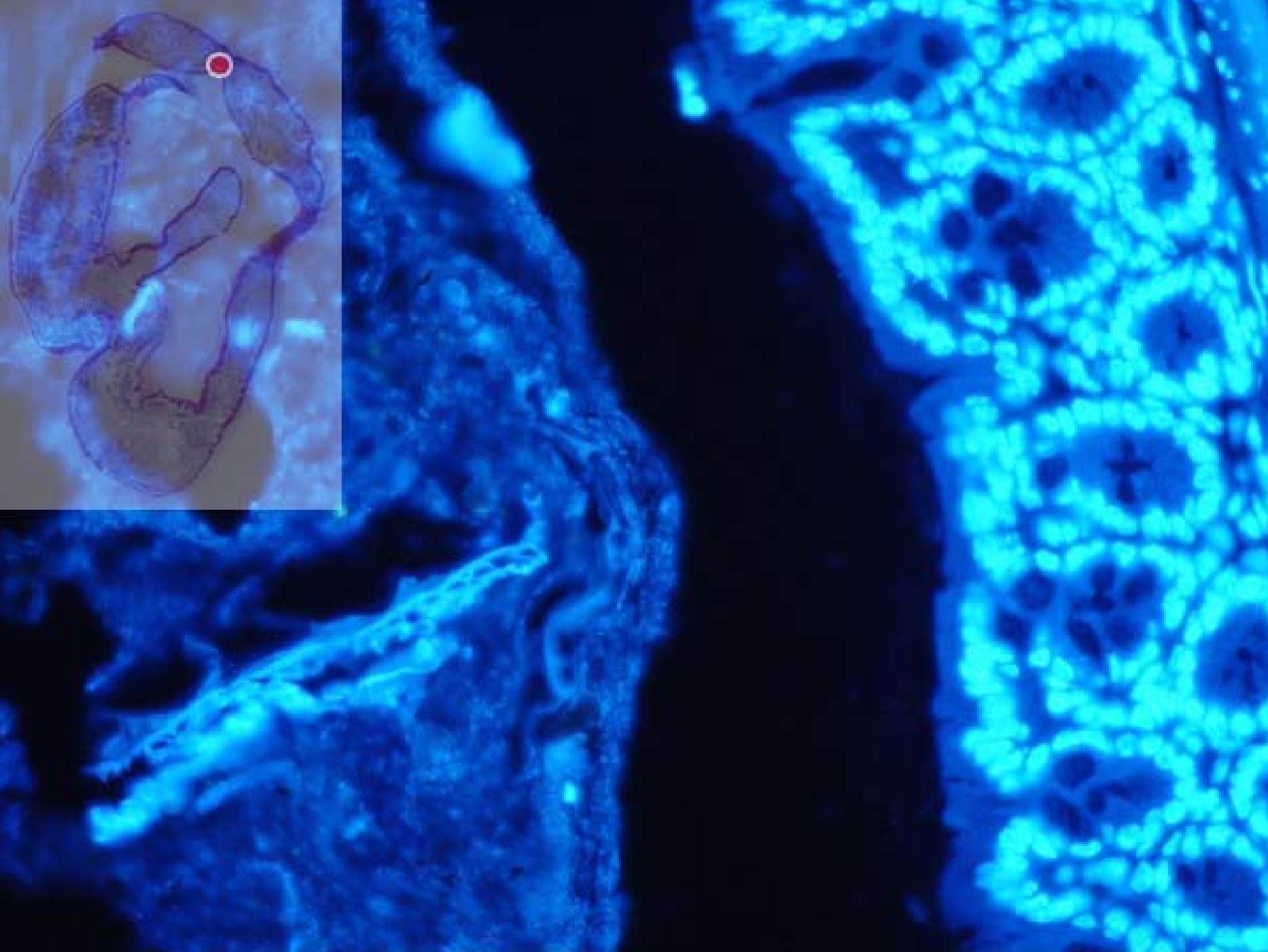


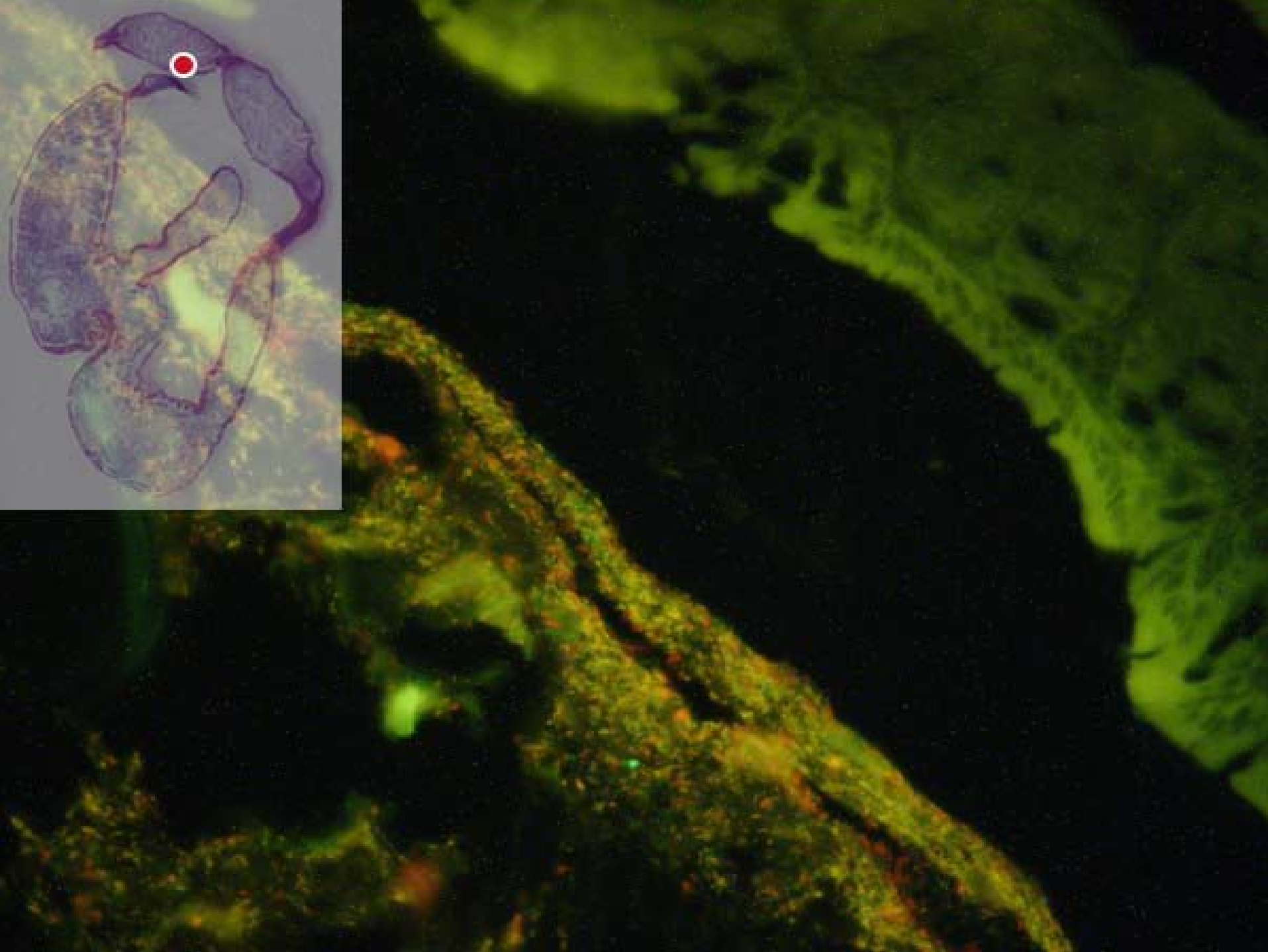


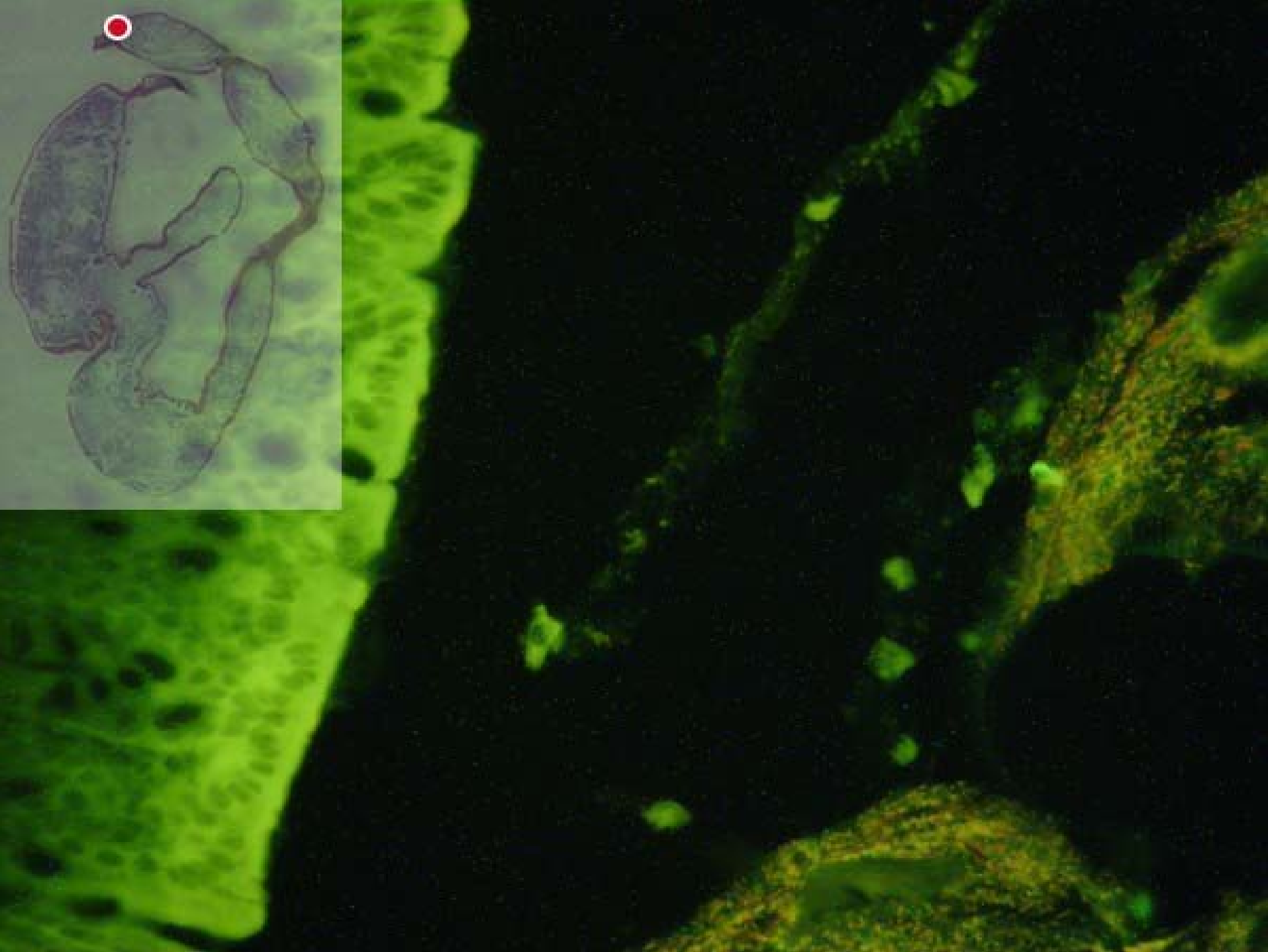


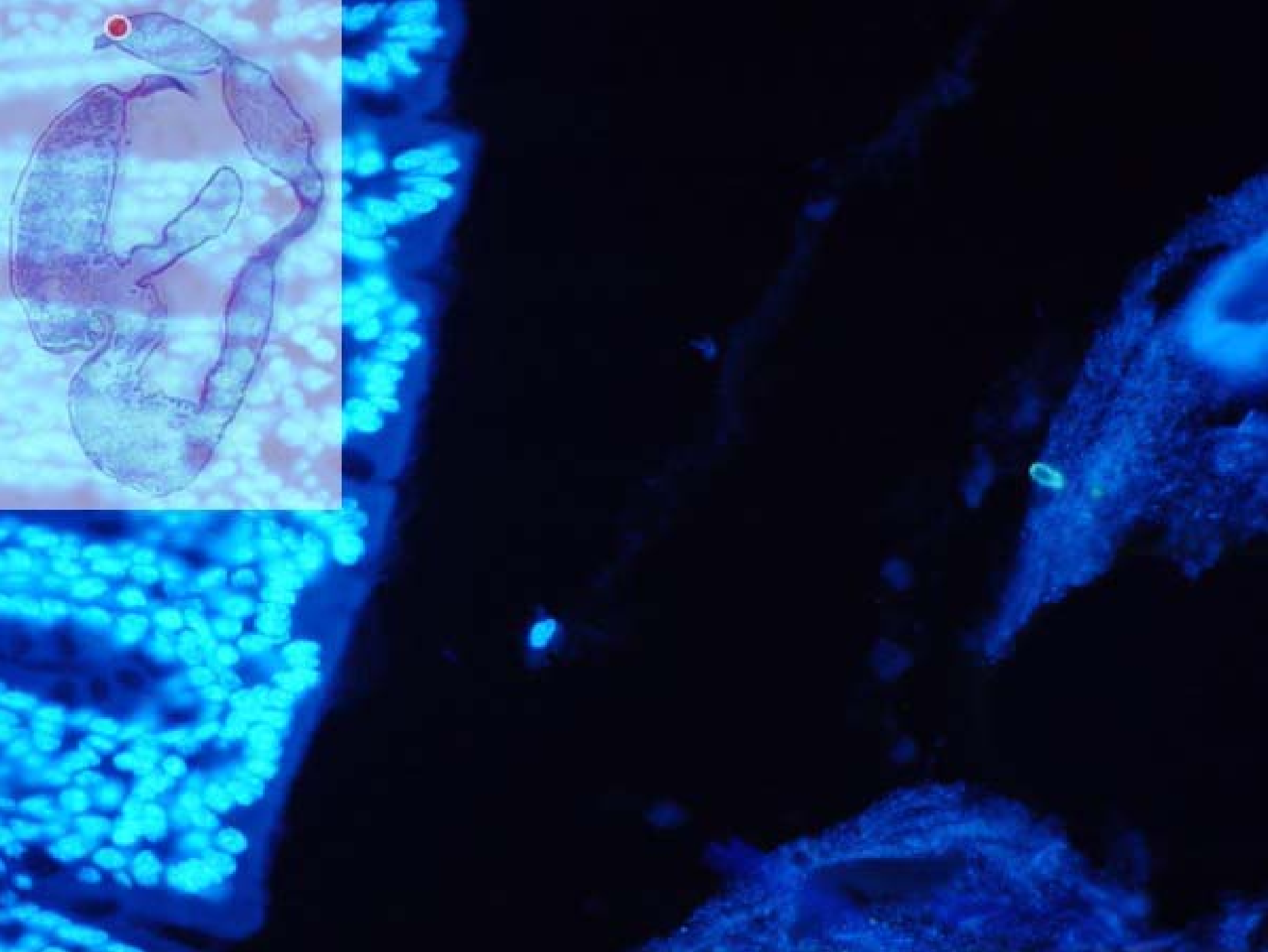


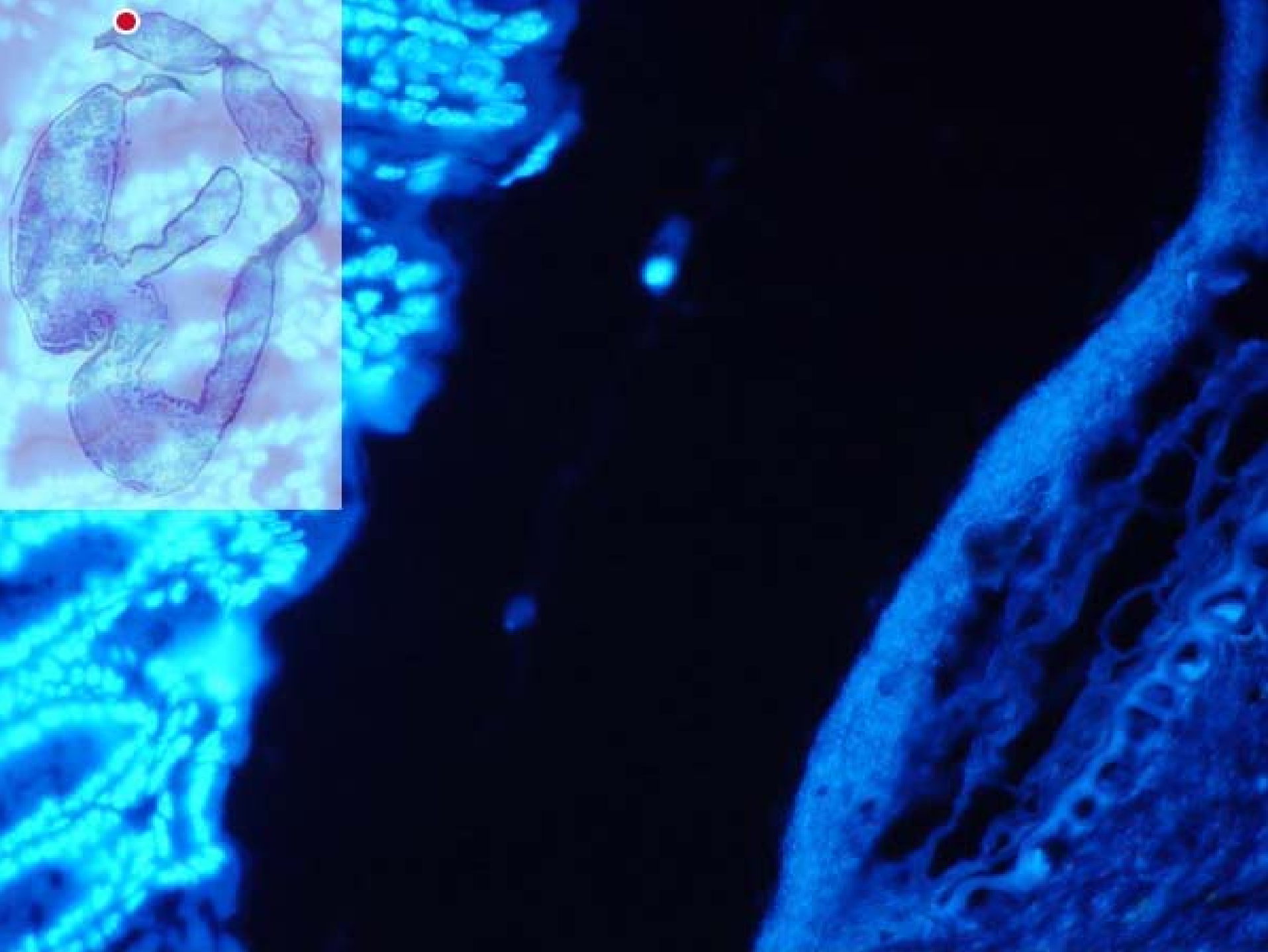


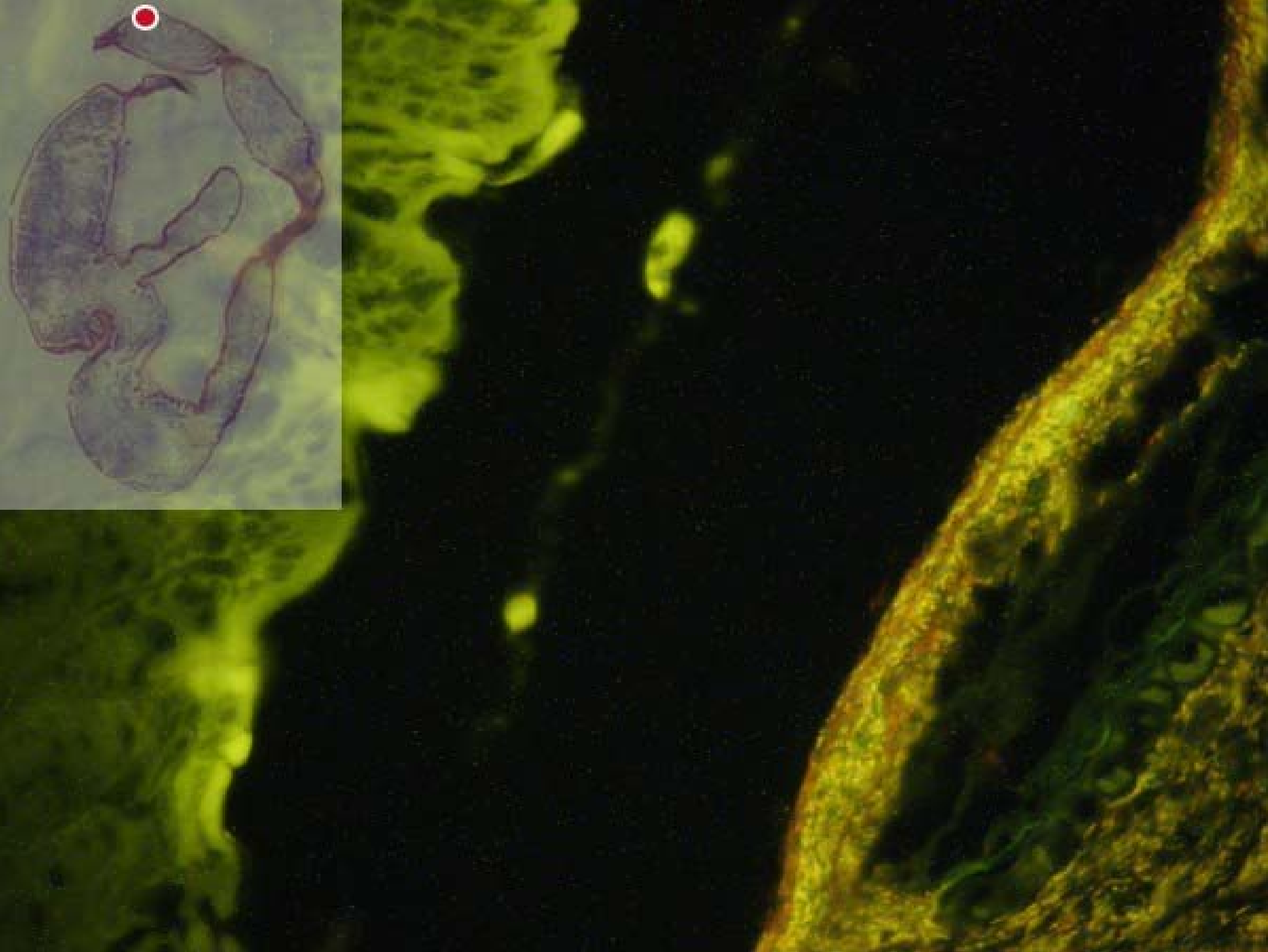


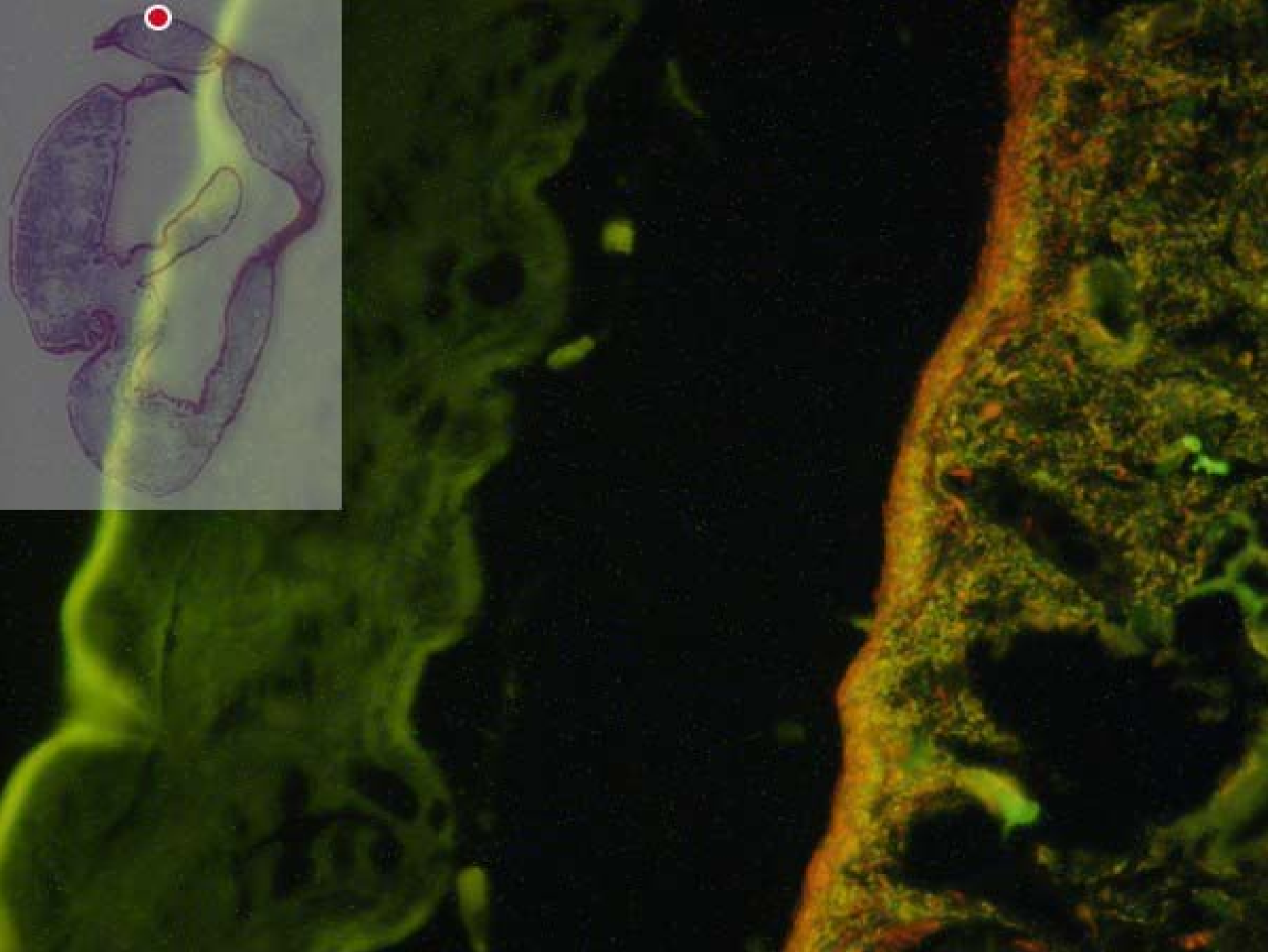


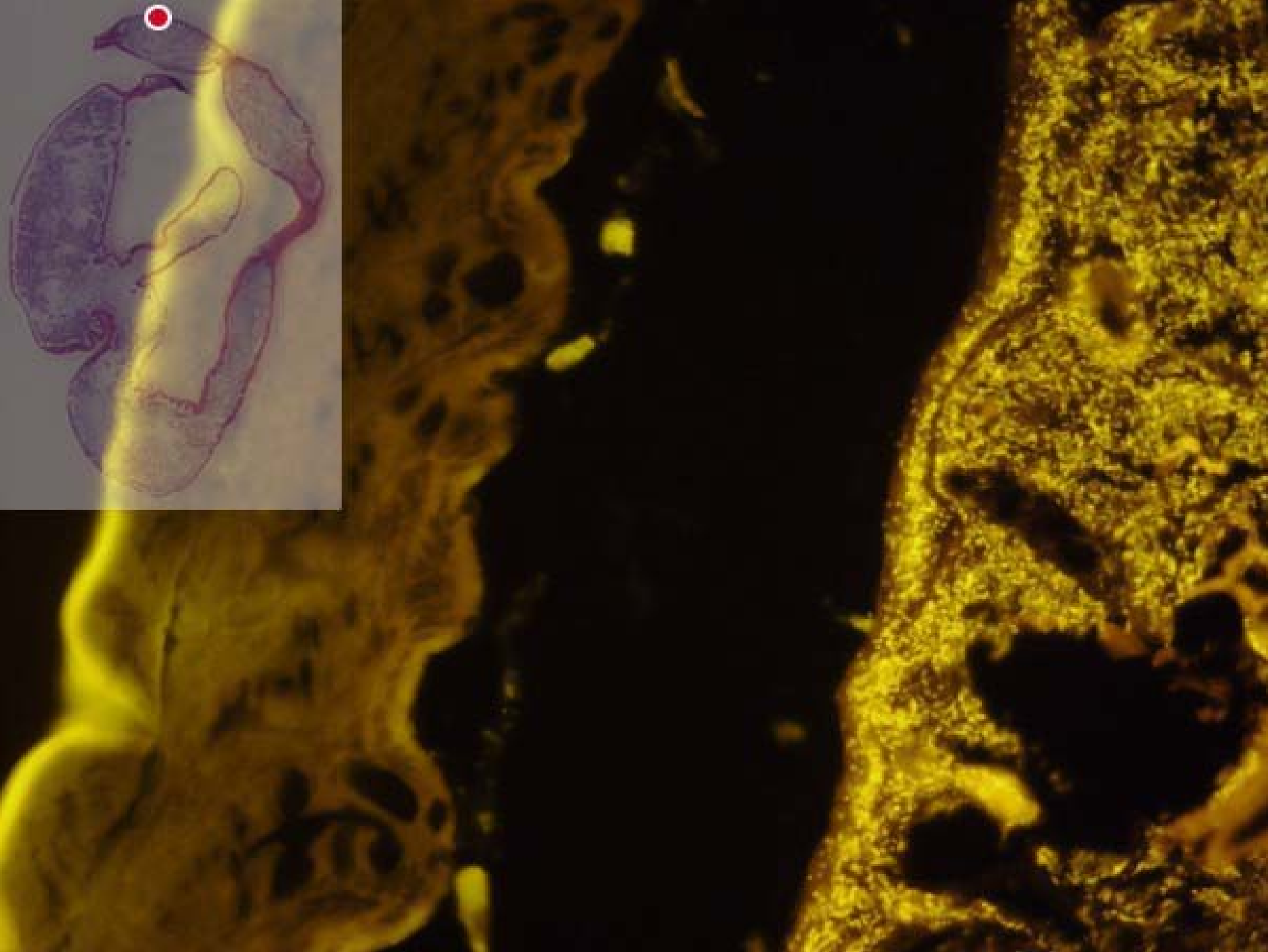


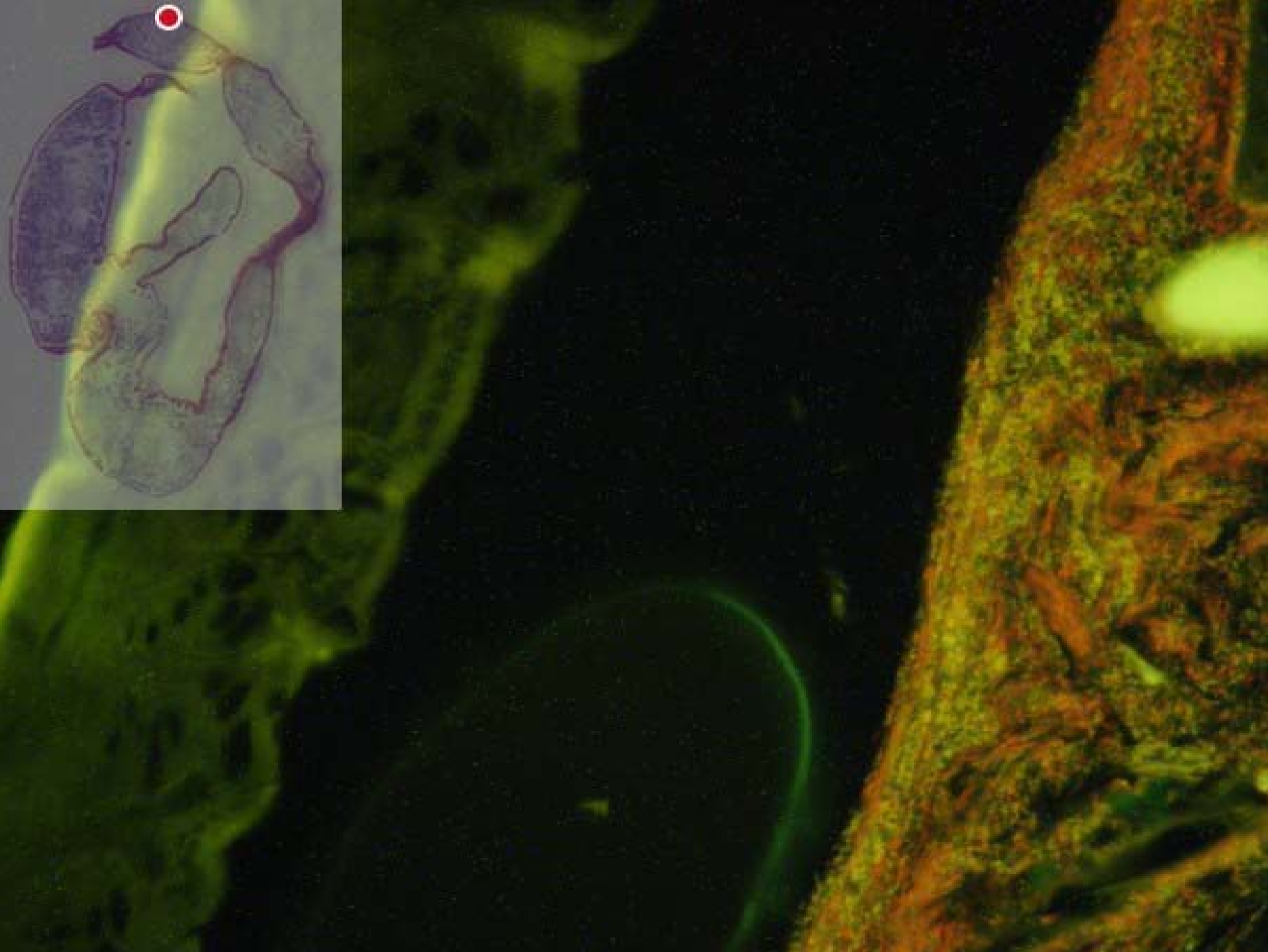


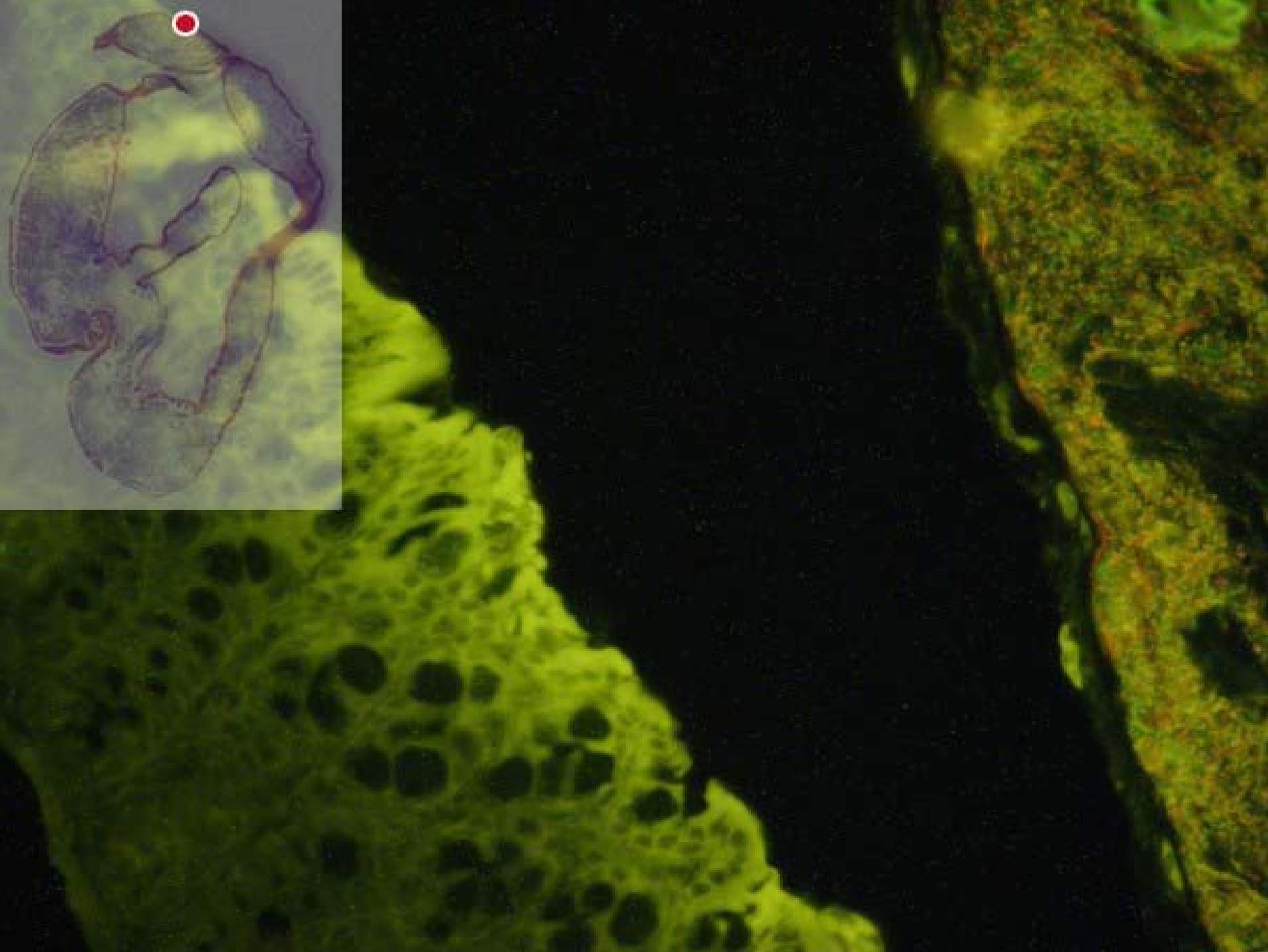


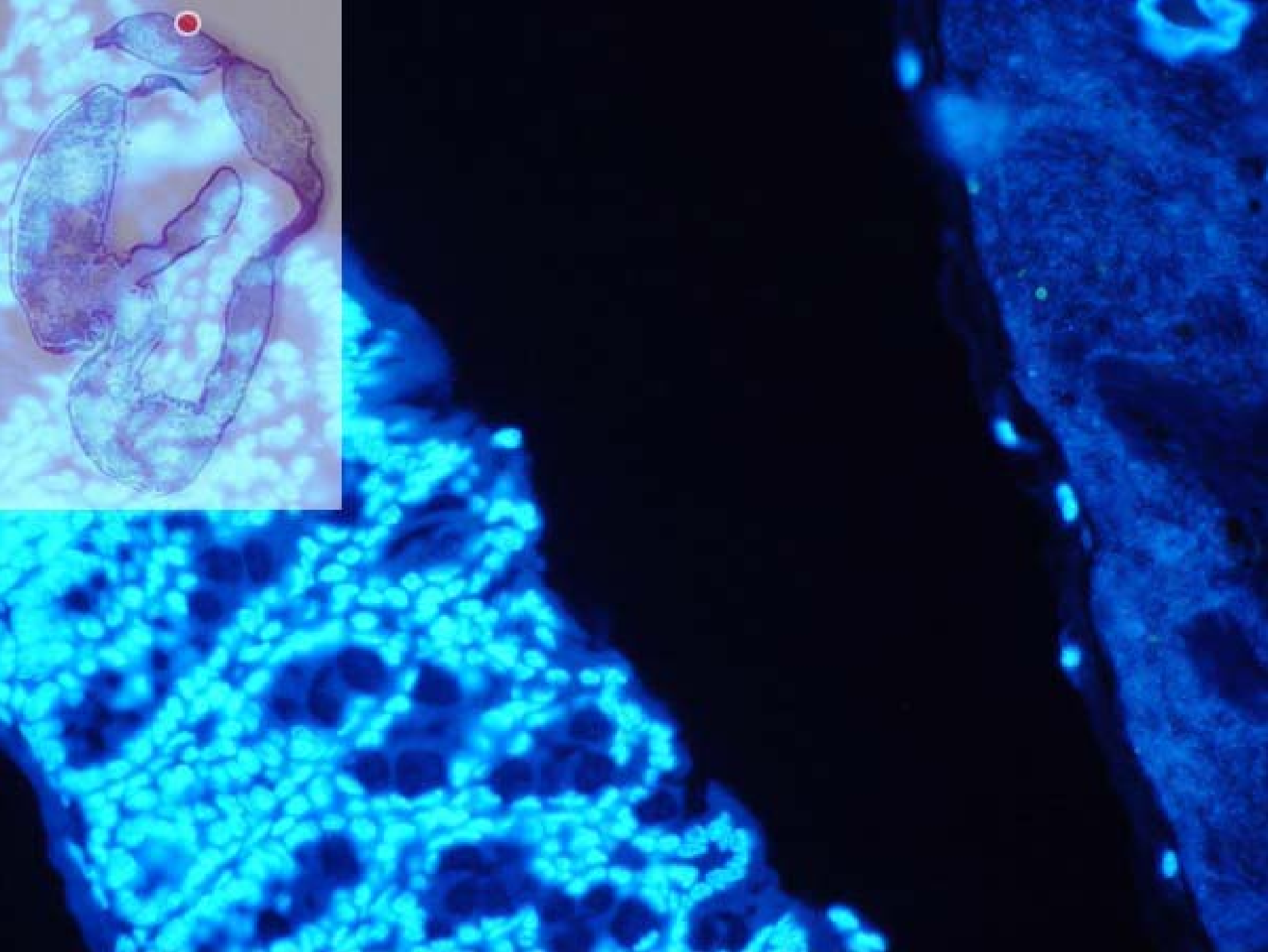


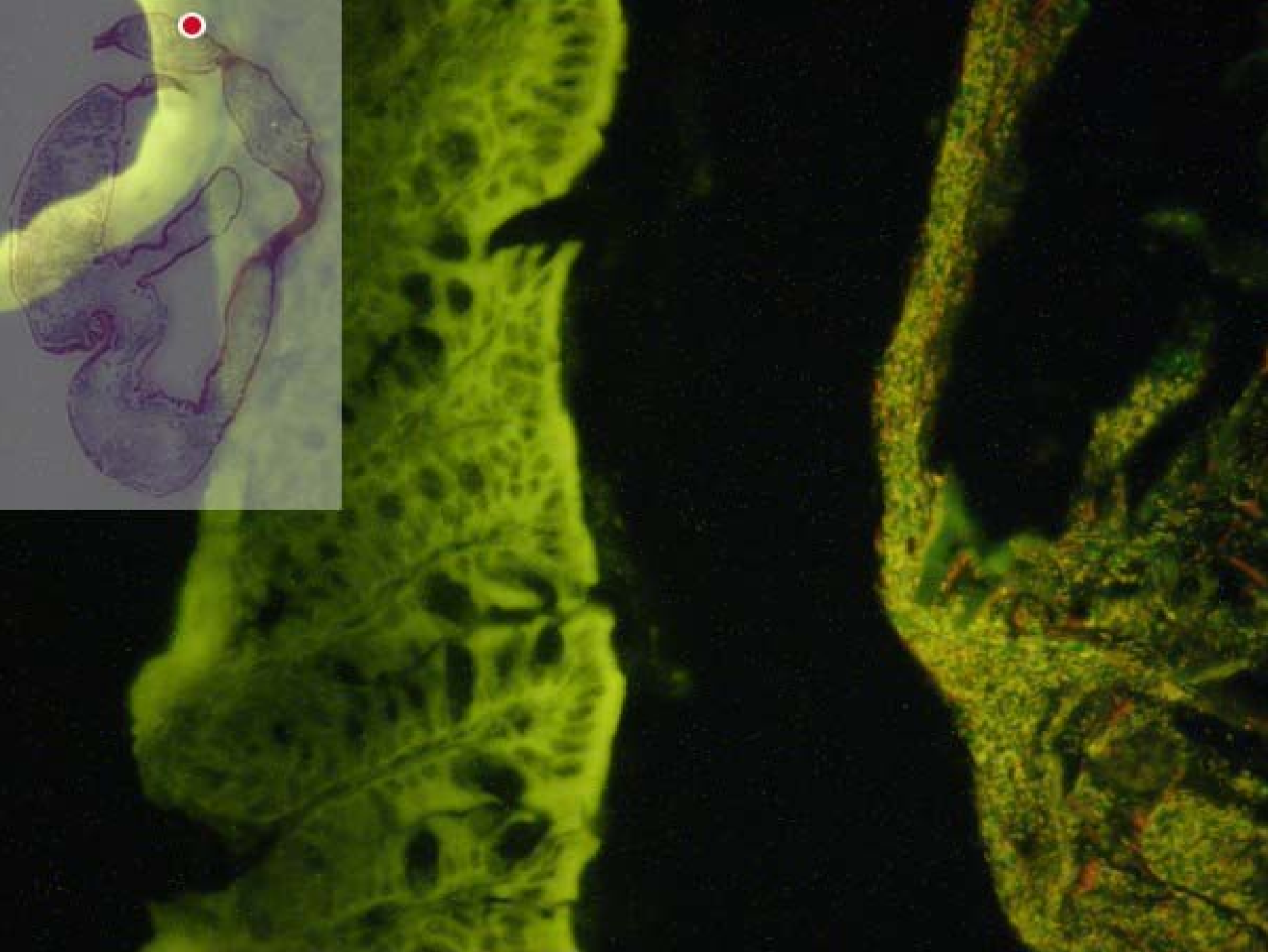


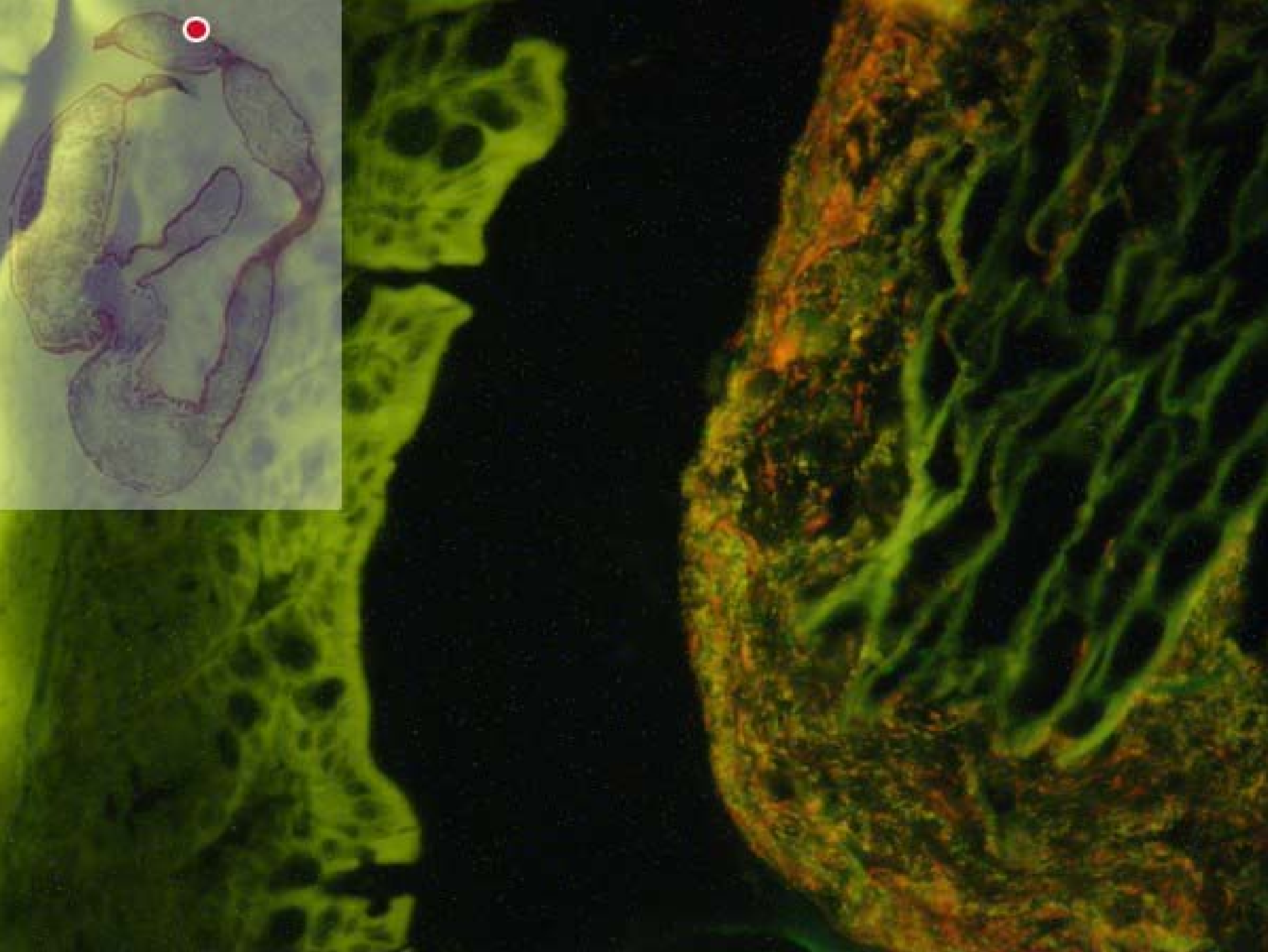


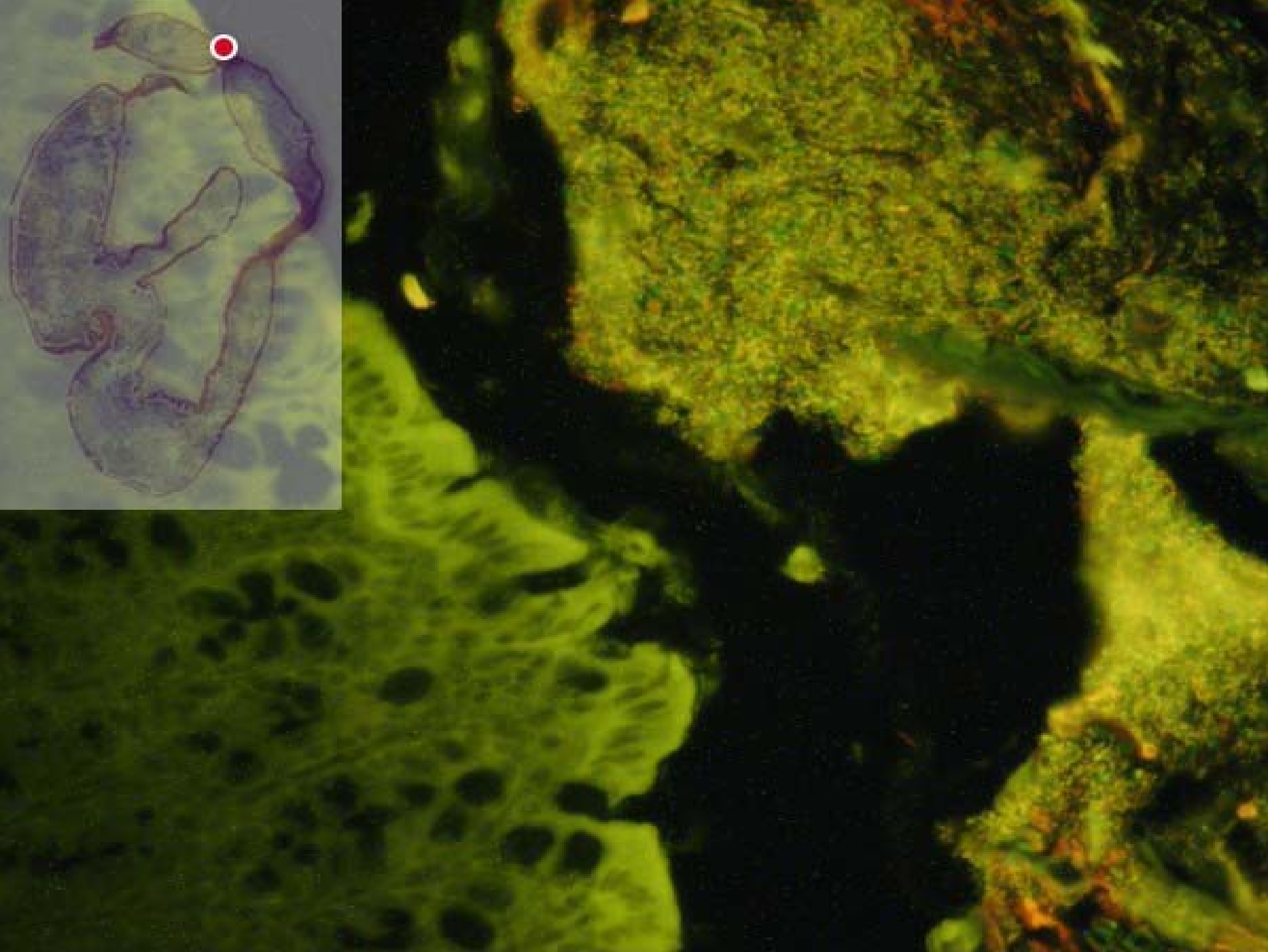


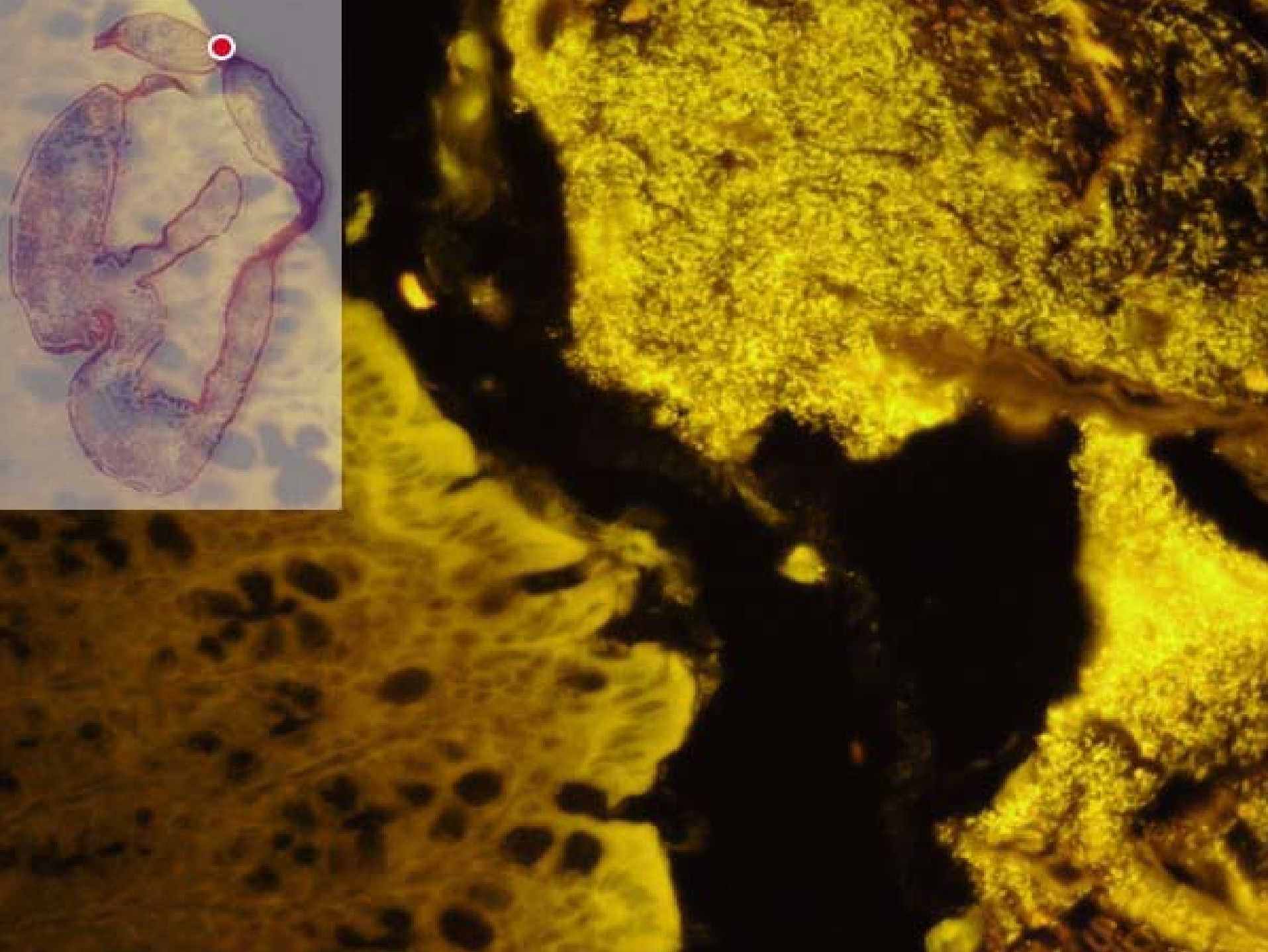


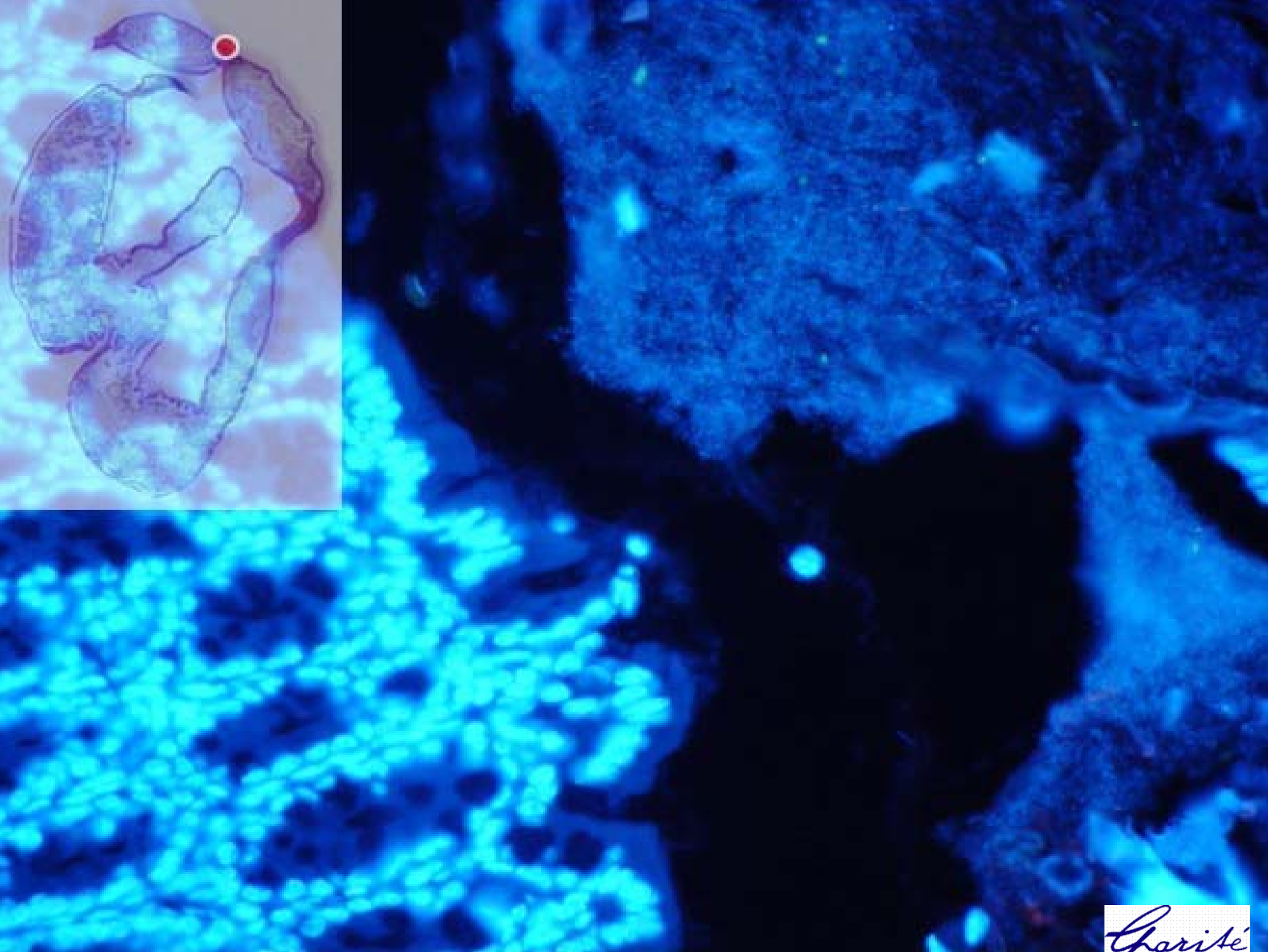


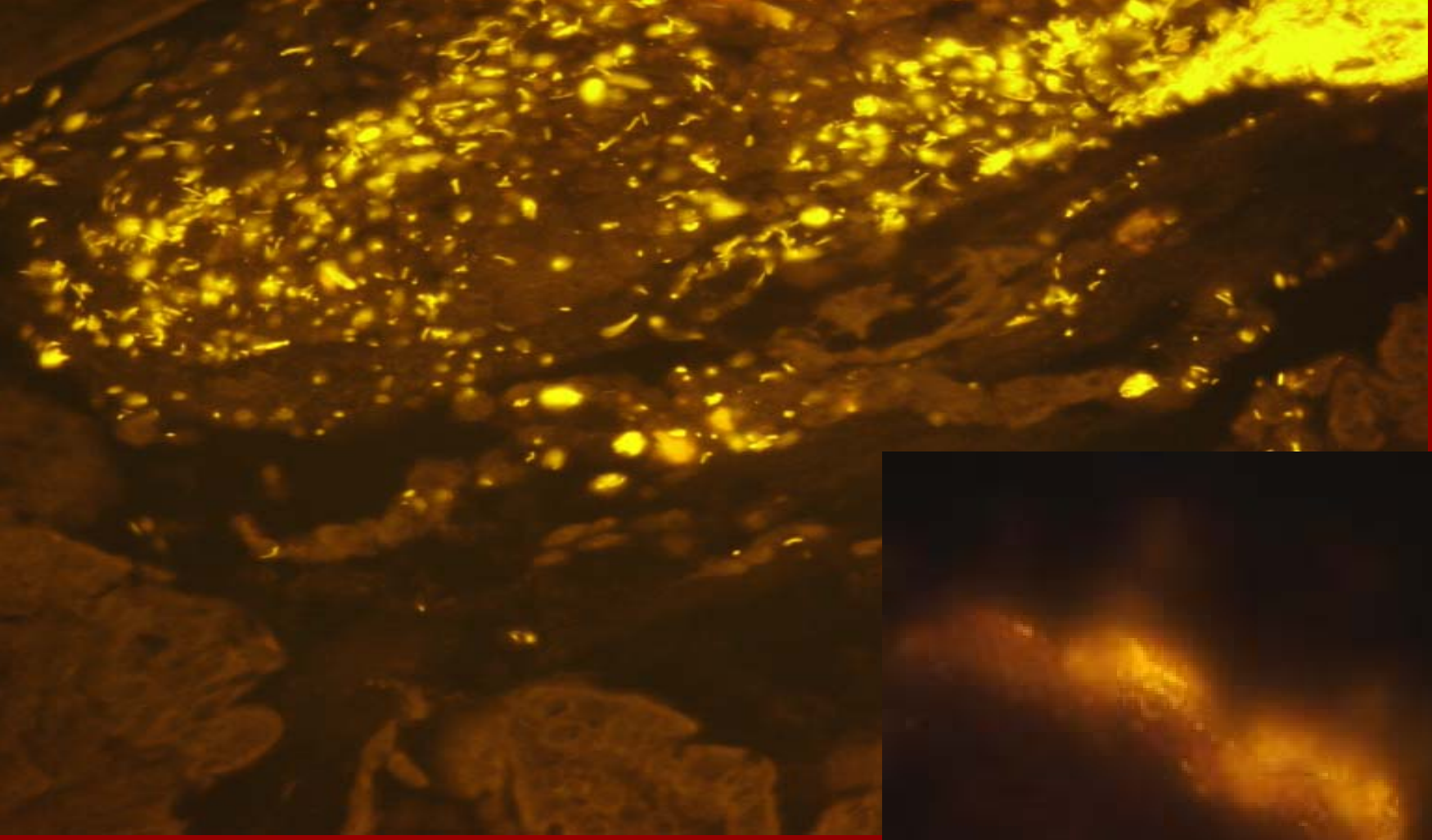






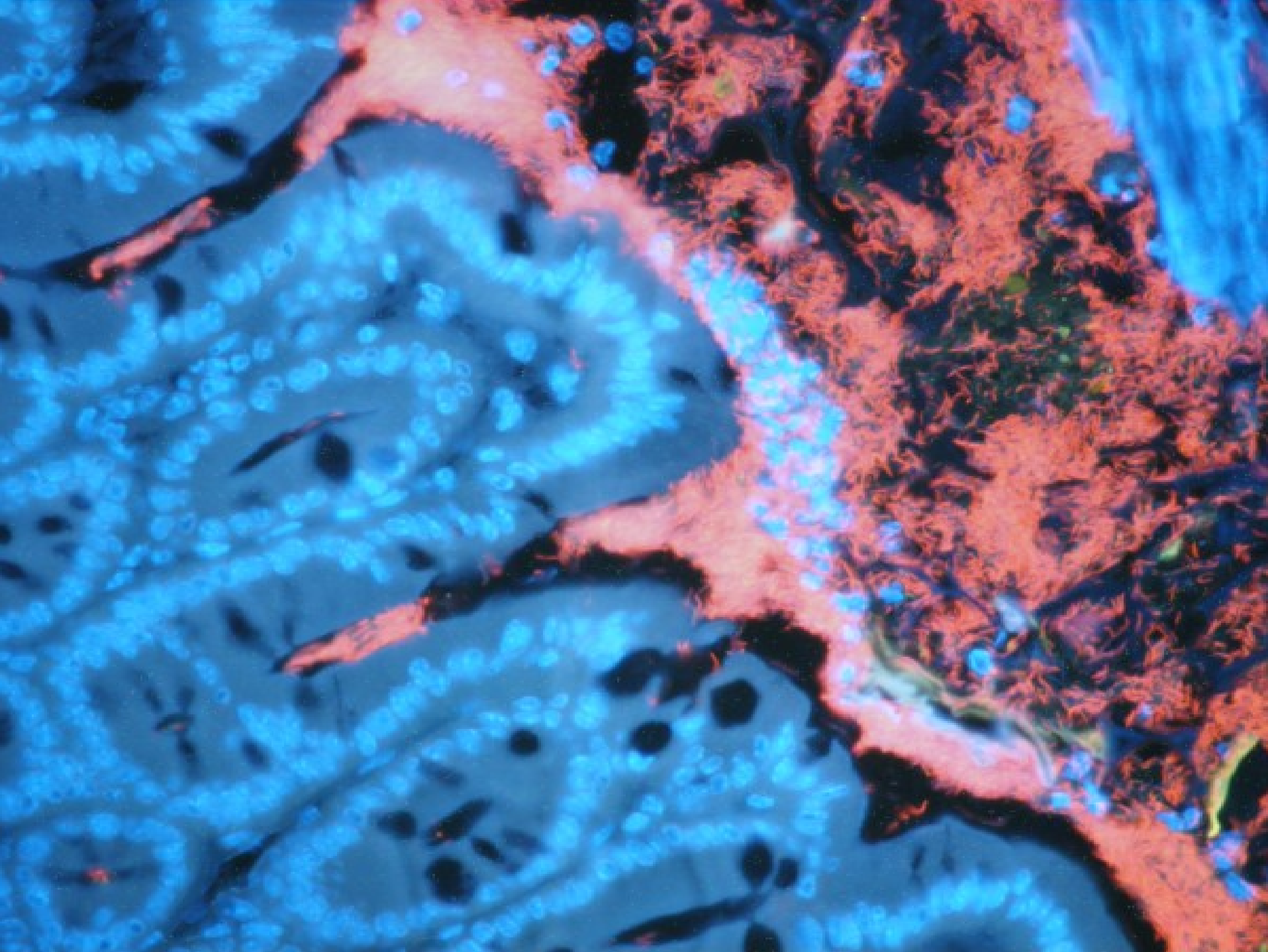


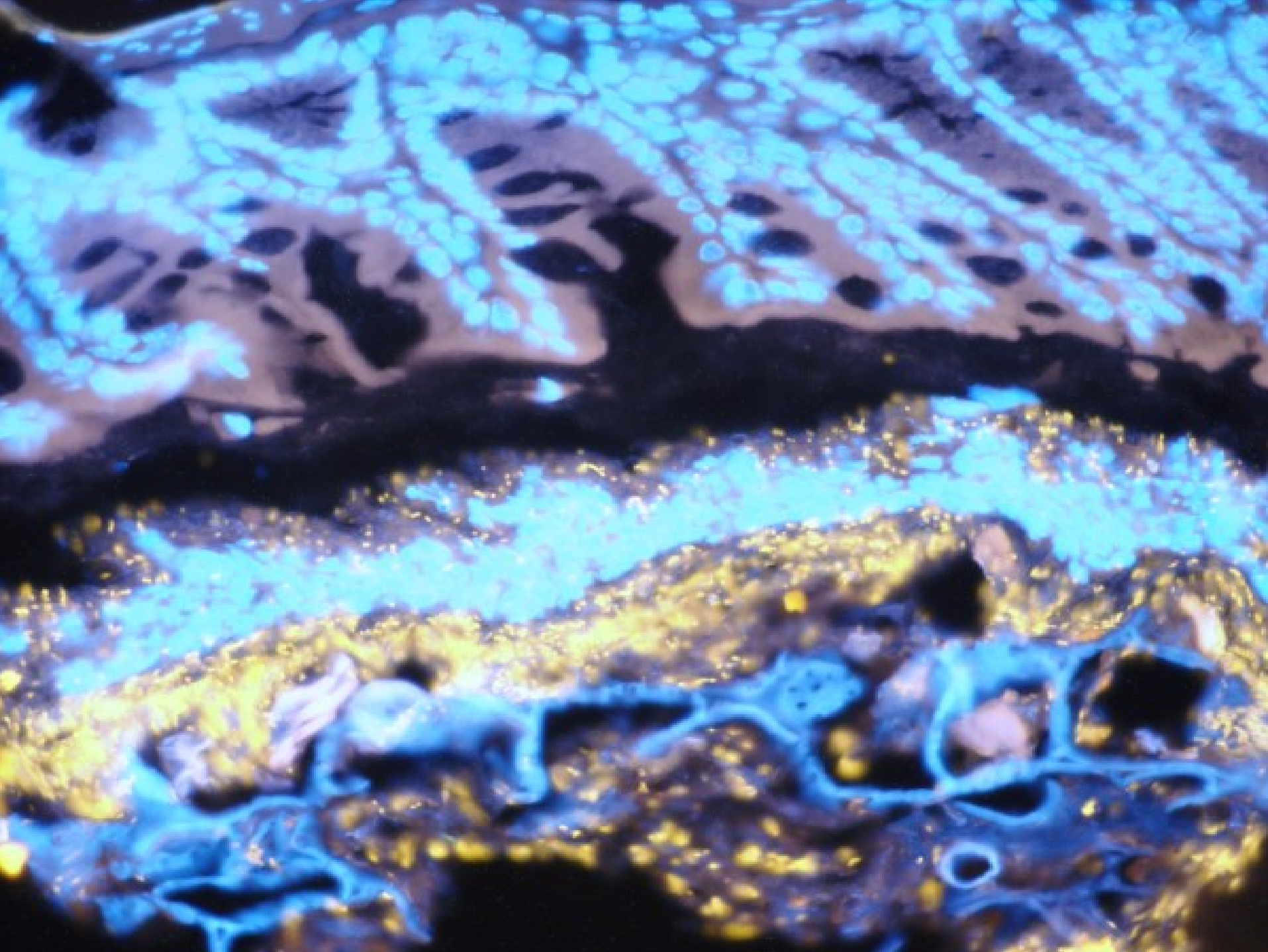

Charité



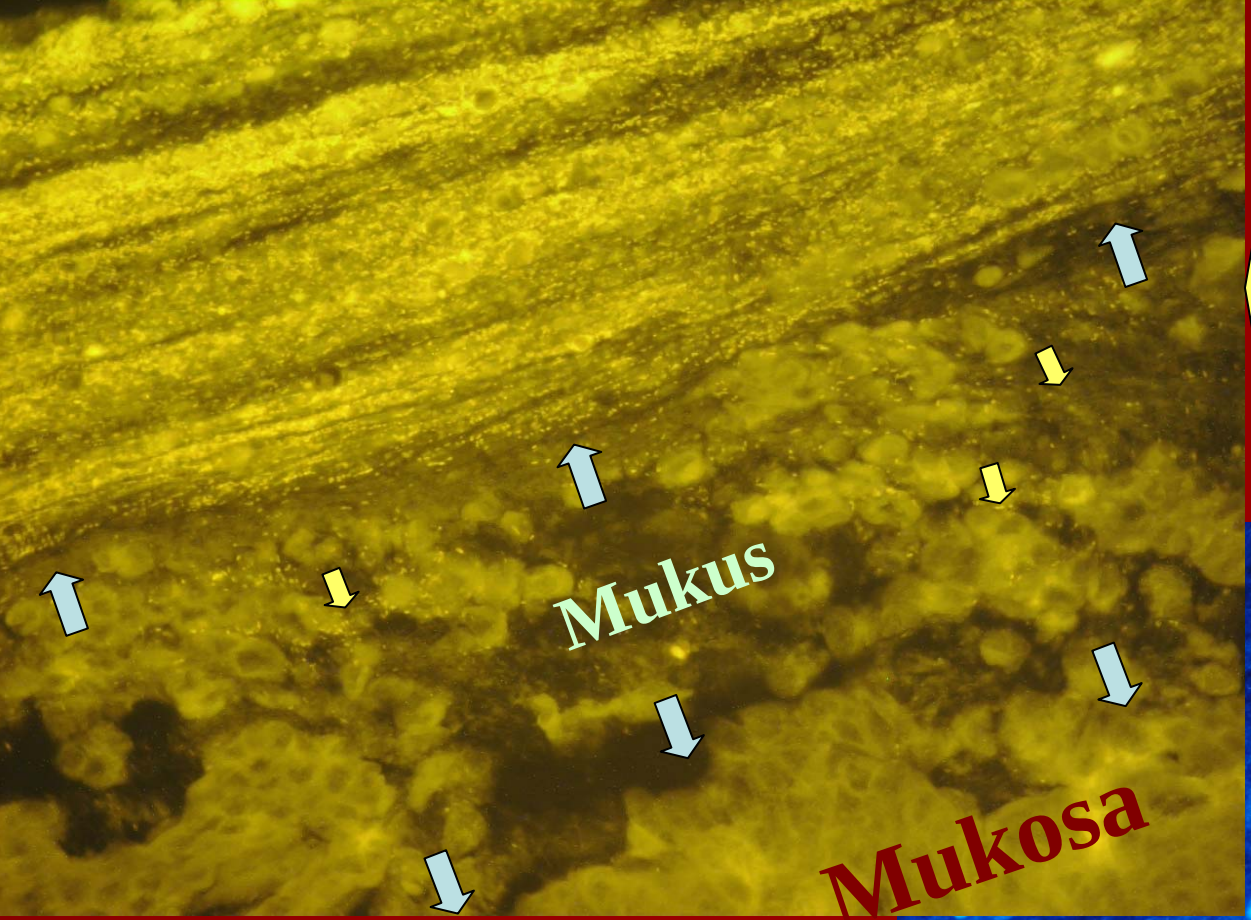
Entzündliche Vorgänge bei
Il 10 ko-Maus
TAG Maus mit Colitis
DSS Colitis


Leukozyten wandern vermehrt
in den Mukus

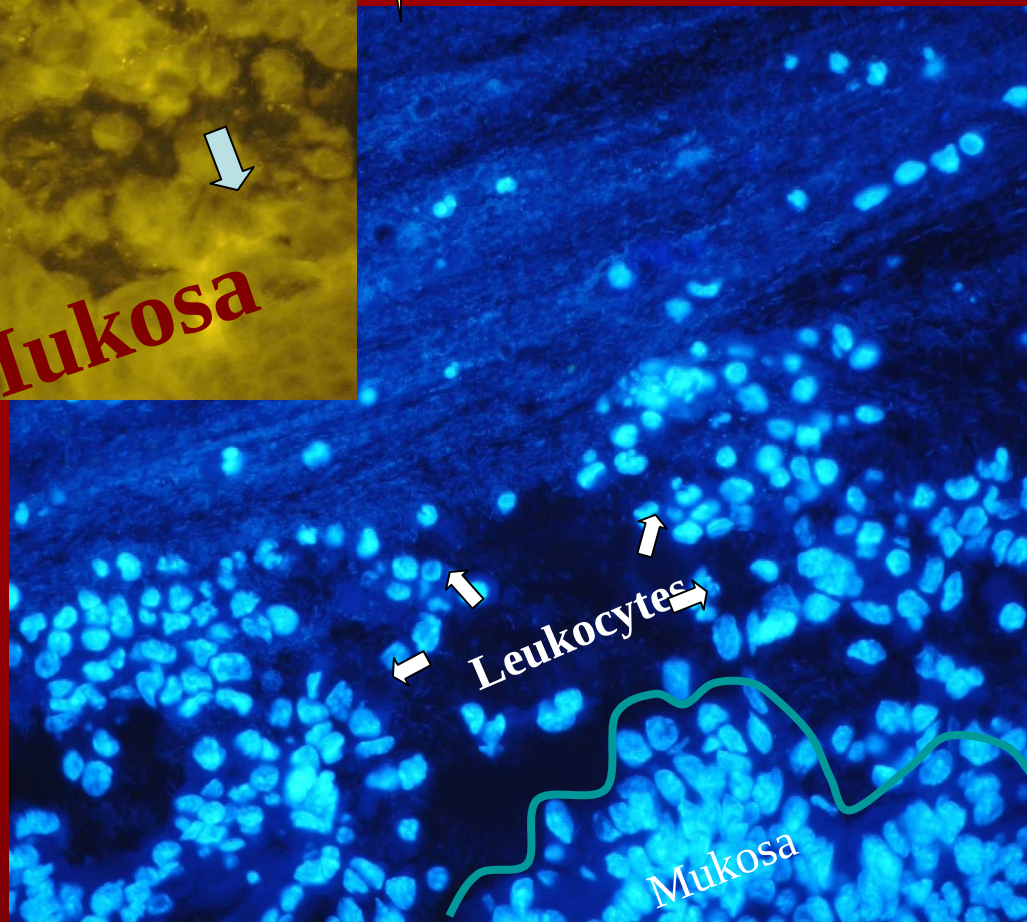


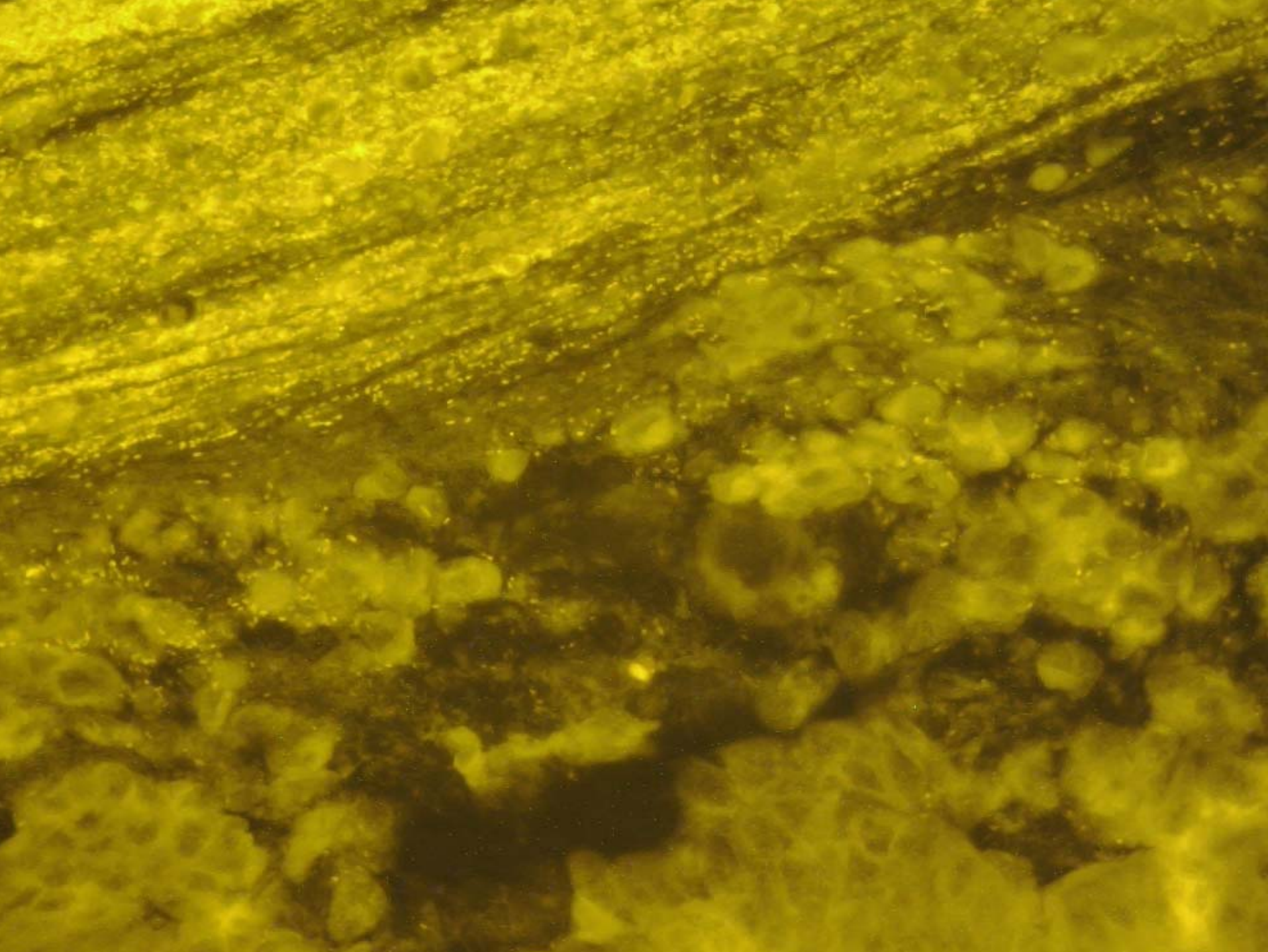


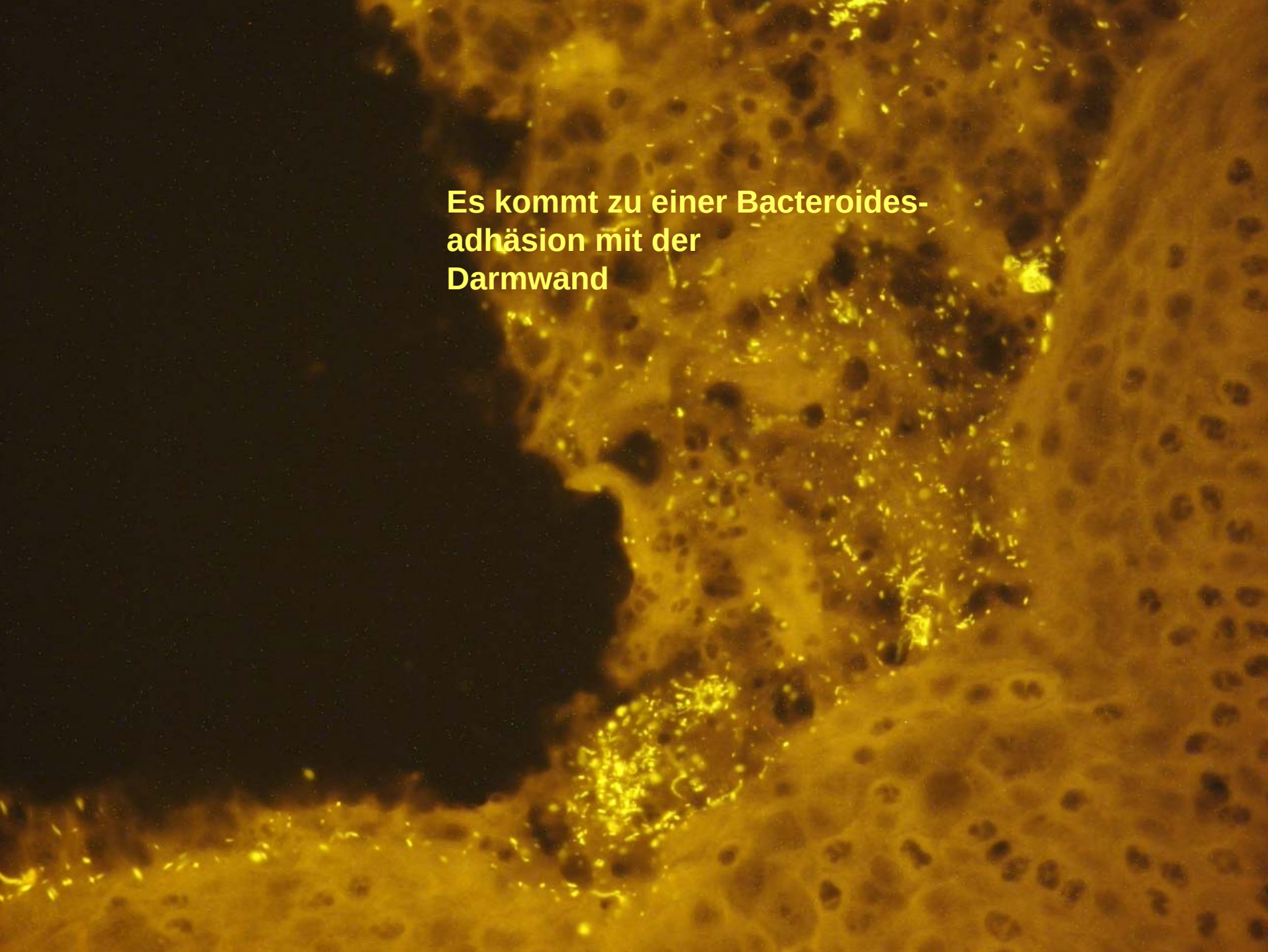
Bacteroides durchdringt Mukus

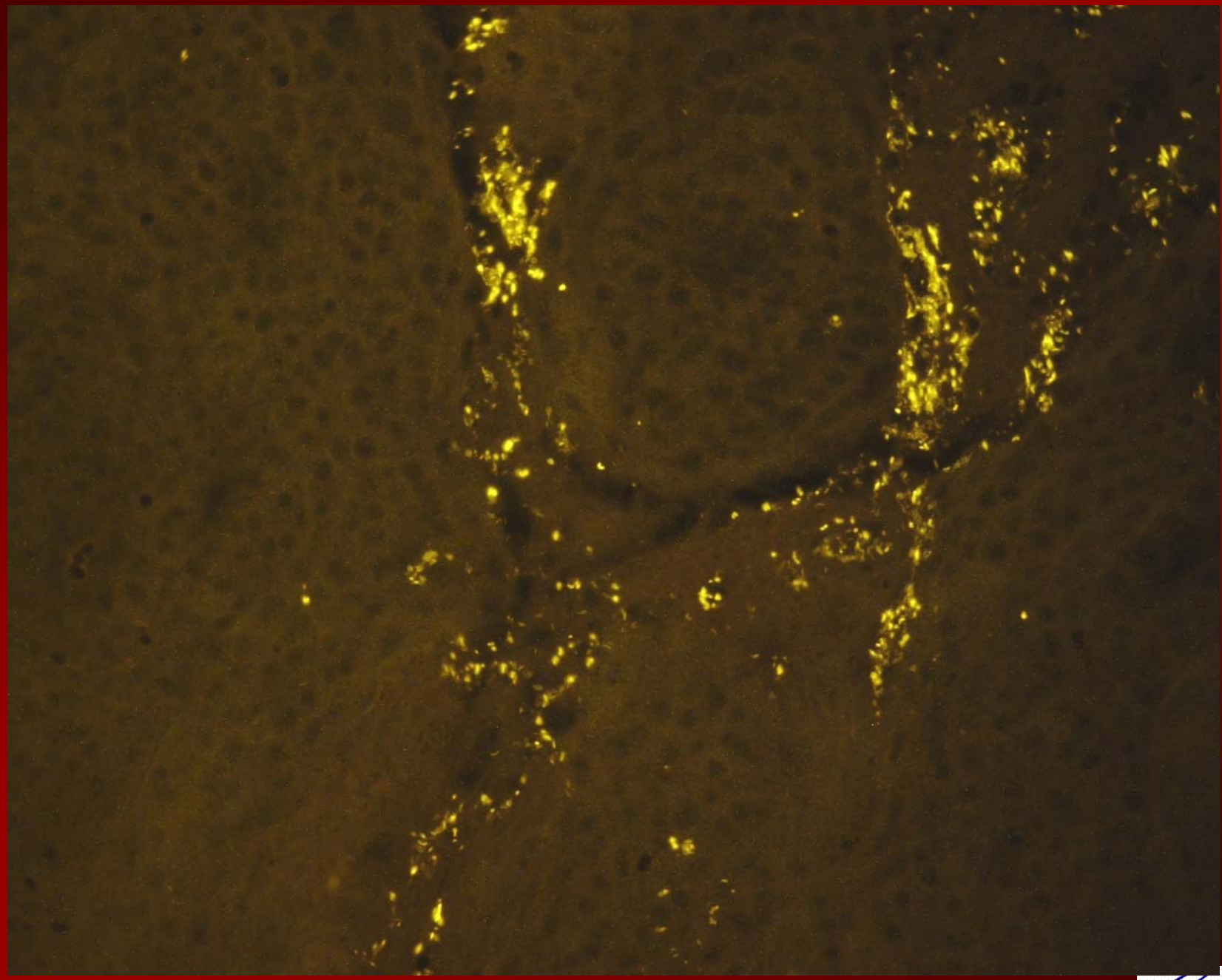


Gleiches Bild in Cy3 (Bacteroides) und Dapi Fluoreszenz zeigt getrennt Bakterien und Leukozyten

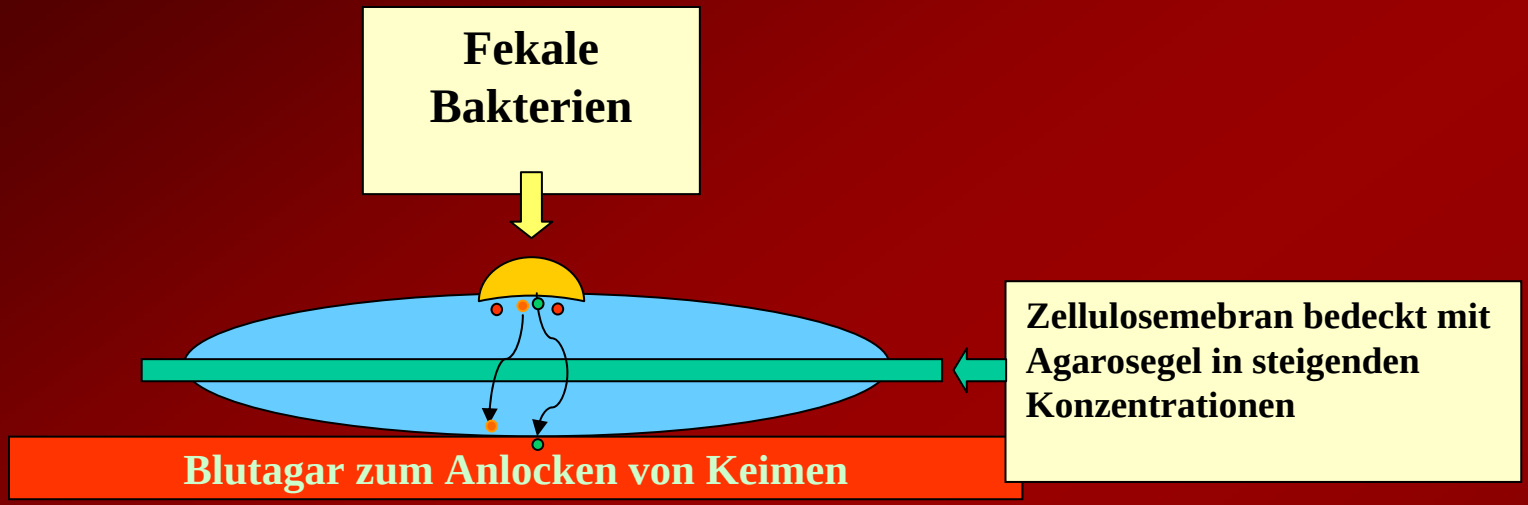


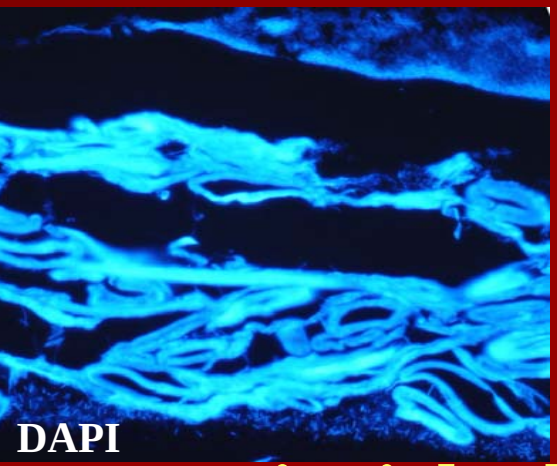


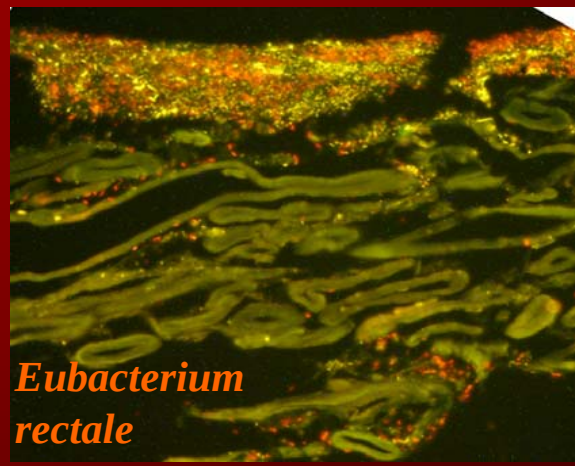

Es kommt zu einer Bacteroides-
adhäsion mit der
Darmwand



Mukussimulation in vitro

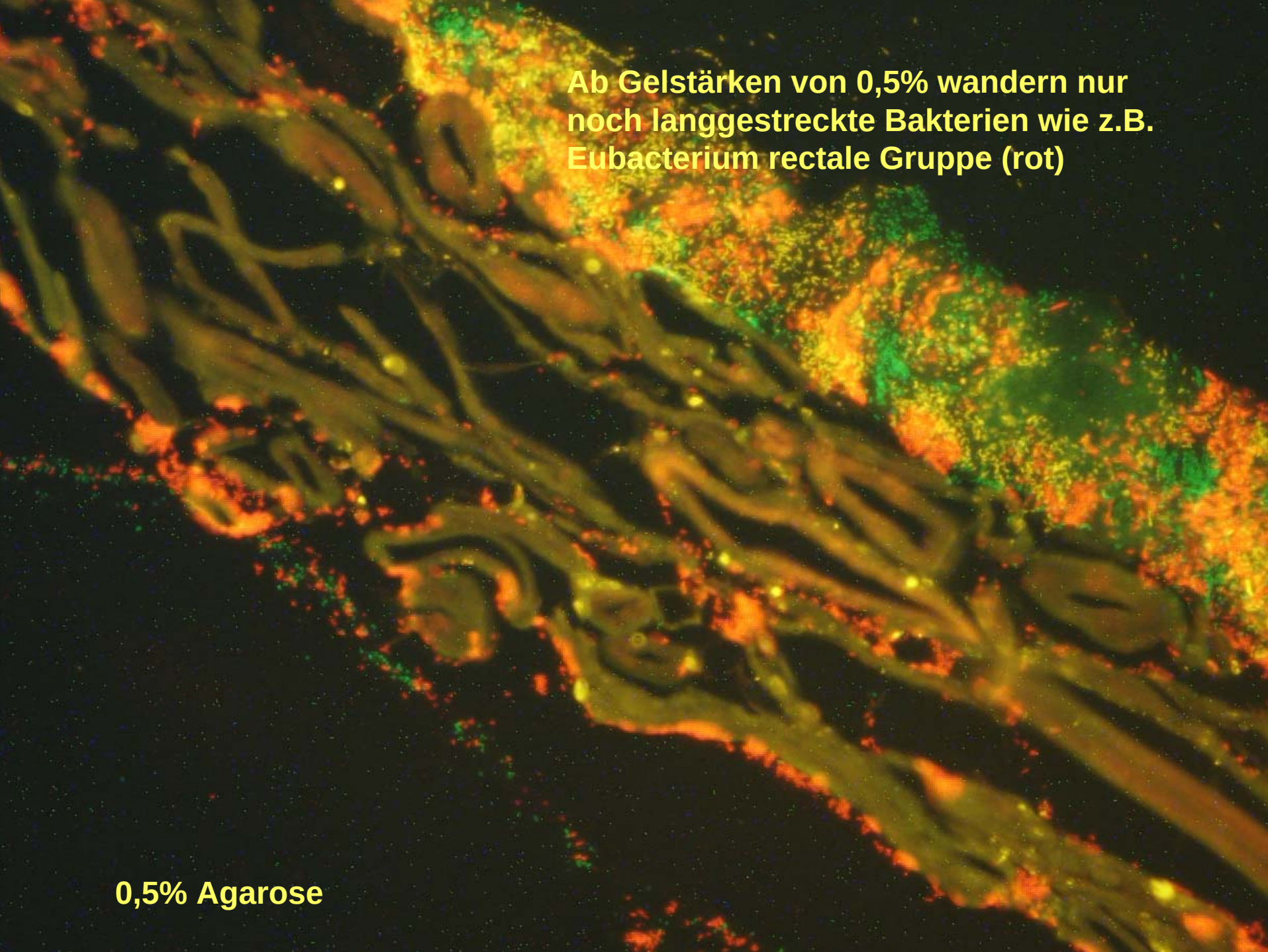




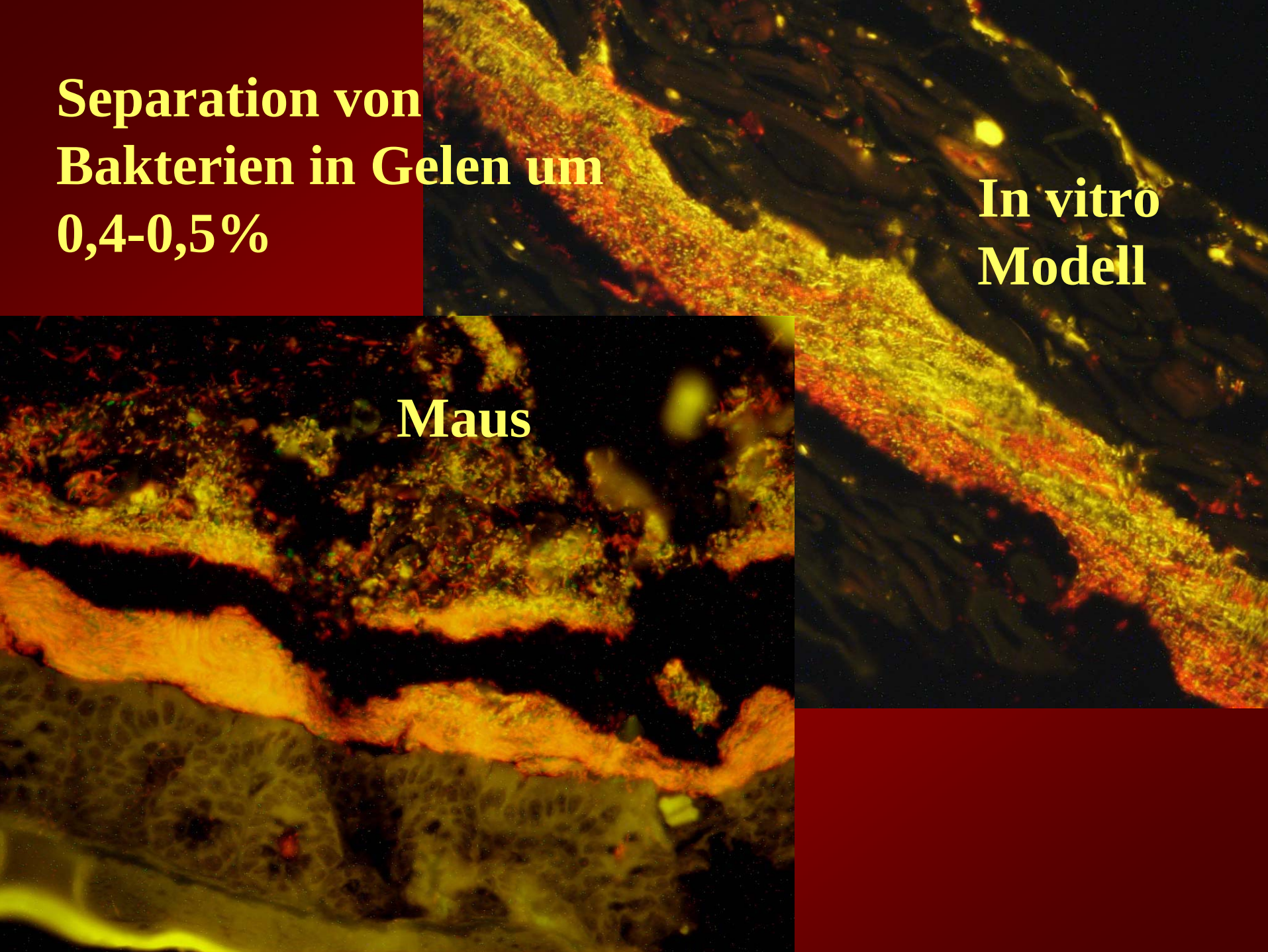


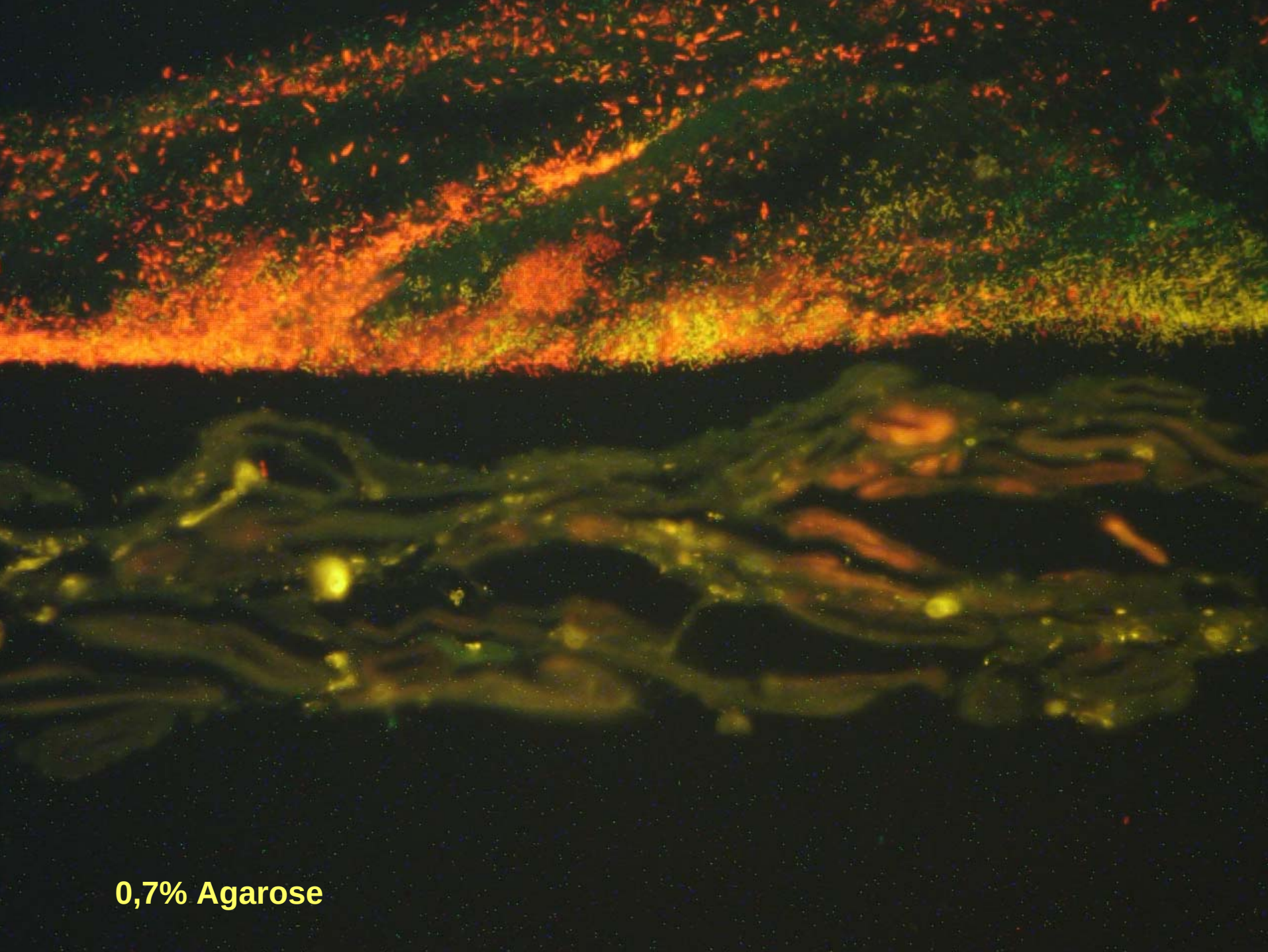
Beispiele der Mobilität


Bakterienbeweglichkeit durch Gele ist speziesspezifisch
und von der Viskosität abhängig
0,2% Agarose


Ab Gelstärken von 0,5% wandern nur noch langgestreckte Bakterien wie z.B. Eubacterium rectale Gruppe (rot)
0,5% Agarose

Separation von Bakterien in Gelen um 0,4-0,5%



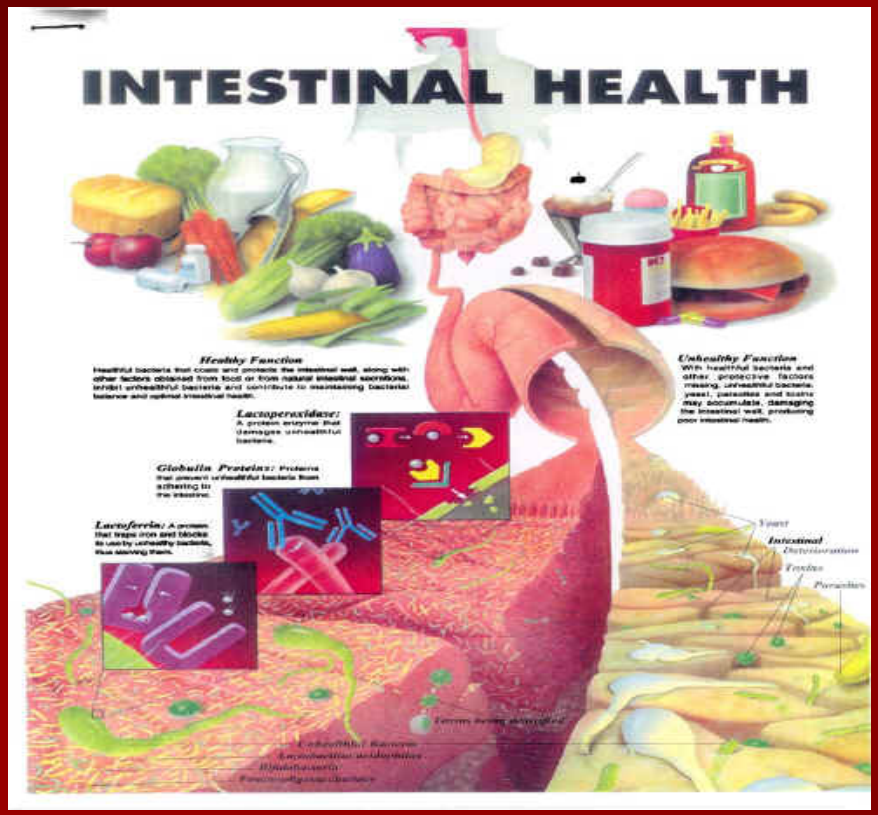

0,7% Agarose

Die Zugabe von DSS zum Gel führt zum Enhancement der Wanderung von Bakterien auch durch Gele mit Agarosedichte über 0,7% (bis 1,0%)
Eine ähnliche, wenn auch schwächere Wirkung zeigt die Zugabe von mukuslösendem DTT zum Stuhl.

Toleranz

Immunantwort

Physiologische Flora



Enterale Pathogene

E. coli

Bacteroides

Clostridium difficile

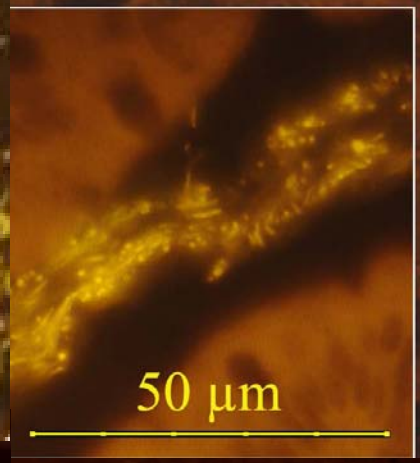
Enterokokken

Salmonellen

Shigellen

Überreaktion?

Der Grund für “Toleranz” ist eine effiziente Abwehr.
Die Mehrzahl fekaler Bakterien ist von der Darmwand getrennt



Viskosität des Mukus
Defensine
Bakterielle Separation/Probiotika
Leukozyten
Antikörper

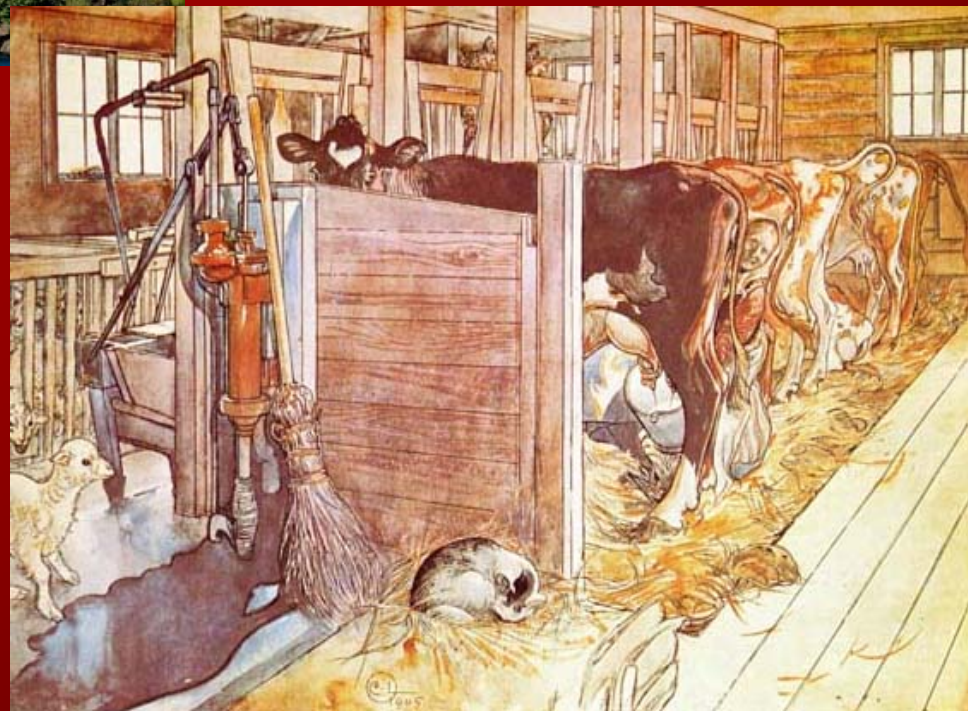
ONE WORLD ONE WAR

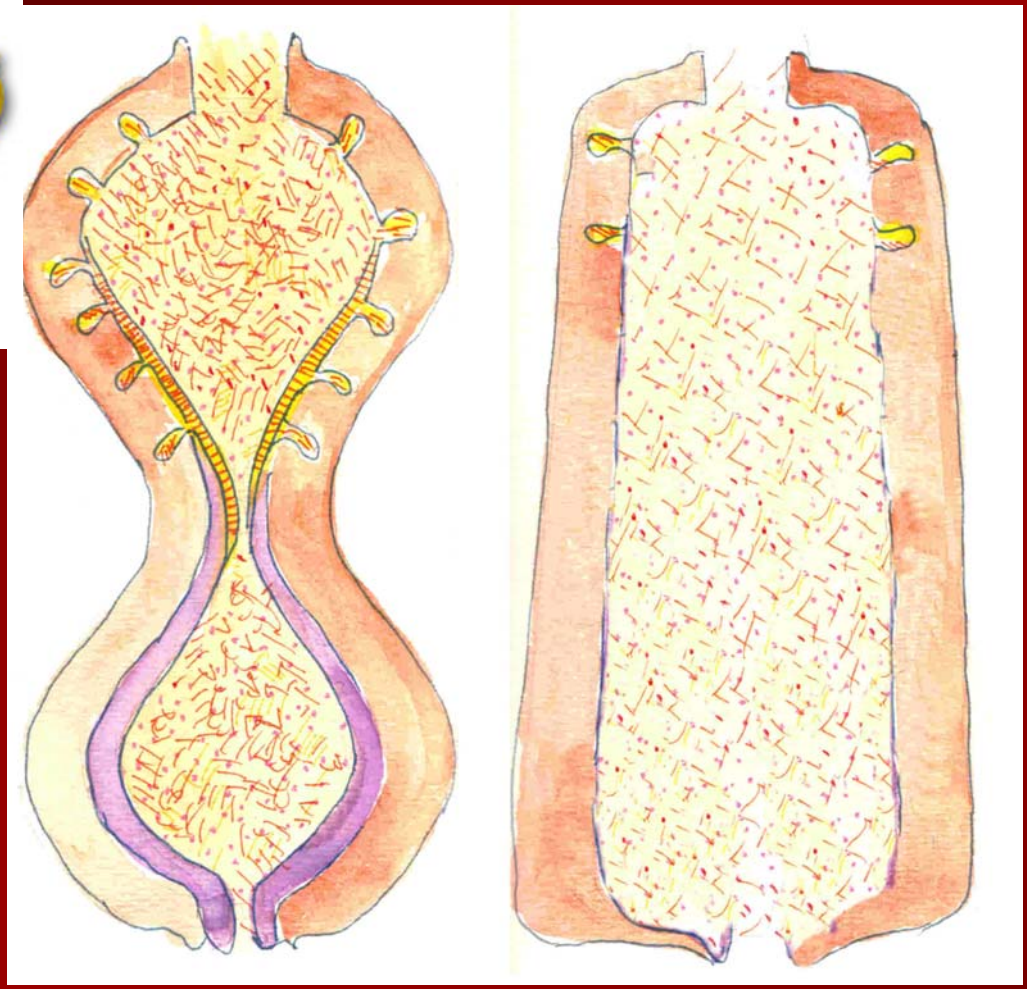
Two diseases

Three life lines connect America with the battle for three continents: (1) across the North Atlantic to the British Isles; (2) through the waist of the Atlantic to West Africa; (3) around South Africa to the Red Sea and the Persian Gulf. Unless Germany succeeds in breaking through the circle that reaches from England to the White Sea, along the Russian front to the Middle East, and across North Africa back to the Atlantic, the United Nations can secure time and space to organize and deploy their gradually growing resources. If Japan and Germany are allowed to join hands in India, the Axis will have the advantages of "the inner line" — on a world scale. Uninterrupted Axis control of Eurasia's huge land masses, from Le Havre to Shanghai, would transform the New World into an island and the two surrounding oceans into highways of invasion.

The tremendous distances in the Pacific, only yesterday appraised as an element of American security, have become the toughest of war problems. While across the relatively narrow Atlantic America has to support Allies who are entrenched on established battlegrounds, across the Pacific we must create, and supply, new fronts in areas that are thousands of miles from industrial centers and safe debarkation points. Unless Soviet Russia, by joining her allies' war against Japan, permits the full use of the most promising line of communication (Alaska - Aleutian Islands - Kamchatka - Vladivostok), the Japanese will enjoy for a long time far greater strategic advantages than Germany ever had in Europe. Even the most distant objective of the Japanese drive is many times nearer to Japan than to the nearest American base. With the China Seas closed to us by the Japanese, American supplies and armed forces will reach South Australia and the Indian coast over a route that is longer than a journey half around the world. Yet in these very regions, stretching out India, the United Nations, ill-prepared though they are, will have to fight one of the great decisive battles of history: The Battle for Asia.

Haben wir zu wenig Bakterien in unserer Umgebung?





Detergenzien halten unsere Umgebung sauber, könnten aber beim Menschen die gleiche Wirkung auf den Mukus wie DSS bei der Maus haben

Einige Faktoren mit potentiellem Einfluss auf die Mukusbarriere

Exogen:

Detergenzien:

Bakterielle Virulenz:

Glutene sind natürliche Emulgatoren. Ihre pathogene Wirkung bedarf einer bakteriellen Kompomente. Da der Dünndarm wenig Bakterien enthält, sind nur wenige Patienten von einer Fehlbesiedlung betroffen.

Rauchen

Endogen:

Gallensäuren sind körpereigene Emulgatoren. Sie wirken im Dünndarm, wo Bakterien fehlen und werden im Ileum resorbiert. Bei unvollständiger Resorption von Gallen-Säuren kommt es zum Durchfall.

Defensine

Probiotika, Prebiotika, Nukleinsaäurenderivate

Entz. Mediatoren der Leukozyten

Genetische

NOD 2 Mutationen

EU zugelassene Emulgatoren für Lebensmitteln

E425, Konjak
E432 bis E436, Polysorbat
- E432, Polyoxyethylen-sorbitan-monolaurat (Polysorbat 20)
- E433, Polyoxyethylen-sorbitan-monooleat (Polysorbat 80)
- E434, Polyoxyethylen-sorbitan-monopalmitat (Polysorbat 40)
- E435, Polyoxyethylen-sorbitan-monostearat (Polysorbat 60)
- E436, Polyoxyethylen-sorbitan-tristearat (Polysorbat 65)

E440, Pektine, Amidiertes Pektin
E442, Ammoniumsalze von Phosphatidsäuren
E444, Saccharose-acetat-isobutyrat
E445, Glycerinester aus Wurzelharz/Kolophonester
E450 bis E452, Phosphate
E459, Beta-Cyclodextrin
E460 bis E469 Cellulose und Celluloseverbindungen
- E460, Cellulose, Mikrokristalline Cellulose, Cellulosepulver
- E461, Methylcellulose
- E463, Hydroxypropylcellulose
- E464, Hydroxypropylmethylcellulose
- E465, Ethylmethylcellulose
- E466, Carboxymethylcellulose, Natriumcarboxymethylcellulose
- E468, Vernetzte Natrium-Carboxymethylcellulose
- E469, Enzymatisch hydrolysierte-Carboxymethylcellulose

E470a und E470b, Salze von Speisefettsäuren
- E470a, Natrium-, Kalium- und Calciumsalze von Speisefettsäuren
- E470b, Magnesiumsalze von Speisefettsäuren

E471 bis E472f, Mono- und Diglyceride von Speisefettsäuren
- E471, Mono- und Diglyceride von Speisefettsäuren, Monoglycerid
- E472a, Essigsäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472b, Milchsäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472c, Citronensäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472d, Weinsäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472e, Mono- und Diacetylweinsäureester von Mono- und Diglyceriden von Speisefettsäuren
- E472f, Gemischte Essig- und Weinsäureester von Mono- und Diglyceriden von Speisefettsäuren

E473, Zuckerester von Speisefettsäuren
E474, Zuckerglyceride
E475, Polyglycerinester von Speisefettsäuren, Polyglycerinester
E476, Polyglycerin-Polyricinoleat
E477, Propylenglycolester von Speisefetten
E479, Thermooxidiertes Sojaöl mit Mono- und Diglyceriden von Speisefettsäuren
E481 bis E483, Natriumstearoyl-2-lactylat, Calciumstearoyl-2-lactylat, Stearyltartrat
E491bis E495, Stearin- und Palmitatverbindungen
E491 Sorbitanmonostearat

Mit der Industrialisierung ist die Belastung des Menschen durch diverse enterale Bakterien nicht gesunken, sondern gestiegen.

Der Anstieg der Häufigkeit chronisch entzündlicher Darmerkrankungen seit Anfang des vorherigen Jahrhunderts kann eine direkte Folge davon sein

Nicht die Mukosa, sondern der anliegende Mukus ist der Ort der primären Auseinandersetzung mit enteralen Pathogenen.

Die Störung der Mukusbarriere ist der Grund für die Entzündung. Solange der Mukus durchlässig für Bakterien bleibt hat die Entzündung keinen Sinn, kann aber nicht erfolgreich beendet werden.

Die Dichte der Mukusbarriere hängt von vielen exogenen und endogenen Faktoren ab. Wir können diese nunmehr Identifizieren und eliminieren.

Morbus Crohn und Colitis sind heilbar.