Image Acquisition and Processing Workflow

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Acquisition :

Below is the proposed data structure for image acquisition :

data

|_ dd/mm/yyyy

      |_ anat-x or koek-x

x is the sample number, anat : recent sample / koek : archaeological sample

Five images are captured per slide:

1. 1 image (4X) in brightfield using the DS-Ri1 camera
2. 4 images (20X) in fluorescence using the spinning disk

1. BrightField

Left panels:

λ : ✓ **DS-Ri1 - DIA:DIA-Couleur**

Large Image :

Scan Area : **8 x 8**

Stitching : Overlap **20%**

    Stitching via **Optimal Path**

Right panels:

Lightpaths : **DS-Ri1 - DIA**

Ti2 Pad :

Nosepiece: **4X**

Lights : **20%**

Exposure time : **4 ms**

Analog gain : **1.0x**

1. Take an initial image at a random location on the sample by clicking "Run now".

2. After acquisition, right-click on the center of the image and select "Move this Point to center".

3. Take another image.

4. Save it as "anat-x-stitch-bf.nd2" or "koek-x-stitch-bf.nd2", where "x" is the slide number. "anat" refers to a recent sample and "koek" to an archaeological sample.

5. Keep the saved image open for the next steps.

2. Fluorescence - Spinning Disk

Left panels: Right panels:

λ : ✓ **Spinning disk - DAPI**   Lightpaths : **Spinning disk**

    ✓ **Spinning disk – Cy3**   Objectives : **20X**
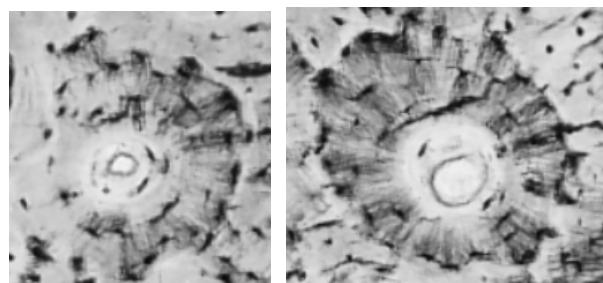
    ✓ **Spinning disk - CFP**   Celesta Pad :

Excitation wavelength	Intensity	Exposure time
405 nm	**45%**	**200 ms**
440 nm	**15%**	**200 ms**
477 nm	**20%**	**500 ms**
517 nm	**25%**	**100 ms**
546 nm	**50%**	**200 ms**

    ✓ **Spinning disk - YFP**

    ✓ **Spinning disk - GFP**

    ✓ **Spinning disk - Brightfield**

Z : Relative

    Step: **3 μm – 7 steps**

A. "Osteon" structure

1. On the brightfield image, right-click on this type of structure, an osteon, and select "Move this Point to center".
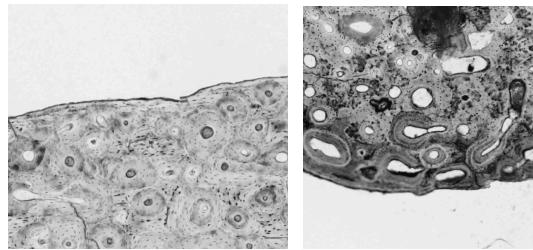

2. Take an image.

3. Save it as "anat-x-fluo-spinning-bernique.nd2" or "koek-x-fluo-spinning-bernique.nd2", where "x" is the slide number, "anat" refers to a recent sample and "koek" to an archaeological sample.

B. "Edge" structure

1. On the brightfield image, right-click on an edge of the section and select "Move this Point to center".
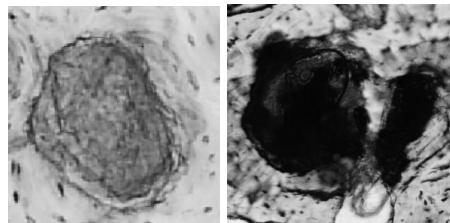

2. Take an image

3. Save it as "anat-x-fluo-spinning-bord.nd2" or "koek-x-fluo-spinning-bord.nd2", where "x" is the slide number, "anat" refers to a recent sample and "koek" to an archaeological sample.

C. "Alteration" structure

1. On the brightfield image, right-click on this type of structure, an alteration, and select "Move this Point to center".
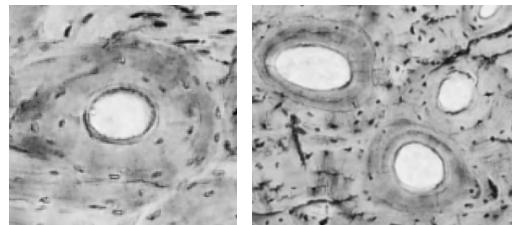

2. Take an image.

3. Save it as "anat-x-fluo-spinning-alteration.nd2" or "koek-x-fluo-spinning-alteration.nd2", where "x" is the slide number, "anat" refers to a recent sample and "koek" to an archaeological sample.

D. "Lacuna" structure

1. On the brightfield image, right-click on this type of structure, a lacuna, and select "Move this Point to center".

2. Take an image.

3. Save it as "anat-x-fluo-spinning-blanc.nd2" or "koek-x-fluo-spinning-blanc.nd2", where "x" is the slide number, "anat" refers to a recent sample and "koek" to an archaeological sample.