A Framework for Organized and Accessible 2D Electrophoresis Data in HCP Assay Development

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Keeping it Together: Organizing 2D Electrophoresis Data Like a Pro!

Access the article and template

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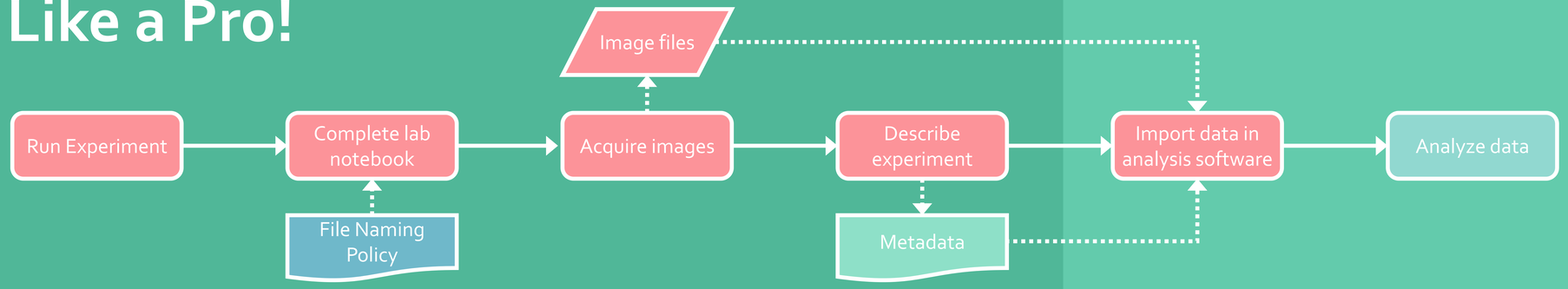

CHALLENGES

Managing 2D electrophoresis data in HCP immunoassay research can be complex, especially when incorporating multiple control images and varied experimental settings.

For instance, a typical 2D-DIBE blot derived from a given IPG strip may necessitate the capturing of multiple images: at different stages (of gels pre- and post-transfer, and membranes pre- and post-probing), across dyes, and with varying image acquisition settings such as resolution or PMT voltage to be optimized. For extensive comparative studies of different antigen and antibody combinations, the image count can quickly explode.

OBJECTIVES

We undertook to design a systematic approach for organizing and naming image files of 2D electrophoresis experiments, and managing their metadata, to enhance process efficiency and ensure data consistency and integrity.

BENEFITS OF THIS APPROACH

- Safeguards data lineage and integrity
- Streamlines consistency and referencing across platforms
- Minimizes labelling and analysis errors
- Reduces file retrieval times
- Facilitates import and reuse of metadata
- Optimizes image analysis setup and results interpretation
- Simplifies auditing procedures

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File Naming Policy

File names should be:

- **Fixed** – Stay consistent across all platforms, including image acquisition devices, lab notebooks, storage systems, analysis software and reports.
- **Unique** – Be distinct to prevent conflicts and enable single-folder storage.
- **Traceable** – Allow tracking of all images originating from an IPG strip, to support data lineage and troubleshooting.
- **Short** – Be brief to simplify reading and retrieval, avoiding long file path issues.
- **Predictable** – Establish names during the planning phase in the lab notebook, independent of acquisition time.
- **Objective** – Follow a straightforward, predefined naming policy without need for (subjective) decisions.

E.g. 5974Gat Cy3 A01(2)

Strip number	Last 4 digits of the IPG strip number (from the same batch for a given experiment).
Object type	Code for object and status (e.g. G - gel before transfer, Gat - gel after transfer, M - membrane before probing, B - membrane after probing).
Stain/label	Name of the stain or label used, important for DIGE or DIBE applications (e.g. Cy3, Cy5, EZ Fluor, ...).
Acquisition code	Code for the set of image acquisition parameters used (optional). It is identical for images of a given DIBE or DIGE object (e.g. A01 = 50 µm resolution, PMT Cy3 490V, PMT Cy5 420V). These codes are kept in the Metadata file. For duplicate acquisitions with the same settings, a replicate number is added.

Aliases in Analyses

File names, e.g. '5974Gat Cy3 A01' often lack usefulness during image analysis but need to stay unchanged to ensure consistency and reference across platforms.

Aliases, or alternate image names used in analysis, should include details about the samples, such as antigen and antibody reagents, along with other analytical parameters. Examples could be 'CHO-HCP LMW' or 'anti-CHO HCP Ab'.

These **aliases** should be flexible, easily adjusted to suit various analysis and reporting needs—for instance, detailed names for internal use and anonymized versions for external publications.

Aliases can either be manually edited or imported from the Metadata file, where they can be constructed automatically based on existing data fields.

Reference across platforms
File names

Analysis software
Aliases in all image labels and reports
File names for external referencing

Reports and publications
Aliases

Metadata

The generated image files of an experiment (or set of related experiments) are always stored in a **single folder**. Subfolders only exist for images from the same acquisition (DIGE/DIBE).

At all times, the image files are accompanied by a simple spreadsheet containing the **metadata**:

- Excel simplifies the completion of a data table (copy, formulas) while being widely accessible.
- Search and Filter can be used to quickly identify and retrieve particular images in the data set.
- The use of picklists improves consistency.
- Formulas and concatenation can help automatically build file names and aliases.
- A file with an anonymized subset of the meta data can easily be created for sharing with collaborators.

File name	Alias	Strip	Object	Dye	Acq.	Ag	Ab	…
1234G Cy3 A01	G1 Ag1	1234	Gel before transfer	Cy3	A01	Ag1	NA	
1234Gat Cy3 A02	G1at Ag1	1234	Gel after transfer	Cy3	A02	Ag1	NA	
1234M Cy3 A03	M1 Ag1	1234	Mem before probing	Cy3	A03	Ag1	NA	
1234M Cy5 A03	M1 Cy5Ctrl	1234	Mem before probing	Cy5	A03	Ag1	NA	
1234B Cy3 A04	B1 Ag1	1234	Mem after probing	Cy3	A04	Ag1	Ab3	
1234B Cy5 A04	B1 Ab3	1234	Mem after probing	Cy5	A04	Ag1	Ab3	
3456G Cy3 A01	G2 Ag2	3456	Gel before transfer	Cy3	A01	Ag2	NA	
3456Gat Cy3 A02	G2at Ag2	3456	Gel after transfer	Cy3	A02	Ag2	NA	
3456M Cy3 A03	M2 Ag2	3456	Mem before probing	Cy3	A03	Ag2	NA	

Images | Reagents | Acquisition

Import desired fields in Melanie analysis software