Complete FLASH Installation Protocol

Muscle Fiber Analyzer - Complete Installation

System Requirements

Minimum configuration required:

- **Operating System:** Windows 10/11 (x64), macOS 10.15+, or Linux Ubuntu 18.04+
- **RAM:** 8 GB minimum (16 GB recommended)
- **Disk Space:** 5 GB free space
- **Graphics Card:** NVIDIA (optional, for GPU acceleration)
- **Administrator Rights:** Required only for GPU/CUDA installation

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STEP 0: GPU Verification (Windows only - optional)

A. Graphics Card Identification

1. **Open Task Manager:**
   - Press `Ctrl + Shift + Esc`
   - Click on the "Performance" tab
   - Select "GPU" in the left panel
2. **Check your graphics card:**
   - **Required:** NVIDIA GTX 10xx, RTX series, or newer
   - **Recommended GPU RAM:** 6 GB or more
   - Note the exact card name (e.g., "NVIDIA GeForce RTX 3070")

Compatibility check:

- ✅ **Compatible:** GTX 1060, RTX 2060, RTX 3070, RTX 4080, etc.
- ❌ **Not compatible:** GTX 900 series or older, AMD cards

B. CUDA Toolkit 11.8 Installation (Administrator rights required)

1. **Download CUDA Toolkit 11.8:**
   - Go to: https://developer.nvidia.com/cuda-11-8-0-download-archive
   - Select: Windows → x86_64 → 10/11 → exe (local)
2. **CUDA Installation:**
   - Close all applications
   - Run the downloaded file as administrator
   - Choose "Custom installation" if offered
   - Restart computer after installation

3. **CUDA Verification:**
    - Open command prompt (cmd)
    - Type: `nvcc --version`
    - Verify that "Cuda compilation tools, release 11.8" is displayed

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STEP 1: FIJI/ImageJ Installation

A. Download and installation

If you already have FIJI, skip to step B.

1. **Download FIJI:**
    - Website: https://imagej.net/software/fiji/downloads
    - Choose the "Stable download" for your OS
2. **Installation:**
    - Extract the archive to a folder (e.g., `C:\Fiji` or `/Applications/Fiji.app`)
    - Launch FIJI to verify installation

B. Initial configuration

1. In FIJI: `Help → Update...`
2. Click "Manage update sites"
3. **Activate the following sites** (check boxes):
    - ✅ IBMP-CNRS
    - ✅ ImageScience
    - ✅ PTBIOP
4. Click `Close → Apply changes`
5. **Restart FIJI** after installation

STEP 2: Python and Cellpose Installation

A. Miniconda Installation (recommended)

1. **Download Miniconda:**
    - Website: https://repo.anaconda.com/miniconda/
    - **Windows:** Miniconda3-latest-Windows-x86_64.exe
    - **macOS:** Miniconda3-latest-MacOSX-x86_64.pkg
    - **Linux:** Miniconda3-latest-Linux-x86_64.sh

2. **Installation:**
    - **Windows:** Double-click executable, check "Add to PATH"
    - **macOS:** Double-click the .pkg
    - **Linux:** `bash Miniconda3-latest-Linux-x86_64.sh`
3. **Restart** your terminal/command prompt

B. Conda Configuration and Cellpose Environment Creation

Open a terminal/command prompt and execute in order:

```
# Initial Conda configuration
conda config --set auto_activate_base false
conda config --set channel_priority flexible
conda config --set solver libmamba

# Accept Conda terms
conda info

# Clean existing environments
conda env remove -n cellpose -y
conda clean --all -y

# Create cellpose environment with Python 3.8
conda create -n cellpose python=3.8 -y

# Activate environment
conda activate cellpose
```

Installation according to your configuration:

For GPU (Windows with NVIDIA):

```
pip install cellpose==3.1.1.2
pip install torch==1.13.1+cu118 torchvision==0.14.1+cu118
torchaudio==0.13.1 --index-url https://download.pytorch.org/whl/cu118 --no-
cache-dir
```

For CPU only (macOS/Linux or Windows without GPU):

```
pip install cellpose==3.1.1.2
pip install torch==1.13.1+cpu torchvision torchaudio --index-url
https://download.pytorch.org/whl/cpu --no-cache-dir
```

Common dependencies:

```
pip install numpy==1.24.3 opencv-python-headless scikit-image imageio
matplotlib scipy numba
```

C. Installation Verification

```
# Basic test
python -c "import cellpose; print('Cellpose successfully installed!')"

# GPU test (Windows with NVIDIA only)
```

```
python -c "import torch; print('CUDA available:',
torch.cuda.is_available()); print('GPU detected:',
torch.cuda.get_device_name(0) if torch.cuda.is_available() else 'None')"

# Cellpose GPU test
python -c "from cellpose import models; model = models.Cellpose(gpu=True);
print('Cellpose GPU: OK' if model.gpu else 'GPU not available')"
```

STEP 3: Cellpose Plugin Configuration in FIJI

Plugin configuration

1. In FIJI: open any image
2. Go to `Plugins → BIOP → Cellpose/Omnipose → Cellpose...`
3. **Check in "to add more parameters:"**
   - ✅ That `--use_gpu` is written (if not, add it)
   - ❌ That `--do_3d` is NOT present (if yes, delete it)

STEP 4: FLASH Macro Installation

A. Macro download

1. Save the macro code in a file named `FLASH.ijm`
2. **Place the file in FIJI's plugins folder:**
   - **Windows:** `C:\Fiji\plugins\`
   - **macOS:** `/Applications/Fiji.app/plugins/`
   - **Linux:** `/path/to/fiji/plugins/`

B. Installation in FIJI

1. **Restart FIJI**
2. The macro appears in `Plugins → FLASH`
3. Alternative: `Plugins → Macros → Run...` → select `FLASH.ijm`

STEP 5: Image Preparation

A. Required format

- **Format:** .tif or .tiff only
- **Type:** Multi-channel images (3-5 channels recommended)
- **Resolution:** Minimum 1024×1024 pixels
- **Depth:** 8-bit or 16-bit

B. Channel organization

Standard organization example:

1. **Channel 1:** Type IIb (MYH4) - optional
2. **Channel 2:** Laminin (membranes) - **required for segmentation**
3. **Channel 3:** Type I (MYH7) - optional
4. **Channel 4:** SDH (Oxidative) - optional
5. **Channel 5:** Type IIa (MYH2) - optional

C. Folder preparation

1. Create a folder for analysis
2. Copy all images to this folder
3. Verify all files are in .tif format
4. **Ensure channel organization is identical for all images**

STEP 6: Using FLASH

A. Analysis launch

1. Open FIJI
2. Launch macro: `Plugins → FLASH`
3. **Fill parameters:**
    - **Total number of channels:** 4 (example)
    - **Laminin channel:** 2 (example)
    - **Fiber type channels** according to your staining
    - ✅ Check "Auto-calibrate diameter" (recommended)

B. Advanced parameters (optional)

- **Cellpose sensitivity:** 1.0 (default)
- **Exclusion threshold:** 200 pixels
- **Adaptive threshold factor:** 0.3
- **GPU:** Check if NVIDIA card available and CUDA installed

C. Folder selection and processing

1. Select folder containing your images
2. Wait for processing completion (progress bar)
3. Check results in source folder

STEP 7: Results Interpretation

A. Automatically generated files (per image)

- **`ImageName_Final.tif`** - Image with colored overlay
- **`ImageName_Classified_Results.csv`** - Classification results
- **`ImageName_ROI_Set.zip`** - Regions of interest
- **`ImageName_cellposeMask.tif`** - Segmentation mask

B. Results analysis

- Open CSV file in Excel/LibreOffice
- **Important columns:**
  - **ROI:** Fiber number
  - **CSA/MinFeret:** Cross-sectional area/MinFeret
  - **Classification:** Identified fiber type
  - **Fiber type statistics** at bottom of file

Troubleshooting

Common problems and solutions

CUDA Error:

- Check graphics card compatibility
- Reinstall CUDA Toolkit 11.8
- Restart computer

Cellpose not working:

- Reinstall cellpose if necessary

Images not detected:

- Check .tif/.tiff format
- Verify channel organization
- Ensure laminin channel is correct

Memory issues:

- Close unnecessary applications
- Use smaller image
- Consider increasing virtual memory

Additional Notes

Performance optimization tips

- Use GPU acceleration when available for faster processing
- Process images in smaller batches if memory is limited

Best practices

- Always backup original images before processing
- Document channel organization for each experiment
- Validate results on a subset of images before batch processing
- Keep detailed records of analysis parameters used

Protocol tested on: Windows 10/11, macOS 12+, Ubuntu 20.04+
Version: FLASH v3.1 – July 2025
